Hickman

Roberts

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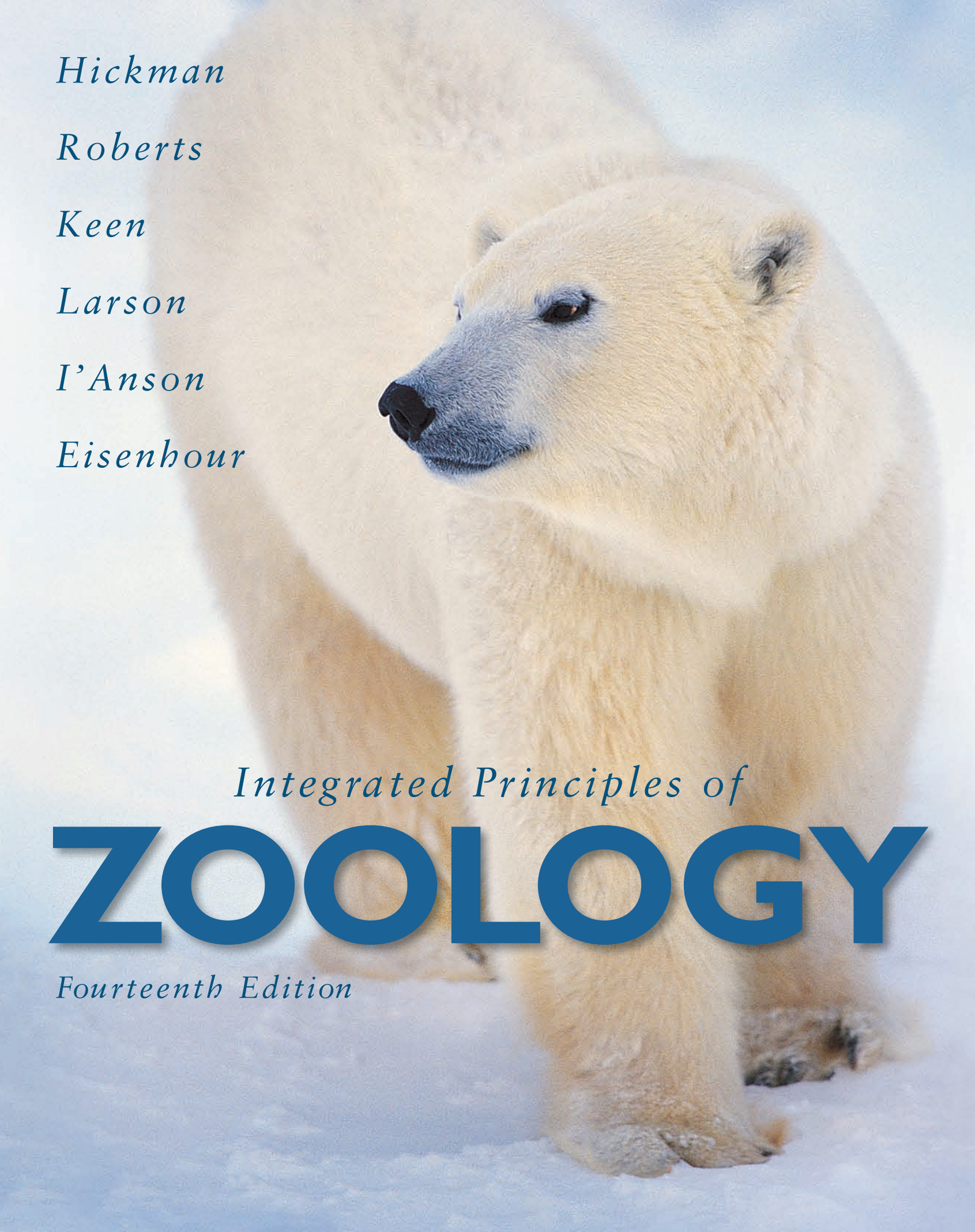


Integrated Principles of

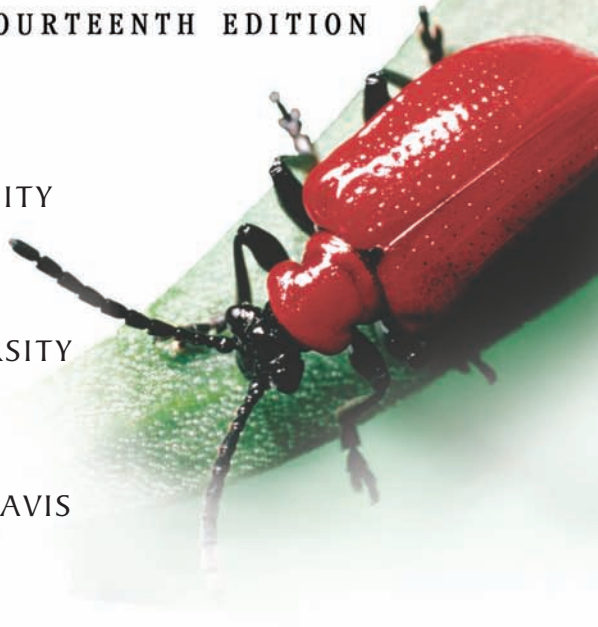
ZOOLOGY

Fourteenth Edition

INTEGRATED PRINCIPLES OF

ZOOLOGY

FOURTEENTH EDITION



Cleveland P. Hickman, Jr.
WASHINGTON AND LEE UNIVERSITY

Larry S. Roberts
FLORIDA INTERNATIONAL UNIVERSITY

Susan L. Keen
UNIVERSITY OF CALIFORNIA AT DAVIS

Allan Larson
WASHINGTON UNIVERSITY

Helen I'Anson
WASHINGTON AND LEE UNIVERSITY

David J. Eisenhour
MOREHEAD STATE UNIVERSITY

Original Artwork by
WILLIAM C. OBER, M.D.
Washington and Lee University and Shoals Marine Laboratory
and
CLAIRE W. GARRISON, B.A.
Shoals Marine Laboratory, Cornell University

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ABOUT THE AUTHORS

CLEVELAND P. HICKMAN, JR.

Cleveland P. Hickman, Jr., Professor Emeritus of Biology at Washington and Lee University in Lexington, Virginia, has taught zoology and animal physiology for more than 30 years. He received his Ph.D. in comparative physiology from the University of British Columbia, Vancouver, B.C., in 1958 and taught animal physiology at the University of Alberta before moving to Washington and Lee University in 1967. He has published numerous articles and research papers in fish physiology, in addition to co-authoring these highly successful texts: *Integrated Principles of Zoology, Biology of Animals, Animal Diversity, Laboratory Studies in Animal Diversity,* and *Laboratory Studies in Integrated Principles of Zoology.*

Over the years Dr. Hickman has led many field trips to the Galápagos Islands. His current research is on intertidal zonation and marine invertebrate systematics in the Galápagos. He has published three field guides in the Galápagos Marine Life Series for the identification of echinoderms, marine molluscs, and marine crustaceans.

His interests include scuba diving, woodworking, and participating in chamber music ensembles.

Dr. Hickman can be contacted at:
hickmanc@wlu.edu

LARRY S. ROBERTS

Larry S. Roberts, Professor Emeritus of Biology at Texas Tech University and an adjunct professor at Florida International University, has extensive experience teaching invertebrate zoology, marine biology, parasitology, and developmental biology. He received his Sc.D. in parasitology at the Johns Hopkins University and is the lead author of Schmidt and Roberts's *Foundations of Parasitology,* sixth edition. Dr. Roberts is also co-author of *Integrated Principles of Zoology, Biology of Animals,* and *Animal Diversity,* and is author of *The Underwater World of Sport Diving.*

Dr. Roberts has published many research articles and reviews. He has served as President of the American Society of Parasitologists, Southwestern Association of Parasitologists, and Southeastern Society of Parasitologists, and is a member of numerous other professional societies. Dr. Roberts also serves on the Editorial Board of the journal, *Parasitology Research*. His hobbies include scuba diving, underwater photography, and tropical horticulture.

Dr. Roberts can be contacted at:
Lroberts1@compuserve.com

SUSAN KEEN

Susan Keen is a lecturer in the Section of Evolution and Ecology at the University of California at Davis. She received her Ph.D. in zoology from the University of California at Davis, following a M.Sc. from the University of Michigan at Ann Arbor. She is a native of Canada and obtained her undergraduate education at the University of British Columbia in Vancouver.

Dr. Keen is an invertebrate zoologist fascinated with jellyfish life histories. She has a particular interest in life cycles where both asexual and sexual phases of organisms are present, as they are in most jellyfishes. Her other research has included work on sessile marine invertebrate communities, spider populations, and Andean potato evolution.

Dr. Keen has been teaching evolution and animal diversity within the Introductory Biology series for 13 years. She enjoys all facets of the teaching process, from lectures and discussions to the design of effective laboratory exercises. In addition to her work with introductory biology, she offers seminars for the Davis Honors Challenge program, and for undergraduate and graduate students interested in teaching methods for biology. She was given an Excellence in Education Award from the Associated Students group at Davis in 2004. She attended the National Academies Summer Institute on Undergraduate Education in Biology in 2005, and was a National Academies Education Fellow in the Life Sciences for 2005–2006. Her interests include weight training, horseback riding, gardening, travel, and mystery novels.

Dr. Keen can be contacted at:
slkeen@ucdavis.edu

ALLAN LARSON

Allan Larson is a professor at Washington University, St. Louis, MO. He received his Ph.D. in genetics at the University of California, Berkeley. His fields of specialization include evolutionary biology, molecular population genetics and systematics, and amphibian systematics. He teaches courses in introductory genetics, zoology, macroevolution, molecular evolution, and the history of evolutionary theory, and has organized and taught a special course in evolutionary biology for high-school teachers.

Dr. Larson has an active research laboratory that uses DNA sequences to examine evolutionary relationships among vertebrate species, especially in salamanders and lizards. The students in Dr. Larson's laboratory have participated in zoological field studies around the world, including projects in Africa, Asia, Australia, Madagascar, North America, South America, the Indo-Pacific Ocean, and the Caribbean Islands. Dr. Larson has authored numerous scientific publications, and has edited for the journals *The American Naturalist, Evolution, Journal of Experimental Zoology, Molecular Phylogenetics and Evolution,* and *Systematic Biology.* Dr. Larson serves as an academic advisor to undergraduate students and supervises the undergraduate biology curriculum at Washington University.

Dr. Larson can be contacted at:
larson@wustl.edu

HELEN I'ANSON

Helen I'Anson, a native of England, is professor of biology at Washington and Lee University in Lexington, Virginia. She received her Ph.D. in physiology at the

University of Kentucky, Lexington, KY, and postdoctoral training at the University of Michigan, Ann Arbor, MI. She teaches courses in animal physiology, microanatomy, neuroendocrinology, general biology, and reproductive physiology. She has an active research program that focuses on the neural regulation of reproductive development. In particular, she is interested in how energy is partitioned in the developing animal, how signals from food and food storage depots are monitored by the brain, and how such signals are transduced to regulate reproductive activity at the onset of puberty in mammals.

Her interests include gardening, hiking, fishing, aromatherapy, music, and participating in choral ensembles.

Dr. I'Anson can be contacted at:
iansonh@wlu.edu

DAVID J. EISENHOUR

David J. Eisenhour is an associate professor of biology at Morehead State University in Morehead, Kentucky. He received his Ph.D. in zoology from Southern Illinois University, Carbondale. He teaches courses in environmental science, human anatomy, general zoology, comparative anatomy, ichthyology, and vertebrate zoology. David has an active research program that focuses on systematics, conservation biology, and natural history of North American freshwater fishes. He has a particular interest in the diversity of Kentucky's fishes and is writing a book about that subject. He and his graduate students have authored several publications. David serves as an academic advisor to prepharmacy students.

His interests include fishing, landscaping, home remodeling, and entertaining his three young children, who, along with his wife, are enthusiastic participants in fieldwork.

Dr. Eisenhour can be contacted at:
d.eisenhour@morehead-st.edu

PREFACE

Integrated Principles of Zoology is a college text designed for an introductory course in zoology. This fourteenth edition, as with previous editions, describes the diversity of animal life and the fascinating adaptations that enable animals to inhabit so many ecological niches.

We retain in this revision the basic organization of the thirteenth edition and its distinctive features, especially emphasis on the principles of evolution and zoological science. Also retained are several pedagogical features that have made previous editions easily accessible to students: opening chapter dialogues drawn from the chapter's theme; chapter summaries and review questions to aid student comprehension and study; concise and visually appealing illustrations; in-text derivations of generic names; chapter notes and essays that enhance the text by offering interesting sidelights to the narrative; literature citations; and an extensive glossary providing pronunciation, derivation, and definition of terms used in the text.

NEW TO THE FOURTEENTH EDITION

The authors welcome to the fourteenth edition Susan Keen, who supervised this revision. Many improvements are the direct result of Susan's new perspectives and those of many zoology instructors who submitted reviews of the thirteenth edition. We revised all chapters to streamline the writing and to incorporate new discoveries and literature citations. Our largest formal revision is to include a cladogram of animal phyla on the inside front cover of the book, and to reorder chapter contents in Part Three (Diversity of Animal Life) to match the arrangement of phyla on the cladogram. Each chapter in Part Three begins with a small image of the zoological cladogram highlighting the phylum or phyla covered in the chapter, followed by an expanded cladogram of the contents of each major phylum. We place stronger emphasis on phylogenetic perspectives throughout the book. Material formerly presented separately as "biological contributions" and "characteristics" of phyla is consolidated in a boxed list of phylum "characteristics" for each chapter in Part Three. New photographs are added to illustrate animal diversity in many phyla.

Material new to the fourteenth edition expands and updates our coverage of eight major principles: (1) scientific process and the role of theory, (2) cellular systems and metabolism, (3) endosymbiotic theory of eukaryotic origins, (4) physiological and ecological systems, (5) populational processes and conservation, (6) evolutionary developmental biology, (7) phylogenetic tests of morphological homologies, and (8) taxonomy. Exciting new fossil discoveries and molecular phylogenies contribute important changes to the last three principles. The primary changes to each major principle are summarized here with references to the relevant chapters.

Scientific Process and the Role of Theory

Many changes throughout the book increase the integration of hypothetico-deductive methodology in discussing new discoveries and controversies. We begin in Chapter 1 with a more detailed explanation of the hypothetico-deductive method of science and the important contrast between the comparative method versus experimental biology as complementary means of testing hypotheses. The role of theory in science is illustrated explicitly using Darwin's theory of common descent in Chapter 6. Uses of Darwin's theory of common descent to test evolutionary hypotheses and to construct taxonomies get expanded treatment in Chapter 10, including a new conceptual distinction between classification and systematization and coverage of DNA barcoding in species identification.

Cellular Systems and Metabolism

We expand in Chapter 3 our coverage of the components of eukaryotic cells, the biological roles of subcellular structures, and specializations of cellular surfaces. Expanded molecular topics include pH (Chapter 2), prions as diseases of protein conformation (Chapter 2), lipid metabolism (Chapter 4), and accumulation of "junk" or "parasitic" DNA in animal genomes (Chapter 5). In Chapter 7, a new boxed essay reports the discovery of actively dividing germ cells in adult female mammals, and a revised boxed essay updates applications of cell biology to contraceptive medicine.

Endosymbiotic Theory of Eukaryotic Origins

The history of the endosymbiotic theory is presented in more detail, including the empirical testing of its original claims and its more recent expansion to cover a broader evolutionary domain (Chapter 2). Important molecular phylogenetic evidence for separate evolutionary origins of nuclear, mitochondrial, and chloroplast genomes is presented in the form of a new global "tree of life" relating prokaryotic and eukaryotic genomes (Chapter 10). The role of endosymbiosis in diversification of unicellular eukaryotes gets new coverage in Chapter 11, and evolutionary loss of mitochondria from some infectious unicellular eukaryotes is added to Chapter 2.

Physiological and Ecological Systems

Numerous revisions address organismal physiology and its ecological consequences, beginning with the addition of "movement" as a general characteristic of life in Chapter 1. We add

new results on tracheal respiration in insects (Chapter 21), respiratory gas transport in terrestrial arthropods and in vertebrates (Chapter 31), and lung ventilation in vertebrates (Chapter 31). Also revised are the plans of vertebrate circulatory systems, coronary circulation, and excitation and control of the heart (Chapter 31). New material appears on regulation of food intake and of digestion (Chapter 32), digestive processes in the vertebrate small intestine (Chapter 32), and foregut fermentation in ruminant mammals (Chapter 28). Evolution of centralized nervous systems, chemoreception, mechanoreception and photoreception in invertebrates gets new coverage in Chapter 33, with expanded explanation of synapses and conduction of action potentials. Endocrinology of invertebrates is expanded, and vertebrate endocrinology is updated to include discussion of white adipose tissue as an endocrine organ, the pancreatic polypeptide (PP) hormone, and controversies regarding medicinal uses of anabolic steroids (Chapter 34). Invertebrate excretory systems, especially arthropod kidneys, get expanded coverage in Chapter 30. We cover regional endothermy in fishes (Chapter 24), and add new explanatory material on the importance of water and osmotic regulation, especially in marine fishes (Chapter 30). Revision of Chapter 35 updates our knowledge of susceptibility and resistance to disease, including acquired immune deficiency. We add a new section on cetacean echolocation (Chapter 28), greater explanation of frog mating systems (Chapter 25), and avian reproductive strategies, including extra-pair copulations (Chapter 27). We provide greater coverage of scientific controversies regarding bee communication, eusociality, and genetics of animal behavior (Chapter 36). Concepts of food chains and food webs are now distinguished, and quantitative data are added to illustrate them using ecological pyramids (Chapter 38).

Populational Processes and Conservation

Modes of speciation receive expanded coverage and explanation (Chapter 6), as do concepts of fitness and inclusive fitness (Chapter 6), and costs and benefits of sexual versus asexual reproduction (Chapter 7). Conservation of natural populations is updated, especially in fishes (Chapter 24), mammals (Chapter 36), and tuataras (Chapter 26). Historical biogeographic processes are illustrated with expanded coverage of explanations for Wallace's Line, the geographic contact between evolutionarily disparate faunas (Chapter 37).

Evolutionary Developmental Biology

This rapidly growing discipline gets updated coverage both in concept and application. New concepts of developmental modularity and evolvability join our general coverage of evolutionary biology in Chapter 6. We discuss in Chapter 8 new evidence that some sponges have two germ layers. Cnidarian development and life cycles get expanded coverage, and the diploblastic status of cnidarians and ctenophores is reconsidered in light of new phylogenetic results (Chapter 13). We provide molecular-genetic interpretations of the diploblast-triploblast distinction (Chapter 13) and updated details of triploblastic development (Chapter 14). Insights from genomic and developmental studies offer new interpretations of metazoan origins (Chapter 12) and suggest that changes in the expression of a single gene underlie alternative developmental pathways of arthropod limbs (uniramous versus biramous; Chapter 19). Developmental differences among chaetognaths, protostomes, and deuterostomes are reevaluated in light of new phylogenetic evidence (Chapter 22). We restructure our general coverage of body plans (Chapter 9) and provide greater explanation of the complex development of gastropod torsion (Chapter 16).

Phylogenetic Tests of Morphological Homologies

New molecular phylogenies and fossil discoveries revise our interpretations of many homologies and reveal independent evolution of similar characters in different groups. In light of these issues, we expand our coverage of the concept of homoplasy in Chapter 10. Chapter 16 incorporates new evidence challenging homology of metamerism in annelids and molluscs and illustrating the scientific process in action. Evidence from *Hox* gene expression is used in Chapter 19 to homologize the cephalothorax of spiders with heads of other arthropods and to support phylogenetic evidence for multiple origins of uniramous limbs from biramous ones in phylum Arthropoda. Homology of diffuse epidermal nervous systems and tripartite coeloms of echinoderms and hemichordates and nonhomology of dorsal hollow nerve chords of hemichordates and chordates change our favored hypotheses for relationships among these groups (Chapter 22). Developmental comparisons demonstrate nonhomology of coelomic compartmentalization of lophophorates with that of echinoderms and hemichordates (Chapter 22). New data and interpretations revise inferred characteristics of the most recent chordate ancestor (Chapter 23), origin and diversification of amniotes and their adaptations for terrestrial life (Chapter 26), evolution of the mammalian middle ear (Chapter 28), and details of hominid morphological evolution (Chapter 28).

Taxonomy

New molecular-phylogenetic and fossil data reject some familiar taxa and suggest new ones. We discuss evidence for a sister-group relationship of choanoflagellates and metazoans in Chapter 12. Chapter 14 discusses new phylogenetic results that underlie recognition of phylum Acoelomorpha and a revised phylogenetic hypothesis for nemertine worms. Acanthocephalans now appear to descend from a rotiferan ancestor (Chapter 15). Clade Clitellata (oligochaetes and leeches), pogonophorans, and vestimentiferans descend from polychaete annelids according to new phylogenetic data, making polychaetes paraphyletic (Chapter 17). Chapter 18 presents new evidence for clade Panarthropoda (Onychophora, Tardigrada, and Arthropoda). Chapters 19 and 21 present evidence supporting

recognition of clade Pancrustacea (crustaceans and hexapods) and rejection of arthropod subphylum Uniramia. Chapter 20 includes new evidence that hexapods derive from a crustacean ancestor, and Pentastomida is subsumed in Crustacea. Entognatha and Insecta form separate clades within subphylum Hexapoda (Chapter 21). We update recognition of insect orders in Chapter 21. We introduce in Chapter 22 clade Ambulacraria (Echinodermata and Hemichordata), which is likely the sister group of chordates (Chapter 23). The fossil genus *Haikouella* gets increased coverage and illustration as the likely sister taxon to craniates (Chapter 23). Changes to fish taxonomy include using the clade name Petromyzontida for lampreys and removing bichirs from chondrosteans (Chapter 24). Early tetrapod evolution is extensively revised with reference to new fossil discoveries, including the genus *Tiktaalik* (Chapter 25). We replace the traditional use of "Reptilia" with one including the traditional reptiles, birds, and all descendants of their most recent common ancestor (Chapter 26). Phylogenetic results place turtles in the clade Diapsida (Chapter 26), contrary to earlier hypotheses. Amphisbaenians are now included within lizards according to their phylogenetic position, and the section on relationships of snakes to lizards is expanded (Chapter 26). Chapter 27 includes fairly extensive revisions of avian taxonomy based upon phylogenetic results from DNA sequence data.

TEACHING AND LEARNING AIDS

To help students in **vocabulary development,** key words are boldfaced and derivations of technical and zoological terms are provided, along with generic names of animals where they first appear in the text. In this way students gradually become familiar with the more common roots that form many technical terms. An extensive **glossary** provides pronunciation, derivation, and definition of each term. Many new terms were added to the glossary or rewritten for this edition.

A distinctive feature of this text is a **prologue** for each chapter that highlights a theme or fact relating to the chapter. Some prologues present biological, particularly evolutionary, principles; those in Part Three on animal diversity illuminate distinguishing characteristics of the group presented in the chapter.

Chapter notes, which appear throughout the book, augment the text material and offer interesting sidelights without interrupting the narrative. We prepared many new notes for this edition and revised several existing notes.

To assist students in chapter review, each chapter ends with a **concise summary,** a list of **review questions,** and **annotated selected references.** The review questions enable a student to self-test retention and understanding of the more important chapter material.

Again, William C. Ober and Claire W. Garrison have strengthened the art program for this text with many new full-color paintings that replace older art, or that illustrate new material. Bill's artistic skills, knowledge of biology, and experience gained from an earlier career as a practicing physician have enriched this text through ten of its editions. Claire practiced pediatric and obstetric nursing before turning to scientific illustration as a full-time career. Texts illustrated by Bill and Claire have received national recognition and won awards from the Association of Medical Illustrators, American Institute of Graphic Arts, Chicago Book Clinic, Printing Industries of America, and Bookbuilders West. They are also recipients of the Art Directors Award.

SUPPLEMENTS

For Students

Online Learning Center

The **Online Learning Center** for ***Integrated Principles of Zoology*** is a great place to review chapter material and to enhance your study routine. Visit **www.mhhe.com/hickmanipz14e** for access to the following online study tools:

- Chapter quizzing
- Key term flash cards
- Web links
- And more!

For Instructors

Online Learning Center

The ***Integrated Principles of Zoology*** **Online Learning Center (www.mhhe.com/hickmanipz14e)** offers a wealth of teaching and learning aids for instructors and students. Instructors will appreciate:

- A password-protected Instructor's Manual
- Access to the new online **Presentation Center** including illustrations, photographs, and tables from the text
- PowerPoint lecture outlines
- Links to professional resources
- Online activities for students such as chapter quizzing, key-term flash cards, web links, and more!

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Instructor's Manual

This helpful ancillary provides chapter outlines, lecture enrichment suggestions, lesson plans, a list of changes from the previous edition, and source materials.

Test Bank

A computerized test bank utilizing testing software to create customized exams is available with this text. The user-friendly software allows instructors to search for questions by topic or format, edit existing questions or add new ones, and scramble questions to create multiple versions of the same test. Word files of the test bank questions are provided for those instructors who prefer to work outside the test-generator software.

Course Management Systems

Online content is available for a variety of course management systems including BlackBoard and WebCT.

Laboratory Studies in Integrated Principles of Zoology by Cleveland Hickman, Jr., and Lee B. Kats

Now in its fourteenth edition, this lab manual was written to accompany *Integrated Principles of Zoology,* and can be easily adapted to fit a variety of course plans.

Biology Digitized Video Clips

McGraw-Hill is pleased to offer digitized biology video clips on DVD! Licensed from some of the highest-quality science video producers in the world, these brief segments range from about five seconds to just under three minutes in length and cover all areas of general biology from cells to ecosystems. Engaging and informative, McGraw-Hill's digitized biology videos will help capture students' interest while illustrating key biological concepts and processes. Includes video clips on mitosis, Darwin's finches, cichlid mouth brooding, sponge reproduction, and much more!

ACKNOWLEDGMENTS

The authors extend their sincere thanks to the faculty reviewers whose numerous suggestions for improvement were of the greatest value in the revision process. Their experience with students of varying backgrounds, and their interest in and knowledge of the subject, helped to shape the text into its final form.

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Jerry Cook, *Sam Houston State University*
Tamara Cook, *Sam Houston State University*
Michael Harvey, *Broward Community College*
Robert Hoyt, *Western Kentucky University*
Timothy Judd, *Southeast Missouri State University*
Robert L. Koenig, *Southwest Texas Jr. College*
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Sharyn Marks, *Humboldt State University*
R. Patrick Randolf, *University of California–Davis*
Anthony Stancampiano, *Oklahoma City Community College*
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Stim Wilcox, *Binghamton University*

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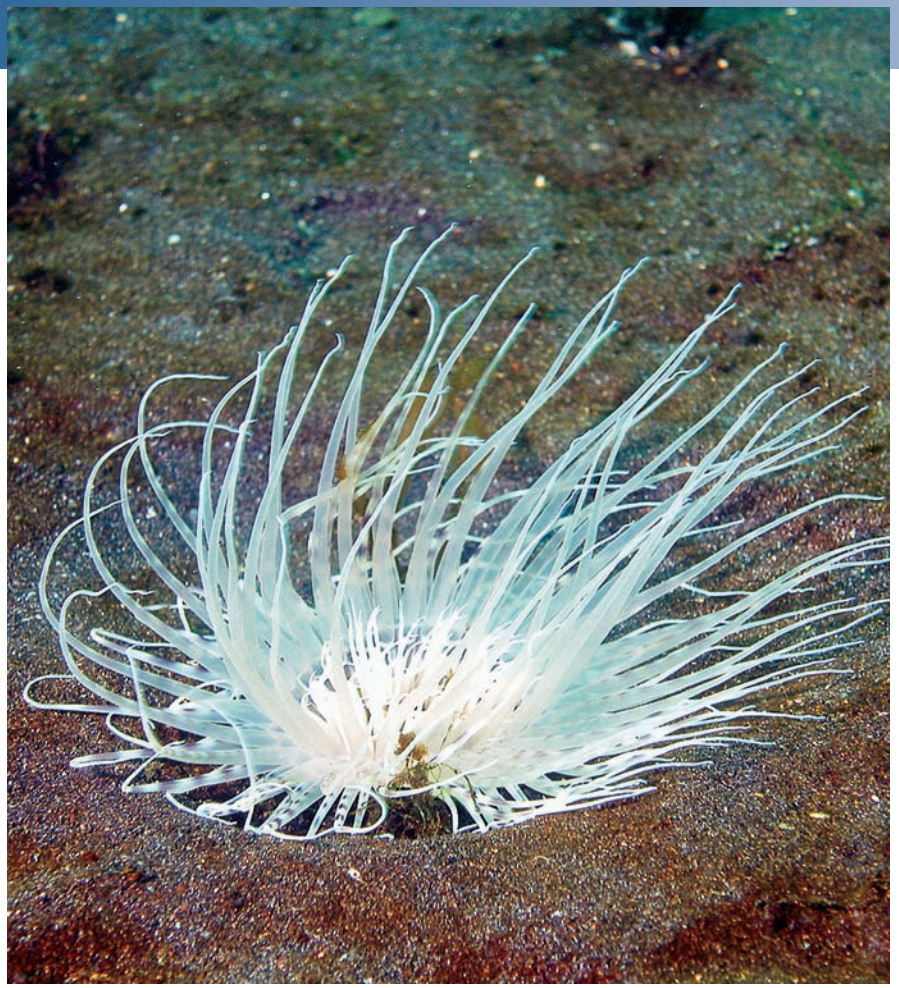
Although we make every effort to bring to you an error-free text, errors of many kinds inevitably find their way into a textbook of this scope and complexity. We will be grateful to readers who have comments or suggestions concerning content to send their remarks to Debra Henricks, Developmental Editor at debra_henricks@mcgraw-hill.com.

Cleveland P. Hickman, Jr.
Larry S. Roberts
Susan Keen
Allan Larson
Helen I'Anson
David J. Eisenhour

PART ONE

1 Introduction to Living Animals

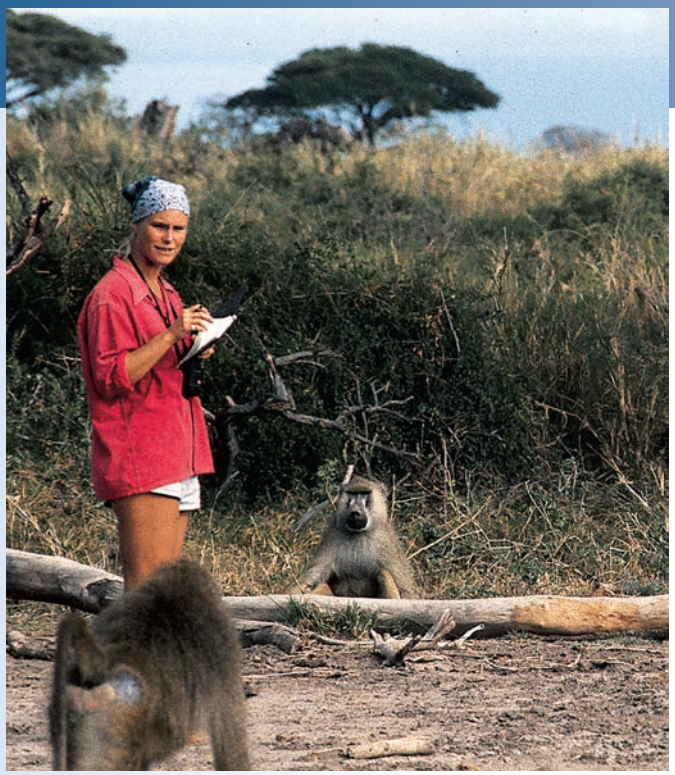


A tube anemone (cerianthid, Botruanthus benedini*) from the eastern Pacific.*

CHAPTER

1

Life: Biological Principles and the Science of Zoology



Zoologist studying the behavior of yellow baboons (Papio cynocephalus) *in the Amboseli Reserve, Kenya.*

The Uses of Principles

We gain knowledge of the animal world by actively applying important guiding principles to our investigations. Just as the exploration of outer space is both guided and limited by available technologies, exploration of the animal world depends critically on our questions, methods, and principles. The body of knowledge that we call zoology makes sense only when the principles that we use to construct it are clear.

The principles of modern zoology have a long history and many sources. Some principles derive from laws of physics and chemistry, which all living systems obey. Others derive from the scientific method, which tells us that our hypotheses regarding the animal world are useless unless they guide us to gather data that potentially can refute them. Many important principles derive from previous studies of the living world, of which animals are one part. Principles of heredity, variation, and organic evolution guide the study of life from the simplest unicellular forms to the most complex animals, fungi, and plants. Because life shares a common evolutionary origin, principles learned from the study of one group often pertain to other groups as well. By tracing the origins of our operating principles, we see that zoologists are not an island unto themselves but part of a larger scientific community.

We begin our study of zoology not by focusing narrowly within the animal world, but by searching broadly for our most basic principles and their diverse sources. These principles simultaneously guide our studies of animals and integrate those studies into the broader context of human knowledge.

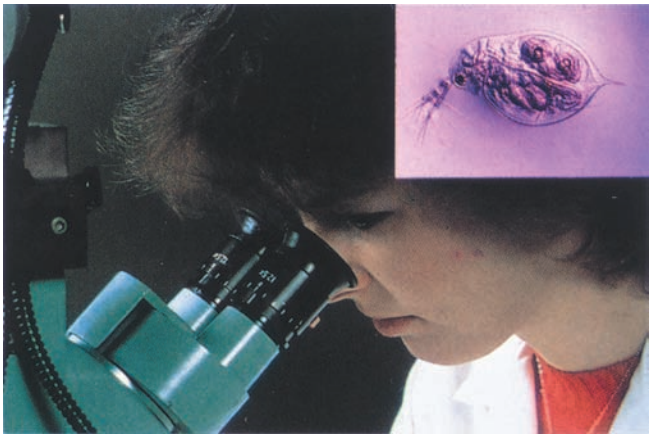
Zoology, the scientific study of animal life, builds on centuries of human inquiry into the animal world. Mythologies of nearly every human culture attempt to solve the mysteries of animal life and its origin. Zoologists now confront these same mysteries with the most advanced methods and technologies developed by all branches of science. We start by documenting the diversity of animal life and organizing it in a systematic way. This complex and exciting process builds on the contributions of thousands of zoologists working in all dimensions of the biosphere (Figure 1.1). We strive through this work to understand how animal diversity originated and how animals perform the basic processes of life that permit them to occupy diverse environments.

This chapter introduces the fundamental properties of animal life, the methodological principles on which their study is based, and two important theories that guide our research: (1) the theory of evolution, which is the central organizing

A

B

C



D

E

Figure 1.1

A few of the many dimensions of zoological research. **A,** Observing moray eels in Maui, Hawaii. **B,** Working with tranquilized polar bears. **C,** Banding mallard ducks. **D,** Observing *Daphnia pulex* (×150) microscopically. **E,** Separating growth stages of crab larvae at a marine laboratory.

principle of biology, and (2) the chromosomal theory of inheritance, which guides our study of heredity and variation in animals. These theories unify our knowledge of the animal world.

FUNDAMENTAL PROPERTIES OF LIFE

Does Life Have Defining Properties?

We begin with the difficult question, What is life? Although many attempts have been made to define life, simple definitions are doomed to failure. When we try to give life a simple definition, we look for fixed properties maintained throughout life's history. However, the properties that life exhibits today (pp. 4–9) are very different from those present at its origin. The history of life shows extensive and ongoing change, which we call *evolution*. As the genealogy of life progressed and branched from the earliest living form to the millions of species alive today, new properties evolved and passed from parents to their offspring. Through this process, living systems have generated many rare and spectacular features that have no counterparts in the nonliving world. Unexpected properties emerge on many different lineages in life's evolutionary history, producing the great organismal diversity observed today.

We might try to define life by universal properties evident at its origin. Replication of molecules, for example, can be traced to life's origin and represents one of life's universal properties. Defining life in this manner faces the major problem that these are the properties most likely to be shared by some nonliving forms. To study the origin of life, we must ask how organic molecules acquired the ability for precise replication. But where do we draw the line between those replicative processes that characterize life and those that are merely general chemical features of the matter from which life arose? Replication of complex crystalline structures in nonliving chemical assemblages might be confused, for example, with the replicative molecular properties associated with life. If we define life using only the most advanced characteristics of the highly evolved living systems observed today, the nonliving world would not intrude on our definition, but we would eliminate the early forms of life from which all others descended and which give life its historical unity.

Ultimately our definition of life must be based on the common history of life on earth. Life's history of descent with modification gives it an identity and continuity that separates it from the nonliving world. We can trace this common history backward through time from the diverse forms observed today and in the fossil record to their common ancestor that arose in the atmosphere of the primitive earth (see Chapter 2). All organisms forming part of this long history of hereditary descent from life's common ancestor are included in our concept of life.

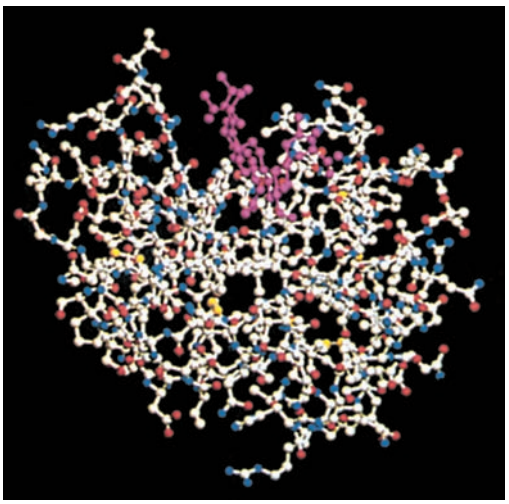
We do not force life into a simple definition, but we can readily identify the living world through its history of common evolutionary descent. Many remarkable properties have arisen during life's history and are observed in various combinations among living forms. These properties, discussed in the next section, clearly identify their possessors as part of the unified historical entity called life. All such features occur in the most highly evolved forms of life, such as those that compose the animal kingdom. Because they are so important for maintenance and functioning of living forms that possess them, these properties should persist through life's future evolutionary history.

General Properties of Living Systems

The most outstanding general features in life's history include chemical uniqueness; complexity and hierarchical organization; reproduction (heredity and variation); possession of a genetic program; metabolism; development; environmental interaction; and movement.

1. **Chemical uniqueness.** *Living systems demonstrate a unique and complex molecular organization.* Living systems assemble large molecules, known as macromolecules, that are far more complex than the small molecules of nonliving matter. These macromolecules are composed of the same kinds of atoms and chemical bonds that occur in nonliving matter and they obey all fundamental laws of chemistry; it is only the complex organizational structure of these macromolecules that makes them unique. We recognize four major categories of biological macromolecules: nucleic acids, proteins, carbohydrates, and lipids (see Chapter 2). These categories differ in the structures of their component parts, the kinds of chemical bonds that link their subunits together, and their functions in living systems.

   The general structures of these macromolecules evolved and stabilized early in the history of life. With some modifications, these same general structures are found in every form of life today. Proteins, for example, contain about 20 specific kinds of amino acid subunits linked together by peptide bonds in a linear sequence (Figure 1.2). Additional bonds occurring between amino acids that are not adjacent to each other in the protein chain give the protein a complex, three-dimensional structure (see Figures 1.2 and 2.15). A typical protein contains several hundred amino acid subunits. Despite the stability of this basic protein structure, the ordering of the different amino acids in the protein molecule is subject to enormous variation. This variation underlies much of the diversity that we observe among different kinds of living forms. The nucleic acids, carbohydrates, and lipids likewise contain characteristic bonds that link variable subunits (see Chapter 2). This organization gives living systems both a biochemical unity and great potential diversity.
2. **Complexity and hierarchical organization.** *Living systems demonstrate a unique and complex hierarchical organization.* Nonliving matter is organized at least into atoms and molecules and often has a higher degree of organization as well. However, atoms and molecules are combined into patterns in the living world that do not exist in the nonliving world. In living systems, we find a hierarchy of levels that includes, in ascending order of complexity, macromolecules, cells, organisms, populations, and species (Figure 1.3). Each level builds on the level below it



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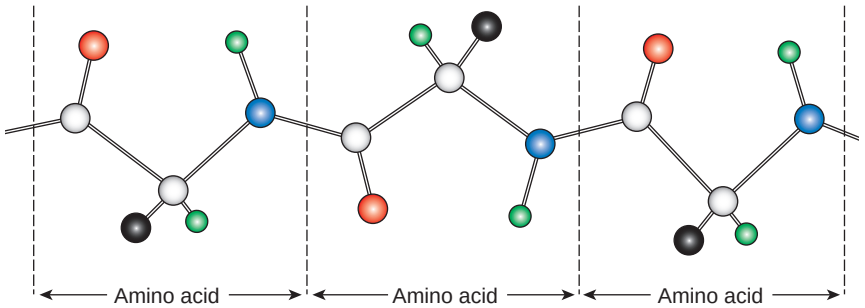


B

Figure 1.2

A computer simulation of the three-dimensional structure of the lysozyme protein **(A),** which is used by animals to destroy bacteria. The protein is a linear string of molecular subunits called amino acids, connected as shown in **B,** which fold in a three-dimensional pattern to form the active protein. The white balls correspond to carbon atoms, the red balls to oxygen, the blue balls to nitrogen, the yellow balls to sulfur, the green balls to hydrogen, and the black balls **(B)** to molecular groups formed by various combinations of carbon, oxygen, nitrogen, hydrogen, and sulfur atoms that differ among amino acids. Hydrogen atoms are not shown in **A.** The purple molecule in **A** is a structure from the bacterial cell wall that is broken by lysozyme.

and has its own internal structure, which is also often hierarchical. Within the cell, for example, macromolecules are compounded into structures such as ribosomes, chromosomes, and membranes, and these are likewise combined in various ways to form even more complex subcellular structures called organelles, such as mitochondria (see Chapters 3 and 4). The organismal level also has a hierarchical substructure; cells combine to form tissues, which combine to form organs, which likewise combine to form organ systems (see Chapter 9).

Cells (Figure 1.4) are the smallest units of the biological hierarchy that are semiautonomous in their ability to conduct basic functions, including reproduction. Replication of molecules and subcellular components occurs only within a cellular context, not independently. Cells

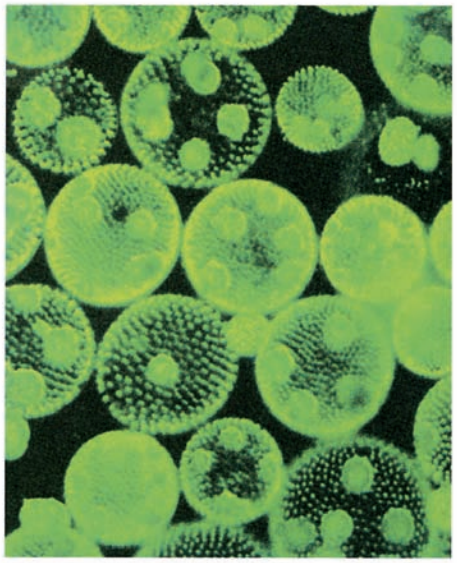


Figure 1.3

Volvox globator (see pp. 229–231) is a multicellular chlorophytan that illustrates three different levels of the biological hierarchy: cellular, organismal, and populational. Each individual spheroid (organism) contains cells embedded in a gelatinous matrix. The larger cells function in reproduction, and the smaller ones perform the general metabolic functions of the organism. The individual spheroids together form a population.

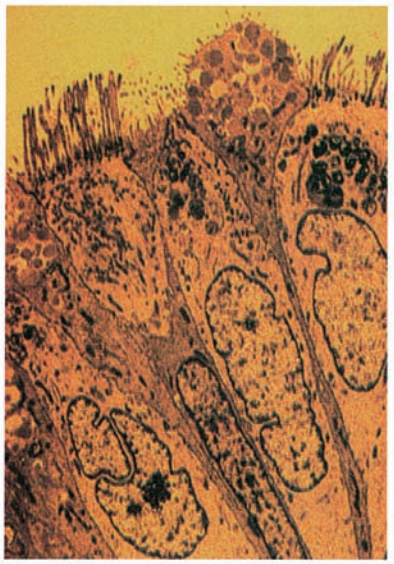


Figure 1.4

Electron micrograph of ciliated epithelial cells and mucus-secreting cells (see pp. 192–195). Cells are the basic building blocks of living organisms.

are therefore considered the basic units of living systems (see Chapter 3). We can isolate cells from an organism and cause them to grow and to multiply under laboratory conditions in the presence of nutrients alone. This semi-autonomous replication is not possible for any individual molecules or subcellular components, which require additional cellular constituents for their reproduction.

Each successively higher level of the biological hierarchy is composed of units of the preceding lower level in the hierarchy. An important characteristic of this hierarchy is that the properties of any given level cannot be inferred even from the most complete knowledge of the properties of its component parts. A physiological feature, such as blood pressure, is a property of the organismal level; it is impossible to predict someone's blood pressure simply by knowing the physical characteristics of individual cells of the body. Likewise, systems of social interaction, as observed in bees, occur at the populational level; it is not possible to infer properties of this social system by studying individual bees in isolation.

The appearance of new characteristics at a given level of organization is called **emergence,** and these characteristics are called **emergent properties.** These properties arise from interactions among the component parts of a system. For this reason, we must study all levels directly, each one being the focus of a different subfield of biology (molecular biology; cell biology; organismal anatomy, physiology and genetics; population biology; Table 1.1). Emergent properties expressed at a particular level of the biological hierarchy are certainly influenced and restricted by properties of the lower-level components. For example, it would be impossible for a population of organisms that lack hearing to develop a spoken language. Nonetheless, properties of parts of a living system do not rigidly determine properties of the whole. Many different spoken languages have emerged in human culture from the same basic anatomical structures that permit hearing and speech. The freedom of the parts to interact in different ways makes possible a great diversity of potential emergent properties at each level of the biological hierarchy.

Different levels of the biological hierarchy and their particular emergent properties are built by evolution. Before multicellular organisms evolved, there was no distinction between the organismal and cellular levels, and this distinction is still absent from single-celled organisms (see Chapter 11). The diversity of emergent properties that we see at all levels of the biological hierarchy contributes to the difficulty of giving life a simple definition or description.

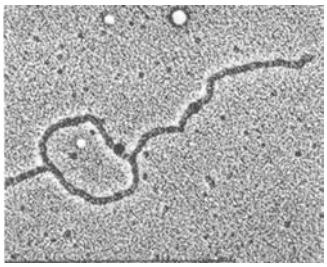
3. **Reproduction.** *Living systems can reproduce themselves.* Life does not arise spontaneously but comes only from prior life, through reproduction. Although life certainly originated from nonliving matter at least once (see Chapter 2), this origin featured enormously long periods of time and conditions very different from the current biosphere. At each level of the biological hierarchy, living forms reproduce to generate others like themselves (Figure 1.5). Genes are replicated to produce new genes. Cells divide to produce new cells. Organisms reproduce, sexually or asexually, to produce new organisms (see Chapter 7). Populations may become fragmented to produce new populations, and species may split to produce new species through a process called speciation. Reproduction at any hierarchical level usually features an increase in numbers. Individual genes, cells, organisms, populations, or species may fail to reproduce themselves, but reproduction is nonetheless an expected property of these individuals.

Reproduction at each of these levels shows the complementary, and yet apparently contradictory, phenomena

TABLE 1.1

Different Hierarchical Levels of Biological Complexity That Display Reproduction, Variation, and Heredity

Level	Timescale of Reproduction	Fields of Study	Methods of Study	Some Emergent Properties
Cell	Hours (mammalian cell = ~16 hours)	Cell biology	Microscopy (light, electron), biochemistry	Chromosomal replication (meiosis, mitosis), synthesis of macromolecules (DNA, RNA, proteins, lipids, polysaccharides)
Organism	Hours to days (unicellular); days to years (multicellular)	Organismal anatomy, physiology, genetics	Dissection, genetic crosses, clinical studies, physiological experimentation	Structure, functions and coordination of tissues, organs and organ systems (blood pressure, body temperature, sensory perception, feeding)
Population	Up to thousands of years	Population biology, population genetics, ecology	Statistical analysis of variation, abundance, geographical distribution	Social structures, systems of mating, age distribution of organisms, levels of variation, action of natural selection
Species	Thousands to millions of years	Systematics and evolutionary biology, community ecology	Study of reproductive barriers, phylogeny, paleontology, ecological interactions	Method of reproduction, reproductive barriers



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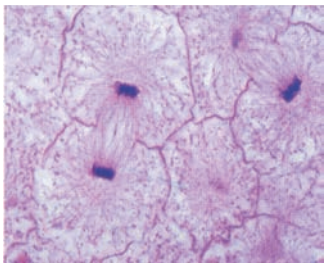
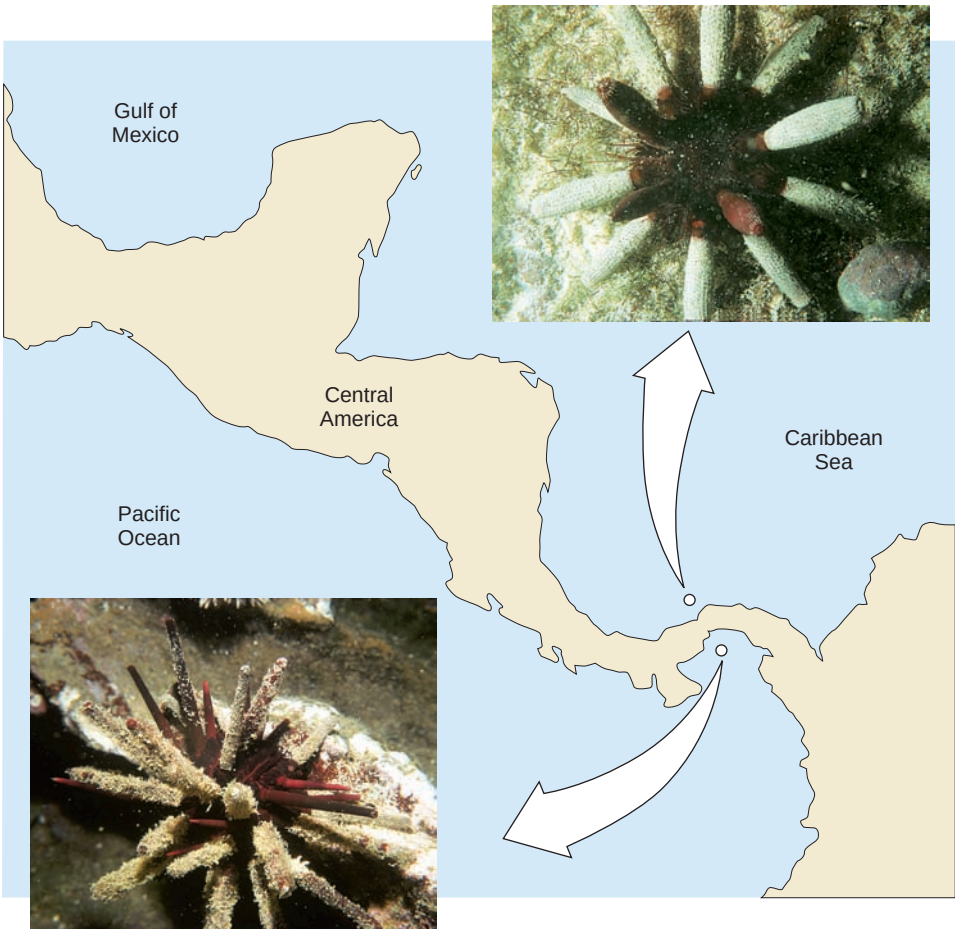


B

C



D

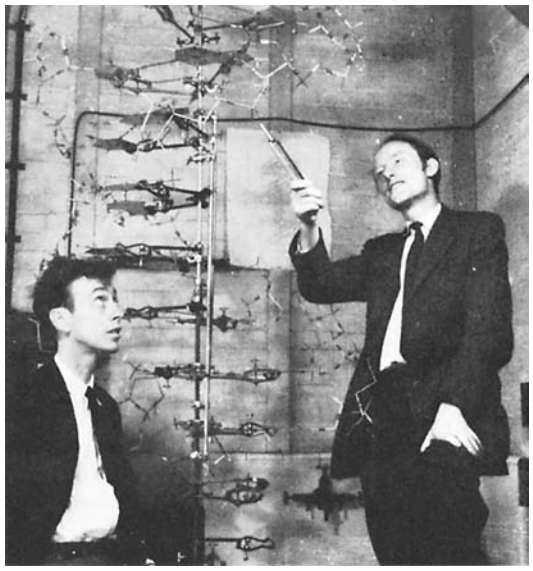
Figure 1.5

Reproductive processes observed at four different levels of biological complexity. **A,** Molecular level—electron micrograph of a replicating DNA molecule. **B,** Cellular level—micrograph of cell division at mitotic telophase. **C,** Organismal level—a king snake hatching. **D,** Species level—formation of new species in the sea urchin (*Eucidaris*) after geographic separation of Caribbean (*E. tribuloides*) and Pacific (*E. thouarsi*) populations by the formation of a land bridge.

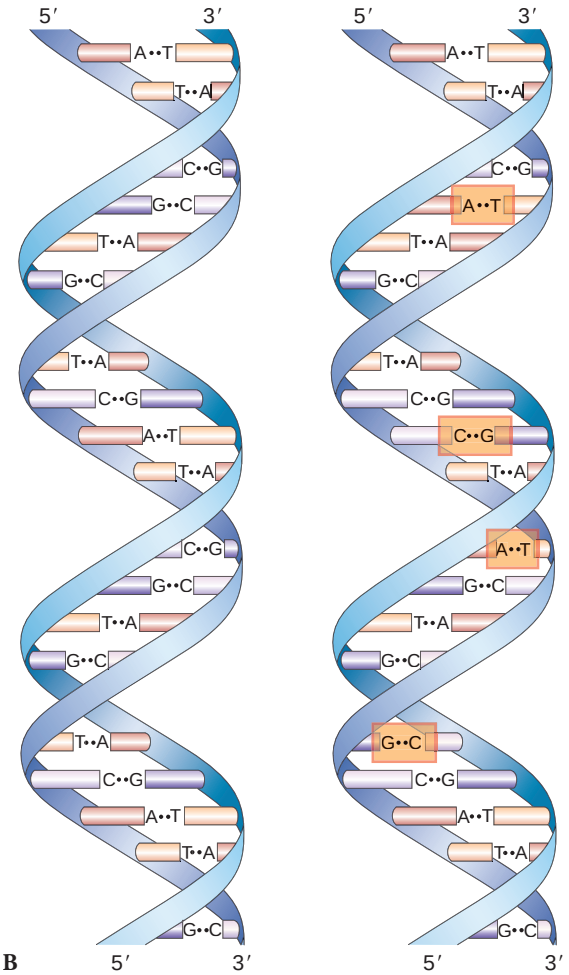
of **heredity** and **variation.** Heredity is the faithful transmission of traits from parents to offspring, usually (but not necessarily) observed at the organismal level. Variation is the production of *differences* among the traits of different individuals. In a reproductive process, properties of descendants resemble those of their parents to varying degrees but usually are not identical to them. Replication of deoxyribonucleic acid (DNA) occurs with high fidelity, but errors occur at repeatable rates. Cell division is exceptionally precise, especially with regard to the nuclear material, but chromosomal changes occur nonetheless at measurable rates. Organismal reproduction likewise demonstrates both heredity and variation, the latter most obvious in sexually reproducing forms. Production of new populations and species also demonstrates conservation of some properties and changes of others. Two closely related frog species may have similar mating calls but differ in the rhythm of repeated sounds.

Interaction of heredity and variation in the reproductive process is the basis for organic evolution (see Chapter 6). If heredity were perfect, living systems would never change; if variation were uncontrolled by heredity, biological systems would lack the stability that allows them to persist through time.

4. **Possession of a genetic program.** *A genetic program provides fidelity of inheritance* (Figure 1.6). Structures of the protein molecules needed for organismal development and functioning are encoded in **nucleic acids** (see Chapter 5). For animals and most other organisms, genetic information is contained in **DNA.** DNA is a very long, linear chain of subunits called nucleotides, each of which contains a sugar phosphate (deoxyribose phosphate) and one of four nitrogenous bases (adenine, cytosine, guanine, or thymine, abbreviated A, C, G, and T, respectively). The sequence of nucleotide bases contains a code for the order of amino acids in the protein specified by the DNA molecule. The correspondence between the sequence of bases in DNA and the sequence of amino acids in a protein is called the **genetic code.**



A



B

Figure 1.6

James Watson and Francis Crick with a model of the DNA double helix **(A).** Genetic information is coded in the nucleotide base sequence inside the DNA molecule. Genetic variation is shown **(B)** in DNA molecules that are similar in base sequence but differ from each other at four positions. Such differences can encode alternative traits, such as different eye colors.

The genetic code arose early in the evolutionary history of life, and the same code occurs in bacteria and in the nuclear genomes of almost all animals and plants. The near constancy of this code among living forms provides strong evidence for a single origin of life. The genetic code has undergone very little evolutionary change since its origin because an alteration would disrupt the structure of nearly every protein, which would in turn severely disrupt cellular functions that require very specific protein structures. Only in the rare instance that the altered protein structures maintain their cellular functions would such a change be able to survive and be reproduced. Evolutionary change in the genetic code has occurred in the DNA contained in animal mitochondria, the organelles that regulate cellular energy. The genetic code in animal mitochondrial DNA therefore is slightly different from the standard code of nuclear and bacterial DNA. Because mitochondrial DNA specifies far fewer proteins than nuclear DNA, the likelihood of getting a change in the code that maintains cellular functions is greater there than in the nucleus.

5. **Metabolism.** *Living organisms maintain themselves by acquiring nutrients from their environments* (Figure 1.7). The nutrients are used to obtain chemical energy and molecular components for building and maintaining the living system (see Chapter 4). We call these essential chemical processes **metabolism.** They include digestion, acquisition of energy (respiration), and synthesis of molecules and structures. Metabolism is often viewed as an interaction of destructive (catabolic) and constructive (anabolic) reactions. The most fundamental anabolic and catabolic chemical processes used by living systems arose early in the evolutionary history of life, and all living forms share them. These reactions include synthesis of carbohydrates, lipids, nucleic acids, and proteins and their constituent parts and cleavage of chemical bonds to recover energy stored in them. In animals, many fundamental metabolic reactions occur at the cellular level, often in specific organelles found throughout the animal kingdom. Cellular respiration occurs, for example, in mitochondria. Cellular and nuclear membranes regulate metabolism by controlling the movement of molecules across the cellular and nuclear boundaries, respectively. The study of complex metabolic functions is called **physiology.** We devote a large portion of this book to describing and comparing the diverse tissues, organs, and organ systems that different groups of animals have evolved to perform the basic physiological functions of life (see Chapters 11 through 36).
6. **Development.** *All organisms pass through a characteristic life cycle.* Development describes the characteristic changes that an organism undergoes from its origin (usually the fertilization of an egg by sperm) to its final adult form (see Chapter 8). Development usually features changes in size and shape, and differentiation of structures within an organism. Even the simplest one-celled organisms grow in size and replicate their component parts until they divide into two or more cells. Multicellular organisms undergo more dramatic changes during their lives. Different developmental

A

B

Figure 1.7

Feeding processes illustrated by **(A)** an ameba surrounding food and **(B)** a chameleon capturing insect prey with its projectile tongue.

A

B

Figure 1.8

A, Adult monarch butterfly emerging from its pupal case. **B,** Fully formed adult monarch butterfly.

stages of some multicellular forms are so dissimilar that they are hardly recognizable as belonging to the same species. Embryos are distinctly different from juvenile and adult forms into which they develop. Even postembryonic development of some organisms includes stages dramatically different from each other. The transformation that occurs from one stage to another is called **metamorphosis.** There is little resemblance, for example, among the egg, larval, pupal, and adult stages of metamorphic insects (Figure 1.8). Among animals, early stages of development are often more similar among organisms of related species than are later developmental stages. In our survey of animal diversity, we describe all stages of observed life histories but concentrate on adult stages in which diversity tends to be greatest.

7. **Environmental interaction.** *All animals interact with their environments.* The study of organismal interaction with an environment is called **ecology.** Of special interest are the factors that influence geographic distribution and abundance of animals (see Chapters 37 and 38). The science of ecology reveals how an organism perceives environmental stimuli and responds in appropriate ways by adjusting its metabolism and physiology (Figure 1.9). All organisms respond to environmental stimuli, a property called **irritability.** The stimulus and response may be simple, such as a unicellular organism moving from or toward a light source or away from a noxious substance, or it may be quite complex, such as a bird responding to a complicated series of signals in a mating ritual (see Chapter 36). Life and environment are inseparable. We cannot isolate the evolutionary history of a lineage of organisms from the environments in which it occurred.
8. **Movement.** *Living systems and their parts show precise and controlled movements arising from within the system.* The energy that living systems extract from their environments permits them to initiate controlled movements. Such movements at the cellular level are essential for reproduction, growth, and many responses to stimuli in all living forms and for development in multicellular ones. Autonomous movement reaches great diversity in animals, and much of this book comprises descriptions of animal movement and the many adaptations that animals have evolved for locomotion. On a larger scale, entire populations or species may disperse from one geographic location to another one over time through their powers of movement. Movement characteristic of nonliving matter, such as that of particles in solution, radioactive decay of nuclei, and eruption of volcanoes is not precisely controlled by the moving objects themselves and often involves forces entirely external to them. The adaptive and often purposeful movements initiated by living systems are absent from the nonliving world.

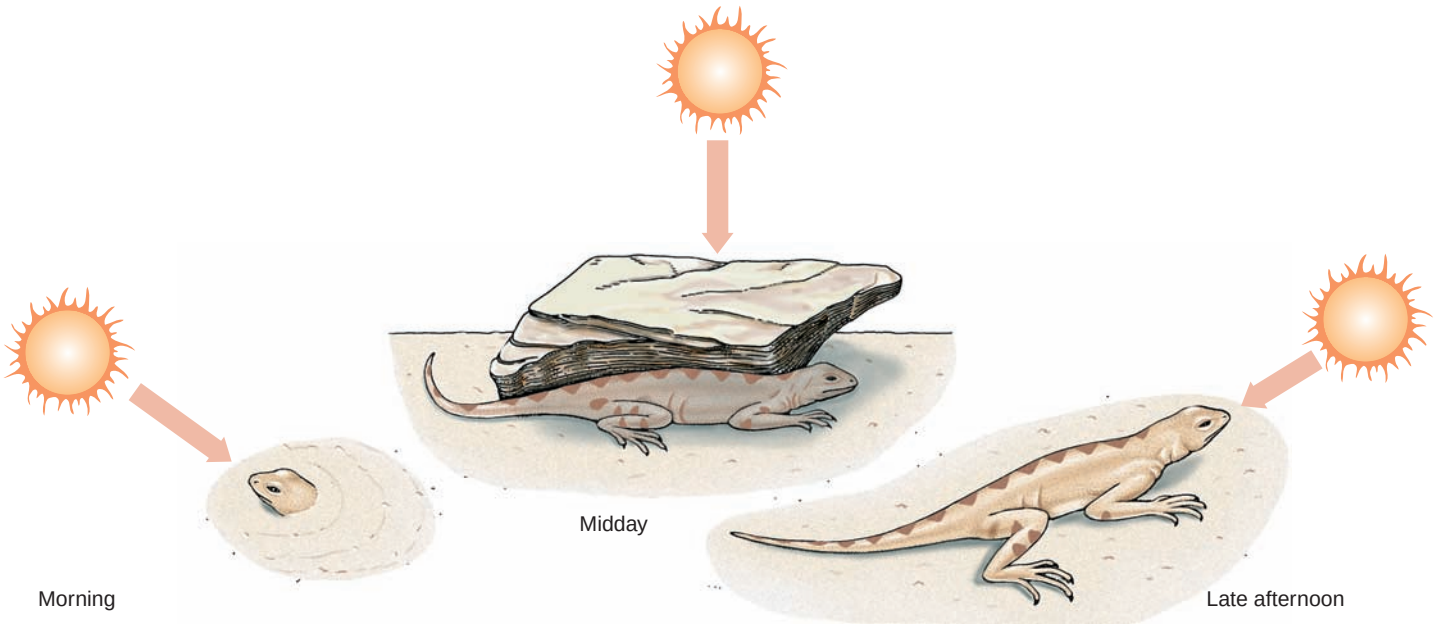


Figure 1.9
A lizard regulates its body temperature by choosing different locations (microhabitats) at different times of day.

Life Obeys Physical Laws

To untrained observers, these eight properties of life may appear to violate basic laws of physics. Vitalism, the idea that life is endowed with a mystical vital force that violates physical and chemical laws, was once widely advocated. Biological research has consistently rejected vitalism, showing instead that all living systems obey basic laws of physics and chemistry. Laws governing energy and its transformations (thermodynamics) are particularly important for understanding life (see Chapter 4). The **first law of thermodynamics** is the law of conservation of energy. Energy is neither created nor destroyed but can be transformed from one form to another. All aspects of life require energy and its transformation. The energy to support life on earth flows from the fusion reactions in our sun and reaches the earth as light and heat. Sunlight captured by green plants and cyanobacteria is transformed by photosynthesis into chemical bonds. Energy in chemical bonds is a form of potential energy released when the bond is broken; the energy is used to perform numerous cellular tasks. Energy transformed and stored in plants is then used by animals that eat the plants, and these animals may in turn provide energy for other animals that eat them.

The **second law of thermodynamics** states that physical systems tend to proceed toward a state of greater disorder, or **entropy.** Energy obtained and stored by plants is subsequently released by various mechanisms and finally dissipated as heat. The complex molecular organization in living cells is attained and maintained only as long as energy fuels the organization. The ultimate fate of materials in the cells is degradation and dissipation of their chemical bond energy as heat. The process of evolution whereby organismal complexity can increase over time may appear at first to violate the second law of thermodynamics, but it does not. Organismal complexity is achieved and maintained only by the constant use and dissipation of energy flowing into the biosphere from the sun. Survival, growth, and reproduction of animals require energy that comes from breaking complex food molecules into simple organic waste. The processes by which animals acquire energy through nutrition and respiration reveal themselves to us through the many physiological sciences.

ZOOLOGY AS A PART OF BIOLOGY

Animals form a distinct branch on the evolutionary tree of life. It is a large and old branch that originated in the Precambrian seas over 600 million years ago. Animals form part of an even larger limb known as **eukaryotes,** organisms whose cells contain membrane-enclosed nuclei. This larger limb includes plants, fungi and numerous unicellular forms. Perhaps the most distinctive characteristic of the animals as a group is their means of nutrition, which consists of eating other organisms. Evolution has elaborated this basic way of life through diverse systems for capturing and processing a wide array of food items and for locomotion.

Animals are distinguished also by the absence of characteristics that have evolved in other eukaryotes. Plants, for example, use light energy to produce organic compounds (photosynthesis), and they have evolved rigid cell walls that surround their cell membranes; photosynthesis and cell walls are absent from animals. Fungi acquire nutrition by absorption of small organic molecules from their environments, and their body plan contains tubular filaments called *hyphae;* these structures are absent from the animal kingdom.

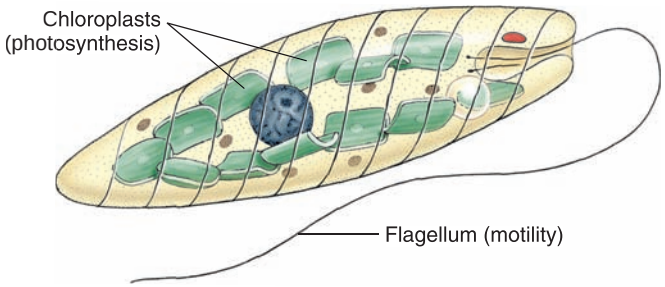


Figure 1.10

Some organisms, such as the single-celled *Euglena* (shown here) and *Volvox* (see Figure 1.3), combine properties that distinguish animals (locomotion) and plants (photosynthetic ability).

Some organisms combine properties of animals and plants. For example, *Euglena* (Figure 1.10) is a motile, single-celled organism that resembles plants in being photosynthetic, but it resembles animals in its ability to eat food particles. *Euglena* is part of a separate eukaryotic lineage that diverged from those of plants and animals early in the evolutionary history of eukaryotes. *Euglena* and other unicellular eukaryotes are sometimes grouped as the kingdom Protista, although this kingdom is an arbitrary grouping of unrelated lineages that violates taxonomic principles (see Chapter 10).

The fundamental structural and developmental features evolved by the animal kingdom are presented in Chapters 8 and 9.

PRINCIPLES OF SCIENCE

Nature of Science

We stated in the first sentence of this chapter that zoology is the scientific study of animals. A basic understanding of zoology therefore requires an understanding of what science is, what it is not, and how knowledge is gained using the scientific method.

Science is a way of asking questions about the natural world and sometimes obtaining precise answers to them. Although science, in the modern sense, has arisen recently in human history (within the last 200 years or so), the tradition of asking questions about the natural world is an ancient one. In this section, we examine the methodology that zoology shares with science as a whole. These features distinguish sciences from activities that we exclude from the realm of science, such as art and religion.

Despite an enormous impact of science on our lives, many people have only a minimal understanding of the nature of science. For example, on March 19, 1981, the governor of Arkansas signed into law the Balanced Treatment for Creation-Science and Evolution-Science Act (Act 590 of 1981). This act falsely presented "creation-science" as a valid scientific endeavor. "Creation-science" is actually a religious position advocated by a minority of the American religious community, and it does not qualify as science. The enactment of this law led to a historic lawsuit tried in December 1981 in the court of Judge William R. Overton, U.S. District Court, Eastern District of Arkansas. The suit was brought by the American Civil Liberties Union on behalf of 23 plaintiffs, including religious leaders and groups representing several denominations, individual parents, and educational associations. The plaintiffs contended that the law was a violation of the First Amendment to the U.S. Constitution, which prohibits "establishment of religion" by government. This prohibition includes passing a law that would aid one religion or prefer one religion over another. On January 5, 1982, Judge Overton permanently enjoined the State of Arkansas from enforcing Act 590.

Considerable testimony during the trial dealt with the nature of science. Some witnesses defined science simply, if not very informatively, as "what is accepted by the scientific community" and "what scientists do." However, on the basis of other testimony by scientists, Judge Overton was able to state explicitly these essential characteristics of science:

1. It is guided by natural law.
2. It has to be explanatory by reference to natural law.
3. It is testable against the observable world.
4. Its conclusions are tentative and therefore not necessarily the final word.
5. It is falsifiable.

Pursuit of scientific knowledge must be guided by the physical and chemical laws that govern the state of existence. Scientific knowledge must explain what is observed by reference to natural law without requiring intervention of a supernatural being or force. We must be able to observe events in the real world, directly or indirectly, to test hypotheses about nature. If we draw a conclusion relative to some event, we must be ready always to discard or to modify our conclusion if further observations contradict it. As Judge Overton stated, "While anybody is free to approach a scientific inquiry in any fashion they choose, they cannot properly describe the methodology used as scientific if they start with a conclusion and refuse to change it regardless of the evidence developed during the course of the investigation." Science is separate from religion, and the results of science do not favor one religious position over another.

Unfortunately, the religious position formerly called "creation-science" has reappeared in American politics with the name "intelligent-design theory." We are forced once again to defend the teaching of science against this scientifically meaningless dogma.

Scientific Method

These essential criteria of science form the **hypothetico-deductive method.** The first step of this method is the generation of hypotheses or potential answers to the question being asked. These hypotheses are usually based on prior observations of nature or derived from theories based on such observations. Scientific hypotheses often constitute general statements about nature that may explain a large number of diverse observations. Darwin's hypothesis of natural selection, for example, explains the observations that many different species have properties that adapt them to their environments.

On the basis of the hypothesis, a scientist must make a prediction about future observations. The scientist must say, "If my hypothesis is a valid explanation of past observations, then future observations ought to have certain characteristics." The best hypotheses are those that make many predictions which, if found erroneous, will lead to rejection, or falsification, of the hypothesis.

The scientific method may be summarized as a series of steps:

1. Observation
2. Question
3. Hypothesis
4. Empirical test
5. Conclusions
6. Publication

Observations illustrated in Figure 1.1A-E form a critical first step in evaluating the life histories of natural populations. For example, observations of crab larvae shown in Figure 1.1E might cause the observer to question whether rate of larval growth is higher in undisturbed populations than in ones exposed to a chemical pollutant. A null hypothesis is then generated to permit an empirical test. A null hypothesis is one worded in a way that would permit data to reject it if it is false. In this case, the null hypothesis is that larval growth rates for crabs in undisturbed habitats are the same as those in polluted habitats. The investigator then performs an empirical test by gathering data on larval growth rates in a set of undisturbed crab populations and a set of populations subjected to the chemical pollutant. Ideally, the undisturbed populations and the chemically treated populations are equivalent for all conditions except presence of the chemical in question. If measurements show consistent differences in growth rate between the two sets of populations, the null hypothesis is rejected. One then concludes that the chemical pollutant does alter larval growth rates. A statistical test is usually needed to ensure that the differences between the two groups are greater than would be expected from chance fluctuations alone. If the null hypothesis cannot be rejected, one concludes that the data do not show any effect of the chemical treatment. The results of the study are then published to communicate findings to other researchers, who may repeat the results, perhaps using additional populations of the same or a different species. Conclusions of the initial study then serve as the observations for further questions and hypotheses to reiterate the scientific process.

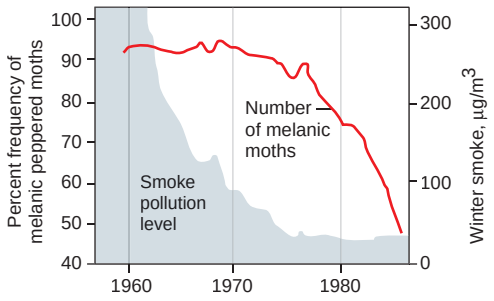
Note that a null hypothesis cannot be proved correct using the scientific method. If the available data are compatible with it, the hypothesis serves as a guide for collecting additional data that potentially might reject it. Our most successful hypotheses are the ones that make specific predictions confirmed by large numbers of empirical tests.

The hypothesis of natural selection was invoked to explain variation observed in British moth populations (Figure 1.11). In industrial areas of England having heavy air pollution, many populations of moths contain primarily darkly pigmented (melanic) individuals, whereas moth populations inhabiting clean forests show a much higher frequency of lightly pigmented individuals. The hypothesis suggests that moths can survive most effectively by matching their surroundings, thereby remaining invisible to birds that seek to eat them. Experimental studies have shown that, consistent with this hypothesis, birds are able to locate and then to eat moths that do not match their surroundings. Birds in the same area frequently fail to find moths that match their surroundings, leaving them to reproduce and to increase their numbers relative to conspicuous moths. Another testable prediction of the hypothesis of natural selection is that when polluted areas are cleaned, the moth populations should demonstrate an increase in frequency of lightly pigmented individuals. Observations of such populations confirmed the result predicted by natural selection.

If a hypothesis is very powerful in explaining a wide variety of related phenomena, it attains the status of a **theory.** Natural selection is a good example. Our example of the use of natural selection to explain observed pigmentation patterns in moth populations is only one of many phenomena to which natural selection applies. Natural selection provides a potential explanation for the occurrence of many different traits distributed among virtually all animal species. Each of these instances constitutes a

A

B



C

Figure 1.11

Light and melanic forms of the peppered moth, *Biston betularia* on **A,** a lichen-covered tree in unpolluted countryside and **B,** a soot-covered tree near industrial Birmingham, England. These color variants have a simple genetic basis. **C,** Recent decline in the frequency of the melanic form of the peppered moth with falling air pollution in industrial areas of England. The frequency of the melanic form still exceeded 90% in 1960, when smoke and sulfur dioxide emissions were still high. Later, as emissions fell and light-colored lichens began to grow again on the tree trunks, the melanic form became more conspicuous to predators. By 1986, only 50% of the moths were still of the melanic form, the rest having been replaced by the light form.

Ethics in Animal Research

The use of animals to serve human needs raises challenging ethical questions. Most controversial is the issue of animal use in biomedical and behavioral research and in testing commercial products.

Congress has passed a series of amendments to the Federal Animal Welfare Act, a body of laws covering care of vertebrate animals in laboratories and other facilities. These amendments are known as the three R's: *Reduction* in the number of animals needed for research; *Refinement* of techniques that might cause stress or suffering; *Replacement* of live animals with simulations or cell cultures whenever possible. As a result, the total number of animals used each year in research and in testing of commercial products has declined. Developments in cellular and molecular biology also have contributed to a decreased use of animals for research and testing. An animal rights movement has created an awareness of the needs of animals used in research and has stimulated researchers to discover more humane alternatives.

Computers and culturing of cells can substitute for experiments on animals only when the basic principles involved are well known. When the principles themselves are being scrutinized and tested, computer modeling is not sufficient. The National Research Council concedes that although the search for alternatives to animals in research and testing will continue, "the chance that alternatives will completely replace animals in the foreseeable future is nil." Realistic immediate goals, however, are reduction in number of animals used, replacement of mammals with other vertebrates, and refinement of experimental procedures to reduce discomfort of the animals being tested.

Medical and veterinary progress depends on research using animals. Every drug and vaccine developed to improve the human condition has been tested first on animals. Research using animals has enabled medical science to eliminate smallpox and polio from at least some parts of the world, and to immunize against diseases previously common and often deadly, including diphtheria, mumps, and rubella. It also has helped to create treatments for cancer, diabetes, heart disease, and depression, and to develop surgical procedures including heart surgery, blood transfusions, and cataract removal. AIDS research is wholly dependent on studies using animals. The similarity of simian AIDS, identified in rhesus monkeys, to human AIDS has permitted the disease in monkeys to serve as a model for the human disease. Recent work indicates that cats, too, may be useful models for development of an AIDS vaccine. Skin grafting experiments, first done with cattle and later with other animals, opened a new era in immunological research with vast contributions to treatment of disease in humans and other animals.

Research using animals also has benefited *other animals* through the development of veterinary cures. The vaccines for feline leukemia and canine parvovirus were first introduced to other cats and dogs. Many other vaccinations for serious diseases of animals were developed through research on animals—for example, rabies, distemper, anthrax, hepatitis, and tetanus. No endangered species is used in general research (except to protect that species from total extinction). Thus, research using animals has provided enormous benefits to humans and other animals. Still, much remains to be learned about treatment of diseases such as cancer, AIDS, diabetes, and heart disease, and research with animals will be required for this purpose.

Despite the remarkable benefits produced by research on animals, advocates of animal rights consider the harm done to animals in some research unethical. The most extreme animal-rights activists advocate total abolition of all forms of research using animals. The scientific community is deeply concerned about the impact of such attacks on the ability of scientists to conduct important experiments that will benefit people and animals. If we are justified to use animals for food and fiber and as pets, are we not justified in experimentation to benefit human welfare when these studies are conducted humanely and ethically?

The Association for Assessment and Accreditation of Laboratory Animal Care International supports the use of animals to advance medicine and science when nonanimal alternatives are not available and when animals are treated in an ethical and humane way. Accreditation by this organization allows research institutions to demonstrate excellence in their standards of animal care. Nearly all major institutions receiving funding from the National Institutes of Health have sought and received this accreditation. See the website at *www.aaalac.org* for more information on accreditation of laboratory animal care.

References on Animal-Research Ethics

Commission on Life Sciences, National Research Council. 1988. Use of laboratory animals in biomedical and behavioral research. Washington, D.C., National Academy Press. *Statement of national policy on guidelines for use of animals in biomedical research. Includes a chapter on benefits derived from use of animals.*

Groves, J. M. 1997. Hearts and minds: the controversy over laboratory animals. Philadelphia, Pennsylvania, Temple University Press. *Thoughtful review of the controversy by an activist who conducted extensive interviews with activists and animal-research supporters.*

Paul, E. F., and J. Paul, eds. 2001. Why animal experimentation matters: the use of animals in medical research. New Brunswick, New Jersey, Social Philosophy and Policy Foundation, and Transaction Publishers. *Essays by scientists, historians, and philosophers that express a defense of animal experimentation, demonstrating its moral acceptability and historical importance.*

specific hypothesis generated from the theory of natural selection. Note, however, that falsification of a specific hypothesis does not necessarily lead to rejection of the theory as a whole. Natural selection may fail to explain origins of human behavior, for example, but it provides an excellent explanation for many structural modifications of the pentadactyl (five-fingered) vertebrate limb for diverse functions. Scientists test many subsidiary hypotheses of their major theories to ask whether their theories are generally applicable. Most useful are theories that explain the largest array of different natural phenomena.

We emphasize that the meaning of the word "theory," when used by scientists, is not "speculation" as it is in ordinary English usage. Failure to make this distinction has been prominent in creationist challenges to evolution. The creationists have spoken

of evolution as "only a theory," as if it were little better than a guess. In fact, the theory of evolution is supported by such massive evidence that biologists view repudiation of evolution as tantamount to repudiation of reason. Nonetheless, evolution, along with all other theories in science, is not proven in a mathematical sense, but it is testable, tentative, and falsifiable. Powerful theories that guide extensive research are called **paradigms.** The history of science shows that even major paradigms are subject to refutation and replacement when they fail to account for our observations of the natural world. They are then replaced by new paradigms in a process called a **scientific revolution.** For example, prior to the 1800s, animal species were studied as if they were specially created entities whose essential properties remained unchanged through time. Darwin's theories led to a scientific revolution that replaced these views with the evolutionary paradigm. The evolutionary paradigm has guided biological research for more than 140 years, and to date there is no scientific evidence that falsifies it; it has strong explanatory power and continues to guide active inquiry into the natural world. Evolutionary theory is generally accepted as the cornerstone of biology.

Chemists and physicists often use the term "law" to denote highly corroborated theories that appear to apply without exception to the physical world. Such laws are considered uniform throughout time and space. Because the biological world is temporally and spatially bounded, and because evolutionary change has produced an enormous diversity of forms with different emergent properties at multiple levels (Table 1.1), biologists now avoid using the term law for their theories. Nearly all of the biological laws proposed in the past have been found to apply only to some of life's diverse forms and not to all. Mendel's laws of inheritance, for example, do not apply to bacteria and often are violated even in animal and plant species that usually follow them. Darwin's theories of perpetual change and common descent of living forms (p. 15) are perhaps the only statements that one meaningfully might call laws of biology.

Experimental versus Evolutionary Sciences

The many questions asked about the animal world since Aristotle can be grouped into two major categories.* The first category seeks to understand the **proximate** or **immediate causes** that underlie the functioning of biological systems at a particular time and place. These include the problems of explaining how animals perform their metabolic, physiological, and behavioral functions at the molecular, cellular, organismal, and even populational levels. For example, how is genetic information expressed to guide the synthesis of proteins? What causes cells to divide to produce new cells? How does population density affect the physiology and behavior of organisms?

*Mayr, E. 1985. Chapter 25 in D. Kohn, ed. *The Darwinian Heritage.* Princeton, Princeton University Press.

The biological sciences that investigate proximate causes are called **experimental sciences,** and they proceed using the experimental method. Our goal is to test our understanding of a biological system. We predict the results of an experimental disturbance of the system based on our current understanding of it. If our understanding is correct, then the predicted outcome should occur. If, after the experimental disturbance, we see an unexpected outcome, we then discover that our understanding is incorrect or incomplete. Experimental conditions are repeated to eliminate chance occurrences that might produce erroneous conclusions. **Controls**—repetitions of the experimental procedure that lack the disturbance—are established to eliminate unknown factors that might bias the outcome of the experiment. The processes by which animals maintain a body temperature under different environmental conditions, digest their food, migrate to new habitats, or store energy are some additional examples of physiological phenomena studied by experiment (see Chapters 29 through 36). Subfields of biology that constitute experimental sciences include molecular biology, cell biology, endocrinology, developmental biology, and community ecology.

In contrast to questions concerning the proximate causes of biological systems are questions of the **ultimate causes** that have produced these systems and their distinctive characteristics through evolutionary time. For example, what are the evolutionary factors that caused some birds to acquire complex patterns of seasonal migration between temperate and tropical areas? Why do different species of animals have different numbers of chromosomes in their cells? Why do some animal species maintain complex social systems, whereas other species have solitary individuals?

The biological sciences that address questions of ultimate cause are called **evolutionary sciences,** and they proceed largely using the **comparative method** rather than experimentation. Characteristics of molecular biology, cell biology, organismal structure, development, and ecology are compared among related species to identify their patterns of variation. The patterns of similarity and dissimilarity are then used to test hypotheses of relatedness, and thereby to reconstruct the evolutionary tree that relates the species being studied. Recent advances in DNA sequencing technology permit detailed tests of relationships among all animal species. The evolutionary tree is then used to examine hypotheses of the evolutionary origins of the diverse molecular, cellular, organismal, and populational properties observed in the animal world. Clearly, evolutionary sciences rely on results of experimental sciences as a starting point. Evolutionary sciences include comparative biochemistry, molecular evolution, comparative cell biology, comparative anatomy, comparative physiology, and phylogenetic systematics.

A scientist's use of the phrase "ultimate cause," unlike Aristotle's usage, does not imply a preconceived goal for natural phenomena. An argument that nature has a predetermined goal, such as evolution of the human mind, is termed teleological. **Teleology** is the mistaken notion that evolution of living organisms is guided by purpose toward an optimal design. A major success of Darwinian evolutionary theory is its rejection of teleology in explaining biological diversification.

THEORIES OF EVOLUTION AND HEREDITY

We turn now to a specific consideration of the two major paradigms that guide zoological research today: Darwin's theory of evolution and the chromosomal theory of inheritance.

Darwin's Theory of Evolution

Darwin's theory of evolution is now over 140 years old (see Chapter 6). Darwin articulated the complete theory when he published his famous book *On the Origin of Species by Means of Natural Selection* in England in 1859 (Figure 1.12). Biologists today are frequently asked, "What is Darwinism?" and "Do biologists still accept Darwin's theory of evolution?" These questions cannot be given simple answers, because Darwinism encompasses several different, although mutually compatible, theories. Professor Ernst Mayr of Harvard University argued that Darwinism should be viewed as five major theories. These five theories have somewhat different origins and different fates and cannot be treated as only a single statement. The theories are (1) perpetual change, (2) common descent, (3) multiplication of species, (4) gradualism, and (5) natural selection. The first three theories are generally accepted as having universal application throughout the living world. The theories of gradualism and natural selection are controversial among evolutionists, although both are strongly advocated by a large portion of the evolutionary community and are important components of the Darwinian evolutionary paradigm. Gradualism and natural selection are clearly part of the evolutionary process, but their explanatory power might not be as widespread as Darwin intended. Legitimate scientific controversies regarding gradualism and natural selection often are misrepresented by creationists as challenges to the first three theories listed, although the validity of those first three theories is strongly supported by all relevant observations.

1. **Perpetual change.** This is the basic theory of evolution on which the others are based. It states that the living world is neither constant nor perpetually cycling, but is always changing. The properties of organisms undergo transformation across generations throughout time. This theory originated in antiquity but did not gain widespread acceptance until Darwin advocated it in the context of his other four theories. "Perpetual change" is documented by the fossil record, which clearly refutes creationists' claims for a recent origin of all living forms. Because it has withstood repeated testing and is supported by an overwhelming number of observations, we now regard "perpetual change" as a scientific fact.
2. **Common descent.** The second Darwinian theory, "common descent," states that all forms of life descended from a common ancestor through a branching of lineages (Figure 1.13). The opposing argument, that the different forms of life arose independently and descended to the present

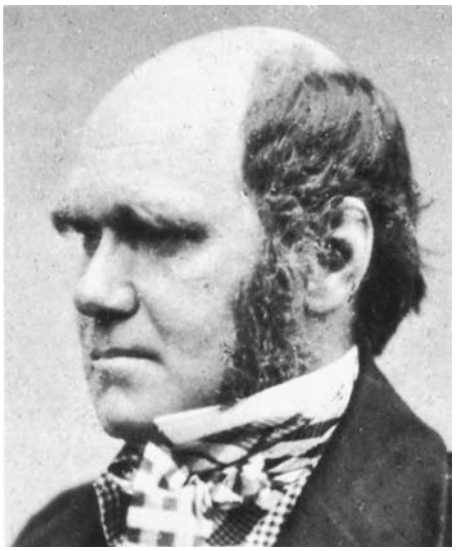


Figure 1.12
Modern evolutionary theory is strongly identified with Charles Robert Darwin, who, with Alfred Russel Wallace, provided the first credible explanation of evolution. This photograph of Darwin was taken in 1854 when he was 45 years old. His most famous book, *On the Origin of Species,* appeared five years later.

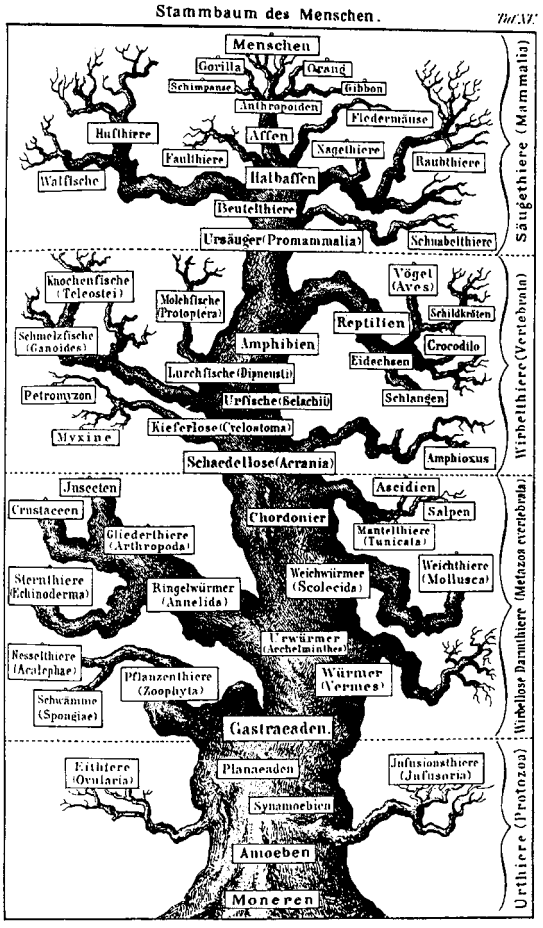


Figure 1.13
An early tree of life drawn in 1874 by the German biologist, Ernst Haeckel, who was strongly influenced by Darwin's theory of common descent. Many of the phylogenetic hypotheses shown in this tree, including the unilateral progression of evolution toward humans (= Menschen, *top*), have been refuted.

in linear, unbranched genealogies, has been refuted by comparative studies of organismal form, cell structure, and macromolecular structures (including those of the genetic material, DNA). All of these studies confirm the theory that life's history has the structure of a branching evolutionary tree, called a **phylogeny.** Species that share recent common ancestry have more similar features at all levels than do species whose most recent common ancestor is an ancient one. Much current research is guided by Darwin's theory of common descent toward reconstructing life's phylogeny using the patterns of similarity and dissimilarity observed among species. The resulting phylogeny serves as the basis for our taxonomic classification of animals (see Chapter 10).

3. **Multiplication of species.** Darwin's third theory states that the evolutionary process produces new species by splitting and transforming older ones. Species are now generally viewed as reproductively distinct populations of organisms that usually but not always differ from each other in organismal form. Once species are fully formed, interbreeding among members of different species does not occur or is too restricted to permit the species' lineages to merge. Evolutionists generally agree that the splitting and transformation of lineages produces new species, although there is still much controversy concerning details of this process (see Chapter 6) and the precise meaning of the term "species" (see Chapter 10). Much active scientific research examines historical processes that generate new species.
4. **Gradualism.** Gradualism states that the large differences in anatomical traits that characterize diverse species originate through the accumulation of many small incremental changes over very long periods of time. This theory is important because genetic changes having very large effects on organismal form are usually harmful to an organism. It is possible, however, that some genetic variants that have large effects are nonetheless sufficiently beneficial to be favored by natural selection. Therefore, although gradual evolution is known to occur, it may not explain the origins of all structural differences that we observe among species (Figure 1.14). Scientists are still actively studying this question.
5. **Natural selection.** Natural selection, Darwin's most famous theory, rests on three propositions. First, there is variation among organisms (within populations) for anatomical, behavioral, and physiological traits. Second, the variation is at least partly heritable so that offspring tend to resemble their parents. Third, organisms with different variant forms are expected to leave different numbers of offspring to future generations. Variants that permit their possessors most effectively to exploit their environments will preferentially survive to be transmitted to future generations. Over many generations, favorable new traits will spread throughout a population. Accumulation of such changes leads, over long periods of time, to production of new organismal characteristics and new species. Natural selection is therefore a creative process that generates novel forms from the small individual variations that occur among organisms within a population.

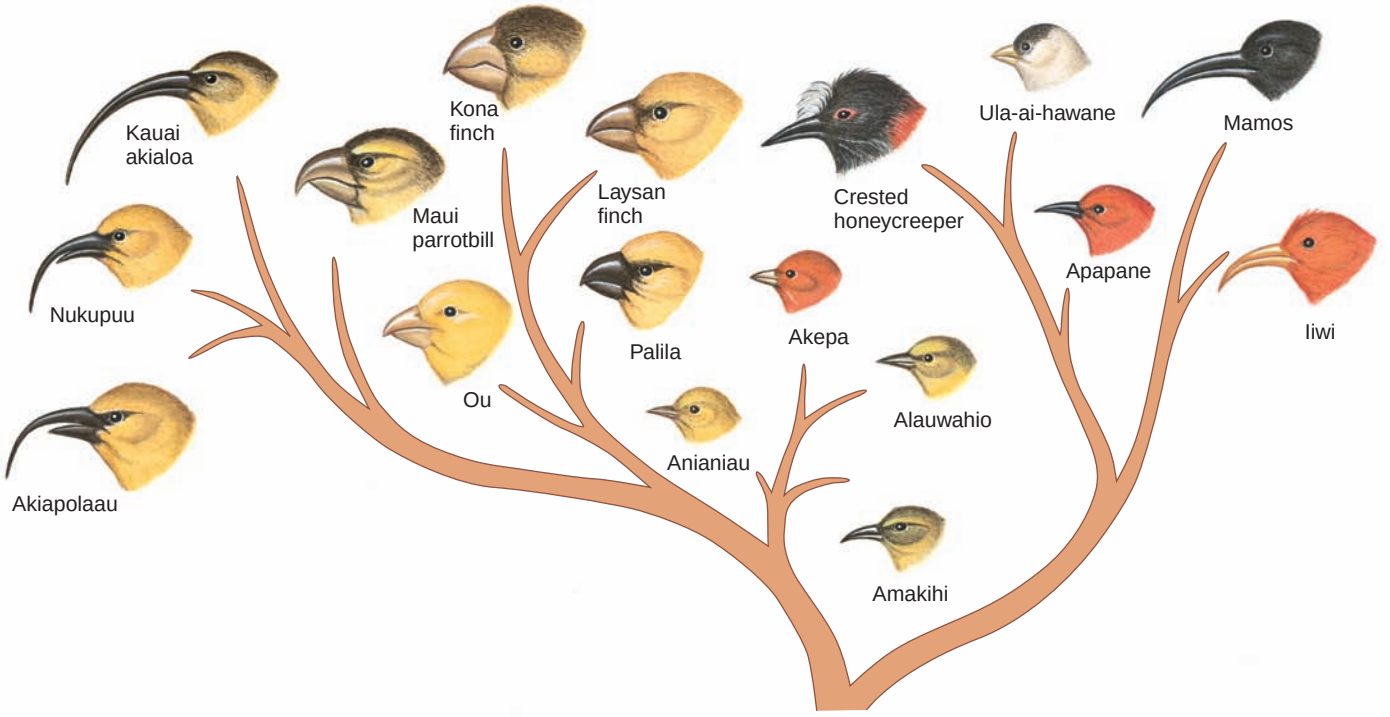


Figure 1.14

Gradualism provides a plausible explanation for the origins of different bill shapes in the Hawaiian honeycreepers shown here. This theory has been challenged, however, as an explanation of the evolution of such structures as vertebrate scales, feathers, and hair from a common ancestral structure. The geneticist Richard Goldschmidt viewed the latter forms as unbridgeable by any gradual transformation series.

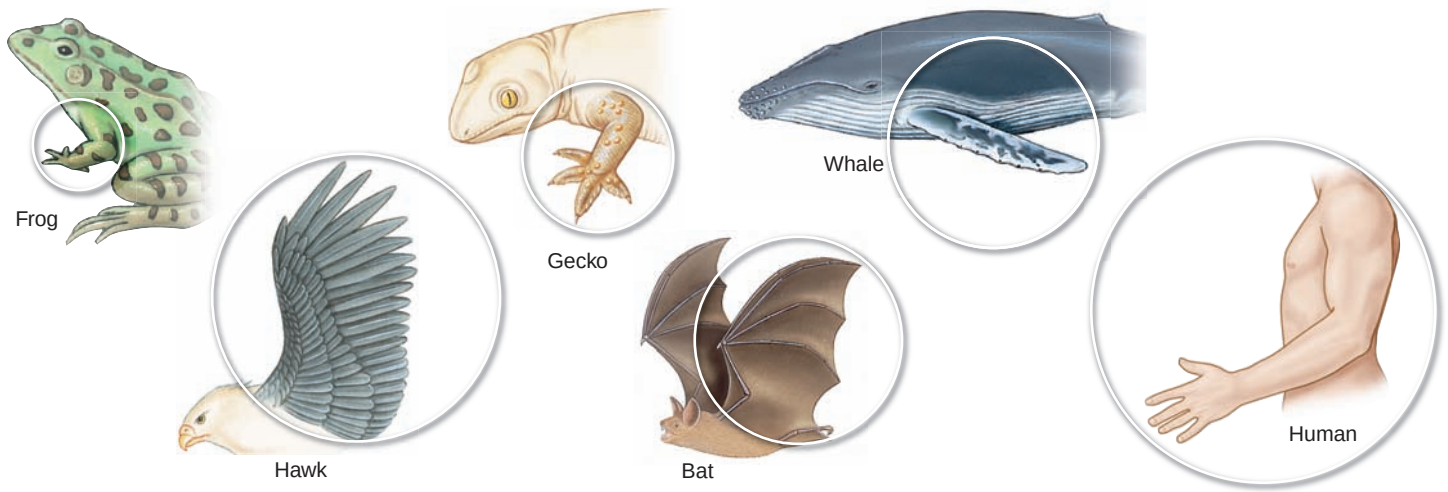


Figure 1.15

According to Darwinian evolutionary theory, the different forms of these vertebrate forelimbs were molded by natural selection to adapt them for different functions. We show in later chapters that, despite these adaptive differences, these limbs share basic structural similarities.

Natural selection explains why organisms are constructed to meet the demands of their environments, a phenomenon called **adaptation** (Figure 1.15). Adaptation is the expected result of a process that accumulates the most favorable variants occurring in a population throughout long periods of evolutionary time. Adaptation was viewed previously as strong evidence against evolution, and Darwin's theory of natural selection was therefore important for convincing people that a natural process, capable of being studied scientifically, could produce new species. The demonstration that natural processes could produce adaptation was important to the eventual acceptance of all five Darwinian theories.

Darwin's theory of natural selection faced a major obstacle when it was first proposed: it lacked a successful theory of heredity. People assumed incorrectly that heredity was a blending process, and that any favorable new variant appearing in a population therefore would be lost. The new variant arises initially in a single organism, and that organism therefore must mate with one lacking the favorable new trait. Under blending inheritance, the organism's offspring would then have only a diluted form of the favorable trait. These offspring likewise would mate with others that lack the favorable trait. With its effects diluted by half each generation, the trait eventually would cease to exist. Natural selection would be completely ineffective in this situation.

Darwin was never able to counter this criticism successfully. It did not occur to Darwin that hereditary factors could be discrete and nonblending and that a new genetic variant therefore could persist unaltered from one generation to the next. This principle is called **particulate inheritance.** It was established after 1900 with the discovery of Gregor Mendel's genetic experiments, and it was eventually incorporated into what we now call the **chromosomal theory of inheritance.** We use the term **neo-Darwinism** to describe Darwin's theories as modified by incorporating this theory of inheritance.

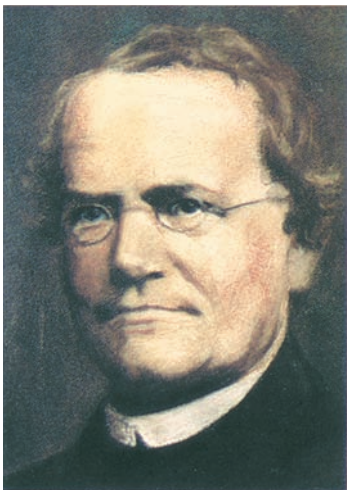
Mendelian Heredity and the Chromosomal Theory of Inheritance

The chromosomal theory of inheritance is the foundation for current studies of genetics and evolution in animals (see Chapters 5 and 6). This theory comes from the consolidation of research done in the fields of genetics, which was founded by the experimental work of Gregor Mendel (Figure 1.16), and cell biology.

Genetic Approach

The genetic approach consists of mating or "crossing" populations of organisms that are true-breeding for alternative traits, and then following hereditary transmission of those traits through subsequent generations. "True-breeding" means that a population maintains across generations only one of the alternative traits when propagated in isolation from other populations. For example, most populations of fruit flies produce only red-eyed individuals, generation after generation, regardless of the environments in which they are raised; such strains are true-breeding for red eyes. Some laboratory strains of fruit flies produce only white-eyed individuals and are therefore true-breeding for white eyes (p. 88).

Gregor Mendel studied the transmission of seven variable features in garden peas, crossing populations that were true-breeding for alternative traits (for example, tall versus short plants). In the first generation (called the F_1 generation, for "filial"), only one of the alternative parental traits was observed; there was no indication of blending of the parental traits. In the example, the offspring (called F_1 *hybrids* because they represent a cross between two different forms) formed by crossing the tall and short plants were tall, regardless of whether the tall trait was inherited from the male or the female parent. These F_1 hybrids were allowed to self-pollinate, and both parental traits were found among their offspring (called the F_2 generation), although the trait observed in the F_1 hybrids (tall plants in this example) was



A

B

Figure 1.16

A, Gregor Johann Mendel. **B,** The monastery in Brno, Czech Republic, now a museum, where Mendel performed his experiments with garden peas.

three times more common than the other trait. Again, there was no indication of blending of the parental traits (Figure 1.17).

Mendel's experiments showed that the effects of a genetic factor can be masked in a hybrid individual, but that these factors are not physically altered during the transmission process. He postulated that variable traits are specified by paired hereditary factors, which we now call "genes." When **gametes** (eggs or sperm) are produced, the two genes controlling a particular feature are segregated from each other and each gamete receives only one of them. Fertilization restores the paired condition. If an organism possesses different forms of the paired genes for a feature, only one of them is expressed in its appearance, but both genes nonetheless are transmitted unaltered in equal numbers to the gametes produced. Transmission of these genes is particulate, not blending. Mendel observed that inheritance of one pair of traits is independent of inheritance of other paired

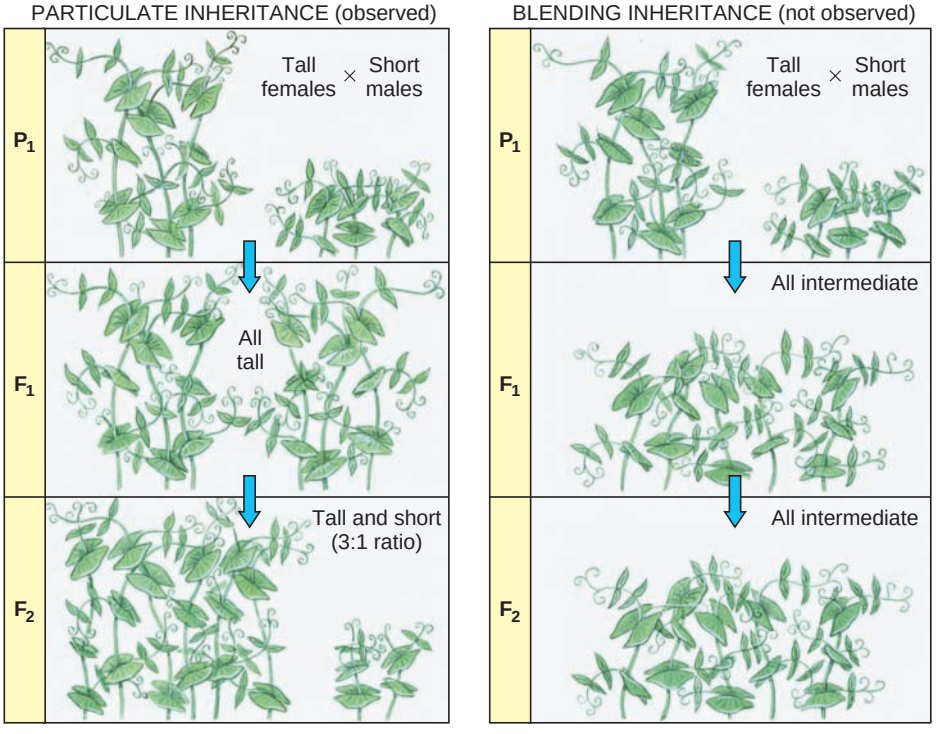


Figure 1.17

Different predictions of particulate versus blending inheritance regarding the outcome of Mendel's crosses of tall and short plants. The prediction of particulate inheritance is upheld and the prediction of blending inheritance is falsified by the results of the experiments. The reciprocal experiments (crossing short female parents with tall male parents) produced similar results. (P_1 = parental generation; F_1 = first filial generation; F_2 = second filial generation.)

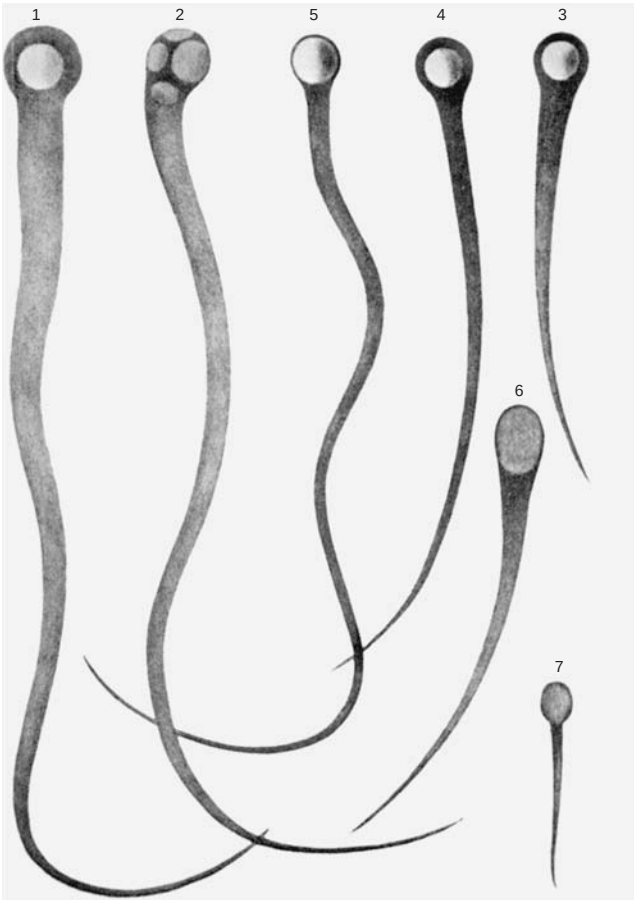


Figure 1.18

An early nineteenth-century micrographic drawing of sperm from (1) guinea pig, (2) white mouse, (3) hedgehog, (4) horse, (5) cat, (6) ram, and (7) dog. Some biologists initially interpreted these as parasitic worms in the semen, but in 1824, Jean Prévost and Jean Dumas correctly identified their role in egg fertilization.

traits. We now know, however, that not all pairs of traits are inherited independently of each other; different traits that tend to be inherited together are said to be genetically linked (p. 88). Numerous studies, particularly of the fruit fly, *Drosophila melanogaster,* have shown that principles of inheritance discovered initially in plants apply also to animals.

Contributions of Cell Biology

Improvements in microscopes during the 1800s permitted cytologists to study the production of gametes by direct observation of reproductive tissues. Interpreting the observations was initially difficult, however. Some prominent biologists hypothesized, for example, that sperm were parasitic worms in semen (Figure 1.18). This hypothesis was soon falsified, and the true nature of gametes was clarified. As the precursors of gametes prepare to divide early in gamete production, the nuclear material condenses to reveal discrete, elongate structures called chromosomes. Chromosomes occur in pairs that are usually similar but not identical in appearance and informational content. The number of chromosomal pairs varies among species. One member of each pair is derived from the female parent and the other from the male parent. Paired chromosomes are physically associated and then segregated into different daughter cells during cell division prior to gamete formation (Figure 1.19). Each resulting gamete receives one chromosome from each pair. Different pairs of chromosomes are sorted into gametes independently of each other. Because the behavior of chromosomal material during gamete formation parallels that postulated for Mendel's genes, Sutton and Boveri in 1903 through 1904 hypothesized that chromosomes were the physical bearers of genetic material. This hypothesis met with extreme skepticism when first proposed. A long series of tests designed to falsify it nonetheless showed that its predictions were upheld. The chromosomal theory of inheritance is now well established.

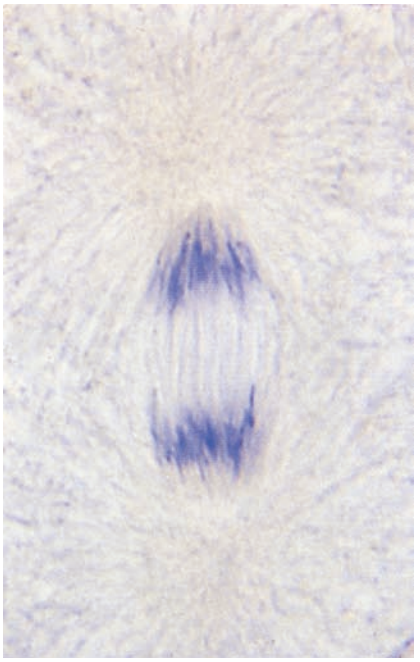


Figure 1.19

Paired chromosomes being separated before nuclear division in the process of forming gametes.

SUMMARY

Zoology is the scientific study of animals, and it is part of biology, the scientific study of life. Animals and life in general can be identified by attributes that they have acquired over their long evolutionary histories. The most outstanding attributes of life include chemical uniqueness, complexity and hierarchical organization, reproduction, possession of a genetic program, metabolism, development, interaction with the environment, and movement. Biological systems comprise a hierarchy of integrative levels (molecular,

cellular, organismal, populational, and species levels), each of which demonstrates a number of specific emergent properties.

Science is characterized by the acquisition of knowledge by constructing and then testing hypotheses through observations of the natural world. Science is guided by natural law, and its hypotheses are testable, tentative, and falsifiable. Zoological sciences can be subdivided into two categories, experimental sciences and evolutionary sciences. Experimental sciences use the experimental method to ask how animals perform their basic metabolic, developmental, behavioral, and reproductive functions, including investigations of their molecular, cellular, and populational systems. Evolutionary sciences use the comparative method to reconstruct the history of life, and then use that history to understand how diverse species and their molecular, cellular, organismal, and populational properties arose through evolutionary time. Hypotheses that withstand repeated testing and therefore explain many diverse phenomena gain the status of a theory. Powerful theories that guide extensive research are called "paradigms." The major paradigms that guide the study of zoology are Darwin's theory of evolution and the chromosomal theory of inheritance.

The principles given in this chapter illustrate the unity of biological science. All components of biological systems are guided by natural laws and are constrained by those laws. Living organisms arise only from other living organisms, just as new cells can be produced only from preexisting cells. Reproductive processes occur at all levels of the biological hierarchy and demonstrate both heredity and variation. Interaction of heredity and variation at all levels of the biological hierarchy produces evolutionary change and has generated the great diversity of animal life documented throughout this book.

REVIEW QUESTIONS

1. Why is life difficult to define?
2. What are the basic chemical differences that distinguish living from nonliving systems?
3. Describe the hierarchical organization of life. How does this organization lead to the emergence of new properties at different levels of biological complexity?
4. What is the relationship between heredity and variation in reproducing biological systems?
5. Describe how evolution of complex organisms is compatible with the second law of thermodynamics.
6. What are the essential characteristics of science? Describe how evolutionary studies fit these characteristics whereas "scientific creationism" or "intelligent-design theory" does not.
7. Use studies of natural selection in British moth populations to illustrate the hypothetico-deductive method of science.
8. How do we distinguish the terms hypothesis, theory, paradigm, and scientific fact?
9. How do biologists distinguish experimental and evolutionary sciences?
10. What are Darwin's five theories of evolution (as identified by Ernst Mayr)? Which are accepted as fact and which continue to stir controversy among biologists?
11. What major obstacle confronted Darwin's theory of natural selection when it was first proposed? How was this obstacle overcome?
12. How does neo-Darwinism differ from Darwinism?
13. Describe the respective contributions of the genetic approach and cell biology to formulating the chromosomal theory of inheritance.

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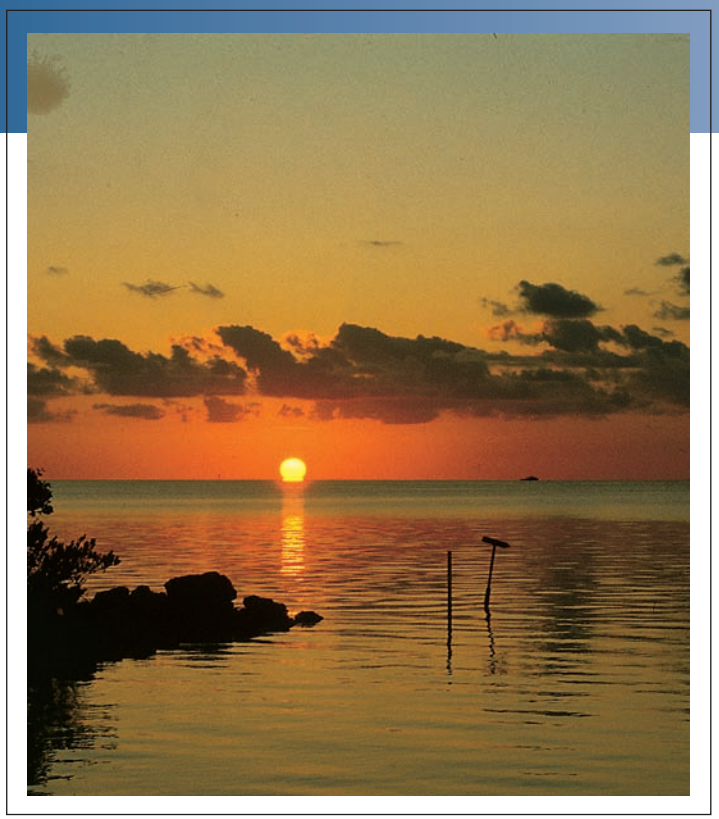
CHAPTER

2

The Origin and Chemistry of Life



Earth's abundant supply of water was critical for the origin of life.

Spontaneous Generation of Life?

From ancient times, people commonly thought that life arose repeatedly by spontaneous generation from nonliving material in addition to parental reproduction. For example, frogs appeared to arise from damp earth, mice from putrefied matter, insects from dew, and maggots from decaying meat. Warmth, moisture, sunlight, and even starlight often were mentioned as factors that encouraged spontaneous generation of living organisms.

Among the efforts to synthesize organisms in the laboratory is a recipe for making mice, given by the Belgian plant nutritionist Jean Baptiste van Helmont (1648). "If you press a piece of underwear soiled with sweat together with some wheat in an open jar, after about 21 days the odor changes and the ferment . . . changes the wheat into mice. But what is more remarkable is that the mice which came out of the wheat and underwear were not small mice, not even miniature adults or aborted mice, but adult mice emerge!"

In 1861, the great French scientist Louis Pasteur convinced scientists that living organisms cannot arise spontaneously from nonliving matter. In his famous experiments, Pasteur introduced fermentable material into a flask with a long S-shaped neck that was open to air. The flask and its contents were then boiled for a long time to kill any microorganisms that might be present. Afterward the flask was cooled and left undisturbed. No fermentation occurred because all organisms that entered the open end were deposited in the neck and did not reach the fermentable material. When the neck of the flask was removed, microorganisms in the air promptly entered the fermentable material and proliferated. Pasteur concluded that life could not originate in the absence of previously existing organisms and their reproductive elements, such as eggs and spores. Announcing his results to the French Academy, Pasteur proclaimed, "Never will the doctrine of spontaneous generation arise from this mortal blow."

All living organisms share a common ancestor, most likely a population of colonial microorganisms that lived almost 4 billion years ago. This common ancestor was itself the product of a long period of prebiotic assembly of nonliving matter, including organic molecules and water, to form self-replicating units. All living organisms retain a fundamental chemical composition inherited from their ancient common ancestor.

According to the big-bang model, the universe originated from a primeval fireball and has been expanding and cooling since its inception 10 to 20 billion years ago. The sun and planets formed approximately 4.6 billion years ago from a spherical cloud of cosmic dust and gases. The cloud collapsed under the influence of its own gravity into a rotating disc. As material in the central part of the disc condensed to form the sun, gravitational energy was released as radiation. The pressure of this outwardly directed radiation prevented a collapse of the nebula into the sun. The material left behind cooled and eventually produced the planets, including earth (Figure 2.1).

In the 1920s, Russian biochemist Alexander I. Oparin and British biologist J. B. S. Haldane independently proposed that life originated on earth after an inconceivably long period of "abiogenic molecular evolution." Rather than arguing that the first living organisms miraculously originated all at once, a notion that formerly discouraged scientific inquiry, Oparin and Haldane argued that the simplest form of life arose gradually by the progressive assembly of small molecules into more complex organic molecules. Molecules capable of self-replication eventually would be produced, ultimately leading to assembly of living microorganisms.

WATER AND LIFE

The origin and maintenance of life on earth depend critically upon water. Water is the most abundant of all compounds in cells, forming 60% to 90% of most living organisms. Water has several extraordinary properties that explain its essential role in living systems and their origin. These properties result largely from hydrogen bonds that form between its molecules (Figure 2.2).

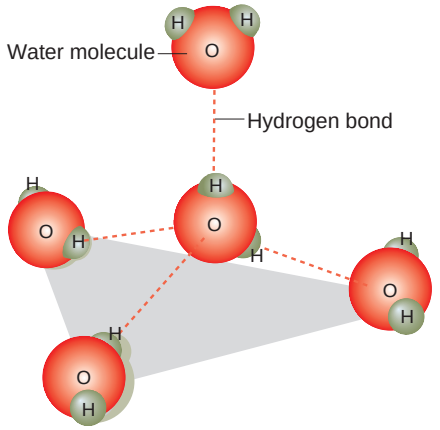


Figure 2.2
Geometry of water molecules. Each water molecule is linked by hydrogen bonds (*dashed lines*) to four other molecules. If imaginary lines connect the water molecules as shown, a tetrahedron is obtained.

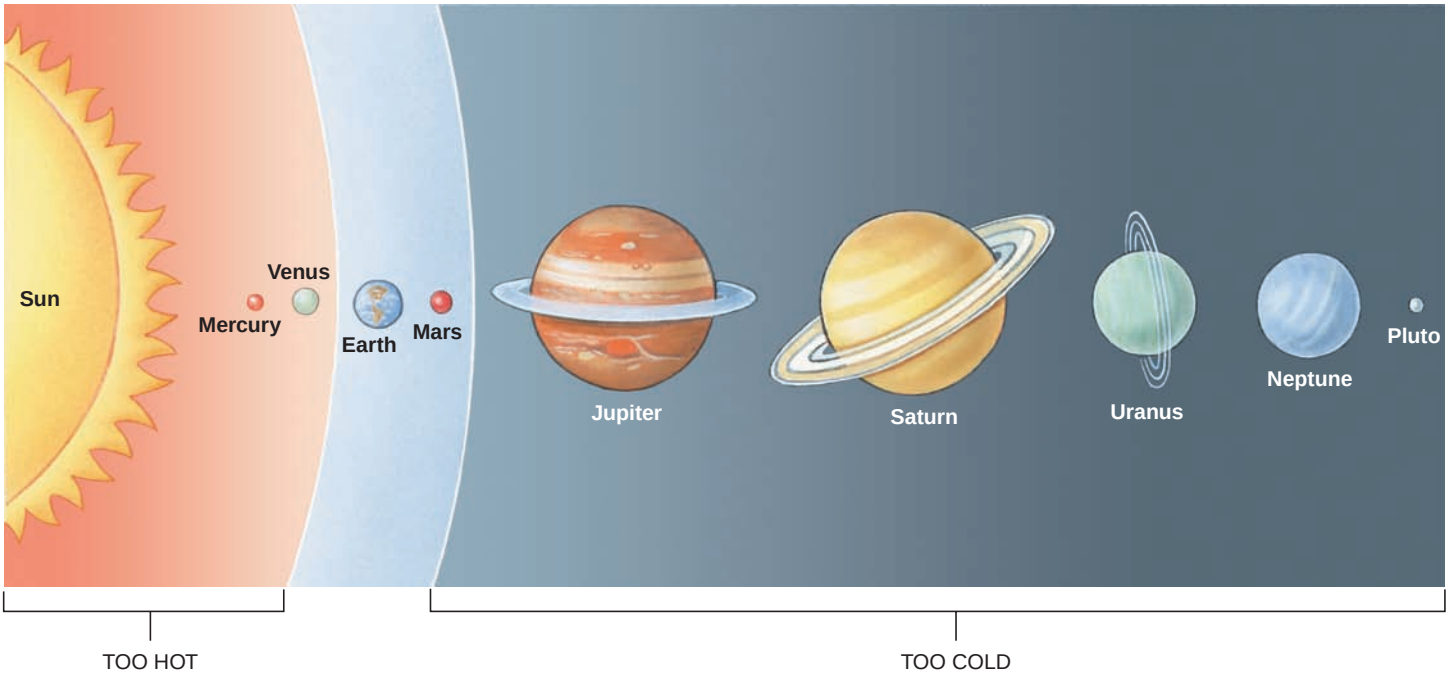


Figure 2.1
Solar system showing narrow range of thermal conditions suitable for life.

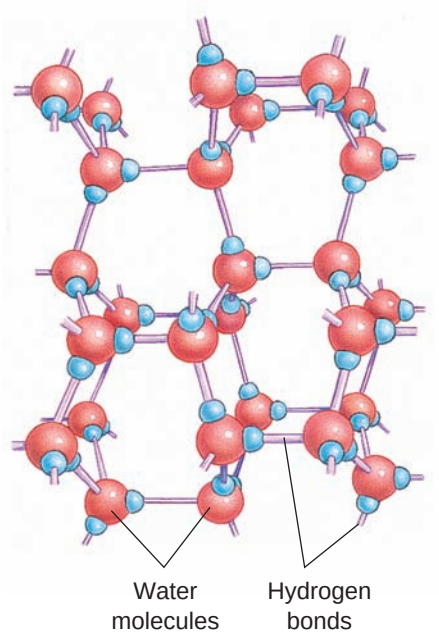


Figure 2.3

When water freezes at 0° C, the four partial charges of each atom in the molecule interact with the opposite charges of atoms in other water molecules. The hydrogen bonds between all the molecules form a crystal-like lattice structure, and the molecules are farther apart (and thus less dense) than when some of the molecules have not formed hydrogen bonds at 4° C.

Water has a **high specific heat capacity:** 1 calorie* is required to elevate the temperature of 1 g of water 1° C, a higher thermal capacity than any other liquid except ammonia. Much of this heat energy is used to rupture some hydrogen bonds in addition to increasing the kinetic energy (molecular movement), and thus the temperature, of the water. Water's high thermal capacity greatly moderates environmental temperature changes, thereby protecting living organisms from extreme thermal fluctuation. Water also has a **high heat of vaporization,** requiring more than 500 calories to convert 1 g of liquid water to water vapor. All hydrogen bonds between a water molecule and its neighbors must be ruptured before that water molecule can escape the surface and enter the air. For terrestrial animals (and plants), cooling produced by evaporation of water is important for expelling excess heat.

Another property of water important for life is its **unique density behavior** during changes of temperature. Most liquids become denser with decreasing temperature. Water, however, reaches its maximum density at 4° C *while still a liquid,* then becomes less dense with further cooling (Figure 2.3). Therefore, ice *floats* rather than sinking to the bottoms of lakes and ponds. If ice were denser than liquid water, bodies of water would freeze solid from the bottom upward in winter and might not melt completely in summer. Such conditions would severely limit aquatic life. In ice, water molecules form an extensive, open, crystal-like network supported by hydrogen bonds that connect all molecules. The molecules in this lattice are farther apart, and thus less dense, than in liquid water at 4° C.

*A calorie is defined as the amount of heat required to heat 1 g of water from 14.5° to 15.5° C. Although the calorie is the traditional unit of heat widely used in publications and tables, it is not part of the International System of Units (the SI system) which uses the joule (J) as the energy unit (1 cal = 4.184 J).

Figure 2.4

Because of hydrogen bonds between water molecules at the water-air interface, the water molecules cling together and create a high surface tension. Thus some insects, such as this water strider, can literally walk on water.

Water has **high surface tension,** exceeding that of any other liquid but mercury. Hydrogen bonding among water molecules produces a cohesiveness important for maintaining protoplasmic form and movement. The resulting surface tension creates an ecological niche (see p. 826) for insects, such as water striders and whirligig beetles, that skate on the surfaces of ponds (Figure 2.4). Despite its high surface tension, water has **low viscosity,** permitting movement of blood through minute capillaries and of cytoplasm inside cellular boundaries.

Water is an excellent **solvent.** Salts dissolve more extensively in water than in any other solvent. This property results from the dipolar nature of water, which causes it to orient around charged particles dissolved in it. When, for example, crystalline NaCl dissolves in water, the Na^+ and Cl^- ions separate (Figure 2.5). The negative zones of the water dipoles attract the Na^+ ions while the positive zones attract the Cl^- ions. This orientation keeps the ions separated, promoting their dissociation. Solvents lacking this dipolar character are less effective at keeping the ions separated. Binding of water to dissolved protein molecules is essential to the proper functioning of many proteins.

Water also participates in many chemical reactions in living organisms. Many compounds are split into smaller pieces by the addition of a molecule of water, a process called **hydrolysis.** Likewise, larger compounds may be synthesized from smaller components by the reverse of hydrolysis, called **condensation reactions.**

$$R — R + H_2O \xrightarrow[\text{Hydrolysis}]{} R — OH + H — R$$

$$R — OH + H — R \xrightarrow[\text{Condensation}]{} R — R + H_2O$$

pH of Water Solutions

In pure liquid water (= distilled water), a small fraction of the water molecules split into ions of hydrogen (H^+) and hydroxide (OH^-); the concentration of both ions is 10^{-7} moles/liter. An acidic substance, when dissolved in water, contributes H^+ ions to solution, thereby increasing their concentration and causing an excess of H^+ ions over OH^- ions in solution. A basic substance does the reverse, contributing OH^- ions to the solution and making OH^- ions more common than H^+ ions. The degree to which a solution is acidic or basic is critical for most cellular processes and requires precise quantification and control; the structure and function of dissolved proteins, for example, depend critically on the concentration of H^+ in the solution.

The **pH** scale quantifies the degree to which a solution is acidic or basic. The scale ranges from 0 to 14 and represents the additive inverse of the logarithm (base 10) of the H^+ concentration (in moles/liter) of the solution. Pure liquid water therefore has a pH of 7 (H^+ concentration = 10^{-7} moles/liter). A solution with pH = 6.0 has an H^+ concentration ten times higher than that of pure water and is acidic, whereas a solution with pH = 8.0 has an H^+ concentration ten times lower than pure water and is basic. A concentrated strong acid, such as hydrochloric acid (HCl, known commercially as "muriatic acid" used to clean masonry) has an H^+ concentration of ~1 = 10^0 mole/liter, giving a pH of 0 (a concentration of H^+ 10,000,000 times that of pure water). A concentrated base, such as sodium hydroxide (NaOH, used commercially in liquid drain cleaners) has an H^+ concentration of approximately 10^{-14} mole/liter, giving a pH of 14.

A buffer is a dissolved substance (solute) that causes a solution to resist changes in pH because the buffer can remove added H^+ and OH^- ions from solution by binding them into compounds. Dissolved carbon dioxide in the form of bicarbonate (HCO_3^-) is a buffer that helps to protect human blood (pH = 7.3 to 7.5) from changes in pH. H^+ ions are removed from solution when they react with bicarbonate ions to form carbonic acid, which then dissociates into carbon dioxide and water. The excess carbon dioxide is removed during exhalation (p. 703). OH^- ions are removed from solution when this reaction is reversed, forming bicarbonate and hydrogen ions. The excess bicarbonate ions are secreted in the urine (p. 676), and the hydrogen ions serve to increase blood pH back to normal levels. Severe health problems occur if the pH of blood drops to 7 or rises to 7.8.

Because water is critical to the support of life, the continuing search for extraterrestrial life usually begins with a search for water. Plans for a human outpost on the moon likewise depend upon finding water there. As we write, NASA is planning to crash a space probe into the moon in 2009 in a search for ice; the moon's south pole is a prime candidate for a human outpost if ice is found there.

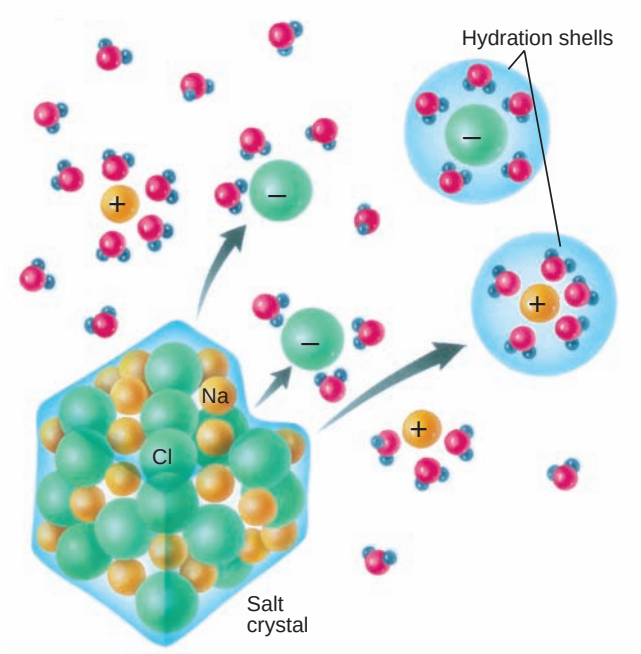


Figure 2.5
When a crystal of sodium chloride dissolves in water, the negative ends of the dipolar molecules of water surround the Na^+ ions, while the positive ends of water molecules face the Cl^- ions. The ions are thus separated and do not reenter the salt lattice.

ORGANIC MOLECULAR STRUCTURE OF LIVING SYSTEMS

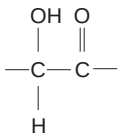
Chemical evolution in the prebiotic environment produced simple organic compounds that ultimately formed the building blocks of living cells. The term "organic" refers broadly to compounds that contain carbon. Many also contain hydrogen, oxygen, nitrogen, sulfur, phosphorus, salts, and other elements. Carbon has a great ability to bond with other carbon atoms in chains of varying lengths and configurations. Carbon-to-carbon combinations introduce the possibility of enormous complexity and variety into molecular structure. More than a million organic compounds are known.

We review the kinds of organic molecules found in living systems, followed by further discussion of their origins in earth's primitive reducing atmosphere.

Carbohydrates: Nature's Most Abundant Organic Substance

Carbohydrates are compounds of carbon, hydrogen, and oxygen. These elements usually occur in the ratio of 1 C: 2 H: 1 O and are grouped as H—C—OH. Carbohydrates function in protoplasm mainly as structural elements and as a source of chemical energy. Glucose is the most important of these energy-storing carbohydrates. Familiar examples of carbohydrates include sugars, starches, and cellulose (the woody structure of plants). Cellulose occurs on earth in greater quantities than all other organic materials combined. Carbohydrates are synthesized by green plants from water and carbon dioxide, with the aid of solar energy. This process, called **photosynthesis,** is a reaction upon which all life depends, for it is the starting point in the formation of food.

Carbohydrates are usually grouped into the following three classes: (1) **monosaccharides,** or simple sugars; (2) **disaccharides,** or double sugars; and (3) **polysaccharides,** or complex sugars. Simple sugars have a single carbon chain containing 4 carbons (tetroses), 5 carbons (pentoses), or 6 carbons (hexoses). Other simple sugars have up to 10 carbons, but these sugars are not biologically important. Simple sugars, such as glucose, galactose, and fructose, all contain a free sugar group,



in which the double-bonded O may be attached to the terminal or nonterminal carbons of a chain. The hexose **glucose** (also called dextrose) is particularly important to the living world. Glucose is often shown as a straight chain (Figure 2.6A), but in water it forms a cyclic compound (Figure 2.6B). The "chair" diagram (Figure 2.7) of glucose best represents its true configuration, but all forms of glucose, however represented, are chemically equivalent. Other hexoses of biological significance include galactose and fructose, which are compared with glucose in Figure 2.8.

Disaccharides are double sugars formed by bonding two simple sugars. An example is maltose (malt sugar), composed of two glucose molecules. As shown in Figure 2.9, the two glucose molecules are joined by removing a molecule of water, causing the sharing of an oxygen atom by the two sugars. All disaccharides are formed in this manner. Two other common disaccharides are sucrose (ordinary cane, or table, sugar), formed by the linkage of glucose and fructose, and lactose (milk sugar), composed of glucose and galactose.

Polysaccharides are composed of many molecules of simple sugars (usually glucose) linked in long chains called polymers. Their empirical formula is usually written $(C_6H_{10}O_5)_n$, where n

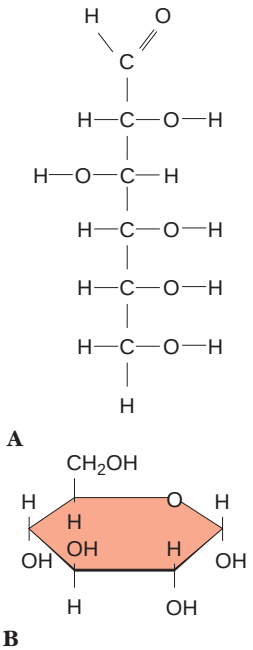


Figure 2.6

Two ways of depicting the simple sugar glucose. In **A,** the carbon atoms are shown in open-chain form. When dissolved in water, glucose tends to assume a ring form as in **B.** In this ring model the carbon atoms located at each turn in the ring are usually not shown.

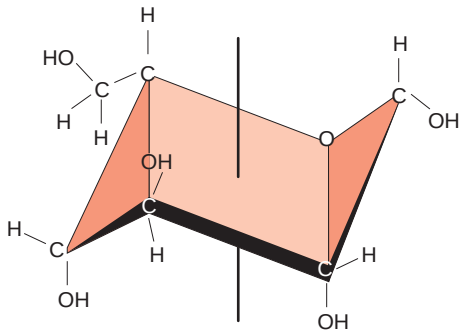


Figure 2.7

"Chair" representation of a glucose molecule.

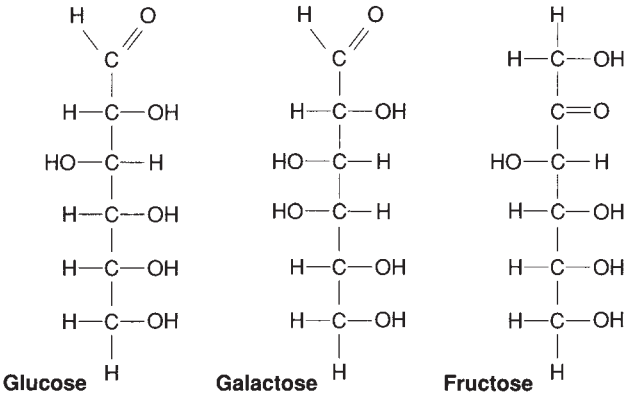


Figure 2.8

These three hexoses are the most common monosaccharides.

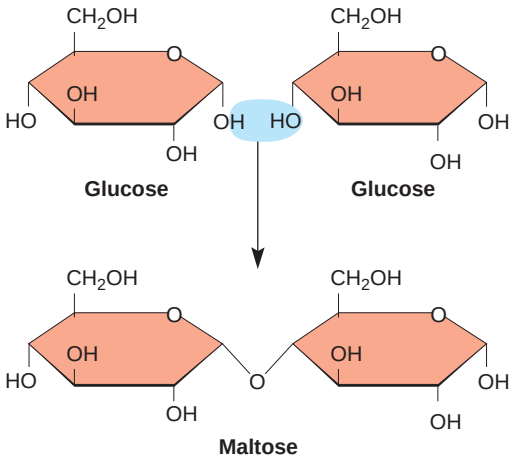


Figure 2.9

Formation of a double sugar (disaccharide maltose) from two glucose molecules with the removal of one molecule of water.

designates the number of simple-sugar subunits in the polymer. Starch is the common polymer in which sugar is stored in most plants and is an important food for animals. **Chitin** is an important structural polysaccharide in the exoskeletons of insects and other arthropods (p. 404). **Glycogen** is an important polymer for storing sugar in animals. It is stored mainly in liver and muscle cells in vertebrates. When needed, glycogen is converted to glucose and delivered by blood to the tissues. Another polymer is **cellulose,** the principal structural carbohydrate of plants.

Lipids: Fuel Storage and Building Material

Lipids are fats and fatlike substances. They are molecules of low polarity; consequently, they are virtually insoluble in water but are soluble in organic solvents, such as acetone and ether. The three principal groups of lipids are neutral fats, phospholipids, and steroids.

Neutral Fats

The neutral or "true" fats are major fuels of animals. Stored fat is derived either directly from dietary fat or indirectly from dietary carbohydrates that the body has converted to fat for storage. Fats are oxidized and released into the bloodstream as needed to meet tissue demands, especially those of active muscle.

Neutral fats include triglycerides, which contain glycerol and three molecules of fatty acids. Neutral fats are therefore esters, a combination of an alcohol (glycerol) and an acid. Fatty acids in triglycerides are simply long-chain monocarboxylic acids; they vary in size but are commonly 14 to 24 carbons long. Production of a typical fat by the union of glycerol and stearic acid is shown in Figure 2.10A. In this reaction, three fatty acid molecules are seen to have united with OH groups of glycerol to form stearin (a neutral fat) plus three molecules of water.

Most triglycerides contain two or three different fatty acids attached to glycerol, and bear ponderous names such as myristoyl stearoyl glycerol (Figure 2.10B). The fatty acids in this triglyceride are **saturated;** every carbon within the chain holds two hydrogen atoms. Saturated fats, more common in animals than in plants, are usually solid at room temperature. **Unsaturated** fatty acids, typical of plant oils, have two or more carbon atoms joined by double bonds; the carbons are not "saturated" with hydrogen atoms and are available to form bonds with other atoms. Two common unsaturated fatty acids are oleic acid and linoleic acid (Figure 2.11). Plant fats, such as peanut oil and corn oil, tend to be liquid at room temperature.

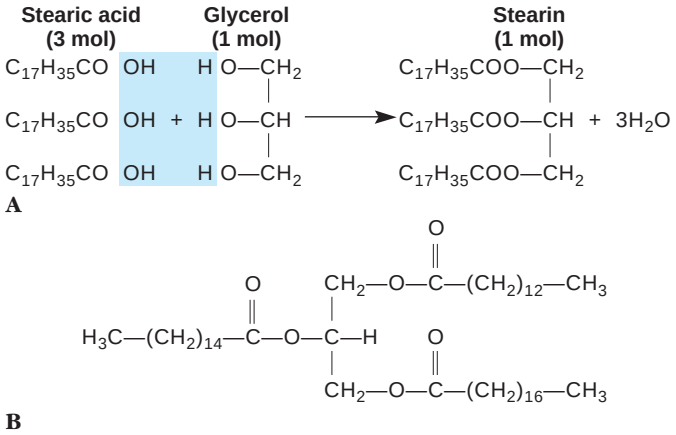


Figure 2.10

Neutral fats. **A,** Formation of a neutral fat from three molecules of stearic acid (a fatty acid) and glycerol. **B,** A neutral fat bearing three different fatty acids.

Phospholipids

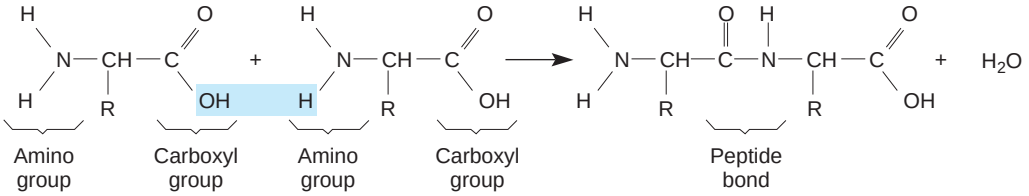
Unlike fats that are fuels and serve no structural roles in the cell, phospholipids are important components of the molecular organization of tissues, especially membranes. They resemble triglycerides in structure, except that one of the three fatty acids is replaced by phosphoric acid and an organic base. An example is lecithin, an important phospholipid of nerve membranes (Figure 2.12). Because the phosphate group on phospholipids is charged and polar and therefore soluble in water, and the remainder of the molecule is nonpolar, phospholipids can bridge two environments and bind water-soluble molecules, such as proteins, to water-insoluble materials.

Steroids

Steroids are complex alcohols. Although they are structurally unlike fats, they have fatlike properties. The steroids are a large group of biologically important molecules, including cholesterol (Figure 2.13), vitamin D3, many adrenocortical hormones, and sex hormones.

Amino Acids and Proteins

Proteins are large, complex molecules composed of 20 kinds of amino acids (Figure 2.14). The amino acids are linked by **peptide bonds** to form long, chainlike polymers. In the formation of a peptide bond, the carboxyl group of one amino acid is linked by a covalent bond to the amino group of another, with elimination of water, as shown here:



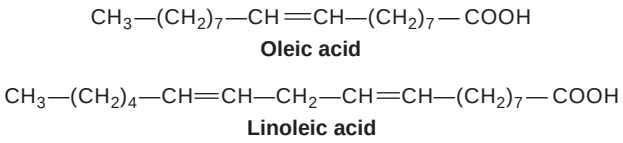


Figure 2.11

Unsaturated fatty acids. Oleic acid has one double bond and linoleic acid has two double bonds. The remainder of both acids is saturated.

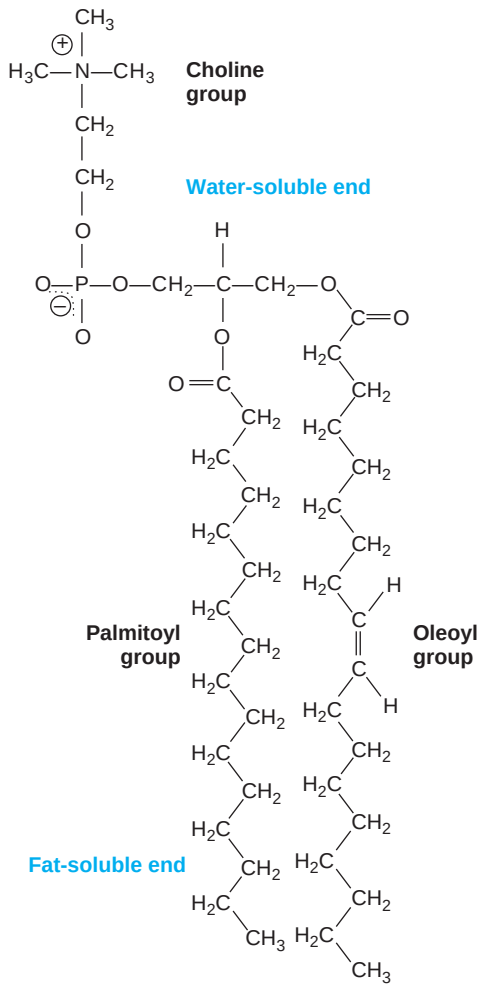


Figure 2.12

Lecithin (phosphatidyl choline), an important phospholipid of nerve membranes.

The combination of two amino acids by a peptide bond forms a dipeptide having a free amino group on one end and a free carboxyl group on the other; therefore, additional amino acids can be joined until a long chain is produced. The 20 different kinds of amino acids can be arranged in an enormous variety of sequences of up to several hundred amino acid units, accounting for the large diversity of proteins found among living organisms.

A protein is not just a long string of amino acids; it is a highly organized molecule. For convenience, biochemists

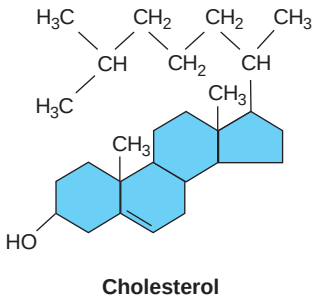


Figure 2.13

Cholesterol, a steroid. All steroids have a basic skeleton of four rings (three 6-carbon rings and one 5-carbon ring) with various side groups attached.

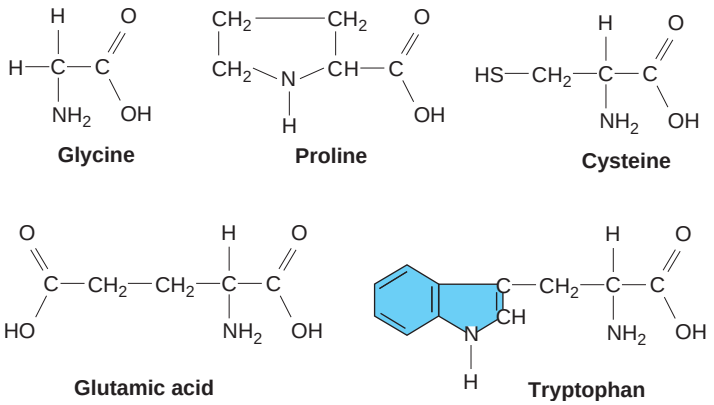
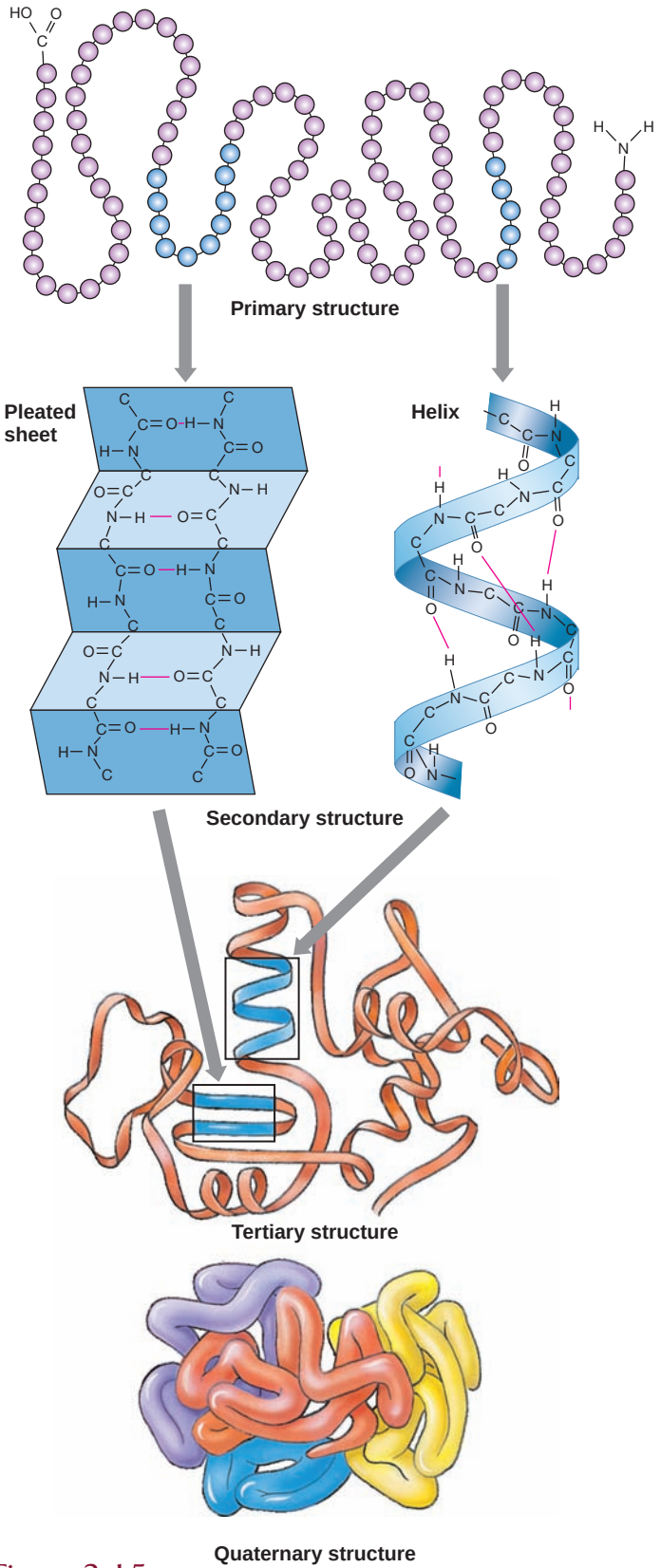


Figure 2.14

Five of the twenty kinds of amino acids.

recognize four levels of protein organization called primary, secondary, tertiary, and quaternary structures.

The **primary structure** of a protein is the sequence of amino acids composing the polypeptide chain. Because bonds between the amino acids in the chain can form only a limited number of stable angles, certain recurring structural patterns are assumed by the chain. These bond angles generate the **secondary structure,** such as the **alpha-helix,** which makes helical turns in a clockwise direction like a screw (Figure 2.15). The spirals of the chains are stabilized by hydrogen bonds, usually between a hydrogen atom of one amino acid and the peptide-bond oxygen of another amino acid from an adjacent turn of the helix. The helical and other configurations formed by the polypeptide chain bend and fold, giving the protein its complex, yet stable, three-dimensional **tertiary structure** (Figure 2.15). The folded chains are stabilized by chemical bonds between pairs of amino acids from different parts of the polypeptide chain. These bonds form between "side groups," parts of the amino acid not involved in a peptide bond. An example is the **disulfide bond,** a covalent bond between the sulfur atoms in two cysteine amino acids that are brought together by folds in the polypeptide chain. Also stabilizing the tertiary structure of proteins are hydrogen bonds, ionic bonds, and hydrophobic bonds.



The term **quaternary structure** describes proteins that contain more than one polypeptide chain. For example, hemoglobin (the oxygen-carrying substance in blood) of higher vertebrates is composed of four polypeptide subunits held together in a single protein molecule (Figure 2.15).

Proteins perform many functions in living organisms. They form the structural framework of protoplasm and many cellular components. Many proteins function as **enzymes,** the biological catalysts required for almost every reaction in the body. Enzymes lower the activation energy required for specific reactions and enable life processes to proceed at moderate temperatures rather than requiring high temperatures. Enzymes control the reactions by which food is digested, absorbed, and metabolized. They promote the synthesis of structural materials for growth and to replace those lost by wear. They determine the release of energy used in respiration, growth, muscle contraction, physical and mental activities, and many other activities. Enzyme action is described in Chapter 4 (p. 60).

A **prion** is an infectious protein particle in which a protein of the host organism is contorted into an abnormal three-dimensional structure. Upon infection, the prion causes its host's normal copies of the protein to be refolded into the abnormal form, with pathological results. In "mad cow disease," a prion infection severely damages brain tissues and is fatal. Fatal neurological diseases associated with transmissible prions occur also in people (for example, kuru), and in sheep and goats (scrapie).

Nucleic Acids

Nucleic acids are complex polymeric molecules whose sequence of nitrogenous bases encodes the genetic information necessary for biological inheritance. They store directions for the synthesis of enzymes and other proteins, and are the only molecules that can (with the help of the right enzymes) replicate themselves. The two kinds of nucleic acids in cells are **deoxyribonucleic acid (DNA)** and **ribonucleic acid (RNA).** They are polymers of repeated units called **nucleotides,** each of which contains a sugar, a nitrogenous base, and a phosphate group. In addition to their role in nucleic acids, nucleotides have an important role as transporters of chemical energy in cellular metabolism (p. 62). Because the structure of nucleic acids is crucial to the mechanism of inheritance and protein synthesis, detailed information on nucleic acids is presented in Chapter 5 (p. 91).

CHEMICAL EVOLUTION

Both Haldane and Oparin proposed that earth's primitive atmosphere consisted of simple compounds such as water, molecular hydrogen, methane, and ammonia, but lacked oxygen gas

Figure 2.15

Structure of proteins. The amino acid sequence of a protein (*primary structure*) encourages the formation of hydrogen bonds between nearby amino acids, producing coils and foldbacks (the *secondary structure*). Bends and helices cause the chain to fold back on itself in a complex manner (*tertiary structure*). Individual polypeptide chains of some proteins aggregate to form a functional molecule composed of several subunits (*quaternary structure*).

(O_2, also called "molecular oxygen"). The nature of the primeval atmosphere is critical for understanding life's origin. The organic compounds that compose living organisms are neither synthesized outside cells nor stable in the presence of molecular oxygen, which is abundant in the atmosphere today. The best evidence indicates, however, that the primitive atmosphere contained not more than a trace of molecular oxygen. The primeval atmosphere therefore was a reducing one, consisting primarily of molecules in which hydrogen exceeds oxygen; methane (CH_4) and ammonia (NH_3), for example, constitute fully reduced compounds. Such compounds are called "reducing" because they tend to donate electrons to other compounds, thereby "reducing" those compounds (p. 64). During this time, the earth was bombarded by large (100 km diameter) comets and meteorites, generating heat that repeatedly vaporized its oceans.

This reducing atmosphere was conducive to the prebiotic synthesis that led to life's beginnings, although totally unsuited for the organisms alive today. Haldane and Oparin proposed that ultraviolet radiation of such a gas mixture caused many organic substances, such as sugars and amino acids, to form. Haldane proposed that the early organic molecules accumulated in the primitive oceans to form a "hot dilute soup." In this primordial broth, carbohydrates, fats, proteins, and nucleic acids could have assembled to form the earliest structures capable of guiding their own replication.

If the simple gaseous compounds present in the early atmosphere are mixed with methane and ammonia in a closed glass system and kept at room temperature, they never react chemically with each other. To produce a chemical reaction, a continuous source of **free energy** sufficient to overcome reaction-activation barriers must be supplied. Ultraviolet light from the sun must have been intense on earth before the accumulation of atmospheric oxygen; ozone, a three-atom form of oxygen located high in the atmosphere, now blocks much of the ultraviolet radiation from reaching the earth's surface. Electrical discharges could have provided further energy for chemical evolution. Although the total amount of electrical energy released by lightning is small compared with solar energy, nearly all of the energy of lightning is effective in synthesizing organic compounds in a reducing atmosphere. A single flash of lightning through a reducing atmosphere generates a large amount of organic matter. Thunderstorms may have been one of the most important sources of energy for organic synthesis.

Widespread volcanic activity is another possible source of energy. One hypothesis maintains, for example, that life did not originate on the surface of the earth, but deep beneath the sea in or around **hydrothermal vents** (p. 816). Hydrothermal vents are submarine hot springs; seawater seeps through cracks in the seafloor until the water comes close to hot magma. The water is then superheated and expelled forcibly, carrying various dissolved molecules from the superheated rocks. These molecules include hydrogen sulfide, methane, iron ions, and sulfide ions. Hydrothermal vents have been discovered in several locations beneath the deep sea, and they would have been much more widely prevalent on the early earth. Interestingly, many heat- and sulfur-loving bacteria grow in hot springs today.

Prebiotic Synthesis of Small Organic Molecules

The Oparin-Haldane hypothesis stimulated experimental work to test the hypothesis that organic compounds characteristic of life could be formed from the simpler molecules present in the prebiotic environment. In 1953, Stanley Miller and Harold Urey in Chicago successfully simulated the conditions thought to prevail on the primitive earth. Miller built an apparatus designed to circulate a mixture of methane, hydrogen, ammonia, and water past an electric spark (Figure 2.16). Water in the flask was boiled to produce steam that helped to circulate the gases. The products formed in the electrical discharge (representing lightning) were condensed in the condenser and collected in the U-tube and small flask (representing an ocean).

After a week of continuous sparking, approximately 15% of the carbon from the reducing "atmosphere" had been converted into organic compounds that collected in the "ocean." The most

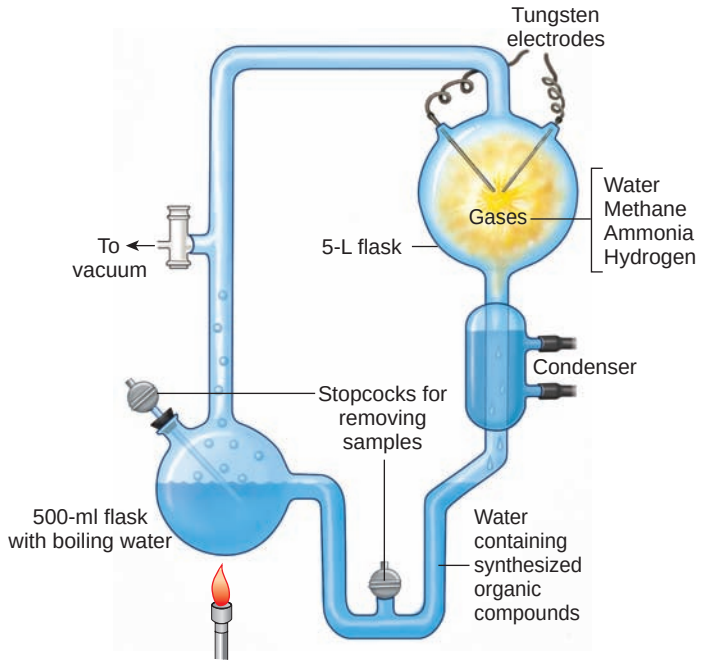


Figure 2.16

Dr. S. L. Miller with a replica of the apparatus used in his 1953 experiment on the synthesis of amino acids with an electric spark in a strongly reducing atmosphere.

striking finding was that many compounds related to life were synthesized. These compounds included four of the amino acids commonly found in proteins, urea, and several simple fatty acids. We can appreciate the astonishing nature of this synthesis when we consider that there are thousands of known organic compounds with structures no more complex than those of the amino acids formed. Yet in Miller's synthesis, most of the relatively few substances formed were compounds found in living organisms. This result was surely no coincidence, and it suggests that prebiotic synthesis on the primitive earth may have occurred under conditions not greatly different from those that Miller simulated.

Miller's experiments have been criticized in light of current opinion that the early atmosphere on earth was quite different from Miller's strongly reducing simulated atmosphere. Nevertheless, Miller's work stimulated many other investigators to repeat and to extend his experiment. Amino acids were found to be synthesized in many different kinds of gas mixtures that were heated (volcanic heat), irradiated with ultraviolet light (solar radiation), or subjected to electrical discharge (lightning). The only conditions required to produce amino acids were that the gas mixture be reducing and that it be subjected violently to a source of energy. In other experiments, electrical discharges were passed through mixtures of carbon monoxide, nitrogen, and water, yielding amino acids and nitrogenous bases. Although reaction rates were much slower than in atmospheres containing methane and ammonia, and yields were poor in comparison, these experiments support the hypothesis that the chemical beginnings of life can occur in atmospheres that are only mildly reducing. The need for methane and ammonia, however, led to proposals that these substances might have been introduced by comets or meteorites, or that they were synthesized near the hydrothermal vents.

Thus the experiments of many scientists have shown that highly reactive intermediate molecules such as hydrogen cyanide, formaldehyde, and cyanoacetylene are formed when a reducing mixture of gases is subjected to a violent energy source. These molecules react with water and ammonia or nitrogen to form more complex organic molecules, including amino acids, fatty acids, urea, aldehydes, sugars, and nitrogenous bases (purines and pyrimidines), all of the building blocks required for the synthesis of the most complex organic compounds of living matter. Further evidence for the natural abiotic synthesis of amino acids comes from finding amino acids in meteorites, such as the Murchison meteorite that landed in Australia in 1969.

Formation of Polymers

The next stage in chemical evolution involved the joining of amino acids, nitrogenous bases, and sugars to yield larger molecules, such as proteins and nucleic acids. Such synthesis does not occur easily in dilute solutions, because excess water drives reactions toward decomposition (hydrolysis). Although the primitive ocean might have been called a "primordial soup," it was probably a rather dilute one containing organic material that was approximately one-tenth to one-third as concentrated as chicken bouillon.

Need for Concentration

Prebiotic synthesis must have occurred in restricted areas where concentrations of the reactants were high. Violent weather on the primitive earth would have created enormous dust storms; impacts of meteorites would have lofted great amounts of dust into the atmosphere. The dust particles could have become foci of water droplets. Salt concentration in the particles could have been high and provided a concentrated medium for chemical reactions. Alternatively, perhaps the surface of the earth was too warm to have oceans but not too hot for a damp surface. This condition would have resulted from constant rain and rapid evaporation. Thus, the earth's surface could have become coated with organic molecules, an "incredible scum." Prebiotic molecules might have been concentrated by adsorption on the surface of clay and other minerals. Clay can concentrate and condense large amounts of organic molecules. The surface of iron pyrite (FeS_2) also has been suggested as a site for the evolution of biochemical pathways. The positively charged surface of pyrite would attract a variety of negative ions, which would bind to its surface. Furthermore, pyrite is abundant around hydrothermal vents, compatible with the hydrothermal-vent hypothesis.

Thermal Condensations

Most biological polymerizations are condensation (dehydration) reactions, in which monomers are linked together by the removal of water (p. 23). In living systems, condensation reactions always occur in an aqueous (cellular) environment containing appropriate enzymes. Without enzymes and energy supplied by ATP, macromolecules (proteins and nucleic acids) of living systems soon decompose into their constituent monomers.

Dehydration reactions could have occurred without enzymes in primitive earth conditions by thermal condensation. The simplest dehydration is accomplished by driving water from solids by direct heating. For example, heating a mixture of all 20 amino acids to 180° C produces a good yield of polypeptides.

The thermal synthesis of polypeptides to form "proteinoids" has been studied extensively by the American scientist Sidney Fox. He showed that heating dry mixtures of amino acids and then mixing the resulting polymers with water forms small spherical bodies. These proteinoid microspheres (Figure 2.17) possess certain characteristics of living systems. Each is not more than 2 μm in diameter, comparable in size and shape to spherical bacteria. The outer walls of the microspheres appear to have a double layer, and they show osmotic properties and selective diffusion. They may grow by accretion or proliferate by budding like bacteria. Proteinoids might have been used to assemble the first cells from macromolecular precursors. Formation of these polymers requires conditions likely to have occurred only in volcanoes. Organic polymers might have condensed on or in volcanoes and then, wetted by rain or dew, reacted further in solution to form polypeptides or polynucleotides.

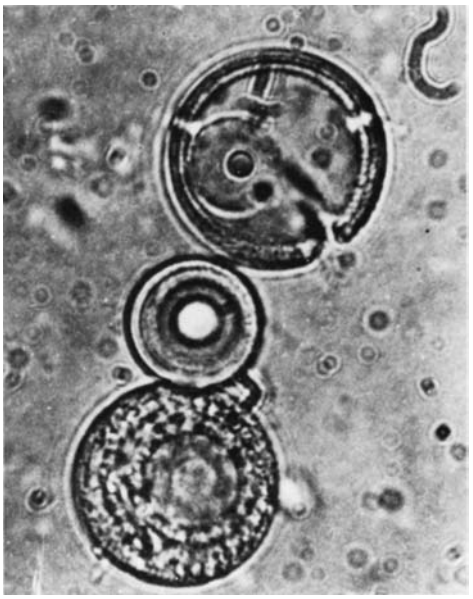


Figure 2.17

Electron micrograph of proteinoid microspheres. These proteinlike bodies can be produced in the laboratory from polyamino acids and may represent precellular forms. They have definite internal ultrastructure. (×1700)

ORIGIN OF LIVING SYSTEMS

The fossil record reveals that life existed 3.8 billion years ago; therefore, the origin of the earliest form of life can be estimated at approximately 4 billion years BP. The first living organisms were protocells, autonomous membrane-bound units with a complex functional organization that permitted the essential activity of self-reproduction. The primitive chemical systems that we have described lack this essential property. The principal problem in understanding the origin of life is explaining how primitive chemical systems could have become organized into living, autonomous, self-reproducing cells.

As we have seen, a lengthy chemical evolution on the primitive earth produced several molecular components of living forms. In a later stage of evolution, nucleic acids (DNA and RNA) began to behave as simple genetic systems that directed the synthesis of proteins, especially enzymes. However, this conclusion leads to a troublesome chicken-egg paradox: (1) How could nucleic acids have appeared without the enzymes that catalyze their synthesis? (2) How could enzymes have evolved without nucleic acids to encode their amino acid sequence? These questions come from a long-accepted notion that only proteins could act as enzymes. Startling evidence presented in the 1980s indicates that RNA in some instances has catalytic activity.

Catalytic RNA (ribozymes) can mediate processing of messenger RNA (removal of introns, p. 95), and can catalyze formation of peptide bonds. Strong evidence suggests that translation of mRNA by ribosomes (p. 95) is catalyzed by their RNA, not protein, content.

Therefore the earliest enzymes could have been RNA, and the earliest self-replicating molecules could have been RNA. Investigators are now calling this stage the "RNA world." Nonetheless, proteins have several important advantages over RNA as catalysts, and DNA is a more stable carrier of genetic information than RNA. The first protocells containing protein enzymes and DNA should have been selectively favored over those with only RNA.

Once this protocellular stage of organization was reached, natural selection (pp. 124–126) would have acted on these primitive self-replicating systems. This stage was critical. Before this stage, biogenesis was shaped by the favorable environmental conditions on the primitive earth and by the nature of the reacting elements themselves. When self-replicating systems became responsive to natural selection, they began to evolve. The more rapidly replicating and more successful systems were favored, thereby gradually evolving efficient replicators. Evolution of the genetic code and fully directed protein synthesis followed. The system now meets the requirements for being the common ancestor of all living organisms.

Origin of Metabolism

Living cells today are organized systems with complex and highly ordered sequences of enzyme-mediated reactions. How did such vastly complex metabolic schemes develop? The exact history of this phase of life's evolution is unknown. We present here a model of the simplest sequence of events that could explain the origin of observed metabolic properties of living systems.

Organisms that can synthesize their food from inorganic sources using light or another source of energy are called **autotrophs** (Gr. *autos,* self, + *trophos,* feeder) (Figure 2.18). Organisms

Figure 2.18

Koala, a heterotroph, feeding on a eucalyptus tree, an autotroph. All heterotrophs depend for their nutrients directly or indirectly on autotrophs, which capture the sun's energy to synthesize their own nutrients.

lacking this ability must obtain their food supplies directly from the environment and are called **heterotrophs** (Gr. *heteros,* another, + *trophos,* feeder). The earliest postulated microorganisms are sometimes called **primary heterotrophs** because they relied on environmental sources for their food and existed prior to the evolution of any autotrophs. They were probably anaerobic organisms similar to bacteria of the genus *Clostridium*. Because chemical evolution had supplied generous stores of organic nutrients in the prebiotic soup, the earliest organisms would not have been required to synthesize their own food.

Autotrophs would have had a tremendous selective advantage over the primary heterotrophs in areas where organic nutrients became depleted. Evolution of autotrophic organisms most likely required acquisition of enzymatic activities to catalyze conversion of inorganic molecules to more complex ones, such as carbohydrates. The numerous enzymes of cellular metabolism appeared when cells started to utilize proteins for catalytic functions.

Carl Woese challenges the traditional view that the first organisms were primary heterotrophs. He finds it easier to visualize membrane -associated molecular aggregates that absorbed visible light and converted it with some efficiency into chemical energy. Thus the first organisms would have been autotrophs. Woese also suggests that the earliest "metabolism" comprised numerous chemical reactions catalyzed by nonprotein cofactors (substances necessary for the function of many of the protein enzymes in living cells). These cofactors would have been associated with membranes.

Appearance of Photosynthesis and Oxidative Metabolism

Autotrophy evolved in the form of photosynthesis. In photosynthesis, hydrogen atoms obtained from water react with carbon dioxide obtained from the atmosphere to generate sugars and molecular oxygen. Energy is stored in the form of covalent bonds between carbon atoms in the sugar molecule. Sugars provide nutrition to the organism and molecular oxygen is released into the atmosphere.

$$6CO_2 + 6H_2O \xrightarrow[\text{light}]{} C_6H_{12}O_6 + 6O_2$$

This equation summarizes the many reactions now known to occur in photosynthesis. Undoubtedly these reactions did not appear simultaneously, and other reduced compounds, such as hydrogen sulfide (H_2S), probably were the early sources of hydrogen.

Gradually, oxygen produced by photosynthesis accumulated in the atmosphere. When atmospheric oxygen reached approximately 1% of its current level, ozone began to accumulate and to absorb ultraviolet radiation, thereby greatly restricting the amount of ultraviolet light that reached the earth. Land and surface waters then were occupied by photosynthetic organisms, thereby increasing oxygen production.

Accumulation of atmospheric oxygen would interfere with anaerobic cellular metabolism that had evolved in the primitive reducing atmosphere. As the atmosphere slowly accumulated oxygen gas (O_2), a new and highly efficient kind of metabolism appeared: **oxidative (aerobic) metabolism.** By using available oxygen as a terminal electron acceptor (p. 68) and completely oxidizing glucose to carbon dioxide and water, much of the bond energy stored by photosynthesis could be recovered. Most living forms became completely dependent upon oxidative metabolism.

Our atmosphere today is strongly oxidizing. It contains 78% molecular nitrogen, approximately 21% free oxygen, 1% argon, and 0.03% carbon dioxide. Although the time course for production of atmospheric oxygen is much debated, the most important source of oxygen is photosynthesis. Almost all oxygen currently produced comes from cyanobacteria (blue-green algae), eukaryotic algae, and plants. Each day these organisms combine approximately 400 million tons of carbon dioxide with 70 million tons of hydrogen to produce 1.1 billion tons of oxygen. Oceans are a major source of oxygen. Almost all oxygen produced today is consumed by organisms for respiration; otherwise, the amount of oxygen in the atmosphere would double in approximately 3000 years. Because Precambrian fossil cyanobacteria resemble modern cyanobacteria, it is reasonable to suppose that oxygen entering the early atmosphere came from their photosynthesis.

PRECAMBRIAN LIFE

The Precambrian period covers the geological time before the beginning of the Cambrian period some 570 to 600 million years BP. Most major animal phyla appear in the fossil record within a few million years at the beginning of the Cambrian period. This appearance has been called the "Cambrian explosion" because before this time, fossil deposits are mostly devoid of any organisms more complex than single-celled bacteria. Comparative molecular studies (p. 206) now suggest that the rarity of Precambrian fossils may represent poor fossilization rather than absence of animal diversity from the Precambrian period. Nonetheless, animals make a relatively late appearance in the history of life on earth. What were the early forms of life that generated both the oxidizing atmosphere critical for animal evolution and the evolutionary lineage from which animals would arise?

Prokaryotes and the Age of Cyanobacteria (Blue-Green Algae)

The earliest bacterium-like organisms proliferated, giving rise to a great variety of forms, some of which were capable of photosynthesis. From these arose the oxygen-producing **cyanobacteria** approximately 3 billion years ago.

Bacteria are called **prokaryotes,** meaning literally "before the nucleus." They contain a single, large molecule of DNA not located in a membrane-bound nucleus, but found in a nuclear region, or **nucleoid.** The DNA is not complexed with histone proteins, and prokaryotes lack membranous organelles such as mitochondria, plastids, Golgi apparatus, and endoplasmic reticulum (see Chapter 3). During cell division, the nucleoid divides and replicates of the cell's DNA are distributed to the daughter cells. Prokaryotes lack the chromosomal organization and chromosomal (mitotic) division seen in animals, fungi, and plants.

The name "algae" is misleading because it suggests a relationship to eukaryotic algae, and many scientists prefer the alternative name "cyanobacteria" rather than "blue-green algae." These organisms were responsible for producing an oxygen-rich atmosphere that replaced earth's primitive reducing atmosphere. Studies of biochemical reactions in extant cyanobacteria suggest that they evolved in a time of fluctuating oxygen concentration. For example, although they can tolerate atmospheric concentrations of oxygen (21%), the optimum concentration for many of their metabolic reactions is only 10%.

Bacteria and especially cyanobacteria ruled earth's oceans unchallenged for 1 to 2 billion years. The cyanobacteria reached the zenith of their success approximately 1 billion years BP, when filamentous forms produced great floating mats on the oceans' surfaces. This long period of cyanobacterial dominance, encompassing approximately two-thirds of the history of life, has been called with justification the "age of blue-green algae." Bacteria and cyanobacteria are so completely different from forms of life that evolved later that they were placed in a separate taxonomic kingdom, Monera.

Carl Woese and his colleagues at the University of Illinois discovered that the prokaryotes actually comprise at least two distinct lines of descent: the Eubacteria ("true" bacteria) and the Archaebacteria also called Archaea (p. 213). Although these two groups of bacteria look very much alike when viewed with the electron microscope, they are biochemically distinct. Archaebacteria differ fundamentally from bacteria in cellular metabolism, and their cell walls lack muramic acid, which is present in the cell walls of all Eubacteria. The most compelling evidence for differentiating these two groups comes from the use of one of the newest and most powerful tools at the disposal of the evolutionist, sequencing of nucleic acids (see note). Woese found that Archaebacteria differ fundamentally from other bacteria in the sequence of bases in ribosomal RNA (p. 95). Woese considers the Archaebacteria so distinct from the true bacteria that they should be considered a separate taxonomic kingdom, Archaea. The Monera then comprise only the true bacteria (see pp. 212–213 for further discussion and criticism of this taxonomy).

Appearance of Eukaryotes

Eukaryotes ("true nucleus"; Figure 2.19) have cells with membrane-bound nuclei containing **chromosomes** composed of **chromatin.** Constituents of eukaryotic chromatin include proteins called **histones** and RNA, in addition to DNA. Some nonhistone proteins are found associated with both prokaryotic DNA and eukaryotic chromosomes. Eukaryotes are generally larger than prokaryotes and contain much more DNA. Cellular division usually is by some form of mitosis. Within their cells are numerous membranous organelles, including mitochondria, in which the enzymes for oxidative metabolism are packaged. Eukaryotes include animals, fungi, plants, and numerous single-celled forms formerly called "protozoans" or "protists." Fossil evidence suggests that single-celled eukaryotes arose at least 1.5 billion years ago (Figure 2.20).

Molecular sequencing has emerged as a very successful approach to unraveling the ancient genealogies of living forms. The sequences of nucleotides in the DNA of an organism's genes are a record of evolutionary relationships, because every gene that exists today is an evolved copy of a gene that existed millions or even billions of years ago. Genes become altered by mutations through the course of time, but vestiges of the original gene usually persist. With modern techniques, one can determine the sequence of nucleotides in an entire molecule of DNA or in short segments of the molecule. When corresponding genes are compared between two different organisms, the extent to which the genes differ can be correlated with the time elapsed since the two organisms diverged from a common ancestor. Similar comparisons are made with RNA and proteins. These methods also permit scientists to synthesize long-extinct genes and proteins and to measure biochemical properties of the extinct proteins.

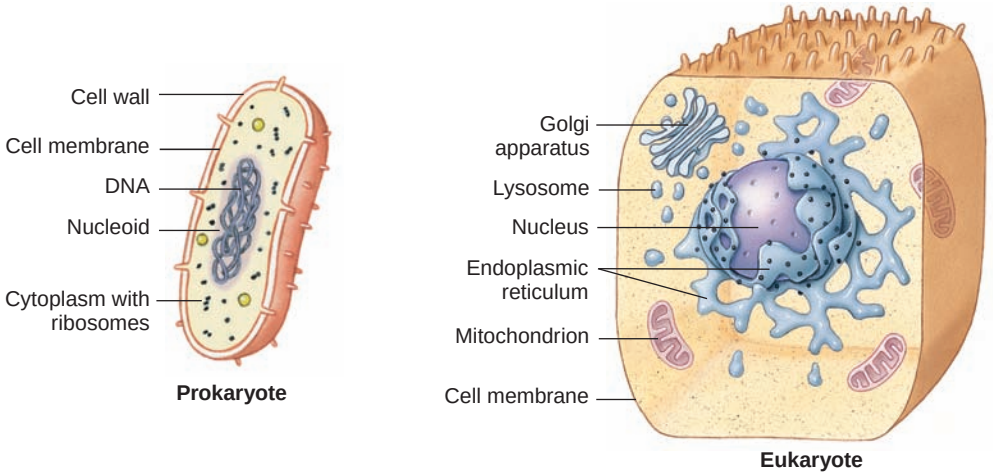


Figure 2.19
Comparison of prokaryotic and eukaryotic cells. Prokaryotic cells are about one-tenth the size of eukaryotic cells.

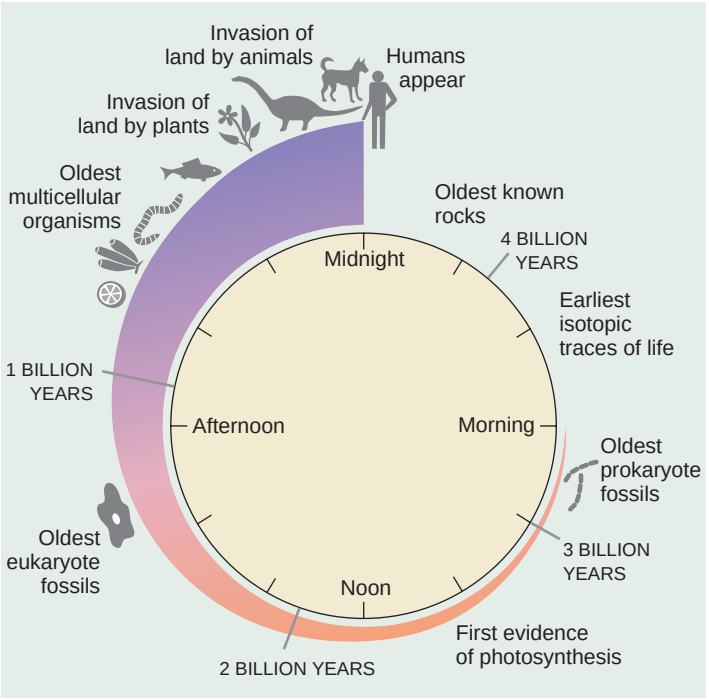


Figure 2.20

The clock of biological time. A billion seconds ago it was 1961, and most students using this text had not yet been born. A billion minutes ago the Roman empire was at its zenith. A billion hours ago Neanderthals were alive. A billion days ago the first bipedal hominids walked the earth. A billion months ago the dinosaurs were at the climax of their radiation. A billion years ago no animal had ever walked on the surface of the earth.

Figure 2.21

Dr. Lynn Margulis, whose endosymbiotic theory of the origins of mitochondria and chloroplasts is strongly supported by molecular evolutionary studies.

Because the organizational complexity of eukaryotes is much greater than that of prokaryotes, it is difficult to visualize how a eukaryote could have arisen from any known prokaryote. The American biologist Lynn Margulis (Figure 2.21) and others have proposed that eukaryotes did not arise from any single prokaryote but were derived from a **symbiosis** ("life together") of two or more types of bacteria. Mitochondria and plastids (photosynthetic organelles found only in plant cells), for example, each contain their own DNA (apart from the nucleus of the cell), which has some prokaryotic characteristics.

Nuclei, plastids, and mitochondria each contain genes encoding ribosomal RNA. Comparisons of the sequences of bases of these genes show that the nuclear, plastid, and mitochondrial DNAs represent distinct evolutionary lineages. Plastid and mitochondrial DNAs are closer in their evolutionary history to bacterial DNAs than to the eukaryotic nuclear DNA. Plastids are closest evolutionarily to cyanobacteria, and mitochondria are closest to another group of bacteria (purple bacteria), consistent with the symbiotic hypothesis of eukaryotic origins. Mitochondria contain the enzymes of oxidative metabolism, and plastids (a plastid with chlorophyll is a chloroplast) conduct photosynthesis. It is easy to see how a host cell able to accommodate such guests in its cytoplasm would have gained enormous evolutionary success.

The endosymbiotic theory proposes that a population ancestral to eukaryotic cells, derived from and resembling **anaerobic** (lacking oxidative metabolism) bacteria, evolved a nucleus and other intracellular membranes (p. 41) from infoldings of the cell membrane. Cells of this population acquired, by ingestion or parasitism, aerobic bacteria that avoided digestion and came to reside in the host cell's cytoplasm (p. 41). The endosymbiotic aerobic bacteria would have metabolized oxygen, which is toxic for their anaerobic host, and the anaerobic host cell would have given its aerobic residents food and physical protection. This mutually beneficial relationship would produce selection for the host cells and their residents to evolve a means of making their relationship a permanent one. Among the evolutionary outcomes of this selection would be compactness of the endosymbiont and its loss of genes redundant with those of its host (or the reverse).

Data collected to test this proposed mechanism show that its conditions are reasonable ones. Fossil data show that both aerobic and anaerobic bacteria were well established by 2.5 billion years ago, and that cells containing nuclei and internal membranes first appeared at this time. Some anaerobic, nucleated forms that lack mitochondria are alive today, including the human parasite *Giardia intestinalis,* although these forms probably represent descendants of lineages that formerly had mitochondria and lost them rather than lineages whose ancestry never

featured mitochondria. Eukaryotic cells containing mitochondria are evident approximately 1.2 billion years ago. Bacteria have been introduced experimentally into single-celled eukaryotes and propagated as a symbiotic unit for many generations. Such experiments have shown further that the host cell can become dependent upon its resident bacteria for proteins whose functions formerly were performed by the host population prior to the experimental endosymbiosis.

In addition to claiming that mitochondria and plastids originated as bacterial symbionts, Lynn Margulis argues that eukaryote flagella, cilia (locomotory structures), and even the spindle of mitosis came from a kind of bacterium like a spirochete. Indeed, she suggests that this association (the spirochete with its new host cell) made evolution of mitosis possible. Margulis's evidence that organelles are former partners of an ancestral cell is now accepted by most biologists. Such merging of disparate organisms to produce evolutionarily novel forms is called symbiogenesis.

The first eukaryotes were undoubtedly unicellular, and many were photosynthetic autotrophs. Some of these forms lost their photosynthetic ability and became heterotrophs, feeding on eukaryotic autotrophs and prokaryotes. As cyanobacteria were cropped, their dense filamentous mats began to thin, providing space for other organisms. Carnivores appeared and fed on herbivores. Soon a balanced ecosystem of carnivores, herbivores, and primary producers appeared. By freeing space, cropping herbivores encouraged a greater diversity of producers, which in turn promoted evolution of new and more specialized croppers. An ecological pyramid developed with carnivores at the top of the food chain (p. 834).

The burst of evolutionary activity that followed at the end of the Precambrian period and beginning of the Cambrian period was unprecedented. Some investigators hypothesize that the explanation for the "Cambrian explosion" lies in the accumulation of oxygen in the atmosphere to a critical threshold level. Larger, multicellular animals required the increased efficiency of oxidative metabolism; these pathways could not be supported under conditions of limiting oxygen concentration.

SUMMARY

Living organisms show a remarkable uniformity in their chemical constituents and metabolism, reflecting their common descent from an ancient ancestor.

Life on earth could not have appeared without water, the primary component of living cells. The unique structure of water and its ability to form hydrogen bonds between adjacent water molecules are responsible for its special properties: solvency, high heat capacity, boiling point, surface tension, and lower density as a solid than as a liquid.

Life also depends critically on the chemistry of carbon. Carbon is especially versatile in bonding with itself and with other atoms, and it is the only element capable of forming the large molecules found in living organisms. Carbohydrates are composed primarily of carbon, hydrogen, and oxygen grouped as H—C—OH. The simplest carbohydrates are sugars, which serve as immediate sources of energy in living systems. Monosaccharides, or simple sugars, may bond together to form disaccharides or polysaccharides, which serve as storage forms of sugar or perform structural roles. Lipids constitute another class of large molecules featuring chains of carbon compounds; fats exist principally as neutral fats, phospholipids, and steroids. Proteins are large molecules composed of amino acids linked by peptide bonds. Many proteins function as enzymes that catalyze biological reactions. Each kind of protein has a characteristic primary, secondary, tertiary, and often, quaternary structure critical for its functioning. Nucleic acids are polymers of nucleotide units, each composed of a sugar, a nitrogenous base, and a phosphate group. They contain the material of inheritance and function in protein synthesis.

Experiments by Louis Pasteur in the 1860s convinced scientists that organisms do not arise repeatedly from inorganic matter. About 60 years later, A. I. Oparin and J. B. S. Haldane provided an explanation for how a common ancestor of all living forms could have arisen from nonliving matter almost 4 billion years ago. The origin of life followed a long period of "abiogenic molecular evolution" on earth in which organic molecules slowly accumulated in a "primordial soup." The atmosphere of the primitive earth was reducing, with little or no free oxygen present. Ultraviolet radiation, electrical discharges of lightning, or energy from hydrothermal vents could have provided energy for early formation of organic molecules. Stanley Miller and Harold Urey demonstrated the plausibility of the Oparin-Haldane hypothesis by simple but ingenious experiments. The concentration of reactants necessary for early synthesis of organic molecules might have been provided by damp surfaces, clay particles, iron pyrite, or other conditions. RNA might have been the primordial biomolecule, performing the functions of both genetic coding of information and catalysis. When self-replicating systems became established, evolution by natural selection could have increased their diversity and complexity.

The first organisms are hypothesized to have been primary heterotrophs, living on energy stored in molecules dissolved in a primordial soup. Later evolution produced autotrophic organisms, which can synthesize their own organic nutrients (carbohydrates) from inorganic materials. Autotrophs are better protected than heterotrophs from depletion of organic compounds from their environments. Molecular oxygen began to accumulate in the atmosphere as an end product of photosynthesis, an autotrophic process that produces sugars and oxygen by reacting water and carbon dioxide. Cyanobacteria appear to be primarily responsible for generation of atmospheric oxygen early in life's history.

All bacteria are prokaryotes, organisms that lack a membrane-bound nucleus and other organelles in their cytoplasm. The prokaryotes consist of two genetically distinct groups, Archaebacteria and Monera.

The eukaryotes apparently arose from symbiotic unions of two or more types of prokaryotes. The genetic material (DNA) of eukaryotes is borne in a membrane-bound nucleus, and also in mitochondria and sometimes plastids. Mitochondria and plastids have resemblances to bacteria, and their DNA is more closely allied to that of certain bacteria than to eukaryotic nuclear genomes.

REVIEW QUESTIONS

1. Explain each of these properties of water, and describe how each is conferred by the dipolar nature of a water molecule: high specific heat capacity; high heat of vaporization; unique density behavior; high surface tension; good solvent for ions of salts.
2. What was the composition of the earth's atmosphere at the time of the origin of life, and how did it differ from the atmosphere of today?
3. Regarding the experiments of Miller and Urey described in this chapter, explain what constituted the following in each case: observations, hypothesis, deduction, prediction, data, control. (The scientific method is described on pp. 11–14.)
4. Explain the significance of the Miller-Urey experiments.
5. Name three different sources of energy that could have powered reactions on early earth to form organic compounds.
6. By what mechanism might organic molecules have been concentrated in a prebiotic world so that further reactions could occur?
7. Name two simple carbohydrates, two storage carbohydrates, and a structural carbohydrate.
8. What characteristic differences in molecular structure distinguish lipids and carbohydrates?
9. Explain the difference between the primary, secondary, tertiary, and quaternary structures of a protein.
10. What are the important nucleic acids in a cell, and of what units are they constructed?
11. Distinguish among the following: primary heterotroph, autotroph, secondary heterotroph.
12. What is the source of oxygen in the present-day atmosphere, and what is its metabolic significance to most organisms living today?
13. Distinguish prokaryotes from eukaryotes.
14. Describe Margulis's view on the origin of eukaryotes from prokaryotes.
15. What was the "Cambrian explosion" and how might you explain it?

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CHAPTER

3

Cells as Units of Life

A humpback whale, Megaptera novaeangliae, *leaps from the water.*

The Fabric of Life

It is a remarkable fact that living forms, from amebas and unicellular algae to whales and giant redwood trees, are formed from a single type of building unit: cells. All animals and plants are composed of cells and cell products. Thus the cell theory is another great unifying concept of biology.

New cells come from division of preexisting cells, and the activity of a multicellular organism as a whole is the sum of activities and interactions of its constituent cells. Energy is required to support life's activities, and virtually all of it flows from sunlight that is captured by green plants and algae and transformed by photosynthesis into chemical bond energy. Chemical bond energy is a form of potential energy that can be released when the bond is broken; the energy is used to perform electrical, mechanical, and osmotic tasks in the cell. Ultimately, all energy is dissipated into heat. This is in accord with the second law of thermodynamics, which states that there is a tendency in nature to proceed toward a state of greater molecular disorder, or entropy. Thus the high degree of molecular organization in living cells is attained and maintained only as long as energy fuels the organization.

CELL CONCEPT

More than 300 years ago the English scientist and inventor Robert Hooke, using a primitive compound microscope, observed boxlike cavities in slices of cork and leaves. He called these compartments "little boxes or cells." In the years that followed Hooke's first demonstration of the remarkable powers of the microscope to the Royal Society of London in 1663, biologists gradually began to realize that cells were far more than simple containers filled with "juices."

Cells are the fabric of life (Figure 3.1). Even the most primitive cells are enormously complex structures that form the basic units of all living organisms. All tissues and organs are composed of cells. In a human an estimated 60 trillion cells interact, each performing its specialized role in an organized partnership. In single-celled organisms all functions of life are performed within the confines of one microscopic package. There is no life without cells. The idea that a cell represents the basic structural and functional unit of life is an important unifying concept of biology.

With the exception of some eggs, which are the largest cells (in volume) known, cells are small and mostly invisible to the unaided eye. Consequently, our understanding of cells paralleled technical advances in the resolving power of microscopes. The Dutch microscopist Antoni van Leeuwenhoek sent letters to the Royal Society of London containing detailed descriptions of the numerous organisms he had observed using high-quality single lenses that he had made (1673 to 1723). In the early nineteenth century, improved design of microscopes permitted biologists to see separate objects only 1 µm apart. This advance was quickly followed by new discoveries that laid the groundwork for the **cell theory**—a theory stating that all living organisms are composed of cells.

In 1838 Matthias Schleiden, a German botanist, announced that all plant tissue was composed of cells. A year later one of his countrymen, Theodor Schwann, described animal cells as being similar to plant cells, an understanding that had been long delayed because animal cells are bounded only by a nearly invisible plasma membrane rather than a distinct cell wall characteristic

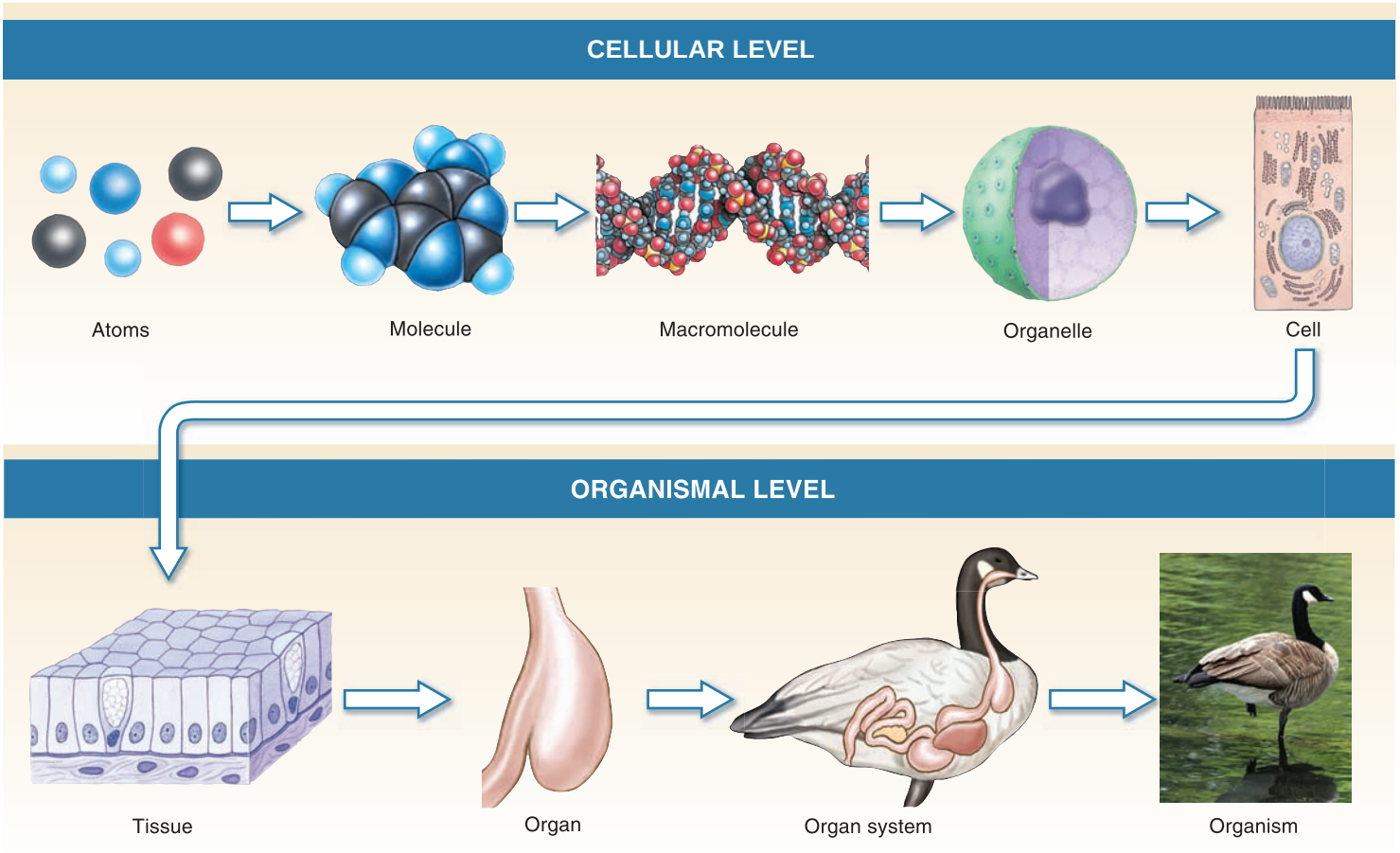


Figure 3.1
Biological organization from simple atoms to complex organisms. Atoms from molecules and macromolecules are assembled into organelles within each cell. Cells are grouped into tissues, organs, and organ systems to form a complex multicellular organism.

of plant cells. Schleiden and Schwann are thus credited with the unifying cell theory that ushered in a new era of productive exploration in cell biology. Another German, Rudolf Virchow, recognized that all cells came from preexisting cells (1858).

In 1840 J. Purkinje introduced the term **protoplasm** to describe cell contents. Protoplasm was at first thought to be a granular, gel-like mixture with special and elusive life properties of its own; cells were viewed as bags of thick soup containing a nucleus. Later the interior of cells became increasingly visible as microscopes were improved and better tissue-sectioning and staining techniques were introduced. Rather than being a uniform granular soup, a cell's interior is composed of numerous **cellular organelles,** each performing a specific function in the life of a cell. Today we realize that the components of a cell are so highly organized, structurally and functionally, that describing its contents as "protoplasm" is like describing the contents of an automobile engine as "autoplasm."

How Cells Are Studied

Light microscopes, with all their variations and modifications, have contributed more to biological investigation than any other instrument. They have been powerful exploratory tools for 300 years, and they continue to be so more than 50 years after invention of the electron microscope. However, electron microscopy has vastly enhanced our appreciation of the delicate internal organization of cells, and modern biochemical, immunological, physical, and molecular techniques have contributed enormously to our understanding of cell structure and function.

Electron microscopes employ high voltages to direct a beam of electrons through or at the surface of objects examined. The wavelength of the electron beam is approximately 0.00001 that of ordinary white light, thus permitting far greater magnification and resolution.

In preparation for viewing using the transmission electron microscope, specimens are cut into extremely thin sections (10 nm to 100 nm thick) and treated with "electron stains" (ions of elements such as osmium, lead, and uranium) to increase contrast between different structures. Electrons pass through a specimen, and images are seen on a fluorescent screen and photographed (Figure 3.2).

In contrast, specimens prepared for scanning electron microscopy are not sectioned, and electrons do not pass through them. The whole specimen is coated with an electron-dense material and bombarded with electrons, causing some electrons to be reflected back and secondary electrons to be emitted. An apparent three-dimensional image is recorded in the photograph. Although the magnification capability of scanning instruments is not as great as transmission microscopes, much has been learned about surface features of organisms and cells, as well as internal, membrane-bound structures. Examples of scanning electron micrographs are shown on pp. 145, 163, and 686.

A still greater level of resolution can be achieved with X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy. These techniques reveal the shapes of biomolecules and the relationships among atoms within them. Both techniques are laborious, but NMR spectroscopy does not require purification and crystallization of a substance, and molecules can be observed in solution.

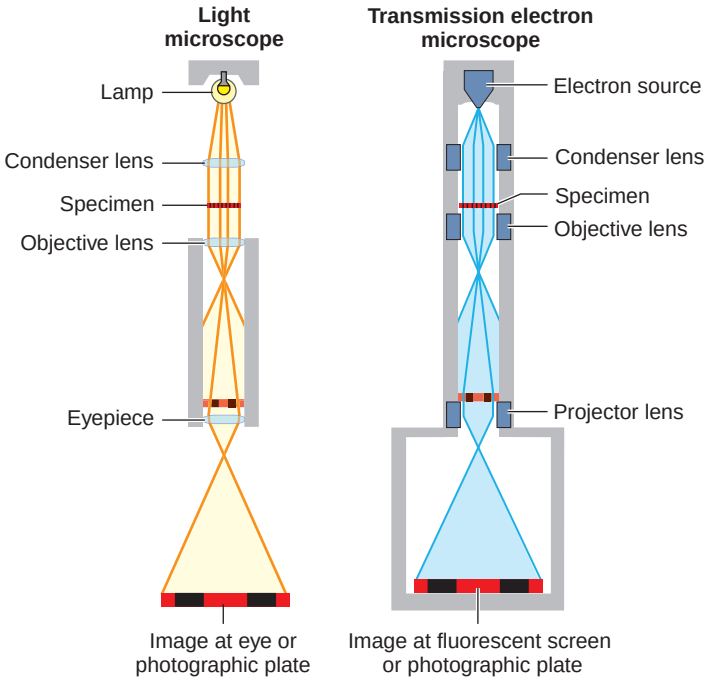


Figure 3.2

Comparison of optical paths of light and transmission electron microscopes. To facilitate comparison, the scheme of the light microscope has been inverted from its usual orientation with light source below and image above. In an electron microscope the lenses are magnets to focus the beam of electrons.

Advances in techniques of cell study (cytology) are not limited to improvements in microscopes but include new methods of tissue preparation, staining for microscopic study, and the great contributions of modern biochemistry and molecular biology. For example, the various organelles of cells have differing, characteristic densities. Cells can be disrupted with most of the organelles remaining intact, then centrifuged in a density gradient (Figure 3.3), and relatively pure preparations of each organelle may be recovered. Thus the biochemical functions of various organelles may be studied separately. DNA and various types of RNA can be extracted and studied. Many enzymes can be purified and their characteristics determined. We use radioactive isotopes to study many metabolic reactions and pathways in cells. Modern chromatographic techniques can separate chemically similar intermediates and products. A particular cellular protein can be extracted and purified, and specific antibodies (see p. 775) can be prepared against the protein. When the antibody is complexed with a fluorescent substance and the complex is used to "stain" cells, the complex binds to the protein of interest, and its precise location in cells can be determined.

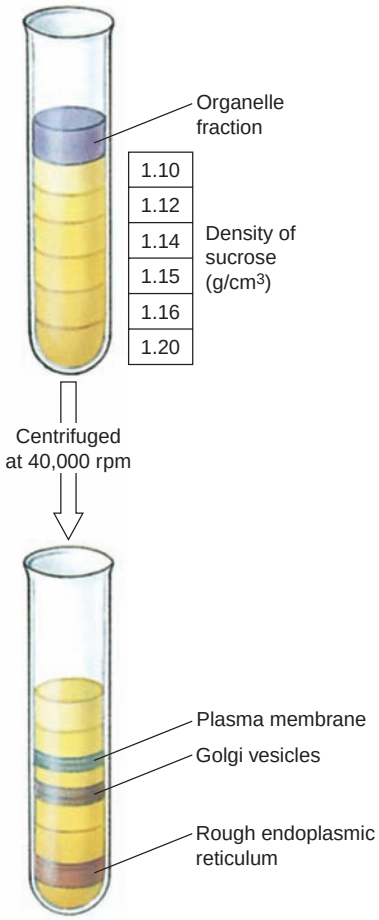


Figure 3.3

Separation of cell organelles in a density gradient by ultracentrifugation. The gradient is formed by layering sucrose solutions in a centrifuge tube, then carefully placing a preparation of mixed organelles on top. The tube is centrifuged at about 40,000 revolutions per minute for several hours, and organelles become separated down the tube according to their density.

ORGANIZATION OF CELLS

If we were to restrict our study of cells to fixed and sectioned tissues, we would be left with the erroneous impression that cells are static, quiescent, rigid structures. In fact, a cell's interior is in a constant state of upheaval. Most cells are continually changing shape; their organelles twist and regroup in a cytoplasm teeming with starch granules, fat droplets, and vesicles of various sorts. This description is derived from studies of living cell cultures with time-lapse photography and video. If we could see the swift shuttling of molecular traffic through gates in the cell membrane and the metabolic energy transformations within cell organelles, we would have an even stronger impression of internal turmoil. However, cells are anything but bundles of disorganized activity. There is order and harmony in cell functioning. Studying this dynamic phenomenon through a microscope, we realize that, as we gradually comprehend more and more about these units of life, we are gaining a greater understanding of the nature of life itself.

Prokaryotic and Eukaryotic Cells

We already described the radically different cell plan of prokaryotes and eukaryotes (p. 32). A fundamental distinction, expressed in their names, is that prokaryotes lack the membrane-bound nucleus present in all eukaryotic cells. Among other differences, eukaryotic cells have many membranous organelles (specialized structures that perform particular functions within cells) (Table 3.1).

Despite these differences, which are of paramount importance in cell studies, prokaryotes and eukaryotes have much in common. Both have DNA, use the same genetic code, and synthesize proteins. Many specific molecules such as ATP perform similar roles in both. These fundamental similarities imply common ancestry. The upcoming discussion is restricted to eukaryotic cells, of which all animals are composed.

TABLE 3.1

Comparison of Prokaryotic and Eukaryotic Cells

Characteristic	Prokaryotic Cell	Eukaryotic Cell
Cell size	Mostly small (1–10 µm)	Mostly large (10–100 µm)
Genetic system	DNA with some DNA-binding protein; simple, circular DNA molecule in nucleoid; nucleoid is not membrane bound	DNA complexed with DNA-binding proteins in complex linear chromosomes within nucleus with membranous envelope; circular mitochondrial and chloroplast DNA
Cell division	Direct by binary fission or budding; no mitosis	Some form of mitosis; centrioles in many; mitotic spindle present
Sexual system	Absent in most; highly modified if present	Present in most; male and female partners; gametes that fuse to form zygote
Nutrition	Absorption by most; photosynthesis by some	Absorption, ingestion, photosynthesis by some
Energy metabolism	No mitochondria; oxidative enzymes bound to cell membrane, not packaged separately; great variation in metabolic pattern	Mitochondria present; oxidative enzymes packaged therein; more unified pattern of oxidative metabolism
Intracellular movement	None	Cytoplasmic streaming, phagocytosis, pinocytosis
Flagella/cilia	If present, not with "9 + 2" microtubular pattern	With "9 + 2" microtubular pattern
Cell wall	Contains disaccharide chains cross-linked with peptides	If present, not with disaccharide polymers linked with peptides

Components of Eukaryotic Cells and Their Functions

Typically, eukaryotic cells are enclosed within a thin, selectively permeable **plasma membrane** (Figure 3.4). The most prominent organelle is a spherical or ovoid **nucleus,** enclosed within *two* membranes to form a double-layered **nuclear envelope** (Figure 3.4). Cellular material located between the cell membrane and nuclear envelope is collectively called **cytoplasm.** Within the cytoplasm are many organelles, such as mitochondria, Golgi complexes, centrioles, and endoplasmic reticulum. Plant cells typically contain **plastids,** some of which are photosynthetic organelles, and plant cells bear a cell wall containing cellulose outside the cell membrane.

The **fluid-mosaic model** is the currently accepted concept describing plasma membrane structure. By electron microscopy, a plasma membrane appears as two dark lines, each approximately 3 nm thick, at each side of a light zone (Figure 3.5). The entire membrane is 8 to 10 nm thick. This image is the result of a phospholipid bilayer, two layers of phospholipid molecules, all oriented with their water-soluble (hydrophilic) ends toward the outside and their fat-soluble portions (hydrophobic) toward the inside of the membrane (Figure 3.6). An important characteristic of the phospholipid bilayer is that it is fluidlike, giving the membrane flexibility and allowing the phospholipid molecules to move sideways freely within their own monolayer. Molecules of cholesterol are interspersed in the lipid portion of the bilayer (Figure 3.6). They make the membrane even less permeable to water-soluble ions and molecules and decrease membrane flexibility.

Glycoproteins (proteins with carbohydrates attached) are essential components of plasma membranes (Figure 3.6). Some of these proteins transport substances such as charged ions across the membrane (see p. 46, membrane function). Others act as specific receptors for various molecules or as highly specific cell markers. For example, the self/nonself recognition that enables the immune system to react to invaders (see Chapter 35) is based on proteins of this type. Some aggregations of protein molecules form pores or channels through which small polar molecules may enter (see gap junctions, p. 46, and channels, p. 49). Like the phospholipid molecules, most glycoproteins can move laterally in the membrane, although more slowly.

Nuclear envelopes contain less cholesterol than cell membranes, and pores in the envelope (Figure 3.7) allow molecules to move between nucleus and cytoplasm. Nuclei contain linear **chromosomes** suspended in **nucleoplasm.** The chromosomes are normally loosely condensed, flexible strands of **chromatin,** composed of a complex of DNA, and DNA-binding proteins. Chromosomal DNA carries the genetic information encoding cellular RNA and protein molecules (see Chapter 5). The linear chromosomes only become condensed and visible as discrete structures during cell division (see p. 52 for mitosis and p. 77 for meiosis). **Nucleoli** are specialized parts of certain chromosomes that stain in a characteristically dark manner. They carry multiple copies of the DNA information used to synthesize ribosomal

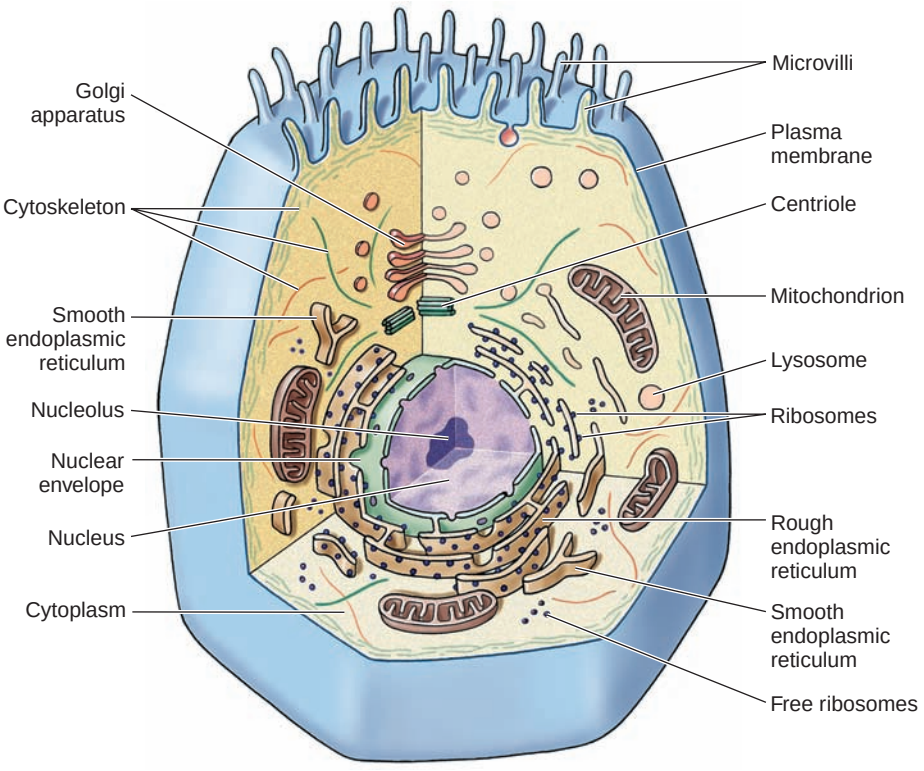


Figure 3.4
Generalized cell with principal organelles, as might be seen with the electron microscope.

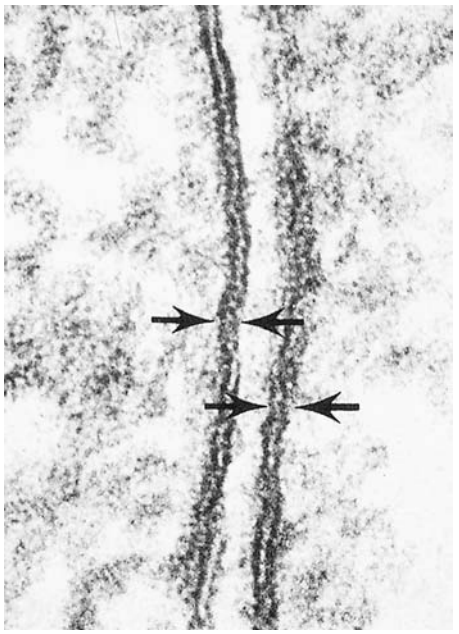


Figure 3.5
Plasma membranes of two adjacent cells. Each membrane (*between arrows*) shows a typical dark-light-dark staining pattern. (×325,000)

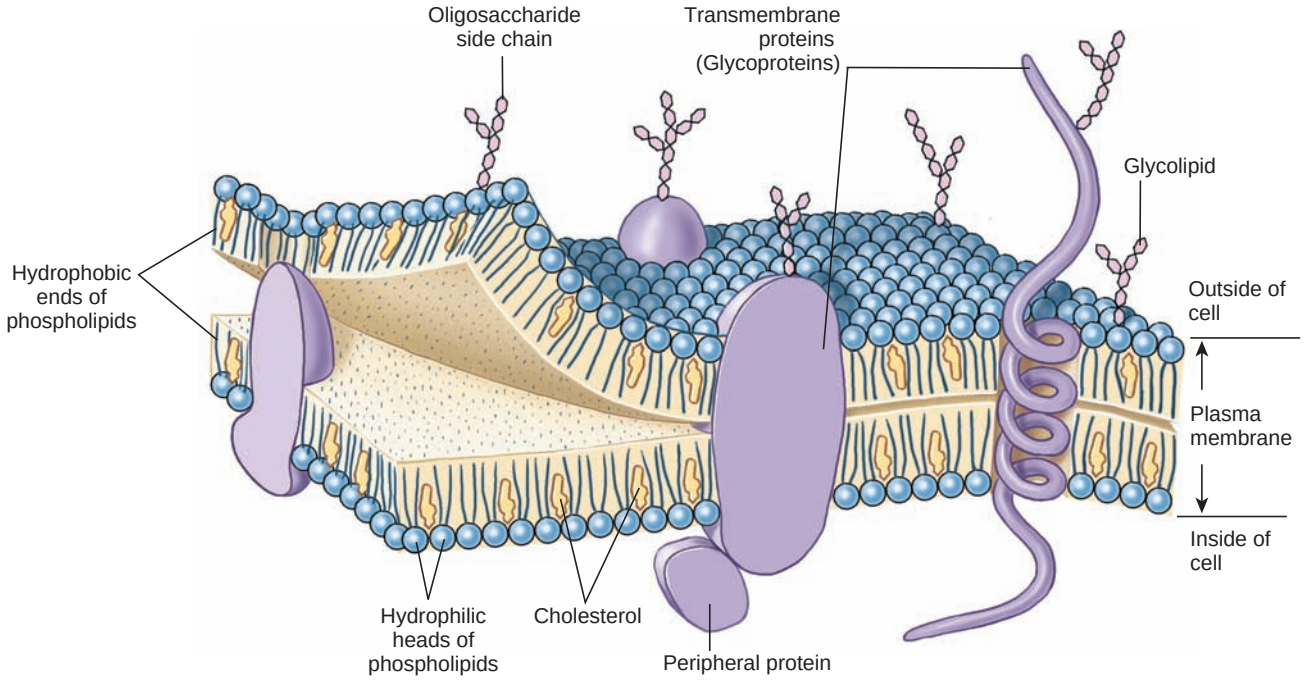


Figure 3.6

Diagram illustrating fluid-mosaic model of a plasma membrane.

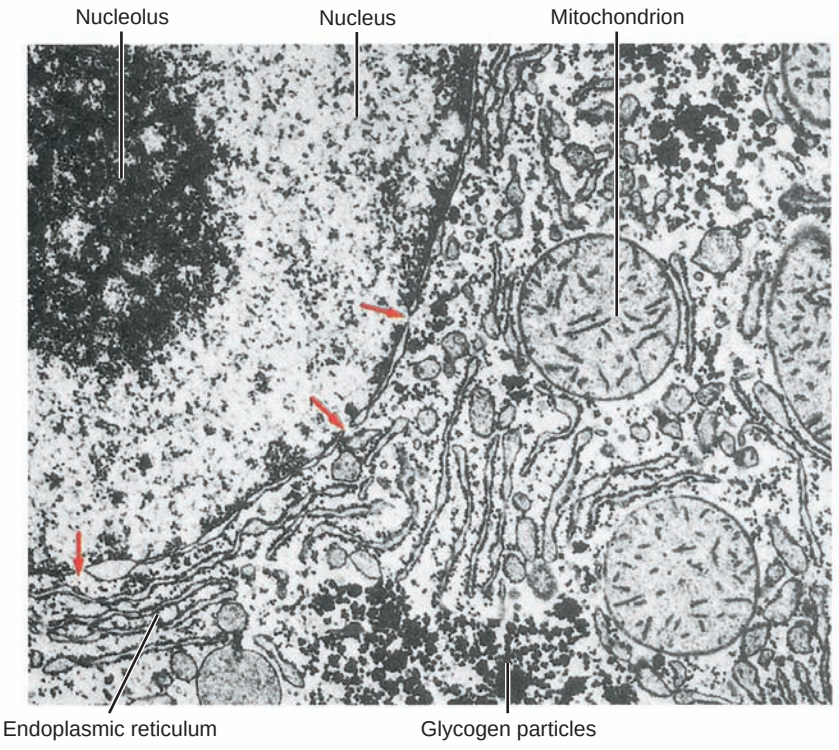


Figure 3.7

Electron micrograph of part of hepatic cell of rat showing portion of nucleus (*left*) and surrounding cytoplasm. Endoplasmic reticulum and mitochondria are visible in cytoplasm, and pores (*arrows*) can be seen in nuclear envelope. (×14,000)

RNA. After transcription from DNA, ribosomal RNA combines with protein to form the two subunits of **ribosomes,** which leave the nucleolus and pass to the cytoplasm through pores in the nuclear envelope. Ribosomes are sites of polypeptide or protein synthesis. They perform this function free, within the cytoplasm, when manufacturing polypeptides for use in the cytoplasm or nucleus. Alternatively, they become attached to **endoplasmic reticulum (ER)** when manufacturing polypeptides destined for the plasma membrane, lysosomes, or for export.

The outer membrane of the nuclear envelope is continuous with a cytoplasmic membranous system called endoplasmic reticulum (ER) (Figures 3.7 and 3.8). The space between the membranes of the nuclear envelope communicates with the space between the ER membranes **(cisterna,** pl., **cisternae).** The ER membranes may be covered on their outer surfaces with ribosomes and are thus designated **rough ER,** or they may lack ribosomal covering and be called **smooth ER.** Ribosomes on rough ER synthesize polypeptides that enter the ER cisternae or membrane and are destined for incorporation into the plasma membrane (Figure 3.9), for export from the cell, or they are bound for the lysosomes. Smooth ER functions in synthesis of lipids and phospholipids.

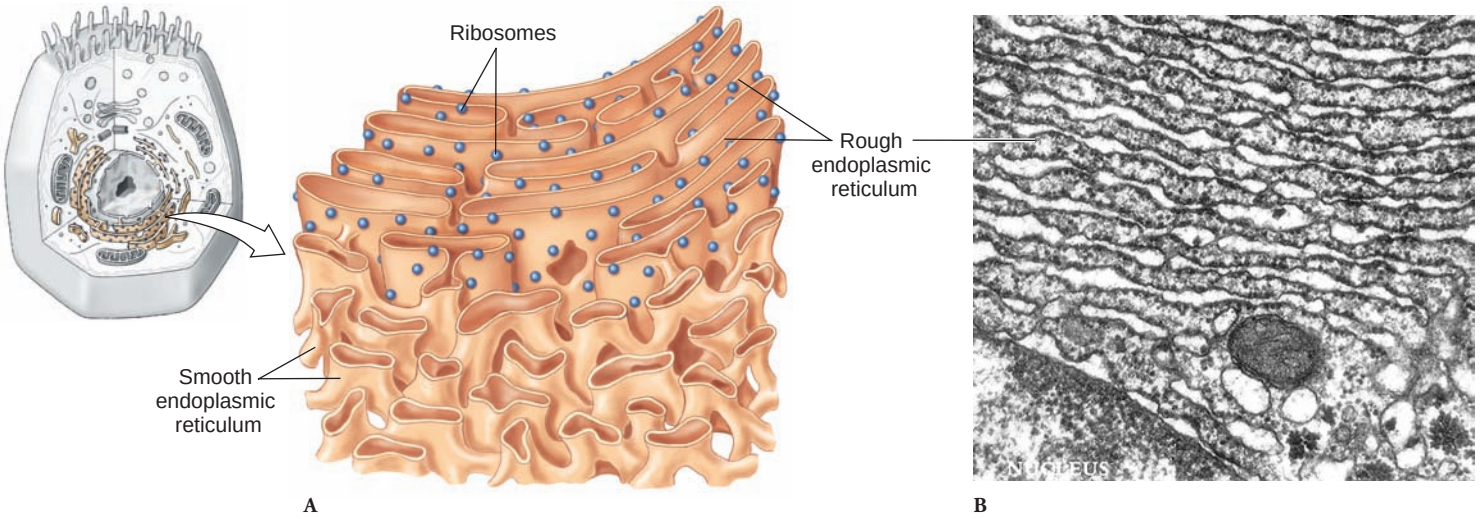


Figure 3.8

Endoplasmic reticulum. **A,** Endoplasmic reticulum is continuous with the nuclear envelope. It may have associated ribosomes (rough endoplasmic reticulum) or not (smooth endoplasmic reticulum). **B,** Electron micrograph showing rough endoplasmic reticulum. (×28,000)

The **Golgi complex** (Figures 3.9 and 3.10) is composed of a stack of membranous vesicles that function in storage, modification, and packaging of polypeptide and protein products produced by rough ER. The vesicles do not synthesize polypeptide or protein but may add complex carbohydrates to the molecules. Small vesicles of ER membrane containing polypeptide or protein detach and then fuse with sacs on the *cis* or "forming face" of a Golgi complex. After modification, the polypeptides or proteins become incorporated into vesicles that detach from the *trans* or "maturing face" of the complex (Figures 3.9 and 3.10). The contents of some of these vesicles may be expelled to the outside of the cell, as secretory products such as from a glandular cell. Some may carry integral polypeptides or proteins for incorporation into the plasma membrane, such as receptors or carrier proteins. Others may contain enzymes that remain in the same cell that produces them. Such vesicles are called **lysosomes** (literally "loosening body," a body capable of causing lysis, or disintegration). Enzymes that they contain are involved in the breakdown of foreign material, including bacteria engulfed by a cell. Lysosomes also are capable of breaking down injured or diseased cells and worn-out cellular components. Their enzymes are so powerful that they kill the cell that formed them if enough of the lysosome membranes rupture. In normal cells the enzymes remain safely enclosed within the protective vesicle membranes. Lysosomal vesicles may pour their enzymes into a larger membrane-bound body containing an ingested food particle, a **food vacuole** or **phagosome** (see Figure 3.21).

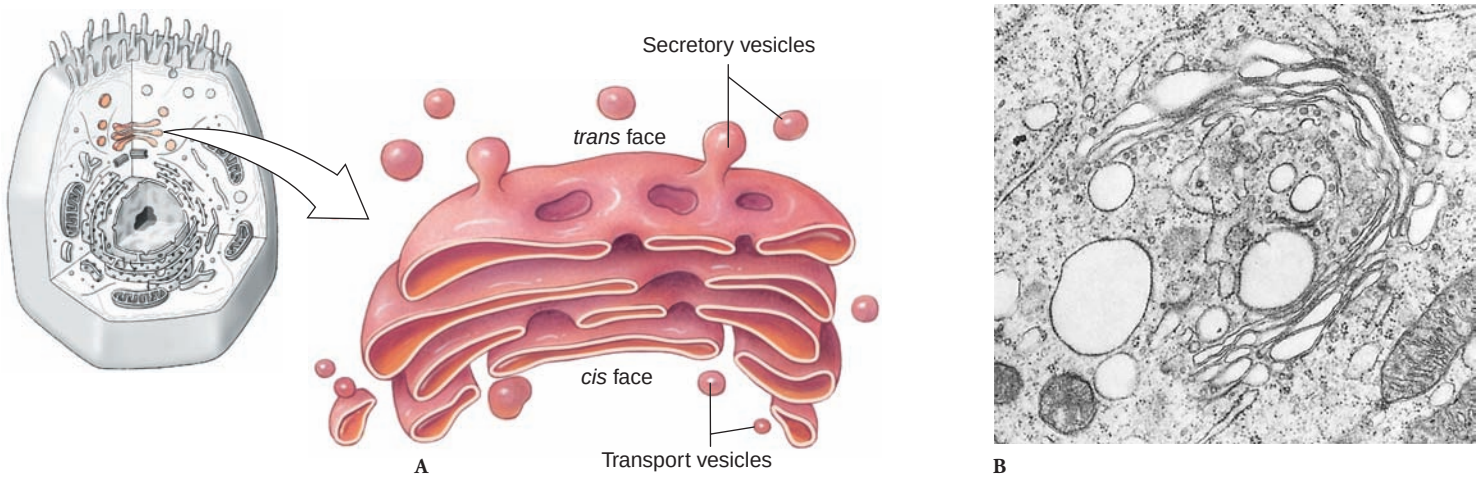


Figure 3.9

Golgi complex (=Golgi body, Golgi apparatus). **A,** The smooth cisternae of the Golgi complex have enzymes that modify polypeptides or proteins synthesized by the rough endoplasmic reticulum. **B,** Electron micrograph of a Golgi complex. (×46,000)

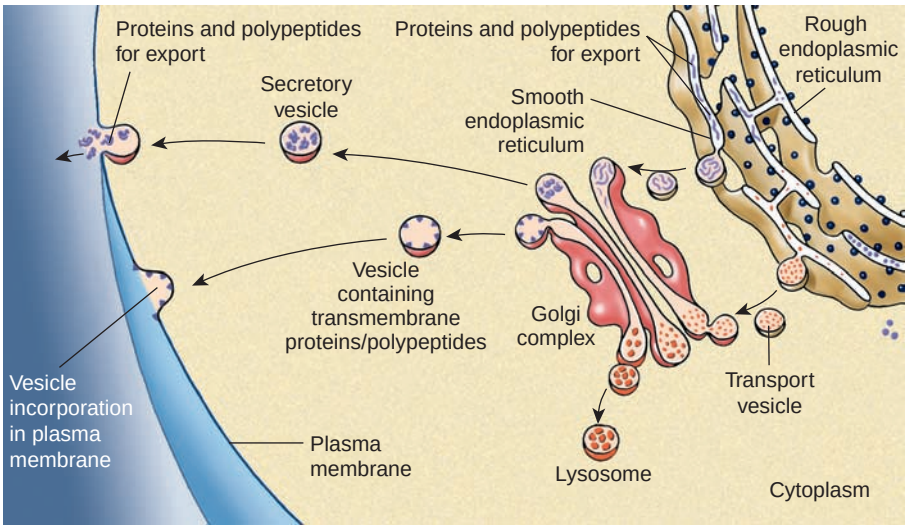


Figure 3.10

System for assembling, isolating, and secreting polypeptides and proteins for export in a eukaryotic cell, for lysosomes, or for incorporation into plasma membrane.

Mitochondria (sing., **mitochondrion**) (Figure 3.11) are conspicuous organelles present in nearly all eukaryotic cells. They are diverse in size, number, and shape; some are rodlike, and others are nearly spherical. They may be scattered uniformly throughout the cytoplasm or localized near cell surfaces and other regions of high metabolic activity. A mitochondrion is composed of a double membrane. The outer membrane is smooth, whereas the inner membrane is folded into numerous platelike or fingerlike projections called **cristae** (sing., **crista;** Figure 3.11), which increase the internal surface area where chemical reactions occur. These characteristic features make mitochondria easy to identify among organelles. Mitochondria are often called "powerhouses of cells," because enzymes located on the cristae catalyze the energy-yielding steps of aerobic metabolism (see Figure 4.14, p. 64). ATP (adenosine triphosphate), the most important energy-transfer molecule of all cells, is produced in this organelle. Mitochondria are self-replicating. They have a tiny, circular genome, much like genomes of prokaryotes except much smaller, which contains DNA specifying some, but not all, proteins of a mitochondrion.

Eukaryotic cells characteristically have a system of tubules and filaments that form a **cytoskeleton** (Figures 3.12 and 3.13). These provide support and maintain the form of cells, and in many cells, they provide a means of locomotion and translocation of macromolecules and organelles within the cell. The cytoskeleton is composed of microfilaments, microtubules, and intermediate filaments. **Microfilaments** are thin, linear structures, first observed distinctly in muscle cells, where they are responsible for the ability of a cell to contract. They are made of a protein called **actin.** Several dozen other proteins are known that bind with actin and determine its configuration and behavior in particular cells. One of these is **myosin,** whose interaction with actin causes contraction in muscle and other cells (p. 658). Actin microfilaments provide a means for movement of molecules and organelles through the cytoplasm, as well as movement of messenger RNA (p. 93) from the nucleus to particular positions within the cytoplasm. Actin and actin-binding proteins are also important in movement of vesicles between the ER, Golgi complex, and plasma membrane or lysosomes. **Microtubules,** somewhat larger than microfilaments, are tubular structures composed of a protein called **tubulin** (Figure 3.13). Each tubulin molecule is actually a doublet composed of two globular proteins. The molecules are attached head-to-tail to form a strand, and 13 strands aggregate to form a microtubule. Because the tubulin subunits in a microtubule are always attached head-to-tail, the ends of the microtubule differ chemically and functionally. One end (called the plus end) both adds and deletes tubulin subunits more rapidly than the other end (the minus end). Microtubules play a vital role in moving chromosomes toward daughter cells during cell division (p. 52), and they are important in intracellular architecture, organization, and transport. In addition, microtubules form essential parts of the structures of cilia and flagella (see next section). Microtubules radiate from a microtubule organizing center, the **centrosome,** near the nucleus. Centrosomes are not membrane bound. Within centrosomes are found a pair of **centrioles** (Figures 3.4 and 3.14), which are themselves

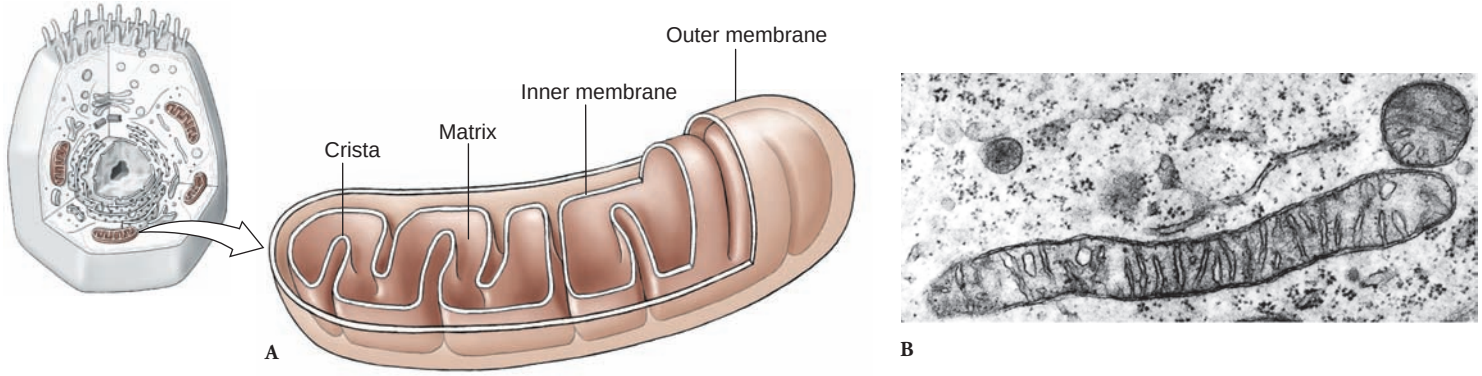


Figure 3.11

Mitochondria. **A,** Structure of a typical mitochondrion. **B,** Electron micrograph of mitochondria in cross and longitudinal section. (×30,000)

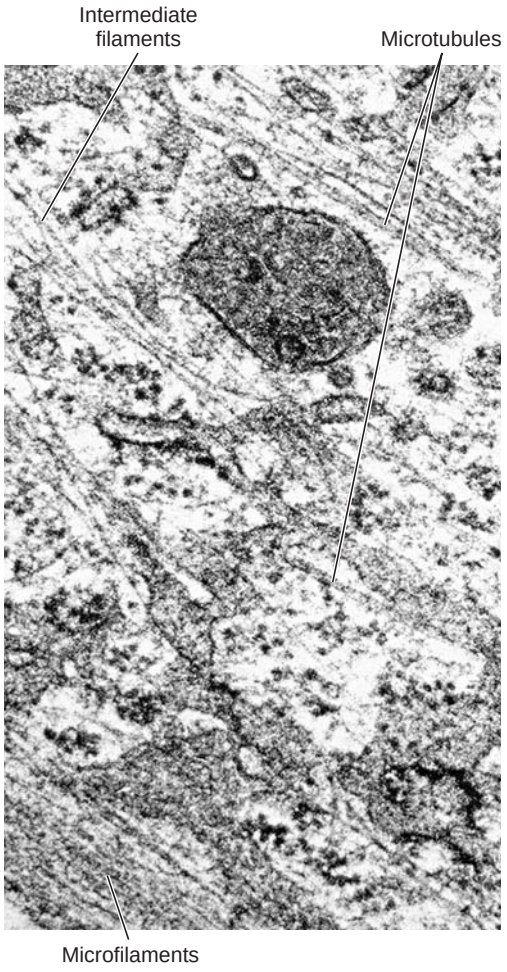


Figure 3.12

Cytoskeleton of a cell, showing its complex nature. Three visible cytoskeletal elements, in order of increasing diameter, are microfilaments, intermediate filaments, and microtubules. (×66,600)

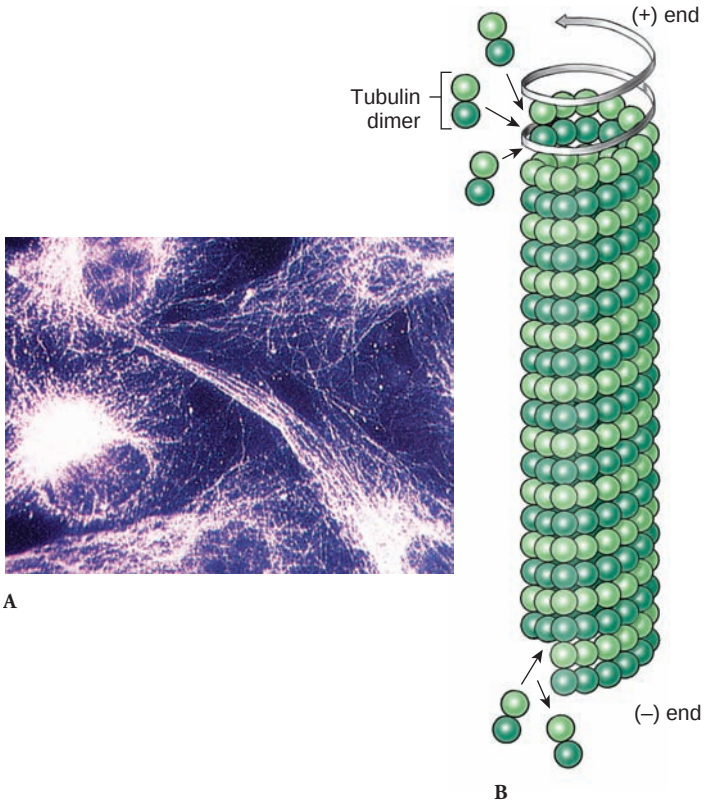


Figure 3.13

A, The microtubules in kidney cells of a baby hamster have been rendered visible by treatment with a preparation of fluorescent proteins that specifically bind to tubulin. **B,** A microtubule is composed of 13 strands of tubulin molecules, and each molecule is a dimer. Tubulin dimers are added to and removed from the (+) end of the microtubule more rapidly than at the (−) end.

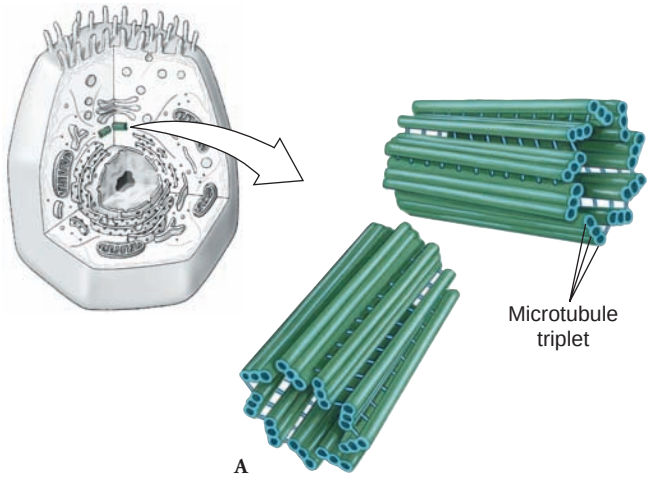
composed of microtubules. Each centriole of a pair lies at right angles to the other and is a short cylinder of nine triplets of microtubules. They replicate before cell division. Although cells of higher plants do not have centrioles, a microtubule organizing center is present. **Intermediate filaments** are larger than microfilaments but smaller than microtubules. There are five biochemically distinct types of intermediate filaments, and their composition and arrangement depend on the cell type in which they occur. The type of intermediate filament is often determined in cancerous cells so that the original cell type can be identified. Knowing the particular cell type can often help in determining treatment options.

Surfaces of Cells and Their Specializations

The free surface of epithelial cells (cells that cover the surface of a structure or line a tube or cavity; see p. 195) sometimes bears either **cilia** or **flagella** (sing., **cilium, flagellum**). These are motile extensions of the cell surface that sweep materials past the cell. In many single-celled organisms and some small multicellular forms, they propel the entire organism through a liquid medium (see pp. 239, 282). Flagella provide the means of locomotion for male reproductive cells (sperm) of most animals (see p. 146) and many plants.

Cilia and flagella have different beating patterns (see p. 656), but their internal structure is the same. With few exceptions, the internal structures of locomotory cilia and flagella are composed of a long cylinder of nine pairs of microtubules enclosing a central pair (see Figure 29.11). At the base of each cilium or flagellum is a **basal body (kinetosome),** which is identical in structure to a single centriole.

Many cells move neither by cilia nor flagella but by **ameboid movement** using **pseudopodia.** Some groups of unicellular eukaryotes (p. 224), migrating cells in embryos of multicellular animals, and some cells of adult multicellular animals, such as white blood cells, show ameboid movement. Cytoplasmic streaming through the action of actin microfilaments extends a lobe (pseudopodium) outward from the surface of the cell. Continued streaming in the direction of a pseudopodium brings cytoplasmic organelles into the lobe and accomplishes movement of the entire cell. Some specialized pseudopodia have cores of microtubules (p. 227), and



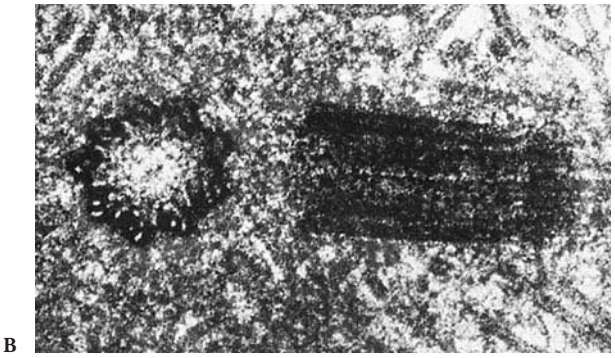


Figure 3.14

The centrosome. **A,** Each centrosome contains a pair of centrioles and each centriole is composed of nine triplets of microtubules arranged as a cylinder. **B,** Electron micrograph of a pair of centrioles, one in longitudinal (*right*) and one in cross section (*left*). The normal orientation of centrioles is at right angles to each other.

movement is effected by assembly and disassembly of the tubulin subunits.

Cells covering the surface of a structure (epithelial cells) or cells packed together in a tissue may have specialized junctional complexes between them. Nearest the free surface, the membranes of two cells next to each other appear to fuse, forming a **tight junction** (Figure 3.15). They are formed from rows of transmembrane proteins that bind tightly between adjacent cells. Tight junctions function as seals to prevent the passage of molecules between cells from one side of a layer of cells to another, because there is usually a space of about 20 nm between the plasma membranes of adjacent cells. The number of rows of transmembrane proteins in the tight junction determines how closely adjacent cells are sealed to each other. Tight junctions between intestinal cells, for example, force molecules from the intestinal contents to pass through epithelial cells during absorption, rather than between them. **Adhesion junctions** (Figure 3.15) occur just beneath tight junctions. These anchoring junctions are similar to tight junctions in that they encircle the cell. They are different from tight junctions in that they do not seal adjacent cells to each other. Rather, the transmembrane proteins link together across a small intercellular space. Inside adjacent cells, the transmembrane proteins are attached to actin microfilaments and thus attach the cytoskeletons of adjacent cells to each other. Modified adhesion junctions are found between cardiac muscle cells, and these hold the cells together as the heart beats throughout the life of an organism (p. 694). At various points beneath tight junctions and adhesion junctions, in epithelial cells, small ellipsoid discs occur, within the plasma membrane in each cell. These appear to act as "spot-welds" and are called **desmosomes** (Figure 3.15). From each desmosome a tuft of intermediate filaments extends into the cytoplasm, and transmembrane linker proteins extend through the plasma membrane into the intercellular space to bind the discs of adjacent cells together. Desmosomes are not seals but seem to increase the strength of the tissue. Many are found between the cells of the skin in vertebrates (p. 645). **Hemidesmosomes** (Figure 3.15) are found at the base of cells and anchor them to underlying connective tissue layers. **Gap junctions** (Figure 3.15), rather than serving as points of attachment, provide a means of intercellular communication. They form tiny canals between cells, so that their cytoplasm becomes continuous, and small molecules and ions can pass from one cell to the other. Gap junctions may occur between cells of epithelial, nervous, and muscle tissues.

Another specialization of cell surfaces is the "lacing together" of adjacent cell surfaces where plasma membranes of the cells infold and interdigitate very much like a zipper. These infoldings are especially common in epithelial cells of kidney tubules and serve to increase the surface area of the cells for absorption or secretion. The distal or apical boundaries of some epithelial cells, as seen by electron microscopy, show regularly arranged **microvilli** (sing., **microvillus**). They are small, fingerlike projections consisting of tubelike evaginations of the plasma membrane with a core of cytoplasm containing bundles of actin microfilaments (Figures 3.15 and 3.16). They are seen clearly in the lining of the intestine where they greatly increase the absorptive and digestive surface. Such specializations appear as brush borders by light microscopy.

Membrane Function

The incredibly thin, yet sturdy, plasma membrane that encloses every cell is vitally important in maintaining cellular integrity. Once erroneously considered to be a rather static entity that defined cell boundaries and kept cell contents in place, plasma membranes (also called the plasmalemma) form dynamic structures having remarkable activity and selectivity. They are a permeability barrier that separates the interior from the external environment. They regulate the flow of molecules into and out of the cell, and provide many of the unique functional properties of specialized cells.

Membranes inside a cell surround a variety of organelles. Indeed, a cell is a system of membranes that divide it into numerous compartments. It has been estimated that if all membranes present in one gram of liver tissue were spread out flat, they would cover 30 square meters! Internal membranes share

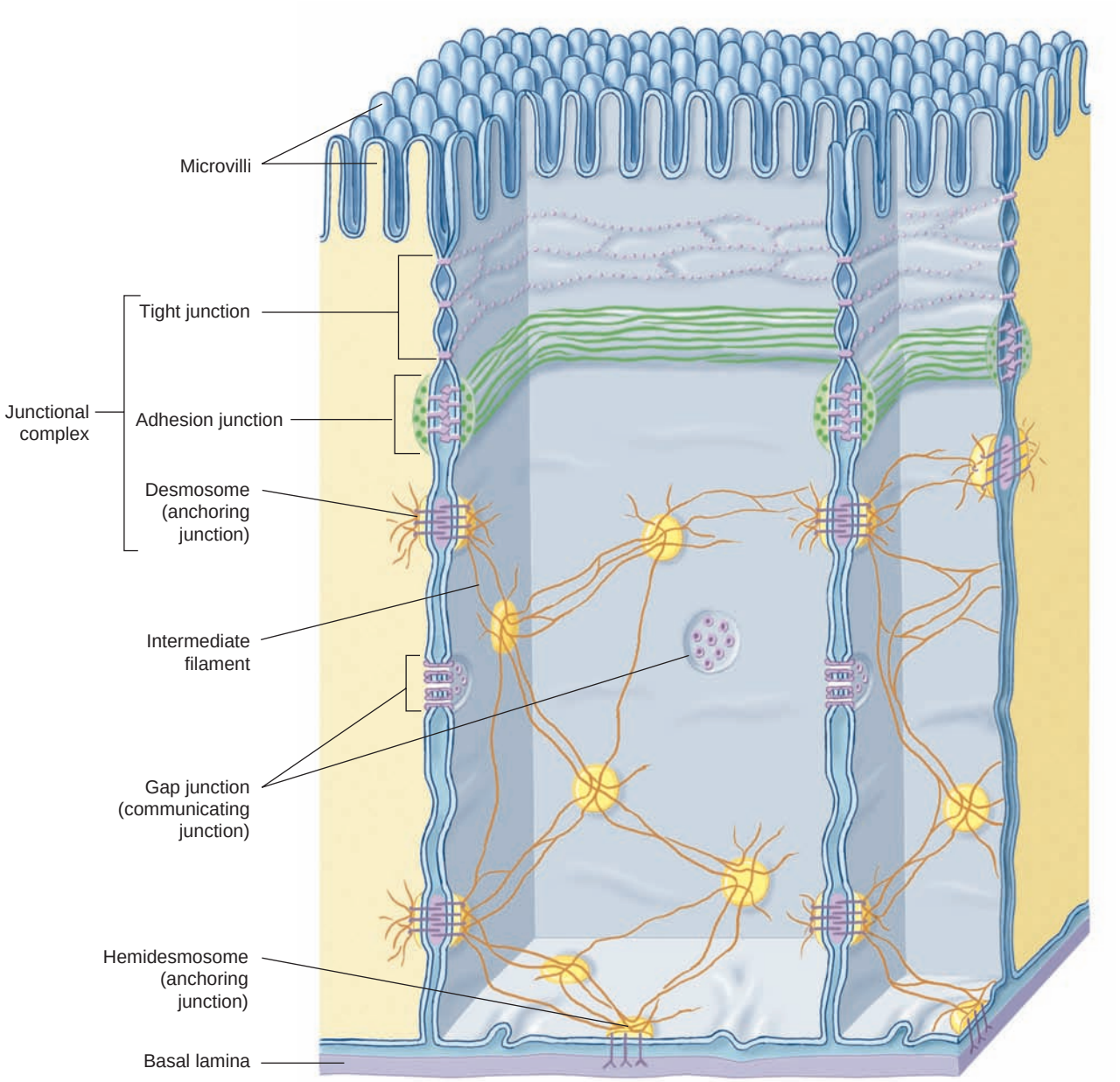


Figure 3.15

Junction types and locations are shown in columnar epithelial cells. Actin microfilaments (shown in green) and intermediate filaments (shown in orange) attach adhesion junctions and desmosomes, respectively, to the cytoskeleton.

many structural features of plasma membranes and are the site for many of a cell's enzymatic reactions.

A plasma membrane acts as a selective gatekeeper for entrance and exit of many substances involved in cell metabolism. Some substances can pass through with ease, others enter slowly and with difficulty, and still others cannot enter at all. Because conditions outside a cell are different from and more variable than conditions within a cell, it is necessary that passage of substances across the membrane be rigorously controlled.

We recognize three principal ways that a substance may enter across a cell membrane: (1) by **diffusion** along a concentration gradient; (2) by a **mediated transport system,** in which the substance binds to a specific site on a transmembrane protein that assists it across the membrane; and (3) by **endocytosis,** in which the substance is enclosed within a vesicle that forms from the membrane surface and detaches inside the cell.

Diffusion and Osmosis

Diffusion is a movement of particles from an area of higher concentration to an area of lower concentration of the particles or molecules, thus tending to equalize the concentration throughout the area of diffusion. If a living cell surrounded by a membrane is immersed in a solution having a higher concentration of solute

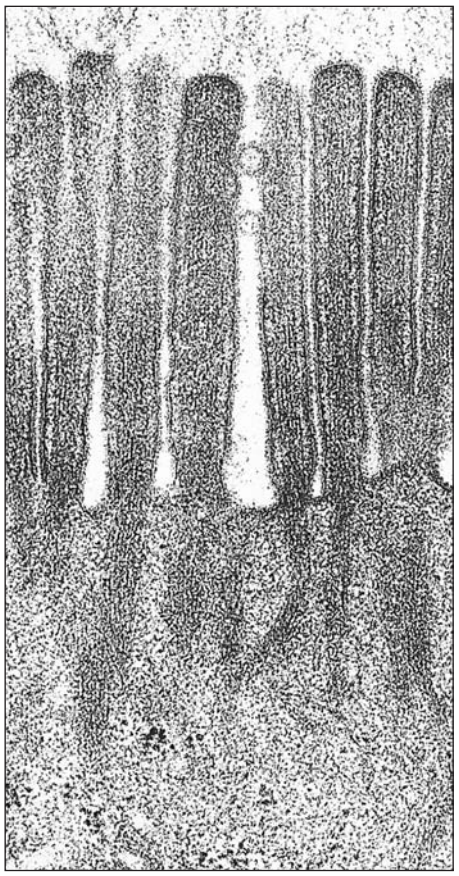


Figure 3.16

Electron micrograph of microvilli. (×59,000)

molecules than the fluid inside the cell, a **concentration gradient** instantly exists between the two fluids across the membrane. Assuming that the membrane is **permeable** to the solute, there is a net movement of solute toward the inside, the side having the lower concentration. The solute diffuses "downhill" across the membrane until its concentrations on each side are equal.

Most cell membranes are **selectively permeable,** that is, permeable to water but variably permeable or impermeable to solutes. In free diffusion it is this selectiveness that regulates molecular traffic. As a rule, gases (such as oxygen and carbon dioxide), urea, and lipid-soluble solutes (such as fats, fatlike substances, and alcohol; see p. 26) are the only solutes that can diffuse through biological membranes with any degree of freedom. Because many water-soluble molecules readily pass through membranes, such movements cannot be explained by simple diffusion. Sugars, many electrolytes, and macromolecules are moved across membranes by carrier-mediated processes that are described in the next section.

If we place a membrane between two unequal concentrations of solutes to which the membrane is impermeable, water flows through the membrane from the more dilute to the more concentrated solution. The water molecules move across the membrane down a concentration gradient from an area where the *water* molecules are more concentrated to an area on the other side of the membrane where they are less concentrated. This is **osmosis.**

We can demonstrate osmosis in a simple experiment by tying a selectively permeable membrane tightly over the end of a funnel. The funnel is filled with a salt solution and placed in a beaker of pure water so that the water levels inside and outside the funnel are equal. In a short time the water level in the glass tube of the funnel rises, indicating a net movement of water through the membrane into the salt solution (Figure 3.17).

Inside the funnel are salt molecules, as well as water molecules, while the beaker contains only water molecules. Thus the concentration of water is less inside the funnel because some of the available space is occupied by the nondiffusible salt ions. A concentration gradient exists for water molecules in the system. Water diffuses from the region of greater concentration of water (pure water in the beaker) to the region of lesser water concentration (salt solution inside the funnel).

As water enters the salt solution, the fluid level in the funnel rises. Eventually the pressure produced by the increasing weight of solution in the funnel pushes water molecules out as fast as they enter. The level in the funnel becomes stationary and the

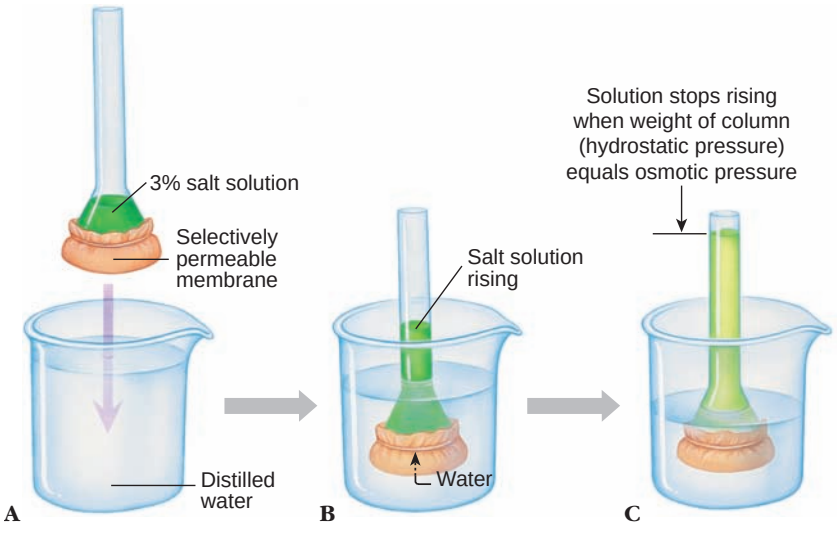


Figure 3.17

Simple membrane osmometer. **A,** The end of a tube containing a salt solution is closed at one end by a selectively permeable membrane. The membrane is permeable to water but not to salt. **B,** When the tube is immersed in pure water, water molecules diffuse through the membrane into the tube. Water molecules are in higher concentration in the beaker because they are diluted inside the tube by salt ions. Because the salt cannot diffuse out through the membrane, the volume of fluid inside the tube increases, and the level rises. **C,** When the weight of the column of water inside the tube exerts a downward force (hydrostatic pressure) causing water molecules to leave through the membrane in equal number to those that enter (osmotic pressure), the volume of fluid inside the tube stops rising. At this point the hydrostatic pressure is equivalent to the osmotic pressure.

system is in equilibrium. The **osmotic pressure** of the solution is equivalent to the **hydrostatic pressure** necessary to prevent further net entry of water.

The concept of osmotic pressure is not without problems. A solution reveals an osmotic "pressure" only when it is separated from solvent by a selectively permeable membrane. It can be disconcerting to think of an isolated bottle of salt solution as having "pressure" much as compressed gas in a bottle (*hydrostatic* pressure) would have. Furthermore, the osmotic pressure is really the hydrostatic pressure that must be applied to a solution to keep it from gaining water *if* the solution were separated from pure water by a selectively permeable membrane. Consequently, biologists frequently use the term **osmotic potential** rather than osmotic pressure. However, since the term "osmotic pressure" is so firmly fixed in our vocabulary, it is necessary to understand the usage despite its potential confusion.

The concept of osmosis is very important in understanding how animals control their internal fluid and solute environment (see Chapter 30). For example, marine bony fishes maintain a solute concentration in their blood about one-third of that in seawater; they are **hypoosmotic** to seawater. If a fish swims into a river mouth and then up a freshwater stream, as salmon do, it would pass through a region where its blood solutes were equal in concentration to those in its environment **(isosmotic),** then enter freshwater, where its blood solutes were **hyperosmotic** to those in its environment. It must have physiological mechanisms to avoid net loss of water in the sea and gain of water in the river.

Diffusion Through Channels

Water and dissolved ions, since they are charged, cannot diffuse through the phospholipid component of the plasma membrane. Instead, they pass through specialized pores or channels created by transmembrane proteins. Ions and water move through these channels by diffusion. Ion channels allow specific ions of a certain size and charge to diffuse through them. They may allow ion diffusion at all times or they may be **gated channels,** requiring a signal to open or close them. Gated ion channels may open or close when a signaling molecule binds to a specific binding site on the transmembrane protein (**chemically-gated ion channels;** Figure 3.18A) or when the ionic charge across a plasma membrane changes (**voltage-gated ion channels;** Figure 3.18B). Ion diffusion through channels is the basis of signaling mechanisms in the nervous system (see Chapter 33, p. 729) and in muscles (see Chapter 29, p. 660). Water channels are called **aquaporins,** and several different types have been discovered. They are especially important in the digestive system for absorption of water from food (see Chapter 32, p. 720), and in the kidney for water reabsorption during urine formation (see Chapter 30, p. 679).

Carrier-Mediated Transport

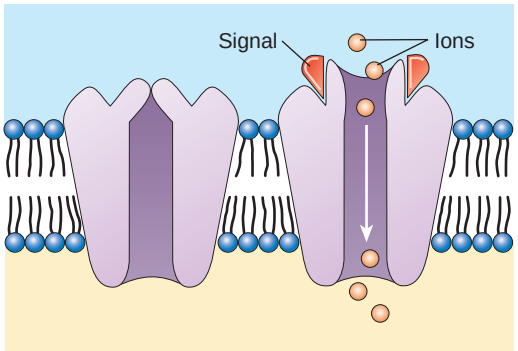
We have seen that a plasma membrane is an effective barrier to free diffusion of most molecules of biological significance, yet it is essential that such materials enter and leave a cell. Nutrients such as sugars and materials for growth such as amino acids must enter a cell, and wastes of metabolism must leave. Such molecules are moved across a membrane by special transmembrane proteins called **transporters,** or carriers. Transporters enable solute molecules to cross the phospholipid bilayer (Figure 3.19A). Transporters are usually quite specific, recognizing and transporting only a limited group of chemical substances or perhaps even a single substance.

At high concentrations of solute, mediated transport systems show a saturation effect. This means simply that the rate of influx reaches a plateau beyond which increasing the solute concentration has no further effect on influx rate (Figure 3.19B). This is evidence that the number of transporters available in a membrane is limited. When all transporters become occupied by solutes, the rate of transport is at a maximum and it cannot be increased. Simple diffusion shows no such limitation; the greater the difference in solute concentrations on the two sides of the membrane, the faster the influx.

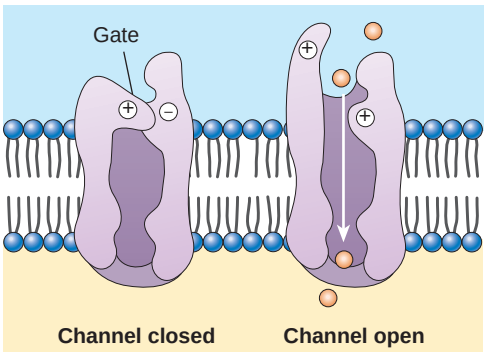
Two distinctly different kinds of mediated transport mechanisms are recognized: (1) **facilitated diffusion,** in which a transporter assists a molecule to diffuse through the membrane that it cannot otherwise penetrate, and (2) **active transport,** in which energy is supplied to the transporter system to transport molecules in the direction opposite to a concentration gradient (Figure 3.20). Facilitated diffusion therefore differs from active

Figure 3.18

Gated channels require a signal to open (or close) them. **A,** Chemically-gated ion channels open (or close) when a signaling molecule binds to a specific binding site on the transmembrane protein. **B,** Voltage-gated ion channels open (or close) when the ionic charge across the membrane changes.



A Chemically-gated ion channel



B Voltage-gated ion channel

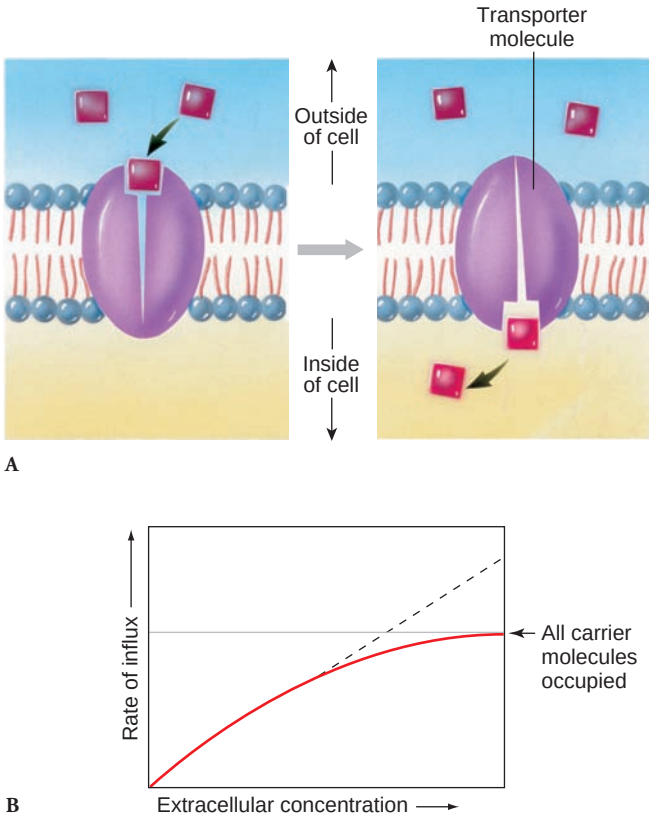


Figure 3.19

Facilitated transport. **A,** A transporter protein molecule binds with a molecule to be transported (substrate) on one side of a plasma membrane, changes shape, and releases the molecule on the other side. Facilitated transport takes place in the direction of a concentration gradient. **B,** Rate of transport increases with increasing substrate concentration until all transporter molecules are occupied.

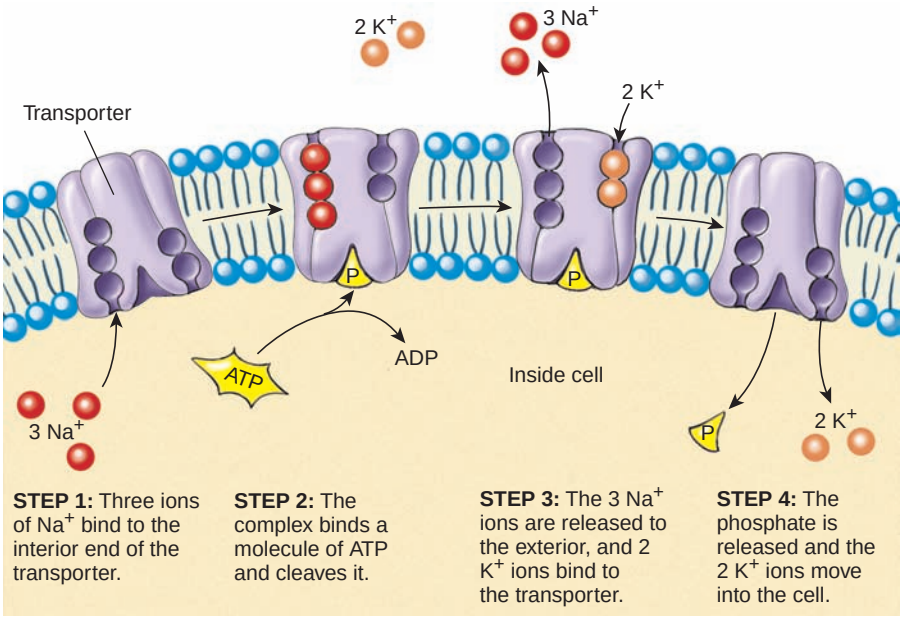
transport in that it sponsors movement only in a downhill direction (in the direction of a concentration gradient) and requires no metabolic energy to drive the transport system.

In many animals facilitated diffusion aids in transport of glucose (blood sugar) into body cells that oxidize it as a principal energy source for the synthesis of ATP. The concentration of glucose is greater in blood than in the cells that consume it, favoring inward diffusion, but glucose is a water-soluble molecule that does not, by itself, penetrate cell membranes rapidly enough to support the metabolism of many cells; the carrier-mediated transport system increases the inward flow of glucose.

In active transport, molecules are moved uphill against the forces of passive diffusion. Active transport always involves an expenditure of energy (from ATP) because materials are transported against a concentration gradient. Among the most important active-transport systems in all animals are those that maintain sodium and potassium ion gradients between cells and the surrounding extracellular fluid or external environment. Most animal cells require a high internal concentration of potassium ions for protein synthesis at the ribosome and for certain enzymatic functions. The potassium ion concentration may be 20 to 50 times greater inside a cell than outside. Sodium ions, on the other hand, may be 10 times more concentrated outside a cell than inside. This sodium gradient forms the basis for electrical signal generation within the nervous system of animals (see Chapter 33, p. 728). Both of these ionic gradients are maintained by active transport of potassium ions into and sodium ions out of the cell. In many cells outward transport of sodium is linked to inward transport of potassium; this same transporter molecule does both. As much as 10% to 40% of all energy produced by cells is consumed by the **sodium-potassium exchange pump** (Figure 3.20).

Figure 3.20

Sodium-potassium pump, powered by bond energy of ATP, maintains the normal gradients of these ions across the cell membrane. The pump works by a series of conformational changes in the transporter: *Step 1.* Three ions of Na^+ bind to the interior end of the transporter, producing a conformational (shape) change in the protein complex. *Step 2.* The complex binds a molecule of ATP, cleaves it, and phosphate binds to the complex. *Step 3.* The binding of the phosphate group induces a second conformational change, passing the three Na^+ ions across the membrane, where they are now positioned facing the exterior. This new conformation has a very low affinity for the Na^+ ions, which dissociate and diffuse away, but it has a high affinity for K^+ ions and binds two of them as soon as it is free of the Na^+ ions. *Step 4.* Binding of the K^+ ions leads to another conformational change in the complex, this time leading to dissociation of the bound phosphate. Freed of the phosphate, the complex reverts to its original conformation, with the two K^+ ions exposed on the interior side of the membrane. This conformation has a low affinity for K^+ ions so that they are now released, and the complex has the conformation it started with, having a high affinity for Na^+ ions.



Endocytosis

Endocytosis, the ingestion of material by cells, is a collective term that describes three similar processes: phagocytosis, pinocytosis, and receptor-mediated endocytosis (Figure 3.21). They are pathways for specifically internalizing solid particles, small molecules and ions, and macromolecules, respectively. All require energy and thus may be considered forms of active transport.

Phagocytosis, which literally means "cell eating," is a common method of feeding among protozoa and lower metazoa. It is also the way in which white blood cells (leukocytes) engulf cellular debris and uninvited microbes or other pathogens in the blood. By phagocytosis, an area of the plasma membrane, coated externally with specific receptors and internally with actin and actin-binding proteins, forms a pocket that engulfs the solid material. The membrane-enclosed vesicle, a food vacuole or phagosome, then detaches from the cell surface and moves into the cytoplasm where it fuses with lysosomes, and its contents are digested by lysosomal enzymes.

Pinocytosis is similar to phagocytosis except that small areas of the surface membrane are invaginated into cells to form tiny vesicles. The invaginated pits and vesicles are called **caveolae** (ka-vee´o-lee). Specific binding receptors for the molecule or ion to be internalized are concentrated on the cell surface of caveolae. Pinocytosis apparently functions for intake of at least some vitamins, and similar mechanisms may be important in translocating substances from one side of a cell to the other (see "exocytosis," next heading) and internalizing signal molecules, such as some hormones or growth factors.

Receptor-mediated endocytosis is a specific mechanism for bringing large molecules within the cell. Proteins of the plasma membrane specifically bind particular molecules (termed **ligands** in this process), which may be present in the extracellular fluid in very low concentrations. The invaginations of the cell surface that bear the receptors are coated within the cell with a protein called **clathrin;** hence, they are described as **clathrin-coated pits.** As a clathrin-coated pit with its receptor-bound ligand invaginates and is brought within the cell, it is uncoated, the receptor and the ligand are dissociated, and the receptor and membrane material are recycled back to the surface membrane. Lysosomes fuse with the remaining vesicle, now called an **endosome,** and its contents are digested and absorbed into the cytoplasm. Some important proteins, peptide hormones, and cholesterol are brought into cells in this manner.

In phagocytosis, pinocytosis, and receptor-mediated endocytosis some amount of extracellular fluid is necessarily trapped in the vesicle and nonspecifically brought within the cell. We describe this as **bulk-phase endocytosis.**

Exocytosis

Just as materials can be brought into a cell by invagination and formation of a vesicle, the membrane of a vesicle can fuse with the plasma membrane and extrude its contents to the surrounding medium. This is the process of **exocytosis.** This process occurs in various cells to remove undigestible residues of substances brought in by endocytosis, to secrete substances such as hormones (Figure 3.10), and to transport a substance completely across a cellular barrier **(transcytosis),** as we just mentioned. For example, a substance may be picked up on one side of the wall of a blood vessel by pinocytosis, moved across the cell, and released by exocytosis.

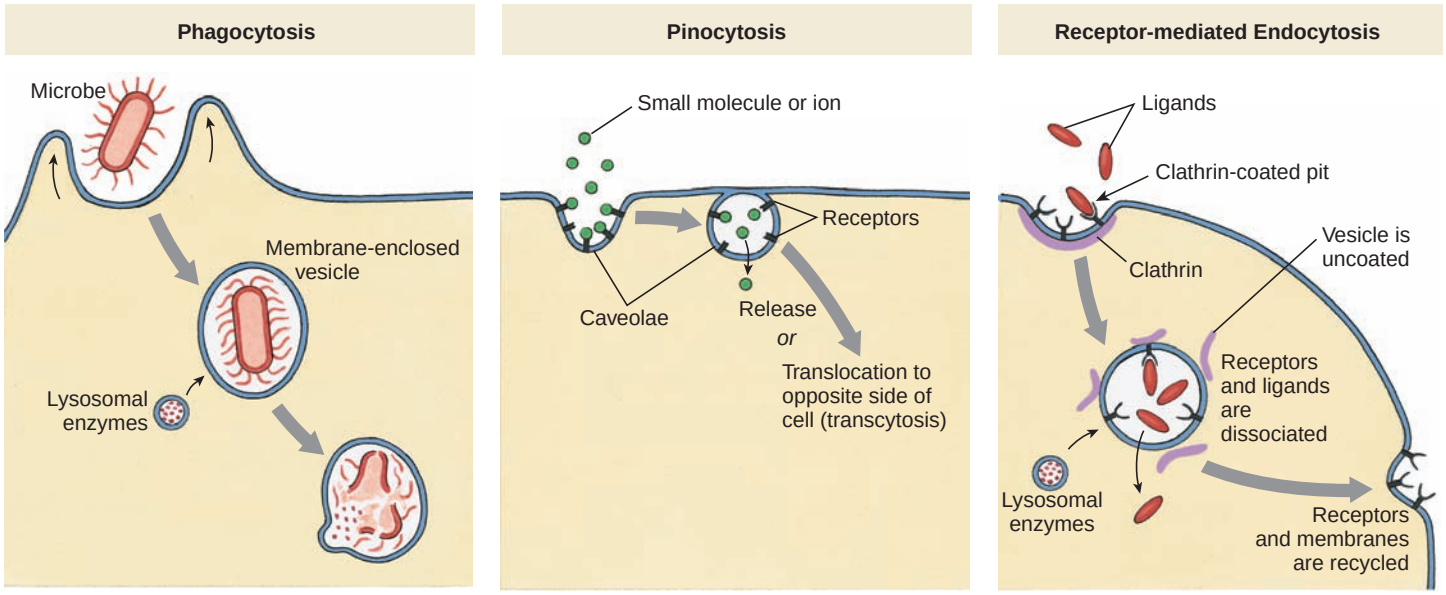


Figure 3.21

Three types of endocytosis. In phagocytosis the cell membrane binds to a large particle and extends to engulf it, forming a membrane-enclosed vesicle, a food vacuole or phagosome. In pinocytosis small areas of cell membrane, bearing specific receptors for a small molecule or ion, invaginate to form caveolae. Receptor-mediated endocytosis is a mechanism for selective uptake of large molecules in clathrin-coated pits. Binding of the ligand to the receptor on the surface membrane stimulates invagination of pits.

Actin and actin-binding proteins are now to be essential cytoskeletal components in the processes of endocytosis and exocytosis.

MITOSIS AND CELL DIVISION

All cells arise from the division of preexisting cells. All cells found in most multicellular organisms originated from the division of a single cell, a **zygote,** which is the product of union (fertilization) of an **egg** and a **sperm (gametes).** Cell division provides the basis for one form of growth, for both sexual and asexual reproduction, and for transmission of hereditary qualities from one cell generation to another cell generation.

In the formation of **body cells (somatic cells)** the process of nuclear division is **mitosis.** By mitosis each "daughter cell" is ensured a complete set of genetic instructions. Mitosis is a delivery system for distributing the chromosomes and the DNA they contain to continuing cell generations. Thus, a single zygote divides by mitosis to produce a multicellular organism, and damaged cells are replaced by mitosis during wound healing. As an animal grows, its somatic cells differentiate and assume different functions and appearances because of differential gene action. Although most of the genes in specialized cells remain silent and unexpressed throughout the lives of those cells, every cell possesses a complete genetic complement. Mitosis ensures equality of genetic potential; later, other processes direct the orderly expression of genes during embryonic development by selecting from the genetic instructions that each cell contains. (These fundamental properties of cells of multicellular organisms are discussed further in Chapter 8.)

In animals that reproduce **asexually** (see Chapter 7), mitosis is the only mechanism for the transfer of genetic information from parent to progeny, and thus the progeny are genetically identical to the parents in this case. In animals that reproduce **sexually** (see Chapter 7), the parents must produce **sex cells** (gametes or germ cells) that contain only half the usual number of chromosomes, so that progeny formed by the union of gametes will not contain double the parental number of chromosomes. This requires a special type of *reductional* division called **meiosis,** described in Chapter 5 (p. 77).

Structure of Chromosomes

As mentioned on page 41, DNA in eukaryotic cells occurs in chromatin, a complex of DNA with associated protein. Chromatin is organized into a number of discrete linear bodies called **chromosomes** (color bodies), so named because they stain deeply with certain biological dyes. In cells that are not dividing, chromatin is loosely organized and dispersed, so that individual chromosomes cannot be distinguished by light microscopy (see Figure 3.24, Interphase). Before division the chromatin becomes more compact, and chromosomes can be recognized and their individual morphological characteristics determined. They are of varied lengths and shapes, some bent and some rodlike. Their number is constant for a species, and every body cell (but not the germ cells) has the same number of chromosomes regardless of a cell's function. A human, for example, has 46 chromosomes in each somatic cell.

During mitosis (nuclear division) chromosomes shorten further and become increasingly condensed and distinct, and each assumes a shape partly characterized by the position of a constriction, the **centromere** (Figure 3.22). The centromere is the location of the **kinetochore,** a disc of proteins that binds with microtubules of the spindle that forms during mitosis.

When chromosomes become condensed, the DNA is inaccessible, such that transcription (see Chapter 5, p. 93) cannot occur. Chromosomal condensation does, however, enable a cell to distribute chromosomal material efficiently and equally to daughter cells during cell division.

Phases in Mitosis

There are two distinct stages of cell division: division of nuclear chromosomes **(mitosis)** and division of cytoplasm **(cytokinesis).** Mitosis (that is, chromosomal segregation) is certainly the most obvious and complex part of cell division and that of greatest interest to cytologists. Cytokinesis normally immediately follows mitosis, although occasionally the nucleus may divide a number of times without a corresponding division of the cytoplasm. In such a case the resulting mass of protoplasm containing many nuclei is called a **multinucleate cell.** An example is the giant resorptive cell type of bone (osteoclast), which may contain 15 to 20 nuclei. Sometimes a multinucleate mass is formed by cell fusion rather than nuclear proliferation. This arrangement is called a **syncytium.** An example is vertebrate skeletal muscle, which is composed of multinucleate fibers formed by fusion of numerous embryonic cells.

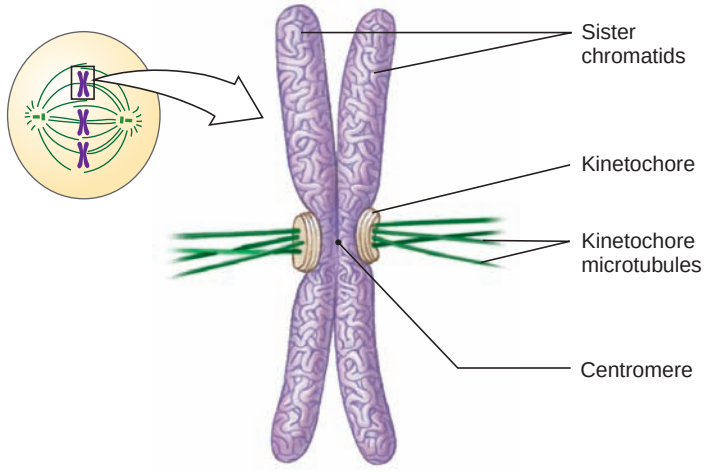


Figure 3.22

Structure of a metaphase chromosome. Sister chromatids are still attached at their centromere. Each chromatid has a kinetochore, to which the kinetochore microtubules or fibers are attached. Kinetochore microtubules from each chromatid run to one of the centrosomes, which are located at opposite poles.

Mitosis is artificially divided into four successive stages or phases, although one stage merges into the next without sharp lines of transition. These phases are prophase, metaphase, anaphase, and telophase (Figures 3.23 and 3.24). When cells are not actively dividing, they are in interphase, a major part of the cell cycle described in some detail on page 54.

Prophase

At the beginning of prophase, centrosomes (along with their centrioles) replicate, the nuclear envelope disintegrates, and the two centrosomes migrate to opposite poles of the cell (Figure 3.23). At the same time, microtubules are manufactured between the two centrosomes to form a football-shaped **spindle,** so named because of its resemblance to nineteenth-century wooden spindles, used to twist thread together in spinning. Other microtubules radiate outward from each centrosome to form **asters.** The asters will develop into the microtubular portion of the cytoskeleton in each new daughter cell formed during cell division.

At this time the diffuse nuclear chromatin condenses to form visible chromosomes. These actually consist of two identical sister **chromatids** (Figure 3.22) formed by DNA replication (see Chapter 5, p. 93) during interphase and joined at their centromere. Dynamic spindle microtubules repeatedly extend and retract from each centrosome. When a microtubule encounters a kinetochore, it binds to the kinetochore, ceases extending and retracting, and is now called a **kinetochore microtubule** or **fiber.** Thus, centrosomes send out "feelers" to find chromosomes.

Metaphase

Each centromere has two kinetochores, and each of the kinetochores is attached to one of the centrosomes by a kinetochore fiber. By a kind of tug-of-war during metaphase, the condensed sister chromatids are moved to the middle of the nuclear region, called the **metaphase plate** (Figures 3.23 and 3.24). The centromeres line up precisely in this region with the arms of the sister chromatids trailing off randomly in various directions.

Anaphase

The single centromere that has held two sister chromatids together now splits so that the two sister chromatids separate to become two independent chromosomes, each with its own centromere. The chromosomes move toward their respective poles, pulled by their kinetochore fibers. The arms of each chromosome trail behind as the microtubules shorten to drag a complete set of chromosomes toward each pole of the cell (Figures 3.23 and 3.24). Present evidence indicates that the force moving the chromosomes is disassembly of the tubulin subunits at the kinetochore end of the each microtubule.

As chromosomes approach their respective centrosomes, the centrosomes move farther apart as the microtubules are gradually disassembled.

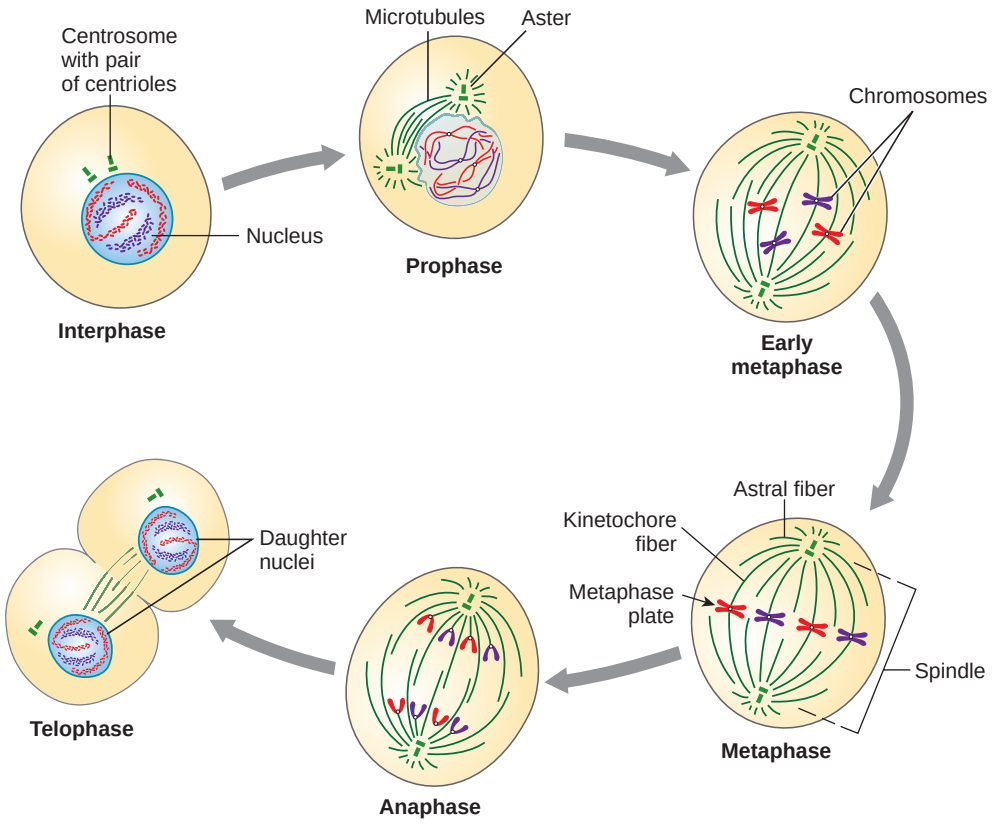


Figure 3.23

Stages of mitosis, showing division of a cell with two pairs of chromosomes. One chromosome of each pair is shown in red.

Telophase

When daughter chromosomes reach their respective poles, telophase has begun (Figures 3.23 and 3.24). The daughter chromosomes are crowded together and stain intensely with histological stains. Spindle fibers disappear and the chromosomes lose their identity, reverting to a diffuse chromatin network characteristic of an interphase nucleus. Finally, nuclear membranes reappear around the two daughter nuclei.

Cytokinesis: Cytoplasmic Division

During the final stages of nuclear division a **cleavage furrow** appears on the surface of a dividing cell and encircles it at the midline of the spindle (Figures 3.23 and 3.24). The cleavage furrow deepens and pinches the plasma membrane as though it were being tightened by an invisible

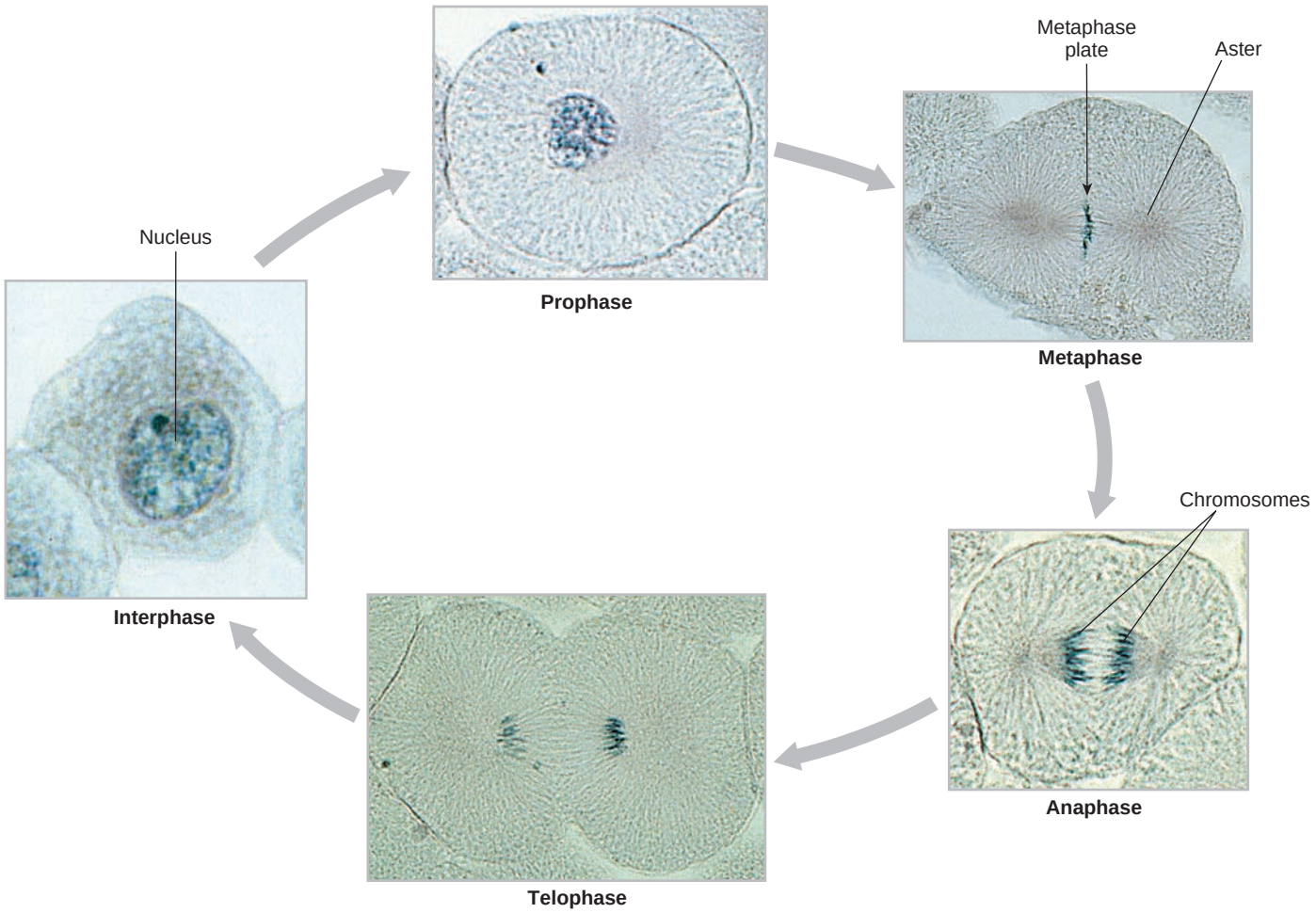


Figure 3.24
Stages of mitosis in whitefish.

rubber band. Actin microfilaments are present just beneath the surface in the furrow between the cells. Interaction with myosin and other actin-binding proteins, similar to muscle contraction mechanisms (p. 658), draws the furrow inward. Finally, the infolding edges of the plasma membrane meet and fuse, completing cell division.

Cell Cycle

Cycles are conspicuous attributes of life. The descent of a species through time is in a very real sense a sequence of life cycles. Similarly, cells undergo cycles of growth and replication as they repeatedly divide. A cell cycle is the interval between one cell division and the next (Figure 3.25).

Actual nuclear division, or mitosis, occupies only about 5% to 10% of the cell cycle; the rest of the cell's time is spent in **interphase,** the stage between nuclear divisions. For many years it was thought that interphase was a period of rest, because nuclei appeared inactive when observed by ordinary light microscopy. In the early 1950s new techniques for revealing DNA replication in nuclei were introduced at the same time that biologists came to appreciate fully the significance of DNA as the genetic material. It was then discovered that DNA replication occurred during the interphase stage. Further studies revealed that many other protein and nucleic acid components essential to normal cell function, growth, and division were synthesized during the seemingly quiescent interphase period.

Replication of DNA occurs during a phase called the S phase (period of synthesis). In mammalian cells in tissue culture, the S phase lasts about six of the 18 to 24 hours required to complete one cell cycle. In this phase both strands of a DNA molecule must replicate; new complementary partners are synthesized for each strand so that two identical DNA molecules are produced from the original strand (see Chapter 5, p. 93). These complementary partners are the sister chromatids that are separated during the next mitosis.

The S phase is preceded and succeeded by G_1 and G_2 phases, respectively (G stands for "gap"), during which no DNA synthesis is occurring. For most cells, G_1 is an important preparatory stage for the replication of DNA that follows. During G_1, transfer RNA, ribosomes, messenger RNA, and many enzymes are synthesized. During G_2, spindle and aster proteins

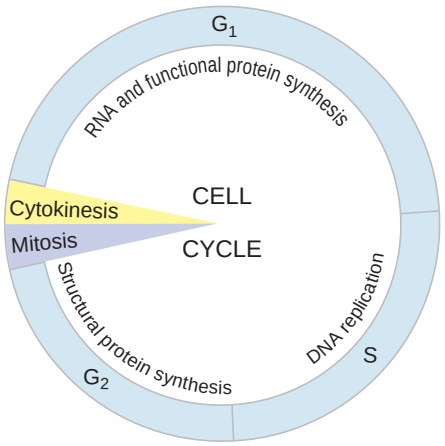


Figure 3.25

Cell cycle, showing relative duration of recognized phases. S, G_1, and G_2 are phases within interphase; S, synthesis of DNA; G_1, presynthetic phase; G_2, postsynthetic phase. After mitosis and cytokinesis the cell may go into an arrested, quiescent stage known as G_0. Actual duration of the cycle and the different phases varies considerably in different cell types.

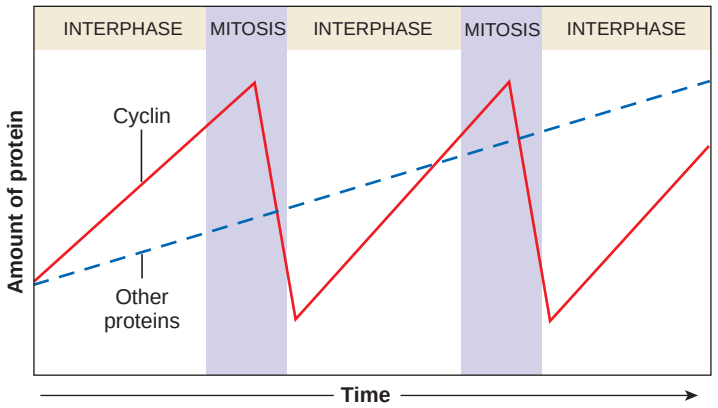


Figure 3.26

Variations in the level of cyclin in dividing cells of early sea urchin embryos. Cyclin binds with its cyclin-dependent kinase to activate the enzyme.

are synthesized in preparation for chromosome separation during mitosis. G_1 is typically of longer duration than G_2, although there is much variation in different cell types. Embryonic cells divide very rapidly because there is no cell growth between divisions, only subdivision of mass. DNA synthesis may proceed a hundred times more rapidly in embryonic cells than in adult cells, and the G_1 phase is very shortened. As an organism develops, the cycle of most of its cells lengthens, and many cells may be arrested for long periods of time in G_1 and enter a nonproliferative or quiescent phase called G_0. Most neurons or nerve cells, for example, divide no further and are essentially in a permanent G_0.

The events in cell cycles are exquisitely regulated. Transitions during cell cycles are mediated by enzymes called **cyclin-dependent kinases (cdk's)** and regulatory protein subunits that activate them, called **cyclins.** In general, kinases are enzymes that add phosphate groups to other proteins to activate or inactivate them, and kinases themselves may require activation. Cdk's become active only when they are bound to the appropriate cyclin, and cyclins are synthesized and degraded during each cell cycle (Figure 3.26). It seems likely that phosphorylation and dephosphorylation of specific cdk's and their interaction with phase-specific cyclins regulates the passage from one cell cycle phase to the next. Current research focuses on the checkpoints that regulate this phase to phase passage since disregulation of these mechanisms has been implicated in cancer.

Flux of Cells

Cell division is important for growth, for replacement of cells lost to natural attrition and for wound healing. Cell division is especially rapid during early development of an organism. At birth a human infant has about 2 trillion cells from repeated division of a single fertilized egg or zygote. This immense number could be attained by just 42 cell divisions, with each generation dividing once every six to seven days. With only five more cell divisions, the cell number would increase to approximately 60 trillion, the number of cells in a human adult weighing 75 kg. Of course no organism develops in this machinelike manner. Cell division is rapid during early embryonic development, then slows with age. Furthermore, different cell populations divide at widely different rates. In some the average period between divisions is measured in hours, whereas in others it is measured in days, months, or even years. Some cells in the central nervous system stop dividing after the early months of fetal development and generally persist without further division for the life of the individual. Muscle cells also stop dividing during the third month of fetal development, and most future growth depends on enlargement of fibers already present.

In other tissues that are subject to abrasion, lost cells must be constantly replaced. It is estimated that in humans about 1% to 2% of all body cells—a total of 100 billion—are shed daily. Mechanical rubbing wears away the outer cells of the skin, and food in the alimentary canal removes lining epithelial cells. In addition, the restricted life cycle of blood cells involves enormous numbers of replacements. Such lost cells are replaced by mitosis.

Normal development, however, does entail cell death in which cells are not replaced. As cells age, they accumulate damage from destructive oxidizing agents and eventually die. Other cells undergo a programmed cell death, or **apoptosis** (a-puh-TOE-sis) (Gr. *apo-,* from, away from; + *ptosis,* a falling), which is in many cases necessary for continued health and development of an organism. For example, during embryonic development of vertebrates, fingers and toes develop as tissues between them die, excess immune cells that would attack the body's own tissues "commit suicide," and nerve cells die to create cerebral convolutions. Apoptosis consists of a well-coordinated and predictable series of events: the cells shrink, disintegrate, and their components are absorbed up by surrounding cells.

SUMMARY

Cells are the basic structural and functional units of all living organisms. Eukaryotic cells differ from the prokaryotic cells of bacteria and Archaebacteria in several respects, the most distinctive of which is presence of a membrane-bound nucleus containing hereditary material composed of DNA, bound to proteins to form chromatin. Chromatin consists of flexible, linear chromosomes, which become condensed and visible only during cell division.

Cells are surrounded by a plasma membrane that regulates the flow of molecules between the cell and its surroundings. The nucleus, enclosed by a double membrane, contains chromatin, associated proteins, and one or more nucleoli. Outside the nuclear envelope is cell cytoplasm, subdivided by a membranous network, the endoplasmic reticulum. Among the organelles within cells are the Golgi complex, mitochondria, lysosomes, and other membrane-bound vesicles. The cytoskeleton is composed of microfilaments (actin), microtubules (tubulin), and intermediate filaments (several types). Cilia and flagella are hairlike, motile appendages that contain microtubules. Ameboid movement by pseudopodia operates by means of the assembly and disassembly of actin microfilaments. Tight junctions, adhesion junctions, desmosomes, and gap junctions are structurally and functionally distinct connections between cells.

Membranes in a cell are composed of a phospholipid bilayer and other materials including cholesterol and transmembrane proteins. Hydrophilic ends of the phospholipid molecules are on the outer and inner surfaces of membranes, and the fatty acid portions are directed inward, toward each other, to form a hydrophobic core.

Substances can enter cells by diffusion, mediated transport, and endocytosis. Osmosis is diffusion of water through channels in a selectively permeable membrane as a result of osmotic pressure. Solutes to which the membrane is impermeable require channels or a transporter molecule to traverse the membrane. Water and ions move through open channels by diffusion (in the direction of a concentration gradient). Transport-mediated systems include facilitated diffusion and active transport (against a concentration gradient, which requires energy). Endocytosis includes bringing droplets (pinocytosis) or particles (phagocytosis) into a cell. In exocytosis the process of endocytosis is reversed.

The cell cycle in eukaryotes includes mitosis, or division of the nuclear chromosomes, and cytokinesis, the division of the cytoplasm, and interphase. During interphase, G_1, S, and G_2 phases are recognized, and the S phase is the time when DNA is synthesized (the chromosomes are replicated).

Cell division is necessary for the production of new cells from preexisting cells and is the basis for growth in multicellular organisms. During this process, replicated nuclear chromosomes divide by mitosis followed by cytoplasmic division or cytokinesis.

The four stages of mitosis are prophase, metaphase, anaphase, and telophase. In prophase, replicated chromosomes composed of sister chromatids condense into recognizable bodies. A spindle forms between the centrosomes as they separate to opposite poles of the cell. At the end of prophase the nuclear envelope disintegrates, and the kinetochores of each chromosome become attached to the centrosomes by microtubules (kinetochore fibers). At metaphase the sister chromatids are moved to the center of the cell, held there by the kinetochore fibers. At anaphase the centromeres divide, and the sister chromatids are pulled apart by the attached kinetochore fibers of the mitotic spindle. At telophase the sister chromatids, now called chromosomes, gather in the position of the nucleus in each cell and revert to a diffuse chromatin network. A nuclear membrane reappears, and cytokinesis occurs. At the end of mitosis and cytokinesis, two cells genetically identical to the parent cell have been produced.

Cells divide rapidly during embryonic development, then more slowly with age. Some cells continue to divide throughout the life of an animal to replace cells lost by attrition and wear, whereas others, such as nerve and muscle cells, complete their division during early development and many do not divide again. Some cells undergo a programmed cell death, or apoptosis.

REVIEW QUESTIONS

1. Explain the difference (in principle) between a light microscope and a transmission electron microscope.
2. Briefly describe the structure and function of each of the following: plasma membrane, chromatin, nucleus, nucleolus, rough endoplasmic reticulum (rough ER), Golgi complex, lysosomes, mitochondria, microfilaments, microtubules, intermediate filaments, centrioles, basal body (kinetosome), tight junction, gap junction, desmosome, glycoprotein, microvilli.
3. Name two functions each for actin and for tubulin.
4. Distinguish among cilia, flagella, and pseudopodia.
5. What are the functions of each of the main constituents of the plasma membrane?
6. Our current concept of the plasma membrane is known as the fluid-mosaic model. Why?
7. You place some red blood cells in a solution and observe that they swell and burst. You place some cells in another solution, and they shrink and become wrinkled. Explain what has happened in each case.
8. Explain why a beaker containing a salt solution, placed on a table in your classroom, can have a high osmotic pressure, yet be subjected to a hydrostatic pressure of only one atmosphere.
9. The plasma membrane is an effective barrier to molecular movement across it, yet many substances do enter and leave the cell. Explain the mechanisms through which this is accomplished and comment on the energy requirements of these mechanisms.
10. Distinguish among phagocytosis, pinocytosis, receptor-mediated endocytosis, and exocytosis.
11. Define the following: chromosome, centromere, centrosome, kinetochore, mitosis, cytokinesis, syncytium.
12. Explain phases of the cell cycle, and comment on important cellular processes that characterize each phase. What is G_0?
13. Name the stages of mitosis in order, and describe the behavior and structure of the chromosomes at each stage.
14. Briefly describe ways that cells may die during the normal life of a multicellular organism.

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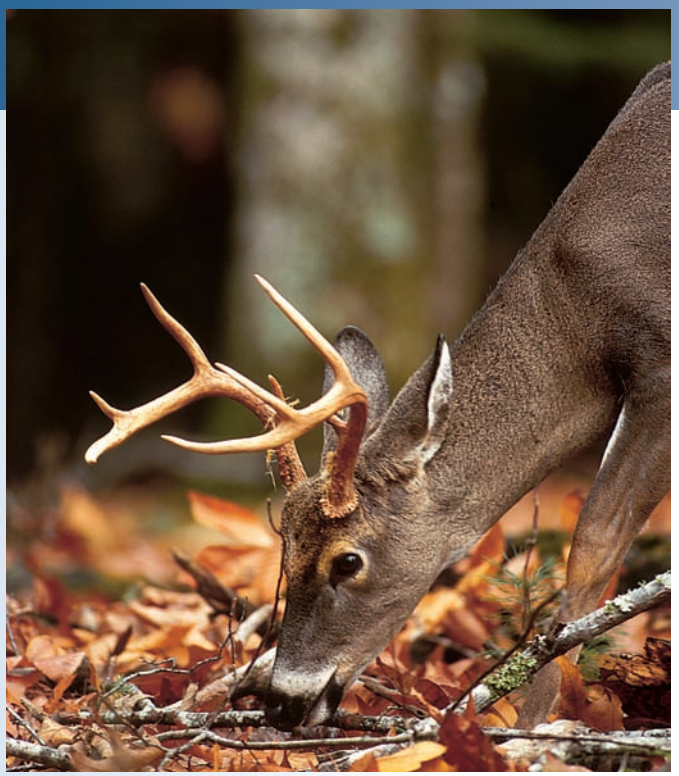
CHAPTER

4

Cellular Metabolism



White-tailed deer (Odocoileus virginianus) *foraging for acorns.*

Deferring the Second Law

Living systems appear to contradict the second law of thermodynamics, which states that energy in the universe has direction and that it has been, and always will be, running down. In effect all forms of energy inevitably will be degraded to heat. This increase in disorder, or randomness, in any closed system is termed entropy. Living systems, however, *decrease* their entropy by *increasing* the molecular orderliness of their structure. An organism becomes vastly more complex during its development from fertilized egg to adult. The second law of thermodynamics, however, applies to closed systems, and living organisms are not closed systems. Animals grow and maintain themselves by borrowing free energy from the environment. When a deer feasts on the acorns and beechnuts of summer, it transfers potential energy, stored as chemical bond energy in the nuts' tissues, to its own body. Then, in step-by-step sequences called biochemical pathways, this energy is gradually released to fuel the deer's many activities. In effect, the deer decreases its own internal entropy by increasing the entropy of its food. The orderly structure of the deer is not permanent, however, but will be dissipated when it dies.

The ultimate source of this energy for the deer—and for almost all life on earth—is the sun (Figure 4.1). Sunlight is captured by green plants, which fortunately accumulate enough chemical bond energy to sustain both themselves and animals that feed on them. Thus the second law is not violated; it is simply held at bay by life on earth, which uses the continuous flow of solar energy to maintain a biosphere of high internal order, at least for the period of time that life exists.

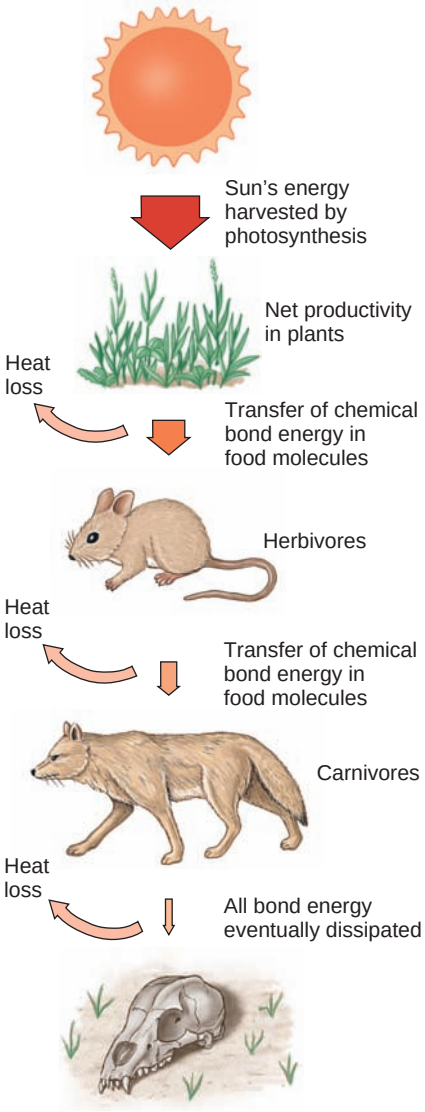


Figure 4.1

Solar energy sustains virtually all life on earth. With each energy transfer, however, about 90% of the energy is lost as heat.

All cells must obtain energy, synthesize their own internal structure, control much of their own activity, and guard their boundaries. **Cellular metabolism** refers to the collective chemical processes that occur within living cells to accomplish these activities. Although the enormous number of reactions in their aggregate are extremely complex, the central metabolic routes through which matter and energy are channeled appear to be conserved by the majority of living organisms.

ENERGY AND THE LAWS OF THERMODYNAMICS

The concept of energy is fundamental to all life processes. We usually express energy as the capacity to do work, to bring about change. Yet energy is a somewhat abstract quantity that is difficult to define and elusive to measure. Energy cannot be seen; it can be identified only by how it affects matter.

Energy can exist in either of two states: kinetic or potential. **Kinetic energy** is the energy of motion. **Potential energy** is stored energy, energy that is not doing work but has the capacity to do so. Energy can be transformed from one state to another. Especially important for living organisms is chemical energy, a form of potential energy stored in the chemical bonds of molecules. Chemical energy can be tapped when bonds are rearranged to release kinetic energy. Much of the work done by living organisms involves conversion of potential energy to kinetic energy.

Conversion of one form of energy to another is governed by the two laws of thermodynamics. The **first law of thermodynamics** states that energy cannot be created or destroyed. It can change from one form to another, but the total amount of energy remains the same. In short, energy is conserved. If we burn gasoline in an engine, we do not create new energy but merely convert the chemical energy in gasoline to another form, in this example, mechanical energy and heat. The **second law of thermodynamics,** introduced in the prologue to this chapter, concerns the transformation of energy. This fundamental law states that a closed system moves toward increasing disorder, or entropy, as energy is dissipated from the system (Figure 4.2). Living systems, however, are open systems that not only maintain their organization but also increase it, as during the development of an animal from egg to adult.

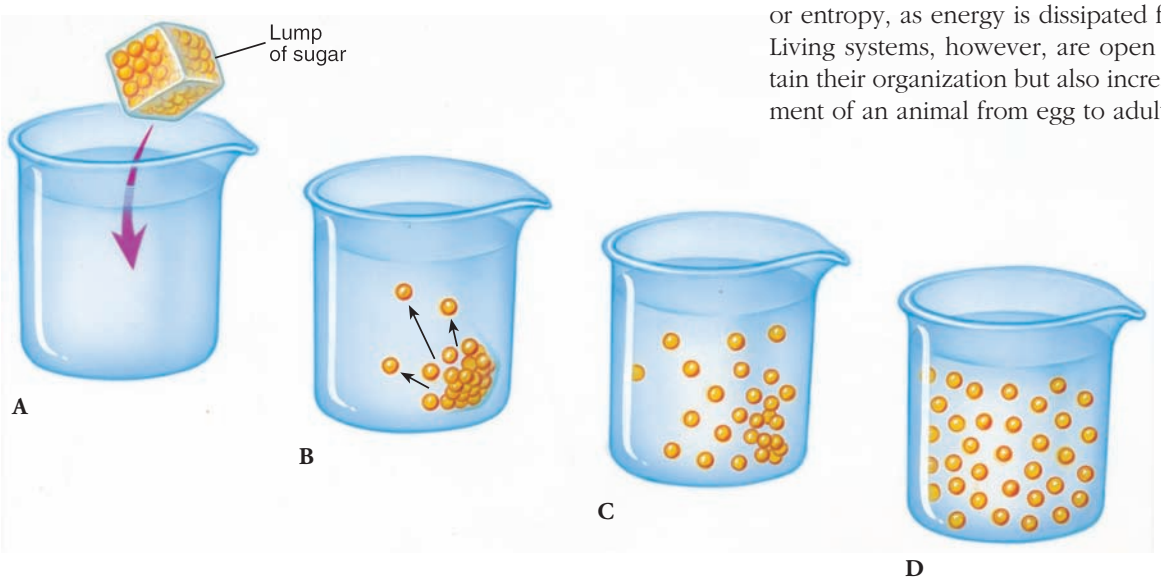
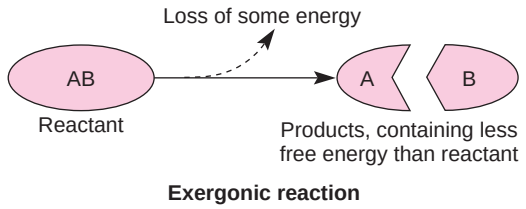


Figure 4.2

Diffusion of a solute through a solution, an example of entropy. When the solute (sugar molecules) is first introduced into a solution, the system is ordered and unstable **(B).** Without energy to maintain this order, the solute particles become distributed into solution, reaching a state of disorder (equilibrium) **(D).** Entropy has increased from left diagram to right diagram.

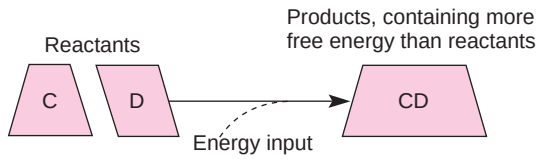
Free Energy

To describe the energy changes of chemical reactions, biochemists use the concept of **free energy.** Free energy is simply the energy in a system available for doing work. In a molecule, free energy equals the energy present in chemical bonds minus the energy that cannot be used. The majority of reactions in cells release free energy and are called **exergonic** (Gr. *ex,* out, + *ergon,* work). Such reactions are spontaneous and always proceed "downhill" since free energy is lost from the system. Thus:



Exergonic reaction

However, many important reactions in cells require the addition of free energy and are said to be **endergonic** (Gr. *endon,* within, + *ergon,* work). Such reactions have to be "pushed uphill" because the products store more energy than the reactants.



Endergonic reaction

Described on page 62, ATP is a ubiquitous, energy-rich intermediate used by organisms to power important uphill reactions such as those required for active transport of molecules across membranes (see Chapter 3, p. 49) and cellular synthesis.

THE ROLE OF ENZYMES

Enzymes and Activation Energy

For any reaction to occur, even exergonic ones that tend to proceed spontaneously, chemical bonds first must be destabilized. Some energy, termed the **activation energy,** must be supplied before the bond is stressed enough to break. Only then will an overall loss of free energy and formation of reaction products occur. This requirement can be likened to the energy needed to push a ball over the crest of a hill before it will roll spontaneously down the other side, the ball liberating its energy as it descends (Figure 4.3, top panel).

One way to activate chemical reactants is to raise the temperature. This increases the rate of molecular collisions and pushes chemical bonds apart. Thus, heat can impart the necessary activation energy to make a reaction proceed. However metabolic reactions must occur at biologically tolerable temperatures, which are usually too low to allow reactions to proceed at a rate capable of sustaining life. Instead, living systems have evolved a different strategy: they employ **catalysts.**

Catalysts are chemical substances that accelerate reaction rates without affecting the products of the reaction and without being altered or destroyed by the reaction. A catalyst cannot make an energetically impossible reaction happen; it simply accelerates a reaction that would proceed at a very slow rate otherwise.

Enzymes are catalysts of the living world. The special catalytic talent of an enzyme is its power to reduce the amount of activation energy required for a reaction. In effect, an enzyme steers the reaction through one or more intermediate steps, each of which requires much less activation energy than that required for a single-step reaction (Figure 4.3). Note that enzymes do not supply the activation energy. Instead they lower the activation energy barrier, making a reaction more likely to proceed. Enzymes affect only the reaction rate. They do not in any way alter the free energy change of a reaction, nor do they change the proportions of reactants and products in a reaction.

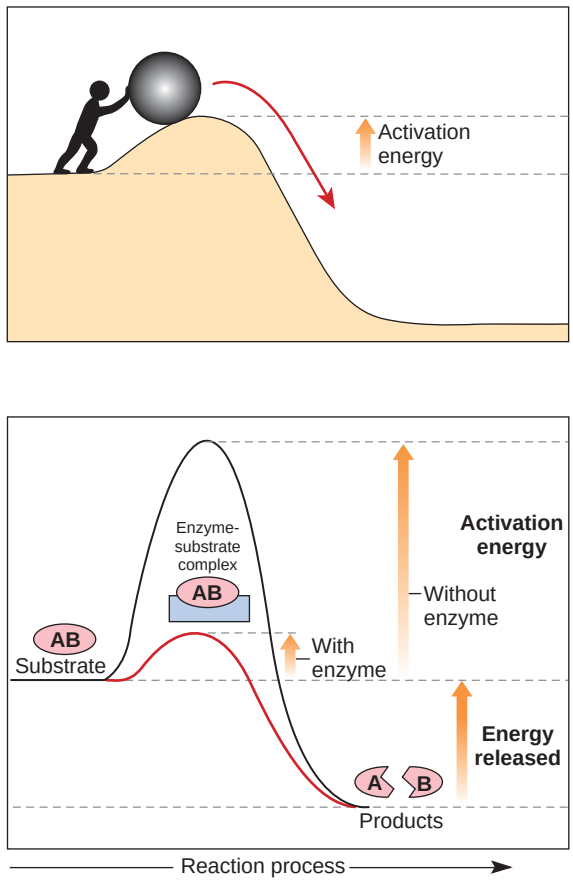


Figure 4.3

Energy changes during enzyme catalysis of a substrate. The overall reaction proceeds with a net release of energy (exergonic). In the absence of an enzyme, substrate is stable because of the large amount of activation energy needed to disrupt strong chemical bonds. The enzyme reduces the energy barrier by forming a chemical intermediate with a much lower internal energy state.

Nature of Enzymes

Enzymes are complex molecules that vary in size from small, simple proteins with a molecular weight of 10,000 to highly complex molecules with molecular weights up to 1 million. Many enzymes are pure proteins—highly folded and interlinked chains of amino acids. Other enzymes require participation of small nonprotein groups called **cofactors** to perform their enzymatic function. In some cases these cofactors are metallic ions (such as ions of iron, copper, zinc, magnesium, potassium, and calcium) that form a functional part of the enzyme. Examples are carbonic anhydrase (see Chapter 31, p. 705), which contains zinc; the cytochromes (some enzymes of the electron transport chain, p. 67), which contain iron; and troponin (a muscle contraction enzyme, see Chapter 29, p. 658), which contains calcium. Another class of cofactors, called **coenzymes,** is organic. Coenzymes contain groups derived from vitamins, most of which must be supplied in the diet. All B-complex vitamins are coenzymatic compounds. Since animals have lost the ability to synthesize the vitamin components of coenzymes, it is obvious that a vitamin deficiency can be serious. However, unlike dietary fuels and nutrients that must be replaced after they are burned or assembled into structural materials, vitamin components of coenzymes are recovered in their original form and are used repeatedly. Examples of coenzymes that contain vitamins are nicotinamide adenine dinucleotide (NAD), which contains the vitamin nicotinic acid (niacin); coenzyme A, which contains the vitamin pantothenic acid; and flavin adenine dinucleotide (FAD), which contains riboflavin (vitamin B_2).

Action of Enzymes

An enzyme functions by associating in a highly specific way with its **substrate,** the molecule whose reaction it catalyzes. Enzymes bear an active site located within a cleft or pocket and that contains a unique molecular configuration. The active site has a flexible surface that enfolds and conforms to the substrate (Figure 4.4). The binding of enzyme to substrate forms an **enzyme-substrate complex (ES complex),** in which the substrate is secured by covalent bonds to one or more points in the active site of the enzyme. The ES complex is not strong and will quickly dissociate, but during this fleeting moment the enzyme provides a unique chemical environment that stresses certain chemical bonds in the substrate so that much less energy is required to complete the reaction.

If the formation of an enzyme-substrate complex is so rapidly followed by dissociation, how can biochemists be certain that an ES complex exists? The original evidence offered by Leonor Michaelis in 1913 is that, when the substrate concentration is increased while the enzyme concentration is held constant, the reaction rate reaches a maximum velocity. This *saturation effect* is interpreted to mean that all catalytic sites become filled at high substrate concentration. A saturation effect is not seen in uncatalyzed reactions. Other evidence includes the observation that the ES complex displays unique spectroscopic characteristics not displayed by either the enzyme or the substrate alone. Furthermore, some ES complexes can be isolated in pure form, and at least one kind (nucleic acids and their polymerase enzymes) has been directly visualized by electron microscopy.

Enzymes that engage in crucial energy-providing reactions of cells often proceed constantly and usually operate in sets rather than in isolation. For example, conversion of glucose to carbon dioxide and water proceeds through 19 reactions, each requiring a specific enzyme. Such crucial enzymes occur in

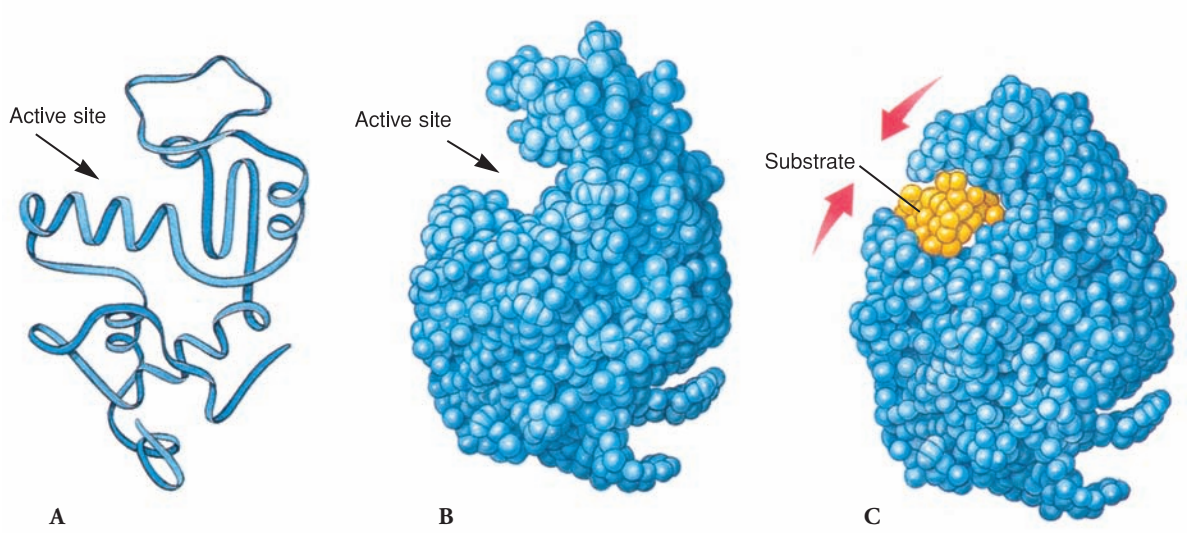


Figure 4.4

How an enzyme works. The ribbon model **(A)** and the space-filling model **(B)** show that the enzyme lysozyme bears a pocket containing the active site. When a chain of sugars (substrate) enters the pocket **(C),** the protein enzyme changes shape slightly so that the pocket enfolds the substrate and conforms to its shape. This positions the active site (amino acids in the protein) next to a bond between adjacent sugars in the chain, causing the sugar chain to break.

relatively high concentrations in cells, and they may implement quite complex and highly integrated enzymatic sequences. One enzyme performs the first step, then another enzyme binds to the product and catalyzes the next step. This process continues until the end of the enzymatic pathway is reached. The reactions are said to be coupled. Coupled reactions are explained in a section on chemical energy transfer by ATP (see this page).

Specificity of Enzymes

One of the most distinctive attributes of enzymes is their high specificity. Specificity is a consequence of the exact molecular fit required between enzyme and substrate. Furthermore, an enzyme catalyzes only one reaction. Unlike reactions performed in an organic chemist's laboratory, no side reactions or by-products result. Specificity of both substrate and reaction is obviously essential to prevent a cell from being swamped with useless by-products.

However, there is some variation in degree of specificity. Some enzymes catalyze the oxidation (dehydrogenation) of only one substrate. For example, succinic dehydrogenase catalyzes the oxidation of succinic acid only (see the Krebs cycle, p. 66). Others, such as proteases (for example, pepsin and trypsin, released into the digestive tract during digestion, pp. 716 and 719), act on almost any protein, although each protease has its particular point of attack in the protein (Figure 4.5). Usually an enzyme binds one substrate molecule at a time, catalyzes its chemical change, releases the product, and then repeats the process with another substrate molecule. An enzyme may repeat this process billions of times until it is finally worn out (after a few hours to several years) and is degraded by scavenger enzymes in the cell. Some enzymes undergo successive catalytic cycles at speeds of up to a million cycles per minute, but most operate at slower rates. Many enzymes are repeatedly activated and inactivated; several mechanisms for regulating enzyme activity are well known (p. 72).

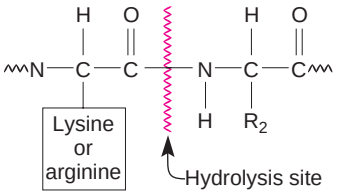


Figure 4.5
High specificity of trypsin. It splits only peptide bonds adjacent to lysine or arginine.

Enzyme-Catalyzed Reactions

Enzyme-catalyzed reactions are reversible, which is signified by double arrows between substrate and products. For example:

$$\text{Fumaric acid} + H_2O \rightleftharpoons \text{Malic acid}$$

However, for various reasons reactions catalyzed by most enzymes tend to go predominantly in one direction. For example, the proteolytic enzyme pepsin degrades proteins into amino acids (a **catabolic** reaction), but it does not accelerate the rebuilding of amino acids into any significant amount of protein (an **anabolic** reaction). The same is true of most enzymes that catalyze the cleavage of large molecules such as nucleic acids, polysaccharides, lipids, and proteins. There is usually one set of reactions and enzymes that degrade them (catabolism; Gr. *kata,* down, + *bole,* throw), but they must be resynthesized by a different set of reactions catalyzed by different enzymes (anabolism; Gr. *ana,* up, + *bole,* throw).

The net **direction** of any chemical reaction depends on the relative energy contents of the substances involved. If there is little change in chemical bond energy of substrate and products, the reaction is more easily reversible. However, if large quantities of energy are released as the reaction proceeds in one direction, more energy must be provided in some way to drive the reaction in the reverse direction. For this reason many if not most enzyme-catalyzed reactions are in practice irreversible unless the reaction is coupled to another one that makes energy available. In cells both reversible and irreversible reactions are combined in complex ways to make possible both synthesis and degradation.

Hydrolysis literally means "breaking with water." In hydrolysis reactions, a molecule is cleaved by the addition of water at the cleavage site. A hydrogen is attached to one subunit and a hydroxyl (—OH) unit is attached to another. This breaks the covalent bond between subunits. Hydrolysis is the opposite of condensation (water-losing) reactions in which subunits of molecules are linked together by removal of water. Macromolecules are built by condensation reactions.

CHEMICAL ENERGY TRANSFER BY ATP

We have seen that endergonic reactions are those that do not proceed spontaneously because their products require an input of free energy. However, an endergonic reaction may be driven by coupling the energy-requiring reaction with an energy-yielding reaction. ATP is one of the most common intermediates in **coupled reactions,** and because it can drive such energetically unfavorable reactions, it is of central importance in metabolic processes.

An ATP molecule consists of adenosine (the purine adenine and the 5-carbon sugar ribose) and a triphosphate group (Figures 4.6 and 4.7). Most free energy in ATP resides in the triphosphate group, especially in two **phosphoanhydride bonds** between the three phosphate groups called **"high-energy bonds."** Usually, only the most exposed high-energy bond is hydrolyzed to release free energy when ATP is converted to adenosine diphosphate (ADP) and inorganic phosphate.

$$ATP + H_2O \rightarrow ADP + P_i$$

where P_i represents inorganic phosphate (i = inorganic). The high-energy groups in ATP are often designated by the

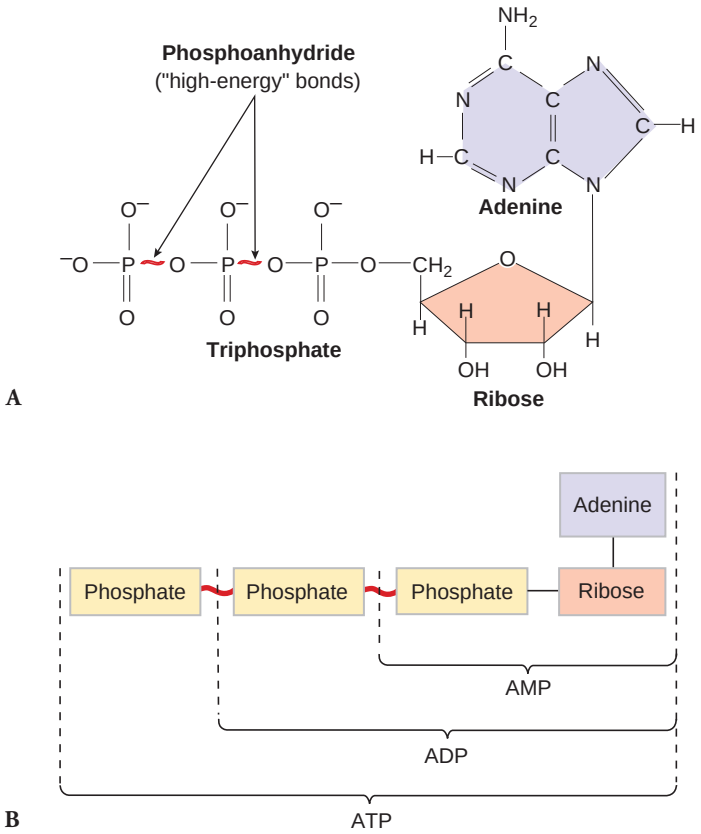


Figure 4.6

A, Structure of ATP. **B,** ATP formation from ADP and AMP. ATP: adenosine triphosphate; ADP: adenosine diphosphate; AMP: adenosine monophosphate.

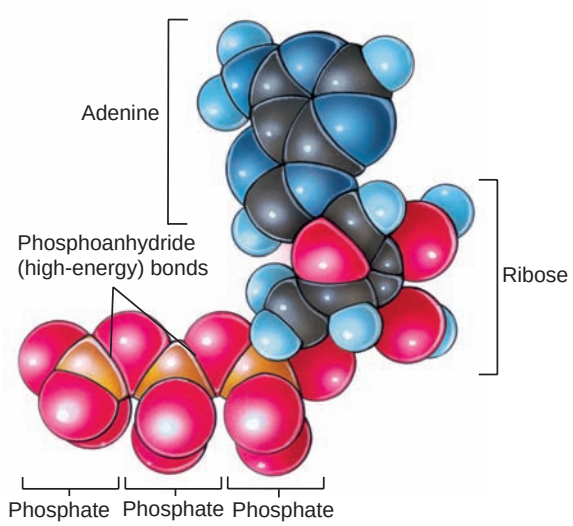


Figure 4.7

Space-filling model of ATP. In this model, carbon is shown in black; nitrogen in blue; oxygen in red; and phosphorus in orange.

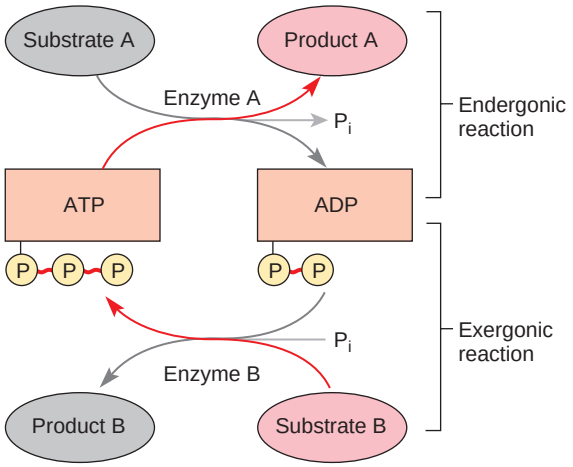


Figure 4.8

A coupled reaction. The endergonic conversion of substrate A to product A will not occur spontaneously but requires an input of energy from another reaction involving a large release of energy. ATP is the intermediate through which the energy is shuttled.

"tilde" symbol ~ (Figure 4.6). A high-energy phosphate bond is shown as ~P and a low-energy bond (such as the bond linking the triphosphate group to adenosine) as —P. Thus, ATP may be symbolized as A—P~P~P and ADP as A—P~P.

The way in which ATP can drive a coupled reaction is shown in Figure 4.8. A coupled reaction is really a system involving two reactions linked by an energy shuttle (ATP). The conversion of substrate A to product A is endergonic because the product contains more free energy than the substrate. Therefore energy must be supplied by coupling the reaction to an exergonic one, the conversion of substrate B to product B. Substrate B in this reaction is commonly called a **fuel** (for example, glucose or a lipid). Bond energy released in reaction B is transferred to ADP, which in turn is converted to ATP. ATP now contributes its phosphate-bond energy to reaction A, and ADP and P_i are produced again.

The high-energy bonds of ATP are actually rather weak, unstable bonds. Because they are unstable, the energy of ATP is readily released when ATP is hydrolyzed in cellular reactions. Note that ATP is an **energy-coupling agent** and *not* a fuel. It is not a storehouse of energy set aside for some future need. Rather it is produced by one set of reactions and is almost immediately consumed by another set. ATP is formed as it is needed, primarily by oxidative processes in mitochondria. Oxygen is not consumed unless ADP and phosphate molecules are available, and these do not become available until ATP is hydrolyzed by some energy-consuming process. *Metabolism is therefore mostly self-regulating.*

CELLULAR RESPIRATION

How Electron Transport Is Used to Trap Chemical Bond Energy

Having seen that ATP is the one common energy denominator by which most cellular machines are powered, we must ask how this energy is captured from fuel substrates. This question directs

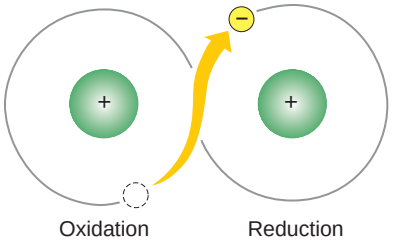


Figure 4.9
A redox pair. The molecule at left is oxidized by the loss of an electron. The molecule at right is reduced by gaining an electron.

us to an important generalization: *all cells obtain their chemical energy requirements from oxidation-reduction reactions.* This means that in the degradation of fuel molecules, hydrogen atoms (electrons and protons) are passed from electron donors to electron acceptors with a release of energy. A portion of this energy can be trapped and used to form the high-energy bonds of molecules such as ATP.

An oxidation-reduction ("redox") reaction involves a transfer of electrons from an electron donor (the reducing agent) to an electron acceptor (the oxidizing agent). As soon as the electron donor loses its electrons, it becomes oxidized. As soon as the electron acceptor accepts electrons, it becomes reduced (Figure 4.9). In other words, a reducing agent becomes oxidized when it reduces another compound, and an oxidizing agent becomes reduced when it oxidizes another compound. Thus for every oxidation there must be a corresponding reduction.

In an oxidation-reduction reaction the electron donor and electron acceptor form a redox pair:

$$\text{Electron donor (reducing agent; becomes oxidized)} \rightleftharpoons e^- + \text{Electron acceptor (oxidizing agent; becomes reduced)} + \text{Energy}$$

When electrons are accepted by the oxidizing agent, energy is liberated because the electrons move to a more stable position.

ATP may be produced in a cell when electrons flow through a series of carriers. Each carrier is reduced as it accepts electrons and then is reoxidized when it passes electrons to the next carrier in the series. By transferring electrons stepwise in this manner, energy is gradually released, and ATP is produced. Ultimately, the electrons are transferred to a **final electron acceptor.** The nature of this final acceptor is the key that determines the overall efficiency of cellular metabolism.

Aerobic Versus Anaerobic Metabolism

Heterotrophs (organisms that cannot synthesize their own food but must obtain nutrients from the environment, including animals, fungi, and many single-celled organisms) are divided into two groups based on their overall efficiency of energy production during cellular metabolism: **aerobes,** those that use molecular oxygen as the final electron acceptor, and **anaerobes,** those that employ another molecule as the final electron acceptor.

As discussed in Chapter 2, life originated in the absence of oxygen, and an abundance of atmospheric oxygen was produced only after photosynthetic organisms (autotrophs) evolved. Some strictly anaerobic organisms still exist and indeed play important roles in specialized habitats. However, evolution has favored aerobic metabolism, not only because oxygen became available, but also because aerobic metabolism is vastly more efficient in energy production than anaerobic metabolism. In the absence of oxygen, only a very small fraction of the bond energy present in nutrients can be released. For example, when an anaerobic microorganism degrades glucose, the final electron acceptor (such as pyruvic acid) still contains most of the energy of the original glucose molecule. An aerobic organism on the other hand, using oxygen as the final electron acceptor, completely oxidizes glucose to carbon dioxide and water. Almost 20 times as much energy is released when glucose is completely oxidized as when it is degraded only to the stage of pyruvic acid. Thus, an obvious advantage of aerobic metabolism is that a much smaller quantity of food is required to maintain a given rate of metabolism.

Overview of Respiration

Aerobic metabolism is more familiarly called **cellular respiration,** defined as the oxidation of fuel molecules to produce energy with molecular oxygen as the final electron acceptor. We emphasize that oxidation of fuel molecules describes the *removal of electrons* and *not* the direct combination of molecular oxygen with fuel molecules. Let us look at this process in general before considering it in more detail.

Hans Krebs, the British biochemist who contributed so much to our understanding of respiration, described three stages in the complete oxidation of fuel molecules to carbon dioxide and water (Figure 4.10). In stage I, food passing through the intestinal tract are digested into small molecules that can be absorbed into the circulation. There is no useful energy yield during digestion, which is discussed in Chapter 32. In stage II, also called **glycolysis,** most of the digested food is converted into two 3-carbon units (pyruvic acid) in the cell cytoplasm. The pyruvic acid molecules then enter mitochondria, where in another reaction they join with a coenzyme (coenzyme A or CoA) to form acetyl coenzyme A or acetyl-CoA. Some ATP is generated in stage II, but the yield is small compared with that obtained in stage III of respiration. In stage III the final oxidation of fuel molecules occurs, with a large yield of ATP. This stage takes place entirely in mitochondria. Acetyl-CoA is channeled into the Krebs cycle where the acetyl group is completely oxidized to carbon dioxide. Electrons released from acetyl groups are transferred to special carriers that pass them to electron acceptor compounds in the electron transport chain. At the end of the chain the electrons (and the protons accompanying them) are accepted by molecular oxygen to form water.

Glycolysis

We begin our journey through the stages of respiration with glycolysis, a nearly universal pathway in living organisms that converts glucose into pyruvic acid. In a series of reactions occurring

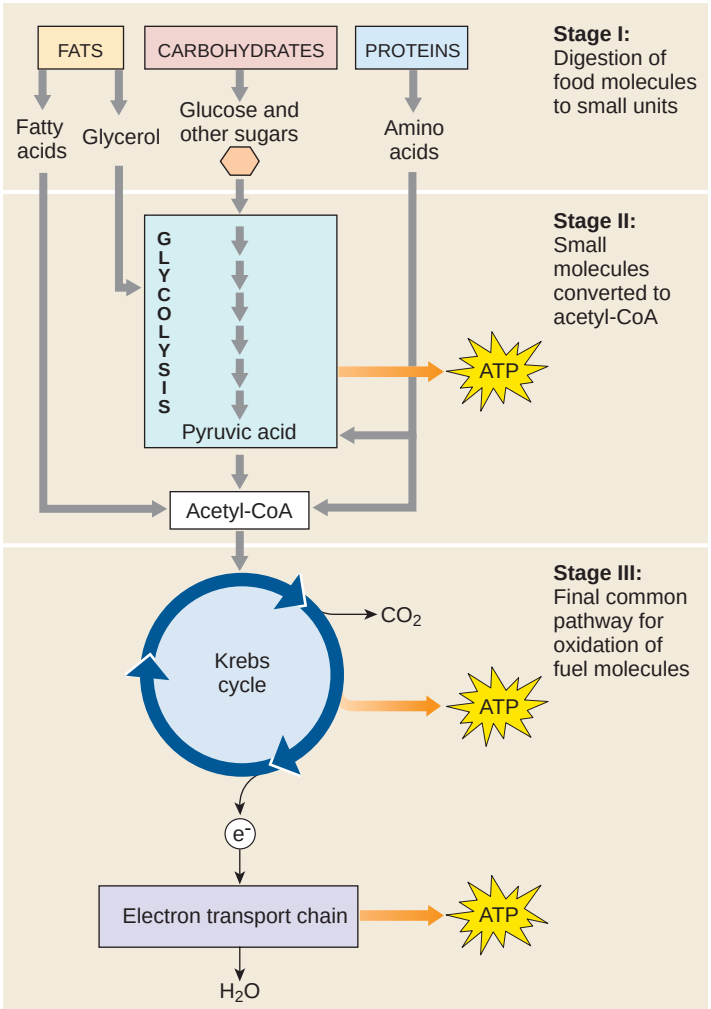


Figure 4.10

Overview of cellular respiration, showing the three stages in the complete oxidation of food molecules to carbon dioxide and water.

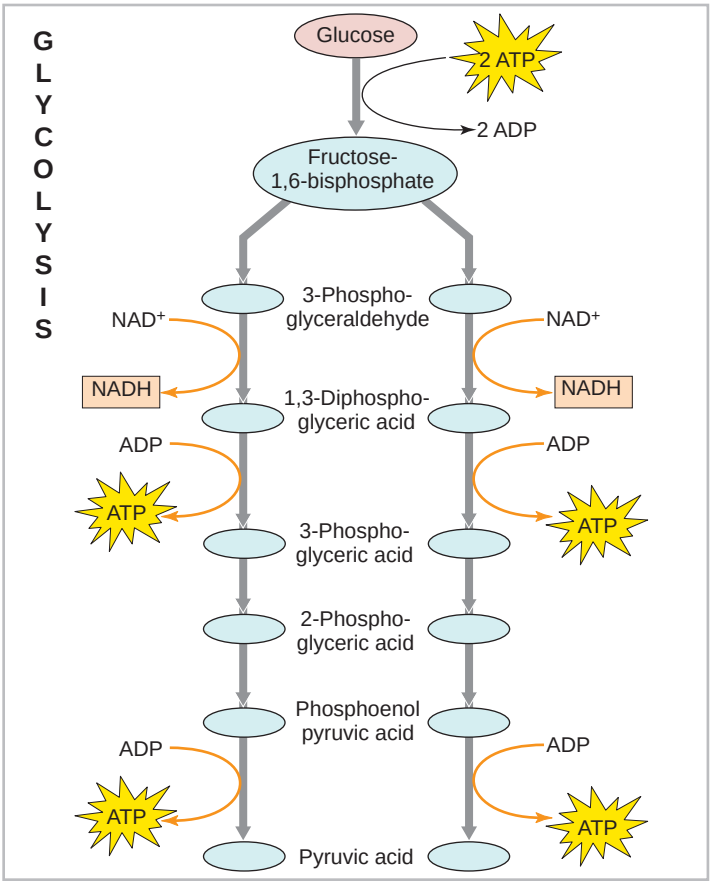


Figure 4.11

Glycolysis. Glucose is phosphorylated in two steps and raised to a higher energy level. High-energy fructose-1,6-bisphosphate is split into triose phosphates that are oxidized exergonically to pyruvic acid, yielding ATP and NADH.

in the cell cytosol, glucose and other 6-carbon monosaccharides are split into 3-carbon molecules of **pyruvic acid** (Figure 4.11). A single oxidation occurs during glycolysis, and each molecule of glucose yields two molecules of ATP. In this pathway the carbohydrate molecule is phosphorylated twice by ATP, first to glucose-6-phosphate (not shown in Figure 4.11) and then to fructose-1,6-bisphosphate. The fuel has now been "primed" with phosphate groups in this uphill portion of glycolysis and is sufficiently reactive to enable subsequent reactions to proceed. This is a kind of deficit financing required for an ultimate energy return many times greater than the original energy investment.

In the downhill portion of glycolysis, fructose-1,6-bisphosphate is cleaved into two 3-carbon sugars, which undergo an oxidation (electrons are removed), with the electrons and one of the hydrogen ions being accepted by **nicotinamide adenine dinucleotide (NAD^+,** a derivative of the vitamin niacin) to produce a reduced form called **NADH.** NADH serves as a carrier molecule to convey high-energy electrons to the final electron transport chain, where ATP is produced.

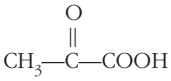
The two 3-carbon sugars next undergo a series of reactions, ending with the formation of two molecules of pyruvic acid (Figure 4.11). In two of these steps, a molecule of ATP is produced. In other words, each 3-carbon sugar yields two ATP molecules, and since there are two 3-carbon sugars, four ATP molecules are generated. Recalling that two ATP molecules were used to prime the glucose initially, the net yield at this point is two ATP molecules. The 10 enzymatically catalyzed reactions in glycolysis can be summarized as:

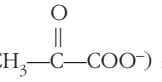
$$\text{Glucose} + 2\ \text{ADP} + 2\ P_i + 2\ NAD^+ \rightarrow 2\ \text{pyruvic acid} + 2\ \text{NADH} + 2\ \text{ATP}$$

Acetyl-CoA: Strategic Intermediate in Respiration

In aerobic metabolism the two molecules of pyruvic acid formed during glycolysis enter a mitochondrion. There, each molecule is oxidized, and one of the carbons is released as carbon dioxide (Figure 4.12). The 2-carbon residue condenses with **coenzyme A (CoA)** to form **acetyl coenzyme A,** or **acetyl-CoA,** and an NADH molecule is also produced.

Pyruvic acid is the undissociated form of the acid:



Under physiological conditions pyruvic acid typically dissociates into pyruvate  and H^+. It is correct to use either term in describing this and other organic acids (such as lactic acid, or lactate) in metabolism.

Acetyl-CoA is a critically important compound. Its oxidation in the Krebs cycle provides energized electrons to generate ATP, and it is also a crucial intermediate in lipid metabolism (p. 70).

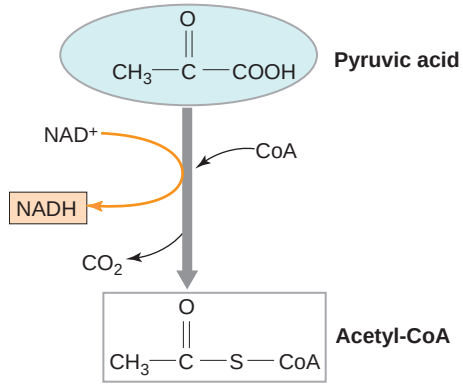


Figure 4.12

Formation of acetyl-CoA from pyruvic acid.

Krebs Cycle: Oxidation of Acetyl-CoA

Degradation (oxidation) of the 2-carbon acetyl group of acetyl-CoA occurs within the mitochondrial matrix in a cyclic sequence called the **Krebs cycle** (also called citric acid cycle and tricarboxylic acid cycle [TCA cycle]) (Figure 4.13). Acetyl-CoA condenses with a 4-carbon acid (oxaloacetic acid), releasing CoA to react again with more pyruvic acid. Through a cyclic series of reactions in the Krebs cycle, the two carbons from the acetyl group

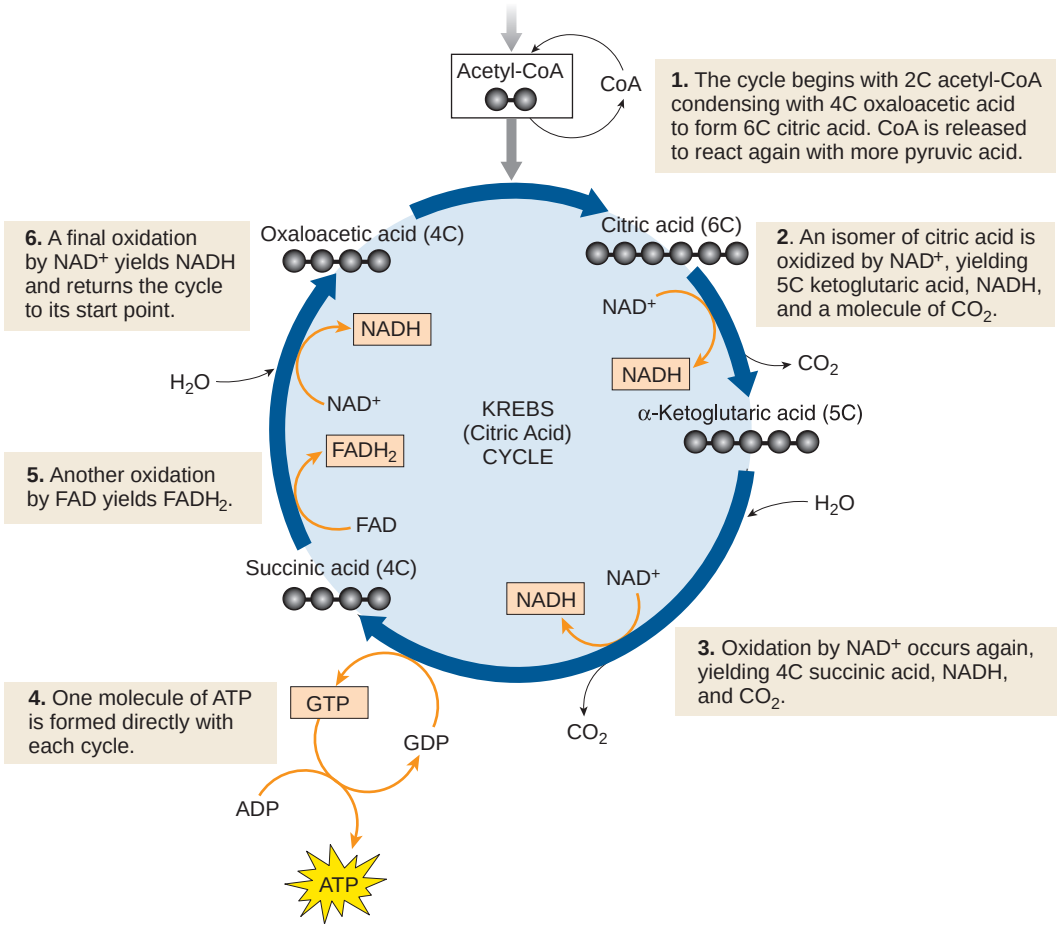


Figure 4.13

The Krebs cycle in outline form, showing production of three molecules of reduced NAD, one molecule of reduced FAD, one molecule of ATP, and two molecules of carbon dioxide. The molecules of NADH and $FADH_2$ will yield 11 molecules of ATP when oxidized in the electron transport system.

are released as carbon dioxide, and oxaloacetic acid is regenerated. Hydrogen ions and electrons in the oxidations transfer to NAD^+ and to FAD (flavine adenine dinucleotide, another electron acceptor), and a pyrophosphate bond is generated in the form of guanosine triphosphate (GTP). This high-energy phosphate readily transfers to ADP to form ATP. Thus, the overall products of the Krebs cycle are CO_2, ATP, NADH, and $FADH_2$:

$$\text{Acetyl unit} + 3\ NAD^+ + FAD + ADP + P_i \rightarrow 2\ CO_2 + 3\ NADH + FADH_2 + ATP$$

The molecules of NADH and $FADH_2$ formed yield 11 molecules of ATP when oxidized in the electron transport chain. The other molecules in the cycle behave as intermediate reactants and products which are continuously regenerated as the cycle turns.

Aerobic cellular respiration uses oxygen as the final electron acceptor and releases carbon dioxide and water from the complete oxidation of fuels. The carbon dioxide that we, and other aerobic organisms, produce is removed from our bodies into the atmosphere during external respiration (see Chapter 31, p. 698). Fortunately for us and other aerobes, oxygen is continuously produced by cyanobacteria (blue-green algae), eukaryotic algae, and plants by the process of photosynthesis. In this process, hydrogen atoms obtained from water react with carbon dioxide from the atmosphere to generate sugars and molecular oxygen. Thus, a balance between oxygen used and produced, and carbon dioxide produced and used, is obtained across our planet. Unfortunately, excessive production of carbon dioxide due to human industrialization, and decreased production of oxygen due to our continuous removal of the world's forests, are threatening this delicate balance. Carbon dioxide levels continue to rise, leading to global atmospheric warming caused by the "greenhouse effect" (see Chapter 37, p. 807).

Electron Transport Chain

Transfer of hydrogen ions and electrons from NADH and $FADH_2$ to the final electron acceptor, molecular oxygen, is accomplished in an elaborate electron transport chain embedded in the inner membrane of mitochondria (Figure 4.14, see also p. 44). Each carrier molecule in the chain (labeled I to IV in Figure 4.14) is a large transmembrane protein-based complex that accepts and releases electrons at lower energy levels than the carrier preceding it in the chain. As electrons pass from one carrier molecule to the next, free energy is released. Some of this energy is used to transport H^+ ions across the inner mitochondrial membrane and in this way creates a H^+ gradient across the membrane. The H^+ gradient produced drives the synthesis of ATP. This process is called chemiosmotic coupling (Figure 4.14). According to this model, as electrons contributed by NADH and $FADH_2$ are carried down the electron transport chain, they activate proton transporting molecules which move protons (hydrogen ions) outward and into the space between the two mitochondrial membranes. This causes the proton concentration in this intermembrane space to rise, producing a diffusion gradient that is used to drive the protons back into the mitochondrial matrix through special proton channels. These channels are ATP-forming transmembrane protein complexes (ATP synthase) that use the inward movement of protons to induce the formation of ATP. By this means, oxidation of one NADH yields three ATP molecules. $FADH_2$ from the Krebs cycle enters the electron transport chain at a lower level than NADH and so yields two ATP molecules. This method of energy capture is called **oxidative phosphorylation** because the formation of high-energy phosphate is coupled to oxygen consumption, and these reactions depend on demand for ATP by other metabolic activities within the cell.

Efficiency of Oxidative Phosphorylation

We can now calculate the ATP yield from the complete oxidation of glucose (Figure 4.15). The overall reaction is:

$$\text{Glucose} + 2\ ATP + 36\ ADP + 36\ P + 6\ O_2 \rightarrow 6\ CO_2 + 2\ ADP + 36\ ATP + 6\ H_2O$$

ATP has been generated at several points along the way (Table 4.1). The cytoplasmic NADH generated in glycolysis requires a molecule of ATP to fuel transport of each molecule of NADH into a mitochondrion; therefore, each NADH from glycolysis yields only two ATP (total of four), compared with the three ATP per NADH (total of six) formed within mitochondria. Accounting for the two ATP used in the priming reactions in glycolysis, the net yield may be as high as 36 molecules of ATP per molecule of glucose. The yield of 36 ATP is a theoretical maximum because some of the H^+ gradient produced by electron transport may be used for other functions, such as transporting substances in and out of the mitochondrion. Overall efficiency of aerobic oxidation of glucose is about 38%, comparing very favorably with human-designed energy conversion systems, which seldom exceed 5% to 10% efficiency.

The capacity for oxidative phosphorylation is also increased by the elaborate folding of the inner mitochondrial membrane (the cristae shown in Figure 4.14 and labeled in Figure 3.11, p. 44), providing a much greater surface area for more electron transport chain and ATP synthase proteins.

Anaerobic Glycolysis: Generating ATP Without Oxygen

Under anaerobic conditions, glucose and other 6-carbon sugars are first converted stepwise to a pair of 3-carbon pyruvic acid molecules during glycolysis, described on page 64 (see also Figure 4.11). This series of reactions yields two molecules of ATP and two molecules of NADH. In the absence of molecular oxygen, further oxidation of pyruvic acid cannot occur because without oxygen as the final electron acceptor in the electron transport chain, the Krebs cycle and electron transport chain cannot operate and cannot, therefore, reoxidize the NADH produced in glycolysis. The problem is neatly solved in most animal

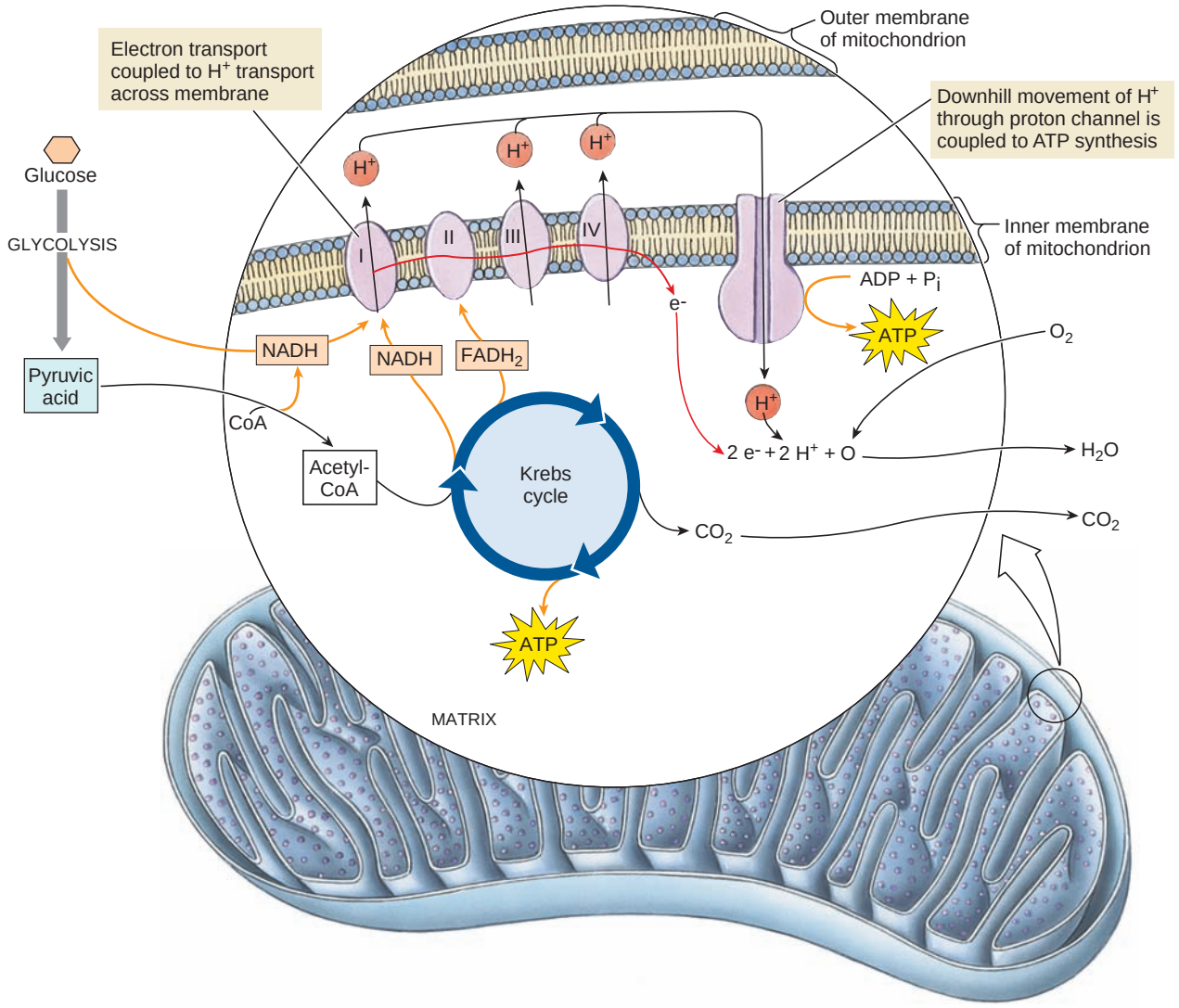


Figure 4.14

Oxidative phosphorylation. Most of the ATP in living organisms is produced in the electron transport chain. Electrons removed from fuel molecules in cellular oxidations (glycolysis and the Krebs cycle) flow through the electron transport chain, the major components of which are four transmembrane protein complexes (I, II, III, and IV). Electron energy is tapped by the major complexes and used to push H^+ outward across the inner mitochondrial membrane. The H^+ gradient created drives H^+ inward through proton channels (ATP synthase) that couple H^+ movement to ATP synthesis.

TABLE 4.1

Calculation of Total ATP Molecules Generated in Respiration

ATP Generated	Source
4	Directly in glycolysis
2	As GTP (→ATP) in Krebs cycle
4	From NADH in glycolysis
6	From NADH produced in pyruvic acid to acetyl-CoA reaction
4	From reduced FAD in Krebs cycle
18	From NADH produced in Krebs cycle
38 Total	
–2	Used in priming reactions in glycolysis
36 Net	

cells by reducing pyruvic acid to lactic acid (Figure 4.16). Pyruvic acid becomes the final electron acceptor and lactic acid the end product of anaerobic glycolysis. This step converts NADH to NAD^+, effectively freeing it to recycle and pick up more H^+ and electrons. In **alcoholic fermentation** (as in yeast, for example) the steps are identical to glycolysis down to pyruvic acid. One of its carbons is then released as carbon dioxide, and the resulting 2-carbon compound is reduced to ethanol, thus regenerating the NAD^+.

Anaerobic glycolysis is only one-eighteenth as efficient as complete oxidation of glucose to carbon dioxide and water, but its key virtue is that it provides *some* high-energy phosphate in situations in which oxygen is absent or in short supply. Many microorganisms live in places where oxygen is severely depleted, such as waterlogged soil, in mud of a lake or sea bottom, or within a decaying carcass. Vertebrate skeletal muscle may rely heavily on glycolysis during short

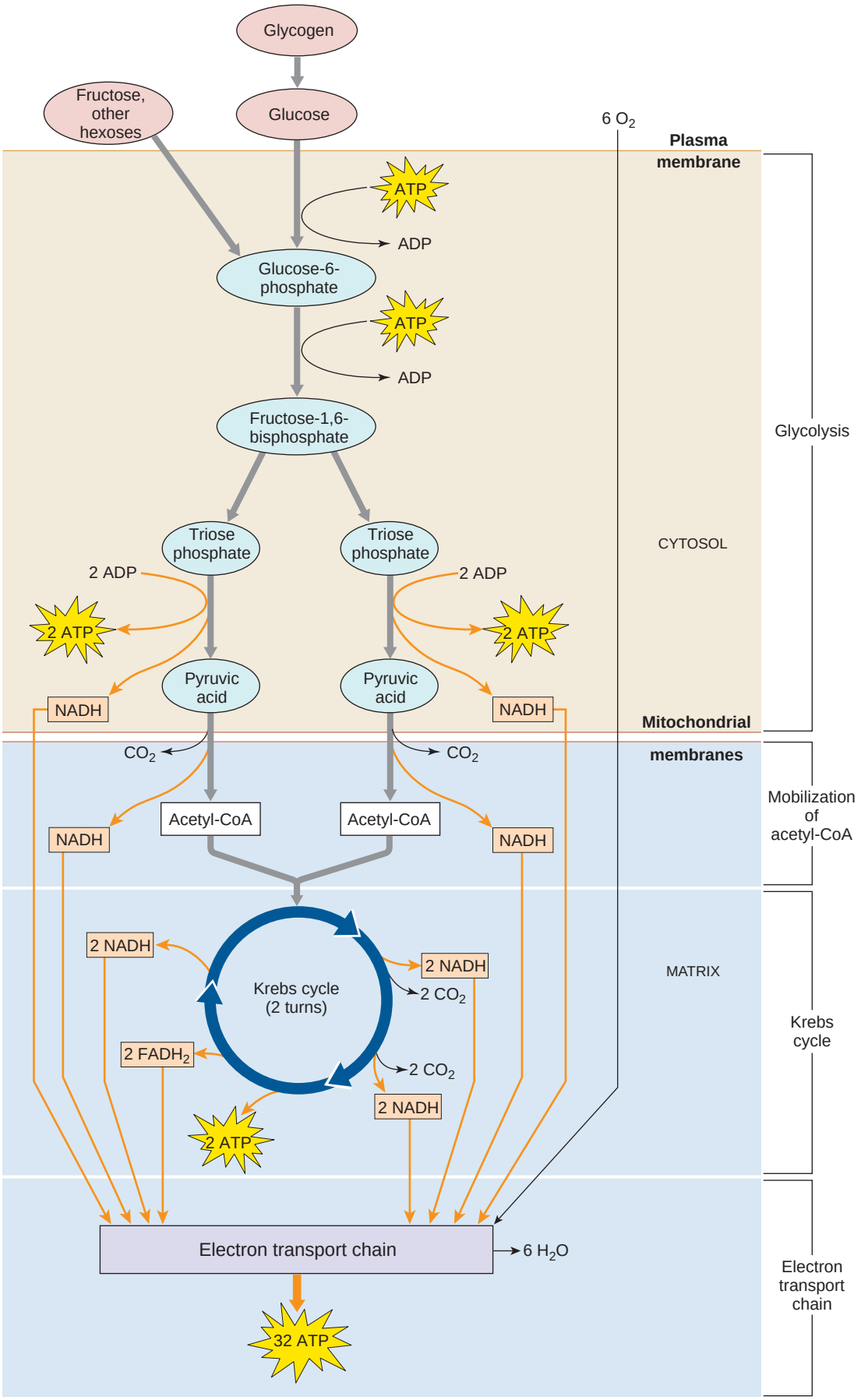


Figure 4.15

Pathway for oxidation of glucose and other carbohydrates. Glucose is degraded to pyruvic acid by cytoplasmic enzymes (glycolytic pathway). Acetyl-CoA is formed from pyruvic acid and is fed into the Krebs cycle. An acetyl-CoA molecule (two carbons) is oxidized to two molecules of carbon dioxide with each turn of the cycle. Pairs of electrons are removed from the carbon skeleton of the substrate at several points in the pathway and are carried by oxidizing agents NADH or $FADH_2$ to the electron transport chain where 32 molecules of ATP are generated. Four molecules of ATP are also generated by substrate phosphorylation in the glycolytic pathway, and two molecules of ATP (initially GTP) are formed in the Krebs cycle. This yields a total of 38 molecules of ATP (36 molecules net) per glucose molecule. Molecular oxygen is involved only at the very end of the pathway as the final electron acceptor at the end of the electron transport chain to yield water.

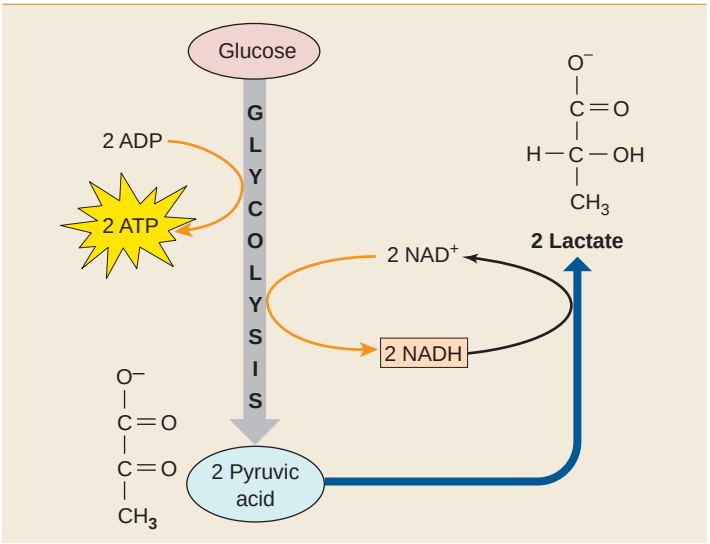


Figure 4.16

Anaerobic glycolysis, a process that proceeds in the absence of oxygen. Glucose is broken down to two molecules of pyruvic acid, with a net production of two molecules of ATP. Pyruvic acid, the final electron acceptor for the hydrogen ions and electrons released during pyruvic acid formation, is converted to lactic acid. Hydrogen and electrons are recycled through the carrier, NAD^+.

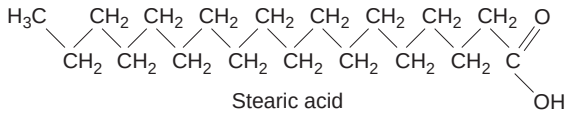
bursts of activity when contraction is so rapid and powerful that oxygen delivery to tissues cannot supply energy demands by oxidative phosphorylation alone. At such times an animal has no choice but to supplement oxidative phosphorylation with anaerobic glycolysis. One kind of muscle fiber (white muscle) has few mitochondria and primarily uses anaerobic glycolysis for ATP production (see Chapter 29, p. 661). In all muscle types, intense or strenuous activity is followed by a period of increased oxygen consumption as lactic acid, the end product of anaerobic glycolysis, diffuses from muscle to the liver where it is metabolized. Because oxygen consumption increases following heavy activity, the animal is said to have acquired an **oxygen debt** during such activity, which is repaid when activity ceases, and accumulated lactic acid is metabolized.

Some animals rely heavily on anaerobic glycolysis during normal activities. For example, diving birds and mammals use glycolysis almost entirely to give them the energy needed to sustain long dives without breathing (that is, without requiring oxygen). Salmon would never reach their spawning grounds were it not for anaerobic glycolysis providing almost all of the ATP used in the powerful muscular bursts needed to carry them up rapids and falls. Many parasitic animals have dispensed with oxidative phosphorylation entirely at some stages of their life cycles. They secrete relatively reduced end products of their energy metabolism, such as succinic acid, acetic acid, and propionic acid. These compounds are produced in mitochondrial reactions that derive several more molecules of ATP than does the path from glycolysis to lactic acid, although such sequences are still far less efficient than the aerobic electron transport chain.

METABOLISM OF LIPIDS

The first step in the breakdown of a triglyceride is its hydrolysis to glycerol and three fatty acid molecules (Figure 4.17). Glycerol is phosphorylated and enters the glycolytic pathway (see Figure 4.10).

The remainder of the triglyceride molecule consists of fatty acids. For example, an abundant naturally occurring fatty acid is **stearic acid.**



The long hydrocarbon chain of a fatty acid is broken down by oxidation, two carbons at a time; these are released from the end of the molecule as acetyl-CoA. Although two high-energy phosphate bonds are required to prime each 2-carbon fragment, energy is derived both from the reduction of NAD^+ and FAD to NADH and $FADH_2$, respectively, and from the acetyl group as it is degraded in the Krebs cycle. The complete oxidation of one molecule of 18-carbon stearic acid nets 146 ATP molecules. By comparison, three molecules of glucose (also totaling 18 carbons) yield 108 ATP molecules. Since there are three fatty acids in each triglyceride molecule, a total of 440 ATP molecules are formed. An additional 22 molecules of ATP are generated in the breakdown of glycerol, giving a grand total of 462 molecules of ATP—little wonder that fat is considered the king of animal fuels! Fats are more concentrated fuels than carbohydrates, because fats are almost pure hydrocarbons; they contain more hydrogen per carbon atom than sugars do, and it is the energized electrons of hydrogen that generate high-energy bonds when they are carried through the mitochondrial electron transport chain.

Fat stores are derived principally from surplus fats and carbohydrates in the diet. Acetyl-CoA is the source of carbon atoms used to build fatty acids. Because all major classes of organic molecules (carbohydrates, fats, and proteins) can be degraded to acetyl-CoA, all can be converted into stored fat. The biosynthetic pathway for fatty acids resembles a reversal of the catabolic pathway already described, but it requires an entirely different set of enzymes. From acetyl-CoA, the fatty acid chain is assembled two carbons at a time. Because fatty acids release energy when they are oxidized,

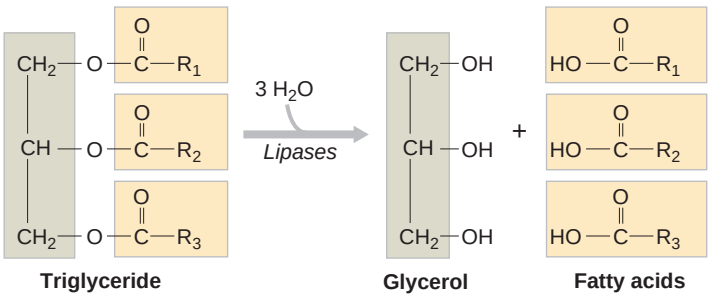


Figure 4.17

Hydrolysis of a triglyceride (neutral fat) by intracellular lipase. The R groups of each fatty acid represent a hydrocarbon chain.

they obviously require an input of energy for their synthesis. This energy is provided principally by electron energy from glucose degradation. Thus the total ATP derived from oxidation of a molecule of triglyceride is not as great as calculated, because varying amounts of energy are required for synthesis and storage.

Stored fats are the greatest reserve fuel in the body. Most usable fat resides in white adipose tissue composed of specialized cells packed with globules of triglycerides. White adipose tissue is widely distributed in the abdominal cavity, in muscles, around deep blood vessels and large organs (for example, heart and kidneys), and especially under the skin. Women average about 30% more fat than men, which is largely responsible for differences in shape between males and females. Humans can only too easily deposit large quantities of fat, generating hazards to health.

Physiological and psychological aspects of obesity are now being investigated by many researchers. There is increasing evidence that food intake, and therefore the amount of fat deposition, is regulated by feeding centers located in the brain (lateral and ventral hypothalamus and brain stem). The set point of these regions determines normal food intake and body weight for an individual, which may be maintained above or below what is considered normal for humans. Although evidence is accumulating for a genetic component to obesity, the epidemic proportions of obesity in the United States are more easily explained by lifestyle and feeding habits. Other developed countries show a similar, but less pronounced, trend toward development of an obesity problem.

Research also reveals that lipid metabolism in obese individuals appears to be abnormal compared to lean individuals. This research has resulted in the development of drugs that act at various stages of lipid metabolism, such as decreasing lipid digestion and absorption from the digestive tract, or increasing metabolism of lipids once they have been absorbed into the body.

METABOLISM OF PROTEINS

Since proteins are composed of amino acids, of which 20 kinds commonly occur (p. 26), the central topic of our consideration is amino acid metabolism. Amino acid metabolism is complex. Each of the 20 amino acids requires a separate pathway for biosynthesis and degradation. Amino acids are precursors to tissue proteins, enzymes, nucleic acids, and other nitrogenous constituents that form the fabric of cells. The central purpose of carbohydrate and fat oxidation is to provide energy, much of which is needed to construct and maintain these vital macromolecules.

Let us begin with the **amino acid pool** in blood and extracellular fluid from which the tissues draw their requirements. When animals eat proteins, most are digested in the digestive tract, releasing their constituent amino acids, which are then absorbed (Figure 4.18). Tissue proteins also are hydrolyzed during normal growth, repair, and tissue restructuring; their amino acids join those derived from protein found in food to enter the amino acid pool. A portion of the amino acid pool is used to rebuild tissue proteins, but most animals ingest a surplus of protein. Since amino acids are not excreted as such in any significant amounts, they must be disposed of in some other way. In fact, amino acids can be and are metabolized through oxidative pathways to yield high-energy phosphate. In short, excess proteins serve as fuel as do carbohydrates and fats. Their importance as fuel obviously depends on the nature of the diet. In carnivores that ingest a diet of almost pure protein and fat, nearly half of their high-energy phosphate comes from amino acid oxidation.

Before an amino acid molecule may enter the fuel depot, nitrogen must be removed by deamination (the amino group splits to form ammonia and a keto acid) or by transamination (the amino group is transferred to a keto acid to yield a new amino acid). Thus amino acid degradation yields two main products, carbon skeletons and ammonia, which are handled in different ways. Once nitrogen atoms are removed, the carbon skeletons

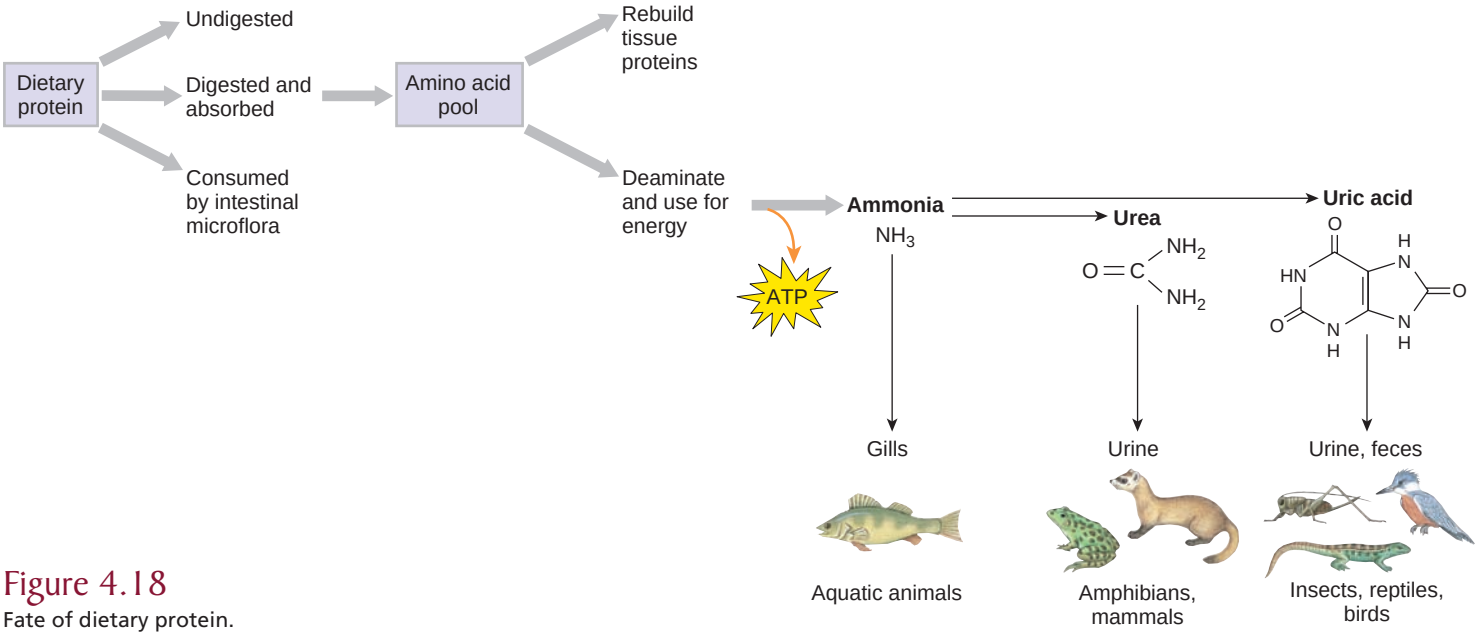


Figure 4.18
Fate of dietary protein.

of amino acids can be completely oxidized, usually by way of pyruvic acid or acetic acid. These residues then enter routes used by carbohydrate and fat metabolism (see Figure 4.10).

The other product of amino acid degradation is ammonia. Ammonia is highly toxic because it inhibits respiration by reacting with α-ketoglutaric acid to form glutamic acid (an amino acid), and effectively removes α-ketoglutarate from the Krebs cycle (see Figure 4.13). Disposal of ammonia offers little problem to aquatic animals because it is soluble and readily diffuses into the surrounding medium, often through respiratory surfaces. Terrestrial animals cannot get rid of ammonia so conveniently and must detoxify it by converting it to a relatively nontoxic compound. The two principal compounds formed are **urea** and **uric acid,** although a variety of other detoxified forms of ammonia are excreted by different animals. Among vertebrates, amphibians and especially mammals produce urea. Reptiles and birds, as well as many terrestrial invertebrates, produce uric acid (the excretion of uric acid by insects and birds is described on pp. 451 and 597, respectively).

The key feature that determines choice of nitrogenous waste is availability of water in the environment. When water is abundant, the chief nitrogenous waste is ammonia. When water is restricted, it is urea. Animals living in truly arid habitats use uric acid. Uric acid is highly insoluble and easily precipitates from solution, allowing its removal in solid form. Embryos of birds and reptiles benefit greatly from excretion of nitrogenous waste as uric acid, because waste cannot be eliminated through their eggshells. During embryonic development, harmless, solid uric acid is retained in one of the extraembryonic membranes. When a hatchling emerges into its new world, accumulated uric acid, along with the shell and membranes that supported development, is discarded.

MANAGEMENT OF METABOLISM

The complex pattern of enzymatic reactions that constitutes metabolism cannot be explained entirely in terms of physicochemical laws or chance happenings. Although some enzymes appear to function automatically, the activity of others is rigidly controlled. In the former case, suppose that the function of an enzyme is to convert A to B. If B is removed by conversion into another compound, the enzyme tends to restore the original ratio of B to A. Since many enzymes act reversibly, either synthesis or degradation may result. For example, an excess of an intermediate in the Krebs cycle would contribute to glycogen synthesis; a depletion of such a metabolite would lead to glycogen breakdown. This automatic compensation (equilibration) is not, however, sufficient to explain regulation of metabolism.

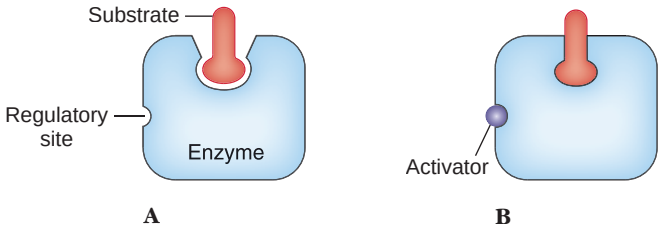


Figure 4.19

Enzyme regulation. **A,** The active site of an enzyme may only loosely fit its substrate in the absence of an activator. **B,** With the regulatory site of the enzyme occupied by an activator, the enzyme binds the substrate, and the site becomes catalytically active.

Mechanisms exist for critically regulating enzymes in both *quantity* and *activity*. In bacteria, genes leading to synthesis of an enzyme are switched on or off, depending on the presence or absence of a substrate molecule. In this way the *quantity* of an enzyme is controlled. It is a relatively imprecise process.

Mechanisms that alter activity of enzymes can quickly and finely adjust metabolic pathways to changing conditions in a cell. The presence or increase in concentration of some molecules can alter the shape (conformation) of particular enzymes, thus activating or inhibiting the enzyme (Figure 4.19). For example, phosphofructokinase, which catalyzes phosphorylation of glucose-6-phosphate to fructose-1,6-bisphosphate (see Figure 4.15), is inhibited by high concentrations of ATP or citric acid. Their presence means that a sufficient amount of precursors has reached the Krebs cycle and additional glucose is not needed. In some cases, the final end product of a particular metabolic pathway inhibits the first enzyme in the pathway. This method is termed **feedback inhibition.**

As well as being subject to alteration in physical shape, many enzymes exist in both an active and an inactive form. These forms may be chemically different. For example, one common way to activate or inactivate an enzyme is to add a phosphate group to the molecule, thus changing its conformational shape and either exposing or blocking the enzyme's active site. Enzymes that degrade glycogen (phosphorylase) and synthesize it (synthase) are both found in active and inactive forms. Conditions that activate phosphorylase tend to inactivate synthase and vice versa.

SUMMARY

Living systems are subject to the same laws of thermodynamics that govern nonliving systems. The first law states that energy cannot be destroyed, although it may change form. The second law states that the structure of systems proceeds toward total randomness, or increasing entropy, as energy is dissipated. Solar energy trapped by photosynthesis as chemical bond energy is passed through the food chain where it is used for biosynthesis, active transport, and motion, before finally being dissipated as heat. Living organisms are able to decrease their entropy and to maintain high internal order because the biosphere is an open system from which energy can be captured and used. Energy available for use in biochemical reactions is termed "free energy."

Enzymes are usually proteins, often associated with nonprotein cofactors, that vastly accelerate rates of chemical reactions in living systems. An enzyme acts by temporarily binding its reactant (substrate) onto an active site in a highly specific fit. In this configuration, internal activation energy barriers are lowered enough to modify the substrate, and the enzyme is restored to its original form.

Cells use the energy stored in chemical bonds of organic fuels by degrading fuels through a series of enzymatically controlled steps. This bond energy is transferred to ATP and packaged in the form of "high-energy" phosphate bonds. ATP is produced as it is required in cells to power various synthetic, secretory, and mechanical processes.

Glucose is an important source of energy for cells. In aerobic metabolism (respiration), the 6-carbon glucose is split into two 3-carbon molecules of pyruvic acid. Pyruvic acid is decarboxylated to form 2-carbon acetyl-CoA, a strategic intermediate that enters the Krebs cycle. Acetyl-CoA can also be derived from breakdown of fat. In the Krebs cycle, acetyl-CoA is oxidized in a series of reactions to carbon dioxide, yielding, in the course of the reactions, energized electrons that are passed to electron acceptor molecules (NAD^+ and FAD). In the final stage, the energized electrons are passed along an electron transport chain consisting of a series of electron carriers located in the inner membranes of mitochondria. A hydrogen gradient is produced as electrons are passed from carrier to carrier and finally to oxygen, and ATP is generated as the hydrogen ions flow down their electrochemical gradient through ATP synthase molecules located in the inner mitochondrial membrane. A net total of 36 molecules of ATP may be generated from one molecule of glucose.

In the absence of oxygen (anaerobic glycolysis), glucose is degraded to two 3-carbon molecules of lactic acid, yielding two molecules of ATP. Although anaerobic glycolysis is vastly less efficient than aerobic metabolism, it provides essential energy for muscle contraction when heavy energy expenditure outstrips the oxygen-delivery system of an animal; it also is the only source of energy generation for microorganisms living in oxygen-free environments.

Triglycerides (neutral fats) are especially rich depots of metabolic energy because the fatty acids of which they are composed are highly reduced and free of water. Fatty acids are degraded by sequential removal of 2-carbon units, which enter the Krebs cycle through acetyl-CoA.

Amino acids in excess of requirements for synthesis of proteins and other biomolecules are used as fuel. They are degraded by deamination or transamination to yield ammonia and carbon skeletons. The latter enter the Krebs cycle to be oxidized. Ammonia is a highly toxic waste product that aquatic animals quickly expel, often through respiratory surfaces. Terrestrial animals, however, convert ammonia into much less toxic compounds, urea or uric acid, for disposal.

Integration of metabolic pathways is finely regulated by mechanisms that control both amount and activity of enzymes. The quantity of some enzymes is regulated by certain molecules that switch on or off enzyme synthesis. Enzyme activity may be altered by the presence or absence of metabolites that cause conformational changes in enzymes and thus improve or diminish their effectiveness as catalysts.

REVIEW QUESTIONS

1. State the first and second laws of thermodynamics. Living systems may appear to violate the second law of thermodynamics because living things maintain a high degree of organization despite a universal trend toward increasing disorganization. What is the explanation for this apparent paradox?
2. Explain what is meant by "free energy" in a system. Will a reaction that proceeds spontaneously have a positive or negative change in free energy?
3. Many biochemical reactions proceed slowly unless the energy barrier to the reaction is lowered. How is this accomplished in living systems?
4. What happens in the formation of an enzyme-substrate complex that favors the disruption of substrate bonds?
5. What is meant by a "high-energy bond," and why might the production of molecules with such bonds be useful to living organisms?
6. Although ATP supplies energy to an endergonic reaction, why is it not considered a fuel?
7. What is an oxidation-reduction reaction and why are such reactions considered so important in cellular metabolism?
8. Give an example of a final electron acceptor found in aerobic and anaerobic organisms. Why is aerobic metabolism more efficient than anaerobic metabolism?
9. Why must glucose be "primed" with a high-energy phosphate bond before it can be degraded in the glycolytic pathway?
10. What happens to the electrons removed during the oxidation of triose phosphates during glycolysis?
11. Why is acetyl-CoA considered a "strategic intermediate" in respiration?
12. Why are oxygen atoms important in oxidative phosphorylation? What are the consequences if they are absent for a short period of time in tissues that routinely use oxidative phosphorylation to produce useful energy?
13. Explain how animals can generate ATP *without* oxygen. Given that anaerobic glycolysis is much less efficient than oxidative phosphorylation, why has anaerobic glycolysis not been discarded during animal evolution?

14. Why are animal fats sometimes called "the king of fuels"? What is the significance of acetyl-CoA to lipid metabolism?
15. The breakdown of amino acids yields two products: ammonia and carbon skeletons. What happens to these products?
16. Explain the relationship between the amount of water in an animal's environment and the kind of nitrogenous waste it produces.
17. Explain three ways that enzymes may be regulated in cells.

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PART TWO

2 Continuity and Evolution of Animal Life

A female Cardinalis cardinalis *(left) and a female* Cardinalis sinuatus *(right).*

CHAPTER

5

Genetics: A Review

The site of Gregor Mendel's experimental garden, Brno, Czech Republic.

A Code for All Life

The principle of hereditary transmission is a central tenet of life on earth: all organisms inherit a structural and functional organization from their progenitors. What is inherited by an offspring is not an exact copy of the parent but a set of coded instructions that a developing organism uses to construct a body resembling its parents. These instructions are in the form of genes, the fundamental units of inheritance. One of the great triumphs of modern biology was the discovery in 1953 by James Watson and Francis Crick of the nature of the coded instructions in genes. The genetic material (deoxyribonucleic acid, DNA) is composed of nitrogenous bases arranged on a chemical chain of sugar-phosphate units. The genetic code lies in the linear order or sequence of bases in the DNA strand.

Because the DNA molecules replicate and pass from generation to generation, genetic variations can persist and spread in a population. Such molecular alterations, called mutations, are the ultimate source of biological variation and the raw material of evolution.

A basic principle of modern evolutionary theory is that organisms attain their diversity through hereditary modifications of populations. All known lineages of plants and animals are related by descent from common ancestral populations.

Heredity establishes the continuity of living forms. Although offspring and parents in a particular generation may look different, there is nonetheless a genetic continuity that runs from generation to generation for any species of plant or animal. An offspring inherits from its parents a set of coded information **(genes),** which a fertilized egg uses, together with environmental factors, to guide its development into an adult bearing unique physical characteristics. Each generation passes to the next the instructions required for maintaining continuity of life.

The gene is the unit entity of inheritance, the germinal basis for every characteristic that appears in an organism. The study of what genes are, how they are transmitted, and how they work is the science of genetics. It is a science that reveals the underlying causes of *resemblance,* as seen in the remarkable fidelity of reproduction, and of *variation,* the working material for organic evolution. All living forms use the same information storage, transfer, and translation system, which explains the stability of all life and reveals its descent from a common ancestral form. This is one of the most important unifying concepts of biology.

MENDEL'S INVESTIGATIONS

The first person to formulate the principles of heredity was Gregor Johann Mendel (1822 to 1884) (Figure 5.1 and p. 18), an Augustinian monk living in Brünn (Brno), Moravia. Brünn was then part of Austria but now lies in the eastern part of the Czech Republic. While conducting breeding experiments in a small monastery garden from 1856 to 1864, Mendel examined with great care the progeny of many thousands of plants. He presented in elegant simplicity the laws governing transmission of characters from parents to offspring. His discoveries, published in 1866, were of great significance, coming just after Darwin's publication of *On the Origin of Species by Means of Natural Selection*. Yet Mendel's discoveries remained unappreciated and forgotten until 1900—35 years after the completion of the work and 16 years after Mendel's death.

Mendel chose garden peas for his classic experiments because they had pure strains differing from each other by discrete characters. For example, some varieties were definitely dwarf and others tall; some strains produced smooth seeds and others wrinkled seeds (Figure 5.1). Mendel studied single characters that displayed sharply contrasting traits. He carefully avoided mere quantitative, continuously varying characteristics. A second reason for selecting peas was that they were self-fertilizing but subject to experimental cross-fertilization.

A giant advance in chromosomal genetics was made when the American geneticist Thomas Hunt Morgan and his colleagues selected a species of fruit fly, *Drosophila melanogaster,* for their studies (1910–1920). Flies were cheaply and easily reared in bottles in the laboratory, fed on a simple medium of bananas and yeast. Most importantly, they produced a new generation every 10 days, enabling Morgan to collect data at least 25 times more rapidly than with organisms that take longer to mature, such as garden peas. Morgan's work led to the mapping of genes on chromosomes and founded the discipline of cytogenetics.

Mendel crossed varieties having contrasting traits, making crosses for each of the seven characters shown in Figure 5.1. He removed the stamens (male part, containing the pollen) from a flower to prevent self-fertilization and then placed on the stigma (female part of flower) pollen from the flower of a plant true-breeding for the contrasting trait. Pollination from other sources such as wind and insects was rare and did not affect his results. Offspring from these crosses are called hybrids, meaning that they contain genetic information from two different parental strains. He collected seeds from the cross-fertilized flowers, planted these hybrid seeds, and examined the resulting plants for the contrasting traits being studied. These hybrid plants then produced offspring by self-pollination.

Mendel knew nothing of the cytological basis of heredity, since chromosomes and genes were not yet discovered. Although we can admire Mendel's power of intellect in his discovery of the principles of inheritance without knowledge of chromosomes, these principles are easier to understand if we first review chromosomal behavior, especially in meiosis.

CHROMOSOMAL BASIS OF INHERITANCE

In sexually reproducing organisms, special **sex cells,** or **gametes** (ova and sperm), transmit genetic information from parents to offspring. A scientific explanation of genetic principles required a study of germ cells and their behavior, and correlations between their transmission and certain visible results of inheritance. Nuclei of sex cells, especially the chromosomes, were early suspected of furnishing the real answer to the hereditary mechanism. Chromosomes are apparently the only entities transmitted in equal quantities from both parents to offspring.

When Mendel's laws were rediscovered in 1900, their parallelism with the cytological behavior of chromosomes was obvious. Later experiments showed that chromosomes carried hereditary material.

Meiosis: Reduction Division of Gametes

Although animal species differ greatly in the characteristic numbers, sizes, and shapes of chromosomes present in their body cells, a common feature is that chromosomes occur in pairs. The two members of a chromosomal pair contain similar genes encoding the same set of characteristics and usually, but not always, have the same size and shape. The members of such a pair are called **homologous** chromosomes; each individual member of a pair is called a **homolog.** One homolog comes from the mother and the other from the father. Meiosis is a special pair of cell divisions in which the genetic material replicates once followed by two rounds of cell division (Figure 5.2). The result is a set of four daughter cells, each of which has only *one* member of each homologous chromosome pair. The chromosomes present in a meiotic daughter cell or gamete are collectively called a single set of chromosomes. The number of chromosomes in a single set, which varies among species, is called the **haploid** (*n*) number of chromosomes. When

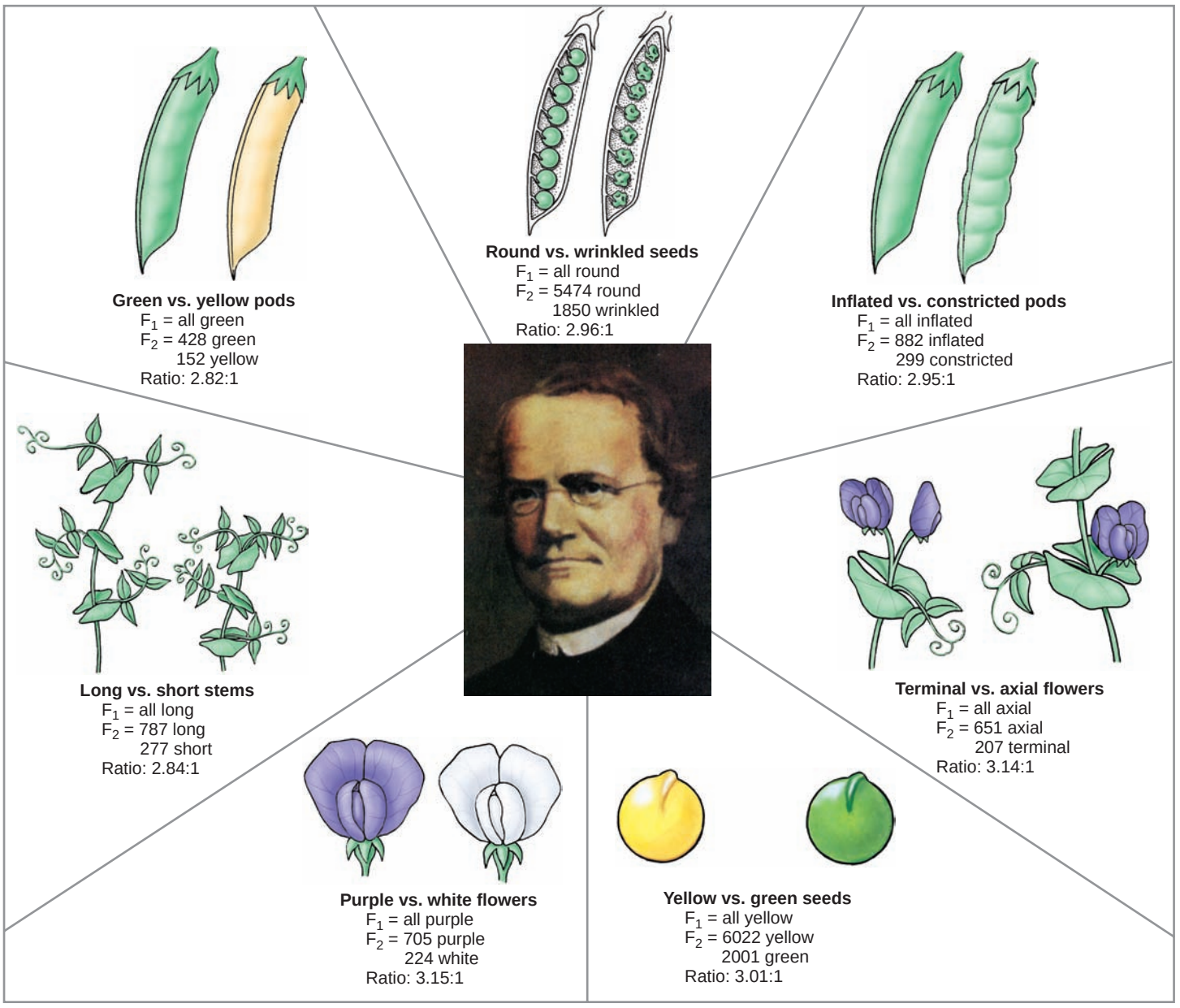


Figure 5.1

Seven experiments on which Gregor Mendel based his postulates. These are the results of monohybrid crosses for first and second generations.

a pair of gametes unites in fertilization, each gamete contributes its set of chromosomes to the newly formed cell, called a **zygote,** which has two complete sets of chromosomes. The number of chromosomes in two complete sets is called the **diploid** ($2n$) number. In humans the zygotes and all body cells normally have the diploid number ($2n$), or 46 chromosomes; the gametes have the haploid number (n), or 23, and meiosis reduces the number of chromosomes per cell from diploid to haploid.

Thus each cell normally has two copies of each gene coding for a given trait, one on each of the homologous chromosomes. Alternative forms of genes for the same trait are **allelic** forms, or **alleles.** Sometimes only one of the alleles has a visible effect on the organism, although both are present in each cell, and either may be passed to progeny as a result of meiosis and subsequent fertilization.

> Alleles are alternative forms of the same gene that have arisen by mutation of the DNA sequence. Like a baseball team with several pitchers, only one of whom can occupy the pitcher's mound at a time, only one allele can occupy a chromosomal locus (position). Alternative alleles for the locus may be on homologous chromosomes of a single individual, making that individual heterozygous for the gene in question. Numerous allelic forms of a gene may be found among different individuals in a population, a condition called "multiple alleles" (p. 85).

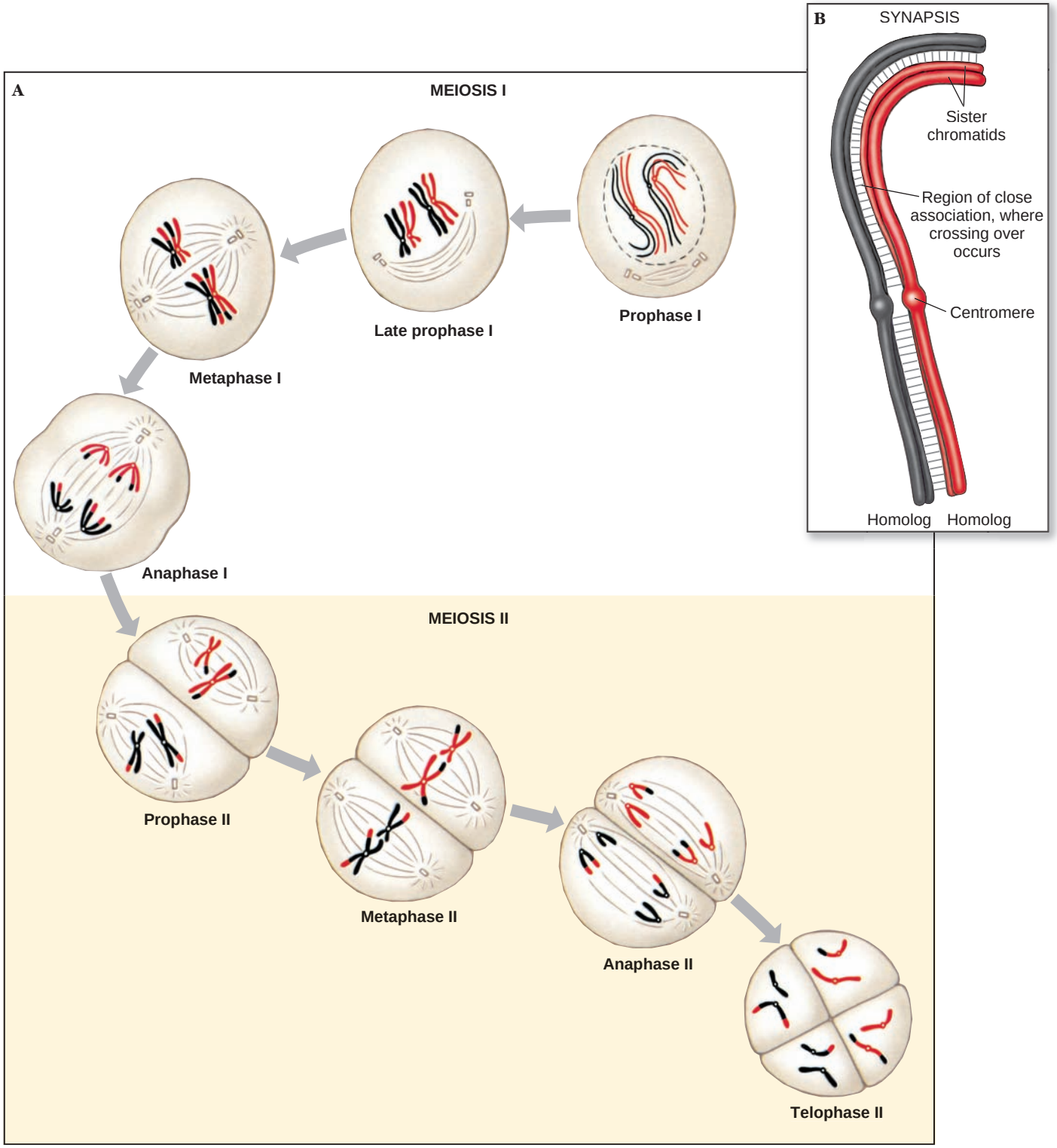


Figure 5.2

A, Meiosis in a sex cell with two pairs of chromosomes. Prophase I, homologous chromosomes come to lie with side-to-side contact, or synapsis, forming bivalents. A bivalent comprises a pair of homologous chromosomes, with each of the chromosomes containing a pair of identical chromatids joined by a centromere. Metaphase I, bivalents align at the spindle equator. Anaphase I, chromosomes of former bivalents are pulled toward opposite poles. Prophase II, daughter cells contain one of each homologous chromosome (haploid) but each chromosome is in replicated form (two chromatids attached at a centromere). Metaphase II, chromosomes align at the spindle equator. Anaphase II, chromatids of each chromosome separate. Telophase II, four haploid cells (gametes) formed, each with unreplicated chromosomes (one chromatid per chromosome). **B,** Synapsis occurs in prophase I, in which homologous chromosomes can break and exchange corresponding portions. The labelled sister chromatids and region of close association extend the full length of the bivalent.

During an individual's growth, all dividing cells contain the double set of chromosomes (mitosis is described on p. 52). In the reproductive organs, gametes (germ cells) are formed after meiosis, which *separates* the chromosomes of each homologous pair. Without this reductional division, the union of ovum (egg) and sperm would produce an individual with twice as many chromosomes as the parents. Continuation of this process in just a few generations could yield astronomical numbers of chromosomes per cell.

Most unique features of meiosis occur during prophase of the first meiotic division (Figure 5.2). Prior to meiosis, each chromosome has already replicated to form two chromatids joined at one point, the centromere. The two members of each pair of homologous chromosomes make side-by-side contact **(synapsis)** to form a **bivalent,** which permits genetic recombination between the paired homologous chromosomes (p. 89). Each bivalent is composed of two pairs of chromatids (each pair is a **dyad,** sister chromatids held together at their centromere), or *four* future chromosomes, and is thus called a **tetrad.** The position or location of any gene on a chromosome is the gene **locus** (pl., **loci**), and in synapsis all gene loci on a chromatid normally lie exactly opposite the corresponding loci on the sister chromatid and both chromatids of the homologous chromosome. Toward the end of prophase, the chromosomes shorten and thicken and then enter the first meiotic division.

In contrast to mitosis, the centromeres holding the chromatids together *do not divide* at anaphase. As a result, each of the dyads is pulled toward one of the opposite poles of the cell by microtubules of the division spindle. At telophase of the first meiotic division, each pole of the cell has one dyad from each tetrad formed at prophase. Therefore at the end of the first meiotic division, the daughter cells contain *one* chromosome of *each* homologous pair from the parent cell, so that the total chromosome number is reduced to haploid. However, because each chromosome contains two chromatids joined at a centromere, each cell contains twice the amount of DNA present in a gamete.

The second meiotic division more closely resembles events in mitosis. The dyads are split at the beginning of anaphase by division of their centromeres, and single-stranded chromosomes move toward each pole. Thus by the end of the second meiotic division, the cells have the haploid number of chromosomes, and each chromatid of the original tetrad exists in a separate nucleus. Four products are formed, each containing one complete haploid set of chromosomes and only one copy of each gene. Only one of the four products in female gametogenesis becomes a functional gamete (p. 146).

Sex Determination

Before the importance of chromosomes in heredity was realized in the early 1900s, genetic control of gender was totally unknown. The first scientific clue to chromosomal determination of sex came in 1902 when C. McClung observed that bugs (Hemiptera) produced two kinds of sperm in approximately equal numbers. One kind contained among its regular set of chromosomes a so-called accessory chromosome lacking in the other kind of sperm. Since all eggs of these species had the same number of haploid chromosomes, half the sperm would have the same number of chromosomes as the eggs, and half of them would have one chromosome less. When an egg was fertilized by a spermatozoon carrying the accessory (sex) chromosome, the resulting offspring was a female; when fertilized by a spermatozoon without an accessory chromosome, the offspring was a male. Therefore a distinction was made between sex chromosomes, which determine sex (and sex-linked traits); and **autosomes,** the remaining chromosomes, which do not influence sex. The particular type of sex determination just described is often called the XX-XO type, which indicates that females have two X chromosomes and males only one X chromosome (the O indicates absence of the chromosome). The XX-XO method of sex determination is depicted in Figure 5.3.

Later, other types of sex determination were discovered. In humans and many other animals each sex contains the same number of chromosomes; however, the sex chromosomes (XX) are alike in females but unlike (XY) in males. Hence a human egg contains 22 autosomes + 1 X chromosome. Sperm are of two kinds; half carry 22 autosomes + 1 X and half bear 22 autosomes + 1 Y. The Y chromosome is much smaller than the X and carries very little genetic information. At fertilization, when the 2 X chromosomes come together, offspring are female; when X and Y come together, offspring are male. The XX-XY kind of sex determination is shown in Figure 5.4.

A third type of sex determination is found in birds, moths, butterflies, and some fish, in which the male has 2 X (or sometimes called ZZ) chromosomes and the female an X and Y (or ZW). Finally, there are both invertebrates (p. 380) and vertebrates (p. 571) in which sex is determined by environmental or behavioral conditions rather than by sex chromosomes, or by genetic loci whose variation is not associated with visible difference in chromosomal structure.

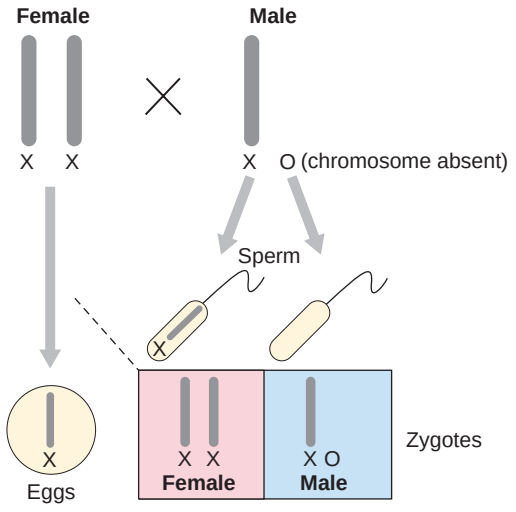


Figure 5.3
XX-XO sex determination. Only the sex chromosomes are shown.

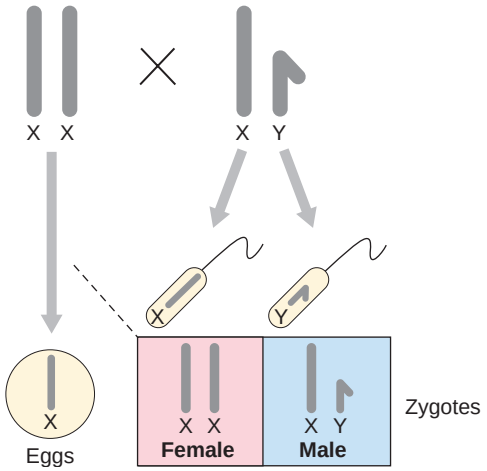


Figure 5.4
XX-XY sex determination. Only the sex chromosomes are shown.

In the case of X and Y chromosomes, homologous chromosomes are unlike in size and shape. Therefore, they do not both carry the same genes. Genes of the X chromosome often do not have allelic counterparts on the diminutive Y chromosome. This fact is very important in sex-linked inheritance (p. 87).

MENDELIAN LAWS OF INHERITANCE

Mendel's First Law

Mendel's **law of segregation** states that *in the formation of gametes, paired factors that may specify alternative phenotypes (visible traits) separate so that each gamete receives only one member of the pair.* In one of Mendel's original experiments, he pollinated pure-line tall plants with the pollen of pure-line dwarf plants. Thus the visible characteristics, or **phenotypes,** of the parents were tall and dwarf. Mendel found that all progeny in the first generation (F_1) were tall, just as tall as the tall parents of the cross. The reciprocal cross—dwarf plants pollinated with tall plants—gave the same result. The tall phenotype appeared in all progeny no matter which way the cross was made. Obviously, this kind of inheritance was not a blending of two traits, because none of the progeny was intermediate in size.

Next Mendel self-fertilized ("selfed") the tall F_1 plants and raised several hundred progeny, the second (F_2) generation. This time, *both* tall and dwarf plants appeared. Again, there was no blending (no plants of intermediate size), but the appearance of dwarf plants from all tall parental plants was surprising. The dwarf trait, seen in half of the grandparents but not in the parents, had reappeared. When he counted the actual number of tall and dwarf plants in the F_2 generation, he discovered that there were almost exactly three times more tall plants than dwarf ones.

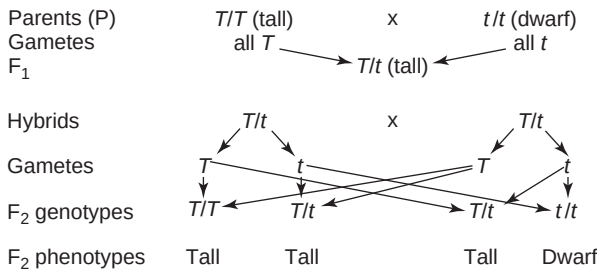
Mendel then repeated this experiment for the six other contrasting traits that he had chosen, and in every case he obtained ratios very close to 3:1 (see Figure 5.1). At this point it must have been clear to Mendel that he was dealing with hereditary determinants for the contrasting traits that did not blend when brought together. Even though the dwarf trait disappeared in the F_1 generation, it reappeared fully expressed in the F_2 generation. He realized that the F_1 generation plants carried determinants (which he called "factors") of both tall and dwarf parents, even though only the tall trait was visible in the F_1 generation.

Mendel called the tall factor **dominant** and the short **recessive.** Similarly, the other pairs of traits that he studied showed dominance and recessiveness. Whenever a dominant factor is present, the recessive one is not visible. The recessive trait appears only when both factors are recessive, or in other words, in a pure condition.

In representing his crosses, Mendel used letters as symbols; a capital letter denotes a dominant trait, and the corresponding lowercase letter denotes its recessive alternative. Modern geneticists still often follow this custom. Thus the factors for pure tall plants might be represented by *T/T,* the pure recessive by *t/t,* and the mix, or hybrid, of the two plants by *T/t.* The slash mark indicates that the alleles are on homologous chromosomes. The zygote bears the complete genetic constitution of the organism. All gametes produced by *T/T* must necessarily be *T,* whereas those produced by *t/t* must be *t.* Therefore a zygote produced by union of the two must be *T/t,* or a **heterozygote.** On the other hand, the pure tall plants (*T/T*) and pure dwarf plants (*t/t*) are **homozygotes,** meaning that the paired factors are alike on the homologous chromosomes and represent copies of the same allele. A cross involving variation at only a single locus is called a **monohybrid cross.**

In the cross between tall and dwarf plants there were two phenotypes: tall and dwarf. On the basis of genetic formulas there are three *hereditary* types: *T/T, T/t,* and *t/t.* These are called **genotypes.** A genotype is an allelic combination present in a diploid organism (*T/T, T/t,* or *t/t*), and the phenotype is the corresponding appearance of the organism (tall or dwarf).

One of Mendel's original crosses (tall plant and dwarf plant) could be represented as follows:



All possible combinations of F_1 gametes in the F_2 zygotes yield a 3:1 phenotypic ratio and a 1:2:1 genotypic ratio. It is convenient in such crosses to use the checkerboard method devised by Punnett (Punnett square) for representing the various combinations resulting from a cross. In the F_2 cross this scheme would apply:

Pollen \ Ova	$^1/_2$ *T*	$^1/_2$ *t*
$^1/_2$ *T*	$^1/_4$ *T*/*T* (homozygous tall)	$^1/_4$ *T*/*t* (hybrid tall)
$^1/_2$ *t*	$^1/_4$ *T*/*t* (hybrid tall)	$^1/_4$ *t*/*t* (homozygous dwarf)

Ratio: 3 tall to 1 dwarf

The next step was an important one because it enabled Mendel to test his hypothesis that every plant contained nonblending factors from both parents. He self-fertilized the plants in the F_2 generation; the pollen of a flower fertilized the stigma of the same flower. The results showed that self-pollinated F_2 dwarf plants produced only dwarf plants, whereas one-third of the F_2 tall plants produced tall and the other two-thirds produced both tall and dwarf in the ratio of 3:1, just as the F_1 plants had done. Genotypes and phenotypes were as follows:

F_2 plants: Tall $^1/_4$ *T*/*T* $\xrightarrow{\text{Selfed}}$ all *T*/*T* (homozygous tall)

Tall $^1/_2$ *T*/*t* $\xrightarrow{\text{Selfed}}$ 1 *T*/*T*: 2 *T*/*t*: 1 *t*/*t* (3 tall: 1 dwarf)

Dwarf $^1/_4$ *t*/*t* $\xrightarrow{\text{Selfed}}$ all *t*/*t* (homozygous dwarf)

This experiment showed that the dwarf plants were pure because they at all times gave rise to short plants when self-pollinated; the tall plants contained both pure tall and hybrid tall. It also demonstrated that, although the dwarf trait disappeared in the F_1 plants, which were all tall, dwarfness appeared in the F_2 plants.

Mendel reasoned that the factors for tallness and dwarfness were units that did not blend when they were together in a hybrid individual. The F_1 generation contained both of these units or factors, but when these plants formed their germ cells, the factors separated so that each germ cell had only one factor. In a pure-breeding plant both factors were alike; in a hybrid they were different. He concluded that individual germ cells were always pure with respect to a pair of contrasting factors, even when the germ cells were formed from hybrid individuals possessing both contrasting factors.

This idea formed the basis for Mendel's law of segregation, which states that whenever two factors are brought together in a hybrid, they segregate into separate gametes produced by the hybrid. The paired factors of the parent pass with equal frequency to the gametes. We now understand that the factors segregate because they occur on different chromosomes of a homologous pair, but the gametes receive only one chromosome of each pair in meiosis. Thus in current usage the law of segregation refers to the parting of homologous chromosomes during meiosis.

Mendel's great contribution was his quantitative approach to inheritance. His approach marks the birth of genetics, because before Mendel, people assumed that traits were blended like mixing together two colors of paint, a notion that unfortunately still lingers in the minds of many and was a problem for Darwin's theory of natural selection when he first proposed it (p. 17). If traits blended, variability would be lost in hybridization. With particulate inheritance, different alleles remain intact through the hereditary process and can be resorted like particles.

In not reporting conflicting findings, which must surely have arisen as they do in any original research, Mendel has been accused of "cooking" his results. The chances are, however, that he carefully avoided ambiguous material to strengthen his central message. Mendel's results have withstood repeated testing by other researchers, which confirms their scientific integrity.

Testcross

When an allele is dominant, heterozygous individuals containing that allele are identical in phenotype to individuals homozygous for it. Therefore one cannot determine the genotypes of these individuals just by observing their phenotypes. For instance, in Mendel's experiment of tall and dwarf traits, it is impossible to determine the genetic constitution of the tall plants of the F_2 generation by mere inspection of the tall plants. Three-fourths of this generation are tall, but which ones are heterozygotes?

As Mendel reasoned, the test is to cross the questionable individuals with pure recessives. If the tall plant is homozygous, all offspring in such a testcross are tall, thus:

Parents *T*/*T* (tall) x *t*/*t* (dwarf)

Pollen \ Ova	*T*	*T*
t	*T*/*t* (hybrid tall)	*T*/*t* (hybrid tall)
t	*T*/*t* (hybrid tall)	*T*/*t* (hybrid tall)

All of the offspring are *T*/*t* (hybrid tall). If the tall plant is heterozygous, half of the offspring are tall and half dwarf, thus:

Parents *T*/*t* (hybrid tall) x *t*/*t* (dwarf)

Pollen \ Ova	*T*	*t*
t	*T*/*t* (hybrid tall)	*t*/*t* (homozygous dwarf)
t	*T*/*t* (hybrid tall)	*t*/*t* (homozygous dwarf)

The **testcross** is often used in modern genetics to assess the genetic constitution of offspring and to make desirable homozygous stocks of animals and plants.

Intermediate Inheritance

In some cases neither allele is completely dominant over the other, and the heterozygous phenotype is distinct from those of the parents, often intermediate between them. This is called

intermediate inheritance, or **incomplete dominance.** In the four-o'clock flower (*Mirabilis*), two allelic variants determine red versus pink or white flowers; homozygotes are red or white flowered, but heterozygotes have pink flowers. In a certain strain of chickens, a cross between those with black and splashed white feathers produces offspring that are not gray but a distinctive color called Andalusian blue (Figure 5.5). In each case, if the F_1s are crossed, the F_2s have a ratio of 1:2:1 in colors, or 1 red: 2 pink: 1 white in four-o'clock flowers and 1 black: 2 blue: 1 white for Andalusian chickens. This phenomenon can be illustrated for the chickens as follows:

Parents	*B/B*	(black feathers)		χ	*B′/B′*	(white feathers)	
Gametes	all *B*				all *B′*		
F_1			*B/B′* (all blue)				
Crossing hybrids		*B/B′*		χ		*B/B′*	
Gametes		*B,B′*				*B,B′*	
F_2 genotypes	*B/B*		*B/B′*		*B/B′*		*B′/B′*
F_2 phenotypes	Black		Blue		Blue		White

When neither of the alleles is recessive, it is customary to represent both by capital letters and to distinguish them by the addition of a "prime" sign (*B′*) or by superscript letters, for example, B^b (equals black feathers) and B^w (equals white feathers).

In this kind of cross, the heterozygous *phenotype* is indeed a blending of both parental types. It is easy to see how such observations would encourage the notion of blending inheritance. However, in the cross of black and white chickens or red and white flowers, only the hybrid phenotype is a blend; its hereditary factors do not blend and homozygous offspring breed true to the parental phenotypes.

Mendel's Second Law

Mendel's second law pertains to studies of two pairs of hereditary factors at the same time. For example, does the inheritance of factors for yellow versus green seeds influence the inheritance of factors for tall versus short plants when the strains being crossed differ for both seed color and plant height? Mendel performed crossing experiments between pea strains that differ by two or more phenotypic characters controlled by variation at different genes located on different chromosomes. According to Mendel's **law of independent assortment,** *genes located on different pairs of homologous chromosomes assort independently during meiosis.*

Mendel had already established that tall plants were dominant to dwarf. He also noted that crosses between plants bearing yellow seeds and plants bearing green seeds produced plants with yellow seeds in the F_1 generation; therefore yellow was dominant to green. The next step was to make a cross between

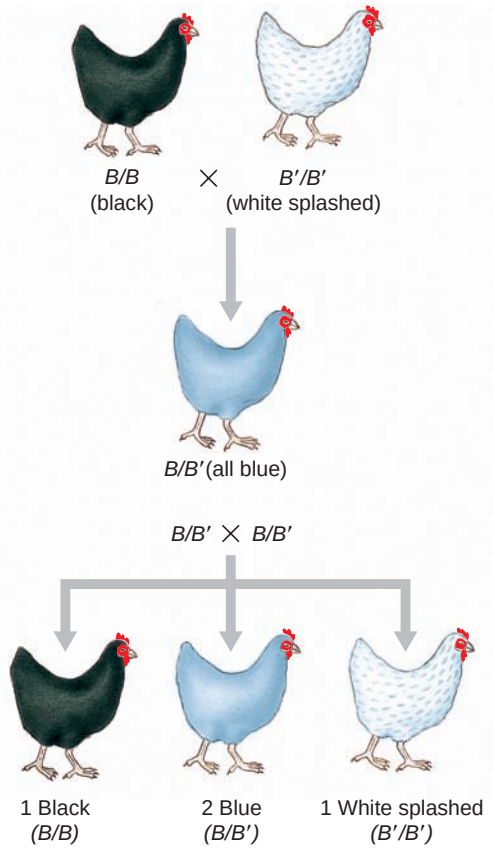


Figure 5.5
Cross between chickens with black and splashed white feathers. Black and white are homozygous; Andalusian blue is heterozygous.

plants differing in these two characteristics. When a tall plant with yellow seeds (*T/T Y/Y*) was crossed with a dwarf plant with green seeds (*t/t y/y*), the F_1 plants were tall and yellow as expected (*T/t Y/y*).

The F_1 hybrids were then self-fertilized giving the F_2 results shown in Figure 5.6.

Parents	*T/T Y/Y*	χ	*t/t y/y*
	(tall, yellow)		(dwarf, green)
Gametes	all *TY*		all *ty*
F_1		*T/t Y/y*	
		(tall, yellow)	

Mendel already knew that a cross between two plants bearing a single pair of alleles of the genotype *T/t* would yield a 3:1 ratio. Similarly, a cross between two plants with the genotypes *Y/y* would yield the same 3:1 ratio. If we examine *only* the tall and dwarf phenotypes expected in the outcome of the dihybrid experiment, they produce a ratio of 12 tall to 4 dwarf, which reduces to a ratio of 3:1. Likewise, a total of 12 plants have yellow seeds for every 4 plants that have green—again a 3:1 ratio. Thus the monohybrid ratio prevails for both traits when they are considered independently. The 9:3:3:1 ratio is nothing more than a combination of the two 3:1 ratios.

$$3:1 \times 3:1 = 9:3:3:1$$

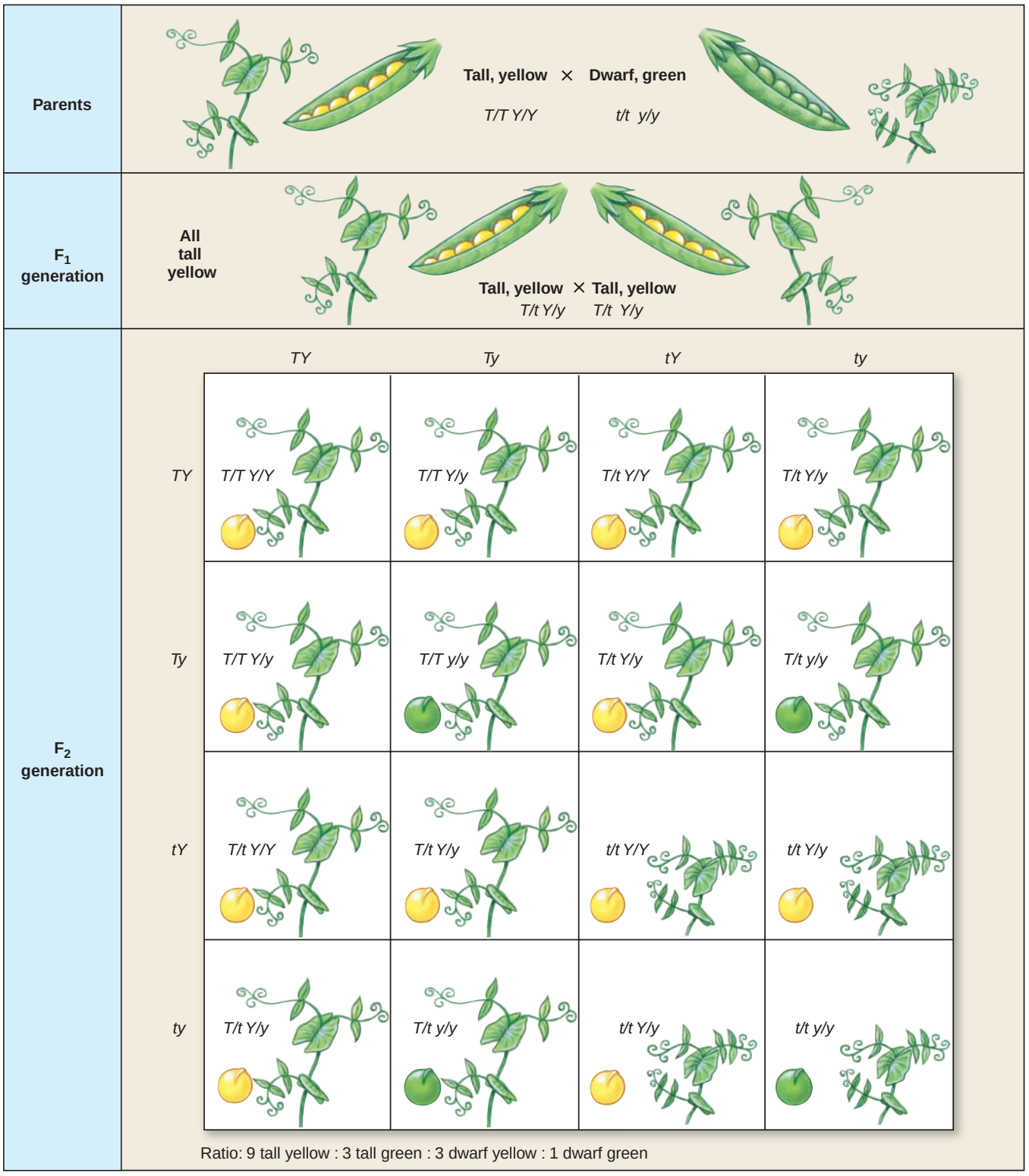


Figure 5.6

Punnett square method for determining ratios of genotypes and phenotypes expected in a dihybrid cross for independently assorting genes.

When one of the alleles is unknown, it can be designated by a dash (*T*/—). This designation is used also when it is immaterial whether the genotype is heterozygous or homozygous, as when we count all of a genetically dominant phenotype. The dash could be either *T* or *t*.

The F_2 genotypes and phenotypes are as follows:

1	*T/T*	*Y/Y*	9 *T/—Y/—*	9 Tall yellow
2	*T/t*	*Y/Y*		
2	*T/T*	*Y/y*		
4	*T/t*	*Y/y*		
1	*T/T*	*y/y*	3 *T/—y/y*	3 Tall green
2	*T/t*	*y/y*		
1	*t/t*	*Y/Y*	3 *t/t—Y/—*	3 Dwarf yellow
2	*t/t*	*Y/y*		
1	*t/t*	*y/y*	1 *t/t y/y*	1 Dwarf green

The results of this experiment show that segregation of alleles for plant height is entirely independent of segregation of alleles for seed color. Thus another way to state Mendel's law of independent assortment is that *paired copies of two different genes located on different (= nonhomologous) chromosomes segregate independently of one another.* The reason is that during meiosis the member of any pair of homologous chromosomes transmitted to a gamete is independent of which member of any other pair of chromosomes it receives. Of course, if the genes were close together on the same chromosome, they would assort together (be linked) unless crossing over occurred. Genes located very far apart on the same chromosome show independent assortment because crossing over occurs between them in nearly every meiosis. Linked genes and crossing over are discussed on p. 88.

One way to estimate proportions of progeny expected to have a given genotype or phenotype is to construct a Punnett square. With a monohybrid cross, this is easy; with a dihybrid cross, a Punnett square is laborious; and with a trihybrid cross, it is very tedious. We can make such estimates more easily by taking advantage of simple probability calculations. The basic assumption is that the genotypes of gametes of one sex have a chance of uniting with the genotypes of gametes of the other sex in proportion to the numbers of each present. This is generally true when the sample size is large enough, and the actual numbers observed come close to those predicted by the laws of probability.

We define probability, which is the expected frequency of an event, as follows:

$$\text{Probability (p)} = \frac{\text{Number of times an event happens}}{\text{Total number of trials or possibilities for the event to happen}}$$

For example, the probability (p) of a coin falling heads when tossed is 1/2, because the coin has two sides. The probability of rolling a three on a die is 1/6, because the die has six sides.

The probability of independent events occurring together (ordered events) involves the **product rule,** which is simply the product of their individual probabilities. When two coins are tossed together, the probability of getting two heads is $1/2 \times 1/2 = 1/4$, or 1 chance in 4. The probability of rolling two threes simultaneously with two dice is as follows:

$$\text{Probability of two threes} = 1/6 \times 1/6 = 1/36$$

We can use the product rule to predict the ratios of inheritance in monohybrid or dihybrid (or larger) crosses if the genes sort independently in the gametes (as they did in all of Mendel's experiments) (Table 5.1).

Note, however, that a small sample size may give a result quite different from that predicted. Thus if we tossed the coin three times and it fell heads each time, we would not be surprised. If we tossed the coin 1000 times and the number of heads diverged greatly from 500, we would strongly suspect something wrong with the coin. However, probability has no "memory." The probability of a coin toss yielding heads remains 1/2, no matter how many times the coin was tossed previously or results of the tosses.

Multiple Alleles

On page 78 we defined alleles as alternate forms of a gene. Whereas an individual can have no more than two alleles at a given locus (one each on each chromosome of the homologous pair, p. 78), many more dissimilar alleles can exist in a population. An example is the set of multiple alleles that affects coat color in rabbits. The different alleles are *C* (normal color), c^{ch} (chinchilla color), c^h (Himalayan color), and *c* (albino). The four alleles form a dominance series with *C* dominant over everything. The dominant allele is always written to the left and the recessive to the right:

$$C/c^h = \text{Normal color}$$
$$c^{ch}/c^h = \text{Chinchilla color}$$
$$c^h/c = \text{Himalayan color}$$
$$c/c = \text{albino}$$

Multiple alleles arise through mutations at the same gene locus at different times. Any gene can mutate (p. 100) if given time and thus can show many different alleles at the same locus.

Gene Interaction

The types of crosses previously described are simple in that the character variation results from the action of a single gene with one phenotypic effect. However, many genes have more than a single effect on organismal phenotypes, a phenomenon called **pleiotropy.** A gene whose variation influences eye color, for instance, could at the same time influence the development of other characters. An allele at one locus can mask or prevent the expression of an allele at another locus acting on the same trait, a phenomenon called **epistasis.** Another case of gene interaction

TABLE 5.1

Use of Product Rule for Determining Genotypic and Phenotypic Ratios in a Dihybrid Cross for Independently Assorting Genes

Parents' genotypes	*T/t Y/y*		×	*T/t Y/y*
Equivalent monohybrid crosses	*T/t* × *T/t*		and	*Y/y* × *Y/y*
Genotype ratios in F_1s of monohybrid crosses		1/4 *T/T*		1/4 *Y/Y*
		2/4 *T/t*		2/4 *Y/y*
		1/4 *t/t*		1/4 *y/y*
Combine two monohybrid ratios to determine dihybrid genotype ratios	1/4 *T/T*	×		1/4 *Y/Y* = 1/16 *T/T Y/Y* 2/4 *Y/y* = 2/16 *T/T Y/y* 1/4 *y/y* = 1/16 *T/T y/y*
	2/4 *T/t*	×		1/4 *Y/Y* = 2/16 *T/t Y/Y* 2/4 *Y/y* = 4/16 *T/t Y/y* 1/4 *y/y* = 2/16 *T/t y/y*
	1/4 *t/t*	×		1/4 *Y/Y* = 1/16 *t/t Y/Y* 2/4 *Y/y* = 2/16 *t/t Y/y* 1/4 *y/y* = 1/16 *t/t y/y*
Phenotype ratios in F_1s of monohybrid crosses				3/4 *T/*—(tall), 1/4 *t/t* (dwarf) 3/4 *Y/*—(yellow), 1/4 *y/y* (green)
Combine two monohybrid ratios to determine phenotype ratios	3/4 *T/*—	×		3/4 *Y/*— = 9/16 *T/*—*Y/*—(tall, yellow) 1/4 *y/y* = 3/16 *T/*—*y/y* (tall, green)
	1/4 *t/t*	×		3/4 *Y/*— = 3/16 *t/t Y/*—(dwarf, yellow) 1/4 *y/y* = 1/16 *t/t y/y* (dwarf, green)

Therefore phenotype ratios = 9 tall, yellow: 3 tall, green: 3 dwarf, yellow: 1 dwarf, green

is one in which several sets of alleles produce a cumulative effect on the same character.

Many cases are known in which the variation of a character results from two or more genes. Mendel probably did not appreciate the real significance of the genotype, as contrasted with the visible character—the phenotype. We now know that many different genes can affect a single phenotype **(polygenic inheritance).**

Several characters in humans are polygenic. In such cases the characters, instead of having discrete alternative phenotypes, show continuous variation between two extremes. Each of several genes has an allele that adds (+) and one that fails to add (−) an incremental dose to the value of the phenotype. This dosage-dependent inheritance is sometimes called **quantitative inheritance.** In this kind of inheritance the children are often phenotypically intermediate between the two parents. Variation at multiple genes influences phenotypic variation, but the different allelic forms of each gene remain unaltered as discrete hereditary factors as they are sorted into various genotypes. As the number of variable genes affecting a quantitative phenotype increases, intermediate conditions between the opposite extreme values of the phenotype become more continuous.

One illustration of such a type is the degree of pigmentation in matings between people having dark and light skin tones. The cumulative genes in such matings have a quantitative expression. Three or four genes are probably involved in skin pigmentation, but we simplify our explanation by referring to only two pairs of independently assorting genes. Thus a person with very dark pigment has two genes for pigmentation on separate chromosomes (*A/A B/B*). Each dominant allele contributes one unit of pigment. A person with very light pigment has alleles (*a/a b/b*) that contribute no color. (Freckles that commonly appear in the skin of very light people represent pigment contributed by entirely separate genes.) The offspring of very dark and very light parents would have an intermediate skin color (*A/a B/b*).

Children of parents having intermediate skin color show a range of skin color, depending on the number of genes for pigmentation that they inherit. Their skin color ranges from very dark (*A/A B/B*), to dark (*A/A B/b* or *A/a B/B*), intermediate (*A/A b/b* or *A/a B/b* or *a/a B/B*), light (*A/a b/b* or *a/a B/b*), to very light (*a/a b/b*). It is thus possible for parents heterozygous for skin color to produce children with darker or lighter colors than themselves.

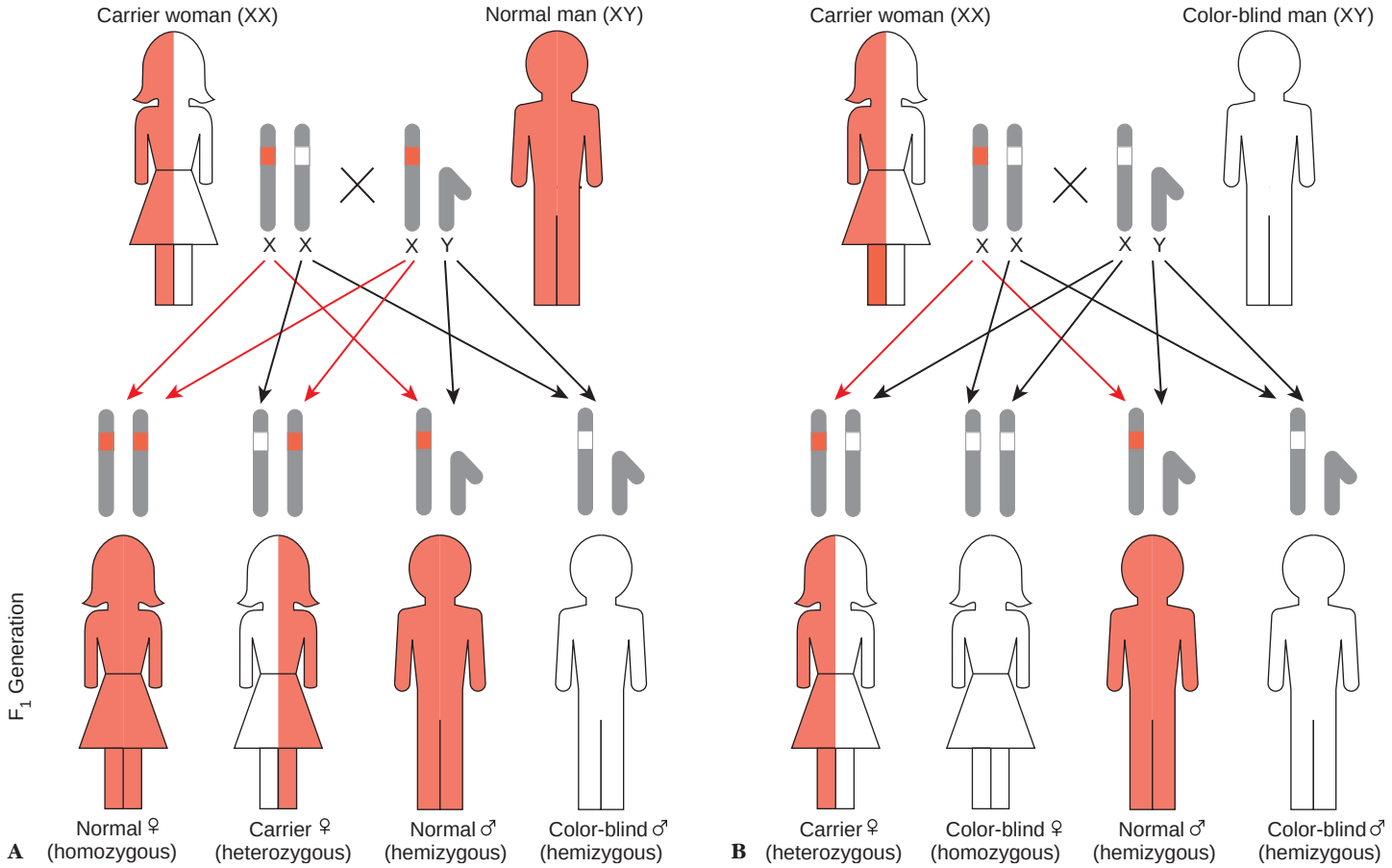


Figure 5.7

Sex-linked inheritance of red-green color blindness in humans. A, Carrier mother and normal father produce color blindness in one-half of their sons but in none of their daughters. **B,** Half of both sons and daughters of carrier mother and color-blind father are color blind.

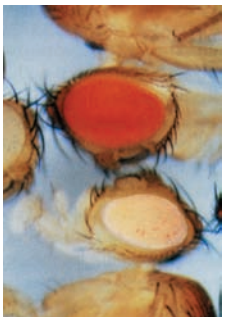
Inheritance of eye color in humans is another example of gene interaction. One allele (*B*) determines whether pigment is present in the front layer of the iris. This allele is dominant over the allele for the absence of pigment (*b*). The genotypes *B/B* and *B/b* pigment generally produce brown eyes, and *b/b* produces blue eyes. However, these phenotypes are greatly affected by many modifier genes influencing, for example, the amount of pigment present, the tone of the pigment, and its distribution. Thus a person with *B/b* may even have blue eyes if modifier genes determine a lack of pigment, thus explaining the rare instances of a brown-eyed child of blue-eyed parents.

Sex-Linked Inheritance

It is known that inheritance of some characters depends on the sex of the parent carrying the gene and the sex of the offspring. One of the best-known sex-linked traits of humans is hemophilia (see Chapter 31, p. 690). Another example is red-green color blindness in which red and green colors are indistinguishable to varying degrees. Color-blind men greatly outnumber color-blind women. When color blindness does appear in women, their fathers are color blind. Furthermore, if a woman with normal vision who is a carrier of color blindness (a **carrier** is heterozygous for the gene and is phenotypically normal) bears sons, half of them are likely to be color blind, regardless of whether the father had normal or affected vision. How are these observations explained?

Color blindness and hemophilia defects are recessive traits carried on the X chromosome. They are phenotypically expressed either when both genes are defective in the female or when only one defective gene is present in the male. The inheritance pattern of these defects is shown for color blindness in Figure 5.7. When the mother is a carrier and the father is normal, half of the sons but none of the daughters are color blind. However, if the father is color blind and the mother is a carrier, half of the sons *and* half of the daughters are color blind (on the average and in a large sample). It is easy to understand then why such defects are much more prevalent in males: a single sex-linked recessive gene in the male has a visible effect because he has only one X chromosome. What would be the outcome of a mating between a homozygous normal woman and a color-blind man?

Another example of a sex-linked character was discovered by Thomas Hunt Morgan (1910) in *Drosophila*. Normal eye



A White and red eyes of *D. melanogaster*

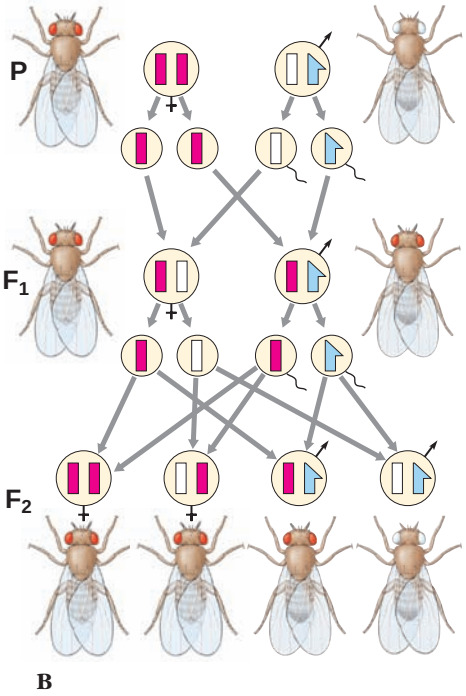


Figure 5.8

Sex-linked inheritance of eye color in fruit fly *Drosophila melanogaster.* **A,** White and red eyes of *D. melanogaster.* **B,** Genes for eye color are carried on X chromosome; Y carries no genes for eye color. Normal red is dominant to white. Homozygous red-eyed female mated with white-eyed male gives all red-eyed in F_1. F_2 ratios from F_1 cross are one homozygous red-eyed female and one heterozygous red-eyed female to one red-eyed male and one white-eyed male.

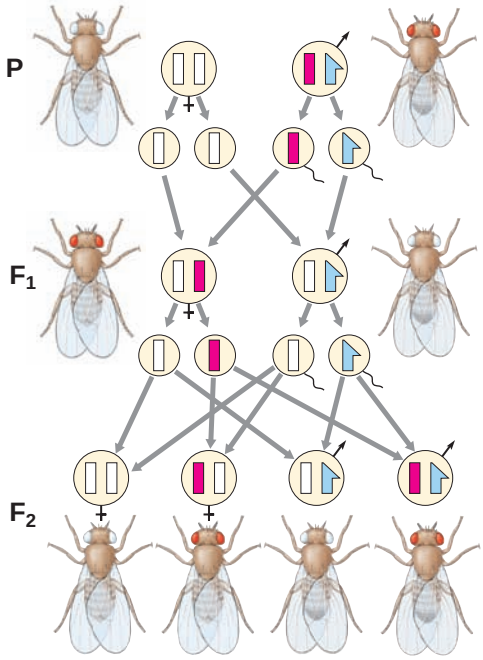


Figure 5.9

Reciprocal cross of Figure 5.8 (homozygous white-eyed female with red-eyed male) gives white-eyed males and red-eyed females in F_1. F_2 shows equal numbers of red-eyed and white-eyed females and red-eyed and white-eyed males.

color of this fly is red, but mutations for white eyes do occur (Figure 5.8). A gene for eye color is carried on the X chromosome. If true-breeding white-eyed males and red-eyed females are crossed, all F_1 offspring have red eyes because this trait is dominant (Figure 5.8). If these F_1 offspring are interbred, all F_2 females have red eyes; half of the males have red eyes and the other half have white eyes. No white-eyed females are found in this generation; only males have the recessive character (white eyes). The allele for white eyes is recessive and should affect eye color only in a homozygous condition. However, since the male has only one X chromosome (the Y does not carry a gene for eye color), white eyes appear whenever the X chromosome carries the allele for this trait. Males are said to be **hemizygous** (only one copy of a genetic locus is present) for traits carried on the X chromosome.

If the reciprocal cross is made in which females are white eyed and males red eyed, all F_1 females are red eyed and all males are white eyed (Figure 5.9). If these F_1 offspring are interbred, the F_2 generation shows equal numbers of red-eyed and white-eyed males and females.

Autosomal Linkage and Crossing Over

Linkage

Since Mendel's laws were rediscovered in 1900, it became clear that, contrary to Mendel's second law, not all factors segregate independently. Indeed, many traits are inherited together. Since the number of chromosomes in any organism is relatively small compared with the number of traits, each chromosome must contain many genes. All genes present on a chromosome are said to be **linked.** Linkage simply means that the genes are on the same chromosome, and all genes present on homologous chromosomes belong to the same linkage groups. Therefore there should be as many linkage groups as there are chromosome pairs.

Geneticists commonly use the word "linkage" in two somewhat different meanings. Sex linkage refers to inheritance of a trait on the sex chromosomes, and thus its phenotypic expression depends on the sex of the organism and the factors already discussed. Autosomal linkage, or simply, linkage, refers to inheritance of genes on a given autosomal chromosome. Letters used to represent such genes are normally written without a slash mark between them, indicating that they are on the same chromosome. For example, *AB/ab* shows that genes *A* and *B* are on the same chromosome. Interestingly, Mendel studied seven characteristics of garden peas that assorted independently because they are on seven different chromosomes. If he had studied eight characteristics, he would not have found independent assortment in two of the traits because garden peas have only seven pairs of homologous chromosomes.

In *Drosophila,* in which this principle has been studied most extensively, there are four linkage groups that correspond to the four pairs of chromosomes found in these fruit flies. Usually,

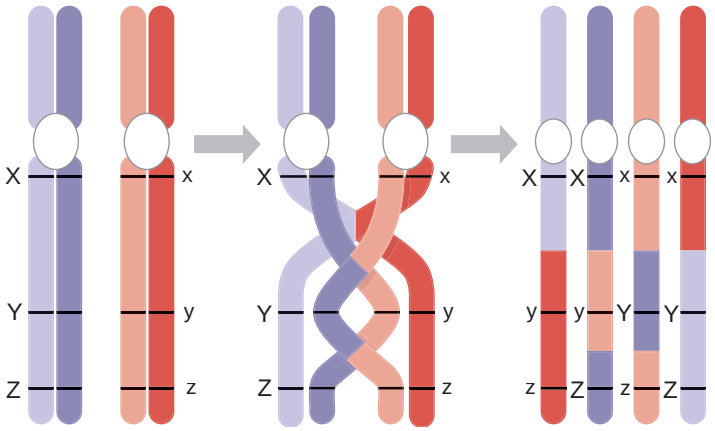


Figure 5.10

Crossing over during meiosis. Nonsister chromatids exchange portions, so that none of the resulting gametes is genetically the same as any other. Gene X is farther from gene Y than Y is from Z; therefore, gene X is more frequently separated from Y in crossing over than Y is from Z.

small chromosomes have small linkage groups, and large chromosomes have large groups.

Crossing Over

Linkage, however, is usually not complete. If we perform an experiment in which animals such as *Drosophila* are crossed, we find that linked traits separate in some percentage of the offspring. Separation of alleles located on the same chromosome occurs because of **crossing over.**

During the protracted prophase of the first meiotic division, paired homologous chromosomes break and exchange equivalent portions; genes "cross over" from one chromosome to its homolog, and vice versa (Figure 5.10). Each chromosome consists of two sister chromatids held together by means of a proteinaceous structure called a **synaptonemal complex.** Breaks and exchanges occur at corresponding points on nonsister chromatids. (Breaks and exchanges also occur between sister chromatids but usually have no genetic significance because sister chromatids are identical.) Crossing over is a means for exchanging genes between homologous chromosomes and thus greatly increases the amount of genetic recombination. The frequency of crossing over varies depending on the species, but usually at least one and often several crossovers occur each time chromosomes pair.

Because the frequency of recombination is proportional to the distance between loci, the relative linear position of each locus can be determined. Genes located far apart on very large chromosomes may assort independently because the probability of a crossover occurring between them in each meiosis is close to 100%. Such genes are found to be carried on the same chromosome only because each one is genetically linked to additional genes located physically between them on the chromosome. Laborious genetic experiments over many years have produced gene maps that indicate the positions of more than 500 genes distributed on the four chromosomes of *Drosophila melanogaster.*

Chromosomal Aberrations

Structural and numerical deviations from the norm that affect many genes at once are called chromosomal aberrations. They are sometimes called chromosomal mutations, but most cytogeneticists prefer to use the term "mutation" to refer to qualitative changes within a gene; gene mutations are discussed on page 100.

Despite the incredible precision of meiosis, chromosomal aberrations do occur, and they are more common than one might think. They are responsible for great economic benefit in agriculture. Unfortunately, they are responsible also for many human genetic malformations. It is estimated that five out of every 1000 humans are born with *serious* genetic defects attributable to chromosomal anomalies. An even greater number of embryos with chromosomal defects abort spontaneously, far more than ever reach birth.

Changes in chromosome numbers are called **euploidy** when there is the addition or deletion of whole sets of chromosomes and **aneuploidy** when a single chromosome is added to or subtracted from a set. A "set" of chromosomes contains one member of each homologous pair as would be present in the nucleus of a gamete. The most common kind of euploidy is **polyploidy,** the carrying of three or more sets of chromosomes by an organism. An organism with three or more complete sets of chromosomes is called a polyploid. Such aberrations are much more common in plants than in animals. Animals are much less tolerant of chromosomal aberrations, especially those in which sex determination requires a delicate balance between the numbers of sex chromosomes and autosomes. Many domestic plant species are polyploid (cotton, wheat, apples, oats, tobacco, and others), and perhaps 40% of flowering plants may have originated in this manner. Horticulturists favor polyploids because they often have more intensely colored flowers and more vigorous vegetative growth.

Aneuploidy is usually caused by failure of chromosomes to separate during meiosis **(nondisjunction).** If a pair of chromosomes fails to separate during the first or second meiotic divisions, both members go to one pole and none to the other. This condition results in at least one gamete or polar body having $n - 1$ chromosomes and another having $n + 1$ chromosomes. If an $n - 1$ gamete is fertilized by a normal n gamete, the result is a **monosomic** animal. Survival is rare because the lack of one chromosome gives an uneven balance of genetic instructions. **Trisomy,** the result of the fusion of a normal n gamete and an $n + 1$ gamete, is much more common, and several kinds of trisomic conditions are known in humans. Perhaps the most familiar is **trisomy 21,** or **Down syndrome.** As the name indicates, it involves an extra chromosome 21 combined with the chromosome pair 21, and it is caused by nondisjunction of that pair during meiosis. It occurs spontaneously, and there is seldom any family history of the abnormality. However, the risk of its appearance rises dramatically with increasing age of the mother;

it occurs 40 times more often in women over 40 years old than among women between the ages of 20 and 30. In cases where maternal age is not a factor, 20% to 25% of trisomy 21 results from nondisjunction during spermatogenesis; it is paternal in origin and is apparently independent of the father's age.

A *syndrome* is a group of symptoms associated with a particular disease or abnormality, although every symptom is not necessarily shown by every patient with the condition. An English physician, John Langdon Down, described in 1866 the syndrome that we now know is caused by trisomy 21. Because of Down's belief that the facial features of affected individuals were mongoloid in appearance, the condition has been called mongolism. The resemblances are superficial, however, and currently accepted names are trisomy 21 and Down syndrome. Among the numerous characteristics of the condition, the most disabling is severe mental retardation. This, as well as other conditions caused by chromosomal aberrations and several other birth defects, can be diagnosed *prenatally* by a procedure involving *amniocentesis*. The physician inserts a hypodermic needle through the abdominal wall of the mother and into fluids surrounding the fetus (*not into* the fetus) and withdraws some of the fluid, which contains some fetal cells. The cells are grown in culture, their chromosomes are examined, and other tests done. If a severe birth defect is found, the mother has the option of having an abortion performed. As an extra "bonus," the sex of the fetus is learned after amniocentesis. How? Alternatively, determination of concentrations of certain substances in the maternal serum, which is less invasive than amniocentesis, can detect about 60% of Down syndrome fetuses. Ultrasound scanning may be more than 80% accurate.

In all diploid species, normal development requires exactly two of each kind of autosome (not sex chromosomes). Nondisjunction can cause trisomies of other chromosomes, but because these lead to imbalance of many gene products, they almost always cause death before or soon after birth. However, each cell requires only one functional X chromosome (the other is inactivated in females). Nondisjunction of sex chromosomes is better tolerated but usually causes sterility and abnormalities of sex organs. For example, a human with XXY (Klinefelter syndrome) is a phenotypic male, usually infertile and with some female sexual characteristics. Presence of only one X (and no Y) is usually lethal in embryos, but the occasional live birth produces a phenotypic female with a variety of developmental abnormalities (Turner syndrome).

Structural aberrations involve whole sets of genes within a chromosome. A portion of a chromosome may be reversed, placing the linear arrangement of genes in reverse order **(inversion);** nonhomologous chromosomes may exchange sections **(translocation);** entire blocks of genes may be lost **(deletion),** usually causing serious developmental defects; or an extra section of chromosome may attach to a normal chromosome **(duplication).** These structural changes often produce phenotypic changes. Duplications, although rare, are important for evolution because they supply additional genetic information that may enable new functions.

GENE THEORY

Gene Concept

The term "gene" (Gr. *genos,* descent) was coined by W. Johannsen in 1909 for the hereditary factors of Mendel. Initially, genes were thought to be indivisible subunits of the chromosomes on which they occurred. Later studies with multiple mutant alleles demonstrated that alleles are in fact divisible by recombination; *portions* of a gene are separable. Furthermore, parts of many genes in eukaryotes are separated by sections of DNA that do not specify a part of the finished product **(introns).**

As the chief unit of genetic information, genes encode products essential for specifying the basic architecture of every cell, details of protein synthesis, cell division, and, directly or indirectly, the entire metabolic function of the cell. Because of their ability to mutate, to be assorted and shuffled in different combinations, genes are important units of heredity and variation in evolution. Genes maintain their identities for many generations despite mutational changes in some parts of their structure.

One Gene–One Enzyme Hypothesis

Since genes act to produce different phenotypes, we may infer that their action follows the scheme: gene → gene product → phenotypic expression. Furthermore, we may suspect that the gene product is usually a protein, because proteins act as enzymes, antibodies, hormones, and structural elements throughout the body.

The first clear, well-documented study to link genes and enzymes was performed on the common bread mold *Neurospora* by Beadle and Tatum in the early 1940s. This organism was ideally suited to a study of gene function for several reasons: these molds are much simpler to handle than fruit flies, they grow readily in well-defined chemical media, and they are haploid organisms unencumbered with dominance relationships among alleles. Furthermore, mutations were readily induced by irradiation with ultraviolet light. Ultraviolet-light-induced mutants, grown and tested in specific nutrient media, had single-gene mutations that were inherited. Each mutant strain was defective in one enzyme, which prevented that strain from synthesizing one or more complex molecules. The ability to synthesize a particular molecule was controlled by a single gene.

From these experiments Beadle and Tatum made an important and exciting formulation: *one gene produces one enzyme.* For this work they received the Nobel Prize for Physiology or Medicine in 1958. The new hypothesis was soon validated by research on many biosynthetic pathways. Hundreds of inherited disorders, including dozens of human hereditary diseases, are caused by single mutant genes causing loss of a specific enzyme. We now know that a particular protein may contain several chains of amino acids (polypeptides), each one specified by a different gene, and not all proteins specified by genes are enzymes (for example, structural proteins, antibodies, transport proteins, and hormones). Furthermore, genes directing the synthesis of various kinds of RNA were not included in Beadle

and Tatum's formulation. Therefore a gene now may be defined more inclusively as *a nucleic acid sequence (usually DNA) that encodes a functional polypeptide or RNA sequence.*

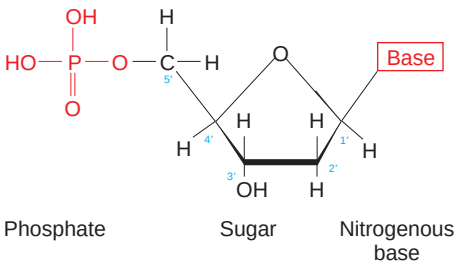
STORAGE AND TRANSFER OF GENETIC INFORMATION

Nucleic Acids: Molecular Basis of Inheritance

Cells contain two kinds of nucleic acids: deoxyribonucleic acid (DNA), which is the genetic material, and ribonucleic acid (RNA), which functions in protein synthesis. Both DNA and RNA are polymers built of repeated units called **nucleotides.** Each nucleotide contains three parts: a **sugar,** a **nitrogenous base,** and a **phosphate group.** The sugar is a pentose (5-carbon) sugar; in DNA it is **deoxyribose** and in RNA it is **ribose** (Figure 5.11).

Nitrogenous bases of nucleotides are also of two types: pyrimidines, whose characteristic structure is a single, 6-membered ring, and purines, which contain two fused rings. Purines and pyrimidines contain nitrogen as well as carbon in their rings, which is why they are called "nitrogenous" bases. The purines in both RNA and DNA are adenine and guanine (Table 5.2). The pyrimidines in DNA are thymine and cytosine, and in RNA they are uracil and cytosine. Carbon atoms in the bases are numbered (for identification) according to standard biochemical notation (Figure 5.12). Carbons in ribose and deoxyribose are also numbered, but to distinguish them from the carbons in the bases, numbers for carbons in the sugars are given prime signs (see Figure 5.11).

The sugar, phosphate group, and nitrogenous base are linked as shown in the generalized scheme for a nucleotide:



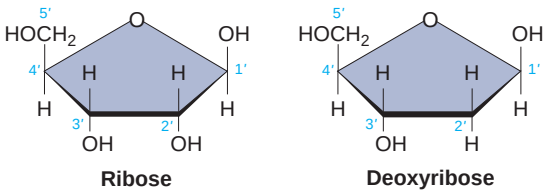


Figure 5.11

Ribose and deoxyribose, the pentose sugars of nucleic acids. A carbon atom lies in each of the four corners of the pentagon (labeled 1′ to 4′). Ribose has a hydroxyl group (—OH) and a hydrogen on the number 2′ carbon; deoxyribose has two hydrogens at this position.

TABLE 5.2

Chemical Components of DNA and RNA

	DNA	RNA
Purines	Adenine	Adenine
	Guanine	Guanine
Pyrimidines	Cytosine	Cytosine
	Thymine	Uracil
Sugar	2-Deoxyribose	Ribose
Phosphate	Phosphoric acid	Phosphoric acid

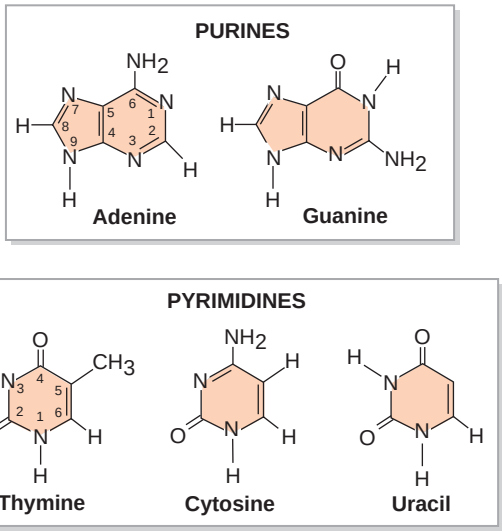


Figure 5.12

Purines and pyrimidines of DNA and RNA.

In DNA the "backbone" of the molecule is built of phosphoric acid and deoxyribose; to this backbone are attached the nitrogenous bases (Figure 5.13). The **5′ end** of the backbone has a free phosphate group on the **5′** carbon of the ribose, and the **3′ end** has a free hydroxyl group on the **3′** carbon. However, one of the most interesting and important discoveries about the nucleic acids is that DNA is not a single polynucleotide chain; it has *two* complementary chains that are precisely cross-linked by specific hydrogen bonding between purine and pyrimidine bases. The number of adenines equals the number of thymines, and the number of guanines equals the number of cytosines. This fact suggested a pairing of bases: adenine with thymine (AT) and guanine with cytosine (GC) (see Figures 1.6 and 5.14).

The result is a ladder structure (Figure 5.15). The upright portions are the sugar-phosphate backbones, and the connecting rungs are the paired nitrogenous bases, AT or GC. However, the ladder is twisted into a **double helix** with approximately 10 base pairs for each complete turn of the helix (Figure 5.16). The two DNA strands run in opposite directions **(antiparallel),** and the 5′ end of one strand is opposite the 3′ end of the other (Figure 5.16). The two strands are also **complementary**—the

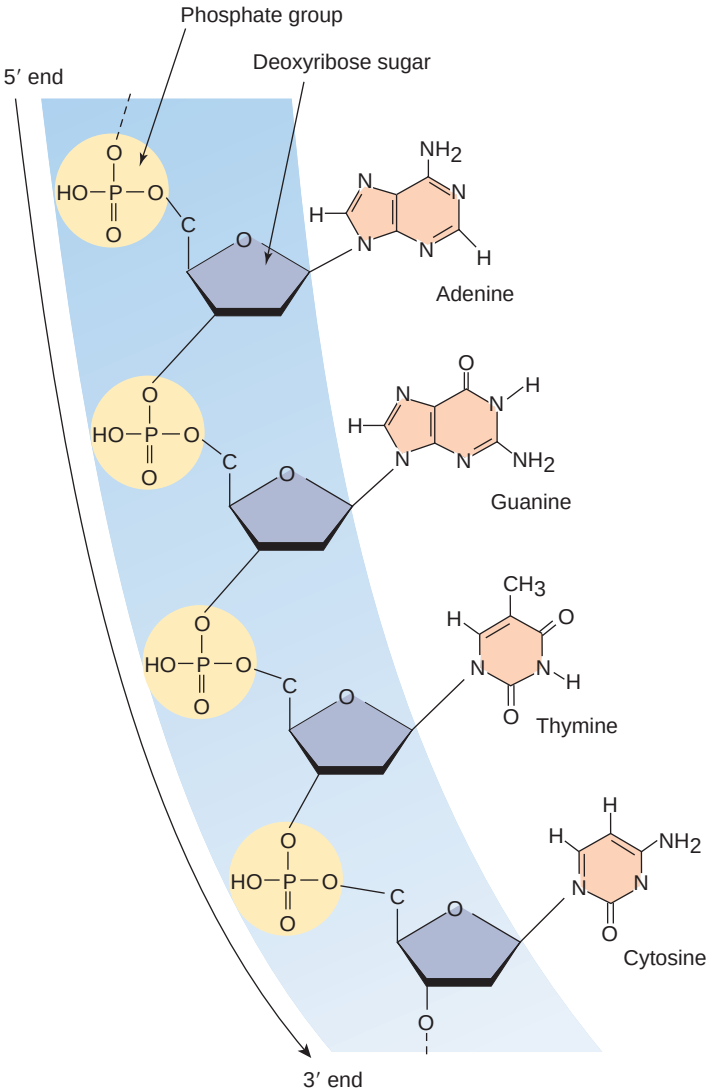


Figure 5.13

Section of a strand of DNA. Polynucleotide chain is built of a "backbone" of phosphoric acid and deoxyribose sugar molecules. Each sugar holds a nitrogenous base. Shown from top to bottom are adenine, guanine, thymine, and cytosine.

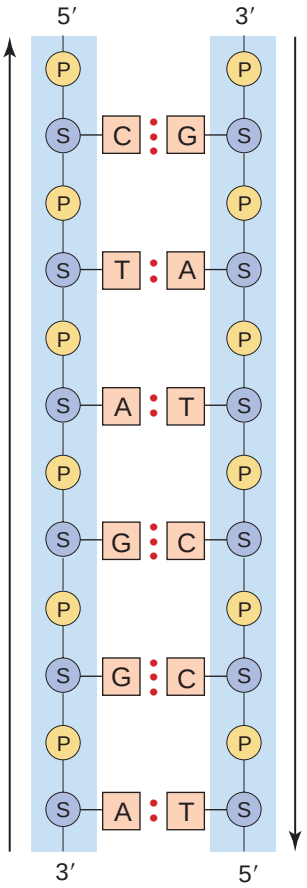


Figure 5.15

DNA, showing how the complementary pairing of bases between the sugar-phosphate "backbones" keeps the double helix at a constant diameter for the entire length of the molecule. Dots represent the three hydrogen bonds between each cytosine and guanine and the two hydrogen bonds between each adenine and thymine.

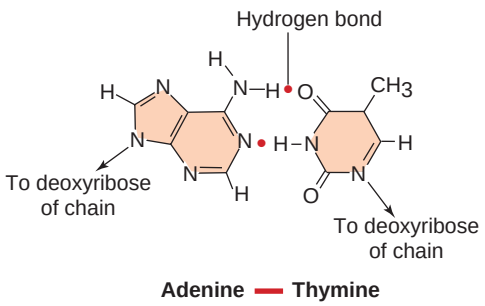
sequence of bases along one strand specifies the sequence of bases along the other strand.

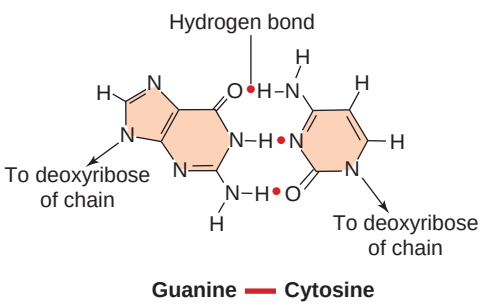
The structure of DNA is widely considered the single most important biological discovery of the twentieth century. It was based on X-ray diffraction studies of Maurice H. F. Wilkins and Rosalind Franklin and on ingenious proposals of Francis H. C. Crick and James D. Watson published in 1953. Watson, Crick, and Wilkins were later awarded the Nobel Prize for Physiology or Medicine for their momentous work. Rosalind Franklin was not included because she died prior to the award.

RNA is similar to DNA in structure except that it consists of a *single* polynucleotide chain (except in some viruses), has ribose instead of deoxyribose, and has uracil instead of thymine.

Figure 5.14

Positions of hydrogen bonds between thymine and adenine and between cytosine and guanine in DNA.





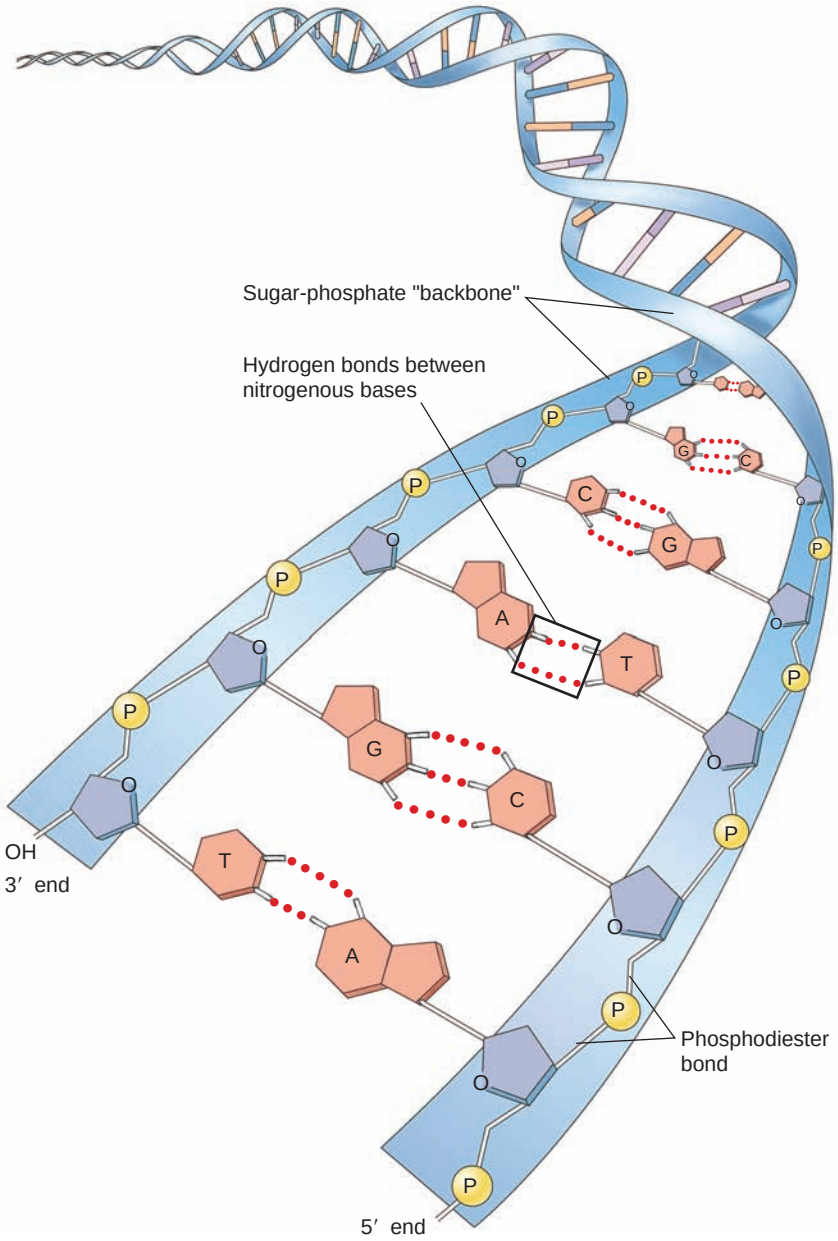


Figure 5.16
DNA molecule.

Ribosomal, transfer, and messenger RNAs are the most abundant and well-known types (function described on pp. 95–96), but many structural and regulatory RNAs, such as micro RNAs, are known.

Every time a cell divides, the structure of DNA must be precisely copied in the daughter cells. This is called **replication** (Figure 5.17). During replication, the two strands of the double helix unwind, and each separated strand serves as a **template** against which a complementary strand is synthesized. An enzyme (DNA polymerase) catalyzes assembly of a new strand of polynucleotides with a thymine group going opposite the adenine group in the template strand, a guanine group opposite the cytosine group, and the two reverse conditions. DNA polymerase synthesizes new strands only in the direction of 5′ to 3′. Because the parent DNA strands are antiparallel, one of which runs 5′ to 3′ and the other running 3′ to 5′, synthesis along one of the strands is continuous, and the other must be formed in a series of fragments, each of which begins with a 5′ end running toward a 3′ end (Figure 5.17).

DNA Coding by Base Sequence

Because DNA is the genetic material and contains a linear sequence of base pairs, an obvious extension of the Watson-Crick model is that the sequence of base pairs in DNA codes for, and is colinear with, the sequence of amino acids in a protein. The coding hypothesis must explain how a string of four different bases—a four-letter alphabet—could specify the sequence of 20 different amino acids.

In the coding procedure, obviously there cannot be a 1:1 correspondence between four bases and 20 amino acids. If a coding unit (often called a word, or **codon**) were two bases, only 16 words (4^2) could be formed, which could not specify 20 amino acids. Therefore the codon must contain at least three bases or three letters, because 64 possible words (4^3) could be formed by four bases when taken as triplets. A triplet code permits considerable redundancy of triplets (codons), because DNA encodes just 20 amino acids. Later work confirmed that nearly all amino acids are specified by more than one triplet code (Table 5.3).

DNA shows surprising stability, both in prokaryotes and in eukaryotes. Interestingly, it is susceptible to damage by harmful chemicals in the environment and by radiation. Such damage is usually not permanent, because cells have an efficient repair system. Various types of damage and repair are known, one of which is **excision repair.** Ultraviolet irradiation often damages DNA by linking adjacent pyrimidines by covalent bonds (dimerize), preventing transcription and replication. A series of several enzymes "recognizes" the damaged strand and excises the pair of dimerized pyrimidines and several bases following them. DNA polymerase then synthesizes the missing strand using the remaining one as a template, according to the base-pairing rules, and the enzyme **DNA ligase** joins the end of the new strand to the old one.

Transcription and the Role of Messenger RNA

Information is coded in DNA, but DNA does not participate directly in protein synthesis. The intermediary molecule between DNA and protein is another nucleic acid called **messenger RNA (mRNA).** The triplet codes in DNA are **transcribed** into mRNA, with uracil substituting for thymine (Table 5.3).

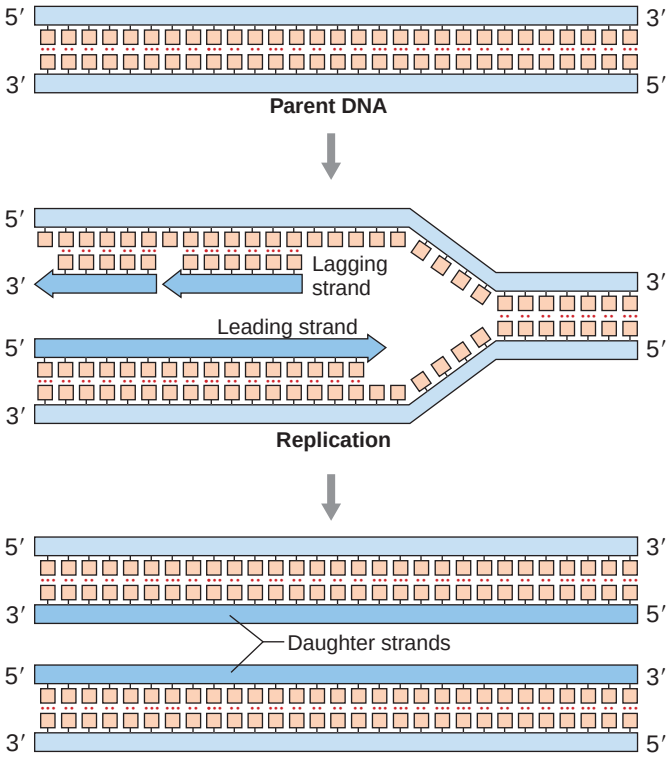


Figure 5.17

Replication of DNA. Parent strands of DNA part, and DNA polymerase synthesizes daughter strands using the base sequence of parent strands as a template. Because synthesis always proceeds in a 5′ to 3′ direction, synthesis of one strand is continuous, and the other strand must be synthesized as a series of fragments.

Ribosomal, transfer, and messenger RNAs are transcribed directly from DNA, each encoded by different sets of genes. RNA is formed as a complementary copy of one strand of the appropriate gene using an enzyme called **RNA polymerase.** (In eukaryotes each type of RNA [ribosomal, transfer, and messenger] is transcribed by a different type of RNA polymerase.) The RNA contains a sequence of bases that complements the bases in one of the two DNA strands, just as the DNA strands complement each other. Thus A in the template DNA strand is replaced by U in RNA; C is replaced by G; G is replaced by C; and T is replaced by A. Only one of the two chains is used as the template for RNA synthesis (Figure 5.18). A codon is referenced as the sequence of bases present in a mRNA molecule (Table 5.3), which is complementary and antiparallel to the template DNA strand (often called the "sense" strand) from which it is made. The DNA strand not used as a template during transcription of a gene is called the "antisense" strand.

A bacterial gene is encoded on a continuous stretch of DNA, transcribed into mRNA, and then translated (see the next section). The hypothesis that eukaryotic genes had a similar structure was rejected by the surprising discovery that some stretches of DNA are transcribed in the nucleus but are not found in the corresponding mRNA in the cytoplasm. In other words, pieces of the nuclear mRNA were removed in the nucleus before the finished mRNA was transported to the cytoplasm (Figure 5.19). Thus many genes are split, interrupted by sequences of bases that do not code for the final product, and mRNA transcribed from them must be edited or "matured" before translation in the cytoplasm. The intervening segments of DNA are called **introns,** and those that encode part of

TABLE 5.3

The Genetic Code: Amino Acids Specified by Codons of Messenger RNA

First Letter	Second Letter: U		Second Letter: C		Second Letter: A		Second Letter: G		Third Letter
U	UUU, UUC	Phenylalanine	UCU, UCC, UCA, UCG	Serine	UAU, UAC	Tyrosine	UGU, UGC	Cysteine	U, C
	UUA, UUG	Leucine			UAA, UAG	End chain	UGA	End chain	A
							UGG	Tryptophane	G
C	CUU, CUC, CUA, CUG	Leucine	CCU, CCC, CCA, CCG	Proline	CAU, CAC	Histidine	CGU, CGC, CGA, CGG	Arginine	U, C, A, G
					CAA, CAG	Glutamine			
A	AUU, AUC, AUA	Isoleucine	ACU, ACC, ACA, ACG	Threonine	AAU, AAC	Asparagine	AGU, AGC	Serine	U, C
	AUG	Methionine*			AAA, AAG	Lysine	AGA, AGG	Arginine	A, G
G	GUU, GUC, GUA, GUG	Valine	GCU, GCC, GCA, GCG	Alanine	GAU, GAC	Aspartic acid	GGU, GGC, GGA, GGG	Glycine	U, C, A, G
					GAA, GAG	Glutamic acid			

*Also, begin polypeptide chain.

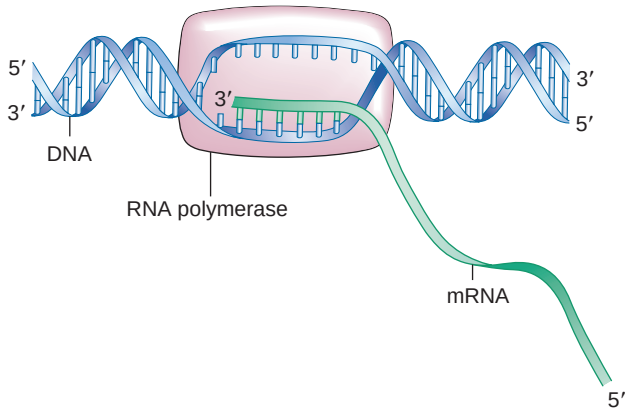


Figure 5.18

Transcription of mRNA from a DNA template. Transcription is similar for mRNA, rRNA, and tRNA except that each kind of RNA uses a different form of the enzyme, RNA polymerase. This diagram shows transcription midway to completion. Transcription began by unwinding the DNA helix, annealing of a RNA primer to the template strand of DNA, and extension of the primer at its 3′ end by adding nucleotides (not shown) complementary to the sequence of bases in the template DNA strand. The primer is at the 5′ end of the mRNA, which continues to grow in length by adding nucleotides at its annealed 3′ end. When transcription is finished, the mRNA will detach completely from the DNA template.

the mature RNA and are translated into protein are called **exons.** Before mRNA leaves the nucleus, a methylated guanine "cap" is added at the 5′ end, and a tail of adenine nucleotides (poly-*A*) is often added at the 3′ end (Figure 5.19). The cap and the poly-*A* tail distinguish mRNA from other kinds of RNA molecules.

In mammals, genes coding for histones and for interferons are on continuous stretches of DNA. However, we now know that genes coding for many proteins are split. In lymphocyte differentiation the parts of the split genes coding for immunoglobulins are actually *rearranged* during development, so that different proteins result from subsequent transcription and translation. This rearrangement partly explains the enormous diversity of antibodies manufactured by descendants of the lymphocytes (p. 775).

Base sequences in some introns are complementary to other base sequences in the intron, suggesting that the intron could fold so that complementary sequences would pair. This folding may be necessary to control proper alignment of intron boundaries before splicing. Most surprising of all is the discovery that, in some cases, RNA can "self-catalyze" the excision of introns. The ends of the intron join; the intron thus becomes a small circle of RNA, and the exons are spliced together. This process does not fit the classical definition of an enzyme or other catalyst because the molecule itself is changed by the reaction.

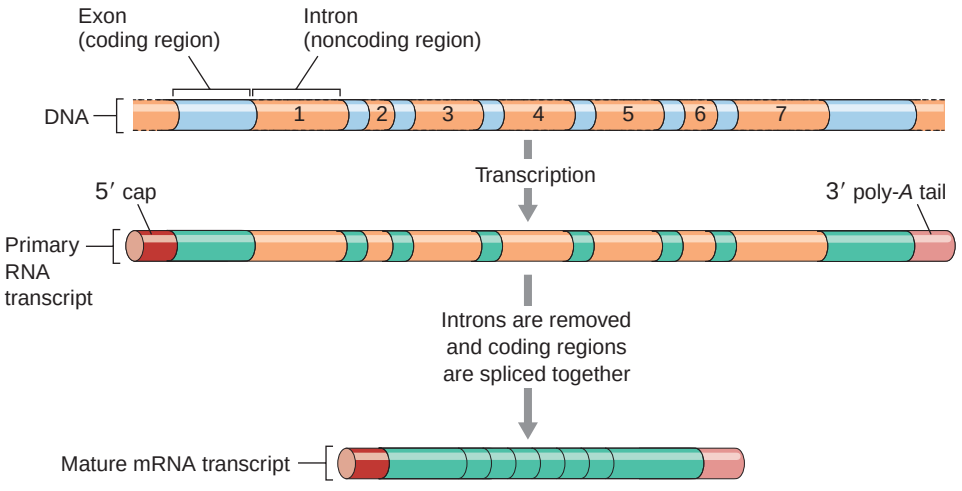
Translation: Final Stage in Information Transfer

The **translation** process occurs on **ribosomes,** granular structures composed of protein and **ribosomal RNA (rRNA).** Ribosomal RNA contains a large and a small subunit, and the small subunit comes to lie in a depression of a large subunit to form a functional ribosome (Figure 5.20). Messenger RNA molecules attach themselves to ribosomes to form a messenger RNA-ribosome complex. Because only a short section of mRNA makes contact with a single ribosome, the mRNA usually has several ribosomes attached along its length, each one at a different stage of synthesizing the encoded polypeptide. The entire complex, called a **polyribosome** or **polysome,** allows several molecules of the same kind of polypeptide to be synthesized concurrently, one on each ribosome of the polysome (Figure 5.20).

Assembly of polypeptides on the mRNA-ribosome complex requires another kind of RNA called **transfer RNA (tRNA).** Transfer RNAs have a complex secondary structure of folded stems and loops, often illustrated in the form of a cloverleaf (Figure 5.21), although the three-dimensional shape is somewhat different. Molecules of tRNA collect free amino acids from the cytoplasm and deliver them to the polysome, where they are assembled into a polypeptide. There are special tRNA molecules

Figure 5.19

Expression of ovalbumin gene of chicken. The entire gene of 7700 base pairs is transcribed to form the primary mRNA, then the 5′ cap of methyl guanine and the 3′ polyadenylate tail are added. After the introns are spliced out, the mature mRNA is transferred to the cytoplasm.



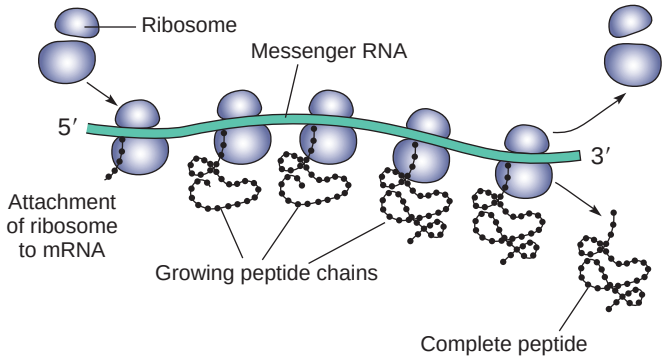


Figure 5.20

How the polypeptide chain is formed. As ribosomes move along messenger RNA in a 5′ to 3′ direction, amino acids are added stepwise to form the polypeptide chain.

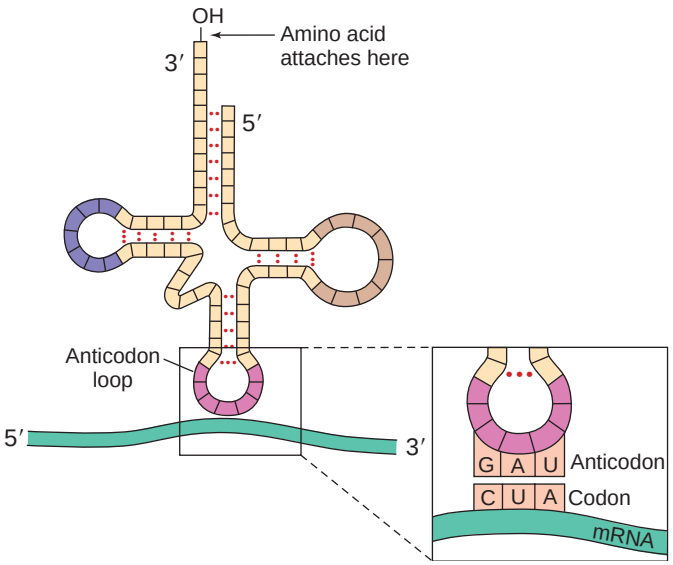


Figure 5.21

Diagram of a tRNA molecule. The anticodon loop bears bases complementary to those in the mRNA codon. The other two loops function in binding to the ribosome in polypeptide synthesis. The amino acid is added to the free single-stranded 3′ end by tRNA synthetase.

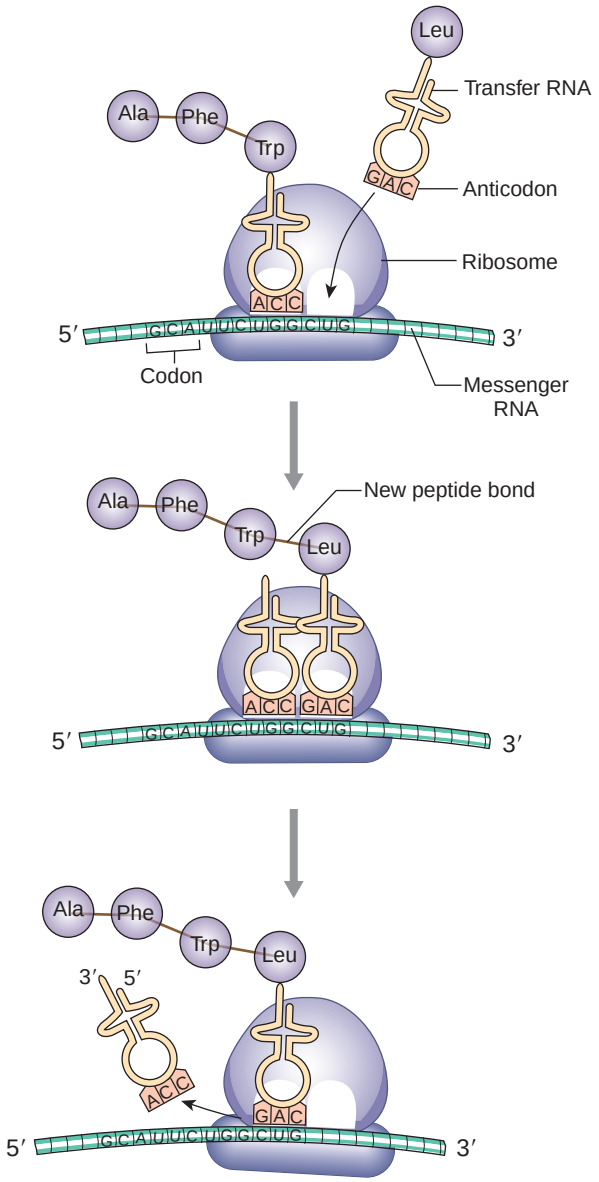


Figure 5.22

Formation of polypeptide chain on messenger RNA. As a ribosome moves down the messenger RNA molecule, transfer RNA molecules with attached amino acids enter the ribosome *(top).* Amino acids are joined together into a polypeptide chain, and transfer RNA molecules leave the ribosome *(bottom).*

for every amino acid. Furthermore, each tRNA is accompanied by a specific tRNA synthetase. Transfer RNA synthetases are enzymes that attach the correct amino acid to the terminal adenine on the 3′ end of each tRNA by a process called **charging.**

On the cloverleaf-shaped molecule of tRNA, a special sequence of three bases (the **anticodon**) is exposed in just the right way to form base pairs with complementary bases (the codon) in the mRNA. The codons are read and polypeptides assembled along the mRNA in a 5′ to 3′ direction. The anticodon of each tRNA is the key to the correct ordering of amino acids in the polypeptide being assembled.

For example, alanine is assembled into a polypeptide when it is signaled by the codon GCG in an mRNA. The translation is accomplished by alanine tRNA in which the anticodon is CGC. An alanine tRNA is first charged with alanine by its tRNA synthetase. The alanine-tRNA complex enters the ribosome where it fits precisely into the right place on the mRNA strand. Then the next charged tRNA specified by the mRNA code (glycine tRNA, for example) enters the ribosome and attaches itself beside the alanine tRNA. The two amino acids are united by a peptide bond, and the alanine tRNA then detaches from the ribosome. The process continues stepwise as the polypeptide chain is built (Figure 5.22). A polypeptide of 500 amino acids can be assembled in less than 30 seconds.

Regulation of Gene Expression

In Chapter 8 we show how the orderly differentiation of an organism from fertilized ovum to adult requires expression of genetic material at every stage of development. Developmental biologists have provided convincing evidence that every cell in a developing embryo is genetically equivalent. Thus as tissues differentiate (change developmentally), each one uses only a part of the genetic instruction present in every cell. Genes express themselves only at certain times and not at others. Indeed, most of the genes are inactive at any given moment in a particular cell or tissue. The problem in development is to explain how, if every cell has a full gene complement, certain genes are "turned on" to produce proteins required for a particular developmental stage while other genes remain silent.

Although developmental changes bring the question of gene activation clearly into focus, gene regulation is necessary throughout an organism's existence. The cellular enzyme systems that control all functional processes obviously require genetic regulation because enzymes have powerful effects even in minute amounts. Enzyme synthesis must respond to the influences of supply and demand.

Gene Regulation in Eukaryotes

Several metabolic stages in eukaryotic cells may serve as control points for gene expression. Transcriptional and translational control are the primary stages for control of gene expression in animals, with gene rearrangement also used in some cases.

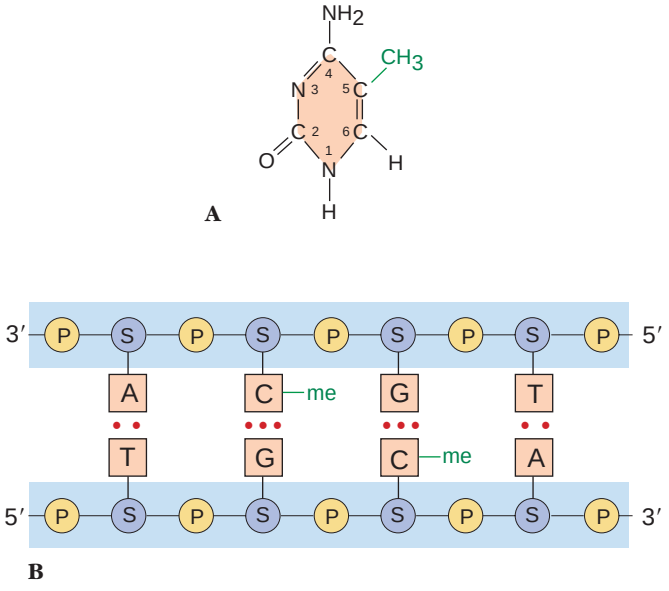


Figure 5.23

Some genes in eukaryotes are turned off by methylation of some cytosine residues in the chain. **A,** Structure of 5-methyl cytosine. **B,** Cytosine residues next to guanine are those that are methylated in a strand, thus allowing both strands to be symmetrically methylated.

Transcriptional Control Control of transcription is probably the most important mechanism for regulating gene expression. **Transcription factors** are molecules that can have a positive or a negative effect on transcription of RNA from the DNA of target genes. The factors in some cases act within cells that produce them and in other cases are transported to different parts of the body prior to action. Examples of transcription factors are steroid receptors when bound to a steroid hormone. Steroid hormones produced by endocrine glands elsewhere in the body enter a target cell and bind with a receptor protein in the nucleus. The steroid-receptor complex then binds with DNA near the target gene (p. 755). Progesterone, for example, binds with a nuclear receptor in cells of the chicken oviduct; the hormone-receptor complex then activates transcription of genes encoding egg albumin and other substances.

An important mechanism for silencing genes is methylation of cytosine bases; a methyl group (CH_3—) binds the carbon in the 5 position in the cytosine ring (Figure 5.23A). This usually happens when the cytosine is next to a guanine base; thus, the bases in the complementary DNA strand would also be a cytosine and a guanine (Figure 5.23B). When the DNA is replicated, an enzyme recognizes the CG sequence and quickly methylates the daughter strand, keeping the gene inactive.

Translational Control Genes can be transcribed and the mRNA sequestered so that translation is delayed. Development of eggs of many animals commonly uses this mechanism. Oocytes accumulate large quantities of messenger RNA during their development; then, fertilization activates metabolism and initiates translation of maternal mRNA.

Gene Rearrangement Vertebrates contain cells called lymphocytes that bear genes encoding proteins called antibodies (p. 775). Each type of antibody binds only a particular foreign substance (antigen). Because the number of different antigens is enormous, diversity of antibody genes must be equally great. One source of this diversity is rearrangement of DNA sequences coding for antibodies during development of lymphocytes.

Molecular Genetics

Progress in our understanding of genetic mechanisms on the molecular level, as discussed in the last few pages, has been almost breathtaking in the last few years. We expect many more discoveries in the near future. This progress results from many biochemical techniques now used in molecular biology. We describe briefly the most important techniques.

Recombinant DNA

An important tool in this technology is a series of enzymes called **restriction endonucleases.** Each of these enzymes, derived from bacteria, cleaves double-stranded DNA at particular sites determined by their base sequences. Many of these endonucleases cut the DNA strands so that one has several bases projecting farther

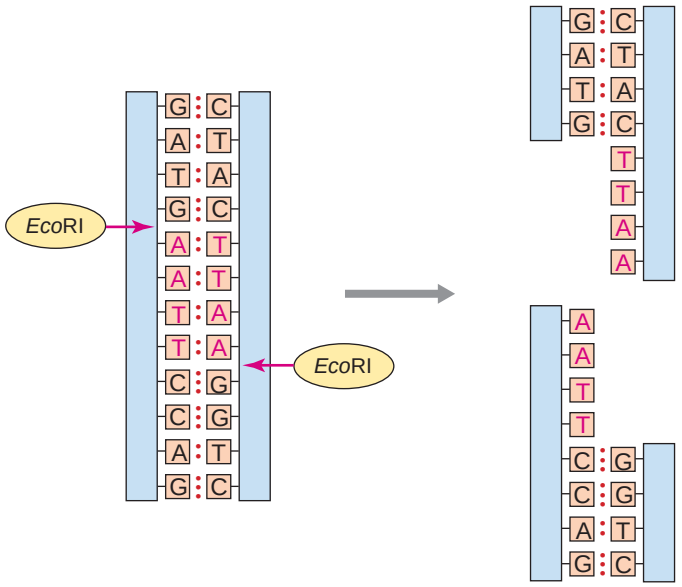


Figure 5.24

Action of restriction endonuclease, *Eco*RI. Such enzymes recognize specific base sequences that are palindromic (a palindrome is a word spelled the same backward and forward). *Eco*RI leaves "sticky ends," which anneal to other DNA fragments cleaved by the same enzyme. The strands are joined by DNA ligase.

than the other strand (Figure 5.24), leaving what are called "sticky ends." When these DNA fragments are mixed with others that have been cleaved by the same endonuclease, their sticky ends tend to anneal (join) by the rules of complementary base pairing. The ends are sealed into their new position by the enzyme **DNA ligase** in a process called **ligation.**

Besides their chromosomes, most prokaryotic and at least some eukaryotic cells have small circles of double-stranded DNA called *plasmids*. Although constituting only 1% to 3% of the bacterial genome, they may carry important genetic information, for example, resistance to an antibiotic. Plastids in plant cells (for example, chloroplasts) and mitochondria, found in most eukaryotic cells, are self-replicating and have their own complement of DNA in the form of small circles reminiscent of plasmids. The DNA of mitochondria codes for some mitochondrial proteins, whereas other mitochondrial proteins are specified by nuclear genes.

If DNA from a foreign source (such as a mammal) is ligated into a plasmid (see preceding note), the product is **recombinant DNA.** To produce the recombinant DNA in large quantities, the modified plasmid must be cloned in bacteria. The bacteria are treated with dilute calcium chloride to make them more susceptible to entry by the recombinant DNA, but plasmids do not enter most bacterial cells. Bacterial cells that have acquired the recombinant DNA can be identified if the plasmid has a marker, for example, resistance to an antibiotic. Then, only bacteria that can grow in the presence of the antibiotic are ones that have absorbed the recombinant DNA. Some bacteriophages (bacterial viruses) also are used as carriers for recombinant DNA. Plasmids and bacteriophages that carry recombinant DNA are called **vectors.** The vectors retain the ability to replicate in the bacterial cells; therefore the recombinant insert is produced in large quantities, a process called amplification.

A clone is a collection of individuals or cells all derived by asexual reproduction from a single individual. When we speak of cloning a gene or plasmid in bacteria, we mean that we isolate a colony or group of bacteria derived from a single ancestor into which the gene or plasmid was inserted. Cloning is used to obtain large quantities of a gene that has been ligated into a bacterial plasmid.

Polymerase Chain Reaction

Recent advances permit a specific gene to be cloned enzymatically from any organism as long as part of the sequence of that gene is known. The technique is called the **polymerase chain reaction (PCR).** Two short chains of nucleotides called primers are synthesized; primers are complementary to different DNA strands in the known sequence at opposite ends of the gene to be cloned. A large excess of each primer is added to a sample of DNA from the organism, and the mixture is heated to separate the double helix into single strands. When the mixture is cooled, there is a much greater probability that each strand of the gene of interest will anneal to a primer than to the other strand of the gene—because the primer is present in a much higher concentration. A heat-stable DNA polymerase and the four deoxyribonucleotide triphosphates are added to the reaction mixture. DNA synthesis proceeds from the 3′ end of each primer, extending the primer in the 5′ to 3′ direction. Primers are designed so that the free 3′ end of each faces toward the gene whose sequence is to be cloned. Entire new complementary strands are synthesized, and the number of copies of the gene has doubled (Figure 5.25). The reaction mixture is then reheated and cooled again to allow more primers to bind original and new copies of each strand. With each cycle of DNA synthesis, the number of copies of the gene doubles. Since each cycle can take less than five minutes, the number of copies of a gene can increase from one to over one million in less than two hours! The PCR allows cloning a known gene from an individual patient, identification of a drop of dried blood at a crime scene, or cloning DNA of a 40,000-year-old woolly mammoth.

Recombinant DNA technology and PCR are currently being used to engineer crop plants, including soybeans, cotton, rice, corn, and tomato. Transgenic mice are commonly used in research, and gene therapy for human genetic diseases is being developed.

Genomics and Proteomics

The scientific field of mapping, sequencing, and analyzing genomes is now called **genomics.** Some researchers divide

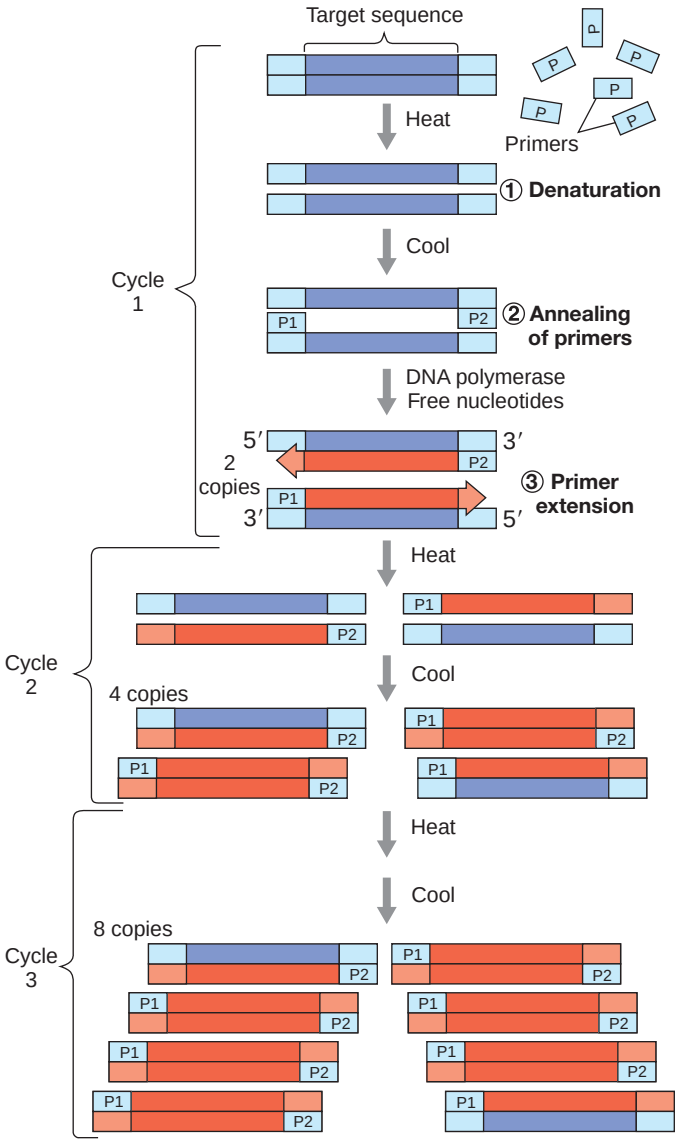


Figure 5.25

Steps in the polymerase chain reaction (PCR). Note that two different primers are required, one for each end of the target sequence.

genomic analysis into "structural genomics" (mapping and sequencing) and "functional genomics" (development of genome-wide or system-wide experimental approaches to understand gene function).

In the 1970s Allan Maxam and Walter Gilbert in the United States and Frederick Sanger in England reported practical techniques for identifying the sequence of bases in DNA. By 1984 and 1985 scientists proposed to sequence and to map the entire human genome, an effort called the Human Genome Project. It was a most ambitious undertaking: the genome was estimated at 50,000 to 100,000 genes and regulatory subunits encoded in a linear sequence of about 3 to 6 billion pairs of bases. Using techniques available in 1988, it would have taken until 2700 to sequence the genome completely, but biologists then expected technical improvements to make it possible by the twenty-second century. In fact, development and improvement of automated sequencers, as well as competition between the publicly supported Human Genome Sequencing Consortium and a large group of privately supported scientists (Celera Genomics and collaborators) led to publication of draft sequences in 2001!

Whether determination of the draft sequence was "the greatest scientific discovery of our time," as claimed by Davies's book (in Selected References), is debatable. Nevertheless, it was very exciting and yielded many surprises. For example, the human genome has fewer genes than thought previously, with 21,724 genes currently known. Only 5% of the 28% of the genome that is actually transcribed into RNA encodes protein. More than half the DNA present is repeated sequences of several types, including 45% in parasitic DNA elements. Parasitic DNA (also called "selfish" and "junk" DNA) is DNA that seems to serve no cellular or organismal function except its own propagation, but it may have utility in ways not yet clear.

Animal species vary by several orders of magnitude in the total amount of DNA present in their nuclear genomes (from slightly less than 10^8 to 10^{11} base pairs in a haploid gamete nucleus). At the low end are sponges (p. 248), some single-celled forms (see Chapter 11) and some arthropods (see Chapter 19), although the latter two groups include a wide range of genome sizes exceeding 10^9 in single-celled forms and reaching 10^{10} base pairs in arthropods. Most vertebrates have genomes of approximately 10^9 base pairs, but salamanders (p. 548), caecilians (p. 548), and lungfishes (p. 529) have genome sizes exceeding 10^{10} base pairs, with some salamanders reaching 10^{11} base pairs. Large genomes should not be considered advantageous, however, because most of the difference in genome size is contributed by accumulation of large amounts of the "selfish" or "junk" DNA in the larger genomes rather than DNA sequences useful to cellular metabolism and organismal function. The metabolic demands of replicating large amounts of DNA and the physical demands of housing it within the cell nucleus produce selection against accumulation of too much parasitic DNA in the genome. Animal groups with the largest genomes are likely those most able to tolerate accumulation of large amounts of parasitic DNA in their nuclear genomes without harming cellular and organismal functions. Because the emphasis of our book is on organismal biology, our review of genetics concentrates on genes that have clear roles in cellular and organismal functions, although these genes are a small minority of the DNA sequences present in animal nuclear genomes. Some DNA sequences considered useless to the organism exhibit variation that is useful in studies of population genetics (p. 126) and evolutionary relationships among species.

A thousand human diseases, such as cystic fibrosis and Huntington's chorea, result from defects in single genes. Almost 300 disease-associated genes are known. Information developed from knowledge of gene sequences can permit new diagnostic tests, treatments, possible preventive strategies, and advances in molecular understanding of genetic diseases. However, to realize

such benefits it is not sufficient simply to know the sequence of amino acids encoded by a nucleotide sequence in a gene. The human genome is responsible for hundreds of thousands of different proteins **(proteome).** The polypeptide encoded by a gene may be cleaved into separate functional parts or associated with polypeptides coded by other genes to produce diverse protein functions. Many scientists are now engaged in the difficult field of **proteomics:** to identify all the proteins in a cell, tissue, or organism; to determine how the proteins interact to accomplish their functions; and to outline the folding structures of the proteins.

GENETIC SOURCES OF PHENOTYPIC VARIATION

The creative force of evolution is natural selection acting on biological variation. Without variability among individuals, there could be no continued adaptation to a changing environment and no evolution (see Chapter 6). Although natural selection acts on varying organismal phenotypes, phenotypic variation within a population in a particular environment is often caused by variation in genotype. Preservation of favored phenotypes by natural selection therefore increases the abundance in a population of alleles associated with favored phenotypes, leading to adaptive evolution of the population. Through this process, a population evolves organismal phenotypes molded for effective use of environmental resources; such phenotypes are termed adaptations.

There are several genetic sources of phenotypic variation, all of which involve mutation at individual genes and the combining of the resulting alleles at variable genes into gametes and zygotes. Independent assortment of chromosomes during meiosis is a random process that creates new chromosomal combinations in gametes. In addition, chromosomal crossing over during meiosis allows recombination of linked genes between homologous chromosomes, further increasing variability. Random fusion of gametes from both parents also produces variation.

Thus sexual reproduction multiplies variation and provides the diversity and plasticity necessary for a species to survive environmental change. Sexual reproduction with its sequence of gene segregation and recombination across generations is what geneticist T. Dobzhansky called the "master adaptation" that makes all other evolutionary adaptations more accessible.

Although sexual reproduction reshuffles and amplifies whatever genetic diversity exists in a population, *new* genetic variation happens through gene mutations, chromosomal aberrations, and possibly by participation of parasitic DNA.

Gene Mutations

Gene mutations are chemicophysical changes that alter the sequence of bases in DNA. These mutations are studied directly by determining the DNA sequence and indirectly through their effects on organismal phenotype, if such effects occur. Some mutations produce a codon substitution as in the human condition called **sickle cell anemia.** Homozygotes for the sickle cell allele often die before the age of 30 because the ability of their red blood cells to carry oxygen is greatly impaired by substitution of only a single amino acid in their hemoglobin. Other mutations involve deletion of one or more bases or insertion of additional bases into a DNA chain. Translation of mRNA is thus shifted, producing codons that specify incorrect amino acids and usually a nonfunctional or dysfunctional protein product.

Once a gene is mutated, it faithfully reproduces its new form. Many mutations are harmful; many are neither helpful nor harmful, and sometimes mutations are advantageous. Helpful mutations are of great significance to evolution because they furnish new possibilities with which natural selection can build adaptations. Natural selection determines which new alleles merit survival; the environment imposes a screening process that accumulates beneficial and eliminates harmful alleles.

When an allele of a gene is mutated to a new allele, the new form tends to be recessive, and its effects are normally masked by its partner allele. Only in the homozygous condition can such mutant alleles influence phenotype. Thus a population carries a reservoir of mutant recessive alleles, some of which are homozygous lethals but which are rarely present in the homozygous condition. Inbreeding encourages formation of homozygotes and increases the probability of recessive mutants being expressed in the phenotype.

Most mutations are destined for a brief existence. There are cases, however, in which mutations harmful or neutral under one set of environmental conditions become helpful under a different set. The earth's changing environment has provided numerous opportunities for favoring new gene mutations, as evidenced by the great diversity of animal life.

Frequency of Mutations

Although mutation occurs randomly with respect to an organism's needs, different mutation rates prevail at different loci. Some *kinds* of mutations are more likely to occur than others, and individual genes differ considerably in length. A long gene (more base pairs) is more likely to have a mutation than a short gene. Nevertheless, it is possible to estimate average spontaneous rates of mutation for different organisms and traits.

Genes are extremely stable. In the well-studied fruit fly, *Drosophila melanogaster,* there is approximately one detectable mutation per 10,000 loci (rate of 0.01% per locus per generation). The rate for humans is one per 10,000 to one per 100,000 loci per generation. If we accept the latter, more conservative figure, then a single normal allele is expected to undergo 100,000 generations before it is mutated. However, since human chromosomes contain approximately 21,724 loci, about every third person carries a new mutation. Similarly, each ovum or spermatozoon contains, on average, one mutant allele.

Since most mutations are deleterious, these statistics are anything but cheerful. Fortunately, most mutant genes are recessive and are not expressed in heterozygotes. Only a few by chance will increase enough in frequency for homozygotes to be produced.

MOLECULAR GENETICS OF CANCER

The crucial defect in cancer cells is that they proliferate in an unrestrained manner **(neoplastic growth).** The mechanism that controls the rate of division of normal cells has somehow been lost, and cancer cells multiply much more rapidly, invading other tissues in the body. Cancer cells originate from normal cells that lose their constraint on division and become dedifferentiated (less specialized) to some degree. Thus there are many kinds of cancer, depending on the original founder cells of the tumor. The change in many cancerous cells, perhaps all, has a genetic basis, and investigation of the genetic damage that causes cancer is now a major thrust of cancer research.

Oncogenes and Tumor-Suppressor Genes

We now recognize that specific genetic changes occurring in a particular clone of cells produce cancer. These genetic changes include alterations in numerous genes of two types, **oncogenes** and **tumor-suppressor genes.**

Oncogenes (Gr. *onkos,* bulk, mass, + *genos,* descent) occur normally in cells, and in their normal form they are called **proto-oncogenes.** One of these encodes a protein called **Ras.** Ras protein is a guanosine triphosphatase (GTPase) located just beneath the cell membrane. When a receptor on the cell surface binds a growth factor, Ras is activated and initiates a cascade of reactions causing cell division. The oncogene form encodes a protein that initiates the cell-division cascade even when the growth factor is absent from the surface receptor.

Of the many ways that cellular DNA can sustain damage, the three most important are ionizing radiation, ultraviolet radiation, and chemical mutagens. The high energy of ionizing radiation (X rays and gamma rays) causes electrons to be ejected from the atoms it encounters, producing ionized atoms with unpaired electrons (free radicals). The free radicals (principally from water) are highly reactive chemically, and they react with molecules in the cell, including DNA. Some damaged DNA is repaired, but if the repair is inaccurate, a mutation results. Ultraviolet radiation is of much lower energy than ionizing radiation and does not produce free radicals; it is absorbed by pyrimidines in DNA and causes formation of a double covalent bond between the adjacent pyrimidines. UV repair mechanisms can also be inaccurate. Chemical mutagens react with the DNA bases and cause mispairing during replication.

Gene products of tumor-suppressor genes act as a constraint on cell proliferation. One such product is called **p53** (for "53-kilodalton protein," a reference to its molecular weight). Mutations in the gene encoding p53 occur in about half of the 6.5 million cases of human cancer diagnosed each year. Normal p53 has several crucial functions, depending on the circumstances of the cell. It can trigger apoptosis (p. 55), act as a transcription activator or repressor (turning genes on or off), control progression from G_1 to S phase in the cell cycle, and promote repair of damaged DNA. Many of the mutations known in p53 interfere with its binding to DNA and thus its function.

SUMMARY

In sexual animals genetic material is distributed to offspring via gametes (ova and sperm), produced by meiosis. Each somatic cell in an organism has two chromosomes of each kind (homologous chromosomes) and is thus diploid.

Meiosis separates homologous chromosomes, so that each gamete has half the somatic chromosome number (haploid). In the first meiotic division, centromeres do not divide, and each daughter cell receives one of each pair of replicated homologous chromosomes with sister chromatids still attached to the centromere. At the beginning of the first meiotic division, replicated homologous chromosomes come to lie alongside each other (synapsis), forming a bivalent. The gene loci on one set of chromatids lie opposite the corresponding loci on the homologous chromatids. Portions of adjacent chromatids can exchange with the nonsister chromatids (crossing over) to produce new genetic combinations. At the second meiotic division, the centromeres divide, completing the reduction in chromosome number and amount of DNA. The diploid number is restored when male and female gametes fuse to form a zygote.

Gender is determined in many animals by the sex chromosomes; in humans, fruit flies, and many other animals, females have two X chromosomes, and males have an X and a Y.

Genes are the unit entities that influence all characteristics of an organism and are inherited by offspring from their parents. Allelic variants of genes might be dominant, recessive, or intermediate; a recessive allele in the heterozygous genotype will not be expressed in the phenotype but requires the homozygous condition for overt expression. In a monohybrid cross involving a dominant allele and a recessive alternative allele (both parents homozygous), the F_1 generation will be all heterozygous, whereas F_2 genotypes will occur in a 1:2:1 ratio, and phenotypes in a 3:1 ratio. This result demonstrates Mendel's law of segregation. Heterozygotes in intermediate inheritance show phenotypes distinct from homozygous phenotypes, sometimes intermediate forms, with corresponding alterations in phenotypic ratios.

Dihybrid crosses (in which genes for two different characteristics are carried on separate pairs of homologous chromosomes) demonstrate Mendel's law of independent assortment, and phenotypic ratios are 9:3:3:1 with dominant and recessive characters. Expected ratios in crosses of two or more characters are calculated from laws of probability.

Genes can have more than two alleles in a population, and different combinations of alleles can produce different phenotypic effects. Alleles of different genes can interact in producing a phenotype, as in polygenic inheritance and epistasis, in which one gene affects the expression of another gene.

A gene on the X chromosome shows sex-linked inheritance and produces an effect in males, even if a recessive allele is present, because the Y chromosome does not carry a corresponding allele.

All genes on a given autosomal chromosome are linked, and their variants do not assort independently unless they are very far apart on the chromosome, in which case crossing over occurs between them in nearly every meiosis. Crossing over increases the amount of genetic recombination in a population.

Occasionally, a pair of homologous chromosomes fails to separate in meiosis causing the gametes to get one chromosome too many or too few. Resulting zygotes usually do not survive; humans with $2n + 1$ chromosomes sometimes live, but they have serious abnormalities, such as Down syndrome.

Nucleic acids in the cell are DNA and RNA, which are large polymers of nucleotides composed of a nitrogenous base, pentose sugar, and phosphate group. The nitrogenous bases in DNA are adenine (A), guanine (G), thymine (T), and cytosine (C), and those in RNA are the same except that uracil (U) is substituted for thymine. DNA is a double-stranded, helical molecule in which the bases extend toward each other from the sugar-phosphate backbone: A always pairs with T and G with C. The strands are antiparallel and complementary, being held in place by hydrogen bonds between the paired bases. In DNA replication the strands part, and the enzyme DNA polymerase synthesizes a new strand along each parental strand, using the parental strand as a template.

A gene can encode either a ribosomal RNA (rRNA), a transfer RNA (tRNA), or a messenger RNA (mRNA); a gene of the latter kind specifies the sequence of amino acids in a polypeptide (one gene–one polypeptide hypothesis). In mRNA, each triplet of three bases specifies a particular amino acid.

Proteins are synthesized by transcription of DNA into the base sequence of a molecule of messenger RNA (mRNA), which functions in concert with ribosomes (containing ribosomal RNA [rRNA] and protein) and transfer RNA (tRNA). Ribosomes attach to the strand of mRNA and move along it, assembling the amino acid sequence of the protein. Each amino acid is brought into position for assembly by a molecule of tRNA, which itself bears a base sequence (anticodon) complementary to the respective codons of the mRNA. In eukaryotic nuclear DNA the sequences of bases in DNA coding for amino acids in a protein (exons) are interrupted by intervening sequences (introns). The introns are removed from the primary mRNA before it leaves the nucleus, and the protein is synthesized in the cytoplasm.

Genes, and the synthesis of the products for which they are responsible, must be regulated: turned on or off in response to varying environmental conditions or cell differentiation. Gene regulation in eukaryotes occurs at several levels, with control of transcription being a particularly important point of regulation.

Molecular genetic methods have made spectacular advances possible. Restriction endonucleases cleave DNA at specific base sequences, and DNA from different sources can be rejoined to form recombinant DNA. Combining mammalian with plasmid or viral DNA, a mammalian gene can be introduced into bacterial cells, which then multiply and produce many copies of the mammalian gene. The polymerase chain reaction (PCR) is used to clone specific genes if the base sequence of short pieces of DNA surrounding the gene are known. Draft sequences of the human genome were published in 2001. Among many exciting results was a revision of the number of genes to 21,724, down from previous estimates of 100,000. These genes are responsible for hundreds of thousands of proteins in a typical cell.

A mutation is a physicochemical alteration in the bases of DNA that may change the phenotypic effect of a gene. Although rare and usually detrimental to survival and reproduction of an organism, mutations are occasionally beneficial and may be accumulated in populations by natural selection.

Cancer (neoplastic growth) results from genetic changes in a clone of cells allowing unrestrained proliferation of those cells. Oncogenes (such as the gene coding for Ras protein) and inactivation of tumor-suppressor genes (such as that coding for p53 protein) are implicated in many cancers.

REVIEW QUESTIONS

1. What is the relationship between homologous chromosomes, copies of a gene, and alleles?
2. Describe or diagram the sequence of events in meiosis (both divisions).
3. What are the designations of the sex chromosomes in males of bugs, humans, and butterflies?
4. How do the chromosomal mechanisms determining sex differ in the three taxa in question 3?
5. Diagram by Punnett square a cross between individuals with the following genotypes: *A/a* × *A/a; A/a B/b* × *A/a B/b.*
6. Concisely state Mendel's law of segregation and his law of independent assortment.
7. Assuming brown eyes (*B*) are dominant over blue eyes (*b*), determine the genotypes of all the following individuals. The blue-eyed son of two brown-eyed parents marries a brown-eyed woman whose mother was brown eyed and whose father was blue eyed. Their child is blue eyed.
8. Recall that red color (*R*) in four-o'clock flowers is incompletely dominant over white (*R′*). In the following crosses, give the genotypes of the gametes produced by each parent and the flower color of the offspring: *R/R′* × *R/R′*; *R′/R′* × *R/R′*; *R/R* × *R/R′*; *R/R* × *R′/R′*.
9. A brown male mouse is mated with two female black mice. In several litters of young, the first female has had 48 black and the second female has had 14 black and 11 brown young. Can you deduce the pattern of inheritance of coat color and the genotypes of the parents?
10. Rough coat (*R*) is dominant over smooth coat (*r*) in guinea pigs, and black coat (*B*) is dominant over white (*b*). If a homozygous rough black is mated with a homozygous smooth white, give the appearance of each of the following: F_1; F_2; offspring of F_1 mated with smooth, white parent; offspring of F_1 mated with rough, black parent.
11. Assume right-handedness (*R*) is genetically dominant over left-handedness (*r*) in humans, and that brown eyes (*B*) are genetically dominant over blue (*b*). A right-handed, blue-eyed man marries a right-handed, brown-eyed woman. Their two children are (1) right handed, blue eyed and (2) left handed, brown eyed. The man marries again, and this time the woman is right handed and brown eyed. They have 10 children, all right handed and brown eyed. What are the probable genotypes of the man and his two wives?
12. In *Drosophila melanogaster,* red eyes are dominant to white and the variation for this characteristic is on the X

chromosome. Vestigial wings (*v*) are recessive to normal (*V*) for an autosomal gene. What will be the appearance of offspring of the following crosses: $X^W/X^w\ V/v \times X^w/Y\ v/v$, $X^w/X^w\ V/v \times X^W/Y\ V/v$.

13. Assume that color blindness is a recessive character on the X chromosome. A man and woman with normal vision have the following offspring: daughter with normal vision who has one color-blind son and one normal son; daughter with normal vision who has six normal sons; and a color-blind son who has a daughter with normal vision. What are the probable genotypes of all individuals?
14. Distinguish the following: euploidy, aneuploidy, and polyploidy; monosomy and trisomy.
15. Name the purines and pyrimidines in DNA and tell which pairs occur in the double helix. What are the purines and pyrimidines in RNA and to what are they complementary in DNA?
16. Explain how DNA is replicated.
17. Why could a codon not consist of only two bases?
18. Explain the transcription and processing of mRNA in the nucleus.
19. Explain the role of mRNA, tRNA, and rRNA in polypeptide synthesis.
20. What are four ways that genes can be regulated in eukaryotes?
21. In modern molecular genetics, what is recombinant DNA, and how is it prepared?
22. Name three sources of genetic recombination that contribute to phenotypic variation.
23. Distinguish between proto-oncogene and oncogene. Describe two mechanisms by which genetic change causes cancer?
24. What are Ras protein and p53? How can mutations in the genes for these proteins contribute to cancer?
25. Outline the essential steps in the polymerase chain reaction.
26. Draft sequences of the human genome have been published. What are some interesting observations based on the results? What are some potential benefits? What is the proteome?

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CHAPTER

6

Organic Evolution

A trilobite fossilized in Paleozoic rock.

A Legacy of Change

Life's history is a legacy of perpetual change. Despite the apparent permanence of the natural world, change characterizes all things on earth and in the universe. Earth's rock strata record the irreversible, historical change that we call organic evolution. Countless kinds of animals and plants have flourished and disappeared, leaving behind a sparse fossil record of their existence. Many, but not all, have left living descendants that bear some resemblance to them.

Life's changes are observed and measured in many ways. On a short evolutionary timescale, we see changes in the frequencies of different genetic traits within populations. Evolutionary changes in the relative frequencies of light- and dark-colored moths were observed within a single human lifetime in the polluted towns of industrial England. The formation of new species and dramatic changes in organismal form, as illustrated by the evolutionary diversification of Hawaiian birds, requires longer timescales covering 100,000 to 1 million years. Major evolutionary trends and episodic mass extinctions occur on even larger timescales, covering tens of millions of years. The fossil record of horses through the past 50 million years shows a series of different species replacing older ones through time and ending with the horses alive today. The fossil record of marine invertebrates shows us a series of mass extinctions separated by intervals of approximately 26 million years.

Because every feature of life as we know it today is a product of evolution, biologists consider organic evolution the keystone of all biological knowledge.

In Chapter 1, we introduced Darwinian evolutionary theory as the dominant paradigm of biology. Charles Robert Darwin and Alfred Russel Wallace (Figure 6.1) first established evolution as a powerful scientific theory. Today the reality of organic evolution can be denied only by abandoning reason. As the noted English biologist Sir Julian Huxley wrote, "Charles Darwin effected the greatest of all revolutions in human thought, greater than Einstein's or Freud's or even Newton's, by simultaneously establishing the fact and discovering the mechanism of organic evolution." Darwinian theory helps us to understand both the genetics of populations and long-term trends in the fossil record. Darwin and Wallace did not originate the basic idea of organic evolution, which has an ancient history. We review the history of evolutionary thinking as it led to Darwin's theory, evidence supporting it, and changes to the theory that have produced our modern synthetic theory of evolution.

ORIGINS OF DARWINIAN EVOLUTIONARY THEORY

Pre-Darwinian Evolutionary Ideas

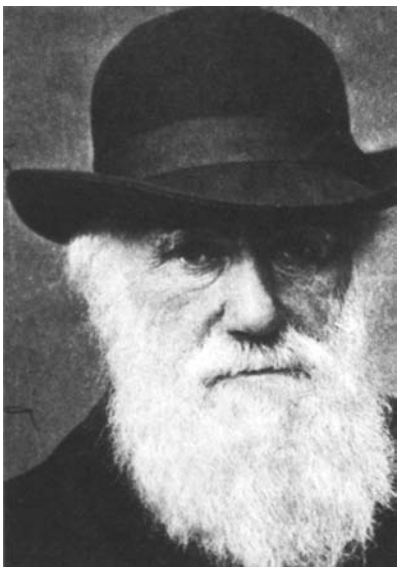
Before the eighteenth century, speculation on origins of species rested on mythology and superstition, not on anything resembling a testable scientific theory. Creation myths often described the world remaining constant after a short period of creation. Nevertheless, some people approached the idea that nature has a long history of perpetual and irreversible change.

Early Greek philosophers, notably Xenophanes, Empedocles, and Aristotle, developed an early idea of evolutionary change. They recognized fossils as evidence for former life that they believed had been destroyed by natural catastrophe. Despite their intellectual inquiry, the Greeks failed to establish an evolutionary concept, and the issue declined well before the rise of Christianity. The opportunity for evolutionary thinking became even more restricted as a biblical account of the earth's creation became accepted as a tenet of faith. The year 4004 B.C. was fixed by Archbishop James Ussher (mid-seventeenth century) as the date of life's creation. Evolutionary views were considered rebellious and heretical, but they refused to die. The French naturalist Georges Louis Buffon (1707 to 1788) stressed the influence of environment on the modifications of animal form. He also extended the age of the earth to 70,000 years.

Lamarckism: The First Scientific Explanation of Evolution

French biologist Jean Baptiste de Lamarck (1744 to 1829; Figure 6.2) authored the first complete explanation of evolution in 1809, the year of Darwin's birth. He made a convincing case that fossils were remains of extinct animals. Lamarck's proposed evolutionary mechanism, **inheritance of acquired characteristics,** was engagingly simple: organisms, by striving to meet the demands of their environments, acquire adaptations and pass them by heredity to their offspring. According to Lamarck, the giraffe evolved its long neck because its ancestors lengthened their necks by stretching to obtain food and then passed the lengthened neck to their offspring. Over many generations, these changes accumulated to produce the long necks of modern giraffes.

We call Lamarck's concept of evolution **transformational,** because it claims that as individual organisms transform their characteristics through the use and disuse of parts, heredity makes corresponding adjustments to produce evolution. We now reject transformational theories because genetic studies show that traits acquired by an organism during its lifetime, such as strengthened muscles, are not inherited by offspring. Darwin's evolutionary theory differs from Lamarck's in being a **variational** theory, based on the distribution of genetic variation in populations. Evolutionary change is caused by differential survival and reproduction among organisms that differ in hereditary traits, not by inheritance of acquired characteristics.



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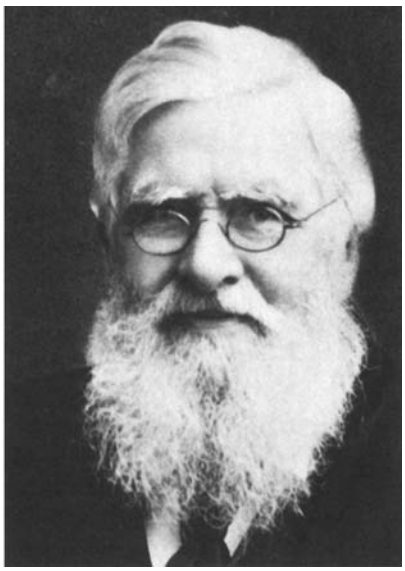


B

Figure 6.1

Founders of the theory of evolution by natural selection. **A,** Charles Robert Darwin (1809 to 1882), as he appeared in 1881, the year before his death. **B,** Alfred Russel Wallace (1823 to 1913) in 1895. Darwin and Wallace independently developed the same theory. A letter and essay from Wallace written to Darwin in 1858 spurred Darwin into writing *On The Origin of Species,* published in 1859.

Charles Lyell and Uniformitarianism

The geologist Sir Charles Lyell (1797 to 1875; Figure 6.3) established in his *Principles of Geology* (1830 to 1833) the principle of uniformitarianism. Uniformitarianism encompasses two important principles that guide scientific study of the history of nature: (1) that the laws of physics and chemistry have not changed throughout the history of the earth, and (2) that past geological events occurred by natural processes similar to those observed today. Lyell showed that natural forces, acting over long periods

Figure 6.2

Jean Baptiste de Lamarck (1744 to 1829), French naturalist who offered the first scientific explanation of evolution. Lamarck's hypothesis that evolution proceeds by inheritance of acquired characteristics has been rejected and replaced by neo-Darwinian theories.

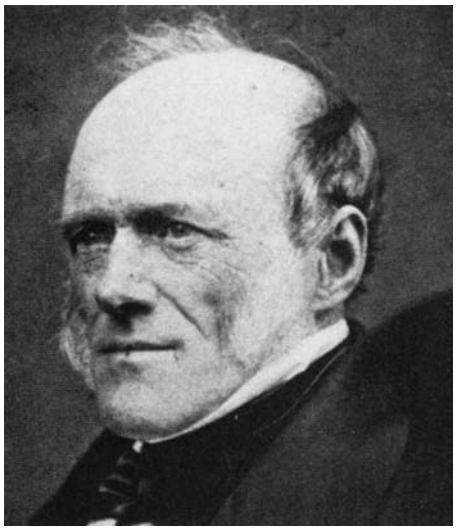


Figure 6.3

Sir Charles Lyell (1797 to 1875), English geologist and friend of Darwin. His book *Principles of Geology* greatly influenced Darwin during Darwin's formative period. This photograph was made about 1856.

of time, could explain the formation of fossil-bearing rocks. Lyell's geological studies led him to conclude that the earth's age must be measured in millions of years. These principles were important for discrediting miraculous and supernatural explanations of the history of nature and replacing them with scientific explanations. Lyell also stressed the gradual nature of geological changes that occur through time, and he argued further that such changes have no inherent tendency to occur in any particular direction. Both of these claims left important marks on Darwin's evolutionary theory.

Darwin's Great Voyage of Discovery

"After having been twice driven back by heavy southwestern gales, Her Majesty's ship *Beagle,* a ten-gun brig, under the command of Captain Robert FitzRoy, R.N., sailed from Devonport on the 27th of December, 1831." Thus began Charles Darwin's account of the historic five-year voyage of the *Beagle* around the world (Figure 6.4). Darwin, not quite 23 years old, had been asked to accompany Captain FitzRoy on the *Beagle,* a small vessel only 90 feet in length, which was about to depart on an

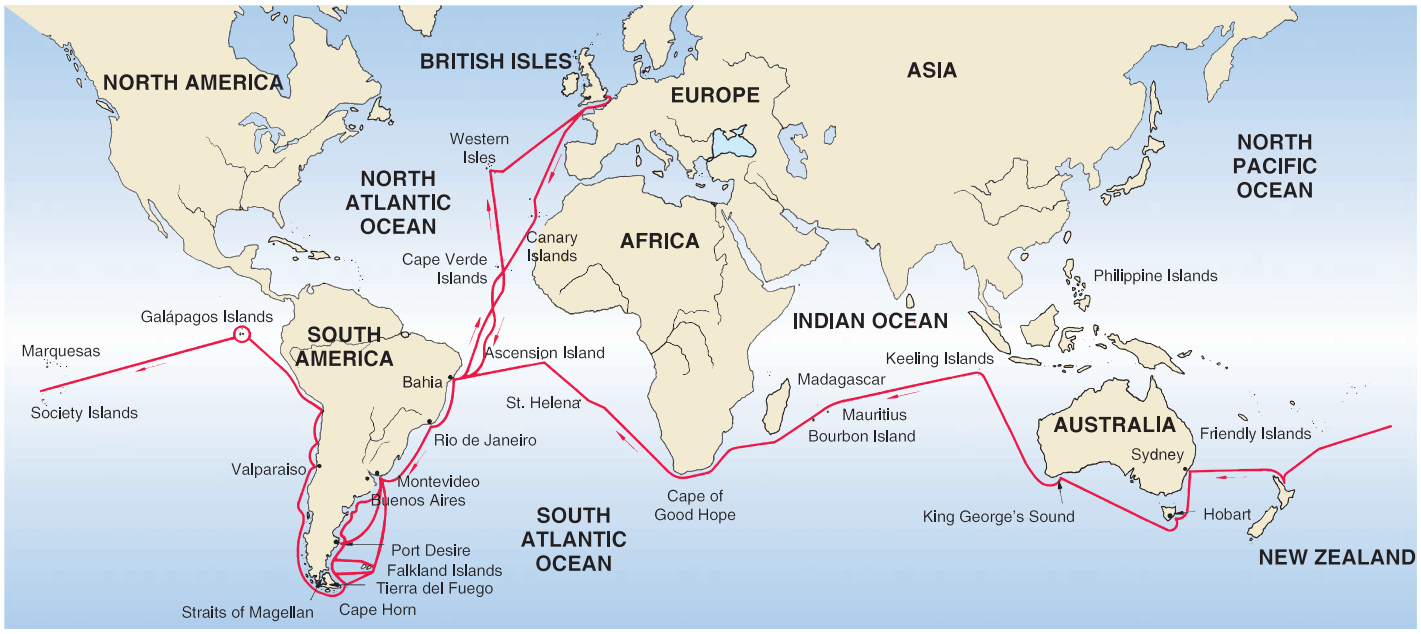


Figure 6.4

Five-year voyage of H.M.S. *Beagle.*

A B

Figure 6.5

Charles Darwin and H.M.S. *Beagle.* **A,** Darwin in 1840, four years after the *Beagle* returned to England, and a year after his marriage to his cousin, Emma Wedgwood. **B,** The H.M.S. *Beagle* sails in Beagle Channel, Tierra del Fuego, on the southern tip of South America in 1833. The watercolor was painted by Conrad Martens, one of two official artists on the voyage of the *Beagle.*

extensive surveying voyage to South America and the Pacific (Figure 6.5). It was the beginning of the most important scientific voyage of the nineteenth century.

During the voyage (1831 to 1836), Darwin endured seasickness and the erratic companionship of Captain FitzRoy, but Darwin's youthful physical strength and early training as a naturalist equipped him for his work. The *Beagle* made many stops along the coasts of South America and adjacent islands. Darwin made extensive collections and observations on the fauna and flora of these regions. He unearthed numerous fossils of animals long extinct and noted the resemblance between fossils of the South American pampas and the known fossils of North America. In the Andes he encountered seashells embedded in rocks at 13,000 feet. He experienced a severe earthquake and watched mountain torrents that relentlessly wore away the earth. These observations, and his reading of Lyell's *Principles of Geology* during the voyage, strengthened his conviction that natural forces could explain the geological features of the earth.

In mid-September of 1835, the *Beagle* arrived at the Galápagos Islands, a volcanic archipelago straddling the equator 600 miles west of Ecuador (Figure 6.6). The fame of the islands stems from their oceanic isolation and rugged volcanic terrain. Circled by capricious currents, surrounded by shores of twisted lava bearing skeletal brushwood baked by the equatorial sun, inhabited by strange reptiles and by convicts stranded by the Ecuadorian government, the islands had few admirers among mariners. By the middle of the seventeenth century, the islands were known to Spaniards as "Las Islas Galápagos"—the tortoise islands. The giant tortoises, used for food first by buccaneers and later by American and British whalers, sealers, and ships of war, were the islands' principal attraction. At the time of Darwin's visit, the tortoises already were heavily exploited.

During the *Beagle*'s five-week visit to the Galápagos, Darwin documented the unique character of the Galápagos plants and animals, including the giant tortoises, marine iguanas, mockingbirds, and ground finches. Darwin later described these studies as the "origin of all my views."

Darwin was struck by the fact that, although the Galápagos Islands and the Cape Verde Islands (visited earlier in this voyage of the *Beagle*) were similar in climate and topography, Galápagos plants and animals were related to those of the South American mainland and were entirely different from the African-derived forms of the Cape Verde Islands. Each Galápagos Island often contained a unique species related to forms on other Galápagos Islands. In short, Galápagos life must have originated in continental South America and then undergone modification in the various environmental conditions of the different islands. He

Figure 6.6

The Galápagos Islands viewed from the rim of a volcano.

Figure 6.7
Darwin's study at Down House in Kent, England, is preserved today much as it was when Darwin wrote *On The Origin of Species.*

concluded that living forms were neither divinely created nor immutable; they were, in fact, products of evolution.

On October 2, 1836, the *Beagle* returned to England, where Darwin conducted most of his scientific work (Figure 6.7). Most of Darwin's extensive collections had preceded him there, as had notebooks and diaries kept during the cruise. Darwin's journal, published three years after the *Beagle*'s return to England, was an instant success and required two additional printings within the first year. Darwin later revised his journal as *The Voyage of the Beagle*, one of the most lasting and popular travel books.

The main product of Darwin's voyage, his theory of evolution, would continue to develop for more than 20 years after the *Beagle*'s return. In 1838, he "happened to read for amusement" an essay on populations by T. R. Malthus (1766 to 1834), who stated that animal and plant populations, including human populations, tend to increase beyond the capacity of the environment to support them. Darwin already had been gathering information on artificial selection of animals under domestication. Darwin was especially fascinated by artificial breeds of pigeons. Many pigeon breeds differed so much in appearance and behavior that they would be considered different species if found in nature, yet all had clearly been derived from a single wild species, the rock pigeon (*Columba livia*). After reading Malthus's article, Darwin realized that a process of selection in nature, a "struggle for existence" because of overpopulation, could be a powerful force for evolution of wild species.

Darwin allowed the idea to develop in his own mind until it was presented in 1844 in a still-unpublished essay. Finally in 1856, he began to assemble his voluminous data into a work on the origin of species. He expected to write four volumes, a very big book, "as perfect as I can make it." However, his plans took an unexpected turn.

In 1858, he received a manuscript from Alfred Russel Wallace (1823 to 1913), an English naturalist in Malaya with whom he corresponded. Darwin was stunned to find that in a few pages, Wallace summarized the main points of the natural selection theory on which Darwin had been working for two decades. Rather than to withhold his own work in favor of Wallace as he was inclined to do, Darwin was persuaded by two close friends, the geologist Lyell and the botanist Hooker, to publish his views in a brief statement that would appear together with Wallace's paper in the *Journal of the Linnean Society*. Portions of both papers were read to an unimpressed audience on July 1, 1858.

For the next year, Darwin worked urgently to prepare an "abstract" of the planned four-volume work. This book was published in November 1859, with the title *On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life*. The 1250 copies of the first printing sold the first day! The book instantly generated a storm that has never abated. Darwin's views were to have extraordinary consequences on scientific and religious beliefs and remain among the greatest intellectual achievements of all time.

> "Whenever I have found that I have blundered, or that my work has been imperfect, and when I have been contemptuously criticized, and even when I have been overpraised, so that I have felt mortified, it has been my greatest comfort to say hundreds of times to myself that 'I have worked as hard and as well as I could, and no man can do more than this.'" *Charles Darwin, in his autobiography, 1876.*

Once Darwin's caution had been swept away by the publication of *On the Origin of Species,* he entered an incredibly productive period of evolutionary thinking for the next 23 years, producing book after book. He died on April 19, 1882, and was buried in Westminster Abbey. The little *Beagle* had already disappeared, having been retired in 1870 and sold for scrap.

DARWINIAN EVOLUTIONARY THEORY: THE EVIDENCE

Perpetual Change

The main premise underlying Darwinian evolution is that the living world is neither constant nor perpetually cycling, but always changing. Perpetual change in the form and diversity of animal life throughout its 600- to 700-million-year history is seen most directly in the fossil record. A **fossil** is a remnant of past life uncovered from the crust of the earth (Figure 6.8). Some fossils constitute complete remains (insects in amber and mammoths), actual hard parts (teeth and bones), and petrified skeletal parts infiltrated with silica or other minerals (ostracoderms and molluscs). Other fossils include molds, casts, impressions, and fossil excrement (coprolites). In addition to documenting organismal evolution, fossils reveal profound changes in the earth's environment, including major changes in the distributions of lands and seas. Because many organisms left no fossils, a complete record of the past is always beyond our reach; nonetheless, discovery of new fossils and reinterpretation of familiar ones expand our knowledge of how the form and diversity of animals changed through geological time.

Fossil remains may on rare occasions include soft tissues preserved so well that recognizable cellular organelles are revealed by electron microscopy! Insects are frequently found entombed in amber, the fossilized resin of trees. One study of a fly entombed in 40-million-year-old amber revealed structures corresponding to muscle fibers, nuclei, ribosomes, lipid droplets, endoplasmic reticulum, and mitochondria (Figure 6.8D). This extreme case of mummification probably occurred because chemicals in the plant sap diffused into the embalmed insect's tissues.

Interpreting the Fossil Record

The fossil record is biased because preservation is selective. Vertebrate skeletal parts and invertebrates with shells and other hard structures left the best records (Figure 6.8). Soft-bodied animals, including jellyfishes and most worms, are fossilized only under very unusual circumstances such as those that formed the Burgess Shale of British Columbia (Figure 6.9). Exceptionally favorable conditions for fossilization produced the Precambrian fossil bed of South Australia, the tar pits of Rancho La Brea (Hancock Park, Los Angeles), the great dinosaur beds (Alberta, Canada, and Jensen, Utah; Figure 6.10), and the Yunnan and Lianoning provinces of China.

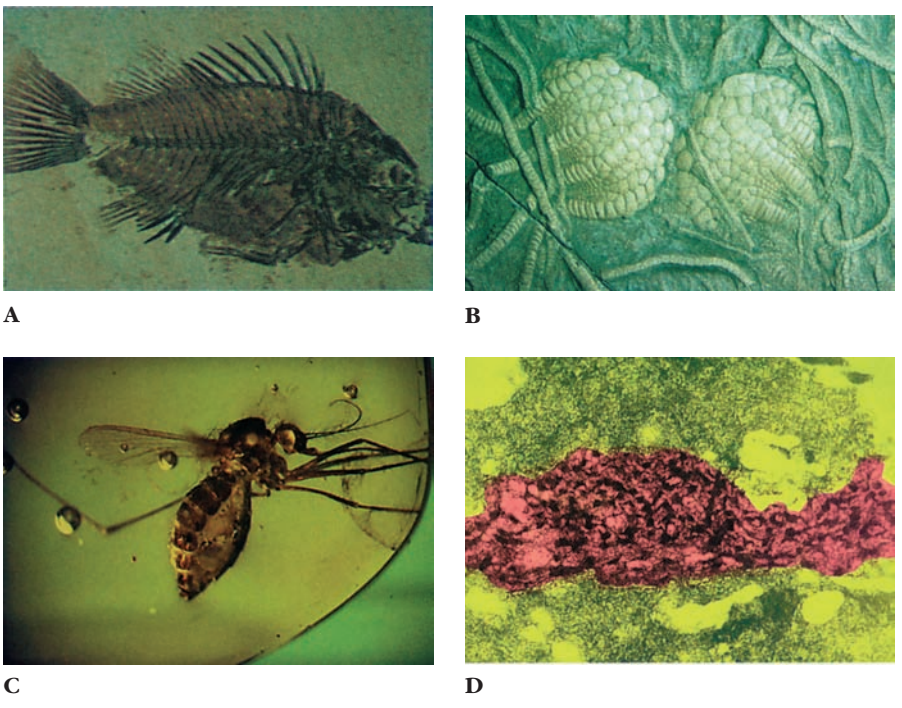
Fossils are deposited in stratified layers with new deposits forming on top of older ones. If left undisturbed, which is rare, a sequence is preserved with the ages of fossils being directly proportional to their depth in the stratified layers. Characteristic fossils often serve to identify particular layers. Certain widespread marine invertebrate fossils, including various foraminiferans (p. 242) and echinoderms (p. 472), are such good indicators of specific geological periods that they are called "index," or "guide," fossils. Unfortunately, the layers are usually tilted or show faults (cracks). Old deposits exposed by erosion may be covered with new deposits in a different plane. When exposed to tremendous pressures or heat, stratified sedimentary rock metamorphoses into crystalline quartzite, slate, or marble, which destroys fossils.

Stratigraphy for two major groups of African antelopes and its evolutionary interpretation are shown on Figure 6.11. Species in this group are identified by characteristic sizes and shapes of horns, which form much of the fossil record of this group. Solid vertical lines in Figure 6.11 denote the temporal distributions of species determined by presence of their characteristic horns in rock strata of various ages. Red lines denote the fossil records of living species, and gray lines denote the fossil records of extinct species. The dotted gray lines show the inferred relationships among living and fossil species based on their sharing of homologous structural features.

Geological Time

Long before the earth's age was known, geologists divided its history into a table of succeeding events based on the ordered layers of sedimentary rock. The "law of stratigraphy" produced a relative dating with the oldest layers at the bottom and the youngest at the top of the sequence. Time was divided into eons, eras, periods, and epochs as shown on the endpaper inside the back cover of this book. Time during the last eon (Phanerozoic) is expressed in eras (for example, Cenozoic), periods (for example, Tertiary), epochs (for example, Paleocene), and sometimes smaller divisions of an epoch.

In the late 1940s, radiometric dating methods were developed for determining the absolute age in years of rock formations. Several independent methods are now used, all based on



A **B**

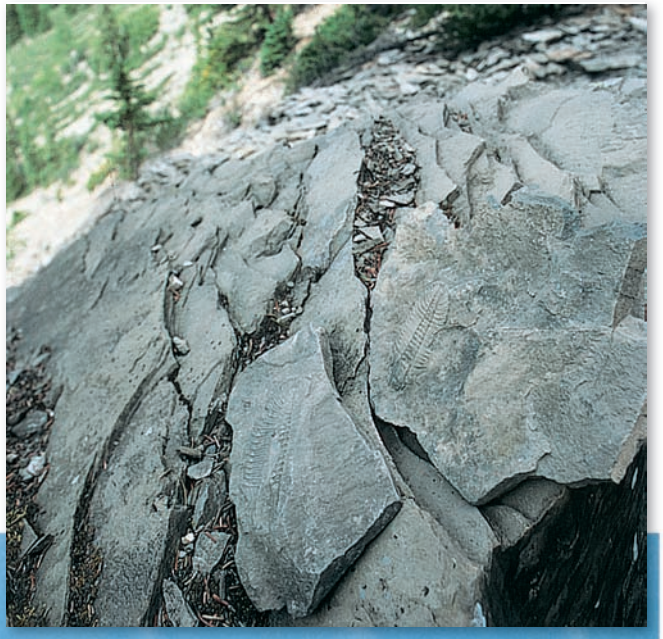
C **D**

Figure 6.8

Four examples of fossil material. **A,** Fish fossil from rocks of the Green River Formation, Wyoming. Such fish swam here during the Eocene epoch of the Tertiary period, approximately 55 million years ago. **B,** Stalked crinoids (class Crinoidea, p. 487) from 85-million-year-old Cretaceous rocks. The fossil record of these echinoderms shows that they reached their peak millions of years earlier and began a slow decline to the present. **C,** An insect fossil that got stuck in the resin of a tree 40 million years ago and that has since hardened into amber. **D,** Electron micrograph of tissue from a fly fossilized as shown in **C;** the nucleus of a cell is marked in red.

Figure 6.9

A, Fossil trilobites visible at the Burgess Shale Quarry, British Columbia. **B,** Animals of the Cambrian period, approximately 580 million years ago, as reconstructed from fossils preserved in the Burgess Shale of British Columbia, Canada. The main new body plans that appeared rather abruptly at this time established the body plans of animals familiar to us today.



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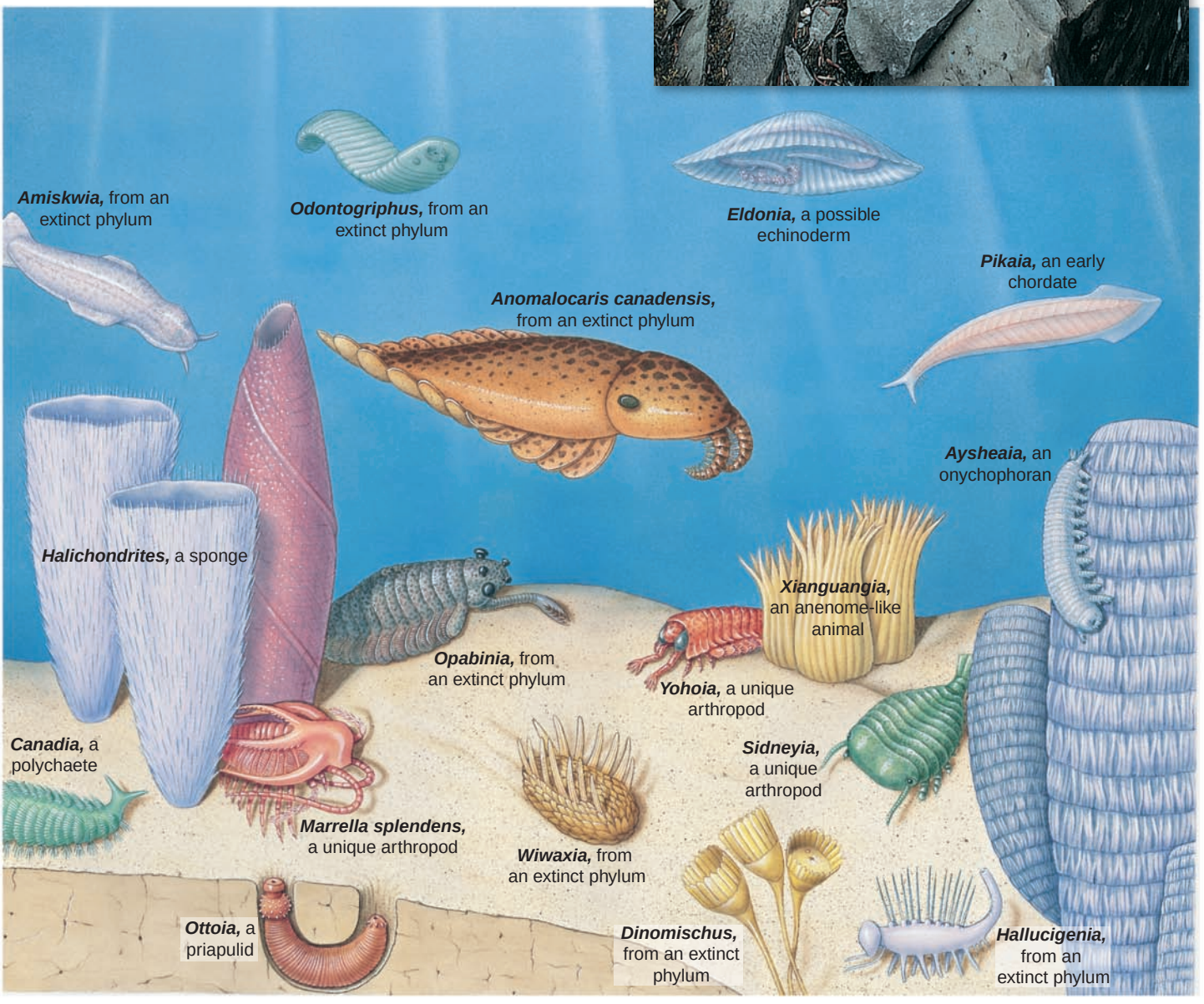


B

Figure 6.10

A dinosaur skeleton partially excavated from rock at Dinosaur Provincial Park, Alberta.

the radioactive decay of naturally occurring elements into other elements. These "radioactive clocks" are independent of pressure and temperature changes and therefore are not affected by often violent earth-building activities.

One method, potassium-argon dating, uses the decay of potassium-40 (^{40}K) to argon-40 (^{40}Ar) (12%) and calcium-40 (^{40}Ca) (88%). The half-life of potassium-40 is 1.3 billion years; half of the original atoms will decay in 1.3 billion years, and half of the remaining atoms will be gone at the end of the next 1.3 billion years. This decay continues until all radioactive potassium-40 atoms are gone. To measure the age of the rock, one calculates the ratio of remaining potassium-40 atoms to the amount of potassium-40 originally there (the remaining potassium-40 atoms plus the argon-40 and calcium-40 into which other potassium-40 atoms have decayed). Several such isotopes exist for dating purposes, some for dating the age of the earth itself. One of the most useful radioactive clocks depends on the decay of uranium into lead. With this method, rocks over 2 billion years old can be dated with a probable error of less than 1%.

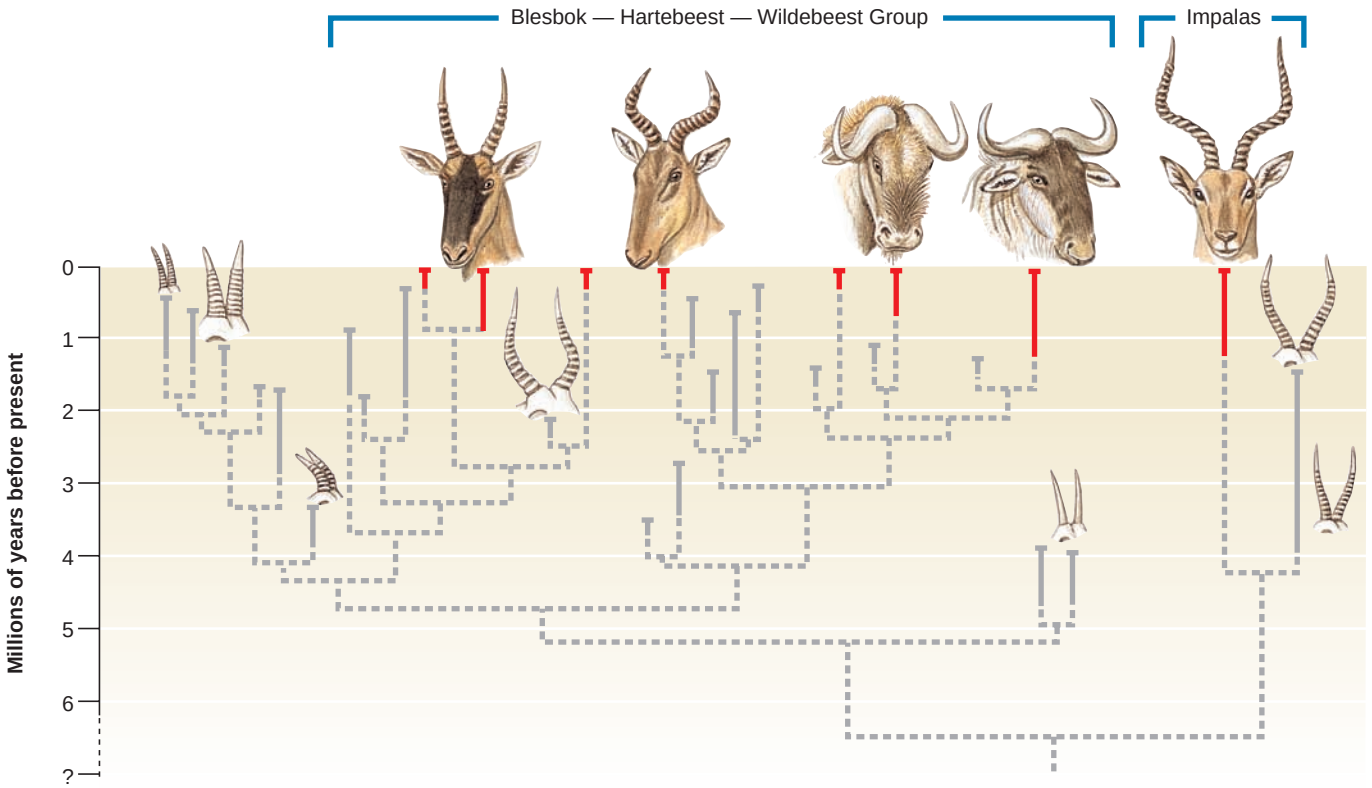


Figure 6.11

Stratigraphic record and inferred evolutionary relationships among alcelaphine (blesboks, hartebeests, wildebeests) and aepycerotine (impalas) antelopes in Africa. Species in this group are identified by characteristic sizes and shapes of horns found in rock strata of various ages. Solid vertical lines show the temporal distribution of species in rock strata whose ages are shown on the scale at the left side of the figure. Red lines show the temporal distributions of living species, and gray lines show the temporal distributions of extinct species in rock strata. Dotted gray lines show the inferred relationships among species based on their sharing of homologous structural features. The relative constancy of horn structure within species through geological time is consistent with the theory of punctuated equilibrium (p. 123). This fossil record shows that rates of speciation and extinction are higher for alcelaphine antelopes than for impalas.

The fossil record of macroscopic organisms begins near the start of the Cambrian period of the Paleozoic era, approximately 600 million years BP. Geological time before the Cambrian is called the Precambrian era or Proterozoic eon. Although the Precambrian era occupies 85% of all geological time, it has received much less attention than later eras, partly because oil, which provides the commercial incentive for much geological work, seldom exists in Precambrian formations. The Precambrian era contains well-preserved fossils of bacteria and algae, and casts of jellyfishes, sponge spicules, soft corals, segmented flatworms, and worm trails. Most, but not all, are microscopic fossils.

Evolutionary Trends

The fossil record allows us to view evolutionary change across the broadest scale of time. Species arise and go extinct repeatedly throughout the geological history recorded by the fossil record. Animal species typically survive approximately 1 to 10 million years, although their durations are highly variable. When we study patterns of species or taxon replacement through time, we observe trends. Trends are directional changes in the characteristic features or patterns of diversity in a group of organisms. Fossil trends clearly demonstrate Darwin's principle of perpetual change.

Our use of the phrase "evolutionary trend" does not imply that more recent forms are superior to older ones or that the changes represent progress in adaptation or organismal complexity. Although Darwin predicted that such trends would show progressive adaptation, many contemporary paleontologists consider progressive adaptation rare among evolutionary trends. Observed trends in the evolution of horses do not imply that contemporary horses are superior in any general sense to their Eocene ancestors.

A well-studied fossil trend is the evolution of horses from the Eocene epoch to the present. Looking back at the Eocene epoch, we see many different genera and species of horses replaced by others through time (Figure 6.12). George Gaylord Simpson (p. 208) showed that this trend is compatible with Darwinian evolutionary theory. The three characteristics that show the clearest trends in horse evolution are body size, foot structure, and tooth structure. Compared to modern horses, those of extinct genera were small; their teeth had a relatively small grinding surface, and their feet had a

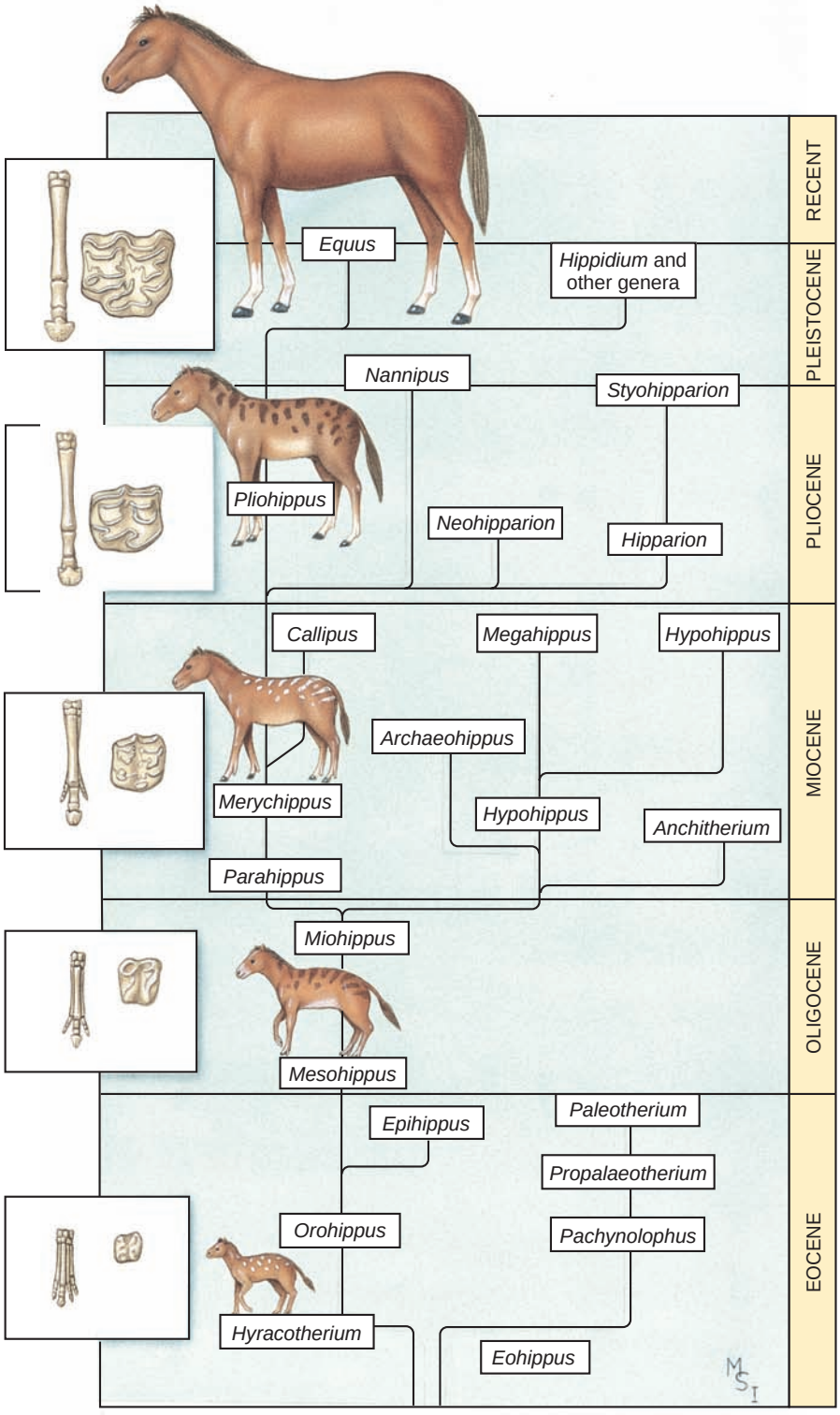


Figure 6.12
A reconstruction of genera of horses from Eocene to present. Evolutionary trends toward increased size, elaboration of molars, and loss of toes are shown together with a hypothetical phylogeny of extant and fossil genera.

relatively large number of toes (four). Throughout the subsequent Oligocene, Miocene, Pliocene, and Pleistocene epochs are continuing patterns of new genera arising and old ones going extinct. In each case, a net increase in body size, expansion of the grinding surface of the teeth, and reduction in the number of toes occurred. As the number of toes was reduced, the central digit became increasingly more prominent in the foot, and eventually only this central digit remained.

The fossil record shows a net change not only in the characteristics of horses but also variation in the numbers of different horse genera (and numbers of species) through time. The many horse genera of past epochs have been lost to extinction, leaving only a single survivor, *Equus*. Evolutionary trends in diversity are observed in fossils of many different groups of animals (Figure 6.13).

Trends in fossil diversity through time are produced by different rates of species formation versus extinction through time. Why do some lineages generate large numbers of new species whereas others generate relatively few? Why do different lineages undergo higher or lower rates of extinction (of species, genera, or taxonomic families) throughout evolutionary time? To answer these questions, we must turn to Darwin's other four theories of evolution. Regardless of how we answer these questions, however, the observed trends in animal diversity clearly illustrate Darwin's principle of perpetual change. Because the remaining four theories of Darwinism rely on the theory of perpetual change, evidence supporting these theories strengthens Darwin's theory of perpetual change.

Common Descent

Darwin proposed that all plants and animals have descended from an ancestral form into which life was first breathed. Life's history is depicted as a branching tree, called a **phylogeny.** Pre-Darwinian evolutionists, including Lamarck, advocated multiple independent origins of life, each of which gave rise to lineages that changed through time without extensive branching. Like all good scientific theories, common descent makes several important predictions that can be tested and potentially used to reject it. According to this theory, we should be able to trace the genealogies of all modern species backward until they converge on ancestral lineages shared with other species, both living and extinct.

We should be able to continue this process, moving farther backward through evolutionary time, until we reach the primordial ancestor of all life on earth. All forms of life, including many extinct forms that represent dead branches, will connect to this tree somewhere. Although reconstructing the history of life in this manner may seem almost impossible, phylogenetic research has been extraordinarily successful. How has this difficult task been accomplished?

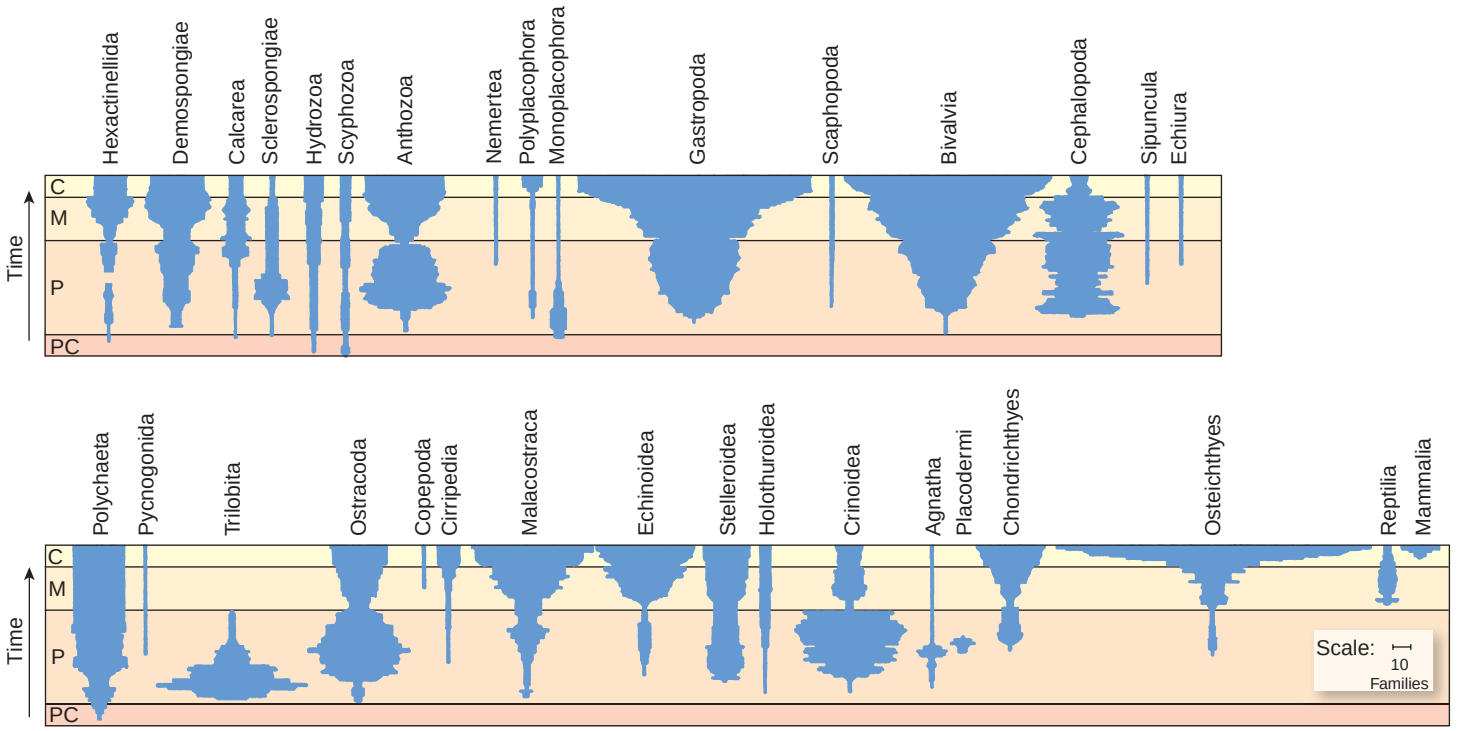


Figure 6.13

Diversity profiles of taxonomic families from different animal groups in the fossil record. The scale marks the Precambrian (PC), Paleozoic (P), Mesozoic (M), and Cenozoic (C) eras. The relative number of families is indicated from the width of the profile.

The Power of a Theory

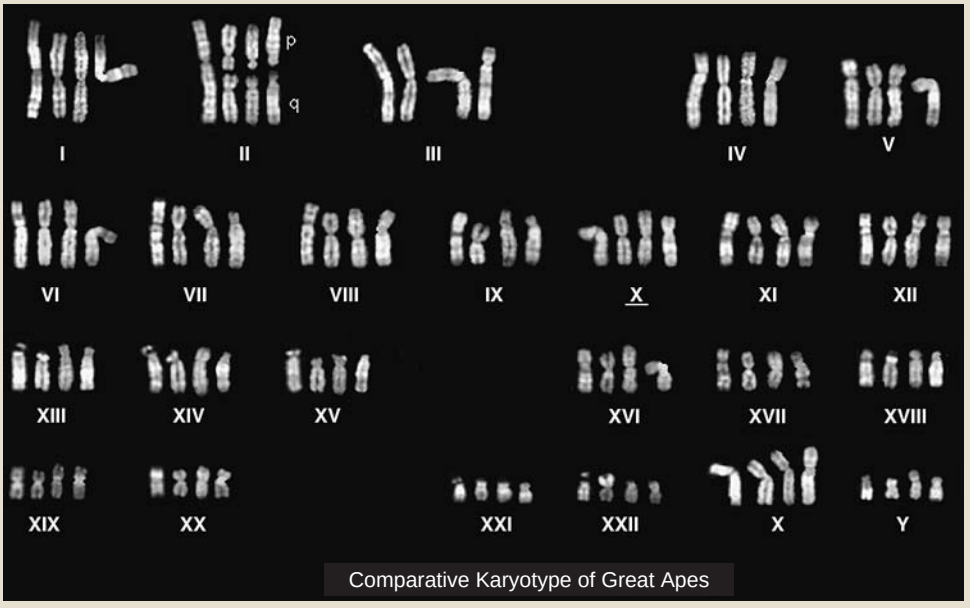
Darwin's theory of common descent illustrates the scientific importance of general theories that give unified explanations to diverse kinds of data. Darwin proposed his theory of descent with modification of all living forms because it explained the patterns of similarity and dissimilarity among organisms in anatomical structures and cellular organization.

Anatomical similarities between humans and apes led Darwin to propose that humans and apes share more recent common ancestry with each other than they do with any other species. Darwin was unaware that his theory, a century later, would provide the primary explanation for similarities and dissimilarities among species in the structures of their chromosomes, sequences of amino acids in homologous proteins, and sequences of bases in homologous genomic DNA.

The accompanying figure shows photographs of a complete haploid set of chromosomes from each of four ape species: human (*Homo sapiens*), bonobo (the pygmy chimpanzee, *Pan paniscus*), gorilla (*Gorilla gorilla*), and orangutan (*Pongo pygmaeus*). Each chromosome in the human genome has a corresponding chromosome with similar structure and gene content in the genomes of other ape species. The most obvious difference between human and ape chromosomes is that the large second chromosome in the human nuclear genome was formed evolutionarily by a fusion of two smaller chromosomes characteristic of the ape genomes. Detailed study of the human and other ape chromosomes shows remarkable correspondence between them in genic content and organization. Ape chromosomes are more similar to each other than they are to chromosomes of any other animals.

Comparison of DNA and protein sequences among apes likewise confirms their close genetic relationships, with humans and the two chimpanzee species being closer to each other than any of these species are to other apes. DNA sequences from the nuclear and mitochondrial genomes independently support the close relationships among ape species and especially the grouping of humans and chimpanzees as close relatives. Homologous DNA sequences of humans and chimpanzees are approximately 99% similar in base sequence.

Studies of variation in chromosomal structure, mitochondrial DNA sequences, and nuclear DNA sequences produced multiple independent data sets, each one potentially capable of rejecting Darwin's theory of common descent. Darwin's theory would be rejected, for example, if the chromosomal structures and DNA sequences of apes were no more similar to each other than to those of other animals. The data in this case support rather than reject predictions of Darwin's theory. The ability of Darwin's theory of common descent to make precise predictions of genetic similarities among these and other species, and to have those predictions confirmed by numerous empirical studies, illustrates its great strength. As new kinds of biological data have become available, the scope and strength of Darwin's theory of common descent have increased enormously. Indeed, nothing in biology makes sense in the absence of this powerful explanatory theory.



The human haploid genome contains 22 autosomes (I–XXII) and a sex chromosome (X or Y). The human chromosome is shown first in each group of four, followed by the corresponding chromosomes of bonobo, gorilla, and orangutan, in that order. Note that the chromatin of human chromosome II corresponds to that of two smaller chromosomes (marked p and q) in other apes.

Homology and Phylogenetic Reconstruction

Darwin recognized the major source of evidence for common descent in the concept of **homology.** Darwin's contemporary, Richard Owen (1804 to 1892), used this term to denote "the same organ in different organisms under every variety of form and function." A classic example of homology is the limb skeleton of vertebrates. Bones of vertebrate limbs maintain characteristic structures and patterns of connection despite diverse modifications for different functions (Figure 6.14). According to Darwin's theory of common descent, the structures that we call homologies represent characteristics inherited with some modification from a corresponding feature in a common ancestor.

Darwin devoted an entire book, *The Descent of Man and Selection in Relation to Sex,* largely to the idea that humans share common descent with apes and other animals. This idea was repugnant to many Victorians, who responded with predictable outrage (Figure 6.15). Darwin built his case mostly on anatomical comparisons revealing homology between humans and apes. To Darwin, the close resemblances between apes and humans could be explained only by common descent.

Throughout the history of all forms of life, evolutionary processes generate new characteristics that are then inherited by subsequent generations. Every time a new feature arises on an evolving lineage, we see the origin of a new homology. That homology gets transmitted to all descendant lineages unless it is subsequently lost. The pattern formed by the sharing of homologies among species provides evidence for common descent and allows us to reconstruct the branching evolutionary history of life. We can illustrate such evidence using a phylogenetic tree for a group of large, ground-dwelling birds (Figure 6.16). A new skeletal homology arises on each of the lineages shown (descriptions of specific homologies are not included because they are highly technical). The different groups of species located at the tips of the branches contain different combinations of these homologies, which reflect ancestry. For example, ostriches show homologies 1 through 5 and 8, whereas kiwis show homologies 1, 2, 13, and 15. Branches of the tree combine these species into a **nested hierarchy** of groups within groups (see Chapter 10). Smaller groups (species grouped near terminal branches) are contained within larger ones (species grouped by basal branches, including the trunk of the tree). If we erase the tree structure but retain patterns of homology observed in the living species, we are able to reconstruct the branching structure of the entire tree. Evolutionists test the theory of common descent by observing patterns of homology present within all groups of organisms. The pattern formed by all homologies taken together should specify a single branching tree that represents the evolutionary genealogy of all living organisms.

The nested hierarchical structure of homology is so pervasive in the living world that it forms the basis for our systematic classification of all forms of life (genera grouped into families, families grouped into orders, and other categories). Hierarchical classification even preceded Darwin's theory

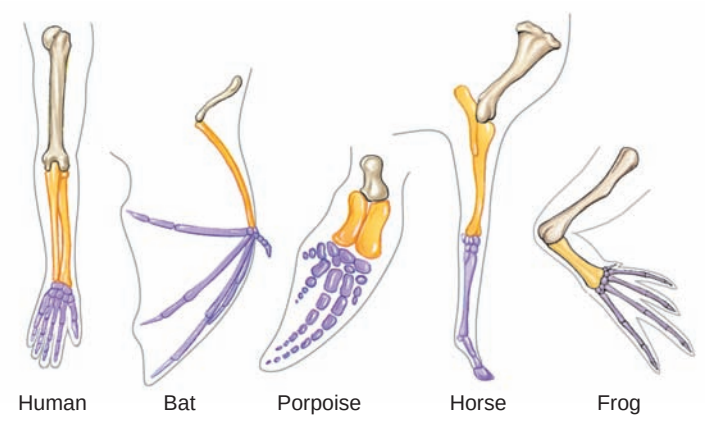


Figure 6.14

Forelimbs of five vertebrates show skeletal homologies: *brown,* humerus; *orange,* radius and ulna; *purple,* "hand" (carpals, metacarpals, and phalanges). Clear homologies of bones and patterns of connection are evident despite evolutionary modification for different functions.

Figure 6.15

This 1873 advertisement for Merchant's Gargling Oil ridicules Darwin's theory of the common descent of humans and apes, which received only limited acceptance by the general public during Darwin's lifetime.

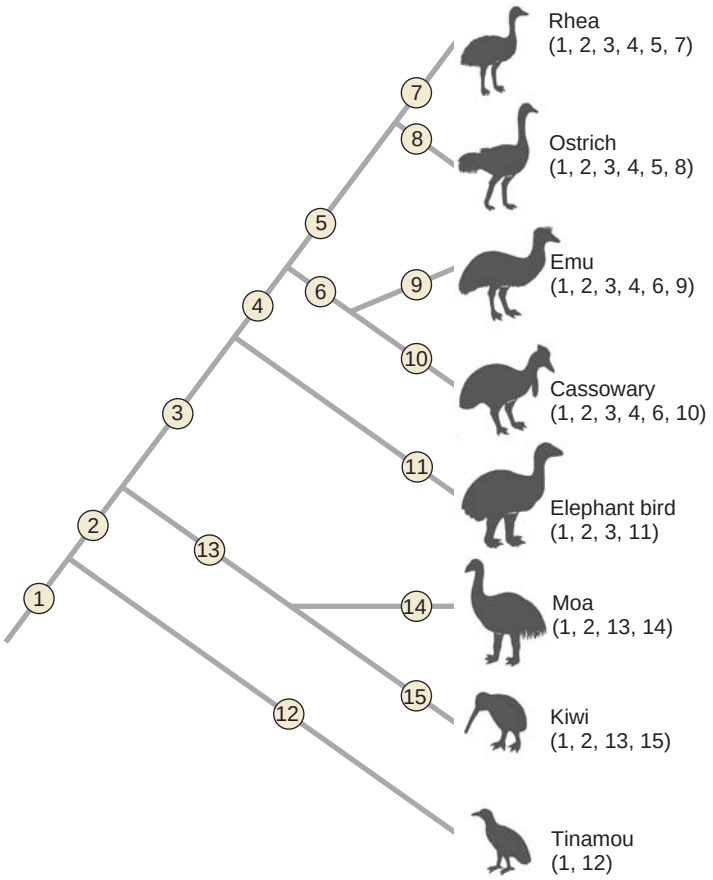


Figure 6.16

The phylogenetic pattern specified by 15 homologous structures in the skeletons of a group of flightless birds. Homologous features are numbered 1 through 15 and are marked both on the branches of the tree on which they arose and on the birds that have them. If you were to erase the tree structure, you would be able to reconstruct it without error from the distributions of homologous features shown for the birds at the terminal branches.

because this pattern is so evident, but it was not explained scientifically before Darwin. Once the idea of common descent was accepted, biologists began investigating the structural, molecular, and chromosomal homologies of animal groups. Taken together, the nested hierarchical patterns uncovered by these studies have permitted us to reconstruct evolutionary trees of many groups and to continue investigating others. Use of Darwin's theory of common descent to reconstruct the evolutionary history of life and to classify animals is the subject of Chapter 10.

Note that the earlier evolutionary hypothesis that life arose many times, forming unbranched lineages, predicts linear sequences of evolutionary change with no nested hierarchy of homologies among species. Because we do observe nested hierarchies of homologies, that hypothesis is rejected. Note also that because the creationist argument is not a scientific hypothesis, it can make no testable predictions about any pattern of homology and therefore fails to meet the criteria of a scientific theory of animal diversity.

Characters of different organisms that perform similar functions are not necessarily homologous. The wings of bats and birds, although homologous as vertebrate forelimbs, are not homologous as wings. The most recent common ancestor of bats and birds had forelimbs, but the forelimbs were not in the form of wings. Wings of bats and birds evolved independently and have only superficial similarity in their flight structures.

Bat wings are formed by skin stretched over elongated digits, whereas bird wings are formed by feathers attached along the forelimb. Such functionally similar but nonhomologous structures are often termed analogues.

Ontogeny, Phylogeny, and Recapitulation

Ontogeny is the history of the development of an organism through its entire life. Early developmental and embryological features contribute greatly to our knowledge of homology and common descent. Comparative studies of ontogeny show how the evolutionary alteration of developmental timing generates new characteristics, thereby producing evolutionary divergence among lineages.

The German zoologist Ernst Haeckel, a contemporary of Darwin, proposed that each successive stage in the development of an individual represented one of the adult forms that appeared in its evolutionary history. The human embryo with gill depressions in the neck corresponded, for example, to the adult appearance of a fishlike ancestor. On this basis Haeckel gave his generalization: *ontogeny (individual development) recapitulates (repeats) phylogeny (evolutionary descent).* This notion later became known simply as **recapitulation** or the **biogenetic law.** Haeckel based his biogenetic law on the flawed premise that evolutionary change occurs by successively adding new features onto the end of an unaltered ancestral ontogeny while condensing the ancestral ontogeny into earlier developmental stages. This notion was based on Lamarck's concept of the inheritance of acquired characteristics (p. 105).

The nineteenth-century embryologist, K. E. von Baer, gave a more satisfactory explanation of the relationship between ontogeny and phylogeny. He argued that early developmental features were simply more widely shared among different animal groups than later ones. Figure 6.17 shows, for example, the early embryological similarities of organisms whose adult forms are very different (see Figure 8.21, p. 176). The adults of animals with relatively short and simple ontogenies often resemble pre-adult stages of other animals whose ontogeny is more elaborate, but embryos of descendants do not necessarily resemble the adults of their ancestors. Even early development undergoes evolutionary divergence among lineages, however, and it is not as stable as von Baer proposed.

We now know many parallels between ontogeny and phylogeny, but features of an ancestral ontogeny can be shifted either to earlier or later stages in descendant ontogenies. Evolutionary change in timing of development is called **heterochrony,**

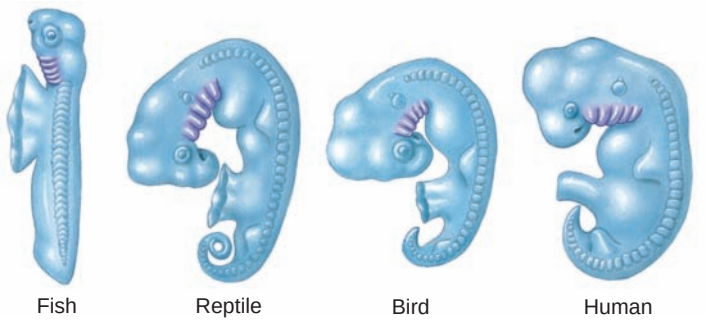


Figure 6.17

Comparison of gill arches of different embryos. All are shown separated from the yolk sac. Note the remarkable similarity of the four embryos at this early stage in development.

a term initially used by Haeckel to denote exceptions to recapitulation. If a descendant's ontogeny extends beyond its ancestral one, new characteristics can be added late in development, beyond the point at which development would have ended in an evolutionary ancestor. Features observed in the ancestor often are moved to earlier stages of development in this process, and ontogeny therefore does recapitulate phylogeny to some degree. Ontogeny also can be shortened during evolution, however. Terminal stages of the ancestor's ontogeny can be deleted, causing adults of descendants to resemble pre-adult stages of their ancestors (Figure 6.18). This outcome reverses the parallel between ontogeny and phylogeny (reverse recapitulation) producing **paedomorphosis** (the retention of ancestral juvenile characters by descendant adults). Because lengthening or shortening of ontogeny can change different parts of the body independently, we often see a mosaic of different kinds of developmental evolutionary change occurring concurrently. Therefore, cases in which an entire ontogeny recapitulates phylogeny are rare.

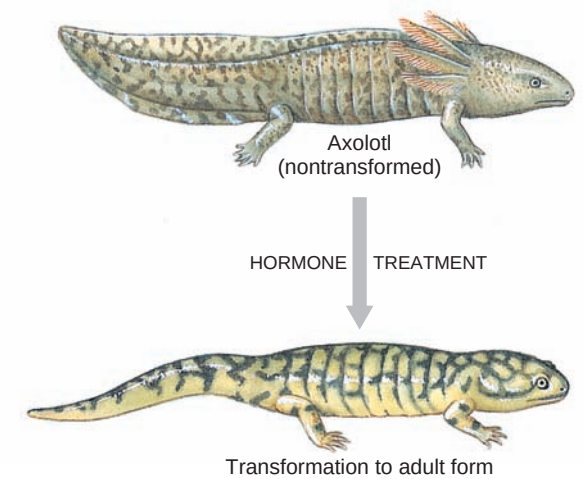


Figure 6.18

Aquatic and terrestrial forms of axolotls. Axolotls retain the juvenile, aquatic morphology (*top*) throughout their lives unless forced to metamorphose (*bottom*) by hormone treatment. Axolotls evolved from metamorphosing ancestors, an example of paedomorphosis.

Developmental Modularity and Evolvability

Evolutionary innovations occur not only by simple changes in rates of developmental processes but by changes in the physical location in the body where a process is activated. **Heterotopy** is the term traditionally used to describe a change in the physical location of a developmental process in an organism's body. For such a change to be successful, the developmental process must be compartmentalized into semiautonomous modules whose expression can be activated in new locations.

An interesting example of modularity and heterotopy occurs in some geckos. Geckos as a group typically have toepads, adhesive structures on the ventral side of the toes that permit climbing and clinging to smooth surfaces. Toepads consist of modified scales containing long protrusions, called setae, which can be molded to the surface of a substrate. A module responsible for toepad development is expressed in an unusual gecko species not only on the toes but also on the ventral side of the tip of the tail. This species has thereby acquired an additional adhesive appendage by ectopic expression of a standard developmental module.

Modularity is evident also in the homeotic mutations of the fruit fly, *Drosophila melanogaster*. Such mutations can substitute a developmental module for a leg in place of one normally specifying an antenna, thereby producing a fly with a pair of legs on its head. Another homeotic mutation in fruit flies transforms the balancer organs on the thorax into a second pair of wings; the balancer module is replaced by activation of the wing module, which in flies is activated normally only in a more anterior portion of the thorax.

Modularity is important in explaining some major evolutionary changes, such as evolution of tetrapod limbs (p. 546). The evolutionary transition from finlike limbs to the standard tetrapod limbs occurred by activating at the site of limb formation a set of homeobox genes (p. 173) whose expression pattern evolved initially as a module for forming part of the vertebral column. Shared patterns of gene expression between the vertebral column and the forelimbs and hindlimbs of tetrapods revealed the genetic and developmental mechanics of this module.

The term **evolvability** has been introduced recently to denote the great evolutionary opportunities created by having semiautonomous developmental modules whose expression can be moved from one part of the body to another. An evolving lineage that contains a large modular developmental toolkit can "experiment" with the construction of many new structures, some of which will persist and give rise to new homologies.

Multiplication of Species

Multiplication of species through time is a logical corollary to Darwin's theory of common descent. A branch point on the evolutionary tree means that an ancestral species has split into two different ones. Darwin's theory postulates that genetic variation present within a species, especially variation that occurs between geographically separated populations, provides the material from which new species are produced. Because evolution is a branching process, the total number of species

produced by evolution increases through time, although most of these species eventually become extinct without leaving descendant species. A major challenge for evolutionists is to discover the process by which an ancestral species "branches" to form two or more descendant species.

Before we explore multiplication of species, we must decide what we mean by "species." As explained in Chapter 10, no consensus exists regarding definition of species. Most biologists agree, however, that important criteria for recognizing species include (1) descent of all members from a common ancestral population forming a **lineage** of ancestor-descendant populations, (2) reproductive compatibility (ability to interbreed) within and reproductive incompatibility between species for sexually reproducing animals, and (3) maintenance within species of genotypic and phenotypic cohesion (lack of abrupt differences among populations in allelic frequencies and organismal characteristics). The criterion of reproductive compatibility has received the greatest attention in studies of species formation, also called **speciation.**

Biological features that prevent different species from interbreeding are called **reproductive barriers.** The primary problem of speciation is to discover how two initially compatible populations evolve reproductive barriers that cause them to become distinct, separately evolving lineages. How do populations diverge from each other in their reproductive properties while maintaining complete reproductive compatibility within each population?

Reproductive barriers between populations usually evolve gradually. Evolution of reproductive barriers requires that diverging populations must be kept physically separate for long periods of time. If diverging populations reunite before reproductive barriers have evolved, interbreeding occurs between the populations and they merge. Speciation by gradual divergence in animals may require extraordinarily long periods of time, perhaps 10,000 to 100,000 years or more. Geographical isolation followed by gradual divergence is the most effective way for reproductive barriers to evolve, and many evolutionists consider geographical separation a prerequisite for branching speciation.

Geographical barriers between populations are not the same thing as reproductive barriers. Geographical barriers refer to spatial separation of two populations. They prevent gene exchange and are usually a precondition for speciation. Reproductive barriers result from evolution and refer to various behavioral, physical, physiological, and ecological factors that prevent interbreeding between different species. Behavioral barriers often evolve faster than other kinds of reproductive barriers. Geographical barriers do not guarantee that reproductive barriers will evolve. Reproductive barriers are most likely to evolve under conditions that include small population size, a favorable combination of selective factors, and long periods of geographical isolation. One or both of a pair of geographically isolated populations may become extinct prior to evolution of reproductive barriers between them. Over the vast span of geological time, however, conditions sufficient for speciation have occurred millions of times.

Allopatric Speciation

Allopatric ("in another land") populations of a species are those that occupy separate geographical areas. Because of their geographical separation, they cannot interbreed, but would be expected to do so if the geographic barriers between them were removed. If populations are allopatric immediately preceding and during evolution of reproductive barriers between them, the resulting speciation is called **allopatric speciation** or geographic speciation. The separated populations evolve independently and adapt to their respective environments, generating reproductive barriers between them as a result of their separate evolutionary paths. Because their genetic variation arises and changes independently, physically separated populations will diverge genetically even if their environments remain very similar. Environmental change between populations also can promote genetic divergence between them by favoring different phenotypes in the separated populations. Ernst Mayr (Figure 6.19) has contributed greatly to our knowledge of allopatric speciation through his studies of speciation in birds.

Allopatric speciation begins when a species splits into two or more geographically separated populations. This splitting can happen in either of two ways: by **vicariant speciation** or by a **founder event.** Vicariant speciation is initiated when climatic or geological changes fragment a species's habitat, producing impenetrable barriers that separate different populations geographically. For example, a mammalian species inhabiting a lowland forest could be divided by uplifting of a mountain barrier, sinking and flooding of a geological fault, or climatic changes that cause prairie or desert conditions to encroach on the forest. Formation of the isthmus of Panama separated populations of the sea urchin genus *Eucidaris*, leading to formation of the pair of species shown in Figure 1.5D.

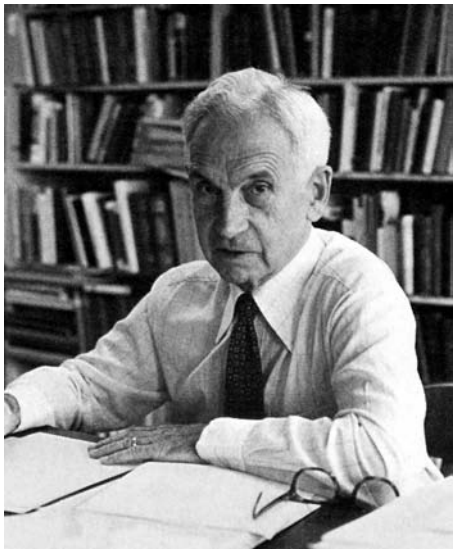


Figure 6.19

Ernst Mayr (1904 to 2005), a major contributor to our knowledge of speciation and of evolution in general.

Vicariant speciation has two important consequences. Although the ancestral population is fragmented, individual fragments are usually left fairly intact. The vicariant process itself does not induce genetic change by reducing populations to a small size or by transporting them to unfamiliar environments. Another important consequence is that the same vicariant events may fragment several different species simultaneously. For example, fragmentation of a lowland forest most likely would disrupt numerous and diverse species, including salamanders, frogs, snails, and many other forest dwellers. Indeed, the same geographic patterns are observed among closely related species in different groups of organisms whose habitats are similar. Such patterns provide strong evidence for vicariant speciation.

An alternative means of initiating allopatric speciation is for a small number of individuals to disperse to a distant place where no other members of their species occur. The dispersing individuals may establish a new population in what is called a founder event. Allopatric speciation caused by founder events has been observed, for example, in the native fruit flies of Hawaii. Hawaii contains numerous patches of forest separated by volcanic lava flows. On rare occasions, strong winds can transport a few flies from one forest to another, geographically isolated forest where the flies are able to start a new population. Sometimes, a single fertilized female may found a new population. Unlike what happens in vicariant speciation, the new population initially has a very small size, which can cause its genetic structure to change quickly and dramatically from that of its ancestral population (see p. 129). When this event happens, phenotypic characteristics that were stable in the ancestral population often reveal unprecedented variation in the new population. As the newly expressed variation is sorted by natural selection, large changes in phenotype and reproductive properties occur, hastening evolution of reproductive barriers between the ancestral and newly founded populations.

The term *founder event* in its most general usage denotes a dispersal of organisms from an ancestral population across a geographic barrier to start a new, allopatric population. A founder event does not always directly cause important changes in the genetic constitution of the new population relative to the old one, although such changes are expected when the number of founding individuals is very small (less than 5 to 10 individuals, for example) and the ancestral population has a large amount of genetic variation. A change in the genetic constitution of a newly founded population because of a small number of founders is termed a *founder effect*, which includes a population bottleneck (p. 129). If a founder effect is so profound that selection acts in new ways on reproductively important characters, the founder event can induce speciation. *Founder-induced speciation* describes the subset of founder events in which a founder effect hastens species-level divergence of the newly founded population. Speciation in Hawaiian *Drosophila* as described in the text illustrates founder-induced speciation. Excluded from founder-induced speciation are founder events whose role in speciation is strictly to establish a new allopatric population capable of independent evolutionary change.

Surprisingly, we often learn most about the genetics of allopatric speciation from cases in which formerly separated populations regain geographic contact following evolution of incipient reproductive barriers that are not absolute. The occurrence of mating between divergent populations is called **hybridization** and offspring of these matings are called **hybrids** (Figure 6.20). By studying the genetics of hybrid populations, we can identify the genetic bases of reproductive barriers.

Biologists often distinguish between reproductive barriers that impair fertilization (premating barriers) and those that impair growth and development, survival, or reproduction of hybrid individuals (postmating barriers). Premating barriers cause members of divergent populations either not to recognize each other as potential mates or not to complete the mating ritual successfully. Details of the horn structures of African antelopes (Figure 6.11) are important for recognizing members of the same species as potential mates. In some other cases, female and male genitalia of the different populations may be incompatible or the gametes may be incapable of fusing to form a zygote. In others, premating barriers may be strictly behavioral, with members of different species being otherwise nearly identical in phenotype. Different species that are indistinguishable in organismal appearance are called **sibling species.** Sibling species arise when allopatric populations diverge in the seasonal timing of reproduction or in auditory, behavioral, or chemical signals required for mating. Evolutionary divergence in these features can produce effective premating barriers without obvious changes in organismal appearance. Sibling species occur in groups as diverse as ciliates, flies, and salamanders.

Nonallopatric Speciation

Can speciation ever occur without prior geographic separation of populations? Allopatric speciation may seem an unlikely

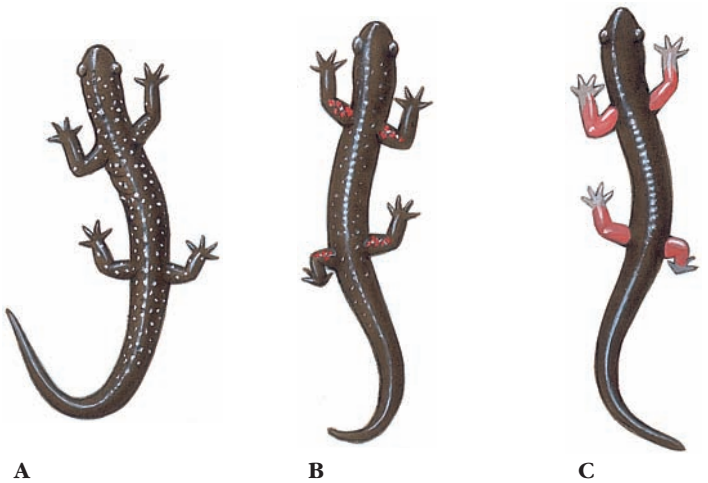


Figure 6.20

Pure and hybrid salamanders. Hybrids are intermediate in appearance between parental populations. **A,** Pure white-spotted *Plethodon teyahalee;* **B,** a hybrid between white-spotted *P. teyahalee* and red-legged *P. shermani,* intermediate in appearance for both spotting and leg color; **C,** pure red-legged *P. shermani.*

explanation for situations where many closely related species occur together in restricted areas that have no traces of physical barriers to animal dispersal. For example, several large lakes around the world contain very large numbers of closely related species of fish. The great lakes of Africa (Lake Malawi, Lake Tanganyika, and Lake Victoria) each contain many species of cichlid fishes that are found nowhere else. Likewise, Lake Baikal in Siberia contains many different species of sculpins that occur nowhere else in the world (Figure 6.21). It is difficult to conclude that these species arose anywhere other than in the lakes they inhabit, and yet those lakes are young on an evolutionary timescale and have no obvious environmental barriers that would fragment fish populations.

To explain speciation of fish in freshwater lakes and other examples like these, **sympatric** ("same land") **speciation** has been hypothesized. According to this hypothesis, different individuals within a species become specialized for occupying different components of the environment. By seeking and using very specific habitats in a single geographic area, different populations achieve sufficient physical and adaptive separation to evolve reproductive barriers. For example, cichlid species of African lakes are very different from each other in their feeding specializations. In many parasitic organisms, particularly parasitic insects, different populations may use different host species, thereby providing the physical separation necessary for reproductive barriers to evolve. Supposed cases of sympatric speciation have been criticized, however, because the reproductive distinctness of the different populations often is not well demonstrated, so that we may not be observing formation of distinct evolutionary lineages that will become different species.

The occurrence of sudden sympatric speciation is perhaps most likely among higher plants. Between one-third and one-half of flowering plant species may have evolved by polyploidy (doubling of chromosome numbers), without prior geographic isolation of populations. In animals, however, speciation through polyploidy is an exceptional event.

Another possible mode of speciation, termed **parapatric speciation**, is geographically intermediate between allopatric and sympatric speciation. Two species are called parapatric with respect to each other if their geographic ranges are primarily allopatric but make contact along a borderline that neither species successfully crosses. In parapatric speciation, a geographically continuous ancestral species evolves within its range a borderline across which populations evolve species-level differences while maintaining geographic contact along the border.

The simplest model of parapatric speciation is one in which a change in environmental conditions splits the geographic range of a species into two environmentally distinct but geographically adjoining parts. An increase in temperature on a Caribbean island, for example, might cause part of a wet tropical forest to become a dry, sandy one. A lizard species occupying the formerly wet forest might become divided into geographically adjacent wet and dry forest populations. Unlike vicariant allopatric speciation, however, the populations in the different habitat types are not isolated by a physical barrier but maintain genetic

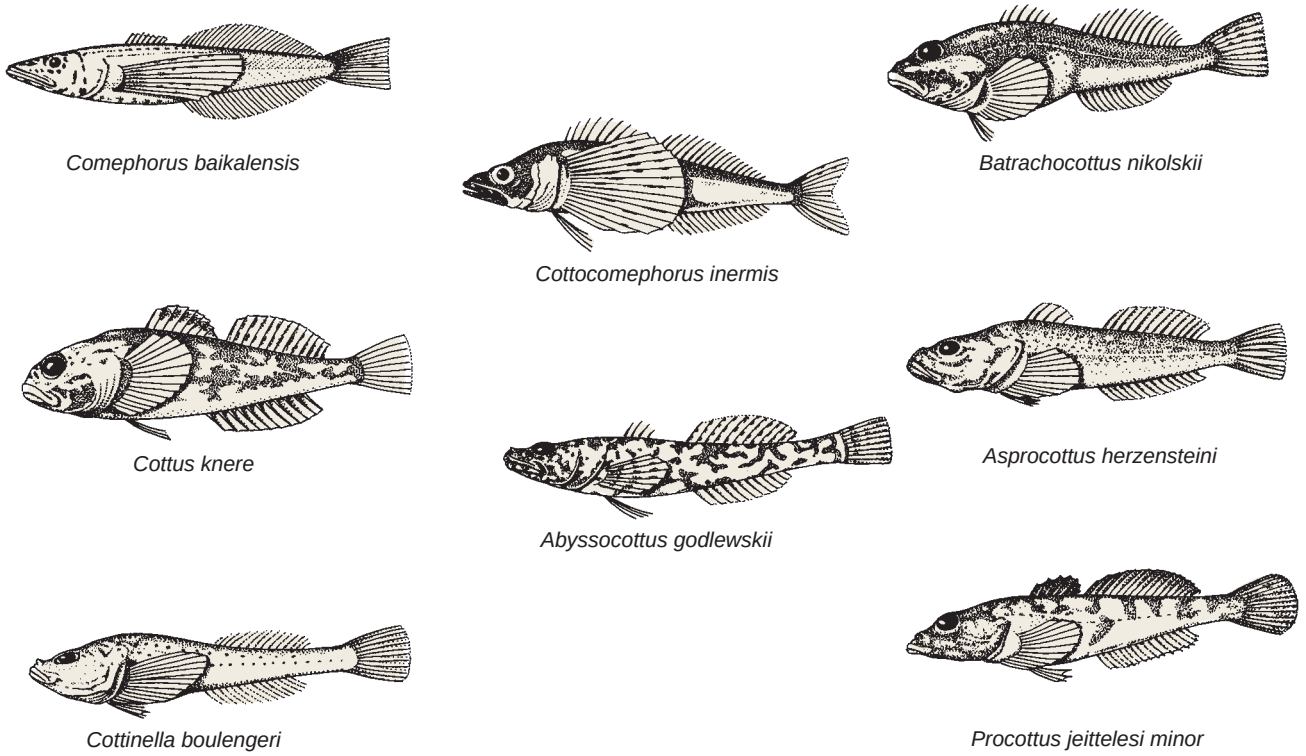


Figure 6.21
The sculpins of Lake Baikal, products of speciation that occurred within a single lake.

interactions along the geographic borderline between the different habitat types. The disparity in environmental conditions across the border, nonetheless, causes the populations to evolve as separate lineages adapted to different environments despite some gene exchange between them.

A parapatric distribution of species does not necessarily imply that speciation occurred parapatrically. Most cases of parapatrically distributed species show evidence of past allopatry, with subsequent removal of a geographic barrier permitting the two species to make geographic contact but with each species excluding the other one from its geographic territory.

The prevalence of parapatric speciation is controversial. This model of speciation predicts that parapatrically distributed populations differ mainly in adaptive features associated with the observed environmental differences but show relative homogeneity for other genetic variation. Comparisons of parapatrically distributed populations, including those of lizards occupying different forest types on Caribbean islands, often show extensive divergence for molecular variation unrelated to adaptive differentiation of the populations; such results are better explained by vicariant allopatric speciation than by parapatric speciation. In some cases, geological evidence shows that what is now a single island was physically fragmented into separate islands during warm periods when the sea level was higher than it is now; such evidence likewise favors the interpretation of allopatric speciation for parapatrically distributed species whose geographic contact occurs in formerly inundated areas.

Adaptive Radiation

The production of several ecologically diverse species from a common ancestral species is called **adaptive radiation,** especially when many disparate species arise within a short interval of geological time (a few million years). Some of our best examples of adaptive radiation are associated with lakes and young islands, which provide new evolutionary opportunities for aquatic and terrestrial organisms, respectively. Oceanic islands formed by volcanoes are initially devoid of life. They are gradually colonized by plants and animals from a continent or from other islands in separate founder events. The founders encounter ideal situations for evolutionary diversification, because environmental resources that were heavily exploited by other species on the mainland are free for colonization on the sparsely populated island. Archipelagoes, such as the Galápagos Islands, greatly increase opportunities for both founder events and ecological diversification. The entire archipelago is isolated from the continent and each island is geographically isolated from the others by sea; moreover, each island is different from every other one in its physical, climatic, and biotic characteristics.

Galápagos finches illustrate adaptive radiation on an oceanic archipelago (Figures 6.22 and 6.23). Galápagos finches (the name "Darwin's finches" was popularized in the 1940s by the British ornithologist David Lack) are closely related to each other, but each species differs from others in size and shape of the beak and in feeding habits. If the finches were specially created, it would require the strangest kind of coincidence for 13 similar kinds of finches to be created on the Galápagos Islands and nowhere else. Darwin's finches descended from a single ancestral population that arrived from the mainland and subsequently colonized the different islands of the Galápagos archipelago. The finches underwent adaptive radiation, occupying habitats that on the mainland were denied to them by the presence of other species better able to exploit those habitats. Galápagos finches thus assumed characteristics of mainland birds as diverse and unfinchlike as warblers and woodpeckers. A fourteenth Darwin's finch, found on isolated Cocos Island far north of the Galápagos archipelago, is similar in appearance to the Galápagos finches and almost certainly descended from the same ancestral founder.

Gradualism

Darwin's theory of gradualism opposes arguments for the sudden origin of species. Small differences, resembling those that we observe among organisms within populations today, are the raw material from which the different major forms of life evolved. This theory shares with Lyell's uniformitarianism the notion that we must not explain past changes by invoking unusual catastrophic events that are not observed today. If new species originated in single, catastrophic events, we should be able to see such events happening today and we do not. Instead, what we usually observe in natural populations are small, continuous changes in phenotypes. Such continuous changes can produce major differences among species only by accumulating over many thousands to millions of years. A simple statement of Darwin's theory of gradualism is that accumulation of quantitative changes leads to qualitative change.

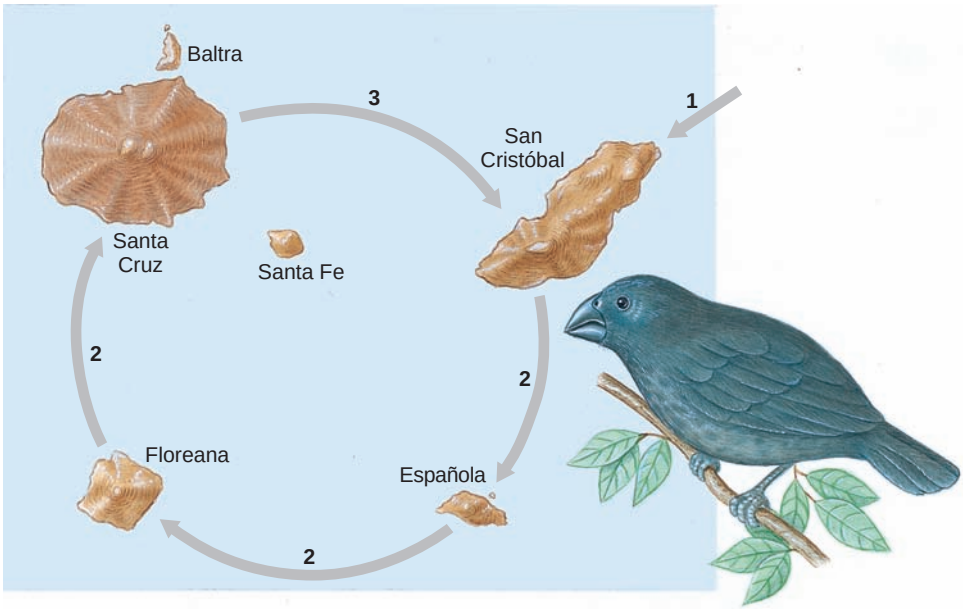
Mayr (see Figure 6.19) makes an important distinction between populational gradualism and phenotypic gradualism. **Populational gradualism** states that new traits become established in a population by increasing their frequency initially from a small fraction of the population to a majority of the population. Populational gradualism is well established and is not controversial. **Phenotypic gradualism** states that new traits, even those that are strikingly different from ancestral ones, are produced in a series of small, incremental steps.

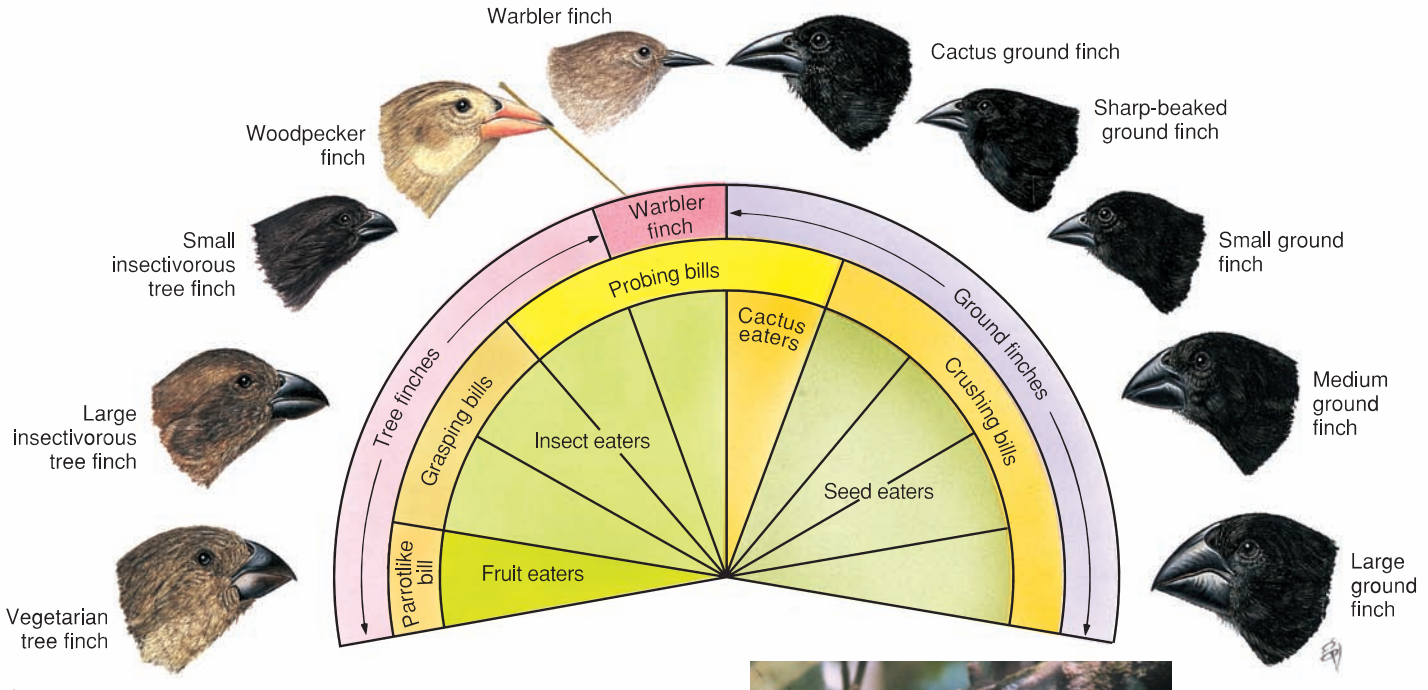
Phenotypic Gradualism

Phenotypic gradualism was controversial when Darwin first proposed it, and it is still controversial. Not all phenotypic changes are small, incremental ones. Some mutations that appear during artificial breeding change the phenotype substantially in a single mutational step. Such mutations traditionally are called "sports." Sports that produce dwarfing are observed in many species, including humans, dogs, and sheep, and have been used by animal breeders to achieve desired results; for example, a sport that deforms the limbs was used to produce Ancon sheep, which cannot jump hedges and are therefore easily contained (Figure 6.24). Many colleagues of Darwin who accepted his other theories considered phenotypic gradualism too extreme. If sporting mutations can be used in animal breeding, why must

Figure 6.22

Model for evolution of the 13 Darwin's finches on the Galápagos Islands. The model postulates three steps: (1) Immigrant finches from the South American mainland reach the Galápagos and colonize an island; (2) once the population becomes established, finches disperse to other islands where they adapt to new conditions and change genetically; and (3) after a period of isolation, secondary contact is established between different populations. The two populations are then recognized as separate species if they cannot interbreed successfully.





A

B

Figure 6.23

A, Adaptive radiation in 10 species of Darwin's finches from Santa Cruz, one of the Galápagos Islands. Differences in bills and feeding habits are shown. All apparently descend from a single common ancestral finch from the South American continent. **B,** Woodpecker finch, one of the 13 species of Galápagos Islands finches, using a slender twig as a tool for feeding. This finch worked for about 15 minutes before spearing and removing a wood roach from a break in the tree.

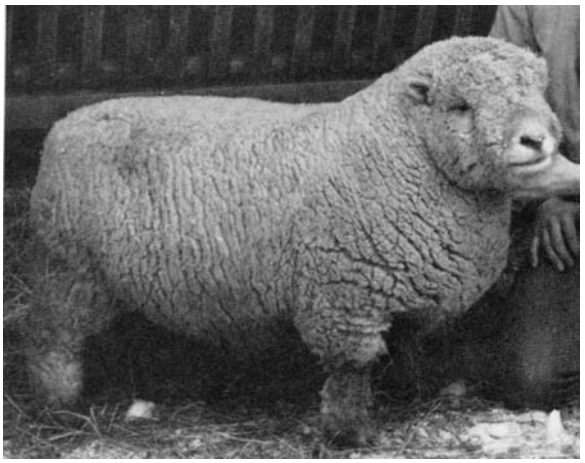


Figure 6.24

The Ancon breed of sheep arose from a "sporting mutation" that caused dwarfing of legs. Many of his contemporaries criticized Darwin for his claim that such mutations are not important for evolution by natural selection.

we exclude them from our evolutionary theory? In favor of gradualism, Darwin and others have replied that sporting mutations always have negative side-effects that would cause selection to eliminate them from natural populations. Indeed, it is questionable whether Ancon sheep, despite their attractiveness to farmers, would propagate successfully in the presence of their long-legged relatives without human intervention. A mutation of large effect appears responsible, however, for an adaptive bill size polymorphism in an African finch species (*Pyrenestes ostrinus*) in which large-billed forms eat hard seeds and small-billed forms eat softer ones. Recent work in evolutionary developmental genetics (p. 174) illustrates the continuing controversy surrounding phenotypic gradualism.

Punctuated Equilibrium

When we view Darwinian gradualism on a geological timescale, we may expect to find in the fossil record a long series of intermediate forms connecting the phenotypes of ancestral and descendant populations (Figure 6.25). This predicted pattern is called **phyletic gradualism.** Darwin recognized that phyletic gradualism is not often revealed by the fossil record. Studies conducted since Darwin's time generally have not revealed the continuous series of fossils predicted by phyletic gradualism. Is the theory of gradualism therefore refuted by the fossil record? Darwin and others claim that it is not, because the fossil record is too imperfect to preserve transitional series. Although evolution is a slow process by our standards, it is rapid relative to the rate at which good fossil deposits accumulate. Others have argued, however, that abrupt origins and extinctions of species in the fossil record force us to conclude that phyletic gradualism is rare.

Niles Eldredge and Stephen Jay Gould proposed **punctuated equilibrium** to explain the discontinuous evolutionary changes observed throughout geological time. Punctuated equilibrium

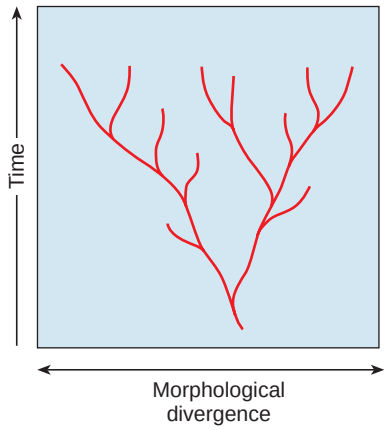


Figure 6.25

The phyletic gradualist model of evolutionary change in morphology, viewed as proceeding more or less steadily through geological time (millions of years). Bifurcations followed by gradual divergence led to speciation. Note that most morphological change accumulates incrementally within species lineages between branch points, which are not accompanied by unusually large amounts of morphological change.

states that phenotypic evolution is concentrated in relatively brief events of branching speciation, followed by much longer intervals of morphological evolutionary stasis (Figure 6.26). Speciation is an episodic event, having a duration of approximately 10,000 to 100,000 years. Because species may survive for 5 to 10 million years, the speciation event is a "geological instant," representing 1% or less of a species's life span. Ten thousand years is plenty of time, however, for Darwinian gradual evolution to accomplish dramatic changes. A small fraction of the evolutionary history of a group therefore contributes most of the morphological evolutionary change that we observe. Punctuated equilibrium contrasts with the views of paleontologist George Simpson, who attributed only moderate rates of morphological

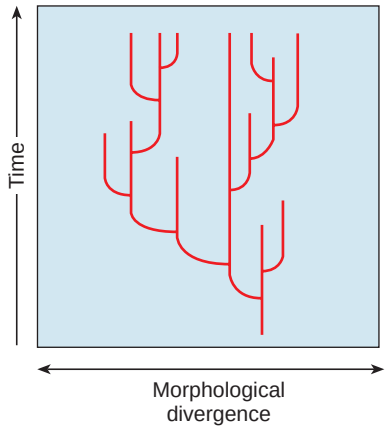


Figure 6.26

The punctuated equilibrium model sees morphological evolutionary change being concentrated in relatively rapid bursts of branching speciation (lateral lines) followed by prolonged periods of no change throughout geological time (millions of years).

evolution to branching speciation and expected most morphological change to accumulate gradually in the "phyletic" mode between events of branching speciation.

Founder-induced allopatric speciation provides a possible explanation for punctuated equilibria. Remember that founder-induced speciation requires the breaking of genetic equilibrium in a small, geographically isolated population. Such small populations have very little chance of being preserved in the fossil record. After a new genetic equilibrium forms and stabilizes, the new population may increase in size, thereby increasing the chance that some of its members will be preserved as fossils. Founder-induced speciation cannot be the exclusive cause of punctuated equilibrium, however, because punctuated equilibrium may be observed in groups where speciation by founder events is unlikely.

Evolutionists who lamented the imperfect state of the fossil record were treated in 1981 to the opening of an uncensored page of fossil history in Africa. Peter Williamson, a British paleontologist working in fossil beds 400 m deep near Lake Turkana, documented a remarkably clear record of speciation in freshwater snails. The geology of the Lake Turkana basin reveals a history of instability. Earthquakes, volcanic eruptions, and climatic changes caused the waters episodically to rise and to fall, sometimes by hundreds of feet. Thirteen lineages of snails show long periods of stability interrupted by relatively brief periods of rapid change in shell shape when snail populations were fragmented by receding waters. These populations diverged to produce new species that then remained unchanged through thick deposits before becoming extinct and being replaced by descendant species. The transitions occurred within 5000 to 50,000 years. In the few meters of sediment where speciation occurred, transitional forms were visible. Williamson's study conforms well to the punctuated equilibrium model of Eldredge and Gould.

Natural Selection

Natural selection is the major process by which evolution occurs in Darwin's theory of evolution. It gives us a natural explanation for the origins of **adaptation,** including all developmental, behavioral, anatomical, and physiological attributes that enhance an organism's ability to use environmental resources to survive and to reproduce. Evolution of color patterns that conceal moths from predators (Figure 1.11, p. 12), and of bills adapted to different modes of feeding in finches (Figure 6.23), illustrate natural selection leading to adaptation. Darwin developed his theory of natural selection as a series of five observations and three inferences drawn from them:

Observation 1—Organisms have great potential fertility. All populations produce large numbers of gametes and potentially large numbers of offspring each generation. Population size would increase exponentially at an enormous rate if all individuals that were produced each generation survived and reproduced. Darwin calculated that, even in slow-breeding animals such as elephants, a single pair breeding from age 30 to 90 and having only six young could produce 19 million descendants in 750 years.

Observation 2—Natural populations normally remain constant in size, except for minor fluctuations. Natural populations fluctuate in size across generations and sometimes go extinct, but no natural populations show the continued exponential growth that their reproductive biology theoretically could sustain.

Observation 3—Natural resources are limited. Exponential growth of a natural population would require unlimited natural resources to provide food and habitat for the expanding population, but natural resources are finite.

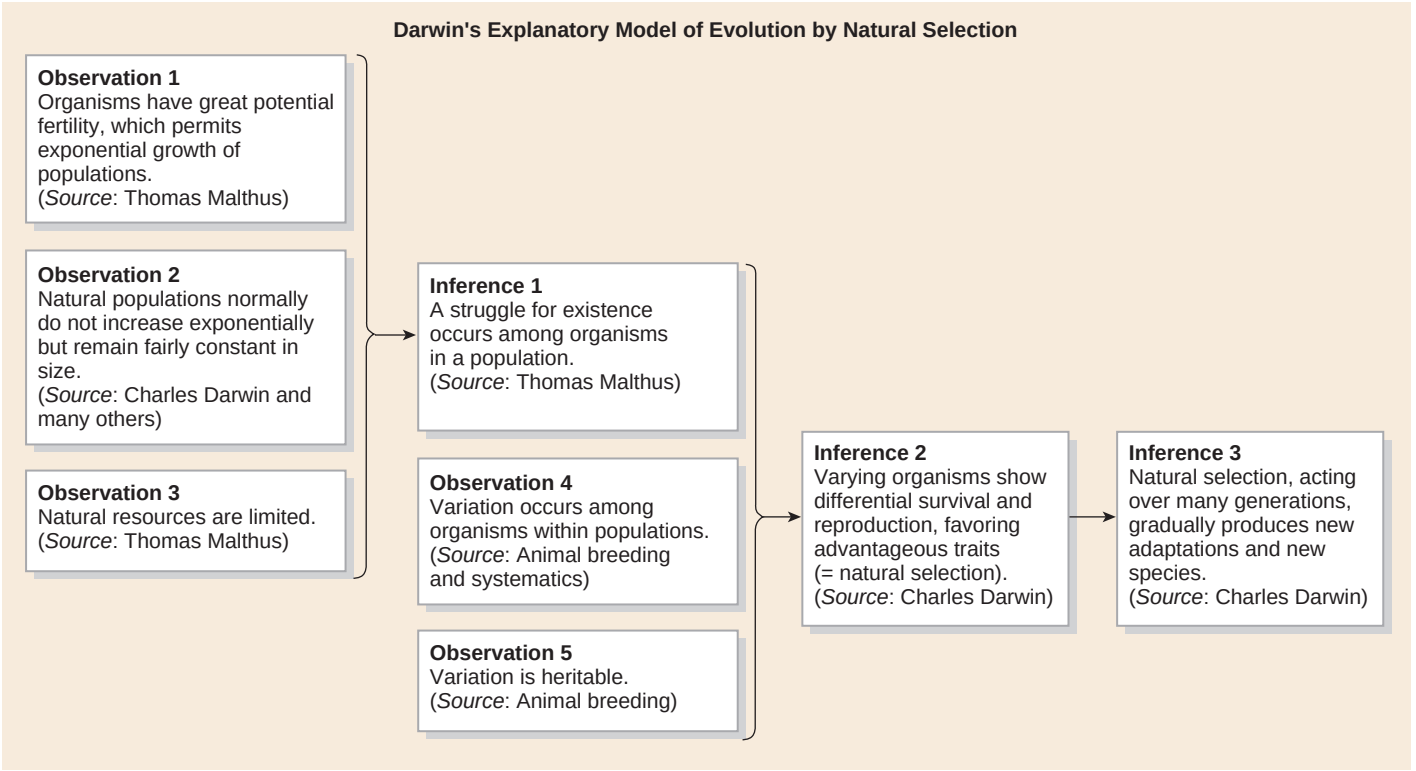
Inference 1—A continuing *struggle* for existence exists among members of a population. Survivors represent only a part, usually a very small part, of the individuals produced each generation. Darwin wrote in *On The Origin of Species* that "it is the doctrine of Malthus applied with manifold force to the whole animal and vegetable kingdoms." The struggle for food, shelter, and space becomes increasingly severe as overpopulation develops.

Observation 4—Populations show *variation* among organisms. No two individuals are exactly alike. They differ in size, color, physiology, behavior, and many other ways.

Observation 5—Some variation is *heritable*. Darwin noted that offspring tend to resemble their parents, although he did not understand how. The hereditary mechanism discovered by Gregor Mendel would be applied to Darwin's theory many years later.

Inference 2—Varying organisms show *differential survival and reproduction* favoring advantageous traits (= natural selection). Survival in the struggle for existence is not random with respect to hereditary variation present in the population. Some traits give their possessors an advantage in using the environment for effective survival and reproduction. Survivors transmit their favored traits to offspring, thereby causing those traits to accumulate in the population.

Inference 3—Over many generations, natural selection generates new adaptations and new species. The differential reproduction of varying organisms gradually transforms species and causes long-term "improvement" of populations. Darwin knew that people often use hereditary variation to produce useful new breeds of livestock and plants. *Natural* selection acting over millions of years should be even more effective in producing new types than the *artificial* selection imposed during a human lifetime. Natural selection acting independently on geographically separated populations would cause them to diverge from each other, thereby generating reproductive barriers that lead to speciation.



The popular phrase "survival of the fittest" was not originated by Darwin but was coined a few years earlier by the British philosopher Herbert Spencer, who anticipated some of Darwin's principles of evolution. Unfortunately the phrase later came to be coupled with unbridled aggression and violence in a bloody, competitive world. In fact, natural selection operates through many other characteristics of living organisms. The fittest animal may be one that enhances the living conditions of its population. Fighting prowess is only one of several means toward survival and reproductive advantage.

Natural selection may be considered a two-step process with a random component and a nonrandom component. Production of variation among organisms is the random component. The mutational process does not preferentially generate traits that are favorable to the organism; new variants are probably more likely to be unfavorable. The nonrandom component is the survival of different traits. This differential survival is determined by the effectiveness of different traits in permitting their possessors to use environmental resources to survive and to reproduce. The phenomenon of differential survival and reproduction among varying organisms is now called **sorting** and should not be equated with natural selection. We now know that even random processes (genetic drift, p. 127) can produce sorting among varying organisms. When selection operates, sorting occurs *because certain traits give their possessors advantages in survival and reproduction* relative to others that lack those traits. Selection is therefore a specific cause of sorting.

Darwin's theory of natural selection has been challenged repeatedly. One challenge claims that directed (nonrandom) variation governs evolutionary change. In the decades around 1900, diverse evolutionary hypotheses collectively called **orthogenesis** proposed that variation has momentum that forces a lineage to evolve in a particular direction that is not always adaptive. The extinct Irish elk was a popular example of orthogenesis. Newly produced variation was considered biased toward enlarging their antlers, thereby generating an evolutionary momentum for producing larger antlers. Natural selection was considered ineffective at stopping the antlers eventually from becoming so large and cumbersome that they forced the Irish elk into extinction (Figure 6.27). Orthogenesis explained apparently nonadaptive evolutionary trends that supposedly forced species into decline. Because extinction is the expected evolutionary fate of most species, disappearance of the Irish elk is not extraordinary and probably not related to large antlers. Subsequent genetic research on the nature of variation clearly has rejected the genetic predictions of orthogenesis.

Another recurring criticism of natural selection is that it cannot generate new structures or species but can only modify old ones. Most structures in their early evolutionary stages could not have performed the biological roles that the fully formed structures perform, and it is therefore unclear how natural selection could have favored them. What use is half a wing or the rudiment of a feather for a flying bird? To answer this criticism, we propose that many structures evolved initially for purposes different from the ones they have today. Rudimentary feathers would have been useful in thermoregulation, for example. The

Figure 6.27

Irish elk, a fossil species that once was used to support the orthogenetic idea that momentum in variation caused the antlers to become so large that the species was forced into extinction.

feathers later became useful for flying after they incidentally acquired aerodynamic properties. Natural selection then could act to improve the usefulness of feathers for flying. **Exaptation** denotes the utility of a structure for a biological role that was not part of the structure's evolutionary origin. Exaptation contrasts with adaptation, which implies that a structure arose by natural selection for a particular biological role. Bird feathers are therefore adaptations for thermoregulation but exaptations for flight. Because structural changes that separate members of different species are similar in kind to variation that we observe within species, it is reasonable to propose that selection can produce new species.

REVISIONS OF DARWIN'S THEORY

Neo-Darwinism

The most serious weakness in Darwin's theory was his failure to identify correctly the mechanism of inheritance. Darwin saw heredity as a blending phenomenon in which the hereditary factors of parents melded together in their offspring. Darwin also invoked the Lamarckian hypothesis that an organism could alter its heredity through use and disuse of body parts and through the direct influence of the environment. August Weismann rejected Lamarckian inheritance by showing experimentally that modifications of an organism during its lifetime do not change its heredity (see Chapter 5), and he revised Darwin's theory accordingly. We now use the term **neo-Darwinism** to denote Darwin's theory as revised by Weismann.

Mendelian genetics eventually clarified the particulate inheritance that Darwin's theory of natural selection required (p. 18). Ironically, when Mendel's work was rediscovered in 1900, it was considered antagonistic to Darwin's theory of natural selection. When mutations were discovered in the early 1900s, most geneticists thought that they produced new species in single large steps. These geneticists relegated natural selection to the role of executioner, a negative force that merely eliminated the obviously unfit.

Emergence of Modern Darwinism: The Synthetic Theory

In the 1930s a new generation of geneticists began to reevaluate Darwin's theory from a mathematical perspective. These were population geneticists, scientists who studied variation in natural populations using statistics and mathematical models. Gradually, a new comprehensive theory emerged that brought together population genetics, paleontology, biogeography, embryology, systematics, and animal behavior in a Darwinian framework.

Population geneticists study evolution as a change in the genetic composition of populations. With the establishment of population genetics, evolutionary biology became divided into two different subfields. **Microevolution** pertains to evolutionary changes in frequencies of different allelic forms of genes (p. 78) within populations. **Macroevolution** refers to evolution on a grand scale, encompassing the origins of new organismal structures and designs, evolutionary trends, adaptive radiation, phylogenetic relationships of species, and mass extinction. Macroevolutionary research is based in systematics and the comparative method (p. 206). Following the evolutionary synthesis, both macroevolution and microevolution have operated firmly within the tradition of neo-Darwinism, and both have expanded Darwinian theory in important ways.

MICROEVOLUTION: GENETIC VARIATION AND CHANGE WITHIN SPECIES

Microevolution is the study of genetic change occurring within natural populations. Occurrence of different allelic forms of a gene in a population is called **polymorphism.** All alleles of all genes possessed by members of a population collectively form the **gene pool** of that population. The amount of polymorphism present in large populations is potentially enormous, because at observed mutation rates, many different alleles are expected for all genes.

Population geneticists study polymorphism by identifying the different allelic forms of a gene present in a population and then measuring the relative frequencies of the different alleles in the population. The relative frequency of a particular allelic form of a gene in a population is called its **allelic frequency.** For example, in the human population, there are three different allelic forms of the gene encoding the ABO blood types (p. 782). Using the symbol I to denote the gene encoding the ABO blood types, I^A and I^B denote genetically codominant alleles encoding blood types A and B, respectively. Allele i is a recessive allele encoding blood group O. Therefore genotypes I^AI^A and I^Ai produce type A blood, genotypes I^BI^B and I^Bi produce type B blood, genotype I^AI^B produces type AB blood, and genotype ii produces type O

blood. Because each individual contains two copies of this gene, the total number of copies present in the population is twice the number of individuals. What fraction of this total is represented by each of the three different allelic forms? In France, we find the following allelic frequencies: $I^A = .46$, $I^B = .14$, and $i = .40$. In Russia, the corresponding allelic frequencies differ ($I^A = .38$, $I^B = .28$, and $i = .34$), demonstrating microevolutionary divergence between these populations (Figure 6.28). Although alleles I^A and I^B are dominant to i, i is nearly as frequent as I^A and exceeds the frequency of I^B in both populations. Dominance describes the *phenotypic effect* of an allele in heterozygous individuals, not its relative abundance in a population of individuals. We will demonstrate that Mendelian inheritance and dominance do not alter allelic frequencies directly or produce evolutionary change in a population.

Genetic Equilibrium

In many human populations, genetically recessive traits, including the O blood type, blond hair, and blue eyes, are very common. Why have not the genetically dominant alternatives gradually supplanted these recessive traits? It is a common misconception that a characteristic associated with a dominant allele increases in frequency because of its genetic dominance. This misconception is refuted by a principle called **Hardy-Weinberg equilibrium** (see box next page), which forms the foundation for population genetics. According to this theorem, the hereditary process alone does not produce evolutionary change. In large biparental populations, allelic frequencies and genotypic ratios attain an equilibrium in one generation and *remain constant* thereafter *unless* disturbed by recurring mutations, natural selection, migration, nonrandom mating, or genetic drift (random sorting). Such disturbances are the sources of microevolutionary change.

A rare allele, according to this principle, does not disappear from a large population merely because it is rare. Certain rare traits, such as albinism and cystic fibrosis, persist for many generations. For example, albinism in humans is caused by a rare recessive allele *a*. Only one person in 20,000 is an albino, and this individual must be homozygous (*a/a*) for the recessive allele. Obviously the population contains many carriers, people with normal pigmentation who are heterozygous (*A/a*) for albinism. What is their frequency? A convenient way to calculate the frequencies of genotypes in a population is with the binomial expansion of $(p + q)^2$ (see box next page). We let p represent the allelic frequency of *A* and q the allelic frequency of *a*.

Assuming that mating is random, the distribution of genotypic frequencies is $p^2 = A/A$, $2pq = A/a$, and $q^2 = a/a$. Only the frequency of genotype *a/a* is known with certainty, 1/20,000; therefore:

$$q^2 = 1/20{,}000$$
$$q = (1/20{,}000)^{1/2} = 1/141$$
$$p = 1 - q = 140/141$$

The frequency of carriers is:

$$A/a = 2pq = 2 \times 140/141 \times 1/141 = 1/70$$

One person in every 70 is a carrier! Although the recessive trait is rare, the recessive allele is surprisingly common in the population. There is a message here for anyone proposing to eliminate a "bad" recessive allele from a population by controlling reproduction. It is practically impossible. Because only the homozygous recessive individuals reveal the phenotype against which artificial selection could act (by sterilization, for example), the allele would persist through heterozygous carriers. For a recessive allele present in 2 of every 100 persons (but homozygous in only 1 in 10,000 persons), it would require 50 generations of complete selection against the homozygotes just to reduce its frequency to one in 100 persons.

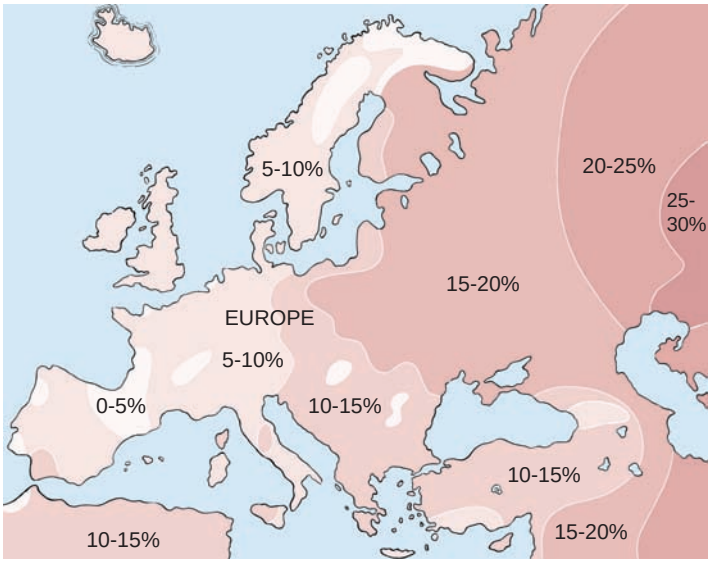


Figure 6.28

Frequencies of the blood-type B allele among humans in Europe. The allele is more common in the east and rarer in the west. The allele may have arisen in the east and gradually diffused westward through the genetic continuity of human populations. This allele has no known selective advantage; its changing frequency probably represents the effects of random genetic drift.

How Genetic Equilibrium Is Upset

Genetic equilibrium is disturbed in natural populations by (1) random genetic drift, (2) nonrandom mating, (3) recurring mutation, (4) migration, (5) natural selection, and interactions among these factors. Recurring mutation is the ultimate source of variability in all populations, but it usually requires interaction with one or more of the other factors to upset genetic equilibrium. We consider these other factors individually.

Genetic Drift

Some species, such as cheetahs (Figure 6.29), contain very little genetic variation, probably because their ancestral lineages

Hardy-Weinberg Equilibrium: Why the Hereditary Process Does Not Change Allelic Frequencies

The Hardy-Weinberg law is a logical consequence of Mendel's first law of segregation and expresses the tendency toward equilibrium inherent in Mendelian heredity.

Let us select for our example a population having a single locus bearing just two alleles *T* and *t*. The phenotypic expression of this gene might be, for example, the ability to taste a chemical compound called phenylthiocarbamide. Individuals in the population will be of three genotypes for this locus, *T/T, T/t* (both tasters), and *t/t* (non-tasters). In a sample of 100 individuals, let us suppose that we have 20 of *T/T* genotype, 40 of *T/t* genotype, and 40 of *t/t* genotype. We could then make a table showing the allelic frequencies (remember that every individual has two copies of the gene):

Genotype	Number of Individuals	Copies of the *T* Allele	Copies of the *t* Allele
T/T	20	40	
T/t	40	40	40
t/t	40		80
Total	100	80	120

Of the 200 copies, the proportion of the *T* allele is 80/200 = 0.4 (40%), and the proportion of the *t* allele is 120/200 = 0.6 (60%). It is customary to use "*p*" and "*q*" to represent the two allelic frequencies. The genetically dominant allele is represented by *p,* and the genetically recessive by *q*. Thus:

$$p = \text{frequency of } T = 0.4$$
$$q = \text{frequency of } t = 0.6$$
$$\text{Therefore } p + q = 1$$

Having calculated allelic frequencies in the sample, let us determine whether these frequencies will change spontaneously in a new generation of the population. Assuming that mating is random (gametes are sampled independently in pairs), each individual will contribute an equal number of gametes to the "common pool" from which the next generation is formed. Frequencies of gametes in the "pool" then will equal the allelic frequencies in the sample: 40% of the gametes will be *T,* and 60% will be *t* (ratio of 0.4:0.6). Both ova and sperm will, of course, show the same frequencies. The next generation is formed:

		Ova	
Sperm		$T = 0.4$	$t = 0.6$
$T = 0.4$		$T/T = 0.16$	$T/t = 0.24$
$t = 0.6$		$T/t = 0.24$	$t/t = 0.36$

Collecting genotypes, we have:

$$\text{frequency of } T/T = 0.16$$
$$\text{frequency of } T/t = 0.48$$
$$\text{frequency of } t/t = 0.36$$

Next, we determine the values of *p* and *q* from the randomly mated populations. From the table above, we see that the frequency of *T* will be the sum of genotypes *T/T,* which is 0.16, and one-half of the genotype *T/t,* which is 0.24:

$$T(p) = 0.16 + .5(0.48) = 0.4$$

Similarly, the frequency of *t* will be the sum of genotypes *t/t,* which is 0.36, and one-half the genotype *T/t,* which is 0.24:

$$t(p) = 0.36 + .5(0.48) = 0.6$$

The new generation bears exactly the same allelic frequencies as the parent population! Note that there has been no increase in the frequency of the genetically dominant allele *T*. Thus, *in a freely interbreeding, sexually reproducing population, the frequency of each allele would remain constant generation after generation in the absence of natural selection, migration, recurring mutation, and genetic drift* (see text). A mathematically minded reader will recognize that the genotype frequencies *T/T, T/t,* and *t/t* are actually a binomial expansion of $(p + q)^2$:

$$(p+q)^2 = p^2 + 2pq + q^2 = 1$$

A statistically minded reader will note that the equilibrium calculations give *expected* frequencies, which are unlikely to be realized exactly in a population of finite size. For this reason, finite population size is a cause of evolutionary change.

passed through periods when the total number of individuals in the population was very small. A small population clearly cannot contain large amounts of genetic variation. Each individual organism has at most two different allelic forms of each gene, and a single breeding pair contains at most four different allelic forms of each gene. Suppose that we have such a breeding pair. We know from Mendelian genetics (see Chapter 5) that chance decides which of the different allelic forms of a gene gets passed to offspring. It is therefore possible by chance alone that one or two of the parental alleles in this example will not be passed to any offspring. It is highly unlikely that the different alleles present in a small ancestral population are all passed to descendants without any change of allelic frequency. This chance fluctuation in allelic frequency from one generation to the next, including loss of alleles from the population, is called **genetic drift.**

Genetic drift occurs to some degree in all populations of finite size. Perfect constancy of allelic frequencies, as predicted by Hardy-Weinberg equilibrium, occurs only in infinitely large populations, and such populations occur only in mathematical models. All populations of animals are finite and therefore experience

Figure 6.29
Cheetahs, a species whose genetic variability has been depleted to very low levels because of small population size in the past.

some effect of genetic drift, which becomes greater, on average, as population size declines. Genetic drift erodes genetic variability of a population. If population size remains small for many generations in a row, genetic variation can be greatly depleted. This loss is harmful to a species's evolutionary success because it restricts potential genetic responses to environmental change. Indeed, biologists are concerned that cheetah populations may have insufficient variation for continued survival.

A large reduction in the size of a population that increases evolutionary change by genetic drift is commonly called a bottleneck. A bottleneck associated with the founding of a new geographic population is called a founder effect and may be associated with formation of a new species (p. 119).

Nonrandom Mating

If mating is nonrandom, genotypic frequencies will deviate from Hardy-Weinberg expectations. For example, if two different alleles of a gene are equally frequent ($p = q = .5$), we expect half of the genotypes to be heterozygous ($2pq = 2[.5]\,[.5] = .5$) and one-quarter to be homozygous for each of the respective alleles ($p^2 = q^2 = [.5]^2 = .25$). If we have **positive assortative mating,** individuals mate preferentially with others of the same genotype, such as albinos mating with other albinos. Matings among individuals homozygous for the same allele generate offspring that are homozygous like themselves. Matings among individuals heterozygous for the same pair of alleles produce on average 50% heterozygous offspring and 50% homozygous offspring (25% of each alternative type) each generation. Positive assortative mating increases the frequency of homozygous genotypes and decreases the frequency of heterozygous genotypes in a population but does not change allelic frequencies.

Preferential mating among close relatives also increases homozygosity and is called **inbreeding.** Whereas positive assortative mating usually affects one or a few traits, inbreeding simultaneously affects all variable traits. Strong inbreeding greatly increases chances that rare recessive alleles will become homozygous and be expressed.

Because inbreeding and genetic drift are both promoted by small population size, they are often confused with each other. Their effects are very different, however. Inbreeding alone cannot change allelic frequencies in the population, only the ways that alleles are combined into genotypes. Genetic drift changes allelic frequencies and consequently also changes genotypic frequencies. Even very large populations have the potential for being highly inbred if there is a behavioral preference for mating with close relatives, although this situation rarely occurs in animals. Genetic drift, however, will be relatively weak in very large populations.

Inbreeding has surfaced as a serious problem in zoos holding small populations of rare mammals. Matings of close relatives tend to bring together genes from a common ancestor and increase the probability that two copies of a deleterious gene will come together in the same organism. The result is "inbreeding depression." Our management solution is to enlarge genetic diversity by bringing together captive animals from different zoos or by introducing new stock from wild populations if possible. Paradoxically, where zoo populations are extremely small and no wild stock can be obtained, deliberate inbreeding is recommended. This procedure selects for genes that tolerate inbreeding; deleterious genes disappear if they kill animals homozygous for them.

Migration

Migration prevents different populations of a species from diverging. If a large species is divided into many small populations, genetic drift and selection acting separately in the different populations can produce evolutionary divergence among them. A small amount of migration in each generation keeps the different populations from becoming too distinct genetically. For example, the French and Russian populations whose ABO allele frequencies were discussed previously show some genetic divergence, but their genetic connection through intervening populations by continuing migration prevents them from becoming completely distinct.

Natural Selection

Natural selection can change both allelic frequencies and genotypic frequencies in a population. Although the effects of selection are often reported for particular polymorphic genes, we must stress that natural selection acts on the whole animal, not on isolated traits. An organism that possesses a superior combination of traits will be favored. An animal may have traits that confer no advantage or even a disadvantage, but it is successful overall if its combination of traits is favorable. When we claim that a genotype at a particular gene has a higher **relative fitness** than others,

we state that on average that genotype confers an advantage in survival and reproduction in the population. If alternative genotypes have unequal probabilities of survival and reproduction, Hardy-Weinberg equilibrium is upset.

Using the genetic theory of natural selection, one can measure relative **fitness** values associated with different genotypes in a population. Geneticists often use *W* to denote the expected average fitness of a genotype in a population, with the genotype of highest fitness given a value of one and fitnesses of other genotypes indicated as fractions.

We illustrate measurement of fitness using genetic variation associated with the disease sickle-cell anemia in human populations. Considering only the alleles for normal hemoglobin (*A*) and sickle-cell hemoglobin (*S*) for the beta-hemoglobin gene in human populations (p. 100), the possible genotypes are *AA*, *AS*, and *SS*. Measurements of viability of individuals of these three genotypes in nonmalarial environments give a fitness value of 1 to genotypes *AA* and *AS* and a fitness of 0.2 to genotype *SS*. People having the *SS* genotype, who are susceptible to severe anemia, are expected to contribute only 20% as many offspring to the next generation on average as are individuals having the *AA* or *AS* genotypes. In malarial environments, genotype *AS* has the highest fitness (=1); genotype *AA* has a slightly decreased fitness (=0.9) because these individuals have a greater incidence of malaria than *AS* individuals, and *SS* has a low fitness (=0.2) because of anemia. From these measured fitness values and knowledge of the frequencies of alleles in a population and its system of mating, one can calculate the **average effect** that an allele has on the phenotype of relative fitness in that population. In the example of sickle-cell anemia, the average effect of allele *S* on fitness in a malarial environment is a balance between the strongly negative effect it has when homozygous and the positive effect that it has when heterozygous with allele *A*.

In Chapter 36, we discuss the related concept of **inclusive fitness**. The average effect of an allele on fitness is expressed not only by its direct contribution to the fitness of its possessors but by aid that its possessors give to close relatives, who are likely also to contain copies of the allele. The term "inclusive fitness" pertains to cases where the average effect of an allele would be calculated incorrectly if only its direct effects on fitness were measured.

Some traits and combinations of traits are advantageous for certain aspects of an organism's survival or reproduction and disadvantageous for others. Darwin used the term **sexual selection** to denote the selection of traits that are advantageous for obtaining mates but not for survival. Bright colors and elaborate feathers can enhance a male bird's competitive ability in obtaining mates while simultaneously increasing his visibility to predators (Figure 6.30). Environmental changes, such as extinction of a predator population, can alter the selective value of different traits. The action of selection on character variation is therefore very complex.

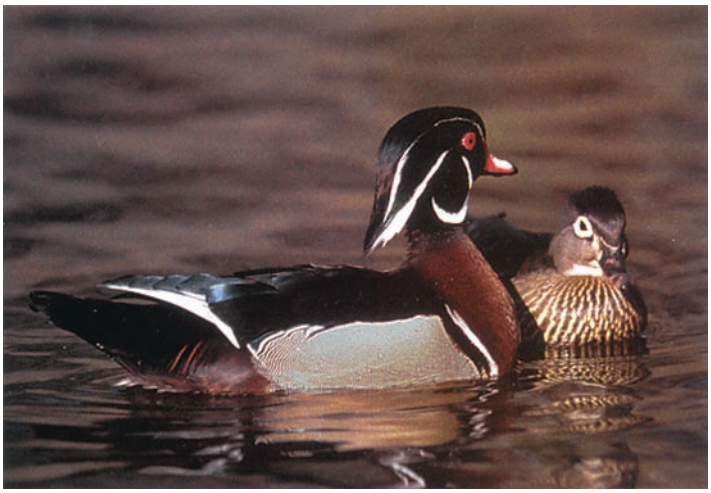


Figure 6.30

A pair of wood ducks. Brightly colored feathers of male birds probably confer no survival advantage and might even be harmful by alerting predators. Such colors nonetheless confer advantage in attracting mates, which overcomes, on average, the negative consequences of these colors for survival. Darwin used the term "sexual selection" to denote evolution of traits that give an individual an advantage in reproduction, even if the traits are neutral or harmful for survival.

Interactions of Selection, Drift, and Migration

Subdivision of a species into small populations that exchange migrants is an optimal situation for promoting rapid adaptive evolution of a species. Interaction of genetic drift and selection in different populations permits many different genetic combinations of many polymorphic genes to be tested against natural selection. Migration among populations permits particularly favorable new genetic combinations to spread throughout the species as a whole. Interaction of selection, genetic drift, and migration in this example produces evolutionary change qualitatively different from what would result if any of these three factors acted alone. Geneticist Sewall Wright called this interaction *shifting balance* because it permits a population to explore different adaptive combinations of variable traits. Natural selection, genetic drift, mutation, nonrandom mating, and migration interact in natural populations to create an enormous opportunity for evolutionary change; perpetual stability, as predicted by Hardy-Weinberg equilibrium, almost never occurs across any significant amount of evolutionary time.

Measuring Genetic Variation Within Populations

How do we measure the genetic variation that occurs in natural populations? Genetic dominance, interactions between alleles of different genes, and environmental effects on a phenotype make it difficult to quantify genetic variation indirectly by observing organismal phenotypes. Variability can be quantified, however, at the molecular level.

Protein Polymorphism

Different allelic forms of genes encode proteins that often differ slightly in their amino acid sequence. This phenomenon is called

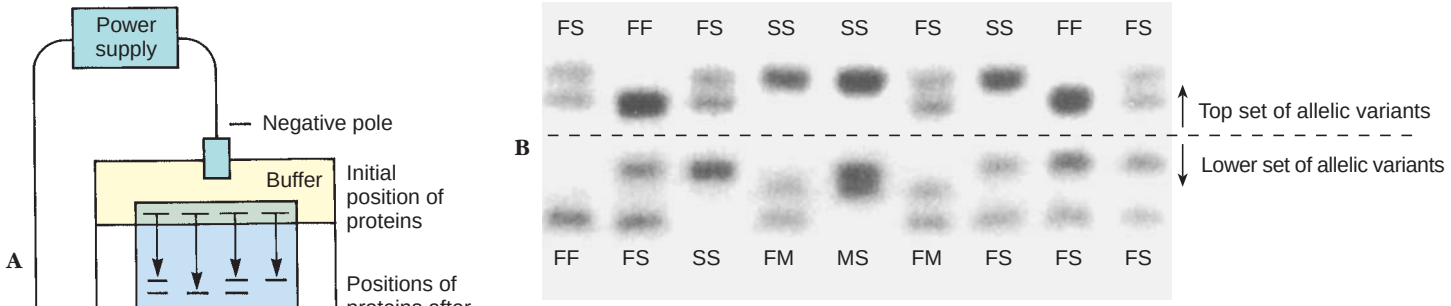


Figure 6.31

Study of genetic variation in proteins using gel electrophoresis. **A,** An electrophoretic apparatus separates allelic variants of proteins that differ in charge because of differences in their sequence of amino acids. **B,** Genetic variation in the protein leucine aminopeptidase for nine brown snails, *Helix aspersa.* Two different sets of allelic variants are revealed. The top set contains two alleles [denoted fast (F) and slow (S) according to their relative movement in the electric field]. Individuals homozygous for the fast allele show only a single fast band on the gel (FF), those homozygous for the slow allele show only a single slow band (SS), and heterozygous individuals have both bands (FS). The lower set contains three different alleles denoted fast (F), medium (M), and slow (S). Note that no individuals shown are homozygous for the medium (M) allele.

protein polymorphism. If these differences affect the protein's net electric charge, the different allelic forms can be separated using protein electrophoresis (Figure 6.31). We can identify the genotypes of particular individuals for protein-coding genes and measure allelic frequencies in a population.

Over the last 40 years, geneticists using this approach have discovered far more variation than was previously expected. Despite the high levels of polymorphism discovered using protein electrophoresis (Table 6.1), these studies underestimate both protein polymorphism and the total genetic variation present in a population. For example, protein polymorphism that does not involve charge differences is not detected. Furthermore, because the genetic code is degenerate (more than one codon for most amino acids, p. 94), protein polymorphism does not reveal all of the genetic variation present in protein-coding genes. Genetic changes that do not alter protein structure sometimes alter patterns of protein synthesis during development and can be very important to an organism. When all kinds of variation are considered, it is evident that most species have an enormous potential for further evolutionary change.

Quantitative Variation

Quantitative traits are those that show continuous variation with no obvious pattern of Mendelian segregation in their inheritance. The values of the trait in offspring often are intermediate between the values in the parents. Such traits are influenced by variation at many genes, each of which follows Mendelian inheritance and contributes a small, incremental amount to the total phenotype. Examples of traits that show quantitative variation include tail length in mice, length of a leg segment in grasshoppers, number of gill rakers in sunfishes, number of peas in pods, and height of adult males of the human species. When trait

TABLE 6.1

Values of Polymorphism (P) and Heterozygosity (H) for Various Animals and Plants as Measured Using Protein Electrophoresis

(a) Species	Number of Proteins	P*	H*
Humans	71	0.28	0.067
Northern elephant seal	24	0.0	0.0
Horseshoe crab	25	0.25	0.057
Elephant	32	0.29	0.089
Drosophila pseudoobscura	24	0.42	0.12
Barley	28	0.30	0.003
Tree frog	27	0.41	0.074
(b) Taxa	**Number of Species**	**P***	**H***
Plants	—	0.31	0.10
Insects (excluding *Drosophila*)	23	0.33	0.074
Drosophila	43	0.43	0.14
Amphibians	13	0.27	0.079
Reptiles	17	0.22	0.047
Birds	7	0.15	0.047
Mammals	46	0.15	0.036
Average		0.27	0.078

Source: Data from P.W. Hedrick, *Population biology.* Jones and Bartlett, Boston, 1984.
***P,** the average number of alleles per gene per species; **H,** the proportion of heterozygous genes per individual.

values are graphed with respect to frequency distribution, they often approximate a normal, or bell-shaped, probability curve (Figure 6.32A). Most individuals fall near the average; fewer fall somewhat above or below the average, and extremes form the "tails" of the frequency curve with increasing rarity. Usually, the larger the population sample, the more closely the frequency distribution resembles a normal curve.

Selection can act on quantitative traits to produce three different kinds of evolutionary response (Figure 6.32B, C, and D). One outcome is to favor average values of the trait and to disfavor extreme ones; this outcome is called **stabilizing selection** (Figure 6.32B). **Directional selection** favors a phenotypic value either above or below the average and causes the population average to shift toward the favored value over time (Figure 6.32C). When we think about natural selection producing evolutionary change, it is usually directional selection that we have in mind, although we must remember that this is not the only possibility. A third alternative is **disruptive selection** in which two different extreme phenotypes are simultaneously favored, but their average is disfavored (Figure 6.32D). The population then becomes bimodal, meaning that two very different phenotypic values predominate.

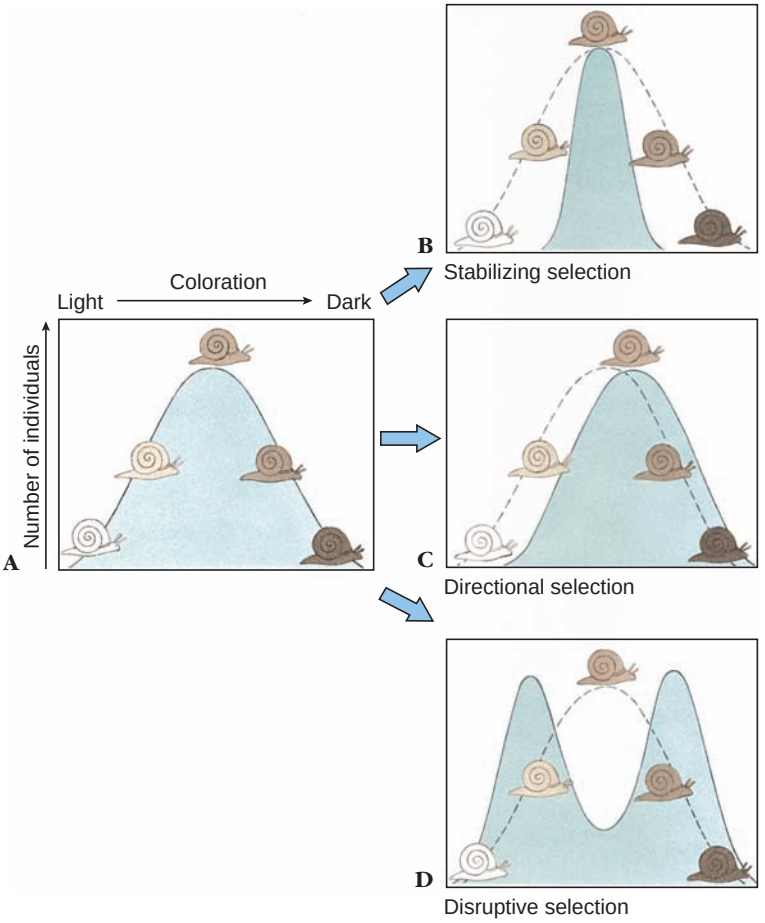


Figure 6.32

Responses to selection on a continuous (polygenic) character, coloration in a snail. **A,** The frequency distribution of coloration before selection. **B,** Stabilizing selection culls extreme variants from the population, in this case eliminating individuals that are unusually light or dark, thereby stabilizing the mean. **C,** Directional selection shifts the population mean, in this case by favoring darkly colored variants. **D,** Disruptive selection favors both extremes but not the mean; the mean is unchanged but the population no longer has a bell-shaped distribution of phenotypes.

MACROEVOLUTION: MAJOR EVOLUTIONARY EVENTS

Macroevolution describes large-scale events in organic evolution. Speciation links macroevolution and microevolution. Major trends in the fossil record (see Figures 6.11, 6.12, and 6.13) are clearly within the realm of macroevolution. Patterns and processes of macroevolutionary change emerge from those of microevolution, but they acquire some degree of autonomy in doing so. The emergence of new adaptations and species, and the varying rates of speciation and extinction observed in the fossil record go beyond the fluctuations of allelic frequencies within populations.

Stephen Jay Gould recognized three different "tiers" of time at which we observe distinct evolutionary processes. The first tier constitutes the timescale of population genetic processes, from tens to thousands of years. The second tier covers millions of years, the scale on which rates of speciation and extinction are measured and compared among different groups of organisms. Punctuated equilibrium is a theory of the second tier, explaining the occurrence of speciation and morphological change and their association over millions of years. The third tier covers tens to hundreds of millions of years, and is marked by occurrence of episodic mass extinctions. In the fossil record of marine organisms, mass extinctions recur at intervals of approximately 26 million years. Five of these mass extinctions have been particularly disastrous (Figure 6.33). The study of long-term changes in animal diversity focuses on the third-tier timescale (see Figures 6.13 and 6.33).

Speciation and Extinction Through Geological Time

Evolutionary change at the second tier provides a new perspective on Darwin's theory of natural selection. Although a species may persist for many millions of years, it ultimately has two possible evolutionary fates: it may give rise to new species or become extinct without leaving descendants. Rates of speciation and extinction vary among lineages, and lineages that have the highest speciation rates and lowest extinction rates produce the greatest number of living species. The characteristics of a species may make it more or less likely than others to undergo speciation or extinction events. Because many characteristics are passed from ancestral to descendant species (analogous to heredity at the organismal level), lineages whose characteristics increase the probability of speciation and confer resistance to extinction should dominate the living world. This species-level process that produces differential rates of speciation and extinction among lineages is analogous in many ways

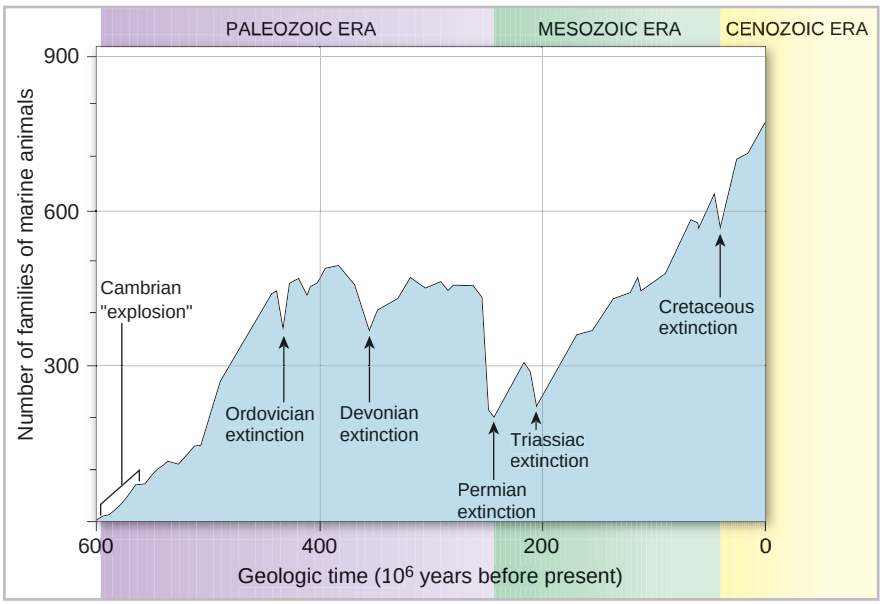


Figure 6.33

Changes in numbers of families of marine animals through time from the Cambrian period to the present. Sharp drops represent five major extinctions of skeletonized marine animals. Note that despite the extinctions, the overall number of marine families has increased to the present.

to natural selection. It represents an expansion of Darwin's theory of natural selection. This expansion is particularly important for macroevolution if one accepts the theory of punctuated equilibrium, which states that the evolutionarily important variation occurs primarily among rather than within species.

Species selection encompasses the differential survival and multiplication of species through geological time based on variation among lineages, especially in emergent, species-level properties. These species-level properties include mating rituals, social structuring, migration patterns, geographic distribution, and all other properties that emerge at the species level (see p. 6). Descendant species usually resemble their ancestors in these properties. For example, a "harem" system of mating in which a single male and several females compose a breeding unit characterizes some mammalian lineages but not others. We expect speciation rates to be enhanced by social systems that promote founding of new populations by small numbers of individuals. Certain social systems may increase the likelihood that a species will survive environmental challenges through cooperative action. Such properties would be favored by species selection over geological time.

Differential speciation and extinction among lineages also can be caused by variation in organismal-level properties (such as specialized versus generalized feeding) rather than species-level properties (see p. 6). Organisms that specialize in eating a restricted range of foods, for example, may be subjected more readily than generalized feeders to geographic isolation among populations, because areas where their preferred food is scarce or absent will function as geographic barriers to dispersal. Such geographic isolation could generate more frequent opportunities for speciation to occur throughout geological time. The fossil records of two major groups of African antelopes suggest this result (Figure 6.11). A group of specialized grazers that contains blesboks, hartebeests, and wildebeests shows high speciation and extinction rates. Since the late Miocene, 33 extinct and 7 living species are known, representing at least 18 events of branching speciation and 12 terminal extinctions. In contrast, a group of generalist grazers and browsers that contains impalas shows neither branching speciation nor terminal extinction during this same interval of time. Interestingly, although these two lineages differ greatly in speciation rates, extinction rates, and species diversity, they do not differ significantly in total number of individual animals alive today.

Paleontologist Elisabeth Vrba, whose research produced the results in Figure 6.11, uses the term **effect macroevolution** to describe differential speciation and extinction rates among lineages caused by organismal-level properties. She reserves the term species selection for cases where species-level emergent properties are of primary importance. Some other evolutionary paleontologists consider effect macroevolution a subset of species selection because fitness differences occur among different species lineages rather than among varying organisms within species.

Mass Extinctions

When we study evolutionary change on an even larger timescale, we observe episodic events in which large numbers of taxa go extinct simultaneously. These events are called **mass extinctions** (see Figure 6.33). The most cataclysmic of these extinction episodes happened about 225 million years ago, when at least half of the families of shallow-water marine invertebrates, and fully 90% of marine invertebrate species disappeared within a few million years. This event was the **Permian extinction.** The **Cretaceous extinction,** which occurred about 65 million years ago, marked the end of dinosaurs, as well as numerous marine invertebrates and many small reptilian taxa.

Causes of mass extinctions and evolutionary timing of mass extinctions at intervals of approximately 26 million years are difficult to explain. Some people have proposed biological explanations for these episodic mass extinctions and others consider many mass extinctions artifacts of our statistical and taxonomic analyses. Walter Alvarez proposed that the earth was periodically bombarded by asteroids, causing these mass extinctions (Figure 6.34). The drastic effects of such bombardment of a planet were observed in July 1994 when fragments of Comet Shoemaker-Levy 9 bombarded Jupiter. The first fragment to hit Jupiter was estimated to have the force of 10 million hydrogen bombs. Twenty additional fragments hit Jupiter within the following week, one of which was 25 times more powerful than the first fragment. This bombardment was the most violent event in the recorded history of the solar system. A similar bombardment on earth would send

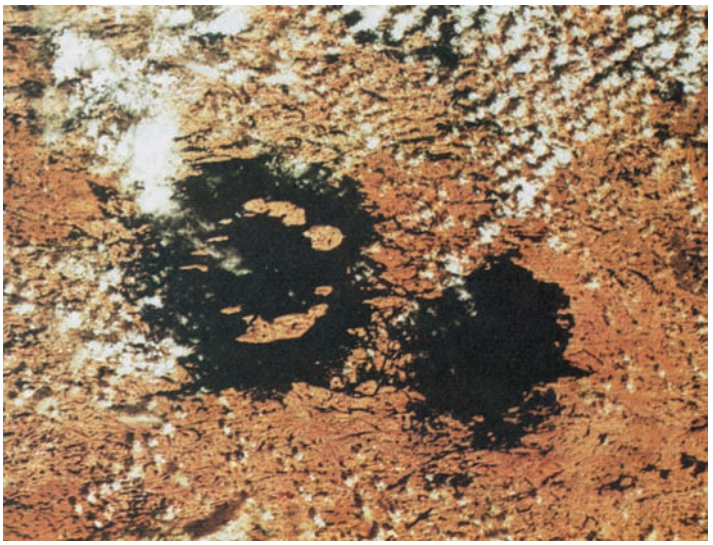


Figure 6.34

Twin craters of Clearwater Lakes in Canada show that multiple impacts on the earth are not as unlikely as they might seem. Evidence suggests that at least two impacts within a short time were responsible for the Cretaceous mass extinction.

debris into the atmosphere, blocking sunlight and causing drastic changes of climate. Temperature changes would challenge ecological tolerances of many species. Alvarez's hypothesis is being tested in several ways, including a search for impact craters left by asteroids and for altered mineral content of rock strata where mass extinctions occurred. Atypical concentrations of the rare-earth element iridium in strata at the Cretaceous-Tertiary boundary imply that this element entered the earth's atmosphere through asteroid bombardment.

Sometimes, lineages favored by species selection are unusually susceptible to mass extinction. Climatic changes produced by the hypothesized asteroid bombardments could produce selective challenges very different from those encountered at other times in the earth's history. Selective discrimination of particular biological traits by events of mass extinction is termed **catastrophic species selection.** For example, mammals survived the end-Cretaceous mass extinction that destroyed the dinosaurs and other prominent vertebrate and invertebrate groups. Following this event, mammals were able to use environmental resources that previously had been denied them, leading to their adaptive radiation.

Natural selection, species selection, and catastrophic species selection interact to produce the macroevolutionary trends seen in the fossil record. Studies of these interacting causal processes have made modern evolutionary paleontology an active and exciting field.

SUMMARY

Organic evolution explains the diversity of living organisms as the historical outcome of gradual change from previously existing forms. Evolutionary theory is strongly identified with Charles Robert Darwin, who presented the first credible explanation for evolutionary change. Darwin derived much of the material used to construct his theory from his experiences on a five-year voyage around the world aboard the H.M.S. *Beagle.*

Darwin's evolutionary theory has five major components. Its most basic proposition is *perpetual change,* the theory that the world is neither constant nor perpetually cycling but is steadily undergoing irreversible change. The fossil record amply demonstrates perpetual change in the continuing fluctuation of animal form and diversity following the Cambrian explosion 600 million years ago. Darwin's theory of *common descent* states that all organisms descend from a common ancestor through a branching of genealogical lineages. This theory explains morphological homologies among organisms as characteristics inherited with modification from a corresponding feature in their common evolutionary ancestor. Patterns of homology formed by common descent with modification permit us to classify organisms according to their evolutionary relationships.

Changes in the timing of developmental processes, termed heterochrony, and changes in their physical location within the body plan, termed heterotopy, explain the evolution of new morphological homologies. A developmental evolutionary module is a set of developmental processes and associated genes that can be expressed as a unit at different parts of the body to produce different structures with some shared developmental properties. Evolution of limbs in terrestrial vertebrates occurred by expressing at the limb bud a set of developmental processes that evolved initially to construct part of the vertebral column. Evolvability denotes the potential of a lineage to evolve new morphological features by using a set of developmental modules as an evolutionary toolkit.

A corollary of common descent is the *multiplication of species* through evolutionary time. Allopatric speciation denotes the evolution of reproductive barriers between geographically separated populations to generate new species. In some animals, especially parasitic insects that specialize on different host species, speciation may occur without geographical isolation, which is called sympatric speciation. Intermediate between allopatric speciation and sympatric speciation is a third mode, parapatric speciation, in which an environmental change splits a species into two environmentally distinct parts that maintain contact along a geographic borderline as they diverge to become separate species.

Adaptive radiation is the proliferation of many adaptively diverse species from a single ancestral lineage within a relatively short period of evolutionary time, such as a few million years. Oceanic archipelagoes, such as the Galápagos Islands, are particularly conducive to adaptive radiation of terrestrial organisms.

Darwin's theory of *gradualism* states that large phenotypic differences between species are produced by accumulation through evolutionary time of many individually small changes. Gradualism

is still controversial. Mutations that have large effects on an organism have been useful in animal breeding, leading some to dispute Darwin's claim that such mutations are not important in evolution. On a macroevolutionary perspective, punctuated equilibrium states that most evolutionary change occurs in relatively brief events of branching speciation, separated by long intervals in which little phenotypic change accumulates.

Darwin's fifth major statement is that *natural selection* is the guiding force of evolution. This principle is founded on observations that all species overproduce their kind, causing a struggle for the limited resources that support existence. Because no two organisms are exactly alike, and because variable traits are at least partially heritable, those organisms whose hereditary endowment enhances their use of resources for survival and reproduction contribute disproportionately to the next generation. Over many generations, the sorting of variation by selection produces new species and new adaptations.

Mutations are the ultimate source of all new variation on which selection acts. Darwin's theory emphasizes that variation is produced at random with respect to an organism's needs and that differential survival and reproduction provide the direction for evolutionary change. Darwin's theory of natural selection was modified around 1900 and in subsequent decades by correction of his genetic errors. This modified theory is called neo-Darwinism.

Population geneticists discovered the principles by which genetic properties of populations change through time. A particularly important discovery, known as Hardy-Weinberg equilibrium, showed that the hereditary process itself does not change the genetic composition of populations. Important sources of evolutionary change include mutation, genetic drift, nonrandom mating, migration, natural selection, and their interactions.

Neo-Darwinism, as elaborated by population genetics, formed the basis for the Synthetic Theory of the 1930s and 1940s. Genetics, natural history, paleobiology, and systematics were unified by the common goal of expanding our knowledge of Darwinian evolution. Microevolution comprises studies of genetic change within contemporary populations. These studies show that most natural populations contain enormous amounts of variation. Macroevolution comprises studies of evolutionary change on a geological timescale. Macroevolutionary studies measure rates of speciation, extinction, and changes of diversity through time. These studies have expanded Darwinian evolutionary theory to include higher-level processes that regulate rates of speciation and extinction among lineages, including species selection and catastrophic species selection.

REVIEW QUESTIONS

1. Briefly summarize Lamarck's concept of the evolutionary process. What is wrong with this concept?
2. What is "uniformitarianism"? How did it influence Darwin's evolutionary theory?
3. Why was the *Beagle*'s journey so important to Darwin's thinking?
4. What was the key idea contained in Malthus's essay on populations that was to help Darwin formulate his theory of natural selection?
5. Explain how each of the following contributes to Darwin's evolutionary theory: fossils; geographic distributions of closely related animals; homology; animal classification.
6. How do modern evolutionists view the relationship between ontogeny and phylogeny? Explain how the observation of paedomorphosis conflicts with Haeckel's "biogenetic law."
7. What are the important differences between the vicariant and founder-event modes of allopatric speciation?
8. What are reproductive barriers? How do premating and postmating barriers differ?
9. Under what conditions is sympatric speciation proposed?
10. What is the main evolutionary lesson provided by Darwin's finches on the Galápagos Islands?
11. How is the observation of "sporting mutations" in animal breeding used to challenge Darwin's theory of gradualism? Why did Darwin reject such mutations as having little evolutionary importance?
12. What does the theory of punctuated equilibrium state about the occurrence of speciation throughout geological time? What observation led to this theory?
13. Describe the observations and inferences that compose Darwin's theory of natural selection.
14. Identify the random and nonrandom components of Darwin's theory of natural selection.
15. Describe some recurring criticisms of Darwin's theory of natural selection. How can these criticisms be refuted?
16. It is a common but mistaken belief that because some alleles are dominant and others are recessive, the dominants will eventually replace all the recessives in a population. How does the Hardy-Weinberg equilibrium refute this notion?
17. Assume that you are sampling a trait in animal populations; the trait is controlled by a single allelic pair *A* and *a,* and you can distinguish all three phenotypes *AA, Aa,* and *aa* (intermediate inheritance). Your sample includes:

Population	***AA***	***Aa***	***aa***	**TOTAL**
I	300	500	200	1000
II	400	400	200	1000

Calculate the distribution of phenotypes in each population as expected under Hardy-Weinberg equilibrium. Is population I in equilibrium? Is population II in equilibrium?

18. If after studying a population for a trait determined by a single pair of alleles you find that the population is not in equilibrium, what possible reasons might explain the lack of equilibrium?
19. Explain why genetic drift is more powerful in small populations.
20. Describe how the effects of genetic drift and natural selection can interact in a subdivided species.
21. Is it easier for selection to remove a deleterious recessive allele from a randomly mating population or a highly inbred population? Why?
22. Distinguish between microevolution and macroevolution, and describe some evolutionary processes evident only at the macroevolutionary level.

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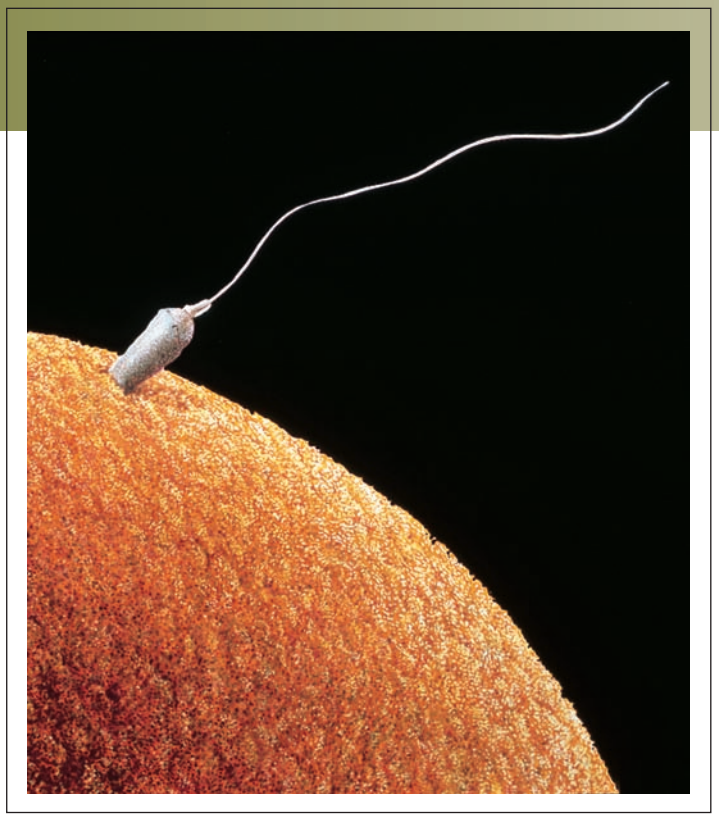
CHAPTER

7

The Reproductive Process



Human egg and sperm at the moment of fertilization.

"Omne vivum ex ovo"

In 1651, late in a long life, William Harvey, the English physiologist who earlier had founded experimental physiology by explaining the circuit of blood, published a treatise on reproduction. He asserted that all life developed from the egg—*omne vivum ex ovo*. This was insightful, since Harvey had no means for visualizing eggs of many animals, in particular the microscopic mammalian egg, which is no larger than a speck of dust to the unaided eye. Further, argued Harvey, eggs are launched into their developmental course by some influence from semen, a conclusion that was either remarkably perceptive or a lucky guess, since sperm also were invisible to Harvey. Such ideas differed sharply from existing notions of biogenesis, which saw life springing from many sources of which eggs were but one. Harvey was describing characteristics of sexual reproduction in which two parents, male and female, must produce gametes that fuse to become a new individual.

Despite the importance of Harvey's aphorism that all life arises from eggs, it was not wholly correct. Life springs from reproduction of preexisting life, and reproduction may not be restricted to eggs and sperm. Asexual reproduction, the creation of new, genetically identical individuals by budding or fragmentation or fission from a single parent, is common, indeed characteristic, among some phyla. Nevertheless, most animals have found sex the winning strategy, probably because sexual reproduction promotes diversity, enhancing long-term survival of the lineage in a world of perpetual change.

Reproduction is one of the ubiquitous properties of life. Evolution is inextricably linked to reproduction, because the ceaseless replacement of aging predecessors with new life gives animal populations the means to adapt to a changing environment. In this chapter we distinguish asexual and sexual reproduction and explore the reasons why, for multicellular animals at least, sexual reproduction appears to offer important advantages over asexual. We then consider, in turn, the origin and maturation of germ cells; plan of reproductive systems; reproductive patterns in animals; and, finally, the endocrine events that orchestrate reproduction.

NATURE OF THE REPRODUCTIVE PROCESS

Two modes of reproduction are recognized: asexual and sexual. In **asexual** reproduction (Figure 7.1A and B) there is only one parent and with no special reproductive organs or cells. Each organism is capable of producing genetically identical copies of itself as soon as it becomes an adult. The production of copies is marvelously simple, direct, and typically rapid. **Sexual** reproduction (Figure 7.1C and D) as a rule involves two parents, each of which contributes special **germ cells** (**gametes** or **sex cells**) that in union (fertilization) develop into a new individual. The **zygote** formed from this union receives genetic material from both parents, and the combination of genes (p. 90) produces a genetically unique individual, bearing characteristics of the species but also bearing traits that make it different from its parents. Sexual reproduction, by recombining parental characters, multiplies variations and makes possible a richer and more diversified evolution.

Mechanisms for interchange of genes between individuals are more limited in organisms with only asexual reproduction. Of course, in asexual organisms that are haploid (bear only one set of genes, p. 77), mutations are immediately expressed and evolution proceeds quickly. In sexual animals, on the other hand, a gene mutation is often not expressed immediately, since it may be masked by its normal partner on the homologous chromosome. (Homologous chromosomes, discussed on p. 77, are those that pair during meiosis and carry genes encoding the same characteristics.) There is only a remote chance that both members of a gene pair will mutate in the same way at the same moment and thus be expressed immediately.

Asexual Reproduction: Reproduction without Gametes

Asexual reproduction (Figure 7.1A and B) is the production of individuals without gametes (eggs or sperm). It includes a number of distinct processes, all without involving sex or a second parent. Offspring produced by asexual reproduction all have the same genotype (unless mutations occur) and are called **clones.**

Asexual reproduction appears in bacteria and unicellular eukaryotes and in many invertebrate phyla, such as cnidarians, bryozoans, annelids, echinoderms, and hemichordates. In

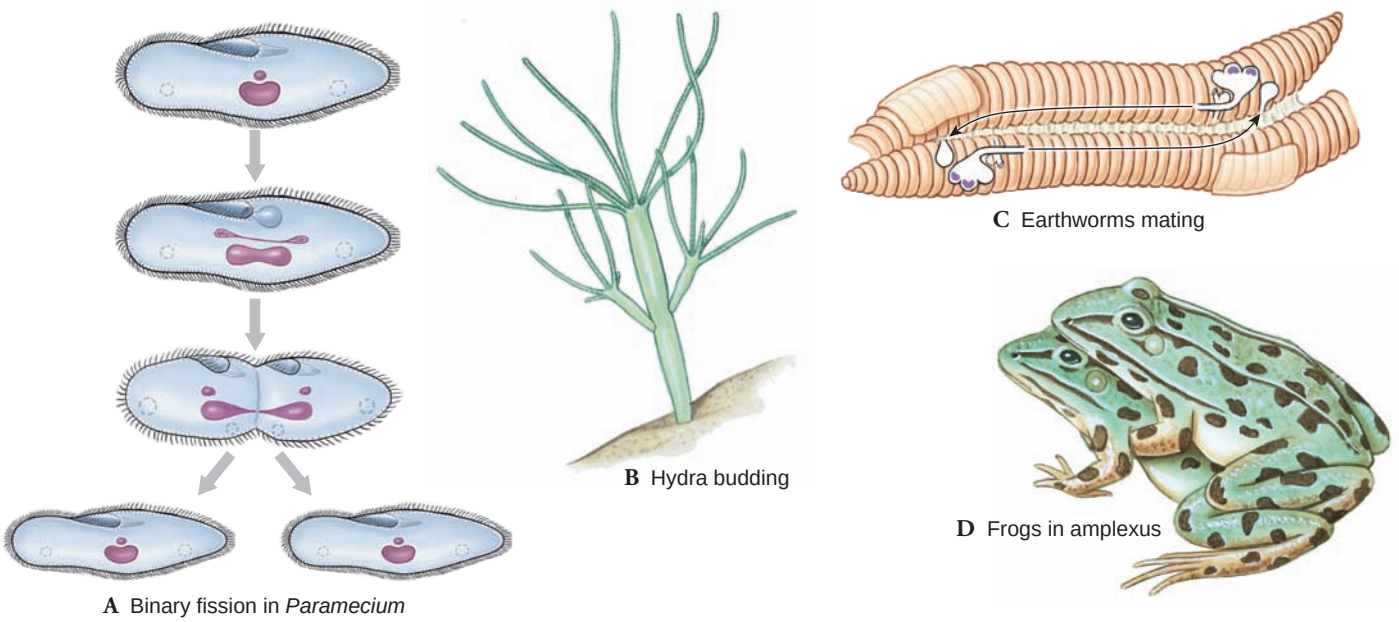


Figure 7.1

Examples of asexual and sexual reproduction in animals. **A,** Binary fission in *Paramecium,* a single-celled eukaryote, results in two individuals. **B,** Budding, a simple form of asexual reproduction as shown in a hydra, a radiate animal. The buds, shown growing out of the central, parent hydra, eventually detach themselves and grow into fully formed individuals. **C,** Earthworms reproduce sexually, but are hermaphroditic, with each individual bearing both male and female organs. Each earthworm passes sperm from genital pores along grooves to seminal receptacles of its mate. **D,** Frogs, here in mating position (amplexus), represent bisexual reproduction, the most common form of sexual reproduction involving separate male and female individuals.

animal phyla in which asexual reproduction occurs, most members also employ sexual reproduction. In these groups, asexual reproduction ensures rapid increase in numbers when development and differentiation of the organism has not advanced to the point of forming gametes. Asexual reproduction is absent among vertebrates (although some forms of parthenogenesis have been interpreted as asexual by some authors; see p. 140).

It would be a mistake to conclude that asexual reproduction is in any way a "defective" form of reproduction relegated to the minute forms of life. Given the facts of their abundance, that they have persisted on earth for 3.5 billion years, and that they form the base of the food chain on which all higher forms depend, single-celled asexual organisms are both resoundingly abundant and supremely important. For these forms the advantages of asexual reproduction are its rapidity (many bacteria divide every half hour) and simplicity (no germ cells to produce and no time and energy expended in finding a mate).

The basic forms of asexual reproduction are fission (binary and multiple), budding, gemmulation, and fragmentation.

Binary fission is common among bacteria and protozoa (Figure 7.1A). In binary fission the body of the unicellular parent divides by mitosis (p. 52) into two approximately equal parts, each of which grows into an individual similar to the parent. Binary fission may be lengthwise, as in flagellate protozoa, or transverse, as in ciliate protozoa. In **multiple fission,** or **schizogony,** the nucleus divides repeatedly before division of the cytoplasm, producing many daughter cells simultaneously. Spore formation, called sporogony, is a form of multiple fission common among some parasitic protozoa, for example, malarial parasites.

Budding is an unequal division of an organism. A new individual arises as an outgrowth (bud) from its parent, develops organs like those of the parent, and then detaches itself. Budding occurs in several animal phyla and is especially prominent in cnidarians (Figure 7.1B).

Gemmulation is the formation of a new individual from an aggregation of cells surrounded by a resistant capsule, called a gemmule. In many freshwater sponges, gemmules develop in the fall and survive the winter in the dried or frozen body of the parent. In spring, the enclosed cells become active, emerge from the capsule, and grow into a new sponge.

In **fragmentation** a multicellular animal breaks into two or more parts, with each fragment capable of becoming a complete individual. Many invertebrates can reproduce asexually by simply breaking into two parts and then regenerating the missing parts of the fragments, for example, most anemones and many hydroids. Many echinoderms can regenerate lost parts, but this is not the same as reproduction by fragmentation.

Sexual Reproduction: Reproduction with Gametes

Sexual reproduction is the production of individuals from gametes. It includes **bisexual** (or **biparental**) reproduction as the most common form, involving two separate individuals. **Hermaphroditism** and **parthenogenesis** are less common forms of sexual reproduction.

Bisexual Reproduction

Bisexual reproduction is the *production of offspring formed by the union of gametes from two genetically different parents* (Figures 7.1C and D, and 7.2). Offspring will thus have a new genotype different from either parent. Individuals sharing parenthood are characteristically of different **sexes,** male and female (there are exceptions among sexually reproducing organisms, such as bacteria and some protozoa in which sexes are lacking). Each has its own reproductive system and produces only one kind of germ cell, spermatozoon or ovum, rarely both. Nearly all vertebrates and many invertebrates have separate sexes, and such a condition is called **dioecious** (Gr. *di,* two, + *oikos,* house). Individual animals that have both male and female reproductive organs are called **monoecious** (Gr. *monos,* single, + *oikos,* house). These animals are called **hermaphrodites** (from a combination of the names of the Greek god Hermes and goddess Aphrodite); this form of reproduction is described on page 140.

Distinctions between male and female are based, not on any differences in parental size or appearance, but on the size and mobility of the gametes they produce. The **ovum** (egg), produced by the female, is large (because of stored yolk to sustain early development), nonmotile, and produced in relatively small numbers. The **spermatozoon** (sperm), produced by the male, is small, motile, and produced in enormous numbers. Each sperm is a stripped-down package of highly condensed genetic material designed for the single purpose of reaching and fertilizing an egg.

There is another crucial event that distinguishes sexual from asexual reproduction: **meiosis,** a distinctive type of gamete-producing nuclear division (described in detail on p. 77). Meiosis differs from ordinary cell division (mitosis) in being a double division. Chromosomes split once, but the cell divides *twice,* producing four cells, each with half the original number of chromosomes (the **haploid** number). Meiosis is followed by **fertilization** in which two haploid gametes are combined to restore the normal (**diploid**) chromosomal number of the species.

The new cell (zygote), which now begins to divide by mitosis (described on p. 52), has equal numbers of chromosomes from each parent and is a unique individual bearing a recombination of parental characteristics. Genetic recombination is the great strength of sexual reproduction that keeps feeding new genetic combinations into the population.

Many unicellular organisms reproduce both sexually and asexually. When sexual reproduction does occur, it may or may not involve male and female gametes. Sometimes two mature sexual parent cells join together to exchange nuclear material or merge cytoplasm (**conjugation,** p. 232 in Chapter 11). Distinct sexes do not exist in these cases.

The male-female distinction is more clearly evident in most animals. Organs that produce germ cells are called **gonads.** The gonad that produces sperm is a **testis** (see Figure 7.12) and

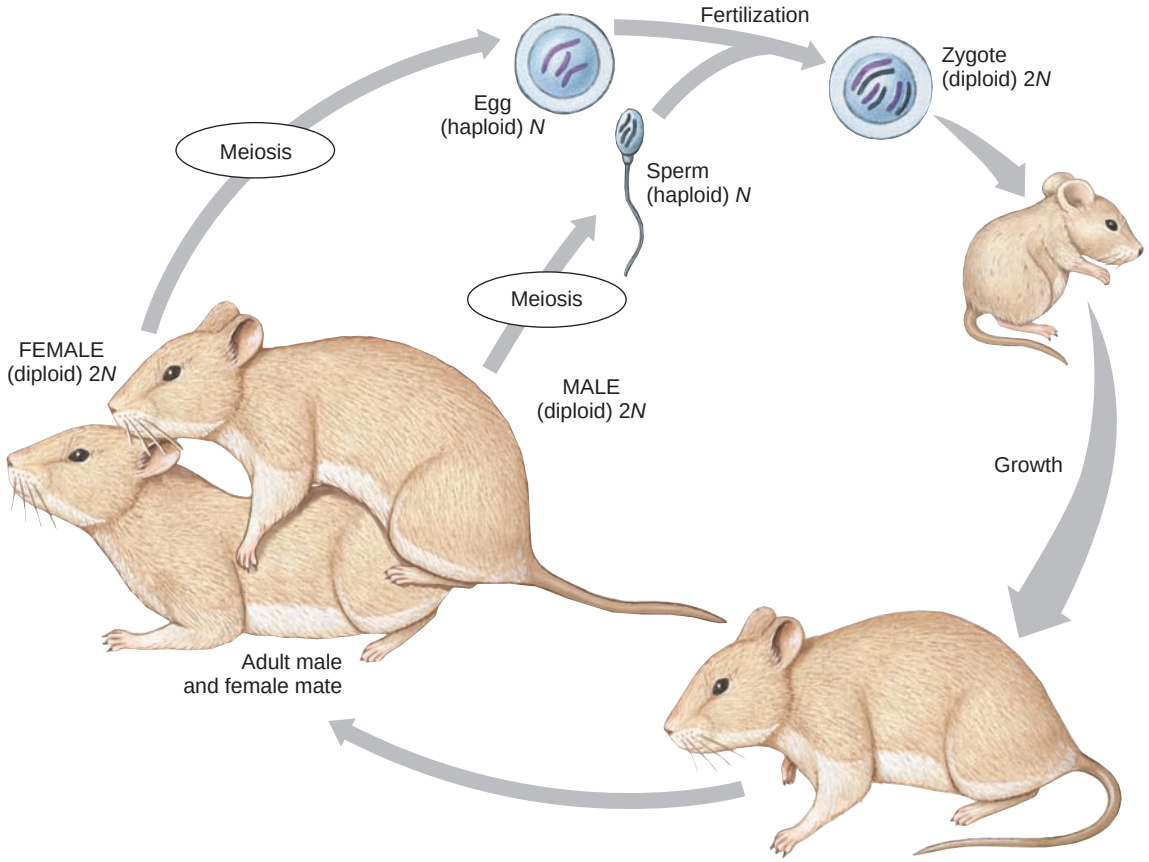


Figure 7.2

A sexual life cycle. The life cycle begins with haploid germ cells, formed by meiosis, combining to form a diploid zygote, which grows by mitosis to an adult. Most of the life cycle is spent as a diploid organism.

that which forms eggs is an **ovary** (see Figure 7.13). Gonads represent the **primary sex organs,** the only sex organs found in certain groups of animals. Most metazoa, however, have various **accessory sex organs** (such as penis, vagina, uterine tubes, and uterus) that transfer and receive germ cells. In the primary sex organs germ cells undergo many complicated changes during their development, the details of which are described on pages 143–146.

Hermaphroditism

Animals that have both male and female organs in the same individual are called **hermaphrodites,** and the condition is called **hermaphroditism.** In contrast to the dioecious state of separate sexes, hermaphrodites are **monoecious,** meaning that the same organism bears both male and female organs. Many sessile, burrowing, or endoparasitic invertebrate animals are hermaphroditic (for example, most flatworms, some hydroids and annelids, and all barnacles and pulmonate snails), as well as a few vertebrates (some fishes). Some hermaphrodites fertilize themselves, but most avoid self-fertilization by exchanging germ cells with another member of the same species (Figures 7.1C and 7.3). An advantage is that with every individual producing eggs, a hermaphroditic species could potentially produce twice as many offspring as could a dioecious species in which half the individuals are nonproductive males. Some fishes are **sequential hermaphrodites,** in which a genetically programmed sex change occurs within an individual organism. In many species of reef fishes, for example, wrasses, an animal begins life as either a female or a male (depending on the species) but later becomes the opposite sex.

Parthenogenesis

Parthenogenesis ("virgin origin") is the development of an embryo from an unfertilized egg or one in which the male and female nuclei fail to unite following fertilization. There are many patterns of parthenogenesis. In one type, called **ameiotic parthenogenesis,** no meiosis occurs, and the egg is formed by mitotic cell division. This "asexual" form of parthenogenesis occurs in some species of flatworms, rotifers, crustaceans, insects, and probably others. In these cases, the offspring are clones of the parent because, without meiosis, the parent's chromosomal complement is passed intact to offspring.

In **meiotic parthenogenesis** a haploid ovum is formed by meiosis, and it may or may not be activated by the influence of

Figure 7.3
Hermaphroditic earthworms mating. Earthworms are "simultaneous" hermaphrodites; during mating each partner passes sperm from genital pores along grooves to seminal receptacles of its mate. They are held together by mucous secretions during this process.

a male's sperm. For example, in some species of fishes, a female may be inseminated by a male of the same or related species, but the sperm serves only to activate the egg; the male's genetic material is rejected before it can penetrate the egg (**gynogenesis**). In several species of flatworms, rotifers, annelids, mites, and insects, the haploid egg begins development spontaneously; no males are required to stimulate activation of an ovum. The diploid condition may be restored by chromosomal duplication or by autogamy (rejoining of haploid nuclei). A variant of this type of parthenogenesis occurs in many bees, wasps, and ants. In honey bees, for example, the queen bee can either fertilize eggs as she lays them or allow them to pass unfertilized. Fertilized eggs become diploid females (queens or workers), and unfertilized eggs develop parthenogenetically to become haploid males (drones); this type of sex determination is known as **haplodiploidy.** In some animals meiosis may be so severely modified that offspring are clones of the parent. Certain populations of whiptail lizards of the American southwest are clones consisting solely of females (Cole, 1984).

Parthenogenesis is surprisingly widespread in animals. It is an abbreviation of the usual steps of bisexual reproduction. It may have evolved to avoid the problem—which may be great in some animals—of bringing together males and females at the right moment for successful fertilization. The disadvantage of parthenogenesis is that if the environment should suddenly change parthenogenetic species have limited capacity to shift gene combinations to adapt to any new conditions. Bisexual species, by recombining parental characteristics, have a better chance of producing variant offspring that can utilize new environments.

Occasionally claims arise that spontaneous parthenogenetic development to term has occurred in humans. A British investigation of about 100 cases in which the mother denied having had intercourse revealed that in nearly every case the child possessed characteristics not present in the mother, and consequently must have had a father. Nevertheless, mammalian eggs very rarely spontaneously start developing into embryos without fertilization. In certain strains of mice, such embryos will develop into fetuses and then die. The most remarkable instance of parthenogenetic development among vertebrates occurs in turkeys in which ova of certain strains, selected for their ability to develop without sperm, grow to reproducing adults.

Why Do So Many Animals Reproduce Sexually Rather Than Asexually?

Because sexual reproduction is so nearly universal among animals, it might be inferred to be highly advantageous. Yet it is easier to list disadvantages to sex than advantages. Sexual reproduction is complicated, requires more time, and uses much more energy than asexual reproduction. Males may waste valuable energy in competition for a mate and often possess sexual characteristics that can be detrimental to survival—for example, the elongated tail feathers of peacocks. Mating partners must come together, and this can be a disadvantage in sparsely populated areas for some species. Males and females must also coordinate their activities to produce young. Many biologists believe that an even more troublesome problem is the "cost of meiosis." A female that reproduces asexually passes all of her genes to her offspring, but when she reproduces sexually the genome is divided during meiosis and only half her genes flow to the next generation. Another cost is wastage in production of males, many of which fail to reproduce and thus consume resources that could be applied to production of females. Whiptail lizards of the American southwest offer a fascinating example of the potential advantage of parthenogenesis (discussed on p. 140). When unisexual and bisexual species of the same genus are reared under similar conditions in the laboratory, the population of the unisexual species grows more quickly because all unisexual lizards (all females) deposit eggs, whereas only 50% of the bisexual lizards do so (Figure 7.4).

Clearly, the costs of sexual reproduction are substantial. How are they offset? Biologists have disputed this question for years. One hypothesis suggests that sexual reproduction, with its separation and recombination of genetic material, keeps producing novel genotypes that *in times of environmental change* may be advantageous to survival and thus the organism may live to reproduce, whereas most others die. An often quoted example is the rapidly changing environment of organisms that is produced by parasites continuously evolving new mechanisms of attack and therefore favoring recombination of their hosts. Variability, advocates of this viewpoint argue, is sexual reproduction's trump card. Another hypothesis suggests that sexual recombination provides a means for the spread of beneficial mutations without a population being held back by deleterious ones. Experimental support for this hypothesis has recently been provided using the fruit fly, *Drosophila,* in which beneficial mutations increased to a greater degree in sexual populations compared with clonal (asexual) ones. These hypotheses are not mutually exclusive,

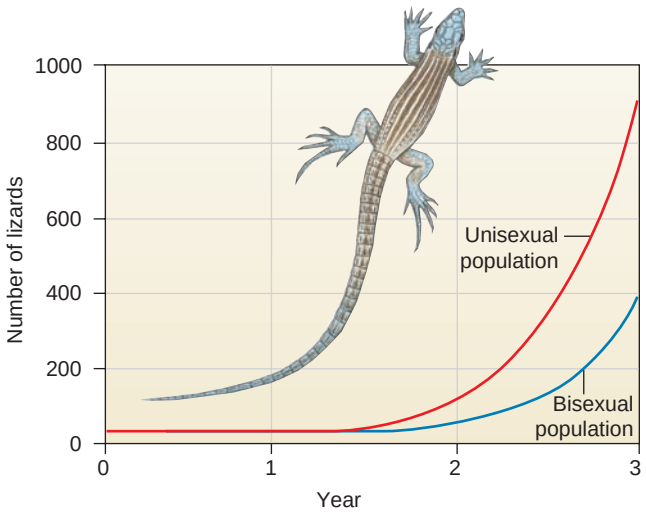


Figure 7.4

Comparison of the growth of a population of unisexual whiptail lizards with a population of bisexual lizards. Because all individuals of the unisexual population are females, all produce eggs, whereas only half the bisexual population are egg-producing females. By the end of the third year the unisexual lizards are more than twice as numerous as the bisexual ones.

however, and both provide possible explanations for the evolution of sexual reproduction.

There still remains the question of why sexual reproduction has been maintained in spite of its costs. Considerable evidence suggests that asexual reproduction is most successful in colonizing new environments. When habitats are empty what matters most is rapid reproduction; variability and increased fitness provided by beneficial genetic recombination matter little. As habitats become more crowded, competition between species for resources increases. Selection becomes more intense, and genetic variability—new beneficial genotypes produced by recombination in sexual reproduction—furnishes the diversity that permits a population to resist extinction. Therefore, on a geological timescale, asexual lineages, because they lack genetic flexibility, may be more prone to extinction than sexual lineages. Sexual reproduction is therefore favored by species selection (species selection is described on p. 133). There are many invertebrates that use both sexual and asexual reproduction, thus enjoying the advantages each has to offer.

THE ORIGIN AND MATURATION OF GERM CELLS

Many sexually reproducing organisms are composed of nonreproductive **somatic cells,** which are differentiated for specialized functions and die with the individual, and **germ cells,** which form the gametes: eggs and sperm. Germ cells provide continuity of life between generations, the **germ cell line.** Germ cells, or their precursors, the **primordial germ cells,** develop at the beginning of embryonic development (described in Chapter 8), usually in the endoderm, and migrate to the gonads. Here they develop into eggs or sperm. The other cells of the gonads are somatic cells. They cannot form eggs or sperm, but they are necessary for support, protection, and nourishment of the germ cells during their development **(gametogenesis).**

A traceable germ cell line, as present in vertebrates, is also distinguishable in some invertebrates, such as nematodes and arthropods. In many invertebrates, however, germ cells develop directly from somatic cells at some period in the life of an individual.

Migration of Germ Cells

In vertebrates, the actual tissue from which gonads arise appears in early development as a pair of **genital ridges,** growing into the coelom from the dorsal coelomic lining on each side of the hindgut near the anterior end of the kidney (mesonephros).

Surprisingly perhaps, primordial germ cells do not arise in the developing gonad, but in the yolk-sac endoderm (p. 175). From studies with frogs and toads, it has been possible to trace the germ cell line back to the fertilized egg, in which a localized area of germinal cytoplasm (called **germ plasm**) can be identified in the vegetal pole of the uncleaved egg mass. This material can be followed through subsequent cell divisions of the embryo until it becomes situated in primordial germ cells in gut endoderm. From here the cells migrate by ameboid movement to the genital ridges, located on either side of the hindgut. A similar migration of primordial germ cells occurs in mammals (Figure 7.5). Primordial germ cells are the future stock of gametes for an animal. Once in the genital ridges and during subsequent gonadal development, germ cells begin to divide by mitosis, increasing their numbers from a few dozen to several thousand.

Sex Determination

At first gonads are sexually indifferent. In normal human males, a "male-determining gene" on the Y chromosome called ***SRY (sex-determining region Y)*** organizes the developing gonad into a testis instead of an ovary. Once formed, the testis secretes the steroid **testosterone.** This hormone, and its metabolite, **dihydrotestosterone (DHT),** masculinizes the fetus, causing the differentiation of penis, scrotum, and the male ducts and glands. It also destroys the incipient breast primordia, but leaves behind the nipples that are a reminder of the indifferent ground plan from which both sexes develop. Testosterone is also responsible for the masculinization of the brain, but it does so indirectly. Surprisingly, testosterone is enzymatically converted to estrogen in the brain, and it is **estrogen** that determines the organization of the brain for male-typical behavior.

Biologists have often stated that in mammals the indifferent gonad has an inherent tendency to become an ovary. Classic experiments performed in rabbits provide support for the idea that the female is the default sex during development. Removal of the fetal gonads before they have differentiated will invariably produce a female with uterine tubes, uterus, and vagina, even if the rabbit is a genetic male. Localization in 1994 of a region on the X chromosome named **DDS (dosage-sensitive sex reversal)** or

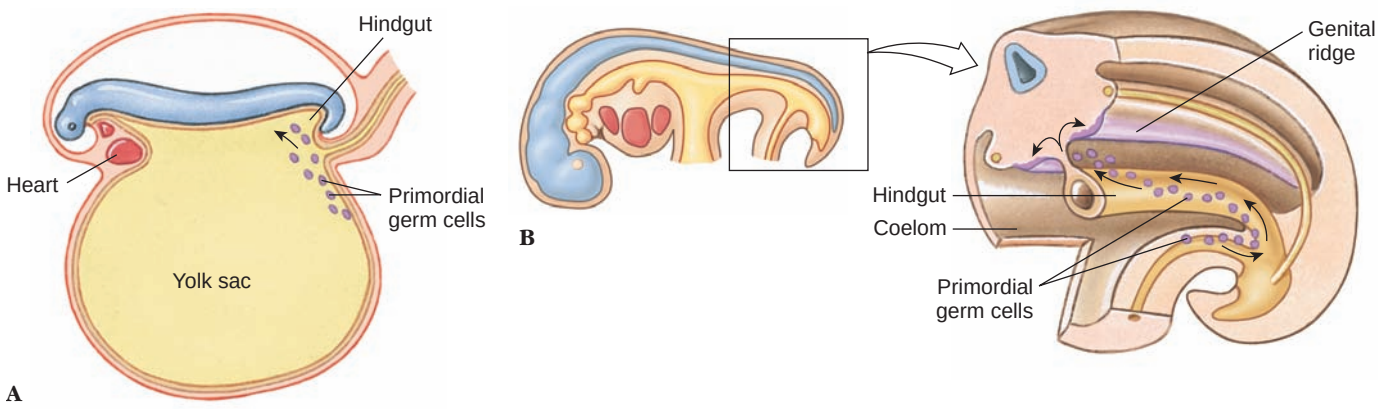


Figure 7.5

Migration of mammalian primordial germ cells. **A,** From the yolk sac the primordial germ cells migrate toward the region where the hindgut develops. **B,** Later-stage embryo in which the hindgut is more developed. Enlarged figure shows the germ cells migrating through the hindgut and into the genital ridges. In human embryos, migration is complete by the end of the fifth week of gestation.

SRVX (sex-reversing X), which promotes ovary formation, has challenged this view. In addition, the presence of such a region may help to explain feminization in some XY males. It is clear, however, that absence of testosterone in a genetic female embryo promotes development of female sexual organs: vagina, clitoris, and uterus. The developing female brain does require special protection from the effects of estrogen because, as mentioned earlier, estrogen causes masculinization of the brain. In rats, a blood protein (alpha-fetoprotein) binds to estrogen and keeps the hormone from reaching the developing female brain. This does not appear to be the case in humans, however, and even though circulating fetal estrogen levels can be quite high, the developing female brain does not become masculinized. One possible explanation is that the level of brain estrogen receptors in the developing female brain is low, and therefore, high levels of circulating estrogen would have no effect.

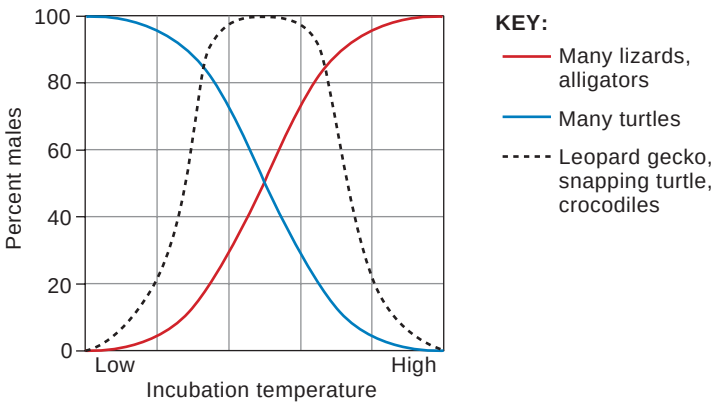


Figure 7.6

Temperature-dependent sex determination. In many reptiles that lack sex chromosomes incubation temperature of the nest determines gender. The graph shows that embryos of many turtles develop into males at low temperature, whereas embryos of many lizards and alligators become males at high temperatures. Embryos of crocodiles, leopard geckos, and snapping turtles become males at intermediate temperatures, and become females at higher or lower temperatures.

Source: Data from David Crews, "Animal Sexuality," Scientific American *270(1):108–114, January 1994.*

The genetics of sex determination are treated in Chapter 5 (p. 80). Sex determination is strictly chromosomal in mammals, birds, amphibians, most reptiles, and probably most fishes. However, some fishes and reptiles lack sex chromosomes altogether; in these groups, gender is determined by nongenetic factors such as temperature or behavior. In crocodilians, many turtles, and some lizards the incubation temperature of the nest determines the sex ratio probably by indirectly activating and/or suppressing genes that direct development of the animals' sex organs. Alligator eggs, for example, incubated at low temperature all become females; those incubated at higher temperature all become males (Figure 7.6). Sex determination of many fishes is behavior dependent. Most of these species are hermaphroditic, possessing both male and female gonads. Sensory stimuli from the animal's social environment determine whether it will be male or female.

Gametogenesis

Mature gametes are produced by a process called gametogenesis. Although the same essential processes are involved in maturation of both sperm and eggs in vertebrates, there are some important differences. Gametogenesis in testes is called **spermatogenesis,** and in ovaries, **oogenesis.**

Spermatogenesis

The walls of the seminiferous tubules contain differentiating germ cells arranged in a stratified layer five to eight cells deep (Figure 7.7). Germ cells develop in close contact with large **Sertoli** (sustentacular) **cells,** which extend from the periphery of the seminiferous tubules to the lumen and provide nourishment during germ-cell development and differentiation (Figure 7.8). The outermost layers contain **spermatogonia,** diploid cells that

Figure 7.7

Section of a seminiferous tubule containing male germ cells. More than 200 meters long, highly coiled seminiferous tubules are packed in each human testis. This scanning electron micrograph reveals, in the tubule's central cavity, numerous tails of mature spermatozoa that have differentiated from germ cells in the periphery of the tubule. (×525)
From R. G. Kessel and R. H. Kardon, Tissues and Organs: A Text-Atlas of Scanning Electron Microscopy, *1979, W. H. Freeman and Co.*

have increased in number by mitosis. Each spermatogonium increases in size and becomes a **primary spermatocyte.** Each primary spermatocyte then undergoes the first meiotic division, as described in Chapter 5 (p. 79), to become two **secondary spermatocytes** (Figure 7.8).

For every structure in the reproductive system of males or females, there is a homologous structure in the other. This happens because during early development male and female characteristics begin to differentiate from the embryonic genital ridge, and two duct systems develop, which at first are identical in both sexes. Under the influence of sex hormones, the genital ridge develops into the testes of males and the ovaries of females. One duct system (mesonephric or Wolffian) becomes ducts of the testes in males and regresses in females. The other duct (paramesonephric or Müllerian) develops into the uterine tubes, uterus, and vagina of females and regresses in males. Similarly, the clitoris and labia of females are homologous to the penis and scrotum of males, since they develop from the same embryonic structures.

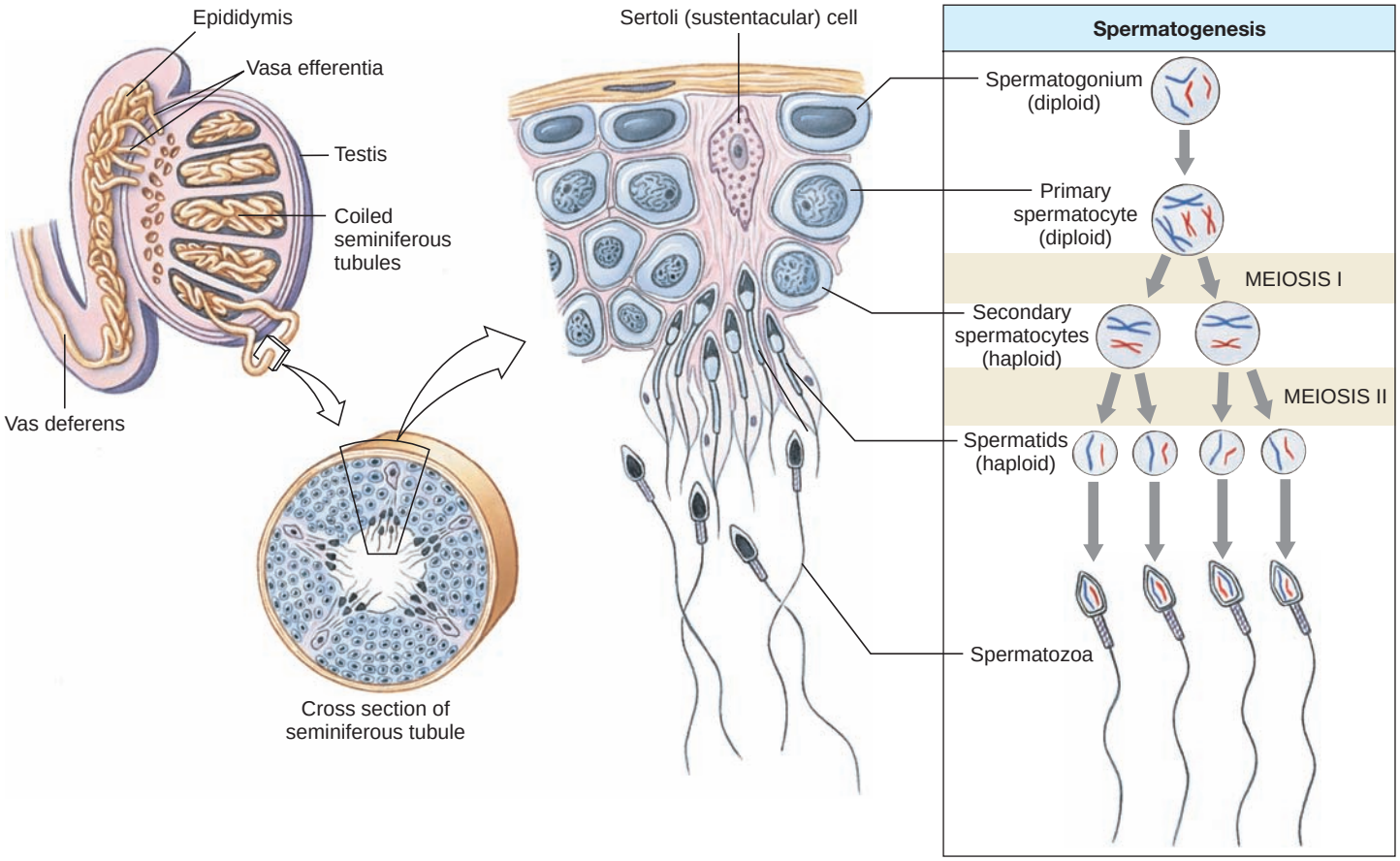


Figure 7.8

Spermatogenesis. Section of seminiferous tubule showing spermatogenesis. Germ cells develop within the recesses of large Sertoli (sustentacular) cells, that extend from the periphery of seminiferous tubules to their lumen, and that provide nourishment to the germ cells. Stem germ cells from which sperm differentiate are the spermatogonia, diploid cells located peripherally in the tubule. These divide by mitosis to produce either more spermatogonia or primary spermatocytes. Meiosis begins when primary spermatocytes divide to produce haploid secondary spermatocytes with double-stranded chromosomes. The second meiotic division forms four haploid spermatids with single-stranded chromosomes. As sperm develop, they are gradually pushed toward the lumen of the seminiferous tubule.

Each secondary spermatocyte enters the second meiotic division without intervention of a resting period. In the two steps of meiosis each primary spermatocyte gives rise to four **spermatids,** each containing the haploid number (23 in humans) of chromosomes. A spermatid usually contains a combination of his parents' chromosomes but may contain all chromosomes that the male inherited from his mother or from his father. Without further divisions the spermatids are transformed into mature **spermatozoa** or **(sperm)** (Figure 7.8). Modifications include great reduction of cytoplasm, condensation of the nucleus into a head, formation of a middle piece containing mitochondria, and a whiplike, flagellar tail for locomotion (Figures 7.8 and 7.9). The head consists of a nucleus containing the chromosomes for heredity and an **acrosome,** a distinctive feature of nearly all metazoa (exceptions are teleost fishes and certain invertebrates). In many species, both invertebrate and vertebrate, the acrosome contains enzymes that are released to clear an entrance through the layers that surround an egg. In mammals at least, one of the enzymes is hyaluronidase, which allows a sperm to penetrate the follicular cells surrounding an egg. A striking feature of many invertebrate spermatozoa is the acrosome filament, an extension of varying length in different species that projects suddenly from the sperm head when the latter first contacts the surface of an egg. Fusion of the egg and sperm plasma membranes is the initial event of fertilization (see Contact and Recognition between Egg and Sperm, p. 160).

The total length of a human sperm is 50 to 70 μm. Some toads have sperm that exceed 2 mm (2000 μm) in length (Figure 7.9) and are easily visible to the unaided eye. Most sperm, however, are microscopic in size (see p. 159 for an early seventeenth-century drawing of mammalian sperm, interpreted by biologists of the time as parasitic worms in the semen). In all sexually reproducing animals the number of sperm in males is far greater than the number of eggs in corresponding females. The number of eggs produced is correlated with the chances of young to hatch and to reach maturity.

Oogenesis

Early germ cells in the ovary, called **oogonia,** increase in number by mitosis. Each oogonium contains the diploid number of chromosomes. After the oogonia cease to increase in number, they grow in size and become **primary oocytes** (Figure 7.10). Before the first meiotic division, the chromosomes in each primary oocyte meet in pairs, paternal and maternal homologues, just as in spermatogenesis. When the first maturation (reduction) division occurs, the cytoplasm is divided unequally. One of the two daughter cells, the **secondary oocyte,** is large and receives most of the cytoplasm; the other is very small and is called the **first polar body** (Figure 7.10). Each of these daughter cells, however, has received half of the chromosomes.

In the second meiotic division, the secondary oocyte divides into a large **ootid** and a small polar body. If the first polar body also divides in this division, which sometimes happens, there are three polar bodies and one ootid (Figure 7.10). The ootid develops into a functional **ovum.** Polar bodies are nonfunctional, and they disintegrate. Formation of nonfunctional polar bodies is necessary to enable an egg to dispose of excess chromosomes,

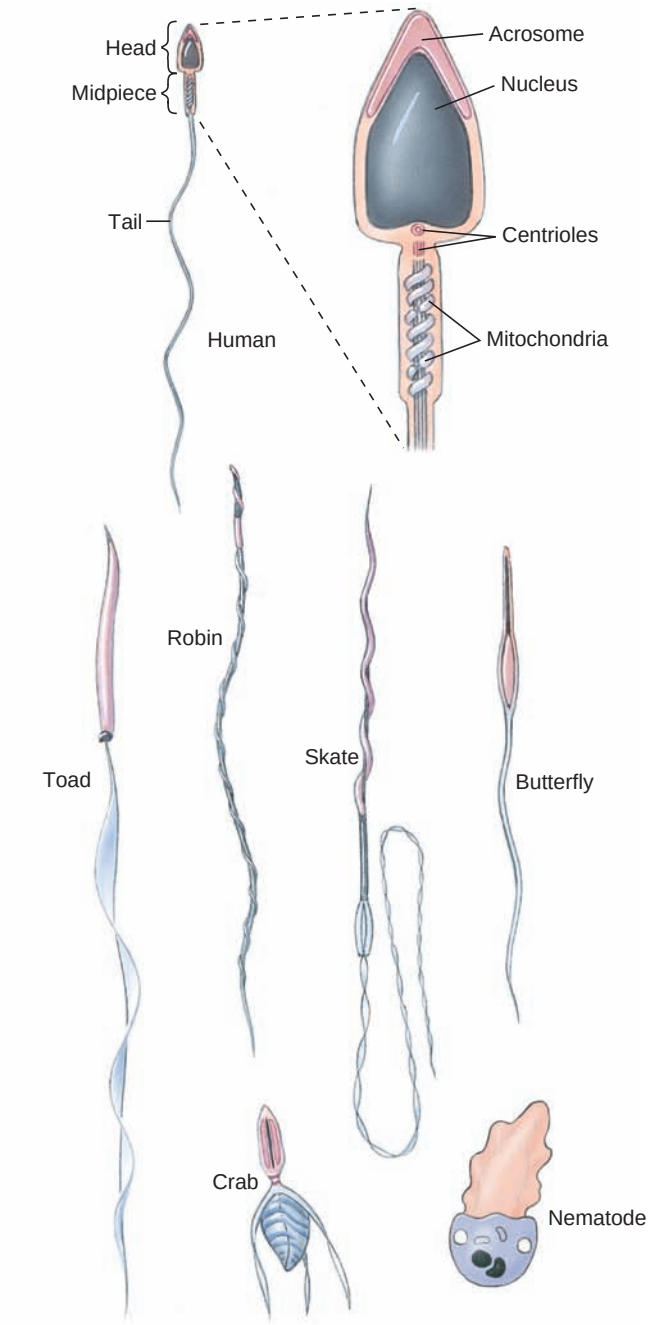


Figure 7.9
Examples of vertebrate and invertebrate sperm. The head and midpiece region of the human sperm is shown in more detail.

and the unequal cytoplasmic division makes possible a large cell with the cytoplasm containing a full set of cytoplasmic components needed for early development. Thus a mature ovum has *N* (haploid) number of chromosomes, the same as a sperm. However, each primary oocyte gives rise to only *one* functional gamete instead of four as in spermatogenesis.

In most vertebrates and many invertebrates the egg does not actually complete meiotic division before fertilization occurs. The general rule is that development is arrested during prophase I of the first meiotic division (in the primary oocyte phase). Meiosis resumes and is completed either at the time of ovulation (birds

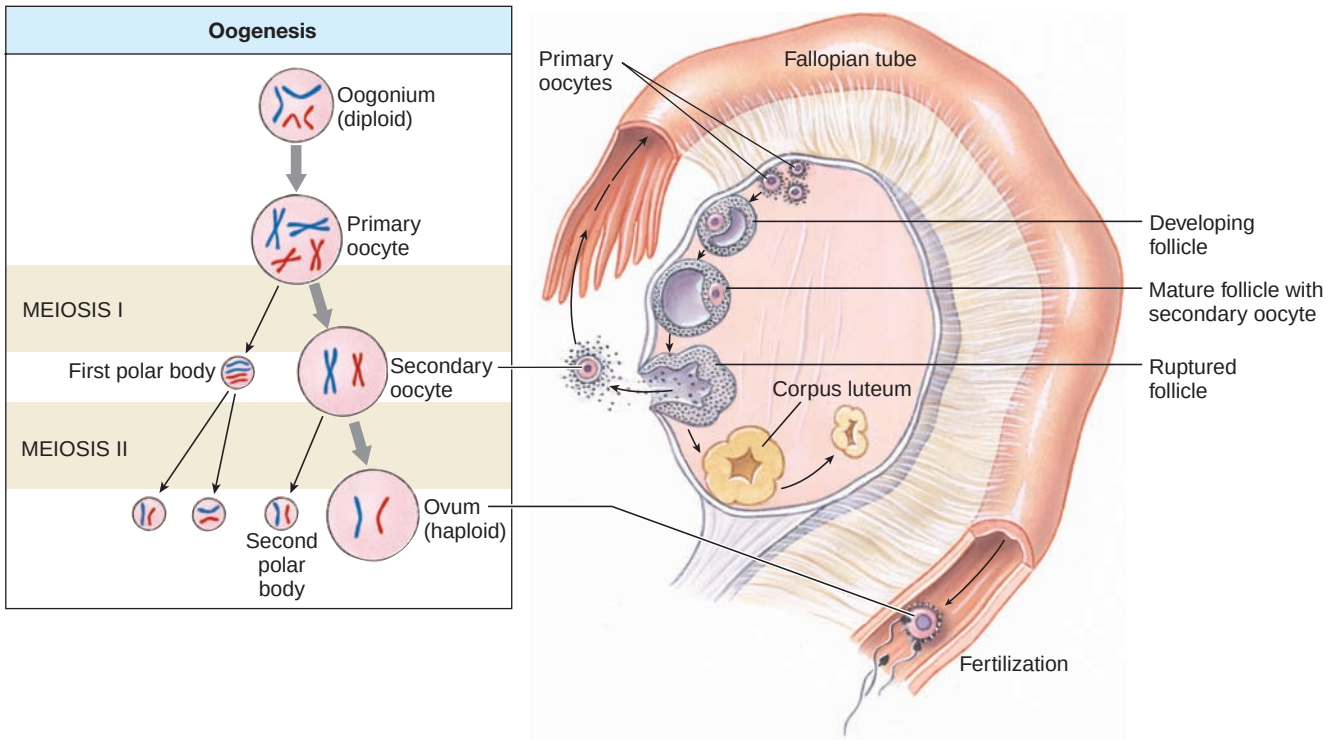


Figure 7.10

Oogenesis in humans. Early germ cells (oogonia) increase by mitosis during embryonic development to form diploid primary oocytes. After puberty, each menstrual month a diploid primary oocyte divides in the first meiotic division into a haploid secondary oocyte and a haploid polar body. If the secondary oocyte is fertilized, it enters the second meiotic division. The double-stranded chromosomes separate into a large ootid and small second polar body. The ootid develops into an ovum. Both ovum and second polar body now contain the *N* amount of DNA. Fusion of the haploid egg nucleus with a haploid sperm nucleus produces a diploid (2*N*) zygote.

and most mammals) or shortly after fertilization (many invertebrates, teleost fishes, amphibians, and reptiles). In humans, the ova begin the first meiotic division at about the thirteenth week of fetal development. Then their development arrests in prophase I as the primary oocyte until puberty, at which time one of these primary oocytes typically develops into a secondary oocyte each menstrual month. Thus, in humans meiosis II is completed only when the secondary oocyte is penetrated by a spermatozoon.

In many animals, the most obvious feature of egg maturation is deposition of yolk. Yolk, usually stored as granules or more organized platelets, is not a definite chemical substance but may be lipid or protein or both. Yolk may be synthesized within an egg from raw materials supplied by surrounding follicle cells, or preformed lipid or protein yolk may be transferred by pinocytosis from follicle cells to the oocyte.

Eggs also contain a large amount of mRNA that in not translated (p. 95) into polypeptides/proteins until fertilization triggers activation of these previously quiescent mRNA molecules. At this time the newly formed polypeptides/proteins begin to orchestrate the developmental process (see Chapter 8, p. 160).

Enormous accumulation of yolk granules, other nutrients (glycogen and lipid droplets), and quiescent mRNA cause an egg to grow well beyond the normal limits that force ordinary body (somatic) cells to divide. A young frog oocyte 50 µm in diameter, for example, grows to 1500 µm in diameter when mature after 3 years of growth in the ovary, and its volume is increased by a factor of 27,000. Bird eggs attain even greater absolute size; a hen egg increases 200 times in volume in only the last 6 to 14 days of rapid growth preceding ovulation.

Thus eggs are remarkable exceptions to the otherwise universal rule that organisms are composed of relatively minute cellular units. An egg's large size creates a problematic surface area-to-cell volume ratio, since everything that enters and leaves the ovum (nutrients, respiratory gases, wastes, and so on) must pass through the cell membrane. As the egg becomes larger, the available surface per unit of cytoplasmic volume (mass) becomes smaller. As we would anticipate, the metabolic rate of an egg gradually diminishes until a secondary oocyte or ovum (depending on the species) is in suspended animation until fertilization.

REPRODUCTIVE PATTERNS

The great majority of invertebrates, as well as many vertebrates, lay their eggs outside the body for development; these animals are called **oviparous** ("egg-birth"). Fertilization may be either internal (eggs are fertilized inside the body of a female before she lays them) or external (eggs are fertilized by a male after a female lays them). While many oviparous animals simply abandon their eggs rather indiscriminately, others display extreme care in finding places that will provide immediate and suitable sources of food for the young when they hatch.

Some animals retain eggs in their body (in the oviduct or uterus) while they develop, with embryos deriving all their nourishment from yolk stored within the egg. These animals are

called **ovoviviparous** ("egg-live-birth"). Ovoviviparity occurs in several invertebrate groups (for example, various annelids, brachiopods, insects, and gastropod molluscs) and is common among certain fishes (p. 538) and reptiles (p. 580).

In the third pattern, **viviparous** ("live-birth"), eggs develop in the oviduct or uterus with embryos deriving their nourishment directly from the mother. Usually some kind of intimate anatomical relationship is established between developing embryos and their mother. In both ovoviviparity and viviparity, fertilization must be internal (within the body of the female) and the mother gives birth to young usually in a more advanced stage of development. Viviparity is confined mostly to lizards, snakes, mammals, and elasmobranch fishes, although viviparous invertebrates (scorpions, for example) and amphibians are known. Development of embryos within a mother's body, whether ovoviviparous or viviparous, obviously affords more protection to the offspring than egg-laying.

STRUCTURE OF REPRODUCTIVE SYSTEMS

The basic components of reproductive systems are similar in sexual animals, although differences in reproductive habits and methods of fertilization have produced many variations. Sexual systems consist of two components: (1) **primary organs,** which are the gonads that produce sperm and eggs and sex hormones; and (2) **accessory organs,** which assist the gonads in formation and delivery of gametes, and may also serve to support the embryo. They are of great variety, and include gonoducts (sperm ducts and oviducts), accessory organs for transferring spermatozoa into the female, storage organs for spermatozoa or yolk, packaging systems for eggs, and nutritional organs such as yolk glands and placenta.

Invertebrate Reproductive Systems

Invertebrates that transfer sperm from male to female for internal fertilization require organs and plumbing to facilitate this function that may be as complex as those of any vertebrate. In contrast, reproductive systems of invertebrates that simply release their gametes into the water for external fertilization may be little more than centers for gametogenesis. Polychaete annelids, for example, have no permanent reproductive organs. Gametes arise by proliferation of cells lining the body cavity. When mature the gametes are released through coelomic or nephridial ducts or, in some species, may exit through ruptures in the body wall.

Insects have separate sexes (dioecious), practice internal fertilization by copulation and insemination, and consequently have complex reproductive systems (Figure 7.11). Sperm from the testes pass through sperm ducts to seminal vesicles (where the sperm are stored) and then through a single ejaculatory duct to a penis. Seminal fluid from one or more accessory glands is added to the semen in the ejaculatory duct. Females have a pair of ovaries formed from a series of egg tubes (ovarioles). Mature ova pass through oviducts to a common genital chamber and then to a short copulatory bursa (vagina). In most insects, the male transfers sperm by inserting the penis directly into the female's genital bursa (vagina); from here they migrate, and are stored in a seminal receptacle. Often a single mating provides sufficient sperm to last the reproductive life of a female.

Vertebrate Reproductive Systems

In vertebrates the reproductive and excretory systems are together called the **urogenital system** because of their close anatomical connection, especially in males. This association is

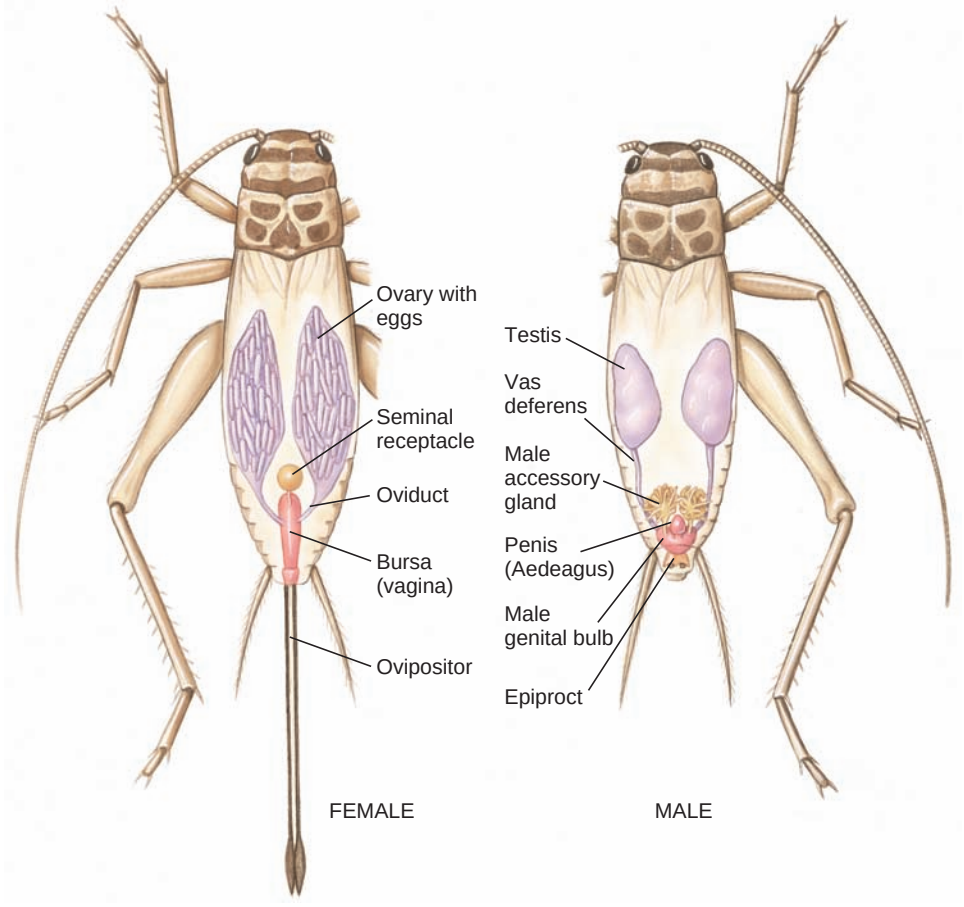


Figure 7.11

Reproductive system of crickets. Sperm from the paired testes of males pass through sperm tubes (vas deferens) to an ejaculatory duct housed in the penis. In females, eggs from the ovaries pass through oviducts to the genital bursa. At mating sperm enclosed in a membranous sac (spermatophore) formed by the secretions of the accessory gland are deposited in the genital bursa of the female, then migrate to her seminal receptacle where they are stored. The female controls the release of a few sperm to fertilize her eggs at the moment they are laid, using the needlelike ovipositor to deposit the eggs in the soil.

very striking during embryonic development. In male fishes and amphibians the duct that drains the kidney **(opisthonephric duct** or **Wolffian duct)** also serves as a sperm duct (see p. 674). In male reptiles, birds, and mammals in which the kidney develops its own independent duct **(ureter)** to carry away waste, the old **mesonephric duct** becomes exclusively a sperm duct or **vas deferens.** In all these forms, with the exception of most mammals, the ducts open into a **cloaca** (derived, appropriately, from the Latin meaning "sewer"), a common chamber into which intestinal, reproductive, and excretory canals empty. Almost all placental mammals have no cloaca; instead the urogenital system has its own opening separate from the anal opening. In females, the **uterine duct** or **oviduct** is an independent duct that opens into the cloaca in animals having a cloaca.

Male Reproductive System

The male reproductive system of vertebrates, such as that of human males (Figure 7.12) includes testes, vasa efferentia, vas deferens, accessory glands, and (in some birds and reptiles, and all mammals) a penis.

Paired **testes** are the sites of sperm production. Each testis is composed of numerous **seminiferous tubules,** in which the sperm develop (Figure 7.8). The sperm are surrounded by **Sertoli cells** (or **sustentacular cells**), which nourish the developing sperm. Between the tubules are **interstitial cells** (or **Leydig cells**), which produce the male sex hormone **(testosterone).** In most mammals the two testes are housed permanently in a saclike scrotum suspended outside the abdominal cavity, or the testes descend into the scrotum during the breeding season. This odd arrangement provides an environment of slightly lower temperature, since in most mammals (including humans) viable sperm do not form at temperatures maintained within the body. In marine mammals and all other vertebrates the testes are positioned permanently within the abdomen.

The sperm travel from the seminiferous tubules to the **vasa efferentia,** small tubes passing to a coiled **epididymis** (one for each testis), where final sperm maturation occurs and then to a **vas deferens,** the ejaculatory duct (Figures 7.8 and 7.12). In mammals the vas deferens joins the **urethra,** a duct that carries both sperm and urinary products through the **penis,** or external intromittent organ.

Most aquatic vertebrates have no need for a penis, since sperm and eggs are liberated into the water in close proximity to each other. However, in terrestrial (and some aquatic) vertebrates that bear their young alive or enclose the egg within a shell, sperm must be transferred to the female. Few birds have a true penis (examples of exceptions are the ostrich and the Argentine lake duck) and the mating process simply involves presenting cloaca to cloaca. Most reptiles and mammals have a true penis. In mammals the normally flaccid organ becomes erect when engorged with blood. Some mammals possess a bone in the penis (baculum), which presumably helps with rigidity.

In most mammals three sets of accessory glands open into the reproductive channels: a pair of **seminal vesicles,** a single **prostate gland,** and the pair of **bulbourethral glands** (Figure 7.12). Fluid secreted by these glands furnishes food to the sperm, lubricates the female reproductive tract for sperm, and counteracts the acidity of the vagina so that the sperm retain their viability longer after being deposited in the female.

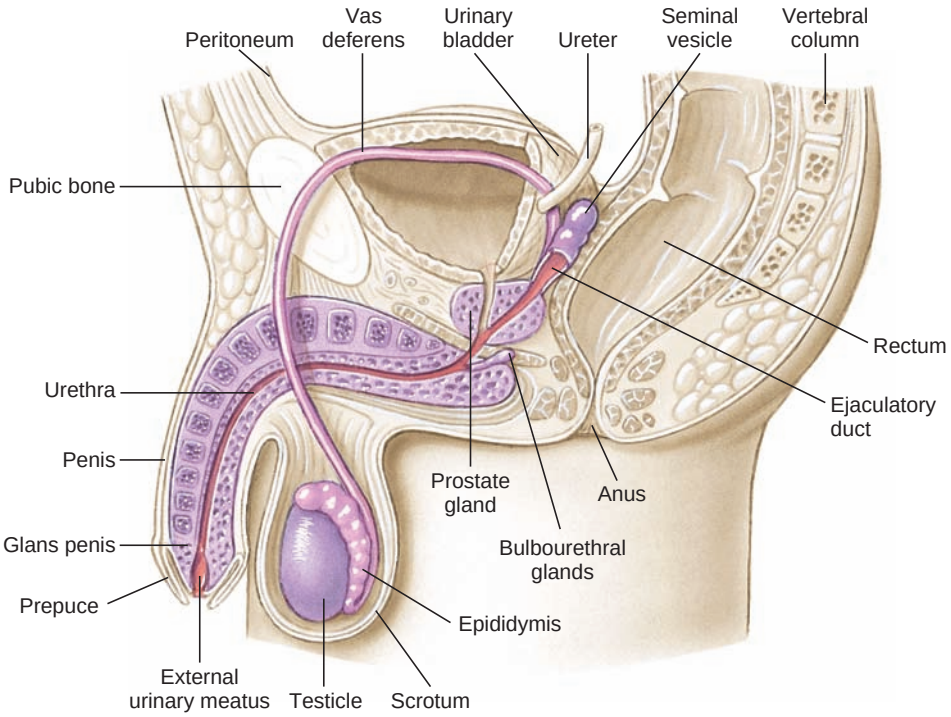


Figure 7.12
Human male reproductive system showing the reproductive structures in sagittal view.

Female Reproductive System

The ovaries of female vertebrates produce both ova and female sex hormones (estrogens and progesterone). In all jawed vertebrates, mature ova from each ovary enter the funnel-like opening of a **uterine tube** or **oviduct,** which typically has a fringed margin (fimbriae) that envelops the ovary at the time of ovulation. The terminal end of the uterine tube is unspecialized in most fishes and amphibians, but in cartilaginous fishes, reptiles, and birds that produce a large, shelled egg, special regions have developed for production of albumin and shell. In amniotes (reptiles, birds, and mammals; see Amniotes and the Amniotic Egg, p. 175) the terminal portion of the uterine tube is expanded into a muscular **uterus** in which shelled eggs are held before laying or in which embryos complete their development. In placental mammals, the

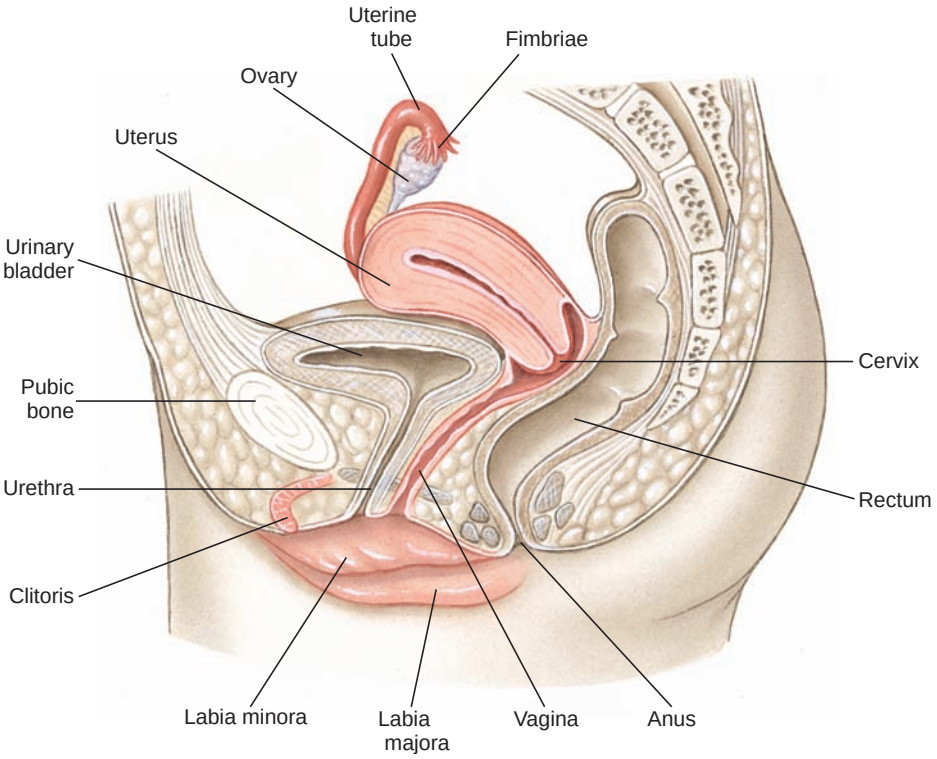


Figure 7.13
Human female reproductive system showing the pelvis in sagittal section.

walls of the uterus establish a close vascular association with the embryonic membranes through a **placenta** (see p. 177).

The paired ovaries of the human female (Figure 7.13), slightly smaller than the male testes, contain many thousands of oocytes. Each oocyte develops within a **follicle** that enlarges and finally ruptures to release a secondary oocyte (Figure 7.10). During a woman's fertile years, except following fertilization, approximately 13 oocytes mature each year, and usually the ovaries alternate in releasing oocytes. Because a woman is fertile for only about 30 years, of the approximately 400,000 primary oocytes in her ovaries at birth, only 300 to 400 have a chance to reach maturity; the others degenerate and are resorbed.

A long-held principle of mammalian reproductive biology has been that in the male, germ cell lines continue to remain functional and form sperm throughout adult life, while females possess a finite number of germ cells, and oocyte production ceases at birth. Indeed, we have just described human follicular development as such a case, where the primary oocytes present at birth provide her only source of follicles. Recently, an exciting discovery in mice has challenged this reproductive dogma. Juvenile and adult mouse ovaries have been shown to possess actively dividing germ cells that replenish the oocyte pool. If this finding can be extended to other mammalian species, it will have significant implications in the management of endangered species, where assisted reproductive techniques could be used to expand pools of oocytes that might mean the difference between extinction and survival.

The **uterine tubes,** or **oviducts,** are lined with cilia for propelling the egg away from the ovary from which it was released. The two ducts open into the upper corners of the **uterus,** or womb, which is specialized for housing the embryo during its intrauterine existence. It consists of thick muscular walls, many blood vessels, and a specialized lining: the **endometrium.** The uterus varies among different mammals, and in many it is designed to hold more than one developing embryo. Ancestrally it was paired but is fused to form one large chamber in many eutherian mammals.

The **vagina** is a muscular tube adapted to receive the male's penis and serves as the birth canal during expulsion of a fetus from the uterus. Where vagina and uterus meet, the uterus projects down into the vagina to form a **cervix.**

The external genitalia of human females, or **vulva,** include folds of skin, the **labia majora** and **labia minora,** and a small erectile organ, the **clitoris** (the female homolog of the glans penis of males). The opening into the vagina is often reduced in size in the virgin state by a membrane, the **hymen,** although in today's more physically active females, this membrane may be much reduced in extent.

ENDOCRINE EVENTS THAT ORCHESTRATE REPRODUCTION

Hormonal Control of Timing of Reproductive Cycles

From fish to mammals, reproduction in vertebrates is usually a seasonal or cyclic activity. Timing is crucial, because offspring should appear when food is available and other environmental conditions are optimal for survival. The sexual reproductive process is controlled by hormones, which are regulated by environmental cues, such as food intake, and seasonal changes in photoperiod, rainfall, or temperature, and by social cues. A region within the forebrain called the hypothalamus (p. 758) regulates the release of anterior pituitary gland hormones, which in turn stimulate tissues of the gonads (neurosecretion and the pituitary gland are described in Chapter 34). This hormonal system controls development of the gonads, accessory sex structures, and secondary sexual characteristics (see next section), as well as timing of reproduction.

The cyclic reproductive patterns of female mammals are of two types: **estrous cycle,** characteristic of most mammals, and **menstrual cycle,** characteristic only of the anthropoid primates (monkeys, apes, and humans). These two cycles differ in two important ways. First, in estrous cycles, females are receptive to males only during brief periods of **estrus,** or "heat," whereas in the menstrual cycle receptivity may occur throughout the cycle.

Second, a menstrual cycle, but not an estrous cycle, ends with breakdown and discharge of the inner portion of the uterus (endometrium). In an estrous cycle, each cycle ends with the endometrium simply reverting to its original state, without the discharge characteristic of the menstrual cycle.

Gonadal Steroids and Their Control

The ovaries of female vertebrates produce two kinds of steroid sex hormones—**estrogens** and **progesterone** (Figure 7.14). There are three kinds of estrogens: estradiol, estrone and estriol, of which estradiol is secreted in the highest amounts during reproductive cycles. Estrogens are responsible for development of female accessory sex structures (oviducts, uterus, and vagina) and for stimulating female reproductive activity. Secondary sex characters, those characteristics that are not primarily involved in formation and delivery of ova (or sperm in males), but that are essential for behavioral and functional success of reproduction, are also controlled or maintained by estrogens. Secondary sexual characteristics include distinctive skin or feather coloration, bone development, body size and, in mammals, initial development of the mammary glands. In female mammals, both estrogen and progesterone are responsible for preparing the uterus to receive a developing embryo. These hormones are controlled by **anterior pituitary gonadotropins: follicle-stimulating hormone (FSH),** and **luteinizing hormone (LH)** (Figure 7.15). The release of these two gonadotropins are in turn governed by **gonadotropin-releasing hormone (GnRH)** produced by neurosecretory cells in the **hypothalamus** (see p. 758 and Table 34.1). Through this control system environmental factors such as light, nutrition, and stress may influence reproductive cycles. Estrogens and progesterone feed back to the hypothalamus and anterior pituitary to keep secretion of GnRH, FSH, and LH in check (see Chapter 34, for a discussion of negative feedback of hormones).

The male sex steroid, **testosterone** (Figure 7.14), is manufactured by the **interstitial cells** of the testes. Testosterone, and its metabolite, **dihydrotestosterone (DHT),** are necessary for the growth and development of the male accessory sex structures (penis, sperm ducts, and glands), development of secondary male sex characters (such as bone and muscle growth, male plumage or pelage coloration, antlers in deer, and, in humans, voice quality), and male sexual behavior. Development of the testes and secretion of testosterone is controlled by FSH and LH, the same anterior pituitary hormones that regulate the female reproductive cycle, and ultimately by GnRH from the hypothalamus. Like estrogens and progesterone in the female, testosterone and DHT feed back to the hypothalamus and anterior pituitary to regulate secretion of GnRH, FSH, and LH.

Recent identification of a peptide in the hypothalamus of birds and mammals that inhibits the secretion of GnRH and LH has led some scientists to believe that a gonadotropin-inhibiting hormone has finally been discovered. Further study is necessary, however, before we can be sure that this peptide antagonizes GnRH in all physiological conditions.

Both the ovary and testes secrete a peptide hormone, **inhibin,** which is secreted by the developing follicles in the female and by the **Sertoli cells** (or sustentacular cells) in the male. This hormone is an additional regulator of FSH secretion from the anterior pituitary in a negative feedback manner.

The Menstrual Cycle

The human menstrual cycle (L. *mensis,* month) consists of two distinct phases within the ovary: follicular phase and luteal phase, and three distinct phases within the uterus: menstrual phase, proliferative phase, and secretory phase (Figure 7.15). Menstruation (the "period") signals the **menstrual phase,** when part of the lining of the uterus (endometrium) degenerates and sloughs off, producing the menstrual discharge. Meanwhile, the **follicular phase** within the ovary is occurring, and by day 3 of the cycle blood levels of FSH and LH begin to rise slowly, prompting some of the ovarian follicles to begin growing and to secrete estrogen. As estrogen levels in the blood increase, the uterine endometrium heals and begins to thicken, and uterine glands within the endometrium enlarge **(proliferative phase).** By day 10 most of the ovarian follicles that began to develop at day 3 now degenerate (become **atretic**), leaving only one (sometimes two or three) to continue developing until it appears as a bulge on the surface of the ovary. This is a mature follicle or **graafian follicle.** During the latter part of the follicular phase, the graafian follicle secretes more estrogen, and also inhibin. As the levels of inhibin rise, the levels of FSH fall.

At day 13 or 14 in the cycle, the now high levels of estrogen from the graafian follicle stimulate a surge of GnRH from the hypothalamus, which induces a surge of LH (and to a lesser extent, FSH) from the anterior pituitary. The LH surge causes the graafian follicle to rupture **(ovulation),** releasing an oocyte

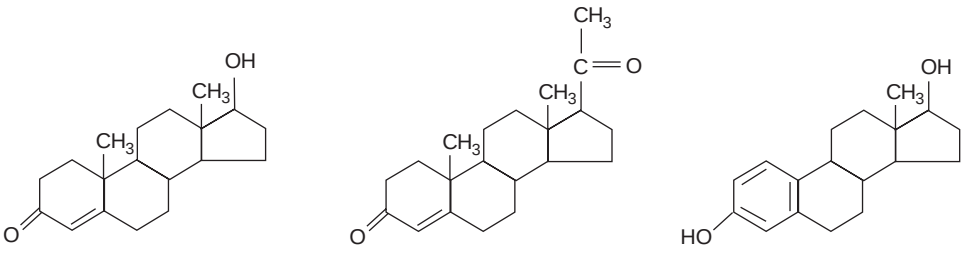
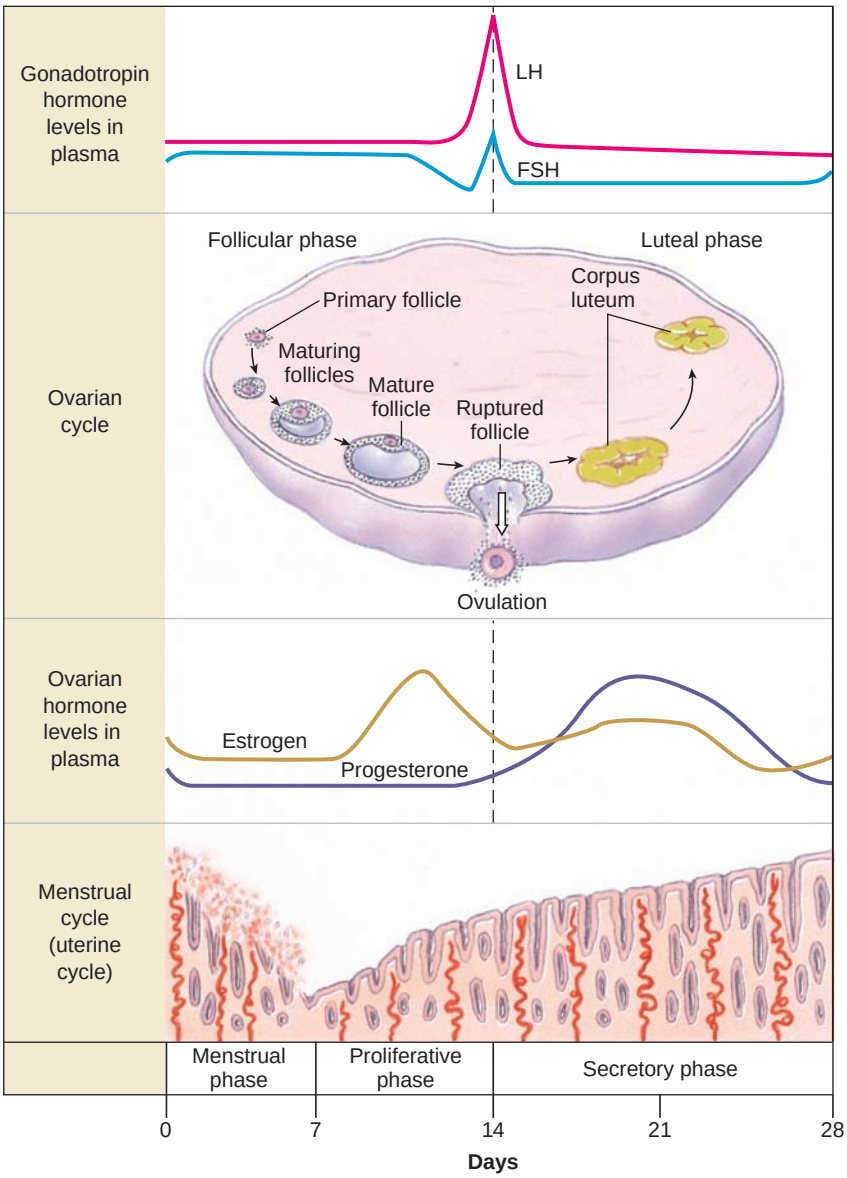


Figure 7.14

Sex hormones. These three sex hormones show the basic four-ring steroid structure. The main female sex hormone, estradiol (an estrogen) is a C_{18} (18-carbon) steroid with an aromatic A ring (first ring to left). The main male sex hormone testosterone (an androgen) is a C_{19} steroid with a carbonyl group (C=O) on the A ring. The female sex hormone progesterone is a C_{21} steroid, also bearing a carbonyl group on the A ring.

Figure 7.15

Human menstrual cycle, showing changes in blood-hormone levels and uterine endometrium during the 28-day ovarian cycle. FSH promotes maturation of ovarian follicles, which secrete estrogen. Estrogen prepares the uterine endometrium and causes a surge in LH, which in turn causes ovulation and stimulates the corpus luteum to secrete progesterone and estrogen. Progesterone and estrogen production will persist only if an ovum is fertilized; without pregnancy progesterone and estrogen levels decline and menstruation follows.



from the ovary. The oocyte remains viable for approximately 12 hours, during which time it may be fertilized by a sperm. During the ovarian **luteal phase,** a **corpus luteum** ("yellow body" for its appearance in cow ovaries) forms from the remains of the ruptured follicle that released the oocyte at ovulation (Figures 7.10 and 7.15). The corpus luteum, responding to continued stimulation by LH, becomes a transitory endocrine gland that secretes progesterone (and estrogen in primates). Progesterone ("before carrying [gestation]"), as its name implies, stimulates the uterus to undergo final maturational changes that prepare it for gestation **(secretory phase).** The uterus is now fully ready to house and to nourish an embryo. If fertilization has *not* occurred, the corpus luteum degenerates, and its hormones are no longer secreted. Since the uterine lining (endometrium) depends on progesterone and estrogen for its maintenance, their declining levels cause the uterine lining to deteriorate, leading to menstrual discharge of the next cycle.

Oral contraceptives (the "Pill") usually are combined preparations of estrogen and progesterone that act to decrease the output of pituitary gonadotropins FSH and LH. This prevents the ovarian follicles from ripening fully and usually prevents ovulation from occurring. Oral contraceptives are highly effective, with a failure rate of less than 1% if the treatment procedure is followed properly. More recently, estrogen and progesterone are administered as a once per month injection (Lunelle), as a skin patch (Ortho Evra), or as a vaginal ring (NuvaRing). Progesterone also acts on the reproductive tract as a whole, making it inhospitable for sperm and any fertilized oocyte. This mechanism has been exploited in progesterone-only contraceptives ("mini-pill," Depo-Provera), which may not block follicular development or ovulation, and in the "morning after pill," a postcoital emergency contraceptive that is now available over the counter in the United States for women aged 18 years or older.

GnRH from the hypothalamus, and LH and FSH from the anterior pituitary, are controlled by **negative feedback** of ovarian steroids (and inhibin). This negative feedback occurs throughout the menstrual cycle, except for a few days before ovulation. As just mentioned, ovulation is due to the *high levels of estrogen* causing a surge of GnRH, LH (and FSH). Such **positive feedback** mechanisms are rare in the body, since they move events away from stable set points. (Feedback mechanisms are described in Chapter 34, p. 756). This event is terminated by ovulation when estrogen levels fall as an oocyte is released from the follicle.

Hormones of Human Pregnancy and Birth

If fertilization occurs, it normally does so in the first third of the uterine tube **(ampulla).** The **zygote** travels from here to the uterus, dividing by mitosis to form a **blastocyst** (see Chapter 8, p. 177) by the time it reaches the uterus. The developing blastocyst adheres to the uterine surface after about 6 days and embeds itself in the endometrium. This process is called **implantation.** Growth of the embryo continues, producing a spherically shaped **trophoblast.** This embryonic stage contains three distinct tissue layers, the amnion, chorion, and embryo proper, the inner cell mass (see Figure 8.25, p. 178). The **chorion** becomes the source of **human chorionic gonadotropin (hCG),** which appears in the bloodstream soon after implantation. hCG stimulates the corpus luteum to continue to synthesize and to release both estrogen and progesterone (Figure 7.16).

The placenta forms the point of attachment between trophoblast and uterus (evolution and development of the placenta is described in Chapter 8, p. 177). Besides serving as a medium for the transfer of materials between maternal and fetal bloodstreams, the placenta also serves as an endocrine gland. The placenta continues to secrete hCG and also produces estrogen (mainly estriol) and progesterone. After about the third month of pregnancy, the corpus luteum degenerates in some mammals, but by then the placenta itself is the main source of both progesterone and estrogen (Figure 7.17).

Preparation of the mammary glands for secretion of milk requires two additional hormones, **prolactin (PRL)** and **human placental lactogen (hPL)** (or **human chorionic somatomammotropin**). PRL is produced by the anterior pituitary, but in nonpregnant women its secretion is inhibited. During pregnancy, elevated levels of progesterone and estrogen depress the inhibitory signal, and PRL begins to appear in the blood. PRL is also produced by the placenta during pregnancy. PRL, in combination with hPL, prepare the mammary glands for secretion. hPL, together with **human placental growth hormone (hPGH)** and maternal growth hormone, also stimulate an increase in available nutrients in the mother, so that more are provided to the developing embryo. The placenta also secretes β-endorphin and other endogenous opioids (see Chapter 33, p. 743) that regulate appetite and mood during pregnancy. Opioids may also contribute to a sense of well-being and help to alleviate some of the discomfort associated with the later months of pregnancy. Later the placenta begins to synthesize a peptide hormone called **relaxin;** this hormone

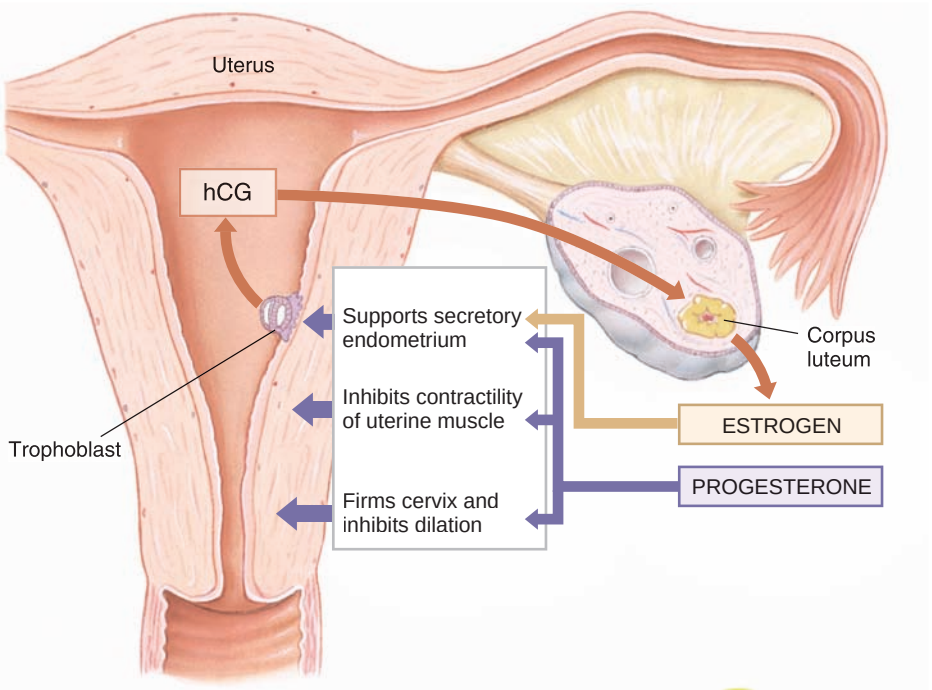


Figure 7.16

The multiple roles of progesterone and estrogen in normal human pregnancy. After implantation of an embryo in the uterus, the trophoblast (the future embryo and placenta) secretes human chorionic gonadotropin (hCG), which maintains the corpus luteum until the placenta, at about the seventh week of pregnancy, begins producing the sex hormones progesterone and estrogen.

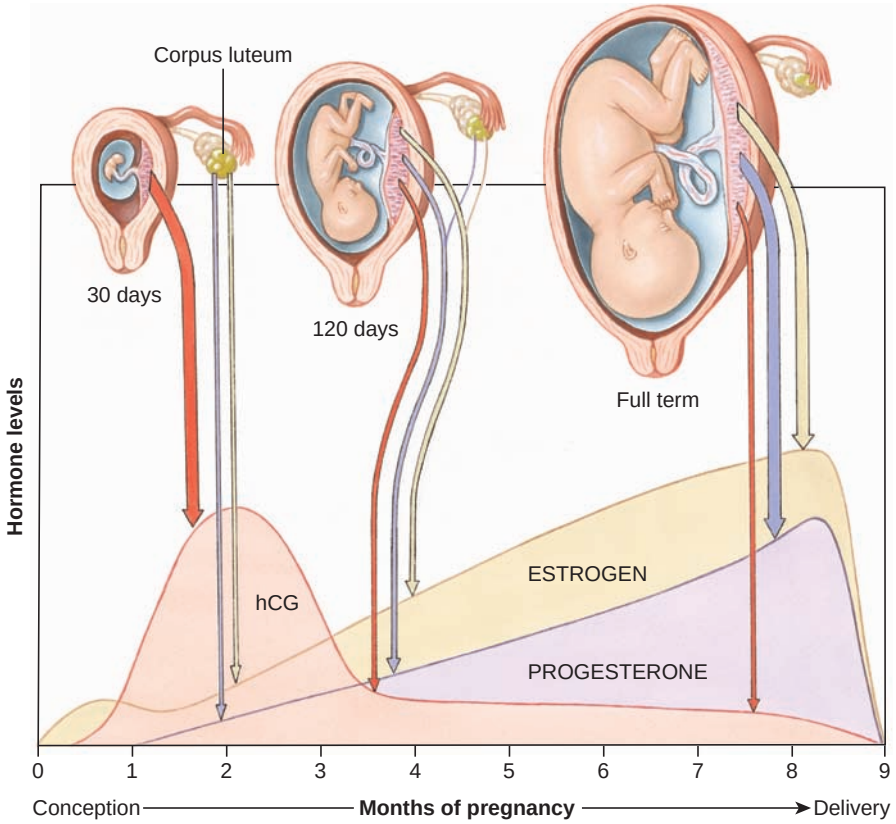


Figure 7.17

Hormone levels released from the corpus luteum and placenta during pregnancy. The width of the arrows suggests the relative amounts of hormone released; hCG (human chorionic gonadotropin) is produced solely by the placenta. Synthesis of progesterone and estrogen shifts during pregnancy from the corpus luteum to the placenta.

allows some expansion of the pelvis by increasing the flexibility of the pubic symphysis, and also dilates the cervix in preparation for delivery.

Birth, or **parturition,** occurs after approximately 9 months in humans and begins with a series of strong, rhythmic contractions of the uterine musculature, called **labor.** The exact signal that triggers birth is not fully understood in humans, but **placental corticotropin-releasing hormone (CRH)** appears to initiate the birth process. Just before birth, secretion of estrogen, which stimulates uterine contractions, rises sharply, while the level of progesterone, which inhibits uterine contractions, declines (Figure 7.17). This removes the "progesterone block" that keeps the uterus quiescent throughout pregnancy. **Prostaglandins,** a large group of hormones (long-chain fatty acid derivatives), also increase at this time, making the uterus more "irritable" (see Chapter 34, p. 762, for more on prostaglandins). Finally, stretching of the cervix sets in motion neural reflexes that stimulate secretion of **oxytocin** from the posterior pituitary. Oxytocin also stimulates uterine smooth muscle, leading to stronger and more frequent labor contractions. Secretion of oxytocin during childbirth is another example of **positive feedback.** This time the event is terminated by birth of the baby.

Childbirth occurs in three stages. In the first stage the cervix is enlarged by pressure from the baby in its bag of amniotic fluid, which may be ruptured at this time (**dilation;** Figure 7.18B). In the second stage, the baby is forced out of the uterus and through the vagina to the outside (**expulsion;** Figure 7.18C). In the third stage, the placenta, or **afterbirth,** is expelled from the mother's body, usually within 10 minutes after the baby is born (**placental delivery;** Figure 7.18D).

Miscarriages during pregnancy, or spontaneous abortions, are quite common and serve as a mechanism to reject prenatal abnormalities such as chromosomal damage and other genetic errors, exposure to drugs or toxins, immune irregularities, or improper hormonal priming of the uterus. Modern hormonal tests show that about 30% of fertile zygotes are spontaneously aborted before or right after implantation; such miscarriages are unknown to the mother or are expressed as a slightly late menstrual period. Another 20% of established pregnancies end in miscarriage (those known to the mother), giving a spontaneous abortion rate of about 50%.

After birth, secretion of milk is triggered when the infant sucks on its mother's nipple. This leads to a reflex release of oxytocin from the posterior pituitary; when oxytocin reaches the mammary glands it causes contraction of smooth muscles lining ducts and sinuses of the mammary glands and ejection of milk.

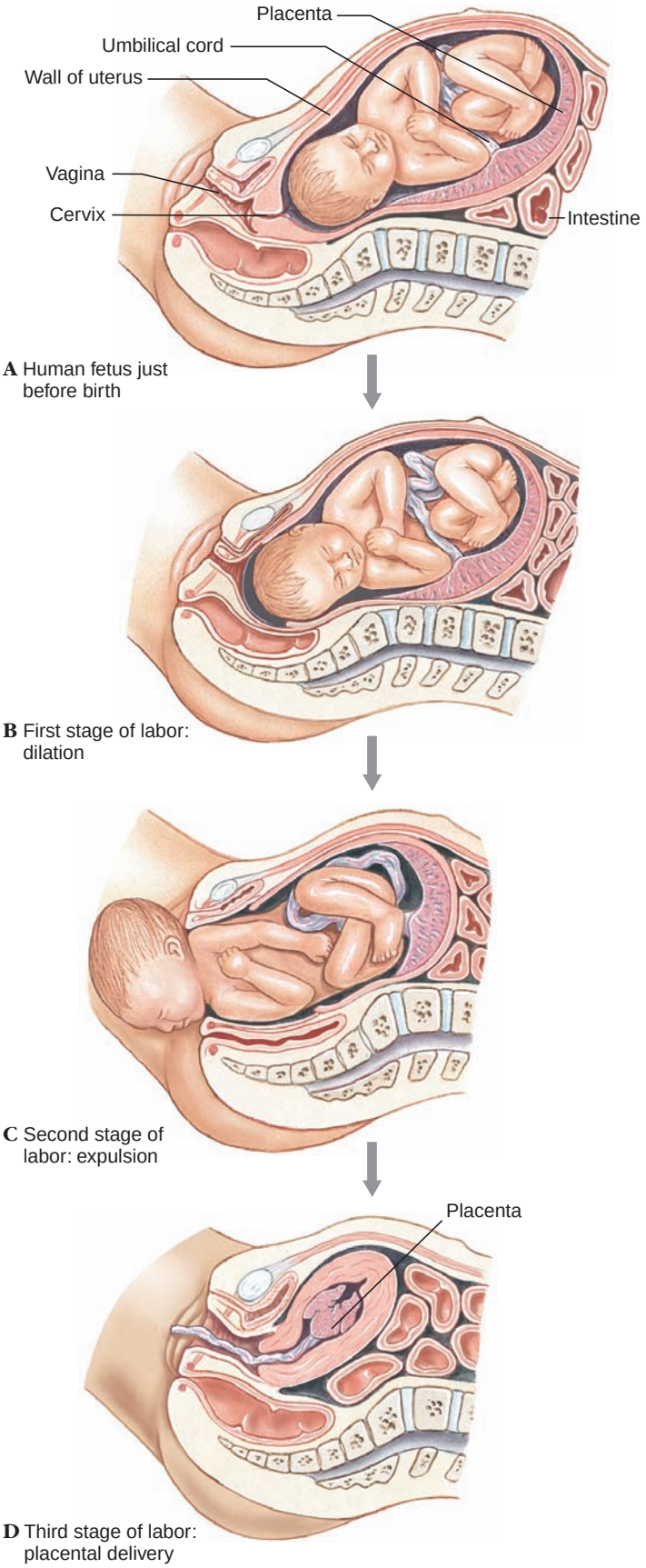


Figure 7.18
Birth, or parturition, in humans.

Suckling also stimulates release of prolactin from the anterior pituitary, which stimulates continued production of milk by the mammary glands.

Multiple Births

Many mammals give birth to more than one offspring at a time or to a litter **(multiparous),** each member of which has come from a separate egg. There are some mammals, however, that have only one offspring at a time **(uniparous),** although occasionally they may have more than one. The armadillo (*Dasypus*) is almost unique among mammals in giving birth to four offspring at one time—all of the same sex, either male or female, and all derived from the same zygote.

Human twins may come from one zygote (**identical,** or **monozygotic** twins; Figure 7.19A) or two zygotes (**nonidentical, dizygotic,** or **fraternal** twins; Figure 7.19B). Fraternal twins do not resemble each other any more than other children born separately in the same family, but identical twins are, of course, strikingly alike and always of the same sex. Triplets, quadruplets, and quintuplets may include a pair of identical twins. The other babies in such multiple births usually come from separate zygotes. About 33% of identical twins have separate placentas, indicating that the blastomeres separated at an early, possibly the two-cell, stage (Figure 7.19A, *top*). All other identical twins share a common placenta, indicating that splitting occurred after formation of the inner cell mass (see Figure 8.25 on p. 180). If splitting were to happen after placenta formation, but before the amnion forms, the twins would have individual amniotic sacs (Figure 7.19A, *middle*), as observed in the great majority of identical twins. Finally, a very small percentage of identical twins share one amniotic sac and a single placenta (Figure 7.19A, *bottom*), indicating that separation occurred after day 9 of pregnancy, by which time the amnion has formed. In these cases, the twins are at risk of becoming conjoined, a condition known as Siamese twinning. Embryologically, each member of fraternal twins has its own placenta and amnion (Figure 7.19B).

The frequency of twin births in comparison to single births is approximately 1 in 86, that of triplets 1 in 86^2, and that of quadruplets approximately 1 in 86^3. Frequency of identical twin births to all births is about the same the world over, whereas frequency of fraternal births varies with race and country. In the United States, three-fourths of all twin births are dizygotic (fraternal), whereas in Japan only about one-fourth are dizygotic. The tendency for fraternal twinning (but apparently not identical twinning) seems to run in family lines; fraternal twinning (but not identical twinning) also increases in frequency as mothers get older.

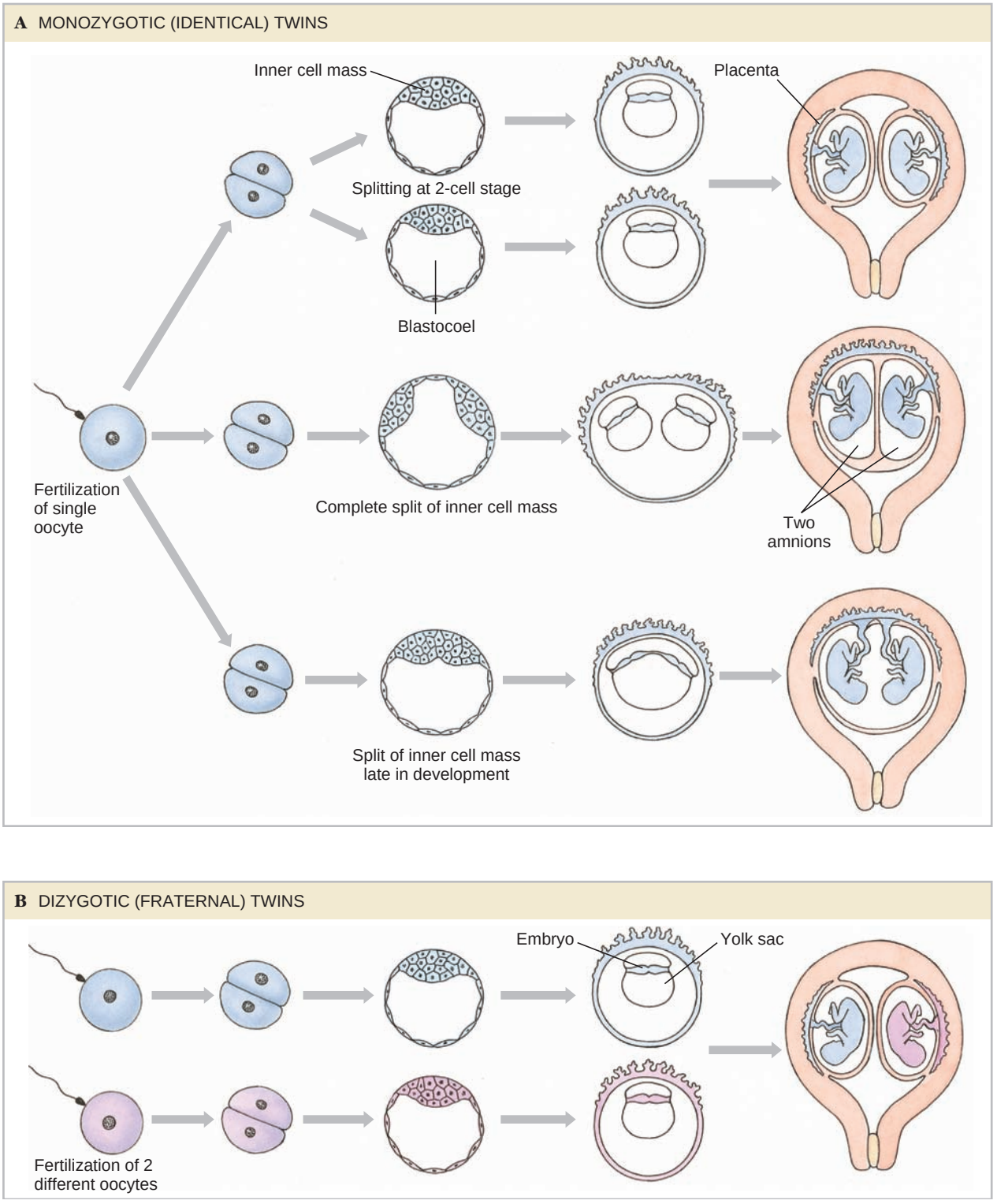


Figure 7.19

Formation of human twins. **A,** Monozygotic (identical) twin formation. **B,** Dizygotic (fraternal) twin formation. See text for explanation.

SUMMARY

Reproduction is the production of new life and provides an opportunity for evolution to occur. Asexual reproduction is a rapid and direct process by which a single organism produces genetically identical copies of itself. It may occur by fission, budding, gemmulation, or fragmentation. Sexual reproduction involves production of germ cells (sex cells or gametes), usually by two parents (bisexual reproduction), which combine by fertilization to form a zygote that develops into a new individual. Germ cells are formed by meiosis, reducing the number of chromosomes to haploid, and the diploid chromosome number is restored at fertilization. Sexual reproduction recombines parental characters and thus reshuffles and amplifies genetic diversity. Genetic recombination is important for evolution. Two alternatives to typical bisexual reproduction are hermaphroditism, the presence of both male and female organs in the same individual, and parthenogenesis, the development of an unfertilized egg.

Sexual reproduction exacts heavy costs in time and energy, requires cooperative investments in mating, and causes a 50% loss of genetic representation of each parent in the offspring. The classical view of why sex is needed is that it maintains variable offspring within the population, which may help the population to survive environmental change.

In vertebrates the primordial germ cells arise in the yolk-sac endoderm, then migrate to the gonad. In mammals, a gonad becomes a testis in response to masculinizing signals encoded on the Y chromosome of the male, and the reproductive tract masculinizes in response to circulating male sex steroids. Female reproductive structures (ovary, uterine tubes, uterus, and vagina) develop in the absence of signals encoded on the Y chromosome, although recent data suggests that a female-determining region on the X chromosome has a role in differentiation of female reproductive organs.

Germ cells mature in the gonads by a process called gametogenesis (spermatogenesis in males and oogenesis in females), involving both mitosis and meiosis. In spermatogenesis, each primary spermatocyte gives rise by meiosis and growth to four motile sperm, each bearing the haploid number of chromosomes. In oogenesis, each primary oocyte gives rise to only one mature, nonmotile, haploid ovum. The remaining nuclear material is discarded in polar bodies. During oogenesis an egg accumulates large food reserves within its cytoplasm.

Sexual reproductive systems vary enormously in complexity, ranging from some invertebrates, such as polychaete worms that lack any permanent reproductive structures to the complex systems of vertebrates and many invertebrates consisting of permanent gonads and various accessory structures for transferring, packaging, and nourishing gametes and embryos.

The male reproductive system of humans includes testes, composed of seminiferous tubules in which millions of sperm develop, and a duct system (vasa efferentia and vas deferens) that joins the urethra, glands (seminal vesicles, prostate, bulbourethral), and penis. The human female system includes ovaries, containing thousands of eggs within follicles; oviducts; uterus; and vagina.

The seasonal or cyclic nature of reproduction in vertebrates has required evolution of precise hormonal mechanisms that control production of germ cells, signal readiness for mating, and prepare ducts and glands for successful fertilization of eggs. Neurosecretory centers within the hypothalamus of the brain secrete gonadotropin-releasing hormone (GnRH), which stimulates endocrine cells of the anterior pituitary to release follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which in turn stimulate the gonads. Estrogens and progesterone in females, and testosterone and dihydrotestosterone (DHT) in males, control the growth of accessory sex structures and secondary sex characteristics, in addition to feeding back to the hypothalamus and anterior pituitary to regulate GnRH, FSH, and LH secretion.

In the human menstrual cycle, estrogen induces the initial proliferation of uterine endometrium. A surge in GnRH and LH, induced by rising estrogen levels from the developing follicle(s), midway in the cycle causes ovulation and the corpus luteum to secrete progesterone (and estrogen in humans), which completes preparation of the uterus for implantation. If an egg is fertilized, pregnancy is maintained by hormones produced by the placenta and mother. Human chorionic gonadotropin (hCG) maintains secretion of progesterone and estrogen from the corpus luteum, while the placenta grows and eventually secretes estrogen, progesterone, hCG, human placental lactogen (hPL), human placental growth hormone (hPGH), prolactin (PRL), endogenous opioids, placental corticotropin-releasing hormone (CRH), and relaxin. Estrogen, progesterone, PRL, and hPL, as well as maternal prolactin, induce development of the mammary glands in preparation for lactation. hPL, hPGH, and maternal growth hormone also increase nutrient availability for the developing embryo.

Birth or parturition (at least in most mammals) appears to be initiated by release of placental CRH. In addition, a decrease in progesterone and an increase in estrogen levels occur so that the uterine muscle begins to contract. Oxytocin (from the posterior pituitary) and uterine prostaglandins continue this process until the fetus (followed by the placenta) is expelled. Placental relaxin makes the birth process easier by enabling expansion of the pelvis and dilation of the cervix.

Multiple births in mammals may result from division of one zygote, producing identical, monozygotic twins, or from separate zygotes, producing fraternal, dizygotic twins. Identical twins in humans may have separate placentas, or (most commonly) they may share a common placenta but have individual amniotic sacs.

REVIEW QUESTIONS

1. Define asexual reproduction, and describe four forms of asexual reproduction in invertebrates.
2. Define sexual reproduction and explain why meiosis contributes to one of its great strengths.
3. Explain why genetic mutations in asexual organisms lead to much more rapid evolutionary change than do genetic mutations in sexual forms. Why might harmful mutations be more deleterious to asexual organisms compared with sexual organisms?

4. Define two alternatives to bisexual reproduction—hermaphroditism and parthenogenesis—and offer a specific example of each from the animal kingdom. What is the difference between ameiotic and meiotic parthenogenesis?
5. Define the terms dioecious and monoecious. Can either of these terms be used to describe a hermaphrodite?
6. A paradox of sexual reproduction is that despite being widespread in nature, the question of why it exists at all is still unresolved. What are some disadvantages of sex? What are some consequences of sex that make it so important?
7. What is a germ cell line? How do germ cells pass from one generation to the next?
8. Explain how a spermatogonium, containing a diploid number of chromosomes, develops into four functional sperm, each containing a haploid number of chromosomes. In what significant way(s) does oogenesis differ from spermatogenesis?
9. Define, and distinguish among, the terms oviparous, ovoviviparous, and viviparous.
10. Name the general location and give the function of the following reproductive structures: seminiferous tubules, vas deferens, urethra, seminal vesicles, prostate gland, bulbourethral glands, mature follicle, oviducts, uterus, vagina, endometrium.
11. How do the two kinds of mammalian reproductive cycles—estrous and menstrual—differ from each other?
12. What are the male sex hormones and what are their functions?
13. Explain how the female hormones GnRH, FSH, LH, and estrogen interact during the menstrual cycle to induce ovulation and, subsequently, formation of the corpus luteum.
14. Explain the function of the corpus luteum in the menstrual cycle. If fertilization of the ovulated egg happens, what endocrine events occur to support pregnancy?
15. Describe the role of pregnancy hormones during human pregnancy. What hormones prepare the mammary glands for lactation and what hormones continue to be important during this process?
16. If identical human twins develop from separate placentas, when must the embryo have separated? When must separation have occurred if the twins share a common placenta but develop within separate amnions?

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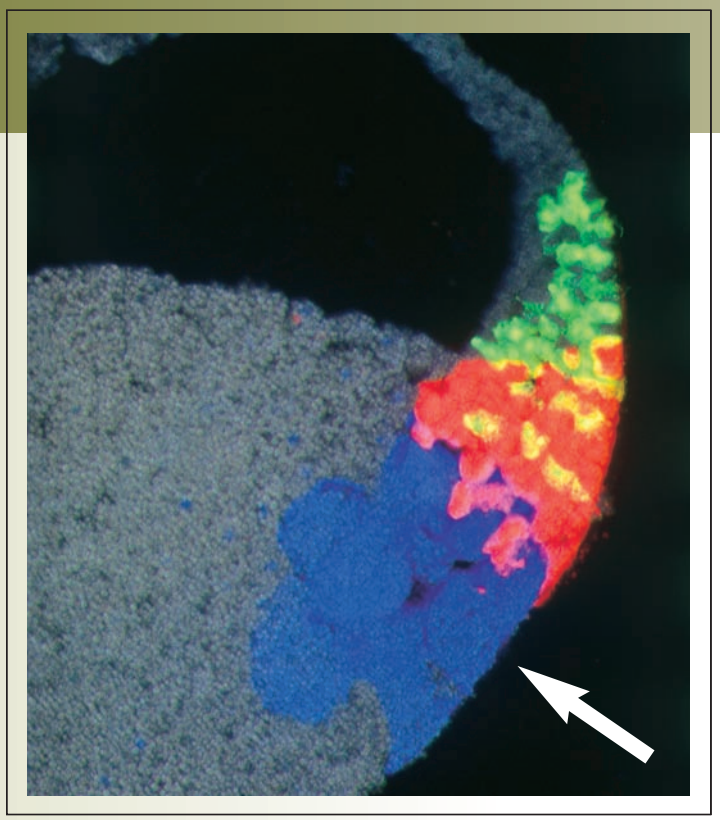
CHAPTER

8

Principles of Development



Spemann organizer cells (color) migrating from the dorsal lip (arrow) of a gastrula.

The Primary Organizer

During the first half of the twentieth century, experiments by the German embryologist Hans Spemann (1869 to 1941) and his student, Hilde Pröscholdt Mangold (1898 to 1924), ushered in the first of two golden ages of embryology. Working with salamanders, they found that tissue transplanted from one embryo into another could induce development of a complete organ, such as an eyeball, at the site of the transplant. This phenomenon is called embryonic induction. Mangold later discovered that one particular tissue, the dorsal lip from an embryonic stage called the gastrula, could induce the development of an entirely new salamander joined to the host salamander at the site of the transplant. (This work earned Spemann the Nobel Prize in Physiology or Medicine in 1935, but Hilde Mangold had died in a household accident only a few weeks after her research was published.) Spemann designated this dorsal lip tissue the **primary organizer,** now often called the **Spemann organizer.** Recent advances in molecular biology have inaugurated the second golden age of embryology, still in progress. During this current golden age we are beginning to understand that induction is due to secretion of certain molecules that trigger or repress the activity of combinations of genes in nearby cells. For example, cells of the Spemann organizer migrate over the dorsal midline, secreting proteins with names like noggin, chordin, and follistatin. These proteins allow nearby cells to develop into the nervous system and other tissues along the middle of the back, and those tissues in turn release other proteins that induce development of other parts of the body. Such organizer proteins do not occur only in salamanders; remarkably similar proteins function in development of other vertebrates and even invertebrates. Because all animals appear to share similar molecular mechanisms for development, it may now be possible to understand how changes in such developmental controls led to the evolution of the great variety of animals. Research in this area has given rise to the exciting new field called evolutionary developmental biology.

How is it possible that a tiny, spherical fertilized human egg, scarcely visible to the naked eye, can develop into a fully formed, unique person, consisting of thousands of billions of cells, each cell performing a predestined functional or structural role? How is this marvelous unfolding controlled? Clearly all information needed must originate from the nucleus and in the surrounding cytoplasm. But knowing where the control system lies is very different from understanding how it guides the conversion of a fertilized egg into a fully differentiated animal. Despite intense scrutiny by thousands of scientists over many decades, it seemed until very recently that developmental biology, almost alone among the biological sciences, lacked a satisfactory explanatory theory. This now has changed. During the last two decades the combination of genetics and evolution with modern techniques of cellular and molecular biology has provided the long-sought explanation of animal development. Causal relationships between development and evolution have also become the focus of research. We do at last appear to have a conceptual framework to account for development.

EARLY CONCEPTS: PREFORMATION VERSUS EPIGENESIS

Early scientists and laypeople alike speculated at length about the mystery of development long before the process was submitted to modern techniques of biochemistry, molecular biology, tissue culture, and electron microscopy. An early and persistent idea was that young animals were preformed in eggs and that development was simply a matter of unfolding what was already there. Some claimed they could actually see a miniature adult in the egg or sperm (Figure 8.1). Even the more cautious argued that all parts of the embryo were in the egg, but too small and transparent to be seen. The concept of **preformation** was strongly advocated by most seventeenth- and eighteenth-century naturalist-philosophers.

In 1759 German embryologist Kaspar Friedrich Wolff clearly showed that in the earliest developmental stages of the chick, there was no preformed individual, only undifferentiated granular material that became arranged into layers. These layers continued to thicken in some areas, to become thinner in others, to fold, and to segment, until the body of the embryo appeared. Wolff called this process **epigenesis** ("origin upon or after"), an idea that a fertilized egg contains building material only, somehow assembled by an unknown directing force. Current ideas of development are essentially epigenetic in concept, although we know far more about what directs growth and differentiation.

Development describes the progressive changes in an individual from its beginning to maturity (Figure 8.2). In sexual multicellular organisms, development usually begins with a fertilized egg that divides mitotically to produce a many-celled embryo. These cells then undergo extensive rearrangements and interact with one another to generate an animal's body plan and all of the many kinds of specialized cells in its body. This generation of cellular diversity does not occur all at once, but emerges sequentially by a **hierarchy of developmental decisions.** The many familiar cell types that make up the body do not simply "unfold" at some point, but arise from conditions created in preceding stages. At each stage of development new structures arise from the interaction of less committed rudiments. Each interaction is increasingly restrictive, and the decision made at each stage in the hierarchy further limits developmental fate. Once cells embark on a course of differentiation, they become irrevocably committed to that course. They no longer depend on the stage that preceded them, nor do they have the option

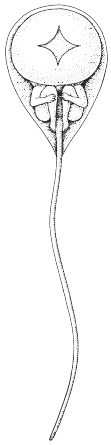


Figure 8.1
Preformed human infant in sperm as imagined by seventeenth-century Dutch histologist Niklaas Hartsoeker, one of the first to observe sperm, using a microscope of his own construction. Other remarkable pictures published during this period depicted the figure sometimes wearing a nightcap!

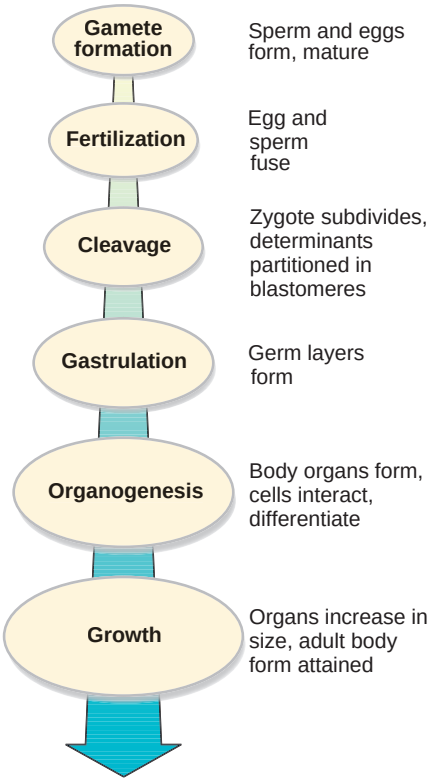


Figure 8.2
Key events in animal development.

of becoming something different. Once a structure becomes committed it is said to be **determined.** Thus the hierarchy of commitment is progressive and it is usually irreversible. The two basic processes responsible for this progressive subdivision are **cytoplasmic localization** and **induction.** We discuss both processes as we proceed through this chapter.

FERTILIZATION

The initial event in development in sexual reproduction is **fertilization,** the union of male and female gametes to form a **zygote.** Fertilization accomplishes two things: it provides for recombination of paternal and maternal genes, thus restoring the original diploid number of chromosomes characteristic of a species, and it activates the egg to begin development. However, sperm are not always required for development. Eggs of some species can be artificially induced to initiate development without sperm fertilization (artificial parthenogenesis), but in the great majority of cases an embryo will not be able to progress very far down the developmental path before lethal developmental abnormalities arise. However, some species have natural parthenogenesis (p. 140). Of these, some have eggs that develop normally in the absence of sperm. In other species (some fishes and salamanders), sperm is required for egg activation, but the sperm contributes no genetic material. Thus neither sperm contact nor the paternal genome is always essential for egg activation.

Oocyte Maturation

During oogenesis, described in the preceding chapter, an egg prepares itself for fertilization and for the beginning of development. Whereas a sperm eliminates all its cytoplasm and condenses its nucleus to the smallest possible dimensions, an egg grows in size by accumulating yolk reserves to support future growth. An egg's cytoplasm also contains vast amounts of messenger RNA, ribosomes, transfer RNA, and other elements required for protein synthesis. In addition, eggs of most species contain **morphogenetic determinants** that direct activation and repression of specific genes later in postfertilization development. The nucleus also grows rapidly in size during egg maturation, becoming bloated with RNA and so changed in appearance that it is given a special name, the **germinal vesicle.**

Most of this intense preparation occurs during an arrested stage of meiosis. In mammals, for example, it occurs during the prolonged prophase of the first meiotic division. The oocyte is now poised to resume meiotic divisions that are essential to produce a haploid female pronucleus that will join a male haploid pronucleus at fertilization. After resumption of meiosis, the egg rids itself of excess chromosomal material in the form of polar bodies (described in Chapter 7, p. 145). A vast amount of synthetic activity has preceded this stage. The oocyte is now a highly structured system, provided with a dowry which, after fertilization, will support nutritional requirements of the embryo and direct its development through cleavage.

Fertilization and Activation

Our current understanding of fertilization and activation derives in large part from more than a century of research on marine invertebrates, especially sea urchins. Sea urchins produce large numbers of eggs and sperm, which can be combined in the laboratory for study. Fertilization also has been studied in many vertebrates and, more recently, in mammals, using sperm and eggs of mice, hamsters, and rabbits.

Contact and Recognition Between Egg and Sperm

Most marine invertebrates and many marine fishes simply release their gametes into the ocean. Although an egg is a large target for a sperm, the enormous dispersing effect of the ocean and limited swimming range of a spermatozoon conspire against an egg and a sperm coming together by chance encounter. To improve likelihood of contact, eggs of numerous marine species release a chemotactic factor that attracts sperm to eggs. The chemotactic molecule is species-specific, attracting to eggs only sperm of the same species.

In sea urchin eggs, sperm first penetrate a jelly layer surrounding the egg, then contact an egg's vitelline envelope, a thin membrane lying just above the egg plasma membrane (Figure 8.3). At this point, egg-recognition proteins on the acrosomal process of the sperm (Figure 8.4) bind to species-specific sperm receptors on the vitelline envelope. This mechanism ensures that an egg recognizes only sperm of the same species. This is important in the marine environment where many closely related species may be spawning at the same time. Similar recognition proteins have been found on sperm of vertebrate species (including mammals) and presumably are a universal property of animals.

Prevention of Polyspermy

At the point of sperm contact with the egg vitelline envelope a **fertilization cone** appears into which the sperm head is later drawn (Figure 8.4). This event is followed immediately by

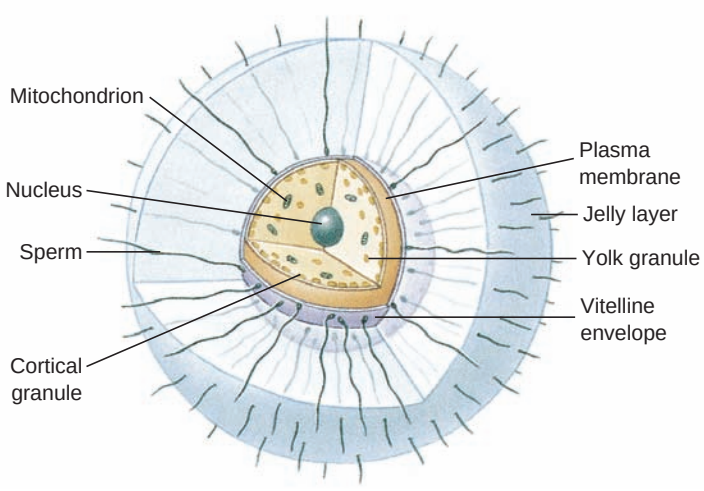


Figure 8.3
Structure of sea urchin egg during fertilization.

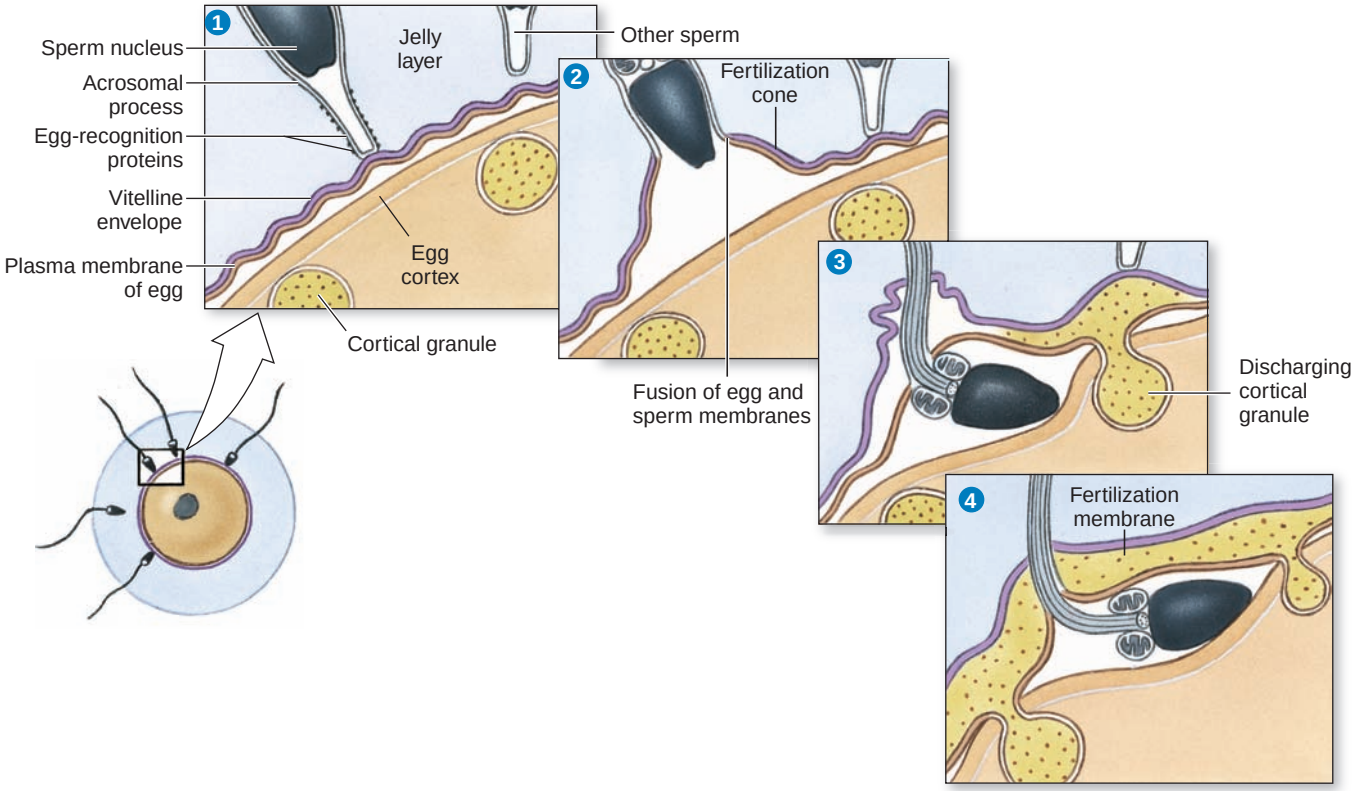


Figure 8.4

Sequence of events during sperm contact and penetration of a sea urchin egg.

important changes in the egg surface that block entrance of additional sperm, which, in marine eggs especially, may quickly surround the egg in swarming numbers (Figure 8.5). Entrance of more than one sperm, called **polyspermy,** must be prevented because union of more than two haploid nuclei would be ruinous for normal development. In a sea urchin egg, contact of the first sperm with the egg membrane is instantly followed by an electrical potential change in the egg membrane that prevents additional sperm from fusing with the membrane. This event, called the **fast block,** is followed immediately by the **cortical reaction,** in which thousands of enzyme-rich cortical granules, located just beneath the egg membrane, fuse with the membrane and release their contents into the space between the egg membrane and the overlying vitelline envelope (see Figure 8.4). The cortical reaction creates an osmotic gradient, causing water to rush into this space, elevating the envelope and lifting away all sperm bound to it, except the one sperm that has successfully fused with the egg membrane. One of the cortical granule enzymes causes the vitelline envelope to harden, and it is now called a **fertilization membrane.** The block to polyspermy is complete. Timing of these early events is summarized in Figure 8.6. Mammals have a similar security system that is erected within seconds after the first sperm fuses with the egg membrane.

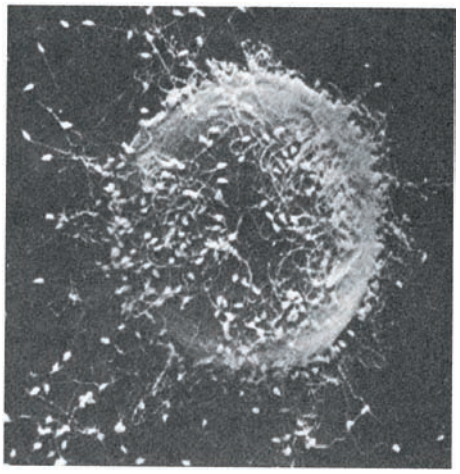


Figure 8.5

Binding of sperm to the surface of a sea urchin egg. Only one sperm penetrates the egg surface, the others being blocked from entrance by rapid changes in the egg membranes. Unsuccessful sperm are soon lifted away from the egg surface by a newly formed fertilization membrane.

Fusion of Pronuclei and Egg Activation

Once sperm and egg membranes have fused, the sperm loses its flagellum, which disintegrates. Its nuclear envelope then breaks apart, allowing the sperm chromatin to expand from its extremely condensed state. The enlarged sperm nucleus, now called a **pronucleus,** migrates inward to contact the female pronucleus. Their fusion forms the diploid **zygote nucleus.** Nuclear fusion takes only about 12 minutes in sea urchin eggs (Figure 8.6), but requires about 12 hours in mammals.

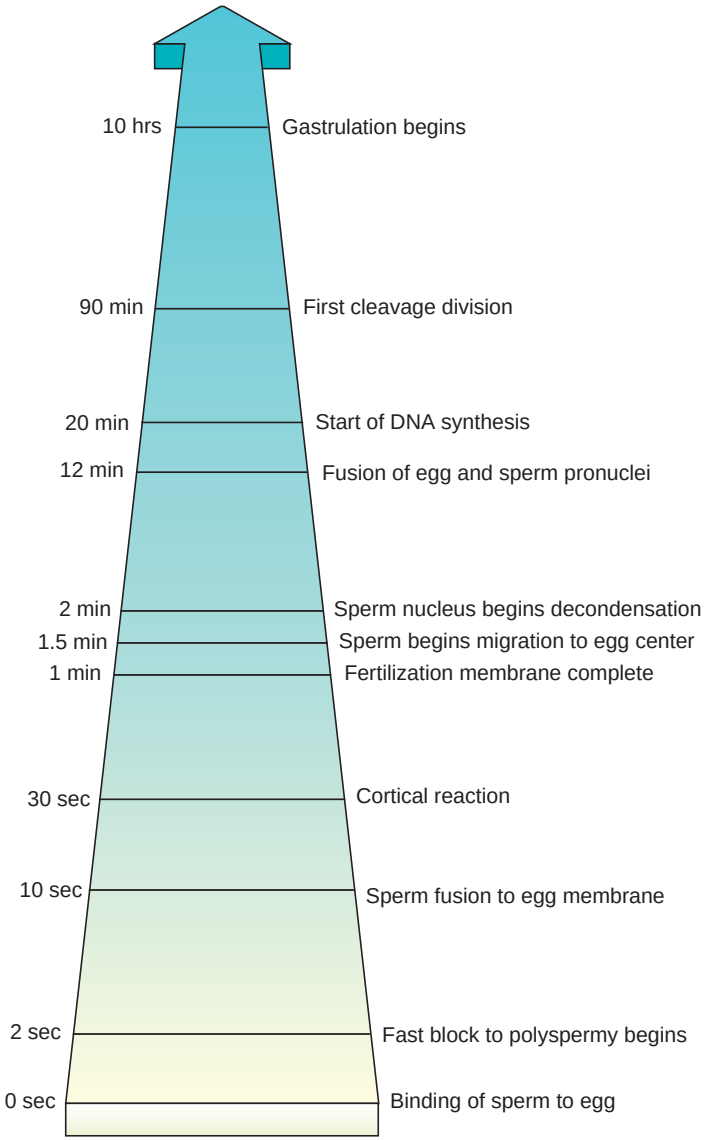


Figure 8.6
Timing of events during fertilization and early development in a sea urchin.

Fertilization sets in motion several important changes in the cytoplasm of an egg—now called a zygote—that prepare it for cleavage. Inhibitors that had blocked metabolism and kept the egg quiescent, in suspended-animation, are removed. Fertilization is immediately followed by a burst of DNA and protein synthesis, the latter utilizing the abundant supply of messenger RNA previously stored in the egg cytoplasm. Fertilization also initiates an almost complete reorganization of the cytoplasm within which are morphogenetic determinants that activate or repress specific genes as development proceeds. Movement of cytoplasm repositions the determinants into new and correct spatial arrangements that are essential for proper development. The zygote now enters cleavage.

In animal eggs, fertilization induces an increase in the amount of free calcium ions inside the egg cytoplasm. This increase in intracellular free calcium regulates later developmental events and is essential for normal development to occur in all taxa studied, but the mechanisms controlling calcium levels vary. In some taxa, calcium ions are released from intracellular stores, whereas in others calcium enters the egg from outside via voltage-gated calcium channels (see Chapter 3, p. 49). Some organisms combine both mechanisms. The calcium signal can occur in a single pulse, as it does in jellyfish, starfish, and frog zygotes, or in a series of closely spaced pulses identified in ribbon worms, polychaetes, and mammals. Researchers once thought that the calcium signaling pattern might vary as part of the developmental dichotomy between protostomes and deuterostomes, but this is not the case. In even the short list of taxa just given, the two chordate deuterostomes exhibit different calcium release patterns, suggesting that the differing patterns are more likely related to the number and duration of developmental events that require calcium signaling.

CLEAVAGE AND EARLY DEVELOPMENT

During cleavage the embryo divides repeatedly to convert the large, unwieldy cytoplasmic mass into a large cluster of small, maneuverable cells called **blastomeres.** No growth occurs during this period, only subdivision of mass, which continues until normal **somatic** cell size is attained. At the end of cleavage the zygote has been divided into many hundreds or thousands of cells and the blastula stage is formed.

Before cleavage begins, an animal-vegetal axis is visible on the embryo. This axis exists because yolk, nutrition for the developing embryo, occurs only at one end, establishing **polarity** in the embryo. The yolk-rich end is the **vegetal pole** and the other end is the **animal pole** (Figure 8.7B); the animal pole contains mostly cytoplasm and very little yolk. The animal-vegetal axis provides a landmark or reference point on the embryo. Cleavage is generally an orderly sequence of cell divisions so that one cell divides to form two cells, these each divide to form four cells, the four make eight cells, and the process continues. During each division, a distinct cleavage furrow is visible in the cell. This **cleavage furrow** can be parallel or perpendicular to the animal-vegetal axis.

How Amount and Distribution of Yolk Affect Cleavage

The amount of yolk at the vegetal pole varies among taxa. Eggs with very little yolk, evenly distributed throughout the egg (Figure 8.7A, C, and E), are called **isolecithal** (Gr. *isos,* equal + *lekithos,* yolk). **Mesolecithal** (Gr. *mesos,* middle + *lekithos,* yolk) eggs have a moderate amount of yolk concentrated at the vegetal pole (Figure 8.7B), whereas **telolecithal**

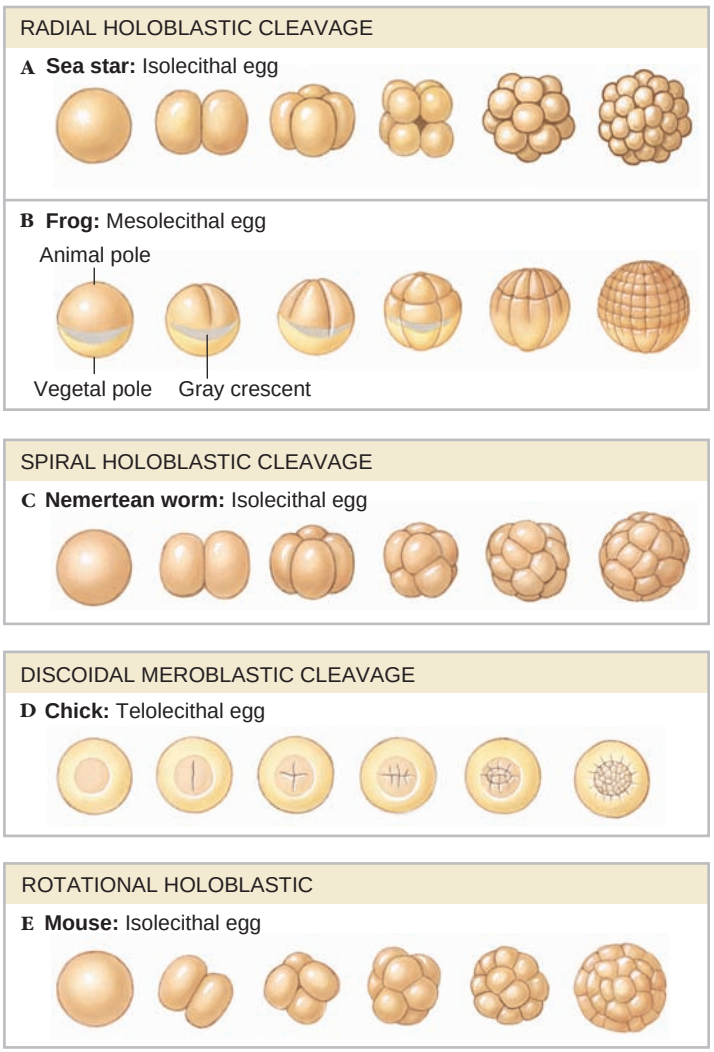


Figure 8.7

Cleavage stages in sea star, frog, nemertean worm, chick, and mouse. Yellow regions represent yolk in each diagram.

eggs (Gr. *telos,* end + *lekithos,* yolk) contain an abundance of yolk densely concentrated at the vegetal pole of the egg (Figure 8.7D). **Centrolecithal** eggs have a large, centrally located, mass of yolk.

The presence of yolk disrupts cleavage to varying degrees; when little yolk is present, cleavage furrows extend completely through the egg in **holoblastic** (Gr. *holo,* whole + *blastos,* germ) cleavage (Figure 8.7A, B, C, and E). When much yolk is present, cleavage is **meroblastic** (Gr. *meros,* part + *blastos,* germ), with cells sitting atop a mass of undivided yolk (Figure 8.7D). Meroblastic cleavage is incomplete because cleavage furrows cannot cut through the heavy concentration of yolk, but instead stop at the border between the cytoplasm and the yolk below.

Holoblastic cleavage occurs in isolecithal eggs and is present in echinoderms, tunicates, cephalochordates, nemerteans, and most molluscs, as well as in marsupial and placental mammals, including humans (Figure 8.7A, C, and E). Mesolecithal eggs also cleave holoblastically, but cleavage proceeds more slowly in the presence of yolk, leaving the vegetal region with a few large, yolk-filled cells, whereas the animal region has many small cells. Amphibian eggs (Figure 8.7B) illustrate this process.

Meroblastic cleavage occurs in telolecithal and centrolecithal eggs. In telolecithal eggs of birds, reptiles, most fishes, a few amphibians, cephalopod molluscs, and monotreme mammals, cleavage is restricted to cytoplasm in a narrow disc on top of the yolk (see chick development in Figure 8.7D). In centrolecithal eggs of insects and many other arthropods, cytoplasmic cleavage is limited to a surface layer of yolk-free cytoplasm, whereas the yolk-rich inner cytoplasm remains uncleaved (see Figure 8.15).

The function of yolk is to nourish the embryo. When much yolk is present, as in telolecithal eggs, young exhibit **direct development,** going from an embryo to a miniature adult. When little yolk is present, as in isolecithal or mesolecithal eggs, young develop into various larval stages capable of feeding themselves. In this **indirect development,** larvae differ from adults and must metamorphose into adult bodies (Figure 8.8). There is another way to compensate for absence of yolk: In most mammals, the mother nourishes the embryos by means of a placenta.

What Can We Learn From Development?

Biologists study development for different reasons. Some studies focus on understanding how the zygote, a single large cell, can produce the multitude of body parts in an organism.

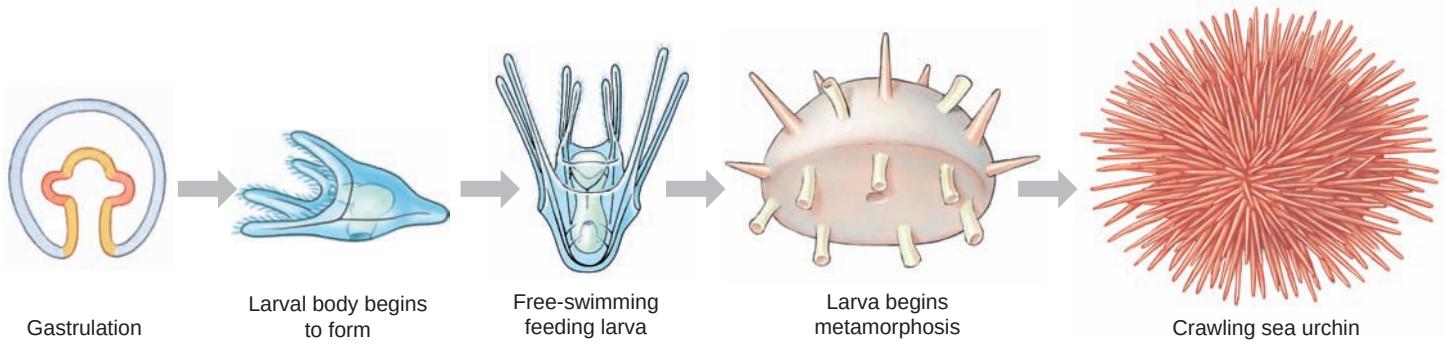


Figure 8.8

Indirect development in a sea urchin. After gastrulation, a free-swimming larva develops; it feeds and grows in ocean surface waters. The larva will metamorphose into a tiny bottom-dwelling sea urchin; the urchin feeds and grows, reaching sexual maturity in this body form.

Understanding the mechanisms of development requires knowledge of how cleavage partitions cytoplasm, how different cells interact, and how gene expression proceeds. These topics are covered on pages 170–174.

Another reason to study development is to search for commonalities among organisms. Commonalities in mechanisms of development are discussed on page 174, but there are also commonalities among organisms in the sequence of developmental events. All multicellular animals begin as zygotes and all go through cleavage and some subsequent developmental stages. Embryos of sponges, snails, and frogs diverge at some point to produce different adults. When does this divergence occur? All zygotes do not cleave in the same way; do certain types of cleavage characterize particular animal groups? Types of cleavage do characterize particular groups of animals, but cleavage type occurs along with other developmental features to form a suite of characters. Therefore, an overview of a developmental sequence is needed to explain other characters in the suite.

Based on these character suites, the 34 multicellular animal phyla fall into several distinct groups. Rather than attempting to grasp the details of 34 phyla, we can understand these phyla as variations on a much smaller number of developmental themes. The character suites are discussed on page 166, and in Chapter 9.

AN OVERVIEW OF DEVELOPMENT FOLLOWING CLEAVAGE

Blastulation

Cleavage subdivides the mass of the zygote until a cluster of cells called a **blastula** (Gr. *blastos,* germ, + *ule,* little) is formed (Figure 8.9). In mammals, the cluster of cells is called a blastocyst (see Figure 8.13E). In most animals, the cells are arranged around a central fluid-filled cavity (Figure 8.9) called a **blastocoel** (Gr. *blastos,* germ, + *koilos,* cavity). (A hollow blastula can be called a coeloblastula to distinguish it from a solid stereoblastula; the general account here assumes the blastula is hollow.) In the blastula stage, the embryo consists of a few hundred to several thousand cells poised for further development. There has been a great increase in total DNA content because each of the many daughter cell nuclei formed by chromosomal replication at mitosis contains as much DNA as the original zygote nucleus. The whole embryo, however, is no larger than the zygote.

Formation of a blastula stage, with its one layer of germ cells, occurs in all multicellular animals. In most animals, development continues beyond the blastula to form one or two more germ layers in a gastrula stage. Sponges were previously thought to complete embryogenesis with only a single layer of blastula cells, but recent work shows that cell migrations produce external and internal layers in embryos of at least some sponges (see Figure 12.12). Whether such cell layers are homologous to the true germ layers of other organisms is debated. The germ layers ultimately produce all structures of the adult body; the germ layer derivatives for vertebrates are shown in Figure 8.26.

Gastrulation and Formation of Two Germ Layers

Gastrulation converts the spherical blastula into a more complex configuration and forms a second germ layer (Figure 8.9). There is variation in the process (see pp. 167–170). In some cases a second layer forms as cells migrate inward without making an internal cavity, but generally one side of the blastula bends inward in a process called invagination. This inward bending continues until the surface of the bending region extends about one-third of the way into the blastocoel, forming a new internal cavity (Figure 8.9). Picture a sphere being pushed inward on one side—the inward region forms a pouch. The internal pouch is the gut cavity, called an **archenteron** (Gr. *archae,* old, + *enteron,* gut) or a **gastrocoel** (Gr. *gaster,* stomach, + *koilos,* cavity). It sits inside the now-reduced blastocoel. The opening to the gut, where the inward bending began, is the **blastopore** (Gr. *blastos,* germ, + *poros,* hole).

The **gastrula** (Gr. *gaster,* stomach, + *ule,* little) stage has two layers: an outer layer of cells surrounding the blastocoel, called **ectoderm** (Gr. *ecto,* out, + *deros,* skin), and an inner layer of cells lining the gut, called **endoderm** (Gr. *endon,* inside, + *deros,* skin). In forming a mental picture of the developmental process, remember that cavities or spaces can only be defined by their boundaries. Thus, the gut cavity is a space defined by the layer of cells surrounding it (Figure 8.9).

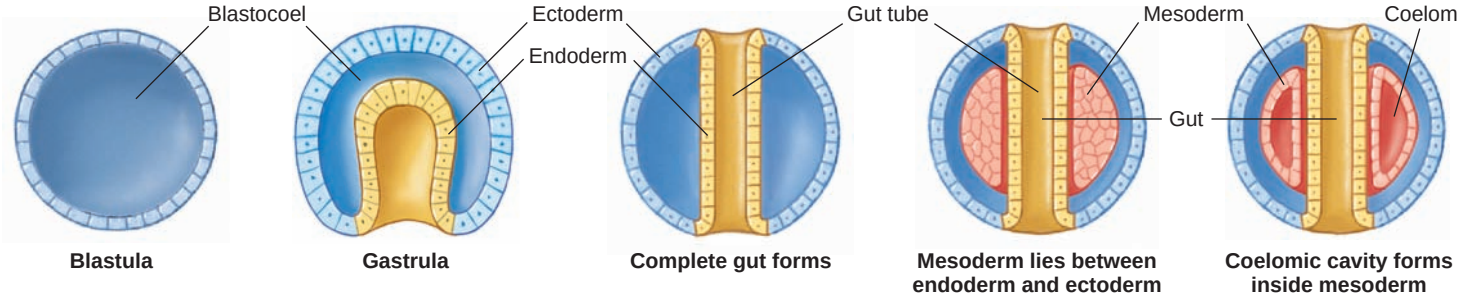


Figure 8.9
A generalized developmental sequence showing formation of three germ layers and two body cavities that persist into adulthood.

This gut opens only at the blastopore; it is called a blind or **incomplete gut.** Anything consumed by an animal with a blind gut must either be completely digested, or the undigested parts egested through the mouth. Certain animals, sea anemones and flatworms, for example, have a blind gut, sometimes called a gastrovascular cavity. However, most animals have a **complete gut** with a second opening, the anus (Figure 8.9). The blastopore becomes the mouth in organisms with one suite of developmental characters, but it becomes the anus in organisms with another such suite of characters (see Figure 8.10).

Formation of a Complete Gut

When a complete gut forms, the inward movement of the archenteron continues until the end of the archenteron meets the ectodermal wall of the gastrula. The archenteron cavity extends through the animal, and the ectoderm and endoderm layers join together. This joining produces an endodermal tube, the gut, surrounded by the blastocoel, inside an ectodermal tube, the body wall (Figure 8.9). The endodermal tube now has two openings, the blastopore and a second, unnamed, opening that formed when the archenteron tube merged with the ectoderm (Figure 8.9).

Formation of Mesoderm, a Third Germ Layer

The vast majority of multicellular animals proceed from a blastula to a gastrula, producing two germ layers. In one of many quirks of biological terminology, there is no term for organisms with only a single germ cell layer, but animals with two germ layers are called **diploblastic** (Gr. *diploos,* twofold, + *blastos,* germ). Diploblastic animals include sea anemones and comb jellies. Most animals have a third germ layer and are **triploblastic** (L. *tres,* three, + *blastos,* germ).

The third layer, **mesoderm** (Gr. *mesos,* middle, + *deros,* skin), eventually lies between the ectoderm and the endoderm (Figure 8.9). Mesoderm can form in two ways: cells arise from a ventral area near the lip of the blastopore and proliferate into the space between the archenteron and outer body wall (see Figure 8.13C), or the central region of the archenteron wall pushes outward into the space between the archenteron and outer body wall (see Figure 8.13A). Regardless of method, initial cells of mesoderm come from endoderm. (In a few groups, such as amphibians, part of a third layer of cells is made from ectoderm; this is called **ectomesoderm** (Gr. *ecto,* out, + *mesos,* middle, + *deros,* skin) to distinguish it from true, endodermally derived, mesoderm).

At the end of gastrulation, ectoderm covers the embryo, and mesoderm and endoderm have been brought inside (Figure 8.9). As a result, cells have new positions and new neighbors, so interactions among cells and germ layers then generate more of the body plan.

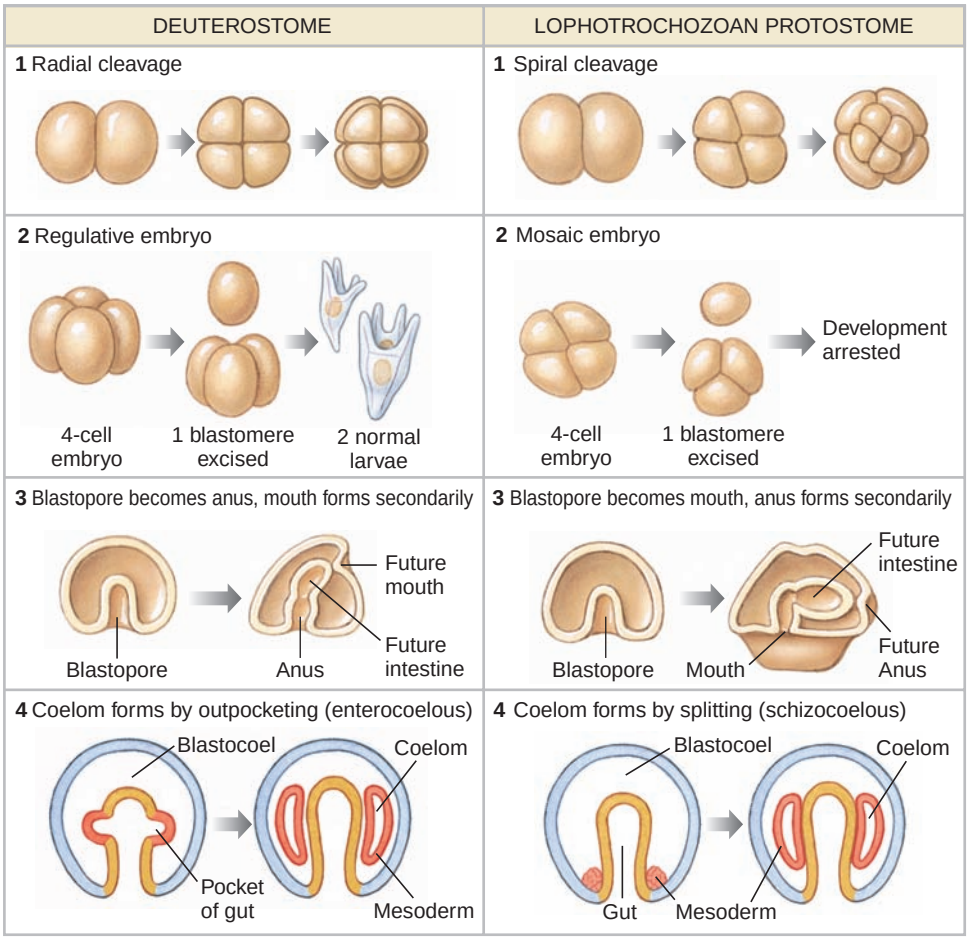


Figure 8.10

Developmental tendencies of lophotrochozoan protostomes (flatworms, annelids, molluscs, etc.) and deuterostomes. These tendencies are much modified in some groups, for example vertebrates. Cleavage in mammals is rotational rather than radial; in reptiles, birds, and many fishes cleavage is discoidal. Vertebrates have also evolved a derived form of coelom formation that is basically schizocoelous.

Formation of the Coelom

A **coelom** (Gr. *koilos,* cavity) is a body cavity completely surrounded by mesoderm; the band of mesoderm with its internal coelom lies inside the space previously occupied by the blastocoel (Figure 8.9). How did this happen? During gastrulation, the blastocoel is filled, partially or completely, with mesoderm. The coelomic cavity appears inside the mesoderm by one of two methods: **schizocoely** or **enterocoely.** These methods are discussed on pages 166 and 169. A coelom made by schizocoely is

functionally equivalent to a coelom made by enterocoely. The method by which the coelom forms is an inherited character useful for grouping organisms into the suites of developmental characters mentioned earlier.

When coelom formation is complete, the body has three germ layers and two cavities (Figure 8.9). One cavity is the gut cavity and the other is the fluid-filled coelomic cavity. The coelom, surrounded by its mesodermal walls, has completely filled the blastocoel. Mesoderm around the coelom will eventually produce layers of muscles, among other structures.

SUITES OF DEVELOPMENTAL CHARACTERS

There are two major groups of triploblastic animals, **protostomes** and **deuterostomes.** The groups are identified by a suite of four developmental characters: (1) radial or spiral positioning of cells as they cleave, (2) regulative or mosaic cleavage of cytoplasm, (3) fate of the blastopore to become mouth or anus, and (4) schizocoelous or enterocoelous formation of a coelom. Snails and earthworms, among others, belong to the protostomes. Sea stars, fishes, and frogs, among others, belong to the deuterostomes.

Deuterostome Development

Cleavage Patterns

Radial cleavage (Figure 8.10) is so named because the embryonic cells are arranged in radial symmetry around the animal-vegetal axis. In radial cleavage of sea stars, the first cleavage plane passes right through the animal-vegetal axis, yielding two identical daughter cells (blastomeres). For the second cleavage division, furrows form simultaneously in both blastomeres, and these are oriented parallel to the animal-vegetal axis (but perpendicular to the first cleavage furrow). Cleavage furrows next form simultaneously in the four daughter blastomeres, this time oriented perpendicular to the animal-vegetal axis, yielding two tiers of four cells each. An upper tier of cells sits directly atop the tier of cells below it (Figure 8.10). Subsequent cleavages yield an embryo composed of several tiers of cells.

A second characteristic of cleavage concerns the fates of isolated blastomeres and the cytoplasm they contain. This issue did not come to light until biologists undertook developmental experiments with embryos early in cleavage. Imagine a four-cell embryo (Figure 8.10). All cells in an organism are ultimately derived from these four cells, but when are the products of each cell decided? If one cell is removed from the mass, can the other cells continue developing to produce a normal organism?

Most deuterostomes have **regulative development** where the fate of a cell depends on its interactions with neighboring cells, rather than on what piece of cytoplasm it acquired during cleavage. In these embryos, at least early in development, each cell is able to produce an entire embryo if separated from the other cells (Figure 8.11). In other words, an early blastomere originally has the ability to follow more than one path of differentiation, but its interaction with other cells restricts its fate. If a blastomere is removed from an early embryo, the remaining blastomeres can alter their normal fates to compensate for the missing blastomere and produce a complete organism. This adaptability is termed regulative development.

Fate of Blastopore

A **deuterostome** (Gr. *deuteros,* second, + *stoma,* mouth) embryo develops through the blastula and gastrula stages, and forms a complete gut. The blastopore becomes the anus, and a second, unnamed, opening becomes the mouth, as is indicated by root words in the name of this group.

Coelom Formation

The final deuterostome feature concerns the origin of the coelom. In **enterocoely** (Gr. *enteron,* gut, + *koilos,* cavity), both mesoderm and coelom are made at the same time. In enterocoely, gastrulation begins with one side of the blastula bending inward to form the archenteron or gut cavity. As the archenteron continues to elongate inward, the sides of the archenteron push outward, expanding into a pouchlike coelomic compartment (see Figure 8.10). The coelomic compartment pinches off to form a mesodermally bound space surrounding the gut (see Figure 8.10). Fluid collects in this space. Notice that the cells

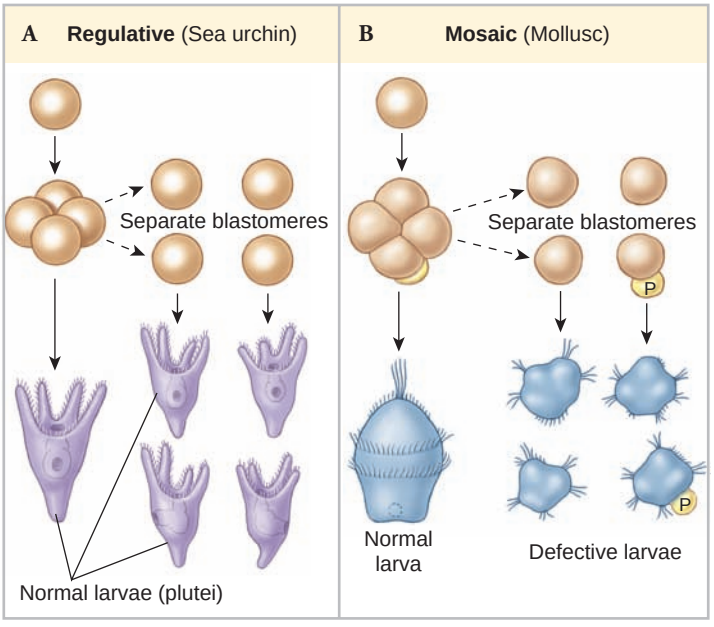


Figure 8.11

Regulative and mosaic cleavage. **A,** Regulative cleavage. Each of the early blastomeres (such as that of a sea urchin) when separated from the others develops into a small pluteus larva. **B,** Mosaic cleavage. In a mollusc, when blastomeres are separated, each gives rise to only a part of an embryo. The larger size of one defective larva is the result of the formation of a polar lobe (P) composed of clear cytoplasm of the vegetal pole, which this blastomere alone receives.

that form the coelom during enterocoely come from a different region of the endoderm than do those making the coelom during schizocoely (see Figure 8.10).

Examples of Deuterostome Development

The general outline of deuterostome development just given varies in some of its details depending upon the animal being studied. The presence of large amounts of yolk in some embryos further complicates the developmental sequence. A few examples of specific developmental sequences illustrate this variation.

Variations in Deuterostome Cleavage The typical deuterostome pattern is radial cleavage, but ascidian chordates (also called tunicates) exhibit **bilateral cleavage.** In ascidian eggs, the anteroposterior axis is established prior to fertilization by asymmetrical distribution of several cytoplasmic components (Figure 8.12). The first cleavage furrow passes through the animal-vegetal axis, dividing the asymmetrically distributed cytoplasm equally between the first two blastomeres. Thus, this first cleavage division separates the embryo into its future right and left sides, establishing its bilateral symmetry (hence the name bilateral holoblastic cleavage). Each successive division orients itself to this plane of symmetry, and the half-embryo formed on one side of the first cleavage is the mirror image of the half embryo on the other side.

Most mammals possess isolecithal eggs and a unique cleavage pattern called **rotational cleavage,** so called because of the orientation of blastomeres with respect to each other during the second cleavage division (see mouse development in Figure 8.7E). Cleavage in mammals is slower than in any other animal group. In humans, the first division is completed about 36 hours after fertilization (compared with about an hour and a half in sea urchins), and the next divisions follow at 12- to 24-hour intervals. As in most other animals, the first cleavage plane runs through the animal-vegetal axis to yield a two-cell embryo. However, during the second cleavage one of these blastomeres divides meridionally (through the animal-vegetal axis) while the other divides equatorially (perpendicular to the animal-vegetal axis). Thus, the cleavage plane in one blastomere is rotated 90 degrees with respect to the cleavage plane of the other blastomere (hence the name rotational cleavage). Furthermore, early divisions are asynchronous; not all blastomeres divide at the same time. Thus, mammalian embryos may not increase regularly from two to four to eight blastomeres, but often contain odd numbers of cells. After the third division, the cells suddenly close into a tightly packed configuration, which is stabilized by tight junctions that form between outermost cells of the embryo. These outer cells form the **trophoblast.** The trophoblast is not part of the embryo proper but will form the embryonic portion of the placenta when the embryo implants in the uterine wall. Cells that actually give rise to the embryo proper form from the inner cells, called the **inner cell mass** (see blastula stage in Figure 8.13E).

Telolecithal eggs of reptiles, birds, and most fish divide by **discoidal cleavage.** Because of the great mass of yolk in these eggs, cleavage is confined to a small disc of cytoplasm lying atop a mound of yolk (see chick development in Figure 8.7D). Early cleavage furrows carve this cytoplasmic disc to yield a single layer of cells called the blastoderm. Further cleavages divide the blastoderm into five to six layers of cells (Figure 8.13D).

Variations in Deuterostome Gastrulation In sea stars, gastrulation begins when the entire vegetal area of the blastula flattens to form a **vegetal plate** (a sheet of epithelial tissue). This event is followed by a process called **invagination,** in which the vegetal plate bends inward and extends about one-third of the way into the blastocoel, forming the archenteron (Figure 8.13A). Coelomic formation is typical of enterocoely. As the archenteron continues to elongate toward the animal pole, and its anterior end expands into two pouchlike **coelomic vesicles,** which pinch off to form left and right coelomic compartments (Figure 8.13A).

The **ectoderm** gives rise to the epithelium of the body surface and to the nervous system. The **endoderm** gives rise to the epithelial lining of the digestive tube. The outpocketing of the archenteron is the origin of **mesoderm.** This third germ layer will form the muscular system, reproductive system, peritoneum (lining of the coelomic compartments), and the calcareous plates of the sea star's endoskeleton.

Frogs are deuterostomes with radial cleavage but morphogenetic movements of gastrulation are greatly influenced by the mass of inert yolk in the vegetal half of the embryo. Cleavage divisions are slowed in this half so that the resulting blastula consists of many small cells in the animal half and a few large cells in the vegetal half (see Figures 8.7B and 8.13B). Gastrulation in amphibians begins when cells located at the future dorsal side of the embryo invaginate to form a slitlike blastopore. Thus, as in sea stars, invagination initiates archenteron formation, but amphibian gastrulation begins in the marginal zone of the blastula, where animal and vegetal hemispheres come together, and where there is less yolk than in the vegetal region. Gastrulation progresses as sheets of cells in the marginal zone turn inward over the blastopore lip and move inside the gastrula to form mesoderm and endoderm (see opening figure of this chapter, p. 158). The three germ layers now formed are the primary structural layers that play crucial roles in further differentiation of the embryo.

Figure 8.12

Bilateral cleavage in tunicate embryos. The first cleavage division divides the asymmetrically distributed cytoplasm evenly between the first two blastomeres, establishing the future right and left sides of the adult animal. Bilateral symmetry of the embryo is maintained through subsequent cleavage divisions.

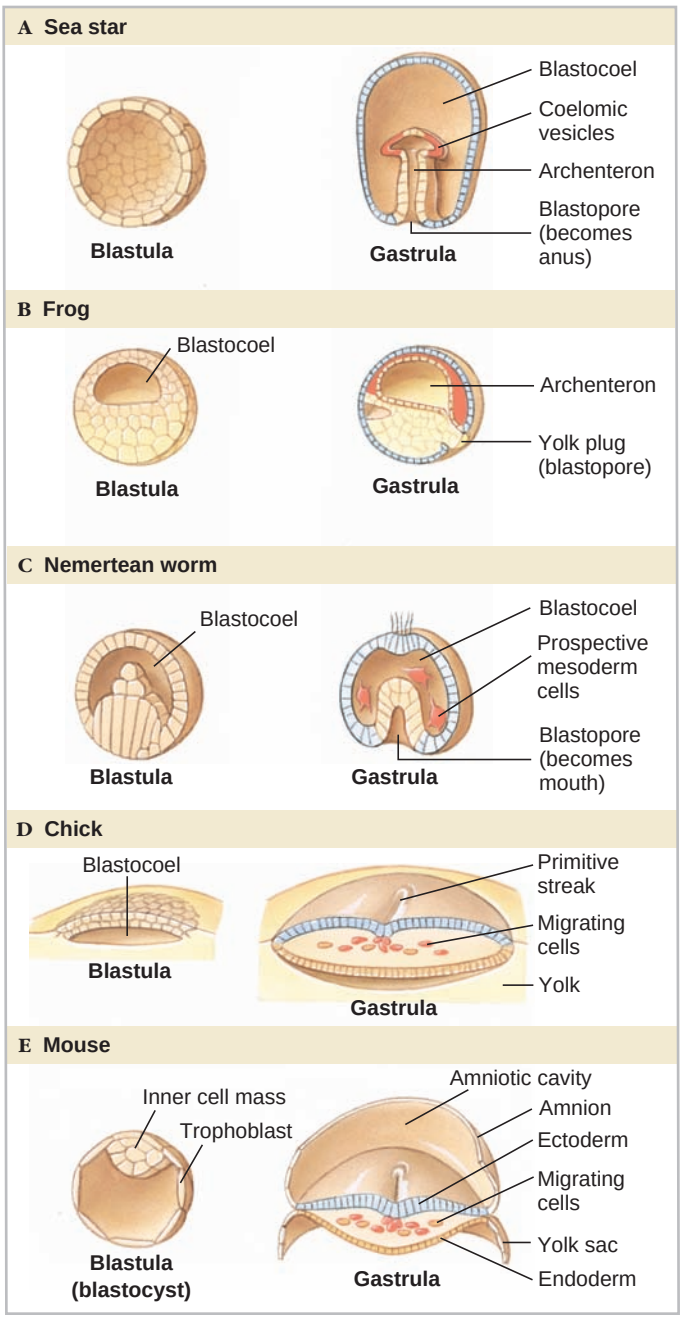


Figure 8.13

Blastula and gastrula stages in embryos of sea star, frog, nemertean worm, chick, and mouse.

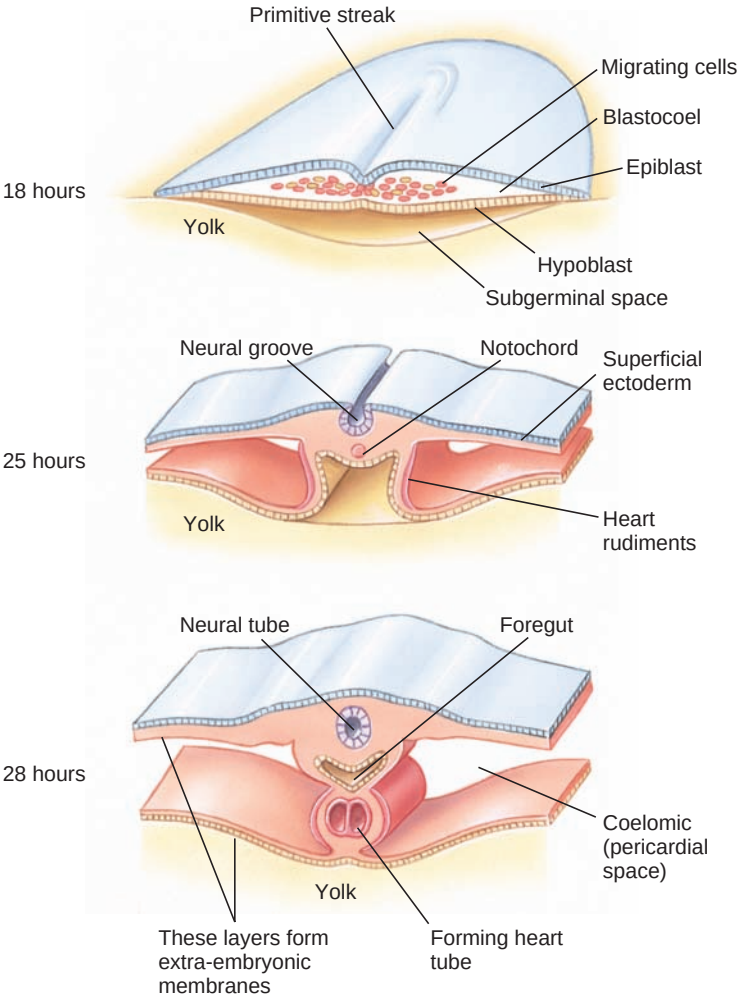


Figure 8.14

Gastrulation in a chick. Transverse sections through the heart-forming region of the chick show development at 18, 25, and 28 hours of incubation.

In bird and reptile embryos (see Figure 8.13D), gastrulation begins with a thickening of the blastoderm at the caudal end of the embryo, which migrates forward to form a **primitive streak** (Figure 8.14). The primitive streak becomes the anteroposterior axis of the embryo and the center of early growth. The primitive streak is homologous to the blastopore of frog embryos, but in chicks it does not open into the gut cavity because of the obstructing mass of yolk. The blastoderm consists of two layers (epiblast and hypoblast) with a blastocoel between them. Cells of the epiblast move as a sheet toward the primitive streak, then roll over the edge and migrate as individual cells into the blastocoel. These migrating cells separate into two streams. One stream of cells moves deeper (displacing the hypoblast along the midline) and forms endoderm. The other stream moves between the epiblast and hypoblast to form mesoderm. Cells on the surface of the embryo compose the ectoderm. The embryo now has three germ layers, at this point arranged as sheetlike layers with ectoderm on top and endoderm at the bottom. This arrangement changes, however, when all three germ layers lift from the underlying yolk (Figure 8.14), then fold under to form a three-layered embryo that is pinched off from the yolk except for a stalk attachment to the yolk at midbody (see Figure 8.22).

Gastrulation in mammals is remarkably similar to gastrulation in reptiles and birds (see Figure 8.13E). Gastrulation movements in the inner cell mass produce a primitive streak. Epiblast cells move medially through the primitive streak into the blastocoel, and individual cells then migrate laterally through the blastocoel to form mesoderm and endoderm. Endoderm cells

(derived from the hypoblast) form a yolk sac devoid of yolk (since mammalian embryos derive nutrients directly from the mother via the placenta).

Amphibians, reptiles, and birds, which have moderate to large amounts of yolk concentrated in the vegetal region of the egg, have evolved derived gastrulation patterns in which the yolk does not participate in gastrulation. Yolk is an impediment to gastrulation and consequently the gastrulation process occurs around (amphibians) or on top (reptiles and birds) of the vegetal yolk. Mammalian eggs are isolecithal, and thus one might expect them to have a gastrulation pattern similar to that of sea stars. Instead they have a pattern more suited to telolecithal eggs. The best explanation for this feature of mammalian egg development is common ancestry with birds and reptiles. Reptiles, birds, and mammals share a common ancestor whose eggs were telolecithal. Thus, all three groups inherited their gastrulation patterns from this common ancestor, and mammals subsequently evolved isolecithal eggs but retained the telolecithal gastrulation pattern.

A further developmental complication in vertebrates is that coelom formation occurs by a modified form of schizocoely (see Figure 8.10), not enterocoely. The nonvertebrate chordates form the coelom by enterocoely, as is typical of deuterostomes.

Protostome Development

Cleavage Patterns

Spiral cleavage (see Figure 8.10) occurs in most protostomes. It differs from radial cleavage in two important ways. Rather than dividing parallel or perpendicular to the animal-vegetal axis, blastomeres cleave obliquely (approximately 45-degree angle) to this axis and typically produce quartets of cells that come to lie, not on top of but in the furrows between cells of the underlying layer. The upper layer of cells appears offset (shifted in a spiral fashion) from the lower (see Figure 8.10). In addition, spirally cleaving blastomeres pack themselves tightly together much like a group of soap bubbles, rather than just lightly contacting each other as do many radially cleaving blastomeres (see Figure 8.10).

Mosaic development characterizes most protostomes (see Figure 8.10). In mosaic development, cell fate is determined by the distribution of certain proteins and messenger RNAs, called **morphogenetic determinants,** in the egg cytoplasm. As cleavage occurs, these morphogenetic determinants are partitioned among the cells unequally. When a particular blastomere is isolated from the rest of the embryo, it still forms the characteristic structures decided by the morphogenetic determinants it contains (see Figure 8.11). In the absence of a particular blastomere, the animal lacks those structures normally formed by that blastomere, so it cannot develop normally. This pattern is called mosaic development because the embryo seems to be a mosaic of self-differentiating parts.

Fate of Blastopore

A **protostome** (Gr. *protos,* first, + *stoma,* mouth) is so named because the blastopore becomes the mouth, and the second, unnamed, opening becomes the anus.

Coelom Formation

In protostomes, a mesodermal band of tissue surrounding the gut forms before a coelom is made. If present, the inner coelomic cavity is made by **schizocoely.** To form mesoderm, endodermal cells arise ventrally at the lip of the blastopore (see Figure 8.10) and move, via **ingression,** into the space between the walls of the archenteron (endoderm) and outer body wall (ectoderm). These cells divide and deposit new cells, called mesodermal precursors, between the two existing cell layers (see Figure 8.13C). The proliferating cells become the mesoderm. Meticulous cell-lineage studies by embryologists established that in many organisms with spiral cleavage, for example, flatworms, snails, and related organisms, these mesodermal precursors arise from one large blastomere, called the 4d cell, that is present in a 29- to 64-cell embryo.

Some protostomes do not develop a coelom. Flatworms, like *Planaria,* develop to an early gastrula stage and then form a mesodermal layer as just described. Mesoderm completely fills the blastocoel and a coelom never forms (see Figure 9.3). Animals without a coelom are called **acoelomate.** In other protostomes, mesoderm lines only one side of the blastocoel, leaving a fluid-filled blastocoel next to the gut (see Figure 9.3). The fluid-filled cavity surrounding the gut is called a **pseudocoelom** (Gr. *pseudés,* false, + *koilos,* cavity); it is bordered on the inner edge by the endodermal gut lining, and on the outer edge by a layer of mesoderm next to the ectoderm. Thus, a pseudocoelom has mesoderm on only one side, whereas a true **coelom** is a fluid-filled cavity completely surrounded by mesoderm (see Figure 9.3). Acoelomate and pseudocoelomate body plans are discussed in more detail in Chapter 9.

For **coelomate** protostomes, like earthworms and snails, the mesodermal layer forms as just described, and a coelom is made by **schizocoely** (Gr. *schizein,* to split, + *koilos,* cavity). A coelom arises, as the name suggests, when the mesodermal band around the gut splits open centrally (see Figure 8.10). Fluid collects in the coelom.

Examples of Protostome Development

The protostomes are divided into two clades. One clade, **lophotrochozoan protostomes,** contains segmented worms, molluscs (snails, slugs, clams, octopus and their kin) and several less familiar taxa. The name of this clade refers to two features present in some members of the group: a horseshoe-shaped whorl of tentacles called a **lophophore** (see pp. 324–328), and a **trochophore larva** (see p. 337). Lophotrochozoans have the four protostome features described previously (see Figure 8.10). They typically form mesoderm from the embryonic 4d cell.

The other clade, **ecdysozoan protostomes,** includes arthropods (insects, spiders, crabs, and related organisms), roundworms, and other taxa which molt the exoskeleton. The name of this clade refers to shedding of the cuticle, **ecdysis** (Gr. *ekdyo,* take off, strip).

Variations in Protostome Cleavage Spiral cleavage is typical of protostomes, but one highly specialized class of molluscs,

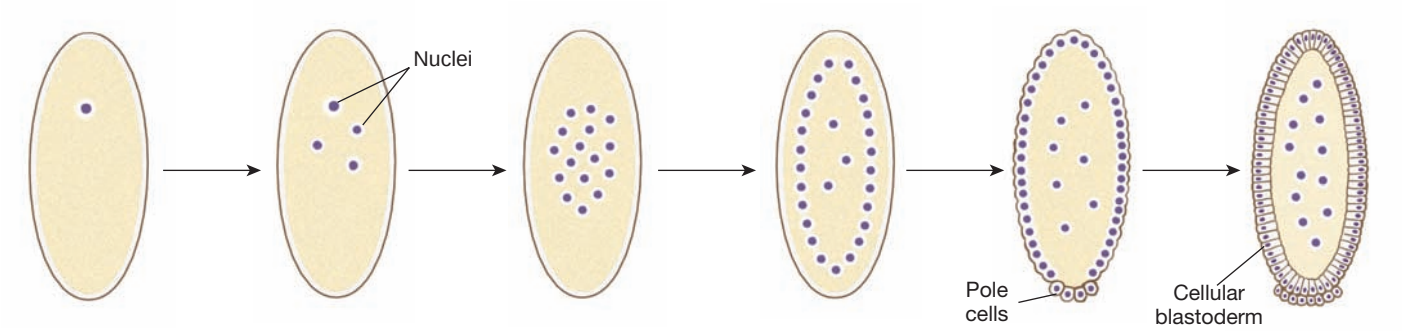


Figure 8.15

Superficial cleavage in a *Drosophila* embryo. The zygote nucleus at first divides repeatedly in the yolk-rich endoplasm by mitosis without cytokinesis. After several rounds of mitosis, most nuclei migrate to the surface where they are separated by cytokinesis into separate cells. Some nuclei migrate to the posterior pole to form the primordial germ cells, called pole cells. Several nuclei remain in the endoplasm where they will regulate breakdown of yolk products. The cellular blastoderm stage corresponds to the blastula stage of other embryos.

cephalopods, has bilateral cleavage like that of ascidian chordates (see p. 167 and Figure 8.12). Octopus, squid, and cuttlefish, among others, are cephalopods.

Many ecdysozoans do not exhibit spiral cleavage; in some, cleavage appears radial, and in others, such as insects, cleavage is neither spiral nor radial.

Centrolecithal eggs of insects undergo **superficial cleavage** (Figure 8.15) where the centrally located mass of yolk restricts cleavage to the cytoplasmic rim of the egg. This pattern is highly unusual because cytoplasmic cleavage (cytokinesis) does not occur until after many rounds of nuclear division. After roughly eight rounds of mitosis in the absence of cytoplasmic division (yielding 256 nuclei), the nuclei migrate to the yolk-free periphery of the egg. A few nuclei at the posterior end of the egg become surrounded by cytoplasm to form pole cells, which give rise to germ cells of the adult. Next, the entire egg cell membrane folds inward, partitioning each nucleus into a single cell, and yielding a layer of cells at the periphery surrounding the mass of yolk (Figure 8.15). Because yolk is an impediment to cleavage, this pattern avoids cleaving the yolk and instead confines cytoplasmic division to small regions of yolk-free cytoplasm.

Variations in Protostome Gastrulation In most protostomes, mesodermal cells all derive from the 4d cell (see p. 169). However, in some nemertean worms (see Figure 8.13C), mesoderm derives from an earlier blastomere. Mesodermal origins are difficult to determine in many ecdysozoan protostomes due to the modified pattern of cleavage.

MECHANISMS OF DEVELOPMENT

Nuclear Equivalence

How does a developing embryo generate a multitude of cell types of a complete multicellular organism from the starting point of a single diploid nucleus of a zygote? To many nineteenth-century embryologists there seemed only one acceptable answer: as cell division ensued, hereditary material had to be parceled unequally to daughter cells. In this view, the genome gradually became broken into smaller and smaller units until finally only the information required to impart the characteristics of a single cell type remained. This became known as the Roux-Weismann hypothesis, after the two German embryologists who developed the concept.

However, in 1892 Hans Driesch discovered that if he mechanically shook apart a two-celled sea urchin into separate cells, both half-embryos developed into normal larvae. Driesch concluded that both cells contained all genetic information of the original zygote. Still, this experiment did not settle the argument, because many embryologists thought that even if all cells contained complete genomes, the nuclei might become progressively modified in some way to dispense with the information not used in forming differentiated cells.

> The efforts of Hans Driesch to disrupt egg development are poetically described by Peattie: "Behold Driesch grinding the eggs of Loeb's favorite sea urchin up between plates of glass, pounding and breaking and deforming them in every way. And when he ceased from thus abusing them, they proceeded with their orderly and normal development. Is any machine conceivable, Driesch asks, which could thus be torn down . . . have its parts all disarranged and transposed, and still have them act normally? One cannot imagine it. But of the living egg, fertilized or not, we can say that there lie latent within it all the potentialities presumed by Aristotle, and all of the sculptor's dream of form, yes, and the very power in the sculptor's arm." From Peattie, D. C. 1935. *An Almanac for Moderns*. New York, G. P. Putnam's Sons.

Around the turn of the century Hans Spemann introduced a new approach to testing the Roux-Weismann hypothesis. Spemann placed minute ligatures of human hair around salamander zygotes just as they were about to divide, constricting them until they were almost, but not quite, separated into two halves. The nucleus lay in one half of the partially divided zygote; the other side was anucleate, containing only cytoplasm. The zygote then completed its first cleavage division on the side containing

the nucleus; the anucleate side remained undivided. Eventually, when the nucleated side had divided into about 16 cells, one of the cleavage nuclei would wander across the narrow cytoplasmic bridge to the anucleate side. Immediately this side began to divide and developed normally.

Sometimes, however, Spemann observed that the nucleated half of the embryo developed only into an abnormal ball of "belly" tissue. The explanation, Spemann discovered, depended on the presence of the gray crescent, a pigment-free area shown in Figure 8.7B. The gray crescent is required for normal development because it is the precursor of the Spemann organizer discussed in the opening essay on page 158.

Spemann's experiment demonstrated that every blastomere contains sufficient genetic information for the development of a complete animal. In 1938 he suggested another experiment that would demonstrate that even somatic cells of an adult contain a complete genome. The experiment, which Spemann characterized as being "somewhat fantastical" at that time, would be to remove the nucleus of an egg cell and replace it with the nucleus from a somatic cell from a different individual. If all cells contained the same genetic information as a zygote, then the embryo should develop into an individual that is genetically identical to the animal from which the nucleus was obtained. It took several decades to solve the technical difficulties, but the experiment was successfully performed on amphibians, and today it is done in a variety of mammals. The procedure is now familiarly called **cloning.** One of the most famous cloned mammals, Dolly the sheep, got the genetic material in her nuclei from the mammary glands of a six-year-old ewe.

If all nuclei are equivalent, what causes some cells to develop into neurons while others develop into skeletal muscle? In most animals (excluding insects), there are two major ways by which cells become committed to particular developmental fates: (1) cytoplasmic partitioning of determinative molecules during cleavage and (2) interaction with neighboring cells (inductive interactions). All animals use both of these mechanisms to some extent to specify different cell types. However, in some animals cytoplasmic specification is dominant, whereas others rely predominantly on inductive interactions.

Cytoplasmic Specification

A fertilized egg contains cytoplasmic components that are unequally distributed within the egg. These different cytoplasmic components are thought to contain morphogenetic determinants that control commitment of a cell to a particular cell type. These morphogenetic determinants are partitioned among different blastomeres as a result of cleavage, and the developmental fate of each cell becomes specified by the type of cytoplasm it acquires during development (see mosaic development, p. 169).

This process is especially striking (and easily visualized) in some tunicate species in which the fertilized egg contains as many as five differently colored types of cytoplasm (see Figure 8.12). These differently pigmented cytoplasms are segregated into different blastomeres, which then proceed to form distinct tissues or organs. For example, yellow cytoplasm gives rise to muscle cells while gray equatorial cytoplasm produces notochord and neural tube. Clear cytoplasm produces larval epidermis and gray vegetal cytoplasm gives rise to the gut.

Embryonic Induction

Induction, the capacity of some cells to evoke a specific developmental response in others, is a widespread phenomenon in development. The classic experiments, cited in the opening essay on page 158, were reported by Hans Spemann and Hilde Mangold in 1924. When a piece of dorsal blastopore lip from a salamander gastrula was transplanted into a ventral or lateral position of another salamander gastrula, it invaginated and developed a notochord and somites. It also induced the *host* ectoderm to form a neural tube. Eventually a whole system of organs developed where the graft was placed, and then grew into a nearly complete secondary embryo (Figure 8.16). This creature was composed partly of grafted tissue and partly of induced host tissue.

It was soon found that *only* grafts from the dorsal lip of the blastopore were capable of inducing the formation of a complete or nearly complete secondary embryo. This area corresponds to the presumptive areas of notochord, somites, and prechordal plate (see p. 500). It was also found that only ectoderm of the host would develop a nervous system in the graft and that the reactive ability was greatest at the early gastrula stage and declined as the recipient embryo got older.

Spemann designated the dorsal lip area the **primary organizer** because it was the only tissue capable of inducing the

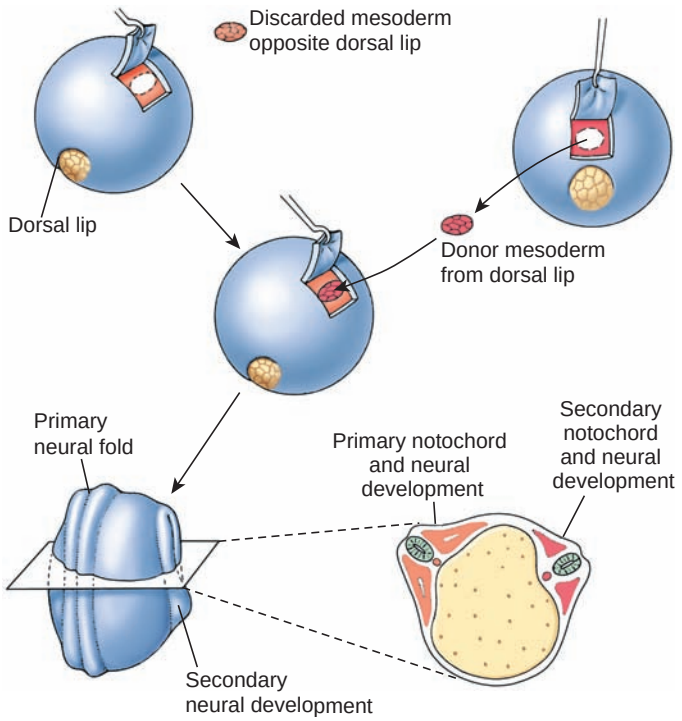


Figure 8.16
The Spemann-Mangold primary organizer experiment.

development of a secondary embryo in the host. It is now often called the Spemann organizer. Spemann also termed this inductive event **primary induction** because he considered it the first inductive event in development. Subsequent studies showed that many other cell types originate by later inductive interactions, a process called **secondary induction.**

Usually cells that have differentiated act as inductors for adjacent undifferentiated cells. Timing is important. Once a primary inductor sets in motion a specific developmental pattern in some cells, numerous secondary inductions follow. What emerges is a sequential pattern of development involving not only inductions but cell movement, changes in adhesive properties of cells, and cell proliferation. There is no "hard-wired" master control panel directing development, but rather a sequence of local patterns in which one step in development is a subunit of another. In showing that each step in the developmental hierarchy is a necessary preliminary for the next, Hans Spemann's induction experiments were among the most significant events in experimental embryology.

GENE EXPRESSION DURING DEVELOPMENT

Since every cell with few exceptions receives the same genetic material, cytoplasmic specification and induction must involve the activation of different combinations of genes in different cells. Understanding development is therefore ultimately a problem of understanding the genetics involved. It is not surprising that developmental genetics was first studied in the geneticists' favorite model organism, the fruit fly *Drosophila*. These studies have been repeated in several other model animals, such as the nematode worm *Caenorhabditis elegans,* zebra fish *Danio rerio,* frog *Xenopus laevis,* chick *Gallus gallus,* and mouse *Mus musculus*. This research suggests that epigenesis proceeds in three general stages: pattern formation, determination of position in the body, and induction of limbs and organs appropriate for that position. Each stage is guided by gradients of gene products that function as **morphogens.**

Pattern Formation

The first step in organizing development of an embryo is pattern formation: determination of the front-to-rear (anteroposterior), left-to-right, and back-to-front (dorsoventral) axes. As Spemann demonstrated in salamanders, the anteroposterior axis of the embryo is determined by the Spemann organizer, located in the gray crescent of a zygote. In *Drosophila* the anteroposterior axis is determined even before an egg is fertilized. Christiane Nüsslein-Volhard and her colleagues in Germany found that this determination is due to a gradient of mRNA that is secreted into the egg by nurse cells in the mother. The end of the egg that receives the highest level of this mRNA is fated to become the anterior of the embryo and eventually of the adult. The mRNA is transcribed from a gene called *bicoid* (pronounced BICK-oyd) in the nurse cells. After an egg is fertilized, *bicoid* mRNA is translated into a protein morphogen called bicoid (not italicized) that binds to certain other genes. The products of these genes in turn activate others in a cascade that ultimately causes the production of an anteroposterior gradient. *Bicoid* is one of about 30 maternal genes that control pattern formation in an embryo. Some of these determine the dorsoventral axis. The gene *short gastrulation* leads to development of ventral structures, such as the nerve cord.

One of the most exciting discoveries in developmental genetics has been that the developmental genes of vertebrates and many other animals are similar to those of *Drosophila;* they are conserved over a wide range of animals. A gene similar to *bicoid* is also important in pattern formation in vertebrates. In vertebrates, however, the gene, called *Pitx2,* determines positioning of certain internal organs to either the left or right side of the body. Mutations in *Pitx2* in frogs, chicks, and mice can place the heart and stomach on the right instead of the left side. Such mutations may explain a reversal of organ position that sometimes occurs in humans. *Pitx2* is in turn activated by a protein produced by the gene *sonic hedgehog (Shh),* which is similar to a *Drosophila* gene called *hedgehog*. (The name *hedgehog* refers to the bristly appearance of fruit flies lacking the gene. The *"sonic"* comes from the video-game character Sonic the Hedgehog.) In vertebrates, *sonic hedgehog* is active in the left side only at the anterior end of the primitive streak (see Figure 8.13). *Short gastrulation* also has a counterpart in vertebrates—the gene *chordin,* which produces one of the proteins from the Spemann organizer.

In *Drosophila,* as well as other arthropods, annelid worms, chordates, and a few other groups, one important aspect of pattern formation along the anteroposterior axis is **segmentation,** also called **metamerism.** Segmentation is a division of the body into discrete segments or metameres (see Fig 9.6, p. 195). The segments are identical early in development, but later activation of different combinations of genes causes each segment to form different structures. For example, the anterior segment of insect embryos will form antennae, eyes, and mouthparts, while segments farther back will form legs. Segments are obvious in insects, but in fishes segmentation is apparent only in somites that produce such structures as vertebrae and repeated muscle bands (myomeres) (see Figure 24.24, p. 531). In *Drosophila* the number and orientation of segments is controlled by **segmentation genes.** There are three classes of segmentation genes: gap, pair-rule, and segment-polarity. **Gap genes** are activated first and divide an embryo into regions such as head, thorax, and abdomen. **Pair-rule genes** divide these regions into segments. Finally, **segment-polarity genes,** such as *hedgehog,* organize the anterior-to-posterior structures within each segment.

Homeotic and *Hox* Genes

Segmentation genes apparently regulate expression of other genes, ensuring that they are active only in appropriate segments. Such segment-specific genes are called homeotic genes. Mutations in homeotic genes, called **homeotic mutations,** place appendages or other structures in the wrong part of the body. For example, in *Drosophila* the homeotic gene *Antennapedia,*

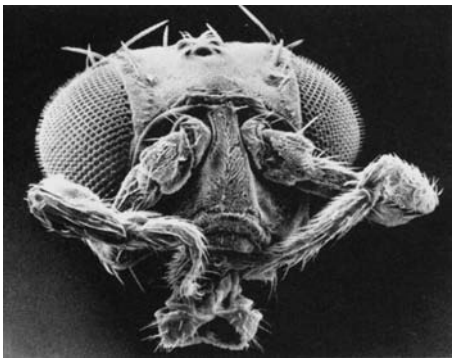


Figure 8.17

Head of a fruit fly with a pair of legs growing out of head sockets where antennae normally grow. The *Antennapedia* homeotic gene normally specifies the second thoracic segment (with legs), but the dominant mutation of this gene leads to this bizarre phenotype.

which helps trigger development of legs, is normally active only in the thorax. If the *Antennapedia* gene is activated by a homeotic mutation in the head of a maggot, the adult will have legs in place of antennae (Figure 8.17). *Antennapedia* and some other homeotic genes, as well as many other genes involved in development, include a sequence of 180 DNA base pairs, called the **homeobox.** The homeobox produces the part of a protein that attaches to the DNA of other genes, activating or blocking their expression.

Several other homeotic and nonhomeotic genes that are clustered close to *Antennapedia* on the same chromosome in *Drosophila* also include a homeobox. Genes in this cluster are called *Hom* genes. *Hom* genes do not encode specific limbs and organs. Instead, they function by specifying the location in the body along the anteroposterior axis. Intriguingly, the order of the *Hom* genes within the cluster on the chromosome is the same as the order in which they are expressed along the length of the body (Figure 8.18). One of the most exciting discoveries of the late twentieth century was that genes similar to *Hom* genes of *Drosophila* occur in other insects, as well as in chordates and unsegmented animals such as hydra and nematode worms. They also occur in plants and yeasts, and perhaps in all eukaryotes. These genes in organisms other than *Drosophila* were called *Hox* genes, but now all such genes are usually called *Hox* genes. Most *Hox* genes occur in a cluster on one chromosome. Mammals have four clusters, each on a different chromosome, with from 9 to 11 *Hox* genes each. As in *Drosophia,* the sequence of *Hox* genes within a cluster is the same as the front-to-rear order in which they are expressed in the body.

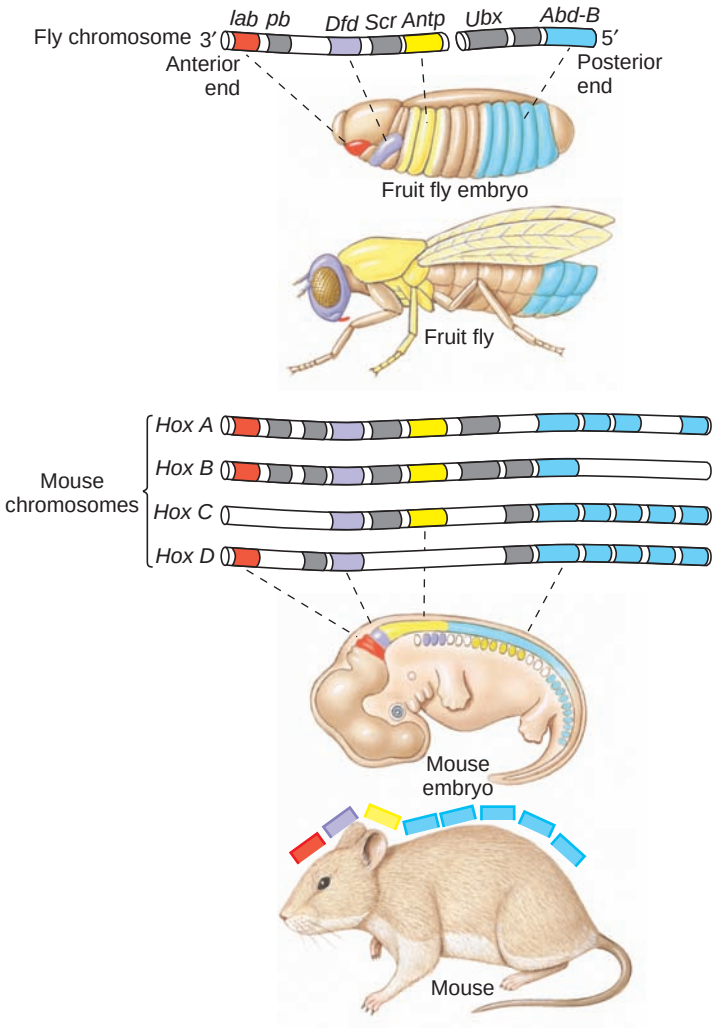
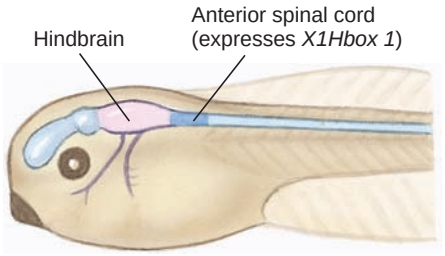


Figure 8.18

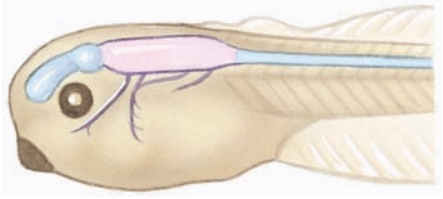
Homology of *Hox* genes in insects and mammals. These genes in both insects (fruit fly) and mammals (mouse) control the subdivision of the embryo into regions of different developmental fates along the anterior-posterior axis. The homeobox-containing genes lie on a single chromosome of the fruit fly and on four separate chromosomes in the mouse. Clearly defined homologies between the two, and the parts of the body in which they are expressed, are shown in color. The open boxes denote areas where it is difficult to identify specific homologies between the two. The *Hox* genes shown here are only a small subset of all the homeobox genes.

Morphogenesis of Limbs and Organs

Hox and other homeobox genes also play a role in shaping individual organs and limbs. As shown in Figures 8.18 and 8.19, for example, regions of the brain and identity of somites are specified by particular *Hox* and homeobox genes. Many other developmental genes that are also involved in pattern formation for the entire body also help shape individual limbs and organs by producing gradients of morphogens. One example, which has been studied by Cheryll Tickle and her coworkers at University College in London, is formation and development of limb buds in chicks. They have found that a new limb bud can be induced to grow from the side of a chick by implanting a bead soaked in fibroblast growth factor (FGF). This result implies that limbs are normally induced to develop by activation of the gene for FGF in appropriate parts of the body. Whether the limb bud develops into a wing or a leg depends on whether the FGF is applied toward the front or the rear of the chick.



Control tadpole



Tadpole injected with antibodies to *X1Hbox 1* protein

Figure 8.19

How the inhibition of a homeodomain regulatory protein alters normal development of the central nervous system of a frog tadpole. When the protein (encoded by a homeobox DNA sequence known as *X1Hbox 1*) was inactivated by antibodies directed against it, the area that should have become anterior spinal cord transformed into hindbrain instead.

FGF also plays a role in shaping the limb. It is secreted by cells in an **apical ectodermal ridge** at the end of the limb bud. FGF acts as a morphogen that forms a gradient from the apical ectodermal ridge to the base of a limb bud. This gradient helps establish a proximodistal axis—one of three axes that guide development of a limb (Figure 8.20). Fingers or toes develop at the end of the proximodistal axis with the highest level of FGF. An anteroposterior axis is established by a gradient of sonic hedgehog and ensures that fingers or toes develop in the appropriate order. Finally, Wnt7a, a protein produced by a gene that is similar to the segment-polarity gene *wingless* in *Drosophila,* helps determine the dorsoventral axis. Wnt7a makes the dorsal side of the wing or foot different from the ventral side.

Evolutionary Developmental Biology

Zoologists have always looked to embryology for clues to the evolutionary history, or phylogeny, of animals. Developmental features such as the number of germ layers and the fate of the blastopore do suggest evolutionary relationships among different phyla. Advances in development genetics, such as those just described in the previous section, have made the relationship between development and evolution even closer and have given rise to an exciting new field called evolutionary

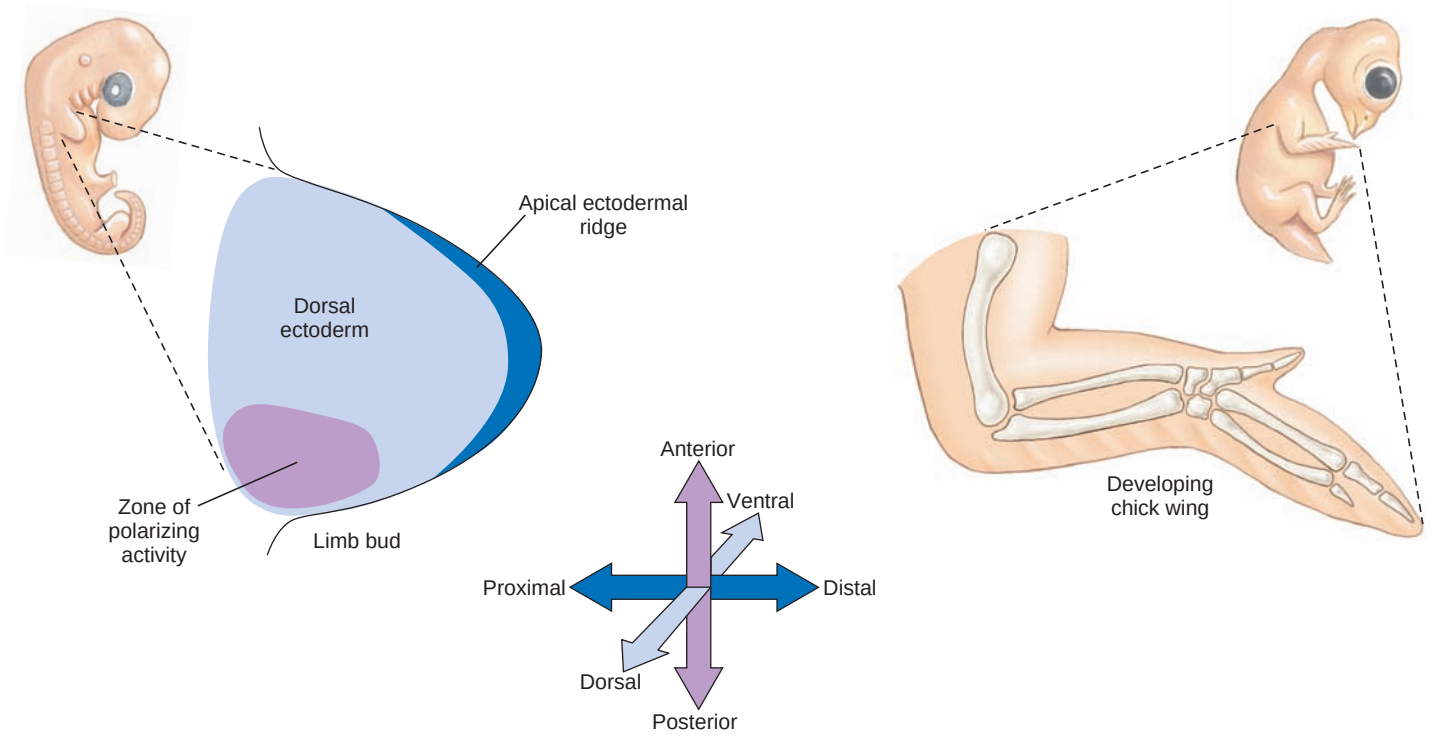


Figure 8.20

Morphogenesis in a vertebrate limb bud. The skeleton of a mature chicken limb is shown for orientation. Three axes are established in the limb bud: a proximal-distal axis by fibroblast growth factor (FGF) from the apical ectodermal ridge; an anterior-posterior axis by sonic hedgehog protein from the zone of polarizing activity; and a dorsal-ventral axis by Wnt7a protein from dorsal ectoderm.

developmental biology. Evolutionary developmental biology, often nicknamed evo-devo, is based on a realization that evolution is essentially a process in which organisms become different as a result of changes in the genetic control of development. The fact that the genes that control development are similar in animals as different as fruit flies and mice offers hope that we can reconstruct the evolutionary history of animals by understanding how functioning of those genes came to differ. Evolutionary developmental biology has already contributed several exciting concepts to our thinking about animal evolution, but the field is so new that it would be premature to accept these concepts as established. It is best to state them as questions for further study.

Are the body plans of all bilaterally symmetric animals fundamentally similar? As noted on page 172, *chordin,* one of the genes responsible for development of the nervous system in the dorsal part of a frog, is similar to *short gastrulation,* which is necessary for development of the ventral nerve cord in *Drosophila*. In addition, the gene *decapentaplegic* promotes dorsal development in *Drosophila,* and the similar gene *bone morphogenetic protein-4* promotes ventral development in frogs. In other words, insects and amphibians, whose body plans look so different, actually share a similar control of dorsoventral patterning, except that one is upside down compared with the other. This finding has prompted a reappraisal of an idea first proposed by the French naturalist Etienne Geoffroy St. Hilaire in 1822 after he noticed that in a dissected lobster on its back the nerve cord was above the gut, and the heart was below it, as in a vertebrate in its normal position. The idea that a vertebrate is like an inverted invertebrate was quickly rejected, but now biologists are once more considering whether the body plans of protostomes and deuterostomes are simply inverted relative to each other.

Can the anatomy of extinct ancestral species be inferred from the developmental genes shared by their descendants? The fact that dorsoventral patterning is similar in protostomes and deuterostomes suggests that the most recent common ancestor of these two branches had a similar dorsoventral patterning with a heart and nervous system separated by the gut. One can also infer from the similarity in *Hom/Hox* clusters in insects and chordates that the most recent common ancestor of protostomes and deuterostomes may have been segmented and that its segments differentiated by similar genes. It may also have had at least rudimentary eyes, judging from the fact that similar genes, *eyeless/Pax-6,* are involved in eye formation in a wide range of both protostomes and deuterostomes.

Instead of evolution proceeding by the gradual accumulation of numerous small mutations, could it proceed by relatively few mutations in a few developmental genes? The fact that formation of legs or eyes can be induced by a mutation in one gene suggests that these and other organs develop as modules (see p. 173). If so, then entire limbs and organs could have been lost or acquired during evolution as a result of one or a few mutations, which would challenge Darwin's theory of gradualism (p. 121). If this is correct, then the apparently rapid evolution of numerous groups of animals during the few million years of the Cambrian explosion and at other times is more easily explained. Instead of requiring mutations in numerous genes, each with a small effect, evolution of different groups could be a result of changes in timing, number, or expression of relatively few developmental genes.

VERTEBRATE DEVELOPMENT

The Common Vertebrate Heritage

A prominent outcome of shared ancestry of vertebrates is their common pattern of development. This common pattern is best seen in the remarkable similarity of postgastrula vertebrate embryos (Figure 8.21). The likeness occurs at a brief moment in the development of vertebrates when shared chordate hallmarks of dorsal neural tube, notochord, pharyngeal gill pouches with aortic arches, ventral heart, and postanal tail are present at about the same stage of development. Their moment of similarity—when the embryos seem almost interchangeable—is all the more extraordinary considering the great variety of eggs and widely different types of early development that have converged toward a common design. Then, as development continues, the embryos diverge in pace and direction, becoming recognizable as members of their class, then their order, then family, and finally their species. The important contribution of early vertebrate development to our understanding of homology and evolutionary common descent is described in Chapter 6 in the section on Ontogeny, Phylogeny, and Recapitulation, page 116.

Amniotes and the Amniotic Egg

Reptiles, birds, and mammals form a monophyletic grouping of vertebrates called **amniotes,** so named because their embryos develop within a membranous sac, the **amnion.** The amnion is one of four **extraembryonic membranes** that compose a sophisticated support system within the **amniotic egg** (Figure 8.22), which evolved when the first amniotes appeared in the late Paleozoic era.

The **amnion** is a fluid-filled sac that encloses the embryo and provides an aqueous environment in which the embryo floats, protected from mechanical shock and adhesions.

Evolution of the second extraembryonic membrane, the **yolk sac,** actually predates appearance of amniotes many millions of years. The yolk sac with its enclosed yolk is a conspicuous feature of all fish embryos. After hatching, a growing fish larva depends on the remaining yolk provisions to sustain it until it can begin to feed itself (Figure 8.23). The yolk sac functions differently in animals that give live birth. In many viviparous vertebrates from diverse groups, the yolk sac becomes vascular and intimately associated with the mother's reproductive tract, allowing transfer of nutrients and respiratory gases

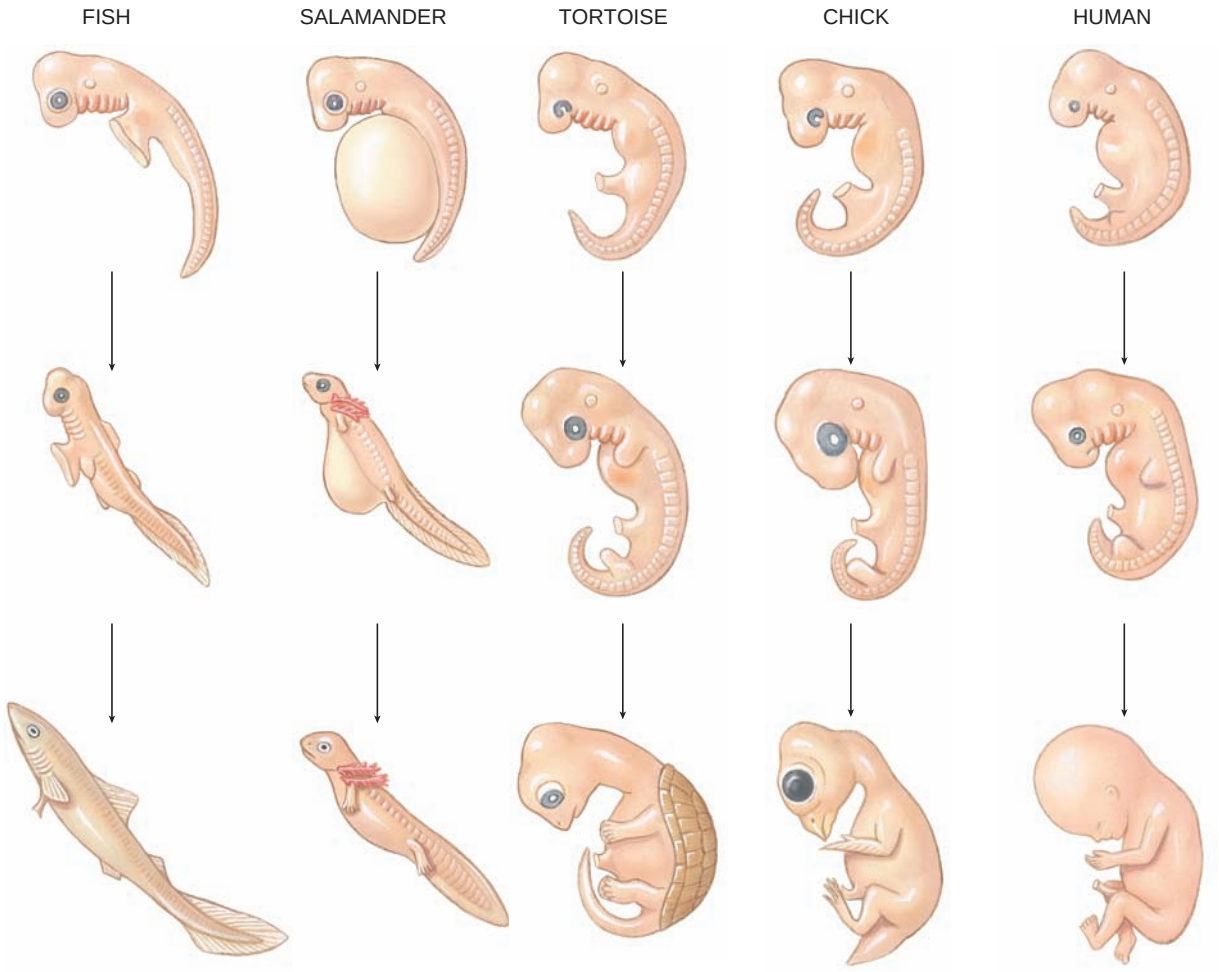


Figure 8.21

Early vertebrate embryos drawn from photographs. Embryos as diverse as fish, salamander, tortoise, bird, and human show remarkable similarity following gastrulation. At this stage (top row) they reveal features common to the entire subphylum Vertebrata. As development proceeds they diverge, each becoming increasingly recognizable as belonging to a specific class, order, family, and finally, species.

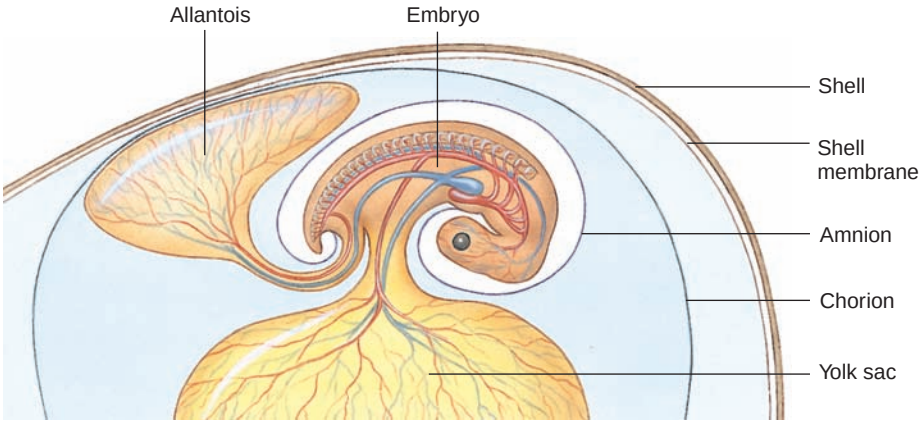


Figure 8.22

Amniotic egg at an early stage of development showing a chick embryo and its extraembryonic membranes.

between mother and fetus. Thus, a yolk sac placenta is formed. The mass of yolk is an extraembryonic structure because it is not a part of the embryo proper, and the yolk sac is an extraembryonic membrane because it is an accessory structure that develops outside the embryo and is discarded after the yolk is consumed.

The **allantois** is a sac that grows out of the hindgut and serves as a repository for metabolic wastes during development. It also functions as a respiratory surface for exchange of oxygen and carbon dioxide.

The **chorion** lies just beneath the eggshell and completely encloses the rest of the embryonic system. As the embryo grows and its need for oxygen increases, the allantois and chorion fuse to form the **chorioallantoic membrane.** This double membrane has a rich vascular network connected to the embryonic circulation.

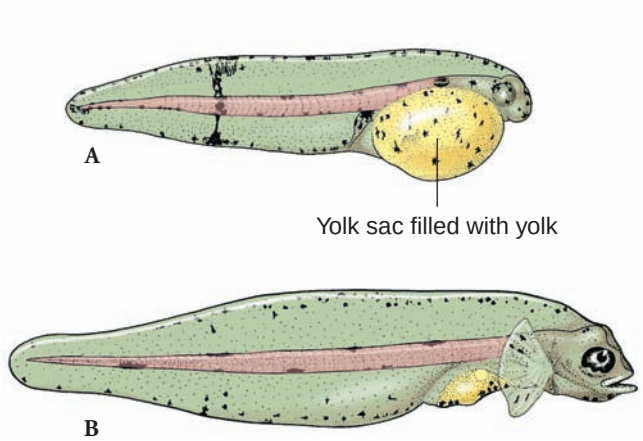


Figure 8.23

Fish larvae showing yolk sac. **A,** The one-day-old larva of a marine flounder has a large yolk sac. **B,** After 10 days of growth the larva has developed mouth, sensory organs, and a primitive digestive tract. With its yolk supply now exhausted, it must capture food to grow and survive.

Lying just beneath the porous shell, the vascular chorioallantois serves as a provisional "lung" across which oxygen and carbon dioxide can freely exchange. Thus an amniotic egg provides a complete life-support system for the embryo, enclosed by a tough outer shell. The amniotic egg is one of the most important adaptations to have evolved in vertebrates.

The evolution of a shelled amniotic egg made internal fertilization a reproductive requirement. A male must introduce sperm directly into the female reproductive tract, since sperm must reach and fertilize the egg before the eggshell is wrapped around it.

The Mammalian Placenta and Early Mammalian Development

Rather than developing within an eggshell like most other vertebrates most mammalian embryos evolved the strategy of developing within the mother's body. We have already seen that mammalian gastrulation closely parallels that of egg-laying amniotes. The earliest mammals were egg layers, and even today some mammals retain this primitive character; **monotremes** (duck-billed platypus and spiny anteater) lay large yolky eggs that closely resemble bird eggs. In **marsupials** (pouched mammals such as opossums and kangaroos), embryos develop for a time within the mother's uterus, but an embryo does not "take root" in the uterine wall, and consequently it receives little nourishment from the mother before birth. The young of marsupials are born at an early stage of development and continue developing sheltered in a pouch in the mother's abdominal wall, nourished with milk (reproduction in marsupials is described on pp. 628–629).

All other mammals, composing 94% of class Mammalia, are **placental mammals.** These mammals have evolved a **placenta,** a remarkable fetal structure through which an embryo is nourished. Evolution of this fetal organ required substantial restructuring, not only of extraembryonic membranes to form the placenta but also of the maternal oviduct, part of which had to expand into long-term housing for embryos, the **uterus.** Despite these modifications, development of extraembryonic membranes in placental mammals is remarkably similar to their development in egg-laying amniotes (compare Figures 8.22 and 8.24). In fact, for some nonmammalian vertebrates that give live birth, the extraembryonic membranes form a placenta. Some viviparous lizards and snakes have either a yolk sac placenta or a chorioallantoic placenta or both.

One of the most intriguing questions the placenta presents is, why is it not immunologically rejected by the mother? Both placenta and embryo are genetically alien to the mother because they contain proteins (called major histocompatibility proteins, p. 775) that differ from those of the mother. We would expect uterine tissues to reject the embryo just as the mother would reject an organ transplanted from her own child. The placenta is a uniquely successful foreign transplant, or **allograft,** because it has evolved measures for suppressing the immune response that normally would be mounted against it and the fetus by the mother. Experiments suggest that the chorion produces proteins and lymphocytes that block the normal immune response by suppressing formation of specific antibodies by the mother.

Early stages of mammalian cleavage, shown in Figure 8.13E, occur while a **blastocyst** is traveling down the oviduct toward the uterus, propelled by ciliary action and muscular peristalsis. When a human blastocyst is about six

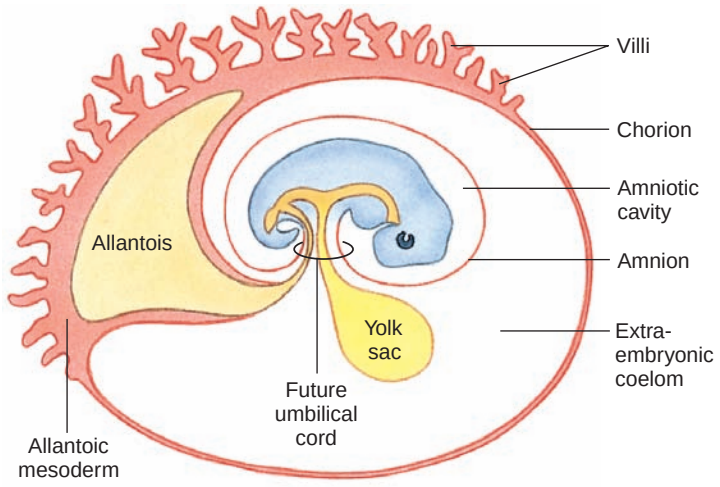


Figure 8.24

Generalized diagram of extraembryonic membranes of a mammal, showing how their development parallels that of a chick (compare with Figure 8.22). Most extraembryonic membranes of mammals have been redirected to new functions.

days old and composed of about 100 cells, it contacts the uterine endometrium (uterine lining) (Figure 8.25). On contact, the trophoblast cells proliferate rapidly and produce enzymes that break down the epithelium of the uterine endometrium. These changes allow the blastocyst to implant in the endometrium. By the eleventh or twelfth day the blastocyst is completely buried and surrounded by a pool of maternal blood. The trophoblast thickens, sending out thousands of tiny, fingerlike projections, the **chorionic villi.** These projections sink like roots into the uterine endometrium after the embryo implants. As development proceeds and embryonic demands for nutrients and gas exchange increase, the great proliferation of chorionic villi vastly increases the total surface area of the placenta. Although a human placenta at term measures only 18 cm (7 inches) across, its total absorbing surface is approximately 13 square meters—50 times the surface area of the skin of the newborn infant.

Since a mammalian embryo is protected and nourished through the placenta rather than with stored yolk, what happens to the four extraembryonic membranes it has inherited from early amniotes? The amnion remains unchanged, a protective water jacket in which the embryo floats. A fluid-filled yolk sac is also retained, although it contains no yolk. It has acquired a new function: during early development it is the source of stem cells that give rise to blood, lymphoid cells, and gametes. These stem cells later migrate into the developing embryo. In organisms such as racoons and mice, a heavily vascularized yolk sac implants in the uterus, along with the typical placenta. The two remaining extraembryonic membranes, allantois and the chorion, are recommitted to new functions. The allantois is no longer needed for storage of metabolic wastes. Instead it contributes to the **umbilical cord,** which links the embryo physically and functionally with the placenta. The chorion, the outermost membrane, forms most of the placenta itself. The rest of the placenta is formed by the adjacent uterine endometrium.

The embryo grows rapidly, and in humans all major organs of the body have begun their formation by the end of the fourth week of development. The embryo is now about 5 mm in length and weighs approximately 0.02 g. During the first two weeks of development **(germinal period)** the embryo is quite resistant to outside influences. However, during the next eight weeks, when all major organs are being established and body shape is forming **(embryonic period),** an embryo is more sensitive to disturbances that might cause malformations (such as exposure to alcohol or drugs taken by the mother) than at any other time in its development. The embryo becomes a **fetus** at approximately two months after fertilization. This ushers in the **fetal period,** which is primarily a growth phase, although organ systems (especially the nervous and endocrine systems) continue to differentiate. The fetus grows from approximately 28 mm and 2.7 g at 60 days to approximately 350 mm and 3000 g at term (nine months).

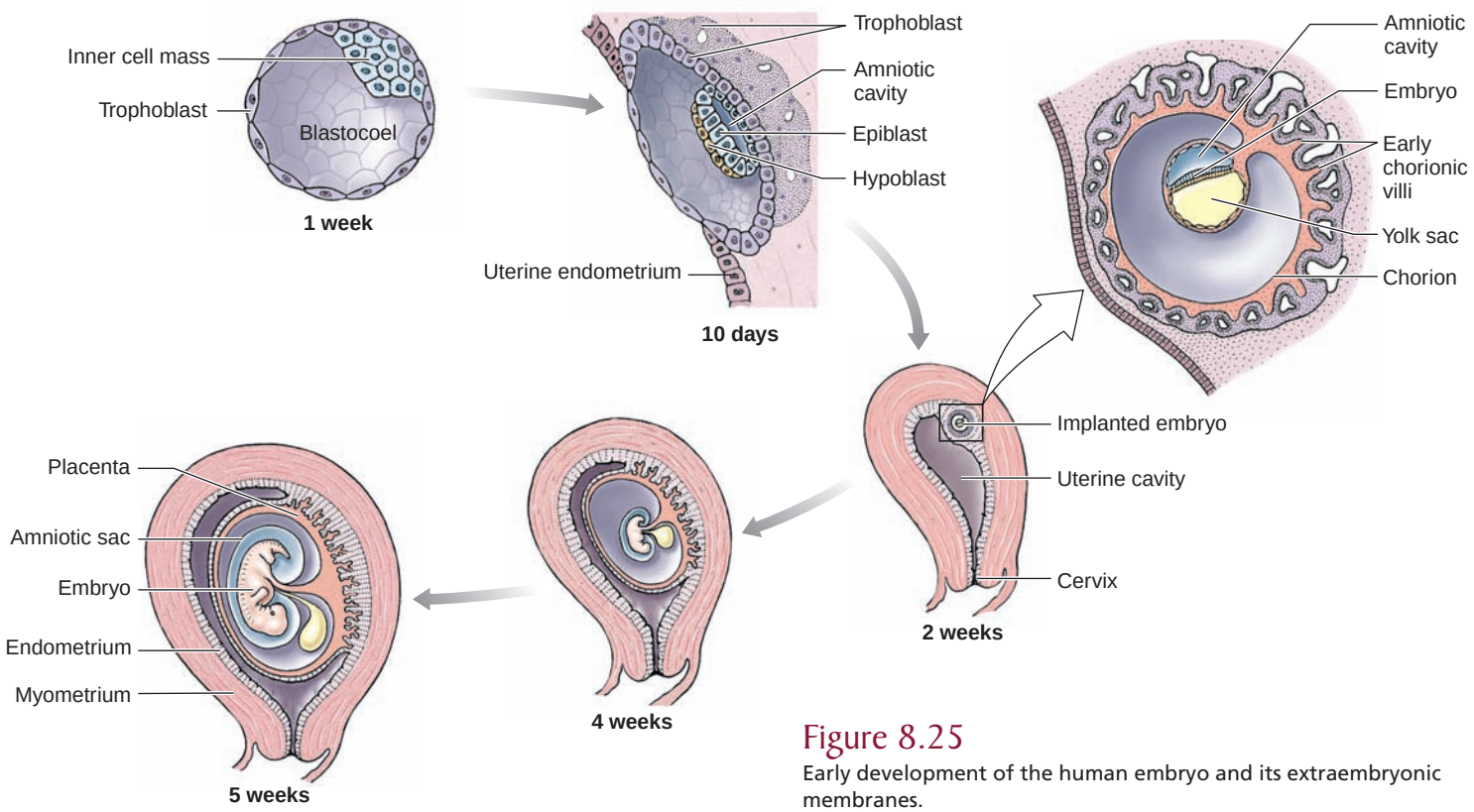


Figure 8.25

Early development of the human embryo and its extraembryonic membranes.

DEVELOPMENT OF SYSTEMS AND ORGANS

During vertebrate gastrulation the three germ layers are formed. These differentiate, as we have seen, first into primordial cell masses and then into specific organs and tissues. During this process, cells become increasingly committed to specific directions of differentiation. Derivatives of the three germ layers are diagrammed in Figure 8.26.

Assignment of early embryonic layers to specific "germ layers" (not to be confused with "germ cells," which are the eggs and sperm) is for the convenience of embryologists and is of no concern to the embryo. Whereas the three germ layers normally differentiate to form the tissue and organs described here, it is not the germ layer itself that determines differentiation, but rather the precise position of an embryonic cell with relation to other cells.

Derivatives of Ectoderm: Nervous System and Nerve Growth

The brain, spinal cord, and nearly all outer epithelial structures of the body develop from primitive ectoderm. They are among the earliest organs to appear. Just above the notochord, the ectoderm thickens to form a **neural plate.** The edges of this plate rise up, fold, and join together at the top to create an elongated, hollow **neural tube.** The neural tube gives rise to most of the nervous system: anteriorly it enlarges and differentiates into the brain and cranial nerves; posteriorly it forms the spinal cord and spinal motor nerves. Much of the rest of the peripheral nervous system is derived from **neural crest cells,** which pinch off from the neural tube before it closes (Figure 8.27). Among the multitude of different cell types and structures that originate with the neural crest are portions of the cranial nerves, pigment cells, cartilage and bone of most of the skull (including jaws), ganglia of the autonomic nervous

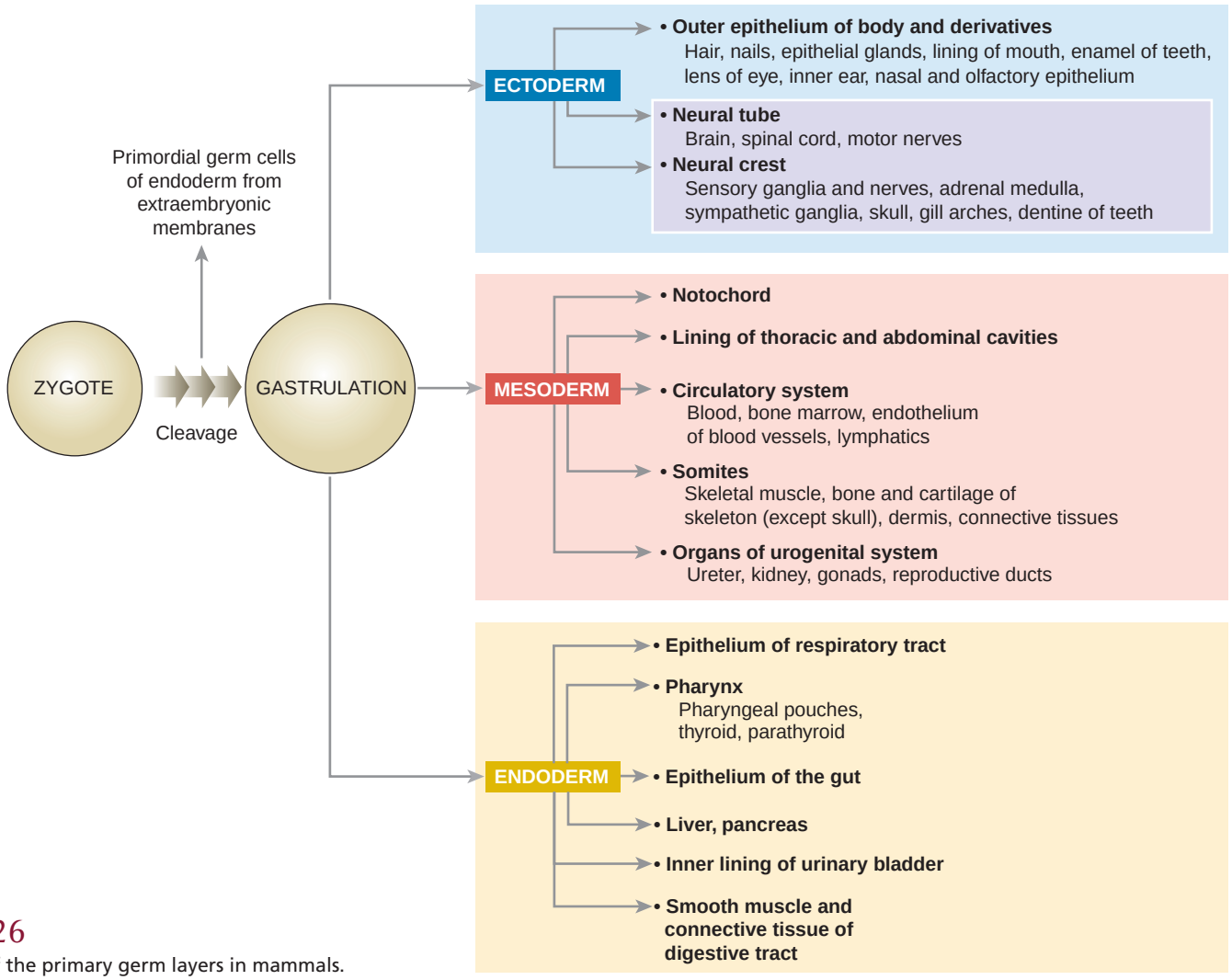


Figure 8.26
Derivatives of the primary germ layers in mammals.

system, medulla of the adrenal gland, and contributions to several other endocrine glands. Neural crest tissue is unique to vertebrates and was probably of prime importance in evolution of the vertebrate head and jaws.

How are the billions of nerve axons in the body formed? What directs their growth? Biologists were intrigued with these questions, which seemed to have no easy solutions. Because a single nerve axon may be more than a meter in length (for example, motor nerves running from the spinal cord to the toes), it seemed impossible that a single cell could reach out so far. The answer had to await the development of one of the most powerful tools available to biologists, the cell culture technique.

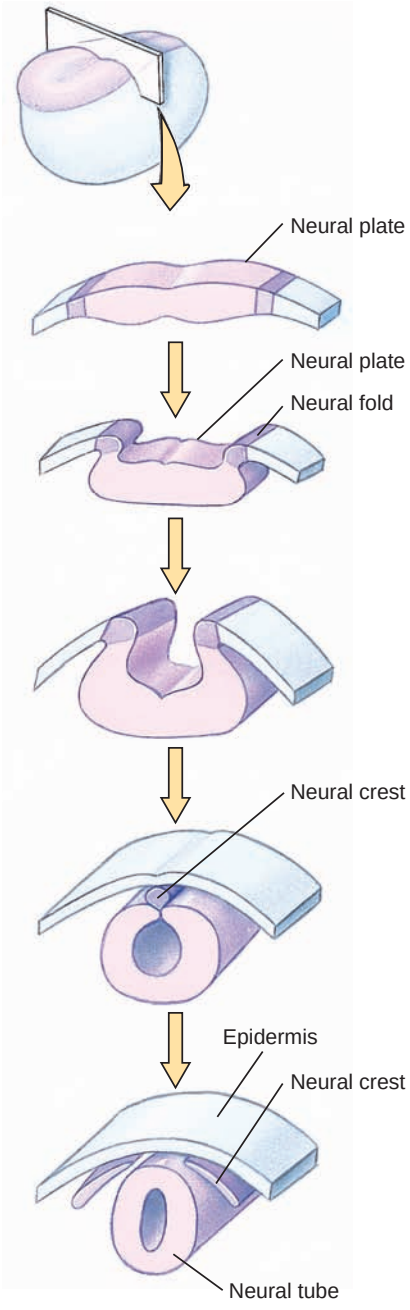


Figure 8.27
Development of neural tube and neural crest cells from neural plate ectoderm.

In 1907 embryologist Ross G. Harrison discovered that he could culture living neuroblasts (embryonic nerve cells) for weeks outside the body by placing them in a drop of frog lymph hung from the underside of a cover slip. Watching nerves grow for periods of days, he saw that each axon was an outgrowth of a single cell. As the axon extended outward, materials for growth flowed down the axon center to the growing tip (growth cone) where they were incorporated into new protoplasm (Figure 8.28).

The second question—what directs nerve growth—has taken longer to unravel. An idea held well into the 1940s was that nerve growth is a random, diffuse process. A major hypothesis proposed that the nervous system developed as an equipotential network, or blank slate, that later would be shaped by usage into a functional system. The nervous system just seemed too incredibly complex for us to imagine that nerve fibers could find their way selectively to so many predetermined destinations, yet it appears that this is exactly what they do! Research with invertebrate nervous systems indicated that each of the billions of nerve cell axons acquires a distinct identity that somehow directs it along a specific pathway to its destination. Many years ago Harrison observed that a growing nerve axon terminated in a growth cone, from which extend numerous tiny threadlike pseudopodial processes (filopodia) (Figure 8.28). Research has shown that the growth cone is steered by an array of guidance molecules secreted along the pathway and by the axon's target. This chemical guidance system, which must, of course, be genetically directed, is just one example of the amazing flexibility that characterizes the entire process of differentiation.

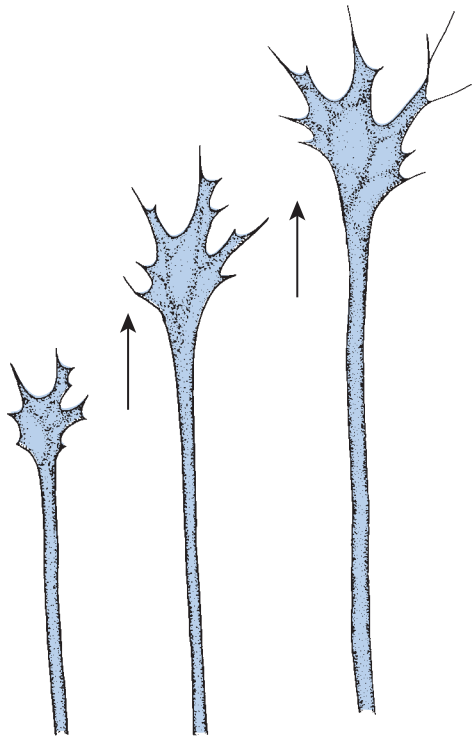


Figure 8.28
Growth cone at the growing tip of a nerve axon. Materials for growth flow down the axon to the growth cone from which numerous threadlike filopodia extend. These serve as a pioneering guidance system for the developing axon. Direction of growth is shown by arrows.

The tissue culture technique developed by Ross G. Harrison is now used extensively by scientists in all fields of active biomedical research, not just by developmental biologists. The great impact of the technique has been felt only in recent years. Harrison was twice considered for the Nobel Prize (1917 and 1933), but he failed ever to receive the award because, ironically, the tissue culture method was then considered "of rather limited value."

Derivatives of Endoderm: Digestive Tube and Survival of Gill Arches

In frog embryos the primitive gut makes its appearance during gastrulation with the formation of the **archenteron.** From this simple endodermal cavity develop the lining of the digestive tract, lining of the pharynx and lungs, most of the liver and pancreas, the thyroid and parathyroid glands, and the thymus (see Figure 8.26).

In other vertebrates the **alimentary canal** develops from the primitive gut and is folded off from the yolk sac by growth and folding of the body wall (Figure 8.29). The ends of the tube open to the exterior and are lined with ectoderm, whereas the rest of the tube is lined with endoderm. **Lungs, liver,** and **pancreas** arise from the foregut.

Among the most intriguing derivatives of the digestive tract are the pharyngeal pouches, which make their appearance in the early embryonic stages of all vertebrates (see Figure 8.21). During development the endodermally-lined pharyngeal pouches interact with overlying ectoderm to form gill arches. In fishes, gill arches develop into gills and supportive structures and serve as respiratory organs. When early vertebrates moved onto land, gills were unsuitable for aerial respiration and respiratory function was performed by independently evolved lungs.

Why then do gill arches persist in embryos of terrestrial vertebrates? Although gill arches serve no respiratory function in either embryos or adults of terrestrial vertebrates, they are necessary primordia for a variety of other structures. For example, the first arch and its endoderm-lined pouch (the space between adjacent arches) form the upper and lower jaws and inner ear of vertebrates. The second, third, and fourth gill pouches contribute to the tonsils, parathyroid glands, and thymus. We can understand then why gill arches and other fishlike structures appear in early mammalian embryos. Their original function has been abandoned, but the structures are retained for new uses. The great conservatism of early embryonic development has conveniently provided us with a telescoped view of the origins of new adaptations.

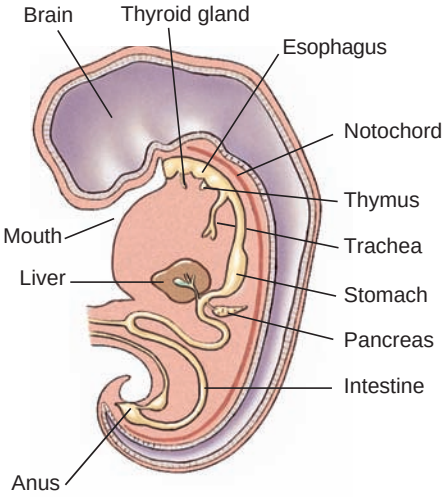


Figure 8.29
Derivatives of the alimentary canal of a human embryo.

Derivatives of Mesoderm: Support, Movement, and Beating Heart

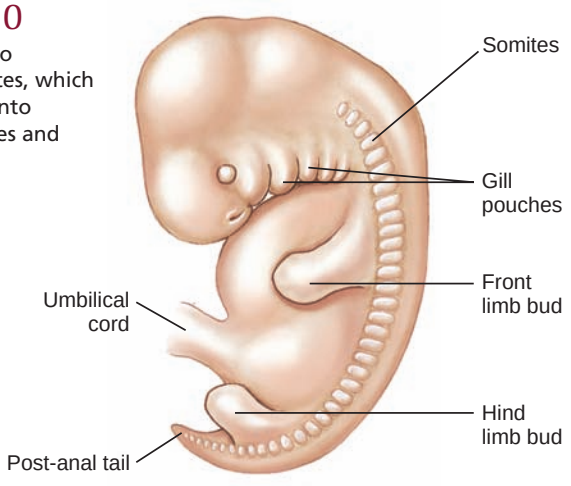
The mesoderm forms most skeletal and muscular tissues, the circulatory system, and urinary and reproductive organs (see Figure 8.26). As vertebrates have increased in size and complexity, mesodermally derived supportive, movement, and transport structures have become an even greater proportion of the body.

Most **muscles** arise from the mesoderm along each side of the neural tube (Figure 8.30). This mesoderm divides into a linear series of blocklike somites (38 in humans), which by splitting, fusion, and migration become the axial skeleton, dermis of the dorsal skin, and muscles of the back, body wall, and limbs.

Mesoderm gives rise to the first functional organ, the embryonic heart. Guided by the underlying endoderm, two clusters of precardiac mesodermal cells move amebalike into position on either side of the developing gut. These clusters differentiate into a pair of double-walled tubes, which later fuse to form a single, thin tube (see Figure 8.14, p. 168).

As the cells group together, the first twitchings are evident. In a chick embryo, a favorite animal for experimental embryological studies, the primitive heart begins to beat on the second day of the 21-day incubation period; it begins beating before any true blood vessels have formed and before there is any blood to pump. As the ventricle primordium develops, the spontaneous cellular twitchings become coordinated into a feeble but rhythmical beat. New heart chambers, each with a beat faster than its predecessor, then develop.

Figure 8.30
Human embryo showing somites, which differentiate into skeletal muscles and axial skeleton.



Finally a specialized area of heart muscle called the **sinoatrial (SA) node** develops and takes command of the entire heartbeat (the role of the SA node in the excitation of the heart is described on p. 694). The SA node becomes the heart's primary **pacemaker.** As the heart builds a strong and efficient beat, vascular channels open within the embryo and across the yolk. Within the vessels are the first primitive blood cells suspended in plasma.

Early development of the heart and circulation is crucial to continued embryonic development, because without circulation an embryo could not obtain materials for growth. Food is absorbed from the yolk and carried to the embryonic body, oxygen is delivered to all tissues, and carbon dioxide and other wastes are carried away. An embryo is totally dependent on these extraembryonic support systems, and the circulation is the vital link between them.

SUMMARY

Developmental biology encompasses the emergence of order and complexity during the development of a new individual from a fertilized egg, and the control of this process. The early preformation concept of development gave way in the eighteenth century to the theory of epigenesis, which holds that development is the progressive appearance of new structures that arise as the products of antecedent development. Fertilization of an egg by a sperm restores the diploid number of chromosomes and activates the egg for development. Both sperm and egg have evolved devices to promote efficient fertilization. The sperm is a highly condensed haploid nucleus provided with a locomotory flagellum. Many eggs release chemical sperm attractants, most have surface receptors that recognize and bind only with sperm of their own species, and all have developed devices to prevent polyspermy.

During cleavage an embryo divides rapidly and usually synchronously, producing a multicellular blastula. Cleavage is greatly influenced by quantity and distribution of yolk in the egg. Eggs with little yolk, such as those of many marine invertebrates, divide completely (holoblastic) and usually have indirect development with a larval stage interposed between the embryo and adult. Eggs having an abundance of yolk, such as those of birds, reptiles, and most arthropods divide only partially (meroblastic), and birds and reptiles have no larval stage.

Based on several developmental characteristics, bilateral metazoan animals are divided into two major groups. The Protostomia have mosaic cleavage and the mouth forms at or near the embryonic blastopore. The Deuterostomia have regulative cleavage and the mouth forms secondarily and not from the blastopore.

At gastrulation, cells on an embryo's surface move inward to form germ layers (endoderm, ectoderm, mesoderm) and the embryonic body plan. Like cleavage, gastrulation is much influenced by the quantity of yolk.

Despite the different developmental fates of embryonic cells, every cell contains a complete genome and thus the same nuclear information. Early development through cleavage is governed by cytoplasmic determinants derived from the maternal genome and placed in the egg cortex. As gastrulation approaches, control gradually shifts from maternal to embryonic as an embryo's own nuclear genes begin transcribing mRNA.

Harmonious differentiation of tissues proceeds in three general stages: pattern formation, determination of position in the body, and induction of limbs and organs appropriate for each position. Each stage is guided by morphogens. Pattern formation refers to determination of the anteroposterior, dorsoventral, and left-to-right body axes. In amphibians the anteroposterior axis is established by morphogens such as chordin from the Spemann organizer in the gray crescent of the zygote. In *Drosophila* that axis is determined by the morphogen bicoid, which is transcribed from maternal mRNA deposited at the anterior of the egg. In these and other segmented animals, such morphogens activate genes that divide the body into head, thorax and abdomen, and then into correctly oriented segments. The structures appropriate to each segment are then induced by homeotic genes, which are characterized by a particular sequence of DNA bases called the homeobox. Mutations in homeotic genes result in the development of inappropriate structures on a segment: legs on the head, for example.

The anteroposterior axis of an embryo is determined by homeotic and other homeobox-containing genes contained in one or more clusters on particular chromosomes. These genes, called *Hox* genes, occur not only in *Drosophila* and amphibians, but apparently in all animals. Each *Hox* gene is active in a particular region of the body, depending on its position within the cluster. Dorsoventral and left-right axes are similarly determined by morphogens that are produced only in the appropriate regions of the embryo. Similarly, morphogens guide the development of limbs along three body axes. Morphogens have been found to be remarkably similar in animals as different as *Drosophila* and amphibians. This realization has given rise to the field of evolutionary developmental biology, which is based on the idea that the evolution of the enormous variety of animals is the result of changes in the position and timing of relatively few genes that control development.

The postgastrula stage of vertebrate development represents a remarkable conservation of morphology when jawed vertebrates from fish to humans exhibit features common to all. As development proceeds, species-specific characteristics are formed.

Amniotes are terrestrial vertebrates that develop extraembryonic membranes during embryonic life. The four membranes are amnion, allantois, chorion, and yolk sac, each serving a specific life-support function for the embryo that develops within a self-contained egg (as in birds and most reptiles) or within the maternal uterus (mammals).

Mammalian embryos are nourished by a placenta, a complex fetal-maternal structure that develops in the uterine wall. During pregnancy the placenta becomes an independent nutritive, endocrine, and regulatory organ for the embryo.

Germ layers formed at gastrulation differentiate into tissues and organs. The ectoderm gives rise to skin and nervous system; endoderm gives rise to alimentary canal, pharynx, lungs, and certain glands; and mesoderm forms muscular, skeletal, circulatory, reproductive, and excretory organs.

REVIEW QUESTIONS

1. What is meant by epigenesis? How did Kaspar Friedrich Wolff's concept of epigenesis differ from the early notion of preformation?
2. How is an egg (oocyte) prepared during oogenesis for fertilization? Why is preparation essential to development?
3. Describe events that follow contact of a spermatozoon with an egg. What is polyspermy and how is it prevented?
4. What is meant by the term "activation" in embryology?
5. How does amount of yolk affect cleavage? Compare cleavage in a sea star with that in a bird.
6. What is the difference between radial and spiral cleavage?
7. What other developmental hallmarks are often associated with spiral or radial cleavage?
8. What is indirect development?
9. Using sea star embryos as an example, describe gastrulation. Explain how the mass of inert yolk affects gastrulation in frog and bird embryos.
10. What is the difference between schizocoelous and enterocoelous origins of a coelom?
11. Describe two different experimental approaches that serve as evidence for nuclear equivalence in animal embryos.
12. What is meant by "induction" in embryology? Describe the famous organizer experiment of Spemann and Mangold and explain its significance.
13. What are homeotic genes and what is the "homeobox" contained in such genes? What is the function of the homeobox? What are *Hox* genes? What is the significance of their apparently universal occurrence in animals?
14. What is the embryological evidence that vertebrates form a monophyletic group?
15. What are the four extraembryonic membranes of amniotic eggs of birds and reptiles and what is the function of each membrane?
16. What is the fate of the four extraembryonic membranes in embryos of placental mammals?
17. Explain what the "growth cone" that Ross Harrison observed at the ends of growing nerve fibers does to influence direction of nerve growth.
18. Name two organ system derivatives of each of the three germ layers.
19. What developmental characters are used to divide animals between protostome and deuterostome groups (clades)?

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PART THREE

3 Diversity of Animal Life

A view of coral reef biodiversity.

CHAPTER

9

Architectural Pattern of an Animal

*Cnidarian polyps have radial symmetry and cell-tissue grade of organization (*Dendronephthya *sp.).*

New Designs for Living

Zoologists today recognize 34 phyla of multicellular animals, each phylum characterized by a distinctive body plan and biological properties that set it apart from all other phyla. All are survivors of perhaps 100 phyla that appeared 600 million years ago during the Cambrian explosion, the most important evolutionary event in the geological history of life. Within the space of a few million years, virtually all major body plans that we see today, together with many other novel plans that we know only from the fossil record, were established. Entering a world sparse in species and mostly free of competition, these new lifeforms diversified, producing new themes in animal architecture. Later bursts of speciation that followed major extinction events produced mainly variations on established themes.

Established themes, in the form of distinctive body plans, are passed down a lineage from an ancestral population to its descendants; molluscs carry a hard shell, bird forelimbs make wings. These ancestral traits limit the morphological scope of descendants no matter what their lifestyle. Although penguin bodies are modified for an aquatic life, the wings and feathers of their bird ancestors might never adapt as well as fish fins and scales. Despite structural and functional evolution, new forms are constrained by the architecture of their ancestors.

The English satirist Samuel Butler proclaimed that the human body was merely "a pair of pincers set over a bellows and a stewpan and the whole thing fixed upon stilts." Most people less cynical than Butler would agree that the body is a triumph of intricate, living architecture. Less obvious, perhaps, is that the architecture of humans and most other animals conforms to the same well-defined plan. The basic uniformity of biological organization derives from the common ancestry of animals and from their basic cellular construction. Despite vast differences of structural complexity of organisms ranging from unicellular forms to humans, all share an intrinsic material design and fundamental functional plan. In this introduction to the diversity chapters (Chapters 11 through 28), we consider the limited number of body plans that underlie the apparent diversity of animal form and examine some of the common architectural themes that animals share.

HIERARCHICAL ORGANIZATION OF ANIMAL COMPLEXITY

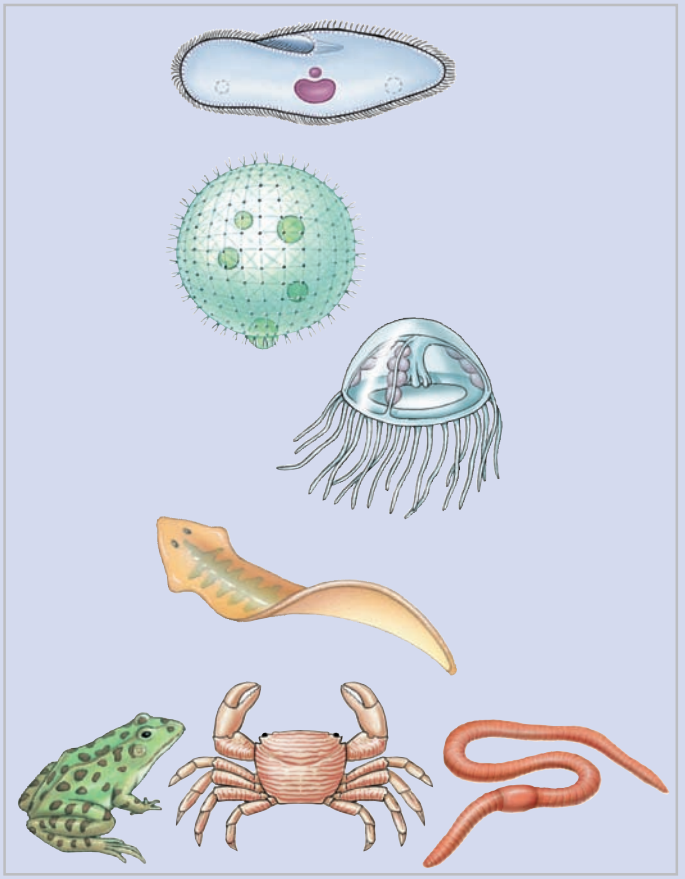
Among the different unicellular and metazoan groups, we recognize five major grades of organization (Table 9.1). Each grade is more complex than the one preceding, and builds on it in a hierarchical manner.

The unicellular groups are the simplest eukaryotic organisms and represent the *protoplasmic* grade of organization. They are nonetheless complete organisms that perform all of the basic functions of life as seen in more complex animals. Within the confines of their cell, they show remarkable organization and division of labor, possessing distinct supportive structures, locomotor devices, fibrils, and simple sensory structures. The diversity observed among unicellular organisms is achieved

TABLE 9.1

Grades of Organization in Organismal Complexity

1. *Protoplasmic grade of organization.* Protoplasmic organization characterizes unicellular organisms. All life functions are confined within the boundaries of a single cell, the fundamental unit of life. Within a cell, protoplasm is differentiated into organelles capable of performing specialized functions.
2. *Cellular grade of organization.* Cellular organization is an aggregation of cells that are functionally differentiated. A division of labor is evident, so that some cells are concerned with, for example, reproduction, and others with nutrition. Some flagellates, such as *Volvox,* that have distinct somatic and reproductive cells are placed at the cellular level of organization. Many authorities also place sponges at this level.
3. *Cell-tissue grade of organization.* A step beyond the preceding is an aggregation of similar cells into definite patterns or layers and organized to perform a common function, to form a **tissue**. Sponges are considered by some authorities to belong to this grade, although jellyfishes and their relatives (Cnidaria) more clearly demonstrate the tissue plan. Both groups are still largely of the cellular grade of organization because most cells are scattered and not organized into tissues. An excellent example of a tissue in cnidarians is the **nerve net,** in which nerve cells and their processes form a definite tissue structure, with the function of coordination.
4. *Tissue-organ grade of organization.* An aggregation of tissues into organs is a further step in complexity. Organs are usually composed of more than one kind of tissue and have a more specialized function than tissues. This is the organizational level of flatworms (Platyhelminthes), in which well-defined organs such as eyespots, proboscis, and reproductive organs occur. In flatworms, the reproductive organs transcend the tissue-organ grade and are organized into a reproductive system.
5. *Organ-system grade of organization.* When organs work together to perform some function, we have the highest level of organization—an organ system. Systems are associated with basic body functions such as circulation, respiration, and digestion. The simplest animals having this type of organization are nemertean worms, which have a complete digestive system distinct from the circulatory system. Most animal phyla demonstrate this type of organization.



by varying the architectural patterns of subcellular structures, organelles, and the cell as a whole (see Chapter 11).

The **metazoa,** or multicellular animals, evolved greater structural complexity by combining cells into larger units. A metazoan cell is a specialized part of the whole organism and, unlike a unicellular organism, it is not capable of independent existence. Cells of a multicellular organism are specialized for performing the various tasks accomplished by subcellular elements in unicellular forms. The simplest metazoans show the *cellular* grade of organization in which cells demonstrate division of labor but are not strongly associated to perform a specific collective function (Table 9.1). In the more complex *cell-tissue* grade of organization, cells are grouped together and perform their common functions as a highly coordinated unit called a **tissue.** Animals at or beyond the cell-tissue grade of organization are termed **eumetazoans.** In animals of the *tissue-organ* grade of organization, tissues are assembled into still larger functional units called **organs.** Usually one type of tissue carries the burden of an organ's chief function, as muscle tissue does in the heart; other tissues—epithelial, connective, and nervous—perform supportive roles. The chief functional cells of an organ are called **parenchyma** (pa-ren´ka-ma; Gr. *para,* beside, + *enchyma,* infusion). The supportive tissues are its **stroma** (Gr. bedding). For instance, in the vertebrate pancreas the secreting cells are the parenchyma; capsule and connective tissue framework represent stroma.

Most metazoa have an additional level of complexity in which different organs operate together as **organ systems.** Eleven different kinds of organ systems are observed in metazoans: skeletal, muscular, integumentary, digestive, respiratory, circulatory, excretory, nervous, endocrine, immune, and reproductive. The great evolutionary diversity of these organ systems is covered in Chapters 14 through 28.

ANIMAL BODY PLANS

As described in the prologue to this chapter, the ancestral body plan constrains the form of its descendant lineage. Animal body plans differ in the grade of organization, in body symmetry, in the number of embryonic germ layers, and in the number of body cavities. Body symmetry can be generally determined from the external appearance of an animal, but other features of a body plan typically require a more detailed examination.

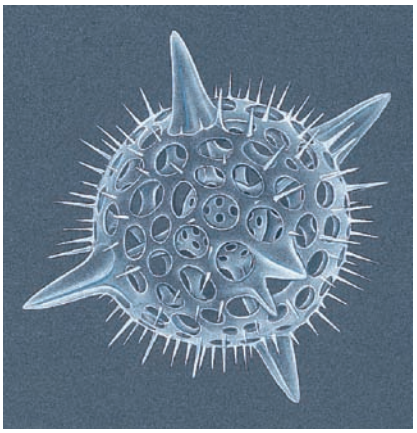
Animal Symmetry

Symmetry refers to balanced proportions, or correspondence in size and shape of parts on opposite sides of a median plane.

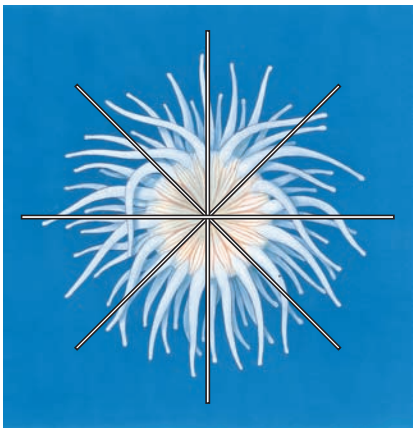
Spherical symmetry means that any plane passing through the center divides a body into equivalent, or mirrored, halves (Figure 9.1, *left*). This type of symmetry is found chiefly among some unicellular forms and is rare in animals. Spherical forms are best suited for floating and rolling.

Radial symmetry (Figure 9.1, *middle*) applies to forms that can be divided into similar halves by more than two planes passing through the longitudinal axis. These are tubular, vase, or bowl shapes found in some sponges and in hydras, jellyfish, sea urchins, and related groups, in which one end of the longitudinal axis is usually the mouth (the **oral** surface). In sessile forms, such as hydra and sea anemones, the basal attachment disc is the **aboral** surface. A variant form is **biradial symmetry** in which, because of some part that is single or paired rather than radial, only two planes passing through the longitudinal axis produce mirrored halves. Comb jellies (phylum Ctenophora, p. 282), which are globular but have a pair of tentacles, are an example. Radial and biradial animals are usually sessile, freely floating, or weakly swimming. Radial animals, with no anterior or posterior end, can interact with their environment in all directions—an advantage to sessile or free-floating forms with feeding structures arranged to snare prey approaching from any direction.

The two phyla that are primarily radial as adults, Cnidaria and Ctenophora, have been called the **Radiata,** although phylogenetic results suggest that this group is not monophyletic (p. 285). Echinoderms (sea stars and their kin) are primarily bilateral animals (their larvae are bilateral) that have become secondarily radial as adults.



Spherical symmetry



Radial symmetry

Bilateral symmetry

Figure 9.1
Animal symmetry. Illustrated are animals showing spherical, radial, and bilateral symmetry.

Bilateral symmetry applies to animals that can be divided along a sagittal plane into two mirrored portions—right and left halves (Figure 9.1, *right*). The appearance of bilateral symmetry in animal evolution was a major innovation, because bilateral animals are much better fitted for directional (forward) movement than are radially symmetrical animals. Bilateral animals form a monophyletic group of phyla called the **Bilateria.**

Bilateral symmetry is strongly associated with **cephalization,** differentiation of a head. The concentration of nervous tissue and sense organs in a head bestows obvious advantages to an animal moving through its environment head first. This is the most efficient positioning of organs for sensing the environment and responding to it. Usually the mouth of an animal is located on the head as well, since so much of an animal's activity is concerned with procuring food. Cephalization is always accompanied by differentiation along an anteroposterior axis, although the evolution of this axis preceded cephalization.

Some convenient terms used for locating regions of bilaterally symmetrical animals (Figure 9.2) are **anterior,** used to designate the head end; **posterior,** the opposite or tail end; **dorsal,** the back side; and **ventral,** the front or belly side. **Medial** refers to the midline of the body; **lateral,** to the sides. **Distal** parts are farther from the middle of the body; **proximal** parts are nearer. A **frontal plane** (sometimes called coronal plane) divides a bilateral body into dorsal and ventral halves by running through the anteroposterior axis and the right-left axis at right angles to the **sagittal plane,** the plane dividing an animal into right and left halves. A **transverse plane** (also called a cross section) would cut through a dorsoventral and a right-left axis at right angles to both the sagittal and frontal planes and would separate anterior and posterior portions (Figure 9.2). In vertebrates **pectoral** refers to the chest region or area associated with the anterior pair of appendages, and **pelvic** refers to the hip region or area associated with the posterior pair of appendages.

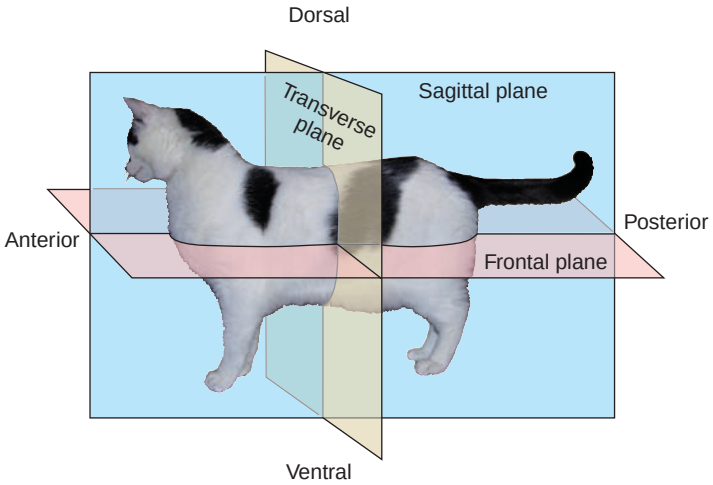


Figure 9.2
The planes of symmetry as illustrated by a bilaterally symmetrical animal.

Body Cavities and Germ Layers

A body cavity is an internal space. The most obvious example is a gut cavity, but the vast majority of animals have a second, less obvious, cavity outside the gut. When this second cavity is fluid-filled, it may cushion and protect the gut from forces exerted on the body. In some animals, such as an earthworm, it also forms part of a hydrostatic skeleton used in locomotion.

Animals differ in the presence and number of body cavities. Sponges, at the cellular grade of organization, have no body cavities, not even a gut cavity. If sponges share the same developmental sequence as other metazoans, why do they lack a gut cavity? Where in the developmental sequence does a gut form? Sponges, like all metazoans, develop from a zygote to a blastula stage. A typical spherical blastula is composed of a layer of cells surrounding a fluid-filled cavity (see Figure 8.9). This cavity, a **blastocoel,** has no external opening, so it could not serve as a gut. In sponges, after the formation of a blastula, the cells reorganize to form an adult animal, although some researchers argue that a gastrula stage does occur before reorganization (see Figure 9.5, *upper pathway* and Chapter 12).

In animals other than sponges, development proceeds from a blastula to a **gastrula** stage, as one side of the blastula pushes inward, making a depression (see Figure 9.3). The depression becomes a gut cavity, also called a **gastrocoel** or **archenteron.** The external opening to the depression is the **blastopore;** it typically becomes the adult mouth or anus. The gut lining is **endoderm** and the outer layer of cells, surrounding the blastocoel, is **ectoderm** (Figure 9.3). The embryo now has two cavities, a gut and a blastocoel. Animals such as sea anemones and jellyfish develop from these two germ layers and are called **diploblastic** (Figure 9.5, *upper pathway*). They typically have radial symmetry as adult animals. The fluid-filled blastocoel persists in diploblasts, but in others it is filled with a third germ layer, **mesoderm.** Animals that possess ectoderm, mesoderm, and endoderm are termed **triploblastic** and the majority are bilaterally symmetrical.

Methods of Mesoderm Formation

Cells forming mesoderm are derived from endoderm, but there are two ways a middle tissue layer of mesoderm can form. In protostomes, mesoderm forms as endodermal cells from near the blastopore migrate into the blastocoel (Figure 9.3A). Following this event, three different body plans—acoelomate, pseudocoelomate, and coelomate—are possible (Figure 9.3A).

In the **acoelomate** plan, mesodermal cells completely fill the blastocoel, leaving a gut as the only body cavity (Figure 9.3A). The region between the ectodermal epidermis and endodermal digestive tract is filled with a spongy mass of space-filling cells, the **parenchyma** (Figure 9.4). Parenchyma is derived from embryonic connective tissue and is important in assimilation and transport of food and in disposal of metabolic wastes.

In the **pseudocoelomate** plan, mesodermal cells line the outer edge of the blastocoel, leaving two body cavities: a persistent blastocoel and a gut cavity (Figures 9.3A and 9.4). The

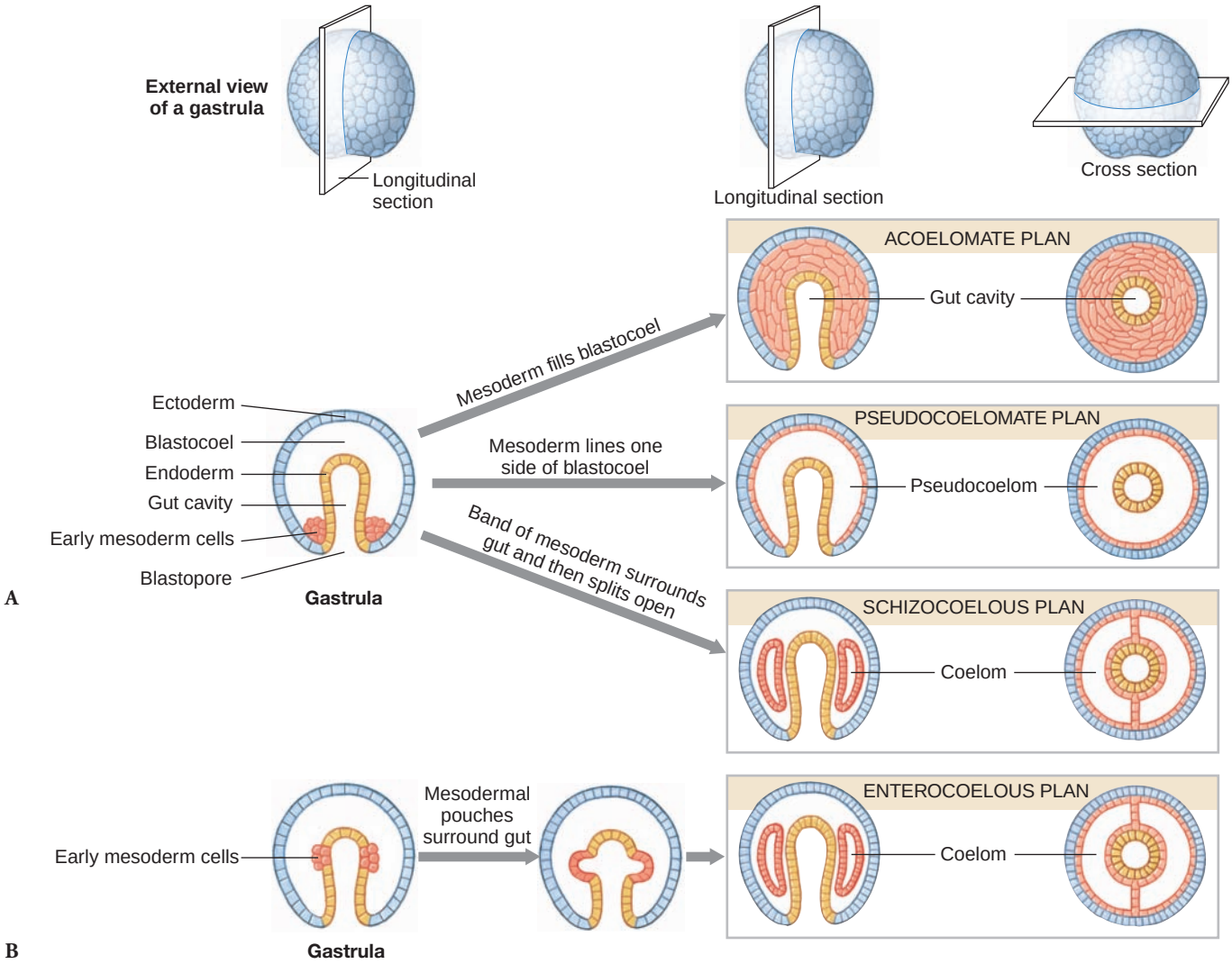


Figure 9.3

Mesoderm resides in different parts of the gastrula during formation of acoelomate, pseudocoelomate, and schizocoelous body plans (A). Mesoderm and a coelom form together in the enterocoelus plan (B).

blastocoel is now called a **pseudocoelom;** the name means false coelom in reference to mesoderm only partially surrounding the cavity, instead of completely surrounding it, as in a true **coelom.**

In the **schizocoelous** plan, mesodermal cells fill the blastocoel, forming a solid band of tissue around the gut. Then, through programmed cell death, space opens *inside* the mesodermal band (Figure 9.3A). This new space is a coelom. The embryo has two body cavities, a gut and a coelom.

In deuterostomes, mesoderm forms by an **enterocoelous** plan, where cells from the central portion of the gut lining begin to grow outward as pouches, expanding into the blastocoel (Figure 9.3B). The expanding pouch walls form a mesodermal ring. As the pouches move outward, they enclose a space. The space becomes a coelomic cavity or coelom. Eventually the pouches pinch off from the gut lining, completely enclosing a coelom bounded by mesoderm on all sides. The coelom completely fills the blastocoel. The embryo has two body cavities, a gut and a coelom.

A coelom made by **enterocoely** is functionally equivalent to a coelom made by **schizocoely,** and are represented as such in the **eucoelomate** body plan (Figure 9.4). Both kinds of coelomic cavities are bounded by mesoderm and lined with a **peritoneum,** a thin cellular membrane derived from mesoderm (Figure 9.4). Mesodermal **mesenteries** suspend organs in the coelom (Figure 9.4). A pseudocoelom lacks a peritoneum.

Developmental Origins of Body Plans in Triploblasts

Triploblastic animals follow one of several major developmental pathways to form a blastula from a zygote (Figure 9.5). The most common pathways are by spiral or radial cleavage (see Figure 8.10, p. 167).

Radial cleavage is typically accompanied by three other traits: The blastopore becomes an anus and a new opening makes the mouth, the coelom forms via enterocoely, and

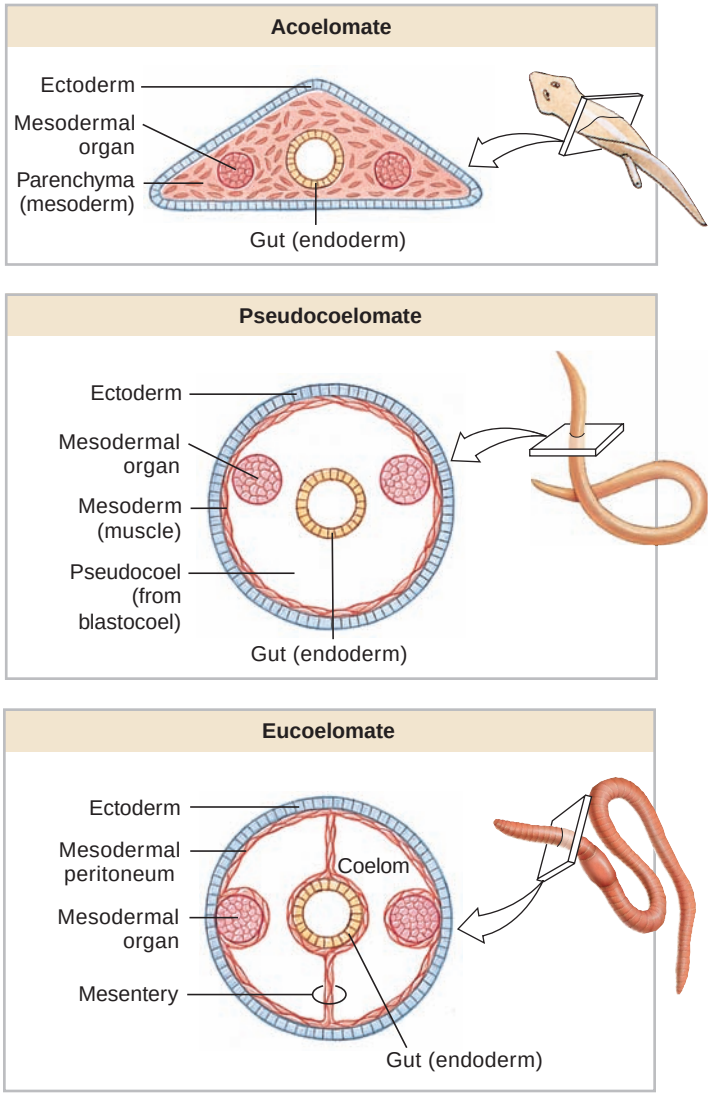


Figure 9.4

Acoelomate, pseudocoelomate, and eucoelomate body plans are shown as cross sections of representative animals. Note the relative positions of parenchyma, peritoneum, and body organs.

cleavage is regulative (see Figure 8.10, p. 165). Animals with these features are called deuterostomes (Figure 9.5, *lower pathway*); this group includes sea urchins and chordates.

Spiral cleavage produces an embryo whose developmental patterns contrast with those described for deuterostomes: the blastopore becomes the mouth, cleavage is mosaic (see Figure 8.10, p. 165), and mesoderm forms from a particular cell in the embryo, the 4d cell (see p. 169). The body may become acoelomate, pseudocoelomate, or coelomate, depending on the taxa (Figure 9.5, *central pathway*). If a coelom is present, it is made via schizocoely. Animals with these features are called lophotrochozoan protostomes; this group includes molluscs, segmented worms, and other taxa (Figure 9.5).

Lophotrochozoans are distinguished from ecdysozoan protostomes (not shown in Figure 9.5) where a range of cleavage patterns have been described. These include spiral cleavage, a superficial cleavage pattern in which nuclei proliferate within common cytoplasm prior to separation by multiple cytoplasmic divisions (see Figure 8.15, p. 170) and a pattern initially resembling radial cleavage. Ecdysozoans may be coelomate or pseudocoelomate. Insects, crabs, and nematodes are among the ecdysozoans.

A Complete Gut Design and Segmentation

A few diploblasts and triploblasts have a blind or incomplete gut where food must enter and exit the same opening, but the majority of forms possess a complete gut (Figure 9.5). A complete gut makes possible a one-way flow of food from mouth to anus. A body constructed in this way is essentially a gut tube within another body tube. A tube-within-a-tube design has proved to be very versatile; members of the most common animal phyla, both invertebrate and vertebrate, have this plan.

Segmentation, also called metamerism, is another common feature of metazoans. Segmentation is a serial repetition of similar body segments along the longitudinal axis of the body. Each segment is called a **metamere,** or **somite.** In forms such as earthworms and other annelids (Figure 9.6), in which metamerism is most clearly represented, the segmental arrangement includes both external and internal structures of several systems. There is repetition of muscles, blood vessels, nerves, and setae of locomotion. Some other organs, such as those of sex, may be repeated in only a few segments. Evolutionary changes have obscured much of the segmentation in many animals, including humans.

The appearance of segmentation in body plans was a highly significant evolutionary event. Segmentation permits greater body mobility and complexity of structure and function. Its potential is amply displayed in phylum Arthropoda, the largest assemblage of animals on earth. Segmentation is found in phylum Chordata in addition to Annelida and Arthropoda (Figure 9.6), although superficial segmentation of ectoderm and body wall may appear among diverse groups of animals. The importance and potential of segmentation is discussed in Chapters 17 and 18.

COMPONENTS OF METAZOAN BODIES

Metazoan bodies consist of cellular components, derived from the three embryonic germ layers—ectoderm, mesoderm, and endoderm—as well as extracellular components.

Extracellular Components

Metazoan animals contain two important noncellular components: body fluids and extracellular structural elements. In all eumetazoans, body fluids are subdivided into two fluid "compartments": those that occupy **intracellular space,** within the body's cells, and those that occupy **extracellular space,** outside the cells. In animals with closed vascular systems

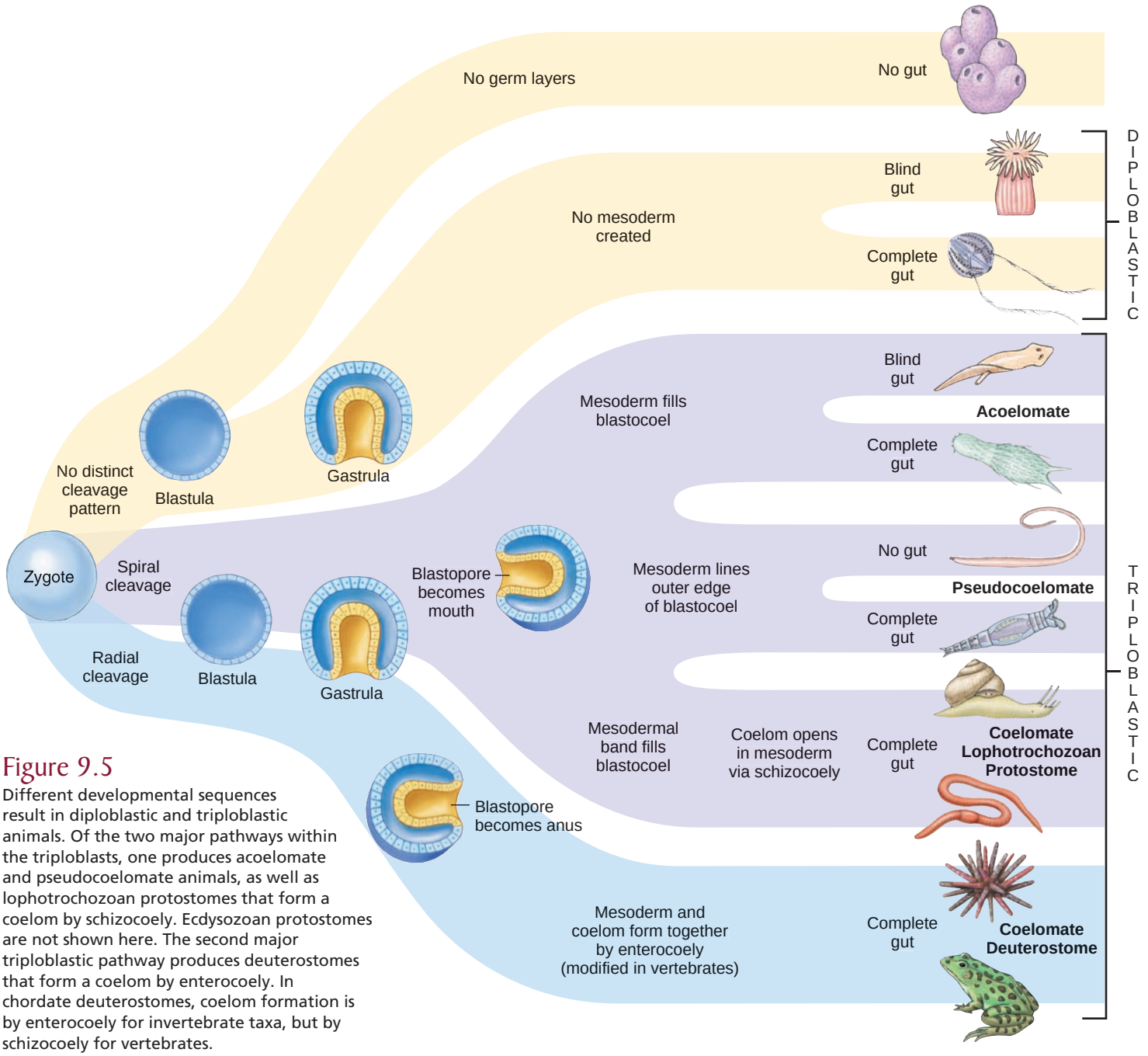


Figure 9.5

Different developmental sequences result in diploblastic and triploblastic animals. Of the two major pathways within the triploblasts, one produces acoelomate and pseudocoelomate animals, as well as lophotrochozoan protostomes that form a coelom by schizocoely. Ecdysozoan protostomes are not shown here. The second major triploblastic pathway produces deuterostomes that form a coelom by enterocoely. In chordate deuterostomes, coelom formation is by enterocoely for invertebrate taxa, but by schizocoely for vertebrates.

(such as segmented worms and vertebrates), the extracellular fluids are subdivided further into **blood plasma** (the fluid portion of blood) and **interstitial fluid.** Interstitial fluid, also called tissue fluid, occupies the space surrounding cells. Many invertebrates have open blood systems, however, with no true separation of blood plasma from interstitial fluid. We explore these relationships further in Chapter 31.

The term "intercellular," meaning "between cells," should not be confused with the term "intracellular," meaning "within cells."

Extracellular structural elements are the supportive material of the organism, including loose connective tissue (especially well developed in vertebrates but present in all metazoa), cartilage (molluscs and chordates), bone (vertebrates), and cuticle (arthropods, nematodes, annelids, and others). These elements provide mechanical stability and protection (see Chapter 29). In some instances, they act also as a depot of materials for exchange between the cells and the interstitial fluid, and serve as a medium for extracellular reactions. We describe diversity of extracellular structural elements characteristic of different groups of animals in Chapters 15 through 28.

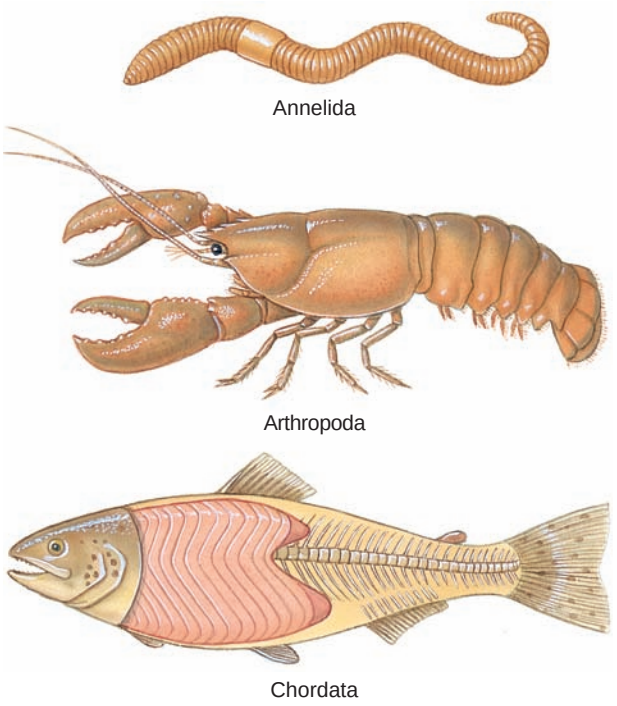


Figure 9.6

Segmented phyla. These three phyla have all made use of an important principle in nature: segmentation (also called metamerism), or repetition of structural units. Segmentation brings more varied specialization because segments, especially in arthropods, have become modified for different functions.

Cellular Components: Tissues

A **tissue** is a group of similar cells (together with associated cell products) specialized for performance of a common function. The study of tissues is called **histology** (Gr. *histos,* tissue, + *logos,* discourse) or microanatomy. All cells in metazoan animals form tissues. Sometimes cells of a tissue may be of several kinds, and some tissues have much extracellular material.

During embryonic development, the germ layers become differentiated into four kinds of tissues. These are epithelial, connective, muscular, and nervous tissues (Figure 9.7). This is a surprisingly short list of only four basic tissue types that are able to meet the diverse requirements of animal life.

Epithelial Tissue

An **epithelium** (pl., epithelia) is a sheet of cells that covers an external or internal surface. Outside the body, epithelium forms a protective covering. Inside, epithelium lines all organs of the body cavity, as well as ducts and passageways through which various materials and secretions move. Thus, ions and molecules must pass through epithelial cells as they move to and from all other cells of the body. Consequently a large variety of transport molecules are located on epithelial cell membranes (see Chapter 3). Epithelial cells are also modified into glands that produce lubricating mucus or specialized products such as hormones or enzymes.

Epithelia are classified by cell form and number of cell layers. Simple epithelia (a single layer of cells; Figure 9.8) are found in all metazoan animals, while stratified epithelia (many cell layers; Figure 9.9) are mostly restricted to vertebrates. All types of epithelia are supported by an underlying basement membrane, which is a condensed region of ground substance of connective tissue, but is secreted by both epithelial and connective tissue cells. Blood vessels never penetrate into epithelial tissues, which depend on diffusion of oxygen and nutrients from underlying tissues.

Connective Tissue

Connective tissues are a diverse group of tissues that serve various binding and supportive functions. They are so widespread in the body that removal of other tissues would still leave the complete form of the body clearly apparent. Connective tissue is composed of relatively few cells, a great many extracellular fibers, and a **ground substance,** in which the fibers are suspended (together called **matrix**). We recognize several different types of connective tissue. Two kinds of **connective tissue proper** occur in vertebrates. **Loose connective tissue** is composed of fibers and both fixed and wandering cells suspended in a viscous fluid ground substance (Figure 9.10). **Dense connective tissue,** such as tendons and ligaments, is composed largely of densely packed fibers and little ground substance (Figure 9.10). Much of the fibers of connective tissue is composed of **collagen** (Gr. *kolla,* glue, + *genos,* descent), a protein of great tensile strength. Collagen is the most abundant protein in the animal kingdom, found in animal bodies wherever both flexibility and resistance to stretching are required. Connective tissue of invertebrates, as in vertebrates, consists of cells, fibers, and ground substance, and show a wide diversity of structure ranging from highly cellular to acellular histologies.

Other types of specialized connective tissue include **blood, lymph** (collectively considered vascular tissue), **adipose** (fat) tissue, **cartilage,** and **bone.** Vascular tissue is composed of distinctive cells in a fluid ground substance, the plasma. Vascular tissue lacks fibers under normal conditions. Blood composition is discussed in Chapter 31.

Cartilage is a semirigid form of connective tissue with closely packed fibers embedded in a gel-like ground substance (Figure 9.10). **Bone** is a calcified connective tissue containing calcium salts organized around collagen fibers (Figure 9.10). Structure of cartilage and bone is discussed in the section on skeletons in Chapter 29.

Muscular Tissue

Muscle is the most abundant tissue in the body of most animals. It originates (with few exceptions) from mesoderm, and its unit is the cell or **muscle fiber,** specialized for contraction. When viewed with a light microscope, **striated muscle** appears transversely striped (striated), with alternating dark and light bands (Figure 9.11). In vertebrates we recognize two types of striated

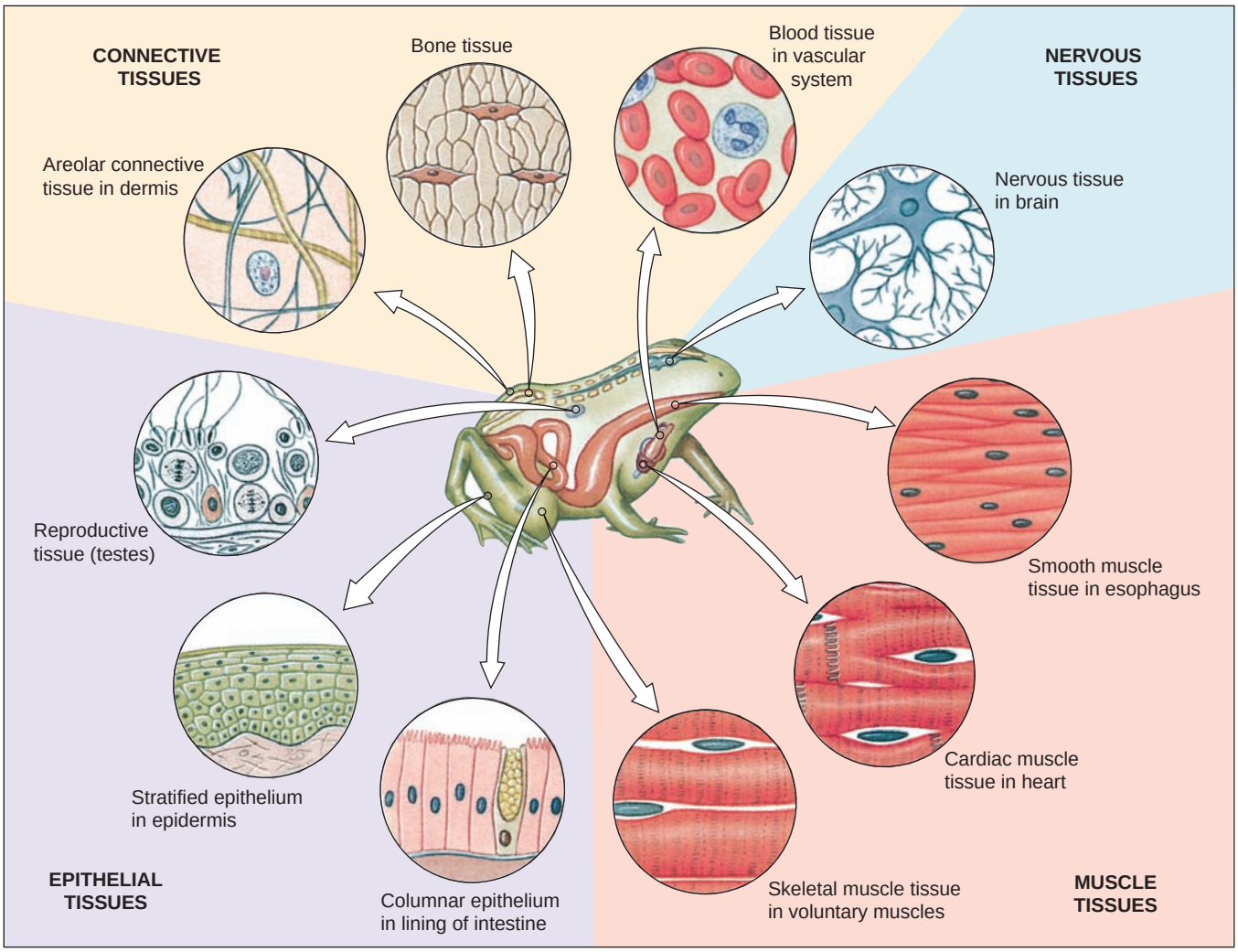


Figure 9.7
Types of tissues in a vertebrate, showing examples of where different tissues are located in a frog.

muscle: **skeletal** and **cardiac muscle.** In invertebrates, a third striated muscle type called **obliquely striated muscle** has been described. **Smooth** (or visceral) **muscle,** which lacks the characteristic alternating bands of the striated type is found in both invertebrates and vertebrates, although major ultrastructural differences have been described between them (Figure 9.11). Unspecialized cytoplasm of muscles is called **sarcoplasm,** and contractile elements within the fiber are **myofibrils.** Muscular movement is covered in Chapter 29.

Nervous Tissue

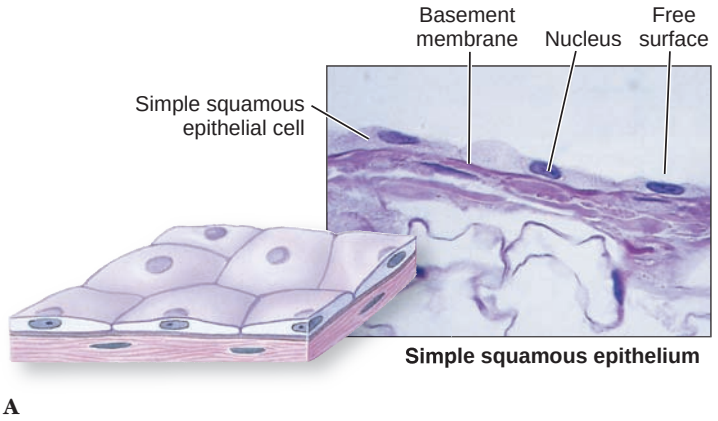
Nervous tissue is specialized for reception of stimuli and conduction of impulses from one region to another. Two basic types of cells in nervous tissue are **neurons** (Gr. nerve), the basic functional unit of nervous systems, and **neuroglia** (nu-rog´le-a; Gr. nerve, + *glia,* glue), a variety of nonnervous cells that insulate neuron membranes and serve various supportive functions. Figure 9.12 shows functional anatomy of a typical nerve cell. The functional roles of nervous tissue are treated in Chapter 33.

COMPLEXITY AND BODY SIZE

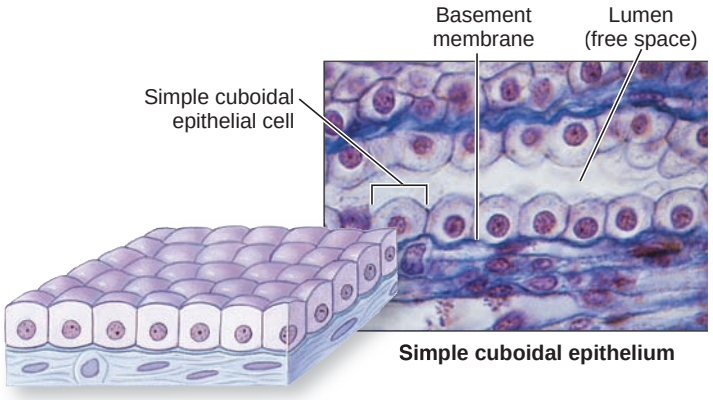
The most complex grades of metazoan organization permit and to some extent even promote evolution of large body size (Figure 9.13). Large size confers several important physical and ecological consequences for an organism. As animals become larger, the body surface increases much more slowly than body volume because surface area increases as the square of body length (length^2), whereas volume (and therefore mass) increases as the cube of body length (length^3). In other words, a large animal has less surface area relative to its volume than does a small animal of the same shape. The surface area of a large animal may be inadequate for respiration and nutrition by cells located deep within its body. There are two possible solutions to this problem. One solution is to fold or invaginate the body surface to increase the surface area or, as exploited by flatworms, flatten the body into a ribbon or disc so that no internal space is far from the surface. This solution allows a body to become large without internal complexity. However, most large animals adopted a second solution; they developed internal transport

Figure 9.8

Types of simple epithelium. **A, Simple squamous epithelium,** composed of flattened cells that form a continuous lining of blood capillaries, lungs, and other surfaces where it permits the diffusion of gases and transport of other molecules into and out of cavities. **B, Simple cuboidal epithelium** is composed of short, boxlike cells. Cuboidal epithelium usually lines small ducts and tubules, such as those of the kidney and salivary glands, and may have active secretory or absorptive functions. **C, Simple columnar epithelium** resembles cuboidal epithelium, but the cells are taller and usually have elongate nuclei. This type of epithelium is found on highly absorptive surfaces such as the intestinal tract of most animals. The cells often bear minute, fingerlike projections called microvilli that greatly increase the absorptive surface. In some organs, such as the female reproductive tract, cells may be ciliated.



A



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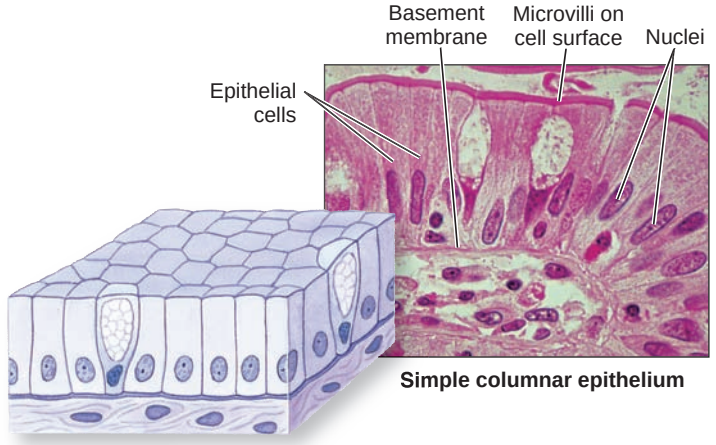
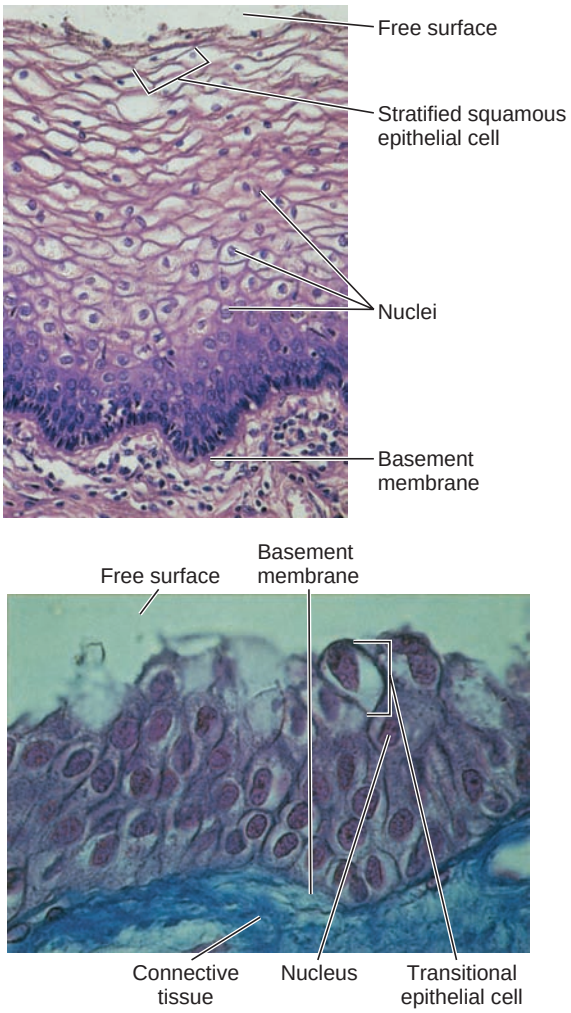


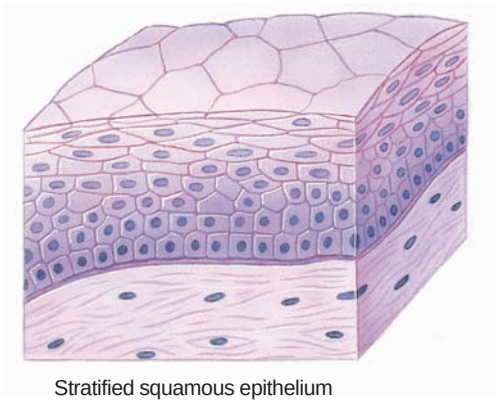
C

systems to shuttle nutrients, gases, and waste products between cells and the external environment.

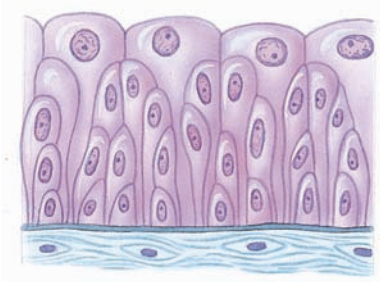
Larger size buffers an animal against environmental fluctuations; it provides greater protection against predation and enhances offensive tactics; and it permits a more efficient use of metabolic energy. A large mammal uses more oxygen than a small mammal, but the cost of maintaining its body temperature is less per gram of weight for a large mammal than for a small one. Large animals also can move at less energy cost than can small animals. For example, a large mammal uses more oxygen in running than does a small mammal, but the energy cost of moving 1 g of its body over a given distance is much less for a large mammal than for a small one (Figure 9.14). For all of these reasons, ecological opportunities of larger animals are very different from those of small ones. In subsequent chapters we describe the extensive adaptive radiations observed in taxa of large animals.

The tendency for maximum body size to increase within lines of descent is known as "Cope's law of phyletic increase," named after nineteenth-century American paleontologist and naturalist Edward Drinker Cope. Cope noted that lineages begin with small organisms that give rise to larger and ultimately to giant forms. Large forms frequently become extinct, providing opportunities for new lineages, which in turn evolve larger forms. Cope's rule holds well for many nonflying vertebrates and invertebrate groups, even though Cope's Lamarckian explanation for the trend—that organisms evolved from an inner urge to attain a higher state of being (and larger size)—was preposterous. Many animal taxa contain lineages that show evolutionary miniaturization, in contrast to Cope's rule (for example, the insects).





Stratified squamous epithelium consists of two to many layers of cells adapted to withstand mild mechanical abrasion and distortion. The basal layer of cells undergoes continuous mitotic divisions, producing cells that are pushed toward the surface where they are sloughed off and replaced by new cells from beneath. This type of epithelium lines the oral cavity, esophagus, and anal canal of many vertebrates, and the vagina of mammals.



Transitional epithelium is a type of stratified epithelium specialized to accommodate great stretching. This type of epithelium is found in the urinary tract and bladder of vertebrates. In the relaxed state it appears to be four or five cell layers thick, but when stretched it appears to have only two or three layers of extremely flattened cells.

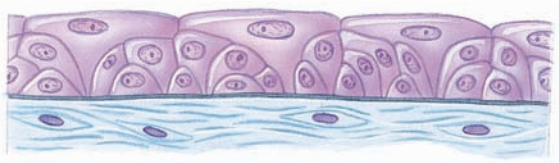


Figure 9.9

Types of stratified epithelium.

SUMMARY

From the relatively simple organisms that mark the beginnings of life on earth, animal evolution has produced more intricately organized forms. Organelles are integrated into cells, cells into tissues, tissues into organs, and organs into systems. Whereas a unicellular organism performs all life functions within the confines of a single cell, a multicellular animal is an organization of subordinate units united at successive levels.

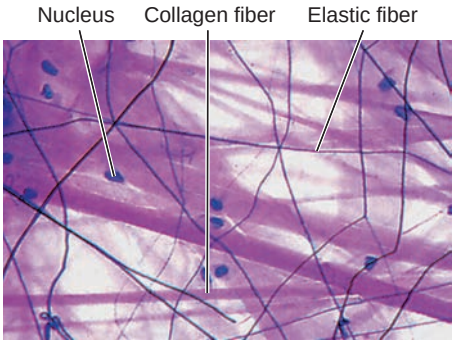
Every organism has an inherited body plan described in terms of body symmetry, number of embryonic germ layers, grade of organization, and number of body cavities. The majority of animals exhibit bilateral symmetry, but spherical and radial symmetry occur in some groups. Most animals are triploblastic and develop from three embryonic germ layers, but cnidarians and a few other forms are diploblastic. Sponges lack germ layers and possess a cellular grade of organization. Most animals have the tissue grade of organization.

All animals other than sponges have a gut cavity. Most animals have a second cavity that surrounds the gut. The second cavity may be a pseudocoelom or a coelom. There are two taxon-specific patterns of coelom formation, schizocoely and enterocoely.

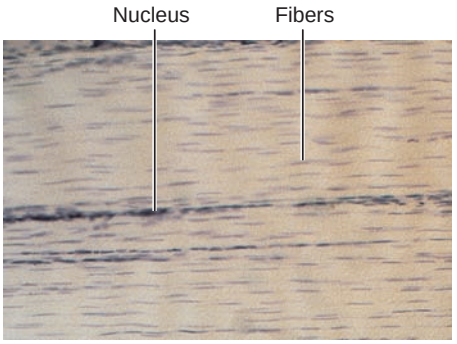
Triploblastic animals are divided among deuterostomes and protostomes according to their particular developmental sequence. Protostomes are further divided into lophotrochozoan and ecdysozoan forms on the basis of more detailed features of development.

A metazoan body consists of cells, most of which are functionally specialized; body fluids, divided into intracellular and extracellular fluid compartments; and extracellular structural elements, which are fibers or formless materials that serve various structural functions in the extracellular space. The cells of metazoa develop into various tissues; basic types are epithelial, connective, muscular, and nervous. Tissues are organized into larger functional units called organs, and organs are associated to form systems.

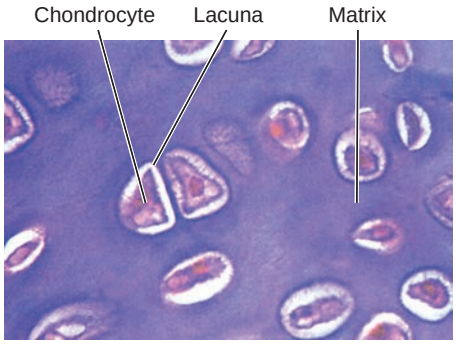
One correlate of increased anatomical complexity is an increase in body size, which offers certain advantages such as more effective predation, reduced energy cost of locomotion, and improved homeostasis.



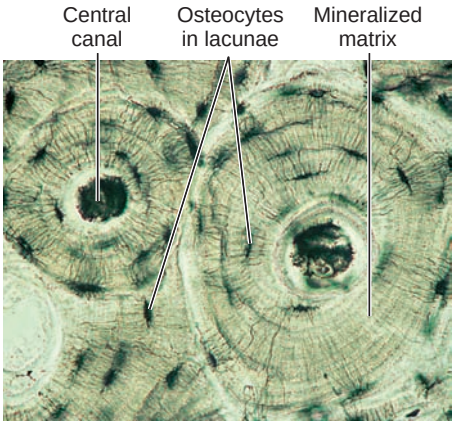
A **Loose connective tissue**



B **Dense connective tissue**



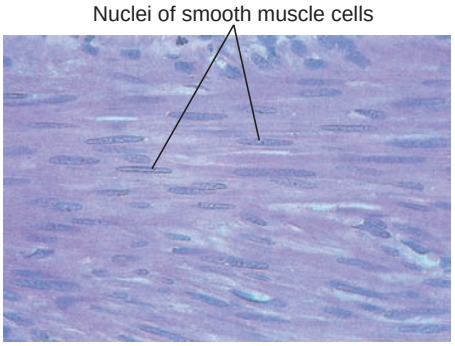
C **Cartilage**



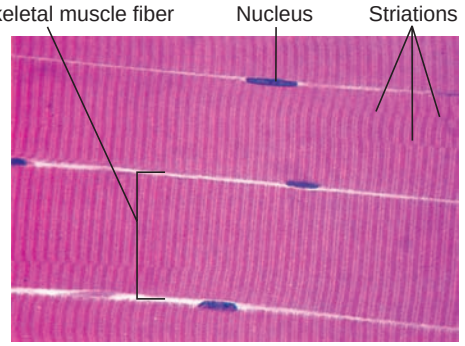
D **Bone**

Figure 9.10

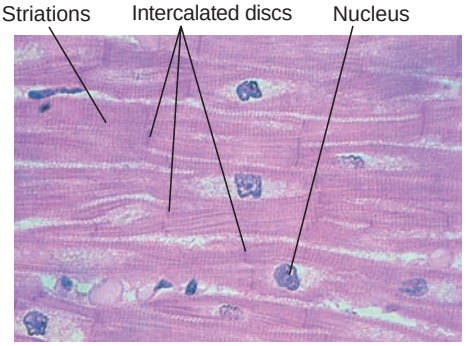
Types of connective tissue. **A, Loose connective tissue,** also called areolar connective tissue, is the "packing material" of the body that anchors blood vessels, nerves, and body organs. It contains fibroblasts that synthesize the fibers and ground substance of connective tissue and wandering macrophages that phagocytize pathogens or damaged cells. The different fiber types include collagen fibers (thick and red in micrograph) and thin elastic fibers (black and branching in micrograph) formed of the protein elastin. **B, Dense connective tissue** forms tendons, ligaments, and fasciae (fa´sha), the latter arranged as sheets or bands of tissue surrounding skeletal muscle. In a tendon (shown here) the collagenous fibers are extremely long and tightly packed together. **C, Cartilage** is a vertebrate connective tissue composed of a firm matrix containing cells (chondrocytes) located in small pockets called lacunae, and collagen and/or elastic fibers (depending on type of cartilage). In hyaline cartilage shown here, both collagen fibers and matrix are stained uniformly purple and cannot be distinguished one from the other. Because cartilage lacks a blood supply, all nutrients and waste materials must diffuse through the ground substance from surrounding tissues. **D, Bone,** strongest of vertebrate connective tissues, contains mineralized collagen fibers. Small pockets (lacunae) within the matrix contain bone cells, called osteocytes. The osteocytes communicate with each other by means of a tiny network of channels called canaliculi. Blood vessels, extensive in bone, are located in larger channels, including central canals. Bone undergoes continuous remodeling during an animal's life, and can repair itself following even extensive damage.



Smooth muscle is nonstriated muscle found in both invertebrates and vertebrates. Smooth muscle cells are long, and tapering, each containing a single nucleus. Smooth muscle is the most common type of muscle in invertebrates in which it serves as body wall musculature and surrounds ducts and sphincters. In vertebrates, smooth muscle surrounds blood vessels and internal organs such as intestine and uterus. It is called involuntary muscle in vertebrates because its contraction is usually not consciously controlled.



Skeletal muscle is a type of striated muscle found in both invertebrates and vertebrates. It is composed of extremely long, cylindrical fibers, which are multinucleate cells that may reach from one end of the muscle to the other. Viewed through the light microscope, the cells appear to have a series of stripes, called striations, running across them. Skeletal muscle is called voluntary muscle (in vertebrates) because it contracts when stimulated by nerves under conscious central nervous system control.



Cardiac muscle is another type of striated muscle found only in the vertebrate heart. The cells are much shorter than those of skeletal muscle and have only one nucleus per cell (uninucleate). Cardiac muscle tissue is a branching network of fibers with individual cells interconnected by junctional complexes called intercalated discs. Cardiac muscle is considered involuntary muscle because it does not require nerve activity to stimulate contraction. Instead, heart rate is controlled by specialized pacemaker cells located in the heart itself. However, autonomic nerves from the brain may alter pacemaker activity.

Figure 9.11

Types of muscle tissue.

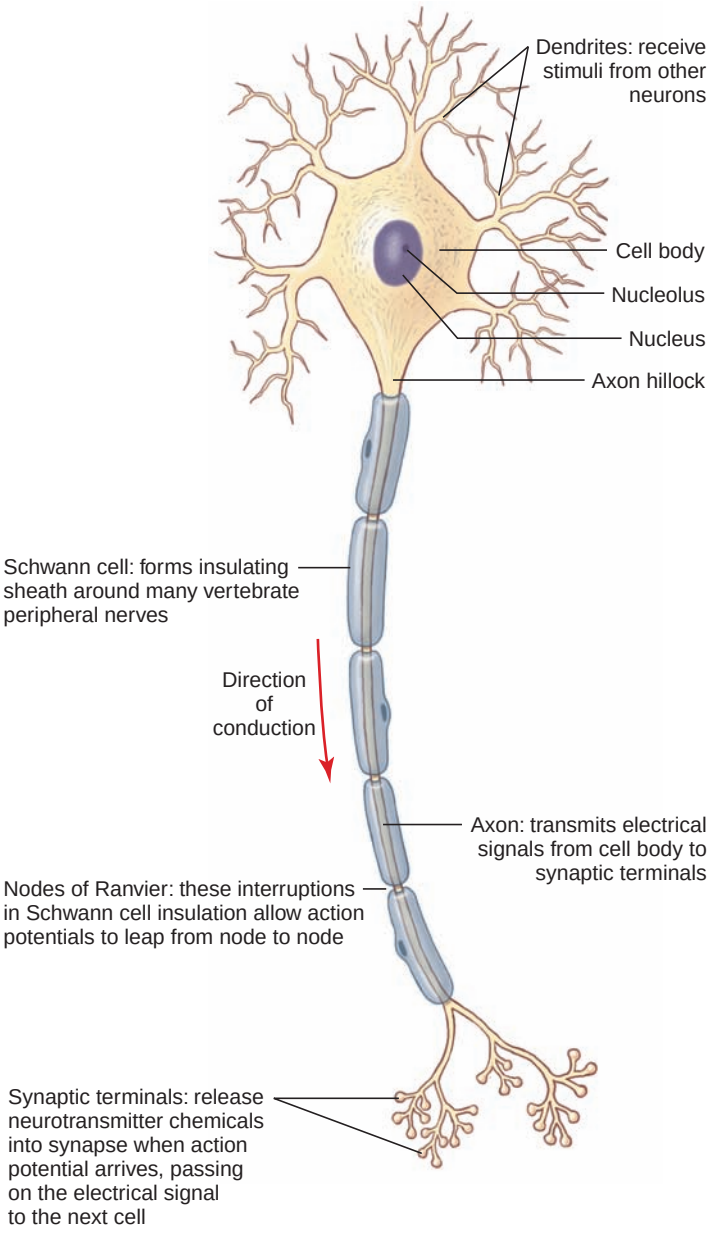


Figure 9.12

Functional anatomy of a neuron. From the nucleated cell body, or **soma,** extend one or more **dendrites** (Gr. *dendron,* tree), which receive electrical signals from receptors or other nerve cells, and a single **axon** that carries signals away from the cell body to other nerve cells or to an effector organ. The axon is often called a **nerve fiber.** Nerves are separated from other nerves or from effector organs by specialized junctions called synapses.

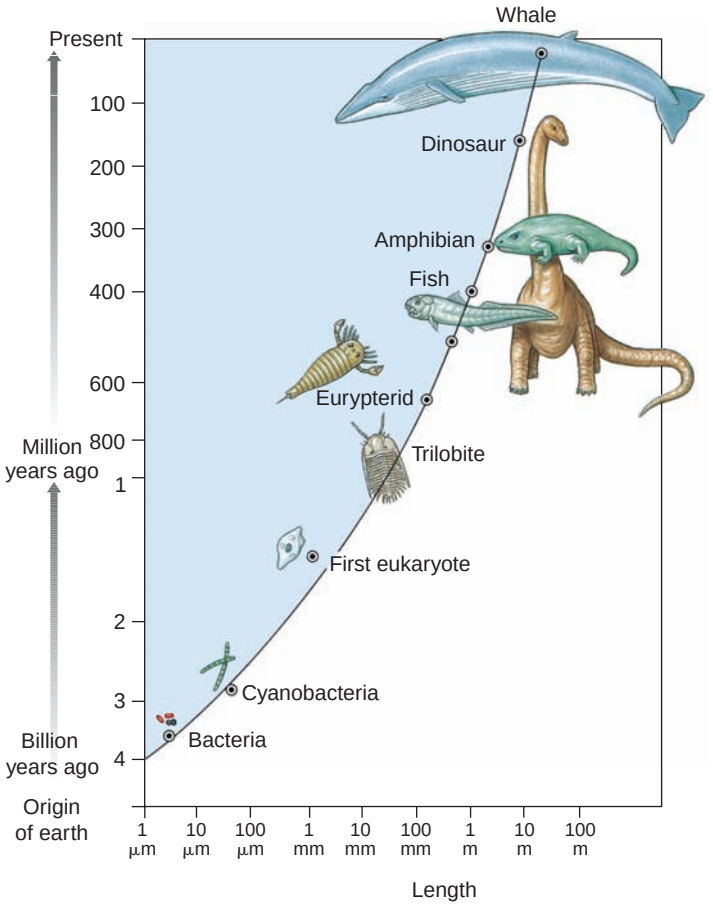


Figure 9.13

Graph showing the evolution of size (length) increase in organisms at different periods of life on earth. Note that both scales are logarithmic.

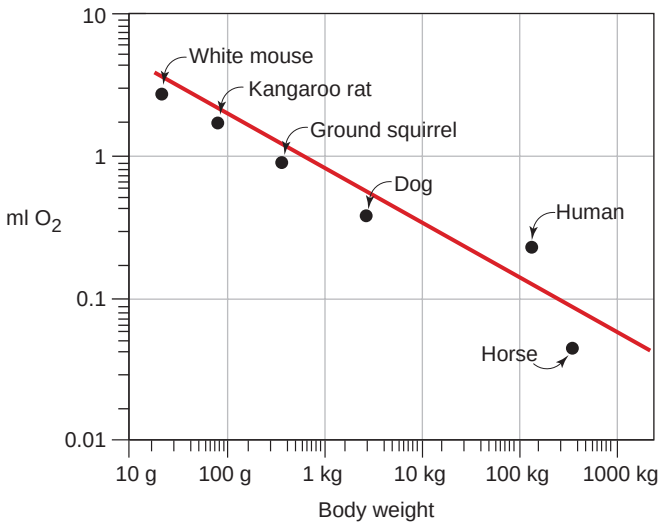


Figure 9.14

Net cost of running for mammals of various sizes. Each point represents the cost (measured in rate of oxygen consumption) of moving 1 g of body over 1 km. Cost decreases with increasing body size.

REVIEW QUESTIONS

1. Name the five grades of organization in organismal complexity and explain how each successive grade is more complex than the one preceding it.
2. Can you suggest why, during the evolutionary history of animals, there has been a tendency for maximum body size to increase? Do you think it inevitable that complexity should increase along with body size? Why or why not?
3. What is the meaning of the terms parenchyma and stroma as they relate to body organs?
4. Body fluids of eumetazoan animals are separated into fluid "compartments." Name these compartments and explain how compartmentalization may differ in animals with open and closed circulatory systems.
5. What are the four major types of tissues in metazoans?
6. How would you distinguish between simple and stratified epithelium? What characteristic of stratified epithelium might explain why it, rather than simple epithelium, is found lining the oral cavity, esophagus, and vagina?
7. What three elements are present in all connective tissue? Give some examples of different types of connective tissue.
8. What are three muscle tissue types found among animals? Explain how each is specialized for particular functions.
9. Describe the principal structural and functional features of a neuron.
10. Match the animal group with its body plan:

____ Unicellular	a. Nematode
____ Cell aggregate	b. Vertebrate
____ Blind sac, acoelomate	c. Protozoan
____ Tube-within-a-tube, pseudocoelomate	d. Flatworm
	e. Sponge
____ Tube-within-a-tube, eucoelomate	f. Arthropod
	g. Nemertean

11. Distinguish among spherical, radial, biradial, and bilateral symmetry.
12. Use the following terms to identify regions on your body and on the body of a frog: anterior, posterior, dorsal, ventral, lateral, distal, proximal.
13. How would frontal, sagittal, and transverse planes divide your body?
14. What is meant by segmentation? Name three phyla showing segmentation.

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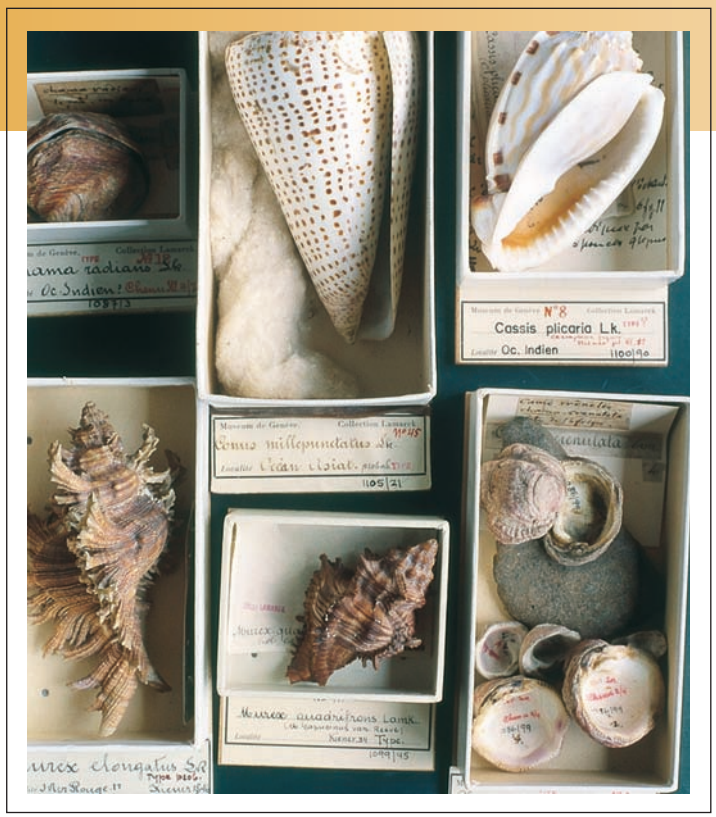
CHAPTER

10

Taxonomy and Phylogeny of Animals



Molluscan shells from the collection of Jean Baptiste de Lamarck (1744 to 1829).

Order in Diversity

Evolution has produced a great diversity of species in the animal kingdom. Zoologists have named more than 1.5 million species of animals, and thousands more are described each year. Some zoologists estimate that species named so far constitute less than 20% of all living animals and less than 1% of all those that have existed.

Despite its magnitude, the diversity of animals is not without limits. Many conceivable forms do not exist in nature, as our myths of minotaurs and winged horses show. Animal diversity is not random but has definite order. Characteristic features of humans and cattle never occur together in a single organism as they do in the mythical minotaurs; nor do characteristic wings of birds and bodies of horses occur together naturally as they do in the mythical horse, Pegasus. Humans, cattle, birds, and horses are distinct groups of animals, yet they do share some important features, including vertebrae and homeothermy, that separate them from even more dissimilar forms such as insects and flatworms.

All human cultures classify familiar animals according to patterns in animal diversity. These classifications have many purposes. Some societies classify animals according to their usefulness or destructiveness to human endeavors; others may group animals according to their roles in mythology. Biologists organize animal diversity in a nested hierarchy of groups within groups according to evolutionary relationships as revealed by ordered patterns in their sharing of homologous features. This ordering is called a "natural system" because it reflects relationships that exist among animals in nature, outside the context of human activity. A systematic zoologists has three major goals: to discover all species of animals, to reconstruct their evolutionary relationships, and to communicate those relationships by constructing an informative taxonomic system.

Darwin's theory of common descent (Chapters 1 and 6) is the underlying principle that guides our search for order in the diversity of animal life. Our science of **taxonomy** ("arrangement law") produces a formal system for naming and grouping species to communicate this order. Animals that have very recent common ancestry share many features in common and are grouped most closely in our taxonomic classification. Taxonomy is part of the broader science of systematics, or comparative biology, in which studies of variation among animal populations are used to understand their evolutionary relationships. The study of taxonomy predates evolutionary biology, however, and many taxonomic practices are remnants of a pre-evolutionary world view. Adjusting our taxonomic system to accommodate evolution has produced many problems and controversies. Taxonomy has reached an unusually active and controversial point in its development in which several alternative taxonomic systems are competing for use. To understand this controversy, it is necessary first to review the history of animal taxonomy.

Figure 10.1
Carolus Linnaeus (1707 to 1778). This portrait was made of Linnaeus at age 68, three years before his death.

LINNAEUS AND TAXONOMY

The Greek philosopher and biologist Aristotle was the first to classify organisms according to their structural similarities. The flowering of systematics in the eighteenth century culminated in the work of Carolus Linnaeus (Figure 10.1), who designed our current scheme of classification.

Linnaeus was a Swedish botanist at the University of Uppsala. He had a great talent for collecting and classifying objects, especially flowers. Linnaeus produced an extensive system of classification for both plants and animals. This scheme, published in his great work, *Systema Naturae,* used morphology (the comparative study of organismal form) for arranging specimens in collections. He divided the animal kingdom into species and gave each one a distinctive name. He grouped species into genera, genera into orders, and orders into "classes" (we use quotation marks or a capital letter to distinguish "class" as a formal taxonomic rank from its broader meaning as a group of organisms that share a common essential property). Because his knowledge of animals was limited, his lower categories, such as genera, often were very broad and included animals that are only distantly related. Much of his classification is now drastically altered, but the basic principle of his scheme is still followed.

Linnaeus's scheme of arranging organisms into an ascending series of groups of ever-increasing inclusiveness is a **hierarchical system** of classification. Major **taxa** (sing., **taxon**), into which organisms are grouped were given one of several standard **taxonomic ranks** to indicate the general degree of inclusiveness of the group. The hierarchy of taxonomic ranks has been expanded considerably since Linnaeus's time (Table 10.1). It now includes seven mandatory ranks for the animal kingdom, in descending

TABLE 10.1

Examples of Taxonomic Categories to Which Representative Animals Belong

Linnaean Rank	Human	Gorilla	Southern Leopard Frog	Katydid
Kingdom	Animalia	Animalia	Animalia	Animalia
Phylum	Chordata	Chordata	Chordata	Arthropoda
Subphylum	Vertebrata	Vertebrata	Vertebrata	Uniramia
Class	Mammalia	Mammalia	Amphibia	Insecta
Subclass	Eutheria	Eutheria	—	Pterygota
Order	Primates	Primates	Anura	Orthoptera
Suborder	Anthropoidea	Anthropoidea	—	Ensifera
Family	Hominidae	Hominidae	Ranidae	Tettigoniidae
Subfamily	—	—	Raninae	Phaneropterinae
Genus	*Homo*	*Gorilla*	*Rana*	*Scudderia*
Species	*Homo sapiens*	*Gorilla gorilla*	*Rana sphenocephala*	*Scudderia furcata*
Subspecies	—	—	—	*Scudderia furcata furcata*

The hierarchical taxonomy of four species (human, gorilla, Southern leopard frog, and katydid). Higher taxa generally are more inclusive than lower-level taxa, although taxa at two different levels may be equivalent in content. Closely related species are united at a lower point in the hierarchy than are distantly related species. For example, humans and gorillas are united at the level of the family (Hominidae) and above; they are united with the Southern leopard frog at the subphylum level (Vertebrata) and with the katydid at the kingdom level (Animalia). Mandatory Linnaean ranks are shown in bold type.

series: kingdom, phylum, "class," order, family, genus, and species. All organisms must be placed into at least seven taxa, one at each of the mandatory ranks. Taxonomists have the option of subdividing these seven ranks further to recognize more than seven taxa (superfamily, subfamily, superorder, suborder, etc.) for any particular group of organisms. In all, more than 30 taxonomic ranks are recognized. For very large and complex groups, such as fishes and insects, these additional ranks are needed to express different degrees of evolutionary divergence. Unfortunately, they also make the system more complex.

Introduction of evolutionary theory into animal taxonomy has changed the taxonomist's role from one of classification to **systematization.** Classification denotes the construction of classes, groupings of organisms that possess a common feature, called an essence, used to define the class. Organisms that possess the essential feature are members of the class by definition, and those that lack it are excluded. Because evolving species are subject always to change, the static nature of classes makes them a poor basis for a taxonomy of living systems. The activity of a taxonomist whose groupings of species represent units of common evolutionary descent is systematization, not classification. Species placed into a taxonomic group include the most recent common ancestor of the group and its descendants and thus form a branch of the phylogenetic tree of life. The species of a group thus formed represent a system of common descent, not a class defined by possession of an essential characteristic.

Because organismal characteristics are inherited from ancestral to descendant species, character variation is used to diagnose systems of common descent, but there is no requirement that an essential character be maintained throughout the system for its recognition as a taxon. The role of morphological or other features in systematization is therefore fundamentally different from the role of such characters in classification. In classification, a taxonomist asks whether a species being classified contains the defining feature(s) of a particular taxonomic class; in systematization, a taxonomist asks whether the characteristics of a species confirm or reject the hypothesis that it descends from the most recent common ancestor of a particular taxon. For example, tetrapod vertebrates descend from a common ancestor that had four limbs, a condition retained in most but not all of its descendants. Although they lack limbs, caecilians (p. 548) and snakes (p. 575) are tetrapods because they are parts of this system of common descent; other morphological and molecular characters group them respectively with living amphibians and lizards.

Although the hierarchical structure of Linnaean classification is retained in current taxonomy, the taxa are groupings of species related by evolutionary descent with modification, as diagnosed by sharing of homologous characters. As one moves up the taxonomic hierarchy from a species toward more inclusive groups, each taxon represents the descendants of an earlier ancestor, a larger branch of the tree of life.

Linnaeus's system for naming species is known as **binomial nomenclature.** Each species has a latinized name composed of two words (hence binomial) printed in italics (or underlined if handwritten or typed). The first word names the **genus,** which is capitalized; the second word is the **species epithet,** which is peculiar to the species within the genus and is written in lowercase (see Table 10.1). The great communicative value of Latin species names is that they are used consistently by scientists in all countries and languages; they are much more precise than "common names," which vary culturally and geographically.

The genus name is always a noun, and the species epithet is usually an adjective that must agree in gender with the genus. For instance, the scientific name of the common robin is *Turdus migratorius* (L. *turdus,* thrush; *migratorius,* of migratory habit). The species epithet never stands alone; the complete binomial must be used to name a species. Names of genera must refer only to single groups of organisms; the same name cannot be given to two different genera of animals. The same species epithet may be used in different genera, however, to denote different species. For example, the scientific name of the white-breasted nuthatch is *Sitta carolinensis*. The species epithet *"carolinensis"* is used in other genera for the species *Poecile carolinensis* (Carolina chickadee) and *Anolis carolinensis* (green anole, a lizard) to mean "of Carolina." All ranks above the species are designated using uninomial nouns, written with a capital initial letter.

Sometimes a species is divided into subspecies using a trinomial nomenclature (see katydid example, Table 10.1, and salamander example, Figure 10.2); such species are called **polytypic.** The generic, specific, and subspecific names are printed in italics (underlined if handwritten or typed). A polytypic species contains one subspecies whose subspecific name is a repetition of the species epithet and one or more additional subspecies whose names differ. Thus, to distinguish geographic variants of *Ensatina eschscholtzii,* one subspecies is named *Ensatina eschscholtzii eschscholtzii,* and different subspecies names are used for each of six other subspecies (Figure 10.2). Both the genus name and species epithet may be abbreviated as shown in Figure 10.2. Formal recognition of subspecies has lost popularity among taxonomists because subspecies are often based on minor differences in appearance that do not necessarily diagnose evolutionarily distinct units. When further study reveals that named subspecies are distinct evolutionary lineages, the subspecies are then often recognized as full species; indeed, many authors argue that the subspecies of *Ensatina eschscholtzii* are in fact separate species. Subspecies designations, therefore, should be viewed as tentative statements indicating that the species status of the populations needs further investigation.

SPECIES

While discussing Darwin's book, *On the Origin of Species,* in 1859, Thomas Henry Huxley asked, "In the first place, what is a species? The question is a simple one, but the right answer to it is hard to find, even if we appeal to those who should know most about it." We have used the term "species" so far as if it had a simple and unambiguous meaning. Actually, Huxley's commentary is as valid today as it was over 140 years ago. Our concepts of species have become more sophisticated, but the

diversity of different concepts and disagreements surrounding their use are as evident now as in Darwin's time.

Despite widespread disagreement about the nature of species, biologists have repeatedly used certain criteria for identifying species. First, **common descent** is central to nearly all modern concepts of species. Members of a species must trace their ancestry to a common ancestral population although not necessarily to a single pair of parents. Species are thus historical entities. A second criterion is that species must be the **smallest distinct groupings** of organisms sharing patterns of ancestry and descent; otherwise, it would be difficult to separate species from higher taxa whose members also share common descent. Morphological characters traditionally have been important in identifying such groupings, but chromosomal and molecular characters now are used extensively for this purpose. A third important criterion is that of **reproductive community.** Members of a species must form a reproductive community that excludes members of other species. For sexually reproducing populations, interbreeding is critical for maintaining a reproductive community. For organisms whose reproduction is strictly asexual, reproductive community entails occupation of a particular ecological habitat in a particular place so that a reproducing population responds as a unit to evolutionary forces such as natural selection and genetic drift.

Any species has a distribution through space, its **geographic range,** and a distribution through time, its **evolutionary duration.** Species differ greatly from each other in both dimensions. Species having very large geographic ranges or worldwide distributions are called **cosmopolitan,** whereas those with very restricted geographic distributions are called **endemic.** If a species were restricted to a single point in space and time, we would have little difficulty recognizing it, and nearly every species concept would lead us to the same decision. We have little difficulty distinguishing from each other the different species of animals that we can find living in our local park or woods. However, when we compare a local population to similar but not identical populations located hundreds of miles away, it may be hard to determine whether these populations represent parts of a single species or different species (Figure 10.2).

Throughout the evolutionary duration of a species, its geographic range can change many times. A geographic range may be either continuous or disjunct, the latter having breaks within it where the species is absent. Suppose that we find two similar but not identical populations living 300 miles apart with no related populations between them. Are we observing a single species with a disjunct distribution or two different but closely related species? Suppose that these populations have been separated historically for 50,000 years. Is this enough time for them to have evolved separate reproductive communities, or can we still view them as parts of the same reproductive community? Clear answers to such questions are very hard to find. Differences among species concepts relate to solving these problems.

Typological Species Concept

Before Darwin, a species was considered a distinct and immutable entity. Species were defined by fixed, essential features (usually morphological) considered as a divinely created pattern or archetype. This practice constitutes the **typological** (or **morphological**) **species concept.** Scientists recognized species formally by designating a **type specimen** that was labeled and

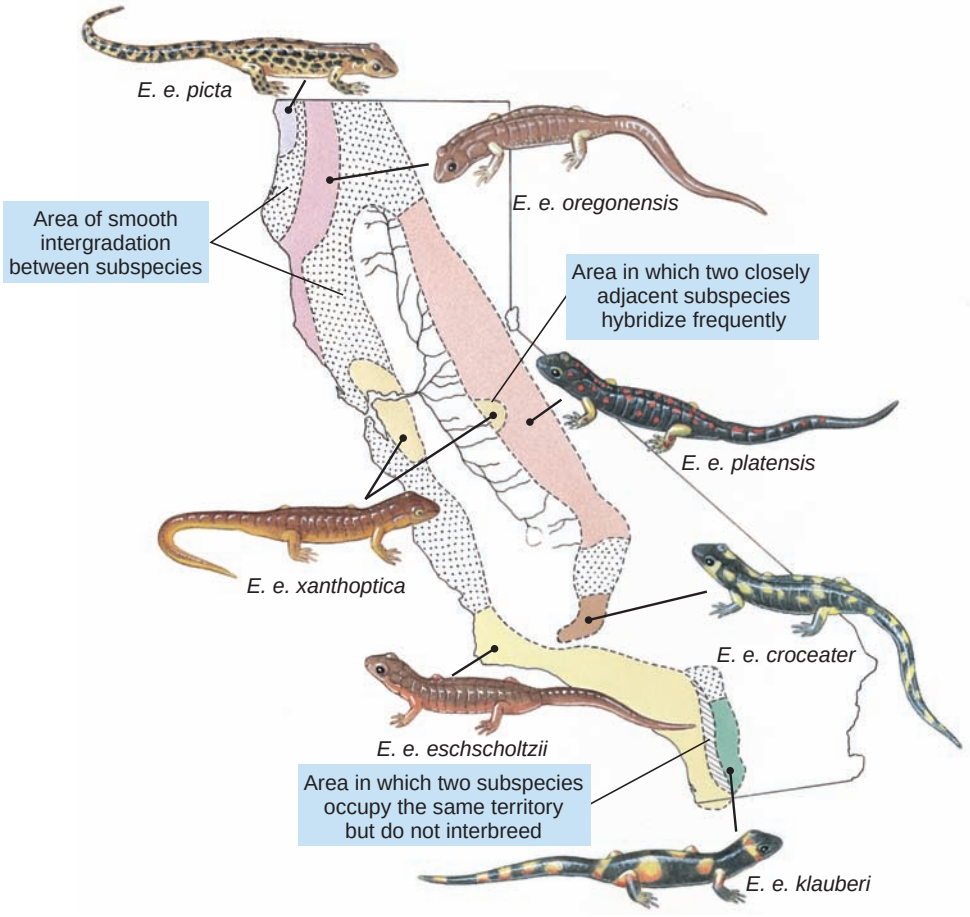


Figure 10.2

Geographic variation of color patterns in the salamander genus *Ensatina.* The species status of these populations has puzzled taxonomists for generations and continues to do so. Current taxonomy recognizes only a single species (*Ensatina eschscholtzii*) divided into subspecies as shown. Hybridization is evident between most adjacent populations, but studies of variation in proteins and DNA show large amounts of genetic divergence among populations. Furthermore, populations of the subspecies *E. e. eschscholtzii* and *E. e. klauberi* can overlap geographically without interbreeding.

deposited in a museum to represent the ideal form or morphology for the species (Figure 10.3). When scientists obtained additional specimens and wanted to assign them to a species, the type specimens of described species were consulted. The new specimens were assigned to a previously described species if they possessed the essential features of its type specimen. Small differences from the type specimen were considered accidental imperfections. Large differences from existing type specimens would lead a scientist to describe a new species with its own type specimen. In this manner, the living world was categorized into species.

Evolutionists discarded the typological species concept, but some of its traditions remain. Scientists still name species by describing type specimens deposited in museums, and the type specimen formally bears the name of the species. Organismal morphology is likewise still important in recognizing species; however, species are no longer viewed as classes of organisms defined by possession of certain morphological features. The basis of the evolutionary world view is that species are historical entities whose properties are subject always to change. Variation that we observe among organisms within a species is not an imperfect manifestation of an eternal "type"; the type itself is only an abstraction taken from the very real and important variation present within the species. A type is at best an average form that changes as organismal variation is sorted through time by natural selection. A type specimen serves only as a guide to the general morphological features that one may expect to find in a particular species as we observe it today.

The person who first describes a type specimen and publishes the name of a species is called the authority. This person's name and date of publication are often written after the species name. Thus, *Didelphis marsupialis* Linnaeus, 1758, tells us that Linnaeus was the first person to publish the species name of the opossum. Sometimes, the generic status of a species is revised following its initial description. In this case, the name of the authority is presented in parentheses. The Nile monitor lizard is denoted *Varanus niloticus* (Linnaeus, 1766) because the species originally was named by Linnaeus as *Lacerta nilotica,* and subsequently placed into a different genus.

Biological Species Concept

The most influential concept of species inspired by Darwinian evolutionary theory is the **biological species concept** formulated by Theodosius Dobzhansky and Ernst Mayr. This concept emerged during the evolutionary synthesis of the 1930s and 1940s from earlier ideas, and it has been refined and reworded several times since then. In 1982, Mayr stated the biological species concept as follows: *"A species is a reproductive community of populations (reproductively isolated from others) that occupies a specific niche in nature."* Note that a species is identified here according to reproductive properties of populations, not according to possession of any specific organismal characteristics.

Figure 10.3
Specimens of birds from the Smithsonian Institution (Washington D.C.), including birds originally collected by John J. Audubon, Theodore Roosevelt, John Gould, and Charles Darwin.

A species is an **interbreeding population** of individuals having common descent and sharing intergrading characteristics. Studies of populational variation in organismal morphology, chromosomal structure, and molecular genetic features are very useful for evaluating the geographical boundaries of interbreeding populations in nature. The criterion of the "niche" (see Chapter 38) recognizes that members of a reproductive community are expected also to have common ecological properties.

Because a reproductive community should maintain genetic cohesiveness, we expect organismal variation to be relatively smooth and continuous within species and discontinuous between them. Although the biological species is based on reproductive properties of populations rather than organismal morphology, morphology nonetheless can help us to diagnose biological species. Sometimes species status can be evaluated directly by conducting breeding experiments. Controlled breeding is practical only in a minority of cases, however, and our decisions regarding species membership usually are made by studying character variation. Variation in molecular characters is very useful for identifying geographical boundaries of reproductive communities. Molecular studies have revealed the occurrence of cryptic or **sibling species** (p. 119), which are too similar in morphology to be diagnosed as separate species by morphological characters alone.

The biological species concept has received strong criticism because of several perceived problems. First, the concept lacks an explicit temporal dimension. It provides a means for diagnosing species status of contemporary populations but gives little guidance regarding the species status of ancestral populations relative to their evolutionary descendants. Proponents of the biological species concept often disagree on the degree of reproductive isolation necessary for considering two populations separate species, thereby revealing some ambiguity in the concept. For example, should occurrence of limited hybridization between populations in a small geographic area cause them to be considered a single species despite evolutionary differences

between them? Another problem is that because the biological species concept emphasizes interbreeding as the criterion of reproductive community, it denies the existence of species in groups of organisms that reproduce only asexually. It is common systematic practice, however, to describe species in all groups of organisms, regardless of whether reproduction is sexual or asexual.

Evolutionary Species Concept

The time dimension creates obvious problems for the biological species concept. How do we assign fossil specimens to biological species that are recognized today? If we trace a lineage backward through time, how far must we go before we have crossed a species boundary? If we could follow the unbroken genealogical chain of populations backward through time to the point where two sister species converge on their common ancestor, we would need to cross at least one species boundary somewhere. It would be very hard to decide, however, where to draw a sharp line between the two species.

To address this problem, the **evolutionary species concept** was proposed by Simpson in the 1940s to add an evolutionary time dimension to the biological species concept. This concept persists in a modified form today. A current definition of the evolutionary species is *a single lineage of ancestor-descendant populations that maintains its identity from other such lineages and that has its own evolutionary tendencies and historical fate.* Note that the criterion of common descent is retained here in the need for a lineage to have a distinct historical identity. Reproductive cohesion is the means by which a species maintains its identity from other such lineages and keeps its evolutionary fate separate from other species. The same kinds of diagnostic features discussed for the biological species concept are relevant for identifying evolutionary species, although in most cases only morphological features are available from fossils. Unlike the biological species concept, the evolutionary species concept applies both to sexually and asexually reproducing forms. As long as continuity of diagnostic features is maintained by the evolving lineage, it is recognized as a species. Abrupt changes in diagnostic features mark the boundaries of different species in evolutionary time.

Phylogenetic Species Concept

The last concept that we present is the **phylogenetic species concept.** The phylogenetic species concept is defined as an *irreducible (basal) grouping of organisms diagnosably distinct from other such groupings and within which there is a parental pattern of ancestry and descent.* This concept emphasizes most strongly the criterion of common descent. Both asexual and sexual groups are covered.

A phylogenetic species is a single population lineage with no detectable branching. The main difference in practice between the evolutionary and phylogenetic species concepts is that the latter emphasizes recognizing as separate species the smallest groupings of organisms that have undergone independent evolutionary change. The evolutionary species concept would group into a single species geographically disjunct populations that demonstrate some phylogenetic divergence but are judged similar in their "evolutionary tendencies," whereas the phylogenetic species concept would treat them as separate species. In general, a greater number of species would be described using the phylogenetic species concept than any other species concept, and many taxonomists consider it impractical for this reason. For strict adherence to cladistic systematics (p. 209), the phylogenetic species concept is ideal because only this concept guarantees strictly monophyletic units at the species level.

The phylogenetic species concept intentionally disregards details of evolutionary process and gives us a criterion that allows us to describe species without first needing to conduct detailed studies on evolutionary processes. Advocates of the phylogenetic species concept do not necessarily disregard the importance of studying evolutionary process. They argue, however, that the first step in studying evolutionary process is to have a clear picture of life's history. To accomplish this task, the pattern of common descent must be reconstructed in the greatest detail possible by starting with the smallest taxonomic units that have a history of common descent distinct from other such units.

Dynamism of Species Concepts

Current disagreements concerning concepts of species should not be considered discouraging. Whenever a field of scientific investigation enters a phase of dynamic growth, old concepts are reevaluated and either refined or replaced with newer, more progressive ones. The active debate occurring within systematics shows that this field has acquired unprecedented activity and importance in biology. Just as Thomas Henry Huxley's time was one of enormous advances in biology, so is the present time. Both times are marked by fundamental reconsiderations of the meaning of species. We cannot predict which concepts of species will remain useful 10 years from now. Researchers whose main interests are branching of evolutionary lineages, evolution of reproductive barriers among populations (p. 118), or ecological properties of species may favor different species concepts. The conflicts among the current concepts will lead us into the future. In many cases, different concepts agree on the locations of species boundaries, and disagreements identify particularly interesting cases of evolution in action. Understanding the conflicting perspectives, rather than learning a single species concept, is therefore of greatest importance for people now entering the study of zoology.

DNA Barcoding of Species

DNA barcoding is a technique for identifying organisms to species using sequence information from a standard gene present in all animals. The mitochondrial gene encoding cytochrome *c* oxidase subunit 1 (*COI*), which contains about 650 nucleotide base pairs, is a standard "barcode" region for animals. DNA sequences of *COI* usually vary among individuals of the same

species but not extensively, so that variation within a species is much smaller than differences among species. DNA barcoding is applied to specimens in nature by taking a small DNA sample from blood or another expendable tissue. The method is useful also for specimens in natural-history museums, zoos, aquaria, and frozen-tissue collections. DNA sequences from such sources are checked against a public reference library of species identifiers to assign unknown specimens to known species. DNA barcoding does not solve the controversies regarding use of different species concepts, but it often permits the origin of a specimen to be identified to a particular local population, which is valuable information regardless of the species status that a taxonomist assigns to that population.

TAXONOMIC CHARACTERS AND PHYLOGENETIC RECONSTRUCTION

A major goal of systematics is to infer the evolutionary tree or **phylogeny** that relates all extant and extinct species. This task is accomplished by identifying organismal features, formally called **characters,** that vary among species. A character is any feature that the taxonomist uses to study variation within and among species. Taxonomists find characters by observing patterns of similarity among organisms in morphological, chromosomal, and molecular features (see p. 206), and less frequently in behavioral and ecological ones. Phylogenetic analysis depends upon finding among organisms shared features that are inherited from a common ancestor. Character similarity that results from common ancestry is called **homology** (see Chapter 6). Similarity does not always reflect common ancestry, however. Independent evolutionary origin of similar features on different lineages produces patterns of similarity among organisms that do not reflect common descent; this occurrence complicates the work of taxonomists. Character similarity that misrepresents common descent is called nonhomologous similarity or **homoplasy.** Endothermy of birds and mammals is an example of homoplasy; this condition arose separately in ancestral lineages of birds and mammals. Variation in other characters shows that birds and mammals are not each other's closest relatives (p. 499). For an example of molecular homoplasy, see the interpretation of character 41 (p. 211) in the boxed essay, Phylogenies from DNA Sequences.

Using Character Variation to Reconstruct Phylogeny

To infer the phylogeny of a group using characters that vary among its members, the first step is to determine which variant form of each character was present in the common ancestor of the entire group. This character state is called **ancestral** for the group as a whole. We presume that all other variant forms of the character arose later within the group, and these are called evolutionarily **derived character states.** Determining the **polarity** of a character refers to identifying which one of its contrasting states is ancestral and which one(s) derived. For example, if we consider as a character the dentition of amniotic vertebrates (reptiles, birds, and mammals), presence versus absence of teeth in the jaws constitute alternative character states. Teeth are absent from modern birds but present in the other amniotes. To evaluate the polarity of this character, we must determine which character state, presence or absence of teeth, characterized the most recent common ancestor of amniotes and which state was derived subsequently within amniotes.

The method used to examine the polarity of a variable character is called **outgroup comparison.** We consult an additional group of organisms, called an **outgroup,** that is phylogenetically close but not within the group being studied. We infer that any character state found both within the group being studied and in the outgroup is ancestral for the study group. Amphibians and different groups of bony fishes constitute appropriate outgroups to the amniotes for polarizing variation in dentition of amniotes. Teeth are usually present in amphibians and bony fishes; therefore, we infer that presence of teeth is ancestral for amniotes and absence of teeth is derived. The polarity of this character indicates that teeth were lost in the ancestral lineage of all modern birds. Polarity of characters is evaluated most effectively when several different outgroups are used. All character states found in the study group that are absent from appropriate outgroups are considered derived.

Organisms or species that share derived character states form subsets within the study group called **clades** (Gr. *klados,* branch). A derived character shared by the members of a clade is formally called a **synapomorphy** (Gr. *synapsis,* joining together, + *morphē,* form) of that clade. Taxonomists use synapomorphies as evidence of homology to infer that a particular group of organisms forms a clade. Among extant amniotes, absence of teeth and presence of feathers are synapomorphies that identify the birds as a clade. A clade corresponds to a unit of evolutionary common descent; it includes all descendants of a particular ancestral lineage. The pattern formed by the derived states of all characters within our study group takes the form of a **nested hierarchy** of clades within clades. The goal is to identify all of the different clades nested within the study group, which would give a complete account of the patterns of common descent among species in the group.

Character states ancestral for a taxon are often called **plesiomorphic** for that taxon, and the sharing of ancestral states among organisms is termed **symplesiomorphy.** Unlike synapomorphies, however, symplesiomorphies do not provide useful information on nesting of clades within clades. In the example just given, we found that presence of teeth in jaws was plesiomorphic for amniotes. If we grouped together mammalian and reptilian groups, which possess teeth, to the exclusion of modern birds, we would not obtain a valid clade. Birds also descend from all common ancestors of reptiles and mammals and must be included within any clade that includes all reptiles and mammals. Errors in determining polarity of characters therefore clearly can produce errors in inference of phylogeny. It is important to note, however, that character states that are plesiomorphic at one taxonomic level can be synapomorphies at a

more inclusive level. For example, the presence of jaws bearing teeth is a synapomorphy of gnathostome vertebrates (p. 511), a group that includes amniotes plus amphibians, bony fishes, and cartilaginous fishes, although teeth have been lost in birds and some other gnathostomes. The goal of phylogenetic analysis therefore can be restated as one of finding the appropriate taxonomic level at which any given character state is a synapomorphy. The character state is then used at that level to identify a clade.

The nested hierarchy of clades is presented as a branching diagram called a **cladogram** (Figure 10.4; see also Figure 6.16, and try to reconstruct this cladogram using only the sharing of numbered synapomorphies among the bird species). Taxonomists often make a technical distinction between a cladogram and a **phylogenetic tree.** The branches of a cladogram are only a formal device for indicating the nested hierarchy of clades within clades. The cladogram is not strictly equivalent to a phylogenetic tree, whose branches represent real lineages that occurred in the evolutionary past. To obtain a phylogenetic tree, we must add to the cladogram important additional interpretations concerning ancestors, the durations of evolutionary lineages, or the amounts of evolutionary change that occurred on the lineages. A cladogram is often used, however, as a first approximation of the branching structure of the corresponding phylogenetic tree.

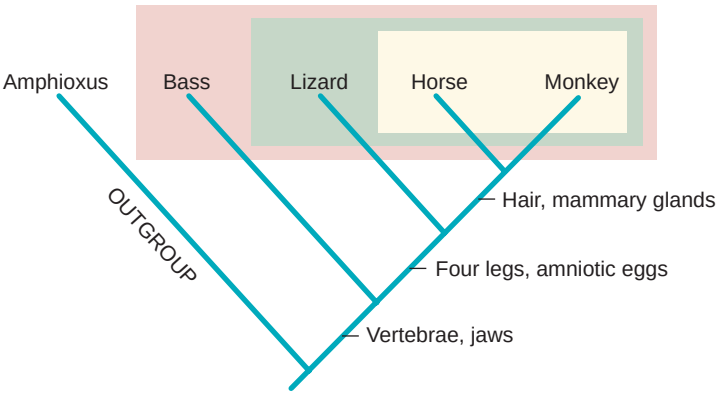


Figure 10.4

A cladogram as a nested hierarchy of taxa among five sampled chordate groups (Amphioxus, bass, lizard, horse, monkey). Amphioxus is the outgroup, and the study group comprises the four vertebrates. Four characters that vary among vertebrates are used to generate a simple cladogram: presence versus absence of four legs, amniotic eggs, hair, and mammary glands. For all four characters, absence is the ancestral state in vertebrates because this is the condition found in the outgroup, Amphioxus; for each character, presence is the derived state in vertebrates. Because they share presence of four legs and amniotic eggs as synapomorphies, the lizard, horse, and monkey form a clade relative to the bass. This clade is subdivided further by two synapomorphies (presence of hair and mammary glands) that unite the horse and monkey relative to the lizard. We know from comparisons involving even more distantly related animals that presence of vertebrae and jaws constitute synapomorphies of vertebrates and that Amphioxus, which lacks these features, falls outside the vertebrate clade.

Sources of Phylogenetic Information

We find characters used to construct cladograms in comparative morphology (including embryology), comparative cytology, and comparative biochemistry. **Comparative morphology** examines the varying shapes and sizes of organismal structures, including their developmental origins. Both macroscopic and microscopic characters are used, including details of cellular structure revealed by histology. As seen in Chapters 23 through 28, the variable structures of skull bones, limb bones, and integument (scales, hair, feathers) are particularly important for reconstructing the phylogeny of vertebrates. Comparative morphology uses specimens obtained from both living organisms and fossilized remains. **Comparative biochemistry** uses sequences of amino acids in proteins and the sequences of nucleotides in nucleic acids (see Chapter 5) to identify variable characters for constructing a cladogram (Figure 10.5). Direct sequencing of DNA is regularly

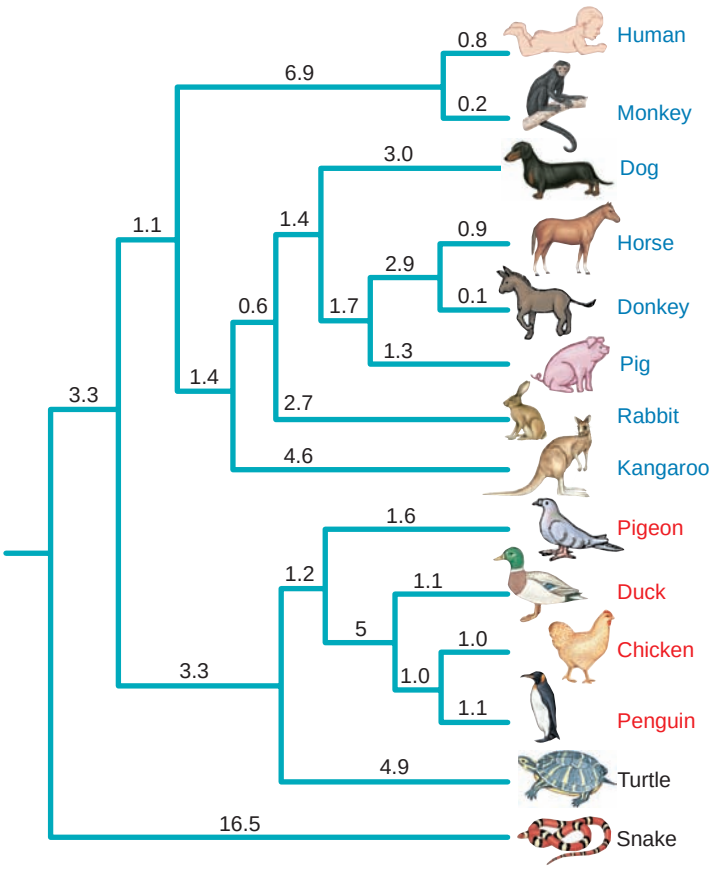


Figure 10.5

An early phylogenetic tree of representative amniotes based on inferred base substitutions in the gene that encodes the respiratory protein, cytochrome *c*. Numbers on the branches are the expected numbers of mutational changes that occurred in this gene along the different evolutionary lineages. Publication of this tree by Fitch and Margoliash in 1967 was influential in convincing systematists that molecular sequences contain phylogenetic information. Subsequent work confirms some hypotheses, including monophyly of mammals (blue) and birds (red) while rejecting others; kangaroo, for example, should be outside a branch containing all other mammals sampled.

applied to phylogenetic studies; however, comparisons of protein sequences are usually indirect, involving immunological or allozymic (see Figure 6.31) methods, or inferences from DNA sequences of protein-coding genes. Recent studies show that comparative biochemistry can be applied to some fossils in addition to living organisms. **Comparative cytology** uses variation in the numbers, shapes, and sizes of chromosomes and their parts (see Chapter 3 and p. 114) to obtain variable characters for constructing cladograms. Comparative cytology is used almost exclusively on living rather than fossilized organisms.

To add an evolutionary timescale necessary for producing a phylogenetic tree, we must consult the fossil record. We can look for the earliest appearance in fossils of derived morphological characters to estimate the ages of clades distinguished by those characters. The age of a fossil showing the derived characters of a particular clade is determined by radioactive dating (p. 109). An example of a phylogenetic tree constructed using these methods is Figure 25.3, page 547.

We can use comparative biochemical data to estimate the ages of different lineages on a phylogenetic tree. Some protein and DNA sequences undergo approximately linear rates of divergence through evolutionary time. The age of the most recent common ancestor of two species is therefore proportional to the differences measured between their proteins and DNA sequences. We calibrate evolution of proteins and DNA sequences by measuring their divergence between species whose most recent common ancestor has been dated using fossils. We then use the molecular evolutionary calibration to estimate ages of other branches on the phylogenetic tree.

THEORIES OF TAXONOMY

A theory of taxonomy establishes the principles that we use to recognize and to rank taxonomic groups. There are two currently popular theories of taxonomy: (1) traditional evolutionary taxonomy and (2) phylogenetic systematics (cladistics). Both are based on evolutionary principles. These two theories differ, however, on how evolutionary principles are used. These differences have important implications for how we use a taxonomy to study evolutionary processes.

The relationship between a taxonomic group and a phylogenetic tree or cladogram is important for both theories. This relationship can take one of three forms: **monophyly, paraphyly,** or **polyphyly** (Figure 10.6). A taxon is monophyletic if it includes the most recent common ancestor of the group and all descendants of that ancestor (Figure 10.6A). A taxon is paraphyletic if it includes the most recent common ancestor of all members of a group and some but not all descendants of that ancestor (Figure 10.6B). A taxon is polyphyletic if it does not include the most recent common ancestor of all members of a group; this condition requires that the group has had at least two separate evolutionary origins, usually requiring independent evolutionary acquisition of similar features (Figure 10.6C). Both evolutionary and cladistic taxonomy accept monophyletic groups and reject polyphyletic groups. They differ on acceptance of paraphyletic groups, however, and this difference has important evolutionary implications.

Traditional Evolutionary Taxonomy

Traditional **evolutionary taxonomy** incorporates two different evolutionary principles for recognizing and ranking higher taxa: (1) common descent and (2) amount of adaptive evolutionary change, as shown on a phylogenetic tree. Evolutionary taxa must have a single evolutionary origin, and must show unique adaptive features.

The mammalian paleontologist George Gaylord Simpson (Figure 10.7) and Ernst Mayr (see Figure 6.19) were highly influential in developing and formalizing the procedures of evolutionary taxonomy. According to Simpson and Mayr, a particular

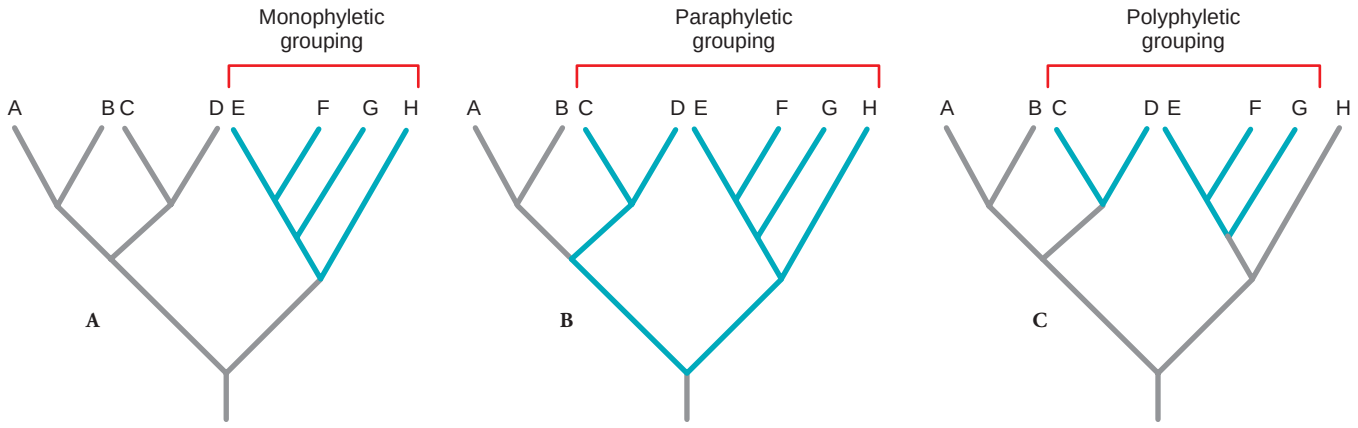


Figure 10.6

Relationships between phylogeny and taxonomic groups illustrated for a hypothetical phylogeny of eight species (A through H). **A,** *Monophyly*—a monophyletic group contains the most recent common ancestor of all members of the group and all of its descendants. **B,** *Paraphyly*—a paraphyletic group contains the most recent common ancestor of all members of the group and some but not all of its descendants. **C,** *Polyphyly*—a polyphyletic group does not contain the most recent common ancestor of all members of the group, thereby requiring that the group have at least two separate phylogenetic origins.

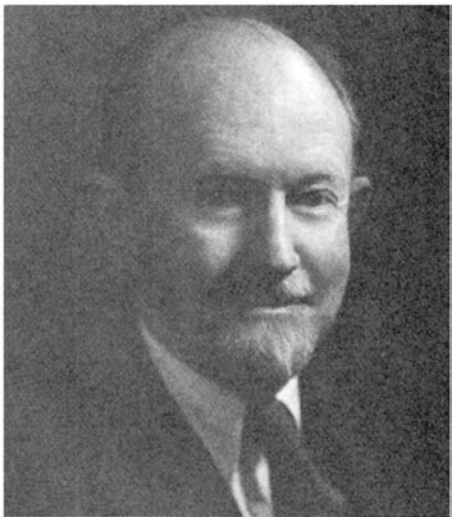


Figure 10.7

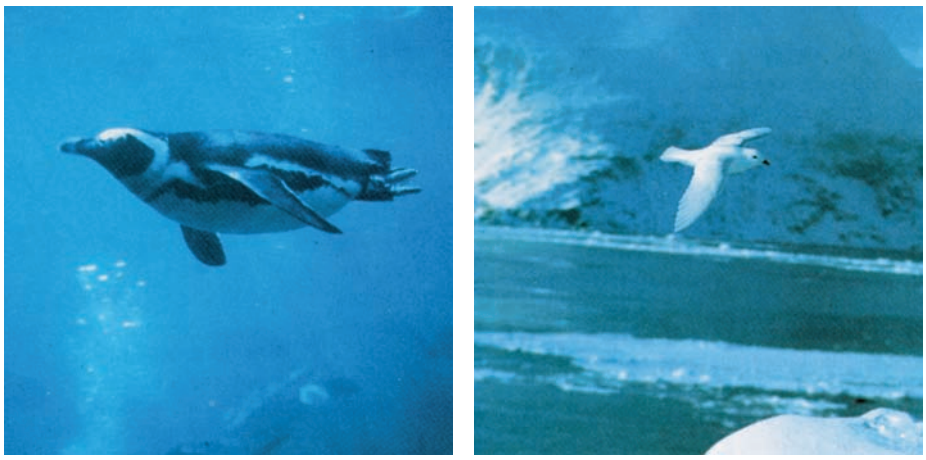
George Gaylord Simpson (1902 to 1984) formulated the principles of evolutionary taxonomy.

branch on an evolutionary tree is considered a higher taxon if it represents a distinct **adaptive zone.** Simpson describes an adaptive zone as "a characteristic reaction and mutual relationship between environment and organism, a way of life and not a place where life is led." By entering a new adaptive zone through a fundamental change in organismal structure and behavior, an evolving population can use environmental resources in a completely new way.

A taxon that constitutes a distinct adaptive zone is termed a **grade.** Simpson gives the example of penguins as a distinct adaptive zone within birds. The lineage immediately ancestral to all penguins underwent fundamental changes in the form of the body and wings to switch from aerial to aquatic locomotion (Figure 10.8). Aquatic birds that can fly both in air and underwater are somewhat intermediate in habitat, morphology, and behavior between aerial and aquatic adaptive zones. Nonetheless, the obvious modifications of the wings and body of penguins for swimming represent a new grade of organization. Penguins are therefore recognized as a distinct taxon within birds, the family Spheniscidae. The broader the adaptive zone when fully occupied by a group of organisms, the higher the rank given to the corresponding taxon.

Evolutionary taxa may be either monophyletic or paraphyletic. Recognition of paraphyletic taxa requires, however, that our taxonomies distort patterns of common descent. An evolutionary taxonomy of the anthropoid primates provides a good example (Figure 10.9). This taxonomy places humans (genus *Homo*) and their immediate fossil ancestors in the family Hominidae, and it places the chimpanzees (genus *Pan*), gorillas (genus *Gorilla*), and orangutans (genus *Pongo*) in the family Pongidae. However, the pongid genera *Pan* and *Gorilla* share more recent common ancestry with the Hominidae than they do with the remaining pongid genus, *Pongo*. This arrangement makes the family Pongidae paraphyletic because it does not include humans, who also descend from the most recent common ancestor of all pongids (Figure 10.9). Evolutionary taxonomists nonetheless recognize the pongid genera as a single, family-level grade of arboreal, herbivorous primates having limited mental capacity; in other words, they show the same family-level adaptive zone. Humans are terrestrial, omnivorous primates who have greatly expanded mental and cultural attributes, thereby forming a distinct adaptive zone at the taxonomic level of the family. Unfortunately, if we want our taxa to constitute adaptive zones, we compromise our ability to present common descent effectively.

Traditional evolutionary taxonomy has been challenged from two opposite directions. One challenge states that because phylogenetic trees can be very difficult to obtain, it is impractical to base our taxonomic system on common descent and adaptive evolution. We are told that our taxonomy should represent a more easily measured feature, the overall similarity of organisms evaluated without regard to phylogeny. This principle is called **phenetic taxonomy.** Phenetic taxonomy contributed some useful analytical methods but did not have a strong impact on animal taxonomy, and scientific interest in this approach has declined. Despite the difficulties of reconstructing phylogeny, zoologists still consider this endeavor a central goal of their systematic work, and they are unwilling to compromise this goal for methodological purposes.



A **B**

Figure 10.8

A, Penguin. **B,** Diving petrel. Penguins (avian family Spheniscidae) were recognized by George G. Simpson as a distinct adaptive zone within birds because of their adaptations for submarine flight. Simpson believed that the adaptive zone ancestral to penguins resembled that of diving petrels, which display adaptations for combined aerial and aquatic flight. Adaptive zones of penguins and diving petrels are distinct enough to be recognized taxonomically as different families within a common order (Ciconiiformes).

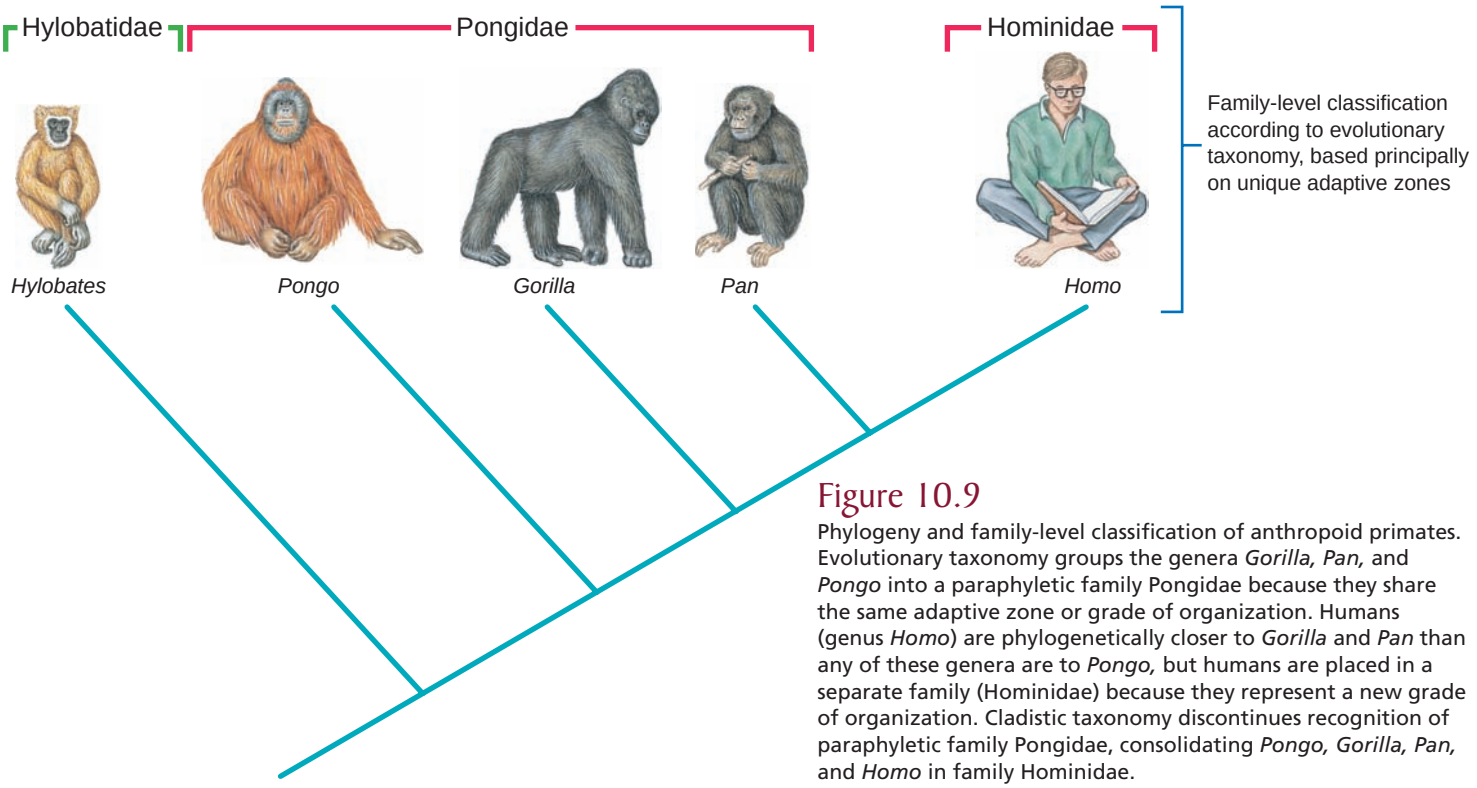


Figure 10.9

Phylogeny and family-level classification of anthropoid primates. Evolutionary taxonomy groups the genera *Gorilla, Pan,* and *Pongo* into a paraphyletic family Pongidae because they share the same adaptive zone or grade of organization. Humans (genus *Homo*) are phylogenetically closer to *Gorilla* and *Pan* than any of these genera are to *Pongo,* but humans are placed in a separate family (Hominidae) because they represent a new grade of organization. Cladistic taxonomy discontinues recognition of paraphyletic family Pongidae, consolidating *Pongo, Gorilla, Pan,* and *Homo* in family Hominidae.

Phylogenetic Systematics/Cladistics

A second and stronger challenge to evolutionary taxonomy is one known as **phylogenetic systematics** or **cladistics.** As the first name implies, this approach emphasizes the criterion of common descent and, as the second name implies, it is based on the cladogram of the group being classified. This approach to taxonomy was first proposed in 1950 by the German entomologist, Willi Hennig (Figure 10.10), and therefore is sometimes called "Hennigian systematics." All taxa recognized by Hennig's cladistic system must be monophyletic. We saw on Figure 10.9 how evolutionary taxonomists' recognition of the primate families Hominidae and Pongidae distorts genealogical relationships to emphasize adaptive uniqueness of the Hominidae. Because the most recent common ancestor of the paraphyletic family Pongidae is also an ancestor of the Hominidae, recognition of the Pongidae is incompatible with cladistic taxonomy. To avoid paraphyly, cladistic taxonomists have discontinued use of the traditional family Pongidae, placing chimpanzees, gorillas, and orangutans with humans in the family Hominidae. We adopt the cladistic classification in this book.

Disagreement on the validity of paraphyletic groups may seem trivial at first, but its important consequences become clear when we discuss evolution. For example, claims that amphibians evolved from bony fish, that birds evolved from reptiles, or that humans evolved from apes might be made by an evolutionary taxonomist but are meaningless to a cladist. We imply by these statements that a descendant group (amphibians, birds, or humans) evolved from part of an ancestral group (bony fish, reptiles, and apes, respectively) to which the descendant does not belong. This usage automatically makes the ancestral group paraphyletic, and indeed bony fish, reptiles, and apes as traditionally recognized are paraphyletic groups. How are such paraphyletic groups recognized? Do they share distinguishing features not shared by the descendant group?

Figure 10.10

Willi Hennig (1913 to 1976), German entomologist who formulated the principles of phylogenetic systematics/cladistics.

Phylogenies from DNA Sequences

A simple example illustrates cladistic analysis of DNA sequence data to examine phylogenetic relationships among species. The study group in this example contains three species of chameleons, two from the island of Madagascar (*Brookesia theili* and *B. brygooi*) and one from Equatorial Guinea (*Chamaeleo feae*). The outgroup is a lizard of genus *Uromastyx,* which is a distant relative of chameleons. Do the molecular data in this example confirm or reject the prior taxonomic hypothesis that the two Madagascan chameleons are more closely related to each other than either one is to the Equatorial Guinean species?

The molecular information in this example comes from a piece of the mitochondrial DNA sequence (57 bases) for each species. Each sequence encodes amino acids 221–239 of a protein called "NADH dehydrogenase subunit 2" in the species from which it was obtained. These DNA base sequences are aligned and numbered as:

```
                10        20        30        40        50
                |         |         |         |         |
Uromastyx       AAACCTTAAAAGACACCACAACCATATGAACAACAACACCAACAATCAGCACACTAC
B. theili       AAACACTACAAAATATAACAACTGCATGAACAACATCAACCACAGCAAACATTTTAC
B. brygooi      AAACACTACAAGACATAACAACAGCATGAACTACTTCAACAACAGCAAATATTACAC
C. feae         AAACCCTACGAGACGCAACAACAATATGATCCACTTCCCCCACAACAAACACAATTT
```

Each column in the aligned sequences constitutes a character that takes one of four states: A, C, G, or T (a fifth possible state, absence of a base, is not observed in this example). Only characters that vary among the three chameleon species potentially contain information on which pair of species is most closely related. Twenty-three of the 57 aligned bases show variation *among chameleons,* as shown here in bold letters:

```
                10        20        30        40        50
                |         |         |         |         |
Uromastyx       AAACCTTAAAAGACACCACAACCATATGAACAACAACACCAACAATCAGCACACTAC
B. theili       AAACACTACAAAATATAACAACTGCATGAACAACATCAACCACAGCAAACATTTTAC
B. brygooi      AAACACTACAAGACATAACAACAGCATGAACTACTTCAACAACAGCAAATATTACAC
C. feae         AAACCCTACGAGACGCAACAACAATATGATCCACTTCCCCCACAACAAACACAATTT
```

To be useful for constructing a cladogram, a character must demonstrate sharing of derived characters (=synapomorphy). Which of these 23 characters demonstrate synapomorphies for chameleons? For each of the 23 variable characters, we must ask whether one of the states observed in chameleons is shared with the outgroup, *Uromastyx.* If so, this state is judged ancestral for chameleons and the alternative state(s) derived. Derived characters are identified for 21 of the 23 characters just identified; derived states are shown in blue:

```
                10        20        30        40        50
                |         |         |         |         |
Uromastyx       AAACCTTAAAAGACACCACAACCATATGAACAACAACACCAACAATCAGCACACTAC
B. theili       AAACACTACAAATATAACAACTGCATGAACAACATCAACCACAGCAAACATTTTAC
B. brygooi      AAACACTACAAGACATAACAACAGCATGAACTACTTCAACAACAGCAAATATTACAC
C. feae         AAACCCTACGAGACGCAACAACAATATGATCCACTTCCCCCACAACAAACACAATTT
```

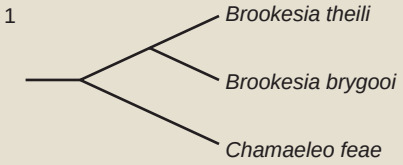
Note that polarity is ambiguous for two variable characters (at positions 23 and 54) whose alternative states in chameleons are not observed in the outgroup.

Of the characters showing derived states, 10 of them show synapomorphies among chameleons. These characters are marked here with numbers 1, 2, or 3 below the appropriate column.

```
                10        20        30        40        50
                |         |         |         |         |
Uromastyx       AAACCTTAAAAGACACCACAACCATATGAACAACAACACCAACAATCAGCACACTAC
B. theili       AAACACTACAAATATAACAACTGCATGAACAACATCAACCACAGCAAACATTTTAC
B. brygooi      AAACACTACAAGACATAACAACAGCATGAACTACTTCAACAACAGCAAATATTACAC
C. feae         AAACCCTACGAGACGCAACAACAATATGATCCACTTCCCCCACAACAAACACAATTT
                  1         1        11         2    1 3    1        11
```

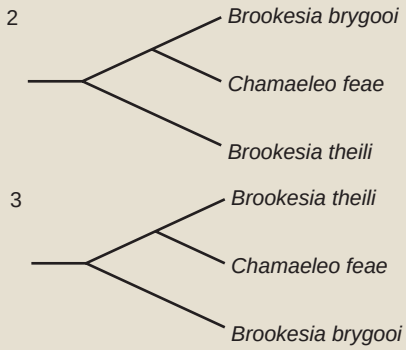
Paraphyletic groups are usually defined in a negative manner. They are distinguished only by lacking features found in a particular descendant group, because any traits that they share from their common ancestry are symplesiomorphies present also in the excluded descendants (unless secondarily lost). For example, apes are those "higher" primates that are not humans. Likewise, fish are those vertebrates that lack the distinguishing characteristics of tetrapods (amphibians and amniotes). What does it mean then to say that humans evolved from apes? To an evolutionary taxonomist, apes and humans are different adaptive zones or grades of organization; to say that humans evolved from apes states that bipedal, tailless organisms of large brain capacity evolved from arboreal, tailed organisms of smaller brain capacity. To a cladist, however, the statement

The eight characters marked 1 show synapomorphies grouping the two Madagascan species (*Brookesia theili* and *B. brygooi*) to the exclusion of the Equatorial Guinean species, *Chamaeleo feae*. We can represent these relationships as a cladogram:

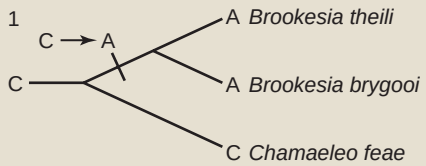


We can explain evolution of all characters favoring this cladogram by placing a single mutational change on the branch ancestral to the two *Brookesia* species. This is the simplest explanation for evolutionary change of these characters.

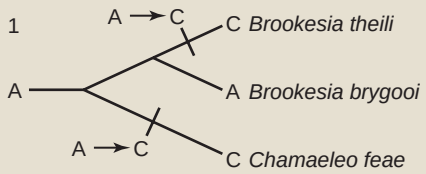
Characters marked 2 and 3 disagree with our cladogram and favor alternative relationships as shown here:



To explain evolutionary changes in characters favoring cladograms 2 or 3 using cladogram 1, we need at least two changes per character. Likewise, if we try to explain evolution of characters favoring cladogram 1 on cladograms 2 or 3, we need at least two changes for each of these characters. These two diagrams show the minimum numbers of changes required for character 5 (which favors cladogram 1) and character 41 (which favors cladogram 3) on cladogram 1; the ancestral state of each character is shown at the root of the tree and the states observed in each species at the tips of the branches:



character 5 (1 change)



character 41 (2 changes)

Systematists often use a principle called **parsimony** to resolve conflicts among taxonomic characters, as seen here. We choose as our best working hypothesis the cladogram that requires the smallest total amount of character change. In our example, cladogram 1 is favored by parsimony. For all 10 phylogenetically informative characters, cladogram 1 requires a total of 12 changes of character state (one for each of the 8 characters favoring it and two for each of the other 2 characters). Cladograms 2 and 3 each require at least 19 character-state changes, 7 steps longer than cladogram 1. By choosing cladogram 1, we claim that characters favoring cladograms 2 and 3 show homoplasy in their evolution.

The molecular sequences shown in this example therefore confirm predictions of the prior hypothesis, based on appearance and geography of these chameleons, that the *Brookesia* species shared a common ancestor with each other more recently than either one did with *Chamaeleo feae*.

As a further exercise, you should convince yourself that the 12 characters that vary among chameleons but which do not demonstrate unambiguous sharing of derived states are equally compatible with each of the three possible cladograms. For each character, find the minimum total number of changes that must occur to explain its evolution on each cladogram. You will see, if you do this exercise correctly, that the three cladograms do not differ in minimum numbers of changes required for each of these characters. For this reason, the characters are phylogenetically uninformative by the parsimony criterion.

Data from Townsend, T., and A. Larson. 2002. Molecular phylogenetics and mitochondrial genomic evolution in the Chamaeleonidae (Reptilia, Squamata). Molecular Phylogenetics and Evolution **23**:22–36.

that humans evolved from apes says essentially that humans evolved from an arbitrary grouping of species that lack the distinctive characteristics of humans, a trivial statement that conveys no useful information. To a cladist, any statement that a particular monophyletic group descends from a paraphyletic one is nothing more than a claim that the descendant group evolved from something that it is not. Extinct ancestral groups are always paraphyletic because they exclude a descendant that shares their most recent common ancestor. Although many such groups have been recognized by evolutionary taxonomists, none are recognized by cladists.

Zoologists often construct paraphyletic groups because they are interested in a terminal, monophyletic group (such as humans), and they want to ask questions about its ancestry.

It is often convenient to lump together organisms whose features are considered approximately equally distant from the group of interest and to ignore their own unique features. It is significant in this regard that humans have never been placed in a paraphyletic group, whereas most other organisms have been. Apes, reptiles, fishes, and invertebrates are all terms that traditionally designate paraphyletic groups formed by combining various "side branches" found when human ancestry is traced backward through the tree of life. Such a taxonomy can give the erroneous impression that all of evolution is a progressive march toward humanity or, within other groups, a progressive march toward whatever species humans designate most "advanced." Such thinking is a relic of pre-Darwinian views that there is a linear scale of nature having "primitive" creatures at the bottom and humans near the top just below angels. Darwin's theory of common descent states, however, that evolution is a branching process with no linear scale of increasing perfection along a single branch. Nearly every branch contains its own combination of ancestral and derived features. In cladistics, this perspective is emphasized by recognizing taxa only by their own unique properties and not grouping organisms only because they lack the unique properties found in related groups.

Fortunately, there is a convenient way to express the common descent of groups without constructing paraphyletic taxa. It is done by finding what is called the **sister group** of the taxon of interest to us. Two different monophyletic taxa are each other's sister group if they share common ancestry more recently than either one does with any other taxa. The sister group of humans appears to be chimpanzees, with gorillas forming the sister group to humans and chimpanzees combined. Orangutans are the sister group of a clade that includes humans, chimpanzees, and gorillas; gibbons form the sister group of the clade that includes orangutans, chimpanzees, gorillas, and humans (see Figure 10.9).

Current State of Animal Taxonomy

The formal taxonomy of animals that we use today was established using the principles of evolutionary systematics and has been revised recently in part using the principles of cladistics. Introduction of cladistic principles initially replaces paraphyletic groups with monophyletic subgroups while leaving the remaining taxonomy mostly unchanged. A thorough revision of taxonomy along cladistic principles, however, will require profound changes, one of which almost certainly will be abandonment of Linnaean ranks. A new taxonomic system called PhyloCode is being developed as an alternative to Linnaean taxonomy; this system replaces Linnaean ranks with codes that denote the nested hierarchy of monophyletic groups conveyed by a cladogram. In our coverage of animal taxonomy, we try to use taxa that are monophyletic and therefore consistent with criteria of both evolutionary and cladistic taxonomy. We continue, however, to use Linnaean ranks. For familiar taxa that are clearly paraphyletic grades, we note this fact and suggest alternative taxonomic schemes that contain only monophyletic taxa.

In discussing patterns of descent, we avoid statements such as "mammals evolved from reptiles" that imply paraphyly and instead specify appropriate sister-group relationships. We avoid referring to groups of organisms as being primitive, advanced, specialized, or generalized because all groups of animals contain combinations of primitive, advanced, specialized, and generalized features; these terms are best restricted to describing specific characteristics and not an entire group.

Revision of taxonomy according to cladistic principles can cause confusion. In addition to new taxonomic names, we see old ones used in unfamiliar ways. For example, cladistic use of "bony fishes" includes amphibians and amniotes (including reptilian groups, birds, and mammals) in addition to finned, aquatic animals that we normally term "fish." Cladistic use of "reptiles" includes birds in addition to snakes, lizards, turtles, and crocodilians; however, it excludes some fossil forms, such as synapsids, that were traditionally placed in Reptilia (see Chapters 26 through 28). Taxonomists must be very careful to specify when using these seemingly familiar terms whether the traditional evolutionary taxa or newer cladistic taxa are being referenced.

MAJOR DIVISIONS OF LIFE

From Aristotle's time to the late 1800s, every living organism was assigned to one of two kingdoms: plant or animal. However, the two-kingdom system had serious problems. Although it was easy to place rooted, photosynthetic organisms such as trees and herbs among the plants and to place food-ingesting, motile forms such as insects, fishes, and mammals among the animals, unicellular organisms presented difficulties (see Chapter 11). Some forms were claimed both for the plant kingdom by botanists and for the animal kingdom by zoologists. An example is *Euglena* (p. 225), which is motile, like animals, but has chlorophyll and photosynthesis, like plants. Other groups, such as bacteria, were assigned rather arbitrarily to the plant kingdom.

Several alternative systems have been proposed to solve the problem of classifying unicellular forms. In 1866 Haeckel proposed the new kingdom Protista to include all single-celled organisms. At first bacteria and cyanobacteria (blue-green algae), forms that lack nuclei bounded by a membrane, were included with nucleated unicellular organisms. Finally, important differences were recognized between the anucleate bacteria and cyanobacteria (prokaryotes) and all other organisms that have membrane-bound nuclei (eukaryotes). In 1969 R. H. Whittaker proposed a five-kingdom system that incorporated the basic prokaryote-eukaryote distinction. The kingdom Monera contained the prokaryotes. The kingdom Protista contained the unicellular eukaryotic organisms (protozoa and unicellular eukaryotic algae). Multicellular organisms were split into three kingdoms by mode of nutrition and other fundamental differences in organization. The kingdom Plantae included multicellular photosynthesizing organisms, higher plants, and multicellular algae. Kingdom Fungi contained molds, yeasts, and fungi that obtain their food by absorption. Invertebrates (except the

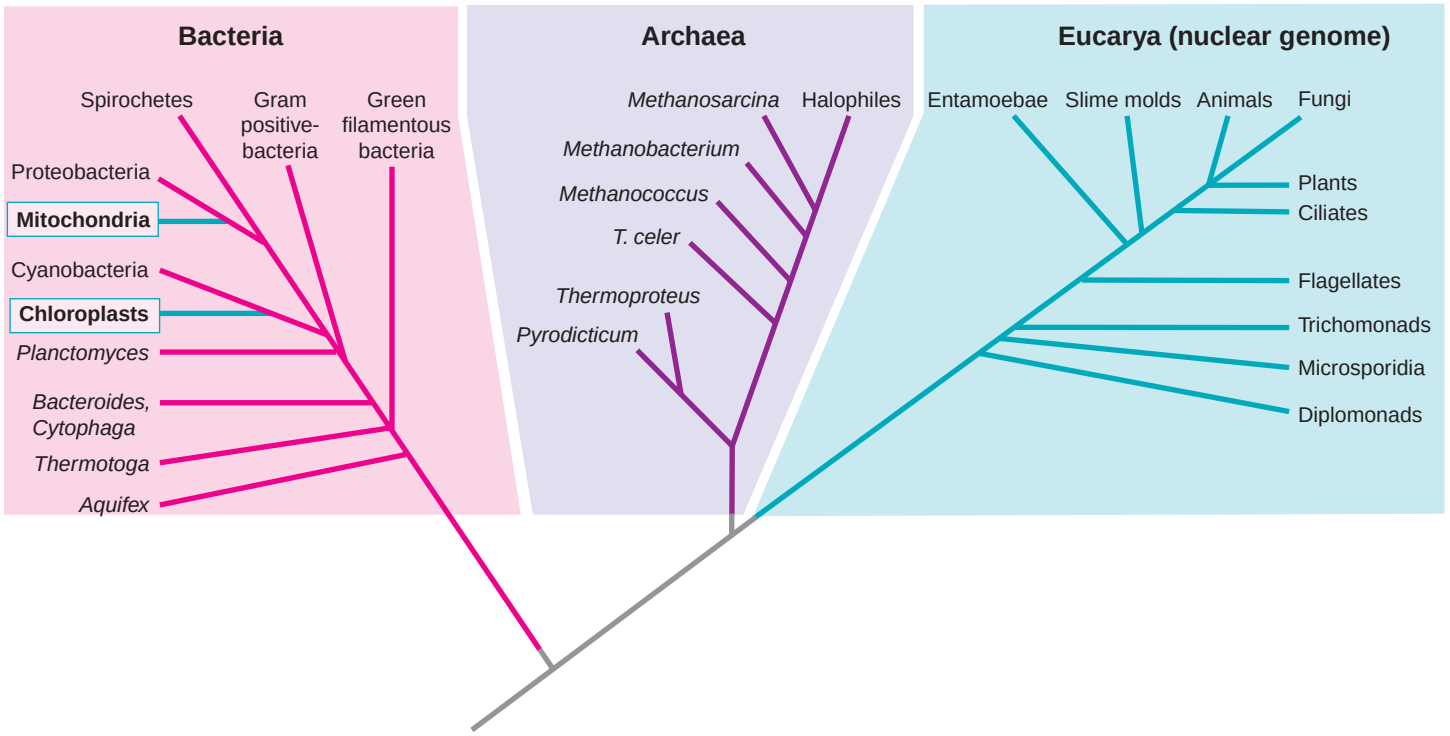


Figure 10.11

Phylogenetic overview of the three domains of life, Archaea, Bacteria and Eucarya, based on analysis of genes encoding ribosomal RNA. Because of their endosymbiotic origin (p. 34), organellar genomes of domain Eucarya (mitochondria, chloroplasts) are phylogenetically within the Bacteria rather than the clade that includes all eukaryotic nuclear genomes. Organisms of domain Eucarya therefore include cellular components of disparate evolutionary origins.

protozoa) and vertebrates compose the kingdom Animalia. Most of these forms ingest their food and digest it internally, although some parasitic forms are absorptive.

These different systems were proposed without regard to the phylogenetic relationships needed to construct evolutionary or cladistic taxonomies. The oldest phylogenetic events in the history of life have been obscure because the different forms of life share very few characters that can be compared among them to reconstruct phylogeny. Recently, however, a cladistic classification of all life-forms has been proposed based on phylogenetic information obtained from molecular data (the nucleotide base sequence of DNA encoding ribosomal RNA). According to this tree (Figure 10.11), Woese, Kandler, and Wheelis (1990) recognized three monophyletic **domains** above the kingdom level: Eucarya (all eukaryotes), Bacteria (the true bacteria), and Archaea (prokaryotes differing from bacteria in membrane structure and ribosomal RNA sequences). They did not divide Eucarya into kingdoms, although if we retain Whittaker's kingdoms Plantae, Animalia, and Fungi, Protista becomes a paraphyletic group (Figure 10.11). To maintain a cladistic classification, Protista must be discontinued by recognizing as separate kingdoms all of the labelled branches of Eucarya as shown in Figure 10.11.

Until a few years ago, animal-like protistans were traditionally studied in zoology courses as animal phylum Protozoa. Given current knowledge and the principles of phylogenetic systematics, this taxonomy commits two errors; "protozoa" are neither animals nor are they a valid monophyletic taxon at any level. Kingdom Protista is likewise invalid because it is not monophyletic. Animal-like protistans, now divided into seven or more phyla, are nonetheless of interest to students of zoology because they provide an important phylogenetic context for the study of animal diversity.

MAJOR SUBDIVISIONS OF THE ANIMAL KINGDOM

The phylum is the largest formal taxonomic category in the Linnaean classification of the animal kingdom. Metazoan phyla are often grouped together to produce additional, informal taxa intermediate between the phylum and the animal kingdom. Phylogenetic relationships among metazoan phyla have been particularly difficult to resolve both by morphological and molecular characters. Traditional groupings based on embryological and anatomical characters that may reveal phylogenetic affinities are:

Branch A (Mesozoa): phylum Mesozoa, the mesozoa
Branch B (Parazoa): phylum Porifera, the sponges, and phylum Placozoa
Branch C (Eumetazoa): all other phyla
  Grade I (Radiata): phyla Cnidaria, Ctenophora
  Grade II (Bilateria): all other phyla

Division A (Protostomia): characteristics in Figure 10.12
Acoelomates: phyla Platyhelminthes, Gnathostomulida, Nemertea
Pseudocoelomates: phyla Rotifera, Gastrotricha, Kinorhyncha, Nematoda, Nematomorpha, Acanthocephala, Entoprocta, Priapulida, Loricifera
Eucoelomates: phyla Mollusca, Annelida, Arthropoda, Echiurida, Sipunculida, Tardigrada, Onychophora
Division B (Deuterostomia): characteristics in Figure 10.12
phyla Phoronida, Ectoprocta, Chaetognatha, Brachiopoda, Echinodermata, Hemichordata, Chordata

Molecular phylogenetic results have challenged the placement of mesozoans (Branch A), suggesting that they might be derived from protostomia (Branch C, Grade II, Division A). As in the outline, bilateral animals are customarily divided into protostomes and deuterostomes by their embryological development (Figure 10.12). However, some of the phyla are difficult to place into one of these two categories because they possess some characteristics of each group (see Chapter 15).

Molecular phylogenetic studies have challenged traditional classification of the Bilateria, but results are not yet strong enough to present a precise hypothesis of phylogenetic relationships among metazoan phyla. Molecular phylogenetic results place four phyla classified above as deuterostomes (Brachiopoda, Chaetognatha, Ectoprocta, and Phoronida) in the Protostomia. This is the scheme shown in Figure 10.12. Furthermore, the traditional major groupings of protostome phyla (acoelomates, pseudocoelomates, and eucoelomates) appear not to be monophyletic. Instead, protostomes are divided into two major

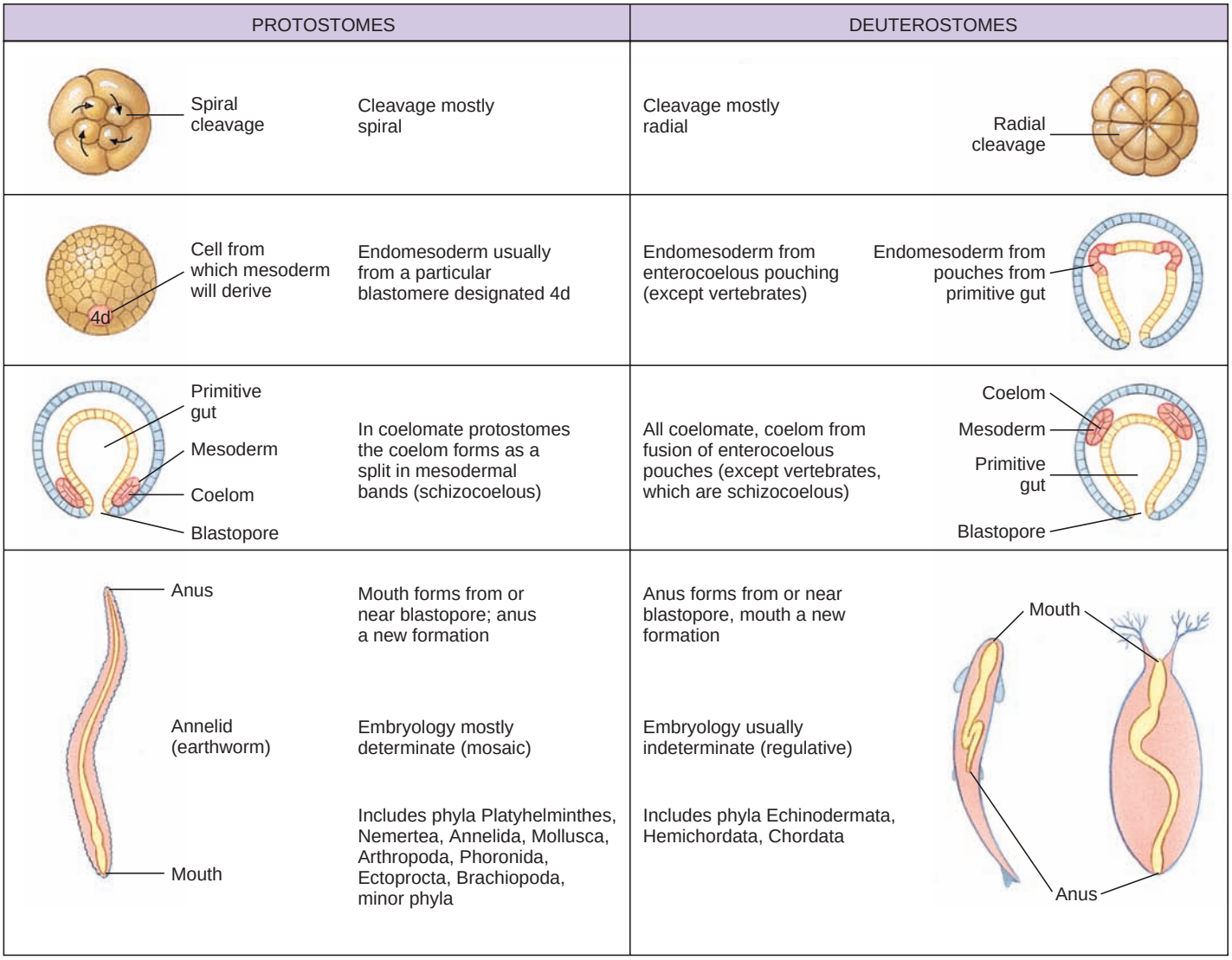


Figure 10.12

Basis for the distinction between divisions of bilateral animals. Traditional classifications often place phyla Brachiopoda, Ectoprocta, and Phoronida with deuterostomes, but recent molecular phylogenetic analyses place them with protostomes as shown here. Phylum Chaetognatha is of uncertain phylogenetic affinity and might lie outside these two groups.

monophyletic groups called the Lophotrochozoa and Ecdysozoa. Reclassification of the Bilateria is summarized:

Grade II: Bilateria
  Division A (Protostomia):
    Lophotrochozoa: phyla Platyhelminthes, Nemertea, Rotifera, Gastrotricha, Acanthocephala, Mollusca, Annelida, Echiurida, Sipunculida, Phoronida, Ectoprocta, Entoprocta, Gnathostomulida, Chaetognatha, Brachiopoda
    Ecdysozoa: phyla Kinorhyncha, Nematoda, Nematomorpha, Priapulida, Arthropoda, Tardigrada, Onychophora, Loricifera
  Division B (Deuterostomia): phyla Chordata, Hemichordata, Echinodermata

Although further study is needed to confirm these new groupings, we use them to organize our survey of animal diversity.

SUMMARY

Animal systematics has three major goals: (1) to identify all species of animals, (2) to evaluate evolutionary relationships among animal species, and (3) to group animal species in a hierarchy of taxonomic groups (taxa) that conveys evolutionary relationships. Taxa are ranked to denote increasing inclusiveness as follows: species, genus, family, order, "class," phylum, and kingdom. All of these ranks can be subdivided to signify taxa that are intermediate between them. Names of species are binomial, with the first name designating the genus to which the species belongs (capitalized) followed by a species epithet (lowercase), both written in italics. Taxa at all other ranks are given single capitalized but nonitalicized names.

The biological species concept has guided the recognition of most animal species. A biological species is defined as a reproductive community of populations (reproductively isolated from others) that occupies a specific niche in nature. It is not immutable through time but changes during the course of evolution. Because the biological species concept may be difficult to apply in spatial and temporal dimensions, and because it excludes asexually reproducing forms, alternative concepts have been proposed. These alternatives include the evolutionary species concept and the phylogenetic species concept. No single concept of species is universally accepted by all zoologists, but zoologists agree that a species should constitute a population lineage with a history of evolutionary descent separate from other such lineages. Because species lineages are expected to differ from each other in the DNA sequence of the rapidly evolving mitochondrial gene *COI,* this gene sequence is used as a diagnostic "barcode" to assign specimens to species.

Two major schools of taxonomy are currently active. Traditional evolutionary taxonomy groups species into higher taxa according to the joint criteria of common descent and adaptive evolution; such taxa have a single evolutionary origin and occupy a distinctive adaptive zone. A second approach, called phylogenetic systematics or cladistics, emphasizes common descent exclusively in grouping species into higher taxa. Only monophyletic taxa (those having a single evolutionary origin and containing all descendants of the group's most recent common ancestor) are used in cladistics. In addition to monophyletic taxa, evolutionary taxonomy recognizes some taxa that are paraphyletic (having a single evolutionary origin but excluding some descendants of the most recent common ancestor of the group). Both schools of taxonomy exclude polyphyletic taxa (those having more than one evolutionary origin).

Both evolutionary taxonomy and cladistics require that patterns of common descent among species be assessed before higher taxa are recognized. Comparative morphology (including development), cytology, and biochemistry are used to reconstruct nested hierarchical relationships among taxa that reflect the branching of evolutionary lineages through time. The fossil record provides estimates of the ages of evolutionary lineages. Comparative studies and the fossil record jointly permit us to reconstruct a phylogenetic tree representing the evolutionary history of the animal kingdom.

Traditionally, all living forms were placed into two kingdoms (animal and plant) but more recently, a five-kingdom system (animals, plants, fungi, protistans, and monerans) has been followed. Neither of these systems conforms to the principles of evolutionary or cladistic taxonomy because they place single-celled organisms into either paraphyletic or polyphyletic groups. Based on our current knowledge of the phylogenetic tree of life, "protozoa" do not form a monophyletic group and they do not belong within the animal kingdom.

Phylogenetic relationships among animal phyla have been clarified by molecular phylogenetic studies, although many of these higher-level groupings remain tentative. Particularly controversial is the grouping of bilaterally symmetrical animals into clades Deuterostomia, Protostomia, Ecdysozoa, and Lophotrochozoa.

REVIEW QUESTIONS

1. List in order, from most inclusive to least inclusive, the principal categories (taxa) in Linnaean classification as currently applied to animals.
2. Explain why the system for naming species that originated with Linnaeus is "binomial."
3. How does the biological species concept differ from earlier typological concepts of a species? Why do evolutionary biologists prefer it to typological species concepts?
4. What problems have been identified with the biological species concept? How do other species concepts attempt to overcome these problems?
5. How are taxonomic characters recognized? How are such characters used to construct a cladogram?
6. How do monophyletic, paraphyletic, and polyphyletic taxa differ? How do these differences affect the validity of such taxa for both evolutionary and cladistic taxonomies?

7. How many different clades of two or more species are possible for species A–H shown in Figure 10.6A?
8. What is the difference between a cladogram and a phylogenetic tree? Given a cladogram for a group of species, what additional information is needed to obtain a phylogenetic tree?
9. How would cladists and evolutionary taxonomists differ in their interpretations of the statement that humans evolved from apes, which evolved from monkeys?
10. What taxonomic practices based on the typological species concept are retained in systematics today? How has their interpretation changed?
11. What are the five kingdoms distinguished by Whittaker? How does their recognition conflict with the principles of cladistic taxonomy?

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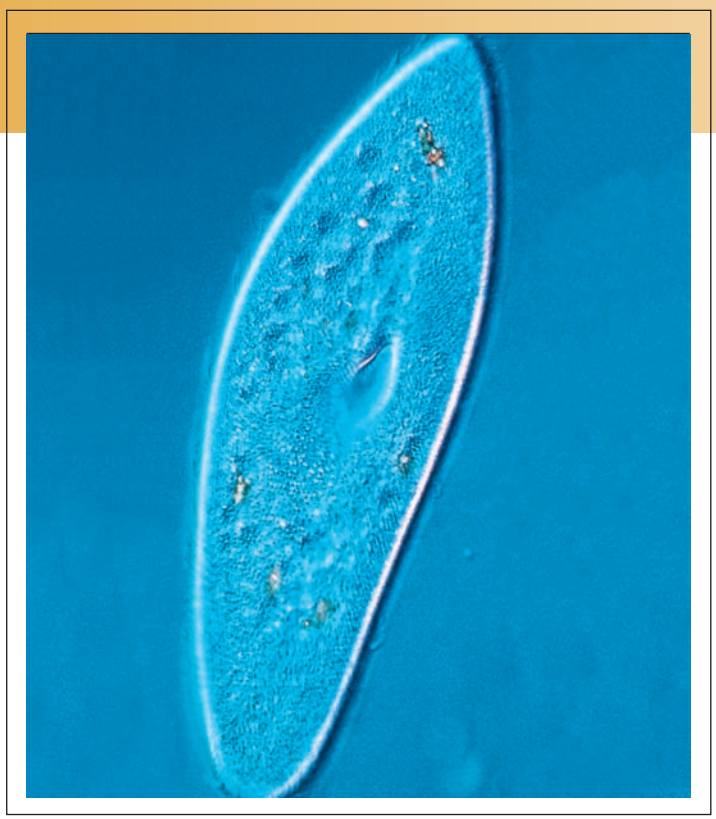
CHAPTER

11

Protozoan Groups

- UNICELLULAR EUKARYOTES



A paramecium.

Emergence of Eukaryotes and a New Life Pattern

The first reasonable evidence for life on earth dates from approximately 3.5 billion years ago. The first cells were prokaryotic bacteria-like organisms. The early prokaryotes diversified greatly over an enormous time span; their prokaryotic descendants now belong to two groups, the Eubacteria and the Archaea. One lineage within the ancient prokaryotes also gave rise to the first eukaryotic form. The key steps in the evolution of a eukaryotic cell from a prokaryotic ancestor involved symbiogenesis, a process whereby one prokaryote engulfed, but did not digest, another. The engulfed cell was eventually reduced to an organelle inside the host cell. The eukaryotic products of symbiogenesis include mitochondria and plastids.

A mitochondrion originated from an aerobic prokaryote capable of deriving energy in the presence of environmental oxygen. An anaerobic bacterium that engulfed such an aerobic form gained the capacity to grow in an oxygen-rich environment. The engulfed aerobic bacterium persisted inside the cell as a mitochondrion with its own genetic material. Over evolutionary time, most, but not all, genes from the mitochondrion came to reside in the host cell nucleus. Almost all present-day eukaryotes have mitochondria and are aerobic.

The eukaryotic plastid originated when a cell engulfed a photosynthetic bacterium. When a prokaryote is engulfed and modified to become a eukaryotic organelle, we say the organelle developed via primary endosymbiosis. The chloroplasts in red algae, and in green algae and multicellular plants, arose this way. However, in some cases, a eukaryotic cell may obtain plastids from another eukaryote. This is secondary endosymbiosis. Two similar cells may have formed very differently, so it is not easy to untangle the evolutionary relationships among the diverse array of unicellular forms we now see.

The assemblage of eukaryotic unicellular organisms is collectively called protozoa. The inclusion of "zoa" in the name refers to two animal-like features: the absence of a cell wall, and the presence of at least one motile stage in the life cycle. However, the plant-animal distinction is not easily made in unicellular forms because many motile unicells carry photosynthetic plastids. The myriad of ways to live as a unicellular organism is fascinating, beguiling, and a little bewildering.

A protozoan, or unicellular eukaryote, is a complete organism in which all life activities occur within the limits of a single plasma membrane. Unicellular eukaryotes are found wherever life exists. They are highly adaptable and easily distributed from place to place. They require moisture, whether they live in marine or freshwater habitats, soil, decaying organic matter, or plants and animals. They may be sessile or free swimming, and they form a large part of the floating plankton. The same species are often found widely separated in time as well as in space. Some species may have spanned geological eras exceeding 100 million years.

Despite their wide distribution, many protozoa can live successfully only within narrow environmental ranges. Species adaptations vary greatly, and successions of species frequently occur as environmental conditions change.

Protozoa play an enormous role in the economy of nature. Their fantastic numbers are attested by the gigantic ocean soil deposits formed over millions of years by their skeletons. About 10,000 species of unicellular eukaryotes are symbiotic in or on animals or plants, sometimes even other protozoa. The relationship may be **mutualistic** (both partners benefit), **commensalistic** (one partner benefits, no effect on the other), or **parasitic** (one partner benefits at the expense of the other), depending on the species involved. Parasitic forms cause some of the most important diseases of humans and domestic animals.

HOW DO WE DEFINE PROTOZOAN GROUPS?

For many years, all protozoans were placed within a single phylum comprised of eukaryotic unicells, but phylogenetic studies showed this group was not monophyletic. Evidence suggests that the origin of the first eukaryote was followed by great diversification, leading some biologists to predict that more than 60 monophyletic eukaryotic clades will eventually emerge. The well-supported clade Opisthokonta (Figure 11.1) includes unicellular choanoflagellates, multicellular animals (metazoans) and fungi, among others (see p. 228). Like the Opisthokonta, the clade Viridiplantae has both unicellular and multicellular members; this group contains green algae, the bryophytes, and the vascular plants. The remaining eukaryotic clades contain less well-known organisms, many of which were considered protozoans.

Protozoans and their relatives have been given several names. Protozoans are usually unicellular, so the name Protoctista was initiated to include unicellular and closely related multicellular organisms in one group. However, protoctistan is far less commonly used than the names protist and protozoan. Protist is a general term that does not distinguish between plantlike and animal-like unicells, whereas protozoan was intended for a subset of animal-like unicellular organisms.

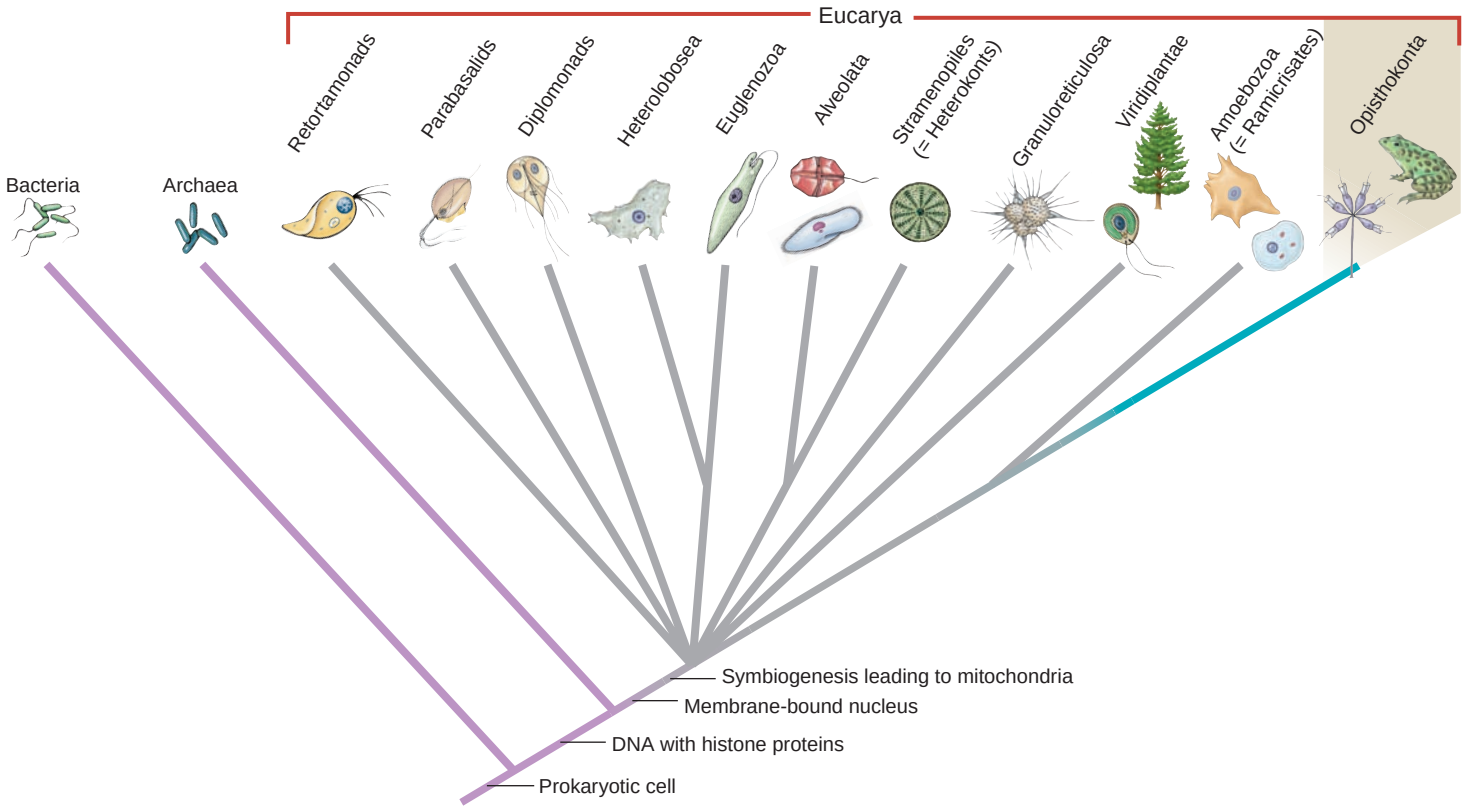


Figure 11.1

Cladogram showing two major prokaryotic branches and diversification of eukaryotes. Major eukaryotic clades containing protists are shown, but several clades of amebas and other forms are not shown. The order of branching remains to be determined for most clades. The very large opisthokont clade contains choanoflagellates, fungi, and all multicellular animals.

The two concepts, plantlike and animal-like, refer in part to the way that food is gathered. Plants are typically **autotrophic,** meaning that they synthesize their own organic constituents from inorganic substrates. Photosynthesis is one kind of autotrophy. Animals are typically **heterotrophic,** meaning that they obtain organic molecules synthesized by other organisms. Heterotrophic protozoa may ingest their food in a soluble form or in a particulate form. Particulate food is acquired by **phagocytosis** via an infolding or invagination of the cell membrane to surround a visible food particle (Figure 11.2). Heterotrophs that feed on visible particles are **phagotrophs** or **holozoic** feeders, whereas those that ingest soluble food are **osmotrophs** or **saprozoic** feeders.

A distinction between plants and animals on the basis of nutrition works well for multicellular forms, but the plant-animal distinction is not so clear among unicells. Autotrophic protozoa (phototrophs) use light energy to synthesize their organic molecules, but they often practice phagotrophy and osmotrophy as well. Even among heterotrophs, few are exclusively either phagotrophic or osmotrophic. A single class Euglenoidea (phylum Euglenozoa) contains some forms that are mainly phototrophs, some that are mainly osmotrophs, and some that are mainly phagotrophs. Species of *Euglena* show considerable variety in nutritional capability. Some species require certain preformed organic molecules, even though they are autotrophs, and some lose their chloroplasts if maintained in darkness, thus becoming permanent osmotrophs. The mode of nutrition employed by unicellular organisms is opportunistic and highly variable, even within a single species, so nutritional features have proved unreliable for defining protozoans, or protozoan subgroups.

Originally, the means of locomotion was used to distinguish three of the four classes in the traditional phylum Protozoa. Members of a parasitic class, once called Sporozoa, lack a distinct locomotory structure, but share an organelle capable of invading host cells. Members of the other three traditional protozoan classes differ in means of locomotion: flagellates (Figure 11.3) use **flagella,** ciliates (Figure 11.4) travel via a ciliated body surface, and amebas extend their **pseudopodia** (Figure 11.5) to move.

Typically, a flagellate has a few long flagella, and a ciliate has many short **cilia,** but no real morphological distinction exists between cilia and flagella. Some investigators have preferred to

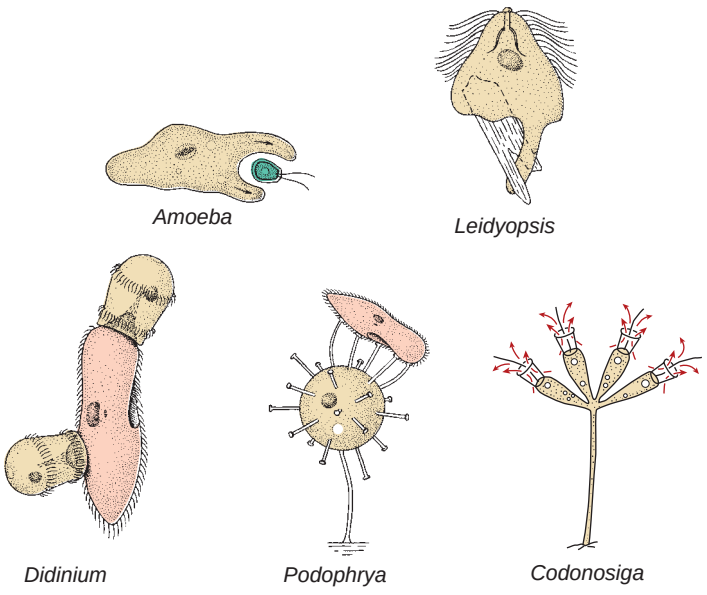


Figure 11.2

Some feeding methods among protozoa. *Amoeba* surrounds a small flagellate with pseudopodia. *Leidyopsis,* a flagellate living in the intestine of termites, forms pseudopodia and ingests wood chips. *Didinium,* a ciliate, feeds only on *Paramecium,* which it swallows through a temporary cytostome in its anterior end. Sometimes more than one *Didinium* feed on the same *Paramecium. Podophrya* is a suctorian ciliophoran. Its tentacles attach to its prey and suck prey cytoplasm into the body of the *Podophrya,* where it is pinched off to form food vacuoles. *Codonosiga,* a sessile flagellate with a collar of microvilli, feeds on particles suspended in the water drawn through its collar by the beat of its flagellum. Technically, all of these methods are types of phagocytosis.

Figure 11.3

One flagellum is clearly visible in the lower left of this photograph of *Euglena.*

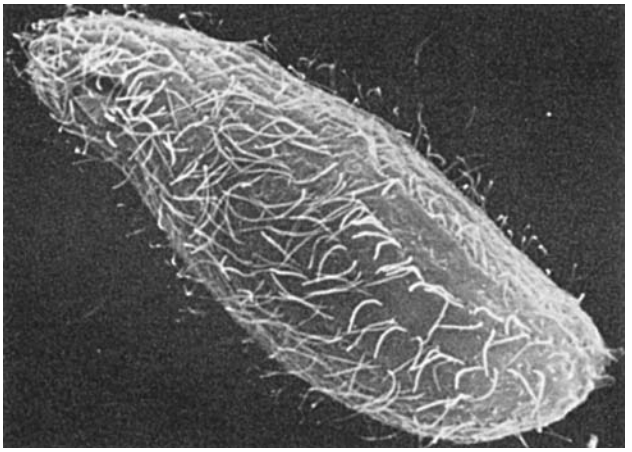


Figure 11.4

Scanning electron micrograph of a free-living ciliate *Tetrahymena thermophila* showing rows of cilia (×2000). Beating of flagella either pushes or pulls the organism through its medium, while cilia propel the organism by a "rowing" mechanism. Their structure is similar, whether viewed by scanning or transmission electron microscopy.