MEDICAL VIROLOGY

OBJECTIVES

1. To familiarize you with the structural components of the virus, which can act as antigens during the infection process.

2. To emphasize the unique nature of viral nucleic acid and its role in the infection process.

3. To familiarize you with the morphological types of virus in order that this information can be used in making a diagnosis.

4. To develop an understanding of the virus replication cycle in order to appreciate how the physician can interrupt this cycle

- ***Learning objectives:***
- ***Definitions in virology***
- ***General properties of viruses and classification basis***
- ***Structure of virus particles***
- ***Cultivation of viruses and preparation of viral antigens***
- ***Replication of viruses***

What are viruses?

- Viruses are filterable agents but are not living organism.

-Viruses are obligate intracellular parasites.

- Viruses cannot produce energy or synthesize protein Independently,

the host cell mechinary is needed

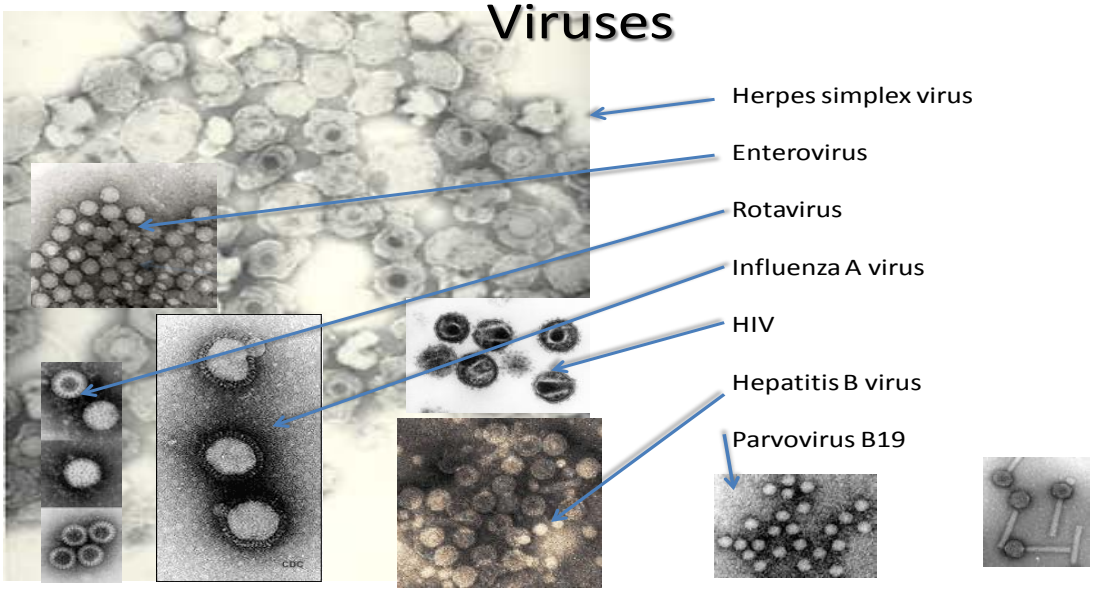
- Viral genome may be RNA or DNA but not both

- Viruses are naked or enveloped

- Viruses do not replicate by division , their components must self assemble .

- Viruses must encode any required process not provided by host cells.

Electron Micrographs of Common Viruses

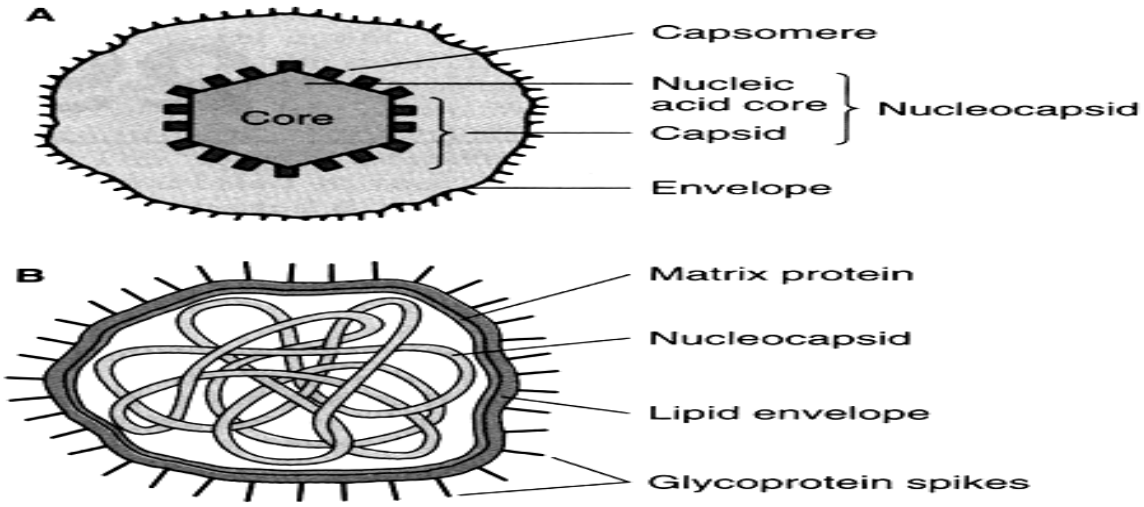


Basis of classification :

1. **Virion morphology : size, shape, type of symmetry, presence or absence of peplomers and membranes**
2. **Physicochemical properties : molecular mass, buoyant density, pH & thermal stability, susceptibility to physical & chemical agents especially ether & detergent**
3. **Virus genome properties : type of nucleic acid (DNA or RNA), size of genome in kb or kbp, strandedness (single or double), linear or circular, sense (positive or negative)**
4. **Virus protein properties (VP,VGP or Lipid containing membrane)**
5. **Genome organization and replication**
6. **Antigenic properties**
7. **Biologic properties : natural host range (animal,plant,bacteria), mode of transmission, vector relationships, pathogenicity, tissue/organ tropisms (for example : enterovirus) and pathology**

General Properties of Viruses

Structure



Virus particle composition:

1. Nucleic acid -contains 3-400 genes

Deoxyribonucleic Acid (DNA) -unique features

- Single and/or double stranded
- Circular or linear
- Bound protein molecules
- Unique purine and/or pyrimidine bases present

Ribonucleic Acid (RNA) - Unique features

- Single or double stranded
- Segmented or unsegmented
- Bound protein molecules
- Unique purine and/or pyrimidine bases present
- Folding pattern

2. Capsid -The capsid accounts for most of the virion mass. It is the protein coat of the virus. It is a complex and highly organized entity which gives form to the virus. Subunits called protomeres aggregate to form capsomeres which in turn aggregate to form the capsid. .

3. Envelope -this is an amorphous structure composed of lipid, protein and carbohydrate which lies to the outside of the capsid. It contains a mosaic of antigens from the host and the virus. A naked virus is one without an envelope.

4. Spikes. These are glycoprotein projections which have enzymatic and/or adsorption and/or hemagglutinating activity. They arise from the envelope and are highly antigenic

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Morphology (Symmetry)

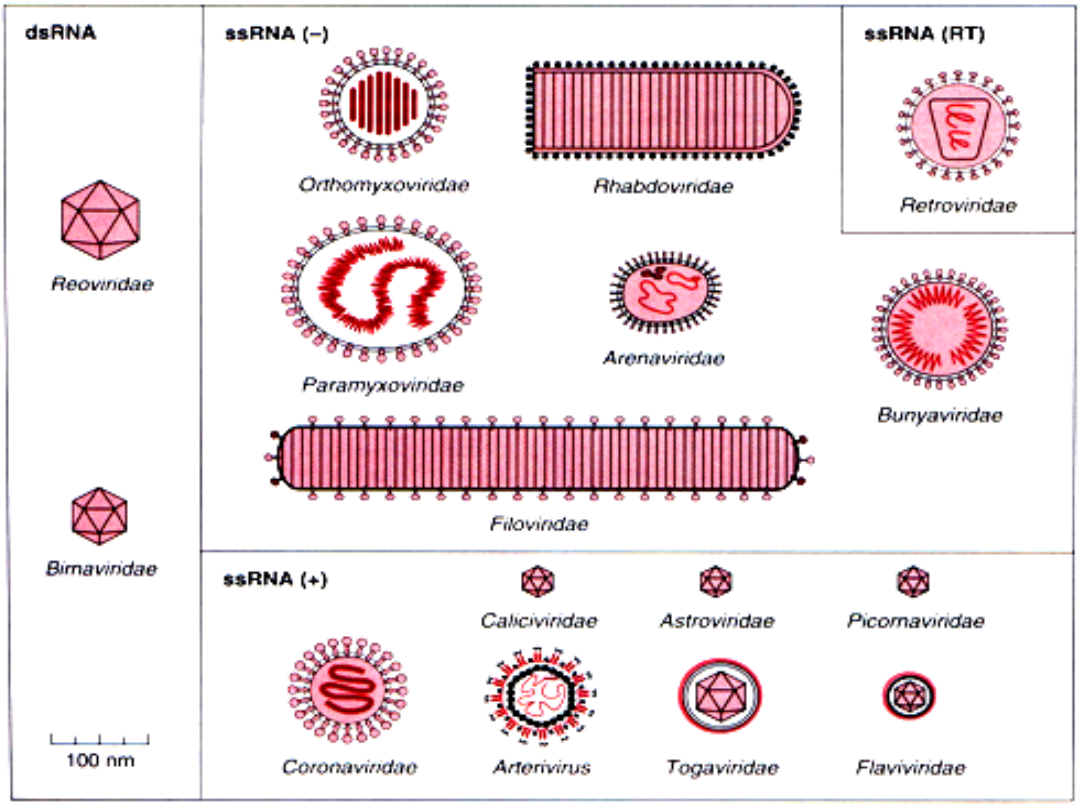
1. Icosahedral -The protomeres aggregate in groups of five or six to form the capsomere. In electron micrographs, capsomeres are recognized as regularly spaced rings with a central hole. The shape and dimensions of the icosahedron depends on characteristics of its protomeres. All icosahedral capsids have 12 corners each occupied by a penton capsomere and 20 triangular faces, each containing the same number of hexon capsomeres. Icosahedral symmetry is identical to cubic symmetry

2. Helical -The protomeres are not grouped in capsomeres, but are bound to each other so as to form a ribbon-like structure. This structure folds into a helix because the protomeres are thicker at one end than at the other. The diameter of the helical capsid is determined by characteristics of its protomeres, while its length is determined by the length of the nucleic acid it encloses

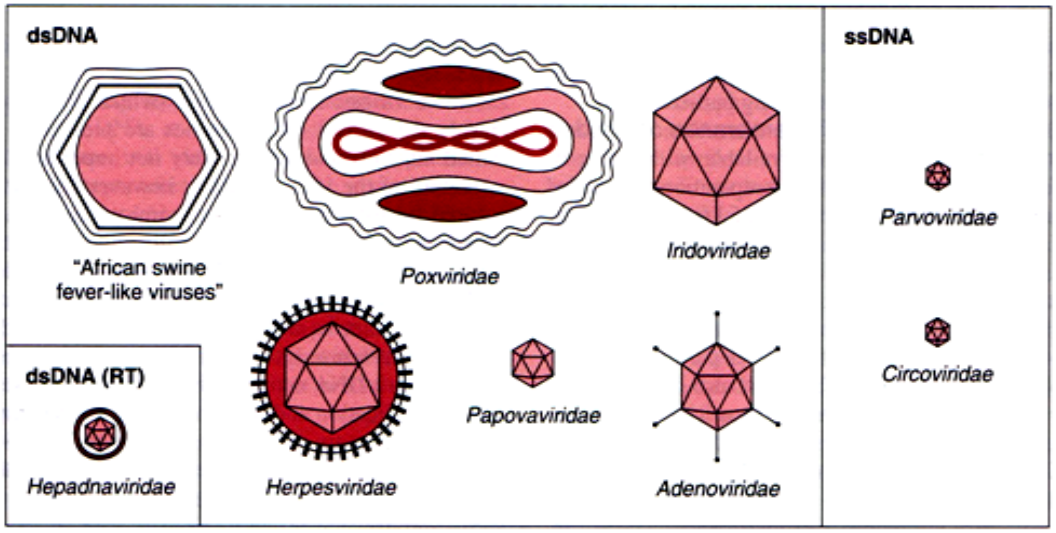
3. Complex -e.g., that exhibited by poxvirus and rhabdovirus. This group comprises all those viruses which do not fit into either of the above two groups

Members and morphologies of viruses

RNA viruses



DNA viruses



Cultivation of viruses

Cell Culture Lines Used in Diagnostic Virology:

- **Primary cell lines (1-2 passes)**
  - Kidney tissues from monkeys, rabbits, etc.
  - Embryos from chickens, guinea pigs, etc.
  - PrRMK (primary rhesus monkey kidney), RK (rabbit kidney)
- **Diploid (limited passage) cell lines (20-50 passes)**
  - Human embryonic lung or human newborn foreskin cells
  - 20-50 passes
- **Heteroploid cell lines (infinite passes)**
  - Human epidermoid carcinoma of larynx (Hep-2), cervix (HeLa), lung (A549)

Virus cultivation assays

1. ANIMAL : type, age, sex, method of inoculation depend on type of viruses

⌦ Herpes simplex virus : rabbit cornea vesicles
⌦ Rabies virus : white mouse (baby/adult) intracerebral

1 – 3 weeks encephalitis/ rabies

⌦ Dengue virus : baby white mouse, 1 – 3 days intracerebral/ subcutaneously': 3 – 7 days tremor, paralysis

⌦ Polio virus : monkey intracutaneously, IM, intraneural, intraspinal paralysis

2.**EMBRYONATED EGG** : inoculation methods depend on the type of viruses

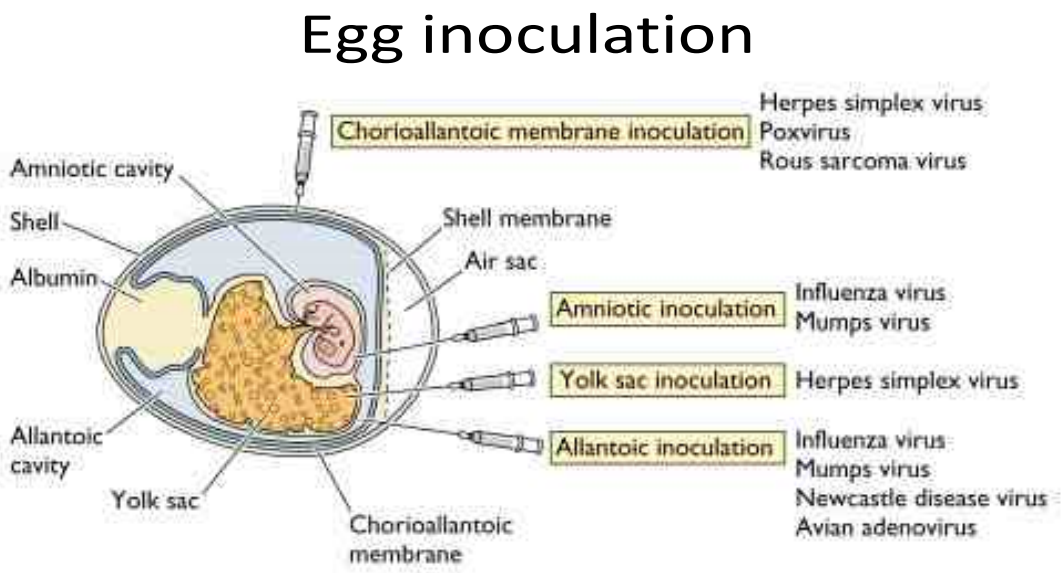
⌦ Chorioallantoic membrane (CAM) : 10 – 12 days old embryo

Pox virus, herpes simplex virus= pocks/plaque

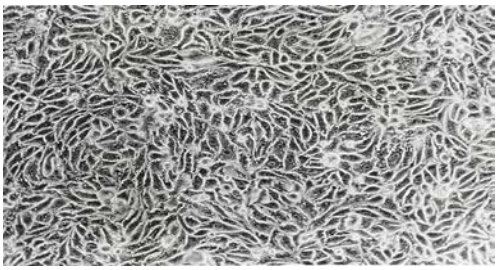
⌦ Amniotic cavity : 10 - 11 days old embryo influenza virus, mumps virus

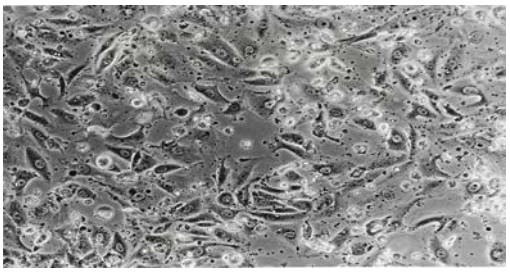
⌦ Allantoic cavity : 10 days embryo can be propagated in much larger quantities, used for vaccine production

⌦ Yolk sac : 3 – 8 days
⌦ Intraembryonal : 8 – 10 days embryo Japanese B encephalitis virus



Virus Detection in Cell Culture by Cytopathogenic Effects





From a public health and risk assessment standpoint, microbial assays based on infectivity are the most relevant and easily interpretable ones

A. Normal cell culture

B. Infected cell culture

Replication Cycle

1. Adsorption -Viruses can enter cells via phagocytosis, viropexis or adsorption. Adsorption is the most common process and the most highly specific process. It requires the interaction of a unique protein on the surface of the virus with a highly specific receptor site on the surface of the cell(cell tropism).

2. Penetration -This occurs by one or more processes.

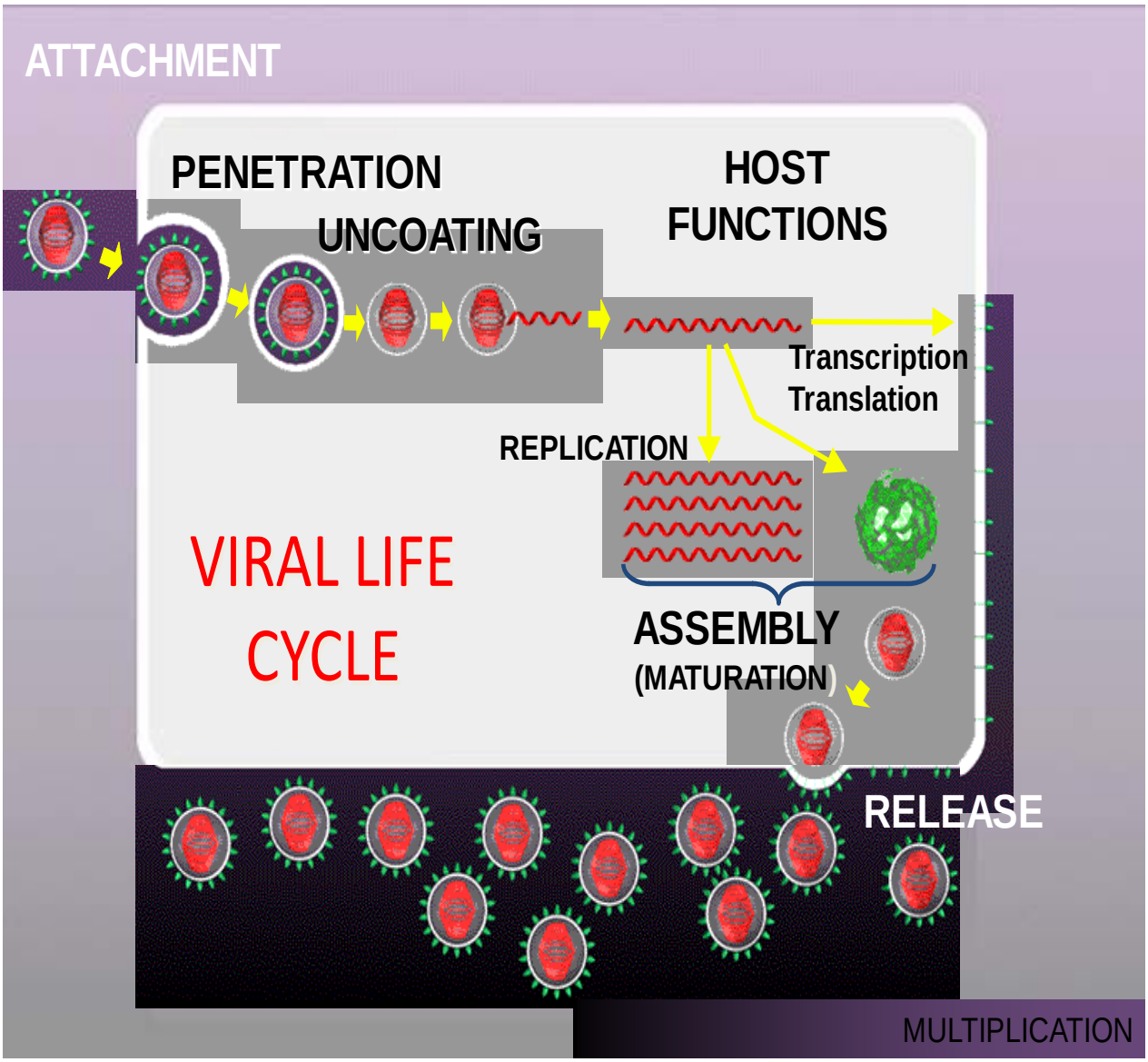
Enveloped viruses fuse their envelope with the membrane of the host cell. This involves local digestion of the viral and cellular membranes, fusion of the membranes and concomitant release of the nucleocapsid into the cytoplasm. Naked viruses bind to receptor sites on the cellular membrane, digest the membrane and enter into the cytoplasm intact.

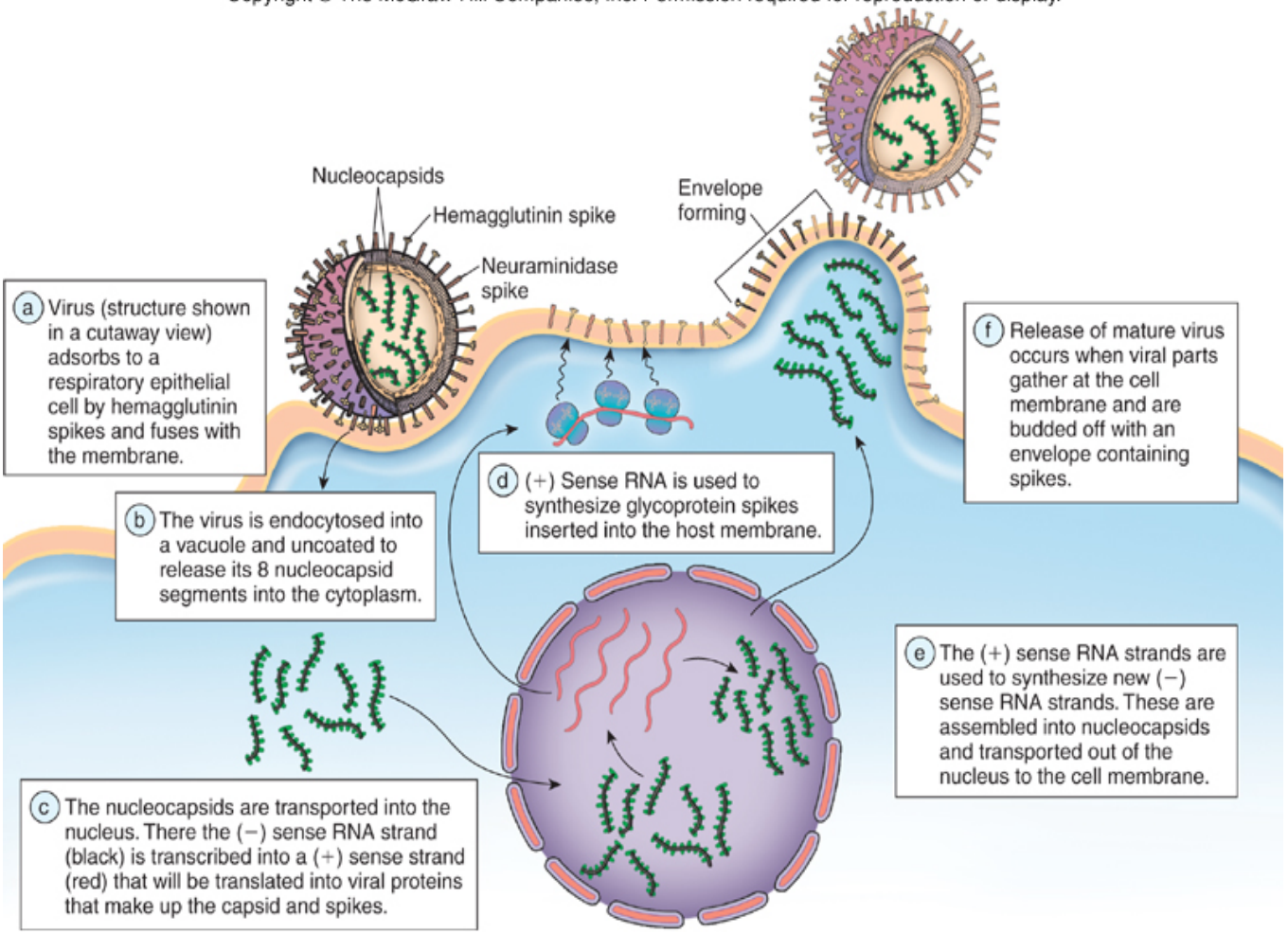
Both naked and enveloped viruses can be ingested by phagocytic cells. However, in this process they enter the cytoplasm enclosed in a cytoplasmic membrane derived from the phagocytic cell.

3. Uncoating -During this stage cellular proteolytic enzymes digest the capsid away from the nucleic acid. This always occurs in the cytoplasm of the host cell. The period of the replication cycle between the end of the uncoating stage and maturation of new viral particles is termed the eclipse. Thus during the eclipse stage, no complete viral particles can be viewed within the cell .

4. Replication of viral nucleic acid is a complex and variable process. The specific process depends on the nucleic acid type :.

DNA virus replication -<u>with the exception of the poxviruses, all DNA viruses replicate in the nucleus</u>. In some cases one of the DNA strands is transcribed (in others both strands of a small part of the DNA may be transcribed) into specific mRNA, which in turn is translated to synthesize virus-specific proteins such as tumor antigen and enzymes necessary for biosynthesis of virus DNA. This period encompasses the early virus functions. Host cell DNA synthesis is temporarily elevated and is then suppressed as the cell shifts over to the manufacture of viral DNA . As the viral DNA continues to be transcribed, late virus functions become apparent. Messenger RNA transcribed during the later phase of infection migrates to the cytoplasm and is translated . Proteins for virus capsids are synthesized and are transported to the nucleus to be incorporated into the complete virion . Assembly of the protein subunits around the viral DNA results in the formation of complete virions , which are released after cell lysis. The single-stranded DNA viruses first form a double stranded DNA, utilizing a host DNA-dependent DNA polymerase. They then undergo a typical replication cycle


ATTACHMENT
PENETRATION
UNCOATING
HOST
FUNCTIONS
Transcription
Translation
REPLICATION
VIRAL LIFE
CYCLE
ASSEMBLY
(MATURATION)
RELEASE
MULTIPLICATION



RNA virus replication -<u>with the exception of the orthomyxoviruses and retroviruses, all RNA viruses replicate in the cytoplasm of the host cell</u>. The exact process varies with the species of virus. The single-stranded RNA that is released after uncoating will act as either: (a) the mRNA to synthesize viral-coded proteins; or (b) a template to synthesize mRNA; or (c) a template to synthesize double stranded RNA, which is then used as a template to synthesize mRNA; or (d) a template to synthesize double-stranded DNA, which is then utilized as a template to synthesize mRNA. This latter process occurs only with the retroviruses (oncornaviruses).

The replication of poliovirus, which contains a single-stranded RNA as its genome, provides a useful example. All of the steps are independent of host DNA and occur in the cell cytoplasm. Polioviruses absorb to cells at specific cell receptor sites (step 1 in the fig.), losing in the process one

virus polypeptide. The sites are specific for virus coat-cell interactions. After attachment, the virus particles are taken into the cell by viropexis (similar to pinocytosis) (step 2), and the viral RNA is uncoated (step 3). The single-stranded RNA then serves as its own messenger RNA. This messenger RNA is translated (step 4), resulting in the formation of an RNA-dependent RNA polymerase that catalyzes the production of a replication intermediate (RI), a partially double-stranded molecule consisting of a complete RNA strand and numerous partially completed strands (step 5). At the same time, inhibitors of cellular RNA and protein synthesis are produced. Synthesis of (+) and (-) strands of RNA occurs by similar mechanisms. The RI consists of one complete (-) strand and many small pieces of newly synthesized (+) strand RNA (step 6). The replicative form (RF) consists of two complete RNA strands, one (+) and one (-).

The single (+) strand RNA is made in large amounts and may perform any one of three functions: (a) serve as messenger RNA for synthesis of structural proteins; b) serve as template for continued RNA replication; or (c) become encapsulated, resulting in mature progeny virions. The synthesis of viral capsid proteins (step 7) is initiated at about the same time as RNA synthesis.

The entire poliovirus genome acts as its own mRNA, forming a polysome of approximately 350S, and is translated to form a single large polypeptide that is subsequently cleaved to produce the various viral capsid polypeptides. Thus, the poliovirus genome serves as a polycistronic messenger molecule. Poliovirus contains four polypeptides.

5. Maturation and Release

- **Naked viruses -Maturation consists of two main processes: the assembly of the capsid, and its association with the nucleic acid. Maturation occurs at the site of nucleic acid replication. After they are assembled into mature viruses, naked virions may become concentrated in large numbers at the site of maturation, forming inclusion bodies. Naked virions are released in different ways, which depend on the virus and the cell type. Generally, RNA-containing naked viruses are released rapidly after maturation and there is little intracellular accumulation; therefore, these viruses do not form predominant inclusion bodies. On the other hand, DNA-containing naked icosahedral viruses that mature in the nucleus do not reach the cell surface as rapidly, and are released when the cells undergo autolysis or in some cases are extruded without lysis. In either case they tend to accumulate within the infected cells over a long period of time. Thus, they generally produce highly visible inclusion bodies.**

- **Enveloped viruses -In the maturation of enveloped viruses, a capsid must first be assembled around the nucleic acid to form the nucleocapsid, which**

is then surrounded by the envelope. During the assembly of the nucleocapsid, virus-coded envelope proteins are also synthesized. These migrate to the plasma membrane (if assembly occurs in the cytoplasm) or to the nuclear membrane (if assembly occurs in the nucleus) and become incorporated into that membrane. Envelopes are formed around the nucleocapsids by budding of cellular membranes. NOTE: Enveloped viruses will have an antigenic mosaicism characteristic of the virus and the host cell. Viruses are slowly and continuously released by the budding process with the results that:

(a) the cell is not lysed; and (b) little intracellular accumulation of virus occurs; and (c) inclusion bodies are not as evident as with naked viruses.

- **Complex viruses -These viruses, of which the poxvirus is a good example, begin the maturation process by forming multilayered membranes around the DNA. These layers differentiate into two membranes: The inner one contains the characteristic nucleoid, while the external one acquires the characteristic pattern of the surface of the virion. These form very characteristic cytoplasmic inclusion bodies. The viruses are generally released from the cell via cell lysis**

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Summary

1. Viruses contain either DNA or RNA as their genetic material, but not both. This nucleic acid usually has unique chemical and/or physical features which makes it distinguishable from human nucleic acid.
2. Viral nucleic acid is enclosed in a capsid made up of protein subunits called protomeres.

3. Some species of viruses have a membrane, the envelope, surrounding the capsid; other species do not have an envelope, i.e., they are naked . Enveloped viruses have glyco-protein spikes arising from their envelope. These spikes have enzymatic, absorptive, hemagglutinating and/or antigenic activity.

4. The morphology of a virus is determined by the arrangement of the protomeres. When protomeres aggregate into units of five or six (capsomeres) and then condense to form a geometric figure having 20 equal triangular faces and 12 apices, the virus is said to have icosahedral (cubic) morphology. When protomeres aggregate to form a capped tube, they are said to have helical

morphology. Any other arrangement of the protomeres results in a complex morphology.

5. All viruses undergo a replication cycle in their human host cell consisting of adsorption, penetration, uncoating, nucleic acid replication, maturation and release stages.

6. During the viral replication cycle, an accumulation of mature viruses, incomplete viruses and viral parts occurs within the cell. This accumulation is the inclusion body. The size, shape, location and chemical properties of the inclusion body are used by the pathologist to diagnose viral infectious disease.

7. A virally-infected cell generally presents three signals that it is infected. The first is the production of double-stranded RNA, which induces interferon; the second is the expression of viral protein on the surface of the plasma membrane, thus causing activation of cytotoxic T-cells, natural killer cells and sometimes induction of antibody synthesis. The third is the formation of an inclusion body either within the cytoplasm or the nucleus or very rarely within both the cytoplasm and nucleus.

8. In general, all DNA-containing viruses replicate in the host cell nucleus. The exceptions to the rule are the poxviruses.

9. In general, all RNA-containing viruses replicate in the host cell cytoplasm. The exceptions to the rule are the retroviruses and the orthomyxoviruses

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