Post-Graduate Degree Programme (CBCS)

in

ZOOLOGY

SEMESTER-I

NON CHORDATE AND INSECT ORGANIZATION

ZHT-101

SELF-LEARNING MATERIAL

DIRECTORATE OF OPEN AND DISTANCE LEARNING

UNIVERSITY OF KALYANI

Kalyani, Nadia

West Bengal, India

COURSE PREPARATION TEAM:

Faculty members of the Department of Zoology,
University of Kalyani and Assistant Professors of Zoology, DODL,
University of Kalyani.

December, 2018

Directorate of Open and Distance Learning, University of Kalyani
Published by the Directorate of Open and Distance Learning,
University of Kalyani, Kalyani-741235, West Bengal and Printed by
East India Photo Composing Centre, 69, Sisir Bhaduri Sarani, Kolkata-700 006

All right reserved. No part of this work should be reproduced in any form without the permission in writing from the Directorate of Open and Distance Learning, University of Kalyani.

Authors are responsible for the academic contents of the course as far as copyright laws are concerned.

Director's Note

Open and Distance Learning (ODL) systems play a threefold role- satisfying distance learners' needs of varying kinds and magnitudes, overcoming the hurdle of distance and reaching the unreached. Nevertheless, this robustness places challenges in front of the ODL systems managers, curriculum designers, Self Learning Materials (SLMs) writers, editors, production professionals and other personnel involved in them. A dedicated team of the University of Kalyani under the leadership of Hon'ble Vice-Chancellor has put its best efforts, professionally and in unison to promote Post Graduate Programmes in distance mode offered by the University of Kalyani. Developing quality printed SLMs for students under DODL within a limited time to cater to the academic requirements of the Course as per standards set by Distance Education Bureau of the University Grants Commission, New Delhi, India under Open and Distance Mode UGC Regulations, 2017 had been our endeavour and we are happy to have achieved our goal.

Utmost care has been taken to develop the SLMs useful to the learners and to avoid errors as far as possible. Further suggestions from the learners' end would be gracefully admitted and to be appreciated.

During the academic productions of the SLMs, the team continuously received positive stimulations and feedback from Professor (Dr.) Sankar Kumar Ghosh, Hon'ble Vice- Chancellor, University of Kalyani, who kindly accorded directions, encouragements and suggestions, offered constructive criticism to develop it within proper requirements. We gracefully, acknowledge his inspiration and guidance.

Due sincere thanks are being expressed to all the Members of PGBOS (DODL), University of Kalyani, Course Writers- who are serving subject experts serving at University Post Graduate departments and also to the authors and academicians whose academic contributions have been utilized to develop these SLMs. We humbly acknowledge their valuable academic contributions. I would like to convey thanks to all other University dignitaries and personnel who have been involved either at a conceptual level or at the operational level of the DODL of University of Kalyani.

Their concerted efforts have culminated in the compilation of comprehensive, learner-friendly, flexible texts that meet the curriculum requirements of the Post Graduate Programme through Distance Mode.

Self Learning Materials (SLMs) have been published by the Directorate of Open and Distance Learning, University of Kalyani, Kalyani-741235, West Bengal and all the copyright is reserved for University of Kalyani. No part of this work should be reproduced in any from without permission in writing from the appropriate authority of the University of Kalyani.

All the Self Learning Materials have been composed by distinguished faculty from reputed institutions, utilizing data from e-books, journals and websites.

Director
Directorate of Open & Distance Learning
University of Kalyani

HARD CORE THEORY PAPER
(ZHT–101)
Group A : Non Chordate

Module	Unit	Content	Credit	Class	Time (h)	Page No.
ZHT–101 (Non Chordate and Insect Organization)	**I**	Cell organelles in protozoa—Golgi, Mitochondria, Kinetoplast, Pellicle and Cuticle. Sensory organelles and reaction for stimuli in protozoa.	**1.5**	**1**	**1**	**7-20**
	II	Osmoregulation in Protozoa Cell association and cellular differentiation in protozoa.		**1**	**1**	**21-24**
	III	Insect flight: structure concerned, functional mechanism.		**1**	**1**	**25-36**
	IV	Insect blood: composition, functions, morphology of circulatory system. Morphology of excretory organs and their function in insects.		**1**	**1**	**37-45**
	V	Insect visual organs, their structure and functional mechanisms. Photogenic organs in insects: structure, mechanism and significance of light production.		**1**	**1**	**46-58**
	VI	Structure and function of sound producing organs in insects. Significance of sound production. Uses of sound in plant protection.		**1**	**1**	**59-63**

Group-A : NON CHORDATE

Unit-I
Cell organelles in protozoa - Golgi, Mitochondria, Kinetoplast, Pellicle and Cuticle and Sensory organelles and reaction for stimuli in protozoa.

Objectives:

In this section we will discuss about Cell organelles in protozoa - Golgi, Mitochondria, Kinetoplast, Pellicle and Cuticle and Sensory organelles and reaction for stimuli in protozoa.

Introduction:

The Protozoa means primitive animal. The basic element of all animal is eukaryotic cell. Generally the protozoans are unicellular. Animal cells are not homogenous. They differ morphologically and physiologically. The various morphological differentiations in structure of the cell are known as cell organelles. Adaptation of Protozoa is extended to all environments open to microorganism. Free-living forms occur in freshwater lakes, ponds, open ocean, river and even in temporary pools. Soil and sands also form natural environment for many Protozoa.

Such Protozoa live in the film of moisture surrounding the soil particles or sand grains. Parasite protozoa occur in many different species of animals, in certain plants and even in a few protozoans themselves. The microsporidian *Nosema notabilis* is a hyperparasite on myxosporidian *Spherospora*.

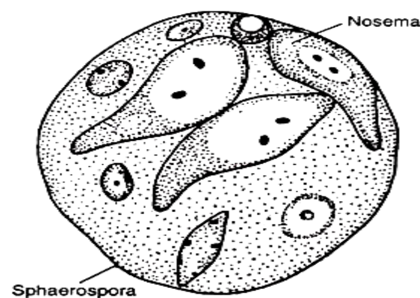


Fig. 10.52: A unique example of hyperparasitism. A trophozoite of *Sphaerospora polymorpha* is infected by trophozoites of *Nosema notabilis* (after Kudo).

In the body of the animal host they occur in the coelom, digestive tract, individual tissue cells and body fluids. Trypanosomes live in the blood plasma and malarial parasites invade

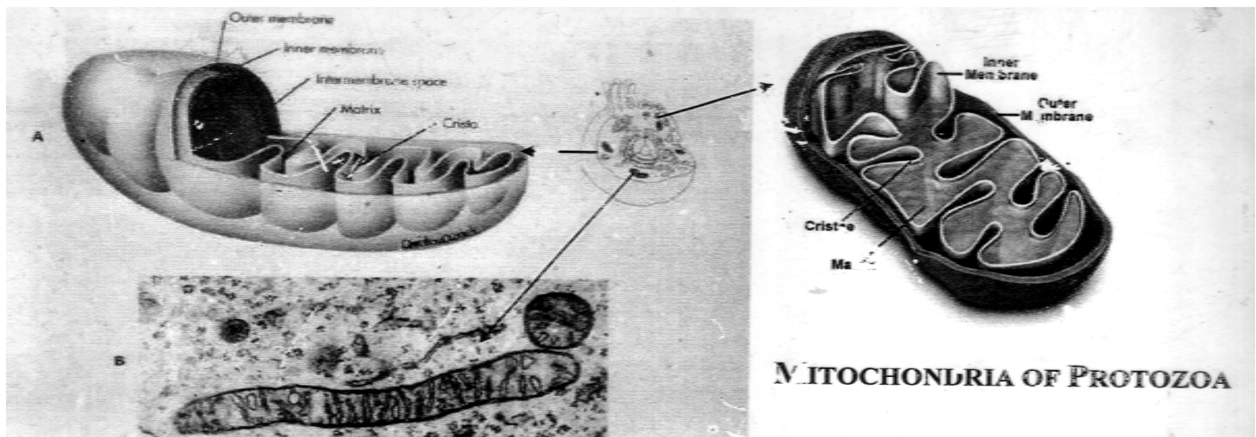
individual red blood cells and liver parenchyma cells and ultimately destroy them. Certain parasitic forms stick to a single kind of host while there are others which can live successfully in a few closely related species of animals, or two or more different and unrelated species of hosts. For such Protozoa that can invade unrelated host species, a change of hosts is essential for the completion of its life cycle. Of all the animals, human beings are most hospitable to parasitic protozoa for as many as twenty five different species of parasitic Protozoa have been encountered in them.

Mitochondria:

Mitochondria are present in all aerobic species. Number of mitochondria present in an organism are dependent on the volume of that particular organism. In *Tetrahymena* there are many mitochondria while in avian malarial parasite Plasmodium lophurae there are one or two mitochondria.

Structurally and functionally the Protozoan mitochondria differ very little from that of higher animals. The mitochondria occur as small spherical, oval, rod-shaped or filamentous bodies. They may be evenly distributed in the cytoplasm or may be localised in position as they are arranged between the kinetostomes of cillia in *Opalina* and *Paramocium*.

- ✖ Spherical or rod shaped.
- ✖ Consist of double membrane.
- ✖ Power house of cell due to ATP production.
- ✖ Have their own DNA



MITOCHONDRIA OF PROTOZOA

Golgi apparatus:

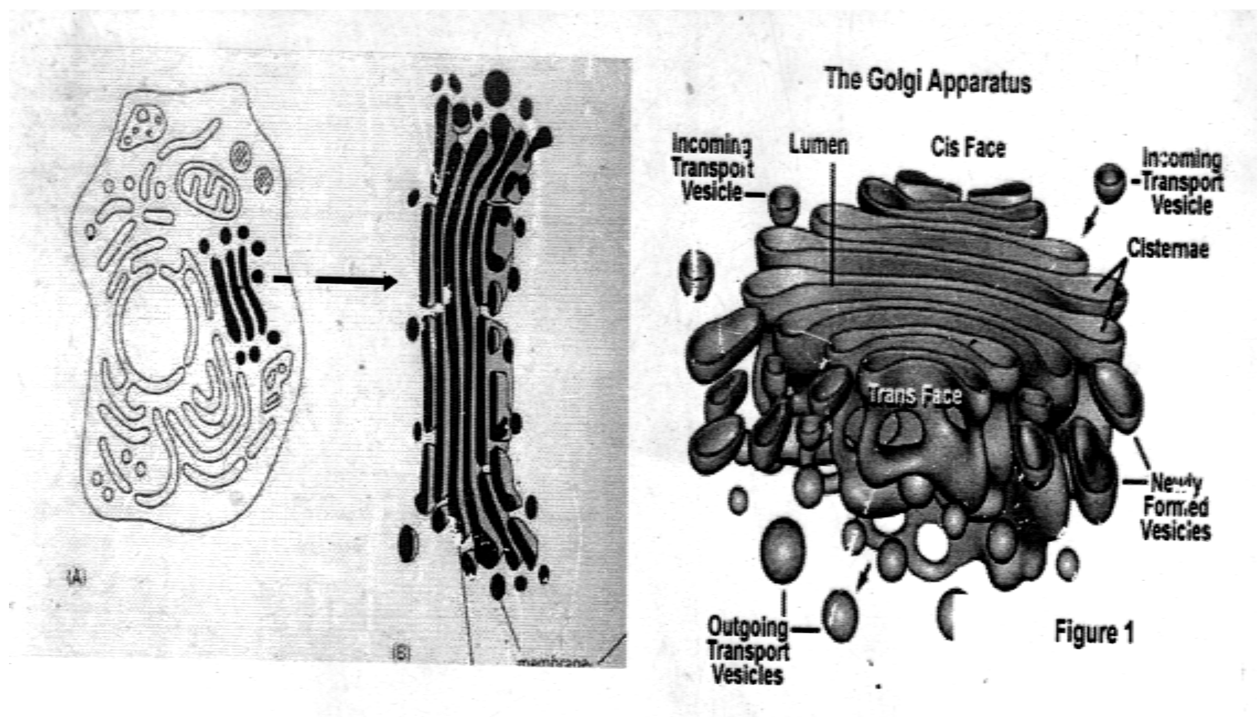
Presence of Golgi bodies in the form of compact, flattened and plate-like vesicles has been reported in Amoeba and Pelomyxa with certainty.

The Golgi apparatus is considered to be a definite cellular inclusion in the Protozoa, although there is still considerable disagreement regarding identification, morphology, distribution, and function in the different classes. The osmic techniques of Weigl (Mann-Kopsch) and Kolatchew, followed by bleaching in hydrogen peroxide or turpentine, are specific for the Golgi material in most organisms. The silver methods of Cajal and Da Fano are seldom successful in the Protozoa. The application of the ultra-centrifuge has proved that the neutral red bodies, i.e. the 'vacuome', are not identical with the Golgi bodies. In the Mastigophora the parabasal bodies of many forms are considered to represent the Golgi apparatus, though the homology may not be said yet to be fully proved. In other flagellates the cortex of the contractile vacuole is impregnated by the osmic Golgi techniques and its behaviour during division in many organisms is similar to that of the metazoan apparatus. These criteria seem sufficient to identify it as Golgi material. In the Sporozoa there is general agreement that the Golgi apparatus is represented by scattered globules or dictyosomes, often possessing osmiophile and osmiophobe regions. In some cells it shows the juxta-nuclear position so characteristic of the Golgi apparatus of many metazoan cells. The homology is also supported by the fact that in the centrifuged sporozoan the osmiophile material occupies the same position relative to the other inclusions as does the Golgi material in higher animal cells. There is still much confusion as to the exact nature of the Golgi apparatus in the Khizopoda, and there is little agreement as to its identity in any of the types studied. Some workers have described the Golgi apparatus as being represented by scattered osmiophilic globules or granules. Others have failed to identify any Golgi material in members of this class. In the Ciliata most workers agree that the osmiophile cortex to the contractile vacuole found in many forms represents the Golgi apparatus. In some instances scattered Golgi bodies are distributed throughout the cytoplasm. These are often present in combination with the Golgi cortex. There is no convincing evidence that any Golgi material is present in seawater ciliates, the freshwater form *Stytonychia*, or the parasitic *Nyctotherus*. Nassonow's view that the osmiophile material *together ivith* the contractile vacuole represented the Golgi apparatus is not supported by modern workers. The osmiophile material alone is believed to represent this inclusion. There is much evidence to suggest that there is a single type of granular Golgi body, from which both the osmiophile cortex type and the dictyosome type are formed. It is

likely that the Golgi apparatus arose in connexion with the base of the flagellum, later becoming associated with the vacuolar system in many forms. In view of the great diversity in form of the protozoan Golgi apparatus any single hypothesis regarding function is inadequate to explain all the facts. There is much evidence, however, to indicate that it is concerned with the mechanism of excretion or secretion, and possibly also with the osmoregulation of the organism. In the Sporozoa it may take part in fat metabolism.

- ✖ Situated near the nucleus
- ✖ Consist of many sacs / cistern like structures.

Receives proteins from ER & sorts, pack & deliver by secretory vesicles internally or externally



Cytoplasmic pigments:

Pigment granules of various colours—violet, blue, green, yellow, pink, red occur in the cytoplasm of phytoflagellates and ciliates, Red pigments occur in Phytomonadina and Euglena. When exposed to bright light these red pigments increase in number and mask the usual green colour of the animals. The pink pigments of *Blepherismaundulans* is toxic to other ciliates.

Contractile vacuoles:

An intracellular, small inconspicuous, membrane bound, spherical, fluid filled vesicle that maintains the osmoregulation in some protozoans.

Occurrence:

They are mainly found in freshwater protozoans such as sarcodines, flagellates and ciliates. It is also found in some marine forms but totally absent in parasitic forms such as Sporozoa.

Position:

The position, number and accessory structures of the contractile vacuoles are different in different Protozoa. In *Amoeba*, the position of the vacuole changes with the movement of the organism. In ciliates and flagellates the position is more or less fixed.

In many Heliozoans contractile vacuoles occur in the ectoplasm. In *Balantidium* and *Nyctotherus* the contractile vacuole is situated close to the cytopyge. Deep seated contractile vacuoles are often provided with a delicate duct which connects the vacuole with the pore on the pellicle as in *Paramecium woodruffi*.

Number:

The number of vacuoles varies in different groups of protozoa but remains constant in a particular species. It is single in *Amoeba* and *Euglena* but two in *Paramoecium*.

Shape:

The contractile vacuoles are generally spherical in shape, and simplest spherical form is found in Amoeba but in many ciliates they have become star-shaped (e.g., *Paramoecium*) because of a number of collecting canals (5-12) which radiate from the main vacuole.

Each canal consists of a narrow elongated terminal part, a swelling ampulla and a short injector canal which opens into the vacuole. In flagellates like Euglena, the contractile vacuole is more complicated and a number of small accessory vacuoles are located around the main contractile vacuole.

Ultrastructure:

The contractile vacuole complex consists of a contractile vacuole proper and a system of vesicles and tubules called the spongiomewhich lies between the mitochondria and plasmalemma and helps in collection of fluid from the surrounding cytoplasm and transfers into the contractile vacuole.

A discharge pore is situated in the plasmalemma through which water expels to the outside. The diameter of vesicles is about 1 μm. The breadth of the boundary membrane of the vacuole is about 0.5 μm. The volume of vesicles varies from 20 nm to 100 nm. Small fluid-filled vesicles contain polyribsomes. Rough walled vesicles are similar in structure to E.R. of mammals.

Types of contractile vacuoles:

The contractile vacuoles may be following type on the basis of complexity of the spongiome:

(i) In the first kind, only collecting tubules are present in the spongiome and collecting fluid is expelled out through the pore of the plasmalemma.

(ii) In the second kind, the tubules and vesicles are present in the formation of spongiome and the tubules collect fluid from the cytoplasm. The fluid is transferred to the permanent collecting canal which is dilated to form ampulla. The above mentioned two types are found in ciliates.

(iii) In the third type, a permanent pore is lacking and vacuoles are formed by the fusion of small fluid filled vesicles. The vacuoles are disappeared after the discharge of solutes (e.g., Amoeba).

(iv) In the fourth type, the vacuole is filled by the conspicuous ampullae and through a permanent pore the fluid is expelled out. The ampullae, permanent pores and bundle of microtubules are absent in the third and fourth types.

Formation of the contractile vacuole:

All the contractile vacuoles pass through a cycle. The origin of a new vacuole involves the fusion of many small vacuoles in the cytoplasm.

The young vacuoles grow in volume (diastole) by fusion of other small vacuoles or by receiving contribution of fluids from visible canals. When the volume reaches its maximum the contents are discharged to the outside (systole) through the pores in the pellicle or into the gullet.

Functions of contractile vacuole:

(i) Osmoregulation:

All freshwater protozoa solve the constant osmotic problem with the help of the contractile vacuoles. The plasmalemma in these organisms is semipermeable.

And as the concentration of water in the cytoplasm is lower than that of the surrounding medium a constant flow of water into the animal body occurs. Water passes more rapidly into

the body than it leaves. The organisms get rid of these excess water by pumping them out with the help of contractile vacuoles and prevent the body from being waterlogged.

(ii) Excretion:

(a) Some amount of nitrogenous wastes are voided along with the discharged water.

(b) Ludwing confirms that the contractile vacuole not only regulates the osmotic pressure but also helps in the excretion of CO_2.

(iii) It is considered by the differences in pulsation frequency that contractile vacuole is mainly excretory in marine protozoa, but excretory and osmotic pressure regulate in freshwater protozoans.

Sensory vacuoles or Concretion vacuoles:

Certain parasitic ciliates of the families Butschliidae and Paraisotrichidae have a number of vacuoles located in the anterior region of the body under a pellicular cap. The vacuolar cavity contains a number of granules, called statoliths and a number of fibrils join the vacuole with the pellicle. These vacuoles are considered as statocysts and excretory vacuoles.

Superficial vacuoles:

Superficial vacuoles are found in passively floating Sarcodina which have a foamy outer cytoplasm. These thin-walled vacuoles presumably containing a light weight fluid or gas maintain the organism at a particular depth. When the vacuoles collapse, the animal sinks. When new vacuoles develop, the organism rises. Thus the superficial vacuoles help in floatation.

D. Chromatophores, Pyrenoids and Stigma*:*

Chromatophores, i.e. chloroplastid and some non-green organelles are restricted to plant-like flagellates. The chromatophores occur in discoid, ovoid, band-like, rod-like or cup-like forms. In *Chlamydomonas* a single cup-shaped chromatophore is found and it is considered as a primitive form. This cup may be subdivided into pairs of lateral lobes or even to separate lobes.

Some of the Euglenidae contain many flattened chromatophores arranged near the surface of the body. In Peridinium chromatophores are arranged near the surface of the body and form anastomosing network. Electron micrograph studies have revealed that chromatophores are double membranes and have a lamellar structure in which electron opaque layers alternate with electron transparent layers.

Electron opaque are believed to be laden with photosynthetic pigments. Chlorophyll is the most predominant pigment in the chromatophores but there are other pigments present in significant amounts.

These pigments are greenish yellow, yellow red, brown and even blue and when present in superabundance they mask the green chlorophyll. The cytoplasmic pink pigment of Blepherisma is toxic to several other ciliates and to small metazoons. Even the annelid worm, Dero is susceptible to it. When exposed to very bright light, Blepherisma falls a victim of its own pigment or a toxic product of the pigment.

Pyrenoids are structures which usually remain associated with the chromatophores though all chromatophores bearing flagellates do not possess them. The structure of the pyrenoids varies from solid bodies to aggregate of granules. In Euglena the pyrenoid is encased in a layer of paramylum while in Chlaydomonas it is often surrounded by starch granules.From this close structural relationship it is suggested that pyrenoids are functionally involved in the synthesis of starch and other polysaccharides. However, there must be other machinery for the synthesis of these substances as there are certain flagellates without pyrenoid, which can synthesise such polysaccharides.

Stigma or eye-spot occurs in many chlorophyll bearing and a few colourless flagellates. The stigma contains reddish pigments presumed to be carotenoid. The stigma of Euglena shows a mass of reddish granules embedded in a matrix. It is a discoid body, placed close to the gullet.

The flagellum which arises from the base of the reservoir through the gullet bears a small granule or a paraflagellar body at the level of the stigma. In Volvox and related colonial types the stigma is made up of a concave mass of pigments and a hyaline lens.

The role of the stigma is to help in the orientation of the flagellates towards a suitable light source. From the work on Euglena it is assumed that the parabasal body of the flagellum is a light sensitive structure and it becomes stimulated by the light energy which the stigma absorbs.

Taxis:

The **cytoplasm of protozoa** can respond to environmental changes by showing behavioural changes. These movements in response to the **stimuli** are called **taxes.**

Taxes – positive ; when movement is towards stimulus and **negative ;** when movement is away. Direct alignment towards or away from stimulus is called **topotaxis.** An escape movement which is merely evasive is **phobotaxis.** Responsive movements in response to the acting stimulus:-

a) **Thermotaxis –** response to temperature.

b) **Chemotaxis –** response to chemicals

c) **Galvanotaxis** – response to electric current

d) **Phototaxis –** response to light

e) **Barotaxis -** response to pressure

f) **Thigmotaxis** – response to water current

g) **Geotaxis –** response to Earth's gravitational pull

Response to Stimuli:

In *Amoeba*, taxis can be shown by the whole cell or certain parts of the cell. When living paramecia are cut up, the pieces continue to be thigmotactic. If the cut is in the front of the cell mouth, the posterior part remains chemotactic, thigmotactic but not thermotactic. Thus, sensitivity to temperature is localized in the anterior part of the cell. If these sort of differences in cellular response to stimuli exist then we can state that these specific areas in the cell are sense organelles. In flagellate amoeboid forms; Rhizomastigina, flagella function as tactile organelles.

The cilia of ciliates are also sensitive to pressure and current, eg. In *Paramecium caudatum,* they help stabilize locomotion. *P. caudatum*is positively rheotactic.

Phototaxis

Positive phototaxis occurs particularly in pigmented phytoflagellates, whose metabolism is light dependent. some of these produce specific photo-sensory organelles, the **stigma or eye-spots.** Stratified hyaline bodies which appear homogeneous by light microscope, but are found to have differentiation when studied using electron microscope form a lens system which produce intensification of light incident on a light-sensitive absorbing pigment.

In ***Euglena*** a pigmented stigma serves merely to cast a shadow in a region of light-sensitive cytoplasm so that the direction of incident light is ascertained. The stigmata are often differentiations of chloroplasts, however in **Euglenoidina,** they lie laterally at the bottom of

the flagellar sac. while autotrophic flagellates are positively phototactile, most heterotrophic **Rhizopoda**, show negative phototaxis provided they do not contain **autotrophic symbionts**, the **zoochlorellae**as in ***Paramecium bursaria.***

Chemotaxis

Dilute fatty acids like butyric acid have a strong positive effect on some flagellates; *Euglena, Astasia*.pH value is important in the chemotaxis of paramecia, optimum being 5.4 -6.4 for *P.caudatum*. If a drop of liquid evoking a negative chemotactic response is added to an uniform suspension of paramecia, they draw away immediately. Conversely, they collect together in a chemotactically positive drop. In case of negative chemotaxis also, paramecia withdraw themselves, but however accumulate in a region of optimal diffusion. If the paramecia swim into a chemically positive drop, there is a **trap effect**. A photobactic evasion motion occurs now and them with an aimless dodging movement and return so that this process is repeated often. **Chemotaxis** is very important in food selection, locating a sexual partner etc. The malarial sporozoites can be stated as a good example; after the oocysts have burst, they circulate with the haemolymph of mosquitoes throughout the whole body and are finally gathered by the trap effect in the salivary glands. In humans however, only the later merozoites have an organotrophy towards erythrocytes.

Thermotaxis

It also proceeds mainly as **phobotaxis,** as in hypotrichous ciliate **Oxytricha.** In this case too, there are species specific optimal ranges, example- **24– 28 °C.** Reaction to electro-stimuli is also species specific, ***Trachelomonas*** **amoebae** move towards **cathode**. ***Spirostomum*** adopts atransverse position across the current. ***Polytoma, Chilomonas*** **and** ***Opalina***migrate to the **anode.** Whilst ***Paramecium*** migrates to the cathode when current is weak, a strong current activates cilia, at the posterior end, which beat in opposition to others causing a return to anode.

Geotaxis

Some ciliates have special **statocysts** which can sense the earth's gravitational pull. These are vesicles, in which enclosed granules exert pressure on whichever is the lower side at that time.

The Muller corpuscles, of genera ***Remanella*** and ***Loxodes***are involved in geotaxis. In the other protozoa, the food and normal excretory vacuoles in the cytoplasm assume the role of geotactic indicators. Most ciliates show a negative geotactic response. Thus ***Paramecium*** rises to the surface. However after phagocytosing finely powdered **iron,** the pressure effect in

its food vacuoles can be altered in the magnetic field, resulting in a motion along the lines of magnetic force.

Neuromotor Organelle

A well-defined system of nerves is lacking in the protozoan. But it has been seen that the cilia of the ciliates are capable of making a well co-ordinated movement. It is known that the ciliary co-ordination is due to the presence of certain fibrillar system in *Epidinium*. The presence of a neuromotor apparatus in the system of *Epidinium* is advocated. This apparatus consists of a central motor mass, called the Motorium, located in the ectoplasm, and from it definite strands radiate to the roots of the membranellae, cytopharynx and other structures. Similar apparatus has been observed in *Balantidium*, *Paramoecium* and many other ciliates. Klein (1926) by silver-impregnation method has demonstrated the presence of such radiating fibrils and has designated the fibres as silver lines and the whole complex as silver line system.

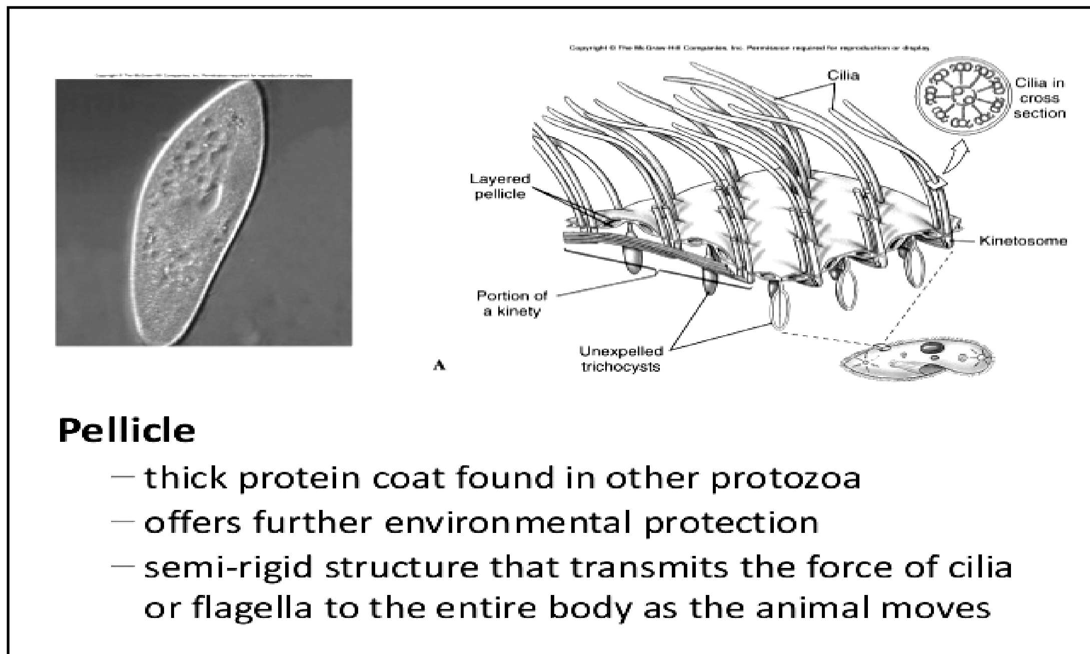
Protective or Supporting Organelles

Pellicle:

Outside the plasma membrane many protozoa have a differentiated pellicle, i.e., a continuous covering which may be more or less flexible. The thick pellicles often show surface decorations in the form of ridges, papillae or pits. The pellicle in ciliates is perforated through which cilia and trichocysts emerge. The chief component of pellicle in case of *Amoeba* is polysaccharide and in *Euglena* the principal component is protein.

Unlike plants, fungi and most types of algae, protozoans do not typically have a rigid cell wall, but are usually enveloped by elastic structures of membranes that permit movement of the cell. In some protozoans, such as the ciliates and euglenozoans, the cell is supported by a composite membranous envelope called the "pellicle." The pellicle gives some shape to the cell, especially during locomotion. Pellicles of protozoan organisms vary from flexible and elastic to fairly rigid. In ciliates and Apicomplexa, the pellicle is supported by closely packed vesicles called alveoli. In euglenids, it is formed from protein strips arranged spirally along the length of the body. Familiar examples of protists with a pellicle are the euglenoids and the ciliate Paramecium. In some protozoa, the pellicle hosts epibiotic bacteria that adhere to the surface by their fimbriae (attachment pili)

In other Protozoa, ***Euglena***, the body covering is in the form of a differentiated pellicle, which is some what thicker and firm. It is underlined by plasma membrane and is formed of proteins . The rigidity of pellicle gives definite shape to the body. The thickened pellicle in some of the more specialized Protozoa is variously ridge and sculptured as in ***Paramoecium***, ***Coleps*** etc.



The pellicle is a thin layer supporting the cell membrane in various protozoa, such as ciliates, protecting them and allowing them to retain their shape, especially during locomotion, allowing the organism to be more hydrodynamic. The pellicle varies from flexible and elastic to rigid. Although somewhat stiff, the pellicle is also flexible and allows the protist to fit into tighter spaces. In ciliates and Apicomplexa, it is formed from closely packed vesicles called alveoli. In euglenids, it is formed from protein strips arranged spirally along the length of the body. Familiar examples of protists with a pellicle are the euglenoids and the ciliate *P*aramecium. In some protozoa, the pellicle hosts epibiotic bacteria that adhere to the surface by their fimbriae or "attachment pili".Pellicle (biology), a thin layer supporting the cell membrane in various protozoa. Pellicle mirror, a thin plastic membrane which may be used as a beam splitter or protective cover in optical systems. Pellicle (dental), the thin layer of salivary glycoproteins deposited on the teeth of many species through normal biologic processes.

Pellicle, the protective cover which can be applied to a photomask used in semiconductor device fabrication. The pellicle protects the photomask from damage and dirt. Pellicle (cooking), a skin or coating of proteins on the surface of meat, fish or poultry, which allow smoke to better adhere the surface of the meat during the smoking process. Pellicle (material), a brand name for a very resistant synthetic material used for covering different surfaces, such as that of the Aeron chair.

Cuticle

Some invertebrates like rotifers have cuticles that are thin and elastic. In crustaceans, arachnids, insects, cuticles are thick and rigid and support the body. Such cuticles consist of chitin and proteins in rigid plates that a flexible membrane links together. Cuticles retard growth. Thus some of invertebrates like arthropods periodically undergo molting or ecdysis. In Cnidarians like Hydra the epidermis is only few cell layers thick. Other cnidarians (e.g. corals) have mucous glands that secrete calcium carbonate ($CaCO_3$) shell. Outer covering of parasitic flukes and tapeworms in a complex syncytuim called tegument (i.e. to cover) host enzymes cannot digest it.

Kinetoplast:

A **kinetoplast** is a network of circular DNA (called kDNA) inside a large mitochondrion that contains many copies of the mitochondrial genome. The most common kinetoplast structure is a disk, but they have been observed in other arrangements. Kinetoplasts are only found in excavates of the class Kinetoplastida. The variation in the structures of kinetoplasts may reflect phylogenic relationships between kinetoplastids. A kinetoplast is usually adjacent to the organism's flagellar basal body, suggesting that it is tightly bound to the cytoskeleton. In *Trypanosoma brucei* this cytoskeletal connection is called the tripartite attachment complex and includes the protein p166.

The kinetoplast contains circular DNA in two forms, maxicircles and minicircles. Maxicircles are between 20 and 40kb in size and there are a few dozen per kinetoplast. There are several thousand minicircles per kinetoplast and they are between 0.5 and 1kb in size. Maxicircles encode the typical protein products needed for the mitochondria which is encrypted. Herein lies the only known function of the minicircles - producing guide RNA (gRNA) to decode this encrypted maxicircle information, typically through the insertion or deletion of uridine residues. The network of maxicircles and minicircles are catenated to form a planar network that resembles chain mail. Reproduction of this network then requires that these

rings be disconnected from the parental kinetoplast and subsequently reconnected in the daughter kinetoplast. This unique mode of DNA replication may inspire potential drug targets.

The best studied kDNA structure is that of *Crithidia fasciculata*, a catenated disk of circular kDNA maxicircles and minicircles, most of which are not supercoiled. Exterior to the kDNA disk but directly adjacent are two complexes of proteins situated 180˚ from each other and are involved in minicircle replication.

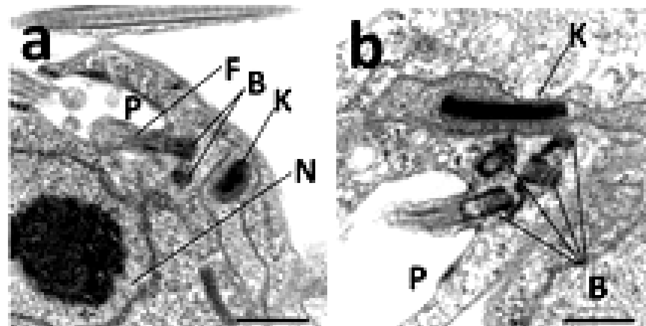


Figure : Electron micrograph of normal kinetoplast (K) of *Trypanosoma brucei*

Probable Questions:

1. What is cristae?
2. Describe the ultra structure of mitochondria?
3. How mitochondria function in the body of a protozoan cell?
4. What do you mean by kinetosome ?
5. What is Golgi bodies?.
6. State the ultra structure of Golgi bodies.
7. What is cistern?
8. State the function of Golgi bodies.
9. What is Pellicle ?.
10. How it protect a protozoan cell?.
11. Describe the electron microscopic structure of pellicle?.
12. What is cuticle?

Suggested Readings / References:

1) Protozoa by Wastphal .

2) Protozoology by Kudo Richard. R.

3) An Introduction to the study of Protozoa by Doris L. Mackinnon & R. S. Hawes.

Unit-II
Osmoregulation in Protozoa and Cell Association and Cellular differentiation in protozoa

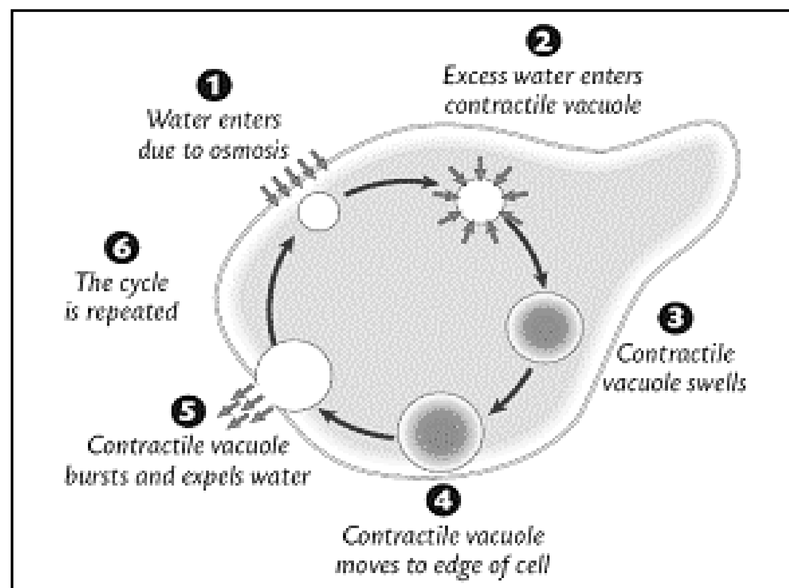
Objectives:

In this Unit we will discuss about Osmoregulation in Protozoa and Cell Association and Cellular differentiation in protozoa

A. Osmoregulation in Protozoa

Introduction:

Water is vital to the chemistry of life. Therefore, must attain a water balance within the body Water ba lance s ystems ar e ba sed on three p rocesses: Diffusion,Osmosis and Active transport.



What is Osmosis?

Osmosis is the move ment of w ater acr oss a pa rtially (semi) pe rmeable membrane from an area of high water potential (low solute concentration) to an area of low water potential (high solute concentration). The solute may be something like salt.

A **semipermeable m embrane** is a ba rrier t hat p ermits t he pa ssage of s ome s ubstances but not others. Cell membranes are described as selectively permeable because not only do they allow t he p assage of water but a lso allow t he pa ssage o f c ertain solutes (dissolved substances). It is a passive process, requiring now energy and is a special case of diffusion.A weak or dilute solution with little dissolved solute in it is a **hypotonic** solution. A strong or concentrated solution is a **hypertonic** solution.

Two solutions with the same concentration (i.e. the same concentration of water and solute) are **isotonic** solutions.Some examples of osmosis are:

- Absorption of water by plant roots.
- Reabsorption of water by the proximal and distal convoluted tubules of the nephron.
- Reabsorption of tissue fluid into the venule ends of the blood capillaries.
- Absorption of water by the alimentary canal — stomach, small intestine and the colon.

Diffusion

Diffusion is the process by which molecules spread from areas of high concentration, to areas of low concentration.When the molecules are even throughout a space - it is called **Equilibrium.** A concentration gradient is a difference between concentrations in a space.The rate (speed) at which the a substance can diffuse in and out of cells can be affected by a number of factors such as temperature and surface area.

1. The medium - diffusion is faster in gases than in liquids
2. The difference between the concentration of a substance inside and outside the cell (concentration gradient)
3. The size of the molecules - smaller diffuse quicker
4. Temperature - molecules move faster when heated which will increase the rate of diffusion.

In addition to these factors, the surface area to volume ratiocan determine the amount of nutrients that can reach the centre of a cell. When you increase the surface area (like in the lungs and the digestive tract), but maintain the volume, you can increase the rate at which nutrients can diffuse throughout a cell. Cells can be different shapes to increase their surface area to volume ratio. E.g. the villi on the cells that line the intestines gives more area for nutrient absorption.

Multicellular organisms use transport epithelia to control water loss and excretion

Platyhelminthes: Protonephridia (flame cells) collect excess water in addition to nitrogenous wastes, empty into nephridiopore, excretes NH_3.

Annelida: Metanephridia organized on a per segment basis collect waste from coelom via the nephrostome, counters water uptake by epidermis, excretes NH_3.

Insecta: Malpighian tubules collect nitrogenous wastes from haemocoel, excretes Uric Acid.

B. Cell Association and Cellular Differentiation in Protozoa:

It was said at the outset that the word "protozoan" does not necessarily imply a unicellular condition. If the Protozoa are nevertheless generally unicellular, this is based on the nature of their organization. They do not form the physiological unity of a centrally controlled multicellular confederation. However, there are first indications of some tendencies towards a cell association. Colonial associations occur in the flagellates *Chrysomonadina, Phytomonadina* and *Protomonadina*. Some genera of the Radiolaria (*Collozoum, Sphaerozoum, Collosphaera*) form 4-6 cm swimming colonies. Many eritrichousciloiates, too form colonies. These associations generally provide greater protection to the individual cells and increase the water current and the procuring of food by means of the action of the flagella and cilia. The protective effect is increased still more when as in *Carchesium* and *Zoothamnium*, a contact stimulus can be transmitted by one individual to the entire colony, thereby leading to a united escape reaction by contraction.

A further advance conducting to organism survival is cell differentiation. This occurs temporally in some amoebae, the Acrasiae, for example in *Dictyostellium discoideum*. When environmental conditions are unfavourable, individual amoebae secretes a hormone, acrasin, which diffuses into the medium and stimulates the amoebae to mass together into a conus structure. The conus tips over; all the constituent amoebae shift their position and finally pile up again to form the so-called sporophore. Cell differentiation occurs in this structure; the amoebae constituting the stalk fuse together into a compact tissue, while the amoebae in the head of the sporophore survive as cysts. In the flagellates, the individuals forming an association of cells usually retain their capacity for independent development. However, in the Pgytomonadina, some volvocidae show differentiation.

The individual colonies contain cell which are still all the same and which can all divide, but in *Pleodorina califonica* there are two types of cell which can be distinguished by their difference in size. The small somatic cells have lost the capacity to divide, so that only the larger generative cells are still capable of forming new colonies. In *Volvox aureus* and *V. globator* only a few generative individuals remain. A corresponding condition is found n the peritrichous ciliate *Zoothamnium alternaus*. Only a few macrozoid individuals can detach themselves and are still able to divide, while the many somatic microzoids have lost the ability to produce daughter colonies. The Cnidosporidia have a particular form of cell differentiation during the production of spores.

The site formation of the spores in the Myxosporidia is multinuclear at first and becomes temporarily multicellular. In this relatively short phase of development, the physiological unity related to that of the metazoan cells is still less marked that in, for example, the colonies of the Phytomonadina. In the Phytomonadina (Volvocidae) not only agamous reproduction but also sexuality is sometimes limited to a few individuals scattered within the colony. This differentiation into pure mortal soma cells and the cells of the ger path achieves at highest development in the genus *Volvox*. A parallel intracellular development occurs in the macronuclei of the eucilliates and of ome Foraminifera which also have only a somatic function. These examples reveal a certain tendency in the Protozoa towards the subsequent development of the Metazoa, but the fact remains that they do not yet have the physiological unity of a multicellular organism as possessed by the true Metazoa.

Probable Questions:

1. Define Osmosis. Write notes on role of osmosis in Protozoa.
2. Define Diffusion. Write notes on role of diffusion in Protozoa.
3. Write a short note on Cell Association and Cellular Differentiation in Protozoa.

Suggested Readings:

1)Protozoa by Wastphal .

2) Protozoology by Kudo Richard. R.

3) An Introduction to the study of Protozoa by Doris L. Mackinnon & R. S. Hawes.

UNIT-III

Insect blood: Composition, functions, morphology of circulatory system. Morphology of Excretory Organs and Their Function in Insects

Objective

In this Unit we will discuss about Insect blood: Composition, functions, morphology of circulatory system. Morphology of Excretory Organs and Their Function in Insects

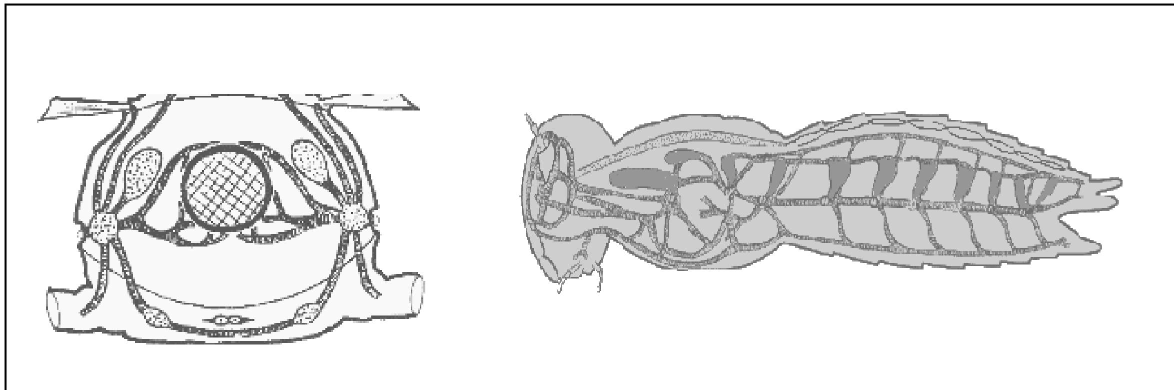
A. Insect blood: Composition, functions, morphology of circulatory system

Introduction:

Insects, like all other arthropods, have an open circulatory system which differs in both structure and function from the closed circulatory system found in humans and other vertebrates. In a closed system, blood is always contained within vessels (arteries, veins, capillaries, or the heart itself). In an open system, blood (usually called hemolymph) spends much of its time flowing freely within body cavities where it makes direct contact with all internal tissues and organs.

The circulatory system is responsible for movement of nutrients, salts, hormones, and metabolic wastes throughout the insect's body. In addition, it plays several critical roles in defense: it seals off wounds through a clotting reaction, it encapsulates and destroys internal parasites or other invaders, and in some species, it produces (or sequesters) distasteful compounds that provide a degree of protection against predators. The hydraulic (liquid) properties of blood are important as well. Hydrostatic pressure generated internally by muscle contraction is used to facilitate hatching, moulting, expansion of body and wings after moulting, physical movements (especially in soft-bodied larvae), reproduction (e.g. insemination and oviposition), and evagination of certain types of exocrine glands. In some insects, the blood aids in thermoregulation: it can help cool the body by conducting excess heat away from active flight muscles or it can warm the body by collecting and circulating heat absorbed while basking in the sun.

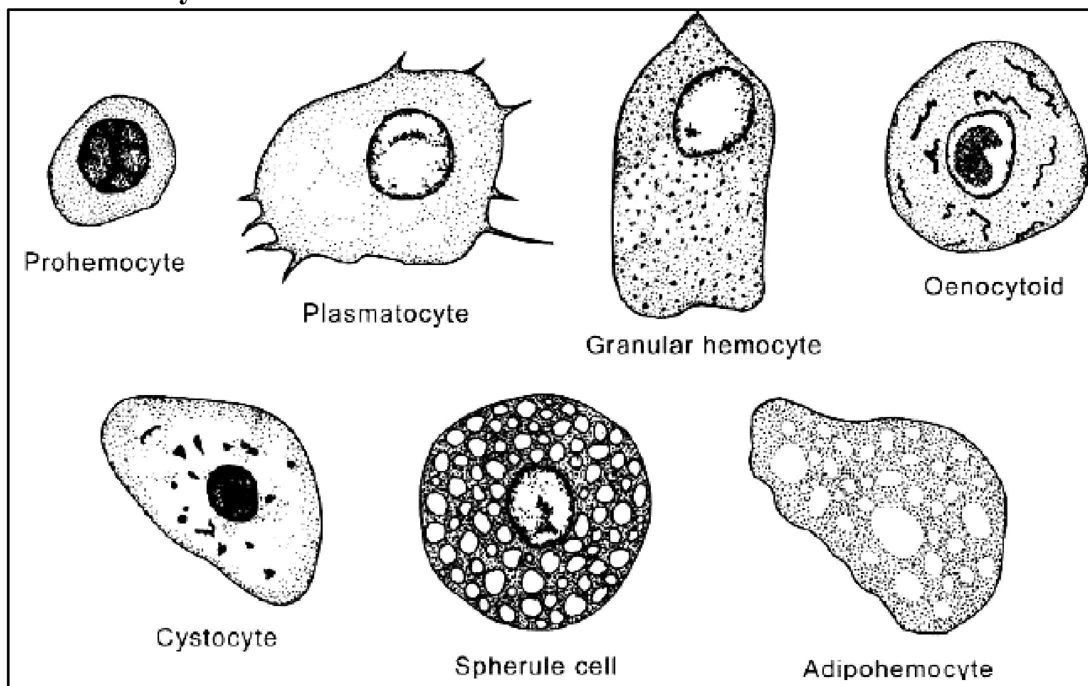
Structural component of insect circulatory system:



A dorsal vessel is the major structural component of an insect's circulatory system. This tube runs longitudinally through the thorax and abdomen, along the inside of the dorsal body wall. In most insects, it is a fragile, membranous structure that collects hemolymph in the abdomen and conducts it forward to the head. In the abdomen, the dorsal vessel is called the heart. It is divided segmentally into chambers that are separated by valves (ostia) to ensure one-way flow of he molymph. A pair of alary muscles is at tached laterally t o the w alls of each chamber.Peristaltic contractions of t he these m uscles f orce t he he molymph f orward from chamber to chamber. During each diastolic phase (relaxation), the ostia open to allow inflow of hemolymph from the body cavity. The heart's contraction rate varies considerably from species to species — typically in the range of 30 to 200 beats per minute. The rate tends to fall as ambient temperature drops and rise as temperature (or the insect's level of activity) increases. In front of the heart, the dorsal vessel lacks valves or musculature. It is a simple tube (called the aorta) which c ontinues f orward t o t he he ad a nd e mpties ne ar t he br ain. Hemolymph bathes the organs and muscles of the head as it emerges from the aorta, and then haphazardly percolates back over the alimentary canal and through the body until it r eaches the abdomen and re-enters the heart. To facilitate circulation of hemolymph, the body cavity is divided into three compartments (called blood sinuses) by two thin sheets of muscle and/or membrane known as the dorsal and ventral diaphragms. The dorsal diaphragm is formed by alary m uscles of t he he art and related structures; i t s eparates t he pericardial sinus from the perivisceral sinus. T he ventral diaphragm usually cov ers t he ne rve cord; i t s eparates the perivisceral sinus from the perineural sinus. In some insects, pulsatile organs are located near the base of the wings or legs. These muscular "pumps" do not usually contract on a regular basis, but they act in conjunction with certain body movements to force hemolymph out into the extremities. About 90% of insect hemolymph is plasma: a watery fluid - usually clear, but sometimes greenish or yellowish in color. Compared to vertebrate blood, it contains

relatively high concentrations of amino acids, proteins, sugars, and inorganic ions. Overwintering insects often sequester enough ribulose, trehalose, or glycerol in the plasma to prevent it from freezing during the coldest winters. The remaining 10% of hemolymph volume is made up of various cell types (collectively known as hemocytes); they are involved in the clotting reaction, phagocytosis, and/or encapsulation of foreign bodies. The density of insect hemocytes can fluctuate from less than 25,000 to more than 100,000 per cubic millimeter, but this is significantly fewer than the 5 million red blood cells, 300,000 platelets, and 7000 white blood cells found in the same volume of human blood. With the exception of a few aquatic midges, insect hemolymph does not contain hemoglobin (or red blood cells). Oxygen is delivered by the tracheal system, not the circulatory system.

Types of haemocytes:



Function of haemocytes: Insect hemocytes originate from mesodermally derived stem cells that differentiate into specific lineages identified by morphology, function, and molecular markers.

1. Phagocytosis is the most common function of blood cells-ingesting foreign matter, bacteria etc.
2. Haemocytes help in formation of fat bodies.
3. Transport of nutrient and waste
4. Temperature control.
5. Protection against parasites and diseases.
6. For maintaining hydrostatic skeletal element.

B. Morphology of Excretory Organs and Their Function in Insects

Introduction:

The removal of waste products of metabolism, especially nitrogenous compounds from the body of insects is known as excretion. This involves the maintenance of a constant level of salts and water and osmotic pressure in the haemolymph and the elimination of toxic nitrogenous wastes derived from protein and purine metabolism and thereby helps the insect to maintain salt water balance and physiological homeostasis.

In most Insects the Malpighian tubules and the rectnum are concerned in excretion and salt and water regulation. Water, salts and excretory products pass into the Malpighian tubules from the haemolymph and controlled resorption takes place in the rectum. Nitrogen is usually excreted as uric acid since this is relatively non-toxic and insoluble. It can therefore be excreted with a minimum of water and the terrestrial insect is thus able to conserve water. Sometimes nitrogenous end products are stored in some relatively non-toxic form rather than being passed out of the body.

I. Excretory organs:

1. Malpighian tubules

The main organ of excretion and osmoregulation in insects are the malpighian tubules, acting in association with rectum or ileum. These tubules are long, thin, blind-ending tubules arising from the gut near the junction of mid and hindgut and lying freely in the body cavity. They may open independently into the gut or may join in groups at an ampulla or a more tubular ureter which then enters the gut. This structure was discovered by Marcello Malpighi.

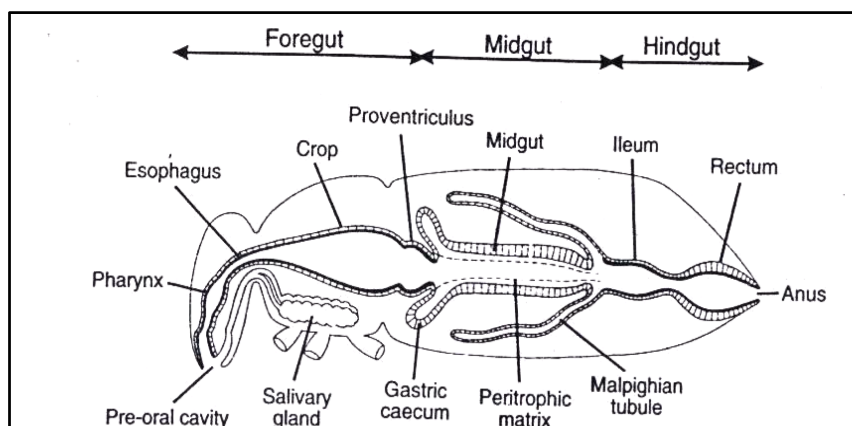


Figure: Location and structure of the Malpighian tubules in a typical insect.

The wall of the tubule consist of a single layer of cells surrounding a blind-ending lumen, they are absent in spring tail and aphids, 2 numbers in scale insects, 4 in bugs, 5 in

mosquitoes, 6 in moths and butterflies, 60 in cockroach and more than 200 in locusts. The cells stand on a tough basement membrane outside which are strands of muscle forming wide spirals round the tubule. The Malpighian tubules of *Rhodnius* and Lepidoptera and Diptera in general have no muscles other than a series of circular longitudinal muscles proximally. These muscles writhing movements of the tubules in the haemolymph ensuring maximum of contact with the blood and at the same time, perhaps, improving the movement of fluid in the tubules themselves. Outside the muscle is a peritoneal sheath formed from tracheoblasts.

The cells of the Malpighian tubules of *Rhodnius* and some other insects are of two types resulting in functional differentiation of the tubules. The distal secretory region and proximal absorptive region. In the distal region the free margins of the cells are produced into cytoplasmic filaments three to ten microns long and packed very close together, forming a honey- comb border. The tips of the filaments are slightly swollen, especially during periods of active secretion. In the proximal region the cells have a typical brush border, this too is formed of cytoplasmic filaments but are separated from each other by their own width or more and are less regularly arranged.

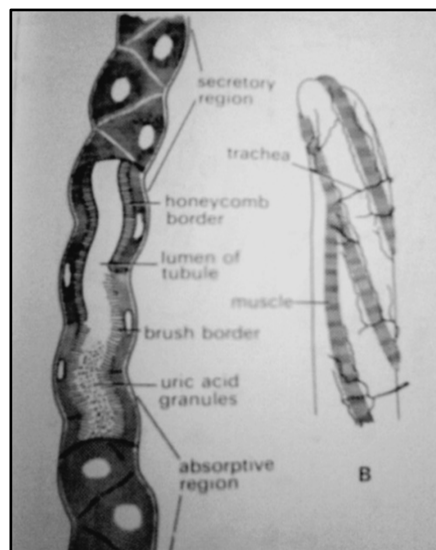


Figure: A) Part of a Malpighian tubule of *Rhodnius*showing the junction of the more distal, secretory regions of cells with honeycomb borders with the proximal regionof absorptive cells with brush borders.B) End of a Malpighian tubule of *Apis* showing the spiral muscle strands and the tracheal supply.

The Plasma membrane of the basal regions of the cells is deeply invaginated within the cells, the invaginations being more complex in the cells with the honey comb borders. Mitochondria are particularly conspicuous in the filaments of the honeycomb border, but in

the more proximal cells they are most numerous within the basal folds of the plasma membrane.

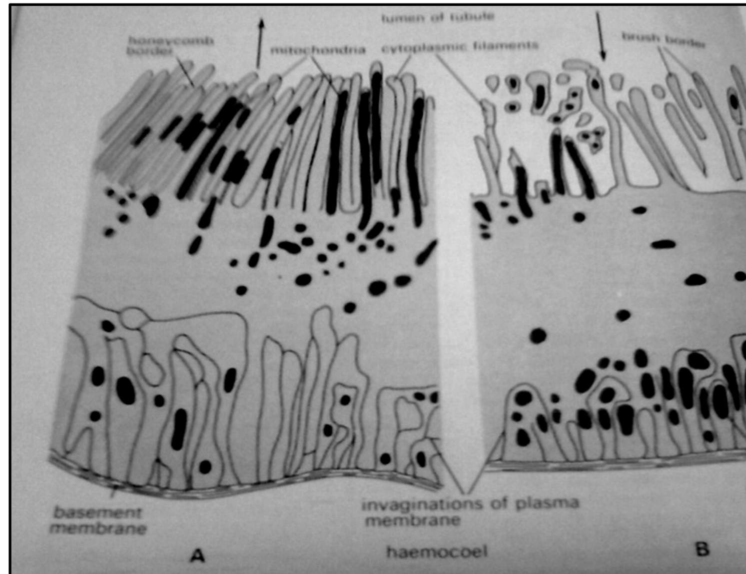


Figure: A) Section of a cell from the distal region of a Malpighian tubule of *Rhodnius* showing the regular cytoplasmic filaments of the honeycomb border. B) Section of a cell from the proximal region showing the irregular filaments of the brush border.

2. Nephrocytes

Cells that sieve the haemolmph for products that they metabolize (pericardial cells).

3. Fat bodies

A loose or compact aggregation of cells, mostly trophocytes, suspended in the haemocoel, responsible for storage and excretion.

4. Oenocytes

The cells of haemocoel, epidermis or fat body with many functions.

5. Integument

The outer covering of the living tissues of an insect.

6.Tracheal system

The insect gas exchange system, comprising tracheae and tracheoles.

7. Rectum

The posterior part of hind gut. Among the above organs, malpighian tubules are the major organ of excretion.

II. The Cryptonephridial arrangement of the malpighian tubules:

In many coleoptera and larval Lepidoptera the distal parts of the Malpighian tubules are closely associated with the rectum, forming a convoluted layer over its surface. This is known as cryptonephridial arrangement of the tubules. In caterpillars the tubules pass beneath the muscle layer of the rectum and then double back on themselves to form a more convoluted outer layer. Inner and outer layers of tubules are separated by a double membrane of thin cells and outside the outer layer are a single membrane, and the muscles of the rectum.

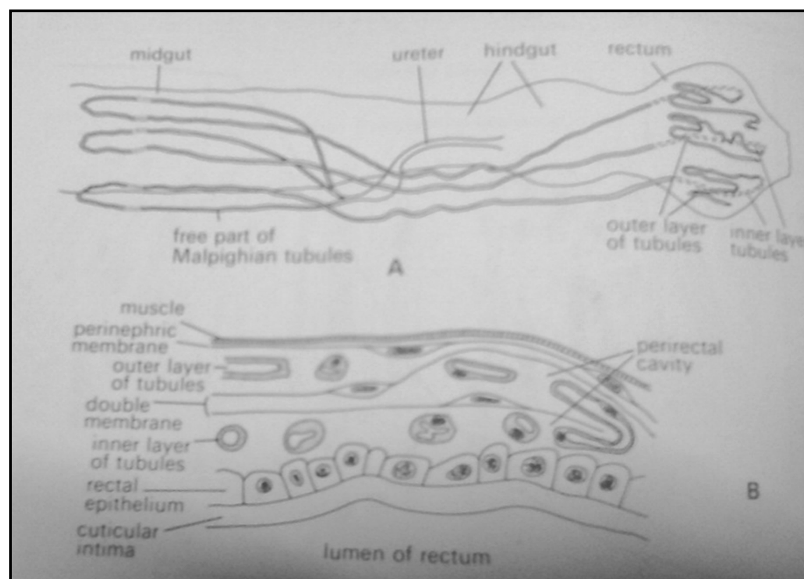


Figure: Cryptonephridial arrangement of the Malpighian tubules.

A. General arrangement showing the close association of the distal ends with the rectum.

B. Section of rectum and associated tubules.

III. Excretion and Osmoregulation

Insect faeces, either in liquid form or solid pellets, contains both undigested food and metabolic excretions. Aquatic insects excrete dilute wastes from their anus directly into water by flushing with water. But, Terrestrial insects must conserve water. This requires efficient waste disposal in a concentrated or even dry form, simultaneously avoiding the toxic effects of nitrogen. Both terrestrial and aquatic insects must conserve ions, such as sodium (Na^+), potassium (K^+) and chloride (Cl^-), that may be limiting in their food or lost into the water by diffusion. Therefore the production of insect excreta (urine or pellets) is a result of two related processes: **excretion and osmoregulation** (maintenance of favourable osmotic pressure and ionic concentration of body fluid). **The system responsible for excretion and osmoregulation is referred to as excretory system** and its activities are performed largely

by the Malpighian tubules and hindgut. However in fresh water insects, haemolymph composition is regulated in response to loss of ions to the surrounding water, with the help of excretory system and special cells. Special cells are called **Chloride cells** which are present in the hindgut, capable of absorbing inorganic ions from the dilute solutions. (e.g. Naids of dragonflies and damselflies).

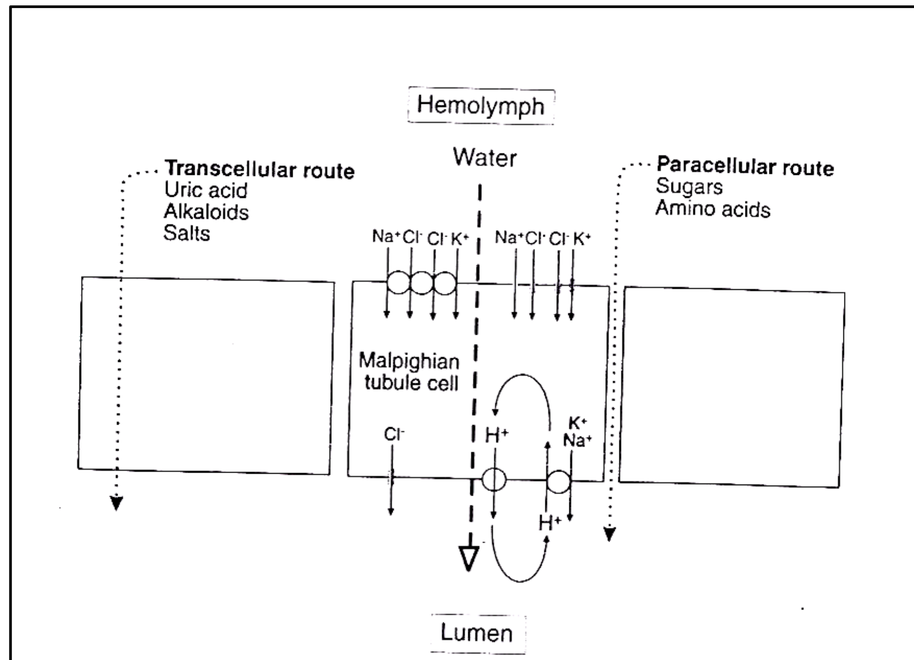


Figure: Diagrammatic illustration of excretion and osmoregulation in insects.

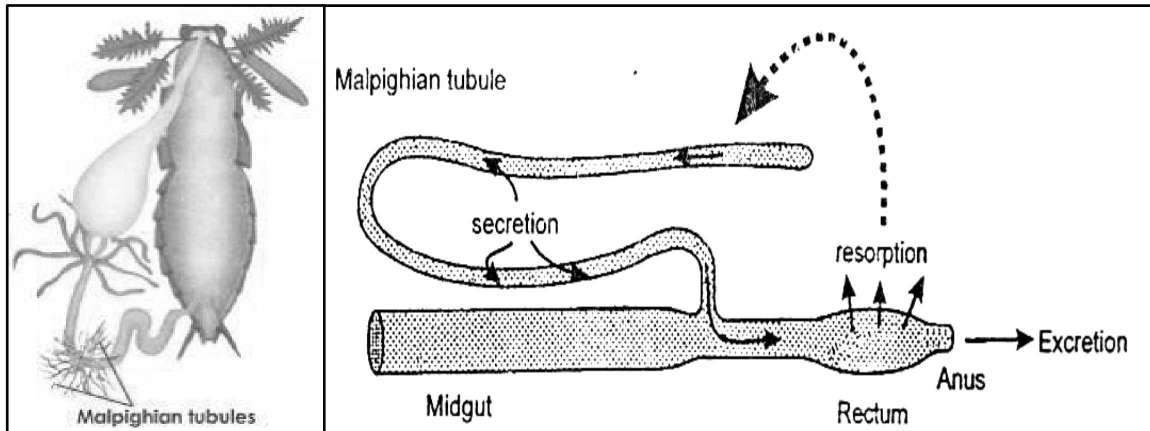
Malpighian Tubules

The main organ of excretion and osmoregulation in insects are the malpighian tubules, acting in association with rectum or ileum. Malpighian tubules are outgrowths of the alimentary canal and consist of long thin tubles formed of a **single layer of cells** surrounding a blind-ending **lumen,** they are absent in spring tail and aphids, 2 numbers in scale insects, 4 in bugs, 5 in mosquitoes, 6 in moths and butterflies, 60 in cockroach and more than 200 in locusts. Generally they are free, waving around in the haemolymph where they filter out solutes. Each tubule is externally covered by **peritonial coat** and supplied with muscle fibres (aiding in peristalsis) and tracheloes. Functional differentiation of the tubules was seen, with the **distal secretory** region and **proximal absorptive** region.

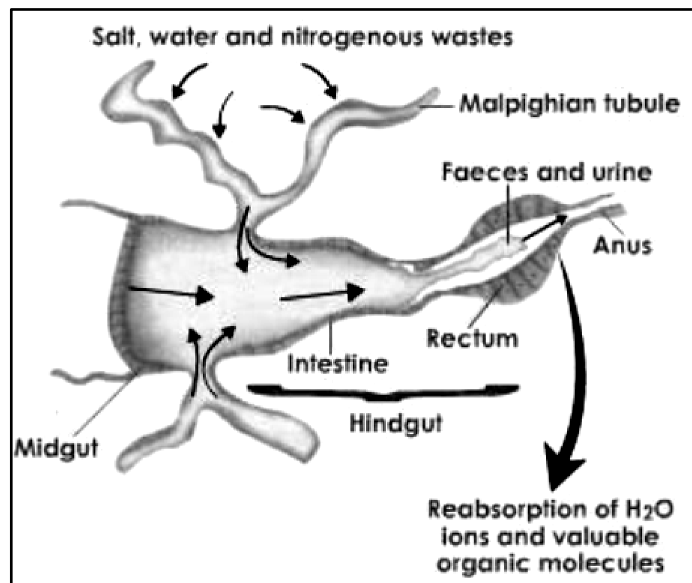
Physiology

The malpighian tubules produce a filtrate (the primary urine) which is isosmotic but ionically dissimilar to the haemolymph and selectively reabsorbs water and certain solutes, but

eliminates others. The malpighian tubules produces an isosmotic filtrate which is high in K^+ and low in Na^+ with Cl^- as major anion. The active transport of ions especially K^+ into the tubule lumen generates an osmotic pressure gradient for the passive flow of **water**.



Sugars and most amino acids are also passively filtered from the haemolymph via junctions between the tubule cells, whereas amino acids and non-metabolizables and toxic organic compounds are actively transported into the tubule lumen. Sugars are reabsorbed from the lumen and returned to the haemolymph. The continuous secretory activity of each Malpighian tubule leads to a flow of primary urine from its lumen towards and into the gut. In the rectum, the urine is modified by removal of solutes and water to maintain fluid and iionic homeostasis of the body.

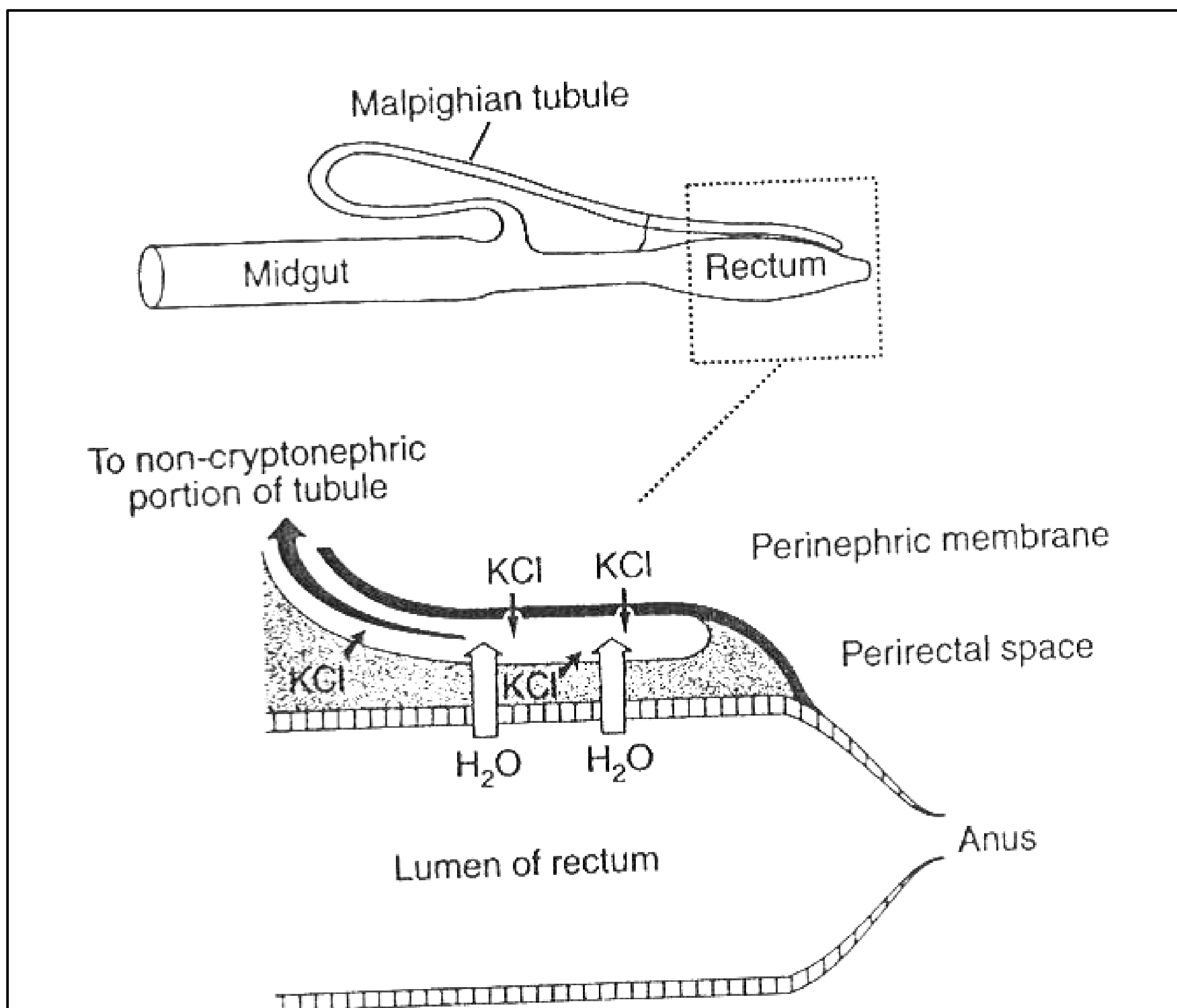


IV. Nitrogen excretion

Terrestrial insects excrete waste products as uric acid or certain of its salts called urates, which were water insoluble and requires less amount of water for waste product removal. This type of excretion is known as **uricotelism**. In aquatic insects ammonia is the excretory product, which is freely soluble in water and requires more amount of water for waste product removal. This type of excretion is known as **ammonotelism**.

Cryptonephry

The distal ends of the Malpighian tubules are held in contact with the rectal wall by the perinephric membrane, which is concerned either with efficient **dehydration of faeces** before their elimination or **ionic regulation**. (e.g. Adult Coleptera, larval Lepidoptera and larval symphyta)



V. Functions of malphighian tubule

Excretory in function, mainly concerned with removal of nitrogenous wastes. The other accessory functions are as follows:

1. Spittle secretion in spittle bug
2. Light production in **Bolitophila**
3. Silk production in larval neuropteran

VI. Storage Excretion

The excretory waste materials are retained within the body in different sites.

Uric acid is stored as urates in the **cells of fat body** e.g., American cockroach.

Uric acid is stored in the **body wall**, giving white colour, e.g., Red cotton bug.

Uric acid is stored in the **male accessory glands** to produce the outer coat of spermatophore, which is excreted during copulation.

Uric acid is stored in the **wing scales** giving white colour. e.g., Pierid butterflies.

Waste products of pupal metabolism (**meconium**) is stored and released during adult emergence.

Probable questions:

1. Describe the structure of Malpighian tubules with proper diagram and comment on the functional differentiation of different regions.
2. What are the two processes of production of urine in insects? Describe the physiology of urine formation.
3. Describe the cryptonephridial arrangement of malpighian tubules and state its function.
4. State the accessory functions of malpighian tubules.
5. What are chloride cells? State their function.
6. Which organs other than the malpighian tubules are concerned with excretion in insects? State their function.
7. What is storage excretion?

Source:

1. Chapman, R. (2012). The Insects: Structure and Function (S. Simpson & A. Douglas, Eds.). Cambridge University Press, 819 p.
2. Gullan, P.J & Cranston, P.S. (2010). The Insects: An outline of Entomology (4th Edition). Wiley-Blackwell, 584 p.

3. https://www.sciencedirect.com/science/article/pii/S0065280608601988.
4. http://eagri.org/eagri50/ENTO231/lec10.pdf.

Suggested readings:

1. Chapman, R. (2012). The Insects: Structure and Function (S. Simpson & A. Douglas, Eds.). Cambridge University Press, 819 p.
2. Evans, H.E. (1984). Insect biology – A textbook of Entomology. Addison-Wesley Publ. Co., 436 p.
3. Gullan, P.J & Cranston, P.S. (2010). The Insects: An outline of Entomology (4th Edition). Wiley-Blackwell, 584 p.
4. Mani, M.S. (1982). General entomology. Oxford & IBH Publ. Co., 912 pages.
5. Richard, O.W. & Davies, R.G. (1977). IMMS' General textbook of Entomology. Part I & II. Chapman & Hall London, 1354 p.
6. Snodgrass, R. E. (1935). Principles of Insect Morphology. Mcgraw-Hill Book Company. 647 p.
7. Tembhare, D.B. (1997). Modern entomology. Himalaya Publ. House, 623 pages.
8. Wigglesworth V. B. (2015). Insect Physiology. Andesite Press. 148p.

UNIT- IV

STRUCTURE AND FUNCTION OF WINGS IN INSECTS

Objective:

In this section we will discuss about Structure and Function of Wings in Insects.

Introduction:

Wings i n l iving i nsects s erve a num ber of functions, including a ctive f lying, gliding, parachuting, altitudestability while jumping, thermoregulation, and soundproduction. Understanding the evolution of wings requires anunderstanding of the adaptive value of the intermediate ortransitional stages in their development.

A. Structure:

- **Cross s ection t hrough t he w ing** –The w ing m embrane consists of two l ayers of integument. Veins include the nerves, blood space and tracheae. Wings do not contain muscle.
- **Venation** -Irregular ne twork of ve ins are found i n pr imitive i nsects. Longitudinal veins w ith limited cross-veins are common i n m any pt erygote groups. Extreme reduction of all veins is common in small insects. Longitudinal veins are concentrated and t hickened t owards t he a nterior m argin o f t he wing. T his gives i ncreased efficiency and support during insect flight.

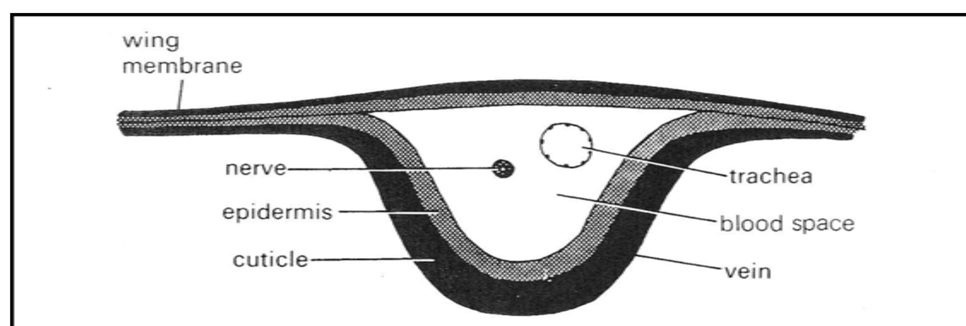


Figure: Diagrammatic section through part of a wing including the TS of a vein.

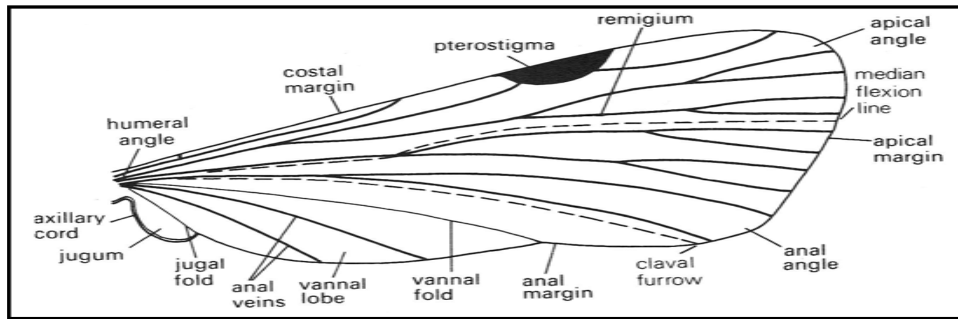


Figure: Diagram of a typical insect wing.

- **Pterostigma -** Darkened area on forewing in Hymenoptera, Pscoptera, Megaloptera and Mecoptera and on both wings in Odonata. Function as inertial mass in flight. Reduces wing flutter during gliding in odonates and thereby increases flight efficiency. Provide passive control of angle of attack in small insects which enhances efficiency during flapping flight.
- **Wing folding -** Flexion lines reduce passive deformation and enhances wing as an aerofoil. Fold lines used in folding of wings over back.

B. Wing movement:

- **Wing coupling -** Orthoptera and Odonata wings are not anatomically coupled. Coordination of forewings and hindwings in flight is accomplished by pattern-generator neurons in the central nervous system. Detail of anatomical wing-coupling varies among taxonomic groups, suggesting that it evolved independently several times.

  Types of Coupling: a) Primitive Mecopteran Pattern, b) Jugate coupling, c) Frenate coupling in female &d) Frenate coupling in male

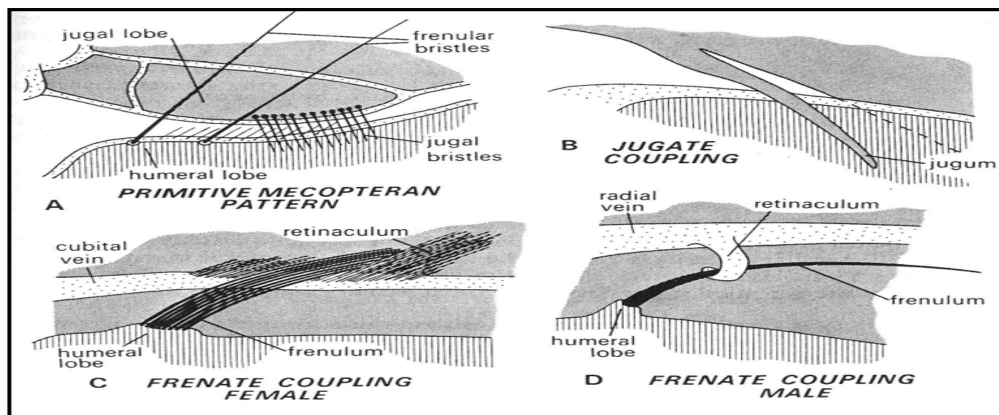


Figure: Types of wing coupling

- **Halteres in Diptera -** Derived from the hindwings. Functions to maintain stability in flight.

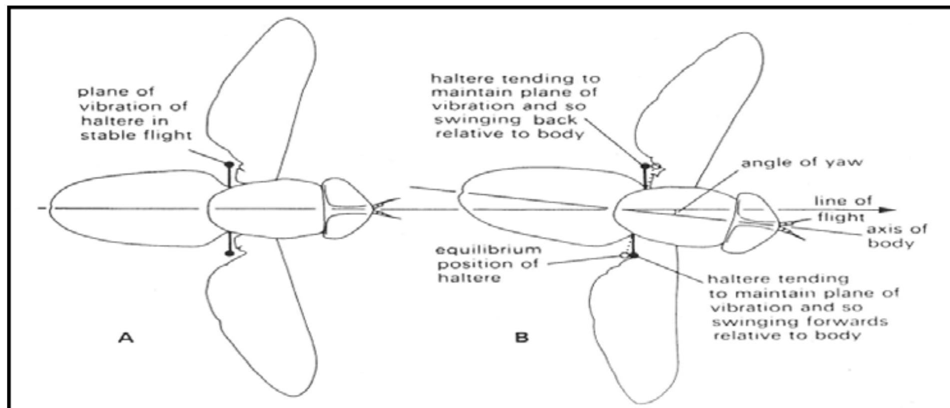


Figure: Halteres in Dipteras

Two general mechanisms of wing movement:

• **Direct mechanism.** Downward movement of the wing is the result of the contraction of muscles attached directlyto the wing. This flight mechanism is under the control of **synchronous flightmuscle**. Because each wingbeat iscontrol by a nervous impulse, the directmechanism of insect flight is said to be**neurogenic**in origin.

• **Indirect mechanism.** Downward movement of the wing is the indirect result of the contraction of musclesattached to the thorax. This flight mechanism is under the control of**asynchronous flight muscle**. Because several to many wing beats occur forevery nervous impulse, the indirect mechanism of insect flight is said to be**myogenic**in origin.

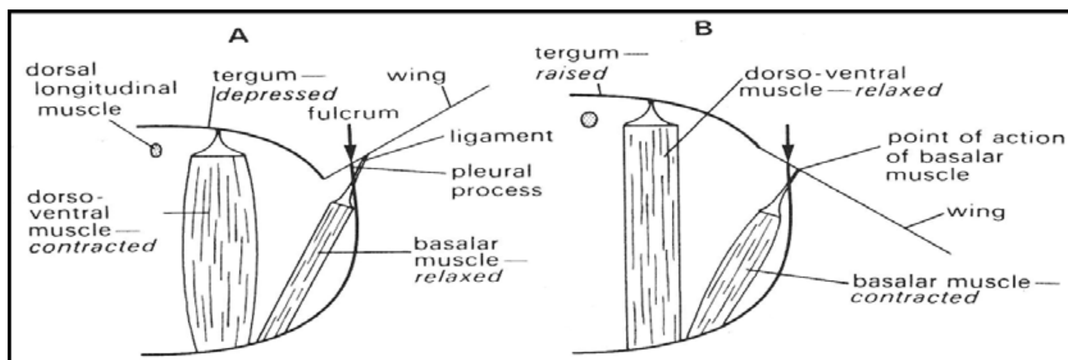


Figure: Diagrammatic cross section of the thorax illustrating the wing movements in an insect, in which the direct wing muscles cause depression of the wings.

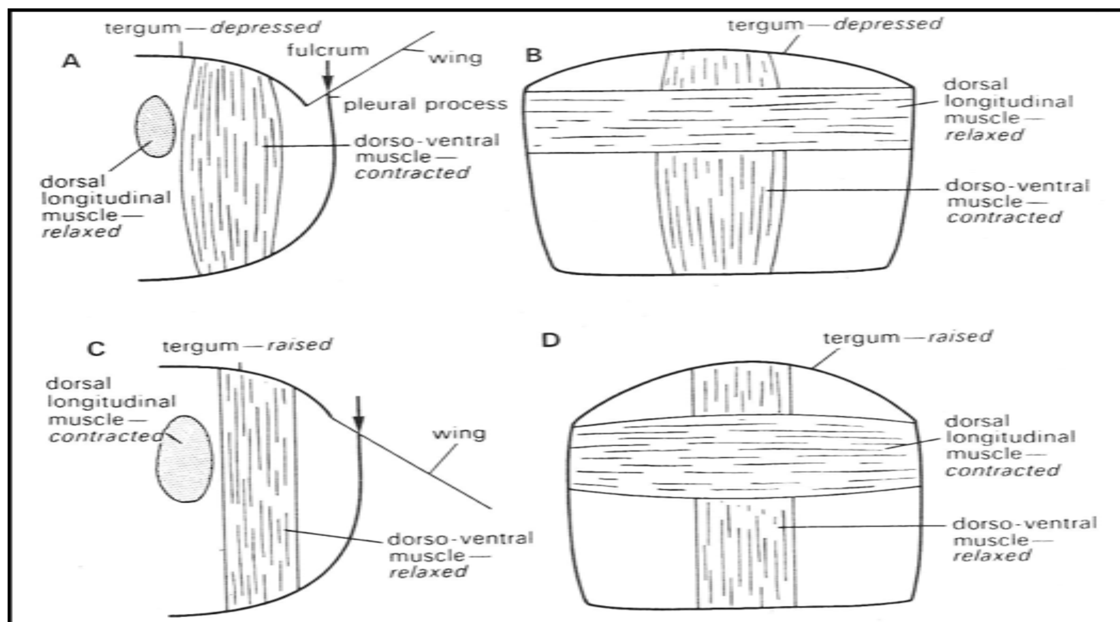


Figure: Diagrams illustrating the movement of wings in an insect in which both up and down movement of the wings are produced by indirect muscles (A and C). Cross section of the thorax, (B and D).

Taxonomic distribution of direct and indirect flight mechanism - Direct mechanism of wing movement is found in the Palaeoptera and the Blatteria. Indirect mechanism of wing movement is found in the Hymenoptera (bees), Diptera, some Coleoptera and some Hemiptera. Other groups (some Coleoptera and Orthoptera) use a combination of direct and indirect mechanisms to move wings.

Efficiency of flight production - Muscles used in flight arise in the coxa in many insects and also function in leg movement during terrestrial locomotion. Elastic properties of wing hinges, wing muscles and thorax greatly enhance flight efficiency. Elasticity of these structures is due to the presence of the protein resilin. In locust, 86% of the energy used in the upstroke can by recovered during the downstroke. Elasticity of the thorax means that wings are in stable position only at the top of the upstroke or at the bottom of the downstroke.

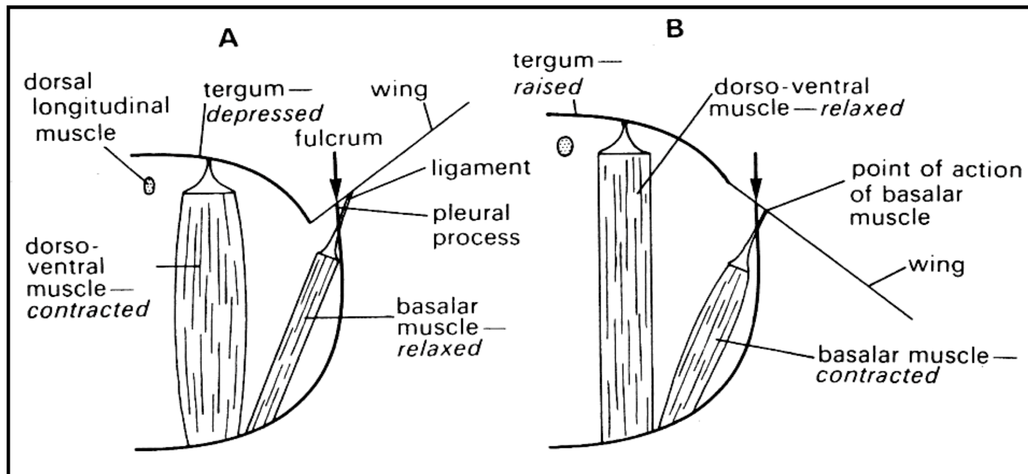


Figure: Diagrammatic cr oss-section of the tho rax illu strating the w ing movements in a n insect in which the direct wing muscles cause depression of the wings.

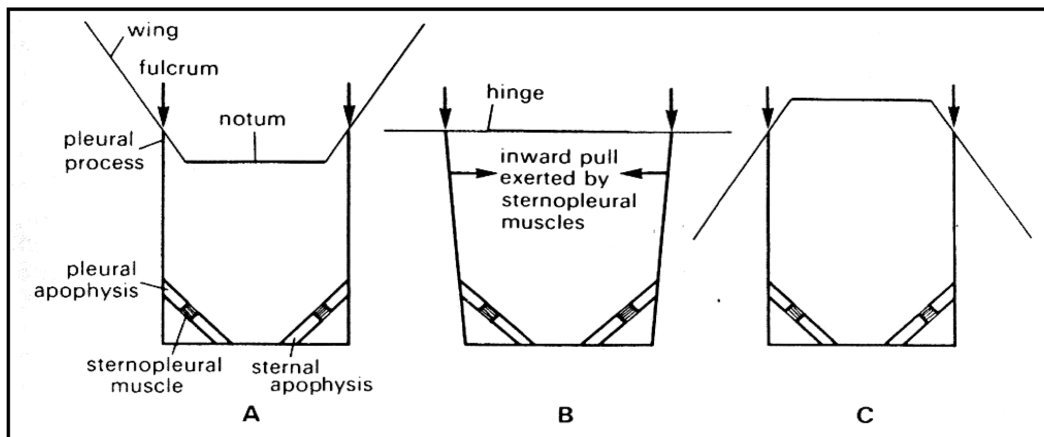


Figure: Diagrammatic c ross-section of the ins ect thor ax il lustrating the di stortion of the thorax produced by wing movement. A. Wings stable in the up position, B. Unstable position due to inward pull of the sternopleural muscles, C. Wings stable in the down position.

Stroke plane is the plane in which wings move relative to the long axis ofthe body. Stroke plane de termines t herate of f orward m ovement dur ing f light. Insects c ontrol t urning movements b ychanging t he s troke pl ane on o ne s ideof t he bod y r elative t o t hat on t he otherside of the body. Stroke plane in locustaverages about 30°. Hovering requiresan average stroke plane of 0°.

• **Amplitude** of wingbeat is the distancein degrees travelled by the wing tipfrom the top of the ups troke t o t he bottom of t he dow nstroke. G reater amplitude pr oduces greater pow er

output. Insect control turning movements by varying the amplitude ofthe wingbeat on both sides of the body.

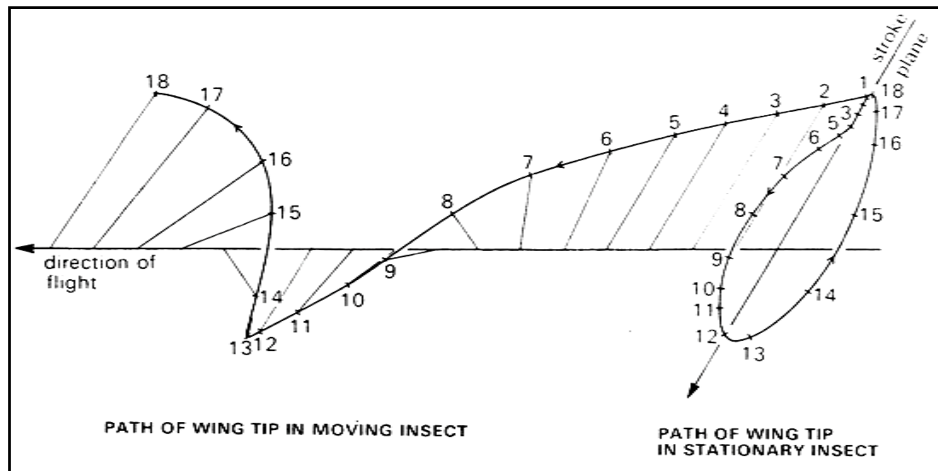


Figure: Movement of the tip of the forewing in a typical insect. The numbers indicate the positions of the wing at regular time intervals throughout the stroke and the lines joining the wing-tip path to the flight axis show the angle which the long axis of the wing makes with the body at different stages of the stroke.

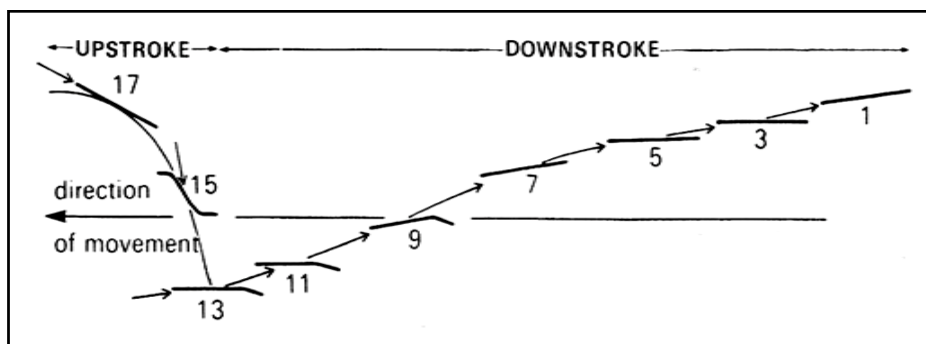


Figure: Changes in the twisting of the mid section of the forewing.

Wingbeat frequency is the number ofwingbeats per second (Hz). Insects withsynchronous flight muscles have lowwingbeat frequencies (≤ 50 Hz) relative toinsects with asynchronous flight muscle(100-1000 Hz). Wingbeat frequency is also negatively correlated with body size.The greater the wingbeat frequency thegreaterwillbethepower out put and the greater thelift production.

Wing twisting occurs when the relative position of the leading and tailing edges ofthe wing changes during the wingbeat. Both passive and active (=muscular)forces are responsible for

changing w ingtwisting. Wing tw isting c ontrols the a ngleof a ttack w hich control lif t and forward movements of the insect in space.

Relative wind is the movement of airrelative to the wing. Its two componentsare due to 1) the airspeed of the insectand 2) the velocity of the wing in the strokeplane.

Angle of attack is the angle at which therelative wind strikes the chord of the wing.Insects control the angle of attack byactive and passive twisting of the wing.Changes in the angle of attack are used tocontrol the force of relative wind.

Force of relative wind has two components:

Lift is the vertical force produced by relative wind. This force iswhat gets insects into the air and keepsthem there.

Thrust is the horizontal force produced by relative wind. This forcemoves insects forward through the air.

Forward thrust is resisted by **profile drag**(the cross-sectional area the insectpresents to the air) and mostly by **induced drag (**development of vortices at the wing tips.)

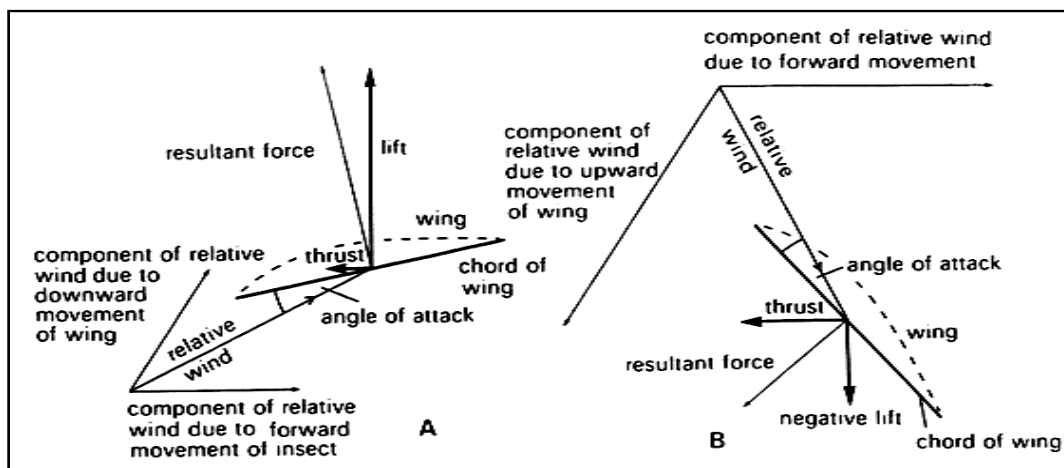


Figure: Diagrams illus trating th e f orces acting at the mid -point of th e w ing a t di fferent phases of the wing beat corresponding roughly with positions 7 and 15 in earlier Figure. A. Down stroke of wing with positive angle of attack. B. Upstroke of wing with negative angle of attack.

Types of flight:

1) **Hovering** in flight is accomplished by changing the stroke plane to nearly horizontal and maintaining a positive angle of attack throughout the wing beat.

2) **Gliding** requires a high lift-to drag ratio. This is accomplished mostly by changing the angle of attack to maximize thrust and minimize drag and negative lift.

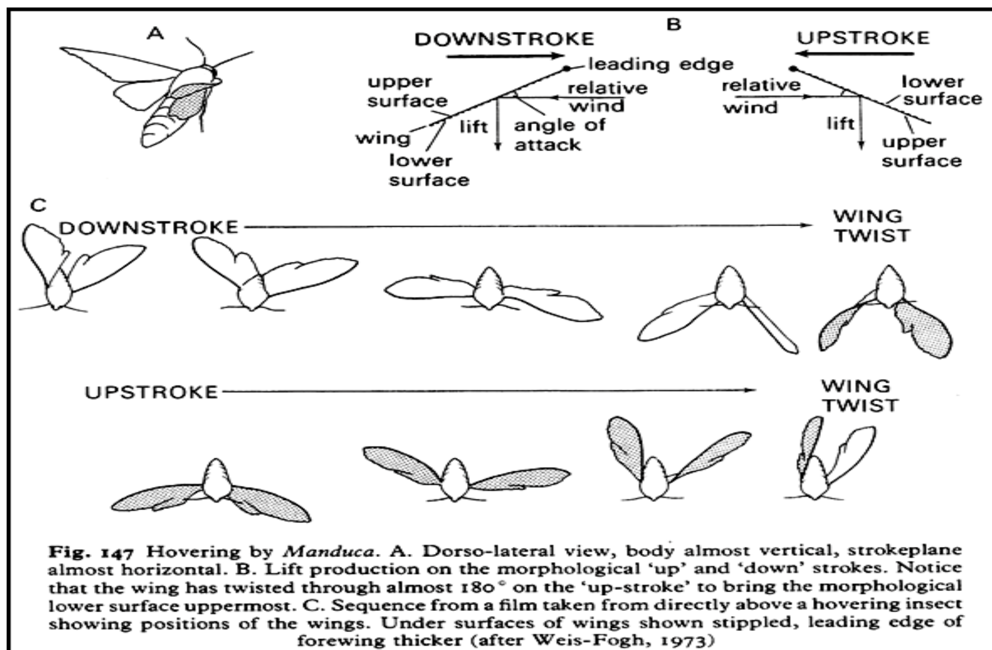


Fig. 147 Hovering by *Manduca*. A. Dorso-lateral view, body almost vertical, strokeplane almost horizontal. B. Lift production on the morphological 'up' and 'down' strokes. Notice that the wing has twisted through almost 180° on the 'up-stroke' to bring the morphological lower surface uppermost. C. Sequence from a film taken from directly above a hovering insect showing positions of the wings. Under surfaces of wings shown stippled, leading edge of forewing thicker (after Weis-Fogh, 1973)

Figure: Hovering in insect. A)Dorso-lateral view, body almost vertical, strokeplane almost horizontal. B) Lift production on the morphological "up" and "down" strokes. C) Sequence from a film taken directly above a hovering insect showing positions of the wings.

Probable questions:

1. Describe the structure of a typical insect wing with proper diagram.
2. What is pterostigma? State its function.
3. Name the different veins of an insect wing.
4. Describe the two general mechanisms of wing movement in insect with diagrammatic illustrations.
5. Describe different types of wing coupling in insects. State the evolutionary significance of wing coupling.
6. What do you mean by stroke plane in regard to insect flight?
7. What do you mean by wing beat frequency and wing twisting?
8. What are the two components of relative wind which forms the angle of attack?
9. Describe different types of insect flight.
10. Write short notes on:

a) Taxonomic distribution of flight mechanism in insects.
b) Efficiency of flight production in different insects.

Source:

1. Chapman, R. (2012). The Insects: Structure and Function (S. Simpson & A. Douglas, Eds.). Cambridge University Press, 819 p.
2. Gullan, P .J & C ranston, P .S. (2010). T he Insects: A n out line of E ntomology (4th Edition). Wiley-Blackwell, 584 p.
3. O.W. Richard & R.G. Davies (1977). IMMS' General textbook of Entomology. Part I & II. Chapman & Hall London, 1354 pages.
4. https://www.si.edu/spotlight/buginfo/insect-flight
5. http://people.virginia.edu/~crk4y/research/flight.PDF
6. http://courses.biology.utah.edu/feener/5445/Lecture/Bio5445%20Lecture%2012.pdf
7. http://w3.impa.br/~jair/1954.pdf

Suggested readings:

9. Chapman, R. (2012). The Insects: Structure and Function (S. Simpson & A. Douglas, Eds.). Cambridge University Press, 819 p.
10. Evans, H.E. (1984). Insect biology – A textbook of Entomology. Addison-Wesley Publ. Co., 436 p.
11. Gullan, P .J & C ranston, P .S. (2010). T he Insects: A n out line of E ntomology (4th Edition). Wiley-Blackwell, 584 p.
12. Mani, M.S. (1982). General entomology. Oxford & IBH Publ. Co., 912 pages.
13. Richard, O.W. & D avies, R.G. (1977). IMMS' G eneral t extbook o f E ntomology. Part I & II. Chapman & Hall London, 1354 p.
14. Snodgrass, R . E. (1935). P rinciples o f Insect M orphology. M cgraw-Hill B ook Company. 647 p.
15. Tembhare, D.B. (1997). Modern entomology. Himalaya Publ. House, 623 pages.
16. Wigglesworth V. B. (2015). Insect Physiology. Andesite Press. 148p.

Unit-V

Insect visual organs: Structure and functional mechanism and Photogenic organ of insects: structure, mechanism and significance of light production

Objectives:

In this Unit will discuss about Insect visual organs: Structure and functional mechanism and Photogenic organ of insects: structure, mechanism and significance of light production.

A. Insect visual organs: Structure and functional mechanism

Introduction:

Responses of light in insects are mediated by:

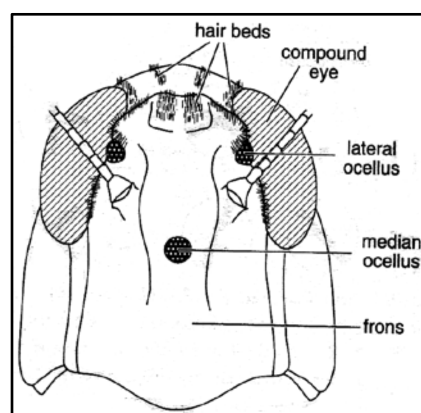
Dermal receptors; Dorsal ocelli (simple eye); Lateral ocelli or Stemmata; Compound eyes

Distribution of ocelli in the insects

Imago possesses compound eyes and dorsal ocelli; Lateral ocelli occur only in endopterygote larvae. Reduction or loss of photoreceptors is common among species which live in dark; eg. Endoparasites, insects inhabiting the nests of termites or ants or burrowing in the soil or plant tissues. It is also characteristic of many ectoparasites (eg.Mallophaga, Siphunculata, Siphonoptera etc.)

Dermal receptors-

Several insects (eg. Lepidopteran larvae, *Periplaneta*) react to light even after the eyes and ocelli have been removed or covered with opaque material.The general body surface appears to be sensitive to light.In *Schistocerca* and *Locusta*, rhythmic deposition of cuticle can be uncoupled from the circadian clock that controls it through a direct, long term effect of light between the wavelengths 435 – 520 nm on the epidermal cells.



Dorsal ocelli (simple eye) -There are generally three dorsal ocelli in the frons and vertex of the head.

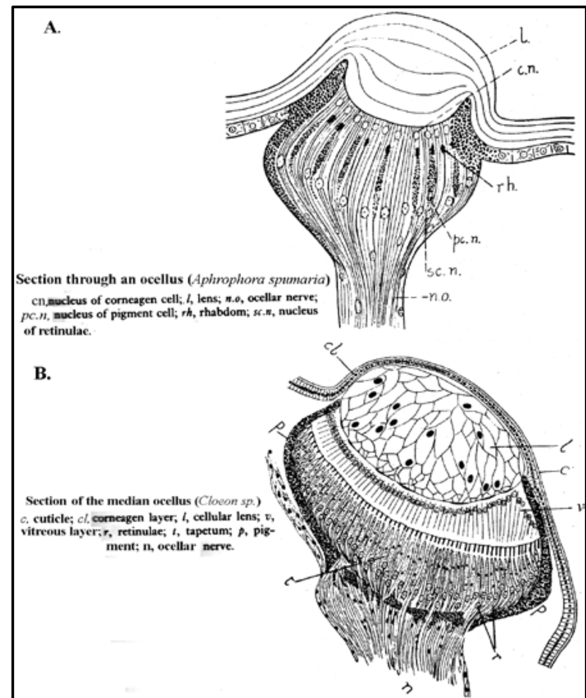
Cornea- usually a m ore t hickened, t ransparent r egion of c uticle t hat s urrounds the oc ellus externally to form a lens. In Ephemeroptera, the cornea is convex, not thickened and the lens is formed from a mass of polyhedral cells lying beneath the corneagen layer.

Corneagen layer consists of modified epidermal cells, colourless, transparent and secrete the lens, sometimes form layer of vitreous cells, dioptric in function.

Retina- consists of some 500-1000 primary sense-cells which form a shallow cup and are sometimes arranged into groups of 2-5 cells, the retinulae.

Rhabdomere- Some p art of t he s urface of e ach retinal c ell is s pecialized as a lig ht s ensitive rhabdomere, c omposed of closely packed microvilli. Within the r etinal c ells the re ma y b e conspicuous RER as well as multivesicular bodies of 50 nm in diameter.

Pigment c ells- These are abs ent i n *Periplaneta* and va ry c onsiderably i n ot her s pecies.They m ay invest the w hole oc ellus or f orm a n iris like r ing of c ells. Their ma in function is to prevent lig ht entering the ocellus except through the lens.



Section through an ocellus (*Aphrophora spumaria*)
cn, nucleus of corneagen cell; *l*, lens; *n.o*, ocellar nerve; *pc.n*, nucleus of pigment cell; *rh*, rhabdom; *sc.n*, nucleus of retinulae.

Section of the median ocellus (*Cloeon sp.*)
c, cuticle; *cl*, corneagen layer; *l*, cellular lens; *v*, vitreous layer; *r*, retinulae; *t*, tapetum; *p*, pigment; n, ocellar nerve.

Central ne rvous c onnections- The o cellar ne rves c onsist of s hort a xons of r etinal c ells a nd longer ones from protocerebral neurons.

Functions of Dorsal ocelli

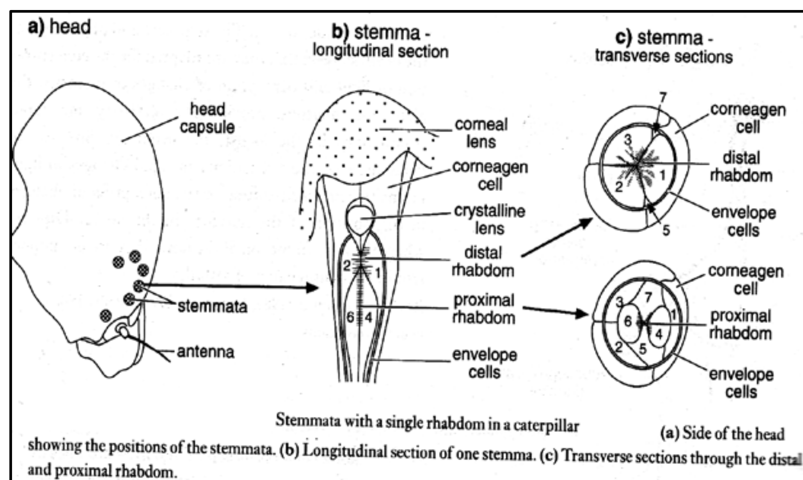
The vi sual fields of t he dorsal oc elli a re ove rlapped b y t hose of t he c ompound e yes, this is because t he pr incipal f ocal pl ane of t heir l ens f alls be low t he level of t he r etinal l ayer and because of the convergence of the many retinal cells on to a much smaller number of ocellar nerve fibers. They s ignal t he l evel o f c hanges i n t he l ight intensity. Occluding the oc elli usually l eads t he i nsects t o m ove l ess r apidly, specially i n br ight l ight. T his i s pr obably because, the do rsal oc elli m ay be ‘stimulatory o rgans’ s erving t o rise the ex citatory l evel of the insects with respect to other visual stimuli perceived through the compound eyes. Another view i s t hat t he o celli a nd c ompound e yes i nteractin m ediating t he phot otactic be havior of insects. I n *Locusta* and *Gryllus*, t hey act t ogether i n pr omoting m ore accurate di rectional orientation; i n di m lig ht the dor sal o celli a nd e yes int eract s ynergistically w hile in brighter light they behave antagonistically.

Stemmata

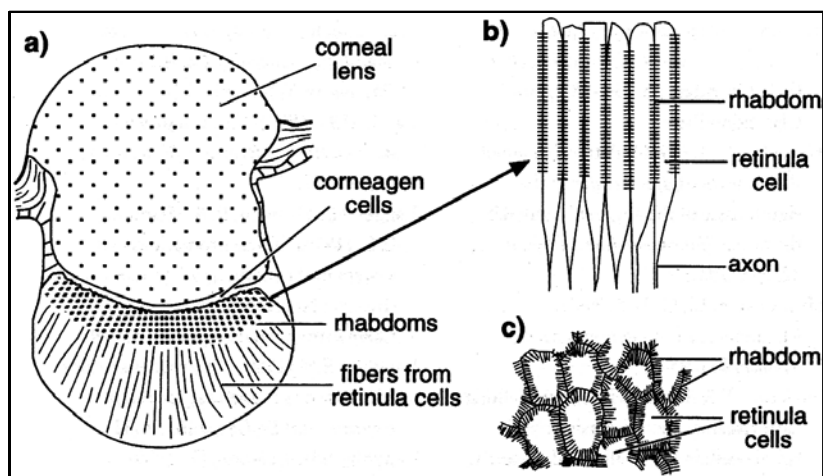
The onl y e yes pr esent i n t he hol ometabolous i nsect l arvae. Located on t he s ides of t he head.The number is variable and not always constant in the same species. Sometimes it can be single or even it can be 6-7 or more. They differ from the dorsal ocelli in being innervated from the optic lobes of the brain and also in that a crystalline refractive body may sometimes be developed beneath the corneal lens and pigment granules are sometimes absent.

Functions of stemmata

Their f unctions a ppear very s imilar t o t hose of c ompound e yes. T hey f orm a r elatively focused image and are important in colour vision, predator avoidance, prey capture etc. They can also detect the plane of polarized light, though no functional significance has yet been reported. Found in Mecoptera, most Neuroptera, Lepidoptera and Trichoptera

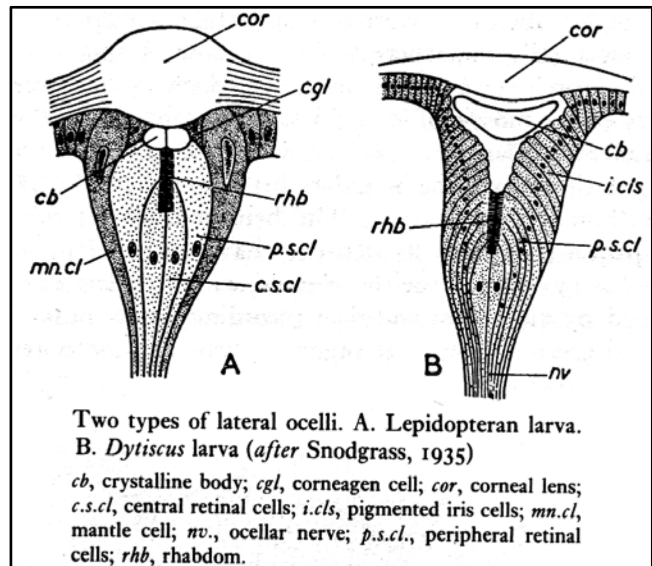


Stemmata with a single rhabdom in a caterpillar (a) Side of the head showing the positions of the stemmata. (b) Longitudinal section of one stemma. (c) Transverse sections through the distal and proximal rhabdom.



Found in larval Symphytans (Hymenoptera), tiger beetle (*Cicindela*)

Types of stemmata found in various insects



Two types of lateral ocelli. A. Lepidopteran larva. B. *Dytiscus* larva (*after* Snodgrass, 1935)
cb, crystalline body; *cgl*, corneagen cell; *cor*, corneal lens; *c.s.cl*, central retinal cells; *i.cls*, pigmented iris cells; *mn.cl*, mantle cell; *nv.*, ocellar nerve; *p.s.cl.*, peripheral retinal cells; *rhb*, rhabdom.

In Saw fly and beetle larvae each stemmata one on either side of the head, comprises a single cuticular lens lying beneath which are groups of photosensitive cells with a central rhabdom.

In *Dytiscus, Euroleon*and*Sialis*, a lens like crystalline body is secreted beneath the cornea but the structure otherwise resembles the first type.

In the Lepidoptera and Trichoptera, each larval ocellus has a cornea and crystalline body with seven retinal cells, forming a single retinula with the rhabdomeres constituting a single axial rhabdom. The resulting organ is strikingly similar to each ommatidium of a compound eye.

In larval Symphytans (Hymenoptera) and tiger beetle (*Cicindela*), stemmata with multiple rhabdoms are reported.In the larvae of cyclorrhaphan Diptera there are no external signs of stemmata, but a pocket of photosensitive cellsoccurs on each side of the pharyngeal skeleton.

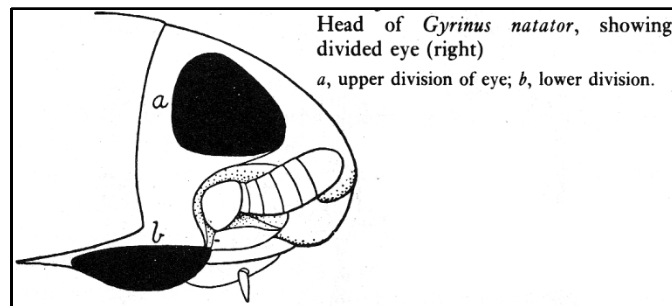
In the unpigmented ocellus of Chironomous the vitreous cells are absent and a single retinula lies directly beneath unmodified cuticle.In*Aedes aegypti* larvae the retinular cells have the kind of ultrastructure expected in insect eyes, with RER, rhabdomeric microvilli and a zonula adherent junction between contiguous cells.In*Musca* there is a small group of light-sensitive cells on each side of the pharyngeal sclerites and invisible externally. They are most sensitive to green light and apparently unable to perceive red.

Compound eyes

The principal feature distinguishing compound eye from ocelli is that in the former the cornea is divided into a number of separate facets, whereas there is only a single facet to each ocellus.Compound eyes are aggregations of separate visual elements known as ommatidia, each corresponding with a single facet of the cornea.They are innervated from the optic lobes of the brain like stemmata.

Variations of compound eye in different insects

The number and size of the facets vary greatly. For example: in the worker ant, *Ponera sp.* each eye is a single facet while among the workers of other ant species, it may vary from 100-600. In *Musca sp.*- 4000; Odonata- 10000-28000.In most insects facets are very closely packed together and hexagonal but where they are fewer and less closely compacted, they are circular.In male *Tabanus*, they are often larger over the anterior and upper part of the eye and the two fields are not sharply demarcated.In males of other Diptera such as *Bibio* and *Simulium*, the two areas of different sized facets are very distinctly separated, each eye appearing double.In certain Coleoptera, *Gyrinus* and Ephemeroptera, *Cloeon*, the two parts of the eye are separated so that the insect appears to possess two pairs of compound eyes. In *Cloeon* the anterior division of each eye is elevated on a pillar like outgrowth of the head while the posterior division is normal.



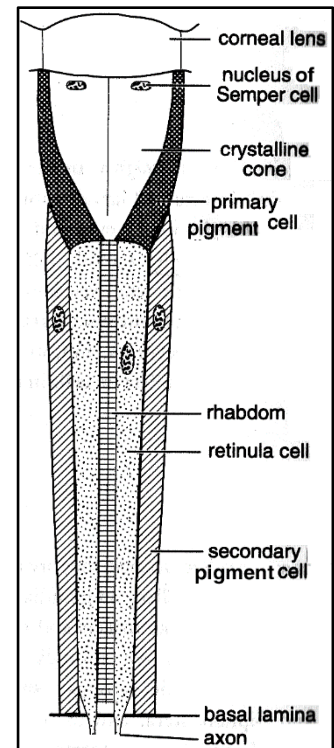
Head of *Gyrinus natator*, showing divided eye (right)
a, upper division of eye; *b*, lower division.

Structure of ommatidium

Cornea - outermost transparent layer of cuticle forming the external facet and acting as lens.It is usually biconvex and may be uniformly refractive or composed of paraboloidal laminae whose refractive index decreases from the centre outwards.

Corneagen layer - The part of the epidermis that extends beneath the cornea. It normally consists of two cells in each ommatidium. In some cases the corneagen cells are said to be absent and the cornea is secreted by the crystalline cone cells.

Crystalline cone cells - Beneath the corneagen layer or cornea lie four cells that secrete the crystalline cone. It contain glycogen inclusions and microtubules. In some insects the crystalline cone cells are produced into six processes which end deep in the ommatidium and may provide support or facilitate the transport of metabolites.



Primary pigment cells - These are densely pigmented, commonly two in number, disposed in a circlet around the crystalline cone and corneagen cells.

Retinula - retinula forms the basal portion of the ommatidium, consisting of a group of more or less elongate visual cells, each continued into an axon, the post retinal fibre, that communicates with the central nervous system. The retinular cells show variation in number, size, arrangement and ultrastructural details.

The cells commonly contain pigment granules or lipid droplets as well as multivesicular bodies and most of the usual organelles.

The cell surface is produced into large numbers of microvilli which collectively form a rhabdomere.

The rhabdomeric microvilli are highly orientated and contain the visual pigment rhodopsin, on which the primary photoreceptor process depends and show signs of pinocytosis at their bases.

Secondary pigment cells - These are numerous, elongated, pigment containing cells that surround the retinula and primary cells, isolating each ommatidium optically from its neighbours.

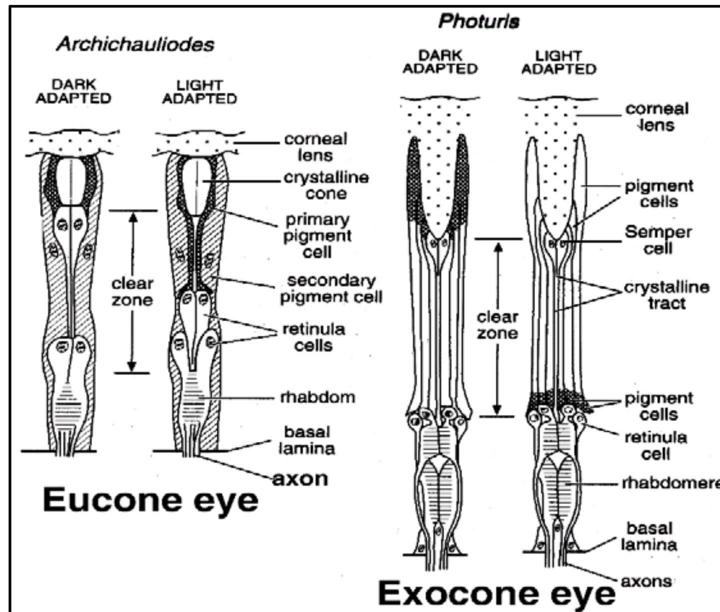
Types of compound eye: according to the presence and nature of the crystalline cone.

Eucone eyes- each ommatidium contains a hard, refractive, conical body secreted with in the crystalline cone cells and forming part of the dioptric apparatus. Occur in Thysanura, Ephemeroptera, Odonata, Orthoptera, some Hemiptera, Lepidoptera, clear zone is bridged by retinula cells]

Pseudocone eyes- the cone cells are filled with a transparent viscous liquid. Ex Brachyceran and Cyclorrhaphan Diptera

Acone eyes- the long, transparent crystalline cone cells do not secrete any refractive material. Eg. Dermaptera, some Hemiptera and Coleoptera.

Exocone eyes- in which the crystalline body is replaced by a cone shaped extension of the inner surface of the cornea, lying distal to the unmodified crystalline cone cells. E x in Dermestidae, Elateridae, Byrrhidae, clear zone is crossed by a crystalline tract formed from the semper cells.



Types of compound eyes on the basis of function

Apposition eye : The rhabdom usually extends the full length of the retinula cells between the crystalline cone and the basal lamina. Eg.*Camponotus*, *Drosophila*

Superposition eye : The rhabdom is restricted to the basal region and hence there is a clear zone between rhabdom and crystalline cone. Eg. *Shrimp*

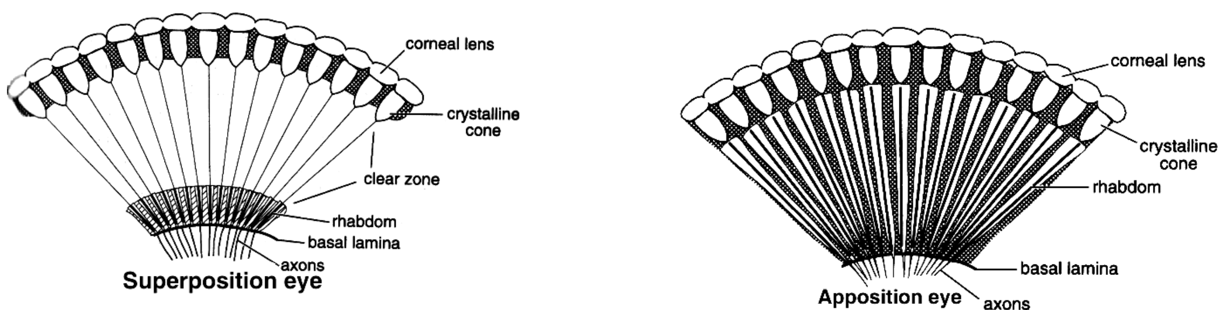
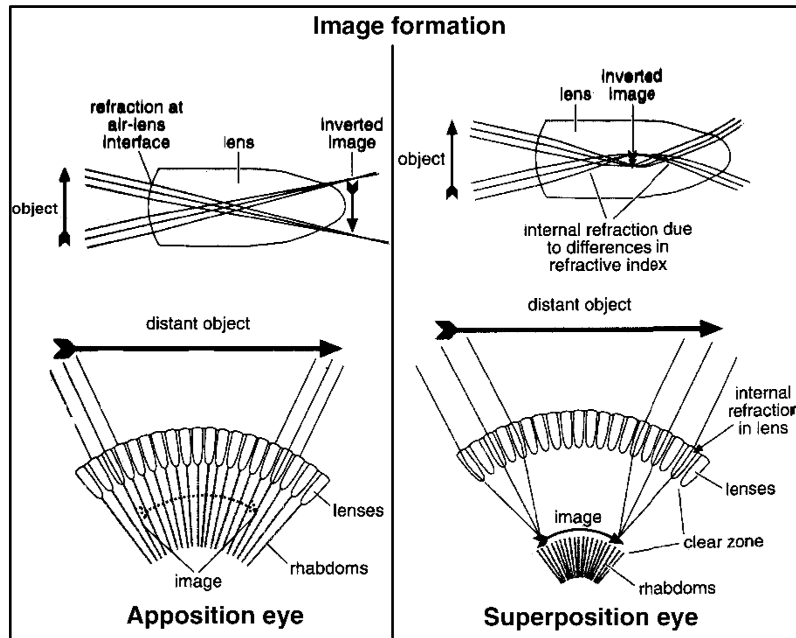


Image formation in apposition eye:

Each lens form an inverted image at the top of the rhabdom, but because the rhabdomeres function as a single unit the impression of this image is not retained. Consequently the output from each rhabdom is a response to the overall intensity of light that reaches it. Information concerning the object is thus represented as a series of spots differing in intensity.

Image formation in superposition eye:

Light rays are refracted internally with in the lens. They are unfocused as they exit the lens, but collectively form a single upright image at the tip of the rhabdoms.



B. P hotogenic or gan of i nsects: s tructure, m echanism an d s ignificance of light production

Introduction:

Bioluminescence in insects: The a bility of c ertain or ganism t o pr oduce light i s know n as Bioluminescence. This phenomenon exists in more than half of Zoological Phyla. The light production is a result of some chemical reaction that does not involve heat production and is brought about half of suitable substrate and an enzyme system.

In many cases the luminescences occur due to some symbiotic bacteria, such as luminescence in some marine fish. But in case of self luminescence, the emersion of light is purely depend on chemical reactions. This luminescence mechanism is highly developed in insects and the production of light in insects is always a phenomenon of Chemiluminescence. Examples-

Onychiurusarmatus- Subclass: C ollembola (Subclass-Apterygota), emits their g eneral from their whole body; *Fulgora lanternaria* –Suborder: H omoptera, l ight or gan i s on t heir he ad; Order: C oleoptera, the i nsects f rom family: Lampyridae (fireflies) e mits the ir lig ht from abdominal segments. In case of male, the light organ is situated on the ventral side of 7th and

8th abdominal segments, in female only in 7th segment and in larvae 1st to 7^{th}sengments; Order: Coleoptera, Family: Elateridae, Some insets from this family emits their light from three organs, A) One is in the lateral side of the prothorax. B) other two organs are in the dorsal side of the 1st abdominal segment; Order: Coleoptera, Family: Phenogodidae, they have 11 pairs if dorsolateral side of thorax, abdomen and another on head.

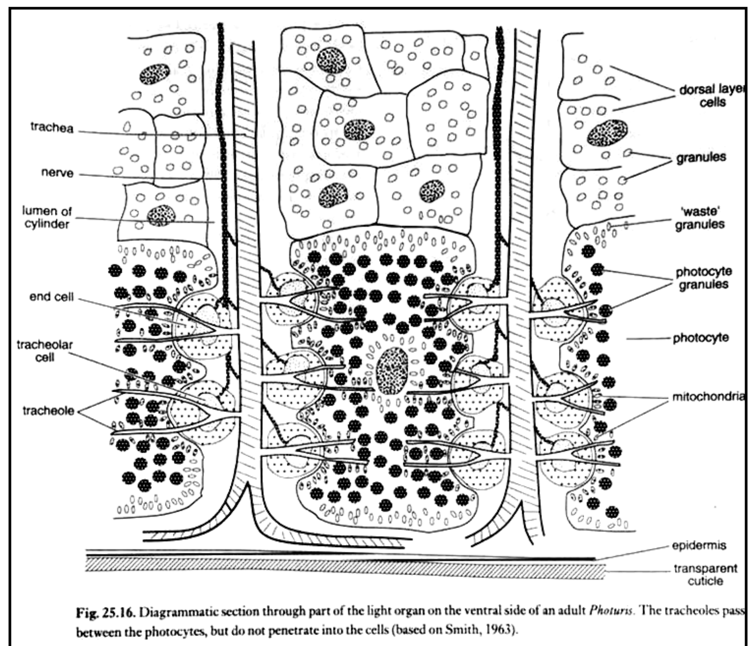


Fig. 25.16. Diagrammatic section through part of the light organ on the ventral side of an adult *Photuris*. The tracheoles pass between the photocytes, but do not penetrate into the cells (based on Smith, 1963).

Types of Light Emission:

1. Continuous Glow – mostly in larvae

2. Intermittent glow – *Photinus* sp. (Order : Coleoptera, Family : Lampyridae)

3. Pulsation glow – *Pyroicoelia*sp. (Order : Coleoptera, Family : Lampyridae)- 6-13 per second & stop

4. Flush glow – *Luciola*sp. (Order: Coleoptera, Family: Lampyridae)- one time high intensity light) Different colour of light:Some of the insects can change the colour of the light and the changing of the pH of enzuyme is responsible for the colour change.

a) Yellow green or Blue Green

b) Pure Green } High intensity
c) Pure Red }

Figure: *Luciola* sp. emitting luminescence.

Image source: Chapman, R.F.(2000). The insects structure and function. *Fourth ed. Cambridge University press*.

Structure of photocytes

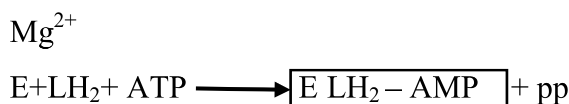
1. Each light producing organ consists of number of large special type of cells the photocytes.
2. Photocytes lie just beneath the epidermis and are backed by several layers of cells called the dorsal layer cells.
3. The cuticle overlying the light organ is transparent.
4. The photocytes from a series of cylinders at right angles to the cuticle.
5. Tracheae and nerve run through the core of each cylinder.
6. Each trachea gives off branches at right angles and as these enter the region of photocytes they break into tracheoles , which run between the photocytes parallel with cuticle.
7. Active ventilation – The tracheoles are spaced 10-15 µm apart and as the photcytes are only 10µm thick the diffusion path for oxygen is short.
8. The origin of the tracheoles is enclosed within a large tracheal end cell, the inner membrane of which is complexly folded where it bounds the tracheolar cell.
9. Mitochondria are sparsely distributed except where the cell adjoins the end cell tracheoles.
10. Prasence of mitochondria i.e. cells very active.
11. Photocytes are packed with photocyte granular, each containing a cavity connecting with outside cytoplasm via a neck.
12. Photocute granules pour its secretion (Luciferase) For this oxidation is necessary, so, tracheal supply is necessary.
13. There are two types of nerve ending
    Big vesicle (larger nerve ending)- Neurosecratory function.
    Small vesicle (Small nerve ending)- Secretion of enzyme , Cholin esterase , which is necessary to form pyrophosphate.
14. Dorsal layer cells contain urate crystal deposit. So, these cells ,become darker and functions as reflective site.

Mechanism of light production in insect

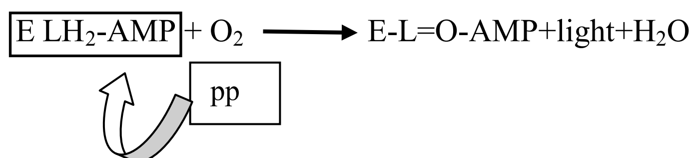
It was William Mcelroy and his colleagues at John Hopkins University first isolated the principal biochemical componenets involved in the firefly light production. Luciferin, a complex carboxylic acid and luciferase is the enzyme.

Basically light is produced by the oxidation of luciferin, in the presence of enzyme luciferase. Luciferin is first activated by ATP in the presence of magnesium and luciferase to produce adenylluciferin. This remains tightly bound to the enzyme and is oxidized to form excited oxyluciferin, which decays spontaneously with the production of light. The reaction is very efficient, some 98% of energy involved being released as light

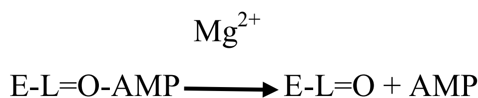
1) Initial Reaction:



2) Final Reaction:



3) Additional Reaction:



E- Enzyme Luciferase

LH_2- Luciferin

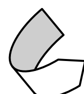
L=O – Oxyluciferin

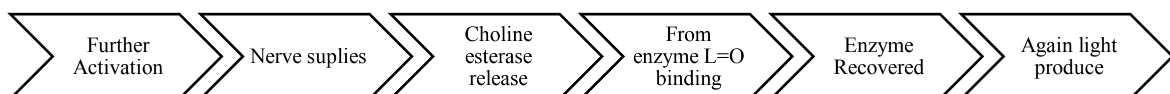
LH_2-AMP = Adenyl luciferin

L=O-AMP= Adenyl oxyluciferin

E-L=O

Light production stop





Significance of bioluminescence:

1. Light production generally related to mating -In Lampyridae, female is sedentary and attracts the male by producing light in a species specific manner. They also release some Phnemenon with light; In case of *Photinus pyratis* generally male on ground climbs steeply at the time of light production after flashing it hovers for about two seconds, then female flash on grasses, it flies towards the female and flashes again and repeated flashing sequences bring the male to female.
2. Food Capturing device- The luminescence of *Bolitophila* larvae serves as a trap, attracting small insects into networks of glutinous silk threads on which they become trapped.

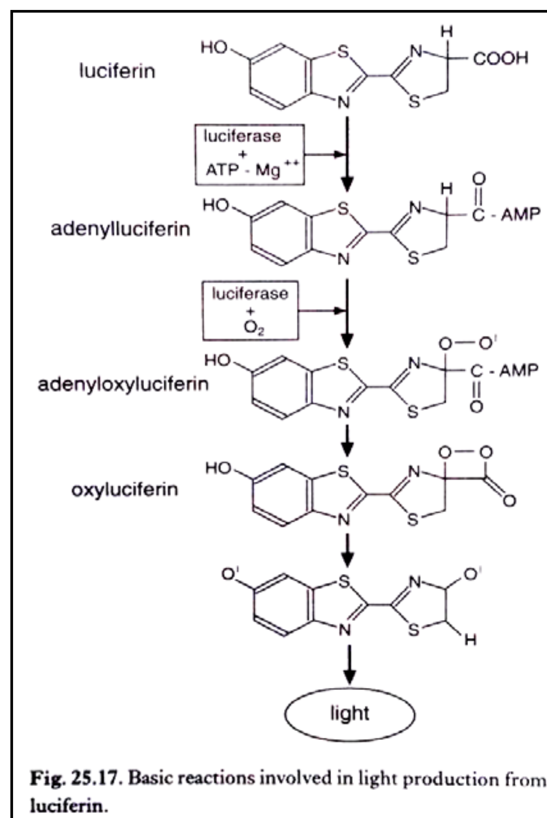


Fig. 25.17. Basic reactions involved in light production from luciferin.

Probable Questions:

1. Give two examples of pterygote insect where bioluminescence is found.
2. Describe the various structural components of dorsal ocelli in insect.
3. Write the significance of light production in bioluminescent insect

Suggested Readings / References:

1. Insect Vision: Ommatidium Structure and Function; You tube presentation by Prof Larry Keeley, Emeritus, Department of Entomology, Texas A & M University
2. Structure and functions of Photoreceptors: Encyclopaedia Britanica.
3. Richards & Davies, Imms general textbook of entomology, 10th Edition
4. The Principles Of Insect Physiology, by Wiggles Worth V.B.
5. The Insects: Structure and Function 5th Edition, by R. F. Chapman

Unit-VI

Structure and functions of sound producing organs in insects.

Objectives:

In this section we will discuss about Structure and functions of sound producing organs in insects.

Introduction:

Insects are preadapted to be noisy animals. With a hard and sclerotised exoskeleton, the segmented form of the body and jointed limbs will inevitably cause vibrations in the surrounding environment when an insect moves. It will be very difficult for insects to move silently without making a noise. It is not surprising that many groups have developed specialised systems of sound production and associated receptors which are used in communication within and between species.

General mechanisms of sound production:

Many authors have attempted to classify sound producing mechanisms in insects. The most useful is probably the entirely mechanistic one in which five categories of sound producing mechanisms are recognized. These are:

1. Vibration

2. Percussion

3. Stridulation

4. Click mechanisms

5. Air expulsion

These categories are not completely exclusive and some insects may use combinations of them.

1. **Vibration — Including Tremulation**

All animal sounds result from the vibration of some structures. However, in this category are included sound emissions which result from vibrations of relatively unspecialized parts of the insect body, most usually oscillations of the abdomen, either dorso-ventrally or laterally. The term tremulation is useful for this type of sound production and differentiates it from the very general term "vibration". Such sounds are usually transmitted through the legs to the substrate on which the insect is walking or standing. These will therefore usually be detected as substrate transmitted vibrations. Such signals have been documented in various insects, but are well known in lacewing flies and their allies.

Vibrations of other body parts may be important in insect signaling, most obviously the wings. Sounds are inevitably produced as byproducts of flapping flight, but many insects have developed the use of wing vibrations in communication. The flight sounds in swarming mosquitoes are known often to be species-specific and to function in part, for species recognition. The use of low frequency wing vibration in the courtship dances of Drosophila species is better known. When in close proximity, the pulsed songs of these flies stimulate antennal receptors of other individuals by air particle vibration in the vicinity.

2. **Percussion**

Tremulation does not involve percussion either of the substrate or of other body parts. This is regarded as a separate mechanism. Percussion of one body part against another may develop as a communication system.

Signaling by percussion of the substrate with the tip of the abdomen is well known in various insect groups, for example in termites and particularly stoneflies. An unusual example among the bush crickets (Tettigoniidae) is the species of Meconema. It is a group otherwise well known for the production of loud stridulatory signals. Males of Meconema lack the distinctive stridulatory mechanism typical of the family, but actively stamp the substrate with one of their hind legs and produce patterned signals in that way.

3. **Stridulation**

The term stridulation has sometimes been used as a general term for any mechanism of sound production in insects, but that negates the utility of the term. It is more usually confined to sounds produced by frictional mechanisms, involving the movements of two specialized body parts against each other in a regular patterned manner. This is an extremely widespread and relatively well-studied mechanism. Such systems have been described in at least seven different insect orders, in most of which it has evolved separately on numerous occasions, as for example in the Coleoptera. Almost all body parts which it is possible to bring into juxtaposition have been modified as stridulatory mechanisms in one group or another. The mechanisms in the groups of Orthoptera sensulato are particularly well-known and documented.

4. **Click Mechanisms**

These rely on the deformation of a modified area of cuticle, usually by contraction and relaxation of special musculature within the body. This results in a series of clicks which may be repeated rapidly in distinctive patterns. Such signals may be amplified in a variety of ways in different insects. The specialized area of cuticle, as exemplified most obviously in the loud singing cicadas (Hemiptera, Cicadidae), is known as a tymbal. Such mechanisms are now well known, though not necessarily well understood, in many other groups, including most, if not all other Auchenorrhyncha, many Heteroptera and various families of Lepidoptera.

5. **Air Expulsion**

This is an unusual and rare mechanism within the Insecta. Various authors have described in a number of insect's exhalatory sounds, often expelled via the tracheal spiracles, but little is known about any function. The best-known example is the large and spectacular European hawkmoth, the Death's Head Hawk, Acherontiaatropos, which expels air forcibly through the mouthparts to make a distinctive piping sound. Thus many sound producing mechanisms have been described for a wide variety of insects, but many exist only as possible mechanisms based simply on surmise from morphological evidence.

Neural regulation of sound production:

The patterns of muscular activity resulting in sound production are generated by pattern generators in the central nervous system. In crickets, where the sound is produced by

movements of the forewings, the pattern generator is in the mesothoracic ganglion; in grasshoppers, where stridulation involves movements of the hind legs, the generator is in the metathorax. The pattern generators are presumed to be networks of interneurons whose output regulates the activity of the motor neurons controlling the muscles.

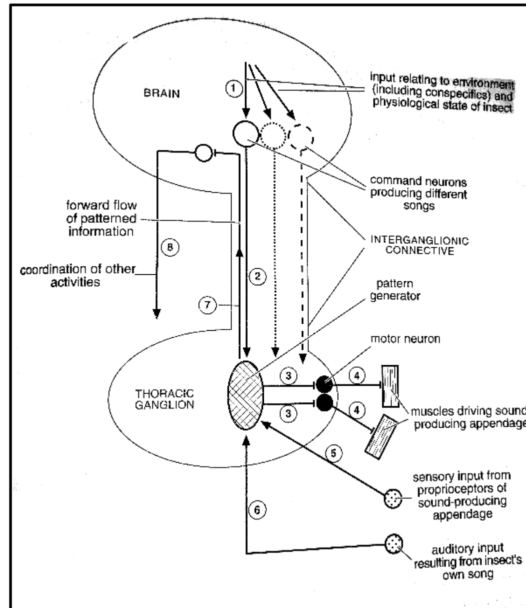


Figure: Neural control of sound production in Orthoptera. Numbers indicate the sequence of neural events.

Probable questions:

1. Discuss the different mechanisms of sound production by insects.
2. What is stridulation? Mention any stridulatory apparatus of insect and write down the mechanism by which it produces.
3. What do you mean by percussion in regard to insect sound production?
4. What do you mean by vibration in regard to insect sound production? Mention the organs by which insect produce this type of sound.
5. Discuss the neural regulation of sound production in insects.
6. What are tymbal organs? State their functions.

Source:

1. Chapman, R. (2012). The Insects: Structure and Function (S. Simpson & A. Douglas, Eds.). Cambridge University Press, 819 p.
2. Gullan, P .J & Cranston, P .S. (2010). The Insects: An outline of Entomology (4th Edition). Wiley-Blackwell, 584 p.
3. Tembhare, D.B. (1997). Modern entomology. Himalaya Publ. House, 623 pp.
4. https://www.ecologycenter.us/insect-sounds/mechanisms-of-sound-production.html
5. http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.329.6459&rep=rep1&type=pdf

Suggested readings:

1. Chapman, R. (2012). The Insects: Structure and Function (S. Simpson & A. Douglas, Eds.). Cambridge University Press, 819 p.
2. Evans, H.E. (1984). Insect biology – A textbook of Entomology. Addison-Wesley Publ. Co., 436 p.
3. Gullan, P .J & Cranston, P .S. (2010). The Insects: An outline of Entomology (4th Edition). Wiley-Blackwell, 584 p.
4. Mani, M.S. (1982). General entomology. Oxford & IBH Publ. Co., 912 pages.
5. Richard, O.W. & Davies, R.G. (1977). IMMS' General textbook of Entomology. Part I & II. Chapman & Hall London, 1354 p.
6. Snodgrass, R . E. (1935). Principles of Insect Morphology. Mcgraw-Hill Book Company. 647 p.
7. Tembhare, D.B. (1997). Modern entomology. Himalaya Publ. House, 623 pages.
8. Wigglesworth V. B. (2015). Insect Physiology. Andesite Press. 148p.

HARD CORE THEORY PAPER (ZHT – 101)
Group B : Insect Organization

Module	Unit	Content	Credit	Class	Time (h)	Page No.
ZHT - 101 (Non Chordate and Insect Organization)	**VII**	Modern classification of insects	1.5	**1**	**1**	**67 – 72**
	VIII	General organization, segmentation, division of body: i) Head and mouth parts in general ii) Thorax and thoracic appendages. Modification of legs and wings. iii) Abdomen and abdominal appendages.		**1**	**1**	**73 – 82**
	IX	Integument: Basic structure and functions		**1**	**1**	**83 – 88**
	X	Digestive organs: Structure and functions; Peritrophic membrane,Filter chamber		**1**	**1**	**89 – 93**
	XI	Morphology of respiratory organs and mechanism of respiration. Morphology of central nervous system		**1**	**1**	**94 – 106**
	XII	Metamorphosis: Basic concept (e.g. House fly) Exocrine glands: Lac gland, Wax gland, Silk gland, Labial gland.		**1**	**1**	**107 – 113**

Group-B: INSECT ORGANIZATION

Unit-VII - MODERN CLASSIFICATION OF INSECTS

Objective: In this Unit we will discuss about Modern Classification of Insects.

Introduction:

Insects are a very large, highly diverse group of small, mostly terrestrial and aquatic animals. They have a three-part body plan with a head, thorax, and abdomen, where six legs and (in most species) four wings are attached to the thorax, and the head has a pair of compound eyes and a pair of antennae.

Insects have by far the largest number of species of all known living things and make up the vast majority of species in Kingdom Animalia. Over a million have been described by science so far. Their relatedness to each other can get rather convoluted, which makes the scientific classification of insects into a Linnaean-style taxonomic tree a challenge.

Insect classification contains 28 extant taxonomic orders - from the primitive wingless silverfish to the evolutionarily advanced butterflies. They belong to the class Insecta, subphylum Hexapoda, phylum Arthropoda, but there are numerous additional levels of subclasses, infraclasses, superorders, and suborders in different schemes of classification.

Classification:

Subclass Apterygota

Order Archaeognatha

Bristletails are a primitive, wingless group of insects with small, moveable appendages along their abdomens, which resemble bristles. They might once have been legs. There are about 350 species in two extant families, Meinertellidae and Machilidae.

Order Thysanura

The other primitive wingless group of insects are the silverfish and firebrats. These have flattened bodies with three long filament tails. There are about 370 species in four families: Lepidothrichidae, Nicoletiidae, Lepismatidae, and Maindroniidae.

Subclass Pterygota

Everything else in class Insecta has two pairs of wings, though in many species one or both pairs have been heavily modified, reduced, or completely lost. It was the evolution of flight

that allowed insects to become so dominant and diverse in terrestrial ecosystems. There are more insect species with wings than all winged vertebrate species (present and past) combined.

Order Ephemeroptera

The 2,500 species of mayflies are aquatic insects with a larval stage that lasts up to a year, then an adult stage that lasts up to a day - just long enough to reproduce.

Order Odonata

Dragonflies and damselflies are large, aerial predators of other insects. They mostly live in aquatic habitats. There are about 6,500 species in two suborders - Zygoptera for dragonflies, Anisoptera for damselflies.

Infraclass (subsubclass) Neoptera

The next evolutionary advancement was the ability to fold the wings flat against the top of the abdomen. Mayflies, dragonflies, and damselflies can't do this, but everything else can (unless they lost the ability later on, such as butterflies).

Order Plecoptera

The stoneflies are aquatic insects that like cold, fast-flowing, very fresh water. The adults look like grasshoppers, but with normal instead of hoppy back legs. There are about 2,000 species in 16 families.

Order Orthoptera

The actual grasshoppers, crickets, locusts, and katydids number about 20,000 species. They're known for chirping, hopping, and being major agricultural pests.

Order Embiidina

The webspinners spin silk webbing, which they use to create tubular living spaces called "galleries." They tend to live in colonies, which expand into new areas by spinning more galleries. Entomologists have described about 360 species out of an estimated 2,000.

Order Zoraptera

The 34 species of angel insects belong to one genus, *Zorotypus*, of family Zorotypidae. These are small colonial insects that look like tiny termites (but aren't related). They are fungivores that live in decaying logs, and are mostly tropical.

Order Dermaptera

The 1,800 species of earwigs like to hide in small, humid crevices. They eat plants and small insects.

Order Phasmida

These are the stick insects and leaf insects - think "walking sticks." They look like sticks or leaves for camouflage. They're mostly nocturnal leaf eaters, and mostly tropical. About 3,000 species have been described.

Order Mantophasmatodea

The 15 species of gladiators look like a cross between stick insects and praying mantises (order Mantodea). They are carnivorous and found only in small parts of Africa, and thought to be most closely related to the Grylloblattodea.

Order Grylloblattodea

The 25 species of ice bugs are nocturnal detritivores found in cold areas, such as the tops of mountains. They look like a cross between a cricket (family Gryllidae), a cockroach (order Blattaria), and a *Campodea* bristletail (subphylum Hexapoda but not class Insecta). All of them are in a single family, Grylloblattidae.

In some insect classification schemes, Phasmida, Mantophasmatodea, and Grylloblattodea are part of the superorder Dictyoptera.

Superorder Dictyoptera

Continuing the scientific classification of insects within Infraclass Neoptera, the cockroaches, termites, and praying mantises are closely related to each other and traditionally thought to be in three orders:

Order **Blattaria** - 4,500 species of cockroaches

Order **Isoptera** - about 4,000 species of termites

Order **Mantodea** - 2,300 species of praying mantises

However, the exact arrangement of their phylogenetic tree is still open to question, with possible intermixing of species between orders. It's also possible that orders Phasmida, Mantophasmatodea, and Grylloblattodea should be included.

Superorder Paraneoptera: Also known as the *hemipteroid assemblage*, this group includes the true bugs, thrips, and lice.

Order Hemiptera

Lots of insects (and non-insects) are commonly called bugs, but these are the scientifically official bugs. There are 50-80 thousand species, including aphids, cicadas, bedbugs, potato bugs, and leafhoppers.

Order Thysanoptera

The 5,000 species of thrips are tiny, cigar-shaped insects with fringed wings. Most are about a millimeter in length. Many are agricultural pests. The word "thrips" is also singular.

Order Psocodea

The 5,500 species of lice are arranged in three suborders. Two of them, Psocomorpha and Trogiomorpha, are bark lice, which are found on trees. Suborder Troctomorpha contains the book lice and the parasitic lice. Book lice are found in old books where they like to eat the binding glue. Parasitic lice are external parasites of mammals and birds that are highly species-specific about their hosts.Parasitic lice traditionally belong to order Phthiraptera.

Superorder Endopterygota

Complete metamorphosis marks the boundary between all previous insects and this next big grouping. Endopterygotes have distinct larval, pupal, and adult stages in their life cycles.

Order Megaloptera

The alderflies, dobsonflies, and fishflies are medium to large aquatic insects, some of them with extra long mandibles. There are about 300 species.

Order Raphidioptera

There are 210 species of snakeflies, which have extra long necks that make them look like snakes.

Order Neuroptera

The lacewings, owlflies, and antlions have large, lace-patterned wings. There are about 6,000 species.

Order Coleoptera

In beetles, the front pair of wings have become a hard covering for the back pair of wings. Fireflies and glowworms are also included in this order. Coleoptera has about 350,000 described species so far, with up to a million possible, which makes it the largest order of known life forms.

Order Strepsiptera

These are 600 species of parasites on other insects, where the females spend their entire lives within the host's abdomen and never develop legs, wings, eyes, antennae, or other important body parts of free-living insects.

Order Diptera

The true flies include house flies, horse flies, fruit flies, and mosquitoes. You can tell they are the scientifically official flies because their common names have "flies" as a separate word (unlike dragonflies, stoneflies, butterflies, etc.). The back pair of wings have become halteres, which are small appendages with knobs that provide balance during flight. There are about 150,000 described species with potentially 240,000 in existence.

Order Mecoptera

There are 550 species of scorpionflies and hangingflies. The scorpion stinger is actually the end of the male abdomen curved upward with an enlarged genital.

Order Siphonaptera

The 2,000 species of fleas are external parasites of mammals and birds that feed on blood. They no longer have wings, but they can jump impressive heights and distances.

Order Trichoptera

Caddisflies are aquatic insects resembling moths. Their larvae make nets, tubes, and casings of sand, pebbles, leaves, twigs, and other debris glued together, which they use as housing. There are about 12,000 described species, with possibly 50,000 in total.

Order Lepidoptera

Butterflies and moths are much loved for their very large, wide, often highly colorful wings. 160,000 species have been described so far.

Order Hymenoptera

Bees, wasps, sawflies, and ants weigh in at 130,000 species. Many species are highly social and form colonies.

Probable questions:

1. Write down the characteristic features of class Insecta. Who are endopterygotes?
2. Classify class Insecta upto living orders.
3. Write down the characteristic features of the following orders and cite examples.
   a) Coleoptera b) Lepidoptera c) Orthoptera d) Diptera c) Heteroptera
4. Write down the evolutionary pattern of insect classification.
5. Insects of which order are known as true fliers? State their advantage over insects belonging to other orders.
6. State the characteristic features of the following insect orders.
   a) Trichoptera b) Hymenoptera c) Dictyoptera c) Siphonaptera

Source:

1. Brues T. C., Melander A. L. & Carpenter F. M. (1954). Classification of Insects. *In Bulletin of the Museum of Comparative Zoology at Harvard College.* Cambridge, 841 p.
2. Wheeler, W. C., M. Whiting, Q. D. Wheeler, and J. M. Carpenter. (2001). The phylogeny of the extant hexapod orders. Cladistics 17:113-169.
3. Tembhare, D.B. (1997). Modern entomology. Himalaya Publ. House, 623 p.
4. http://guaminsects.myspecies.info/sites/guaminsects.myspecies.info/files/Insect%20Orders.pdf
5. http://www.uwyo.edu/entomology/_files/_docs/slideshows/2007-major-insect-orders-of-horticultural-importance.pdf

Suggested readings:

1. Gullan, P.J & Cranston, P.S. (2010). The Insects: An outline of Entomology (4th Edition). Wiley-Blackwell, 584 p.
2. Mani, M.S. (1982). General entomology. Oxford & IBH Publ. Co., 912 pages.
3. Richard, O.W. & Davies, R.G. (1977). IMMS' General textbook of Entomology. Part I & II. Chapman & Hall London, 1354 p.
4. Tembhare, D.B. (1997). Modern entomology. Himalaya Publ. House, 623 p.
5. Wheeler, W. C., M. Whiting, Q. D. Wheeler, and J. M. Carpenter. 2001. The phylogeny of the extant hexapod orders. Cladistics 17:113-169.

Unit VIII

General organization, segmentation and division of body in insect

Objectives:

In this Unit we will discuss about General organization, segmentation and division of body in insect

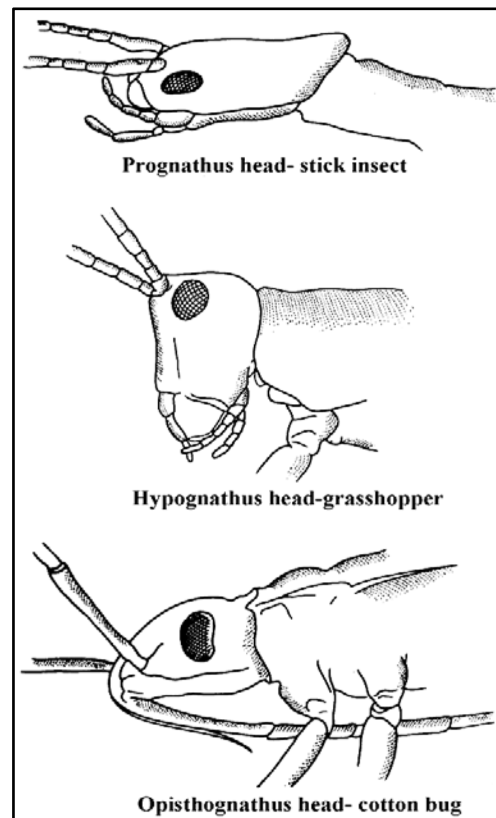
i) Head and mouth parts in general

Head of insect:

The head is entirely encased in rigid exoskeleton (head capsule) which supports the mouth parts, antennae and eyes and within it lies the brain.

Orientation of the head:

Based on the inclination of the long axis and the position of mouth parts, the heads of insects are oriented into three types:



Prognathus head- stick insect

Hypognathus head-grasshopper

Opisthognathus head- cotton bug

Prognathus- The long axis of the head is horizontal, or slightly inclined ventrally and this is in line with the long axis of the insect's body. The mouth parts are pointed forward. E.g. Stick insects, mostly Coleoptera.

Hypognathus- The long axis of the head is at right angle (vertical) to the long axis of the body. The mouth parts are directed downwards ventrally. Eg. Cricket, Grasshoppers

Opisthognathus- The long axis of the head is horizontal and the mouth parts are directed backward so that they arise between the anterior legs. E.g. Plant sucking bug.

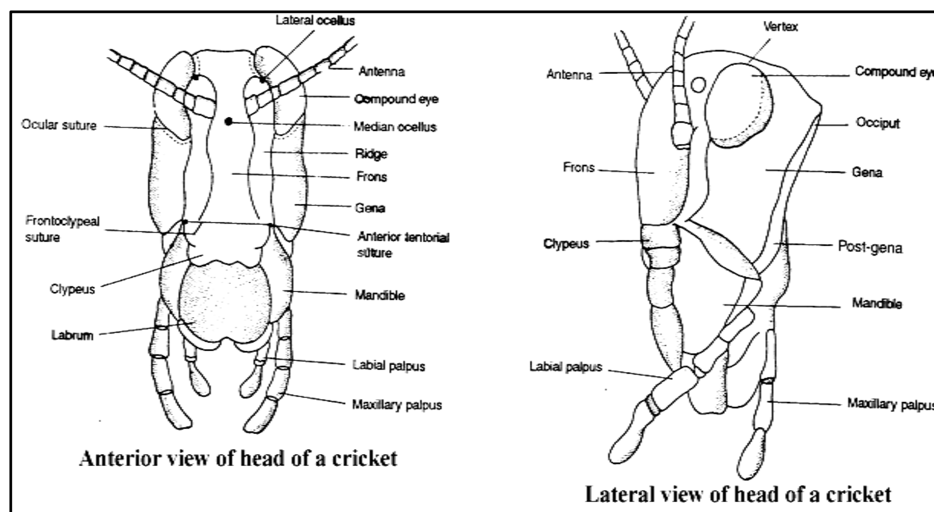
Structural components of insect head:

The head has lost all the traces of segmentation and forms a strongly sclerotized capsule. However, it is a well known fact that at least 6 anterior segments are fused in the head. In the head of a generalized insect the following structures are clearly recognized:

Frons- It is a large unpaired upper sclerite that occupies the frontal area of the head which extends from the frontal sutures to the clypeus and it laterally extends up to the eyes and to the genal suture running from the eyes to the anterior condyle of the mandible. The frons bears the median ocellus.

Clypeus- It is separated from the frons by the epistomial or fronto- clypeal or clypeal suture and the upper lip or labrum is attached along its anterior border.

Labrum- It is an unpaired sclerite movably articulated with the clypeus with the clypeo-labral suture.



Anterior view of head of a cricket

Lateral view of head of a cricket

Vertex- It is the upper part of the head which lies between the compound eyes and behind the frontal suture. This area includes the dorsal portion of the two sclerites separated by the coronal suture or ecdysial cleavage line. These lateral sclerites are called the parietals, which extend back to the occipital sclerite and down each side of the head to include the compound eyes and the genae.

Occiput- It is a narrow, arching, sclerite lying behind the vertex and extending downward on each side to form postgenae. Behind the occiput lies a narrow rim which attaches the head to the neck membrane. A few small cervicle sclerites are present in the neck membrane.

Genae- These represent the parietals or lateral areas that extend below the eye and between the genal suture and the post genae.

Post genae- The part of the occiput below the level of the compound eyes and behind the occipital suture are known as post genae.

Compound eyes-These are located in the lateral areas (parietal) and each eye is surrounded by a narrow ocular sclerite.

Ocelli- These are simple eyes, 3 in number. The median ocellus is located in the frons and the lateral ocelli are placed near the compound eyes.

Antennae- These arise in front of the eyes. Each is set in a membranous area, the margin of which is reinforced by a ridge, the antennal ridge.

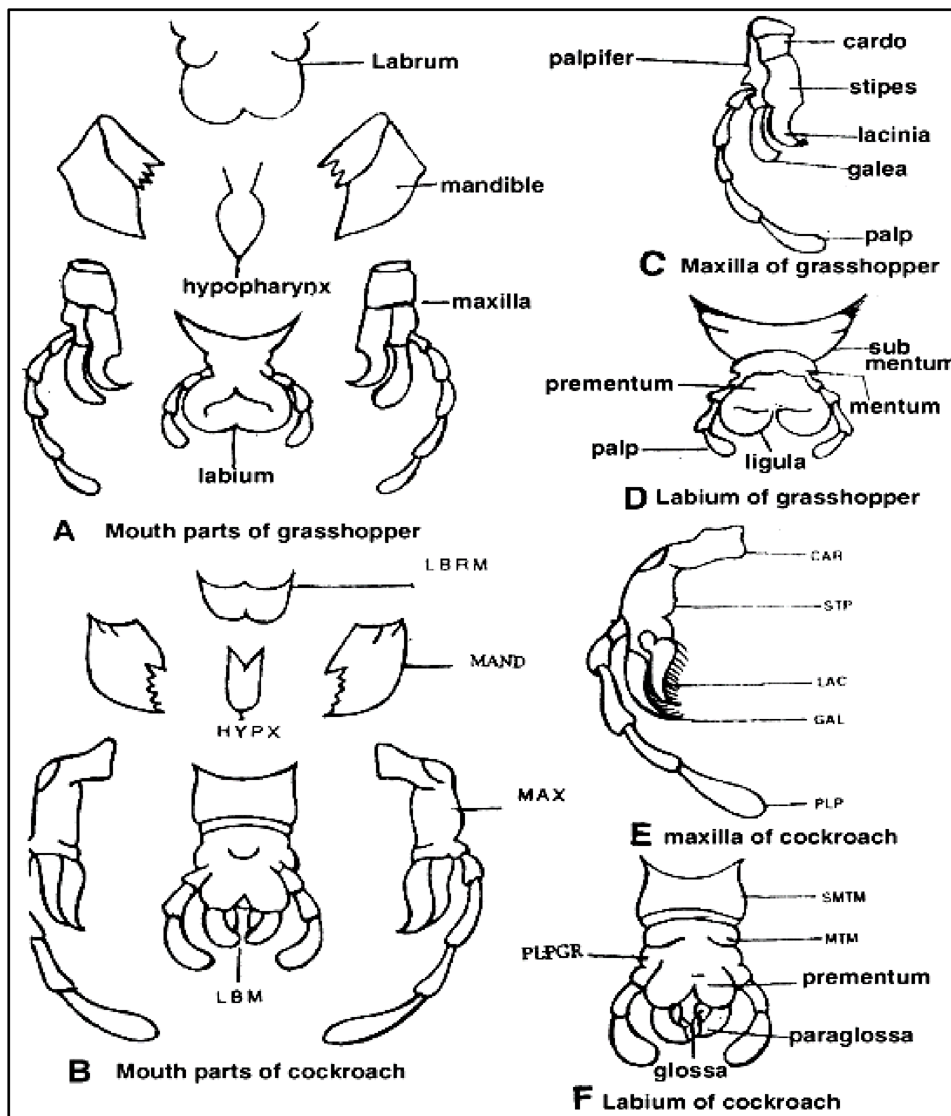
Tentorium- It is an endoskeletal structure which braces the head internally. The tentorium is composed of 2 pairs of invaginations which unite within the head to form a strong frame, overlying on the ventral nerve cord and supporting the the foregut which passes above it. It has anterior, posterior and dorsal arms. The anterior arms arise as invagination at each end of the clypeal suture, the posterior arms arise from the lower part of the post occipital suture and the dorsal arms extend from the anterior arms to the bases of the antennal sclerite.

Mouth parts of insects:

The structures on the lower part of the head are known as mouth parts, which are variously adapted to the diet of the insects. The mouth parts are formed from modified appendages of head segments. They surround the mouth opening and in most cases are quite exposed and visible. However in a few insect groups eg. Collembola the mouth parts are enclosed by the lower part of the head. Mouth parts in a generalized insect comprise of the following structures:

Labrum- flap like structure attached to the clypeus and is capable of a limited up and down movement, it acts as upper lip and helps to pull the food into the mouth.

Epipharynx- it is continuous with the roof of the mouth and to the oesophagus. It is a sensitive area which contains the organs of taste.



Mandible- important structure in biting and chewing type of mouth parts; are stiff, solid and compact structures articulating with the head possessing about three faces and are pyramidal shaped. The upper or outer surface is articulated and connected with a gena, the inner face is provided with large number of teeth and the hinder ends are articulated by a round process that fits into a socket of the genae, post genae or subgenae. They are regarded as 1st pair of jaws and they move from side to side in a transverse plane. They also help in carrying the food particles and cutting of leaf or tissues; they act as organ of offence and defense.

Maxillae- 2nd pair of jaws, work side to side just like mandible, shape varies with the kind of food and mode of feeding, composed of cardo, stripes and palpifer.

The palpifer forms the maxillary palp which is 1-7 segmented and antennae like in shape. The palp functions as olfactory and gustatory organ. Maxilla is distally composed of two lobes- an

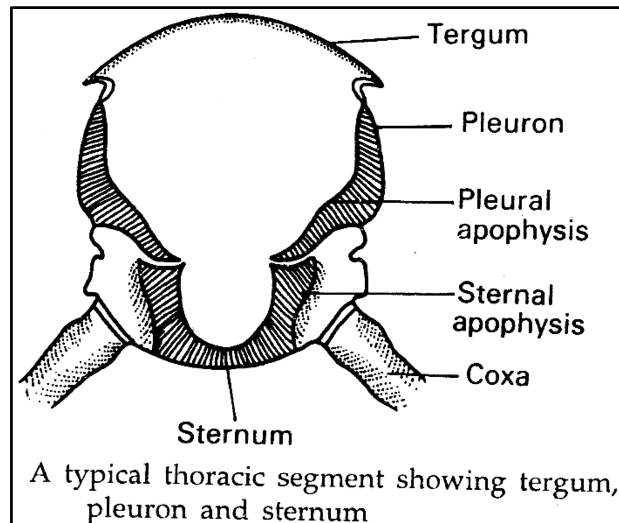
outer lobe galea and an inner lobe lacinia, the inner border of lacinia is spined and often modified for cutting, grasping and grinding the food.

Hypopharynx - it is a fleshy tongue, arising from the floor of mouth or preoral cavity and is usually attached to the inner wall of the labium. It is more or less covered with hairs. The salivary glands open through it.

Labium - it is formed by the fusion of a pair of appendages homologous to maxillae. It form the lower lip of the mouth. The labium is divided into two primary regions- proximal-postmentum and a distal-prementum. The line of division between the two portions is known as labial suture. On either side near the base of prementum is the labial palp consisting of 1- 4 segments and functioning as sensory organs. From the distal margin of prementum arises 2 pairs of lobes which collectively constitute the ligula. Ligula comprises 4 unsegmented appendages- a median or Inner pair of glossae and a lateral or outer pair of paraglossae.

ii) Thorax and thoracic appendages, modification of legs

Thoracic segments:



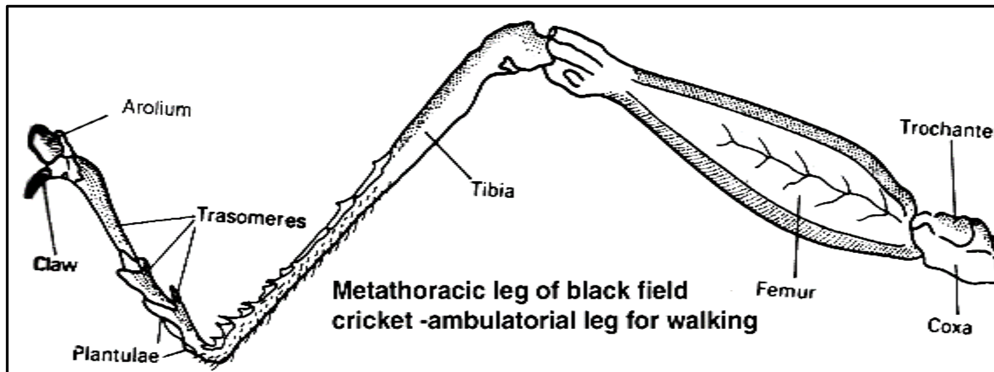
A typical thoracic segment showing tergum, pleuron and sternum

Thorax is the 2nd region of the body of the insects. It is connected to the head by a membranous neck region that help in the movement of the head.Thorax comprises of three segments: Prothorax, Mesothorax and Metathorax. Each of these segments consists of a dorsal sclerite called the tergum or notum, a ventral sclerite called the sternum and on the either side is the pleuron which is membranous. Three pairs of jointed legs are attached to the thorax, one pair to each segment. 2 pairs of wings arise from the 2nd and 3rd segments of the thorax.Within the thorax are the powerful muscles which operate the wings and legs.

Insect leg:

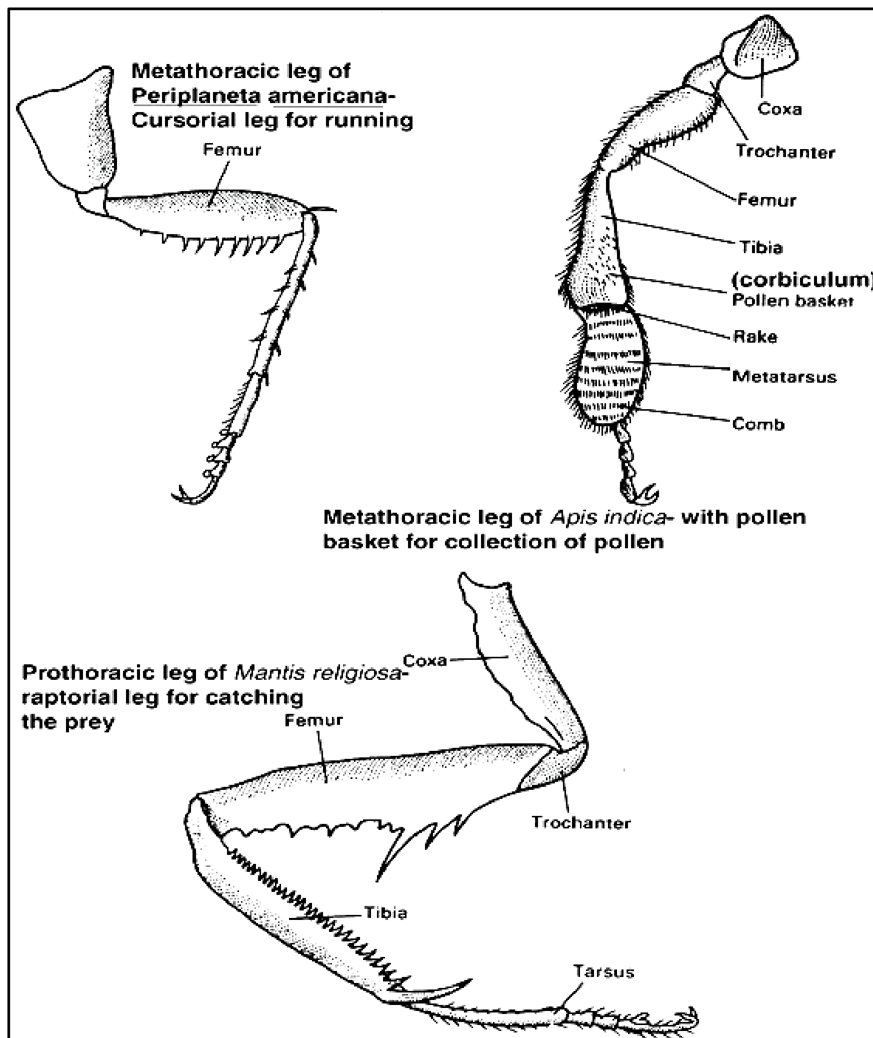
Insect legs have great structural variations adapted to life habits of insects. However the basic plan of all the legs is similar. Each leg consists of a coxa (articulating with the pleuron and

sternum by a membrane), trochanter, femur, tibia tarsus pretarsus and two claws and a pad (called arolium).



Modification of insect legs:

The functions for which the legs have become modified include jumping, swimming, grasping, digging, sound production and cleaning.



iii) Abdomen and abdominal appendages

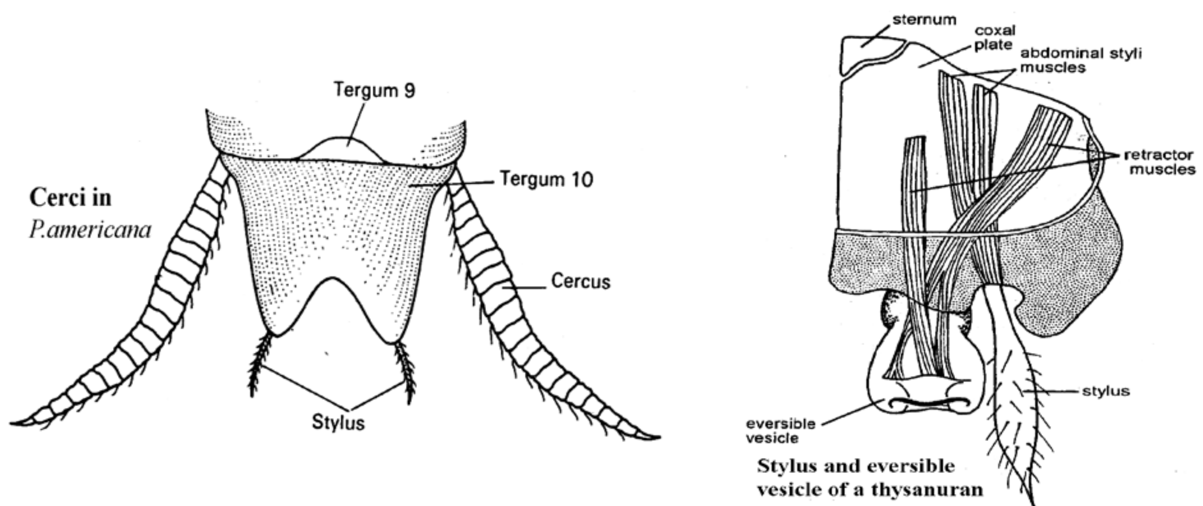
The 3rd division of the insect body, the abdomen, consists of 10-11 segments. In most insects the actual number of segments present is less and it is not possible to recognize more than 5-6 segments (reduction at the posterior end).Each abdominal segment consists of a tergum or dorsal plate, a sternum or ventral plate, separated by a membranous area at each lateral side. The dorsal and ventral plates are joined together by membranous intersegmental membranes.There are small dark openings, the spiracles, set into the soft membranous area along each side of the abdomen. There is 1 pair in each segment.

Abdominal appendages:

1**. Cerci:** These tapering appendages arise from the tip of the abdomen (generally segment 11). Paired cersi occur in Diptera, Ephemeroptera, Zygoptera, Plecoptera, Dictyoptera. They are elongate multi segmented structures that function as sense organs. In *Periplaneta americana* (Dictyoptera), cerci are simple, jointed and clothed in fine hair while in Dermaptera, these are sclerotized and form unjointed forceps. The cerci of nymphs of Zygoptera are modified to form the lateral caudal lamellae where as in adult male the cerci form claspers for grasping the female during copulation.

2. **Styli:** Styli occur on most abdominal segments of Microcoryphia, Zygentoma and Diplura and on the 9th sternum of some male Orthopteroids. In some bristletails the styli are articulated with a distinct coxal plate and helps in locomotion but generally the original coxal segment is fused with the sternum

3. **Eversible vesicles:** they are short cylindrical structures found on some pregenital segments of apterygotes. They are closely associated with the styli when present and believed to have the ability to take up water from the environment.

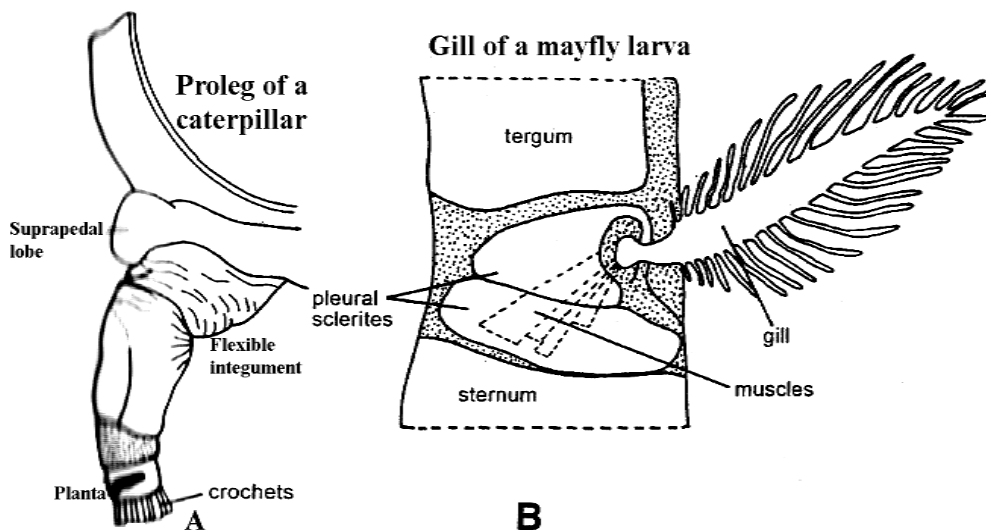


Cerci in *P.americana*

Stylus and eversible vesicle of a thysanuran

4. Prolegs (pseudopods or larvapods): Segmentally arranged, leg like structures are present on the abdomen of many endopterygote larvae. Their structure is varied, though typically (eg. in caterpillars) three regions can be distinguished; a basal membranous articulation, followed by a longer section having a sclerotized plate on the outer wall and an apical protractile lobe, planta, which bears claws, crochets, peripherally. The planta is protracted by means of blood pressure. Above the leg there is a swollen area in the body wall, suprapedal lobe.

5. Gills: A large number of aquatic larvae possess segmentally arranged gills on a varied number of abdominal segments. These are flattened, filamentous structures, frequently articulated at the base.

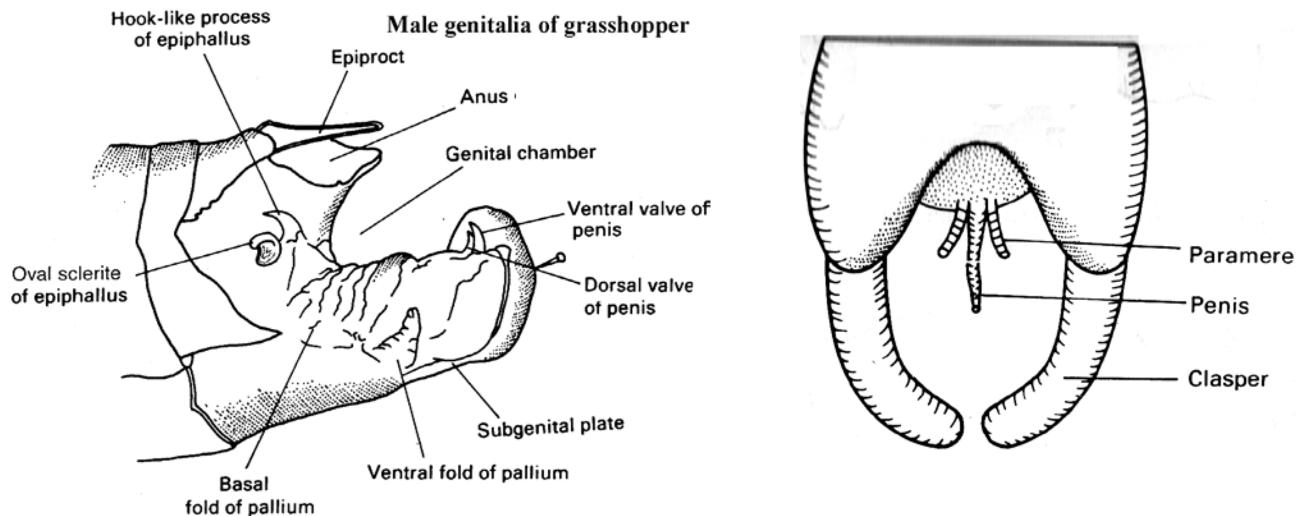


6. **Non-segmental appendages**: These are typically a mediodorsal projection on the last abdominal segment. eg. The median lamella of Zygopteran larvae and the caudal filament of Microcoryphia, Zygentoma and Ephemeroptera. Occasionally these structures are paired (e.g. the urogomphi of some larval Coleoptera) and are easily mistaken for cerci. The anal papillae of certain dipteran larvae also fit in this category.

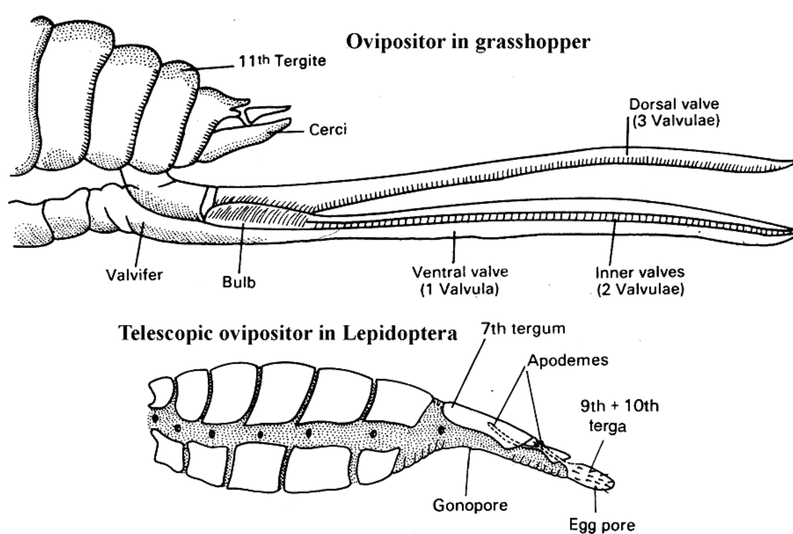
7. **External Genitalia or reproductive appendages:** The 9th abdominal segment in the male and the 8th and 9th in the female insects form the genital apparatus.

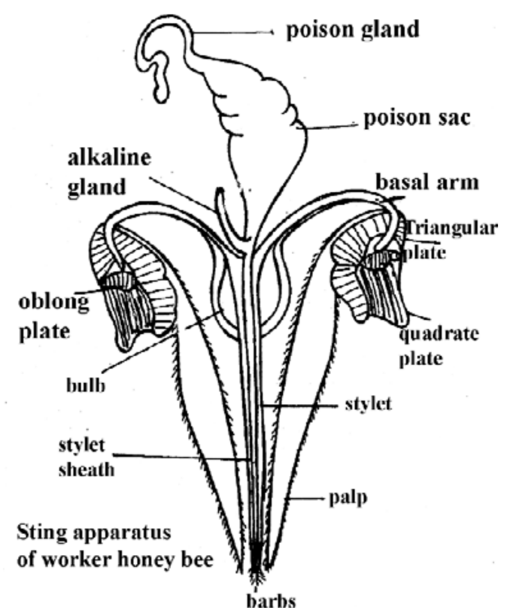
8. **Male genitalia:** Helps in distinguishing species. In a typical male Pterygote insect, the complete external genitalia are composed of – Penis; a pair of parameres or the inner processes; and a pair of claspers. The penis is formed by the fusion of paired outgrowths and it bears the male gonopore. The original paired condition is found only in Ephemeroptera and in certain Dermaptera.In several apterygote and pterygote insects, gonopodsare present which are unmodified limb bases bearing either long

styli but perform no genital functions as in Thysanura, or short styli as in Dictyoptera. In many insects e.g. Ephemeroptera, Mecoptera and Trichoptera, the styli along with their limb bases form functional clasping organs. Absent in Coleoptera.



Female genitalia: They take the form of a sword-like projection, the ovipositor, directed backwards. Ovipositor is composed of 3 pairs of valves- anterior or ventral (1st pair); posterior or inner (2nd pair); and lateral or dorsal (3rd pair). The 1st pair arise from the valvifer-1 of the 8th segment, 2nd pair are the median outgrowths of the valvifer-2 of the 9th segment and the 3rd pair arise from the valvifer-3 of the 9th segment. These three pairs of valves are variously modified in several insects and are well developed in grasshoppers, crickets and cicadas. It is used for depositing eggs (in soil) which are squeezed along its length between the shafts. In some insect groups such as Diptera, Lepidoptera and Coleoptera, the hind end of the abdomen is telescopic in form and acts as an ovipositor





instead. In higher Hymenoptera such as wasps, bees the ovipositor has become modified to form a poison-injecting apparatus, the sting, instead of lying eggs. At rest the sting lies in a pocket within 7th abdominal segment, from which it is exerted when in use. There are 3 pairs of plate-like sclerites- the quadrate, oblong and triangular plates at the base of the sting which articulate with each other.

Probable Questions:

1. Classify orientation of insect head.

2. Describe different structural components of insect head.

3. Describe different components of insect mouth parts.

4. Write down the structure of insect leg.

5. Describe different types of abdominal appendages of insect.

Suggested Reading:

1. Chapman, R. (2012). The Insects: Structure and Function (S. Simpson & A. Douglas, Eds.). Cambridge University Press, 819 p.
2. Evans, H.E. (1984). Insect biology – A textbook of Entomology. Addison-Wesley Publ. Co., 436 p.
3. Gullan, P.J & Cranston, P.S. (2010). The Insects: An outline of Entomology (4th Edition). Wiley-Blackwell, 584 p.
4. Mani, M.S. (1982). General entomology. Oxford & IBH Publ. Co., 912 pages.
5. Richard, O.W. & Davies, R.G. (1977). IMMS' General textbook of Entomology. Part I & II. Chapman & Hall London, 1354 p.
6. Snodgrass, R. E. (1935). Principles of Insect Morphology. Mcgraw-Hill Book Company. 647 p.
7. Tembhare, D.B. (1997). Modern entomology. Himalaya Publ. House, 623 pages.
8. Wigglesworth V. B. (2015). Insect Physiology. Andesite Press. 148p.

Unit-IX

INSECT INTEGUMENT STRUCTURE AND FUNCTION

Objectives:

In this unit we will discuss about Insect Integument Structure and Function

Introduction:

Insect body wall is called as Integument or Exoskeleton. It is the external covering of the body which is ectodermal in origin. It is rigid, flexible, lighter, stronger and variously modified in different body parts to suit different modes of life.

I. Structure

Insect Body wall consists of a **basement membrane,** an inner cellular layer (**Epidermis**) and an outer non cellular part (**Cuticle**).

a) Basement Membrane

It is a continuous sheet of mucopolysaccharide, as much as 0.5 mm in thickness; initially secreted by hemocytes

b) Epidermis

It is an inner unicellular layer resting on basement membrane with the following function.

i. Cuticle secretion

ii. Digestion and absorption of old cuticle

iii. Wound repairing

iv. Gives surface look

c) Cuticle

It is an outer non cellular layer comprising of three sub layers.

i. Endocuticle

Compared to others it is the inner and thickest layer. This layer is made up of **Chitin** and **arthropodin**. This layer is colourless, soft and flexible.

ii.**Exocuticle**

Outer layer, much thicker with the composition of **Chitin** and **sclerotin**. This layer is dark in colour and rigid.

iii. **Epicuticle:** Outer most layer which is very thin. Pore canals present in the exocuticle helps in the deposition of epiculticle. This layer is differentiated into the following layers.

a. Inner epicuticle: It contains **wax filaments**

b. Outer epicuticle: It makes the contact with **cuticulin**

c. Cuticulin : Non chitinous polymerised lipoprotein layer.

d. Wax layer: It contains closely packed wax molecules which prevents desiccation.

e. Cement layer: Outer most layer formed by lipid and tanned protein. It protects wax layer.

Oenocytes:

The oenocytes are large polyploid cells associated with the basement membrane.

- some oenocytes might be involved in the production of cuticular lipid that are deposited in the epicuticle.
- other types of oenocytes may secrete ecdysteroid hormones.

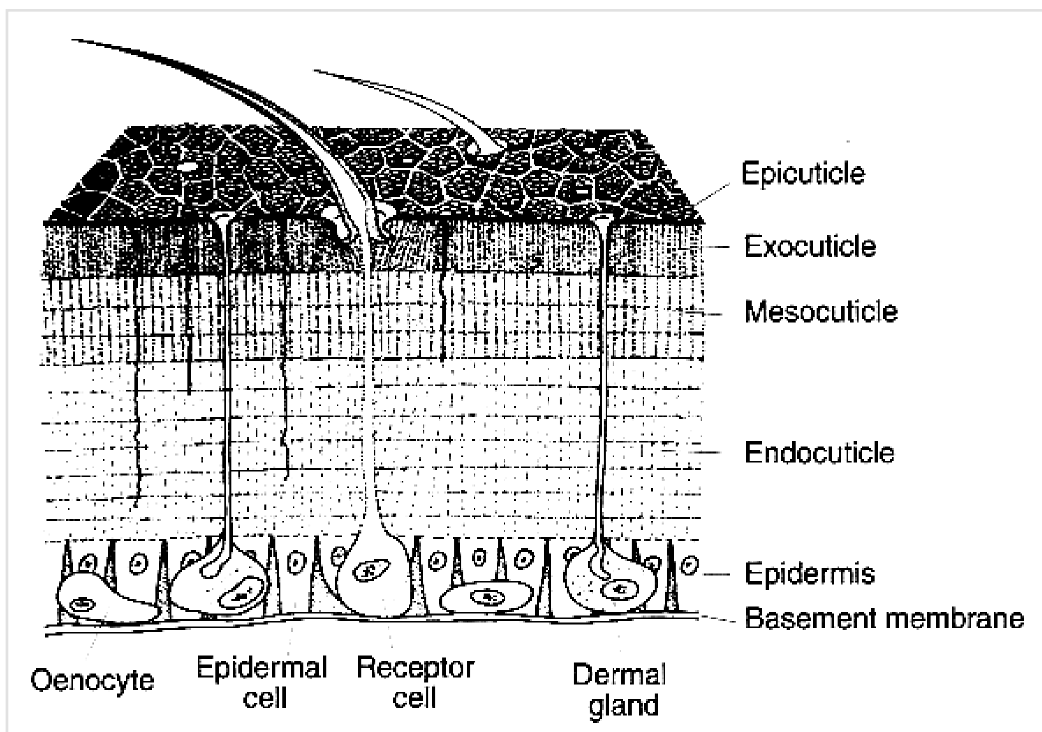


Figure: Section of Insect integument

Composition of cuticle

i. **Chitin:** It is the main constituent of cuticle, which contains Nitrogenous polysacharide and polymer of **N-acetylglucosamine**. It is water insoluble but soluble in dilute acids, alkalies and organic solvents.

ii.**Arthropodin:** An untanned cuticular protein, which is water soluble.

iii.**Sclerotin:** Tanned cuticular protein, which is water insoluble.

iv.**Resilin:** An elastic cuticular protein responsible for the flexibility of sclerites, e.g., wing articulatory sclerites.

ENDOSKELETON

Cuticular in growth of body wall providing space for muscle attachment is known as endoskeleton. There are two types

i.**Apodeme:** Hollow invagination of body wall.

ii.**Apophysis:** Solid invagination of body wall.

CUTICULAR APPENDAGES

Non-cellular: Non-cellular appendages have no epidermal association, but rigidly attached. e.g. minute hairs and thorns.

Cellular: Cellular appendages have eipdermal association.

Unicellular

a. Clothing hairs, plumose hairs. e.g. Honey bee. Bristles. e.g. flies.

b. Scales - flattened out growth of body wall e.g. Moths and butterflies

c. Glandular seta. e.g. caterpillar

d. Sensory setae - associated with sensory neuron or neurons

e. Seta - hair like out growth (Epidermal cell generating seta is known as **Trichogen**, while the socket forming cell housing trichogen is known as **Tormogen**. Study of arrangement of seta is known as **Chaetotaxy**).

Multicellular

e.g. Spur - movable structure

Spine- Immovable structure

GLANDS

Cuticular glands are either unicellular or multicellular. Following are some of the examples.

i. Wax gland - e.g. Honey bee and mealy bug

ii. Lac gland - e.g. Lac insects

iii. Moulting gland secreting moulting fluid.

iv. Androconia or scent scale - e.g.moth

v. Poison gland - e.g. slug caterpillar

Functions of the Integument:

i. Acts as external armour and strengthen external organs like jaws and ovipositor

ii. Protects the organs against physical aberation, injurious chemicals, parasites, predators and pathogen.

iii. Internally protects the vital organs, foregut, hindgut and trachea.

iv. Provides space for muscle attachment and gives shape to the body.

v. Prevents water loss from the body.

vi. Cuticular sensory organs helps in sensing the environment.

vii. Cuticular pigments give colour.

MOULTING (Ecdysis)

Ecdysis

Periodical process of shedding the old cuticle accompanied by the formation of new cuticle is known as **moulting** or **ecdysis**. The cuticular parts discarded during moulting is known as **Exuvia**. Moulting occurs many times in an insect during the immatured stages before attaining the adult-hood. The time interval between the two subsequent moulting is called as **Stadium** and the form assumed by the insect in any stadium is called as **Instar**.

Steps in moulting

1. Behaviroual changes: Larva stops feeding and become inactive.
2. Changes in epidermis: In the epidermis cell size, its activity, protein content and enzyme level increases. Cells divide miotically and increases the tension, which results in loosening of cells of cuticle.
3. Aolysis: Detachment of cuticle from epidermis
4. Formation of Sub cuticular space
5. Secretion of moulting gel in the sub cuticular space which is rich with chitinase and protease.
6. New epicuticle formation: Lipoprotein layer (cuticulin) is laid over the epidermis.
7. Procuticle formation: Procuticle is formed below the epicuticle.
8. Activation of moulting gel: Moulting gel is converted into moulting fluid rich in enzymes. This activates endocuticle digestion and absorption.
9. Wax layer formation: Wax threads of pore canals secrete wax layer.
10. Cement layer formation: Dermal glands secretes cement layer (Tectocuticle).
11. **Moulting:** This involves two steps

i. **Rupturing of old cuticle:** Insect increases its body volume through intake of air or water which enhances the blood flow to head and thorax. There by the old cuticle ruptures along prede-termined line of weakness known as **ecdysial line**

ii. **Removal of old cuticle:** Peristaltic movement of body and lubricant action of moulting fluid helps in the removal of old cuticle. During each moulting the cuticular coverings discarded are the cuticular of legs, internal linings of foregut and hindgut and trachea.

12. Formation of exocuticle: The upper layer of procuticle develops as exocuticle through addition of protein and tanning by phenolic substance.

13. Formation of endocuticle: The lower layer of procuticle develops as endocuticle through addition of chitin and protein. This layer increases in thickness.

Control of Moulting: It is controlled by endocrine gland like prothoracic gland which secrete moulting hormone. Endocrine glands are activated by prothoracico-tropic hormones produced by neurosecretory cells of brain.

Probable questions:

1. Describe the structure of insect integument with proper diagram and comment on the roles of different regions.
2. Write down the chemical composition of cuticle. How exocuticle and endocuticle are formed?
3. Describe the different steps of Moulting. Add a note on control of moulting.
4. What are apodemes and apophysis?
5. What are cuticular appendages? Name different cuticular appendages of insects.
6. Write down the functions of integument.
7. What are oenocytes? State their function.
8. Name the different glands of the cuticle and state their function.
9. What are pore canals? State their function.

Source:

1. Chapman, R. (2012). The Insects: Structure and Function (S. Simpson & A. Douglas, Eds.). Cambridge University Press, 819 p.

2. Gullan, P.J & Cranston, P.S. (2010). The Insects: An outline of Entomology (4th Edition). Wiley-Blackwell, 584 p.
3. Snodgrass, R. E. (1935). Principles of Insect Morphology. Mcgraw-Hill Book Company. 647 pp.
4. http://eagri.org/eagri50/ENTO231/lec04.pdf

Suggested readings:

9. Chapman, R. (2012). The Insects: Structure and Function (S. Simpson & A. Douglas, Eds.). Cambridge University Press, 819 p.
10. Evans, H.E. (1984). Insect biology – A textbook of Entomology. Addison-Wesley Publ. Co., 436 p.
11. Gullan, P.J & Cranston, P.S. (2010). The Insects: An outline of Entomology (4th Edition). Wiley-Blackwell, 584 p.
12. Mani, M.S. (1982). General entomology. Oxford & IBH Publ. Co., 912 pages.
13. Richard, O.W. & Davies, R.G. (1977). IMMS' General textbook of Entomology. Part I & II. Chapman & Hall London, 1354 p.
14. Snodgrass, R. E. (1935). Principles of Insect Morphology. Mcgraw-Hill Book Company. 647 p.
15. Tembhare, D.B. (1997). Modern entomology. Himalaya Publ. House, 623 pages.
16. Wigglesworth V. B. (2015). Insect Physiology. Andesite Press. 148p.

Unit- X:

Digestive organs: Structure and functions; Peritrophic membrane, Filter chamber

Objectives:

In this section we will discuss about Digestive organs: Structure and functions; Peritrophic membrane, Filter chamber

Introduction:

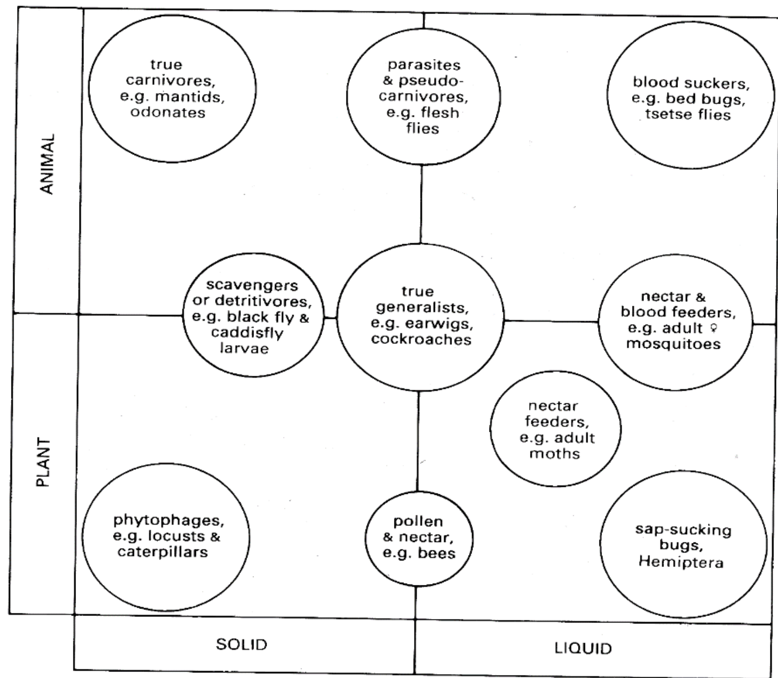
Insects of different groups consume an astonishing variety of foods. There are four major feeding specializations that can be identified depending on whether the food is solid or liquid or of plant or animal origin.Hence gut morphology and physiology relate to these dietary differences in the following ways:

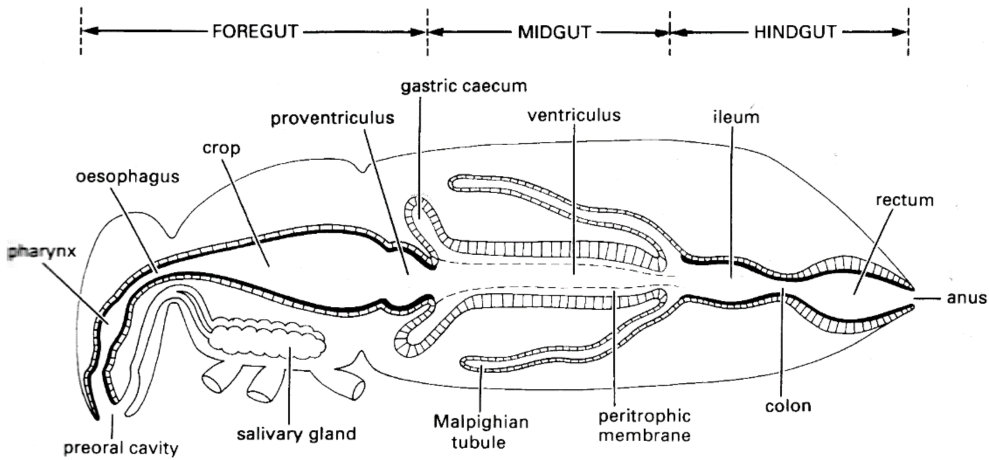
Insects that take solid food typically have wide, straight, short gut with strong musculature and obvious protection from abrasion especially in the midgut which has no cuticular lining. e.g. Plant feeding caterpillars

Insects feeding on blood sap or nectar usually have long, narrow, convoluted guts to allow maximal contact with the liquid food. It requires the mechanism for removing excess water to concentrate nutrient substances prior to digestion. e.g. Hemiptera

From a nutritional viewpoint most plant feeding insects need to process large amounts of food because nutrient levels in leaves and stems are often low. The gut is usually short and without storage areas as food is available continuously.

A diet of animal tissue is nutrient rich and well balanced. However the food may be available only intermittently and hence gut has large storage capacity.





Generalized insect alimentary canal showing division into three regions. The cuticular lining of the foregut and hindgut are indicated by thicker black lines.

Salivary glands-The salivary glands are usually paired tubular, racemose or branched structures with a number of lobes situated on either side of the foregut, generally in the thoracic region. The saliva secreted by these glands is often stored- as in the case of cockroaches, in reservoirs called salivary receptacles, whose ducts open by a common duct into the median salivary duct formed by the union of the salivary ducts of each gland. The salivary glands of the larvae of Lepidoptera and Hymenoptera are modified into silk producing organs. In mosquitoes, bed bugs etc the saliva contains anticoagulant substances,

while in some ants the saliva contains formic acid and toxic substances in some others. Other well defined glands also are present in many insects like mandibular glands, accessory labial glands etc which are modifications of glands associated with the gut.

Fore gut (stomodaeum)-ectodermal in origin, the foregut and the hind guts possess a chitinous lining. A stomodeal valve and a proctodaeal valve are generally present at the opening of the foregut into the midgut and that between the midgut and hindgut respectively.

The true mouth is situated in the cibarium and leads into a comparatively narrow tube, the pharynx. The pharynx directly leads into the oesophagus, often enlarged posteriorly into a simple, sac-like crop which serves for food storage. The crop is not always present but when present may be only an enlargement of the oesophagus or a diverticulum from it. The crop is followed by a muscular proventriculus often provided with strong cuticular plates or teeth serving for trituration and mixing of food. The proventriculus is pushed into the cavity of the mesenteron to form the stomodaeal valve generally consisting of four proventricular lips which serve to regulate the passage of food into the mesenteron preventing the regurgitation of food from the ventriculus to the crop. The foregut in grasshoppers or acridids is provided with numerous chitinised teeth arranged in various patterns.

Mid gut (mesenteron) endodermal in origin, the mesenteron or the midgut is physiologically the most active part of the alimentary canal being concerned with digestion. Opening anteriorly into the midgut are usually a variable number of gastric caecae, varying in number from 2 -6. Lying at the junction of the midgut and hindgut are the malpighian tubules discharging their products into the alimentary tract.

Hind gut (proctodaeum) ectodermal in origin, is usually differentiated into an anterior ileum and a posterior large intestine ending in an enlarged rectum.

Structure and Function of Filter chamber

In many homopterans, which feed on plant sap, the mid gut is modified both morphologically and anatomically so that excess water present in the food can be removed, thus preventing dilution of the hemolymph. Though details vary among different groups of homopterans, the anterior end of the midgut or the posterior part of the oesophagus is brought into close contact with the posterior region of the midgut or anterior hindgut and the region of contact becomes enclosed within a sac called the 'Filter chamber'.

Such an arrangement facilitates rapid movement of water by osmosis from the lumen of the anterior midgut across the wall of the posterior midgut and possibly also the malpighian tubules and thus only little of the original water in the food actually passes along the full length of the midgut.

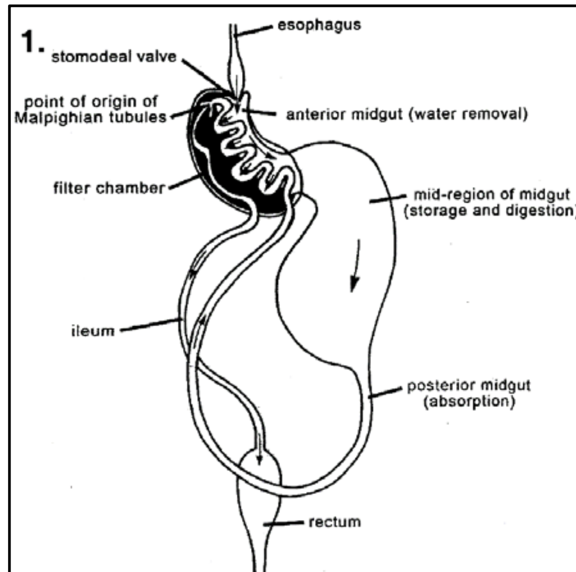


Figure: Alimentary canal of cercopid (cercopoidea) showing filter chamber arrangement.

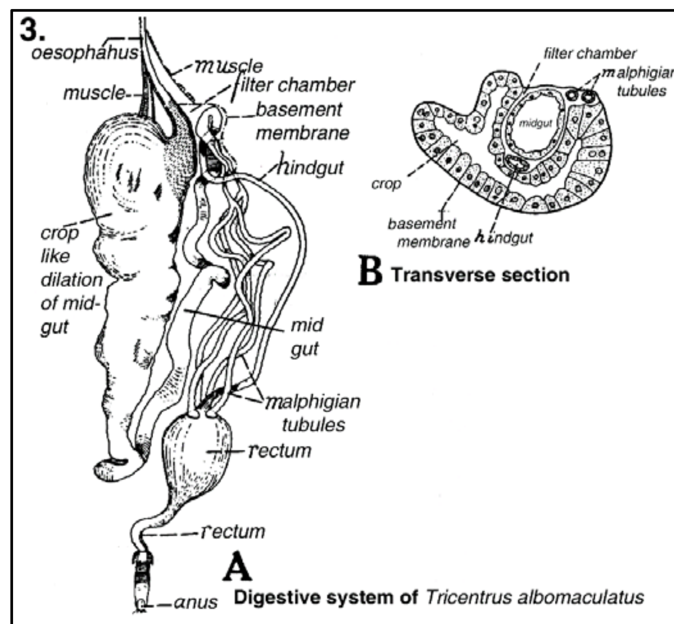


Figure:Structure and function of Peritrophic membrane

The epithelial cells of the mid gut are devoid of cuticle, but in most insects they are protected from the contents of the gut by a delicate detached sheath called peritrophic membrane (PM).

This consists of a mucoprotein in which chitin fibrils are arranged irregularly or in hexagonal or orthogonal arrays.

PM protect the midgut from abrasion by food particles and are said to be absent from most insects feeding on a liquid diet eg. the Hemiptera except Corixidae and many adult Lepidoptera and blood-sucking insects. However they occur in Cicadella, mosquitoes and Glossina.

The PM has been considered to arise in two ways:

- In some Lepidoptera, Diptera and Dermaptera it is secreted by cells near the junction of fore and mid gut and extruded in tubular form by a muscular press in this region.
- In other insects it arises by delamination from part or all of the general surface of the mid gut and aseries of concentric membranes, formed by successive delaminations is often present. In Apis and Periplanata a membrane formed predominantly in the anterior part of the mid gut is supplemented by delamination further back.

Function of PM-

Protect the mid gut from abration by hard fragments in the foods.

Provides permeability to the dies with large colloidal particles such as congo red or Berlin blue.

Probable Questions:

1. Give the structure of generalized digestive system of an insect.

2. Explain the structural and functional significance of filter chamber found in the mid gut of insect.

3. Explain with proper diagram the various structural components of a typical insect mouth parts.

Suggested Readings / References:

1. Richards & Davies, Imms general textbook of entomology, 10th Edition
2. The Principles Of Insect Physiology, by Wiggles Worth V.B.
3. The Insects: Structure and Function 5th Edition, by R. F. Chapman
4. Entomology by Cedric Gillot, Springer

Unit-XI

Morphology of respiratory organs and mechanism of respiration in insect and Morphology of Central Nervous System

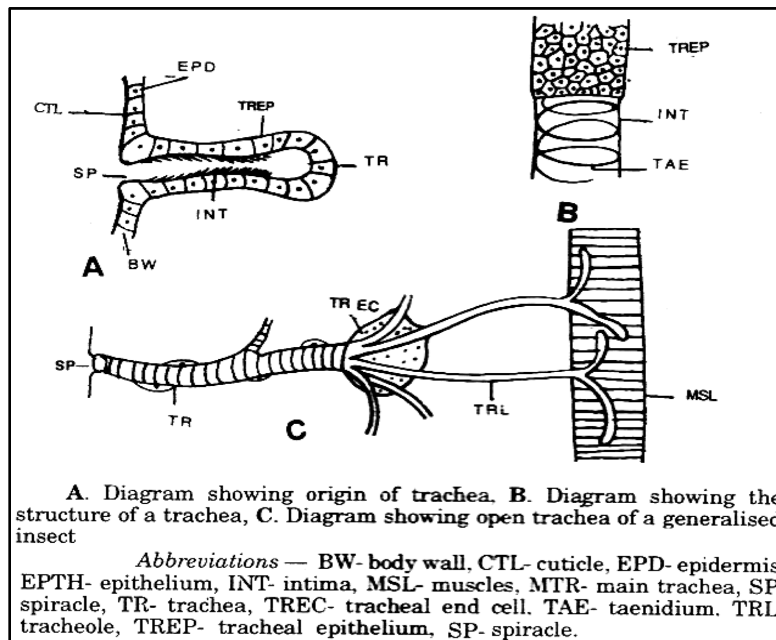
Objective:

In this unit we will discuss on Morphology of respiratory organs and mechanism of respiration in insect and Morphology of Central Nervous System.

A. Morphology of respiratory organs and mechanism of respiration in insect

Introduction:

Respiration in insects mainly takes place by means of internal air-tubes called tracheae.These ramify into finest branches termed as tracheoles.The air enter the tracheae through spiracles, which are paired, segmentally arranged along the thorax and abdomen.In the immature stages of many aquatic insects special respiratory organs known as gills or branchiae are present.Rarely some insects have imperfectly developed system or no trachea, where the integument acts as a respiratory organ. e.g. majority of Collembola, Protura, parasitic insect larvae, aquatic Chironomidae.



A. Diagram showing origin of trachea. **B**. Diagram showing the structure of a trachea, **C**. Diagram showing open trachea of a generalised insect

Abbreviations — BW- body wall, CTL- cuticle, EPD- epidermis, EPTH- epithelium, INT- intima, MSL- muscles, MTR- main trachea, SP- spiracle, TR- trachea, TREC- tracheal end cell. TAE- taenidium. TRL- tracheole, TREP- tracheal epithelium, SP- spiracle.

Tracheal respiration

Development of trachea - These tubes arise as simple invagination of ectoderm along the side of the body in insects. The external openings of these invaginations become the

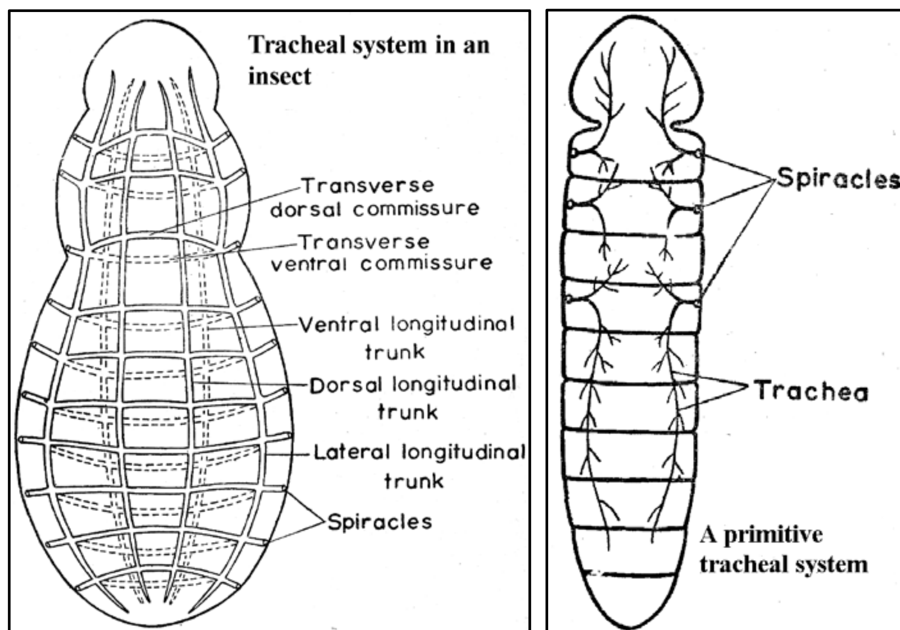
spiracles.These invaginated tubes later extend inward and branch into ramifying structures that ultimately become a well developed tracheal system in insects.The wall of the tracheae contains the same contents as that of the body wall but in the reverse order.

Structure of trachea

The trachea consists of a layer of flat epithelial cells (ectotrachea) which secretes a cuticular substance called the intima (endotrachea).

The surface of the intima is thickened and ensures that it remains round and opens even under conditions of bending pressure. The tracheae divide and redivide, becoming smaller and smaller, finally each ends in a cluster of minute branches, the tracheoles. They are less than 2 un in diameter and possess taenidia but no regular layer of epithelial cells. The base of each cluster of tracheoles has a web like cell known as end cell or tracheole cell with extremely thin protoplasmic extensions. The tips of tracheoles lie alongside between and actually within the tissue cells of the body. Most of the respiratory gas exchange in the tissues occurs through these tracheole tips.

Arrangement of trachea (net like structure)

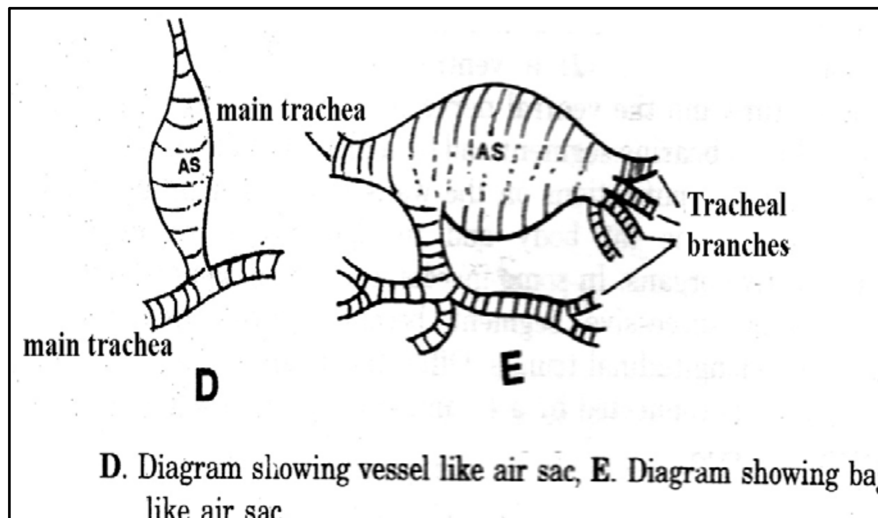


Organization of tracheal system attains a definite fundamental pattern in higher groups of insects. In primitive insects, the tracheae are not anastomosing. This condition is found in Diplura and Thysanura.In highly developed insects, there is well developed system of the fundamental pattern by uniting some of the branches from consecutive and opposite spiracles.There are three main trunks, lateral longitudinal, dorsal longitudinal connected with the lateral by palisade tracheae, and less frequently with ventral longitudinal trunks.

Transverse, dorsal or ventral commissures connect the systems of each side.The dorsal long trunks give off segmental branches which pass to the heart and dorsal musculature. Visceral branches, which supply the digestive canaland reproductive organs take their origin from the palisade tracheae or directly from the spiracular longitudinal trunks in the thoracic region and the basal tracheae of the developing wings usually take origin in close association with those of the legs of the meso and metathorax. The head and mouth parts are principally supplied by branches derived from the anterior most spiracle and dorsal longitudinal trunks.

Modifications of the trachea

Tracheae are expanded or widened to form thin walled swellings known as air sacs. The taenidia are either absent or poorly developed and are often irregularly arranged in air sacs. Consequently the air sacs will collapse under pressure and they play very important part in ventilation of the tracheal system. The air sacs vary in size from minute vesicles to large bags and may be widely distributed in the body, head and appendages of the insect e.g. in *Melanoplus* there is a pair of large thoracic air sac and 5-pairs in the abdomen. In certain forms such as *Musca*, *Apis*, *Bombus* etc. the air sacs attain greatest development and they occur as dilations of the main trachea.



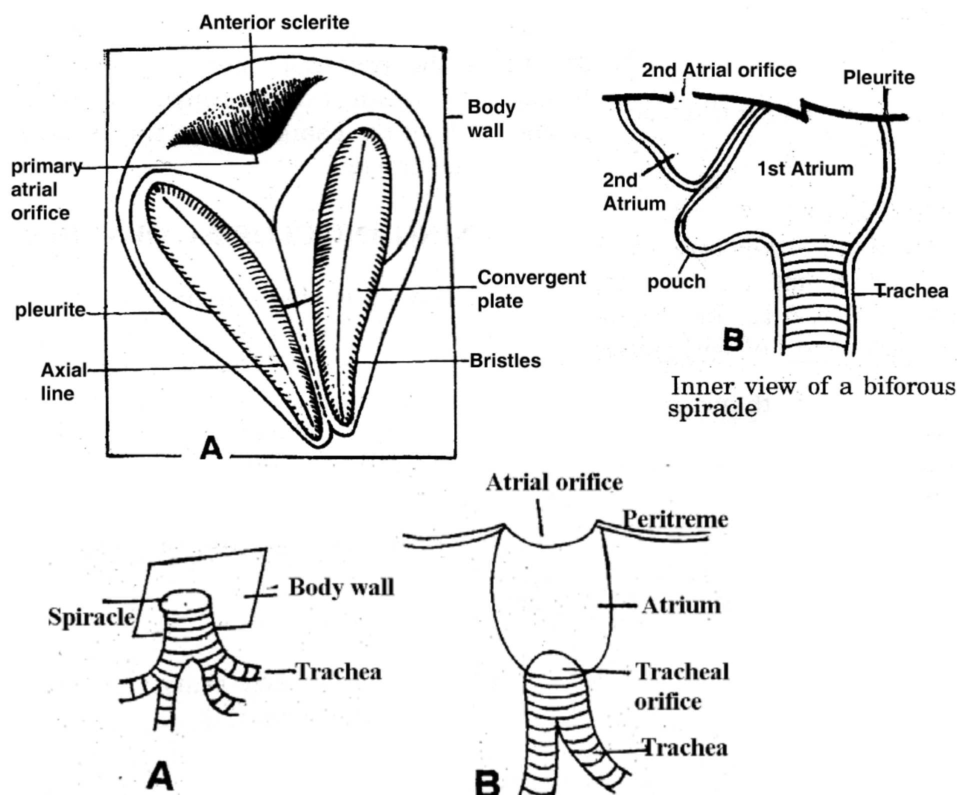
D. Diagram showing vessel like air sac, **E.** Diagram showing bag like air sac.

Spiracles - In many insects the spiracles are more or less sunk into the secondary depressions of the integument, thus the external part of each spiracle becomes a pit-like or tubular chamber called spiracular atrium. This atrium opens on one hand to the out side by the secondary atrial orifice and on the other into the main trachea, through the primitive tracheal orifice. The atrial orifice is usually situated in a small sclerotic plate of the body wall called peritreme.

Types of Spiracles

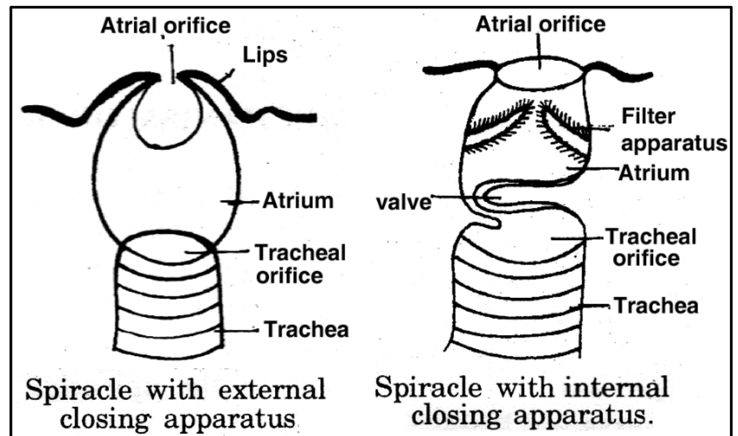
Simple spiracles - they are just openings from the integument into the tracheae, they do not have a ny pr ovision f or r egulating t he s ize of t he t racheal a perture. e.g. A pterygotes, Plecoptera.

Biforous s piracle - Two ope nings are t here, t he f unctional ope nings are s econdary formations, s ince t he pr imary a trial or ifice i s c losed e xcept dur ing m oulting a nd t he c lose atrial c hamber l eading p osteriorly i nto a wide m embranous pouch. Found i n t he l arvae o f certain Coleoptera. It s hows an ovate pe ritremal ar ea h aving anteriorly ad ark sclerotic thickening and posteriorly two elongate convergent plates. This thickening marks the site of the cl osed primary ap erture of t he s piracle. Each conve rgent pl ate ha s a cl ear m edian area traversed by an axial line.



Air from outside enters the posterior chamber i.e. 2nd atrial chamber through the openings of the c onvergent pl ates a nd t hen di ffuses t hrough the ope nings o f t he c onvergent pl ates and then di ffuses t hrough t he w alls of t he c hamber i nto t he 1st atrium f rom where it e nters th e trachea.

Mechanism of opening and closing of spiracles



Spiracle with external closing apparatus
Spiracle with internal closing apparatus.

Spiracles with external closing apparatus - Closing or opening of the atrial aperture is effected by outer lips of atrium. Common occurrence on the thorax in most groups of insects.

Spiracles with internal closing apparatus - Tracheal orifice is regulated at the inner end of the atrium by a special structure called Filter apparatus. The function of this apparatus is to freely permit the passage of air but to prevent the entrance of foreign particles or water into the trachea. Common occurrence on the abdomen in most groups of insects.

B. Morphology of Central Nervous System of Insects

Introduction:

An insect's nervous system is a network of specialized cells (called **neurons**) that serve as an "information highway" within the body. These cells generate electrical impulses (action potientials) that travel as waves of depolarization along the cell's membrane. Every neuron has a nerve cell body (where the nucleus is found) and filament-like processes (dendrites, axons, or collaterals) that propagate the action potential. Signal transmission is always unidirectional -- moving toward the nerve cell body along a dendrite or collateral and away from the nerve cell body along an axon.

Like most other arthropods, insects have a relatively simple central nervous system with a dorsal brain linked to a ventral nerve cord that consists of paired segmental ganglia running along the ventral midline of the thorax and abdomen. Ganglia within each segment are linked to one another by a short medial nerve (commissure) and also joined by intersegmental connectives to ganglia in adjacent body segments. In general, the central nervous system is rather ladder-like in appearance. Commissures are the rungs of the ladder and intersegmental connectives are the rails. In more "advanced" insect orders there is a tendency for individual

ganglia to combine (both laterally and longitudinally) into larger ganglia that serve multiple body segments.

Individual nerve cells connect with one another through special junctions, called **synapses**. When a nerve impulse reaches the synapse, it releases a chemical messenger (neurotransmitter substance) that diffuses across the synapse and triggers a new impulse in the dendrite(s) of one or more connecting neurons. Acetylcholine, 5-hydroxytryptamine, dopamine, and noradrenaline are examples of neurotransmitters found in both vertebrate and invertebrate nervous systems.

Nerve cells are typically found grouped in bundles. A nerve is simply a bundle of dendrites or axons that serve the same part of the body. A ganglion is a dense cluster of interconnected neurons that process sensory information or control motor outputs.

The Central Nervous System

An insect's **brain** is a complex of six fused ganglia (three pairs) located dorsally within the head capsule. Each part of the brain controls (innervates) a limited spectrum of activities in the insect's body:

Protocerebrum: The first pair of ganglia is largely associated with vision; they innervate the compound eyes and ocelli. The **optic lobes** of the fly (an insect with particularly good vision) contains about 76% of the brain's neurons. The optic lobe connects directly to the sensory bodies')are best developed in social insects, making up 20% of the brain of the bee and 50% of the brain of worker ants (*Formica*). These are thought to function as higher centres responsible for the most sophisticated computations occurring in the insect brain. Each consists of a topmost cap and a stalk or peduncle (which branches into at least two lobes). The cap consists of a pair of cup-like structures, the medial calyx and the lateral calyx (plural of calyx is calyces). The mushroom bodies receive sensory inputs from the lobula of the optic lobe and from the antennal lobes of the deutocerebrum. Most sensory inputs enter the MB through the calyx. There are about 1000 to 100 000 specialised neurons, called **Kenyon cells**, in each mushroom body. These neurons have tree-like branching dendrites which receive inputs in the calyces of the MB, a single axon which extends down the stalk of the

MB and then gives off branches to two lobes of the MB. Dragonfly mushroom bodies have no calyces and no Kenyon cells. The mushroom bodies are also involved in learning, and in the honeybee have been shown to process memories, transferring data from **short-term memory (STM)** into **long-term memory (LTM)**. The **central body** receives inputs from the mushroom bodies and integrates sensory inputs from different sensory modalities (such as smell and vision) - so-called **multimodal sensory perception**. It functions as an **activating centre**, switching on appropriate locomotor activity patterns which are **central programs** located in the thoracic ganglia. That is it instructs the thoracic ganglia which programs to run - programs that control the legs and wings. These hard-wired programs are sometimes called **central pattern generators** and require no sensory input for their execution, though sensory inputs may start and stop these programs or modify them slightly.

The **pars intercerebralis** is a mass of cell bodies, **including neurosecretory cells** which send their axons to the pair of corpora cardiaca (see the neuroendocrine system in insect development). The corpora cardiac are sometimes fused into a single medial ganglion. They send out nerves to innervate the dorsal blood vessel, forming a **cardio-aortic system**, which controls the rate of heart beat, as well as having a secretory hormonalfunction.

Biological Clocks

Another function associated with the protocerebrum is time-keeping. Insect activity is timed with the daily light/dark cycle - the **circadian cycle** ('ciracdian' means 'about a day', the exact time being set each day according to environmental cues such as the length of daylight). This timing is due to internal clocks within the insect, which update themselves according to external cues from the environment (**zeitgeibers** or time-givers) such as the number of hours of light and dark.

Deutocerebrum: The second pair of ganglia process sensory information collected by the antennae. This consists of two nerve centres - the main **antennal lobe (AL)** and the smaller **antennal mechanosensory and motor centre (AMMC)** or dorsal lobe. The AL receives inputs from the third (terminal) antennal segment (the flagellum, which is made-up of sub-segments called flagellomeres) via the antennal nerves. It contains from less than 10 to

more than 200 sub-centres called **glomeruli** (singular glomerulus). Inputs to the AL appear to be mainly or exclusively from chemoreceptors(i.e. chemical sensors - olfactory and gustatory, smell and taste) on the flagellum. Each antenna sends signals to the AL on the same side of the head(ipsilateral pathways) although some may also send signals to the AL on the opposite side (contralateral pathways).Each glomerulus is a region of neuropil (nerve cell processes and synapses) where computations occur. It is thought that each glomerulus may, in some species at least, receive inputs from a specific class of receptor (sensor) on the antenna. For example, in the males of some species there is a specially large glomerulus, called the **macroglomerular complex (MGC)** which receives inputs from pheromone olfactory sensors on the antenna.The AL does not receive one input line from each chemoreceptor, as sensors of the same type converge - their axons fuse into a smaller number of axons in the antennal nerve (typically inputs from 15 sensors are combined, a 15:1 ratio). These sensory input axons, and also input axons from the CB of the protocerebrum, synapse with local interneurones within the AL (amacrine cells). Outputs from the AL are carried along the axons of output neurons to the MB of the protocerebrum.The AMMC receives mechanosensory inputs from mechanosensors (mechanoreceptors)on the first two antennal segments (scape and pedicel) via the antennal nerves. It also sends motor outputs to the muscles of the scape. It also receives inputs from mechanosensors on the labial palps, some tegument (body wall) mechanosensors, and some inputs from the flagellum (possibly from the mechanosensors found on the flagellum). The antennal nerve is therefore a mixed nerve - containing bothsensory and motor axons. Some of the antennal mechanoreceptors also send outputs to the SOG, the protocerebrum and the thoracic ganglia.

Tritocerebrum and Stomatogastric System: The third pair of ganglia innervate the labrum and integrate sensory inputs from proto- and deutocerebrums. They also link the brain with the rest of the ventral nerve cord and the stomodaeal nervous system (see below) that controls the internal organs. The commissure for the tritocerebrum loops around the digestive system, suggesting that these ganglia were originally located behind the mouth and migrated forward (around the esophagus) during evolution.

Located ventrally in the head capsule (just below the brain and esophagus) is another complex of fused ganglia (jointly called the subesophageal ganglion). Embryologists believe this structure contains neural elements from the three primitive body segments that merged with the head to form mouthparts. In modern insects, the subesophageal ganglion innervates not only mandibles, maxillae, and labium, but also the hypopharynx, salivary glands, and neck muscles. A pair of circumesophageal connectives loop around the digestive system to link the brain and subesophageal complex together.

In the thorax, three pairs of thoracic ganglia (sometimes fused) control locomotion by innervating the legs and wings. Thoracic muscles and sensory receptors are also associated with these ganglia. Similarly, abdominal ganglia control movements of abdominal muscles. Spiracles in both the thorax and abdomen are controlled by a pair of lateral nerves that arise from each segmental ganglion (or by a median ventral nerve that branches to each side). A pair of terminal abdominal ganglia (usually fused to form a large **caudal ganglion**) innervate the anus, internal and external genitalia, and sensory receptors (such as cerci) located on the insect's back end.

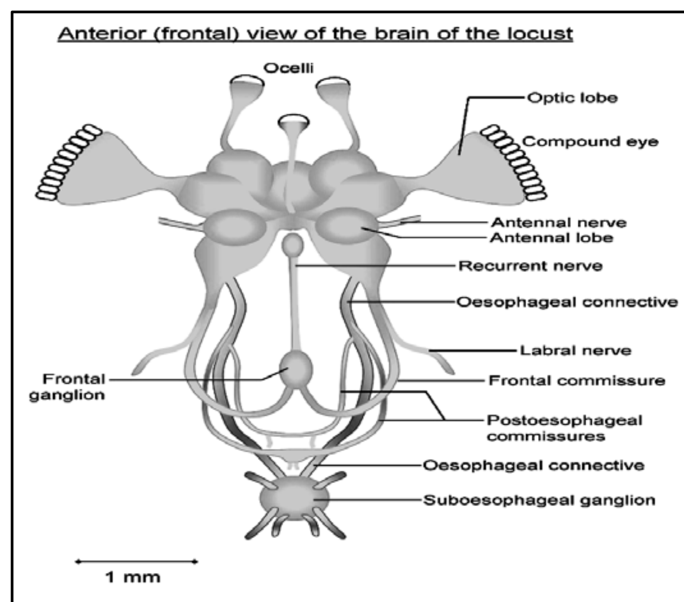


Figure: Insect Brain- frontal view.

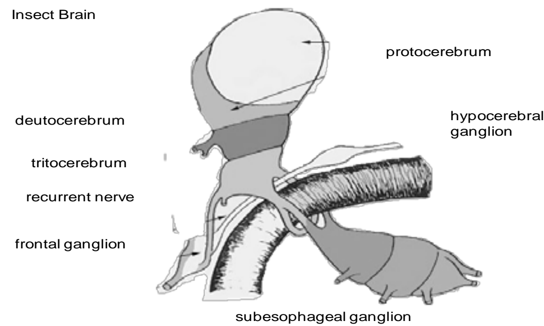


Figure: Insect Brain- lateral view.

The Stomodaeal Nervous System

An insect's internal organs are largely innervated by a stomodaeal (or stomatogastric) nervous system. A pair of frontal nerves arising near the base of the tritocerebrum link the brain with a frontal ganglion (unpaired) on the anterior wall of the esophagus. This ganglion innervates the pharynx and muscles associated with swallowing. A recurrent nerve along the anterio-dorsal surface of the foregut connects the frontal ganglion with a hypocerebral ganglion that innervates the heart, corpora cardiaca, and portions of the foregut. Gastric nerves arising from the hypocerebral ganglion run posteriorly to ingluvial ganglia (paired) in the abdomen that innervates the hind gut.

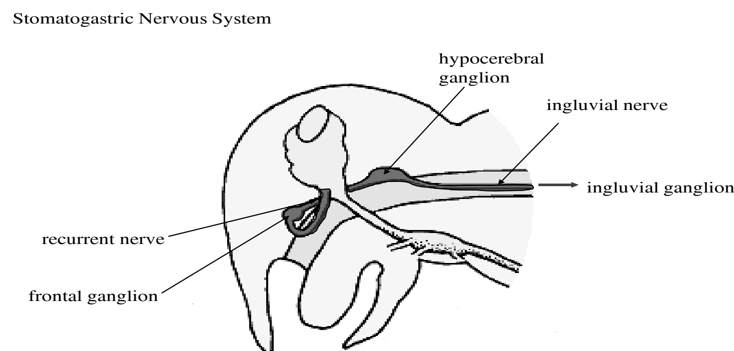


Figure: Stomodaeal Nervous System of Insect

In comparison to vertebrates, an insect's nervous system is far more de-centralized. Most overt behavior (*e.g.* feeding, locomotion, mating, etc.) is integrated and controlled by

segmental ganglia instead of the brain. In some cases, the brain may stimulate or inhibit activity in segmental ganglia but these signals are not essential for survival. Indeed, a headless insect may survive for days or weeks (until it dies of starvation or dehydration) as long as the neck is sealed to prevent loss of blood!

Suboesophageal Ganglion

The suboesophageal ganglion (SOG) and the segmental ganglia of the double ventral nerve-cord each send out pairs of nerves, one of which innervate the pair of spiracles on that segment and so help regulate breathing. (In some insects the segmental ganglia are absent, e.g. in *Dytiscus*, in which case the lateral abdominal nerves send out nerves to innervate the spiracles). The SOG is a composite ganglion, formed by fusion of the ganglia from the mandibular, maxillary and labial segments of the head and the SOG also sends out nerves to the mouthparts (mandibles, palps, etc.) and so controls feeding behaviours.

The Ventral Nerve Cord

From the suboesophageal ganglion two connectives or nerve cords run back along the ventral side (underside) of the insect. These connect to the thoracic ganglion of the first thoracic segment, T1, which is actually a pair of ganglia, more-or-less fused into a single structure. T1 then gives off two connectives to the second thoracic ganglion, T2 and the sequence continues with a chain of connected ganglia running throughout the length of the insect, in the basic plan. Thus, we say that insects have a double ganglionated ventral nerve cord (VNC). Each ganglion functions as a local processor, regulating the functions of its body segment. The thoracic ganglia are especially well-developed as they have to carry out complex computations to generate patterns of movement in the legs and wings. These **output patterns or central programs** are contained in the ganglia, but the brain is normally required to switch them on and off. Sensory inputs have little effect on the basic patterns, but do modify them. For example, stress sensors in the wings feedback information to allow fine-adjustments to the wings and control of the angle of attack and wing-twisting. Typically, however, the basic pattern of movement is pre-coded.

Probable questions:

1. What are the differences between tracheae and tracheoles?
2. What are air sacs? State its significance in insect.
3. Discuss about structure and modifications in Trachea.
4. Write a short note on opening and closing mechanism of spiracles.
5. Name the different parts of insect brain and draw a labeled diagram of the frontal view.
6. Describe the protocerebrum of insect brain and state the functions of different cells of this region.
7. Describe the deutocerebrum of insect brain and state the functions of different centres of this region.
8. Describe the tritocerebrum of insect brain and state the functions of different centres of this region.
9. What is stomodaeal nervous system? Describe stomodaeal nervous system with proper diagram.
10. What are Kenyon cells? State their function.
11. What do you mean by AMMC & AL in respect of insect brain? State their function.
12. Describe the structure of insect ventral nerve cord and state its function.

Source:

1) Chapman, R. (2012). The Insects: Structure and Function (S. Simpson & A. Douglas, Eds.). Cambridge University Press, 819 p.
2) Gullan, P .J & C ranston, P .S. (2010). T he Insects: A n out line of E ntomology (4th Edition). Wiley-Blackwell, 584 p.
3) Evans, H.E. (1984). Insect biology – A textbook of Entomology. Addison-Wesley Publ. Co., 436 pp.
4) https://genent.cals.ncsu.edu/bug-bytes/nervous-system/
5) http://cronodon.com/BioTech/insect_nervous_systems.html

Suggested readings:

1. Chapman, R. (2012). The Insects: Structure and Function (S. Simpson & A. Douglas, Eds.). Cambridge University Press, 819 p.
2. Evans, H.E. (1984). Insect biology – A textbook of Entomology. Addison-Wesley Publ. Co., 436 p.
3. Gullan, P.J & Cranston, P.S. (2010). The Insects: An outline of Entomology (4th Edition). Wiley-Blackwell, 584 p.
4. Mani, M.S. (1982). General entomology. Oxford & IBH Publ. Co., 912 pages.
5. Richard, O.W. & Davies, R.G. (1977). IMMS' General textbook of Entomology. Part I & II. Chapman & Hall London, 1354 p.
6. Snodgrass, R.E. (1935). Principles of Insect Morphology. Mcgraw-Hill Book Company. 647 p.
7. Tembhare, D.B. (1997). Modern entomology. Himalaya Publ. House, 623 pages.
8. Wigglesworth V. B. (2015). Insect Physiology. Andesite Press. 148p.

Unit-XII

Metamorphosis : Basic Concept (House fly) and Exocrine Glands : Lac gland, Wax gland, Silk Gland and Labial Gland.

Objectives:

In this Unit we will discuss on Metamorphosis : Basic Concept (House fly) and Exocrine Glands : Lac gland, Wax gland, Silk Gland and Labial Gland.

A. Metamorphosis : Basic Concept (House fly)

Introduction:

The house fly has a complete metamorphosis with distinct egg, larval or maggot, pupal and adult stages. The house fly overwinters in either the larval or pupal stage under manure piles or in other protected locations. Warm summer conditions are generally optimum for the development of the house fly, and it can complete its life cycle in as little as seven to ten days. However, under suboptimal conditions the life cycle may require up to two months. As many as 10 to 12 generations may occur annually in temperate regions, while more than 20 generations may occur in subtropical and tropical regions.

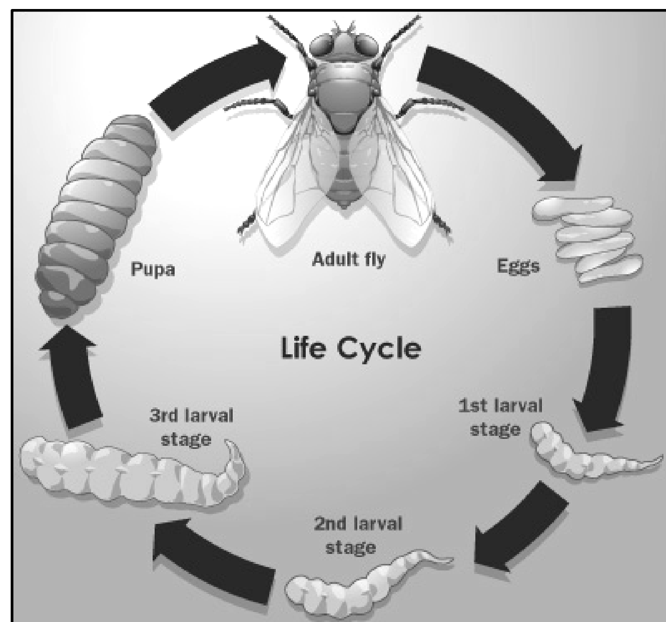


Figure: Life cycle of the house fly *Musca domestica* Linnaeus.

Life Cycle:

Egg: The white egg, about 1.2 mm in length, is laid singly but eggs are piled in small groups. Each female fly can lay up to 500 eggs in several batches of 75 to 150 eggs over a three to

four day period. The number of eggs produced is a function of female size which, itself, is principally a result of larval nutrition. Maximum egg production occurs at intermediate temperatures, 25 to 30° C. Often, several flies will deposit their eggs in close proximity, leading to large masses of larvae and pupae. Eggs must remain moist or they will not hatch.

Larva: Early instar larvae are 3 to 9 mm long, typical creamy whitish in color, cylindrical but tapering toward the head. The head contains one pair of dark hooks. The posterior spiracles are slightly raised and the spiracular openings are sinuous slits which are completely surrounded by an oval black border. The legless **maggot** emerges from the egg in warm weather within eight to 20 hours. Maggots immediately begin feeding on and developing in the material in which the egg was laid.

The larva goes through three instars and a full-grown maggot, 7 to 12 mm long, has a greasy, cream-colored appearance. High-moisture manure favours the survival of the house fly larva. The optimal temperature for larval development is 35 to 38°C, though larval survival is greatest at 17 to 32°C. Larvae complete their development in four to 13 days at optimal temperatures, but require 14 to 30 days at temperatures of 12 to 17°C.

Nutrient-rich substrates such as animal manure provide an excellent developmental substrate. Very little manure is needed for larval development, and sand or soil containing small amounts of degraded manure allows for successful belowground development. When the maggot is full-grown, it can crawl up to 50 feet to a dry, cool place near breeding material and transform to the pupal stage.

Pupa: The pupal stage, about 8 mm long, is passed in a pupal case formed from the last larval skin which varies in colour from yellow, red, brown, to black as the pupa ages. The shape of the pupa is quite different from the larva, being bluntly rounded at both ends. Pupae complete their development in two to six days at 32 to 37°C, but require 17 to 27 days at about 14°C. The emerging fly escapes from the pupal case through the use of an alternately swelling and shrinking sac, called the ptilinum, on the front of its head which it uses like a pneumatic hammer to break through the case.

Adult: The house fly is 6 to 7 mm long, with the female usually larger than the male. The female and can be distinguished from the male by the relatively wide space between the eyes (in males, the eyes almost touch). The head of the adult fly has reddish-eyes and sponging

mouthparts. The thorax bears four narrow black stripes and there is a sharp upward bend in the fourth longitudinal wing vein. The abdomen is gray or yellowish with dark midline and irregular dark markings on the sides. The underside of the male is yellowish.

Adults usually live 15 to 25 days, but may live up to two months. Without food, they survive only about two to three days. Longevity is enhanced by availability of suitable food, especially sugar. Access to animal manure does not lengthen adult life and they live longer at cooler temperatures. They require food before they will copulate, and copulation is completed in as few as two minutes or as long as 15 minutes. Oviposition commences four to 20 days after copulation. Female flies need access to suitable food (protein) to allow them to produce eggs, and manure alone is not adequate.

Hormones in Metamorphosis:

1) **Prothoracicotropic Hormone** – Secreted from two pairs of cells in the brain of larva – helps in molting and Pupation.
2) **Ecdysone** – Secreted from Prothoracic glands – helps in larval instar and larva to pupa molts.
3) **Juvenile Hormone** – Secreted from Corpora allata – Controls the action of Ecdysone. Absence of JH results in the formation of adults.

B.INSECT EXOCRINE GLANDS

Introduction:

The variety of chemical signals and defenses vary among different group of insects. These chemicals are produced by the exocrine glands which show immense diversity in location, structure and function. Some of these glands are the modified labial/salivary glands while others are dedicated glands (wax, poison) having specific functions.

Types of Insect exocrine glands:

1. **Salivary Glands/ Labial glands**

The salivary glands of most insects are labial glands. There is great variation in the structure of these glands considering the variation in mode of feeding (e.g., chewing, piercing-sucking, non-piercing-sucking, sponging, etc.) and types of food consumed by different insect species. Several aspects of structure and function are common to most or all variations of insect labial salivary glands. The glands occur in pairs, and the ducts from each gland usually join to form

a single common duct that opens to the oral cavity at a single orifice. Even though the glands originate in the labial segment, the orifice usually occurs just behind or on the hypopharynx, and the glands often extend back into the thorax and even as far back as the abdomen. The glands are suspended in the hemocoel and are constantly bathed in hemolymph. The glands generally have at least two regions: a secretory region and a reabsorptive region. Generally, the lumen of the salivary duct is lined with cuticle, at least at the end closest to its opening.
The secretory region produces the primary saliva. The major component of saliva is water. Water is transported from the hemo-lymph across cells of the salivary gland and into the lumen of the gland. Movement of water from the blood to the gland lumen is accomplished by active transport of potassium or sodium ions from the hemolymph to the lumen, causing water to move from the hemolymph to the lumen down an osmotic gradient. Cells responsible for water transport generally have deep infoldings of the cell membrane and/or dense microvilli on the side of the cell adjacent to the lumen of the gland. This serves to greatly increase the cell's luminal surface area, and also serves to enclose very narrow extracellular spaces into which ions are pumped. The enclosed nature of the spaces helps contain the ions to keep their concentration high, thus facilitating the osmotic movement of water from the cell into the space. The infoldings and microvilli usually are associated with abundant mitochondria to provide the energy for the ion pumps. The secretory region of the gland also synthesizes proteins, such as salivary enzymes and other organic components of the saliva. Cells responsible for secretion of these components generally possess extensive endoplasmic reticulum, Golgi bodies, and secretory granules that synthesize and transport (intracellularly) the secretions. There may be one or several different types of cell in the secretory region. It should be noted that salivary components are not necessarily produced by the salivary glands themselves, but may be produced elsewhere in the body and transported to the salivary glands via the hemolymph.
The reabsorptive region of the salivary gland reabsorbs potassium or sodium ions from the saliva and transports them back into the hemolymph. As a result, potassium and sodium ions are conserved, and the saliva is usually hypotonic to the hemolymph. Reabsorptive cells often have infoldings, especially on their basal side (hemolymph side), to increase surface area. These infoldings, however, tend not to be tightly enclosed (unlike the lumen side of water-secreting cells in the secretory region), to facilitate movement of secreted ions into the hemolymph and away from the cells, thus reducing the osmotic gradient, which would cause the cells to lose water. Reabsorptive cells also have abundant mitochondria to power the active transport of ions from saliva to hemolymph.

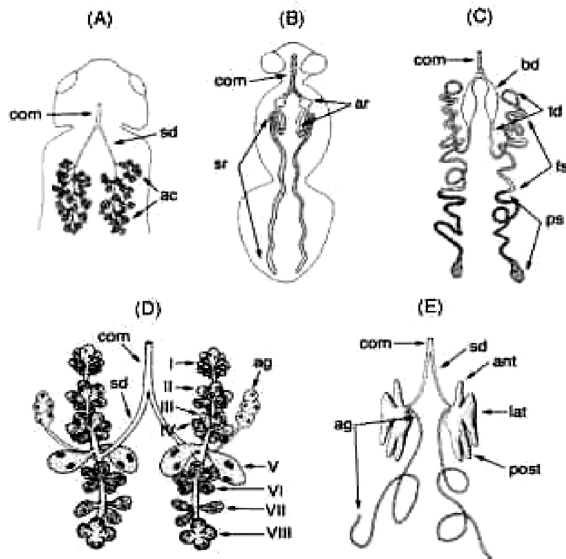


Figure: Salivary glands of representative insects. (A) The locust (B) Adult blowfly (C) The tobacco hornworm moth (D) The beet leafhopper showing cell types I through VIII. (E) The large milkweed bug

Abbreviations: ac, acini; ag, accessory gland; ant, anterior lobe; ar, absorptive region; bd, bulbous duct region; com, common salivary duct; fs, fluid secretion region; lat, lateral lobe; post, posterior lobe; ps, protein secretion region; sd, salivary duct; sr, secretory region; td, thin duct region.

2. Silk Glands

In some insects the labial glands (or in some cases an additional pair of them) are used to produce the protein silk. Lepidopterans (moths and butterflies) and hymenopterans (wasps and their allies) produce silk from their labial glands. Embiopterans ('webspinners' or 'lively wings') produce silk from **tarsal glands** in their feet, as do some dipterans (true flies). Some beetles (coleopterans) and neuropterans (lacewings and their allies) secrete silk from their **Malpighian tubules**, structures associated with the midgut of insects and which generally have an excretory function in removing nitrogenous waste. The larvae of some cockroaches produce silk from the cerci (sensory projections at the rear of the abdomen). Silk is extremely strong but very light, which has made it the object of research in the development of body armour. However, it is also very flexible (which has hampered the aforementioned research!). Silk is about 3 quarters the tough protein fibroin and one quarter the gelatinous protein sericin. generally, one strand is produced by each labial gland and then cemented together into a ribbon once they reach the common secretory duct of the labial glands.

3. Wax Glands

The **dermal glands** of insects are modified epidermal cells which send fine secretory ducts through the cuticle (the exoskeleton covering the epidermis). These secrete wax, mixtures of long-chain alcohols, carboxylic acids and hydrocarbons, which waterproof the cuticle. Shellac (or lac) is a secretion of wax, resins, sugars and pigment secreted by certain coccids

(scale insects). Dissolved in ethanol, lac forms liquid shellac, which is used as a wood finish, varnish and food glaze.

4. **Poison Glands**

Venom is secreted by modified **accessory glands** and released through the sting (a modified ovipositor) of certain Hymenoptera including stinging wasps, ants and bees. The accessory glands normally have a role in reproduction. **Dufour's gland** also releases secretion into the ovipositor or sting. This may serve to lubricate the ovipositor or to cement the egg to the wall of the comb cell. In some ants, the Dufour's gland secretion is used in trail-laying, in which a worker who finds a suitable food source lays a trail with its sting back to the nest, so that other workers may follow the trail to the food source. Bee venom contains the protein elittin which causes cells to lyse (burst open).

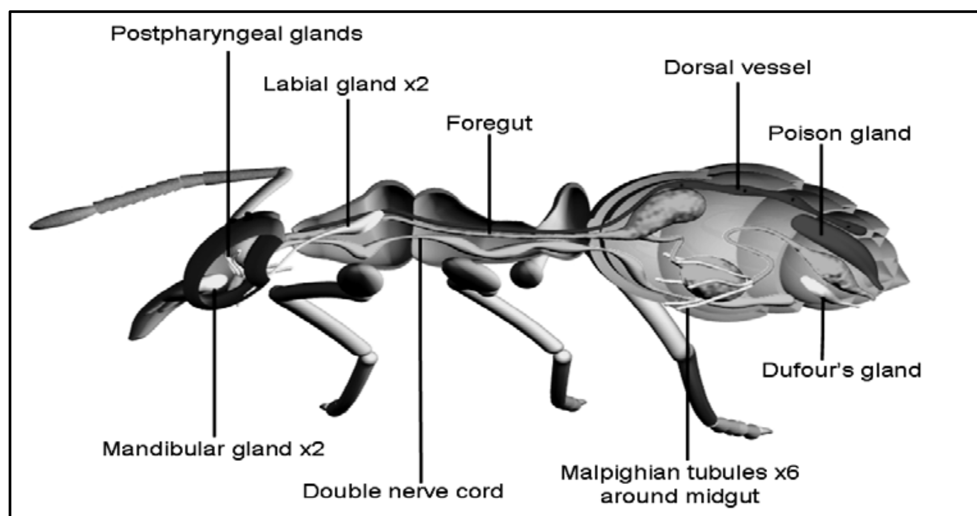


Figure: Location of Major Exocrine glands in an insect body.

Probable questions:

1. Describe metamorphosis in house fly and add a note on the hormonal control of metamorphosis.
2. What do you mean by holometabolous and hemimetabolous insects? Cite examples. .
3. Name the different hormones which aid in insect metamorphosis. State their functions.
4. What is ecdysis? Discuss the controlling factors of this phenomenon.

5. Mention the location of major exocrine glands in an insect body. What is the general function of these glands?

6. Describe the structure of labial glands of insects and state their role in different insect orders.
7. What is Dufour's gland? State its location and function.
8. Discuss different types of silk glands found among insects. Cite examples.
9. What is a wax gland? State its location and function.
10. What is a poison gland? State its location and function.

Source:

1. Chapman, R. (2012). The Insects: Structure and Function (S. Simpson & A. Douglas, Eds.). Cambridge University Press, 819 p.
2. Gullan, P .J & C ranston, P .S. (2010). T he Insects: A n out line of E ntomology (4th Edition). Wiley-Blackwell, 584 p.
3. Snodgrass, R . E. (1935). P rinciples o f Insect M orphology. M cgraw-Hill B ook Company. 647 p.
4. Tembhare, D.B. (1997). Modern entomology. Himalaya Publ. House, 623 pages.
5. Wigglesworth V. B. (2015). Insect Physiology. Andesite Press. 148 p.

Suggested readings:

1. Chapman, R. (2012). The Insects: Structure and Function (S. Simpson & A. Douglas, Eds.). Cambridge University Press, 819 p.
2. Evans, H.E. (1984). Insect bi ology – A t extbook of E ntomology. Addison-Wesley Publ. Co., 436 p.
3. Gullan, P .J & Cranston, P .S. (2010). T he Insects: A n out line of E ntomology (4th Edition). Wiley-Blackwell, 584 p.
4. Mani, M.S. (1982). General entomology. Oxford & IBH Publ. Co., 912 pages.
5. Richard, O .W. & D avies, R .G. (1977). IMMS' G eneral t extbook o f E ntomology. Part I & II. Chapman & Hall London, 1354 p.
6. Snodgrass, R . E. (1935). P rinciples o f Insect M orphology. M cgraw-Hill B ook Company. 647 p.
7. Tembhare, D.B. (1997). Modern entomology. Himalaya Publ. House, 623 pages.
8. Wigglesworth V. B. (2015). Insect Physiology. Andesite Press. 148p.

Disclaimer:

The study materials of this book have been collected from various books, e-books, journals and other e-sources.

POST GRADUATE DEGREE PROGRAMME (CBCS)

IN

ZOOLOGY

(M. Sc. Programme)

SEMESTER-I

PARASITOLOGY AND ECOLOGY AND ENVIRONMENT

ZHT-102

Self-Learning Material

DIRECTORATE OF OPEN AND DISTANCE LEARNING

UNIVERSITY OF KALYANI

Kalyani, Nadia

West Bengal, India

COURSE PREPARATION TEAM:

Faculty members of the Department of Zoology,
University of Kalyani and Assistant Professors of Zoology, DODL,
University of Kalyani.

December, 2018

Directorate of Open and Distance Learning, University of Kalyani

Published by the Directorate of Open and Distance Learning,
University of Kalyani, Kalyani-741235, West Bengal and Printed by
East India Photo Composing Centre, 69, Sisir Bhaduri Sarani, Kolkata-700 006

All right reserved. No. part of this work should be reproduced in any form without the permission in writing from the Directorate of Open and Distance Learning, University of Kalyani.

Authors are responsible for the academic contents of the course as far as copyright laws are concerned.

Director's Note

Open and Distance Learning (ODL) systems play a threefold role- satisfying distance learners' needs of varying kinds and magnitudes, overcoming the hurdle of distance and reaching the unreached. Nevertheless, this robustness places challenges in front of the ODL systems managers, curriculum designers, Self Learning Materials (SLMs) writers, editors, production professionals and other personnel involved in them. A dedicated team of the University of Kalyani under the leadership of Hon'ble Vice-Chancellor has put its best efforts, professionally and in unison to promote Post Graduate Programmes in distance mode offered by the University of Kalyani. Developing quality printed SLMs for students under DODL within a limited time to cater to the academic requirements of the Course as per standards set by Distance Education Bureau of the University Grants Commission, New Delhi, India under Open and Distance Mode UGC Regulations, 2017 had been our endeavour and we are happy to have achieved our goal.

Utmost care has been taken to develop the SLMs useful to the learners and to avoid errors as far as possible. Further suggestions from the learners' end would be gracefully admitted and to be appreciated.

During the academic productions of the SLMs, the team continuously received positive stimulations and feedback from Professor (Dr.) Sankar Kumar Ghosh, Hon'ble Vice- Chancellor, University of Kalyani, who kindly accorded directions, encouragements and suggestions, offered constructive criticism to develop it within proper requirements. We gracefully, acknowledge his inspiration and guidance.

Due sincere thanks are being expressed to all the Members of PGBOS (DODL), University of Kalyani, Course Writers- who are serving subject experts serving at University Post Graduate departments and also to the authors and academicians whose academic contributions have been utilized to develop these SLMs. We humbly acknowledge their valuable academic contributions. I would like to convey thanks to all other University dignitaries and personnel who have been involved either at a conceptual level or at the operational level of the DODL of University of Kalyani.

Their concerted efforts have culminated in the compilation of comprehensive, learner-friendly, flexible texts that meet the curriculum requirements of the Post Graduate Programme through Distance Mode.

Self Learning Materials (SLMs) have been published by the Directorate of Open and Distance Learning, University of Kalyani, Kalyani-741235, West Bengal and all the copyright is reserved for University of Kalyani. No part of this work should be reproduced in any from without permission in writing from the appropriate authority of the University of Kalyani.

All the Self Learning Materials have been composed by distinguished faculty from reputed institutions, utilizing data from e-books, journals and websites.

Director
Directorate of Open & Distance Learning
University of Kalyani

HARD CORE THEORY PAPER (ZHT – 102)

Group A (Parasitology)

Module	Unit	Content	Credit	Class	Time (h)	Page No.
ZHT - 102 (Parasitology, Ecology and Environment)	**I**	Classification of Protozoa and Helminths. Zoonosis with particular reference to *Toxoplasma, Balantidium, Entamoeba, Schistosoma*.	**1**	**1**	**1**	**7 – 55**
	II	Mode of transmission of (*Plasmodium, Trypanosoma, Piroplasm*). Microspora: Structure and life history of *Nosema bombycis* - impact on sericulture.		**1**	**1**	**56 – 69**
	III	Life cycle, biology, pathogenesis, epidemiology and control of important human and veterinary helminthes - *Diphyllobothrium latum, Paragonimus westermani, Trichinella spiralis*		**1**	**1**	**70 – 85**
	IV	Salient features of plant parasitic nematodes and life cycle patterns of , *i) Anguina tritici* ii) *Meloidogyne hapla,*		**1**	**1**	**86 – 95**

Group-A: Parasitology

Unit-I:

Classification of Protozoa and Helminths and Zoonosis with particular reference to *Toxoplasma*, *Balantidium*, *Entamoeba*, *Schistosoma*

Objectives:

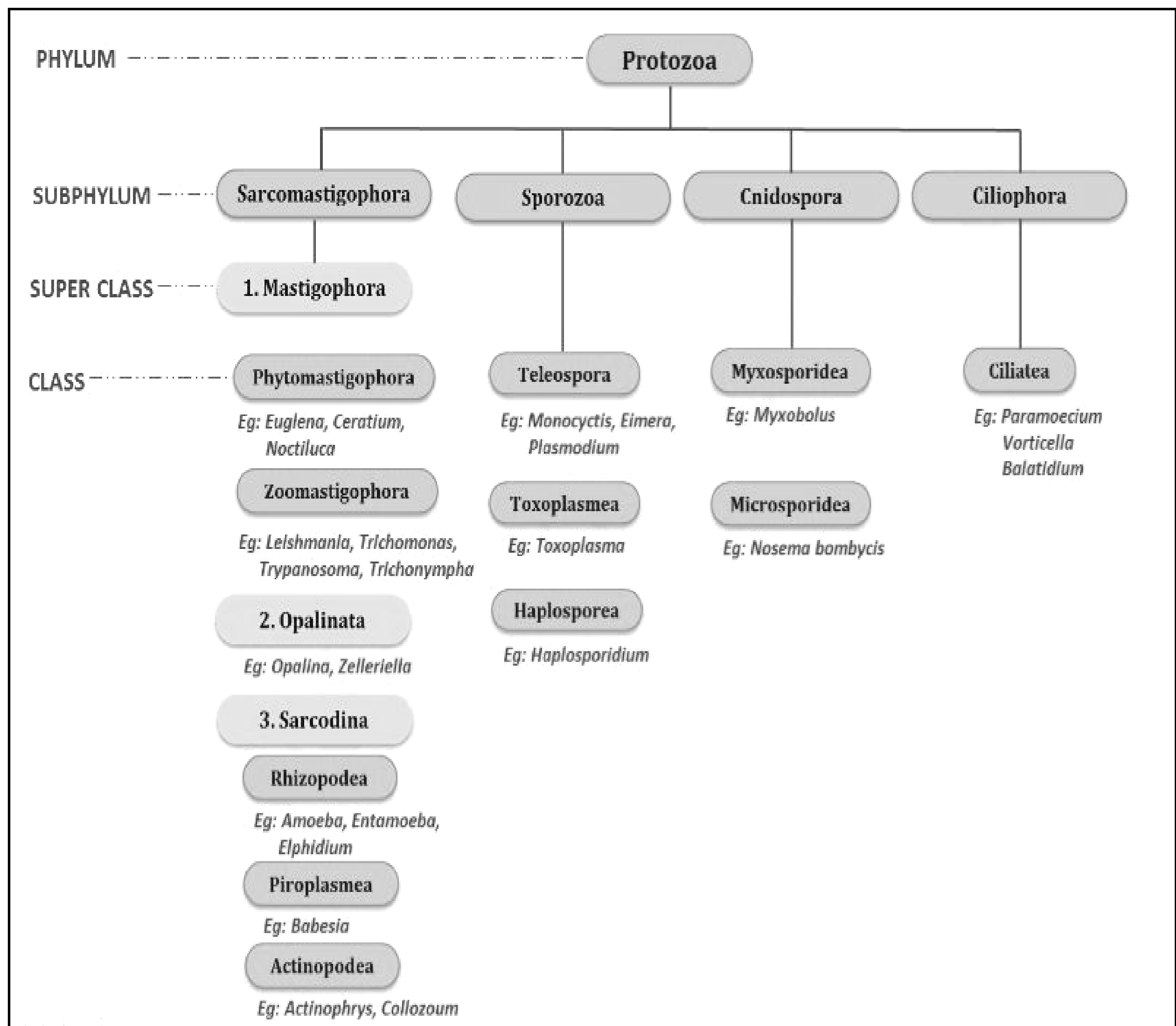
In this section we will discuss on Classification of Protozoa and Helminths and Zoonosis with particular reference to *Toxoplasma, Balantidium, Entamoeba* and *Schistosoma.*

Introduction:

Unicellular or acellular, eukaryotic and commonly motile heterotrophic organisms are called Protozoa. It is a loose assemblage of different groups of unicellular organisms and they do not belong to a monophyletic group, probably shares a member of polyphyletic lineages. Dobell (1911) first introduced the term 'acellular' or non-cellular, instead of unicellular and Hyman (1940) extensively used the term acellular or non-cellular.

General Characteristic Features:

1. Protozoans are usually microscopic and unicellular individuals.
2. They exhibit all types of symmetry.
3. Most species occur as single but many are colonial.
4. Body is bounded by a cell membrane or plasmalemma.
5. Body may be naked or is covered by a pellicle or a test, made of silica or calcium carbonate.
6. A filamentous network of the cytoskeleton may form a dense supportive structure, called the epiplasm.
7. Usually uninucleate, but may be more than single nucleus in some forms.
8. Locomotor organelles may be flagella (e.g.*Euglena*), cilia (e.g.*Paramoecium*), pseudopodium (e.g.*Amoeba*) or absent in parasitic forms (contractile myonemes are present in the body).
9. Nutrition may be holozoic, e.g.*Amoeba*(animal-like), holophytic (e.g.*Euglena*), saprophytic, mixotrophic or parasitic.
10. Intracellular type of digestion occurs within the food vacuoles.
11. Respiration performs generally through the outer surface of the body, but may be few obligatory or facultative anaerobes.



12. Excretion performs generally through the body surface, and water regulation of the body is accomplished by contractile vacuole.

13. Asexual reproduction occurs by fission (mitosis), plasmotomy or budding. In certain forms sexual reproduction may occur either by conjugation or fusion by gametes (syngamy).

14. They never develop from blastula stage during development.

15. Mainly aquatic but many are parasitic, commensal or mutualistic.

Classification in Outline:

Previously, the protozoan classification was done mainly on the basis of locomotor organs but recently the electron microscopic findings have added new dimension to the study of Protozoa and the scheme of protozoan classification has been changed considerably.

The following classification is based on Honigberg Report.

It is subdivided into four subphyla— Sarcomastigophora, Sporozoa, Cnidospora and Ciliophora.

Phylum Protozoa:
I. Subphylum Sarcomastigophora
A. Superclass mastigophora:
(i) Class Phytomastigophorea
Order – Chrysomonadida, e.g.*Ochromonas*
Order – Silicoflagellida, e.g.*Dictyocha*
Order – Coccolithophorida, e.g.*Coccolithus*
Order – Heterochlorida, e.g.*Heterochloris*
Order – Cryptomonadida, e.g.*Cryptomonas*
Order – Dinoflagellida, e.g.*Noctiluca*
Order – Ebriida, e.g.*Ebria*
Order – Euglenida, e.g.*Euglena*
Order – Chloromonadida, e.g.*Gonyostomum*
Order – Volvocida, e.g.*Volvox*

(ii) Class Zoomastigophorea:
Order – Choanoflagellida, e.g.*Proterospongia*
Order- Bicosoecida, e.g.*Bicosoeca*
Order – Rhizomastigida, e.g.*Dimorpha*
Order – Kinetoplastida, e.g.*Trypanosoma*
Order – Retortamonadida, e.g.*Retortamonas*
Order – Diplomonadida, e.g.*Giardia*
Order – Oxymonadida, e.g.*Oxymonas*
Order – Trichomonadida, e.g.*Trichomonas*
Order – Hypemastigida, e.g.*Lophomonas*

B. Superclass opalinata:
Order- Opalinida, e.g.*Opalina*

C. Superclass sarcodina:
(iii) Class Rhizopodea:
1. Subclass Lobosia:
Order- Amoebida, e.g. *Amoeba*

Order- Arcellinida, e.g.*Arcella*

2. Subclass Filosia:
Order – Aconchulinida, e.g.*Penardia*
Order – Gromiida, e.g.*Gromia*

3. Subclass Granuloreticulosia:
Order – Athalamida, e.g.*Biomyxa*
Order – Foraminiferida, e.g.*Elphidium*
Order – Xenophyophorida, e.g.*Stannoma*

4. Subclass Mycetozoia:
Order – Acrasida, e.g.*Dictyostelium*
Order – Eumycetozoida, e.g.*Physarum*
Order – Plasmodiophorida, e.g.*Plasmodiophora*

5. Subclass Labyrinthulia:
Order Labyrinthulida, e.g.*Labyrinthula*

(iv) Class Piroplasmea:
Order – Piroplasmida, e.g.*Babesia*

(v) Class Actinopodea:
1. Subclass Radiolaria:
Order – Porulosida, e.g.*Bathysphaera*
Order – Oculosida, e.g.*Cystidium*

2. Subclass Acantharia:
Order – Aqinthometrida, e.g.*Acanthometron*
Order – Acanthophractida, e.g.*Challengeron*

3. Subclass Heliozoia:
Order – Actinophryida, e.g.*Actinophrys*
Order – Centrohelida, e.g.*Acanthocysitis*
Order – Desmothoracida, e.g.*Clathrulina*

4. Subclass Proteomyxidia:
Order – Proteomyxida, e.g.*Leptomyxa*

II. Subphylum sporozoa:
(vi) Class Telosporea:
1. Subclass Gregarinia:
Order – Archigregarinida, e.g., Selenidium
Order – Eugregarinida, e.g., Monocystis
Order – Neogregarinida, e.g., Ophryocystis

2. Subclass Coccidia:
Order – Protococcida, e.g.*Eucoccidium*
Order – Eucoccida, e.g.*Eimeria*

(vii) Class Toxoplasmea:
Order – Toxoplasmida, e.g.*Toxoplasm*

(viii) Class Haplosporea:
Order – Haplosporida, e.g. *Haplosporidium*

III. Subphylum cnidospora:
(ix) Class Myxosporidea:
Order – Myxosporida, e.g.*Leptotheca*
Order – Actinomyxida, e.g.*Triactinomyxon*
Order – Helicosporida, e.g.*Helicosporidium*

(x) Class Microsporidea:
Order – Microsporida, e.g.*Nosema*

IV. Subphylum Ciliophora:
(xi) Class Ciliatea:
1. Subclass Holotrichia:
Order – Gymnostomatida, e.g.*Didinium*
Order – Trichostomatida, e.g.*Balantidium*
Order – Chonotrichida, e.g.*Spirochona*
Order – Apostomatida, e.g.*Foettingeria*
Order – Astomatida, e.g.*Anoplophrya*
Order – Hymenostomatida, e.g.*Tetrahymena, Paramoecium*
Order – Thigmotrichida, e.g.*Ancistrocoma*

2. Subclass Peritrichia:
Order – Peritrichida, e.g.*Vorticella*

3. Subclass Suctoria:
Order – Suctorida, e.g.*Podophrya*

4. Subclass Spirotrichia:
Order – Heterotrichida, e.g.*Spirostomum*
Order – Oligotrichida, e.g.*Halteria*
Order – Tintinnida, e.g.*Tintinnidium*
Order – Entodiniomorphida, e.g.*Entodinium*
Order – Odontostomatida, e.g.*Discomorphella*
Order – Hypotrichida, e.g.*Aspidisca*

Classification with Characters:
A. Subphylum Sarcomastigophora:
Features:
1. Locomotory structures are present either in the form of flagella, pseudopodia or both.

2. Nucleus is usually of single type (excepting the developmental stages of certain Foraminiferida).

3. No spore formation.

4. Sexual reproduction when present is through syngamy.

It includes three super-classes—Mastigophora, Opalinata and Sarcodina.

Superclass Mastigophora:
Features:
1. Either solitary or colonial.

2. Presence of one or more flagella in trophozoite stage.

3. Sexual reproduction is uncommon.

4. Nutrition may be either phototrophic or heterotrophic or both.

It is subdivided into two classes— Phytomastigophorea and Zoomastigophorea.

I. Class Phytomastigophorea:

Features:

1. Usually possess chromatophores which may be secondarily lost.
2. Presence of one or two emergent flagella.
3. In some groups amoeboid forms occur.
4. Most members are free-living and certain forms exhibit sexual reproduction.

There are ten orders in this class.

Order 1. Chrysomonadida:

Features:

1. Presence of one to three flagella.
2. One or two yellow or yellow green or brown coloured chromatophores are usually present.
3. Amoeboid stages are frequent.
4. Food reserves are present as leucosin and lipids.
5. Cyst wall is always siliceous.

Examples. *Ochromonas, Chromulina*.

Order 2. Silicoflagellida:

Features:

1. Flagellum is either absent or only one.
2. Chromatophores are brown coloured.
3. Inner skeleton is made up of silica.

Examples. *Dictyocha, Clathropyxidella*.

Order 3. Coccolithophorida

Features:

1. Flagella and chromatophores are always two in number.
2. Presence of calcareous plates as external covering.
3. Usually marine.

Examples. *Discoaster, Coccolithus*.

Order 4. Heterochlorida:

Features:

1. Two flagella are of unequal length.
2. Yellow green coloured chromatophores vary from two to several.
3. Usually amoeboid forms are present.
4. Lipids are common food reserves.
5. Walls of cysts are made up of silica

Examples. *Heterochloris, Rhizochloris*.

Order 5. Cryptomonadida:

Features:

1. Body is compressed.

2. Two flagella usually originate from a depression.

3. Chromatophores are two and usually brown but may be red, olive green or blue green in colour.

4. Amoeboid stages are absent.

5. Starch and amyloid bodies are the usual food reserves.

Examples. *Chilomonas, Cryptomonas*.

Order 6. Dinoflagellida:

Features:

1. Body is divided into cingulum and sulcus by transverse and longitudinal grooves.

2. Each part contains a flagellum.

3. Of these two flagella, one is transverse and causes both rotation and forward movement, and the other longitudinal one drives water posteriorly and helps in forward movement.

4. Chromatophores are either yellow or dark brown, but may be green or blue green.

5. A theca, a thickened pellicle is present in many forms.

6. Reserve foods are starch and lipids.

Examples. *Noctiluca, Gymnodinium, Glenodinium, Amphidinium, Ceratium, Dinophysis, Zooanthella*.

Order 7. Ebriida:

Features:

1. Chromatophores are absent and usually with two flagella.

2. Internal skeleton is siliceous.

Example. *Ebria*.

Order 8. Euglenida:

Features:

1. One or two flagella arise from an anterior reservoir.
2. Chromatophores are green and their shapes may vary.
3. Though body form may change yet no amoeboid movement occurs.
4. Food reserves are present as paramylum.

Examples. *Euglena, Peranema*.

Order 9. Chloromonadida:

Features:

1. Two flagella originate from the side of a superficial apical cleft or furrow.

2. Body is dorsoventrally flattened.

3. Chromatophores green and numerous.

4. Food reserves present as lipids and glycogen.

Example. *Gonyostomum*.

Order 10. Volvocida (Phytomonadida):

Features:

1. Either solitary or colonial.

2. Flagella are two to four and apical.

3. Chromatophores, when present, are leaf green.

4. Appearance more or less like a shell or cup.

5. Amoeboid forms usually absent.

6. Food reserves in the form of starch.

Examples. *Pandorina, Volvox*.

II. Class Zoomastigophorea:

Features:

1. Usually live in association.

2. Chromatophores are absent.

3. Presence of one to many flagella.

4. Amoeboid forms, when present, may not have flagella.

It includes nine orders.

Order 1. Choanoflagellida:

Features:

1. Free-living and may be solitary or colonial.

2. A peduncle for attachment may be present in some forms.

3. Flagellum is single, anteriorly placed and enclosed posteriorly by a thin collar.

Examples. *Proterospongia, Codosiga*.

Order 2. Bicosoecida:

Features:

1. Free-living, with two flagella—one is free and the other is attached to the posterior end.

Examples. *Bicosoeca, Poteriodendron*.

Order 3. Rhizomastigida:

Features:

1. Usually free-living.

2. Flagella and pseudopodia occur either at the same time or at different times.

Examples. *Dimorpha, Histomonas*.

Order 4. Kinetoplastida:

Features:

1. Most members live in association.

2. Number of flagella varies from one to four.

3. A self-replicating (DNA), Feulgen positive organella, called Kinetoplast, is located within a single large mitochondrion.

Examples. *Trypanosoma, Leishmania, Herpetomonas, Bodo*.

Order 5. Retortamonadida:

Features:

1. Usually live in association.

2. Presence of a ventral cytostome with fibrillar border.

3. Number of flagella ranges from two to four, and one of them is turned posteriorly to remain attached with cytostomal region.

Examples. *Retortamonas, Chilomastix*.

Order 6. Diplomonadida:

Features:

1. Most members live in association.

2. Body is bilaterally symmetrical and possesses two karyomastigonts each having four flagella and set of accessory organelles.

Example. *Giardia*.

Order 7. Oxymonadida:

Features:

1. Presence of one or more karyomastigonts, each having two pairs of flagella.

2. A few flagella are turned posteriorly and attach for some distances to the body surface.

3. Axostyles vary one to many.

Examples. *Oxymonas, Pyrsonympha*.

Order 8. Trichomonadida:

Features:

1. Presence of four to six flagella.

2. Undulating membrane, when present, is associated with recurrent flagellum.

3. Axostyle and parabasal apparatus are present.

4. Spindle during division is extra-nuclear.

5. Sexual reproduction and cyst formation are absent.

6. Usually live in association.

Examples. *Trichomonas, Tritrichomonas*.

Order 9. Hypermastigida:

Features:

1. Presence of six multiple flagella and numerous parabasal apparatus.

2. Golgi apparatus and filament associated with basal body constitutes the parabasal body.

3. Kinetosomes are distributed in various ways and meet anteriorly in a central structure.

4. Uninucleated and extra-nuclear spindle formation occurs during division.

5. Occurrence of sexual reproduction is observed in some forms.

Examples. *Lophomonas, Trichonympha*.

Superclass Opalinata:

It includes a single order Opalinida.

Order Opalinida:

Features:

1. Presence of cilium-like organelles in oblique rows over entire body surface.

2. Cytostome is absent, more than one nucleus of same type.

3. Sexual reproduction happens through the production of anisogamous flagellated gametes.

4. Always live in association.

Examples. *Opalina, Zelleriella*.

Superclass Sarcodina:

Features:

1. Usually free-living locomotor organella in the form of pseudopodia.

2. Flagella appear in some forms during development.

3. Cortical cytoplasm is undifferentiated.

4. Body may or may have various types or exo or endo-skeleton.

5. Asexual reproduction occurs by fission.

6. Sometimes sexual reproduction with flagellate or amoeboid gametes is noted. The members of this superclass are again subdivided into three classes— Rhizopodea, Piroplasmea and Actinopodea.

III. Class Rhizopodea:

Features:

1. Nutrition is phagotrophic.

2. Pseudopodia may be lobopodia, filo-podia or reticulopodia.

There are five subclasses—Lobosia, Filosia, Granuloreticulosia, Mycetozoia and Labyrinthulia.

Subclass 1. Lobosia:

Features:
1. Locomotion by characteristic lobose type of pseudopodia, occasionally becoming filiform or anastomosing.

It consists of the orders, Amoebida and Arcellinida.

Order 1. Amoebida:

Features:
1. Uninucleate and without any covering.

2. Majority, are free-living.

Examples. *Amoeba, Pelomyxa, Entamoeba, Chaos*.

Order 2. Arcellinida:

Features:
1. Free-living forms having a test or rigid membranes.

2. Pseudopodia protrude through definite aperture.

Examples. *Arcella, Difflugia*.

Subclass 2. Filosia:

Features:
1. Filopods are tapering and branching but the branches rarely anastomose.

Two orders, Aconchulinida and Gromiida are included within this subclass.

Order 1. Aconchulinida:

Features:
1. Filosia with naked body.

Example. *Penardia*.

Order 2. Gromiida:

Features:
1. Presence of test with definite aperture.

2. Certain members possess uniflagellate gametes.

Examples. *Gromia, Euglypha*.

Subclass 3. Granuloreticulosia:

Features:
1. Pseudopods are thin, reticular and granular.

Three orders, Athalamida, Foraminiferida and Xenophyophorida are present within this subclass.

Order 1. Athalamida:

Features:

1. Without any test and pseudopodia may originate from any part of the body.

Example. *Biomyxa*.

Order 2. Foraminiferida:

Features:

1. Presence of a test having one or more chambers.
2. Pseudopodia appear from aperture or perforations or both.
3. Life cycle involves definite alteration of sexual and asexual forms.
4. Gametes are with flagella and sometimes they may be amoeboid.
5. Presence of sexual dimorphism in some.

Examples. *Elphidium, Rosalina, Globigerina*.

Order 3. Xenophyophorida:

Features:

1. Body is multinucleated Plasmodium i.e. the amoeboid syncytial mass, and network of pseudopodia passes through a hollow organic tube.
2. Many foreign particles are present in the interstices of pseudopodial network.

Example. *Stannoma*.

Subclass 4. Mycetozoia:

Features:

1. Trophic amoeboid forms either form an aggregate or a multinucleate Plasmodium.
2. Complicated life cycle involves sexual reproduction and ends in sporangia form.
3. Spore gives rise to amoeboid form.
4. Nutrition may be heterotrophic or osmotrophic.

This subclass includes three orders— Acrasida, Eumycetozoida and Plasmodiophorida.

Order 1. Acrasida:

Features:

1. Never forms true plasmodium.
2. Flagellated stage absent.
3. Free-living.
4. No sexual reproduction.

Example. *Dictyostelium*.

Order 2. Eumycetozoida:

Features:

1. Free-living.
2. Flagellated stage present.
3. Presence of true plasmodium and typical sporangia with peridia (pi.), i.e., the covering of the spore-bearing organ and capillitia (pi.), i.e., a net-work of filaments in which spores are embedded within sporangia.

Examples. *Physarum, Ceratiomyxa*.

Order 3. Plasmodiophorida:

Features:

1. Live in association with plants.
2. Occurrence of large plasmodium with host tissue.
3. Presence of flagellated stages.
4. Sporangia without peridia and capillitia.

Example. *Plasmodiophora*.

Subclass 5. Labyrinthulia:

Only one order Labyrinthulida represents the subclass.

Order Labyrinthulida:

Features:

1. Individuals are spindle-shaped and form a net along filamentous tracks.
2. Either live on marine plants or in soil.
3. True amoeboid stage lacking.

Example. *Labyrinthula*.

IV. Class Piroplasmea:

It includes a single order piroplasmida.

Order Piroplasmida:

Features:

1. Small forms of various shapes.
2. Spores, flagellal and cilia absent.
3. Locomotion by gliding.
4. Binary fission takes place.
5. Lives as parasite in vertebrate blood and are carried by ticks.

Examples. *Theileria, Babesia*.

V. Class Actinopodea:

Features:

1. Usually floating with spherical body and delicate pseudopodia.
2. Pseudopodia may be axopodia, filose or reticulate.
3. Usually naked, when test present it is either membranous or chitinoid or silicious or strontium.
4. Both asexual and sexual reproduction occur.
5. Gametes are flagellated.

There are four subclasses, Radiolaria, Acanthacia, Heliozoia and Proteomyxida.

Subclass 1. Radiolaria:

Features:

1. Marine forms having one to many pores in the central capsule.
2. Presence of siliceous spicules or skeleton.
3. Locomotor organelles are either filopod or reticulopod or axopod.

Two orders, Porulosida and Oculosida are included within this subclass.

Order 1. Porulosida:

Features:

1. The round central capsule bears pores all around.

Examples. *Pipetta, Thalassicolla*.

Order 2. Oculosida:

Features:

1. The central capsule has pores only at one pole.

Examples. *Cystidium, Eucyrtidium*.

Subclass 2. Acantharia:

Features:

1. Thin central capsule with membraneous poreless covering.
2. Strontium sulphate forms regularly oriented radial spines.
3. Pseudopodia as axopod.
4. All are marine.

Two orders included are—Acanthometrida and Acanthophractida.

Order 1. Acanthometrida:

Features:

1. Rod-like skeleton without lattice shell.

Example. *Acanthometron*.

Order 2. Acanthophractida:

Features:

1. Skeleton completely latticed.

Example. *Challengeron*.

Subclass 3. Heliozoia:

Features:

1. Central capsule is absent.
2. Sometimes skeletons are present as siliceous scales or spines, but usually naked.
3. Locomotion through axopods or filopods.
4. Most of the members are freshwater.

It has three orders—Actinophryida, Centrohelida and Desmothoracida.

Order 1. Actinophryida:

Features:

1. Skeleton and centroplast are absent.

Examples. *Actinophrys, Actino- sphaerium*.

Order 2. Centrohelida

Features:

1. Centroplast is present.
2. Plate or spine-like skeletons are siliceous.

Example. *Acanthocysitis*.

Order 3. Desmothoracida:

Features:

1. Centroplast is absent.
2. Chitinous skeleton has siliceous impregnation.

Example. *Clathrulina*.

Subclass 4. Proteomyxidia:

Features:

1. Filopodia and reticulopodia are present in some species.
2. Marine and freshwater parasites of algae and higher plants.

Order Proteomyxida:

Features:

1. Only a few forms are free-living.
2. No test, filopodia, reticulopodia and flagellated forms.
3. Cysts are seen in some cases.

Examples. *Pseudospora, Leptomyxa*.

B. Subphylum Sporozoa:

Features:

1. Simple spores without polar filaments carry one to many sporozoites.

2. Cilia absent but flagellated gametes may occur.

3. Sexual reproduction, when occurs, is syngamous.

4. All the forms live in association.

It is subdivided into three classes— Telosporea, Toxoplasmea and Hoplosporea.

VI. Class Telosporea:

Features:

1. Spores are seen.

2. Both asexual and sexual reproduction take place.

3. Locomotion by gliding or body flexion.

4. Pseudopodia are usually absent but sometimes used only for food capture.

5. Microgametes are flagellated in some. Two subclasses Gregarinia and Coccidia are included in this class.

Subclass 1. Gregarinia:

Features:

1. Live as extracellular parasites in the digestive tract and body cavity of invertebrates.

It consists of three orders— Archigregarinida, Eugregarinida and Neogregarinida.

Order 1. Archigregarinida

Features:

1. Presence of three schizogony.

2. Live as parasites of ascidians, enteropneusids, sipunculids and annelids.

Example. *Selenidium.*

Order 2. Eugregarinida:

Features:

1. Live as parasites of annelids and arthropods and have no schizogony.

Examples. *Monocystis, Gregarina, Nina*.

Order 3. Neogregarinida:

Features:

1. Presence of secondary schizogony.

2. Lives as parasite of insects.

Example. *Ophryocystis*.

Subclass 2. Coccidia:
Features:
1. Always live as intracellular parasite and have small trophozoites.

Two orders, Protococcida and Eucoccida are present in this subclass.

Order 1. Protococcida:
Features:
1. Parasites of marine annelids and do not have schizogony.

Example. *Eucoccidium*.

Order 2. Eucoccida:
Features:
1. Live as parasite in epithelial and blood cells of invertebrates and vertebrates.

2. Presence of schizogony.

3. Alternation of asexual and sexual phases in life cycle.

Examples. *Eimeria, Plasmodium*.

VII. Class Toxoplasmea:
It includes single order Toxoplasmida under this class.

Order Toxoplasmida:
Features:
1. No spore formation.

2. Asexual reproduction by binary fission.

3. Locomotion is effected by gliding or body flexion.

4. Structures like pseudopodia and flagella are absent.

5. Cysts include naked trophozoites.

Examples. *Toxoplasma, Sarcocystis*.

VIII. Class Haplosporea:
It includes a single order Haplosporida.

Order Haplosporida:
Features:
1. Presence of spores, only asexual reproduction takes place.

2. Schizogony is present.

3. Though pseudopodia may appear in some cases, yet flagella are absent.

Examples. *Haplosporidium, Coelosporidium*.

C. Subphylum Cnidospora:
Features:
1. Presence of spores having one or more spore filaments and sporoplasms.

2. All the members live as parasite.

Two classes—Myxosporidea and Microsporidea are present.

IX. Class Myxosporidea:
Features:
1. Multicellular state gives rise to spore.

2. Presence of one or more sporoplasms and more than one valve.

It comprises of three orders— Myxosporida, Actinomyxida and Helicosporida.

Order 1. Myxosporida:
Features:
1. Presence of one or two sporoplasms and one to six polar capsules.

2. Each capsule having a coiled polar filament for anchoring.

3. Spore membrane may have up to six valves.

4. Live as parasite in poikilothermal vertebrates.

Examples. *Leptotheca, Myxidium*.

Order 2. Actinomyxida:
Features:
1. Presence of three polar capsules in a spore.

2. Each capsule with a polar filament.

3. Three valves are present in the membrane.

4. Many sporoplasms occur.

6. Live in annelids and other invertebrates.

Example. *Triactinomyxon*.

Order 3. Helicosporida:
Features:
1. Three sporoplasms in a spore are enclosed by coiled thick filament.

2. Spore membrane possesses one valve.

3. Parasites in insects.

Example. *Helicosporidium*.

X. Class Microsporidea:
Order Microsporida:
Features:
1. Spores originate from a single cell.

2. Presence of single sporoplasm, valve and an elongated tubular polar filament.

3. Parasites in invertebrates.

Examples. *Caudospora, Nosema*.

D. Subphylum Ciliophora:
A single class Ciliatea, constitutes the subphylum.

XI. Class Ciliatea:
Features:
1. Free living forms with cilia or ciliated organelle at least in some part of the life cycle.

2. Sub-pellicular infra-ciliature always present, even during the absence of cilia.

3. Usually two types of nuclei are seen.

4. Both asexual and sexual reproductions occur.

5. Sexual reproduction involves either conjugation or autogamy or cytogamy.

6. Nutrition is heterotrophic.

It is divided into four subclasses— Holotrichia, Peritrichia, Suctoria and Spirotrichia.

Subclass 1. Holotrichia:
Features:
1. Ciliature on the surface is uniform and simple.

2. Buccal ciliature present in a few cases.

It includes seven orders:
Order 1. Gymnostomatida:
Features:
1. Larger-sized forms with no oral ciliature.

2. Cytostome communicates directly to the outside.

3. Presence of rods in the cytopharyngeal wall.

Examples. *Didinium, Urotricha*.

Order 2. Trichostomatida:
Features:
1. Generally body ciliation is uniform but may be asymmetrical in some cases.

2. No buccal ciliation in oral area.

Examples. *Balantidium, Colpoda*.

Order 3. Chonotrichida:
Features:
1. Body ciliature absent in mature forms which are vase-shaped and cling to the crustacean body by means of non- contractile stalk.

2. Reproduction is asexual and by budding.

Examples. *Spirochona, Chilodochona*.

Order 4. Apostomatida:
Features:
1. Body ciliature in mature forms is spiral.

2. Cytostome is inconspicuous.

3. Life cycle exhibits polymorphism.

Examples. *Foettingaria, Polyspira*.

Order 5. Astomatida:
Features:
1. Body ciliature is uniform.

2. Cytostome is absent.

3. Usually of large size, some have endoskeletons or structures as holdfast.

4. Usually parasites in oligochaetes.

Examples. *Anoplophrya, Haptophrya*.

Order 6. Hymenostomatida:
Features:
1. Small-sized forms.

2. Uniform body ciliature.

3. Buccal cavity is ventral and presence of one undulating membrane on the right.

4. Three membranelles on the left.

Examples. *Tetrahymena, Paramoecium*.

Order 7. Thigmotrichida:
Features:
1. Tuft of cilia is present near the anterior end.

2. Buccal ciliature either ventral or posteriorly placed.

3. Usually live in association with bivalve molluscs.

Examples. *Ancistrocoma, Concho- phthirus*.

Subclass 2. Peritrichia:
The only order belonging to this subclass is Peritrichida.

Order Peritrichida:
Features:
1. Cilia is usually absent in matured forms.

2. Presence of either contractile stalk or adhesive disc for attachment to the substrate.

3. Ciliary arrangement in the oral region is conspicuous.

4. It coils here around apical pole counter-clockwise to cytostome.

Examples. *Vorticella, Epistylis*.

Subclass 3. Suctoria:
It includes a single order Suctorida.

Order Suctorida:
Features:
1. Absence of external ciliature in mature forms.

2. Usually sessile with non-contractile stalk for attachment.

3. Presence of suctorial tentacles for nutrition.

4. Reproduction by budding.

5. Larva is free-swimming and with external ciliation.

Examples. *Podophyra, Acineta*.

Subclass 4. Spirotrichia:
Features:
1. External ciliature is sparse in most.

2. Presence of cirri in some.

3. Elaborate buccal ciliature.

4. Presence of adoral zone with many membranelles.

5. Oral cilia coil around apical pole in clockwise to cytostome.

It includes six orders under this subclass:

Order 1. Heterotrichida
Features:
1. External ciliature is uniform.

2. Large sized body in some cases bears pigments.

Examples. *Stentor, Spirostomum*.

Order 2. Oligotrichida:
Features:
1. External ciliature is absent.

2. Prominent buccal membranelles are present.

3. Small size.

4. Usually marine.

Examples. *Halteria, Tontonia*.

Order 3. Tintinnida:
Features:
All with varied coverings, called lorica, from where prominent oral membranelles extend.

2. Marine.

Examples. *Tintinnus, Codonella*.

Order 4. Entodiniomorphida:
Features:
1. External ciliature is absent.

2. Oral membranelles are restricted.

3. Presence of membranellar tufts or zones.

4. Pellicle stiff and extended posteriorly in some forms as spine.

Examples. *Entodinium, Diplodinium*.

Order 5. Odontostomatida:
Features:
1. Eight membranelles represent oral ciliature.

2. Laterally compressed miniature body sometimes possesses spines on the pellicle.

Example. *Saprodinium*.

Order 6. Hypotrichida:
Features:
1. Various types of cirri are ventrally placed.

2. Dorso-ventrally flattened body.

3. Membranelles are prominent in adoral zone.

Examples. *Aspodisca, Gastrostyla, Euplotes.*

Zoonosis

A zoonosis is any disease or infection that is naturally transmissible from vertebrate animals to humans. Animals thus play an essential role in maintaining zoonotic infections in nature. Zoonoses may be bacterial, viral, or parasitic, or may involve unconventional agents. As well as being a public health problem, many of the major zoonotic diseases prevent the efficient production of food of animal origin and create obstacles to international trade in animal products.

Animals provide many benefits to people. Many people interact with animals in their daily lives, both at home and away from home. Pets offer companionship and entertainment, with millions of households having one or more pets. We might come into close contact with animals at a county fair or petting zoo, or encounter wildlife while enjoying outdoor activities. Also, animals are an important food source and provide meat, dairy, and eggs.

Zoonosis refers to diseases that can be passed from animals to humans. They are sometimes called zoonotic diseases.

Animals can carry harmful germs, such as bacteria, fungi, parasites, and viruses. These are then shared with humans and cause illness. Zoonotic diseases range from mild to severe, and some can even be fatal.

Zoonotic diseases are widespread both in the U.S. and worldwide. The World Health Organization (WHO) estimates that 61 percent of all human diseases are zoonotic in origin, while 75 percent of new diseases discovered in the last decade are zoonotic.

Before the introduction of new hygiene regulations around 100 years ago, zoonotic diseases such as bovine tuberculosis, bubonic plague, and glanders caused millions of deaths. They are still a major problem in developing countries.

However, some animals can carry harmful germs that can be shared with people and cause illness – these are known as zoonotic diseases or zoonoses. Zoonotic diseases are caused by harmful germs like viruses, bacterial, parasites, and fungi. These germs can cause many different types of illnesses in people and animals ranging from mild to serious illness and even death. Some animals can appear healthy even when they are carrying germs that can make people sick.

Zoonotic diseases are very common, both in the United States and around the world. Scientists estimate that more than 6 out of every 10 known infectious diseases in people are spread from animals, and 3 out of every 4 new or emerging infectious diseases in people are spread from animals. Every year, tens of thousands of Americans will get sick from harmful germs spread between animals and people. Because of this, CDC works 24/7 to protect people from zoonotic diseases.

How do germs spread between animals and people?

Because of the close connection between people and animals, it's important to be aware of the common ways people can get infected with germs that can cause zoonotic diseases. These can include:

- **Direct contact**: Coming into contact with the saliva, blood, urine, mucous, feces, or other body fluids of an infected animal. Examples include petting or touching animals, and bites or scratches.
- **Indirect contact**: Coming into contact with areas where animals live and roam, or objects or surfaces that have been contaminated with germs. Examples include aquarium tank water, pet habitats, chicken coops, plants, and soil, as well as pet food and water dishes.
- **Vector-borne**: Being bitten by a tick, or an insect like a mosquito or a flea.

- **Foodborne**: Each year, 1 in 6 Americans get sick from eating contaminated food. Eating or drinking something unsafe (such as unpasteurized milk, undercooked meat or eggs, or raw fruits and vegetables that are contaminated with feces from an infected animal).

Types

Common zoonotic illnesses include:

- **Rabies**

  Rabies is a disease that affects the nervous system of mammals. It is usually caused by a virus and is transmitted if an infected animal bites a person or other animal.

  Rabies is almost always fatal once symptoms appear. However, rabies vaccines exist and are commonly available.

- **Lyme disease and Rocky Mountain spotted fever**

  Lyme disease is transmitted through tick bites. Symptoms can range from mild to severe, but it can be treated using antibiotics.

- **Dengue, malaria, and chikungunya**

  These are mosquito-borne diseases and are more common in certain areas, such as the Caribbean.

  Symptoms include fever, vomiting, and headaches. It is vital to treat these conditions as soon as possible, as they can be fatal.

- ***Salmonella* infection**

  Salmonella is often caused by handling reptiles or amphibians that carry *Salmonella*, or by handling baby chicks or ducks.

  The illness usually lasts for between 4 and 7 days, and symptoms include diarrhoea, fever, and abdominal cramps. People can usually recover without medical treatment, although conservative measures are recommended.

- ***E. coli* infection**

  This infection is often caused by touching infected animals or handling contaminated food. Cows also have *E. coli* germs on their udders.

  Often associated with food poisoning, salmonella can cause vomiting, abdominal cramps, and diarrhoea. It is essential that infected people rest and drink plenty of fluids.

- **Psittacosis**

  Also known as ornithosis or parrot fever, psittacosis is a bacterial disease that most often affects birds. Humans can get it from feathers, secretions, and droppings.

Symptoms include fever, headache, and dry cough. In serious cases, it may cause pneumonia and require a hospital visit.

Other types

There are hundreds of zoonotic diseases, but many are rare. Other well-known types include:

- anthrax
- avian influenza or bird flu
- bovine tuberculosis
- brucellosis
- cat scratch fever
- Ebola
- West Nile virus
- leprosy
- Zika fever
- trichinosis
- swine influenza
- histoplasmosis

Toxoplasmosis

Toxoplasmosis is caused by the protozoan parasite *Toxoplasma gondii*. In the United States it is estimated that 11% of the population 6 years and older have been infected with *Toxoplasma*. In various places throughout the world, it has been shown that up to 95% of some populations have been infected with *Toxoplasma*. Infection is often highest in areas of the world that have hot, humid climates and lower altitudes.

Toxoplasmosis is not passed from person-to-person, except in instances of mother-to-child (congenital) transmission and blood transfusion or organ transplantation. People typically become infected by three principal routes of transmission.

- Foodborne
- Animal-to-human (zoonotic)
- Mother-to-child (congenital)
- Rare instances

Foodborne transmission

The tissue form of the parasite (a microscopic cyst consisting of bradyzoites) can be transmitted to humans by food. People become infected by:

- Eating undercooked, contaminated meat (especially pork, lamb, and venison).
- Accidental ingestion of undercooked, contaminated meat after handling it and not washing hands thoroughly (*Toxoplasma* cannot be absorbed through intact skin).
- Eating food that was contaminated by knives, utensils, cutting boards, or other foods that had contact with raw, contaminated meat.

Animal-to-human (zoonotic) transmission

Cats play an important role in the spread of toxoplasmosis. They become infected by eating infected rodents, birds, or other small animals. The parasite is then passed in the cat's feces in an oocyst form, which is microscopic.

Kittens and cats can shed millions of oocysts in their feces for as long as 3 weeks after infection. Mature cats are less likely to shed *Toxoplasma* if they have been previously infected. A *Toxoplasma*-infected cat that is shedding the parasite in its feces contaminates the litter box. If the cat is allowed outside, it can contaminate the soil or water in the environment as well.

People can accidentally swallow the oocyst form of the parasite. People can be infected by:

Accidental ingestion of oocysts after cleaning a cat's litter box when the cat has shed *Toxoplasma* in its feces.

- Accidental ingestion of oocysts after touching or ingesting anything that has come into contact with a cat's feces that contain *Toxoplasma.*
- Accidental ingestion of oocysts in contaminated soil (e.g., not washing hands after gardening or eating unwashed fruits or vegetables from a garden).
- Drinking water contaminated with the *Toxoplasma* parasite.

Mother-to-child (congenital) transmission

A woman who is newly infected with *Toxoplasma* during pregnancy can pass the infection to her unborn child (congenital infection). The woman may not have symptoms, but there can be severe consequences for the unborn child, such as diseases of the nervous system and eyes.

Rare instances of transmission

Organ transplant recipients can become infected by receiving an organ from a *Toxoplasma*-positive donor. Rarely, people can also become infected by receiving infected blood via transfusion. Laboratory workers who handle infected blood can also acquire infection through accidental inoculation.

Causal Agent:

Toxoplasma gondii is a protozoan parasite that infects most species of warm blooded animals, including humans, and can cause the disease toxoplasmosis.

Life Cycle:

The only known definitive hosts for *Toxoplasma gondii* are members of family Felidae (domestic cats and their relatives). Unsporulated oocysts are shed in the cat's feces . Although oocysts are usually only shed for 1-2 weeks, large numbers may be shed. Oocysts take 1-5 days to sporulate in the environment and become infective. Intermediate hosts in nature (including birds and rodents) become infected after ingesting soil, water or plant material contaminated with oocysts. Oocysts transform into tachyzoites shortly after ingestion. These tachyzoites localize in neural and muscle tissue and develop into tissue cyst bradyzoites. Cats become infected after consuming intermediate hosts harboring tissue cysts. Cats may also become infected directly by ingestion of sporulated oocysts. Animals bred for human consumption and wild game may also become infected with tissue cysts after ingestion of sporulated oocysts in the environment. Humans can become infected by any of several routes:

- eating undercooked meat of animals harboring tissue cysts .
- consuming food or water contaminated with cat feces or by contaminated environmental samples (such as fecal-contaminated soil or changing the litter box of a pet cat) .
- blood transfusion or organ transplantation .
- transplacentally from mother to fetus .

In the human host, the parasites form tissue cysts, most commonly in skeletal muscle, myocardium, brain, and eyes; these cysts may remain throughout the life of the host. Diagnosis is usually achieved by serology, although tissue cysts may be observed in stained

biopsy specimens. Diagnosis of congenital infections can be achieved by detecting *T. gondii* DNA in amniotic fluid using molecular methods such as PCR.

Healthy people (nonpregnant)

Healthy people who become infected with *Toxoplasma gondii* often do not have symptoms because their immune system usually keeps the parasite from causing illness. When illness occurs, it is usually mild with "flu-like" symptoms (e.g., tender lymph nodes, muscle aches, etc.) that last for weeks to months and then go away. However, the parasite remains in their body in an inactive state. It can become reactivated if the person becomes immunosuppressed.

Mother-to-child (congenital)

Generally if a woman has been infected before becoming pregnant, the unborn child will be protected because the mother has developed immunity. If a woman is pregnant and becomes newly infected with *Toxoplasma* during or just before pregnancy, she can pass the infection to her unborn baby (congenital transmission). The damage to the unborn child is often more severe if the transmission occurs earlier in pregnancy. Potential results can be

- a miscarriage
- a stillborn child
- a child born with signs of toxoplasmosis (e.g., abnormal enlargement or smallness of the head)

Infants infected before birth often show no symptoms at birth but may develop them later in life with potential vision loss, mental disability, and seizures.

Persons with ocular disease

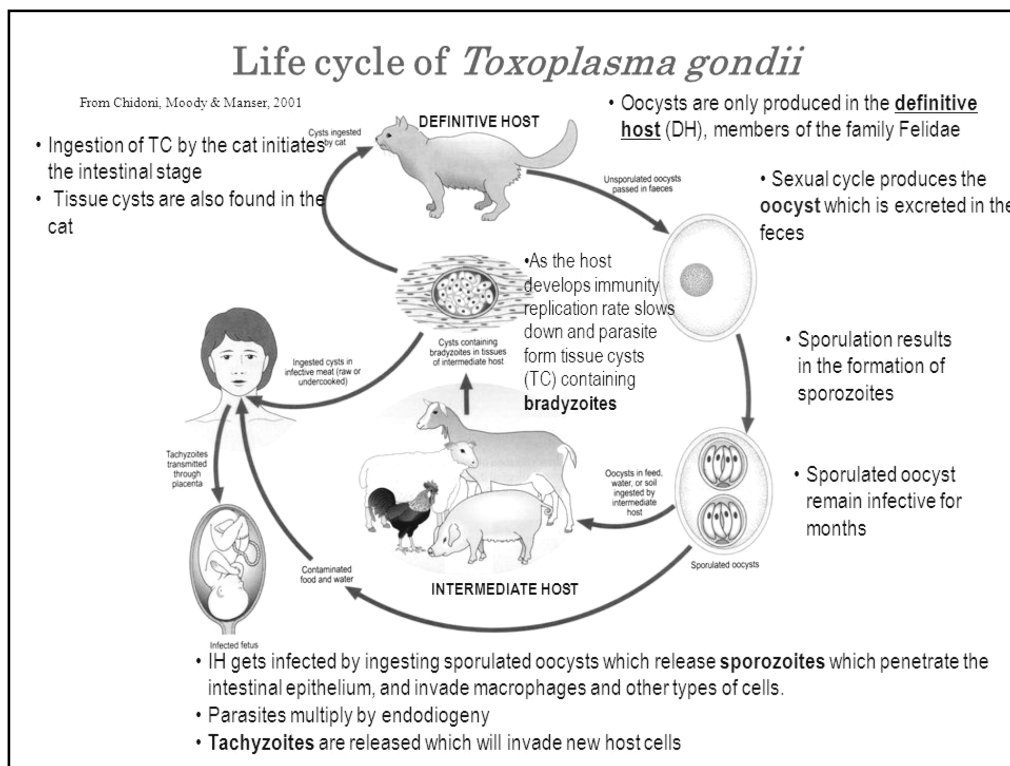
Eye disease (most frequently retinochoroiditis) from *Toxoplasma* infection can result from congenital infection or infection after birth by any of the modes of transmission discussed on the epidemiology and risk factors page. Eye lesions from congenital infection are often not identified at birth but occur in 20-80% of infected persons by adulthood. However, in the U.S. <2% of persons infected after birth develop eye lesions. Eye infection leads to an acute inflammatory lesion of the retina, which resolves leaving retinochoroidal scarring. Symptoms of acute disease include

- eye pain
- sensitivity to light (photophobia)
- tearing of the eyes
- blurred vision

The eye disease can reactivate months or years later, each time causing more damage to the retina. If the central structures of the retina are involved there will be a progressive loss of vision that can lead to blindness.

Persons with compromised immune systems

Persons with compromised immune systems may experience severe symptoms if they are infected with *Toxoplasma* while immune suppressed. For example, a person who is HIV-



infected and who has reactivated *Toxoplasma* infection can have symptoms that include fever, confusion, headache, seizures, nausea, and poor coordination. Persons who acquire HIV infection and were not infected previously with *Toxoplasma* are more likely to develop a severe primary infection.

Immunocompromised persons who were infected with *Toxoplasma* at some point before they become immunosuppressed are particularly at risk for developing a relapse of toxoplasmosis.

Toxoplasma infection can reactivate in immunocompromised pregnant women who were infected with *Toxoplasma* before their pregnancy and this can lead to congenital infection.

Diagnosis

The diagnosis of toxoplasmosis is typically made by serologic testing. A test that measures immunoglobulin G (IgG) is used to determine if a person has been infected. If it is necessary to estimate the time of infection, which is of particular importance for pregnant women, a test which measures immunoglobulin M (IgM) is also used along with other tests such as an avidity test.

Diagnosis can be made by direct observation of the parasite in stained tissue sections, cerebrospinal fluid (CSF), or other biopsy material. These techniques are used less frequently because of the difficulty of obtaining these specimens.

Parasites can also be isolated from blood or other body fluids (for example, CSF) but this process can be difficult and requires considerable time.

Molecular techniques that can detect the parasite's DNA in the amniotic fluid can be useful in cases of possible mother-to-child (congenital) transmission.

Ocular disease is diagnosed based on the appearance of the lesions in the eye, symptoms, course of disease, and often serologic testing.

Treatment

Healthy people (nonpregnant)

Most healthy people recover from toxoplasmosis without treatment. Persons who are ill can be treated with a combination of drugs such as pyrimethamine and sulfadiazine, plus folinic acid.

Pregnant women, newborns, and infants

Pregnant women, newborns, and infants can be treated, although the parasite is not eliminated completely. The parasites can remain within tissue cells in a less active phase; their location makes it difficult for the medication to completely eliminate them.

Persons with ocular disease

Persons with ocular toxoplasmosis are sometimes prescribed medicine to treat active disease by their ophthalmologist. Whether or not medication is recommended depends on the size of

the eye lesion, the location, and the characteristics of the lesion (acute active, versus chronic not progressing).

<u>Persons with compromised immune systems</u>

Persons with compromised immune systems need to be treated until they have improvement in their condition. For AIDS patients, continuation of medication for the rest of their lives may be necessary, or for as long as they are immunosuppressed.

Prevention and Control

People who are healthy should follow the guidelines below to reduce risk of toxoplasmosis. If you have a weakened immune system, please see guidelines for Immunocompromised Persons.

Reduce Risk from Food

To prevent risk of toxoplasmosis and other infections from food:

Cook food to safe temperatures. A food thermometer should be used to measure the internal temperature of cooked meat. Do not sample meat until it is cooked. USDA recommends the following for meat preparation.

For Whole Cuts of Meat (excluding poultry)
Cook to at least 145° F (63° C) as measured with a food thermometer placed in the thickest part of the meat, then allow the meat to rest for three minutes before carving or consuming.

For Ground Meat (excluding poultry)
Cook to at least 160° F (71° C); ground meats do not require a rest time.

For All Poultry (whole cuts and ground)
Cook to at least 165° F (74° C), and for whole poultry allow the meat to rest for three minutes before carving or consuming.

According to USDA, "A 'rest time' is the amount of time the product remains at the final temperature, after it has been removed from a grill, oven, or other heat source. During the three minutes after meat is removed from the heat source, its temperature remains constant or continues to rise, which destroys pathogens."

- Freeze meat for several days at sub-zero (0° F) temperatures before cooking to greatly reduce chance of infection.

- Peel or wash fruits and vegetables thoroughly before eating.
- Wash countertops carefully.
- Wash cutting boards, dishes, counters, utensils, and hands with hot soapy water after contact with raw meat, poultry, seafood, or unwashed fruits or vegetables.

The U.S. Government and the meat industry continue their efforts to reduce *T. gondii* in meat.

Reduce Risk from the Environment
To prevent risk of toxoplasmosis from the environment:

- Avoid drinking untreated drinking water.
- Wear gloves when gardening and during any contact with soil or sand because it might be contaminated with cat feces that contain *Toxoplasma*. Wash hands with soap and warm water after gardening or contact with soil or sand.
- Teach children the importance of washing hands to prevent infection.
- Keep outdoor sandboxes covered.
- Feed cats only canned or dried commercial food or well-cooked table food, not raw or undercooked meats.
- Change the litter box daily if you own a cat. The *Toxoplasma* parasite does not become infectious until 1 to 5 days after it is shed in a cat's feces. If you are pregnant or immunocompromised:
  1. Avoid changing cat litter if possible. If no one else can perform the task, wear disposable gloves and wash your hands with soap and warm water afterwards.
  2. Keep cats indoors.
  3. Do not adopt or handle stray cats, especially kittens. Do not get a new cat while you are pregnant.

Balantidiasis

Balantidium coli, though rare in the US, is an intestinal protozoan parasite that can infect humans. These parasites can be transmitted through the fecal-oral route by contaminated food and water. *Balantidium coli* infection is mostly asymptomatic, but people with other serious illnesses can experience persistent diarrhoea, abdominal pain, and sometimes a perforated

colon. When travelling to endemic tropical countries, *Balantidium coli* infection can be prevented by following good hygiene practices. Wash all fruits and vegetables with clean water when preparing or eating them, even if they have a removable skin.

What is *Balantidium coli?*

Balantidium coli is an intestinal protozoan parasite that causes the infection called balantidiasis. While this type of infection is uncommon in the United States, humans and other mammals can become infected with *Balantidium coli* by ingesting infective cysts from food and water that is contaminated by feces. Mostly asymptomatic, *Balantidium* infection can cause such symptoms as diarrhea and abdominal pain.

Where is *Balantidium coli* endemic?

Balantidium coli infection in humans is rare in the United States. *Balantidium coli* is found throughout the world, but it is most prevalent in tropical and subtropical regions and developing countries. Because pigs are an animal reservoir, human infections occur more frequently in areas where pigs are raised, especially if good hygiene is not practiced.

How is *Balantidium coli* transmitted?

Balantidium coli is transmitted through the fecal-oral route. Humans can become infected by eating and drinking contaminated food and water that has come into contact with infective animal or human fecal matter. Infection can occur in several ways, including the following examples:

- eating meat, fruits, and vegetables that have been contaminated by an infected person or contaminated by fecal matter from an infected animal,
- drinking and washing food with contaminated water, or
- having poor hygiene habits.

Biology

Causal Agent:

Balantidium coli, a large ciliated protozoan parasite

Life Cycle:

Cysts are the parasite stage responsible for transmission of balantidiasis. The host most often acquires the cyst through ingestion of contaminated food or water. Following ingestion, excystation occurs in the small intestine, and the trophozoites colonize the large intestine. The trophozoites reside in the lumen of the large intestine of humans and animals, where they replicate by binary fission, during which conjugation may occur. Trophozoites undergo encystation to produce infective cysts. Some trophozoites invade the wall of the colon and multiply. Some return to the lumen and disintegrate. Mature cysts are passed with feces.

Symptoms:

Most people infected with *Balantidium coli* experience no symptoms. *Balantidium coli* infects the large intestine in humans and produces infective microscopic cysts that are passed in the feces, potentially leading to re-infection or infection of others. People who are immune-compromised are the most likely to experience more severe signs and symptoms. These include persistent diarrhoea, dysentery, abdominal pain, weight loss, nausea, and vomiting. If left untreated, perforation of the colon can occur.

Treatment

Three medications are used most often to treat *Balantidium coli*: **tetracycline**, **metronidazole**, and **iodoquinol**.

Tetracycline*: adults, 500 mg orally four times daily for 10 days; children ≥ 8 years old, 40 mg/kg/day (max. 2 grams) orally in four doses for 10 days. (Note: Tetracyclines are contraindicated in pregnancy and in children < 8 years old. Tetracycline should be taken 1 hour before or 2 hours after meals or ingestion of dairy products.)

Alternatives:

Metronidazole*: adults, 500-750 mg orally three times daily for 5 days; children, 35-50 mg/kg/day orally in three doses for 5 days.

OR

Iodoquinol*: adults, 650 mg orally three times daily for 20 days; children, 30-40 mg/kg/day (max 2 g) orally in three doses for 20 days. (Note: iodoquinol should be taken after meals.)

Nitazoxanide*: has been tried in small studies, which suggest some therapeutic benefit (adults, 500 mg orally twice daily for 3 days; children age 4-11 years old 200 mg orally twice daily for 3 days; children 1-3 years old 100 mg orally twice daily for 3 days).

*Not FDA-approved for this indication

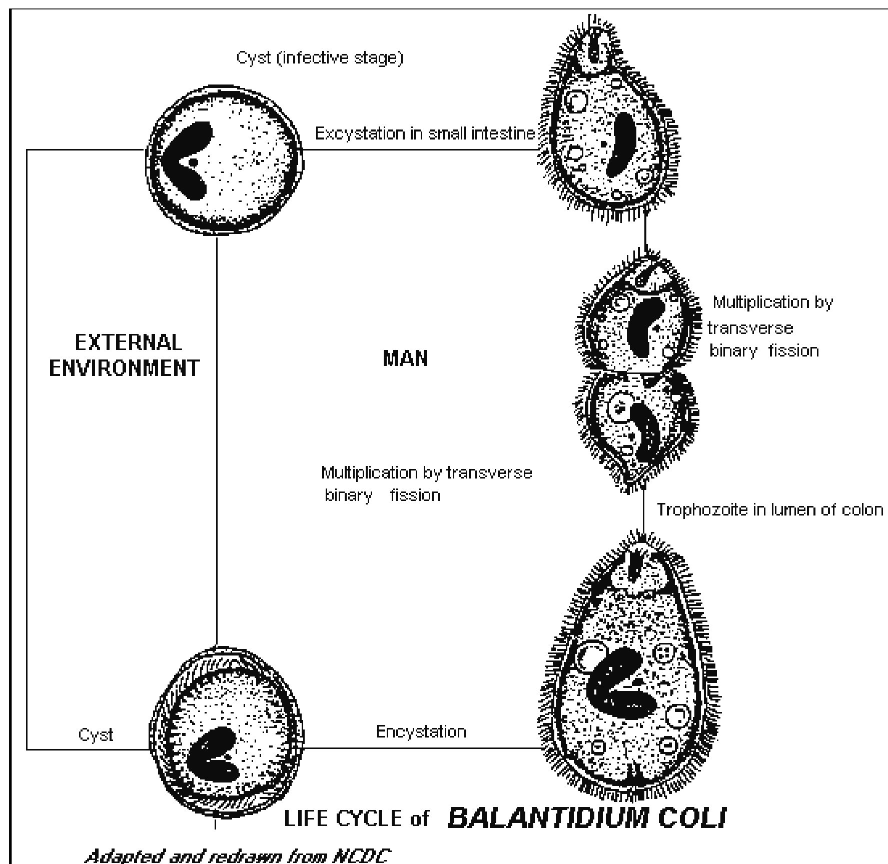
* Tetracycline is available for human use in the United States.

* Metronidazole is available for human use in the United States.

* Iodoquinol is available for human use in the United States.

Prevention of *Balantidium coli*

Balantidium coli infection can be prevented when travelling by following good hygienic practices. Wash your hands with soap and warm water after using the toilet, changing diapers, and before handling food. Teach children the importance of washing hands to prevent infection. Wash all fruits and vegetables with clean water when preparing or eating them, even if they have a removable skin.



Entamoeba

Entamoeba histolytica was first of all reported by a Russian Zoologist, Losch (1875), as *Amoeba coli* from the faeces of a patient suffering from dysentery. The genus *Entamoeba* was established by Cosagrandi and Barbagallo (1895); it should not, be confused with the genus *Endamoeba* which was established by Leidy (1879). Concilman and Loffteur (1901) worked out the pathogenecity of amoebiasis and amoebic ulcers.

The species *Entamoeba histolytica* was established by Schaudinn (1903) and he differentiated the pathogenic and non-pathogenic types. But, Walker and Sellards (1930) are credited for attributing pathogenic effects of this parasite. Craig (1962) has estimated that more than 10 percent of world population is suffering from the infection of this parasite.

Distribution of *Entamoeba histolytica*:

Entamoeba histolytica is world-wide (cosmopolitan) in distribution. But it is commonly found in epidemic form in tropical and sub-tropical regions than in the temperate region. The more epidemic condition of this parasite is reported from Mexico, China, India, Philippines, South America and Thailand.

Its incidence is relatively higher in rural and densely-populated urban areas particularly in those areas where the sanitary conditions are poor. In India, its effect is on higher level in humid climates as compared to dry and cold climates. The children and adults are more frequently infected; surprisingly, the males are more commonly infected than the females.

Habit and Habitat of *Entamoeba histolytica*:

Entamoeba histolytica is microscopic and lives as an endoparasite in the upper part of the large intestine, i.e., colon of man. It inhabits the mucous and sub-mucous layers of the large intestine. It feeds mainly on the tissues of the intestinal wall and often produces severe ulcers and abscesses.

In chronic cases, it may enter the blood circulation to reach the liver, lungs, brain and other organs. It causes a serious and often fatal disease known as amoebic dysentery or amoebiasis.

Structure of *Entamoeba histolytica*:

Entamoeba histolytica exists in two distinct forms the magna or trophozoite form and the minuta or precystic form.

Trophozoite:

The mature parasite or active and motile adult is known as trophozoite. The trophozoites of *E. histolytica* are large, hence, called magna, usually 20-30 *Entamoeba histolytica* trophozoite, microns in diameter. It is feeding form which is pathogenic to man.

It resembles *Amoeba* in all structural details. The cytoplasm of trophozoite is differentiated into two distinct portions, the outer ectoplasm and inner endoplasm. The ectoplasm is clear, non-granular and hyaline, while the endoplasm is granular and fluid-like.

The pseudopodia may be short, broad and rounded, long and finger-like, mainly composed of ectoplasm. During locomotion, the rapidly advancing end of the body consists of a single clear pseudopodium, i.e., monopodial. With this single pseudopodium, it moves in a crawling fashion like garden slug, hence, the movement is also sometimes called limax-type movement.

The endoplasm contains the nucleus and food vacuoles containing the tissue fragments, erythrocytes, leucocytes and ingested bacteria, etc.

The nucleus is rounded, 4-6 microns in diameter and vesicular. The nucleus is composed of a delicate membrane, small peripheral chromatin granules and a centrally located small dot-like nucleolus or endosome or karyosome and chromatin granules arranged in spoke-like striations.

The nucleolus is surrounded by an indefinite clear area called halo. The presence of red blood corpuscles (RBCs) in food vacuoles is an important characteristic feature of this parasite, as the capacity of ingesting RBCs. is not seen in other intestinal amoebae of man.

Contractile vacuole is entirely absent because *E. histolytica* lives in an environment which is isotonic. Since, the osmotic concentration of its body remains equal to its surroundings and, hence, no water enters in its body by osmosis. Therefore, there is no need of contractile vacuole.

The magna or trophozoic form of *E. histolytica* develops from small minuta form; it enters into the mucosa and sub-mucosa layers of the intestinal wall by dissolving its tissues. Thus, it makes small wounds in the intestinal lining which later develop into ulcers. After reaching into the intestinal tissues, ingests RBCs and grows up to 60 microns in size.

Minuta:

It is the pre-cystic form which is smaller, spherical, non-feeding, non-motile and non-pathogenic. It measures to about 7-10 microns in diameter and resembles the trophozoite

form in its structure except that it is smaller in size having no pseudopodium and contractile vacuole. It lives only in the lumen of intestine and rarely found in the tissues. It undergoes encystation and helps in the transmission of the parasite from one host to another.

Nutrition of *Entamoeba histolytica*:

In *Entamoeba histolytica*, the nutrition is holozoic. It feeds mainly upon the blood corpuscles, other host elements, bacteria and yeasts. It also absorbs substances saprozoically from the surrounding medium.

Reproduction:

The trophozoite of *Entamoeba histolytica* reproduces normally by a process of simple binary fission in the intestinal wall and by a modified form of mitosis. The exact nature of the division of the nucleus is controversial but it is believed by many authors that it is probably a modified type of mitosis. Kofoid and Swezy observed six chromosomes in it. *Entamoeba histolytica* also has the capacity to encyst.

In fact, the nucleus divides by mitosis but without the disappearance of the nuclear membrane. It is, then, followed by the division of the cytoplasm (cytokinesis) resulting into two daughter *Entamoeba*. These start feeding upon bacteria and host tissues, grow in size and again multiply by binary fission. Some of these forms may invade fresh intestinal tissues, while some of them become precystic or minuta form.

How does this organism cause disease?

- Trophozoites of *E. histolytica* adhere to colonic epithelial cells via a specific lectin, the galactose/N-acetylgalactosamine lectin. This lectin also seems to play a role in immunity, with one study from Bangladesh indicating that children with a mucosal IgA response against the lectin had 86% fewer new infections during a 1-year period than children without this response.
- Colitis results after penetration of the trophozoite through the intestinal mucous layer. *E. histolytica* trophozoites are cytolytic and are able to kill both epithelial cells and inflammatory cells. This is thought to be medicated by a number of mechanisms, including:
  - Secretion of proteinases by the trophozoites
  - Lysis of target cells via a contact-dependent mechanism

- Induction of programmed cell death (apoptosis)
- Formation of amoebapores, small peptides capable of forming pores in lipid bilayers
- Changes in intestinal permeability, probably via disruption of tight-junction proteins

Causal Agents

Several protozoan species in the genus *Entamoeba* colonize humans, but not all of them are associated with disease. *Entamoeba histolytica* is well recognized as a pathogenic *Amoeba*, associated with intestinal and extraintestinal infections. The other species are important because they may be confused with *E. histolytica* in diagnostic investigations.

Life Cycle of *Entamoeba histolytica*:

Entamoeba histolytica is monogenetic (Gr., mono = single; genos = race), i.e., its life cycle is completed in one host only; the host being the man.

Encystment:

The pre-cystic forms, under certain circumstances which are not well understood, remain small (7-10 microns in diameter) and live only in the intestinal lumen.

They undergo encystment but before encystment, the parasites round up, eliminate food vacuoles and accumulate considerable amount of food materials in the form of glycogen and black rod-like chromatoid granules. Soon each parasite secretes a thin, rounded, resistant, colourless and transparent cyst wall around it.

The cysts of *Entamoeba histolytica* vary in size from 10-20 microns (average 12 microns) in diameter. Its cytoplasm is clear and each cyst is mononucleate at this stage. Presence of chromatoid bodies is the characteristic of the cysts of *Entamoeba histolytica*. They occur either singly or in the multiples of two or more.

There is a controversy about the exact nature of these bodies. Some authorities consider them as nutrient material of the cysts, while others believe them as excess of chromatin thrown off during nuclear division. The chromatoid bodies are found in the early stages of the cysts but they disappear in the mature tetranucleate cysts.

Pitelka (1963) has suggested that the chromatoid bodies are made of ribonucleoprotein and Neal (1966) believes that the disappearance of chromatid granules occurs because of the dispersion of their nucleoprotein in the substance of mature tetranucleate cystic form.

The nucleus of the cysts divides twice so that each cyst now becomes tetranucleate. At this stage, the cyst is infective to a new host. Encysted forms pass out with the faecal matter of the host.

Transfer to new host:

The infective cysts remain viable for a considerable length of time outside the human intestine, if environmental conditions are favourable. Infection of fresh human host takes place by swallowing the infective cysts with contaminated food and drinks. Contamination of food and drinks is brought about by houseflies, cockroaches and food-handlers.

Houseflies generally carry the cysts from the faeces to the foods. Cockroaches have also been found to transport cysts from faeces to food. Food-handlers are also sometimes responsible for the contamination of food through touch by dirty fingers carrying the cysts under the nails. Through contaminated food or drinks, the infective cysts pass into the lower portion of the small intestine of the new host.

Excystment:

The excystment of cysts and metacystic development have been observed and studied specially by Dobell (1924) and Cleveland and Sanders (1930) in cultures. According to Dobell, in the process of excystation, a single tetranucleate amoeba (metacystic form) emerges from a cyst through a minute pore in the cyst wall.The tetranucleate metacystic form produces a new generation of trophozoites by a diverse series of nuclear and cytoplasmic divisions which result in the production of eight uninucleate amoebulae.

These are called metacystic trophozoites. No sexual phenomena have been observed during these changes. The metacystic trophozoites feed on the contents of the intestine and grow in size to form the trophozoites of the next generation. The trophozoites stay in the lumen of the intestine for a particular period when they may attack the wall of the intestine and start the life cycle again.

Cysts and trophozoites are passed in feces. Cysts are typically found in formed stool, whereas trophozoites are typically found in diarrheal stool. Infection by *Entamoeba histolytica* occurs by ingestion of mature cysts in fecally contaminated food, water, or hands. Excystation occurs in the small intestine and trophozoites are released, which migrate to the large intestine. The trophozoites multiply by binary fission and produce cysts, and both stages

are passed in the feces. Because of the protection conferred by their walls, the cysts can survive days to weeks in the external environment and are responsible for transmission. Trophozoites passed in the stool are rapidly destroyed once outside the body, and if ingested would not survive exposure to the gastric environment. In many cases, the trophozoites remain confined to the intestinal lumen (noninvasive infection) of individuals who are asymptomatic carriers, passing cysts in their stool. In some patients the trophozoites invade the intestinal mucosa (intestinal disease), or, through the bloodstream, extraintestinal sites such as the liver, brain, and lungs (extraintestinal disease), with resultant pathologic manifestations. It has been established that the invasive and noninvasive forms represent two separate species, respectively *E. histolytica* and *E. dispar*. These two species are morphologically indistinguishable unless *E. histolytica* is observed with ingested red blood cells (erythrophagocystosis). Transmission can also occur through exposure to fecal matter during sexual contact (in which case not only cysts, but also trophozoites could prove infective).

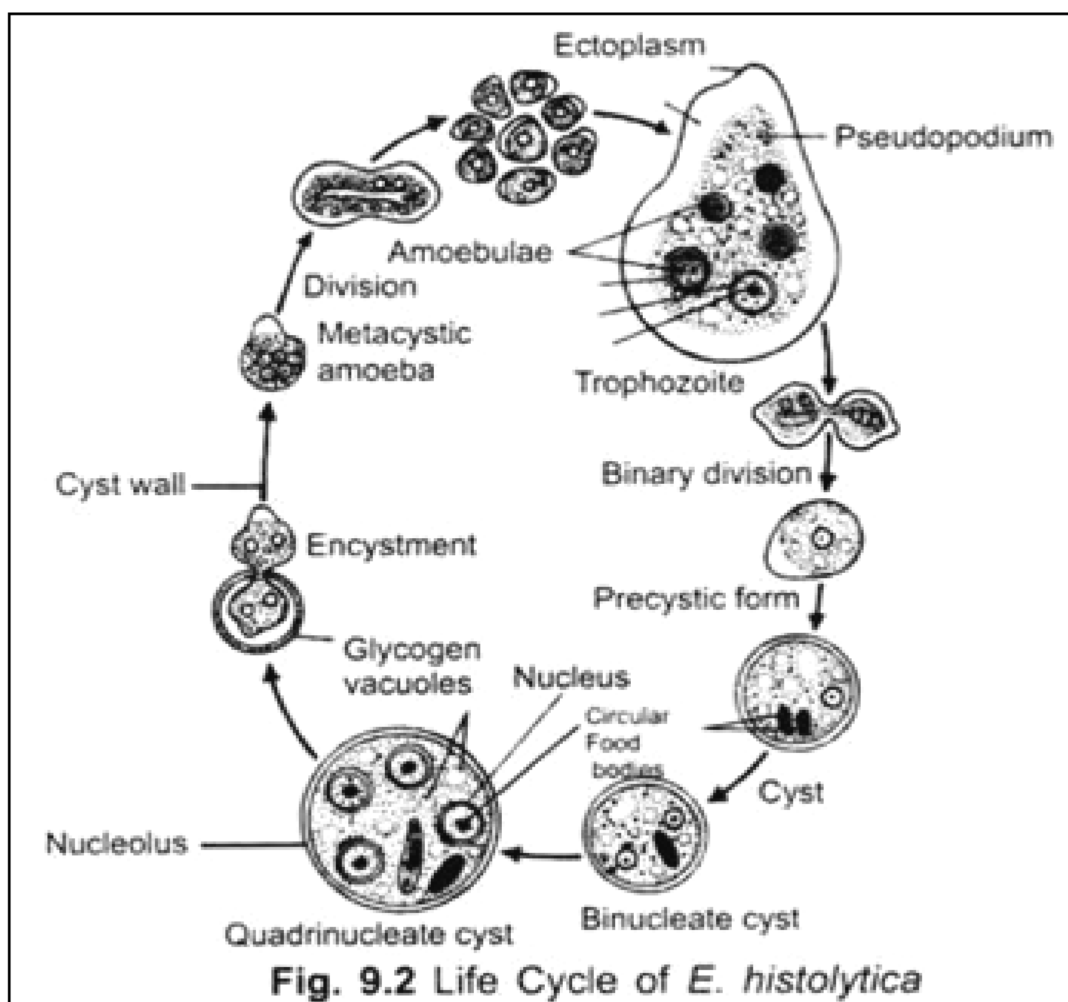


Fig. 9.2 Life Cycle of *E. histolytica*

Geographic Distribution

Worldwide, with higher incidence of amebiasis in developing countries. In industrialized countries, risk groups include male homosexuals, travellers and recent immigrants, and institutionalized populations.

Pathogenicity (Pathogenic Effects) of *Entamoeba histolytica*:

Entamoeba histolytica causes amoebic dysentery, abscesses in liver, lungs and brain and non-dysenteric infections.

1. Amoebic Dysentery:

Entamoeba histolytica secretes a tissue dissolving enzyme (probably of histolysin nature) that destroys the epithelial lining of the colon and causes its necrosis and forms the abscesses (small wounds) which later become flask- shaped bleeding ulcers. The cavity of these ulcers is generally filled with mucus, bacteria, amoeba and cell debris. The abscesses pour their contents into the lumen of the intestine.

The ulcers vary greatly in number and size; in severe cases almost the entire colon is undermined. The ulceration of colon may produce severe dysentery. In amoebic dysentery, the stools are acidic and contain pure blood and mucus, in which swarms of amoeba and blood corpuscles, are usually present. The patient feels discomforted due to the rectal straining and intense gripping pains with the passage of blood and mucus stools every few minutes.

2. Abscesses in Liver, Lungs and Brain:

Sometimes *Entamoeba histolytica* may be drawn into the portal circulation and carried to the liver. In liver the parasites settle, attack the liver tissue and form abscesses. The patient has pain in liver region, fever and high leucocyte number, a condition referred to as amoebic hepatitis.

Lung abscesses are fairly frequent; these are usually caused by direct extension from a liver abscess through the diaphragm. The lung abscesses usually rupture into a bronchial tube and discharge a brown mucoid material which is coughed out with the sputum. Sometimes the parasite also forms abscesses in the brain. Abscesses elsewhere are rare.

3. Non-Dysenteric Infections:

Although amoebiasis is usually thought of as the cause of dysentery with blood and mucus containing stools or of liver abscesses, these conditions are actually the exception rather than rule and some workers have reported that as many as 90% of dysentery cases in temperate climates are apparently symptomless. Even in tropics, dysentery is exceptional.

Although about 10% of the general population is infected with *Entamoeba histolytica*, yet most of them are carriers or passers. The symptoms commonly associated with chronic amoebiasis are abdominal pain, nausea, and bowel irregularity, with headaches, fatigability and nervousness in minority of cases.

Symptoms, Diagnosis and Treatment of Infection Caused by *Entamoeba histolytica*:

As referred, the infection of *Entamoeba histolytica* causes amoebiasis. The common symptoms are the passing out of stool with blood and mucus, abdominal pain, nausea, flatulence and bowel irregularity with headache and fatigability, etc.

Diagnosis of *Entamoeba histolytica*:

The microscopic examination of the stool of an infected man shows the presence of trophozoites and cysts in it. The presence of stone-shaped, white coloured crystals of Charcot- Leyden suggests the infection of *Entamoeba histolytica*.

Treatment (Therapy) of *Entamoeba histolytica*:

For prompt relief of acute or sub-acute dysentery the injections of Emetin are given. But certain antibiotics, such as Fumagillin, Terramycin, Erythromycin and Aureomycin are more effective and may be given orally.

For eradication of intestinal infections or in chronic cases, certain arsenic compounds (Carbarsone, Thiocarbarsone and Vioform) and a number of iodine compounds (Yatren, Diodoquin and Vioform) are effective. For amoebiasis of liver or lungs, Chloroquine is quite effective. The most significant advancement in the treatment of amoebiasis is the use of Metronidazole and Tinidazole as both luminal and tissue amoebicide.

Prevention (Prophylaxis) of Infection Caused by *Entamoeba histolytica*:

Following measures are essential in the prevention of the disease:

1. Sanitary disposal of faecal matter.
2. Perfect sanitation and protection of water and vegetables from pollution.
3. Washing of hands with antiseptic soap and water before touching the food.
4. Cleanliness in preparing the food.

5. Protection of foods and drinks from houseflies, cockroaches, etc.
6. Raw and improperly washed and cooked vegetables should be avoided.

Schistosomiasis

Schistosomiasis, also known as bilharzia, is a disease caused by parasitic worms. Although the worms that cause schistosomiasis are not found in the United States, more than 200 million people are infected worldwide. In terms of impact this disease is second only to malaria as the most devastating parasitic disease. Schistosomiasis is considered one of the Neglected Tropical Diseases (NTDs).

The parasites that cause schistosomiasis live in certain types of freshwater snails. The infectious form of the parasite, known as cercariae, emerge from the snail, hence contaminating water. You can become infected when your skin comes in contact with contaminated freshwater. Most human infections are caused by *Schistosoma mansoni, S. haematobium*, or *S. japonicum*.

Epidemiology and Risk Factors

Schistosomiasis is an important cause of disease in many parts of the world, most commonly in places with poor sanitation. School-age children who live in these areas are often most at risk because they tend to spend time swimming or bathing in water containing infectious cercariae.
If you live in, or travel to, areas where schistosomiasis is found and are exposed to contaminated freshwater, you are at risk.

Areas where human Schistosomiasis is found include:

- ***Schistosoma mansoni***
  - distributed throughout Africa: There is risk of infection in freshwater in southern and sub-Saharan Africa–including the great lakes and rivers as well as smaller bodies of water. Transmission also occurs in the Nile River valley in Sudan and Egypt
  - South America: including Brazil, Suriname, Venezuela
  - Caribbean (risk is low): Dominican Republic, Guadeloupe, Martinique, and Saint Lucia.

- ***S. haematobium***
  - distributed throughout Africa. There is risk of infection in freshwater in southern and sub-Saharan Africa–including the great lakes and rivers as well as smaller bodies of water. Transmission also occurs in the Nile River valley in Egypt and the Mahgreb region of North Africa.
  - found in areas of the Middle East.
- ***S. japonicum***
  - found in Indonesia and parts of China and Southeast Asia.
- ***S. mekongi***
  - found in Cambodia and Laos.
- ***S. intercalatum***
  - found in parts of Central and West Africa.

Biology

Causal Agents:

Schistosomiasis is caused by digenetic blood trematodes. The three main species infecting humans are *Schistosoma haematobium, S. japonicum,* and *S. mansoni.* Two other species, more localized geographically, are *S. mekongi* and *S. intercalatum.* In addition, other species of schistosomes, which parasitize birds and mammals, can cause cercarial dermatitis in humans.

Life Cycle:

Eggs are eliminated with feces or urine. Under optimal conditions the eggs hatch and release miracidia , which swim and penetrate specific snail intermediate hosts . The stages in the snail include 2 generations of sporocysts and the production of cercariae. Upon release from the snail, the infective cercariae swim, penetrate the skin of the human host, and shed their forked tail, becoming schistosomulae. The schistosomulae migrate through several tissues and stages to their residence in the veins. Adult worms in humans reside in the mesenteric venules in various locations, which at times seem to be specific for each species. For instance, *S. japonicum* is more frequently found in the superior mesenteric veins draining the small intestine, and *S. mansoni* occurs more often in the superior mesenteric veins draining the large intestine. However, both species can occupy either location, and they are capable of moving between sites, so it is not possible to state unequivocally that one species only occurs

in one location. *S. haematobium* most often occurs in the venous plexus of bladder, but it can also be found in the rectal venules. The females (size 7 to 20 mm; males slightly smaller) deposit eggs in the small venules of the portal and perivesical systems. The eggs are moved progressively toward the lumen of the intestine (*S. mansoni* and *S. japonicum*) and of the bladder and ureters (*S. haematobium*), and are eliminated with feces or urine, respectively. Pathology of *S. mansoni* and *S. japonicum* schistosomiasis includes: Katayama fever, hepatic perisinusoidal egg granulomas, Symmers' pipe stem periportal fibrosis, portal hypertension, and occasional embolic egg granulomas in brain or spinal cord. Pathology of *S. haematobium* schistosomiasis includes: hematuria, scarring, calcification, squamous cell carcinoma, and occasional embolic egg granulomas in brain or spinal cord. Human contact with water is thus necessary for infection by schistosomes. Various animals, such as dogs, cats, rodents, pigs, hourse and goats, serve as reservoirs for *S. japonicum*, and dogs for *S. mekongi*.

Disease

Infection occurs when skin comes in contact with contaminated freshwater in which certain types of snails that carry the parasite are living. Freshwater becomes contaminated by *Schistosoma* eggs when infected people urinate or defecate in the water. The eggs hatch, and if the appropriate species of snails are present in the water, the parasites infect, develop and multiply inside the snails. The parasite leaves the snail and enters the water where it can survive for about 48 hours. *Schistosoma* parasites can penetrate the skin of persons who come in contact with contaminated freshwater, typically when wading, swimming, bathing, or washing. Over several weeks, the parasites migrate through host tissue and develop into adult worms inside the blood vessels of the body. Once mature, the worms mate and females produce eggs. Some of these eggs travel to the bladder or intestine and are passed into the urine or stool.

Symptoms of schistosomiasis are caused not by the worms themselves but by the body's reaction to the eggs. Eggs shed by the adult worms that do not pass out of the body can become lodged in the intestine or bladder, causing inflammation or scarring. Children who are repeatedly infected can develop anemia, malnutrition, and learning difficulties. After years of infection, the parasite can also damage the liver, intestine, spleen, lungs, and bladder.

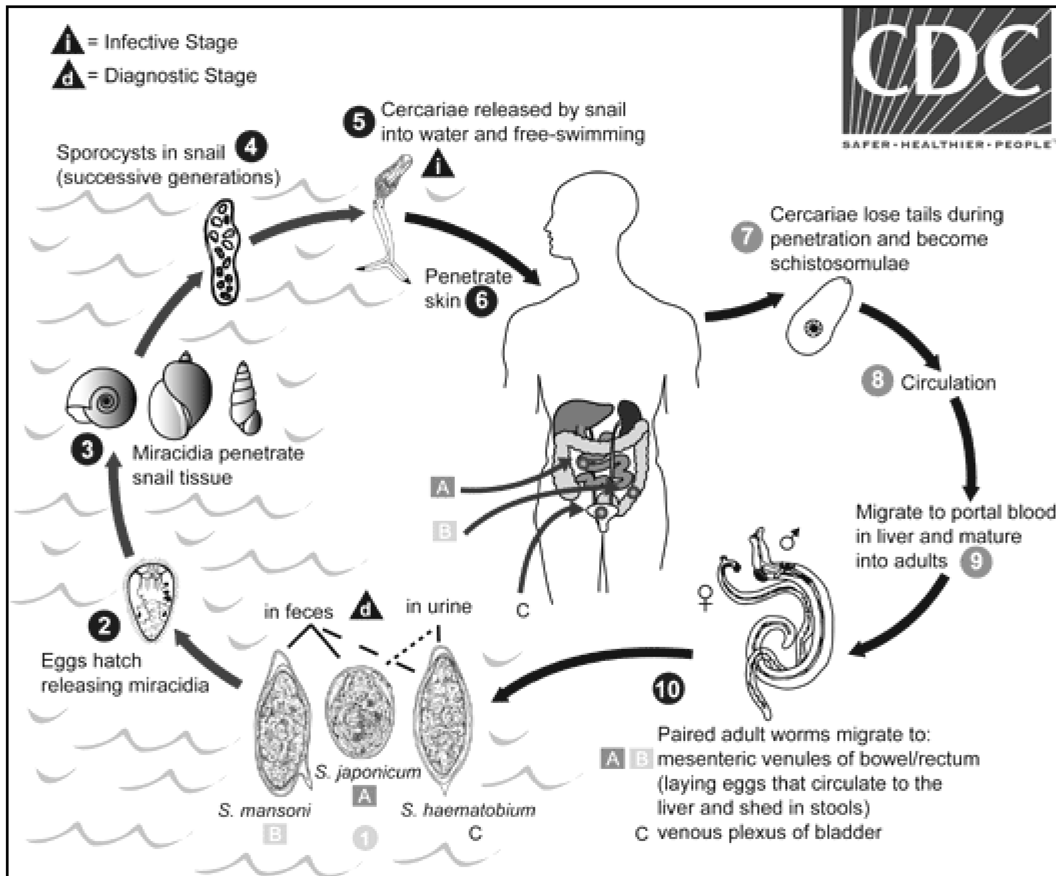


Figure: General life cycle of *Schistosoma* sp.

Common Symptoms

Most people have no symptoms when they are first infected. However, within days after becoming infected, they may develop a rash or itchy skin. Within 1-2 months of infection, symptoms may develop including fever, chills, cough, and muscle aches.

Chronic schistosomiasis

Without treatment, schistosomiasis can persist for years. Signs and symptoms of chronic schistosomiasis include: abdominal pain, enlarged liver, blood in the stool or blood in the urine, and problems passing urine. Chronic infection can also lead to increased risk of bladder cancer.

Rarely, eggs are found in the brain or spinal cord and can cause seizures, paralysis, or spinal cord inflammation.

Diagnosis

Stool or urine samples can be examined microscopically for parasite eggs (stool for *S. mansoni* or *S. japonicum* eggs and urine for *S. haematobium* eggs). The eggs tend to be passed intermittently and in small amounts and may not be detected, so it may be necessary to perform a blood (serologic) test.

Treatment

Safe and effective medication is available for treatment of both urinary and intestinal schistosomiasis. Praziquantel, a prescription medication, is taken for 1-2 days to treat infections caused by all *Schistosoma* species.

Prevention and Control

Prevention

No vaccine is available.

The best way to prevent schistosomiasis is to take the following steps if you are visiting or live in an area where schistosomiasis is transmitted:

- Avoid swimming or wading in freshwater when you are in countries in which schistosomiasis occurs. Swimming in the ocean and in chlorinated swimming pools is safe.
- Drink safe water. Although schistosomiasis is not transmitted by swallowing contaminated water, if your mouth or lips come in contact with water containing the parasites, you could become infected. Because water coming directly from canals, lakes, rivers, streams, or springs may be contaminated with a variety of infectious organisms, you should either bring your water to a rolling boil for 1 minute or filter water before drinking it. Bring your water to a rolling boil for at least 1 minute will kill any harmful parasites, bacteria, or viruses present. Iodine treatment alone will not guarantee that water is safe and free of all parasites.
- Water used for bathing should be brought to a rolling boil for 1 minute to kill any cercariae, and then cooled before bathing to avoid scalding. Water held in a storage tank for at least 1 - 2 days should be safe for bathing.
- Vigorous towel drying after an accidental, very brief water exposure may help to prevent the *Schistosoma* parasite from penetrating the skin. However, do not rely on vigorous towel drying alone to prevent schistosomiasis.

Those who have had contact with potentially contaminated water overseas should see their health care provider after returning from travel to discuss testing.

Control

In countries where schistosomiasis causes significant disease, control efforts usually focus on:

1. reducing the number of infections in people and/or
2. eliminating the snails that are required to maintain the parasite's life cycle.

For all species that cause schistosomiasis, improved sanitation could reduce or eliminate transmission of this disease. In some areas with lower transmission levels, elimination of schistosomiasis is considered a "winnable battle" by public health officials.

Control measures can include mass drug treatment of entire communities and targeted treatment of school-age children. Some of the problems with control of schistosomiasis include:

1. Chemicals used to eliminate snails in freshwater sources may harm other species of animals in the water and, if treatment is not sustained, the snails may return to those sites afterwards.
2. For certain species of the parasite, such as *S. japonicum*, animals such as cows or water buffalo can also be infected. Runoff from pastures (if the cows are infected) can contaminate freshwater sources.

Unit-II

Mode of transmission of *Plasmodium*, *Trypanosoma*, Piroplasm. Microspora: Structure and life history of *Nosema bombycis* - impact on sericulture

Objective:

In this Unit we will discuss on Mode of transmission of *Plasmodium*, *Trypanosoma*, Piroplasm and Microspora: Structure and life history of *Nosema bombycis* - impact on sericulture.

A. Mode of transmission of *Plasmodium*, *Trypanosoma*, Piroplasm

Piroplasmosis:

Piroplasmosis a disease of horses and other equids, is caused by one of two protozoan parasites: *Theileria equi* or *Babesia caballi*, says Glen Scoles, PhD, a research entomologist with the U.S. Department of Agriculture (USDA) Animal Disease Research Unit (ADRU). These organisms can be transmitted by ticks or through contaminated blood from infected horses, whether transmitted iatrogenically or via blood transfusions. Though biologically different, the two parasites share similar pathologies, life cycles and tick vector relationships.
T. equi and *B. caballi* must undergo sexual-stage development in ticks to complete their life cycle, making ticks the definitive hosts and vectors of the disease-causing parasites. Though relatively few species of ticks can support *T. equi* and *B. caballi*, competent tick vectors in the United States include:

- *Amblyomma mixtum* (the Cayenne tick, formerly known as *Amblyomma cajennese*) is probably one of the primary U.S. vectors for *T. equi*.
- *Dermacentor variabilis* (the American dog tick) transmits *T. equi*.
- *Dermacentor nitens* (the tropical horse tick) transmits *B. caballi*.
- *Dermacentor albipictus* (the winter tick) transmits *B. caballi*.

Incubation Period

Incubation period can last anywhere between 1-6 weeks.

Life Cycle

T. equi undergoes four stages of development. First, asexual replication occurs in the equine host's peripheral blood mononuclear cells (PBMCs), followed by asexual replication in the host's erythrocytes. Once a tick obtains erythrocytes infected with *T. equi* during blood feeding, the parasites sexually reproduce in the the tick's midgut, followed by a round of asexual replication in the tick's salivary glands. Sporozoites develop in the tick's salivary glands and are transferred to the horse during the feeding process, thereby infecting it with piroplasmosis.

According to Scoles, infection is transmitted in one of two ways: transtadial (or interstadial) transmission or intrastadial transmission. In the former, larval or nymphal ticks take on infected erythrocytes when they feed on an infected host. The ticks then drop off the equine host, molt, and find and feed on a new host once they reach their next development stage. In intrastadial transmission, an adult male tick feeds on an infected host before moving to another horse to transmit.

The cycle begins when an infected tick sends sporozoites into a mouse while taking a blood meal. The sporozoites then go into red blood cells, where they asexually reproduce by budding. The babesia then differentiate into male and female gametes. The gametes are once again ingested by the tick, where the they join and undergo the sporogonic, producing sporozoites. Vertical transmission occurs in some types of Babesia, but not *B. microti*.

The life cycle of *B. caballi* is similar to that of *T. equi* in many respects, but there are some fundamental differences, Scoles says. For example, *B. caballi* does not replicated in the equine host's PBMCs, and it invades the tick's ovaries rather than its salivary glands. *B. caballi* is therefore transmitted when a female tick that has fed on an infected host lays infected eggs—a process called transovarial transmission. Within the tick embryo, the parasite invades the salivary glands. After the larvae hatch, the parasites develop into sporozoites that are then shed into the saliva during blood feeding to infect naive equines.

Because horses are social animals that tend to cluster together, Scoles says, male ticks, which take blood in smaller amounts than females (females engorge themselves before dropping off to lay eggs), are easily able to move from horse to horse, primarily in search of mates. Once

on another horse, they may transmit the infection while feeding. When an infected tick bites a human for a blood meal, Babesia sporozoites are introduced into the human. Just as in the mouse, sporozoites then go into erythrocytes, where they asexually reproduce by budding. As the parasites multiply within the blood, the disease begins to clinically manifest itself. Once within the human, the parasite cycle cannot continue, and is only transmitted human-to-human by blood transfusions.

Signs

B. caballi may clear their infection in three to five years, such horses are reservoirs of infection during that period.

Diagnostics

The number of parasites present in infected horses is often too low to detect on blood smears, but there are other methods of diagnosis, Scoles says. For example, infected animals develop an immune response that can be detected using a serological assay. However, the presence of an immune response doesn't necessarily mean the horse is currently infected, Scoles says. It could just mean that the animal was infected and has cleared the infection without a change to its serology results.

That's why Scoles also uses polymerase chain reaction (PCR) assay to detect the presence of a certain parasite DNA sequence. According to Scoles, PCR is a method for chemically amplifying that sequence many times so you can get enough to detect. "PCR confirms the presence of parasite DNA," Scoles says, "but not necessarily living parasites. However, if the animal is seropositive *and* positive by PCR, the combined results may confirm that you have an active parasite infection present."

Malaria:

Plasmodium vivax is a protozoal parasite and a human pathogen. This parasite is the most frequent and widely distributed cause of recurring (Benign tertian) malaria, *P. vivax* is one of the five species of malaria parasites that commonly infect humans. Although it is less virulent than *Plasmodium falciparum*, the deadliest of the five human malaria parasites, *P. vivax* malaria infections can lead to severe disease and death, often due to splenomegaly (a pathologically enlarged spleen). *P. vivax* is carried by the female *Anopheles* mosquito, since it is only the female of the species that bites.

Epidemiology

Plasmodium vivax is found mainly in Asia, Latin America, and in some parts of Africa. *P. vivax* is believed to have originated in Asia, but latest studies have shown that wild chimpanzees and gorillas throughout central Africa are endemically infected with parasites that are closely related to human *P. vivax.* These findings indicate that human P. vivax is of African origin. *Plasmodium vivax* accounts for 65% of malaria cases in Asia and South America. Unlike *Plasmodium falciparum*, *Plasmodium vivax* is capable of undergoing sporogonic development in the mosquito at lower temperatures. It has been estimated that 2.5 billion people are at risk of infection with this organism.

Although the Americas contribute 22% of the global area at risk, high endemic areas are generally sparsely populated and the region contributes only 6% to the total population at risk. In Africa, the widespread lack of the Duffy antigen in the population has ensured that stable transmission is constrained to Madagascar and parts of the Horn of Africa. It contributes 3.5% of global population at risk. Central Asia is responsible for 82% of global population at risk with high endemic areas coinciding with dense populations particularly in India and Myanmar. South East Asia has areas of high endemicity in Indonesia and Papua New Guinea and overall contributes 9% of global population at risk.

P. vivax is carried by at least 71 mosquito species. Many vivax vectors live happily in temperate climates—as far north as Finland. Some prefer to bite outdoors or during the daytime, hampering the effectiveness of indoor insecticide and bed nets. Several key vector species have yet to be grown in the lab for closer study, and insecticide resistance is unquantified.

Clinical representation

Pathogenesis results from rupture of infected red blood cells, leading to fever. Infected red blood cells may also stick to each other and to walls of capillaries. Vessels plug up and deprive tissues of oxygen. Infection may also cause the spleen to enlarge.

Unlike *P. falciparum*, *P. vivax* can populate the bloodstream with sexual-stage parasites—the form picked up by mosquitoes on their way to the next victim—even before a patient shows symptoms. Consequently, prompt treatment of symptomatic patients doesn't necessarily help stop an outbreak, as it does with falciparum malaria, in which fevers occur as sexual stages develop. Even when symptoms appear, because they are usually not immediately fatal, the parasite continues to multiply.

The parasite can go dormant in the liver for days to years, causing no symptoms and remaining undetectable in blood tests. They form what are called hypnozoites (the name derives from "sleeping organisms"), a small form that nestles inside an individual liver cell. The hypnozoites allow the parasite to survive in more temperate zones, where mosquitoes bite only part of the year.

Sign and Symptoms

A single infectious bite can trigger six or more relapses a year, leaving sufferers more vulnerable to other diseases. Other infectious diseases, including falciparum malaria, appear to trigger relapses

Uncomplicated malaria

This is diagnosed when symptoms are present, but there are no signs to indicate severe infection or dysfunction of the vital organs. This form can become severe malaria if left untreated, or if the host has poor or no immunity. Symptoms of uncomplicated malaria typically last 6 to 10 hours and recur every second day. Some strains of the parasite can have a longer cycle or cause mixed symptoms. As symptoms resemble those of flu, they may be undiagnosed or misdiagnosed in areas where malaria is less common. In uncomplicated malaria, symptoms progress as follows, through cold, hot, and sweating stages:

- a sensation of cold with shivering
- fever, headaches, and vomiting
- seizures sometimes occur in younger people with the disease
- sweats, followed by a return to normal temperature, with tiredness

In areas where malaria is common, many patients recognize the symptoms as malaria and treat themselves without visiting a doctor.

Severe malaria

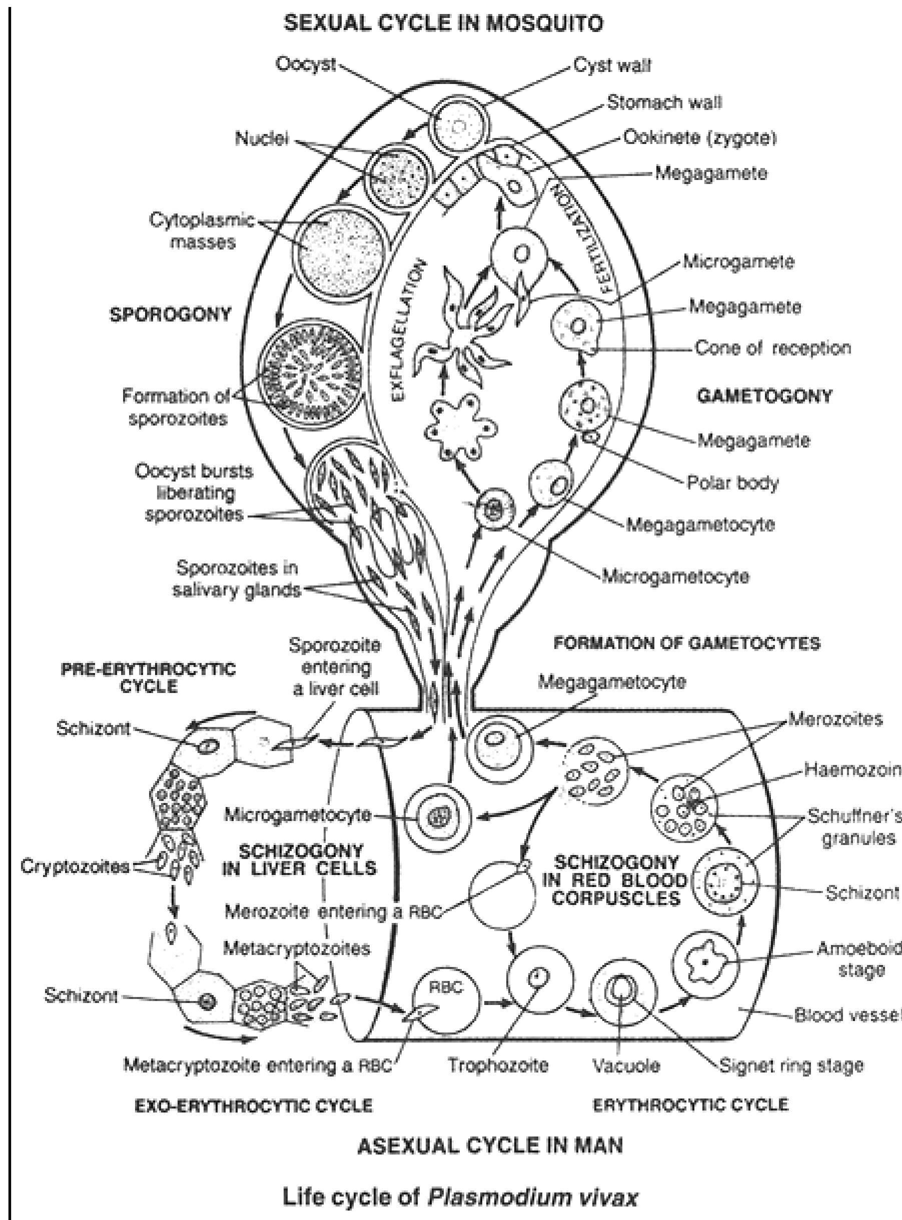
In severe malaria, clinical or laboratory evidence shows signs of vital organ dysfunction.

Symptoms of severe malaria include:

- fever and chills
- impaired consciousness
- prostration, or adopting a prone position
- multiple convulsions

- deep breathing and respiratory distress
- abnormal bleeding and signs of anemia
- clinical jaundice and evidence of vital organ dysfunction

Severe malaria can be fatal without treatment.



Life cycle of *Plasmodium vivax*

Causes

Malaria happens when a bite from the female *Anopheles* mosquito infects the body with Plasmodium. Only the *Anopheles* mosquito can transmit malaria. The successful development of the parasite within the mosquito depends on several factors, the most important being humidity and ambient temperatures. When an infected mosquito bites a human host, the parasite enters the bloodstream and lays dormant within the liver. The host will have no

symptoms for an average of 10.5 days, but the malaria parasite will begin multiplying during this time.

The new malaria parasites are then released back into the bloodstream, where they infect red blood cells and multiply further. Some malaria parasites remain in the liver and are not released until later, resulting in recurrence. An unaffected mosquito becomes infected once it feeds on an infected individual. This restarts the cycle.

Transmission routes

The main mode of transmission of the disease is by bites from infected Anopheles mosquitoes that have previously had a blood meal from an individual with parasitemia. The human infection begins when an infected female anopheles mosquito bites a person and injects infected with **sporozoites** saliva into the **blood circulation**. That is the first life stage of *plasmodium* (**stage of infection**).

The next stage in malaria life cycle is the one of asexual reproduction that is divided into different phases: the pre- erythrocytic (or better, **exoerythrocytic**) and the erythrocytic **phase**. Within only 30- 60 minutes after the parasites inoculation, sporozoites find their way through blood circulation to their first target, the **liver**. The sporozoites enter the liver cells and start dividing leading to **schizonts** creation in 6- 7 days. Each schizont gives birth to thousands of **merozoites** (**exoerythrocytic schizogony**) that are then released into the blood stream marking the end of the exoerythrocytic phase of the asexual reproductive stage. The liver phase occurs only once while the erythrocytic phase undergoes multiple cycles; the merozoites release after each cycle creates the febrile waves. A second scenario into the RBCs is the parasite differentiation into male and female **gametocytes** that is a non pathogenic form of parasite. When a female anopheles mosquito bites an infected person, it takes up these gametocytes with the blood meal (mosquitoes can be infected only if they have a meal during the period that gametocytes circulate in the human's blood). The gametocytes, then, mature and become **microgametes** (male) and **macrogametes** (female) during a process known as gametogenesis. The time needed for the gametocytes to mature differs for each plasmodium species: 3- 4 days for *P. vivax* and *P. ovale*, 6- 8 days for *P. malariae* and 8- 10 days for *P. falciparum*.

In the mosquito gut, the microgamete nucleus divides three times producing eight nuclei; each nucleus fertilizes a macrogamete forming a **zygote**. The zygote, after the fusion of nuclei and the fertilization, becomes the so- called **ookinete**. The ookinete, then, penetrates the midgut wall of the mosquito, where it encysts into a formation called oocyst. Inside the oocyst, the ookinete nucleus divides to produce thousands of **sporozoites** (**sporogony**). That is the end of the third stage (stage of sexual reproduction/ sporogony). Sporogony lasts 8- 15 days**.**

The oocyst ruptures and the sporozoites are released inside the mosquito cavity and find their way to its salivary glands but only few hundreds of sporozoites manage to enter. Thus, when the above mentioned infected mosquito takes a blood meal, it injects its infected saliva into the next victim marking the beginning of a new cycle.

Prevention

The main way to prevent malaria is through vector control. There are mostly three main forms that the vector can be controlled: (1) insecticide-treated mosquito nets, (2) indoor residual spraying and (3) antimalarial drugs. Long-lasting insecticidal nets (LLNs) are the preferred method of control because it is the most cost effective. The WHO is currently strategizing how to ensure that the net is properly maintained to protect people at risk. The second option is indoor residual spraying and has been proven effective if at least 80% of the homes are sprayed. However, such method is only effective for 3-6months. A drawback to these two methods, unfortunately, is that mosquito resistance against these insecticides has risen. National malaria control efforts are undergoing rapid changes to ensure the people are given the most effective method of vector control. Lastly, antimalarial drugs can also be used to prevent infection from developing into a clinical disease. However, there has also been an increase resistance to antimalarial medicine.

In 2015 the World Health Organization (WHO) drew up a plan to address vivax malaria, as part of their Global Technical Strategy for Malaria.

Diagnosis

P. vivax and *P. ovale* that has been sitting in EDTA for more than 30 minutes before the blood film is made will look very similar in appearance to *P. malariae*, which is an important reason to warn the laboratory immediately when the blood sample is drawn so they can process the sample as soon as it arrives. Blood films are preferably made within 30 minutes

of the blood draw and must certainly be made within an hour of the blood being drawn. Diagnosis can be done with the strip fast test of antibodies.

Treatment

Chloroquine remains the treatment of choice for *vivax* malaria,except in Indonesia's Irian Jaya (Western New Guinea) region and the geographically contiguous Papua New Guinea, where chloroquine resistance is common (up to 20% resistance). Chloroquine resistance is an increasing problem in other parts of the world, such as Korea and India.

When chloroquine resistance is common or when chloroquine is contraindicated, then artesunate is the drug of choice, except in the U.S., where it is not approved for use. Where an artemisinin-based combination therapy has been adopted as the first-line treatment for *P. falciparum* malaria, it may also be used for *P. vivax* malaria in combination with primaquine for radical cure. An exception is artesunate plus sulfadoxine-pyrimethamine (AS+SP), which is not effective against *P. vivax* in many places. Mefloquine is a good alternative and in some countries is more readily available. Atovaquone-proguanil is an effective alternative in patients unable to tolerate chloroquine. Quinine may be used to treat *vivax* malaria but is associated with inferior outcomes.

32–100% of patients will relapse following successful treatment of *P. vivax* infection if a radical cure (eradication of liver stages) is not given.

Eradication of the liver stages is achieved by giving primaquine. Patients with glucose-6-phosphate dehydrogenase risk haemolysis. G6PD is an enzyme important for blood chemistry. No field-ready test is available. Recently, this point has taken particular importance for the increased incidence of vivax malaria among travelers. At least a 14-day course of primaquine is required for the radical treatment of *P. Vivax.*

Trypanosomiasis:

Trypanosome is a flagellate pathogenic parasite growing in man and domestic animals causing fatal diseases known as Trypanosomiasis. Trypanosome has clourless, elongated and flattened leaf like body. It is spindle shaped about 10 to 40 long and 1 to 5 broad.

A firm but elastic pellicle, supported by fine microtubules covers the body and maintain its shape. A long thread like flagellum project from the front end of the body. All along its

length over the body, the flagellum is connected by a fin-like undulating membrane formed of cytoplasm and folded pellicle. There is a large oval nucleus and a long oval narrow band like mitochondrion in the cytoplasm. A conspicuous mass of DNA called Kinetoplast is embedded in the mitochondrion near the gullet.

In between the reservoir and the nucleus, Golgi apparatus is found. In trypanosomes, large deeply-staining volutin granules are found scattered in the cytoplasm. These granules represent the stored glycogen or protein or nucleic acids. Endoplasmic reticulum with ribosome's is also present

Mode of Transmission:

The life cycle of most trypanosomes species is digenetic. Man and domestic animals serve as primary host and blood-sucking insect, the tsetse fly serve as the intermediate host. Man and domestic animals becomes infected by the bite of tsetse fly. The injected parasite undergo prepatent period of active multiplication in lymph, intercellular spaces and tissue cells. Finally the parasite invades blood. It undergoes extensive multiplication.

During multiplication it changes shape of its body several times but finally changes into normal trypanosomes. At this stage, it is ready for transmission into the intermediate host. After sometime the pararits disappear completely from the blood due to formation of antibodies in host body. In pathogenic forms, the parasites invade vital organs from the blood causing serious disease.

In invertebrate host also, the parasite undergo extensive multiplication in stomach. Ultimately they migrate into salivary glands. When tsetse fly bites the skin of vertebrate host for its blood- meal, if pours a drop of saliva into the wound to prevent blood coagulation. With the drop of saliva numerous trypanosomes are inoculated into the blood of final host.

During a blood meal on the mammalian host, an infected tsetse fly (genus *Glossina*) injects metacyclic trypomastigotes into skin tissue. The parasites enter the lymphatic system and pass into the bloodstream. Inside the host, they transform into bloodstream trypomastigotes, are carried to other sites throughout the body, reach other blood fluids (e.g., lymph, spinal fluid), and continue the replication by binary fission. The entire life cycle of African Trypanosomes is represented by extracellular stages. The tsetse fly becomes infected with bloodstream trypomastigotes when taking a blood meal on an infected mammalian host. In

the fly's midgut, the parasites transform into procyclic trypomastigotes, multiply by binary fission, leave the midgut, and transform into epimastigotes. The epimastigotes reach the fly's salivary glands and continue multiplication by binary fission. The cycle in the fly takes approximately 3 weeks. Humans are the main reservoir for *Trypanosoma brucei gambiense*, but this species can also be found in animals.

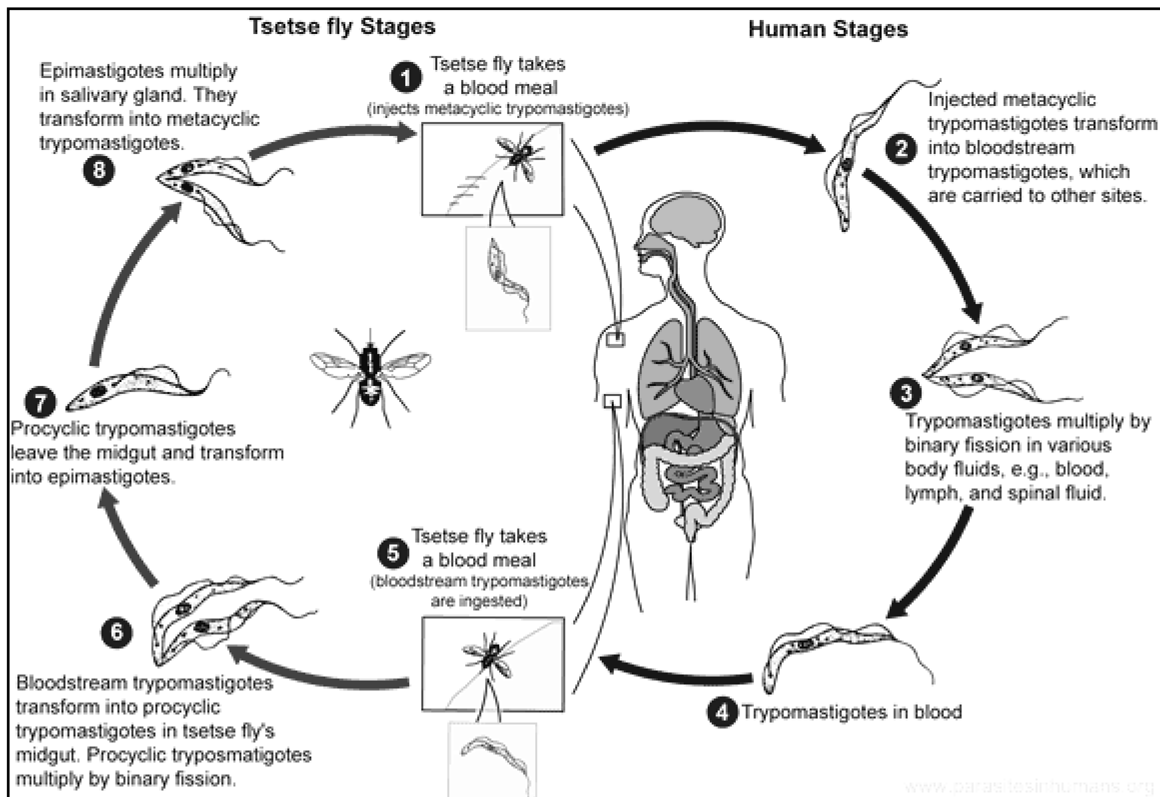


Figure: Life cycle of *Trypanosoma* sp.

B. Microspora: Structure and life history of *Nosema bombycis* - impact on sericulture.

Nosema Bombycis:

Nosema is a genus of microsporidian parasites. The genus, circumscribed by Swiss botanist Karl Wilhelm von Nägeli in 1857, contains 81 species.Most parasitise insects and other arthropods, and the best-known *Nosema* species parasitise honeybees, where they are considered a significant disease by beekeepers, often causing a colony to fail to thrive in the spring as they come out of their overwintering period. Eight species parasitize digeneans, a group of parasitic flatworms, and thus are hyperparasites, i.e., parasites of a parasite.

Pébrine, or "pepper disease," is a disease of silkworms, which is caused by protozoan microsporidian parasites, mainly *Nosema bombycis* and, to a lesser extent, *Vairimorpha*, *Pleistophora* and *Thelohania* species. The parasites infect eggs and are therefore transmitted to next generation.

The silkworm larvae infected by pébrine are usually covered in brown dots and are unable to spin silkworm thread. Louis Pasteur was the first one to recognize the cause of this disease when a plague of the disease spread across France.

Nosema bombycis is a microsporidium that kills all of the silkworms hatched from infected eggs and comes from the food that silkworms eat. If silkworms acquire this microsporidium in their larval stage, there are no visible symptoms; however, mother moths will pass the microsporidium onto the eggs, and all of the worms hatching from the infected eggs will die in their larva stage. Therefore, it is extremely important to rule out all eggs from infected moths by checking the moth's body fluid under a microscope.

Life cycle stages

Nosema bombycis, like other microsporidians, has two major life cycle stages, a spore stage and a vegetative stage. In most cases, the spore is ingested by the host, infecting host cells in the gut lumen and the Malpighian tubules. During reproduction and proliferation the parasite spreads within the host. Fresh spores are then released into the environment via feces or a decaying host.

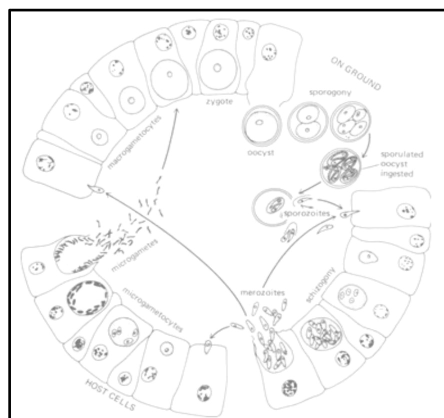


Figure: Life cycle of *Nosema bombysis*

Transmission

The parasite is believed to rely mainly on horizontal transmission between colonies via infected workers contaminating shared food sources such as pollen or nectar, but there is some evidence that it may also be transmitted vertically.Males may transmit the infection to new queens during mating. *N. bombycis* infection prevalence has been reported to vary widely over time. For example, Manlik et al. (2017) reported that *N. bombycis* infection prevalence in buff-tailed bumblebees (*Bombus terrestris*) fluctuated between 2% (2010) and 81% (2003) in Neunforn, Switzerland.

---

1. The infective form of microsporidia is the resistant spore and it can survive for an extended period of time in the environment.
2. The spore extrudes its polar tubule and infects the host cell.
3. The spore injects the infective sporoplasm into the eukaryotic host cell through the polar tubule.
4. Inside the cell, the sporoplasm undergoes extensive multiplication either by merogony (binary fission) or schizogony (multiple fission).
5. This development can occur either in direct contact with the host cell cytoplasm (*E. bieneusi*) or inside a vacuole called a parasitophorous vacuole (*E. intestinalis*). Either free in the cytoplasm or inside a parasitophorous vacuole, microsporidia develop by sporogony to mature spores.
6. During sporogony, a thick wall is formed around the spore, which provides resistance to adverse environmental conditions. When the spores increase in number and completely fill the host cell cytoplasm, the cell membrane is disrupted and releases the spores to the surroundings.
7. These free mature spores can infect new cells thus continuing the cycle

Effects on host

Studies have found conflicting results as to the effects of *N. bombycis* on bumble bee health. Some field studies have found little to no negative effects on colony size or success, while others have found that infected queens produce smaller colony sizes and reduced sexual offspring.Under laboratory conditions, the fungus has been found to affect the survival and efficiency of adult individuals as well as the sperm counts of male offspring.

Impact on Silk industry

The commercial rearing of polyphagous Indian tasar silkworm*, Antheraea mylitta* Drury being practiced on naturally grown primary food plants like *Terminalia arjuna* (Arjun) *Terminalia tomentosa* (Asan), and *Shorea robusta* (Sal) available in the tropical forests of central India , at times, is seriously affected by the disease- Pebrine, caused by *Nosema* sp., a microsporidian pathogen. The present investigation on comparative larval, silk gland weight and also cocoon parameters in Pebrine-free and Pebrine- infected ecorace of tasar silkworm Antheraea mylitta Drury (Daba TV), illustrates the tasar silkworm larvae infected with pebrine disease causing heavy losses to the economy of the silk industry. It plays a major role in improving socio-economic status of tribal, weaker sections, landless people and women.

The tasar food plant leaf quality in terms of nutrition can influence the health and growth of larvae, effective rate of rearing (ERR) and crop yields as it has correlation with the weights of cocoon, shell and silk ratio and can influence the crop economics. The leaf nutrient status of tasar food plant is fundamental not only for silk productivity, but also for its metamorphosis during life cycle and subsequent parental moth reproductive efficiency. The larval feeding status of any polyphagous commercial insect has impact on food storage and budgeting for biological activities so as to combat the adverse or to excel during favourable conditions. The diseases in silkworm are the major constraintsin tasar culture, which adversely affect the economics of this culture by causing 35-40% crop loss. Among the diseases, pebrine is causing most devastating effect on the rearing of the tasar silkworm accounts for 20-25% yield loss Even though some work has been done on the breeding aspects of Tasar silkworm, not much work has been published so far on pathological aspects of tasar silkworm.

Tasar silkworm is often infected with the intracellular parasite of the genus *Nosema*. Pebrine can be acquired from the mother moth (primary infection) or from the environment through food (secondary infection). Infected larvae show black pepper like spots on the integument. These infected hypodermal cells become enlarged and vacuolated and blackened due to the formation of melanin. Larvae infected with *Nosema* sp. show extended development period, reduced size and larval weight in comparison to uninfected one.

Unit-III

Life cycle, biology, pathogenesis, epidemiology and control of important human and veterinary helminthes - *Diphyllobothrium latum, Paragonimus westermani, Trichinella spiralis*

Objectives:

In this Unit we will discuss on Life cycle, biology, pathogenesis, epidemiology and control of important human and veterinary helminthes - *Diphyllobothrium latum, Paragonimus westermani, Trichinella spiralis.*

Diphyllobothrium latum

Diphyllobothrium is a genus of tapeworms which can cause diphyllobothriasis in humans through consumption of raw or undercooked fish. The principal species causing diphyllobothriasis is *Diphyllobothrium latum*, known as the **broad** or **fish tapeworm**, or **broad fish tapeworm**. *D. latum* is a pseudophyllid cestode that infects fish and mammals. *D. latum* is native to Scandinavia, western Russia, and the Baltics, though it is now also present in North America, especially the Pacific Northwest.

Causal Agent

The cestode *Diphyllobothrium latum* (the fish or broad tapeworm), the largest human tapeworm. Several other *Diphyllobothrium* species have been reported to infect humans, but less frequently; they include *D. pacificum*, *D. cordatum*, *D. ursi*, *D. dendriticum*, *D. lanceolatum*, *D. dalliae*, and *D. yonagoensis*.

Morphology of *Diphyllobothrium Latum* (Fish Tapeworm):

The adult worm is ivory or yellowish grey in colour, measuring 3-10 metres in length. The head (scolex) is small, spatulated or spoon shaped, has a pair of slit grooves (bothria) ventrally and dorsally and has no rostellum (a beaklike projection on the head) and no hooklets. Scolex is followed by "**neck**" and 3,000 segments

Signs and symptoms

Most persons with diphyllobothriasis are asymptomatic. In symptomatic persons, the following are the most common symptoms:

- Abdominal pain

- Indigestion or dyspepsia
- Passage of proglottids

Other, less common, symptoms include the following:

- Fatigue
- Diarrhea
- Dizziness
- Weakness (rare)
- Numbness of extremities
- Sensation of hunger
- Pruritus ani

Most patients with diphyllobothriasis have no signs of illness . Rare physical findings that may be noted include the following:

- Pallor
- Glossitis
- Dyspnea
- Tachycardia
- Weakness
- Hypoesthesia
- Paresthesias
- Disturbances of movement and coordination, loss of vibratory sense and proprioception

In patients who present with obstruction, the following physical findings may be noted:

- Abdominal tenderness
- Abdominal distention
- Peritoneal signs

Life Cycle:

Adult tapeworms may infect humans, canids, felines, bears, pinnipeds, and mustelids, though the accuracy of the records for some of the nonhuman species is disputed. Immature eggs are passed in feces of the mammal host (the definitive host, where the worms reproduce). After ingestion by a suitable freshwater crustacean such as a copepod (the first intermediate host), the coracidia develop into procercoid larvae. Following ingestion of the copepod by a suitable second intermediate host, typically a minnow or other small freshwater fish, the procercoid larvae are released from the crustacean and migrate into the fish's flesh where they develop

into a plerocercoid larvae (sparganum). The plerocercoid larvae are the infective stage for the definitive host (including humans).

Because humans do not generally eat undercooked minnows and similar small freshwater fish, these do not represent an important source of infection. Nevertheless, these small second intermediate hosts can be eaten by larger predator species, for example trout, perch, walleye, and pike. In this case, the sparganum can migrate to the musculature of the larger predator fish and mammals can acquire the disease by eating these later intermediate infected host fish raw or undercooked. After ingestion of the infected fish, the plerocercoids develop into immature adults and then into mature adult tapeworms which will reside in the small intestine. The adults attach to the intestinal mucosa by means of the two bilateral grooves (bothria) of their scolex. The adults can reach more than 10 m (up to 30 ft) in length in some species such as *D. latum,* with more than 3,000 proglottids. One or several of the tape-like proglottid segments (hence the name tape-worm) regularly detach from the main body of the worm and release immature eggs in fresh water to start the cycle over again. Immature eggs are discharged from the proglottids (up to 1,000,000 eggs per day per worm) and are passed in the feces. The incubation period in humans, after which eggs begin to appear in the feces is typically 4–6 weeks, but can vary from as short as 2 weeks to as long as 2 years. The tapeworm can live up to 20 years

Immature eggs are passed in feces. Under appropriate conditions, the eggs mature (approximately 18 to 20 days) and yield oncospheres which develop into a coracidia . After ingestion by a suitable freshwater crustacean (the copepod first intermediate host) the coracidia develop into procercoid larvae. Following ingestion of the copepod by a suitable second intermediate host, typically minnows and other small freshwater fish, the procercoid larvae are released from the crustacean and migrate into the fish flesh where they develop into a plerocercoid larvae (sparganum). The plerocercoid larvae are the infective stage for humans. Because humans do not generally eat undercooked minnows and similar small freshwater fish, these do not represent an important source of infection. Nevertheless, these small second intermediate hosts can be eaten by larger predator species, e.g., trout, perch, walleyed pike . In this case, the sparganum can migrate to the musculature of the larger predator fish and humans can acquire the disease by eating these later intermediate infected host fish raw or undercooked. After ingestion of the infected fish, the plerocercoid develop into immature adults and then into mature adult tapeworms which will reside in the small intestine. The adults of *D. latum* attach to the intestinal mucosa by means of the two bilateral

groves (bothria) of their scolex. The adults can reach more than 10 m in length, with more than 3,000 proglottids. Immature eggs are discharged from the proglottids (up to 1,000,000 eggs per day per worm) and are passed in the feces. Eggs appear in the feces 5 to 6 weeks after infection. In addition to humans, many other mammals can also serve as definitive hosts for *D. latum*.

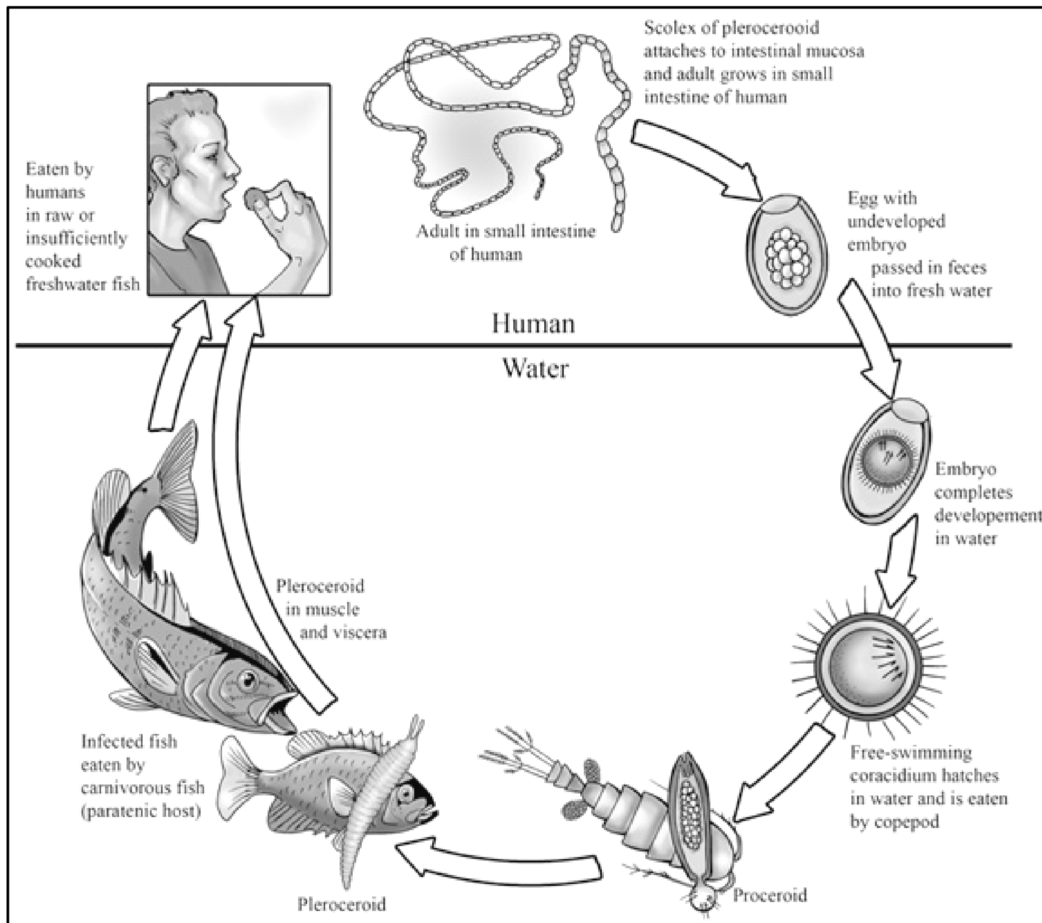


Figure: Life Cycle of *Diphyllobothrium Latum*

Geographic Distribution

Diphyllobothriasis occurs in the Northern Hemisphere (Europe, North America, and Asia) and in South America (Uruguay and Chile). Freshwater fish infected with *Diphyllobothrium* sp. larva may be transported to and consumed in geographic areas where active transmission does not occur, resulting in human diphyllobothriasis. For example, cases of *D. latum* infection associated with consumption of imported fish have been reported in Brazil.

Diagnosis

Diagnosis is usually made by identifying proglottid segments, or characteristic eggs in the feces. These simple diagnostic techniques are able to identify the nature of the infection to the genus level, which is usually sufficient in a clinical setting. However, when the species needs to be determined (in epidemiological studies, for example), restriction fragment length polymorphisms can be effectively used. PCRcan be performed on samples of purified eggs, or native fecal samples following sonication of the eggs to release their contents. Another interesting potential diagnostic tool and treatment is the contrast medium, Gastrografin, introduced into the duodenum, which allows both visualization of the parasite, and has also been shown to cause detachment and passing of the whole worm.

Clinical Features

Diphyllobothriasis can be a long-lasting infection (decades). Most infections are asymptomatic. Manifestations may include abdominal discomfort, diarrhea, vomiting, and weight loss. Vitamin B12 deficiency with pernicious anemia may occur. Massive infections may result in intestinal obstruction. Migration of proglottids can cause cholecystitis or cholangitis. The presence of adult worm in the intestinal tract causes no symptom, but sometimes, non-specific abdominal symptoms have been ascribed. If the worms attach themselves to the jejunum, clinical vitamin B_{12} deficiency develops. In the laboratory, mircoscopical examination of the faeces will reveal the eggs; sometimes proglottids can be observed in the stool.

Pathogenesis

Infection with *Diphyllobothrium latum* is often asymptomatic and long-lasting (decades). Infected persons may experience abdominal pain, vomiting, diarrhoea, and weight loss. In some infections there may be a severe Vitamin B_{12} deficiency and anaemia caused by obstruction of Vitamin B_{12} absorption coupled with high absorption rates by the tapeworm.

Treatment

Quinacrine hydrochloride, niclosamide and paromomycin are found effective. Pernicious anaemia can be treated with folic acid.

Paragonimus westermani

Paragonimus westermani is the major species of lung fluke that infects humans, causing paragonimiasis. The species sometimes is called the **Japanese lung fluke** or **oriental lung fluke**. Human infections are most common in eastern Asia and in South America. *Paragonimus westermani* was discovered when two Bengal tigers died of paragonimiasis in zoos in Europe in 1878. Several years later, infections in humans were recognised in Formosa

In size, shape, and color, *Paragonimus westermani* resembles a coffee bean when alive. Adult worms are 7.5 mm to 12 mm long and 4 mm to 6 mm wide. The thickness ranges from 3.5 mm to 5 mm. The skin of the worm (tegument) is thickly covered with scalelike spines. The oral and ventral suckers are similar in size, with the latter placed slightly pre-equatorially. The excretory bladder extends from the posterior end to the pharynx. The lobed testes are adjacent from each other located at the posterior end, and the lobed ovaries are off-centered near the center of the worm (slightly postacetabular). The uterus is located in a tight coil to the right of the acetabulum, which is connected to the vas deferens. The vitelline glands, which produce the yolk for the eggs, are widespread in the lateral field from the pharynx to the posterior end. Inspection of the tegumental spines and shape of the metacercariae may distinguish between the 30-odd species of *Paragonimus spp.* but the distinction is sufficiently difficult to justify suspicion that many of the described species are synonyms.

- **Eggs:** *Paragonimus westermani* eggs range from 80 to 120 µm long by 45 to 70 µm wide. They are yellow-brown, ovoid or elongate, with a thick shell, and often asymmetrical with one end slightly flattened. At the large end, the operculum is clearly visible. The opposite (abopercular) end is thickened. The eggs are unembryonated when passed in sputum or feces.
- **Cercaria**: Cercariae are often indistinguishable between species. There is a large posterior sucker, and the exterior is spined.
- **Metacercaria:** Metacercariae are usually encysted in tissue. The exterior is spined and has two suckers
- **Adults:** Adult flukes are typically reddish brown and ovoid, measuring 7 to 16 mm by 4 to 8 mm, similar in size and appearance to a coffee bean.They are hermaphroditic, with a

lobed ovary located anterior to two branching testes. Like all members of the Trematoda, they possess oral and ventral suckers.

Causal Agent

More than 30 species of trematodes (flukes) of the genus *Paragonimus* have been reported which infect animals and humans. Among the more than 10 species reported to infect humans, the most common is *P. westermani*, the oriental lung fluke.

Epidemiology

Paragonimus spp. is a common parasite of crustacean-eating mammals such as tigers, leopards, domestic cats, dogs, mongooses, opossums and monkeys (reservoir final hosts). The adult flukes live in the lungs and lay eggs that are coughed up through the airways and either expectorated in the sputum or swallowed and defecated. When they reach freshwater, the eggs develop into miracidia that penetrate various species of aquatic snails, where they further develop and reproduce asexually, giving rise to cercariae (larvae).

Cercariae released into water swim to penetrate suitable species of freshwater crabs, crayfish and other crustaceans and encyst the gills, liver and muscles as metacercariae. When such animals are eaten, the metacercariae hatch in the intestine: young worms penetrate the intestinal wall and the peritoneum, then the diaphragm and the pleura; they finally reach the lungs, where they live in pairs surrounded by a capsula, thus completing the cycle.

Life Cycle

Paragonimus has a quite complex life-cycle that involves two intermediate hosts as well as humans. Eggs first develop in water after being expelled by coughing (unembryonated) or being passed in human feces. In the external environment, the eggs become embryonated.

The eggs are excreted unembryonated in the sputum, or alternately they are swallowed and passed with stool . In the external environment, the eggs become embryonated , and miracidia hatch and seek the first intermediate host, a snail, and penetrate its soft tissues . Miracidia go through several developmental stages inside the snail: sporocysts, rediae, with the latter giving rise to many cercariae, which emerge from the snail. The cercariae invade the second intermediate host, a crustacean such as a crab or crayfish, where they encyst and become metacercariae. This is the infective stage for the mammalian host. Human infection with *P. westermani* occurs by eating inadequately cooked or pickled crab or crayfish that harbor metacercariae of the parasite. The metacercariae excyst in the duodenum, penetrate through

the intestinal wall into the peritoneal cavity, then through the abdominal wall and diaphragm into the lungs, where they become encapsulated and develop into adults (7.5 to 12 mm by 4 to 6 mm). The worms can also reach other organs and tissues, such as the brain and striated muscles, respectively. However, when this takes place completion of the life cycles is not achieved, because the eggs laid cannot exit these sites. Time from infection to oviposition is 65 to 90 days.

In the next stage, the parasite miracidia hatch and invades the first intermediate host such as a species of freshwater snail. Miracidia penetrate its soft tissues and go through several developmental stages inside the snail but mature into cercariae in 3 to 5 months. Cercariae next invade the second intermediate host such as crabs or crayfish and encyst to develop into metacercariae within 2 months. Infection of humans or other mammals (definitive hosts) occurs via consumption of raw or undercooked crustaceans. Human infection with *P. westermani* occurs by eating inadequately cooked or pickled crab or crayfish that harbor metacercariae of the parasite. The metacercariae excyst in the duodenum, penetrate through the intestinal wall into the peritoneal cavity, then through the abdominal wall and diaphragm into the lungs, where they become encapsulated and develop into adults. The worms can also reach other organs and tissues, such as the brain and striated muscles, respectively. However, when this takes place completion of the life cycles is not achieved, because the eggs laid cannot exit these sites.

Infections may persist for 20 years in humans. Animals such as pigs, dogs, and a variety of feline species can also harbor *P. westermani*.

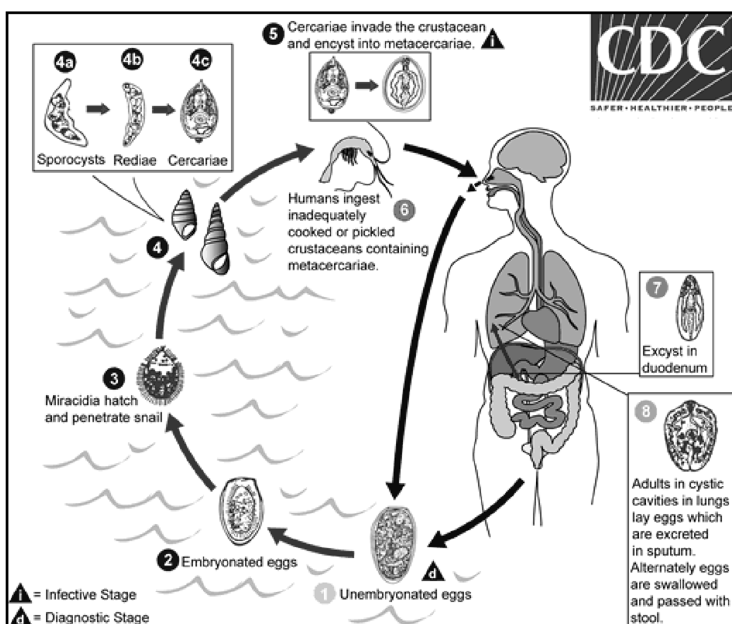


Fig: Life cycle of *Paragonimus westermani*

Disease

Humans may substitute reservoir hosts in the transmission cycle when they eat raw, salted, pickled, smoked, marinated, dried, partially cooked or poorly processed crustaceans, thus ingesting the metacercariae. In humans, the earliest stages of paragonimiasis may present an elusive clinical picture, and be asymptomatic or scarcely symptomatic. Conversely, when worms reach the lungs, symptoms may be significant and typically include chronic cough with blood-stained sputum; chest pain with dyspnoea and fever; pleural effusion and pneumothorax are possible complications.

Symptoms and signs mimic those of tuberculosis, and paragonimiasis should always be suspected in patients with tuberculosis who are non-responsive to treatment. Ectopic paragonimiasis may result from erratic migration of the juvenile worms: the most frequent locations include the abdominal cavity and subcutaneous tissues and, most frequently, the brain: cerebral paragonimiasis is a severe condition that may be associated with headache, visual impairment and epileptic seizures.

Pathology

Once in the lung or ectopic site, the worm stimulates an inflammatory response that allows it to cover itself in granulation tissue forming a capsule. These capsules can ulcerate and heal over time. The eggs in the surrounding tissue become pseudotubercles. If the worm becomes disseminated and gets into the spinal cord, it can cause paralysis; capsules in the heart can cause death. The symptoms are localized in the pulmonary system, which include a bad cough, bronchitis, and blood in sputum (hemoptysis).

Diagnosis, treatment and control

Diagnosis of paragonimiasis is suspected on the basis of the clinical picture, on the anamnestic recall of consuming raw crustaceans, on the detection of eosinophilia, and on typical findings of ultrasound, X-ray, computed tomography or magnetic resonance imaging scans. Tests to rule out tuberculosis should always be conducted. Confirmation of diagnosis relies on different types of diagnostic techniques:

- **parasitological techniques** to detect *Paragonimus* eggs in sputum or stool samples.; the cost and sensitivity of these techniques may vary according to the type of technique used; they can only be employed once worms have reached the lungs and started laying eggs;

some quantify the intensity of infection (allowing an estimation of the severity of the infection);

- **immunological techniques** to detect worm-specific antibodies in serum samples or worm-specific antigens in serum or stool samples; these techniques are usually more sensitive than the commonly used parasitological techniques; detection of antibodies does not distinguish between current, recent and past infections; their ability to quantify intensity of infection is disputed; these techniques are still at an experimental stage;
- **molecular techniques** such as the polymerase chain reaction are also still at an experimental stage.

Triclabendazole, 20 mg/kg, in two divided doses of 10 mg/kg, to be administered on the same day, and praziquantel 25 mg/kg of body weight, 3 times a day for 3 days, are both WHO-recommended medicines for treatment of paragonimiasis. The former is preferred for the simplicity of its regimen, which ensures higher compliances to treatment.

The most basic public health measure that should be implemented is making triclabendazole or praziquantel available at peripheral health centres in all endemic areas for clinical management of confirmed cases.

In areas where cases appear to be clustered, treatment should be also offered to people with suspected paragonimiasis. Suspected cases are defined as individuals coming from an endemic district with a history of consuming raw crustaceans who present with any of the following characteristics:

- cough lasting for more than 3 weeks;
- bloody or rusty sputum;
- clinically or radiologically diagnosed tuberculosis with a negative sputum smear (smear-negative tuberculosis);
- poor or no response to tuberculosis treatment.

In communities and villages where cases of paragonimiasis appear to be significantly clustered, mass drug administration with triclabendazole should also be considered. The recommended regimen is 20 mg/kg of body weight in a single administration.

Complementary interventions such as information, education and communication on safe food practices, improved sanitation and veterinary public health measures should also be implemented in order to decrease rates of transmission.

Trichinella spiralis

Trichinella spiralis is an ovoviviparous nematode parasite, occurring in rodents, pigs, horses, bears, and humans, and is responsible for the disease trichinosis. It is sometimes referred to as the "pork worm" due to it being typically encountered in undercooked pork products. It should not be confused with the distantly related pork tapeworm.

Description

Trichinella species, the smallest nematode parasite of humans, have an unusual lifecycle, and are one of the most widespread and clinically important parasites in the world. The small adult worms mature in the small intestine of a definitive host, such as a pig. Each adult female produces batches of live larvae, which bore through the intestinal wall, enter the blood (to feed on it) and lymphatic system, and are carried to striated muscle. Once in the muscle, they encyst, or become enclosed in a capsule. Humans can become infected by eating infected pork, horsemeat, or wild carnivores such as fox, cat, or bear.

Morphology

Males of *T. spiralis* measure between 1.4 and 1.6 mm long, and are more flat anteriorly than posteriorly. The anus can be found in the terminal end, and they have a large copulatory pseudobursa on each side. The females of *T. spiralis* are about twice the size of the males, and have an anus found terminally. The vulva is located near the esophagus. The single uterus of the female is filled with developing eggs in the posterior portion, while the anterior portion contains the fully developed juveniles.

Life Cycle

Trichinella spiralis can live the majority of its adult life in the intestines of humans. To begin its life cycle, *Trichinella spiralis* adults will invade the intestinal wall of a pig, and produce larvae that invade the pig's muscles. The larval forms are encapsulated as a small cystic structure within a muscle cell of the infected host. When another animal (perhaps a human) eats the infected meat, the larvae are released from the nurse cells in the meat (due to stomach pH), and migrate to the intestine, where they burrow into the intestinal mucosa, mature, and reproduce. Juveniles within nurse cells have an anaerobic or facultative anaerobic metabolism, but when they become activated, they adopt the aerobic metabolism characteristics of the adult.

Female *Trichinella* worms live for about six weeks, and in that time can produce up to 1,500 larvae; when a spent female dies, she passes out of the host. The larvae gain access to the

circulation and migrate around the body of the host, in search of a muscle cell in which to encyst. The migration and encystment of larvae can cause fever and pain, brought on by the host inflammatory response. In some cases, accidental migration to specific organ tissues can cause myocarditis and encephalitis that can result in death.

Trichinellosis is acquired by ingesting meat containing cysts (encysted larvae) of *Trichinella*. After exposure to gastric acid and pepsin, the larvae are released from the cysts and invade the small bowel mucosa where they develop into adult worms (female 2.2 mm in length, males 1.2 mm; life span in the small bowel: 4 weeks). After 1 week, the females release larvae that migrate to the striated muscles where they encyst. *Trichinella pseudospiralis*, however, does not encyst. Encystment is completed in 4 to 5 weeks and the encysted larvae may remain viable for several years. Ingestion of the encysted larvae perpetuates the cycle. Rats and rodents are primarily responsible for maintaining the endemicity of this infection. Carnivorous/omnivorous animals, such as pigs or bears, feed on infected rodents or meat from other animals. Different animal hosts are implicated in the life cycle of the different species of *Trichinella*. Humans are accidentally infected when eating improperly processed meat of these carnivorous animals (or eating food contaminated with such meat).

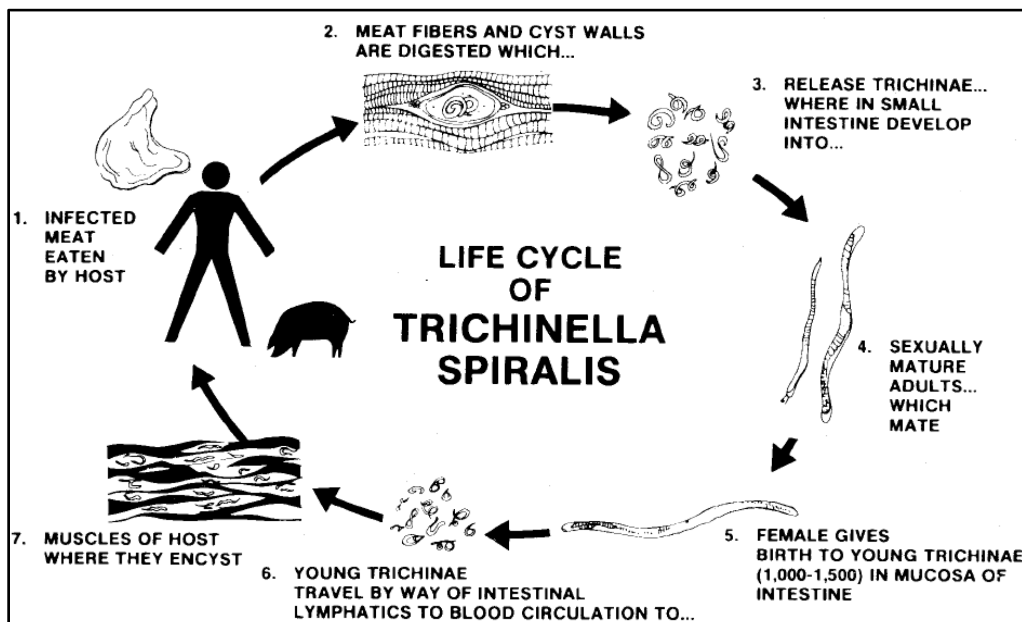


Figure : Life cycle of *Trichinella spiralis*

Infectious dose, incubation, and colonization

The infectious dose of trichinosis is still unknown, however predictions estimate consuming between 100 and 300 of live cysts will result in the disease. A severe infection may result

from the ingestion of 1,000 larvae, however these estimates have also not been scientifically proven.

Development and colonization of *T. spiralis* occurs entirely within the host during two larval stages and one adult stage . When a host ingests the pathogen, the larvae will spend between one to seven days developing into an adult in the gastrointestinal tract. Gastric symptoms of the disease will occur during this time frame. Incubation period of larvae in the host tissue may range from one week to eight weeks, however encapsulated larvae may persist in the host for months or even years. Incubation periods vary depending on the severity and initial infectious dose. Initial colonization begins as the host ingests cysts containing larvae. During digestion, acidic compounds such as hydrochloric acid dissolve the cystic shell. The liberated larvae will pass through the stomach and invade and occupy epithelial tissues of the small intestine. Once established, the larvae will undergo four molting events to become an adult worm and mate. The female will potentially produce between 500-1,500 larvae before being expelled from the body in the stool . The larvae will then move across the intestinal wall and migrate to the rest host by lymphatic blood vessels, specifically the striated muscles. Exact mechanisms of this transfer across the intestine are unknown. Once *T. spiralis* larvae invade individual muscle cells, they adjust cellular function to accommodate their nutrient requirements thereby turning the host cell into a nurse cell. These mechanisms are still unknown today. The nurse cell will stop its lifecycle as the worm stimulates new blood vessel formation around the host cell for nutrients. *T. spiralis* larvae will also encapsulate itself during development . This incubation period generally lasts 15 days, however this is also based on severity of the infection. If left untreated, these larvae will remain in nurse cells for as long as 40 years.

Epidemiology

Outbreaks of trichinosis occur worldwide, however infections are is more common in Europe, Asia, and Southeast Asia. This disease is now endemic in Japan and China. Outbreaks are generally linked to cultural dietary preferences. The number of cases globally is estimated to be approximately 10,000, however these statistics are possibly higher due to unreported cases in countries without proper identification techniques. The mortality rate for trichinosis is approximately 0.2%. Rates of infection have dramatically decreased in the United States over the past decades. In the late 1940s, the CDC reported 400 cases of Trichinosis yearly. Based on statistics from 2010, the number of cases yearly has dropped to 20. This change can be

attributed to increased awareness of the pathogen as well as strict regulations enacted about meat preparation as well the adoption of safer pig raising methods. Currently, most reported cases of trichinosis in the United States stem from personal preparation of wild game instead of commercial production error. Outbreaks have recently occurred in Europe within the last 20 years. In 2003, Poland experienced an outbreak that caused 124 people to be hospitalized. Romania experienced a similar outbreak in 2008 that caused 108 people to be hospitalized. These outbreaks were all linked to contaminated and undercooked meat

Clinical Features

Severity and signs of symptoms vary on the number of larvae ingested by host. If the infection is minor based on the low amount of larvae ingested, *T. spiralis* may not be able to colonize and the immune system can free the infection. Symptoms may never appear or slowly intensify as larvae move to the muscles. If infectious dose is large enough, within the first week following infection gastrointestinal problems may arise including diarrhea, vomiting, cramps, or abdominal pain. As larvae migrate through the lymphatic system during the second week of infection, symptoms may include muscle pain, fever, swelling of the face or eyes, weakness, constipation or diarrhea, and splinter hemorrhages under the fingernail. These symptoms may persist for up to eight weeks with no medical attention, however larvae may survive in cells for up to 40 years. Many patients do not seek treatment due to symptoms being similar to the flu. Severe trichinosis may also become extremely debilitating. Loss of motor functions including walking, swallowing, and breathing can result from this agonizing pain . In the case of severe trichinosis, complications may arise because migrating larvae have access to the entire body through the blood stream. *T. spiralis* larvae stimulate inflammation at major organ sites including the brain, lungs, and the heart. This may result in life threatening conditions such as myocarditis, encephalitis, meningitis, nephritis, pneumonia, or bronchopneumonia.

Diagnosis

Diagnosis of the parasite during the first week of infection is challenging due to the similar secretion of enzymes, including creatine kinase and lactate dehydrogenase, which also elevate during other infections. Levels of eosinophil cells also elevate, however this is also nonspecific to trichinosis and could indicate other parasitic infections or even allergies.

Detection of antibodies developed to this parasite through tests, such as indirect immunofluorescence and latex agglutination, are the least invasive tests available. A muscle biopsy, however, is the most effective testing in diagnosing trichinosis. The deltoid muscle is most commonly used to test for *T.spiralis* larvae formation. The biopsy can also indicate the severity and stage of the disease. Complications with this test include an incubation period between 17 to 24 days while the larvae develop, as well as a false negative test if infection rates are too low.

Treatment

Depending on the severity of the infection, trichinosis may not require medical intervention. Moderate to severe infections require medication. Within the first week of infection, the main goal of medical intervention is to limit the spread of the larvae which would lead to a systemic infection. Patients are generally administered albendazole, mebendazole, or thiabendazole, an anti-parasitic medication. This treatment is less effective after larvae invasion of the muscles. If this occurs, pain relievers may be prescribed for sore muscles or antipyretics to counteract fever. The main purpose for medication after larvae have migrated is to control and decrease muscle tissue damage.

In a case where this infection results in an allergic reaction due to chemicals being released in the muscles after larvae death, corticosteroids are usually prescribed. While steroids are helpful in regards to controlling inflammation, they may delay the expulsion of the adult worms in the intestinal lining which would result in a longer infection.

Prevention

Due to meat being the main vector of transfer, meat preparation must be monitored and controlled. This would require careful preparation of meat as well as animal feed which would include heating to higher temperatures to ensure larvae death. According to standards set by the USDA, a food thermometer should be used when cooking whole cut meats excluding wild game chicken in order to ensure the temperature reaches 145° F. This would also require a rest time of three minutes where the meat should be set out to before consumption in order to kill all pathogens as the temperature remains constant. For ground meat and wild that also excludes poultry, the meat should be cooked above 160° F with no rest time required afterwards. Poultry should be cooked above 165 °F with a three minute rest time before consumption. Regulations about freezing meats are also in place. Deep freezing

meat for three weeks is shown to deactivate larvae, however larvae in bear meat are more resistant to this method. Other methods of preserving meat including curing or smoking will not ensure the meat is pathogen free. Heating meat is the most effective way to ensure larval death.

Animals destined for human consumption should be carefully managed to ensure they are not in contact with wild animals such as rats, which would put them at risk of contracting trichinosis.

Unit-IV:

Salient features of plant parasitic nematodes and life cycle patterns of

i) *Anguina tritici* ii) *Meloidogyne hapla*

Objectives:

In this Unit we will discuss on Salient features of plant parasitic nematodes and life cycle patterns of i) *Anguina tritici*, ii) *Meloidogyne hapla*.

i) *Anguina tritici*

Anguina tritici (ear-cockle nematode, seed-gall nematode, seed and leaf gall nematode, wheat gall nematode, wheat seed gall nematode, wheat seed-gall nematode, wheat seed and leaf gall nematode) is a plant pathogenic nematode.

Hosts

Emmer (*Triticum monococcum*), rye (*Secale cereale*), spelt (*T. spelta*), and wheat (*T. aestivum*). Barley (*Hordeum vulgare*) is a very poor host. There is no evidence that this nematode reproduces on oats (*Avena sativa*) and other grasses.

Morphology

It is a large nematode, ranging from 3–5 mm in length. *Anguina tritici has a three part* esophagus and the esophageal glands do not overlap with intestine. The female body tends to be thickened and curved ventrally. It has a short stylet (8-11 µm). Females have one ovary and the vulva located posterior. Males possess small spicules and small bursae or alae.

Notes on Taxonomy and Biology

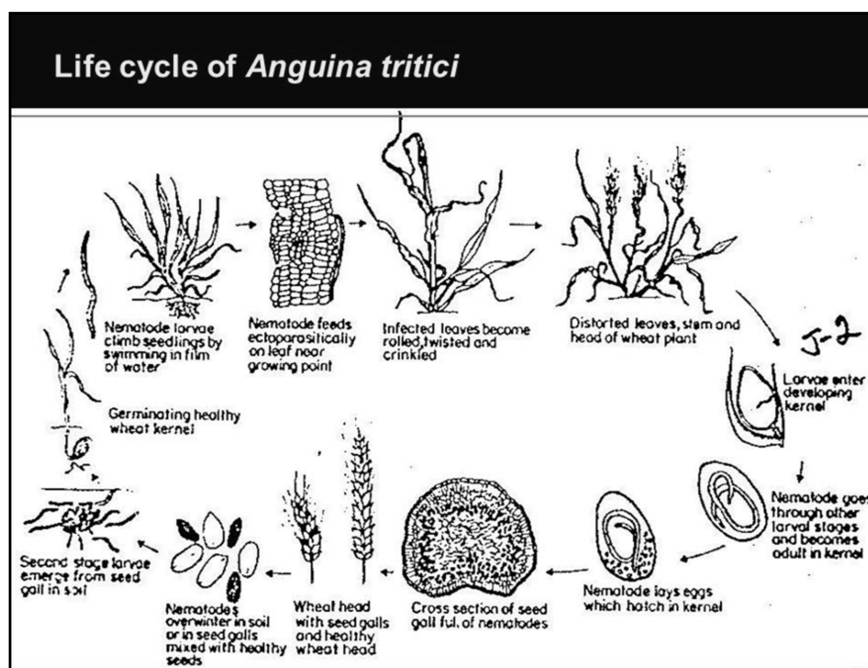
Anguina tritici female show a well developed anterior branch of the ovary which is folded in two or more flexures and a conoid tail, tapered to an obtuse or round tip. This species is closely related to *A. funesta* and *Subanguina wevelli*. The morphological separation of these three species is difficult. Recent molecular diagnostic techniques have facilitated the separation of these three species. J2 emerge from the seed galls in the soil and crawl onto the newly germinated seedlings. They establish infection sites between young leaves where they feed as an ectoparasite causing leaf distortion and crinkling. Later, they penetrate the flower

buds at the time of flower bud initiation. J2 stimulate the formation of galls in floral tissues in place of seed development. Juvenile development is completed inside the galls. Newly formed females deposit eggs, which hatch producing J2, which remain, encased in the galls (cockle) and perpetuate plant infection in following years. Dried cockles are harvested with developed seeds. *Anguina tritici* vectors a bacterium *Clavibacter tritici*, which is the causal agent of yellow ear rot or tonduof wheat. Freshly harvested infected wheat cockles containing the bacterium are toxic to cattle and sheep.

Juveniles find host and move up the plant in a film of water, they invade meristems and penetrate inflorescence. Once in the developing seed they molt, become adults, mate, and reproduce. Eggs laid by the female develop and hatch as J2 within the seed gall where they desiccate and become dormant. Dormant J2 overwinter in the seed galls until spring. They are released when galls come in contact with moist soil and hydrate. Total life cycle is completed in 113 days.

Host parasite relationship

It is an ectoparasite that becomes endoparasitic invading inflorescence and developing seeds. It causes a disease called "ear-cockle", "gout" or seed gall on wheat and rye. It is not a host of oat, maize and shorgum. On wheat it causes stunted plants, distorted leaves, seeds are transformed into galls which contain a dried mass of nematodes. If compared to normal wheat seeds, galls are smaller in size, lighter, and their color ranges from light brown to black (normal wheat seeds are tan in color).



Symptoms

The absence of symptoms does not mean absence of *A. tritici*. Slight elevations occur on the upper leaf surface with indentations on the lower side. Other symptoms include wrinkling, twisting, curling of the margins towards the midrib, distortion, buckling, swelling and bulging. A tight spiral coil evolves, and dwarfing, loss of colour or a mottled, yellowed appearance and stem bending may also occur. In severe infection, the entire above-ground plant is distorted to some degree and a disease problem is usually obvious. Heads (spikes).Wheat heads are reduced with glumes protruding at an abnormal angle exposing the galls to view. This does not occur in rye heads.

Galls

Young galls are short-thick, smooth, light to dark green, turning brown to black with age, 3.5-4.5 mm long and 2-3 mm wide. Rye galls are small, buff-coloured and longer than wide, 2-4.5 mm long by 1-2.5 mm wide.

Prevention and Control

Ear-cockles are the only source for perpetuation of the disease and their removal from contaminated seed lots can completely eradicate the disease. *A. tritici* has been eliminated, or reduced to a minimal number of infestations, in Europe and the USA by seed cleaning, crop rotation and fallow.

Cultural Control and Sanitary Methods

Seed cleaning:

Salt brine method: seed is poured into a salt solution (8 lbs salt in 5 gallons water) and stirred vigorously. Sound galls sink, and debris and galls float to the surface. The galls and debris are skimmed from the surface and steamed, boiled or chemically treated to kill the nematodes. The salt solution is drained into another container and the cleaned seed is rinsed several times in fresh water to remove salt and then spread in thin layers on a clean surface to dry. The cleaned seed is ready to sow when dry. It is important that the seed is washed two or three times in plain water after brine treatment to remove salt particles which may impair germination.

Crop rotation or fallow

A. tritici cannot survive in soil for more than 1 year if the soil is left fallow or planted to a non-host crop. The pest will be eliminated in more than a year.

Physical Control

Hot-water treatments:

Hot-water treatments may be used to eradicate *A. tritici* from seed lots. Marcinowski (1910) demonstrated that nematode galls in a seed lot could be destroyed by keeping the mixture in water at 54-56°C for 10-12 minutes. Pre-soaking the seeds before the hot-water treatment has also been advocated.

Another hot-water treatment involves pre-soaking the seed at 21-27°C for 2-4 hours, then placing them in water for 30 minutes at 50°C. The seeds are rinsed in tap water, then spread in thin layers on a clean surface till dry.

Mechanical separation:

Jones et al. (1938) developed an indented cylinder machine which separated oval wheat seeds from globular nematode galls; the device was claimed to be 98% effective in removing the seed galls. Chu (1945) also designed a machine to separate nematode galls from healthy grain.

Nematicidal Plants

Nematicidal plants are not as effective as the clean seed or fallow method and offer little hope as an effective method of controlling *A. tritici*.

Biological Control

There are few reports concerning biological control of *A. tritici*.

Host-Plant Resistance

A large number of plants have been evaluated for resistance to *A. tritici* over a period of more than 60 years. A few resistant plants have been found, such as the wheat cultivar however, resistance does not appear to be a viable solution to the problem of seed gall nematodes.

Geographical Distribution: Reported from Afghanistan, Australia, Brazil, Bulgaria, China, Egypt, Ethiopia, Hungary, India, Iran, Iraq, Israel, Lithuania, New Zealand, Pakistan, Poland, Romania, Russian Federation, Russian Far East, Syria, Switzerland, Turkey, and Yugoslavia. Early records of nematode detection in the US include California, Georgia, Maryland, New York, North and South Carolina, Virginia and West Virginia. Recent surveys of the wheat

seed gal nematode in stored grain harvested from states with records of this nematode have not provided any evidence that nematodes are still occurring in the USA.

Crop Losses: Nematode damage is negligible in countries adopting modern mechanical and cleaning procedures to separate the nematode galls from visible wheat seeds. The use of high quality seeds has nearly eradicated this nematode from developed countries. However, the nematodes causes severe crop losses to rye (35- 65%) and wheat (20-50%) in 3rd world countries, where poor agricultural practices, monoculture, and the use of poor quality seeds are widespread. In spite of the insignificant damage caused by the nematode in modern agricultural production systems of developed countries, their ability to export grains in the international markets is severely hampered if historical records still exist of the presence of this pest in grain production areas due to the quarantines imposed by many countries because of this pest.

Means of Movement and Dispersal: Through the characteristic dark seed galls harboring the nematode juveniles in harvested grains.

ii) Root knot nematode (*Meloidogyne hapla*)

Meloidogyne hapla was first described from the USA by Chitwood (1949). The type host was Solanum tuberosum and the type locality was Long Island, New York, USA. No synonyms are known, although some of the records attributed to this species refer to other species such as *Meloidogyne chitwoodi*. **Root-knot nematodes** are plant-parasitic nematodes from the genus ***Meloidogyne***. They exist in soil in areas with hot climates or short winters. About 2000 plants worldwide are susceptible to infection by root-knot nematodes and they cause approximately 5% of global crop loss.[1] Root-knot nematode larvae infect plant roots, causing the development of root-knot galls that drain the plant's photosynthate and nutrients. Infection of young plants may be lethal, while infection of mature plants causes decreased yield. **Northern root-knot nematode** (*Meloidogyne hapla*) is a species of vegetable pathogens which produces tiny galls (a sore caused by friction and abrasion) on around 550 crop and weed species. They invade root tissue after birth. Females are able to lay up to 1,000 eggs at a time in a large egg mass. By surviving harsh winters, they can survive in cold climates (hence, the name, **Northern**).

Description

Female: Body pyroid with short neck. Cuticle becoming thicker in posterior half of body, sometimes considerably. Head with two annules behind head-cap. Spear knobs rounded, inconspicuous. Excretory pore 14-20 annules behind head, hemizonid just posterior to pore. Posterior cuticular pattern roughly circular, composed of closely spaced smooth or slightly wavy striae. Dorsal arch low. Lateral fields may be unmarked, may be marked only by slight irregularities in the striae, or dorsal and ventral striae may meet at a slight angle along the fields. Some forking of striae at lateral fields may also occur. In some cases ventral striae may extend laterally on one or both sides to form 'wings' which the dorsal striae meet almost at right angles. Tail with few striae but distinct punctuations forming a stippled area between the anus and tail terminus. Sometimes the stippling may be more diffuse over the inner part of the pattern. Phasmids fairly widely spaced.

Male: Numerous in some populations, absent in others. Head not offset, a truncate cone to hemispherical in outline. Usually only one annule behind head-cap. Spear slender, spear knobs rounded and not offset. Anterior cephalid on second body annule, posterior cephalid just anterior to level of relaxed spear. Hemizonid 45-58 annules behind head, 0-4 annules anterior to excretory pore. Lateral field with four incisures. Tail terminus bluntly rounded; phasmids at about cloacal level. One or two testes. Spicules slightly curved, with small sharp processes projecting from the spicule wall at the junction of head and shaft into the spicule head. Gubernaculum crescentic, proximal end thicker than distal end.

Distribution

M. hapla is widely distributed, particularly in temperate regions and the cooler, higher altitude areas of the tropics. According to Whitehead (1969), *M. hapla* only flourishes at high altitudes above 6000 feet in East Africa (Kenya, Tanzania and Uganda), despite the abundance of host plants at lower altitudes. In Queensland, Australia, *M. hapla* was not found as far north as *M. javanica* (Colbran, 1958). Taylor and Buhrer (1958) reported that in the USA, *M. hapla* was the commonest root-knot nematode north of 39°N.

The distribution in this summary table is based on all the information available. When several references are cited, they may give conflicting information on the status. Further details may be available for individual references in the Distribution Table Details section which can be selected by going to Generate Report.

Biology and Ecology

All nematodes pass through an embryonic stage, four juvenile stages (J1–J4) and an adult stage. Juvenile *Meloidogynes* parasites hatch from eggs as vermiform, second-stage juveniles (J2), the first moult having occurred within the egg. Newly hatched juveniles have a short free-living stage in the soil, in the rhizosphere of the host plants. They may reinvade the host plants of their parent or migrate through the soil to find a new host root. J2 larvae do not feed during the free-living stage, but use lipids stored in the gut.

An excellent model system for the study of the parasitic behaviour of plant-parasitic nematodes has been developed using *Arabidopsis thaliana* as a model host. The *Arabidopsis* roots are initially small and transparent, enabling every detail to be seen. Invasion and migration in the root was studied using *M. incognita*. Briefly, second stage juveniles invade in the root elongation region and migrate in the root until they became sedentary. Signals from the J2 promote parenchyma cells near the head of the J2 to become multinucleate to form feeding cells, generally known as giant cells, from which the J2 and later the adults feed Concomitant with giant cell formation, the surrounding root tissue gives rise to a gall in which the developing juvenile is embedded. Juveniles first feed from the giant cells about 24 hours after becoming sedentary.

After further feeding, the J2s undergo morphological changes and become saccate. Without further feeding, they moult three times and eventually become adults. In females, which are close to spherical, feeding resumes and the reproductive system develops. The life span of an adult female may extend to three months, and many hundreds of eggs can be produced. Females can continue egg laying after harvest of aerial parts of the plant and the survival stage between crops is generally within the egg.

The length of the life cycle is temperature-dependent. The relationship between rate of development and temperature is linear over much of the root-knot nematode life cycle, though it is possible the component stages of the life cycle, e.g. egg development, host root invasion or growth, have slightly different optima. Species within the *Meloidogyne* genus also have different temperature optima. In *M. javanica*, development occurs between 13 and 34 °C, with optimal development at about 29 °C.

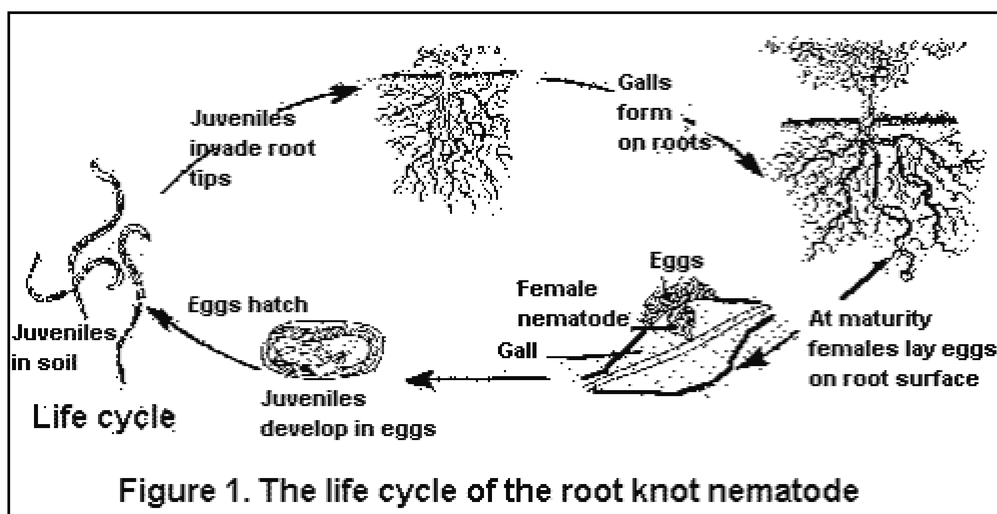


Figure 1. The life cycle of the root knot nematode

List of symptoms/signs

Leaves- abnormal colours

Roots- galls along length

Roots- reduced root system

Whole plant- dwarfing

Whole plant- early senescence

Symptoms

Typical symptoms of attack include a galling of the root system, the galls being relatively small and subspherical, often with a marked proliferation of small roots at the site of the gall (this is in contrast to the symptoms caused by other common species of *Meloidogyne*). In potato tubers, brown spots appearing in the tubers after the females commence egg production may identify infection sites. Severe attack by *M. hapla* results in impaired root function and concomitant stunting of the above ground parts leading to a reduction in yield.

Prevention and control

Various methods have been used to cleanse planting material, including hot water treatment and a range of nematicidal drenches. Treatment in the field also relies upon the application of nematicides although frequent rotation with cereals or other graminaceous non-host crops may also be efficacious. Glasshouse soils may be fumigated to eradicate the pest. Many crops have potential for development of resistant or tolerant varieties.

Economic Impact

Root-knot nematodes (*Meloidogyne* spp.) are one of the three most economically damaging genera of plant-parasitic nematodes on horticultural and field crops. Root-knot nematodes are distributed worldwide, and are obligate parasites of the roots of thousands of plant species, including monocotyledonous and dicotyledonous, herbaceous and woody plants. The genus includes more than 90 species, with some species having several races. Four *Meloidogyne* species (*M. javanica, M. arenaria, M. incognita, and M. hapla*) are major pests worldwide, with another seven being important on a local basis. *Meloidogyne* occurs in 23 of 43 crops listed as having plant-parasitic nematodes of major importance, ranging from field crops, through pasture and grasses, to horticultural, ornamental and vegetable crops. If root-knot nematodes become established in deep-rooted, perennial crops, control is difficult and options are limited.

Meloidogyne spp. were first reported in cassava by Neal in 1889. Damage on cassava is variable depending on cultivar planted, and can range from negligible to serious. Early-season infection leads to worse damage. In most crops, nematode damage reduces plant health and growth; in cassava, though, nematode damage sometimes leads to increased aerial growth as the plants try to compensate. This possibly enables the plant to maintain a reasonable level of production. Therefore, aerial correlations to nematode density can be positive, negative or not at all. Vegetable crops grown in warm climates can experience severe losses from root-knot nematodes, and are often routinely treated with a chemical nematicide. Root-knot nematode damage results in poor growth, a decline in quality and yield of the crop and reduced resistance to other stresses (e.g. drought, other diseases). A high level of damage can lead to total crop loss. Nematode-damaged roots do not use water and fertilisers as effectively, leading to additional losses for the grower. In cassava, it has been suggested that levels of *Meloidogyne* spp. that are sufficient to cause injury rarely occur naturally. However, with changing farming systems, in a disease complex or weakened by other factors, nematode damage is likely to be associated with other problems.

Probable questions:

1. Classify phylum Protozoa up to order with examples.
2. Classify Nematoda, Trematoda and Cestoda.
3. State the mode of transmission of protozoan parasites.
4. Define zoonosis. Clarify it. Discuss any one of the disease studied by you in the light of zoonotic potential.
5. Describe the life history of *Nosema bombycis* with reference to autoinfection.
6. Biology, Pathogenesis, epidemiology and of some important human parasitic helminths studied by you.
7. Life cycle pattern of different plant parasitic nematodes studied by you.

Suggested Readings /References:

1. Animal Parasitology by J. D. Smyth.
2. Parasitology by William C. Marquuardt and Richard S. Demaree, jr.
3. Foundation of Parasitology by Roberts and Janovy.
4. Fundamentals of Parasitic Zoonoses by Dr. K.M.L. Pathak.
5. Medical microbiology by Gilbart A.Castro.
6. The physiology of Trematodes, by J.D.Smyth and D. W. Halton.
7. Advances in Parasitology by J.D.Smyth.
8. Introduction to Parasitology Nobel and Nobel.

HARD CORE THEORY PAPER (ZHT – 102)
Group B (Ecology)

Module	Unit	Content	Credit	Class	Time (h)	Page No
ZHT - 102 (Parasitology, Ecology and Environmet)	**V**	The Ecosystem: concept, Gaia hypothesis, cybernetic nature and stability of the ecosystem, ecosystem management and optimization. Biogeochemical cycle: Nitrogen and phosphorus cycle	**1**	**1**	**1**	**99 –121**
	VI	Community: Structure and Gradient analysis. Niche theory : Niche concepts,niche width		**1**	**1**	**122 –149**
	VII	Population attributes: Growth forms and mathematics of growth, Life Table - (Cohort and Static); survivorship curves, generation time, net reproductive rate. Life history strategies: Evolution of life history traits, strategies related to longevity; clutch size; life history optimization.		**1**	**1**	**150 –157**
	VIII	Metapopulation: Concept, models , structure and dynamics. Major terrestrial biomes; major biogeographical zones of India		**1**	**1**	**158–171**

Group-B: Ecology

Unit-V

The Ecosystem: concept, Gaia hypothesis, cybernetic nature and stability of the ecosystem, ecosystem management and optimization. Biogeochemical cycle: Nitrogen and phosphorus cycle.

Objective:

In this Unit we will discuss about The Ecosystem:concept, Gaia hypothesis, cybernetic natureand stability of the ecosystem, ecosystem management and optimization. In this Unit we will also discuss about Biogeochemical cycle: Nitrogen and Phosphorus Cycle.

Introduction:

The ecosystem is the basic functional unit in ecology, since it includes both biotic and abiotic environment each influencing the properties of the other & both necessary for maintenance of life as well as we have it on the earth.

Some examples of the natural ecosystems are ponds, lakes, oceans, grasslands, forests, deserts tundra & so on. An ecosystem be it a pond, forest, desert or at tundra has the following four components:

1. **The non living environment:**

   Which is called abiotic environment includes air, water, soil and the basic elements. There, substances enter into the body of the environment and performs metabolic activity and again return to the environment. Abiotic factor could be divided into three-

   a. Physical (climate) factors: Such as temperature, relative humidity.
   b. In organic substances like water, C, N_2, S, P and so on.
   c. Organic substances: such as proteins, carbohydrates, lipids and humic substances.

The organic substances linked the living body with the biotic and abiotic components. In the terrestrial ecosystem the diversity of the organisms determined by the climate, soil etc. In high altitude the availability of O_2 is important. In aquatic ecosystem temperature, salinity, dissolved gases, chemical substances etc determine the specific diversity. In arctic & tropics climatic factors greatly affect the distribution of the organisms.

2. **Producers:** Producers are energy transducers which converts solar energy into chemical energy with the help of inorganic substances such as water, $CO_{2,}$ and enzymes. These producers are autotrophic (auto-self, troph - nourishing) organisms mainly green plants like trees, grasses, phytoplanktons etc. They possess a green pigment (Chlorophyll) and transducts solar energy and are known as photoautotrophs, while the chemoautotrophs use energy generated in an oxidation reduction process. eg: S-bacteria .

3. **Consumers:** Which are heterotrophic (hetero = other, trophic = nourishing) organisms. Depending upon their food habits consumer may be herbivores (Plant eaters) and Carnivores (Flesh eaters).

   Macro consumers or phagotrophs (Phago = to eat) ingest other organisms or particulate matters.

   Micro consumers are saprotrophs (sapro = to decompose) or osmotrophs (Osmo = to pass through membrane). They are mainly bacteria & fungi, which breakdown the complex compounds of dead. Their protoplasm absorb some of the decomposition products.

   Herbivores include insects, zooplanktons, deer, cattle elephants etc. The carnivores are grouped into 1st order, 2nd order and so on depending upon their food habit.

4. **Decomposer:** Decomposers are actually the micro consumers. They breakdown complex organic matter like cellulose, hemicelluloses, chitin etc of plant and animal body and fungi. But some invertebrates like protozoa, oligochaetes (earthworm) euchytraeid etc use the dead organic matter for their food and hence they are also grouped into decomposers.

   Another two category subdivisions for heterotrophs suggested by Wiegert and Owens (1970) is as follows.

   (i) Biophages, organisms consuming other living organisms.

   (ii) Saprophages, organisms feeding on dead organic matter.

From the functional standpoint an ecosystem may be conveniently analysed in terms of following:

(i) Energy circuits.

(ii) Food chain.

(iii) Diversity patterns in time & space.

(iv) Nutrient cycles.

(v) Development and evolution.

(vi) Cybernetics.

The Gaia hypothesis

The Gaia means The Greek goddess "Mother Earth". James Lovelock was the pioneer of the concept. But James Lovelock and Redfield contributed independently the gaia concept. Lovelock published a readable little book entitled "Gaia: A New look at life on earth" which is his own words, is a personal account of journey through space & time in search of evidence to substantiate this model of earth.

In develops word, "The Gaia hypothesis states that the biosphere is a self regulating entity with the capacity to keep our planet healthy by controlling the chemical and physical environment." In other words, the earth is a super-ecosystem with numerous interacting functions and general feedback loops that tolerate extremes of temperature and oceans relatively constant.

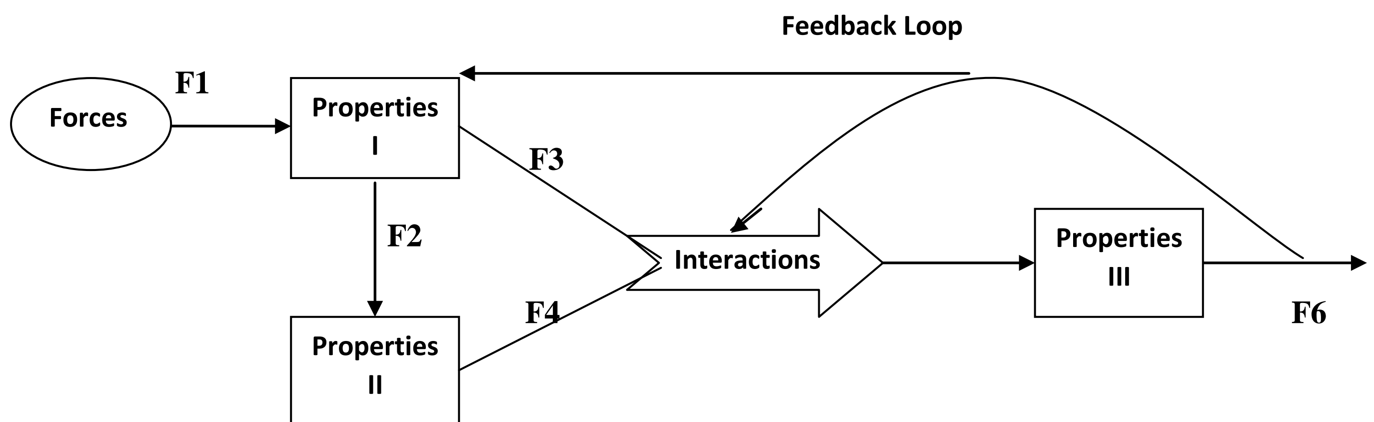


Figure: Diagram showing the five basic components that are of primary intern is modelling ecosystems.

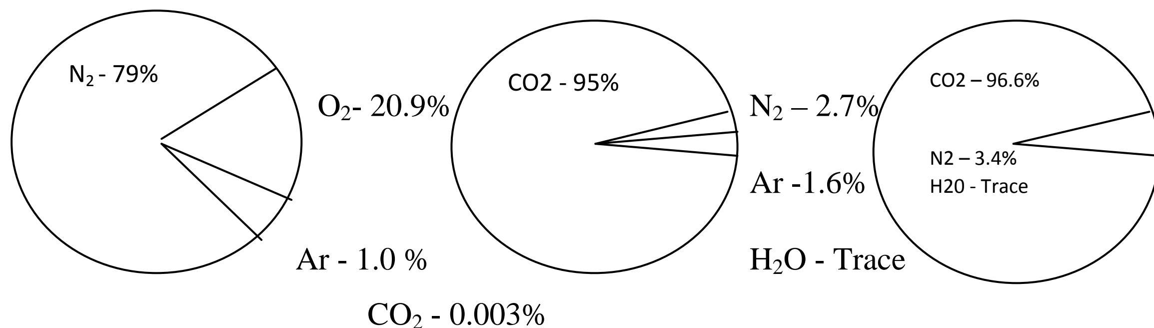
The most controversial past of the hypothesis is that the biotic community plays the major role in biosphere homeostasis and organisms began to establish soon after the first life appeared more than a billion years ago. The contrary hypothesis is that purely geological (abiotic) processes produced condition favorable for life, which then merely adapted to these conditions.

The question is that did physical conditions evolve first then life or did both evolve together? The primary atmosphere was formed from gases (Outgas rising from the hot core of the earth by the process called outgassing).

While volcanoes continue to affect climates, the secondary atmosphere is produced and according to Gaia hypothesis, is a biological product.

This reconstruction, as it were, began with the first life and thus the primitive microbes are that do not require oxygen – the anaerobes. When the green anaerobic microbes began to put oxygen into the air, the plants and animal that require gaseous oxygen, the aerobes, evolved. The anaerobes retreated to the O_2 less depths of soils & sediments, where they continue to thrive & play a major role in various ecosystems. The cooling of earth that resulted from the removal of CO_2 from the atmosphere by lime-stone forming marine organisms.

Comparison of the atmosphere of the earth with that of the planets Venus & Mars, where of there in life it is restricted to anaerobic microbes, provides strong indirect evidence for the Gaia hypothesis.



Earth **Mars** **Venus**

The low CO_2, High O_2 and N_2 atmosphere of the earth is completely opposite from the conditions of the nearby planets. Since photosynthesis, which evolved soon from after the first appearance of life, removes CO_2 and add O_2 in the atmosphere. It is logical to conclude that the biotic community is responsible for the building up of O_2 & reduction in CO_2 over time. Recently, many geochemical geochemist assumed without much direct evidence that O_2 came solely from the breakdown of water vapour & the escape of H_2 into space, leaking an exces of O_2 behind.

It is also difficult to explain how gaseous N_2 could accumulate in the atmosphere in absence of life. But biological transformations, nitrogen would go its most stable form, nitrate ion dissolved in the oceans. The N_2 cycle clearly demonstrated that biotic community does not just borrow gases from the atmosphere and return them unchanged but after their chemistry in ways that are beneficial to life.

A variety of special microorganisms (N_2-fire and denitrification) play major roles in keeping the vital compounds (such as NH_3, H_2O, N_2 & H_2) moving in an orderly manner between biotic and abiotic states.

Without the critical buffering activities (to the acedic environment) of early life forms and the continued coordinated activities of plants & microbes that dampen fluctuations in physical factors, conditions on earth, according to Lovelok & Margulis, would be similar to current conditions on Venus: very hot, with no O_2 in the atmosphere.

Cybernetic Structure & Stability of the Ecosystem

Ecosystems are capable of self maintenance and self regulation as their component populations and organisms. Thus cybernetics (from kybernetes = pilot or governor). The science of control, has important application in ecology especially since man increasingly tends to disrupt natural controls or attempts to substitute artificial mechanisms for natural ones. Homeostasis (Homeo = same, Stasis = Standing) is the term generally applied to the tendency for biological systems to resist change and toremain in a state of equilibrium.

According to the Gaia hypothesis, the biosphere is a highly integrated self organised cybernetic or controlled system. But cybernetics at the biosphere level is not accomplished by external goal oriented set point controls (like thermostat, chemostat or other mechanical feed back) but the control is internal & diffuse, involving hundreds of thousands of feedback loops & synergistic interactions in subsystems such as the microbial network controls the N_2 cycle.

Waddington (1975) coined the term HOMEORHESIS (from the Greek meaning "Maintaining the flow") to denote evolutionary & ecological stability as opposed to homeostasis, the widely used term for physiological stability at the organism level.

The simplest form of control system consists of two black boxes (A black box may be defined as any unit whose functions may be evaluated without specifying the internal contents).

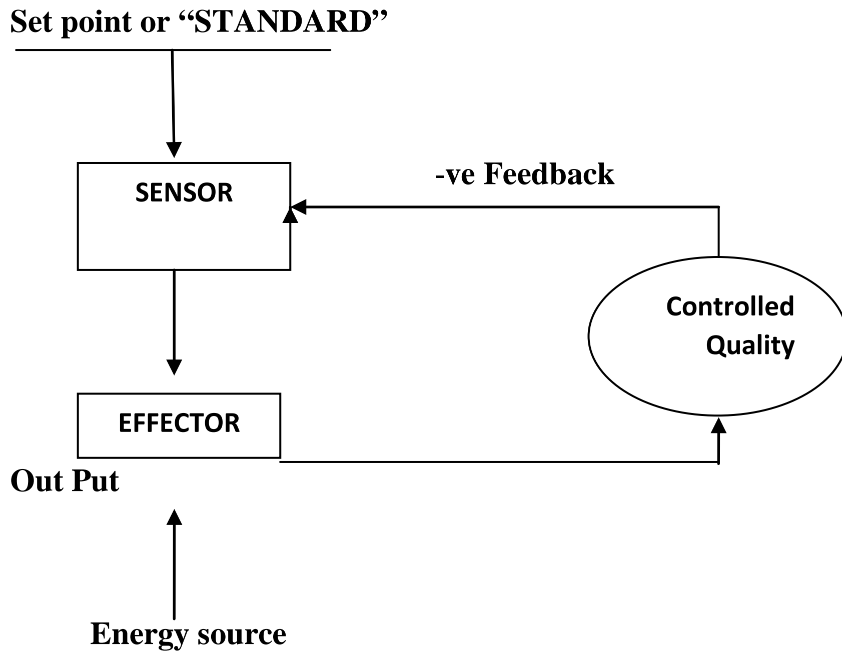


Figure: A simple control system, analogus to a house hold thermostat, in which some of the output is used as –ve feedback to maintain some kind of equilibrium into controlled quantity.

And a controlled quantity interconnected by output & input circuit or signals. When this feedback input is +ve. The quantity grows which is necessary for growth & survival of organisms. Again –ve feedback and the limits of homeostatic control can be plotted.

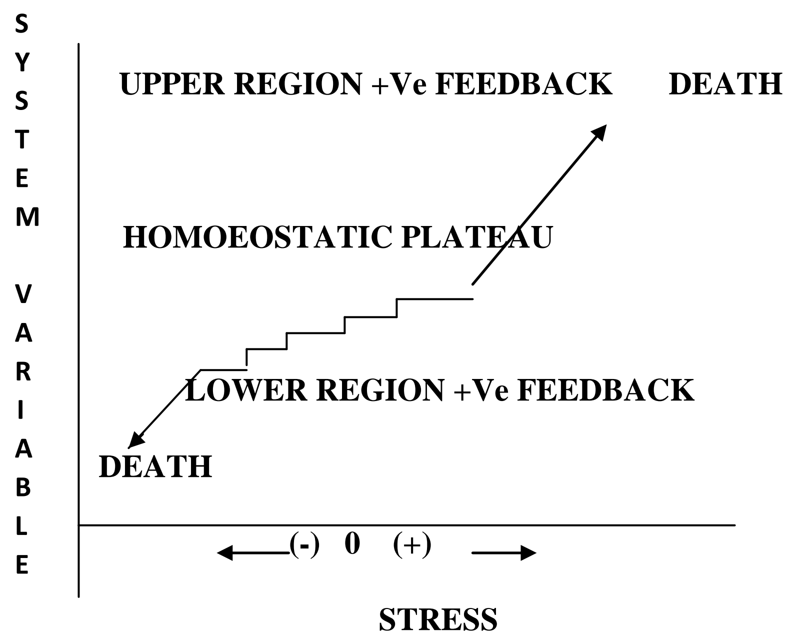


Figure: The concept of the homeostatic plateau within which relative constancy is maintained by –ve feed back despite tendency of stress to cause deviation. Beyond limits of homeostasis +ve feed back results in rapid destruction of the system (Hardin 1963).

The +ve feedback involved in the expansion of knowledge power and productivity threatens the quality of human life and environment unless adequate –ve feedback controls can be found. The science of controls or cybernetics, thus becomes one of the most important subjects in practice.

Some population are regulated by density which "feedback" by way of behavioral mechanisms to reduce or increase the reproductions rate (the effectors) and thus maintain the population size (controlled quality) within set limits. But in other populations like human do not seem to be capable of self limitation but are controlled by outside factors.

The enter play of material cycles (storage & release of nutrients) and production & decomposition of organic substances) and energy flows in large ecosystems generate & a self correcting homeostasis with no outside control or required set point.

The homeostatic mechanisms have limits beyond which unrestricted +ve feedback leads to death. There is a series of levels of steps in the homeostatic plateau.

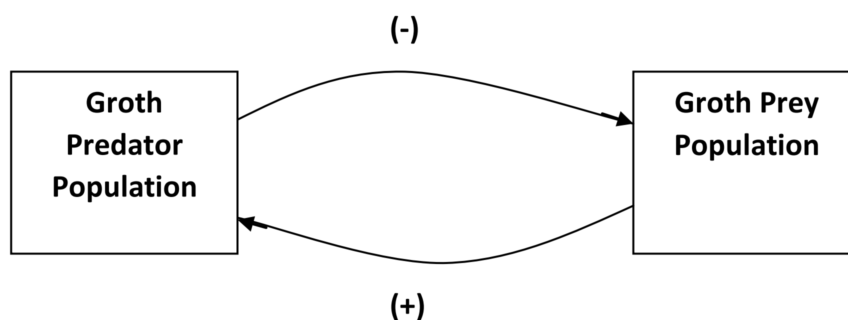


Figure: The interaction of +ve & -ve feedback in a predator prey feedback loop system.

Really good homeostatic control comes only after a period of evolutionary adjustment and thus the system actually becomes stable. New ecosystems or new predator prey assemblage tend to oscillate violently.

As a result of the evolution of the central nervous system, mankind has gradually become the most powerful organism and are able to modify the operation of ecosystem.

Because of the absence of set point controls, many scientistsare doubting that ecosystems and the biosphere really function as cybernetic systems, although most accept the concept that organisms play major roles in the control of the chemistry of the atmosphere and the oceans (Kerr 1988). The fact that catastrophic events, such as come to crushing into the earth, massive volcanic eruptions, and glaciers, have occurred from time to time rises questions

about global homeostasis. Yet despite a loss of species during these geological & cosmic upheavals, life has not only persisted but has continued to diversify & play a role in restoring favorable conditions for it self. However, just become the biosphere has exhibited the resilience stability to recover in past ages is no reason to be complacent or satisfied about the resilience (flexibility) of our current life support system. Humans as a species might not survive a human-made catastrophic such as nuclear war or fortification (defense) of the oceans, and even if we did survive, all our hardened cultural & life style gains would be wiped out.

Ecosystem Management & Optimization

The proper functioning of natural ecosystem as interacting & indicated biological units in liable to artificial change and disruption as a consequence of various anthropogenic activities such as agriculture, forestry and fisheries. Man made activities can cause changes in the nature and extent of the 4 dynamic phenomena of organic productions and breakdown energy flow, nutrient cycling and water movement involved in the ecosystem functioning. As a result of man's activities the plant, animal and microbial communities of a given ecosystem can be significantly modified and show also the environment characteristic of the system. For example – soil erosion is a main consequence of indiscriminate deforestation.

Proper management of the ecosystem is often hampered by lack of adequate knowledge of the functional relationships within ecosystems and also of their numbers form and kind etc. Most ecosystems at some stages on the other are subjected to actual or man made shocks, calamities, traumatic stresses etc. Those who can survive such stresses are the one's ended within the capacity to tolerate these shocks, that is they manifest a kind of internal resilience. This resilience is a determinate of the magnitude of the disturbance that can be tolerated by the ecosystem before it would shift into a basically different behavior.

The concept of persistence or resistance & resilience are the component parts of the concept of ecosystem stability. Desert ecosystem in general show low persistence or resistance but high resilience. Ecosystem stability constitutes one of the main unifying concepts of ecology. There are several features which contribute to stability of ecosystem.

i. Tolerance to extreme & harsh conditions.
ii. Ability for rapid recovery upon recurrence of favorable growth condition.
iii. Flexible & opportunistic feeding habits.
iv. Nomadic migrations of animals etc.

Likewise some destabilizing features include:

i) Sensitivity to damage to reserves.

ii) Sensitivity to lagging components.

iii) Low density, biomass & productivity.

iv) Sensitivity to soil erosion.

Of course biotic & human features are highly significant in affecting the stability of the ecosystem.

Optimizations:

The term 'optimizations' implies that as a consequence of Natural Selection & evolution, organisms tend to have a combination of form & function, that is optimal for growth & reproduction in their particular environment. So, the principle of optimization may not however become translated into reality in all cases. Parkhurst & Loucks (1972) have listed 3 reasons that may possibly explain such a failure to attain optimization-

i) Inadequate time in a given environment.

ii) The environment does not remain constant for a long enough period of time.

iii) Omission or lack of certain important variables.

According to Parkhurst & Loucks (1972) optimization works mostly at the organic level but according to Cody(1974) it is applicable at the level of the ecosystem. In fact it is only to be expected that Natural selection would ensure survival of the fittest phenotypes. Cody's model to explain optimization explain the following areas:

i. Higher resource availability tends to favour specialist feature over generalist where as lower resources favour generalist over specialist.

ii. A mix of similar resources favors generalist over specialist, where as dissimilar resources favor specialist.

iii. The addition of a competing species favors a wide range of specialist.

iv. At higher premium or breeding greater co-existence occurs.

Holling (1973) believes that natural system could have multiple domex (habitats) or attraction and that system persistence possibly is more dependent on the ability to move from one domain to another rather than on the dynamics of a given domain.

The principle of optimization is however not universally accepted based on work of bioenergetics and dynamics of birds in a grassland biome. Innis (1974) finds it difficult to

rationalize optimization and states. The determination of an objective function such that observed phenomena optimize that function subjected to can strains is not a 'vacuous enterprise'. On the other hand Katz and Batnick (1974) distinguished between dynamic long term and static (instantaneous) optimizations in ecosystems, the former differing from the later in that it considers the effect of decisions made at one time upon the status of the system at a later time and optimizes a long term objectives by choosing these variables which change throughout the long period of time. In static optimizations, only a single time period is considered and the relationship between this particular time period and other time period is ignored. Fisheries, pest management and phorasing are the example of dynamic optimizations.

Resistance stability indicates the ability of an ecosystem to resist perturbation.

Resilience stability indicates ability to recover when the system disrupted by a perturbation.

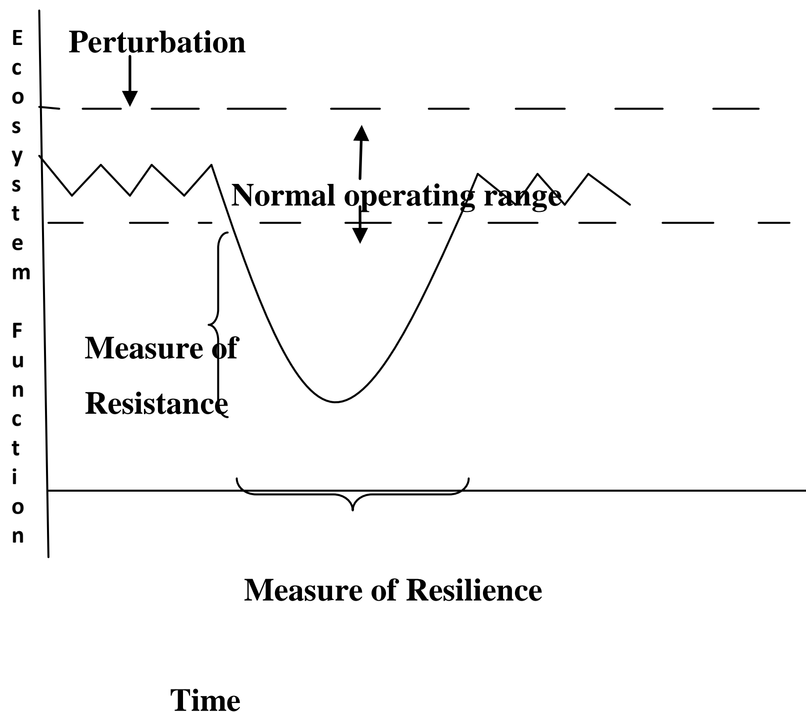


Figure: Resistance & resilience stability

When a perturbation causes a major ecosystem function to deviate from the normal operations range, the degree of deviation is a measure of relative resistance. While time required for recovery is a measure of relative resilience.

Biogeochemical cycle:

Introduction:

Biogeochemical cycles are components of the broader cycle that govern the functioning of planet Earth. The Earth is a system open to electromagnetic radiation from the sun and outer space, but is a virtually closed system with regard to matter. This means that the planet has minimal flux of matter, other than meteorite collisions and minor amounts of intergalactic particle trapping (or loss) by the upper atmosphere. Therefore, matter that Earth contained from the time of its birth is transformed and circulated geographically. This is in line with the law of conservation of matter which states that matter cannot be created nor destroyed but can be transformed including the transformation between matter and energy.

The transfer of matter involves biological, geological and chemical processes; hence the name biogeochemical cycles derives. Biogeochemical cycles may also be referred to as cycles of nature because they link together all organisms and abiotic features on earth. Matter is continually recycled among living and abiotic elements on earth. Biogeochemical cycles facilitate the transfer of matter from one form to another and from one location to another on planet earth. Additionally, biogeochemical cycles are sometime (Figure es called nutrient cycles, because they involve the transfer of compounds that provide nutritional support to living organisms.

Pathways of biogeochemical cycles

Parts that comprise planet earth have been categorized into four spheres (regions). One is the sphere which has life and it is called the biosphere (it is the region occupied by living organisms such as plants, animals, fungi) and the other three spheres are largely devoid of life, they include; lithosphere (region occupied bysoil, land and the earth crust), atmosphere (air and space) and hydrosphere (areas covered by water such as rivers, lakes and oceans). However, where the biosphere overlaps the lithosphere, atmosphere or hydrosphere, there is a zone occupied by living organisms.

Categories and examples of biogeochemical cycles

Biogeochemical cycles differ in their pathways, and on this basis the biogeochemical cycles have been categorized into two:

• **Sedimentary cycles:** these cycles involve the transportation of matter through the ground to water; that is to say from the lithosphere to the hydrosphere. Common examples of cycles under the sedimentary category are:

• **Phosphorus cycle:** Phosphorus is commonly found in water, soil and sediments. Phosphorus cannot be found in air in the gaseous state. This is because phosphorus is usually a liquid at standard temperatures and pressures. Phosphorus is mainly cycled trough water, soil and sediments. However, very small particles in the atmosphere may contain phosphorus or its compounds. Phosphorus moves slowly from deposits on land and in sediments, to living organisms, and much more slowly back into the soil and water sediment. The phosphorus cycle is the slowest one of the sedimentary cycles.

• **Sulphur cycle:** Sulphur in its natural form is a solid, and restricted to the sedimentary cycle in this form. It is transported by physical processes like wind, erosion by water, and geological events like volcanic eruptions. However, in its compounds such as sulphur dioxide, sulphuric acid, salts of sulphate or organic sulphur, sulphur can be moved from the ocean to the atmosphere, to land and then to the ocean through rainfall and rivers.

• **Gaseous cycles:** these involve the transportation of matter through the atmosphere. Common example of gaseous cycles are:

• **Carbon cycle:** Carbon is one of the most important elements that sustain life on earth. Carbon dioxideand methane gases (compounds of carbon) in the earth's atmosphere has a substantial effect on earth's heat balance. It absorbs infrared radiation and hence may contribute to global warming and climate change.

• **Nitrogen cycle:** Nitrogen gas is the most abundant element in the atmosphere and all the nitrogen found in terrestrial ecosystems originate from the atmosphere. The nitrogen cycle is by far the most important nutrient cycle for plant life.

• **Oxygen cycle:** The oxygen cycle describes the movement of oxygen within and between its three main reservoirs: the atmosphere, the biosphere, and the lithosphere. The main driving factor of the oxygen cycle is photosynthesis and because of this, oxygen and carbon cycles are usually linked and the two cycles are collectively called oxygen-carbon cycle.

• **Hydrological cycle:** This is some times called the water cycle. Water is the most important chemical of life for all living organisms on earth. Water in the atmosphere is usually in form of vapor but condenses to liquid water and can solidify when temperatures are 0°C to form

ice. Ninety three percent of water on earth is in solid state mainly comprising the ice caps and glaciers of Polar Regions.

Nature of elements transported in biogeochemical cycles

When living organisms die and decay, their body structures disintegrate and may be reduced to constituent molecules. Depending on the region where disintegration of the organisms occurred, the component molecular elements then join the biogeochemical cycle. Elements transported in the biogeochemical cycles have been categorized as:

1. Microelements – these are elements required by living organisms in smaller amounts. Examples of such elements include boron used mainly by green plants, copper used by some enzymes and molybdenum used by nitrogen-fixing bacteria.
2. Macroelements – these are elements required by living organisms in larger amounts. Examples of such elements include carbon, hydrogen, oxygen, nitrogen, phosphorous, sulphur.

Macroelements are the commonly cited examples of elements that constitute major biogeochemical cycles. The molecules may be further reduced to ionized or hydrated ions in aqueous solutions or to ions in the atmosphere. These molecules or ions are then cycled and metabolized by new organisms, and the organism may pass them on through excreta or death/decay to be cycled again. Therefore, the whole process becomes a cycle.

The importance of biogeochemical cycles

Biogeochemical cycles serve as a variety of functions at ecosystem level and in ensuring survival of various organisms including humans. Below are some of the importance of biogeochemical cycles.

• Biogeochemical cycles enable the transformation of matter from one form to another. This transformation enables the utilization of matter in a form specific to particular organisms. For example humans utilize water in liquid form. Through the hydrological cycle, water vapour is condensed to liquid and ice converted to liquid water. Nitrogen, despite its abundance in the atmosphere it's often the most limiting nutrient for plant growth. This problem occurs because most plants can only take up nitrogen in two solid forms: ammonium ion (NH_4^+) and the ion nitrate (NO_3^-). Therefore, biogeochemical cycles enable the provision of elements to organisms in utilizable forms.

• Biogeochemical cycles enable the transfer of molecules from one locality to another. Some elements such as nitrogen a re highly concentrated in the atmosphere, but some of the

atmospheric nitrogen is transferred to soil through the nitrogen cycle (which is a biogeochemical cycle).

• Biogeochemical cycles facilitate the storage of elements. Elements carried through the biogeochemical cycles are stored in their natural reservoirs, and are released to organisms in small consumable amounts. For example through the nitrogen cycle and with the help of the nitrogen fixing bacteria, green plants are able to utilize nitrogen in bits though it is abundant in the atmosphere.

• Biogeochemical cycles assists in functioning of ecosystems. An ecosystem is a system that properly functions in a state of equilibrium, and when ever any imbalances occur, the ecosystem through the biogeochemical cycles restores to the equilibrium state; this may take a few days or many years. The adjustment is such that the disturbing factor is eliminated.

• Biogeochemical cycles link living organisms with living organisms, living organisms with the non living organisms and nonliving organisms with non living organism. This is because all organisms depend on one another and most especially, the biotic (living component) and a biotic component of the ecosystem are linked by flow on nutrients engineered by the biogeochemical cycles.

• Biogeochemical cycles regulate the flow of substances. Since the biogeochemical cycles pass through different spheres, the flow of elements is regulated because each sphere has a particular medium and the rate at which elements flow is determined by the viscosity and density of the medium. Therefore elements in the biogeochemical cycles flow at differing rates with in the cycle and this regulates the flow of the elements in those cycles.

Human activities and their influence on biogeochemical cycles and climate change

Life on earth is inextricably linked to climate through a variety of interacting cycles and feedback loops. In recent years there has been a growing awareness of the extent to which human activities, such as deforestation and fossil fuel burning, have directly or indirectly modified the biogeochemical and physical processes involved in determining the earth's climate. These changes in atmospheric processes can disturb a variety of {C}ecosystem services that humans depend upon. In addition to helping to maintain relative climate stability ecosystem services protect living organisms on earth from the sun's harmful ultraviolet rays, mediate runoff and evapotranspiration and regulate nutrient cycling.

Humans clearly disrupt many, if not all biogeochemical cycles and in the process threaten many ecosystems. In resent years human activities have directly or indirectly affected the biogeochemical cycles that determine climatic conditions of earth. It is imperative to mention

that, managing and understanding environmental problems caused by climate change would require an understanding of the biogeochemical cycles. Biogeochemical cycles always involve equilibrium states: a balance in the cycling of the element between spheres. However, overall balance may involve elements distributed on a global scale and that is why a disruption in one cycle causes a disruption in all other cycles. Below is a summary of how human activities have contributed to disruption of biogeochemical cycles. For impacts on specific cycles, the reader should refer to the sites where these cycles are presented.

Use of phosphorus fertilizers:

Human influences on the phosphorus cycle come mainly from the introduction and use of commercial synthetic fertilizers. Use of fertilizers mainly has affected the phosphorus and nitrogen cycles. Plants may not be able to utilize all of the phosphate fertilizer applied; as a consequence, much of it is lost from the land through the water run-off. The phosphate in the water is eventually precipitated as sediments at the bottom of the water body. In certain lakes and ponds this may be redissolved and recycled as a problem nutrient. Animal wastes or manure may also be applied to land as fertilizer. If misapplied on frozen ground during the winter, much of the fertilizer may be lost when ice melts and forms runoff. In certain areas very large feed lots of animals, may result in excessive run-off of phosphate and nitrate into streams. Other human sources of phosphate are in the out flows from municipal sewage treatment plants. Without an expensive tertiary treatment, the phosphate in sewage is not removed during various treatment operations. Again an extra amount of phosphate enters the water.

Mining of Fossil fuels:

Humans have interfered with the carbon cycle where fossil fuels have been mined from the earth crust. Had fossils not been discovered prior to industrial revolution, they could have remained there until now. Carbon dioxide is number one green house gas contributing to global warming and climate change. Additionally, clearing of vegetation that serve as carbon sinks has increased the concentration of carbondioxide in the atmosphere.

Production of Sulphur dioxide: Human impact on the sulphur cycle is primarily in the production of sulphur dioxide (SO_2) from industry (e.g. burning coal) and the internal combustion engine. Sulphur dioxide can precipitate onto surfaces where it can be oxidized to sulphate in the soil (it is also toxic to some plants), reduced to sulphide in the atmosphere, or

oxidized to sulphate in the atmosphere as sulphuric acid (a principal component of acid rain). Sulphur compounds play a big role in the climate system because they are important for the formation of clouds. Additionally, a lot of sulphur is brought into the air by volcanic eruptions. A strong eruption can emit particles up to the stratosphere hence leading to cooling down of the planet.

Cultivation of legumes and use of nitrogen fertilizers:

As a result of extensive cultivation of legumes, creation of chemical fertilizers, and pollution emitted by vehicles and industrial plants, human beings have more than doubled the annual transfer of nitrogen into biologically available forms. Humans have significantly contributed to the transfer of nitrogen gases from Earth to the atmosphere, and from the land to aquatic systems through four main processes:

• The application of nitrogen fertilizers to crops has caused increased rates of denitrification and leaching of nitrate into groundwater. The additional nitrogen entering the groundwater system eventually flows into streams, rivers, lakes, and estuaries. In these systems, the added nitrogen can lead to eutrophication.

• Increased deposition of nitrogen from atmospheric sources because of fossil fuel combustion and forest burning. Both of these processes release a variety of solid forms of nitrogen through combustion.

• Livestock ranching. Livestock release a large amounts of ammonia into the environment from their wastes. This nitrogen enters the soil system and then the hydrologic system through leaching, groundwater flow, and runoff.

• Sewage waste and septic tank leaching.

Nitrogen Cycle:

The nitrogen cycle is the shift between different chemical forms of nitrogen through biologic, physical, and geologic processes on Earth. Nitrogen is an essential element for all living things. It is a building block of biological molecules such as proteins and nucleic acids. The majority of nitrogen on the planet is in the form of molecular nitrogen in the air. Only certain bacteria can convert nitrogen into biological molecules that occur mainly inside living cells. Humans are interfering with the nitrogen cycle by making nitrogen fertilizers and by oxidizing atmospheric molecular nitrogen through the extensive burning of fossil fuels.

Cycling of Nitrogen on Earth:

The majority of Earth's chemical elements are circulating through biological, physical, chemical, and geological processes. These processes operate in circles and are called biogeochemical cycles. Nitrogen is one of the key elements in human activities and in biological, physical, chemicalal, and geological processes. Estimates show that more than 20 million tons of nitrogen exists on every square mile of the planet. The atmosphere contains up to 78 percent molecular nitrogen (N_2), and this nitrogen is mainly cycling through biologic processes. Four major nitrogen-transformation (biological) processes exist in nature: nitrogen fixation, ammonification, nitrification, and denitrification. Mineralization is the only geologic process that is involved in the circulation of nitrogen. The main mineral sources of nitrogen on Earth are Bengal salt peter (KNO_3) in India and other Asian countries and Chile saltpeter ($NaNO_3$) in South America. Natural gas also contains nitrogen. Vast amounts of nitrogen are circulated by physical and chemical processes. Nitric oxide (NO) is formed in the air from N_2 and O_2 (molecular oxygen) during thunderstorms by lightning. Nitric oxide oxidizes further to nitrogen dioxide (NO_2) and later reacts with water to form nitric (or nitrous (HNO_3) acids. Acids fall to the ground during rain and form nitrates (NO_3) and nitrites (NO_2) in the soil (acid rain). Living things require nitrogen as a component for proteins, nucleic acids (deoxyribonucleic acid, or DNA, and ribonucleic acid), and other organic compounds. Nitrogen is often a limiting plant nutrient. Plants take up nitrogen from soil mainly as ammonium ions, nitrate, or nitrogenous organic compounds and incorporate nitrogen into organic molecules such as proteins or nucleic acids. The nitrogen then follows food webs from plant eaters (herbivores) to decomposers (mainly microbes). Animals use only organic forms of nitrogen.

Nitrogen Fixation:

The utilization of molecular nitrogen (N_2) by particular bacteria is called nitrogen fixation. Some of these bacteria (Rhizobium) live in symbiosis with certain legume plants and others are free-living bacteria such as cyanobacteria or Azotobacter. Legume plants include soybeans, clover, alfalfa, beans, and pears. Symbiotic nitrogen-fixing cyanobacteria provide nitrogen to other plant species such as the water-fern Azolla and liverworts and cycads. The nitrogen fixation or reduction of N_2 to NH_3 (ammonia) is a complicated, multistep process ($N_2 + 8e + 8H+ + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16P$). Ammonia produced by this process is further con verted to proteins, nucleic acids (DNA), and other nitrogen-containing organic molecules ($NH_3 \rightarrow$ nitrogenous organic molecules: proteins, nucleic acids, and so

forth). The nitrogen fixation is catalyzed by the enzyme nitrogenase. Nitrogenase is sensitive to molecular oxygen (O_2). Nitrogen-fixing organisms possess a number of morphological and biochemical modifications designed to protect enzymes from oxygen inactivation. For example, the bacterium *Rhizobium* controls the oxygen level in cells by the protein leghemoglobin, which catches oxygen. In the case of cyanobacteria, there are specialized cells (called heterocysts) for nitrogen fixation. Heterocysts show high rates of respiration, which ultimately reduces oxygen levels in these cells. Nitrogen fixation is an energy-consuming process, which explains why cyanobacteria normally have only 5 to 10 percent of heterocysts among their cells. To maintain nitrogen fixation, other cyanobacterial cells (vegetative cells) work to generate enough energy for heterocysts. All life on Earth depends on nitrogen fixation be cause the main reservoir of nitrogen on Earth is in the air as molecular nitrogen (N_2). The main path of nitrogen from the air into biologic nitrogen-containing molecules of different organisms is through nitrogen fixation. Nitrogen fixation also is of enormous importance to agriculture because it supports the nitrogen needs of many crops. This process was discovered by Russian microbiologist Sergei Winogradsky. Apart from natural nitrogen fixation, the industrial Haber-Bosch process converts molecular nitrogen to ammonia. In this process nitrogen fertilizers are made for agriculture. Haber-Bosch is an energy-consuming route, and the process of manufacturing nitrogenous fertilizers consumes up to 50 percent of the energy input in modern agriculture.

Ammonification:

Ammonification is the process of making ammonia or ammonium ions (NH_4^+) by living things. Ammonium ions are produced as a waste of such animals as fish and during decomposition of organic nitrogen wastes by bacteria and by metabolism of some bacteria. Bacteria, for example, can convert nitrate into ammonia in soils or in the human gut. Globally, only a small amount (15 %) of nitrogen reaches the atmosphere as ammonia, com pared with N_2 and N_2O. The majority of ammonium ions are quickly consumed in soil and water by microorganisms and plants. At different points in the food web, ammonium ions are returned to the environment.

Nitrification:

Nitrification is caused by the sequential action of two separate groups of soil bacteria: the ammonia-oxidizing bacteria (the nitrosifyers) and the nitrite-oxidizing bacteria (nitrifying bacteria). These bacteria obtain energy by consuming nitrogen compounds and can feed only

on inorganic compounds. The end product of nitrification is nitrate, a valuable nitrogen source for plants. Nitrification is a two-step process. Nitrosifyers, such as the bacterium Nitrosomonas, convert ammonium ion into nitrite first ($NH_4^+ + O_2 \rightarrow NO_2 + H_2O + H^+$). Later, nitrifying bacteria, such as the bacterium Nitrobacter, oxidize nitrite into nitrate (NO_2 + O2 → NO3). Nitrosifyers and nitrifying bacteria are common in soil and water. They live especially in areas where ammonia is present in high amounts, such as sites of ammonification and in wastewater and manure. Nitrification does not contribute significantly to agriculture. Although liked by plants, nitrate is not always available for plants in soils. Nitrate is quickly consumed by microorganisms during denitrification. Additionally, one species of Archaea (microorganisms similar to bacteria) undergoes nitrification by oxidizing ammonia in the oceans.

Denitrification:

The conversion of nitrate into gaseous nitrogen compounds such as N_2O, NO, and N_2 by different bacteria in soils is called denitrification, or nitrate reduction. Bacteria use nitrate as a substitute for oxygen during respiration and convert it to different nitrogenous compounds according to the following chain of reactions: $NO_3 \rightarrow NO_2 \rightarrow NO \rightarrow N_2O \rightarrow N_2$. Eventually, nitrogen is released into the atmosphere as N_2O and NO or as N_2. Simultaneously bacteria decompose significant amounts of organic matter within the soil. Denitrification has a negative effect on agriculture, as it removes nitrogen from soils. In contrast, denitrification can be useful in wastewater treatment.

The Phosphorus Cycle:

Phosphorus, Crops and the Environment

This agronomy fact sheet provides a brief overview of the important components of the phosphorus (P) cycle. Understanding the P cycle can help producers make decisions regarding P management on the farm, both for farm profitability and protection of theenvironment. Most plants are only about 0.2% P by weight, but that small amount is critically important. Phosphorus is an essential component of adenosine triphosphate (ATP), which is involved in most biochemical processes in plants and enables them to extract nutrients from the soil. Phosphorus also plays a critical role in cell development and DNA formation. Insufficient soil P can result in delayed crop maturity, reduced flower development, low seed quality, and decreased crop yield. Too much P, on the other hand, can be harmful in some situations; when P levels increase in fresh water streams and lakes, algae

blooms can occur. When algae die, their decomposition results in oxygen depletion which can lead to the death of aquatic plants and animals. This process is called “eutrophication”.

Crop Uptake

One goal with field crop management is to optimize crop uptake of available P. A typical corn silage crop will remove about 4.3 lbs of P_2O_5 per ton of silage (35% dry matter). Soil testing of available P can help avoid application of fertilizer P that is not needed for optimum production. Applying fertilizer beyond crop needs is a waste of time and money, and can be harmful to the environment.

Phosphorus exists in many different forms in soil. For practical purposes, we can group these sources into four general forms: (1) plant available inorganic P, and three forms which are not plant available: (2) organic P, (3) adsorbed P, and (4) primary mineral P. The P cycle in Figure 1 shows these P forms andthe pathways by which P may be taken up by plants or leave the site as P runoff or leaching. The general P transformation processes are: weathering and precipitation, mineralization and immobilization, and adsorption and desorption. Weathering, mineralization and desorption increase plant available P. Immobilization, precipitation and adsorption decrease plant available P.

Weathering and Precipitation

Soils naturally contain P-rich minerals, which are weathered over long periods of time and slowly made available to plants. Phosphorus can become unavailable through precipitation, which happens if plant available inorganic P reacts with dissolved iron, aluminum, manganese (in acid soils), or calcium (in alkaline soils) to form phosphate minerals.

Mineralization and Immobilization

Mineralization is the microbial conversion of organic P to H2PO4- or HPO4 2- , forms of plant available P known as orthophosphates. Immobilization occurs when these plant available P forms are consumed by microbes, turning the P into organic P forms that are not available to plants. The microbial P will become available over time as the microbes die. Maintaining soil organic matter levels is important in P management. Mineralization of organic matter results in the slow release of P to the soil solution during the growing season, making it available for plant uptake. This process reduces the need for fertilizer applications and the risk of runoff and leaching that may result from additional P. Soil temperatures between 65 and 105°F favor P mineralization.

Adsorption and Desorption

Adsorption is the chemical binding of plant available P to soil particles, which makes itunavailable to plants. Desorption is the release of adsorbed P from its bound state into the soil solution.

- Adsorption (or "fixing" as it is sometimes called) occurs quickly whereas desorption isusually a slow process.
  - Adsorption differs from precipitation: adsorption is reversible chemical binding of P to soil particles while precipitation involves a more permanent change in the chemical properties of the P as it is removed from the soil solution.
  - Soils that have higher iron and/or aluminium contents have the potential to adsorb more P than other soils.
  - Phosphorus is in its most plant available form when the pH is between 6 and 7. At higher pH, P can precipitate with Ca. At lower pH, P tends to be absorbed to Fe and Al compounds in the soil. Every soil has a maximum amount of P that it can adsorb. Phosphorus losses to the environment through runoff and/or leaching increase with P saturation level.
  - Precise fertilizer placement can decrease P adsorption effects by minimizing P contact with soil and concentrating P into a smaller area. Band application of fertilizer is a common example of this.

Runoff

Runoff is a major cause of P loss from farms. Water carries away particulate (soil-bound) P in eroded sediment, as well as dissolved P from applied manure and fertilizers. Erosion control practices decrease P losses by slowing water flow over the soil surface and increasing infiltration.

Leaching

Leaching is the removal of dissolved P from soil by vertical water movement. Leaching is a concern in relatively high P soils (near or at P saturation), especially where preferential flow or direct connections with tile drains exist.

Summary

Crop uptake is the goal of applying P fertilizer or manure to the soil. If soil test P levels are already optimum, P additions through fertilizer or manure should not exceed crop removal. If additional P is needed (soils testing low or medium in P), P adsorption can be minimized by band applications and by maintaining an optimum pH. Naturally occurring immobilization of P by microbes can help ration plant available P to crops over the course of a growing season. Steps should be taken to reduce losses in order to maximize the efficiency of fertilizer and manure application.

Probable Questions:

1. Describe the components of ecosystem?
2. Who proposed the term Gaia Hypothesis ? Illustrate it.
3. How did the aerobic organisms thrieve within the primitive earth condition?
4. Who coined the term 'Homeorhesis'? Describe with suitable diagram?
5. How does a control system work to impart stability in an ecosystem?
6. How does the concept of 'Resistance' & 'Resilience' control the concept of 'Ecosystem stability'.
7. Explain "Cody's Model" in the light of 'Optimization'.
8. Desert ecosystem shows low resistance but high resilence – Justify with suitable example?
9. What is the classical definition of 'Optimization'? Why it is hard to achieve? What is cody's idea of explaning 'optimization'?
10. Describe Phosphorous cycle.
11. Describe Nitrogen Cycle.
12. What are ammonification, nitrification and denrification.
13. Write short notes on leaching, runoff.
14. Differentiate between Adsorption and Desorption

Suggested Readings/ References:

1. J. E. Lovelock (1972). "Gaia as seen through the atmosphere". *Atmospheric Environment*. 6 (8):579–580. Bibcode:1972AtmEn...6..579L. doi:10.1016/0004-6981(72)90076-5
2. Lovelock, J.E.; Margulis, L. (1974). "Atmospheric homeostasis by and for the biosphere: the Gaia hypothesis". *Tellus*. Series A. Stockholm: International Meteorological

Institute. 26 (12):210. Bibcode:1974Tell...26....2L. doi:10.1111/j.21533490.1974.tb0194 .x. ISSN 1600-0870. Retrieved 20 October 2012.

3. McNaughton, S. J. and Coughenour, M. B. (1981). The Cybernetic Nature of Ecosystems. *The American Naturalist*.117(6). pp. 985-990.
4. Michael Begon, Colin R. Townsend, and John L. Harper. (2006). *Ecology: From Individuals to Ecosystems.*Blackwell Publishing.ISBN 978-1-4051-1117-1
5. Ricklefs, R.E. and Miller, G.L. (2000) Ecology. 4th Edition, W.H. Freeman, New York.
6. Global Biogeochemical Cycles. Samuel S Butcher. 2012.
7. Fundamentals of Ecology. Eugene Odum. 5th Edition 2006.
8. Elements of Ecology. Smith and Smith. 9th Edition.2018.

Unit-VI

Community: Structure and Gradient analysis. Niche theory: Niche concepts, niche width

Objective:

In this Unit we will discuss about Community: Structure and Gradient analysis. In this Unit we will also discuss about Niche Theory, Niche Concept and Niche width

Introduction:

The group of species that occupy a given area, interacting either directly or indirectly, is called a **community.** The definition of community also recognizes that species living in close association may interact. They may compete for a shared resource, such as food, light, space, or moisture. One may depend on the other as a source of food. They may provide mutual aid, or they may not directly affect each other at all. A community also has several attributes. These attributes include the number of species, their relative abundances, the nature of their interactions (mutualism, commensalism, symbiosis, parasitism, predation and competition) and their physical structure (defined primarily by the growth forms of the plant components of the community).

Community Structure:

A community is composed of individuals and populations. The species that assemble to make up a community are determined by dispersal constraints, environmental constraints and internal dynamics (Belyea& Lancaster, 1999). Different ecological communities can be pretty different in terms of the types and numbers of species they contain. For instance, some Arctic communities include just a few species, while some tropical rainforest communities have huge numbers of species packed into each cubic meter. One way to describe this difference is to say that the communities have different structures. Community structure is essentially the composition of a community, including the number of species in that community and their relative numbers. It can also be interpreted more broadly, to include all of the patterns of interaction between these different species. Some ecologists also use the term "community structure" for the pattern of a community in space – how the populations that make it up are distributed in the physical environment. For instance, a community may have sharp edges or

may blend gradually into the neighboring community. Also, different populations may occupy different areas inside the community's boundaries.

Ecologists use to describe the composition of a community by two important measures i.e. species richness and species diversity.

- **Species richness:**

  Species richness is the number of different species in a particular community. Species richness is simply a count of species, and it does not take into account the abundances of the species or their relative abundance distribution. For instance, if we found 100 species in one community and 1000 species in another community, the second community would have much higher species richness than the first. Communities with the highest species richness tend to be found in areas near the equator, which have lots of solar energy (supporting high primary productivity), warm temperatures, large amounts of rainfall, and little seasonal change. Communities with the lowest species richness lie near the poles, which get less solar energy and are colder, drier, and less amenable to life. This pattern is illustrated below for mammalian species richness (species richness calculated only for mammal species, not for all species). Many other factors in addition to latitude can also affect a community's species-richness.

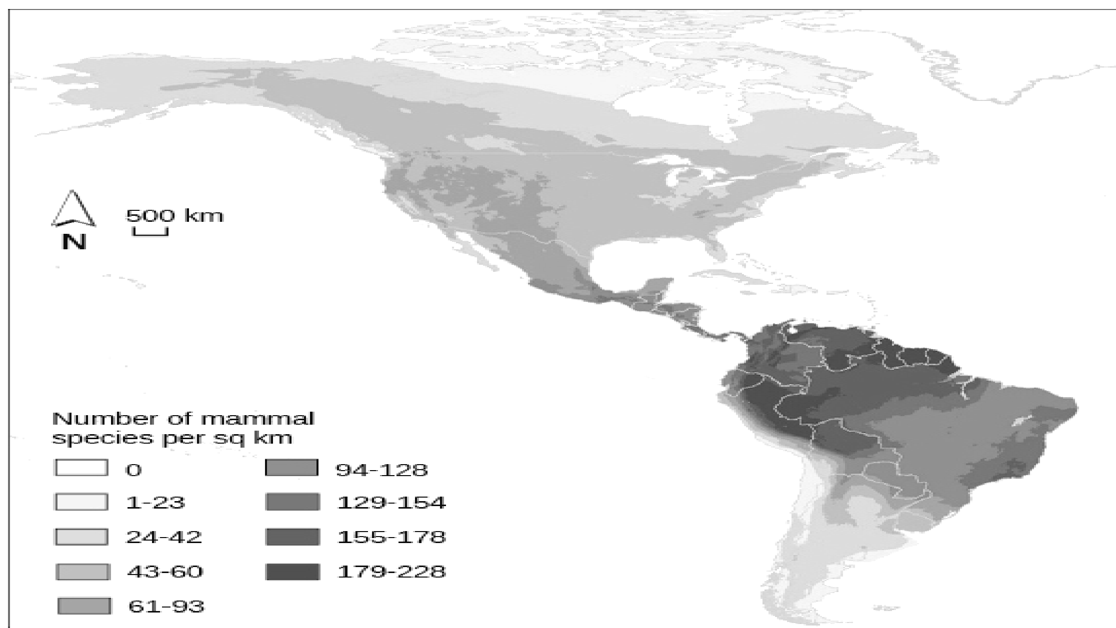


Figure: Map shows the spatial distribution of mammal species richness in North and South America. The highest number of mammal species, 179-228 per square kilometer, occurs in

the Amazon region of South America. Species richness is generally highest in tropical latitudes, and then decreases to the north and south, with zero species in the Arctic regions. Global species richness as calculated for mammal species.

- **Species diversity:**

  Species diversity is a measure of community complexity. It is a function of both the number of different species in the community (species richness) and their relative abundances (species evenness). Larger numbers of species and more even abundances of species lead to higher species diversity. For example, a forest community with 200500 different kinds of trees would have greater species diversity than a forest community with only 5000 kinds of trees. Communities in which the species are all more or less equal in abundance exhibit evenness, whereas communities with one or a few abundant species and many rare one show dominance.

Diversity Index:

A diversity index is a mathematical measure of species diversity in a community. It takes into account the number of species present (species richness), as well as the abundance of each species (evenness).

Simpson's diversity index (D):

One of the simplest and most widely used indices of diversity is the **Simpson's index (D)**. Simpson's index (*D*)measures the probability that two individuals randomly selected from a sample will belong to the same species (category). It is actually the measurement of dominance.

$$D = \sum \left(\frac{n}{N}\right)^2 \text{ or } D = \frac{\sum n(n-1)}{N(N-1)}$$

Where n is the total number of organisms of a particular species and N is the total number of organisms of all species.

The value of *D* ranges between 0 and 1. In the absence of diversity, where only one species is present, the value of *D* is 1. As both species richness and evenness increase, the value approaches 0. Because the greater the value of *D,* the lower the diversity, *D* is often

subtracted from 1 to give Simpson's index of diversity = 1 – *D*. The value of this index also ranges between 0 and 1, but now the value increases with diversity. In this case, the index represents the probability that two individuals randomly selected from asample will belong to different species.

Shannon's diversity index (H'):

Shannon's diversity index (H') is another index that is commonly used to characterize species diversity in a community. Shannon's index accounts for both abundance and evenness of the species present.

$$H' = \sum_{i=1}^{s} -P_i In P_i$$

Where, S = Total number of species in the sample (species richness).

P_i = Relative abundance of each species, calculated as the proportion of individuals of a given species to the total number of individuals in the community (n/N).

n = The number of individuals of a species.

N = Total numbers of individuals in the sample.

Factors influencing community structure:

The structure of a community is the result of many interacting factors, both abiotic (non-living) and biotic (living organism-related). Here are some important factors that influence community structure:

- The climate patterns of the community's location.
- The geography of the community's location.
- The heterogeneity (patchiness) of the environment.
- The frequency of disturbances, or disruptive events.
- Interactions between organisms.

A community's structure can also be shaped by the chance events that happened during its history. For instance, suppose that a single seed blows into the dirt of a particular area. If it

happens to take root, the species may establish itself and, after some period of time, become dominant (excluding similar species). If the seed fails to sprout, another similar species may instead be the lucky one to establish itself and become dominant.

Foundation Species:

A **foundation species** plays a unique, essential role in creating and defining a community. Often, foundation species act by modifying the environment so that it can support the other organisms that form the community.

Example:

Kelp (brown algae) is a foundation species that forms the basis of the kelp forests off the coast of California. Kelps create environments that allow the survival of other organisms that make up the kelp forest. The corals of a coral reef are another foundation species. The exoskeletons of living and dead coral make up most of the reef structure, which protects other species from waves and ocean currents. Beavers, which modify their environment by building dams, can also be seen as a foundation species.

Image: Kelp (brown algae) of kelp forests of California.

Image: Beaver building dams.

Keystone species: A **keystone species** is a species that has a disproportionately large effect on community structure relative to its biomass or abundance. Keystone species differ from foundation species in two main ways: they are more likely to belong to higher trophic levels (to be top predators), and they act in more diverse ways than foundation species, which tend

to modify their environment. Keystone species are species that function in a unique and signify cant manner through their activities and their removal initiates changes in community structure and often results in a significant loss of diversity. Their role in the community may be to create or modify habitats or to influence the interactions among other species.

Example:

The intertidal sea star *Pisastero chraceus*, which is found in the northwestern United States, is perhaps the most famous example of a keystone species. In a classic experiment of community ecology, the sea stars were experimentally removed from the intertidal zone where they lived. As a result, populations of their prey (mussels) increased, altering the species composition of the community and sharply reducing species diversity. When the sea stars were present, about 25 species of barnacles and algae were found in the lower part of the intertidal zone, but when they were missing, the mussel population expanded downward and almost entirely replaced these other species. This type of sharp reduction in diversity or collapse of community structure commonly occurs when a keystone species is removed. In this case, the loss of diversity happened because the mussels crowded out other species, which could normally persist because the sea stars kept the mussels in check.

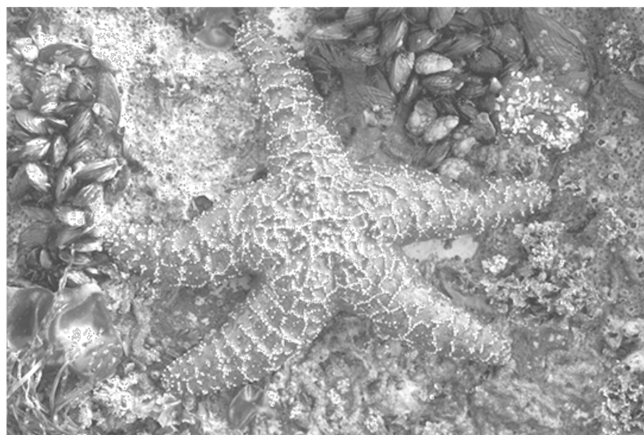


Figure: Photograph of sea star.

Functional groups within a community:

- **Guilds:**

The grouping of species into trophic levels is a functional classification, defining groups of species that derive their energy in a similar manner. Another approach is to subdivide each

trophic level into groups of species that exploit a common resource in a similar fashion; these groups are termed guilds.

For example, humming birds and other nectar-feeding birds form a guild of species that exploit the common resource of flowering plants in a similar fashion. Likewise, seed-eating birds could be grouped into another feeding guild within the broader community. Because species within a guild draw upon a shared resource, there is potential for strong interactions between the members.

- **Functional type:**

The term **functional type** is now commonly used to define a group of species based on their common response to the environment, life history characteristics, or role within the community.

For example, plants may be classified into functional types based on their photosynthetic pathway (C3, C4, and CAM) which, as we have seen earlier, relates to their ability to photosynthesize and grow under different thermal and moisture environments.

Qualitative characters of communities:

The qualitative character is a analytic attribute of community. Qualitative characters are difficult to measure. Some important qualitative characters include:

a) Stratification: Tropical forests are characterized typically by a marked vertical stratification. Stratification results in the upper strata or canopy, receiving a larger proposition of solar energy, and in instances of particularly dense foliage, little sunrise reaches the ground reducing photosynthesis and plant growth at that level. Stratification is also seen in the underground plant parts, that is the roof and the rhizome systems. Root systems of different plant species tap moisture and nutrients from different soil depths enable them to avoid competition and too much exploitation of a particular soil layer.

Aquatic ecosystems also have marked stratification. In lake ecosystems light penetration, temperature, and availability of oxygen vary with depth.

b) Vitality and Vigour: Vitality is related to the condition of a plant and its capacity to complete its life cycle, while vigour refers more specifically to the health or development within a certain stage. A number of criteria may be used in determining the vigour of plants such as the rate and total amount of growth especially in height; rapidity of growth renewal in

spring or following mowing or grazing; area of foliage, colour and turbidity of leaves and stems; degree of damage causes by disease or insects etc.

c) Periodicity: It refers to the study of seasonal changes in the community. Periodicity is a strong fixed characters in the plants. Different species of plants has different periods of seed germination, vegetative growth, flowering and fruiting, leaf fall, seed and fruit dispersal. Study of the date and time of these events is termed as phenology. The appearance of the community as a whole at different season is called aspection.

d) Physiognomy: Physiognomy is the assessment of characters from their outer appearance. The general appearance of vegetation can be determined by the growth form of the dominant species and may be described as grassland, forest etc.

e) Sociability: Sociability refers to the nature of grouping of individual plants, that is, whether they grow singly, in patches, in colonies etc. Sociability expresses the degree of association between species.

Physical Structure of communities:

The physical structure of the community reflects abiotic factors, such as the depth and flow of water in aquatic environments. It also reflects biotic factors, such as the spatial arrangement of the resident organisms. For example, the size and height of the trees and the density and spatial distribution of their populations help define the physical attributes of the forest community. The form and structure of terrestrial communities are defined primarily by their vegetation. Plants may be tall or short, evergreen or deciduous, herbaceous or woody. Such characteristics can describe growth forms.

In aquatic environments, the dominant organisms are also used to classify and name communities. Kelp forests, sea grass meadows, and coral reefs are examples. The physical structure of aquatic communities is more often defined by features of the abiotic environment, such as water depth, flow rate, or salinity.

The vertical structure of the plant community also provides a physical framework in which many forms of animal life are adapted to live. A well-developed forest ecosystem, for example, has multiple layers of vegetation. From top to bottom, they are the canopy, the understory, the shrub layer, the herb or ground layer, and the forest floor. The upper layer, the canopy, is the primary site of energy fixation through photosynthesis. The canopy structure plays as a major influence on the rest of the forest. If the canopy is fairly open, considerable

sunlight will reach the lower layers. If ample water and nutrients are available, a well-developed understory and shrub strata will form. If the canopy is dense and closed, light levels are low, and the understory and shrub layers will be poorly developed.

The nature of the herb layer depends on the soil moisture and nutrient conditions, slope position, density of the canopy and understory, and exposure of the slope, all of which vary from place to place throughout the forest. The final layer, the forest floor, is where the important process of decomposition takes place and where microbial organisms feeding on decaying organic matter release mineral nutrients for reuse by the forest plants.

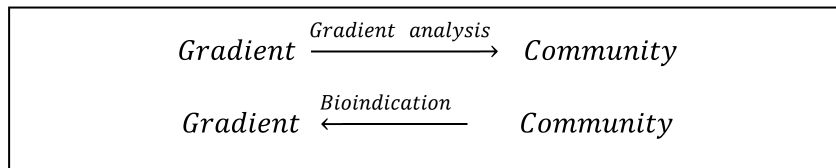
Aquatic ecosystems such as lakes and oceans have strata determined largely by light penetration through the water column. They also have distinctive profiles of temperature and oxygen. In the summer, well-stratified lakes have a layer of well-mixed water, the epilimnion; a second layer, the metalimnion, which is characterized by a thermocline (a steep and rapid decline in temperature relative to the waters above and below); the hypolimnion, a deep, cold layer of dense water at about 4°C (39°F), often low in oxygen; and a layer of bottom sediments. Two other structural layers are also recognized, based on light penetration: an upper zone, the photic zone, where the availability of light supports photosynthesis primarily by phytoplankton; and in deeper waters, the aphotic zone, an area without light. The bottom zone, where decomposition is most active, is referred to as the benthic zone.

Gradient analysis:

Introduction: It is often difficult to determine where one community ends and the next community begins. Many communities grade continuously into each other with no sharp boundaries. The zone of vegetation separating two different types of communities called ecotone. It is also known as a transition zone. Ecotone is a region where the influence of two different patterns of environment works together and hence the vegetation of ecotone is highly specialized. A general characteristic of ecotone is that it has sufficiently greater number of species and high diversity as compared to neighbouring communities. The phenomenon of increased species diversity at the boundary (edge) is called the edge effect. Species that uses the edges for the purposes of reproduction or survival termed as edge species. Gradient analysis is the small step on the way to the objective description of the communities. Choice of gradient is always subjective. The environmental factor that is

chosen as a gradient is more or less loosely correlated with whatever really matters in the lives of the species involved.

So, Gradient analysis is the relation of the species and environmental variation or gradients.



Types of Gradients: Austin, Smith (1989) discussed the current issues in continuum theory and offered a new model. The authors distinguish three types of gradients:

Indirect or complex gradients – These are complex gradients such as elevation gradient, for example. The influence of elevationis indirect, through variables like temperature and precipitation, which have direct effect on plant growth. These variables, depending on the site location, are in complex correlation with the elevation. Relationships between vegetation and these environmental gradients cannot be extrapolated beyond the studied territory.

Resource gradients –These are variable resources consumed by plants for their growth. For the autotrophy plants these resources are with limited number: light, water, CO_2, O_2, nutrients. Plant reaction to the resource gradients is relaxed. Plants react only to the toxic levels, exceeding normal concentrations in the environment.

Direct gradients – These are gradients having direct physiological effect on plant growth but are not consumed by plants, for example, air temperature and soil pH. Direct gradients regulate plant growth and support plant physiological integrity. Plant physiological response shows different adaptations in different temperature regimes and their fundamental niches are dispersed along the gradient.

Organismic and continuum concept of communities:

Organismic concept:

Frederick Clements proposed this concept. Clements likened associations to organisms, with each species representing an interacting, integrated component of the whole. Development of the community through time (a process termed *succession*) was viewed as development of the

organism in this concept. This view of the community suggests mutualism and co evolution play an important role in the evolution of species that make up the association. The community has evolved as an integrated whole; species interactions are the "glue" holding it together.

Continuum or individualistic concept:

It is H. A. Gleason's view on community. The continuum concept states that the relationship among coexisting species (species within a community) is due to similarities in their requirements and tolerances, not to strong interactions or common evolutionary history. Gleason concluded that changes in species abundance along environmental gradients occur so gradually that it is not practical to divide the vegetation (species) into associations. Species distributions along environmental gradients do not form clusters but represent the independent responses of species. Transitions are gradual and difficult to identify.

Different Approaches in Gradient Analysis:

Goodall(1954) first introduced the term 'ordination', relevant to methods, which arrange samples or species in 'multidimensional series'. Ordination in vegetation ecology is the process of sample (or species) arrangement along one or more environmental gradients, or along abstract axes, which may represent important environmental gradients.

Two different approaches are used-

Direct gradient analysis or **ecological ordination**:

Inthis technique vegetation data are investigated graphically or mathematicallyin the context of selected environmental gradients. This method is a simple graphical technique for representation of species distribution along the environmental gradients, which are *a priori* considered as important for plants.

Indirect gradient analysis or vegetation ordination:

In this gradient analysis mathematical methods are used for summarizing the emergent data structure of species and samples in a few abstract dimensions. In indirect gradient analysis there is not *a priori* assumptions about which are the most important environmental gradients influencing vegetation. Graphical result from the analysis shows which samples are located in extreme and intermediate parts of the environmental gradient. Next, appropriate statistical

tests are used, which on the basis of statistically significant correlations, show the environmental variables having sense in the species and sample arrangement.

Models of Gradient analysis:

Gaussian response model: The most popular model that gives symmetric responses, and is the basis of much of theory of ordination and gradient analysis.

$$\mu = h\exp(-\frac{(x-u)^2}{2t^2})$$

Three interpretable parameters of Gaussian response are

1. Location of optimum *u* on gradient *x*.
2. Expected height *h* at the optimum.
3. Width *t* of the response.

Evidences of Gaussian response:

- Whittaker described many response types: multimodal, skewed, flat, plateau and symmetric.
- Only a small part of responses were regarded as symmetrical, still became the standard.
- First canonized in coenocline simulations.
- Species packing is the theoretical basis of (canonical) correspondence analysis.

Other Popular response models:

Beta response: Able to produce responses of varying skewness and kurtosis, and challenges the Gaussian dominance.

$$\mu = k(x-p_1)^{\alpha}(p_2-x)^{\gamma}$$

- Responses with varying skewness and kurtosis.
- Simulated coenoclines to test robustness of ordination.
- Commonly fitted fixing endpoints p_1 and p_2 and using GLM.
- Must be fitted with non linear regression.

HOF (Huisman-Olff-Fresco model) response: A family of hierarchic models which can be produce skewed, symmetric or different monotone responses, and can be used to analyse the response shape.

- Alternative models differ only in response shape.
- Selection of parsimonious model with statistical criteria.
- 'Shape' is a parametric concept, and parametric HOF models may be the best way of analyzing in response shape.

GAM models: Can find any smooth shape and fit any kind of smooth response.

$$g(\mu) = smooth\ (x)$$

- Generalized from GLM: Linear predictor replaced with smooth predictor.
- Smoothening by regression splines or by other smoothers.
- Degree of smoothing is controlled by degree of freedom
- Enormous use in ecology- also outside gradient modelling.

NICHE CONCEPT:

Introduction- For a species to maintain its population, its individuals must survive and reproduce. Certain environmental conditions are necessary for individuals of each species to tolerate the physical environment, obtain energy and nutrients, and avoid predators. The total requirements of a species for all resources and physical conditions determine where it can live and how abundant it can be at any one place within its range. These requirements are termed abstractly the ecological niche.

Definition of Niche- G.E. Hutchinson (1958) suggested that the niche could be modelled as an imaginary space with many dimensions, in which each dimension or axis represents the range of some environmental condition or resource that is required by the species. Thus, the niche of a plant might include the range of temperatures that it can tolerate, the intensity of light required for photosynthesis, specific humidity regimes, and minimum quantities of essential soil nutrients for uptake.

Ecological Niche-

An ecological niche is the role and position a species has in its environment; how it meets its needs for food and shelter, how it survives, and how it reproduces. A species' niche includes all of its interactions with the biotic and abiotic factors of its environment. Biotic factors are living things, while abiotic factors are non-living things. It is advantageous for a species to

occupy a unique niche in an ecosystem because it reduces the amount of competition for resources that species will encounter.

In otherwords ecological niche is all of the physical, chemical and biological conditions required by a species for survival, growth and reproduction. Two further abstractions of this concept are the fundamental niche and the realized niche. A useful extension of the niche concept is the distinction between fundamental and realized niches. The fundamental niche of a species includes the total range of environmental conditions that are suitable for existence without the influence of interspecific competition or predation from other species. The realized niche describes that part of the fundamental niche actually occupied by the species.

Difference between Niche and Habitat: Niche vs habitat

Every organism in the planet has a significant role to play in the ecosystem of the earth. They have their own places to live, functions, and amazing ways of reproduction to multiply their species. To help maintain the biological world people must be informed, not only on the physical aspect of it, but on a deeper level of how they live as one.The ecological study or the way of learning about the way these organisms interact with each other in the ecosystem is through studying its ecological niche and habitat. The niche and the habitat may have common attributes, but they are totally different from each other. To have a deeper knowledge of the biological world, it is best that these two be defined and differentiated.

The most basic and the most difficult thing to understand in this study is the ecological niche. This is the study of how of adaption of these organisms to their ecological community is also very important in studying the ecological niche concept. The habitat, on the other hand, is focused more on the location or the place in the organisms relate or fit in the ecosystem that it belongs to. The physical structure of the organisms is one of the very important factors for this concept or study. The physical attributes of the organisms are called the morphology. The psychological and behavioural ways environment where the organisms live. The resources available to each species, whether physical or biological, are very important in this study or aspect.

The niche is the study that is more focused on the response of the organism to the limited resources of the environment and their competitors. On the other hand, the habitat is more focussed on where the organisms live and how their environment affects them. To describe niche easier, it is the study of how an organism makes a living in the ecological community it belongs to, while the habitat is defined as where the organisms live. With this simple definition, it will be easy to differentiate the two. Niche and habitat is correlated in the study

of biodiversity. By studying these two, people may be able to help keep the homes of different organisms healthy and ultimately maintaining the numbers of the organisms in the ecosystem. In today's fast developing world, there are tendencies that the bio-diversities of this world are taken for granted. This is why studying the concept of niche and habitat is very important in today's modern world.

Summary of Niche vs habitat:

1.The study or the concept of the ecological niche is more difficult to understand than that of the habitat.

2. The niche is more on how the organisms behave in the places where they belong, while the habitat is focussed on the places where each organism belongs.

3. The niche is more on how organisms react to their environment while habitat is more on how their environment affects them.

4. The short definition for niche is how an organism makes a living in the place it belongs, while the habitat is where the organisms live.

Examples of Niche:

a) Pandas

Giant panda bears (*Ailuropoda melanoleuca*) are niche specialists. They have a very limited diet, 99% of which consists of bamboo. They have evolved specially adapted thumbs, which allow them to grip the bamboo. Bamboo does not provide much nutrition, and so the pandas must spend most of their time eating, consuming around 70lbs of bamboo every day to support their large bodies.

To conserve energy, pandas do not move very far, and so have a home range of around only 3 miles, near streams so that fresh drinking water is available, and where there are caves suitable for raising their young. Their highly specialized diet means that they do not usually encounter interspecific competition, and they live solitary lives so as to avoid intraspecific competition. Adults have no natural predators, so their populations are kept to suitable levels through their life cycles; in the wild they reproduce only once every two years, which means natural populations do not get too dense and intraspecific competition does not occur frequently. Because the niche of pandas is so specialized, they are exceptionally vulnerable to human impact and their populations have experienced dramatic declines.

The biggest threat has been deforestation for farmland, mining and logging, which has destroyed most of their habitat. They are now restricted to the humid bamboo forests of a few

mountains in South Western China where they prefer the cool temperatures at high altitudes of around 4,000 to 10,000 ft.

b) The Birds of New Zealand

The island of New Zealand is a geographically isolated landmass off the southeast coast of Australia. Because of the great distance between New Zealand and any other large landmass, the only organisms that were able to colonize the land were those which were able to fly or float across the sea. Because migration on to the island was so difficult, the island community completely lacked mammals, except for three species of bat and mammals that were able to swim, such as seals and sea lions.

In the absence of mammals, the native animals filled ecological niches of predation, scavenging and grazing, which are filled by mammals in most other ecosystems. This resulted in a diverse set of morphologically distinct birds, insects and reptiles, which are like no others seen on Earth. For example, the South Island takahē (*Porphyrio hochstetteri*) and the Kakapo Parrot (*Strigops habroptilus*) evolved to assume the role of grazers such as sheep, feeding on grass, shoots and leaves. The Giant Moa (*Dinornis robustus* and *Dinornis novaezelandiae*), although now extinct, were large birds, growing up to around 12ft tall and over 500lb in weight. These birds fed on twigs, leaves and other various plant parts, assuming the niche that in other parts of the world is filled by deer and other ungulate browsing herbivores. The Kiwi, a nocturnal bird of the genus Apteryx, assumes the niche that small mammals such as mice and moles usually fill, feeding on seeds, fruit, invertebrates and grubs. The only predators on the island were flying birds, so in the absence of land-based predators, and as a result of their terrestrial niche roles, many of New Zealand's birds evolved to become flightless; even the bats spend most of their time on the ground.

When humans first arrived on the islands around 700 years ago, they brought with them rats and dogs, and later, Europeans brought stoats, pigs, dogs, cats, sheep, cattle, deer and many other animals. This has been devastating to the native wildlife; they are either outcompeted by other organisms that are adapted to fill particular niches or they are directly predated on because they lack protection against land-based predators. The case of New Zealand's birds is not entirely an anomaly. Islands that have been isolated for a long time often develop their own unique flora and fauna which have evolved to fill niches in the absence of other organisms. For example, the lemurs of Madagascar evolved diverse adaptions to fill many niches not usually filled by primates, due to Madagascar's varied terrains and habitats and a lack of other types of mammal. Another famous example of niche adaption is Darwin's

finches on the Galapagos Islands. These birds evolved different beak shapes and body sizes on each island, according to the types of food that was available.

Importance of Niche:

Niche concept has several significances. In ecology, a niche is a term describing the relational position of a species or population in an ecology and how a population responds to the abundance of its resources and enemies (e. g., by growing when resources are abundant, and predators, parasites and pathogens are scarce) and how it affects those same factors (e. g., by reducing the abundance of resources through consumption and contributing to the population growth of enemies by falling prey to them).

1) The niche includes a biotic or physical environment which is also a part of the ecosystem.

2) More formally, the niche includes niche because it influences how populations affect, and are affected by, resources and enemies.

3) The description of a niche may include descriptions of the organism's life history, habitat, and place in the food chain.

4) According to the competitive exclusion principle, no two species can occupy the same niche in the same environment for a long time.

5) A species' niche is determined by the traits that allow it to gather resources, evade enemies and any other factor that influences its relative birth and death rates.

6) The niche is often heralded as one of the most important concepts in community ecology, studies of the niche are often considered tautological, owing to the seemingly insurmountable task of defining the dimensions of the niche, especially those that help maintain species diversity.

7) Data on niches can be used to: (i). Make comparisons of the composition and organization of communities.

(ii) Examine shifts in the behaviour or ecology of one species in response to another species. (In particular, niche shifts are commonly used to study interspecific competition, based on Gause’s Principle of Competitive Exclusion).

Types of Niche - Niche is generally four types. Such as- A] Fundamental Niche , B] Realized Niche C] Spatial Niche and D] Multidimensional Niche

Fundamental Niche:

Every species has a role that it plays in nature. That role is defined by a combination of the organism's behaviours, habitat, and interaction with other species. For example, a garden spider is a predator that hunts for prey among plants, while an oak tree grows to dominate a forest canopy, turning sunlight into food. The role that a species plays is called its ecological niche.A niche includes more than what an organism eats or where it lives. Environmental factors, such as climate, soil chemistry, and elevation, also play a role in defining a niche.Sometimes other species will compete for the same niche. Lions on the African savanna compete with hyenas for food. Daisies and dandelions in a field compete for sunlight and soil. Competition from other species for the same niche is called interspecific competition. In order to better understand an organism, ecologists try to determine what sort of niche it would have in the absence of such competition. They might ask, 'How would a lion behave if there were no other predators competing for a zebra's flesh?' or 'What would a stand of water lilies look like if there was no duckweed living in the same area of the pond?' A fundamental niche is the term for what an organism's niche would be in the absence of competition from other species. Generally, however, there are competitors for the same lifestyle. Rabbits compete with groundhogs for food. Grasses compete with shrubs for soil, and bacteria compete with mold for nutrients among the leaf litter. The niche that a species actually inhabits, taking into account interspecific competition, is its realized niche.

Definition of fundamental niche

The full range of environmental conditions and resources an organism can possibly occupy and use, especially when limiting factors are absent in its habitat. The fundamental niche describes the potential area and resources an organism is capable of using. But the presence of limiting factors such as direct competition with other organisms, the organism tends to occupy a niche narrower than this.

In another word the fundamental niche is the theoretical niche of an organism given that there are no limiting factors on the environment or resources the organism can use/inhabit.

Explanation of the above definition:

A fundamental niche is the niche the organism would have if there were no limiting factors, such as predators, competitors, parasites, and disease. A fundamental niche differs from a realized niche because limiting factors often exist in the real world. The niche the organism actually occupies in the wild is called the realized niche.

For example, let's say an animal has a diverse, omnivorous diet. It can travel in many habitats and find sufficient food. However, if a predator is introduced in one of those habitats, the animal will avoid that area and no longer feed in that habitat. Thus, its realized niche differs from its fundamental niche.

Below we have an example where competition is the limiting factor for a species of barnacle:

Examples of Fundamental Niches

The male red-winged blackbird's mating call can be heard in the marshes in early spring. At that time, they hold the prime real estate in the marsh. However, as the season progresses, the more aggressive tri-color blackbirds move in. The tri-colors take over the best territory and force the red-wings to choose the leftovers. The entire marsh represents the red-winged blackbirds' fundamental niche

Spartina alterniflora is a grass species that is very tolerant of salt. It lives in salt marshes along the eastern North American coast. These marshes go underwater at high tide. While it is found all along the coast, it does not live in nearby freshwater environments.

Tolerance to Environmental Conditions-

Each species is tolerant of a range of certain environmental conditions, such as temperature, light and moisture, which are essential for their survival mechanisms and for growth, reproduction and feeding.

Organisms which live in the littoral zone of a coastal habitat are exposed to extreme changes in these conditions on a daily basis. A study on two species of barnacle within the littoral zone demonstrates how the niche of two organisms could be affected by slight changes in their environmental tolerance, in the presence of another species. are species removed, the larger *Semibalanus balanoides* is a species of large barnacle, adapted to living within deep water where it is rarely exposed to air. *Chthamalus stellatus* is a slightly smaller species, living in both deep and shallow water and capable of withstanding exposure to air which causes dehydration. Systematic removal of each species from an area is able to show that the smaller species could occupy shallow and deep zones in the absence of the large species. However, when smaller species do not occupy the shallow zone. In the natural environment when both species are present, the larger *S.balanoides* outcompete the smaller *C.stellatus* for resources and space, and the smaller species do not inhabit the deeper zone; this is called competitive exclusion. The fundamental niche of the two barnacles overlaps in the deeper

zone, although the actual niche of the smaller species can only be realized in the shallow zone. In contrast, the fundamental niche of the large species is met in its realized niche.

Realized niche-General concept:

The role that an organism plays in nature is called ecological niche. For an animal, that niche includes things like its behavior, the food it eats, and whether it is active at night or in the day. For a plant, it includes how much direct sunlight it can tolerate and the sort of soil on which it thrives.

Every organism, whether an apex predator like the lion or a bacterium living in a hippo's intestinal tract, is likely to face competition from other species. The most direct form of competition comes from those organisms that try to make a living in almost the exact same way. For example, a lion does not worry much about the presence of a mongoose, but it is certainly concerned when a pack of hyenas moves in. This direct form of competition for an ecological niche is called interspecific competition.

If all the lions' competitors, such as hyenas, leopards, and hunting dogs, were to leave the African savanna, the lions would have the prey to themselves. Their niche would be wide open, limited only by their adaptations to their habitat and lifestyle. This ideal niche that would exist in the absence of competition from other species is called a species' fundamental niche. However, organisms like the lion are generally forced to play a more limited role thanks to competition. The actual niche that a species fills in the face of interspecific competition is called its realized niche.

Definition of Realized Niche-

The realized niche is the part of fundamental niche that an organism occupies as a result of limiting factors present in its habitat.The presence of competing species in an environment is one example of a limiting factor that restrains or narrows an organism's ecological niche. In a realized niche, the organism tends to occupy and play an ecological role where it is mostly highly adapted.

Realized niche width

It is a phrase relating to ecology defining the actual space that an organism inhabits and the resources it can access as a result of limiting pressures from other species (e.g. superior competitors).

Niche width vs. realized niche width

The niche width of an organism refers to a theoretical range of conditions that a species could inhabit and successfully survive and reproduce with no competition. The niche width is defined as the parameters of this range which are determined by biotic and abiotic factors such as appropriate food sources and suitable climate respectively.

The niche width often differs from the area that a species actually inhabits, with the area a species actually persists in referred to as its realized niche width. This is due to interspecific competition with other species within their ecosystem and other biotic and abiotic limiting factors. A species realized niche is usually much narrower than its theoretical niche width as it is forced to adapt its niche around superior competing species.

The physical area where a species lives, is its "habitat." The abstract hypercube that defines the limits of environmental features essential to that species' survival, is its "niche." (ECOLOGY, Begon,Harper,Townsend).

Example in comparison to Fundamental Niche:

To mention just one issue, there is a difference between fundamental niches and realized niches. What does that mean? Well, it could be that a plant, for example, could happily grow under wet, mesic and dry conditions if it were the only plant around. However, there is another plant that can also grow under mesic conditions but it is much more competitive than the first plant. That means that if they occur in different areas, the first plant will grow in all three habitats and the second one only in the mesic habitat, but if they happen to occur in the same area, you will find the first species in wet and dry places and the second one inthe mesic places where it excludes the first.

For illustration, this is how well both species do along a moisture gradient, which we could call their fundamental niches:

And this is how many individuals of each species you would really be able to find along the moisture gradient if they both occur together, which would show their realized niches:

What this shows is first that no organism should be expected to come with a clearly defined role but that it has to settle into one depending on what other organisms are around. Also, which of these two is the niche relevant for the Ecological Species Concept?

If we go with the realized niche, for example because we have so far only observed the species together in nature and don't realize that the orange species would feel even happier under mesic conditions than where it is actually forced to live, we would probably intuit that the orange populations in dry and the wet habitats are two Ecospecies.

Spatial Niche – General Concept

Spatial resource partitioning occurs when two competing species use the same resource by occupying different areas or habitats within the range of occurrence of the resource. Spatial partitioning can occur at small scales (microhabitat differentiation) or at large scales (geographical differentiation).

Definition:

Spatial ecology represents the ultimate distributional or spatial unit occupied by a species. If a particular habitat is shared by several species, each of the species is usually confined to its own micro habitat or spatial niche because two species in the same general territory cannot usually occupy the same ecological niche

Multidimensional Niche – General Concept

Hutchinson (1957) included in a species' (fundamental) niche "all ecological factors", "all environmental variables, both physical and biological, relative to [the] species", that is, both abiotic conditions, abiotic and/or biotic resources, and (though this may not have been his intention) other biotic factors (predators, parasites, competitors, etcetera). Thus, Hutchinson's niche concept is very comprehensive: it says just about everything about the ecology of a species. It is probably for that reason that the concept has become so popular. In my view, however, it is inadequate as a niche concept. In order to show this, I will have to extend a bit on modern niche theory and in particular competition theory.

Fundamental vs Realized Niche.

Fundamental niche is the entire set of conditions under which an animal (population, species) can survive and reproduce itself. Realized niche is the set of conditions actually used by given animal (population, species), after interactions with other species (predation and especially competition) have been taken into account. Sometimes FN and RN are termed precompetitive and postcompetitive niches, reflecting a traditional focus on interspecific competition's effect on niches.

Note that:

1. FN $\geq$ RN

2. RN for different populations of same species may differ, because of differences in competitors and predators between locations.

Summary: 1. Fundamental and realized niche refers to the environmental conditions or positions of different species in an ecosystem.

3. A fundamental niche can be defined as the range of environmental conditions in which each of the species survives.

4. The realized niche can be termed as the range of environmental conditions in which a species is really found.

5. The fundamental niche is larger than the realized niche. The realized niche can be called a subset of the fundamental niche. It can be said that as the realized niche grows, the fundamental niche also grows accordingly.

6. While a fundamental niche elaborates on the various roles of species, the realized niche elaborates on what the species actually do.

7.It is in the realized niche that a species will be well adapted, and so this niche is where the species actually exist.

8.The fundamental niche refers to a range of conditions, roles, and resources under which a species survives, grows, and reproduces.

Niche differentiation:

The term niche differentiation (synonymous with niche segregation, niche separation and niche partitioning), as it applies to the field of ecology, refers to the process by which competing species use the environment differently in a way that helps them to coexist. The competitive exclusion principle states that if two species with identical niches (i.e., ecological roles) compete, then one will inevitably drive the other to extinction. When two species differentiate their niches, they tend to compete less strongly, and are thus more likely to coexist. Species can differentiate their niches in many ways, such as by consuming different foods, or using different parts of the environment.

As an example of niche partitioning, several anole lizards in the Caribbean islands share common food needs—mainly insects. They avoid competition by occupying different physical locations. For example, some live on the leaf litter floor while others live on branches. Species who live in different areas compete less for food and other resources, which minimizes competition between species. However, species who live in similar areas compete strongly.

Types of Niche differentiation

Below is a list of ways that species can partition their niche. This list is not exhaustive, but illustrates several classic examples.

Resource partitioning:

When species use different resources, this can help them to coexist. For example, some lizard species appear to coexist because they consume insects of differing sizes.Alternatively, species can coexist on the same resources if each species is limited by different resources, or differently able to capture resources. For example, different types of phytoplankton can coexist when different species are differently limited by nitrogen, phosphorus, silicon, and light. In the Galapagos Islands, finches with small beaks are more able to consume small seeds, and finches with large beaks are more able to consume large seeds. If a species' density declines, then the food it most depends on will become more abundant (since there are so few individuals to consume it). As a result, the remaining individuals will experience less competition for food.Although "resource" generally refers to food, species can partition other non-consumable objects, such as parts of the habitat. For example, warblers are thought to coexist because they nest in different parts of trees. Species can also partition habitat in a way that gives them access to different types of resources. As stated in the introduction, anole lizards appear to coexist because each uses different parts of the forests as perch locations. This likely gives them access to different species of insects.

Predator partitioning:

Predator partitioning occurs when species are attacked differently by different predators (or natural enemies more generally). For example, trees could differentiate their niche if they are consumed by different species of specialist herbivores, such as herbivorous insects. If a species density declines, so too will the density of its natural enemies, giving it an advantage. Thus, if each species is constrained by different natural enemies, they will be able to coexist. Early work focused on specialist predators however, more recent studies have shown that predators do not need to be pure specialists, they simply need to affect each prey species differently. The Janzen–Connell hypothesis represents a form of predator partitioning.

Conditional differentiation

Conditional differentiation (sometimes called temporal niche partitioning) occurs when species differ in their competitive abilities based on varying environmental conditions. For

example, in the Sonoran Desert, some annual plants are more successful during wet years, while others are more successful during dry years. As a result, each species will have an advantage in some years, but not others. When environmental conditions are most favourable, individuals will tend to compete most strongly with member of the same species. For example, in a dry year, dry-adapted plants will tend to be most limited by other dry-adapted plants.This can help them to coexist through a storage effect.

Competition-predation trade-off

Species can differentiate their niche via a competition-predation trade-off if one species is a better competitor when predators are absent, and the other is better when predators are present. Defences against predators, such as toxic compounds or hard shells, are often metabolically costly. As a result, species that produce such defences are often poor competitors when predators are absent. Species can coexist through a competition-predation trade-off if predators are more abundant when the less defended species is common, and less abundant if the well-defended species is common.This effect has been criticized as being weak, because theoretical models suggest that only two species within a community can coexist because of this mechanism.

Niches and competition

Humans compete with other humans all the time – for jobs, athletic prizes, dates, you name it. But do we compete with other species? If you've ever gone camping and had you food stolen by an enterprising raccoon, bear, or other critter, you've had a little taste of interspecific competition – competition between members of different species that use overlapping, limited resources. Resources are often limited in a habitat, and many species may compete to get ahold of them. For instance, plants in a garden may compete with each other for soil nutrients, water, and light. The overall effect of interspecific competition is negative for both species that participate (a -/- interaction). That is, each species would do better if the other species weren't there.In this article, we'll look at the concept of an ecological niche and see how species having similar niches can lead to competition. We'll also see how species can evolve by natural selection to occupy more different niches, thus divvying up resources and minimizing competition.

A species' niche is its ecological role or "way of life," which is defined by the full set of conditions, resources, and interactions it needs. Each species fits into an ecological community in its own special way and has its own tolerable ranges for many environmental

factors. For example, a fish species' niche might be defined partly by ranges of salinity (saltiness), pH (acidity), and temperature it can tolerate, As we'll see, two organisms with exactly the same niche can't survive in the same habitat (because they compete for exactly the same resources, so one will drive the other to extinction). However, species whose niches only partly overlap may be able to coexist. Also, over long periods of time, they may evolve to make use of more different, or less overlapping, sets of resources. as well as the types of food it can eat.

Competitive exclusion principle

The competitive exclusion principle tells us that two species can't have exactly the same niche in a habitat and stably coexist. That's because species with identical niches also have identical needs, which means they would compete for precisely the same resources.

A famous example of the competitive exclusion principle is shown in the Figurebelow, which features two types of single-celled microorganisms, *Paramecium aurelia* and *Paramecium caudatum*. When grown individually in the lab, both species thrive. But when they are grown in the same test tube (habitat) with a fixed amount of nutrients, both grow more poorly and P. *aurelia* eventually outcompetes *P. caudatum* for food, leading to *P. caudatum's* extinction.

In nature, it's rarely the case that two species occupy exactly identical niches. However, the greater the extent to which two species' niches overlap, the stronger the competition between them will tend to be.

Resource partitioning

Competitive exclusion may be avoided if one or both of the competing species evolves to use a different resource, occupy a different area of the habitat, or feed during a different time of day. The result of this kind of evolution is that two similar species use largely non-overlapping resources and thus have different niches. This is called resource partitioning, and it helps the species coexist because there is less direct competition between them.The anole lizards found on the island of Puerto Rico are a good example of resource partitioning. In this group, natural selection has led to the evolution of different species that make use of different resources. Each species lives in its own preferred habitat, which is defined by type and height of vegetation (trees, shrubs, cactus, etc.), sunlight, and moisture, among other factors.

Niche width and overlap

Identifying the niche width and overlap of a species depends on the distribution of the species across.

Resource availability: A species that can live on diverse resources normally will have a wider width. In natural systems organisms prefer to have smaller, realized width as this ensures better resource within a small area. This assists in improving the ecological efficiency of the species. Niche overlap is the collective utilization of resources by two or more species in the same region. Overlap brings in interspecific competition. Organisms constantly try to minimize competition in sharing resources either by temporal or spatial variation. Niche breadth is the "distance through" a niche along some particular line in niche space. Niche overlap is simply the joint use of a resource, or resources, by two or more species. The measures of niche breadth and overlap we will discuss are all based on the distribution of individual organisms, by species, within a set of resource states. The table formed by using species as rows, and resource states as columns will be called the "resource matrix." A heterogeneous habitat, for example, might be subdivided into sunny-dry, sunny-wet, shady-dry, and shady-wet resource states, or alternatively, into unnamed random quadrats, which would then be considered the resource states. When two distinct habitats merge each other one can observe more number of plant and animal species this is known as ecotone. This zone having different types of food/ different stages of mineralized food supporting saprophytes, autotrophs and phagotropes. This is an example of resource partitioning.

Conclusion:

Ultimately we can say that niche is one of the most important part of ecology.The term niche was for the first time used by Joseph Grinnel(1971) to explain microhabitats of organsims.It has various types, difference and specific importance in ecology. Niche overlap, competition and other term like resource partitioning are related with eachother.While habitat and niche are not same but they both are related with each other. In very simple way we can define niche asIn ecology, a niche is a term describing the relational position of a species or population in an ecosystem. The description of a niche may include descriptions of the organism's life history, habitat, and place in the food chain. According to the competitive exclusion principle, no two species can occupy the same niche in the same environment for a long time.

Probable Questions:

1. What is community?
2. Define Species richness with suitable example.
3. Write a short note on species diversity with proper example.
4. What is diversity index?
5. Write a short note on Simpson's diversity index.
6. What is Shannon's diversity index?
7. What are the factors that influence the structure of a community?
8. Write a short note on keystone species.
9. What are foundation species? Site example.
10. What are guilds?
11. What is functional type?
12. Write a short note on the qualitative structure of a community.
13. What do you mean by phenology?
14. What is stratification?
15. What is benthic zone?
16. What are the vegetative layers of a forest ecosystem?
17. Define ecotone.
18. What is edge effect? What are edge species?
19. Write a short note on different type of gradients.
20. What are organismic and continuum concept of communities?
21. Differentiate between direct and indirect gradient analysis.
22. Write a short note on Gaussian response model of gradient analysis.
23. Write briefly about the beta response model and GAM model of gradient analysis.
24. Define Niche. State the difference between realized niche and fundamental niche.
25. State the importance of niche. What is Resource partitioning.
26. Define Competitive exclusion theory. What is multidimensional niche.

Suggested Readings / References:

1. Kumar, P., and Mina, U. Life Sciences: Fundamental and Practice-II. Fifth ed. 2016.p- 596-601. ISBN:978-81-906427-7-4.
2. Smith, T. M., and Smith, R. L. Elements of Ecology.Eight ed. 2012.p- 330-350. ISBN: 13: 978-0-321-73607-9.
3. Fundamentals of Ecology. Eugene Odum. 5th Edition 2006.
4. Elements of Ecology. Smith and Smith. 9th Edition.2018
5. Ecology. Robert Ricklefs. 1990

Unit-VII

Population attributes: Growth forms and mathematics of growth, Life Table - (Cohort and Static); survivorship curves, generation time, net reproductive rate. Life history strategies: Evolution of life history traits, strategies related to longevity; clutch size; life history optimization.

Objectives:

In this Unit we will discuss about Population attributes: Growth forms and mathematics of growth, Life Table - (Cohort and Static); survivorship curves, generation time, net reproductive rate. Life history strategies: Evolution of life history traits, strategies related to longevity; clutch size; life history optimization.

Population Biology

A population is a group of individual of same species that inhibit a particular geographical area and have the capability of interbreeding. Population ecology is the study of populations (especially population abundance) and how they change over time.

Population Attributes

A population has several characteristics or attributes which is a function of whole group not of the individual. Different population can be compared by measuring these attributes. These attributes are population density, natality, mortality, survivorship, age structure, growth forms, emigration, immigration etc. The study of group characteristics or parameters of the population, their changes over time and prediction of future changes is known as demography.

Growth Forms

The population growth refers to the change in the number of individuals in a population with time. This growth is controlled by the rate at which new individuals are added to the population through the process of birth and immigration and the rate at which individual leave the population through the process of death and emigration. Population growth can be exponential growth or logistic growth.

Exponential growth

A population shows exponential growth if all members have abundant resource availability and are free to reproduce at their physiological capacity. This is known as geometric growth. This form of growth may be represented by the model based on the exponential equation:

$$dN/dt = rN$$

Where N is the population size and the value r is the instantaneous per capita rate of growth (sometimes called the intrinsic rate of population growth), and the resulting equation is referred to as the model of exponential population growth.

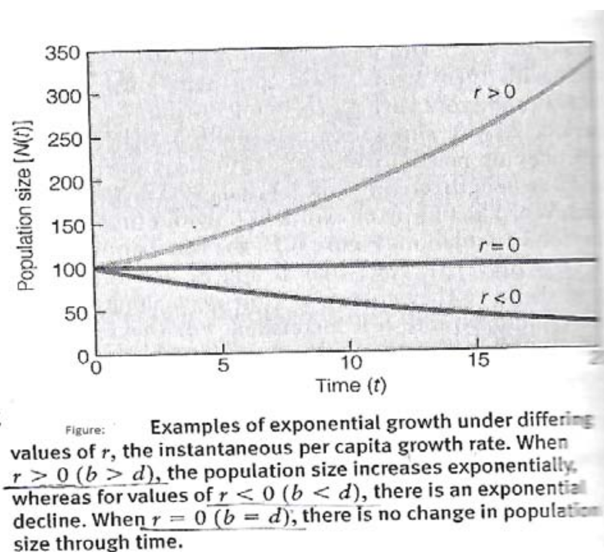


Figure: Examples of exponential growth under differing values of r, the instantaneous per capita growth rate. When $r > 0$ $(b > d)$, the population size increases exponentially, whereas for values of $r < 0$ $(b < d)$, there is an exponential decline. When $r = 0$ $(b = d)$, there is no change in population size through time.

The model of exponential growth ($dN/dt = rN$) predicts the rate of population change through time. If we wish to define the equation to predict population size, N(t), under conditions of exponential growth, it is necessary to integrate the differential equation presented earlier. The result is

$$N(t) = N(0)e^{rt}$$

Where $N(0)$ is the initial population size at $t = 0$, and e is the base of the natural logarithms; its value is approximately 2.72.

The population size that increases exponentially at a constant rate, results in a J-shaped growth curve when the population size is plotted over time.

Logistic growth

The exponential growth assumes resources are unlimited but it is never in the real situation. As population increases, each individual has access to fewer resources. It means a particular environment can only support a maximum population size. The maximum number of individuals of a particular species that a particular environment can sustain indefinitely is defined as carrying capacity. It is not fixed and determined by various factors including predation, competition as well as climatic conditions. Since such factors are varied, it is clear that the carrying capacity of any area for a population may vary over a period of time. So, the growth of a population eventually slows as the population reaches the carrying capacity for environment. This results in an S-shaped curve of population growth known as logistic growth curve. This growth form may be represented by the model:

$dN/dt = rN (K-N)/K$

Where, N is the population size; K means Carrying capacity and r is the intrinsic rate of natural increase.

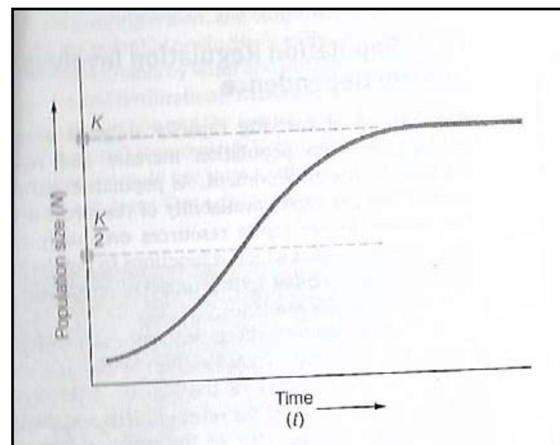


Figure: The logistic growth model of population (Ref: Smith and Smith)

In sigmoid growth form, the population increases slowly at first (establishment or positive acceleration phase), then more rapidly (possibly approaching a logistic phase) but it slows down gradually as the environmental resistance increases (the negative acceleration phase), until equilibrium is reached and maintained

Life Table

Life history tables, or life tables, are a method of quantifying population structure that addresses all of the above population traits. Life tables provide age-specific information on survival and fecundity rates for a particular population. An ecologist can collect two very

different types of life history data for individuals in a population, which can lead to two kinds of life tables:

Cohort (horizontal or dynamic) life tables require ecologists to follow all the individuals of a single cohort in a population from birth to death. A cohort is a group of individuals all born during the same time interval. Construction of cohort life tables frequently depends on the recapture of marked individuals for mobile species or repeated, representative samples of sessile species. Since individuals must be followed from birth to death, the horizontal life table technique is not well suited for the study of long-lived individuals.

Static (vertical or time-specific) life tables consist of data on individuals of all ages in a population from a single point in time. In vertical life table studies, it is important to work with a large, random sample of individuals to ensure that the data is representative of the entire population. For example, the age distribution of your sample of individuals should reflect the age distribution of the whole population. Non-destructive sampling methods are particularly useful for the construction of vertical life tables as they minimize the impact of large sampling efforts on population dynamics.

Both cohort and static observations of population structure can be used 1) to quantify the age structure of a population; 2) to estimate an optimal age of sexual maturity; and 3) to predict population growth rates.

Survivorship curves

Survivorship curves plot the number of surviving individuals to a particular age. Theses curves are of three general types:

A highly convex curve (type I) is characteristic of the species in which the population mortality rate is low near the end of the life span. Many species of the large animals such as deer, mountain sheep and man, sow such curves.

A highly concave curve (type III) is characteristic of those species where the mortality rate is high during the young stages. Oyster or shell fish show this type of curve. In oysters, mortality is extremely high during free swimming larval stages but once an individual is well established on a favourable substrate, life expectancy improves considerably.

In type II curve, the rate of mortality is at all age groups that means mortality is more or less constant throughout the life span. This curve is typical of several birds and of human beings exposed to poor nutrition and hygiene.

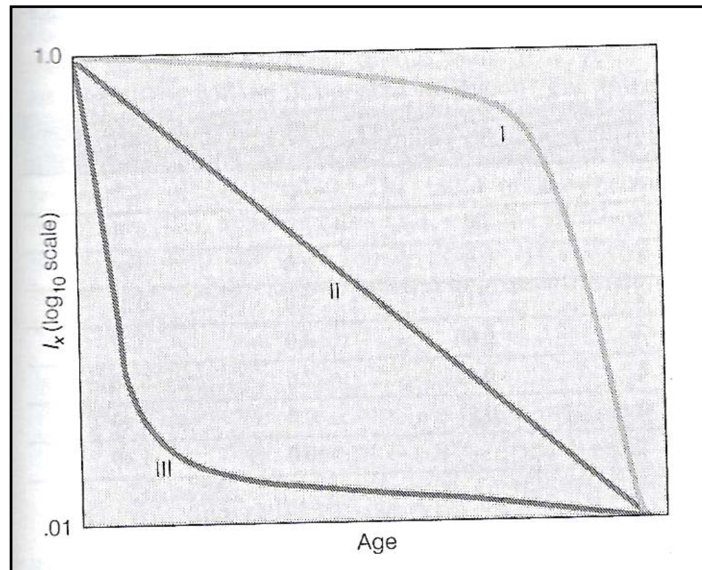


Figure: Three basic types of survivorship curve (Ref: Smith and Smith)

Type I survivorship curves are typical of organisms which are likely to breed several times during the course of their lifespan. This strategy is referred to as iteroparous. On the other hand, species with a type III curve are more likely to breed only once during their life time, a strategy referred to as semelparous.

The three types of survivorship curves are generalizations and few population exactly fit one of the three curves. Some species have one type of curve early in life and another type as adults. Herring gulls have a type III survivorship curve early in life and type II as adults.

Net Reproductive Rate (R_0)

The net reproductive rate is the reproductive potential of the average female, adjusted for survival. We can calculate it by multiplying the standardized survivorship of each age (lx) by its fecundity (bx), and summing these products:

$$R_0 = \sum l_x b_x$$

Assuming survival and fertility schedules remain constant over time (an unreasonable assumption in most situations), if $R_0 > 1$, then the population will grow exponentially, if R0 < 1, the population will shrink exponentially, and if $R_0 = 1$, the population size will not change over time. You may be tempted to conclude that $R_0 = r$. However, this is not quite correct: r measures population change in absolute units of time (e.g., years); R_0 measures population change in terms of generation time.

Generation Time

The generation time is the average time between two consecutive generations in the lineages of a population. Generation time is calculated as

$$G = \sum l_x b_x x / \sum l_x b_x$$

For organisms that live only 1 year, the numerator and denominator will be equal, and generation time will equal 1 year. For all longer-lived organisms, generation time will be >1 year, but exactly how much greater will depend on the survival and fertility.

Life History strategies

Life history strategy is correlated with many aspects of an organism's reproductive strategy and life history, as well as with demographic variables such as generation time, life span and population density and population dynamics. The concept of r-strategies and k-strategies links population dynamics to life history.

A high intrinsic growth rate r which is achieved by a distinctive strategy consisting of rapid development, a small body size, early reproduction, semelparity and a short life span (less than a year). Species with this reproductive pattern overcome the massive loss of their offspring by producing so many unprotected young that a few will survive to reproduce to begin the cycle again. Species with these characteristics are called r-strategists or r-selected species. Algae, bacteria, rodent, annual plants (such as dandelions) and most insects are r-selected species.

On the other hand, relatively constant or predictable environmental conditions allow a population to reach its carrying capacity k and thus a high average population density. The concept says that these conditions select for high k, which is achieved by slow development (associated with great competitive ability), a large body size, delayed reproduction, iteroparity and a long life span. Species with these characteristics are called k-strategists or k-selected species.

Table 1: Differences between r-selection and k-selection

Characteristics	**r-selection**	**k-selection**
Environment	Variable and unpredictable	Fairly constant or predictable
Survivorship	Often type III	Usually types I and II
Population size	Variable in time, nonequilibrium; Usually well below carrying capacity of environment	Fairly constant in time, equilibrium; at or near carrying capacityof the environment
Selection favors	Rapid development Early reproduction High rate of increase Small body size Semelparity: single reproduction Many offsprings Low level of social organization	Slower development Delayed reproduction Low rate of increase Larger body size Iteroparity: repeated reproduction Fewer and larger offsprings High level of social organization
Life span	Short, less than one year	Longer, usually more than one year
Successional stage	Early	Late, climax

Source: Pianka ER (1970) r- and k-selection. American Natuaralist, 102: 592-597

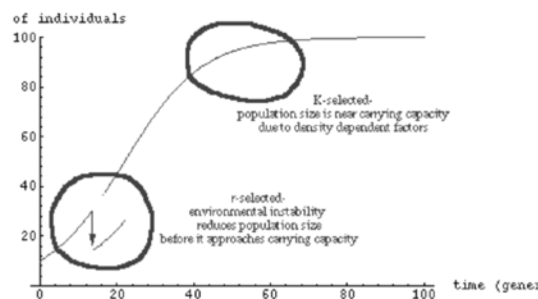


Figure: r and k selection of life history strategies (Ref: Smith and Smith)

Evolution of life history traits

Life history evolution: A general framework based on quantitative genetics, population ecology and physiology to understand variation and adaptation in life history strategies.

Life history strategies (demographic tactics): Ensemble of life history traits of a given individual, population, species or higher taxa.

Life history traits: Traits that are directly involved into a characteristic equation describing individual fitness; such age at maturity, survival and reproduction. Life history traits are coupled into a life cycle and their interactions determine individual fitness, population growth or the species growth/competitive ability.

Clutch Size

Clutch size refers to the number of eggs produced by birds, amphibians or reptiles, often at a single time, particularly those laid in a nest. Clutch size differs not only among major taxonomic groups of birds among species, but also among populations and individuals of the same species.

Probable Questions

1. Definition- Population attributes.
2. Explain the exponential growth curves of population biology.
3. Describe the logarithmic growth curves with suitable diagram.
4. What do life table represents?
5. What is cohort life table?
6. State the difference between cohort and strategic life table.
7. What are the 3 types of survivorship curves? Explain it with proper diagram and examples.
8. What types of survivorship curve do humans have?
9. What is net reproductive rate (with equation)?
10. Define generation time.
11. What are r- and k-strategies?
12. Show the differences between R and K selected population.
13. What is clutch size?

Suggested readings /References

1. Kumar P and Mina U. Life Science Fundamentals and Practice (part-II), Fourth edition. ISBN: 978-81-906427-7-4.
2. Lack D (1947). The significance of clutch size (part I-II). Ibis 89:302-352.
3. Pianka ER (1970) r- and k-selection. American Naturalist, 102: 592-597
4. Smith TM and Smith RL. Elements of Ecology, Eighth edition. ISBN: 978-0-321-73607-9.

Unit-VIII

Metapopulation: Concept, models, structure and dynamics. Major terrestrial biomes; major biogeographical zones of India

Objective:

In this Unit we will discuss about Metapopulation: Concept, models, structure and dynamics. We will also discuss about Major Terrestrial Biomes and major biogeographical zones of India.

Meta population:

Meta population is a set of local population occupying an array of habitat patches and connected to one another by the movement of individuals among them.

Theory of metapopulation:

The term metapopulation was first used by Richard Levins, 1970. A metapopulation of species is thought of as a set of small populations occupying an array of similar small habitat patches situated in a matrix of uniform suitable habitat. At any particular time, some suitable habitat patches may contain no individual of the species and thus may be subjected to colonization from other patches that are inhabited.

The local populations are assumed to be small. Local catastrophes and chance fluctuations in number of individuals have important effects on population dynamics, so there is a high probability of extinction of a local population during a particular time interval. Metapopulation dynamics indicate a balance between extinction and colonization.

Simple metapopulation model:

It is conceptualized as a group of local population each having a density of either zero (extinct) or K(equilibrium density), where “K” is the patch carrying capacity. The carrying capacity of patch is the number of individuals that can be supported by the recourses in the patch for a indefinite period of time. At any time, some proportions (P) of the total number of patches in the metapopulations will be occupied and the remaining fraction (1-P), will be unoccupied or become extinct.

$$\frac{dP}{dt} = [mP(1-P)] - eP$$

Where, m = rate of patch

e =rate of patch extinction.

When the rate of change of occupancy is zero $\frac{dP}{dt}$ = 0 and P= 1-$\frac{e}{m}$is the equilibrium proportion of occupied populations.

The main prediction of this model is that species will not perish i.e p < 0 when extinction rate is greater than colonization rate, m is the metapopulation and to put it in an another way metapopulation persistence require $\frac{e}{m}$< 1.

Characteristics of metapopulation:

The major characteristics of metapopulation are patch size & density and compensation of patch size and density.

Patch size and density: The dynamics of metapopulation are affected not only by the extinction and colonization rates but also by the relationship of those rates to the spatial arrangement and density of the habitat patches for a metapopulation to persist the overall colonization rate must be greater than the extinction rate (1-$\frac{e}{m}$). But successful colonization requires that individuals move from an occupied site to one that is unoccupied and such movements may be prevented if there is a great distance between the occupied and unoccupied sites. The distance barrier may be overcome in time if potential colonized arise continuously from an occupied site. Thereby increasing the chance that one will make it to the distant unoccupied site. But, in order for this to happen, the colonizing population must be persist. In general, the population persistence is related to population size. Small population suffer a higher risk of extinction than the large populations.

Compensation of patch size and density: Patch size and density may interact in a compensatory way to affect population persistence. This interaction can be shown with a modification of a basic metapopoulation model.

Suppose, migration rate (m) dependent on degree to which a patch is isolated, measured as some distance (D), that is migration to an unoccupied patch that is isolated from a colonising patch [high(D)] is less likely than if the patch is in the proximity of the colonizing patch(low D).

The exact relationship between “E” and “m” is not that important so long as “m” declines as “D” increases. One possible relationship between two is a negative potential function.

$$m = m_0 e^{-\alpha D}$$

Fluctuation in meta population:

The metapopulation are characterized by frequent extinctions of population in patches followed by the colonization of those patches (turn over). Thus at any point in time, some patches area unoccupied. In metapopulation, it is the level of patch occupancy and the rate of turnover (extinction, colonization events) that are important rather than the density of populations in particular patch.

Migration & Demographic feature of Metapopulation:

The more isolated patches in a metapopulation are less likely to be colonized. The reason is that the greater distance represents a barrier to the movement of individuals. Although receives a reasonable basic relationship, other factors such as patch-quality, environment & demo graphic patterns affect the immigration & emigration of the individuals in a patch.

Rescue effect in Metapopulation:

One of the assumptions of simple metapopulation model, is that local patches either contain a population (occupied) or do not (extinct). Some population model relay this assumption and take into consideration of demographic of the individual population.

This more advance model gave us an avenue for understanding the fluctuation or stability of local populations, in the context of regional process.

Stacey et. al (1992) identified a natural population that appear to show rescue effects. In the case of checker spot butterflies (*Euphytryas*) of North America, this distribution reflects the patchy distribution of planets of plant on which the butterflies suggest that relatively high level of heterozygocity are maintained in local populations.

Differences in habitat quality among patches, rather than patches on population size, may produce a similar rescue effects, giving rise to source and sink populations (of habitat patches). Source populations are defined by their ability to maintain a positive growth rate ($\pi>0$), whereas sink populations cannot support negative population growth ($\pi<0$), because of their four qualities-

(1) The suitable habitat occurs in discrete patches that may be occupiedby local breeding populations.
(2) Even the largest populations have a substantial risk of extinction.
(3) Habitat patches must not be too isolated to prevent recolonization after local extinction; and
(4) The dynamics of the local populations are not synchronized.

Metapopulation dynamics:

The fundamental idea of metapopulation persistence is a dynamic balance between the extinction of local populations and recolonization of empty habitat patches. Levins proposed a simple model of metapopulation dynamics, where metapopulation size is defined by the fraction of (discrete) habitat patches (P) occupied at any given time (t). Within a given time interval, each subpopulation occupying a habitat patch has a probability of going extinct (e). Therefore, if P is the fraction of patches that is occupied during the time interval, the rate at which subpopulations will go extinct (E) is defined as

$$E = eP$$

The rate of colonization of empty patches (C) depends on the fraction of empty patches ($1-P$) available for colonization and the fraction of occupied patches, providing colonists (P), multiplied by the probability of colonization (m), a constant that reflects the rate of movement (dispersal) of individuals between habitat patches. Therefore, the colonization rate will be $C = [mP(1 - P)]$

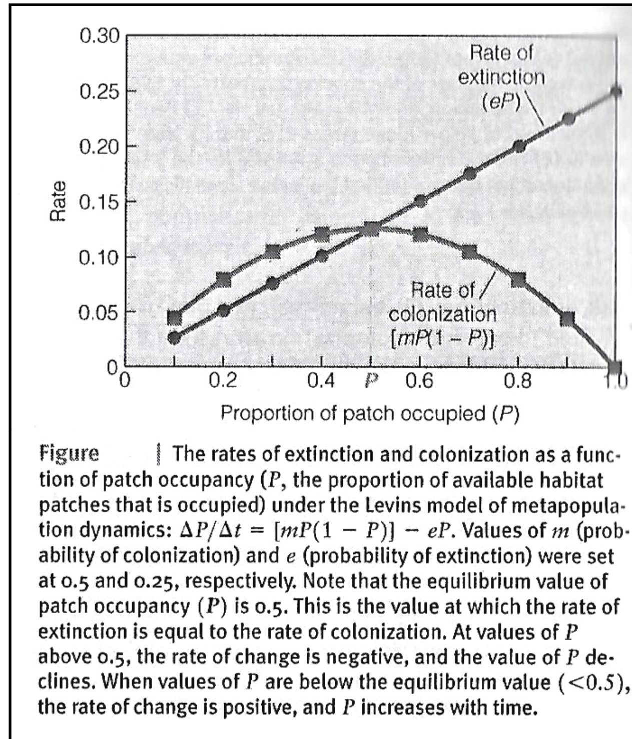


Figure | The rates of extinction and colonization as a function of patch occupancy (P, the proportion of available habitat patches that is occupied) under the Levins model of metapopulation dynamics: $\Delta P/\Delta t = [mP(1-P)] - eP$. Values of m (probability of colonization) and e (probability of extinction) were set at 0.5 and 0.25, respectively. Note that the equilibrium value of patch occupancy ($\boldsymbol{P}$) is 0.5. This is the value at which the rate of extinction is equal to the rate of colonization. At values of P above 0.5, the rate of change is negative, and the value of P declines. When values of P are below the equilibrium value (<0.5), the rate of change is positive, and P increases with time.

Figure: Metapopulation dynamics (Ref: Smith and Smith)

We can think of metapopulation growth in a manner analogous to the normal population growth, where the change in the population (ΔN) over a given time interval (Δt) can be expressed as the difference between the rates of birth and death ($\frac{\Delta N}{\Delta t} = b - d$). The change in metapopulation, defined as the fraction of habitat patches occupied by local population through time ($\frac{\Delta P}{\Delta t}$), can therefore be defined as the difference between the rates of colonization (*C)* and extinction (*E)* :

$$\frac{\Delta P}{\Delta t} = C - E$$

Or

$$\frac{\Delta P}{\Delta t} = [mP(1-P)] - eP$$

The model of metapopulation growth functions in a manner similar to the logistic model, in that growth is regulated in a density dependent fashion. For any given values of "*e*" and "*m*", we can plot the rates of extinction (*E*) and colonization (*C*) as a function of the proportion of habitat patches occupied(P). The rate of extinction increases linearly with *P,* and the colonization rate forms a convex curve, initially rising with the proportion of patches occupied, then declining as the proportion approaches 1 (all patches are occupied). The value of *P* where the lines cross represents the equilibrium value, ***P*** and at this value of *P,* the

extinction and colonization rates are equal ($E = C$) and the metapopulation growth rate is zero ($\frac{\Delta P}{\Delta t} = 0$). It is an equilibrium value because when the fraction of patches occupied (P) is below this value ($\boldsymbol{P}$), the rate of colonization exceeds the rate of extinction and the number of occupied habitat patches increases. Conversely, if the value of P exceeds P (total patches) ,the rate of extinction exceeds the rate of colonization and the size of the metapopulation (number of occupied patches) declines. So just as in the logistic model—in which the population density (N) tends to the equilibrium population size represented by the carrying capacity (K)—in the metapopulation model, the metapopulation density, P (proportion of patches occupied), will tend to the equilibrium metapopulation size represented by $\boldsymbol{P}$**.** The equilibrium value of P is a function of the probabilities of extinction (e) and colonization (m):

$$P = 1 - \frac{e}{m}$$

Terrestrial Biome:

The word biome is formed from two Greek words: bios = life and oma = group or mass. The biome as a biological unit which is a type of vegetation, climate, soil and altitude of that specific place. The word was first used with this meaning by the American ecologist Clements in 1916.

Definition:

A biome is a community of plants and animals that have common characteristics for the environment they exist in. They can be found over a range of continents. Biomes are distinct biological communities that have formed in response to a shared physical climate.

Terrestrial Biomes:

Terrestrial biomes are usually distinguished on the basis of the major components of their mature or climax vegetation, while aquatic biomes, especially marine ones, are often characterized by their dominant animals.

9 major terrestrial biomes are -

a. Tundra
b. Boreal forest
c. Temperate rain forest
d. Temperate deciduous forest
e. Tropical rain forest
f. Chaparral
g. Temperate grassland
h. Savanna
i. Desert

a. Tundra:

- The treeless biome in the far north that consists of boggy plains covered by lichens and mosses.
- Average annual rainfall -25 cm.
- Average temperature-summer:38°C;winter-7°C.
- Climate-very dry and hot. It is hot during the day and cold at night.
- Landform-flat sandy plains.

b. Boreal Forest/Taiga:

- A region of coniferous forest (such as pine, spruce, and fir) in the

  Northern Hemisphere.

  located just south of the tundra.
- Average annual rainfall-30cm-83cm
- Average temperature-summer-14°c,winter-10°c.
- Climate-It is cold with wind blown from the arctic, most rainfall occurs during the hot

  summer. winter are freezing cold.
- Landform-mountaintops, valleys and forests.

c. Temperate Rain Forest:

- A coniferous biome with cool weather. dense fog, and high precipitation

- Average annual rainfall-507cm
- Average temperature - 27°C
- Climate-seasonal variation, near freezing in winter.

d. Temperate Deciduous Forest:

- A forest biome that occurs in temperature areas where annual precipitation ranges from
  about 75 cm to 126 cm
- Average temperature-summer :28°C;Winter :6°C
- Climate-four distinct seasons with mild summer and cool winter. Rainfalls throughout the year.
- Landform-coastal plains, piedmont and mountains.

e. Tropical Rain Forest:

- Dense tall evergreen forest.
- A lush, species-rich forest biome.
- Average annual rainfall-400cm
- Average temperature-34°C.
- Climate-very humid, it rains everyday and is warm throughout the year.
- Landform-the terrain consist of both mountains and flat plains.

f. Chaparral:

- A biome with mild, moist winters and hot, dry summers;
- vegetarian is typically small-leaved evergreen shrubs and small trees.

g. Temperate Grassland:

- A grassland with hot summers, cold winters, and less rainfall than is found in the temperature deciduous forest biome
- Average annual rainfall-25cm-75cm
- Average temperature in summer :30°C,in winter-0°C
- Landform-gently rolling hills and flat plains.

h. Savanna:

- A tropical grassland with widely scattered trees or clumps of trees
- Average annual rainfal-150cm
- Average temperature-34°C
- Climate-there are 2 season, wet in the summer and dry in the winter.
- Landform-flat plain with small hills

i. Desert:

- A biome in which the lack of precipitation limits plants growth; deserts are found in both temperature and tropical regions
- Average annual rainfall -25cm
- Average temperature-summer:38°C;winter :7°C.
- Climate-very dry and hot. It is hot during the day and cold at night.
- Landforms-flat sandy plains

Major biogeographical zones of India

Biogeographic zones of India are the division of India according to biogeographic characteristics. Biogeography is the study of the distribution of species, organism, and ecosystems in geographic space and through geological time.

India is a megadiverse country with only 2.4% of the total land area of the world, the known biological diversity of India contributes 8% to the known global biological diversity. Biogeographically, India has been divided into 10 biogeographic zones as follows:

a. Trans-Himalayan region

b. Himalayan zone

c. Indian Desert Zone

d. Semi-Arid Region

e. Western Ghats

f.. Deccan Plateau

g. Gangetic Plain

h. North-East Region

i. Coastal region

j. Andaman and Nicobar Islands

a. Trans-Himalayan region: The Himalayan ranges immediately north of the great Himalayan ranges are called the trans-himalayan.it constitutes 5.6% of the total geographical area,includes the high altitude, cold and arid mountain areas of Kazakh, Jammu and Kashmir,North Sikkim,lahaul and spirit areas of Himachal Pradesh. This zone has sparse alpine steppe vegetarian that harbours several endemic species and is a favourable habitat for the biggest population of wild sheep and goats in the world and other rare fauna that includes Snow Leopard and the migratory Blacknecked Crane(Grus nigricollis).The cold dry desert of this zones represent an extremely fragile ecosystem.

b. Himalayan zone: It constitutes 6.4% of the total geographical areas includes some of the highest peaks in the world.The Himalayan zone makes India one of the richest areas in terms of habitats and species.

The Himalayas consists of the youngest and loftiest mountain chains in the world .The Himalayas have attained a unique personality owing to their high altitude ,steep gradient and rich temperate flora.The forests are very dense with extensive growth of grass and evergreen tall trees.Oaktu,chestnut,conifer,ash,pine,said at are abundant in Himalayas. Chief species includes wild sheep,mountain goats, ibex, shrew, Tapir, Panda and snow leopard.

The alpine and sub-alpine forests ,grassy meadows and moist mixed deciduous forests provide diverse habitat for endangered species of voids such as Bharal (*Pseudois nayaur*), Ibex(*captain ibex*),Markhor(*Capra falconeri*),Himalayan Tahr(*Hemitragus jemlabicus*), and Takin(*Budoreas taxicolor*). Other rare and endangered species restricted to this zone include Hangul (*Verbis elfineldi*) and Mask Deer(*Moschus mpschiferus*).

c. Indian Desert Zone: It constitutes 6.6% of the total geographical areas, includes the Thar and the Kutch deserts and has large expanses of grassland. The climate is characterized by very hot and dry summer and cold winter. Rainfall is less than 70cm.The plants are mostly Xerophytic. Bavul, Korar, wild plants grows in areas of moderate rainfall.

It supports several endangered species of mammals such as Wolf (*Canis lupus*),Caracal (*Felix caracal*),Desert Cat(*Felix libyca*) and birds of conservation interest viz., Houbara Bustard (*Chamydotis undulate*) and the Great Indian Bustard(*Ardeotis nigriceps*).

d. Semi-Arid Region: It constitutes 16.6% of total geographical area,is a transition zone between the desert and the dense forest's of western Ghats.

Peninsula India has two large regions, which are climatically semi-arid. This region has several artificial and natural lakes and marshy lands.

The dominant grass and palatable shrub layer in this zone supports the highest wildlife biomass. The cervix species of samber (*Cervix unicolor*) and Chital(*Axis axis*) are restricted to the better wooded hills and mioster valley areas respectively. The Lion (*Leo parsica*), an endangered species ,Caracal(*Felis caracal*),Jackal(*Canis aureus*) and Wolf (*Canis lupus*)are some of the endangered species that are characteristic of this region.

e. Western Ghats: It constitutes 4.0% of the total geographic area. The mountains along the Western Ghat extend from the southern tip of the peninsula (8 degree N) Northwards about 1600km to the mouth of the tapti (21 degree N).

It is one of the major tropical evergreen forest region in India and represents one of the two biodiversity 'hotspots'. Western Ghats are home to viable populations of most of the vertebrate species found in peninsular India, besides an endemic faunal element of its own.

Significant species endemic to this region include Nilgiri langur (*Presbytis jobni*), Lion tailed macaque (*Macaca silenus*), Grizzled Giant Squirrel (*Ratufa macroura*),Malabar Civet (*Viverricula megaspila*), Nilgiri Tahr (*Hemitragus bylocriud*) and Malabar Grey Hornbill (*Ocycerous griseus*).The Travancore Tortoise (*Indotestudo forstem*) are two endangered taxa restricted to a small area in central Western Ghats.

f. Deccam Plateau: Beyond the ghats is Deccan Plateau, a semi- arid region laying in the rain shadow of the Wester Ghats. This is India is largest biogeographic region, constitutes 42% of the total geographical area.

This zone of peninsular India is most extensive zone, covering India's finest forests, particularly in the states of Madhya Pradesh, Maharashtra and Odisha.

The highlands of the plateau are covered with different types of forests, which provide a large variety of forest products. Majority of the forests are deciduous in nature but there are regions of greater biological diversity in the hill ranges. The zone comprising of deciduous forests, thorn forest and degraded scrubland support diverse wildlife species .

Species found in this region are Chital (*Axis axis*),Sambar(Cervus unicolor), Nilgai (*Boselapbus tragocamelus*) and Chousingha (*Tetracerus quadricornis*) .Barking deer (*Muntiacus muntjak*),Gaur (*Antilope cervicapra*), Elephant (*Elephus maximus*) in Bihar-Orissa and Karnataka-Tamil Nadu belts, wild Buffalo (*Bubalus bubalis*) in a small area at the junction of Orissa, Madhya Pradesh and Maharashtra and the hard ground swamp Deer (*Cervus duvauceli*), now restricted to a single locality in Madhya Pradesh.

g. Gangetic Plain: Gangetic plain constitutes around 10.8% of the total geographical area. In the north is the Gangetic plain extending upto the Himalayan foothills. This is the largest unit

of the Great Plain of India. Ganga is the main river after whose name this plain is named. The tree belonging to these forests are are teak, sal, shisham, mahua, khair etc.

The characteristic fauna of this region include Rhino (*Rhinoceros unicornis*), Elephant (*Elephas maximus*), Buffalo(*Bubalus bubalis*), Swamp Deer *(Cervus duvauceli*), Hog-Deer (*Axis porcinus*) and Hispid Hare (*Carprolagus bispidus*).

h. North-East Region: It constitutes 5.2% of the total geographical area, represents the transition zone between the India, Indo- Malayan and Indo-Chinese biogeographical regions as well as being a melting point of the Himalayan mountains and peninsular India. Thus North-East is the ‘gateway' for much of India's flora and fauna and also a biodiversity hotspot(Easter Himalaya). It is one of the richest flora region in the country It has several species includes orchids, bamboos, ferns and other plants. Many species are either restricted to this biological diversity, or to the smaller region itself or to the Kashi Hills of the smaller localized areas.

i. Coastal region: It constitutes 2.5% of the total geographical area which includes sandy beaches, mangroves ,mud flats, coral reefs and marine angiosperm pastures make them the wealth and health zones of India. The coastline extending from Gujrat to Sundarbans is estimated to be 5,500 km long. Total of 25 islets constitutes the Lakshadweep, which are of coral origin, and have a typical reef lagoon system, rich in biodiversity .However the densely populated Lakshadweep islands virtually have no natural vegetation.

The backwaters are the characteristic features of south cost and east coast are broader due to depositional activities of the eat -flowing river owning to the change in their base levels .

j. Andaman and Nicobar Islands: This constitutes 0.3% of the total geographical are.it is one of the three tropical moist evergreen forests zones in India. The islands house an array of fauna and flora not found elsewhere.

These islands are centers of high endemism and contain some of India’s finest evergreen forests and support a wide diversity of corals. In India endemic island biodiversity is found only in the Andaman and Nicobar Islands. Some of the Island are fringed with coral and some of the endemic fauna of Andaman and Nicobar islands include Narcondam hornbill, South Andaman krait etc.

Probable Questions:

1. What is metapopulation?
2. What four conditions are necessary for the term metapopulation to be applied to a system of local populations?
3. Discuss the role of local extinction and colonization on metapopulation dynamics.
4. Describe briefly the simple metapopulation model.
5. What are the major characteristics of a metapopulation?
6. How fluctuation in metapopulation occurs?
7. What is rescue effect in metapopulation?
8. Write a short notes on metapopulation dynamics.
9. Define Biome. State the characteristics of each biome.
10. Describe the characteristics of different biogeographical zones of India.

Suggested Readings/ References:

1. Smith, T. M., and Smith, R. L. Elements of ecology. Eight ed. 2012.p- 330-350. ISBN: 13: 978-0-321-73607-9.
2. Essentials of Ecology & Environmental Science, S.V.S. Rana. 5th Edition.2013
3. Elements of Ecology. Smith and Smith. 9th Edition.2018.

HARD CORE THEORY PAPER (ZHT – 102)
Group C (Environment)

Module	Unit	Content	Credit	Class	Time (h)	Page No
ZHT - 102 **(Parasitology, Ecology and Environment)**	**IX**	Concept of Environment: Structure, radiation balance, climate cycle	**1**	**1**	**1**	**175 –189**
	X	Anthropogenic impact on environment: Green house gases, global warming, ozone depletion, UN movements on environment		**1**	**1**	**190 – 200**
	XI	Environment and agriculture: Green revolution and its impact on environment, organic farming, participatory approach inagriculture		**1**	**1**	**201 –212**
	XII	Theory and analysis of conservation: Stochastic perturbations, population viability analysis, recovery strategy for threatened species		**1**	**1**	**213 –218**

Group-C: Environment

Unit-IX

Concept of Environment: Structure, radiation balance, climate cycle.

Objective:

In this Unit we will discuss about Concept of Environment: Structure, Radiation Balance and Climate Cycle.

Structure of environment:

Earth is the only planet in our solar system that supports life as we know it, and its distance from the Sun provides favourable temperatures for life. Planet Earth is a sphere about 25,000 miles (40,000 kilometers) in circumference, the fifth largest in the solar system. About 70 percent of its surface is covered by large bodies of salty water called oceans, which are never still, but flow and change all the time. About 30 percent is dry land. The great land masses, known as continents, are surrounded by the oceans. The formation and evolution of the Earth and its environment have occurred over the long, long course of millennia. The principal components of the planet are:

The atmosphere: The mass of air surrounding the Earth like a transparent wrapping.

The lithosphere: the solid outer crust of rocks about 80 km thick, which is the outer solid shell of the planet body. This also includes the pedosphere, where the soils are present and the soil-forming processes occur.

The hydrosphere: the water portion of Earth as distinguished from the solid part (the lithosphere), and from the gaseous outer envelope (the atmosphere). This part of the environment also includes the cryosphere, which is that part of the Earth's body that is predominantly frozen and mainly consists of different forms of ice.

The biosphere: the life zone of the planet which permeates all the above as life is widely spread around the planet. The biosphere includes the lower atmosphere, the whole hydrosphere, the pedosphere, and the outer portion of the lithosphere to a depth of about 2 km; in short, all regions in which living organisms exist. These structural components are interlinked and unified into a holistic concept of the Earth system. While the hydrosphere is included here to provide a complete overview of the physical environment as a whole, the subject is considered in far greater detail in other themes. Besides covering the hydrological

cycle, the hydrosphere topic includes articles on the oceans, freshwater (surface water and groundwater), and the linkages between surface water and groundwater. The cryosphere topic deals with all forms of ice on Earth. This has an important effect on the global climate, which is also considered in other themes. In the topic on the lithosphere, discussions deal with the genesis of the zone, the geologic processes occurring there, and the mineral resources it contains. Although the pedosphere is the outer layer of the lithosphere, it is discussed here as a separate entity because of the special circumstances of its genesis and its important role in the functioning of many life-supporting systems.

i) Atmosphere:

In everyday life and activities, human society interacts closely with the atmosphere through climate and weather, which are intimately related to the state of the atmosphere. Quite minor in its mass, as compared to that of the whole planet (it amounts to only about one millionth of the latter) the atmosphere is an absolutely indispensable environment for all life forms. Without it the Earth would be a lifeless planet. Weather has a powerful effect on agricultural productivity; it controls human needs in the production and consumption of all forms of energy, and it is critical for aviation safety and the efficiency of ground transportation. Many aspects of human activities are critically affected by sharp changes in weather and the oscillations of climate. History knows many cases when severe winters, or extensive summer droughts afflicting large territories, brought catastrophe to the economies of whole countries. The atmosphere—an air envelope of our planet—is studied in the science of meteorology, which considers atmospheric processes in all their complexity, including the interaction of the atmosphere with the hydrosphere and the lithosphere (the Earth’s surface), and investigates the origin and causes of various atmospheric phenomena, partly with the objective of developing forecasting techniques.

Structure of Atmosphere:

Troposphere:

Almost 70% of the mass of the atmosphere is contained in this layer. It is more or less homogeneous in composition where there is no air pollution. The water content here also depends on the hydrological cycle. The troposphere is a region of turbulence. This is because of the global energy flow that arises from imbalances of heating and cooling rates between

the equator and the poles. The air is far from uniform, depending on density and temperature. In the troposphere, temperature decreases uniformly with increasing altitude. Near the ground level, the air is heated by radiation from the earth. At the top of the troposphere is the tropopause. This is the cold layer (-56°C) which marks temperature inversion, i.e. transition from negative to positive lapse rates.

Stratosphere:

This is the quiescent layer marked by a positive lapse rate. The temperature increases with increase in altitude. At the upper limit of the stratosphere it is -2°C. This region has the maximum concentration of Ozone. The gas absorbs ultraviolet radiation of the sun, raising the temperature and causing a positive lapse rate.

The importance of ozone lies in the fact that it acts as a protective shield for life on earth and from the detrimental effects of the scorching ultraviolet rays. It also supplies the heat for dividing the earth into a quiescent stratosphere and turbulent troposphere.

Since mixing in the stratosphere is very slow, the molecules or particles here persist for a long time. Therefore, once the pollutants are able to reach the stratosphere, they pose long-term global hazards, compared to their impact in the more dense troposphere.

Mesosphere:

This layer has been so named because of its situation in the middle of the five layers that is divided according to the thermal conditions of the atmosphere. The mesosphere stretches at altitudes between 50 and 80 km. It is also known as upper Troposphere since, due to the absence of ultraviolet absorbing species, temperature falls with increasing altitude, i.e. negative lapse rate prevails, basically similar to what happens in the troposphere.

During summer, the temperature of the mesopause over Arctic region can drop to as low as -100°C. The upper part of this layer remains colder while the lower part is warm. Due to this, there arises a connective motion of air. A significant feature of this layer is the formation of noctilucent cloud or an ice-cloud, formed by the deposition of a very small amount of water vapour on the nickel containing cosmic dust (by depositing, it means here the process by which water vapour turns into ice-crystals directly on the condensation nuclei).

The noctilucent cloud is silver-white and light blue in colour. It is cirrus-like in shape and appears over higher latitudes before sunset and disappears after sunset. A series of intense photochemical reactions, i.e. dissociations and recombination's of different elements of the atmosphere, take place in the mesosphere under the action of the sun's ultraviolet radiation.

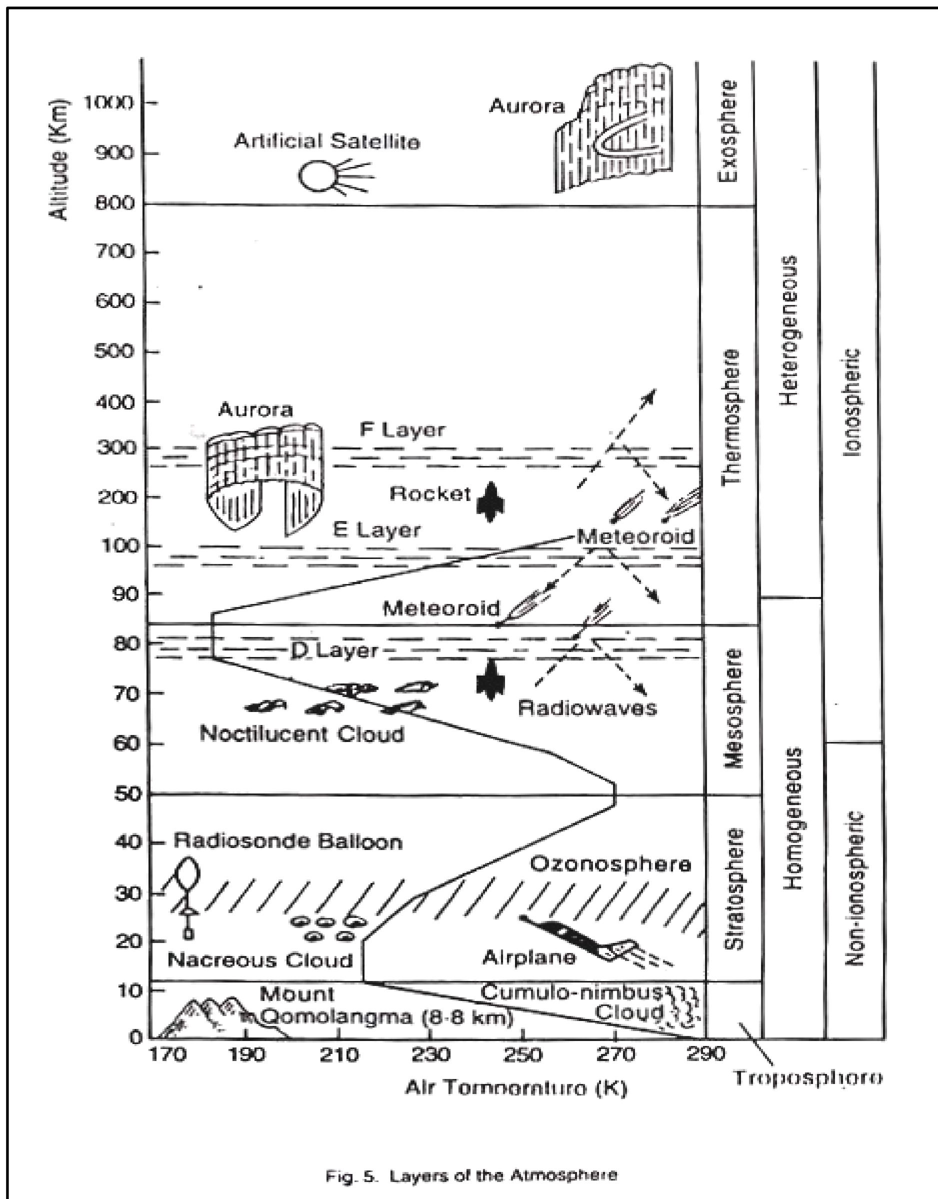


Fig. 5. Layers of the Atmosphere

In the layer below the mesosphere, the air is homogeneously mixed since both horizontal and vertical motions are present. For this reason, the atmosphere up to 90 km is termed as the homogeneous atmosphere.

Thermosphere:

This layer that extends from the mesopause up to about 800 km is noted for two striking features:

(a) Temperature rises with increasing altitude i.e. positive lapse rate prevails here. Satellite data shows that at 200 km temperature stays around 700°C, and at 300 km it exceeds 1,000°C. The high temperature results from the heat released by the dissociation of oxygen and nitrogen molecules and atoms. The dissociation is caused by the sun's ultraviolet radiation with wavelengths shorter than 0.175 microns that can absorb the gases in the layer.

(b) The air is highly ionized. The gases present here, especially nitric oxide and oxygen, split into atoms and undergo ionization, following absorption of solar radiation in the far ultraviolet region. Over higher latitudes, the resplendent aurora appears in the Thermosphere. It is the outcome of the collision of some of the charged particles thrown out of the sun with the molecules and atoms of gases in the thermosphere, under the action of the earth's magnetic field.

Exosphere:

This is the outermost layer of the atmosphere, and also the transition zone between the earth's atmosphere and interplanetary space. The air is inconceivably thin and almost completely ionized. It consists of helium and hydrogen, the lightest constituents of air.

Based on its electric properties, the atmosphere can be divided into two layers:

(a) The non-ionosphere is the section of the atmosphere up to 60 km. Here, the constituents remain in neutral state.

(b) The Ionosphere is the section of the atmosphere between 60 and 2,000 km. The ultraviolet radiation from the sun ionizes the air and produces ions and free electrons. The air thus becomes a mixture of charged particles and non-ionized neutral particles. These free electrons act as mirror and reflect certain types of radio-waves.

ii) Hydrosphere:

It includes water in the oceans, lakes, ponds, etc., and covers about 73% area of the earth's surface. Water is the major inorganic nutrient needed by all living organisms, hence, water is essential to all life. First life originated in water.

The means of obtaining and conserving water have shaped the nature of terrestrial life; means of living within the water have the overwhelming influence on aquatic life. Water is one of the main agents in pedogenesis and is also the medium for several different ecosystems.

Water continuously circulates between atmosphere and the earth's surface; this cycle is referred to as the water cycle. The energy for driving the cycle and, thus, ensuring a constant supply of fresh water on land comes from the sun. Solar heat evaporates water from the ocean which is the great reservoir of water.

A lesser amount of water is also evaporated from the surface of the land from plants, a process known as evapotranspiration. All this vaporized water forms clouds which moved by winds, may pass over land where they are cooled enough to precipitate the water as rain or snow. Some of the precipitated water soaks into ground, some runs off the surface into stream and goes directly back to the seas.

The ground water is returned to the surface by springs, by pumps and the transpiration—the movement of water in plants from roots to leaves. Water inevitably ends up back in the sea, but it may become incorporated into the bodies of several different organisms, one after another, en route.

iii) **Lithosphere:**

The solid component of earth is called lithosphere. It is multilayered and includes following three main layers: crust, mantle and core (outer and inner). The core is the central fluid or vapourized sphere having diameter of about 2500 km from the centre and is possibly composed of nickel-iron. The mantle extends about 2900 km above the core. This is in a molten state.

The crust is the outermost solid zone of the earth and it is about 8 to 40 km above mantle. The crust is very complex and its surface is covered with the soil supporting rich and varied

Cross section of earth showing different layers, biotic communities. The soil provides food, shelter, anchorage and concealment from predators to living organisms.

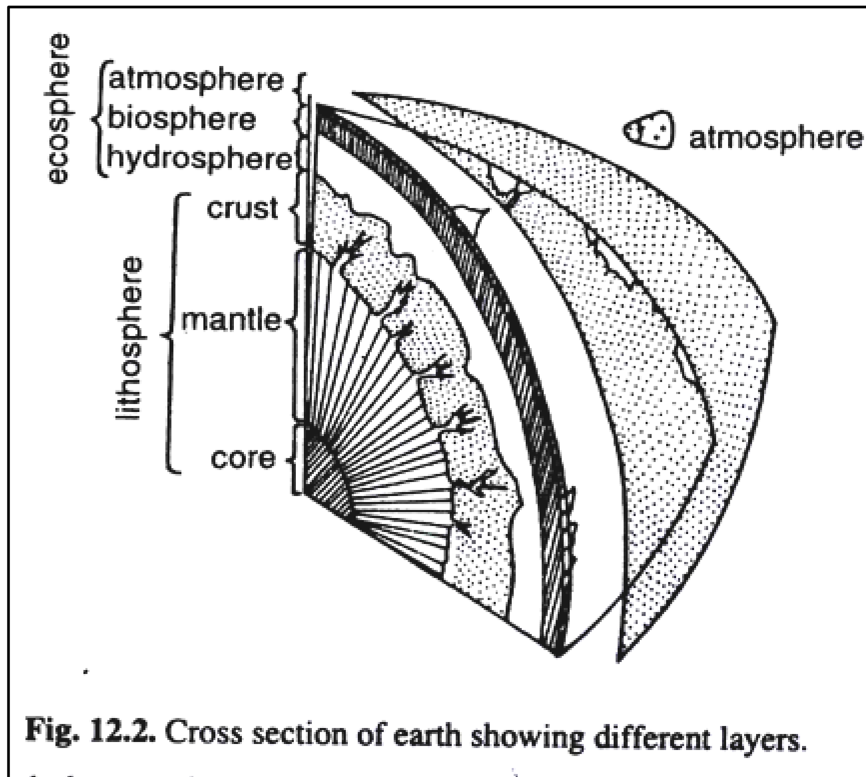


Fig. 12.2. Cross section of earth showing different layers.

The soil is the most characteristic feature of terrestrial environment. It is the top layer of earth's crust and is the mixture of weathered rock materials (i.e., minerals) and organic detritus, both of which are formed through the physical, chemical and biological processes occurring slowly and slowly for a long period at the earth's surface.

Besides being the source for the entry of nutrients and water in plants, the soil is the medium for the detritus food chain: nutrients released in detritus are decomposed by various soil microbes like bacteria, algae, fungi, protozoa, etc., bound in or on soil particles and taken back into plants through their roots.

Soil (mud) is also the main source of nutrients for all aquatic plants; rooted, submerged or free-floating. In addition, soil is the means to support for all terrestrial organisms: plants are anchored to the soil by their root systems; animals walk upon it and are supported by it, as many animals like nematodes, polychaetes, arachnids, insects, rodents, etc., live under the soil.

However, the chemical composition of earth's crust has been shown in Table below. In addition, soil also contains organic matters like humus, etc.

Table 12.2. Composition of dry atmosphere.

S.No.	*Constituent*	*Percentage*
1.	Nitrogen (N_2)	78.084
2.	Oxygen (O_2)	20.947
3.	Argon (Ar)	0.934
4.	Carbon dioxide (CO_2)	0.0314*
5.	Other gases	0.003

* These constituents are highly variable.

Table 12.3. Chemical composition of earth's crust.

S.No.	*Constituent*	*Percentage*
1.	Oxygen (O)	46.6
2.	Silicon (Si)	27.7
3.	Aluminium (Al)	8.1
4.	Iron (Fe)	5.0
5.	Calcium (Ca)	3.6
6.	Sodium (Na)	2.8
7.	Potassium (K)	2.6
8.	Magnesium (Mg)	2.1
9.	Compounds of elements like B, Mn, Cu, Zn, Mo, Co, I, F, etc.	1.4

Thus, these three ecological components and the biotic component of the world constitute the biosphere. All these four components, thus, represent the four major global components of the world ecosystem.

These four spheres continuously exchange matter with one another in a cyclical manner. Thus, biosphere is that part of the earth in which life exists. More specifically, the sum of those portions of the hydrosphere, lithosphere and atmosphere into which life penetrates, is the biosphere.

However, together with the geological, chemical features of the totality of our habitats, these (air, water, earth and organisms) are sometimes grouped under the term ecosphere. Biosphere or ecosphere may be thought as a biochemical system capable of capturing, converting, storing and utilizing the energy of the sun.

Approximately three hundred thousand species of green plants and microorganisms are recognized as primary producers which utilize inorganic elements and compounds to synthesize the organic minerals of life.

Their productivity is consumed by more than a million other species of organisms which convert this organic store-house into animal form, adding to the beauty, and value of the biosphere as well as its complexity. Still other species, primarily bacteria and fungi, accomplish the recycling process by returning plant and animal wastes and residues to inorganic form so the process may be renewed.

In this cyclic process of life, many elements are shared form a common global pool and are converted from inorganic to organic form and back again. Examples of such elements which commonly shuttle among the air, water, earth and organism are carbon, oxygen, hydrogen, nitrogen, phosphorus, sulphur, sodium, potassium, calcium, magnesium, iron, manganese, cobalt, copper, and zinc.

In physical terms, the biosphere is a relatively thin and incomplete envelope covering most of the world. It represents a mosaic of different biotic communities from simple to complex, aquatic to terrestrial, and tropical to polar. It does not exist in the extremities of the Polar Regions, the highest mountains, the deepest ocean troughs, the most extreme deserts, or the most highly polluted areas of land and water.

Its total thickness, including all portions of the earth where living organisms can exist, is less than 26 kilometres. Its zone of active biological production, in terms of photosynthesis, is much narrower, and varies from a few centimetres to over 100 metres.

This zone would, for instance, be only a few centimetres in muddy or turbid water, whereas in very clear ocean water, it could be more than 100 metres in thickness. On land, the zone of biological production might be only a few millimetres in a desert or rock environment, whereas it might again be more than 100 metres in a sequoia or tropical rain forest.

Living organisms can exist, of course, beyond the range of active biological production; some insects or birds may be airborne to altitudes above 20.000 feet, and viable spores, seeds of plants, and microorganisms may be found in the atmosphere and mountain tops above 25,000 feet.

In the ocean depths, many animals can exist well below a thousand feet—one hydra-like animal has been photographed at a depth of 15,900 feet in the south Atlantic.

However, in both extreme altitude and extreme depth, the organisms depend upon the much thinner zone of active biological production, that portion of the system which converts the energy of sunlight into the chemical and physical energy of living organisms.

Radiation Balance:

Apart from a small amount of energy that comes from inside the Earth, the energy that feeds the climate system of our planet comes mainly from the Sun. In fact the Earth receives the radiant energy of the Sun (i.e. transported by electromagnetic radiation), about half of which is visible light, a small part is ultraviolet light, and the remaining part is infrared light. The solar radiation that hits the Earth's surface in one hour is equal to approximately 342 w/m^2; out of which only 235 w/m^2 are actually absorbed by the Earth's surface, while the remaining 107 are immediately reflected into space. The percentage of total incident radiation reflected from the Earth's surface is known as albedo. The Earth's albedo, therefore, is equal to 30% (342/107=30%). Out of these 107 w/m^2, 77 are reflected by the clouds, by gases and by the micro-particles that are present in the atmosphere (aerosol), while the remaining 30 w/m^2 return to the atmosphere as they are reflected by light-coloured surfaces present on the Earth, consisting prevalently of glaciers, snow and deserts. Snow has a very high albedo, equal to 0.9, which means that 90% of the radiation that hits the snow is reflected.

The energy that is not reflected into space, equal to 235 w/m^2, is absorbed by the Earth's surface and by the atmosphere, and is re-emitted in the form of infrared radiations (heat). The atmosphere, which consists prevalently of nitrogen and oxygen, that are transparent to infrared thermal radiation, let these radiations escape into space. However there are some gases (known as greenhouse gases) that absorb thermal radiation and prevent their dispersion, and this causes a warming of the atmosphere. This natural physical phenomenon, called greenhouse effect is very important for life on Earth because it allows the Earth's surface to have an average temperature of 14°C instead of -18°C which would be the case without greenhouse gases in the atmosphere.

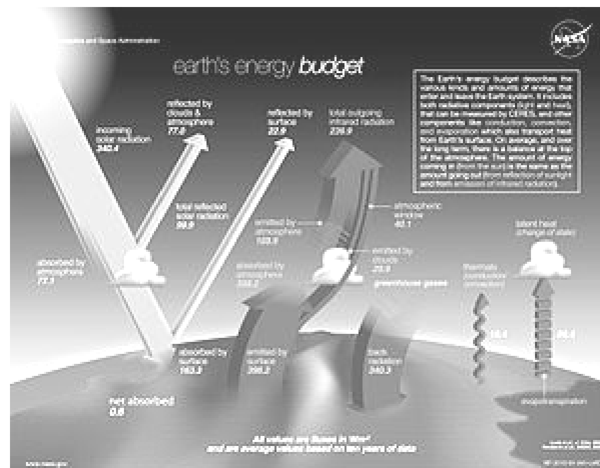


Figure: Earth's Energy Budget.

Climate Cycle:

The word climate derives from the Greek word κλιμα, or inclination. Climate, in fact, is partly determined by the inclination of the sun's rays on the Earth's surface, which varies with the latitude and seasons. The term "climate" is often used as a synonym of meteorological "weather" even though their meanings are quite different. Meteorological weather is the set of meteorological elements that characterize the atmosphere at a precise moment. If we look out of the window we can immediately see what the weather is like. The climate, instead, is defined as the "set of meteorological phenomena (e.g. temperature, precipitation, winds, etc.) that characterize the average state of the atmosphere at a particular point of the Earth's surface", (according to the definition given by J. Hann an Austrian meteorologist). Due to the extreme variability of meteorological parameters, the World Meteorological Organization has established that, in order to identify climatic characteristics, and therefore the "average state of the atmosphere", of a particular location, the minimum duration of the historical time-series of meteorological data must be at least 30 years. The discipline that studies the climate, its elements and its factors, and classifies the types of climate is called climatology.

If the Earth rotated around the sun in a perfectly vertical way like a spinning top, the weather wouldn't vary during the year, in other words there wouldn't be any seasons, because the amount of solar radiation reaching the planet' surface would always be the same. If the earth's axis wasn't at an angle with respect to the orbital plane, not only would there be no seasons, but neither could there be any differences between one hemisphere and the other and obviously the tropics would not exist. Instead, fortunately for us, during the year the inclination of earth's axis with respect to the solar rays changes thus varying the angle at

which they reach the planet. Therefore on the 21st of June, summer solstice, the solar rays are perpendicular to the imaginary line called Tropic of Cancer in our hemisphere; while the 22nd of December, which is winter solstice in our hemisphere whereas it is summer in the Austral one, solar rays are perpendicular with respect to the imaginary line called Tropic of Capricorn which is in the southern hemisphere. During each equinox, instead, solar rays are perpendicular to the Equator. Rays that reach the ground perpendicularly have the maximum atmosphere penetration power and the maximum thermal effect at both tropics, which is why the tropical belt is the area with the hottest and most constant climate all year round. Climate variation increases as one goes north towards the boreal hemisphere and south towards the Austral one. The temperature difference among the various latitudes trigger the complex climatic thermodynamics which actually are influenced by many other factors: local geology, the presence of large water masses or high altitudes and atmospheric conditions. A clear example of how the local geography influences climate is the Assuan dam that created the Nasser Lake, PIC21 a huge artificial basin that had deep implications not only on the region's weather, but also on the southern Mediterranean area. Before it was built it was impossible to imagine that it would affect the weather so heavily and from that we have learned that weather dynamics are really complex phenomena to foresee with mathematical precision.

A climate oscillation or climate cycle is any recurring cyclical oscillation within global or regional climate, and is a type of climate pattern. These fluctuations in atmospheric temperature, sea surface temperature, precipitation or other parameters can be quasi-periodic, often occurring on inter-annual, multi-annual, decadal, multidecadal, century-wide, millennial or longer timescales. They are not perfectly periodic and a Fourier analysis of the data does not give a sharp spectrum.

A prominent example is the El Niño Southern Oscillation, involving sea surface temperatures along a stretch of the equatorial Central and East Pacific Ocean and the western coast of tropical South America, but which affects climate worldwide. Records of past climate conditions are recovered through geological examination of proxies, found in glacier ice, sea bed sediment, tree ring studies or otherwise.

Many oscillations on different time-scales are hypothesized, although the causes may be unknown. (Some of them are more like a random walk than an oscillation.) Here is a list of known or proposed climatic oscillations:

the Quasi-biennial oscillation – about 30 months

the El Niño Southern Oscillation – 2 to 7 years

the Pacific decadal oscillation – 8 to 12 years? (not clear)

the Interdecadal Pacific Oscillation – 15 to 30 years? (not clear)

the Atlantic Multidecadal Oscillation – around 50 to 70 years, but unpredictable

a 60-year climate cycle recorded in many ancient calendars[1]

North African climate cycles – tens of thousands of years

the glacial periods of the current ice age – period around 100 000 years

the Arctic oscillation – no particular periodicity

the North Atlantic Oscillation – no particular periodicity

Some natural periodicities in the sun exist, and these may or may not show up as periodicities in climate:

the Schwabe Cycle or sunspot cycle – about 11 years (may be discernible in climate records)

the Hale Cycle or double sunspot cycle - about 22 years

the Gleissberg Cycle - a solar cycle of about 88 years

the Suess Cycle or De Vries Cycle - a solar cycle of about 200 years

the Hallstadtzeit/Hallstatt Cycle - a solar cycle of about 2,200 to 2,400 years

Anomalies in oscillations sometimes occur when they coincide, as in the Arctic dipole anomaly (a combination of the Arctic and North Atlantic oscillations) and the longer-term Younger Dryas, a sudden non-linear cooling event that occurred at the onset of the current Holocene interglacial. In the case of volcanoes, large eruptions such as Mount Tambora in 1816, which led to the Year Without a Summer, typically cool the climate, especially when the volcano is located in the tropics. Around 70,000 years ago the Toba super volcano eruption created an especially cold period during the ice age, leading to a possible genetic bottleneck in human populations. However, outgassing from large igneous provinces such as the Permian Siberian Traps can input carbon dioxide into the atmosphere, warming the climate. Triggering of other mechanisms, such as methane clathrate deposits as during the

Paleocene-Eocene Thermal Maximum, increased the rate of climatic temperature change and oceanic extinctions.

Another longer-term near-millennial oscillation involves the Daansgard-Oeschger cycles, occurring on roughly 1,500-year cycles during the last glacial maximum. They may be related to the Holocene Bond events, and may involve factors similar to those responsible for Heinrich events.

There are close correlations between Earth's climate oscillations and astronomical factors (barycenter changes, solar variation, cosmic ray flux, cloud albedo feedback, Milankovic cycles), and modes of heat distribution between the ocean-atmosphere climate system. In some cases, current, historical and paleo-climatological natural oscillations may be masked by significant volcanic eruptions, impact events, irregularities in climate proxy data, positive feedback processes or anthropogenic emissions of substances such as greenhouse gases.

Effects:

Extreme phases of short-term climate oscillations such as ENSO can result in characteristic patterns of floods and droughts (including megadroughts), monsoonal disruption and extreme temperatures in the form of heat waves and cold waves. Shorter-term climate oscillations typically do not directly result in longer-term climate change in temperatures. However, the effects of underlying climate trends such as recent global warming and oscillations can be cumulative to global temperature, producing shorter-term fluctuations in the instrumental and satellite temperature records. Collapses of past civilizations such as the Maya may be related to cycles of precipitation, especially drought, that in this example also correlates to the Western Hemisphere Warm Pool. One example of possible correlations between factors affecting the climate and global events, popular with the media, is a 2003 study on the correlation between wheat prices and sunspot numbers

Probable questions:

1. What is atmosphere? Describe the different layers of Atmosphere.

2. How hydrosphere is formed?

3. What is radiation balance.

4. Describe the zonation in lithosphere.

5. Name some of the oscillations in climate cycle. What is the effect of climate cycle?

Suggested Readings:

1.http://www.biologydiscussion.com/atmosphere/atmosphere-zones-5-main-zones-of-atmosphere-environment/16623

2. http://www.eniscuola.net/en/argomento/climate-change/climate/radiation-balance-on-earth/

3.http://www.eniscuola.net/wpcontent/uploads/2013/11/migrazione/assets/6293/pdf_climate_changes_1.pdf

4. Essentials of Ecology & Environmental Science, S.V.S. Rana. 5th Edition.2013

Unit-X

Anthropogenic impact on environment: Green House gases, global warming, ozone depletion, UN movements on environment.

Objective:

In this Unit we will discuss about Anthropogenic impact on environment: Green House Gases, Global warming, Ozone depletion and UN movements on environments.

Green House gases and Global warming:

The green house effect is a naturally occurring phenomenon which is responsible for heating the earth surface and atmosphere. Due to green house effect, the average temperature of earth surface is 15°C and without green house effect the average temperature would have been –18°C. A green house (also called as glasshouse) is a building in which plants are grown. These structures range in size from small sheds to industrialized buildings.

A greenhouse has different type of covering materials, such as glass or plastic roof and walls. It accumulates temperature and heats up because incoming visible solar radiation from the sun is absorbed by plants, soil and other things inside the building. The absorbed radiation gets accumulated and converted to heat energy (lower frequencies of infrared thermal radiation). Infrared radiation is absorbed by green house gasoes and water vapours. Some of the heat rays one reflected by the glass panes and again come back to the surface. Warming effect found in green house is due to accumulation of heat rays. Green house warm up is similar to the inside of a car parked in the sun for some time.

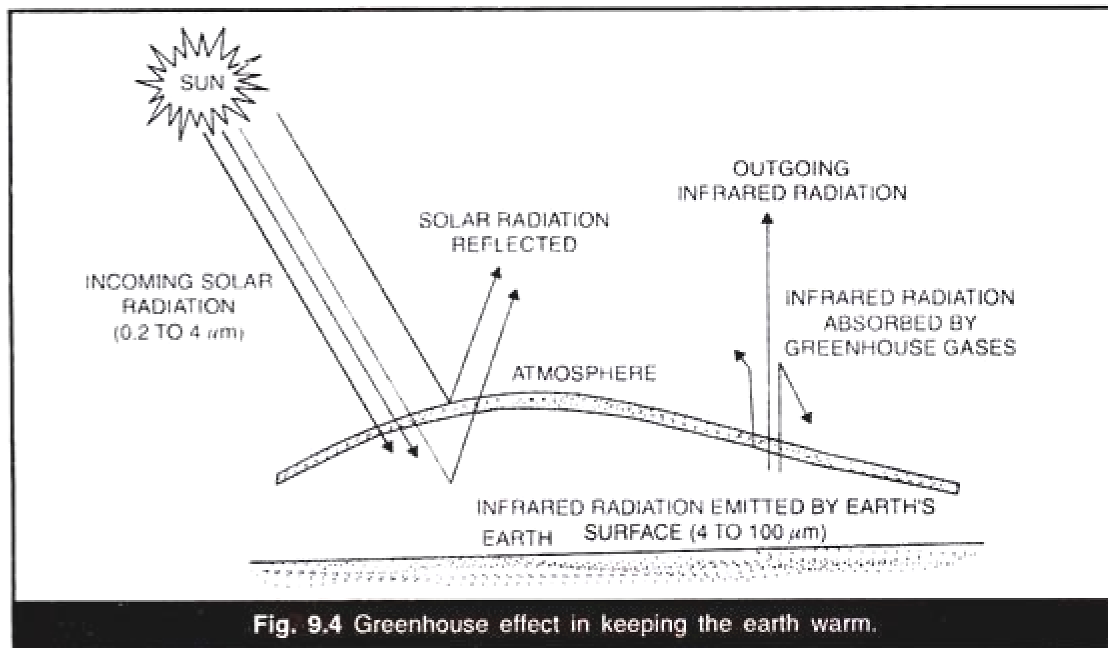


Fig. 9.4 Greenhouse effect in keeping the earth warm.

The gases which allow the solar radiations to pass through but retain the long wave heat radiations are called green house gases. The various green house gases are CO_2, CH_4, CFCs and N_20 and others of minor significance are water vapours and ozone. They prevent a substantial part of long wave radiations emitted by earth to escape into space.

Contribution of Greenhouse Gases to Global Warming:
The gases in the atmosphere that act like glass in a greenhouse are called greenhouse gases.

These are as follows:

1. Carbon dioxide (causes 9—26%)

2. Water vapour (causes about 36 – 70%) of the greenhouse effect (not including clouds)

3. Methane (causes 4 – 9%)

4. Ozone (causes 3 – 7%)

5. Chlorofluorocarbons (CFCs)

6. Oxides of Nitrogen.

These gases act like the glass in a botanical greenhouse, trapping the reradiated heat near the earth's surface and warning the planet. These gases along with water vapour and clouds absorb the infrared radiation, trapping heat near the earth's surface.

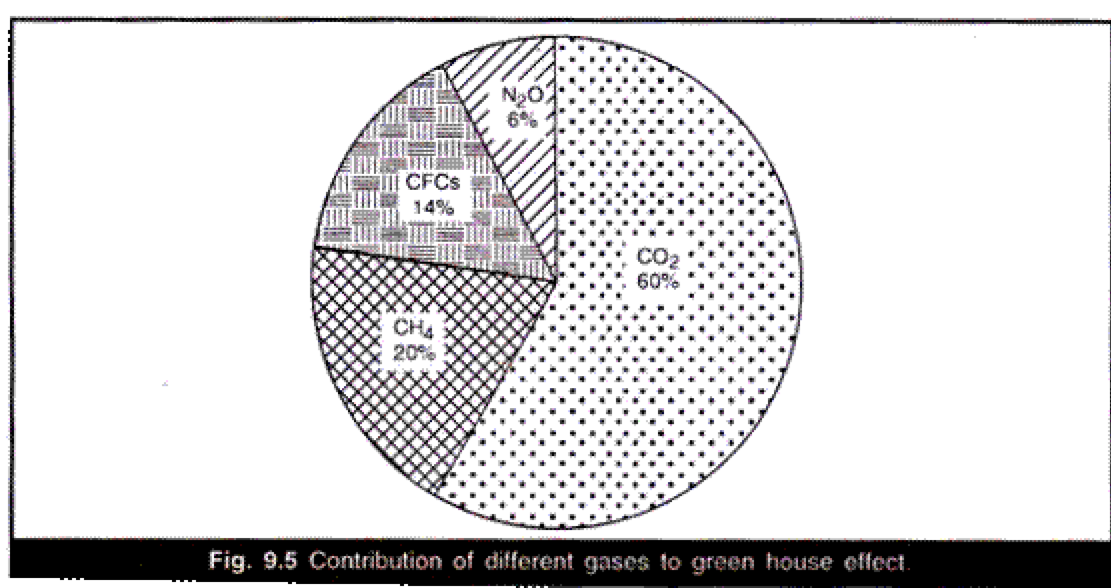


Fig. 9.5 Contribution of different gases to green house effect.

Carbon Dioxide (CO_2):

The CO_2 is considered as the most dominant factor responsible for the Gh effect. The troposphere or lower atmosphere contains only 0.0325% CO_2 (by volume) and its amount is controlled by a cycle, often called the carbon cycle.

There are four major 'pools' or reservoirs of carbon:

(a) Fossil fuels,
(b) The atmosphere,
(c) The biosphere, and
(d) The oceans.

Carbon dioxide concentration in the atmosphere has increased from 290 ppm (1860) to 350 ppm (today) and is expected to be 700 ppm in 2100. It was estimated in 1976 that the world consumption of about 5000 million tons of fossil fuels per year was contributing the equivalent of 2.3 ppm of CO_2 to the atmosphere. The net annual increase of CO_2 in the air is 0.77 ppm, which indicates that 1.6 ppm of fuel generated CO_2 is being absorbed elsewhere perhaps into the oceans and the biosphere.

A continued increase in world fossil fuel combustion in the future can be expected to produce a further increase of CO_2 in the troposphere (about 340 ppm in 1981), and this has been estimated at 20% by the year 2000. Thus it is widely accepted that during the last couple of decades the fossil fuels may have contributed more CO_2 to the atmosphere than the terrestrial biosphere and that the oceans may not be able to absorb CO_2 that is building up in the atmosphere. Clearance of forests is another factor for the increase of CO_2.

Nitrous oxide (N_2O):

It is emitted to the atmosphere from biomass burning and artificial fertilizers, rising by 0.2% per year. Since it absorbs 259 times more heat than CO_2, it contributes to global warming by increasing the greenhouse effect. It also destroys tropospheric ozone, which protects the earth from UV radiation.

Methane (CH_4):

It is produced in a number of ways including the action of anaerobic bacteria on vegetation, decomposition of organic matter, incomplete combustion of vegetation, natural gas pipeline leaks and is rising by 2% per year. It absorbs 20-25 times more heat than CO_2.

CFCs:

There are large scale industrial uses of CFCs, which include aerosol propellants (CFC-11), refrigerants (CFC-12) and a wide range of solvents. The concentration of CFCs is rising nearly 5% per year. Like nitrous oxides, CFCs trap heat 20,000 times more efficiently than $CO_2$and also destroy ozone layer, thus posing a serious twofold environmental problem. The effect of CFCs is so powerful that by the year 2050 they alone could contribute more than CO_2 to global warming (Dickson and Cicerone, 1986).

Ozone:

It comes mostly from hydrocarbons (HC) and nitrogen oxides (NO_x). It causes irritation to eyes and respiratory organs. It also decreases the resistance power to infections and aggravates illness.

Infra-red Absorption:

The CO_2 is a greenhouse gas, that is, it remains transparent to the incoming shortwave solar radiation and absorbs the outgoing long wave radiation (infra-red or IR radiation). This absorptive property of CO_2 prevents long wave IR, particularly the earth's thermal radiation, being emitted to the outer atmospheric layers and lost into outer space. The warming effect of this upon the atmospheric temperature has been linked to a "blanket" or 'greenhouse effect'. The increasing concentration of CO_2 in the atmosphere results in a 'heat frap' around the earth raising its temperature. Thus, Gh effect is a phenomenon based on well established principles of infra-red (IR) absorption characteristics of gases. Besides CO_2, a number of trace gases exhibit IR-absorption properties and contribute to the Gh effect. The dominant

role of CO_2 in producing the net Gh effect has been important due to its role in the evolution of the earth's climate in the past billion years, and as a major pollutant released into the atmosphere as a result of man's activities.

What determines the contribution of a greenhouse gas to global warming?

1. Concentrations of H_2O and CO_2 are the two biggest contributors to the atmospheric warming because of their higher concentrations.

2. Lifetime of each greenhousegas.

3. The longer-lived a gas is, the higher the contribution, e.g., N_2O contribution > CH_4.

4. Effectiveness as an infrared absorber CFC- 11 and CFC-12.

Among the greenhouse gas, the contribution of CO_2 is very significant. The concentration atmospheric CO_2 level over a century has increased in alarming level as recorded from various observatories.

Global warming:

Increase in the level of green house gases has led to considerable heating effect on Earth, so that there has been constant rise in atmospheric temperature during past 4-5 decades. During the past century the temperature of earth has increased by 0.6°C. According to IPPC (intergovernment Panel on Climatic Change) average temperature of Earth would rise by 1.4°C to 5.8°C. According to the forecast of World Climate Programme (WCP) 1988, there will be slight rise in temperature in tropics, moderate in middle latitudes and maximum in Polar Regions.

Causes of Global warming:

Global warming occurs when carbon dioxide (CO_2) and other air pollutants and greenhouse gases collect in the atmosphere and absorb sunlight and solar radiation that have bounced off the earth's surface. Normally, this radiation would escape into space—but these pollutants, which can last for years to centuries in the atmosphere, trap the heat and cause the planet to get hotter. That's what's known as the greenhouse effect.

In the United States, the burning of fossil fuels to make electricity is the largest source of heat-trapping pollution, producing about two billion tons of CO2 every year. Coal-burning power plants are by far the biggest polluters. The country's second-largest source of carbon pollution is the transportation sector, which generates about 1.7 billion tons of CO2 emissions a year.

Impact of Global Warming on Climate and Living Organisms:

Some of the major impact of global warming on climate and living organisms are as follows:

(A) Climate Change (B) Climate Change and Plant Communities (C) Effect on Sea Levels (D) Reduction of Biodiversity (E) Effect on Agriculture (F) Effect on Arctic Ecosystems (G) Overall Effect.

(A) Climate Change:

It is believed that increased levels of greenhouse gases that cause global warming, have affected the global climate already and these effects will increase in future. According to IPCC (1996), the world climate has warmed from 0.3 to 0.6°C during the last century. Complex computer models of global climate predict that temperatures will increase further by 1°C to 3.5°C over the next century as a result of increased concentrations of carbon dioxide and other greenhouse gases.

The increase in temperature will be greatest at high latitudes and over large continents (Myneni et al, 1997). However, some scientists also predict an increase in extreme weather events such as flooding, regional drought and hurricanes associated with this warming (Karl et al. 1997). It seems likely that many species will be unable to adjust quickly to global warming and associated climate change.

As a result, biological communities may suffer profoundly. More than 10% of plant species in many temperate parts wall not be able to survive the new climatic conditions, they must migrate northward or die. This change has already been observed with alpine plants found growing higher on mountains and migratory birds spending longer times on their summer breeding grounds.

However, the effects of global climate change on rainfall and temperature are expected to be less drastic in the tropics than in the temperate zones. But even small changes in the amount

and timing of rainfall will effect species composition and plant reproduction cycles. Changes in temperature and global climate can be expected to influence the biogeochemical cycles, which have already been perturbed by anthropogenic disturbances.

(B) Climate Change and Plant Communities:

Climatic changes as a result of global warming will naturally affect the biotic communities on this earth. Some plant species may utilize the increased CO_2 concentrations and high temperature to increase their growth rates, but less adaptable species will decrease in abundance. Such unpredictable fluctuations in plant communities and associated herbivorous insect species could lead to the extinction of many rare species and great population increases in some other species.

As a result, the global climate change may restructure biological communities and change the distributional ranges of many animal and plant species. Some species may be in danger of going extinct in the wild and therefore new conservation strategies including captive breeding, will have to be adopted.

(C) Effect on Sea Levels:

Rising temperatures will cause glaciers to melt and the polar ice caps to shrink. As a result of this, sea levels may rise by 0.2 to 1.5 m and flood low lying coastal areas and their biotic communities. There is evidence that this process has already started. Sea levels have already risen by 10 to 25 cm over the last 100 years, possibly due to rising global temperatures (IPCC, 1996). If the trend continues, many low lying areas may be submerged in near future.

It is possible that rising sea levels may significantly change or destroy 20% -80% of the coastal wetlands. In tropical areas, mangroves will be adversely affected as seawater will be too deep in existing mangrove areas to allow the seedlings to develop. Rising sea levels are detrimental to coral reef species, which grow at a precise depth with optimum temperature and water movement.

It is possible that slow growing coral reefs will be unable to keep pace with the rise in sea level and will be gradually submerged and die and only fast growing coral reef species will be able to survive. This threat to coral reefs may be further compounded by increasing seawater temperatures. Abnormally high water temperatures in the Pacific Ocean during 1982 and 1983 caused the death of symbiotic algae that live inside the coral. Subsequently, the

"bleached" coral suffered a massive dieback of 70%-95% coral cover of die area to depths of 18m (Brown and Ogden, 1993).

(D) Reduction of Biodiversity:

As mentioned above increased temperatures, inundation of some coastal biological communities and changes in the pattern of distribution of many species over a long period of time are likely to cause reduction in biodiversity in aquatic and terrestrial ecosystems.

(E) Effect on Agriculture:

The global climate change may have important effects on agriculture (Rosenweig and Parry, 1994). However, the effects of this change will vary for C_3 (e.g., wheat, rice, beans) and C_2 (e.g., maize, millet, sugarcane) plants. As temperatures increase with rising levels of CO_2, some crop plants may no longer be grown in certain regions. According to Ricklefs and Miller (2000), under the most common models of global climate change, global temperature increases will have negative effects on both C_2 and C_4 plants unless the higher levels of CO_2 in the atmosphere increase plant growth.

(F) Effect on Arctic Ecosystems:

Global climate change will have profound effects on arctic ecosystems. Studies on the response of arctic Tundra to elevated CO_2 indicated that the Tundra is more sensitive to global climate change than most other ecosystems on earth. According to Shaver et. al (1992), warmer temperatures may increase primary production, thereby increasing carbon input and soil respiration, thereby increasing carbon output. The extent to which production may be increased is constrained by the availability of nitrogen.

(G) Overall Effect:

The overall effect of global warming on world climate has many dimensions, some of which are discussed above. The natural greenhouse maintains the earth's temperatures within the limits for physiological functions. But studies suggest that even a moderate increase in the average global temperature could result in significant changes in biotic communities including reduction in biodiversity both in terrestrial and aquatic ecosystems.

International Concern for Mitigating Global Change:

Global warming is the global concern. The long term challenge of stabilizing the atmospheric concentrations of greenhouse gases requires that global emissions be significantly lowered than what they are today. In this context, the representatives of many countries assembled from time to time to pass some resolutions, which can be implemented.

Montreal Protocol:

In 1987, seventy seven industrially developed countries signed the Montreal Protocol. This conference was held at Montreal. This was a landmark international agreement to protect the stratospheric ozone by agreeing to limit the production and use of ozone- depleting substances.

It was decided in the conference to limit the use of CFCs and cut level of CFCs production to 50 per cent by 1999. To-date, more than 175 countries have signed the Montreal Protocol.

However, India and China did not sign the Protocol. Developed countries have also agreed to establish a multilateral find under Montreal Protocol to provide financial support to developing countries for that purpose.

London Protocol:

In 1990, London Protocol was signed. In London Protocol, India, China and several other countries demanded more time to reduce CFCs consumption. However, the developed countries agreed to stop CFCs consumption completely by 2000 AD, and for developing nations the time limit was extended to 2010 AD.

Toronto Conference:

In 1988, the world conference on the changing atmosphere was held in Toronto (Canada). There it was decided to reduce 20 per cent carbon dioxide emissions by the year 2005.

Earth Summit:

In 1992, The First Earth Summit was held in Rio de Janeiro, Brazil. The Earth Summit was held under the banner of UNCED (i.e., The United Nations Conference on Environment and Development). This summit, established the principles for reducing greenhouse gas emission.

Kyoto Protocol:

The Kyoto Protocol, approved by a follow-up conference held in Kyoto, Japan, during December 1997, has specified the commitments of different countries to mitigate climate change. This protocol has established the rules to reduce overall greenhouse gas emissions to a level at least 5 per cent below the 1990 level by 2008-2012.

At present, on the basis of Montreal Protocol, India has established ozone cell under the Ministry of Forests and Environment with the help of UNDP (United Nations Development Programme). By now, India has developed R-22 and R-134 as CFCs substitutes which are presently at trial stage.

In 2015, the U.S. Environmental Protection Agency pledged to reduce carbon pollution from our power plants by nearly a third by 2030, relative to 2005 levels, through its Clean Power Plan. But fast-forward to 2017, and under the Trump Administration, the EPA proposed repealing this critical tool for curbing climate change. Likewise, while under the Obama administration, the U.S. Department of Transportation proposed carbon pollution and fuel economy standards intended to cut emissions through the 2020s, under Trump administration, the DOT is working to roll back those clean vehicle safeguards that protect the climate and our health.

Fortunately, state leaders—including in car country itself—recognize that clean transportation must remain a priority if we are to address the costly risks of climate change and protect public health. And regional efforts around the country are helping to boost the electric car market, which saw an increase in sales for 2017 over 2016. Our clean energy economy is growing too, despite federal efforts to derail it. In 2016, wind employment grew by 32 percent and solar jobs increased by 25 percent.

Globally, at the United Nations Conference on Climate Change in Paris, 195 countries—including the United States, at the time—agreed to pollution-cutting provisions with a goal of preventing the average global temperature from rising more than 1.5 degrees Celsius above preindustrial times. (Scientists say we must stay below a two-degree increase to avoid catastrophic climate impacts.)

To help make the deal happen, the Obama administration pledged $3 billion to the Green Climate Fund, an international organization dedicated to helping poor countries adopt cleaner energy technologies. Under the terms of the Paris agreement, participating nations will meet

every five years, starting in 2020, to revise their plans for cutting CO_2 emissions. Beginning in 2023, they will also have to publicly report their progress.

While in 2017, President Trump announced the country's withdrawal from the Paris climate agreement and to eliminate "harmful and unnecessary policies such as the Climate Action Plan," Americans are forging ahead without him. Through initiatives like the United States Climate Alliance, the Regional Greenhouse Gas Initiative, We Are Still In, and Climate Mayors, state, business, and local leaders have pledged to honor and uphold the goals of the Paris Agreement. More than 25 cities in 17 states, with populations totaling more than 5 million have adopted resolutions that will enable them to get 100 percent of their electricity from renewable sources like wind and solar.

Even better, a new initiative by former New York City mayor Michael Bloomberg gives the urban layer of this movement a boost. He's asked mayors from the 100 most populous cities in the country to share their plans for making their buildings and transportation systems run cleaner and more efficiently. The 20 that show the greatest potential for cutting the dangerous carbon pollution that's driving climate change will share a total of $70 million in technical assistance funding provided by Bloomberg Philanthropies and partners.

To come to a conclusion on climate change, the United Nations formed a group of scientists called the Intergovernmental Panel on Climate Change, or IPCC. The IPCC meets every few years to review the latest scientific findings and write a report summarizing all that is known about global warming. Each report represents a consensus, or agreement, among hundreds of leading scientists.

Probable Questions:

1. What is Green House effect? Name some of the green house gases and their role in global warming.

2. What are the causes of Global warming. Describe the effects of Global warming on different systems.

3. Give a note on UN measures relating to controlling Global Warming.

Suggested Readings:

1. http://www.biologydiscussion.com/speech/speech-on-global-warming/5496

2.http://www.biologydiscussion.com/ecosystem/impact-of-global-warming-on-climate-and-living-organisms/4814

3. http://www.biologydiscussion.com/notes/study-notes-on-green-house-effect/5517

Unit-XI

Environment and agriculture: Green revolution and its impact on environment, organic farming, participatory approach in agriculture.

Objective:

In this Unit we will discuss about Environment and Agriculture: Green Revolution and its impact on environment. Organic Farming, Participatory approach in agriculture

Introduction:

Green revolution usually refers to the transformation and diversified agriculture that started in 1945 in Mexico. In 1943, Mexico imported half of its wheat from other countries to meet up their hunger. But due to taking scientific technology in agricultural field, specially for wheat production, through establishment of advanced agricultural research pilot project- "Green Revolution", By which in 1956 made Mexico self-sufficient in wheat production and by 1964, it exported half a million tons of wheat to other countries. This is possible due to transformation of advance agricultural technology obtain from research work funded by The Rockefeller Foundation along with the Ford Foundation in USA. Many agronomist state that the green revolution has allowed food production to keep balance with the world wide population growth. Thus the green revolution has the major social & ecological importance making it a popular topic of social science.

Brief History of Green Revolution:

The term 'Green revolution' was first used in 1968 by former US-AID director WILLIAM GAUD. He noted that it is a spread of new technologies followed by developments in the field of agriculture with a new revolution. It is not a violent-red revolution occurred in Soviet Union, nor it is a white revolution which occurred in Denmark, but it is called the green revolution in the field of agriculture.

Green Revolution in India:

In 1961, India was on the brink of mass famine. During that time Indian Agricultural Minister M.S.Swaminathan with the help of Ford Foundation of USA imported wheat seed and started culture in Punjab provinces.

Thus India began its own green revolution programme in the state of Punjab through cultivation of wheat, irrigation development and financing of agrochemicals. Thus India soon adopted IR-8 – a rice of semi dwarf variety developed by the International Rice Research Institute (IRRI) which would produce more grains of rice per plant when grown properly with fertilizer and irrigation. It is reported that IR-8 rice yielded about 5 ton per hectare with no fertilizer and 10 ton per hectare with fertilizer, proper irrigation, and use of agrochemicals. Thus IR-8 was a great success throughout Asia for ten times production which is called as "miracle rice". In this way the green revolution was established in India in the field of agriculture.

To increase agricultural production and productivity, the Government of India invited a team of experts sponsored by the Ford Foundation. The team submitted its report entitled India's Food Crisis and Steps to Meet It. in April 1959. This report suggested the means of improving production and productivity of the country with stress on modern inputs, especially fertilizers, credit, marketing facilities etc.

On the basis of the recommendation of this team Government introduced Intensive Area Development Programme (lADP) in 1960 in seven selected districts. The seven selected districts were West Godavari (AP), Shahabad (Bihar), Raipur (Chhattisgarh), Thanjavaur (T.N.), Ludhiana (Punjab), Aligarh in U.P. and Pali (Rajasthan). As a result of high-yielding varieties of wheat the production of wheat rose to high level of 5000 to 6000 kg. These seeds required proper irrigation facilities and extensive use of fertilizers, pesticides and insecticides.

This new 'agriculture strategy' was put into practice for the first time in India in the Kharif season of 1966 and was termed HIGH-YIELDING VARIETIEIS PROGRAMME (HYVP). This programme was introduced in the form of a package programme since it depended crucially on regular and adequate irrigation, fertilizers, high yielding varieties of seeds, pesticides and insecticides.

Impact of Green Revolution on Environment:

(i) Increase in Production and Productivity:

As a result of new agricultural strategy, food grains output substantially increased from 81.0 million tonnes in the Third Plan (annual average) to 203 million tonnes in the Ninth Plan (annual average) and further to 212. 0 million tonnes in 2003-04. HYVP was restricted to

only five crops – wheat, rice jowar, bajra and maize. Therefore, non- food grains were excluded from the ambit of the new strategy.

Wheat has made rapid strides with its production increasing from 11.1 million tonnes (Third Five Year Plan) to 71.3 million tonnes in the Ninth Plan. The production of wheat touched a high level of 72.1 million tonnes in 2003-04, the overall contribution of wheat to total food grains has increased from 13 per cent in 1950 – 51 to 34 per cent in 2003-04. The average annual production of rice rose from 35. 1 million tonnes in the Third Plan to 87.3 million tonnes in the Ninth Plan. It stood at 87.0 million tonnes in 2003-04.

(ii) Scientific Cultivation:

A very important effect of Green Revolution is that traditional agricultural inputs and practices have given way to new and scientific practices. Instead of farm seeds, farmers are now using HYV seeds. Traditional fertilizers are replaced by chemical fertilizers. Consequently under HYV seeds increased sharply from 1.66 million hectares in 1966-67 (when green revolution came to India) to about 78.4 million hectares in 1998-99.

(iii) Change in Cropping Pattern:

Two changes are significant. First, the proportion of cereals in the food grains output has increased and the proportion of pulses has declined. Second, the proportion of wheat cereals has increased while that of coarse grains has declined.

(iv) Development of Industries:

Green revolution has benefited the industrial development. Many industries producing agriculture, machinery, chemical fertilizers, pesticides, insecticides etc., have come up to meet the growing demand for these commodities.

(v) Change in Attitudes:

A healthy contribution of green revolution is the change in the attitudes of farmers. Our farmers have now begun to think that they can change their misfortunes by adopting new technology. Unlike past, they are now giving up traditional agricultural practices for scientific practices.

Package Programme:

The new technology adopted in the Indian agriculture during mid-1960s consists of several ingredients like HYV seeds, chemical, fertilizers, pesticides, irrigation and improved machines and tools like tractors, pump sets etc. All these things are together termed as, package programme.

If any one of these elements are missing there will be no significant remarkable impact on productivity per hectare of land. In this case, we cannot then call it Green Revolution or the New Agricultural technology. Thus, in other words this new technology is known as Package Programme, i.e., it insists the adoption of total package.

The new technology was tried out in 1960 – 61 as a pilot project in seven selected districts of India and this programme was named Intensive Area Development Programme extended to other districts on an experimental basis and was called Intensive Agriculture Areas Programme (lAAP). Thus, as a result both production and productivity per hectare have increased considerably. This qualitative and quantitative improvement in Indian Agriculture is scientifically termed as “Green Revolution ”.

The Government took several steps to improve irrigation facilities in rural areas. The number of tractors used for cultivation increased from 0.3 lakh in 1960-61 to about 20 lakhs in 1999-2000. The gross irrigated area was 22.56 million in 1950-51 and went up to 94.7 million hectares in 1999-2000.

The important achievements of the package programme are:

(i) Increase in the total production of crops.

(ii) Increase in food crops per hectare.

(iii) Increase in use of chemical fertilizers

(iv) Increase in use of HYV seeds,

(v) Increase in use of power tillers and tractors,

(vi) Expansion of irrigational facility.

Limitations of the Green Revolution:

In spite of several achievements, the green revolution has several defects:

(i) More inequality among farmers (Inter-personal inequalities):

The new technology requires a huge amount of investment which can be only afforded by the big farmers. Hence, these farmers are getting the absolute benefits of the green revolution and became comparatively more rich than farmers. This increases inequality in rural India

(ii) Regional inequality:

Benefits of the new technology remained concentrated in wheat growing area since green revolution remained limited to wheat for a number of years. These were thy regions of Punjab, Haryana and Western Uttar Pradesh. On account of the above reasons new agricultural strategy has led to an increase in regional inequalities.

(ii) The Question of Labour Absorption:

There is a general consensus that the adoption of new technology had reduced labour absorption in agriculture. The uneven regional growth was mainly responsible for the low absorption of labour within agriculture. The growth of output was also slow to generate adequate employment opportunities. The sudden rise in the demand for labour in these areas induced mechanisation and labour-saving practices in general.

(iv) Undesirable Social Consequences:

Some micro level socio-economic studies of green revolution areas have revealed certain undesirable social consequences of the green revolution. Many large farmers have evicted tenants as they now find it more profitable to cultivate land themselves.

Thus, a large number of tenants and share-croppers have lost their lands and have been forced to join the ranks of agricultural labourers. Wetlands have also attracted outsiders (non-agriculturists from nearby towns to invest capital in buying farms.

(v) Health Hazards:

The health hazards of the new technology can also not be lost sight of. Increased mechanization that has accompanied the modernisation of farm technology in green revolution areas carries with it the risk of incapitation due to accidents. The attitude of the

Government towards the problems of treatment and rehabilitation of victims of accidents on farm machines is that of total ambivalence. Meagre compensation is provided to victims.

(vi) Change in Attitudes:

A healthy contribution of green revolution is the change in the attitudes of fanners in areas where the new agricultural strategy was practiced. Increase in productivity in these areas has enhanced the status of agriculture from a low level subsistence activity to a money- making activity. The desire for better farming methods and better standard of living is growing up.

Organic Farming:

What is organic farming?

Organic farming system in India is not new and is being followed from ancient time. It is a method of farming system which primarily aimed at cultivating the land and raising crops in such a way, as to keep the soil alive and in good health by use of organic wastes (crop, animal and farm wastes, aquatic wastes) and other biological materials along with beneficial microbes (biofertilizers) to release nutrients to crops for increased sustainable production in an eco friendly pollution free environment.

As per the definition of the United States Department of Agriculture (USDA) study team on organic farming "organic farming is a system which avoids or largely excludes the use of synthetic inputs (such as fertilizers, pesticides, hormones, feed additives etc) and to the maximum extent feasible rely upon crop rotations, crop residues, animal manures, off-farm organic waste, mineral grade rock additives and biological system of nutrient mobilization and plant protection".

FAO suggested that "Organic agriculture is a unique production management system which promotes and enhances agro-ecosystem health, including biodiversity, biological cycles and soil biological activity, and this is accomplished by using on-farm agronomic, biological and mechanical methods in exclusion of all synthetic off-farm inputs".

Need of organic farming

With the increase in population our compulsion would be not only to stabilize agricultural production but to increase it further in sustainable manner. The scientists have realized that the 'Green Revolution' with high input use has reached a plateau and is now sustained with diminishing return of falling dividends. Thus, a natural balance needs to be maintained at all cost for existence of life and property. The obvious choice for that would be more relevant in the present era, when these agrochemicals which are produced from fossil fuel and are not renewable and are diminishing in availability. It may also cost heavily on our foreign exchange in future.

Differences Between Organic and Conventional Farming Methods

In conventional farming method, before seeds are sown, the farmer will have to treat or fumigate his farm using harsh chemicals to exterminate any naturally existing fungicides. He will fertilize the soil using petroleum based fertilizers. On the flip side, the organic farmer will prepare and enrich his land before sowing by sprinkling natural based fertilizers such as manure, bone meal or shellfish fertilizer.

Before planting seeds, the organic farmer will soak the seeds in fungicides and pesticides to keep insects and pests at bay. Chemical are also incorporated in the irrigation water to prevent insects from stealing the planted seeds. On the other hand, the organic farmer will not soak his seeds in any chemical solution nor irrigate the newly planted seeds using water with added chemicals. In fact, he will not even irrigate with council water, which is normally chlorinated to kill any bacteria. He will depend on natural rain or harvest and stored rainwater to use during dry months.

When the seeds have sprung up, and it's time to get rid of weeds, the conventional farmer will use weedicide to exterminate weeds. The organic farmer will not use such chemicals to get rid of the weed problem. Instead, he will physically weed out the farm, although it's very labor intensive. Better still, the organic farmer can use a flame weeder to exterminate weeds or use animals to eat away the weeds.

When it comes to consumption, it's a no-brainer that anyone consuming products from the conventional farmer will absorb the pesticide and weedicide residues into the body, which

could lead to developing dangerous diseases like cancer. People understand that health is important to them and that's why they are going organic in record numbers today.

Reasons For Organic Farming

The population of the planet is skyrocketing and providing food for the world is becoming extremely difficult. The need of the hour is sustainable cultivation and production of food for all. The Green Revolution and its chemical based technology are losing its appeal as dividends are falling and returns are unsustainable. Pollution and climate change are other negative externalities caused by use of fossil fuel based chemicals.

1. To accrue the benefits of nutrients

Foods from organic farms are loaded with nutrients such as vitamins, enzymes, minerals and other micro-nutrients compared to those from conventional farms. This is because organic farms are managed and nourished using sustainable practices. In fact, some past researchers collected and tested vegetables, fruits, and grains from both organic farms and conventional farms.

The conclusion was that food items from organic farms had way more nutrients than those sourced from commercial or conventional farms. The study went further to substantiate that five servings of these fruits and vegetables from organic farms offered sufficient allowance of vitamin C. However, the same quantity of fruits and vegetable did not offer the same sufficient allowance.

2. Stay away from GMOs

Statistics show that genetically modified foods (GMOs) are contaminating natural foods sources at real scary pace, manifesting grave effects beyond our comprehension. What makes them a great threat is they are not even labeled. So, sticking to organic foods sourced from veritable sources is the only way to mitigate these grave effects of GMOs.

3. Natural and better taste

Those that have tasted organically farmed foods would attest to the fact that they have a natural and better taste. The natural and superior taste stems from the well balanced and nourished soil. Organic farmers always prioritize quality over quantity.

4. Direct support to farming

Purchasing foods items from organic farmers is a sure-fire investment in a cost-effective future. Conventional farming methods have enjoyed great subsidies and tax cuts from most governments over the past years. This has led to the proliferation of commercially produced foods that have increased dangerous diseases like cancer. It's time governments invested in organic farming technologies to mitigates these problems and secure the future. It all starts with you buying food items from known organic sources.

5. To conserve agricultural diversity

These days, it normal to hear news about extinct species and this should be a major concern. In the last century alone, it is approximated that 75 percent of agricultural diversity of crops has been wiped out. Slanting towards one form of farming is a recipe for disaster in the future. A classic example is a potato. There were different varieties available in the marketplace. Today, only one species of potato dominate.

This is a dangerous situation because if pests knock out the remaining potato specie available today, we will not have potatoes anymore. This is why we need organic farming methods that produce disease and pest resistant crops to guarantee a sustainable future.

6. To prevent antibiotics, drugs, and hormones in animal products

Commercial dairy and meat are highly susceptible to contamination by dangerous substances. A statistic in an American journal revealed that over 90% of chemicals the population consumes emanate from meat tissue and dairy products. According to a report by Environmental Protection Agency (EPA), a vast majority of pesticides are consumed by the population stem from poultry, meat, eggs, fish and dairy product since animals and birds that produce these products sit on top of the food chain.

Key Features of Organic Farming

- Protecting soil quality using organic material and encouraging biological activity
- Indirect provision of crop nutrients using soil microorganisms
- Nitrogen fixation in soils using legumes

- Weed and pest control based on methods like crop rotation, biological diversity, natural predators, organic manures and suitable chemical, thermal and biological intervention
- Rearing of livestock, taking care of housing, nutrition, health, rearing and breeding
- Care for the larger environment and conservation of natural habitats and wildlife

Four Principles of Organic Farming

- **Principle of Health:** Organic agriculture must contribute to the health and well being of soil, plants, animals, humans and the earth. It is the sustenance of mental, physical, ecological and social well being. For instance, it provides pollution and chemical free, nutritious food items for humans.

- **Principle of Fairness:** Fairness is evident in maintaining equity and justice of the shared planet both among humans and other living beings. Organic farming provides good quality of life and helps in reducing poverty. Natural resources must be judiciously used and preserved for future generations.

- **Principle of Ecological Balance:** Organic farming must be modelled on living ecological systems. Organic farming methods must fit the ecological balances and cycles in nature.

- **Principle of Care:** Organic agriculture should be practiced in a careful and responsible manner to benefit the present and future generations and the environment.

As opposed to modern and conventional agricultural methods, organic farming does not depend on synthetic chemicals. It utilizes natural, biological methods to build up soil fertility such as microbial activity boosting plant nutrition.

Secondly, multiple cropping practiced in organic farming boosts biodiversity which enhances productivity and resilience and contributes to a healthy farming system. Conventional farming systems use mono cropping that destroys the soil fertility.

Benefits of Organically Grown Food Items and Agricultural Produce

Better Nutrition: As compared to a longer time conventionally grown food, organic food is much richer in nutrients. Nutritional value of a food item is determined by its mineral and vitamin content. Organic farming enhances the nutrients of the soil which is passed on to the plants and animals.

Helps us stay healthy: Organic foods do not contain any chemical. This is because organic farmers don’t use chemicals at any stage of the food-growing process like their commercial counterparts. Organic farmers use natural farming techniques that don’t harm humans and environment. These foods keep dangerous diseases like cancer and diabetes at bay.

Free of poison: Organic farming does not make use of poisonous chemicals, pesticides and weedicides. Studies reveal that a large section of the population fed on toxic substances used in conventional agriculture have fallen prey to diseases like cancer. As organic farming avoids these toxins, it reduces the sickness and diseases due to them.

Organic foods are highly authenticated: For any produce to qualify as organic food, it must undergo quality checks and the creation process rigorously investigated. The same rule applies to international markets. This is a great victory for consumers because they are getting the real organic foods. These quality checks and investigations weed out quacks who want to benefit from the organic food label by delivering commercially produced foods instead.

Lower prices: There is a big misconception that organic foods are relatively expensive. The truth is they are actually cheaper because they don’t require application of expensive pesticides, insecticides, and weedicides. In fact, you can get organic foods direct from the source at really reasonable prices.

Enhanced Taste: The quality of food is also determined by its taste. Organic food often tastes better than other food. The sugar content in organically grown fruits and vegetables provides them with extra taste. The quality of fruits and vegetables can be measured using Brix analysis.

Organic farming methods are eco-friendly: In commercial farms, the chemicals applied infiltrate into the soil and severely contaminate it and nearby water sources. Plant life, animals, and humans are all impacted by this phenomenon. Organic farming does not utilize these harsh chemicals so; the environment remains protected.

Longer shelf–life: Organic plants have greater metabolic and structural integrity in their cellular structure than conventional crops. This enables storage of organic food for a longer time.

Organic farming is preferred as it battles pests and weeds in a non-toxic manner, involves less input costs for cultivation and preserves the ecological balance while promoting biological diversity and protection of the environment.

Probable Questions:

1. Discuss about Green Revolution in India.
2. What are the positive effects of Green Revolution?
3. What are the negative effects of Green Revolution?
4. What is Organic farming?
5. What are the differences between Organic and Conventional Farming Methods?
6. Discuss about key features of organic farming.
7. What are the benefits of organic farming?
8. Discuss the principles of organic farming.

Suggested Readings :

1. http://agritech.tnau.ac.in/org_farm/orgfarm_introduction.html

2. https://www.conserve-energy-future.com/organic-farming-benefits.php

3. http://www.economicsdiscussion.net/essays/green-revolution-effects-and-limitations-of-the-green-revolution/2096.

Unit-XII

Theory and analysis of conservation: Stochastic perturbations, population viability analysis, recovery strategy for threatened species.

Objectives:

In this Unit we will discuss about Theory and Analysis of Conservation: Stochastic perturbations, Population viability analysis and Recovery strategy for threatened species

Stochastic perturbations:

Definition

Stochastic perturbations or fluctuations refer to changes in populations owing to chance events.

This is felt more strongly in small populations (Shaffer 1981, Pimm et al. 1988).

Types

These can be divided into two types –

1. Demographic Stochasticity:

Stochastic (random) variations in birth and death rates that occurs in populations from year to year are called **demographic stochasticity**. They cause populations to deviate from the predictions of population growth based on the deterministic models.

2. Environmental Stochasticity:

Random variations in the environment, such as annual variations in climate (temperatureand precipitation) or the occurrence of natural disasters such as fire, flood, and drought, are referred to as **environmental stochasticity.** They can **directly** influence the birth and death rates within the population.

Allele effect

Allele effect, proposed by W. C. Allele, is the decline in either reproduction or survival in conditions of low population density.

Gene

A potential cause of extinction in a small population is reduced genetic diversity. Small population will support less genetic variation than larger population and thus will have reduced ability to adapt to a new disease, predators or changes in physical environment.

There are two mechanisms that operating on a small population and can function to reduce genetic variation –

1. Genetic drift
2. Inbreeding

Genetic drift

The resulting random change in the frequency of allele within the populations (gene pool) as a result of chance is called genetic drift.

Inbreeding

When a population is small, the choice of mates is limited, creating the potential called inbreeding i.e. breeding between genetically closely related individuals. This frequently leads to expression of deleterious recessive genes and the population as a result suffers loss of fitness. This phenomenon of reduced fitness due to inbreeding is called **inbreeding depression**.

Core and satellite population

Populations that persist for relatively long period are often called core subpopulation, where as those that are more likely to wink on and off are called satellite subpopulation. Core subpopulation is likely to be large and serve as net source of individuals while satellite subpopulation is likely to be small and serve as sink.

Population viability analysis (PVA):

Population viability analysis (PVA) is quantitative model-based approach that uses demographic and abundance data to estimate the probability of extinction of a population within a given number of years. It is a species-specific method of risk assessment and thus, plays an important part in recovery plan of endangered species. It involves strategic analysis of the ecological, economic, social and cultural, and political issues and challenges related to the conservation of an endangered species, community or ecosystem.

PVA seeks to determine the minimum viable population size of the concerned species and also to identify restoration procedures that will maintain genetic variability in the population. The smaller the population is the greater the probability that it will become of extinct within a given span of time. PVA model relates a dependant variable (e.g. population size) with the independent variable that influences it (e.g. weather) through parameters like survival rates and reproductive rates of individuals.

Recovery strategies for threatened species:

Because endangered species consists of a few or even a single population, protecting populations is the key to preserving these species. These populations are restricted to a small area and an adequate conservation plan requires the preservation of as many individuals as possible within the greatest possible area.

Minimum viable population size (MVP) and **Minimum dynamic area (MDA):**

Minimum viable population size (MVP) is the smallest population that can persist for a specified time, usually taken to be 1000 years. It allows a quantitative assessment of the number of individuals necessary to ensure long term survival of a species. The MVP is dependent both on the demographics of the population and the genetic diversity within the population.

Once a minimum viable population (MVA) size for the concern species is established, the area required to support the population must be considered. The area of suitable habitat necessary for maintaining the minimum viable population is called Minimum **dynamic area (MDA)**. An estimation of MDA for a species begins with an understanding of the home range size of the individuals, family, groups or colonies.

Habitat restoration ecology:

In recent years, considerable efforts are underway to restore natural communities affected by human activities, stimulating a new approach to human intervention that is termed **restoration ecology**. The goal of restoration ecology is to return an ecosystem to a close approximation of its condition prior to disturbance through the application of ecological principals. It involves a continuum of approaches from the reintroduction of species and the restoration of habitats to attempts to re-establish whole communities as functioning ecosystems and often takes years to get back to previous undisturbed condition.

Generally, the restoration effort involves,

- Rejuvenation of existing communities by eliminating invasive species
- Replanting and reintroducing native species
- Reintroduction of natural disturbances such as short term periodic fires in grasslands etc.
- Reduction of nutrient inputs, especially phosphorus, (that stimulate algae growth) from the surrounding lands, in case of lake restoration
- Restoration of hydrological conditions in case of wetland restoration

Effective population size (N_e):

The **effective population size** is the size of an ideal population (i.e. one that follows all Hardy – Weinberg assumptions) that would undergo the same amount of random genetic drift as the natural population under study.

It is expressed in terms of the number of individuals assumed to contribute genes (through offspring) equally to the next generation. Generally N_e is smaller than the actual size of the population, though it varies depending on the variation in reproductive success among individuals. It is sometimes used to measure the amount of inbreeding in a finite, randomly mating population.

$$N_e = \frac{4\, N_m \times N_f}{N_m + N_f}$$

N_e = Effective population size

N_m = no of breeding males

N_f = no of breeding females

When the no of breeding individuals is small relative to the total number of individuals in the population, N_e is small and vice-versa. It has been suggested that N_e of at least 500 is required in order to maintain the genetic diversity in the face of genetic drift, though it is likely that the minimum number will vary widely among populations having different sex ratios, age structures, and social arrangements (Lande and Barrowclough, 1987).

Reintroduction:

In some cases, saving a species from inevitable decline to extinction requires direct action like establishing new populations through transplants and reintroduction. In these cases captive breeding technique is applied to increase the number of individuals before reintroduction in a habitat or individuals are relocated from high density area to low density area.

Habitat conservation :

Habitat conservation i.e. protecting habitats, functions to protect whole ecological community or overall biological diversity. It requires an understanding of the relationship between overall patterns of biological diversity and features of the landscape. As large areas can support a greater number of species than do smaller areas, it is better to protect as much large area as possible for the conservation purpose.

Protected areas :

The preservation of biological diversity involves the establishment of protected areas i.e. legally and scientifically designed habitat. Protected lands differ in their degree of protection, with many categories serving multiple purposes, including resource extraction.

IUCN has developed a classification system for protected areas which is as follows,

- CATEGORY Ia: Strict Nature Reserve (managed mainly forscientific research)
- CATEGORY Ib: Wilderness Area (wilderness protection)
- CATEGORY II: National parks (ecosystem protection and recreation)
- CATEGORY III: Natural Monuments (conservation of specific natural features)
- CATEGORY IV: Habitat/Species Management Area (conservation through management intervention)
- CATEGORY V: Protected Landscape/ Seascape
- CATEGORY VI: Managed Resource Protected Area (Multiple & sustainable use of natural ecosystem)

Current conservation efforts focuses on providing buffer zones and corridors in existing protected areas that enhance their conservation value.

Probable Questions

1. What is stochastic perturbation? Describe the types of stochastic perturbation.
2. What is allele effect?
3. Describe the mechanisms that operate on small population to reduce genetic variation?
4. Explain inbreeding depression.
5. What is the deference between core and satellite population?
6. What is PVA? How it is used in conservation of threatened species?
7. Discuss about recovery strategies for threatened species.
8. What is MVA?
9. What is effective population size?

Suggested Readings / References

1. Robert E. Ricklefs and Gary L. Miller. ECOLOGY. *Fourth Edition*.
2. Thomas M. Smith and Robert Leo Smith. Elements of ECOLOGY. *Eighth Edition*. ISBN 978-0-321-73607-9.

<u>Disclaimer</u>:

The study materials of this book have been collected from various books, e-books, journals and other e-sources.

POST GRADUATE DEGREE PROGRAMME (CBCS)

IN

ZOOLOGY

(M. Sc. Programme)

SEMESTER-I

DEVELOPMENTAL BIOLOGY AND CYTOGENETICS

ZHT-103

Self-Learning Material

DIRECTORATE OF OPEN AND DISTANCE LEARNING

UNIVERSITY OF KALYANI

Kalyani, Nadia

West Bengal, India

COURSE PREPARATION TEAM:

Faculty members of the Department of Zoology,
University of Kalyani and Assistant Professors of Zoology, DODL,
University of Kalyani.

December, 2018

Directorate of Open and Distance Learning, University of Kalyani

Published by the Directorate of Open and Distance Learning,
University of Kalyani, Kalyani-741235, West Bengal and Printed by
East India Photo Composing Centre, 69, Sisir Bhaduri Sarani, Kolkata-700 006

All right reserved. No part of this work should be reproduced in any form without the permission in writing from the Directorate of Open and Distance Learning, University of Kalyani.

Authors are responsible for the academic contents of the course as far as copyright laws are concerned.

Foreword

Open and Distance Learning (ODL) systems play a threefold role- satisfying distance learners' needs of varying kinds and magnitudes, overcoming the hurdle of distance and reaching the unreached. Nevertheless, this robustness places challenges in front of the ODL systems managers, curriculum designers, Self Learning Materials (SLMs) writers, editors, production professionals and other personnel involved in them. A dedicated team of the University of Kalyani under the leadership of Hon'ble Vice-Chancellor has put its best efforts, professionally and in unison to promote Post Graduate Programmes in distance modeoffered by the University of Kalyani. Developing quality printed SLMs for students under DODL within a limited time to cater to the academic requirements of the Course as per standards set by Distance Education Bureau of the University Grants Commission, New Delhi, India underOpen and Distance Mode UGC Regulations, 2017 had been our endeavour and we are happy to have achieved our goal.

Utmost care has been taken to develop the SLMs useful to the learners and to avoid errors as far as possible. Further suggestions from the learners' end would be gracefully admitted and to be appreciated.

During the academic productions of the SLMs, the team continuously received positive stimulations and feedback from Professor (Dr.) Sankar Kumar Ghosh, Hon'ble Vice- Chancellor, University of Kalyani, who kindly accorded directions, encouragements and suggestions, offered constructive criticism to develop it within proper requirements. We gracefully, acknowledge his inspiration and guidance.

Due sincere thanks are being expressed to all the Members of PGBOS (DODL), University of Kalyani, Course Writers- who are serving subject experts serving at UniversityPost Graduate departments and also to the authors and academicians whose academic contributions have been utilized to develop these SLMs. We humbly acknowledge their valuable academic contributions. I would like to convey thanks to all other University dignitaries and personnel who have been involved either at a conceptual level or at the operational level of the DODL of University of Kalyani.

Their concerted efforts have culminated in the compilation of comprehensive, learner-friendly, flexible texts that meet the curriculum requirements of the Post Graduate Programme through Distance Mode.

Self Learning Materials (SLMs) have been published by the Directorate of Open and Distance Learning, University of Kalyani, Kalyani-741235, West Bengal and all the copyright reserved for University of Kalyani. No part of this work should be reproduced in any from without permission in writing from the appropriate authority of the University of Kalyani.

All the Self Learning Materials are self writing and collected from e-book, journals and websites.

Director
Directorate of Open & Distance Learning
University of Kalyani

HARD CORE THEORY PAPER

(ZHT–103)

DEVELOPMENTAL BIOLOGY AND CYTOGENETICS

Group A : Developmental Biology

Module	Unit	Content	Credit	Class	Time (h)	Page No.
ZHT–103 (Developmental Biology and Cytogenetics)	I	Basic concepts in Developmental Biology: potency, commitment, specification, induction, competence, determination and differentiation, morphogenetic gradient and fate map	1.5	1	1	7-43
	II	Stem cells: embryonic stem cells and adult stem cells; stem cell niches		1	1	44-61
	III	Sex, Gametes and Fertilization: i) Germ cell migration ii) Gametogenesis iii) Gamate recognition, contact and fertilization, prevention of polyspermy.		1	1	62-73
	IV	Axis specification in vertebrates: i) Early patterning in vertebrates —Symmetry breaking, Nieuwkoop center. Wnt and cadherin signalling ii) Left-right asymmetry in vertebrates—Asymmetric gene expression		1	1	74-86
	V	Metamorphosis and organogenesis: Axes, compartment formation and pattern formation in Drosophila; Homeobox genes and development; development and metamorphosis of tadpole larve; limb development and regeneration in vertebrates.		1	1	87-115
	VI	Concept on aging and senescence		1	1	116-130

Group-A : DEVELOPMENTAL BIOLOGY

Unit-I

Basic concepts in Developmental Biology: potency, commitment, specification, induction, competence, determination and differentiation, morphogenetic gradient and fate map.

Objective:

In this Unit we will discuss about Basic concepts in Developmental Biology: potency, commitment, specification, induction, competence, determination and differentiation, morphogenetic gradient and fate map.

Potency:

Cell potency is a cell's ability to differentiate into other cell types. The more cell types a cell can differentiate into, the greater its potency. Potency is also described as the gene activation potential within a cell which like a continuum begins with totipotency to designate a cell with the most differentiation potential, pluripotency, multipotency, oligopotency and finally unipotency.

Totipotency

Totipotency (Lat. totipotentia, "ability for all things") is the ability of a single cell to divide and produce all of the differentiated cells in an organism. Spores and zygotes are examples of totipotent cells. In the spectrum of cell potency, totipotency represents the cell with the greatest differentiation potential, being able to differentiate into any embryonic cell, as well as extraembryonic cells. In contrast, pluripotent cells can only differentiate into embryonic cells.

It is possible for a fully differentiated cell to return to a state of totipotency. This conversion to totipotency is complex, not fully understood and the subject of recent research. Research in 2011 has shown that cells may differentiate not into a fully totipotent cell, but instead into a "complex cellular variation" of totipotency. Stem cells resembling totipotent blastomeres from 2-cell stage embryos can arise spontaneously in mouse embryonic stem cell cultures and

also can be induced to arise more frequently in vitro through down-regulation of the chromatin assembly activity of CAF-1.

The human development model is one which can be used to describe how totipotent cells arise. Human development begins when a sperm fertilizes an egg and the resulting fertilized egg creates a single totipotent cell, a zygote. In the first hours after fertilization, this zygote divides into identical totipotent cells, which can later develop into any of the three germ layers of a human (endoderm, mesoderm, or ectoderm), or into cells of the placenta (cytotrophoblast or syncytiotrophoblast). After reaching a 16-cell stage, the totipotent cells of the morula differentiate into cells that will eventually become either the blastocyst's Inner cell mass or the outer trophoblasts. Approximately four days after fertilization and after several cycles of cell division, these totipotent cells begin to specialize. The inner cell mass, the source of embryonic stem cells, becomes pluripotent.

Research on Caenorhabditis elegans suggests that multiple mechanisms including RNA regulation may play a role in maintaining totipotency at different stages of development in some species.Work with zebrafish and mammals suggest a further interplay between miRNA and RNA-binding proteins (RBPs) in determining development differences.

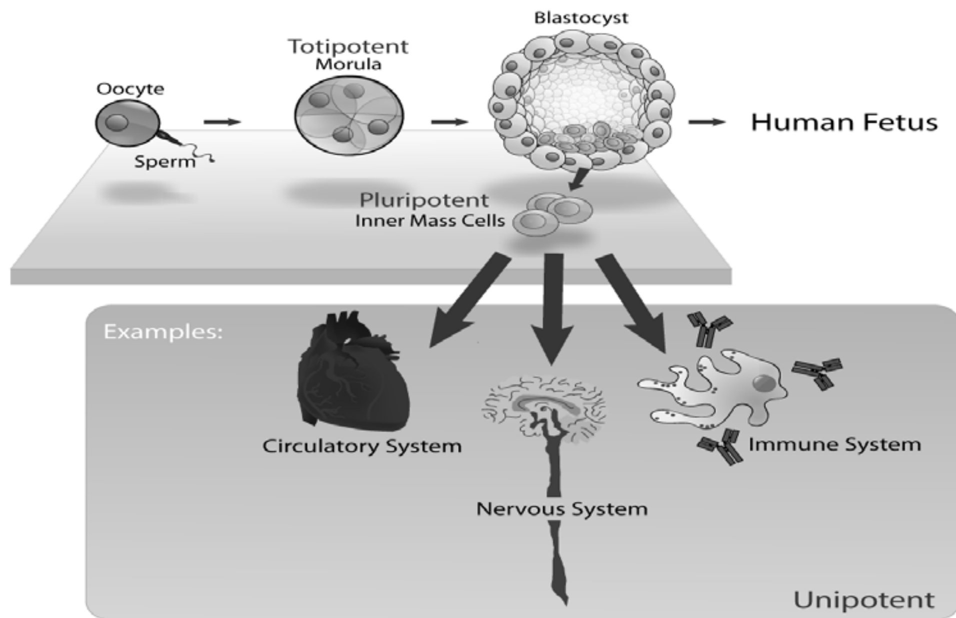


Figure : Pluripotent, embryonic stem cells originate as inner mass cells within a blastocyst. These stem cells can become any tissue in the body, excluding a placenta. Only the cells of the morula are totipotent, able to become all tissues and a placenta.

Pluripotency

In cell biology, pluripotency (Lat. pluripotentia, "ability for many things") refers to a stem cell that has the potential to differentiate into any of the three germ layers: endoderm (interior stomach lining, gastrointestinal tract, the lungs), mesoderm (muscle, bone, blood, urogenital), or ectoderm (epidermal tissues and nervous system). However, cell pluripotency is a continuum, ranging from the completely pluripotent cell that can form every cell of the embryo proper, e.g., embryonic stem cells and iPSCs, to the incompletely or partially pluripotent cell that can form cells of all three germ layers but that may not exhibit all the characteristics of completely pluripotent cells.

Multipotency

Multipotency describes progenitor cells which have the gene activation potential to differentiate into discrete cell types. For example, a multipotent blood stem cell —and this

cell type can differentiate itself into several types of blood cell types like lymphocytes, monocytes, neutrophils, etc., but it is still ambiguous whether HSC possess the ability to differentiate into brain cells, bone cells or other non-blood cell types.

New research related to multipotent cells suggests that multipotent cells may be capable of conversion into unrelated cell types. In another case, human umbilical cord blood stem cells were converted into human neurons. Research is also focusing on converting multipotent cells into pluripotent cells.

Multipotent cells are found in many, but not all human cell types. Multipotent cells have been found in cord blood, adipose tissue,cardiac cells, bone marrow, and mesenchymal stem cells (MSCs) which are found in the third molar. MSCs may prove to be a valuable source for stem cells from molars at 8–10 years of age, before adult dental calcification. MSCs can differentiate into osteoblasts, chondrocytes, and adipocytes.

Oligopotency

In biology, oligopotency is the ability of progenitor cells to differentiate into a few cell types. It is a degree of potency. Examples of oligopotent stem cells are the lymphoid or myeloid stem cells. A lymphoid cell specifically, can give rise to various blood cells such as B and T cells, however, not to a different blood cell type like a red blood cell. Examples of progenitor cells are vascular stem cells that have the capacity to become both endothelial or smooth muscle cells.

Unipotency

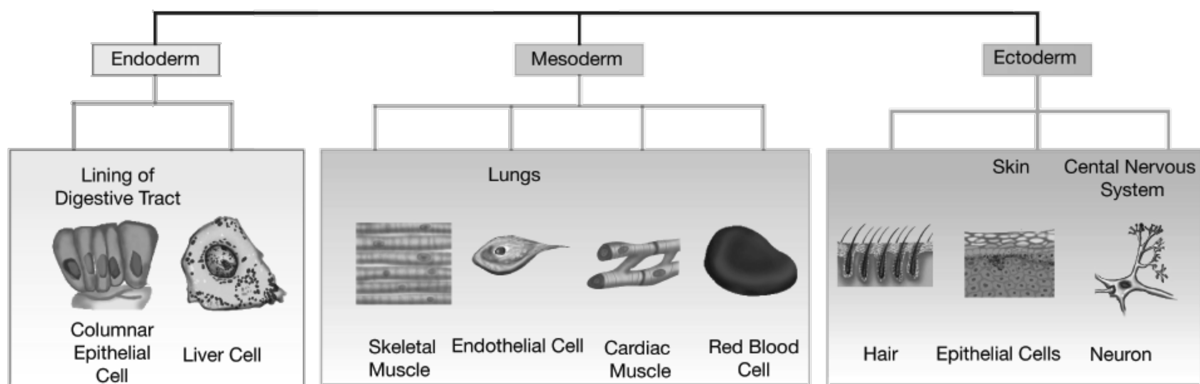
In cell biology, a unipotent cell is the concept that one stem cell has the capacity to differentiate into only one cell type. It is currently unclear if true unipotent stem cells exist. Hepatoblasts, which differentiate into hepatocytes (which constitute most of the liver) or cholangiocytes (epithelial cells of the bile duct), are bipotent. A close synonym for unipotent cell is precursor cell.

Differentiation, determination, and stem cells

Over the course of development, cells tend to become more and more restricted in their developmental potential. That is, the types of other cells they can make by cell division (or directly turn into) become fewer and fewer.

For instance, a human zygote can give rise to all the cell types of the human body, as well as the cells that make up the placenta. To use vocab from the stem cell field, this ability to give rise to all cell types of the body and placenta makes the zygote totipotent. However, after multiple rounds of cell division, the cells of the embryo lose their ability to give rise to cells of the placenta and become more restricted in their potential (pluripotent). These changes are due to alterations in the set of genes expressed in the cells.

Eventually, the cells of the embryo are split into three different groups known as germ layers: mesoderm, endoderm, and ectoderm. Each germ layer will, under normal conditions, give rise to its own specific set of tissues and organs.

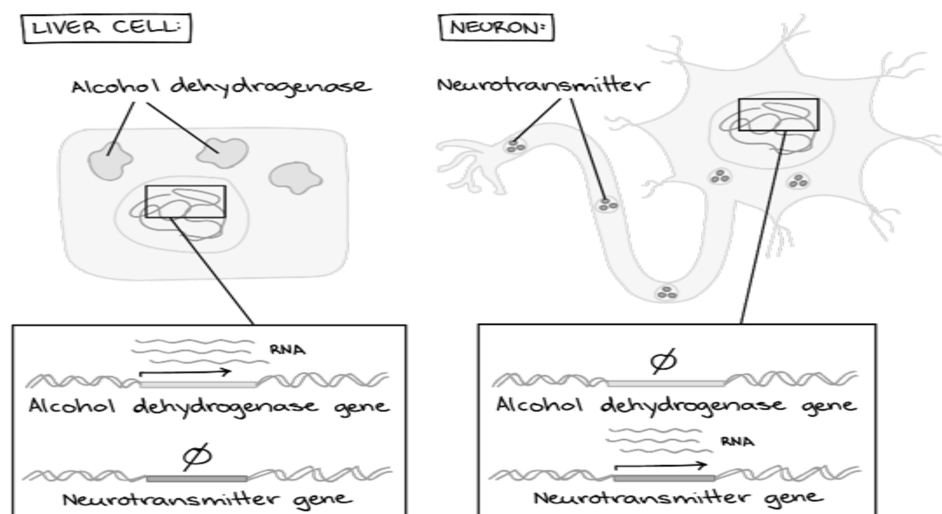


As the cells of a germ layer continue to divide, interacting with their neighbours and reading out their own internal information, their cell fate "options" will get narrower and narrower. At first, cells may be specified, earmarked for a certain fate but able to switch given the right cues. Next, they may become determined, meaning that they are irreversibly committed to a

certain fate. Once a cell is determined, even if it's moved to a new environment, it will differentiate as the cell type to which it's become committed.

Eventually, most cells in the body differentiate, or take on a stable, final identity. Examples of differentiated cell types in the human body include neurons, the cells lining the intestine, and the macrophages that gobble up bacterial invaders in the immune system. Each differentiated cell type has a specific gene expression pattern that it maintains stably. The genes expressed in a cell type specify proteins and functional RNAs needed by that particular cell type, giving it the right structure and function to do its job.

For example, the diagram below shows two genes that are differently expressed between a liver cell and a neuron. One gene, encoding part of an enzyme that breaks down alcohol and other toxins, is expressed only in the liver cell (and not in the neuron). The other gene, encoding a neurotransmitter, is expressed only in the neuron (and not in the liver cell). Many other genes would also be expressed differently between these two cell types.



Competence and Induction:

In amphibian embryos, the dorsal ectodermal cells in a mid-longitudinal region differentiate to form a neural plate, only when the chorda-mesoderm is below it. Chorda-mesoderm is the layer formed by invagination cells from the region of the dorsal blastoporal lip, which form

the roof of archenteron. Mangold (1927) selected a small part of dorsal blastoporal lip from an early gastrula of *Triturus cristatus* and grafted it at a place near the lateral lip of the blastopore of the host gastrula of *Triturus taeniatus*. The graft cells grew in number and spread inside the host gastrula to form an additional chorda-mesoderm at this place. This chorda-mesoderm, subsequently induced the ectoderm of the host gastrula to form an additional neural tube.

The graft cells themselves formed an additional notochord. As the host gastrula developed further, it grew into a double embryo joined together. One of the embryos was the regular one, while the second was the induced one. The latter did not develop a complete head.

This experiment clearly showed that the dorsal blastoporal lip of the blastula had the ability to induce the formation of the neural plate in the ectoderm of the host. This phenomenon is called neural induction. Other parts of an embryo can similarly induce the formation of other structures. This influence of one structure in the formation of another structure is called embryonic induction. In fact, the entire development of an organism is due to a series of inductions. The structure, which induces the formation of another structure, is called the inductor or organizer. The chemical substance that is emitted by an inductor is called an evacuator. The tissue on which an evacuator or inductor acts is called the responsive tissue.

Historical Background of Embryonic Induction:

For the discovery of neural induction, the German embryologist, Hans Spemann and his student, Hilde Mangold (1924) worked a lot and for his work Spemann received Nobel Prize in 1935. These two scientists performed certain heteroblastic transplantations between two species of newt, i.e., *Triturus cristatus* and *Triturus taeniatus* and reported that the dorsal lip of their early gastrula has the capacity of induction and organization of presumptive neural ectoderm to form a neural tube and also the capacity of evocation and organization of ectoderm, mesoderm and endoderm to form a complete secondary embryo.

They called the dorsal lip of the blastopore the primary organizer since it was first in the sequence of inductions and as it had the capacity to organize the development of a second

embryo. Later on, the primary organizer was reported to exist in many animals, e.g. in frogs; in cyclostomes; in bony fishes; in birds and in rabbit.

Primary organizer and neural induction have been reported in certain pre-vertebrate chordates, such as ascidians and Amphioxus. In 1960 and 1963 Curtis investigated and reported that the organizer of gastrula of *Xenopus laevis* can be distinguished in the cortex of grey crescent of a fertilized egg.

Holtfreter (1945) gave an account of how an enormous variety of entirely unspecific substances-organic acids, steroids, kaolin, methylene blue, sulphhydryl compounds, which had nothing in common except the property of being toxic to sub-ectodermal cells-produced neurulation in explants. Barth and Barth (1968, 69) provided further information about the chemical nature of embryonic induction.

Types of embryonic induction:

Lovtrup (1974) classified different types of embryonic induction into two basic categories-endogenous and exogenous inductions.

1. Endogenous induction:

Certain embryonic cells gradually assume new diversification pattern through the inductors that are produced by them endogenously. Due to these inductors, these cells undergo either self-transformation or self-differentiation. Examples of such induction were reported in Mesenchymal cells of ventral pole of Echinoid and in small sized, yolk-laden cells of dorsal lip of amphibian blastopore.

2. Exogenous induction:

When some external agent or a cell or a tissue is introduced into an embryo, they exert their influence by a process of diversification pattern upon neighbouring cells through contact induction. This phenomenon is called exogenous induction. It may be homotypic or heterotypic depending on the fact that whether the inductor provokes the formation of same or different kind of tissues respectively.

In homotypic induction, a differentiated cell produces an inductor. The inductor not only serves to maintain the state of the cell proper, but also induces adjacent cells to differentiate according to it, after crossing the cell boundaries. Best example of the heterotypic exogenous induction is the formation of a secondary embryonic axis by an implanted presumptive notochord in amphibians.

Experimental evidences to induction:

Spemann and Mangold (1924) transplanted heteroplastically a piece of the dorsal lip of the blastopore of an early gastrula of pigmented newt, *Triturus cristatus* and grafted it near the ventral or lateral lip of the blastopore of the early gastrula of pigmented newt *T. taeniatus*. Most of the graft invaginated into the interior and developed into notochord and somite's and induced the host ectoderm to form a neural tube, leaving a narrow strip of tissue on the surface.

With the development of host embryo, an additional whole system of organs was induced at the graft – placement area. Except for the anterior part of the head, almost a complete secondary embryo comprising of the additional organs was formed. Posterior part of the head was present as indicated by a pair of ear rudiments.

Since in this experiment the type of transplantation involved was heteroplastic, it was found that notochord of secondary embryo consisted exclusively of graft cells; the somites consisted partly of graft and partly of host cells. Few cells, which did not invaginate during gastrulation, were left in the neural tube. The bulk of the neural tube, part of the somites, kidney tubules and the ear rudiments of the secondary embryo consisted of host cells. The graft becomes self-differentiated and at the same time induces the adjoining host tissue to form spinal cord and other structures including somites and kidney tubules. Spemann (1938) described dorsal lip of the early gastrula as a "primary organizer" of the gastrulative process.

However, organization of the secondary embryo results from a series of both inductive interactions and self-differentiative changes in the host and donor tissues. Hence, now a days the term "embryonic induction" or "inductive interactions" is preferred. The part, which is the source of induction, is called "inductor".

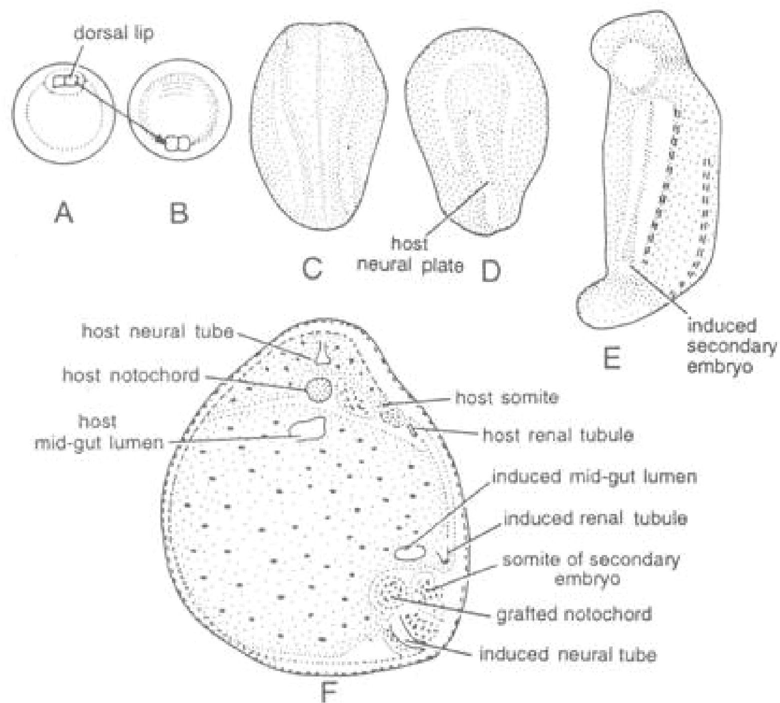


Fig. 1. Induction of secondary embryo in *Triturus* by transplanting a piece of dorsal lip to the future belly region of another gastrula (A–B). C–E, are stages of resulting primary embryo, with a secondary embryo attached to it. F–T.S. of the same embryo.

Characteristics of the organizer:

Organizer has the ability for self-differentiation and organization. It also has the power to induce changes within the cell and to organize surrounding cells, including the induction and early organization of neural tube. Primary organizer determines the main features of axiation and organization of the vertebrate embryo.

Induction is a tool-like process, utilized by this center of activity through which it affects changes in surrounding cells and as such influences organization and differentiation. These surrounding cells, changed by the process of induction, may in turn act as secondary inductor centers with abilities to organize specific sub-areas.Thus, the transformation of the late blastula into an organized condition of the late gastrula appears to be dependent upon a number of separate inductions, all integrated into one coordinated whole by the “formative stimulus” of the primary organizer located in the pre-chordal plate area of the endodermal - mesodermal cells and adjacent chorda-mesodermal material of the early gastrula.

Regional specificity of the organizer:

Vital-staining experiments of Vogt with newt eggs have shown that the material successively forming the dorsal blastoporal lip moves forward as the archenteron roof. Transplants taken from this region are also able to induce a secondary embryo or the belly of a new host i.e. the archenteron roof acts as a primary inductor in essentially the same way as does the dorsal lip tissue proper. The inductions of neural inductor are found to be regionally specific and the regional specificity is imposed on the induced organ by the inductor.

Therefore, the inductive capacity of the blastoporal lip varies both regionally and temporally. Most of the dorsal and dorso-lateral blastoporal material is necessary for a graft to induce a more or less complete secondary embryo. Spemann (1931) demonstrated that during gastrulation anterior part of the archenteric roof invaginates over the dorsal lip of the blastopore earlier.

Dorsal blastopore lip of the early gastrula contains the archenteric and deuterocephalic organizer and the dorsal blastopore lip of the late gastrula contains the spinocaudal organizer. Inductions produced by the dorsal lip of the blastopore taken from the early and the late gastrula differ in accordance with exception; the first tends to produce head organs and the second tends to produce trunk and tail organs (Fig. below).

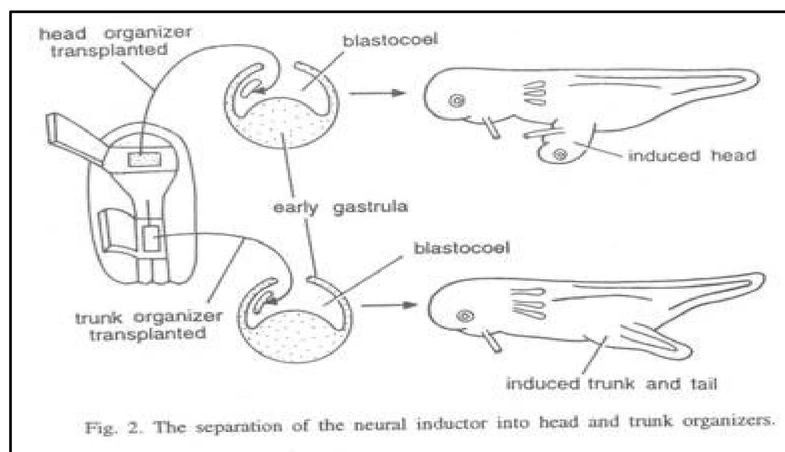


Fig. 2. The separation of the neural inductor into head and trunk organizers.

As invagination continues and the dorsal lip no longer consists of prospective head endo-mesoderm but progressively becomes prospective trunk mesoderm; it acts as a trunk-tail inductor. The most caudal region of the archenteron roof, in fact, specifically induces tail somites and probably other mesodermal tissues. The archenteron roof induces entirely

different class of tissues; various neural and meso-ectodermal tissues by its anterior region and various mesodermal tissues by its most posterior region.

Therefore, differences in specific induction capacities exist between head and trunk level of archenteron roof and are related to the regional differentiation of the neural tissue into archencephalic (including fore-brain, eye, nasal pit), deuterencephalic (including hind-brain, ear vesicle) and spinocaudal components. Thus, archenteron roof consists of an anterior head inductor including an archencephalic inductor and a deuterencephalic inductor and a trunk or spinocaudal inductor.

Primary induction and grey crescent:

The dorsal lip region of the blastopore at the onset of gastrulation can be traced back to the grey-crescent of the undivided fertilized amphibian egg. It was conceived by some developmental biologists that the crescent material of egg cortex initiated gastrulation and has the capacity of neural induction. A.S.G. Curtis (1963) performed a series of experiments of transplanting parts of the cortex of the fertilized egg of the clawed toad, *Xenopus laevis* at the beginning of cleavage.

In one experiment, the grey-crescent cortex was excised from the fertilized egg and it was observed that the cell division though proceeded undisturbed, the gastrulation failed to take place (Fig. 3A). In another experiment, the grey crescent cortex of uncleaved fertilized egg was excised and transplanted into a ventral position of a second egg, so that the egg receiving the graft had two grey crescents on opposite sides.As a result, egg cleaved to form a blastula, which underwent two separate gastrulation movements to produce two separate primary nervous systems, notochord and associated somites (Fig. 3D). Similar experiments conducted on the eight-cell stage showed that something had happened during the short – interval represented by the first three cleavages.

Grey crescent cortex of the eight-cell stage still retained its inductive capacity when grafted to younger stages. Removal of the grey crescent at this stage no longer inhibits subsequent gastrulation and normal development, the missing crescent properties being replaced from adjacent cortical regions.

According to Curtis, a change in cortical organization spreads across the surface of the egg during the second and third cleavages, starting from the grey crescent; when this change is completed, interactions, probably of a biophysical nature, can take place among various parts of the cortex.

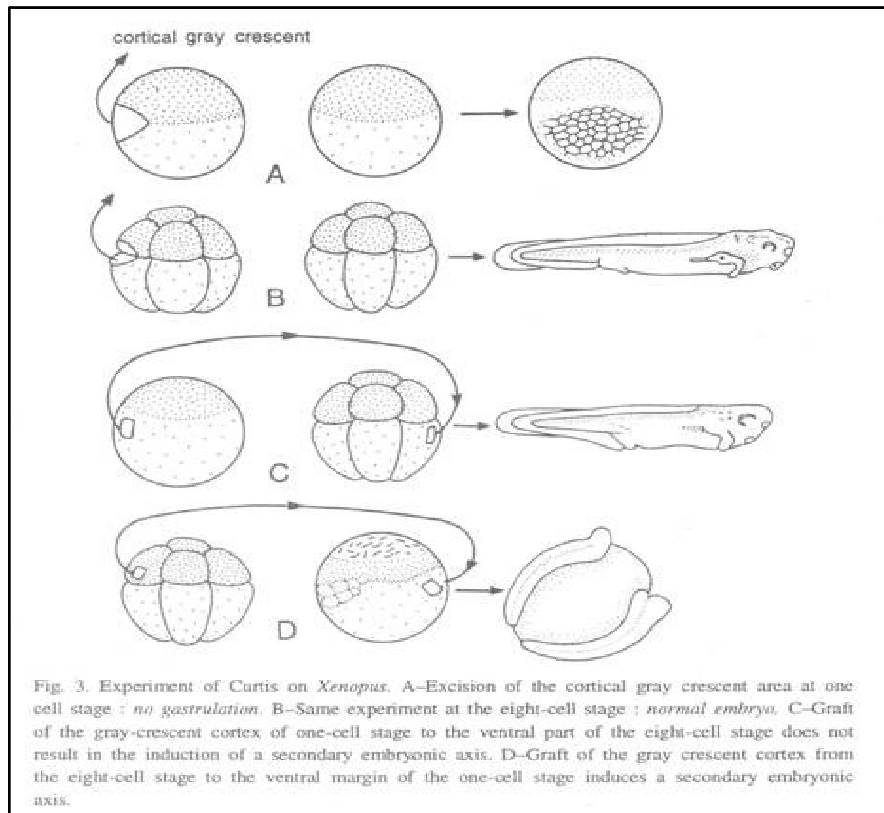


Fig. 3. Experiment of Curtis on *Xenopus*. A–Excision of the cortical gray crescent area at one cell stage : *no gastrulation*. B–Same experiment at the eight-cell stage : *normal embryo*. C–Graft of the gray-crescent cortex of one-cell stage to the ventral part of the eight-cell stage does not result in the induction of a secondary embryonic axis. D–Graft of the gray crescent cortex from the eight-cell stage to the ventral margin of the one-cell stage induces a secondary embryonic axis.

Mechanism of neural induction:

Development of the ectoderm overlying the roof of the archenteron into neural tissue suggests a direct action upon the ectodermal cells, either by surface interaction or by chemical mediation.

(1) One of the broad possibility is surface interaction of the cells at the inductive interface. The contact of the two cellular layers may provide a device whereby the structural pattern or geometry or behaviour of the ectodermal cell membranes is altered directly by the underlying chorda mesodermal cells.

Thus, the spatial configuration of the latter membranes might induce a change in the spatial configuration of the ectodermal cell membranes, this in turn producing in the interior of the

cell changes that determine its development into neural plate. A morphological arrangement of this kind could account for quick and effective transmission of the inductive effect.

(2) Another broad possibility is a chemical mediation of the inductive effect. Therefore, a chemical substance or substances produced and released by inducing chorda mesoderm cells at the archenteron -ectoderm interface may act upon or enter the ectodermal cells to initiate cellular activities leading to neural development. A great deal of evidence favours the idea of an exchange of material between cells and also suggests that a diffusible substance may act as effective inductive stimulus.

Chemical basis of neural induction:

The results of numerous studies to elucidate the mechanism of induction and to identify the chemical substance or substances presumed to be involved have not yielded good results. It was found that many different tissues, embryonic or adult, from a great variety of different species, were capable of inducing nervous tissue in amphibian embryos. Moreover, some foreign tissues were found to be much more potent inductors after they had been killed by heat or alcohol treatment.

This fact remains against the concept of a universally present ‘masked organizer’, released in the primary inductor region. Few inorganic agents as iodine and kaolin, local injury, exposure to saline solutions of excessively high or low pH, cause neural differentiation in ectoderm. These findings establish the early grand concept of master-chemical embryonic organizer of Holtfreter’s sublethal cytolysis. It has the concept of reversible cell injury liberating neural inductor. Different chemical substances of either grey crescent or dorsal lip or chordamesoderm are separated by different biochemical methods to find out the molecule which causes the neural induction and then the inductive capacity of each molecule was tested separately. Few experiments show that evocator or inducing substance is a protein.Exhaustive attempts were made by different embryologists to understand the real mechanism of neural induction. Some theories have been put forward to understand the mechanism of neural induction, out of which the most important are as follows:

1. Protein denaturation theory of neural induction:

According to Ranzi (1963) neural induction and notochord formation are related to protein denaturation. Site of notochord formation is amphibian grey crescent, which is a center of high metabolic activity. Such centers of greater metabolic activity correspond to sites of protein denaturation.

2. Gradient theory of neural induction:

Toivonen (1968) and Yamada (1961) stated that two chemically distinct factors are involved in the action of the primary inductor. Out of these two factors, one is neuralizing agent and the other is mesodermalizing agent. These experiments were conducted with denatured bone marrow and liver as the inductors.

Regional specificity of the embryonic axis arises from the interaction between two gradients: neutralizing principle has its highest concentration in the dorsal side of the embryo and diminishes laterally, while the mesodermalizing principle is present as an antero-posterior gradient with its peak in the posterior region.

Anteriorly the neutralizing principle acts alone to induce forebrain structures, more posteriorly the mesodermalizing principle acts along with the neutralizing one to induce mid-brain and hind-brain structures, while even more posteriorly the high concentration level of the mesodermal gradient produces spino-caudal structures (Fig. below).

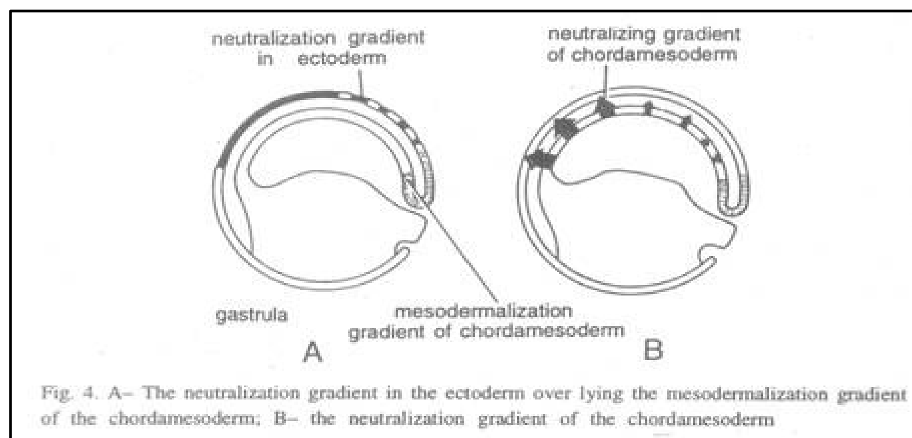


Fig. 4. A– The neutralization gradient in the ectoderm over lying the mesodermalization gradient of the chordamesoderm; B– the neutralization gradient of the chordamesoderm

3. One factor hypothesis of neural induction:

Nieuwkoop (1966) using living notochord as the inductor, postulated that only one factor which first evokes ectoderm to form neural tissue and later causes ectoderm to transform into more posterior and mesodermal structure (Fig. 5) is involved.

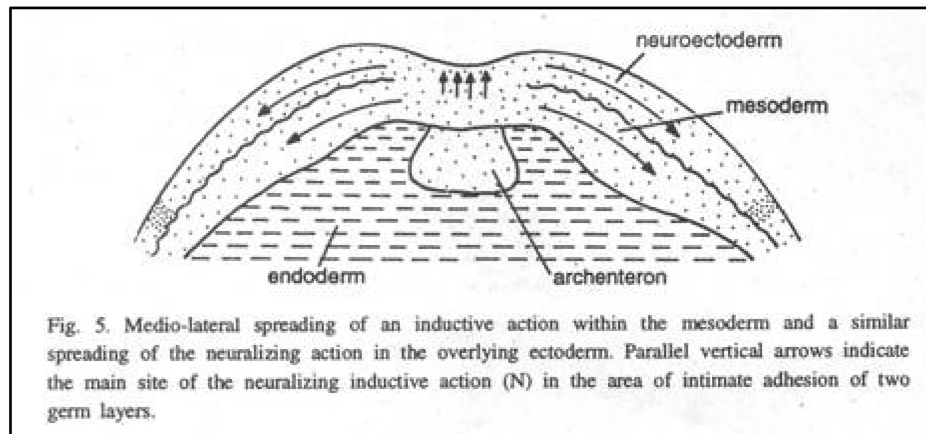


Fig. 5. Medio-lateral spreading of an inductive action within the mesoderm and a similar spreading of the neuralizing action in the overlying ectoderm. Parallel vertical arrows indicate the main site of the neuralizing inductive action (N) in the area of intimate adhesion of two germ layers.

In one experiment, consisting of combining isolated gastrular ectoderm with a piece of notochord and then removing the notochord tissue after varying lengths of time, it was found that only 5 minutes exposure to inductor caused a part of the ectoderm to transform into brain and eye structures.

4. Ionic theory of neural induction:

According to Barth and Barth (1969), the actual process of induction may be initiated by release of ions from bound form, representing a change in the ratio between bound to free ions within the cell of the early gastrula. Induction of nerve and pigment cells in small aggregates of prospective epidermis of the frog gastrula were found to be dependent on the concentration of the sodium ions.

Normal induction of nerve and pigment cells by mesoderm in small explants from the dorsal lip and lateral marginal zones of the early gastrula is dependent on the external concentration of sodium. Thus, normal embryonic induction depends on an endogenous source of ions and that an intracellular release of such ions occurs during late gastrulation.

Genic basis of neural induction:

There are evidences that the component tissues of neural inductor become differentiated prior to ectodermal cells. During this process, the rate of transcription of mRNA and differential activation of genes becomes many fold, while the differentiation of ectodermal cells is set in only after mid-gastrulation.

According to experiments conducted by Tiedemann (1968), after 2 to 7 days of cultivation of dorsal blastopore lip of young *Triturus* gastrula with adjacent ectoderm in a medium containing sufficient quantities of Actinomycin-D to inhibit RNA synthesis, induction could not take place, but some differentiation of muscle and notochord occurred. It shows that mRNA by transcription from the DNA was required, which also requires the presence of Actinomycin-D. Therefore, no neural induction could be detected in this experiment.

Time of neural induction:

Neural induction occurs at the time when the material of chordamesoderm moves from the dorsal lip of blastopore inward and forward. The inductive stimuli exhibit a time gradient, which may be crucial with regard to action and reaction events.

Embryonic induction in different chordates:

Although neural induction was first discovered in urodele amphibians, it was found that the dorsal lip of the blastopore and the roof of the archenteron of other vertebrates have the same function. The chordamesoderm in all vertebrates induces the nervous system and sense organs. Neural inductor has been investigated in the following chordates:

(1) In Cyclostomes, especially in lampreys, the property of neural induction lies in the presumptive chorda mesodermal cells of dorsal lip of the blastopore. Prior to cyclostomes, in Ascidians different blastomeres of eight cell stage have the following presumptive fates-(i) the two anterior animal pole blastomeres produce head epidermis, palps and the brain with its two pigmented sensory structures, (ii) two posterior animal pole blastomeres produce epidermis, (iii) two anterior vegetal blastomeres produce notochord, spinal cord and part of the intestine (iv) two posterior vegetal cells produce mesenchyme, muscles and part of the intestine.From these experiments, Raverberi (1960) concluded that the formation and differentiation of brain by two anterior animal blastomeres is dependent on the induction of

two anterior vegetal blastomeres, which act as neural inductors. It was further concluded that the two anterior vegetal blastomeres gave rise to diverse tissues, namely, endoderm, notochord and spinal cord.

(2) Wu and Tung (1962) proved the existence of the primary organizer and neural induction in *Amphioxus*. They transplanted pieces of tissues from the inner surface of the dorsal blastopore lip of an early gastrula of Amphioxus into the blastocoel of another embryo in the same stage (Fig. 6) and observed that secondary embryo developed in the ventral region of the host with a notochord and mesoderm produced by the graft and the neural tube from host tissue.

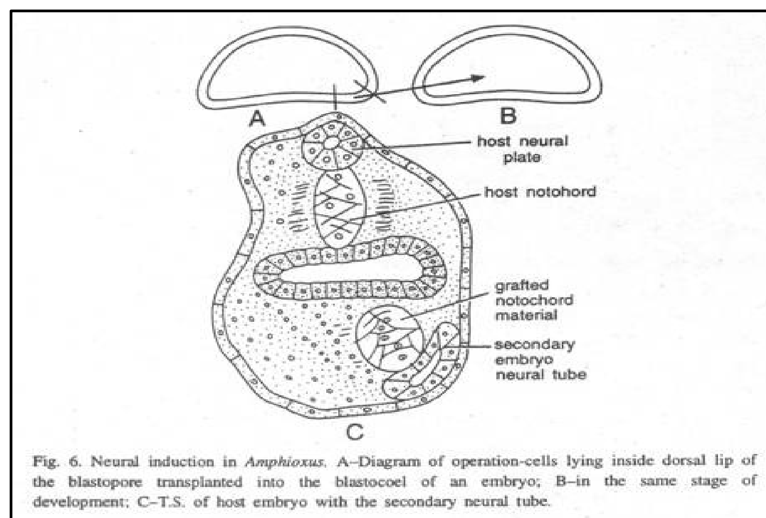


Fig. 6. Neural induction in *Amphioxus*. A–Diagram of operation-cells lying inside dorsal lip of the blastopore transplanted into the blastocoel of an embryo; B–in the same stage of development; C–T.S. of host embryo with the secondary neural tube.

Thus, the chordal tissue of Amphioxus gastrula possesses the power of neural induction, while mesodermal and endodermal tissues have little such inductive power.

(3) In bony fishes, induction of secondary well developed embryos were produced by transplanting the posterior edge of the blastodisc which corresponds to the dorsal lip of the blastopore, into the blastocoel of another embryo (Fig. below) or by transplanting the chordamesoderm and ectoderm. Neural inductions were also obtained by transplanting the dorsal lip of the blastopore in the sturgeon.

(4) In frogs, the induction of secondary embryo can be produced by the dorsal lip of the blastopore transplanted into the blastocoel of a young gastrula, in very much the same way as in newts and salamanders.

(5) In reptiles archenteron has the same inducing activity as in other vertebrates but there is no experimental proof of occurrence of neural inductor.

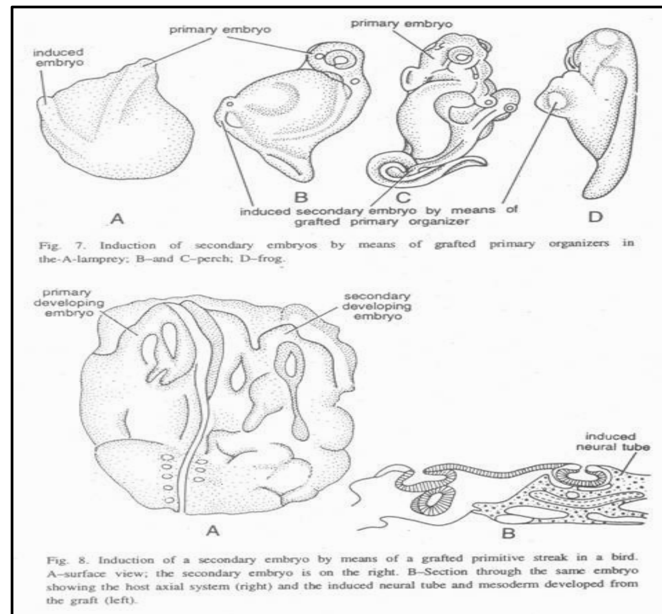


Fig. 7. Induction of secondary embryos by means of grafted primary organizers in the-A-lamprey; B–and C–perch; D–frog.

Fig. 8. Induction of a secondary embryo by means of a grafted primitive streak in a bird. A–surface view; the secondary embryo is on the right. B–Section through the same embryo showing the host axial system (right) and the induced neural tube and mesoderm developed from the graft (left).

(6) In birds, the existence of primary organizer was established by Waddington and co-workers. Anterior half of the primitive streak was the inducing part similar to the lips of the blastopore in amphibians. In the experiment whole blastoderms were removed from the egg in early gastrulation and cultivated *in vitro* on the blood plasma clot.

From another embryo, parts of the primitive streak were then inserted between epiblast and hypoblast, inductions of secondary embryos obtained. Primitive streak was found dependent on the underlying hypoblast for its formation (Fig. above).

(7) A successful neural induction was performed in a rabbit embryo by cultivating the early blastodisc on a plasma clot and implanting the primitive streak of the chick as inductor. Tissues of the mammalian gastrula were found having competence for neural induction. Anterior end of a rabbit embryo, with two pairs of somites, induced a neural plate in a chick embryo when placed under a chick blastoderm.

Other types of embryonic inductions:

Along with gastrulation growth, various organ systems of the embryo begin to differentiate and acquire the power of inducing the differentiation of later formed structures or organs such as eyes, ears, limbs and lungs, etc. These organs develop organizing property and become the source of induction. Therefore, this series of organizers can be called as secondary, tertiary and quaternary organizers. Progressive development of embryonic organs is dependent on sequential induction. One embryonic tissue interacts with the adjacent one and induces it to develop and this process continues in sequence.

Development of eye:

Chorda mesoderm, the primary organizer induces the formation of fore-brain and optic area in the anterior part of the embryo. The optic area evaginates forming the optic vesicle. By invagination it changes into a double walled cup-like structure, the optic cup which acts as secondary organizer to induce the formation of tertiary organizer to form cornea.

The layer of mesenchyme left in front of the anterior chamber of eye combines with the overlying somatic ectoderm (epidermis) and forms cornea, choroid and sclera. Thus, the whole process of development seems to be a cause of induction and interaction only. Number of inductions are secondary or tertiary such as nasal-groove, optic vesicle, lens, cornea and so on involve ectodermal reactions.

Morphogenetic gradient:

The cytoplasm of the egg, and particularly its cortex, starts the chain of reactions which eventually leads to the differentiation of parts of the embryo, it should not be assumed that the structure of the future embryo is rigidly determined by local differences in the cytoplasm of the egg. The local peculiarities of the egg cytoplasm are only some of the factors necessary for the formation of organ rudiments. That this is so can be shown by examining the development of the sea urchin, for instance.

In the 16-cell stage of the sea urchin, the blastomeres are of three different sizes. The animal hemisphere consists of eight blastomeres of medium size, the mesomeres, which are destined

to produce most of the ectoderm of the larva. The vegetal hemisphere consists of four very large blastomeres, the macromeres, and of four very small blastomeres, the micromeres, which lie at the vegetal pole of the egg.

The macromeres also contain some material for the ectoderm and all the material for the endoderm. In a subsequent cleavage the future ectoderm and endoderm are segregated from each other into an upper tier of macromeres and a lower tier of macromeres. The upper tier, lying immediately under the equator of the embryo, contains the ectodermal material; the lower tier, lying nearer to the vegetal pole, contains the endodermal material.

The micromeres develop into mesenchyme, which later produces the larval skeleton consisting of calcareous spicules. In the sea urchin *Paracentrotus lividus*, the cytoplasm of the macromeres possesses a surface layer of red pigment granules, making the macromeres easily distinguishable from the other cleavage cells. The red pigment is already present in the egg at the beginning of cleavage as a broad subequatorial zone.

In the blastula stage, the cytoplasmic substances are found in the same arrangement as at the beginning of cleavage. Subsequently, the descendants of the micromeres migrate into the blastocoele where they develop the skeletal spicules; the descendants of the lower tier of macromeres invaginate, forming a pocket-like cavity—the gut; and the ectoderm produces a ciliary band, serving for locomotion, and a tuft of rigid cilia at the former animal pole.

The ectoderm also sinks inward to produce a stomodeum, coming into communication with the endodermal gut at its anterior end, while the original opening of the pocket-like invagination (the blastopore) becomes the anal opening.

The embryo of the sea urchin develops into a larva called a pluteus. If the blastomeres of the sea urchin are separated in the two-cell stage or in the four-cell stage, each of them develops into a complete pluteus of diminished size. This may be related to the fact that the first two cleavage planes are meridional, passing through the main axis of the egg.

All of the first four blastomeres therefore get equal portions of the three cytoplasmic regions of the egg (ectodermal, endodermal, and mesenchymal). A different result is observed if the egg is separated into the animal and vegetal halves after the third cleavage, the third cleavage plane being equatorial. In this case both halves produce, as a rule, defective embryos. The animal half differentiates as an ectodermal vesicle; it does not produce a gut.

Even purely ectodermal structures are abnormally differentiated- the ciliary band is not developed, whereas the tuft of long cilia on the animal pole develops excessively, growing over a much greater surface than it normally does. The vegetal half is differentiated into an ovoid embryo with a disproportionately large endodermal gut but without a mouth. There may be a few irregular skeletal spicules but no arms and no ciliary band.

In later cleavage stages, it is possible to cut the morula transversely below the equator. In such cases the vegetal part develops still more abnormally; it produces a large endodermal gut and a small ectodermal vesicle. The gut does not lie inside the ectodermal vesicle but is evaginated to the exterior (turned inside out), owing to interference with the normal processes of gastrulation. This phenomenon is known as exogastrulation. The mesenchyme cells migrate into the interior, but they usually produce no skeletal spicules or only very small and abnormal ones.

The result of this experiment is obviously due to each of the two halves lacking some parts contained in the other half. However, each half does not simply produce what would normally have been the fate of the respective part. Instead, the differentiation of each half seems to be “exaggerated” as compared with its normal fate. This is especially clear in the case of the increased animal tuft of cilia.

The same “exaggeration” of the ectodermal or endodermal differentiations can be achieved by exposing the developing eggs to certain chemical substances, even without removing any parts of the egg or embryo. If the fertilized sea urchin egg is exposed to seawater containing some lithium salts in solution, the embryo develops just as if it were only the isolated vegetal half.

The gut is increased and tends to exogastrulate instead of invaginating toward the interior. The skeleton is absent or abnormal, and the ectoderm is represented by an epithelial vesicle and fails to differentiate further. The increase in the size of the gut occurs at the expense of

the ectoderm, and in extreme cases, most of the embryo differentiates as an enormous everted gut, with the ectodermal vesicle reduced to a tiny appendage.

The opposite effect is achieved if, before fertilization, the egg is exposed to artificial seawater lacking calcium ions but with sodium thiocyanate (NaSCN) added to it. In this case, the gut is diminished or completely absent, the ciliary bands in the ectoderm fail to develop, and the tuft of stiff cilia at the animal pole is increased in size.

It appears that all these phenomena may be accounted for by assuming that in the sea urchin's egg two factors or principles exist which are mutually antagonistic and yet interact with each other at the same time, and that normal development is dependent on a certain equilibrium between the two principles. Each has its center of activity at one of the poles of the egg. The activity diminishes away from the center, producing a gradient of activity.

The two gradients of activity decline in opposite directions – the one from the animal pole, the other from the vegetal pole. Taking into account this type of distribution of activities, the factors or principles themselves have been called gradients. The two gradients are therefore the animal gradient, with a center of activity at the animal pole, and the vegetal gradient, with a center of activity at the vegetal pole.

According to this theory normal development depends on the presence of both gradients and on an equilibrium between them. If the animal gradient is weakened or suppressed, the vegetal gradient becomes preponderant, and the embryo is vegetalized; i.e., it develops, in excess, parts pertaining to the vegetal gradient, such as the gut.

If the vegetal gradient is weakened or suppressed, the animal gradient becomes preponderant, and the embryo is animalized; i.e., it develops, in excess, parts pertaining to the animal gradient, such as the tuft of stiff cilia at the animal pole. Other structures of the embryo, such as the ciliary bands and the skeleton, can develop only if both gradients are active, and the development is the more nearly normal the more the two gradients approach a correct equilibrium.

The equilibrium between the two gradients may be upset in various ways. The animal gradient may be weakened, with concomitant vegetalization of the embryo, by removing its center of activity (the blastomeres at the animal pole of the egg) or by the action of lithium salts. The vegetal gradient may be weakened by removing its center of activity (the vegetal part of the egg) or by the action of sodium thiocyanate.

The gradient concept aptly covers the results of various experiments on sea urchins' eggs. In addition, we will consider the following experiments. It is possible to separate from one another the three groups of blastomeres in the 16-cell stage—the mesomeres, the macromeres, and the micromeres—and then to recombine them at will.

In isolation the three groups develop as follows:

1. Isolated mesomeres develop into a vesicle with a tuft of cilia owing to a preponderance of the animal gradient and animalization.

2. Isolated macromeres plus micromeres develop into an extremely vegetalized embryo with evaginated gut, the result of a preponderance of the vegetal gradient.

3. Mesomeres plus macromeres develop into a practically normal embryo; the macromeres bear a sufficiently strong vegetal gradient to counterbalance the animal gradient. Mesenchyme and skeleton develop in such embryos in spite of the absence of micromeres.

4. Micromeres alone are not capable of any development, since they do not keep together but fall apart.

5. Mesomeres (ectoderm) plus micromeres (mesenchyme) develop into a complete and more or less normal pluteus. This combination is especially illuminating since the gut of such embryos develops in spite of the absence of the macromeres, which should normally have supplied the material for the gut. However, the embryo possesses the two gradients, the animal gradient borne by the mesomeres and the vegetal gradient borne by the micromeres,

and the possession of the two gradients seems to create the necessary conditions for normal development.

What has been said is sufficient to show that the presence of different cytoplasmic substances in the egg does not necessarily mean that there is a direct relation between these substances and certain specific organs, in the sense that the cells containing the particular kind of cytoplasm develop directly into the corresponding organ. The absence of the cytoplasm with red pigment did not prevent the development of the gut because the necessary conditions for gut development were provided for in another way.

Physicochemical Nature of the Animal-Vegetal Gradient System in Sea urchin Eggs:

The existence of animal and vegetal gradients is proved not only by the patterns of morphogenetic processes occurring after certain interventions in the normal development of the egg, but also by the direct demonstration of the gradients as peculiar physicochemical states of the cells of the developing embryo.

One way of proving their existence is to study the reduction of dyes by the embryo under conditions of anaerobiosis. Sea urchin embryos (or embryos of other animals for that matter), slightly stained with the vital dye Janus green (diethyl safranin azodimethylaniline), are placed in a small chamber sealed off with petroleum jelly. After some time all the free oxygen contained in the chamber is used up as a result of the respiration of the embryos.

The embryos then, respires using Janus green as an acceptor of hydrogen, which is first reduced to a red dye, diethylsafranin, and further to a colorless substance, leucosafranin. That is, the light greyish-blue color of Janus green first changes to red, and then, as a second step in the reduction of the dye, the color disappears completely.

The reduction of Janus green, shown by the color change, does not occur simultaneously in the whole embryo but follows a characteristic sequence. In the late blastula and early gastrula of the sea urchin, the change in color is first noticeable at the vegetal pole, at the point where

the primary mesenchyme is given off. Then the color change spreads, involving the whole vegetal hemisphere, and reaches the equator.

At this stage a spot of changed color appears at the animal pole and also gradually increases, so that the bluish color remains only in the form of a ring lying well above the equator in the animal hemisphere. Even this ring eventually disappears. The red color then begins to fade in the same sequence, starting first from the vegetal pole and then from the animal pole.

The order in which the dye is reduced in the embryo shows such a nice correspondence to the postulated animal and vegetal gradients that this alone would justify our mentioning these experiments. However, the connection goes much further. When either the animal or vegetal gradient is suppressed, the corresponding gradient of reduction also disappears.

In isolated animal halves of sea urchins' eggs, the animal tendencies of development are predominant, and it is found that such animalized embryos start reducing Janus green at the animal pole only; there is no center of reduction at the vegetal pole.

In isolated vegetal halves the animal tendencies of development are suppressed and the vegetal tendencies are supreme. Correspondingly, the only center of reduction is at the vegetal pole; no center of reduction appears at the animal pole. Embryos animalized or vegetalized chemically show reduction gradients similar to isolated animal and vegetal halves of the egg.

The micromeres are carriers of the highest point of the vegetal gradient, and they preserve this property after transplantation. Accordingly, the micromeres can also serve as the center of a reduction gradient.

If a group of four micromeres is implanted laterally and the embryo is tested for reduction of Janus green, it can be seen that in addition to the two normal gradients, one from the vegetal pole and one from the animal pole, a third gradient appears, having the implanted micromeres as its center but spreading out from these to the adjacent cells.

In short, every modification of the gradient system of the embryo that is postulated from the morphogenetic behavior of the embryo is reflected in the gradients of reduction of Janus green.

If the micromeres are able to establish a vegetal gradient in the adjoining parts of the blastoderm, we would expect that they do so by producing some substance which diffuses into the nearby cells. To prove the existence of such a transmission, advantage was taken of an unusual peculiarity of the micromeres. After the fourth division of the egg, the micromeres lag behind in cleavage.

While the mesomeres and macromeres continue rapid division and accordingly synthesize DNA, the micromeres pause with the next divisions and start synthesizing RNA instead. This synthesis can be shown very clearly if the embryo is supplied with radioactively tagged uridine; only the micromeres take up the label.

If the micromeres are then transplanted into an isolated animal half of a 16- or 32-cell embryo, radioactivity can be detected as being widely spread in the cytoplasm of the host half-embryo. The RNA synthesized by the micromeres thus diffused into the cytoplasm of the adjoining cells. It is known that micromeres implanted in an animal half-embryo cause the development of an endodermal gut from cells which normally would have produced only ectoderm.

The foregoing experiment does not prove, in itself, that the RNA diffusing from the micromeres is responsible for the newly established vegetal gradient in the animal hemisphere cells. This explanation, however, is shown to be more likely by another modification of the labeling experiment. If a 16-cell embryo, instead of being provided with uridine, is supplied with 8-azaguanine, an analogue of uridine, this substance is incorporated into the RNA by the micromeres.

An abnormal RNA is produced. After the treatment the progeny of the micromeres—the primary mesenchyme—develop quite normally, but the gut completely fails to develop. This experiment has been interpreted as proving that the RNA diffusing from the micromeres is essential for gut development and that if this RNA is of an abnormal composition, it cannot perform its function.

From the setup of the preceding experiments it appears highly probable that the spreading of substances from the micromeres into the other parts of the blastoderm occurs directly from

cell to cell and not through the fluids surrounding the cells. The fluid filling the blastocoel in particular does not transmit any morphogenetic influences.

In this connection it is pertinent to refer to experiments that prove the possibility of substances moving directly from cell to cell without passing into the intercellular medium. It was first shown that electric currents may flow from cell to cell, which involves essentially the transmission of sodium and potassium ions.

Subsequently it was found that larger molecules, of molecular weight over 1000, but not greater than 10,000, can also pass from cell to cell by simple diffusion. The passage of these molecules (as well as of simple ions) is affected through a special type of junction, which can be established between most kinds of cells where they come in contact with one another.

The junctions are the so-called “gap junctions”. These are in fact tiny channels (20 Å in diameter) which connect the interior cytoplasm of adjacent cells. It has been found that gap junctions, permeable to intracellular substances, are established in the morula-early blastula stage in amphibians, fishes, birds and squids.

Gap junctions are established between cells of the embryo very rapidly – within seconds of contact between cells of an early newt embryo. There is every reason to believe that wherever the interaction between cells and groups of cells is in the form of gradients; this interaction is performed through the medium of gap junctions.

The animal and vegetal gradients in the sea urchin embryo can be considered to be definite metabolic processes or systems of metabolic reactions which involve oxidation and which have their points of highest intensity at the animal and vegetal poles respectively.

The nature of these reactions is probably very complex and is not, as yet, fully understood, but some indications concerning these reactions may be deduced from a comparison of the chemical agents which may cause vegetalization or animalization.

Apart from the use of lithium ions, vegetalization may be caused by sodium azide and dinitrophenol. Both of these substances belong to a group of enzyme poisons; the azide is known to inactivate the cytochrome oxidase system, and the dinitrophenol disturbs

respiration by preventing oxidative phosphorylation, that is, the formation of energy-rich bonds between phosphoric acid and adenosine diphosphate (resulting in the formation of adenosine triphosphate). This might mean that vegetalization is based essentially on a disturbance of oxidative processes in the embryo and, perhaps even more specifically, a disturbance of the processes of phosphorylation.

That the action of lithium ions is along the same lines may be deduced from a number of observations of which we shall single out the following. Lithium salt treatment suppresses the rise of oxygen consumption which occurs normally at the beginning of gastrulation, that is, at the same time as the morphological effects of vegetalization begin to be apparent.

Furthermore, inorganic phosphate accumulates in the seawater during the development of lithium ion-treated embryos—again a hint that these embryos are incapable of utilizing the energy of oxidation for phosphorylation and synthesis of adenosine triphosphate.

The whole sequence of reactions involved in vegetalization must, of course, be far more complicated. Thus, it has been found that the relative amounts of various amino acids change as a result of vegetalization in lithium ion-treated embryos.

Furthermore, if a modification of the respiratory system of the embryo is the essential feature of vegetalization, it remains to be discovered why the processes of morphogenesis at the animal pole of the embryo (essentially the ectodermal organs) are more severely affected than those of endodermal parts developing at the vegetal pole.

With regard to animalizing agents, sodium thiocyanate, has been found to be rather unreliable. Some batches of eggs treated by this chemical do not react at all. In other batches individual embryos become animalized to greatly varying degrees. Subsequently, many other substances have been found whose animalizing action is much more predictable.

These belong to several distinct groups:

i. Some metals – zinc, mercury.

ii. Some proteolytic enzymes – trypsin, chymotrypsin.

iii. Many acidic dyes – in particular some possessing sulfonic (HSO_3) groups in their molecules, such as Evans blue, chlorazol sky blue, trypan blue, and Congo red; and others possessing carboxyl groups (COOH), such as uranin and rose bengal.

iv. Some other sulfonated organic compounds – germanin and others.

v. Some anionic (acid) detergents.

Animalization may be achieved by extreme dilutions of a chemical; for instance, 1:50,000 in the case of Evans blue.

The common property of most, if not all, of the preceding agents seems to be their ability to attack proteins or to form compounds with proteins, especially basic proteins. In the case of the many sulfonated and carboxylated dyestuffs, it is fairly certain that their acidic groups become attached to the functional side groups of protein molecules.

The two metals, zinc and mercury, also become easily bonded to side groups of protein molecules. The case of mercury is very remarkable – mercuric chloride, a powerful poison used therefore as a fixative for proteins, causes animalization when applied for a very short time in great dilutions (1:90,000).

By blocking the active side groups of the protein molecules, the agents referred to probably “immobilize” these molecules, preventing them from interacting normally with other cell constituents. Even the steric configurations of protein molecules may thus be altered, since they are based on lateral bonding between parts of the polypeptide chains. A far-reaching change in the properties of proteins may thus be brought about.

Fortunately, in the case of animalizing agents, their point of attack can be clearly demonstrated. The dyes which have been used for this purpose stain the cells of the embryo, and it can be seen that the first cells to be stained are the primary mesenchyme cells at the vegetal pole.

With higher concentrations of the dye or with a longer duration of the treatment, the adjoining presumptive endodermal cells show the staining too. The zinc taken into the cells of embryos may be made visible because it yields a pink coloration with dithizone.

In embryos first treated with zinc and later immersed in a dithizone solution, the pink color is detectable in the same position as in the case of animalizing dyes. It is thus quite clear that animalization is due to damage to the center of the vegetal gradient.

While the treatment of embryos with various chemicals of simple and known composition (and, in part, of known mode of action) is useful in establishing the existence of gradients, it does not reveal the chemical mechanism of the gradients under normal conditions. A more direct approach is here indicated. Such an approach was made in attempts to isolate animalizing and vegetalizing substances from the embryo itself.

By homogenizing sea urchin unfertilized eggs or early cleavage stages and by separation of the materials by centrifugation and column chromatography, some fractions were obtained which had a distinct animalizing action on developing embryos. Several fractions with a slightly different effect could be detected.

Less success was obtained with vegetalizing substances, though some vegetalizing activity was also recorded. The chemical nature of the fractions has not, as yet, been determined, but the absorption curve of one fraction in ultraviolet light resembles that of the amino acid tryptophan, while the absorption curve of another suggests nucleotide structure. Further characterization of the sub-stances is to be expected.

Fate map:

In developmental biology, fate mapping is a method of understanding the embryonic origin of various tissues in the adult organism by establishing the correspondence between individual cells (or groups of cells) at one stage of development, and their progeny at later stages of development. When carried out at single-cell resolution, this process is termed cell lineage tracing. The first attempts at fate mapping were made by inferences based on the examination of embryos that had been fixed, sectioned, and stained at different developmental timepoints. The disadvantage of this technique was that observation of single points in developmental

time provide only snapshots of what cell movements are actually occurring and what fates are being assigned. Early embryologists thus had to infer which cells became what tissues at later stages.

Early embryologists used "vital dyes" (which would stain but not harm the cells) to follow movements of individual cells or groups of cells over time in *Xenopus* frog embryos. The tissue(s) to which the cells contribute would thus be labeled and visible in the adult organism. The first person to develop and use this technique to study cell fate was embryologist Walter Vogt in 1929. Vogt used small chips of agar impregnated with a vital dye, (such as Nile Blue or Nile Red) which he placed on a particular cell or population of cells in *Xenopus* embryos until the dye absorbed into the yolk platelets within the desired cell(s). Once the cells were effectively labelled, the agar chip could be removed and the embryo was allowed to develop normally. With this method, Vogt was able to discern movements of particular cell populations and the ultimate organ or tissue into which they integrated. Although innovative for the time, this technique is limiting in that the size of a chip of agar may not accommodate single-cell resolution studies at later stages of development, since successive cell divisions will yield smaller cells (until the embryo develops into a larval form that can eat, and thereby grow larger). Additionally, the cell or cell population of interest must be superficial, since the agar chip with the dye must be placed on the surface of the embryo.

The information Vogt gathered from his tracing experiments of distinct cells and populations of cells in *Xenopus* was then pooled to construct a fate map. The map was a representation of an early-stage embryo (such as a blastula) that has particular regions highlighted which are known to give rise to specific tissues in the adult organism. For instance, Nile Blue staining of a 32-cell blastula at the dorsal side of the animal pole yields a blue-stained brain and (depending on the size of the agar chip) may also stain the anterior portion of the notochord.

In 1978, David Weisblat and colleagues in Gunther Stent's lab at Berkeley improved the technique of single-cell resolution fate mapping by injection of horseradish peroxidase (HRP) enzyme, and later fluorescent peptides (1980), into individual cells in *Helobdella triserialis* (leech) embryos during early development. All progeny of the injected cells could later be discerned by staining for HRP using benzidine substrate or visualized by fluorescence microscopy. This technique allowed the experimenter greater control and selectivity over

what cell was labeled and traced. However, the opaque character of the HRP stain prevented use of vital dye nuclear counter-stains such as Hoechst 33258 (blue) to observe the mitotic state of the injected cell's progeny. Also, embryos had to be fixed in order to stain for the HRP, thus allowing only a single timepoint view of each individual leech embryo injected. The use of fluorescent peptides such as Rhodamine-D-protein (red, RDP) and Fluorescein-D-protein (yellow/green, FDP) conjugated to large carrier molecules to prevent diffusion through cell gap junctions, alleviated several of the shortcomings of HRP injection. Leech embryos injected with the fluorescent tracers could be visualized, and images collected of the same specimen at multiple timepoints, without fixation. The fluorescent tracers could also be combined with nuclear Hoechst staining to visualize the mitotic status of the progeny of injected cells. Seth Blair, also in Stent's lab, introduced a novel ablation technique that could be used in tandem with lineage tracing to pursue the questions relating to developmental potential changes in cell fate in experimentally perturbed embryos that were first raised by Roux and Driesch (1980). For this purpose, specific cells were ablated by microinjection with Pronase (an enzyme that digests proteins) to ablate the cell; later modifications of this technique employed DNase or the ricin A chain. The single-cell injection technique is now also in use by researchers studying other model organisms such as Xenopus (frogs), Danio rerio (zebrafish), and Caenorhabditis elegans (worms).

Genetic cell lineage tracing became an extremely powerful approach when Nat Sternberg and Daniel Hamilton identified a topoisomerase in P1 bacteriophage called Cre recombinase, which has site-specific (loxP site) recombination activity (1981). Subsequent studies have used the Crelox system in transgenic mice and zebrafish to create tissue specific conditional knockouts. In order to conditionally ablate a tissue or cell population of interest, one can create a transgenic mouse using a construct containing the promoter of a gene that is specific to the target cell population, so that the Cre recombinase is only expressed in that tissue type. A second transgenic line must be created, in which loxP recognition sites flank a portion of a particular critical "housekeeping" gene such as DNA polymerase-β. Each transgene is completely harmless by itself; however, when the two strains are crossed, the Cre expressed in the target cell population will cause the excision of DNA pol-β in those cells, which will die. Use of the Cre system instead of injected Pronase ensures that when the targeted cell(s) dies, its intracellular contents will not harm neighboring cells since the Cre is innocuous by

itself and outside the cell, whereas Pronase can damage other cells and the extracellular matrix and thus may have nonspecific effects on development. This approach allows tissue-specific targeted ablation studies, which are important for understanding the importance of particular progenitor cells in the establishment and functionality of adult tissues.

However, in some cases a particular gene of interest is turned on multiple times during development, and Cre-mediated ablation during that first wave of expression is lethal. In this scenario, it is possible to use an inducible version of the transgene: the Cre-ERt loxP approach. Here, the Cre-ERt is a fusion of the recombinase and Tamoxifen-responsive estrogen receptor (ERt). Cre-ERt will be expressed in the cytoplasm of cells expressing its upstream promoter, but will not translocate to the nucleus to conduct recombination until the animal is dosed with Tamoxifen. This tightly-regulated genetic approach to ablation studies has been an invaluable tool for learning about the hierarchy of cell fate during embryogenesis – as well as many other fields of research.

Cre-lox can also be used to permanently, fluorescently label cells by using a transgenic which contains a ubiquitous promoter such as β-actin driving a super-stop sequence flanked by loxP sites, upstream of a fluorescent protein sequence such as RFP. In this fashion, Julien Bertrand from David Traver's lab and Neil Chi discovered the endothelial-origin of hematopoietic (blood) stem cells in zebrafish by using a transgenic with an endothelial promoter, kdrl, driving Cre recombinase crossed to β-actin loxP transgenic fish (2010). Thus, in the presence of Cre, all endothelium-derived cells became indelibly fluorescent red since the super-stop was removed from the genome, and RFP will be expressed under the control of β-actin in all cellular progeny. Indeed, they found that nearly all hematopoietic stem cells and differentiated blood cells were RFP+, indicating their embryonic endothelial origin. Similar site-directed recombination technology such as FLP-FRT recombination (Flippase and Flippase recognition target sites) is extremely powerful in cell fate mapping, ablation studies, and genetic mosaic analysis; this tool is also used heavily in studies in animal systems as divergent as mice and flies. In a similar fashion to reporter gene activation, Cre-lox recombination system can also be used to activate or inactivate a gene of interest, creating gain-of-function or loss-of-function models. As a matter of fact, researchers often need to perform lineage tracing whist perturbing a gene of interest. To this purpose, one could design

a genetic model where within a cell one recombination event is designed for manipulating the gene of interest and the other recombination event is designed for activating a reporter gene. The fate of the cell whose gene is perturbed can then be followed and the function of the gene be revealed. Interpretation of this kind of cell lineage tracing experiments should be made with caution because the two recombination events may not occur simultaneously.

Work in other animal models such as the nematode *C. elegans* was done with single-cell resolution prior to the widespread use of injectable cell tracers. The rapid embryonic development and transparent nature of the nematode allowed the construction of a hierarchical fate map of each mitotic event, from the single-cell zygote to the multicellular adult worm by John Sulston and colleagues in 1982. This cell lineage map was based entirely on observation using Nomarski optics, with no dyes.

New Techniques

More recently, scientists have developed new tools inspired by past approaches. The use of fluorescent peptide tracers can be helpful, but in order to extend fate mapping to later stages when cells are smaller and thus difficult to consistently and selectively inject (unlike the 0.5mm leech embryo), modifications were made. Chemically-"caged" fluorescent peptides, such as caged Rhodamine-dextran or caged Fluorescein-dextran (FITC), have been developed to be non-fluorescent until hit with an ultraviolet (450 nm) laser, which "uncages" the compound and causes it to fluoresce. This tool was particularly well received in the zebrafish community since it is ideal to inject caged compound into a freshly fertilized, single-cell embryo that will rapidly develop over 24 hours into a transparent swimming larvae with approximately 30,000 cells. Injection of the compound into the single-cell embryo allows uniform distribution throughout all the cells of the developing embryo, and the dextran carrier, developed by Jochen Braun and Bob Glimich prevents diffusion between cells through gap junctions, which are common during embryogenesis. At a given stage of development, one can use a UV laser to uncage the compound in a distinct cell or set of cells, effectively labeling them red (Rhodamine) or green (FITC). The embryos can be allowed to develop normally until a later time, at which point they can be imaged for red or green

fluorescence in the progeny of the uncaged cells. However, it is important to note that properly focusing the UV laser beam on an individual cell deep within the embryo is difficult. Sub-optimal focussing can lead to unintentional uncaging of cells outside the focal plane of the target cell. Also, uncaged Rhodamine has a short half-life, and must be imaged within 48 hours or the signal may be difficult to see. Similarly uncaged FITC is sometimes difficult to image later in development, and thus detection by immunostaining is often performed. Injected embryos must also be kept in the dark to avoid non-specific uncaging from ambient light.

Aside from chemical tracers, we can also lineage trace with GFP mRNA injection, over-express a protein of interest by injecting mRNA, knockdown expression by shRNA injection or mutant protein construct injection to see what cell types and tissues are affected during embryogenesis. Particularly mRNAs encoding histone tagged with green, cyan, yellow or red fluorescent proteins co-injected with mRNA for a membrane-bound fusion protein conjugated to another fluorophore, greatly enhance our ability to obtain high-resolution images of individual cell movements over time. Such microinjection experiments allow highly specific and selective cell manipulations superior to gross ablation experiments. Thus, specificity will facilitate the effective observation of injected cells and their neighbors and resultant deviations from normal development.

Fate mapping is therefore an extremely powerful tool for biologists, with new and improved tools constantly evolving to allow great resolution of what goes on during embryogenesis in various model organisms. Many genetic and chemical tools have been generated that allow long-term cell lineage tracing, bringing insight into the longevity of embryonic stem cells for various tissues. The ability to over-express and knockdown putative cell-fate patterning molecules and fluorescently label them, will also enhance our understanding of the extrinsic and intrinsic molecular cues required by various cell types during embryogenesis.

Probable Questions:

1. Define Potency of a cell. Write short notes on Pluripotency, multipotency, unipotency and totipotency.

2. What is embryonic induction. What is exogenous and endogenous induction? Give experimental proof of induction.

3. What is organizer concept. Give notes on grey crescent.

4. Describe neural induction .briefly explain the chemical basis and genetic basis of it.

5. What do you mean by morphogenetic gradient?

6. What is fate map? Briefly describe the importance of fate map in vertebrate development.

Suggested readings / References:

1.https://www.khanacademy.org/science/biology/developmental-biology/development-and-differentiation/a/introduction-to-development.

2.http://www.yourarticlelibrary.com/biology/embryonic-induction-its-types-experimental-evidence-characteristics-and-mechanism/5045.

3. https://en.wikipedia.org/wiki/Fate_mapping

4.http://www.notesonzoology.com/embryology/fertilization/morphogenetic-gradients-in-the-egg-cytoplasm-cleavage-embryology/13380

5. Developmental Biology. Gilbert SF. 6th edition.

Unit-II

Stem Cells: Embryonic Stem cells and adult stem cells

Objectives:

In this section we will discuss on Stem Cells: Embryonic Stem cells and adult stem cells

Introduction:

Human embryonic stem (ES) cells capture the imagination because they are immortal and have an almost unlimited developmental potential. After many months of growth in culture dishes, these remarkable cells maintain the ability to form cells ranging from muscle to nerve to blood—potentially any cell type that makes up the body. The proliferative and developmental potential of human ES cells promises an essentially unlimited supply of specific cell types for basic research and for transplantation therapies for diseases ranging from heart disease to Parkinson's disease to leukemia. Here we discuss the origin and properties of human ES cells, their implications for basic research and human medicine, and recent research progress since August 2001, when President George W. Bush allowed federal funding of this research for the first time. A previous report discussed progress prior to June 17, 2001.

What Are Embryonic StemCells?

Embryonic stem cells are derived from embryos at a developmental stage before the time that implantation would normally occur in the uterus. Fertilization normally occurs in the oviduct, and during the next few days, a series of cleavage divisions occur as the embryo travels down the oviduct and into the uterus. Each of the cells (blastomeres) of these cleavage-stage embryos are undifferentiated, i.e. they do not look or act like the specialized cells of the adult, and the blastomeres are not yet committed to becoming any particular type of differentiated cell. Indeed, each of these blastomeres has the potential to give rise to any cell of the body.

The first differentiation event in humans occurs at approximately five days of development, when an outer layer of cells committed to becoming part of the placenta (the trophectoderm) separates from the inner cell mass (ICM). The ICM cells have the potential to generate any cell type of the body, but after implantation, they are quickly depleted as they differentiate to other cell types with more limited developmental potential. However, if the ICM is removed from its normal embryonic environment and cultured under appropriate conditions, the ICM-derived cells can continue to proliferate and replicate themselves indefinitely and still maintain the developmentalpotential to form any cell type of the body(pluripotency). These pluripotent, ICM-derived cells are ES cells.

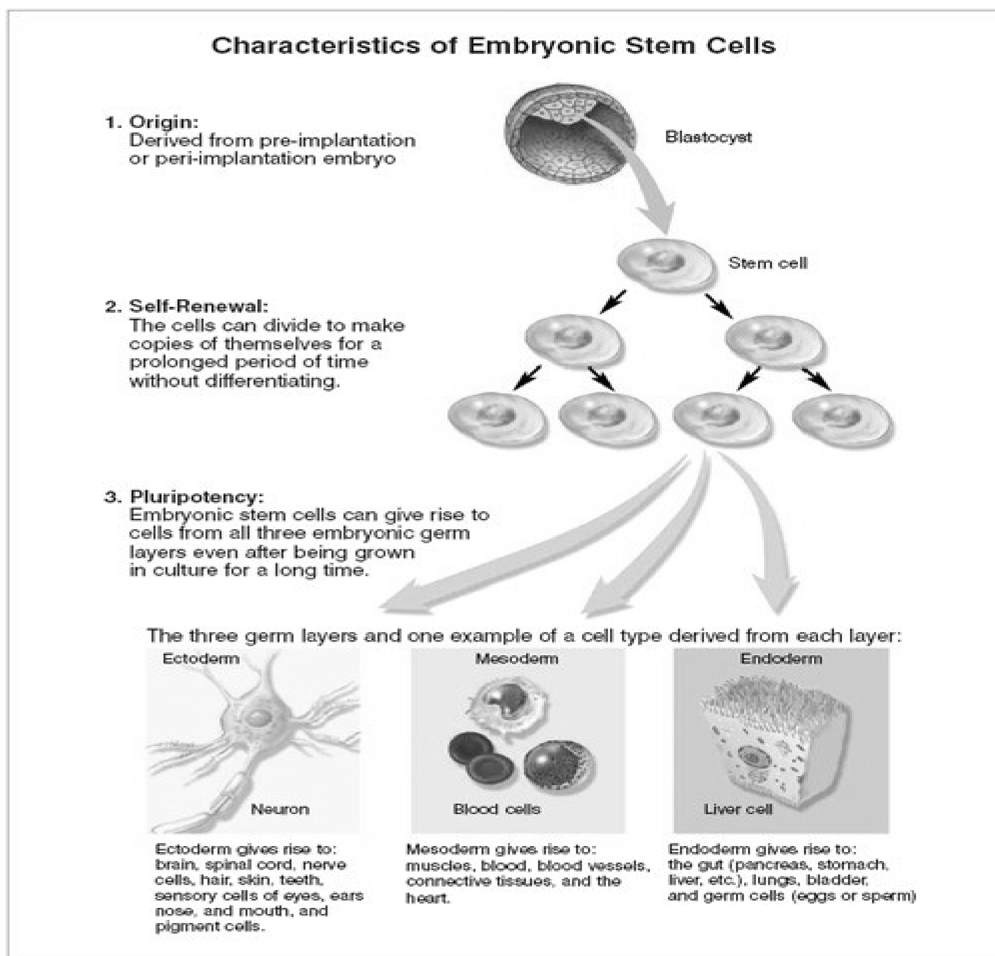


Figure: Characteristics of Embryonic Stem Cells. (© 2006 Terese Winslow)

The derivation of mouse ES cells was first reported in 1981, but it was not until 1998 that derivation of human ES cell lines was first reported. Human ES cell lines are derived from

embryos produced by in vitro fertilization (IVF), a process in which oocytes and sperm are placed together to allow fertilization to take place in a culture dish. Clinics use this method to treat certain types of infertility, and sometimes, during the course of these treatments, IVF embryos are produced that are no longer needed by the couples for producing children. Currently, there are nearly 400,000 IVF-produced embryos in frozen storage in the United States alone, most of which will be used to treat infertility, but some of which (~2.8%) are destined to be discarded. IVF-produced embryos that would otherwise have been discarded were the sources of the human ES cell lines derived prior to President Bush's policy decision of August 2001. These human ES cell lines are now currently eligible for federal funding. Although attempts to derive human ES cells were made as early as the 1980s, culture media for human embryos produced by IVF were suboptimal. Thus, it was difficult to culture single-cell fertilized embryos long enough to obtain healthy blastocysts for the derivation of ES cell lines. Also, species-specific differences between mice and humans meant that experience with mouse ES cells was not completely applicable to the derivation of human ES cells. In the 1990s, ES cell lines from two non-human primates, the rhesus monkey and the common marmoset,6 were derived, and these offered closer models for the derivation of human ES cells. Experience with non-human primate ES cell lines and improvements in culture medium for human IVF- produced embryos led rapidly to the derivation of human ES cell lines in1998.

Because ES cells can proliferate without limit and can contribute to any cell type, human ES cells offer an unprecedented access to tissues from the human body. They will support basic research on the differentiation and function of human tissues and provide material for testing that may improve the safety and efficacy of human drugs For example, new drugs are not generally tested on human heart cells because no human heart cell lines exist. Instead, researchers rely on animal models. Because of important species-specific differences between animal and human hearts, however, drugs that are toxic to the human heart have occasionally entered clinical trials, sometimes resulting in death. Human ES cell-derived heart cells may be extremely valuable in identifying such drugs before they are used in clinical trials, thereby accelerating the drug discovery process and leading to safer and more effective treatments.

Such testing will not be limited to heart cells, but to any type of human cell that is difficult to obtain by othersources.

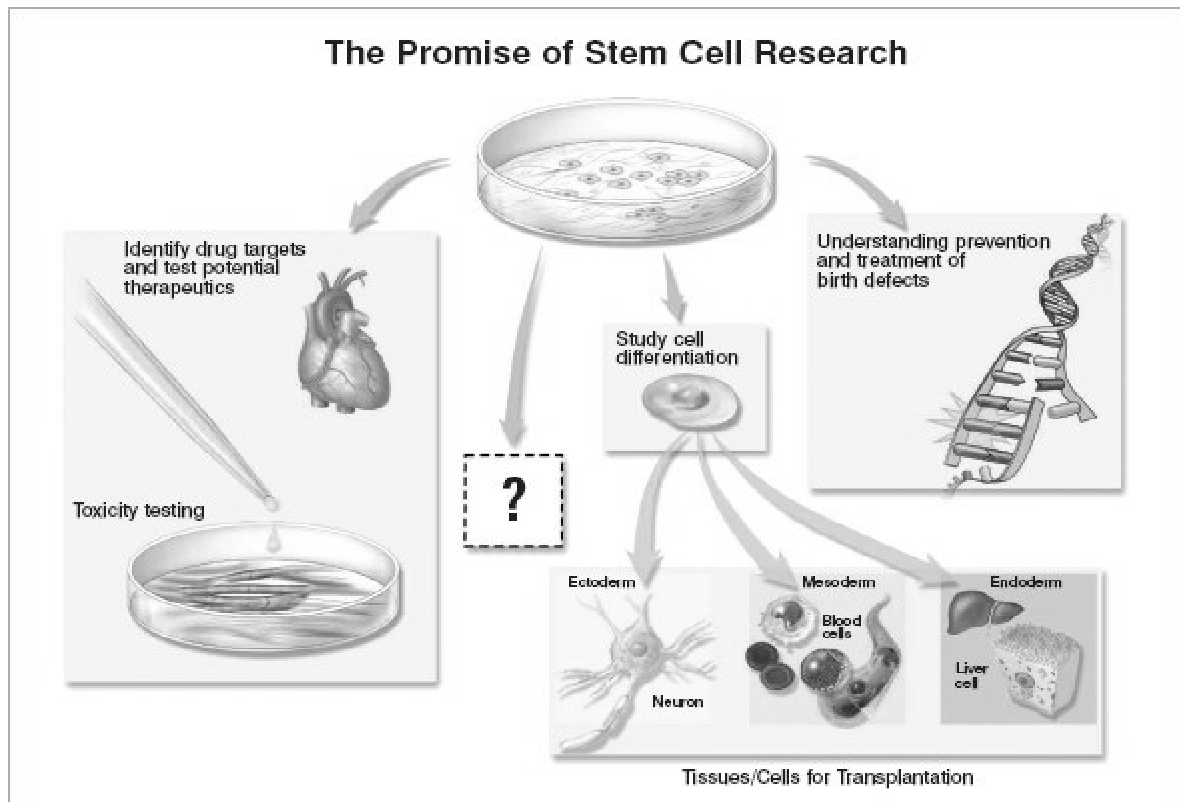


Figure: The Promise of Stem Cell Research. (© 2006 Terese Winslow)

Human ES cells also have the potential to provide an unlimited amount of tissue for transplantation therapies to treat a wide range of degenerative diseases. Some important human diseases are caused by the death or dysfunction of one or a few cell types, e.g., insulin-producing cells in diabetes or dopaminergic neurons in Parkinson's disease. The replacement of these cells could offer a lifelong treatment for these disorders. However, there are a number of challenges to develop human ES cell-based transplantation therapies, and many years of basic research will be required before such therapies can be used to treat patients. Indeed, basic research enabled by human ES cells is likely to impact human health in ways unrelated to transplantation medicine. This impact is likely to begin well before the widespread use of ES cells in transplantation and ultimately could have a more profound long-term effect on human medicine. Since August 2001, improvements in culture of human ES cells, coupled with recent insights into the nature of pluripotency, genetic manipulation of human ES cells, and differentiation, have expanded the possibilities for these unique cells.

Culture of ES Cells

Mouse ES cells and human ES cells were both originally derived and grown on a layer of mouse fibroblasts in the presence of bovine serum. However, the factors that sustain the growth of these two cell types appear to be distinct. The addition of the cytokine, leukemia inhibitory factor (LIF), to serum-containing medium allows mouse ES cells to proliferate in the absence of feeder cells. LIF modulates mouse ES cells through the activation of STAT3 (signal transducers and activators of transcription) protein. In serum-free culture, however, LIF alone is insufficient to prevent mouse ES cells from differentiating into neural cells. Recently, Ying et al. reported that the combination of bone morphogenetic proteins (BMPs) and LIF is sufficient to support the self-renewal of mouse ES cells. The effects of BMPs on mouse ES cells involve induction of inhibitor of differentiation (Id) proteins, and inhibition of extracellular receptor kinase (ERK) and p38 mitogen-activated protein kinases (MAPK). However, LIF in the presence of serum is not sufficient to promote the self-renewal of human ES cells,3 and the LIF/STAT3 pathway appears to be inactive in undifferentiated human ES cells. Also, the addition of BMPs to human ES cells in conditions that would otherwise support ES cells leads to the rapid differentiation of human EScells.

Several groups have attempted to define growth factors that sustain human ES cells and have attempted to identify culture conditions that reduce the exposure of human ES cells to non human animal products. One important growth factor, bFGF, allows the use of a serum replacement to sustain human ES cells in the presence of fibroblasts, and this medium

allowed the clonal growth of human ES cells. Human ES cell culture system has been developed, in which human ES cells are grown on a protein matrix (mouse Matrigel or Laminin) in a bFGF-containing medium that is previously conditioned by co-culture with fibroblasts. Although this culture system eliminates direct contact of human ES cells with the fibroblasts, it does not remove the potential for mouse pathogens being introduced into the culture via the fibroblasts. Several different sources of human feeder cells have been found to support the culture of human ES cells, thus removing the possibility of pathogen transfer from mice to humans. However, the possibility of pathogen transfer from human to human in these culture systems still remains. More work is still needed to develop a culture system that eliminates the use of fibroblasts entirely, which would also decrease much of the variability associated with the current culture of human ES cells. Sato et al. reported that activation of the Wnt pathway by 6-bromoindirubin 3'-oxime (BIO) promotes the self-renewal of ES cells in the presence of bFGF, Matrigel, and a proprietary serum replacement product. Amit et al. reported that bFGF, TGFβ, and LIF could support some human ES cell lines in the absence of feeders. Although there are some questions about how well these new culture conditions will work for different human ES cell lines, there is now reason to believe that defined culture conditions for human ES cells, which reduce the potential for contamination by pathogens, will soon be achieved.

Once a set of defined culture conditions is established for the derivation and culture of human ES cells, challenges to improve the medium will still remain. For example, the cloning efficiency of human ES cells—the ability of a single human ES cell to proliferate and become a colony—is very low (typically less than 1%) compared to that of mouse ES cells. Another difficulty is the potential for accumulation of genetic and epigenetic changes over prolonged periods of culture. For example, karyotypic changes have been observed in several human ES cell lines after prolonged culture, and the rate at which these changes dominate a culture may depend on the culture method. The status of imprinted (epigenetically modified) genes and the stability of imprinting in various culture conditions remain completely unstudied in human ES cells. The status of imprinted genes can clearly change with culture conditions in other cell types. These changes present potential problems if human ES cells are to be used in cell replacement therapy, and optimizing medium to reduce the rate at which genetic and epigenetic changes accumulate in culture represents a long-term endeavor. The ideal human ES cell medium, then, (a) would be cost-effective and easy to use so that many more investigators can use human ES cells as a research tool; (b) would be composed entirely of defined components not of animal origin; (c) would allow cell growth at clonal densities; and (d) would minimize the rate at which genetic and epigenetic changes accumulate in culture. Such a medium will be a challenge to develop and will most likely be achieved through a series of incremental improvements over a period of years.

Among all the newly derived human ES cell lines, twelve lines have gained the most attention. In March 2004, a South Korean group reported the first derivation of a human ES cell line (SCNT-hES-1) using the technique of somatic cell nuclear transfer (SCNT). Human somatic nuclei were transferred into human oocytes (nuclear transfer), which previously had been stripped of their own genetic material, and the resultant nuclear transfer products were cultured in vitro to the blastocyst stage for ES cell derivation. Because the ES cells derived through nuclear transfer contain the same genetic material as that of the nuclear donor, the intent of the procedure is that the differentiated derivatives would not be rejected by the donor's immune system if used in transplantation therapy. More recently, the same group reported the derivation of eleven more human SCNT-ES cell lines with markedly improved efficiency. However, given the abnormalities frequently observed in cloned animals, and the costs involved, it is not clear how useful this procedure will be in clinical applications. Also, for some autoimmune diseases, such as type I diabetes, merely providing genetically-matched tissue will be insufficient to prevent immunerejection. Additionally, new human ES cell lines were established from embryos with genetic disorders, which were detected during the practice of preimplantation genetic diagnosis (PGD). These new cell lines may provide an excellent in vitro model for studies on the effects that the genetic mutations have on cell proliferation anddifferentiation.

To date, more than 120 human ES cell lines have been established worldwide, 67 of which are included in the National Institutes of Health (NIH) Registry. As of this writing, 21 cell lines are currently available for distribution, all of which have been exposed to animal products during their derivation. Although it has been eight years since the initial derivation of human ES cells, it is an open question as to the extent that independent human ES cell lines differ from one another. At the very least, the limited number of cell lines cannot represent a reasonable sampling of the genetic diversity of different ethnic groups in the United States, and this has consequences for drug testing, as adverse reactions to drugs often reflect a complex genetic component. Once defined culture conditions are well established for human ES cells, there will be an even more compelling need to derive additional cell lines.

Pluripotency of ES Cells

The ability of ES cells to develop into all cell types of the body has fascinated scientists for years, yet remarkably little is known about factors that make one cell pluripotent and another more restricted in its developmental potential. The transcription factor Oct4 has been used as a key marker for ES cells and for the pluripotent cells of the intact embryo, and its expression

must be maintained at a critical level for ES cells to remain undifferentiated. The Oct4 protein itself, however, is insufficient to maintain ES cells in the undifferentiated state. Recently, two groups identified another transcription factor, Nanog, that is essential for the maintenance of the undifferentiated state of mouse ES cells. The expression of Nanog decreased rapidly as mouse ES cells differentiated, and when its expression level was maintained by a constitutive promoter, mouse ES cells could remain undifferentiated and proliferate in the absence of either LIF or BMP in serum-free medium. Nanog is also expressed in human ES cells, though at a much lower level compared to that of Oct4, and its function in human ES cells has yet to beexamined.

By comparing gene expression patterns between different ES cell lines and between ES cells and other cell types such as adult stem cells and differentiated cells, genes that are enriched in the ES cells have been identified. Using this approach, Esg-1, an uncharacterized ES cell-specific gene, was found to be exclusively associated with pluripotency in the mouse. Sperger et al. identified 895 genes that are expressed at significantly higher levels in human ES cells and embryonic carcinoma cell lines, the malignant counterparts to ES cells. Sato et al. identified a set of 918 genes enriched in undifferentiated human ES cells compared with their differentiated counterparts; many of these genes were shared by mouse ES cells. Another group, however, found 92 genes, including Oct4 and Nanog, enriched in six different human ES cell lines, which showed limited overlap with those in mouse ES cell lines. Care must be taken to interpret these data, and the considerable differences in the results may arise from the cell lines used in the experiments, methods to prepare and maintain the cells, and the specific methods used to profile gene expression.

Genetic Manipulation of ES Cells

Since establishing human ES cells in 1998, scientists have developed genetic manipulation techniques to determine the function of particular genes, to direct the differentiation of human ES cells towards specific cell types, or to tag an ES cell derivative with a certain marker gene. Several approaches have been developed to introduce genetic elements randomly into the human ES cell genome, including electroporation, transfection by lipid-based reagents, and lentiviral vectors. However, homologous recombination, a method in which a specific gene inside the ES cells is modified with an artificially introduced DNA molecule, is an even more precise method of genetic engineering that can modify a gene in a defined way at a specific locus. While this technology is routinely used in mouse ES cells, it has recently been

successfully developed in human ES cells, thus opening new doors for using ES cells as vehicles for gene therapy and for creating *in vitro* models of human genetic disorders such as Lesch-Nyhan disease. Another method to test the function of a gene is to use RNA interference (RNAi) to decrease the expression of a gene of interest. In RNAi, small pieces of double-stranded RNA (siRNA; small interfering RNA) are either chemically synthesized and introduced directly into cells, or expressed from DNA vectors. Once inside the cells, the siRNA can lead to the degradation of the messenger RNA (mRNA), which contains the exact sequence as that of the siRNA. mRNA is the product of DNA transcription and normally can be translated into proteins.

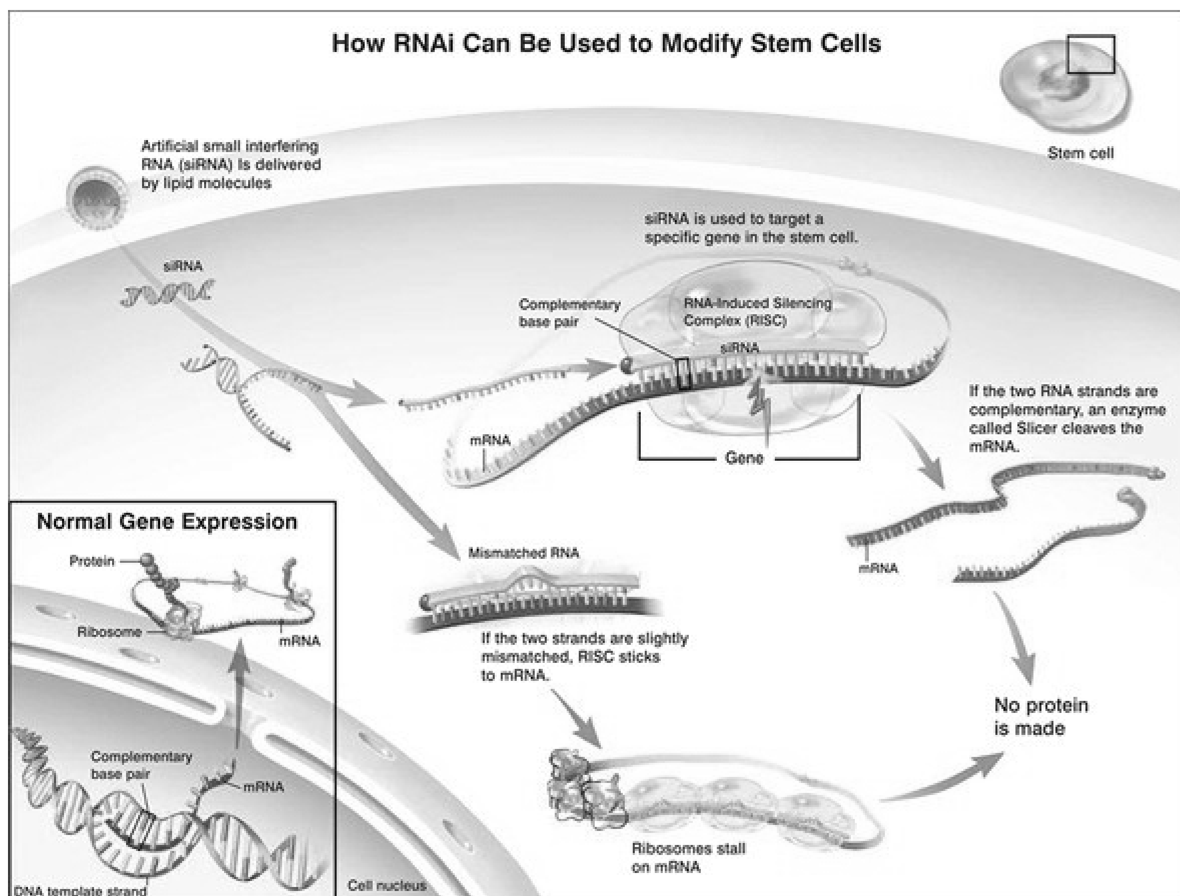


Figure . How RNAi Can Be Used To Modify Stem Cell.

Differentiation of Human ES Cells

The pluripotency of ES cells suggests possible widespread uses for these cells and their derivatives. The ES cell-derived cells can potentially be used to replace or restore tissues that have been damaged by disease or injury, such as diabetes, heart attacks, Parkinson's disease or spinal cord injury. The recent developments in these particular areas are discussed in detail in other chapters, and Table 1 summarizes recent publications in the differentiation of specific cell lineages.

The differentiation of ES cells also provides model systems to study early events in human development. Because of possible harm to the resulting child, it is not ethically acceptable to experimentally manipulate the postimplantation human embryo. Therefore, most of what isknown about the mechanisms of early human embryology and human development, especially in the early postimplantation period, is based on histological sections of a limited number of human embryos and on analogy to the experimental embryology of the mouse. However, human and mouse embryos differ significantly, particularly in the formation, structure, and function of the fetal membranes and placenta, and the formation of an embryonic disc instead of an egg cylinder. For example, the mouse yolk sac is a well-vascularized, robust, extraembryonic organ throughout gestation that provides important nutrient exchange functions. In humans, the yolk sac also serves important early functions, including the initiation of hematopoiesis, but it becomes essentially a vestigial structure at later times or stages in gestation. Similarly, there are dramatic differences between mouse and human placentas, both in structure and function. Thus, mice can serve in a limited capacity as a model system for understanding the developmental events that support the initiation and maintenance of human pregnancy. Human ES cell lines thus provide an important new in vitro model that will improve our understanding of the differentiation of human tissues, and thus provide important insights into processes such as infertility, pregnancy loss, and birth defects.

Human ES cells are already contributing to the study of development. For example, it is now possible to direct human ES cells to differentiate efficiently totrophoblast, the outer layer of the placenta that mediates implantation and connects the conceptus to the uterus. Another use of human ES cells is for the study of germ cell development. Cells resembling both oocytes and sperm have been successfully derived from mouse ES cells in vitro. Recently, human ES cells have also been observed to differentiate into cells expressing genes characteristic of germ cells. Thus it may also be possible to derive oocytes and sperm from human ES cells, allowing the detailed study of human gametogenesis for the first time. Moreover, human ES cell studies are not limited to early differentiation, but are increasingly being used to

understand the differentiation and functions of many human tissues, including neural, cardiac, vascular, pancreatic, hepatic, and bone (see Table 1). Moreover, transplantation of ES-derived cells has offered promising results in animalmodels.

Although scientists have gained more insights into the biology of human ES cells since 2001, many key questions remain to be addressed before the full potential of these unique cells can be realized. It is surprising, for example, that mouse and human ES cells appear to be so different with respect to the molecules that mediate their self-renewal, and perhaps even in their developmental potentials. BMPs, for example, in combination with LIF, promote the self- renewal of mouse ES cells. But in conditions that would otherwise support undifferentiated proliferation, BMPs cause rapid differentiation of human ES cells. Also, human ES cells differentiate quite readily to trophoblast, whereas mouse ES cells do so poorly, if at all. One would expect that at some level, the basic molecular mechanisms that control pluripotency would be conserved, and indeed, human and mouse ES cells share the expression of many key genes. Yet we remain remarkably ignorant about the molecular mechanisms that control pluripotency, and the nature of this remarkable cellular state has become one of the central questions of developmental biology. Of course, the other great challenge will be to continue to unravel the factors that control the differentiation of human ES cells to specific lineages, so that ES cells can fulfill their tremendous promise in basic human biology, drug screening, and transplantation medicine.

Cell types	**Publications**	**References**
Neural	8	61, 66, 68–73
Cardiac	6	9–11, 74–76
Endothelial (Vascular)	2	77, 78
Hematopoietic (Blood)	8	79–86
Pancreatic (Islet-like)	2	87, 88
Hepatic (Liver)	3	89–91
Bone	1	92
Trophoblast	2	17, 53
Multilineages	9	16, 57, 93–99

Table 1. Publications on Differentiation of Human Embryonic Stem Cells since 2001.

Adult Stem Cells

Adult stem cells are undifferentiated cells, found throughout the body after development, that multiply by cell divisionto replenish dying cells and regenerate damaged tissues. Also known as somatic stem cells, they can be found in juvenile as well as adult animals and humans, unlike embryonic stem cells.

Scientific interest in adult stem cells is centered on their ability to divide or self-renew indefinitely, and generate all thecell types of the organ from which they originate, potentially regenerating the entire organ from a few cells.Unlike for embryonic stem cells, the use of human adult stem cells in research and therapy is not considered to be controversial, as they are derived from adult tissue samples rather than human embryos designated for scientific research. They have mainly been studied in humans and model organisms such as mice andrats.

A stem cell possesses two properties:

- **Self-renewal**, which is the ability to go through numerous cycles of cell division while still maintaining its undifferentiatedstate.

- **Multipotency** or **multi differentiative potential**, which is the ability to generate progeny of several distinct cell types, (for example glial cells and neurons) as opposed to unipotency, which is the term for cells that are restricted to producing a single-cell type. However, some researchers do not consider multipotency to be essential, and believe that unipotent self-renewing stem cells can exist. These properties can be illustrated with relative ease *in vitro*, using methods such as clonogenic assays, where the progeny of a single cell is characterized. However, it is known that *in vitro* cell culture conditions can alter the behavior of cells, proving that a particular subpopulation of cells possesses stem cell properties *in vivo* is challenging, and so considerable debate exists as to whether some proposed stem cell populations in the adult are indeed stemcells.

Types:

Hematopoietic stem cell

Hematopoietic stem cells are found in the bone marrow and umbilical cord blood and give rise to all the blood cell types.

Mammary stem cells

Mammary stem cells provide the source of cells for growth of the mammary gland during puberty and gestation and play an important role in carcinogenesis of the breast. Mammary stem cells have been isolated from human and mouse tissue as well as from cell lines derived from the mammary gland. Single such cells can give rise to both the luminal and myoepithelial cell types of the gland, and have been shown to have the ability to regenerate the entire organ in mice.

Intestinal stem cells

Intestinal stem cells divide continuously throughout life and use a complex genetic program to produce the cells lining the surface of the small and large intestines. Intestinal stem cells reside near the base of the stem cell niche, called the crypts of Lieberkuhn. Intestinal stem cells are probably the source of most cancers of the small intestine and colon.

Mesenchymal stem cells Endothelial stem cells

Endothelial stem cells are one of the three types of multipotent stem cells found in the bone marrow. They are a rare and controversial group with the ability to differentiate into endothelial cells, the cells that line blood vessels.

Neural stem cells

The existence of stem cells in the adult brain has been postulated following the discovery that the process of neurogenesis, the birth of new neurons, continues into adulthood in rats. The presence of stem cells in the mature primate brain was first reported in 1967. It has since been shown that new neurons are generated in adult mice, songbirds and primates, including humans. Normally, adult neurogenesis is restricted to two areas of the brain – the subventricular zone, which lines the lateral ventricles, and the dentate gyrus of the hippocampal formation. Although the generation of new neurons in the hippocampus is well established, the presence of true self-renewing stem cells there has been debated.Under certain circumstances, such as following tissue damage in ischemia, neurogenesis can be induced in other brain regions, including theneocortex. Neural stem cells are commonly cultured in vitro as so called neurospheres – floating heterogeneous aggregates of cells, containing a large proportion of stem cells. They can be propagated for extended periods of time and differentiated into both neuronal and glia cells, and therefore behave as stem

cells. However, some recent studies suggest that this behaviour is induced by the culture conditions in progenitor cells, the progeny of stem cell division that normally undergo a strictly limited number of replication cycles in vivo. Furthermore, neurosphere-derived cells do not behave as stem cells when transplanted back into thebrain. Neural stem cells share many properties with haematopoietic stem cells (HSCs). Remarkably, when injected into the blood, neurosphere-derived cells differentiate into various cell types of the immunesystem.

Olfactory adult stem cells

Olfactory adult stem cells have been successfully harvested from the human olfactory mucosa cells, which are found in the lining of the nose and are involved in the sense of smell. If they are given the right chemical environment these cells have the same ability as embryonic stem cells to develop into many different cell types. Olfactory stem cells hold the potential for therapeutic applications and, in contrast to neural stem cells, can be harvested with ease without harm to the patient. This means they can be easily obtained from all individuals, including older patients who might be most in need of stem cell therapies.

Neural crest stem cells

Hair follicles contain two types of stem cells, one of which appears to represent a remnant of the stem cells of the embryonic neural crest. Similar cells have been found in the gastrointestinal tract, sciatic nerve, cardiac outflow tract and spinal and sympathetic ganglia. These cells can generate neurons, Schwann cells, myofibroblast, chondrocytes and melanocytes.

Testicular cells

Multipotent stem cells with a claimed equivalency to embryonic stem cells have been derived from spermatogonial progenitor cells found in the testicles of laboratory mice by scientists in Germany and the United States, and, a year later, researchers from Germany and the United Kingdom confirmed the same capability using cells from the testicles of humans. The extracted stem cells are known as human adult germline stem cells(GSCs) Multipotent stem cells have also been derived from germ cells found in human testicles.

Development:

To ensure self-renewal, stem cells undergo two types of cell division. Symmetric division gives rise to two identical daughter cells, both endowed with stem cell properties, whereas asymmetric division produces only one stem cell and a progenitor cell with limited self-renewal potential. Progenitors can go through several rounds of cell division before finally

differentiating into a mature cell. It is believed that the molecular distinction between symmetric and asymmetric divisions lies in differential segregation of cell membrane proteins (such as receptors) between the daughter cells.

Plasticity

Discoveries in recent years have suggested that adult stem cells might have the ability to differentiate into cell types from different germ layers. For instance, neural stem cells from the brain, which are derived from ectoderm, can differentiate into ectoderm, mesoderm, and endoderm. Stem cells from the bone marrow, which is derived from mesoderm, can differentiate into liver, lung, GI tract and skin, which are derived from endoderm and mesoderm. This phenomenon is referred to as stem cell transdifferentiation or plasticity. It can be induced by modifying the growth medium when stem cells are cultured in vitro or transplanting them to an organ of the body different from the one they were originally isolated from. There is yet no consensus among biologists on the prevalence and physiological and therapeutic relevance of stem cell plasticity. More recent findings suggest that pluripotent stem cells may reside in blood and adult tissues in a dormant state. These cells are referred to as "Blastomere Like Stem Cells"[and "very small embryonic like" – "VSEL" stem cells, and display pluripotency in vitro. As BLSC's and VSEL cells are present in virtually all adult tissues, including lung, brain, kidneys, muscles, and pancreas Co-purification of BLSC's and VSEL cells with other populations of adult stem cells may explain the apparent pluripotency of adult stem cell population.

Aging

Stem cell function becomes impaired with age, and this contributes to progressive deterioration of tissue maintenance and repair.A likely important cause of increasing stem cell dysfunction is age-dependent accumulation of DNA damage in both stem cells and the cells that comprise the stem cell environment.

Adult stem cells can, however, be artificially reverted to a state where they behave like embryonic stem cells (including the associated DNA repair mechanisms). This was done with mice as early as 2006[with future prospects to slow down human aging substantially. Such cells are one of the various classes of induced stem cells.

Function

Signalling pathways

Adult stem cell research has been focused on uncovering the general molecular mechanisms that control their self-renewal and differentiation.

Notch

The Notch pathway has been known to developmental biologists for decades. Its role in control of stem cell proliferation has now been demonstrated for several cell types including haematopoietic, neural, and mammary stem cells.

Wnt

These developmental pathways are also strongly implicated as stem cell regulators.

TGFβ

The TGFβ family of cytokines regulate the stemness of both normal and cancer stem cells.

Clinical significance

Early regenerative applications of adult stem cells has focused on intravenous delivery of blood progenitors known as Hematopetic Stem Cells (HSC's). CD^{34+} hematopoietic Stem Cells have been clinically applied to treat various diseases including spinal cord injury, liver cirrhosis and Peripheral Vascular disease. Research has shown that CD^{34+} hematopoietic Stem Cells are relatively more numerous in men than in women of reproductive age group among spinal cord Injury victims. Other early commercial applications have focused on Mesenchymal Stem Cells (MSCs). For both cell lines, direct injection or placement of cells into a site in need of repair may be the preferred method of treatment, as vascular delivery suffers from a "pulmonary first pass effect" where intravenous injected cells are sequestered in the lungs. Clinical case reports in orthopedic applications have been published. Wakitani has published a small case series of nine defects in five knees involving surgical transplantation of mesenchymal stem cells with coverage of the treated chondral defects. Centeno et al. have reported high field MRI evidence of increased cartilage and meniscus volume in individual human clinical subjects as well as a large n=227 safety study. Many other stem cell based treatments are operating outside the US, with much controversy being reported regarding these treatments as some feel more regulation is needed as clinics tend to exaggerate claims of success and minimize or omit risks.

Therapies

The therapeutic potential of adult stem cells is the focus of much scientific research, due to their ability to be harvested from the patient. In common with embryonic stem cells, adult stem cells have the ability to differentiate into more than one cell type, but unlike the former they are often restricted to certain types or "lineages". The ability of a differentiated stem cell of one lineage to produce cells of a different lineage is called trans differentiation. Some types of adult stem cells are more capable of transdifferentiation than others, but for many there is no evidence that such a transformation is possible. Consequently, adult stem therapies require a stem cell source of the specific lineage needed, and harvesting and/or culturing them up to the numbers required is a challenge. Additionally, cues from the immediate environment (including how stiff or porous the surrounding structure/extracellular matrix is) can alter or enhance the fate and differentiation of the stem cells.

Sources

Pluripotent stem cells, i.e. cells that can give rise to any fetal or adult cell type, can be found in a number of tissues, including umbilical cord blood. Using genetic reprogramming, pluripotent stem cells equivalent to embryonic stem cells have been derived from human adult skin tissue. Other adult stem cells are multipotent, meaning they are restricted in the types of cell they can become, and are generally referred to by their tissue origin (such as mesenchymal stem cell, adipose-derived stem cell, endothelial stem cell, etc. A great deal of adult stem cell research has focused on investigating their capacity to divide or self-renew indefinitely, and their potential for differentiation. In mice, pluripotent stem cells can be directly generated from adult fibroblastcultures

Cancer

In recent years, acceptance of the concept of adult stem cells has increased. There is now a hypothesis that stem cells reside in many adult tissues and that these unique reservoirs of cells not only are responsible for the normal reparative and regenerative processes but are also considered to be a prime target for genetic andepigenetic changes, culminating in many abnormal conditions including cancer.

Multidrug resistance

Adult stem cells express transporters of the ATP-binding cassette family that actively pump a diversity of organic molecules out of the cell. Many pharmaceuticals are exported by these transporters conferring multidrug resistance onto the cell. This complicates the design of drugs, for instance neural stem cell targeted therapies for the treatment of clinical depression.

Probable questions:

1. What are embryonic stem cells?
2. What is Pluripotency of stem cells?
3. Write short notes in genetic manipulation of embryonic stem cells.
4. what is adult stem cells?
5. How differentiation of stem cells occur/
6. Discuss about clinical significance of stem cells.

Suggested Readings / References:

1. Developmental Biology. Gilbert SF. 6th edition.

Unit-III

Sex, Gametes and fertilization: Germ cell migration; Gametogenesis; fertilization and prevention of polyspermy

Objective:

In this Unit we will discuss on Sex, Gametes and fertilization: Germ cell migration; Gametogenesis; fertilization and prevention of polyspermy

Introduction:

Gametogenesis: The origin and development of gametes (reproductive cells- Ovum & Spermatozoon) is called gametogenesis This may be divided into spermatogenesis and oogenesis.

A. Spermatogenesis: Spermatogenesis deals with the development of male sex-cells called sperms in the male gonad or testis.

The male gonad known as testis is the site of spermatogenesis. In each vertebrate a pair of testes remains attached to dorsal body wall by a connective tissue called mesorchium. Each testis is formed of thousands of minute elongated and coiled tubules called seminiferous tubules. The inner lining of seminiferous tubules is called as germinal epithelium and is made of primordial germ cells (Primary germ cells) as well as some supporting nutritive cells. The primordial germ cells give rise to spermatids through the following three successive steps

Multiplication Phase: The primary germ cells multiply by repeated mitotic division. The cells produced after the final mitotic divisions are known as spermatogonia or sperm mother cells.

Growth Phase: The spermatogonia do not divide for sometime but increase in size by accumulating nutritive materials from the supporting cells. In mammals such supporting cells are called cells of Sertoli. The enlarged spermatogonia are now called primary spermatocytes.

Maturation Phase: During the phase of maturation, the primary spermatocytes divide by meiosis consisting of two successive divisions. The first division is reductional or disjunctional reducing the chromosome number from '2n' to 'n'. These cells are celled secondary spermatocytes. Second division is equational resulting in formation of four daughter cells called spermatids.

Spermiogenesis (Spermatoleosis):

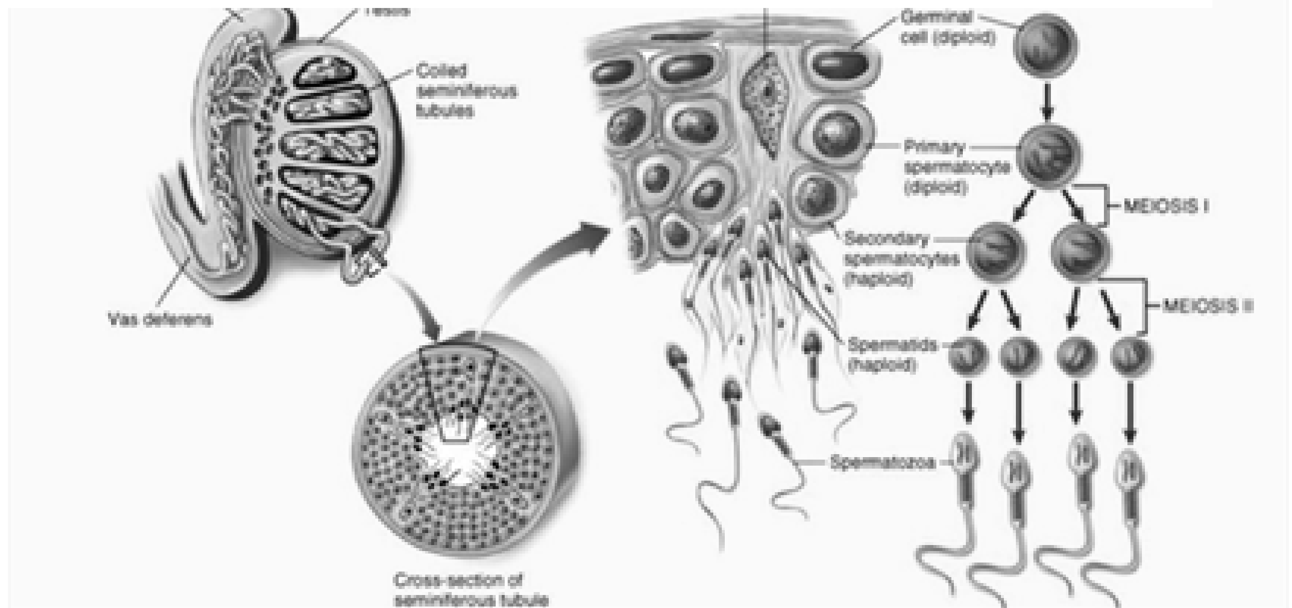
This is the second phase of spermatogenesis during which the spermatids produced at the end of first phase are metamorphosed into sperm cells. The spermatid is a typical cell containing a nucleus and cytoplasmic organelles such as mitochondria, Golgi bodies, centriole etc, but the nucleus only contains haploid number of chromosomes. During spermiogenesis or spermatoleosis the following transformations occur in the spermatids:

1. The large spherical nucleus becomes smaller by losing water and usually changes its shape into elongated structure.

2. The Golgi bodies condense into a cap called acrosome in front of the nucleus.

3. Nucleus and the acrosome combinedly form the head of the developing sperm while the cytoplasm with mitochondria and centrioles move downwards and form the cylindrical middle piece behind the head

4. The two centrioles of middle piece develop axial filaments which are bunched into a single thread and extend behind in the form of a long vibratile tail. Thus, spermatid is transformed into a motile sperm divisible into head, middle piece and tail.



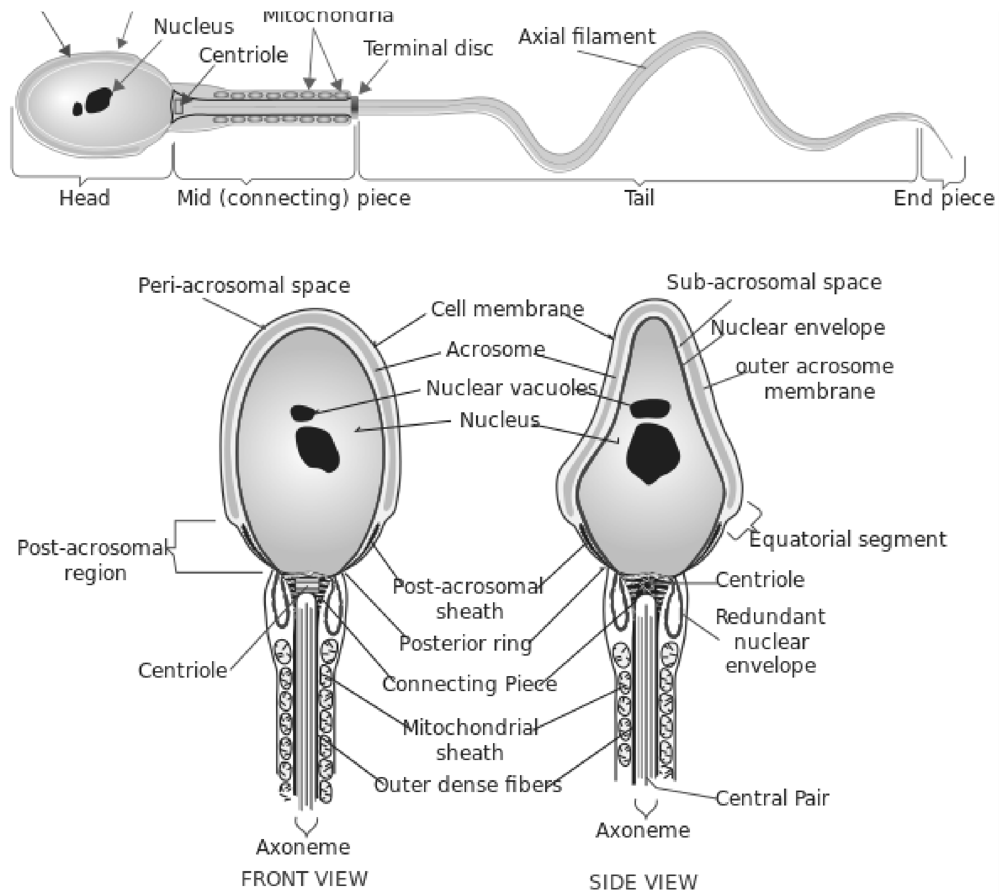
Structure of spermatozoa

Head: It has a compact nucleus with only chromatic substance and is surrounded by only a thin rim of cytoplasm. Above the nucleus lies a cap-like structure called the acrosome, formed by modification of the Golgi body and which secretes enzyme spermlysin (hyaluronidase, corona-penetrating enzyme, zona eyesin, or aerosin.). On the surface of the head lies a decapcitating substance which is removed before fertilisation.

Neck: It is the smallest part (0.03×10^{-6} m), and has a proximal and distal centriole. The proximal centriole enters into the egg during fertilisation and starts the first cleavage division of the egg, which has no centriole. The distal centriole gives rise to axial filament which forms the tail and has (9+2) arrangement. A transitory membrane called Manchette lies in middle piece.

Middle piece: It has 10-14 spirals of mitochondria surrounding axial filament in the cytoplasm. It provides motility, and hence is called the powerhouse of the sperm. It also has a ring centriole (annulus) with unknown function.

Tail: It is the longest part(50×10^{-6} m) having axial filament surrounded by cytoplasm and plasma membrane, but at the posterior end axial filament is naked.



B. Oogenesis: Oogenesis is the development of female sex-cells called ova or eggs in the female gonad or ovary.

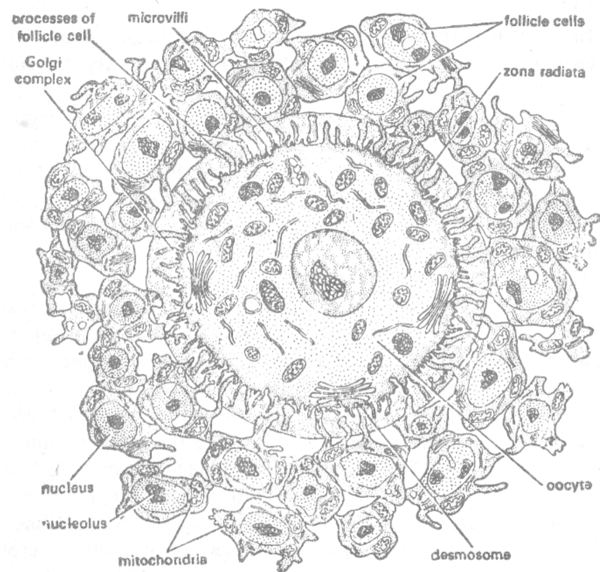
Three successive stages of oogenesis

Multiplication Phase: The primordial germinal cells divide repeatedly to form the oogonia (oon = egg). The oogonia multiply by the mitotic divisions and form the primary oocytes which pass through the growth phase.

Growth Phase: The growth phase of the oogenesis is comparatively longer than the growth phase of the spermatogenesis. In the growth phase, the size of the primary oocyte increases enormously. For instance, the primary oocyte of the frog in the beginning has the diameter about 50μ but after growth phase the diameter of the mature egg reaches about 1000μ to 2000μ. In the primary oocyte, large amount of fats and proteins becomes accumulated in the form of yolk and due to its heavy weight (or gravity), it is usually concentrated towards the lower portion of the egg forming the vegetative pole. The portion of the cytoplasm containing the egg pro-nucleus remains often separated from the yolk and occurs towards the upper side of the egg forming the animal pole. The cytoplasm of the oocyte becomes rich in RNA, DNA, ATP and enzymes. Moreover, the mitochondria, Golgi apparatus, ribosomes, etc., become concentrated in the cytoplasm of the oocyte.

In certain oocytes (Amphibia and birds), the mitochondria become accumulated at some place in the oocyte cytoplasm and form the mitochondrial clouds. During the growth phase, tremendous changes also occur in the nucleus of the primary oocyte. The nucleus becomes large due to the increased amount of the nucleoplasm and is called germinal vesicle. The nucleolus becomes large or its number is multiplied due to excessive synthesis of ribosomal RNA (rRNA) by ribosomal DNA (rDNA) of nucleolar organizer region of chromosomes. Thus, the nucleus or germinal vesicle of primary oocyte of *Triturus* has 600 nucleoli, of Siredon has 1000 nucleoli and of Xenopus has 600 to 1200 nucleoli due to synthesis of ribosomal RNA (rRNA).The chromosomes change their shape and become giant lamp-brush chromosomes which are directly related with increased transcription of messenger RNA (mRNA) molecules and active protein synthesis in the cytoplasm. When the growth of the cytoplasm and nucleus of the primary oocyte is completed, it becomes ready for maturation phase.

Maturation Phase: The maturation phase is accompanied by the maturation or meiotic division. The maturation division of the primary oocyte differs greatly from the maturation division of the spermatocyte. Here after the meiotic division of the nucleus, the cytoplasm of the oocyte divides unequally to form a single large-sized haploid egg and three small haploid polar bodies or polocytes at the end. This type of unequal division has the great significance for the egg. If the equal divisions of the primary oocyte might have been resulted, the stored food amount would have been distributed equally to the four daughter cells and which might prove insufficient for the developing embryo.



Young oocyte of a mammal surrounded by follicle cells Balinsky, 1970).

Therefore, these unequal divisions allow one cell out of the four daughter cells to contain most of the cytoplasm and reserve food material which is sufficient for the developing embryo.

(i) First maturation division:

During the first maturation division or first meiosis, the homologous chromosomes of the primary oocyte nucleus pass through the pairing or synapsis, duplication, chiasma formation and crossing over. Soon after, the nuclear membrane breaks and the bivalent chromosomes move towards the opposite poles due to contraction of chromonemal fibres. A new nuclear envelope is developed around the daughter chromosomes by the endoplasmic reticulum. After the karyokinesis, the unequal cytokinesis occurs and a small haploid polar body or polocyte and a large haploid secondary oocyte or ootid are formed

(ii) Second meiotic division:

The haploid secondary oocyte and first polocyte pass through the second meiotic division. Due to the second meiotic division, the secondary oocyte forms amature egg and a second polocyte. By the second meiotic division, the first polocyte also divides into two secondary

polocytes. These polocytes ooze out from the egg and degenerate while the haploid egg cell becomes ready for the fertilisation.

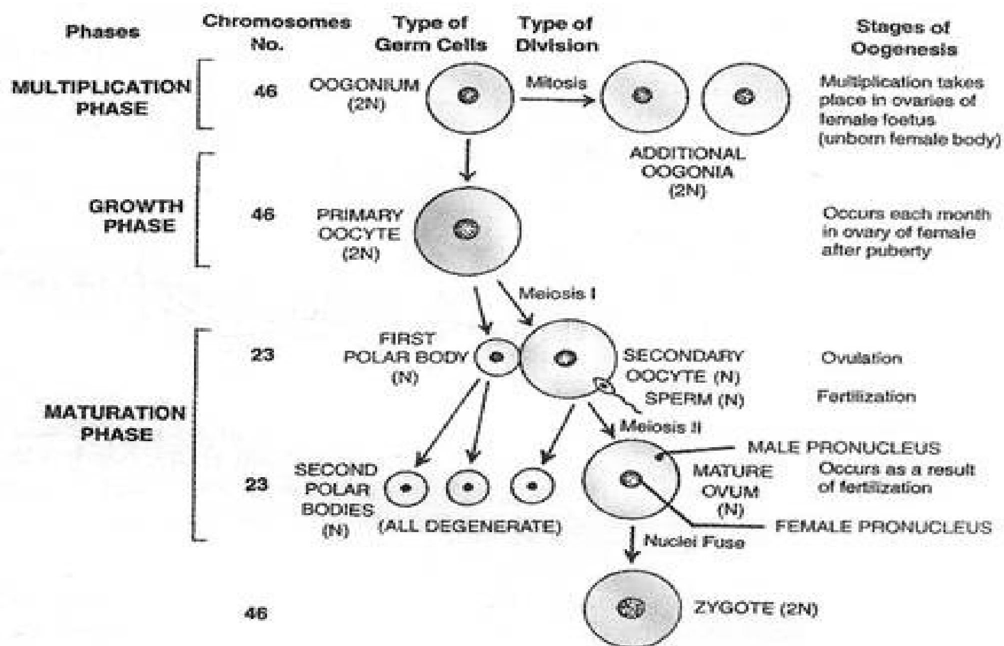
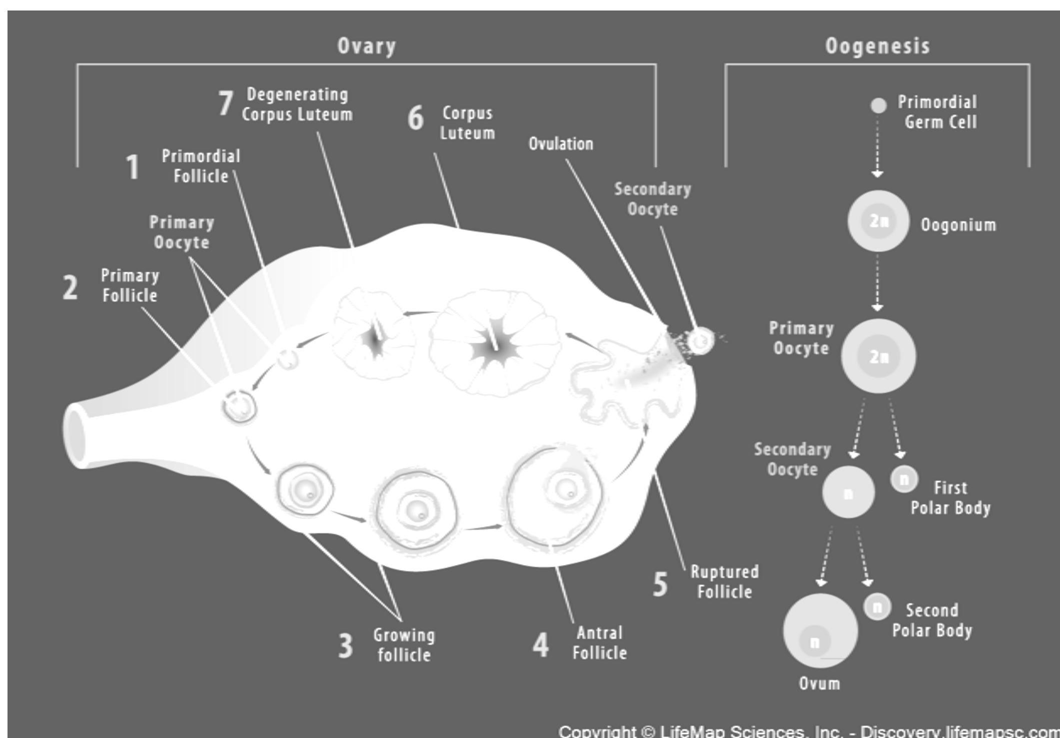


Fig. 3.18. Stages in oogenesis (diagrammatic).



Copyright © LifeMap Sciences, Inc. - Discovery.lifemapsc.com

Fertilization:

Fertility Window

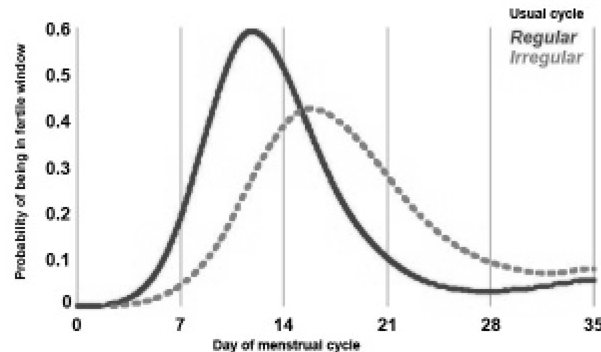


Fig: Probability of women with regular or irregular cycles being in their fertile window.

Clinical guidelines have typically identified the "fertile window" between days 10 and 17 within the typical 28 day menstrual cycle. Fertile window occurred during a broad range of days in the menstrual cycle. between days 6 and 21 women had at minimum a 10% probability of being in their fertile window. Women cannot predict a sporadic late ovulation; 4 - 6% of women whose cycles had not yet resumed were potentially fertile in the fifth week of their cycle. Only about 30% of women is the fertile window entirely within the days of the menstrual cycle identified by clinical guidelines (between days 10 and 17) women should be advised that the timing of their fertile window can be highly unpredictable, even if their cycles are usually regular.

Fertilization Site

Fertilization usually occurs in first 1/3 of oviduct . Fertilization can also occur outside oviduct, associated with *In Vitro* Fertilization (IVF, GIFT, ZIFT...) and ectopic pregnancy. The majority of fertilized eggs do not go on to form an embryo.

Fertilization - Spermatozoa

Sperm Binding - zona pellucida protein ZP2 acts as receptor for sperm.

Acrosome Reaction - exocytosis of acrosome contents (Calcium mediated), enzymes to digest the zona pellucida.

exposes sperm surface proteins to bind ZP2.

Membrane Fusion - between sperm and egg, allows sperm nuclei passage into egg cytoplasm.

Contact between spermatozoa and oocyte egg coat (zona pellucida [ZP]) glycoproteins triggers increases in intracellular calcium ion (iCa^{2+}) concentration in spermatozoa.

CATSPER channels on the distal portion of sperm (the principal piece) are required for the ZP-induced iCa^{2+} increases.

iCa^{2+} increase starts from the spermatozoa tail and propagates toward the head.

Store depletion-activated Ca^{2+} entry is thought to mediate the sustained phase.

Capacitation –final step of sperm maturation- changes in acrosome, preparing the enzyme release (in female genital tract), changes in sperm membrane, Sperm attraction and hyperactivation.

Acrosome reaction – fusion of the acrosome with plasma membrane, extension of the acrosomal process.

Fertilization - Oocyte

Membrane Depolarization - in non-mammalian species, caused by sperm membrane fusion, acts as a primary block to polyspermy.

Cortical Reaction - IP3 pathway elevates intracellular Calcium, exocytosis of cortical granules, enzyme alters ZP2 so it will no longer bind sperm plasma membrane.

Meiosis 2 - completion of 2nd meiotic division

forms second polar body (a third polar body may be formed by meiotic division of the first polar body)

Formation of the Zygote

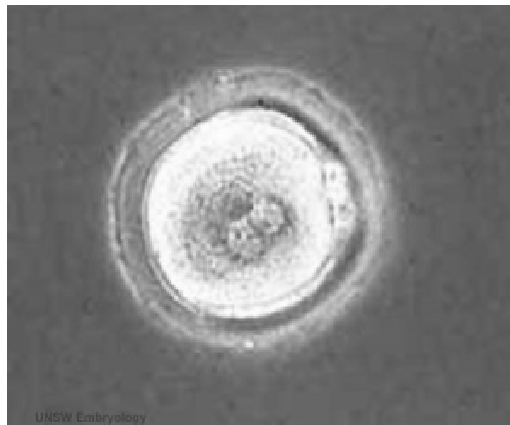


Fig: Early human zygote showing Pronuclei

Pronuclei - Male and Female haploid nuclei approach each other and nuclear membranes break down

chromosomal pairing, DNA replicates, first mitotic division

Spermatozoa contributes - centriole which organizes mitotic spindle

Oocyte contributes - mitochondria (maternally inherited)

Sex Determination

based upon whether an X or Y carrying sperm has fertilized the egg, should be 1.0 sex ratio, actually 1.05 i.e. 105 males for every 100 females, some studies show more males 2+ days after ovulation, cell totipotent (equivalent to a stem cell, can form any tissue of the body).

Men - Y Chromosome-Y Chromosome carries Sry gene, protein product activates pathway for male gonad (covered in genital development).

Women - X Chromosome-Gene dosage, one X chromosome in each female embryo cell has to be inactivated, process is apparently random and therefore 50% of cells have father's X, 50% have mother's X, note that because men only have 1 X chromosome, if abnormal, this leads to X-linked diseases more common in male that female where bothe X's need to be abnormal.

Fertilization Protein Changes

A recent study in mice has shown that after fertilization the maternal proteins present in the original oocyte are quickly degraded by the zygote stage. MII oocytes have 185,643 different peptides while zygotes contain only 85,369 peptides.

MII oocyte **Zygote**

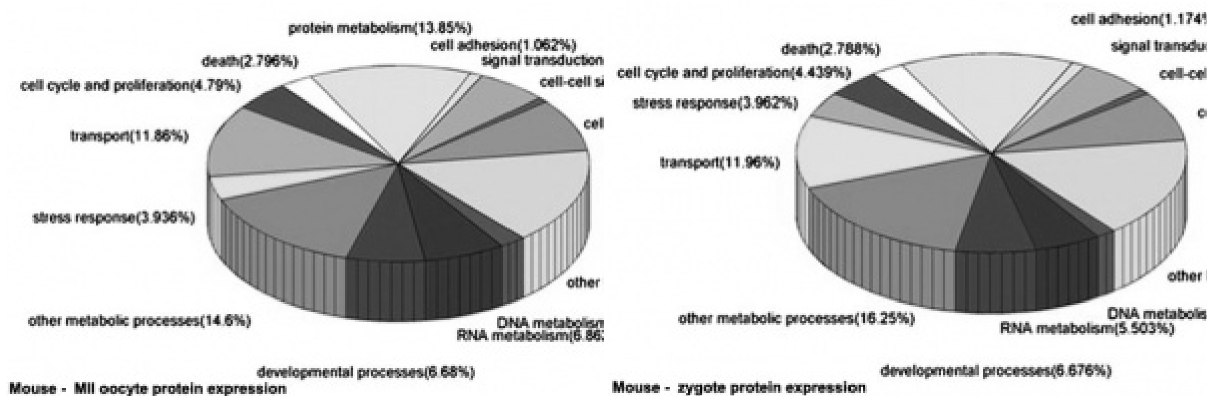


Figure. Protein Expression Classified by Molecular Functions

Prevention of polyspermy

Fertilization is an interaction between sperm and oocyte.

Spermatozoon binds to specific sperm receptor in the zona pellucida (ZP3). It induces release of enzymes from acrosome.

Penetration of the zona pellucida

Sperm and oocyte fuse

Cortical reaction – cortical granules release to perivitelline space (between oocyte and zona pellucida) – alteration of receptors for sperms – prevent polyspermy

Fast block of polyspermy – change the electrical potential

Slow block of polyspermy - cortical granules -enzymes – proteases – clip off binding receptor

Fertilization envelope formed – space between zona pellucida and egg - GAG, peroxidase, and hyalin – zona reaction

Probable Questions:

1. Give a schematic diagram showing analogies in the process of maturation of the ovum and the development of the spermatids.
2. Why only single sperm among many is able to fertilize the egg?
3. What is the role of proximal centriole of the spermatozoa?
4. What is spermiogenesis?
5. What is the chromosome number of primary and secondary spermatocytes?
6. What are Sertoli cells?
7. Which phase of spermatogenesis constitutes the meiotic division?
8. Explain with proper diagram the different phases of oogenesis.

Suggested Readings / References:

Developmental biology, 11th Edition; S. F. Gilbert

Anatomy and Physiology by Rice University; licensed under a Creative Commons Attribution 4.0 International License; OpenStrax, 2013.

Spermatogenesis physiology; Britannica.com

Oogenesis physiology; Britannica.com

Unit- IV

Axis specification in vertebrates:

i) Early patterning in vertebrates - Symmetry breaking, Nieuwkoop center. Wnt and cadherin signaling

ii) Left- right asymmetry in vertebrates - Asymmetric gene expression

Objective:

In this Unit we will discuss about the Axis specification in vertebrates:

i) Early patterning in vertebrates - Symmetry breaking, Nieuwkoop center. Wnt and cadherin signalling

ii) Left- right asymmetry in vertebrates - Asymmetric gene expression

Early patterning in vertebrates

In vertebrates, the development of the nervous system is triggered by signals from a powerful 'organizing' region of the early embryo during gastrulation. This phenomenon—neural induction—was originally discovered and given conceptual definition by experimental embryologists working with amphibian embryos. Work on the molecular circuitry underlying neural induction, also in the same model system, demonstrated that elimination of ongoing transforming growth factor-β (TGFβ) signalling in the ectoderm is the hallmark of anterior neural-fate acquisition. This observation is the basis of the 'default'model of neural induction. Endogenous neural inducers are secreted proteins that act to inhibit TGFβ ligands in the dorsal ectoderm. In the ventral ectoderm, where the signalling ligands escape the inhibitors, a non-neural fate is induced. Inhibition of the TGFβ pathway has now been demonstrated to be sufficient to directly induce neural fate in mammalian embryos as well as pluripotentmouse and human embryonic stem cells. Hence the molecular process that delineates neural from non-neural ectoderm is conserved across a broad range of organisms in the evolutionary tree. The availability of embryonic stem cells from mouse, primates, and humans will facilitate further understanding of the role of signaling pathways and their downstream mediators in neural induction in vertebrate embryos.

Establishment of the neuroectoderm in vertebrates:

In all vertebrates, the fertilized egg divides to generate a blastocyst (or blastula). Three different territories called embryonic germ layers, ectoderm, mesoderm, and endoderm, emerge in the blastula. In the amphibian embryo, where the dorsal (D) and ventral (V) sides of the embryo are specified during fertilization, each germ layer has a distinct D–V polarity and is fated to generate different tissues as the embryo matures. Subsequently during gastrulation, the primitive ectoderm (called epiblast) covers the outside of the embryo and forms different tissue derivatives depending on position along the embryonic D–V axis. The central nervous system (CNS) derives from the most dorsal region of the ectoderm, which thickens and flattens after gastrulation to form the neural plate. During subsequent stages, the plate rolls into a tube, separates from the overlying epidermis, and goes on to form the brain at the anterior, and spinal cord at the posterior end. In contrast, on the ventral side, most of the remaining ectoderm forms the epidermis. The neural crest forms where the dorsal and ventral boundaries meet at the edge of the neural plate. This progenitor cell population detaches and migrates throughout the embryo to form the peripheral nervous system, cranium, and cartilage of branchial arches. Ectodermal cells at the most anterior edge of the neural–epidermal boundary give rise to placodal areas that will form sensory organs—such as the ear and nose—as well as some cranial sensory ganglia (Figure 1). At the start of gastrulation, cells from any part of the ectoderm can still develop as either epidermis or neural tissue, but by the end of gastrulation commitment has occurred. These events are characteristic of all vertebrates although the timing and geometry vary across phylogeny. Thus, the first step in the establishment of the nervous system invertebrates involves the partition of the ectoderm into epidermal and neuroectodermal primordia during gastrulation.

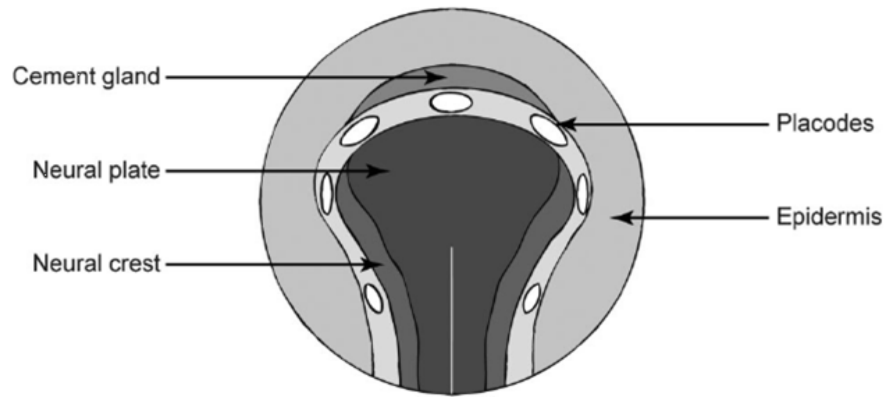


Figure 1: Fate map of the anterior border of the neural plate in *Xenopus* embryos. Schematic of dorsal–anterior (head-on) view of a *Xenopus* neurula (the ventral side is up, and the dorsal side is down). Different colors highlight different fates.

Symmetry breakage in the vertebrate embryo

Asymmetric development of the vertebrate embryo has fascinated embryologists for over a century. Much has been learned since the asymmetric Nodal signalling cascade in the left lateral plate mesoderm was detected, and began to be unravelled over the past decade or two. When and how symmetry is initially broken, however, has remained a matter of debate. Two essentially mutually exclusive models prevail. Cilia-driven leftward flow of extracellular fluids occurs in mammalian, fish and amphibian embryos. A great deal of experimental evidence indicates that this flow is indeed required for symmetry breaking. An alternative model has argued, however, that flow simply acts as an amplification step for early asymmetric cues generated by ion flux during the first cleavage divisions. In this review, we critically evaluate the experimental basis of both models. Although a number of open questions persist, the available evidence is best compatible with flow-based symmetry breakage as the archetypical mode of symmetry breakage.

Establishment of left–right asymmetry of animal body plans is of the utmost importance for embryonic development and adult health. During vertebrate embryogenesis, the cardiovascular system, the organs of the chest and abdomen, and even the brain, develop morphological and/or functional asymmetries. Developmental defects in laterality

specification and asymmetric morphogenesis are sometimes compatible with embryogenesis, and occasionally, fully mirror-image individuals develop to term. Left–right (L–R) defects are often much less pervasive and usually strike organs at random, resulting in severe visceral misalignment, organ malformations and malfunctions. Asymmetric organ morphogenesis is preceded by an asymmetric signalling cascade, which initiates during neurulation in the left lateral plate mesoderm (LPM). This so-called Nodal cascade consists of the TGFβ-type growth factor Nodal, its secreted feedback repressor Lefty (also known as Antivin) and the homeodomain transcription factor Pitx2. Expression of this cascade is both necessary and sufficient to induce the correct asymmetric placement of organs.

How the Nodal cascade becomes asymmetrically expressed constitutes a conceptual cell-biological problem, because zygotes typically lack recognizable morphological or functional asymmetries that could initiate it. Brown and Wolpert (1990) proposed the concept of an intrinsic biochemical-structural chirality (represented in their model by an "F-molecule"), even though uniformly distributed, such a molecule would operate by undergoing chiral alignment against the A–P and D–V axes of the embryo. Then other molecular interactions feeding off the deduced L–R vector would eventually lead (via an unknown number of steps) to the morphogenetic process of asymmetric organ development. Amongst the animals classified as the bilateria, two cytoskeleton-dependent chirality have been identified, whose mechanism of action fulfill the conceptual nature of the F-molecule hypothesis (though not being single molecules in the initial invocation of the model). Interestingly, although expressed at two different developmental stages, both of these instances result in asymmetric activation of the Nodal pathway. In spirally cleaving snail embryos, asymmetric positioning of the spindle apparatus during cleavage induces Nodal asymmetry, ostensibly by repositioning maternally synthesized factors. In embryos of most vertebrates, including fish, frogs and mammals, chiral rotation of cilia polarized to the posterior pole of cells produces a vectorial leftward flow of extracellular fluids. This flow is necessary and sufficient for Nodal-dependent symmetry breakage, and substantial enough to sweep fluorescent latex microbeads across from one side of the ciliated epithelium to the other. Although both mechanisms lead to asymmetric Nodal activity, they seem to have little else in common, raising the question as

to why and how flow-type symmetry breakage has evolved. We have recently addressed this problem in a hypothesis article, and will not repeat this issue here. One major difference between the two strategies is that one (spiral cleavage in spiralian protostomes) is determined maternally and acts during very early cleavage stages, while the other (leftward flow) operates much later, and depends on zygotic gene expression during neurula stages.

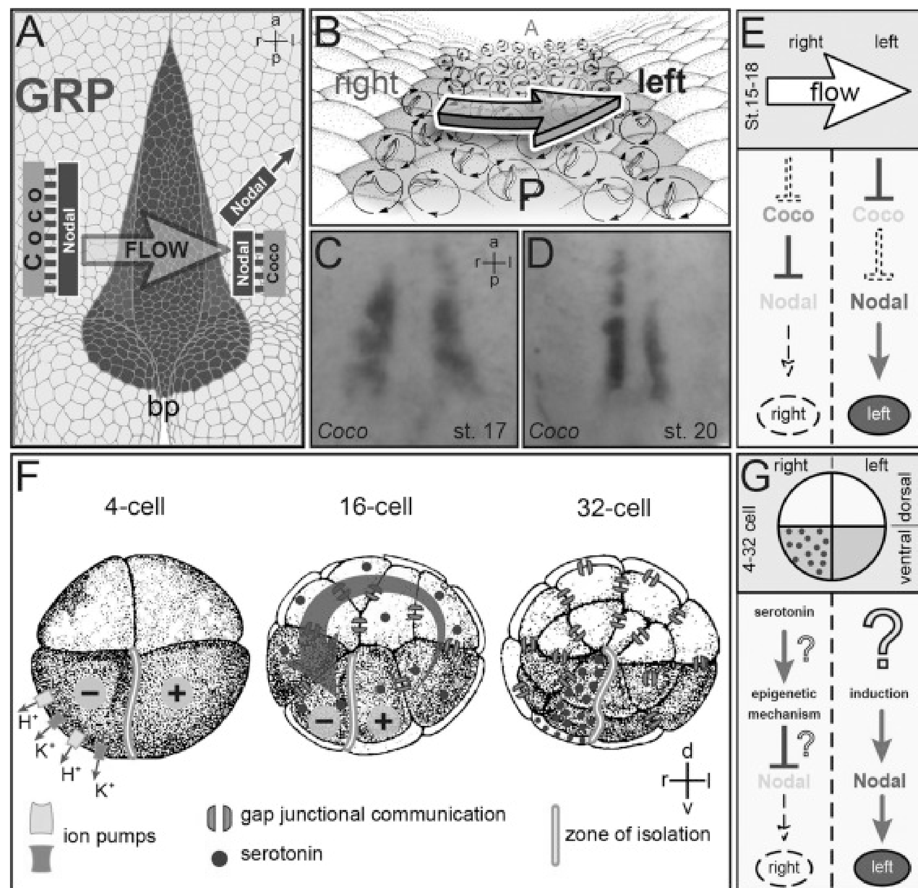


Figure. Prevailing models of symmetry breakage in the frog Xenopus. (A–E) Leftward flow. (A) Schematic representation of a stage 17 archenteron roof in ventral perspective. Flow occurs from the right to the left side of the ciliated gastrocoel roof plate (GRP; red). Nodal and Coco are co-expressed at the lateral GRP margins on both sides (purple). Flow represses Coco, activating Nodal by release of repression. bp, blastopore. (B) GRP at higher magnification. Polarized and flow-producing cilia at the GRP center are bordered by Nodal/Coco-positive cells (purple) which harbor unpolarized, sensory cilia. (C and D) Coco expression during (C) and following (D) leftward flow. Note the decrease in signal intensity on the left at post-flow stage 20 (D). (E) Schematic depiction of events on the left and right

side leading up to asymmetric Nodal cascade induction in the left lateral plate mesoderm (LPM). (F and G) Ion-flux. (F) Asymmetrically expressed ion pumps create a voltage gradient in the 4-cell embryo which initiates the electrogenic transfer of serotonin through gap junctional communication to the ventral-right lineage at the 32-cell stage. Serotonin accumulates in this lineage because the ventral midline is devoid of GJC. (F) Schematic depiction of events on the left and right side leading up to asymmetric Nodal cascade induction in the left LPM. Question marks indicate unproven interactions and mechanisms.

Although most of the vertebrates examined so far utilize cilia-generated flow to initiate the asymmetric Nodal cascade, two alternative strategies have also been observed. First, in the chick, large-scale whole-cell repositioning during gastrulation results in a significant asymmetry in the morphology of Hensen′s node, and this appears to play a role in the asymmetric expression of specific intercellular signalling molecules. Second, in amphibians, asymmetric localization of determinants has been proposed to act during early cleavage stages. According to this view, cytoskeletal motor proteins asymmetrically transport a maternal deposit of the ion pump ATP4 (as mRNA and/or translated protein), changing its distribution from a symmetric to an asymmetric one. This asymmetry is hypothesized to generate an intracellular pH and voltage gradient, along which the small charged monoamine, serotonin, transfers via gap junctional communication (GJC) to blastomeres on the right side of the cleavage stage embryo. Almost one day later, when the embryo consists of thousands of cells, this right-sided serotonin asymmetry, by an unknown epigenetic mechanism, is proposed to repress Nodal activity on the right side, thereby initiating the left-asymmetric activation of the Nodal cascade. This mechanism for symmetry breakage will be referred to herein as the “ion-flux” model. Consistent with this model, asymmetries in serotonin, ATP4 and ATP6 were reported in early cleavage stage embryos. In addition, blockage of GJC or mild interference with cytoskeletal dynamics reportedly disrupts L–R development.

When cilia-driven leftward flow was found in the neurula of the *Xenopus* embryo, as observed previously in mouse, rabbit, zebrafish and medaka, the question arose as to which mechanism is principally instructive for breaking symmetry in the amphibian embryo. Advocates of the ion-flux model have suggested that, throughout the animal kingdom, symmetry breakage occurs very early, i.e. in the zygote or during the first two cell divisions, and that the function of cilia-driven fluid flow must therefore be restricted to a later-stage amplification step. Here, we present our view of the conceptual problems with the ion-flux model, and evaluate the salient experimental support for each of the two opposing models.

For detailed reviews on other aspects of the two models we refer to recent comprehensive reviews.

Embryological considerations: Spemann′s organizer and left–right asymmetry

A mechanism that breaks symmetry during early cleavage stages is likely to be independent of L–R orientational cues that derive from the gastrula, or Spemann′s organizer. In contrast, a mechanism that operates during or after gastrulation is likely to be strongly influenced by, or even be mandatorily dependent on, the organizer. Experimental analysis of organizer function on L–R asymmetry should thus provide an answer as to when symmetry is broken.

Spemann′s and the left–right organizer: how are they related?

In frogs, symmetry breakage via cilia-driven leftward flow is intrinsically tied to Spemann′s organizer. The ciliated gastrocoel roofplate (GRP), where leftward flow develops during neurulation, is derived from the superficial mesoderm (SM) of the gastrula. The SM constitutes the superficial cell layer of a region that sits above Spemann′s organizer during early gastrulation (Fig. 2A and B). It is thus sandwiched between prospective neuroectoderm and the more vegetally located epithelial layer of the organizer. In the early gastrula, the SM expresses foxj1, the main control gene for motile cilia. In addition, both the organizer and the central part of the GRP contain cells with eventual notochordal fate, reflecting their close relationship. Ciliated, flow-generating epithelia in other vertebrates, despite their common function, display a wide morphological variety. In this review they will be referred to as left–right organizers (LRO). In rabbit and frog, for example, the LRO develops as a flattened epithelial plate, while it appears as a concavity in the mouse, as a raised dome in medaka, and as a completely enclosed, hollow vesicle in zebrafish.

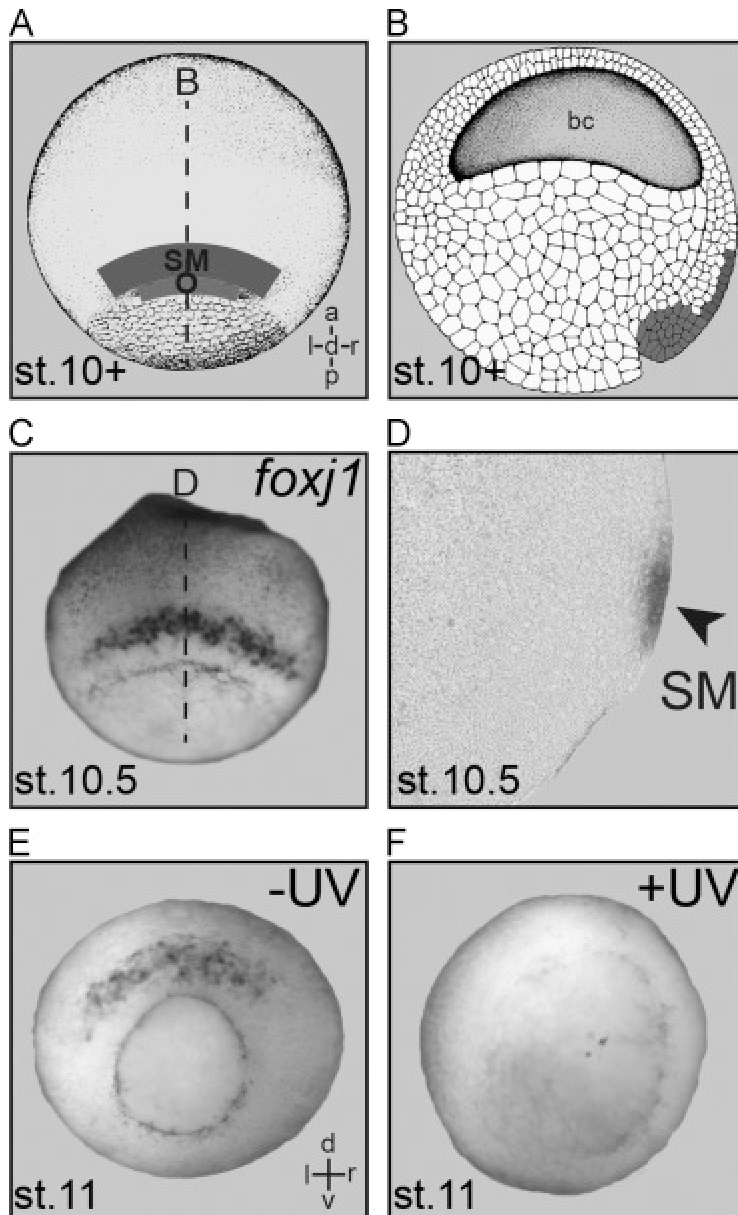


Fig. 2. Structural and functional relationship of Spemann's organizer and superficial mesoderm. (A) Schematic depiction of superficial mesoderm (SM; red) and organizer (O; green) in whole-mount stage 10^+ gastrula embryo shown in dorsal view. (B) Arrangement of organizer and SM in a sagittal section. (C) SM *foxj1* expression in a whole-mount gastrula embryo. (D) Sagittal section (plane indicated by dashed line in C) demonstrates *foxj1* mRNA in the SM (arrowhead). (E and F) Loss of SM *foxj1* expression (E) in UV-ventralized gastrula embryo (F), demonstrating the dependence of SM specification on organizer function.

Nieuwkoop Center

Nieuwkoop Center is the Primary Organizer forming centre seen in the developing egg of some Amphibians. In *Xenopus*, the Nieuwkoop Center is the dorsal- and vegetal-most region. It gives rise to the Primary Organizer called Spemann-Mangold Organizer which is the region known as the dorsal lip of the blastopore (DLB). Spemann and Mangold's experiments found that the DLB dorsalizes surrounding tissue, thus forming the dorsal-ventral axis. In addition to dorsalizing surrounding tissue, the Primary Organizer fates overlying ectoderm as neural

plate tissue; and is determined to be notochord tissue. Dorsalized tissue gives rise to somites and pronephric tubules. Spemann- Mangold Experiment revealed that

1. A transplanted vegetal dorsal cell (from the DLB) induces a new axis.

2. A transplanted vegetal dorsal cell does not itself give rise to new dorsal tissues.

3. A transplanted vegetal dorsal cell restores other cells to correct fates.

The DLB uses induction via secreted diffusible signals. A cell that can be induced is competent; embryonic tissues are only competent during gastrulation. The use of diffusible substances was proven when dorsal lip tissue and ectoderm were cultured together, but separated by a filter with a 0.5μm pore; the ectoderm was induced into neural tissue, despite no cell processes seen to pass through the filter.

The importance of the Nieuwkoop Centre in development is

1.The Nieuwkoop Centre sets up D/V polarity in the blastula and is essential for normal development.

2. The 1st cleavage cuts through the site of sperm entry and the Nieuwkoop Centre.

3. The 2nd cleavage splits the embryo into 4 cells.

Wnt Signalling and Cadherins Compete for beta-Catenin

The concept that canonical Wnt signalling and cadherin-mediated cell adhesion depend on the same pool of β-catenin is based on genetic and overexpression experiments in embryos and cultured cells. Heasman et al. showed in 1994 that overexpression of cadherins in *Xenopus* embryos inhibited dorsal axis formation, which is a clear function of canonical Wnt signalling. Peifer and collaborators showed that armadillo (beta-catenin) mutant embryos of Drosophila, which harbor only one E-cadherin allele, showed a less severe segment polarity phenotype than embryos with two cadherin alleles. Segment polarity is controlled by Wnt signalling. Similarly, cadherin overexpression mimicked the wingless (Wnt) phenotype in Drosophila embryos. These data from the mid 1990s are thus consistent with a model in which there is crosstalk between β-catenin in two different compartments, the adhesion complex at the plasma membrane and a signalling complex in the nucleus. Shortly after these findings in model organisms, Geiger and Ben-Ze'ev and collaborators showed an interplay between cadherin mediated cell adhesion and canonical Wnt signalling in cell culture

experiments. In colon cancer cells, expression of N-cadherin or an interleukin receptor-cadherin hybrid (in which the β-catenin binding region of N-cadherin was maintained) triggered a relocation of b-catenin from the nucleus to the plasma membrane and inhibited LEF1-mediated transcription. Inducible expression of the Fos proto-oncogene in mammary gland epithelial cells resulted in the loss of E-cadherin and cell polarization, the colocalization of b-catenin with LEF1 in the nucleus, and increased Wnt/b-catenin signalling. Moreover, the absence of E-cadherin in E-cadherin -/- embryonic stem (ES) cells led to an accumulation of b-catenin with LEF1 in the nucleus and activation of a Wnt reporter. This could be antagonized by expression of E-cadherin. Behrens and collaborators have recently shown that siRNA-mediated knockdown of E-cadherin augments b-catenin-dependent transcription in colon cancer cells in which the Wnt pathway is active. On the other hand, the same procedure has no effect in nontransformed keratinocytes that do not display Wnt signalling. These data indicate that the mere loss of E-cadherin does not activate Wnt signalling—except in cases in which the b-catenin degradation machinery is compromised. These results are consistent with data from breast cell cancer lines showing that the absence of E-cadherin alone does not result in activation of Wnt sigalling. Nor does a loss of E-cadherin function in Rip1Tag2 transgenic mice contribute to Wnt/b-catenin sigalling. Weinberg and collaborators recently showed that in rastransformed mammary gland cells (HMLER), shRNA down-regulation of E-cadherin results in translocation of β-catenin from cell–cell junctions to the cytoplasm and nucleus. This type of β-catenin was nonphosphorylated and thus was not targeted for ubiquitination and degradation. However, in this system, the loss of E-cadherin affected numerous other sigalling pathways that have been implicated in metastasis formation. Gottardi and Gumbiner have performed precise studies to determine what controls b-catenin targeting to cadherin adhesion or to TCF transcriptional complexes. They showed that Wnt sigalling generates a monomeric, intramolecularly folded-back form of b-catenin that binds TCF but not cadherins. In contrast, the cadherin binding form of b-catenin builds a dimer with a-catenin. X-ray crystallographic studies have shown that cadherin-binding involves all 12 armadillo repeats of b-catenin, whereas TCF binding requires only the central eight repeats. Thus, it is possible that the carboxyl terminus of Wnt-produced b-catenin folds back over armadillo repeats, affecting binding to cadherin but not TCF. The selective binding of b-catenin induced by Wnt could also involve posttranslational

modifications or the activation of further proteins. Overall, these data suggest that b-catenin's selectivity between adhesion and transcription are not always coupled; in other words, they might be regulated independently. BCL9 protein, the product of a human proto-oncogene, also acts in the switch between cadherin cell adhesion and b-catenin signalling. This story has been worked out through work on BCL9 and its ortholog legless, a Drosophila segment polarity gene. Legless, which was isolated in 2002 by the group of Konrad Basler, is required for Wnt signalling in the fly. It acts by binding directly to b-catenin. Human BCL9 was discovered in a B-cell lymphoma because of a translocation to the immunoglobulin locus, which caused BCL9 overexpression in the tumors. Remarkably, human BCL9 could rescue the segment polarity phenotype of the legless mutation, indicating functional identity. Vertebrates have a second homolog BCL9-2, which also binds to b-catenin like BCL9. BCL9-2 promotes nuclear location of bcatenin, increased b-catenin signalling, and triggers EMT in vertebrate cells and Zebra fish embryos. BCL9-2 cannot colocalize with the E-cadherin/bcatenin/a-catenin complex at the plasmamembrane, but following tyrosine phosphorylation of b-catenin, it is translocated to the nucleus and promotes b-catenin signalling. Thus BCL9 proteins may act in the switch between cadherin-mediated cell adhesion and Wnt signalling.

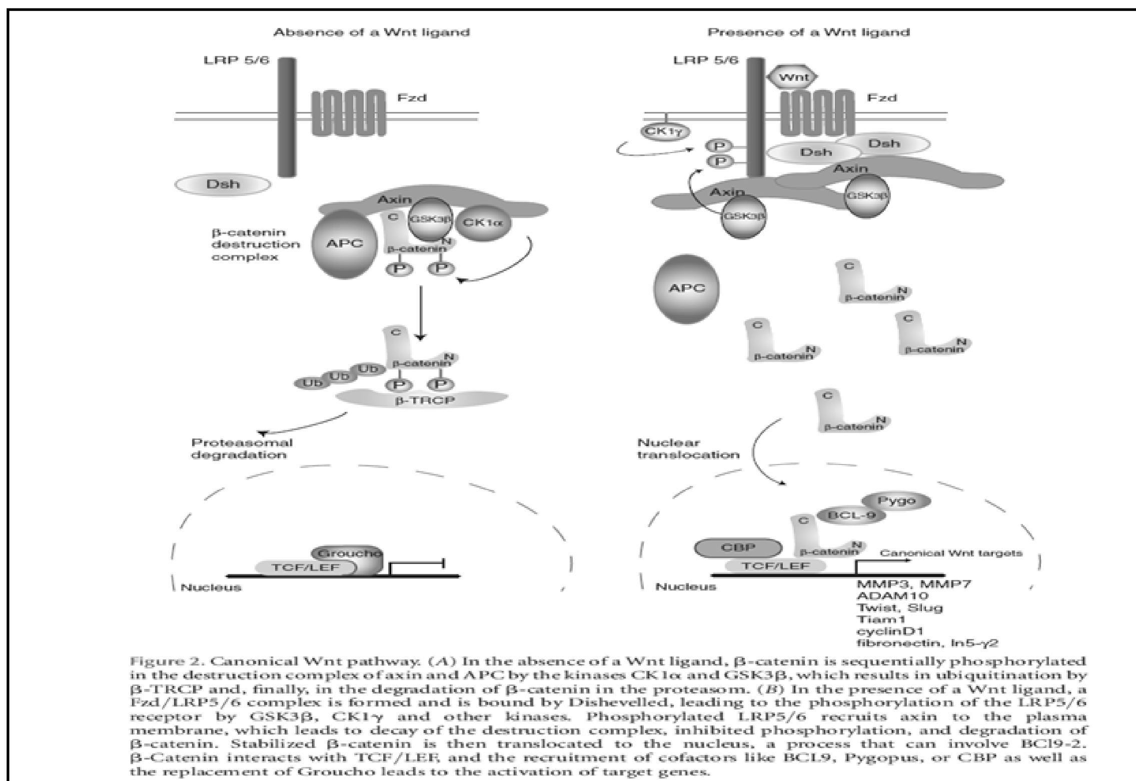


Figure 2. Canonical Wnt pathway. (*A*) In the absence of a Wnt ligand, β-catenin is sequentially phosphorylated in the destruction complex of axin and APC by the kinases CK1α and GSK3β, which results in ubiquitination by β-TRCP and, finally, in the degradation of β-catenin in the proteasom. (*B*) In the presence of a Wnt ligand, a Fzd/LRP5/6 complex is formed and is bound by Dishevelled, leading to the phosphorylation of the LRP5/6 receptor by GSK3β, CK1γ and other kinases. Phosphorylated LRP5/6 recruits axin to the plasma membrane, which leads to decay of the destruction complex, inhibited phosphorylation, and degradation of β-catenin. Stabilized β-catenin is then translocated to the nucleus, a process that can involve BCl9-2. β-Catenin interacts with TCF/LEF, and the recruitment of cofactors like BCL9, Pygopus, or CBP as well as the replacement of Groucho leads to the activation of target genes.

Proteolysis of Cadherins Affects Wnt Sigalling

Competition for b-catenin is one mechanism by which cells can modulate Wnt signals. Another is the cleavage of E- and N-cadherin by proteases, which can lead to the release of b-catenin and to up-regulated b-catenin signalling. This occurs in normal development but also in processes such as wound healing, Ca2þ-influx, and apoptosis. Cadherins are cleaved intracellularly by proteases like caspase 3 or presenilin, or extracellularily by ADAM10. Intracellular cleavage of cadherins releases nearly the entire cytoplasmic domain, whereas extracellular cleavage releases the adhesion domain (Fig. 3). ADAM10 belongs to the family of disintegrins and metalloproteases (ADAM), type I transmembrane proteins that combine cell–cell adhesion and proteinase activity. ADAM10 was recently identified as a b-catenin/TFC target gene. The group of Saftig has shown that cleavage of E- and N-cadherin by ADAM10 in keratinocytes and neuronal cells, respectively, reduced cell adhesion, increased cell migration and led to translocation of b-catenin to the nucleus. Translocated b-catenin activated Wnt/b-catenin target genes like c-Myc and cyclinD1. These are clear examples of an immediate crosstalk between a loss of cadherin-mediated cell adhesion and Wnt/b-catenin signalling. Presenilin1 (PS1) is an integral membraneprotein and a component of the g-secretase complex, which mediates an 1-cleavage of type I membrane proteins such as the amyloid precursor protein (APP). A mutant form of PS1 (PS1 FAD, familial Alzheimer's disease) is known to process APP, leading to increased production of the b-amyloid peptide (Ab42), which is responsible for Alzheimer's disease. Mechanistically, PS1 acts in several ways to influence cell adhesion and transcription. PS1 can bind to E-cadherin and b-catenin and promotes their association to the cytoskeleton, and the overexpression of PS1 results in enhanced cadherin-mediated cell adhesion. Under imbalanced calcium conditions or apoptosis, PS1 does not stabilize cell–cell adhesion but cleaves E-cadherin, which leads to the disassembly of adherens junctions, to the release of a cytoplasmic E-cadherin fragment, and to an increase of soluble b-catenin. It is possible that the release of b-catenin from adherens junctions caused by PS-1 modulates gene expression. It has been shown that the released N-cadherin fragments generated by PS1 favour nuclear localization of b-catenin and promote b-catenin signalling. The released fragments of E-cadherin also translocate to the nucleus and affect transcriptional activity.

Probable Questions:

1. Describe the process of Early patterning in vertebrates with special reference to symmetry breaking.
2. What is Nieuwkoop center ?
3. Elucidate the mechanism of Wnt and cadherin sigalling in Axis specification in vertebrates.

Suggested Readings/ References:

1. Heuberger, J., Birchmeier, W., 2009. Interplay of Cadherin-Mediated Cell Adhesion and Canonical Wnt Signalling. Cold Spring Harbor Perspectives in Biology 2, a002915-a002915.
2. Ozair MZ, Kintner C, Brivanlou AH. 2013. Neural induction and early patterning in vertebrates. Wiley Interdiscip Rev Dev Biol. 2(4):479-98.
3. Blum, M., Schweickert, A., Vick, P., Wright, C., Danilchik, M., 2014. Symmetry breakage in the vertebrate embryo: When does it happen and how does it work?. Developmental Biology 393, 109-123.

Unit-V

Metamorphosis and organogenesis: Axes, compartment formation and pattern formation in *Drosophila;* Homeobox genes and development; development and metamorphosis of tadpole larve; limb development and regeneration in vertebrates

Objective:

In this Unit we will discuss about the Metamorphosis and organogenesis: Axes, compartment formation and pattern formation in *Drosophila;* Homeobox genes and development; development and metamorphosis of tadpole larve; limb development and regeneration in vertebrates.

Subject-Matter of Cellular Differentiation:

Differentiation is the process by which the genes are preferentially active and the gene products are utilised to bring some phenotypic changes in the cell. It is not the only property of multicellular organisms. Many unicellular organisms undergo phenotypic changes along with changes in physiological processes.

Any change in the environment of unicellular organisms whether—physical or at the nutrient level—can undergo remarkable physical cellular changes like the formation of different types of spores, sporulation in bacteria, fungi etc.

These are the example of cellular differentiation in unicellular organisms. The differentiation observed in higher organisms, particularly animals, is different and complicated. It has attracted developmental bi-ologists to study the development of an embryo from a single cell, i.e., zygote.

This process takes place in several steps:

i. Fertilisation: Fusion of sperm and egg.

ii. Cleavage: Development of zygote to form blastula (group of undifferentiated cells).

iii. Gastrulation: Differentiation and movement of cells to form specialised cell layers.

iv. Differentiation: Development of specialised cells into tissues, organs and growth of the embryo.

v. Growth, Maintenance and Regeneration of some cells.

Molecular Changes During Oogenesis and Fertilisation:

The process of female gamete formation is known as oogenesis. The primordial germ cells are called oogonia. When the oogonia start meiosis they are called oocytes; where an exten-sive growth phase occurs.

During this growth phase of oocyte, large number of metabolic and morphological changes occur.

These are:

i. Increase of RNA synthesis—along with polytene-like changes, are found in the chromosome.

ii. Amplification of Ribosomal genes takes place leading to the increased synthesis of ribosomal RNA. Associated cytological changes are the occurrence of many nucle-oli in the nucleus.

iii. Size of the nucleus is increased indicating the metabolic changes of the nucleus.

iv. Accumulation of protein, lipid and carbohydrate takes place which will be utilised during the formation of embryo. In higher animals all those nutrients are produced by the liver and follicle cells of the developing oocyte. However, mammalian oocytes do not accumulate yolk proteins as they come through mother’s bloodstream and thus storage of nutrients is not necessary here.

v. In non-mammalian oocyte the asymmetry in polarity is found during its development. One end of the cell is called the Vegetal pole containing most of the nutrients and yolk platelets. The other end is called the Animal pole containing ribosomes and mitochondria besides nucleus.

The embryo is developed at this end. After these developmental changes, the meiosis takes place in the oocyte to produce mature egg which is a highly differentiated specialised cell.

The Spermatogenesis—Spermatogonia:

increase their number by mitosis and then undergoes meiosis to form sperms. Cellular and other molecular changes are not so significant like oogenesis. Considerable changes are found after fertilisation during the development of embryo.

Role of Cytoplasm in Cellular Differentiation:

The importance of cytoplasm on cell differentiation has been demonstrated in large number of experiments.

The egg of snail produces a lobe-like structure at the vegetal end during cell differentiation which is known as Polar lobe. If this lobe is excised, defective embryo is produced. Again, a coloured area is produced in the amphibian egg during cell differentiation after fertilisation. This is known as Grey Crescent. If the grey crescent is injured it induces abnormality in the nervous system.

In case of amphibian egg, if the first cleavage is perpendicular to the grey crescent and the resulting blastomere is separated, each blastomere will produce a normal animal. But if the cleavage takes place parallel to the grey crescent and if the two blastomeres are separated—the one having grey crescent will produce normal animal. Thus the differentiation of cell depends on the partitioning of substances in the cytoplasm.

Another observation has been made in case of the embryonic development of egg of the round worm, *Ascaris*. During the development of embryo, the first cleavage occur perpendicular to the animal vegetal axis.

When the animal pole of the new blastomere starts dividing, the heterochromatic portion of the chromosome becomes degenerated. The euchromatic portion of the chromosome is fragmented into numerous small chromosomes by a process known as chromosome diminution.

Thus chromosome diminution occurs during embryonic develop-ment. Now Theodor Boveri made an exper-iment in which eggs are centrifuged before cleavage in order to disturb the polarity of the cell.

By centrifugation, the mixture of both animal and vegetal cytoplasm occurs and the first cleavage occurs along the animal vegetal axis not perpendicular to it. No chromosome diminution occurs—this indicates the role of cytoplasm in chromosomal behaviour.

Again, in case of embryonic development of Drosophila, the primordial germ cells generally arise from the posterior end of the egg. Illmensee and Mahowald made an experiment by removing some cytoplasm from the posterior end of the egg of Drosophila and injected then into the anterior end of another egg. It has been noted that germ ceils are then produced from the anterior end of the injected egg.

Thus it can be said that the cytoplasm has an important role in inducing differentiation in cells. Its role has also been noted in adult cells. When the inactive nuclei of mature erythrocytes are injected into the cytoplasm of active cells, the inactive nuclei become transcriptionally active.

Similar effect has also been noted in other types of cells. All these results clearly reveal that cytoplasmic factors are responsible for cell differentiation. But in case of mammals, embryonic cells are totipotent after the first cleavage division till the development of the embryo.

This has been evidenced by segregating mouse or rabbit embryo at 8 or 16 cells stage. Each blastomere gives rise to blastocyst if the proper cultural conditions are provided and these blastocysts will form normal animal after implantation into the uterus of another female rat. This property of totipotency of egg cell is due to the homogeneity of its cytoplasm.

The retention of totipotency by an individual cell has been demonstrated in case of plant systems. Any cell from any part of the plant can be grown in culture where they form at first the mass of undifferentiated tissue, known as callus.

This callus can be regenerated into a whole plant if the proper concentration of the hormone is supplemented in the media. But single animal cells after the first cleavage division will not form full animal even if the proper nutrients and other conditions are given in culture.

Genetic totipotency of animal cell has been demonstrated by John Gurdon in the nuclear transplantation experiment in toad; showing that each and every cell possesses all the genes required for the development of the whole organism.

Still the development or differentiation into whole organism has not been in animal system. Thus researchers thought that the pattern of gene expression in each cell type is different, although all the genes are present in each cell. So we see that the control of gene expression in higher organisms, particularly animals, is very complicated.

In other words, many types of post-transcriptional modifications of the primary transcript of the gene are regulated in the formation of the final functional gene product necessary for a particular cell differentiation or development.

Two model organisms are used for the study of the specific genes involved for a particular cellular development. One is the round-worm, *Caenorhabditis elegans*, and another is *Drosophila, C. elegans*.

These are used as a model in the developmental genetics for the following reasons:

i. Short life cycle (3 days).

ii. Ease of maintenance like *E. coli*.

iii. Can reproduce by self or by cross- fertilization.

iv. Hermaphrodite (XX)—contains 5 pairs of autosomes and 1 pair of X chromosomes.

v. The male (XO) contains 5 pairs of auto-somes and a single X chromosome.

vi. Haploid genome is about 8 x 107 bp.

vii. More than 600 genes have been identified.

viii. Easy to obtain homozygous populations, as self-fertilization is possible.

ix. In-breeding is automatic in hermaphrodite population.

x. DNA transformation through microinjec-tion at the selected stage of development is possible in this animal.

The reproductive system of the hermaphrodite animal produces a bilobed structure in early stages of development. The oocytes and embryos in each lobe start developing from the distal

end to the uterus. All stages can be detected easily and thus microinjection of DNA can be done at the selected stage of development.

Another important work on *Drosophila* made the discovery of new class of genes called Homeotic genes. These genes were identified through mutations where one part of the body is replaced by a structure that is found somewhere else. This unusual thing occurs during development of the embryo. The mutations of homeotic gene (Antp) result in the formation of middle legs in place of antenna of *Drosophila*.

The molecular analysis of homeotic gene has resulted in the discovery of a 180bp sequence present in other homeotic genes. This sequence is known as Homeobox. Since the homeobox is present in higher animals also, gene cloning and sequencing has been done from mice to man. The function of these genes has been found to be the same as in *Drosophila*.

The homeobox genes are expressed in highly differentiated cells in case of Amphibians and Mammals. Homeobox genes are found to play very important role in developmental processes and they are highly conserved during evolution.

Homeotic Genes of Cellular Differentiation:

During embryonic development in *Drosophila*, the identification of different segments of the body has been found to be under the control of many genes. Two complexes of these genes have been identified in *Drosophila*.

One is Antennapedia Complex (ANT-C) located in the chromosomal position 84AB. Another group of genes is known as Bi-thorax complex (BX-C) located at chromosomal position 89E. The first group (ANT-C) is responsible for the develop-ment of the head and thoracic segments while the BX-C are responsible for the development of the trunk segments.

The analysis of this homeotic complex has been done in Beetle also. One of the important features of these genes is the large size—about 50 to 100 kb and very large introns. The next important feature is the presence of con-served region, i.e., Homeobox.

The homeobox generally codes for a DNA binding protein domain, whose product binds to DNA. Another important characteristic is the presence of cis- acting regulatory regions.

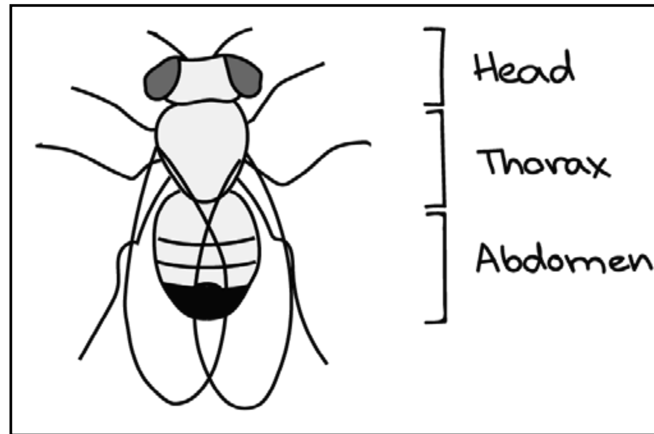
Cellular differentiation, pattern formation and morphogenesis were studied in detail in case of plant *Arabidiopsis* as model system. It involves some factors which help in causing different cell types, organs etc. to originate at specific locations. This is also done by cell shape changes and planes of cell division. Cell differentiation is clearly noted during embryo-genesis.

Different molecular observations were studied in maize and rice in case of monocotyledonous plants. In dicots, several genes involved in storage proteins have been cloned and their expressions noted in Soybean. However, detailed studies have been done in Crucifers, particularly in *Arabidiopsis thaliana*, in identifying embryo developmental genes but mutagenesis.

Homeotic genes:

Homeotic genes are master regulator genes that direct the development of particular body segments or structures. When homeotic genes are overactivated or inactivated by mutations, body structures may develop in the wrong place—sometimes dramatically so! Most animal homeotic genes encode transcription factor proteins that contain a region called the homeodomain and are called Hox genes. Hox genes are turned on by a cascade of regulatory genes; the proteins encoded by early genes regulate the expression of later genes. Hox genes are found in many animals, including fruit flies, mice, and humans. Mutations in human Hox genes can cause genetic disorders.

How many legs does a fruit fly have? Even if you're not particularly into fruit flies, you may know that insects tend to have six legs total—as compared to, say, the eight legs of spiders. Also, you may have noticed that a fly's legs usually grow out of the middle part of its body—its thorax—and not, say, out of its head.



What's responsible for this orderly organization of body parts in something as tiny as a fly? As it turns out, a set of master regulator genes are expressed in different regions of a fly's body during development. These genes turn on the right genetic "program" for development of each section of the body. They make sure, for example, that the fly's thorax carries legs while its head does not.

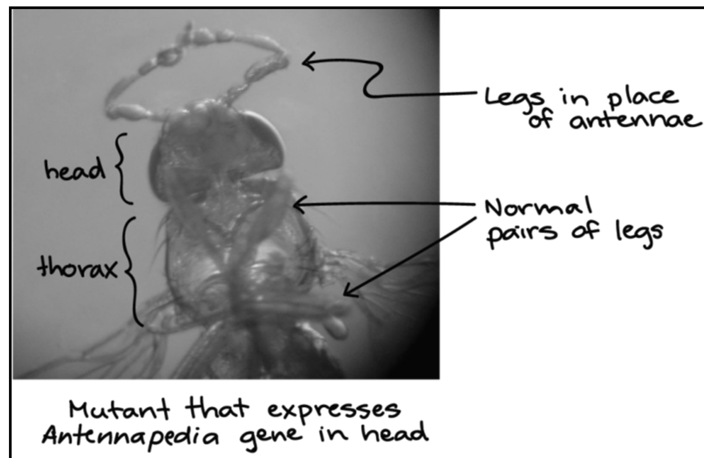
Homeotic mutations in fruit flies

Homeotic genes are responsible for determining the identity of particular segments or structures of the body. So, when homeotic genes are inactivated or expressed in unusual locations due to mutations, they may cause body segments to take on new—and sometimes startling!—identities.

As an example, let’s look at a homeotic gene called *Antennapedia*. Normally, *Antennapedia* is expressed in what will become the second segment of a fly's thorax, starting when the fly is a tiny embryo and persisting into the adult fly. There, the gene acts as a master regulator, turning on the genetic program that makes the fly's second pair of legs and other segment-specific structures.

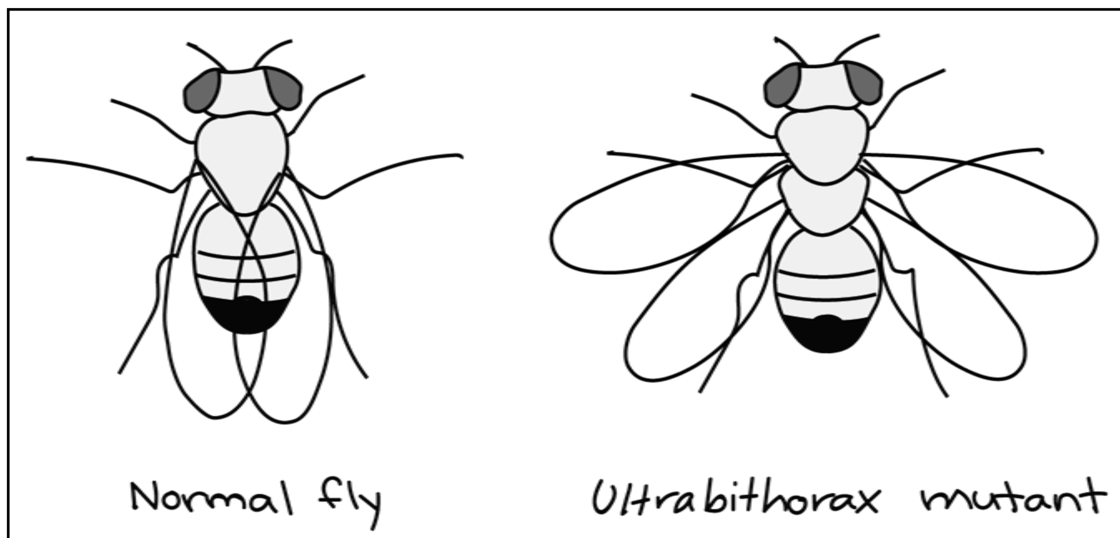
If *Antennapedia* stays where it's supposed to and does its job, we get a nice, normal-looking fly with all its appendages in the right place. But what happens if a genetic mutation causes

expression of the *Antennapedia* gene to expand into the fly's head? This type of mutation causes legs to grow from the fly's head in place of antennae! In other words, the gene activates its normal, second-segment leg development program, but in the wrong part of the fly.



Another fly homeotic gene with dramatic effects is the *Ultrabithorax* gene. This gene is expressed strongly in the third segment of the thorax, which bears the fly's rearmost pair of legs. *Ultrabithorax* expression in this region of the fly starts early in development and continues throughout the fly's life.

Wings usually form only in the second segment of the thorax, not in the third, which instead makes small structures called halteres that help the fly balance. The job of *Ultrabithorax* is to repress second-segment identity and formation of wings in the third segment. When *Ultrabithorax* is inactivated in the developing third segment due to mutations, the halteres will be converted to a second set of wings, neatly positioned behind the normal set.



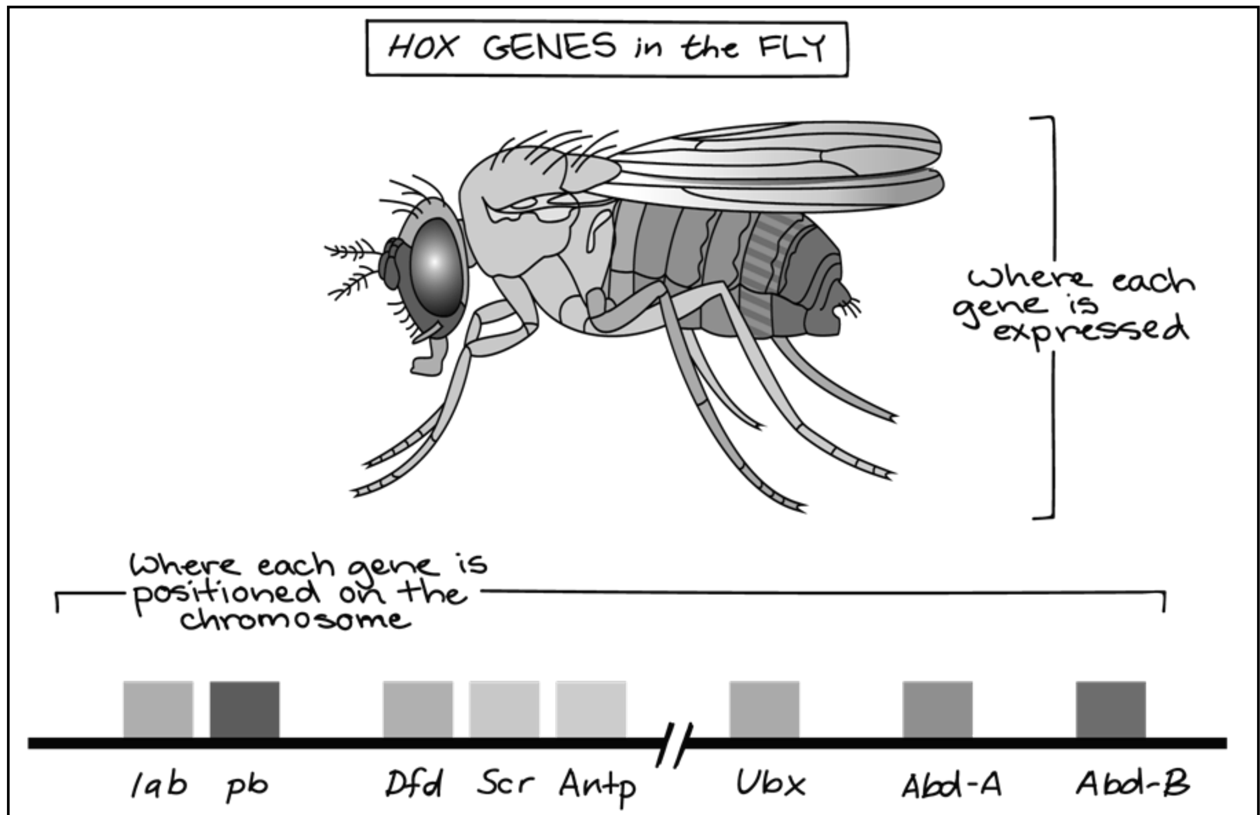
Overview of fruit fly *Hox* genes

Antennapedia and *Ultrabithorax* are not the only homeotic genes in a fruit fly. In fact, a whole set of different homeotic genes act in different regions of the fly's body, ensuring that each segment takes on its correct identity. These genes are typically expressed in the regions they regulate, starting early in embryonic development, and they continue to be expressed in the adult fly.

The diagram below shows eight major homeotic genes in flies. The upper part of the diagram shows where each gene is most strongly expressed in the mature fly, while the lower part of the diagram shows where the genes are located on the chromosome. The order of the genes on the chromosome more or less mirrors their order of expression along the head-tail axis of the fly.

What exactly are these homeotic genes? Each gene encodes a transcription factor that is expressed in a specific region of the fly starting early in its development as an embryo. The transcription factors change the expression of target genes to enact the genetic “program” that's right for each segment.

The homeotic transcription factors shown in the diagram above all contain a DNA-binding protein region called the homeodomain, which is encoded by a segment of DNA called the homeobox. Because they contain a homeobox, homeotic genes of this class are sometimes called *Hox genes* for short.



How are fly *Hox* genes turned on?

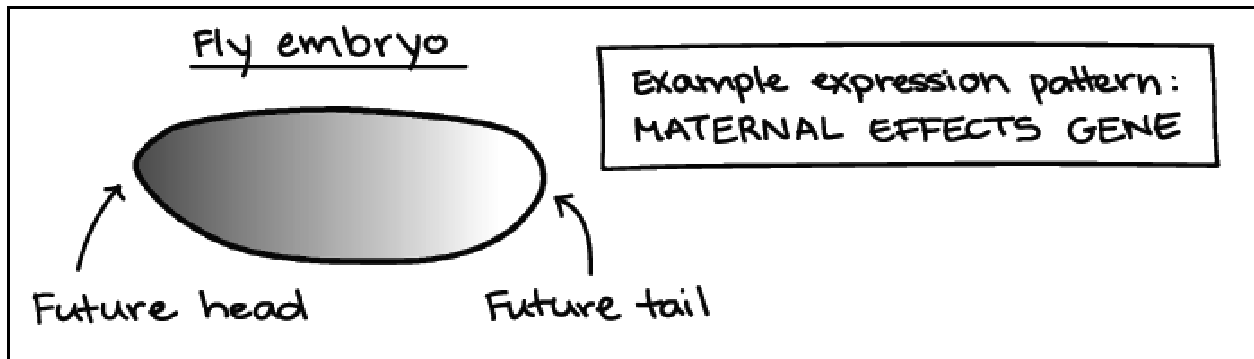
Hox genes need to be carefully regulated. As you learned above, a little sloppy regulation can result in things like extra wings or legs instead of antennae—both of which would be pretty bad for the survival of a fruit fly in the wild! So, how are these genes expressed in the right parts of the developing embryo?

To answer this question, let's take a quick look at the early steps of fly embryo development. Genetic patterns laid out in the fly egg—before the embryo is even an embryo—lay the groundwork for the fly's body plan. During development, the fly's body is first roughed out very generally, starting with head end over here, tail end over there. Then, the structure is gradually refined, first into broad sections, then smaller sections, then finally into actual body segments.

This process involves different classes of genes with increasingly narrow and specific patterns of expression. Broadly speaking, earlier-acting groups regulate later-acting groups in a sort of molecular domino effect. *Hox* genes are turned on in specific places through the activity of genes in this cascade.

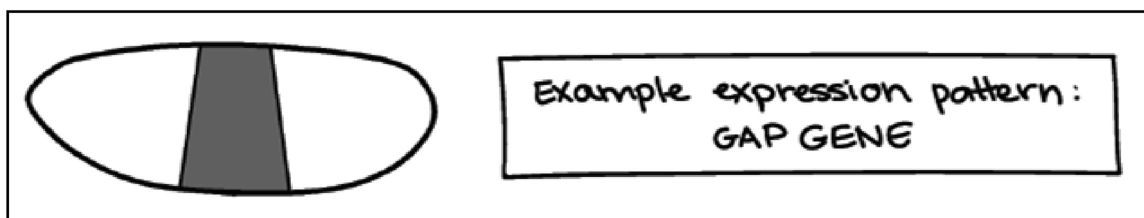
Genes in the early developmental cascade include the following groups:

- **Maternal effects genes,** which are genes whose mRNAare placed in the egg cell by the mother fly before fertilization. Some of the mRNA are “tied” to the head or tail end of the embryo and are responsible for setting up the head-tail polarity. The maternal effects genes encode regulators of transcription or translation that control each other as well as othe**r** genes.

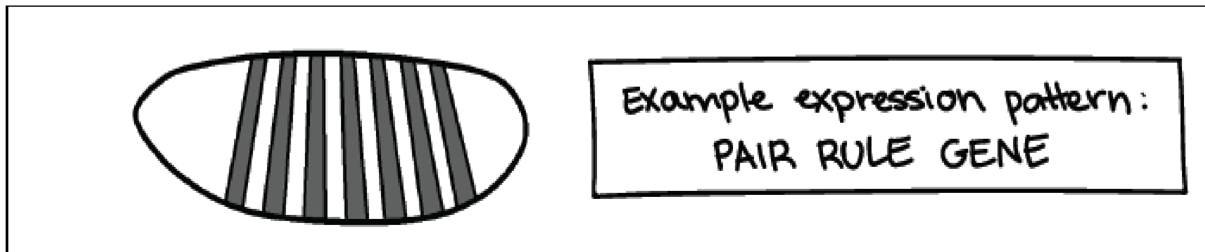


-

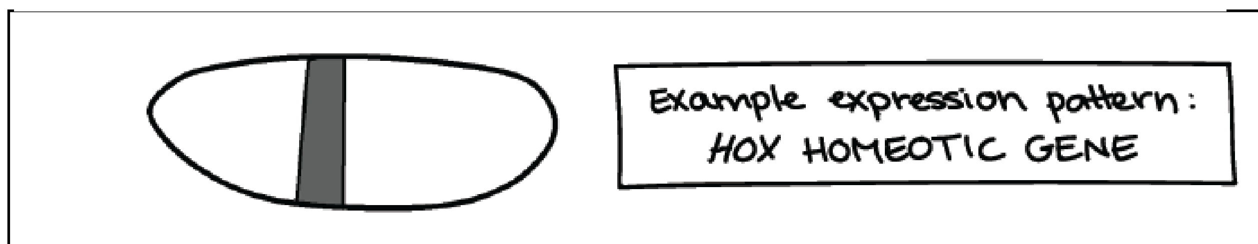
Gap genes are named appropriately. If gap genes are missing due to a mutation, there is a big gap in the fly larva—it is missing a large chunk of its normal segments.Gap genes are activated through interactions between the protein products of the maternal effects genes, and they also regulate each other. They're responsible for defining large, multi-segment regions of the fly, the ones that are missing when the gene is mutated.



Pair-rule genes are turned on by interactions between gap genes, and their expression patterns are refined by interactions with one another. They appear in multiple “stripes” along the embryo, similar in pattern to the segments of the mature fly but slightly offset. When a pair-rule gene is missing due to mutation, there is a loss of structures in the segment regions where the gene is normally expressed.



So, where do the *Hox* genes come in? *Hox* genes are turned on in specific patterns by the protein products of the gap genes and pair-rule genes. Their expression patterns are refined—by the products of these genes and through interactions with other Hox proteins—as the embryo develops.

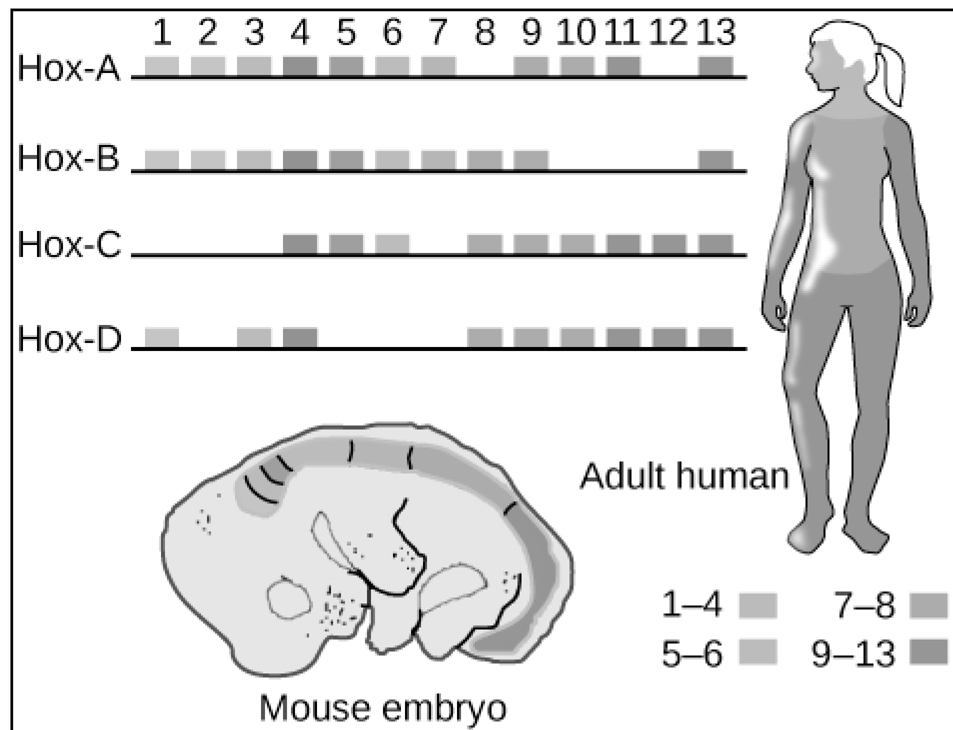


Many animal species have *Hox* genes.

Hox genes are not unique to fruit flies. In fact, *Hox* genes are found in many different animal species, including mice and humans. Yes, you have your very own *Hox* genes! The presence of similar *Hox* genes in different species reflects their common ancestry: a *Hox* gene cluster was likely already present in a common ancestor of mice, flies, humans, and other animal groups.

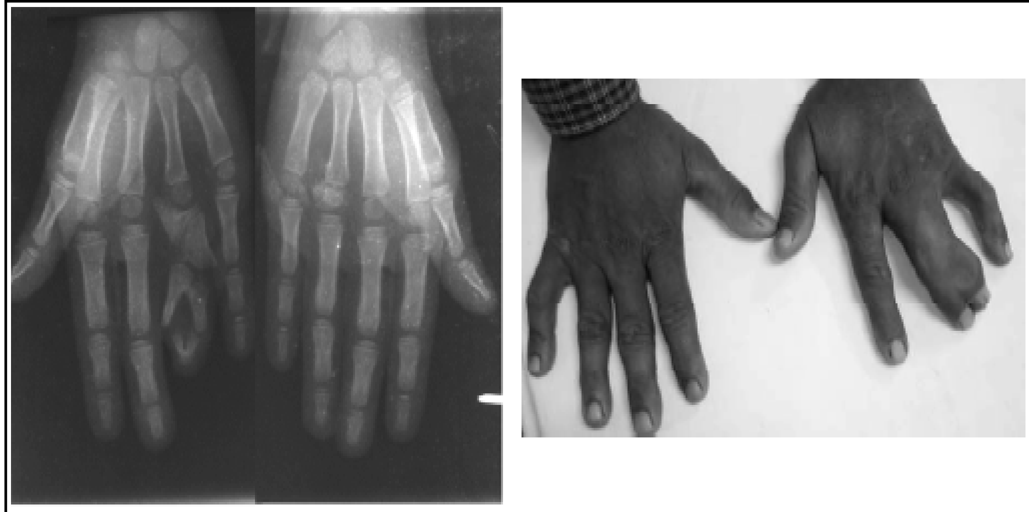
Not only are *Hox* genes found in many different animal species, but they also tend to have the same order on the chromosome in all of these species. As in flies, this order roughly maps to the parts of the body whose development is controlled by each gene. Because this is so consistently the case, scientists think it is likely not a coincidence and may have functional importance.

In vertebrates like humans and mice, *Hox* genes have been duplicated over evolutionary history and now exist as four similar gene clusters labelled A through D:



In general, the genes of the different clusters work together to establish the identity of body segments along the head-tail axis. That is, the genes towards the beginning of the cluster—closer to one in the diagram—tend to specify structures at the head end of the organism, and the genes toward the end of the cluster—closer to 13 in the diagram—tend to specify structures near the tail end.

However, gene duplication has allowed some *Hox* genes to take on more specialized roles. For instance, many *Hox* genes towards the end of the cluster act specifically in the development of vertebrate limbs—arms, legs, or wings—as shown in the diagram of the woman above. Mutations in *HoxD13* in humans can cause a genetic condition called synpolydactyly, in which people are born with extra fingers or toes that may also be fused together.



The Hox cluster is a great example of how developmental genes can be both preserved and modified through evolution, particularly when they are copied by a duplication. Hox genes also show just how powerful a developmental gene can be, especially when it is a transcription factor that that turns many target genes on or off to activate a particular genetic "program."

Meaning of Metamorphosis:

Metamorphosis may be defined as "a rapid differentiation of adult characters after a relatively prolonged period of slow or arrested differentiation in a larva". According to Duellman and Trueb (1986) Metamorphosis can be defined as **"a radical transformation from larval life to the adult stage involving structural, physiological, biochemical and behavioural changes"**.

Types of Amphibian Metamorphosis:

1. Progressive metamorphosis:

During metamorphosis if the animal progresses in the evolutionary grades, the metamorphosis is considered as a progressive metamorphosis; e.g., in most anurans of Amphibia.

2. Retrogressive metamorphosis:

When metamorphosis takes place in lower direction, i.e., by metamorphosis the animal retrogresses or shows indication of degeneration in the scale of evolution, called retrogressive metamorphosis; e.g., Ascidia of urochordates or in neotenic forms like salamanders.

Metamorphic changes of amphibians:

Etkin (1968) have divided three stages:

a. Premetamorphic stage:

The stage is characterized by the considerable growth and development of larval structures but metamorphosis does not occur.

b. Prometamorphosis:

The stage is characterised by the continuous growth specially the development of limbs and initiation of metamorphic changes.

c. Metamorphic climax:

The stage is characterised by the radical changes in the features of the larva, and climax is considered by the loss of most larval features.

Structure of a freshly hatched tadpole larva:

1. A freshly hatched tadpole larva has a limbless body.
2. The body is divided into an ovoid head, a short trunk and a slender tail.
3. A small opening situated ventrally at the root of the tail is known as anus.
4. An adhesive sucker is present on the ventral side of the head by which the tadpole larva attaches itself to the aquatic weeds.
5. The mouth is lacking and as a result it cannot take anything from outside.
6. The yolk material provides the nutrition.
7. The respiratory organs comprise of three pairs of highly vascular and branched feathery external gills.
8. After a few days the mouth is formed near the sucker.
9. A pair of horny jaws surrounds the mouth.
10. The tail becomes more elongated and develops a dorsal and a ventral fin.
11. V-shaped myotomes develop on both the sides of the tail.

12. At this time this free-swimming tadpole larva ingests aquatic weeds, as a result of which the alimentary canal becomes extremely elongated.

13. To accommodate such a long alimentary canal inside the cavity of the short trunk, it becomes spirally coiled like the spring of a watch.

Structure of an advanced tadpole larva:

1. In the advanced stage, the pharynx of the tadpole larva becomes perforated by gill- slits.
2. External gills disappear and the internal gills are formed between the gill slits.
3. The gills and the gill-slits are covered by the operculum (or gill-cover).
4. Thus the tadpole larva has three pairs of external gills at the start which are subsequently replaced by three pairs of internal gills.
5. In the larval stages, the arterial arches also show modifications in terms of both external and internal gills (Fig. 7.28).

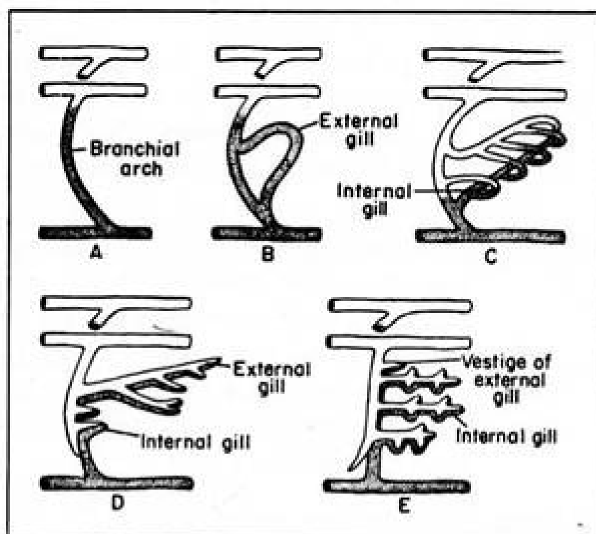


Fig. 7.28: Showing the stages (A–E) of development of an aortic arch in relation to external and internal gills in larval phase of *Bufo*.

6. The operculum fuses with the trunk on all sides except a small opening, called spiracle on the left side.
7. Water enters into the pharynx through the mouth and goes out through the spiracle.

8. During this transit of water the internal gills are bathed with water containing oxygen dissolved in it.

9. While the internal gills are functioning, a pair of lungs develops as outgrowths from the pharynx on the ventral surface.

10. The hind limbs appear prior to the forelimbs.

11. The forelimbs remain first hidden under the operculum and subsequently emerge through it.

12. At this stage both the internal gills as well as the newly formed lungs are functional.

13. When the lungs become fully developed, the internal gills become degenerated.

14. At this stage it looks like a miniature toad except having a tail.

15. As the limbs are developing, the animal enters into a period of starvation.

16. The material of the tail becomes eventually absorbed into the body.

Structure of a freshly formed toad:

1. After the absorption of tail, the young toad leaves the primal aquatic home and comes to the land and hops.

2. The mouth becomes wider and a pair of true bony jaws replaces the horny jaws.

3. It now changes its food habit to become carnivorous type, as a result the alimentary canal becomes short and less coiled.

The changes that take place in the tadpole can be divided into four groups.

They are:

1. Changes of tadpole in habit and habitat:

(i) With the metamorphosis, the metamorphosed larva leaves aquatic medium and frequently visits the land.

(ii) The herbivorous tadpole larva changes into carnivorous specially consume the insects (insectivorous).

(iii) The praying habits develop by the adults and the adult animals become more active and swift moving.

(iv) In the first stage of adult toad, they jump into nearby pond and in other aquatic medium, and then jump on the land by their elongated hind limbs.

2. Morphological metamorphic changes:

a. Regressive changes:

(i) The tissues of tail and tailfin are completely absorbed into the body.

(ii) The horny jaws with teeth are shed and mouth becomes a large transverse slit.

(iii) The external gills disappear and the gill slits communicate to the pharyngeal cavity.

(iv) The length of the alimentary canal much reduces.

(v) The changes of the blood vascular system take place and ultimately some blood vessels are reduced.

(vi) The lateral line sense organ disappears.

(vii) Operculum and spiracle disappear.

b. Progressive changes:

(i) The fore and hind limbs increase in size.

(ii) The tongue becomes long and more elastic which is free and bifid posteriorly.

(iii) The eyes become large and prominent and develop eye-lids and nictitating membrane.

(iv) External nostrils communicate with buccal cavity through internal nostrils.

(v) Tympanum and middle ear develop.

(vi) Liver becomes more enlarged.

(vii) Three chambered heart develops from two-chambered heart.

(viii) Pronephros is replaced by mesonephros.

3. Biochemical changes during metamorphosis:

(i) The concentration of serum protein becomes about double during metamorphosis.

(ii) Biosynthesis and concentration of haemoglobin are greater in adult than in larvae.

(iii) In the liver, DNA synthesis, lipid synthesis, enzymes for ornithine urea cycle increase during adult stage.

iv) Alkaline phosphatase and hydrolase decrease in adult stage of the anurans.

4. Changes in Physiology:

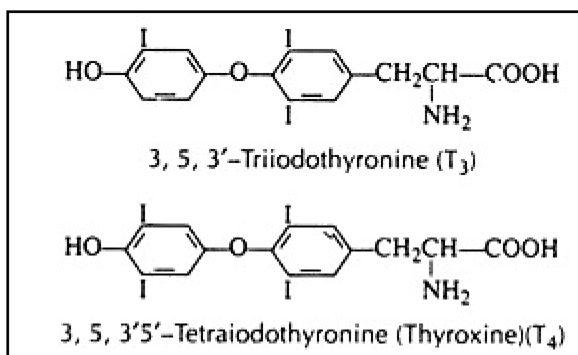
(i) At the beginning of metamorphosis, the pancreas starts to secrete insulin and glucagon hormones. This is related to the increased role of the liver.

(ii) During the larval stage, the end product of nitrogen metabolism is ammonia. But after metamorphosis, the toads and frogs excrete most of their nitrogen in the form of urea. This is a shift from ammonotelism to ureotelism with the change of environment from aquatic medium to land.

Hormonal Control for Metamorphosis:

Two hormones such as Triiodothyronine (T_3) and Tetraiodothyronine (T_4) or thyroxine are necessary for biochemical and morphological changes during anuran metamorphosis. These thyroid hormones are produced by the induction of anterior pituitary lobe or pars distalis when it reaches certain degree of differentiation.

Then it is capable to synthesize a hormone, thyrotropin (Thyroid Stimulating Hormone, TSH) which acts on the thyroid, stimulating the production and secretion of triiodothyronine (T_3) and thyroxine.



In pre-metamorphic stage the prolactin level is high but levels of thyroid stimulating hormone (TSH) and thyroid hormone (T_3, T_4) are low. The hypothalamus – pituitary link is poorly developed. In pro-metamorphosis, the hypothalamus and pituitary link develops.
The prolactin level is low but the levels of thyroid stimulating hormone (TSH) and thyroid hormones (T_3, T_4) are high. In metamorphic climax, the prolactin level increases suddenly, then maintains steady low level. The TSH is high until end of climax and the thyroid hormone (T_4) level becomes low.

Metamorphosis of Toad:

The young tadpole larva resembles a fish. It leads an independent and self-supporting life. This fish like tadpole larva completely metamorphoses into toad, is exclusively a progressive process. According to Mohanty-Hejmadi and Dutta (1978) – development is rapid being completed in 34-52 days. Daniel (1963) reports the hatching in about 4 days after laying.

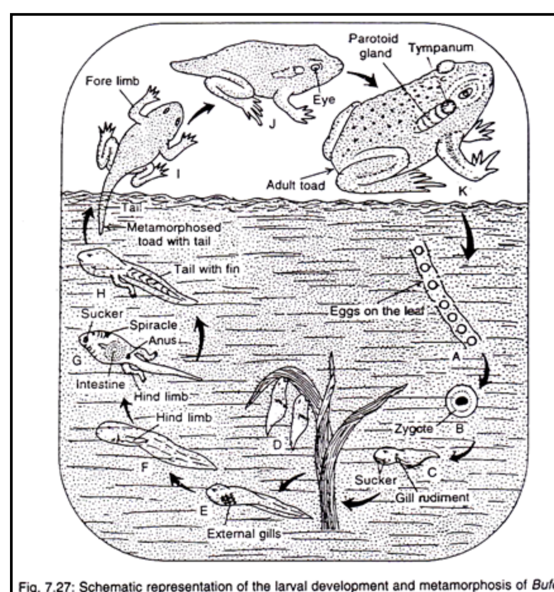


Fig. 7.27: Schematic representation of the larval development and metamorphosis of *Bufo*.

Regeneration

Regeneration—the reactivation of development in later life to restore missing tissues—is so "unhuman" that it has been a source of fascination to humans since the beginnings of biological science. It is difficult to behold the phenomenon of limb regeneration in newts or starfish without wondering why we cannot grow back our own arms and legs. What gives salamanders this ability we so sorely lack? Experimental biology was born in the efforts of eighteenth-century naturalists to document regeneration and to answer this question. The regeneration experiments of Tremblay (hydras), Réaumur (crustaceans), and Spallanzani (salamanders) set the standard for experimental research and for the intelligent discussion of one's data. Réaumur, for instance, noted that crayfish had the ability to regenerate their limbs because their limbs broke easily at the joints. Human limbs, he wrote, were not so vulnerable, so Nature provided us not with regenerable limbs, but with "a beautiful opportunity to admire

her foresight.” Tremblay's advice to researchers who would enter this new field is pertinent to read even today. He tells us to go directly to nature and to avoid the prejudices that our education has given us.* Moreover, “one should not become disheartened by want of success, but should try anew whatever has failed. It is even good to repeat successful experiments a number of times. All that is possible to see is not discovered, and often cannot be discovered, the first time.”

More than two centuries later, we are beginning to find answers to the great problem of regeneration, and we may soon be able to alter the human body so as to permit our own limbs, nerves, and organs to regenerate. This would mean that severed limbs could be restored, that diseased organs could be removed and regrown, and that nerve cells altered by age, disease, or trauma could once again function normally. To bring these benefits to humanity, we first have to understand how regeneration occurs in those species that have this ability. Our new knowledge of the roles of paracrine factors in organ formation, and our ability to clone the genes that produce those factors, has propelled what Susan Bryant (1999) has called “a regeneration renaissance.” Since “renaissance” literally means “rebirth,” and since regeneration can be seen as a return to the embryonic state, the term is apt in many ways.

There are three major ways by which regeneration can occur. The first mechanism involves the dedifferentiation of adult structures to form an undifferentiated mass of cells that then becomes respecified. This type of regeneration is called **epimorphosis** and is characteristic of regenerating limbs. The second mechanism is called **morphallaxis**. Here, regeneration occurs through the repatterning of existing tissues, and there is little new growth. Such regeneration is seen in hydras. A third type of regeneration is an intermediate type, and can be thought of as **compensatory regeneration**. Here, the cells divide, but maintain their differentiated functions. They produce cells similar to themselves and do not form a mass of undifferentiated tissue. This type of regeneration is characteristic of the mammalian liver. We discussed regeneration of flatworms and of the amphibian eye earlier in the book. Here we will concentrate on salamander limb, hydras, and mammalian liver regeneration.

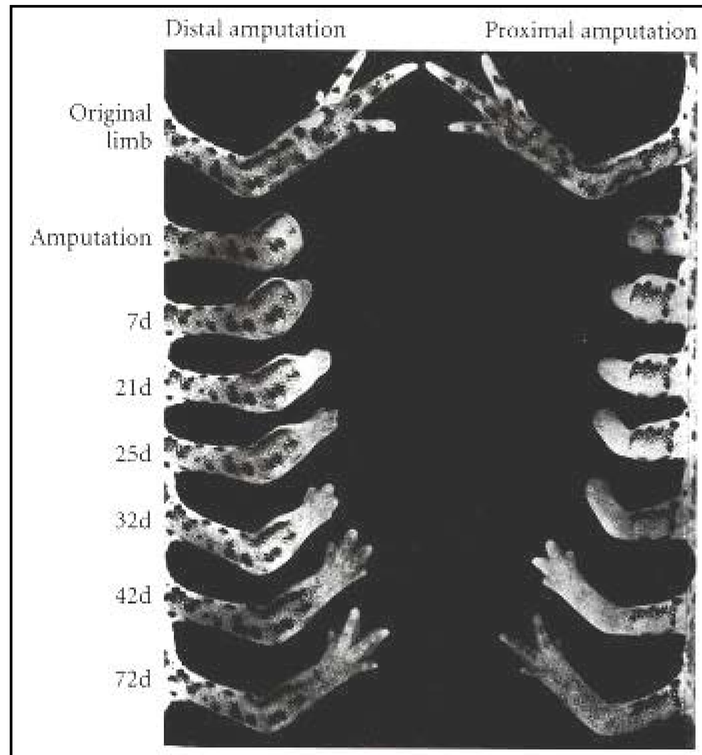


Figure: Regeneration of a salamander forelimb. The amputation shown on the left was made below the elbow; the amputation shown on the right cut through the humerus. In both cases, the correct positional information is respecified.

Epimorphic Regeneration of Salamander Limbs

When an adult salamander limb is amputated, the remaining cells are able to reconstruct a complete limb, with all its differentiated cells arranged in the proper order. In other words, the new cells construct only the missing structures and no more. For example, when a wrist is amputated, the salamander forms a new wrist and not a new elbow. In some way, the salamander limb “knows” where the proximal-distal axis has been severed and is able to regenerate from that point on.

Formation of the apical ectodermal cap and regeneration blastema

Salamanders accomplish this feat by dedifferentiation and respecification. Upon limb amputation, a plasma clot forms, and within 6 to 12 hours, epidermal cells from the remaining stump migrate to cover the wound surface, forming the wound epidermis. This single-layered structure is required for the regeneration of the limb, and it proliferates to form the apical ectodermal cap. Thus, in contrast to wound healing in mammals, no scar forms, and the dermis does not move with the epidermis to cover the site of amputation. The nerves

innervating the limb degenerate for a short distance proximal to the plane of amputation. During the next 4 days, the cells beneath the developing cap undergo a dramatic dedifferentiation: bone cells, cartilage cells, fibroblasts, myocytes, and neural cells lose their differentiated characteristics and become detached from one another. Genes that are expressed in differentiated tissues (such as the MRF4 and myf5 genes expressed in the muscle cells) are downregulated, while there is a dramatic increase in the expression of genes, such as msx1, that are associated with the proliferating progress zone mesenchyme of the embryonic limb. The formerly well-structured limb region at the cut edge of the stump thus forms a proliferating mass of indistinguishable, dedifferentiated cells just beneath the apical ectodermal cap. This dedifferentiated cell mass is called the regeneration blastema. These cells will continue to proliferate, and will eventually redifferentiate to form the new structures of the limb.

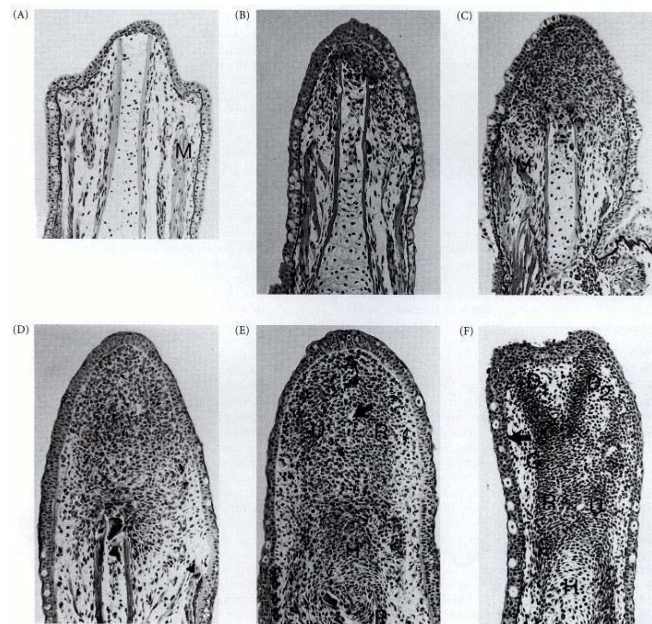


Figure. Regeneration in the larval forelimb of the spotted salamander *Ambystoma maculatum*. (A) Longitudinal section of the upper arm, 2 days after amputation. The skin and muscle have retracted from the tip of the humerus. (B) At 5 days after amputation, a thin accumulation of blastema cells is seen beneath a thickened epidermis. (C) At 7 days, a large population of mitotically active blastema cells lies distal to the humerus. (D) At 8 days, the blastema elongates by mitotic activity; much dedifferetiation has occured. (E) At 9 days, early redifferentiation can be seen. Chondrogenesis has begun in the proximal part of the

regenerating humerus, H. The letter A marks the apical mesenchyme of the blastema, and U and R are the precartilaginous condensations that will form the ulna and radius, respectively. P represents the stump where the amputation was made. (F) At 10 days after amputation, the precartilaginous condensations for the carpal bones (ankle, C), and the first two digits (D1, D2) can also be seen.

The creation of the blastema depends upon the formation of single, mononucleated cells. It is probable that the macrophages that are released into the wound site secrete metalloproteinases that digest the extracellular matrices holding epithelial cells together. But many of these cells are differentiated and have left the cell cycle. How do they regain the ability to divide? Microscopy and tracer dye studies have shown that when multinucleated myotubes (whose nuclei are removed from the cell cycle) are introduced into a blastema, they give rise to labeled mononucleated cells that proliferate and can differentiate into many tissues of the regenerated limb. It appears that myotube nuclei are forced to enter the cell cycle by a serum factor created by thrombin, the same protease that is involved in forming clots. Thrombin is released when the amputation is made, and when serum is exposed to thrombin, it forms a factor capable of inducing cultured newt myotubes to enter the cell cycle. Mouse myotubes, however, do not respond to this chemical. This difference in responsiveness may relate directly to the difference in regenerative ability between salamanders and mammals.

Proliferation of the blastema cells: the requirement for nerves

The proliferation of the salamander limb regeneration blastema is dependent on the presence of nerves. Singer (1954) demonstrated that a minimum number of nerve fibers must be present for regeneration to take place. It is thought that the neurons release mitosis-stimulating factors that increase the proliferation of the blastema cells. There are several candidates for these neural-derived mitotic factors, and each may be important. Glial growth factor (GGF) is known to be produced by newt neural cells, is present in the blastema, and is lost upon denervation. When this peptide is added to a denervated blastema, the mitotically

arrested cells are able to divide again. A fibroblast growth factor may also be involved. FGFs infused into denervated blastemas are able to restore mitosis. Another important neural agent necessary for cell cycling is transferrin, an iron-transport protein that is necessary for mitosis in all dividing cells (since ribonucleotide reductase, the rate-limiting enzyme of DNA synthesis, requires a ferric ion in its active site). When a hindlimb is severed, the sciatic nerve transports transferrin along the axon and releases large quantities of this protein into the blastema. Neural extracts and transferrin are both able to stimulate cell division in denervated limbs, and chelation of ferric ions from a neural extract abolishes its mitotic activity.

Pattern formation in the regeneration blastema

The regeneration blastema resembles in many ways the progress zone of the developing limb. The dorsal-ventral and anterior-posterior axes between the stump and the regenerating tissue are conserved, and cellular and molecular studies have confirmed that the patterning mechanisms of developing and regenerating limbs are very similar. By transplanting regenerating limb blastemas onto developing limb buds, Muneoka and Bryant (1982) showed that the blastema cells could respond to limb bud signals and contribute to the developing limb. At the molecular level, just as Sonic hedgehog is seen in the posterior region of the developing limb progress zone mesenchyme, it is seen in the early posterior regeneration blastema. The initial pattern of Hox gene expression in regenerating limbs is not the same as that in developing limbs. However, the nested pattern of Hoxa and Hoxd gene expression characteristic of limb development is established as the limb regenerates.

Retinoic acid appears to play an important role both in the dedifferentiation of the cells to form the regeneration blastema and in the respecification processes as the cells redifferentiate. If regenerating animals are treated with sufficient concentrations of retinoic acid (or other retinoids), their regenerated limbs will have duplications along the proximal-distal axis. This response is dose-dependent and at maximal dosage can result in a complete new limb regenerating (starting at the most proximal bone), regardless of the original level of

amputation. Dosages higher than this result in inhibition of regeneration. It appears that the retinoic acid causes the cells to be respecified to a more proximal position .

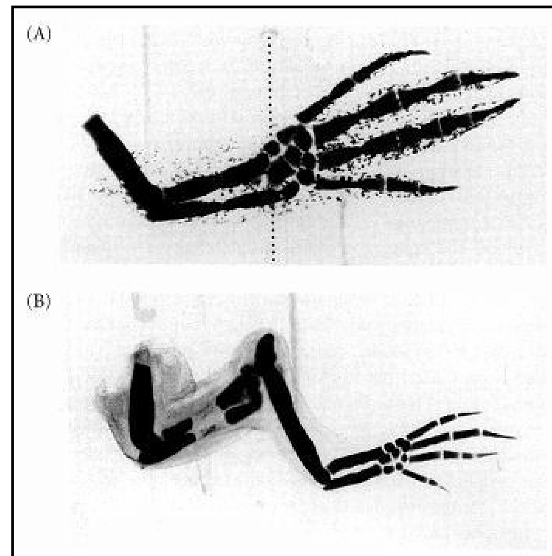


Figure: Effects of vitamin A (a retinoid) on regenerating salamander limbs. (A) Normal regenerated axolotl limb (9×) with humerus, paired radius and ulna, carpals, and digits. Dotted line shows plane of amputation through the carpal area. (B) Regeneration after amputation through the carpal area, but after the regenerating animal had been placed in retinol palmitate (vitamin A) for 15 days. A new humerus, ulna, radius, carpal set, and digit set have emerged (5×).

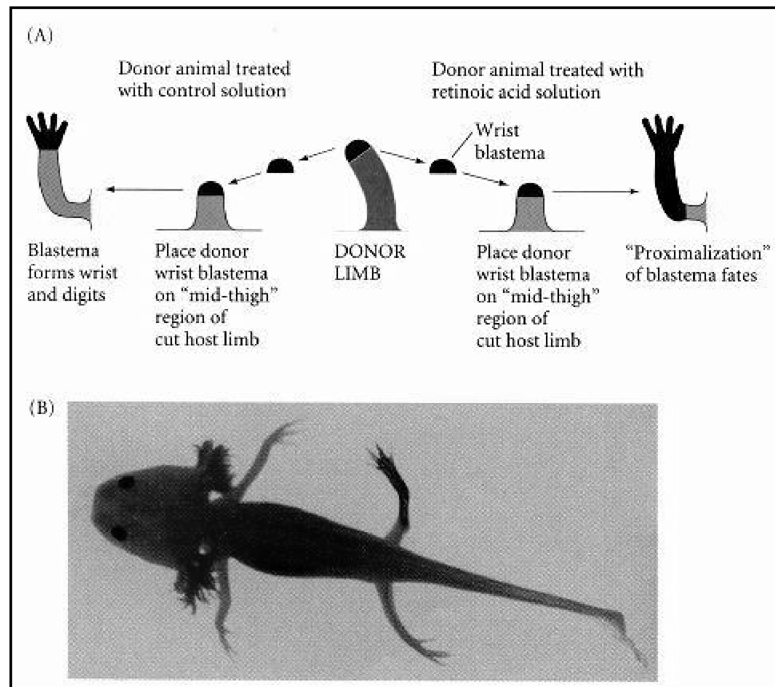


Figure: Proximalization of blastema respecification by retinoic acid. (A) When a wrist blastema from a recently cut axolotl forelimb is placed onto a host hindlimb cut at the mid-thigh level, it will generate only the wrist. The host (whose own leg was removed) will fill the gap and regenerate up to the wrist. However, if the donor animal is treated with retinoic acid, the wrist blastema will regenerate a complete limb and, when grafted, will fail to cause the host to fill the gap. (B) Wrist blastema from a darkly pigmented axolotl was treated with retinoic acid and placed onto the amputated mid-thigh region of a golden axolotl. The treated blastema regenerated a complete limb.

Retinoic acid is synthesized in the regenerating limb wound epidermis and is seen to form a gradient along the proximal-distal axis of the blastema. This gradient of retinoic acid is thought to activate genes differentially across the blastema, resulting in the specification of pattern in the regenerating limb. One of these retinoic acid-responsive genes is the msx1 gene that is associated with mesenchyme proliferation. Another set of genes that may be respecified by retinoic acid is the Hoxa genes. Gardiner and colleagues (1995) have shown that the expression pattern of certain Hoxa genes in the distal cells of the regeneration blastema is changed by exogenous retinoic acid into an expression pattern characteristic of more proximal cells. It is probable that during normal regeneration, the wound epidermis/apical ectodermal cap secretes retinoic acid, which activates the genes needed for

cell proliferation, downregulates the genes that are specific for differentiated cells, and activates a set of Hox genes that tells the cells where they are in the limb and how much they need to grow. The mechanism by which the Hox genes do this is not known, but changes in cell-cell adhesion and other surface qualities of the cells have been observed. Thus, in salamander limb regeneration, adult cells can go “back to the future,” returning to an “embryonic” condition to begin the formation of the limb anew.

Probable questions:

1. Describe the process of pattern formation in *Drosophila.*
2. What is Homeobox genes and mention its role in development?
3. Describe the process of development and metamorphosis of tadpole larve.
4. Comment on the process of limb development and regeneration in vertebrates.

Suggested Readings/ References:

http://www.biologydiscussion.com/cell/cellular-differentiation/notes-on-cellular-differentiation-cell-biology/27091

https://www.khanacademy.org/science/biology/developmental-biology/sigalling-and-transcription-factors-in-development/a/homeotic-genes

http://www.biologydiscussion.com/zoology/amphibians/metamorphosis-of-amphibians-phylum-chordata/40933

Unit-VI

Concept on Aging and Senescence

Objectives: In this Unit we will discuss on Aging and Senescence concept.

Aging:

Aging is characterized by a gradual functional decline. In mammals, aging occurs heterogeneously across multiple organ systems, causing a progressive deterioration that eventually results in tissue dysfunction. Consequently, age is a risk factor for many diseases, such as cardiovascular disease, dementia, osteoporosis, osteoarthritis, cancer, type 2 diabetes, idiopathic pulmonary fibrosis and glaucoma.

Although its biological causes remain largely unknown, studies in the past few decades have identified common cellular and molecular traits associated with aging.

Aging hallmarks can be divided into three categories:

(1) primary, or the causes of age-associated damage; (2) antagonistic, or the responses to the damage; and (3) integrative, or the consequences of the responses and culprits of the aging phenotype. Senescence, a cellular response that limits the proliferation of aged or damaged cells, belongs to the antagonistic

class. Although senescence plays physiological roles during normal development and it is needed for tissue homeostasis, senescence constitutes a stress response triggered by insults associated with aging such as genomic instability and telomere attrition, which are primary aging hallmarks themselves. There is also an intimate link between senescence and the other antagonistic hallmarks of aging. For example, senescent cells display decreased mitophagy, resulting in an "old," defective mitochondrial network that may contribute to metabolic dysfunction in age.

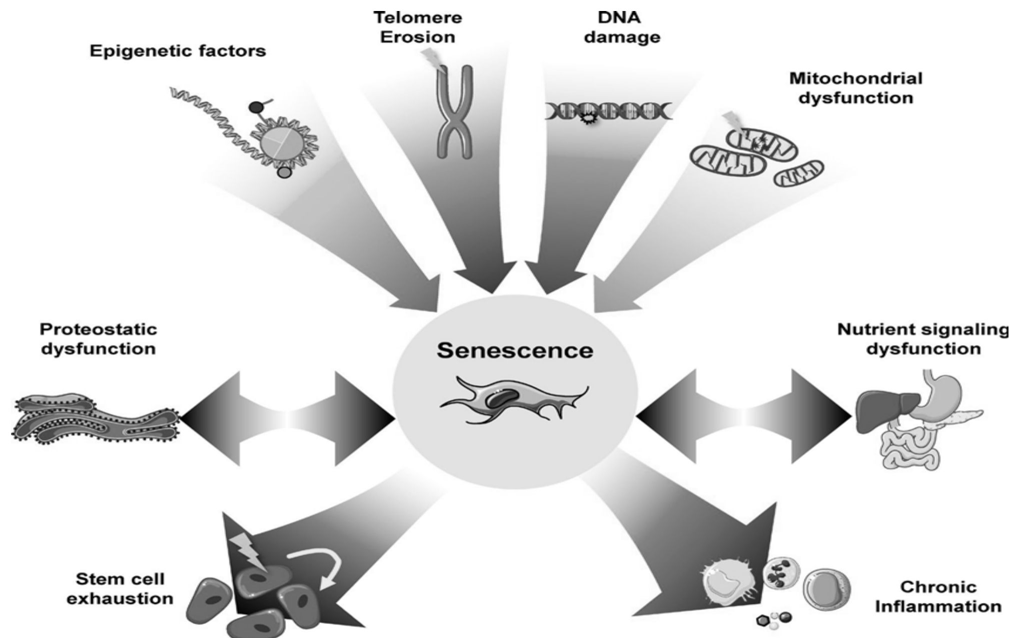


Figure : Senescence as a central hallmark of aging. Telomere damage, epigenetic dysregulation, DNA damage, and mitochondrial dysfunction are primary drivers of damage in aging. Several of these drivers of damage can induce senescence. Senescence can in turn drive the consequential aging hallmarks in response to damage: stem cell exhaustion and chronic inflammation. Other responses to damage, such as proteostatic dysfunction and nutrient sigalling disruption, are also integrally linked with the senescence response.

Senescence also influences the integrative aging hallmarks. Somatic multipotent stem cells facilitate tissue homeostasis; for example, hematopoietic stem cells (HSCs) renew the blood system. Stem cell exhaustion occurs with age, and the consequent decline in stem cell functionality and their capacity for renewal leads to tissue deterioration. For example, HSCs display a decreased success rate of transplantation when isolated from elderly patients. This decline correlates with increased numbers of senescent HSCs and diminished immunity, decreased numbers of naive B and T cells, and reduced natural killer cell activity. Somatic stem cell decline is not limited to high-turnover tissues. Neural stem cells (NSCs) experience reduced functionality, with limited neurogenesis capacity with age. This is marked by a twofold reduction in NSC numbers and a decreased proliferation, which correlates with increased expression of senescence markers in the regions where NSCs reside. Mesenchymal stem cells and their descendants, satellite cells, chondrocytes, adipocytes, and osteoclasts, also display a reduced ability to self-renew with age that correlates with increased levels of

senescence markers. This may have an impact in age-associated pathologies such as sarcopenia, cachexia, osteoporosis, and osteoarthritis.

Altered intracellular communication is another of the integrative hallmarks of aging. In particular, chronic low-level inflammation is a serious complicating factor for many diseases in which risk increases with age. This detrimental role of inflammation is supported by inflammatory markers such as interleukin-1 (IL-1) and IL-6 acting as prognostic markers for diseases such as type 2 diabetes, atherosclerosis, and breakdown in stem cell function. Inflammatory responses are one of the major extrinsic effects of senescent cells, which suggests that there is a link between senescence and altered intracellular communication. Aging influences a broad range of disease etiologies. Therefore, targeting the underlying aging machinery may provide broad-spectrum protection against many pathologies.

What is senescence?

Senescence is cellular program that induces a stable growth arrest accompanied by distinct phenotypic alterations, including chromatin remodeling, metabolic reprogramming, increased autophagy, and the implementation of a complex proinflammatory secretome. These complex changes to the cell largely serve to implement various aspects of senescence such as growth arrest and the senescence secretome. Despite the many facets of senescence, stable growth arrest is its defining characteristic. A permanent arrest is effective to ensure that damaged or transformed cells do not perpetuate their genomes. This growth arrest is implemented by the activation of p16INK4a/Rb and p53/p21CIP1 tumor suppressor networks.

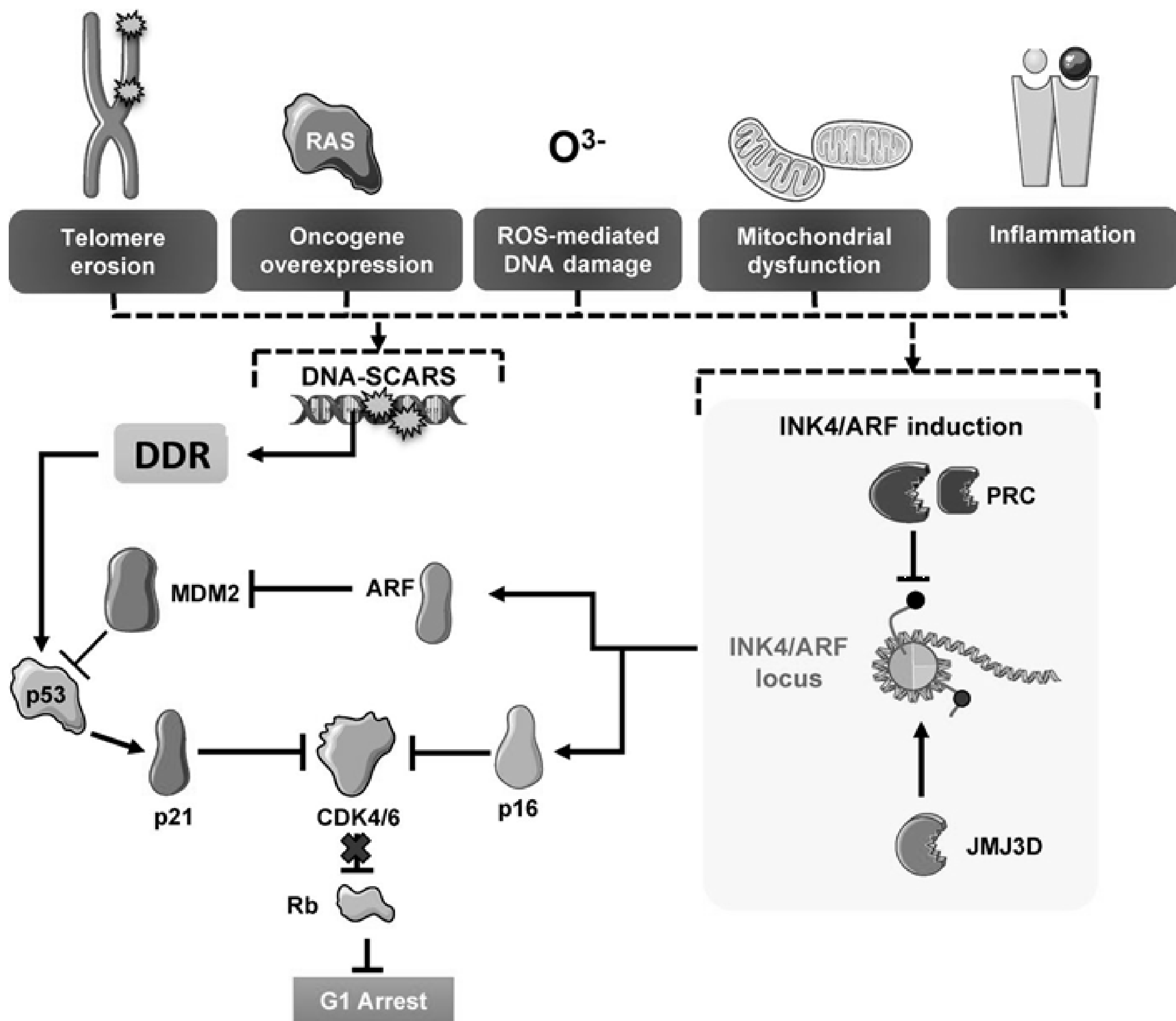


Figure : Pathways regulating senescence- mediated arrest. The senescence growth arrest is regulated through two main pathways, p16INK4a/Rb and p53/p21CIP1, both which converge on repression of CDK4/6. The INK4A/ ARF locus is normally silenced by Polycomb repressive complexes (PRCs) and becomes activated during senescence. The p53/p21CIP1 pathway is activated downstream of the DNA damage response (DDR) from repair-resistant DNA segments with chromatin alterations reinforcing senescence (DNA-SCA RS).

Historically, senescence was first identified by Hayflick and Moorhead (1961) during serial passage of human fibroblasts. The limit to proliferation that senescence imposes was hypothesized as a barrier to cancer initiation. Senescence is indeed a powerful mechanism of tumor suppression. Senescence has also physiological roles during normal development, acting in concert with apoptosis to facilitate embryonic morphogenesis. In adult tissues,

senescence is triggered primarily as a response to damage, allowing for suppression of potentially dysfunctional, transformed, or aged cells. The aberrant accumulation of senescent cells with age results in potential detrimental effects. In balance, although senescence is a biologically necessary process, it may come at a cost. The early research of Hayflick and Moorhead (1961) hinted at a relationship between senescence and aging, but the consequent discovery that senescent cells accumulate in aged tissues has substantiated the hypothesis that senescence itself can drive aging.

Factors driving senescence:

Telomere damage driving senescence in aging

In adult tissues, senescence is engaged in response to different types of damage. One of the insults causing senescence is damage of the telomeres, highly repetitive DNA structures located at the end of chromosomes. Telomeres are protected by a multiprotein complex known as shelterin. By coating the telomere, shelterin prevents the activation of a DNA damage response, thereby preventing end-to-end chromosome fusions that would result in a telomere crisis. Moreover, cells lacking shelterin components, such as POT1 or TRF2, suffer an aberrant DNA damage response and premature induction of senescence. The end-replication problem is a consequence of the inability of DNA polymerases to synthesize DNA without a template, which occurs at telomeres. This results in telomeres that shorten progressively with each cell cycle division. Embryonic tissues circumvent this erosion by expressing telomerase, a ribonucleoprotein complex that serves to concatenate DNA to the ends of chromosomes, thus providing a template for DNA synthesis. Repeated cell division in adult tissues that lack telomerase, however, results in progressive erosion of DNA, reduced shelterin binding, and senescence. As an organism ages, cells accumulate more divisions. This results in increased telomere erosion and senescence. But the extent to which telomere erosion drives senescence during aging and contributes to the aging process itself remains unknown.

Supporting the causative role of telomere erosion in aging, deletion of telomerase in mice eventually results in premature aging. This phenotype can be rescued by transient activation of telomerase reverse transcription expression in mice using a telomerase reverse transcription estrogen receptor construct. Cells isolated from these mice proliferate normally *in vitro*, and deterioration in multiple tissues is reduced. This evidence correlates with studies showing that fibroblasts or T cells derived from centenarians reset their telomeres, which results in rejuvenation and sustained proliferation. Similarly, stimulation of T cells derived from serially transplanted HSCs results in telomerase expression and rejuvenation. Shortened telomeres are associated with many pathologies such as liver cirrhosis and correlate with an increase in mortality in people older than 60 years. Correlative evidence supports telomere erosion as a major driver of aging decline, yet this is challenged by mammals such as laboratory mice (*Mus musculus*), whose telomeres do not reach a critical limit during normal aging. Telomere length is also not predictive of aging deterioration in mice, highlighting that alternative factors could also drive aging.

Metabolic dysfunction as a driver of senescence

Several lines of evidence suggest that aging is the result of a complex amalgam of damages such as metabolic and proteostatic dysfunction. Metabolic dysfunction relates to aging at the organismal and molecular level. Multiple studies have demonstrated that caloric restriction can retard the aging decline. Molecularly, pathways finetuning metabolic regulation, such as the mTOR or insulin pathway, have also been linked to increased health span and life span. mTORC1 integrates inputs from nutrient and growth signals to regulate general cellular processes such as protein and lipid synthesis, autophagy, and metabolism. In this regard, mTOR is able to regulate the senescence-associated secretory phenotype (SASP), autophagy, and senescent growth arrest. The connection between autophagy and senescence is complex; although there is an increase in autophagy during senescence that serves to regulate SASP production, inhibition of autophagy can induce senescence through metabolic and

proteostatic dysfunction, further emphasizing the intricate connection between metabolic stress andsenescence in aging.

Sirtuins constitute another molecular link between metabolism and senescence. Sirtuins are ribosyltransferases with a wide array of functions, such as metabolism regulation and DNA repair. Their role in senescence is antagonistic; SIRT1 deacetylates p53, promotes its degradation and facilitates senescence bypass, whereas SIRT6 deacetylates H3K18 to prevent mitotic errors and suppress senescence.

In addition to these forms of damage, general stress is sensed by other mechanisms such as activation of MAPK p38 or induction of p16INK4a. These pathways are up-regulated in response to oxidative stress, DNA damage, telomere attrition, or oncogene activation. Substantiating their role in aging, activation of MAPK p38 or induction of p16INK4a limits the proliferative potential of HSCs and yields proaging phenotypes. Overall, it is likely that the accumulation of senescent cells during aging reflects a gradual increase of different types of damage in different tissues.

Pathways regulating the senescence growth arrest

Despite the multifaceted nature of senescence, the induction of stable growth arrest is the defining characteristic of senescence. Moreover, stable arrest is paramount to halt the propagation of dysfunctional cells. Two tumor suppressor pathways, p53 and the p16/Rb, are responsible for the implementation of this growth arrest.

p53 and senescence

Senescence inducers such as telomeric attrition and oncogenic or oxidative stress cause DNA damage. DNA damage results in increased deposition of γH2Ax and 53BP1 in chromatin that in turn activates a kinase cascade involving first ATM and ATR and then CHK1 and CHK2, eventually resulting in p53 activation. p53 induces transcription of the cyclin-dependent kinase inhibitor p21CIP1. In turn, p21CIP1 blocks CDK4/6 activity, resulting in hypophosphorylated Rb and cell cycle exit. Although transient increases in p53 levels can enact a quiescent state and activate DNA repair processes, during senescence, there is a

sustained induction of p53. This is a result of damage occurring in repair-resistant regions of the genome known as DNA segments with chromatin alterations reinforcing senescence, such as telomeres, that allow for a permanent arrest of the cell cycle by persistent induction of p21cip1. Given the key roles of p53, additional regulatory layers exist. For example, the induction of ARF, a product of the INK4/ARF locus, sequesters the ubiquitin ligase MDM2, contributing to increased levels of p53. Recently, the interaction between Forkhead box protein O4 (FOXO4) and p53 has been shown to play an important role in modulating p53 localization and transcriptional activity during senescence. Interestingly FOXO transcription factors regulate aging, with FOXO activity in Drosophila melanogaster leading to delayed aging in response to disrupted protein homeostasis and oxidative stress.

The INK4/ARF locus in senescence

Three tumor suppressors reside in the INK4/ARF locus: p16INK4a and ARF, which are both encoded by the CDKN2A gene, and p15INK4b, which is encoded by CDKN2B. Two of these, p15INK4b and p16INK4a, are CDKIs, like p21CIP1, that affect the cell cycle by binding and inhibiting CDK4 and CDK6. In contrast, ARF inhibits MDM2, thereby allowing cross talk with the p53/p21CIP1 pathways. Conversely, p53 can regulate expression of ARF through a negative feedback loop, as demonstrated by elevated ARF expression in p53−/− mouse embryonic fibroblasts.

Given this unusual concentration of three tumor suppressors in barely 35 kb, this locus plays a key regulatory role and is frequently mutated in cancer. Genome-wide association studies have also identified various genomic variants occurring at the INK4/ARF locus as major risk factors for atherosclerosis, stroke, and diabetes, among other pathologies. However, most of these are found in noncoding regions, and the precise mechanism of action is unclear. The INK4/ARF locus behaves as a senescence sensor. In young, normal cells, the INK4/ARF locus is epigenetically silenced through deposition of repressive H3K27me3 marks. H3K27

methylation is controlled by Polycomb repressive complexes (PRC2 and PRC1). Disrupting PRC1 or PRC2 activity by depleting the expression of some of their components, such as BMI1, CBX7, or EZH2, derepresses p16INK4a and induces senescence. There is still debate over how Polycomb is recruited to the INK4/ARF locus. It has been proposed that a long noncoding RNA, ANRIL, divergently transcribed from the INK4/ARF locus, and transcription factors such as those of the homeobox family can contribute to recruiting PRCs.

Conversely, during senescence, the H3K27 histone demethylase JMJD3 plays a role in removing the repressive marks around the INK4/ARF locus, facilitating its induction. INK4/ARF induction can be observed in tissues during natural aging. In particular, p16INK4a is considered an aging biomarker. With exceptions (such as during senescence-induced during development), p16INK4a is also one of the best markers of senescence. An analysis of the pathways regulating p16INK4a shows coincidences with those controlling development. This has been argued to formulate the theory that aging might be driven by gradual functional decay of developmental pathways.

The SASP

Besides growth arrest, the production of a complex mixture of secreted factors, termed the SASP or senescence-messaging secretome, is the most relevant phenotypic program implemented in senescent cells. Senescent cells secrete hundreds of factors that include proinflammatory cytokines, chemokines, growth factors, and proteases.

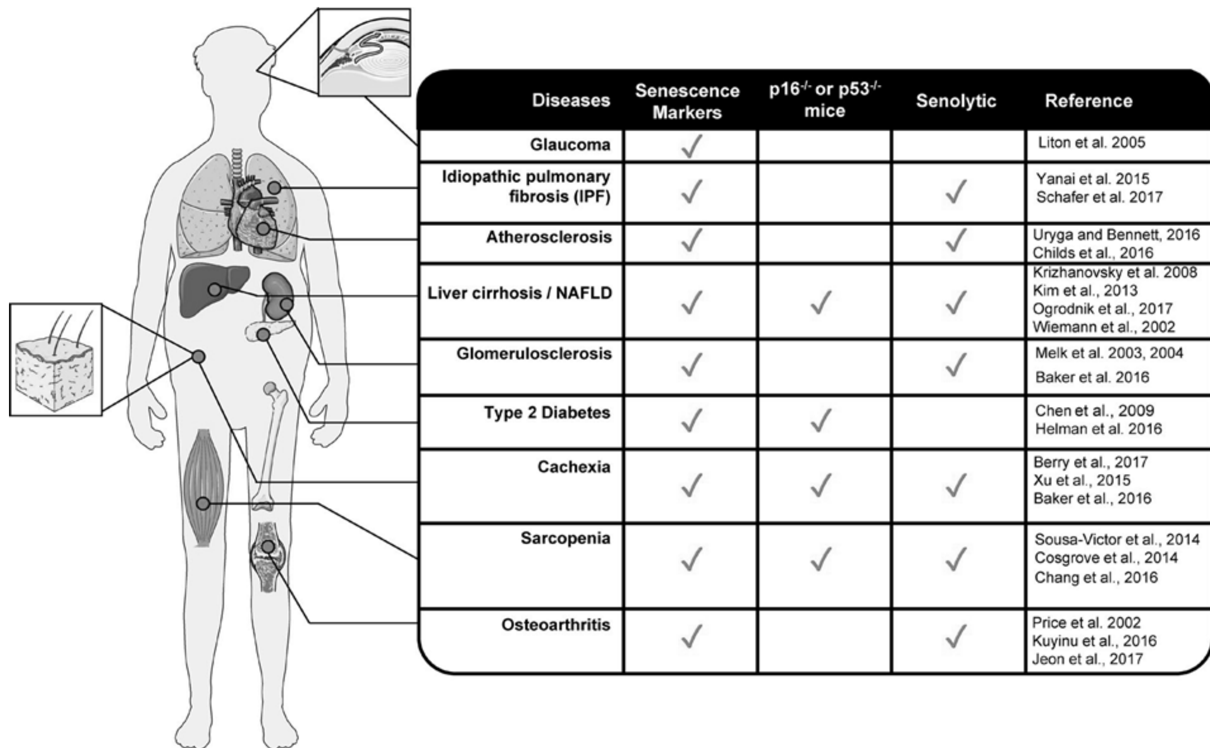
Regulation of the SASP

The specific combination of secreted factors is thought to depend on the cell type and the senescent inducer. However, many of the key effectors of the SASP and its regulatory mechanism seemed to be shared. Nuclear factor κB (NF-κB) and CCAAT/enhancer–binding protein beta are the key transcriptional SASP regulators. DNA damage, p38α MAPK, mTOR, mixed lineage leukemia 1, and GATA4 are also able to regulate the SASP. Recently, sensing of cytoplasmic chromatin by the cGAS/STING pathway has been suggested as a trigger for SASP induction. There are additional layers of SASP regulation. For example, mTOR controls IL-1α translation to regulate the SASP. In addition, mTOR indirectly regulates the activity of ZFP36L1, an RNA-binding protein that binds to AU-rich elements in the 5′-end of inflammatory transcripts, targeting them for degradation. There is also a global remodeling of enhancers in senescent cells, and the recruitment of BRD4 to superenhancers adjacent to SASP genes is needed for their induction.

The complex composition of the SASP means that different subsets of the SASP, such as the proinflammatory and TGF-β secretomes, can be regulated independently. The proinflammatory arm of the SASP is regulated by IL-1 sigalling. IL-1α partially recapitulates the inflammatory SASP in vitro, and inhibiting the NLRP3 inflammasome, which processes IL-1β, can blunt the SASP. Conversely, the juxtacrine Notch sigalling pathway promotes the secretion of a TGF-β–enriched secretome.

Contribution of senescence to age-related diseases

Now that a general causative role for senescence during aging has been established, the next step is to identify how senescence contributes to different age-related pathologies such as glaucoma or osteoarthitis. Thanks to the use of senolytic drugs and genetic models for senescence ablation, we are progressing quickly in that task.



Diseases	Senescence Markers	$p16^{-/-}$ or $p53^{-/-}$ mice	Senolytic	Reference
Glaucoma	✓			Liton et al. 2005
Idiopathic pulmonary fibrosis (IPF)	✓		✓	Yanai et al. 2015 Schafer et al. 2017
Atherosclerosis	✓		✓	Uryga and Bennett, 2016 Childs et al., 2016
Liver cirrhosis / NAFLD	✓	✓	✓	Krizhanovsky et al. 2008 Kim et al., 2013 Ogrodnik et al., 2017 Wiemann et al., 2002
Glomerulosclerosis	✓		✓	Melk et al. 2003, 2004 Baker et al. 2016
Type 2 Diabetes	✓	✓		Chen et al., 2009 Helman et al. 2016
Cachexia	✓	✓	✓	Berry et al., 2017 Xu et al., 2015 Baker et al., 2016
Sarcopenia	✓	✓	✓	Sousa-Victor et al., 2014 Cosgrove et al., 2014 Chang et al., 2016
Osteoarthritis	✓		✓	Price et al. 2002 Kuyinu et al., 2016 Jeon et al., 2017

Figure. Involvement of senescence in disease. Establishment of robust biomarkers of senescence, usage of genetic knockout models and senolytic models are expanding our knowledge on the age-related diseases in which senescence plays a role.

Opposing roles for senescence in cancer

Age is a strong prognostic marker of reduced survival across many cancers. Senescence is a strong tumor suppressor mechanism that limits cancer initiation through both cell-intrinsic and cell-extrinsic mechanisms. However, there is strong evidence suggesting that through the SASP, aged tissues provide a supportive niche for cancer. Senescent cells can contribute to tumor progression by enhancing the proliferative potential of cancer cells or contributing to epithelial to mesenchymal transition. Therefore, the increased numbers of senescent cells present in aged tissues could contribute to the increased incidence of cancer with age. Supporting this, a delayed onset in tumor formation is observed when senescent cells are eliminated. Senolytic therapy also reduces the incidence of metastasis, the leading cause of cancer-related deaths.

Renal dysfunction

Aged individuals often display a reduced glomerular filtration rate and cortical volume that can result in glomerulosclerosis and nephron atrophy, both of which are associated with increased expression of p16INK4a and p53. Senescence has detrimental effects in most renal diseases analyzed. Ablation of senescent cells protects against glomerulosclerosis and improves kidney function in aged mice.

Type 2 diabetes

One of the largest risk factors for the development of type 2 diabetes is age. Several genome-wide association studies of type 2 diabetes have highlighted variants at the INK4/ARF locus, suggesting a possible link between senescence and diabetes. In addition, senescence markers and IL-1β are elevated in β cells from diabetic mice. Surprisingly, although p16INK4a expression drove a decline in β cell regenerative capacity and predisposed mice to mild diabetes, senescent β islets increased insulin secretion, making it unclear how senescence contributes to maintain glucose homeostasis.

IPF

Fibrosis is a pathological condition whereby tissue accumulates ECM proteins such as collagen, resulting in tissue scarification, usually in response to damage. Senescence appears to have both beneficial and detrimental roles during fibrosis and wound healing. Secretion of MMPs, which occurs as part of the SASP, could help in the resolution of fibrotic plaques. Conversely, fibroblasts and tissues isolated from IPF patients display increased levels of SA-β-Gal staining and p21CIP1, suggesting a link with senescence. The detrimental nature of senescence in IPF was recently demonstrated using senolytics. Elimination of senescent

fibroblasts in a mouse model of lung fibrosis reduced expression of profibrotic SASP components and improved pulmonary function.

Nonalcoholic fatty liver disease

Cirrhosis is the pathological outcome from liver fibrosis and nonalcoholic fatty liver disease, which in turn is a result of hepatic steatosis, the abnormal accumulation of lipids in hepatocytes. Senescence is associated with liver fibrosis and cirrhosis. The risk of developing nonalcoholic fatty liver disease increases with age and is predicted by the presence of senescent hepatocytes. The elimination of senescent cells using INK-ATTC mice reduces liver fat accumulation. The role of senescence in the liver is complex, however, because knocking out p53 or p16INK4a increases liver fibrosis. Moreover, senescent hepatic stellate cells down-regulate collagen and up-regulate MMPs and cytokines that could remodel fibrotic plaques and recruit macrophages.

Cardiovascular disease

The risk of developing atherosclerosis and cardiomyopathy and their respective conditions, coronary heart disease and heart failure, increases with age. In the case of atherosclerosis, the role of senescence has been confirmed using senolytic models. Ablation of senescent cells improved the stability of plaques and reduced both the incidence and progression of plaque formation. Senescent cells were initially identified in atherosclerosis in vascular smooth muscle cells at the site of the plaque. Subsequent studies showed that macrophages were the primary senescent cell present with higher levels of SA-β-Gal staining and SASP production, suggesting their key contribution to coronary heart disease. Cardiomyocyte atrophy is one of the underlying causes of myocardial infarction in the elderly. It is unclear how ablation of senescent cells protects against cardiomyocyte hypertrophy in aged mice and provides resistance to cardiac stress.

Osteoarthritis

Lifelong wear and tear on ligaments is a significant risk factor for the development of arthritis. Failure of chondrocytes to produce cartilage results in degradation of joints and immobilization. Expression of p16INK4a in these cells correlates with severity and progression of the disease. Moreover, when mice were subjected to an acute trauma to model osteoarthritis, senescent cells accumulated in the site of the injury. Clearance of these senescent cells using senolytics resulted in the increased functionality of the remaining chondrocytes with rejuvenation of cartilage soon after.

Decline in immune function with age

One of the primary risk factors for complications in end-of life care is infection. The inability of the body to raise a response to immune offenses is caused by a functional decline in HSCs. The accumulation of senescent HSCs with age contributes to immune decline and senescence bypass allows for stem cell rejuvenation. Interestingly, the removal of these cells restored the functionality of HSCs and increased myeloid, B, and T cell numbers in transplant experiments.

Sarcopenia

Muscle stem cells (MuSCs) undergo a decline in their ability to differentiate and facilitate repair of muscle tissue, which is hypothesized to be the underlying cause of age-dependent muscle wasting or sarcopenia. MuSCs are quiescent unless stimulated to repair muscle. However, with age, they become senescent, up-regulating p16INK4a. The elimination of senescent MuSCs increases the ability of the remaining MuSCs to form muscle cell colonies. Additionally, inhibition of p38 or p16INK4a bypasses MuSC senescence and strengthens muscle in geriatric mice.

Age-related cachexia

Loss of adiposity and loss of muscle mass in aged individuals are primary contributors to age-dependent wastage or cachexia. White adipose tissue isolated from aged mice display SA-β-Gal activity. Removal of senescent cells from mice leads to increased adiposity and prevents mass loss in aged mice. Recently it has been shown that bypass of senescence or senolysis restores adipose beiging and adipogenesis and improves metabolic function in aged mice. This suggests that senescent cells prevent adipocyte differentiation and contribute to an age-dependent loss of adaptive thermogenic capacity and metabolic dysfunction.

Probable questions:

1. What is aging? Describe different hallmarks of aging?

2. What is senescence? How different pathways regulates senescence?

3. Describe the role of p53 and INK4/ARF locus in senescence.

4. How aging and senescence is related to various diseases?

Suggested Readings:

Senescence and aging: Causes, consequences, and therapeutic avenues. A Review. Domhnall McHugh,and Jesús Gil. 2017.

Group-B : Cytogenetics

Module	Unit	Contents	Credit	Class	Time (h)	
ZHT - 103 (Developmental Biology and Cytogenetics)	**VII**	Size, Complexity, Organization and nature of nuclear DNA in eukaryotes,	1.5	**1**	**1**	**133-141**
	VIII	Transposable elements, retro-transposons, SINE, LINE, Alu and other repeat elements, pseudogenes, segmental duplications ; super coiling of DNA		**1**	**1**	**142-172**
	IX	Organelle genome, architecture of mitochondrial genome, conserved chloroplast DNA;		**1**	**1**	**173-185**
	X	Cell cycle, apoptosis and cancer : Phases of cell cycle. Regulation of cell cycle: Discovery of MPF, cyclins and cyclin dependent kinases, Check points- role of Rb and p53		**1**	**1**	**186-217**
	XI	Cancer: Types and stages. Tumor suppressor genes and protooncogenes. Molecular basis of cancer.; Apoptosis: Neurotrophic factors, caspases, Pathways of apoptosis; cell senescence, telomerase		**1**	**1**	**218-273**
	XII	DNA replication: nature, enzymology of replication, replication fork; fidelity of replication; extrachromosomal replicons; leading and lagging strands; Okazaki fragments; termination of replication		**1**	**1**	**274-297**

Group-B : Cytogenetics

Unit-VII

Size, Complexity, Organization and nature of nuclear DNA in eukaryotes

Objectives:

In general, eukaryotic genomes are larger and more complex when compared to prokaryotic genomes. One obvious belief for this was the sheer number of genes present in complex organisms. However, over the years, scientists have learnt that the size of the genome is not related to the genetic complexity of the organism. For example, salamanders and lilies have 10 fold more DNA than humans, although they are less complex than humans. This difference in size is because of the large amounts of noncoding sequences that are present in the genome.

Genome Size and Complexity

A genome is all the genetic information of an organism. It consists of DNA (or RNA in RNA viruses). The genome includes the genes (the coding regions) and the noncoding DNA, as well as the genetic material of extrachromosomal origins like mitochondriaand chloroplasts. Many bacterial species have only one chromosome per cell and, in nearly all cases; each chromosome contains only one copy of each gene. A very few genes, such as those for rRNAs, are repeated several times. Genes and regulatory sequences account for almost all the DNA in bacteria. Moreover, almost every gene is precisely collinear with the amino acid sequence (or RNA sequence) for which it codes. The organization of genes in eukaryotic DNA is structurally and functionally much more complex. The study of eukaryotic chromosome structure, and more recently the sequencing of entire eukaryotic genomes, has yielded many surprises.

The genomes of most eukaryotes are larger and more complex than those of prokaryotes. This larger size of eukaryotic genomes is not inherently surprising, since one would expect to find more genes in organisms that are more complex. Many, if not most, eukaryotic genes have a distinctive and puzzling structural feature: their nucleotide sequences contain one or more intervening segments of DNA that do not code for the amino acid sequence of the polypeptide product. These nontranslated inserts interrupt the otherwise colinear relationship

between the nucleotide sequence of the gene and the amino acid sequence of the polypeptide it encodes. Such nontranslated DNA segments in genes are called intervening sequences or introns, and the coding segments are called exons (Figure 1).

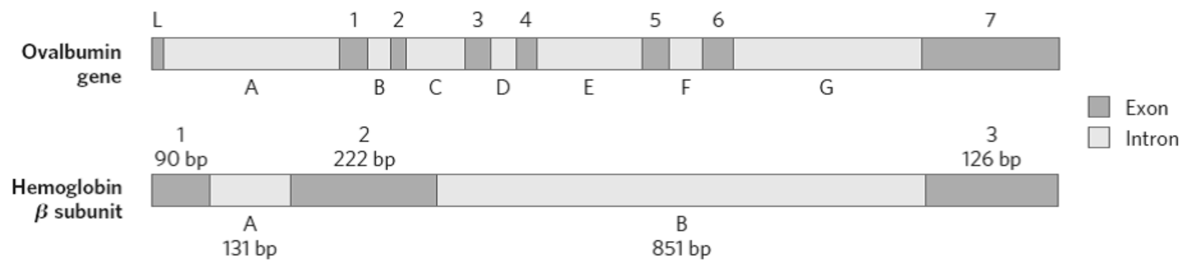


Figure 1: Structure of two eukaryotic genes.

However, the genome size of many eukaryotes does not appear to be related to genetic complexity (Figure 2 and 3). For example, the genomes of salamanders and lilies contain more than ten times the amount of DNA that is in the human genome, yet these organisms are clearly not ten times more complex than humans. This apparent paradox was resolved by the discovery that the genomes of most eukaryotic cells contain not only functional genes but also large amounts of DNA sequences that do not code for proteins. The difference in the sizes of the salamander and human genomes thus reflects larger amounts of noncoding DNA, rather than more genes, in the genome of the salamander. The presence of large amounts of noncoding sequences is a general property of the genomes of complex eukaryotes. Thus the thousand fold greater size of the human genome compared to that of *E. coli* is not due solely to a larger number of human genes. The human genome is thought to contain 20,000-25,000 genes- only about 5 times more than *E.coli* has. Much of the complexity of eukaryotic genomes thus results from the abundance of several different types of noncoding sequences, which constitute most of the DNA of higher eukaryotic cells. Thus several kinds of noncoding DNA contribute to the genomic complexity of higher eukaryotes. The lack of precise correlation between the complexity of an organism and the size of its genome was looked on as a bit of a puzzle, the so called **C-value paradox**. In fact the answer is quite simple: space is saved in the genomes of less-complex organisms because the genes are more closely packed together (Figure 2 and 3).

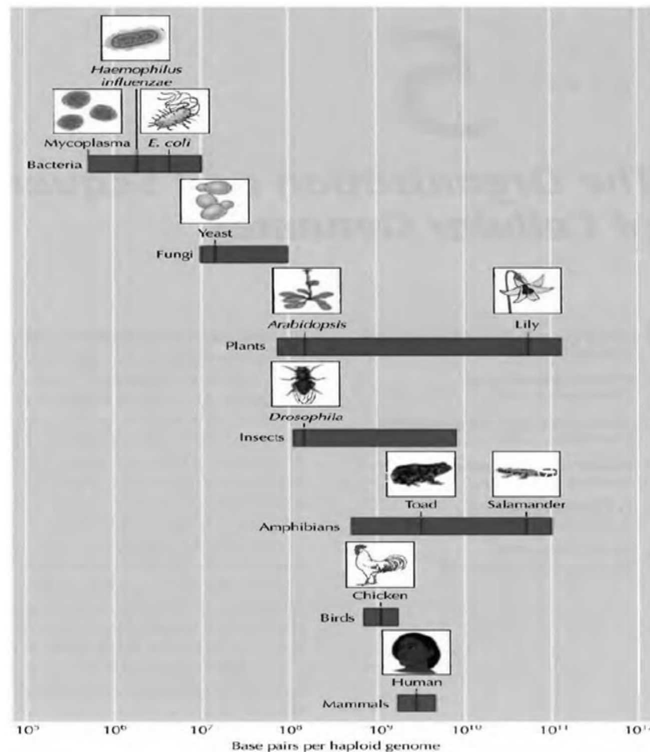


Figure 2: Genome size of representative groups of organisms.

In bacterial genomes, most of the DNA encodes proteins. For example, the genome of *E. coli* is approximately 4.6 x 10^6 base pairs long and contains about 4000 genes, with nearly 90% of the DNA used as protein-coding sequence. The yeast genome, which consists of 12 x 10^6 base pairs, is about 2.5 times the size of the genome of *E. coli,* but is still extremely compact. Only 4% of the genes of *Saccharomyces cerevisiae* contain introns, and these usually have only a single small intron near the start of the coding sequence. Approximately 70% of the yeast genome is used as protein-coding sequence, specifying a total of about 6000 proteins. The relatively simple animal genomes of C. *elegans* and *Drosophila* are about 10 times larger than the yeast genome, but contain only 2- 3 times more genes. Instead, these simple animal genomes contain more introns and more repetitive sequence, so that protein-coding sequences correspond to only about 25% of the C. *elegans* genome and about 13% of the genome of *Drosophila.* The genome of the model plant *Arabidopsis* contains a similar number of genes, with approximately 26% of the genome corresponding to protein-coding sequence. The genomes of higher animals (such as humans) are approximately 20-30 times larger than those of C. *elegans* and *Drosophila.* However, a major surprise from deciphering the human genome sequence was the discovery that the human genome contains only 20,000 to 25,000 gene (Figure 2 and 3). It appears that only about 1.2% of the human genome consists of

protein-coding sequence. Approximately, 20% of the genome consists of introns, and more than 60% is composed of various types of repetitive and duplicated DNA sequences, with the remainder corresponding to pseudogenes, to nonrepetitive spacer sequences between genes, and to exon sequences that are present at the 5' and 3' ends of mRNAs but are not translated into protein. The increased size of the genomes of higher eukaryotes is thus due far more to the presence of large amounts of repetitive sequences and introns than to an increased number of genes (Figure 1 and 2).

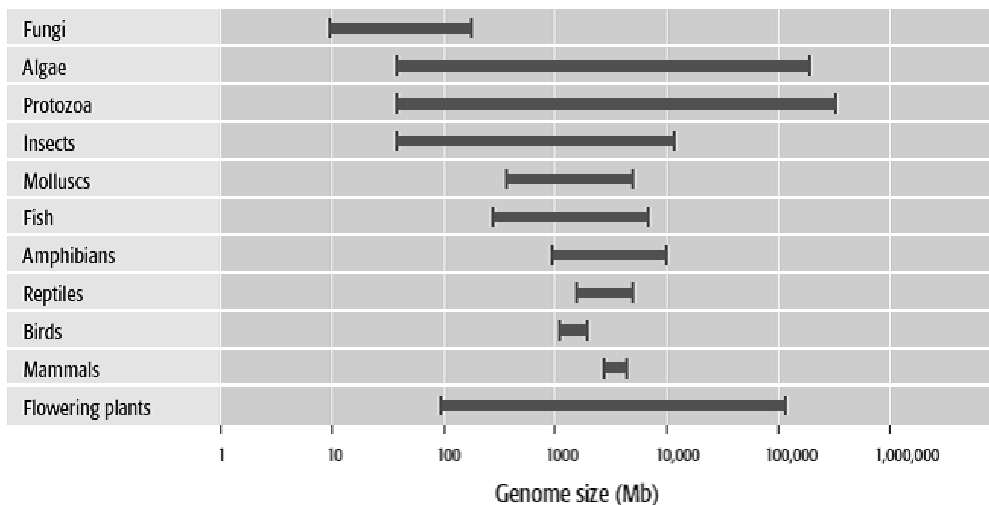


Figure 3: Genome size of eukaryotes.

Complexity of eukaryotic DNA sequences and repetitive DNA sequence

The term **sequence complexity** refers to the number of times a particular base sequence appears throughout the genome. Unique or nonrepetitive sequences are those found once or a few times within the genome. Structural genes are typically unique sequences of DNA. The vast majority of proteins in eukaryotic cells are encoded by genes present in one or a few copies. In the case of humans, unique sequences make up roughly 41% of the entire genome. Apart from unique DNA sequences there are repetitive DNAs, that is, sequences that are similar or identical to sequences elsewhere in the genome. Most large genomes are filled with repetitive sequences; for example, nearly half of the human genome is covered by repeats, many of which have been known about for decades. Although some repeats appear to be non-functional, others have played a part in human evolution at times creating novel functions, but also acting as independent, ‘selfish’ sequence elements. Repeats arise from a variety of biological mechanisms that result in extra copies of a sequence being produced and inserted into the genome. Repeats come in all shapes and sizes: they can be widely interspersed

repeats, tandem repeats or nested repeats, they may comprise just two copies or millions of copies, and they can range in size from 1–2 bases (mono-and dinucleotide repeats) to millions of bases.

One approach that has proven useful in understanding genome complexity has come from renaturation studies. These kinds of experiments were first carried out by Roy Britten and David Kohne in 1968. In a renaturation study, the DNA is broken up into pieces containing several hundred base pairs. The double-stranded DNA is then denatured (separated) into single-stranded pieces by heat treatment. When the temperature is lowered, the pieces of DNA that are complementary can reassociate, or renature, with each other to form double-stranded molecules.

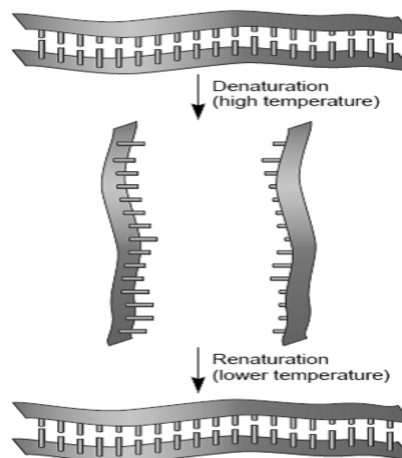


Figure 4: Denaturation and renaturation of DNA strands

The rate of renaturation of complementary DNA strands provides a way to distinguish between unique, moderately repetitive, and highly repetitive sequences. For a given category of DNA sequences, the renaturation rate depends on the concentration of its complementary partner. Highly repetitive DNA sequences renature much faster because many copies of the complementary sequences are present. In contrast, unique sequences, such as those found within most genes, take longer to renature because of the added time it takes for the unique sequences to find each other (Figure 5).

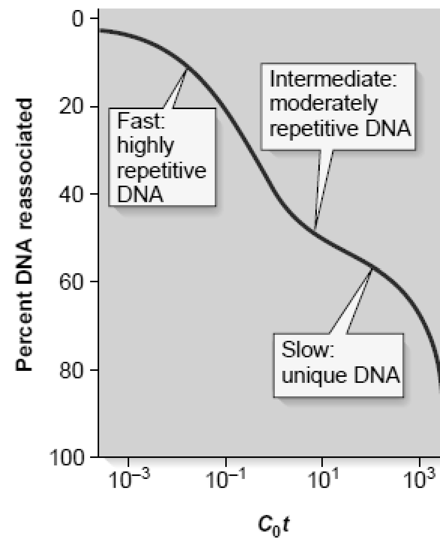


Figure 5:Renaturation of human DNA sequence complex.

Highly repeated DNA sequences

The highly repeated fraction (also called **tandem repeats**) constitutes anywhere from about 1 to 10 percent of the total DNA (Figure 6). These sequences are typically short (a few hundred nucleotides at their longest) and present in clusters in which the given sequence repeats itself over and over again without interruption. A sequence arranged in this end-to-end manner is said to be present *in tandem.* Approximately 3% of the human genome consists of **highly repetitive** sequences, also referred to as **simplesequence DNA** or **simple sequence repeats (SSR)**. These short sequences, generally less than 10 bp long, are sometimes repeated millions of times per cell. The simple sequence DNA has also been called **satellite DNA**, so named because its unusual base composition often causes it to migrate as "satellite" bands (separated from the rest of the DNA) when fragmented cellular DNA samples are centrifuged in a cesium chloride density gradient (Figure 7).

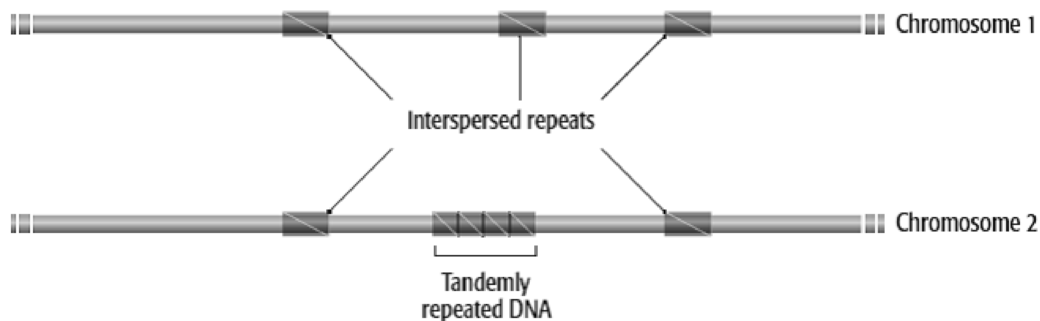


Figure 6: Two different types of repetitive sequence.

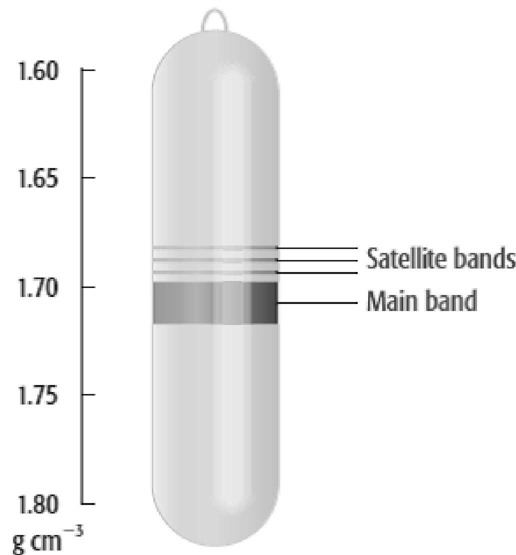


Figure 7: Satellite DNA from human genome.

Highly repeated sequences fall into several overlapping categories, including satellite DNAs, minisatellite DNAs, and microsatellite DNAs. The localization of satellite DNAs are found mostly within the centromeres and telomeres of chromosomes.

Satellite DNAs: Satellite DNAs consist of short sequences (about five to a few hundred base pairs in length) that form very large linear arrays, each containing up to several million base pairs of DNA. In many species, the base composition of these DNA segments is sufficiently different from the bulk of the DNA that fragments containing the sequence can be separated into a distinct "satellite" band during density gradient centrifugation (Figure 7). Satellite DNAs tend to evolve very rapidly, causing the sequences of these genomic elements to vary even between closely related species.

Minisatellite DNAs: Minisatellite sequences range from about 10 to 100 base pairs in length and are found in sizeable clusters containing as many as 3000 repeats. Thus, minisatellite sequences occupy considerably shorter stretches of the genome than do satellite sequences. Minisatellites tend to be unstable, and the number of copies of a particular sequence often increases or decreases from one generation to the next, most likely as the result of unequal crossing over. Consequently, the length of a particular minisatellite locus is highly variable in the population, even among members of the same family. Because they are so variable (or *polymorphic*) in length, minisatellite sequences form the basis for the technique of *DNA fingerprinting* , which is used to identify individuals in criminal or paternity.

Microsatellite DNAs: Microsatellites are the shortest sequences (1 to 9 base pairs long) and are typically present in small clusters of about 10 to 40 base pairs in length, which are scattered quite evenly through the genome. DNA replicating enzymes have trouble in copying regions of the genome that contain these small, repetitive sequences, which causes these stretches of DNA to change in length through the generations. Because of their variable lengths within the population, microsatellite DNAs have been used to analyze the relationships between different human populations.

Moderately repeated DNA sequence

The moderately repeated fraction of the genomes of plants and animals can vary from about 20 to more than 80 percent of the total DNA, depending on the organism. This fraction includes sequences that are repeated within the genome anywhere from a few times to tens of thousands of times (Figure 5). Included in the moderately repeated DNA fraction are some sequences that code for known gene products, either RNAs (such as rRNAs) or proteins (including histones), but the bulk of this DNA fraction lacks a coding function. Rather than occurring as clusters of tandem sequences, these noncoding elements are scattered (i.e., *interspersed*) throughout the genome (Figure 6). Most of these repeated sequences can be grouped into two classes that are referred to as SINEs (short interspersed elements) or LINEs (long interspersed elements). SINEs and LINEs sequences are discussed later.

Nonrepeated DNA sequence

As initially predicted by Mendel, classical studies on the inheritance patterns of visible traits led geneticists to conclude that each gene was present in one copy per single (haploid) set of chromosomes. When denatured eukaryotic DNA is allowed to reanneal, a significant fraction of the fragments are very slow to find partners, so slow in fact that they are presumed to be present in a single copy per genome (Figure 5). This fraction comprises the nonrepeated (or single-copy) DNA sequences, which includes the genes that exhibit Mendelian patterns of inheritance. Because they are present in a single copy in the genome, nonrepeated sequences localize to a particular site on a particular chromosome. Included within the nonrepeated fraction are the DNA sequences that code for virtually all proteins other than histones. Even though these sequences are not present in multiple copies, genes that code for polypeptides are usually members of a family of related genes. This is true for the globins, actins, myosins,

collagens, tubulins, integrins, and most other proteins in a eukaryotic cell. Each member of a multigene family is encoded by a different but related sequence.

Probable questions:

1. What is C-Value paradox?
2. What is Satellite DNA?
3. Distinguish between microsatellite and minisatellite DNA?
4. What is moderately repeated and non repeated DNA sequence?
5. The yeast genome is 0.004 times the size of the human genome and yet it contains approximately 0.2 times fewer genes. Give explanation.
6. What are introns and exons?
7. What differences in gene distribution and repetitive DNA content are seen when yeast and human chromosomes are compared?

Suggested Readings/References:

1. James D. Watson, Tania A. Baker, Stephen P. Bell-Molecular Biology of Gene
2. Robert J. Brooker-Genetics Analysis and Principles
3. Benjamin Lewin-Genes IX
4. Harvey F Lodish et al-Molecular Cell Biology
5. Gerald Karp-Cell and Molecular Biology
6. Geoffrey M. Cooper, Robert E. Hausman-The Cell:A Molecular Approach
7. T.A. Brown-Genomes
8. William S. Klug, Michael R. Cummings, Charlotte A. Spencer, Michael A. Palladino-Concepts of Genetics.
9. Anthony J.F. Griffiths, Susan R. Wessler, Sean B. Carroll, John Doebley-An Introduction to Genetic Analysis.
10. Other Internet Websites and Sources.

Unit-VIII

Transposable elements, retro- transposons, SINE, LINE, Alu and other repeat elements, pseudogenes, segmental duplications ; super coiling of DNA.

Objectives:

Factors contributing to the size and complexity of the genome are: Introns and exons, Pseudogene, repetitive sequences, gene families etc. In addition in eukaryotic cells the length of DNA in the nucleus is far greater than the size of the compartment in which it is contained. To fit into this compartment the DNA is not packaged directly into final structure of chromatin. It contains several hierarchies of organization. The first level of packing is achieved by the winding of DNA around a protein core to produce a "bead-like" structure called a nucleosome. Subsequently, a series of levels of compaction and condensation produce the structural chromosomes in the nucleus. These levels of organization are dynamic. Often the dynamic nature of DNA in its organization pattern produce a coiled-coiled structure which should be properly segregated to maintain its structural and functional property. Supercoiling is introduced into DNA molecules when the double helix is twisted around its own axis in three-dimensional space. Generally, DNA molecules are negatively supercoiled inside cells, although the level of supercoiling is not equal throughout the genome and many supercoils may be constrained by bound proteins. When the ends of a linear DNA molecule are ligated to produce a covalently closed circle, the two strands become intertwined like the links of a chain, and will remain so unless one of the strands is broken.

Transposable Elements and retro transposons:

Definition of Transposons:

Presence of transposable elements was first predicted by Barbara McClintock in maize (corn) in late 1940s. After several careful studies, she found that certain genetic elements were moving from one site to an entirely different site in the chromosome. She called this phenomenon of changing sites of genetic elements as transposition and those genetic elements were called by her as controlling elements.

These controlling elements were later on called as transposable elements by Alexander Brink. In late 1960s this phenomenon was also discovered in bacteria.

Consequently, the molecular biologists called them as Transposons. A transposon may be defined as: "a DNA sequence that is able to move or insert itself at a new location in the genome." The phenomenon of movement of a transposon to a new site in the genome is referred to as transposition.

Transposons are found to encode a special protein named as transposase which catalyses the process of transposition. Transposons are particular to different groups of organisms. They constitute a fairly accountable fraction of genome of organisms like fungi, bacteria, plants, animals and humans. Transposons have had a major impact on changing or altering the genetic composition of organisms.

Transposons or transposable genetic elements are often referred to as 'mobile genetic elements' also. They can be categorized on different bases like their mode of transposition or on the basis of the organisms in which they are present.

Types of Transposons:

Different transposons may change their sites by following different transposition mechanisms.

On the basis of their transposition mechanism, transposons may be categorized into following types:

(i) Cut-and-Paste Transposons:

They transpose by excision (cutting) of the transposable sequence from one position in the genome and its insertion (pasting) to another position within the genome (Fig. 1).

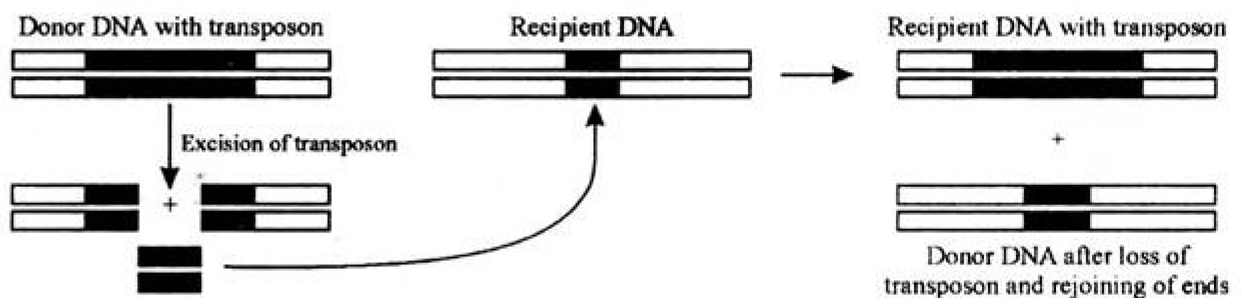


Fig. 1. Cut and Paste Transposons.

The cut-and-paste transposition involves two transposase subunits. Each transposase submit binds to the specific sequences at the two ends of transposon. These subunits of transposase protein then come together and lead to the excision of transposon.

This excised 'transposon-Transposase Complex' then gets integrated to the target recipient site. In this manner, the transposon is cut from one site and then pasted on other site by a mechanism mediated by transposase protein (Fig. 2).

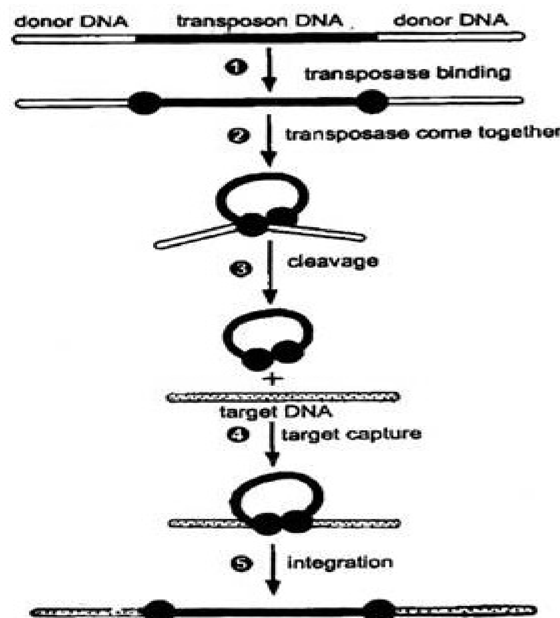


Fig. 2. Role of Transposase protein in cut-and-paste transposition.

Examples of cut-and-paste type of transposons are IS-elements, P-elements in maize, hobo-elements in *Drosophila* etc.

(ii) Replicative Transposons:

They transpose by a mechanism which involves replication of transposable sequence and this copy of DNA, so formed, is inserted into the target site while the donor site remains unchanged (Fig. 3). Thus, in this type of transposition, there is a gain of one copy of transposon and both-the donor and the recipient DNA molecule are having one-one transposable sequence each, after transposition.

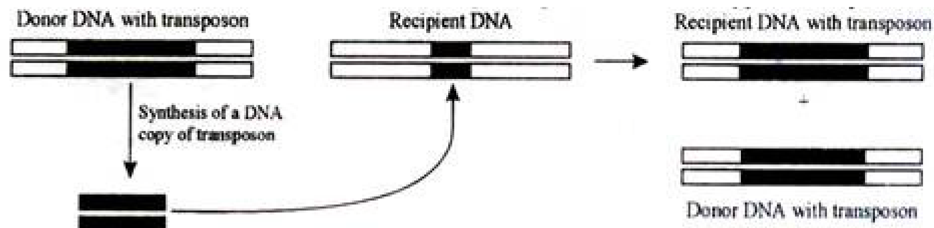


Fig. 3. Replicative Transposons.

Tn3-elements found in bacteria are good examples of such type of transposons.

(iii) Retro Elements:

Their transposition is accomplished through a process which involves the synthesis of DNA by reverse transcription (i.e. RNA to DNA) by using elements RNA as the template (Fig. 4). This type of transposition involves an RNA intermediate, the transposable DNA is transcribed to produce an RNA molecule.

This RNA is then used as a template for producing a complementary DNA by the activity of enzyme reverse transcriptase. This single stranded DNA copy so formed, is then made double stranded and then inserted into the target DNA site. The transposable elements which require reverse transcriptase tor their movement are called retro transposons.

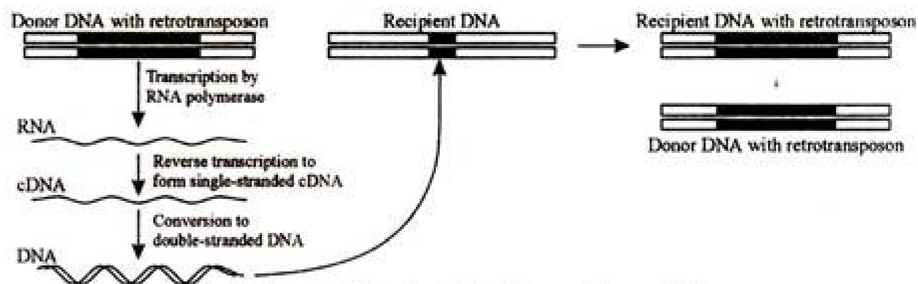


Fig. 4. Transposition Involving Reverse Transcription.

The Retro elements may be viral or non-viral. Out of these two, the non-viral retro elements are important and may further be classified as:

(A) Retrovirus like elements:

They carry long terminal repeats (LTR). Examples are copia, gypsy elements in *Drosophila*.

Retroposons:

LTR are absent. Examples are LINEs and SINEs in humans.

Transposable Elements in Prokaryotes:

Although the presence of transposons was predicted in eukaryotes but first observation at molecular level was done in bacteria, which is a prokaryote.

Bacterial transposable elements are of the following types:

(a) Insertion Sequences or IS Elements:

They are the transposable sequences which can insert at different sites in the bacterial chromosomes.

IS-elements contain ITRs (Inverted Terminal Repeats), these were first observed in *E.coli*. IS elements are relatively short usually not exceeding 2500 bp. The ITRs present at the ends of IS-elements are an important feature which enables their mobility. The ITRs present in the IS-elements of E.coli usually range between 18-40 bp.

The term 'Inverted Terminal Repeat' (ITR) implies that the sequence at 5 end of one strand is identical to the sequence at 5 end of th e other strand but they run in inverse opposite direction (Fig. 5). In *E.coli* chromosome, a number of copies of several IS-elements like IS1, IS2, IS3, IS4 and IS5 are present.

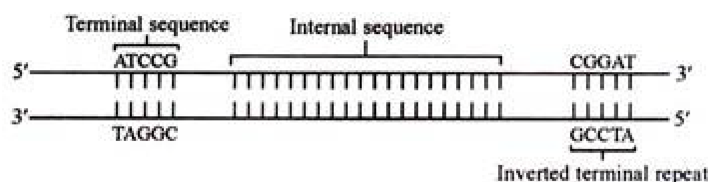


Fig. 5. An Insertion sequence (IS-element) with inverted terminal repeats (ITRs)

(b) Prokaryotic Transposon Element:

These are also called composite transposons and are shown by the symbol Tn. It is made up of two IS elements, one present at each end of a DNA sequence which contains genes whose functions are not related to the transposition process. These transposons have been found to have inverted repeats at the ends. The length of these inverted repeats ranges from a few nucleotides to about 1500 bp.

It can be said that these are the large transposons which are formed by capturing of an immobile DNA sequence within two insertion sequences thus enabling it to move. Examples of such transposons include the members of Tn series like Tn1, Tn5, Tn9, Tn10, etc.

Transposable Elements in Eukaryotes:

(a) Transposons in Maize:

Different types of transposons present in maize are described below:

Ac-Ds system:

This system of transposable elements in maize was analysed and given by Barbara Mc. Clintock. Here Ac stands for Activator and Ds for Dissociation. Barbara found that Ds and Ac genes were sometimes mobile and moved to different chromosomal locations thus resulting in different kernel phenotypes.

Ds element is activated by Ac and on activation it serves as the site provider for breakage in chromosome. Ac can move autonomously while Ds can move only in the presence of Ac (Fig. 6). The transposition involving this Ac-Ds system produces altered kernel phenotypes.

Other transposable elements of maize are:

i. spm (suppressor mutator) system,

ii. dt (dotted) system,

iii. Mu (Mutator) system, etc.

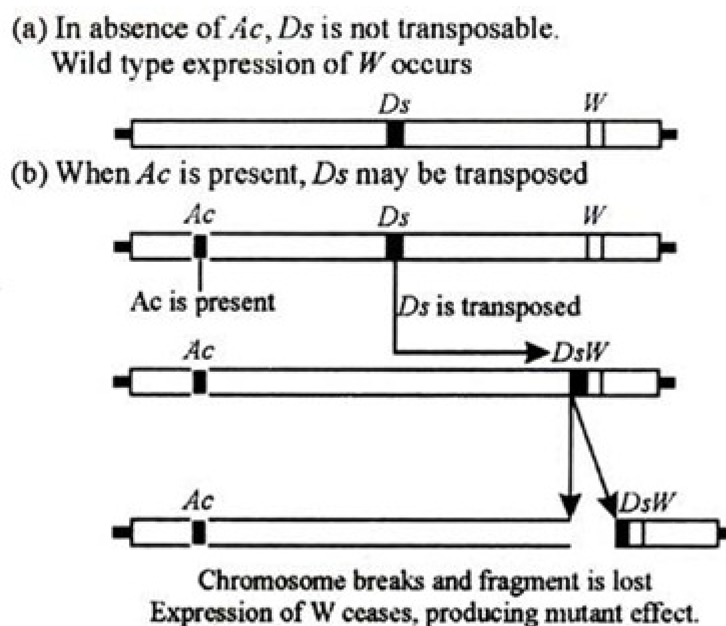


Fig. 6. Effects of Transposition involving *Ac-Ds* system in maize.

(b) Transposons in *Drosophila*:

A number of transposable elements are found in Drosophila which are of different types and account for a quite high fraction of *Drosophila* genome.

Some of these transposons are given below:

P-elements:

These were discovered during the study of 'hybrid-dysgenesis'which is a sterility causing condition. They are 2.9 kb long and contain 31 bp long inverted terminal repeats High rate of P-element transposition causes hybrid dysgenesis. P-elements encode transposase enzyme which helps in their transposition. These are also useful as vectors for introducing foreign genes into Drosophila.

Copia-elements:

Their transposition causes mutations for eye-colour in *Drosophila*. They are of size approximately 5-8 kb with direct terminal repeat (DTR) of about 276 bp at each end. Within each of this direct repeats is present short inverted repeat (IR) of about 17 bp length. About 10-80 copia- elements are present in cell-genome (Fig. 7).

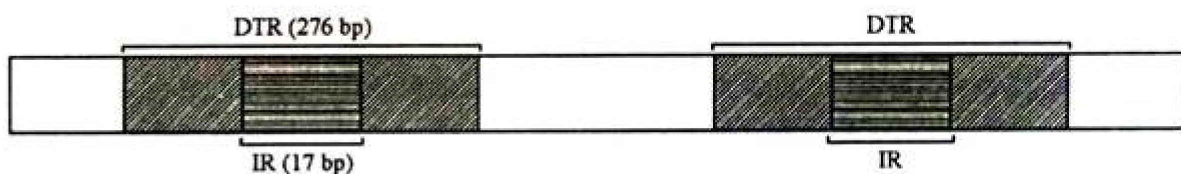


Fig. 7. Organization of a copia transposable element in *D. melanogaster*.

FB Elements:

These are the fold back elements present in *Drosophila* genome. These have ability to fold back to form a stem and loop structure due to the presence of long inverted terminal repeats. Their transposition results into a changed expression by causing mutation by insertion or by affecting the normal gene expression.

Other important types of transposable elements found in *Drosophila* are:

i. I elements,

ii. Mariner elements,

iii. Gypsy elements,

iv. Hobo elements, etc.

(c) Transposons in Humans:

Transposons in humans are in the form of repetitive DNA which consists of sequences that are interspersed within the entire human genome. These sequences are transposable and can move to different locations within the genome.

Significance of Transposable Elements:

1. Transposons may change the structural and functional characteristics of genome by changing their position in the genome.

2. Transposable elements cause mutation by insertion, deletion, etc.

3. Transposons make positive contribution in evolution as they have tremendous impact on the alteration of genetic organisation of organisms.

4. They are useful as cloning vectors also, in gene cloning. For example, P-elements are frequently used as vector for introducing transgenes into *Drosophila*.

5. Transposons may also be used as genetic markers while mapping the genomes.

6. Transposon-mediated gene tagging is done for searching and isolation of a particular gene.

LINEs, SINEs and Alu repeats

There are other repetitive DNA sequences which are scattered throughout the genome rather than being clustered as tandem repeats. These interspersed repetitive elements are a major contributor to genome size, accounting for approximately 45% of human genomic DNA (Table 1). The two most prevalent classes of these sequences are called SINEs (short interspersed elements) and LINEs (long interspersed elements). Both SINEs and LINEs are examples of transposable elements, which are capable of moving to different sites in genomic DNA.

Type of sequence	Number of copies	Fraction of genome
Simple-sequence repeats[a]	>1,000,000	~10%
Retrotransposons		
LINEs	850,000	21%
SINEs	1,500,000	13%
Retrovirus-like elements	450,000	8%
DNA transposons	300,000	3%

Table 1: Type of repetitive sequence and their percentage in human genome.

LINEs (long interspersed nuclear elements) have been very successful transposons. They have a comparatively long evolutionary history, occurring in other mammals, including mice. As autonomous transposons, they can make all the products needed for retrotransposition, including the essential reverse transcriptase. Human LINEs consist of three distantly related families: LINE-1, LINE-2, and LINE-3, collectively comprising about 20% of the genome. They are located primarily in euchromatic regions and are located preferentially in the dark AT-rich G bands (Giemsa-positive) of metaphase chromosomes. Of the three human LINE families, LINE-1 (or L1) is the only family that continues to have actively transposing members. LINE-1 is the most important human transposable element and accounts for a higher fraction of genomic DNA (17%) than any other class of sequence in the genome.

Full-length LINE-1 elements are more than 6 kb long and encode two proteins: an RNA-binding protein and a protein with both endonuclease and reverse transcriptase activities (Figure 8). The 6.1 kb LINE-1 element has two open reading frames: ORF1, a 1 kb open reading frame, encodes p40, an RNA-binding protein that has a nucleic acid chaperone activity; the 4 kb ORF2 specifies a protein with both endonuclease and reverse transcriptase activities. A bidirectional internal promoter lies within the 5′ untranslated region (UTR). At the other end, there is an A*n*/T*n* sequence, often described as the 3′ poly(A) tail (pA). Unusually, an internal promoter is located within the 5′ untranslated region. Full-length copies therefore bring with them their own promoter that can be used after integration in a permissive region of the genome. After translation, the LINE-1 RNA assembles with its own encoded proteins and moves to the nucleus. To integrate into genomic DNA, the LINE-1 endonuclease cuts a DNA duplex on one strand, leaving a free 3′ OH group that serves as a primer for reverse transcription from the 3′ end of the LINE RNA. The endonuclease's preferred cleavage site is TTTT ↓A; hence the preference for integrating into AT-rich regions. AT-rich DNA is comparatively gene-poor, and so because LINEs tend to integrate

into AT-rich DNA they impose a lower mutational burden, making it easier for their host to accommodate them. During integration, the reverse transcription often fails to proceed to the 5′ end, resulting in truncated, nonfunctional insertions. Accordingly, most LINE-derived repeats are short, with an average size of 900 bp for all LINE-1 copies, and only about 1 in 100 copies are full length. The LINE-1 machinery is responsible for most of the reverse transcription in the genome, allowing retrotransposition of the nonautonomous SINEs and also of copies of mRNA, giving rise to processed pseudogenes and retrogenes. Of the 6000 or so full-length LINE-1 sequences, about 80–100 are still capable of transposing, and they occasionally cause disease by disrupting gene function after insertion into an important conserved sequence.

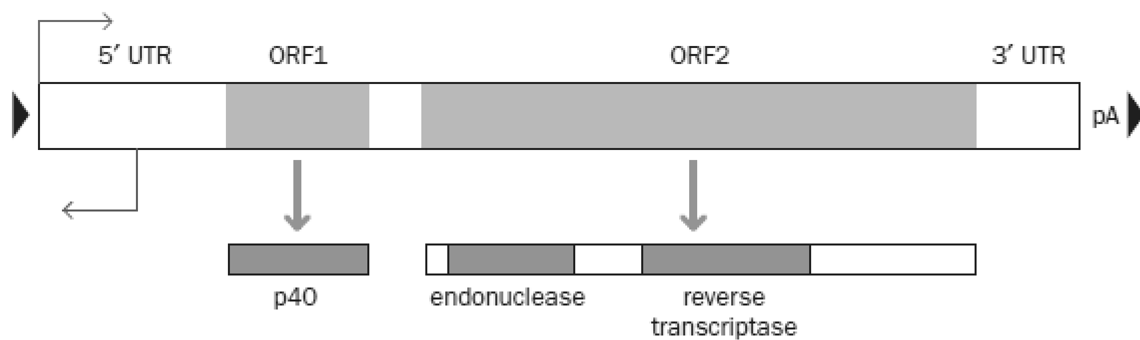


Figure 8: LINE-1 repeats element

SINES and Alu repeats

SINEs (short interspersed nuclear elements) are retrotransposons about 100–400 bp in length. They have been very successful in colonizing mammalian genomes, resulting in various interspersed DNA families, some with extremely high copy numbers. Unlike LINEs, SINEs do not encode any proteins and they cannot transpose independently. However, SINEs and LINEs share sequences at their 3′ end, and SINEs have been shown to be mobilized by neighboring LINEs. By parasitizing on the LINE element transposition machinery, SINEs can attain very high copy numbers. The human **Alu family** is the most prominent SINE family in terms of copy number, and is the most abundant sequence in the human genome, occurring on average more than once every 3 kb. The full-length Alu repeat is about 280 bp long and consists of two tandem repeats, each about 120 bp in length followed by a short A*n*/T*n* sequence. The tandem repeats are asymmetric: one contains an internal 32 bp sequence that is lacking in the other (Figure 9). Monomers containing only one of the two tandem repeats, and various truncated versions of dimers and monomers are also common, giving a genome wide average of 230 bp. Like other mammalian SINEs,

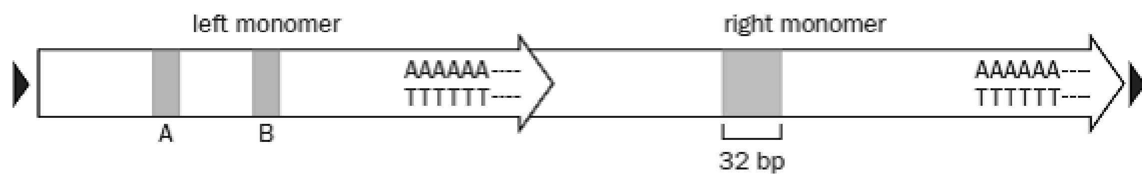


Figure 9: Alu repeats elements

Alu repeats originated from cDNA copies of small RNAs transcribed by RNA polymerase III. Genes transcribed by RNA polymerase III often have internal promoters, and so cDNA copies of transcripts carry with them their own promoter sequences. Both the Alu repeat and, independently, the mouse B1 repeat originated from cDNA copies of 7SL RNA, the short RNA that is a component of the signal recognition particle, using a retrotransposition mechanism. Other SINEs, such as the mouse B2 repeat, are retrotransposed copies of tRNA sequences. Alu repeats have a relatively high GC content and, although dispersed mainly throughout the euchromatic regions of the genome, are preferentially located in the GC-rich and gene-rich R chromosome bands, in striking contrast to the preferential location of LINEs in AT-rich DNA.

Segmental duplication

The human genome contains numerous blocks of highly homologous duplicated sequence. Segmental duplications (also termed "low-copy repeats") are blocks of DNA that range from 1 to 400 kb in length, occur at more than one site within the genome, and typically share a high level of (>90%) sequence identity. Both in situ hybridization and *in silico* analyses have shown that ~5% of the human genome is composed of duplicated sequence and many studies have noted a significant association between the location of segmental duplications and regions of chromosomal instability or evolutionary rearrangement. Indeed, segmental duplications have been implicated as the probable mediators of >25 recurrent genomic. Molecular studies have shown that the presence of large, highly homologous flanking repeats predisposes these regions to recurrent rearrangement by nonallelic homologous recombination, resulting in deletion, duplication, or inversion of the intervening sequence.

Large-scale subgenomic duplications can arise as a result of chromosome translocations. Euchromatic regions close to human centromeres and to telomeres (pericentromeric and subtelomeric regions, respectively) are comparatively unstable and are prone to

recombination with other chromosomes. As a result, large segments of DNA containing multiple genes have been duplicated. Within the past 40 million years of primate evolution, this process has led to the duplication of about 400 large (several megabases long) DNA segments, accounting for more than 5% of the euchromatic genome. This type of duplication, known as segmental duplication, results in very high (often more than 95%) sequence identity between the DNA copies and can involve both intrachromosomal duplications and also interchromosomal duplications. Segmental duplications are important contributors to copy-number variation and to chromosomal rearrangements leading to disease and rapid gene innovation. Segmental duplication at its simplest involves the tandem duplication of some region within a chromosome (typically because of an aberrant recombination event at meiosis). However, in many cases the duplicated regions are on different chromosomes, implying that either there was originally a tandem duplication followed by a translocation of one copy to a new site or that the duplication arose by some different mechanism altogether. The extreme case of a segmental duplication is when a whole genome is duplicated, in which case the diploid genome initially becomes tetraploid. As the duplicated copies evolve differences from one another, the genome may gradually become effectively a diploid again, although homologies between the diverged copies leave evidence of the event. Locating and characterizing these segmental duplications is of great interest because these recent genomic changes might have significantly contributed to the species divergence between human and the apes or Old World monkeys and because some genomic rearrangements have been found to be the causes of several genetic diseases in humans.

Pseudogene

Pseudogenes are usually thought of as defective copies of a functional gene to which they show significant sequence homology. They typically arise by some kind of gene duplication event that produces two gene copies. Selection pressure to conserve gene function need only be imposed on one gene copy; the other copy can be allowed to mutate more freely (*genetic drift*) and can pick up inactivating mutations, producing a pseudogene (Figure 10).

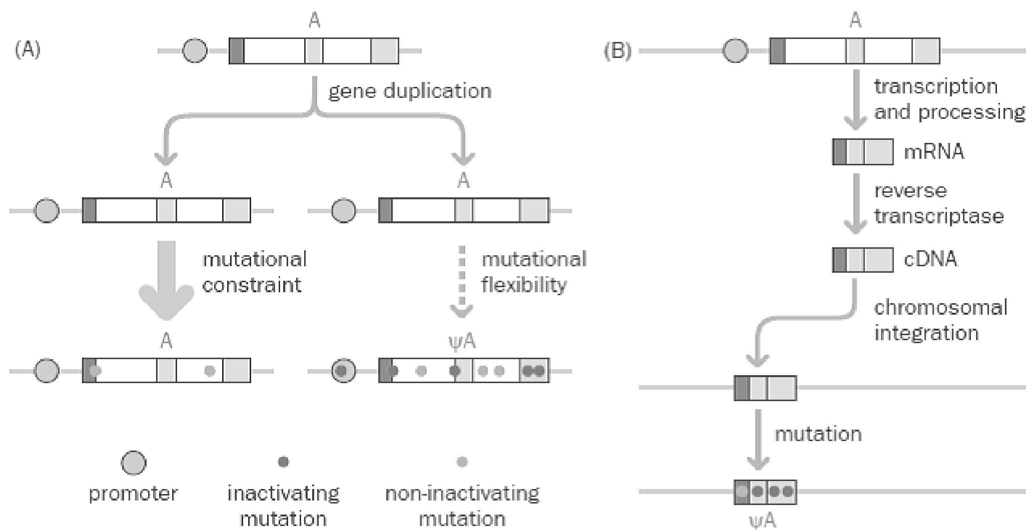


Figure 10. Mechanism of origin of pseudogene (A) Non-processed pseudogene (B) Processed pseudogene

However, some sequences are referred to as pseudogenes even though they have not originated by DNA copying. Humans have rare *solitary pseudogenes* that are clearly orthologs of functional genes in the great apes and became defective after acquiring harmful mutations in the human lineage. Different gene duplication mechanisms can give rise to multiple functional gene copies and defective pseudogenes. Either the genomic DNA sequence is copied, or a cDNA copy is made (after reverse transcription of a processed RNA transcript) that integrates into genomic DNA (Figure 10). Gene families frequently have defective gene copies in addition to functional genes (Figure 11). They are represented by the symbol Ψ corresponding to the active gene.

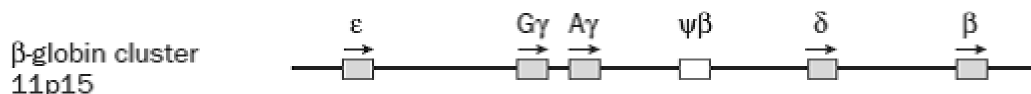


Figure 11: Pseudogene in β-globin gene cluster.

For a protein-coding gene, copying at the genomic DNA level can result in duplication of the promoter and upstream regulatory sequences as well as of all exons and introns. A defective gene that derives from a copy of a genomic DNA sequence is known as a *nonprocessed pseudogene* (Figure 10). Such pseudogenes usually arise by tandem duplication so that they are located close to functional gene counterparts, but some are dispersed as a result of recombination. Copying at the cDNA level produces a gene copy that typically lacks introns,

promoter elements, and upstream regulatory elements. Very occasionally, a processed gene copy can retain some function (a *retrogene*). However, because they lack important sequences needed for expression, most processed gene copies degenerate into *processed pseudogene. Processed pseudogenes* are defective copies of a gene that contain only exonic sequences and lack an intronic sequence or upstream promoter sequences. They arise by *retrotransposition*: cellular reverse transcriptases can use processed gene transcripts such as mRNA to make cDNA that can then integrate into chromosomal DNA (Figure 10). Processed pseudogenes are common in interspersed gene families. Processed pseudogenes lack a promoter sequence and so are typically not expressed. Sometimes, however, the cDNA copy integrates into a chromosomal DNA site that happens, by chance, to be adjacent to a promoter that can drive expression of the processed gene copy. Selection pressure may ensure that the processed gene copy continues to make a functional gene product, in which case it is described as a retrogene. A variety of intronless retrogenes are known to have testis-specific expression patterns and are typically autosomal homologs of an intron-containing X-linked gene. Eukaryotic genomes typically have many pseudogenes. A longstanding rationale for their abundance is that gene duplication is evolutionarily advantageous. New functional gene variants can be created by gene duplication, and pseudogenes have long been viewed as unsuccessful by-products of the duplication mechanisms. Although some prokaryotic genomes seem to have many pseudogenes, pseudogenes are generally rare in prokaryotes because their genomes are generally designed to be compact. The great majority of what are conventionally recognized as human pseudogenes are copies of protein-coding genes simply because it is relatively easy to identify them (by looking for frameshifting, splice site mutations, and so on). There are more than 8000 different processed pseudogene copies of protein-coding genes in the human genome, plus more than 4000 nonprocessed pseudogenes

DNA Supercoiling

"Supercoiling" means the coiling of a coil. An old fashioned telephone cord, for example, is typically a coiled wire. The path taken by the wire between the base of the phone and the receiver often includes one or more supercoils **(Figure 12)**.

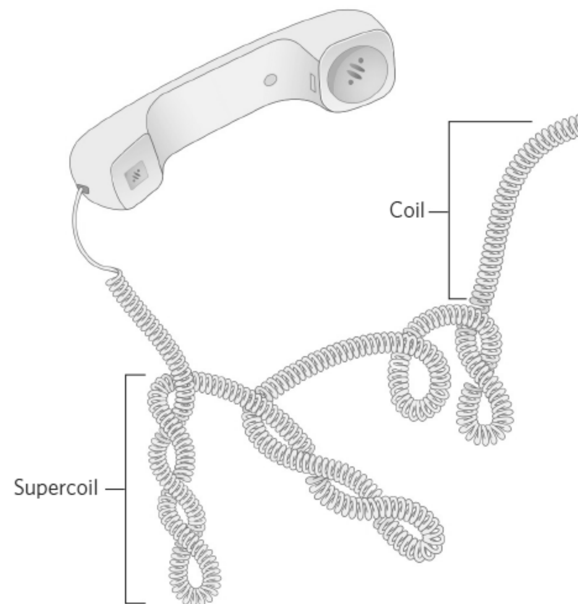


Figure 12: Supercoiling

DNA is coiled in the form of a double helix, with both strands of the DNA coiling around an axis. The further coiling of that axis upon itself (Figure 13) produces DNA supercoiling. As detailed below, DNA supercoiling is generally a manifestation of structural strain. When there is no net bending of the DNA axis upon itself, the DNA is said to be in a relaxed state.

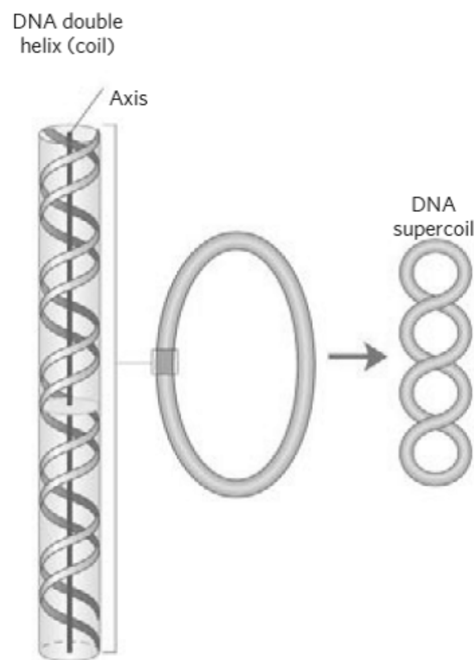


Figure 13: Supercoiling of DNA

As DNA is a flexible structure, its exact molecular parameters are a function of both the surrounding ionic environment and the nature of the DNA-binding proteins with which it is complexed. Because their ends are free, linear DNA molecules can freely rotate to accommodate changes in the number of times the two chains of the double helix twist about each other. But if the two ends are covalently linked to form a circular DNA molecule and if there are no interruptions in the sugar-phosphate backbones of the two strands, then the absolute number of times the chains can twist about each other cannot change. Such a covalently closed, circular DNA is said to be topologically constrained. Even the linear DNA molecules of eukaryotic chromosomes are subject to topological constraints due to their extreme length, entrainment in chromatin, and interaction with other cellular components.

Covalently closed, circular DNA, which is referred to as cccDNA has no interruptions in either polynucleotide chain. The two strands of cccDNA cannot be separated from each other without the breaking of a covalent bond. If we wished to separate the two circular strands without permanently breaking any bonds in the sugar-phosphate backbones, we would have to pass one strand through the other strand repeatedly. The number of times one strand would have to be passed through the other strand in order for the two strands to be entirely separated from each other is called the linking number.

The linking number is the sum of two geometric components called the twist and the writhe. Twist is simply the number of helical turns of one strand about the other, that is, the number of times one strand completely wraps around the other strand. Consider a cccDNA that is lying flat on a plane. In this flat conformation, the linking number is fully composed of twist. Indeed, the twist can be easily determined by counting the number of times the two strands cross each other (Figure 14). The helical crossovers (twist) in a right-handed helix are defined as positive such that the linking number of DNA will have a positive value. But cccDNA is generally not lying flat on a plane. Rather, it is usually torsionally stressed such that the long axis of the double helix crosses over itself, often repeatedly, in three-dimensional space (Figure 14). This is called writhe.

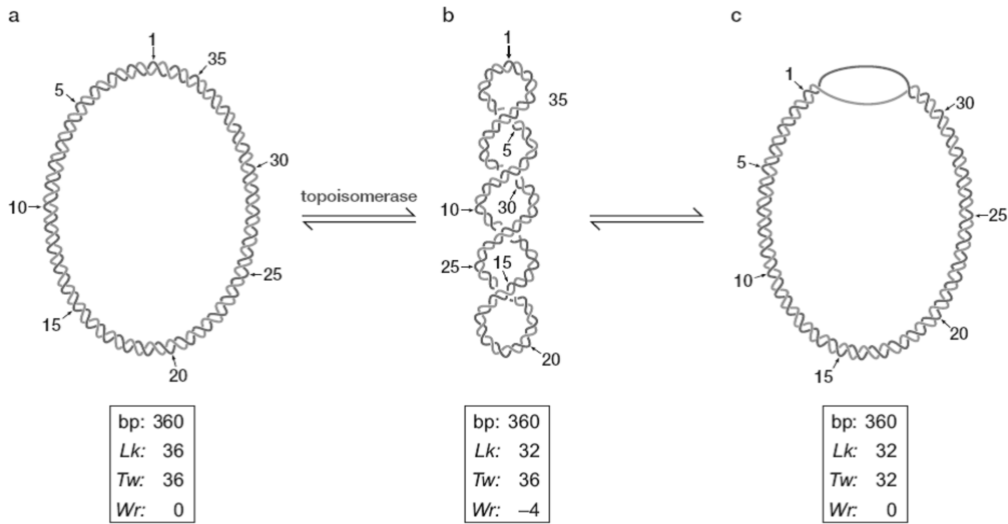


Figure 14: Topological states of covalently closed circular DNA (cccDNA)

To visualize the distortions caused by torsional stress, think of the coiling of a telephone cord that has been overtwisted. Writhe can take two forms. One form is the interwound or plectonemic writhe, in which the long axis is twisted around itself. The other form of writhe is a toroid or spiral in which the long axis is wound in a cylindrical manner, as often occurs when DNA wraps around protein (Figure 15). The writhing number (Wr) is the total number of interwound and/or spiral writhes in cccDNA Interwound writhe and spiral writhe are topologically equivalent to each other and are readily interconvertible geometric properties of cccDNA. Also, twist and writhe are interconvertible. A molecule of cccDNA can readily undergo distortions that convert some of its twist to writhe or some of its writhe to twist without the breakage of any covalent bonds. The only constraint is that the sum of the twist number (Tw) and the writhing number (Wr) must remain equal to the linking number (Lk). This constraint is described by the equation:

$$\mathbf{Lk = Tw + Wr}$$

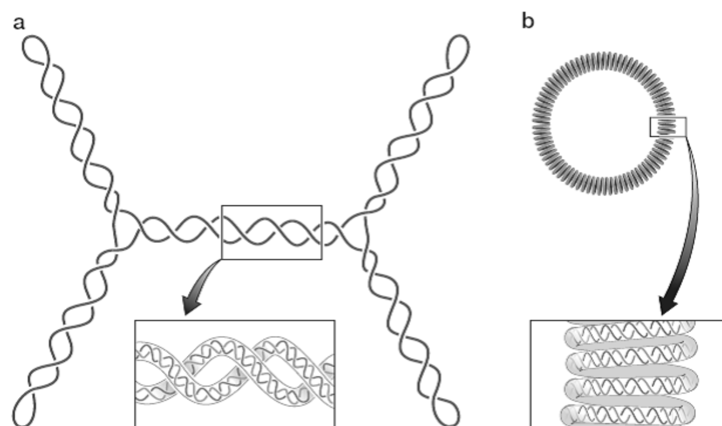


Figure 15: Two forms of Writhe (a) Toroid or spiral and (b) Interwound or Plectonemic

The cccDNA that is free of supercoiling (i.e., relaxed) and whose twist corresponds to that of the B-form of DNA in solution under physiological conditions (~10.5 bp per turn of the helix), the linking number (Lk) of such cccDNA under physiological conditions is assigned the symbol Lk^0. Lk^0 for such a molecule is the number of base pairs divided by 10.5. For a cccDNA of 10,500 base pairs, Lk= +1000. (The sign is positive because the twists of DNA are right-handed). One way to see this is to imagine pulling one strand of the 10,500-bp cccDNA out into a flat circle. If we did this, then the other strand would cross the flat circular strand 1000 times.

To remove supercoils from cccDNA (if it is not already relaxed), one procedure is to treat the DNA mildly with the enzyme DNase I, so as to break on average one phosphodiester bond (or a small number of bonds) in each DNA molecule. Once the DNA has been "nicked" in this manner, it is no longer topologically constrained, and the strands can rotate freely, allowing writhe to dissipate (Fig. 16).

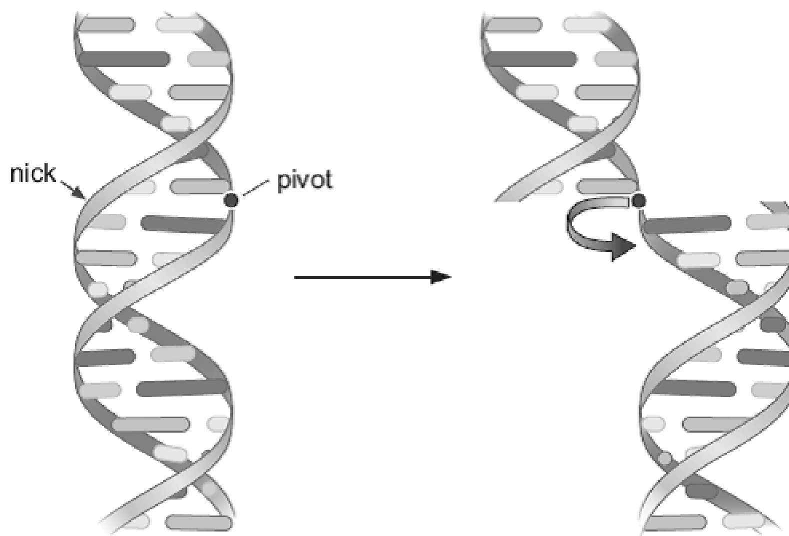


Figure 16: Relaxing DNA with DNase I

If the nick is then repaired, the resulting cccDNA molecules will be relaxed and will have on average an Lk that is equal to Lk^0.

The extent of supercoiling is measured by the difference between Lk and Lk^0, which is called the linking difference. The equation is given below

$$\mathbf{\Delta Lk = Lk - Lk^0}$$

If the ΔLk of a cccDNA is significantly different from 0, then the DNA is torsionally strained, and hence it is supercoiled. If $Lk < Lk0$ and $\Delta Lk < 0$, then the DNA is said to be "negatively supercoiled." Conversely, if $Lk > Lk^0$ and $\Delta Lk > 0$, then the DNA is "positively supercoiled." For example, the molecule shown in Figure 15b is negatively supercoiled and has a linking difference of –4 because its Lk (32) is 4 less than that (36) for the relaxed form of the molecule shown in Figure 15a. Because ΔLk and Lk^0 are dependent on the length of the DNA molecule, it is more convenient to refer to a normalized measure of supercoiling. This is the superhelical density, which is assigned the symbol σ and is defined as

$$\sigma = \Delta Lk / Lk0$$

The linking number is an invariant property of DNA that is topologically constrained. It can be changed only by introducing interruptions into the sugar–phosphate backbone. A remarkable class of enzymes known as topoisomerases are able to do just this by introducing transient single-strand or double-strand breaks into the DNA. Topoisomerases are of two general types. Type II topoisomerases change the linking number in steps of two. They make transient double-strand breaks in the DNA through which they pass a segment of uncut duplex DNA before resealing the break. Type II topoisomerases require the energy of ATP hydrolysis for their action. Type I topoisomerases, in contrast, change the linking number of DNA in steps of one. They make transient single-strand breaks in the DNA, allowing the uncut strand to pass through the break before resealing the nick. In contrast to the type II topoisomerases, type I topoisomerases do not require ATP.

Organization and nature of nuclear DNA in eukaryotes

Deoxyribose nucleic acid (DNA)

As a chemical, DNA is quite simple. It contains three types of chemical components: (1) phosphate, (2) a sugar called deoxyribose, and (3) four nitrogenous bases-adenine, guanine, cytosine, and thymine. The sugar in DNA is called "deoxyribose" because it has only a hydrogen atom (H) at the 2′-carbon atom, unlike ribose (a component of RNA), which has a hydroxyl (OH) group at that position. Two of the bases, adenine and guanine, have a double-ring structure characteristic of a type of chemical called a purine. The other two bases, cytosine and thymine, have a single-ring structure of a type called a pyrimidine. The carbon

atoms in the bases are assigned numbers for ease of reference. The carbon atoms in the sugar group also are assigned numbers-in this case, the number is followed by a prime (1′, 2′, and so forth). The chemical components of DNA are arranged into groups called nucleotides, each composed of a phosphate group, a deoxyribose sugar molecule, and any one of the four bases. It is convenient to refer to each nucleotide by the first letter of the name of its base: A, G, C, or T. The nucleotide with the adenine base is called deoxyadenosine 5′-monophosphate, where the 5′ refers to the position of the carbon atom in the sugar ring to which the single (mono) phosphate group is attached.

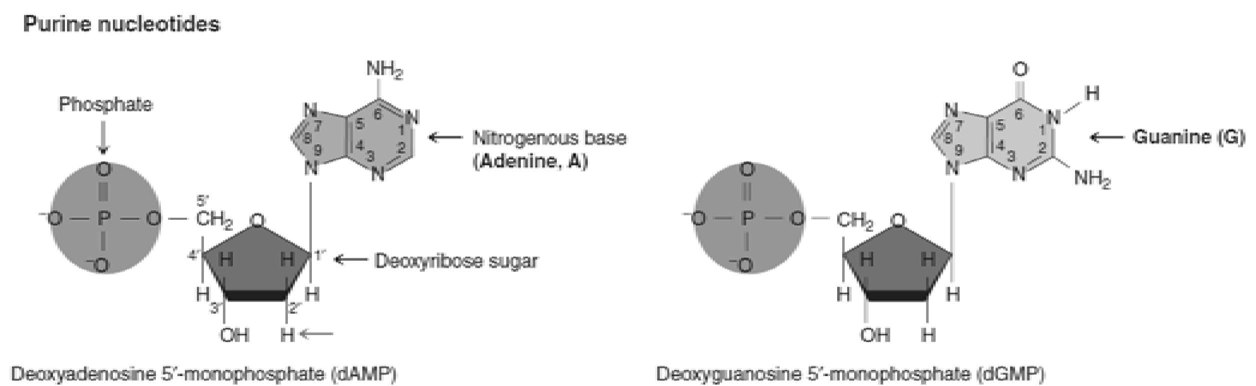


Figure 17: Purine nucleotide of DNA

The three-dimensional structure derived by Watson and Crick is composed of two side-by-side chains (“strands”) of nucleotides twisted into the shape of a double helix(Figure 18). The two nucleotide strands are held together by hydrogen bonds between the bases of each strand, forming a structure like a spiral staircase (Figure 18). The backbone of each strand is formed of alternating phosphate and deoxyribose sugar units that are connected by phosphodiester linkages (Figure 18). As already mentioned, the carbon atoms of the sugar groups are numbered 1′ through 5′. A phosphodiester linkage connects the 5′-carbon atom of one deoxyribose to the 3′-carbon atom of the adjacent deoxyribose. Thus, each sugar–phosphate backbone is said to have a 5′-to-3′ polarity, or direction, and understanding this polarity is essential in understanding how DNA fulfills its roles.

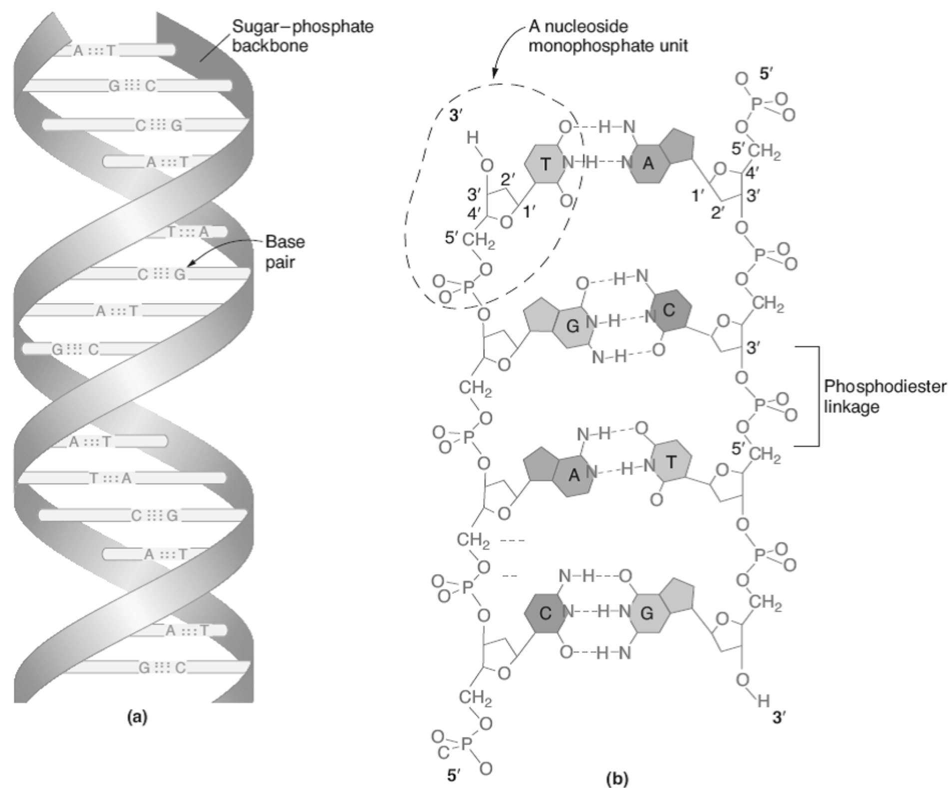


Figure 18: Structure of DNA

In the double-stranded DNA molecule, the two backbones are in opposite, or **antiparallel,** orientation (Figure 18). Each base is attached to the 1′-carbon atom of a deoxyribose sugar in the backbone of each strand and faces inward toward a base on the other strand. Hydrogen bonds between pairs of bases hold the two strands of the DNA molecule together. Two complementary nucleotide strands paired in an antiparallel manner automatically assume a double-helical conformation (Figure 18), mainly through the interaction of the base pairs. The base pairs, which are flat planar structures, stack on top of one another at the center of the double helix. Stacking adds to the stability of the DNA molecule by excluding water molecules from the spaces between the base pairs. The most stable form that results from base stacking is a double helix with two distinct sizes of grooves running in a spiral: the **major groove** and the **minor groove,** which can be seen in both the ribbon and the space-filling models. Most DNA–protein associations are in major grooves (Figure 19). A single strand of nucleotides has no helical structure; the helical shape of DNA depends entirely on the pairing and stacking of the bases in the antiparallel strands. DNA is a right-handed helix; in other words, it has the same structure as that of a screw that would be screwed into place by using a clockwise turning motion.

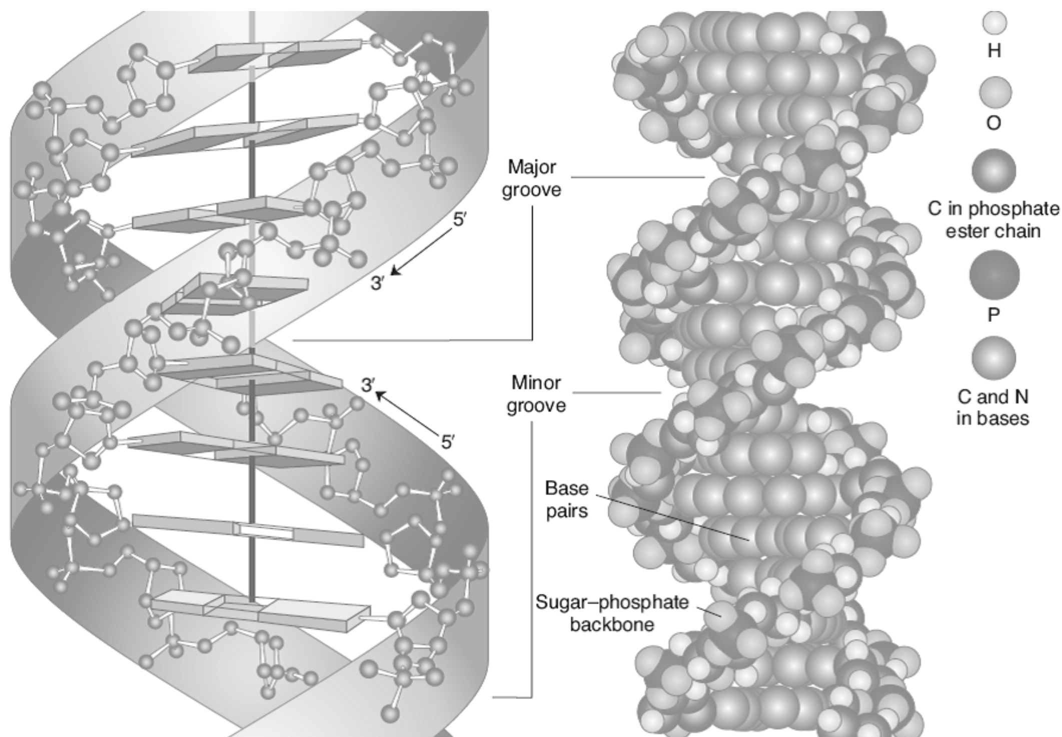


Figure 19: The ribbon diagram of DNA structure

Eukaryotic Chromosome and organization of DNA

Eukaryotic species have one or more sets of chromosomes; each set is composed of several different linear chromosomes. Humans, for example, have two sets of 23 chromosomes each, for a total of 46. A distinguishing feature of eukaryotic cells is that their chromosomes are located within a separate cellular compartment known as the **nucleus.** To fit within the nucleus, the length of DNA must be compacted by a remarkable amount. The term **chromatin** is used to describe the DNA-protein complex found within eukaryotic chromosomes. Chromatin is a dynamic structure that can change its shape and composition during the life of a cell. In this section, we will examine the organization of DNA sequences along the length of eukaryotic chromosomes and the levels of compaction of eukaryotic chromosomes during different stages of the cell cycle. Our discussion of chromatin compaction in this largely focuses on structural features between DNA and DNA-binding proteins that occur in eukaryotic chromosomes. In addition chromatin is a dynamic structure that can alternate between loose and compact conformations in a way that regulates gene expression.

Eukaryotic Chromosome

Individual eukaryotic chromosomes contain enormous amounts of DNA. Each eukaryotic chromosome consists of a single, extremely long molecule of DNA. For all of this DNA to fit into the nucleus, tremendous packing and folding are required, the extent of which must

change in the course of the cell cycle. The chromosomes are in an elongated, relatively uncondensed state during interphase of the cell cycle, but the term *relatively* is an important qualification here. Although the DNA of interphase chromosomes is less tightly packed than the DNA of mitotic chromosomes, it is still highly condensed; it's just *less* condensed. In the course of the cell cycle, the level of DNA packing changes: chromosomes progress from a highly packed state to a state of extreme condensation. DNA packing also changes locally in replication and transcription, when the two nucleotide strands must unwind so that particular base sequences are exposed. Thus, the packing of eukaryotic DNA (its tertiary chromosomal structure) is not static but changes regularly in response to cellular processes.

Chromatin Eukaryotic DNA in the cell is closely associated with proteins. This combination of DNA and protein is called chromatin. The two basic types of chromatin are euchromatin, which undergoes the normal process of condensation and decondensation in the cell cycle, and heterochromatin, which remains in a highly condensed state throughout the cell cycle, even during interphase. Euchromatin constitutes the majority of the chromosomal material and located where most transcription takes place. All chromosomes have heterochromatin at the centromeres and telomeres. Heterochromatin is also present at other specific places on some chromosomes, along the entire inactive X chromosome in female mammals and throughout most of the Y chromosome in males. In addition to remaining condensed throughout the cell cycle, heterochromatin is characterized by a general lack of transcription, the absence of crossing over, and replication late in the S stage.

The most abundant proteins in chromatin are the *histones*, which are small, positively charged proteins of five major types: H1, H2A, H2B, H3, and H4 (Table 2). All histones have a high percentage of arginine and lysine, positively charged amino acids that give the histones a net positive charge. The positive charges attract the negative charges on the phosphates of DNA; this attraction holds the DNA in contact with the histones. A heterogeneous assortment of nonhistone chromosomal proteinsalso are found in eukaryotic chromosomes. At times, variant histones, with somewhat different amino acid sequences, are incorporated into chromatin in place of one of the major histone proteins.

Histone Protein	Molecular Weight	Number of Amino Acids
H1	21,130	223
H2A	13,960	129
H2B	13,774	125
H3	15,273	135
H4	11,236	102

Table 2: Characteristics of histone protein

The nucleosome: Chromatin has a highly complex structure with several levels of organization (Figure 20). The simplest level is the double-helical structure of DNA as discussed earlier. At a more complex level, the DNA molecule is associated with proteins and is highly folded to produce a chromosome.

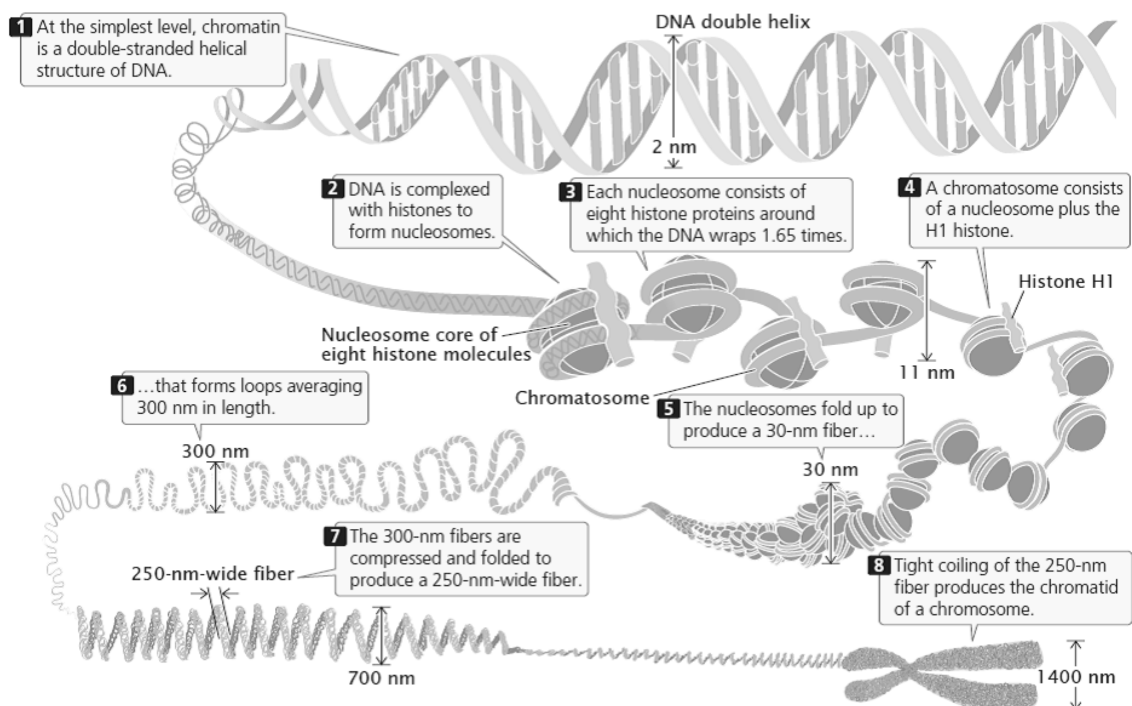


Figure 20: Chromatin and several levels of organization into chromosome

When chromatin is isolated from the nucleus of a cell and viewed with an electron microscope, it frequently looks like beads on a string (Figure 21a). If a small amount of nuclease is added to this structure, the enzyme cleaves the “string” between the “beads,”

leaving individual beads attached to about 200 bp of DNA (Figure 21b). If more nuclease is added, the enzyme chews up all of the DNA between the beads and leaves a core of proteins attached to a fragment of DNA (Figure 21c). Such experiments demonstrated that chromatin is not a random association of proteins and DNA but has a fundamental repeating structure.

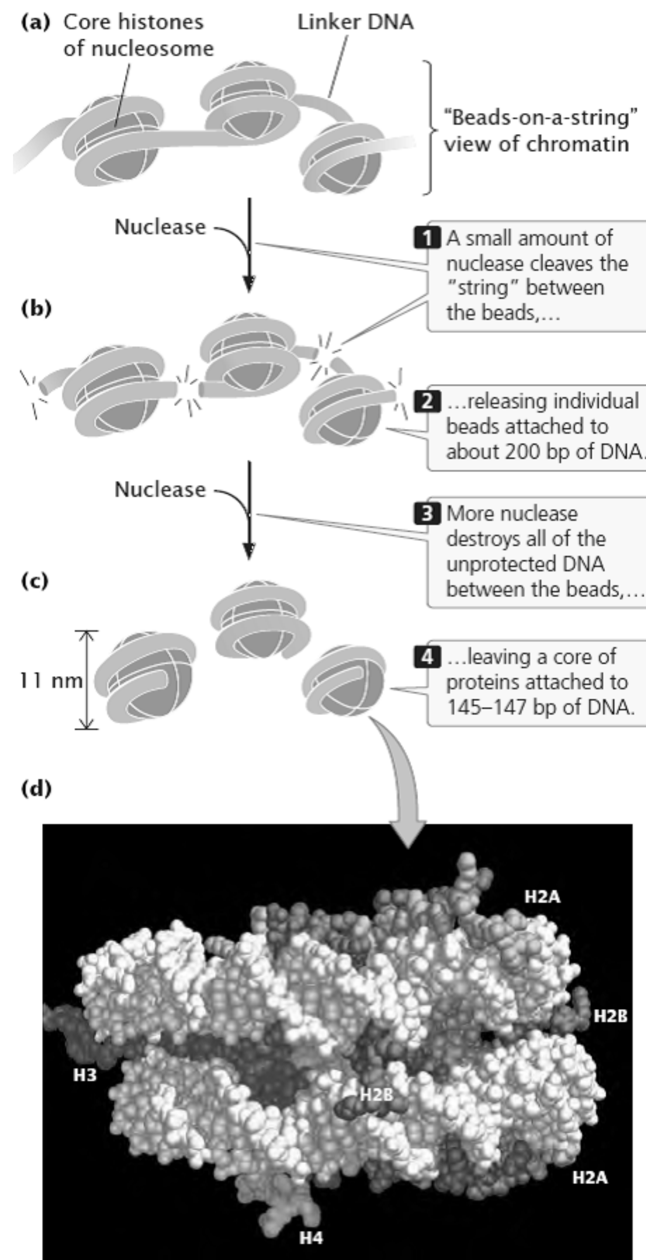


Figure 21: Nucleosome is the fundamental unit of Chromatin

The repeating core of protein and DNA produced by digestion with nuclease enzymes is the simplest level of chromatin structure, the nucleosome (Figure 22). The nucleosome is a core particle consisting of DNA wrapped about two times around an octamer of eight histone

proteins (two copies each of H2A, H2B, H3, and H4), much like thread wound around a spool (Figure 22). The DNA in direct contact with the histone octamer is between 145 and 147 bp in length.

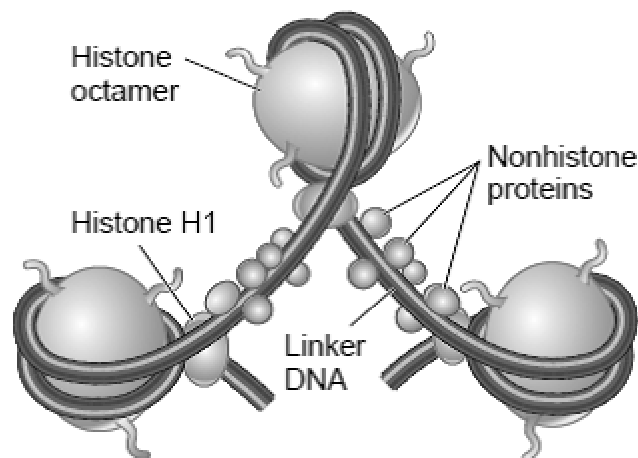


Figure 22: Nucleosome with histone and non-histone protein

Levels of organization of DNA in eukaryotic chromosome

Each of the histone proteins that make up the nucleosome core particle has a flexible "tail," containing from 11 to 37 amino acids, that extends out from the nucleosome. Positively charged amino acids in the tails of the histones interact with the negative charges of the phosphates on the DNA, keeping the DNA and histones tightly associated. The tails of one nucleosome may also interact with neighboring nucleosomes, which facilitates compaction of the nucleosomes themselves. Chemical modifications of the histone tails bring about changes in chromatin structure that are necessary for gene expression. The fifth type of histone, H1, is not a part of the core particle but plays an important role in nucleosome structure. H1 binds to 20 to 22 bp of DNA where the DNA joins and leaves the octamer and helps to lock the DNA into place, acting as a clamp around the nucleosome octamer. Together, the core particle and its associated H1 histone are called the **chromatosome (**Figure 22), the next level of chromatin organization. Each chromatosome encompasses about 167 bp of DNA. Chromatosomes are located at regular intervals along the DNA molecule and are separated from one another by linker DNA, which varies in size among cell types; in most cells, linker DNA comprises from about 30 to 40 bp. Nonhistone chromosomal proteins may be associated with this linker DNA, and a few also appear to bind directly to the core particle.

Higher-order chromatin structure : When chromatin is in a condensed form, adjacent nucleosomes are not separated by space equal to the length of the linker DNA; rather,

nucleosomes fold on themselves to form a dense, tightly packed structure (igure 21) that makes up a fiber with a diameter of about 30 nm (Figure 23). Two different models have been proposed for the 30-nm fiber: a solenoid model, in which a linear array of nucleosomes are coiled, and a helix model, in which nucleosomes are arranged in a zigzag ribbon that twists or supercoils. Recent evidence supports the helix model (Figure 23).

The next-higher level of chromatin structure is a series of loops of 30-nm fibers, each anchored at its base by proteins in the nuclear scaffold (Figure 21). On average, each loop encompasses some 20,000 to 100,000 bp of DNA and is about 300 nm in length, but the individual loops vary considerably. The 300-nm loops are packed and folded to produce a 250-nm-wide fiber. Tight helical coiling of the 250-nm fiber, in turn, produces the structure that appears in metaphase-individual chromatids approximately 700 nm in width.

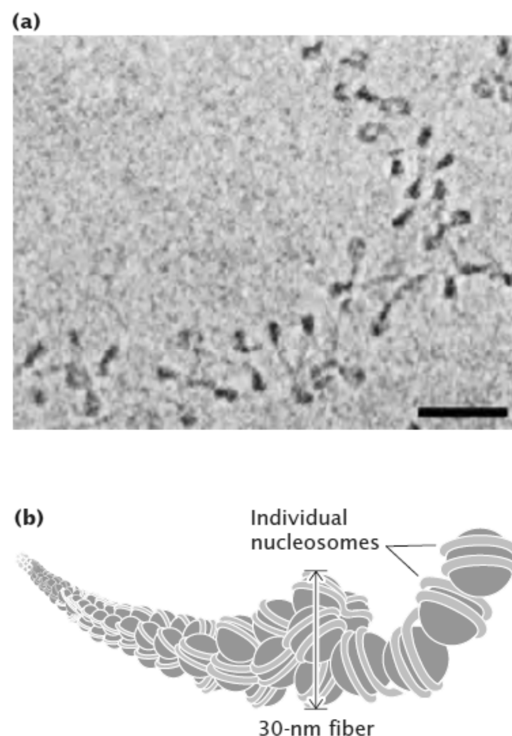


Figure 23: (a) Electron micrograph of nucleosome. (b) The model of 30-nm chromatin fiber.

Non-histone proteins in chromatin organization

In highly condensed chromosomes, such as those found in metaphase, the radial loops are highly compacted and remain anchored to a **scaffold,** which is formed from nonhistone proteins of the nuclear matrix. Experimentally, researchers can delineate the nonhistone proteins of the scaffold that hold the loops in place. In this condition, the radial loops of DNA are in a very compact configuration. If this chromosome is treated with a high concentration

of salt to remove both the core and linker histones, the highly compact configuration is lost, but the bottoms of the elongated loops remain attached to the scaffold composed of nonhistone proteins. Remarkably, the scaffold retains the shape of the original metaphase chromosome when chromosomes are treated with high salt to remove histone proteins, even though the DNA strands have become greatly elongated. These results illustrate that the structure of metaphase chromosomes is determined by the nuclear matrix proteins, which form the scaffold, and by the histones, which are needed to compact the radial loops. Researchers found that cells contain two multiprotein complexes called condensin and cohesin, which play a critical role in chromosomal condensation and sister chromatid alignment, respectively. Condensin and cohesin are two completely distinct complexes, but both contain a category of proteins called SMC proteins. SMC stands for structural maintenance of chromosomes. These proteins use energy from ATP to catalyze changes in chromosome structure. Together with topoisomerases, SMC proteins have been shown to promote major changes in DNA structure. An emerging theme is that SMC proteins actively fold, tether, and manipulate DNA strands. They are dimers that have a V-shaped structure. The monomers, which are connected at a hinge region, have two long coiled arms with a head region that binds ATP (Figure 25). The length of each monomer is about 50 nm, which is equivalent to approximately 150 bp of DNA.

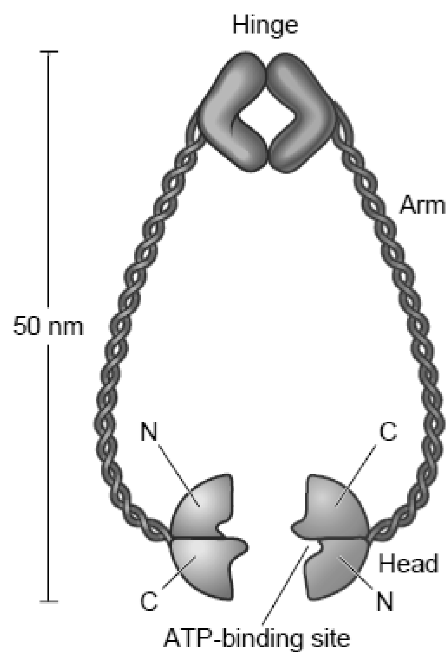


Figure 25: Structure of SMC protein

As their names suggest, condensin and cohesin play different roles in metaphase chromosome structure. Prior to M phase, condensin is found outside the nucleus (Figure 26).However, as M phase begins, condensin is observed to coat the individual chromatids as euchromatin is

converted into heterochromatin. The role of condensin in the compaction process is not well understood. Although condensin is often implicated in the process of chromosomal condensation, researchers have been able to deplete condensin from actively dividing cells, and the chromosomes are still able to condense. However, such condensed chromosomes show abnormalities in their ability to separate from each other during cell division. These results suggest that condensin is important in the proper organization of highly condensed chromosomes, such as those found during metaphase. In comparison, the function of cohesin is to promote the binding (i.e., cohesion) between sister chromatids. After S phase and until the middle of prophase, sister chromatids remain attached to each other along their length. This attachment is promoted by cohesin, which is found along the entire length of each chromatid. In certain species, such as mammals, cohesins located along the chromosome arms are released during prophase, which allows the arms to separate. However, some cohesions remain attached, primarily to the centromeric regions, leaving the centromeric region as the main linkage before anaphase. At anaphase, the cohesins bound to the centromere are rapidly degraded by a protease aptly named separase, thereby allowing sister chromatid separation.

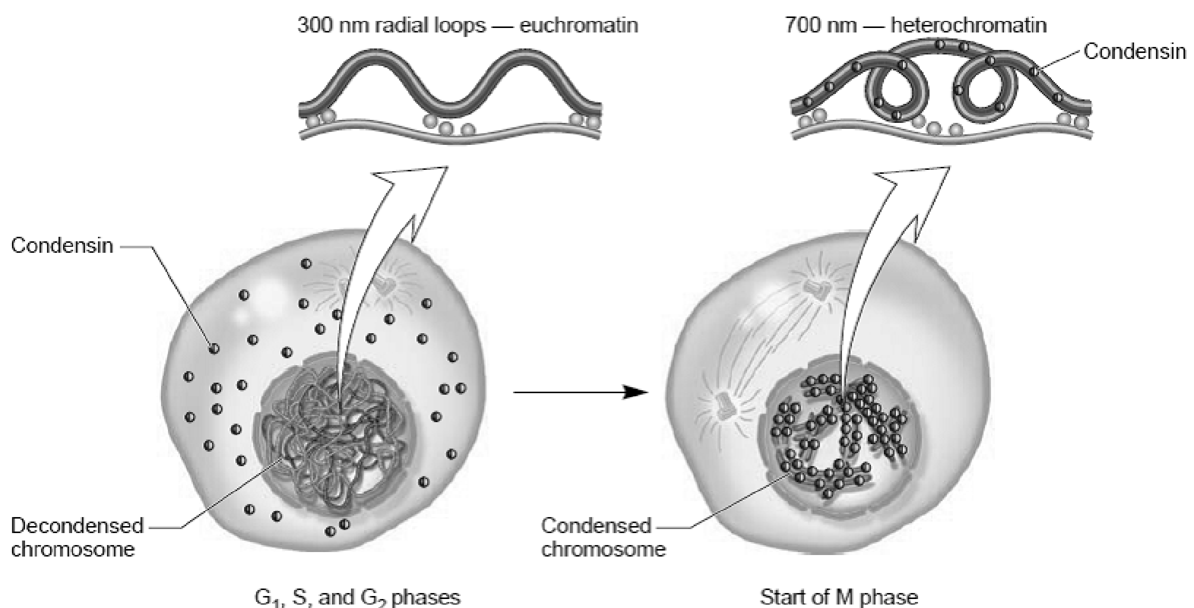


Figure 26: Localization of Condensin during Interphase and start of M phase

Changes in Chromatin Structure

Although eukaryotic DNA must be tightly packed to fit into the cell nucleus, it must also periodically unwind to undergo transcription and replication.

Polytene chromosomes : Giant chromosomes found in certain tissues of *Drosophila* and some other organisms (Figure 27). Polytene chromosomes have provided researchers with evidence of the changing nature of chromatin structure. These large, unusual chromosomes arise when repeated rounds of DNA replication take place without accompanying cell divisions, producing thousands of copies of DNA that lie side by side. When polytene chromosomes are stained with dyes, numerous bands are revealed. Under certain conditions, the bands may exhibit chromosomal puffs-localized swellings of the chromosome. Each puff is a region of the chromatin having a relaxed structure and, consequently, a more open state.

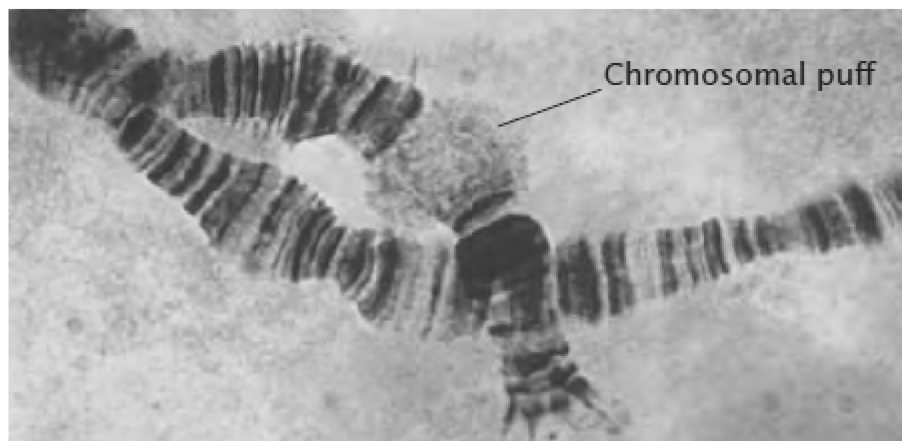


Figure 27: Polytene Chromosome showing chromosome puff-site of active transcription

If radioactively labelled uridine (a precursor to RNA) is briefly added to a *Drosophila* larva, radioactivity accumulates in chromosomal puffs, indicating that they are regions of active transcription. Additionally, the appearance of puffs at particular locations on the chromosome can be stimulated by exposure to hormones and other compounds that are known to induce the transcription of genes at those locations. This correlation between the occurrence of transcription and the relaxation of chromatin at a puff site indicates that chromatin structure undergoes dynamic change associated with gene activity (Figure 27).

Probable Questions

1. What is the difference between SINEs and tandem repeats?
2. Explain why a packaging system is needed to fit a DNA molecule into its chromosome in eukaryotes.
3. Describe the role of histones in packaging DNA into eukaryotic chromosomes.
4. Why is negative supercoiling preferred over positive supercoiling?

5. Name the enzyme that negatively supercoils bacterial chromosomes
6. What are catenanes? How are they unlinked?
7. What is a linking number (L)?
8. What are topoisomerases? What is the difference between type I and type II topoisomerases?
9. What differences in gene distribution and repetitive DNA content are seen when yeast and human chromosomes are compared?
10. The human genome contains about 50,000 fewer genes than was predicted by many researchers. Why were these initial predictions so high?
11. Define pseudogene. What is the difference between a non-processed pseudogene and a processed pseudogene?
12. Define chromatin and nucleosome.
13. Name the core histones and the linker histone and describe how they are arranged in a nucleosome.
14. What are the two types of chromatin and how do they differ?
15. What is the linking number, Lk, for a relaxed, closed-circular DNA with 3675 base pairs?
16. What is Lk for negatively supercoiled 3675 bp DNA if it is underwound by 2 complete turns?

Suggested Readings / References:

1. James D. Watson, Tania A. Baker, Stephen P. Bell-Molecular Biology of Gene
2. Robert J. Brooker-Genetics Analysis and Principles
3. Benjamin Lewin-Genes IX
4. Harvey F Lodish et al-Molecular Cell Biology
5. Gerald Karp-Cell andMolecularBiology
6. Geoffrey M. Cooper, Robert E. Hausman-The Cell:A Molecular Approach
7. T.A. Brown-Genomes
8. William S. Klug, Michael R. Cummings, Charlotte A. Spencer, Michael A. Palladino-Concepts of Genetics
9. Anthony J.F. Griffiths, Susan R. Wessler, Sean B. Carroll, John Doebley-An Introduction to Genetic Analysis.
10. http://www.biologydiscussion.com/biotechnology/transposons-definition-and-types-with-diagram/17769

Unit-IX

Organelle genome, architecture of mitochondrial genome, conserved chloroplast DNA

Objectives:

In this Unit we will discuss about Organelle genome, architecture of mitochondrial genome, conserved chloroplast DNA

Organelle Genome:

Introduction

There are many exceptions to the rule in genetics. One of them is that not all inherited characters are determined by genes located in the nucleus. Few among them are controlled by genes located in cell organelles in the cytoplasm i.e. cytoplasmic genes, and these of course are exceptions to the chromosome theory of inheritance. Since they are extrachromosomal (i.e. outside the chromosomes), such genes are not subject to the normal rules of Mendelian heredity.

Extrachromosomal circular DNA (eccDNA) is ubiquitous in eukaryotic organisms, and has been noted for more than 3 decades. eccDNA occurs in normal tissues and in cultured cells, is heterogeneous in size, consists of chromosomal sequences and reflects plasticity of the genome. Recent findings indicate that this eccDNA can vary in size, sequence complexity, and copy number. However, the best characterized eccDNAs contain sequences homologous to chromosomal DNA. These findings may indicate that eccDNA may arise from genetic rearrangements, for example, from homologous recombination events. Elevated levels of eccDNA are now thought to correlate with genomic instability and exposure to carcinogens.
In contrast to the human nuclear genome, which consists of 3.3 billion base pairs of DNA, the human mitochondrial genome is built of a mere 16,569 base pairs. Despite its small size, the mitochondrial genome can be used to establish maternal family ties, thanks to its maternal pattern of inheritance. Mutations in the mitochondrial genome have also been associated with diverse forms of human disease and aging.

Extrachromosomal inheritance or cytoplasmic inheritance is the transmission of genes that occur outside the nucleus or a form of non-Mendelian inheritance in which a trait is

transmitted from the parent to offspring through non-chromosomnal cytoplasmic means. Mendelian inheritance patterns involve genes that directly influence the outcome of an organism's traits and obey Mendel's laws. Most genes in eukaryotic species follow a Mendelian pattern of inheritance however, there are many that don't and these genes are present in mitochondria and chloroplast.

Mitochondrial Genome:

Animal mitochondrial genomes are 13-18 kb in size.

Fungal mitochondrial genomes are ~75 kb.

Higher plant mitochondrial genomes are 300-500 kb.

Each mitochondrion has 5-20 copies of the mitochondrial chromosomes. Human cells have a range of numbers of mitochondria: Liver cells have 1000 mitochondria per cell, Skin cells have 100, Egg cells have up to 10 million.

Nuclear DNA is inherited from both parents. However, all the mitochondria for an individual are provided by the egg cell. It means that all mitochondrial DNA is inherited only from one's mother. Nuclear DNA resides in the membrane bound cell nucleus whereas mitochondrial DNA molecules are found in the mitochondria which are scattered throughout the cell cytoplasm. Nuclear DNA is organized into linear strands that make up the 23 pairs of chromosomes in the human genome. There are about 3.3 billion base pairs in the nuclear DNA of a cell. Buried in these linear strands are about 23,000 genes that code for proteins along with other sections that control which genes are expressed in each cell and when.

Mitochondrial DNA is circular in shape and contains only about 16,569 base pairs: There are only 37 genes and a "non-coding" region (also called the D loop) (Fig. 1) that does not code for any gene products (protein, various forms of RNA). Of the 37 genes, 22 code for transfer RNA, two code for ribosomal RNA and 13 code for proteins that are necessary for cellular energy production. The mitochondria are not totally autonomous. Most of the proteins necessary for their function are coded by nuclear DNA.

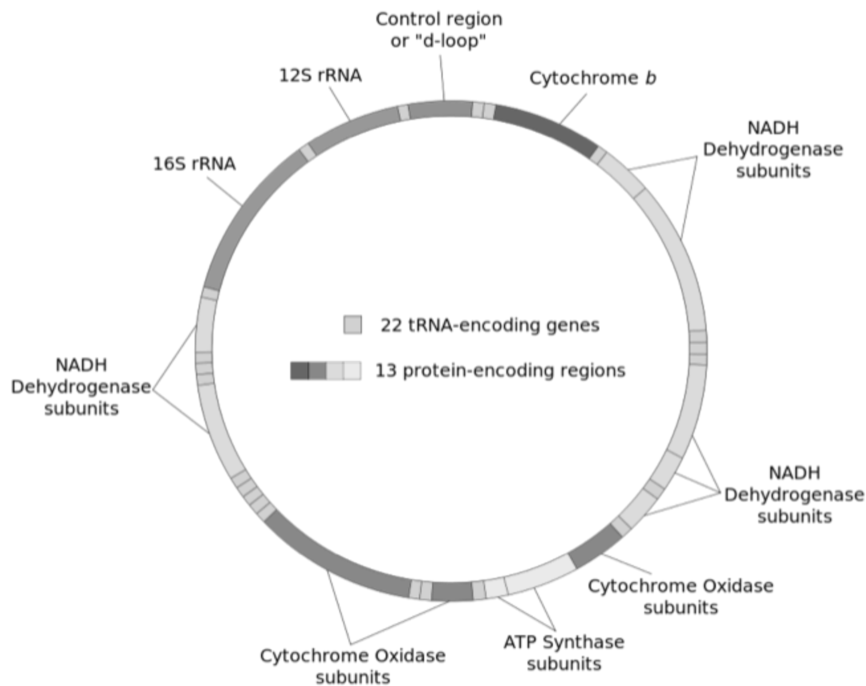


Figure. 1**:** Human mitochondrial DNA "chromosome" showing the arrangement of regions that code for proteins and RNA molecules. For genetic genealogy, note also the blue "control region" that does not code for any gene products.

Each cell contains only 23 pairs of chromosomal DNA. A cell may have tens to hundreds of mitochondria each with hundreds (or more) of DNA molecules. For example, the average human liver cell contains more than a thousand mitochondria each with hundreds of circular DNA molecules. The chromosomes that comprise nuclear DNA are paired. Each member of a chromosome pair carries the same genes and codes for the same traits (Differences in the nucleotide sequences of the genes are responsible for the variety of traits found in biological organisms). We say that paired chromosomes are diploid. Though mitochondria have multiple copies of their circular DNA molecules, they are not paired and we say mitochondria are haploid. When nuclear chromosomes are replicated during the process of creating new sex cells, cross-over or recombination between the members of the chromosome pairs insures variety and uniqueness to the new organism. No recombination occurs in mitochondrial DNA because there is no pairing. Mitochondrial DNA lacks the repair mechanisms and proofreading capabilities that insure the integrity of the nuclear DNA during replication. This leads to a mutation rate for mitochondria that is about 10 times higher than for nuclear DNA.Unlike nuclear DNA, mitochondrial DNA is densely packed with functional genes with

no duplicates for backup in case one gene is disrupted by mutation. Mutations in these coding areas will result in mitochondria deficient in gene products (proteins mostly) necessary to their function.They will most likely be unable to reproduce and pass on their mutated genes. Consequently, despite the high mutation rate, the functional part of the mitochondrial genome varies slowly among individuals over time. In contrast, mutations in the control or non-coding region of mtDNA do no harm to the mitochondria nor do they affect the survival of the organism. They accumulate and are passed down through the maternal line. These mutations provide a source of variability that is useful in human identity testing and in investigating evolutionary and genealogical relationships.

The mitochondrial genetic code differs some from the code of nuclear DNA. For example, in mitochondria, the triplet codon AUA codes for the amino acid methionine whereas the same codon in nuclear DNA codes for isoleucine. Similarly the mitochondrial triplet UGA codes for tryptohphan and is NOT a STOP codon as it is in the nuclear DNA.Nuclear DNA has long stretches of non-coding DNA that is interspersed throughout the genome, sometimes even separates nucleotide sequences that code for a specific protein. Introns make up as much as 93% of nuclear DNA. Mitochondrial DNA has no introns. Nuclear DNA produces messenger RNA that codes for only one protein. If there are more than one polypetide chains coded for in a consecutive string of nuclear DNA nucleotides the resulting mRNA is cut apart into separate mRNA's before binding to a ribosome. Nuclear DNA is monocistronic meaning there is a separate strand of mRNA for each protein. Mitochondrial DNA has a scheme more like that of the eukaryotic cells responsible for its origin. If an uninterrupted segment of mitochondrial DNA that consecutively codes for say three proteins, is transcribed to mRNA, that mRNA is NOT cut into three different mRNA's. Rather, the whole mRNA proceeds to bind to a ribosome where it is directs the formation of all three proteins. In contrast to nuclear DNA, mRNA is polycistronic meaning one strand of mRNA may be responsible for the production of more than one type of mRNA.

Key Differences Between Nuclear DNA and Mitochondrial DNA	
Nuclear DNA	Mitochondrial DNA
Inherited from both parents	Inherited from mother only
Linear	Circular
3.2 billion base pairs	16,569 base pairs
20,000 genes	37 genes
23 pairs in each cell	Hundreds to thousands in each cell
Paired or diploid	Not paired or haploid
Varied by recombination	No recombination
Repair and proof reading mechanisms	No repair or proof reading mechanisms
Lower mutation rate	Higher mutation rate
Genetic code differs	Genetic code differs
Has introns	No introns
Monocistronic	Polycistronic

Table 1: Differences between Nuclear DNA and Mitochondrial DNA

Mitochondrial Inheritance

Ex. 1: The pattern of inheritance associated with alterations in the mtDNA involves both males and females, but always with the condition passed on through the female line (maternal inheritance). Since many mitochondria are passed into the egg from the cells in the ovary, all the offspring of an affected woman would be expected to inherit the condition. An affected male does not pass his mitochondria on to his children, so his children will be unaffected.

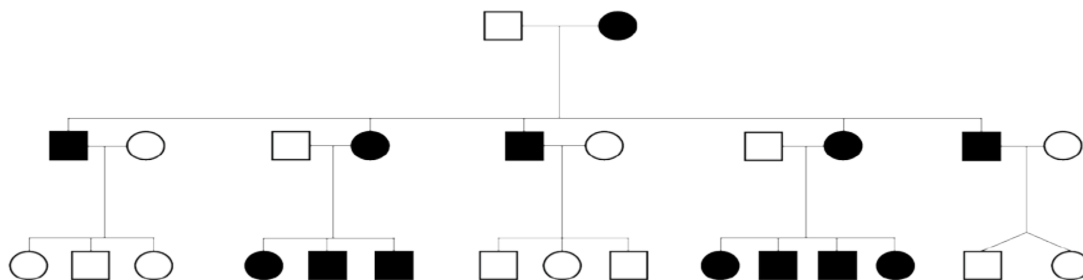
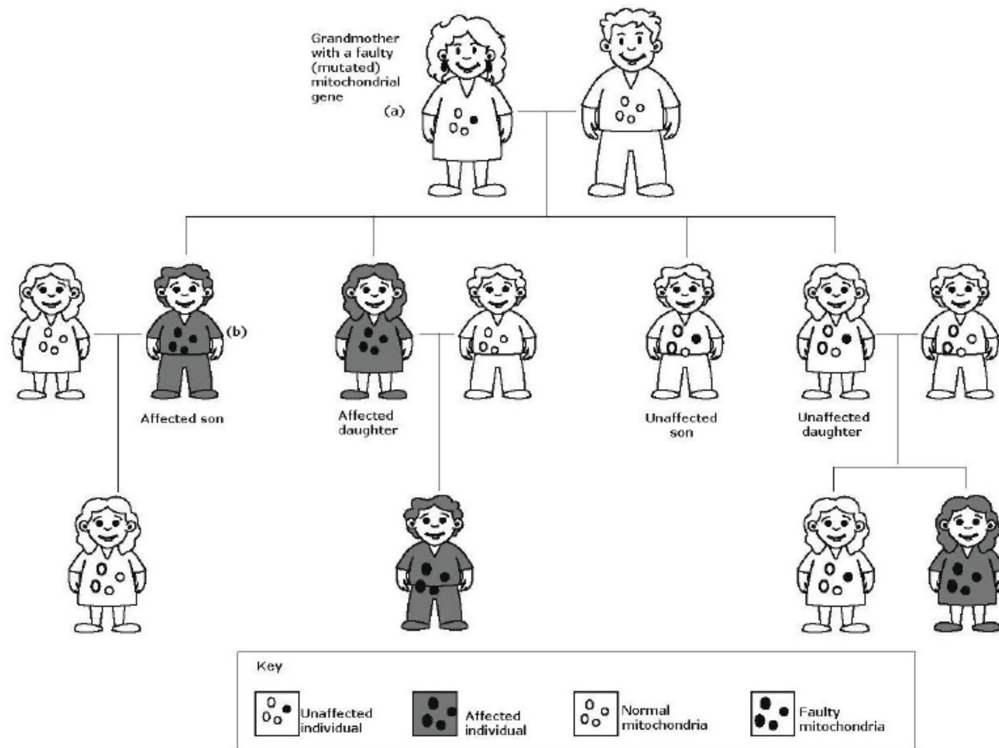


Figure 2: In some cases, the variation in the mitochondrial gene occurs for the first time in the egg or at the time of fertilization of the egg.



As previously mentioned, one cell contains numerous mitochondria, and each mitochondrion contains dozens of copies of the mitochondrial genome. Moreover, the mitochondrial genome has a higher mutation rate (about 10-fold higher) than the nuclear genome. This leads to a heterogeneous population of mitochondrial DNA within the same cell, and even within the same mitochondrion; as a result, mitochondria are considered heteroplasmic. When a cell divides, its mitochondria are partitioned between the two daughter cells. However, the process of mitochondrial segregation occurs in a random manner and is much less organized than the highly accurate process of nuclear chromosome segregation during mitosis. As a result, daughter cells receive similar, but not identical, copies of their mitochondrial DNA.

Why do mitochondria have such a high mutation rate? A nuclear gene, called DNA polymerase gamma (*POLG*), encodes the DNA polymerase responsible for replicating the mitochondrial genome. The POLG protein consists of two domains: a catalytic domain that exhibits polymerase activity and an exonuclease domain that is involved in the recognition and removal of DNA base-pair mismatches that occur during DNA replication. A recent study suggests that mitochondria may have a nucleotide imbalance that leads to decreased POLG fidelity and higher mitochondrial DNA mutation rates (Song *et al.*, 2005).

Clinical Manifestations of Mitochondrial Mutations

Due to the maternal pattern of mitochondrial inheritance, males with a mitochondrial disease are not considered to be at risk for transmitting the disorder to their offspring. It's important to remember that there are many mitochondria within a cell, each with its own mtDNA and potential mutations. Thus, when discussing mitochondrial mutations, it is necessary to think of mutations present across the entire mitochondrial population rather than in a single mitochondrion. Although mitochondrial populations are considered heteroplasmic, with variations among the many mtDNA genomes, mothers can have mitochondrial populations that are homoplasmic for a given mitochondrial mutation; in this case, the majority of their mitochondrial genome would harbor the mutation. Homoplasmic mitochondrial mutations will be transmitted to all maternal offspring; however, due to the complex interplay between the mitochondrial and nuclear genomes, it is often difficult to predict disease outcomes, even with homoplasmic mitochondrial populations.

Classic Mitochondrial Syndromes

A list of clinical disorders associated with mitochondrial mutations is provided in Table 2. One of these mitochondria-associated disorders is Leber hereditary optic neuropathy (LHON), which leads to a loss of vision in both eyes and is most commonly associated with a homoplasmic mitochondrial DNA mutation, although heteroplasmic transmission also occurs (Man *et al.*, 2003). While all of the children of a homoplasmic mother will inherit the LHON mutation, not all will develop the disease; in fact, only 50% of the male offspring and 10% of female offspring will suffer from optic nerve disease. These findings point to the likely involvement of other genes and environmental factors.

Adult

- Neurological: migraine | strokes | epilepsy | dementia | myopathy | peripheral neuropathy | DIPLOPIA | ATAXIA | speech disturbances | sensorineural deafness
- Gastrointestinal: constipation | irritable bowel | DYSPHAGIA
- Cardiac: heart failure | heart block | cardiomyopathy
- Respiratory: respiratory failure | nocturnal hypoventilation | recurrent aspiration | pneumonia
- Endocrinal: diabetes | thyroid disease | parathyroid disease | ovarian failure
- Ophthalmological: optic atrophy | cataract | ophthalmoplegia | PTOSIS

Paediatric

- Neurological: epilepsy | myopathy | psychomotor retardation | ataxia | spasticity | DYSTONIA | sensorineural deafness
- Gastrointestinal: vomiting | failure to thrive | dysphagia
- Cardiac: biventricular hypertrophic cardiomyopathy | rhythm abnormalities
- Respiratory: central hypoventilation | apnoea
- Haematological: anaemia | PANCYTOPAENIA
- Renal: renal tubular defects
- Liver: hepatic failure
- Endocrinal: diabetes | adrenal failure
- Ophthalmological: optic atrophy

Table 2: Mitochondrial gene mutation and clinical disorders

Similarly, a homoplasmic mutation in a mitochondrial genome-encoded ribosomal RNA, called RNR1, causes postlingual deafness (deafness that occurs after three years of age, when a child has already learned to speak). The clinical symptoms of this disease are associated with the administration of a particular type of antibiotic. Therefore, environmental factors also contribute to the phenotypes associated with this mitochondrial mutation.

Clinical Syndromes with a High Probability of Mitochondrial DNA Involvement

What are some clues that may suggest a mitochondrial link to disease? Some clinical features include a maternal family history and the involvement of several different tissues. Furthermore, because mitochondria function as the powerhouses of our cells, mitochondrial mutations often lead to more pronounced phenotypes in tissues that have high energy demands, such as brain, retinal, skeletal muscle, and cardiac muscle tissues. A number of clinical syndromes are currently believed to be associated with mitochondrial disease. Possible examples include Pearson syndrome, Leigh syndrome, progressive external ophthalmoplegia, exercise-induced muscle pain, fatigue, and rhabdomyolysis.

Mitochondrial Mutations That Contribute to Common Disease Phenotypes

Mitochondrial mutations are also likely to contribute to a number of common clinical diseases. One example is diabetes, which is the most prevalent metabolic disease affecting human. It is also likely that mitochondrial mutations may predispose individuals to Alzheimer's disease and Parkinson's disease.

Chloroplast DNA

In 1909 German botanist Karl Erich Correns discovered a trait in the four-o'clock plants (*Mirabilisjalapa*) that appeared to be inconsistent with Mendelian inheritance patterns. He discovered that four-o'clock plants had a mixture of leaf colors on the same plant: Some were all green, many were partly green and partly white (variegated), and some were all white. Since Correns's discovery, many other such traits have been discovered. It is now known that the reason these traits do not follow Mendelian inheritance patterns is that their genes are not on the chromosomes in the nucleus of the cell where most genes are located. Instead, the gene for the four o'clock leaf color trait is located on the single, circular chromosome found in chloroplasts. Because chloroplasts are specialized for photosynthesis, many of the genes on the single chromosome produce proteins or ribonucleic acid (RNA) that either directly or indirectly affects synthesis of chlorophyll, the pigment primarily responsible for trapping energy from light. Because chlorophyll is green and because mutations in many chloroplast genes cause chloroplasts to be unable to make chlorophyll, most mutations result in partially or completely white or yellow leaves. Correns discovered that seed produced by flowers carried on the green branches gave progeny which were all normal green. It made no difference whether the phenotype of the branch which carried the flower used for pollen was green, white or variegated. Seed taken from white branches likewise gave all white progeny, regardless of the pollen donor phenotype. These of course died in the seedling stage. Seeds from flowers on variegated branches gave three kinds of progeny, green, white and variegated, in varying proportions; again regardless of the pollen donor phenotype. In other words, the phenotype of the progeny always resembled the female parent and the male made no contribution at all to the character. The effect is seen quite clearly in the difference which Correns found between reciprocal crosses:

♀ green × white ♂ → green progeny
♀ white × green ♂ → white progeny

The explanation for this unusual pattern of inheritance is that the genes concerned are located in the chloroplasts within the cytoplasm, not in the nucleus, and are therefore transmitted only through the female parent. In eukaryote organisms the zygote normally receives the bulk of its cytoplasm from the egg cell (Fig. 3) and the male gamete contributes little more than a nucleus. Any genes contained in the cell organelles of the cytoplasm will therefore show maternal inheritance.

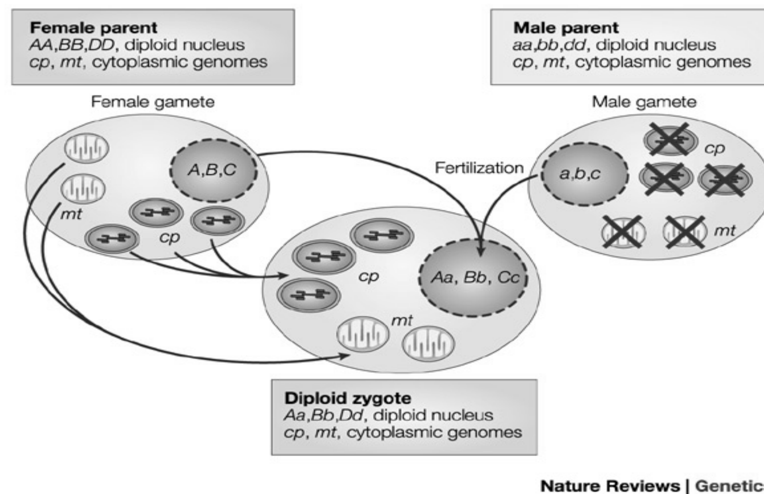


Figure .3: The cytoplasmic organelles characteristically contain multiple, homogeneous genomes that are usually inherited from one parent only (in this example, and most commonly, the female parent). In tobacco and many other plants, the mitochondrial and chloroplast genomes are specifically degraded before fertilization. There are many exceptions to this common inheritance pattern of genes in mitochondria and chloroplasts.

The leaf variegation is due to two kinds of chloroplasts: normal green ones and defective ones lacking in chlorophyll pigment. Chloroplasts are genetically autonomous (i.e. self-determining) and have their own system of heredity in the form of chloroplast ‘chromosomes’. These are small circular naked DNA molecules which carry genes controlling some aspects of chloroplast structure and function. A mutation in one of these

genes, which affects the synthesis of chlorophyll as in *Mirabilis*, will therefore follow the chloroplast in its transmission and will not be inherited in the same way as a nuclear gene.

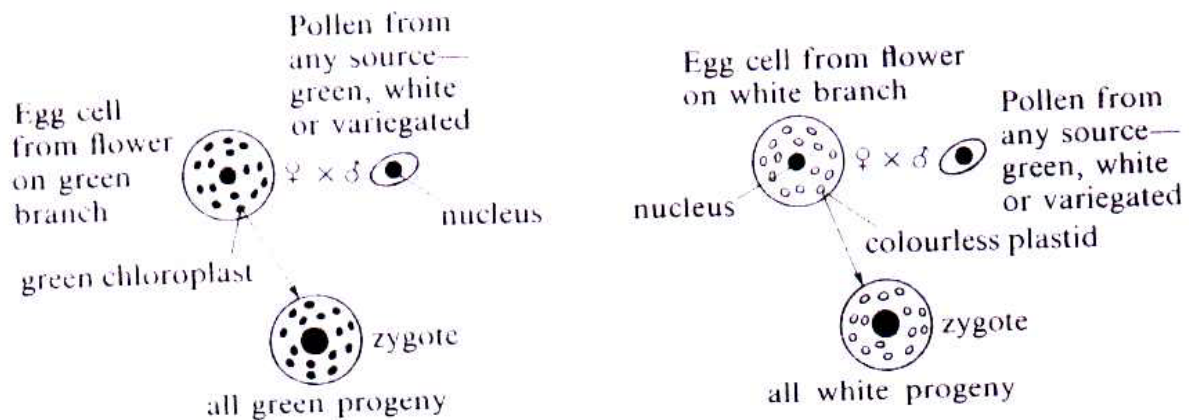
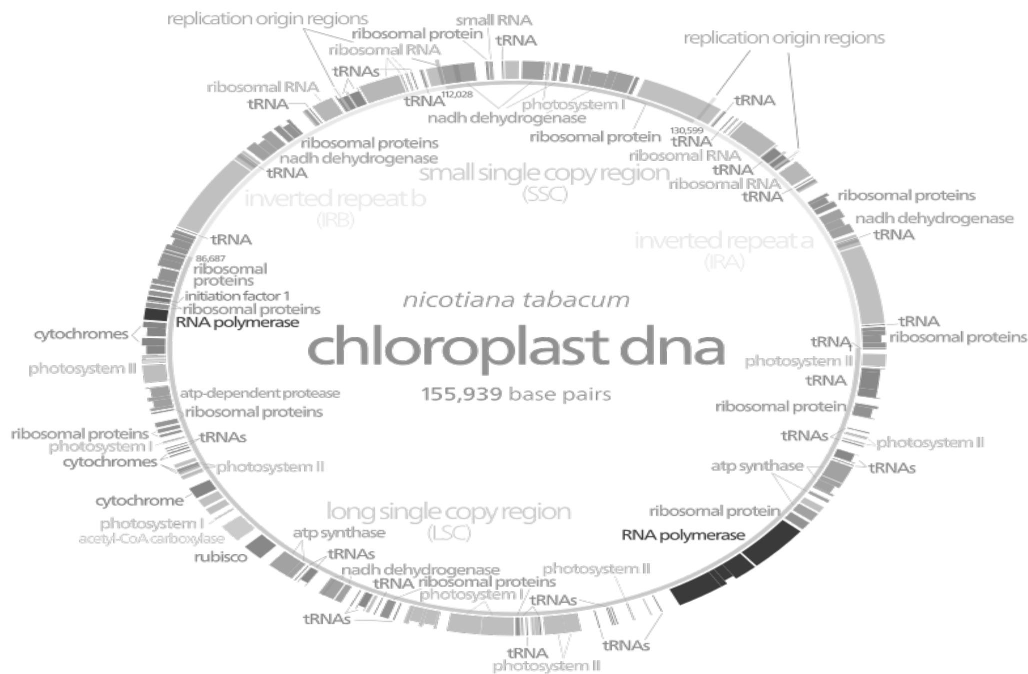


Figure. 4: Inheritance of leaf variegation in *Mirabilis jalapa*. The character is controlled by cytoplasmic genes located in the chloroplast 'chromosome'. Chloroplasts are self-perpetuating cell organelles and during sexual reproduction they are only transmitted through the cytoplasm of the egg cell, as undifferentiated protoplastids. They are not inherited through the pollen. The progeny of crosses therefore have the characters of the female parent and show maternal inheritance.

The other important point to note about the inheritance of chloroplasts is that they have no regular means of distribution, such as chromosomes do at mitosis, where they can be equally shared out to the daughter cells following division. A plant that begins life as a zygote containing a mixture of normal and mutant chloroplasts cannot therefore maintain the same mixture in all of its somatic cells. The two kinds of plastids are shared out randomly during cell division, according to the way they happen to be placed in the cytoplasm when it is partitioned. Some branches of variegated plants may therefore remain mosaic while others, by chance, may turn out to contain all white or all green chloroplasts in all of their cells. In a similar way the flowers on variegated branch may be of three kinds. Some will have egg cells with all green chloroplasts, some egg cells with all white and others will retain a mixture.

It is now known that the chloroplasts of plants carry their genetic information in the form of small circular DNA molecules, similar in size and form to the chromosomes of bacteria. Advances in molecular genetics have allowed scientists to take a much closer look at the

chloroplast genome. The size of the genome has been determined for a number of plants and algae and ranges from 85 to 292 kilobase pairs, with most being between 120 kb and 160 kb (Fig.4).



These DNA molecules contain genes which code for some of the proteins and RNAs used in chloroplast structure and function; and it is mutations in these genes which are most likely to be responsible for the leaf variegation effects described above. It must also be emphasised that chloroplasts are not totally independent of the nucleus in their heredity; most of their proteins are coded by nuclear genes, and mutations in these show normal Mendelian patterns of inheritance.The DNA molecules which make up the chloroplast genome are ‘naked’ ones and bear no resemblance to the chromosomes of the nucleus, which are much larger and are composed of both protein and DNA. The really surprising thing about the chloroplast DNA is the large number of copies which are present: up to 300 in a mature plastid. Since an average of 160 chloroplasts are present in a mesophyll cell of the mature leaf of a cereal such as wheat, this means that there may be as many as 48,000 chloroplast ‘chromosomes’ per mesophyll cell. The reason for this enormous redundancy of genetic information is unknown.

Probable Questions:

1. What do you mean by organelle genome?

2. Write down the characteristics of Mitochondrial DNA?

3. Discuss about mitochondrial inheritance.

4. Write down the characteristics of Chloroplast DNA.

5. Discuss the relation between mitochondrial mutations and disease.

Suggested Readings:

1. Lodish, H. (2016). Molecular cell biology. New York, NY: Freeman.

2. Alberts, B. (2008). Molecular biology of the cell. New York, NY [u.a.]: Garland Science Taylor & Francis.

3. Lewin, B., Krebs, J., Goldstein, E. and Kilpatrick, S. (2014). Lewin's genes XI. Burlington, MA: Jones & Bartlett Learning.

4. Karp, G. and Patton, J. (2015). Cell and molecular biology. Brantford, Ont.: W. Ross MacDonald School Resource Services Library.

5. Cooper, G. and Hausman, R. (n.d.). The cell.

Unit -X:

Cell cycle, apoptosis and cancer : Phases of cell cycle. Regulation of cell cycle: Discovery of MPF, cyclins and cyclin dependent kinases, Check points- role of Rb and p53

Objectives:

In this Unit we will discuss on Cell cycle, apoptosis and cancer : Phases of cell cycle. Regulation of cell cycle: Discovery of MPF, cyclins and cyclin dependent kinases, Check points- role of Rb and p53

Cell Cycle:

Definition

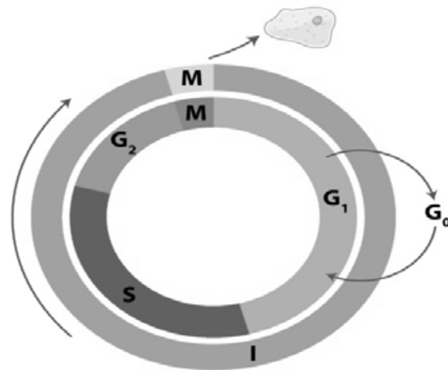
The cell cycle is a cycle of stages that cells pass through to allow them to divide and produce new cells. It is sometimes referred to as the "cell division cycle" for that reason.

Daughter cells start life, containing only half of the parent cell's cytoplasm and only one copy of the DNA that is the cell's "blueprint" or "source code" for survival. In order to divide and produce "daughter cells" of their own, the newborn cells must grow and produce more copies of vital cellular machinery – including their DNA.The two main parts of the cell cycle are mitosis and interphase.

Mitosis is the phase of cell division, during which a "parent cell" divides to create two "daughter cells."The longest part of the cell cycle is called "interphase" – the phase of growth and DNA replication between mitotic cell divisions. Both mitosis and interphase are divided into smaller sub-phases which need to be executed in order for cell division, growth, and development to proceed smoothly. Here we will focus on interphase, as the phases of mitosis have been covered in our "Mitosis" article. Interphase consists of at least three distinct stages during which the cell grows, produces new organelles, replicates its DNA, and finally

divides. Only after the cell has grown by absorbing nutrients, and copied its DNA and other essential cellular machinery, can this "daughter cell" divide, becoming "parent" to two "daughter cells" of its own.

The graphic below shows a visual representation of the cell cycle. The small section labeled "M" represents mitosis, while interphase is shown subdivided into its major components: the G_1, S, and G_2 phases.



This cell cycle is used by all eukaryotic cells to produce new cells. Prokaryotic cells such as bacteria use a process called "binary fission."

For some unicellular eukaryotes, the cell cycle is the same as the reproductive cycle. Their "daughter cells" are independent organisms that will go on to reproduce themselves through mitosis.In other organisms, the cell cycle is used for growth and development of a single organism, while other methods are used to reproduce the organism.Animals and some plants, for example, create new offspring through a process of sexual reproduction which involves the creation and combination of special sex cells.

But animals and plants still use the cell cycle to produce new cells within their tissues. This allows these multicellular organisms grow and heal throughout their lifespans.

Phases of Cell Cycle

Mitosis

Let's start this cell cycle with "birth."During mitosis, the "parent" cell goes through a complex series of steps to ensure that each "daughter" cell will get the materials it needs to survive, including a copy of each chromosome. Once the materials are properly sorted, the "parent" cell divides down the middle, pinching its membrane in two.

Each of the new “daughters” are now independently living cells. But they’re small, and have only one copy of their genetic material.

This means they can’t divide to produce their own “daughters” right away. First, they must pass through “interphase” – the phase between divisions, which consists of three distinct phases.

G_1 Phase

In G_1 phase, the newly formed daughter cell grows. The “G” is most often said to stand for “gap,” since these phases appear to an outside observer with a light microscope to be relatively inactive “gaps” in the cell’s activity.However given what we know today, it might be more accurate to say the “G” stands for “growth” – for the “G” phases are flurries of protein and organelle production as well as literal increase in the size of the cell.

During the first “growth” or “gap” phase, the cell produces many essential materials such as proteins and ribosomes. Cells that rely on specialized organelles such as chloroplasts and mitochondria make a lot more of those organelles during G_1 as well. The cell’s size may increase as it assimilates more material from its environment into its machinery for life.This allows the cell to increase its energy production and overall metabolism, preparing it for S Phase.

S Phase

During S phase, the cell replicates its DNA. The “S” stands for “synthesis” – referring to the synthesis of new chromosomes from raw materials.This is a very energy-intensive operation, since many nucleotides need to by synthesized. Many eukaryotic cells have dozens of chromosomes – huge masses of DNA – that must be copied.Production of other substances and organelles is slowed greatly during this time as the cell focuses on replicating its entire genome.When the S phase is completed, the cell will have two complete sets of its genetic material. This is crucial for cell division, as it ensures that both daughter cells can receive a copy of the “blueprint” they need to survive and reproduce.

G_2 Phase

Just like the first “gap” phase of the cell cycle, the G_2 phase is characterized by lots of protein production.

During G_2, many cells also check to make sure that both copies of their DNA are correct and intact. If a cell's DNA is found to be damaged, it may fail its "G_2/M checkpoint" – so named because this "checkpoint" happens at the end of the G_2 phase, right between G_2 and "M phase" or "Mitosis."

This "G_2/M checkpoint" is a very important safety measure for multicellular organisms like animals. Cancers, which can result in the death of the entire organism, can occur when cells with damaged DNA reproduce. By checking to see if a cells' DNA has been damaged immediately before replication, animals and some other organisms reduce the risk of cancer.

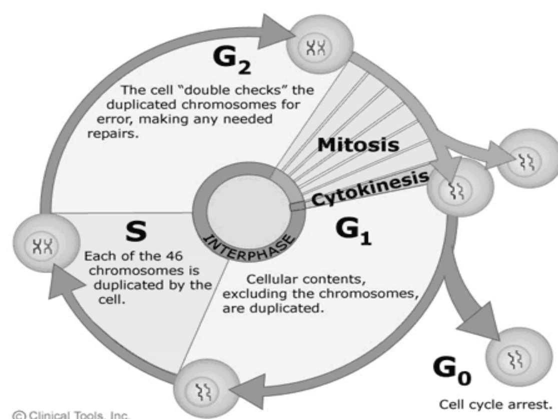
Interestingly, some organisms can skip G_2 altogether and go straight into mitosis after DNA is synthesized during S phase. Most organisms, however, find it safer to use G_2 and its associated checkpoint!

If the G_2/M checkpoint is passed, the cell cycle begins again. The cell divides through mitosis, and new daughter cells begin the cycle that will take them through G_1, S, and G_2 phases to produce new daughter cells of their own.

An Alternative Path: G_0 Phase

After being born through mitosis, some cells are not meant to divide themselves to produce daughter cells.Neurons, for example – animal nerve cells – do not divide. Their "parent cells" are stem cells, and the "daughter" neuron cells are programmed not to go through the cell cycle themselves because uncontrolled neuron growth and cell division could be very dangerous for the organism.

So instead of entering G_1 phase after being "born," neurons enter a phase scientists call "G_0 phase." This is a metabolic state meant only to maintain the daughter cell, not prepare for cell division.Neurons and other non-dividing cell types may spend their whole lives in G_0 phase, performing their function for the overall organism without ever dividing or reproducing themselves.



Mitosis:

Prophase

In the phase to follow, called prophase, the duplicated chromosomes from the previous phase condense, meaning they become compacted and more tightly wound. An apparatus known as a mitotic spindle forms on the edges of the dividing cell. The mitotic spindle is made up of proteins called microtubules that gradually lengthen during prophase, which drives the division of the cell by elongating it.

Metaphase

Preceding metaphase is a period called prometaphase, during which the membrane, or nuclear envelope, surrounding the chromosomes breaks down, allowing the condensed chromosomes to come into direct contact with the microtubules of the mitotic spindle. Upon entering metaphase, the pairs of condensed chromosomes line up along the equator of the elongated cell. Because they are condensed, they move more easily without becoming tangled.

Anaphase

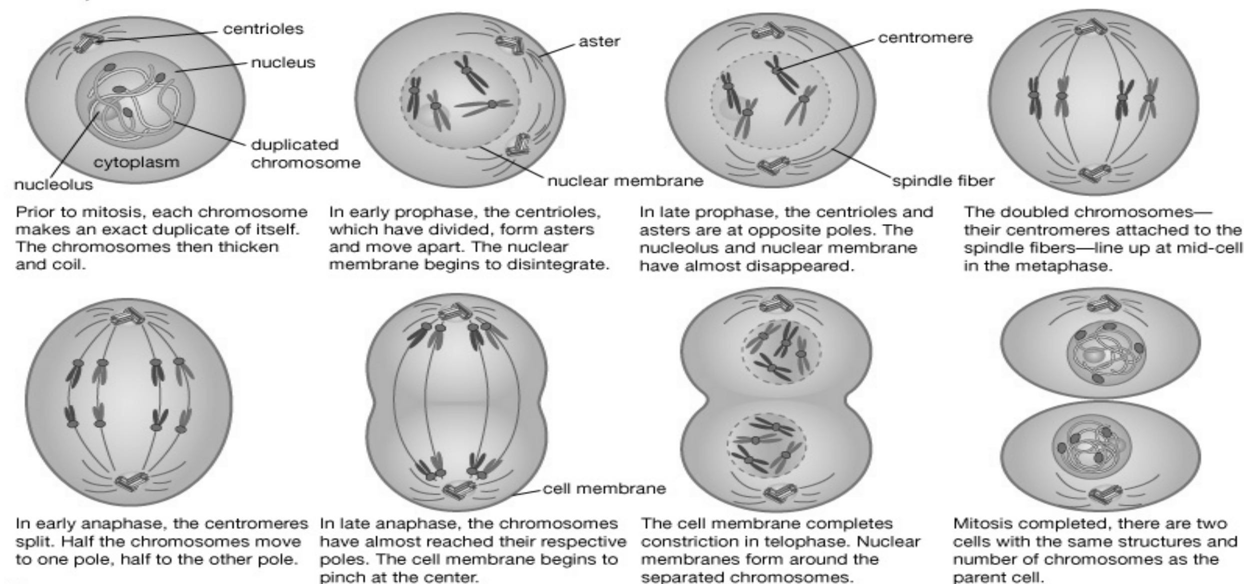
During anaphase, the pairs of chromosomes, also called sister chromatids, are drawn to opposite poles of the elongated cell. Therefore, duplicate copies of the cell's DNA are now on either side of the cell and are ready to divide completely. At this stage the microtubules get shorter, which begins to allow the cell to separate.

Telophase

The most important characteristic of telophase is that the nuclear envelope, which had previously broken down to allow the microtubules to access and recruit the chromosomes to the equator of the dividing cell, reforms as two new nuclear envelopes around the separated sister chromatids. The complete division of the cell, however, is not complete until cytokinesis takes place. Cytokinesis is the process by which the elongated cell is finally pinched into two brand new cells by a ring of proteins called actin and myosin, the same proteins found in muscle. At this point, the cytoplasm, or fluid in which all cell components are bathed, is equally divided between the two new daughter cells. Each daughter cell is identical, containing it's own nucleus and a complete copy of the organism's DNA.

Cytokinesis

Cytokinesis, or the separating of the two daughter cells, begins during or after the final phases of mitosis. It occurs in animal cells happens when a fiber ring containing a protein called actin, located in the cell's center, contracts, pulling the cell membrane to it, and



subsequently squeezing the two nuclei apart. Because cell walls are so rigid in plant cells, cytokinesis in plant cells occurs when a cell plate takes the place of the metaplate and grows between the two new cells. This plate becomes a membrane that inculcates itself into the cell wall in each of the daughter cells.

Meiosis:

Prophase I

Prophase I, the first step in meiosis I, is similar to prophase in mitosis in that the chromosomes condense and move towards the middle of the cell. The nuclear envelope degrades, which allows the microtubules originating from the centrioles on either side of the cell to attach to the kinetochores in the centromeres of each chromosome. Unlike in mitosis, the chromosomes pair with their homologous partner. This step does not take place in mitosis.

Metaphase I

In metaphase I of meiosis I, the homologous pairs of chromosomes line up on the metaphase plate, near the center of the cell. This step is referred to as a *reductional division*. The homologous chromosomes that contain the two different alleles for each gene, are lined up to be separated. As seen in the diagram above, while the chromosomes line up on the metaphase plate with their homologous pair, there is no order upon which side the maternal or paternal chromosomes line up. This process is the molecular reason behind the law of segregation.

The law of segregation tells us that each allele has the same chance at being passed on to offspring. In metaphase I of meiosis, the alleles are separated, allowing for this phenomena to happen. In meiosis II, they will be separated into individual gametes. In mitosis, all the chromosomes line up on their centromeres, and the sister chromatids of each chromosome separate into new cells. The homologous pairs do not pair up in mitosis, and each is split in half to leave the new cells with 2 different alleles for each gene. Even if these alleles are the same allele, they came from a maternal and paternal source. In meiosis, the lining up of homologous chromosomes leaves 2 alleles in the final cells, but they are on sister chromatids and are clones of the same source of DNA.

Also during metaphase I, the homologous chromosomes can swap parts of themselves that are the same parts of the chromosome. This is called *crossing-over* and is responsible for the other law of genetics, the law of independent assortment. This law states that traits are inherited independently of each other. For traits on different chromosomes, this is certainly true all of the time. For traits on the same chromosome, it makes it possible for the maternal and paternal DNA to recombine, allowing traits to be inherited in an almost infinite number of ways.

Anaphase I

Much like anaphase of mitosis, the chromosomes are now pulled towards the centrioles at each side of the cell. However, the centrosomes holding the sister chromatids together do not dissolve in anaphase I of meiosis, meaning that only homologous chromosomes are separated, not sister chromatids.

Telophase I

In telophase I, the chromosomes are pulled completely apart and new nuclear envelopes form. The plasm membrane is separated by *cytokinesis* and two new cells are effectively formed.

Results of Meiosis I

Two new cells, each haploid in their DNA, but with 2 copies, are the result of meiosis I. Again, althgough there are 2 alleles for each gene, they are on sister chromatid copies of each other. These are therefore considered haploid cells. These cells take a short rest before entering the second division of meiosis, meiosis II.

Phases of Meiosis II

Prophase II

Prophase II resembles prophase I. The nuclear envelopes disappears and centrioles are formed. Microtubules extend across the cell to connect to the kinetochores of individual chromatids, connected by centromeres. The chromosomes begin to get pulled toward the metaphase plate.

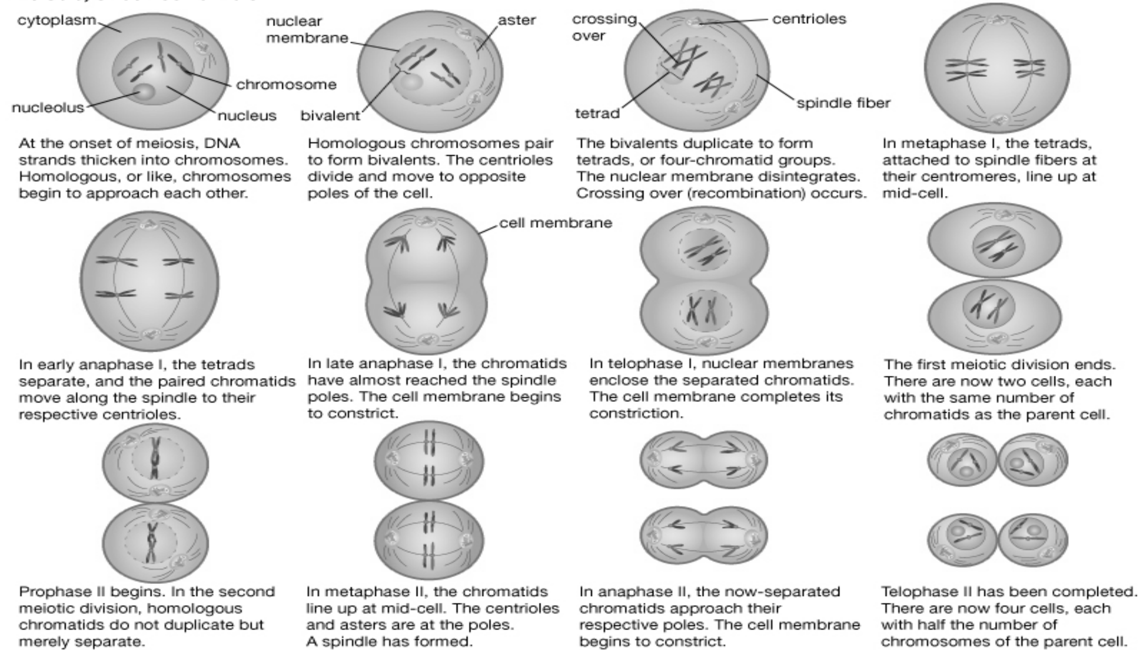
Metaphase II

Now resembling mitosis, the chromosomes line up with their centromeres on the metaphase plate. One sister chromatid is on each side of the metaphase plate. At this stage, the centromeres are still attached by the protein *cohesin*.

Anaphase II

The sister chromatids separate. They are now called sister chromosomes, and are pulled toward the centrioles. This separation marks the final division of the DNA. Unlike the first division, this division is known as an *equational division*, because each cell ends up with the same quantity of chromosomes as when the division started, but with no copies.

Telophase II



As in the previous telophase I, the cell is now divided in two and the chromosomes are on opposite ends of the cell. Cytokinesis, or plasma division occurs, and new nuclear envelopes are formed around the chromosomes.

Results of Meiosis II

At the end of meiosis II, there are 4 cells, each haploid, and each with only 1 copy of the genome. These cells can now be developed into gametes, eggs in females and sperm in males.

Cell Cycle Regulation:

It’s very important for the survival of cells and organisms that the cell cycle be regulated.

Organisms need to be able to stop cell division when the cell in question is damaged, or when there isn’t enough food to support new growth; they must also be able to start up cell division when growth or wound healing are needed.

To accomplish this, cells use a variety of chemical “signal cascades” where multiple links in a chain create complex effects based on simple signals.

In these regulatory cascades, a single protein may change the function of many other proteins, bringing about widespread changes to the functioning or even structure of the cell.

This allows these proteins – such as cyclins and cyclin-dependent kinases – to act as "stop points." If the cyclins or cyclin-dependent kinases don't give the go-ahead, the cell cannot progress to subsequent stages of the cell cycle.

Some examples of cell cycle regulators are given below:

Here we'll discuss common examples of how cells regulate their cell cycles, using a complex cascade of signal molecules, protein-activating enzymes, and signal-destroying molecules.

p53:

p53 is a protein that is well-known to scientists for its role in stopping cells with severe DNA damage from reproducing. When DNA is damaged, p53 works with cyclin-dependent protein kinases and other proteins to initiate repair and protection functions – and can also stop the cell from entering mitosis, ensuring that cells with DNA damage do not reproduce.

Cyclins:

Cyclins are a group of proteins that are produced at different points in the cell cycle. There are cyclins unique to most phases of the cell cycle – G_1 cyclins, G_1 /S cyclins that regulate the transition from G_1 into S, S cyclins, and M cyclins that regulate the progress through the stages of mitosis.Most cyclins are found in the cell at very low concentrations during other phases of the cell cycle, but then spike suddenly when they're needed to give the go-ahead to the next stage of the cell cycle. Certain types of DNA damage may prevent these cyclins from appearing to move the cell cycle forward, or may prevent them from activating their cyclin-dependent protein kinases. A few others, such as G_1 cyclins, remain high as a constant "go ahead" signal from G_1 until mitosis.

Cyclin-Dependent Protein Kinases:

The cell's cyclins ultimately do their jobs by interacting with Cyclin-Dependent Protein Kinases i.e., kinases that activate certain enzymes and proteins when they bind to a cyclin.

This allows cyclins to function as the “go” signal for many changes in cellular activity that happens throughout the cell cycle.Protein kinases are a special set of enzymes that “activate” other enzymes and proteins by affixing phosphate groups to them. When an enzyme or other protein is “activated” by a kinase, its behavior changes until it returns to its inactivated form.

The system by which one protein kinase can change the activities of many other proteins allows simple signals, such as cyclins, to produce complex changes to cellular activity. Signal-dependent protein kinases are used to coordinate many complex cellular activities.

Cyclins and cyclin-dependent kinases (CDKs):

CDKs are important master regulators of the cell cycle. Their role is to phosphorylate proteins on either S or T amino acids and thereby regulate the activity of those proteins. Yeast have just one CDK (Cdk1), while ‘metazoans’ (animals) like us have nine, of which four are really critical to the cell cycle and will be introduced today.

How are the CDKs themselves regulated?

The levels of these proteins remain pretty constant throughout the cell cycle, yet their levels of *activity* rise and fall cyclically. CDKs need to hydrolize ATP for energy in order to perform phosphorylation. They have an ATP binding cleft whose ability to bind ATP is regulated by two mechanisms. First, CDKs have a ‘flexible T loop’ which contains a threonine (T) residue which normally blocks the ATP binding cleft, but not when the T is phosphorylated. Second, cyclins bind CDKs and induce a conformational change that also helps to expose the ATP binding cleft. Therefore a fully active CDK is one which is both phosphorylated at the T on the T loop *and* is bound to a cyclin.The various activities of the cell cycle, then, are determined by the combination of cyclins and CDKs that are active at each stage, as shown in the following table.

cell cycle stage	cyclins	CDKs	Comments
G1	Cyclin D	CDK4&6	Can react to outside signals such as growth factors or mitogens.
G1/S	Cyclins E & A	CDK2	Regulate centrosome duplication; important for reaching START
S	Cyclins E & A	CDK2	Targets are helicases and polymerases
M	Cyclins A & B	CDK1	Regulate G2/M checkpoint. The cyclins are synthesized During S but not active until synthesis is complete. Phosphorylate lots of downstream targets.

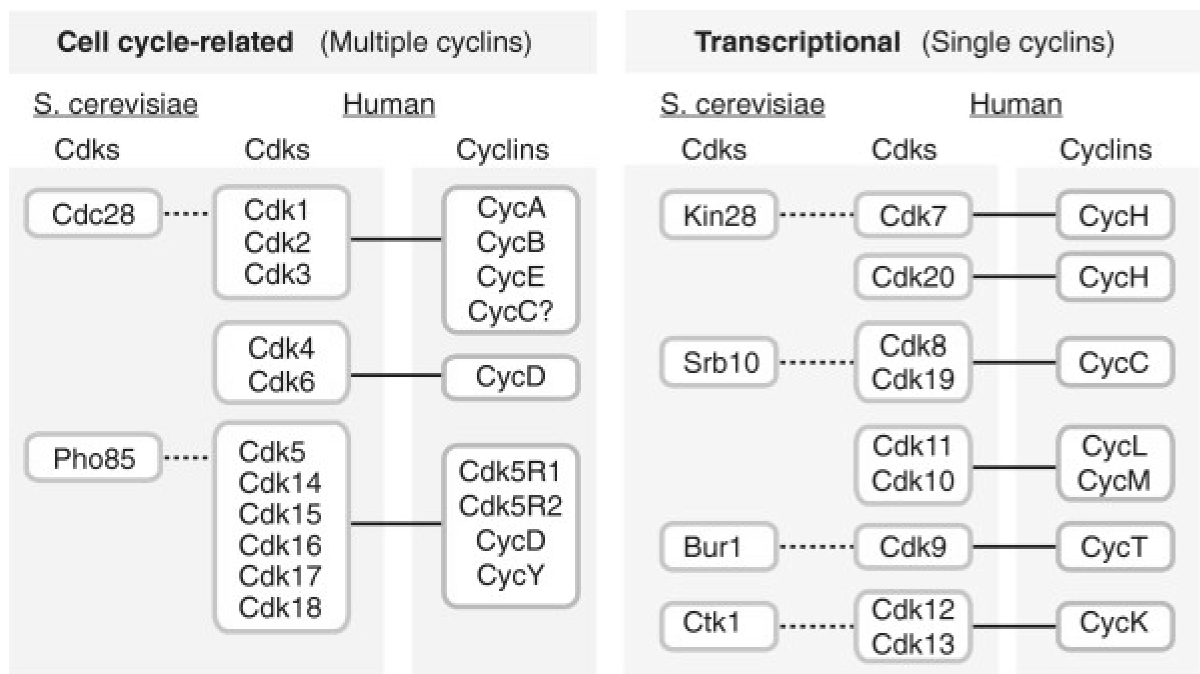
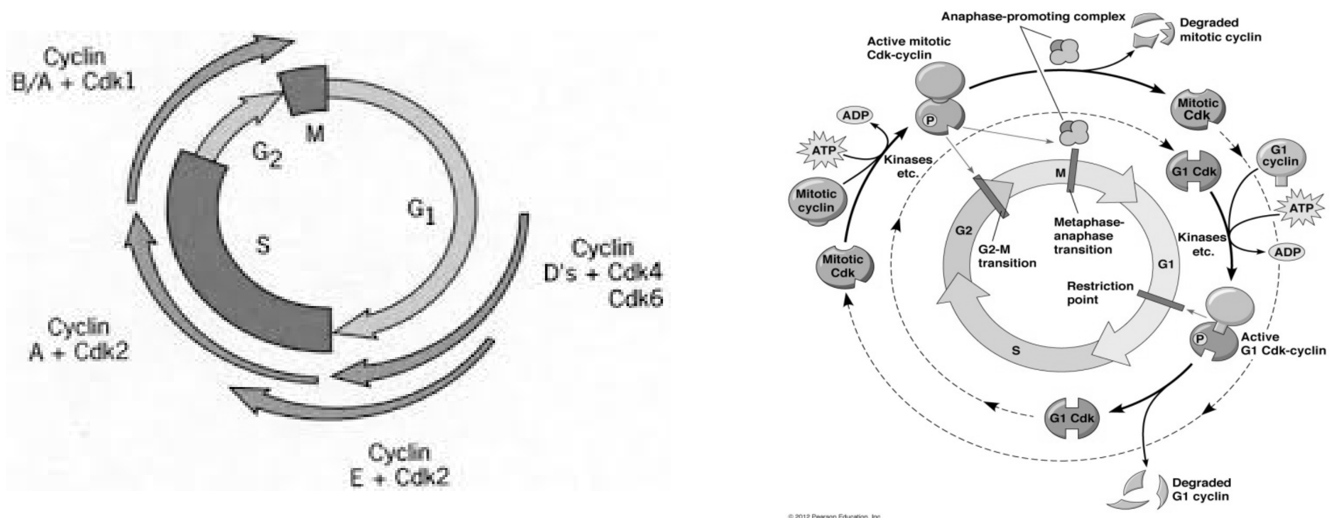


Figure: Comparison of Yeast and Mammalian Cdks and Cyclins.



CYCLIN-DEPENDENT KINASE INHIBITORS

The cyclin-dependent kinase inhibitors are a family of cell cycle regulators. Their primary function seems to be the formation of stable complexes with cyclin-dependent kinase proteins and the subsequent inhibition of the cell cycle. These complexes inactivate the catalytically operative units. Among the most well known and clinically relevant are p21, p27, and p16.

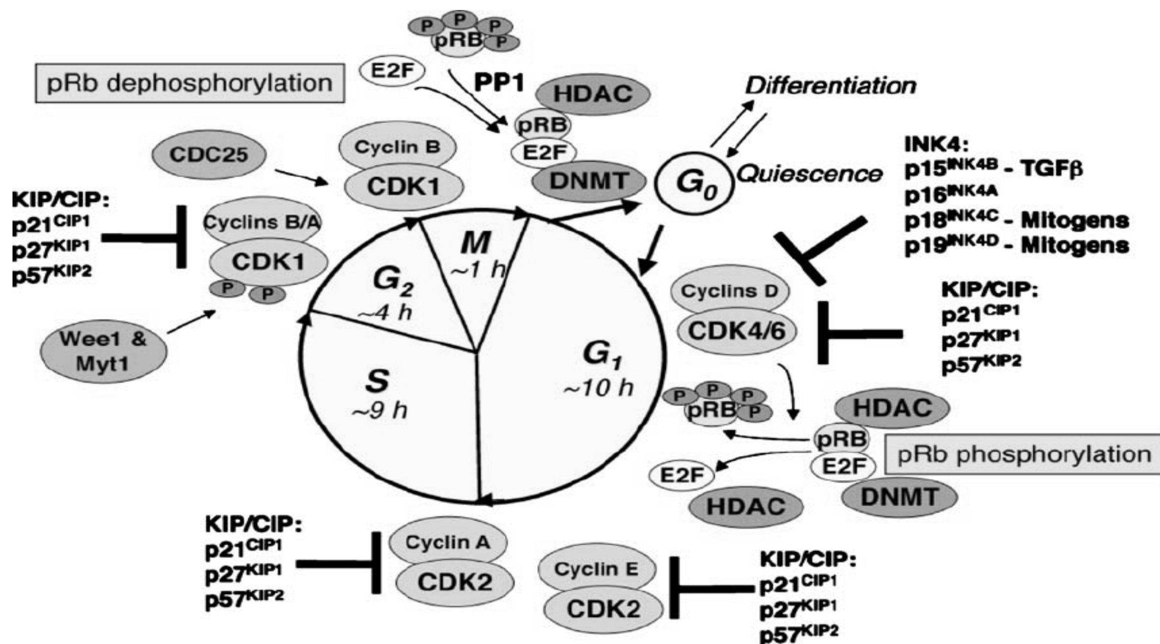
p21

A member of the WAF/CIP/KIP family of cyclin-dependent kinase inhibitors, p21 is probably the best characterized. It acts as a regulator of epithelial carcinogenesis and differentiation and is thought to play an important role in tumor suppression by regulating cell cycle progression, DNA replication, and DNA repair.The protein expression of p21 has been studied in a variety of tumor types, including breast, gastric, ovary, colorectal, and bladdercarcinomas. The alteration of protein expression assessed by immunohistochemical methods has been associated with higher tumor grade and worse prognosis in patients with bladder cancer.

p27

The p27 inhibitor is involved in the regulation of the cell cycle at the G_1-S transition, ultimately through the inhibition of pRb phosphorylation. Mutations in the human *p27* gene appear to be rare. Loss of p27 expression is associated with colon, breast, prostate, and gastric cancer progression.

p16

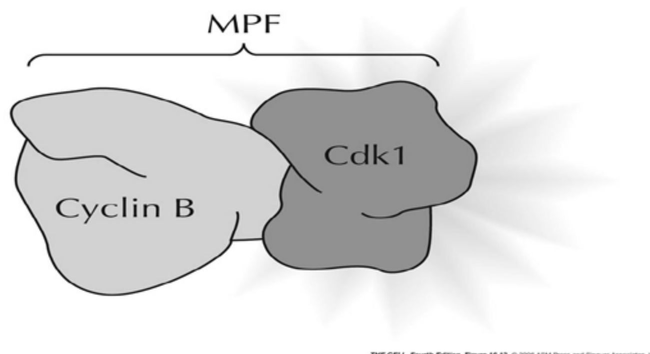


Also known as $p16^{INK4}$ and CDKN2A, p16 is a tumor suppressor protein encoded on the INK4a/ARF locus of chromosome 9p21, which is one of the most frequent sites of genetic loss in human cancer. Numerous studies have found abnormal p16 protein in a variety of tumor types, including melanomas; gliomas; esophageal, pancreatic, lung, and bladder carcinomas; and certain types of lymphomas. In addition, p16 is known to regulate *Rb*, and immunohistochemical expression of pRb and p16 is inversely correlated in a variety of tumors

Maturation-Promoting Factor:

Maturation-promoting factor (abbreviated **MPF**, also called **mitosis-promoting factor** or **M-Phase-promoting factor**) is the cyclin-Cdk complex that was discovered first in frog eggs. It stimulates the mitotic and meiotic phases of the cell cycle. MPF promotes the entrance into mitosis (the M phase) from the G_2 phase by phosphorylating multiple proteins needed during mitosis. MPF is activated at the end of G_2 by a phosphatase, which removes an inhibitory phosphate group added earlier.One example of a protein kinase at work is the Maturation-Promoting Factor, or MPF. MPF is a protein kinase that is activated by an M cyclin, meaning that it is activated during mitosis.When MPF is activated, it in turn activates several different proteins in the nuclear envelop of its host cell. The changes to these proteins result in the disintegration of the nuclear envelope.This is something that would be very dangerous at other points in the cell cycle, but which is necessary during mitosis so that the chromosomes can be sorted to ensure that each daughter cell receives a copy of each chromosome.

If M cyclins do not appear, MPF does not activate, and mitosis cannot go forward. This is a good example of how cyclins and cyclin-dependent kinases work together to coordinate – or stop – the cell cycle.



Discovery:

Before dividing, eukaryotic cells undergo a highly ordered series of events called the cell cycle. These events include S-phase, when the cell's DNA is replicated, and mitosis (M-phase), when the replicated chromosomes are separated. These phases are separated by periods called gap (G) phases, with the G1 phase preceding S phase and the G2 phase

occurring between S phase and mitosis. How the cell controls this essential series of events is one of the fundamental problems of cell biology.

One prominent early approach to studying control of the cell cycle was to introduce a nucleus from one phase of the cell cycle into a cytoplasm from another. Techniques for making such nucleo-cytoplasmic hybrid cells, including nuclear transplantation and cell fusion, became available in the 1950s, but it was not until the late 1960s that they were used to study cell cycle activities such as the initiation of DNA synthesis and the condensation of chromosomes. This type of experiment included nuclear transplantation by injection in frog oocytes and eggs, excision and transplantation of cytoplasmic fragments in protozoa, and virus-mediated fusion between tissue culture cells. In all cases the nucleus conformed to the cell cycle stage of the cytoplasm, indicating that cytoplasmic factors control nuclear activities during the cell cycle. Evidence for the existence of factors which might control the initiation of cell cycle events came from other types of experiments. For example, experiments done with Tetrahymena showed that heat shock synchronized the cell cycles of a population of cells, presumably because of the heat lability of a component that promotes cell division.

Despite what these experiments revealed about the organization of the cell cycle, none of them provided any clue to the identity of the factors responsible for cytoplasmic control over the nucleus. Nor could any of the experiments be readily adapted to provide an assay with which to identify those factors and study their biochemical mechanisms. That would require an experimental system in which cytoplasm from particular stages of the cell cycle could be isolated and used to cause a transition from one phase of the cell cycle to another. The frog oocyte provided this system and played an essential role in identifying the molecular machinery that drives the cell cycle.

Role in the cell cycle:

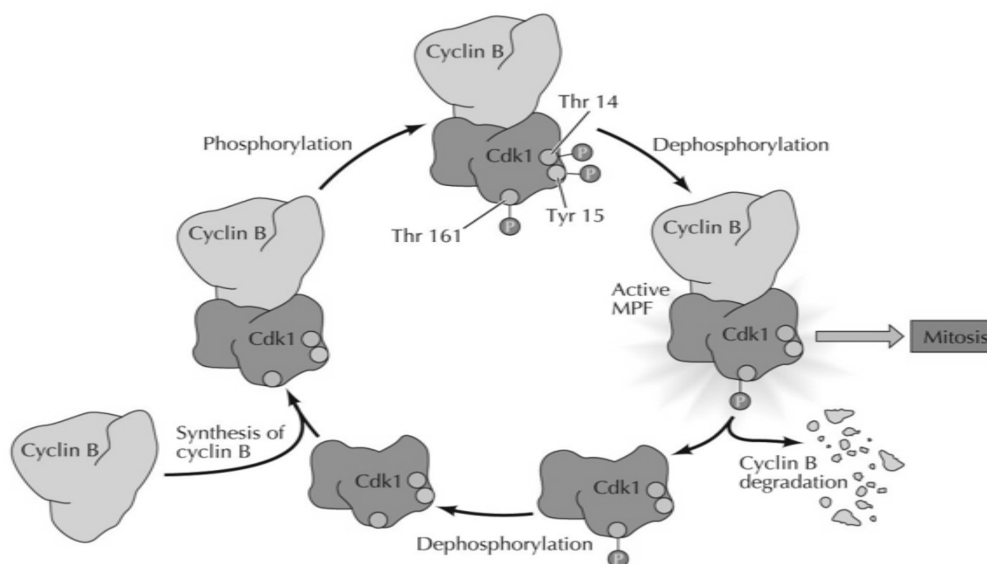
During G_1 and S phase, the CDK1 subunit of MPF is inactive due to an inhibitory enzyme, Wee1. Wee1 phosphorylates the Thr-14 residues in yeast and Tyr-15 residues in humans of CDK1, rendering MPF inactive. During the transition of G_2 to M phase, cdk1 is de-

phosphorylated by CDC25. The CDK1 subunit is now free and can bind to cyclin B, activate MPF, and make the cell enter mitosis. There is also a positive feedback loop that inactivates wee1.

Activation:

MPF must be activated in order for the cell to transition from G_2 to M phase. There are three amino acid residues responsible for this G_2 to M phase transition. The Threonine-161 (Thr-161) on CDK1 must be phosphorylated by a Cyclin Activating Kinase (CAK). CAK only phosphorylates Thr-161 when cyclin B is attached to CDK1.

In addition, two other residues on the CDK1 subunit must be activated by dephosphorylation. CDC25 removes a phosphate from residues Threonine-14 (Thr-14) and Tyrosine-15 (Tyr-15) and adds a hydroxyl group. Cyclin B/CDK1 activates CDC25 resulting in a positive feedback loop.



Overview of functions

- Triggers the formation of mitotic spindle through microtubule instability.
- Promotes mitosis i.e. chromatin condensation through phosphorylation of condensins.
- The three lamins present in the nuclear lamina, lamin A, B & C, are phosphorylated

by MPF at serine amino residues. This leads to depolymerisation of the nuclear lamina & breakdown of nuclear envelope into small vesicles.

- Causes phosphorylation of GM130, which leads to the fragmentation of the Golgi and the ER

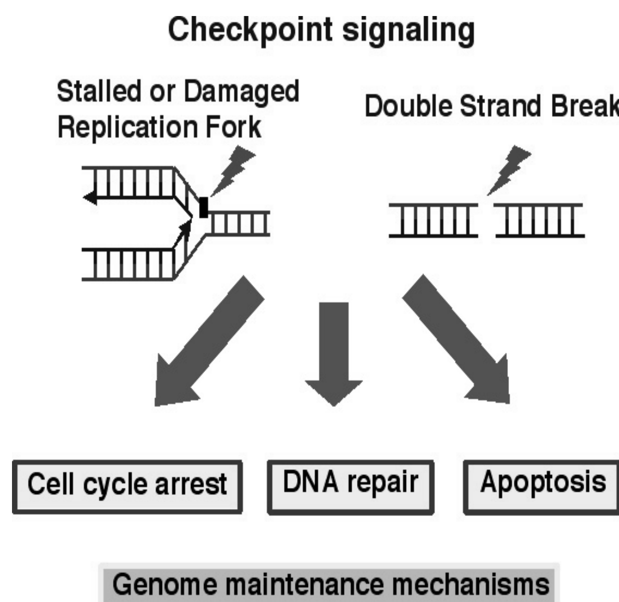
Anaphase-Promoting Complex/Cyclosome

Ingeniously, the protein kinase MPF doesn't just ensure that the nuclear envelope breaks down during mitosis – it also ensures that MPF levels will fall after the nuclear envelope is broken down. It does this by activating the Anaphase-Promoting Complex/Cyclosome, or "APC/C" for short.

As its name suggests, the APC/C promotes passage into Anaphase – and one of the ways it does that is by breaking down MPF, a messenger from a previous phase. So MPF actually activates the very proteins that destroy it.

The destruction of MPF by the APC/C ensures that the actions MPF promotes – such as the disintegration of the nuclear envelope – do not happen again until the daughter cell makes more MPF after passing through G_1 phase, S phase, and G_2 phase.

Cell Cycle Checkpoint:



The cell cycle proceeds by a defined sequence of events where late events depend upon completion of early events. The aim of the dependency of events is to distribute complete and accurate replicas of the genome to daughter cells. To monitor this dependency, cells are equipped with the **checkpoints** that are set at various stages of the cell cycle. When cells have DNA damages that have to be repaired, cells activate **DNA damage checkpoint** that arrests cell cycle. According to the cell cycle stages, DNA damage checkpoints are classified into at least 3 checkpoints:

G1/S (G1) checkpoint, intra-S phase checkpoint, and G2/M checkpoint. Upon perturbation of DNA replication by drugs that interfere with DNA synthesis, DNA lesions, or obstacles on DNA, cells activate **DNA replication checkpoint** that arrests cell cycle at G2/M transition until DNA replication is complete. There are more checkpoints such as **Spindle checkpoint** and Morphogenesis checkpoint. The spindle checkpoint arrests cell cycle at M phase until all chromosomes are aligned on spindle. This checkpoint is very important for equal distribution of chromosomes. Morphogenesis checkpoint detects abnormality in cytoskeleton and arrests cell cycle at G2/M transition.

DNA maintenance checkpoint:

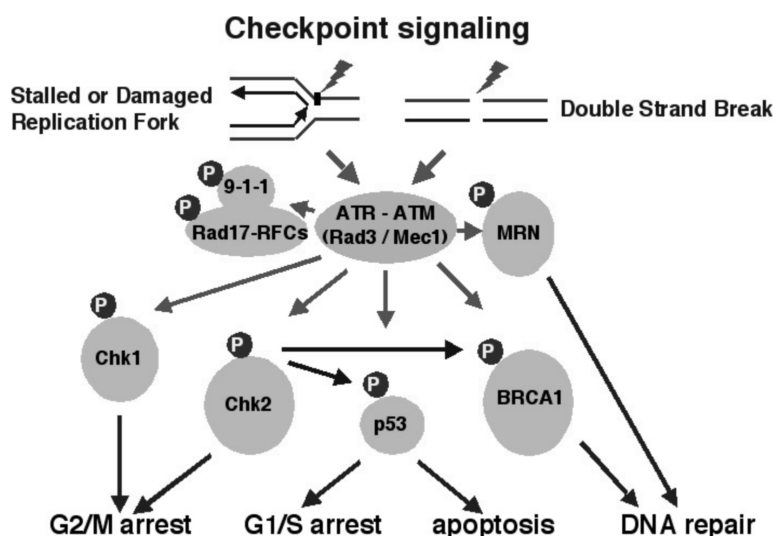
Accurate duplication of eukaryotic genome is a challenging task, given that environment of cell growth and division is rarely ideal. Cells are constantly under the stress of intrinsic and extrinsic agents that cause DNA damage or interference with DNA replication. To cope with these assaults, cells are equipped with **DNA maintenance checkpoints** to **arrest cell cycle** and facilitate **DNA repair** pathways. DNA maintenance checkpoints include (a) the **DNA damage checkpoints** that recognize and respond to DNA damage, and (b) the **DNA replication checkpoint** that monitors the fidelity of copying DNA.

(a) DNA damage checkpoint

DNA damage checkpoints ensure the fidelity of genetic information both by arresting cell cycle progression and facilitating DNA repair pathways. Studies on many different species have uncovered a network of proteins that form the DNA damage checkpoints. Central to this network are **protein kinases** of **ATM/ATR** family known as **Tel1/Mec1** in budding yeast and **Tel1/Rad3** in fission yeast. These kinases sense DNA damages and

phosphorylate number of proteins that regulate cell cycle progression and DNA repair pathways.

(b) DNA replication checkpoint



Accurate replication of the millions or billions of DNA base pairs in a eukaryotic genome is a remarkable achievement. This accomplishment is even more astonishing when one considers for DNA synthesis are rarely ideal. Damaged template, protein complexes bound to DNA, and poor supply of dNTPs are among the many obstacles that must be overcome to replicate genome. All of these situations can stall **replication forks**. **Stalled forks** pose grave threats to **genome integrity** because they can rearrange, break, or collapse through disassembly of the replication complex. The pathways that respond to replication stress are signal transduction pathways that are conserved across evolution.Atop the pathways are also ATM/ATR family kinases. These kinases together with a trimeric checkpoint clamp (termed 9-1-1 complex) and five-subunit checkpoint clamp loader (Rad17-RFC2-RFC3-RFC4-RFC5) senses stalled replication forks and transmit a checkpoint signal. One of major functions of replication checkpoint is to prevent the onset of mitosis by regulating mitotic control proteins such as Cdc25.

But perhaps the most important activity of replication checkpoint is to stabilize and protect replication forks. The protein kinase **Cds1** (human **Chk2** homolog; in human, **Chk1** is a functional Cds1 homolog) is a critical effector of the replication checkpoint in the fission yeast *Schizosaccharomyces pombe*. Cds1 is required to prevent stabilization of replication

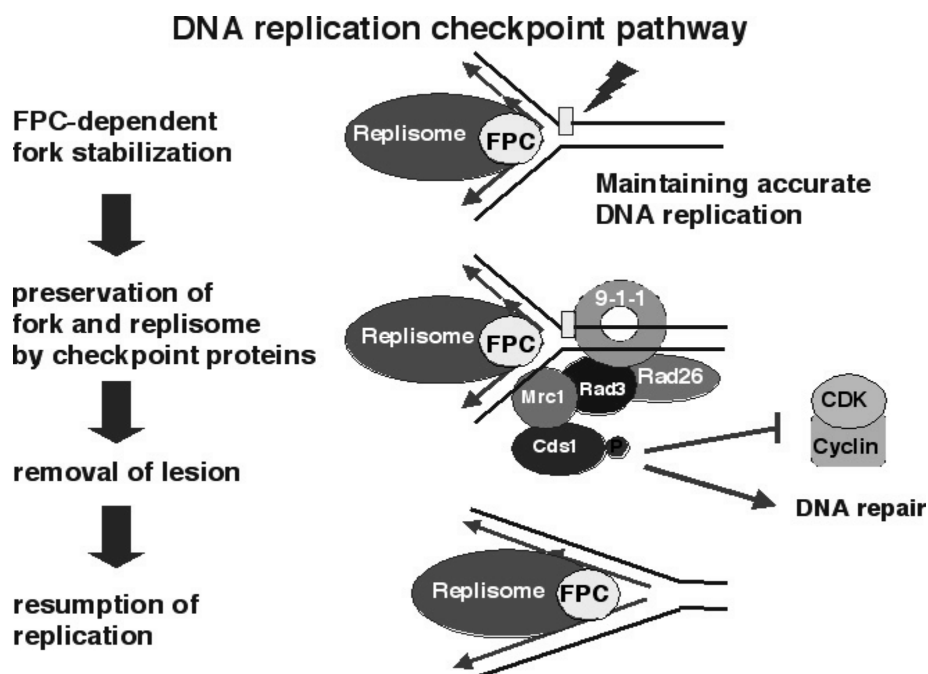
fork in cells treated with hydroxyurea (HU), a ribonucleotide reductase inhibitor that stalls replication by depleting dNTPs. In the budding yeast *Saccharomyces cerevisiae*, a failure to activate **Rad53** (Chk2 homolog) is associated with collapse and regression of replication forks and gross chromosomal rearrangements in cells treated with HU.

Replication fork protection complex (FPC):

The DNA replication checkpoint stabilizes replication forks that have stalled at DNA adducts and other lesions that block DNA polymerases. In the absence of DNA replication checkpoint, stalled forks are thought to collapse, creating **strand break** that threatens genome stability and cell viability. Therefore, discovering how cells cope with aberrant replication forks is essential for understanding mechanisms of genome maintenance.

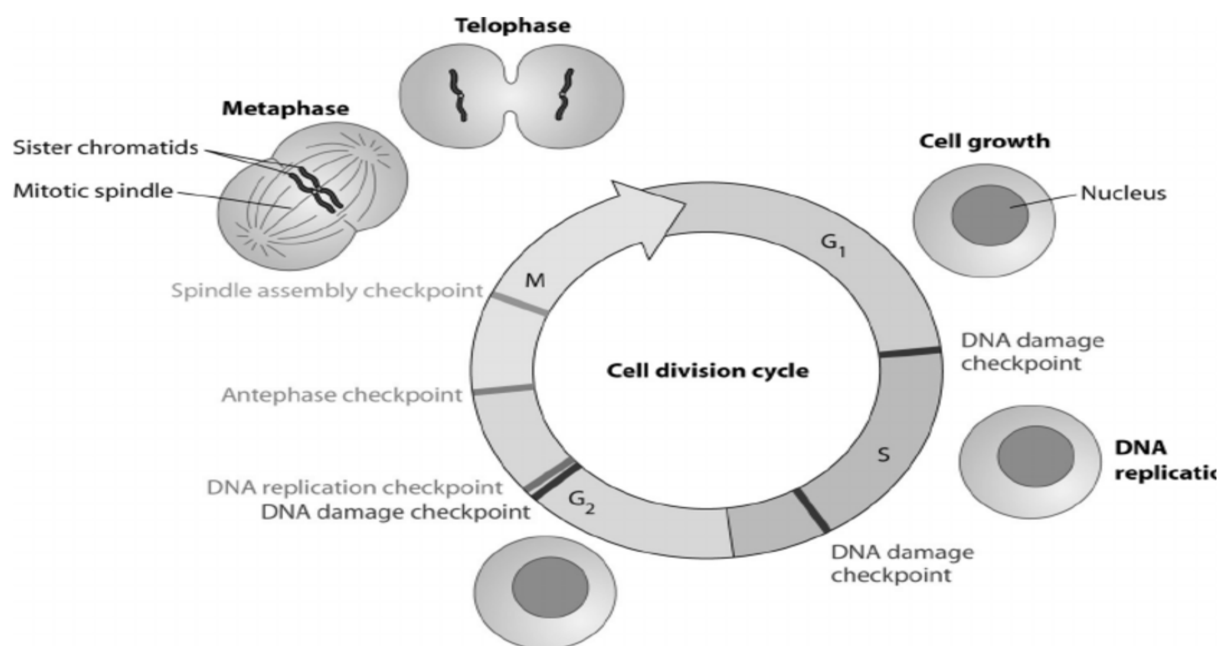
The Chk1 and Chk2/Cds1 checkpoint kinases, which are key mediators of DNA damage and DNA replication checkpoints, are thought to be involved in **cancer development**. We found the **Swi1** protein is required for survival of replication fork arrest and effective activation of Chk2 kinase in fission yeast.

Swi1 forms tight complex with **Swi3** protein and moves with replication forks. **Swi1-Swi3 complex** is also important for proficient DNA replication even in the absence of agents that cause genotoxic stress, creating **single-strand DNA gaps** at replication forks.



These results led us to propose Swi1-Swi3 define a **replication fork protection complex (FPC)** that stabilizes replication forks in a configuration that is recognized by replication checkpoint sensors. Interestingly, **Tof1** protein (Budding yeast Swi1 homolog) has been reported to have similar functions. Tof1 is also involved in Rad53 (Chk2 homolog) activation and travels with replication fork.

Tof1 is needed to restrain fork progression when DNA synthesis is inhibited by HU indicating that Tof1 is required for coordination of DNA synthesis and **replisome** (replication machinery) movement.



Role of Rb and P53 protein in cell cycle:

Rb Inactivation:

Since the 1990s, Rb was known to be inactivated via phosphorylation. Until, the prevailing model was that Cyclin D- Cdk 4/6 progressively phosphorylated it from its unphosphorylated

to it hyperphosphorylated state (14+ phosphorylations). However, it was recently shown that Rb only exists in three state: un-phosphorylated, mono-phosphorylated, and hyper-phosphorylated. Each has a unique cellular function.

Before the development of 2D IEF, only hyper-phosphorylated Rb was distinguishable from all other forms, i.e. un-phosphorylated Rb resembled mono-phosphorylated Rb on immunoblots. As Rb was either in its active "hypo-phosphorylated" state or inactive "hyperphosphorylated" state. However, with 2D IEF, it is now known that Rb is un-phosphorylated in G0 cells and mono-phosphorylated in early G1 cells, prior to hyper-phosphorylation after the restriction point in late G1.

Cyclin D - Cdk 4/6 Mono-phosphorylates Rb:

When a cell enters G1, Cyclin D- Cdk4/6 phosphorylates Rb at a single phosphorylation site. No progressive phosphorylation occurs because when HFF cells were exposed to sustained cyclin D- Cdk4/6 activity (and even deregulated activity) in early G1, only mono-phosphorylated Rb was detected. Furthermore, triple knockout, p16 addition, and Cdk 4/6 inhibitor addition experiments confirmed that Cyclin D- Cdk 4/6 is the sole phosphorylator of Rb. Throughout early G1, mono-phosphorylated Rb exists as 14 different isoforms (the 15th phosphorylation site is not conserved in primates in which the experiments were performed). Together, these isoforms represent the "hypo-phosphorylated" active Rb state that was thought to exist. Each isoform has distinct E2F binding preferences which suggest that mono-phosphorylated Rb has a diversity of functions and can be "active" to varying degrees.It is currently unknown how such specificity is achieved.

Passing a bifurcation point induces hyper-phosphorylation by Cyclin E - Cdk2:

After a cell passes the restriction point, Cyclin E - Cdk 2 hyper-phosphorylates all mono-phosphorylated isoforms. While the exact mechanism is unknown, one hypothesis is that binding to the C-terminus tail opens the pocket subunit, allowing access to all phosphorylation sites. This process is hysteretic and irreversible, and it is thought accumulation of mono-phosphorylated Rb induces the process. The bistable, switch like behavior of Rb can thus be modeled as a bifurcation point. Hyper-phosphorylation of mono-phosphorylated Rb is an irreversible event that allows entry into S phase.

Un-phosphorylated and mono-phosphorylated Rb have unique functional roles:

Presence of un-phosphorylated Rb drives cell cycle exit and maintains senescence. At the end of mitosis, PP1 dephosphorylates hyper-phosphorylated Rb directly to its un-phosphorylated state. Furthermore, when cycling C2C12 myoblast cells differentiated (by being placed into a differentiation medium), only un-phosphorylated Rb was present. Additionally, these cells had a markedly decreased growth rate and concentration of DNA replication factors (suggesting G0 arrest).

This function of un-phosphorylated Rb gives rise to a hypothesis for the lack of cell cycle control in cancerous cells: Deregulation of Cyclin D - Cdk 4/6 phosphorylates un-phosphorylated Rb in senescent cells to mono-phosphorylated Rb, causing them to enter G1. The mechanism of the switch for Cyclin E activation is not known, but one hypothesis is that it is a metabolic sensor. Mono-phosphorylated Rb induces an increase in metabolism, so the accumulation of mono-phosphorylated Rb in previously G0 cells then causes hyper-phosphorylation and mitotic entry. Since any un-phosphorylated Rb is immediately phosphorylated, the cell is then unable to exit the cell cycle, resulting in continuous division.

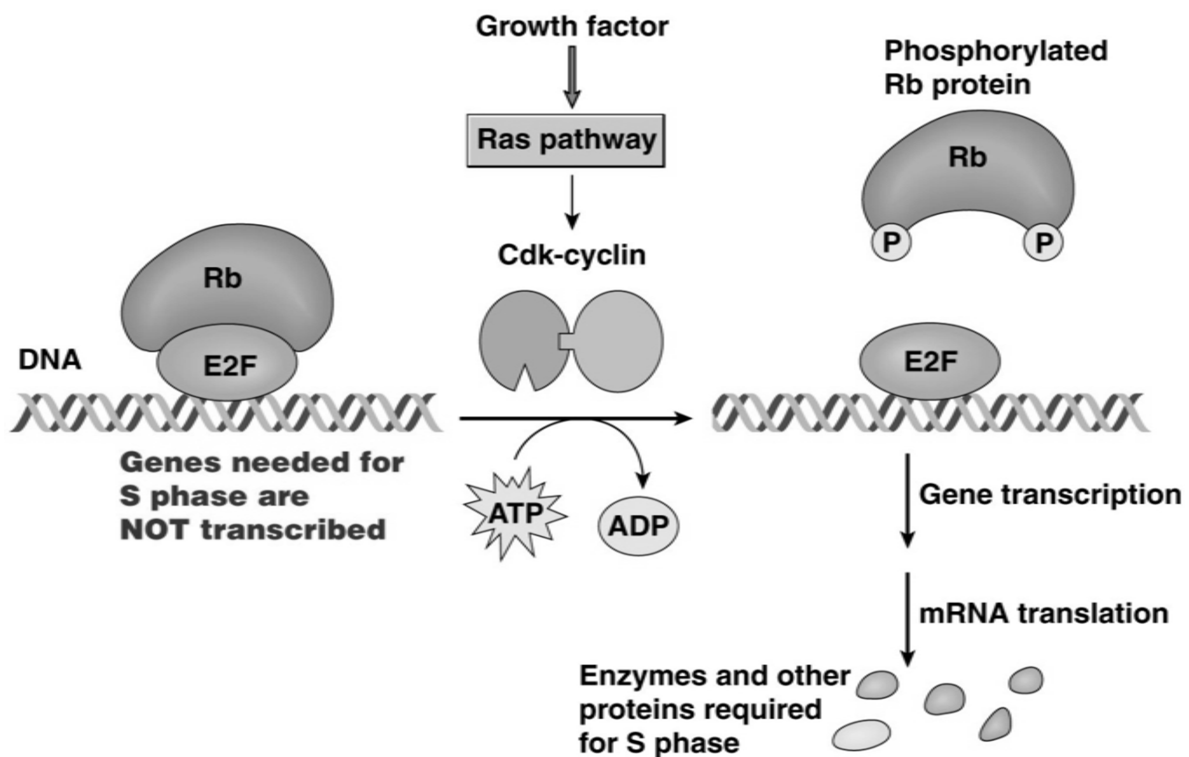
DNA damage to G0 cells activates Cyclin D - Cdk 4/6, resulting in mono-phosphorylation of un-phosphorylated Rb. Then, active mono-phosphorylated Rb causes repression of E2F-targeted genes specifically. Therefore, mono-phosphorylated Rb is thought to play an active role in DNA damage response, so that E2F gene repression occurs until the damage is fixed and the cell can pass the restriction point. As a side note, the discovery that damages causes Cyclin D - Cdk 4/6 activation even in G0 cells should be kept in mind when patients are treated with both DNA damaging chemotherapy and Cyclin D - Cdk 4/6 inhibitors.

Activation:

During the M-to-G1 transition, pRb is then progressively dephosphorylated by PP1, returning to its growth-suppressive hypophosphorylated state Rb. Rb family proteins are components of the DREAM complex (also named LINC complex), which is composed of LIN9, LIN54,

LIN37, MYBL2, RBL1, RBL2, RBBP4, TFDP1, TFDP2, E2F4 and E2F5. There is a testis-specific version of the complex, where LIN54, MYBL2 and RBBP4 are replaced by MTL5, MYBL1 and RBBP7, respectively. In Drosophila both DREAM versions also exist, the components being mip130 (lin9 homolog, replaced by aly in testes), mip120 (lin54 homolog, replaced by tomb in testes), and Myb, Caf1p55, DP, Mip40, E2F2, Rbf and Rbf2.

The DREAM complex exists in quiescent cells in association with MuvB (consisting of HDAC1 or HDAC2, LIN52 and L3mbtl1, L3mbtl3 or L3mbtl4) where it represses cell cycle-dependent genes. DREAM dissociates in S phase when LIN9, LIN37, LIN52 and LIN54 form a subcomplex that binds to MYBL2.



Probable questions:

1. Define Cell cycle. Describe different phases of Cell Cycle.

2. How cell cycles are regulated?

3. Write a note on Cyclin Dependent Kinase inhibitors?

4. What are Cell Cycle Checkpoints? How they regulate cell cycle?

Suggested Readings:

1. Lodish, H. (2016). Molecular cell biology. New York, NY: Freeman.

2. Alberts, B. (2008). Molecular biology of the cell. New York, NY [u.a.]: Garland Science Taylor & Francis.

3. Lewin, B., Krebs, J., Goldstein, E. and Kilpatrick, S. (2014). Lewin's genes XI. Burlington, MA: Jones & Bartlett Learning.

4. Karp, G. and Patton, J. (2015). Cell and molecular biology. Brantford, Ont.: W. Ross MacDonald School Resource Services Library.

5. Cooper, G. and Hausman, R. (n.d.). The cell.

Unit-XI

Cancer: Types and stages. Tumor suppressor genes and protooncogenes. Molecular basis of cancer.; Apoptosis: Neurotrophic factors, caspases, Pathways of apoptosis; cell senescence, telomerase.

Objectives:

In this Unit we will discuss on Cancer: Types and stages. Tumor suppressor genes and protooncogenes. Molecular basis of cancer.; Apoptosis: Neurotrophic factors, caspases, Pathways of apoptosis; cell senescence, telomerase.

Introduction

Cancer is a disease of cells. Despite of several types, two things are very common in these cells: division at inappropriate time and locations. It means there is "no need of division and this is not a right time of division". Normal cells are constantly subjected to signals that dictate whether the cell should divide, differentiate into another cell or die. Cancer cells develop a degree of autonomy resulting in above mentioned problems. If the proliferations of dividing cells are continued, then it results into metastasis of those cells. The metastasis is a fatal condition because almost 90 % of cancer-related deaths are due to this metastasis.

Initiation and progression of cancer depends on both external factors in the environment (tobacco, chemicals, radiation, infectious organisms) and factors within the cell (inherited mutations, hormones, immune conditions, and mutation that occur due to metabolism). These factors can act together or in sequence resulting in abnormal cell behavior and rapid proliferation. However it is important to remember that most common cancers take months and years for these DNA mutations to accumulate and result in a detectable cancer.

#Note: Cancers arise approximately 1 among 3 individuals. DNA mutations arise normally at a frequency of 1 in every 20 million base pairs per gene per cell division. The average number of cells formed in any individual during an average lifetime is 10^{16} (10 million cells being replaced every second!). It would therefore be logical to assume that human populations anywhere in the world would show similar frequencies of cancer. However, cancer incidence rates (number of individuals diagnosed) vary dramatically across countries.

Evidently, some factors seem to intervene to dramatically increase cancer incidences in some populations. The obvious inference is that contributory factors that cause cancer are either hereditary or environmental. It means that either certain populations carry a large number of cancer-susceptibility genes or that the environments in which populations live largely contribute to the cancer incidence rates.

Cancer Statistics in India

One woman dies of cervical cancer every 8 minutes in India.

For every 2 women newly diagnosed with breast cancer, one woman dies of it in India.

As many as 2,500 persons die every day due to tobacco-related diseases in India.

Smoking accounts for 1 in 5 deaths among men and 1 in 20 deaths among women, accounting for an estimated 9,30,000 deaths in 2010.

Estimated number of people living with the disease: around 2.5 million

Every year, new cancer patients registered: Over 7 lakh

Cancer-related deaths: 5,56,400

Deaths in the age group between 30-69 years

Total: 3,95,400 (71% of all cancer related deaths)

Men: 2,00,100

Women: 1,95,300

Cancers of oral cavity and lungs in males and cervix and breast in females account for over 50% of all cancer deaths in India.

The top five cancers in men and women account for 47.2% of all cancers; these cancers can be prevented, screened for and/or detected early and treated at an early stage. This could significantly reduce the death rate from these cancers. (Source: http://cancerindia.org.in)

	Men	Women
1	LIP,ORAL CAVITY	BREAST
2	LUNG	CERVIX
3	STOMACH	COLORECTUM
4	COLORECTUM	OVARY
5	PHARYNX	LIP,ORAL CAVITY

Classification:

Cancers are classified in two ways: by the type of tissue in which the cancer originates (histological type) and by primary site, or the location in the body where the cancer first developed.

From a histological standpoint there are hundreds of different cancers, which are grouped into **six major categories:**

- Carcinoma
- Sarcoma
- Myeloma
- Leukemia
- Lymphoma
- Mixed Types

Carcinoma

Carcinoma refers to a malignant neoplasm of epithelial origin or cancer of the internal or external lining of the body. Carcinomas, malignancies of epithelial tissue, account for 80 to 90 percent of all cancer cases. Carcinomas are divided into two major subtypes: adenocarcinoma, which develops in an organ or gland, and squamous cell carcinoma, which originates in the squamous epithelium.

Adenocarcinomas generally occur in mucus membranes and are first seen as a thickened plaque-like white mucosa. They often spread easily through the soft tissue where they occur. Squamous cell carcinomas occur in many areas of the body. Most carcinomas affect organs or glands capable of secretion, such as the breasts, which produce milk, or the lungs, which secrete mucus, or colon or prostate or bladder.

Sarcoma

Sarcoma refers to cancer that originates in supportive and connective tissues such as bones, tendons, cartilage, muscle, and fat. Generally occurring in young adults, the most common sarcoma often develops as a painful mass on the bone. Sarcoma tumors usually resemble the tissue in which they grow. Examples of sarcomas are: Osteosarcoma or osteogenic sarcoma (bone), Chondrosarcoma (cartilage), Leiomyosarcoma (smooth muscle), Rhabdomyosarcoma (skeletal muscle), Mesothelial sarcoma or mesothelioma (membranous lining of body cavities), Fibrosarcoma (fibrous tissue), Angiosarcoma or hemangioendothelioma (blood vessels), Liposarcoma (adipose tissue), Glioma or astrocytoma (neurogenic connective tissue found in the brain), Myxosarcoma (primitive

embryonic connective tissue), Mesenchymous or mixed mesodermal tumor (mixed connective tissue types)

Myeloma

Myeloma is cancer that originates in the plasma cells of bone marrow. The plasma cells produce some of the proteins found in blood.

Leukemia

Leukemias ("liquid cancers" or "blood cancers") are cancers of the bone marrow. The disease is often associated with the overproduction of immature white blood cells. These immature white blood cells do not perform as well as they should, therefore the patient is often prone to infection. Leukemia also affects red blood cells and can cause poor blood clotting and fatigue due to anaemia. Examples of leukaemia include:

- Myelogenous or granulocytic leukemia (malignancy of the myeloid and granulocytic white blood cell series)
- Lymphatic, lymphocytic, or lymphoblastic leukemia (malignancy of the lymphoid and lymphocytic blood cell series)
- Polycythemia vera or erythremia (malignancy of various blood cell products, but with red cells predominating)

Lymphoma

Lymphomas develop in the glands or nodes of the lymphatic system, a network of vessels, nodes, and organs (specifically the spleen, tonsils, and thymus) that purify bodily fluids and produce infection-fighting white blood cells, or lymphocytes. Unlike the leukemias which are sometimes called "liquid cancers," lymphomas are "solid cancers." Lymphomas may also occur in specific organs such as the stomach, breast or brain. These lymphomas are referred to as extranodal lymphomas. The lymphomas are subclassified into two categories: Hodgkin lymphoma and Non-Hodgkin lymphoma. The presence of Reed-Sternberg cells in Hodgkin lymphoma diagnostically distinguishes Hodgkin lymphoma from Non-Hodgkin lymphoma.

Mixed Types

The type components may be within one category or from different categories. Some examples are: adenosquamous carcinoma, mixed mesodermal tumor, carcinosarcoma, teratocarcinoma.

CANCER TYPES BY SITE

Medical professionals frequently refer to cancers based on their histological type. However, the general public is more familiar with cancer names based on their primary sites. The most common sites in which cancer develops include: Skin, Lungs, Female Breasts, Prostate, Colon and Rectum, Cervix and Uterus.

Compared with those based on histological type, cancers named after the primary site may not be as accurate. Take lung cancer for example; the name does not specify the type of tissue involved. It simply indicates where the cancer is located. In fact, depending on how the cells look under a microscope, there are two major types of lung cancer: non-small cell lung cancer and small cell lung cancer. Non-small cell lung cancer can be further divided into various types named for the type of cells in which the cancer develops, typically: squamous cell carcinoma, adenocarcinoma, and large cell carcinoma. However, it's important to know that cancer can be classified either by the cell type or its primary site. Saying that a woman has uterine carcinoma or uterine cancer is the same thing as saying that she has cancer (or carcinoma) of the uterus.

Hanahan and Weinberg (2000) listed the seven attributes of cancer: 1) Self sufficiency in growth signals, 2) Insensitivity to anti-growth signals, 3) Evading apoptosis, 4) Limitless replicative potential, telomerase and telomeres 5) Sustained angiogenesis, 6) Tissue invasion and metastasis, and 7) Genome instability.

Cancer stages

There are 2 main types of staging systems for cancer. These are the TNM system and the number system.

The TNM staging system: TNM stands for Tumor, Node, Metastasis. This system describes the size of the initial cancer (the primary tumor), whether the cancer has spread

to the lymph nodes, and whether it has spread to a different part of the body (metastasized). The system uses letters and numbers to describe the cancer:

T refers to the size of the cancer and how far it has spread into nearby tissue – it can be 1, 2, 3 or 4, with 1 being small and 4 large

N refers to whether the cancer has spread to the lymph nodes – it can be between 0 (no lymph nodes containing cancer cells) and 3 (lots of lymph nodes containing cancer cells)

M refers to whether the cancer has spread to another part of the body – it can either be 0 (the cancer hasn't spread) or 1 (the cancer has spread)

So for example, a small cancer that has spread to the lymph nodes but not to anywhere else in the body may be T2 N1 M0. Or a more advanced cancer that has spread may be T4 N3 M1.

Sometimes doctors use the letters a, b or c to further divide the categories. For example, stage M1a lung cancer is a cancer that has spread to the other lung. Stage M1b lung cancer has spread to other parts of the body.

The letter p is sometimes used before the letters TNM. For example, pT4. This stands for pathological stage. It means that doctors based the staging on examining cancer cells in the lab, after surgery to remove a cancer.

The letter c is sometimes used before the letters TNM. For example, cT2. This stands for clinical stage. It means that the stage is based on what the doctor knows about the cancer before surgery. Doctors may look at your tests results and use the clinical information from examining you.

Number staging systems: Number staging systems usually use the TNM system to divide cancers into stages. Most types of cancer have 4 stages, numbered from 1 to 4. Often doctors write the stage down in Roman numerals. So you may see stage 4 written down as stage IV.

Here is a brief summary of what the stages mean for most types of cancer:

Stage 1 usually means that a cancer is relatively small and contained within the organ it started in

Stage 2 usually means that the tumor is larger than in stage 1, but the cancer has not started to spread into the surrounding tissues. Sometimes stage 2 means that cancer cells have spread into lymph nodes close to the tumor. This depends on the particular type of cancer

Stage 3 usually means the cancer is larger. It may have started to spread into surrounding tissues and there are cancer cells in the lymph nodes in the area

Stage 4 means the cancer has spread from where it started to another body organ. This is also called secondary or metastatic cancer

Sometimes doctors use the letters A, B or C to further divide the number categories. For example, stage 3B cervical cancer.

Carcinoma in situ: Carcinoma in situ is sometimes called stage 0 cancer or 'in situ neoplasm'. It means that there is a group of abnormal cells in an area of the body. The cells may develop into cancer at some time in the future. The changes in the cells are called dysplasia. The number of abnormal cells is too small to form a tumor.

Some doctors and researchers call these cell changes 'precancerous changes' or 'non invasive cancer'. But many areas of carcinoma in situ will never develop into cancer. So some doctors feel that these terms are inaccurate and they don't use them.

Because these areas of abnormal cells are still so small they are usually not found unless they are somewhere easy to spot, for example in the skin. A carcinoma in situ in an internal organ is usually too small to show up on a scan. But tests used in cancer screening programmes can pick up carcinomas in situ in the breast or the neck of the womb (cervix).

Tumor Suppressor Genes

Tumor suppressor genes represent the opposite side of cell growth control, normally acting to inhibit cell proliferation and tumor development. In many tumors, these genes are lost or inactivated, thereby removing negative regulators of cell proliferation and contributing to the abnormal proliferation of tumor cells.

Identification of Tumor Suppressor Genes

The first insight into the activity of tumor suppressor genes came from somatic cell hybridization experiments, initiated by Henry Harris and his colleagues in 1969. The fusion

of normal cells with tumor cells yielded hybrid cells containing chromosomes from both parents. In most cases, such hybrid cells were not capable of forming tumors in animals. Therefore, it appeared that genes derived from the normal cell parent acted to inhibit (or suppress) tumor development. The first tumor suppressor gene was identified by studies of retinoblastoma, a rare childhood eye tumor. Consequently, it was recognized that some cases of retinoblastoma are inherited. In these cases, approximately 50% of the children of an affected parent develop retinoblastoma, consistent with Mendelian transmission of a single dominant gene that confers susceptibility to tumor development. Although susceptibility to retinoblastoma is transmitted as a dominant trait, inheritance of the susceptibility gene is not sufficient to transform a normal retinal cell into a tumor cell. All retinal cells in a patient inherit the susceptibility gene, but only a small fraction of these cells give rise to tumors. Thus, tumor development requires additional events beyond inheritance of tumor susceptibility. In 1971, Alfred Knudson proposed that the development of retinoblastoma requires two mutations, which are now known to correspond to the loss of both of the functional copies of the tumor susceptibility gene (the Rb tumor suppressor gene) that would be present on homologous chromosomes of a normal diploid cell. In inherited retinoblastoma, one defective copy of Rb is genetically transmitted. The loss of this single *Rb* copy is not by itself sufficient to trigger tumor development, but retinoblastoma almost always develops in these individuals as a result of a second somatic mutation leading to the loss of the remaining normal *Rb* allele. Noninherited retinoblastoma, in contrast, is rare, since its development requires two independent somatic mutations to inactivate both normal copies of *Rb* in the same cell.

The functional nature of the Rb gene as a negative regulator of tumorigenesis was initially indicated by observations of chromosome morphology. Visible deletions of chromosome 13q14 were found in some retinoblastomas, suggesting that loss (rather than activation) of the *Rb* gene led to tumor development. Gene-mapping studies further indicated that tumor development resulted from loss of normal *Rb* alleles in the tumor cells, consistent with the function of *Rb* as a tumor suppressor gene. Isolation of the *Rb* gene as a molecular clone in 1986 then firmly established that *Rb* is consistently lost or mutated in retinoblastomas. Gene transfer experiments also demonstrated that introduction of a normal *Rb* gene into retinoblastoma cells reverses their tumorigenicity, providing direct evidence for the activity of *Rb* as a tumor suppressor.

Rb deletions in retinoblastoma:

Many retinoblastomas have deletions of the chromosomal locus (13q14) that contains the *Rb* gene. Although Rb was identified in a rare childhood cancer, it is also involved in some of the more common tumors of adults. In particular, studies of the cloned gene have established that *Rb* is lost or inactivated in many bladder, breast, and lung carcinomas. The significance of the *Rb* tumor suppressor gene thus extends beyond retinoblastoma, apparently contributing to development of a substantial fraction of more common human cancers. In addition the Rb protein is a key target for the oncogeneproteins of several DNA tumor viruses, including SV40, adenoviruses, and human papillomaviruses, which bind to Rb and inhibit its activity. Transformation by these viruses thus results, at least in part, from inactivation of Rb at the protein level rather than from mutational inactivation of the *Rb* gene.

Characterization of Rb as a tumor suppressor gene served as the prototype for the identification of additional tumor suppressor genes that contribute to the development of many different human cancers. Some of these genes were identified as the causes of rare inherited cancers, playing a role similar to that of *Rb* in hereditary retinoblastoma. Other tumor suppressor genes have been identified as genes that are frequently deleted or mutated in common noninherited cancers of adults, such as colon carcinoma. In either case, it appears that most tumor suppressor genes are involved in the development of both inherited and noninherited forms of cancer. Indeed, mutations of some tumor suppressor genes appear to be the most common molecular alterations leading to human tumor development.

Table 1: Tumor Suppressor Genes

Familial Cancer Syndrome	Tumor Suppressor Gene	Function	Chromosomal Location	Tumor Types Observed
Li-Fraumeni Syndrome	P53	cell cycle regulation,apoptosis	17p13.1	brain tumors, sarcomas, leukemia, breast cancer
Familial Retinoblastoma	RB1	cell cycle regulation	13q14.1-q14.2	retinoblastoma, osteogenic sarcoma
Wilms Tumor	WT1	transcriptional regulation	11p13	pediatric kidney cancer, most common form of childhood solid tumor
Neurofibromatosis Type 1	NF1, protein = neurofibromin 1	catalysis of RAS inactivation	17q11.2	neurofibromas, sarcomas, gliomas
Neurofibromatosis Type 2	NF2, protein = merlin or neurofibromin 2	linkage of cell membrane to actin cytoskeleton	22q12.2	Schwann cell tumors, astrocytomas, meningiomas, ependymonas
Familial Adenomatous Polyposis	APC	sigalling through adhesion molecules to nucleus	5q21-q22	colon cancer
Tuberous sclerosis 1	TSC1, protein = hamartin	forms complex with TSC2 protein, inhibits sigalling to	9q34	seizures, mental retardation, facial angiofibromas

		downstream effectors of mTOR		
Tuberous sclerosis 2	TSC2, protein = tuberin	see TSC1 above	16p13.3	benign growths (hamartomas) in many tissues, astrocytomas, rhabdomyosarcomas
Deleted in Pancreatic Carcinoma 4, Familial juvenile polyposis syndrome	DPC4, also known as SMAD4	regulation of TGF-β/BMP signal transduction	18q21.1	pancreatic carcinoma, colon cancer
Deleted in Colorectal Carcinoma	DCC	transmembrane receptor involved in axonal guidance via netrins	18q21.3	colorectal cancer
Familial Breast Cancer	BRCA1	functions in transcription, DNA binding, transcription coupled DNA repair, homologous recombination, chromosomal stability, ubiquitination of proteins, and centrosome	17q21	breast and ovarian cancer

		replication		
Familial Breast Cancer	BRCA2: same as the FANCD1 locus	transcriptional regulation of genes involved in DNA repair and homologous recombination	13q12.3	breast and ovarian cancer

The second tumor suppressor gene to have been identified is p53, which is frequently inactivated in a wide variety of human cancers, including leukemias, lymphomas, sarcomas, brain tumors, and carcinomas of many tissues, including breast, colon, and lung. In total, mutations of p53 may play a role in up to 50% of all cancers, making it the most common target of genetic alterations in human malignancies. It is also of interest that inherited mutations of p53 are responsible for genetic transmission of a rare hereditary cancersyndrome, in which affected individuals develop any of several different types of cancer. In addition, the p53 protein (like Rb) is a target for the oncogene proteins of SV40, adenoviruses, and human papillomaviruses.

Like p53, the *INK4* and PTEN tumor suppressor genes are very frequently mutated in several common cancers, including lung cancer, prostate cancer, and melanoma. Two other tumor suppressor genes (*APC*and *MADR2*) are frequently deleted or mutated in colon cancers. In addition to being involved in noninherited cases of this common adult cancer, inherited mutations of the *APC* gene are responsible for a rare hereditary form of colon cancer, called familial adenomatous polyposis. Individuals with this condition develop hundreds of benign colon adenomas (polyps), some of which almost inevitably progress to malignancy. Inherited mutations of two other tumor suppressor genes, *BRCA1* and *BRCA2*, are responsible for hereditary cases of breast cancer, which account for 5 to 10% of the total breast cancer incidence.

Additional tumor suppressor genes have been implicated in the development of brain tumors, pancreatic cancers, and basal cell skin carcinomas, as well as in several rare inherited cancer syndromes, such as Wilms' tumor. The number of identified tumor

suppressor genes is rapidly expanding, and the characterization of these genes remains an active area of cancer research

Functions of Tumor Suppressor Gene Products

In contrast to proto-oncogene and oncogene proteins, the proteins encoded by most tumor suppressor genes inhibit cell proliferation or survival. Inactivation of tumor suppressor genes therefore leads to tumor development by eliminating negative regulatory proteins. In several cases, tumor suppressor proteins inhibit the same cell regulatory pathways that are stimulated by the products of oncogenes.

The protein encoded by the PTEN tumor suppressor gene is an interesting example of antagonism between oncogene and tumor suppressor gene products. The PTEN protein is a lipid phosphatase that dephosphorylates the 3 position of phosphatidylinositides, such as phosphatidylinositol 3,4,5-bisphosphate (PIP_3). By dephosphorylating PIP_3, PTEN antagonizes the activities of PI 3-kinase and Akt, which can act as oncogenes by promoting cell survival. Conversely, inactivation or loss of the PTEN tumor suppressor protein can contribute to tumor development as a result of increased levels of PIP_3, activation of Akt, and inhibition of programmed cell death.

Proteins encoded by both oncogenes and tumor suppressor genes also function in the Hedgehog sigalling pathway. The receptor Smoothened is an oncogene in basal cell carcinomas, whereas Patched (the negative regulator of Smoothened) is a tumor suppressor gene. In addition, the Gli proteins (the mammalian homologs of the *Drosophil* Ci transcription factor activated by Smoothened) were first identified as the products of an amplified oncogene.

Several tumor suppressor genes encode transcriptional regulatory proteins. A good example is provided by the product of *WT1*, which is frequently inactivated in Wilms' tumors (a childhood kidney tumor). The WT1 protein is a repressor that appears to suppress transcription of a number of growth factor-inducible genes. One of the targets of WT1 is thought to be the gene that encodes insulin-like growth factor II, which is overexpressed in Wilms' tumors and may contribute to tumor development by acting as an autocrine growth factor. Inactivation of WT1 may thus lead to abnormal growth factor expression, which in turn drives tumor cell proliferation. Two other tumor suppressor

genes, *DPC4* and *MADR2*, encode SMAD family transcription factors that are activated by TGF-β sigalling and lead to inhibition of cell proliferation.

The products of the Rb and *INK4* tumor suppressor genes regulate cell cycle progression at the same point as that affected by cyclin D1. Rb inhibits passage through the restriction point in G_1 by repressing transcription of a number of genes involved in cell cycle progression and DNA synthesis . In normal cells, passage through the restriction point is regulated by Cdk4/cyclin D complexes, which phosphorylate and inactivate Rb. Mutational inactivation of *Rb* in tumors thus removes a key negative regulator of cell cycle progression. The *INK4* tumor suppressor gene, which encodes the Cdk inhibitor p16, also regulates passage through the restriction point. p16 inhibits Cdk4/cyclin D activity. Inactivation of *INK4* therefore leads to elevated activity of Cdk4/cyclin D complexes, resulting in uncontrolled phosphorylation of Rb.

The p53 gene product regulates both cell cycle progression and apoptosis (Figure 15.39). DNA damage leads to rapid induction of p53, which activates transcription of the Cdk inhibitor p21. The inhibitor p21 blocks cell cycle progression, both by acting as a general inhibitor of Cdk/cyclin complexes and by inhibiting DNA replication by binding to PCNA (proliferating cell nuclear antigen). The resulting cell cycle arrest presumably allows time for damaged DNA to be repaired before it is replicated. Loss of p53 prevents this damage-induced cell cycle arrest, leading to increased mutation frequencies and a general instability of the cell genome. Such genetic instability is a common property of cancer cells, and it may contribute to further alterations in oncogenes and tumor suppressor genes during tumor progression.

Action of p53: Wild-type p53 is required for both cell cycle arrest and apoptosis induced by DNA damage. In addition to mediating cell cycle arrest, p53 is required for apoptosis induced by DNA damage. Unrepaired DNA damage normally induces apoptosis of mammalian cells, a response that is presumably advantageous to the organism because it eliminates cells carrying potentially deleterious mutations (e.g., cells that might develop into cancer cells). Cells lacking p53 fail to undergo apoptosis in response to agents that damage DNA, including radiation and many of the drugs used in cancer chemotherapy. This failure to undergo apoptosis in response to DNA damage contributes to the resistance of many tumors to chemotherapy. In addition, loss of p53 appears to interfere with apoptosis induced by other stimuli, such as growth factor deprivation and oxygen deprivation. These effects of p53

inactivation on cell survival are thought to account for the high frequency of *p53* mutations in human tumors.

Roles of Oncogenes and Tumor Suppressor Genes in Tumor Development

As discussed earlier, the development of cancer is a multistep process in which normal cells gradually progress to malignancy. The complete sequence of events required for the development of any human cancer is not yet known, but it is clear that both the activation of oncogenes and the inactivation of tumor suppressor genes are critical steps in tumor initiation and progression. Accumulated damage to multiple genes eventually results in the increased proliferation, invasiveness, and metastatic potential that are characteristic of cancer cells.

The role of multiple genetic defects is best understood in the case of colon carcinomas, which have been studied extensively by Bert Vogelstein and his colleagues. These tumors frequently involve mutation of *ras*K oncogenes and inactivation or deletion of three distinct tumor suppressor genes—*APC*, *MADR2,* and p53. Lesions representing multiple stages of colon cancer development are regularly obtained as surgical specimens, so it has been possible to correlate these genetic alterations with discrete stages of tumor progression.

Introduction to Proto-oncogenes

Proto-oncogenes are a group of genes that cause normal cells to become cancerous when they are mutated. Mutations in proto-oncogenes are typically dominant in nature, and the mutated version of a proto-oncogene is called an oncogene. Often, proto-oncogenes encode proteins that function to stimulate cell division, inhibit cell differentiation, and halt cell death. All of these processes are important for normal human development and for the maintenance of tissues and organs. Oncogenes, however, typically exhibit increased production of these proteins, thus leading to increased cell division, decreased cell differentiation, and inhibition of cell death; taken together, these phenotypes define cancer cells.

From Good to Bad: How Proto-Oncogenes Become Oncogenes

Today, more than 40 different human proto-oncogenes are known. But what types of mutations convert these proto-oncogenes into oncogenes? The answer is simple: Oncogenes

arise as a result of mutations that increase the expression level or activity of a proto-oncogene. Underlying genetic mechanisms associated with oncogene activation include the following:

a) Point mutations, deletions, or insertions that lead to a hyperactive gene product
b) Point mutations, deletions, or insertions in the promoter region of a proto-oncogene that lead to increased transcription
c) Gene amplification events leading to extra chromosomal copies of a proto-oncogene
d) Chromosomal translocation events that relocate a proto-oncogene to a new chromosomal site that leads to higher expression
e) Chromosomal translocations that lead to a fusion between a proto-oncogene and a second gene, which produces a fusion protein with oncogenic activity

A number of proto-oncogenes code for cell surface receptors that span the plasma membrane and bridge communication between the extracellular environment and the inside of the cell. These transmembrane receptors consist of three parts: an extracellular region that is exposed to the outside of the cell and acts like an antenna to collect outside signals; a transmembrane region that spans the plasma membrane; and an intracellular region that often has its own enzymatic activity and can associate with other proteins located inside the cell.

In order to grow and divide, cells respond to outside signals through the binding of extracellular ligands to the extracellular region of these transmembrane receptors (Figure 2). Often, these ligands are growth factors that stimulate cell division and growth, or angiogenic factors that stimulate new blood vessel formation. When a ligand binds to a receptor, the receptor will frequently undergo a change in its conformation (shape), which in turn leads to activation of the intracellular domain and a chain of intracellular events that regulate cell growth, proliferation, angiogenesis, or death. Examples of proto-oncogenic receptors include EGFR, the receptor of the epidermal growth factor (EGF) that is involved in growth factor-mediated sigalling, and KDR, the receptor of the vascular endothelial growth factor (VEGF) that is involved in angiogenesis.

Proto-oncogenes can also code for intracellular proteins that normally act downstream of cell surface receptor pathways to stimulate cell growth and division. Examples of these downstream sigalling proteins include HRAS and KRAS. Additionally, some proto-oncogenes, including cyclin D1 (*CCND1*) and cyclin E1 (*CCNE1*), normally act to push cells through distinct stages of the cell cycle when the cells receive the appropriate signals. When

these proto-oncogenes are expressed at higher than normal levels, or when their expression is inappropriately turned on, cancer can occur.

Oncogene activation can also arise through chromosomal translocation events. The Philadelphia chromosome, discovered in 1960 in the Philadelphia laboratories of Peter Nowell and David Hungerford, is the best-known example of an oncogenic chromosomal translocation. In this case, one end of chromosome 9 is exchanged with one end of chromosome 22. At the broken end of chromosome 22 lies the *BCR* gene, which fuses with a fragment of chromosome 9 that carries the *ABL1* gene; this fused chromosome is called the Philadelphia chromosome. When the chromosome ends fuse, the two genes also fuse with each other to become *BCR-ABL*. The fused gene is expressed, and it encodes a protein that exhibits high protein tyrosine kinase activity, courtesy of the *ABL1* half of the protein. The unregulated expression of this protein activates a repertoire of other proteins that are involved in cell cycle regulation and stimulation of cell division. As a result, the Philadelphia chromosome is associated with chronic myelogenous leukemia (CML) and several other forms of leukemia.

Examples of (proto)Oncogenes

Currently, about 40 genes are known to be protooncogenes. In 16 of them direct correlation with tumor proliferation was shown, such as:

- *ERBB2 (HER2)*: breast cancer, by amplification;
- *KRAS*: tumors of the eosophagus, colon, pancreas, by point mutation;
- beta-Catenin: Pancreatic cancer;
- Cyclin E: liver tumors;
- *BRAF*: melanomas;
- *BCR-ABL*: chronic myeloid leukemia.

Molecular basis of cancer

Although the genetic basis of tumorigenesis may vary greatly between different cancer types, the cellular and molecular steps required for metastasis are similar for all cancer cells. Not surprisingly, the molecular mechanisms that propel invasive growth and metastasis are also found in embryonic development, and to a less perpetual extent, in adult tissue repair processes. It is increasingly apparent that the stromal microenvironment, in which neoplastic cells develop, profoundly influences many steps of cancer progression, including the ability of tumor cells to metastasize. In carcinomas, the influences of the microenvironment are mediated, in large part, by bidirectional interactions (adhesion, survival, proteolysis, migration, immune escape mechanisms lymph-/angiogenesis, and homing on target organs) between epithelial tumor cells and neighboring stromal cells, such as fibroblasts as well as endothelial and immune cells. Affected proteins include growth factor sigalling molecules, chemokines, cell–cell adhesion molecules (cadherins, integrins) as well as extracellular proteases (matrix metalloproteinases).

Metastatic cascade:

The process of metastasis involves an intricate interplay between altered cell adhesion, survival, proteolysis, migration, lymph-/angiogenesis, immune escape mechanisms, and homing on target organs. However, there is still very little knowledge of how these events are coordinated by the cancer cell, with conspirational help by the stromal component (microenvironment) of the host. This process is usually said to be ‘uncontrolled’. However, it is by no means purely stochastic, but rather a finely tuned molecular machinery with active tumor cell–host collaboration. Thus, all explanations of ‘success’ of the metastatic axis contain a strong element of determinism. Whereas the early steps in the metastastic campaign are completed very efficiently, metastasis is an inefficient process in its later steps, especially the regulation of cancer cell growth at the secondary sites. Given that spread of the tumor to distant organs is usually lethal, more intense studies of these molecular mechanisms assume general importance to develop more effective anticancer strategies. In the following discussion of specific molecular mechanisms, we have often

chosen to draw mainly from examples that pertain to melanoma progression, although similar processes are most likely also operating during oncogenesis of a wide range of cancers.

Redefining the metastatic cascade/axis: dominant plasticity of cancer cells

The classical metastatic cascade encompasses intravasation by tumor cells, their circulation in lymph and blood vascular systems, arrest in distant organs, extravasation, and growth into metastatic foci. Recently, this extravasation model has been challenged by Ruth Muschel and co-workers, who showed that tumor cells can readily proliferate after arrest in blood vessels, suggesting that extravasation is not a prerequisite for metastatic growth (Figure 1b). In a separate series of experiments, Mary Hendrix and co-workers described that tumor cells can even have endothelial cell-like functions and form channels that allow fluid flow (Figure 1c). The group has identified some of the players, such as EphA2 and VE-cadherin, on aggressive melanoma cells that are shared with endothelial cells and that are likely involved in 'vasculogenic mimicry'.

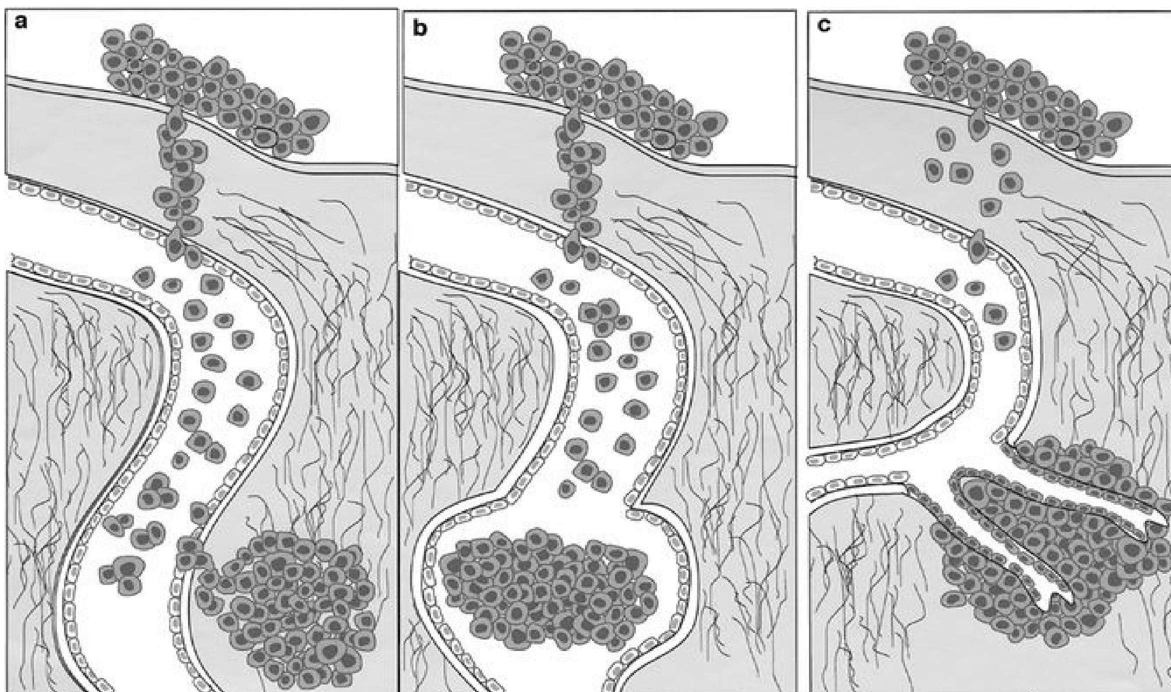


Figure. 1**:** Models of metastasis. (**a**) According to Chambers and co-workers, only a very small population of injected cells (2%) form micrometastases, although over 87% are arrested in the liver. Furthermore, not all of the micrometastases persist, and the

progressively growing metastases that kill the mice arise only from a small subset (0.02%) of the injected cells. (**b**) Muschel and co-workers recently proposed a new model for pulmonary metastasis in which endothelium-attached tumor cells that survived the initial apoptotic stimuli proliferate intravascularly. Thus, a principal tenet of this new model is that the extravasation of tumor cells is not a prerequisite for metastatic colony formation and that the initial proliferation takes place within the blood vessels. (**c**) The unique ability of aggressive tumor cells generate patterned networks, similar to the patterned networks during embryonic vasculogenesis, and concomitantly to express vascular markers associated with endothelial cells, their precursors and other vascular cells has been termed 'vasculogenic mimicry' by Hendrix and co-workers

Cell–cell interactions and adhesion molecules: linking proliferation, survival, and motility

At the core of the metastatic process lie the changing adhesive preferences of the cancer cells (i.e. from epithelial cells to fibroblasts and endothelial cells) that dictate their reciprocal interactions with the ECM and neighboring stromal cells. The majority of cell adhesion molecules (CAMs) fall into three gene families, the integrins, the immunoglobulin superfamily, and the cadherins, all of which have been implicated in metastasis.

Integrins: outside-in and inside-out sigalling

This large group of membrane proteins is formed from at least 18α and 8β subunits, which dimerize to yield at least 24 different integrin heterodimers, each with distinct ligand binding and sigalling properties. Integrins are essential for progression because of their ability to mediate physical interactions with ECMs and their ability to regulate sigalling pathways that control actin dynamics and cell movement. At the molecular level, adhesion and deadhesion as well as cytoskeletal remodeling are not only a prerequisite for cellular motility, but are also linked to proliferation and survival (antiapoptotic) pathways through integrins. Integrin engagement activates a battery of downstream molecules crucial for motile function and survival. Focal adhesion kinase (FAK), whose phosphorylation is necessary for functional adhesion sigalling and migration, was shown to be an early component of prosurvival pathways.

One of the best-studied integrins is $\alpha v\beta 3$, the vitronectin receptor. In melanoma, for example, it is currently the best molecular marker correlating with the change from radial growth phase (RGP) to the metastatically competent vertical growth phase (VGP). Introduction of $\beta 3$-integrin into RGP melanoma cell lines converted them into VGP-like melanoma cells. A series of studies have shown that this integrin is a good prognostic indicator of poor survival and short disease-free interval.

CAMs of the immunoglobulin superfamily: not just superglue

CAMs of the immunoglobulin superfamily mediate cation-independent adhesion with themselves (homophilic) or via a heterophilic ligand, but also act as receptors for integrins and ECM proteins. Hence, these molecules do not simply function as a molecular 'superglue' organizing cells into static structures. Instead, they support and direct the dynamic interchange of information between two cells by actively transducing signals into the cells through interaction of their cytoplasmatic regions with kinases as well as through interaction with growth factor receptors. One of the best-characterized CAMs is MCAM (MUC18/Mel-CAM/CD146) in cutaneous melanoma. MCAM is overexpressed in advanced primary and metastatic melanomas when compared to benign nevus cells. More than 80% of melanoma metastases express this molecule, and among primary melanomas expression increases with vertical thickness (Breslow's index). Moreover, overexpression of MCAM in melanoma cells correlates with the ability to grow and form metastases in nude mice.

Cadherin expression and function. Cadherin switch during cancer progression

In functional adherens junctions, the extracellular domain of E-cadherin interacts in a homophilic manner with E-cadherin molecules on the surface of neighboring cells. Loss or reduction of E-cadherin expression correlates with enhanced aggressiveness and dedifferentiation in most carcinomas and serves as a prognostic indicator. The cytoplasmic domain of E-cadherin is the site of interaction with the catenin molecules, which mediate its binding to the actin cytoskeleton. Alterations of proteins involved in the E-cadherin–catenin complex are early incidents in cancer development. These include reduction or loss of E-cadherin expression, induced by genetic and epigenetic events (i.e. mutation or reduced transcription of the genes), redistribution of E-cadherin to different sites within the cell,

shedding of E-cadherin, and competition for binding sites from other proteins (Jiang, 1996). Indeed, reconstitution of a functional E-cadherin adhesion complex suppresses the invasive phenotype of many different tumor cell types.

Recent studies highlight the role of other members of the cadherin family in tumor cell invasion and metastasis. N-cadherin, in particular, enhances cell motility of various tumor cell types, in some cases even overcoming E-cadherin-dependent cell–cell adhesion (Nieman et al., 1999). In addition, *de novo* expression of N-cadherin in tumor cells, which have lost functional E-cadherin, has been repeatedly reported. These data raise the intriguing possibility that a ‘cadherin switch’ from proadhesive, epithelial cadherins (e.g. E-cadherin) to mesenchymal, promigratory cadherins (e.g. N-cadherin) promotes tumor invasion and metastasis. It is thus conceivable that a change in cadherin repertoire results in a change of cellular partners, to support the interaction of tumor cells with stromal cells such as fibroblasts and endothelial cells.

β-catenin: a dual role in cell–cell adhesion and Wnt-mediated signal transduction

β-catenin, one of the components of the adhesion complex, also plays a significant role in cell signal transduction, gene activation, apoptosis inhibition, and increased cellular proliferation and migration. *β*-catenin exerts a dual role: Besides being important for cadherin-mediated cell–cell adhesion, it plays a key role in Wnt-mediated signal transduction. *β*-catenin is usually sequestered in the E-cadherin adherens junction or in tight-junction complexes. Nonsequestered, free *β*-catenin is rapidly phosphorylated by glycogen synthetase kinase 3*β*(GSK-3*β*) in the adenomatous polyposis coli (APC)/GSK-3*β*/axin complex and subsequently degraded by the ubiquitin-proteasome pathway. If the tumor suppressor APC is nonfunctional, as for example is the case in many colon cancer cells, or GSK-3*β* activity is blocked by activated Wnt-sigalling, *β*-catenin accumulates at high levels in the cytoplasm. Subsequently, it translocates to the nucleus, where it binds to a member of the TCF/LEF-1 family of transcription factors and possibly activates the expression of TCF/LEF-1-target

genes. Target genes of TCF/β-catenin that could be relevant for tumor progression include the proto-oncogene c-Myc and cyclin D1.

Ephrins/Eph receptors: control of cell behavior by bidirectional communication

Eph receptors, the largest subfamily of receptor tyrosine kinases (RTKs), and their ephrin ligands are important mediators of cell–cell communication regulating cell shape, attachment, and mobility (reviewed in Himanen and Nikolov, 2003a,2003b). Eph sigalling is crucial for the development of many tissues and organs including the nervous and cardiovascular systems. Eph receptors and ephrins are unique in that they mediate bidirectional sigalling. Both Ephs and ephrins are membrane bound and their interactions at sites of cell–cell contact occur, where information is transduced in both the receptor- and the ligand-expressing cells. Both Eph receptors and ephrins are divided into two subclasses, based on sequence conservation and their binding affinities. In general, the 80 different EphA RTKs (EphA1–A8) promiscuously interact with five A-ephrins (ephrinA1–A5). The EphB subclass receptors (EphB1–B6) interact with three different B-ephrins (ephrinB1–B3) (Himanen and Nikolov, 2003a,b).

Increasing evidence also implicates Eph family proteins in cancer. Eph–ephrin interactions regulate critical steps in tumor angiogenesis and possibly metastasis. Eph receptors and ephrins are frequently overexpressed in a wide variety of cancers, including breast, small-cell lung and gastrointestinal cancers, melanomas, and neuroblastomas. Ephrin-A1 is upregulated during melanoma progression and may correlate with invasion by endothelial cells. Ephrin-B2 is also overexpressed in melanoma and correlates with tumorigenicity and metastatic potential. EphA2 is overexpressed in many cancers, including 40% of breast cancers. EphA2 can also transform breast epithelial cells *in vitro* to display properties commonly associated with the development of metastasis. More specifically, oncogenecity may depend on the capacity of unactivated (unphosphorylated) EphA2 to interact with a variety of signalling

molecules, while stimulation of EphA2 by its ligand (ephrin-A1) results in EphA2 autophosphorylation, the stimulation reverses the oncogenic transformation.

Manipulation of the microenvironment and ECM proteolysis: Vanguard of the metastatic campaign?

The ECM provides a mechanical support for migration and prevents the induction of apoptosis (anoikis). Remodeling ('landscaping') of the tumor microenvironment and ECM through lysis of matrix proteins is a necessary step in local invasion and metastasis (Chang and Werb, 2001). The principal classes of enzymes that degrade the ECM- and cell-associated proteins are (1) the matrix metalloproteinases (MMPs), a family of membrane-anchored and -secreted proteinases; (2) tissue serine proteinases, including urokinase plasminogen activator, thrombin, and plasmin; (3) the adamalysin-related membrane proteases (ADAM family); (4) the bone morphogenetic protein (BMP)-1-type metalloproteinases; (5) heparanase; and (6) cathepsins.

Migration/motility

From proteolytic mesenchymal toward nonproteolytic 'amoeboid' movement

Collective cell movement represents an efficient dissemination strategy in epithelial and mesenchymal cancers. Recently, Friedl and colleagues have shown that interference with β1-integrin function induces complex changes in cell polarity and cohesion, including development of two or several opposing leading edges, cluster disruption, and the detachment of individual cells followed by β1-integrin-independent 'amoeboid' crawling and dissemination. Hence, the conversion from β1-integrin-dependent collective movement to β1-integrin-independent single-cell motility suggests an efficient cellular and molecular plasticity in tumor cell migration strategies. The transition from proteolytic mesenchymal toward nonproteolytic 'amoeboid' movement highlights a supramolecular plasticity mechanism in cell migration and further represents a putative escape mechanism in tumor cell dissemination after abrogation of pericellular proteolysis.

Met-SF/HGF-sigalling: a specific mediator of invasive growth

Local attractants include scatter factor/hepatocyte growth factor (SF/HGF), also termed plasminogen-related growth factor-1 (PRGF-1), which binds to the Met receptor (c-Met), a tyrosine kinase receptor. SF/HGF-binding to the Met receptor stimulates tyrosine phosphorylation of FAK and its association with the signal-transducing adaptor Grb2, thereby connecting c-Met to the Ras pathway, which promotes growth. Moreover, the autophosphorylation of c-Met is followed by activation of the PI3K/AKT pathway, thereby impinging on survival (suppressed apoptosis) and motility. Generally speaking, SF/HGF stimulates the facets of invasive growth in virtually every tissue of the body.

ECM proteins

Another group of motility factors corresponds to ECM proteins surrounding the tumor cells (microenvironment). As they are cleaved by proteolytic enzymes secreted by the tumors, the soluble matrix proteins can stimulate the tumor cells to migrate. Thus, the motility stimulation by matrix proteins may be an important component of tumor metastasis, coupling matrix protein degradation to motility. A recent study, based on a whole-genomic analysis of metastasis, reveals that enhanced expression of several genes normally involved in ECM assembly correlates with progression to a metastatic phenotype.

Matrix proteins that are known to induce motility are vitronectin, fibronectin, laminin, type I collagen, type IV collagen, and thrombospondin. These proteins stimulate chemotaxis (motility toward a chemical gradient) and haptotaxis (motility stimulation toward a bound substrate). Several of these matrix proteins stimulate motility through integrin receptors. Type I collagen, which comprises 90% of the bone matrix, has been shown to stimulate the motility of tumor cells. In addition, many cytokines, such as members of the EGF family, TGF-β1, platelet-derived growth factor (PDGF), b-FGF, and IFN-γ bind to various components of the ECM.

Growth factors and cytokines: heterotypic sigalling

Another group of motility factors are host-secreted growth factors. Many of these paracrine motility factors also act as mitogens for the tumor cells in which they cause increased motility, once again supporting the 'conspirational' hypothesis in tumor cell–host interaction during cancer progression. Two potent growth factors expressed in the stroma of carcinomas are the insulin-like growth factors (IGF-I and IGF-II). IGFs probably exert both autocrine and paracrine effects on tumor growth. In breast cancers, IGF-II in particular correlates with tumor progression. However, while both IGF-I and IGF-II are typically expressed in fibroblasts of carcinomas, cell lines derived from metastases may acquire the ability to express IGF-II, which may assist in their ability to proliferate in a stroma-independent fashion.

Several studies demonstrate that the biological effects of tumor-derived growth factors and cytokines are biphasic, depending on the level of secretion and the tumor progression stage. For example, we could recently show that intermediate levels of monocyte chemoattractant protein-1 (MCP-1) elicit an angiogenic effect mediated through monocyte activation that results in melanoma growth, whereas high levels of MCP-1 lead to massive monocyte/macrophage accumulation and tumor destruction. Similarly, there is a differential response of primary and metastatic melanomas to neutrophils attracted by IL-8. Nontumorigenic primary melanomas depend on IL-8 stimulation *in vivo* for growth. Tumor growth depends on the level of neutrophil infiltration, as at high IL-8 transduction levels tumor growth was impaired due to massive neutrophil infiltration. In contrast, highly tumorigenic and metastatic melanoma cells proliferate *in vivo* independent of infiltrating neutrophils and show marked increases in tumor growth and number of metastatic foci in the lungs, depending on the expression levels of IL-8.

Angiogenesis and lymphangiogenesis

Tumor metastasis to regional lymph nodes is a crucial step in the progression of cancer. Clinicopathological data suggest that the lymphatics are an initial route for the spread of solid tumors. Detection of sentinel lymph nodes by biopsy provides significant information for staging, prognostic information, and designing therapeutic regimens. However, the molecular

mechanisms that control the spread of cancer to the lymph nodes were unknown until recently. The proliferation of new lymphatic vessels (lymphangiogenesis) is controlled, in part, by members of the vascular endothelial growth factor (VEGF) family – namely, VEGF C, and VEGF D – and their cognate receptor on the lymphatic endothelium, VEGFR3.

Chemokines/chemokine receptors

It has been recognized for over a century that the organ preference for metastatic colonization is influenced by interactions between the circulating tumor cell (the 'seed') and the target host tissue (the 'soil'). Circulating immune and stem cells are known to use chemokine-mediated sigalling to home on specific organs. Chemokines are growth-factor-like molecules that bind to G-coupled receptors. They induce leukocytes to adhere tightly to endothelial cells and to migrate towards a gradient. Tumor cells co-opt the same mechanisms to direct metastatic organ preference. For example, the receptor/ligand pairs CXCR4/CXCL12 and CCR7/CCL21 fit the profile expected for breast cancer metastasis homing to bone, lung, and liver. Similarly, melanoma cells would find a CCL27/CCR10 chemokine-receptor 'match' in the skin.

However, it has been argued that this metastatic organ specificity is due to efficient organ-specific growth rather than preferential homing of cells. Accordingly, the initial delivery and arrest of cancer cells to specific metastatic organs seems to be primarily mechanical. Subsequently, molecular factors present in individual organs then influence whether or not a specific cancer cell will grow there. This model is based on *in vivo* videomicroscopy observations that most circulating cancer cells arrest by size restriction, and that both the lung and liver are very efficient at arresting the flow of cancer cells. Further, while leukocytes often arrest by adhering to the walls of vessels that are much larger than themselves, cancer cells usually do not arrest in this manner when injected into mice.

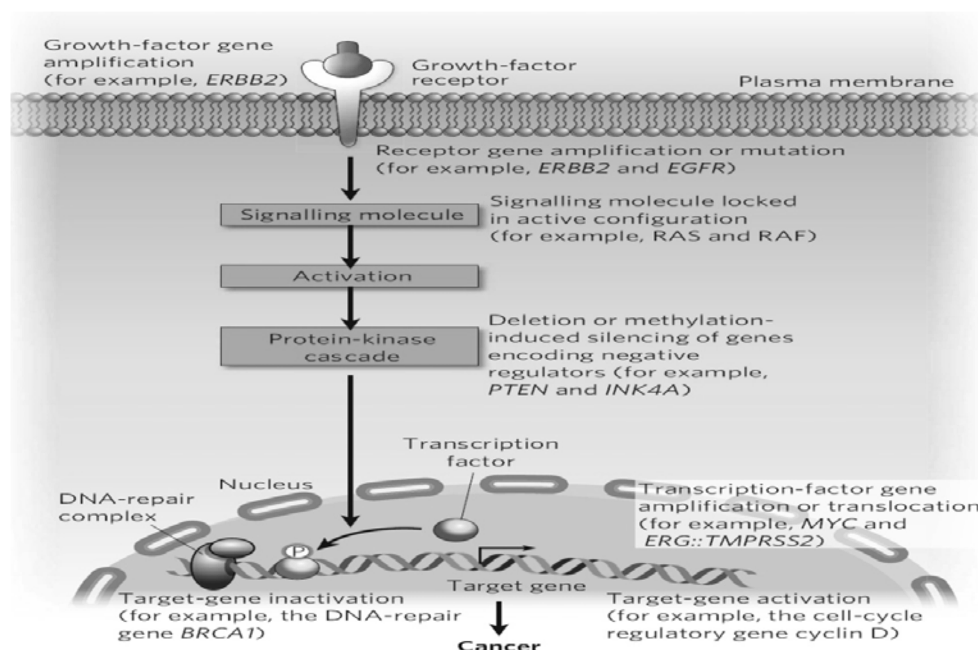
Metastasis to the bone

Bone is the third most common site of metastasis. Of the patients who die of breast cancer, 90% have bone metastases, and very often the bulk of the tumor burden at the time of death will be in the bone. Bone metastases cause severe pain and a dramatic reduction of the quality

of life of affected patients. The mechanisms responsible for bone pain are poorly understood, but seem to be a consequence of osteolysis.

Cancer cells that metastasize to the bone are capable of having effects on osteoblasts that are stimulated to form new bone (osteoblastic or osteosclerotic metastasis) or on osteoclasts that cause bone resorption (osteolytic metastasis) (Mundy, 2002). In turn, the growth of bone metastases can be influenced by growth factors derived from the bone marrow, osteoblasts, or from the bone matrix products released by osteoclastic bone resorption, thereby causing a vicious circle. In most patients, however, bone metastases have both osteolytic and osteoblastic elements.

In breast cancer, bone metastases are predominantly osteolytic and the main mediator is parathyroid hormone-related peptide (PTHrP). PTHrP stimulates osteoclast activity by enhancing production of the cytokine receptor activator of nuclear factor-κB ligand (RANKL), which binds and activates its receptor RANK, which is expressed by osteoclasts. In prostate cancer, bone metastases are frequently osteoblastic and known mediators include TGF-β, IGFs, FGFs, PDGF, endothelin-1, and uPA.



Disruption of cell sigalling can occur at several points and ultimately lead to cancer. Growth factor binding and the sigalling pathway following receptor activation can be

altered by growth factor gene amplification, such as increased EGF or TGF-alpha expression, or by receptor gene amplification or mutation, such as alterations in EGFR or ERBB2. In the cytoplasm, sigalling molecules, such as RAS or RAF, may become locked in the active configuration. In the protein kinase cascade, disruptions may occur due to deletion or methylation-induced silencing of genes encoding negative regulators, such as PTEN and INK4A. Disruptions may also occur in transcription factor gene amplification or translocation, such as alterations in MYC and ERG-TMPRSS2. Within the nucleus, target gene inactivation, such as inactivation caused by mutations of the DNA repair gene BRCA1, and target-gene activation, such as activation of the cell cycle regulatory gene cyclin D, can disrupt intracellular sigalling and lead to cancer.

Apoptosis

Introduction

The term apoptosis (a-po-toe-sis) was first used in a now-classic paper by Kerr, Wyllie, and Currie in 1972 to describe a morphologically distinct form of cell death, although certain components of the apoptosis concept had been explicitly described many years previously.

Our understanding of the mechanisms involved in the process of apoptosis in mammalian cells transpired from the investigation of programmed cell death that occurs during the development of the nematode *Caenorhabditis elegans* . In this organism 1090 somatic cells are generated in the formation of the adult worm, of which 131 of these cells undergo apoptosis or “programmed cell death.” These 131 cells die at particular points during the development process, which is essentially invariant between worms, demonstrating the remarkable accuracy and control in this system. Apoptosis has since been recognized and accepted as a distinctive and important mode of “programmed” cell death, which involves the genetically determined elimination of cells.

Apoptosis occurs normally during development and aging and as a homeostatic mechanism to maintain cell populations in tissues. Apoptosis also occurs as a defence mechanism such as in immune reactions or when cells are damaged by disease or noxious agents. Although there are a wide variety of stimuli and conditions, both physiological and

pathological, that can trigger apoptosis, not all cells will necessarily die in response to the same stimulus. Irradiation or drugs used for cancer chemotherapy results in DNA damage in some cells, which can lead to apoptotic death through a *p53*-dependent pathway.

Some cells express Fas or TNF receptors that can lead to apoptosis via ligand binding and protein cross-linking. Other cells have a default death pathway that must be blocked by a survival factor such as a hormone or growth factor. There is also the issue of distinguishing apoptosis from necrosis, two processes that can occur independently, sequentially, as well as simultaneously. In some cases it's the type of stimuli and/or the degree of stimuli that determines if cells die by apoptosis or necrosis. At low doses, a variety of injurious stimuli such as heat, radiation, hypoxia and cytotoxic anticancer drugs can induce apoptosis but these same stimuli can result in necrosis at higher doses. Finally, apoptosis is a coordinated and often energy-dependent process that involves the activation of a group of cysteine proteases called "caspases" and a complex cascade of events that link the initiating stimuli to the final demise of the cell.

Morphology of Apoptosis

Light and electron microscopy have identified the various morphological changes that occur during apoptosis. During the early process of apoptosis, cell shrinkage and pyknosis are visible by light microscopy. With cell shrinkage, the cells are smaller in size, the cytoplasm is dense and the organelles are more tightly packed. Pyknosis is the result of chromatin condensation and this is the most characteristic feature of apoptosis. On histologic examination with hematoxylin and eosin stain, apoptosis involves single cells or small clusters of cells. The apoptotic cell appears as a round or oval mass with dark eosinophilic cytoplasm and dense purple nuclear chromatin fragments (Figure below). Electron microscopy can better define the subcellular changes. Early during the chromatin condensation phase, the electron-dense nuclear material characteristically aggregates peripherally under the nuclear membrane although there can also be uniformly dense nuclei.

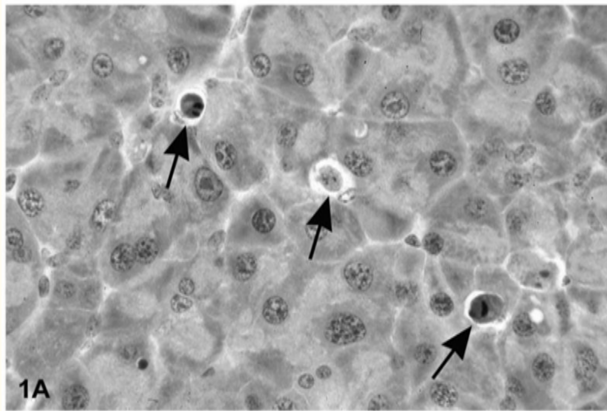
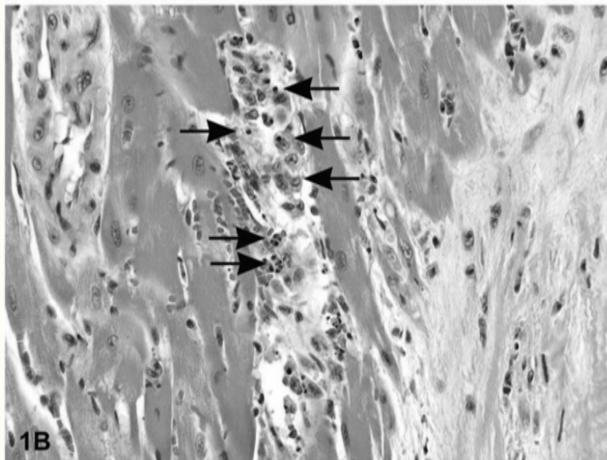
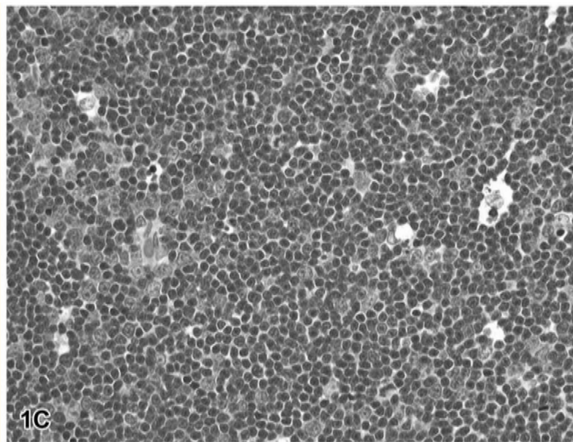
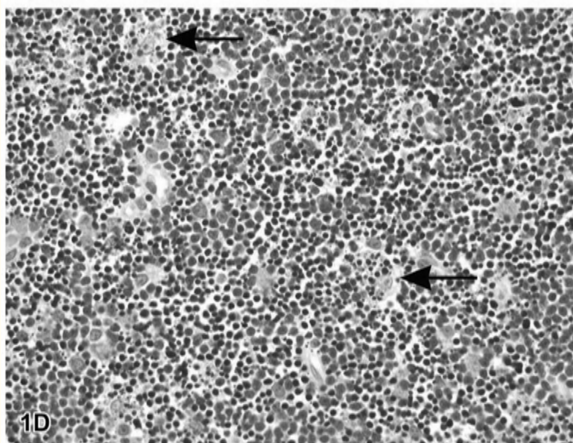

1A

1B

1C

1D

Figure : **Figure 1A** is a photomicrograph of a section of exocrine pancreas from a B6C3F1 mouse. The arrows indicate apoptotic cells that are shrunken with condensed cytoplasm. The nuclei are pyknotic and fragmented. Note the lack of inflammation. **Figure 1B** is an image of myocardium from a 14 week-old rat treated with ephedrine (25 mg/kg) and caffeine (30 mg/kg). Within the interstitial space there are apoptotic cells with condensed cytoplasm, condensed and hyperchromatic chromatin and fragmented nuclei (long arrows). Admixed with the apoptotic bodies are macrophages, some with engulfed apoptotic bodies (arrowheads). **Figure 1C** is a photomicrograph of normal thymus tissue from a control Sprague–Dawley rat for comparison. **Figure 1D** illustrates sheets of apoptotic cells in the thymus from a rat that was treated with dexamethasone to induce lymphocyte apoptosis. Under physiological conditions, apoptosis typically affects single cells or small clusters of cells. However, the degree of apoptosis in this treated rat is more severe due to the amount of dexamethasone administered (1 mg/kg body weight) and the time posttreatment (12 hours). The majority of lymphocytes are apoptotic although there are a few interspersed cells that are morphologically normal and most likely represent lymphoblasts or macrophages. The apoptotic lymphocytes are small and deeply basophilic with pyknotic and often-fragmented nuclei. Macrophages are present with engulfed cytoplasmic apoptotic bodies (arrows).

Distinguishing Apoptosis from Necrosis

Apoptosis	**Necrosis**
Single cells or small clusters of cells	Often contiguous cells
Cell shrinkage and convolution	Cell swelling

Pyknosis and karyorrhexis	Karyolysis, pyknosis, and karyorrhexis
Intact cell membrane	Disrupted cell membrane
Cytoplasm retained in apoptotic bodies	Cytoplasm released
No inflammation	Inflammation usually present

Mechanisms of Apoptosis

The mechanisms of apoptosis are highly complex and sophisticated, involving an energy-dependent cascade of molecular events (Figure). To date, research indicates that there are two main apoptotic pathways: the extrinsic or death receptor pathway and the intrinsic or mitochondrial pathway. However, there is now evidence that the two pathways are linked and that molecules in one pathway can influence the other. There is an additional pathway that involves T-cell mediated cytotoxicity and perforin-granzyme-dependent killing of the cell. The perforin/granzyme pathway can induce apoptosis via either granzyme B or granzyme A. The extrinsic, intrinsic, and granzyme B pathways converge on the same terminal, or execution pathway. This pathway is initiated by the cleavage of caspase-3 and results in DNA fragmentation, degradation of cytoskeletal and nuclear proteins, cross-linking of proteins, formation of apoptotic bodies, expression of ligands for phagocytic cell receptors and finally uptake by phagocytic cells. The granzyme A pathway activates a parallel, caspase-independent cell death pathway via single stranded DNA damage.

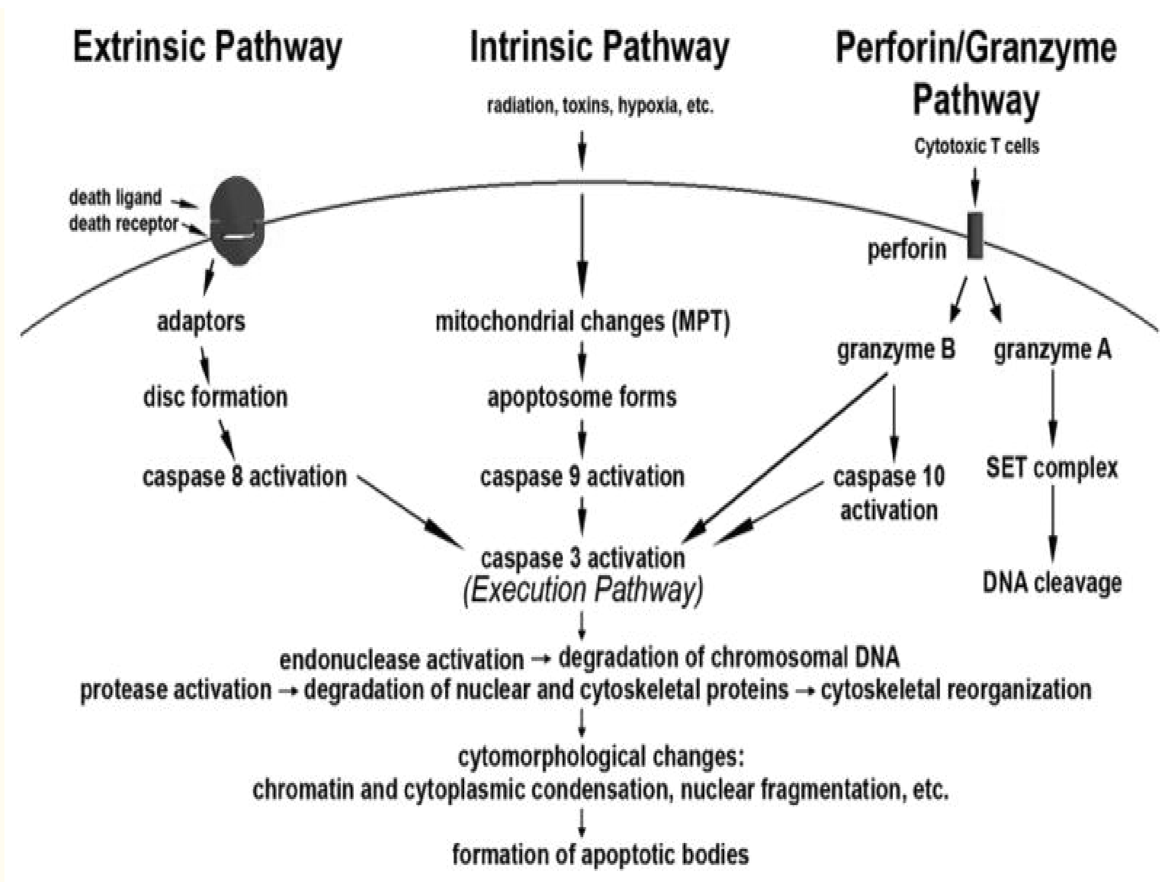


Figure : Schematic representation of apoptotic events. The two main pathways of apoptosis are extrinsic and intrinsic as well as a perforin/granzyme pathway. Each requires specific triggering signals to begin an energy-dependent cascade of molecular events. Each pathway activates its own initiator caspase (8, 9, 10) which in turn will activate the executioner caspase-3. However, granzyme A works in a caspase-independent fashion. The execution pathway results in characteristic cytomorphological features including cell shrinkage, chromatin condensation, formation of cytoplasmic blebs and apoptotic bodies and finally phagocytosis of the apoptotic bodies by adjacent parenchymal cells, neoplastic cells or macrophages.

Biochemical Features

Apoptotic cells exhibit several biochemical modifications such as protein cleavage, protein cross-linking, DNA breakdown, and phagocytic recognition that together result in the distinctive structural pathology described previously. Caspases are widely expressed in an inactive proenzyme form in most cells and once activated can often activate other procaspases, allowing initiation of a protease cascade. Some procaspases can also aggregate

and autoactivate. This proteolytic cascade, in which one caspase can activate other caspases, amplifies the apoptotic sigalling pathway and thus leads to rapid cell death.

Caspases have proteolytic activity and are able to cleave proteins at aspartic acid residues, although different caspases have different specificities involving recognition of neighboring amino acids. Once caspases are initially activated, there seems to be an irreversible commitment towards cell death. To date, ten major caspases have been identified and broadly categorized into initiators (caspase-2,-8,-9,-10), effectors or executioners (caspase-3,-6,-7) and inflammatory caspases (caspase-1,-4,-5)). The other caspases that have been identified include caspase-11, which is reported to regulate apoptosis and cytokine maturation during septic shock, caspase-12, which mediates endoplasmic-specific apoptosis and cytotoxicity by amyloid-*β*, caspase-13, which is suggested to be a bovine gene, and caspase-14, which is highly expressed in embryonic tissues but not in adult tissues.

Extensive protein cross-linking is another characteristic of apoptotic cells and is achieved through the expression and activation of tissue transglutaminase. DNA breakdown by Ca^{2+}- and Mg^{2+}-dependent endonucleases also occurs, resulting in DNA fragments of 180 to 200 base pairs. A characteristic “DNA ladder” can be visualized by agarose gel electrophoresis with an ethidium bromide stain and ultraviolet illumination.

Another biochemical feature is the expression of cell surface markers that result in the early phagocytic recognition of apoptotic cells by adjacent cells, permitting quick phagocytosis with minimal compromise to the surrounding tissue. This is achieved by the movement of the normal inward-facing phosphatidylserine of the cell’s lipid bilayer to expression on the outer layers of the plasma membrane. Although externalization of phosphatidylserine is a well-known recognition ligand for phagocytes on the surface of the apoptotic cell, recent studies have shown that other proteins are also be exposed on the cell surface during apoptotic cell clearance. These include Annexin I and calreticulin.

Annexin V is a recombinant phosphatidylserine-binding protein that interacts strongly and specifically with phosphatidylserine residues and can be used for the detection of apoptosis. Calreticulin is a protein that binds to an LDL-receptor related protein on the engulfing cell and is suggested to cooperate with phosphatidylserine as a recognition signal. The adhesive glycoprotein, thrombospondin-1, can be expressed on the outer surface of activated microvascular endothelial cells and, in conjunction with CD36, caspase-3-like proteases and other proteins, induce receptor-mediated apoptosis.

Extrinsic Pathway

The extrinsic sigalling pathways that initiate apoptosis involve transmembrane receptor-mediated interactions. These involve death receptors that are members of the tumor necrosis factor (TNF) receptor gene superfamily. Members of the TNF receptor family share similar cyteine-rich extracellular domains and have a cytoplasmic domain of about 80 amino acids called the "death domain". This death domain plays a critical role in transmitting the death signal from the cell surface to the intracellular sigalling pathways. To date, the best-characterized ligands and corresponding death receptors include FasL/FasR, TNF-α/TNFR1, Apo3L/DR3, Apo2L/DR4 and Apo2L/DR5.

The sequence of events that define the extrinsic phase of apoptosis are best characterized with the FasL/FasR and TNF-α/TNFR1 models. In these models, there is clustering of receptors and binding with the homologous trimeric ligand. Upon ligand binding, cytplasmic adapter proteins are recruited which exhibit corresponding death domains that bind with the receptors. The binding of Fas ligand to Fas receptor results in the binding of the adapter protein FADD and the binding of TNF ligand to TNF receptor results in the binding of the adapter protein TRADD with recruitment of FADD and RIP. FADD then associates with procaspase-8 via dimerization of the death effector domain. At this point, a death-inducing sigalling complex (DISC) is formed, resulting in the auto-catalytic activation of procaspase-8.

Once caspase-8 is activated, the execution phase of apoptosis is triggered. Death receptor-mediated apoptosis can be inhibited by a protein called c-FLIP which will bind to FADD and caspase-8, rendering them ineffective. Another point of potential apoptosis regulation involves a protein called Toso, which has been shown to block Fas-induced apoptosis in T cells via inhibition of caspase-8 processing.

Intrinsic Pathway

The intrinsic sigalling pathways that initiate apoptosis involve a diverse array of non-receptor-mediated stimuli that produce intracellular signals that act directly on targets within the cell and are mitochondrial-initiated events. The stimuli that initiate the intrinsic pathway produce intracellular signals that may act in either a positive or negative fashion. Negative signals involve the absence of certain growth factors, hormones and cytokines that can lead to failure of suppression of death programs, thereby triggering apoptosis. In other words, there

is the withdrawal of factors, loss of apoptotic suppression, and subsequent activation of apoptosis. Other stimuli that act in a positive fashion include, but are not limited to, radiation, toxins, hypoxia, hyperthermia, viral infections, and free radicals.

All of these stimuli cause changes in the inner mitochondrial membrane that results in an opening of the mitochondrial permeability transition (MPT) pore, loss of the mitochondrial transmembrane potential and release of two main groups of normally sequestered pro-apoptotic proteins from the intermembrane space into the cytosol. The first group consists of cytochrome *c*, Smac/DIABLO, and the serine protease HtrA2/Omi. These proteins activate the caspase-dependent mitochondrial pathway. Cytochrome *c* binds and activates Apaf-1 as well as procaspase-9, forming an “apoptosome”.

The clustering of procaspase-9 in this manner leads to caspase-9 activation. Smac/DIABLO and HtrA2/Omi are reported to promote apoptosis by inhibiting IAP (inhibitors of apoptosis proteins) activity. Additional mitochondrial proteins have also been identified that interact with and suppress the action of IAP however gene knockout experiments suggest that binding to IAP alone may not be enough evidence to label a mitochondrial protein as “pro-apoptotic”.

The second group of pro-apoptotic proteins, AIF, endonuclease G and CAD, are released from the mitochondria during apoptosis, but this is a late event that occurs after the cell has committed to die. AIF translocates to the nucleus and causes DNA fragmentation into ~50–300 kb pieces and condensation of peripheral nuclear chromatin. This early form of nuclear condensation is referred to as “stage I” condensation. Endonuclease G also translocates to the nucleus where it cleaves nuclear chromatin to produce oligonucleosomal DNA fragments. AIF and endonuclease G both function in a caspase-independent manner. CAD is subsequently released from the mitochondria and translocates to the nucleus where, after cleavage by caspase-3, it leads to oligonucleosomal DNA fragmentation and a more pronounced and advanced chromatin condensation. This later and more pronounced chromatin condensation is referred to as “stage II” condensation.

The control and regulation of these apoptotic mitochondrial events occurs through members of the Bcl-2 family of proteins. The tumor suppressor protein *p53* has a critical role in regulation of the Bcl-2 family of proteins, however the exact mechanisms have not yet been completely elucidated. The Bcl-2 family of proteins governs mitochondrial membrane permeability and can be either pro-apoptotic or anti-apoptotic. To date, a total of 25 genes have been identified in the Bcl-2 family. Some of the anti-apoptotic proteins include Bcl-2,

Bcl-x, Bcl-XL, Bcl-XS, Bcl-w, BAG, and some of the pro-apoptotic proteins include Bcl-10, Bax, Bak, Bid, Bad, Bim, Bik, and Blk. These proteins have special significance since they can determine if the cell commits to apoptosis or aborts the process. It is thought that the main mechanism of action of the Bcl-2 family of proteins is the regulation of cytochrome *c* release from the mitochondria via alteration of mitochondrial membrane permeability.

A few possible mechanisms have been studied but none have been proven definitively. Mitochondrial damage in the Fas pathway of apoptosis is mediated by the caspase-8 cleavage of Bid. This is one example of the "cross-talk" between the death-receptor (extrinsic) pathway and the mitochondrial (intrinsic) pathway). Serine phosphorylation of Bad is associated with 14-3-3, a member of a family of multifunctional phosphoserine binding molecules. When Bad is phosphorylated, it is trapped by 14-3-3 and sequestered in the cytosol but once Bad is unphosphorylated, it will translocate to the mitochondria to release cytochrome C.

Bad can also heterodimerize with Bcl-Xl or Bcl-2, neutralizing their protective effect and promoting cell death. When not sequestered by Bad, both Bcl-2 and Bcl-Xl inhibit the release of cytochrome C from the mitochondria although the mechanism is not well understood. Reports indicate that Bcl-2 and Bcl-XL inhibit apoptotic death primarily by controlling the activation of caspase proteases. An additional protein designated "Aven" appears to bind both Bcl-Xl and Apaf-1, thereby preventing activation of procaspase-9. There is evidence that overexpression of either Bcl-2 or Bcl-Xl will down-regulate the other, indicating a reciprocal regulation between these two proteins.

Puma and Noxa are two members of the Bcl2 family that are also involved in pro-apoptosis. Puma plays an important role in *p53*-mediated apoptosis. It was shown that, in vitro, overexpression of Puma is accompanied by increased BAX expression, BAX conformational change, translocation to the mitochondria, cytochrome *c* release and reduction in the mitochondrial membrane potential. Noxa is also a candidate mediator of *p53*-induced apoptosis. Studies show that this protein can localize to the mitochondria and interact with anti-apoptotic Bcl-2 family members, resulting in the activation of caspase-9.

Execution Pathway

The extrinsic and intrinsic pathways both end at the point of the execution phase, considered

the final pathway of apoptosis. It is the activation of the execution caspases that begins this phase of apoptosis. Execution caspases activate cytoplasmic endonuclease, which degrades nuclear material, and proteases that degrade the nuclear and cytoskeletal proteins. Caspase-3, caspase-6, and caspase-7 function as effector or "executioner" caspases, cleaving various substrates including cytokeratins, PARP, the plasma membrane cytoskeletal protein alpha fodrin, the nuclear protein NuMA and others, that ultimately cause the morphological and biochemical changes seen in apoptotic cells.

Caspase-3 is considered to be the most important of the executioner caspases and is activated by any of the initiator caspases (caspase-8, caspase-9, or caspase-10). Caspase-3 specifically activates the endonuclease CAD. In proliferating cells CAD is complexed with its inhibitor, ICAD. In apoptotic cells, activated caspase-3 cleaves ICAD to release CAD . CAD then degrades chromosomal DNA within the nuclei and causes chromatin condensation. Caspase-3 also induces cytoskeletal reorganization and disintegration of the cell into apoptotic bodies. Gelsolin, an actin binding protein, has been identified as one of the key substrates of activated caspase-3.

Assays for Apoptosis

However, some of the most commonly employed assays are mentioned and briefly described. Apoptosis assays, based on methodology, can be classified into six major groups and a subset of the available assays in each group is indicated and briefly discussed:

1. Cytomorphological alterations
2. DNA fragmentation
3. Detection of caspases, cleaved substrates, regulators and inhibitors
4. Membrane alterations
5. Detection of apoptosis in whole mounts
6. Mitochondrial assays.

Neurotrophic Factors

Neurotrophins are an important class of neurotrophic factors, but they are by no means the only ones that play important roles. Glial cell line derived neurotrophic factor (GDNF) family has turned out to be crucially important in neuronal development and maintenance as well as the ciliary neurotrophic factor (CNTF) family. Since the first member of the family was

discovered by its ability to promote neuronal survival the name of "neurotrophic factor" was acquired. However, all neurotrophic factors also regulate important processes in the non-neuronal cells that are essential for the development and function of the organisms. The multiple functions of neurotrophic factors in different tissues provide an interesting example of how an organism uses the same biologically active factors for several purposes. Many other growth factors, mainly known for their non neuronal activities (e.g. some fibroblast growth factors, insuline-like growth factors) have some trophic effects on selected neuronal populations, but are not called neurotrophic factors.

The family of neurotrophins and their receptors: Neurotrophins are polypeptide molecules that regulate the survival, development and maintenance of specific functions in different populations of nervous cells. This family includes four closely related factors: nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophins-4/5 (NT4/5). These four factors share more than 30% sequence homology and are relatively well conserved in all mammals. The most recently discovered neurotrophin is NT-6 whose receptor remains to be defined, although there is evidence for NT-6 binding to proteoglycans on the cell surface and/or to extracellular matrix molecules. Neurotrophins exert their biological activities by activation of two unrelated types of transmembrane receptors. The first type is the low-affinity NGF receptor, also known as low-affinity neurotrophin receptor (LANR, p75NGFR) which binds neurotrophins with varying affinities and does not possess intrinsic tyrosine kinase activity. The second type of receptors has specificity for the binding of individual neurotrophins and is conferred by the Trk family of tyrosine kinases known as TrkA, TrkB, TrkC.

Anti-apoptotic action of Neurotrophins

Neurons, like any other differentiated cells, require the presence of survival factors to suppress the intrinsic cell death machinery and thereby avoid apoptosis. The regulation of apoptosis by survival factors is therefore critical for normal development an proper functioning of the nervous system. In addition, apoptotic-like death was described in injured neurons following such insults as β-amyloid exposure, excitotoxicity, DNA damage, or oxidative stress. These observations implicated apoptosis as an important element of several major neurological diseases booting the interest in identifying both anti- and proapoptotic signal transduction pathways in neurons. Neurotrophins promote survival and suppress apoptosis in many populations of neurons. It has been suggest that the major pathways

transducing the antiapoptotic effects of neurotrophins are phosphatidylinositol3kinase (PI-3K) and Erk1/ 2. The nature of the apoptotic stimulus seems to be a critical determinant of pathway preference. Thus, it appears that in most cases of trophic deprivation, PI-3K is the main player, whereas Erk1/ 2 dominates as a major neuroprotective mechanism in damaged cells. In addition to Erk1/2 and PI-3K pathways, there are many more signalling small ,circuits regulated by neurotrophins such as phospholipase Cγ (PLCγ), GTPases ras and rho, protein kinase C, p38, Erk5, Jnk, transcription factor NFκ and tyrosine phosphatase Shp. Some of them including PLCγ, proteinkinase C or protein Kinase p38, a relative of Erk1/2 were implicated in survival responses to such stimuli as NGF, serum Phorbol esters or membrane depolarization. One should also emphasize that although the p75 receptor for neurtrophins is generally considered to be pro-apoptotic, it can activate a protective transcription factor NFκ. The main challenge in the field is to learn how these pathways protect neurons. This knowledge may lead to new strategies for effective neuroprotection in diseases. For example, a substrate for PI-3K is an emerging candidate drug target for neuroprotection since it mediates the suppression of apoptosis.

Neurotrophins regulating neuronal apoptosis:

As we have previously mentioned, neurotrophins mediate the survival, differentiation, growth and apoptosis of neurons by binding to two types of cell surface receptors, the Trk and p75 neurotrophin receptor. These receptors, often present on the same cell, coordinate and modulate the responses of neurons to neurotrophins. The functions of the neurotrophin receptors vary markedly, from sculpting the developing nervous system to regulation of the survival and regeneration of injured neurons. While Trk receptors largely transmit positive Neurotrophins: Responsible for death and survival signals that promote neuronal survival, p75 transmits both positive and negative signals and, in particular, can cause neuronal apoptosis. Since it is known that p75 could mediate neural cell line apoptosis, this has been extended to a large number of primary neural cells in culture. A number of studies indicate that this proapoptotic function is essential for rapid and appropriate apoptosis during developmental cell death. In particular, apoptosis is significantly reduced in certain neuronal populations in mice lacking p75 or its neurotrophin ligands. The proapoptotic function of p75 has also been implicated in injury-induced apoptosis. The first study to support this idea, involved the neuron-specific expression of the p75 intracellular domain, which led to the death of the injured facial motor neurons in transgenic mice. Although p75 mechanisms of

apoptosis have been associated to Trk receptors, a number of studies indicate that p75 can also signal apoptosis in a Trk-independent fashion. For example, p75 activation caused apoptosis when sympathetic neurons were maintained in KCl, when sensory neurons were maintained in ciliary neurotrophic factor, and when Schwann cells were maintained in insulin-like growth factor plus nurteulin, all Trk-independent survival signals. Recent surprising findings have demonstrated that for at least some developing neurons, p75 mediates a constitutive death signal, and that one of the primary ways that Trk receptors mediate neuronal survival is by silencing this constitutive signal. This conclusion derives from studies showing that p75 is essential for apoptosis of some cells following growth factor withdrawal.

Caspase

Caspases, Key Apoptotic Proteins

Virtually all animal cells contain caspases, but they occur as inactive zymogens that can do no harm. There are various triggers that can lead to their activation, which usually occurs through proteolytic processing of the zymogen at conserved aspartic acid residues. Needless to say, their activation and suicidal function is highly regulated. Once activated caspases act as cysteine proteases, using a cysteine side chain for catalysing peptide bond cleavage at aspartyl residues in their substrates. The name "caspase" denotes their function: Cysteine-dependent ASPartyl-specific proteASE. There are many such caspases within an organism, which work together in a proteolytic cascade to activate themselves and one other. Cascades are effective means of amplifying a signal to give a much larger response than could be achieved through a single enzymatic reaction. The high degree of specificity of caspases enables a precisely controlled cascade process, rather than indiscriminate proteolysis. Caspases have several roles within the cascade: as triggers of the cell death process, as regulatory elements within it, and as effectors of cell death itself, the latter usually being activated by caspases acting earlier in the cascade. At the end of this cascade, caspases act on a variety of signal transduction proteins, cytoskeletal and nuclear proteins, chromatin-modifying proteins, DNA repair proteins and endonucleases to target a cell for destruction by disintegrating its contents, including its DNA.

Caspases can have roles other than in apoptosis, such as caspase-1 (interleukin-1 beta convertase), which is involved in the inflammatory process. The activation of apoptosis can

sometimes lead to caspase-1 activation, providing a link between apoptosis and inflammation, such as during the targeting of infected cells.

Classification

Caspases involved in apoptosis are classified into two groups, the initiator caspases, such as caspase-9 in mammals or its functional ortholog Dronc in *Drosophila*, and the effector caspases, such as caspases-3 and -7 in mammals and their homolog DrICE in *Drosophila*. An initiator caspase invariably contains an extended N-terminal prodomain (>90 amino acids) important for its function, whereas an effector caspase frequently contains 20–30 residues in its prodomain sequence. All caspases are synthesized in cells as catalytically inactive zymogens, and must undergo an activation process.

Caspases and chromatin breakdown

One of the hallmarks of apoptosis is the cleavage of chromosomal DNA into nucleosomal units. The caspases play an important role in this process by activating DNases, inhibiting DNA repair enzymes and breaking down structural proteins in the nucleus.

1) Inactivation of enzymes involved in DNA repair.

The enzyme poly (ADP-ribose) polymerase, or PARP, was one of the first proteins identified as a substrate for caspases. PARP is involved in repair of DNA damage and functions by catalyzing the synthesis of poly (ADP-ribose) and by binding to DNA strand breaks and modifying nuclear proteins. The ability of PARP to repair DNA damage is prevented following cleavage of PARP by caspase-3.

2) Breakdown of structural nuclear proteins.

Lamins are intra-nuclear proteins that maintain the shape of the nucleus and mediate interactions between chromatin and the nuclear membrane. Degradation of lamins by caspase 6 results in the chromatin condensation and nuclear fragmentation commonly observed in apoptotic cells.

3) Fragmentation of DNA.

The fragmentation of DNA into nucleosomal units - as seen in DNA laddering assays - is caused by an enzyme known as CAD, or caspase activated DNase. Normally CAD exists as an inactive complex with ICAD (inhibitor of CAD). During apoptosis, ICAD is cleaved by

caspases, such as caspase 3, to release CAD. Rapid fragmentation of the nuclear DNA follows.

How is an effector caspase activated? An effector caspase is known to exist constitutively as a homodimer, both before and after the intrachain activation cleavage. However, as a consequence of the intrachain cleavage, the catalytic activity of an effector caspase is increased by several orders of magnitude . The mechanism of activation for a representative effector caspase, caspase-7, is revealed by the conformational changes of the active site following the activation cleavage.

How is an initiator caspase activated? For the initiator caspases, however, the definition of activation carries an entirely different meaning. Although an initiator caspase undergoes an autocatalytic intrachain cleavage, this cleavage appears to have only modest effect on its catalytic activity, the fully processed caspase-9 in isolation is only marginally active, much the same way as the unprocessed caspase-9 zymogen. In sharp contrast, association with the apoptosome leads to an enhancement of three orders of magnitude in the catalytic activity for the processed as well as the unprocessed caspase-9. Thus, for caspase-9, activation has little to do with the intrachain cleavage; rather, it refers to the apopto some-mediated enhancement of the catalytic activity of caspase-9.

At present, we do not understand the molecular mechanism for the activation of any initiator caspase. Nonetheless, two models have been proposed. Based on results using heterologous fusion proteins, an Induced Proximity model was proposed to provide a general explanation for the activation of initiator caspases. It states that the initiator caspases autoprocess themselves when they are brought into close proximity of each other. However, this model merely summarizes what have been observed experimentally in laboratories, and does not reveal the molecular mechanisms for the activation of initiator caspases.

Cellular Senescence

Cellular senescence was formally described more than 40 years ago as a process that limited the proliferation (growth) of normal human cells in culture. Thus, nearly half a century ago, the process now known as cellular senescence was linked to both tumor suppression and aging. Recent data validate the early idea that cellular senescence is important for tumor suppression.

Cellular senescence: a primer

Cellular senescence refers to the essentially irreversible growth arrest that occurs when cells that can divide encounter oncogenic stress. With the possible exception of embryonic stem cells, most division-competent cells, including some tumor cells, can undergo senescence when appropriately stimulated.

What causes cellular senescence?

Senescence inducing stimuli are myriad. It is known now that the limited growth of human cells in culture is due in part to telomere erosion, the gradual loss of DNA at the ends of chromosomes. Telomeric DNA is lost with each S phase because DNA polymerases are unidirectional and cannot prime a new DNA strand, resulting in loss of DNA near the end of a chromosome; additionally, most cells do not express telomerase, the specialized enzyme that can restore telomeric DNA sequences de novo. We also know that eroded telomeres generate a persistent DNA damage response (DDR), which initiates and maintains the senescence growth arrest. In fact, many senescent cells harbor genomic damage at nontelomeric sites, which also generate the persistent DDR sigalling needed for the senescence growth arrest. DNA double strand breaks are especially potent senescence inducers. In addition, compounds such as histone deacetylase inhibitors, which relax chromatin without physically damaging DNA, activate the DDR proteins ataxia telangiectasia mutated (ATM) and the p53 tumor suppressor, and induce a senescence response. Finally, many cells senesce when they experience strong mitogenic signals, such as those delivered by certain oncogenes or highly expressed pro-proliferative genes. Notably, these mitogenic signals can create DNA damageand a persistent DDR due to misfired replication origins and replication fork collapse. Thus, many senescence-inducing stimuli cause epigenomic disruption or genomic damage.

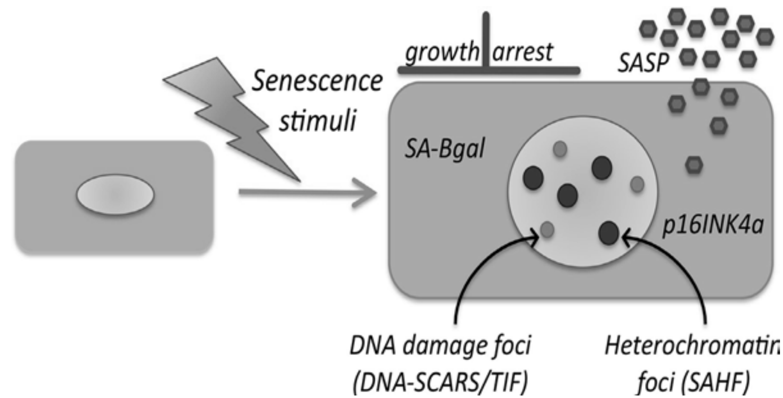


Figure: Hallmarks of senescent cells. Senescent cells differ from other nondividing (quiescent, terminally differentiated) cells in several ways, although no single feature of the senescent phenotype is exclusively specific. Hallmarks of senescent cells include an essentially irreversible growth arrest; expression of SA-Bgal and p16INK4a; robust secretion of numerous growth factors, cytokines, proteases, and other proteins (SASP); and nuclear foci containing DDR proteins (DNA-SCARS/TIF) or heterochromatin (SAHF). The pink circles in the nonsenescent cell (left) and senescent cell (right) represent the nucleus.

Senescence can also occur, however, without detectable DDR sigalling. "Culture stress," the natural and in vivo equivalent of which are unknown, causes a senescence arrest without significant telomere erosion. These stresses could include inappropriate substrata (e.g., tissue culture plastic), serum (most cells experience plasma, not serum, in vivo), and oxidative stress (e.g., culture in atmospheric O2, which is hyperphysiological). Cells also senesce without a DDR upon loss of the Pten tumor suppressor, a phosphatase that counteracts pro proliferative/pro-survival kinases. Additionally, ectopic expression of the cyclin-dependent kinase inhibitors (CDKis) that normally enforce the senescence growth arrest, notably p21WAF1 and p16INK4a, cause senescence without an obvious DDR.

What defines a senescent cell?

(a) Senescent cells are not quiescent or terminally differentiated cells, although the distinction is not always straightforward. No marker or hallmark of senescence identified

thus far is entirely specific to the senescent state. Further, not all senescent cells express all possible senescence markers. Nonetheless, senescent cells display several phenotypes, which, in aggregate, define the senescent state. Salient features of senescent cells are: a)The senescence growth arrest is essentially permanent and cannot be reversed by known physiological stimuli. However, some senescent cells that do not express the CDKi p16INK4a can resume growth after genetic interventions that inactivate the p53 tumor suppressor. So far, there is no evidence that spontaneous p53 inactivation occurs in senescent cells (whether in culture or in vivo), although such an event is not impossible.

(b) Senescent cells increase in size, sometimes enlarging more than twofold relative to the size of nonsenescent counterparts.

(c) Senescent cells express a senescence-associated β-galactosidase, which partly reflects the increase in lysosomal mass.

(d) Most senescent cells express p16INK4a, which is not commonly expressed by quiescent or terminally differentiated cells. In some cells, p16INK4a, by activating the pRB tumor suppressor, causes formation of senescence-associated heterochromatin foci (SAHF), which silence critical pro-proliferative genes (Narita et al., 2003). p16INK4a, a tumor suppressor, is induced by culture stress and as a late response to telomeric or intrachromosomal DNA damage Moreover, p16INK4a expression increases with age in mice and humans and its activity has been functionally linked to the reduction in progenitor cell number that occurs in multiple tissues during aging.

(e) Cells that senesce with persistent DDR sigalling harbor persistent nuclear foci, termed DNA segments with chromatin alterations reinforcing senescence (DNA-SCARS). These foci contain activated DDR proteins, including phospho-ATM and phosphorylated ATM/ataxia telangiectasia and Rad3 related (ATR) substrates and are distinguishable from transient damage foci. DNA-SCARS include dysfunctional telomeres or telomere dysfunction–induced foci.

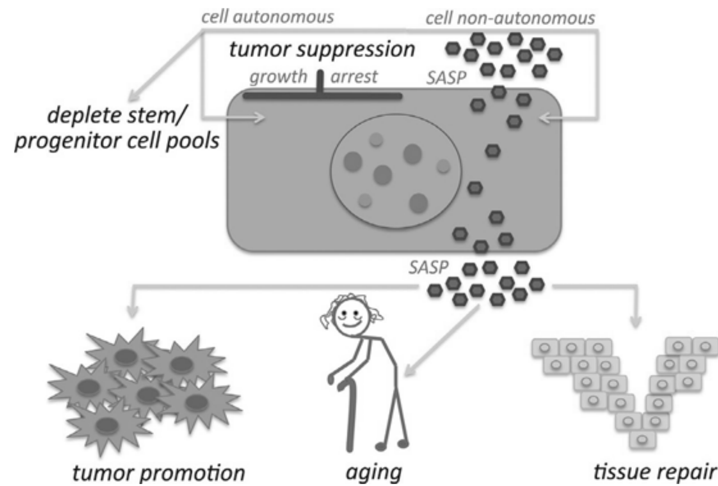


Figure**.** Biological activities of cellular senescence. Senescent cells arrest growth owing to cell autonomous mechanisms, imposed by the p53 and p16INK4a/pRB tumor suppressor pathways, and cell nonautonomous mechanisms, imposed by some of the proteins that comprise the SASP. The growth arrest is the main feature by which cellular senescence suppresses malignant tumorigenesis but can contribute to the depletion of proliferative (stem/progenitor) cell pools. Additionally, components of the SASP can promote tumor progression, facilitate wound healing, and, possibly, contribute to aging.

(f) Senescent cells with persistent DDR sigalling secrete growth factors, proteases, cytokines, and other factors that have potent autocrine and paracrine activities. As we discuss later, this senescence-associated secretory phenotype SASP) helps explain some of the biological activities of senescent cells.

Telomerase

Introduction

Telomerase is the enzyme responsible for maintenance of the length of telomeres by addition of guanine-rich repetitive sequences. Telomerase is a ribonucleoprotein complex. The core enzyme includes telomerase reverse transcriptase and telomerase RNA containing a template site for DNA elongation. The telomerase complex also contains a number of auxiliary components that provide for functioning of telomerase *in vivo*. Some proteins are necessary for maturation of the telomerase complex and degradation of its components. The existence of the enzyme preventing telomere shortening was predicted long before its

discovery by the Russian scientist A. M. Olovnikov. He suggested naming this enzyme telomerase. Telomere length correlates with the cell proliferative potential. A hypothesis by Olovnikov suggests that maintenance of telomere length is responsible for the proliferative potential. Telomeres are DNA–protein structures that are localized at the ends of eukaryotic chromosomes. They protect the linear ends of eukaryotic chromosomes against degradation and fusion, thus maintaining genome stability. The cell replication apparatus is not able to provide for complete replication of chromosome ends (See Fig. below); also, telomeres are subject to the action of nucleases and other destructive factors. As a result, telomeres shorten during each cell division. In most organisms the main mechanism of telomere length maintenance is completion of DNA telomere repeats by telomerase. This enzyme elongates the chromosome $3'$-end, whereas the complementary strand is completed by DNA polymerases.

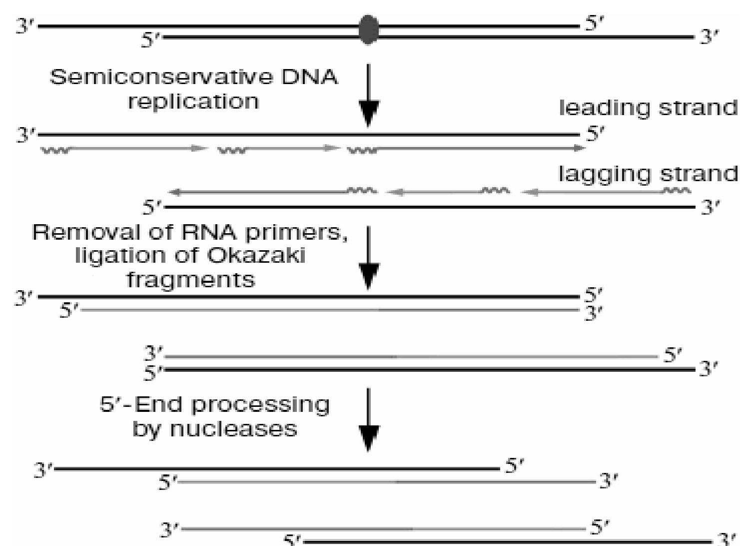


Figure**.** Telomere shortening due to under-replication and processing in each cell division.

In humans, somatic cells proliferation potential is strictly limited and senescence follows approximately 50-70 cell divisions. No doubt, DNA polymerase is not capable to completely copy DNA at the very ends of chromosomes; therefore, approximately 50 nucleotides are lost during each cell cycle, which results in gradual telomere length shortening. Critically short telomeres cause senescence, following crisis, and cell death. However, in tumor cells the system of telomere length maintenance is activated. Besides catalytic telomere elongation, independent telomerase functions can be also involved in cell cycle regulation. Inhibition of the telomerase catalytic function and resulting cessation of telomere length maintenance will help in restriction of tumor cell replication potential.

On the other hand, formation of temporarily active enzyme via its intracellular activation or due to stimulation of expression of telomerase components will result in telomerase activation and telomere elongation that can be used for correction of degenerative changes.

There are data showing that activation of telomerase is associated with the development of cancer, and that it is active in cells exhibiting potential for unlimited division. It is known that telomerase is active in 85% of cancer tumors, while in the other 15% of cases different mechanisms of telomere length maintenance based on recombination are active. It should be noted that telomerase activity is not found in usual somatic tissues.

The main criterion of telomerase efficiency is the number of telomeric repeats at the ends of telomeres. Telomere length reduction is a symptom of many diseases and can be both the result of primary telomerase dysfunction (like those caused by mutations in the main telomerase components, hTERT, hTR, or by disturbance in telomere-organizing systems) and the result of premature telomere loss induced by different factors. Inborn dyskeratosis is of the first type. It was the first identified human genetic disease caused by disturbance in the system of telomere length maintenance. This disease is characterized by skin hyperpigmentation, epithelium keratinization, nail dystrophy, and progressive aplastic anemia. In most cases autosomal diseases are due to mutations in the H/ACA region of human telomerase RNA, while X-chromosome-linked cases emerge due to mutations in protein dyskerin leading to disturbance in telomerase complex assembly.

Telomerase structure and function:

As mentioned above, telomerase is a particular reverse transcriptase working in a complex with special telomerase RNA (See Fig below). Telomerase substrates in its reaction are deoxynucleotide 5′-triphosphates and the telomere 3 terminus (in tests *in vitro* it is DNA-oligonucleotide containing the sequence corresponding to telomeric repeats of chromosomes). The particular property distinguishing telomerase from different RNA-dependent DNA polymerases is the use of a fixed region of special telomerase RNA as template for telomere elongation.

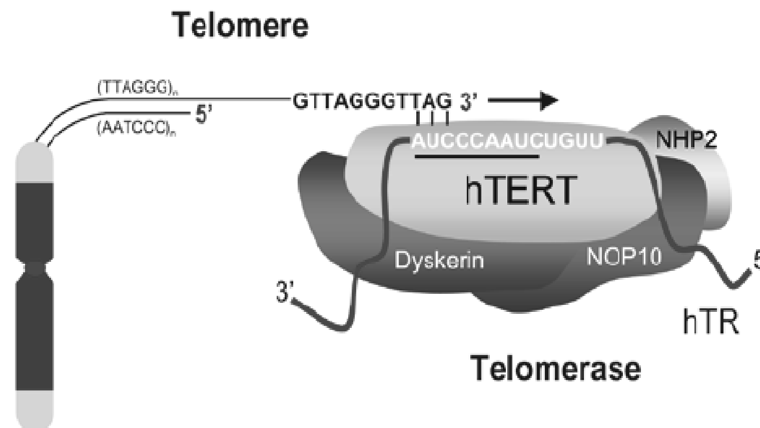


Figure. Structure and function of telomerase. The human telomerase complex consists of a catalytic sub- unit (hTERT), an RNA component (hTR), dyskerin, NOP2, NHP2, and additional associated proteins.

Telomerase RNA interacts with telomere not only at this template region, but additionally in the so-called “anchor site”. Telomerase is able to add several telomeric repeats during a single act of attachment to oligonucleotide substrate.

Main Components of Telomerase Complex

Telomerase RNA (TER) contains template region and different functionally important secondary structure elements involved in template region restriction, protein subunit binding, and partially carrying out catalytic and other functions. Telomerase reverse transcriptase (TERT) contains a catalytically important domain resembling that of reverse transcriptases, as well as only telomerase-specific domains necessary for TER and DNA substrate binding and for functional activity of telomerase. TER and TERT form the core enzyme. These components are enough to provide functional activity of telomerase *in vitro*. *In vivo* functioning requires auxiliary proteins, some of which are included in the holoenzyme. Despite high interest in telomerase and importance of its study in applied aspect, structural data on TERT, TER, and other telomerase proteins have become available relatively recently due to complication of telomerase investigation (very low intracellular enzyme content, difficulties in isolation of its components in soluble form and in sufficient amount, etc.).

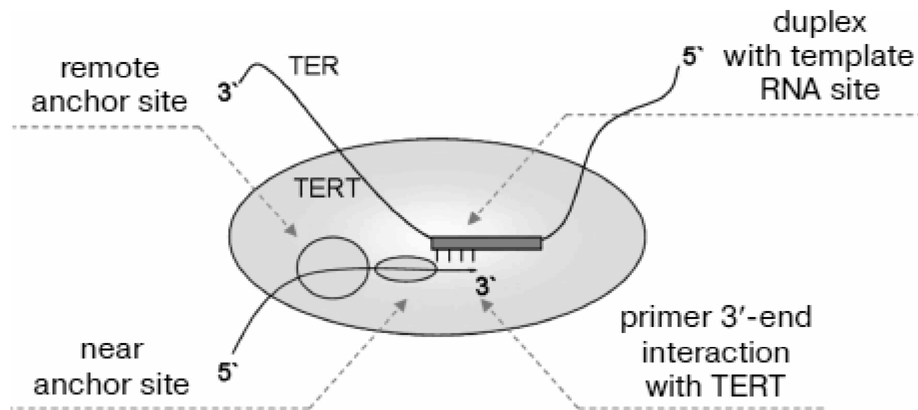


Figure: Interaction of primer with telomerase.

Telomerase binding proteins

Proteins specifically binding telomeric sequence and factors interacting with these proteins form a dynamic ribonucleoprotein structure. This structure has a protective role; it participates in telomere length regulation and is responsible for gene silence at telomeric and telomere-side sites. Also, telomere structures serve as targets for inhibitors that prevent telomerase binding to telomere.

Yeast telomere DNA consists of a 250-350 bp long double-stranded region with $C_{1-3}A/TG_{1-3}$ sequence and a short single-stranded protruding end with TG_{1-3} sequence. Yeast telomeric repeats $((TG)_{0-6}TGGGTGTG(G))_n$ are heterogeneous, unlike the homogeneous repeats of mammalian telomeres $(TTAGGG)_n$.

In yeasts the telomere double-stranded region directly binds protein Rap1p, and the single-stranded region binds Cdc13p. Rap1p interacts with complex of Sir (silent information regulators) proteins that are responsible for heterochromatin formation in the subtelomeric region. Protein Rap1p also interacts with telomere proteins Rif1p and Rif2p. These proteins are associated with the telomere during the whole cell

In mammals telomeric DNA is more closely packed in nucleosomes compared to other eukaryotes, and some nucleosomes carry heterochromatin markers. Proteins TRF1 and TRF2 interact with double-stranded telomeric DNA, and protein POT1 and its partner

TPP1 interact with the single-stranded region. Protein TRF2 binds RAP1. Proteins binding single-stranded and double-stranded DNA interact with each other via TIN2 protein interacting with TRF1 and TRF2 as well as with TPP1 protein.

Inhibition of telomerase

All presently available inhibitors of telomerase activity can be divided into three groups based on their action and chemical properties. They are nucleoside and nucleotide analogs working as substrate inhibitors, various low molecular weight compounds with different mechanisms of action, and oligonucleotide-based inhibitor. Let us consider all three groups.

Nucleos(t)ide analogs are well known inhibitors for DNA-polymerases. Inhibition of DNA polymerization is due to the impossibility of incorporation of the next nucleotide residue because of competitive binding of these substances in the enzyme active site. Since the catalytic subunit of telomerase is RNA-depended DNA-polymerase or a reverse transcriptase and there are antivirus preparations such as those for therapy of HIV-infections blocking reverse transcriptase, the idea to check inhibitory activity of such preparations towards telomerase became apparent. The most widely used inhibitor of HIV reverse transcriptase is azidothymidine (AZT). AZT was the first nucleos(t)ide analog tested for telomerase inhibition.

Another class of telomerase inhibitors consists of a group of low molecular weight compounds with structures different from nucleos(t)ides. This group includes substances influencing mainly hTERT. Some of them are used in HIV therapy. Rubromycins and purpuromycin appeared to be powerful inhibitors (50% inhibitory concentrations are as low as 3 μM). It was reported that some quinolines form a family of integrase inhibitors. These inhibitors block telomerase either due to disturbance of protein–nucleic acid interactions supporting telomere structure, or due to blocking the substrate (telomere) binding to enzyme (telomerase).

In 2005 it was shown that the natural lactone helenalin is a telomerase inhibitor. The mode of action of this cytostatic agent is not clear. Perhaps helenalin affects telomerase activity

through its interaction with nuclear factor κB and regulation of hTERT level. Curiously, polyunsaturated fatty acids inhibit telomerase via direct interaction with the catalytic subunit, and at the same time they switch off expression of the *hTERT* gene.

A unique approach to telomerase inhibition was recently proposed—the use of low molecular weight substances involved in recognition of RNA/DNA heteroduplexes formed upon the interaction of telomerase RNA with chromosome ends.

It was shown that the main catechin of green tea (*Epigallocatechin gallate*) not only directly inhibits telomerase in a concentration-dependent manner, but also induces apoptosis in cells of tumor of head and neck via inhibiting the telomerase activity.

The most interesting potential inhibitor was found upon screening of 16,000 organic compounds. It is an isothiozolin derivative (50% inhibition is achieved at 1 μM concentration) that is a noncompetitive inhibitor relative to substrate and deoxynucleotide triphosphates. It possesses remarkable selectivity. It simultaneously has no effect on DNA polymerase and HIV reverse transcriptase. Glutathione and dithiothreitol enhance its inhibitory activity. This fact suggests telomerase inhibition by affecting cysteine residues in the catalytic subunit.

The third class of inhibitors is oligonucleotides. The main components for targeting telomerase by oligonucleotides are hTR and messenger RNA for hTERT.

Probable Questions:

1. Classify Cancer on the basis of invasiveness and location.

2. Discuss Tumor suppressor gene's role in cancer control

3. Write short notes on Integrins.

4. Discuss about Cadherin switch during cancer progression

5 What is the difference between necrosis and apoptosis.

6. Discuss the mechanism of apoptosis.

7. Name some of the assay methods of apoptosis.

8. Discuss about Anti-apoptotic action of Neurotrophins.

9. Write about structure and Function of Telomerase.

10. Write about the role of caspase proteins.

Suggested Readings:

1. Lodish, H. (2016). Molecular cell biology. New York, NY: Freeman.

2. Alberts, B. (2008). Molecular biology of the cell. New York, NY [u.a.]: Garland Science Taylor & Francis.

3. Lewin, B., Krebs, J., Goldstein, E. and Kilpatrick, S. (2014). Lewin's genes XI. Burlington, MA: Jones & Bartlett Learning.

4. Karp, G. and Patton, J. (2015). Cell and molecular biology. Brantford, Ont.: W. Ross MacDonald School Resource Services Library.

5. Cooper, G. and Hausman, R. (n.d.). The cell.

Unit-XII

DNA replication: nature, enzymology of replication, replication fork; fidelity of replication; extrachromosomal replicons; leading and lagging strands; Okazaki fragments; termination of replication

Objectives:

In this Unit we will discuss on DNA replication: nature, enzymology of replication, replication fork; fidelity of replication; extrachromosomal replicons; leading and lagging strands; Okazaki fragments; termination of replication

Introduction:

Genetic materials are transmitted from parent to offspring and from cell to cell. For transmission to occur, the genetic material must be copied. Each DNA strand serves as a template for the synthesis of a new strand, producing two new DNA molecules, each with one new strand and one old strand. This process is known as DNA replication.

The fundamental properties of the DNA replication process and the mechanisms used by the enzymes that catalyze it have proved to be essentially identical in all species. This mechanistic unity is a major theme as we proceed from general properties of the replication process in *E. coli* to replication in eukaryotes.

Nature of DNA Replication

DNA replication relies on the complementarity of DNA strands according to the AT/GC rule. During the replication process, the two complementary strands of DNA come apart and serve as template strands, or parental strands, for the synthesis of two new strands of DNA. After the double helix has separated, individual nucleotides have access to the template strands. Hydrogen bonding between individual nucleotides and the template strands must obey the AT/GC rule. To complete the replication process, a covalent bond is formed between the phosphate of one nucleotide and the sugar of the previous nucleotide. The two newly made strands are referred to as the daughter strands. Note that the base sequences are identical in both double-stranded molecules after replication (Figure 1). Therefore, DNA is replicated so that both copies retain the same information-the same base sequence-as the original molecule.

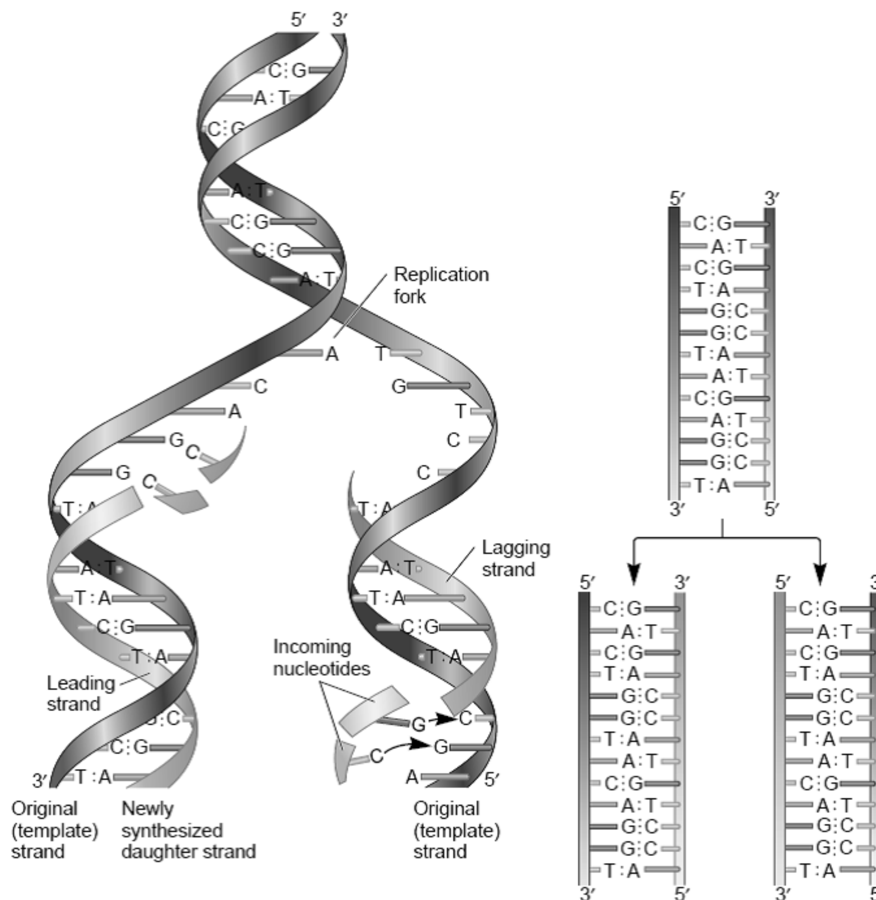


Figure 1: Mechanism of replication and Product of replication

Scientists in the late 1950s had considered three different mechanisms to explain the net result of DNA replication. These mechanisms are shown in Figure 2. The first is referred to as a conservative model.According to this hypothesis, both strands of parental DNA remain together following DNA replication. In this model, the original arrangement of parental strands is completely conserved, while the two newly made daughter strands also remain together following replication. The second is called a semiconservative model. In this mechanism, the double stranded DNA is half conserved following the replication process. In other words, the newly made double-stranded DNA contains one parental strand and one daughter strand. The third, called the dispersive model, proposes that segments of parental DNA and newly made DNA are interspersed in both strands following the replication process. Only the semiconservative model is actually the correct model of replication.

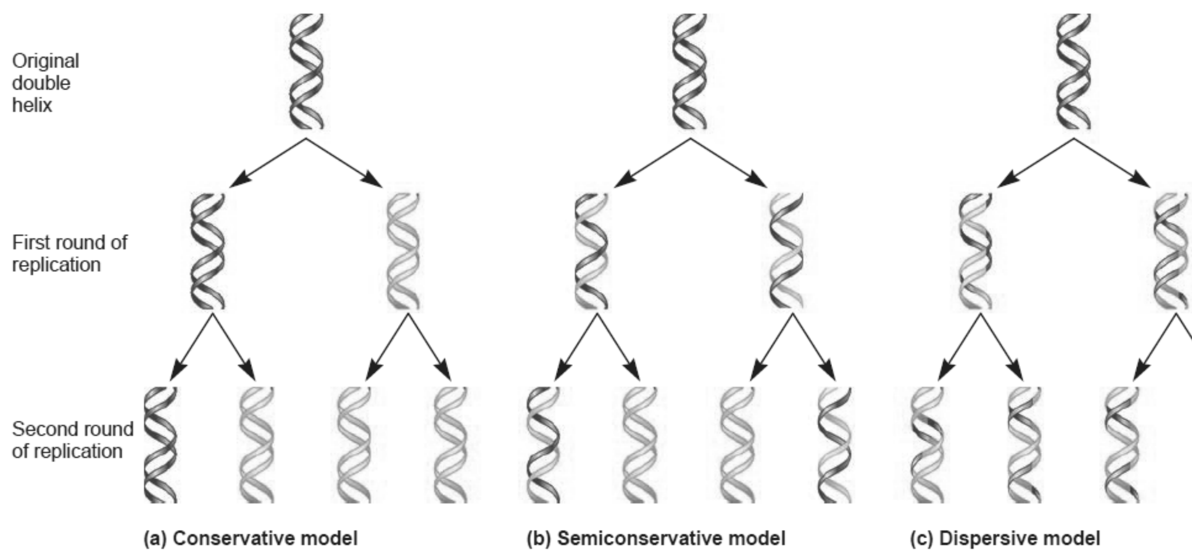


Figure 2: Possible models of DNA replication

Replication Fork, Okazaki fragments, Leading and Lagging Strands of DNA replication:

Like many processes in molecular biology, DNA replication is also regarded as being made up of three phases-initiation, elongation and termination. **Initiation** involves recognition of the position(s) on a DNA molecule where replication will begin. **Elongation** concerns the events occurring at the replication fork, where the parent polynucleotides are copied. **Termination** which in general is only vaguely understood, occurs when the parent molecule has been completely replicated.

The site on the chromosome where DNA synthesis begins is known as the **origin of replication.** The synthesis of new daughter strands is initiated within the origin and proceeds in both directions, or **bidirectionally.** This means that two **replication forks** move in opposite directions outward from the origin. A replication fork is the site where the parental DNA strands have separated and new daughter strands are being made.

A new strand of DNA is always synthesized in the 5'→3' direction, with the free 3' OH as the point at which the DNA is elongated (the 5' and 3' ends of a DNA strand are defined in Fig. 3). Because the two DNA strands are antiparallel, the strand serving as the template is read from its 3' end toward its 5' end. If synthesis always proceeds in the 5'→3' direction, how can both strands be synthesized simultaneously? If both strands were synthesized continuously while the replication fork moved, one strand would have to undergo 3'→5' synthesis. This problem was resolved by Reiji Okazaki and colleagues in the 1960s. Okazaki found that one

of the new DNA strands is synthesized in short pieces, now called **Okazaki fragments**. This work ultimately led to the conclusion that one strand is synthesized continuously and the other discontinuously (Fig. 4). The continuous strand, or **leading strand**, is the one in which 5'→3' synthesis proceeds in the samedirection as replication fork movement. The discontinuous strand, or **lagging strand**, is the one in which 5'→3' synthesis proceeds in the direction oppositeto the direction of fork movement. Okazaki fragments range in length from a few hundred to a few thousand nucleotides, depending on the cell type.

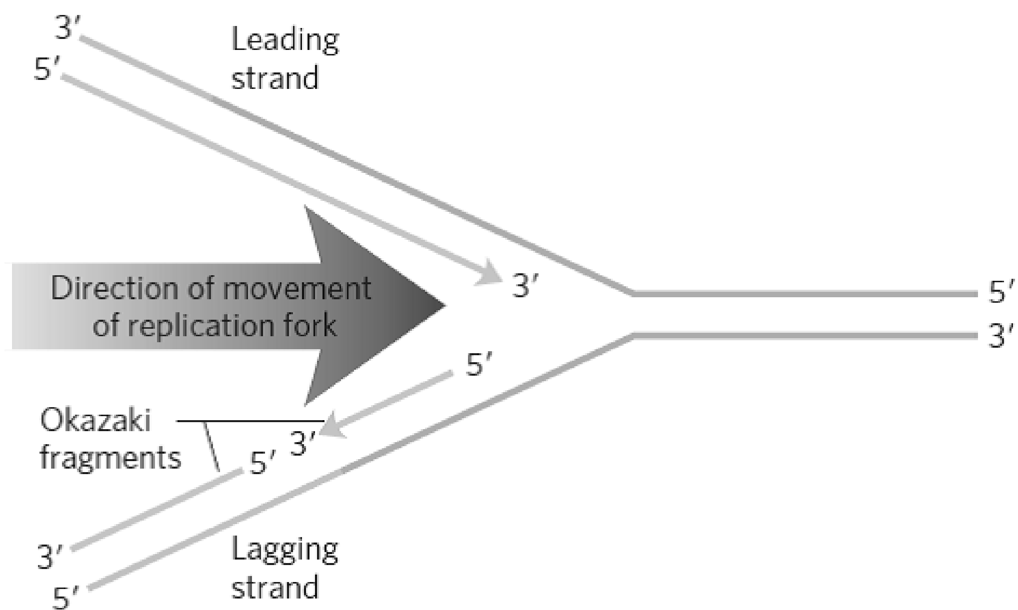


Fig: 3. DNA synthesis in Leading and Lagging strand

DNA replication and Enzymology in *E. coli*

DNA replication requires many types of proteins especially enzymes. The origin of replication in *E. coli* is named *oriC* (Figure 4). Three types of DNA sequences are found within *oriC:* an AT-rich region, DnaA box sequences, and GATC methylation sites. DNA replication is initiated by the binding of **DnaA proteins** to sequences within the origin known as **DnaA box sequences.** The DnaA box sequences serve as recognition sites for the binding of the DnaA proteins.

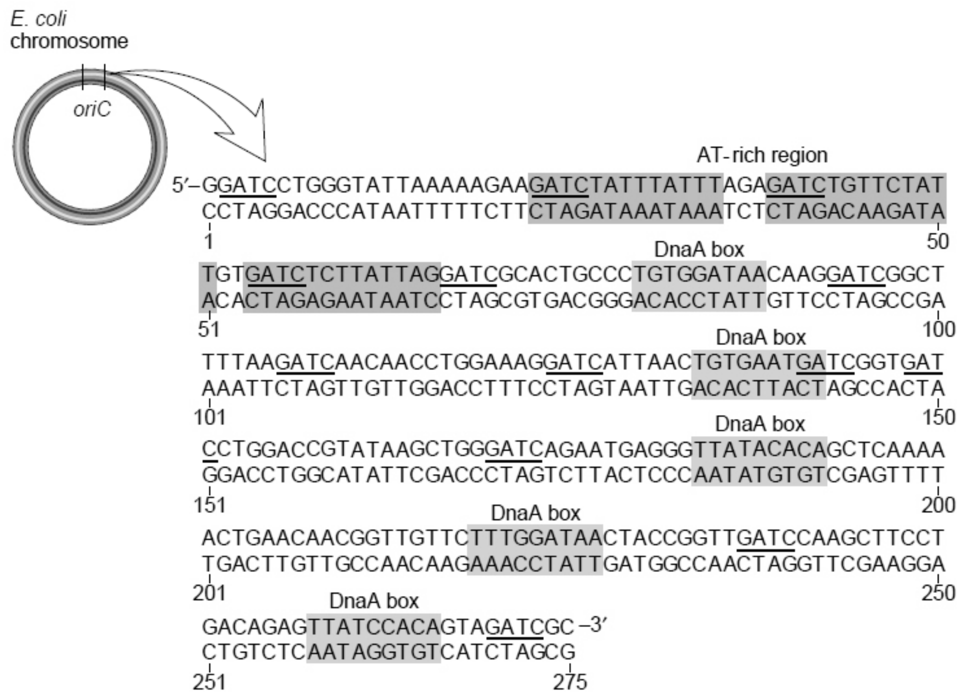


Figure 4. The sequence of o*riC* in *E.coli*

When DnaA proteins are in their ATP-bound form, they bind to the five DnaA boxes in *oriC* to initiate DNA replication. DnaA proteins also bind to each other to form a complex (**Figure 5**). With the aid of other DNA-binding proteins, such as HU and IHF, this causes the DNA to bend around the complex of DnaA proteins and results in the separation of the AT-rich region. AT-rich region, the DnaA proteins, with the help of the DnaC protein, recruit **DNA helicase** proteins to this site. DNA helicase is also known as DnaB protein. When a DNA helicase encounters a double-stranded region it breaks the hydrogen bonds between the two strands, thereby generating two single strands. Two DNA helicases begin strand separation within the *oriC* region and continue to separate the DNA strands beyond the origin. In *E. coli,* DNA helicases bind to single stranded DNA and travel along the DNA in a 5to 3´ direction to keep the replication fork moving. The action of DNA helicases promotes the movement of two replication forks outward from *oriC* in opposite directions. This initiates the replication of the bacterial chromosome in both directions, an event termed **bidirectional replication.**

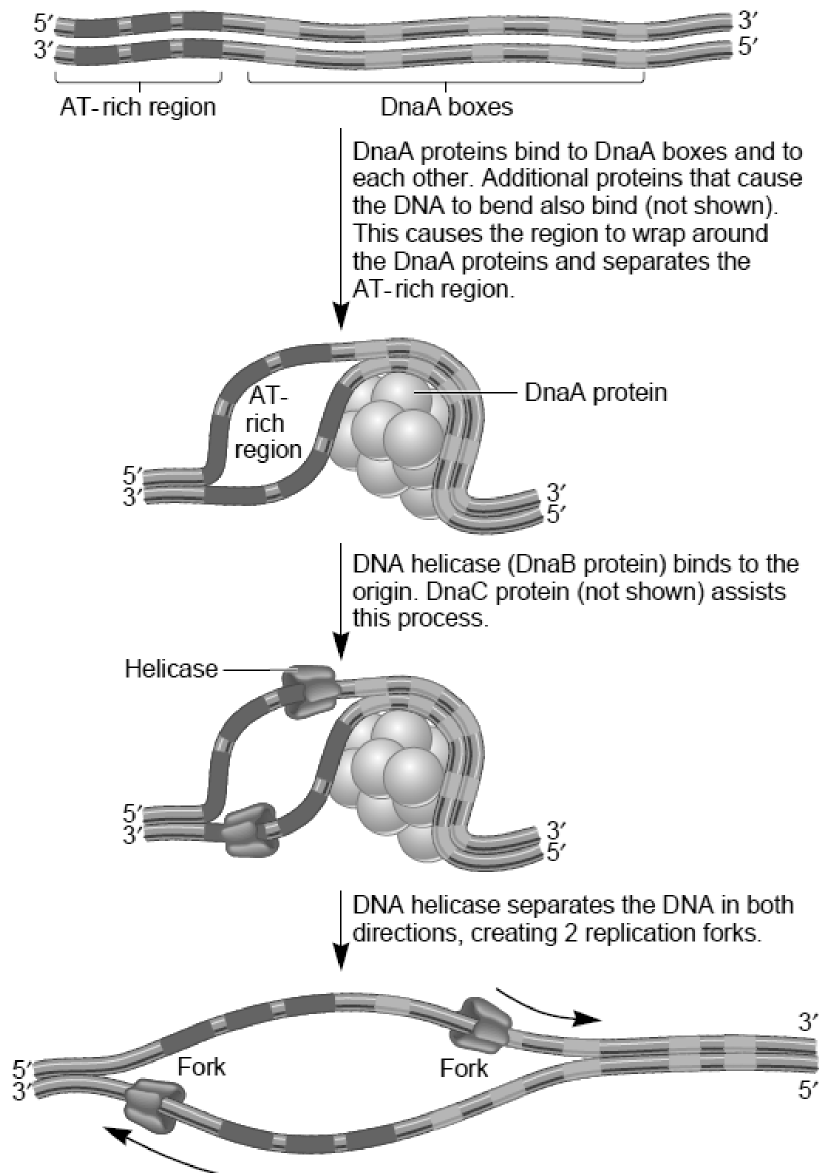


Figure 5: Initiation of replication in *E.coli*

The DNA helicase break the hydrogen bonds between base pairs and thereby unwind the strands and this action generates positive supercoiling ahead of each replication. An enzyme known as a **topoisomerase (type II),** also called **DNA gyrase,** travels in front of DNA helicase and alleviates positive supercoiling. After the two parental DNA strands have been separated and the supercoiling relaxed, they must be kept that way until the complementary daughter strands have been made. DNA replication requires **single-strand binding proteins** that bind to the strands of parental DNA and prevent them from re-forming a double helix.

The next event in DNA replication involves the synthesis of short strands of RNA (rather than DNA) called **RNA primers.** These strands of RNA are synthesized by the linkage of

ribonucleotides via an enzyme known as **primase.** This enzyme synthesizes short strands of RNA, typically 10 to 12 nucleotides in length. These short RNA strands start, or prime, the process of DNA replication. In the **leading strand,** a single primer is made at the origin of replication. In the **lagging strand,** multiple primers are made. A type of enzyme known as **DNA polymerase** is responsible for synthesizing the DNA of the leading and lagging strands. In *E. coli*, five distinct proteins function as DNA polymerases and are designated polymerase I, II, III, IV, and V. DNA polymerase III is responsible for most of the DNA replication. It is a large enzyme consisting of 10 different subunits (Table 1) that play various roles in the DNA replication process.

Subunit Composition of DNA Polymerase III Holoenzyme from *E. coli*

Subunit(s)	Function
α	Synthesizes DNA
ε	3′ to 5′ proofreading (removes mismatched nucleotides)
θ	Accessory protein that stimulates the proofreading function
β	Clamp protein, which allows DNA polymerase to slide along the DNA without falling off
τ, γ, δ, δ′, ψ, and χ	Clamp loader complex, involved with helping the clamp protein bind to the DNA

Table 1: Subunits of DNA polymerase III from *E. coli*

The α subunit actually catalyzes the bond formation between adjacent nucleotides, and the remaining nine subunits fulfill other functions. The complex of all 10 subunits together is called DNA polymerase III holoenzyme. By comparison, DNA polymerase I is composed of a single subunit. In *E. coli*, the RNA primers are removed by the action of DNA polymerase I. This enzyme has a 5′ to 3′ exonuclease activity, which means that DNA polymerase I digests away the RNA primers in a 5′ to 3′ direction, leaving a vacant area. DNA polymerase I then synthesizes DNA to fill in this region. It uses the 3′ end of an adjacent Okazaki fragment as a primer by attaching nucleotides to the 3 end of the second Okazaki fragment. After the gap has been completely filled in, a covalent bond is still missing between the last nucleotide added by DNA polymerase I and the adjacent DNA strand that had been previously made by DNA polymerase III. An enzyme known as **DNA ligase** catalyzes a

covalent bond between adjacent fragments to complete the replication process in the lagging strand (Figure 6 and 7).

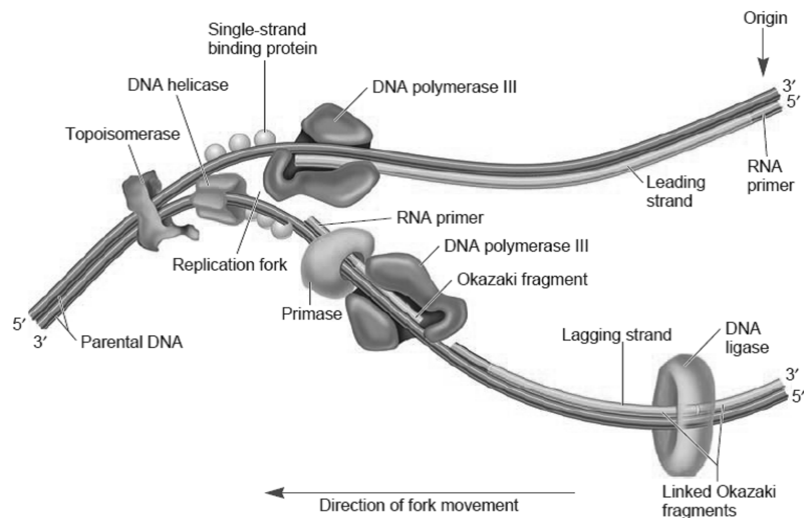


Figure 6: Mechanism of replication in *E.coli*

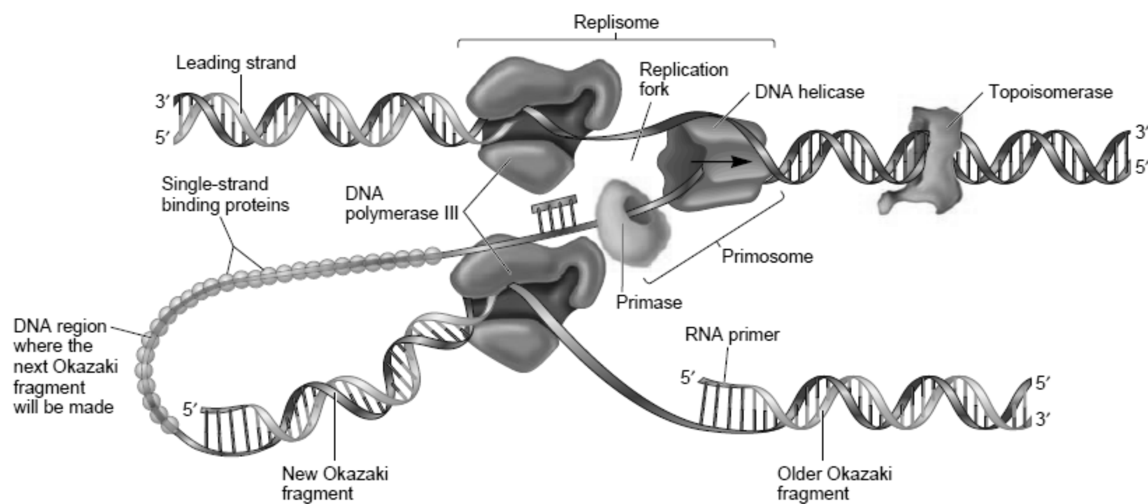


Figure 7: A three dimensional view of replication in *E.coli*

Termination of Replication:

Replication is terminated in *E.coli* when the replication forks meet at the termination sequences. On the opposite side of the *E. coli* chromosome from *oriC* is a pair of **termination sequences** called ter sequences. A protein known as the termination utilization substance (Tus) binds to the ter sequences and stops the movement of the replication forks. As shown in **Figure 8**, one of the ter sequences designated T1 prevents the advancement of the fork moving left to right, but allows the movement of the other fork. Alternatively, T2 prevents the advancement of the fork moving right to left, but allows the advancement of the

other fork. In other words, DNA replication ends when oppositely advancing forks meet, usually at T1 or T2. Finally, DNA ligase covalently links the two daughter strands, creating two circular, double-stranded molecules. After DNA replication is completed, it often results in two intertwined DNA molecules known as **catenanes.** In *E. coli*, topoisomerase II introduces a temporary break into the DNA strands and then rejoins them after the strands have become unlocked. This allows the catenanes to be separated into individual circular molecules.

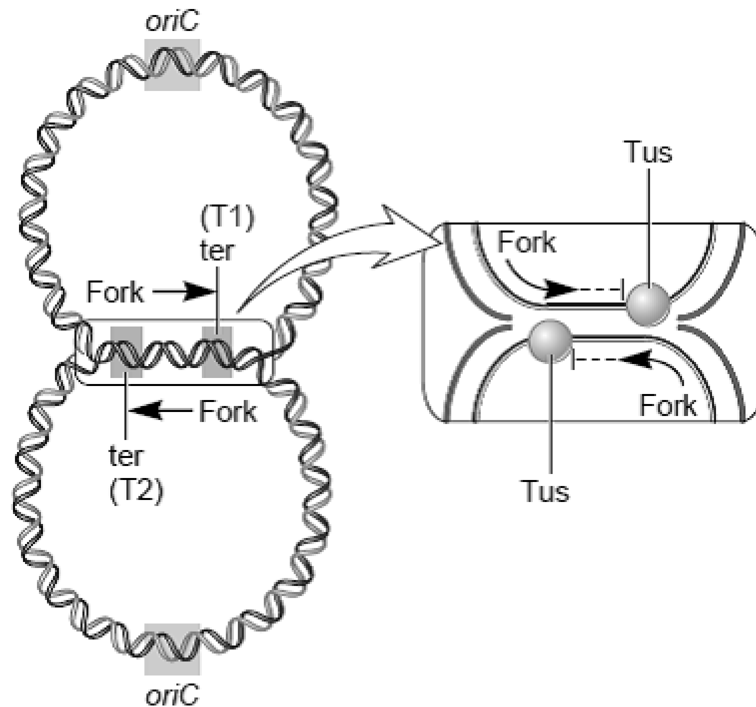


Fig: 8. Termination of replication in *E.coli*

DNA Replication and Enzymology in Eukaryotes

Eukaryotic DNA replication is not as well understood as bacterial replication. Many of these studies on experimental organisms, particularly yeast and mammalian cells have found extensive similarities between the general features of DNA replication in prokaryotes and eukaryotes. For example, DNA helicases, topoisomerases, single-strand binding proteins, primases, DNA polymerases, and DNA ligases-the types of bacterial enzymes described in bacterial replication have also been identified in eukaryotes (Table 2). On the other hand eukaryotic cells have larger, linear chromosomes, the chromatin is tightly packed within nucleosomes, and cell cycle regulation is much more complicated.

E. coli protein	Eukaryotic protein	Function
DnaA	ORC proteins	Recognition of origin of replication
Gyrase	Topoisomerase I/II	Relieves positive supercoils ahead of replication fork
DnaB	Mcm	DNA helicase that unwinds parental duplex
DnaC	Cdc6, Cdt1	Loads helicase onto DNA
SSB	RPA	Maintains DNA in single-stranded state
γ-complex	RFC	Subunits of the DNA polymerase holoenzyme that load the clamp onto the DNA
pol III core	pol δ/ε	Primary replicating enzymes; synthesize entire leading strand and Okazaki fragments; have proofreading capability
β clamp	PCNA	Ring-shaped subunit of DNA polymerase holoenzyme that clamps replicating polymerase to DNA; works with pol III in *E. coli* and pol δ or ε in eukaryotes
Primase	Primase	Synthesizes RNA primers
———	pol α	Synthesizes short DNA oligonucleotides as part of RNA–DNA primer
DNA ligase	DNA ligase	Seals Okazaki fragments into continuous strand
pol I	FEN-1	Removes RNA primers; pol I of *E. coli* also fills gap with DNA

Table 2. Proteins and enzymes of Replication

Because eukaryotes have long, linear chromosomes, the chromosomes require multiple origins of replication so the DNA can be replicated in a reasonable length of time. DNA replication proceeds bidirectionally from many origins of replication during S phase of the cell cycle. The multiple replication forks eventually make contact with each other to complete the replication process (Figure 9).

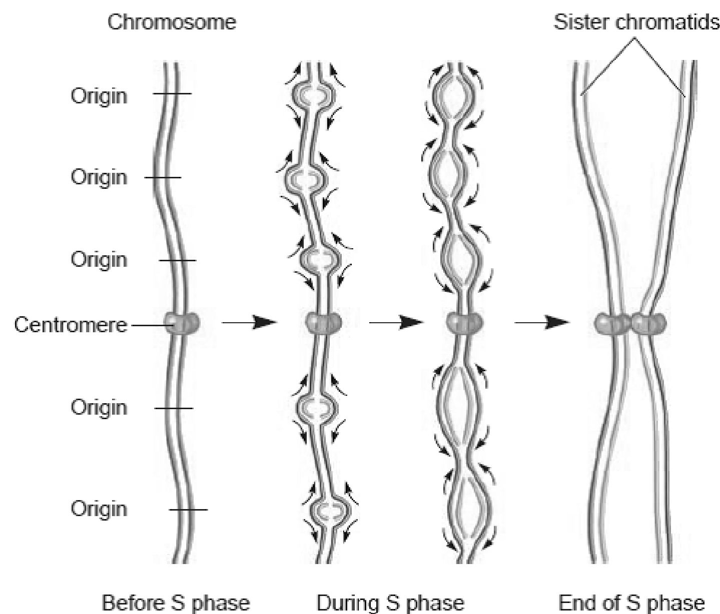


Figure 9: The replication of eukaryotic chromosome

At the molecular level, eukaryotic origins of replication have been extensively studied in the yeast *Saccharomyces cerevisiae*. In this organism, several replication origins have been identified and sequenced. They have been named **ARS elements** (for autonomously replicating sequence). ARS elements, which are about 50 bp in length, are necessary to initiate chromosome replication. ARS elements have unique features of their DNA sequences. First, they contain a higher percentage of A and T bases than the rest of the chromosomal DNA. In addition, they contain a copy of the ARS consensus sequence (ACS),

ATTTAT(A or G)TTTA, along with additional elements that enhance origin function. This arrangement is similar to bacterial origins. In *S. cerevisiae*, origins of replication are determined primarily by their DNA sequences. In animals, the critical features that define origins of replication are not completely understood.

DNA replication in eukaryotes begins with the assembly of a **prereplication complex (preRC)** consisting of at least 14 different proteins. Part of the preRC is a group of six proteins called the **origin recognition complex (ORC)** that acts as the initiator of eukaryotic DNA replication. The ORC was originally identified in yeast as a protein complex that binds directly to ARS elements. The ORC has been described as a "molecular landing pad" because of its role in binding the proteins required in subsequent steps. DNA replication at the origin begins with the binding of ORC, which usually occurs during G1 phase. Other proteins of the preRC then bind, including a group of proteins called **MCM helicase.** At the pre-RC stage, each of the origins contains a *double* hexameric MCM complex, that is, two complete replicative helicases, which remain inactive at this stage of the cell cycle. Each of these replicases will travel in opposite directions away from the origin once replication begins (Figure 10).

Assembly of a pre-RC marks that site on the genome as a potential origin of replication. Just before the beginning of S phase of the cell cycle, the activation of key protein kinases leads to the phosphorylation of the MCM complex and other proteins and to the initiation of replication at selected sites in the genome. One of these protein kinases is a cyclin-dependent kinase (Cdk). Cdk activity remains high from S phase through mitosis, which suppresses the formation of new prereplication complexes. Consequently, each origin can only be activated once per cellcycle. Cessation of Cdk activity at the end of mitosis permits the assembly of pre-RCs for the next cell cycle (Figure 10).

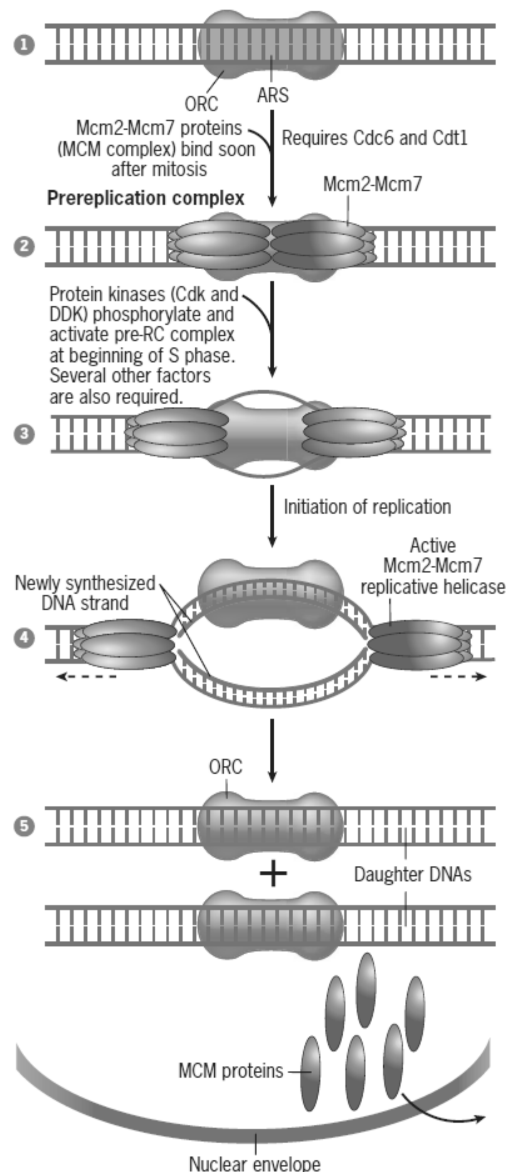


Figure 10: Steps of Replication Initiation in Yeast

The DNA of eukaryotic cells is synthesized in a semidiscontinuous manner, although the Okazaki fragments of the lagging strand are considerably smaller than in bacteria, averaging about 150 nucleotides in length. As in *E. coli* , the leading and lagging strands are thought to be synthesized in a coordinate manner by a single replicative complex, or *replisome.* To date, five “classic” DNA polymerases have been isolated from eukaryotic cells, and they are designated α, β, γ, δ and ε (Table 3). Of these enzymes, polymerase γ replicates mitochondrial DNA, and polymerase β functions in DNA repair. The other three polymerases have replicative functions. Polymerase is tightly associated with the primase, and together they initiate the synthesis of each Okazaki fragment. The polymerase-primase complex

recognizes and binds to unwound DNA that is coated by a single stranded DNA-binding protein called RPA. Primase initiates synthesis by assembly of a short RNA primer, which is then extended by the addition of about 20 deoxyribonucleotides by polymerase α. Polymerase δ is thought to be the primary DNA-synthesizing enzyme during replication of the lagging strand, whereas polymerase ε is thought to be the primary DNA-synthesizing enzyme during replication of the leading strand.

Eukaryotic DNA Polymerases	
Polymerase Types*	Function
α	Initiates DNA replication in conjunction with primase
ε	Replication of the leading strand during S phase
δ	Replication of the lagging strand during S phase
γ	Replication of mitochondrial DNA
η, κ, ι, ξ (lesion-replicating polymerases)	Replication of damaged DNA
α, β, δ, ε, σ, λ, μ, φ, θ, η	DNA repair or other functions†

Table 3: Eukaryotic DNA Polymerases and their functions

Like the major replicating enzyme of *E. coli*, both polymerase and 5′ require a "sliding clamp" that tethers the enzyme to the DNA, allowing it to move processively along a template. In eukaryotes, the sliding clamp is called PCNA. The clamp loader that loads PCNA onto the DNA is called RFC and is analogous to the *E. Coli* polymerase III clamp loader complex. After synthesizing an RNA-DNA primer, polymerase α is replaced at each template-primer junction by the PCNA-polymerase complex, which completes synthesis of the Okazaki fragment. When polymerase δ reaches the 5 ′ end of the previously synthesized Okazaki fragment, the polymerase continues along the lagging-strand template, displacing the primer. The displaced primer is cut from the newly synthesized DNA strand by an endonuclease (FEN-1) and the resulting nick in the DNA is sealed by a DNA ligase. FEN-1 and DNA ligase are thought to be recruited to the replication fork through an interaction with the PCNA sliding clamp (Figure 11).

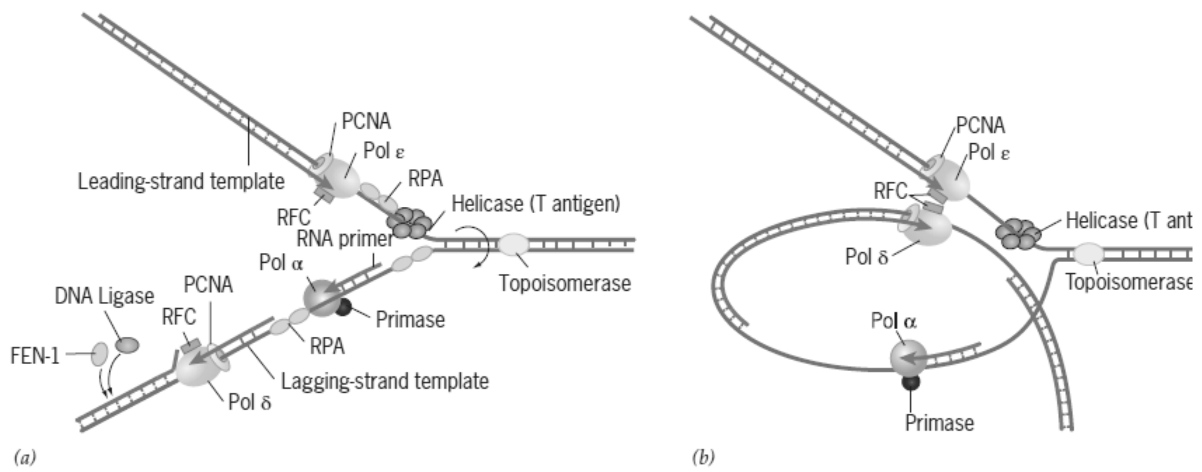


Figure 11: A Schematic view of major components of eukaryotic replication fork. (a) Proteins required for replication. (b) Proposed mechanism of leading and lagging strand synthesis.

Like bacterial polymerases, all of the eukaryotic polymerases elongate DNA strands in the 5′→ 3′ direction by the addition of nucleotides to a 3 ′ hydroxyl group, and none of them is able to initiate the synthesis of a DNA chain without a primer. Polymerases γ, δ and ε. and possess a 3′ → 5′ exonuclease, whose proofreading activity ensures that replication occurs with very high accuracy. Several other DNA polymerases (including η, κ, ι) have a specialized function that allows cells to replicate damaged DNA.

Termination of Replication: Little is known about termination of replication in eukaryotes. No sequences equivalent to bacterial terminators are known in eukaryotes, and proteins similar to Tus have not been identified. Quite possibly, replication forks meet at random positions and termination simply involves ligation of the ends of the new polynucleotides (Fig. 9). We do know that the replication complexes do not break down, because these factories are permanent features of the nucleus. Rather than concentrating on the molecular events occurring when replication forks meet, attention has been focused on the difficult question of how the daughter DNA molecules produced in a eukaryotic nucleus do not become impossibly tangled up. Initially, the two daughter molecules are held together by **cohesin** proteins. These are attached to the DNA immediately after passage of the replication fork by a process that appears to involve DNA polymerase κ, an enigmatic enzyme that is essential for replication but whose role does not obviously require a DNA polymerase activity. The cohesins maintain the alignment of the daughter DNA molecules until the cell divides, when they are cleaved by cutting proteins, enabling the daughter chromosomes to separate.

Fidelity of replication

With replication occurring so rapidly, mistakes can happen during DNA replication, they are extraordinarily rare. In the case of DNA synthesis via DNA polymerase III in *E.coli*, only one mistake per 100 million nucleotides is made. Therefore, DNA synthesis occurs with a high degree of accuracy or **fidelity.** The replication involves several mechanisms to serve the fidelity.First, the hydrogen bonding between G and C or A and T is much more stable than between mismatched pairs. However, this stability accounts for only part of the fidelity, because mismatching due to stability considerations accounts for 1 mistake per 1000 nucleotides. Second, the active site of DNA polymerase preferentially catalyzes the attachment of nucleotides when the correct bases are located in opposite strands. Helix distortions caused by mispairing usually prevent an incorrect nucleotide from properly occupying the active site of DNA polymerase. By comparison, the correct nucleotide occupies the active site with precision and undergoes induced fit, which is necessary for catalysis. The inability of incorrect nucleotides to undergo induced fit decreases the error rate to a range of 1 in 100,000 to 1 million. A third way that DNA polymerase decreases the error rate is by the enzymatic removal of mismatched nucleotides. DNA polymerase can identify a mismatched nucleotide and remove it from the daughter strand. This occurs by exonuclease cleavage of the bonds between adjacent nucleotides at the ′3end of the newly made strand. The ability to remove mismatched bases by this mechanism is called the **proofreading function** of DNA polymerase. Proofreading occurs by the removal of nucleotides in the 3 to 5′ direction at the 3′ exonuclease site. After the mismatched nucleotide is removed, DNA Polymerase resumes DNA synthesis in the 5′ to 3′ direction (Figure 12).

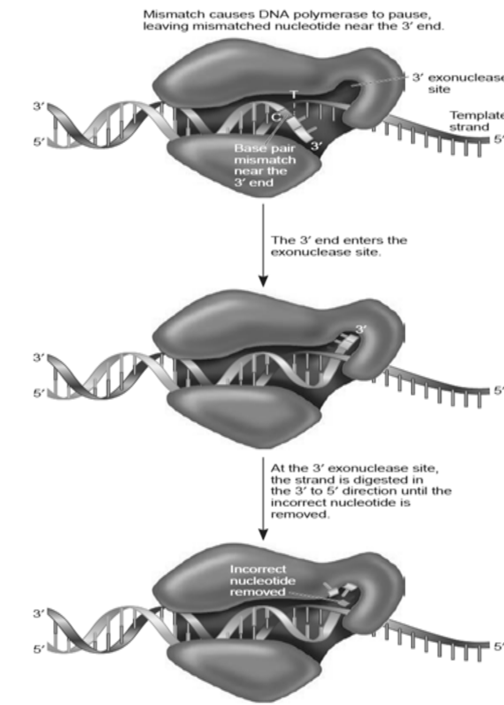


Figure 12. Proof reading function of DNA polymerase

Extrachromosomal Replication

Extrachromosomal DNA is any DNA that is found outside the nucleus of a cell. It is also referred to as extranuclear DNA or cytoplasmic DNA. Most DNA in an individual genome is found in chromosomes but DNA found outside the nucleus also serves important biological functions. In prokaryotes, extrachromosomal DNA is primarily found in plasmids whereas in eukaryotes extrachromosomal DNA is primarily found in organelles. Mitochondrial DNA is a main source of this extrachromosomal DNA in eukaryotes. Extrachromosomal DNA is often used in research of replication because it is easy to identify and isolate. In addition to DNA found outside the nucleus in cells, infection of viral genomes also provides an example of extrachromosomal DNA. Several models and mechanisms are associated with the replication of extrachromosomal DNA. Some of the replication mechanisms found in plasmid, mitochondria and virus are given below.

Replication of Plasmid DNA

In general, bacterial plasmids replicate independently of the host chromosome, although usually they rely on some host-encoded factors for their replication. They are present in bacterial cells replicating at a specific number of copies per cell, which can range from one or two to several hundreds. Several host- and plasmid-encoded functions are required for

plasmid replication. Initiation of plasmid replication is molecule specific and of great importance for the propagation process, copy number, and incompatibility properties of plasmids in both Gram-positive and -negative bacteria. In general, plasmid replicons contain one or several origins (*ori*) of replication and one or more regulatory elements, located in a DNA fragment no larger than 4 kb. In addition, most plasmid replicons harbor a gene encoding either a protein or an RNA molecule that functions as a primer for DNA replication. The Rep proteins can often act in *trans* on a specific *ori*, but in some cases they may only function in *cis*. However, in all cases examined so far the preprimer RNA acts in *cis* with the replication initiation sequences. Composite multi-replicon plasmids were also described. One example is R6K, in which three origins are able to function *in vivo* independently, although the rate of initiation from each origin is different. It was reported that many plasmid origins follow a molecular mechanism similar to *ori*C, the origin of replication of the *E. coli* chromosome. However, the major difference is that plasmids require an origin-specific plasmid-encoded protein for the initiation step, generally called Rep proteins. These plasmid-encoded Rep proteins act in place of or in combination with DnaA, the replication initiation protein for chromosomal DNA. Some plasmids require additional host-gene products such as *dam* methylases, integration host factor (IHF), and heat shock proteins to replicate. Other plasmids, such as the ColE1-type encode an RNA-specific plasmid molecule and require the host-encoded DNA polymerase I (PolI or PolA), RNA polymerase, and ribonuclease H (RNase H). Two types of mechanisms basically control the replication of plasmid DNA. One utilizes a series of repeated sequences, designated iterons, located at *ori* and capable of interacting with the replicator protein. In the other, small complementary RNA molecules (antisense) hybridize with the transcript responsible for the initiation process, either directly or indirectly by encoding the Rep protein.

There are three general replication mechanisms for circular plasmids, namely, theta type, strand displacement and rolling circle.

Theta type:

Replication by the theta-type mechanism has been most extensively studied among the prototype circular plasmids of gram-negative bacteria. Replication through the theta mechanism involves melting of the parental strands, synthesis of a primer RNA (pRNA), and initiation of DNA synthesis by covalent extension of the pRNA. DNA synthesis is continuous on one of the strands (leading strand) and discontinuous on the other (lagging strand),

although synthesis of the two strands seems to be coupled. Theta-type DNA synthesis can start from one or from several origins, and replication can be either uni- or bidirectional (Figure 13).Under electron microscopy (EM), the replication intermediates are seen as typical θ ("theta")-shaped molecules that, when digested with enzymes that cleave within the replicated region, yield Y-shaped molecules ("forks"). With few exceptions, plasmids using the theta mechanism of replication require a plasmid-encoded Rep initiator protein. Some replicons may require the host DNA polymerase I (DNA Pol I) during the early stages of leading-strand synthesis. With some exceptions, initiation of plasmid DNA replication requires a specific plasmid-encoded Rep initiator protein. This is reflected by the presence, at the origin of replication, of specific sequences with which the Rep protein interacts. Additional features found in many origins of theta-replicating plasmids are (i) an adjacent AT-rich region containing sequence repeats, where opening of the strands and assembly of host initiation factors occur, and (ii) one or more sites (*dnaA* boxes) where the host DnaA initiator protein binds. Multiple Dam methylation sequences, which are present in the origin of replication of the *Escherichia coli* chromosome, *oriC*, can also be found at the origin of replication ofplasmids.

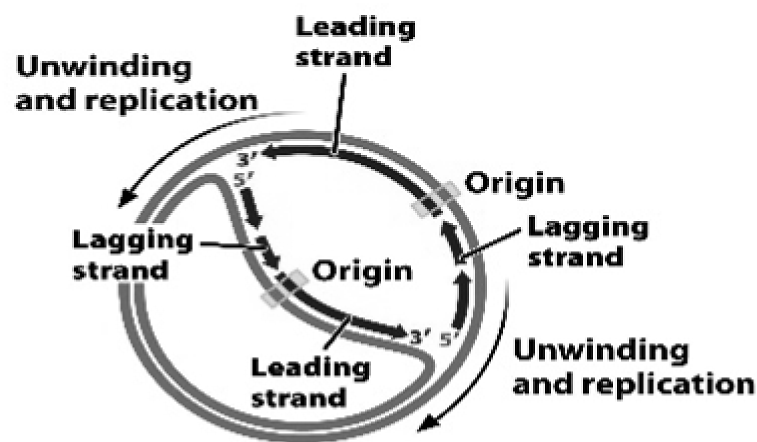


Figure 13: Theta model of replication

Strand displacement replication: Replication of RSF1010 DNA plasmid follow this mechanism and it is independent of the host-encoded DnaA, DnaB, DnaC, and DnaG proteins, whose roles are played by the combined action of the plasmid-encoded RepA, RepB, and RepC proteins. DNA Pol III-HE and SSB are required for replication. The first stage of this process involves the binding of the RepC protein to the origin also called iterons. It is assumed that the RepA helicase binds to both DNA strands in the AT-rich region, close to the site of interaction of RepC. Subsequent translocation in the 5′→3′ direction of the

RepA helicase bound to the L strand melts the duplex, exposing and activating the *ssi* sites which is palindromic inverted repeat in the origin. Alternatively, the interaction of RepC with the iterons could induce the opening of the duplex near the *ssi* sites. The exposure of the stem-loop structure in the *ssi* sites is probably required for the assembly of the RepB-primase to initiate replication. Initiation at either *ssi* site can occur independently, and replication proceeds continuously, with the RepA helicase facilitating displacement of the nonreplicated parental strand as a D loop. Continuous replication from each *ssi* signal in opposite directions would originate a double stranded DNA theta-shaped structure in the overlapping region and two D loops beyond this region. The helicase activity of the RepA protein is required during the elongation. The end products of the strand displacement replication mechanism are single strand-displaced circles and double-stranded supercoiled circles. The ssDNA molecules are used to initiate synthesis of the complementary strand, which converts the ssDNA templates into double stranded supercoiled circles. Therefore, double-stranded DNA (dsDNA) molecules, displaced single-stranded circular molecules, and partial double-stranded circles can be formed in this mode of replication (Figure 14).

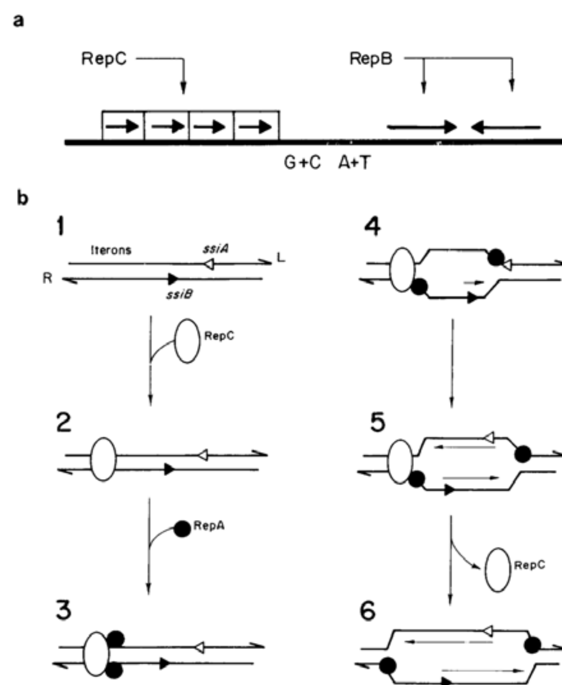


Figure 14: Strand displacement replication

Rolling circle (RC) replication: Replication by the RC mechanism has to be unidirectional, and it is considered to be an asymmetric process because synthesis of the leading strand and synthesis of the lagging strand are uncoupled. One of the most relevant features of RC replication is that the newly synthesized leading plus strand remains covalently bound to the

same parental plus strand. Replication is initiated by the plasmid-encoded Rep protein, which introduces a site-specific nick on the plus strand, at a region termed doublestranded origin (*dso*). The nick leaves a 3′-OH end that is used as a primer for leading-strand synthesis, which most probably involves host replication proteins (at least DNA Pol III, SSB, and a helicase). Elongation from the 3′-OH end, accompanied by the displacement of the parental plus strand, continues until the replisome reaches the reconstituted *dso*, where a DNA strand transfer reaction(s) takes place to terminate leading strand replication (see below). Thus, the end products of leading-strand replication are a dsDNA molecule constituted by the parental minus strand and the newly synthesized plus strand, and a ssDNA intermediate which corresponds to the parental plus strand. Unlike replication by the strand displacement mechanism, the ssDNA intermediates generated by the RC replication mode correspond to only one of the plasmid DNA strands. Finally, the parental plus strand is converted into dsDNA forms by host proteins initiating at the single-strand origin (*sso*), which is physically distant from the *dso*. The last step would be the supercoiling of the replication products by the host DNA gyrase (Figure 15).

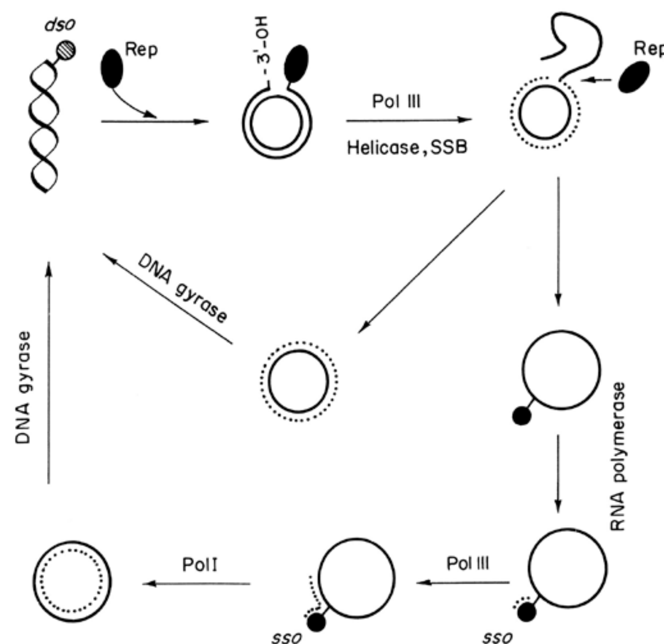


Figure 15: Rolling circle replication

Mitochondrial DNA (mtDNA) replication

Replication of mtDNA may be stochastic because there is no control over which particular

copies are replicated, so that in any cycle some mtDNA molecules may replicate more times than others. The mtDNA replication undergoes D-loop formation. Replication starts at a specific origin in the circular duplex DNA. Initially, though, only one of the two parental strands (the H strand in mammalian mitochondrial DNA) is used as a template for synthesis of a new strand. Synthesis proceeds for only a short distance, displacing the original partner (L) strand, which remains single-stranded. The condition of this region gives rise to its name as the displacement loop, or D loop (Figure 16).

Replication at the H –strand origin is initiated when RNA polymerase transcribes a primer. The 3′ ends are generated in the primer by an endonuclease that cleaves the DNA-RNA hybrid at several discrete sites. The endonuclease is specific for the triple structure of DNA-RNA hybrid plus the displaced DNA single strand. The 3' end is then extended into DNA by the DNA polymerase. A single D loop is found as an opening of 500 to 600 bases in mammalian mitochondria. The short strand that maintains the D loop is unstable and turns over; it is frequently degraded and resynthesized to maintain the opening of the duplex at this site. Some mitochondrial DNAs possess several D loops, reflecting the use of multiple origins. The same mechanism is employed in chloroplast DNA, where (in complex plants) there are two D loops.

To replicate mammalian mitochondrial DNA, the short strand in the D loop is extended.

The displaced region of the original L strand becomes longer, expanding the D loop. This expansion continues until it reaches a point about two-thirds of the way around the circle.

Replication of this region exposes an origin in the displaced L strand. Synthesis of an H strand initiates at this site, which is used by a special primase that synthesizes a short RNA. The RNA is then extended by DNA polymerase, proceeding around the displaced single-stranded L template in the opposite direction from L-strand synthesis.

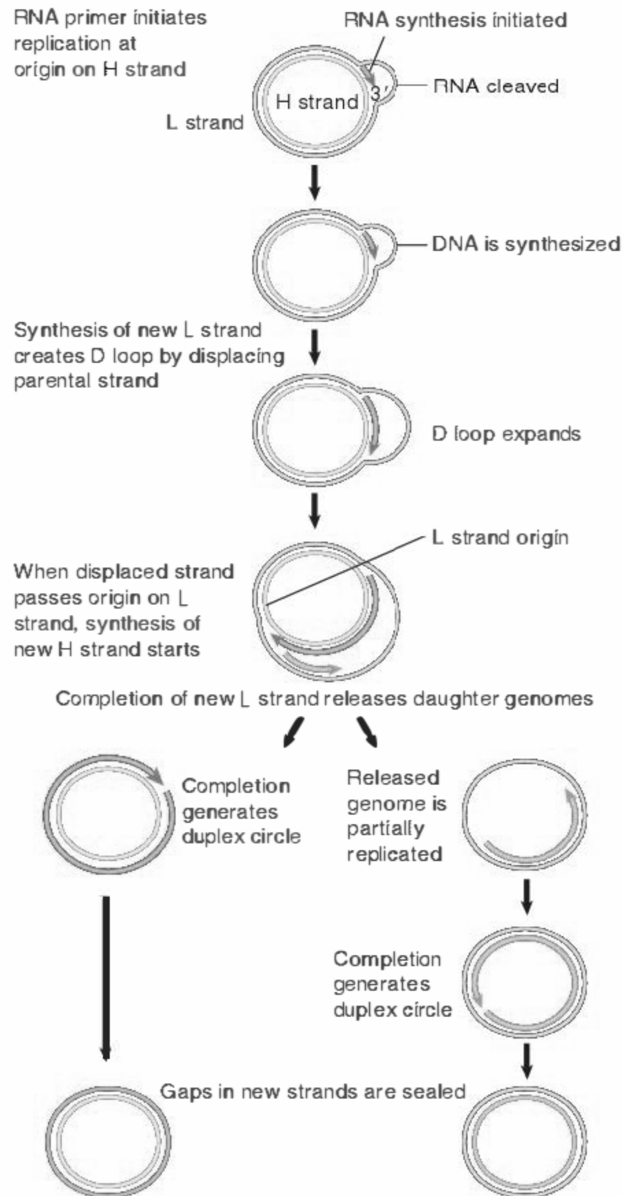


Figure 16: Mitochondrial DNA replication

Virus DNA replication

Replication by rolling circles is common among bacteriophages. Unit genomes can be cleaved from the displaced tail, generating monomers that can be packaged into phage particles or used for further replication cycles. Phage ΦX 174 consists of a single-stranded circular DNA known as the plus (+) strand. A complementary strand, called the minus (-) strand, is synthesized. The duplex circle is converted to a covalently closed form, which becomes supercoiled (Figure 17).

A protein coded by the phage genome, the A protein, nicks the (+) strand of the duplex DNA at a specific site that defines the origin for replication. After nicking the origin, the A protein

remains connected to the 5 ' end that it generates, while the 3 ' end is extended by DNA polymerase (Figure 17).

The nick generates a 3'-OH end and a 5'-phosphate end (covalently attached to the A protein), both of which have roles to play in ΦX 174 replication. Using the rolling circle, the 3' -OH end of the nick is extended into a new chain. The chain is elongated around the circular (-) strand template until it reaches the starting point and displaces the origin. Now the A protein functions again. It remains connected with the rolling circle as well as to the 5' end of the displaced tail, and is therefore in the vicinity as the growing point returns past the origin. Thus, the same A protein is available again to recognize the origin and nick it, now attaching to the end generated by the new nick. Following this nicking event, the displaced single (+) strand is freed as a circle. The A protein is involved in the circularization. In fact, the joining of the 3' and 5 ' ends of the (+) strand product is accomplished by the A protein as part of the reaction by which it is released at the end of one cycle of replication, and starts another cycle.

The displaced (+) strand may follow either of two fates after circularization. First, during the replication phase of viral infection, it may be used as a template to synthesize the complementary (-) strand. The duplex circle may then be used as a rolling circle to generate more progeny. Second, during phage morphogenesis, the displaced (+) strand is packaged into the phage virion.

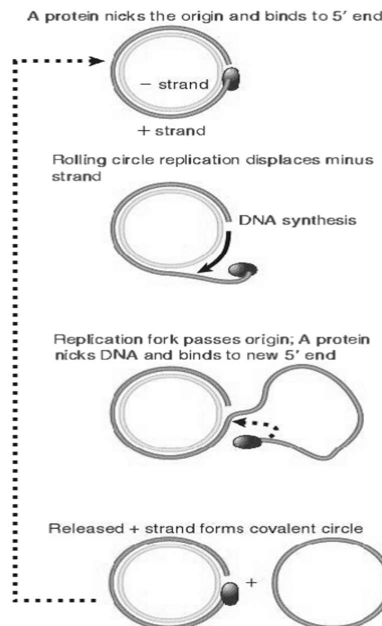


Figure 17. Bacteriophage ΦX174 replication

Probable questions:

1. What is the nature of DNA Replication. Explain with examples.

2. Write short notes on a) Replication Fork, b) Okazaki fragments c) Leading strand and Lagging strand

3. How initiation of replication occurs.

4. Discuss enzymology of DNA replication.

5. How termination of replication occurs?

6. Discuss fidelity of Replication.

7. What is Theta replication?

8. Discuss mitochondrial DNA replication.

9. What is rolling circle mode of DNA replication.

10. Discuss viral DNA replication.

Suggested Readings:

1. Lodish, H. (2016). Molecular cell biology. New York, NY: Freeman.

2. Alberts, B. (2008). Molecular biology of the cell. New York, NY [u.a.]: Garland Science Taylor & Francis.

3. Lewin, B., Krebs, J., Goldstein, E. and Kilpatrick, S. (2014). Lewin's genes XI. Burlington, MA: Jones & Bartlett Learning.

4. Karp, G. and Patton, J. (2015). Cell and molecular biology. Brantford, Ont.: W. Ross MacDonald School Resource Services Library.

5. Cooper, G. and Hausman, R. (n.d.). The cell

Disclaimer:

The study materials of this book have been collected from various books, e-books, journals and other e-sources.

POST GRADUATE DEGREE PROGRAMME (CBCS)

IN

ZOOLOGY

(M. Sc. Programme)

SEMESTER-I

ANIMAL PHYSIOLOGY AND BIOCHEMISTRY AND METABOLIC PROCESSES

ZHT-104

Self-Learning Material

DIRECTORATE OF OPEN AND DISTANCE LEARNING

UNIVERSITY OF KALYANI

Kalyani, Nadia
West Bengal, India

COURSE PREPARATION TEAM:

Faculty members of the Department of Zoology,
University of Kalyani and Assistant Professors of Zoology, DODL,
University of Kalyani.

December, 2018

Directorate of Open and Distance Learning, University of Kalyani

Published by the Directorate of Open and Distance Learning,
University of Kalyani, Kalyani-741235, West Bengal and Printed by
East India Photo Composing Centre, 69, Sisir Bhaduri Sarani, Kolkata-700 006

All right reserved. No. part of this work should be reproduced in any form without the permission in writing from the Directorate of Open and Distance Learning, University of Kalyani.

Authors are responsible for the academic contents of the course as far as copyright laws are concerned.

Foreword

Open and Distance Learning (ODL) systems play a threefold role- satisfying distance learners' needs of varying kinds and magnitudes, overcoming the hurdle of distance and reaching the unreached. Nevertheless, this robustness places challenges in front of the ODL systems managers, curriculum designers, Self Learning Materials (SLMs) writers, editors, production professionals and other personnel involved in them. A dedicated team of the University of Kalyani under the leadership of Hon'ble Vice-Chancellor has put its best efforts, professionally and in unison to promote Post Graduate Programmes in distance modeoffered by the University of Kalyani. Developing quality printed SLMs for students under DODL within a limited time to cater to the academic requirements of the Course as per standards set by Distance Education Bureau of the University Grants Commission, New Delhi, India underOpen and Distance Mode UGC Regulations, 2017 had been our endeavour and we are happy to have achieved our goal.

Utmost care has been taken to develop the SLMs useful to the learners and to avoid errors as far as possible. Further suggestions from the learners' end would be gracefully admitted and to be appreciated.

During the academic productions of the SLMs, the team continuously received positive stimulations and feedback from Professor (Dr.) Sankar Kumar Ghosh, Hon'ble Vice- Chancellor, University of Kalyani, who kindly accorded directions, encouragements and suggestions, offered constructive criticism to develop it within proper requirements. We gracefully, acknowledge his inspiration and guidance.

Due sincere thanks are being expressed to all the Members of PGBOS (DODL), University of Kalyani, Course Writers- who are serving subject experts serving at UniversityPost Graduate departments and also to the authors and academicians whose academic contributions have been utilized to develop these SLMs. We humbly acknowledge their valuable academic contributions. I would like to convey thanks to all other University dignitaries and personnel who have been involved either at a conceptual level or at the operational level of the DODL of University of Kalyani.

Their concerted efforts have culminated in the compilation of comprehensive, learner-friendly, flexible texts that meet the curriculum requirements of the Post Graduate Programme through Distance Mode.

Self Learning Materials (SLMs) have been published by the Directorate of Open and Distance Learning, University of Kalyani, Kalyani-741235, West Bengal and all the copyright reserved for University of Kalyani. No part of this work should be reproduced in any from without permission in writing from the appropriate authority of the University of Kalyani.

All the Self Learning Materials are self writing and collected from e-book, journals and websites.

Director
Directorate of Open & Distance Learning
University of Kalyani

HARD CORE THEORY PAPER

(ZHT–104)

ANIMAL PHYSIOLOGY AND BIOCHEMISTRY AND METABOLIC PROCESSES

Group A : Animal Physiology

Module	Unit	Content	Credit	Class	Time (h)	Page No.
ZHT–104 (Animal Physiology, Biochemistry and Metabolic Processes)	**I**	Respiratory function of blood: a) Respiratory pigments—distribution and brief chemistry b) Function of hemoglobin— i) in adult and ii) during embryonic life c) Environmental influences.	**1.5**	**1**	**1**	**7-18**
	II	Physiology of muscles: a) Chemical nature of contractile elements b) Role of structural and regulatory proteins in muscular contraction c) ATP and signal molecules in muscular contraction.		**1**	**1**	**19-31**
	III	Physiology of excretion: Formation of urine: glomerular filtration; tubular function; counter current mechanism and urine formation.		1	1	**32-41**
	IV	Synaptic and functional transmission; a) Pre-and postsynaptic structure and function b) Chemical transmission of synaptic activity		1	1	**42-50**

Group A : Animal Physiology

Unit I

Respiratory function of blood:

a) Respiratory pigments - distribution and brief chemistry

b) Function of hemoglobin-i) in adult and ii) during embryonic life

c) Environmental influences.

Objectives :

1. To have a precise knowledge about different types of respiratory pigments present in different animals and to know their functions.
2. To study the structure and function of hemoglobin in detail which is the only respiratory pigment in human beings.
3. To understand the physiological mechanism of gaseous transport in human body through hemoglobin.

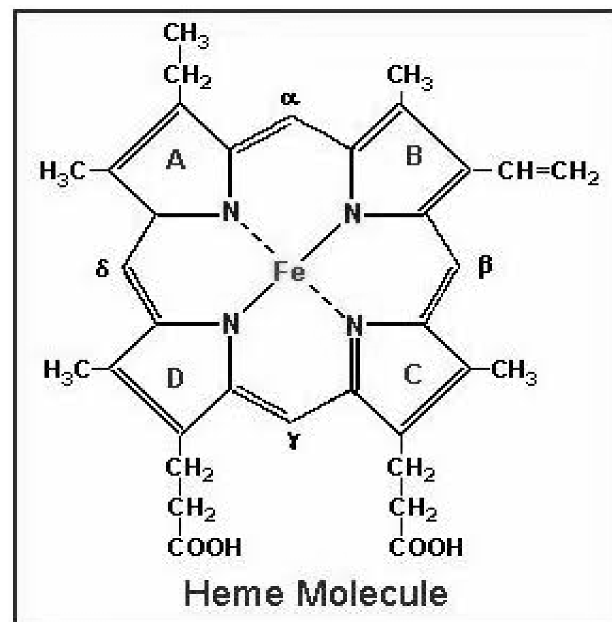
Introduction :

A respiratory pigment is a kind of substance which increases the oxygen carrying capacity of blood in human (hemoglobin) and in other vertebrates. These are metal containing proteins, combine reversibly with oxygen. The four common respiratory pigments are –1) Hemocyanin, 2) Hemoglobin 3) Chlorocruorin and 4) Haemerythrin.

1. **Hemocyanin-**It is the primary respiratory pigment or oxygen transporter throughout the body of some invertebrate animals. (Abbreviation-Hc).

Molecular weight-ranges from 17,000-30,00,000KDa.

Chemistry and Structure-Hb made up of globulin chains (connected together).In normal adult Hb (HbA) two α $(141\ amino\ acid\ residues)$ and two β(146 amino acid residues) globulin chains are present. In fetus, the beta chains are normaly absent, instead of two beta chains, two γ chains are present. Each globulin chains contain an important iron containing porphyrin compound-known as Heme. Heme group contains an iron (Fe) ion (coordinate with 4 nitrogen atoms) in a heterocyclic ring. The porphyrin ring consists of four pyrrole molecules joined by methane bridge.



Heme Molecule

Oxyhemoglobin-is a form when oxygen binds with heme group.

Oxygenetion of hemoglobin-deoxygenetivehemoglobin is purplish, but bright red in oxygenative state.

Distribution-all vertebrates possesHb except Lepidocephalous larva.

Mollusca-purpura, chiton, etc.

Annelida-*Lumbricus, Arenicota, Tubifex.*

Echinodermata-Sea cucumber.

Arthropods-insects (Chironmas and Gastrophilus).

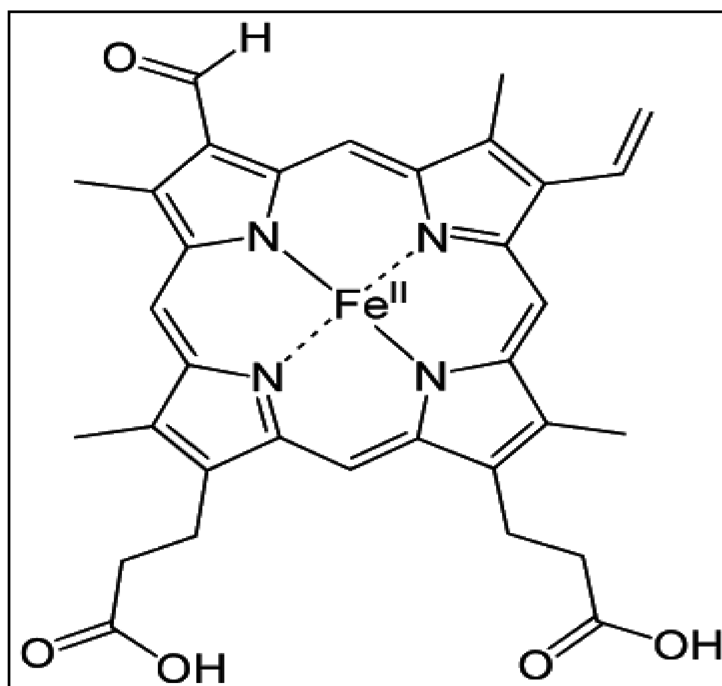
Nematoda-*Ascaris sp.*

In addition, some protozoa, yeast and root nodules of leguminous plants also contain Hb.

1) **Chlorocruorin-**It is an hemeprotein , similar to erythrocruorin (found in annelids and arthropods).

Molecular weight-20075000 Da

Chemistry and structure-It consists of many myoglobinlikesubunits, arranged in a huge complex of over a hundred subunits. It contains abnormal heme group.



Oxygenation-Oxygenated chlorocruorin turns from green to red.

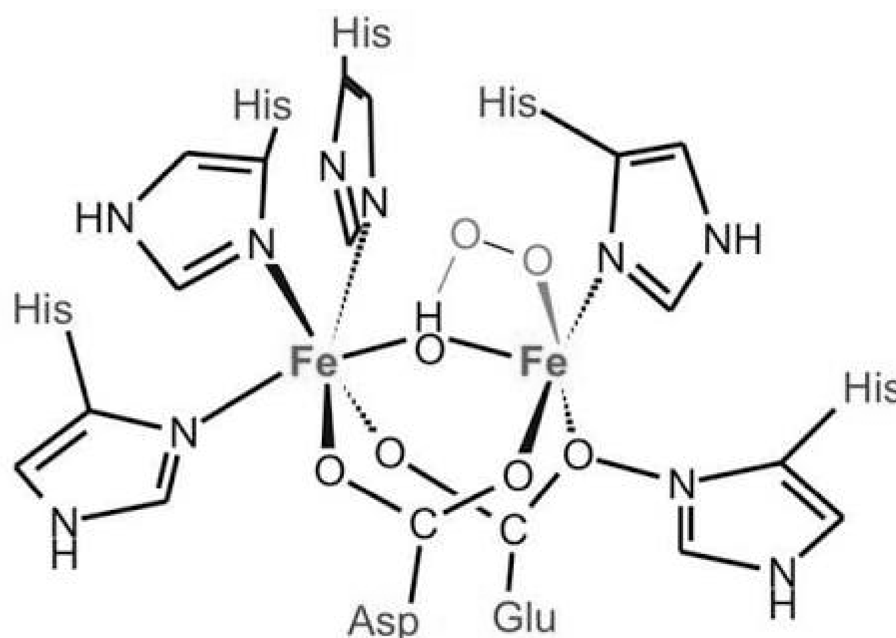
Distribution-restricted in 4 families of polychaetes- Sabellidae (all sp.), Surpulidae, Chloronaemidae.

4)Hemerythrin-The pigment is mainly responsible for oxygen transport in the marine invertebrate phyla.

Molecular weight-1,08,000KDa.

Chemistry and structure-The pigment is a homooctamer or heterooctamer in structure, composed of alpha and beta type subunits .Each subunit has four alpha helix fold binding

a binuclear iron centre. It lacks cooperative binding of to oxygen, roughly ¼ 0f hemoglobin.



Oxygenation-oxygenated haemerythrin is violet to pink coloured. But colourless when deoxygenated.

Distribution-Sypunculids-all sp.Polychaetes-Megelona, Brachiopods-Lingnea.

HEMOGLOBIN

1. Hemoglobin, the O_2 carrying pigment found within the cytoplasm of RBC.

2. In RBM,Hb begins to appear from the stage of intermediate or early normoblast stage, leads to new Hb synthesis.

3. Mature RBC cannot synthesize new Hb.

Normal values-In normal adult male it is about 15gm/100ml, and in female 11-13gm/100ml. Over the age of 75 yrs. Hb concentration in males about 12.5 gm%.

4. **Shape-**spherical.

5. **Molecular weight**-64,500.

Structure-

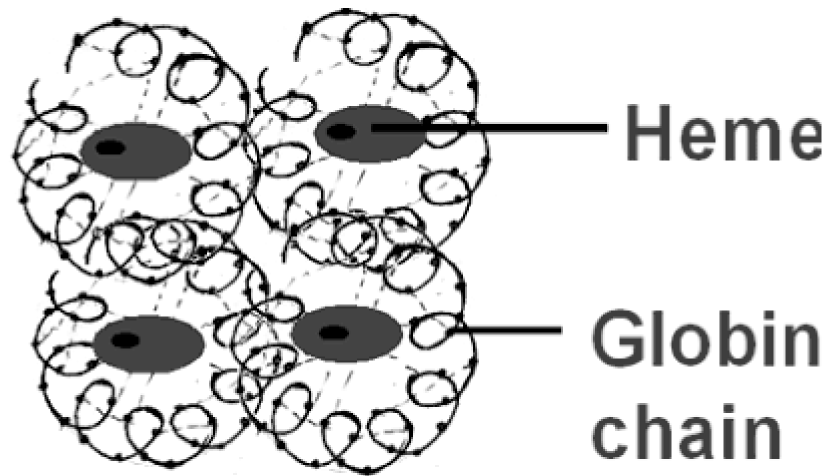
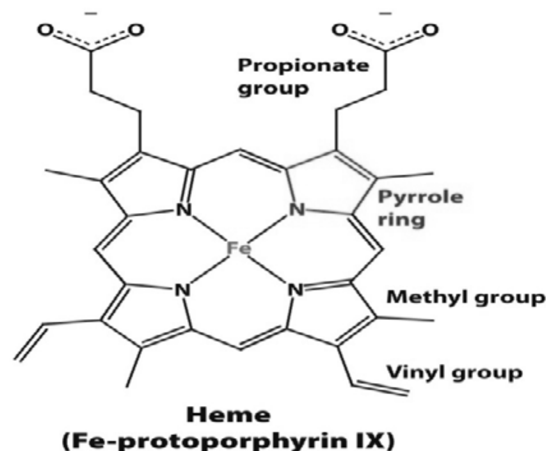


Figure : Structure of hemoglobin.

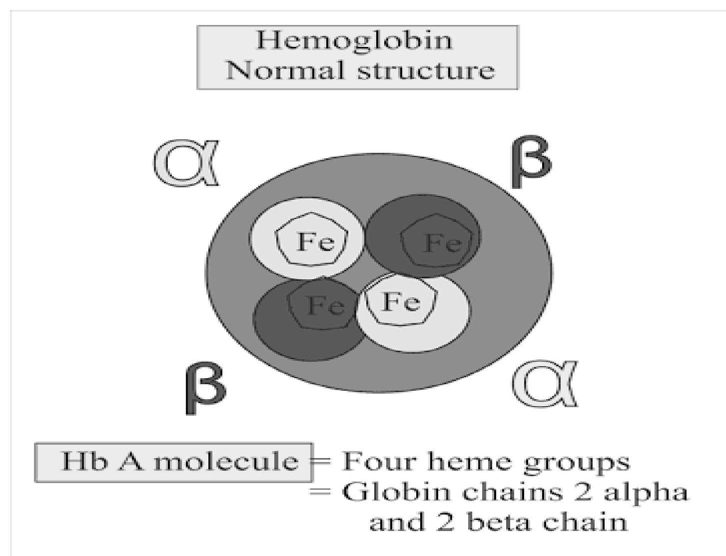
6. Each hemoglobin molecule contains two pairs of polypeptide chains andtwo each polypeptide chain, a coloured prosthetic group, called heme, is attached.In each heme group there are 4 pyrole rings, linked together by methine group, known as porphyrine. The particular type of porphyrin found in Hb is called iron photoporphyrin. Each hemepart, there is one iron (held in a porphyrin ring) and with each ion one molecule of oxygen can bind.



7. The polypeptide chains are two α chains (having 141 amino acids each chain) and two β chains (having 146 amino acids in each chains). The 4 polypeptide chains are together called globin. So Hemoglobin has two parts–heme (4%) and globin (96%).

8. Physiological varieties of hemoglobin-

Adult haemoglobin (HbA)-In normal adults, 98% Hb is HbA, α_2 β_2, i.e.two alpha and two beta chains. About 2% of adult Hb is HbA_2 (α_2 and δ_2 chains). There are two δ chains instead of two β chains. There are some differences between the two chains, but the δ chains also have 146 amino acid residues.



Foetal hemoglobin (HbF)-Embryo/foetus has distinct Hb, embryo synthesizes ζ chains which are α like chains and ε chains which are βlike chains. In developmental stage ζ chains are replaced by α chains and the ε chains are replaced by γ chains. HbF has subunit components $\alpha_2\,\gamma_2$.

Difference between Adult and Fetal Hemoglobin

HbA	HbF
1. Composed of two α and two β chains.	2. Composed of two α and two γ chains.
3. Life span is a about 120 days.	4. Life span is less than 80 days.
5. Affinity for oxygen less than HbF.	6. Greater affinity for oxygen binding.

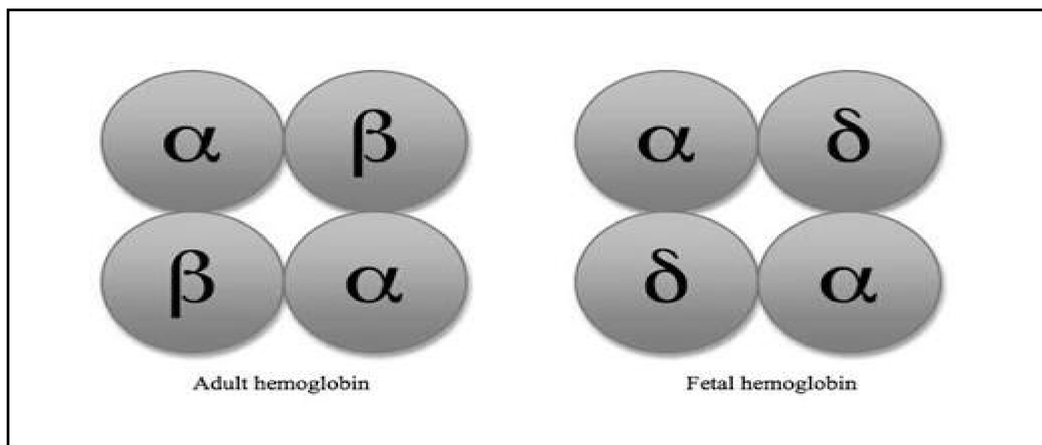


Figure : difference between HbA and HbF.

Functions of Hb-1) carriage of oxygen (co factor helps is Fe). Oxygenated Hb abbreviated as HbO2.

2) CO_2 transport.

3) act as blood buffer.

ROLE OF Hb IN OXYGEN TRANSPORT

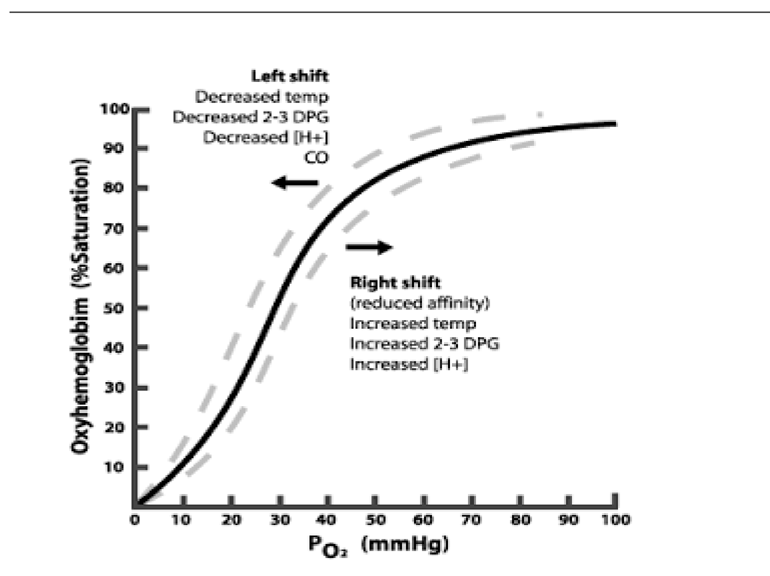
Normally 97% of O_2 transported from lungs to tissues is carriedin chemical combination with Hb in the RBC. 3% is transported in the dissolved state in water of plasma and blood cells.

Oxygen molecule can bind loosely or reversibly with the prosthetic group of Hb. When PO2 is high oxygen binds withHb, but when PO2 is low, in tissue capillaries oxygen is released from the Hb. This is the basis of oxygen transport from lungs to tissues.

Oxygen Hemoglobin dissociation curve-

The curve demonstrate a progressive increase in percentage of hemoglobin bound with oxygen as blood PO2 increases, which is called percent saturation of hemoglobin. Because the blood leaving the lungs and entering systemic arteries usually has a PO2 about 95 mmHg. Conversely in normal venous blood returning from the peripheral tissues the PO2 is about 40 mmHg and the saturation of Hb averages 75%. [each gm of Hb can bind with a maximum 1.34 milliliters of oxygen]. Under normal condition, about 5 milliliters of oxygen are transported by each 100ml of blood flow.

Factors that shift Oxygen Hemoglobin curve-



The figure shows that when blood becomes slightly acidic (decreasing PH 7.4-7.2) the normal curve is about 15 percent to the right. When the pH increases(7.4-7.6), the curve is similar to the left.

Bohr effect-A right shift of the curve in response to the increase in CO2 and H^+ ions have a significant effect by enhancing the release of O2 from blood in the tissues and enhancing oxygenation of blood in lungs, called Bohr effect.

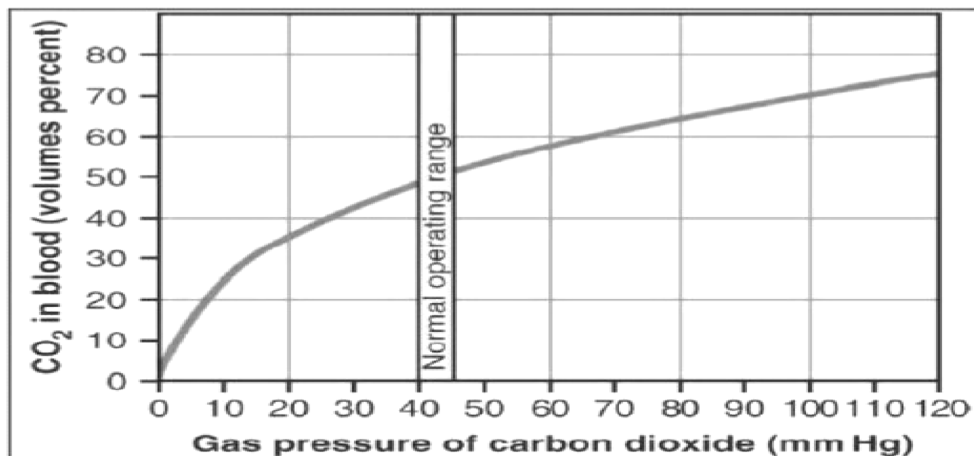
Normal 2,3DPG in the blood keeps the O2-Hb dissociation curve shifted slightly to the right all the time. In hypoxic condition that last longer than a few hours, the quantity of DPG in the blood increases considerably thus shifting the dissociation curve even farther to the right.

Increasing in blood temperature (2° to 3° C) increases the oxygen delivery, shifted the curve rightward.

ROLE OF Hb IN CO_2 TRANSPORT-

CO_2 reacts directly with the amine radical of Hb molecule to form carbaminohemoglobin (CO_2Hgb) This combination is a reversible reaction that occurs within a loose bond, so CO_2 easily released into the alveoli, where Pco_2 lower than in the pulmonary capillaries. About 23% of carbon dioxide transported with the help of Hb.

CO_2 Dissociationcurve-



Binding of oxygen with Hb tends to displace carbon dioxide from blood, this is called Haldane effect. There to ways to displace carbon dioxide-

The more highly acidic hemoglobin has less tendency to combine with CO_2 to form carbaminohemoglobin, thus displacing much of the CO_2.

The increased acidity of the hemoglobin also causes it to release an excess of H^+ ions and these bind with bicarbonate ions to form carbonic acid, this then dissociates into water and carbon dioxide. Carbon dioxide is released from blood into alveoli and finally into air.

ENVIRONMENTAL INFLUENCES

Availability of oxygen-

Insufficient oxygen in atmosphere (in high altitude and polluted area), causes inadequate oxygenation of blood in the lungs, leads to **Hypoxia**. In this condition the oxygen binding capacity of hemoglobin is influenced by the partial pressure of oxygen in the environment. The partial pressure of oxygen falls to 60 mmHg or below. causes problems with blood flow in the tissues, leads to breathing problem.

The respiratory function of hemocyanin containing blood of Libiniaemarginataand Ocypodeguadratawas studied, exposed to hypoxic condition. During progressive hypoxia convection initially increases on both sides of the gill in *L.emarginata* , while in *O.quadrata* cardiac output decreases. Blood pH increases with decreasing ambient Po_2 below 60 torr in *L. emarginata* inducing a greater hemocyanin O_2 affinity.

CO Poisoning-

CO binds with Hb hundred times tighter than oxygen and disrupts the oxygen carrying capacity of blood.

The ranges of CO –4-6ppm at a resting level.

In urban areas 7-13ppm and for smokers-20-40ppm. The level of 40ppm is equivalent to a reduction in hemoglobin levels of 10 g/L.

It removes the allosteric shift of the oxygen dissociation curve and shifting the foot of the curve to the left. So the Hb is less likely to release its oxygens at the tissues. So the poor supply of oxygen occurs.

Higher concentration of CO_2-

In polluted areas, there are excess carbon dioxide. Increase in carbon di oxide, increases alveolar Pco_2, rises about 60 to 75 mmHg. Due to greater binding affinity of carbon dioxaide with Hb leads to rapid and deep breathing, called air hunger leads to dyspnea.

If partial pressure of carbondioxaide increases 80-100 mmHg, the person becomes lethargic and even semi comatose. Above 120 mmHg rising partial pressure depresses the respiration and causes respiratory death.

Temperature-

Decreased temperature causes the oxygen hemoglobin dissociation curve shift to leftward, tends to lower oxygen supply in the tissues.

Exposure of slightly increasing temperature on hermit crab associated problem of dehydration. Changes in enthalpy (>39 kj/mol) changes the hemocyanin concentration, which affect the oxygen affinity.

Others-Some inflammatory agents (air born viruses, bacteria, moulds) infects the membrane of pulmonary and walls of alveoli, raptures the red blood cells to leak out of blood into the alveoli leads to infection spreads. This infection causes reduction in the area of respiratory membrane and decreased ventilation perfusion ratio. The both causes low blood oxygen and high concentration of carbon dioxide, which causes respiratory problems.

Probable Questions-

1. Enlist the different types of respiratory pigments found throughout the animal kingdom.
2. How is foetal hemoglobin different from that of adult hemoglobin?
3. Explain briefly how oxygen binds with hemoglobijn and transported to the tissues.
4. What is oxygen-hemoglobin dissociation curve? What will happen to the curve if the pH of blood is increased temperature is increased? Justify your answer.
5. What is Bohr's effect? How is the concentration of carbon dioxide related to this phenomenon?

Suggested Readings/References-

1. Animal physiology-Mohan P. Arora.
2. Textbook of medical physiology/Arthur C. Guyton, John E. Hall.
3. Ganong's review of medical physiology.

Unit II

Physiology of muscles :

a) Chemical nature of contractile elements

b) Role of structural and regulatory proteins in muscular contraction

c) ATP and signal molecules in muscular contraction.

Objectives :

1. To study the structural component of muscle fibre.
2. To understand the molecular mechanism of muscle contraction.
3. To understand the role of various proteins in muscle contraction.

Introduction :

Muscle is the contractile tissue of the body and derived from mesodermal layer of embryonic germ cells. There are 3 types of muscles : skeletal or voluntary muscle, smooth or involuntary and cardiac (involuntary) muscles.

Cardiac and skeletal muscles are striated, i.e., they contain sarcomeres and packed into regular arrangement of bundles, smooth muscle has neither.

Characteristics of Muscles

1. **Irritability :** The ability of muscle tissue to receive and respond to stimuli.
2. **Contractility :** The ability to shorten and thicken or contract when a sufficient stimulus is received.
3. **Conductibility :** Once a part of the muscle fibre is stimulated by a stimulus of adequate strength, it is conducted within no time to all its other parts.
4. **Extensibility :** The stretching ability of muscle.

5. **Elasticity :** The ability of muscle to return to its original shape after contraction or extension.

6. **Tonicity :** It can be defined as involuntary resistance to passive stretch. All the muscles in the body in a given time are never found in a perfect relaxed state. They are in a state of mild contraction which causes them to resist being stretched. This is the tonicity of muscles.

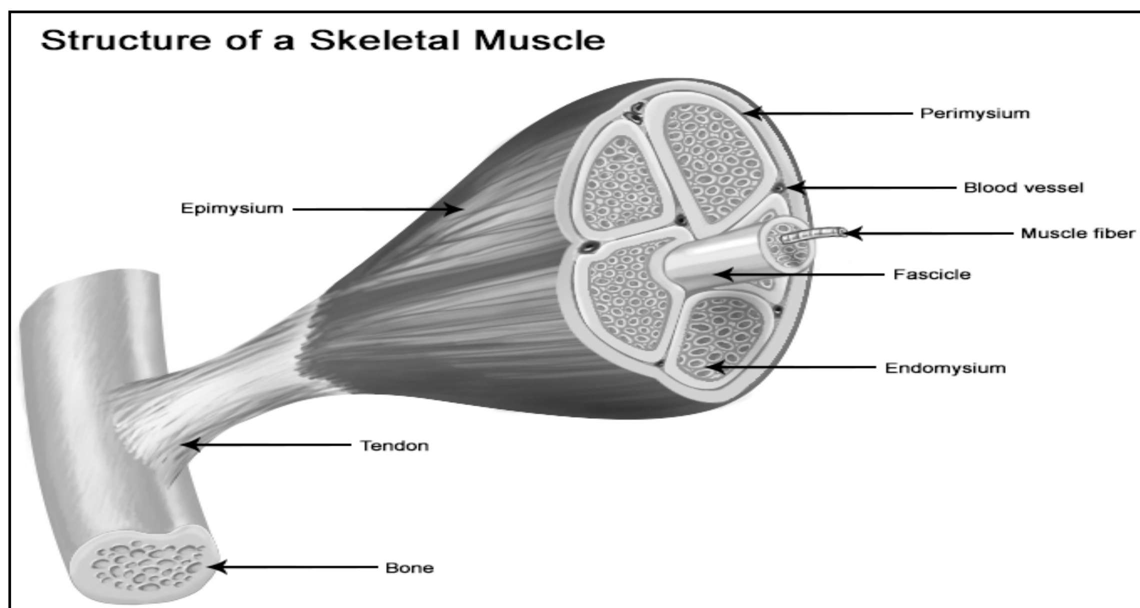
Anatomy and Histology of a Muscle

1. Each muscle is covered by a layer of connective tissue called epimysium. A single muscle is in turn made of several fascicles(bundles).

2. The individual fascicles are covered by a layer of connective tissue called perimysium. Each fascicle is again divided into several muscle fibre.

3. Each muscle fibre is covered by alayer of thin connective tissue called endomysium.

4. Each muscle fibre is composed of several myofibrils (myofilaments)



5. **Structure of myofibrils/myofilaments** :

Myofibrils are small contractile filaments located within the cytoplasm of striated muscle cells. They are actually bundles of actomyosin.

i) Each myofibril is covered by a membrane called sarcolemma.

ii) The sarcoplasm contains bundles of myofibrils, multiple nuclei, numerous mitochondria, Golgi Apparatus, myoglobin, sarcoplasmic reticulum, etc.

iii) Each myofibril consists of longitudinally repeated units called sarcomeres which are bounded by Z-lines.

iv) Myofibrils are long cylindrical structure of about 1μm in diameter and have characteristic dark (anisotropic) A-bands and light (isotropic) I-band.

v) Extending in both direction from Z-line are thin filaments made primarily of protein actin, coiled with nebulin filaments.

vi) Thin filaments interdigitate with thick filaments which consists primarily of protein myosin, held in place by titin filaments.

vii) Surrounding the Z-line is the region of I-band and following I-band is A-band. The portion of sarcomere between 2 A-bands is called the I-bands.

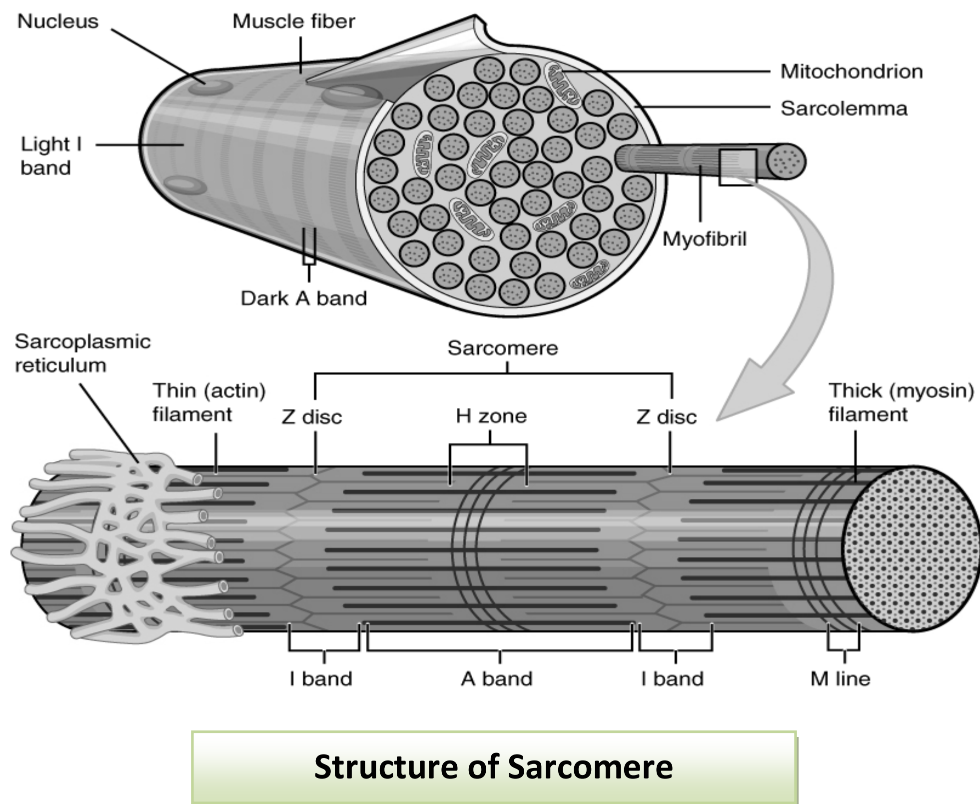
viii) A-band is made of both actin and myosin filaments and I-band contains only actin filaments.

ix) Within the A-band is a paler region called the H-band.

x) Finally, inside the H-band is a thin M-line.

xi) Close inspection with electron microscope shows small projections called "cross bridges" from the myosin filaments. These make contact with the actin filaments during contraction.

In the region of overlap, each myosin filament is surrounded by 6 action filaments and each actin filament is surrounded by 3 myosin filaments.



Structure of Sarcomere

CHEMICAL COMPOSITION OF MUSCLE :

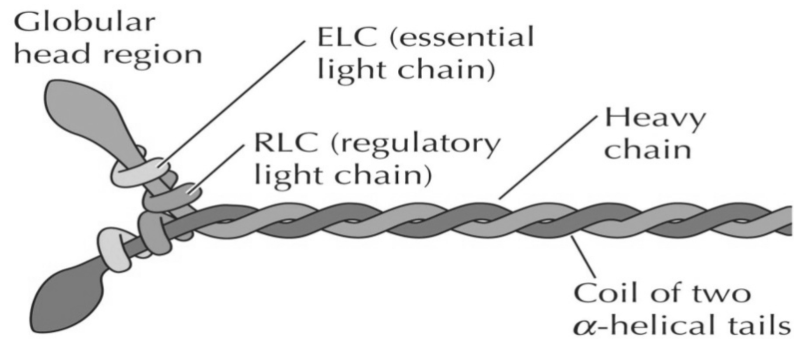
1. **WATER-**A muscle contains about 75-80% water which plays an important role in contraction. The amount of water in muscle is maintained by osmotic forces. It prevents muscle dehydration. Considerable amount of water has also been reported in the interspaces between the muscle fibres.

2. **PROTEINS-**The proteins constitute about 80% of the dry weight of muscle. The muscle proteins, actin and myosin, associated with the contractile mechanism comprise of 60% of this and the remaining two-fifth of the muscle of protein is shared equally by protein enzymes and by stroma proteins.

Composition and Structure Of Proteins In The Myofibril :

1. **THICK FILAMENT-MYOSIN:**Each thick filament is composed of several hundred myosin molecules packed together in specific arrangement.

1. It is a globular protein with molecular weight of 460 KDa.
2. Myosin has a characteristically shaped molecule-like a golf club with a short compact head and a long shaft. It is an asymmetrical hexamer composed of a head, neck and a tail domain.
3. It is made up of 2 heavy chains with molecular weight of 200 KDa and 4 light chains with molecular weight of 20 KDa.
4. The 2 heavy chains coil around each other to form a fibrous tail. One end of these chains are folded into a globular protein called myosin head. The four light chains are also the part of the head.
5. The head domain binds to actin and use ATP hydrolysis to change its conformation and helps in muscle sliding.
6. Myosin filament is made of 200 myosin molecules. The tails are bundled together to form body of filament, while heads hang outward. There is an arm made of portion of double helix. The protruding head and arm together form CROSS-BRIDGES.
7. ATPase activity of myosin head: The myosin head is essential for muscle contraction. It can function as an ATPase enzyme. This allows the head to break ATP and use the ATP to energize the contraction process.
8. When myosin is digested with trypsin, 2 myosin fragments (meromyosins) are generated. Light meromyosin consists of insoluble α-helical fibres from the tail. It has no ATPase activity. Another fraction is called heavy mero-myosin which is a soluble protein that has both a fibrous and globular portion. It exhibits ATPase activity and binds to F-actin. Digestion of heavy meromyosin with papain produces 2 sub-fragments, S-1 and S-2. The S-2 is fibrous with no ATPase activity. S-1 exhibits ATPase activity.



9. **THIN FILAMENT** :

Thin filament is actually composed of 3 components: actin, tropomyosin and troponin.

Actin (25% of myofibril proteins) :

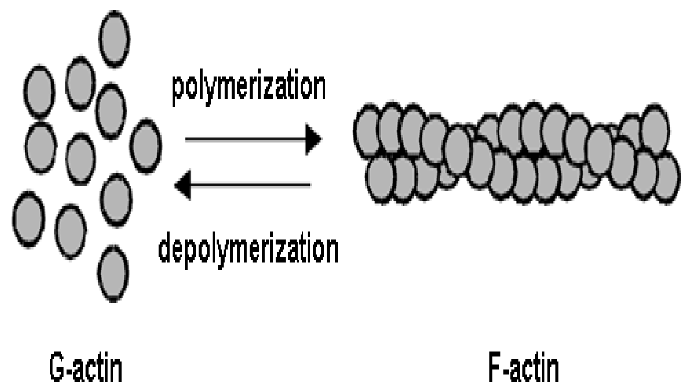
1. The backbone of actin filament is composed of double stranded F-actin protein molecules. The two strands are wound in a helix.

2. Each strand of F-actin is composed of polymerized G-actin.

3. G-actin forms the actual globular monomer with molecular weight of 43 KDa with 375 amino acids. In presence of Mg^{2+} and at physiological ionic strength, G-actin polymerises to form F-actin fibre (6-7 nm thick).

4. There are approximately 13 G-actin molecules in each turn of helix.

5. Attached to each G-actin molecule is one GDP molecule.



1. **Function**- a) Forms major structural component of microfilament.

   b) In association with myosin helps in muscle contraction.

c) There are atleast 6 isoforms of actin. For example, type of actin in striated muscle differs from that in heart muscle.

Some Actin-Binding Proteins

ADF/Cofilin

Members of this family are expressed in all eukaryotes, where they are generally involved in the recycling of actin monomers, mainly by filament depolymerization, during processes involving rapid cytoskeleton turnover, such as membrane ruffling and cytokinesis.

Profilin

Profilin (together with Tβ4) contributes to maintaining a large fraction (~50%) of the cellular actin in the unpolymerized pool. Profilin-actin constitutes the main source of actin monomers for polymerization.

Gelsolin

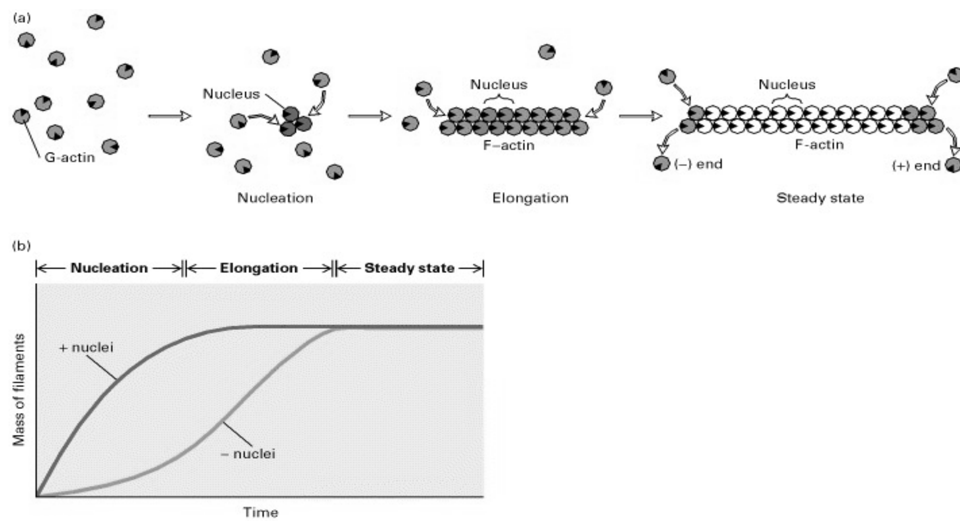
Gelsolin belongs to a family of actin-severing and actin-capping proteins, which includes adseverin, villin, capG, advillin, and supervillain.

Actin Polymerization

Addition of salts to a solution of G-actin induces polymerization, creating F-actin filaments.

The polymerization of actin filaments proceeds in three sequential phases.

The first phase is marked by a **lag period in which G-actin aggregates into short, unstable oligomers**. Once the oligomer reaches a certain length (three or four subunits) it can act as a stable seed, or nucleus, which in the second phase rapidly **elongates into a filament by the addition of actin monomers to both of its ends**. As F-actin filaments grow, the concentration of G-actin monomers decreases until it is in equilibrium with the filament. **This third phase is called steady state** because G-actin monomers exchange with subunits at the filament ends but there is no net change in the total mass of filaments. This is known as **actin treadmilling**.



Courtesy : Molecular Biology of the Cell, Lodish et al., 5th Edition

Tropomyosin (5-10% of myofibril protein) :

This protein constitutes of about 3-8% of the total protein contents of the muscle filament. It has a molecular weight of the order of 70,000. The amino acid composition reveals the absence of proline and tryptophan. It lacks ATPase activity. There are two known forms of tropomyosin: tropomyosin A and tropomyosin B. Tropomyosin A, also known as paramyosin, is mixed with actomyosin, it can inhibit calcium activated ATPase. It does not inhibit the magnesium activated ATPase. There is one tropomyosin for every 7 actin monomers. It plays an integral role in contraction by regulation of acto-myosin interaction.

Troponin (2% of myofibril protein) :

1. It is a complex of 3 globular protein molecules with molecular weight of 73,000-80,000.
2. The 3 polypeptides are-

   a) Troponin T-binds to tropomyosin and two other troponin components; MW=37,000.

   b) Troponin C-binds to Ca^{2+} , MW=17,000-18,000

c) Troponin I-binds to actin and inhibits F actin-myosin interaction; MW=22,000-24,000

3. **Function-**This complex along with tropomyosin mediates regulation of muscle contraction by Ca^{2+}.

Other proteins of myofilament :

Titin-

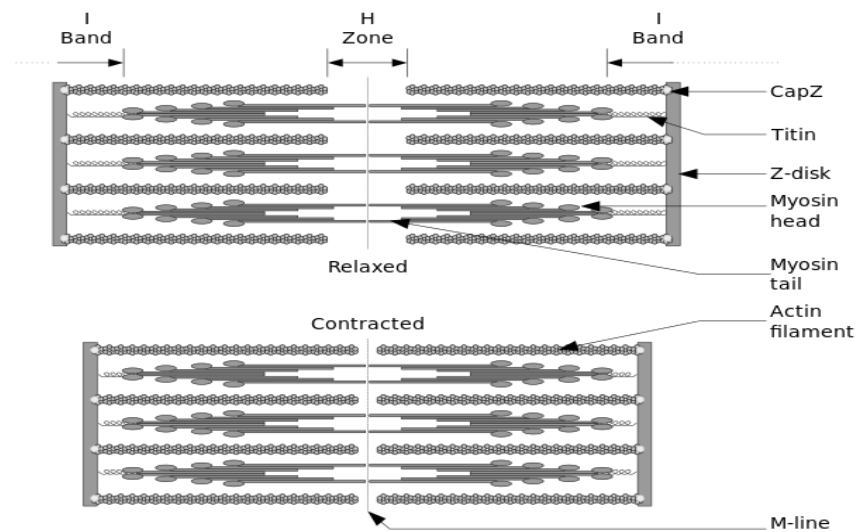
1. It is the largest known protein with MW= 3000 KDa and controls the length of sarcomere.
2. It is the 3rd most abundant muscle protein which makes 10% of combined muscle protein content. It is elastic in nature.
3. Titin connects the end of myosin filaments to Z-lines and extend along the thick filament upto the H-zone.

Nebulin-

1. It is another large protein of molecular weight of 600-900 KDa
2. It is a non-elastic actin binding protein. It extends from each side of 2-disc and localized in I-band.
3. It regulates the thin filament length during sarcomere assembly

Myomesin-

1. It has a molecular weight of 190 KDa and can bind both myosin and titin.
2. M-line also contain the enzyme creatine kinase which is vital to energetic of muscle contraction.



Structure of sarcomere; relaxed and contracted

Muscle Contraction :

SLIDING FILAMENT THEORY :

In 1954, this theory was independently formulated by H.E Huxley and Jean Hanson and A.F Huxley and R. Nieddergerke.

1. It states that during muscle contraction shortening of sarcomere occurs due to sliding of thin filaments (actin) into A and H bands, i.e. actin filaments slide inward towards myosin filaments.

2. Myosin filaments gradually approach the Z-line and the actin filaments, by altering the site of attachment of cross bridges.

3. Each flexion of a cross-bridge produces only a very small movement in actin filament; so many cross bridges throughout the muscle must flex repeatedly and rapidly for movement to occur.

Molecular Mechanism :

With the elucidation of structure of actin and myosin, the molecular mechanism of muscle contraction was defined. The globular heads of myosin form cross-bridges with the actin monomers, hence the theory was called 'CROSS-BRIDGE THEORY'. Here, myosin acts like an active ratchet, hence it is also called 'RATCHET THEORY'.

THE STEPS :

1. Action potential arrives at the end of amotor neuron, at the neuro-muscular junction. This causes release of acetylcholine from synapse. This initiates an action potential in muscle cell membrane.

2. The action potential spreads via T-tubule which causes sarcoplasmic reticulum to release its store of calcium ions into myofibrils.

3. In absence of calcium ion, the tropomyosin blocks the myosin binding site of actin and therefore myosin cross-bridge cannot attach to actin.

4. As soon as muscle is excited, the Ca^{2+} binds to Troponin C, which causes a conformational change in whole troponin-tropomyosin complex. This results in the tropomyosin moving away from the myosin binding site on actin, which allows myosin to bind with actin.

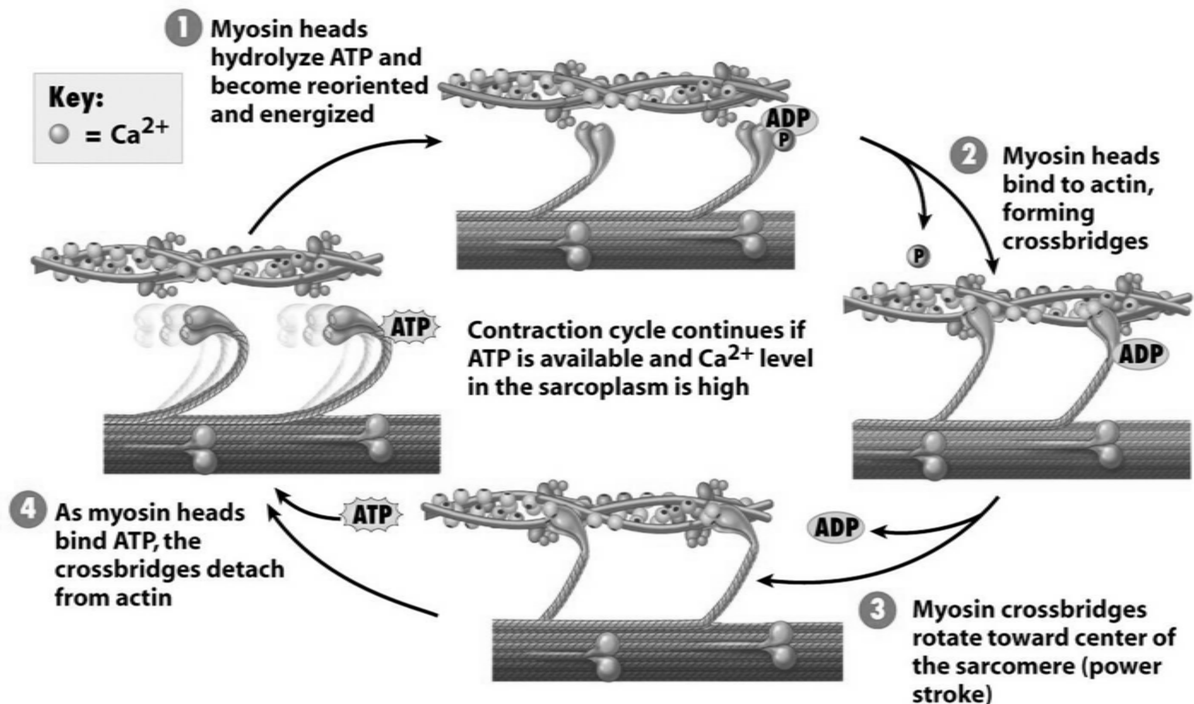
5. Myosin head acts as an ATPase (the S-1 subunit). In the presence of Ca^{2+}, ATPase is activated. The ATP bound to myosin S1 head is hydrolysed into ADP and Pi, forming myosin-ADP-Pi complex.

6. The myosin head bind with an actin with the help of Pi and forming myosin-ADP-actin complex. This binding occurs at an angle of 90°.

7. Immediately after binding of myosin with actin, the ADP is released from myosin and gives a "powerv stroke", which results in the movement of actin filaments about 100Å. This movement occurs like a sliding filament, pulling the Z-bands towards each other, thus shortening sarcomere and I-band. Therefore the process is called "SLIDING FILAMENT THEORY". At this time the myosin remains attached with actin at an angle of 45°

8. A new ATP molecule attaches to the myosin head, and causes its detachment from actin, bringing the myosin back to its original weak binding state.

As long as Ca^{2+} and ATP is available, the process continues.



Role of Calcium and ATP In Muscle Contraction :

1. In resting state, sarcoplasmic reticulum takes up calcium which keeps the calcium concentration low in the cytoplasm inhibiting its binding with troponin and activation of tropomyosin. When action potential reaches sarcoplasmic reticulum, it releases calcium. Calcium binds with troponin which induces a conformational change in troponin-tropomyosin complex, exposing the actin-binding site of the myosin filament. Thus binding of calcium to troponin allows cross-bridge activation.

2. ATP provides the immediate source of energy. It is broken down to ADP and Pi to energize contractile process and pumping calcium during relaxation and maintaining sodium-potassium gradient across sarcolemma.

The available amount of ATP is enough for only a few twitches. Therefore, the ADP formed is immediately resynthesised to form ATP with addition of high energy PO_4 radical. This is provided by creatine phosphate which is abundant in resting muscle.

Probable Questions-

1. Explain with the help of a diagram the structure of a sarcomere.
2. What effect does the deficiency of calcium ion has on muscle contraction?
3. Describe with the help of diagram the sliding filament theory of muscle contraction.

Suggested Readings / References-

1. Animal physiology- Mohan P. Arora.
2. Textbook of medical physiology/Arthur C. Guyton, John E. Hall.
3. Ganong's review of medical physiology.

Unit III

Physiology of excretion :

Formation of urine : glomerular filtration; tubular function; counter current mechanism and urine formation.

Objectives :

1. To understand the structure and function of kidney.
2. To have a clear concept about the mechanism of urine formation and excretion by the kidney.
3. Understanding the role of kidney in osmoregulation.

Introduction

In the kidneys, a fluid that resembles plasma is filtered through the glomerular capillaries into the renal tubules. As the glomerular filtrate passes down the tubules, its volume is reduced, and its composition altered by the processes of tubular reabsorption and tubular secretion to form the urine that enters the renal pelvis. From the renal pelvis, the urine passes to the bladder and is expelled to the exterior by the process of urination or micturition.

General Organization of the Kidneys and Urinary Tract

The two kidneys lie on the posterior wall of the abdomen, outside the peritoneal cavity. Each kidney of the adult human weighs about 150 grams and is about the size of a clenched fist. The medial side of each kidney contains an indented region called the hilum through which pass the renal artery and vein, lymphatics, nerve supply, and ureter, which carries the final urine from the kidney to the bladder, where it is stored until emptied. The kidney is surrounded by a tough, fibrous capsule that protects its delicate inner structures. If the kidney is bisected from top to bottom, the two major regions that can be visualized are the outer cortex and the inner medulla regions. The medulla is divided into 8 to 10 cone-shaped masses of tissue called renal pyramids. The base of each pyramid originates at the border between the cortex and medulla and terminates in the papilla, which projects into the space of the renal

pelvis, a funnel-shaped continuation of the upper end of the ureter. The outer border of the pelvis is divided into open-ended pouches called major calyces that extend downward and divide into minor calyces, which collect urine from the tubules of each papilla. The walls of the calyces, pelvis, and ureter contain contractile elements that propel the urine toward the bladder, where urine is stored.

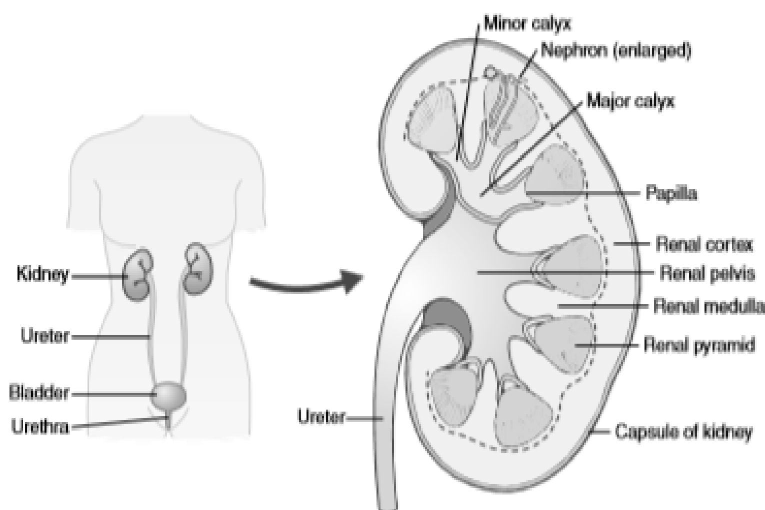
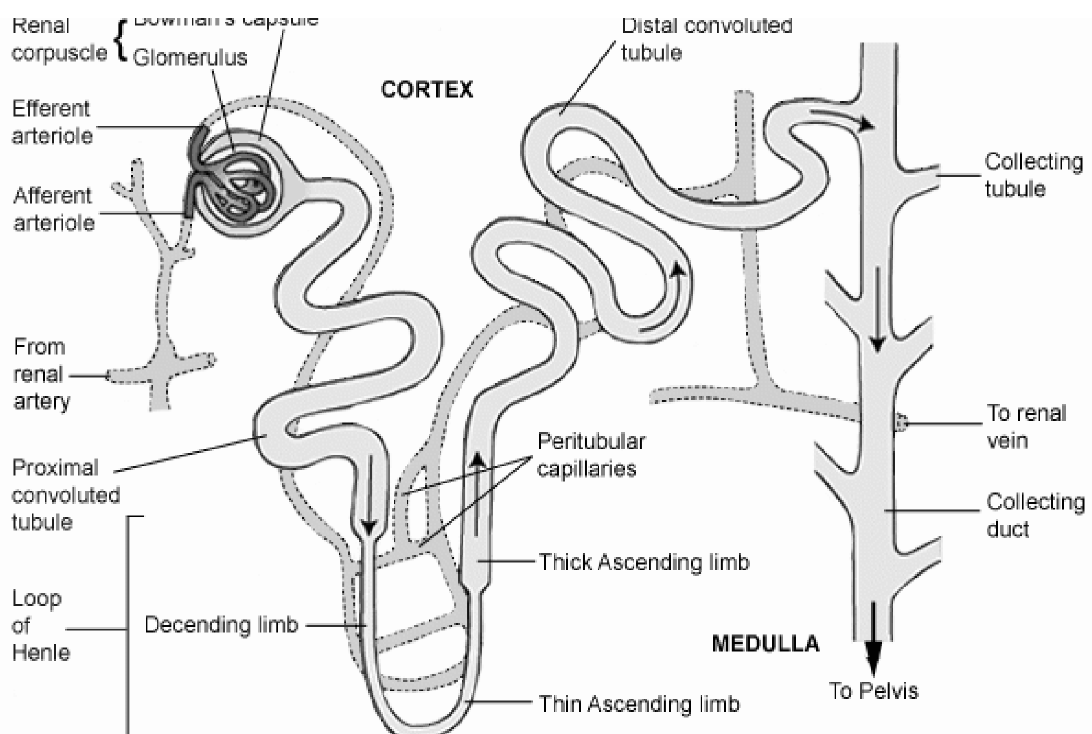


Figure: General Organization of Kidney

The Nephron Is the Functional Unit of the Kidney

Each kidney in the human contains about 800,000 to 1,000,000 nephrons, each capable of forming urine. The kidney cannot regenerate new nephrons. Therefore, with renal injury, disease, or normal aging, there is a gradual decrease in nephron number. After age 40, the number of functioning nephrons usually decreases about 10 percent every 10 years; thus, at age 80, many people have 40 percent fewer functioning nephrons than they did at age 40. This loss is not life threatening because adaptive changes in the remaining nephrons allow them to excrete the proper amounts of water, electrolytes, and waste products. Each nephroncontains (1) a tuft of glomerularcapillaries called the glomerulus, through which large amounts of fluid are filtered from the blood, and (2) a long tubule in which the filtered fluid is converted into urine on its way to the pelvis of the kidney. The glomerulus contains a network of branching and anastomosing glomerular capillaries that, compared with other capillaries, have high hydrostatic pressure (about 60 mm Hg). The glomerular capillaries are covered by epithelial cells, and the total glomerulus is encased in Bowman’s capsule. Fluid filtered from the glomerular capillaries flows into Bowman’s capsule and then into the

proximal tubule, which lies in the cortex of the kidney. From the proximal tubule, fluid flows into the loop of Henle, which dipsin to the renal medulla. Each loop consists of a descending and an ascending limb. The walls of the descending limb and the lower end of the ascending limb are very thin and therefore are called the thin segment of the loop of Henle. After the ascending limb of the loop returns partway back to the cortex, its wall becomes much thicker, and it is referred to as the thick segment of the ascending limb. At the end of the thick ascending limb is a short segment that has in its wall a plaque of specialized epithelial cells, known as the macula densa. The macula densa plays an important role in controlling nephron function. Beyond the macula densa, fluid enters the distal tubule, which, like the proximal tubule, lies in the renal cortex. This is followed by the connecting tubule and the cortical collecting tubule, which lead to the cortical collecting duct. The initial parts of 8 to 10 cortical collecting ducts join to form a single larger collecting duct that runs downward into the medulla and becomes the medullary collecting duct. The collecting ducts merge to form progressively larger ducts that eventually empty into the renal pelvis through the tips of the renal papillae. In each kidney, there are about 250 of the very large collecting ducts, each of which collects urine from about 4000 nephrons.



Overview of renal physiology

To produce urine, nephrons and collecting ducts perform three basic process-

1. **Glomerular filtration :** In the first step of urine production, water and most solutes in the blood plasma move across the wall of glomerular capillaries into the glomerular capsule and then into the renal tubule.
2. **Tubular reabsorption :** As filtered fluid flows along the renal tubule and through the collecting duct, tubule cells reabsorb about 99% of the filtered water and many useful solutes.
3. **Tubular secretion :** As fluid flows along the renal tubule and through the collecting duct, the tubule and duct cell secrete other materials, such as wastes, drugs and excess ions, into the fluid.

Glomerular filtration

Glomerular filtration occurs when the whole blood enters the afferent arteriole and the glomerulus. Due to glomerular blood pressure, water and small molecule move from the glomerulus to the inside of the glomerular capsule. This is a filtration process because large molecule and formed elements are unable to pass through the capillary wall. In effect, then, blood in the glomerulus has two portions: the filtrable components (such as water, nutrients, ions, nitrogenous waste) and the nonfilterable components (such as blood cells, platelets, proteins). On average, the daily volume of glomerular filtrate in adults is 150 liters in females and 180 liters in male. More than 99% of the glomerular filtrate returns to the bloodstream via tubular reabsorption, so only 1-2 liters are excreted as urine.

Glomerular Ultrafiltration

The fluid that enters the glomerular capsule is called ultrafiltrate because it is formed under pressure—the hydrostatic pressure of the blood. This process is similar to the formation of tissue fluid by other capillary beds in the body in response to Starling forces. The force favoring filtration is opposed by a counterforce developed by the hydrostatic pressure of fluid in the glomerular capsule. Also, since the protein concentration of the tubular fluid is low (less than 2 to 5 mg per 100 ml) compared to that of plasma (6 to 8 g per 100 ml), the greater

colloid osmotic pressure of plasma promotes the osmotic return of filtered water. When these opposing forces are subtracted from the hydrostatic pressure of the glomerular capillaries, a net filtration pressure of only about 10 mmHg is obtained.

Glomerular blood hydrostatic pressure (GBHP), is the blood pressure in glomerular capillaries. Generally GBHP is about 55 mmHg.

Capsular hydrostatic pressure(CHP*)*, is the hydrostatic pressure exerted against the filtration membrane by fluid already in the capsular space and renal tubule. CHP opposes filtration and represents back a back pressure of about 15mmHg.

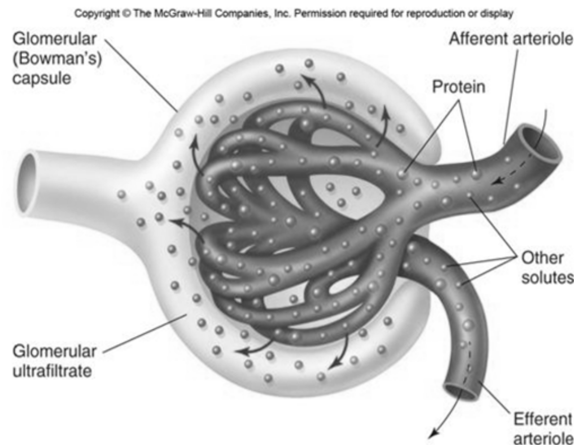
Blood colloid osmotic pressure(BCOP), which is due to the present of protein such as albumin, globulin, and fibrinogen in blood plasma, also opposes filtration. The average BCOP in glomerular capillaries is 30mmHg.

Net filtration pressure (NFP), the total pressure that promotes filtration=GBHP-CHP-BCOP

NFP= 55mmHg - 15mmHg – 30mmHg = 10mmHg.

Glomerular filtration rate

The amount of filtrate formed in all the renal corpuscles of both kidneys per minute is the glomerular filtration rate(GFR). GFR is calculated as GFR = $k_f \times$ EFP (effective filtration pressure),where k_f is filtration coefficient. Therefore, GFR is 12.5ml $\times$ 10 = 125ml.The mechanism that regulate the glomerular filtration rate operate in two main ways: 1. By adjusting blood flow into and out of the glomerulus and 2. By altering the glomerular capillary surface area available for filtration. GFR increases when blood flow into the glomerular capillaries increases. Another mechanism for controlling GFR is renal autoregulation, neural regulation and hormonal regulation.



Tubular reabsorption and tubular secretion

Tubular reabsorption is a selective process that reclaims materials from tubular fluid and returns them to the bloodstream. Reabsorbed substances include water, glucose, amino acids, urea, ions such as sodium, chloride, potassium, bicarbonate and phosphate.

Some substances not need by the body are removed from the blood and discharged into the urine via tubular secretion. This includes urea, creatinine and certain drugs.

Glomerular filtration :

Rate- 105-125mL/min of fluid that is isotonic to blood.

Filtration substances – water and all solutes present in blood including ions, glucose, amino acids, creatinine, uric

Distal convoluted tubule :

Reabsorption of :

Water, Na^+, Cl^-, Ca^{2++}

Proximal convoluted tubule :

Reabsorption of filtered :

Water, Na^+,K^+, glucose, amino acid, cl^- ,HCO_3,urea, Ca^{2+}, Mg^{2+}

Secretion into urine :

H^+,NH^{4+},urea,creatine

Principle cells in the late distal tubule and collecting duct :

Reabsorption of :

Water, Na^+, urea

Secretion of :

K^+

Loop of Henle :

Reabsorption of filtered :

Water, Na^+, K^+,cl^-,$HCO3^+$,urea, Ca^{2+}, $Mg2^+$.

Secretion of : Urea.

Intercalated cells in late distal tubule and collecting duct :

Reabsorption of : HCO_3^-, urea.

Secretion of : H^+

Hormonal regulation of tubular reabsorption and tubular section

Four hormones affect the extent of sodium, chloride, and water reabsorption as well as potassium secretion by the renal tubules. The most important hormonal regulation of electrolytes reabsorption and secretion and angiotensin 2 and aldosterone. The major hormone that regulates water reabsorption is antidiuretic hormone.

Renin-angiotensin–aldosterone system

When blood volume and blood pressure decrease, the juxtaglomerular cells secrete the enzyme renin into the blood. Renin clips off a 10-amino-acid peptide called angiotensin1 from angiotensinogen, which is synthesized by hepatocytes. By clipping off two more amino acid, angiotensin converting enzyme converts angiotensin I to angiotensin II, which is the active form of the hormone.

Antidiuretic hormone (ADH) and its mechanism of action

Antidiuretic hormone or vasopressin released by the posterior lobe of the pituitary plays a role in water reabsorption at the collecting duct. When more ADH is present, more water is reabsorbed and the decreased amount of urine formed. Blood borne ADH binds with its receptor. Present on the basolateral membrane of the cell in the distal or collecting tubule. This binding activates the cAMP signaling. ADH regulates the water channel in the membrane. water enters the tubular cell from the tubular lumen through the inserted water channel. water exits the cell through a different, always open water channel permeability positioned at the basolateral border, and then enters the blood, in this way being reabsorbed.

The Countercurrent Multiplier System

Water cannot be actively transported across the tubule wall, and osmosis of water cannot occur if the tubular fluid and surrounding interstitial fluid are isotonic to each other. In order

for water to be reabsorbed by osmosis, the surrounding interstitial fluid must be hypertonic. The osmotic pressure of the interstitial fluid in the renal medulla is, in fact, raised to over four times that of plasma by the juxtamedullary nephrons. This results partly from the fact that the tubule bends; the geometry of the loop of Henle permits interaction between the descending and ascending limbs. Since the ascending limb is the active partner in this interaction, its properties will be described before those of the descending limb.

Countercurrent Multiplication

Countercurrent flow (flow in opposite directions) in the ascending and descending limbs and the close proximity of the two limbs allow for interaction between them. Since the concentration of the tubular fluid in the descending limb reflects the concentration of surrounding interstitial fluid, and since the concentration of this fluid is raised by the active extrusion of salt from the ascending limb, a positive feedback mechanism is created. The more salt the ascending limb extrudes, the more concentrated will be the fluid that is delivered to it from the descending limb. This positive feedback mechanism multiplies the concentration of interstitial fluid and descending limb fluid, and is thus called the countercurrent multiplier system. First, assume that the loop of Henle is filled with fluid with a concentration of 300 mOsm/L, the same as that leaving the proximal tubule. Next, the active ion pump of the thick ascending limb on the loop of Henle reduces the concentration inside the tubule and raises the interstitial concentration; this pump establishes a 200-mOsm/L concentration gradient between the tubular fluid and the interstitial fluid (step 2). The limit to the gradient is about 200 mOsm/L because paracellular diffusion of ions back into the tubule eventually counterbalances transport of ions out of the lumen when the 200-mOsm/L concentration gradient is achieved. Step 3 is that the tubular fluid in the descending limb of the loop of Henle and the interstitial fluid quickly reach osmotic equilibrium because of osmosis of water out of the descending limb. The interstitial osmolarity is maintained at 400 mOsm/L because of continued transport of ions out of the thick ascending loop of Henle. Thus, by itself, the active transport of sodium chloride out of the thick ascending limb is capable of establishing only a 200-mOsm/L concentration gradient, much less than that achieved by the countercurrent system. Step 4 is additional flow of fluid into the loop of Henle from the proximal tubule, which causes the hyperosmotic fluid previously formed in the descending limb to flow into the ascending limb. Once this fluid is in the ascending limb, additional ions are pumped into the interstitium, with water remaining in the tubular fluid,

until a 200-mOsm/L osmotic gradient is established, with the interstitial fluid osmolarity rising to 500 mOsm/L (step 5). Then, once again, the fluid in the descending limb reaches equilibrium with the hyperosmotic medullary interstitial fluid (step 6), and as the hyperosmotic tubular fluid from the descending limb of the loop of Henle flows into the ascending limb, still more solute is continuously pumped out of the tubules and deposited into the medullary interstitium. These steps are repeated over and over, with the net effect of adding more and more solute to the medulla in excess of water; with sufficient time, this process gradually traps solutes in the medulla and multiplies the concentration gradient established by the active pumping of ions out of the thick ascending loop of Henle, eventually raising the interstitial fluid osmolarity to 1200 to 1400 mOsm/L as shown in step 7. Thus, the repetitive reabsorption of sodium chloride by the thick ascending loop of Henle and continued inflow of new sodium chloride from the proximal tubule into the loop of Henle is called the countercurrent multiplier. The sodium chloride reabsorbed from the ascending loop of Henle keeps adding to the newly arrived sodium chloride, thus "multiplying" its concentration in the medullary interstitium.

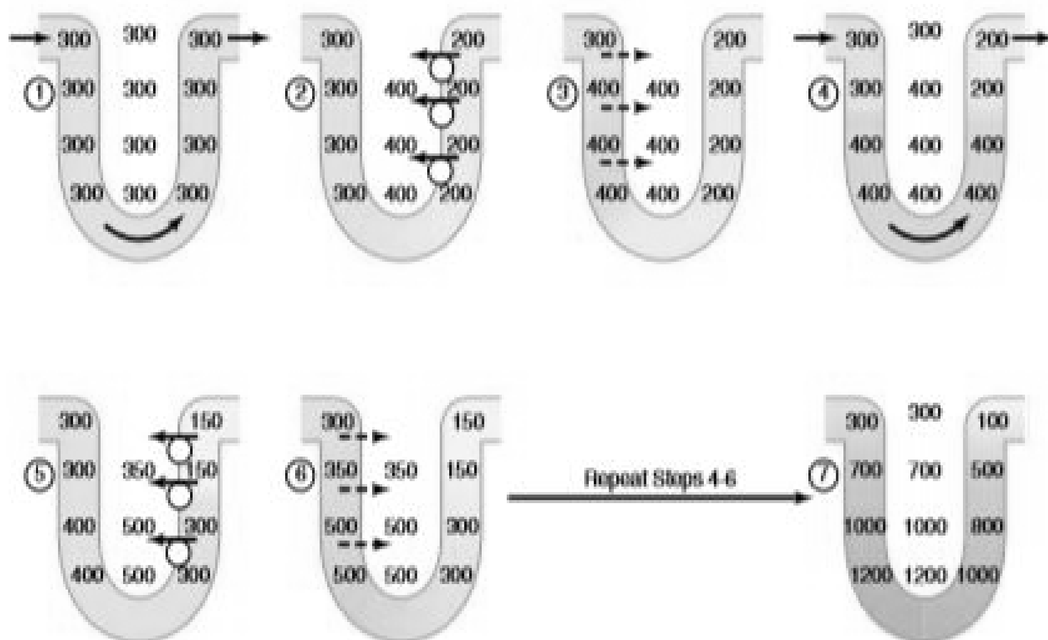


Figure : Countercurrent multiplier system in the loop of Henle for producing a hyperosmotic renal medulla.

Probable Questions

1. Write a short note on juxta-glomerular apparatus.
2. How does ADH and aldosterone regulate the salt water balance in our body?
3. How does the counter-current mechanism help in the formation of hypertonic urine? Elaborate.

Suggested readings / References-

1. Animal physiology- Mohan P. Arora.
2. Textbook of medical physiology / Arthur C. Guyton, John E. Hall.
3. Ganong's review of medical physiology.

Unit IV

Synaptic and functional transmission

a) Pre-and postsynaptic structure and function

b) Chemical transmission of synaptic activity

Objectives :

1. Understanding the mechanism of nerve impulse transmission through synapse.
2. Understanding the mechanism of release of neurotransmitter from the synaptic vesicles.
3. To understand the fate of Neurotransmitters after synaptic transmission

Introduction :

Neurons receive information from sensory organs, send information to motor organs, or share information with other neurons. The process of communicating information is very similar, whether it is to another neuron or to a muscle or gland cell. However, by far the largest number of neuronal connections is with other neurons. The rest of this tutorial therefore focuses on inter-neuronal communication. The transmission is accomplished in two ways :

Electrically : the neuron is directly adjacent to other neurons. Small holes in each cell's membrane, called gap junctions, are juxtaposed so that as the action potential reaches the end of the axon (at the terminal boutons), the depolarization continues across the membrane to the postsynaptic neuron directly.

Chemically : there is a space (the synaptic cleft) between the axon terminus and adjacent neuron. As the action potential reaches the end of the axon, a chemical is released that travels across the synaptic cleft to the next neuron to after its electric potential.

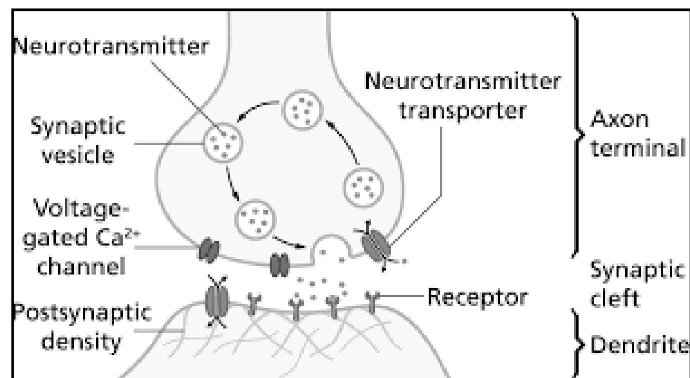
Structure of synapse :

1. The part of the synapse that belongs to the initiating neuron is called the *presynaptic membrane*.

2. The part of the synapse that belongs to the receiving neuron is called the *postsynaptic membrane*.

3. The space between the two is called the *synaptic cleft*. It is approximately 20nm wide.

4. Presynaptic terminals contain numerous synaptic vesicles. These vesicles contain neurotransmitters, chemical substances which ultimately cause postsynaptic changes in the receiving neuron, is contained within the synaptic vesicles. Common neurotransmitters include : Acetylcholine, Dopamine, Norepinephrine, Serotonin, etc.



Transmission :

Electrical transmission occurs by virtue of the fact that the cells are in direct contact with each other: depolarization of the presynaptic membrane causes a depolarization of the postsynaptic membrane, and the action potential is propagated further. Here the transmission of information is always excitatory : the conduction of information always causes a depolarization of the adjacent cell’s membrane.

Chemical transmission, being more complex than electrical synapse, allows for far more control, including the ability to excite or inhibit the postsynaptic cell. Here the conduction of

information can cause either depolarization or hyperpolarization, depending on the nature of the chemical substance.

The sequence of events that lead to postsynaptic changes is as follows:

1. The action potential signal arrives at the axon terminal.
2. The local depolarization causes Ca^{2+} channels to open.
3. Ca^{2+} enters the presynaptic cell because its concentration is greater outside the cell than inside.
4. The Ca^{2+}, by binding with calmodulin, causes vesicles filled with neurotransmitter to migrate towards the presynaptic membrane.
5. The vesicle merges with presynaptic membrane.
6. The resynaptic membrane and the vesicle now forms a continuous membrane, so that the neurotransmitter is released into the synaptic cleft. This process is called exocytosis.
7. The neurotransmitter diffuses through the synaptic cleft and binds with receptor channel membranes that are located in both presynaptic and postsynaptic membranes.
8. The time period from neurotransmitter release to receptor channel binding is less than a million of a second.

Synaptic vesicle cycle :

The synaptic vesicles functions in the synaptic knob and also is recycled for further use.

1. **Trafficking to the synapse :** Synaptic vesicle components are initially trafficked to the synapse using members of the kinesin motor family. In *C. elegans*, the major motor for synaptic vesicle is UNC-104. There is also evidence that other proteins such as UNC-16/Sunday Driver regulate the use of motor for transport of synaptic vesicles. Transport vesicles used to traffic synaptic vesicle components probably differ in composition from mature synaptic vesicles though these differences have not been well defined. Another question that has not been well resolved is how motors release cargo st the synapses. Finally, though there is substantial evidence that active zone

components are also trafficked to synapses on vesicles, the nature of the motors that performs this transport remains unclear.

2. **Transmitter loading :** Once at synaptic sites, vesicles are loaded with neurotransmitter. Loading of transmitter is an active process requiring a neurotransmitter transporter and a vacuolar-type proton pump ATPase that provides a pH and electrochemical gradient. These transporters are selective for different classes of transmitters. Interestingly, the identity of many of these transporters was determined through the molecular characterization of *C. elegans* mutants. Characterizations of *unc*-17 and *unc-47*, which encode the vesicular acetylecholine transporter and vesicular GABA transporter, defined the founding members of these two families of transporters. To what extent loading is regulated to modulate transmitter release is not known.

3. **Docking:** Next, loaded vesicles must dock near release sites. Docking is a step of the cycle that we know little about. Though many proteins on vesicles and at release sites have been identified, none of the identified protein interactions that occur between vesicle proteins and proteins at release sites can account for docking. Mutants in *rab-3* and *unc-18* alter vesicle docking or vesicle organization at release sites, but they do not completely disrupt docking. Perhaps surprisingly, the SNAREs, which are thought to mediate fusion, do not appear to be involved in the docking process.

4. **Priming:** When vesicles initially dock they are not fusion competent. Vesicles first need to be primed so that they are able to fuse rapidly in response to calcium influx. This priming step is thought to involve the formation of partially assembled SNARE complexes. SNARE proteins must assemble into trans-SNARE complexes so that they can provide the force that is necessary for vesicle fusion. The four α-helix domains (1 each from synaptobrevin and syntaxin, and 2 from SNAP-25) come together to form a coiled-coil motif. The rate-limiting step in the assembly process is the association of the syntaxin SNARE domain, since it is usually found in a “closed” state where it is incapable of interacting with other SNARE proteins. When syntaxin is in an open state, trans-SNARE complex formation begins with the association of

the four SNARE domains at their N-termini. The SNARE domains proceed in forming a coiled-coil motif in the direction of the C-termini of their respective domains. The proteins UNC—13 and Rim participate in this event. UNC-13 is thought to stimulate the change of the t-SNARE syntaxin from a closed conformation to an open conformation, which stimulates the assembly of v-SNARE/t-SNARE complexes. Rim also appears to regulate the priming, but it is not essential for the step.

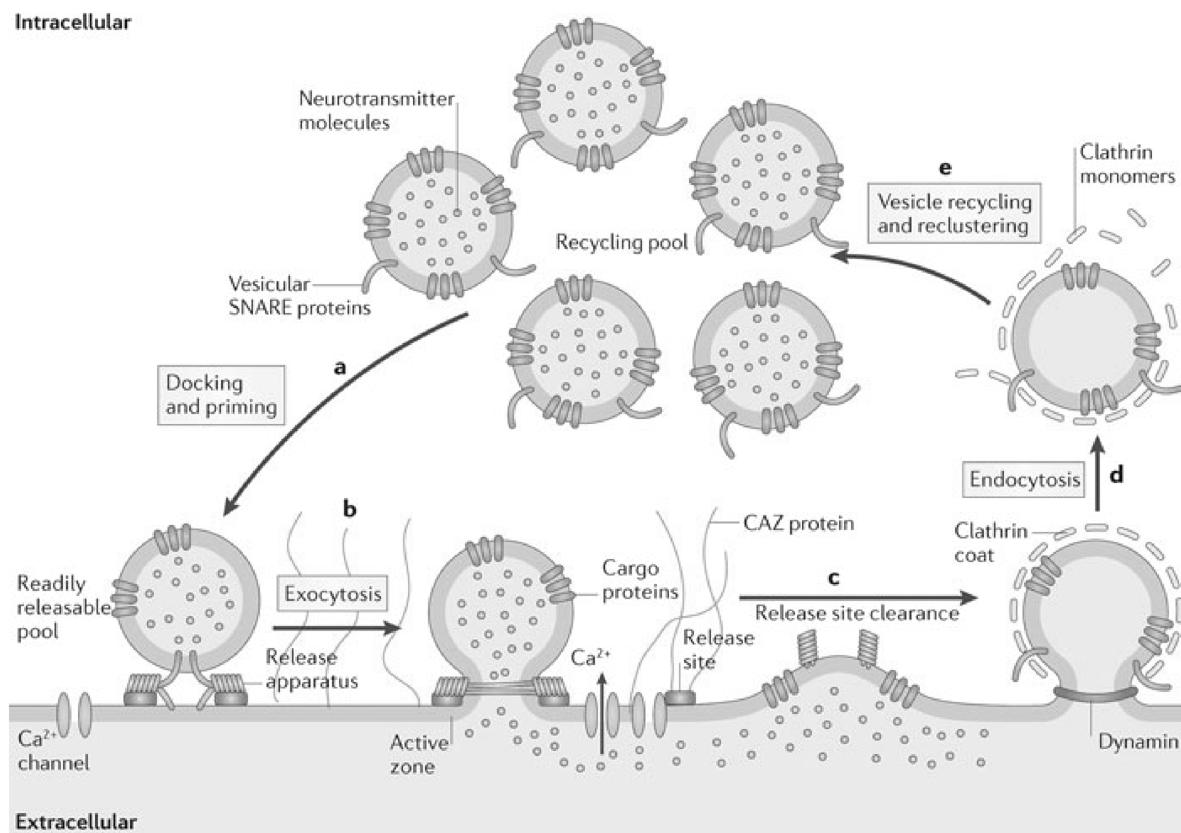


Figure: Synaptic vesicle cycle (source: Nature review)

5. **Fusion :** Primed vesicles fuse very quickly in response to calcium elevations in the cytoplasm. This fusion event is thought to be medicated directly by the SNAREs and driven by the energy provided from SNARE assembly. The calcium-sensing trigger for this event is the calcium-binding synaptic vesicle protein synaptotagmin. The ability of SNAREs to mediate fusion in a calcium-dependent manner recently has

been reconstituted *in vitro*. Consistent with SNAREs being essential for the fusion process, v-SNARE and t-SNARE mutants of *C. elegans* are lethal and even viable hypomorphic mutants exhibit almost no evoked release. Similarly, mutants are *Drosophila* and knockouts in mouse indicate that these SNAREs play a critical role in synaptic exocytosis. One model hypothesizes that the force required to bring two membranes together during fusion comes from the conformational change in trans-SNARE complexes to form cis-SNARE complexes. The current hypothesis that describes this process is referred to as SNARE "zippering". Several models to explain the subsequent step – the formation of stalk and fusion pore – have been proposed. However, the exact nature of these processes remains debated. In accordance with the "zipper" hypothesis as the SNARE complex forms, the tightening helix bundle puts torsional force on the transmembrane (TM) domains of synaptobrevin and syntaxin. This causes the TM domains to tilt within the separate membranes as the proteins coil more tightly. The unstable configuration of the TM domains eventually causes the two membranes to fuse and the SNARE proteins come together within the same membrane, which is referred to as a "cis"-SNARE complex. As a result of the lipid rearrangement, a fusion pore opens and allows the chemical contents of the vesicle to leak into the outside environment. However, our understanding of fusion is farfrom complete. One major issue in the field is defining the contribution of incomplete fusion events (also referred to as kiss-and-run) where a fusion pore forms briefly to allow exit of transmitter without complete fusion of the vesicle and plasma membranes. There is substantial evidence that kiss and run occurs at least at some synapses. Studies examining the rates of endocytosis suggest that both very rapid and slow endocytosis occur at synapses. Analysis of SNAP-25 and synaptobrevin mouse knockout mutant revealed that although evoked release was completely disrupted, that some spontaneous fusion events continued. Similarly, *C. elegans* null mutants lacking the v-SNARE synaptobrevin and the t-SNARE SNAP-25 still are capable of some movements, in contrast to completely paralysis seen in t-SNARE syntaxin mutants.

The energy input that is required for SNARE-mediated fusion to take place comes from SNARE-complex disassembly. The suspected energy source is N-

ethylmaleimide-sensitive factor (NSF), an ATPase that is involved with membrane fusion. NSF homohexamers, along with the NSF cofactor α-SNAP, bind and dissociate the SNARE complex by coupling the process with ATP hydrolysis. This process allows for reuptake of synaptobrevin for further use in vesicles, whereas the other SNARE proteins remain associated with the cell membrane.

The dissociated SNARE proteins have a higher energy state than the more stable cis-SNARE complex. It is believed that the energy that drives fusion is derived from the transition to a lower energy cis-SNARE complex. The ATP hydrolysis-coupled dissociation of SNARE complexes is an energy investment that can be compared to "cocking the gun" sothat, once vesicle fusion is triggered, the process takes place spontaneously and at optimum velocity. A comparable process takes place in muscles, in which the myosin heads must first hydrolyze ATP in order to adapt the necessary conformation for interaction with actin and the subsequent power stroke to occur.

6. **Endocytosis :** Synaptic vesicle proteins that have been incorporated into the plasma membrane after fusion are retrieved by endcytosis. A large cohort of proteins have been identified which participate in these events including endophilin, synaptojanin, synaptotagmin, dynamin, clathrin, AP180, as well as others. The span of presynaptic membrane containing the primed vesicles and dense collection of SNARE proteins is referred to as active zone. Voltage-gated calcium channels are highly concentrated around active zones and open in response to membrane depolarization at the synapse. The influx of calcium is sensed by synaptotagmin 1, which in turn dislodges complexin protein and allows the vesicle to fuse with the presynaptic membrane to release neurotransmitter. It has also been shown that the voltage-gated calcium channels directly interact with the t-SNAREs syntaxin 1A and SNAP-25, as well as with synaptotagmin 1. The interactions are able to inhibit calcium channel activity as well as tightly aggregate the molecules around the release site. Rate of endocytosis vary widely in different preparations and also vary depending on the stimulus intensity. A variety of evidence suggests that different pathways may be utilized even at the same synapse. One of the major outstanding questions remains whether synaptic vesicle membranes are selectively endocytosed as synaptic vesicle entities or

as precursors with other membrane proteins which must then traffic through as endosomal compartment to yield mature vesicles.

7. **Neurotransmitter clearance :** After transmitter is released and binds to receptor on the postsynaptic membrane, it must be cleared to permit subsequent signalling. Some transmitters like dopamine are transported back into the neuron using plasma membrane transporters. Other transmitter including acetylcholine and some neuropeptides are broken down in the synaptic cleft. Acetyl cholinesterase does this job at the neuromuscular junction.

Probable Questions-

1. Explain the structure of a typical synapse with proper diagram.
2. What is the role of SNARE proteins in the release of neurotransmitter from the synapse?
3. Describe how neurotransmitter is released in a calcium-dependent manner.

Suggested Readings/References-

1. Animal physiology-Mohan P. Arora.
2. Textbook of medical physiology/Arthur C. Guyton, John E. Hall.
3. Ganong's review of medical physiology.

HARD CORE THEORY PAPER (ZHT – 104)

Group B: Biochemistry and Metabolic Processes

Module	Unit	Content	Credit	Class	Time (h)	Page No
ZHT - 104 (Animal Physiology & Biochemistry and Metabolic Processes)	**V**	Proteins: Protein folding and protein stability		**1**	**1**	**53-67**
	VI	Bioenergetics and oxidative metabolism		**1**	**1**	**68-79**
	VII	Thermodynamic principles and steady-state conditions of living organism; standard free energy change in a reacting system; energy change for ATP hydrolysis.	1.5	**1**	**1**	**76 -79**
	VIII	Amino acid metabolism a. Amino acid classification b. Urea cycle		**1**	**1**	**80 -88**
	IX	Carbohydrate metabolism		**1**	**1**	**89-94**

	Glycolysis, glycogenolysis, gluconeogenesis, interrelationship between different carbohydrate metabolism			
X	Lipid metabolism Biosynthesis and transport of cholesterol	**1**	**1**	**95-101**
XI	Enzymes: a. Kinetic analysis of enzyme-catalyzed reaction b. Regulation of enzyme activity c. Allosteric control of enzyme activity.	**1**	**1**	**102-113**
XII	Intracellular protein traffic for secretary and non-secretary cells: protein synthesis, intracellular transport, packaging, storage and release	**1**	**1**	**114 -134**

Group –B : BIOCHEMISTRY AND METABOLIC PROCESSESS

Unit V : Proteins : Protein folding and protein stability

Objectives : In this unit we will discuss on Protein folding and protein stability

Introduction :

Protein folding occurs in a cellular compartment called the endoplasmic reticulum. This is a vital cellular process because proteins must be correctly folded into specific, three-dimensional shapes in order to function correctly. Unfolded or misfolded proteins contribute to the pathology of many diseases. Cells rely on a very sensitive system known as the unfolded protein response (UPR) to guard against the cellular stress caused by protein folding problems. The UPR is a cell's way to ensure its ability to secrete proteins is working properly. Its role is to turn on genes that help the endoplasmic reticulum properly fold proteins. With these genes turned on, the cell is better equipped to handle the stress of protein folding problems that may arise. However, severe stress can overwhelm the UPR and lead to abnormal cellular function.

The classic principle of protein folding is that all the information required for a protein to adopt the correct three-dimensional conformation is provided by its amino acid sequence. The classic experiment, carried out by Christian Anfinsen in the 1950s, provided the first evidence that the amino acid sequence of a polypeptide chain contains all the information required to fold the chain into its native, three-dimensional structure. Purified ribonuclease can be completely denatured by exposure to a concentrated urea solution in the presence of a reducing agent. The reducing agent cleaves the four disulfide bonds to yield eight Cys residues, and the urea disrupts the stabilizing hydrophobic interactions, thus freeing the entire polypeptide from its folded conformation. Denaturation of ribonuclease is accompanied by a complete loss of catalytic activity. When the urea and the reducing agent are removed, the randomly coiled, denatured ribonucleasespontaneously refolds into its correct tertiary structure, with full restoration of its catalytic activity.

Protein folding thus appeared to be a self-assembly process that did not require additional cellular factors. More recent studies, however, have shown that this is not an adequate description of protein folding within the cell. The proper folding of proteins within cells is mediated by the activities of other proteins.

In 1958 John Kendrew was the first person to determine the three-dimensional structure of a protein, myoglobin- a relatively simple protein of 153 amino acids (Figure 1). Since then, the three-dimensional structures of several thousand proteins have been analyzed. Most, like myoglobin, are globular proteins with polypeptide chains folded into compact structures, although some (such as the structural proteins of connective tissues) are long fibrous molecules. Analysis of the three-dimensional structures ofthese proteins has revealed several basic principles that govern proteinfolding, although protein structure is so complex that predicting the three dimensional structure of a protein directly from its amino acid sequence isimpossible.

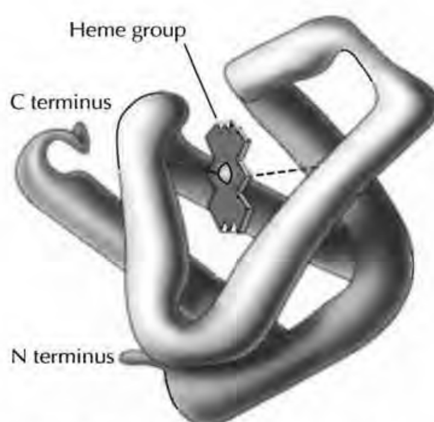


Figure 1**:** Three-dimensional structure of myoglobin**:** Myoglobinis a protein of 153 amino acids that isinvolved in oxygen transport. The polypeptide chain is folded around aheme group that serves as the oxygen binding site.

Protein structure is generally described as having four levels. The primary structure of a protein is the sequence of amino acids in its polypeptide chain. The secondary structureis the regular arrangement of amino acids within localized regions of the polypeptide.Two types of

secondary structure, which were first proposed by Linus Pauling and Robert Corey in 1951, are particularly common: the αhelix and the β sheet. Both of these secondary structures are held together by hydrogen bonds between the CO and NH groups of peptide bonds. Anαhelix is formed when a region of a polypeptide chain coils around itself, with the CO group of one peptide bond forming a hydrogen bond with the NH group of a peptide bond located four residues downstream in the linear polypeptide chain (Figure 2). In contrast, βsheet is formed when two parts of a polypeptide chain lie side by side with hydrogen bonds between them. Such βsheets can be formed between several polypeptide strands, which can be oriented either parallel or antiparallel to each other.

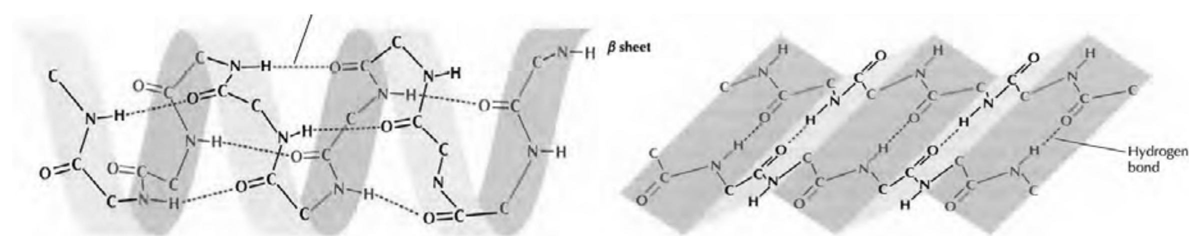


Figure 2:Secondary structure of proteins**:**The most common types are thehelix andthe βsheet. In an *a* helix, hydrogenbonds form between CO and NH groups of peptide bonds separated by 4amino acid residues. In a βsheet,hydrogen bonds connect two parts of apolypeptide chain lying side by side

In most proteins, combinations of αhelices and β sheets, connected by loop regions of the polypeptide chain, fold into compact globular structures called domains, which are the basic units of tertiary structure. Small proteins, such as ribonuclease or myoglobin, contain only a single domain; larger proteins may contain a number of different domains, which are frequently associated with distinct functions. A critical determinant of tertiary structure is the localization ofhydrophobic amino acids in the interior of the protein and of hydrophilic amino acids on the surface, where they interact with water. The interiors offolded proteins thus consist mainly of hydrophobic amino acids arranged in *a* helices and βsheets; these secondary structures are found in the hydrophobic cores of proteins because hydrogen bonding neutralizes the polar character of the CO and NH groups of the polypeptide backbone. The loop regions connecting the elements of secondary structure are found onthe surface of folded

proteins, where the polar components of the peptide bonds form hydrogen bonds with water or with the polar side chains of hydrophilic amino acids. Interactions between polar amino acid side chains (hydrogen bonds and ionic bonds) on the protein surface are also important determinants of tertiary structure. In addition, the covalent disulfide bonds between the sulfhydryl groups of cysteine residues stabilize the folded structures of many cell-surfaces or secreted proteins.

The fourth level of protein structure, quaternary structure, consists ofthe interactions between different polypeptide chains in proteins composed of more than one polypeptide. Hemoglobin, for example, is composed offour polypeptide chains held together by the same types of interactions that maintain tertiary structure (Figure. 3).

The distinct chemical characteristics of the 20 different amino acids thuslead to considerable variation in the three-dimensional conformations of folded proteins. Consequently, proteins constitute an extremely complexand diverse group of macromolecules, suited to the wide variety of tasks they perform in cell biology.

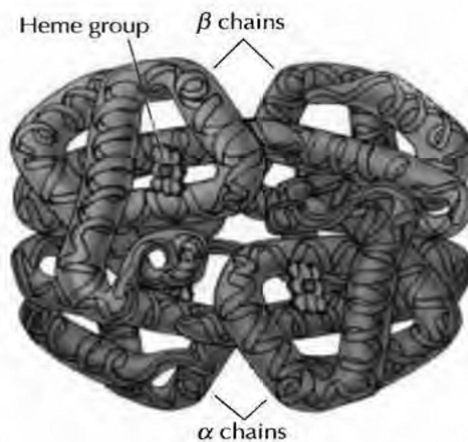


Figure. 3: Quaternary structure of hemoglobin: Hemoglobin is composed of four polypeptide chains, each of which is bound to a heme group. The two $a$chains and the two β chains are identical.

Amino Acid Sequence Determines Tertiary Structure

Anfinsen's experiment : The refolding of ribonuclease is so accurate that the four intrachain disulfide bonds are re-formed in the same positions in the renatured molecule as in the native ribonuclease (Figure 4). As calculated mathematically, the eight Cys residues could recombine at random to form up to four disulfide bonds in 105 different ways. In fact, an essentially random distribution of disulfide bonds is obtained when the disulfides are allowed to reform in the presence of denaturant, indicating that weak bonding interactions are required for correct positioning of disulfide bonds and assumption of the native conformation.

Later, similar results were obtained using chemically synthesized, catalytically active ribonuclease. This eliminated the possibility that some minor contaminant in Anfinsen's purified ribonuclease preparation might have contributed to the renaturation of the enzyme, thus dispelling any remaining doubt that this enzyme folds pontaneously.

Renaturation of unfolded, denatured ribonuclease

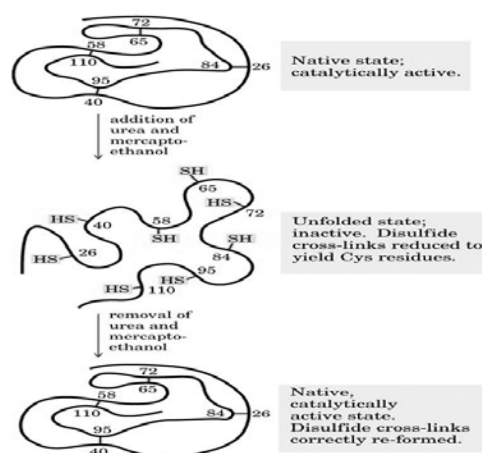


Figure 4 : Renaturation of unfolded, denatured ribonuclease : Urea is used to denature ribonuclease, and mercaptoethanol (HOCH2CH2SH) to reduce and thus cleave the disulfide

bonds to yield eight Cys residues. Renaturation involves reestablishment of the correct disulfide cross-links. So, It is now evident that the tertiary structure of a globular protein is determined by its amino acid sequence.

Polypeptides Fold Rapidly by a Stepwise Process

In living cells, proteins are assembled from amino acids at a very high rate. For example, *E. coli* cells can make a complete, biologically active protein molecule containing 100 amino acid residues in about 5 seconds at 37°C. How does such a polypeptide chain arrive at its native conformation? Let’s assume conservatively that each of the amino acid residues could take up 10 different conformations on average, giving 10^{100} different conformations for the polypeptide. Let’s also assume that the protein folds itself spontaneously by a random process in which it tries out all possible conformations around every single bond in its backbone until it finds its native, biologically active form. If each conformation were sampled in the shortest possible time (~10^{-13} second, or the time required for a single molecular vibration), it would take about 1077 years to sample all possible conformations. Thus protein folding cannot be a completely random, trial-and-error process. There must be shortcuts. This problem was first pointed out by Cyrus Levinthal in 1968 and is sometimes called Levinthal’s paradox.

The folding pathway of a large polypeptide chain is unquestionably complicated, and not all the principles that guide the process have been worked out. However, extensive study has led to the development of several plausible models. In one, the folding process is envisioned as hierarchical. Local secondary structures form first. Certain amino acid sequences fold readily into α helices or β sheets, guided by constraints. This is followed by longer-range interactions between, say, two α helices that come together to form stable supersecondary structures. The process continues until complete domains form and the entire polypeptide is folded (Figure 5).

Figure 5 : A simulated folding pathway. The folding pathway of a 36-residue segment of the protein villin (an actin-binding proteinfound principally in the microvilli lining the intestine) was simulated by computer. The process started with the randomly coiled peptide and 3,000 surrounding water molecules in a virtual "water box." The molecular motions of the peptide and the effects of the water molecules were taken into account in mapping the most likely paths to the final structure among the countless alternatives. The simulated folding took place in a theoretical time span of 1 ms; however, the calculation required half a billion integration steps on two Cray supercomputers, each running for two months.

In an alternative model, folding is initiated by a spontaneous collapse of the polypeptide into a compact state, mediated by hydrophobic interactions among nonpolar residues. The state resulting from this "hydrophobic collapse" may have a high content of secondary structure, but many amino acid side chains are not entirely fixed. The collapsed state is often referred to as a molten globule.

Most proteins probably fold bya process that incorporates features of both models. Instead of following a single pathway, a population of peptide molecules may take a variety of routes to the same end point, with the number of different partly folded conformational species decreasing as folding nears completion.

Some Proteins Undergo Assisted Folding

Proteins that facilitate the folding of other proteins are called molecular chaperones. The term "chaperone" was first used by Ron Laskey and his colleagues to describe a protein (nucleoplasmin) that is required for the assembly of nucleosomes from histones and DNA. Nucleoplasmin binds to histones and mediates their assembly into nucleosomes, but nucleoplasmin itself is not incorporated into the final nucleosome structure. Chaperones thus act as catalysts that facilitate assembly without being part of the assembled complex. Subsequent studies have extended the concept to include proteins that mediate a variety of other assembly processes, particularly protein folding.

Not all proteins fold spontaneously as they are synthesizedin the cell. Folding for many proteins is facilitatedby the action of specialized proteins. Molecular chaperones are proteins

that interact with partially folded or improperly folded polypeptides, facilitating correct folding pathways or providing microenvironments in which folding can occur.

Two classes of molecular chaperoneshave been well studied. Both are found in organisms ranging from bacteria to humans. The first class, a family of proteins called **Hsp70,** generally havea molecular weight near 70,000 and are more abundantin cells stressed by elevated temperatures (hence, *h*eat*s* hock *p*roteins of *M*r 70,000, or Hsp70). Its mode of action is depicted in Figure 7. Hsp70 proteins bind to regions of unfolded polypeptides that are richin hydrophobic residues, preventing in appropriate aggregation. These chaperones thus "protect" proteins that have been denatured by heat and peptides that arebeing synthesized (and are not yet folded). Hsp70 proteins also block the folding of certain proteins thatmust remain unfolded until they have been translocated across membranes. Some chaperones also facilitate the quaternary assembly of oligomeric proteins. The Hsp70 proteins bind to and release polypeptides in a cycle that also involves several other proteins (including a class called Hsp40) and ATP hydrolysis.

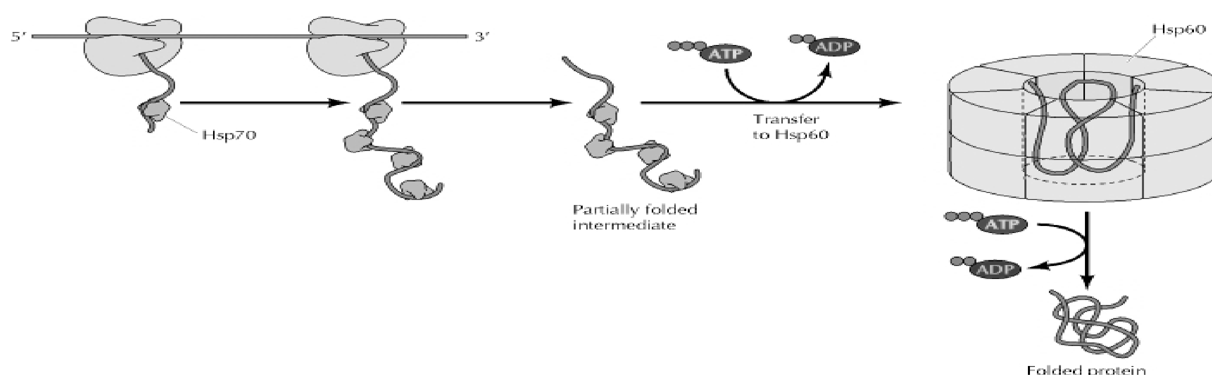


Figure. 6 : Sequential actions of Hsp70 and Hsp60 chaperones

Chaperones of the Hsp70 family bind to and stabilize unfolded polypeptide chains during translation. The unfolded polypeptide is then transferred to chaperones of the Hsp60 family, within which protein folding takes place. ATP hydrolysis is required for release of the unfolded polypeptide from Hsp70 as well as for folding within Hsp60.

The second class of chaperones is called chaperonins. These are elaborate protein complexes required for the folding of a number of cellular proteins that donot fold spontaneously. In *E. coli* an estimated 10% to15% of cellular proteins require the resident chaperonin system,

called GroEL/GroES, for folding under normal conditions (up to 30% require this assistance when the cells are heat stressed). These proteins first becameknown when they were found to be necessary for the growth of certain bacterial viruses (hence the designation “Gro”). Unfolded proteins are bound within pockets in the GroEL complex, and the pockets are capped transiently by the GroES “lid”. GroEL undergo substantial conformational changes, coupled toATP hydrolysis and the binding and release of GroES, which promote folding of the bound polypeptide. Although the structure of the GroEL/GroES chaperonin isknown, many details of its mechanism of action remain unresolved.

Finally, the folding pathways of a number of proteins require two enzymes that catalyze isomerisation reactions. Protein disulfide isomerase (PDI) is a widely distributed enzyme that catalyzes the interchange or shuffling of disulfide bonds until the bonds ofthe native conformation are formed (Figure 7). Among its functions, PDI catalyzes the elimination of folding intermediates with inappropriate disulfide cross-links.

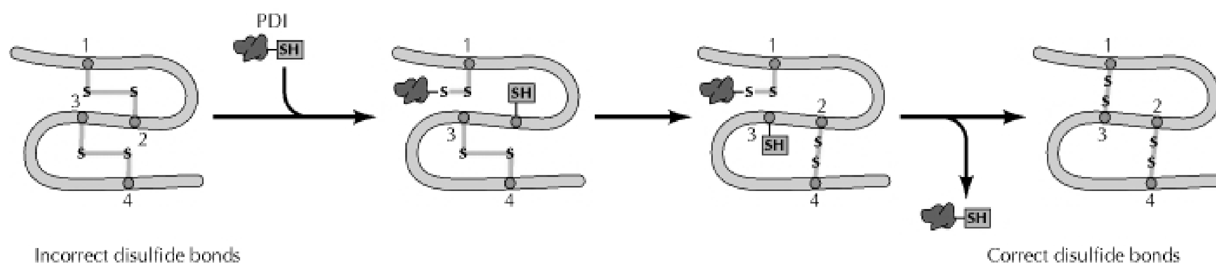


Figure. 7: The action of protein disulfide isomerase

Protein disulfide isomerase (PDI) catalyzes the breakage and rejoining of disulfide bonds, resulting in exchanges between paired disulfides in a polypeptide chain. The enzyme forms a disulfide bond with a cysteine residue of the polypeptide and then exchanges its paired disulfide with another cysteine residue. In this example, PDI catalyzes the conversion of two incorrect disulfide bonds (1-2 and 3-4) to the correct pairing (1-3 and 2-4).

Peptideprolyl cis-trans isomerase (PPI) catalyzes the interconversionof the cis and trans isomers of Pro peptidebonds (Figure 8), which can be a slow step in thefolding of proteins that contain some Pro residue peptidebonds in the cis conformation.

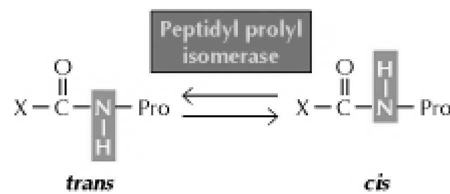


Figure 8 : The action of peptidyl prolyl isomerase:Peptidyl prolyl isomerase catalyzes the isomerization of peptide bonds that involve proline between the *cis* and *trans* conformations.

Protein Misfolding and Degenerative Diseases

For many proteins, the most prominent structural motif of the functional protein in its native conformation is known as the alpha helix, a right-handed spiral coil. When a protein becomes toxic, an extensive conformational change occurs and it acquires a motif known as the beta sheet. Note that the beta sheet conformation also exists in many functional native proteins, such as the immunoglobulins, but the transition from alpha helix to beta sheet is characteristic of amyloid deposits. The abnormal conformational transition from alpha helix to beta sheet exposes hydrophobic amino acid residues and promotes protein aggregation.

Most of the time, only the native conformation is produced in the cell. But as millions and millions of copies of each protein are made during our lifetimes, sometimes a random event occurs and one of these molecules follows the wrong path, changing into a toxic configuration. This kind of conformational change is most likely to occur in proteins that have repetitive amino acid motifs, such as polyglutamine; such is the case in Huntington's disease.

Remarkably, the toxic configuration is often able to interact with other native copies of the same protein and catalyze their transition into the toxic state. Because of this ability, they are known as infective conformations. The newly made toxic proteins repeat the cycle in a self-sustaining loop, amplifying the toxicity and thus leading to a catastrophic effect that eventually kills the cell or impairs its function. A prime example of proteins that catalyze their own conformational change into the toxic form is the prion proteins,

Under normal circumstances, the cell has mechanisms to prevent proteins from folding incorrectly, as well as to get rid of misfolded proteins. Proteins that have problems achieving

their native configuration are helped by chaperones to fold properly, using energy from ATP. Chaperones can avoid the conformational change to beta sheet structure and the aggregation of these altered proteins; thus they seem fundamental to the prevention of protein misfolding. Despite chaperone actions, some proteins still misfold, but there is a remedy: The misfolded proteins can be detected by quality-control mechanisms in the cell that tags them to be sent to the cytoplasm, where they will be degraded (Figure 9).

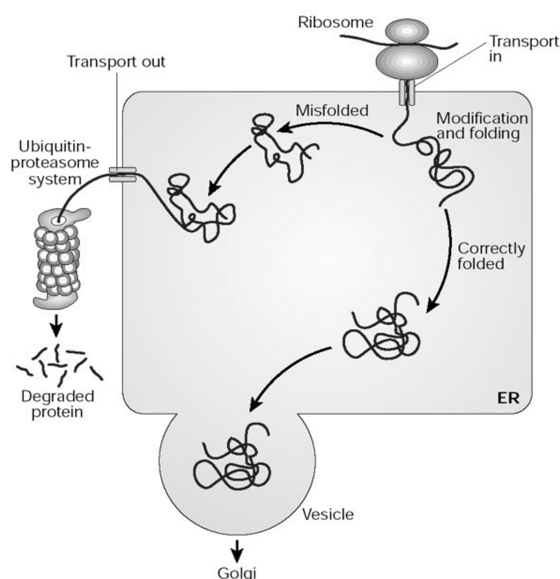


Figure 9 : A schematic diagram shows how a newly synthesized protein is transported from a ribosome into the lumen of the endoplasmic reticulum (ER) where modification and folding of the nascent polypeptide occurs. A newly translated protein, represented by a purple squiggly line, is exiting the ribosome and entering the ER through the pore. After the protein enters the ER lumen, it can follow two pathways to different destinations in the cell, depending on whether it is folded correctly or misfolded. A correctly folded protein is shown entering a budding vesicle at the bottom edge of the ER, where it will be transported to the Golgi apparatus. A misfolded protein is exported from the ER to the cytosol where it is degraded by the ubiquitin-proteasome system.

Misfolded Proteins and Neurodegenerative Diseases

Accumulation of misfolded proteins can cause disease, and unfortunately some of these diseases, known as amyloid diseases, are very common. The most prevalent one is Alzheimer's disease, which affects about 10 percent of the adult population over sixty-five years old in North America. Parkinson's disease and Huntington's disease have similar amyloid origins. These diseases can be sporadic (occurring without any family history) or familial (inherited). Regardless of the type, the risk of getting any of these diseases increases dramatically with age. The mechanistic explanation for this correlation is that as we age (or as a result of mutations), the delicate balance of the synthesis, folding, and degradation of proteins is perturbed, resulting in the production and accumulation of misfolded proteins that form aggregates (Figure 10).

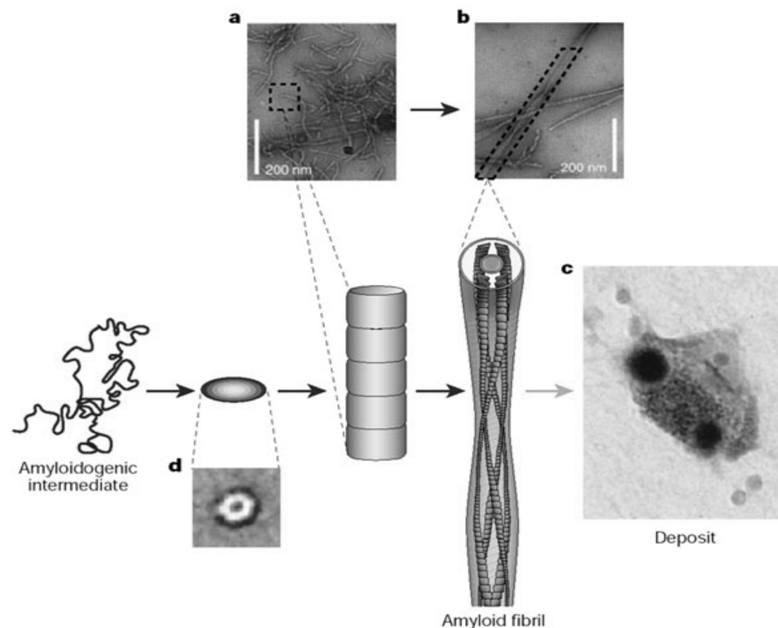


Figure.10 : Unfolded or misfolded amyloid proteins associate with each other in different stages, starting with the formation of a small, soluble aggregate, which is shown on the far left side of this diagram as a purple squiggly line that is labeled "amyloidogenic intermediate." A black arrow points to the right to indicate that the soluble aggregates can

form a circular early aggregate, which is shown as a blue oval shape. Another black arrow points to the right to indicate that the early aggregates can combine to form a protofilament, which is shown as five vertically stacked cylinders. Another black arrow points to the right to indicate that protofilaments can combine to form a mature amyloid fibril, which is shown as four protofilaments that are twisted around each other with a green-colored border around them. A green arrow points to the right to indicate that mature fibrils can combine to create plaques or other structures, which is shown as an electron micrograph of Lewy body structures that are associated with Parkinson's disease. A series of panels extend from the illustrations to additional electron micrographs of the corresponding structures to show how they appear in a cell. Panel A is an electron micrograph of a protofibril that extends from the illustration of the protofibril; it shows grey filamentous structures that are approximately 10 nm in diameter. Panel B is an electron micrograph of a mature amyloid fibril that extends from the illustration of the same structure; they look much like the protofibrils shown in panel A, but the mature amyloid fibrils are thicker.

Among the environmental factors known to increase the risk of suffering degenerative diseases is exposure to substances that affect the mitochondria, increasing the amount of oxidative damage to proteins. However, it is clear that no single environmental factor determines the onset of these disorders. In addition, there are genetic factors. For example, in the simplest forms of familial Parkinson's disease, mutations are associated with dominant forms of the disease. This means that an individual with a single copy of a defective gene will develop the disease, yet two copies of the defective gene are required for recessive forms of the disease to develop. In the case of Alzheimer's disease, and for other less common neurodegenerative diseases, the genetics can be even more complicated, since different mutations of the same gene and combinations of these mutations may differently affect disease risk.

Misfolding in Non-neurological Diseases :

Protein aggregation diseases are not exclusive to the central nervous system; they can also appear in peripheral tissues. In general, the genes and protein products involved in these kinds of diseases are called amyloidogenic. Such diseases include type 2 diabetes, inherited cataracts, some forms of atherosclerosis, hemodialysis-related disorders, and short-chain

amyloidosis, among many others. All these diseases have in common the expression of a protein outside its normal context, leading to an irreversible change into a sticky conformation rich in beta sheets that make the protein molecules interact with each other.

The general pattern that emerges in all these diseases is an abnormal tendency of proteins to aggregate as a result of misfolding. The aggregation can be caused by chance; by protein hyperphosphorylation (a condition where multiple phosphate groups are added to the protein), by prion self-catalytic conformational conversion, or by mutations that make the protein unstable. Aggregation can also be caused by an unregulated or pathological increase in the intracellular concentration of some of these proteins. Such imbalances in protein concentration can be a consequence of mutations such as duplications of the amyloidogenic gene or changes in the protein's amino acid sequence. Imbalances can also be caused by deficiencies in the proteasome, the cellular machinery involved in the degradation of aging proteins. Inhibition of autophagy (a process by which cells engulf themselves) also promotes amyloid aggregation. In addition, some evidence suggests that the severity of these diseases correlates with an increase in oxidative stress, mitochondrial dysfunction, alteration of cytoplasmic membrane permeability, and abnormal calcium concentration (Table 1).

Table 1

Disease	**Genetic causes**	**Function**
Alzheimer's disease	APP	Gives rise to Aβ, the primary component of senile plaques
Parkinson's disease	PS1 and PS2	A component of γ-secretase, which cleaves APP to yield Aβ
Parkinson's disease	α-Synuclein	The primary component of Lewy bodies
Parkinson's disease	Parkin	A ubiquitin E3 ligase
Parkinson's disease	DJ-1	Protects the cell against oxidant-induced cell death

Parkinson's disease	PINK1	A kinase localized to mitochondria. Function unknown. Seems to protect against cell death
Parkinson's disease	LRRK2	A kinase. Function unknown
Parkinson's disease	HTRA2	A serine protease in the mitochondrial intermembrane space. Degrades denatured proteins within mitochondria. Degrades inhibitor of apoptosis proteins and promotes apoptosis if released into the cytosol
Amyotrophic lateral sclerosis	SOD1	Converts superoxide to hydrogen peroxide. Disease-causing mutations seem to confer a toxic gain of function
Huntington's disease	Huntingtin	Function unknown. Disease-associated mutations produce expanded polyglutamine repeats

Probable Questions :

1. How amino acid sequence determine tertiary sequence of a protein.

2. Discuss protein folding.

3. Discuss interrelation between protein misfolding and degenerative diseases.

4. Misfolded Proteins and Neurodegenerative Diseases

Suggested Readings :

Lin, M. T. & Beal, M. F. Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. *Nature* 443, 787–795 (2006) doi:10.1038/nature05292.

Unit VI

Bioenergetics and Oxidative Metabolism

Objectives:

In this Unit we will discuss about Bioenergetics and Oxidative Metabolism

Introduction :

Bioenergetics or biochemical thermodynamics is the study of energy changes in biochemical reactions. Non-biologic systems use heat energy to accomplish work but biologic systems are isothermic and utilize chemical energy for the living process.

Free Energy and the Laws of Thermodynamics :

i. Free energy (ΔG) is the useful energy also known as the chemical potential.

ii. The first law of thermodynamics states that "the total energy of a system plus its surroundings remains constant". This is also the laws of conservation of energy. Energy may be transferred from one part to another or may be transformed into another form of energy.

iii. The second law of thermodynamics states that "the total entropy of a system must increase if a process is to occur spontaneously".

Entropy represents the extent of disorder of the system and becomes maximum when it approaches true equilibrium. Under constant temperature and pressure, the relationship between the free energy change (ΔG) and the change in entropy (ΔS) is given by the following equation which combines the two laws of thermodynamics.

$$\Delta G = \Delta H - T\Delta S$$

where ΔH = the change in enthalpy (heat) and T = the absolute temperature. Under biochemical reactions AH is approximately equal to AE.

So the above relationship may be expressed in the following manner :

$\Delta G = \Delta H - T\Delta S$

If ΔG is negative in sign, the reaction proceeds spontaneously with loss of free energy i.e. it is exergonic. On the other hand, if ΔG is positive, the reaction proceeds with the gain of energy

i.e. it is endergonic. If the magnitude of ΔG is great, the system is stable. If ΔG is zero, the system is at equilibrium.

Coupling of Endergonic to Exergonic Processes:

i. The vital processes (Synthetic reactions, muscular contraction, nerve impulse conduction, and active transport) obtain energy by chemical linkage or coupling to oxidative reactions.

ii. Metabolite A is converted to metabolite B with the release of free energy. It is coupled to another reaction in which free energy is required to convert metabolite C to metabolite D. Some of the energy liberated in the degradative reaction is transferred to the synthetic reaction. The exergonic reactions are termed Catabolism (the breakdown or oxidation of fuel molecules), whereas the synthetic reactions are termed Anabolism.

iii. Reaction shown in Fig. 11.1 has to go from left to right, then the overall process must be accompanied by loss of free energy as heat.

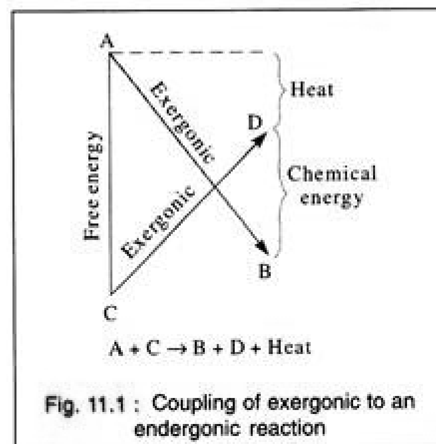
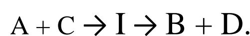


Fig. 11.1 : Coupling of exergonic to an endergonic reaction

One possible mechanism of coupling is shown :


A + C → I → B + D.

Some exergonic and endergonic reactions in biologic systems are coupled in this way. An extension of the coupling concept is provided by dehydrogenation reactions which are coupled to hydrogenations by an intermediate carrier.

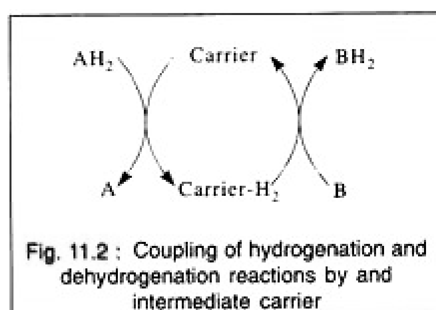


Fig. 11.2 : Coupling of hydrogenation and dehydrogenation reactions by and intermediate carrier

iv. The alternative process of coupling from an exergonic to an endergonic process is to synthesize a compound of high energy potential in the exergonic reaction and to incorporate this new compound into the endergonic reaction, thus effecting a transference of free energy from the exergonic to the endergonic pathway

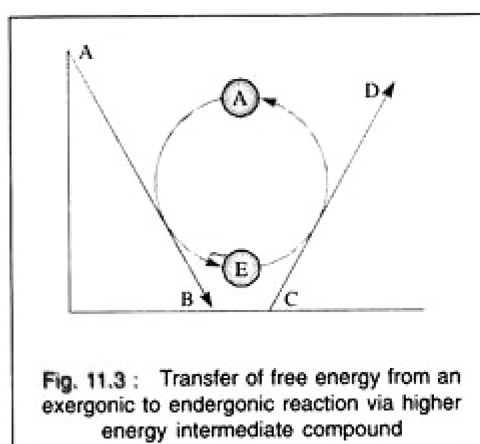


Fig. 11.3 : Transfer of free energy from an exergonic to endergonic reaction via higher energy intermediate compound

In the living cell, the principal high energy intermediate or carrier compound (designated ~ Ⓔ is ATP).

Role of energy Phosphates in Bioenergetics & energy capture:

a. Autotrophic organisms couple their metabolism to some simple exergonic process in their surroundings e.g., green plants utilize the energy of sunlight, and some autotrophic bacteria utilize the reaction Fe^{++} Fe^{+++}.

b. Heterotrophic organisms obtain free energy by coupling their metabolism to the breakdown of complex organic molecules in their environment.

c. In all these processes, ATP plays an important role in the transfer of free energy from the exergonic to the endergonic processes. ATP is a nucleotide consisting of adenine, ribose, and three phosphate groups. In its reaction in the cell, it functions as the Mg^{++} complex.

d. ATP was considered to be a means of transferring phosphate radicals in the process of phosphorylation. Lipmann introduced the concept of “high-energy phosphates” and the “high-energy phosphate bond” :

High-energy Phosphates :

Lipmann introduced the symbol ~ ℗, indicating high-energy phosphate bond. The term group transfer potential is preferred to “high-energy bond”. Thus, ATP contains 2 high-energy phosphate groups and ADP contains one. The phosphate bond in AMP is of the low energy type, since it is a normal ester link

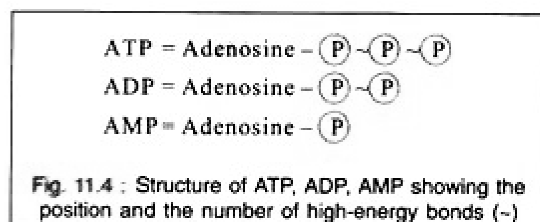


Fig. 11.4 : Structure of ATP, ADP, AMP showing the position and the number of high-energy bonds (~)

Role of High-energy Phosphates as the “Energy Currency” of the Cell :

ATP is the donor of high-energy phosphate and ADP can accept high-energy phosphate to form ATP. ATP/ADP cycle connects these processes which generate ~ ℗ to those processes that utilize ~ ℗.

There are three major sources of taking part in energy conservation or energy capture :

(a) Oxidative phosphorylation :

This is the greatest quantitative source of ~℗ aerobic organisms. The free energy comes from respiratory chain oxidation within mitochondria.

(b) Glycolysis :

A net formation of 2 ~ ℗ results from the formation of lactate from one molecule of glucose, generated in two reactions catalyzed by phosphoglycerate kinase and pyruvate kinase.

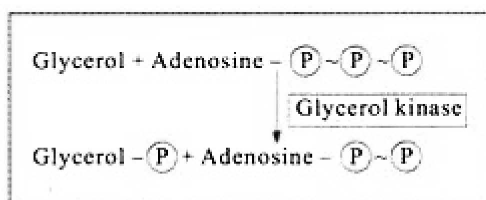
(c) Citric acid cycle :

One ~ ℗ is generated directly in the cycle at the succinyl thiokinase step.

Another group of compounds (Phosphagens) act as storage forms of high-energy phosphate. These include creatine phosphate in vertebrate muscle and brain, arginine phosphate in invertebrate muscle.

In physiologic conditions, phosphagens permit ATP concentrations to be maintained in muscle when ATP is being rap idly used as a source of energy for muscular contraction. When ATP is abundant, its concentration can cause the reverse reaction to take place and allow the concentration of creatine phosphate to increase abundantly so as to act as a store of high-energy phosphate.

When ATP acts as a phosphate donor to form those compounds of lower free energy of hydrolysis, the phosphate group is invariably converted to one of low energy e.g. :



Inter Conversion of Adenine Nucleotides :

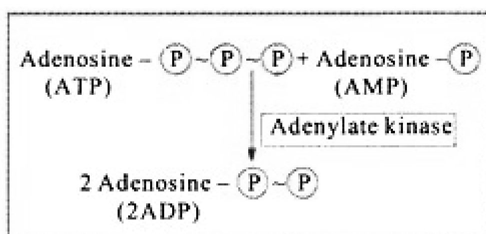
The enzyme Adenylate Kinase (myokinase) is present in most cells. It catalyzes the inter-conversion of ATP and AMP on the one hand and ADP on the other.

The reaction has three functions:

i. It allows high-energy phosphate in ADP to be used in the synthesis of ATP.

ii. It allows AMP to be recovered by re-phosphorylation to ADP.

iii. It allows AMP to increase in concentration when ATP becomes depleted and act as a metabolic signal to increase the rate of catabolic reactions which, in turn leads, to the generation of more ATP :



This reaction is accompanied by loss of free energy as heat which ensures that the activation reaction will go to the right. This is further aided by the hydrolytic splitting of PPi by inorganic Pyrophosphatase. The activation via the pyrophosphate pathway results in the loss of 2 ~®rather than one ~ ®as occurs when ADP and Pi are formed.

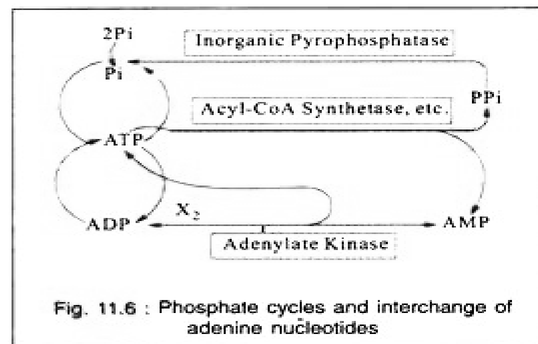


Fig. 11.6 : Phosphate cycles and interchange of adenine nucleotides

Mitochondria acts as the respiratory organelle of the cell where foodstuffs i.e. carbohydrates and fats are completely oxidized into CO_2 and H_2O. The energy released during the above process is utilized by the mitochondria for the synthesis of energy rich compound i.e. ATP.

The process of energy transformation that occurs in mitochondria is based on four steps :

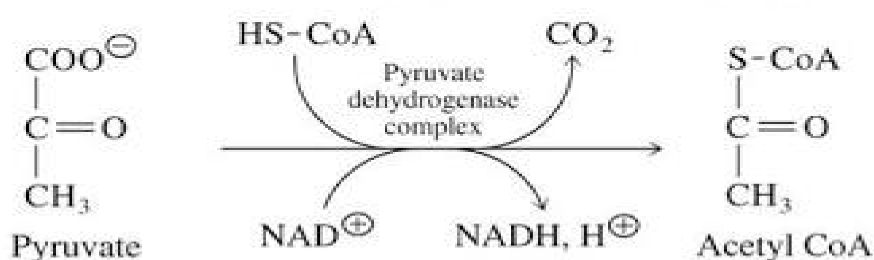
1. Oxidative decarboxylation of pyruvic acid to acetyl CoA.
2. Krebs cycle.
3. Electron transport system.
4. Phosphorylating system that synthesizes ATP

Oxidative decarboxylation of pyruvic acid :

The first step of cellular metabolism is glycolysis that occurs in the cytosol. The resulting end product pyruvic acid undergoes oxidative decarboxylation that is catalyzed by a multienzyme complex known as the pyruvate dehydrogenase complex. Pyruvate is decarboxylated in presence of lipoic acid and thiamine pyrophosphate (TPP) to form S-acetyl lipoate. This

reaction is catalyzed by pyruvate dehydrogenase. TPP acts as a prosthetic group of pyruvate dehydrogenase complex and α-ketogluterate dehydrogenase complex.

This S-acetyl lipoate reacts with coenzyme A to form Acteyl CoA and reduced lipoate. This reduced lipoate is oxidized by FAD. The reduced FAD is oxidized by NAD^+. Now this reduced NAD ($NADH + H^+$) enters the electron transport chain to produce 3 molecules of ATP.

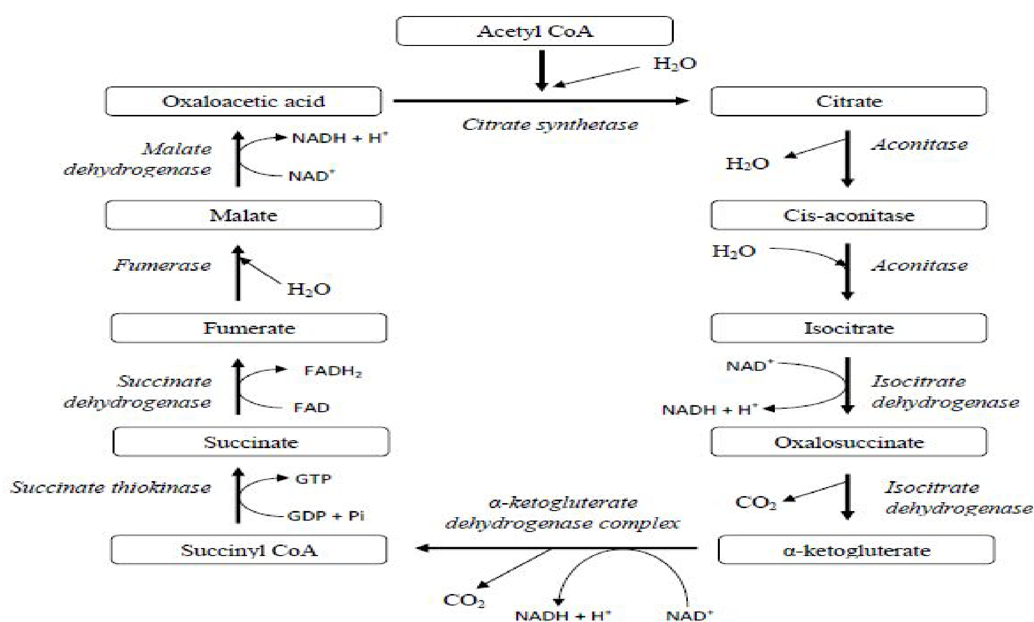


The acetyl CoA now enters the Krebs cycle where it is utilized to synthesize ATP. If ATP level is high in the cellular environment, acetyl CoA is sent to another metabolic pathway like production of fatty acid.

Krebs cycle :

This is a cyclic reaction that oxidizes the acetyl CoA within the mitochondria to form CO_2 and H_2O in aerobic condition. In this process, acetyl CoA combines with oxaloacetate to form citric acid (tricarboxylic acid). So, this process is also known as Tricarboxylic acid cycle (TCA cycle) or citric acid cycle.

This cyclic reaction is described in the flow chart given below :



Significance of the Krebs cycle :

1. It acts as the common metabolic pathway for the oxidation of carbohydrates, lipids and proteins because glucose, fatty acid and many amino acids are metabolized into pyruvate that takes part in the TCA cycle.

2. The reducing equivalents in form of hydrogen or electrons enter the respiratory chain or the electron transport system where ATP is formed by oxidative phosphorylation.

3. The TCA cycle is amphibolic in nature that is both catabolic and anabolic reactions take place in the cycle.

4. The enzymes required for the TCA cycle are located in the mitochondria, either free or attached to the inner surface of the inner mitochondrial membrane, that helps the reducing equivalents to be easily transferred to the adjacent enzymes of the respiratory chain.

Electron transport system :

The hydrogen attached with $FADH_2$ and NADH breaks up to form one proton (H^+) and one electron (e^-). These electrons are utilized by the electron transport chain to form ATP, that is

the main source of cellular energy. The electron carriers in the electron transport chain are arranged in an order of decreasing energy level. So, the transfer of electron from one carrier to another release some of their potential energy that is used to form ATP.

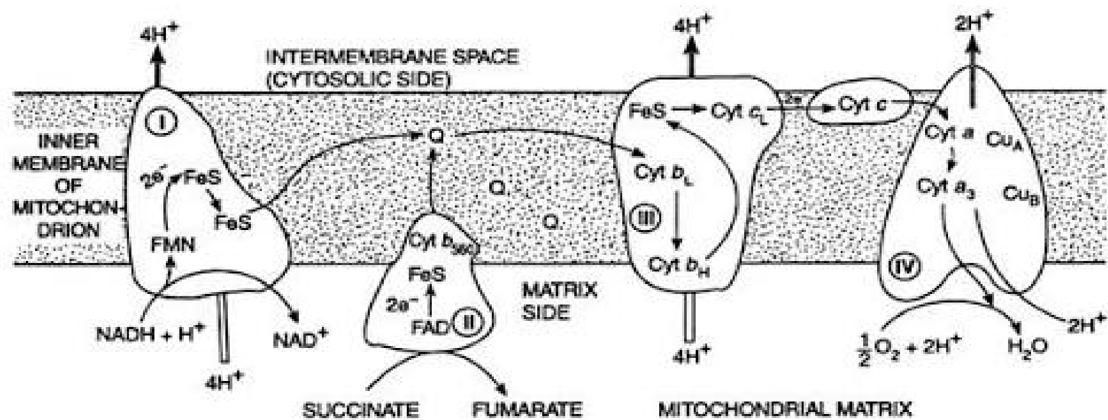
The ETS is composed of flavins, FeS complex, quinines and cytochromes. Common quinine is ubiquinone or CoQ. It is mobile in the membranes. The cytochromes involved are cyt*b*, cyt*c*, cyte_1, cyt*a* and cyta_3. The contain iron which can undergo $Fe^{2+} \rightarrow Fe^{3+} \rightarrow Fe^{2+}$ change during passage of electrons. Cyta_3 additionally contains copper, which helps in transferring electrons to oxygen.

1. **Complex I :** NADH + H^+ hands over its H^+ and e^- to to FMN, that forms $FMNH_2$. $FMNH_2$ then passes the proton (H^+) to outer chamber and transfers the electron to FeS.

2. **Complex II :** Coenzyme Q or CoQ receives electrons from FeS, picks up H^+ from matrix and produces QH_2. $FADH_2$ also transfers its protons and electrons to CoQ. QH_2 passes protons to outer chamber and electrons to cyt*b*.

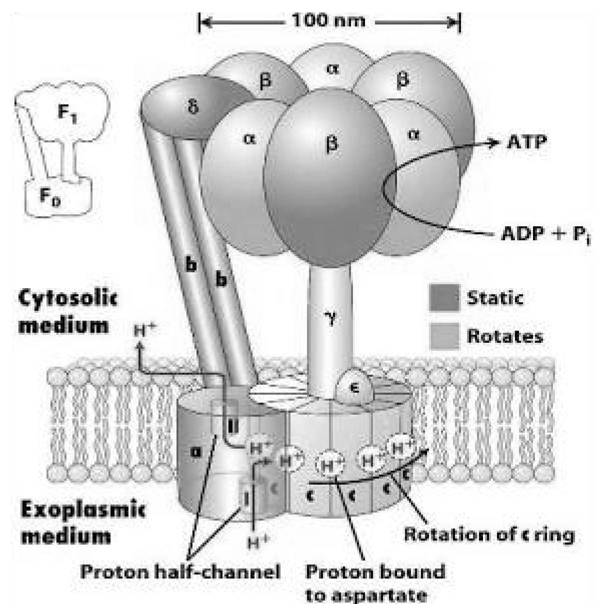
3. **Complex III :** cyt*b* passes its electrons to FeS which in turn hands over the same to CoQ. The later repeats the process of picking protons from mitochondrial matrix and passing them to the outer chamber.

4. **Complex IV:** Electrons are now handed over to cytc_1. The path of electron transfer of electrons within this comples is cyt$c_1 \rightarrow$cyt*c*$\rightarrow$cyt*a*$\rightarrow$cyta_3. Cyta_3 contains two copper centres. Both cyta_3 and cyt*a* function as cytochrome oxidase and hands over electrons to oxygen. Oxygen is able to diffuse into inside of mitochondria, where it is converted to anionic form (O^{2-}). This O^{2-} combines with $2H^+$ and forms metabolic water. Reduced coenzyme NADH + H^+ helps in pushing out 3 pairs of H^+ to outer chamber while $FADH_2$ sends 2 pairs of H^+ to the outer chamber.



Oxidative phosphorylation :

It is the synthesis of ATP from ADP and inorganic phosphate, which occurs with the help of energy obtained from oxidation of reduced coenzymes formed in cellular respiration.In 1961, Mitchell proposed a mechanism for the coupling of electron transfer through mitochondria to ATP synthesis. He suggested that, as electrons are passed down the electron transport chain, protons are pumped across the membrane. This results in a pH and electrical gradient. The protons move back into the matrix through a pore created by ATP synthase allowing the enzyme to produce ATP at the expense of this gradient. This has been called the “chemiosmotic theory”.



The ATP synthase, which is embedded in cristae of the inner mitochondrial membrane, includes two major subunits:

1. F_1: the catalytic subunit, made of 5 polypeptides with stoichiometry $\alpha_3\beta_3\gamma\delta\varepsilon$.

2. F_0 : a complex of integral membrane proteins that mediated proton transport.

The F_1F_0 complex couples ATP synthesis to H^+ transport into the mitochondrial matrix. Transport of at least $3H^+$ per ATP synthesized is required. The phosphorus/oxygen ratio (P/O) that is ATP made per oxygen atom reduced is about 3 for NADH and 2 for $FADH_2$.

ATP produced in the mitochondria must exit to the cytosol to be used by transport pumps, kinases, etc. ADP and Pi, arising from ATP hydrolysis in the cytosol, must re-enter the mitochondria to be converted again to ATP. Two carrier proteins in the inner mitochondrial membrane are required for this metabolic cycle. The outer membrane is considered to be not a permeability barrier. The large VDAC channels in the outer membrane are assumed to allow passage of adenine nucleotides and Pi. The Adenine Nucleotide Translocase (ADP/ATP carrier) is an antiporter that catalyzes exchange of ADP for ATP across the inner mitochondrial membrane.

Total ATP production from TCA cycle:

Isocitrate to Oxalosuccinate (1 molecule of NADH) →	3 molecules of ATP
α-ketogluterate to Succinyl CoA (1 molecule of NADH) →	3 molecules of ATP
Succinyl CoA to Succinate (substrate level phosphorylation)→	1 molecule of ATP
Succinate to Fumerate (1 molecule of $FADH_2$) →	2 molecules of ATP
Malate to Oxaloacetate (1 molecule of NADH) →	3 molecules of ATP
Total →	12 molecules of ATP

Total ATP production in complete oxidation of one molecule of glucose :

One molecule of glucose forms 2 molecules of pyruvic acid by glycolysis

From glycolysis (2 molecules of NADH 2 molecule of ATP) →	8 molecules of ATP
From oxidation of pyruvate (1 molecule of NADH) →	6 molecules of ATP (3 x 2)
From TCA cycle →	24 molecules of ATP (12x2)
Total →	**38 molecules of ATP**

Probable questions :

1. Define Bioenergetics. State the laws of thermodynamics.

2. Describe the role of energy Phosphates in Bioenergetics & energy capture.

3. Write a note on Role of High-energy Phosphates as the "Energy Currency" of the Cell.

4. Briefly describe the oxidative decarboxylation of pyruvic acids in cell.

5. What is Krebs Cycle. Describe the significance of Krebs cycle.

6. What is Electron Transport Chain? Describe the different complex of ETC.

7. What is Oxidative Phosphorylation. Describe the structure of F_0F_1 particle.

8. How many ATP are produced in complete oxidation of one molecule of glucose?

Suggested Readings and References :

1.http://www.biologydiscussion.com/biochemistry/bioenergetics/essay-on-bioenergetics-biochemistry/42121

2. Lehninger Principles of Biochemistry. Nelson and Cox. Seventh edition.

3. Fundamentals of Biochemistry. J L Jain, Nitin Jain, Sunjay Jain. S. Chand Publications.

4. Biochemistry, by Jeremy M. Berg and John L. Tymoczko. Seventh Edition

Unit VII

Thermodynamic principles and steady-state conditions of living organism

Objectives :

1. Understanding the laws of thermodynamics
2. Elucidating how the energy of a chemical reaction changes
3. How the laws of thermodynamics are conserved in a chemical reaction within the biological system

The three laws of thermodynamics define fundamental physical quantities (temperature, energy, entropy) that characterize a thermodynamic system. The three laws are as follows :

First law : It states that energy cannot be created or destroyed in an isolated system, but it can be transformed from one form to another. This law is also known as the law of conservation of energy.

Second law : It states that the entropy of an isolated system always increases, where entropy refers to the measure of a system's thermal energy per unit of temperature that is unavailable for doing useful work.

Third law : This law states that the entropy of a system approaches constant value as the temperature approaches absolute zero, which is the lowest temperature theoretically possible.

Living organisms consist of collection of molecules much more highly organized than the surrounding materials from which they are constructed. This may seem like that the living organisms violate the second law of thermodynamics, but actually they operate strictly within it. To discuss the application of the second law of thermodynamics in biological system, we must first define the system and the universe in which they occur. The reacting system is a collection of matter that may be an organism, a cell or two reacting compounds. The reacting system and its surrounding together constitute the universe. Living cells and organisms are

open systems which exchange both material and energy with the surrounding. Living systems are never at equilibrium with their surroundings.

Energy changes occurring in a chemical reaction :

1. Gibb's free energy (G) expresses the amount of energy capable of doing work during a reaction at constant temperature and pressure.

2. When a reaction proceeds with the release of free energy, the free energy change (ΔG) has a negative sign and the reaction is said to be exergonic.

3. In endergonic reaction, the system gains the energy and the ΔG is positive.

4. Enthalpy (H) is the heat content of the reacting system. It reflects the number and kinds of chemical bonds formed or broken during the chemical reaction in the reactants and products. When a chemical reaction releases heat, it is said to be exothermic, i.e. the heat content of the product is less than the reactants and ΔH is negative. Reacting system that take up heat from the surroundings are termed as endothermic and there the ΔH is positive.

5. Entropy is a quantitative expression of randomness or disorder of a system. When the products of a reaction are less complex and more disordered than the reactants, the reaction is said to proceed with a gain of entropy.

6. The unit of ΔG and ΔH are Joules/mole or Calories/mole. Under the condition existing in biological system, the changes in free energy, enthalpy and entropy are related with each other quantitatively by an equation: $\Delta G = \Delta H - T\Delta S$. In which, ΔG is the change in Gibb's free energy of the reacting system and ΔH is the change in enthalpy. T is the absolute temperature and ΔS is the change in entropy of the reacting system.

By convention, ΔS has a positive sign when entropy increases and ΔH has a negative sign when heat is released by the system into the surroundings. Either of these conditions which are typical for favourable processes will tend to make ΔG negative. In fact, ΔG of spontaneously reacting system is always negative.

The second law of thermodynamics states that entropy of the universe increases during all chemical and physical processes but it doesn't require that the increase in entropy take place in the reacting system. The order produced within the cells as they grow and divide is more than the disorder created by their surroundings in the course of growth and division. In short, living organisms preserve their internal order by taking from their surrounding free energy in the form of nutrients or sunlight and returning to their surrounding an equal amount of energy as heat and entropy.

Cells are isothermal system. They function at essentially constant temperature. Heat flow is not a source of energy for the cells because heat can do work only as it passes from a zone or object at one temperature to a zone of object at a lower temperature. The energy that cells can and must use is free energy described by Gibb's free energy function, which allows prediction of the direction of the chemical reaction, theor exact equilibrium position and the amount of work they can in theory at constant temperature and pressure. Heterotrophic cells acquire its energy from nutrient molecules and photosynthetic cells acquire it from sunlight. Both kind of cells transform this free energy into ATP and other energy-rich compound (NADH), capable of providing energy for biological functions at constant temperature.

An important thermodynamic fact is that overall free energy of a chemically coupled series of reactions is equal to the sum of the free energy changes of individual states. This fact can describe the spontaneous nature of many biological reactions that seems not to be spontaneous. As for example, the free energy change (ΔG) of the synthesis of Glucose-6-phosphate from Glucose, which is the first step for utilization of glucose in many organisms, is +13.8 kJ/mole. The positive value for ΔG predicts that the reaction will not proceed spontaneously at standard condition. Another cellular reaction, the hydrolysis of ATP to ADP + Pi is very much exergonic and the free energy change (ΔG) is – 30.5 kJ/mole. These to reactions couple with each other and the overall change in free energy becomes (13.8 kJ/mole + (- 30 kJ/mole)) = - 16.7 kJ/mole. So, in standard condition, the reaction transformation of glucose to glucose-6-phosphate is a spontaneous reaction.

Probable questions :

1. State the laws of thermodynamics.
2. How does the energy state change during a chemical reaction?
3. Describe with example that how the laws of thermodynamics are conserved during a favourable and unfavourable chemical reaction.

Suggested Readings/ References :

1. Cox, M.M and Nelson, D.L. (2008). Lehninger's Principles of Biochemistry, V Edition, W.H. Freeman and Co., New York.

2. Berg, J.M., Tymoczko, J.L. and Stryer, L.(2007). Biochemistry, VI Edition, W.H. Freeman and Co., New York.

3. Murray, R.K., Bender, D.A., Botham, K.M., Kennelly, P.J., Rodwell, V.W. and Well, P.A. (2009). Harper's Illustrated Biochemistry, XXVIII Edition, International Edition, The McGraw- Hill Companies Inc.

4. Donald Voet and Judith G. Voet Biochemistry

Unit VIII

Protein Metabolism: Amino acid Classification and Urea Cycle

Objectives :

1. Understanding different types of amino acids and their structures
2. Understanding the importance of urea cycle
3. To learn the intermediates, enzymes for the conversion of NH_3 to urea
4. To understand the regulation of urea cycle

Amino acids

Amino acids are a group of organic compounds containing two functional groups amino and carboxyl. The amino group (-NH2) is basic while the carboxyl group (-COOH) is acidic in nature.

General structure of amino acids

The amino acids are termed as α-amino acids, if both the carboxyl and amino groups are attached to the same carbon atom, as depicted below. The α-carbon atom binds to a side chain represented by R which is different for each of the 20 amino acid found in proteins. The amino acids mostly exist in the ionized form in the biological system.

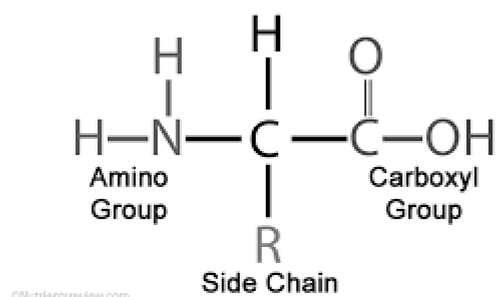


Figure: Optical isomers of amino acids

If a carbon atom is attachedto four different groups, it is asymmetricand therefore exhibits optical isomerism. The amino acids (except glycine) possess four distinct groups (R, H, COO-, NH-) held by c-carbon.Thus all the amino acids (except glycine where R = H) have optical isomers. The structures of L- and D-amino acids are written based on the configuration of L- and D-glyceraldehyde.The proteins are composed of L-α-amino acids.

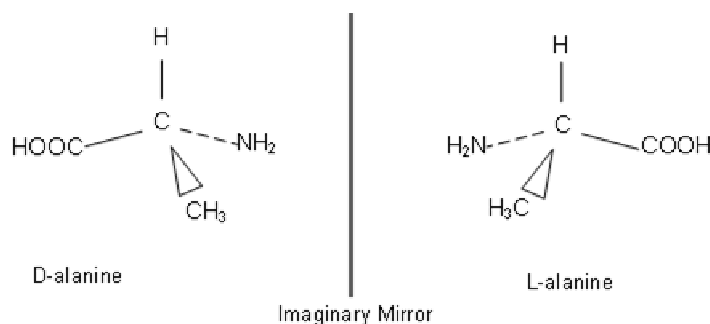


Figure: Optical isomerism of Amino acids

Amino acids classification

Knowledge of the chemical properties of the common amino acids is central to an understanding of biochemistry. The topic can be simplified by grouping the amino acids into five main classes based on the properties of their R groups in particular, their polarity, or tendency to interact with water at biological pH (near pH 7.0). The polarity of the R groups varies widely, from nonpolar and hydrophobic (water-insoluble) to highly polar and hydrophilic (water-soluble). The structures of the 20 common amino acids are shown in Figure 1. Within each class there are gradations of polarity, size, and shape of the R groups.

Nonpolar, Aliphatic R Groups

The R groups in this class of amino acids are nonpolar and hydrophobic.The side chains of alanine, valine, leucine, and isoleucine tend to cluster together within proteins, stabilizing protein structure by means of hydrophobic interactions. Glycine has the simplest structure. Although it is most easily grouped with the nonpolar amino acids, its very small side chain makes no real contribution to hydrophobic interactions. Methionine, one of the two sulfur-containing amino acids, has a nonpolar thioether group in its side chain. Proline has an

aliphatic side chain with a distinctive cyclic structure. The secondary amino (imino) group of proline residues is held in a rigid conformation that reduces the structural flexibility of polypeptide regions containing proline.

Aromatic R Groups Phenylalanine, tyrosine, and tryptophan, with their aromatic side chains are relatively nonpolar (hydrophobic). All can participate in hydrophobic interactions. The hydroxyl group of tyrosine can form hydrogen bonds, and it is an important functional group in some enzymes. Tyrosine and tryptophan are more polar than phenylalanine, because of the tyrosine hydroxyl group and the nitrogen of the tryptophan indole ring.

Polar, Uncharged R Groups

The R groups of these amino acids are more soluble in water, or more hydrophilic, than those of the nonpolar amino acids, because they contain functional groups that form hydrogen bonds with water. This class of amino acids includes serine, threonine, cysteine, asparagine, and glutamine. The polarity of serine and threonine is contributed by their hydroxyl groups; that of cysteine by its sulfhydryl group, which is a weak acid and can make weak hydrogen bonds with oxygen or nitrogen; and that of asparagine and glutamine by their amide groups. Asparagine and glutamine are the amides of two other amino acids also found in proteins, aspartate and glutamate, respectively, to which asparagine and glutamine are easily hydrolyzed by acid or base. Cysteine is readily oxidized to form a covalently linked dimeric amino acid called cystine, in which two cysteine molecules or residues are joined by a disulfide bond. The disulfide-linked residues are strongly hydrophobic (nonpolar). Disulfide bonds play a special role in the structures of many proteins by forming covalent links between parts of a polypeptide molecule or between two different polypeptide chains.

Positively Charged (Basic) R Groups

The most hydrophilic R groups are those that are either positively or negatively charged. The amino acids in which the R groups have significant positive charge at pH 7.0 are lysine, which has a second primary amino group at the position on its aliphatic chain; arginine, which has a positively charged guanidinium group; and histidine, which has an aromatic imidazole group. As the only common amino acid having an ionizable side chain with pKa

near neutrality, histidine may be positively charged (protonated form) or uncharged at pH 7.0. His residues facilitate many enzyme-catalyzed reactions by serving as proton donors/acceptors.

Negatively Charged (Acidic) R Groups The two amino acids having R groups with a net negative chargeat pH 7.0 are aspartate and glutamate, each of which has a second carboxyl group.

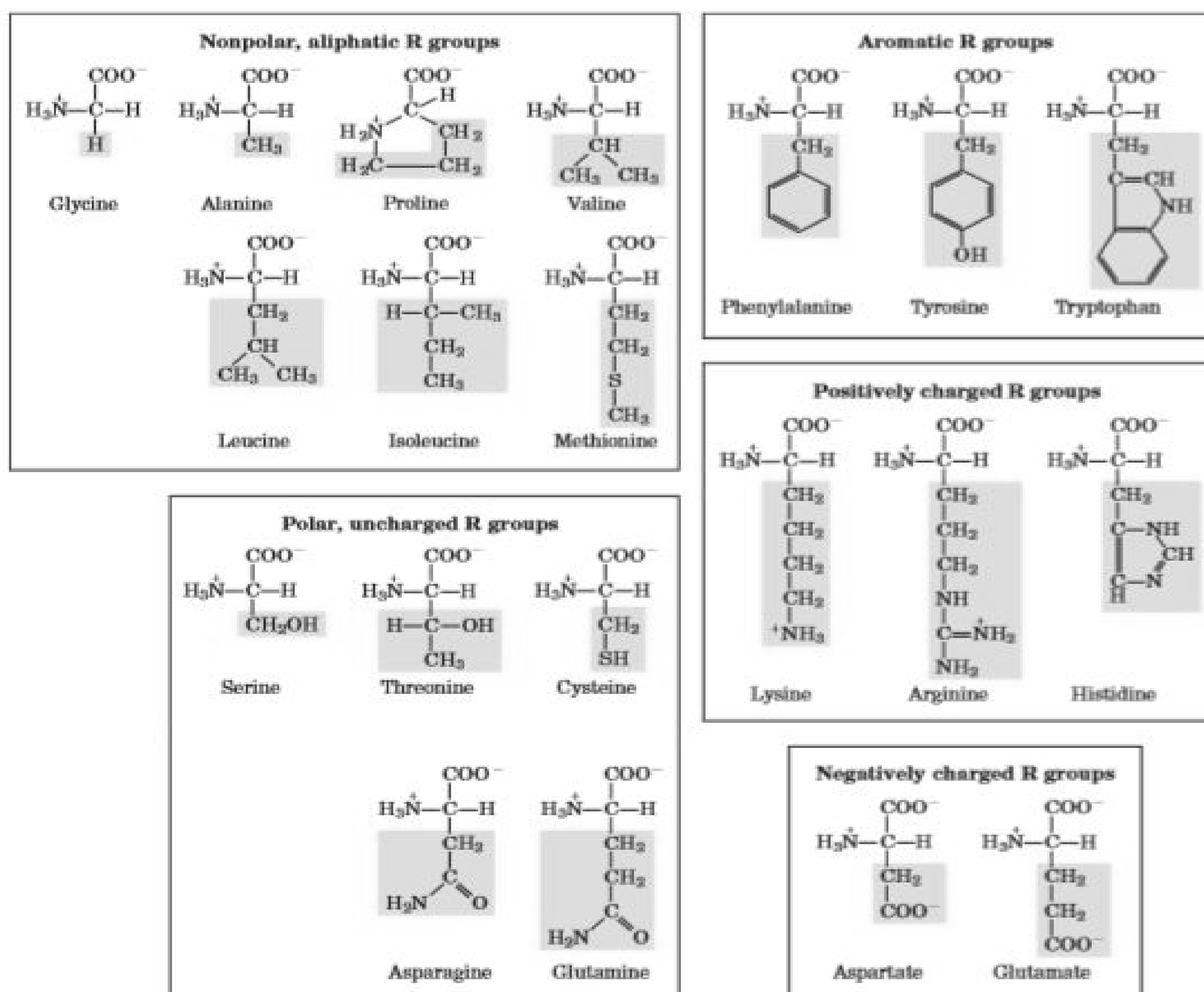


Figure : Chemical structure pf twenty common amino acids

Urea cycle

Living organism excrete the excess nitrogen resulting from the metabolic breakdown of the amino acids in one of three ways. Urea is formed from ammonia, co_2 and aspartate in a cyclic pathway referred to as the urea cycle. Because the urea cycle is discovered by Krebs and Henseleit, it is often referred to as **Krebs-Henseleit cycle**.

Why is Urea Cycle important to us?

Through urea nitrogenous waste of the body are excreted. Urea is less toxic than ammonia and can be effectively excreted by kidneys. The urea cycle mediates the removal of ammonia as urea in the amount of 10 to 20 g per day in the healthy adult. In the absence of a fully functional urea cycle, hyperammonemic encephalopathy and irreversible brain injury in occur. Hyperammonemia also may affect brain volume control; cell swelling is sometimes observed, perhaps because of the marked increase of brain glutamine. Hyperammonemia also affects neurotransmitter metabolism. Major effects on the handling of GABA and serotonin have been observed.

The Synthesis of Urea

Urea synthesis which occurs in the hepatocytes in liver, consists of five sequential enzymatic reactions. The first two reactions occur in the mitochondria and the remaining reactions takes place in the cytosol. Urea cycle begins with the formation of carbamoyl phosphate in the mitochondria. The substrate for this reaction, catalyzed by carbamoyl phosphate synthetase I, are NH^{+4} and HCO_3^-. Because two molecules of ATP are required in carbamoyl phosphate synthesis, this reaction is essentially irreversible. Carbamoyl phosphate subsequently reacts with ornithine to form citrulline. Citrulline passes into the cytosol.

Next three steps that occur in cytosol involves :

1. Formation of argininosuccinate by ATP dependent by ATP dependent reaction of citrulline with aspartate.

2. Formation of arginine from argininosuccinate. This reaction release fumarate, which enters the citric acid cycle.

3. Formation of urea and regeneration of ornithine.

Regulation of urea cycle

The urea cycle operates only o eliminate excess nitrogen. On high-protein diets the carbon skeletons of the amino acids are oxidized for energy for stored as fat and glycogen, but the amino nitrogen must be excreted. To facilitate this process, enzyme of the urea cycle are control at the gene level. with long term changes in the quantity of dietary protein, Changes of 20-fold or greater in concentration of cycle enzyme are observed. When dietary proteins increases significantly, enzyme concentrations rise. On return to balanced diet, enzyme levels decline. Under conditions of starvation, enzyme levels rise as protein are degraded and amino acid carbon skeletons are used to provide energy, thus increasing the quantity of nitrogen that must be excreted.

Short-term regulation of the cycle occurs principally at CPS-I, which is relatively inactive in absence of its Allosteric activator n-acetyleglutamate. This metabolite is synthesized from glutamate and acetyl CoA by N-acetylglutamate synthase. Increased urea synthesis is required when amino acid breakdown rates increase, generating excess nitrogen that must be excreted. Increased in these breakdown rates are signaled by an increase in glutamate concentration through transmission reactions. This situation, in turn, cause an increase in N-acetyl-glutamate synthesis, stimulating carbamoyl phosphate synthetase and thus entire urea cycle.

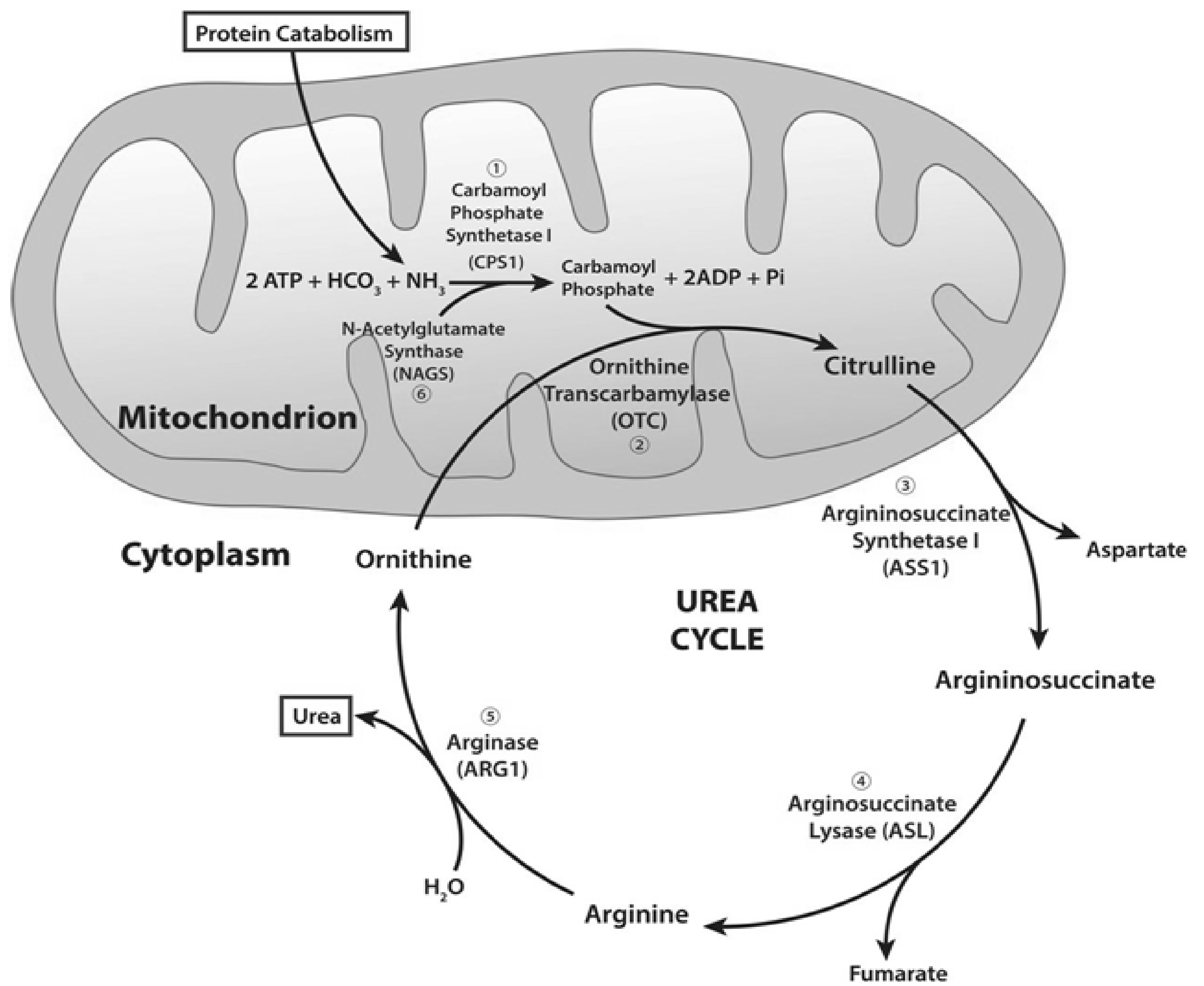


Figure: Urea Cycle: Source BMJ Practical Neurology

Probable questions :

1) With the help of a schematic diagram describe the synthesis of urea in the liver.

2) What is the rate limiting step in urea cycle?

3) How is urea cycle regulated by n-acetylglutamate?

4) Write a short note on optical isomerism of amino acids.

5) Schematically represent the steps of urea cycle.

Suggested Readings/ References :

1. Cox, M.M and Nelson, D.L. (2008). Lehninger's Principles of Biochemistry, V Edition,

W.H. Freeman and Co., New York.

2. Berg, J.M., Tymoczko, J.L. and Stryer, L.(2007). Biochemistry, VI Edition, W.H.

Freeman and Co., New York.

3. Murray, R.K., Bender, D.A., Botham, K.M., Kennelly, P.J., Rodwell, V.W. and Well,

P.A. (2009). Harper's Illustrated Biochemistry, XXVIII Edition, International Edition,

The McGraw- Hill Companies Inc.

4. Donald Voet and Judith G. Voet Biochemistry

Unit IX

Carbohydrate metabolism: Glycolysis, glycogenolysis, gluconeogenesis, interrelationship between different carbohydrate metabolism.

Objectives :

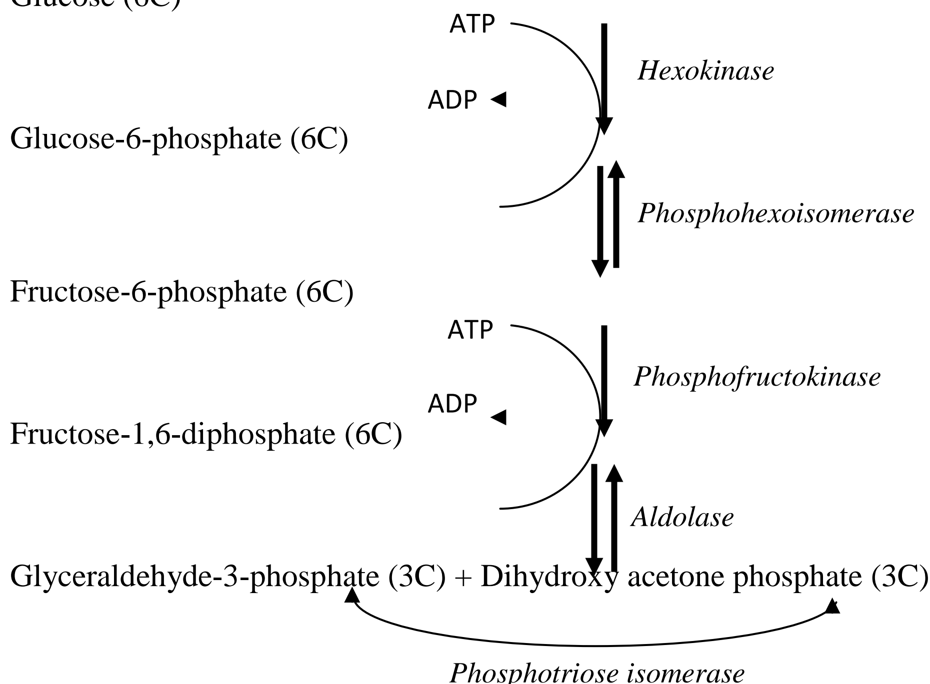
1. Understanding the mechanism and role of glycolysis and to know the total ATP production
2. Understanding the mechanism of glycogenolysis
3. To learn the mechanism of glucose production of different sources within the body, other than carbohydrate
4. To know the interrelationship between different carbohydrate metabolism pathways

Glycolysis

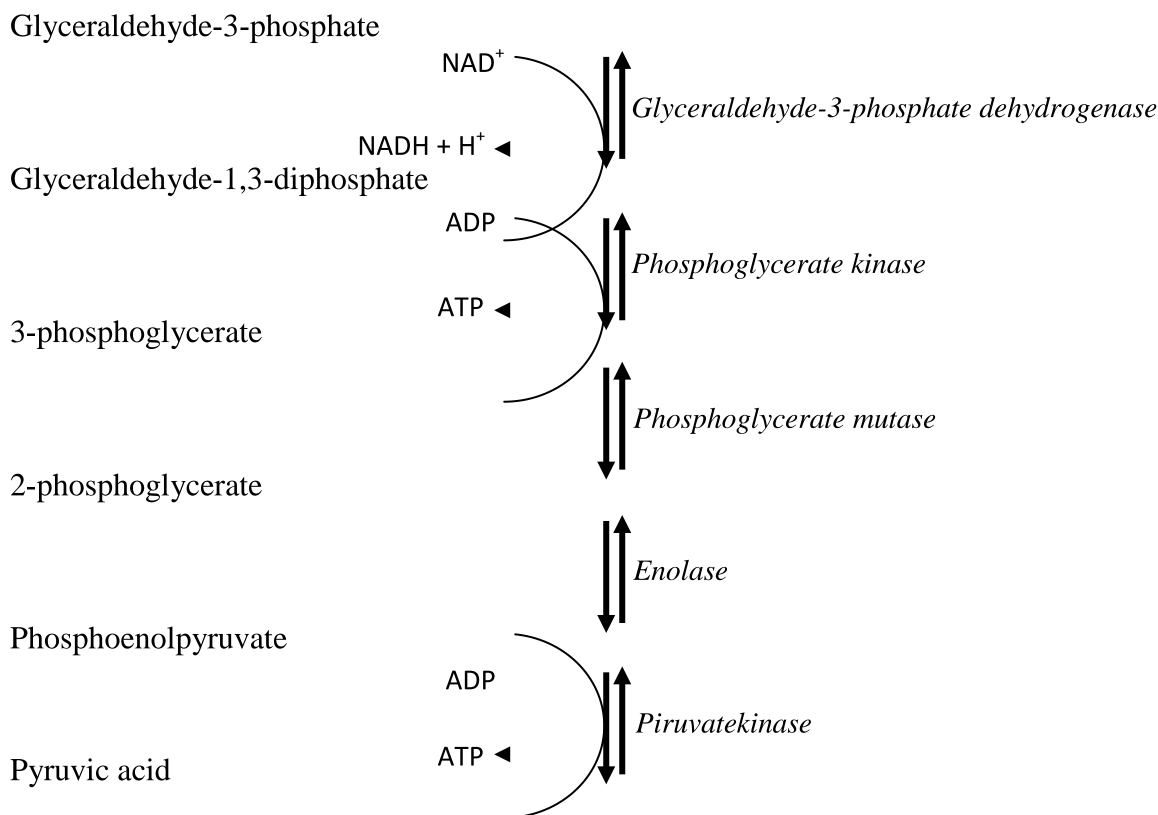
It is the process of anaerobic breakdown of glucose to pyruvic acid. Sugars other than glucose are similarly converted to pyruvic acid after their conversion to one of the intermediates in this glycolytic pathway. This series of reactions take place in the cytosol of the cell and is also known as the EMP (Embden, Mayerhof and Parnas) pathway.

The entire reaction can be differentiated into two phases: preparatory phase and pay off phase.

Preparatory Phase : This 5 step reaction consumes energy to transform glucose into two 3C sugar phosphates. This step is referred to as preparatory phase because it actually consumes energy in form of ATP. The steps of the phase are given below:



Pay off phase : The second phase of glycolysis is known as the pay off phase which is characterized by net gain in energy by production of ATP and NADH. In the preparatory phase, one molecule of glucose produces two 3C compounds, as a result two pay off phase occur for one molecule of glucose. The steps of this phase are given below:



Total ATP production in glycolysis :

Total ATP consumed in preparatory phase:

Glucose to Glucose-6-phosphate →	1 molecule of ATP
Fructose-6-phosphate to Fructose-1,6-diphosphate→	1 molecule of ATP
Total→	2 molecules of ATP

Total ATP formed in pay off phase: (the reactions occurs twice for one molecule of glucose)

1,3-bisphosphoglycerate to 3-phosphoglycerate →	1 x 2= 2 molecules of ATP
Phosphoenolpyruvate to pyruvic acid →	1 x 2= 2 molecules of ATP
Glyceraldehyde-3-PO_4 to 1,3-bisPO_4glycerate (1 NADH) →	3 x 2= 6 molecules of ATP
Total→	8 molecules of ATP

So, the net gain of ATP per molecule of glucose is (8 – 2) = 6 molecules of ATP.

Significance of Glycolysis :

1. It is the principal route of glucose metabolism.
2. It is also the main pathway for the metabolism of fructose and galactose.
3. This process is capabe of producing ATP in the absence of oxygen that is helpful for skeletal muscles to perform at very high levels when aerobic oxidation become insufficient.
4. In pathological conditions like fast growing cancer cells, glycolysis proceeds at a higher rate and provides the required energy for those cells. Ths they can live in anaerobic conditions. Moreover, the high amount if pyruvate produced during this is converted into lactate, producing an acidic environment in tumors.

Glycogenolysis

Glycogenolysis is the breakdown of glycogen into glucose-6-phosphate and glycogen. Glycogen branches are catabolised by the enzyme glycogen phosphorylase through the sequential removal of glucose monomer.

Here the enzyme glycogen phosphorylase cleaves the bond linking the terminal glucose residue to a glycogen branch by substitution of a phosphoryl group for the α[1→4] linkage. Glucose-1-phosphate is converted to glucose-6-phosphate by the enzyme phosphor-glucomutase.

Glucose residues are phosphorolysed from branches of glycogen until four residues before a glucose that is branched with a α[1→6] linkage.

The glycogen debranching enzyme now transfers the three of the remaining four glucose units to the end of another glycogen branch, exposing the α[1→6] branching point, which is hydrolized by α[1→6] glucsidase enzyme, removing the final glucose residue.

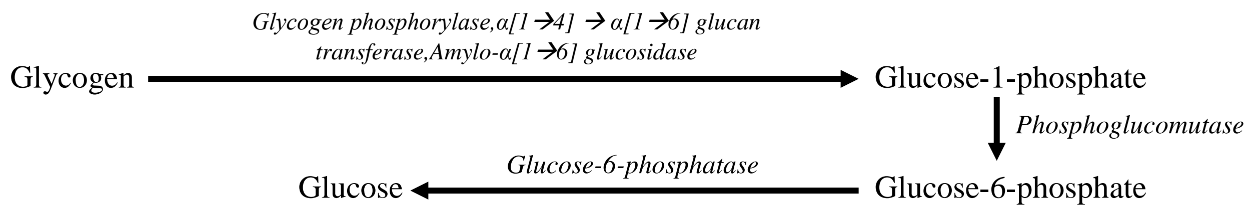


Figure : Gluconeogenetic pathways (source: http://www.namrata.co/semester-examination-november-2012-model-answers-set-2-gluconeogenesis/)

Gluconeogenesis is the metabolic pathway that produces glucose from non-carbohydrate carbon substrate such as lactate, glycerol (from breakdown of lipids) and glucogenic amino

acids (from breakdown of proteins). It is one of the several main mechanisms used by living organisms to maintain the glucose level inside the body. In vertebrates, this process takes pace mainly in liver and to a lesser extent in the cortex of the kidneys. In some animals such as ruminants, this tends to be a continuous process, but in many other animals, this process occurs during periods of fasting, starvation, low-carbohydrate diets or intensive exercise. The process is highly endergonic until it is coupled to the hydrolysis of ATP or GTP, effectively making the process exergonic.

Interrelationship between different carbohydrate metabolisms :

The carbohydrate metabolism pathways of our body are extremely essential because glucose acts as the main source of energy in our system. So, coordinated production, degradation and proper storage of glucose and other carbohydrates are very necessary. Blood glucose undergoes glycolysis when there is a need of energy in our body. Glycolysis produces ATP from glucose that can be used in various cellular functions. For more ATP, the products of glycolysis, that is pyruvate, undergoes Krebs cycle.

If there is excess glucose in the body, then it undergoes glycogenesis that results in production of glycogen from glucose. Glycogen can be stored in the body (for example, in the muscles) for further use. If there is lack of glucose in the body due to fasting or starvation or low-carbohydrate diet, then glycogenolysis occurs. This process leads to breakdown of glycogen to produce glucose that can be used to synthesize ATP for cellular functions. Moreover, excessive scarcity of glucose leads to the process of gluconeogenesis, where glucose is synthesized from non-carbohydrate carbon substrates. Thus, proper coordination of all these metabolic processes results in the maintenance of glucose level in the body.

Probable questions :

1. What is the significance of the preparatory phase and pay off phase of glycolysis?
2. State the significance of glycolysis.
3. Mention the steps of glycolysis where ATP is produced or consumed and calculate the total ATP production in glycolysis.
4. State how glucose is produced from glycogen (or Schematically represent the steps of glycogenolysis)
5. What is gluconeogenesis? Schematically represent how glucose is produced from glycerol.
6. State the interrelationship between different carbohydrate metabolism pathways and mention their significance in brief.

Suggested Readings/ References :

1. Cox, M.M and Nelson, D.L. (2008). Lehninger's Principles of Biochemistry, V Edition, W.H. Freeman and Co., New York.

2. Berg, J.M., Tymoczko, J.L. and Stryer, L.(2007). Biochemistry, VI Edition, W.H. Freeman and Co., New York.

3. Murray, R.K., Bender, D.A., Botham, K.M., Kennelly, P.J., Rodwell, V.W. and Well, P.A. (2009). Harper's Illustrated Biochemistry, XXVIII Edition, International Edition, The McGraw- Hill Companies Inc.

4. Donald Voet and Judith G. Voet Biochemistry

Unit X

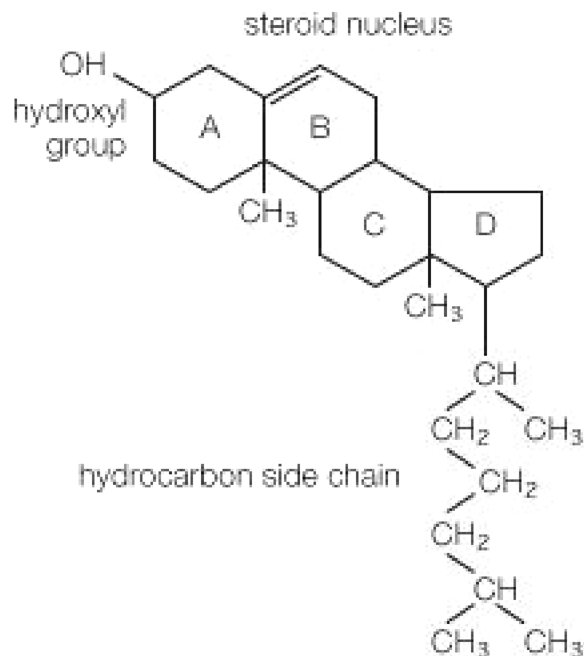
Biosynthesis and transport of Cholesterol

Objectives :

Understanding the mechanism, regulation of biosynthesis and degradation of cholesterol within the body and also to learn about the transport of cholesterol in different parts of the body

Cholesterol and its structure :

Cholesterol, the characteristic steroid alcohol of animal tissues, performs a number of essential functions in the body. For example, cholesterol is a structural component of all cell membranes, modulating their fluidity, and in specialized tissues, cholesterol is a precursor of bile acids, steroid hormones, and vitamin D. It is therefore of critical importance that the cells of the body be assured an appropriate supply of cholesterol. To meet this need, a complex series of transport, biosynthetic, and regulatory mechanisms has evolved. The liver plays a central role in the regulation of the body's cholesterol homeostasis. For example, cholesterol enters the liver's cholesterol pool from a number of sources including dietary cholesterol, as well as cholesterol synthesized de novo by extrahepatic tissues and by the liver itself. Cholesterol is eliminated from the liver as unmodified cholesterol in the bile, or it can be converted to bile salts that are secreted into the intestinal lumen. It can also serve as a component of plasma lipoproteins sent to the peripheral tissues. In humans, the balance between



cholesterol influx and efflux is not precise, resulting in a gradual deposition of cholesterol in the tissues, particularly in the endothelial linings of blood vessels. This is a potentially life-threatening occurrence when the lipid deposition leads to plaque formation, causing the narrowing of blood vessels (atherosclerosis) and increased risk of cardio-cerebro and peripheral vascular disease.

Cholesterol is a very hydrophobic compound. It consists of four fused hydrocarbon rings called the “steroid nucleus”, and it has an eight-carbon, branched hydrocarbon chain attached to carbon 17. Ring A has a hydroxyl group at carbon 3, and ring B has a double bond between carbon 5 and carbon 6.

CHOLESTEROL BIOSYNTHESIS

About 1g of cholesterol is synthesized per day in adults. Almost all the tissues of the body participate in cholesterol biosynthesis. The largest contribution is made by liver (5O%), intestine (15%), skin, adrenal cortex, reproductive tissue etc.

The enzymes involved in cholesterol synthesis are found in the cytosol and microsomal fractions of the cell. Acetate of acetyl CoA provides all the carbon atoms in cholesterol. The reducing equivalents are supplied by NADPH while AIP provides energy. For the production of one mole of cholesterol, 18 moles of acetyl CoA, 36 moles of ATP and 16 moles of NADPH are required. By administering acetate with 14C isotope label. Either on the methyl (-CH3) group or carboxyl (-COO) group, the origin of carbon atoms in the entire molecule of cholesterol has been established. The sources of carbon atoms and the key intermediates of cholesterol formation.

The synthesis of has 5 stages

1. Synthesis of HMG CoA

2. Formation of mevalonate (6 C)

3. Production of isoprenoid units (5 C)

4. Synthesis of squalene (30 C)

5. Conversion of squalene to cholesterol (27 C).

1. Synthesis of p-hydroxy p-methylglutaryl CoA (HMG CoA): Two moles of acetyl CoA condense to form acetoacetyl CoA. Another molecule of acetyl CoA is then added to produce HMG CoA. These reactions are similar to that of ketone body synthesis. However, the two pathways are distinct, since ketone bodies are produced in mitochondria while cholesterol synthesis occurs in cytosol. Thus, there exist two pools of HMG CoA in the cell. Further, two isoenzymes of HMG CoA synthase are known. The cytosomal enzyme is involved in cholesterol synthesis whereas the mitochondrial HMC CoA synthase participates in ketone body formation.

2. Formation of mevalonate: HMG CoA reductase is the rate limiting enzyme in cholesterol biosynthesis. This enzyme is present in endoplasmic reticulum and catalyses the reduction of HMC CoA to mevalonate. The reducing equivalents are supplied by NADPH.

3. Production of isoprenoid units: In a three-step reaction catalysed by kinases mevalonate is converted to 3-phospho 5-pyrophosphomevalonate which on decarboxylation forms isopentenyl pyrophosphate (IPP). The latter isomerizes to dimethylallyl pyrophosphate (DPP). Both IPP and DPP are 5-carbon isoprenoid units.

4. Synthesis of squalene: IPP and DPP condense to produce a 1O-carbon geranyl pyrophosphate (GPP). Another molecule of IPP condenses with CPP to form a 1S-carbon farnesyl pyrophosphate (FPP). Two units of farnesyl pyrophosphate unite and get reduced to produce a 30-carbon squalene.

5. Conversion of squalene to cholesterol: Squalene undergoes hydroxylation and cyclization utilizing 0_2 and NADPH and gets converted to lanosterol. The formation of cholesterol from lanosterol is a multistep process with a series of about 19 enzymatic reactions. The following are the most important reactions

● Reducing the carbon atoms from 30 to 27.

● Removal of two methyl groups from C_4 and one methyl group from C_{14}.

● Reduction in the double bond present between C_{24} and C_{25}. The enzymes involved in the conversion of lanosterol to cholesterol are associated with endoplasmic reticulum. 14-

Desmethyl lanosterol, zymosterol cholestadienol and desmosterol are among the intermediates in the cholesterol biosynthesis. The penultimate product is 7-dehydrocholesterol which, on reduction, finally yields cholesterol.

Cholesterol biosynthesis is now believed to be a part of a major metabolic pathway concerned with the synthesis of several other isoprenoid compounds. These include ubiquinone (coenzyme Q of electron transport chain) and dolichol (found in glycoprotein). Both of them are derived from farnesyl pyrophosphate.

Regulation of cholesterol synthesis

Cholesterol biosynthesis is controlled by the rate limiting enzyme HMG CoA reductase at the beginning of the pathway. HMC CoA reductase is found in association with endoplasmic reticulum and is subjected to different metabolic controls.

1. **Feedback control :** The end product cholesterol controls its own synthesis by a feedback mechanism. Increase in the cellular concentration of cholesterol reduces the synthesis of the enzyme HMG CoA reductase. This is achieved by decreasing the transcription of the gene responsible for the production of HMC CoA reductase. Feedback regulation has been investigated with regard to LDL-cholesterol taken up by the cells, and the same mechanism is believed to operate whenever cellular cholesterol level is elevated.

2. **Hormonal regulation :** The enzyme HMG CoA reductase exists in two interconvertible forms. The dephosphorylated form of HMC CoA reductase is more active while the phosphorylated form is less active. The hormones exert their influence through cAMP by a series of reactions which are comparable with the control of the enzyme glycogen synthase. The net effect is that glucagon and glucocorticoids favor the formation of inactive HMC CoA reductase (phosphorylated form) and, thus, decrease cholesterol synthesis. On the other hand, insulin and thyroxine increase cholesterol production by enhancing the formation of active HMC CoA reductase (dephosphorylated form).

3. **Inhibition by drugs :** The drugs compactin and lovastatin (mevinolin) are fungal products. They are used to decrease the serum cholesterol level in patients with hypercholesterolemia. Compactin and lovastatin are competitive inhibitors of the enzyme HMG CoA reductase and,

therefore, reduce cholesterol synthesis. About 50 to 60% decrease in serum cholesterol level has been reported by a combined use of these two drugs.

4. HMG CoA reductase activity is inhibited by bile acids. Fasting also reduces the activity of this enzyme.

Degradation of Cholesterol

The steroid nucleus (ring structure) of the cholesterol cannot be degraded to CO_2 and H_2O. Cholesterol (50%) is converted to bile acids (excreted in feces), serves as a precursor for the synthesis of steroid hormones, vitamin D, coprostanol and cholesterol. The latter two are the fecal sterols, besides cholesterol.

l. Synthesis of bile acids

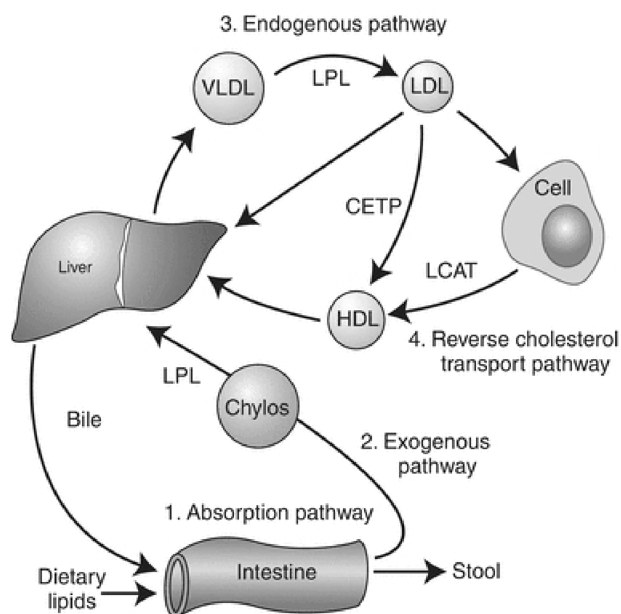
The bile acids possess 24 carbon atoms, 2 or 3 hydroxyl groups in the steroid nucleus and a side chain ending in carboxyl group. The bile acids are amphipathic in nature since they possess both polar and non-polar groups. They serve as emulsifying agents in the intestine and actively participate in the digestion and absorption of lipids.

The synthesis of primary bile acids takes place in the liver and involves a series of reactions. The step catalyzed by 7 a-hydroxylase is inhibited by bile acids and this is the rate limiting reaction. Cholic acid and chenodeoxycholic acid are the primary bile acids and the former is found in the largest amount in bile. On conjugation with glycine or taurine, conjugated bile acids (glycocholic acid, taurocholic acid etc.) are formed which are more efficient in their function as surfactants. In the bile, the conjugated bile acids exist as sodium and potassium salts which are known as bile salts.

2. Synthesis of steroid hormones from cholesterol

Cholesterol is the precursor for the synthesis of all the five classes of steroid hormones (a) Glucocorticoids (e.g. cortisol) (b) Mineralocorticoids (e.g. aldosterone) (c) Progestins (e.g. progesterone).

3. Synthesis of vitamin D7-Dehydrocholesterol, an intermediate in the synthesis of cholesterol, is converted to cholecalciferol (vitamin O3) by ultraviolet rays in the skin.



Transport of cholesterol

Cholesterol is present in the plasma lipoproteins in two forms

1. About 70-75% of it is in an esterified form with long chain fatty acids.

2. About 25-30% as free cholesterol. This form of cholesterol readily exchanges between different lipoproteins and also with the cell membranes.

Role of ICAT : High density lipoproteins (HDL) and the enzyme lecithin-cholesterol acyltransferase (LCAT) are responsible for the transport and elimination of cholesterol from the body. LCAT is a plasma enzyme, synthesized by the liver. It catalyzes the transfer of fatty acid from the second position of phosphatidyl choline (lecithin) to the hydroxyl group of cholesterol. HDL-cholesterol is the real substrate for LCAT and this reaction is freely reversible. LCAT activity is associated with apo-A1 of HDL.

The cholesterol (cholesteryl) ester forms an integral part of HDL. In this manner, the cholesterol from the peripheral tissues is trapped in HDL, by a reaction catalyzed by LCAT and then transported to liver for degradation and excretion. This mechanism is commonly known as reverse cholesterol transport.

Probable Questions :

1. Draw the chemical structure of cholesterol. Describe the process of cholesterol biosynthesis in our body.

2. State the different modes of regulation of the cholesterol biosynthesis in our body.

3. Give a short note on transport of cholesterol.

Suggested Readings/ References :

1. Cox, M.M and Nelson, D.L. (2008). Lehninger's Principles of Biochemistry, V Edition, W.H. Freeman and Co., New York.

2. Berg, J.M., Tymoczko, J.L. and Stryer, L.(2007). Biochemistry, VI Edition, W.H. Freeman and Co., New York.

3. Murray, R.K., Bender, D.A., Botham, K.M., Kennelly, P.J., Rodwell, V.W. and Well, P.A. (2009). Harper's Illustrated Biochemistry, XXVIII Edition, International Edition, The McGraw- Hill Companies Inc.

4. Donald Voet and Judith G. Voet Biochemistry

Unit XI

Enzymes: Kinetic Analysis of Enzyme-Catalyzed Reaction; Regulation of Enzyme Activity; Allosteric Control of Enzyme Activity

Objectives :

1. Understanding the different modes of enzyme-catalyzed reactions and their regulation
2. Understanding the different mode of inhibition of enzyme-catalyzed reactions
3. Derivation of the Michaelis-Menten Equation of enzyme kinetics
4. To learn about the alosteric modulation of enzymes

Enzyme Kinetics

Enzyme kinetics is the quantitative study of enzyme catalysis. Kinetic study measure reaction rates and the affinity of enzymes for substrates and inhibitors. Kinetics also provides insight into reaction mechanism. The general principles of chemical reaction apply to enzyme catalyzed reactions as well. In chemical reaction of the form $A+B \rightarrow P$, where A and B are the reactants and P is the product, the rate of reaction can be expressed in terms of either the rate of disappearance of one of the reactants or the rate of appearance of a product.

The rate at which an enzyme works is influenced by several factors, e.g.,

1. **The concentration** of substrate molecules (the more of them available, the quicker the enzyme molecules collide and bind with them). The concentration of substrate is designated **[S]** and is expressed in units of molarity.
2. **The temperature.** As the temperature rises, molecular motion — and hence collisions between enzyme and substrate — speed up. But as enzymes are proteins, there is an upper limit beyond which the enzyme becomes denatured and ineffective.
3. **The presence of inhibitors.**

A. **Competitive inhibitors** are molecules that bind to the same site as the substrate — preventing the substrate from binding as they do so — but are not changed by the enzyme.

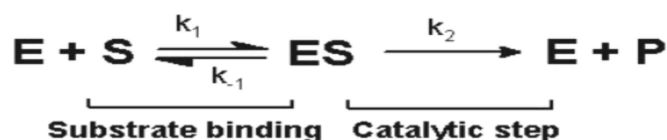
B. **Noncompetitive inhibitors** are molecules that bind to some other site on the enzyme reducing its catalytic power.

4. **pH.** The conformation of a protein is influenced by pH and as enzyme activity is crucially dependent on its conformation, its activity is likewise affected.

Kinetics of Enzyme Catalyzed Reaction :

A particularly useful model for the kinetics of enzyme-catalyzed reaction was devised in 1913 by Leonor Michaelis and Maud Menten when they studied the reaction in which the invertase hydrolyzes sucrose, a disaccharide, into monosaccharides, glucose and fructose.

Michaelis-Menten described the reaction velocity and substrate concentration. To explain their results in the conversion of sucrose to glucose and fructose by the enzyme invertase, Michaelis and Menten proposed the following scheme of reactions:



The Michaelis-Menten kinetic model of a single-substrate reaction is shown that there is an initial bimolecular reaction between the enzyme E and substrate S to form the enzyme–substrate complex ES. In this reaction k_1 is the rate constant for the formation of enzyme-substrate complex and for the unimolecular reaction $ES \rightarrow E + P$rate-determining enzymatic step that allows the mechanism to be modeled as a single kinetic step of rate constant k_2.

According to the Michaelis-Menten approach when the rate (also called velocity) of an enzyme catalyzed reaction is measured at varying substrate concentration, the rate depends on the substrate concentrations [S]. At a relatively low concentration of substrate, initial

velocity (V) increases almost linearly with an increase in substrate concentration and it does not increase any further by increasing the concentration of substrate.

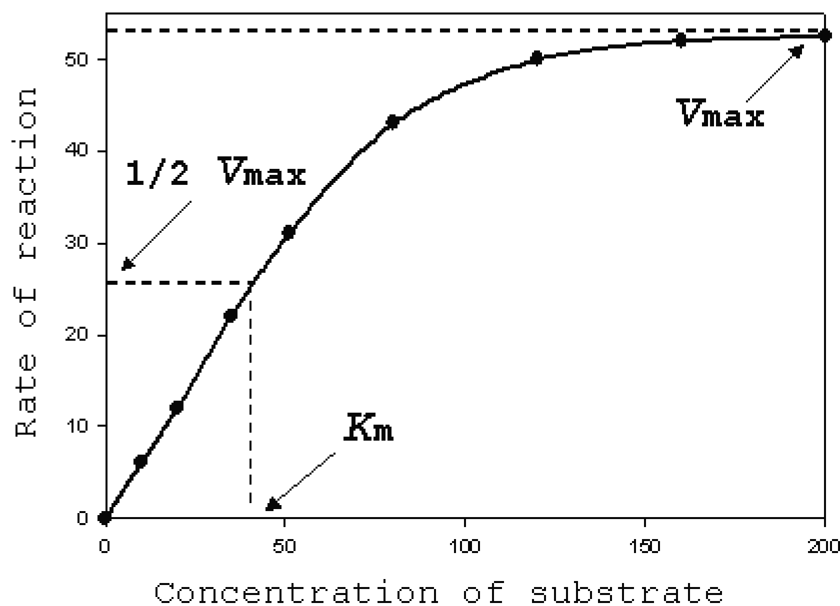


Figure: The hyperbolic relationship between initial velocity (V) and substrate concentration (S) of an Enzyme catalyzed reaction

Concentration of substrate at which reaction velocity reaches half its maximum velocity is called **Michaelis constant (K_m).**Lower value of K_m describes the greater affinity of the enzyme for the substrate.

Michaelis-Menten put forward a mathematical equation to establish the mathematical relationship among the quantities [E], [S], V_{max} and $K_{m.}$

$$v = \frac{V_{max}[S]}{K_m + [S]}$$

1. When $[S] << K_m$, then $V \propto [S]$
2. When $[S] >> K_m$, then $V = [S]$
3. When $[S] = K_m$, then $V = 1/2 V_{max}$

1. Since at low concentration of the substrate, velocity is proportional to the substrate concentration [S], the enzyme catalyzed reaction is first order reaction. At high [S],

velocity becomes virtually independent of [S] and approaches a maximum limit. Since rate is no longer dependent on [S] at high concentration, the enzyme catalyzed reaction obey zero order kinetics.

2. Michaelis-Menten model is based on the assumptions. The following assumptions are made in driving the Michaelis-Menten rate equation:

1. The concentration of substrate[S] is much greater than enzyme[E].
2. The rate of formation of ES is equal to that of the breakdown of ES.
   (steady state assumption)
3. Very little accumulation of P, so the formation of enzyme-substrate complex from E+P is negligible.

Steady-state assumption: The interpretation of the Michelis-Menten model were refined by an assumption termed the steady state assumption. The steady state hypothesis states that the concentration of enzyme-substrate complex remains constant through much of the reaction. When the enzyme substrate are first mixed, the concentration [ES] will rise rapidly from zero to a so called steady state level.

The more normal situation where $k_2 > k_{-1}$ is sometimes called Briggs- Haldane kinetics. The Michaelis–Menten equation still holds under these more general conditions, as may be derived from the **steady-state assumption**. During the initial-rate period, the reaction rate v is roughly constant, indicating that [ES] is similarly constant.

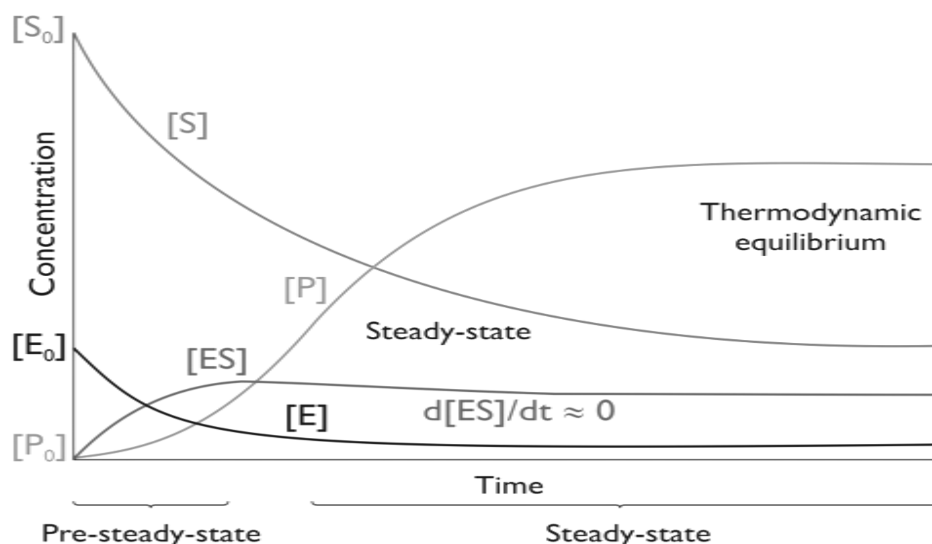


Figure: change in concentrations over time for enzyme E, substrate S, complex ES and product P

$$\frac{d}{dt}[\mathrm{ES}] = k_1[\mathrm{E}][\mathrm{S}] - k_2[\mathrm{ES}] - k_{-1}[\mathrm{ES}] \approx 0$$

Therefore, the concentration [ES] is given by the formula

$$[\mathrm{ES}] \approx \frac{[\mathrm{E}]_{tot}[\mathrm{S}]}{[\mathrm{S}] + K_m}$$

where the Michaelis constant K_m is defined

$$K_m \stackrel{\mathrm{def}}{=} \frac{k_2 + k_{-1}}{k_1} \approx \frac{[\mathrm{E}][\mathrm{S}]}{[\mathrm{ES}]}$$

([E] is the concentration of *free* enzyme). Taken together, the general formula for the reaction rate *v* is again the Michaelis-Menten equation:

$$v = k_2[\mathrm{ES}] = \frac{k_2[\mathrm{E}]_{tot}[\mathrm{S}]}{[\mathrm{S}] + K_m} = \frac{V_{max}[\mathrm{S}]}{[\mathrm{S}] + K_m}$$

Turn over number (K_{cat}): The number of substrate molecules converted into product by an enzyme molecule per unit time when the enzyme is fully saturated with substrate . The term K_{cat} represents the kinetic efficiency of the enzyme. Its unit is sec^{-1}.

At saturating [s], $V=V_{max}=K_2\ [E_t]$

Thus, $K_{2} = V_{max}/\ E_{t} = K_{cat}$

The specificity constant $k_{cat}\ /\ K_m$ is a measure of how efficiently an enzyme converts a substrate into product. Using the definition of the Michaelis constant K_m, the Michaelis-Menten equation may be written in the form

$$v = k_2[\mathrm{ES}] = \frac{k_2}{K_m}[\mathrm{E}][\mathrm{S}]$$

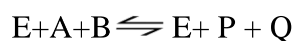
where [E] is the concentration of free enzyme. Thus, the specificity constant is an effective bimolecular rate constant for free enzyme to react with free substrate to form product. The specificity constant is limited by the frequency with which the substrate and enzyme encounter each other in solution, roughly 10^{10} M^{-1} s^{-1} at 25°C. Remarkably, this maximum does not depend on the size of either the substrate or the enzyme. The ratio of the specificity constants for two substrates is a quantitative comparison of how efficient the enzyme is in converting those substrates. The slope of the Michaelis-Menten equation at low substrate concentration [S] (when [S] $<<$ K_m) also yields the specificity constant.

Kinetics of bi or multi-reactant system :

So, far we have considered enzyme catalyzed reactions with single substrate. This situation is not common. Usually, enzymes catalyze reactions are those in which two (or more) substrates take part. Consider the case of An enzyme catalyzing a reaction involving two substrates A and B, and yielding the products P and Q:

Such a reaction is termed as bisubstrate reaction. In general, bisubstrate reactions proceed by one of the two possible routes:

1. Both A and B are to the enzyme and then reaction occurs to give P+Q:

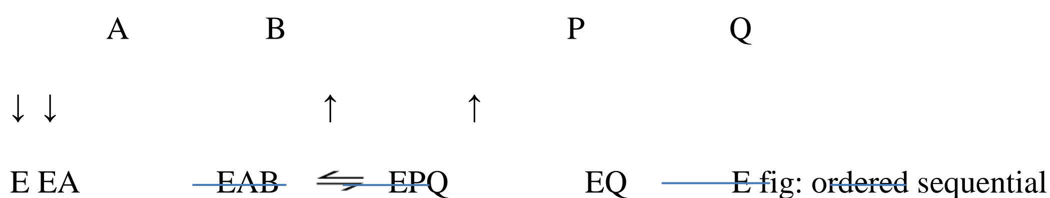


Reaction of this type are defined as sequential or single-displacement reactions.

Sequential reactions can be either of two distinct classes :

a .If there is no obligatory order of addition of substrates or release of products, it is called random sequential.

b. If substrates add in an obligatory order, the mechanism is called ordered sequential.



In order sequential reactions, one substrate is obligated to bind to the enzyme before a second substrate. In random sequential mechanisms, there is no preference.

In practice, there is usually some degree of order in binding.

2. The other general possibility is that one substrate, A, binds to the enzyme and reacts with it to yield a chemically modified form of the enzyme (E') plus the product, P. The second substrate, B, then reacts with E', regenerating E and forming the other product, Q. Reactions that fit this model are called ping-pong or double-displacement reactions.

INHIBITION OF ENZYME-CATALYSED REACTION :

Enzyme inhibitor is defined as a substance which binds with the enzyme and brings about a decrease in catalytic activity of the enzyme. They may be organic or inorganic in nature.

There are 3 broad categories of enzyme inhibition :

1. Reversible inhibition

2. Irreversible inhibition

3. Allosteric modulation

Reversible Inhibition : Here the inhibitors bind non-covalently with the enzyme and the inhibition can be reversed if the inhibitors are removed.

Types- Competitive, non-competitive and un-competitive.

Competitive inhibition-A competitive inhibitor competes with the substrate for the active site of the enzyme. Here,the substrate and the inhibitor are similar in structure. The inhibitor occupies the active site of the enzyme and an enzyme-inhibitor complex is formed. Substrate cannot bind and reaction does not occur. Example-succinic acid dehydrogenase is competitively inhibited by malonic acid and glutaric acid.

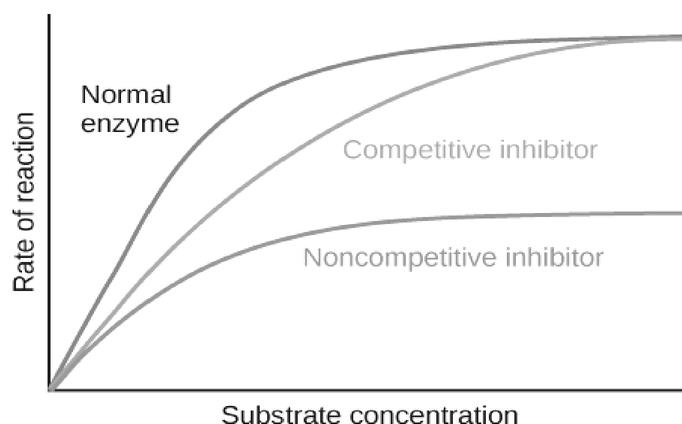
Non-competitive inhibition-The inhibitor does not occupy the active site of the enzyme. Instead it attaches with enzyme at some other point. Binding of the inhibitor changes the shape of the enzyme, making it non-functional.

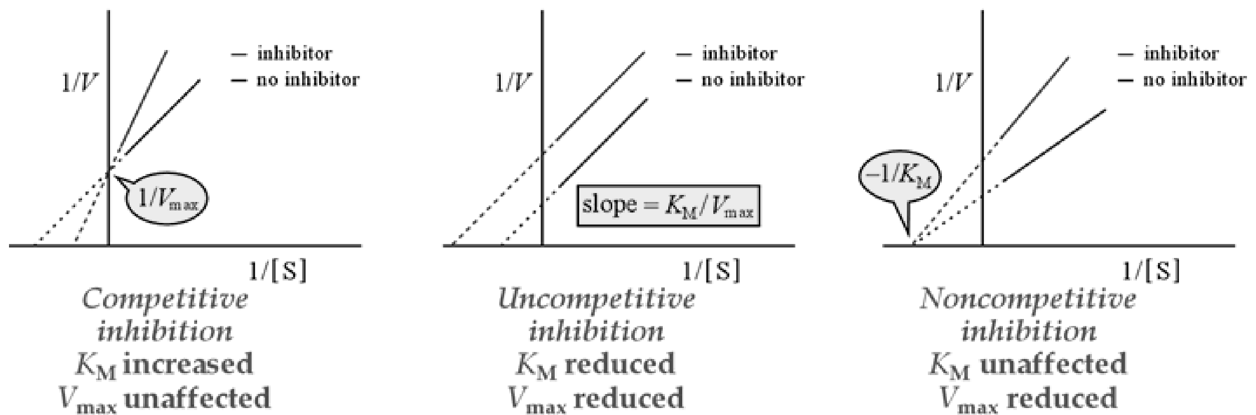
Example-Non-competitive inhibitors

of CYP2C9 enzyme include nifedipine, tranylcypromine, phenethyl isothiocyanate, and 6-hydroxyflavon.

Uncompetitive inhibition-The inhibitor has no structural similarity with the substrate and does not compete with the substrate for occupying the binding site of the enzyme. The inhibitor do not bind with the enzyme alone, but only with the enzyme-substrate complex. The inhibitor does not affect ES complex formation nut deforms the conformation of the enzyme in such a way that its catalytic power is suppressed, which prevents for,ation and release of the product.

Example-This behavior is found in the inhibition of acetylcholinesterase by tertiary amines (R_3N). Such compounds bind to the enzyme in its various forms, but the acyl-intermediate-amine complex cannot break down into enzyme plus product.





IRREVERSIBLE INHIBITION :

Irreversible inhibitors are those that bind covalently with the enzyme and inactivate them. Such inhibition is irreversible. They destroy the functional groups of the enzyme which are the key catalytic groups of the enzyme. Example- Iodoacetate is an irreversible inhibitor of glyceraldehydes-3-phosphate dehydrogenase (it combines with sulfydryl groups at the active site and the enzyme becomes irreversibly inactive).

ALLOSTERIC MODULATION OF ENZYMES :

Sometimes, the activity of an enzyme may be either enhanced or inhibited due to its conformational changes brought abouit by non-covalent binding of some specific low molecular weight ligands, to specific sites of the enzyme other than the active site. Such modification of enzyme activity is called allosteric modulation or allosterism.

Positive modulation

Positive allosteric modulation occurs when the binding of one ligand enhances the attraction between substrate molecules and other binding sites. An example is the binding of oxygen molecules to hemoglobin, where oxygen is effectively both the substrate and the effector.

Negative modulation

Negative allosteric modulation occurs when the binding of one ligand decreases the affinity for substrate at other active sites. For example, when 2,3-BPG binds to an allosteric site on hemoglobin, the affinity for oxygen of all subunits decreases.

ALLOSTERIC MODULATORS :

The substances which bind at the allosteric site of the enzyme and regulate their activity are called allosteric modulators or effectors. These are generally small molecular weight by products of that particular reaction pathway. The binding site of allosteric modulators on enzyme is called allosteric site and the enzyme thus being regulated is called allosteric enzyme.

Example- 1) Citrate enhances the activity of AcetylCoA carboxylase hich produces malonyl CoA from acetyl CoA (positive regulation).

2) Palmitoyl CoA suppresses the activity of Acetyl CoA carboxylase (negative regulation).

The activation state of an enzyme is often referred to as **R**, or the relaxed state, where the enzyme is on, and its activity is turned up. In the **T**, or the tense state, the enzyme is off, and its activity is turned down.

One molecule may bind the allosteric site and make the enzyme change from the T to R state, while a different molecule can bind the same enzyme and change it from the R to T state. The state of the enzyme will also affect its function. An example of this can be found in respiration, where a specific enzyme, phosphofructokinase-1, is activated by adenosine diphosphate (ADP), but inactivated by adenosine triphosphate (ATP).

Probable questions :

1. State how the rate of enzyme activity is influenced by several factors.
2. What are the assumptions of the Michaelis-Menten equation? Derive the Michaelis-Menten equation.
3. What is Lineweaverburk plot? Explain graphically.
4. Describe the different modes of reversible inhibition of enzymatic reactions.
5. What do you mean by allosteric modulation of enzymes?

Suggested Readings/References

1. Cox, M.M and Nelson, D.L. (2008). Lehninger's Principles of Biochemistry, V Edition, W.H. Freeman and Co., New York.

2. Berg, J.M., Tymoczko, J.L. and Stryer, L.(2007). Biochemistry, VI Edition, W.H. Freeman and Co., New York.

3. Murray, R.K., Bender, D.A., Botham, K.M., Kennelly, P.J., Rodwell, V.W. and Well, P.A. (2009). Harper's Illustrated Biochemistry, XXVIII Edition, International Edition, The McGraw- Hill Companies Inc.

4. Donald Voet and Judith G. Voet Biochemistry

Unit XII

Intracellular protein traffic for secretory and non-secretory cells, protein synthesis, intracellular transport, packaging, storage and release

Objective :

The secretory and endocytic pathways of eukaryotic organelles consist of multiple compartments, each with a unique set of proteins and lipids. Specific transport mechanisms are required to direct molecules to defined locations and to ensure that the identity, and hence function, of individual compartments are maintained. The localization of proteins to specific membranes is complex and involves multiple interactions. The topic summarises the general principles of protein sorting in the secretory and endocytic pathways and highlight the dynamic nature of these processes. The molecular mechanisms involved in this transport along the secretory and endocytic pathways are discussed along with the signals responsible for targeting proteins to different intracellular locations.

Intracellular protein traffic for secretory and non-secretory cells

Before a eukaryotic cell divides, it must duplicate its membrane-enclosed organelles. Even in cells that are not dividing, proteins are being produced continually. These newly synthesized proteins must be accurately delivered to their appropriate organelle-some for eventual secretion from the cell and some to replace organelle proteins that have been degraded. For some organelles, including mitochondria, chloroplasts, peroxisomes, and the interior of the nucleus, proteins are delivered directly from the cytosol. For others, including the Golgi apparatus, lysosomes, endosomes, and the inner nuclear membrane, proteins and lipids are delivered indirectly via the ER, which is itself a major site of lipid and protein synthesis. Proteins enter the ER directly from the cytosol: some are retained there, but most are transported by vesicles to the Golgi apparatus and then onward to the plasma membrane or other organelles. Peroxisomes acquire some of their membrane proteins from the ER, but the bulk of their enzymes enter directly from the cytosol.

Proteins made in the cytosol are dispatched to different locations in the cell according to specific address labels contained in their amino acid sequence. Once at the correct address, the protein enters either the membrane or the interior lumen of its designated organelle.

Protein synthesis and sorting signal : The synthesis of virtually all proteins in the cell begins on ribosomes in the cytosol. The exceptions are the few mitochondrial and chloroplast proteins that are synthesized on ribosomes inside these organelles; most mitochondrial and chloroplast proteins, however, are made in the cytosol and subsequently imported. The fate of any protein molecule synthesized in the cytosol depends on its amino acid sequence, which can contain a sorting signal that directs the protein to the organelle in which it is required. Proteins that lack such signals remain as permanent residents in the cytosol; those that possess a sorting signal move from the cytosol to the appropriate organelle. Different sorting signals direct proteins into the nucleus, mitochondria, chloroplasts (in plants), peroxisomes, and the ER. When a membrane-enclosed organelle imports a water-soluble protein to its interior-either from the cytosol or from another organelle-it faces a problem: how can it transport the protein across its membrane (or membranes), which are normally impermeable to hydrophilic macromolecules?

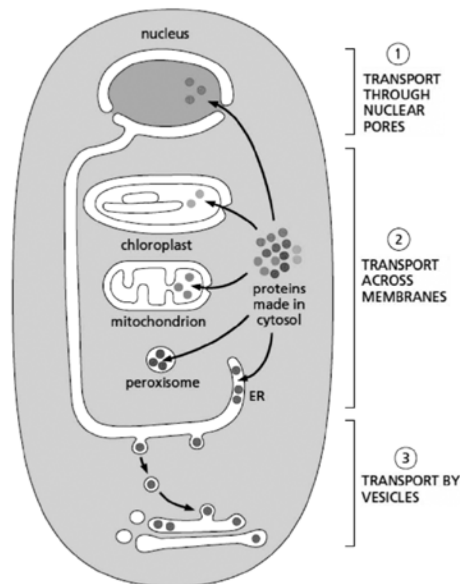
This task is accomplished in different ways by different organelles.

A. Proteins moving from the cytosol into the nucleus are transported through the nuclear pores, which penetrate both the inner and outer nuclear membranes. The pores function as selective gates that actively transport specific macromolecules but also allow free diffusion of smaller molecules (mechanism 1 in Figure 1).

B. Proteins moving from the cytosol into the ER, mitochondria, or chloroplasts are transported across the organelle membrane by *protein translocators* located in the membrane. Unlike transport through nuclear pores, the transported protein must usually unfold in order to snake across the membrane through the translocator (mechanism 2 in Figure 1).

C. Proteins moving onward from the ER-and from one compartment of the endomembrane system to another-are transported by a mechanism that is fundamentally different. These proteins are ferried by *transport vesicles*, which pinch off from the membrane of one compartment and then fuse with the membrane of a second compartment (mechanism 3 in

Figure below). In this process, transport vesicles deliver soluble cargo proteins, as well as the proteins and lipids that are part of the vesicle membrane.



-

Figure 1 : Mechanism of protein import in organelles

Signal Sequence :

The typical sorting signal on a protein is a continuous stretch of amino acid sequence, typically 15–60 amino acids long. This signal sequence is often (but not always) removed from the finished protein once it has been sorted. Some of the signal sequences used to specify different destinations in the cell are shown in Table 1. Signal sequences are both necessary and sufficient to direct a protein to a particular destination. Deleting a signal sequence from an ER protein, for example, converts it into a cytosolic protein, while placing an ER signal sequence at the beginning of a cytosolic protein redirects the protein to the ER. The signal sequences specifying the same destination can vary greatly even though they have the same function: physical properties such as hydrophobicity compartment (mechanism 3 in Figure 1). In this process, transport vesicles deliver soluble cargo proteins, as well as the proteins and lipids that are part of the vesicle membrane

Function of Signal	Example of Signal Sequence
Import into ER	$^{+}H_3N$-Met-Met-Ser-Phe-Val-Ser-Leu-Leu-Leu-Val-Gly-Ile-Leu-Phe-Trp-Ala-Thr-Glu-Ala-Glu-Gln-Leu-Thr-Lys-Cys-Glu-Val-Phe-Gln-
Retention in lumen of ER	-Lys-Asp-Glu-Leu-COO^{-}
Import into mitochondria	$^{+}H_3N$-Met-Leu-Ser-Leu-Arg-Gln-Ser-Ile-Arg-Phe-Phe-Lys-Pro-Ala-Thr-Arg-Thr-Leu-Cys-Ser-Ser-Arg-Tyr-Leu-Leu-
Import into nucleus	-Pro-Pro-Lys-Lys-Lys-Arg-Lys-Val-
Export from nucleus	-Met-Glu-Glu-Leu-Ser-Gln-Ala-Leu-Ala-Ser-Ser-Phe-
Import into peroxisomes	-Ser-Lys-Leu-
Positively charged amino acids are shown in *red* and negatively charged amino acids in *blue*. Important hydrophobic amino acids are shown in *green*. $^{+}H_3N$ indicates the N-terminus of a protein; COO^{-} indicates the C-terminus.	

Table 1 : Some typical signal sequence

Proteins Transport in Nucleus

The nuclear envelope, which encloses the nuclear DNA and defines the nuclear compartment, is formed from two concentric membranes. The inner nuclear membrane contains some proteins that act as binding sites for the chromosomes and others that provide anchorage for the *nuclear lamina*, a finely woven meshwork of protein filaments that lines the inner face of this membrane and provides structural support for the nuclear envelope. The composition of the *outer nuclear membrane* closely resembles the membrane of the ER, with which it is continuous. The nuclear envelope in all eukaryotic cells is perforated by nuclear pores that form the gates through which molecules enter or leave the nucleus. A nuclear pore is a large, elaborate structure composed of a complex of about 30 different proteins. Many of the proteins that line the nuclear pore contain extensive, unstructured regions in which the polypeptide chains are largely disordered. These disordered segments form a soft, tangled meshwork-like a kelp forest-that fills the center of the channel, preventing the passage of large molecules but allowing small, water-soluble molecules to pass freely and nonselectively between the nucleus and the cytosol (Figure 2).

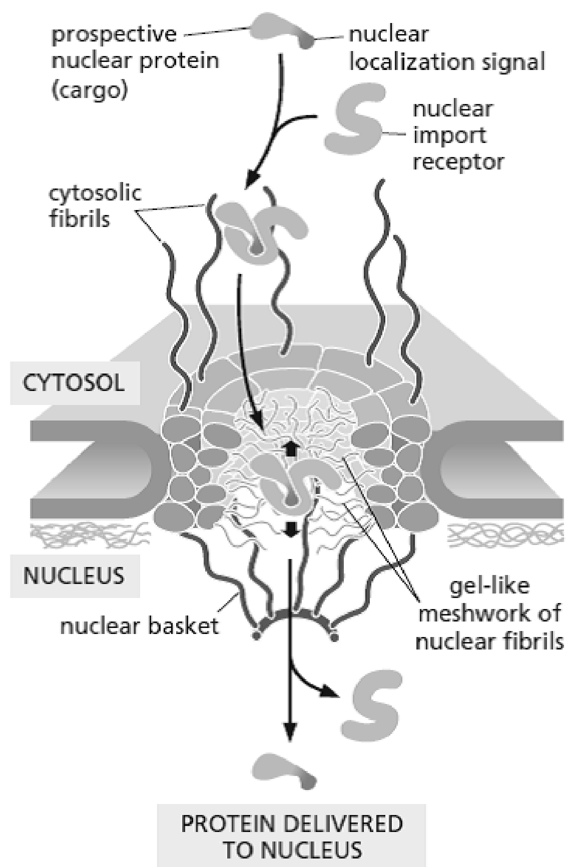


Figure 2 : Protein transport in nucleus

Selected larger molecules and macromolecular complexes also need to pass through nuclear pores. RNA molecules, which are synthesized in the nucleus, and ribosomal subunits, which are assembled there, must be exported to the cytosol. And newly mad proteins that are destined for the nucleus must be imported from the cytosol. To gain entry to a pore, these large molecules and macromolecular complexes must display an appropriate sorting signal. The signal sequence that directs a protein from the cytosol into the nucleus, called a *nuclear localization signal*, typically consists of one or two short sequences containing several positively charged lysines or arginines (Table 1).

The nuclear localization signal on proteins destined for the nucleus is recognized by cytosolic proteins called *nuclear import receptors*. These receptors help direct a newly synthesized protein to a nuclear pore by interacting with the tentacle-like fibrils that extend from the rim of the pore into the cytosol Once there, the nuclear import receptor penetrates the pore by

grabbing onto short, repeated amino acid sequences within the tangle of nuclear pore proteins that fill the center of the pore. When the nuclear pore is empty, these repeated sequences bind to one another, forming a loosely packed gel. Nuclear import receptors interrupt these interactions, and they open a local passageway through the meshwork. The import receptors simply bump along from one repeat sequence to the next, until they enter the nucleus and deliver their cargo (Figure 2). The empty receptor then returns to the cytosol via the nuclear pore for reuse.

Protein transport in Mitochondria and Chloroplast

Both mitochondria and chloroplasts are surrounded by inner and outer membranes, and both organelles specialize in the synthesis of ATP. Chloroplasts also contain a third membrane system, the thylakoid membrane. Although both organelles contain their own genomes and make some of their own proteins, most mitochondrial and chloroplast proteins are encoded by genes in the nucleus and are imported from the cytosol. These proteins usually have a signal sequence at their N-terminus that allows them to enter their specific organelle. Proteins destined for either organelle are translocated simultaneously across both the inner and outer membranes at specialized sites where the two membranes contact each other. Each protein is unfolded as it is transported, and its signal sequence is removed after translocation is complete. Chaperone proteins inside the organelles help to pull the protein across the two membranes and to fold it once it is inside. Subsequent transport to a particular site within the organelle, such as the inner or outer membrane or the thylakoid membrane in chloroplasts, usually requires further sorting signals in the protein, which are often only exposed after the first signal sequence has been removed. The insertion of transmembrane proteins into the inner membrane, for example, is guided by signal sequences in the protein that start and stop the transfer process across the membrane, as observed in transmembrane proteins in the ER membrane (Figure 3).

The growth and maintenance of mitochondria and chloroplasts also require the incorporation of new lipids into the organelle membranes. Most of their membrane phospholipids are thought to be imported from the ER, which is the main site of lipid synthesis in the cell. Phospholipids are transported to these organelles by lipid-carrying proteins that extract a phospholipid molecule from one membrane and deliver it into another. Such transport may

occur at specific junctions where mitochondrial and ER membranes are held in close proximity. Thanks to these lipid-carrying proteins, the different cell membranes are able to maintain different lipid compositions.

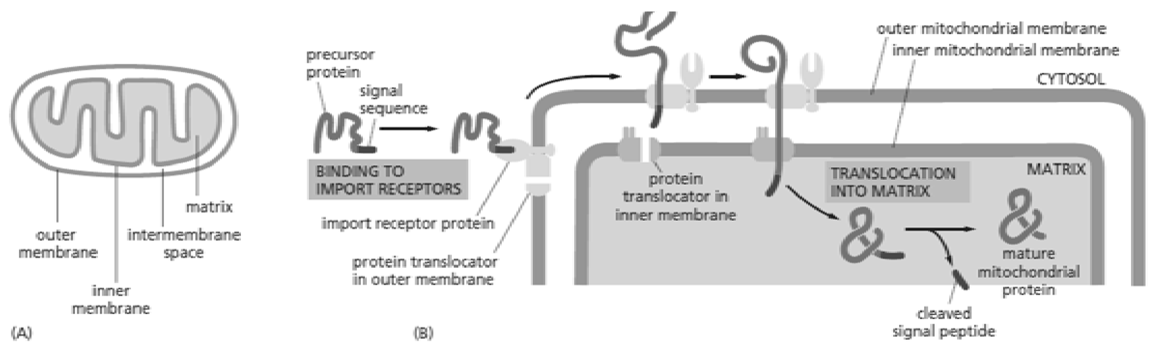


Figure 3: Protein transport in Mitochondria and Chloroplast

Protein Transport in Peroxisome

Peroxisomes generally contain one or more enzymes that produce hydrogen peroxide, hence their name. These organelles are present in all eukaryotic cells, where they break down a variety of molecules, including toxins, alcohol, and fatty acids. They also synthesize certain phospholipids, including those that are abundant in the myelin sheath that insulates nerve cell axons. Peroxisomes acquire the bulk of their proteins via selective transport from the cytosol. A short sequence of only three amino acids serves as an import signal for many peroxisomal proteins. This sequence is recognized by receptor proteins in the cytosol, at least one of which escorts its cargo protein all the way into the peroxisome before returning to the cytosol. Like the membranes of mitochondria and chloroplasts, the peroxisomal membrane contains a protein translocator that aids in the transport. Unlike the mechanism that operates in mitochondria and chloroplasts, however, proteins do not need to unfold to enter the peroxisome-and the transport mechanism is still mysterious.

Although most peroxisomal proteins-including those embedded in the peroxisomal membrane-come from the cytosol, a few membrane proteins arrive via vesicles that bud from the ER membrane. The vesicles either fuse with preexisting peroxisomes or import peroxisomal proteins from the cytosol to grow into mature peroxisomes. The most severe peroxisomal disease, called Zellweger syndrome, is caused by mutations that block peroxisomal protein import.

Protein Transport in Endoplasmic reticulum (ER)

The endoplasmic reticulum serves as an entry point for proteins destined for other organelles, as well as for the ER itself. Proteins destined for the Golgi apparatus, endosomes, and lysosomes, as well as proteins destined for the cell surface, all first enter the ER from the cytosol. Once inside the ER lumen, or embedded in the ER membrane, individual proteins will not re-enter the cytosol during their onward journey. They will instead be ferried by transport vesicles from organelle to organelle within the endomembrane system, or to the plasma membrane.

Two kinds of proteins are transferred from the cytosol to the ER: (1) water soluble proteins are completely translocated across the ER membrane and are released into the ER lumen; (2) prospective transmembrane proteins are only partly translocated across the ER membrane and become embedded in it. The water-soluble proteins are destined either for secretion (by release at the cell surface) or for the lumen of an organelle of the endomembrane system. The transmembrane proteins are destined to reside in the membrane of one of these organelles or in the plasma membrane. All of these proteins are initially directed to the ER by an *ER signal sequence*, a segment of eight or more hydrophobic amino acids, which is also involved in the process of translocation across the membrane.

Unlike the proteins that enter the nucleus, mitochondria, chloroplasts, or peroxisomes, most of the proteins that enter the ER begin to be threaded across the ER membrane before the polypeptide chain has been completely synthesized. This requires that the ribosome synthesizing the protein be attached to the ER membrane. There are, therefore, two separate populations of ribosomes in the cytosol. *Membrane-bound ribosomes* are attached to the cytosolic side of the ER membrane (and outer nuclear membrane) and are making proteins that are being translocated into the ER. *Free ribosomes* are unattached to any membrane and are making all of the other proteins encoded by the nuclear DNA. When a ribosome happens to be making a protein with an ER signal sequence, the signal sequence directs the ribosome to the ER membrane.

Two protein components help guide ER signal sequences to the ER membrane: (1) a *signal-recognition particle* (*SRP*), present in the cytosol, binds to both the ribosome and the ER

signal sequence when it emerges from the ribosome, and (2) an *SRP receptor*, embedded in the ER membrane, recognizes the SRP. Binding of an SRP to a ribosome that displays an ER signal sequence slows protein synthesis by that ribosome until the SRP engages with an SRP receptor on the ER. Once bound, the SRP is released, the receptor passes the ribosome to a protein translocator in the ER membrane, and protein synthesis recommences. The polypeptide is then threaded across the ER membrane through a *channel* in the translocator (Figure 4). Thus the SRP and SRP receptor function as molecular matchmakers, uniting ribosomes that are synthesizing proteins with an ER signal sequence and available translocation channels in the ER membrane (Figure 4).

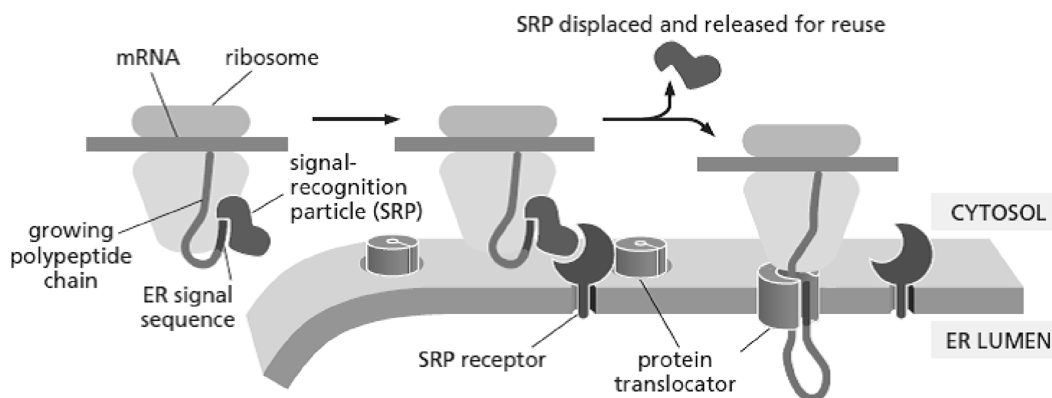


Figure 4: Protein transport in ER

Some protein remain embedded in the ER membrane as transmembrane proteins. Such proteins has some parts of the polypeptide chain that must be translocated completely across the lipid bilayer, whereas other parts remain fixed in the membrane. In the simplest case, that of a transmembrane protein with a single membrane-spanning segment, the N-terminal signal sequence initiates translocation-as it does for a soluble protein. But the transfer process is halted by an additional sequence of hydrophobic amino acids, a *stop-transfer sequence*, further along the polypeptide chain. At this point, the translocation channel releases the growing polypeptide chain sideways into the lipid bilayer. The N-terminal signal sequence is cleaved off, whereas the stop-transfer sequence remains in the bilayer, where it forms an α-helical membrane-spanning segment that anchors the protein in the membrane. As a result,

the protein ends up as a single-pass transmembrane protein inserted in the membrane with a defined orientation-the N-terminus on the lumenal side of the lipid bilayer and the C-terminus on the cytosolic side (Figure 5).

Some transmembrane proteins, an internal, rather than an N-terminal, signal sequence is used to start the protein transfer; this internal signal sequence, called a *start-transfer sequence,* is never removed from the polypeptide. This arrangement occurs in some transmembrane proteins in which the polypeptide chain passes back and forth across the lipid bilayer. In these cases, hydrophobic signal sequences are thought to work in pairs: an internal start-transfer sequence serves to initiate translocation, which continues until a stop-transfer sequence is reached; the two hydrophobic sequences are then released into the bilayer, where they remain as membrane-spanning α helices (Figure 5). In complex multipass proteins, in which many hydrophobic α helices span the bilayer, additional pairs of start- and stop-transfer sequences come into play: one sequence reinitiates translocation further down the polypeptide chain, and the other stops translocation and causes polypeptide release, and so on for subsequent starts and stops. Thus, multipass membrane proteins are stitched into the lipid bilayer as they are being synthesized, by a mechanism resembling the workings of a sewing machine (Figure 5).

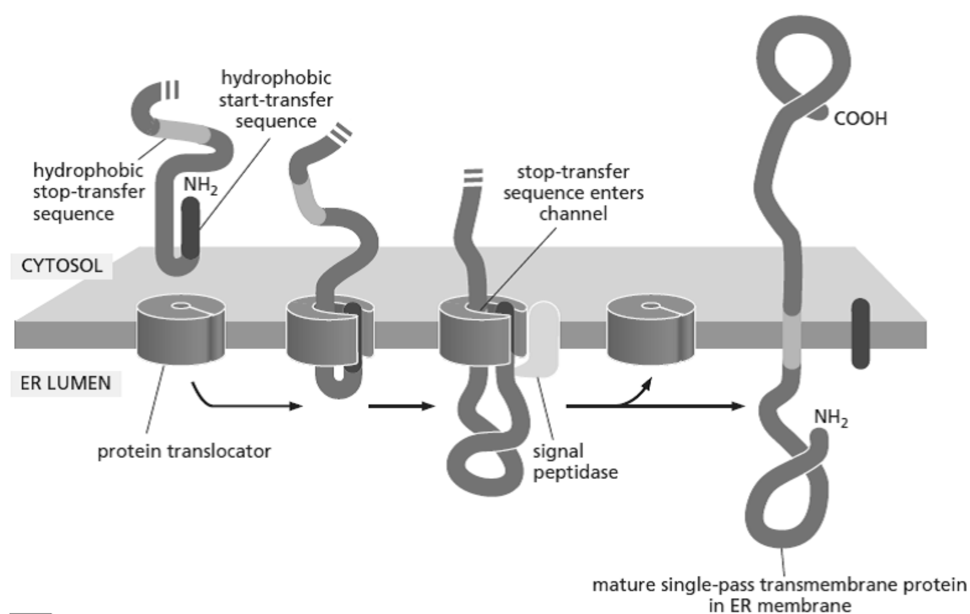


Figure 5: Transmembrane protein transport in ER

Vesicular Protein Transport

Entry into the ER lumen or membrane is usually only the first step on a pathway to another destination. That destination, initially at least, is generally the Golgi apparatus; there, proteins and lipids are modified and sorted for shipment to other sites. Transport from the ER to the Golgi apparatus and from the Golgi apparatus to other compartments of the endomembrane system is carried out by the continual budding and fusion of transport vesicles. This vesicular transport extends outward from the ER to the plasma membrane, and inward from the plasma membrane to lysosomes, and thus provides routes of communication between the interior of the cell and its surroundings. As proteins and lipids are transported outward along these pathways, many of them undergo various types of chemical modification, such as the addition of carbohydrate side chains.

Vesicular transport between membrane-enclosed compartments of the endomembrane system is highly organized. A major outward *secretory pathway* starts with the synthesis of proteins on the ER membrane and their entry into the ER, and it leads through the Golgi apparatus to the cell surface; at the Golgi apparatus, a side branch leads off through endosomes to lysosomes. A major inward *endocytic pathway*, which is responsible for the ingestion and degradation of extracellular molecules, moves materials from the plasma membrane, through endosomes, to lysosomes (Figure 6). To function optimally, each transport vesicle that buds off from a compartment must take with it only the proteins appropriate to its destination and must fuse only with the appropriate target membrane. A vesicle carrying cargo from the Golgi apparatus to the plasma membrane, for example, must exclude proteins that are to stay in the Golgi apparatus, and it must fuse only with the plasma membrane and not with any other organelle. While participating in this constant flow of membrane components, each organelle must maintain its own distinct identity, that is, its own distinctive protein and lipid composition. All of these recognition events depend on proteins displayed on the surface of the transport vesicle.

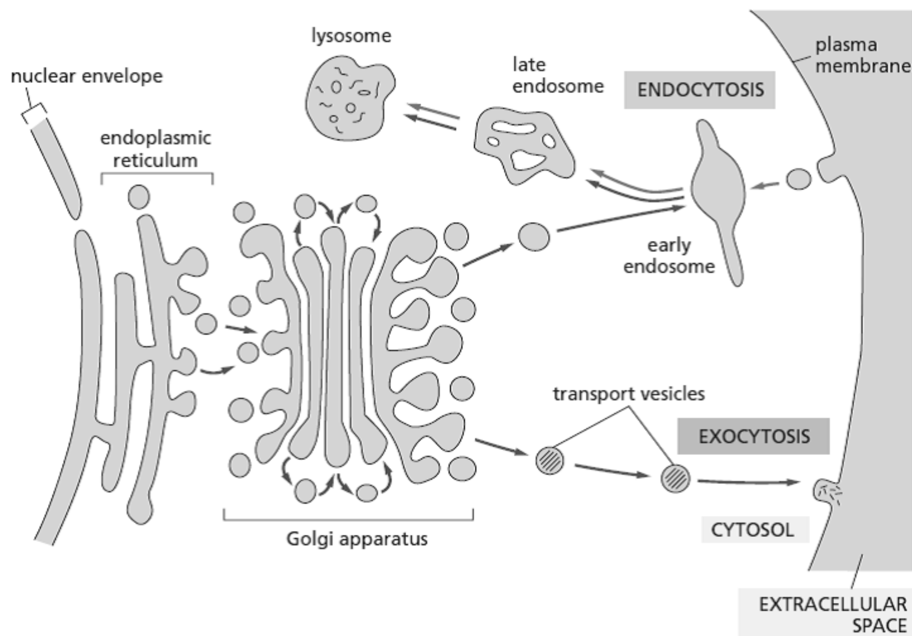


Figure 6 : Transport in Endomembrane system

Protein Packaging via Coated Vesicle : Vesicles that bud from membranes usually have a distinctive protein coat on their cytosolic surface and are therefore called coated vesicles. After budding from its parent organelle, the vesicle sheds its coat, allowing its membrane to interact directly with the membrane to which it will fuse. Cells produce several kinds of coated vesicles, each with a distinctive protein coat. The coat serves at least two functions: it helps shape the membrane into a bud and captures molecules for onward transport. The best-studied vesicles are those that have an outer coat made of the protein clathrin. These *clathrin-coated vesicles* bud from both the Golgi apparatus on the outward secretory pathway and from the plasma membrane on the inward endocytic pathway. At the plasma membrane, for example, each vesicle starts off as a *clathrin-coated pit*. Clathrin molecules assemble into a basketlike network on the cytosolic surface of the membrane, and it is this assembly process that starts shaping the membrane into a vesicle (Figure 7). A small GTP-binding protein called *dynamin* assembles as a ring around the neck of each deeply invaginated coated pit. Together with other proteins recruited to the neck of the vesicle, the dynamin causes the ring to constrict, thereby pinching off the vesicle from its parent membrane. Other kinds of transport vesicles, with different coat proteins, are also involved in vesicular transport. They

form in a similar way and carry their own characteristic sets of molecules between the endoplasmic reticulum, the Golgi apparatus, and the plasma membrane.

The mechanism of selecting its particular cargo in transport vesicle is best understood for clathrin-coated vesicles. Clathrin itself plays no part in choosing specific molecules for transport. This is the function of a second class of coat proteins called *adaptins,* which both secure the clathrin coat to the vesicle membrane and help select cargo molecules for transport. Molecules for onward transport carry specific *transport signals* that are recognized by *cargo receptors* in the Golgi or plasma membrane. Adaptins help capture specific cargo molecules by trapping the cargo receptors that bind them. In this way, a selected set of cargo molecules, bound to their specific receptors, is incorporated into the lumen of each newly formed clathrin-coated vesicle (Figure 7). There are different types of adaptins: the adaptins that bind cargo receptors in the plasma membrane, for example, are not the same as those that bind cargo receptors in the Golgi apparatus, reflecting the differences in the cargo molecules to be transported from each of these sources.

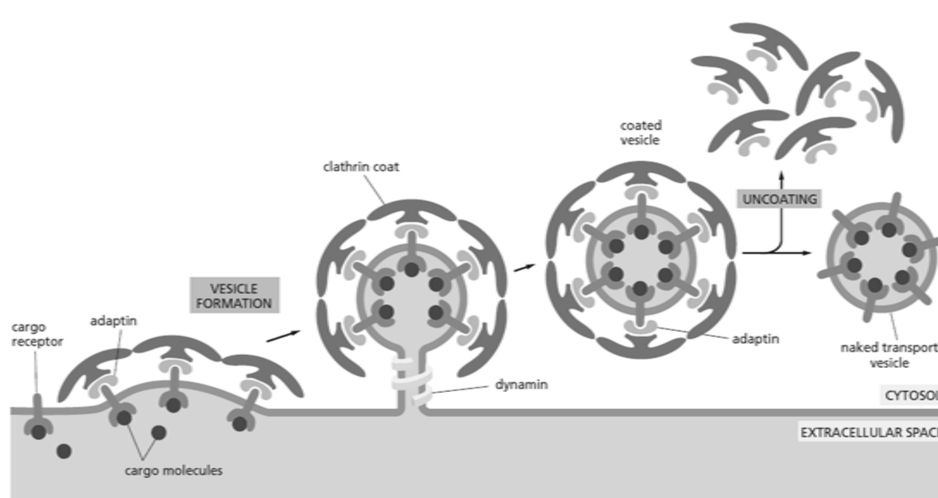


Figure 7: Clathrin coated vesicle transport system

Another class of coated vesicles, called *COP-coated vesicles* (COP being shorthand for "coat protein"), is involved in transporting molecules between the ER and the Golgi apparatus and from one part of the Golgi apparatus to another (Table 2).

Type of Coated Vesicle	Coat Proteins	Origin	Destination
Clathrin-coated	clathrin + adaptin 1	Golgi apparatus	lysosome (via endosomes)
Clathrin-coated	clathrin + adaptin 2	plasma membrane	endosomes
COP-coated	COP proteins	ER Golgi cisterna Golgi apparatus	Golgi apparatus Golgi cisterna ER

Table 2: Type of Coated vesicles

Coated vesicle recognition, docking and unloading of cargo into destination membrane : Once a transport vesicle has reached its target, it must recognize and dock with its specific organelle and finally fuse with the target membrane to unload the vesicle's cargo. The impressive specificity of vesicular transport suggests that each type of transport vesicle in the cell displays molecular markers on its surface that identify the vesicle according to its origin and cargo. These markers must be recognized by complementary receptors on the appropriate target membrane, including the plasma membrane. The identification process depends on a diverse family of monomeric GTPases called Rab proteins. Specific Rab proteins on the surface of each type of vesicle are recognized by corresponding *tethering proteins* on the cytosolic surface of the target membrane. Each organelle and each type of transport vesicle carries a unique combination of Rab proteins, which serve as molecular markers for each membrane type. The coding system of matching Rab and tethering proteins helps to ensure that transport vesicles fuse only with the correct membrane (Figure 8).

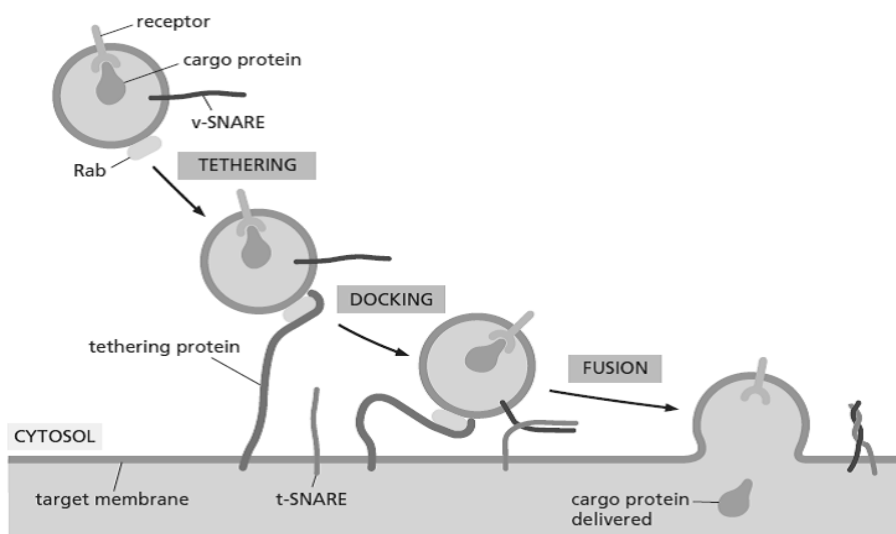


Figure 8: Transport of vesicle into destination membrane

Additional recognition is provided by a family of transmembrane proteins called SNAREs. Once the tethering protein has captured a vesicle by grabbing hold of its Rab protein, SNAREs on the vesicle (called v-SNAREs) interact with complementary SNAREs on the target membrane (called t-SNAREs), firmly docking the vesicle in place (Figure 8). The same SNAREs involved in docking also play a central role in catalyzing the membrane fusion required for a transport vesicle to deliver its cargo. Fusion not only delivers the soluble contents of the vesicle into the interior of the target organelle, but it also adds the vesicle membrane to the membrane of the organelle (Figure 8). After vesicle docking, the fusion of a vesicle with its target membrane sometimes requires a special stimulatory signal. Whereas docking requires only that the two membranes come close enough for the SNAREs protruding from the two lipid bilayers to interact, fusion requires a much closer approach: the two bilayers must come within 1.5 nm of each other so that their lipids can intermix. For this close approach, water must be displaced from the hydrophilic surfaces of the membranes-a process that is energetically highly unfavorable and thus prevents membranes from fusing randomly. All membrane fusions in cells must therefore be catalyzed by specialized proteins that assemble to form a fusion complex, which provides the means to cross this energy barrier. The SNARE proteins themselves catalyze the fusion process: once fusion is triggered, the v-SNAREs and t-SNAREs wrap around each other, thereby acting like a winch that pullsthe two lipid bilayers into close proximity.

Protein Storage and Release in Secretory Pathway

Secretory Pathway: Vesicular traffic extends to and from the plasma membrane. Newly made proteins, lipids, and carbohydrates are delivered from the ER, via the Golgi apparatus, to the cell surface by transport vesicles that fuse with the plasma membrane in the process of *exocytosis* (Figure 6). Each molecule that travels along this route passes through a fixed sequence of membrane-enclosed compartments and is often chemically modified en route.

The outward path of proteins travels from the ER, where they are made and modified, through the Golgi apparatus, where they are further modified and sorted, to the plasma membrane. As a protein passes from one compartment to another, it is monitored to check that it has folded properly and assembled with its appropriate partners, so that only correctly

built proteins make it to the cell surface. Incorrect assemblies, which are often in the majority, are degraded inside the cell.

Most proteins that enter the ER are chemically modified there. *Disulfide bonds* are formed by the oxidation of pairs of cysteine side chains, a reaction catalyzed by an enzyme that resides in the ER lumen. Many of the proteins that enter the ER lumen or ER membrane are converted to glycoproteins in the ER by the covalent attachment of short branched oligosaccharide side chains composed of multiple sugars.

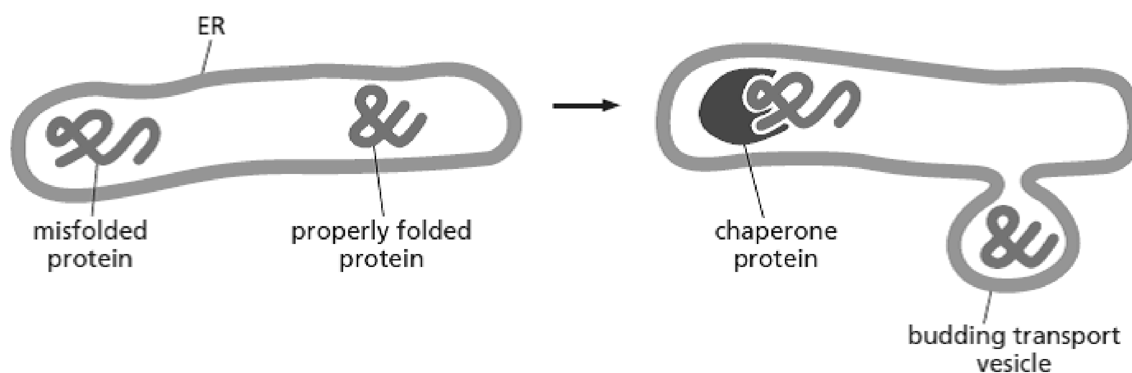


Figure 9: Chaperone protein in ER

Some proteins made in the ER are destined to function there. They are retained in the ER (and are returned to the ER whenever they escape to the Golgi apparatus) by a C-terminal sequence of four amino acids called an *ER retention signal*. This retention signal is recognized by a membrane-bound receptor protein in the ER and Golgi apparatus. Most proteins that enter the ER, however, are destined for other locations; they are packaged into transport vesicles that bud from the ER and fuse with the Golgi apparatus Exit from the ER is highly selective. Proteins that fail to fold correctly, and dimeric or multimeric proteins that do not assemble properly, are actively retained in the ER by binding to *chaperone proteins* that reside there. The chaperones hold these proteins in the ER until proper folding or assembly occurs. Chaperones prevent misfolded proteins from aggregating, which helps steer proteins along a path toward proper folding (Figure 9); if proper folding and assembly still fail, the proteins are exported to the cytosol, where they are degraded. If the misfolded proteins accumulate in the ER, the buildup is large enough, it triggers a complex program called the unfolded protein response (UPR). This program prompts the cell to produce more ER, including more chaperones and other proteins concerned with quality control (Figure 9). The

UPR allows a cell to adjust the size of its ER according to the load of proteins entering the secretory pathway. In some cases, however, even an expanded ER cannot cope, and the UPR directs the cell to self-destruct by undergoing apoptosis.

Golgi Apparatus in Secretory Pathway: The Golgi apparatus consists of a collection of flattened, membrane-enclosed sacs called cisternae, which are piled like stacks of pita bread. Each stack contains 3–20 cisternae (Figure 10). Each Golgi stack has two distinct faces: an entry, or *cis*, face and an exit, or *trans,* face. The *cis* face is adjacent to the ER, while the *trans* face points toward the plasma membrane. The outermost cisterna at each face is connected to a network of interconnected membranous tubes and vesicles. Soluble proteins and membrane enter the *cis Golgi network* via transport vesicles derived from the ER. The proteins travel through the cisternae in sequence by means of transport vesicles that bud from one cisterna and fuse with the next. Proteins exit from the *trans Golgi network* in transport vesicles destined for either the cell surface or another organelle of the endomembrane system. Both the *cis* and *trans* Golgi networks are thought to be important for protein sorting: proteins entering the *cis* Golgi network can either move onward through the Golgi stack or, if they contain an ER retention signal, be returned to the ER; proteins exiting from the *trans* Golgi network are sorted according to whether they are destined for lysosomes (via endosomes) or for the cell surface.

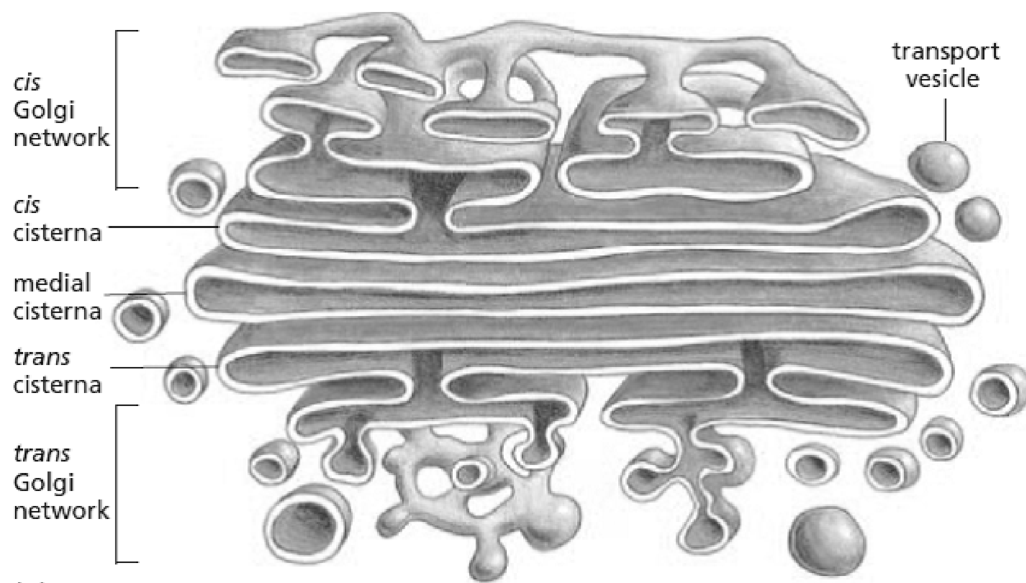


Figure 10 : Golgi Apparatus

Exocytosis of Protein : In all eukaryotic cells, a steady stream of vesicles buds from the *trans* Golgi network and fuses with the plasma membrane in the process of exocytosis. This *constitutive exocytosis pathway* supplies the plasma membrane with newly made lipids and proteins, enabling the plasma membrane to expand prior to cell division and refreshing old lipids and proteins in nonproliferating cells. The constitutive pathway also carries soluble proteins to the cell surface to be released to the outside, a process called secretion. Some of these proteins remain attached to the cell surface; some are incorporated into the extracellular matrix; still others diffuse into the extracellular fluid to nourish or signal other cells. Entry into the constitutive pathway does not require a particular signal sequence like those that direct proteins to endosomes or back to the ER.

In addition to the constitutive exocytosis pathway, which operates continually in all eukaryotic cells, there is a *regulated exocytosis pathway*, which operates only in cells that are specialized for secretion. Each specialized *secretory cell* produces large quantities of a particular product-such as a hormone, mucus, or digestive enzymes-which is stored in secretory vesicles for later release. These vesicles, which are part of the endomembrane system, bud off from the *trans* Golgi network and accumulate near the plasma membrane. There they wait for the extracellular signal that will stimulate them to fuse with the plasma membrane and release their contents to the cell exterior by exocytosis (Figure 11). An increase in blood glucose, for example, signals insulin-producing endocrine cells in the pancreas to secrete the hormone (Figure 11).

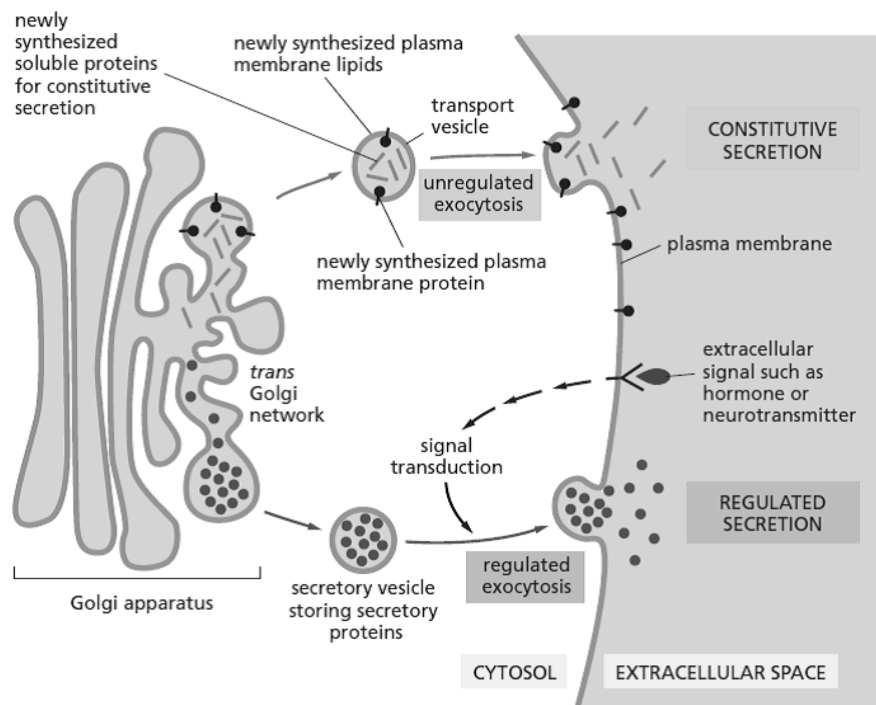


Figure 11 : Secretory pathway in secretory cells

Proteins destined for regulated secretion are sorted and packaged in the *trans* Golgi network. Proteins that travel by this pathway have special surface properties that cause them to aggregate with one another under the ionic conditions (acidic pH and high Ca2+) that prevail in the *trans* Golgi network. The aggregated proteins are packaged into secretory vesicles, which pinch off from the network and await a signal instructing them to fuse with the plasma membrane. Proteins secreted by the constitutive pathway, on the other hand, do not aggregate and are therefore carried automatically to the plasma membrane by the transport vesicles of the constitutive pathway. Selective aggregation has another function : it allows secretory proteins to be packaged into secretory vesicles at concentrations much higher than the concentration of the unaggregated protein in the Golgi lumen. This increase in concentration can reach 200-fold, enabling secretory cells to release large amounts of the protein promptly when triggered to do so (Figure 11). When a secretory vesicle or transport vesicle fuses with the plasma membrane and discharges its contents by exocytosis, its membrane becomes part of the plasma membrane. Although this should greatly increase the surface area of the plasma membrane, it does so only transiently because membrane components are removed from other regions of the surface by endocytosis almost as fast as they are added by exocytosis. This removal returns both the lipids and the proteins of the vesicle membrane to the Golgi network, where they can be used again.

Probable Questions :

1. How are particular proteins targeted to particular subcellular compartments?
2. What are the major morphological differences between the RER and SER? What are the major differences in their functions?
3. Describe the steps that occur between the time a ribosome attaches to a messenger RNA encoding a secretory protein and the time the protein leaves the RER.
4. How are newly synthesized integral proteins inserted into a membrane?
5. Contrast the roles of COPI and COPII coated vesicles in protein trafficking.
6. How does protein transport occur in Nucleus, Mitochondria, ER and Golgi Apparatus?
7. What is Signal sequence? Give an account on the characteristic feature of Signal sequence of protein transported to mitochondria, nucleus and ER.
8. How do retrieval signals ensure that proteins are kept as residents of a particular membrane compartment?
9. Describe the steps that ensure that a lysosomal enzyme will be targeted to a lysosome rather than a secretory vesicle.
10. Describe the molecular structure of clathrin and the relationship between its structure and function.
11. What is the fate of a protein with no sorting signal?

Suggested Readings/References :

1. Gerald Karp-Cell and Molecular Biology
2. Geoffrey M. Cooper, Robert E. Hausman-The Cell:A Molecular Approach
3. Bruce Alberts, Alexander Johnson, Julian Lewis, David Morgan, Martin Raff, Keith Roberts, Peter Walter-Molecular Biology of Cell
4. Harvey F Lodish-Molecular Cell Biology

Disclaimer :

The study materials of this book have been collected from books, various e-books, journals and other e-sources.