College of
Veterinary Medicine, Animal Resources
& BioSecurity

BIOMEDICAL MICROBIOLOGY (BLT 1202)

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Overview of DNA viruses associated with:

a) Tumour:

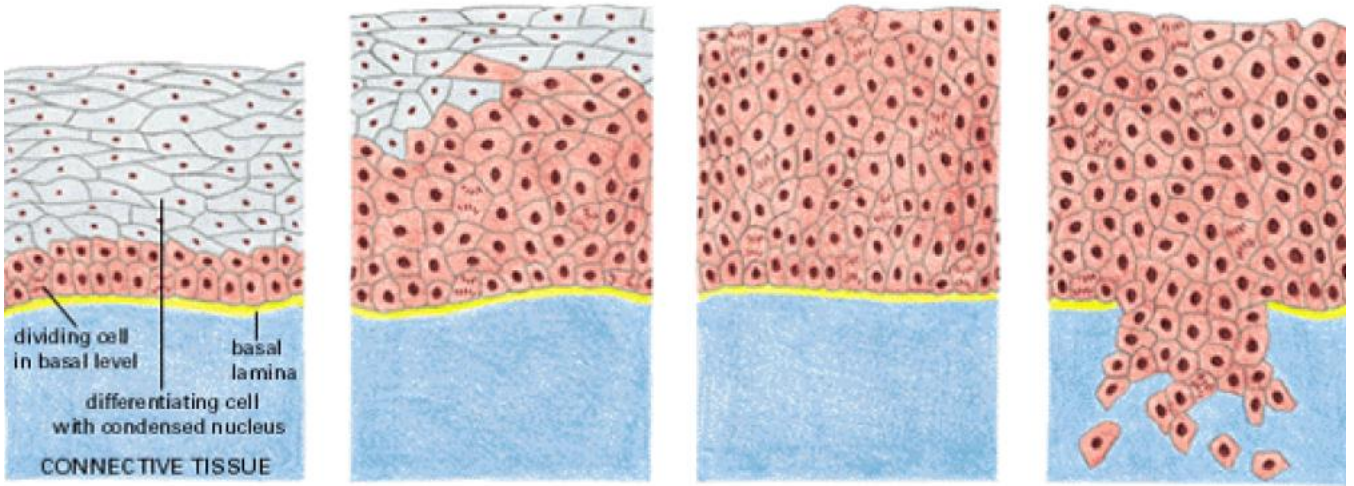
Papilloma virus,
Polyomavirus,
Herpes virus,
Hepatitis B virus

b) Systemic diseases:

Adenovirus
Pox virus
Parvovirus

Tumour associated with DNA viruses

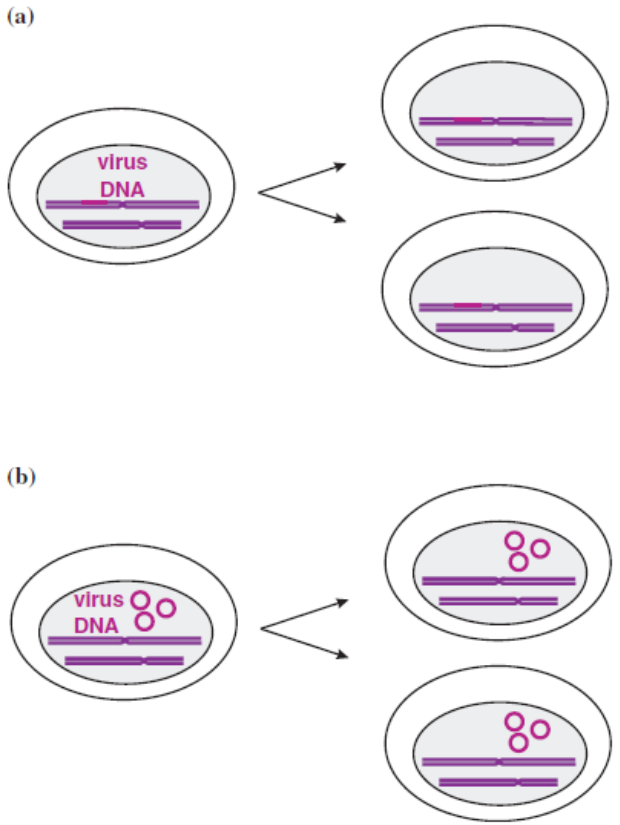
- A cancer is a malignant tumour and involves continuous proliferation of a clone of cells derived from one of the body's normal cells.



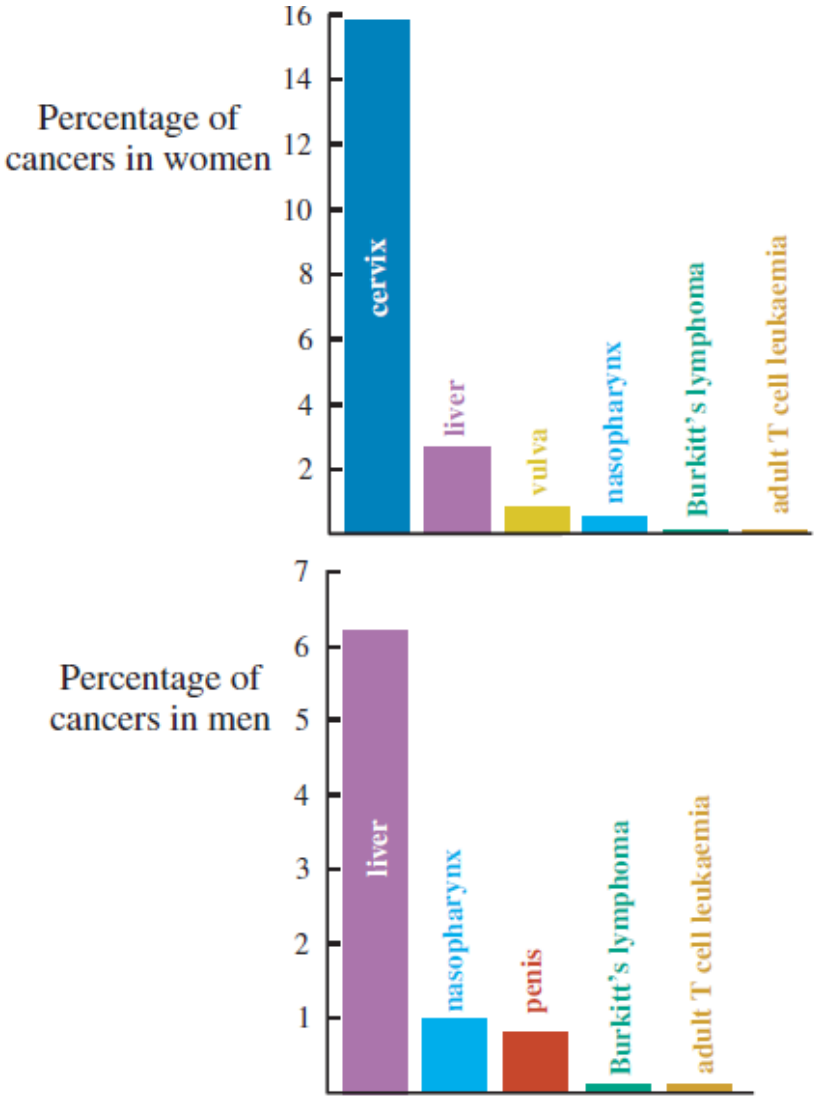
- The cell undergoes changes known as <u>transformation</u> as a result of events that include **mutation**, activation of **oncogenes** (tumour genes) and inactivation of **tumour suppressors**.

- This **transformation** can be triggered by **environmental factors** (e.g. chemicals and irradiation), and **viruses**.
- A virus that causes cancer is known as **an oncogenic virus**.
- DNA of oncogenic viruses is regularly present in the tumour cells.

•The viral DNA can be found **integrated** into a cell chromosome (Fig. a) or sometimes present as multiple copies of **covalently closed circular DNA** (Fig. b).



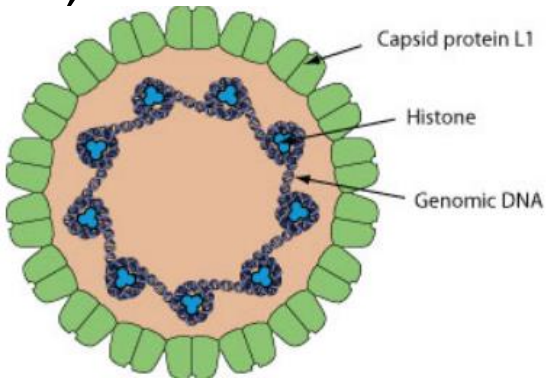
•Virus-associated cancers in women and men. *Adapted from Microbiology Today, August 2005*

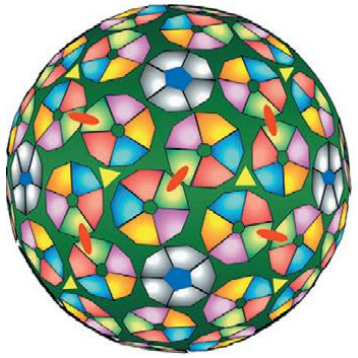


a) Papilloma virus

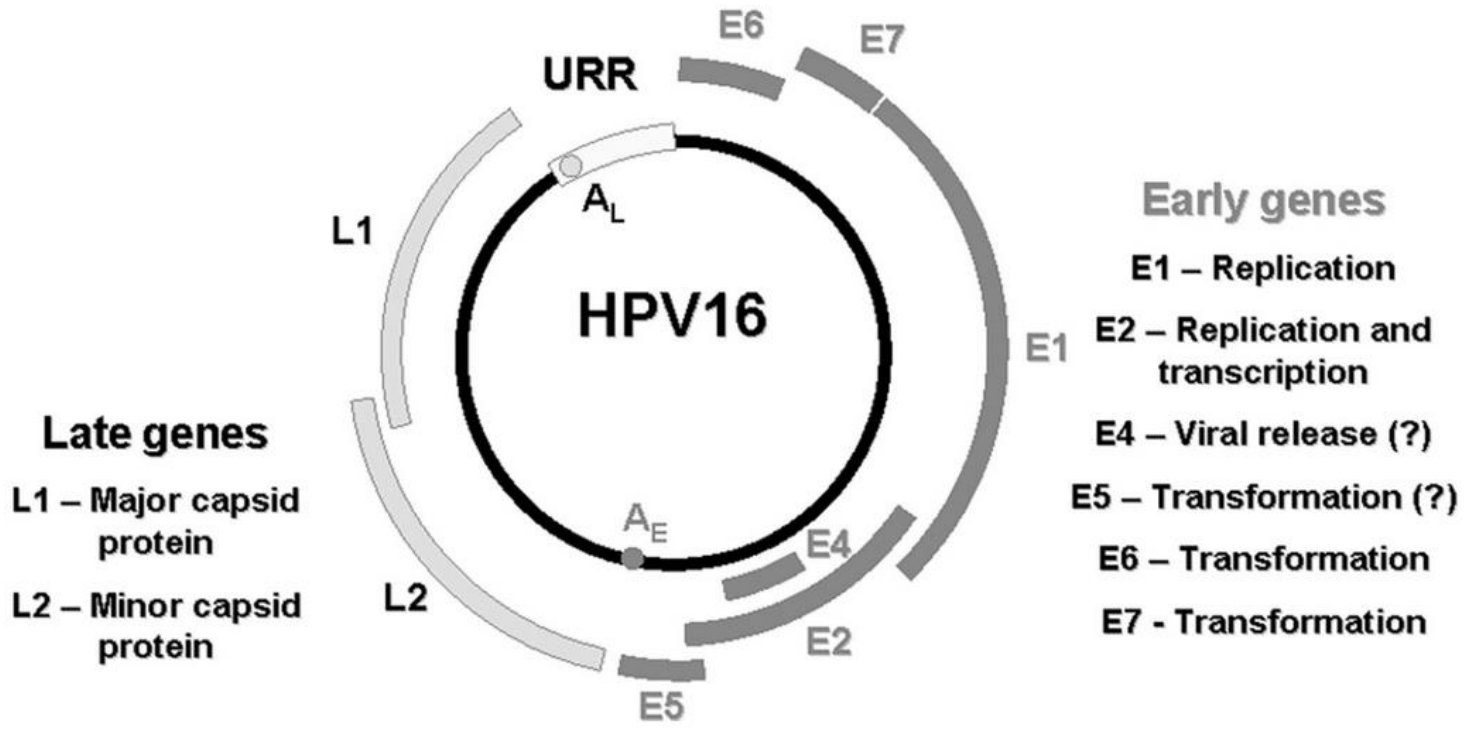
Properties:

- Papillomavirus virions are non-enveloped, spherical, 50-55 nm in diameter, with icosahedral symmetry.





- Virions are composed of 72 hexavalent (six-sided) capsomers arranged in pentameric (five-sided) arrays.
- The **genome** consists of a single molecule of **covalently closed circular double-stranded DNA**, 6.8–8.4 kb in size.
- The genome **encodes some 8–10 proteins**, two of which (L1 and L2) form the **capsid** and the remainder are **non-structural proteins** (designated E1–E8). **Resistant** to lipid solvents and detergents, low pH, and high T°


E6
E7
URR
L1
A_L
HPV16
E1
Late genes
L1 – Major capsid protein
L2 – Minor capsid protein
L2
A_E
E4
E2
E5
Early genes
E1 – Replication
E2 – Replication and transcription
E4 – Viral release (?)
E5 – Transformation (?)
E6 – Transformation
E7 - Transformation

Replication:

- Replication is **divided in two distinct steps** that are linked to the differentiation state of the host epithelial cell:

a) The plasmid replication takes place in the **basal squamous epithelial cells**. It corresponds to viral DNA replication in synchrony with the host cell chromosome in order to ensures an average of one viral genome per basal cell.

i. **Attachment** of the viral proteins to host receptors mediates endocytosis into vesicles in the **basal squamous epithelial cell**.

ii. **Transport to the nucleus** and uncoating of the viral DNA.

iii. **Early-genes** transcription and translation of the **early proteins**.

iv. **Steady-state** viral DNA nuclear replication.

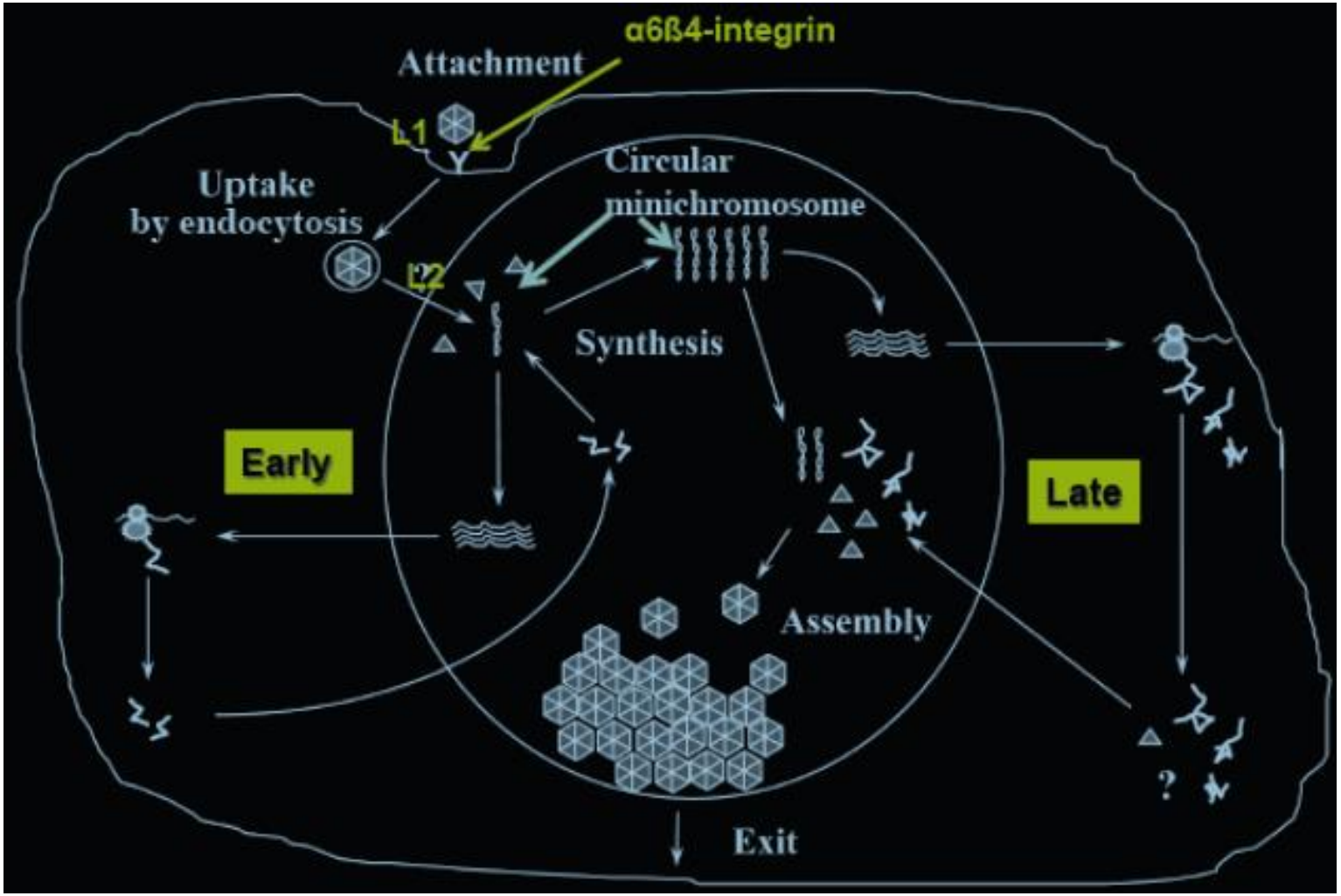
b) The vegetative replication, occurs in **differentiated keratinocytes**. In these cells, which no longer undergo cellular DNA synthesis, there is **a burst of viral DNA synthesis** with active **production of virions**.

i. Transcription of the late region.

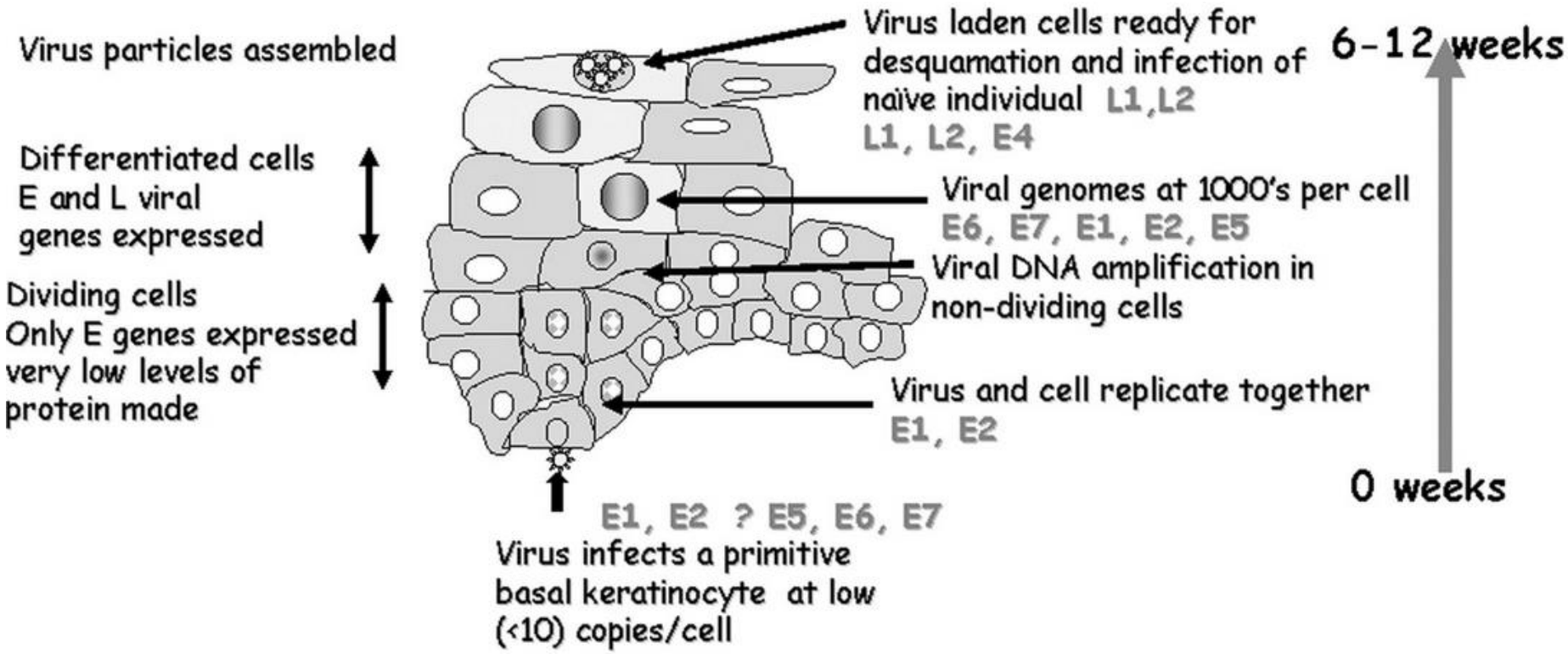
ii. Capsid proteins L1 and L2 synthesis.

iii. Nuclear capsid assembly and release of viruses.

Replication cycle of papillomaviruses



Replication of HPV in synchrony with maturation of epithelia cells



Epidemiology:

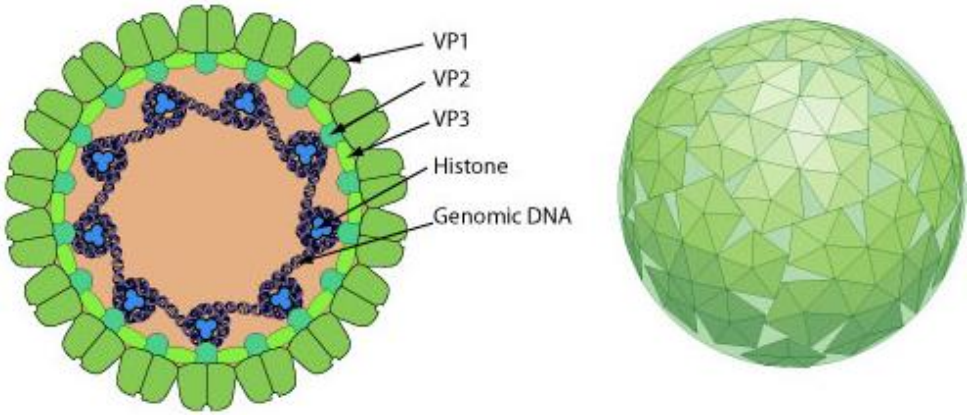
- ❑ Distribution: It occurs worldwide.
- ❑ Associated diseases: Warts, papilloma, malignant tumours.
  - Genital-type, **high-risk** of malignancy (cervical cancer): HPV-16, 18, 31, 33, 35, 45, 51, 52, 58.
  - Genital-type, **low-risk** of malignancy (genital warts): HPV-6, 11.
  - Skin-type: HPV-1, 2, 3, 4, 5, 7, 8, 10, 27, 57, 60, ...
- ❑ *Transmission*:
  - Sexual, or close contact.

Vaccine:

- HPV vaccine against HPV types 6, 11, 16 and 18.

b) Polyoma (many types of tumour) virus

- The virus belongs to the family *Polyomaviridae* with natural hosts being **mammals** and **birds**.
- It is **non-enveloped** capsid with **icosahedral** symmetry, about 50 nm in diameter.



- **Genome** is circular dsDNA and 5 kb in size, associated with cellular histones. Encodes for **5-9 proteins**.

- The **viral genome** can be **integrated in host chromosome** and can **give the host cell a replicative advantage** sometimes leading to malignant **tumours**.

REPLICATION

- It occurs in the **nucleus** and it involves the following:

1. Viral proteins **attach** to host receptors triggering lipid-mediated **endocytosis** of the virus into the host cell.
2. Virion transits through **endoplasmic reticulum** and its capsid structure is rearranged.
3. Export of misfolded virion to the **cytoplasm**.
4. **Loss of VP1** in the low-calcium conditions of the cytosol
5. Import of genomic DNA into host **nucleus**.
6. Transcription of **early genes**.
7. **Replication** of the DNA genome in the nucleus.
8. Transcription of **late genes** encoding for structural proteins (VP1, VP2 and VP3).
9. **Assembly** of new virions in nuclear viral factories.
10. Virions are released by **lysis** of the cell.

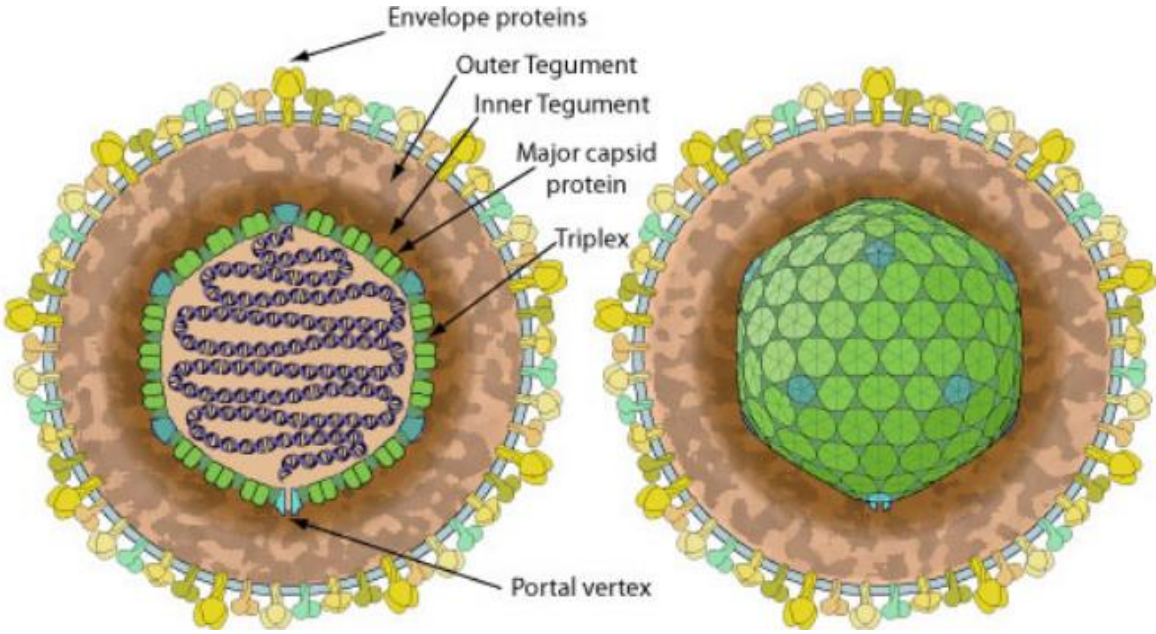
Epidemiology

- ❑ Geographic distribution: Worldwide occurrence
- ❑ Associated diseases:
  - JCPyV: progressive multifocal leukoencephalopathy.
  - BKPyV: mild respiratory infection.
  - Some cancers may be associated with polyoma viruses
  - In birds: Budgerigar Fledgling Disease
- ❑ Transmission:
  - BKPyV: contaminated faeces and aerosolized dust. Egg transmission.
- ❑ Currently neither vaccines nor drugs are available.

c) Herpes virus

- It is an **enveloped**, **spherical** to **pleomorphic**, 150-200 nm in diameter, capsid is **icosahedral symmetry**.
- Capsid consists of **162 capsomers** and is surrounded by an amorphous **tegument**.

•Glycoproteins complexes are embedded in the lipid envelope.



•The **genome** is single, linear, dsDNA genome of 120-240 kb.

Replication:

- Occurs in the nucleus

i) Lytic replication

1. **Attachment** of the viral proteins to host receptors mediates endocytosis of the virus into the host cell.
2. **Fusion** with the plasma membrane to release the core and the tegument proteins into the host cytoplasm.
3. The capsid is **transported to the nuclear pore** where viral DNA is released into the nucleus.
4. Transcription of **immediate early genes** which promote transcription of **early genes** and protect the virus against innate host immunity.
5. Transcription of **early viral mRNA by host polymerase II**, encoding proteins involved in replication of the viral DNA.
6. A first round of circular genome amplification occurs by **bidirectional replication**

7. Synthesis of linear concatemer copies of viral DNA by **rolling circle**.
8. Transcription of **late mRNAs by host polymerase II**, encoding structural proteins.
9. **Assembly** of the virus in nuclear viral factories and **budding** through the inner lamella of the **nuclear membrane** which has been modified by the insertion of herpes glycoproteins, throughout the Golgi and final **release at the plasma membrane**.

ii) Latent replication

- Replication of circular viral episome in tandem with the host cell DNA using the host cell replication machinery.

Epidemiology:

❑ Geographical distribution:

- Worldwide occurrence

❑ Associated diseases:

- HHV-1 and HHV-2: skin vesicles or mucosal ulcers (oral and/or genital). Rarely, encephalitis and meningitis.
- HHV-3: chickenpox (Varicella) and shingles. Congenital varicella syndrome may be caused by infection in utero during the first trimester.
- GaHV-2: Marek's disease.
- HHV-5: congenital CMV infection.
- HHV-6: "sixth disease" (roseola infantum, exanthem subitum).
- HHV-7: symptoms analog to the "sixth disease".
- HHV-4: B lymphocytes (Burkitt's lymphoma).
- HHV-8: B lymphocytes.

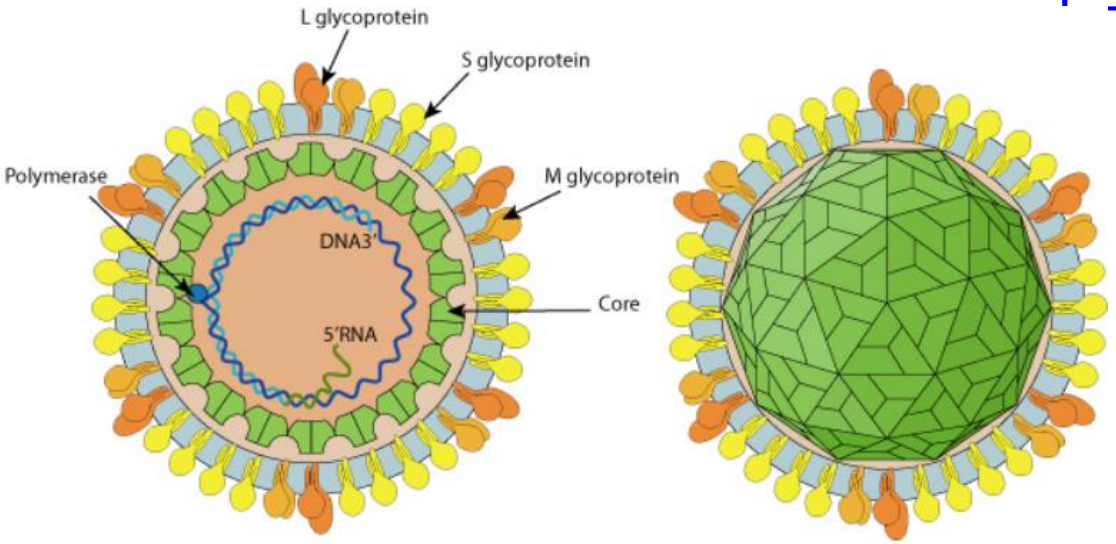
- Transmission:

- HHV-1: contact with lesions and body fluids.
- HHV-2: sexual. Infection at birth by a genitally-infected mother.
- HHV-3: contact, respiratory route.
- HHV-5:Infected body fluids (urine, saliva), transplacentary, transplantation, blood transfusion.
- HHV-4: Saliva, sexual (probable), transplacentary.
- HHV-8: Saliva, sexual.

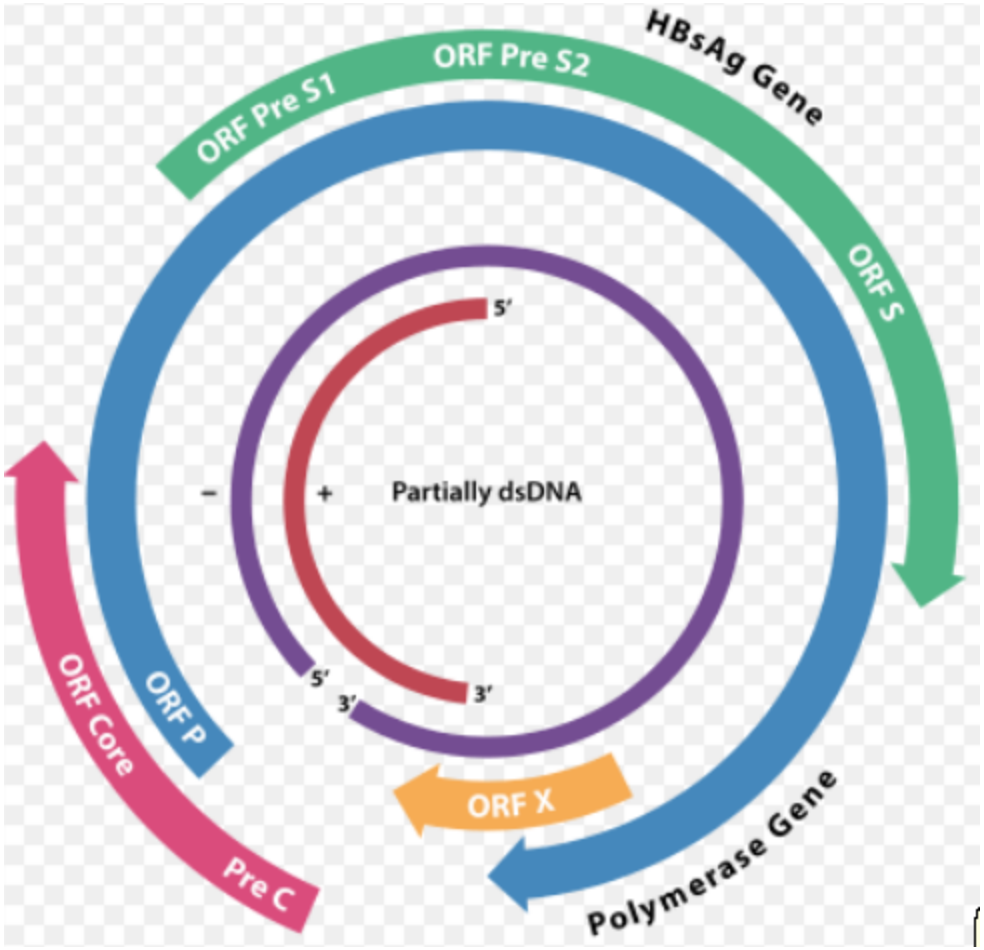
- No vaccine
- Antiviral Drugs: Nucleoside analogs (Acyclovir, famcyclovir, valacyclovir…). These drugs are activated by the viral specific enzyme, thymidine kinase, and are therefore specific to herpes-infected cells. These drugs act against the replicating virus (they are incorporated into the DNA as it is copied) and are ineffective against a latent virus.

d) Hepatitis B virus

- Virus infects humans, apes and birds. It is enveloped, spherical. Diameter is about 42nm. Icosahedric capsid.



- **Partially dsDNA circular genome**, about 3.2 kb in size. Encodes for **7 proteins**.
- The viral genome can be **integrated in host chromosome**. This can give the host cell a **replicative advantage** sometimes leading to **hepatocarcinoma**.


HBsAg Gene
ORF Pre S1
ORF Pre S2
ORF S
5′
−
+
Partially dsDNA
5′
3′
3′
ORF P
ORF Core
Pre C
ORF X
Polymerase Gene

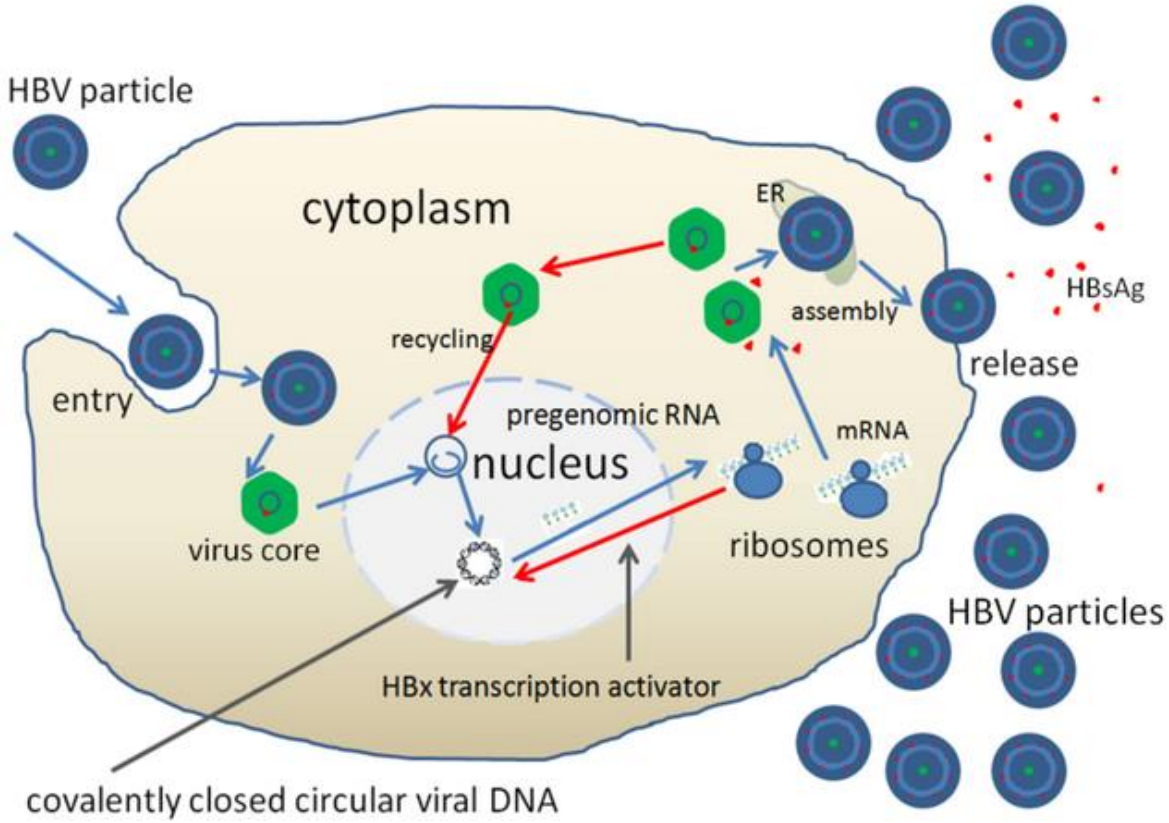
Replication:

- The replication is **cytoplamic/nuclear**.

1. **Virus attaches** to host receptors through major surface antigen and enters the cell by an unknown mechanism.
2. Relaxed circular DNA (RC-DNA) and capsid are **transported via microtubules to the nucleus** where **DNA is released** through the nuclear pore, and repaired to form covalently closed circular DNA (cccDNA).
3. Transcription by RNA polymerase II of the pregenomic RNA (pgRNA) and subgenomic mRNAs, inducing synthesis of all the viral proteins.
4. pgRNA is encapsidated, together with the P protein, and reverse-transcribed inside the nucleocapsid in (-)DNA covalently linked to P protein.

6. (+)DNA synthesis from the (-)DNA template generates new RC-DNA.

7. Transport to the nucleus of new RC-DNA leads to cccDNA amplification; alternatively, the RC-DNA containing nucleocapsids are enveloped at the ER, and new virions are released by exocytosis.



Epidemiology

- ❑ Geography:
- Worldwide occurrence
- ❑ Associated diseases:
- Hepatitis, hepatocellular carcinomas (chronic infections), liver cirrhosis.
- ❑ Transmission:
- Parental, sexual, blood.
- ❑ Vaccines:
- Subunit vaccine for Human Hepatitis B virus.
- ❑ Antiviral drugs:
- Lamivudine, Interferon

TABLE 35-8 Interpretation of Hepatitis B Virus Serologic Markers in Patients with Hepatitis[a]

Assay Results			
HBsAg	Anti-HBs	Anti-HBc	Interpretation
Positive	Negative	Negative	Early acute HBV infection. Confirmation is required to exclude nonspecific reactivity
Positive	(±)	Positive	HBV infection, either acute or chronic. Differentiate with IgM anti-HBc. Determine level of replicative activity (infectivity) with HBeAg or HBV DNA
Negative	Positive	Positive	Indicates previous HBV infection and immunity to hepatitis B
Negative	Negative	Positive	Possibilities include: HBV infection in remote past; "low-level" HBV carrier; "window" between disappearance of HBsAg and appearance of anti-HBs; or false-positive or nonspecific reaction. Investigate with IgM anti-HBc. When present, anti-HBe helps validate the anti-HBc reactivity
Negative	Negative	Negative	Never infected with HBV. Possibilities include another infectious agent, toxic injury to liver, disorder of immunity, hereditary disease of the liver, or disease of the biliary tract
Negative	Positive	Negative	Vaccine-type response

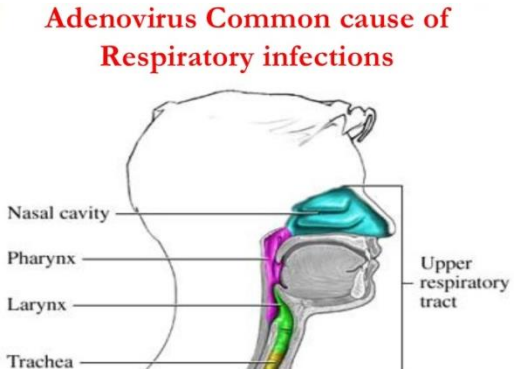
[a]Modified and reproduced with permission from Hollinger FB: Hepatitis B virus. In Fields BN, Knipe DM, Howley PM (editors-in-chief). *Fields Virology,* 3rd ed. Lippincott-Raven, 1996.

Anti-HBc,antibody to hepatitis B core antigen; anti-HBe, antibody to hepatitis B e antigen; anti-HBs, antibody to hepatitis B surface antigen (HBsAg); HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; IgM, immunoglobulin M.

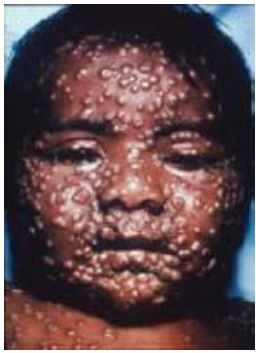
Jawetz et al..Medical Microbiology; 2013 (Pg, 516)

DNA viruses causing systemic diseases

- Adenovirus

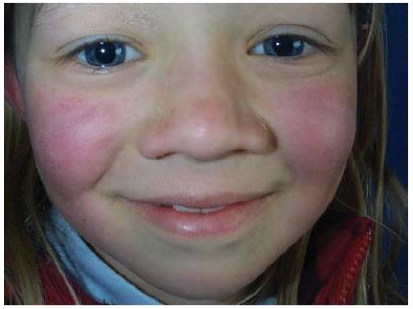


- Poxvirus



E.g. Smallpox

- Parvovirus

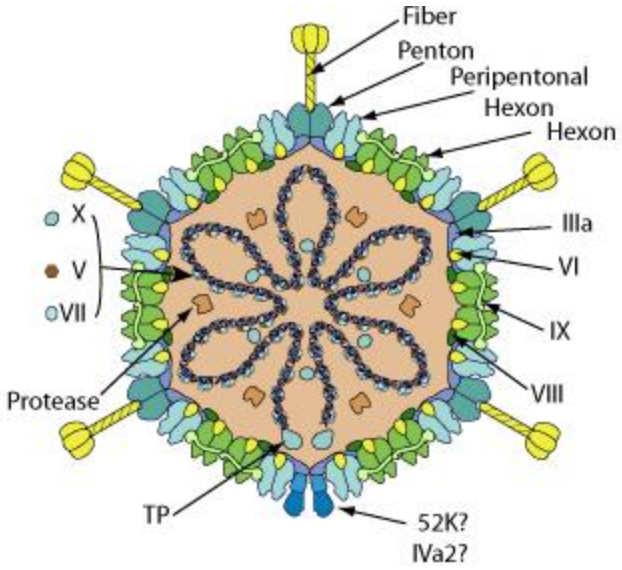


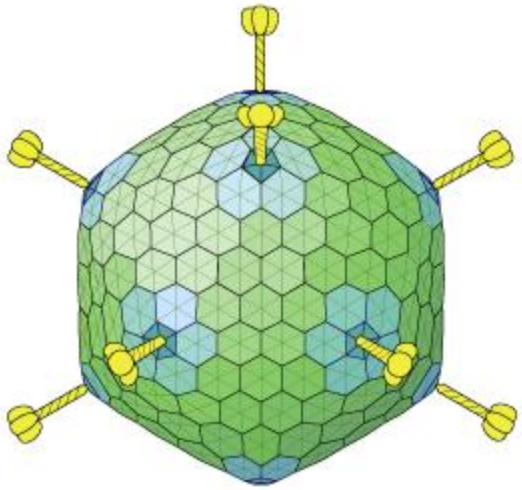
E.g. Erythema infectiosum or Fifth disease

a) Adenovirus

Properties:

- The virus infects vertebrate hosts.
- Viral capsid is icosahedral and non-enveloped. The capsid diameter is about 90 nm. The capsid shell consists of 720 hexon subunits arranged as 240 trimers and 12 vertex penton capsomers each with a fiber protruding from the surface.





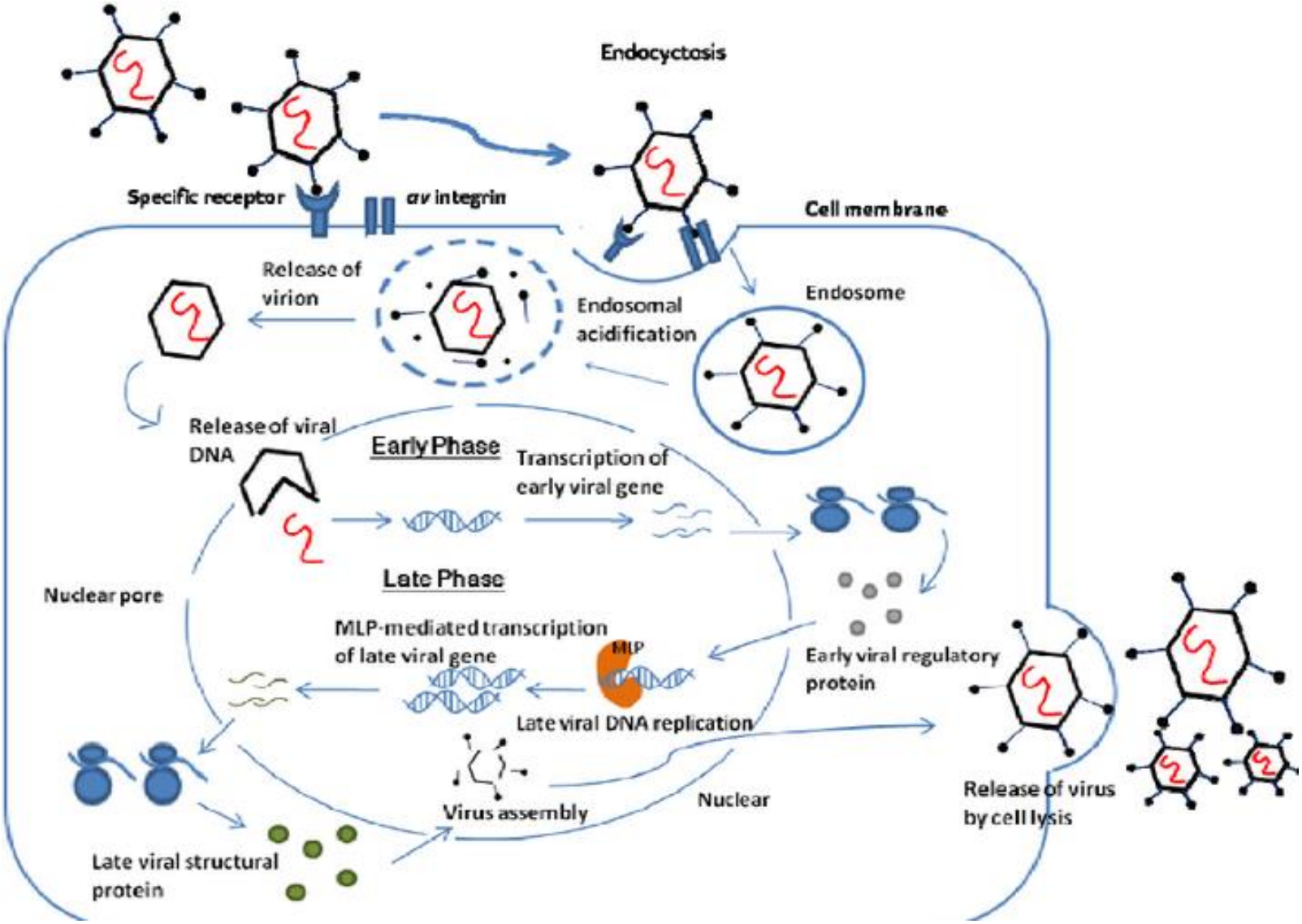
- Genome: Non-segmented, linear double-stranded DNA of 35-36kb.
- The genome has terminally redundant nucleotide sequences which have inverted terminal repetitions (ITR).
- The terminal protein (TP) is covalently attached to each end of the genome.

Replication

- Occurs in the nucleus and it proceeds as follows:

1. Attachment of the viral fiber glycoproteins to host receptors mediates endocytosis of the virus into the host cell and fiber shedding.

2. **Disruption of the endosome** by lytic protein VI releases the viral capsid in the cytosol.
3. Viral penetration into **host nucleus**.
4. **Transcription of early genes by host RNA pol II**, replication of the DNA genome by DNA strand displacement in the nucleus.
5. **Transcription of late genes by host RNA pol II**, mostly encoding for structural proteins.
6. Assembly of new virions in the nucleus.
7. Virions are **released by lysis** of the cell.


Endocytosis
Specific receptor
αv integrin
Cell membrane
Release of virion
Endosomal acidification
Endosome
Release of viral DNA
Early Phase
Transcription of early viral gene
Late Phase
Nuclear pore
MLP-mediated transcription of late viral gene
MLP
Early viral regulatory protein
Late viral DNA replication
Virus assembly
Nuclear
Release of virus by cell lysis
Late viral structural protein

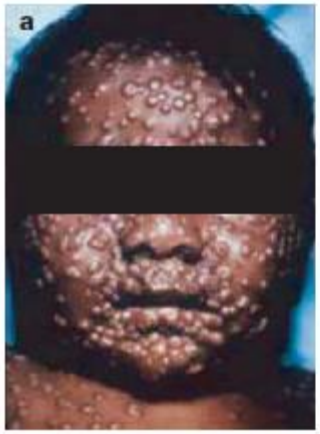
Epidemiology

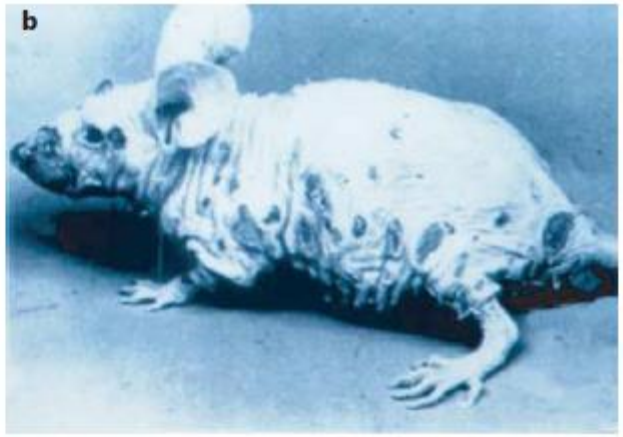
- Geography:
- Worldwide occurrence
- Associated diseases:
- Human adenoviruses: mainly respiratory diseases, croup, and bronchitis, pneumonia. Also keratoconjunctivitis, cystitis and gastroenteritis.
- Fowl adenovirus C: hydropericardium syndrome (HPS) also known as Angara disease.
- Transmission:
- Respiratory droplets, fecal-oral route.
- Vaccines:
- Human adenovirus E serotype 4 and Human adenovirus B serotype 7 for the military.
  Vaccine for hydropericardium syndrome, made from inactivated fowl adenovirus C serotype 4.

b) Poxvirus

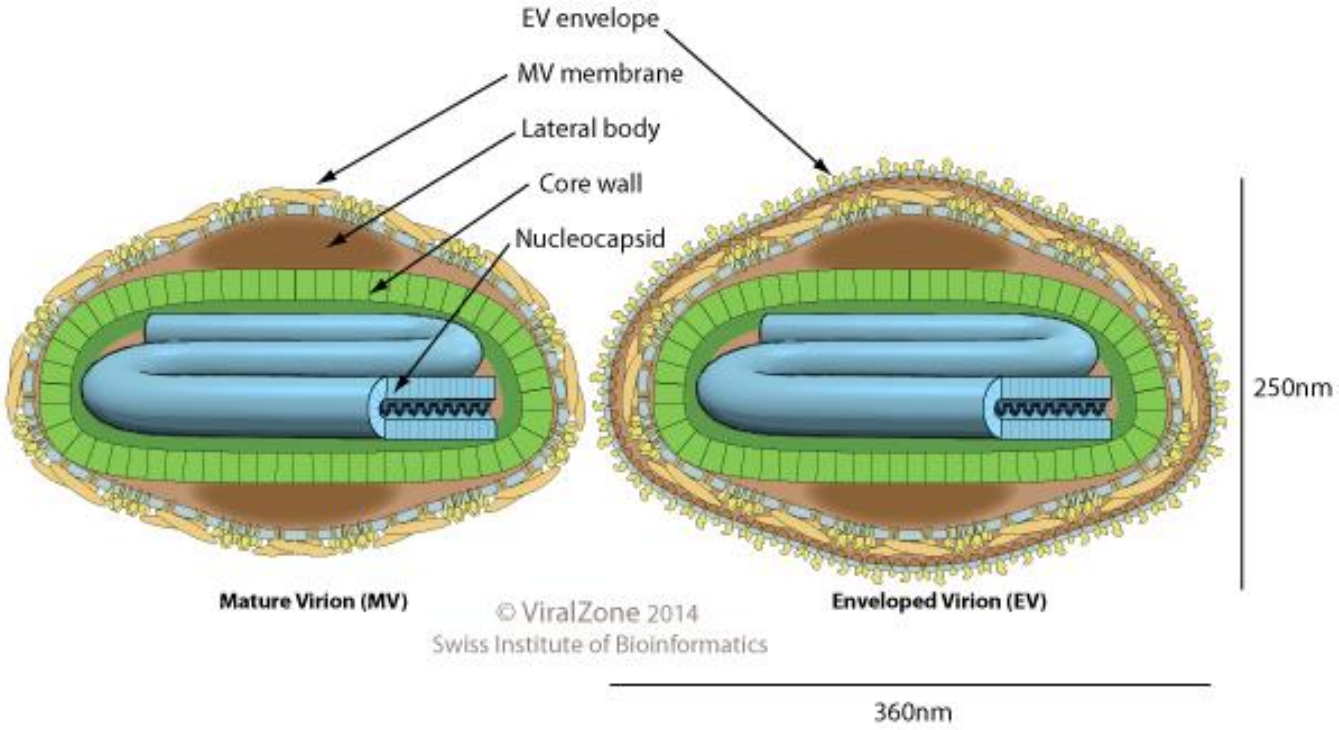
Properties:

- **Pox** from English pock, ‘pustule’, referring to skin lesions.
- There are species that infect Human, vertebrates, arthropods.





•Virion is enveloped, brick-shaped or ovoid, 220-450 nm long and 140-260 nm wide. The surface membrane displays surface tubules or surface filaments. Two distinct infectious virus particles exists: the intracellular mature virus (IMV) and the extracellular enveloped virus (EEV).



- Genome is linear, dsDNA of 130-375kb. The linear genome is flanked by ITR sequences which are covalently-closed at their extremities.

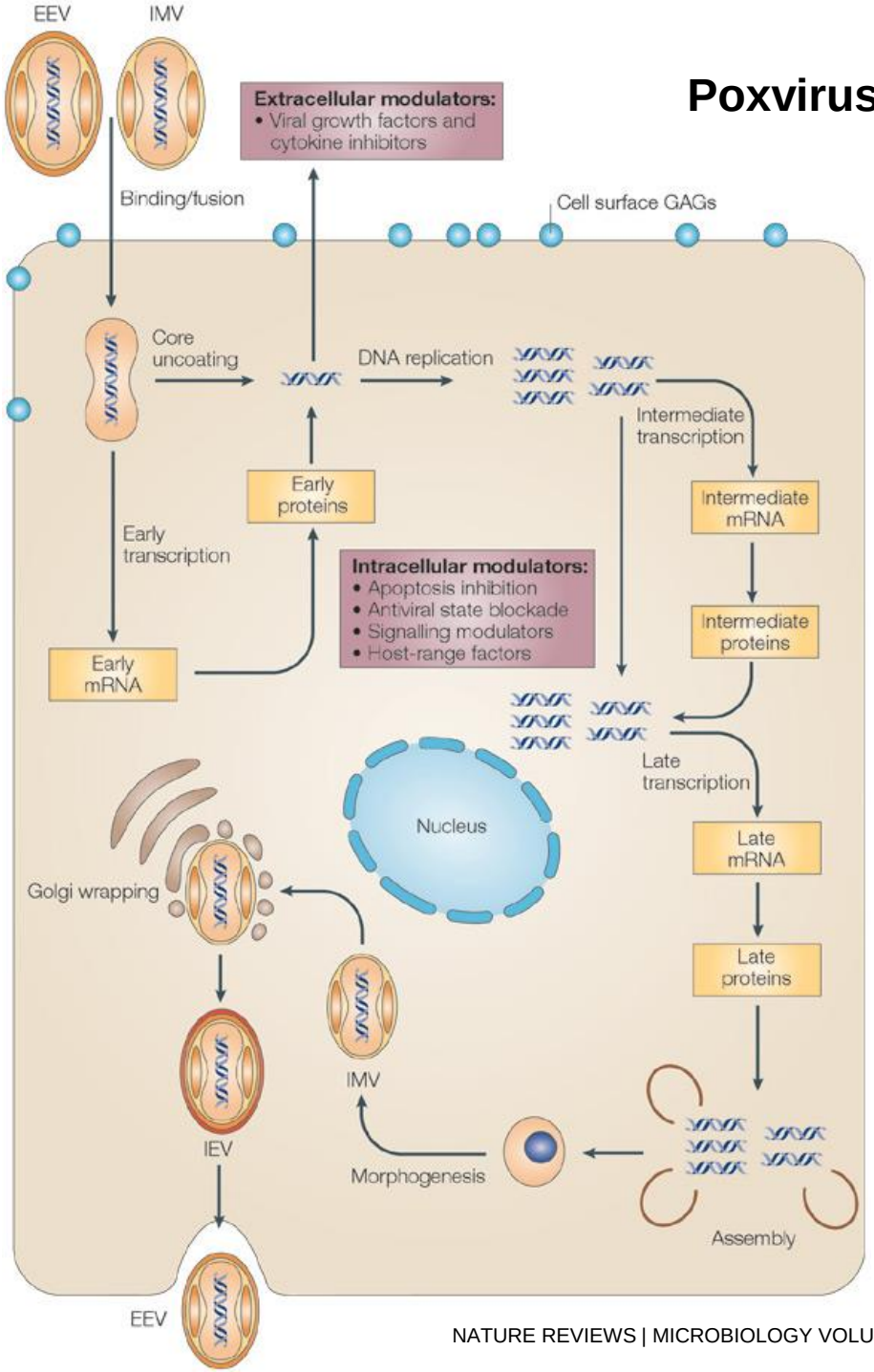
Replication

- Occurs in the cytoplasm.

1. Attachment of the viral proteins to host glycosaminoglycans (GAGs) mediates endocytosis of the virus into the host cell.
2. Fusion with the plasma membrane to release the core into the host cytoplasm.
3. **Early phase**: early genes are transcribed in the cytoplasm by viral RNA polymerase. Early expression begins at 30 minutes post-infection.
4. Core is completely uncoated as early expression ends, viral genome is now free in the cytoplasm.

5. **Intermediate phase**: Intermediate genes are expressed, triggering genomic DNA replication at approximately 100 minutes post-infection.
6. **Late phase**: Late genes are expressed from 140 min to 48 hours post-infection, producing all structural proteins.
7. Assembly of progeny virions starts in cytoplasmic viral factories, producing an spherical immature particle. This virus particle matures into brick-shaped intracellular mature virion (IMV).
8. IMV virion can be released upon cell lysis, or can acquire a second double membrane from *trans*-Golgi and bud as external enveloped virion (EEV).

Poxvirus replication cycle



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Epidemiology

❑ Geography:

- Worldwide occurrence. Natural Smallpox has been eradicated.

❑ Associated disease:

- Smallpox.

❑ Transmission:

- Direct contact or fomites. Smallpox: respiratory droplets, direct contact or fomites.

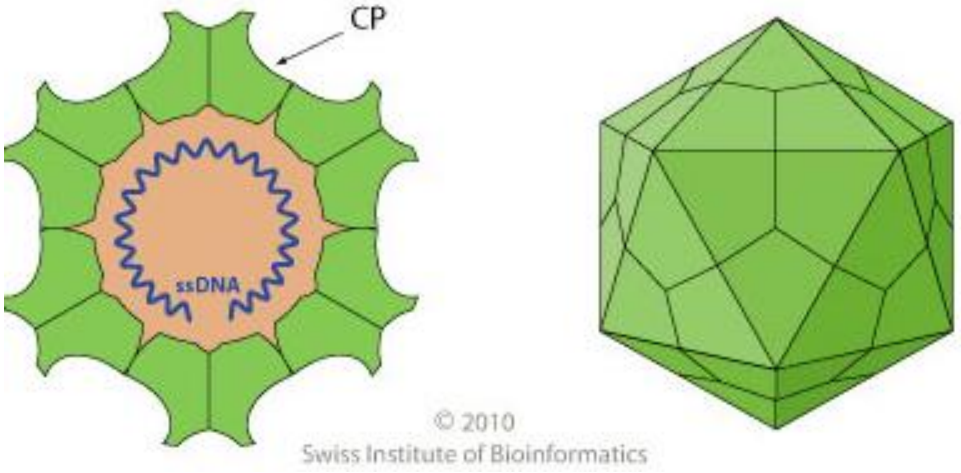
❑ Vaccine:

- Live vaccine against Smallpox (Vaccinia virus).

c) Parvovirus

Properties:

- **Parvo**: from Latin, 'small'. Belongs to the family *Parvoviridae.*
- Have got species infects vertebrates as well as insects.
- Human parvovirus B19: has tropism for mitotically active erythroid precursor cells in bone marrow.
- It is non-enveloped, round, with icosahedral capsid symmetry of about 18-26 nm in diameter. The capsid consists of 60 copies of CP protein.



- Genome is linear, ssDNA and it is about 4 to 6 kb in size.

Replication:

1. Attachment to host receptors initiates clathrin-mediated endocytosis of the virion into the host cell.
2. The virion penetrates into the cytoplasm via permeabilization of host endosomal membrane.
3. Microtubular transport of the virion toward the nucleus.
4. The viral ssDNA genome penetrates into the nucleus.
5. The ssDNA is converted into dsDNA by cellular proteins.
6. dsDNA transcription gives rise to viral mRNAs when host cell enters S phase and translated to produce viral proteins.
7. Replication occurs through rolling-hairpin mechanism, with NS1 endonuclease binding covalently to the 5' genomic end.
8. Individual ssDNA genomes are excised from replication concatemers by a process called junction resolution.

9. These newly synthesized ssDNA can either
   a) be converted to dsDNA and serve as a template for transcription/replication
   b) be encapsidated to form new virions that are released by cell lysis.

Epidemiology

- Geography:
- Worldwide occurrence.
- Associated disease:
- Human parvovirus B19: erythema infectiosum (fifth disease) in children.
- Polyarthropathy syndromes in adults.
- Canine parvovirus: gastrointestinal tract damage in puppies, about 80% fatal .

- Transmission:
- Respiratory, oral droplets of fecal oral-route
- Vaccine:
- Vaccination is widely practiced, with both inactivated and live-attenuated virus vaccines available..

References

- https://viralzone.expasy.org/
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