

HISTORY OF VIROLOGY STRUCTURE AND CLASSIFICATION OF VIRUSES

- **Neolithic period** around **12,000 years** ago- change in human behaviour from hunter-gatherers to agricultural communities.
- Lead to rapid spread of many plant viruses such as potyviruses that infect potatoes, and other fruits and vegetables, began about **6,600 years** ago.
- First written record of a virus infection, provided by the ancient **Egyptian civilization** indicated by the photos on the walls of the temples.

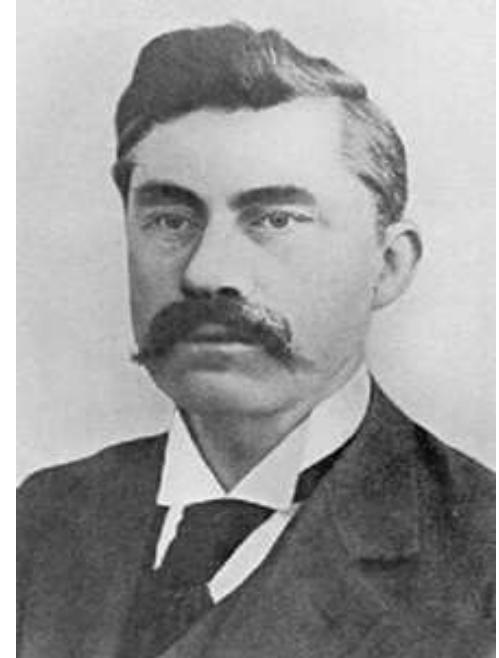
- Ramesses V's preserved mummy shows that he died of **smallpox** at about the age of 35 in 1143 BC.
- Smallpox is endemic in China by **1000BC**. In response, the practice of **variolation** is developed.
- Variolation involves inhalation of the dried crusts from smallpox lesions like snuff, or in later modifications, inoculation of the pus from a lesion into a scratch on the forearm

- 1796: **Edward Jenner** used cowpox to vaccinate against smallpox.
- 1886: **Louis Pasteur** experimented with rabies vaccination, using the term "**virus**" (Latin, poison) to describe the agent.
- 1886: **John Buist** (a Scottish pathologist) stained lymph from skin lesions of a smallpox patient and saw "elementary bodies" which he thought were the spores of micrococci.
- These were in fact smallpox virus particles - just large enough to see with the light microscope.

1892: **Dmitri Ivanovski** described the **first "filterable"** infectious agent - **tobacco mosaic virus (TMV)** - smaller than any known bacteria. He showed that extracts from diseased tobacco plants can transmit disease to other plants after passage through ceramic filters fine enough to retain the smallest known bacteria.

This is generally recognized as the beginning of Virology.

- **1898:** **Martinus Beijerinick** confirmed and extended Ivanovski's work with TMV and formed the first clear concept of the virus "*contagium vivum fluidum*" - soluble living germ.
- He was the person who **developed the concept of the virus as a distinct entity**. The question of whether the agent was a "living fluid" or a particle was however still open.



Martinus Beijerinick

- 1898: **Freidrich Loeffler** and **Paul Frosch** identified the first animal virus which causes **foot and mouth disease (FMD)**
- **Loeffler and Frosch** were the first to prove that viruses could infect animals as well as plants.
- 1900: **Walter Reed** demonstrated that yellow fever was caused by a virus, spread by mosquitoes.

- **1908:Karl Landsteiner** and **Erwin Popper** proved that poliomyelitis was caused by a virus. Landsteiner and Popper were the first to prove that viruses could infect humans as well as animals.
- **1911: Francis Peyton Rous** (1879-1970) demonstrated that a virus (Rous sarcoma virus) can cause cancer in chickens (Nobel Prize, 1966) . He was the **first person to show that a virus could cause cancer**. It was later called Rous sarcoma virus 1 and understood to be a retrovirus. Several other cancer-causing retroviruses have since been described.

- **1915: Frederick Twort** The existence of viruses that infect bacteria (**bacteriophages**) was first discovered.
- **1917: Felix d'Herelle** independently discovered viruses of bacteria and coined the term **bacteriophage**.
- **1935: Wendell Stanley** crystallized (TMV) and showed that it remains infectious (Nobel Prize, 1946).
- Stanley's work was the first step towards describing the molecular structure of any virus and helped to further illuminate the nature of viruses.

- 1937: **Max Theiler** was the first to propagate yellow fever virus in chick embryos and successfully produced live attenuated vaccine - the 17D strain. (Nobel Prize, 1951).
- 1939(1937): **Emory Ellis** and **Max Delbrück** established the concept of the "**one step virus growth cycle**" essential to the understanding of virus replication (Nobel Prize, 1969). - that virus particles do not "grow" but are instead assembled from preformed components.

- 1940: **Helmut Ruska** used an electron microscope to take the first pictures of virus particles. Along with other physical studies of viruses, direct visualization of **virions** was an important advance in understanding virus structure.
- 1941: **George Hirst** demonstrated that influenza virus agglutinates red blood cells. **This was the first rapid, quantitative method of measuring eukaryotic viruses.**
- 1945: **Salvador Luria** (1912-1991) and **Alfred Hershey**(1908-1997) demonstrated that **bacteriophages** mutate (Nobel Prize, 1969).

- **1949: John Enders, Thomas Weller and Frederick Robbins** were able to grow **poliovirus in vitro** using human (embryonal cells) tissue culture (Nobel Prize, 1954).
- This development led to the isolation of many new viruses in tissue culture.
- This work aided **Jonas Salk** in deriving a polio vaccine from killed polio viruses; this vaccine was shown to be effective in 1955.

- 1950: André Lwoff (1902-1994) and colleagues discovered lysogenic bacteriophage in *Bacillus megaterium* irradiated with ultra-violet light and coined the term **prophage** (Nobel Prize, 1965).
- 1952: Renato Dulbecco showed that animal viruses can form plaques in a similar way to **bacteriophages** (Nobel Prize, 1975). Dulbecco's work allowed rapid quantitation of animal viruses using plaque assays.

- **1957:** The first interferon was discovered by **Alick Isaacs and Jean Lindenmann**
- **1963:** **Baruch Blumberg** discovered hepatitis B virus (HBV) (Nobel Prize, 1976). Blumberg went on to develop the first vaccine against the HBV, considered by some to be the first vaccine against cancer because of the strong association of hepatitis B with liver cancer.

- **1970:Howard Temin (1934-1994) and David Baltimore** independently discovered **reverse transcriptase** in retroviruses. (Nobel Prize, 1975).
- The discovery of reverse transcription established a pathway for genetic information flow from RNA to DNA, refuting the so-call "central dogma" of molecular biology.

- **1972:** Paul Berg created the first recombinant DNA molecules, circular SV40 DNA genomes containing I phage genes and the galactose operon of *E.coli* (Nobel prize, 1980). **This was the beginning of recombinant DNA technology.**
- **1979:** Smallpox was officially declared to be eradicated by the World Health Organization (WHO). The last naturally occurring case of smallpox was seen in Somalia in 1977.
- This was the first microbial disease ever to be completely eliminated.

- **1982:** **Stanley Prusiner** discovered prions & demonstrates that infectious proteins cause scrapie, a fatal neurodegenerative disease of sheep (Nobel Prize, 1997).
- The first cases of AIDS were reported in 1981.
- **1983:** **Luc Montaigner and Robert Gallo** announced the discovery of human immunodeficiency virus (HIV), the causative agent of AIDS. In only 2-3 years since the start of the AIDS epidemic the agent responsible was identified.

- **1989:** Hepatitis C virus (HCV), the source of most cases of non-A, non-B hepatitis, was identified
- **2003-** The newly discovered **Mimivirus** became the largest known virus, with a diameter of 400 nm and a genome of 1.2 Mbp.
- Severe acute respiratory syndrome (SARS) broke out in China and subsequently spread around the world.
- **2010:** The United Nations Food and Agriculture Organization (FAO) declared **Rinderpest virus to be globally eradicated.**

COMMON TERMS

- The term virus was coined by Pasteur, and is from the Latin word for poison.
- **Virus particle or virion-** An infectious agent composed of nucleic acid (RNA or DNA), a protein shell (capsid) and, in some cases, a lipid envelope. Virions have full capacity for replication when a susceptible target cell is encountered.
- **A virion is an infectious virus particle - not all virus particles are infectious**

- **Viroids** are infectious agents consisting of a low molecular weight RNA that contains no protein capsid responsible for many plant diseases.
- **Prions-** small infectious particle composed of abnormally folded protein that causes progressive neurodegenerative conditions. The term prion was coined to mean proteinaceous infectious particle [Prusiner 1982]
- **Satellite or Defective Viruses.** Viruses which require a second virus (helper virus) for replication. Hepatitis delta virus is the major human pathogen example. It requires the presence of hepatitis B virus to complete its replication cycle.

Properties of Unicellular Microorganisms and Viruses

	Growth on artificial media	Division by binary fission	Whether they have both DNA and RNA	Whether they have ribosomes	Whether they have muramic acid	Their sensitivity to antibiotics
Bacteria	Yes	Yes	Yes	Yes	Yes	Yes
Mycoplasma	Yes	Yes	Yes	Yes	No	Yes
Rickettsia	No	Yes	Yes	Yes	Yes	Yes
Chlamydia	No	Yes	Yes	Yes	No	Yes
Viruses	No	No	No	No *	No	No

* The arenavirus family (an RNA virus family) appears to package ribosomes 'accidentally'. The packaged ribosomes appear to play no role in viral protein synthesis.

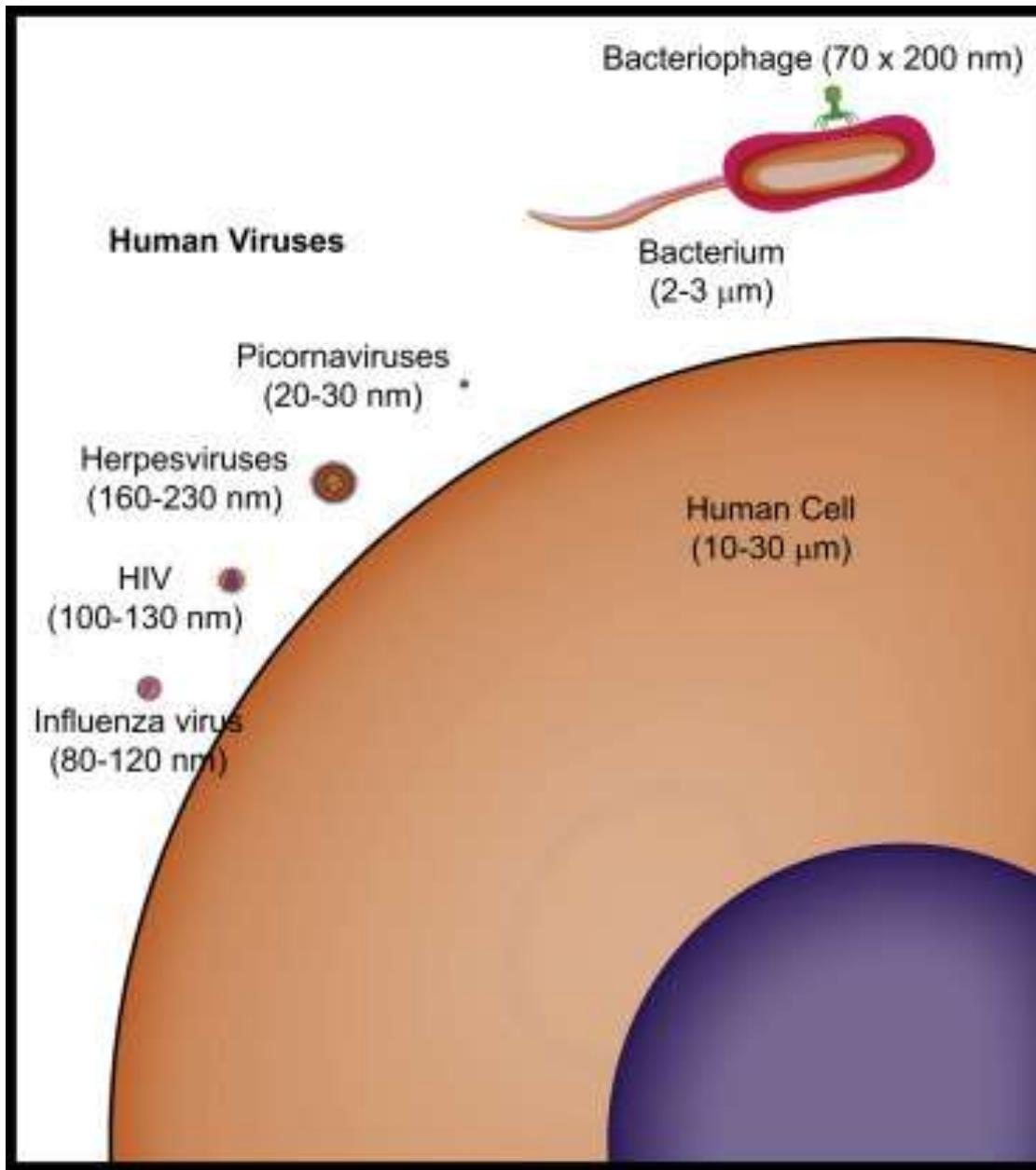
PROPERTIES OF VIRUSES

- Viruses are not cells, do not have nuclei or mitochondria or ribosomes or other cellular components.
- They lack metabolic activity (i.e., metabolically inert) and essential constituents required for independent growth and multiplication.
- **Viruses are obligate intracellular parasites.**

- Viruses do not grow.
- Because viruses are non-motile, they are entirely dependent on external physical factors for chance movement and spread to infect other susceptible cells.
- Some viruses can induce latent infections.
- DNA genome of some viruses could get integrated in the host genome-prophage
- Some viruses could lead to transformation of the host cells.

Virus size

- Small in size
- Smallest of viruses are about 20 nm in diameter
- Average human cells are 10–30 µm (microns) in diameter- 100 to 1000 times larger than the viruses that are infecting them.
- Some viruses are >100 nm. Eg Poxviruses can approach 400 nm in length, and filoviruses, are only 80 nm in diameter but extend into long threads that can reach lengths of over 1000 nm.
- Recently discovered –megavirus- 400 nm in diameter, and pandoraviruses have an elliptical or ovoid structure approaching 1000 nm in length.



VIRUS STRUCTURE

- Viruses have:
- a nucleic acid genome (RNA or DNA)
- a protective protein coat (called the **capsid**)
- The nucleic acid genome plus the protective protein coat =
nucleocapsid

- Viral capsids - icosahedral or helical or complex symmetry
- Viruses may have an envelope made of lipid derived from the host cell membranes.
- Virus without envelope-**naked** virus or Non-enveloped virus
- In some enveloped viruses the glycoprotein projects out in the form of spike called **peplomers**

- **Enveloped viruses**
- The envelope is a lipid membrane that is derived from one of the cell's membranes, most often the plasma membrane,
- Endoplasmic reticulum, Golgi complex, or nuclear membrane.
- **Matrix proteins** function to connect the envelope to the capsid inside.

- Enveloped viruses do not necessarily have to kill their host cell in order to be released, since they can bud out of the cell hence some budding viruses can set up persistent infections.
- Enveloped viruses are readily infectious only if the envelope is intact (since the viral attachment proteins which recognize the host cell receptors are in the viral envelope). This means that agents that damage the envelope, such as alcohols and detergents, reduce infectivity.

- **Virus attachment protein** embedded in outer-most layer of virus (in the capsid, in the case of a naked virus, or the envelope, in the case of an enveloped virus).
- The virus attachment protein is the viral protein that facilitates the docking of the virus to the plasma membrane of the host cell, the first step in gaining entry into a cell.

- The **Capsid (coat) protein** is the basic unit of structure made up of protein subunits called **capsomeres**. Subunits called **protomers** aggregate to form capsomeres.
- The number of capsomeres in a capsid varies from virus to virus.

Functions

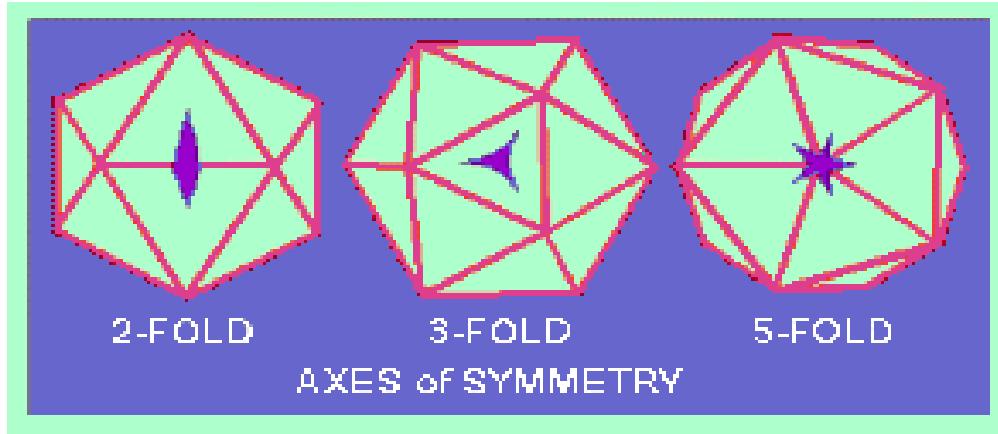
- Protect viral nucleic acid
- Interact specifically with the viral nucleic acid for packaging
- Interact with host receptors for entry to cell
- Allow for release of nucleic acid upon entry into new cell

VIRION NUCLEOCAPSID STRUCTURES

- 1. Icosahedral symmetry**
- 2. Helical symmetry**
- 3. Complex**

- Symmetry refers to the way in which capsomere units are arranged in viral capsid.

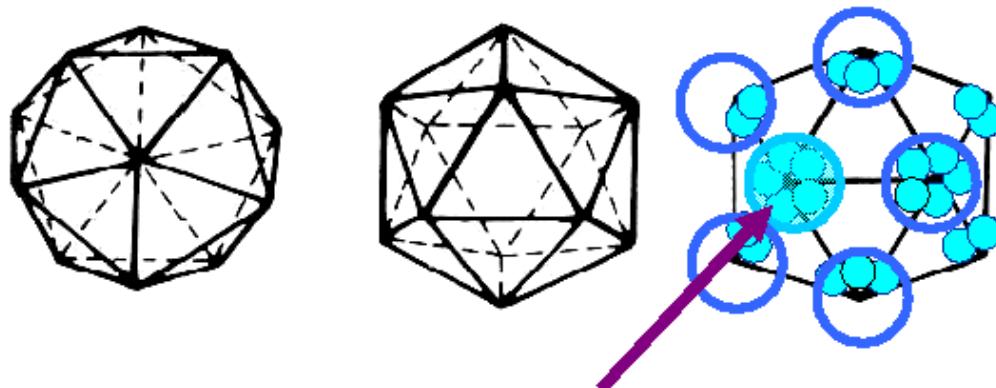
An **ICOSAHEDRON** is composed of 20 faces, each an equilateral triangle, 12 vertices and 30 edges, and because of the axes of rotational symmetry is said to have **5:3:2 symmetry. Mostly appear spherical in shape.**



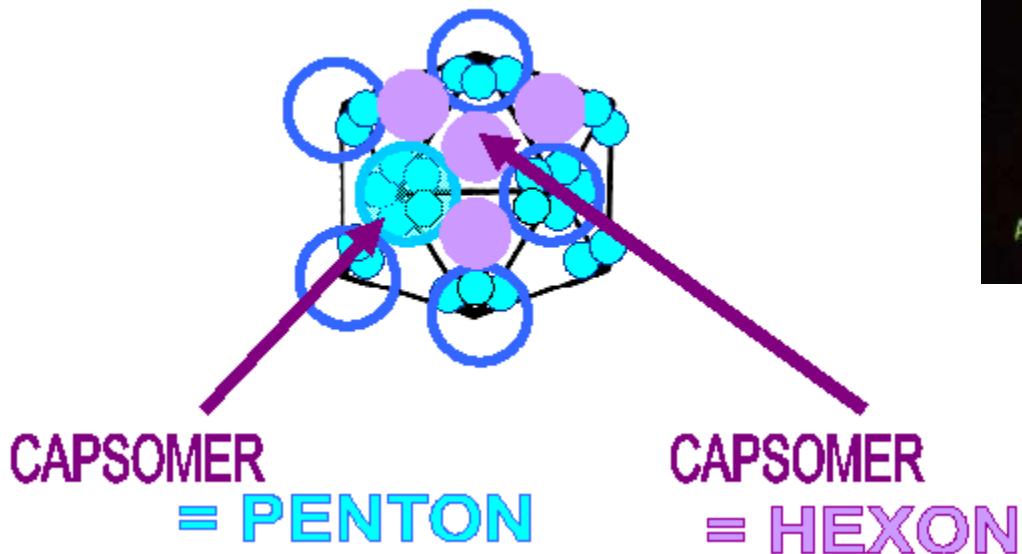
Axes of Symmetry : Six 5-fold axes of symmetry passing through the vertices, ten 3-fold axes extending through each face and fifteen 2-fold axes passing through the edges of an icosahedron.

- Capsomers at the 12 corners have a 5-fold symmetry and interact with 5 neighboring capsomers, and are thus known as **pentons or pentamer**.
- Larger viruses contain more capsomers; extra capsomers are arranged in a regular array on the **faces** of the icosahedrons. They have six neighbors and are called **hexons or hexamers**
- The size of such an icosahedron depends on the size and number of capsomers, there will always be 12 pentons, but the number of hexons may increase.

ICOSAHEDRAL SYMMETRY

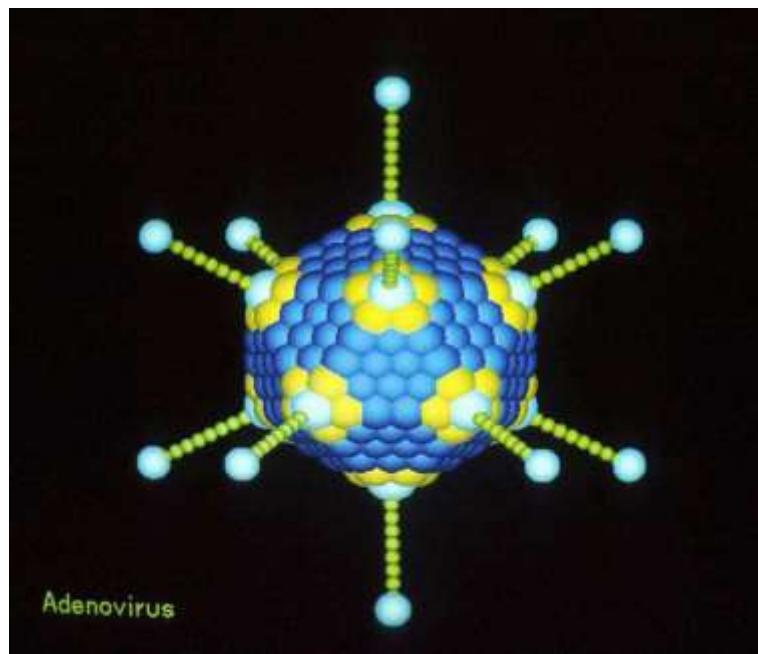


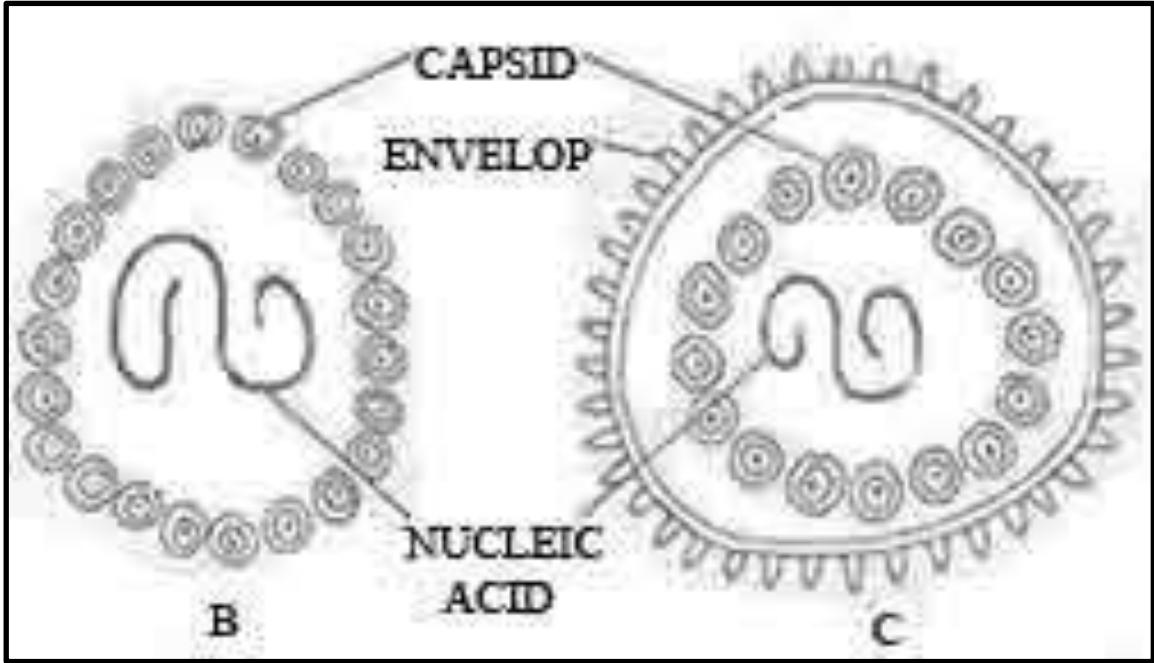
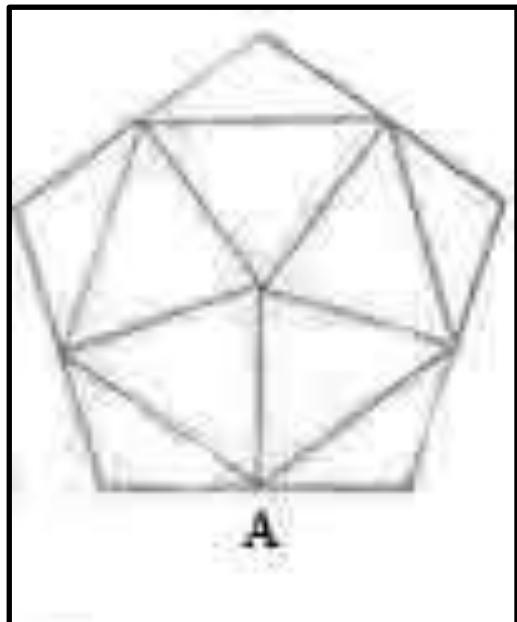
CAPSOMER
= PENTON (pentamer)



CAPSOMER
= PENTON

CAPSOMER
= HEXON



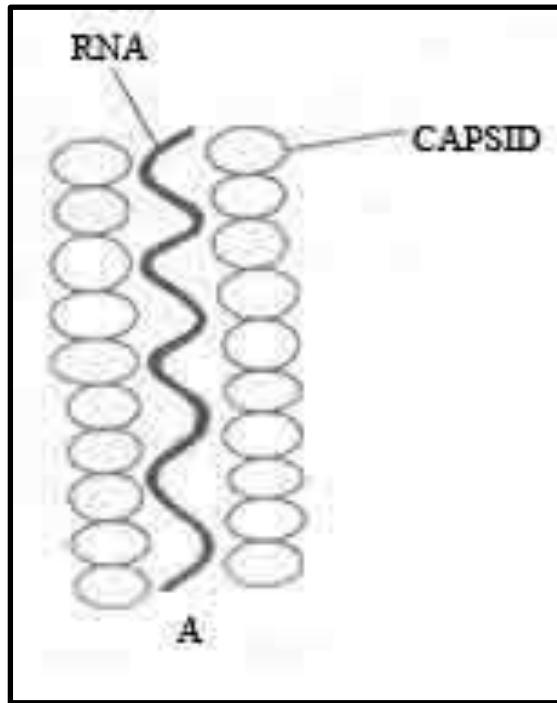


Icosahedral

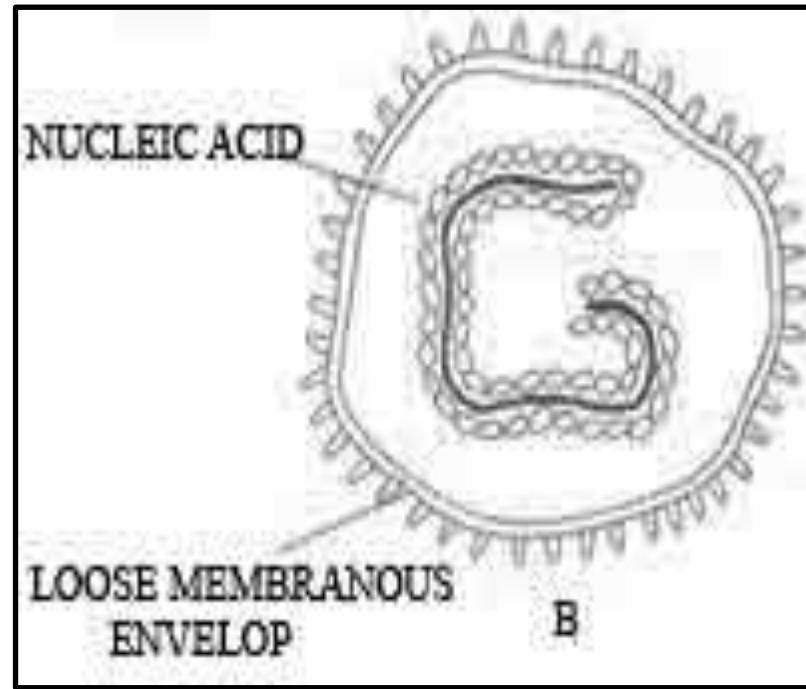
- B. Naked Icosahederal**
C. Enveloped Icosahederal

Helical symmetry

- The helix is a spiral shape that curves cylindrically around an axis.
- The viral nucleic acid coils into a helical shape and the capsid proteins wind around the inside or outside of the nucleic acid, forming a long tube or rod-like structure.
- One type of capsid protein (only 1 gene required hence small size genomes) present so simple in structure.
- The capsid length will be the size of the coiled nucleic acid (helical structure can continue indefinitely)
- **First non-enveloped helical symmetry plant virus - TMV**
- In enveloped, helically symmetrical viruses (e.g. influenza virus, rabies virus), the capsid is more flexible (and longer)
- **All helical animal viruses are enveloped and all are RNA viruses.**



**Naked Virus
(TMV)**

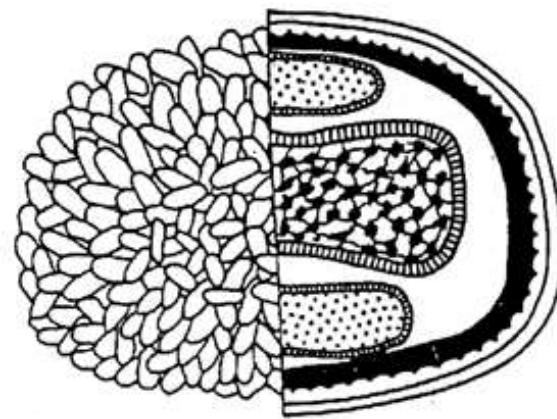


**Enveloped helical virus
(Influenza virus)**

Complex symmetry

- Symmetry that does not strictly conform to a simple helical or icosahedral shape.
- Example: **Poxviruses and many bacteriophages**
- They may posses tails and icosahedral head (e.g., many bacteriophages) or
- have complex, multilayered walls surrounding the nucleic acid (e.g., poxviruses such as vaccinia).

COMPLEX SYMMETRY



POXVIRUS FAMILY

FIVE BASIC STRUCTURAL FORMS OF VIRUSES IN NATURE

1. **Naked icosahedral** e.g. poliovirus, adenovirus, hepatitis A virus
2. **Naked helical** e.g. tobacco mosaic virus. So far no human viruses with this structure are known
3. **Enveloped icosahedral** e.g. herpes virus, yellow fever virus, rubella virus
4. **Enveloped helical** e.g. rabies virus, influenza virus, parainfluenza virus, mumps virus, measles virus
5. **Complex** e.g. poxvirus

CLASSIFICATION OF VIRUSES

- The process of naming viruses and placing them into a taxonomic system
- 1966 the International Committee for the Nomenclature of Viruses formed.
- Changed to the **International Committee for the Taxonomy of Viruses (ICTV)** in 1973
- Viruses are only classified using **order, family, genus, and species**

- The general taxonomic structure is as follows:

Order (-virales)

Family (-viridae)

Subfamily (-virinae)

Genus ("virus")

Species ("virus")

Primary characteristics used in classification

VIRAL CLASSIFICATION

Nucleic acid

RNA or DNA

single-stranded or double-stranded

non-segmented or segmented

linear or circular

if genome is single stranded RNA, can it function as mRNA?

whether genome is diploid (such as in retroviruses)

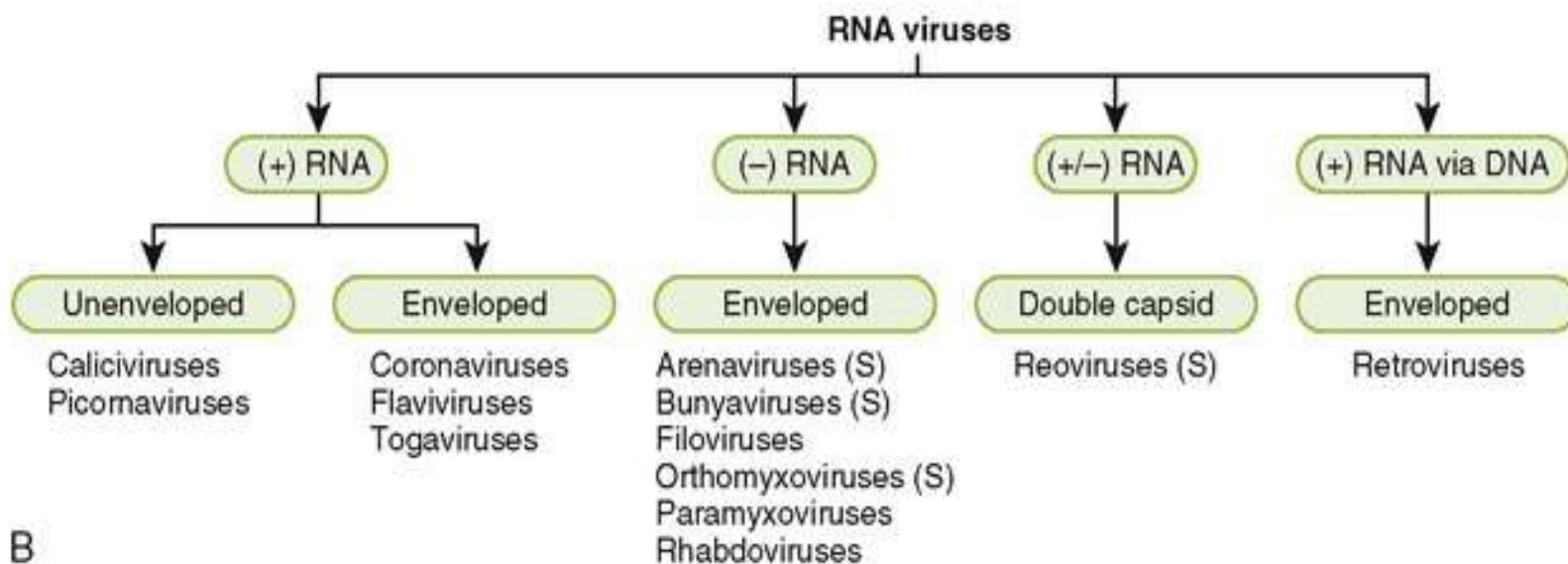
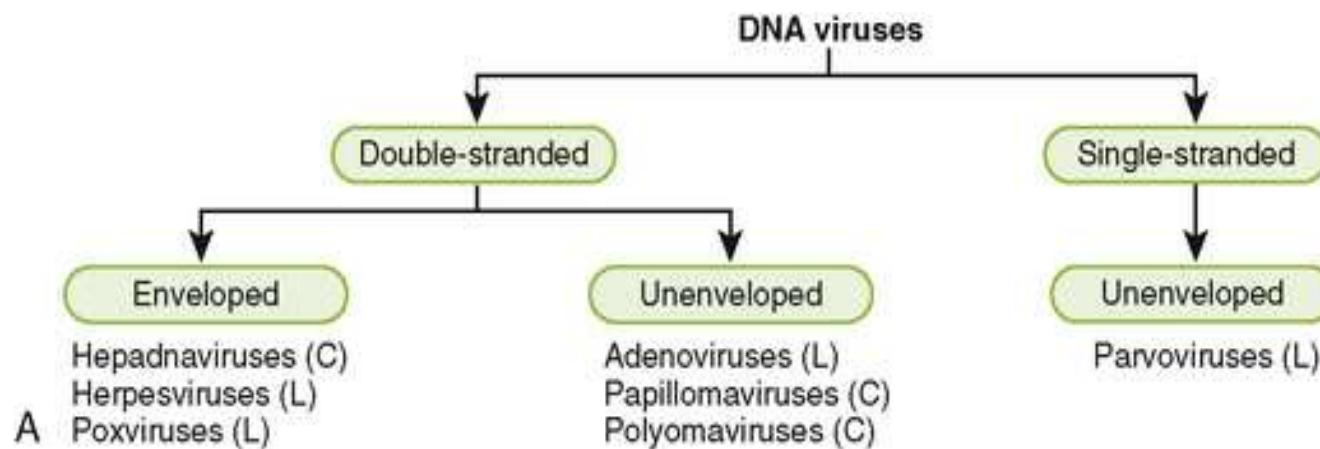
symmetry (icosahedral, helical, complex)

Virion structure

enveloped or non enveloped

number of capsomeres

Classification of virus on the basis of nucleic acid



BALTIMORE CLASSIFICATION

- Nobel Prize-winning biologist **David Baltimore** in the early 1970s.
- The central theme - all viruses must generate positive strand mRNAs from their genomes, in order to produce proteins and replicate themselves.
- The precise mechanisms whereby this is achieved differ for each virus family.
- Either the genes are stored in the $5' \rightarrow 3'$ direction (positive or + polarity), analogous to the direction in which genes are represented in mRNA in cells, or the genes are stored in the opposite, $3' \rightarrow 5'$ direction (negative or - polarity).

- In other words: + or - polarity of RNA:
 - "+" sense is able to serve as mRNA.
 - "-" sense is the complement of "+", must function as template to make a complementary strand of + RNA before any translation can occur.
- **Ambisense viruses** contain at least one **ambisense** RNA segment, i.e. an RNA that is in part of positive and in part of negative polarity.

- 7 classes or groups:
 1. • dsDNA viruses
 2. • ssDNA viruses
 3. • dsRNA viruses
 4. • (+)-sense ssRNA viruses
 5. • (-)-sense ssRNA viruses
 6. • RNA reverse transcribing viruses
 7. • DNA reverse transcribing viruses

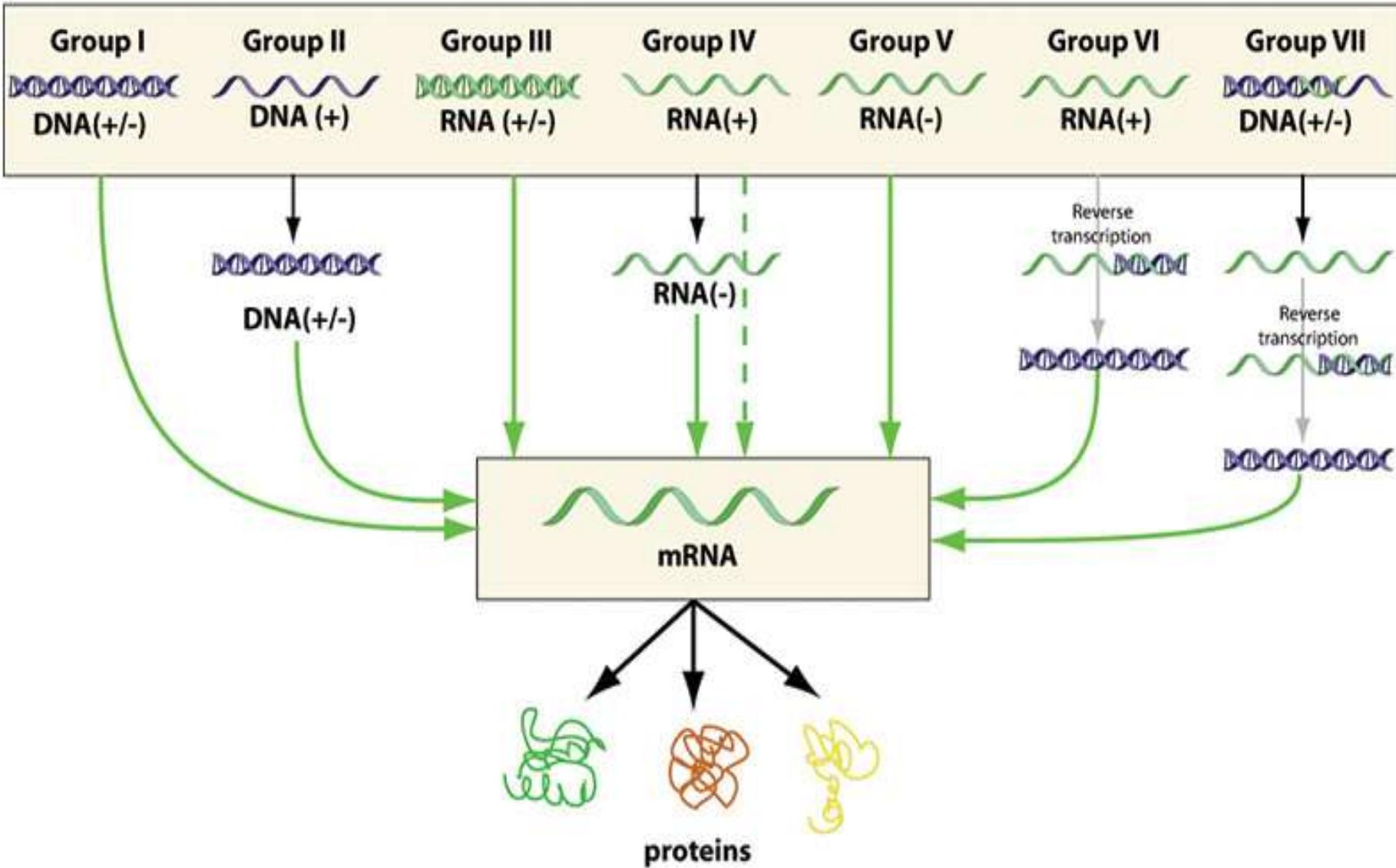
- **Group I (Adenoviruses; Herpesviruses; Poxviruses, etc)** viruses contain double-stranded DNA (**dsDNA**) as their genome. Their mRNA is produced by transcription in much the same way as with cellular DNA.
- **Group II (Parvoviruses)** viruses have single-stranded DNA (**ssDNA**) as their genome. They convert their single-stranded genomes into a dsDNA intermediate before transcription to mRNA can occur.

- **Group III (Reoviruses; Birnaviruses) dsRNA genome.** The strands separate, and one of them is used as a template for the generation of mRNA using the RNA-dependent RNA polymerase encoded by the virus.
- **Group IV (Picornaviruses; Togaviruses, etc) viruses** have **ssRNA** as their genome with a positive polarity. Intermediates of dsRNA, called replicative intermediates, are made in the process of copying the genomic RNA. Multiple, full-length RNA strands of negative polarity (complementary to the positive-stranded genomic RNA) are formed from these intermediates, which may then serve as templates for the production of RNA with positive polarity, including both full-length genomic RNA and shorter viral mRNAs.

- **Group V (Orthomyxoviruses, Rhabdoviruses, etc)** viruses contain **ssRNA genomes with a negative polarity**, meaning that their sequence is complementary to the mRNA.
- As with Group IV viruses, dsRNA intermediates are used to make copies of the genome and produce mRNA. In this case, the negative-stranded genome can be converted directly to mRNA. Additionally, full-length positive RNA strands are made to serve as templates for the production of the negative-stranded genome.

Group VI (Retroviruses) viruses have **diploid (two copies) ssRNA** genomes that must be converted, using the enzyme reverse transcriptase, to dsDNA; the dsDNA is then transported to the nucleus of the host cell and inserted into the host genome. Then, mRNA can be produced by transcription of the viral DNA that was integrated into the host genome.

Group VII (Hepadnaviruses) viruses have **partial dsDNA** genomes (longer negative-sense strand and a shorter positive-sense strand of variable length) and make ssRNA intermediates that act as mRNA, but are also converted back into dsDNA genomes by reverse transcriptase, necessary for genome replication.



VIRAL GENETICS

Purpose

- Generation of different strain, type, variant within a particular virus
- Generation of viruses with increased or decreased pathogenic potential
- Genetic and molecular basis of viral disease process
- Effective diagnosis, prevention and treatment of viral infection

Mutations

- A mutation is a change in the nucleic acid sequence of an organism.
- In viruses, errors in copying the viral nucleic acid during replication cycles results in mutation.
- **In general, mutation rates are considerably higher in most RNA viruses than in DNA viruses. This is due to higher error rates in RNA-dependent RNA polymerases than DNA-dependent DNA polymerases.**
- The organism possessing a mutation is referred to a **mutant**. This change is based on comparison with the wild type (reference) virus.
- The term “strain,” “type,” “variant,” and “mutant” are used to designate a virus that differs in certain heritable characteristics from the parental or wild-type virus.
- **Strain:** Different lines or isolates of the same virus (e.g., from different geographical locations or patients)
- **Type:** Different serotypes of the same virus (e.g., various antibody neutralization phenotypes)
- **Variant:** A virus whose phenotype differs from the original wild-type strain but the genetic basis for the difference is not known

Mutagenesis

1. **Spontaneous mutation** - Mutation in the absence of any known mutagen. These are **endogenous**, being the result of DNA or RNA polymerase errors or the result of incorporation of naturally occurring tautomeric forms of nucleotides. **DNA viruses are typically more genetically stable than RNA viruses**
2. **Induced mutation** – are **exogenous** and derived from mutagen-treated population of the wild-type virus.

Mutagens (chemicals or radiation) are used to increase the frequency of mutants in the population that is subsequently screened by using appropriate selective pressures.

- Chemical mutagens act either directly on the bases or indirectly by enhancing mispairing. Eg. nitrous acid or nitrosoguanidine. Nucleotide analogs such as 5-fluorouracil (for RNA viruses) or 5'-bromodeoxyuridine (for DNA viruses) are mutagenic only when virus replicates in their presence, because they are incorporated into the viral nucleic acid and produce mutations by miscoding during replication.
- Uv radiation can induce the formation of pyrimidine dimers, ionizing radiation can damage DNA directly by breaking chemical bonds or indirectly by forming free radicals that in turn damage DNA.

Types of Mutations

- Mutations results in change in **viral phenotype** (the kind of change they produce in the properties of the virus or the infection it causes) or
- **Viral genotype** (the kind of change they produce in the viral genome)
- **Phenotypic change** may be evidenced-
 - by a change in a physical characteristic of the virion,
 - by a change in a replicative characteristic seen during infection in cell culture, or
 - by a change in a pathogenic property in an infected animal

Phenotypic classification of mutants

- 1. Plaque morphology mutations:** Mutants have altered morphology due to one of a variety of metabolic differences. e.g. syncytial (*syn*) *mutants of viruses like herpesvirus, cause neighboring cells to fuse rather than undergo typical cytolytic changes.*
- 2. Host range mutations:** Mutation allows for a change in the host range of a particular virus from the original one associated with the wild type virus. This type of change is believed to have occurred with feline parvovirus, which extended its host range and became capable of infecting dogs.
- 3. Temperature-sensitive mutations:** This includes a series of mutations that replicate under a specific range of conditions, beyond this range wild type viruses are capable of replication but the conditional is not.

Examples of conditional lethal mutants include **temperature-sensitive mutants and cold-sensitive mutants.**

Temperature-sensitive (ts) mutants have conditional-lethal phenotype. The ts mutants are produced by alteration in the nucleotide sequence of a gene so that the resulting protein product of the gene is unable to assume or maintain its functional configuration at the non-permissive (39°C) temperature. The protein, however, is able to assume a functional configuration at permissive temperature (32 °C). e.g. herpesviruses, adenoviruses.

Genotypic Classification of Mutants

- **Deletion mutations:** These mutants have a deletion in their genomes. e.g. pseudorabies virus gene deleted vaccine.
- **Point mutations:** Alteration of a single nucleotide in the viral genome. This can be achieved by an *in vitro mutagenesis technique*.
- The phenotypic expression of a mutation may be reversed not only by a back-mutation in the affected nucleotide(s) but also by a **suppressor mutation** occurring elsewhere in the same gene, or even in a different gene, which leads to the reappearance of the wild-type phenotype.
- Mutations based on nucleotide substitutions revert most frequently, those based on small deletions or insertions less frequently, and those based on large deletions rarely if ever.

Defective Interfering (DIP) particles or Mutants

- Defective interfering particles (DIPs), caused by the critical absence of part the viral genome, are unable to replicate on their own.
- They need the presence of the parental wild-type virus; at the same time they interfere with and usually decrease the yield of the parental virus.
- All DI particles of RNA viruses that have been characterized are deletion mutants.
- Hepatitis delta virus or HDV is a defective virus, requires infection of the same cell with HBV, which contributes the HBsAg that HDV uses for virion assembly.

Antigenic shift and drift

- **Antigenic shift** refers to the change in antigen associated with a viral pathogen due to the acquisition of a novel entire gene or a change in an existing one.
- Typically antigenic shift is observed readily with those viruses that possess multipartite genomes, such orthomyxoviruses, arenaviruses and bunyaviruses.
- Coinfection of different strains in the same cell may result in packaging mixed genomes, containing some segments from one virus and other from the other.
- In influenza viruses, antigenic shift involves the sudden acquisition of the gene for a completely new hemagglutinin(HA) or neuraminidase (N), giving rise to a novel subtype that may spread rapidly around the world, unencumbered by any herd immunity.

Pig serves as a "mixing vessel"

- Influenza A viruses from birds grow very poorly in humans, and vice versa.
- Both avian and human influenza viruses can replicate in swine, and genetic reassortment between them occurs in host.
- An antigenic shift in nature occurs when the prevailing human strain of influenza A virus and an avian influenza virus concurrently infect a pig, which serves as a "mixing vessel." Every 10-20 years a reassortant virus from the pig, containing genes encoding replicative functions from a human virus and a hemagglutinin gene derived from an avian virus, emerges.
- H1N1 replaced by H2N1 or H3N2

Antigenic drift

- It is a result of accumulation of point mutations (single bases substitutions) and has been identified as the mechanism associated with the antigenic variation observed with influenza viruses and may be the mechanism associated with the variability observed with rhinoviruses.
- Produce strains each antigenically slightly different from its predecessor.
- In influenza viruses, mutations in the gene encoding the hemagglutinin sometimes alter its antigenic sites. When antiserum against the formerly prevalent strain no longer neutralizes the variant, a new strain has emerged.

S.N.	Antigenic Shift	Antigenic Drift
1	Major Antigenic Change	Minor Antigenic Change
2	Forming new sub-type (Subtype A + Subtype B → New Subtype)	Forming new strain of virus
3	One or Two Viruses are Involved	Only one virus is involved
4	Occurs once in a time	Occurs frequently
5	May jump from one species to another (animal-human)	May infect animals of the same species
6	Large change in nucleotides of RNA	Small mutation of RNA
7	Occurs as a result of genome reassortment between different subtypes.	Occurs as a result of the accumulation of point mutations in the gene.

S.N.	Antigenic Shift	Antigenic Drift
8.	An antigenic change which results in drastic or dramatic alternation in HA (hemagglutinin) or NA (neuraminidase) subtypes	An antigenic change can alter antigenic sites on the molecule such that a virion can escape recognition by the host's immune system
9	Large and sudden mutation	Random and Spontaneous Mutation
10	Difficult to treat (need new vaccine)	Easy to treat (antibody and drugs available)
11	Occurs only in Influenza Virus A	Occurs in Influenza Virus A, B and C
12	Give rise to pandemics, which occurs irregularly and unpredictably.	Usually responsible for epidemics in between pandemics

Genetic Interaction Between Viruses

- Viral infections with two or more different viruses are known to occur in nature as well as in culture. These are referred to as **mixed infections** and can result in new viral combinations, and thus new variants of the virus. Some of the interactions that can occur during mixed infections are:

1. Complementation- interactions between viral proteins in doubly infected cells that result in rescue or increased yields of one or both viruses.

This can occur between two strains of the same virus, two related viruses, or two unrelated viruses **while their genotypes remain unchanged.**

Complementation can occur during a mixed infection when one of the two viruses is deficient in a particular gene product. Without this protein, the virus is incapable of transmission and replication and is therefore a defective particle.

In a mixed infection, if the second virus involved does make the product (thus complementing of the defect), the defective particle is capable of completing the transmission and replication processes

2. Genetic recombination

- When two different viruses simultaneously infect the same cell, genetic recombination may occur between the nucleic acid molecules during or after their synthesis. As a result, the progeny are genetically distinct from the two "parental" viruses. Three mechanisms:
 1. Intramolecular recombination
 2. Copy-choice Recombination
 3. Genetic reassortment
 4. Genetic reactivation

Intramolecular recombination

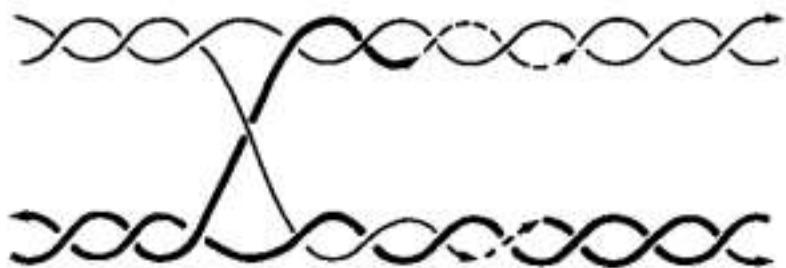
- Involves the exchange of nucleotide sequences between different but usually closely related viruses during replication
- It occurs with all double-stranded DNA viruses, presumably because of template switching by the polymerase. Intramolecular recombination also occurs among RNA viruses (e.g., picornaviruses, coronaviruses and togaviruses)
- In all DNA viruses and RNA viruses that replicate via DNA intermediates, recombination involves the breakage and reformation of covalent bonds within the nucleic acid.
- Recombination between two regions on a single dsDNA molecule can result in looping out of the intermediate region, yielding a shorter dsDNA molecule and a separate circular dsDNA molecule

Copy-choice Recombination

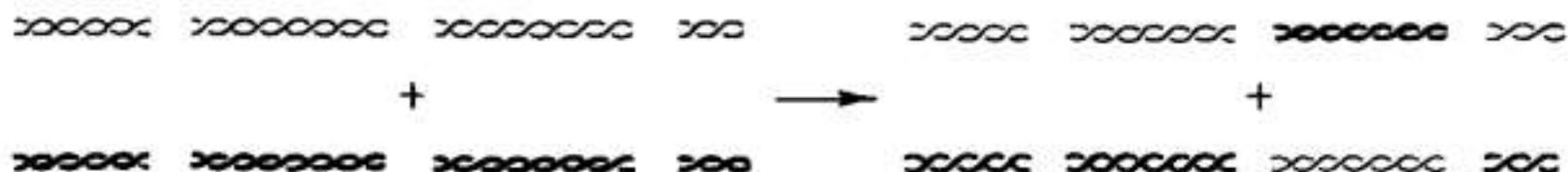
- A genetic recombination in which the new nucleic acid molecule comes about by replicating selected parts of each parental molecule and by alternating between the two (maternal and paternal).
- Polymerase switches template strand during RNA synthesis
- This mechanism is poorly understood and occurs in monopartite RNA viruses.

Genetic Reassortment

- This occurs in mixed infections with variant viruses having segmented genomes infecting a single cell.
- The progeny virions can contain some segments from one parent, some from the other.
- This is an efficient process observed with orthomyxoviruses, reoviruses, arenaviruses, and bunyaviruses.
- Reassortment has been implicated in the appearance of new, highly virulent influenza virus strains throughout the 20th century.



A. Intramolecular recombination



B. Genetic reassortment

Genetic Reactivation

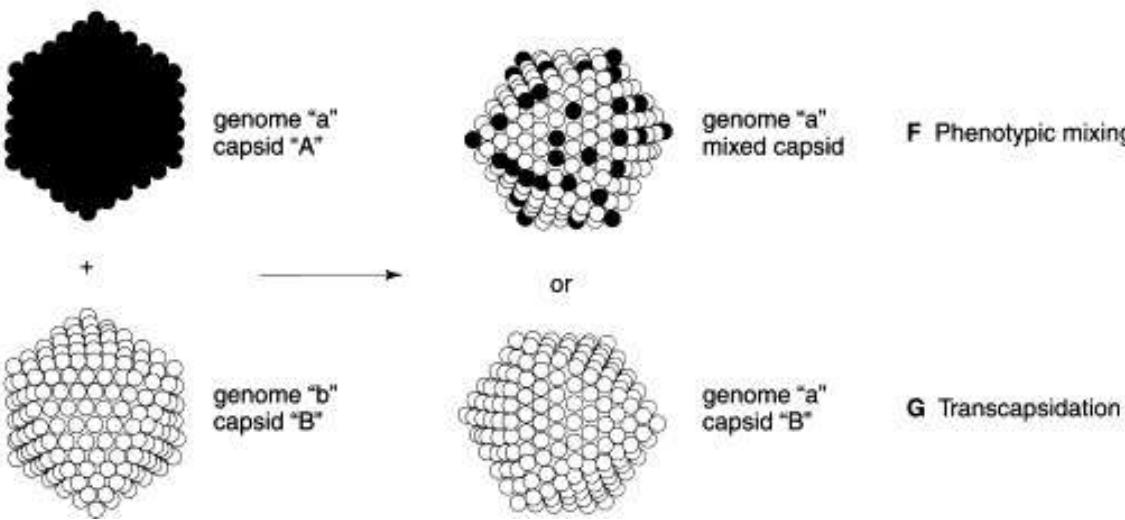
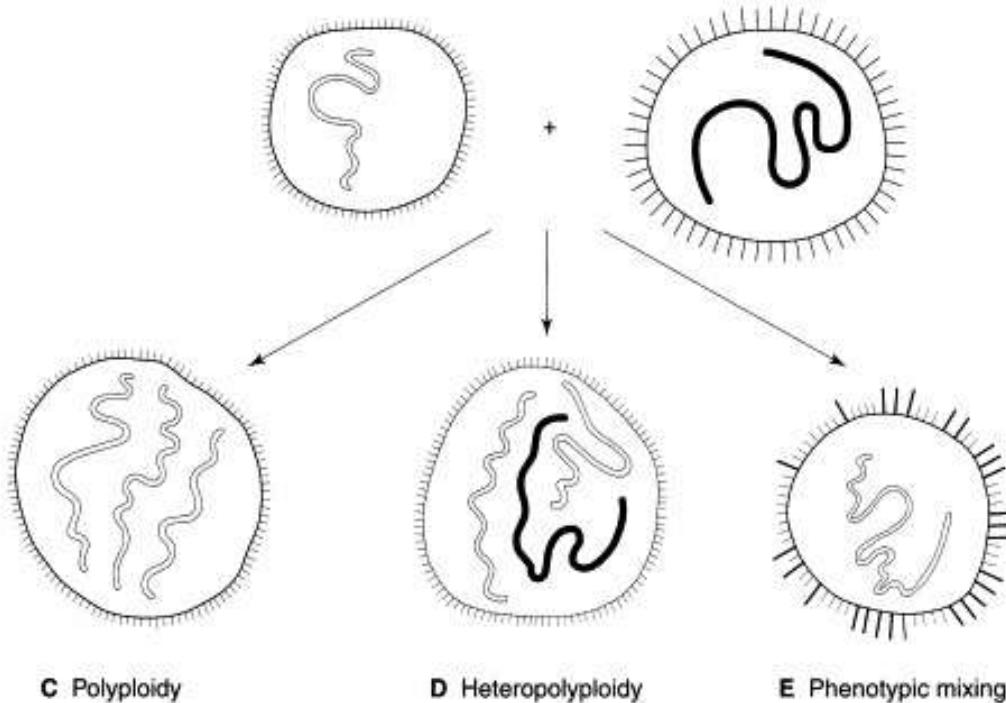
- Genetic reactivation is a special case recombination/reassortment that occurs in mixed infections when one or both of the viruses is non-infectious.
- The progeny, resulting from either recombination or reassortment, are now infectious and carry markers of both parents.
- If only one parent was non-infectious, the process is called **cross-reactivation or marker rescue**.
- If both parents were non-infectious, the process is called **multiplicity reactivation**.

Genotypic mixing

- This is the incorporation of more than one complete genome into the same virus capsid.
- If the genomes are from the same virus species, the phenomenon is **polyploidy**;
- if the genomes are from different virus species, the phenomenon is **heteropolyploidy or genotypic mixing**.
- There is no recombination between the genomes and cells singly infected with the genotypically mixed viruses will yield progeny identical to both original parents as well as more genotypically mixed viruses.

Phenotypic mixing

- A process by which individual progeny of a mixed infection contains structural proteins (capsid or envelope) derived from both parental viruses.
- Phenotypic mixing is an example of non-genetic interactions between two viruses.
- A progeny genome identical to one parent is enclosed within a capsid or envelope entirely specified by the other parent and it is referred to as **pseudotype formation or the envelopes of some of the progeny particles display antigens derived from both parents.**
- With nonenveloped viruses, phenotypic mixing can take the form of **transcapsidation**, in which there is partial or usually complete exchange of capsids . For example, poliovirus nucleic acid may be enclosed within a coxsackievirus capsid, or adenovirus 7 genome may be enclosed within an adenovirus 2 capsid.



VIRUS -CELL INTERACTIONS

The range of changes induced in more than 200 different kinds of cells in the typical animal host by different viruses is remarkably diverse.

- Disruption of cellular function
- Induction of cell death
- Transformation
- Activation of an inappropriate immune response
- **These changes are manifested as disease.**

Types of Virus-Cell Interactions

- **Cytolytic or cytocidal infection** – Cell die
- **Non-cytolytic infection** – Cell remain intact
- **Productive Infections** – Virus replicates and fully infectious virions are produced
- **Non productive Infections/ abortive infections** – Virus replicate inside the cell but defective virus or incomplete progeny is produced. Infections of non-permissive cells yield no infectious progeny virus
- **Permissive cells** – They support the complete replication of viruses
- **Non permissive cells** – Not allow the replication of virus. viral replication may be blocked at any point from viral attachment through to the final stages of virion assembly and release.

Types of Virus-Cell Interaction

TYPE OF INFECTION	EFFECTS ON CELL	PRODUCTION OF INFECTIOUS VIRIONS	EXAMPLES
Cytocidal	Morphologic changes in cells (cytopathic effects); inhibition of protein, RNA and DNA synthesis; cell death	Yes	Alphaherpesviruses, enteroviruses, reoviruses
Persistent, productive	No cytopathic effect; little metabolic disturbance; cells continue to divide; may be loss of the special functions of some differentiated cells	Yes	Pestiviruses, arenaviruses, rabies virus, most retroviruses
Persistent, nonproductive	Usually nil	No, but virus may be induced ^a	Canine distemper virus in brain
Transformation	Alteration in cell morphology; cells can be passaged indefinitely; may produce tumors when transplanted to experimental animals	No, oncogenic DNA viruses	Polyomavirus, adenoviruses
		Yes, oncogenic retroviruses	Murine, avian leukosis, and sarcoma viruses

Cytocidal changes in virus-infected cells

- Cytopathic viruses kill the cells in which they replicate and leads to cell damage.
- Morphological changes in cells caused by viral infection are called **cytopathic effects (CPE)**; the responsible virus is said to be cytopathogenic.
- CPE can usually be observed by low-power light microscopy of unstained cell cultures.
- The microscopic appearance of the CPE caused by some of these cytoidal viruses may be sufficiently characteristic to allow provisional identification of an unknown virus.
- Cytopathic effect is often characteristic of the particular virus involved

Mechanisms of Cell Damage

- Inhibition of Host Cell Nucleic Acid Synthesis
- Inhibition of Host Cell RNA Transcription
- Inhibition of Processing of Host Cell mRNAs
- Inhibition of Host Cell Protein Synthesis
- Inviting immune cells

Inhibition of Host Cell Nucleic Acid Synthesis

- Common in viral infections.
- It is an inevitable consequence of viral inhibition of host cell protein synthesis
- Eg. poxviruses produce a DNase that degrades cellular DNA,
- Herpes viruses specifically displace the synthesis of host cell DNA with their own synthetic processes.

Inhibition of Host Cell RNA Transcription

- Poxviruses, rhabdoviruses, reoviruses, paramyxoviruses, and picornaviruses, inhibit host cell RNA transcription.
- May be indirect consequence of viral effects on host cell protein synthesis which decrease availability of transcription factors required for RNA polymerase activity
- Viruses encode specific transcription factors for the purpose of regulating the expression of their own genes and, in some instances, these factors modulate the expression of cellular genes as well
- Eg. herpesviruses encode proteins that bind directly to specific viral DNA sequences, thereby regulating the transcription of viral genes.

Inhibition of Processing of Host Cell mRNAs

- Interference with the splicing of cellular primary mRNA transcripts
- Ex. vesicular stomatitis viruses, influenza viruses, and herpesviruses.
- Spliceosomes are formed, but subsequent catalytic steps are inhibited. Spliceosomes are complexes composed of small nuclear RNA (snRNA) that remove introns in protein-encoding genes (splicing)
- Eg: a protein synthesized in herpesvirus- infected cells suppresses RNA splicing and leads to reduced amounts of cellular mRNAs and the accumulation of primary mRNA transcripts.

Inhibition of Host Cell Protein Synthesis

- The shutdown of host cell protein synthesis, while viral protein synthesis continues, is a characteristic of many virus infections.
- Eg. Picorna virus, togavirus, influenza virus, rhabdovirus, poxvirus, and herpesvirus infections.
- Mechanisms :
- viral enzymes degrade cellular mRNAs
- Factors that bind to ribosomes and inhibit cellular mRNA translation
- alteration of the intracellular environment favouring the translation of viral mRNAs .
- Viral proteins may also inhibit the processing and transport of cellular proteins from the endoplasmic reticulum, and this inhibition may lead to their degradation. Ex. lentiviruses

Inviting immune cells

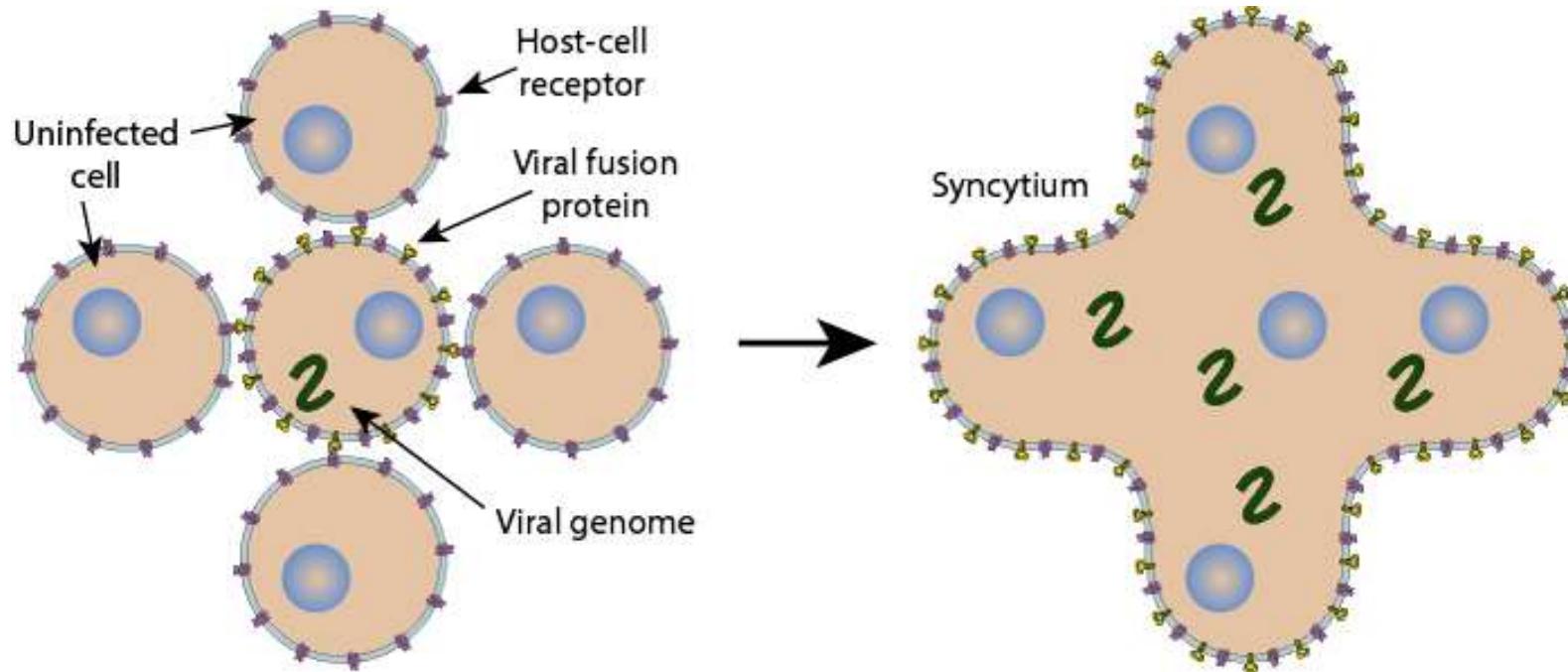
- Virus infect healthy cells
- During replication of virus some viral protein leaks
- Leaked protein attach to the cell membrane
- Natural Killer cells detect that protein in abnormal cells
- Perforin released by killer cells and make hole in mb
- Cytoplasm will come out and cell dies

Cytopathic Changes Involving Cell Membranes

- Viruses may alter plasma membrane permeability,
- affect ion exchange and membrane potential,
- induce the synthesis of new intracellular membranes, and
- induce the rearrangement of previously existing membranes.
- Enveloped viruses also direct the insertion of their surface glycoproteins, including fusion proteins, into host cell membranes as part of their budding process, often leading to membrane fusion and syncytium formation.

Cell Membrane Fusion and Syncytium Formation

- Infection of cell monolayers by lentiviruses, paramyxoviruses, morbilliviruses, pneumoviruses, some herpesviruses, and some other viruses is the production of **syncytia** which result from the fusion of an infected cell with neighbouring infected or uninfected cells.
- Such multinucleate syncytia may also be seen in the tissues of animals infected with these viruses
- Such syncytia may represent an important mechanism of viral spread in tissues: fusion bridges may allow subviral entities, such as viral nucleocapsids and nucleic acids, to spread while escaping the effects of host defenses.



Hemadsorption

- Infective cell produce some proteins which have the ability to adsorb erythrocytes phenomenon known as Haemadsorption .
- Incorporation of viral glycoprotein peplomers into the plasma membrane of infected cells where they serve as receptors for ligands on the surface of erythrocytes. Eg. orthomyxoviruses, paramyxoviruses, and togaviruses,

Haemagglutination

- Glycoprotein peplomers (enveloped virus) are responsible for hemagglutination, *in vitro*, i.e., the agglutination of erythrocytes.
- Virions added to an erythrocyte suspension form cell-virus-cell bridges involving large numbers of erythrocytes.
- Both phenomena are used extensively in laboratory diagnostics
- Haemadsorption – by Infected host cell
Haemagglutination – by virus

Cytopathic Changes Involving the Cytoskeleton

- The cytoskeleton is responsible for the structural integrity of the cell, for the transport of organelles through the cell, and for certain cell motility activities.
- Damage to the cytoskeleton results in changes in cell shape
- Particular viruses are known to damage specific filament systems.
- Canine distemper virus, vesicular stomatitis viruses, vaccinia virus → depolymerization of actin-containing microfilaments
- Enteroviruses -----extensive damage to microtubules

Non-cytocidal Changes in Virus-Infected Cells

- Non-cytocidal viruses usually do not kill the cells in which they replicate.
- Infected cells even continue to grow and divide.
- Found in cells infected with several kinds of RNA viruses: pestiviruses, arenaviruses, retroviruses, and some paramyxoviruses.
- Persistent infection
- Inclusion bodies
- Cell transformation

Persistent infections

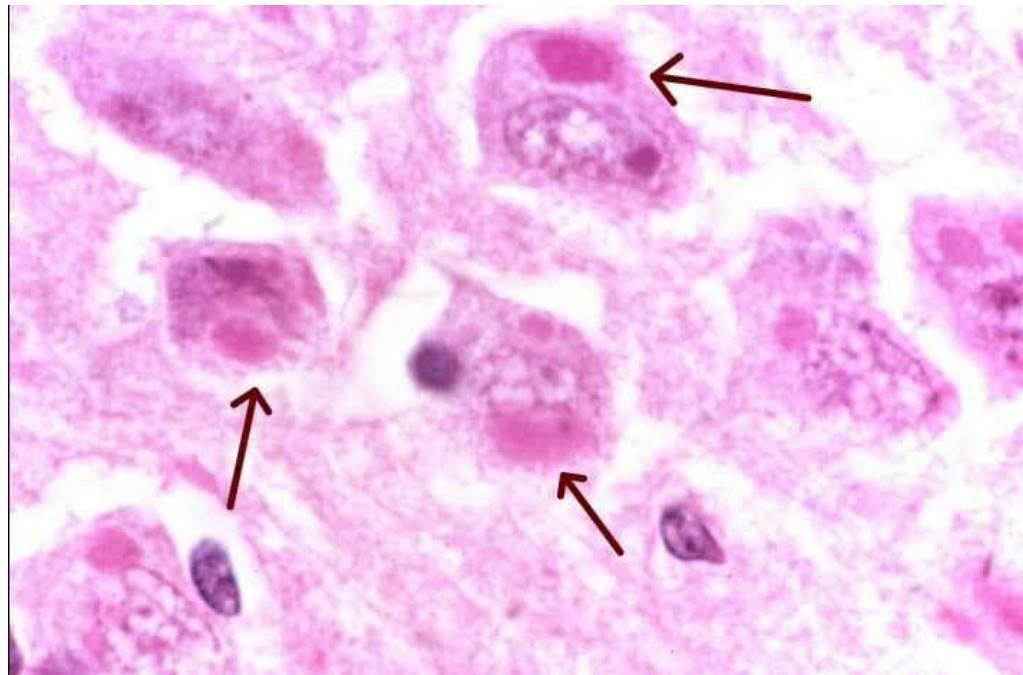
- Non-productive virus cell interactions
- The term persistent infection simply describes an infection that lasts a long time.
- The term latent infection describes an infection that "exists but is not exhibited," i.e., an infection in which infectious virions are not formed.
- In either case, the virus or its genome is maintained indefinitely in the cell, either by the integration of the viral nucleic acid into the host cell DNA or by carriage of the viral nucleic acid in the form of an episome

Inclusion bodies

- Inclusion bodies are aggregates of virus particles or virus-induced proteins or special structures characteristic of infection or degenerative cellular changes by viruses either in the cytoplasm or the nucleus.
- Intranuclear/ Intracytoplasmic, also found both
- single / multiple, Large/ small, round/ irregular in shape
- Acidophilic (pink, stained by eosin) or basophilic (blue, stained by hematoxylin).
- Intracytoplasmic Inclusion bodies – Pox virus, Reovirus, Paramyxo virus and Rabies virus
- Intranuclear Inclusion bodies – Herpes virus, Adeno virus and Parvo virus
- Both IB – Canine distemper virus and porcine cytomegalo virus

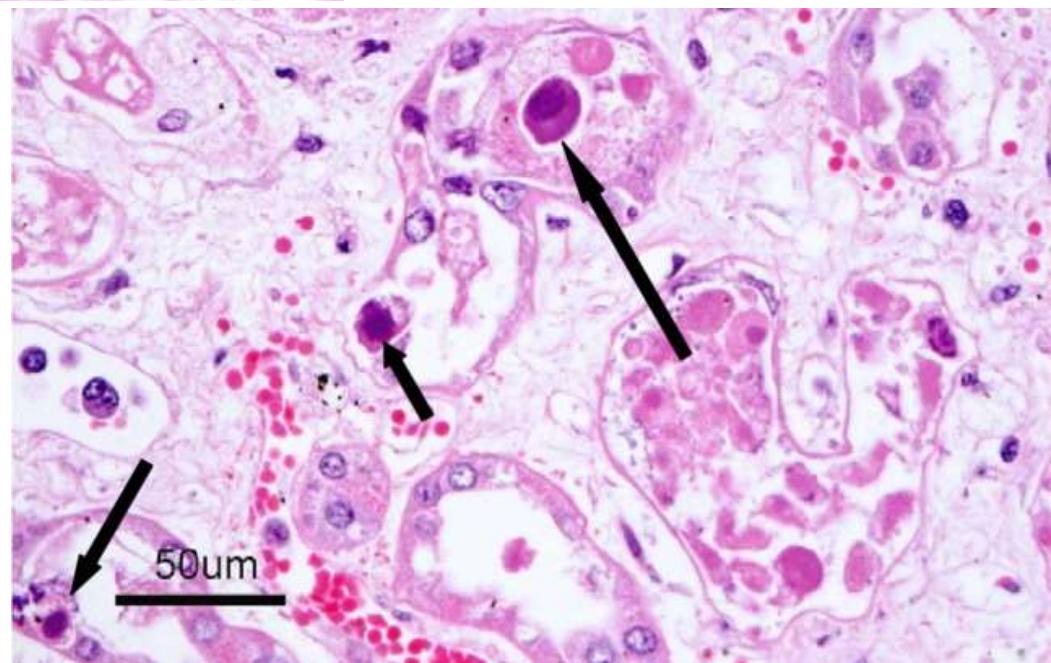
Examples of viral inclusion bodies in animals

- **Cytoplasmic eosinophilic (acidophilic)-**
 - Downie bodies in cowpox
 - **Negri bodies in rabies**
 - Guarnieri bodies in vaccinia, variola (smallpox)
 - Paschen bodies in variola (smallpox)
 - **Bollinger bodies in fowlpox**
- **Nuclear eosinophilic (acidophilic)-**
 - Cowdry bodies type A in Herpes simplex virus and Varicella zoster virus
 - Torres bodies in yellow fever
 - Cowdry bodies type B in polio and adenovirus
- **Nuclear basophilic-**
 - Cowdry bodies type B in adenovirus
 - "Owl's eye appearance" in cytomegalovirus



Negri body

Cowdry A



Ultrastructural Changes in Virus-Infected Cells

- Specific and nonspecific changes in virus infected cells that may vary.
- **Specific changes-**
- Early changes in cell structure often are dominated by proliferation (herpes viruses cause increased synthesis or reduplication, of nuclear membranes)
- Disruption of cytoskeletal elements, mitochondrial damage, and changes in the density of the cytosol.
- Many cytopathic viruses cause nuclear, organelle, and cytoplasmic rarefaction and/or condensation, with terminal loss of host cell membrane integrity.

- **Non-specific changes**
- **Cloudy swelling**- a reversible pathological change associated with increasing permeability of the plasma membrane resulting in diffuse swelling of the nucleus, distention of the endoplasmic reticulum and mitochondria, and rarefaction of the cytoplasm.
- Cell destruction can be the consequence of further loss of osmotic integrity and leakage of lysosomal enzymes into the cytoplasm. This progression, overall, is called the common terminal pathway to cell death.

Virus-Induced Cell Death

- By triggering cellular "suicide," or programmed cell death or **apoptosis**.
- By chromatin condensation and margination and activation of cellular endonuclease during apoptosis that cleaves cellular DNA (not seen in necrosis).
- Some viruses induce apoptosis by the direct action of a specific protein--adenoviruses, alphaviruses, and the circovirus, chicken anemia virus, have proteins that by themselves are sufficient to induce apoptotic cell death.
- Or indirectly through their effects on cellular processes.
- Some viruses have acquired one or more anti-apoptotic genes and gene products to prolong cell survival

VIRAL ONCOGENESIS

TERMINOLOGY

- **Oncology-** study of tumors or cancers.
- A **tumor** is an abnormal growth or mass of tissue (also known as lump, lesion, or neoplasm).
- **Cancer** is a group of diseases caused by the uncontrolled growth and spread of abnormal cells.
- **Tumors can be cancerous BUT not all tumors are cancerous**
- Tumors can be-
- **Benign tumor-** a growth produced by abnormal cell proliferation that remains localized and does not invade adjacent tissue. Non-cancerous
- **Malignant tumor-** is locally invasive and may also be metastatic, i.e., spread to other parts of the body. Growth that is not encapsulated and infiltrates into surrounding tissues, replacing normal cells Malignant tumors are often referred to as **cancers**.

- **Carcinomas**- Malignant tumors of epithelial cell origin
- **Sarcomas**- Malignant tumors of cells of mesenchymal origin.
- Solid tumors arising from leukocytes are known as **lymphomas**, or if circulating cells are involved **leukemias**. Collectively, especially in veterinary medicine, lymphomas and leukemias are known as **leukoses**.
- **Oncogenesis**- The process through which healthy cells become transformed into cancer cells.
- **Synonyms** tumorigenesis and carcinogenesis.
- It is characterized by a series of genetic and cellular changes, including oncogene activation, that lead the cell to divide in an uncontrolled manner.

Cell Transformation

- The changes in the biological functions of a cell that result from **REGULATION** of the cell's metabolism by viral genes and that confer on the infected cell certain properties characteristic of **NEOPLASIA**.
- Oncogenic virus- has the ability to induce cell transformation and to form tumors.
- Transformation by DNA viruses is always nonproductive (i.e., the transformed cells do not yield infectious progeny virus);
- Transformation by retroviruses, on the other hand, is usually productive.
- Viral (or proviral) DNA in transformed cells is integrated into the cell DNA, except in the case of papillomavirus and herpesvirus DNAs, which remain episomal.

Properties of Transformed Cells

- Loss of growth control (loss of contact inhibition in cultured cells)
- Cell morphology changes
- Altered cell metabolism and membrane changes
- Transformed cells frequently exhibit chromosomal aberrations
- Capacity to grow in suspension or in semisolid agar (anchorage independence).
- Tumor formation when transformed cells injected into suitable animal.
- Capacity to produce malignant neoplasms when inoculated into isologous or severely immunosuppressed animals.
- **Tumor-specific antigens (TSA)**, found on cancer cells only, not on healthy cells.
- **Tumor-associated antigens (TAA)**, which have elevated levels on tumor cells, but are also expressed at lower levels on healthy cells. Self expressed antigens by tumor cells

Discovery

- **1911:** **Francis Peyton Rous** (1879-1970) demonstrated that a virus (Rous sarcoma virus) can cause cancer in chickens (Nobel Prize, 1966) . He was the **first person to show that a virus could cause cancer**. It was later called Rous sarcoma virus 1.
- **1976-** J. Michael Bishop and H. Varmus identified the **oncogene** (v-SRC) carried by Rous sarcoma virus.
- Nobel Prize to both in 1989 for this discovery

Mechanisms of Oncogenesis

- Activation of cellular proto-oncogenes or cellular oncogenes (c-onc)
- Presence of viral oncogene (v-onc)
- Inactivation of tumor suppressor genes

Proto-oncogenes

- Normal cellular proteins found in all cells, involved in control of cell growth, division, and differentiation.
- Highly regulated proteins.
- Abbreviated as c-ONCS, eg c-MYC, c-MOS, c-RAS.
- Certain retroviruses isolated from tumors carry altered copies of c-ONCS abbreviated as v-ONCS, eg v-MYC, v-MOS, v-RAS.
- Proto-oncogenes are partially or even completely silenced after cellular differentiation is complete.
- When they suffer alterations (e.g., amplification, mutations or epigenetic modifications), which are able to completely reactivate them, **the proto-oncogenes become oncogenes**, and, if overexpressed, they lead to abnormal cell proliferation, dedifferentiation, and immortality, turning them to malignant tumoral cells

Classes of proto-oncogenes

- The proteins c-oncogenes encode, called **oncoproteins**, act in five major ways-
 - (1) **Extracellular growth factors**- homologues of normal growth factors
 - (2) **Growth factor receptors**- Membrane proteins that capture extracellular signals stimulating cell growth.
 - (3) **Intracellular signal transducers**- Transmit signal initiated by growth factor binding to receptors.
 - (4) **Nuclear transcription factors**- regulate gene expression
 - (5) **Cell cycle control proteins**- negative regulators of cell growth

Activation of Cellular Oncogenes

- Abnormal c-onc gene expression may be responsible for some oncogenic transformations.
1. **Insertional mutagenesis-** The presence upstream from a c-onc gene of an integrated provirus which lacks the v-onc gene, with its strong promoter and enhancer elements, may amplify the expression of the c-onc gene greatly.
Eg. Integrated avian leukosis provirus increases the synthesis of the normal c-myc oncogene product 30- to 100-fold.
 2. **Transposition or translocation-** Transposition of c-onc genes may result in their enhanced expression by bringing them under the control of strong promoter and enhancer elements.

3. Gene Amplification- The increase in gene copy number leads to a corresponding increase in the amount of oncogene product, thus producing cancer.

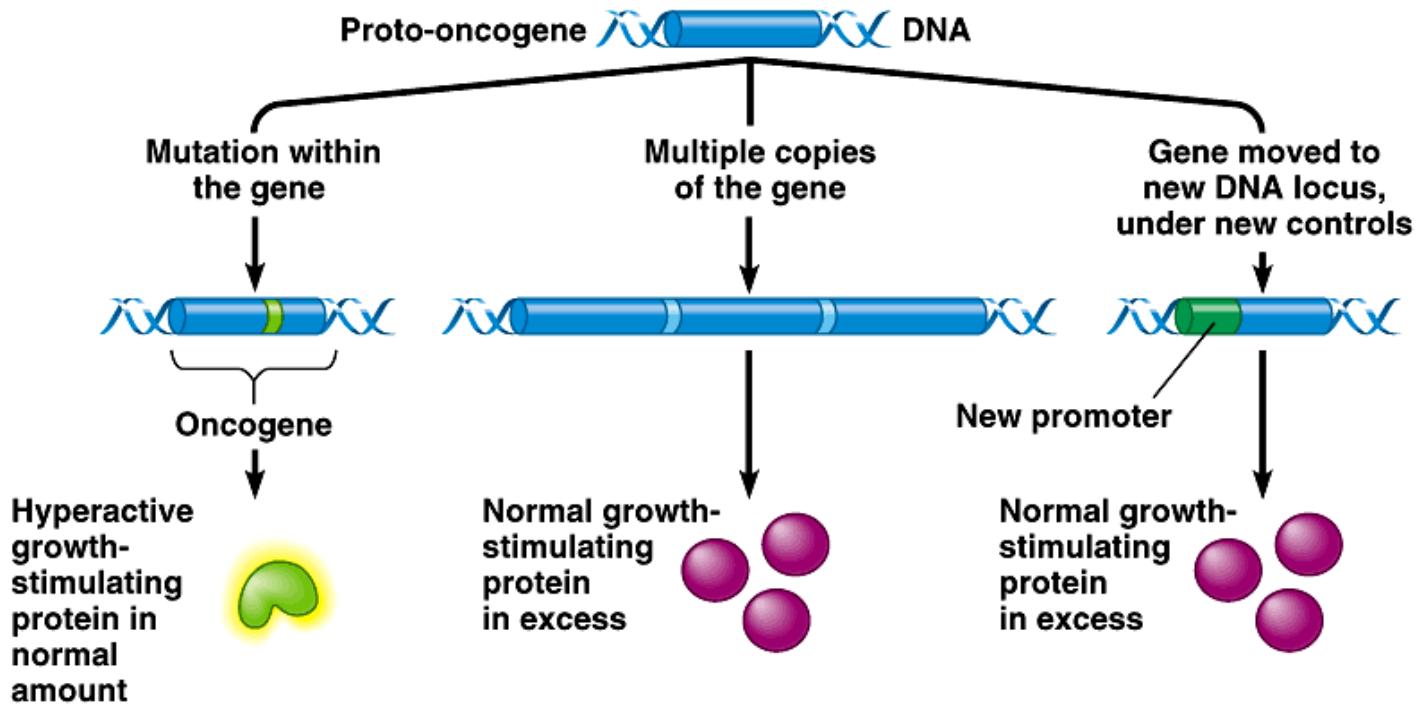
Eg. Amplification of *c-ras* and *c-myc* genes in human tumors.

4. Mutation- Mutation in a c-onc gene, e.g., *c-ras*, may alter the function of the corresponding oncoprotein. Such mutations can occur either in situ as a result of chemical or physical mutagenesis or in the course of recombination with integrated retroviral DNA.

Given the high error rate of reverse transcription, v-onc gene homologues of c-onc genes will always carry mutations and the strongly promoted production of the viral oncoprotein will readily exceed that of the normal cellular oncoprotein resulting in uncontrolled cell growth.

Activation of proto-oncogenes

- mutation
- amplification
- translocation
- insertion of viral genome

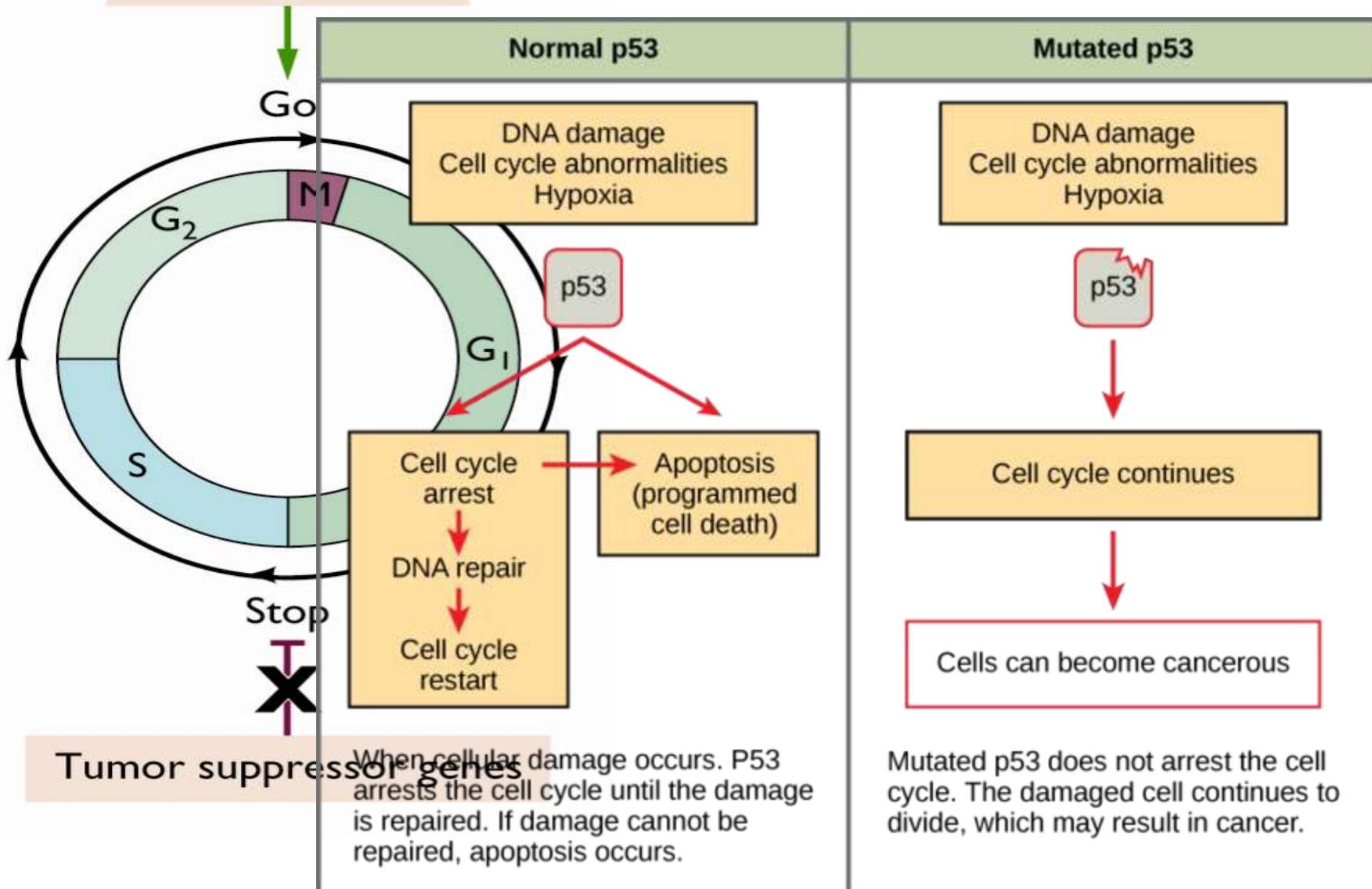


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Tumor Suppressor Genes and Cell Cycle Control Proteins

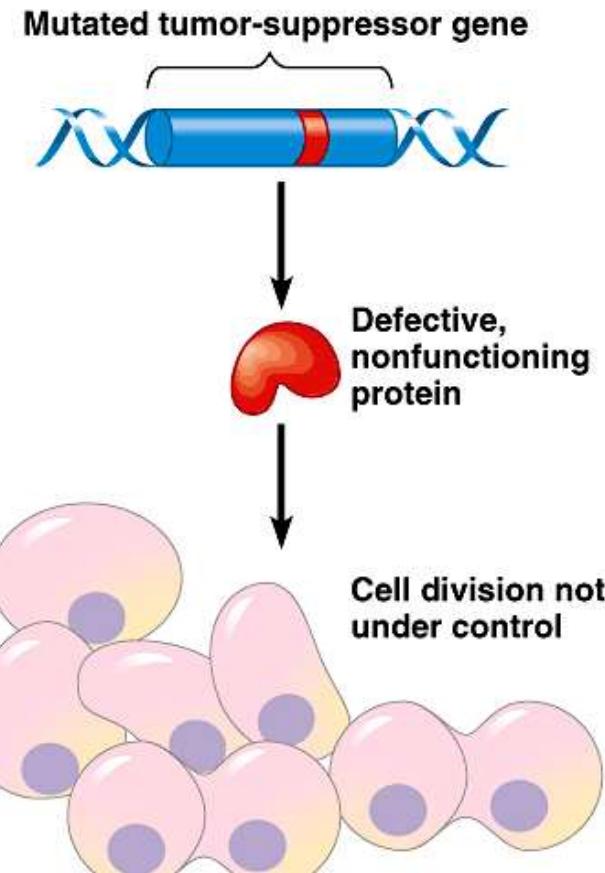
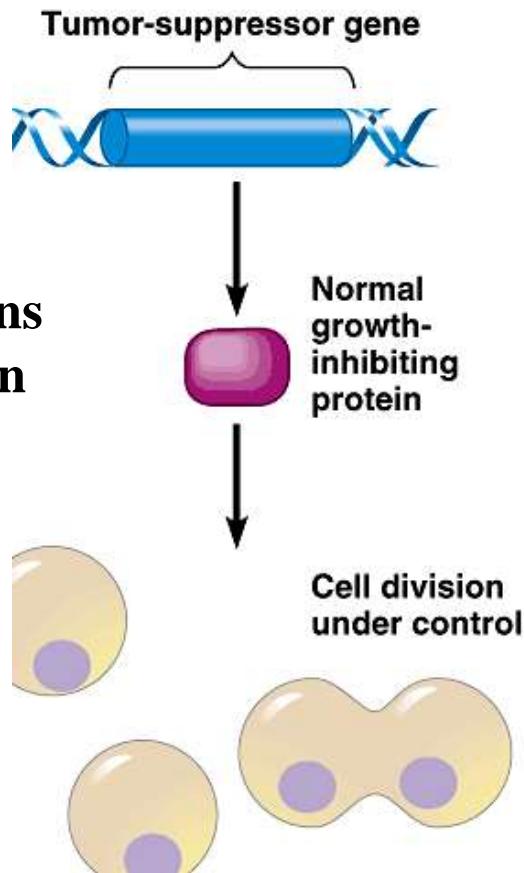
- Their protein products are involved in negative regulation of the cell cycle in that they hold the cell at the G1 phase (**antioncogenes**).
- This regulatory role may be ablated by mutations in tumor suppressor genes or by the binding of other oncoproteins to these genes.
- **Retinoblastoma (Rb)** and p53 tumor suppressor proteins each block the progression of the cell cycle at G1.
- The p53 protein prevents propagation of genetically damaged cells and also plays a role in triggering apoptosis.

Proto-oncogenes



Inactivation of tumor suppressor genes

- mutation
- viral oncogenes
- viral degradation



v-onc genes

- The genes in the viral genome that change host cell proliferation control, lead to the synthesis of new proteins, and are responsible for transformation characteristics are called viral oncogenes (v-onc genes).
- Retroviral v-onc genes are not necessary for replication but play role in cellular transformation.
- Proto-oncogenes (c-onc genes) are the cellular counterparts of v-onc genes.

v-onc genes

- The cellular oncogenes or proto-oncogenes have multiple exons separated by introns, whereas the viral oncogenes are single exons.
- **v-onc genes are under the control of the viral long terminal repeats (LTRs) , which are not only strong promoters but are also influenced by cellular regulatory factors.**
- v-onc genes may be joined to other viral genes in such a way that their functions are modified.
- v-onc genes may undergo mutations (deletions and rearrangements) that alter the structure of their protein products; such changes can interfere with normal protein-protein interactions leading to escape from normal regulation.

Classification of viral oncogenes

- **1. Growth factors**-Growth factors are secreted polypeptides that stimulate the proliferation of target cells and have extracellular signal functions. Target cells must have a specific receptor to be able to respond to a specific type of growth factor.

Eg. The **v-sis oncogene** and its cellular homolog c-sis encode chain B of platelet-derived growth factor (PDGF). Cells transformed by v-sis produce a platelet-derived growth factor-related molecule which is able to stimulate the platelet-derived growth factor receptor in an autocrine fashion. Incessant expression of the sis gene product (PDGF- β) leads to significant neoplastic transformation in **fibroblasts**.

2. Growth factor receptors and hormone receptors-

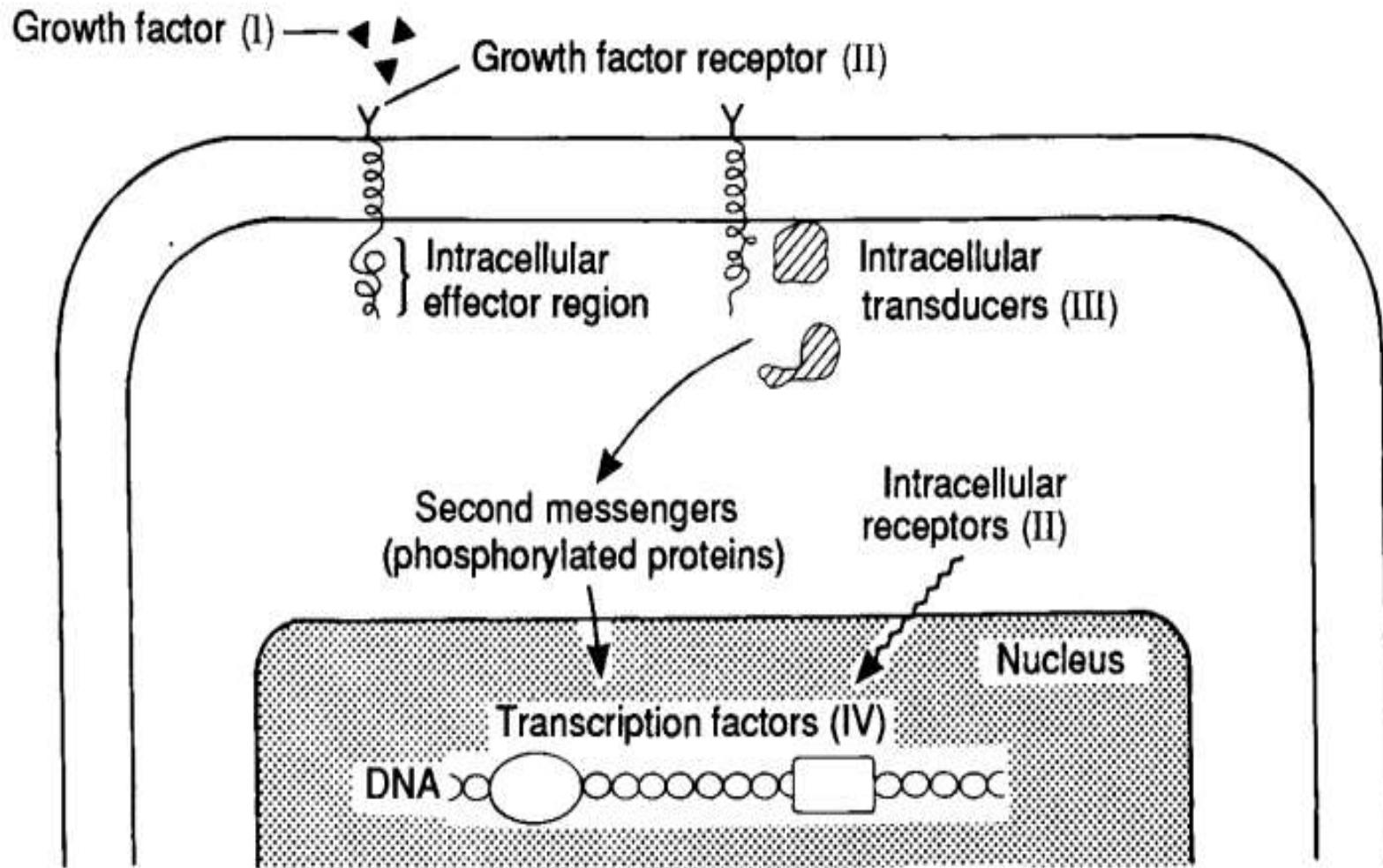
- **v-erbB oncogene** product is a modified epidermal growth factor (EGF) receptor that retains tyrosine kinase activity. In normal cells this kinase becomes activated only after the binding of circulating epidermal growth factor to the receptor.
- However, in the presence of the **v-erbB** gene product, ErbB, the enzyme is activated permanently, phosphorylating any intracytoplasmic protein in its vicinity, thereby initiating a cascade of events culminating in the transmission of conflicting signals to the nucleus. In some cells this results in uncontrolled growth.
- The product of the **v-erbA** gene mimics the intracellular receptor for thyroid hormone. This oncoprotein, ErbA, competes with the natural receptor for the hormone, causing uncontrolled growth.

3. Intracellular Signal Transducers

- Mitogenic signals are transmitted from growth factor receptors on the cell surface to the cell nucleus through a series of interconnected complex processes called the signal transduction path.
- This regulator information is completed with the gradual phosphorylation of proteins that interact with each other in the cytosol.
- The ultimate recipients of the propagated signal are transcription factors that up-regulate specific sets of genes and start a cascade of synthesis that leads to cell growth.
- v-onc genes that act as signal transducers are **Ha-ras and K-ras**
- Signal transducers are transformed into oncogenes by mutations that lead to irregular activities, frequently causing uncontrollable cellular proliferation.

4. Transcription factors

- Transcription factors are nuclear proteins which regulate the expression of target genes or gene families.
- Transcriptional regulation is induced by the binding of protein to specific DNA sequences or DNA structural motifs that are located above the target genes.
- Transcription factors are the final step of the signal transducer process that changes extracellular signals into modulated changes in gene expression.
- Many c-onc genes are transcription factors and they were discovered by studies on retroviruses that have homology with proto-oncogenes. Some examples of these factors are **erb A, ets, fos, jun, myb, and c-myc**.

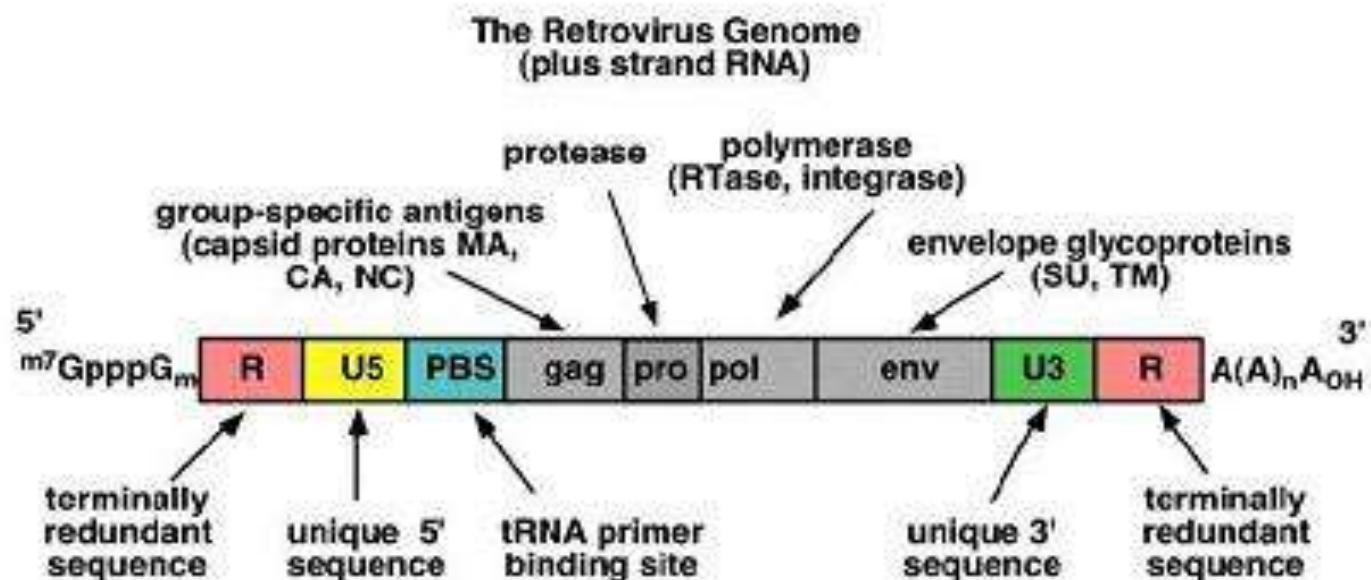


Tumor induction by retroviruses

- Replication competent
- Replication defective
- Endogenous retroviruses
- Exogenous Retroviruses (rapidly transforming or trans-activating and slowly transforming or cis-activating viruses)
- Retroviral oncogenes have very recently been derived from the host, most likely from the species from which the virus was isolated.

Replication-Competent and Replication-Defective Retroviruses

- The genome of a typical replication-competent **slowly transforming retrovirus** consists of two identical copies of a positive sense, single-stranded RNA molecule, each of which has three genes:
- ***gag***, encoding core proteins
- ***pol***, encoding the reverse transcriptase, integrase, RNase H,
- ***pro –protease*** gene
- ***Env***, encoding envelope glycoproteins.



- It has a cap at the 5' end and a poly(A) tail at the 3' end.
- The RNA genome has **terminal non-coding regions**, which are important in replication, and **internal regions** that encode virion proteins for gene expression.
- The **5' end** includes four regions, which are R, U5, PBS, and L.

The R region is a short repeated sequence at each end of the genome during the reverse transcription in order to ensure correct end-to-end transfer in growing chain.

U5 is a short unique sequence between R and PBS.

PBS (primer binding site) consists of 18 bases complementary to 3' end of the tRNA primer, which supplies an 'OH group to initiate reverse transcription.

The L region is an un-translated leader region that give the signal for packaging of genomic RNA.

- The **3' end** includes 3 regions, which are PPT (polypurine tract), U3, and R.

PPT (or PP), polypurine tract is the primer for plus-strand DNA synthesis during reverse transcription.

U3 is a sequence between PPT and R, which has signal that provirus can use in transcription.

R is the terminal repeated sequence at 3' end, the same as the R (i.e., repeat region) of the 5' end.

- **Replication defective retrovirus-** v-onc gene is usually incorporated into the viral RNA in place of part of one or more normal viral genes.
- These viruses are usually defective, i.e., they are dependent on non defective helper retroviruses for their replication.
- They are **rapidly transforming retrovirus**.

Some retroviruses have an extra gene

"typical retrovirus"

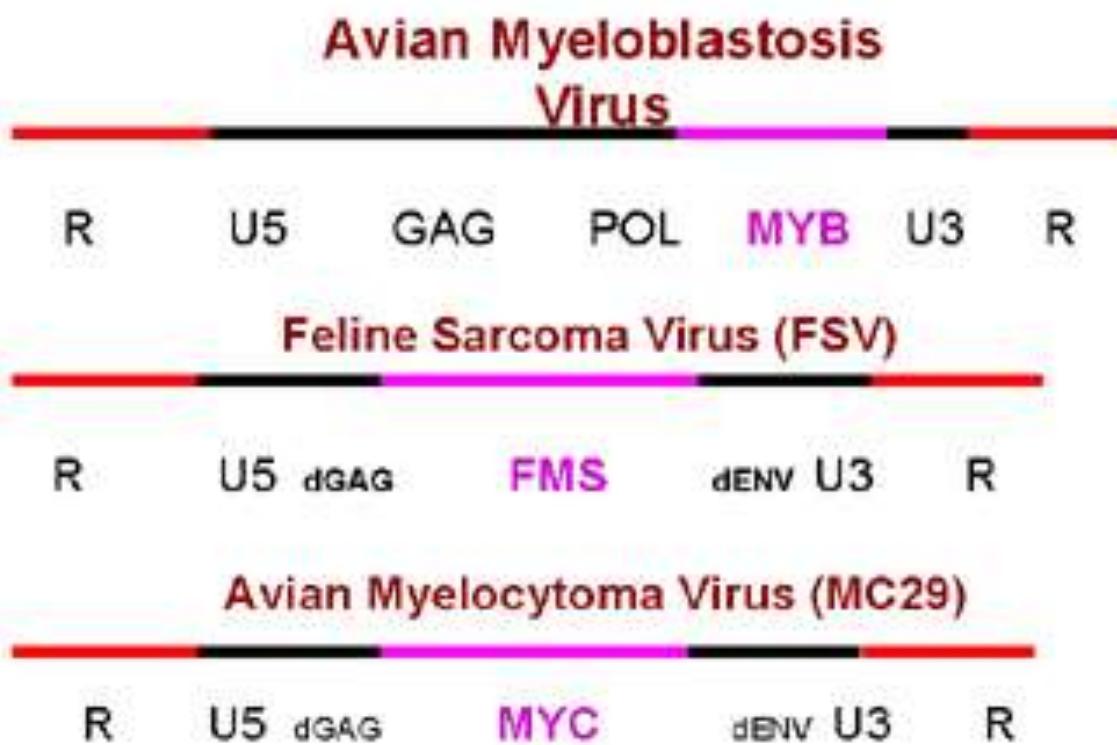
R U5 GAG POL ENV U3 R

Rous Sarcoma Virus

R U5 GAG POL ENV SRC U3 R

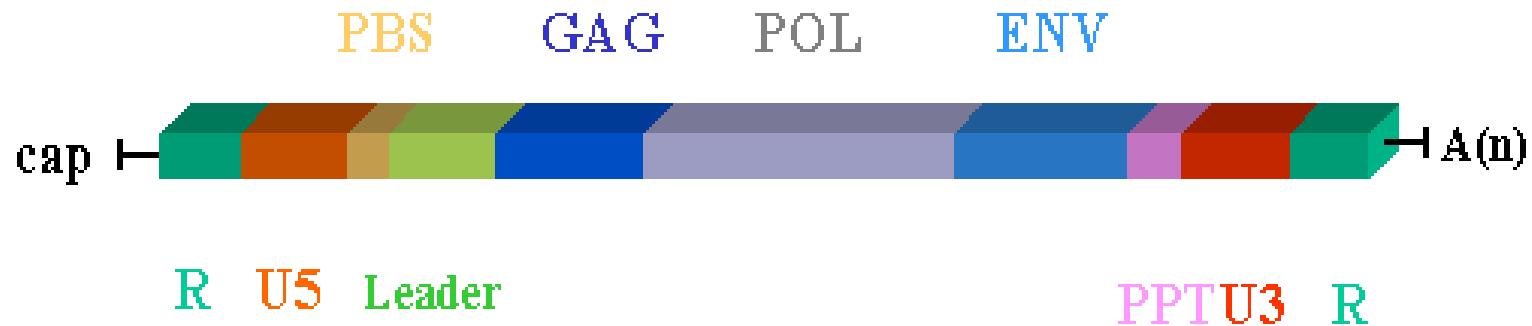
Rous sarcoma virus is replication competent and rapidly oncogenic

Some retroviruses have an oncogene instead of their regular genes

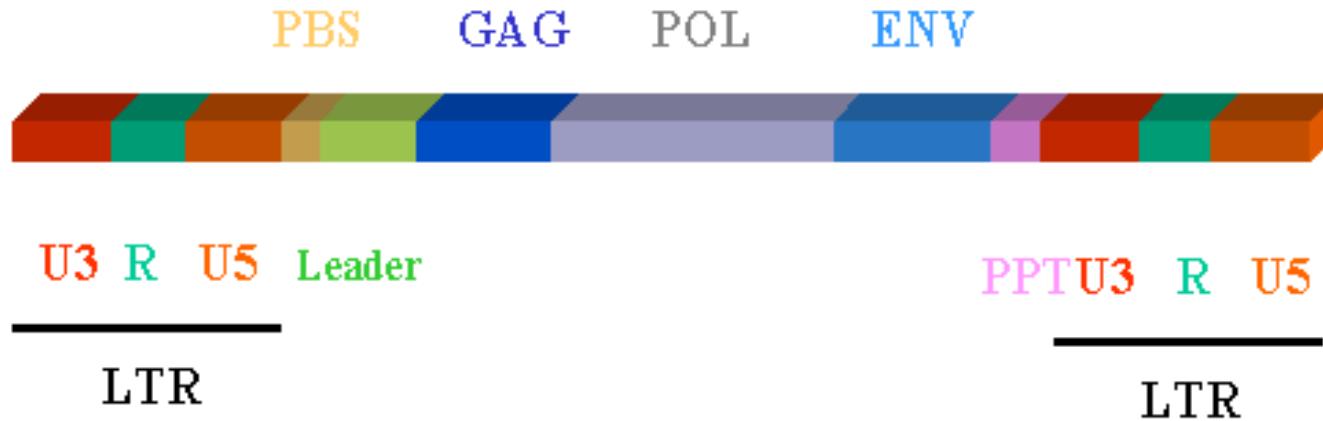


- In the **integrated** form, the **provirus** is more complicated.
- Part of the 3' unique region (called U3) of the RNA genome has been copied and transposed to the opposite end of the genome.
- Conversely, part of the 5' end of the unique region (called U5) has been copied and transposed to the other end.
- The **U3-R-U5 regions** are known as **long terminal repeats or LTRs**.
- The U3 region contains all of the **promotor** information that is necessary to start RNA transcription at the **beginning of the R (repeat) region** while the U5 region contains all of the information necessary to **terminate** after the other R repeat.

- In addition, the LTRs contain information that enhances the degree of transcription of the three retroviral genes (**enhancer regions**).
- These enhancers can be up or downstream from the protein-encoding part of the genes.
- The expression of mRNA from the provirus is under the control of the viral transcriptional regulatory sequences, which include promoter and enhancer elements that are located in the long terminal repeats.



The structure of the RNA genome of the mature retrovirus



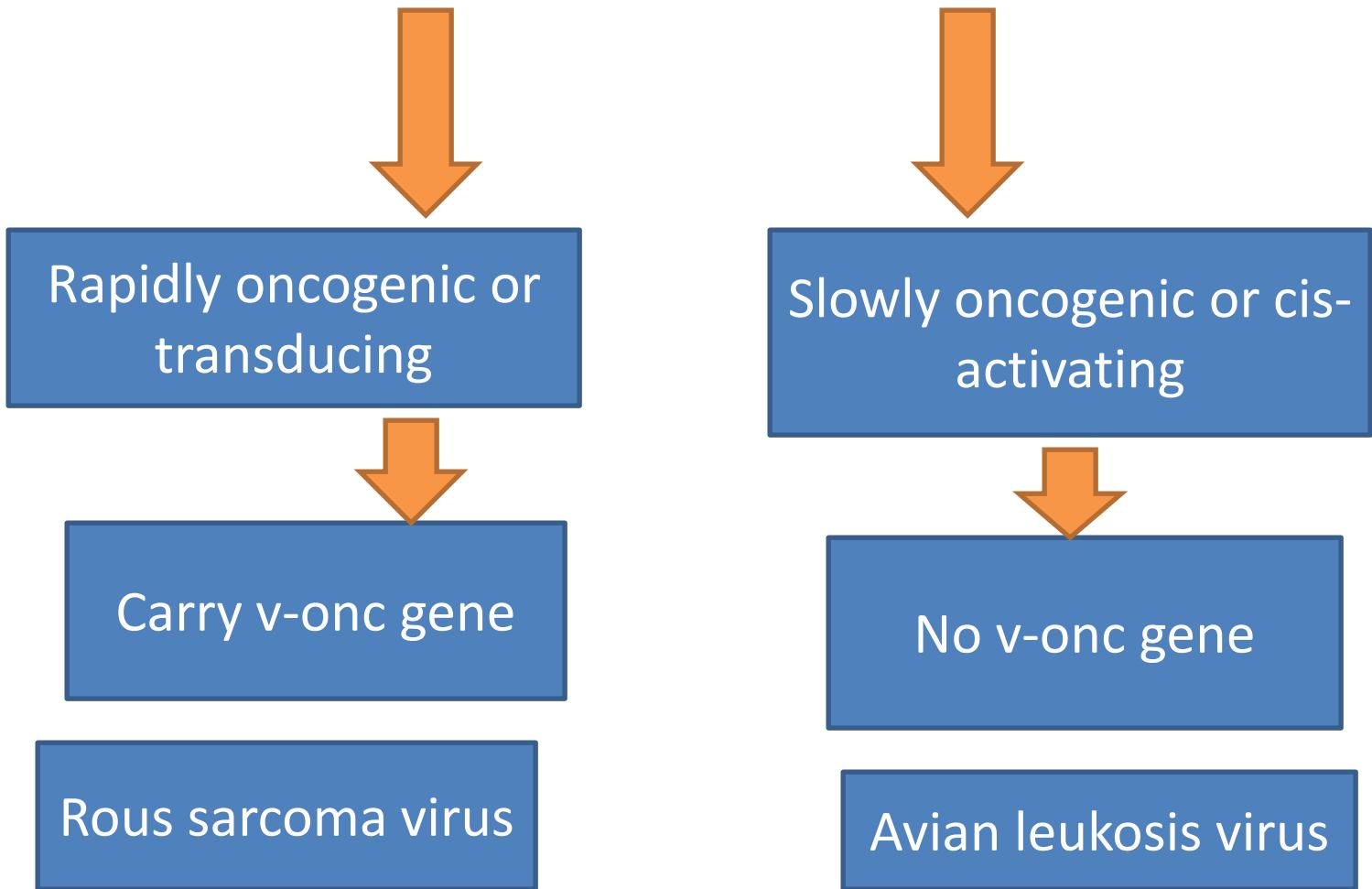
The genome structure of the DNA proviral form of a retrovirus

Endogenous and exogenous Retroviruses

- A complete DNA copy of the genome (known as the provirus) of one, or sometimes more than one, retrovirus may be **transmitted in the germ line DNA from parent to progeny** (i.e., via ova or sperm) and may thus be perpetuated in every cell of every individual of certain vertebrate species.
- Such proviral genomes are under the control of cellular regulatory genes and are normally completely silent in normal animals. Such retroviruses are said to be **endogenous**.
- Expression of such proviruses can be induced by various factors such as irradiation and exposure to mutagenic or carcinogenic chemicals or hormonal or immunological stimuli, so that virions may be produced in some circumstances with some viruses

- **Exogenous retroviruses** behave as more typical infectious agents, spreading horizontally to contacts.
- Most endogenous retroviruses never produce disease, cannot transform cultured cells, and contain no oncogene in their genome.
- Most exogenous retroviruses, however, are oncogenic; some characteristically induce leukemias or lymphomas, others sarcomas, and yet others carcinomas, usually displaying a predilection for a particular target cell.

- Exogenous retroviruses



CHARACTERISTIC	EXOGENOUS RETROVIRUSES		
	ENDOGENOUS RETROVIRUSES	SLOWLY TRANSFORMING OR <i>cis</i> -ACTING RETROVIRUS	RAPIDLY TRANSFORMING OR TRANSDUCING RETROVIRUS
Transmission	Vertical (germ line)	Horizontal	Horizontal
Expression	Usually not, but inducible	Yes	Yes
Genome	Complete	Complete	Defective ^a
Replication	Independent	Independent	Requires helper ^a
Oncogene	Absent	Absent	Present
Tumorigenicity	Nil, or rarely leukemia	Leukemia after long incubation period	Sarcoma, leukemia, or carcinoma, after short incubation period
<i>In vitro</i> transformation	No	No	Yes

Mechanisms of Tumor Induction by Retroviruses

- (1) **Transducing retroviruses** introduce a v-onc gene into the chromosome of the cell. Are replication defective transform cells rapidly both in vitro and in vivo.
- (2) **cis-activating retroviruses**, which lack a v-onc gene, transform cells by becoming integrated in the host cell DNA close to a c-onc gene and thus usurping normal cellular regulation of this gene. Are replication competent, induce tumors more slowly, and do not transform cells in culture,
- (3) **trans-activating retroviruses** contain a gene that codes for a regulatory protein that may either increase transcription from the viral long terminal repeat or interfere with the transcriptional control of specific cellular genes. Either have no oncogenic activity or induce tumors very late by affecting cellular transcription

Tumor Induction by DNA Viruses

- DNA tumor viruses interact with cells in one of two ways:
- (1) **productive infection**, in which the virus completes its replication cycle, resulting in cell lysis, or
- (2) **non-productive infection**, in which the virus transforms the cell without completing its replication cycle. During such non-productive infection, the viral genome or a truncated version of it is integrated into the cellular DNA or, alternatively, the complete genome persists as an autonomously replicating plasmid (**episome**). The genome continues to express early gene functions.
- Eg polyoma viruses, papilloma viruses, Herpes virus, Hepadna viruses, and adenoviruses

- DNA virus oncogenes act primarily in the nucleus, where they alter patterns of gene expression and regulation of cell growth.
- In every case the relevant genes encode **early proteins** having a dual role in viral replication and cell transformation.
- With a few possible exceptions, the **oncogenes of DNA viruses have no homologue or direct ancestors (c-onc genes) among cellular genes of the host.**
- The protein products of DNA virus oncogenes are multifunctional, with particular functions that mimic functions of normal cellular proteins.

Viral Interference

- When a virus-infected cell resists superinfection with the same or a different virus or virus-virus interaction in which the infection and/or replication of one virus is altered by the presence of another virus within the same host
- HOW?
 - (1) interference mediated by defective interfering mutants, operating only against the homologous virus , and
 - (2) interference mediated by interferon

INTERFERONS

- Discovery by **Isaacs and Lindenmann** in 1957.
- Interferon is so named because of its ability **to interfere** with virus reproduction or replication
- Interferons (IFNs) are low molecular weight proteins made and released by lymphocytes in response to the presence of pathogens—such as viruses, bacteria, or parasites—or tumor cells.
- They allow communication between cells to trigger the protective defences of the immune system that eradicate pathogens or tumors.

Types of IFNs

- Three main classes- type I IFNs, type II IFN and type III IFNs.
- The two main **type I IFNs** includes **IFN- α** (further classified into 13 different subtypes such as IFN- α 1, - α 2, - α 4, - α 5, - α 6, - α 7, - α 8, - α 10, - α 13, - α 14, - α 16, - α 17 and - α 21), and **IFN- β** .
- The **type II IFN** family consists of just **IFN- γ** .
- The **type III IFN** family comprises IFN- λ subtypes with similar functions to type I IFN cytokines but restricted activity because their receptor is restricted to epithelial cell surfaces.

Properties of Interferons

PROPERTY	INTERFERON α	INTERFERON β	INTERFERON γ
Principal source	Leukocytes, many other cells	Fibroblasts Epithelial cells	T lymphocytes, NK cells
Inducing agent	Virus infection	Virus infection	Antigen (or mitogen)
Number of subtypes	At least 22 in humans, fewer identified in animals	1	1
Glycosylation	No (most subtypes)	Yes	Yes
Functional form	Monomer	Dimer	Tetramer
Principal activity	Antiviral	Antiviral	Immunomodulation
Mechanism of action	Inhibits protein synthesis	Inhibits protein synthesis	Enhances MHC antigens; activates cytotoxic T cells, macrophages, and NK cells

- Being **cytokines**, IFNs have other functions:
 1. They activate immune cells, such as natural killer cells & macrophages;
 2. They increase recognition of infection or tumor cells by up-regulating antigen presentation to T-lymphocytes;
 3. They increase the ability of uninfected host cells to resist new infection by virus.
- Interferon itself is not directly the anti-viral agent, but it is the inducer of one or many anti-viral mechanisms

Antiviral actions

- initiate an antiviral state in cells
- block viral protein synthesis
- inhibit cell growth

Immunomodulatory actions

- IFN alpha and IFN-beta activate NK cells
- IFN alpha and gamma activates macrophages
- increase MHC antigen expression
- regulate the activities of T cells

Other actions

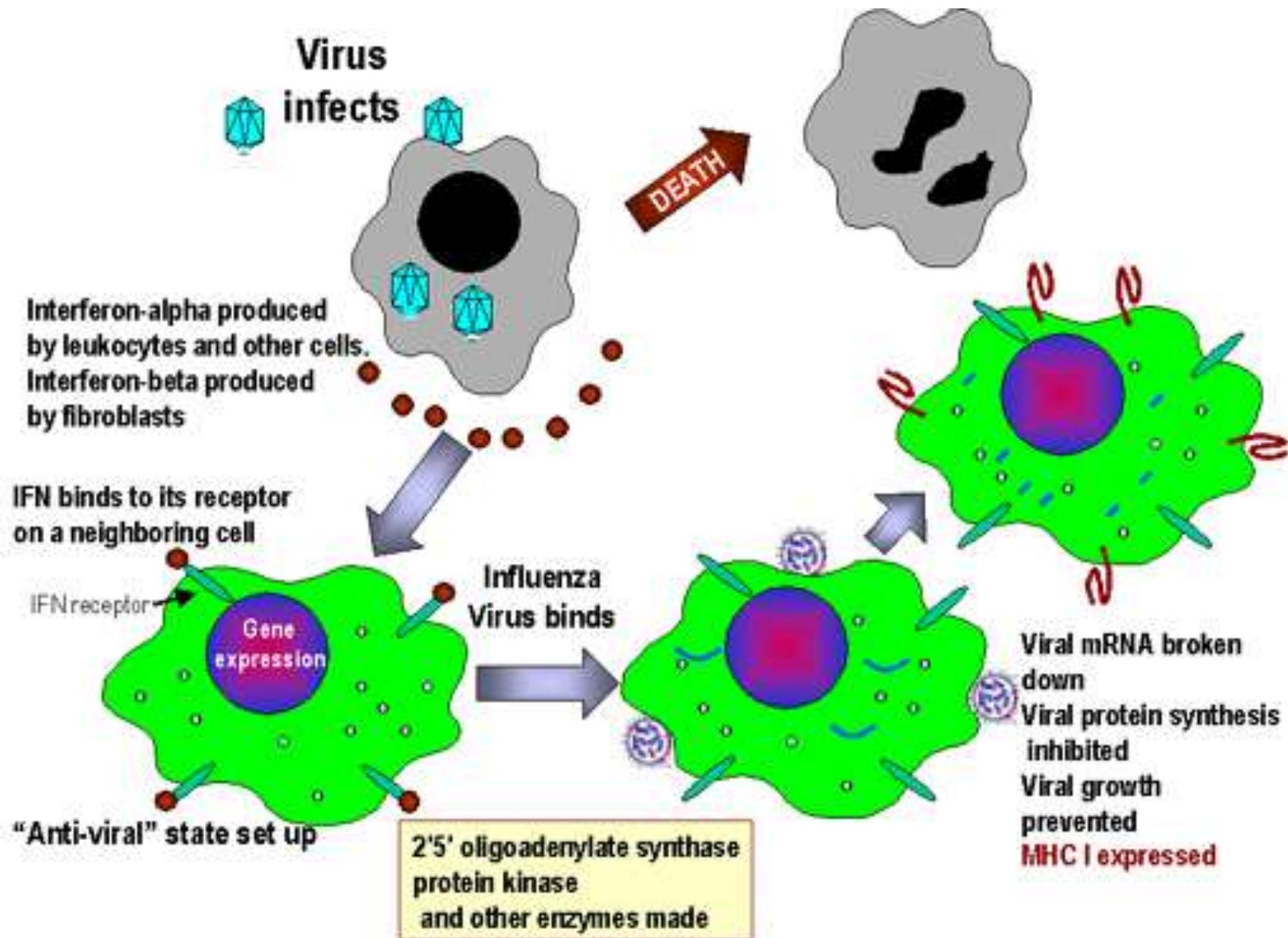
- regulate inflammatory processes
- regulate tumor growth

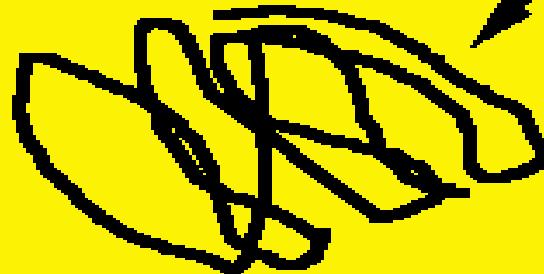
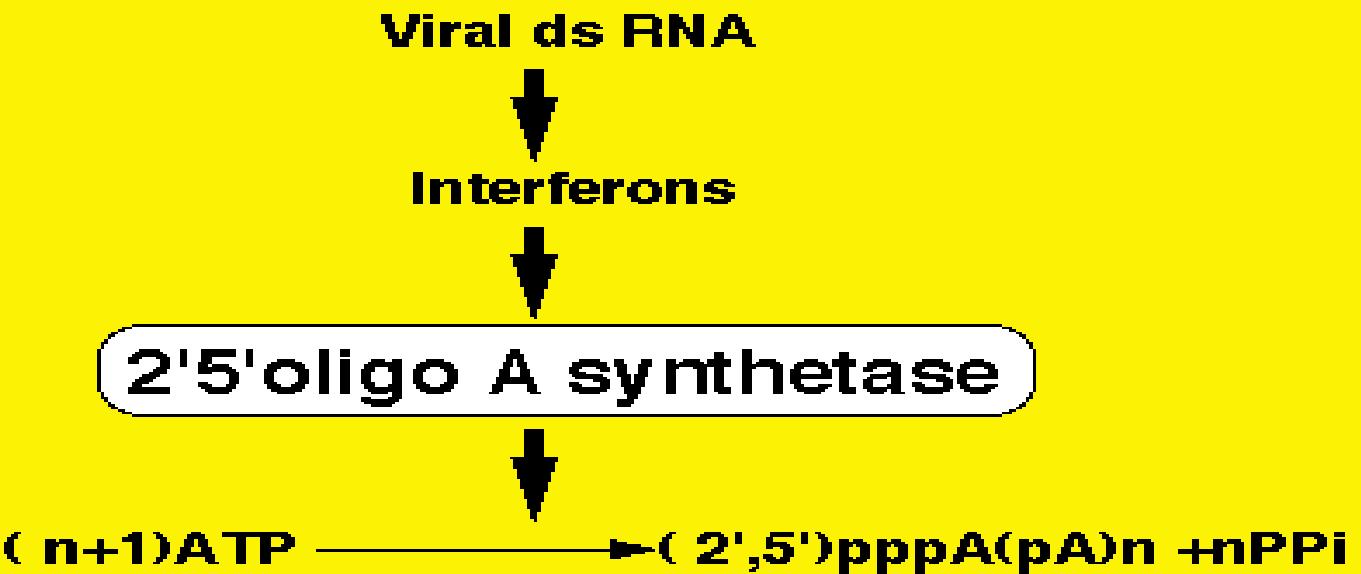
Interferon inducing agents

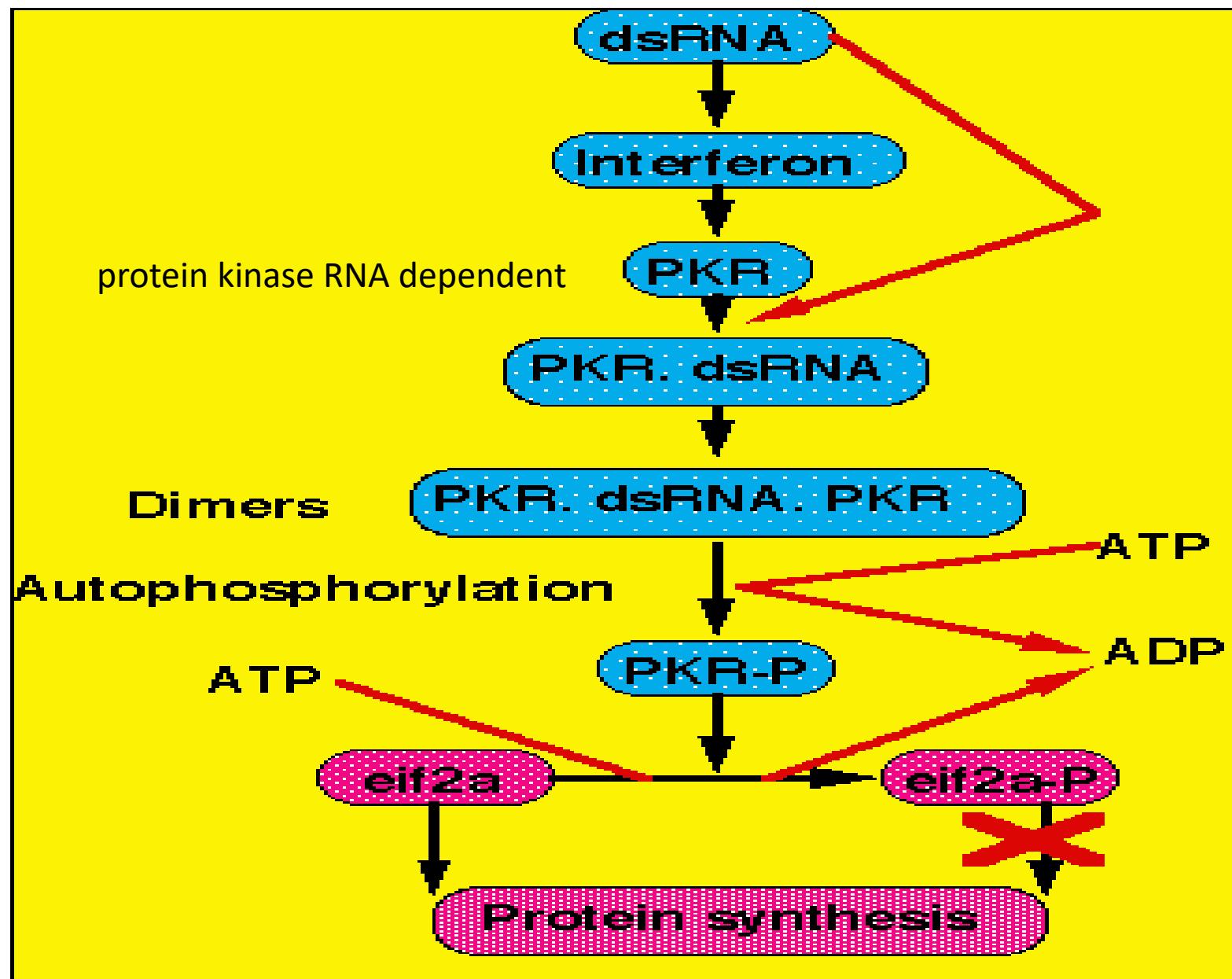
- (1) Viruses.
- (2) dsRNA is a potent inducer, both viral intermediates, and synthetic poly-C.
- (3) Certain Bacterial infections, and the production of endotoxin.
- (4) Metabolic activators/inhibitors.

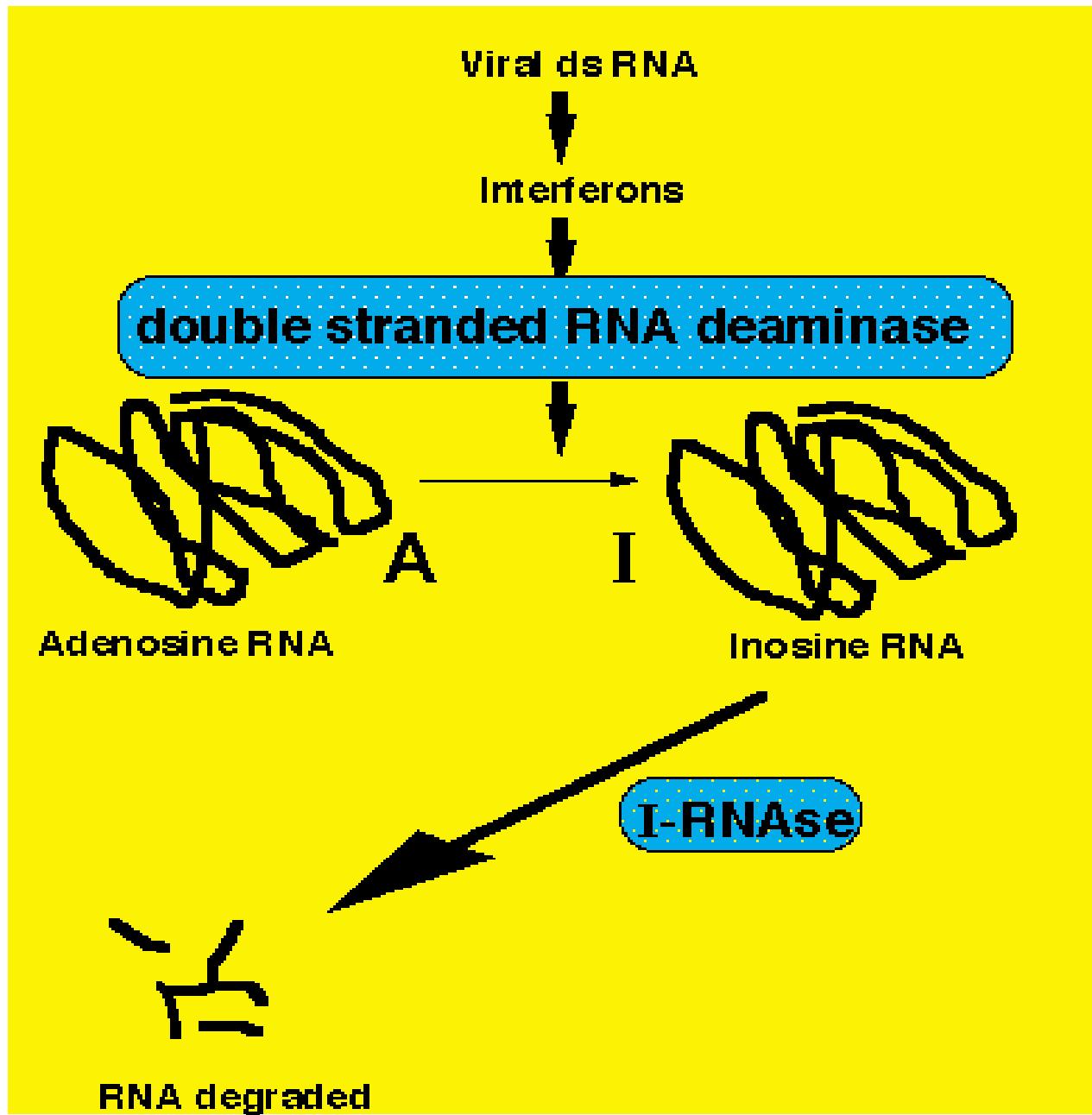
Mechanism of action

- IFN release from an initially infected cell occurs
- IFN binds to a specific cell surface receptor on plasma membrane of other cells- one receptor for interferons α and β and another for interferon γ .
- IFN induces the “antiviral state” : synthesis of **protein kinase, 2'5' oligoadenylate synthetase, and ribonuclease L**
- Viral infection of the cell activates these enzymes
- Inhibition of viral and cellular protein synthesis occurs.









The molecular basis of interferon action

Bcl-2 and caspase cascade

Virus-infected cells



IFN α/β



protects neighboring uninfected cells

= resistant status

intracellular defense

of uninfected cells

Increase expression of MHC class I and II, and thus antigen presentation

PKR
(protein kinase)

2'5'-oligoadenylate synthetase

Mx protein

Killing signal for CTLs

apoptosis

eIF-2

blocks viral protein synthesis

Blocks translation

RNase L
(latent endonuclease)

blocks viral transcription

degrades viral mRNA

VIRAL PATHOGENESIS

Viral pathogenesis describes the processes by which viral infections cause diseases and involves virus–host interactions at the cellular and systemic level that determine whether a virus will cause a disease, what form that disease takes, and how severe the disease will be.

- **Tropism** - The spectrum of tissues infected by a virus is called tropism.
- For example, an enterotropic virus replicates in the gut, whereas a neurotropic virus replicates in cells of the nervous system.
- Some viruses are **pantropic**, infecting and replicating in many cell types and tissues.
- Tropism is governed by at least four parameters.
 - by the distribution of receptors for entry (**susceptibility**), or
 - by a requirement of the virus for differentially expressed intracellular gene products to complete the infection (**permissivity**).

- However, even if the cell is permissive and susceptible, infection may not occur because the virus is physically prevented from interacting with the tissue (**accessibility**).
- Rhinoviruses multiply exclusively in the upper respiratory tract because they are adapted to multiply best at low temperature and pH and high oxygen tension.
- Finally, an infection may not occur even when the tissue is accessible and the cells are susceptible and permissive because of the local intrinsic and innate immune defenses.
- Even if virus initiates infection in a susceptible organ, replication of sufficient virus to cause disease may be prevented by host defenses.

- Disease occurs only if the virus replicates sufficiently to damage essential cells directly, to cause the release of toxic substances from infected tissues, to damage cellular genes or to damage organ function indirectly as a result of the host immune response to the presence of virus antigens.
- Pathogenesis at the cellular level occurs in progressive stages leading to cellular disease.
- Since viruses must use the cell's machinery to synthesize their own nucleic acids and proteins, they have evolved various mechanisms to subvert the cell's normal functions to those required for production of viral macromolecules and eventually viral progeny.

Obligatory Steps in Viral Infection

STEP IN INFECTION PROCESS	REQUIREMENT FOR VIRAL SURVIVAL AND PROGRESSION OF INFECTION
Entry into host and primary viral replication	Evade host's natural protective and cleansing mechanisms
Local or general spread in the host, cell and tissue tropism, and secondary viral replication	Evade immediate host defenses and natural barriers to spread; at the cellular level the virus takes over necessary host cell functions for its own replication processes
Evasion of host inflammatory and immune defenses	Evade host inflammatory, phagocytic, and immune defenses long enough to complete the viral transmission cycle
Shedding from host	Exit host body at site and at concentration needed to ensure infection of the next host
Cause damage to host	Not necessary, but this is the reason we are interested in the virus and its pathogenetic processes

Viral Pathogenesis

Pathogenic mechanisms of viral disease include

- (1) implantation of virus at the portal of entry,
- (2) local replication,
- (3) spread to target organs (disease sites), and
- (4) spread to sites of shedding of virus into the environment.

Factors that affect pathogenic mechanisms:

- (1) accessibility of virus to tissue,
- (2) cell susceptibility to virus multiplication, and
- (3) virus susceptibility to host defenses.

Entry Points for a virus into a host

Viral Entry -Three requirements must be satisfied to ensure successful infection in an individual host:

- Sufficient virus must be available to initiate infection
- Cells at the site of infection must be accessible, susceptible, and permissive for the virus
- Local host anti-viral defense systems must be absent or initially ineffective.

Routes of Entry

1. Skin - poxviruses, togaviruses, flaviviruses, rhabdoviruses

- By injury
- Mechanical transfer by arthropod vectors -**arboviruses**
- Piercing through skin- **iatrogenic**
- Animal bites

2. Respiratory Tract - herpesviruses, adenoviruses, myxoviruses, paramyxoviruses, rhinoviruses

- Through droplets or fomites
- Spread via coughing and sneezing
- Environmental and physicochemical properties of virus particles influence virus survival in aerosol

3. Gastrointestinal Tract - picornaviruses, rotaviruses, coronaviruses, parvoviruses, caliciviruses, astroviruses, adenoviruses.

- By contaminated food and water

4. Genitourinary Tract - retroviruses, herpesviruses, papillomaviruses.

- Due to trauma or abrasions to epithelium
- Infections of epithelium

5. Conjunctiva - adenoviruses, herpesviruses

- Usually leads to conjunctivitis
- Sometimes may lead to systemic infection, e.g. Infectious canine hepatitis virus

Viremia - presence of infectious virus particles in the blood. These virions may be free in the blood or contained within infected cells such as lymphocytes.

Virus dissemination within the host

- An infection that spreads beyond the primary site of infection is called **disseminated**.

1. Localized versus systemic infection:

- Infection of polarized epithelial cells
- If virus is budding at the luminal surface- the disease is localized
- If the virus is released at the basal surface- systemic infection is a strong possibility

2. Hematogenous spread. Occurs through:

- The bite of an arthropod vector
- Iatrogenic inoculation
- Use of contaminated needles
- Blood transfusion
- Infections lead to viremia, then spread to other susceptible sites in body

3. Neural spread

- A **neurotropic virus** can infect neural cells; infection may occur by neural or hematogenous spread initiating from a peripheral site.
- A **neuroinvasive virus** can enter the central nervous system (spinal cord and brain) after infection of a peripheral site.
- A **neurovirulent virus** can cause disease of nervous tissue, manifested by neurological symptoms and often death.

Examples:

- Herpes simplex virus has low neuroinvasiveness of the central nervous system, but high neurovirulence. It always enters the peripheral nervous system but rarely enters the central nervous system. When it does, the consequences are almost always severe, if not fatal.
- Mumps virus has high neuroinvasiveness but low neurovirulence. Most infections lead to invasion of the central nervous system, but neurological disease is mild.
- Rabies virus has high neuroinvasiveness and high neurovirulence. It readily infects the peripheral nervous system and spreads to the central nervous system with 100% lethality unless antiviral therapy is administered shortly after infection.

Incubation Period

- The incubation period is **the time between exposure to virus and onset of disease.**
- During this usually asymptomatic period, implantation, local multiplication, and spread (for disseminated infections) occur.
- The incubation period tends to be brief (1 to 3 days) in infections in which virus travels only a short distance to reach the target organ (i.e., in infections in which disease is due to virus replication at the portal of entry).
- Conversely, incubation periods in generalized infections are longer because of the stepwise fashion by which the virus moves through the body before reaching the target organs.

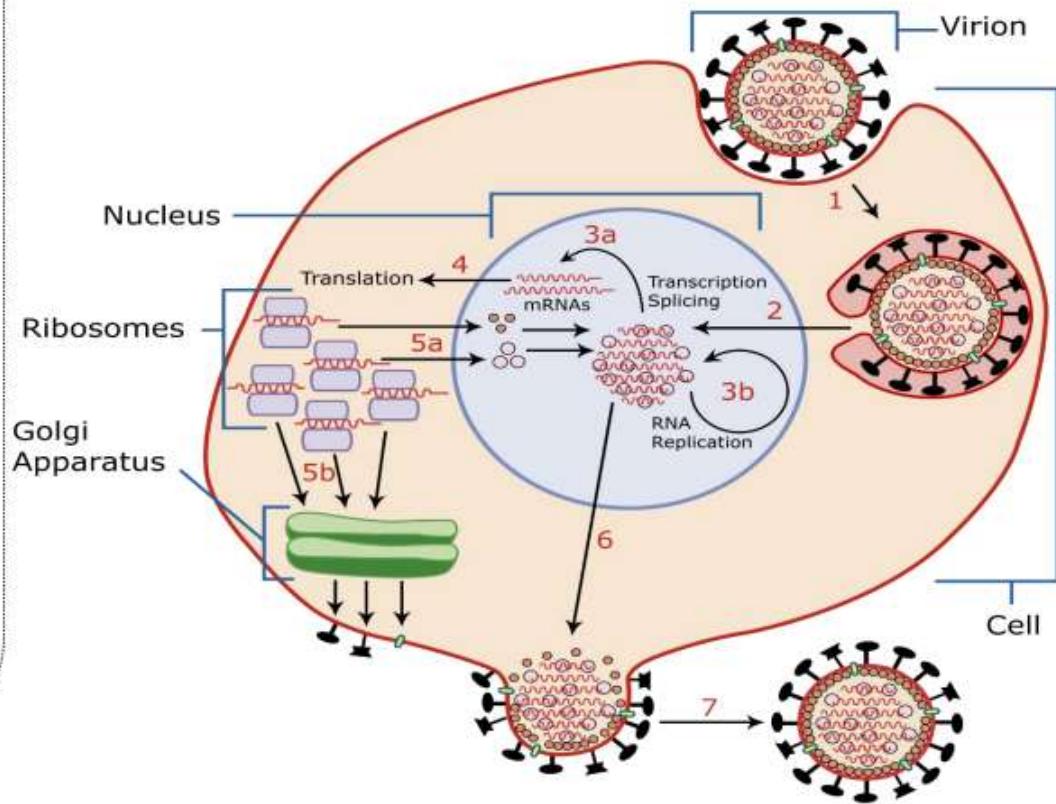
Multiplication in Target Organs

- Virus replication in the target organ resembles replication at other body sites except that
 - (1) the target organ in systemic infections is usually reached late during the stepwise progression of virus through the body, and
 - (2) clinical disease originates there.
- At each step of virus progression through the body, the local recovery mechanisms (local body defenses, including interferon, local inflammation, and local immunity) are activated.
- Thus, when the target organ is infected, the previously infected sites may have reached various stages of recovery.
- Depending on the balance between virus and host defenses, virus multiplication in the target organ may be sufficient to produce dysfunction manifested by disease or death.

Virus shedding

Viral shedding refers to a type of spreading Mechanism of a virus through the body.

it is expulsion and release of virus progeny "off spring" following successful reproduction during a host-cell infection. Once replication has been completed and the host cell is exhausted of all resources in making viral progeny, the viruses may begin to leave the cell by several methods



Although the respiratory tract, alimentary tract, urogenital tract and blood are the most frequent sites of shedding, diverse viruses may be shed at virtually every site.

- Virions can be released from the apical surface, from the basolateral surface, or from both.
- After replication, virus released from the apical surface is outside the host. Such directional release facilitates the dispersal of many newly replicated enteric viruses in the feces (e.g., poliovirus).
- In contrast, virus particles released from the basolateral surfaces of polarized epithelial cells have been moved away from the defenses of the luminal surface.
- Directional release is therefore a major determinant of the infection pattern.
- In general, **viruses released at apical membranes establish a localized or limited infection.**
- **Release of viruses at the basal membrane provides access to the underlying tissues and may facilitate systemic spread.**

Pathogenesis Of Viral Infection:

Viral Disease At The Cellular Level (Cytopathogenesis):

Abortive
"Vs Not Produced"

Productive
"Vs Produced"

Non-productive
"Vs Not Produced But Viral NA Present"

Viral Disease At The Host Level:

Asymptomatic Infection
"Most Common"

Persistent Infection:

Late Complication Of Acute Infection

Chronic Infection

Acute Infection

Latent Infection

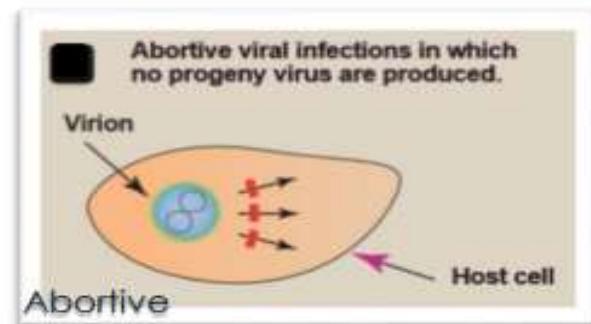
Extra:

Only viral nucleic acid is present, the virus itself is not produced and that is why it is called non-productive.

1- Abortive Infection:

- Viruses don't complete the replication cycle.
- Due to: mutation, defective interfering particles & the action of IFNs.

(Extra: ifns or interferons are proteins released by animal cells, usually in response to the entry of a virus, which has the property of inhibiting virus replication.)



2- Productive Infection:

Non-cytolytic Infections:

Viruses Replicate & Produce Progeny

Viruses Releases By Cell Budding & Little Or No CPE

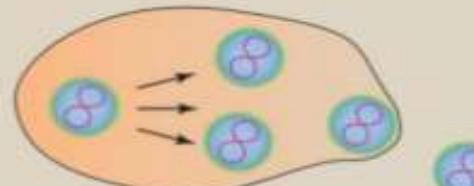
Identified By Hemadsorption & Direct IF

Virus Replicate & Produce Progeny (Progeny Offspring)

Cause Of Cell Death & Cytopathic Effects

Inhibition Of Cellular Protein & NA Synthesis

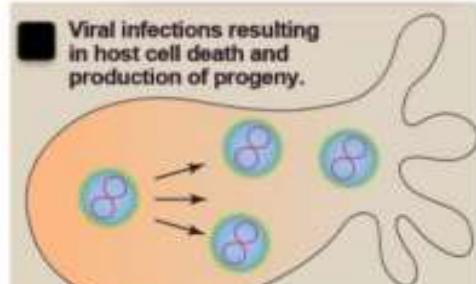
Productive viral infections in which the host cell is not killed, although progeny virus are released.



Productive non-cytolytic infection

Hemadsorption is the adherence of red blood cells to other cells, particles, or surfaces

Viral infections resulting in host cell death and production of progeny.



Productive cytolytic infection

3- Non-productive Infections:

- Viruses infect cells that restrict or lack the machinery for transcribing viral genes.
- Viral genome is found either integrated into cell DNA or as a circular episome or both.

Latent infection:

The cell retains its normal properties

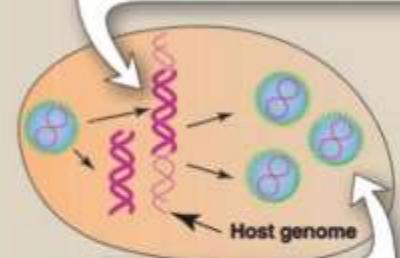
There is limited expression of viral genes
e.g. HSV

Transformation:

Cause tumor in animals & human and it can transform cell culture
e.g. EBV, HPV & HTLV

Viral infections that result in a latent viral state in the host cell.

Some viral infections result in the persistence of the viral genome inside a host cell with no production of progeny virus. The viral nucleic acid may or may not be integrated into the host chromosome, depending on the virus.



Such latent viruses can be reactivated months or years in the future, leading to a productive infection.

Non-Productive Latent infection

Viral infections that result in transformation of the host cell.

Some viral infections result in the persistence of the viral genome inside a host cell with no production of progeny virus.



Non-Productive Transformation

Virus can stimulate uncontrolled cell growth causing transformation
by: alternating the balance between growth activators & growth suppressors gene products.

Cytopathic effects

Cytopathic effects

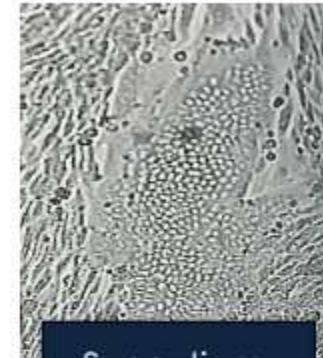
seen in several forms:

Cell lysis
"cell disintegration"
(non-enveloped viruses)

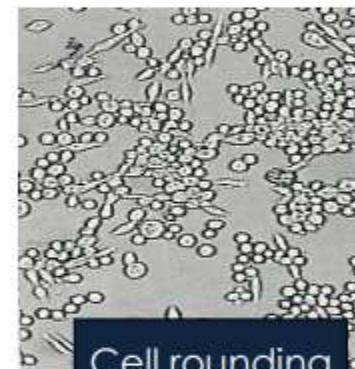
Cell rounding
(enveloped)

Syncytium formation
"Cell fusion"
Ex: Herpes Paramyxo Viruses
Respiratory Syncytial virus (RSV)

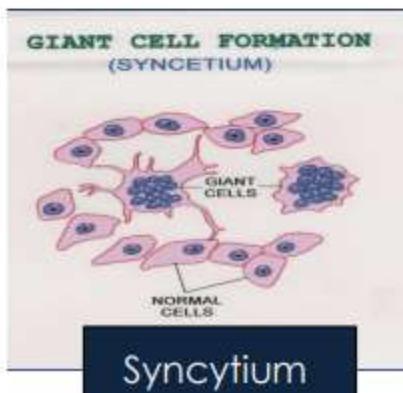
Inclusion Body formation



Syncytium



Cell rounding



Syncytium



Uninfected cc

Genetic Determinants of Virulence

1. Gene products that alter the ability of the virus to replicate.
2. Gene products that modify the host's defense mechanisms.
3. Genes that enable the virus to spread in the host
4. Toxic viral proteins

1. Gene products that alter the ability of the virus to replicate

- Genes that encode proteins affecting both viral replication and virulence can be placed in one of two subclasses:
 1. Mutation including a general defect in replication in the animal host and in many cultured cell types. Reduced virulence results from failure to produce sufficient numbers of virus particles to cause disease. Such a phenotype may be caused by mutations in any viral gene.
 2. Mutation in a gene specifically required for virulence but no replication defects in cells in culture

2. Gene products that modify the host's defense mechanisms

- The study of viral virulence genes has identified a diverse array of viral proteins that sabotage the body's intrinsic defenses and innate and adaptive systems.
- Some of these viral proteins are called **virokines** (secreted proteins that mimic cytokines, growth factors, or similar extracellular immune regulators) or
- **viroceptors** (homologs of cytokine receptors).
- Mutations in genes encoding either class of protein affect virulence, but these genes are not required for growth in cell culture.
- Most virokines and viroceptors have been discovered in the genomes of large DNA viruses.

Virokines

- Virokines comprise a class of virus-coded proteins that are not required for viral replication in vitro, but influence the pathogenesis of infection in vivo by sabotaging the body's innate resistance or immune response.
- Virokines can be grouped as
 - (1) inhibitors of T cell cytotoxicity that bind nascent class I MHC protein;
 - (2) inhibitors of cytokines, such as interleukin 1, interferon 2' and tumor necrosis-factor a;
 - (3) inhibitors of the complement cascade;
 - (4) inhibitors of antibody-mediated cytolysis; and
 - (5) cytokine mimics, e.g., interleukin 10.

3. Genes that enable the virus to spread in the host

- The mutation of some viral genes disrupts spread from peripheral sites of inoculation to the organ where disease occurs.
- For example, after intramuscular inoculation in mice, reovirus type 1 spreads to the central nervous system through the blood, while type 3 spreads by neural routes.
- Studies of viral recombinants between types 1 and 3 indicate that the gene encoding the viral outer capsid protein s1, which recognizes the cell receptor, determines the route of spread.

4. Toxic viral proteins

- Some viral gene products cause cell injury directly, and alterations in these genes reduces viral virulence.
- Evidence of their intrinsic activity is usually obtained by adding purified proteins to cultured cells, or by synthesis of the proteins from plasmids or viral vectors.
- The most convincing example of a viral protein with intrinsic toxicity relevant to the viral disease is the **NSP4 protein** of rotaviruses, which cause gastroenteritis and diarrhea.
- NSP4 acts as a viral enterotoxin and triggers a signal transduction pathway in the intestinal mucosa.

Viral infections and Immunopathology

- Most symptoms and many diseases caused by viral infection are a consequence of the immune response
- Damage caused by the immune system is called **immunopathology**, and it may be the price paid by the host to eliminate a viral infection.
- For **non-cytolytic viruses** it is likely that the immune response is the sole cause of disease.

Immunopathological Lesions

1. **Lesions caused by cytotoxic T lymphocytes (CTLs)-** eg Myocarditis caused by coxsackievirus B infection of mice requires the presence of CTLs. Acute and often fatal respiratory disease caused by hantaviruses is characterized by prominent infiltration of CTLs into the lung.
2. **Lesions caused by CD4+ T-cells-** CD4+ T lymphocytes elaborate far more cytokines than do CTLs and recruit and activate many nonspecific effector cells. Such inflammatory reactions are usually called **delayed-type hypersensitivity responses**.

Most of the recruited cells are neutrophils and mononuclear cells, which are protective and cause tissue damage.

Immunopathology is the result of release of proteolytic enzymes, reactive free radicals such as peroxide and nitric oxide, and cytokines such as TNF- α .

3. Immunopathological Lesions Caused by B-cells –

- Virus-antibody complexes accumulate to high concentrations when extensive viral replication occurs at sites inaccessible to the immune system or continues in the presence of an inadequate immune response.
- Such complexes are not efficiently cleared by the reticuloendothelial system and continue to circulate in the blood.
- They become deposited in the smallest capillaries and cause lesions that are exacerbated when the complement system is activated.
- Deposition of these immune complexes in blood vessels, kidney, and brain may result in vasculitis, glomerulonephritis, and mental confusion, respectively.
- Antibodies may also enhance viral infection. This mechanism probably accounts for the pathogenesis of dengue hemorrhagic fever.

4. Injury Mediated by Free Radicals-

Nitric oxide (NO) is produced in virus-infected tissues during inflammation as part of the innate immune response.

This gas has been shown to inhibit the replication of many viruses in cultured cells and in animal models.

While low concentrations of NO have a protective effect, high concentrations or prolonged production have the potential to contribute to tissue damage.

VIRAL REPLICATION

Introduction

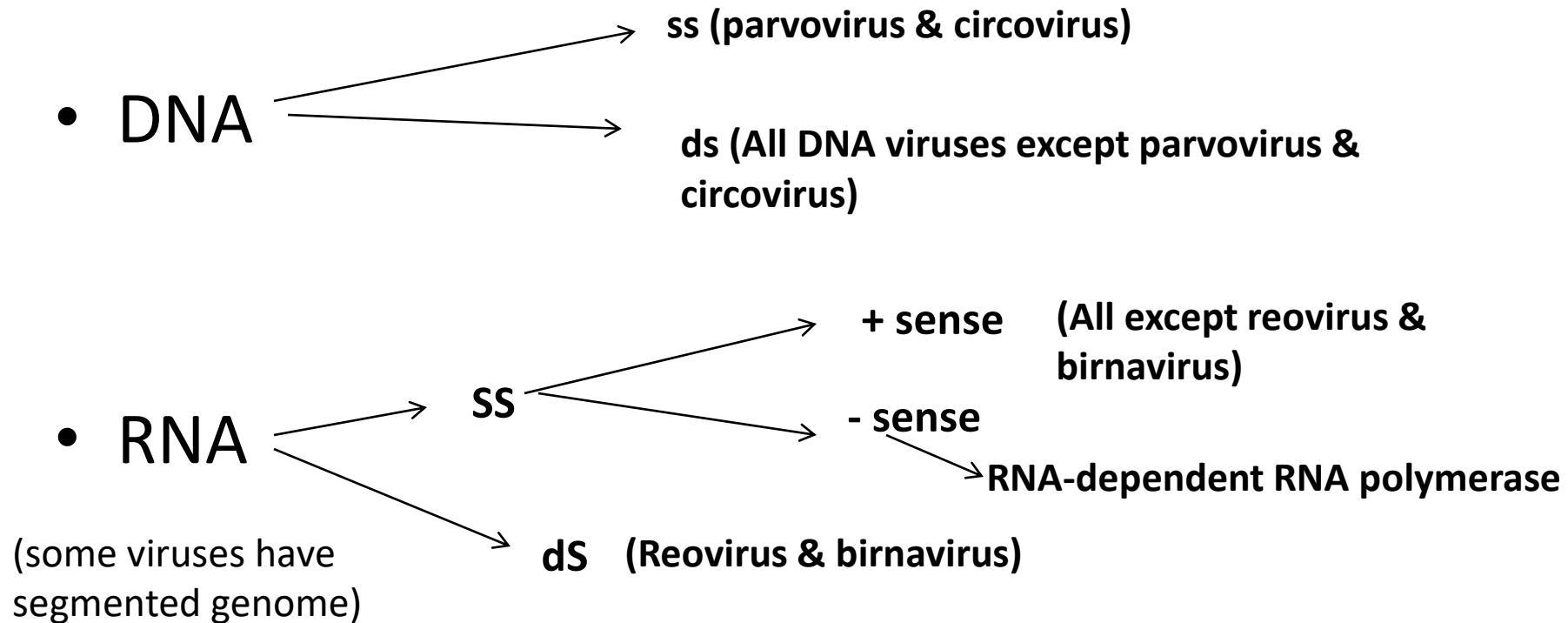
- Replication is to create new, infectious virions that are able to infect other cells of the body or subsequent hosts.
- Or is the process by which a virus makes copies of itself.
- Replication cycle produces –
 - ✓ Functional RNA's and proteins
 - ✓ Genomic RNA or DNA and structural proteins.
- **One-step growth curves** are used to study the replication cycle of a virus infection. Ellis & Delbrück carried out this procedure for the first time in 1939.

- **Multiplicity of infection (MOI)** refers to the number of virions that are added per cell during infection.
- The **burst size** is the number of infectious virions that are released per cell.
- **Eclipse period:** represents the amount of time it takes to form infectious virions within the cell. Eclipse is characterized by the incapacity to detect free virions since viruses are actively transcribing and replicating inside the host. The eclipse usually lasts from minutes (bacteriophages) to hours or days (animal or plant viruses).

- A **susceptible cell** has a functional receptor for a given virus. The cell may or may not be able to support viral replication.
- A **resistant cell** has no receptor- it may or may not be competent to support viral replication
- A **permissive cell** has the capacity to replicate virus- it may or may not be susceptible
- A **susceptible and permissive cell** can take up a virus particle and replicate it.
- **Transfection** – Introduction of viral genetic material into permissive cells artificially (abnormal route of entry) and recovery of infectious virus
- **Infection** – Replication of virus through normal route of entry into susceptible cells

- The **tropism** of a virus refers to the specificity of a virus for a particular host cell or tissue.
- Viruses will only be able to infect the cells that display the molecules to which their virus attachment proteins bind.
- **Narrow host range-** different host species may lack the cell surface proteins that a particular virus uses for attachment.
- **Broad host range-** viruses can successfully infect multiple hosts, either multiple strains of the same species eg Rabies

Genetic material of viruses



Location of Replication:

- **RNA viruses:** cytoplasm except retrovirus and orthomyxovirus;
- **DNA virus:** nucleus except poxvirus and iridovirus.

Enzymes or host functions for initiation of viral replication

A. Normal eukaryotic host cell enzymes for nucleic acid replication:

- For mRNA synthesis: DNA-dependent RNA polymerase.
- For DNA synthesis: DNA-dependent DNA polymerase.

B. Virus specified enzymes for nucleic acid replication:

- Reverse transcriptase (RNA dependent DNA polymerase),
- RNA dependent RNA polymerase (RdRp)
- DNA dependent RNA polymerase, if the DNA virus replicates in cytoplasm.

Early and late genes

- **Early:** refers to the period of a viral infection before the start of nucleic acid replication.
- **Late:** refers to the period of a viral infection after the start of nucleic acid replication.
- **Early gene:** viral genes expressed before nucleic acid replication; most early genes are enzymes and factors to turn on and off the host functions.
- **Late gene :** viral genes expressed after nucleic acid replication; most late genes are viral structural genes and factors for virus assembly.

Stages of Viral Replication

- **Phase – I Initiation:** This stage is characterized by introduction of genetic material of the virus into the cell
- Attachment
- Penetration
- Uncoating
- **Phase – II Replication:** There is no single pattern of replication. But all make proteins with 3 sets of functions to:
 - Ensure replication of the genome
 - Package the genome into virus particles
 - Alter the metabolism of the infected cell so that viruses are produced.
- **Phase – III Assembly, Maturation, Release**

Viral Replication

The seven stages of virus replication are categorized as follows:

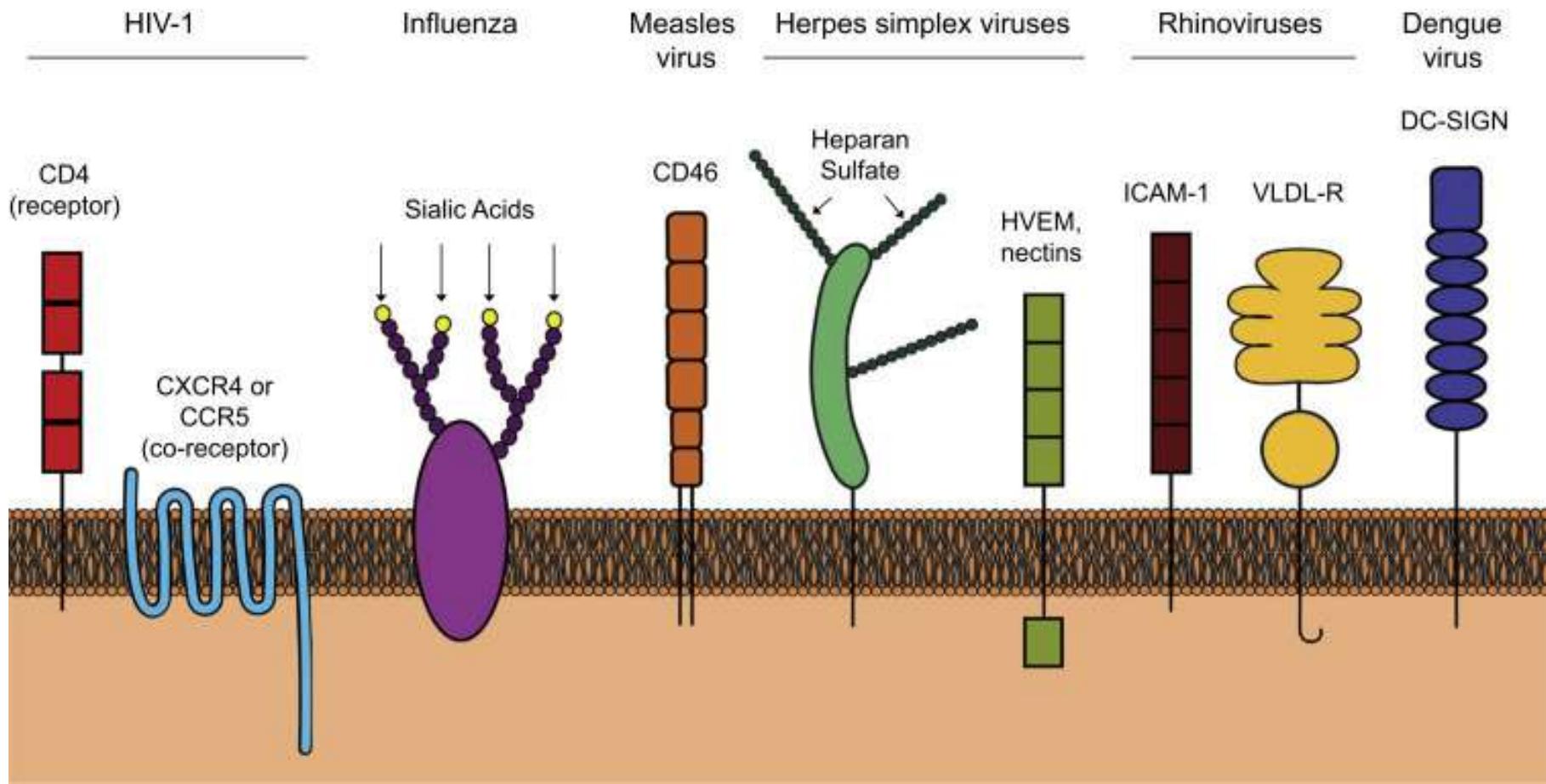
- 1. Attachment**
- 2. Penetration/Entry**
- 3. Uncoating**
- 4. Replication**
- 5. Assembly**
- 6. Maturation**
- 7. Release**

Early phase

Late phase

1. Attachment

- Binding of the virus to the host cell.
- This interaction is specific: the virus contains a **virus attachment protein** that adsorbs to a **cell surface receptor** on the cell
- **Receptors** include cell surface glycoproteins, components of the extracellular matrix and receptors involved in cell signaling and activation.
- Eg - sialic acid, heparan sulfate proteoglycans, ICAM-1, CD46, CD4 and certain integrins.
- **Co-receptors**- used by many viruses (these viruses require the presence of two essential molecules).
- Initial interaction between the virus and its primary receptor brings conformational change in the virus which exposes a binding site for the coreceptor. eg. HIV-1 virus



Cell surface receptors.

2.Penetration or Entry

- **Penetration** refers to Entry of entire virion particle or its genetic material into the cell.
- Virus cross the plasma membrane without killing the cell.

Mechanisms:

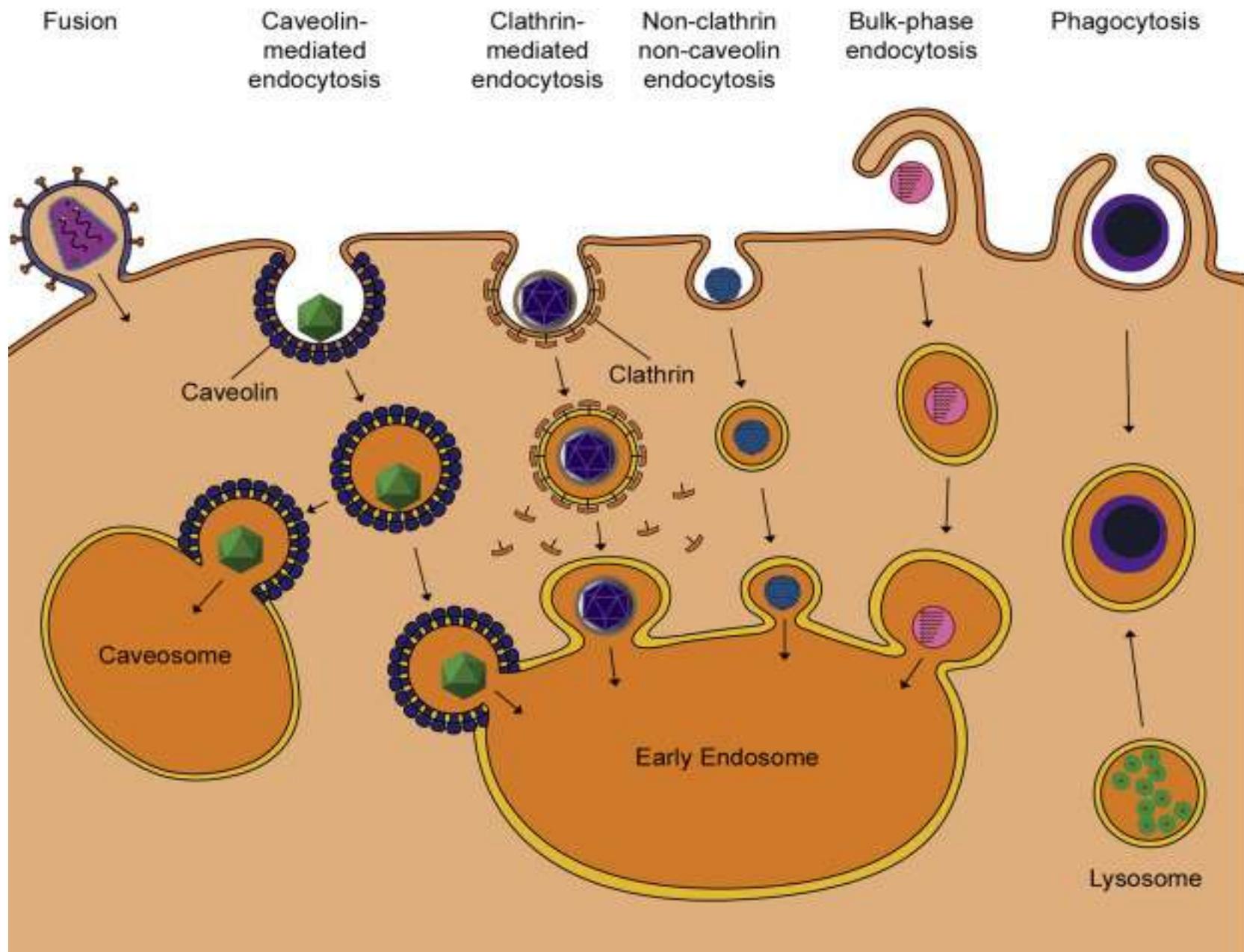
- i. **Translocation**--in some non-enveloped viruses such as FMD virus, the entire virus crosses the cytoplasmic membrane, entering the cell.
- ii. **Receptor-mediated endocytosis** --**commonly used** by viruses (Both enveloped and non-enveloped) to penetrate the plasma membrane. Most types of viruses use clathrin-mediated endocytosis to enter the cell, like dengue virus, hepatitis C virus, and reoviruses. Virion attachment to receptors, which cluster at clathrin-coated pits, is followed by endocytosis into clathrin-coated vesicles. Vesicles enter the cytoplasm and, after removal of the clathrin coat, fuse with endosomes

Acidification within the vesicle triggers changes in virion proteins and surface structures. The configuration of capsid protein VP4 of picornaviruses, for example, leads to release of the viral RNA from the virion into the cytosol.

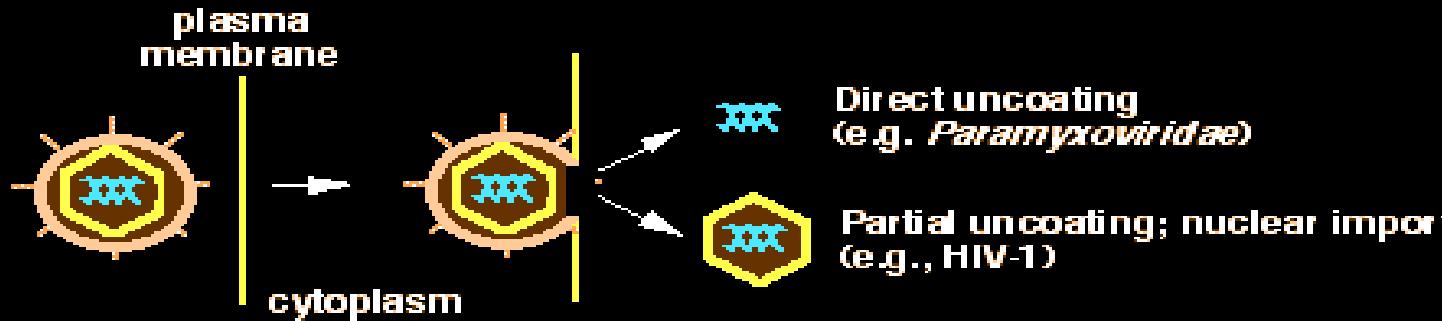
Similarly, at the acidic pH of the endosome, the hemagglutinin molecule of influenza virus undergoes a conformational change, which enables fusion to occur between the viral envelope and the endosomal membrane, leading to release of the viral nucleocapsid into the cytoplasm.

iii. **Fusion**---in most enveloped viruses, such as paramyxoviruses and herpesviruses, fusion of plasma membrane with the viral envelope occurs. This allows the nucleocapsid to be released directly into the cytoplasm.

iv. **Injection**---in bacterial viruses, only the nucleic acid enters the cell.

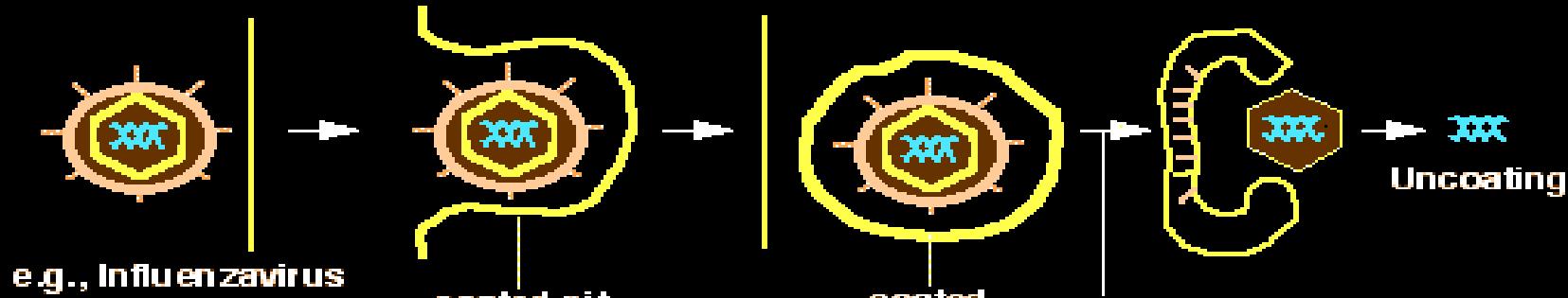


Surface Fusion

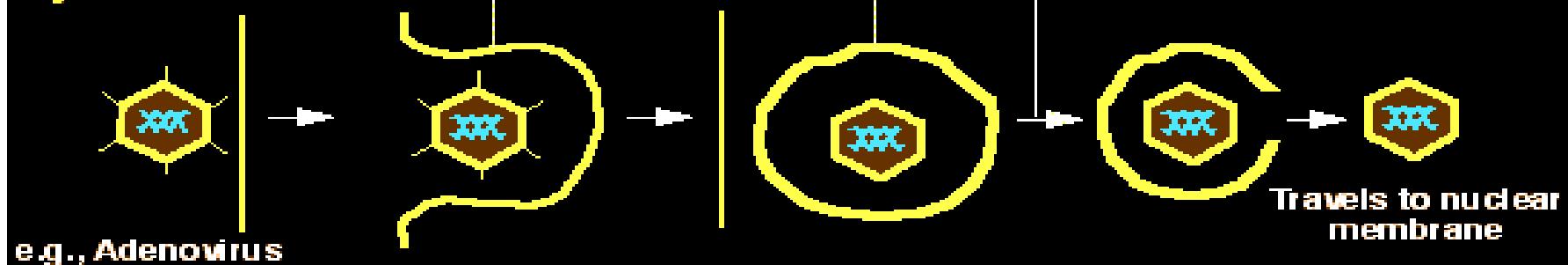


Receptor-mediated endocytosis

Fusion in endosome



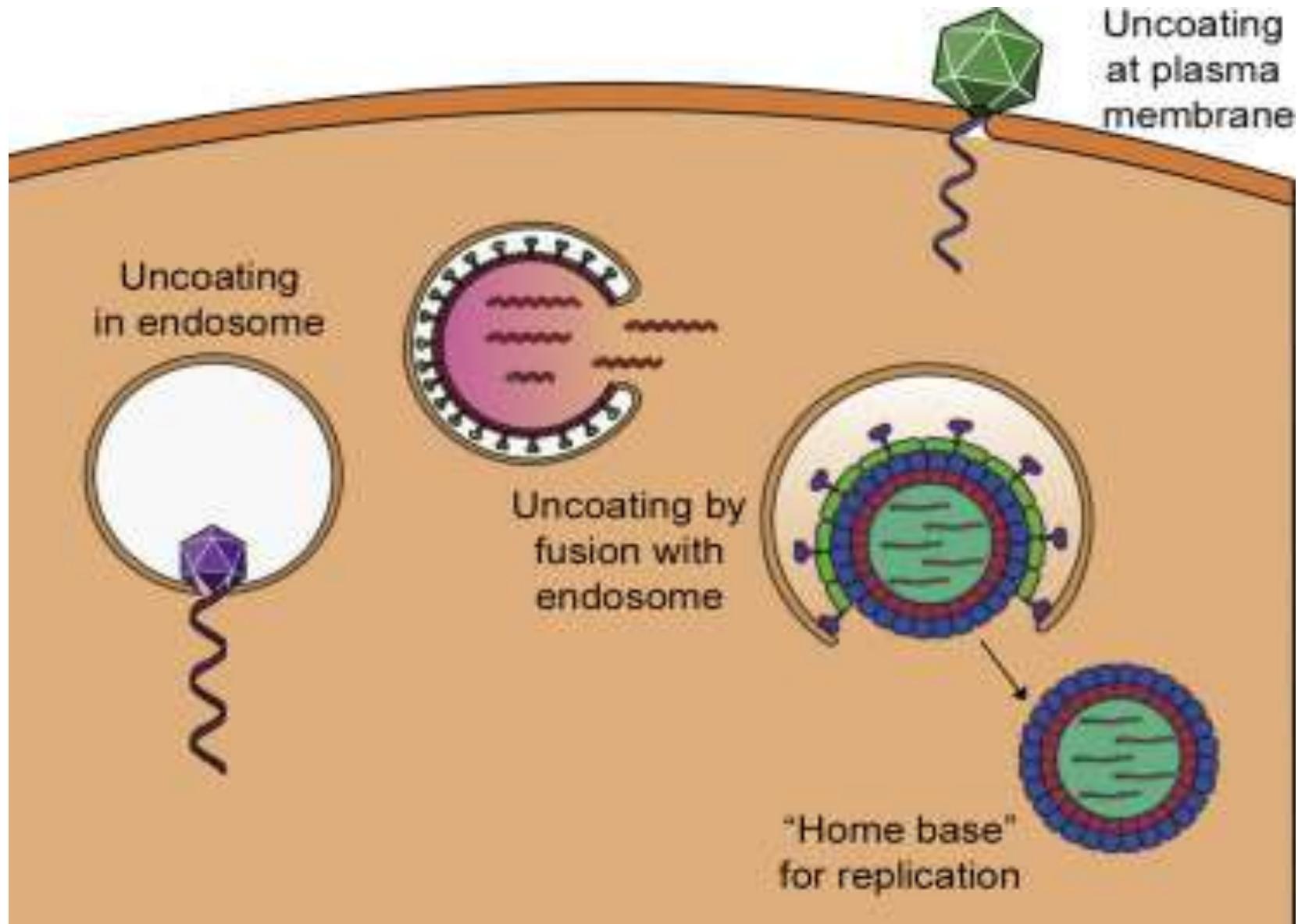
Lysis of endosome



3. Uncoating

- Partial exposure or complete release of viral nucleic acids for gene expression or
- Breakdown or removal of the capsid, causing the release of the virus genome into the cell to wherever genome replication and transcription will take place.
- The lysosomal enzymes play a major role in uncoating.
- Different viruses have different mechanisms.
- Certain viruses, including rhinoviruses, expand to form pores in the endosome through which the viral genome can escape.
- Influenza virus and other enveloped viruses induce fusion of the virion envelope with the endosomal membrane, releasing the viral genome.

- **Non-enveloped viruses-** like adenoviruses- As the endosome acidifies further, the penton base protein is released which triggers endosome lysis leading to escape of the partially disassembled core particle into the cytosol.
- **Pore formation-** Some non-enveloped viruses, such as picornaviruses, form pores in the cell membrane through which the viral RNA is then released into the cytosol.
- **Lysosomal uncoating-** Most viruses which enter the cell via endosomes escape before the endosomes arrive at the lysosome. Except Reoviruses which rely on enzymes found in lysosomes (proteases) to carry out their uncoating. The lysosomal proteases convert the reovirus particle into an infectious subviral particle (ISVP) with partial intact capsids which then penetrates the cytosol.



4. Replication

- Once uncoating has taken place, synthesis of viral NA starts. This occurs as three different stages with differences between different families of the viruses.
- **Early transcription and translation:** The proteins derived from this stage is mostly the enzymes required for virus replication.
- **Replication of Nucleic acid:** making copies of genome
- **Late transcription and translation :**The proteins produced during this stage are structural proteins and other proteins involved in assembly, maturation, and release from the cell.

5. Assembly

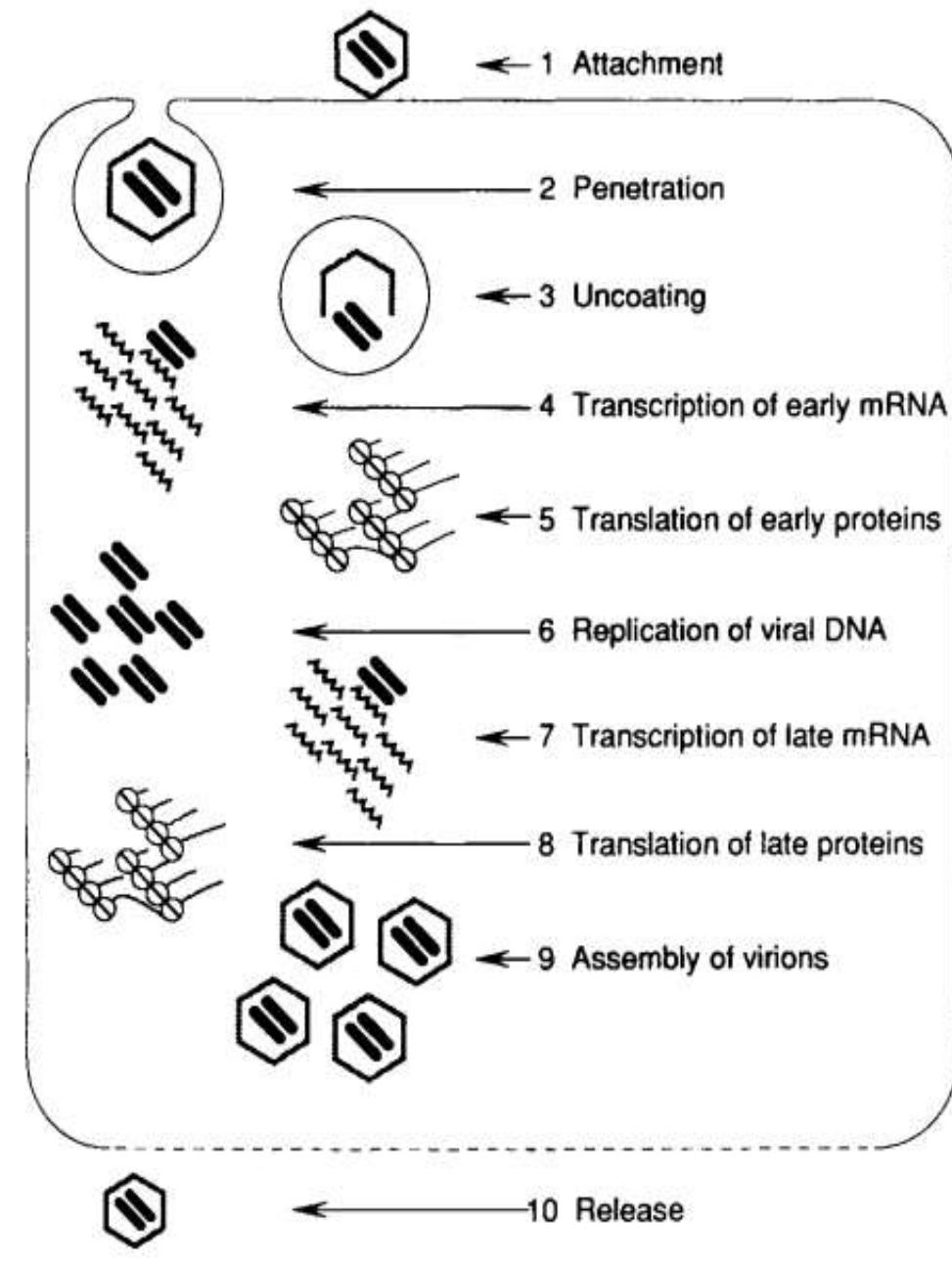
- Viruses are created from newly synthesized components, and to be released from the cell, those components must be collected at a particular site of the cell and undergo assembly to form an immature virus particle.
- Assembly can often occur alongside maturation and release.
- The location of virion assembly will depend upon the particular virus.
- Most non-enveloped DNA viruses assemble their nucleocapsid in the nucleus. Viral proteins are imported through nuclear pores to reach the site of assembly.
- DNA viruses with envelopes derived from the plasma membrane usually assemble there.
- Most RNA viruses assemble in the cytoplasm.

6. Maturation

- Final changes within an immature virion that result in an infectious virus particle.
- Structural capsid changes often resulting from specific cleavage of capsid proteins to form the mature products, which frequently leads to a conformational change in the capsid, or the condensation of nucleoproteins with the genome and these can be mediated by host enzymes or virus-encoded enzymes.
- For eg cleavage of HA proteins of influenza virus by cellular proteases.
- For some viruses, assembly and maturation are inseparable, whereas for others, maturation may occur after the virus particle has left the cell.

7. Release

- Virion released into the extracellular environment, where it can continue the cycle of infection with new cells.
- **By budding-** Enveloped viruses acquire the lipid membrane as the virus buds out through the cell membrane. Virion envelope proteins are picked up during this process as the virus is extruded. Budding may or may not kill the cell.
- **By exocytosis-** Viruses can bud from any of the membrane systems within the cell, including the rER, Golgi complex, or even the nuclear envelope. These viruses are not released by budding but it generally undergoes exocytosis to leave the cell.
- Non-enveloped viruses can also exit the cell via exocytosis.
- **Lytic viruses- most NE viruses** disrupt the plasma membrane and cause the lysis, or bursting, of the cell. This releases the nascent virions to infect new cells.



Replication of DNA viruses

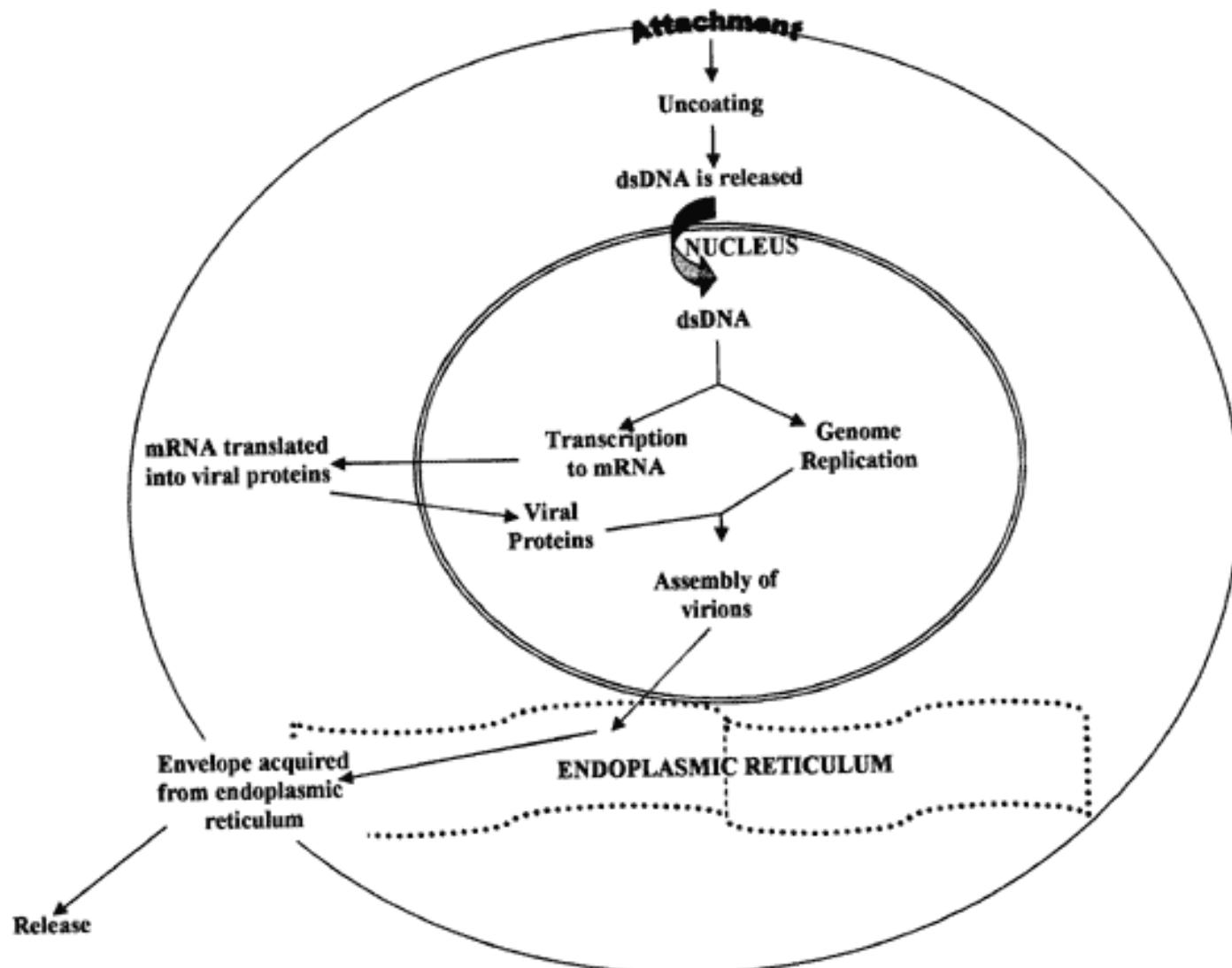
- **Location: nucleus.**
- Use host DNA-dependent RNA polymerase for transcription.
- The majority of poxviruses and iridoviruses have virion-encoded transcriptases that allow them to replicate in the cytoplasm.
- Replication of viral DNA is semi-conservative and symmetrical with both strands being replicated.
- Host DNA polymerases may be involved in replicating small- to moderate-sized genome viruses (papillomaviruses, polyomaviruses), whereas larger sized genome viruses usually code for their own polymerases (adenoviruses, herpesviruses, poxviruses).
- Viruses with no DNA polymerase of their own require actively dividing cells to replicate, since they must use the cellular DNA polymerase eg *parvovirus B19*

- Maturation of DNA viruses, with the exception of poxviruses and iridoviruses, occurs in the nucleus.
- Structural proteins are transported from the cytoplasm to the nucleus, where they interact with each other and with the genome and are assembled into capsids that surround the nucleic acid.
- Enveloped viruses complete maturation by budding through the nuclear membrane (iridoviruses) or the plasma membrane.

Double-Stranded DNA Virus Replication

- **Asfarviridae, Poxviridae, Iridoviridae, Herpesviridae, Papillomaviridae, and Adenoviridae**
- In general, replication takes place in the nucleus by host enzymes (for small viruses such as polyoma and papillomaviruses) or by virus-encoded replicases (adenovirus, herpesvirus).
- Replication of poxviruses and some iridoviruses takes place in the **cytoplasm resulting in the formation of inclusion bodies**, which contain necessary enzymes of viral origin associated with replication, such as **viral DNA-dependent DNA polymerases**.
- The dsDNA may be in the form of circular, linear, circularly permuted, or linear with covalently closed ends.
- The small circular genomes replicate bi-directionally in a manner similar to plasmids.

dsDNA Virus Replication

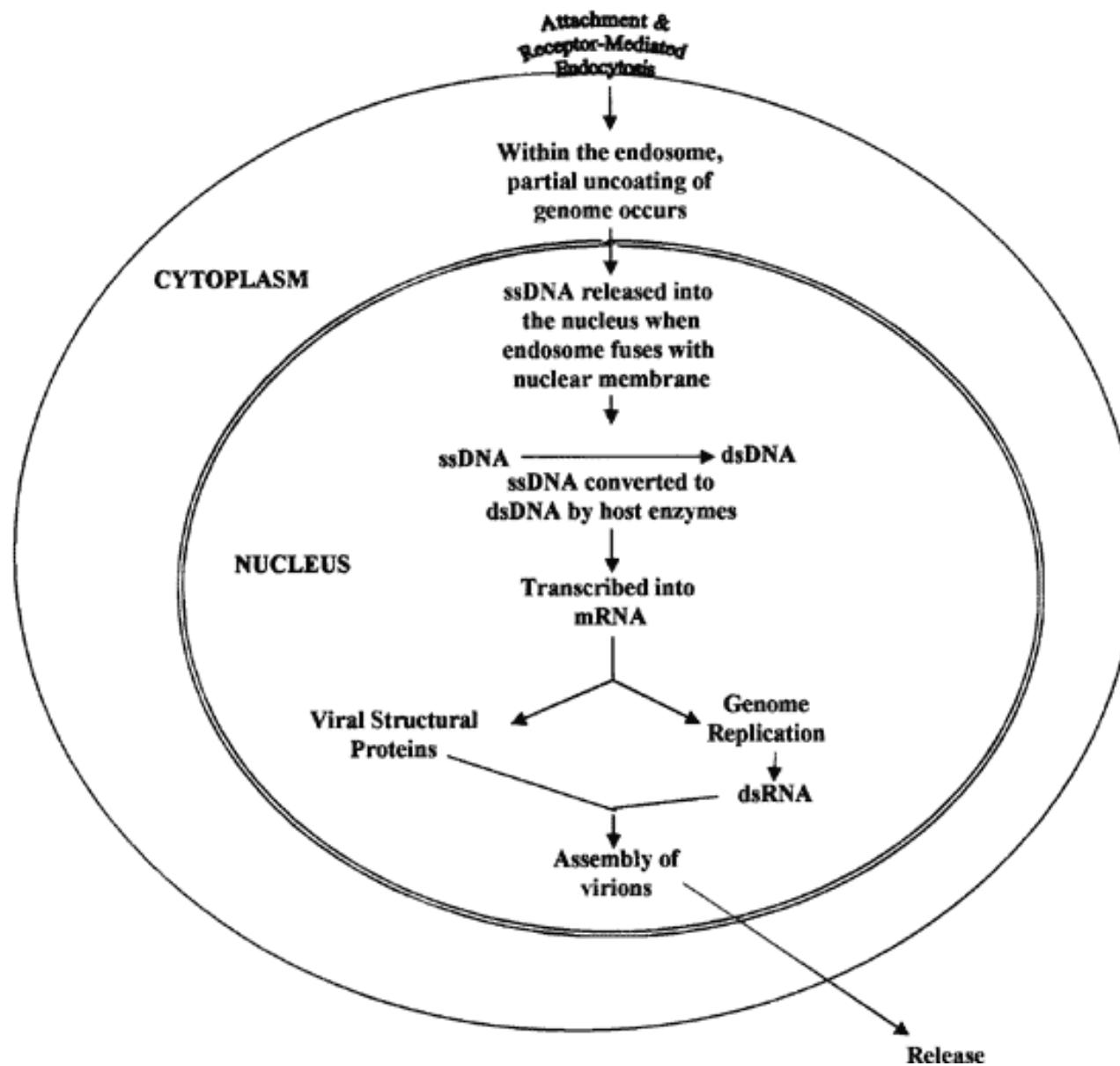


Single-Stranded DNA Virus Replication

- Includes **Circoviridae** and **Parvoviridae**.
- The ssDNA may be in the forms of **linear** single-component (parvoviridae), **circular** single-component (circoviruses).
- The single-stranded circular DNA of circoviruses replicates by a **rolling circle mechanism**.
- Replication occurs in the nucleus and involves the generation of a - sense DNA strand to serve as a template for the + sense DNA genome for the progeny virions. This involves the production of a dsDNA intermediate, known as the replicative form.

- Entry of the viral ssDNA into the nucleus stimulates its "repair" by host enzymes into the replicative form.
- In the case of the circular forms, the replicative form is associated with host histones and other nuclear proteins and thus "treated" as a host chromosome.
- Linear forms have derived mechanisms that allow the genome to be replicated without a loss of DNA following each replication.

ssDNA Virus Replication



Double-Stranded DNA Viruses with Reverse Transcriptase

- Includes the **Hepadnaviridae** with Genome partially ds non-covalently closed circular DNA,
- Following attachment, penetration, and partial uncoating of the virion, the partially dsDNA enters the nucleus and is completed by viral polymerase/ and/or cellular enzymes. Once completed, the backbone is sealed by the action of host ligase.
- In the nucleus, the viral DNA acts like a "mini-chromosome" following its association with host histones, etc. However, host DNA polymerase cannot replicate it.

- In the replication cycle a large mRNA called the **pg-RNA (pre-genomic RNA)**, which is longer than the DNA template from which it was transcribed due to the addition of a poly A tail is produced. It is this RNA intermediate that serves as the template for the virion DNA.
- Smaller mRNAs are also produced, leave the nucleus, and serve as template for translation, giving origin for viral polymerase and capsid protein. Partial assembly of capsids ensues.
- Some of the pgRNA is encapsidated into these recently assembled, immature virions.

- Within the capsid, a cDNA copy of the pgRNA is made by encapsidated reverse transcriptase (viral polymerase).
- Following synthesis of the first complementary cDNA strand, the viral polymerase degrades the pgRNA template and begins synthesizing the second DNA strand.
- The virions are then released from the cells by budding, containing a DNA genome that is only partially double stranded.

Replication of RNA Viruses

- Replication of most RNA viruses occurs strictly within the cytoplasm of cells
- Exceptions are orthomyxoviruses (bunyaviruses) that require factors from host DNA transcription and retroviruses that replicate via a DNA intermediate.
- Attachment is an electrostatic interaction between the virions and specific cell receptors.

- Viruses then enter the cell by receptor-mediated endocytosis or by fusing with the cell membrane or with the endocytic vesicle (enveloped viruses).
- Uncoating occurs in the cytoplasm, or during passage (translocation) through the cell membrane,(picornaviruses).
- The RNA of reoviruses, however, is never completely uncoated, but remains in viral cores during genome expression and replication.
- The genome of some RNA viruses is a single molecule of RNA (monopartite); in others, the genome is segmented (multipartite).

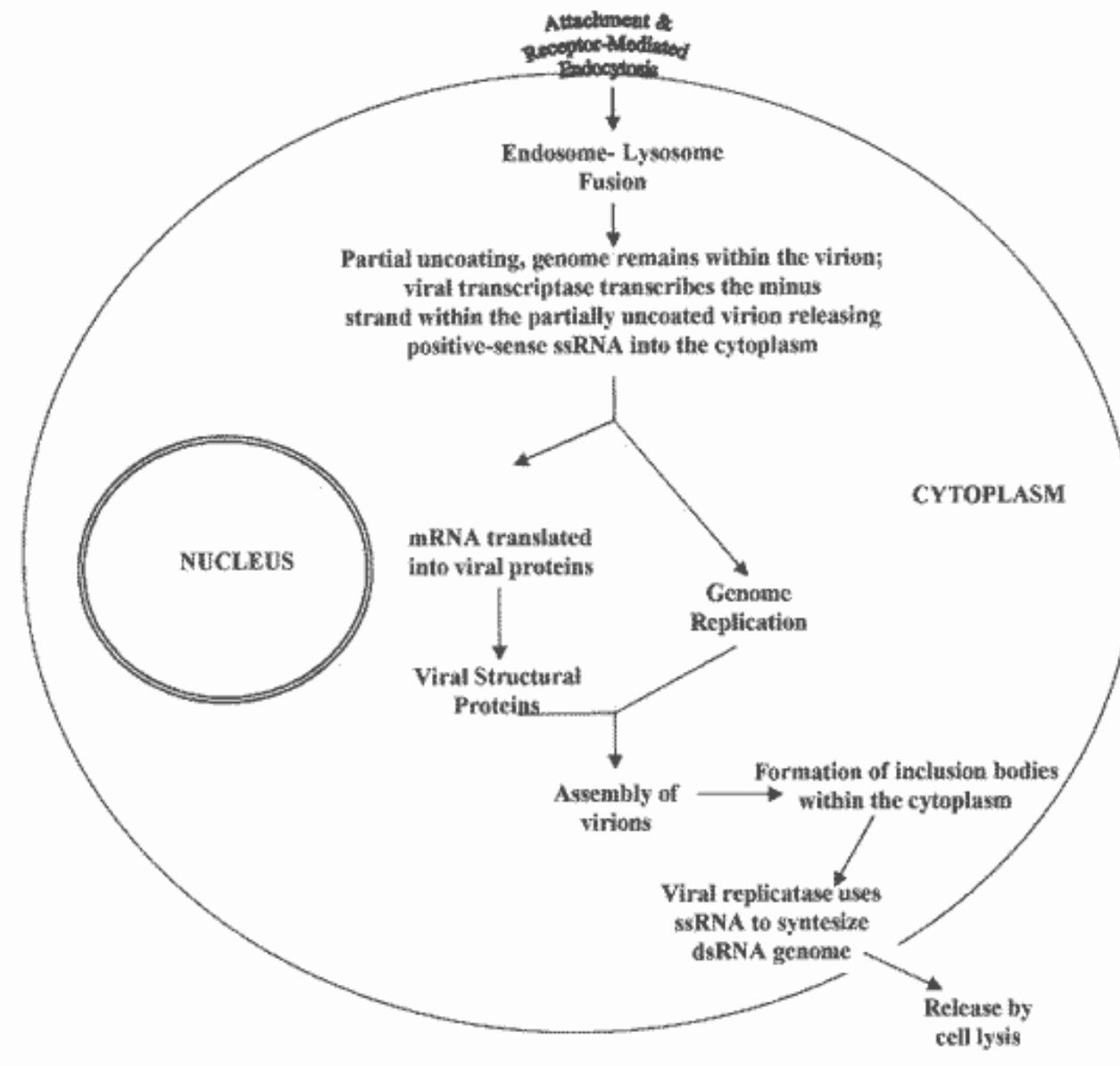
- The RNA of some animal viruses has mRNA function (+ sense) and can be directly translated, whereas the genome in others is antisense (- sense), and must first be transcribed into + RNAs by a viral-encoded RNA-dependent RNA polymerase (transcriptase).
- Retroviruses have the enzyme **reverse transcriptase** (RNA-dependent DNA polymerase) permitting the formation of a dsDNA intermediate (provirus DNA), which becomes incorporated into the host genome, and is subsequently transcribed into mRNA by the host DNA-dependent RNA polymerase.

- In general, replication of viral RNA is semi-conservative and proceeds via a replicative intermediate (R1). The R1 consists of parental viral RNA that serves as a template for the transcription of several RNA strands, which eventually "peel off" and serve as templates for the synthesis of viral RNA.
- Replication of double-stranded RNA of reoviruses is conservative and asymmetrical; only one strand is replicated, unlike double-stranded DNA. The replication processes requires RNA-dependent RNA polymerases (replicases) that are virus encoded.
- Maturation occurs in the cytoplasm with the viral RNA becoming associated with the capsid proteins forming the nucleocapsid. Enveloped viruses complete maturation by budding through the endoplasmic reticulum, Golgi apparatus, or the plasma membrane.

Double-Stranded RNA Viruses

- Includes **Reoviridae** and **Birnaviridae**.
- Attachment is via receptor-mediated endocytosis. The virion is partially uncoated and the core particle remains in the endocytic vesicle (ISVP)
- Replication is by a conservative mechanism, the dsRNA serves as a template for the production of mRNA by a viral RNA-dependent RNA polymerase.
- Replication does not involve the formation of R1 intermediates. **No free dsRNA is formed in the cytoplasm of the host cell.**
- All have segmented, linear genomes. Each segment corresponds to a monocistronic mRNA.
- All of the genomes are linear, but may be two-component (Birnaviridae), or multi-component (reoviruses have 10 – 12 components).

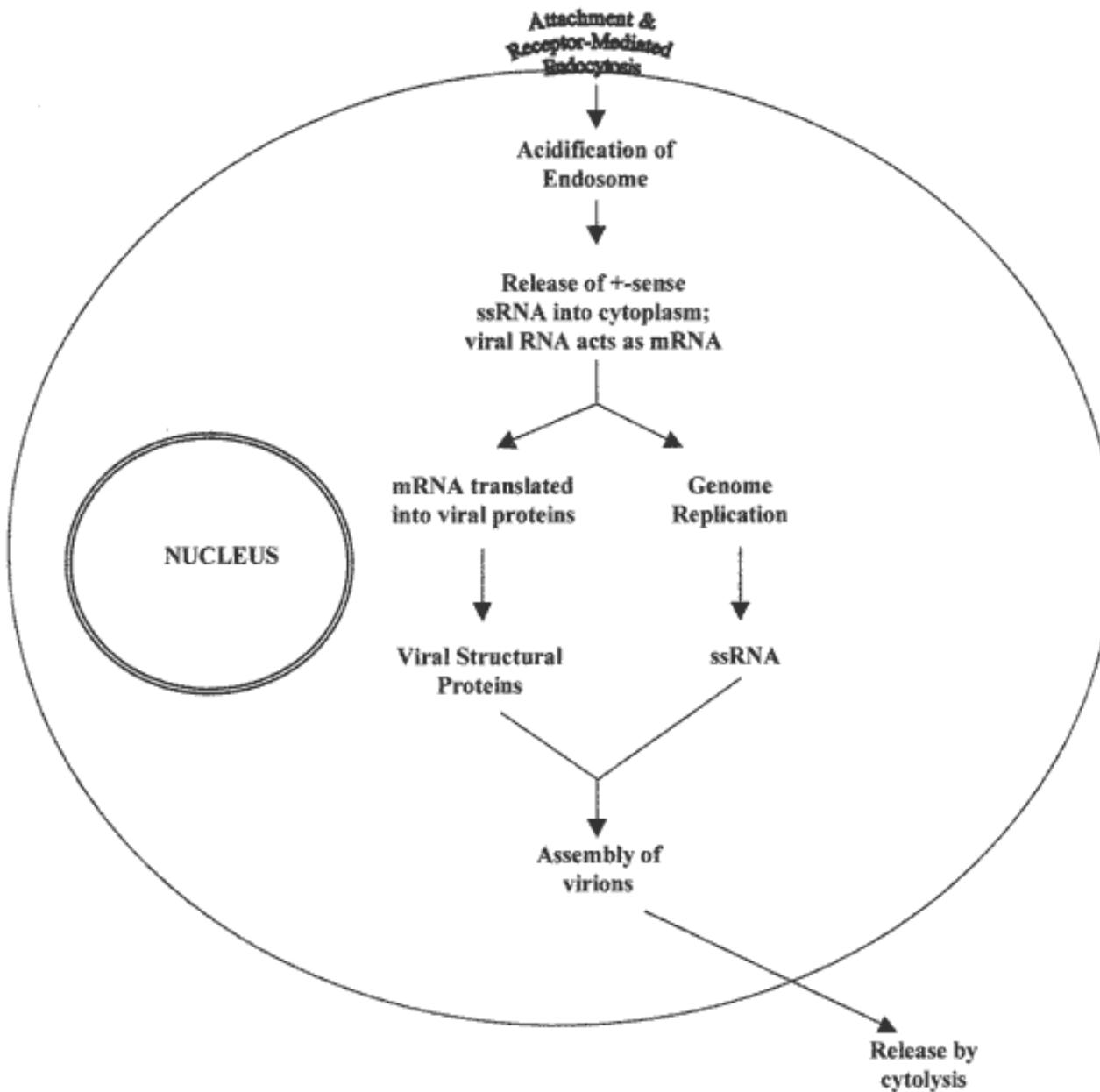
Double-stranded RNA Virus Replication



Single-Stranded Positive Sense RNA Viruses

- Includes :**Caliciviridae, Picornaviridae, Astroviridae, Flaviviruses, Coronaviridae, Togaviridae, and Arteriviridae.**
- Attachment is via receptor-mediated endocytosis. There, the virion is uncoated and the ssRNA released to the cytoplasm.
- The viral genomes that are messenger-sense are totally or partially translated into proteins as the first step of virus replication.
- Picornaviruses and Flaviviruses possess a + sense RNA as genome, which behaves as a polycistronic mRNA. The genome is directly translated into one large polypeptide, which is co-translationally cleaved into a number of proteins by viral encoded or host cell proteases.
- Coronaviruses have a complex transcription pattern, involving several rounds of translation in order to complete the replication cycle.

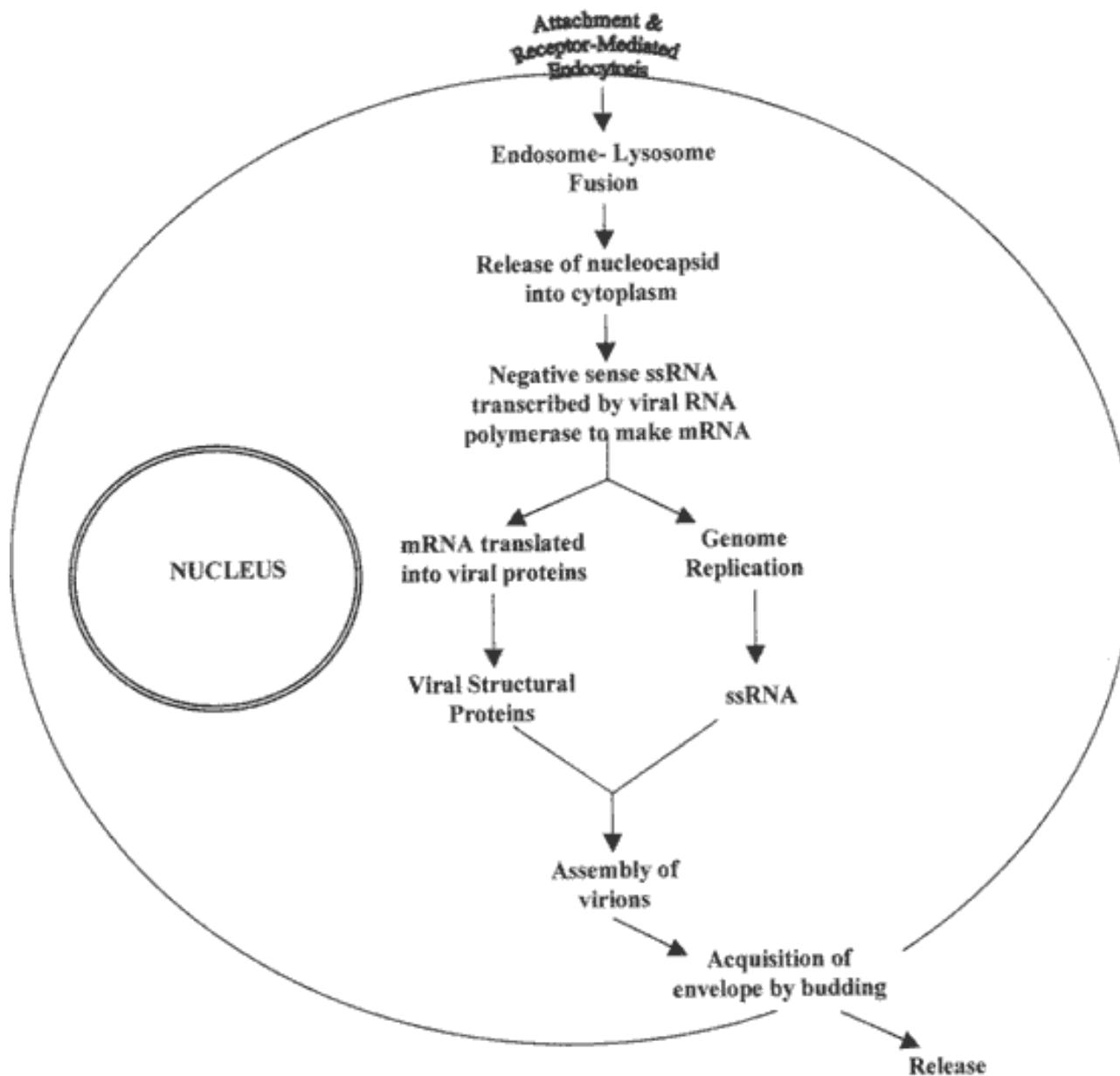
Positive-Sense ssRNA Virus Replication



Single-Stranded Negative Sense RNA Viruses

- Includes: **Orthomyxoviridae, Rhabdoviridae, Paramyxoviridae, Bornaviridae, Filoviridae, Deltavirus, Arenaviridae, and Bunyaviridae.**
- As the genomes are negative-sense, they are not translated. Therefore, these viruses need to bring their replicases in the virion to proceed with transcription and replication of the genome.
- Orthomyxoviruses have segmented genomes. The first step in the replication process is the transcription of – sense RNA by a virus encoded RdRp
- Rhabdoviruses have non-segmented genomes. Replication still requires the transcription via a viral RdRp.
- In the case of the ambisense viruses, the transcriptase is encoded within the positive-sense portion that will eventually mediate the transcription of the negative-sense regions.

Negative-Sense ssRNA Virus Replication



Single-Stranded Positive Sense RNA Viruses with Reverse Transcriptase

- Includes the vertebrate-associated viruses in the family **Retroviridae having** diploid linear ssRNA held together by protein.
- It is 5'-capped and has a 3' poly A tail, and has four characteristic coding regions (*gag-pro-pol-env*)-*gag* (*group specific antigen: matrix protein, nucleoprotein, capsid*) genes; *pro (protease) gene*; *pol (reverse transcriptase and RNase-H)*; and *env (envelope, receptor binding) genes*.
- The conversion of RNA to ssDNA and then to dsDNA is mediated by the viral enzyme reverse transcriptase. The resulting dsDNA, called **provirus DNA**, is integrated into the host chromosome by the viral enzyme integrase.
- Once integrated into the host genome, the viral dsDNA remains latent until "triggered" into active virion production. The provirus is then transcribed into mRNA by cellular RNA polymerase II.

ADENOVIRIDAE

Baltimore Group I

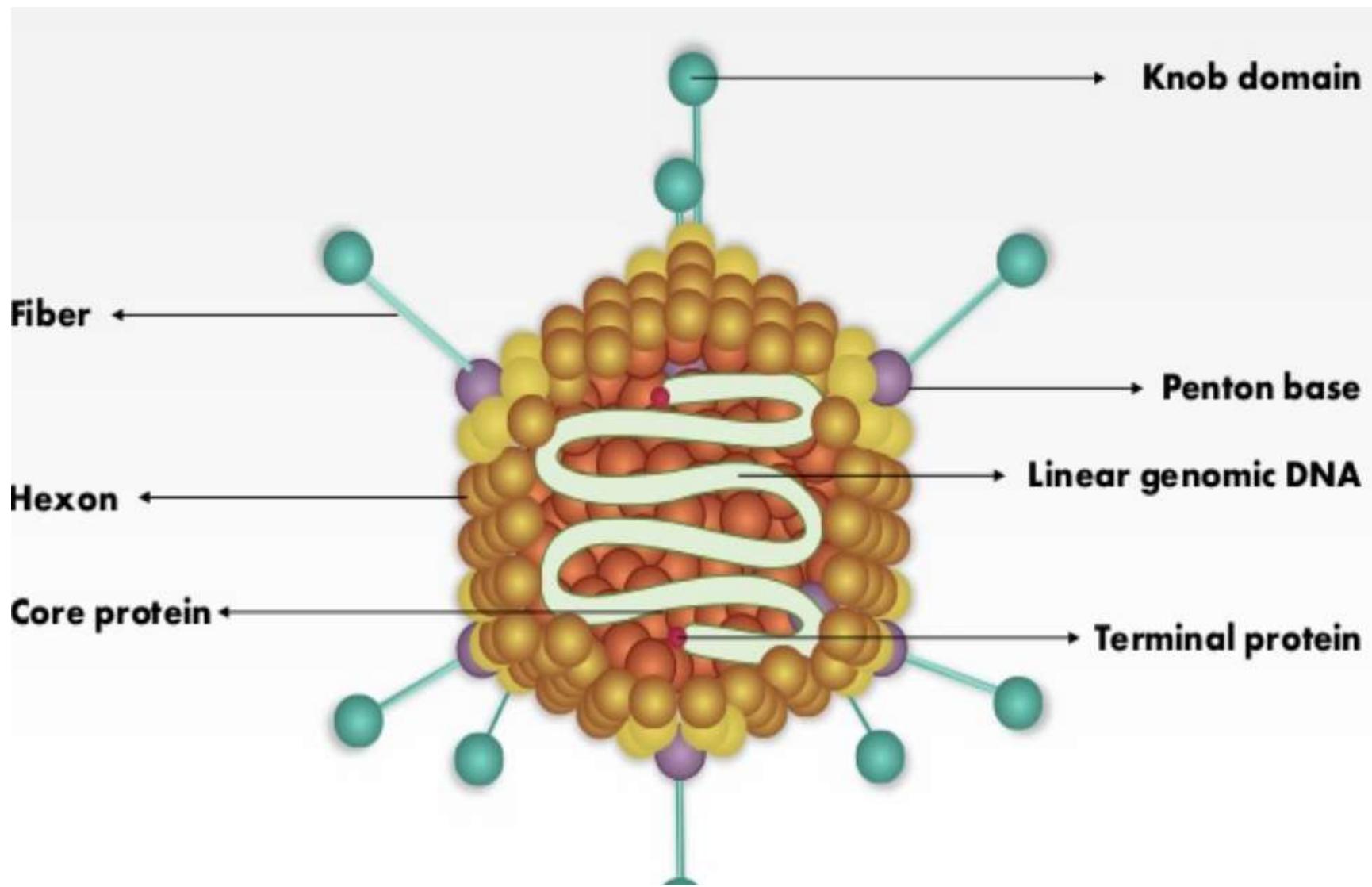
- 1. *Barthadenovirus (formerly Atadenovirus)*
occur in squamate reptiles, birds, ruminants,
marsupials and tortoises**
- 2. *Aviadenovirus (Egg drop syndrome virus)***
- 3. *Ichtadenovirus- single fish adenovirus***
- 4. *Mastadenovirus (Canine adenovirus 1- ICH)***
- 5. *Siadenovirus - infect birds, frog and tortoise***
- 6. *Testadenovirus occur in turtles***

Adenoviridae

- Genera –
- **Mastadenovirus** – Mammalian adenoviruses
- Single penton fiber from vertex
- **Aviadenovirus** – Avian adenoviruses
- Each penton is bifurcated – giving the appearance of 2 fibers extending from each penton base
- **The adenoviruses are named after the human adenoids, from which they were first isolated.**
- Adenoviruses are popular virus vectors, for example in vaccination against the coronavirus SARS-CoV-2.

Virion Morphology

- Virion shows penton fiber peplomers from the vertices
- Penton fiber knobs contain cell binding ligands that are responsible for viral attachment to host cell receptors.
- Associated with hemagglutinating activity.
- **Non-enveloped, icosahedral**, 80-100 nm in diameter, 252 capsomers, 240 hexons occupying faces, and edges & 12 pentons occupying vertices.
- Virus codes for about 40 different proteins and there are 13 structural proteins



Viral Genome

- The genome is not segmented and consists of a **single molecule of linear double-stranded DNA**.
- The genome has a guanine + cytosine content of 48-61% for *Mastadenovirus* and 54-55% for *Aviadenovirus*.
- The genome has terminally redundant sequences with **inverted terminal repeats (ITR)** varying in length from ~ 40 – 200 bp, where the origin of replication is present.
- The genome has a virus coded **terminal protein (TP)** which is covalently linked to the 5'-end of each DNA strand.
- The genetic organization of the **central part** of the genome is **conserved** throughout the family, whereas the **terminal parts** show **large variations** in length and gene content.

Viral Replication

- Fiber attaches to specific receptor on the cell surface.
- **Receptors**-Major histocompatibility complex (MHC) molecules and sialic acid residues
- Virus is then taken into endocytotic pits (endocytosis) where it enters the cytoplasm.
- In the cytoplasm pentons are removed and core moves to the nucleus via microtubules attached to the hexon capsomers.

- In the nucleus the genome is transcribed by **cellular RNA polymerase II** according to a complex program involving both DNA strands and a number of primary transcripts are made.
- The transcripts are spliced, capped, and polyadenylated, giving several different mRNAs.
- These mRNA move to the cytoplasm.
- The transcription is in two phases Early and late.

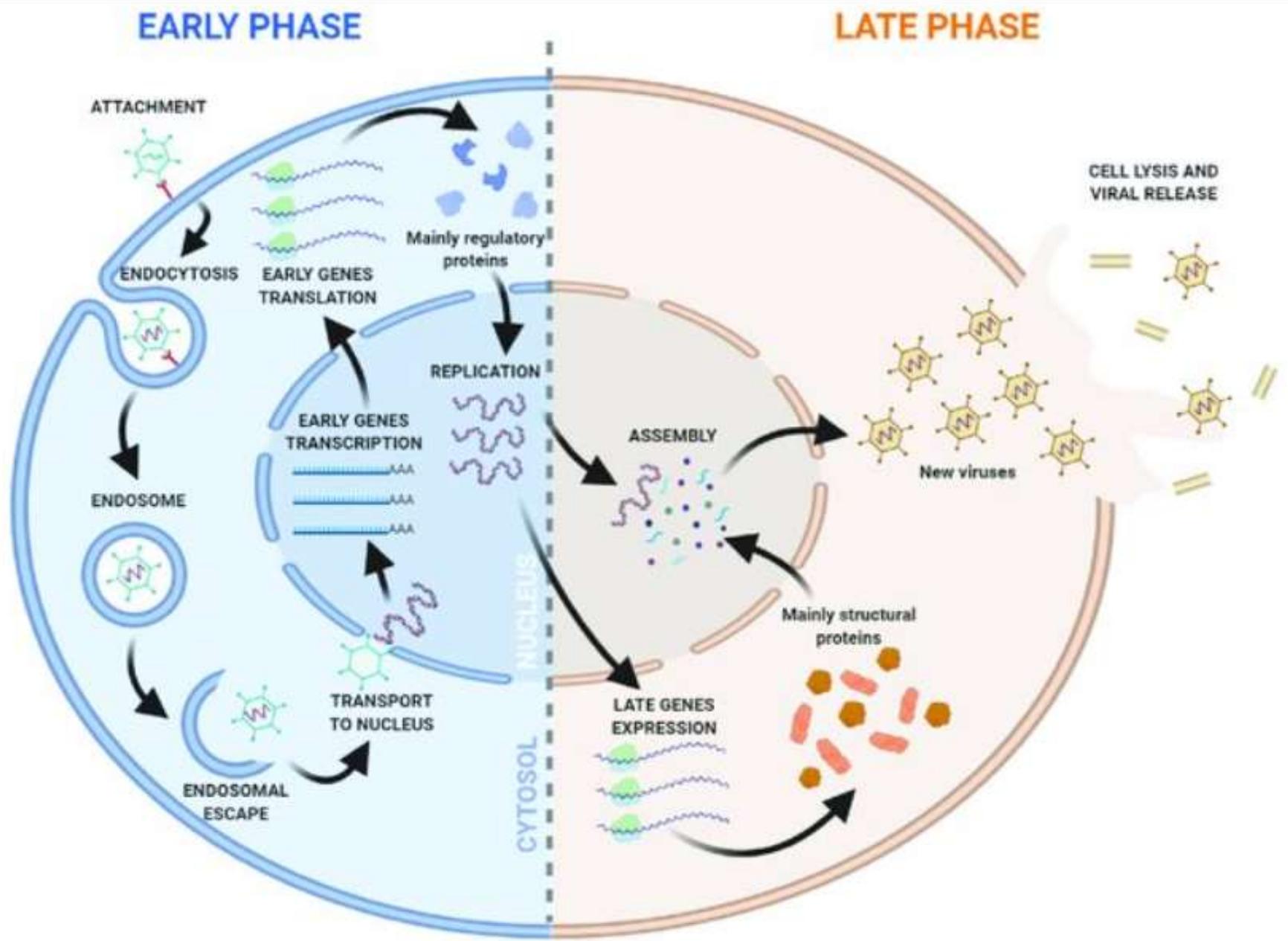
- Adenovirus genes are organized into **transcription units (TU)**.
- The **five early transcription units** include early region 1A (E1A), E1B, E2, E3, and E4.
- **Intermediate transcription units**, including IX, IVa2, L4 intermediate, and E2 late, are transcribed at the onset of DNA replication.
- A single **late transcription unit** (major late) generates five populations of late mRNAs, L1–L5.
- Most of the adenovirus transcription units are transcribed by RNA polymerase II and give rise to multiple mRNAs that are differentiated by alternative splicing or alternative poly(A) sites

E1A region of the viral genome codes for protein which has following three properties:

- Induction of mitosis of cell.
 - To protect infected cells from host defence.
 - Synthesis of viral proteins for DNA replication.
-
- **Protein E1B** targets a tumor suppressor protein p53
 - **E1A and E1B products can cause cell transformation and oncogeneity.**
-
- The **E3** region is not essential for adenovirus replication in cell cultures and can be deleted or replaced without disrupting viral replication *in vitro*.
 - **E3** proteins interact with host immune defense mechanisms thus modulating the host response to adenovirus infection

- Once the early genes have liberated adequate virus proteins, replication of the adenovirus genome can occur.
- **Viral DNA replication uses a virus-encoded protein (TP) as a primer and another virus-encoded protein as DNA Polymerase.**
- In **primate mastadenoviruses**, there are one or two virus-associated (VA) RNA genes, which are transcribed by RNA polymerase III. These encode RNA products that facilitate translation of late mRNAs and block the cellular interferon response.
- The late phase of the adenovirus life cycle is focused on producing sufficient quantities of structural protein to pack all the genetic material produced by DNA replication.

- Once the viral components have successfully been replicated the virus is assembled in the nucleus into its protein shells.
- Viruses are then released from the cell as a result of viral induced cell lysis.
- Viral DNA replication, mRNA transcription and virion assembly occur in the nucleus, utilizing both host and virus-encoded factors. This results in the formation of **basophilic and / or acidophilic intranuclear inclusions**.



Adenoviridae - Pathogenesis

- All adenoviruses- NARROW HOST RANGE – HIGHLY HOST SPECIFIC.
- Numerous adenoviruses can cross host species barriers (reported recently)
- Mostly subclinical infections with occasional URT infections, GI tract diseases
- Virus persists in the pharyngeal region, particularly associated with the lymphoid tissues of respiratory tract and in the GALT tissues – enteric infections

Adenovirus – Infectious Canine Hepatitis (ICH)

- The infection as encephalitis in enzootic form was reported by Green in 1925.
- ICH was first described in dogs from Sweden by the veterinary scientist **Carl Swen Rubarth** in the 1940s and then worldwide reported in the following years also as “**Rubarth’s disease**”
- He also distinguished it from Canine Distemper virus using ferrets that are resistant to ICH, but not to Canine Distemper.
- **Hosts** – Family *Canidae* – domestic and wild,
- *Ursidae* – bears and *Mustelidae* families (Otter, mink, ferrets)

- *Canine adenovirus* species has been recently taxonomically renamed as ***Canine mastadenovirus A***.
- There are two important canine adenovirus infections caused by different canine adeno virus serotypes-
- **CAV-1:** It causes **Infectious Canine Hepatitis** which may be acute or chronic, respiratory or ocular disease, encephalopathy and interstitial nephritis.
- **CAV-2:** CAV-1 is antigenically related to but distinct from CAV-2 - causes **Infectious Canine Tracheobronchitis** or **kennel cough**.

Disinfecting agents

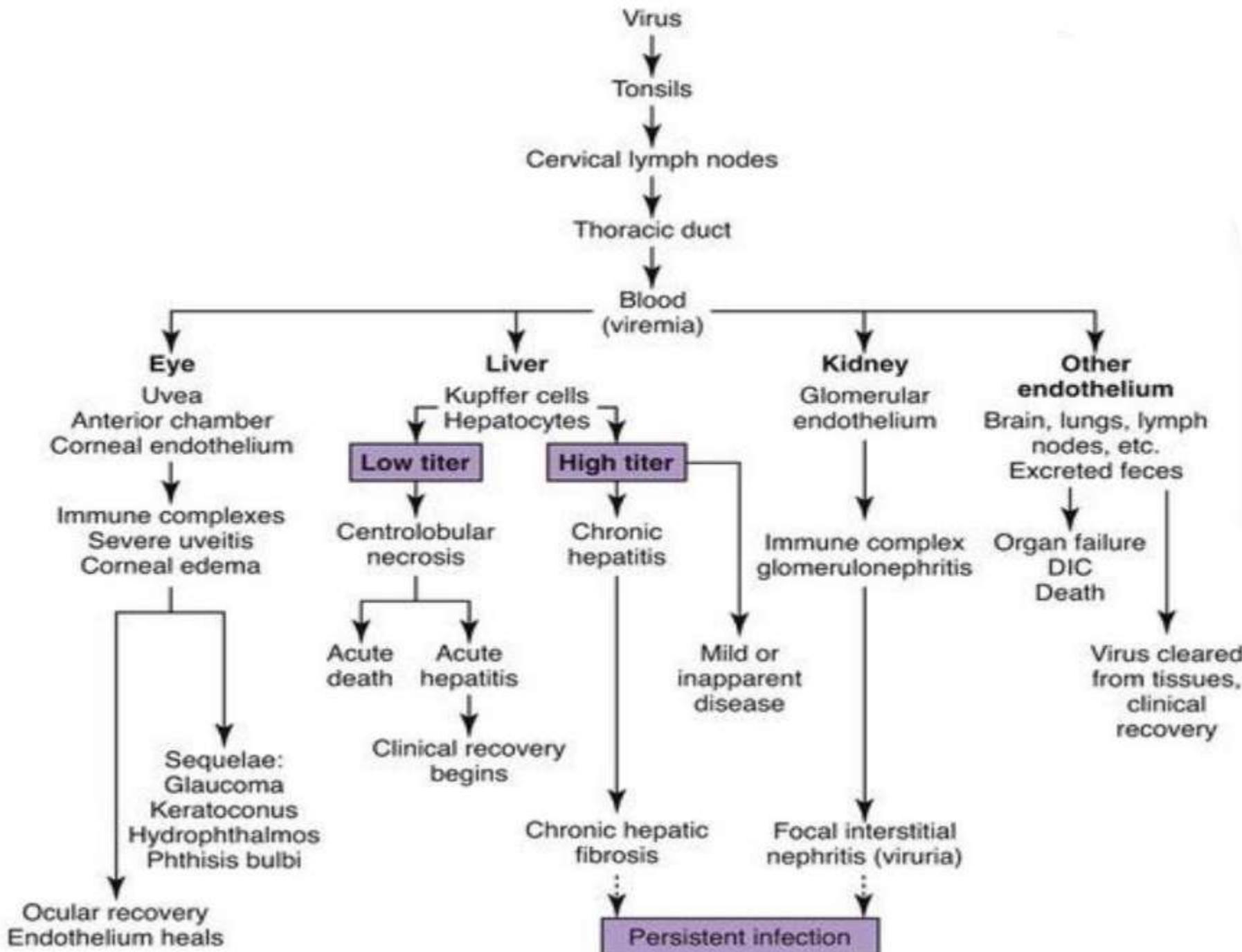
- **STABLE VIRUS –**
- Stable in the environment .
- Relatively resistant to disinfection (Alcohol, chlorhexidine, detergents) .
- Stable in GI tract- can withstand low pH, bile acids and proteolytic enzymes
- Susceptible to heat (steam cleaning) and disinfection by quaternary ammonium compounds.
- CAV-1 is susceptible to iodine, phenol, NaOH and bleach

Transmission

- Present in respiratory secretions, urine, feces, and saliva
- Infection is by inhalation and ingestion. Spread is by direct and indirect contact.
- Ingestion of urine, feces or saliva of infected dogs is the main route of infection.
- It may be present in kidney and excreted in urine for 6-9 months post infection.
- However, virus may be acquired via CONJUNCTIVAL OR AEROSOL ROUTES.

Pathogenesis

- Upon entry through mouth or nose, the virus replicates initially in tonsils and Peyer's patches (oropharyngeal lymph nodes) producing a viremia with secondary localization and replication in the liver (hepatocytes), endothelial cells within a variety of tissues occurs, such as the lungs, liver, kidneys, spleen, and eye with resultant hemorrhage, necrosis, and inflammation. .
- The virions are released by cell lysis, which leads to tissue injury and disseminated intravascular coagulation (DIC)
- Recovery from infectious canine hepatitis (ICH) results in lasting immunity.



Clinical Forms

- I.P from 4-10 days.
- Course 5-7 days in uncomplicated cases and is long in presence of concurrent infection and in dogs with chronic active hepatitis.
- Morbidity rate (less than 5%)
- Mortality rate 10%–30%

1. Per acute form: Sudden death due to damage of vital organs as brain and lungs or due to shock or hepatic coma.

2. Acute form: Biphasic Fever “Saddle type curve“, anorexia, and thirst.

- Abdominal pain, vomiting and diarrhea.
- Petechiae of the oral mucosa, as well as enlarged tonsils.
- S/C edema of the head, neck, and trunk.
- Dogs with acute disease either recover or die within a 2-week period.

- **Hepatic involvement:** Abdominal tenderness, distention due to serosanguinous ascites and hepatomegaly & icteric mucous membrane.
- **Non-suppurative encephalitis** (uncommon) due to vascular damage of the brain tissue.
- **Eye involvement:** Corneal edema, ulceration or perforation and anterior uveitis result in blepharospasm, photophobia and serous ocular discharge (transient uni or bilateral corneal opacity or **blue eye**).
- Corneal edema (“blue eye”) occurs in the first week of illness and results from replication of virus within corneal endothelial cells
- Conjunctivitis, serous discharge from the eyes and nose.
- Death due to hepatic insufficiency and hepato-encephalopathy.

- The **chronic form** - occurs in dogs with partial immunity, with death due to hepatic failure weeks (subacute disease) or months (chronic infection) after initial infection.
- The antibody response appears 7 days after infection and limits tissue damage.
- Viral persistence within the renal glomeruli, uveal structures of the eye (the iris and ciliary body), and the cornea can trigger immune complex formation in dogs that recover from acute illness.
- This leads to **glomerulonephritis** with proteinuria, severe uveitis, and persistent corneal edema in some surviving dogs.
- Glomerulonephritis usually occurs about 1 to 2 weeks after the acute signs resolve.

Clinical Signs



Diagnosis

- **Diagnosis** – Based on clinical signs, lab findings
- **Sample collection** :- Urine, faces, saliva, ocular and nasal discharge in case of live animals
- Spleen,lungs,lymph node,kidney in case of dead animals
- **Virus Cultivation**:- Madin-Darby canine kidney cells (MDCK)
- Adenoviruses infecting susceptible cells cause similar cytopathic effects, i.e., early rounding of cells and aggregation or lysis of chromatin, followed by the later appearance of characteristic eosinophilic or basophilic nuclear inclusions.
- **Serology**:- ELISA test, VNT test, HI test
- **Molecular technique**:- PCR

Immunity

- Recovered animals are immune to the systemic form of the disease but may not resist an aerosol challenge and may develop respiratory disease
- Maternal antibody interferes with active immunization until puppies are 9-12 weeks of age.
- Vaccines should be administered every 3 to 4 weeks from 6 weeks of age, with the last vaccine given no earlier than 16 weeks of age
- **Both inactivated virus and attenuated/modified virus CAV-1 vaccines are used**
- **Annual revaccination is recommended**
- Attenuated CAV-2 vaccines provide cross-protection against CAV-1



live attenuated **canine distemper** virus, live attenuated canine **adenovirus 2** and live attenuated **parainfluenzavirus**, live attenuated canine **parvovirus1&2**, inactivated **Leptospira canicola** and inactivated **Leptospira icterohaemorrhagiae**.



Canine Infectious Tracheobronchitis or Kennel cough

- Kennel cough is mostly self-limiting respiratory disease of dogs
- Etiologic agents – CAV-2, Canine parainfluenza 2, *Bordetella bronchiseptica*, *Mycoplasma canis*
- Other contributing factors include – CAV-1 and Canine morbillivirus (canine distemper)
- Transmission – highly contagious – via aerosols

- **Clinical Features –**
- **IP – 30 days**
- **Uncomplicated ITB**
- **Mild respiratory disease – laryngitis, tonsilitis, pharyngitis, tracheitis, bronchitis**
- **Paroxysms of dry, hacking, mostly nonproductive cough, followed by retching**
- **Rhinitis with serous to mucopurulent nasal discharges**

Canine Infectious Tracheobronchitis - ITB



- Usually resolves in 2 weeks
- **Diagnosis** – similar to CAV-1
- Palpation of trachea stimulates coughing attack
- **Immunity** – **CAV-2 vaccination**
–MLV is routinely incorporated into vaccine protocols
recommended for all dogs

Egg drop syndrome' 76

- Egg drop syndrome '76 is caused by **duck atadenovirus A** (formerly duck adenovirus A), a member of the genus **Atadenovirus**
- The lesions are minimal and are **confined to the reproductive tract of hens**.
- In these birds there may be inactive ovaries, atrophy of the oviducts, and edema and white exudates in the uterus (shell gland).
- Laid eggs may be paler than normal, rough, thin-shelled, soft-shelled, or shell-less by apparently healthy chickens

- Lesions in pouch shell glands and oviduct where epithelial cells of the uterus, isthmus, and vaginal gland region become necrotic and contain **intranuclear inclusions**
- The main route of transmission is through contaminated eggs. Droppings also contain virus, and contaminated fomites such as crates or trucks can spread virus.
- Vertical transmission thru eggs so detection of source is difficult.



Abnormal eggs



Diagnosis

- Loss of egg production with abnormal eggshells suggest EDS
- Serologic tests include hemagglutination inhibition using fowl RBC, ELISA, and serum neutralization.
- **Cultivation of Virus** : Duck and goose embryonated eggs (preferred) and also in duck kidney or fibroblast cell lines.
- The presence of virus is indicated by hemagglutination of avian red cells (HA test).
- Diagnosis is made with the hemagglutination inhibition test (HI test). It is used to screen flocks but negative tests do not indicate that birds are necessarily free of infection.

Inclusion body hepatitis (Litchi disease) and Hepatitis Hydropericardium syndrome (HHS) (Angara disease)

- It is a condition caused by **Fowl adenovirus 4**, possibly in combination with an RNA virus and immunosuppression caused by Chick Anaemia Virus or Infectious Bursal Disease.
- The disease was initially reported from Angara Goth, Pakistan, and then from India during 1994, in the poultry belt of Jammu and Kashmir, and thereafter, from almost all parts of the country, causing heavy economic losses to the poultry industry.
- The disease occurs predominantly in **broilers of the age group of 3-5 weeks**, characterized by sudden onset of high mortality up to 80%.
- It is transmitted both vertically and horizontally

Signs

- Sudden increase in mortality.
- Lethargy.
- Huddling with ruffled feathers.
- Yellow mucoid droppings.

Post-mortem lesions

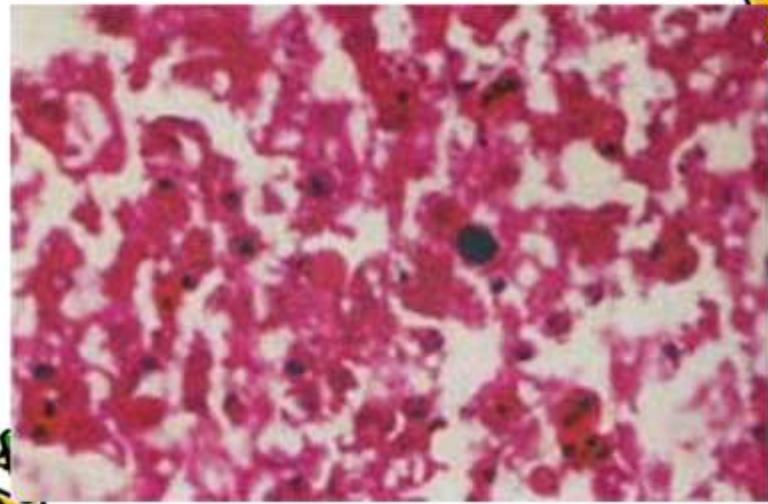
- Excessive **straw-coloured fluid** distending the pericardium (up to 10 mls) -hydropericardium
- Enlarged, pale friable liver containing multiple pale or hemorrhagic foci and enlarged kidney.
- Congestion of the carcass.
- Lungs oedematous.



Haemorrhage on pericardium



Liver: Hepatocytes showing basophilic intranuclear inclusion bodies. (H & E $\times 400$)



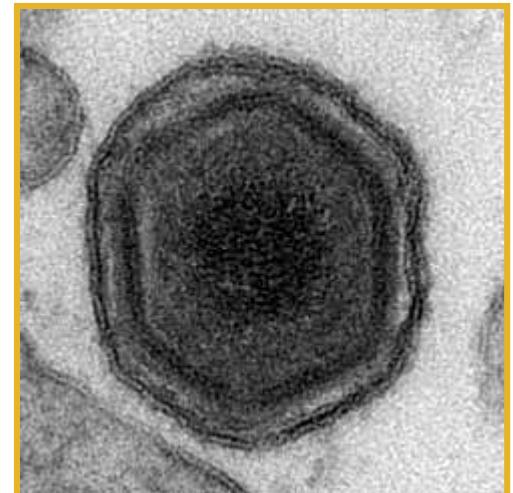
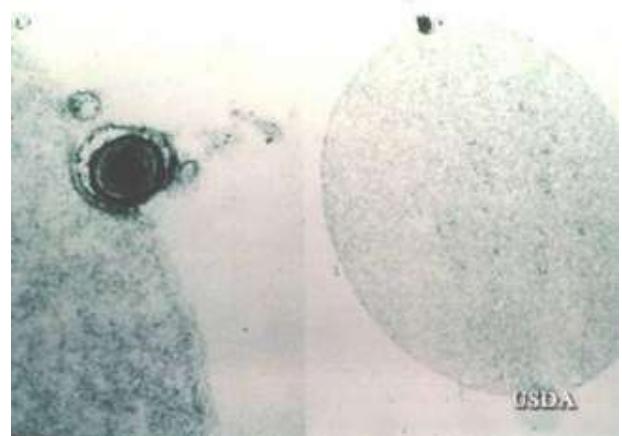
FAMILY ASFARVIRIDAE

(African Swine Fever virus)

- **Baltimore group I**
- The name Asfarviridae is derived from **African Swine Fever And Related viruses**.
- ASFV was first classified as a member of the family *Iridoviridae* because of its large size, cytoplasmic location, and double-stranded DNA genome.
- However, studies of replication strategy and the genome revealed similarities with the *Poxviridae*, although the viruses differ structurally.
- ASFV was therefore placed as the species *African swine fever virus* into a separate virus family, the *Asfarviridae*, of which it is the sole member of the single genus, the ***Asfivirus genus***.

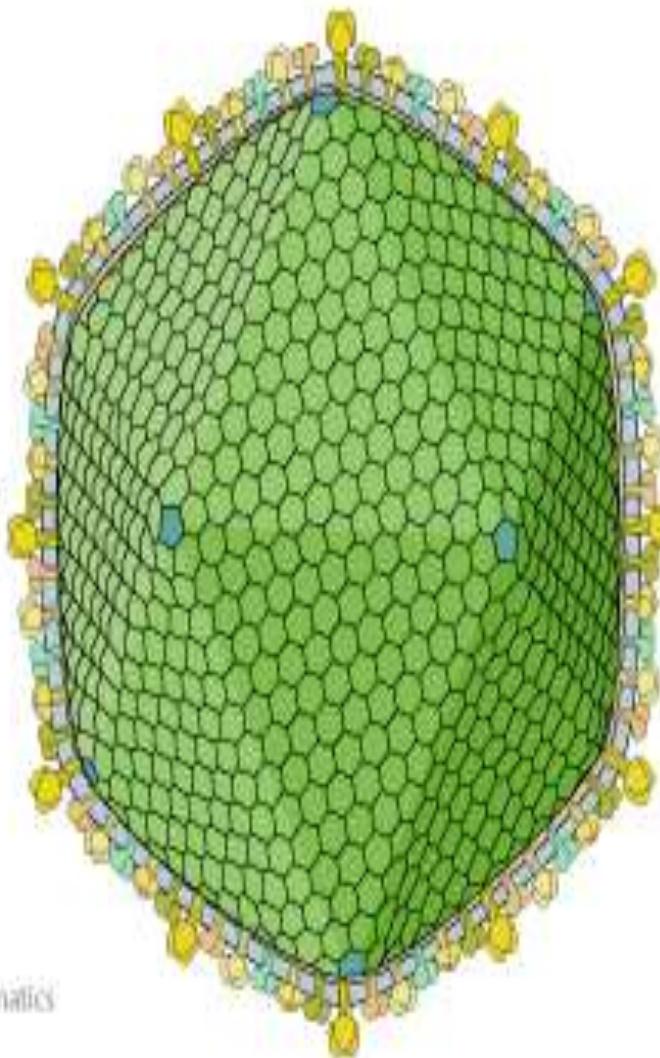
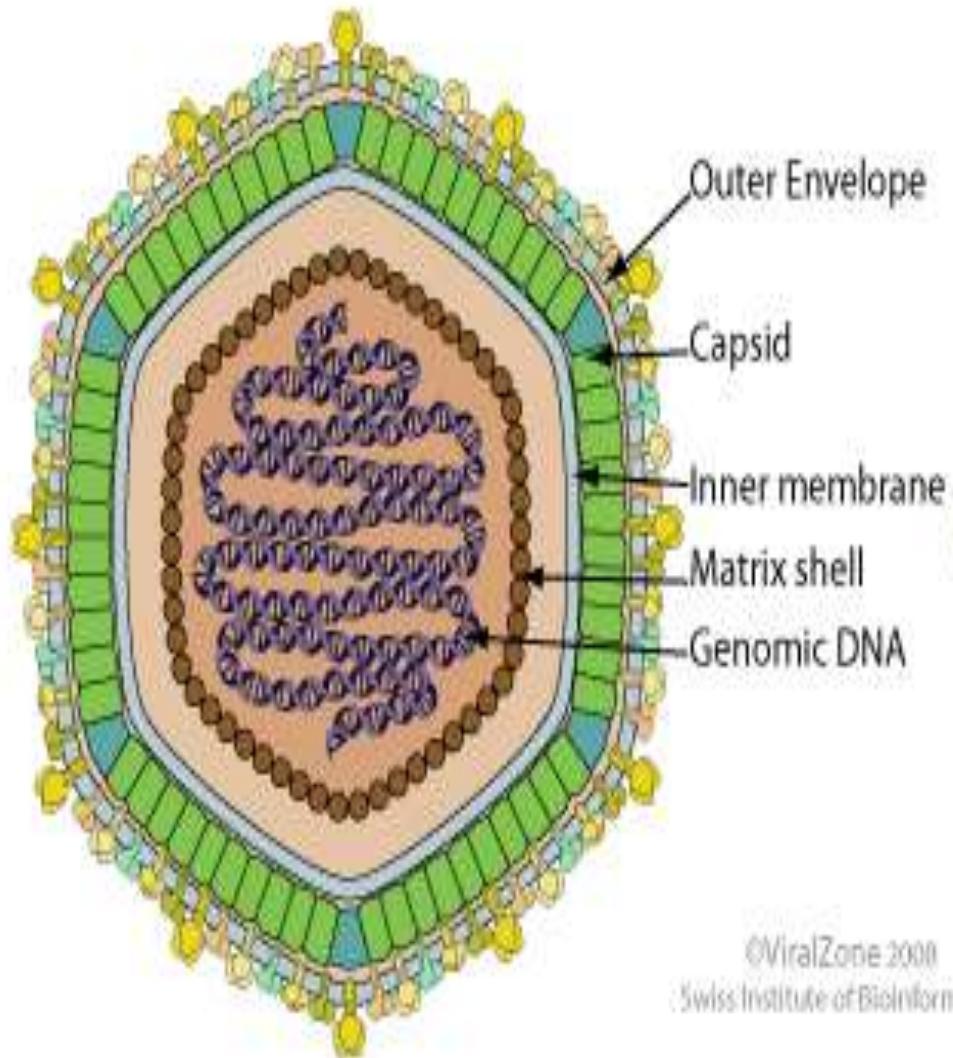
ASF virus

- Large (~ 200 nm) spherical or pleomorphic, lipoprotein-enveloped, icosahedral symmetry double-stranded DNA virus
- ASFV is the only DNA virus that can qualify as an **arbovirus**, soft ticks of the *Ornithodoros* genus
- **Replicates in the cytoplasm** and produces **acidophilic** intracytoplasmic inclusion bodies



Morphology

- The ASFV particle has an icosahedral morphology composed of several concentric domains: the internal core formed by the central genome contains the nucleoid, which is coated by a thick protein layer named **core or matrix shell**;
- an **inner lipid membrane** surrounding the core;
- and finally the **capsid**, which is the outermost layer of the intracellular virions.
- The extracellular virions possess an additional **external envelope** that is obtained when the virus buds out through the plasma membrane.
- The **viral genome** -single molecule of **linear, covalently close-ended, double stranded DNA**.



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Swiss Institute of Bioinformatics

VIRAL REPLICATION

- Replication occurs primarily in the cytoplasm, although the nucleus is needed for viral DNA synthesis.
- Viral attachment is via a specific receptor and entry into host cells is via endocytosis.
- After entry into the cytoplasm, virions are uncoated and their DNA is transcribed by a virion-associated, DNA-dependent RNA polymerase (transcriptase) – early mRNA synthesis

- Viral DNA replication and assembly take place in perinuclear areas.
- Parental genomic DNA serves as the template for the first round of DNA replication, the product of which then serves as a template for the synthesis of large replicative complexes that are cleaved to produce mature virion DNA.
- Formation of the capsid takes place on two layers of membranes derived from the endoplasmic reticulum (ER); extracellular virus acquires a membrane by budding through the cellular plasma membrane.

African Swine Fever

- African Swine Fever is a tick-borne, contagious, febrile, systemic viral disease of swine.
- Highly contagious viral disease of domestic pigs with up to 100% mortality
- Pigs die as a result of a hemorrhagic fever.
- Is a serious **transboundary animal disease** with the potential for rapid international spread by live or dead pigs, domestic or wild, and pork products; also via contaminated feed and fomites (non-living objects) such as shoes, clothes, vehicles, knives, equipment etc. due to the high environmental resistance of ASF virus.



African Swine Fever

Morbidity:

High morbidity — usually 100% in pigs that have contact with one another; 100% in naïve pigs

Mortality:

Highly virulent isolates have about 100% mortality

Moderately virulent isolates range from low percentage to 60-70%.

There is no vaccine for African Swine Fever

Host Range

Ornithodoros or soft ticks are believed to be the original host. Numerous isolates have been made from the soft tick *Ornithodoros moubata*



Host Range

- ASFV is believed to be a tick virus with domestic pigs and wild pigs as accidental hosts.



Host Range

In Africa:

- Warthogs
- Bush pigs
- Giant forest hogs



In Europe:

- Wild pigs



Epidemiology

- Two distinct epidemiologic patterns occur: a sylvatic cycle in warthogs in Africa and epidemic and endemic cycles in domestic swine.
- **Sylvatic Cycle** involving asymptomatic infection in wild pigs (warthogs and, to a lesser extent, bush pigs) and argasid ticks (soft ticks, genus *Ornithodoros*), *which occur in* the burrows used by these animals.
- After feeding on viremic swine, the virus replicates in the gut of the tick and subsequently infects its reproductive system, which leads to **transovarial** and venereal transmission of the virus.

- The virus is also transmitted between developmental stages of the tick-**transstadial** transmission-and is excreted in tick saliva, coxal fluid, and Malpighian excretion.
- After primary infection, young warthogs develop viremia sufficient to infect at least some of the ticks feeding on them.
- Older warthogs are persistently infected but are seldom viremic.
- It is therefore likely that the virus is maintained in a cycle involving young warthogs and ticks.

Domestic Cycle

- Primary outbreaks of African swine fever in domestic swine in Africa probably result from the bite of an infected tick, although tissues of acutely infected warthogs, if eaten by domestic swine, can also cause infection.
- Introduction of the virus into a previously non-infected country may result in indigenous ticks becoming infected and acting as biological vectors and reservoirs of disease—a feature of great epidemiologic significance.

- Once the virus has been introduced into domestic swine, either by the bite of infected ticks or through infected meat, infected animals form the most important source of virus for susceptible swine.
- Disease spreads rapidly by contact and within buildings by aerosol.
- Mechanical spread by people, vehicles, and fomites is possible because of the stability of the virus in swine blood, feces, and tissues.

Transmission

- Transmission by contact and ticks

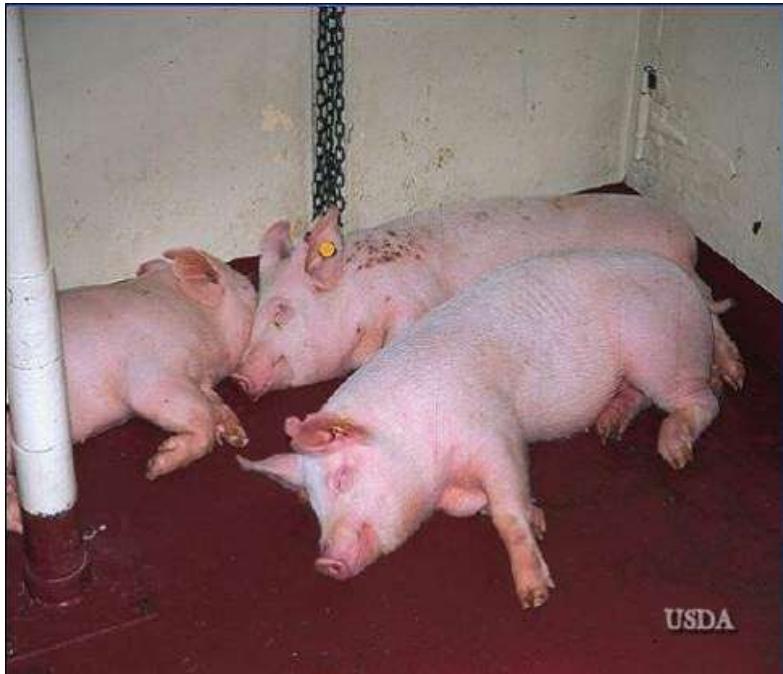


Transmission

- Ingestion → Tonsil → Local LNs → Viremia
- Virus in excretions and secretions, blood.
- Carrier pigs incriminated in maintaining infection in herds.
- Pigs with mild forms of ASF may shed virus for ~ 30 days.
- Bites of infected ticks.

General Clinical Signs

- HOT, SICK, RED pigs



USDA



J. Lubroth

General Clinical Signs

- In contrast to pigs with hog cholera:
 - African Swine Fever pigs **do not** develop conjunctivitis or encephalitis
 - Despite high fever, ASF infected pigs stay in good condition, whereas hog cholera infected pigs drastically lose weight

Abortion

- Occurs whether isolates are high, moderate or low in virulence.
 - Fetuses may be anasarcaous.
 - May find petechiae in placenta, skin, and myocardium, and a mottled liver.

Clinical Signs

- The predilection of ASF viruses for cells of the monocyte/ macrophage lineage results in major defects in the immune system of infected pigs
- Coagulopathy, abnormal clotting
- Thrombocytopenia
- Hemorrhages
- Sudden death in peracute cases
- High fever, low appetite, huddling, shallow breathing, reluctant to move

Clinical Signs

- These signs are influenced by the virulence and the physiological state (age, pregnancy status)
- There are three categories:
 - Highly Virulent Isolate
 - Moderately Virulent Isolate
 - Low-Virulent Isolate

Clinical Signs: Highly Virulent

- Pigs eat and move less
- Most die between 7 and 10 DPI.
- It is not unusual to see a pig walking and find it dead a short time later



http://www.defra.gov.uk/animalh/diseases/images/v2/asfn_8.jpg

Clinical Signs

- Peracute
 - Sudden death
- Acute
 - Fever (105-107°F) –
 - Discolored skin
 - Huddling
 - Diarrhea / melena
 - Abortions
 - Death



Clinical Signs

- Erythema of skin:



Clinical Signs: Moderately Virulent

- Infected pigs usually have high fever for 10 to 12 DPI. Some mortality occurs at this time.
- After 12 to 14 DPI, temperatures and leukocyte count begins to return to normal levels.

Clinical Signs: Moderately Virulent

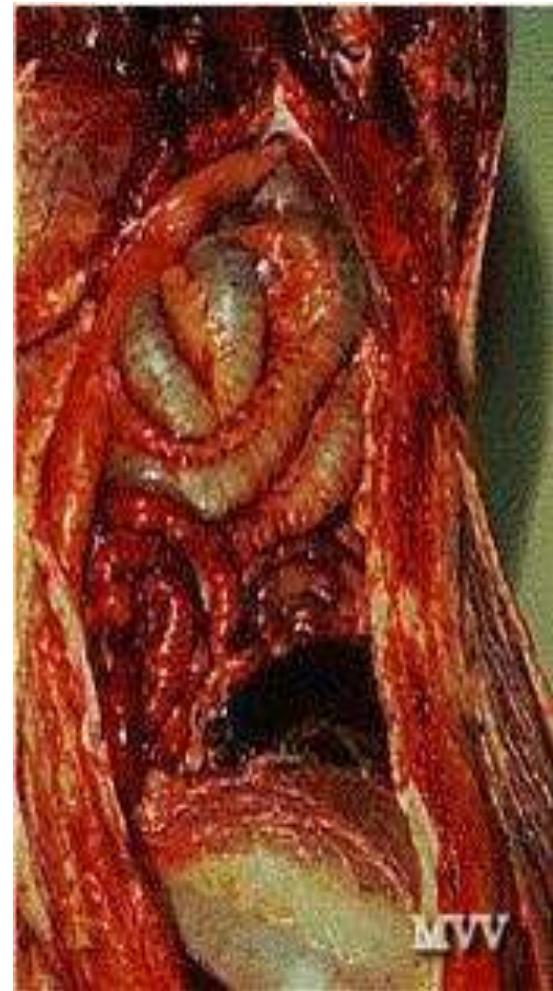
- Very young pigs may have high mortality rate and lesions similar to those caused by highly virulent isolates



Clinical Signs: Moderately Virulent

Some pigs will die at 7 to 8
DPI, frequently caused by
hemorrhage into the
stomach

Underlying causes: ASF
infection causes prolonged
bleeding time



Clinical Signs: **Low-virulence**

- Many non-pregnant animals infected with low-virulence isolates may seroconvert but not show other signs of infection
- Pregnant animals will abort

Clinical Signs:

Chronic

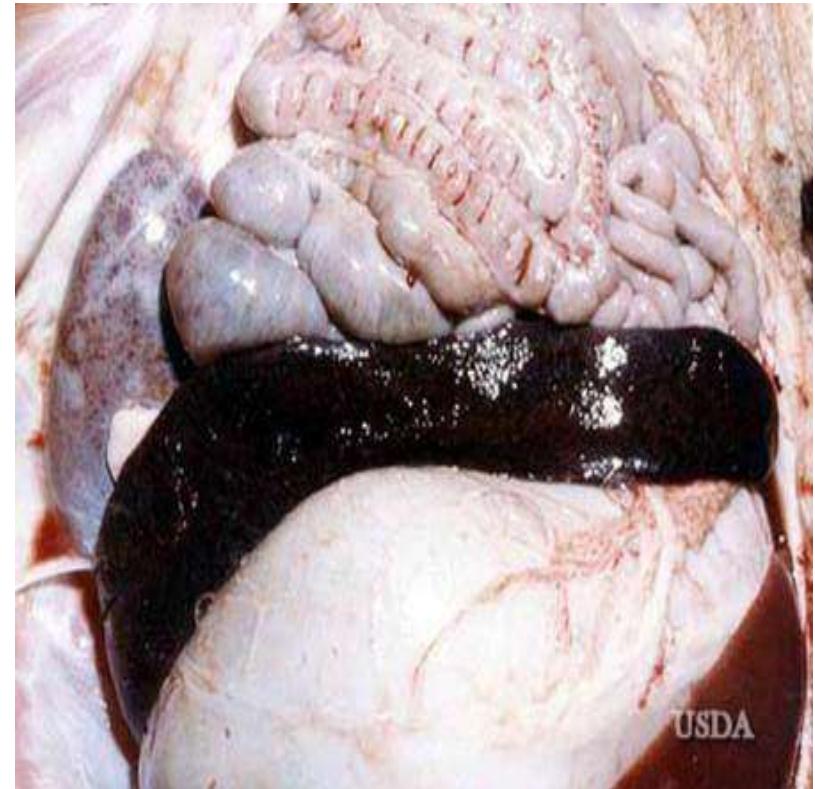
- Transient / recurrent fever
- Stunting / emaciation
- Pneumonia
- Skin ulcers



Gross Lesions

Highly Virulent Virus

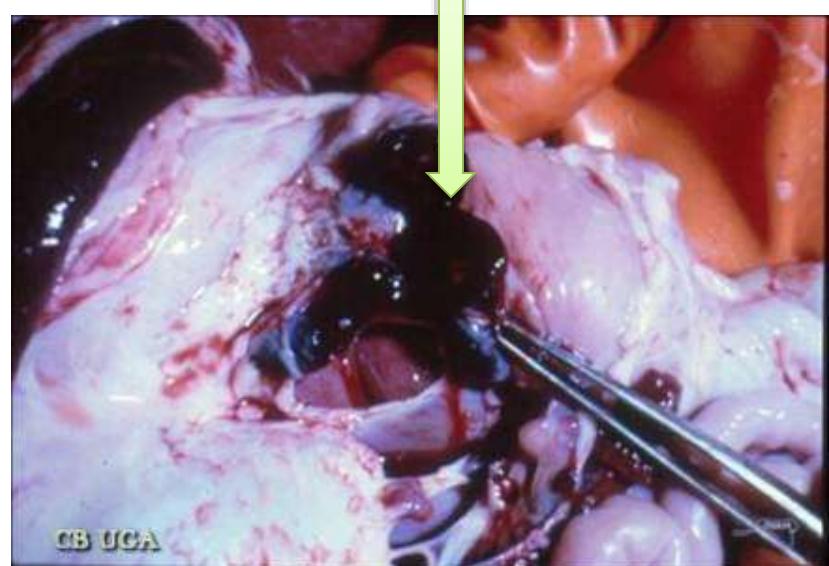
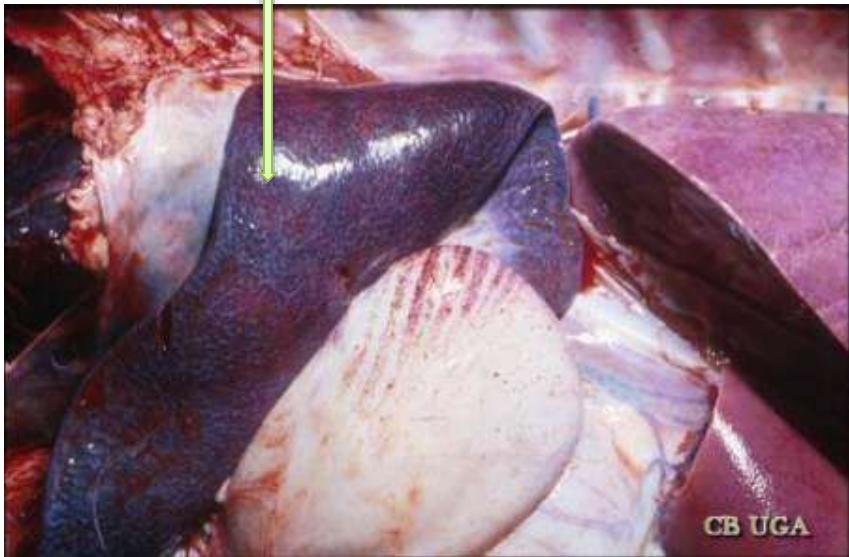
- Peracute deaths
 - Lesions may be poorly developed
- Animals that die 7 or more DPI
 - Classic lesions likely.



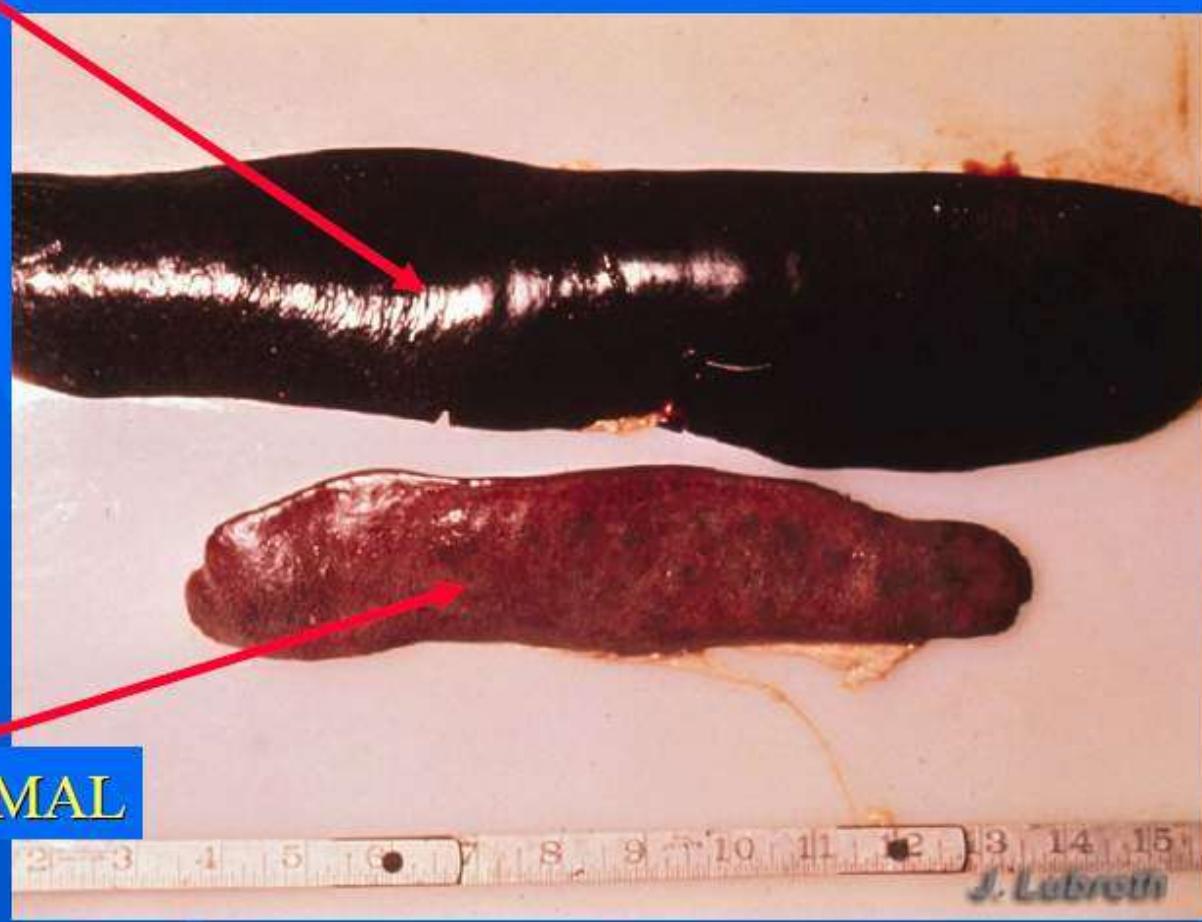
Lesions

Swollen necrotic spleen

Hemorrhagic gastro-hepatic lymph nodes



AFS INFECTED



Splenomegaly

Gross Lesions Moderately Virulent Virus

- From 8-12 DPI
 - Gross lesions are similar whether pigs are infected with a moderately virulent or highly virulent ASFV.
- The main difference between these two types of isolates:
 - Splenomegaly is still present,
 - More normal color and is not friable.

Gross Lesions

Low Virulence Virus

- The most common lesions in chronic ASF:
 - Necrotic skin lesions
 - Consolidated lung lobules
 - Generalized lymphadenopathy
 - Swollen joints
 - Pericarditis



Chronic ASF: Necrotic skin lesions

Raised reddened areas with central areas of necrosis



Raised reddened area behind the ear.



Diagnosis

- African Swine Fever should always be suspected where there are febrile pigs
- Necropsy findings include:
 - Greatly enlarged spleen, dark red to black in color, friable spleen
 - Very enlarged, hemorrhagic gastrohepatic lymph nodes
 - Very enlarged, hemorrhagic renal lymph nodes

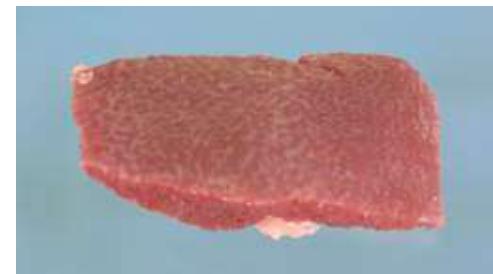
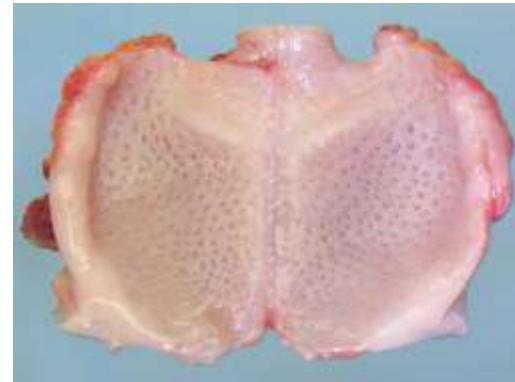
Diagnosis

- Hog Cholera vs. African Swine Fever
 - ETIOLOGY ?
 - Hog cholera infected pigs become depressed and lose weight, whereas ASF infected pigs have neither symptoms
 - Hog cholera is also characterized by a foul-smelling diarrhea

Diagnosis

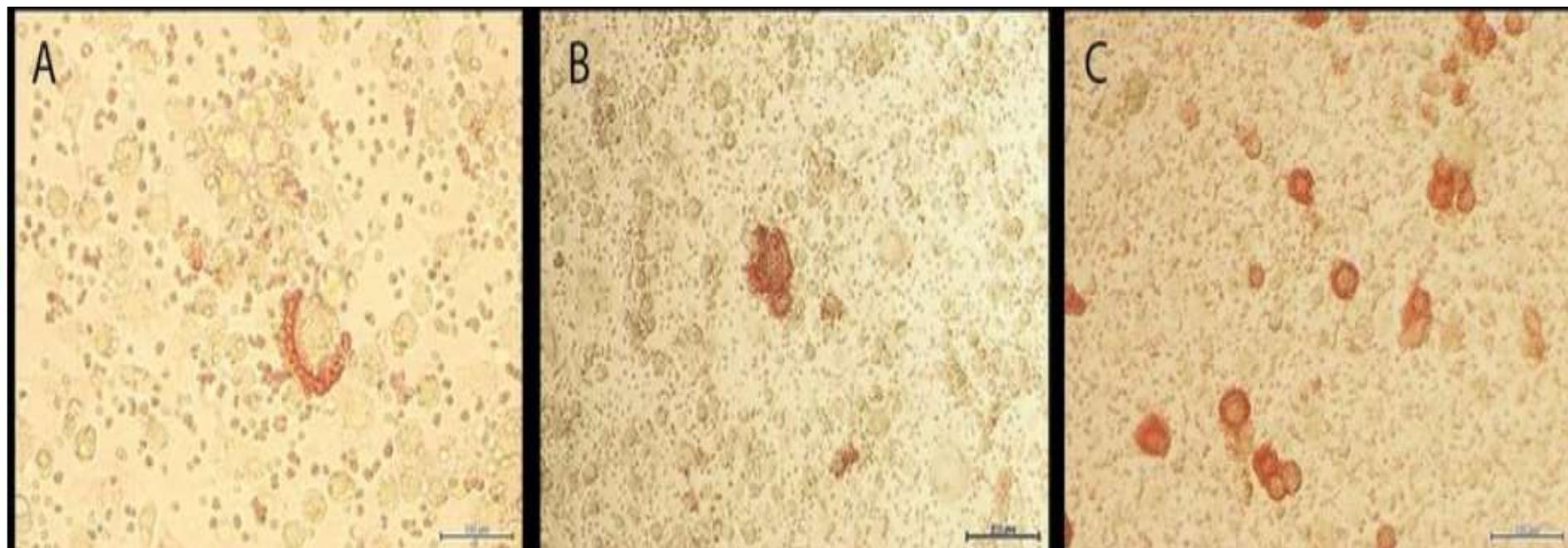
Laboratory Specimens

- Serum / clotted blood
- EDTA, heparin blood
- Lymph nodes
- Spleen
- Tonsil
- Lung
- Liver
- Kidney



Laboratory Diagnosis

- Virus isolation
 - Haemadsorption test (HAD) of leukocyte cultures.
 - Haemadsorption autorosette test of Peripheral Blood Leucocytes(PBL) of suspect pigs.
- Pig inoculation
 - Requires inoculation of naïve and CSF- vaccinated pigs.
 - Not recommended with newer tests available.



2. Haemadsorption with rosette formation in ASFV infected PBM cells with a virulent genotype I ASF strain. Details of the haemadsorption on the surface of swine peripheral blood monocytes infected with the virulent genotype I ASFV Spanish strain E70 at: (A) 12 hours after the infection, (B) 24 hours after the infection, and (C) 48 hours after the infection where complete rosette formation on the periphery of the cells can be observed.

Diagnosis

- Virus antigen detection
 - Direct fluorescent antibody test (DFAT)
- Virus genome detection
 - Polymerase Chain Reaction (PCR)
 - PCR-based sequencing method which permits detection and characterization of ASFV variants.
 - Useful for molecular epidemiological clarification of ASFV

(ORTHO)HERPESVIRIDAE

Herpes means Creeping

Family of enveloped, double-stranded DNA viruses with relatively large complex genomes

Latency i.e. survival in the host is by restricted/no gene expression

Genera

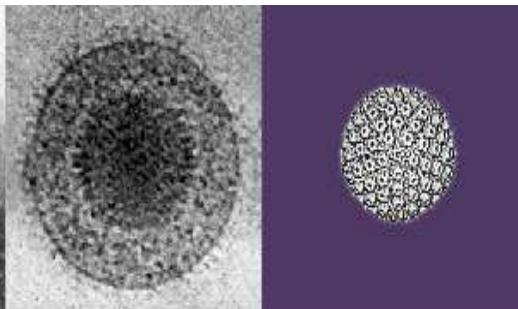
Herpesviruses

Virus classification

Group: Group I (dsDNA)

Order: *Herpesvirales*

Family: ***Herpesviridae***



Subfamily *Alphaherpesvirinae*

- *Iltovirus*
- *Mardivirus* (*Gallid herpes virus*)
- *Simplexvirus* (*human herpes virus I*)
- *Varicellovirus* (*human herpesvirus III*)

Subfamily *Betaherpesvirinae*

- *Cytomegalovirus*
- *Muromegalovirus*
- *Proboscivirus*
- *Roseolovirus*

Subfamily *Gammaherpesvirinae*

- *Lymphocryptovirus* (*Epstein barr virus*)
- *Macavirus*
- *Percavirus*
- *Rhadinovirus* (*Ateline herpes virus-2*)

Varicellovirus (*Alpha herpesvirinae*)

- *Bovine herpesvirus 1* (Infectious bovine rhinotracheitis virus)
- *Bovine herpesvirus 5* (Bovine encephalitis virus)
- *Canid herpesvirus 1* (Canine herpesvirus)
- *Caprine herpesvirus 1* (Goat herpesvirus)
- *Equid herpesvirus 1* (Equine abortion virus)
- *Equid herpesvirus 3* (Equine coital exanthema virus)
- *Equid herpesvirus 4* (Equine rhinopneumonitis virus)
- *Equid herpesvirus 8* (Asinine herpesvirus 3)
- *Equid herpesvirus 9*
- *Felid herpesvirus 1* (Feline rhinotracheitis virus)
- *Suid herpesvirus 1* (Pseudorabies virus)

Mardivirus

- *Gallid herpesvirus 2* (Marek's disease virus type 1)
 - *Gallid herpesvirus 3* (Marek's disease virus type 2)
 - *Meleagrid herpesvirus 1* (Turkey herpesvirus)
-
- *Iltovirus*
 - *Gallid herpesvirus 1* (Infectious laryngotracheitis virus)
-

Gamma-herpesvirinae

Lymphocryptovirus - Human herpesvirus

Rhadinovirus -

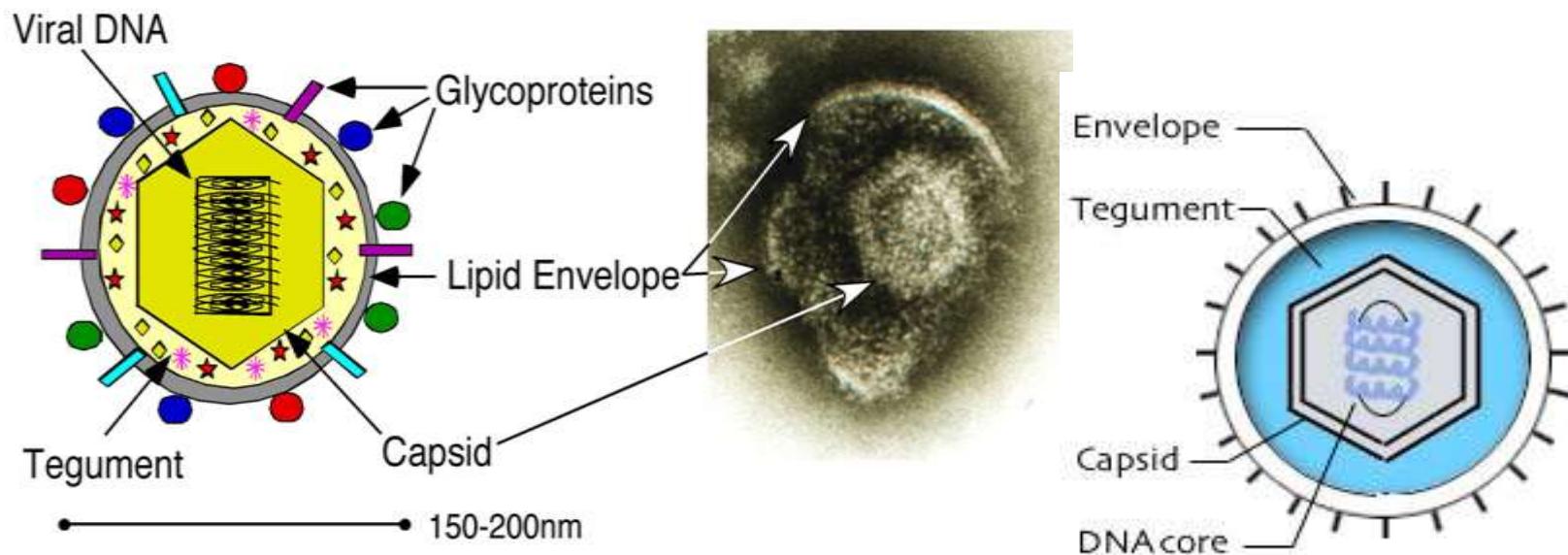
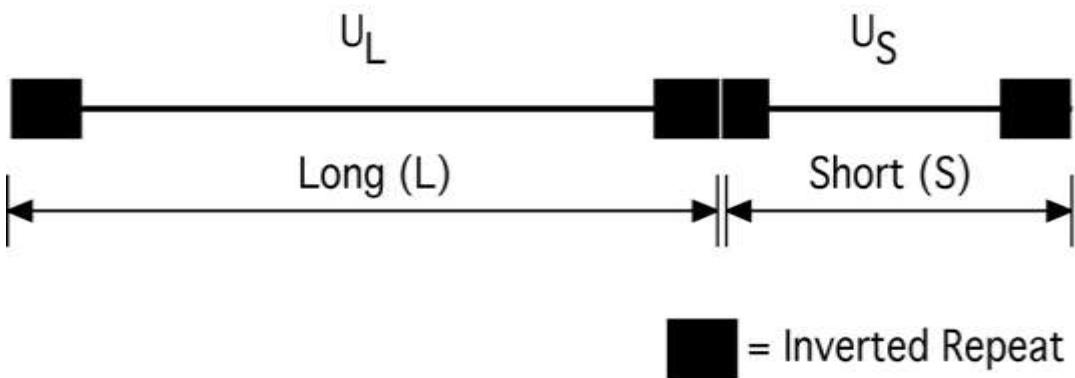
- *Bovine herpesvirus 4*
- *Equid herpesvirus 2*
- *Equid herpesvirus 5*
- *Equid herpesvirus 7*

Macavirus

- *Ovine herpesvirus 2*
(Sheep-associated malignant catarrhal fever virus)

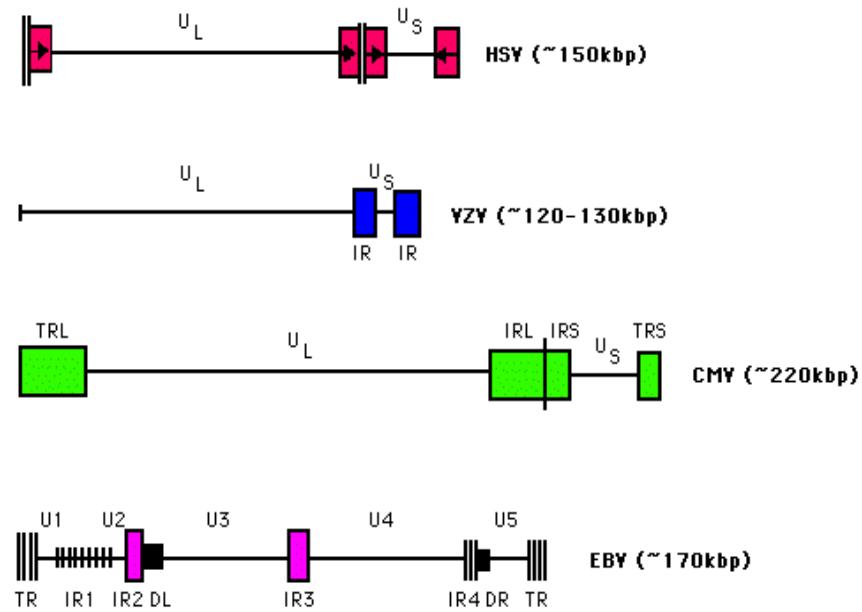
Virion Morphology

- **Core.** The core consists of a single linear molecule of dsDNA in the form of a torus.
- **Capsid.** Surrounding the core is an **icosahedral capsid** with a 100 nm diameter constructed of 162 capsomeres.
- **Tegument.** Present between the capsid and envelope is an amorphous, sometimes asymmetrical, It consists of viral enzymes, some of which are needed to take control of the cell's chemical processes and subvert them to virion production, some of which defend against the host cell's immediate responses, and others for which the function is not yet understood.
- **Envelope.** The envelope is the outer layer of the virion and is composed of altered host membrane and a dozen unique viral glycoproteins. They appear in electron micrographs as short spikes embedded in the envelope.

A.**B.**

Genome

- There are two covalently linked segments i.e. L and S.
- Each segment has Unique long and Unique short sequences.
- These are flanked by repeat sequences located internally (IRL and IRS) and terminally (TRL and TRS)



Because replication takes place inside the nucleus, herpes viruses can use both the host's transcription machinery and DNA repair enzymes to support a large genome with complex arrays of genes

Biological properties

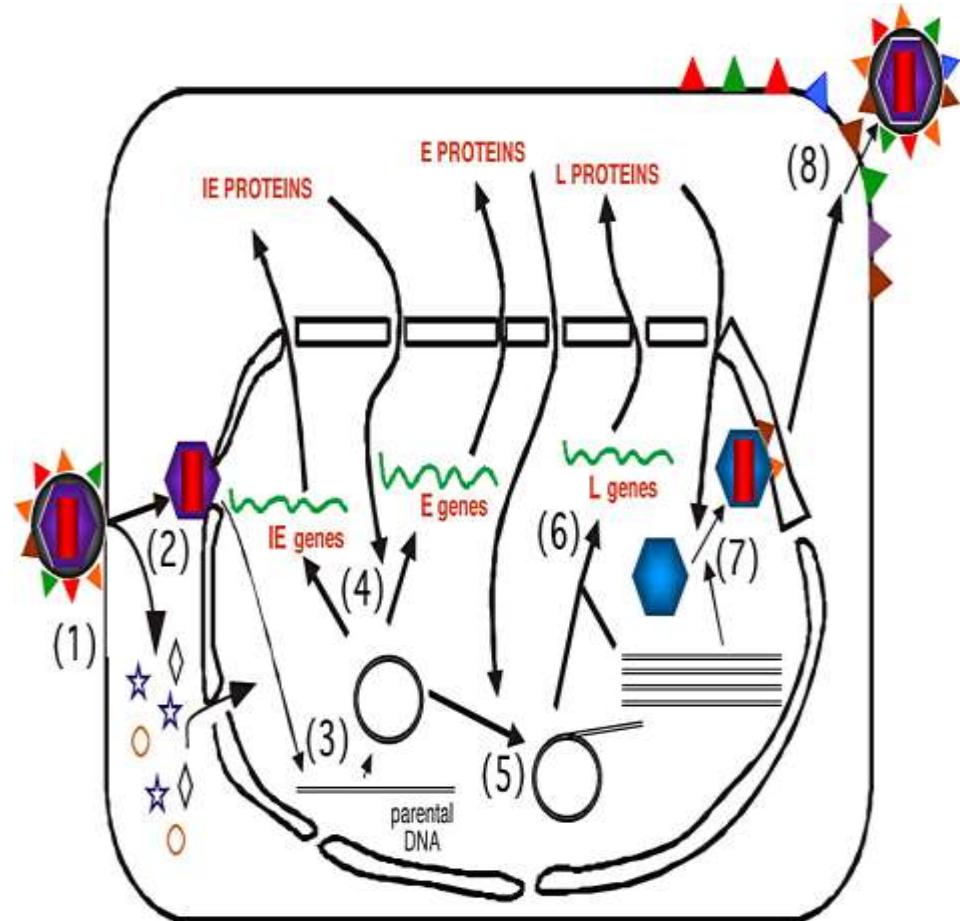
- Herpesviruses express a large number of enzymes involved in metabolism of nucleic acid (e.g. thymidine kinase), DNA synthesis (e.g. DNA helicase/primase) and processing of proteins (e.g. protein kinase).
- The synthesis of viral genomes and assembly of capsids occurs in the nucleus.
- Productive viral infection is accompanied by inevitable cell destruction.
- **Eosinophilic Intranuclear inclusion bodies are characteristic of herpesvirus infections**
- Herpes viruses are able to establish and maintain a **latent state** in their host and reactivate following cellular stress.
- **Latency** involves stable maintenance of the viral genome in the nucleus with limited expression of a small subset of viral genes.

Herpesviridae

- ***Alphaherpesvirinae*** (BHV-1, EHV-1,4, Feline HV, Canine HV)
 - Grow rapidly and are cytopathic and give CPE in 8-12 hrs.
 - Latency in sensory neurons so neurotropic
 - Wide host range
- ***Betaherpesvirinae eg*** Cytomegaloviruses (large balloon-like cells)
 - Grow slowly and produce CPE in 12-20 hrs and increase the size of the cell.
 - Latency in epithelial cells of salivary glands and kidneys, lymphocytes
 - Narrow host range.
- ***Gammaherpesvirinae*** (malignant catarrhal fever virus, EBV)
 - Lymphoproliferative diseases (lymphotropic)
 - Latency in lymphoid cells (some oncogenic)
 - Very slow growing and produce CPE in days.
 - Host range limited to those in which cause natural infections- restricted host range

Viral Replication

- **Attachment and Entry:**
- Viral envelope proteins bind to receptors on the plasma membrane of the host cell
→ **Fusion**
- Nucleocapsids enter the cytoplasm of the host cell and are transported to the nucleus
- Linear viral DNA is released into the nucleus and circularizes (exception: HSV)



- **Transcription and Replication:**
- Viral genes are transcribed and translated into 3 proteins:
immediate-early (α), early(β), late (γ)
 - Immediate-early proteins participate in further transcription of β gene products
 - Early proteins synthesize new viral DNA molecules using circular DNA as a template (exception: HSV uses linear DNA)

Assembly, Encapsidation and Nuclear Egress:

- Late proteins assemble into capsids, which incorporate newly replicated viral DNA
- **Nucleocapsids leave the nucleus by budding through the inner nuclear membrane (envelopment)**
- Mature virus particles reach as vesicles in the cytoplasm which fuse with the host cell plasma membrane → new virus particles are released into the extracellular space

Infection Characteristics

- Transmission is generally associated with mucosal contact, but droplet infection is also common.
- Many alpha herpesviruses produce localized lesions.
- In animals less than 3 months of age infected without the protection provided by maternal antibody-- Generalized alphaherpesvirus infections.
- Pregnant animals – abortion
- Betaherpesviruses and Gammaherpesviruses are not highly lytic for infected cells, they typically cause chronic infections lasting several months before clinical recovery.

- **Persistent infection with periodic or continuous shedding occurs in all herpesvirus infections.**
- In alphaherpes virus infections, multiple copies of viral DNA are demonstrable either as episomes or more rarely integrated into the chromosomal DNA of latently infected neurons.
- The latent genome is essentially silent except for the production of a **latency-associated transcript (LAT)**
- This RNA transcript is not known to code for any protein.
- Reactivation is usually associated with stress.
- Shedding of virus in nasal, oral, or genital secretions provides the source of infection for other animals, including transfer from dam to offspring

Diseases caused by Alpha Herpesvirus

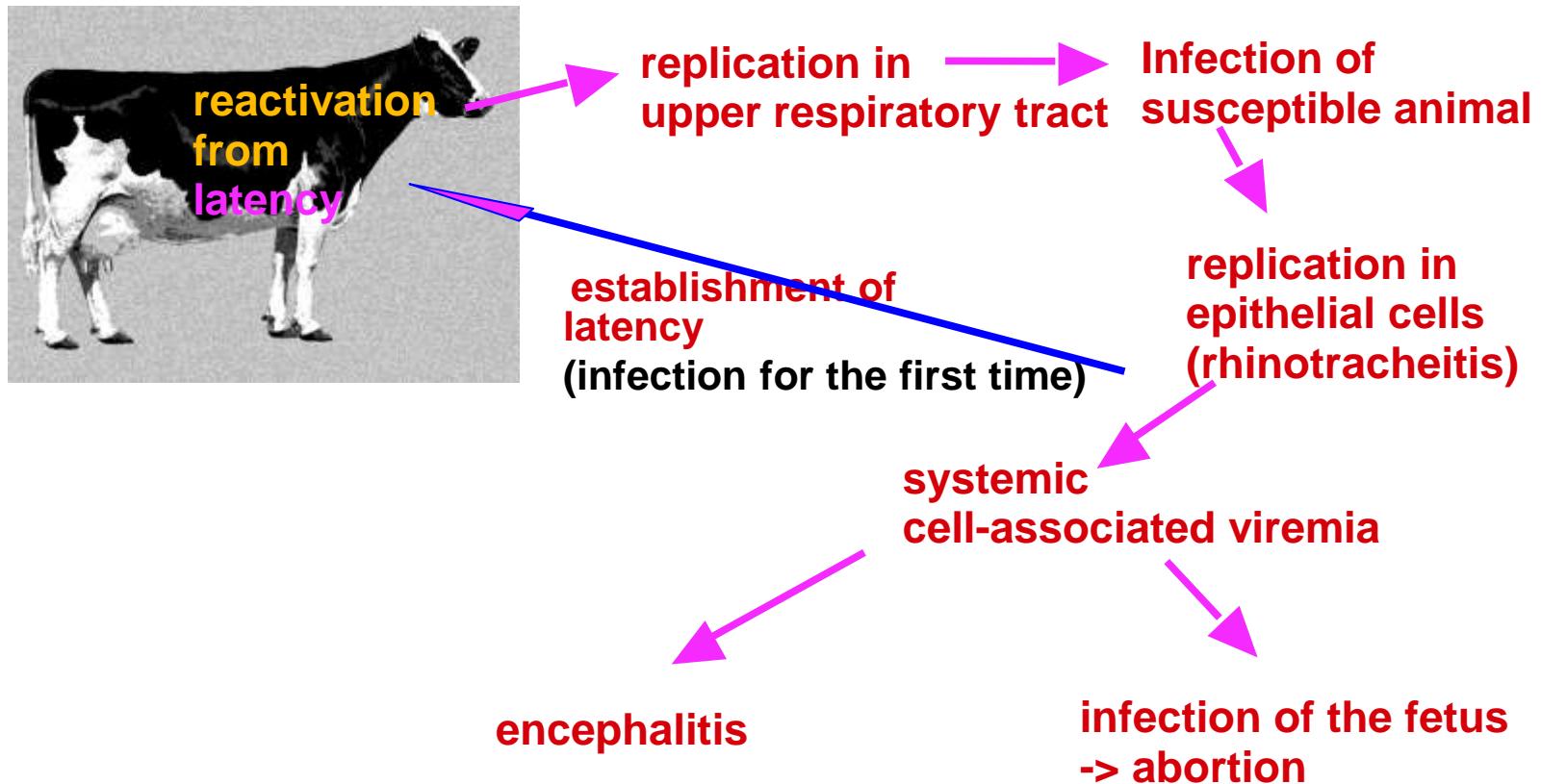
- **Bovine Herpesvirus 1 or BoHV-1, BHV1 or BHV-1 of genus *Varicellovirus***
 - Infectious bovine rhinotracheitis (IBR)—respiratory form
 - Infectious pustular vulvovaginitis (IPV)
 - Infectious balanoposthitis (IBP)
 - Conjunctivitis
 - Abortions in 3rd trimester
 - Mastitis
 - Metritis
 - **IBR/IPV is a OIE List B notifiable disease**
- } Genital disease

Virus transmission

- Viruses exist only in relationships with hosts and **cannot replicate outside their hosts.**
- BoHV-1 is inactivated by normal environmental conditions outside the host and by common disinfectants and solvents.
- Transmission of BoHV-1 occurs by contact with mucosal droplets from infected cattle ; infectious virus is nasally shed for 10–14 days during acute respiratory infection, and virus is also shed following reactivation from latency.
- Contaminated materials, including semen, can transmit the virus.

- **Genital disease** may result from coitus or artificial insemination .
 - **Respiratory disease** and conjunctivitis result from droplet transmission.
 - BoHV-1 can cross placental barrier to infect foetus causing abortions
-
- Lifelong latent infection with periodic virus shedding occurs after bovine herpesvirus 1 infection; the **sciatic and trigeminal ganglia** are the sites of latency following genital and respiratory disease, respectively

Infection in the animal



Respiratory disease -Clinical Signs

- Infectious Bovine Rhinotracheitis (IBR) or “**Red Nose**”.
- Pyrexia, Nasal discharge may contain blood.
- Focal pustular necrosis, haemorrhages and ulcerated mucosa.
- Foul smelling breath, Depression, loss of appetite and increased respiration rate.
- Necrotic areas have cream coloured diphtheritic membrane and virus is shed with coughing.
- Profuse salivation and muzzle becomes encrusted with dried exudates. This when peeled off leads to **red nose**.
- Unilateral or bilateral conjunctivitis, often with profuse lacrimation
- Symptoms remain for one week.
- Recovery after 1 to 4 days of peak temperature.

Genital form-Clinical signs

- IPV/IBP
- In this form the cows stand with legs apart and tail held away.
- Micturition is frequent and painful
- Small pustules are seen on vulva which may coalesce with ulcerated mucosa.
- The disease lasts for 4-5 days.
- In bulls the clinical disease is similar and semen from recovered bulls may be contaminated with virus.
- The virus may infect the foetus and may cause abortions and mummification.
- Abortion may occur at **4-7 months gestation**, and the virus has also been reported to cause mastitis

Diagnosis of BHV

- In both the genital and the respiratory forms of the disease the lesions are focal areas of **epithelial cell necrosis** in which there is ballooning of epithelial cells.
- Virus isolation
- In cell cultures, rapid cytopathic effect, with syncytia and typical herpesvirus inclusions may be present in nuclei at the periphery of necrotic foci.
- Immunofluorescence
- Immunohistochemistry
- PCR
- Serology
 - virus neutralization
 - ELISA

Vaccination against BHV-1

- **Recombinant DNA vaccines-** in which the thymidine kinase and other glycoprotein "marker" genes have been deleted. Although they do not prevent infection, vaccines significantly reduce the incidence and severity of disease.
- modified-live (attenuated)
 - intra nasal
 - intra muscular
- inactivated
 - intra muscular

Bovine Herpesvirus 2

- Similar in structure to human herpes simplex virus.
- Causes Bovine mamillitis and Pseudo-lumpy skin disease
- **Bovine mamillitis** - Lesions on teats and udders
- **Pseudo-lumpy skin disease** - characterized by transient moderate fever and exudative cutaneous plaques on face, neck, back, and perineum.
- The benign nature of pseudo-lumpy skin disease, the characteristic central depression on the surface of the nodules, the superficial necrosis of the epidermis, and the shorter course of the disease are helpful in differentiating the condition from true lumpy skin disease in countries where both occur.

Equine Herpesviruses (EHVs)

- Group of **nine agents** that cause diseases ranging in severity from a neurological disease, which can be fatal in more than 50% of infected animals (equid herpesvirus 1, EHV-1), to mild or subclinical infection of B-lymphocytes (equid herpesvirus 5, EHV-5).
- Among the nine equid herpesviruses, **three** are referred to as **asinine herpesviruses, which primarily infect donkeys.**
- The **six ‘true’ equid herpesviruses (EHV-1, EHV-2, EHV-3, EHV-4, EHV-5, and EHV-9)** infect horses primarily and generally have a very narrow host range *in vivo*.
- However, EHV-9 is able to infect many different species and often causes lethal encephalitis in non-equine hosts.

- EHV represent two subfamilies -
Alphaherpesvirinae and *Gammaherpesvirinae*, and
two genera, *Varicellovirus* and *Radinovirus* in these
subfamilies respectively.
- The most important EHV clinically and economically are
EHV-1 and EHV-4, whose genomes have been
sequenced in their entirety

Equine Herpesviruses

- EHV 1, 3 and 4 are of veterinary importance

Type	Clinical
EHV-1	Respiratory, abortions, myeloencephalitis, Neonatal death
EHV-4	Respiratory (Rhinopneumonitis)
EHV-3	Genital (coital exanthema)

Transmission

- Equine herpes virus (EHV-1 and EHV-4) is spread via nose to nose contact, contaminated equipment (water and feed buckets, tack and grooming supplies, and shoes) and respiratory secretions within stalls/stables.
- Aborted fetuses and after-birth can also contain the virus.
- **EHV-3** is spread through venereal transmission or contaminated equipment used for breeding.

EHV-1

- Equine herpesvirus 1 (EHV-1) and equine herpesvirus 4 (EHV-4) comprise two genetically and antigenically distinct groups of viruses previously referred to as subtypes 1 and 2 of EHV-1 with different disease profiles
- EHV-1 is one of the major pathogens affecting horses worldwide.
- EHV-1 is responsible for respiratory disorders, abortion, neonatal foal death and **equine herpes myeloencephalopathy (EHM)**.
- Immunity short lived
- Reinfection or reactivation can occur.

EHV-1 (Respiratory disease)

- Incubation period (2-10 days)
- Primary infection in **young horses**
- **Biphasic fever**
- Nasal discharge, cough
- Uncomplicated cases -> complete recovery 1-2 weeks

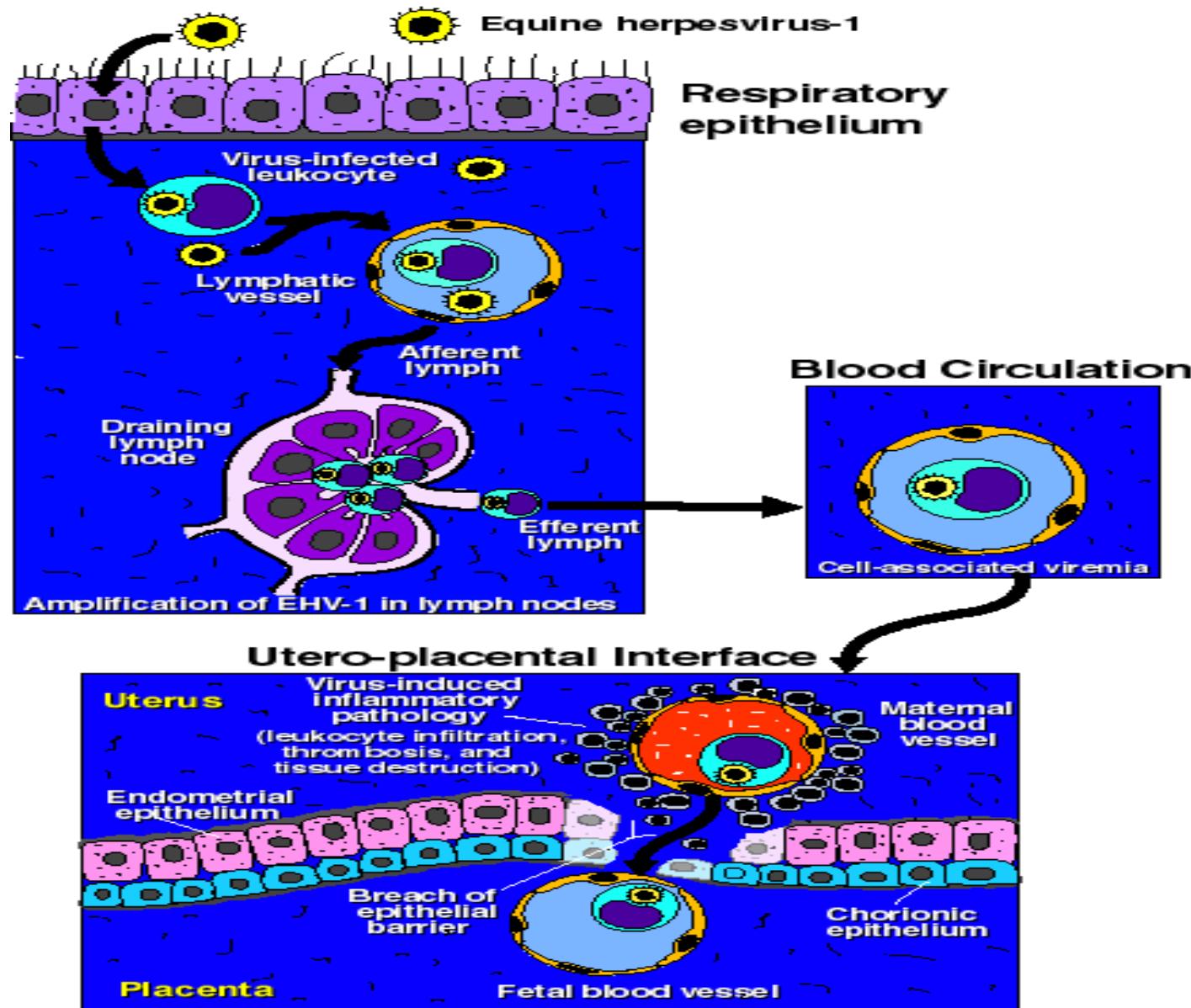
Myeloencephalopathy by EHV-1

- Often but not always associated with respiratory disease
- Sudden onset rapid progression, early stabilization
- Ataxia, paresis, urinary incontinence, cystitis
- little evidence of viral replication in neural tissues (immune mediated?)
- Vasculitis, thrombosis, hemorrhages

EHV-1 abortions

- May occur as early as the fourth month of gestation,
- Common late in gestation (7th to 11th month)
- few weeks to several months after respiratory outbreak
- Equine herpesvirus I carrier mare can reactivate virus and infect a large number of non-immune contact mares, leading to devastating "**abortion storms.**"

Figure 1. Pathogenesis of EHV-1 Abortion



Control

- Inactivated cell culture vaccine given at 5th, 7th and 9th months of gestation.
- EHV-1/EHV-4 combined inactivated vaccine is used



Equine rhinopneumonitis (Equine Herpes virus 4)

- A common infection of horses, world wide.
- Occurs in **foals over 2 months old, weanlings, and yearlings** and results in acute febrile respiratory disease characterized by rhinitis, pharyngitis, tracheitis, bronchitis, serous nasal discharge, anorexia, enlarged mandibular and pharyngeal lymph nodes.
- More severe disease including bronchopneumonia and death may occur
- Virus may be transmitted to brain and may cause weakness, in coordination and paralysis.

Equine Coital Exanthema (Equid alphaherpesvirus 3 (EHV-3)).

- INCIDENCE AND OCCURRENCE:
Worldwide in distribution. Acute but mild. Persistence and recurrence in mares and stallions have been observed.
- CLINICAL FINDINGS: Vesicles on the skin of the vulva or penis, progress to erosions, scabs, heal in about 2 weeks. Secondary bacterial infection common.
May leave white depigmented areas.
- **Abortion or infertility is not associated with EHV-3 infection**
- TRANSMISSION: Venereal, possibly also other forms of contact.



Malignant Catarrhal Fever

- These “malignant catarrhal fever viruses” are all included in the genus ***Macavirus***, of subfamily **Gammaherpesvirinae**.
- The two most important viruses are **Alcelaphine herpesvirus 1 (AlHV-1)**, which is endemic in wildebeast populations worldwide causing wildebeast-associated MCF; and **ovine herpesvirus 2 (OvHV-2)**, which causes sheep-associated MCF, and is endemic in most sheep populations worldwide
- A fatal **lymphoproliferative disease** of cattle and wild beast affecting lymphoid tissue and epithelial cells of respiratory and gastrointestinal tract.
- Presented as alimentary, encephalitis, or skin forms; all three may occur in an animal.

- **Corneal edema** starting at the limbus and progressing centripetally is a nearly **pathognomonic sign**; photophobia, severe keratoconjunctivitis, and ocular involvement may follow.
- Other signs include prolonged fever, oral mucosal erosions, salivation, lacrimation, purulent nasal discharge, encephalitis, and pronounced lymphadenopathy.
- Death in a week after the onset of infection.

Pseudorabies (Aujeszky's Disease)

- By **Suid herpesvirus 1 in the genus Varicellovirus, subfamily Alphaherpesvirinae**
- Disease primarily of pigs but horses, cattle, sheep, goat, dogs may get infected.
- Disease is endemic in most parts of the world.
- Spread through excretions.

Clinical signs

- Incubation period of 30hrs to 8 days
- There is coughing, sneezing, fever, constipation and vomiting.
- Pigs become listless and recumbent.
- By fifth day there is incoordination, circling, intermittent convulsions along with excessive salivation. Then there is death.
- In adult animals mortality is low but there is poor growth rate.
- **In pregnant sows -abortion.**
- If infection occurs before 30th day of pregnancy there is foetal death and reabsorption. If infection occurs late in pregnancy it may lead to mummified foetus, still birth and weak piglets.
- In piglets of non immune sows there may be 100% mortality.

- Disease in secondary hosts (cattle and dogs) is sporadic and occurs where there is direct or indirect contact with swine.
- **In dogs it is called “Pseudorabies”**- there is intense pruritis and along with it there is paralysis of jaws and pharynx accompanied with drooling saliva, howling but there is no tendency for dogs to attack other animals.
- **In cattle it causes “Mad Itch” characterized by intense itching and licking and gnawing of flanks.**

Marek's disease

- **Jozseb Marek:** 1907: first described about the disease as Fowl paralysis, range paralysis
- Herpes viral etiology was established in 1967 only
- By **Gallid herpes virus-2 of genus *Mardivirus* belonging to Alpha herpesvirinae.**
- **Syn.- Range paralysis, Fowl paralysis, Neural leukosis, Gray eye**
- OIE, List B disease

Serotypes of MDV

- Presently members of the genus Mardivirus can be divided immunologically into three serotypes:
- **Serotype 1 (GaHV-2):** Includes all pathogenic and nonpathogenic strains. It varies markedly in pathogenicity and are further divided into pathotypes that include mild (m)MDV, virulent MDV (vMDV), very virulent MDV (vvMDV) and very virulent plus MDV (vv+MDV).
- **Serotype 2 (GaHV-3):** Contains naturally avirulent and non-oncogenic strains.
- **Serotype 3 (meleagrid herpesvirus 1):** Includes avirulent HVT

- Susceptible species- **gallinaceous birds** -Chicken, quails and turkeys.
- Distribution- Worldwide.
- Birds are infected **by inhalation** of contaminated dust from the poultry houses, and, following complex pathogenic pathways, the virus is shed from the feather follicle of infected birds.
- MD can occur at any time, beginning at 3–4 weeks of age or older, sometimes even well after the onset of egg production

Marek's disease

- A lymphoproliferative disease, slowly cytopathogenic and remains highly cell associated (except in dander of feather follicles).
- Epithelial cells at the base of feather follicles are exceptional in that productive infection of these cells is also associated with the release of cell-free infectious virus.
- There are four progressive syndromes known to occur after infection with Marek's disease. These syndromes may overlap.
- **Classical Marek's disease or neurolymphomatosis**
- **Acute Marek's disease**
- **Ocular lymphomatosis**
- **Cutaneous Marek's disease**

1. Classical Marek's disease or Neurological form: Asymmetric paralysis of one or both legs or wings, incoordination, one leg forward and one backward, wing drooping, lowering of head and neck, if vagus is paralysed dilation of crop and gasping

2. Acute Marek's disease or Visceral form:

- Tumors in gonads, liver, spleen, muscles, outbreaks of ataxia, mortality
- Other signs :Depression, weight loss, loss of appetite, and potentially death without any obvious clinical signs.

3. Ocular lymphomatosis:

- Graying of iris of one or both because of lymphoblastoid cell infiltration;
- The pupil is irregular and eccentric and there is partial or total blindness.
- Less common and affects adult birds.

4. Cutaneous Marek's disease - round, firm lesions at the feather follicles.

Epithelial cells at the base of feather follicles have productive infection

Pathogenesis

- Involves early cytolytic infection, latency, reactivation, and ultimately, tumor formation.
 - MDV infection starts with the inhalation of dust containing the infectious virus.
- 1. Early cytolytic infection-** Initial virus replication in macrophages and B cells in the lung of infected animals .
- Phagocytic cells, such as macrophages, transport the virus to regional lymphatic tissues, the bursa of Fabricius, and the spleen, where other immune cells become infected
 - This initial infection is characterized by a cytolytic phase, where the virus replicates and damages cells.
 - T cells are also infected during this phase, and the virus establishes latency in both B and T cell

2. Latency:

- MDV establishes latency in infected T cells, meaning the virus remains dormant within the host.

3. Reactivation and Tumor Formation:

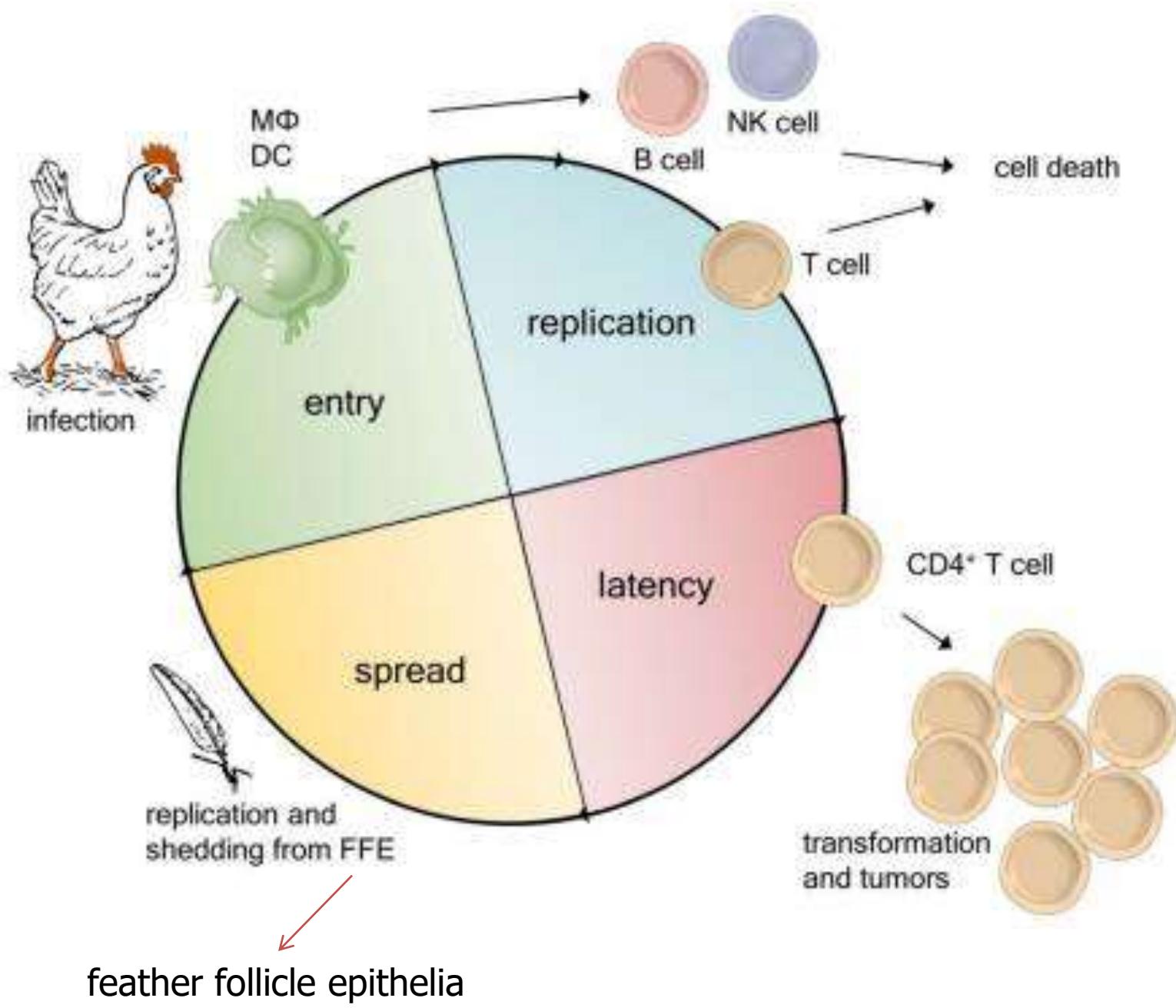
- Under certain conditions, latently infected T cells can reactivate, leading to a second phase of cytotropic infection.
- This reactivation is accompanied by permanent immunosuppression, impairing the host's ability to fight off the virus and tumor development.
- The virus can transform latently infected T cells, resulting in the formation of **T-cell lymphomas**, which are the hallmark of Marek's disease.
- These lymphomas can infiltrate nerves and organs, causing paralysis, blindness, and other neurological and visceral signs.

4. Immunosuppression:

- MDV infection leads to a significant immunosuppression, making the host more susceptible to secondary infections.
- This immunosuppression is thought to be due to the cytopathic effects of the virus on B and T lymphocytes during the early lytic phase

MDV

Oncogenic , Lymphotrophic and exhibits persistency



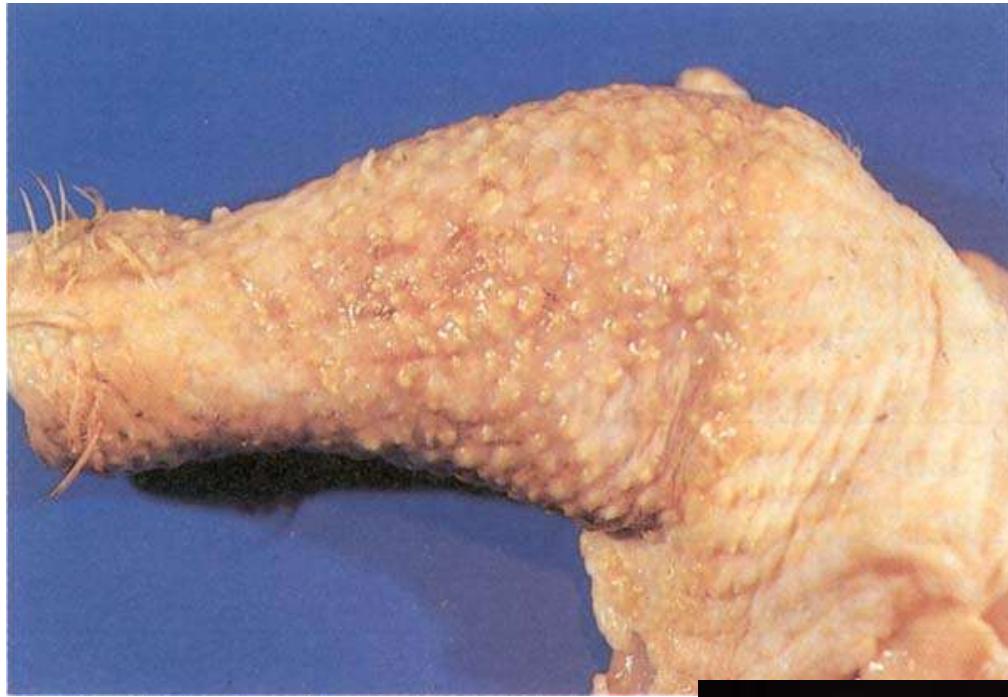
Transmission

- The route of infection is **usually respiratory** and the disease is highly contagious being spread by infective feather-follicle dander, fomites, etc.
- Infected birds remain viraemic for life.
- Vertical transmission is not considered to be important.
- The virus survives at ambient temperature for a long time (65 weeks) when cell associated and is resistant to some disinfectants (quaternary ammonium and phenol).
- It is inactivated rapidly when frozen and thawed.
- **Marek's disease is not zoonotic.**

Marek's disease

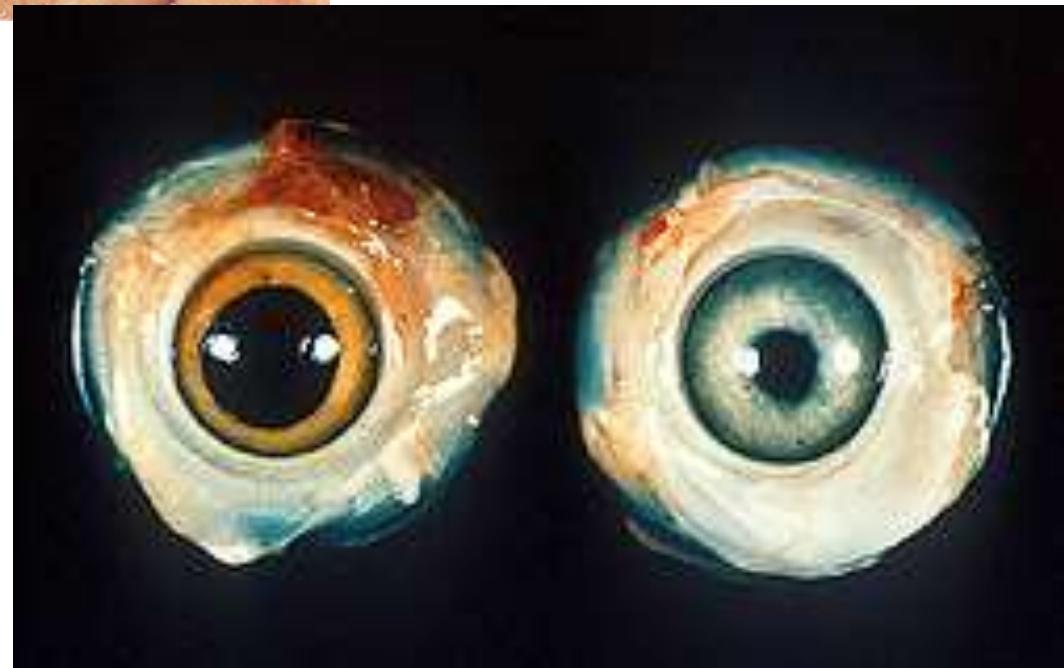
Signs

- Paralysis of legs, wings and neck.
- Loss of weight.
- Grey iris or irregular pupil.
- Vision impairment.
- Skin around feather follicles raised and roughened.



Enlargement of feather
follicles

Occular form





Post-mortem lesions

- Grey-white foci of neoplastic tissue (visceral tumors) in liver, spleen, kidney, lung, gonads, heart, and skeletal muscle.
- Enlargement of one or more peripheral nerve trunks, celiac, cranial, intercostal, mesenteric, brachial, sciatic, greater splanchnic nerves, usually unilateral
- Nerves have diameter 3 times more than normal with loss of striations, edematous, grey, yellow or translucent
- Microscopically - lymphoid infiltration is polymorphic.

Diagnosis

- Identification of agent can be done by inoculation in monolayer cultures of chicken kidney cells or duck embryo fibroblasts (chicken embryo fibroblasts (CEF))
- For that purpose specimens can be heparinised blood, lymph nodes or spleen.
- MD viral antigen can also be detected in the feather tips of infected birds using a radial precipitin test.
- Serological diagnosis is possible 1 to 2 days after infection.
- Tests include agar gel immunodiffusion test, the indirect fluorescent antibody test, and ELISA.
- Detection by immuno-fluorescence of activated T cell antigens present on the surface of MD tumour cells (MD tumour-associated surface antigen or **MATSA**),

Prevention

Vaccination: Vaccines are extremely effective (90%+).

- There are **three serotypes**: Serotype 1 which is available commercially as attenuated virulent or attenuated mildly virulent,
- Serotype 2 vaccines which are naturally non-pathogenic strains of MDV, or
- Serotype 3 "Herpes Virus Turkey (HVT) which are effective against virulent MDV but less effective against very virulent MDV.
- *Bivalent and Trivalent Vaccines*: Synergistic effect and good protection can be achieved by combining the serotype vaccines 1,2, or 3 as bivalent or trivalent vaccines. These have become standard for the layer chick hatcheries, administered subcutaneously at hatching. Broiler chicks are given vaccine *in ovo* at the time of egg transfer.
- *Genetic selection*: MD resistant chicks are obtained.

Infectious Laryngotracheitis (ILT)

- **Gallid herpesvirus 1 (GaHV-1)** belonging to the genus *Itovirus*, subfamily Alphaherpesvirinae
- Virus may live for 8 to 10 days in droppings and up to 70 days in carcasses, hence correct disposal is essential.
- It is believed that the virus may survive for up to 80 days in tracheal exudate (throat exudate) if not disturbed.
- **Susceptible Species:**
- Fowls, pheasants and turkeys.
- Water fowl, such as ducks and geese, show no signs, but ducks are known to carry ILT.
- Wild birds may act as carriers.

Transmission

- **Introduction of infected birds** or carrier birds or birds which are incubating the disease at the time of introduction.
- **People and contaminated equipment**
- **Airborne spread**
- **Litter and manure**

Clinical signs

- The classical signs are gasping, coughing and sticking the neck forward and upwards with each breath in an effort to clear the mucus which builds up in the trachea (windpipe) – in fact, many birds die from the disease due to suffocation, ie, the windpipe becomes completely blocked.
- In acute cases, there has been up to 70% mortality.
- There is a marked variation in the pathogenicity (potency) of various strains of the virus.
- Three major forms – the peracute, the subacute and mild or chronic forms

Diagnosis

- Acutely (severely) affected birds will show free blood in the trachea, generally associated with a mucus plug which inhibits normal breathing. The symptoms will rapidly spread throughout the flock.
- Birds with subacute and mild infections may show only slight difficulty in breathing and perhaps a mild watering of one or both eyes. However, the disease can still be easily transmitted from one bird to another. Mild ILT infection may look like any other respiratory or virus infection.
- Laboratory diagnosis will always be required to determine whether ILT virus is present.

Duck plague virus

- Duck plague (DP) or duck viral enteritis (DVE), caused by the **alpha herpes virus anatid herpesvirus-1**, is an acute, highly contagious disease of ducks, geese, and swans of all ages.
- It is an OIE-listed notifiable disease.
- Disease is characterized by sudden death and high mortality.
- Although disease is widespread in distribution and frequent in captive waterfowl, it is infrequently seen in wild waterfowl.
- Ingestion of contaminated water is believed to be the major mode of transmission, but the virus may also be transmitted by contact.
- Birds are typically found dead. Those exhibiting clinical signs may be photophobic, lethargic, anorexic, ataxic, and have a serosanguinous nasal discharge and watery or bloody diarrhoea.

FAMILY *PAPILLOMAVIRIDAE*

Papillomatosis

INTRODUCTION

- Non-enveloped with circular double-stranded DNA
- **Baltimore group I**
- **Highly host-and tissue-tropic,**
- All known papillomavirus types infect a particular body surface, typically the skin or mucosal epithelium of the genitals, anus, mouth, or airways
- Replicate exclusively in the basal layer of the body surface tissues.
- Produce proliferative lesions or warts in many mammalian and avian species.
- **Have not been cultured *in vitro***

- The *Papillomaviridae* family includes two subfamilies, the *First papillomavirinae* and the *Secondpapillomavirinae*.
- The *Firstpapillomavirinae* currently contains **51 genera** while only one genus is currently classified within the *Secondpapillomavirinae* subfamily.
- Genera in the *Firstpapillomavirinae* are named using the Greek alphabet (*Alpha-*, *Beta-*, *Gamma-*, *Deltapapillomavirus*, etc., and derivatives thereof, eg, *Dyodeltapapillomavirus*)

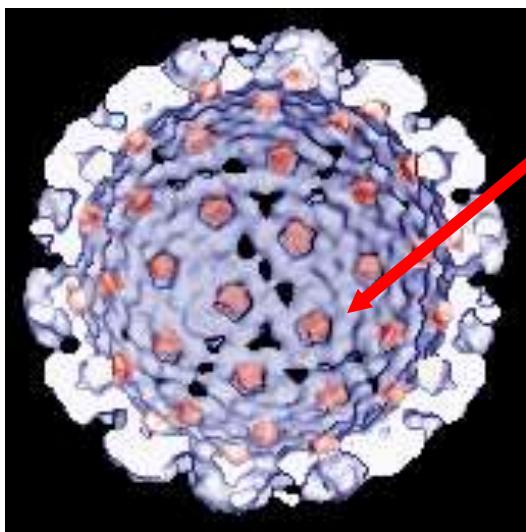
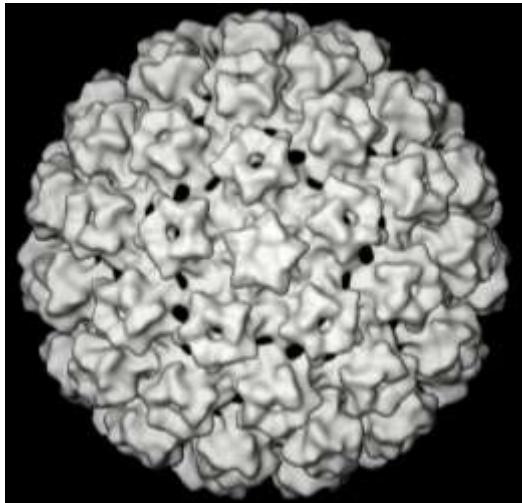
- Within genera, the papillomaviruses are further subdivided into numerically-named species. Currently there are **133 papillomavirus species** within the 51 papillomavirus genera.
- Within the species, papillomaviruses are subclassified into individual papillomavirus types. Each papillomavirus type is named sequentially using the scientific name of the host species.
 - Around 500 individual papillomavirus types have been fully classified, including around 175 from the non-human species.
 - Each animal species may be infected with multiple genetically distinct papillomavirus types that are included in different genera.

- Important species causing disease in animals
BPV-*Bos taurus* papillomavirus;
OaPV, *Ovis aries* papillomavirus;
EcPV, *Equus caballus* papillomavirus;
CPV, *Canis familiaris* papillomavirus;
FcaPV, *Felis catus* papillomavirus

Species	Papillomavirus genus	Papillomavirus types	Predominant associated lesions
Cattle	<i>Delta</i>	BPV-1, -2, -13, -14	Cutaneous and esophageal fibropapillomas Bladder neoplasia
	<i>Xi</i>	BPV-3, -4, -6, -9, -10, -11, -12, -15, -17, -20, -23, -24	Cutaneous and upper alimentary papillomas
	<i>Epsilon</i>	BPV-5, -8	Cutaneous papilloma
	<i>Dyoxi</i>	BPV-7	Cutaneous papilloma
	<i>Dyokappa</i>	BPV-16, -18, -22	Cutaneous papilloma
	Unclassified	BPV-19, -21	Cutaneous papilloma
Sheep	<i>Delta</i>	OaPV-1, -2, -4	Cutaneous fibropapilloma
	<i>Dyolambda</i>	OaPV-3	Cutaneous SCC
Horses	<i>Zeta</i>	EcPV-1	Cutaneous papilloma
	<i>Dyoiota</i>	EcPV-2, -4, -5	Penile papilloma Aural plaque
	<i>Dyorho</i>	EcPV-3, 6, 7	Aural plaque
	Unclassified	EcPV-8	Cutaneous papilloma
Dogs	<i>Delta</i>	BPV-1, -2, 13	Equine sarcoid
	<i>Lambda</i>	CPV-1, -6	Oral papillomas Cutaneous papillomas
	<i>Tau</i>	CPV-2, -7, -13, -17, -19	Cutaneous papillomas
	<i>Chi</i>	CPV-3, -4, -5, -8, -9, -10, -11, -12, -14, -15, -16, -18, -20	Viral pigmented plaques
Cats	<i>Lambda</i>	FcaPV-1	Oral papillomas
	<i>Dyotheta</i>	FcaPV-2	Viral plaques/BISC Cutaneous SCC
	Unclassified	FcaPV-3, -4, -5	Viral plaques/BISC
	<i>Delta</i>	BPV-14	Feline sarcoid

- Papillomaviruses can be categorized according to their tissue tropism and the lesions that they cause.
- The vast majority of papillomaviruses **only infect keratinocytes** and induce a **squamous cell papilla**oma comprised of thickened folded epithelium.
- In contrast, the **deltapapillomaviruses of ruminants** infect and cause proliferation of both keratinocytes and the underlying fibroblasts to produce ***fibropapillomas***.
- While these papillomaviruses are able to infect both epithelial and mesenchymal cells, productive virus replication is restricted to the epithelial component of fibropapillomas.

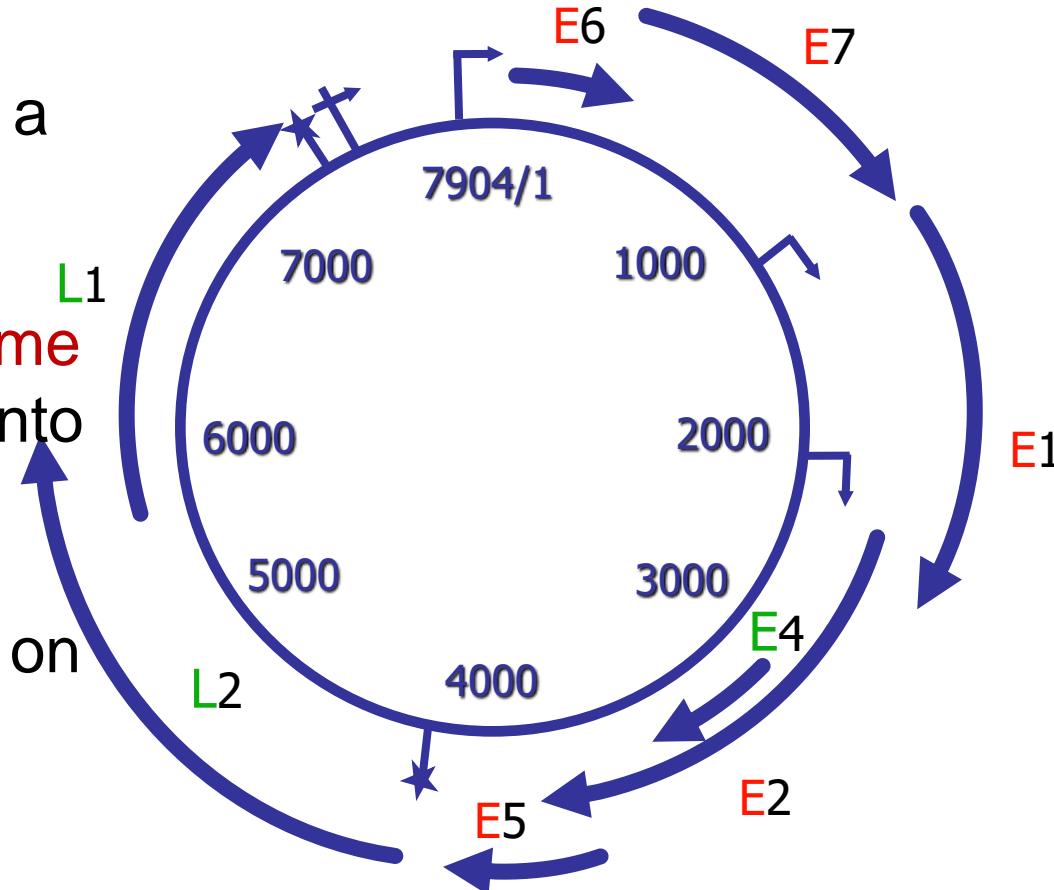
Papillomavirus Virion



- Non-enveloped
- 60 nanometer diameter
- Two capsid proteins L1 and L2
- Icosahedral shell formed by 72 star shaped capsomeres (12 pentons and 60 hexons) of mostly protein, L1

Genome

- 8 kilobase
- circular dsDNA
- associated with cellular histones in a chromatin-like complex.
- Persists as **episome** (doesn't integrate into cellular DNA)
- Virus uses overlapping genes on one strand to pack different genes



GENE EXPRESSION

- Only one strand of the genome is transcribed and yield two classes of proteins expressed by **alternative splicing** :
- a) Early Proteins: non-structural regulatory proteins (E1-E7).
- b) Late Proteins: the structural proteins L1 and L2.

Various proteins coded by Papilloma viruses

- E1- Helicase (Helps in virus replication)
- E2- Transcriptase (Helps in viral replication)
- E4- Destabilizes cytoskeleton
- E5- Mediates mitogenic signals
- E6- Cell transformation (binding to p53)
- E7- cell transformation (binding to pRB)
- L1- Major capsid protein
- L2- Minor capsid protein

REPLICATION

Site-Nuclear

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 ensure an average of one viral genome per basal cell.

- Attachment of the viral proteins to host receptors mediates endocytosis into vesicles in the basal squamous epithelial cell.
- Transport to the nucleus and uncoating of the viral DNA.
- Early-region transcription with the help of **DNA dependent RNA polymerase** early transcription occurs and translation of the early proteins
- Steady-state viral DNA nuclear replication.
- **At this stage, expression of viral capsid proteins cannot be detected.**

b) **The vegetative replication**, which 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.

Vegetative viral DNA synthesis.

Transcription of the late region.

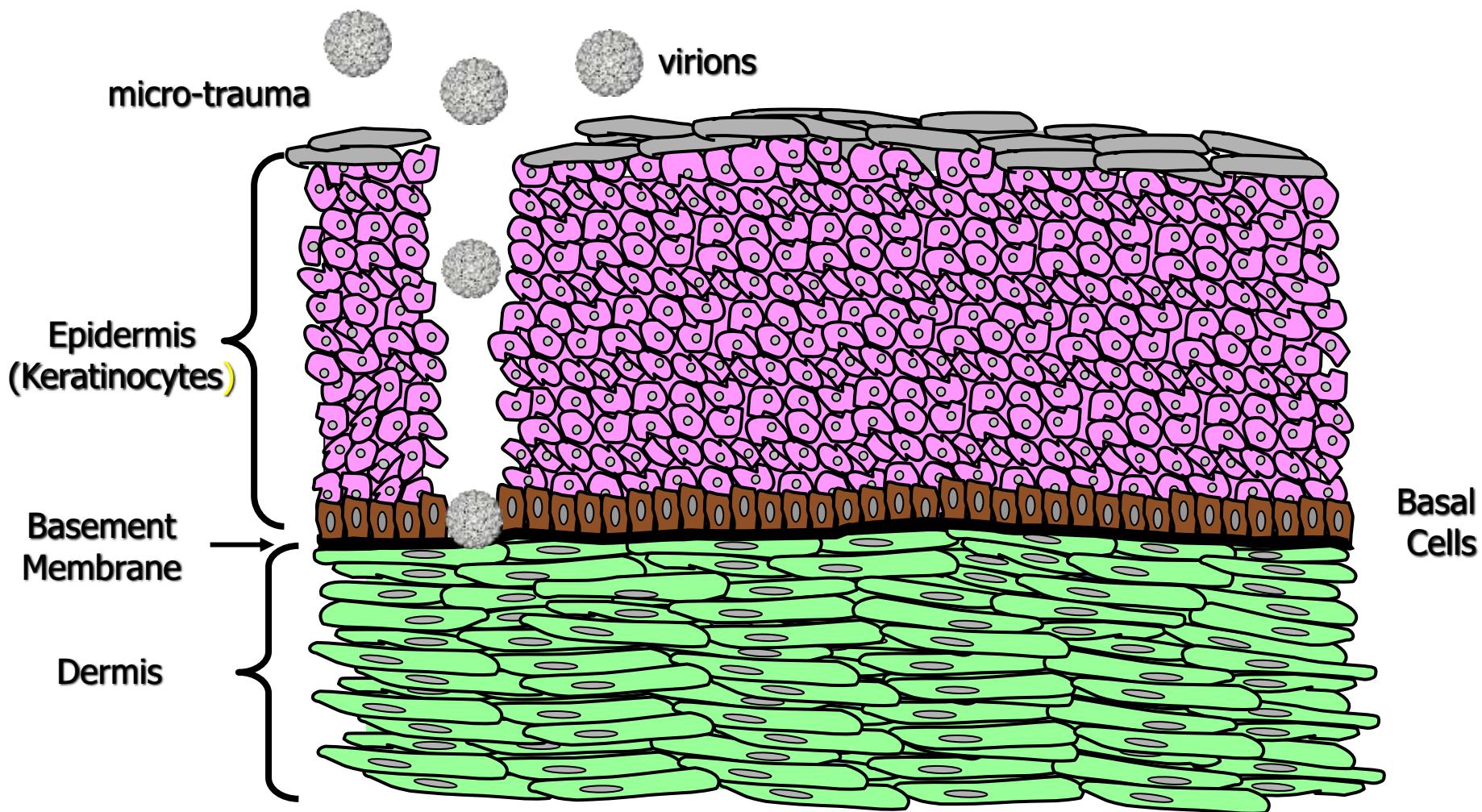
Capsid proteins L1 and L2 synthesis.

Nuclear capsid assembly and release of viruses.

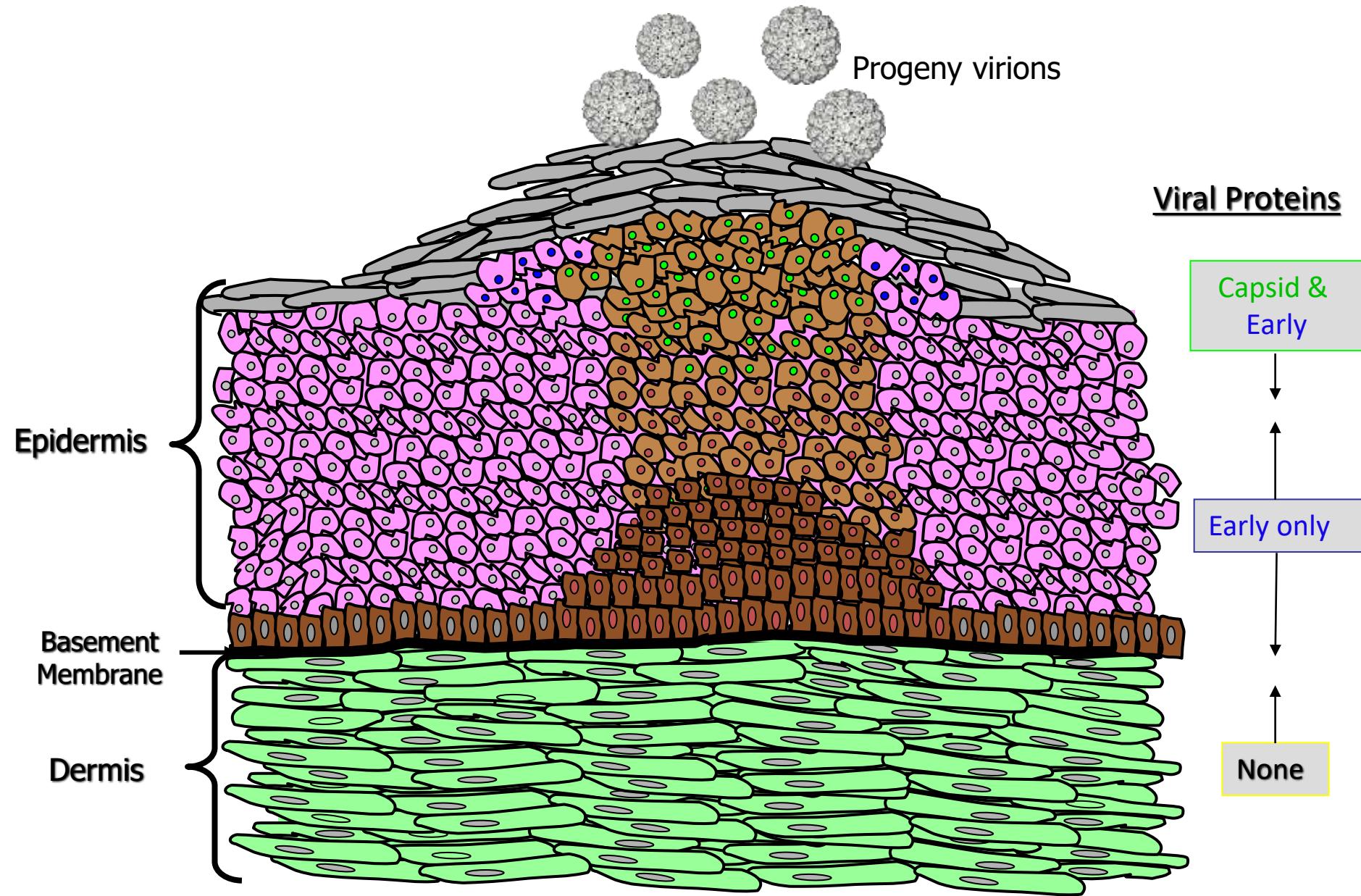
PATHOGENESIS

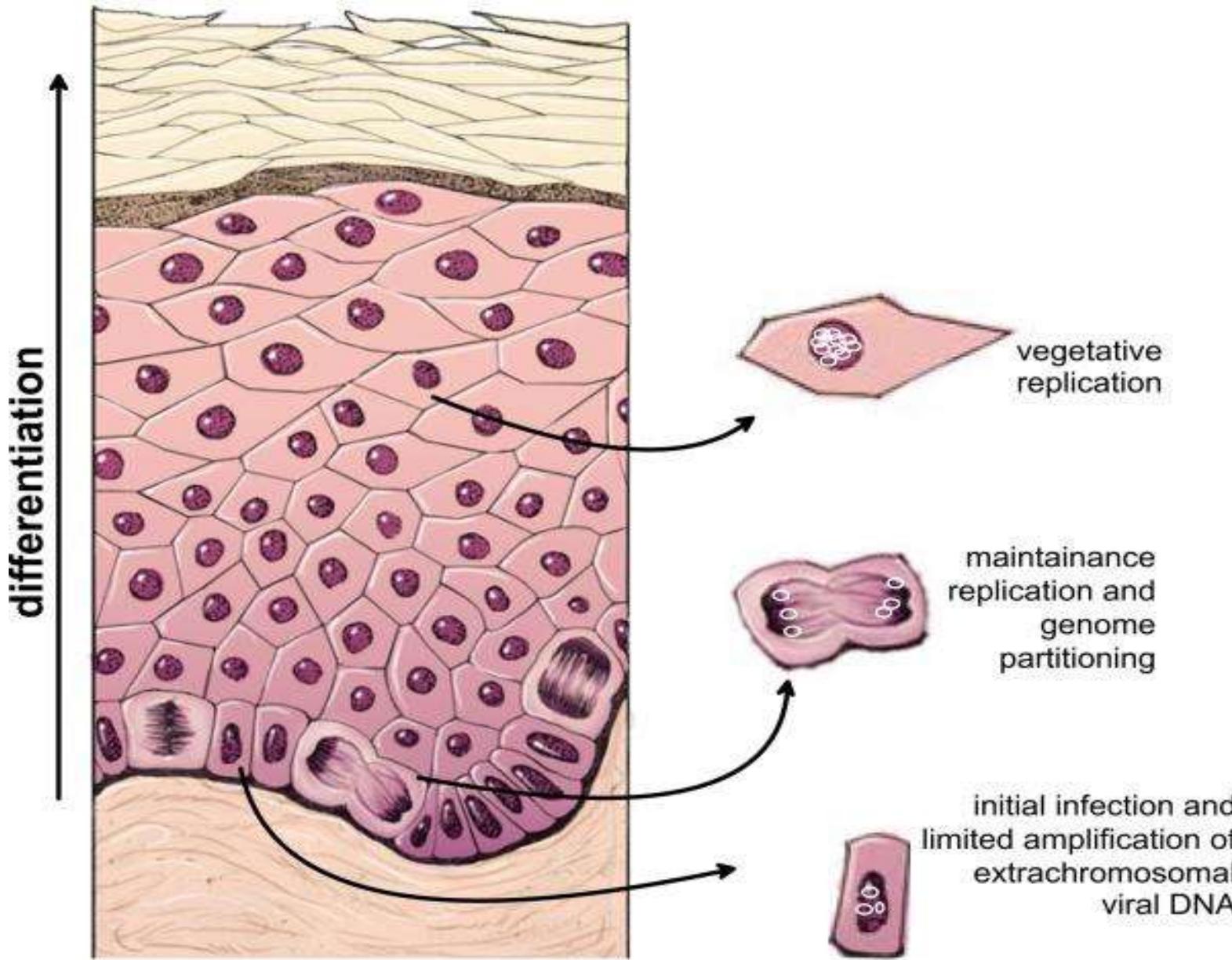
- Papillomas develop after introduction of the virus through skin abrasions.
- In the cells of ***stratum germinativum*** virus proliferate excessively.
- Early proteins delay the maturation of the cells and there is hyperplasia of basal cells.
- There is piling of the cells.
- Slowly differentiation starts and late proteins are expressed in ***stratum spinosum*** and complete virus particles are seen in the cells of ***stratum granulosum***.
- Infection of epithelial cells result in hyperplasia of cells of the surface epithelium with subsequent degeneration and hyperkeratinization.

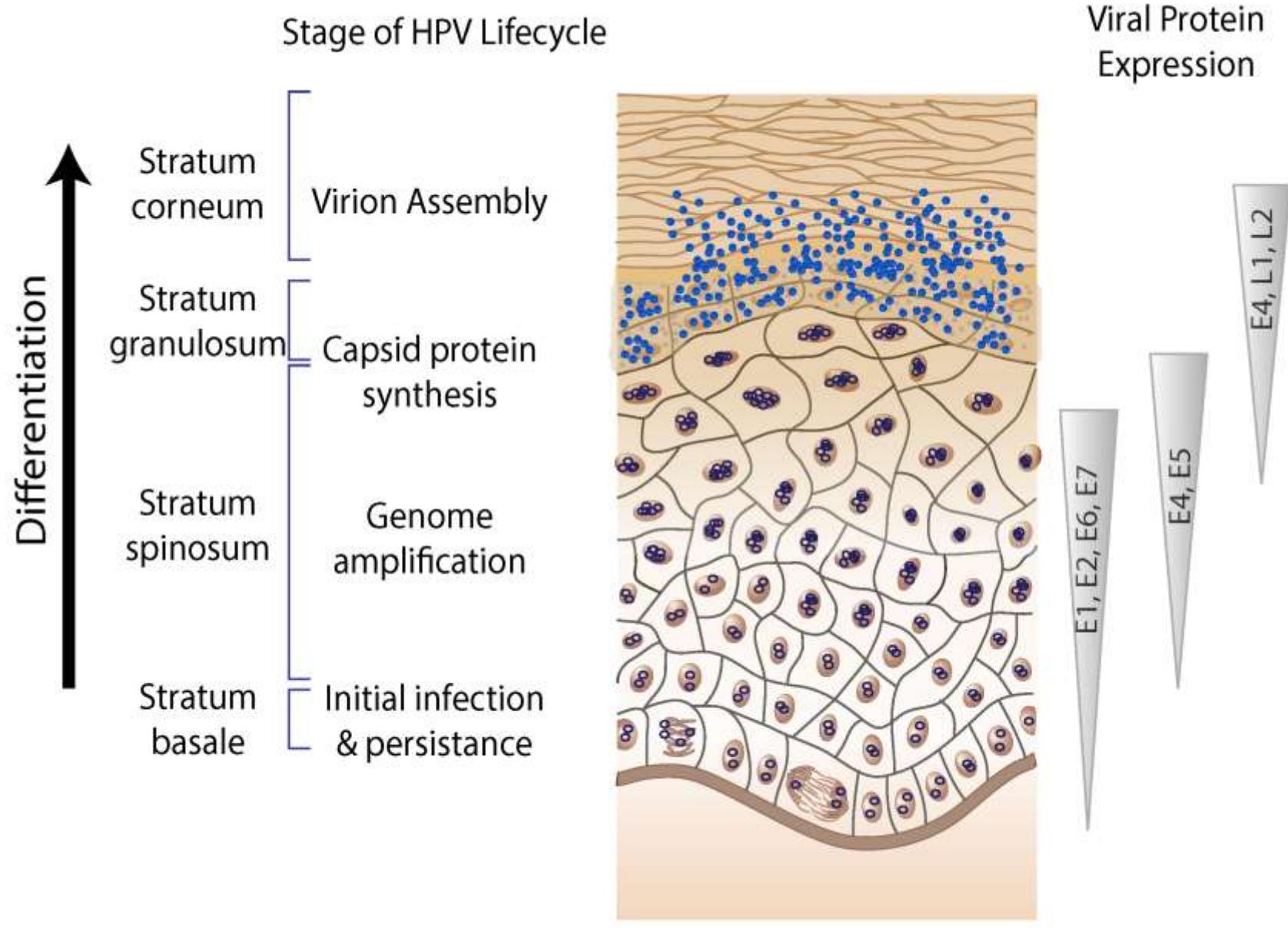
Life Cycle - Initial Infection (Skin)



Gene Expression (Wart)







Maintenance and latency

- After successful infection of a keratinocyte, the virus expresses very low levels of the early viral proteins E1 and E2, which are responsible for replicating and maintaining the viral DNA as a circular episome.
- The viral oncogenes E6 and E7, which promote cell growth by inactivating the tumor suppressor proteins p53 and pRb, respectively, may also be expressed at very low levels.
- Keratinocyte stem cells in the epithelial basement layer can maintain papillomavirus genomes in a dormant or "latent" state for decades.

Bovine papilloma virus (BPV)

- BPV cause both benign and malignant epithelial and mesenchymal tumors or warts in cows and equids.
- They are strictly **species-specific** and, even in experimental conditions, do not infect any other host than the natural one.
- The only known case of cross-species infection is the infection of horses and other equids by BPV type 1 (BPV-1) or BPV type-2 (BPV-2)
- To date, a variety of different papillomavirus types ($n = 25$) from five genera (*Delta-*, *Dyoxi-*, *Dyokappa-*, *Epsilon-*, and *Xipapillomavirus*) have been identified from clinical specimens obtained from cutaneous or mucosal epithelial lesions from infected cattle

- Six BPV types (BPV 1-6) have been characterised associated with different histopathological lesions and classified into three groups:
- **Xi-papillomaviruses** encompassing the pure epitheliotropic BPV-3;BPV-4 and BPV-6;
- BPV-3 infects skin
- BPV-4 infects the upper alimentary tract
- BPV-6 infects teats and udders

Delta papillomaviruses encompassing BPV-1 and BPV-2 associated to fibropapillomas (i.e. benign tumors of both epithelium and underlying derma) and

- BPV-1 infects paragenital areas, including penis, teats and udders
- BPV-2 infects skin, alimentary canal and urinary bladder
- **Epsilon-papillomavirus** comprising the BPV-5 whose genome seems to share similarities with the formers two BPV groups.

PATHOLOGY

- Cutaneous warts are most common in younger animals (under 2 years) and usually spontaneously regress due to the animal's immune response without significant scarring.
- Warts caused by the ***Xipapillomavirus*** group have a **cauliflower-like appearance** and can attain the size of a fist; most common on the head, neck and shoulders, they may also occur in other locations.

- **Cutaneous fibropapillomas - caused by *Deltapapillomavirus* group have a nodular appearance**
- The most common lesion in dairy cattle is the flat “rice-grain” fibropapilloma caused by BPV5
- Warts are contagious and spread primarily by milking machines and milkers’ hands that carry the virus, which then infects the skin in areas of abrasion.



Large warts, showing the cauliflower-like appearance

Equine sarcoid

- The sarcoids are benign tumors of fibroblastic skin origin affecting horses, mules and donkeys. They are locally invasive often occurring at sites of previous injury or scarring.
- Most common tumour in equids worldwide
- Clinically, **six** different types of sarcoids can be distinguished:
- **Occult sarcoid**: is an hairless circular area of the skin;
- **Verrucous**: tumors with wart-like appearance;
- **Fibroblastic** sarcoids present as a fleshy mass;

- **Nodular** sarcoids consist of firm masses lying under the skin and
- **Mixed** sarcoid show a combination of features of verrucous, fibroblastic and nodular types
- **Malevolent** sarcoids are the most infrequent form and are aggressive, invasive tumours that proliferate rapidly and may spread along fascial planes and vessels
- Common sites of appearance is the skin of the head, ventral abdomen, legs and the paragenital region .
- **Both BPV-1 and BPV-2 have been detected in sarcoid tumours with the BPV-1 being the predominant type**



Diagnosis

- By histopathology, thus a biopsy sample is needed (excisional biopsies).
- BPV DNA can be detected by *in situ* hybridization and PCR on formalin fixed and paraffin embedded tissue sections of biopsy samples

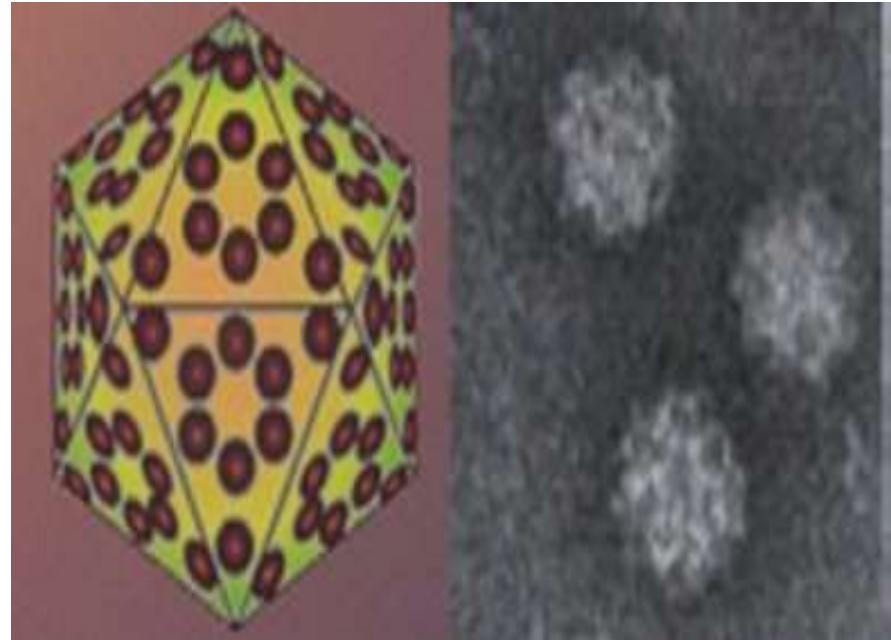
FAMILY CIRCOVIRIDAE

- The family's name, "circo," is derived from the Greek root "gyro," which means ring or circular. This refers to the characteristic circular shape of the circovirus genome
- The family Circoviridae is comprised of viruses with circular, covalently closed, **single-stranded DNA (ssDNA)** genomes, including the **smallest known viral pathogens of animals.**
- **Baltimore group II**
- **Genera in the Family: *Circovirus* and *Cyclovirus***
- Members from the genus Circovirus mainly restricted to vertebrate hosts, whereas cycloviruses have been identified in both vertebrates and invertebrates

- The **genus Circovirus** includes beak and feather disease virus, canary circovirus, goose circovirus, pigeon circovirus, duck circovirus, finch circovirus, gull circovirus, and porcine circoviruses 1 and 2.
- **Porcine circovirus 1 is the type species of the genus Circovirus**
- **Psittacine beak and feather disease virus and porcine circovirus are 17 nm in diameter and are the smallest viruses of vertebrates known.**
- Chicken anemia virus (Gyrovirus) is 22 nm in diameter.
- Characteristically, virions often appear in infected cells and in diagnostic specimens in rather stable linear arrays "strings of pearls."

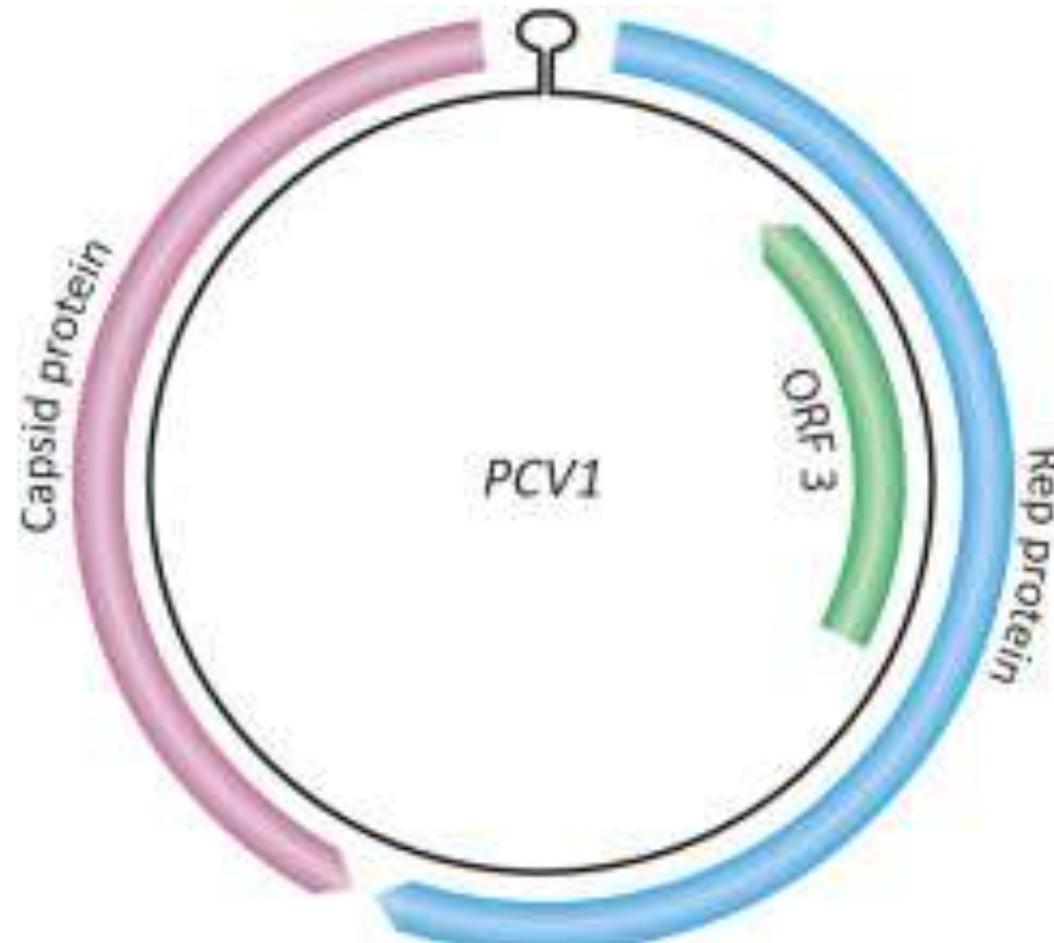
Virion morphology

- Small, 17-22 nm in diameter.
- Non-enveloped viruses with icosahedral nucleocapsids.
- The capsid consists of 12 pentagonal **trumpet shaped pentamers**



GENOME

- **Non-segmented** and **single-stranded DNA**, in the shape of a covalently-closed circle.
- The genomic DNA is made up of 1800-2000 nucleotides.
- Psittacine beak and feather disease virus and porcine circovirus employ an **ambisense** transcription strategy, i.e., ORFs are transcribed in opposite directions from a single promoter site.
- Chicken anemia virus genes are all encoded in the positive-sense strand.
- Viral replication occurs in the **nucleus** and depends on cellular proteins produced during the **S phase** of the cell cycle.

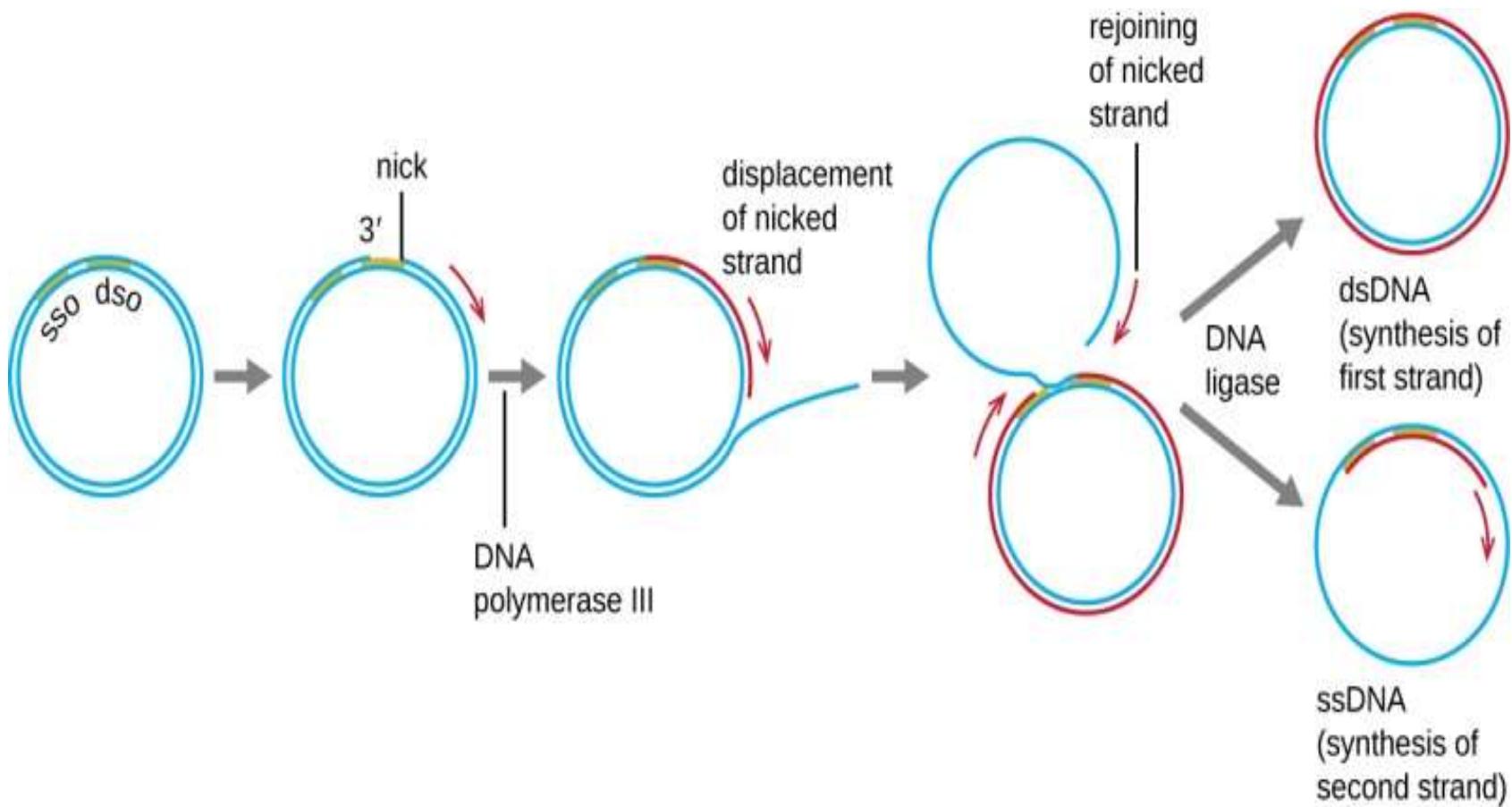


REPLICATION

- Virus penetrates into the host cell.
- Uncoating, the viral ssDNA genome penetrates into the nucleus.
- Viral ssDNA is converted into dsDNA with the participation of cellular factors. dsDNA transcription gives rise to viral mRNAs.
- Viral mRNAs are translated to produce viral proteins.
- Replication may be mediated by a Rep protein, and occur by **rolling circle replication** producing ssDNA genomes.
- These newly synthesized ssDNA can either
 - a) be converted to dsDNA and serve as a template for transcription/replication
 - b) be encapsidated by the capsid proteins and form virions released from the cell by budding.

Rolling circle replication

- The ssDNA virus is converted to transcriptionally active, covalently closed circular DNA by host DNA polymerase
- The Rep protein expressed from the viral genome cleaves at specific sites (origin of replication *v-ori*) at the virion strand to produce replicative form of DNA.
- The free 3' OH end is used by host DNA polymerase to synthesize the new strand using the circular strand as template.
- As the new strands are synthesized, old strands are progressively displaced, after one or two circles, the old displaced strands are ligated to yield circular ssDNA virion strands.
- The circularized new strands are either encapsidated or converted into replicative forms to enter another round of replication



CHICKEN ANEMIA VIRUS

Chicken Infectious Anemia, Blue Wing Disease

- Non-enveloped, icosahedral virus with a very small (2.3 kb), single-stranded, negative sense, circular DNA genome, is the **only recognized member of the Gyrovirus genus of the Anelloviridae family.**
- **It was previously classified as a Circovirus,** but important differences in genome organization led to its reclassification into the new Anellovirus family.

Introduction

- Chicken anemia virus genome has **three overlapping open reading frames**,
- VP1 major capsid protein- vital role in the growth and transmission of CAV
- VP2 non-structural protein with phosphatase activity that plays a key role in the virus's assembly during the infectious cycle.
- VP3 apoptin protein which induces apoptosis of T lymphocytes. It induces severe lympho-atrophy and anemia in infected chickens, and can trigger apoptosis independent of p53 activation

- **Chickens** are the natural hosts of CAV.
- CAV has also been isolated from other species such as **turkeys** and CAV antibodies have been detected in quails.
- CAV mainly infects **10–14 days-old chickens (less than two weeks old)**, leading to severe anemia, yellow bone marrow, aplasia of the bone marrow and atrophy of the lymphoid organ by damaging erythroblastoid cells, resulting in depletion of thymocytes, which makes the chickens immunodeficient .
- It causes atrophy of the thymus and bone marrow, which also causes immunosuppression and weight loss in 2–4 weeks-old chicks.

The importance of this virus comes from its:

1. Trans-ovarian transmission (vertical) and Horizontal transmission
2. Potential for inducing immunosuppression alone or in combination with other infectious agents.

All Chicken Anemia Virus isolates belong to **one serotype**

Transmission

- CAV is highly contagious and it takes few weeks to spread through an entire flock.
- Disease usually occurs during the first 3 week of life through;
 1. Vertical transmission.
 2. Contact exposure close to hatch.
- Chickens at any age are susceptible to infection by:
 1. Oral route.
 2. Respiratory route.

TRANSMISSION

- **Horizontal transmission** is through inhalation or oral exposure, and virus is shed in feces and feather dander.
- Breeder flocks may become infected before they begin to lay fertile eggs, and virus subsequently is transmitted vertically for as long as the hen is viremic.
- If hens are seropositive, maternal antibody generally protects chicks from disease.
- Many flocks of otherwise **specific-pathogen-free** (SPF) chickens carry chicken anemia virus, and it is often difficult to eradicate the virus once it is present.

- The principal sites of CAV replication are **hemocytoblasts in the bone marrow, precursor T cells in the cortex of the thymus, and dividing CD4 and CD8 T cells in the spleen.**
- Replication in and destruction of the hemocytoblasts leads to **anemia**, whereas replication in and destruction of the T cells causes **immunosuppression**.
- Under experimental conditions, the virus is found present in **most organs after one day** (brain, liver, spleen, bursa, bone marrow, rectal contents and serum).
- **Morbidity and mortality rates are high** dependent on the age of the chickens when infected

Clinical Signs

1. Young chickens are **depressed** and **huddle** under the heat source.
2. Exhibit a marked **pallor** that may extend to the internal organs.
3. In chicken anemia virus-infected chickens with severe anemia, **hemorrhagic-aplastic anemia syndrome** as characterized by intracutaneous, subcutaneous, and intramuscular hemorrhages with the wing tips frequently affected, can also occur (**blue wing disease**)
4. The bone marrow is pale or yellow in color and may have a fatty consistency.

5. **Thymic atrophy** and congestion is common and is considered diagnostic when associated with other typical signs or lesions.
6. **Bursal atrophy** is generally modest and transitory, typically occurring at 10 to 14 days of age in chickens vertically infected.
7. All of the aforementioned lesions are exacerbated and more persistent in chickens coinfected with infectious bursal disease virus or other lymphocidal agents.
8. Severely affected birds generally die within 2 to 4 week of age
9. Survivors are often stunted.



Haemorrhages on the wing of a young chicken with Chicken Infectious Anemia, hence the name Blue Wing Disease.



Subcutaneous haemorrhage in a young chicken with Chicken Infectious Anemia, resulting in blue discoloration of the hock joint.



Pale anemic carcass of a young chicken with Chicken Infectious Anemia

Diagnosis

- Diagnosis of chicken anemia virus infection in chickens is based on history, clinical signs, and gross and microscopic pathologic findings.
- Anemia, leukocytopenia, and thrombocytopenia.
- Packed cell volumes are low, and blood smears often reveal anemia and leukopenia.
- Blood may be watery and clot slowly as a consequence of thrombocytopenia.

Diagnosis

Laboratory tests

- Laboratory tests to identify the viral genome, antigen or antibodies are required for a definitive diagnosis.
- Testing serum samples for example at the time of the clinical signs and 2-3 weeks later provide the best basis for serological diagnosis.
- Chicken anemia virus is the only avian gyrovirus that can be efficiently propagated in cell culture, specifically in lymphoblastoid T cell (MDCC-MSB1 and MDCC-JP2) and B cell lines (LSCC-1104B1)

- No specific treatment is available. Control is best achieved by vaccination.
- Chicken anemia virus poses **no known zoonotic risk to humans**, nor does it infect other mammals.

Porcine circovirus (PCV)

- Common virus of pigs found throughout the world
- **The smallest virus to infect mammals**
- Two porcine circoviruses have been isolated from pigs.
- **Porcine circovirus 1 (PCV1)** is ubiquitous, and nonpathogenic in swine.
- **Porcine circovirus 2 (PCV2)** emerged as a major swine pathogen about 20 years ago and is now endemic worldwide.
- PCV2 is associated with **postweaning multisystemic wasting syndrome (PMWS)**, a disease characterized by jaundice, diarrhea, respiratory disease, failure to thrive, and sudden death.

FAMILY PARAMYXOVIRIDAE

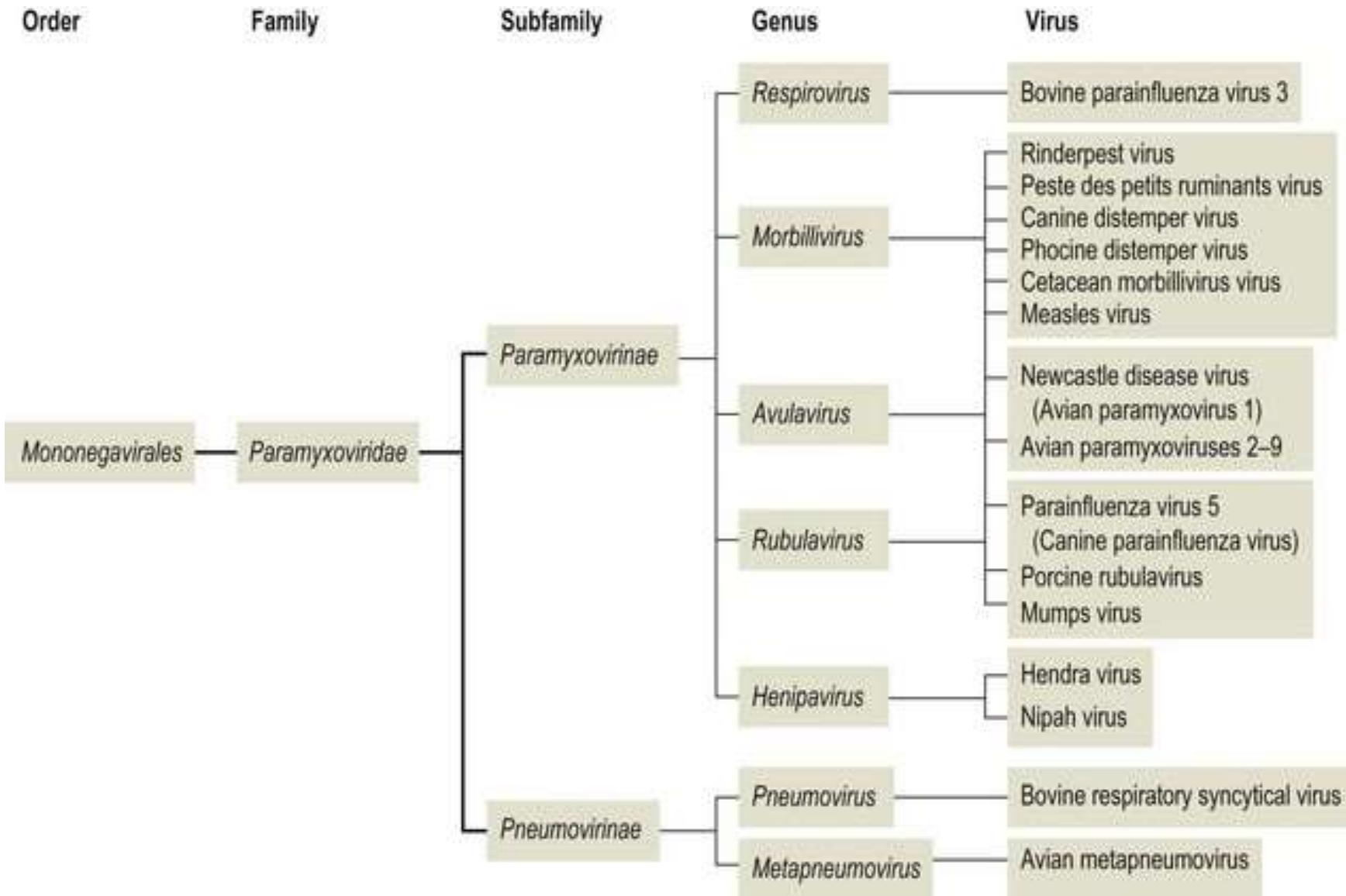
Order Mononegavirales- four families-
Bornaviridae, Rhabdoviridae, Filoviridae
and Paramyxoviridae

Introduction

- The name paramyxovirus is derived from two Greek words, *para* meaning by the side of and *myxa* meaning mucus
- Belongs to **order Mononegavirales**- All the viruses are enveloped, covered with peplomers, and all have genomes consisting of a **single molecule of negative-sense, single-stranded RNA**
- **Baltimore group V**

- Includes important agents of disease, causing age-old diseases of humans and animals (measles, rinderpest, PPR, canine distemper, New Castle disease, mumps, respiratory syncytial virus (RSV), the parainfluenza viruses), and newly recognized emerging diseases (Nipah, Hendra, morbilliviruses of aquatic mammals)
- Most viruses have a **narrow host range** in nature, many are **host-specific**
- **The transmission of paramyxoviruses is horizontal, mainly through airborne routes; no vectors are known**

Classification



International Committee on Taxonomy of Viruses (ICTV)

Report on the family Paramyxoviridae by Rima *et al.* (2019)

- Currently, *Paramyxoviridae* has **four subfamilies**, 17 genera, and 77 species.
- Subfamily: ***Avulavirinae***, which contains three genera (Meta, ortho and Para avulavirus) and 21 species
- Subfamily: ***Metaparamyxovirinae***, which contains one genus and one species
- Subfamily: ***Orthoparamyxovirinae***, which contains eight genera and 34 species
- Subfamily: ***Rubulavirinae***, which contains two genera (Ortho and para rubulavirus) and 18 species

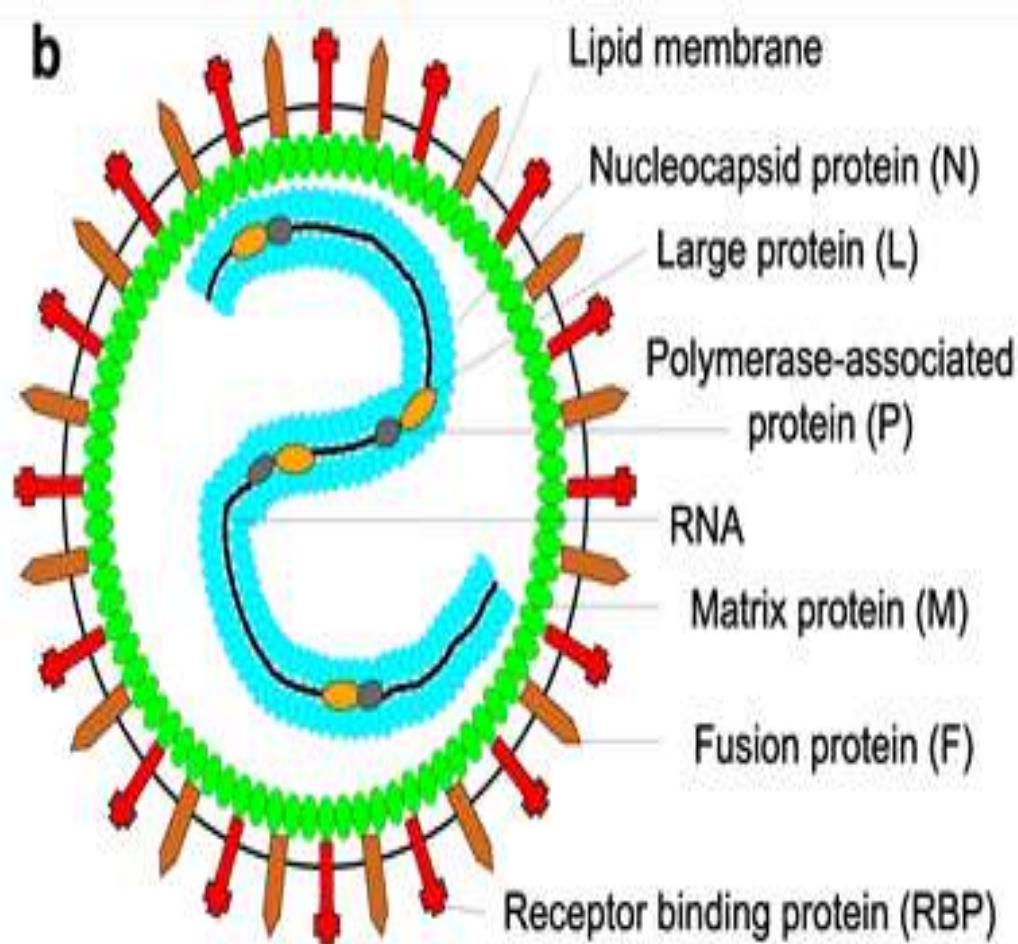
Virion Properties

- **Pleomorphic** in shape (spherical as well as filamentous forms occur), 150-300 nm in diameter and **enveloped**.
- The lipid envelope is covered in glycoprotein spikes (**peplomers**) of two types, an attachment protein (G or H or HN) which is a tetramer and a trimer or tetramer of fusion protein (F).
- Proteins are designated **H (hemagglutinin) for morbilliviruses** as they possess haemagglutination activity, (ability to cause red blood cells to clump in laboratory tests).

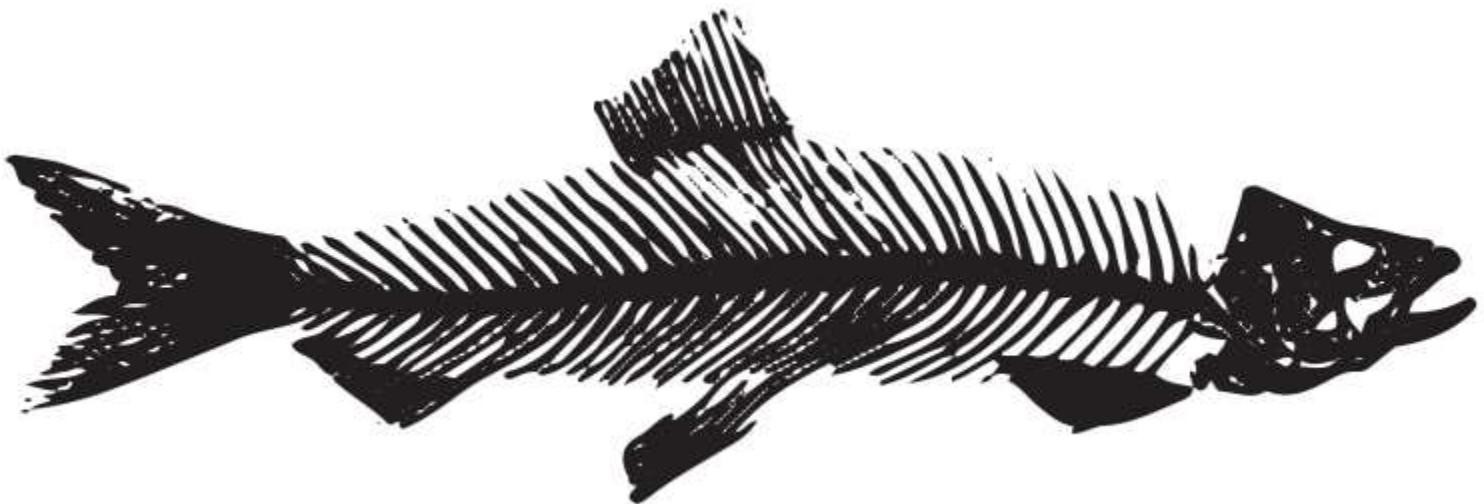
- **HN (Hemagglutinin-neuraminidase)** attachment proteins occur in **respiroviruses, rubulaviruses and avulaviruses**. These possess both haemagglutination and neuraminidase activity, which cleaves sialic acid on the cell surface, preventing viral particles from reattaching to previously infected cells.
- Attachment proteins with neither haemagglutination nor neuraminidase activity are designated **G (glycoprotein)**. These occur in **henipaviruses**.

- Cell attachment is mediated via the hemagglutinin-neuraminidase (HN) protein.
- This glycoprotein elicits neutralizing antibodies that inhibit adsorption of virus to cellular receptors.
- The **fusion protein** is present on newly formed virions in an inactive precursor form (F_0) that is activated by proteolytic cleavage by a cellular protease which is essential for viral infectivity.
- A neutral pH for fusogenic activity is also a requirement.

- Because the fusion protein is essential for viral infectivity and for direct cell-to-cell spread via fusion between the viral envelope and the cell membrane, it plays a **key role** in the pathogenesis of paramyxovirus infections, including persistent infections.
- There is also an envelope-associated non-glycosylated **matrix (M)** protein between the envelope and the nucleocapsid core.

a**b**

- The nucleocapsid has **helical symmetry**, 13 to 18 nm in diameter, has a characteristic ‘herringbone’ appearance
- A single molecule of linear, non-segmented, negative-sense, single-stranded RNA present
- **Three** nucleocapsid-associated proteins, i.e., an RNA-binding **nucleocapsid protein (N)** protects the RNA from nuclease digestion
- a polymerase-associated **phosphoprotein (P)** that binds to N and L proteins and forms part of the RNA polymerase complex
- and a **large protein (L)**, is the catalytic subunit of RNA-directed RNA polymerase (RdRP) (capping and cap methylation activities)



Herring bone - named for the resemblance to the bone structure of the herring fish skeleton

- Virions **are labile**, being sensitive to heat, desiccation, lipid solvents, non-ionic detergents and many disinfectants.
- Paramyxoviruses can possess haemagglutinating, haemolytic and neuraminidase activities.
- Infection of cultured cells is generally lytic, but temperate or persistent infections are common in this family in vitro and in vivo.
- Also there is the formation of inclusion bodies and syncytia in infected cells.

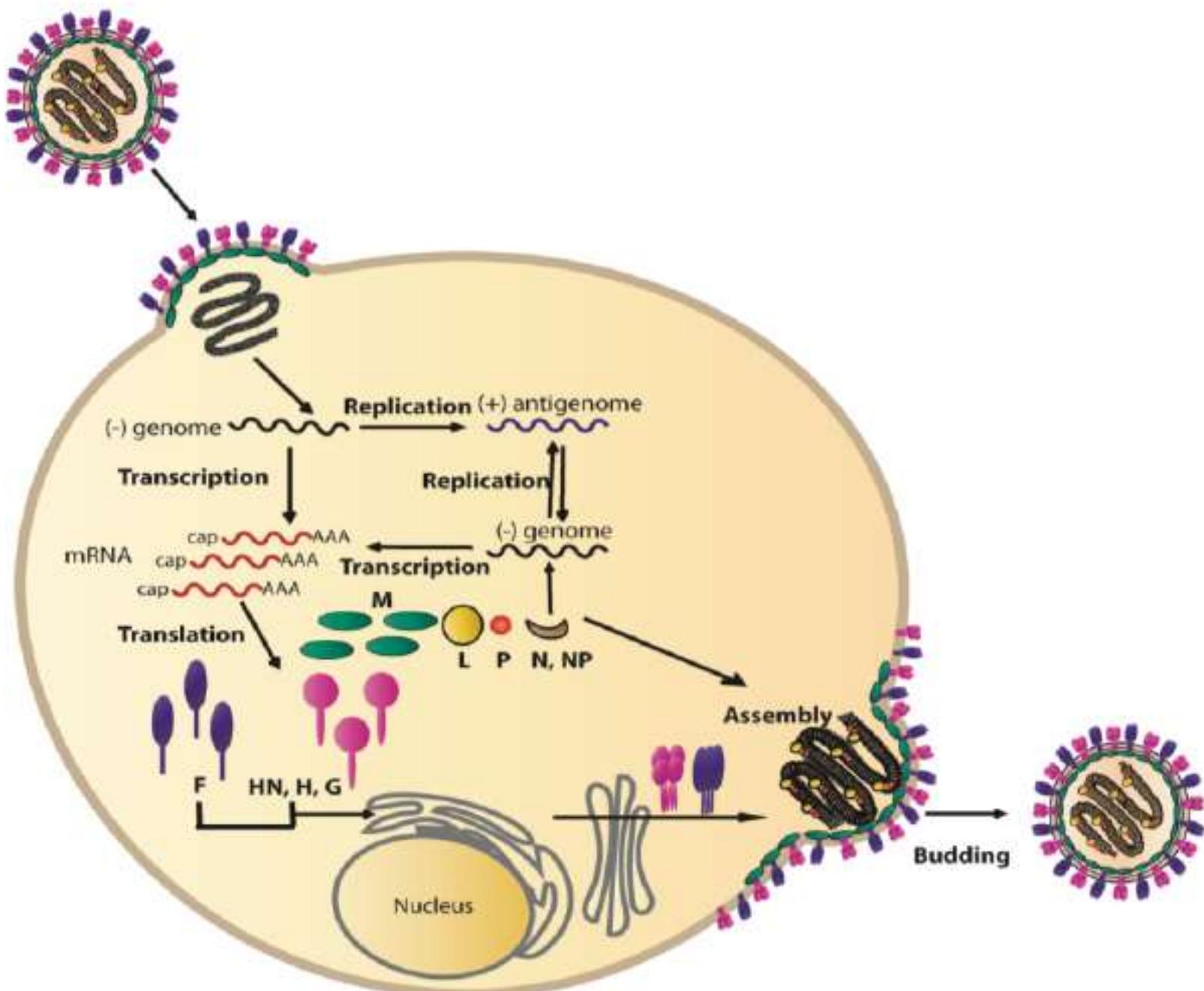
Viral replication

- **Site- cell cytoplasm**
- Even though their replication is entirely cytoplasmic, Morbilliviruses produce acidophilic intranuclear inclusion bodies
- Virions attach via their hemagglutinin- neuraminidase protein to cellular sialoglycoprotein or glycolipid receptors.
- The fusion protein then mediates fusion of the viral envelope with the plasma membrane, at physiologic pH.

- The liberated nucleocapsid remains intact, with all three of its associated proteins (N, P, and L) being required for transcription by the virion-associated, RNA-dependent RNA polymerase (transcriptase).
- The viral genome is transcribed from the 3' end by viral polymerase (P-L) which generate mRNAs for secondary transcription.

- Replication of the full-length genome occurs efficiently only after accumulation of viral proteins and involves production of positive sense anti-genomes which act as templates for the synthesis of new negative-sense genomic RNA.
- Matrix proteins are considered the key organizers of virus particle assembly since they act as bridges between the envelope glycoproteins and the ribonucleoprotein complexes, can self-assemble into higher order structures, and bind cellular membranes as well as several cellular factors.

- This new RNA associates with the NP and transcriptase to form the nucleocapsid and then the M, F, and HN proteins migrate to the surface of the plasma membrane.
- Paramyxoviruses bud off of the cell membrane, leaving them enveloped.



New Castle disease virus

- The taxonomic name for Newcastle disease virus (NDV) has recently changed from Avian paramyxovirus 1 (APMV-1) to **Avian Avulavirus 1 (AAvV-1)**.
- **Belongs to subfamily- Avulavirinae**

Pathotypes of NDV

- **Five pathotypes** based on clinical signs in infected chickens:
 - a) viscerotropic velogenic (Doyle's form) - hemorrhagic intestinal lesions
 - b) neurotropic velogenic (Beache's form)- respiratory and neurologic signs
 - c) mesogenic, (Beaudett's form).
 - d) lentogenic or respiratory (Hitchner form).
 - e) asymptomatic. .

- Velogens and mesogens are now classified as **virulent NDV (vNDV)**, the cause of Newcastle disease and reportable infection,
- whereas infections with lentogens, the **low virulence NDV (loNDV)** widely used as live vaccines, are not reportable.
- Members of all species of the subfamily Avulavirinae have hemagglutinin and neuraminidase activities.

New Castle disease

or Ranikhet disease or Avian Distemper

- The disease was first observed in Java in 1926 and in the same year it spread to England, where it was first recognized in Newcastle, hence the name.
- The disease is one of the most contagious of all viral diseases, spreading rapidly among susceptible birds
- **OIE list A** disease
- **Zoonotic**- Humans may become infected; manifested by unilateral or bilateral reddening, excessive lacrimation, oedema of the eyelids, conjunctivitis and sub-conjunctival haemorrhage

Transmission

- By direct contact with diseased or carrier birds.
- Infected birds may shed the virus in their feces, contaminating the environment.
- Transmission can then occur by direct contact with feces and respiratory discharges or by contaminated food, water, equipment, and human clothing.
- Newcastle disease viruses can survive for several weeks in the environment, especially in cool weather.

- Generally, virus is shed during the incubation period and for a short time during recovery.
- Birds in the pigeon family can shed the virus intermittently for a year or more.
- The virus is present in all parts of the carcass of an infected bird.
- When the virus is introduced into a susceptible flock, virtually all the birds will be infected within two to six days.

Pathogenesis

- Initially the virus replicates in the mucosal epithelium of the upper respiratory and intestinal tracts; shortly after infection, virus spreads via the blood to the spleen and bone marrow, producing a secondary viremia.
- This leads to infection of other target organs: lung, intestine, and central nervous system.
- Respiratory distress and dysponea result from congestion of the lungs and damage to the respiratory center in the brain.
- The most prominent histologic lesions are necrotic foci in the intestinal mucosa and the lymphatic tissue and hyperemic changes in most organs, including the brain.

In vivo Pathogenicity tests

1. Intravenous Pathogenicity Index (IVPI)-

- 6 wks susceptible chicks
- Birds are examined at 24 hour intervals for 10 days and scored at each observation:
 - 0 if normal,
 - 1 if sick,
 - 2 if paralysed and
 - 3 if dead.
- The IVPI is the mean score per bird per observation over the 10 day period. Lentogenic strains and some mesogenic strains will have IVPI values of 0, whereas the indices for virulent strains will approach 3.0.

2. Mean death time (MDT)- in 9 days embryonated eggs.

Each egg is examined twice daily for 7 days and the times of any embryo deaths recorded.

- The minimum lethal dose is the highest virus dilution which causes all the embryos inoculated with that dilution to die.
- The MDT is the mean time in hours for the minimum lethal dose to kill embryos.
- The MDT has been used to classify NDV strains into velogenic, taking less than 60 hours to kill; mesogenic, taking between 60-90 hours; and lentogenic, taking more than 90 hours.

3. Intracerebral pathogenicity index (ICPI)-0.05ml
of the diluted virus is injected intracerebrally into each of 10 one-day-old chicks hatched from an SPF flock. The birds are examined every 24 hours for 8 days.

- At each observation the birds are scored: 0 if normal, 1 if sick, and 2 if dead.
- The ICPI is the mean score per bird per observation over the 8-day period.
- The most virulent viruses will give indices which approach the maximum score of 2.0, whereas lentogenic strains will give values close to 0.0.

Clinical signs

- Dependent on factors such as: the virus/pathotype, host species, age of host, co-infection with other organisms, environmental stress and immune status.
- **Lentogenic strains** Usually associated with subclinical disease marked by mild respiratory disease; coughing, gasping, sneezing and rales
- Mortality is negligible
- **Mesogenic strains** May cause acute respiratory disease and neurologic signs in some species
- Mortality rate is usually low (<10%)
- **Velogenic strains** Most commonly cause severe disease in chickens with 100% mortality; signs principally respiratory and/or nervous

- Initial clinical signs vary but include: lethargy, inappetence, ruffled feathers, oedema and infection of conjunctiva.
- As the disease progresses birds may develop: **greenish or white watery diarrhoea**, dyspnoea and inflammation of the head and neck often with cyanotic discoloration
- In later stages of disease neurologic signs may be manifested as: tremors, tonic/clonic spasms, wing/leg paresis or paralysis, **torticollis**, and aberrant circling behaviour

- Sharp drop in egg production; eggs contain a watery albumin and appear misshapen with abnormally coloured, rough or thin shells
- These strains often result in sudden death, with few or no signs
- Birds that survive serious infection may develop neurologic and partial or complete cessation of egg production

Lesions

- Only velogenic strains produce significant gross lesions
- **Predominantly hemorrhagic lesions**, in particular at the esophagus/proventriculus and proventriculus/gizzard junctions and in the posterior half of the duodenum, the jejunum, and ileum.
- In severe cases, hemorrhages are also present in subcutaneous tissues, muscles, larynx, tracheal/esophageal tissues, serous membranes, trachea, lungs, airsacs, pericardium, and myocardium.
- **Spleen may appear enlarged, friable and dark red or mottled**

- In adult hens, hemorrhages are present in ovarian follicles.
- Lesions can develop into diphtheroid inflammatory foci and later into necrotic foci.
- In the **central nervous system**, lesions are those of encephalomyelitis--neuronal necrosis, perivascular cuffing, and interstitial inflammatory infiltration



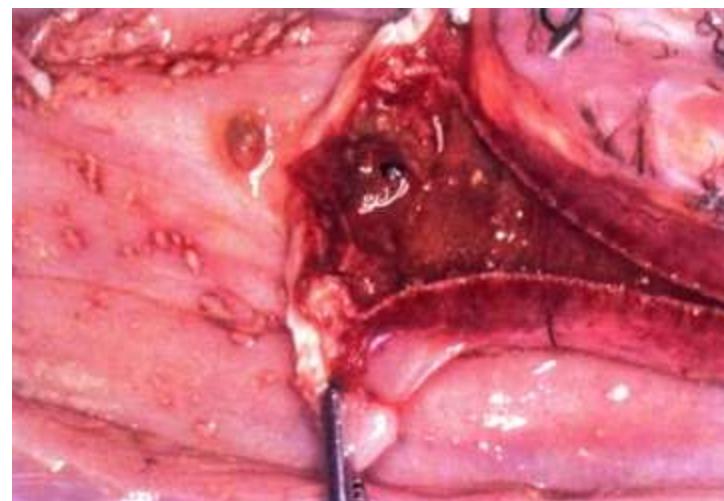
Opisthotonus in the neurotropic form



The haemorrhages of gizzard epithelium



The mucous coat of gizzard is oedematous, covered with thick mucus



The haemorrhagic necrotic and focal diphtheroid lesions in the mucosa of the buccal cavity

Diagnosis

Samples

- **Samples should be collected from recently dead birds or moribund birds that have been killed humanely.**
- **Dead birds:** oro-nasal swabs; lung, kidneys, intestine (including contents), caecal tonsils, spleen, brain, liver and heart tissues, separately or as a pool
- **Live birds:** tracheal or oropharyngeal and cloacal swabs (visibly coated with faecal material) from live birds or from pools of organs and faeces from dead birds
- Clotted blood samples or serum

Identification of the agent

- **Virus isolation** : by **allantoic inoculation** of 10-day-old embryonated eggs, and tested for haemagglutination (HA) activity
- **Virus identification:** use of specific antiserum in a haemagglutination inhibition (HI) test
- Pathogenicity index determined by intracerebral pathogenecity test.
- Molecular techniques- RT-PCR

Serological tests

- Haemagglutination and haemagglutination inhibition tests: most widely used and detects antibody response to virus glycoprotein (predictor of protection against disease)
- Enzyme-linked immunosorbent assay (ELISA): as whole virus is used as antigen, detects antibody to all of the virus proteins

Vaccination

- **Conventional live virus vaccines:** 2 groups
- **Ientogenic vaccines** (e.g. Hitchner-B1, La Sota, V4, NDW, I2 and F)
- **mesogenic vaccines** (e.g. Roakin, Mukteswar and Komarov)
- Live virus vaccines administered to birds by incorporation in the drinking water, delivered as a coarse spray (aerosol), or by intranasal or conjunctival instillation; some mesogenic strains are given by wing-web intradermal inoculation

- **Inactivated vaccines**- prepared from allantoic fluid that has had its infectivity inactivated by formaldehyde or beta-propiolactone incorporated into an emulsion with mineral oil or vegetable oil, and is administered intramuscularly or subcutaneously;
- **New recombinant vaccines**: fowlpox virus, vaccinia virus, pigeonpox virus, turkey herpesvirus and avian cells in which the HN gene, the F gene, or both, of NDV are expressed

Prevention

When the disease appears in a previously disease free area, a stamping out policy is practiced in most countries. This includes:

- strict isolation or quarantine of outbreaks;
- humane destruction of all infected and exposed birds
- thorough cleaning and disinfection of premises;
- proper carcass disposal;
- pest control in flocks;
- depopulation followed by 21 days without poultry before restocking;
- avoidance of contact with birds of unknown health status;
- control of access to poultry farms.

Canine distemper virus

- **Canine distemper virus**, or CDV, is a **morbillivirus** closely related to the viruses of measles and rinderpest.
- Morbillivirus belongs to **subfamily orthoparamyxovirinae** and are able to infect a wide range of hosts to cause varied types of disease, many of which are severe.
- Wild animals like raccoons, foxes, wolves, coyotes, skunks, ferrets, and mink can also get distemper.

Canine distemper

- Syn. - Canine influenza; Carres; Hard pad disease; footpad disease
- It is an **acute highly contagious** viral disease of carnivorous animals characterized by **diphasic fever**, ocular and nasal catarrh and frequent cutaneous eruptions.
- **Affects dogs most commonly aged between 3 and 6 months** when maternal antibody level is declining, but can occur **in older dogs** that have been vaccinated infrequently or improperly, especially after stress, immunosuppression, or contact with other affected dogs.

- CD is a **multisystemic disease** that can present with one or more of the following:
 - Respiratory disease with severe pneumonia
 - Gastrointestinal disease with vomiting and diarrhoea
 - Neurological disease including seizures
 - Severe immunosuppression leading to infection by normally innocuous bacteria and viruses
- The mortality rate of all forms of the disease, taken together, ranges between 30 and 80%.

Transmission

- Indigenous breeds are quite resistant.
- In nature, the transmission of the virus takes place by inhalation (droplet or aerosol).
- The disease may indirectly be transmitted through ingestion of contaminated food and water.

Sources of virus

- The virus is discharged through secretions and excretions-conjunctival, nasal, and oral exudates, urine, feces, and skin dander.
- The virus is present in all the excretions during the systemic reaction phase of the disease.
- virus is sensitive to lipid solvents, such as ether, and most disinfectants, including phenols and quaternary ammonium compounds. It is relatively unstable outside the host.

Pathogenesis

- CDV is considered a multi-cell pathogen that has the ability to infect three different types of host cells including **epithelial, lymphoid, and neurological cells.**
- The pathogenesis is as follows:
Virus – inhalation – Pharynx – Palatine tonsil –
Macrophages–Lymphoid vessels – Blood vessels –
Bone marrow –Spleen – Lymphoid tissues –
Leukopenia – Leukocytosis.

- Infected cells carry the virus to the draining lymph node where the resident activated T-cells and B-cells are infected, resulting in virus amplification and the initiation of primary viremia.
- The virus gets disseminated to secondary lymphoid organs, including the spleen, the thymus, the tonsils and subsequently a systemic spread through the entire immune system.

Clinical signs

- **In acute form-** There is a high rise of temperature. This temperature reaction is characteristic and is known **as biphasic reaction.**
- The temperature rises upto 103 to 104°F. In this stage, the nose of the animal will turn dry and hot and eyes will turn congested. The animal is markedly depressed and refuses to take food.
- The first fever peak, often 3-6 days after infection, is accompanied by lymphoid depletion.

- Within few days yellowish green discharge is voided from the eyes and nose.
- This temperature usually come down in 96 hours to normal level and remains so till 11 to 12 days.
- Afterwards, temperature rises to a second peak which remains continuous during the course of the systemic infection.
- The second rise of temperature is accompanied by widespread viremia and dog shows rhinitis, conjunctivitis, gastroenteritis and bronchopneumonia.

- **The pulmonary disease** is characterized by catarrhal inflammation of the larynx and bronchi, tonsillitis, and a cough. Later, bronchitis or catarrhal bronchopneumonia develop, sometimes with pleuritis.
- **The gastrointestinal disease** is characterized by severe vomiting and watery diarrhea.
- **Occular form** -swollen eye lids, congestion of conjunctival mucosa (conjunctivitis), purulent discharge from the eyes. The pus may lead to ulceration of the cornea.

- **Nervous form –subacute form or follow any of the acute manifestation.**

These are characterized by restlessness, excitement, chewing movements (“**chewing-gum fits**”), excessive salivation and convulsion. The dog may fall and show epileptic form fits. Lymphopenia is the distinct feature of distemper

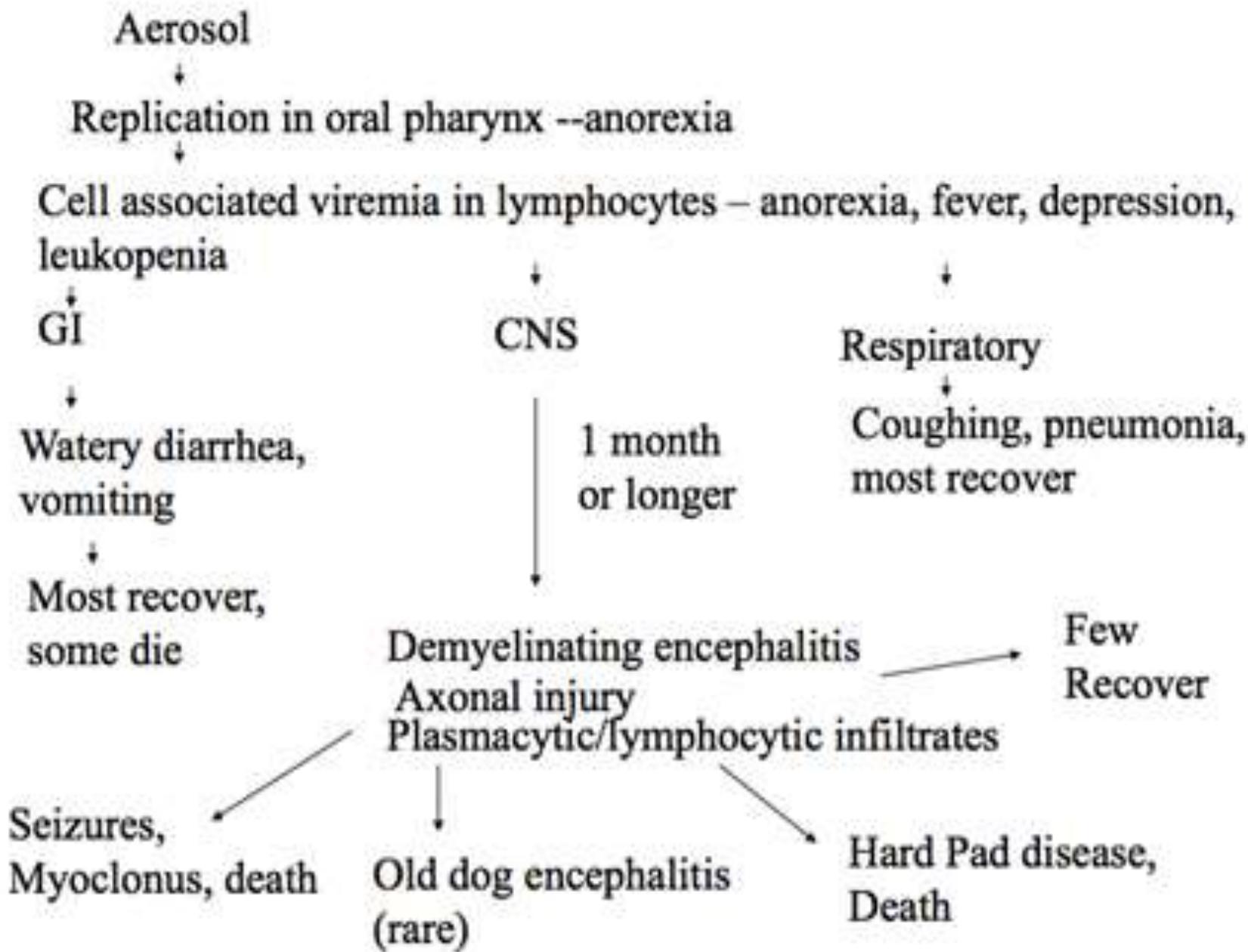
- The nervous form may be characterized by **chorea**.
- Chorea indicates jerky movements of group of muscles. The muscular spasms may be observed in the lips, cheeks, jaws, head, neck or limb muscles.
- **Paresis or paralysis**, often beginning in the hind limbs and evident as ataxia, followed by ascending paresis and paralysis.

- **Cutaneous form**

There is appearance of rash, vesicles and pustules, In some cases, the **skin of the foot pads and nose** may become hard due to hyper keratosis and the condition is ascribed as “**hard pad disease**”.

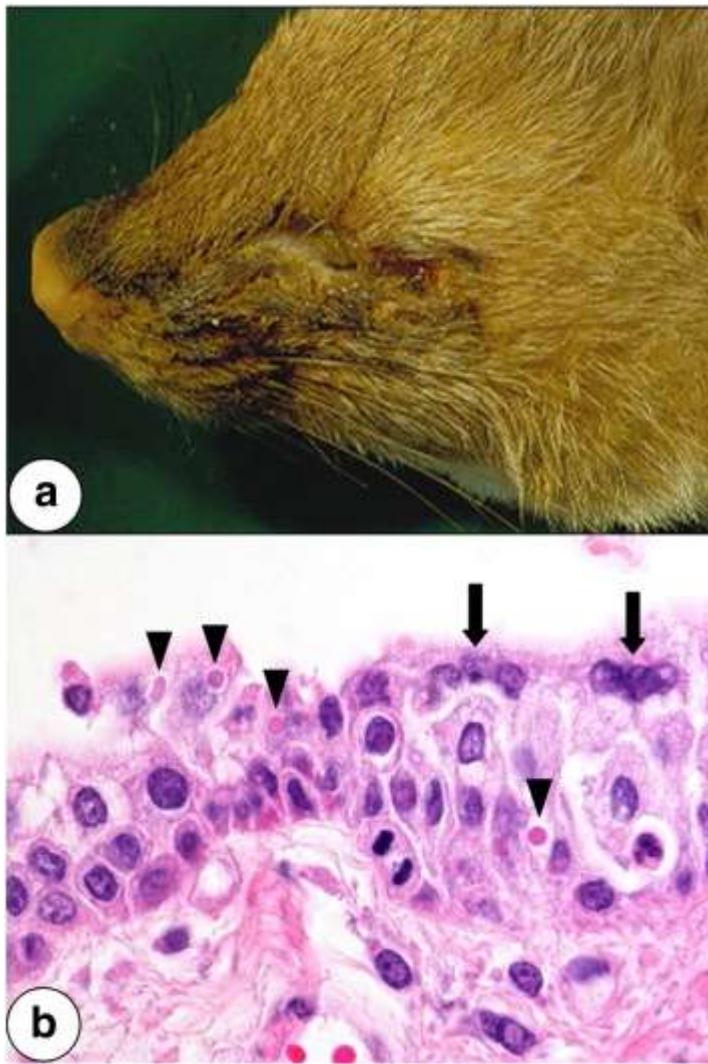
- When the foot pads are thickened, the dog may experience some difficulty in walking on hard objects.
- There may be vesicopustular eruptions on the ventral aspect of the abdomen and on the inner parts of the thighs of skin. This type of distemper eruptions are known as **distemper exanthema**.

- There are **two late forms of the disease seen in old dogs: old dog encephalitis and hard-pad disease**
- “**Old dog encephalitis**” in old dogs in which there is slow progressive loss of neurologic functions and characterized by mental disorders, motor deterioration and death





Hard pad disease



a) Severe muco-suppurative conjunctivitis; b) Conjunctiva with epithelial syncytial cells (arrows) and cytoplasmic eosinophilic viral inclusion bodies (arrowheads); hematoxylin–eosin

Lesions

- **Hyperkeratosis** of the nose and footpads
- **Thymic atrophy** is a consistent pm finding in infected young puppies.
- Necrosis of lymphatic tissues, interstitial pneumonia, and **cytoplasmic and intranuclear inclusion bodies** in respiratory, urinary, and GI epithelium.
- **Lesions found in the brains** of dogs with neurologic complications include:
 - neuronal degeneration, gliosis
 - Non-inflammatory demyelination
 - perivascular cuffing
 - Non-suppurative leptomeningitis, and
 - **Intranuclear inclusion bodies** predominately within glial cells

Diagnosis

- Reverse-transcriptase polymerase chain reaction (RT-PCR)
- Virus isolation
- Immuno-histochemistry
- Fluorescent antibody staining
- Faecal enzyme-linked immunosorbent assay (ELISA)
- Electron microscopy
- Histopathology
- **Serological tests**
- Indirect fluorescent antibody tests
- Haemagglutination inhibition
- ELISA

Vaccination

- A dog after recovery from an attack of distemper generally acquire a long lasting immunity
- Vaccination is very effective at preventing distemper.
- Young dogs require 2 vaccinations with modified-live virus (MLV) vaccine which can be given from 6 weeks of age, with the second being given 3-4 weeks after the first vaccination.
- Vaccination against distemper is then repeated one year later and thereafter is given as part of the booster vaccination regime.

Peste des Petits Ruminants or PPR

- **Syn- goat plague, kata, syndrome of stomatitis-pneumoenteritis, and ovine rinderpest**
- French name for "plague of small ruminants".
- Highly contagious disease of goats and sheep characterized by fever, sores in the mouth, diarrhea, pneumonia, and sometimes death.
- It is caused by a **morbillivirus** that is related to rinderpest, measles and canine distemper. The official name of this virus was changed in 2016 to **Small ruminant morbillivirus (SRM)**

- Cattle and several wild ruminants have been infected most often experimentally, but goats and sheep are the usual targets.
- **PPR is WOAH list A disease**
- The disease occurs in a band that spreads across Africa between the equator and the Sahara, through the Arabian Peninsula, the Middle East, south-west Asia.
- Reported in 1989 in India from Tamil Nadu.

Transmission

- The virus is secreted in tears, nasal discharge, secretions from coughing, and in the faeces of infected animals.
- Close contact between animals, especially through inhalation of fine droplets that are released into the air when affected animals cough and sneeze will spread the disease.
- Water, feed troughs, and bedding can also be contaminated with secretions and become additional sources of infection, however the virus does not survive for a long time outside the body of a host animal.

- Since animals excrete the virus before showing signs of the disease, it can spread by movement of infected animals.
- Morbidity rate in susceptible populations can reach 90–100%
- Mortality rates vary among susceptible animals but can reach 50–100% in more severe instances
- Both morbidity and mortality rates are lower in endemic areas and in adult animals when compared to young.

Pathogenesis

- PPR virus, like other morbilliviruses, is **lymphotropic** and **epitheliotropic**
- It induces the most severe lesions in organ systems rich in lymphoid and epithelial tissues.
- After the entry of the virus through the respiratory tract system, it localizes first replicating in the pharyngeal and mandibular lymph nodes as well as tonsil.
- Viremia may develop 2-3 days after infection and 1-2 days before the first clinical sign appears.
- Subsequently viremia results in dissemination of the virus to spleen, bone marrow and mucosa of the gastro-intestinal tract and the respiratory system

Clinical signs

- Disease severity depends on various factors: PPRV strain, host species and breed, and the health status of host animals.
- The disease is particularly seen in **young** animals
- The incubation period is 4–6 days
- High fever (41°C/106F), erosive stomatitis, conjunctivitis, gastroenteritis, and pneumonia, dry muzzle, oculo-nasal discharges,
- Watery blood-stained diarrhea.

- Erosions-small pin-point red-greyish areas on the gums, dental pad, palate, lips, inner aspects of the cheeks and upper surface of the tongue.
- Eye, nose and mouth discharges with scabs or nodules around the mouth
- Death usually occurs 4–6 days after the onset of fever.

Lesions

- The carcass of an affected animal is usually emaciated, the hindquarters soiled with soft/watery faeces and the eyeballs sunken. The eyes and nose contain dried-up discharges.
- Prominent **crusty scabs along the outer lips and severe interstitial pneumonia frequently occur with PPR**
- Erosive lesions may extend from the mouth to the reticulo–rumen junction
- Erosive or haemorrhagic enteritis

- Peyer's patches may be necrotic.
- Lymph nodes are enlarged, and the spleen and liver may show necrotic lesions.
- The large intestine is usually more severely affected, with congestion around the ileo-cecal valve, at the ceco-colic junction and in the rectum.
- In the posterior part of the colon and the rectum, discontinuous streaks of congestion “**zebra stripes**” form on the crests of the mucosal folds



Diagnosis

- A tentative diagnosis of PPR infection can be made based on characteristic signs like mucopurulent nasal discharge, pneumonia, diarrhea, scabby lesions on tongue, around mouth and death of many animals in flock should be suspected of PPR.
- Swabs of the eyes, nasal, mouth, tongue and rectal discharges.
- Nucleic acid detection and identification- Reverse-transcription PCR (RT-PCR)
- Immunocapture enzyme-linked immunosorbent assay, AGID, CIE, virus neutralization test

Prevention and control

- Farm Disinfection: PPR virus can be killed by most common Disinfectants-Phenols, Sodium hydroxide 2%
- Vaccination:
 - Live attenuated PPR vaccine is available.
- Names: PPR vaccine, Raksha PPR.
- Dose: 1 ml by s/c
- Age group: 3 months' kids.
- Immunity: 3 years.

Rinderpest or cattle plague

- Rinderpest was declared as eradicated worldwide by the WOAH and FAO in May and June 2011.
- India was declared rinderpest-free in May 2006 by WOAH.

PARVOVIRIDAE

**Latin *parvus* or
parvum, meaning small or tiny,
referring to the small size
of parvovirus virions**

Parvoviruses

Virus classification

Group: **Group II (ssDNA)**

Family: **Parvoviridae**

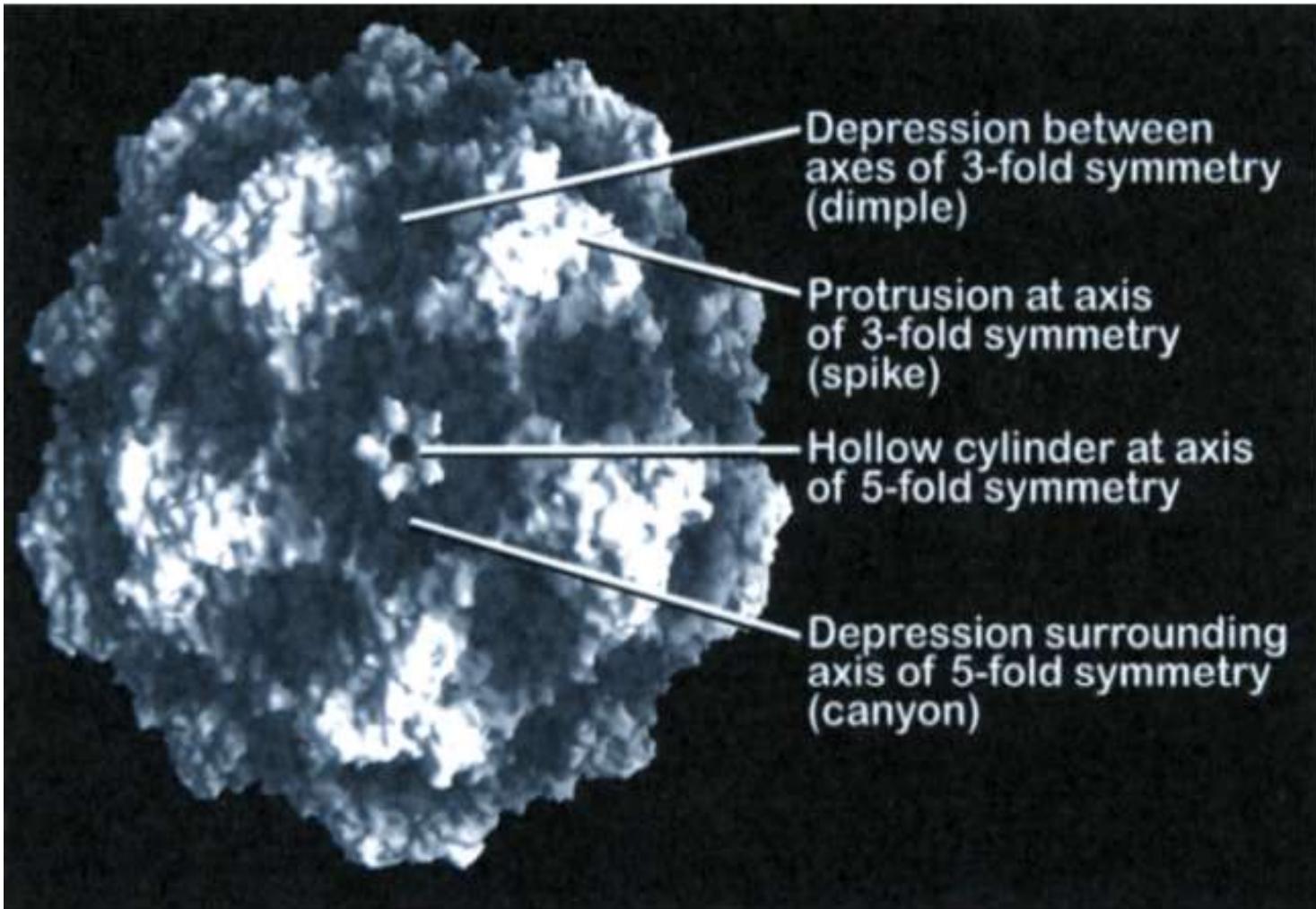
Subfamilies

Densovirinae (Viruses of insects)- Densovirus, Iteravirus,
Contravirus

Parvovirinae- Parvovirus (Feline panleukopenia, Canine
parvovirus)
Erythrovirus,
Dependovirus (Adeno Associated Virus)

Morphology

- **Non-enveloped**, 25 nm in diameter, and have icosahedral symmetry.
- The surface features of the capsid revealed by X-ray crystallography include a hollow cylinder at each fivefold axis of symmetry that is surrounded by a circular depression (called the **canyon**).
- The capsid is composed of **60 molecules of VP2 protein**, along with a few molecules of VP1. VP1 and VP2 are formed by alternative splicing of the same mRNA, and the entire sequence of VP2 is encoded within the VP1 gene.
- A third structural protein, VP3, is formed only in "full" (DNA-containing) capsids by cleavage of 15-20 amino acids from the amino terminus of VP2.
- VP2 is the most immunogenic.



Capsid confers much stability upon the virus particles, allowing for resistance to inactivation by pH, solvents, or temperatures up to 50 degrees C.

Parvoviruses are thus among the most resistant viruses known.

Genome

- The genome consists of a single molecule of **linear single-stranded DNA**, 5.2 kb in size.
- Some parvoviruses encapsidate only the negative-sense or antisense DNA strand (e.g., canine parvovirus 2, minute virus of mice), whereas others encapsidate different proportions of either strand.
- The genome has 6 to 10 terminal palindromic sequences, enabling each end to form hairpin structures.
- Replication occurs in the nucleus of dividing cells; infection leads to large intranuclear inclusion bodies
- Most viruses hemagglutinate red blood cells

Viral Replication

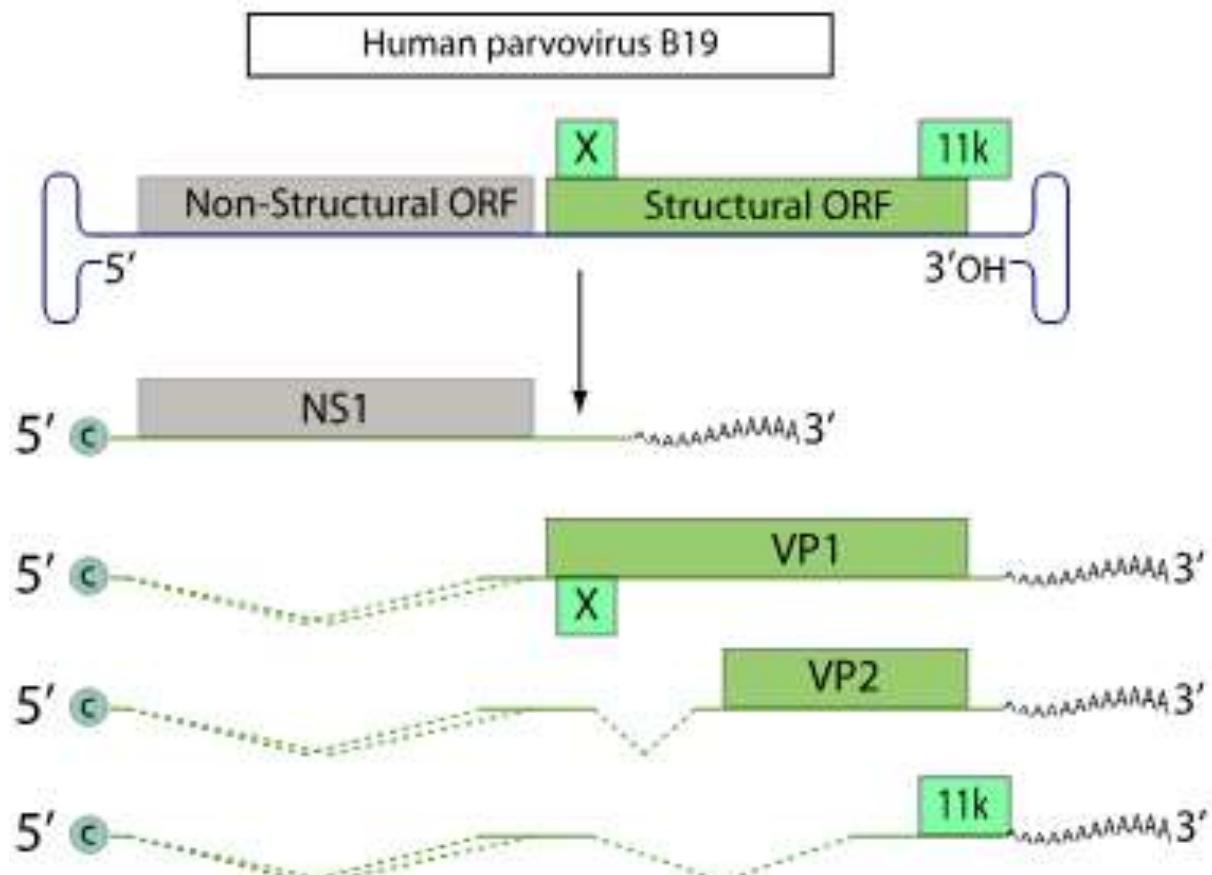
- Viral replication takes place in the nucleus and requires host cell functions of **late S phase** (i.e. period of DNA replication) **or early G2 phase** (i.e. The stage of cell cycle between the end of DNA synthesis and beginning of mitosis) of the cell division cycle.
- This requirement for cycling cells for viral replication is the basis for many aspects of the pathogenesis of parvovirus infections.
- Specifically, there is no polymerase enzyme in the virion itself nor does the virus encode any such enzyme.
- Instead, **cellular DNA polymerase II** is employed to transcribe viral DNA into a double stranded DNA intermediate, which is then used as a template as other cellular enzymes catalyze the transcription of viral mRNAs.

Viral Replication

- The viral ssDNA genome penetrates into the nucleus.
- The ssDNA is converted into dsDNA by cellular proteins.
- dsDNA transcription gives rise to viral mRNAs when host cell enters S phase and translated to produce viral proteins.
- Replication occurs through **rolling-hairpin mechanism**, with NS1 endonuclease binding covalently to the 5' genomic end.
- Individual ssDNA genomes are excised from replication concatemers.
- 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.

Viral Replication

- Cellular DNA dependent RNA polymerase synthesizes three mRNA.
- These mRNA have a common 3' terminus.
- Most abundant mRNA which is encoded in the 3'-half of the genome, codes for structural proteins (VP1 and VP2) late in infection.
- The non structural (NS1) proteins involved in DNA replication reside in left half, the 5'-half, of DNA.

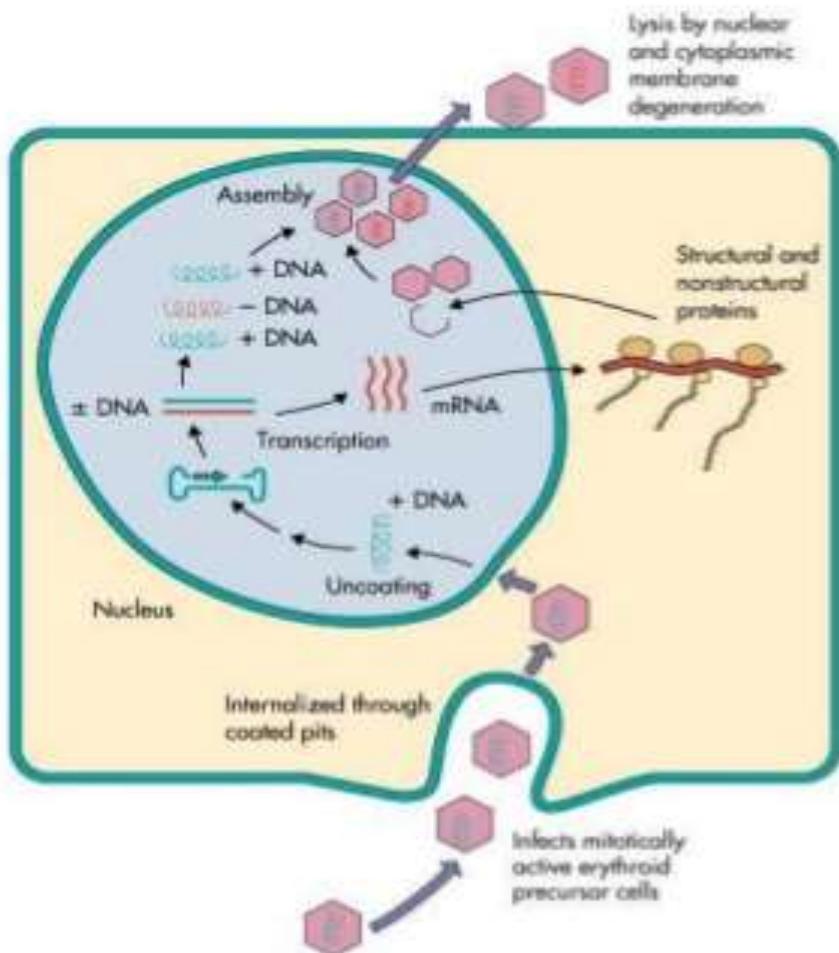


The nonstructural protein (NS1), is also produced in very large amounts and serves a number of functions:

- (1) it binds to DNA and is required for viral DNA replication,
- (2) it serves as a helicase,
- (3) it serves as an endonuclease
- (4) it interferes with cellular DNA replication by producing nicks in cellular DNA.

This latter activity leads to the arrest of the cell division cycle in S phase.

Autonomous parvovirus replication



Postulated replication of parvovirus (B19) based on information from related viruses (minute virus of mice). The internalized parvovirus delivers its genome to the nucleus, where the single-stranded (plus or minus) DNA is converted to double-stranded DNA by host factors and DNA polymerases present only in growing cells. Transcription, replication, and assembly occur in the nucleus. Virus is released by cell lysis. (From Medical Microbiology, 5th ed., Murray, Rosenthal, Kobayashi & Pfaffer, Mosby Inc., 2002, Fig. 56-2.)

CANINE PARVOVIRUS

History

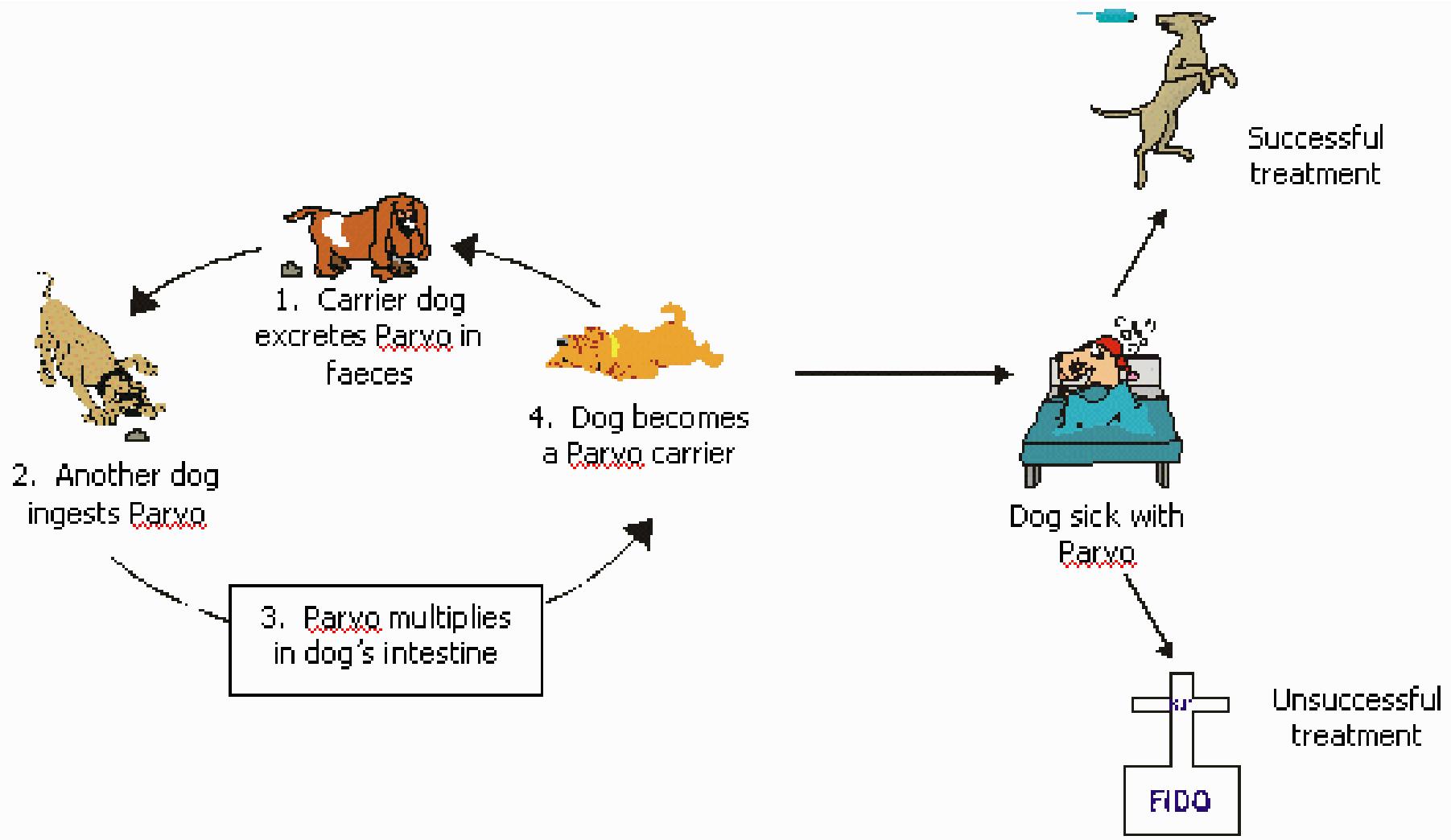
- There are two types of canine parvovirus called canine minute virus (CPV1) and CPV2.
- CPV2 causes the most serious disease and affects domesticated dogs and wild canids.
- Variants of CPV type 2 - CPV-2a, CPV-2b and CPV-2c. The antigenic patterns of 2a and 2b are quite similar to the original CPV type 2. Variant 2c however has a unique pattern of antigenicity.
- **CPV2 virus is very similar to feline panleukopenia virus; they are 98% identical, differing only in two amino acids in the viral capsid protein VP2 (determinant of Host range difference).**
- The canine virus can replicate in both canine and feline cells but the feline virus replicates only in feline cells.
- It is also highly similar to mink enteritis, and the parvoviruses of raccoons and foxes.

- Three distinct age-related canine parvovirus disease syndromes have been recognized in dogs.
 1. The generalized **neonatal disease syndrome**- rare.
 2. The **myocarditis syndrome** is usually recognized in pups by sudden death, usually without preceding clinical signs. Even though damage to the myocardium may be extensive, some pups may survive with lifelong cardiac problems.
- Seen in pups at 4 to 8 weeks of age and was common when the virus first emerged in 1978-1979.
- Widespread use of vaccine and its induction or boosting of maternal antibody has made the generalized neonatal disease syndrome and the myocarditis syndrome less common.

3. The **leukopenia/enteritis syndrome** parallels that seen in cats – feline panleukopenia or feline distemper.

- The incidence of the leukopenia/enteritis syndrome has fallen since the virus first emerged, but is still an important cause of morbidity.
- Most commonly in **pups at 8 to 12 weeks of age.**
- Vomiting is often the initial sign and can be severe and protracted. There is anorexia, lethargy, and diarrhea leading to rapid dehydration.
- The feces are often streaked with blood or frankly hemorrhagic and remain fluid until recovery or death.
- Death is uncommon except in young pups.

Life cycle of Parvovirus

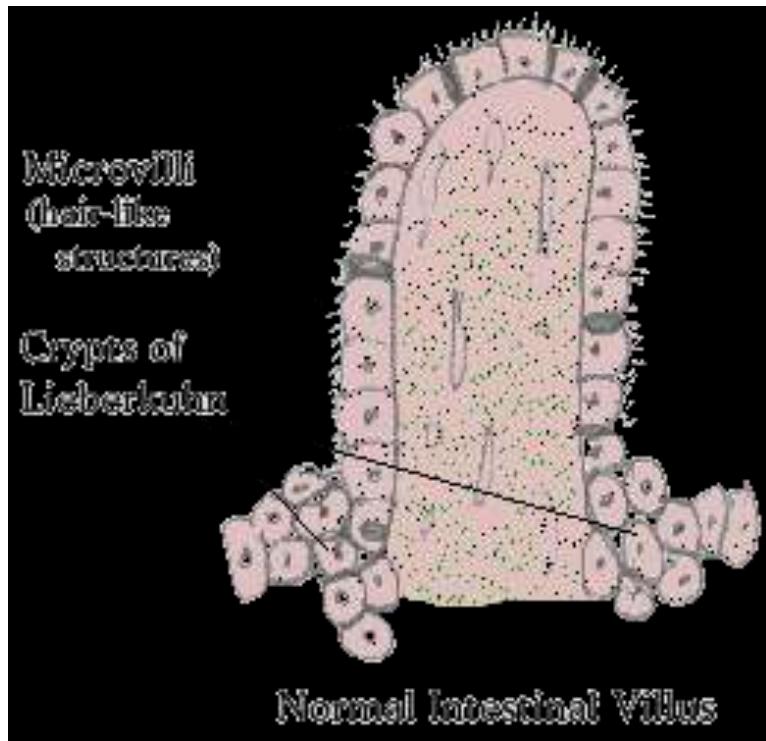


Pathogenesis

- Following viral entry in the oropharynx, initial viral replication occurs in pharyngeal lymphoid tissue.
- From here the virus is distributed to other organs and tissues via the bloodstream.
- **Cells that have appropriate receptors and are in the S phase of the cell cycle are infected and killed or prevented from entering mitosis.**
- The **characteristic profound leukopenia** involves all white blood cell elements: lymphocytes, neutrophils, monocytes, and platelets.
- These cells are destroyed, both those present in the circulation and those in lymphoid organs, including the thymus, bone marrow, lymph nodes, spleen, and Peyer's patches.
- Resting peripheral leukocytes may be stimulated to proliferate, thereby becoming permissive for viral replication.
- Alternatively, the presence of virus bound to the surface of cells may render them targets for cytotoxic lysis.

Hemorrhagic enteritis

- Villi become blunt and unable to absorb nutrients.
- Barrier to GIT flora is broken down.



- Rapidly dividing intestinal epithelial cells in the **crypts of Lieberkuhn** are very susceptible to infection.
- The loss of cells from villus tips continues in normal fashion, but the failure in replacing these cells with cells from the crypts leads to greatly shortened, non absorptive villi and hence to diarrhea.
- **At all ages, the continuous division of cells in lymphoid tissues and the intestinal epithelium leads to the common occurrence of leukopenia and enteritis.**

Mechanisms of Death

1. Diarrhea and vomiting
→ extreme dehydration → SHOCK
2. Loss of intestinal barrier
→ bacterial invasion → SEPTIC SHOCK

Clinical features

- Fever, lethargy, anorexia
- Vomiting
- Diarrhea: watery, bloody, with a tell-tale odor
- Severely dehydrated

Diagnosis

- History and PE
- Clinical signs
- Haemagglutination of pig and rhesus monkey RBC by virus present in fecal extracts.
- Electron microscopy, virus isolation, enzyme immunoassay, and amplification of viral DNA using PCR
- Biopsy
- Necropsy
 - Lower and middle small intestines
 - Bone marrow

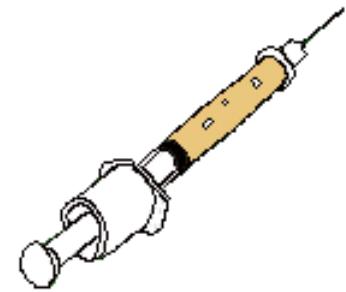
Prognosis

- Most recover with intensive therapy
- Dobermans and Rottweilers
 - Sensitive to the virus
- Poor prognosis
 - Intussusception
 - Hypoproteinemia
 - No improvement after 4th day of intensive care

Protection

- Colostrum
 - Maternal antibodies protective until 2 month of age
 - Regular Vaccination
 - Keep indoors
 - Until the vaccination series are complete
- Immunity after natural infection appears to be lifelong.**

CPV Vaccine History



- Original vaccines incorporated feline panleukopenia virus or mink enteritis viruses
 - Similar to the original CPV
- Killed and MLV were developed, but variable efficacy
 - Low titer
 - Interference of Maternal antibodies

Vaccines

- The **DHPP** vaccine, also known as the **5-in-1** vaccine, protects dogs against five highly contagious viral diseases that cause severe illness and may be fatal: distemper, canine adenovirus-1 (CAV-1), canine adenovirus-2 (CAV-2), parainfluenza, and parvovirus.
- Other abbreviations for the vaccine are DA2PP and DAPP.
- **Nobivac® DHPPi**
- Live attenuated vaccine for immunization of dogs against canine distemper virus, canine adenovirus, canine parvovirus and canine parainfluenza virus

CPV Vaccination Recommendations

- Use modified live vaccines for puppies
- Start vaccinations at 6-8 weeks of age
- Vaccinate every 3-4 weeks until 15-16 weeks of age
 - Giving a vaccine more frequently than every 2 weeks will cause interference
 - This includes vaccines for different infections

Other Parvoviruses...

- Feline Panleukopenia Virus
- Porcine Parovirus
- Aleutian Mink Diseases

PICORNAVIRIDAE

Picornaviridae: an acronym from **poliovirus**, **i**nsensitivity to ether, **c**oxsackievirus, **o**rphan virus, **r**hinovirus, ribonucleic **a**cid;

Also “pico” - small

“rna” - RNA

Baltimore group IV

Genera in Picornaviridae

- *Aphthovirus* - Foot and Mouth disease virus
- *Enterovirus* - enteroviruses of various species, polio virus (humans), hand, foot and mouth disease (humans), **Swine vesicular disease**
- **Avihepatovirus - Duck Hepatitis virus**
- *Cardiovirus*
- *Rhinovirus* - various species, human “common cold” viruses
- *Hepatovirus* - hepatitis A (humans)
- *Parechovirus- human*
- *Erbovirus- Equine rhinitis virus*
- *Kobu virus- human and cattle*
- *Teschovirus- swine*

Vesicular diseases in swine

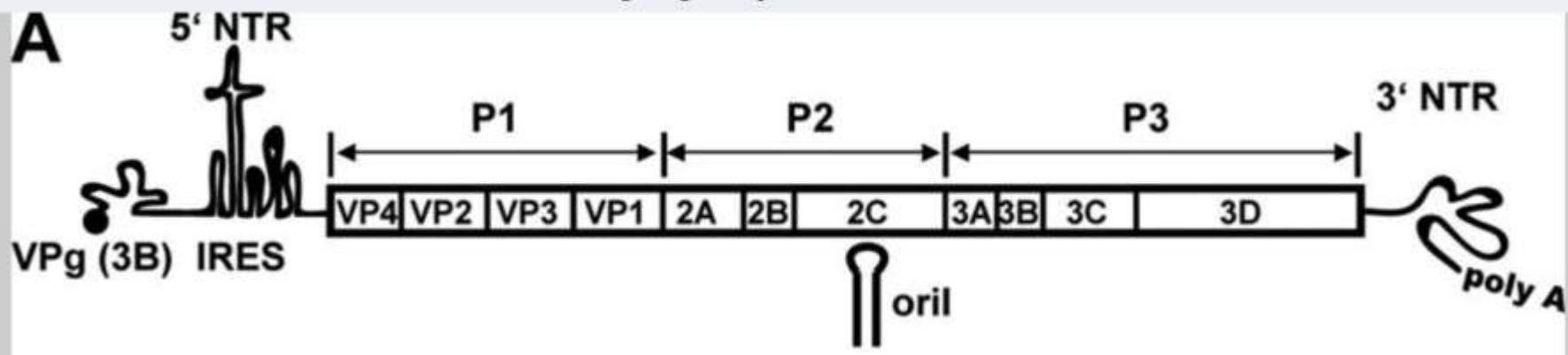
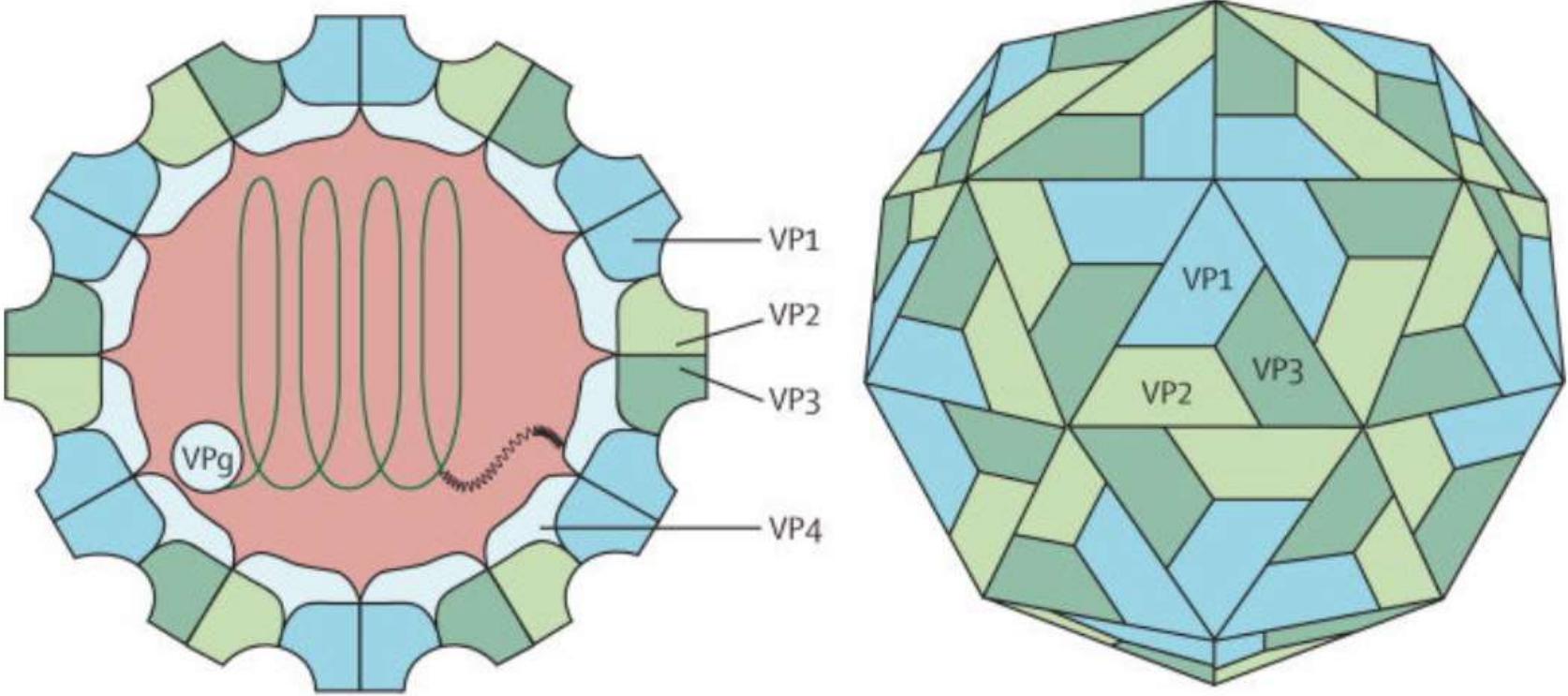
- FMD
- Swine vesicular disease – Enterovirus
- Vesicular stomatitis- Vesiculovirus (Rhabdoviridae)
- Vesicular exanthema of swine- Calicivirus

Structure

- **Non-enveloped**, relatively stable
- Capsid has icosahedral symmetry
- 60 capsomeres are there made of 4 viral proteins VP1, VP2, VP3 and VP4.
- All are derived from a single poly protein.
- Particle is 27-30nm in diameter and full length genome is 2500nm thus tightly packed in the virion.
- **Single stranded, positive sense RNA**
- RNA is infectious

Genome

- RNA lacks a cap at the 5' end.
- On 5' end a covalently attached small basic protein is there called **VPg** and on 3' end there is a poly A tail.
- Genome has a long 600-1200 base untranslated region (UTR) at 5'end and shorter 3' untranslated region of 50-100 bases.
- 5'UTR contains a clover leaf secondary structure also called as the **IRES (internal ribosome entry site)**. Internal ribosomal entry sites (IRESS) are regions in the mRNAs that allow the internal initiation of translation.
- Rest of the genome codes a single poly protein of around 2100-2400 aa.



- The single open reading frame encodes a polyprotein.
- P1 encodes virion structural proteins
- P2 encodes proteins thought to participate in virus-host interactions required for genome replication.
- P3 encodes proteins thought to participate directly in genome replication.
- Polyprotein processing is mediated by protease activity residing in 2A, 3C, and/or 3CD proteins.

Susceptibility to inactivation

- pH
 - stable between pH 7 and 9
 - inactivated by:
 - 5% acetic acid or other acids
 - 1-2% sodium hydroxide or other alkalis
- Phenolic and quarternary ammonium compounds - not effective
- Oxidizing agents (bleach) effective when environment not contaminated with organic substances.
- Detergents increase effectiveness.
- The virus is sensitive to calcium chlorate lime, chloramine, formalin, potassium permanganate, and hydrogen peroxide solutions.

- It is rapidly killed on boiling.
- By Heat:
- in suspension 80° C for 1 hr, 50°C for 2 days,
37°C for seven days
- stable when associated with dried organic matter.

Stability

- The virus is extremely resistant to photodynamic inactivation.
- It survives in sterile water at room temperature for a period of more than 100 days, in milk for 90 days, in faeces in the cold for more than 6 months, and in sewage for several months.
- It withstands exposure to 0.5-1 per cent phenol solutions.
- Aphtho virus is unstable below pH 7.0
- Rhino virus is unstable below pH 5.0
- Enterovirus is unstable below pH 3.0

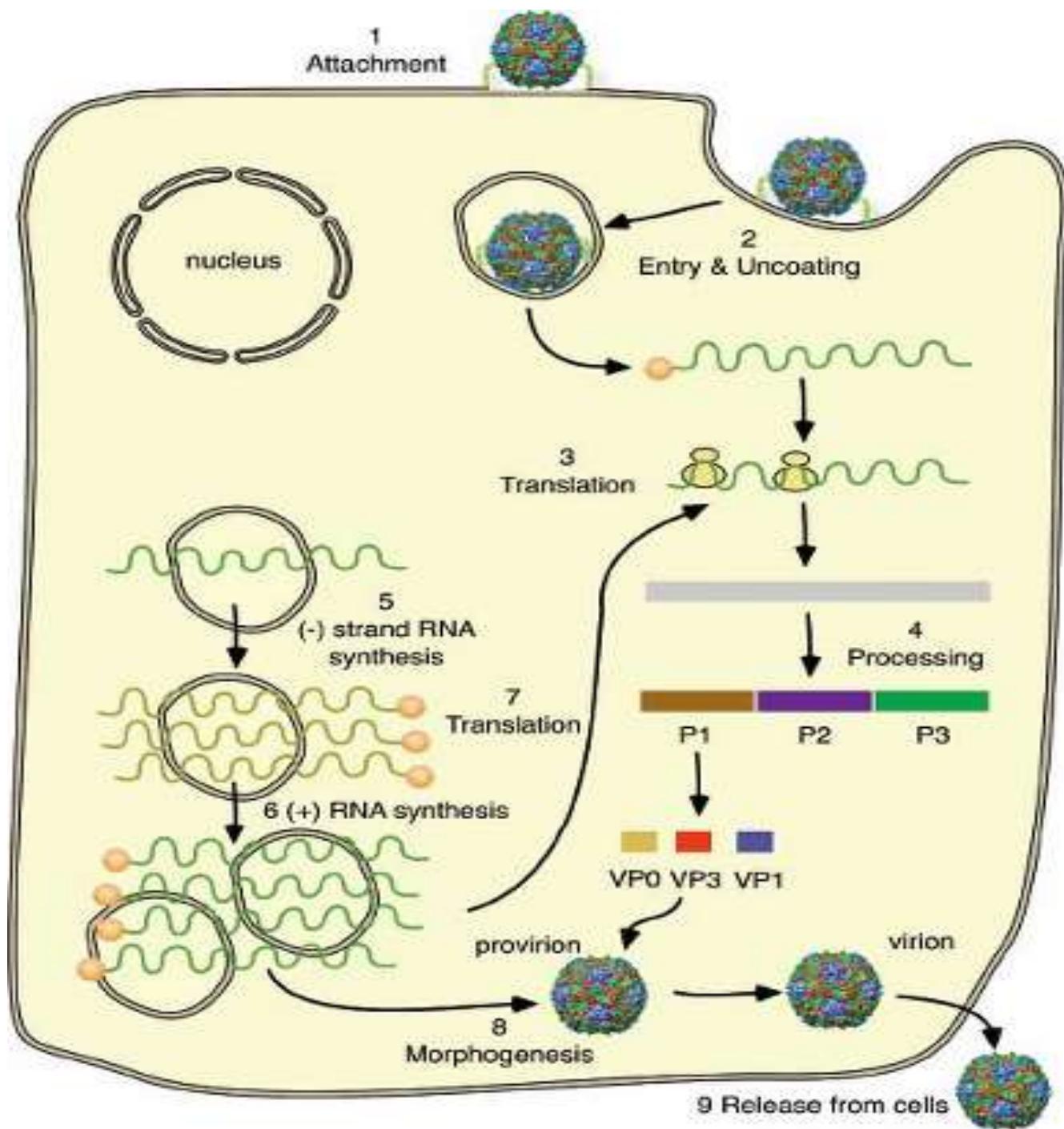
Replication

- **Poliovirus-** a major model for studying RNA viruses
- Site: cytoplasm
- The cellular receptors used by different picornaviruses are diverse.
- Polioviruses, Coxsackie B viruses, and some rhinoviruses use members of the immunoglobulin (Ig) superfamily, while other picornaviruses attach via other cell surface molecules including integrins, heparin sulfate, low-density lipoproteins, or extracellular matrix-binding proteins.
- Some foot-and-mouth disease viruses, employ two receptors: initial binding involving heparin sulfate followed by high-affinity binding via integrins.

- Following attachment, entry and viral genome uncoating, the VPg protein covalently bound to the 5' end of the RNA is removed by cellular enzymes, and translation of the RNA begins.
- Due to the absence of a 7-methyl guanosine cap, picornaviruses initiate translation in a **cap-independent manner**.
- Initiation of translation relies on binding of ribosomes to an internal ribosomal entry site (IRES) within the 5' UTR.
- This region of RNA can fold to form cloverleaf structures that bind host proteins, allowing the initiation of viral protein and RNA synthesis.

- Because the poliovirus genome does not contain internal translational stop codons, a single polyprotein is generated and this is subsequently cleaved at specific sites by virus-coded proteinases to generate 11 or 12 discrete proteins.
- The structural proteins that make up the virion capsid (VP4, VP2, VP3, and VP1 respectively) are coded by the 5' end of the genome,
- while the various cleavage products of proteins from the 3' end provide replicative functions, including protease and RNA-dependent RNA polymerase activities and the genome-associated protein VPg.

- Virus replication takes place in a replication complex, consisting of RNA templates, the virus-coded RNA polymerase and several other viral and cellular proteins, all tightly associated with newly assembled smooth cytoplasmic membrane structures.
- Synthesis of the minus RNA strand is initiated at the 3' end of the virion RNA, using the protein VPg or 3B as a primer.
- The completed minus strand is then used as the template for synthesis of the virion plus strand RNA.
- Newly synthesized plus strands and the structural capsid proteins then self-assemble into virions,
- Picornaviruses have evolved various ways to rapidly and preferentially inhibit the translation, and the synthesis, of cellular mRNA.



Foot and Mouth disease (FMD)

- By FMDV - the type species of the *Aphthovirus* genus.
- Highly infectious disease of **cloven-footed animals** (e.g., cattle, goats, pigs, and sheep)
- Systemic disease with high fever, vesicles on epithelial surfaces
- Not usually fatal in adults but causes economic losses
- Can be fatal in young animals – myocarditis.
- In 1897, **Loeffler and Frosch** demonstrated that a filterable agent caused FMD. This was the first demonstration that a disease of animals was caused by a filterable agent and ushered in the era of virology.
- FMD is in the OIE **list A** of infectious diseases of animals

Serotypes

- Basically 7 serotypes –
- O, A, C, South African territories (SAT)-1, SAT-2, SAT-3, and Asia-1.
- **Currently, FMDV serotypes O, A, Asia1, Southern African Territories (SAT)-1, SAT-2, and SAT-3 are the six antigenically distinct serotypes globally prevalent.**
- **FMDV serotype C has not been reported from any part of the globe since 2004**
- **Serotypes O, A, and Asia1 are currently prevalent in India.**
- **Serotype O is responsible for the majority of FMD outbreaks in the country, followed by serotypes Asia1 and A.**

Susceptible species

- Domestic ruminants (cattle, buffalo, sheep, goats, camelids)
 - Wild or exotic ruminants (African buffalo, various antelope and deer species)
 - Others (pigs, rabbits, mice, guinea pigs, chickens, humans)
-
- **Pigs (amplifying hosts)** - secrete large amounts of virus in breath, air borne spread
 - **Cattle (sentinel or indicator hosts)** - highly sensitive to infection by respiratory route
 - **Sheep (maintenance hosts)** - mild-asymptomatic disease, can spread through flocks before detection

Important factors for spread of infection

- Short incubation period
- Release of virus prior to appearance of clinical signs
- Massive quantities of virus released
- Antigenic variation (7 serotypes with no cross protection and many antigenic variants with limited cross reactivity)
- Extended survival in the environment
- Multitude of routes of virus transmission
- Minimal size of the infective dose
- Aerosol transmission possible up to 250 km depending on strain and environmental conditions (10km, 170km, 250km reported over water)
- **Agroterrorism**

Transmission

- FMD is found in all excretions and secretions from infected animals.
- These animals breathe out a large amount of aerosolised virus, which can infect other animals via the respiratory or oral routes.
- The virus may be present in milk and semen for up to 4 days before the animal shows clinical signs of disease.

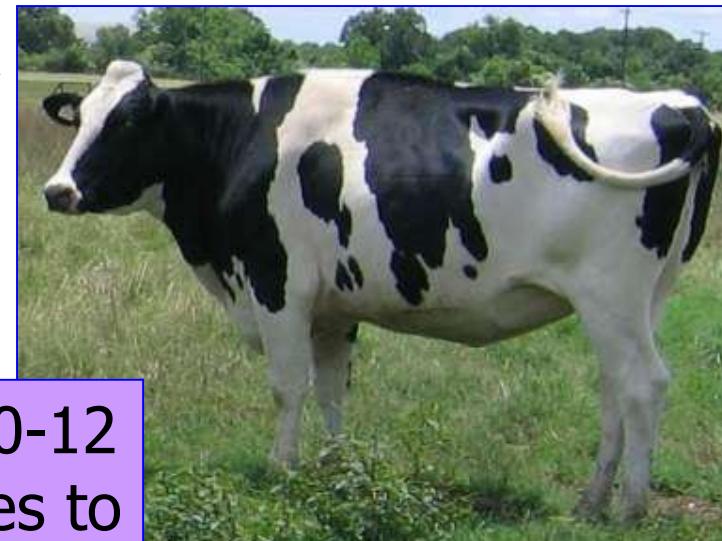
Routes of transmission

- Aerosols/Airborne, Inhaled
- Direct or indirect contact-droplets,
- Ingestion
- Artificial Insemination
- Vectors (vehicles, equipment, or humans)
- Carrier state

Transmission

Aerosol droplets spread from infected animal

Sheds 400,000,000 virus particles per day

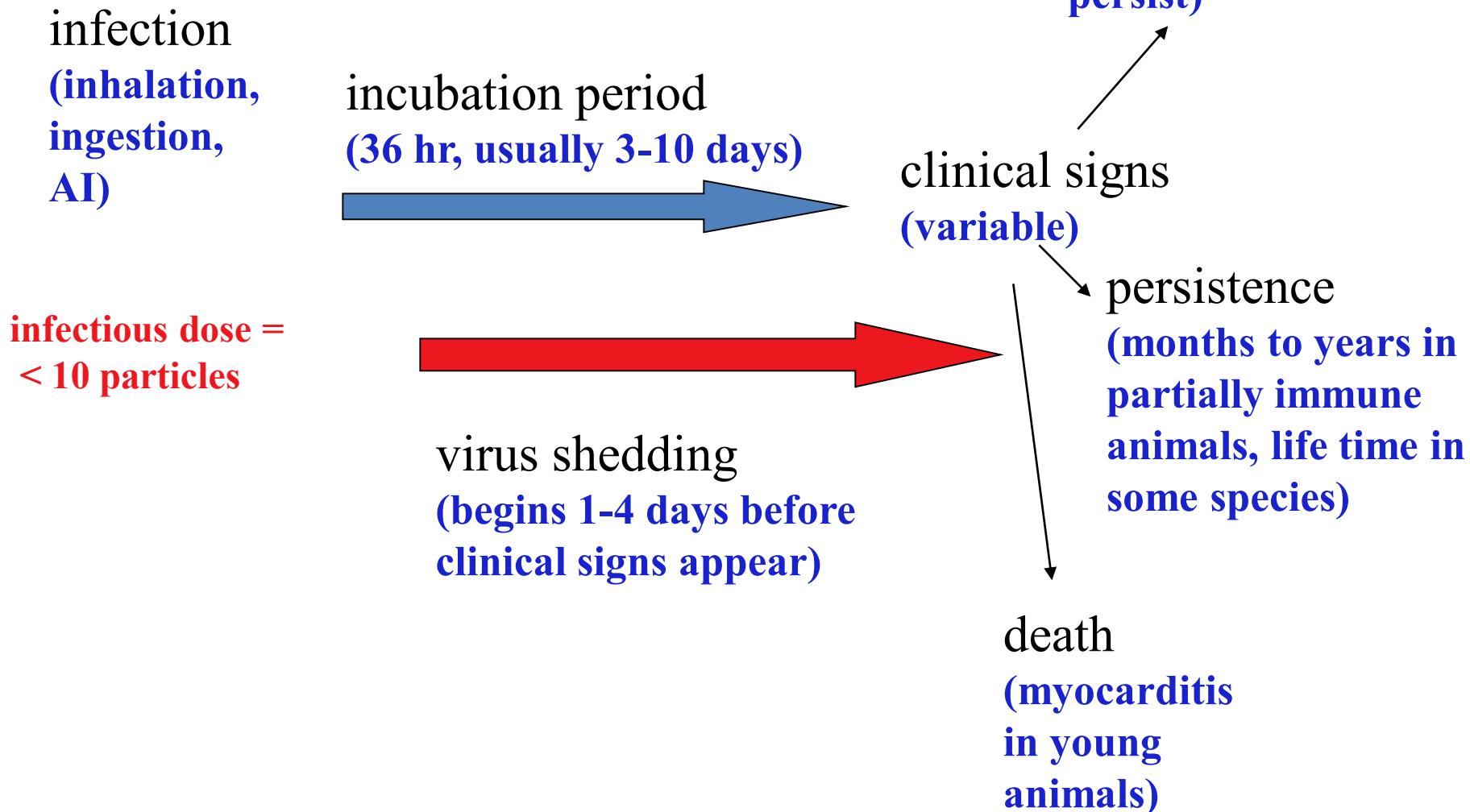


Only takes 10-12 virus particles to infect one cow

Transmission by humans

- A person in contact with infected animals may retain and exhale virus for up to 36 hours and serve as source of infection.
- Humans serve as a mechanical vector when moving from infected animals to susceptible animals.

Pathogenesis



Pathogenesis

- The pharynx is the primary site of infection, unless the virus enters the skin through a wound.
- The virus needs access to live cells on the surface and does not enter through cornified tissue
- After initial replication, the virus enters the circulation and disseminates to the areas of amplification such as the skin, tongue, and mouth (spread to **stratified squamous epithelium-target cells**).
- Virus can be detected in most cells during viremia but vesicles or blisters form in areas of rapid cell growth and where friction occurs naturally such as in the mouth or between toes

Progression of disease

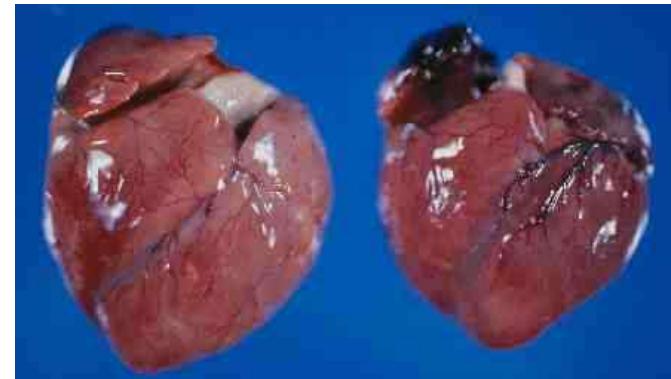
- Blisters at infection site initially appear as blanched area in the epithelium
- Area fills with serous fluid forming a vesicle
- Vesicles enlarge and coalesce
- Vesicles crack or rupture leaking fluid
- The epithelium necroses off leaving raw ulcer or erosion
- Grey fibrinous coating forms over lesions
- The coating becomes discolored, yellow, brown, green
- As epithelium is restored, lines of demarcation are evident
- Sometimes but not always, permanent scars form

Progression of disease

- When blisters are present, cattle salivate profusely withropy viscous material hanging from mouth
- Also severe lacrimation and nasal discharge
- When vesicles rupture, fever ends followed by end of viremia
- Start to finish, signs last 15-30 days.
- Recovered animals are permanently unthrifty

Infection of heart muscle

- In young animals (up to 6 months of age in cattle)
- 50 % mortality by myocardial damage in young animals
- In calves, myocarditis is considered a fatal form of FMD that occurs without developing the characteristic blister lesions noted in adult cattle
- The acute myocarditis of young animals is distinguished by hyaline degeneration, necrosis of muscle fibers and an intense infiltration mainly of lymphocytes.
- The mottled myocardial lesions referred to as “**tiger-heart**” lesions are useful in diagnosis



General Clinical Signs

- Listless
- Lifting feet alternately
- Lameness
- Clear nasal discharge progressing to mucopurulent



Clinical Signs in Cattle

- Fever (103°-106°F)
- Depression
- Anorexia
- Milk production ceases
- Blisters start to form in the mouth
- Excessiveropy, viscous salivation
- Smacking of the lips and sucking of the sore tongue is characteristic
- Blisters rupture within 24 hrs leaving raw, painful ulcers
- Mouth lesions usually heal in 10 - 14 days
- Mastitis
- Abortion in pregnant cows.

Clinical Signs in Cattle

Blisters form on:

Mouth

Tongue

Dental pad

Gums

Soft palate

Muzzle

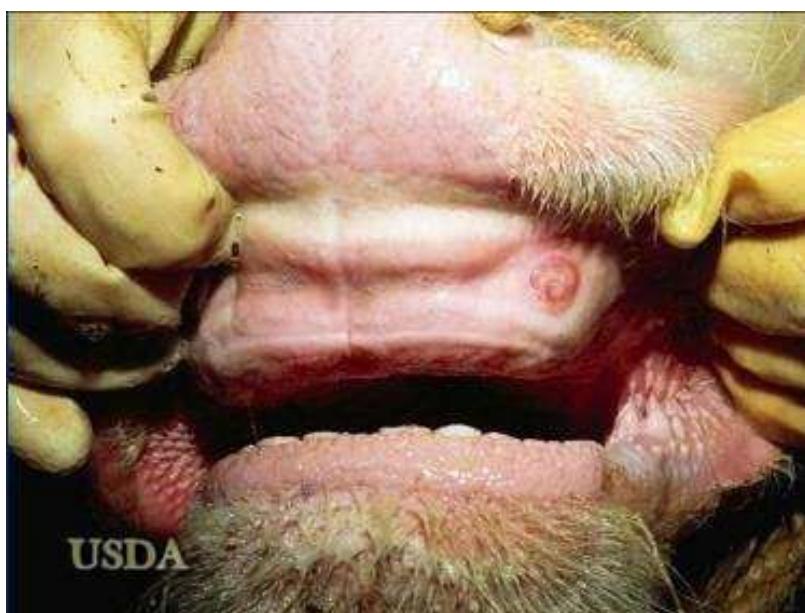
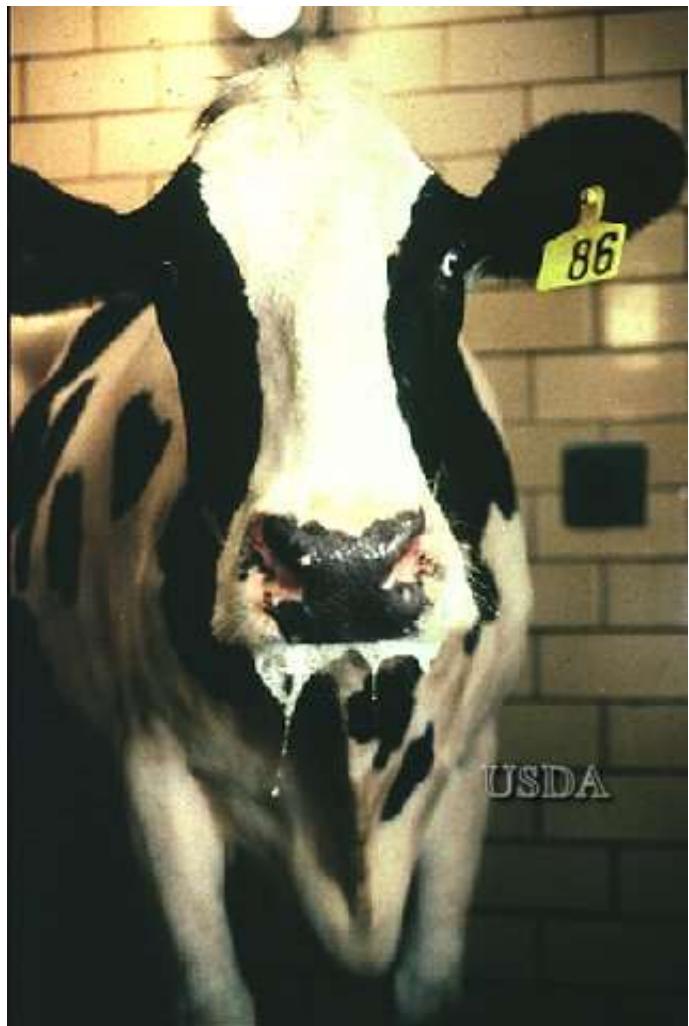
Nostrils

Feet

Inter-digital space

Coronary band

Teats



Foot-and-Mouth Disease



Foot-and-Mouth Disease

■ Lameness

- Coronary band lesion first appears blanched
- Blisters form between the digits
- Stamping and shaking of feet
- Trembling
- Excretion of virus from foot lesions tends to last a day or two longer than from mouth lesions, so foot lesions may be a better source of virus for diagnostic purposes in older cases.



Prevention

- In countries with endemic FMD
 - vaccination
 - vaccination and slaughter
- FMD free countries
 - prevent introduction
 - in face of outbreak
 - test and slaughter
 - ring-vaccination and slaughter
 - ring-vaccination and slaughter only sick animals

- Both Polyvalent and Monovalent Vaccines are available.
- Monovalent vaccines provide immunity for longer periods.
- Vaccines are cell culture based.
- In endemic areas otherwise vaccination is done every six months.
- Under greater infection pressure four monthly vaccination schedules can be followed.

Vaccines

- Killed/Inactivated Oil Adjuvanted vaccine widely used in all species
- **Raksha**
- **Raksha-Ovac-trivalent –against FMD 0, A, Asia**
- **Raksha-Biovac- FMD and HS**
- **Raksha-Triovac- FMD, HS and BQ**

- **Futvac**

Problems with vaccination

- no cross protection if wrong serotype
- short-lived immunity
- partial protection if variant
 - does not prevent infection
 - persistent infection
- cannot distinguish between vaccinated and infected animals
- detection easier if no vaccination

Diagnosis

- Clinical signs - can be confused with other vesicular diseases
- Laboratory
 - sample collection
 - vesicle fluid, skin at edge of ruptured vesicle, excretions and secretions
 - inoculated onto susceptible cells
 - Serum neutralization test
 - Complement fixation test
 - ELISA

Laboratory Diagnosis

For virus	For antibodies
ELISA	Virus neutralization
Virus isolation	Agar gel immunodiffusion
PCR*	

Duck Viral Hepatitis (DVH)

- Typically associated with an acute, contagious infection in susceptible ducklings **less than 6 weeks of age** and frequently under 3 weeks of age.
- **It does not occur in older birds.**
- The disease, DVH, has traditionally been subdivided into types I, II and III.
- **DVH type I** can be caused by at least three different genotypes of duck hepatitis A virus (DHAV) virus, a member of the genus *Avihepatovirus*, of the family Picornaviridae.
- The most pathogenic and widespread is DHAV type 1 (DHAV-1), which was formerly designated as duck hepatitis virus type 1.

- DHAV-2 and DHAV-3 are two additional genotypes within the genus Avihepatovirus that have subsequently been identified as additional aetiological agents of DVH in ducklings.
- **DVH type II** is caused by **duck astrovirus type 1 (DAstV-1)**, a member of the Astroviridae family. DAstV-1 has been reported primarily in the **United Kingdom**. It has been reported in ducklings from 10 days to 6 weeks of age, and causes pathological changes similar to those of DHAV-1.
- **DVH type III** is caused by **duck astrovirus type 2 (DAstV-2)**, a member of the Astroviridae. It is considered distinct from DAstV-1 and has been reported only in the **United States of America**. It causes similar liver lesions in young ducklings, but is less virulent than DHAV.

Signs

- The clinical disease is characterised by lethargy and ataxia followed by opisthotonus and death.
- Ducklings lose their balance, fall on their sides and kick spasmodically prior to death.
- The whole disease sequence is rapid and can take as little as 1–2 hours.
- Practically all mortality in a flock will occur within 3–4 days, with most deaths on the second day
- **Post-mortem lesions**
- Liver swollen with Punctate/diffuse haemorrhages.
- Kidneys and spleen swollen.
- Microscopically - hepatocyte necrosis, bile duct hyperplasia

Diagnosis

- History, lesions, SN serology, isolation in CE (causes stunting of 9 day embryo).
- Differentiate from Duck plague (viral enteritis), Duck septicaemia (*anatipestifer*), coccidiosis, Newcastle disease, Influenza and a 'Type II Variant' hepatitis caused by Astrovirus
- DHAV-1 infections can be controlled by the use of live attenuated virus vaccines and an inactivated virus vaccine

POXVIRIDAE

Group I viruses

**Large enveloped ds DNA viruses with Complex
symmetry**

- **Baltimore classification-7 groups**
 1. dsDNA viruses
 2. ssDNA viruses
 3. dsRNA viruses
 4. (+)-sense ssRNA viruses
 5. (-)-sense ssRNA viruses
 6. RNA reverse transcribing viruses
 7. DNA reverse transcribing viruses

Classification

- Subfamily ***Chordopoxvirinae*** (8 genera)
 - Genus *Orthopoxvirus*; type species: *Vaccinia virus*; diseases: cowpox, vaccinia, smallpox
 - Genus *Parapoxvirus*; type species: *Orf virus*
 - Genus *Avipoxvirus*; type species: *Fowlpox virus*
 - Genus *Capripoxvirus*; type species: *Sheeppox virus*
 - Genus *Leporipoxvirus*; type species: *Myxoma virus*
 - Genus *Suipoxvirus*; type species: *Swinepox virus*
 - Genus *Molluscipoxvirus*; type species: *Molluscum contagiosum virus*
 - Genus *Yatapoxvirus*; type species: *Yaba monkey tumor virus*
- Subfamily ***Entomopoxvirinae*** –insect poxviruses
 - Genus *Entomopoxvirus A; B and C*

Family Poxviridae

Subfamilies	Genera	Member viruses	Features
<i>Chordopoxvirinae</i>	<i>Orthopoxvirus</i>	Camelpox, cowpox, ectromelia, monkeypox, raccoonpox, skunkpox, ^a Uasin Gishu, ^{b,c} vaccinia, ^c variola, volepox	Brick-shaped virion, DNA ~200 kbp, G + C ~36%, wide to narrow host range, variola (smallpox), vaccinia (smallpox vaccine)
	<i>Parapoxvirus</i>	^a Auzduk disease, ^a chamois contagious ecthyma, ^b orf, pseudocowpox, parapox of deer, ^a sealpox	Ovoid virion, DNA ~140 kbp, G + C ~64%
	<i>Avipoxvirus</i>	Canarypox, ^{b,c} fowlpox, juncopox, mynahpox, pigeonpox, psittacinepox, quailpox, ^a peacockpox, ^a penguinpox, sparrowpox, starlingpox, turkeypox	Brick shaped, DNA ~260 kbp, G + C ~35%, birds, arthropod transmission
	<i>Capripoxvirus</i>	Goatpox, lumpy skin disease, ^b sheeppox	Brick-shaped, DNA ~150 kbp, ungulates, arthropod transmission
	<i>Leporipoxvirus</i>	Hare fibroma, ^{b,c} myxoma, ^c rabbit fibroma, squirrel fibroma	Brick-shaped, DNA ~160 kbp, G + C ~40%, leporids and squirrels
	<i>Suipoxvirus</i>	Swinepox	Brick-shaped, DNA ~170 kbp, narrow host range
	<i>Molluscipoxvirus</i>	^a Molluscum contagiosum	Brick-shaped, DNA ~180 kbp, G + C ~60%, human host, localized tumors, contact spread
	<i>Yatapoxvirus</i>	Tanapox, ^b Yaba monkey tumor	Brick-shaped, DNA ~145 kbp, G + C ~33%, primates and ? rodents
	<i>Entomopoxvirus A</i>	^b <i>Melontha melontha</i>	Ovoid virion, DNA ~260–370 kbp, Coleoptera
<i>Entomopoxvirinae</i>	<i>Entomopoxvirus B</i>	^{b,c} <i>Amsacta moorei</i> , ^c <i>Melanoplus sanguinipes</i>	Ovoid, DNA ~236 kbp, G + C ~18%, Lepidoptera and Orthoptera
	<i>Entomopoxvirus C</i>	^b <i>Chironimus luridus</i>	Brick-shaped, DNA ~250–380 kbp, Diptera

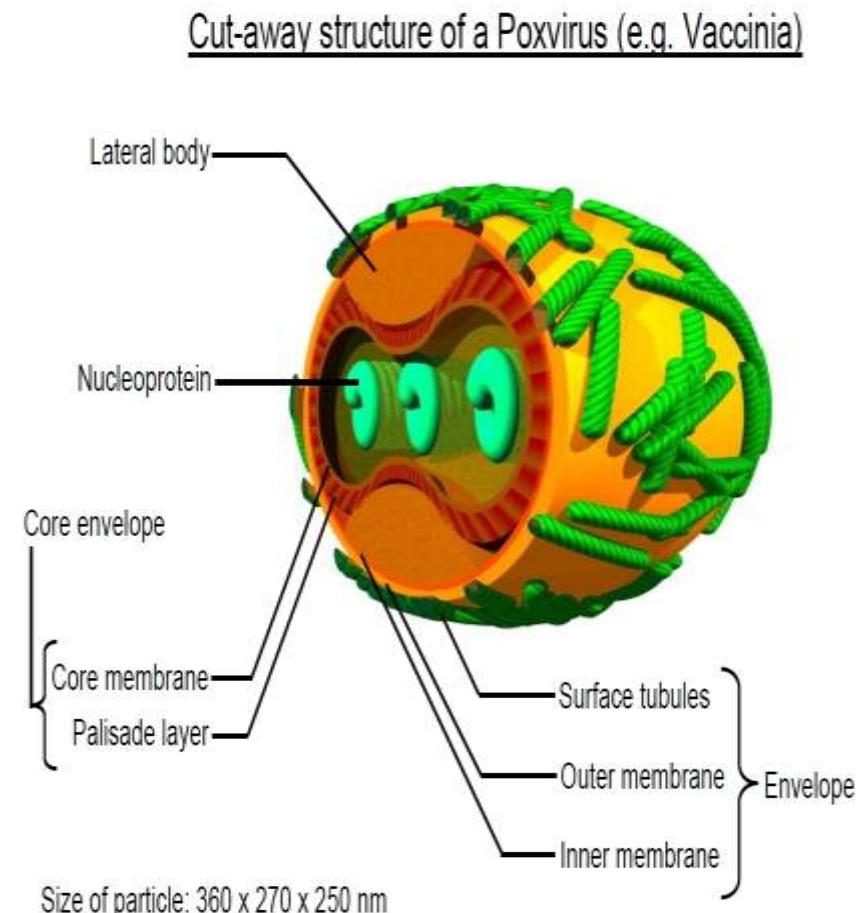
^aProbable member of genus.

^bPrototypal member.

^cCompletely sequenced.

Virus Morphology

- Pox viruses do not have helical or icosahedral symmetry. It is called as “**Complex Symmetry**”.
- The outer envelope of the virus consists of two layers:
- The thick outer layer contains surface tubules or short filaments of helically wound protein subunits (with a hollow channel running through the centre of each tubule)
- The thinner inner layer (which is possibly a lipid bilayer)



- Virions in most genera are **brick-shaped**, 250 x 200 x 200 nm, with an irregular arrangement of surface tubules.
- Virions of members of the genus *Parapoxvirus* are **ovoid**, 260 x 160 nm, with regular spiral arrangement of surface tubules wound around the virus giving a shape of “**Ball of Yarn**” or **cocoon shaped**.
- In some poxviruses the tubules are replaced by globular protein structures.
- Inside the envelope is the **Dumbbell shaped core** and **two lateral bodies**.
- The core consists of the core wall - an outer thick palisade layer, 17 nm thick (so called because it is striated, giving the appearance of many tiny columns) and the thinner, smooth inner layer or core membrane, 8 nm thick.

Surface layer:

Outer membrane

Inner membrane

Core wall

Core

DNA genome

Virion enzymes

A

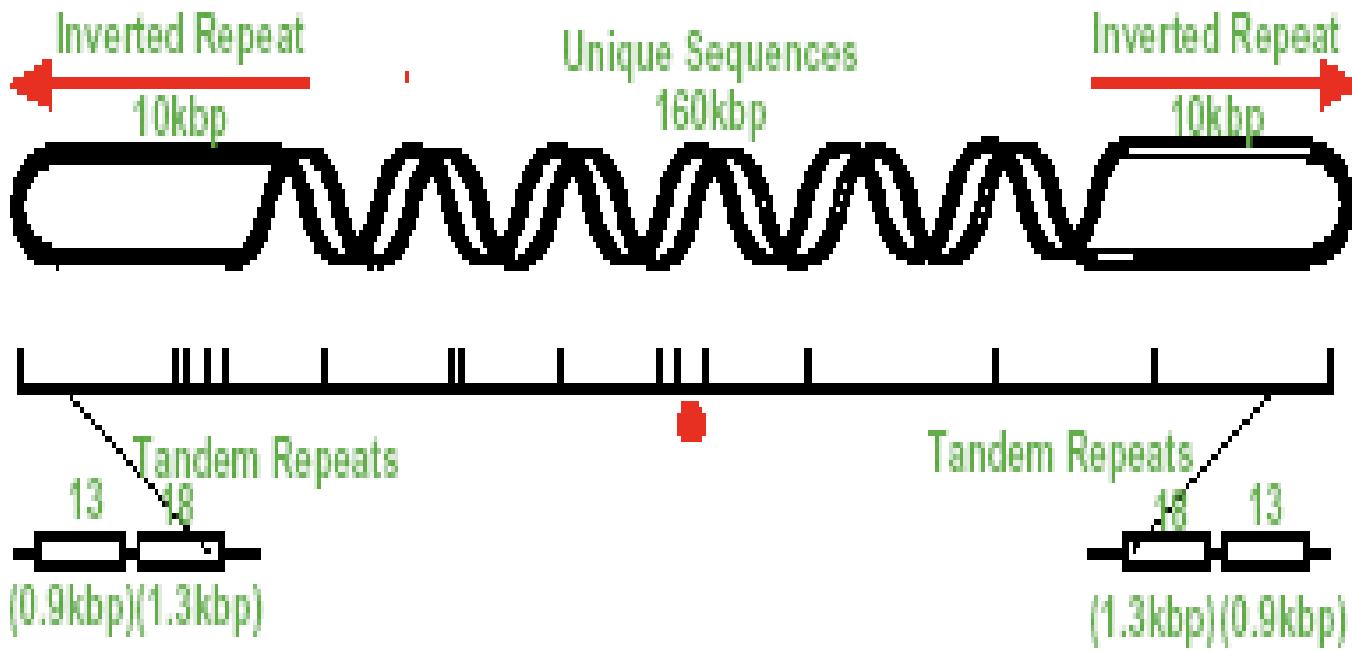
Fig. A: Structure of Poxvirus



Fig. B: Smallpox

Viral Genome

- The nucleoprotein consists of the tightly-packed **linear double-stranded DNA** genome coated by at least 4 different types of proteins, maintaining the DNA in a superhelical state.
- A DNA-dependent RNA polymerase is also present in the nucleoprotein or core.
- Genomes have the capacity to encode about 200 proteins, as many as 100 of which are contained in virions.



- It contains **no introns** and contains more than 150 open reading frames that do not overlap.
- The genome ends are **palindromic tandem repeats and is covalently closed at each end; the ends are hairpin-like telomeres.**
- Unlike other DNA viruses, poxviruses encode all of the enzymes required for transcription and replication, many of which are carried in the virion
- **Cytoplasmic replication**
- Enveloped virions released by exocytosis;
- Non-enveloped virions released by cell lysis.

Replication

- After fusion of the virion with the plasma membrane or after endocytosis, the viral core is released into the cytoplasm.
- Transcription is characterized by a cascade in which the transcription of each temporal class of genes ("early, intermediate," and "late" genes) requires the presence of specific transcription factors that are made by the preceding **temporal class of genes**.
- Intermediate gene transcription factors are encoded by early genes, whereas late transcription factors are encoded by intermediate genes.
- Transcription is initiated by the viral transcriptase and other factors carried in the core of the virion that enable the production of mRNAs within minutes after infection.

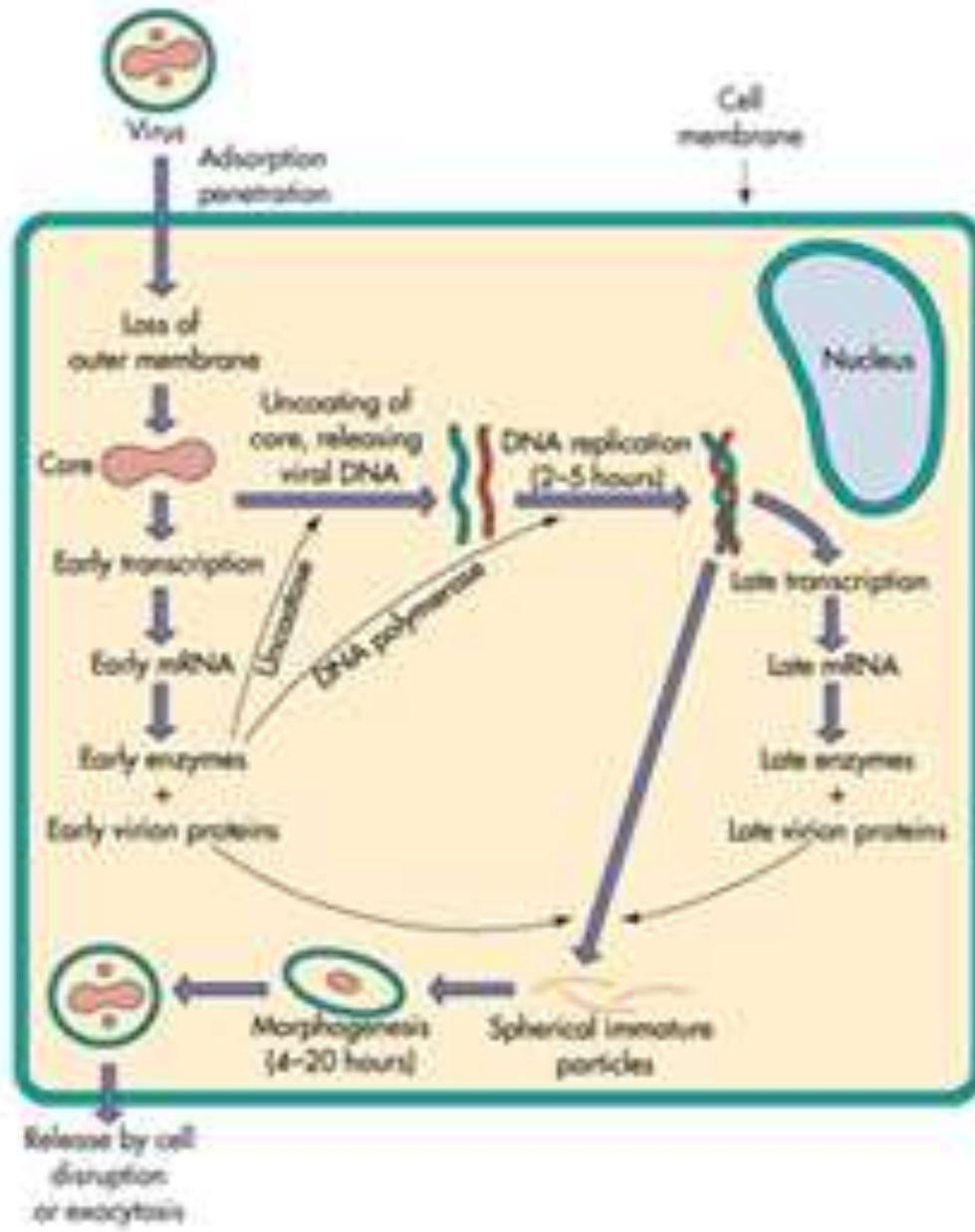
- Proteins produced by these mRNAs complete the uncoating of the core and transcription of about 100 early genes; all this occurs before viral DNA synthesis begins.
- Early proteins include DNA polymerase, thymidine kinase, and several other enzymes required for replication of the genome.
- Poxvirus DNA replication involves the synthesis of long concatemeric intermediates, which are subsequently cut into unit-length genomes.
- With the onset of DNA replication there is a dramatic shift in gene expression.
- Transcription of "intermediate" and "late" genes is controlled by binding of specific viral proteins to promoter sequences in the viral genome.
- Some early gene transcription factors are made late in infection, packaged in virions, and used in the subsequent round of infection.

- The pox viral genes are expressed in Three phases.
- **Early Phase:** Genes that encode the **non-structural protein**, including proteins necessary for replication of the viral genome, and are expressed before the genome is replicated.
- **Intermediate Phase:** The intermediate genes are switched-on, **triggering replication** of the viral DNA genome. Viral synthesis occurs inside virally-induced double-membrane bound vesicles (derived from the host endoplasmic reticulum) called **virosomes**. DNA replication occurs inside these virosomes, which later disassemble prior to virion assembly in the late phase.
- **Late Phase:** Late phase genes are expressed after the genome has been replicated and encode the **structural proteins** to make the virus particle.

- Virion formation involves coalescence of DNA within crescent shaped immature core structures, which then mature by the addition of outer coat layers.
- Replication and assembly occur in discrete sites within the cytoplasm (called **viroplasm** or **viral factories**), and virions are released by budding (enveloped virions) or by exocytosis, or cell lysis (nonenveloped virions).
- Both enveloped and non-enveloped virions are infectious, but enveloped virions are taken up by cells more readily and appear to be more important in the spread of virions through the body of the animal

- **Two major forms of infectious virions:**
- The **mature virion (MV)** contains more than 80 proteins and consists of a nucleoprotein core surrounded by a lipid membrane with about 20 proteins. Approximately 20 proteins within the core are devoted by synthesis and modification of mRNA.
- The **enveloped virion (EV)** consists essentially of a MV with an additional membrane containing about 6 proteins distinct from those in the MV membrane.

Poxvirus Replication



-cytoplasmic life cycle unique for DNA viruses.

Transmission

- By aerosol and droplets (variola virus),
- By introduction of virus into small skin abrasions after direct or indirect contact with an infected animal (orf virus, milker's nodule virus), and
- In the case of some animal poxviruses mechanically by biting arthropods. .
- The viruses generally have **narrow host ranges**.
- Poxviruses are resistant in the environment under ambient temperatures and may survive for many years in dried scabs or other virus-laden material.

Skin lesions

- Skin lesions are the principal feature.
- Several viral encoded proteins are released from infected cells including a homologue of epidermal growth factor (virokines) which stimulates cell proliferation.
- Typically pox lesions begins as **macules** and progress through **papules**, **vesicles** and **pustules** to **scabs** which detach leaving a scar

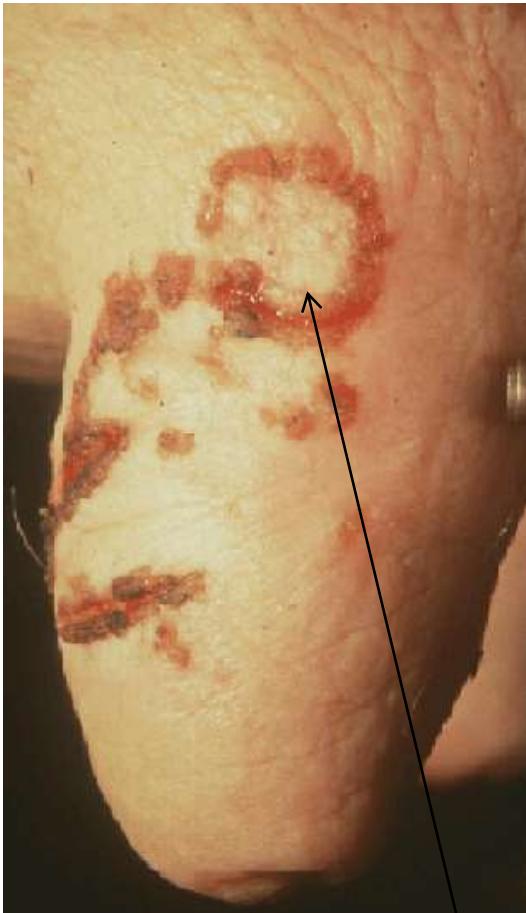
Cow/Buffalo Pox

- **Orthopox virus**
- It is a mild eruptive disease of dairy cows and buffaloes localized on the **udder and teats**.
- The disease spreads by contact during milking.
- After an incubation period of 3–7 days, during which cows may be mildly febrile, papules appear on the teats and udder.
- Vesicles may not be evident or may rupture readily, leaving raw, ulcerated areas that form scabs.
- Lesions heal within 1 month.
- Most cows in a milking herd may become affected.
- Milkers may develop fever and have lesions on the hands, arms, or face.
- Occasionally, cowpox in people can cause generalized disease, and fatalities have been recorded



Pseudocowpox

- **Parapox virus.**
- They don't produce pock on the CAM.
- It is a mild infection of the **udder and teats** of cows.
- Lesions begin as small, red papules on the teats or udder.
- These may be followed rapidly by scabbing, or small vesicles or pustules may develop before scabs form.
- Granulation occurs beneath the scabs, resulting in a raised lesion that heals from the center and leaves a characteristic **horseshoe or circular ring of small scabs**. Similar lesions can occur on the muzzles and within the mouths of nursing calves
- The infection spreads slowly throughout milking herds, and a variable percentage of cows shows lesions at any time.



This virus can also infect humans and the condition is commonly referred to as **milker's nodule**



Horseshoe or circular ring of small scabs

Orf (Contagious Pustular dermatitis, Contagious Ecthyma, Scabby Mouth)

- The disease is primarily of sheep and goat caused by **Parapox virus**.
- There is development of pustular and scabby lesions on muzzle and lips.
- The incidence in the flock may reach upto 90%.
- The disease is common in lambs 2-3years of age.
- The lesions develop as papules, pustules and then thick scabs and spread from oral commisures to muzzle and nostrils.
- The scabs are often friable and mild trauma causes the lesions to bleed

- Systemic invasion may lead to lesions on coronet, ears, anus and vulva. May extend to alimentary tract and trachea.
- Spread of infection can be by direct contact or through exposure to contaminated feeding troughs and similar fomites, including wheat stubble and thorny plants.
- Ewes can be vaccinated several weeks before lambing.
- Vaccine is live virus derived from scabs collected from sheep.
- **Orf virus is zoonotic**



Lumpy skin disease (LSD)

- Infectious, eruptive, occasionally fatal disease of cattle characterized by nodules on the skin and other parts of the body.
- **The Lumpy skin disease virus is related to capripox virus. Also called as Neethling virus**
- **Vector borne -transmitted mechanically between cattle by biting insects.**
- The virus can be transmitted through blood, nasal discharge, lacrimal secretions, semen and saliva. The disease can also be transmitted through infected milk to suckling calves.
- It is characterized by fever, reduced milk production and skin nodules. Mastitis, swelling of peripheral lymph nodes, loss of appetite, increased nasal discharge and watery eyes are also common.
- Temporary or permanent infertility occur among infected cows and bulls. Recovery is slow and affected cattle often remain debilitated for several months.
- Morbidity is high but mortality is low.

LSD

- LSDV attacks the circulatory system of an animal and causes vasculitis or inflammation of blood vessels and lesions in organs like liver, lungs, spleen, lymph nodes
- The epidermis, the outer surface of the skin, gets separated from the dermis or inner layer, leading to the formation of **lumps or nodules** on an animal's body.
- **PM lesions-** necrotising vasculitis, or the death of tissues, and fibrosis in various organs of the infected cattle, leading to their death.
- **Vaccines-** Lumpi-ProVacInd (ICAR)
- **Due to cross-protection within the Capripoxvirus genus, sheep pox virus (SPPV) or goat pox virus vaccines have been widely used for cattle against lumpy skin disease virus (LSDV).**



Sheep & goat Pox

- Often fatal, diseases characterized by widespread skin eruptions.
- The incubation period of sheeppox is 4–8 days and that of goatpox 5–14 days.
- Sheep and goats of all ages may be affected, but the disease is generally **more severe in young animals**.
- Clinical cases vary from mild to severe:
 - fever, depression, polypnoea
 - conjunctivitis, lacrimation, rhinitis, oedema of eyelids, photophobia
 - cutaneous eruption beginning with erythematous areas especially noticeable in hair or wool-free parts of the body, such as the perineum, inguinal area, scrotum, udder, muzzle, eyelids and axillae
 - lesions evolve into papules

- ***Papulo-vesicular form***
 - Papules become a white-grey colour, desiccate and form crusts that are easy to remove
 - Rarely, papules may transform into vesicles. After rupture of vesicles, a thick crust covers the lesions
- ***Nodular form ('stone pox')***
 - Papules give rise to nodules involving all the layers of the skin and the subcutaneous tissue
 - Necrosis and sloughing of the nodules leaves a hairless scar
- In both forms, nodules develop in the lungs causing bronchopneumonia with cough, abundant nasal discharge, depression, anorexia and emaciation
- Animals may recover within 20-30 days
- Death is frequent when complications occur (abortion, which is rare, secondary infections, fly strike, septicaemia)

Lesions

- **Skin lesions:** congestion, haemorrhage, oedema, vasculitis and necrosis. All the layers of epidermis, dermis and sometimes musculature are involved
- **Lymph nodes draining infected areas:** enlargement (up to eight times normal size), lymphoid proliferation, oedema, congestion, haemorrhage
- **Pox lesions:** on mucous membranes of the eyes, mouth, nose, pharynx, epiglottis, trachea, on the rumenal and abomasal mucosae, and on the muzzle, nares, in the vulva, prepuce, testicles, udder, and teats. Lesions may coalesce in severe cases
- **Lung lesions:** severe and extensive pox lesions, focal and uniformly distributed throughout the lungs; congestion, oedema, focal areas of proliferation with necrosis, lobular atelectasis. Enlargement, congestion, oedema and haemorrhages of mediastinal lymph nodes

Prevention

- Non-endemic areas – keep free with import restrictions and proper quarantine
- Prevent introduction of infected animal products
 - Meat, hair, wool, hides must be subjected to suitable decontamination procedures before entry into non-endemic areas

Control and Eradication

- Endemic areas
 - Vaccinate
 - The most widely employed vaccine is probably the **Romanian strain** that has been used effectively for many years .
 - The **Kenya O 180** strain is possibly the vaccine with the best safety and efficacy.
- Outbreak in endemic area -small scale
 - Quarantine, slaughter infected and exposed, clean and disinfect
 - Ring vaccination
- Outbreak in endemic area –large scale
 - Massive vaccination
 - Movement restrictions

Control and Eradication

- Outbreak in non-endemic areas
 - Quarantine, slaughter infected and exposed, clean and disinfect
 - Ring vaccination
- **A carrier state has not been shown for SGPV**
- Isolate infected herds and sick animals for at least 45 days after recovery

Disinfection

- Sodium hypochlorite
- Phenol 2% in 15 minutes
- Detergents (SDS)
- Virus can survive
 - For 2 months in wool
 - For 6 months in the environment
 - for many years in dried scabs



Vaccination

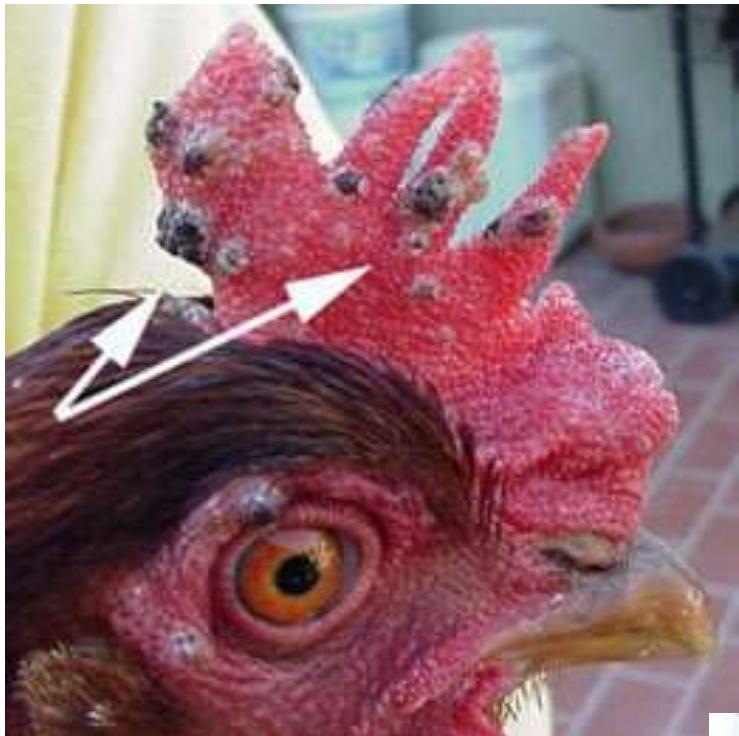
- Vaccination can provide effective control in endemic areas
- Killed vaccines do not provide long lasting immunity
- Attenuated virus vaccines give immunity up to 2 years
- Sheep Pox vaccine can protect against lumpy skin disease and vice versa.
- Goat pox vaccine gives protection against sheep pox but not vice versa.
- Eg Raksha-SP vaccine

Fowl Pox

- A common disease of domestic and wild birds caused by **Avipox virus**
- Avipoxvirus is **highly host specific**; therefore, infection with one type of poxvirus does not protect against others.
- Poxviruses are named according to the susceptible host species (i.e., Fowlpox virus, Canarypox virus, Pigeonpox virus, Psittacinepox virus, Turkeypox virus, etc.)
- Disease course generally 3 to 4 weeks.
- Avipoxvirus is transmitted by means of desquamation of skin lesions or by the biting of insects that sting the unfeathered skin areas, producing the characteristic papular, vesicular, and crusty lesions visible in the nares, eyelids, and legs.
- Birds can also ingest or inhale viral particles, producing diphtheritic lesions in the oral cavity, pharynx, larynx, and trachea

Clinical Signs

- Two forms of the disease:
- **Skin or cutaneous or Dry form** - development of proliferative lesions, ranging from small nodules to spherical wart-like masses on the skin of the comb, wattle and other unfeathered areas
- **Diphtheritic or 'Wet' form**- slightly elevated white opaque nodules develop on the mucous membranes. They rapidly increase in size to become a **yellowish diphtheritic membrane**. Lesions occur on the mucous membranes of the mouth, oesophagus, larynx or trachea.
- The mortality rate is higher in the diphtheritic form than in the cutaneous form, sometimes nearing 50% particularly in young birds
- Birds have difficulty breathing.
- Weight loss, reduced egg production



- **Post Mortem Lesions**
- **Skin form:** small whitish or yellowish areas, nodules or scabs
- **Diphtheritic form:** raised white or opaque nodules which may join to form yellow, cheesy, necrotic lesion

Diagnosis

- Characteristic gross and microscopic lesions and PCR
- Fowlpox virus multiplies in the cytoplasm of epithelial cells with the formation of large intracytoplasmic inclusion bodies (**Bollinger bodies**) that contain smaller elementary bodies (**Borrel bodies**). The inclusions can be demonstrated in sections of cutaneous and diphtheritic lesions
- Viral particles with typical poxvirus morphology can be demonstrated by negative-staining electron microscopy
- The virus can be isolated by inoculating **chorioallantoic membrane** of developing chicken embryos, susceptible birds, or cell cultures of avian origin.

Prevention and Control

- Management – do not introduce new birds with lesions
- Virus survives in dried scabs for months or years.
- Burn infected chicken houses and dispose of all infected birds.
- Burn or bury all parts of the birds that are unused.
- After all infected birds have been removed and no new cases occur, build another chicken house on a new site.
- Fowlpox and pigeonpox virus vaccines of chicken embryo or cell culture origin are available.
- The vaccines are used in susceptible flocks where the disease has been endemic or has been diagnosed in previous flocks
- No zoonotic risk.

PRIONS

Proteinaceous Infectious Particle



No nucleic acid

Introduction

- **Stanley B. Prusiner** coined the term proin from **Proteinaceous infective particle** and changed to prion to sound it rhythmic.
- The word prion derived from the initial letters of the words "proteinaceous" and "infectious".
- In 1997, Pruisner was awarded Nobel Prize in Medicine for discovery of infectious proteins (prion) and their mechanism of amplification
- A **prion** is an infectious agent, composed primarily of protein in **misfolded form**.

- Not classified like viruses--there are no families, genera, or species.
- They first are identified by their host species and disease association Then, they are characterized by their molecular and biological properties.
- Non-immunogenic, evoke no detectable acquired immune response in their host
- extremely resistant to inactivation by heating,
- chemicals and ultraviolet & γ -irradiation irradiation
- Prions are stable at a wide pH range

Prion Diseases (Transmissible Spongiform Encephalopathy)

Human

- Kuru
- Fatal Familial Insomnia (FFI)
- Creutzfeldt-Jakob disease (CJD)
- Gerstmann-Straussler-Scheinker syndrome (GSS)

Animals

- Scrapie
- Bovine Spongiform Encephalopathy (BSE)
- Chronic Wasting Disease (CWD)
- Feline transmissible encephalopathy

- The basic lesion is a spongiform degeneration in the gray matter of the brain and astrogial hypertrophy and proliferation.
- Scrapie is the prototype of prion disease with the longest history of the known animal prion diseases
- **The first prion disease evidenced to be both infectious and transmissible in natural settings**
- Scrapie is endemic in sheep in all countries except Australia and New Zealand.

Classification of Prion diseases

- **Infectious/Exogenous**
 - e.g., Kuru, BSE (mad cow disease), Scrapie
 - Spread by
 - Consumption of infected material containing PrP^{Sc} gene
 - Transfusion.
- **Sporadic-** Rarely, random spontaneous conversion of native PrP^C to PrP^{Sc}
- **Familial/Hereditary**
 - Due to autosomal dominant mutation of PrP^c gene.
 - ‘**Species barrier**’- The resistance of some species to infection by prions derived from another species

Prion Structure

Isoforms

The protein that prions are made of (PrP) is found throughout the body,

The normal form of the protein is called PrP^C, the infectious form is called PrP^{Sc}

PrPC

- The cellular prion protein (PrPC) is a cell surface protein expressed in a variety of different organs and tissues with high expression levels in the central and peripheral nervous systems
- It has **209 amino acids** and mainly **alpha-helical** structure.
- It is anchored to the membrane through a glycosyl-phosphatidyl inositol link, with the protein chain on the outside of the neuronal plasma membrane.
- The PrPc (without the PI link) is water soluble, protease sensitive, and consists of **42% alpha helix and 3% beta sheet**.

- PrP^C binds copper (II) ions with high affinity.
- Related to PrP structure or function.
- PrP^C play important roles in cell-cell adhesion and intracellular signaling '*in vivo*'.
- Therefore is involved in cell-cell communication in the brain.

PrP^{Sc}

- The infectious isoform of PrP, known as PrP^{Sc}, "Sc" refers to 'scrapie',
- It is able to convert normal PrP^C proteins into the infectious isoform by changing their conformation, or shape; alters the way the proteins interconnect.
- Occurs as a result of post-translational processing of a normal cellular protein.
- It has a **higher proportion of β-sheet structure.**
- Aggregations of these abnormal isoforms form amyloid fibers, which accumulate to form plaques.
- The end of each fiber acts as a template onto which free protein molecules may attach, allowing the fiber to grow.

The Conversion of PrP^c to PrP^{se}

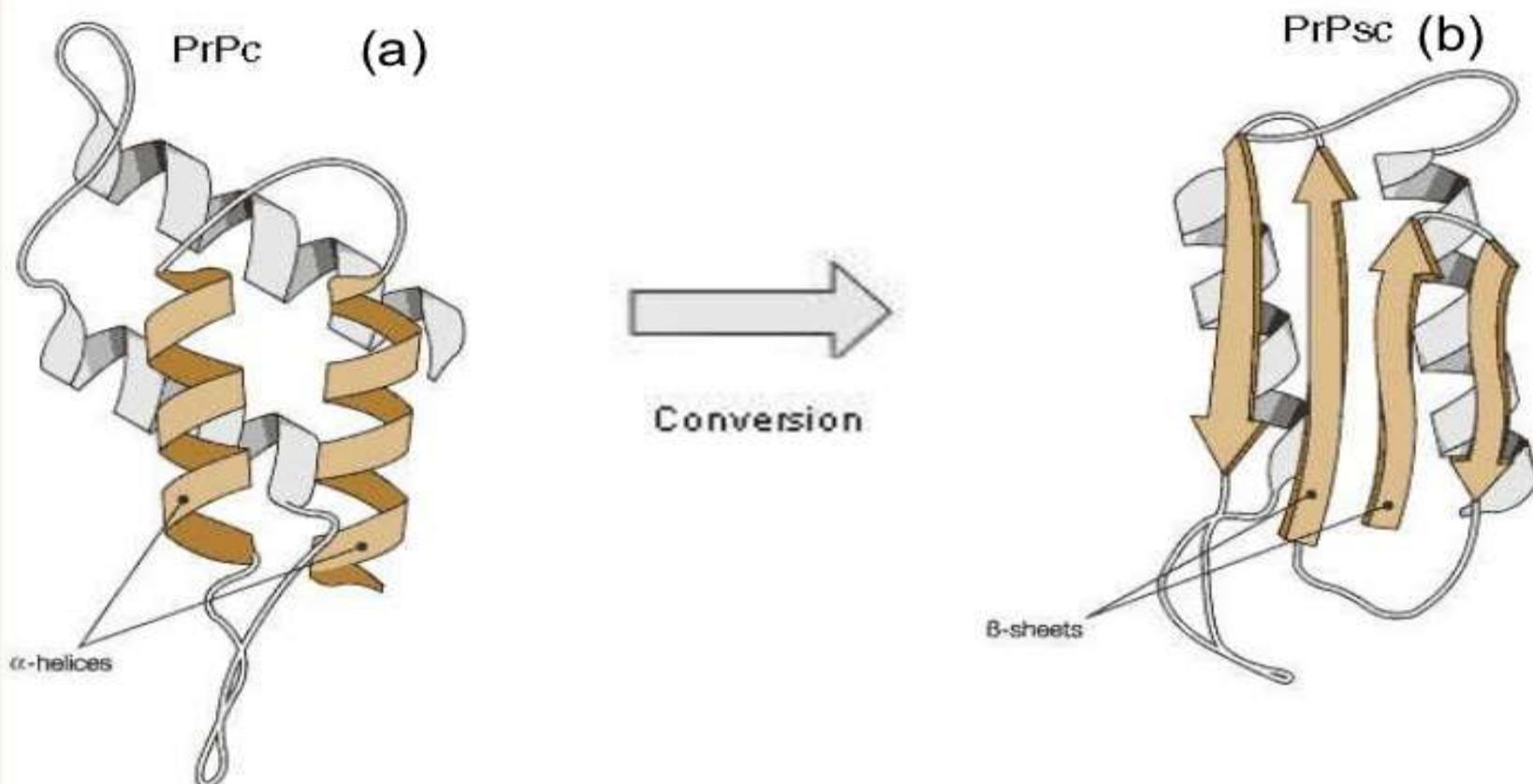
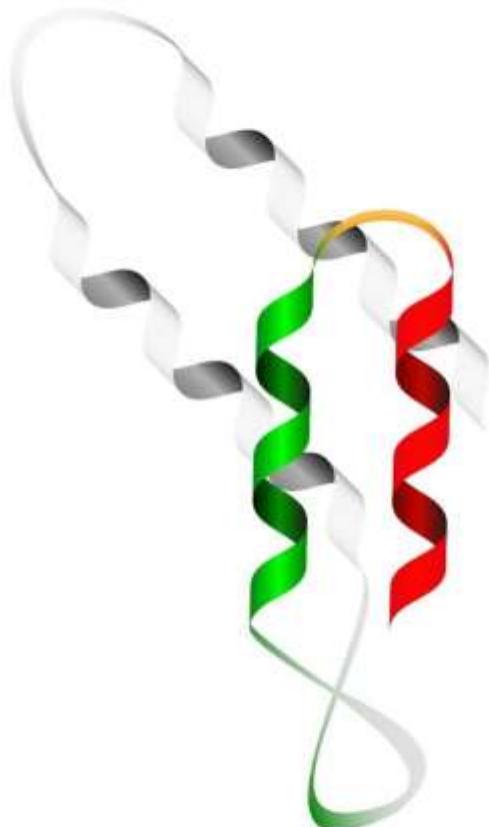


Figure 1: Proposed three-dimensional structure (a) PrPC and (b) PrPSc

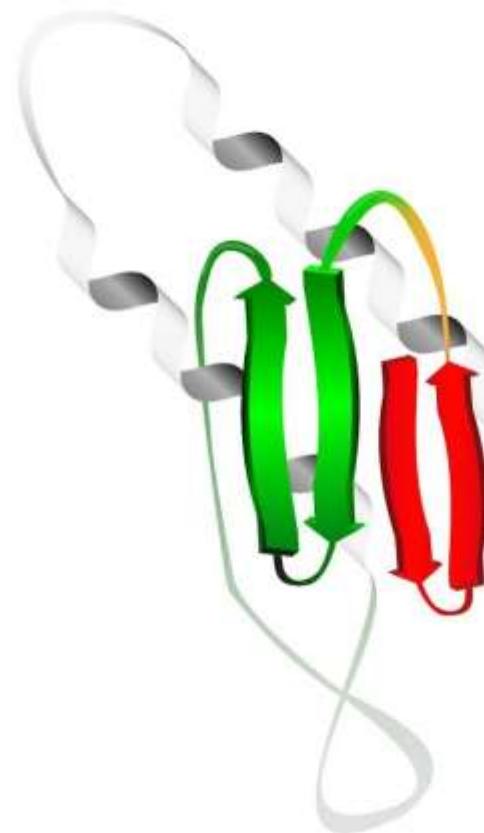
PrP^C

is a normal protein



PrP^{Sc}

the disease-causing form of the
prion protein



•Prion Function

It has been proposed that neurodegeneration caused by prions may be related to abnormal function of PrP that causes brain damage

Normal function is maintenance of long term memory

Prion replication mechanism

- **Heterodimer model**
 - A single PrP^{Sc} molecule binds to a single PrP^C molecule and catalyzes its conversion into PrP^{Sc}.
 - The two PrP^{Sc} molecules then come apart and can go on to convert more PrP^C
- **Fibril model**
 - Assumes PrP^{Sc} exists only as fibrils, fibril ends bind PrP^C and convert it into PrP^{Sc}
 - The quantity of prions would increase linearly, forming ever longer fibrils.
 - Exponential growth of both PrP^{Sc} and the quantity of infectious particles is explained by taking into account fibril breakage

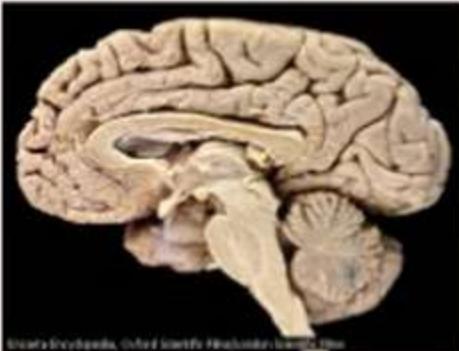
- The mechanism of prion replication has implications for designing drugs.

An effective drug does not need to eliminate all prions,

But simply needs to slow down the rate of exponential growth.

By binding to fibril ends and blocks them from growing any further

How PrPsc attack the Brain?



Brain consists of a mass of nerve tissue (Hundred billions of neuron) and neuroglia, supporting neural tissue.

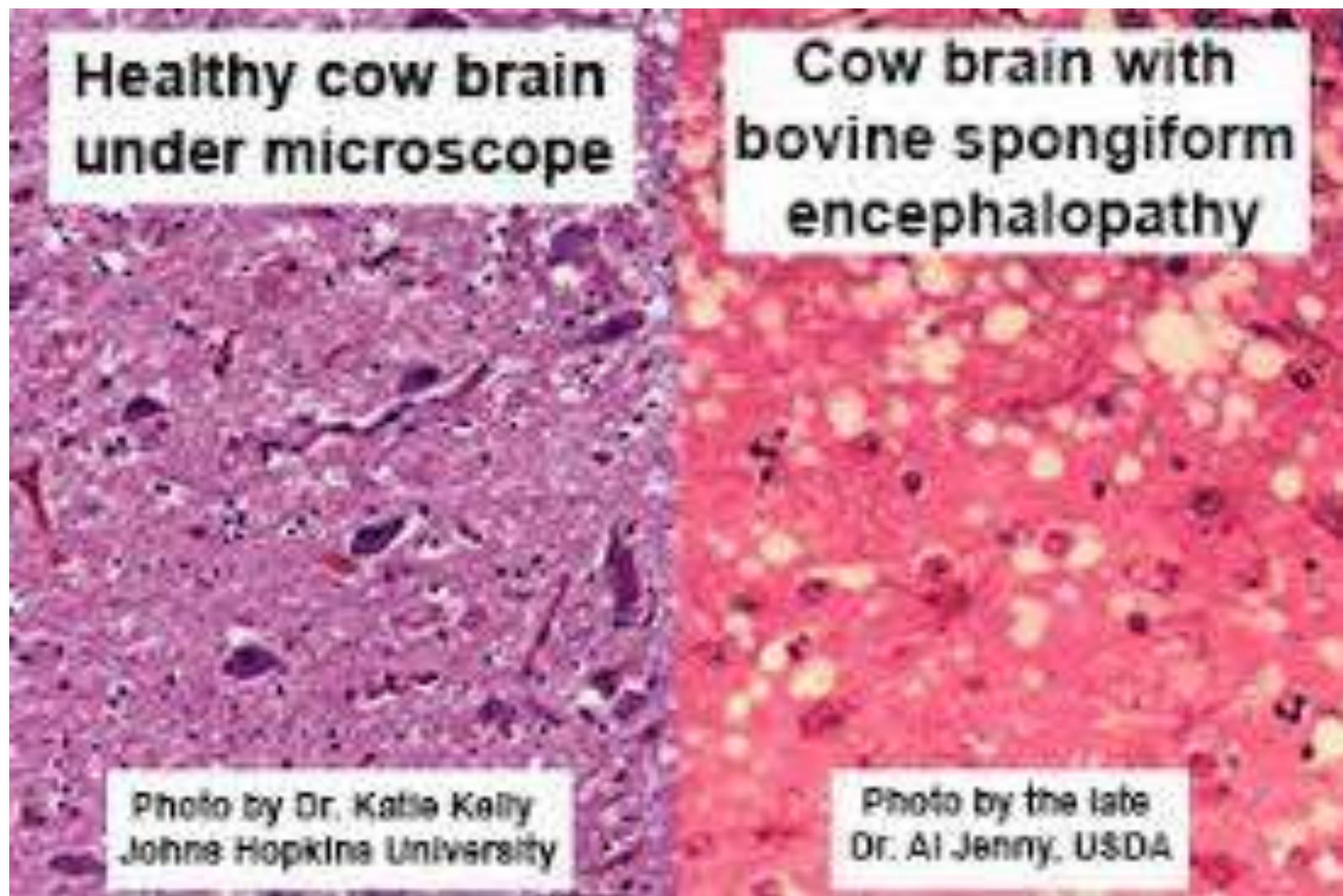
When enough PrPSc proteins have been made they form long filamentous aggregates that gradually damage neuronal tissue.

When neuron in the brain are all dead, the appearance of the brain will become sponge-like appearance. And this eventually lead to death.

The harmful PrPSc form is very resistant to high temperatures, UV-irradiation and strong degradative enzymes.

Prion Disease

- Prions cause neurodegenerative disease by aggregating extracellularly within the CNS to form plaques known as **amyloid**, disrupt the normal tissue structure characterized by "holes" in it with resultant **spongy architecture due to the vacuole formation in the neurons**.
- Other histological changes include
Astrogliosis
Absence of an inflammatory reaction.
- ‘**Species barrier**’- The resistance of some species to infection by prions derived from another species is species barrier.



- IP is long but once symptoms appear the disease progresses rapidly, leading to brain damage and death.
- Neurodegenerative symptoms can include Convulsions, Dementia, Ataxia (balance and coordination dysfunction), Behavioural or personality changes

BOVINE SPONGIFORM ENCEPHALOPATHY

- Commonly known as **Mad cow disease**.
- A fatal, neurodegenerative disease causes a spongy degeneration in the brain and spinal cord.
- BSE has a long incubation period, about 4 years.
- usually affecting adult cattle at a peak age onset of four to five years.
- All breeds being equally susceptible.
- The disease may be transmitted to human beings who eat the brain or spinal cord of infected carcasses.
- In humans, it is known as Creutzfeldt–Jakob disease

Types of BSE

1. Classical BSE:

- occurs through the consumption of contaminated feed

2. Atypical BSE:

- refers to naturally and sporadically occurring forms
- believed to occur in all cattle populations at a very low rate
- have only been identified in older cattle

Clinical signs

- The onset of disease is insidious, with tremors, hyperesthesia with kicking during milking,
- abnormal posture,
- hindlimb ataxia,
- progressive apprehensive behavior,
- aggression and even frenzy,
- reduced milk yield, and weight loss.

BSE

Way of infection



The cow eat offal of the infected sheep



Prions are taken up from the gut and transported along nerve fibers to the brain stem.



Prions accumulate and convert normal prion proteins to the disease-causing form, PrPSc.



Years later, BSE results when a sufficient number of nerve cells have become damaged, affecting the behaviour of the cows. And eventually the cow is dead.

SCRAPIE

- A fatal, degenerative disease that affects the nervous systems of **sheep and goats**.
- The name scrapie is derived from one of the symptoms of the condition, wherein affected animals will compulsively scrape off their fleece against rocks, trees or fences.
- The disease expresses:
 - severe itching sensation
 - lip-smacking
 - strange gaits,
 - convulsive collapse.
- Scrapie is infectious and transmissible among similar animals

Pathogenesis

Following natural infection, PrPSc is usually first detected in tissues of the lymphoreticular system including spleen, palatine tonsil and retropharyngeal and mesenteric lymph nodes

- In lymph nodes, replication apparently occurs in follicular dendritic cells
- Following ingestion of prions, infection is initiated in gut lymphoid tissues
- Prions produced in these tissues then move to the central nervous system

Diagnosis

Diagnosis can be made by:

- 1. Clinical signs and Symptoms.**
- 2. Detection of Scrapie Associated fibrils.**
- 3. Detection of Abnormal Prion protein (PrP^{sc}) by Western blotting.**
- 4. Two dimensional Gel Electrophoresis.**
- 5. Immunodiagnosis of Prion disease.**
- 6. Bioassay in Mice.**



Scrapie Associated fibrils.

RHABDOVIRIDAE

Rhabdos (greek)-rod

Pathogens of mammals, birds, fish, plants

In the order **Mononegavirales** which also includes families *Bornaviridae*, *Filoviridae*,

Paramyxoviridae

Classification

Group:

Group V (-ssRNA)

Order:

Mononegavirales

Family:

Rhabdoviridae

Genera

Cytorhabdovirus

Lyssavirus

Rabies

Ephemerovirus

Bovine ephemeral fever

Nucleorhabdovirus

Novirhabdovirus

Hematopoetic necrosis virus of fish

Vesiculovirus

Vesicular Stomatitis Virus

Dichorhavirus

Viruses of plants

- The family ***Rhabdoviridae*** includes **4 subfamilies**, 56 genera and 246 species of viruses.
- **Subfamily *Alpharhabdovirinae*** includes 33genera for viruses infecting only vertebrates, only invertebrates or vertebrate hosts and arthropod vectors. Important genera *includes* Genus *Ephemerovirus*, Genus *Lyssavirus*, Genus *Vesiculovirus*.
- **Subfamily *Betarhabdovirinae***
- The subfamily includes 9 genera for viruses infecting plant hosts and arthropod vectors.
- Eg. Genus *Cytorhabdovirus*, *Dichorhavirus*
- **Subfamily *Gammarhabdovirinae***
- The subfamily includes 2 genera infecting finfish
- **Subfamily *Deltarhabdovirinae- 11 genera***

Morphology

- **Bullet-shaped** or cone shaped or bacilliform virions are 70nm in diameter and 170nm in length.
- Enveloped with large peplomers and within this is a **helically coiled cylindrical nucleocapsid**.
- **Negative, single-stranded RNA** 11 to 15 kbp in size.
- Prototype for (-) RNA viruses
- **Vesicular stomatitis Indiana virus – type species**
- Replication in the cytoplasm.

- They possess a three-layered structure: an outer lipid bilayer, a middle matrix protein layer, and an inner nucleocapsid composed of RNA and associated proteins.
- Peplomers consist of trimers of the viral envelope glycoprotein (G)
- A honeycomb pattern of peplomers is observed on the surface of some viruses

Genome

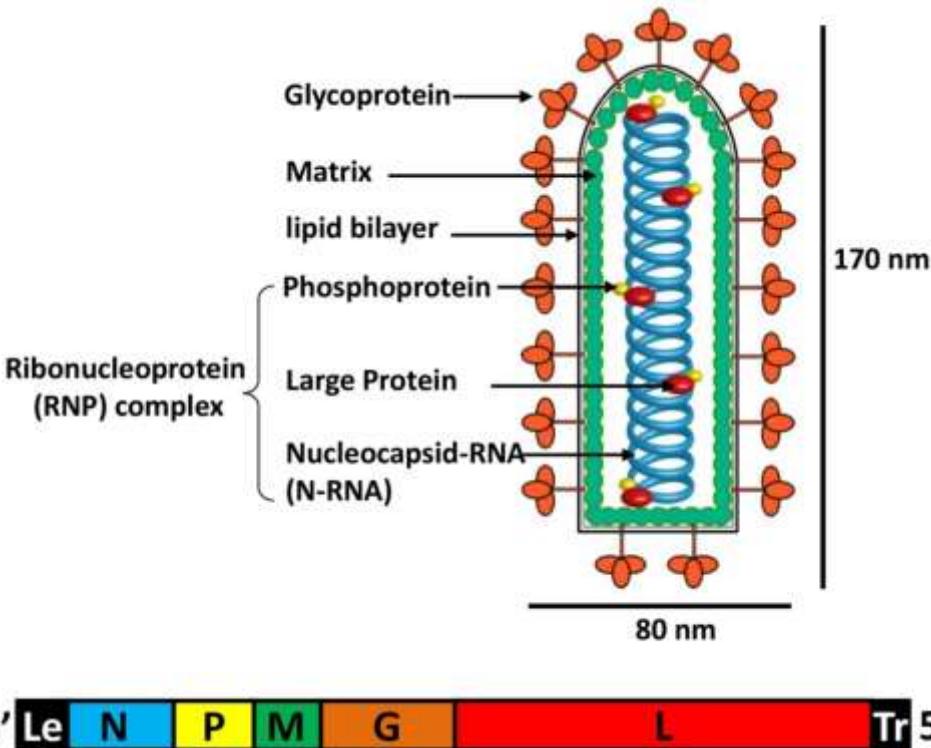
- The RNA has a 3'-terminal free hydroxyl group and a 5'-triphosphate and is not polyadenylated
- The ends have inverted complementary sequences encoding transcription and replication initiation signals.
- The nucleocapsid consists of a ribonucleoprotein (RNP) complex comprising the genomic RNA and tightly bound nucleoprotein (N) together with an RNA-dependent RNA polymerase (L) and polymerase-associated phosphoprotein (P).
- The individual genes are flanked by conserved transcription stop and start signals separated by short untranscribed intergenic sequences

The genome encodes **five genes** in the order **3'-N-P-M-G-L-5'**.

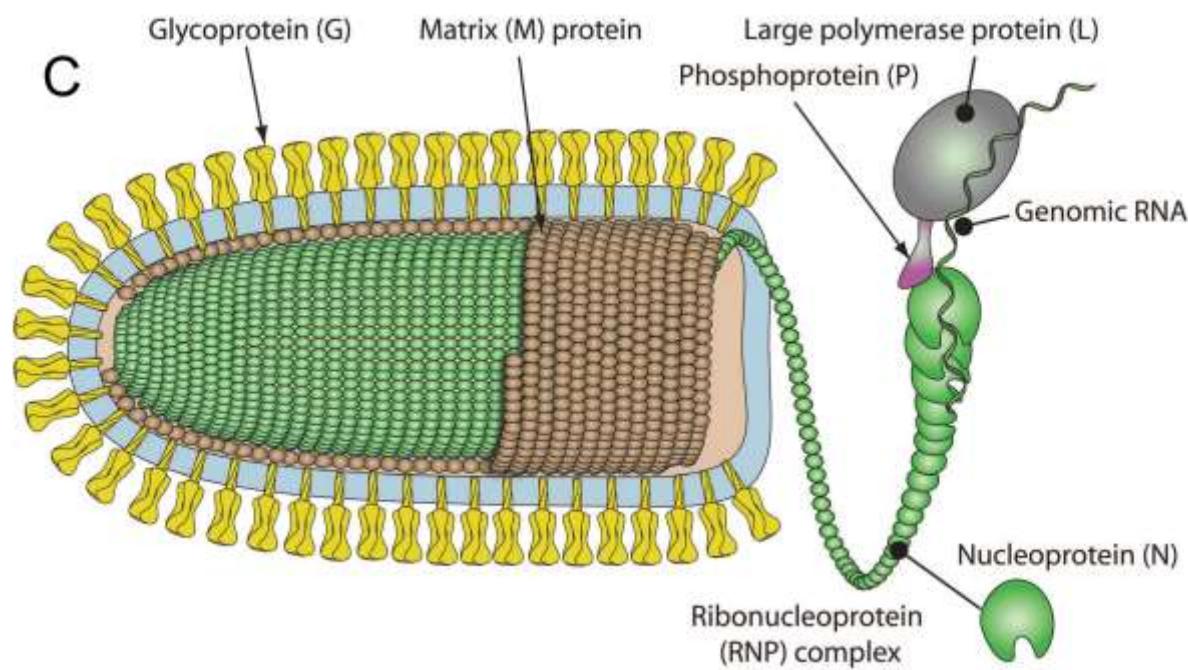
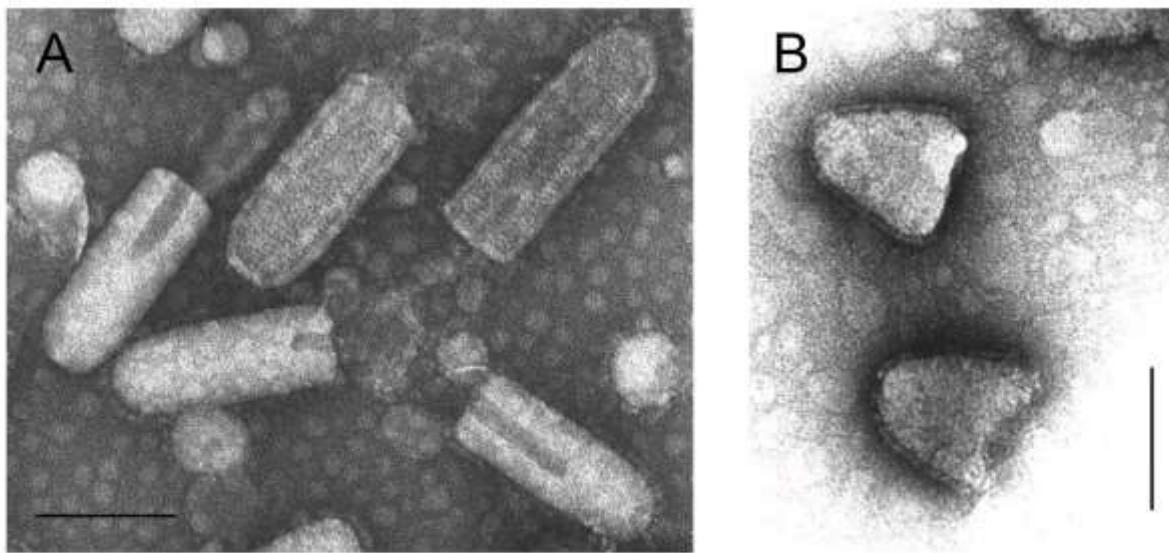
- **L** -the RNA-dependent RNA polymerase that functions in transcription and RNA replication;
- **G** -the glycoprotein that forms trimers that make up the peplomers
- **M** (or M2 for rabies virus)- Matrix protein that facilitates virion budding by binding to the nucleocapsid and to the cytoplasmic domain of the glycoprotein.
- **P**-(also called NS or M1), a component of the viral polymerase;
- **N** -the nucleoprotein, the major component of the viral nucleocapsid
- Three proteins (N, P, and L), in association with viral RNA, constitute the nucleocapsid and comprise the transcription complex



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rabies virus (*left*) and vesicular
stomatitis virus (*right*).



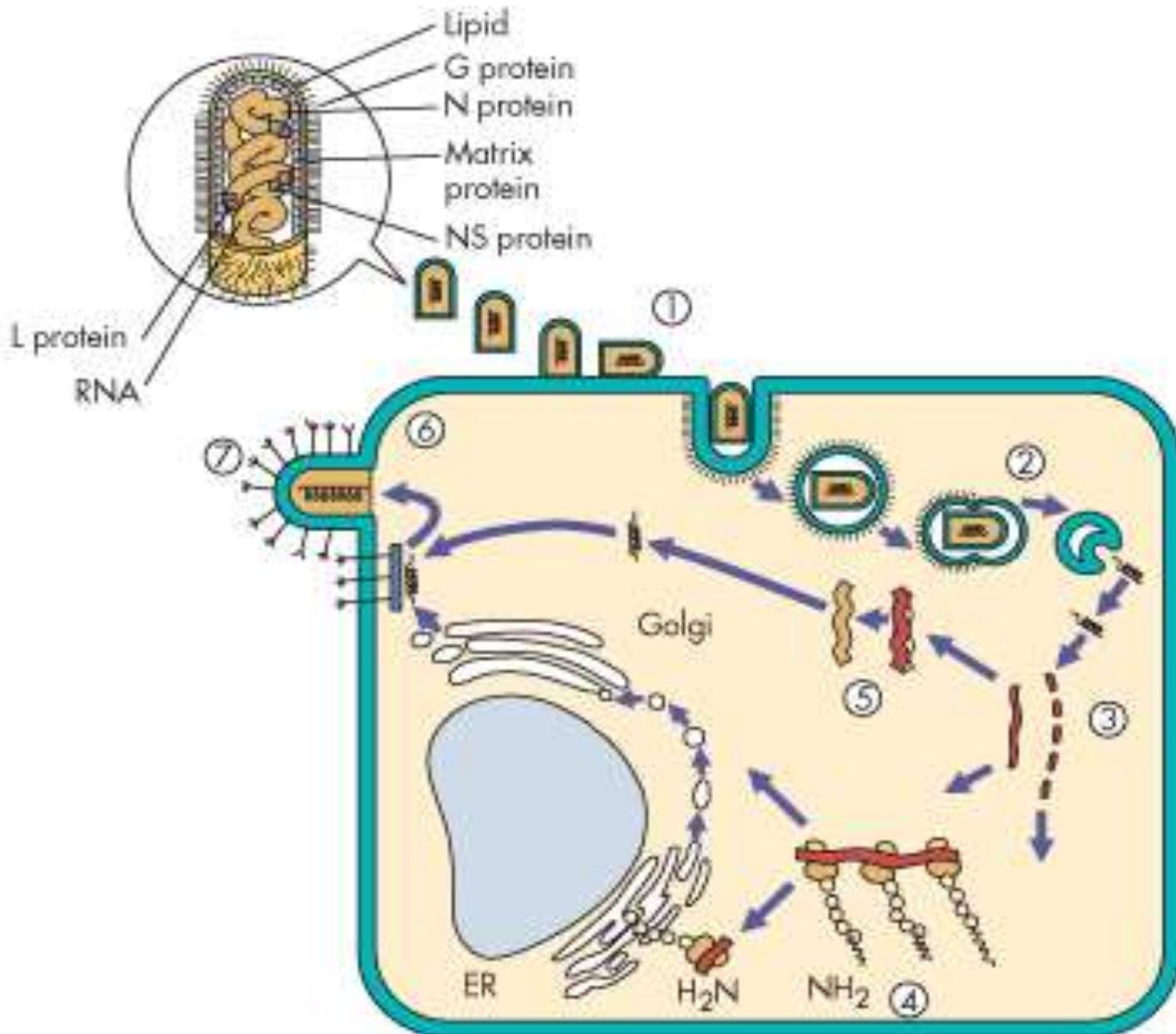
Transcription

- There is only a single promoter site, located at the 3' end of the viral genome; the polymerase (transcriptase) attaches to the genomic RNA template at this site and, as it moves along the viral RNA, it encounters stop/start signals at the boundaries of each of the viral genes.
- The stop signal present at the end of each gene comprises a stretch of U on which the viral polymerase acquires a stuttering behaviour.
- This is called **stop-start or stuttering transcription**- it also accounts for the addition of poly(A) tails on the 3' ends of each mRNA

Viral replication

- 1, Rhabdoviruses bind to the cell surface and are
- (2) endocytosed. The envelope fuses with the endosome vesicle membrane to deliver the nucleocapsid to the cytoplasm.
The virion must carry a polymerase, which (3) produces five individual messenger RNAs (mRNAs) and a full-length (+) RNA template.
- 4, Proteins are translated from the mRNAs, including one glycoprotein (G), which is co-translationally glycosylated in the endoplasmic reticulum (ER), processed in the Golgi apparatus, and delivered to the cell membrane.

- 5, The genome is replicated from the (+) RNA template, and N, L, and NS proteins associate with the genome to form the nucleocapsid.
- 6, The matrix protein associates with the G protein-modified membrane, which is followed by assembly of the nucleocapsid.
- 7, The virus buds from the cell in a bullet-shaped virion.



Rabies

- Rabies otherwise ‘rabere’ in Latin means ‘to be mad.’
- **Syn.** Hydrophobia
- Rabies is a **zoonotic**, fatal and progressive neurological infection caused by rabies virus of the genus *Lyssavirus*. Most rabies cases are caused by genotype 1 / serotype 1 strains.
- Identified by **Louis Pasteur** in 1880's
- It affects **all warm-blooded animals** and the disease is prevalent throughout the world and endemic in many countries **except in Islands like Australia and Antarctica.**
- The natural hosts are terrestrial carnivores and bats. Most mammals can be experimentally infected.
- **Disease has long asymptomatic period**

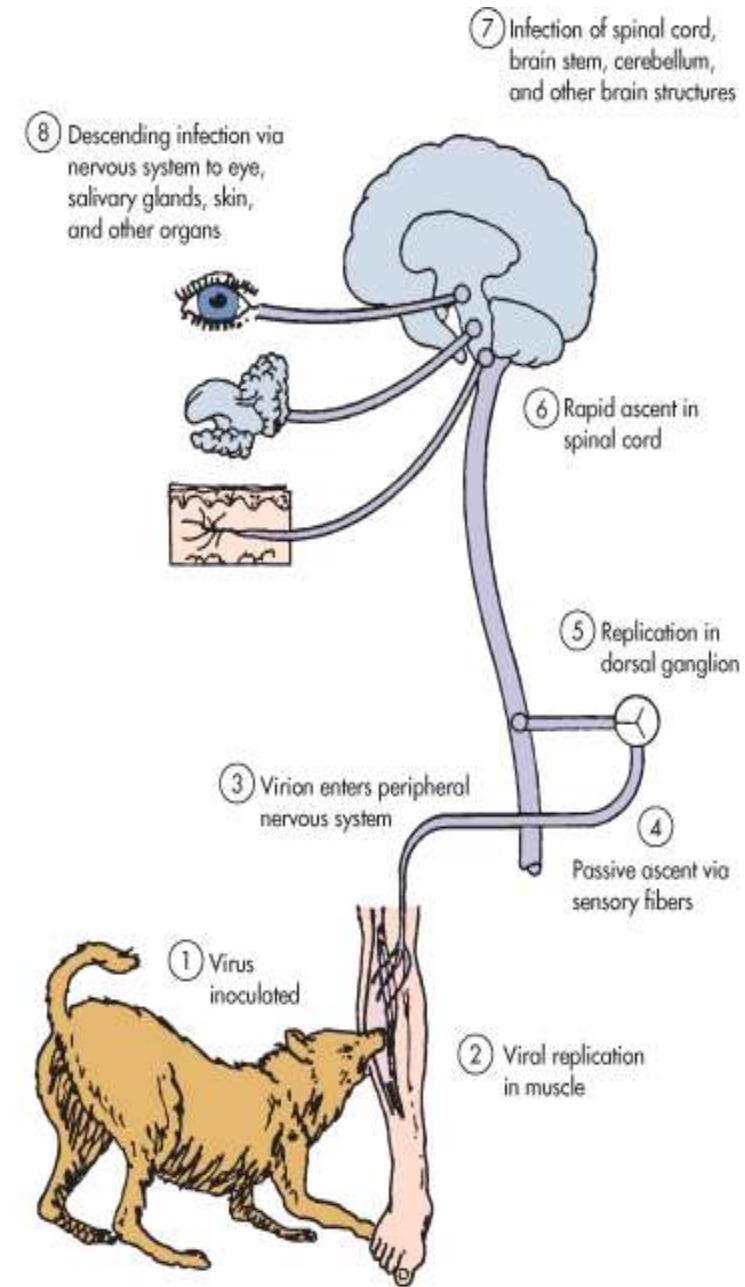
Transmission

- Spread to humans and animals is almost always **by bites** of a rabid animal via introduction of virus-laden saliva into tissues.
- Vampire bats, insectivorous, and fruit bats also transmit the virus by bites.
- There are rare instances of human infection by inhalation, e.g., in bat-infected caves and laboratories and by infected tissues used as transplants.
- Saliva is infectious at, or before, the time clinical signs occur.
- Domestic dogs, cats, and ferrets may shed virus up to 10 days before onset of clinical signs.
- Viral shedding in wildlife has been reported for several weeks before onset of signs.

Pathogenesis

- Virus is not very cytopathic and seems to remain cell-associated
- Virus replicates in the smooth muscle at the site of the bite with minimal or no symptoms
- The length of the incubation phase is determined by the infectious dose and the proximity of the infection site to the CNS and brain
- Moves to nerves and binds to acetylcholine receptors at the neuromuscular junctions.
- Moves centripetally to the spinal cord and then moves to brain.

- An ascending neuronal dysfunction occurs.
- In brain virus multiplies extensively leading to behavioral changes.
- In reservoir host virus moves from the brain centrifugally to adrenals, pancreas, salivary glands.
- For the movement of the virus amino acid at position 333 of the rabies virus glycoprotein (G) is critical. If here arginine or lysine is there, the virus is virulent.



Pathogenesis

- After weeks to months, the virus infects the peripheral nerves and travels up the CNS to the brain (*prodrome phase*)
- Infection of the brain causes classic symptoms, coma, and death (*neurologic phase*)
- During the neurologic phase, the virus spread to the glands, skin, and other body parts, including the salivary glands, from where it is transmitted.
- Replication in the neocortex leads to **dumb form** of the disease, there is paralysis and finally death.
- Antibody response occurs at the late stages
- Antibody can block the progression of the virus
- The long incubation period allows active immunization as a post-exposure treatment.

Clinical features

- Incubation period from 14 to 90 days but as long as 7 years has been reported.
- **The prodromal phase:** Involves change in behavior and lasts 2 - 3 days. Anxiety, irritability and unease are characteristic. Some are more alert, restless and sensitive to light and noise.
- **The excitive or furious phase:** Signs include restlessness, depraved appetite, hiding, wandering, aggressive biting, excessive salivation, dysphagia, muscle tremors, incoordination and staggering.
- **The paralytic or dumb phase:** This develops in several days with seizures, paralysis, coma and death in 3 - 4 days.
- **In horses and cattle the paralytic phase appears to be predominant**

Rabies virus/Epidemiology

- **At risk:**
 - Veterinarians and animal handlers
 - Person bitten by a rabid animal
 - Inhabitants of countries with no pet vaccination program

Modes of control

- Vaccination
- For pets
- For at-risk personnel
- “Vaccination programs have been implemented to control rabies in forest mammals”

Diagnosis

- **Clinical specimens:** Brain.
- The **fluorescent antibody procedure (FAT)** is widely used and is the preferred method for rabies diagnosis.
- The FA test is used occasionally on formalin fixed brain tissue (when fresh tissue is unavailable) to confirm a rabies diagnosis based on the microscopic finding of **Negri bodies**.
- Rabies virus can be propagated in cell cultures and in suckling mice inoculated intracerebrally.
- RT-PCR

Rabies virus/Treatment & Prophylaxis

- Clinical rabies is almost always fatal unless treated
- Only hope:
 - Post exposure prophylaxis
 - For anyone exposed by bite or by contamination of an open wound or mucous membrane to the saliva or brain tissue of an animal suspected to be infected with the virus

Rabies virus/Treatment & Prophylaxis

- First protective measure
 - Local treatment
 - Washing with soap and water.
 - Rabies antiserum Purified human antirabies immunoglobulin at a dose rate of 20 IU/Kg body weight.
- Then
 - Vaccination

Rabies virus/Treatment & Prophylaxis

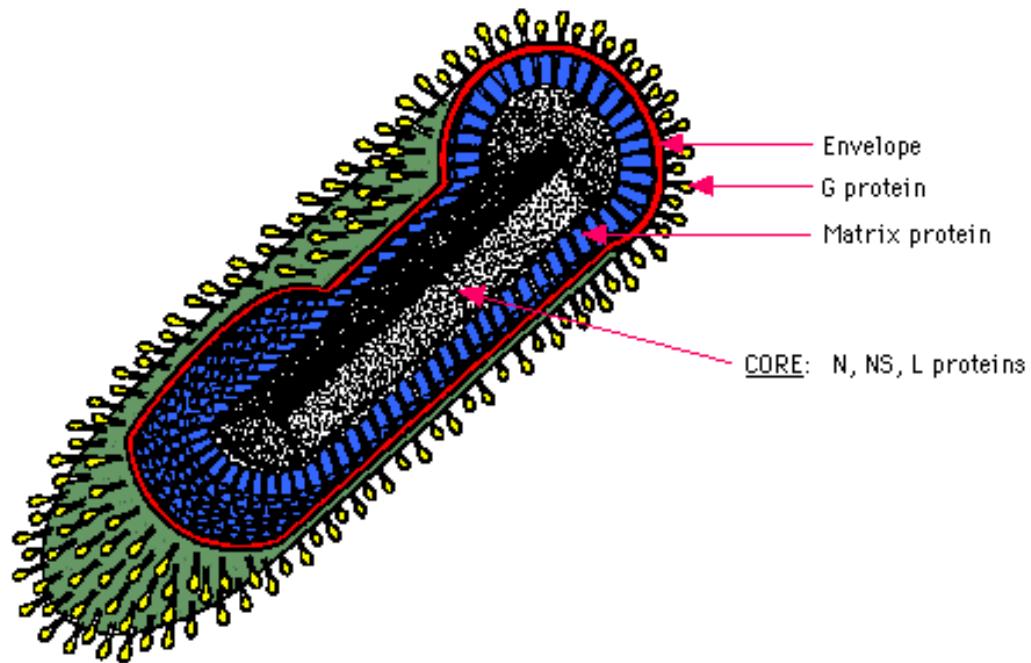
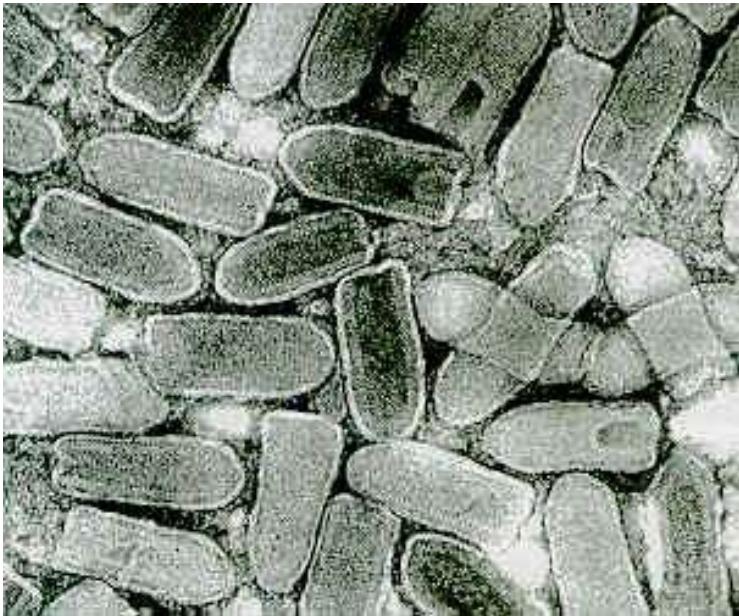
- Vaccine (inactivated virus)
 - Human diploid cell vaccine (HDCV), purified Vero cell rabies vaccine (PVRV), purified chick-embryo cell vaccine (PCECV) and purified duck embryo vaccine (PDEV).
 - **Post exposure On, 0-3-7-14-28 days.** The person should also receive another shot called rabies immune globulin (RIG).
 - In humans at high risk three injections at 0-7 and 21 days are given as **pre-exposure** vaccine.
 - Pets should be vaccinated at 12 to 16 weeks with a booster 9 months later.
 - Then annual vaccination is done.

BOVINE EPHEMERAL FEVER

(Three-day sickness, Bovine Enzootic
Fever, Three-day stiff sickness,
Dragon boat disease)

Definition

- A **non-contagious epizootic arthropod-borne viral disease**
- Bovine ephemeral fever virus (BEFV) an arthropod-borne rhabdovirus which is classified as the **type species** of the **genus *Ephemerovirus***.
- It causes an acute febrile illness of cattle and water buffalo with a sudden episode of fever accompanied by muscle involvement with arthritis, stiffness of the limbs, and lameness, followed by rapid recovery.
- It is of economic importance because it reduces milk production and fertility and causes abortion.



- Bullet-shaped morphology, although virions (~185 nm x ~75 nm) appear to be more tapered at one end
- BEFV genome is much larger and more complex

Transmission

- **Insect bite**
- not spread from cow to cow
- In enzootic areas, ephemeral fever is a seasonal disease that occurs in the summer and autumn, especially in the rainy season.
- Bovine ephemeral fever virus most probably is transmitted by arthropod vectors
- Potential vectors include **culicine and anopheline mosquitoes**, and possibly **Culicoides midges**; both enzootic and epizootic spread is limited by the distribution of appropriate vectors.

Clinical Signs

- Depressed
- High fever (105-107 F) with biphasic or triphasic fever
- Serous ocular and nasal discharge
- Anorexia
- Decreased milk production
- Weight loss
- Stiffness and lameness
- **More severe in high BW animals**

Clinical Signs

- **Severe cases**
 - Muscle stiffness
 - Drag feet when forced to walk
 - Lying down, with hide limbs outstretched- to relieve muscle cramp
 - Lie down for three days
 - cessation of rumination- constipation
 - abortion

Clinical Signs

- Morbidity may reach to 30%
- Usually, recovery is dramatic and complete in 3 days (range 2–5 days), with the exception of a return of milk production.
- Low mortality
- Causes of the death
 - Pneumonia from secondary infection
 - Muscle damaged and inflammation from long period lying down
 - Pregnancy toxemia (fatty liver syndrome)

Pathogenesis

- The pathogenesis of the disease is complex and probably reflects pathophysiologic and immunologic effects mediated by the release and activity of various inflammatory mediators (so-called “**cytokine storm**”).
- Injury to the endothelial lining of small blood vessels is central to the expression of bovine ephemeral fever, but there is no evidence that the virus causes widespread tissue destruction.

- In all cases, there is an early neutrophilia with an **abnormal level of immature neutrophils in the circulation (left shift)**.
- There is **an increase in plasma fibrinogen and a significant decrease in plasma calcium**.
- Therapeutically, there is a dramatic response to anti-inflammatory drugs, and often to calcium infusion.
- **Gross (macroscopic) lesions** include serofibrinous polyserositis and synovitis, pulmonary and lymph node edema, and focal necrosis of selected muscles.

Diagnosis

- Clinical signs
- Sero-conversion: paired serum
 - SN test
 - ELISA
- Gross lesion

Prevention and Control

- Vector control
- Vaccine: Modified-live virus and killed vaccines are available
- Infection results in solid, long-lasting immunity.

Vesicular Stomatitis

Vesicular Stomatitis Virus

- **Vesiculovirus**
 - Major serotypes are Indiana and New Jersey variants
 - **VSV-NJ** and **VSV-I**
- Affects horses, cattle, South American camelids
swine, humans
 - Sheep and goats resistant
- Vesicular stomatitis closely resembles three vesicular diseases exotic to the U.S.: FMD, swine vesicular disease, and vesicular exanthema of swine.

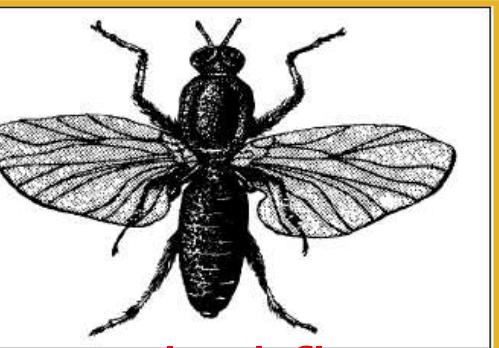
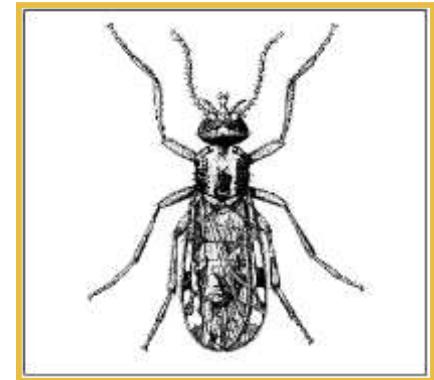
Morbidity/ Mortality

- Morbidity
 - Range: 5 to 90%
 - Most animals seroconvert
- Mortality
 - Higher in adults
 - Death rare in cattle and horses

Animal Transmission

- Vectors
 - Sandflies
 - Blackflies
 - Seasonal outbreaks
- Direct contact
 - Infected animals-saliva, exudate, epithelium of open vesicles
 - Contaminated objects

Sandfly



Blackfly

Human Transmission

- Direct contact
 - Infected tissues, vesicular fluid, saliva
- Insect bites
 - Blackfly, sandfly
- Aerosol
 - Laboratory settings

Clinical Signs

- Incubation period
 - 3 to 5 days
- Fever and vesicles that resemble FMD
- Horses severely affected
 - Oral lesions
 - Drooling, chomping, mouth rubbing, lameness
 - Coronary band lesions



Clinical Signs

- Cattle, pigs
 - Vesicular lesions
 - Oral, mammary gland, coronary band, interdigital region
 - Vesicles usually isolated to one body area
 - Salivation, lameness
- Recover within 2 weeks



Post Mortem Lesions

- Gross lesions
 - Erosive, ulcerative lesions
 - Oral cavity, nostrils, teats, coronary band
- Histopathology
 - Degeneration of epithelial cells



Clinical Diagnosis

- Vesicular diseases are clinically indistinguishable!
- But, symptoms in horses are suggestive
 - Salivation and lameness
- VSV vs. FMD
 - VSV less contagious
 - VSV lesions generally found in one area of the body

Laboratory Diagnosis

- Virus isolation
- Viral antigen detection
 - Vesicular fluid or epithelium
 - ELISA, complement fixation,
virus neutralization
- Antibody tests
 - Paired serum samples
 - ELISA, complement fixation,
virus neutralization