

Module 6: DNA viruses

Lecture 35: Small DNA viruses: parvo- and polyomaviruses

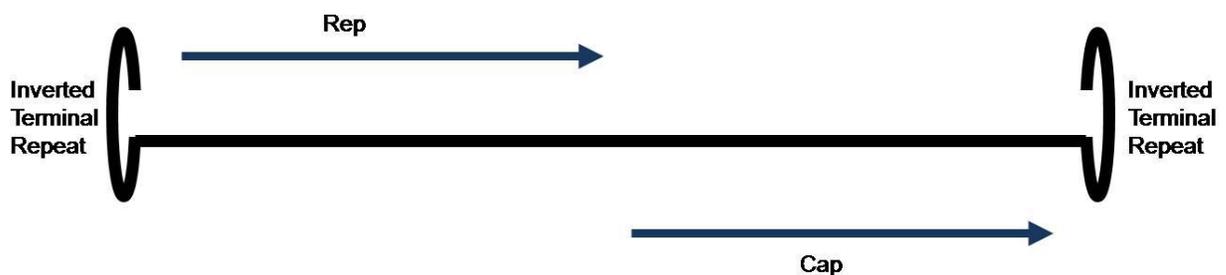
Cellular DNA synthesis occurs only during the S phase of the cell cycle, so the viruses which depend on host cell DNA polymerase must either wait for cells to enter S phase or express some protein early during infection to regulate the cell cycle (many small DNA viruses).

35.1 Parvoviruses

Parvo in latin stands for small. The virion is icosahedral, non-enveloped, and around 25 nm in diameter. These are the smallest of all animal viruses. They do not contain any viral or host enzyme and virion is made up of 80% protein and 20% DNA by weight.

The genome of parvovirus is linear, ssDNA which contains approximately 4500 to 5500 nucleotides. All parvoviruses contain terminal palindromic sequences at their 5' and 3' ends which allow the formation of Y or T shaped structures. In addition, they also contain inverted repeats at 3' and 5' ends which allow the circularization of the genome. **Rep** gene is required for replication of DNA while **cap** gene forms the capsid. The virion also contains 3 coat proteins namely, VP1, VP2, and VP3. VP3 is the most abundant among all three and it is made by the proteolytic cleavage of VP2. VP2 constitutes about 80% of capsid protein.

Figure 35.1 Genome map of parvoviruses:



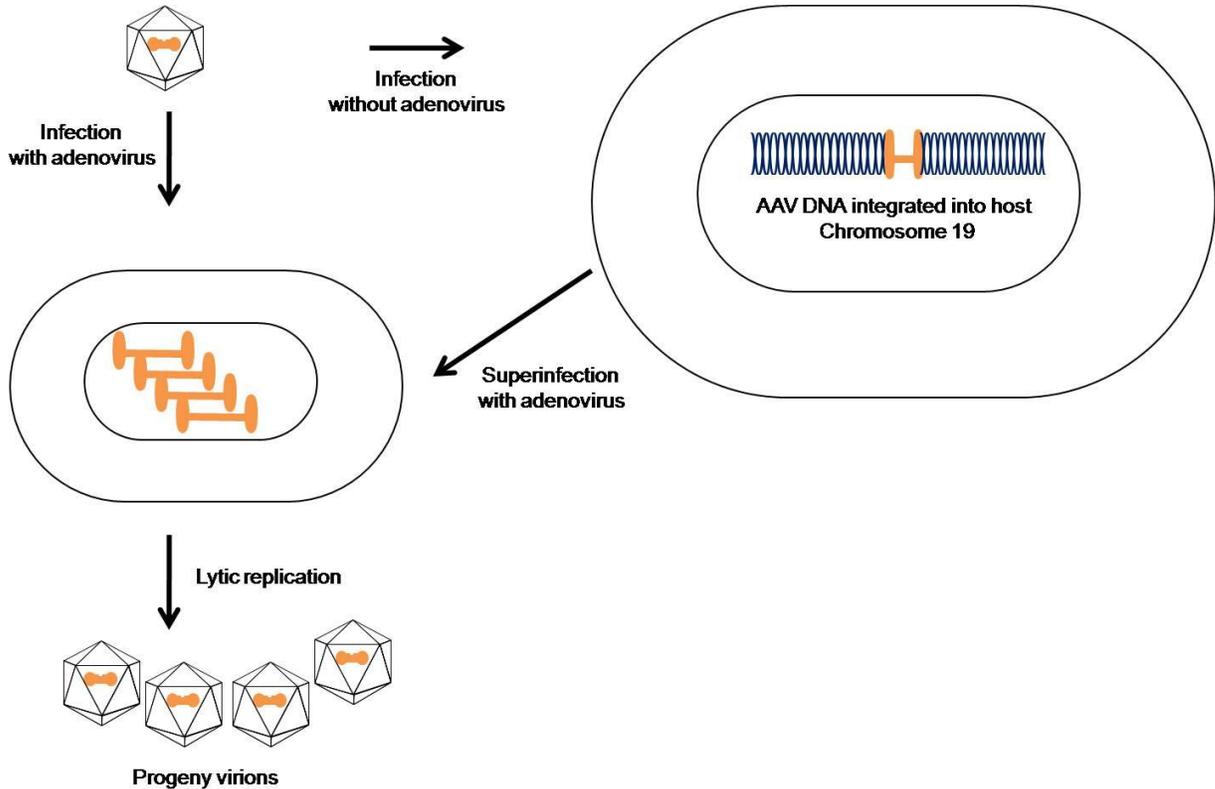
There are 3 genomes of Parvoviruses.-

- 1) Parvovirus
 - 2) Dependovirus
 - 3) Densovirus
- 1) **Parvovirus** – They only replicate in actively dividing cells. These viruses are highly resistant to heat, nucleases, detergents, proteases, and mild acid. They generally spread

through body secretions. The most common strain of parvovirus that infects humans is B19.

- 2) **Dependovirus** – They are also called as adeno associated virus (AAV). They require adenoviruses to replicate. Upon infection in absence of helper virus they can establish latent infection by integrating into the host genome. They are one of the very important tools for targeted gene therapy viral vector.

Figure 35.2 Helper dependent replication strategy of adeno-associated viruses:



- 3) **Densovirus** – They infect only invertebrates.

35.1.1 Replication

The receptor for parvovirus has not been identified yet. It replicates with the help of host DNA polymerase and its assembly occurs in nucleus. Palindromic sequences at the termini are the initiation sites for DNA replication. Following replication, transcription is carried out by host cell RNA polymerase II. The genome of parvovirus codes for two non- structural proteins NS1 and NS2 apart from 3 coat proteins VP1, VP2 and VP3. Translation of the mRNA occurs in the cytoplasm and protein enters into the nucleus

where assembly occurs. The virion exits the cell by lysis and the whole process is completed in 24hrs.

35.2 Papovaviruses

The name is derived from **papillomas** (warts), **polyomas** (multifocal tumors), and **vacuoles** in infected cells. These viruses contain dsDNA and are non- enveloped and spherical. They replicate and assemble in the nucleus of the infected cell and are released out following the lysis of the cell.

There are two genera

- 1) Papilloma
- 2) Polyoma

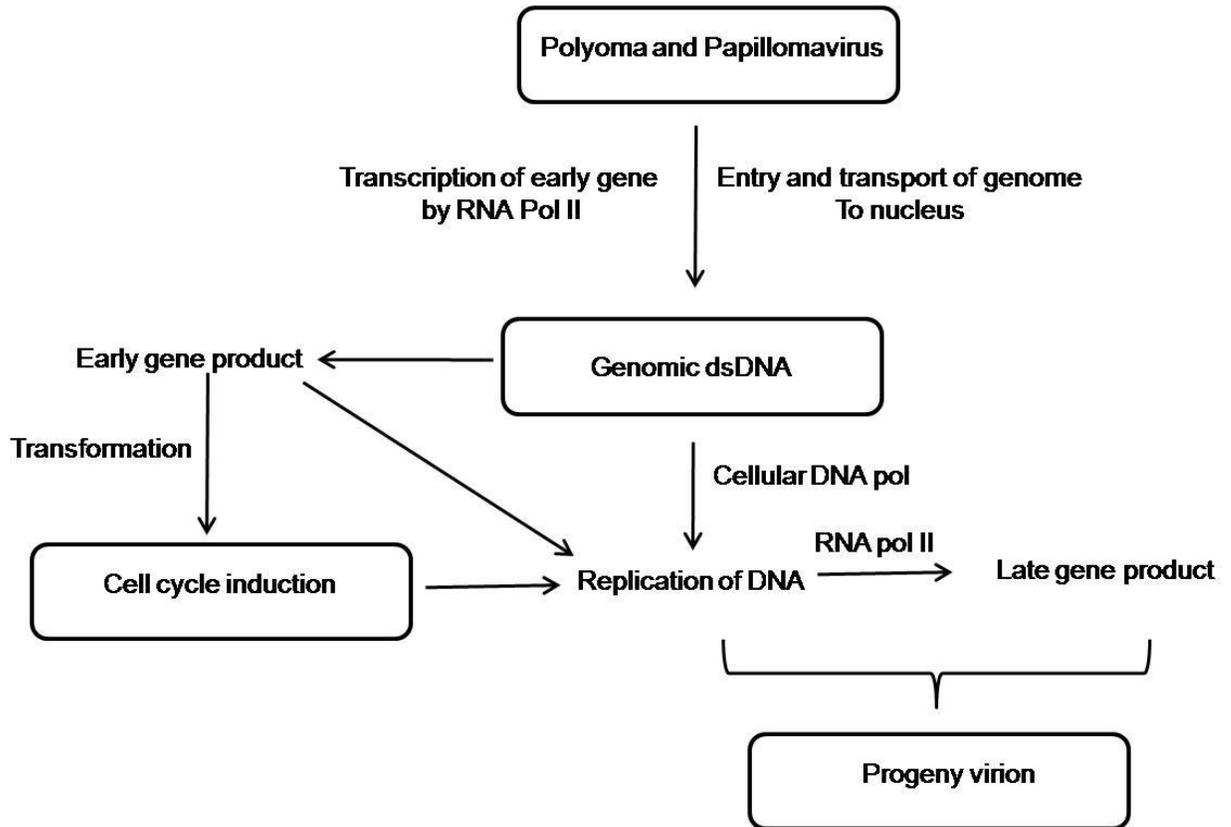
Papilloma - Genome is around 8000 bp long. They depend upon the replication machinery of the host cell. They often infect basal cell layers of the skin and hence are associated with warts. Papilloma viruses do not grow in tissue culture.

Polyoma - Genome is around 6000 bp long. They are associated with leukoencephalopathy and immunosuppression in humans. The most common virus SV40 that has been used to study mammalian replication belongs to this group. SV 40 makes large and small T-antigens which are required for viral DNA replication as it binds to origin of replication and is known to possess helicase and ATPase activity.

35.2.1 Replication

- I. Adsorption of virions to the cell surface and entry by endocytosis.
- II. Transport to the cell nucleus and uncoating
- III. Transcription to produce early gene mRNAs and translation to produce early proteins (T antigens).
- IV. Viral DNA replication and transcription of late gene mRNAs.
- V. Translation to produce late proteins (capsid proteins) and assembly of progeny virions in the nucleus.
- VI. Release of virions from the cell

Figure 35.3 General replication strategies of polyoma and papillomaviruses:



Lecture 36: Large DNA viruses

The viruses with large DNA have different strategies for their genome replication. The virus having intermediate size DNA (Adenoviruses) carry their own DNA replication machinery including DNA polymerase and other regulatory proteins. However, they still depend on the host cell RNA pol-II for the transcription of the viral RNA. The virus having large genome size like pox and herpesviruses have their own machinery to fulfill the requirement of RNA transcription and genomic DNA replication.

Virus type	Genome	Replication	Example
Adenovirus	dsDNA, 35 kb	Nucleus with DNA polymerase	Many serotypes infecting humans as well as animals
Herpesvirus	dsDNA, 120-230 kbp	Nucleus with DNA polymerase	HSV-1 and -2, Varicella/Zoster, HHV-5, 6, &7, Epstein-Barr
Poxvirus	dsDNA, 200 kbp	cytoplasm with DNA polymerase	Smallpox, Sheeppox, ORF, cowpox, vaccinia

Figure 36.1 General replication strategies of Adenoviruses:

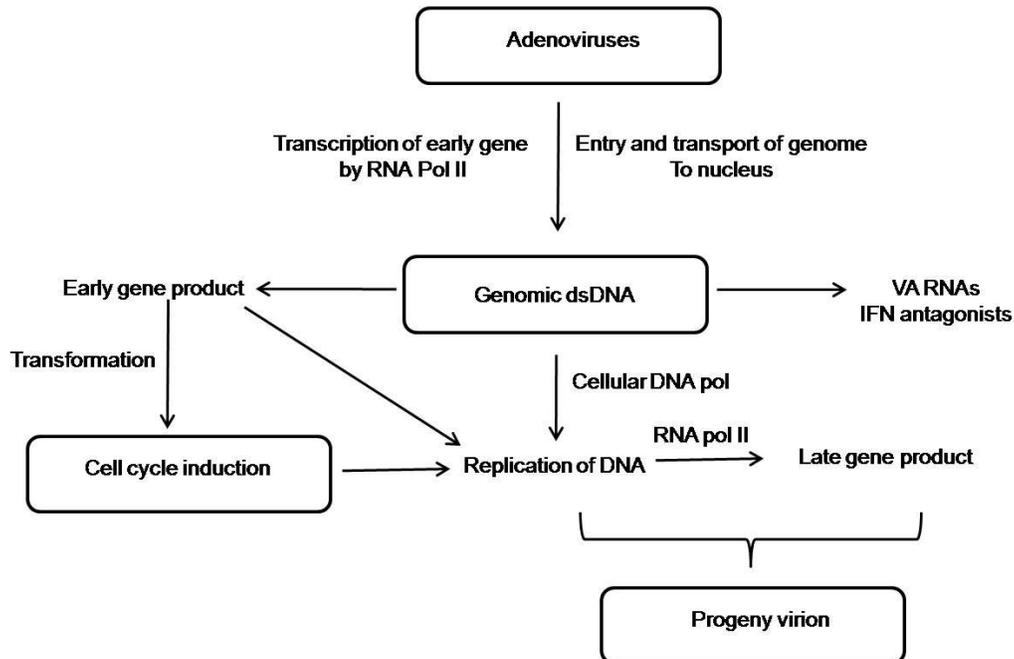
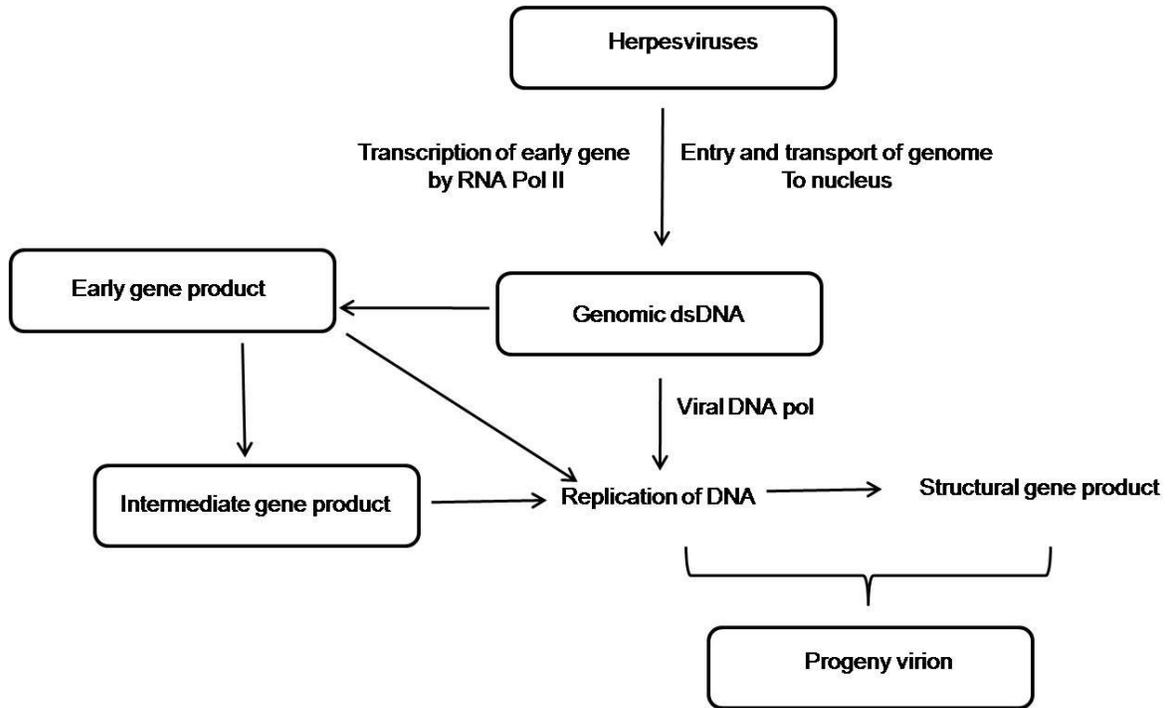


Figure 36.2 General replication strategies of herpesviruses:



36.1 Oncogenic DNA viruses

Epstein-Barr virus, *Hepatitis B* virus, papillomaviruses and Human Herpesvirus type 8 (HHV-8) or Kaposi's sarcoma are associated with one or other type of cancer in humans. In addition, HIV induces severe immunosuppression and facilitates development of cancer by other persisting infections, especially by HHV-8, Epstein-Barr virus and human papillomaviruses. Thus these agents contribute indirectly to human cancer. The mechanism about how these DNA viruses induce cancer is quite well known now. E6 and E7 gene of human papillomavirus modulate large array of cellular gene making cells vulnerable to undergo uncontrolled multiplication. Similarly, Epstein-Barr virus nuclear antigen 2 (EBNA-2) results in the induction of viral oncogenes that modulate many cell proteins. Many liver associated malignancies are caused by Hepatitis B virus. Bovine papillomaviruses can induce tumors (sarcoids) in horses and donkeys.

Lecture 37: Herpesviruses

The herpes name is derived from the Greek word *herpein*, meaning to creep. Many of the herpesviruses were isolated from different species of animal and at least eight from human. One of the important characteristics of this virus is to cause latency in the infected individuals.

37.1 Herpesvirus virion

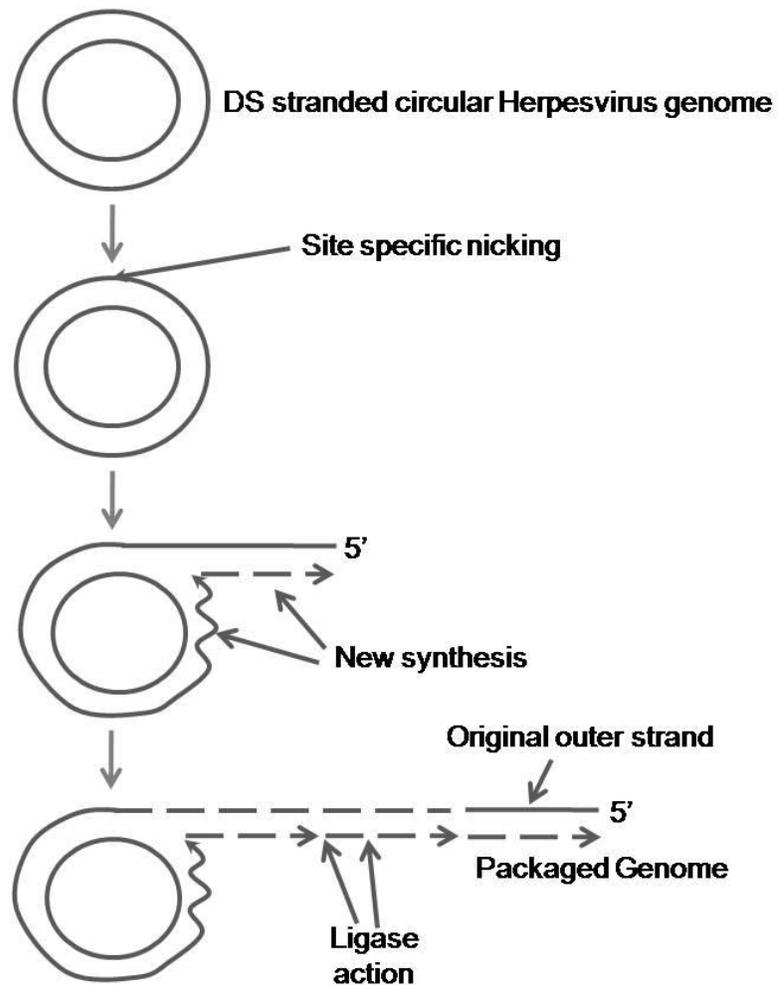
Herpesviruses have a complex structure because of its large size, multiple proteins, and tegument. The viral genome is a linear dsDNA of about 125-250 Kbp. The DNA is encapsidated inside an icosahedral capsid which is surrounded by a tegument. The tegument of herpesvirus contains many proteins while envelope is composed of 10 or more glycoproteins. The structural proteins of the herpesviruses are called as viral protein (VP) and VP5 is the most abundant protein present in the capsid. The envelope glycoproteins such as gB, gC and gD are the antigenic determinants and are involved in mounting the host immune response. In addition, the virion also contains the hexon and penton fibers. Genome of the herpesvirus contains two unique sequences; large and small and both are flanked by the repeat sequences. The genome encodes more than 75 proteins and many mRNA subspecies. Both strands of the DNA are used for the coding purpose. As some genes are present in the inverted repeats so the genome contains a pair of those genes in each strand.

37.2 Herpesvirus replication

Herpesvirus largely infects humans, many animals and lab animal in research laboratories. The virus binds first to **heparan sulfate** and then to cell adhesion molecules such as **nectins**. The virus then fuses with the cell membrane and enters the cell following endocytosis. The nucleocapsid and the tegument are released into the cytoplasm. The replication of the genome occurs in the nucleus and therefore nucleocapsid is first transported into the nucleus. The linear DNA molecule is converted into closed and circular in the nucleus of the host cells. The closed circular DNA then binds with the histones. The major tegument protein VP16 helps in modulating the viral gene expression and is transported along with the viral DNA to the nucleus. The herpesvirus genes express as immediate early (IE), early (E), and late (L). VP16 acts as a

transcription factor to recruit RNA polymerase II to activate the immediate early genes. Early proteins have their role in viral DNA replication and late proteins are formed during the assembly of the virion. Most of the late proteins are structural proteins. DNA replication in herpesvirus starts with a θ mode and later switches to rolling circle mode. Rolling circle mode of replication is the major form observed in the herpesviruses.

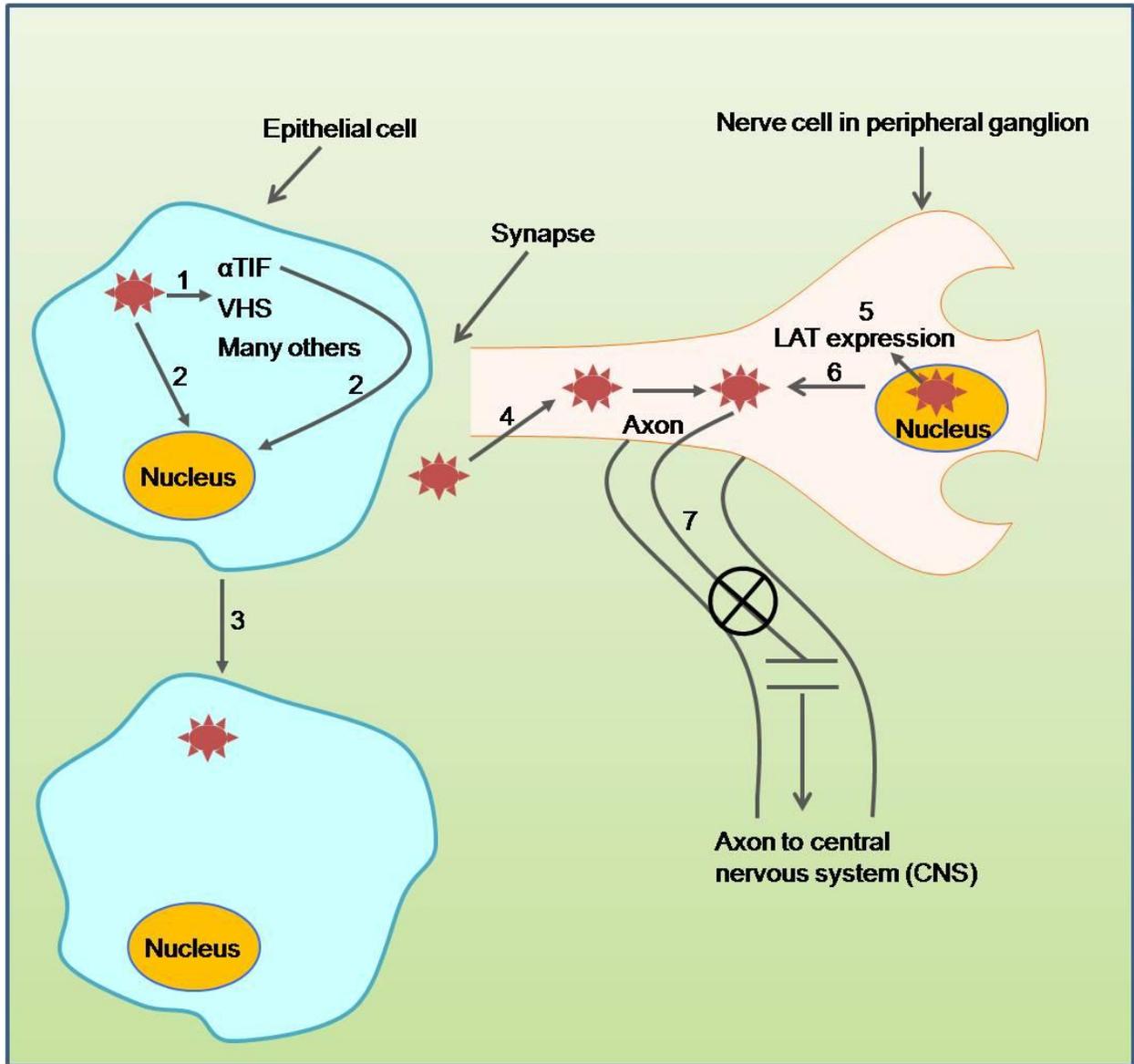
Figure 37.1 Rolling circle replication in herpesviruses:



37.3 Latency during herpesvirus infections

- I. After entry virus releases many factors that initiate infection. The factors include **virion host shut-off (VHS)**, **α -trans inducing factor (α TIF)** and many others.
 α -TIF- helps in the synthesis of 5 α mRNA.
VHS- favors to shut off host protein synthesis by degrading cellular mRNA.
- II. The viral genome and α TIF migrates to the nucleus where viral gene expression begins.
- III. New viruses bud out from the cell and infect other neighboring epithelial cells.
- IV. Some newly formed virions cross the synapses and travel downwards the axon to the nerve cells towards peripheral ganglion.
- V. The virions become latent inside neurons with the expression of **Latency associated transcript (LAT)**.
LAT- It is an mRNA made during latency by viruses.
- VI. During the process of reactivation new viruses are made in the nerve cell. They travel back downwards the axon to infect the epithelial cells again.
- VII. Nerve cells in the ganglion are well connected to the brain but virus rarely goes in that direction.

Figure 37.2 Latency in herpesviruses:



37.4 Important herpesviruses

37.4.1 Herpes simplex viruses 1 and 2

Herpes simplex viruses 1 and 2 (HSV-1 and HSV-2) infect epithelial cells of the buccal cavity, genital mucosa membrane, skin and cornea. Generally the virus migrates to central nervous system via neurons and initiates a latent infection. HSV-1 is mostly transmitted by lips and nasal contacts mostly to the young ones (1-2 years). HSV-2 is mostly transmitted by sexual contact and hence called as **genital herpes**.

37.4.2 Varicella-zoster virus

Varicella-zoster virus leads to a condition commonly called as **chicken pox** (varicella) where virus spreads to the skin and produces rashes. The rashes are mostly towards the face and trunk area. It may spread to CNS to produce a latent infection and reactivates during stress or administration of corticosteroids leading to a condition called as **shingles**. The symptoms include rashes in different body parts, fever, headache, joint pain, and swollen lymph nodes.

37.4.3 Epstein-Barr virus

Epstein-Barr virus is generally transmitted by saliva from an infected individual and spread in the body by its multiplication in the B cells. The virus infects the young ones with asymptomatic infection which activates during adolescence. The virus leads to a condition called as **infectious mononucleosis** or glandular fever. Epstein-Barr virus is also associated with different kind of cancers in humans. In medical science the infection is referred as **kissing disease** since it is transmitted by kissing through saliva.

37.4.4 Human cytomegalovirus

Human cytomegalovirus is transmitted vertically from mother to foetus. The infection at birth can cause reduced brain size and enlargement of the liver and spleen. During the later phase of life virus can cause hearing loss and mental retardation. HIV positive patients who are immunocompromised can easily be infected by cytomegalovirus which terminates into life threatening pneumonia or hepatitis.

Lecture 38: Adenoviruses

Adenoviruses are one of the major causative agents of upper respiratory tract or common cold infection in humans. In addition, they also cause conjunctivitis (eye inflammation), tonsillitis (inflammation of tonsils), gastroenteritis (inflammation of intestine), urinary tract infections, and infection to brain.

There are four genera of adenoviruses

1. Aviadenovirus- Infecting to avian species
2. Mastadenovirus- Infecting to mammals
3. Atadenovirus- Infecting to avian and humans
4. Siadenovirus- Infecting to avian, mammals, and reptiles.

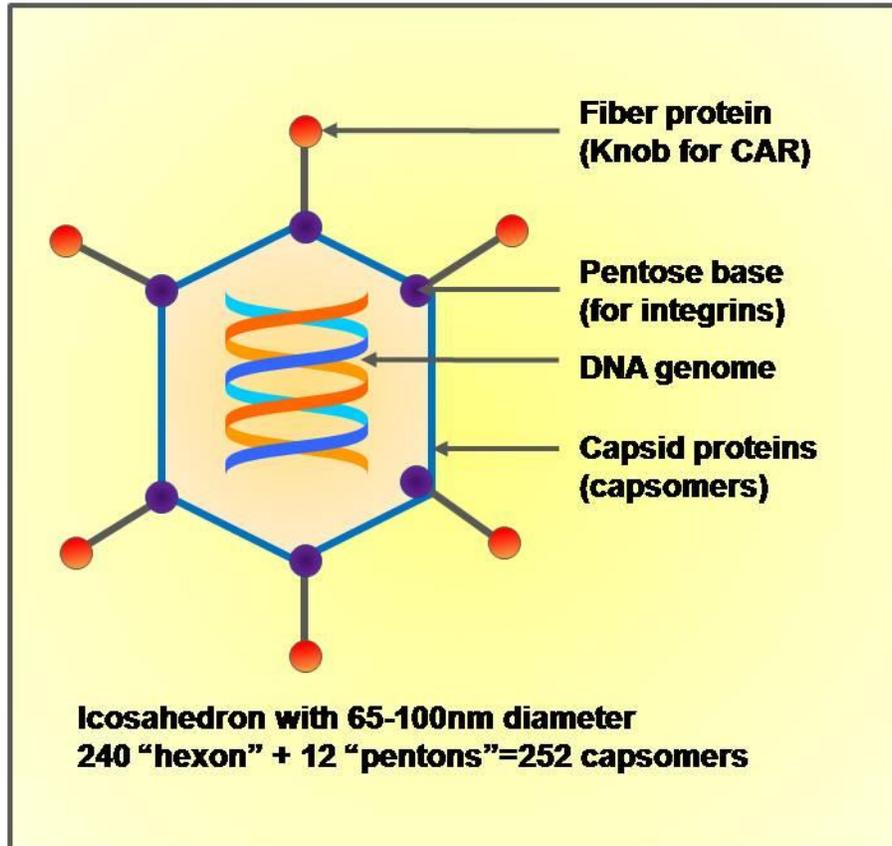
38.1 Adenovirus structure and genome organization

Adenoviruses are non-enveloped and icosahedral particles. They are 60-90 nm in diameter and contain 252 capsomers (240 hexons and 12 pentons) in the vertices of the icosahedrons.

The **hexons** on the virions are involved in the stabilization and assembly of the viral particle.

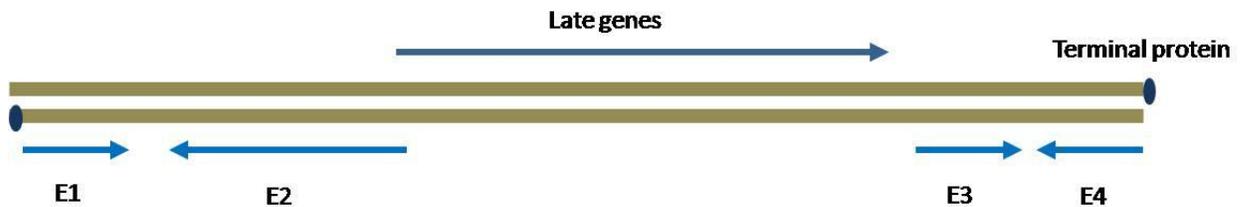
They contain a **penton** fiber that projects from each apex from the virion surface. The penton fiber consists of a shaft and a globular head. They are involved in the attachment of the virus to the surface of the host cell. They are very fragile and usually detached during preparation for electron microscopy.

Figure 38.1 Schematic representation of adenovirus virion:



Genome of adenovirus contains linear dsDNA of about 35 kb which encodes approximately 40 different proteins. The genome has inverted terminal repeats which are required during the replication process. The adenovirus DNA contains terminal protein at its 5' end. The early genes (E1-E4) are present towards either of the ends and are required to control the transcription and viral DNA replication. The late genes are generally associated with the viral structural proteins.

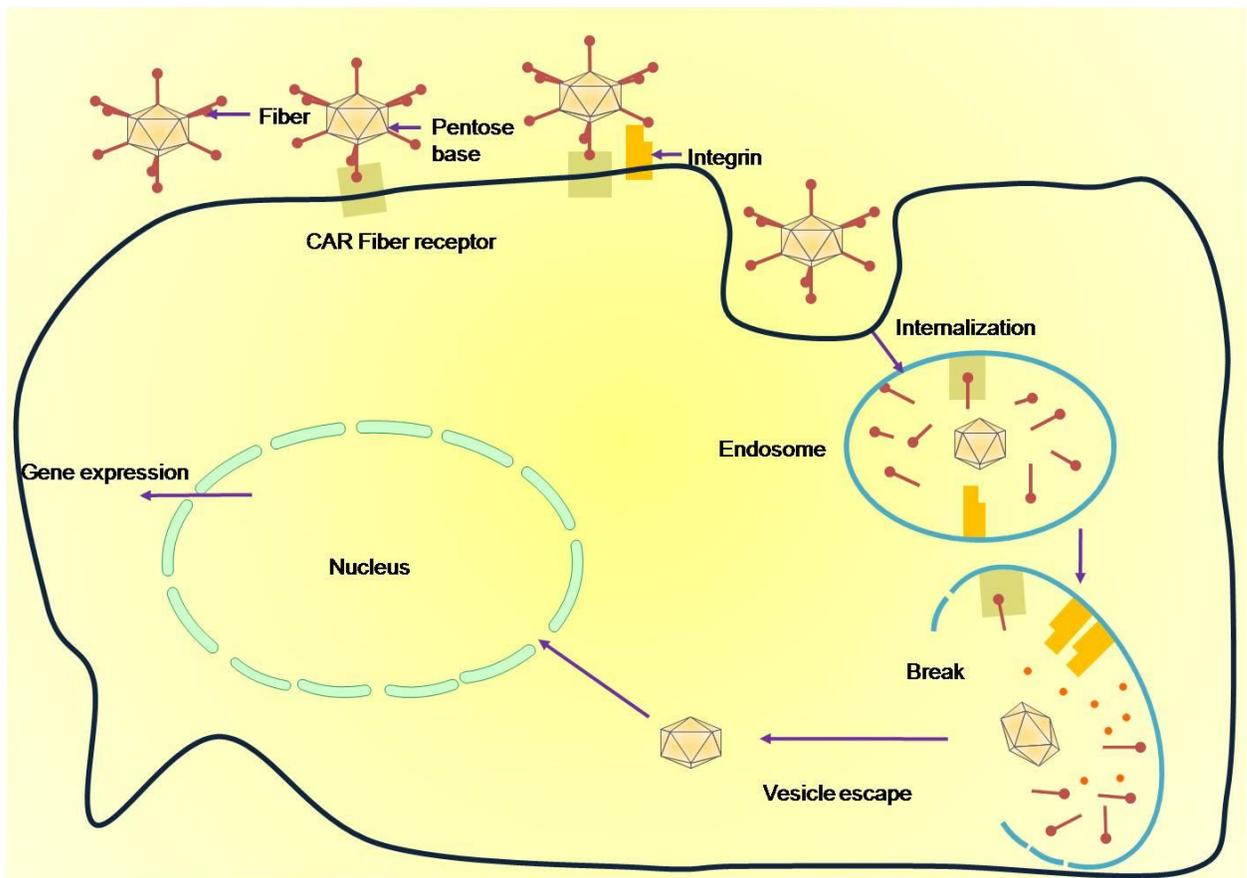
Figure 38.2 Adenovirus genome:



38.2 Adenovirus replication

To enter the cells they use a receptor present in the host cell called as **CAR** (coxsackie and adenovirus receptor). The internalization of the virus particles occur through receptor mediated endocytosis. After entry the endosome containing the virus particle migrates to nucleus and the genetic information of the virus is released into the nucleus. Transcription of the first gene is done by the terminal protein attached with the viral DNA. Viral mRNA is then transported to the cytoplasm and translated into the viral proteins. Virus assembly takes place in the cytoplasm and the mature viral particles get released from the infected cells after killing them by accumulated adenoviral death proteins.

Figure 38.3 Adenovirus life cycle:



38.3 Adenovirus associated diseases

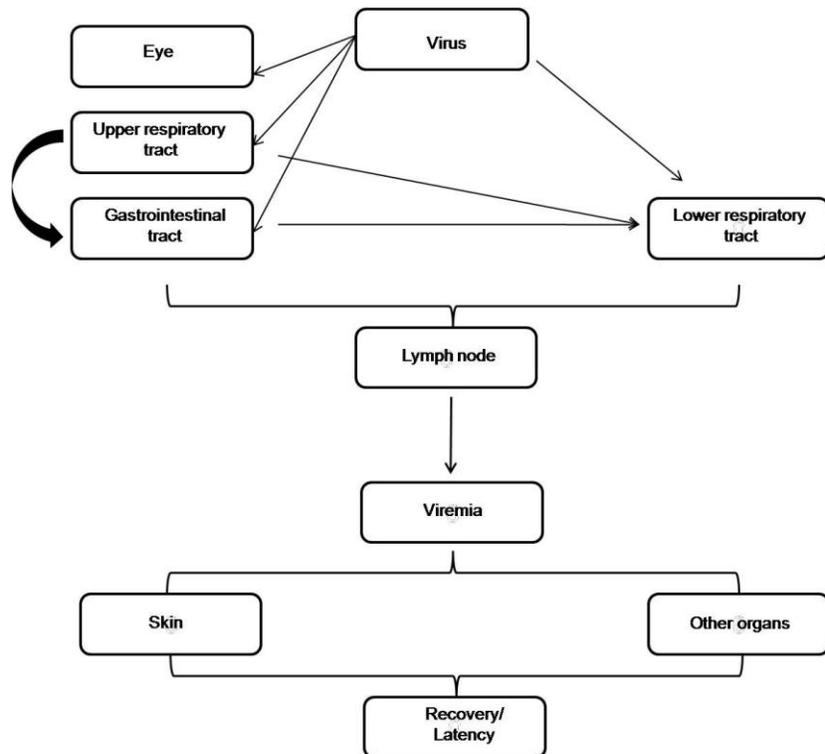
38.3.1 Respiratory diseases

In young children and infants it causes an acute febrile upper respiratory tract infection. It may progress to pneumonia and pharyngeal infection in untreated cases and in immuno-compromised individuals. In adults the symptoms include fever with pneumonia and pharyngitis.

38.3.2 Other diseases

In children the virus can cause acute gastroenteritis and hemorrhagic cystitis (inflammation of urinary bladder). Occasionally they may cause condition like meningoencephalitis in immuno-compromised patients. Sometimes they infect liver and eye leading to hepatitis and keratoconjunctivitis, respectively.

38.4 Adenovirus pathogenesis



38.5 Prevention and control

Currently no vaccine is available to protect against adenoviruses.

Good hygienic practices can prevent the infection.

Hand washing is still the best way to avoid adenovirus infection.

Wear protective clothing.

Heat and bleach will kill adenoviruses.

Adenoviruses are unusually stable to chemicals, physical agents, and adverse pH, causing them to survive longer in environment.

Lecture 39: Poxviruses

Poxviruses belong to family *Poxviridae* and are among the complex viruses in the field of virology. The disease has a great historical impact; the first case of the poxvirus was reported about 2000 years ago in China. The virus produces a characteristic pock like lesions in the body (small pox). Last naturally occurring outbreak was reported in Somalia in 1977.

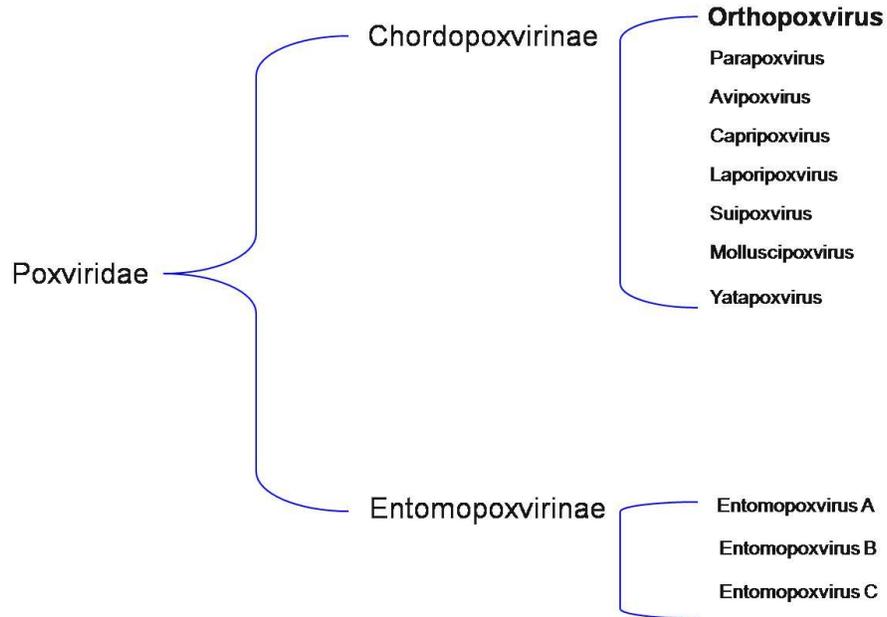
What characteristics of small pox made its eradication possible?

- ◆ Short incubation period
- ◆ No animal reservoir
- ◆ High morbidity and mortality
- ◆ Clinically apparent disease
- ◆ Mode of transmission
- ◆ An effective vaccine
- ◆ Social and economic factors

39.1 Classification

All human pox viruses are in the Chordopoxovirinae subfamily, and most of them belong to either the Orthopoxvirus (variola, vaccinia, cow pox) or the Parapoxvirus (Orf virus) genus.

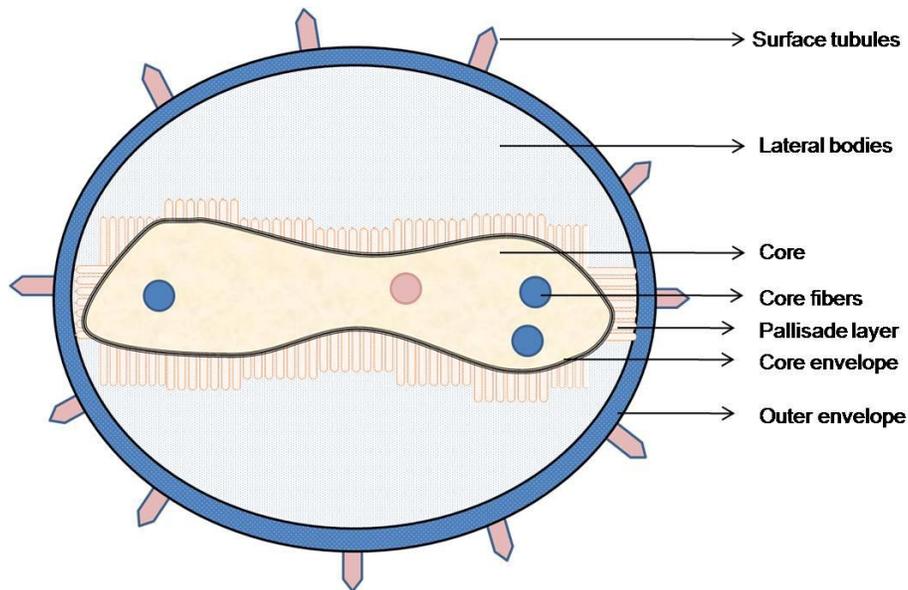
Figure 39.1 Classification of poxviruses:



39.2 Morphology

Virus is brick or oval shaped and around 300-400 nm in diameter. The viruses contain many proteins and are highly complex. The virus contains a lipid envelope that surrounds the core which is dumbbell shaped or biconcave. The virion may be beaded or smooth based on the presence or absence of surface tubules. Beaded form is converted into smooth form by the treatment of non-ionic detergent. The virus is present in both extracellular and intracellular form. The intracellular form contains a single envelope and is called as intracellular envelope virion (IEV) while the extracellular form has two envelopes and is called as extracellular envelope virion (EEV). Either side of the core (dumbbell shape) contains lateral bodies. The core is compactly packed with the genomic DNA. Antigenically, poxviruses are complex and produce a strong antibody response together with a long lasting memory. The genome of the virus contains dsDNA of about 130-300kbp. The terminal end of the viral genome contains inverted terminal repeats. More than 200 genes have been identified for the poxviruses; many of the essential genes are located in the center of the genome while non-essentials lie towards the ends.

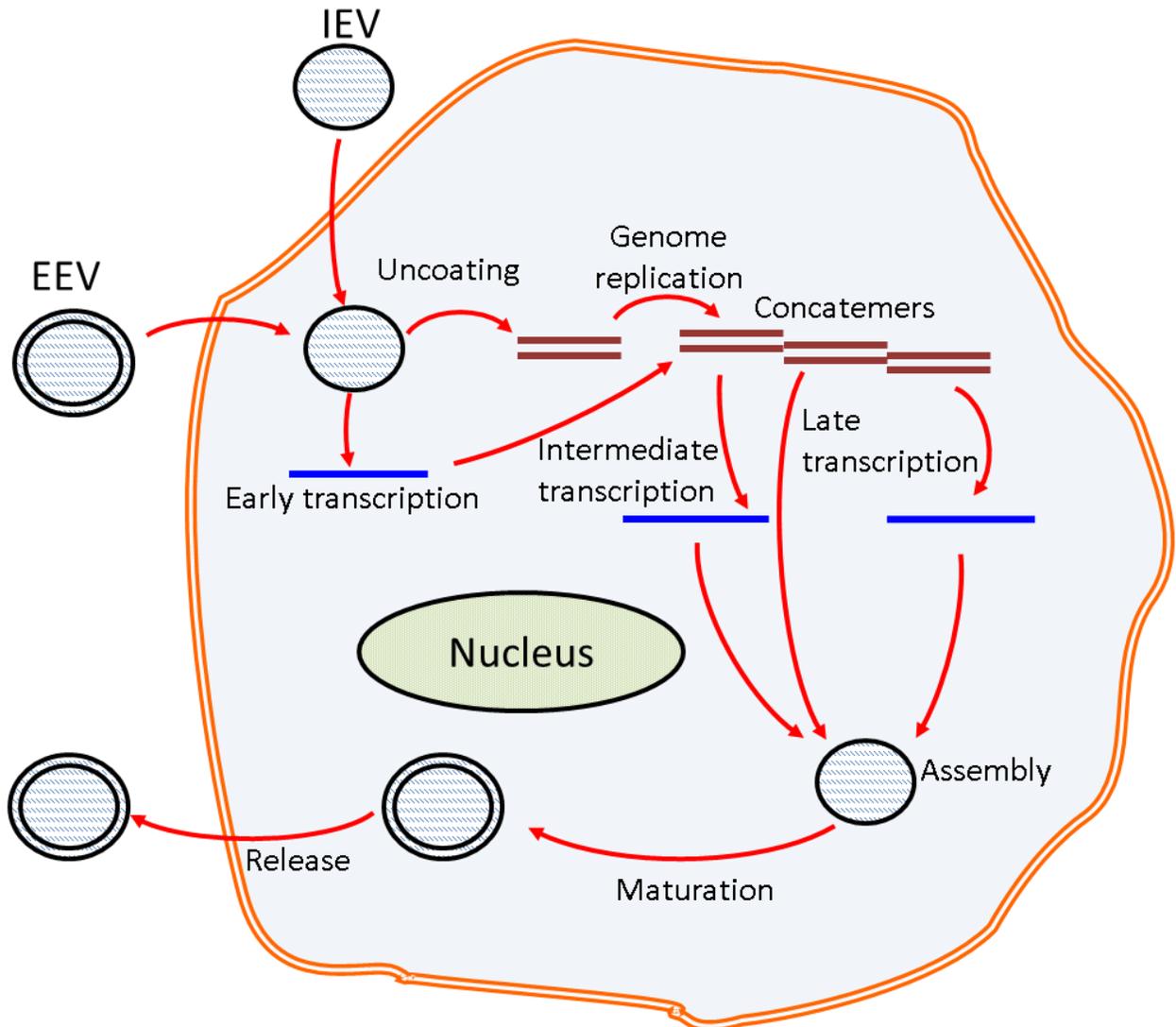
Figure 39.2 Poxvirus virion:



39.3 Replication

The replication of the genomes occurs in the cytoplasm. Many of the poxviruses attach to the cells with the help of **epidermal growth factor** as a receptor. Uncoating of the outer membrane occurs in the cytoplasm and genomic DNA is released into the cytoplasm. The virus contains both early and late gene based on its transcription preference. More than 50% of the early genes are transcribed before the DNA replication while late genes are transcribed after the completion of DNA replication. Many virus encoded enzymes help in the replication of DNA, concatemers are formed during the replication that later on cleave to form viral genome.

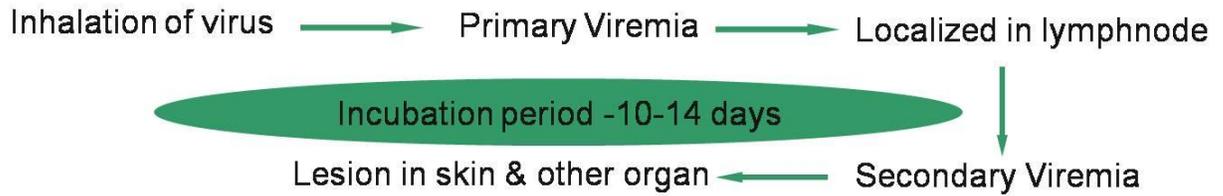
Figure 39.3 Life cycle of poxviruses in infected cell:



39.4 Transmission

In poxviruses, transmission is through direct contact. In case of small pox, the virus is found in lesions in the upper respiratory tract, which can be transmitted to others in droplet secretions, and in skin lesions. Route of transmission makes its spread relatively slow. The mechanical transmission of the virus by flies is also reported.

39.5 Pathogenicity



39.6 Important poxviruses

39.6.1 Vaccinia virus:

The virus causes a wide spread infection in animal and humans. The causative agent is an Orthopoxvirus. Symptoms of the disease includes pustular lesion in the teat and udder of the dairy cattle. Outbreaks in human produce lesions in hands and face of milkers who are not protected from smallpox.

39.6.2 Monkeypox virus

Monkey pox virus is a zoonotic agent with a wide host range. The virus was first reported in Democratic Republic of Congo. The signs of the disease include pustular rashes in the body, high fever and enlargement of lymph nodes.

Lecture 40: Miscellaneous viruses

Infectious diseases have played a significant role throughout the history of mankind. Investigation of diseases dates back to ancient times and the query to understand it through science has led to the discovery of viruses and bacteria as the causative agents of various types of infection and illness. Pathogenicity of viruses and susceptibility of host to infectious agents have constantly appeared through the emergence of new diseases and reappearance of pre-existing diseases. **Emerging infectious viral** diseases are those that have recently appeared in a population as a result of a new virus or the recognition of a previously undetected virus and are often zoonotic. Emergence of an infectious viral disease may occur due to the extension of the geographic or host range of the virus. Recently, bats have been implicated as an important reservoir and source of many emerging viruses. As new technology for detection of viruses becomes increasingly available, more viruses are likely to be detected. Enhanced molecular biology techniques will allow faster and more complete characterization of new and miscellaneous viruses.

40.1 Bat paramyxoviruses

Bats have been shown to be the reservoir hosts of a variety of viruses responsible for severe disease outbreaks in humans and animals, including filoviruses, coronaviruses and paramyxoviruses. Recently Hendra and Nipah viruses were also isolated from bats.

Hendra and Nipah viruses are zoonotic viruses of the genus *Henipavirus* under the family *Paramyxoviridae*. The natural reservoirs for both the viruses are fruit bats or flying foxes of the genus *Pteropus*. Hendra virus was first isolated from an acute febrile illness in horses and subsequently in humans with a sign of fatal encephalitis. The first known human infections with Nipah virus were detected during an outbreak of severe febrile encephalitis in peninsular Malaysia and Singapore.

Menangle virus was isolated during an outbreak of reproductive disease in pigs in New South Wales, Australia in 1997. Symptoms of the disease included malaise, chills, fever, sweating, headache, weight loss and decrease in farrowing rate (birth giving process in pigs).

Tioman virus was isolated from the urine of fruit bats (*Pteropus hypomelanus*). Tioman virus is lethal in suckling mice 8-12 days post intracerebral inoculation. Their role in human and animal infection is still under debate.

Mapuera virus was isolated from the salivary glands of an asymptomatic fruit bat (*Sturnira lilium*) in 1979 in Brazil.

40.2 Canine Distemper Virus

Canine distemper virus is an important pathogen which naturally infects a broad range of terrestrial and marine carnivores. Canine distemper virus is a member of genus *Morbillivirus* of the family *Paramyxoviridae*. The disease is characterized by skin rash, fever, gastrointestinal and respiratory signs, and a profound immune-suppression as well as by frequent neurological complications.

40.3 Rift Valley Fever Virus

Rift Valley Fever Virus (RVFV) is a member of the genus *Phlebovirus* (family *Bunyaviridae*). The RVFV is transmitted by the bite of mosquitoes. The disease was first reported in sheep in Kenya in 1918. Infection of RVFV is characterized by febrile illness with hemorrhages and inflammation of brain.

40.4 Hantavirus

Hantavirus belongs to the family *Bunyaviridae* (negative-sense, single-stranded RNA viruses). Hantavirus is transmitted by rodents (deer mice) via their urine and feces. Hantavirus is a cause of hemorrhagic fever with a renal (Kidney) syndrome. The early symptoms of the disease are similar to flu and include fever, chills, cough and muscle ache. The disease can progress to Hantavirus pulmonary syndrome.

40.5 Ebola Virus

Ebola virus belongs to family of RNA viruses called the *Filoviridae*. The virus leads to fatal hemorrhagic disease in humans and nonhuman primates. The transmission of the virus occurs by direct contact with the blood and/or secretions of an infected person. Sudden onset of illness is characterized by fever, sore throat, headache, joint and muscle

pain, and weakness, followed by diarrhea, vomiting, and abdominal pain. In highly fatal cases internal and external bleeding may be seen in the patients.

40.6 Arenaviruses

The family *Arenaviridae* contains the viruses which are usually associated with rodent-transmitted disease in humans. The virus particles are spherical with a diameter of around 110-130 nm. The virus contains negative strand RNA as a genetic material. Infection of Arenaviruses leads to hemorrhagic disease in humans that are often fatal.

Table 40.1 Different Arenavirus diseases:

Virus	Disease
Junin virus	Argentine hemorrhagic fever
Lassa virus	Lassa fever
Guanarito virus	Venezuelan hemorrhagic fever
Machupo virus	Bolivian hemorrhagic fever

40.7 Prions

Prions are the infectious agents made up of only proteins (No DNA or RNA) and were discovered by **Stanley Prusiner**. Prions are propagated by transmitting the misfolded form of the protein. Prion diseases or transmissible spongiform encephalopathies (TSEs) are a family of rare progressive neurodegenerative disorders of humans and animals. They are characterized by long incubation periods, spongiform changes in brain, and a failure to induce inflammatory response.

Table 40.2 Different prion diseases in human and animals:

Human Prion disease	Animal Prion disease
Creutzfeldt- Jakob Disease (CJD)	Scrapie
Kuru	Mad Cow Disease (Bovine Spongiform Encephalopathy)
Gerstmann- Straussler- Scheinker Syndrome	Chronic Wasting Disease Transmissible mink encephalopathy

40.8 Viroids

Viroids are plant pathogen that contains circular single stranded RNA as a genetic material. They are discovered by **Theodor Diener** in 1971. Viroids contain small RNA of around 250 to 500 nt and do not encode any proteins. The **Potato spindle tuber viroid** was the first viroid to be identified.