LECT.3 REPLICATION OF ANIMAL VIRUSES

By Dr. Agharid Ali Hussein

,Department Microbiology, Veterinary College of Veterinary Medicine, Tikrit University

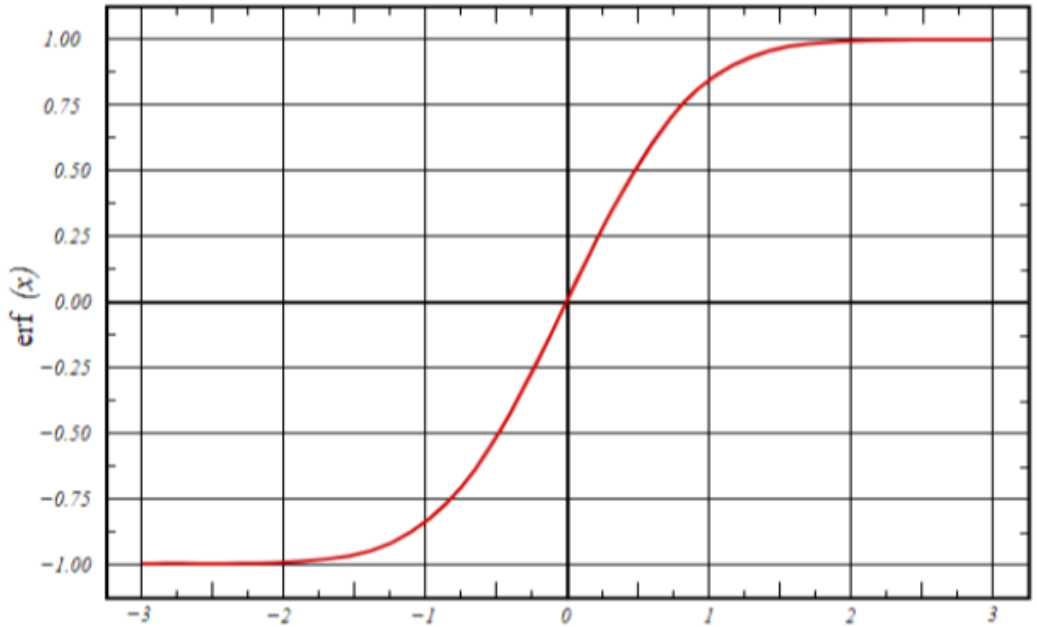
A definition of how viruses are unique:

1. **Virus particles are produced from the assembly of preformed components, while other biological agents grow from an increase in the integrated sum of their components and reproduce by division.**
2. **Virus particles (virions) do not grow or undergo division.**
3. **Viruses lack the genetic information that encodes the tools needed to generate metabolic energy or to synthesize protein (ribosomes).**

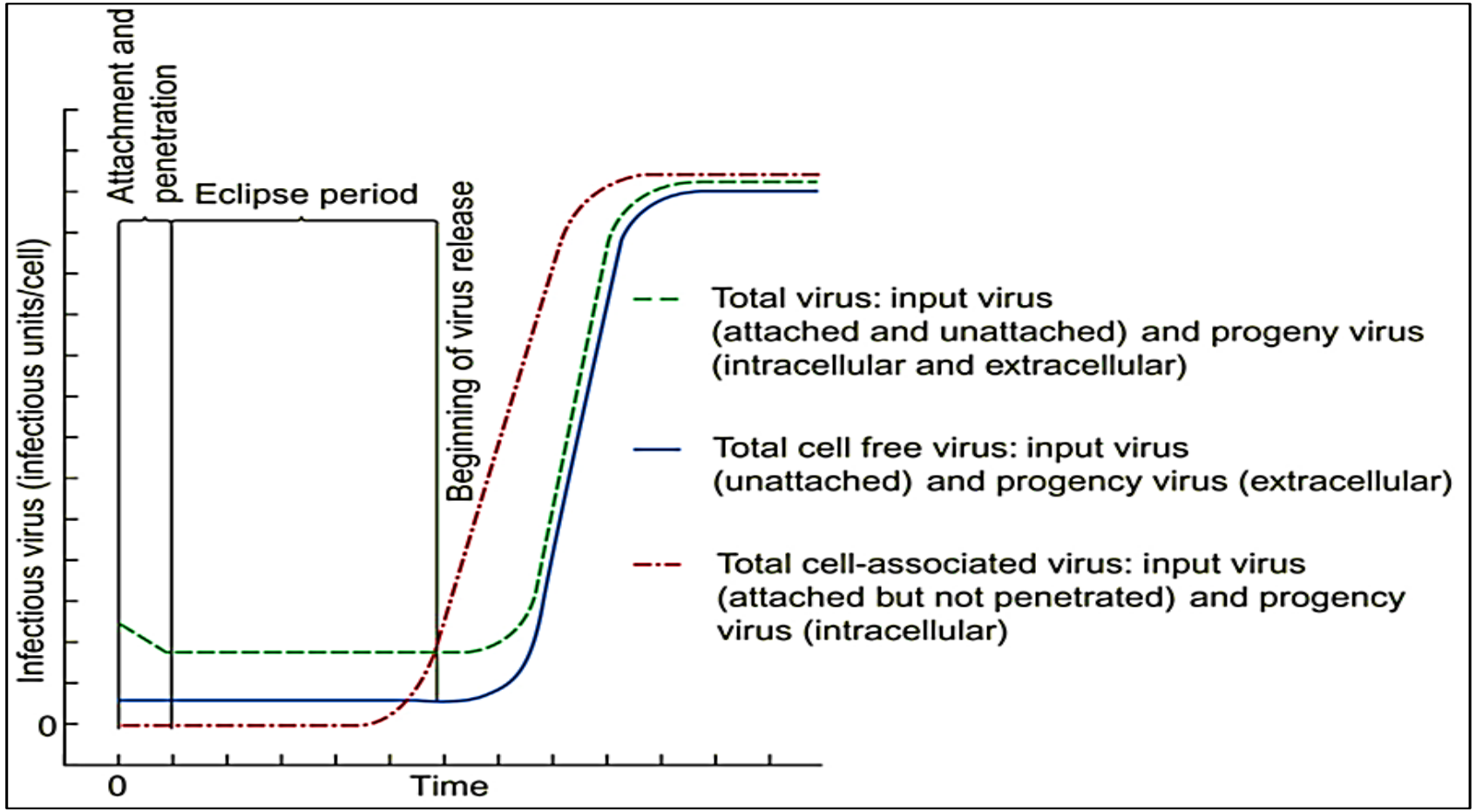
Viral replication

Unlike the growth curve for a bacteria, the growth curve for a viruses do not follow a sigmoidal curve(S shape).

Viral replication or multiplication is often presented as including the following steps: Attachment, entry and uncoating, biosynthesis, maturation, and release. Unlike eukaryotic and prokaryotic cells, which increase their numbers through the processes of mitosis and binary fission.



S-shaped growth curve(sigmoid growth curve). A pattern of growth in which, in a new environment, the population density of an organism increases slowly initially, in a positive acceleration phase; thenincreases rapidly, approaching an exponential growth rate as in the J-shaped curve; but then declinesin a negative acceleration phase until at zero growth rate the population stabilizes



The one-step multiplication curve for a bacteriophage population follows three steps: 1)inoculation, during which the virions attach to host cells; 2) eclipse, during which entry of the viral genome occurs; and 3) burst, when sufficient numbers of new virions are produced and emerge from the host cell. The burst size is the maximum number of virions produced per bacterium

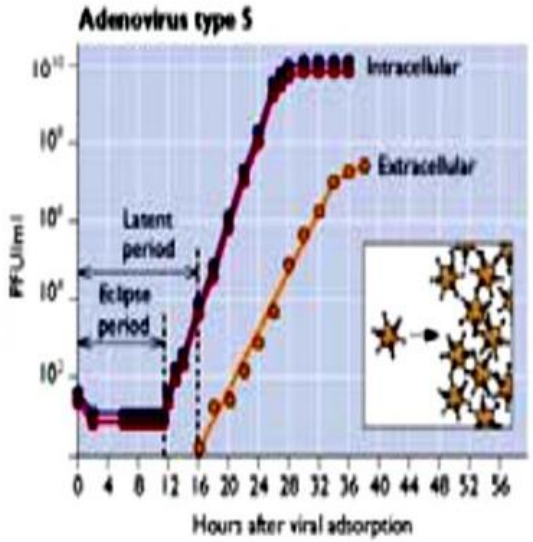
One-step growth curve :

The replication of viruses was early conducted by using bacteriophage and can preformed with any virus that can be replicate in cell culture
Plaque assay is a technique to enumerate virus particles based on their ability to kill cultured cells and therefore produce holes, or plaques in the cell layer that became a keystone for defining the properties of viruses

(1) add a chloroform-resistant phage to a culture of bacteria for several minutes; (2) rinse the bacteria to remove nonattached phage; (3) incubate the culture and remove samples at various periods of time; (4) treat sampled bacterial cultures with chloroform to stop growth; (5) quantify the amount of phage at each of the time periods.

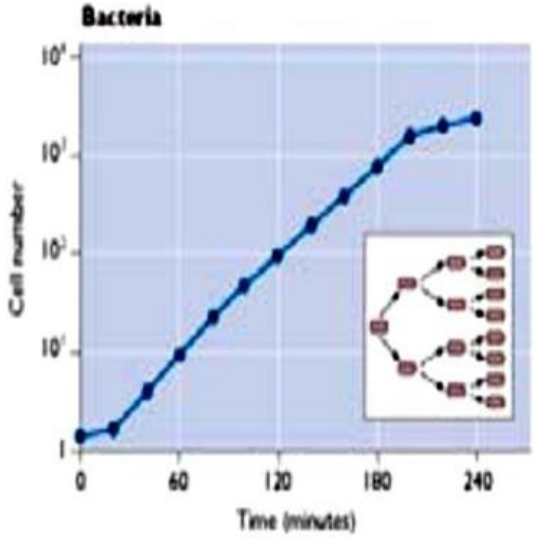
Plaque counting is considered the golden standard for phage enumeration. The double agar overlay assay (DLA) allows localized phage-host contact in a confined environment (Petri dish) containing two layers of agar on top of each other.

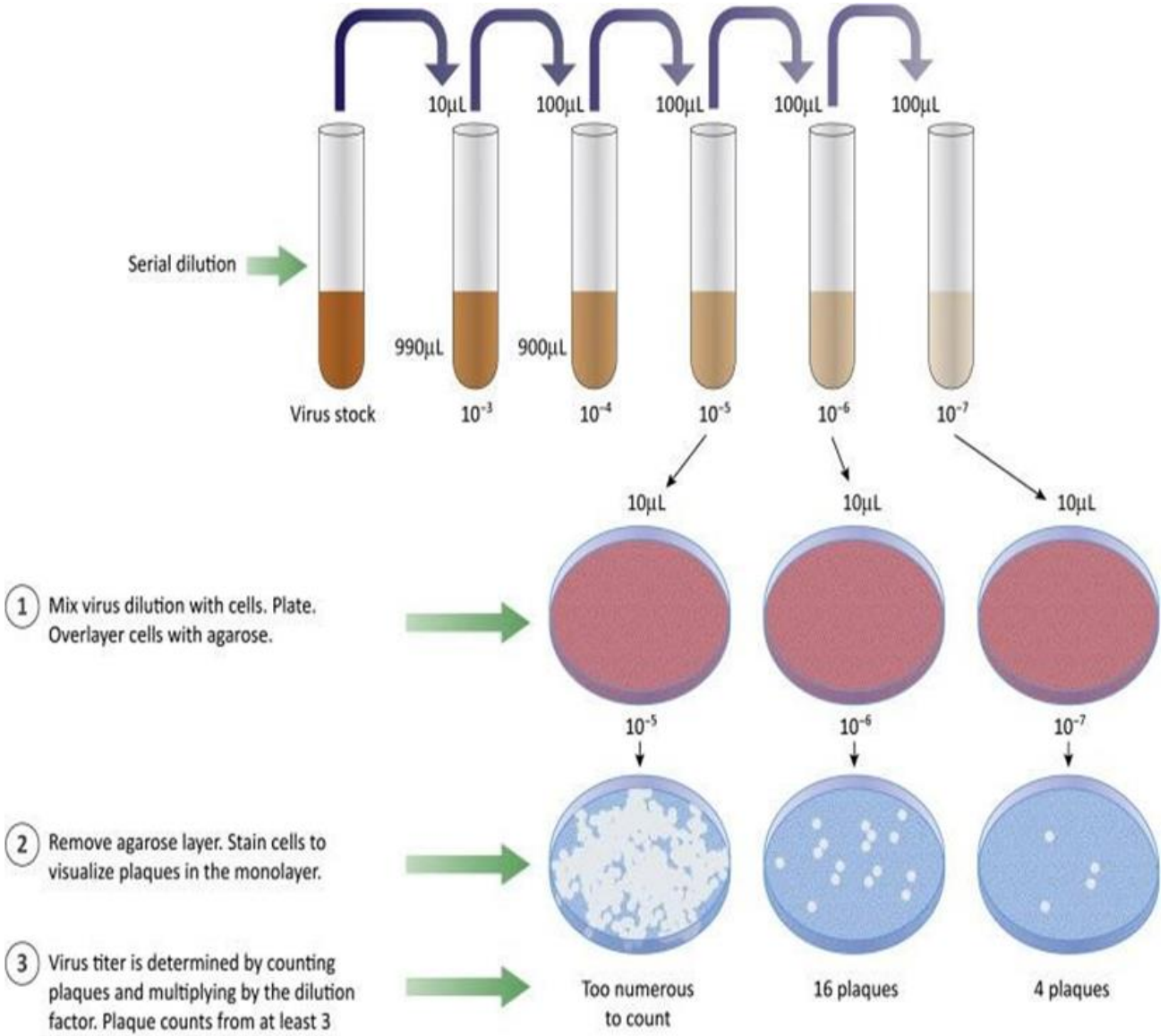
Viruses replicate by assembly of pre-formed components into many particles



First make the parts, then assemble the final product.

Not binary fission like cells





The remarkable findings compared to a bacterial growth

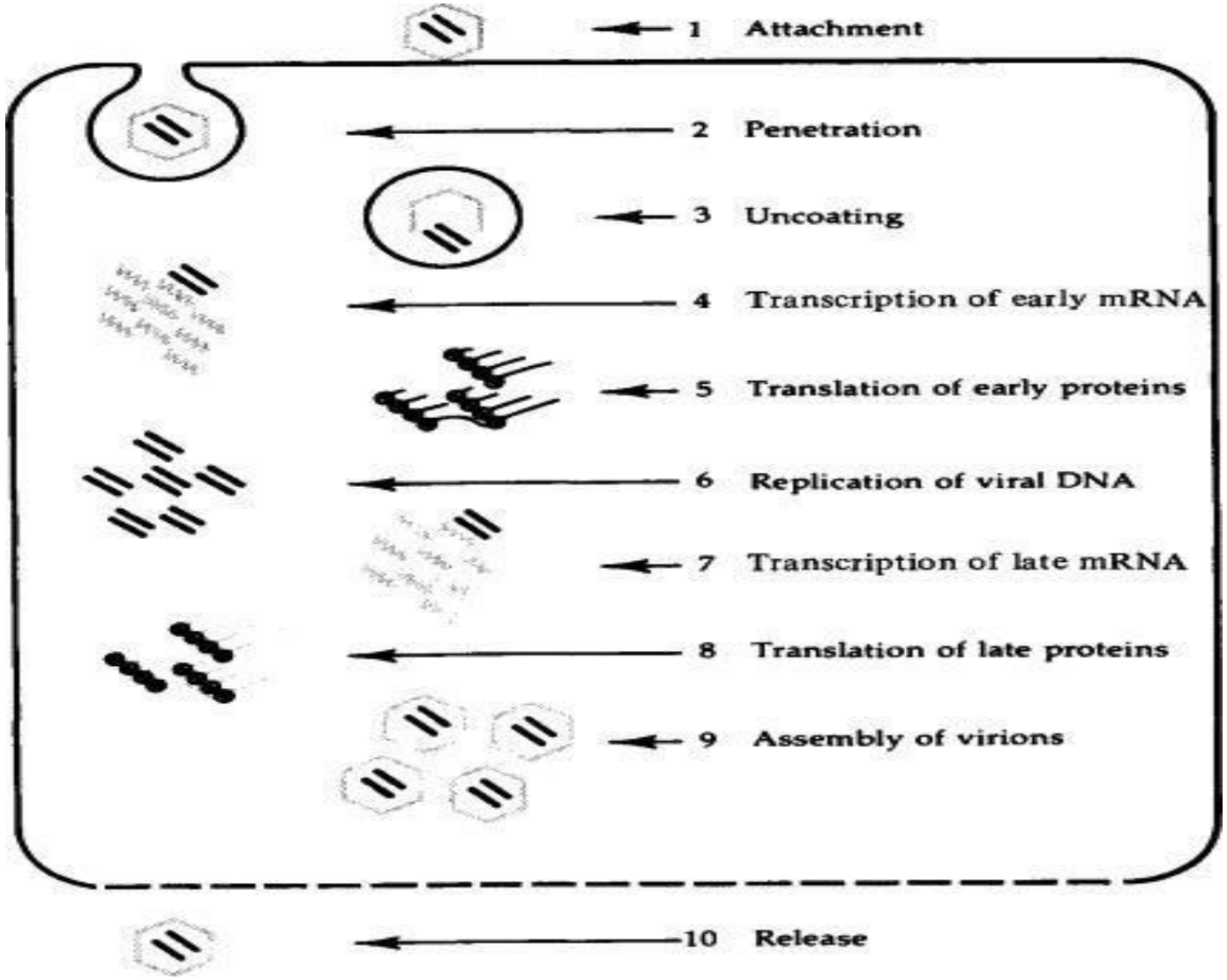
- **Viruses bind and penetrate the cells with no virions detected in the medium.**

The viruses were disappeared from the infected cultures for a variable period of time, depending on the virus-host-cell system (Virus–host interactions). This period called eclipse phase.

- Next appears in the viral growth curve occurs when virions are released from the lysed host cell at the same time. **Such an occurrence is called a burst**, and the number of virions per bacterium released is described **as the burst size.**

- The exponential increase in production of infectious virus particles until the host cell is unable to maintain its metabolic integrity

Viral Replication Cycle



I. Attachment

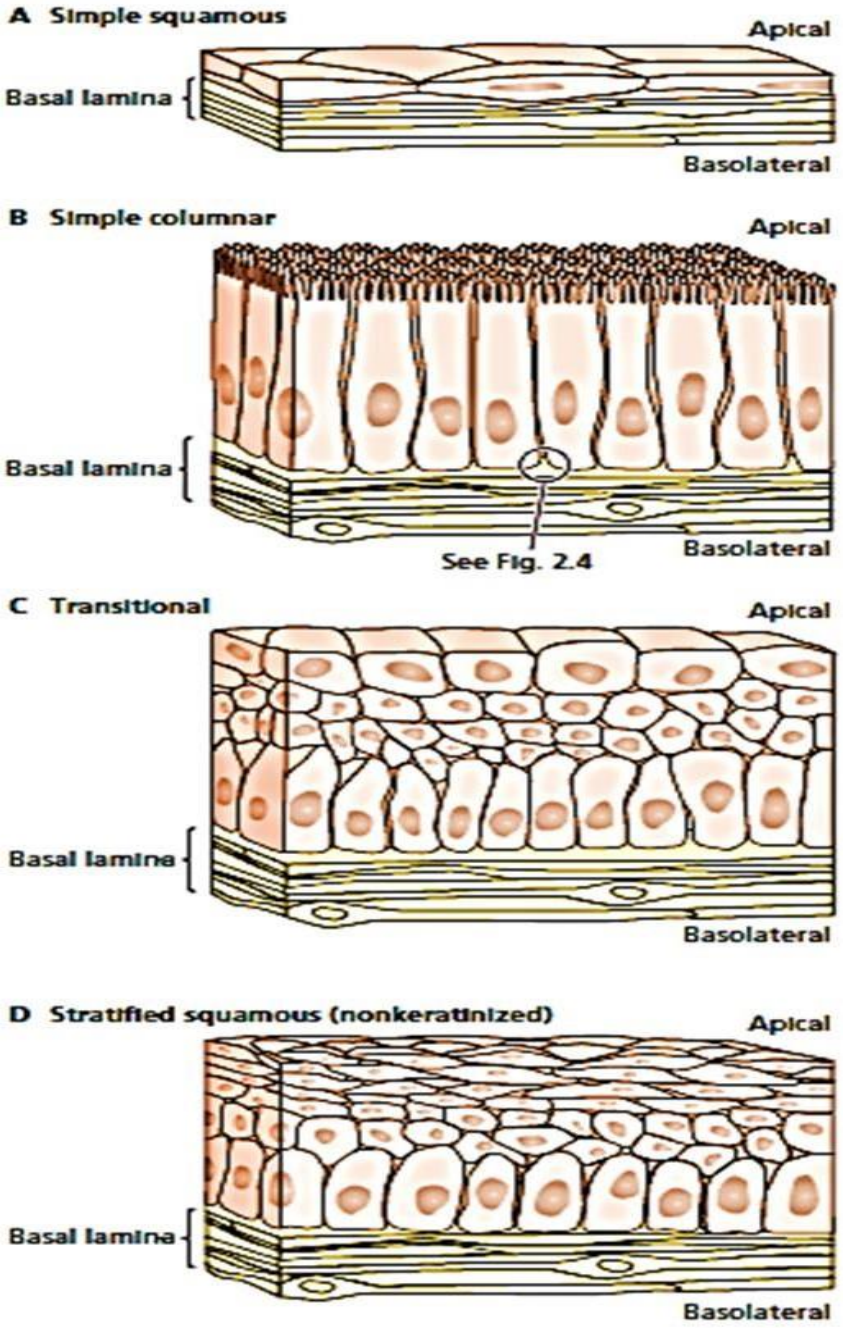
- **Viruses are obligate intracellular pathogens that rely on host cell machinery in order to carry out an infectious life cycle and ultimately spread to new host cells.**

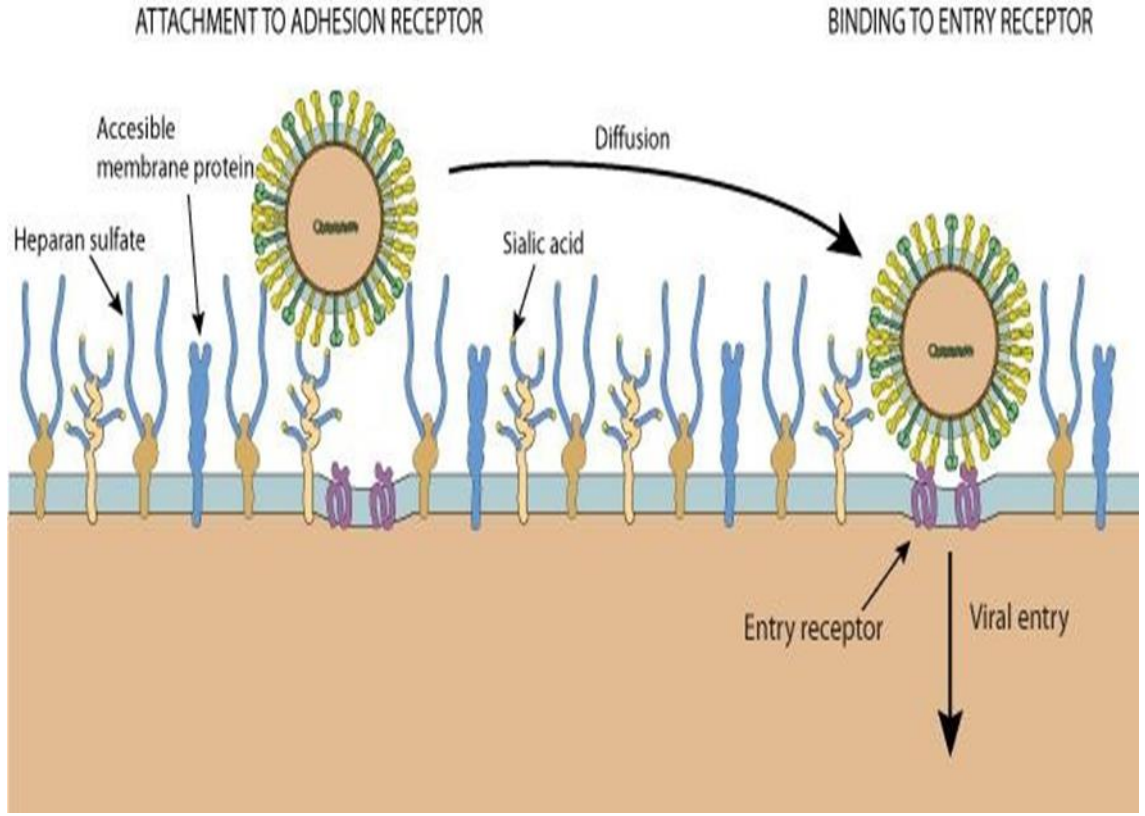
- **The critical first step in the virus replication cycle is the attachment of the virus particle to a host cell.**
- **Attachment requires specific interactions between components of the virus particle (eg, capsid proteins or envelope glycoproteins) and components of the host cell (eg, a glycoprotein or carbohydrate moiety).**

- **Virus particles interact with cell-surface molecules which are referred to as receptors, coreceptors, attachment factors, or entry factors depending on the role(s) that they play in the attachment and entry processes. Its required for the final stages of theentry/ uncoating process.**
- **interactions is a critical initial step in the infectious viral life cycle and plays a key regulatory role in host range, tissue tropism, and viral pathogenesis.**

- **interactions involve short-distance electrostatic interactions with charged molecules and <u>important for understanding the molecular details of specific virus replication cycles</u>, and <u>estimate the design of antiviral drugs.</u>**

- **For example, binding of influenza A virus to a host cell requires only an interaction between the viral hemagglutinin (HA) glycoprotein and a sialic acid residue on the cellsurface.**


A Simple squamous
Apical
Basal lamina
Basolateral
B Simple columnar
Apical
Basal lamina
See Fig. 2.4
Basolateral
C Transitional
Apical
Basal lamina
Basolateral
D Stratified squamous (nonkeratinized)
Apical
Basal lamina
Basolateral


ATTACHMENT TO ADHESION RECEPTOR
BINDING TO ENTRY RECEPTOR
Accesible membrane protein
Diffusion
Heparan sulfate
Sialic acid
Entry receptor
Viral entry

II. Penetration and Uncoating

Viruses enter host cells via several mechanisms, including endocytosis, macropinocytosis, and phagocytosis.
Uptake (Penetration) Following attachment, virions(infectious particles) can enter cells
There are several factors determine which entry mechanism will be active, including **the cell type and the cellular receptors it display**s. Viruses enter host cells via one of three major pathways:

A. Fusion: Viral proteins promote the fusion of the virion with the plasma membrane, which then form a pore, and the virion becomes uncoated. Its genomic cargo or elements is then transferred into the cytoplasm. The proteins involved in fusion, so-called fusogens, can be divided into three classes: (i) class I fusogens, (ii) class II fusogens,and (iii) class III fusogens.

B. Cell-cell fusion: Some viruses such as vaccinia virus (VV) and herpes simplex virus (HSV) induce the expression of proteins on the surfaces of infected cells that attract uninfected cells and cause them to fuse with the infected cell at low pH values to form a multinuclear cell known as a syncytium. Syncytium formation represents a very efficient way for a virus to spread within a host: it circumvents the immune response and creates a good site of replication for a nuclear-replicating virus. fusogenic oncolytic viruses
C. Endocytosis: Once the cell internalizes the virus, it is then delivered to an acidic pit, a so-called early endosome. The virus then may be transferred into a late endosome and then to a lysosome. Alternatively, due to the low pH value in the lumen of endosomes, the viral membrane can fuse with the endosomal membrane, releasing the viral genome into the cytoplasm . Once in cytoplasm, some viruses move toward the nucleus to deliver their cargo inside the nucleus,.

There are several major endocytosis-based pathways that viruses can use to enter cells and evade the host's immune system. The most important viral entry pathways are as follows:

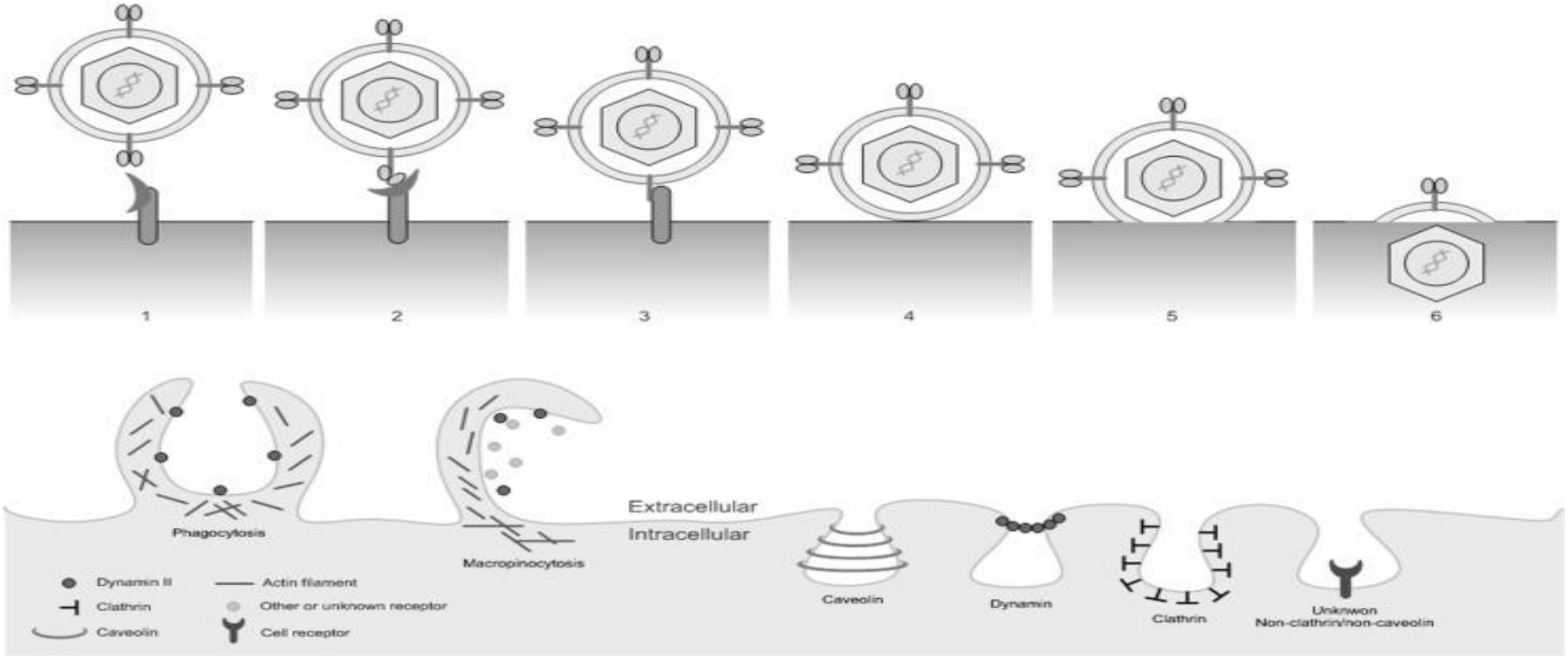
1) **Phagocytosis** (cell eating), which occurs in specialized mammalian cells (so-called professional phagocytes, e.g., dendritic cells and macrophages) that engulf large and essential particles. Viral entry by this pathway typically involves the formation of large extracellular projections, and the internalized virus is taken into a phagosome.

2) **Pinocytosis** (cell drinking), which is the process by which cells take up solutes and fluids. When it is exploited by viruses, interactions between viral proteins and cell receptors activate intracellular signaling and actin rearrangements that form ruffles or filopodia on the external surface of the host cell. The ruffles then close up to form a vesicle known as a macropinosome, which carries the virus into the cytosol.

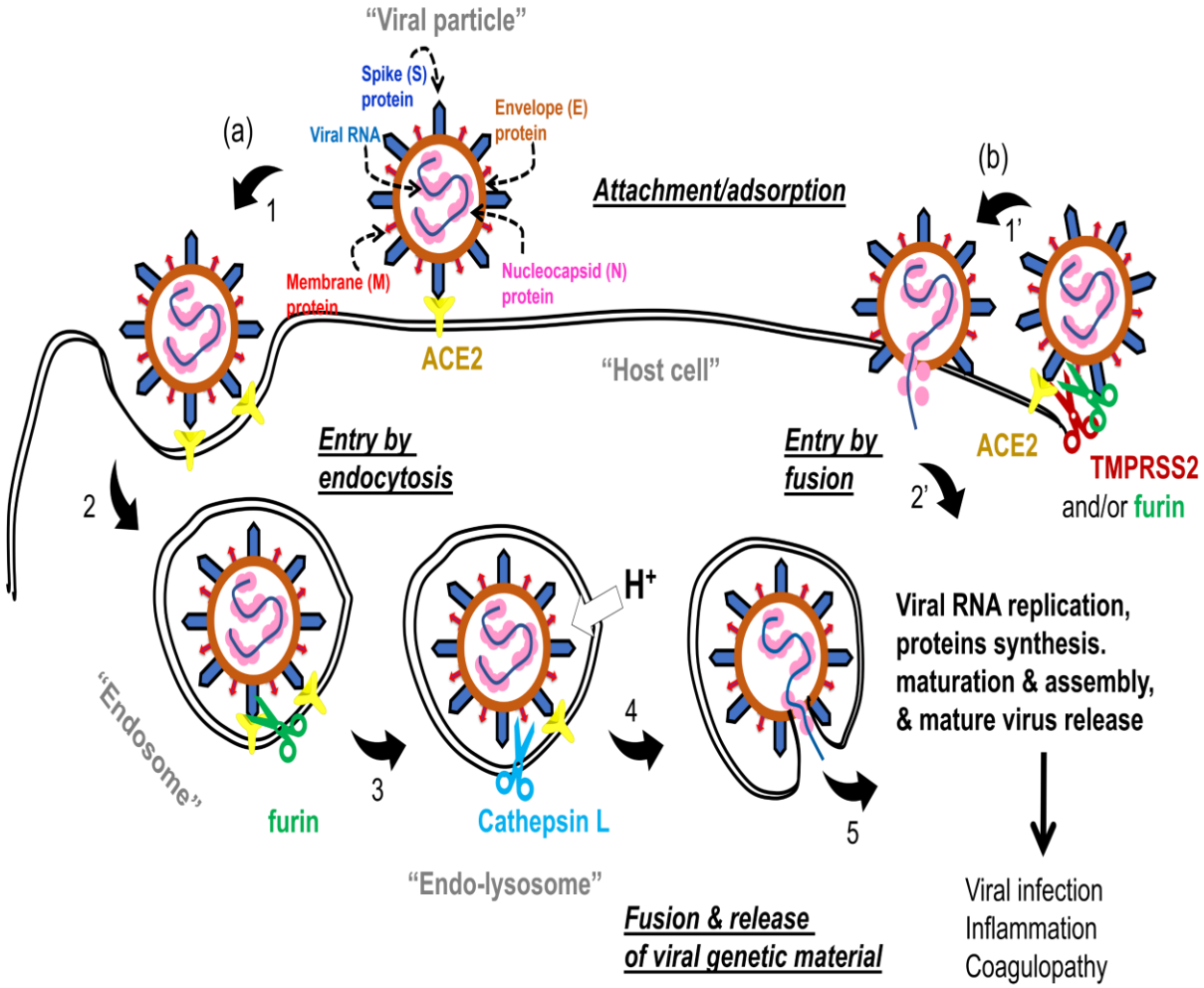
3) **Clathrin-mediated endocytosis**, which is the process by which the cell internalizes the virus in a clathrin-rich flask-shaped invagination/cavity (vesicle) known as a clathrin-coated pit. The virus is then delivered into the cytoplasm via endosomes. Clathrin and cholesterol are required, and dynamin and transferrin are usually involved in pit formation.

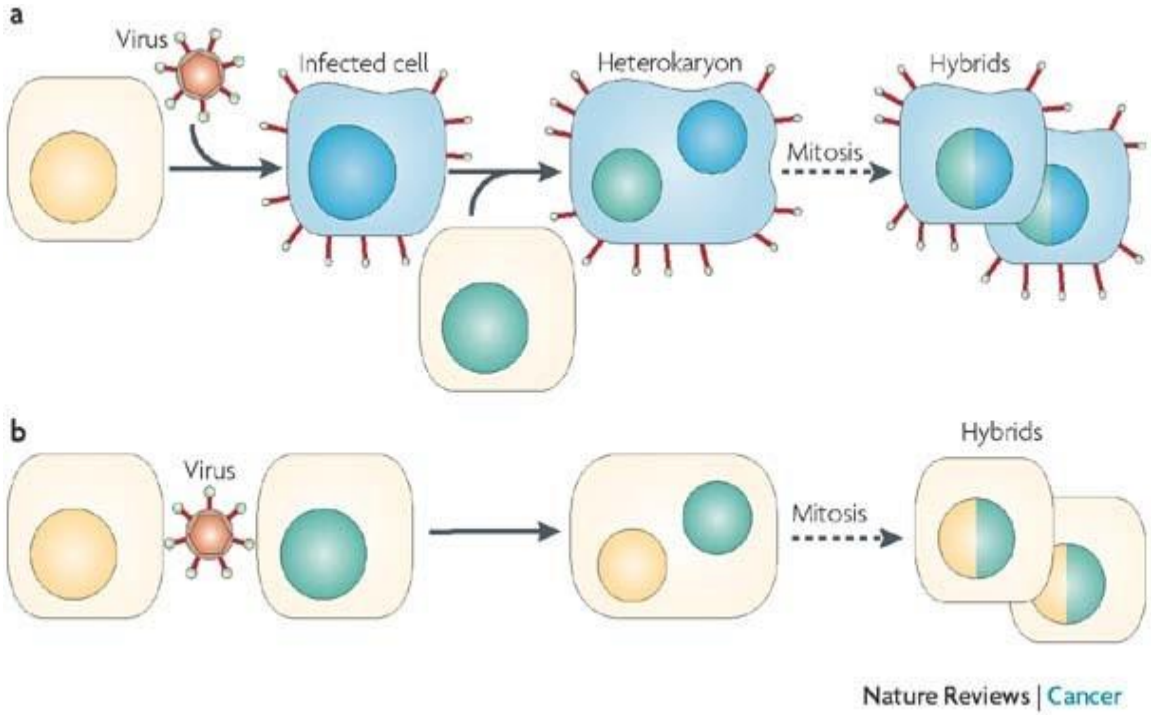
4) **Caveolar/raft endocytosis**, which is similar to clathrin-mediated endocytosis but involves pits containing caveolin-1 rather than clathrin. The internalized virus is delivered to the cytoplasm in cave-like bodies known as caveolae or caveosomes, whose internal pH is neutral.

5) **Endocytosis based on other routes.** These pathways involve vesicles that contain neither clathrin nor caveolin. However, like the clathrin- and caveolin-based pathways, they generally require dynamin, cholesterol and/or lipids.



viral attachment and fusion (upper panel) and entry mechanisms (lower panel)





Early Stage of SARS-CoV-2 Life Cycle
(Targets for Potential Therapeutics in Clinical Trials)

III.Uncoating

- **Viral genes to become available for transcription, it is necessary that virions be at least partially uncoated.**

- **In the case of enveloped RNA viruses that enter by fusion of their envelope with either the plasma membrane or an endosomal membrane, the nucleocapsid is discharged directly into the cytoplasm and transcription commences from viral nucleic acid still associated with this structure.**

- **With the nonenveloped icosahedral reoviruses, only certain capsid proteins are removed and the viral genome expresses all its functions without ever being released from the virion core. For most viruses, however, uncoating proceeds to completion.**
  Other viruses that replicate in the nucleus, the later stages of uncoating occur there rather than in the cytoplasm.

IV. Replication of viral nucleic acid and protein synthesis

- One universal function of viral genomes once inside a cell is to specify proteins. However, viral genomes do not encode the machinery needed to carry out protein synthesis.
- An important principle is that all viral genomes must be copied to produce messenger RNAs (mRNAs) that can be read by host ribosomes.
- Literally, all viruses are parasites of their host cells translation system.

Strategies of Replication

Once uncoating has taken place, synthesis of viral nucleic acid starts. This occurs asthree different stages with differences between different families of the viruses.

1. Early transcription and translation – The proteins derived from this stage is mostly the enzymes required for virus replication.
1. Replication of Nucleic acid
2. Late transcription and translation – The proteins produced this stage are structural proteins.

V. Assembly and Release

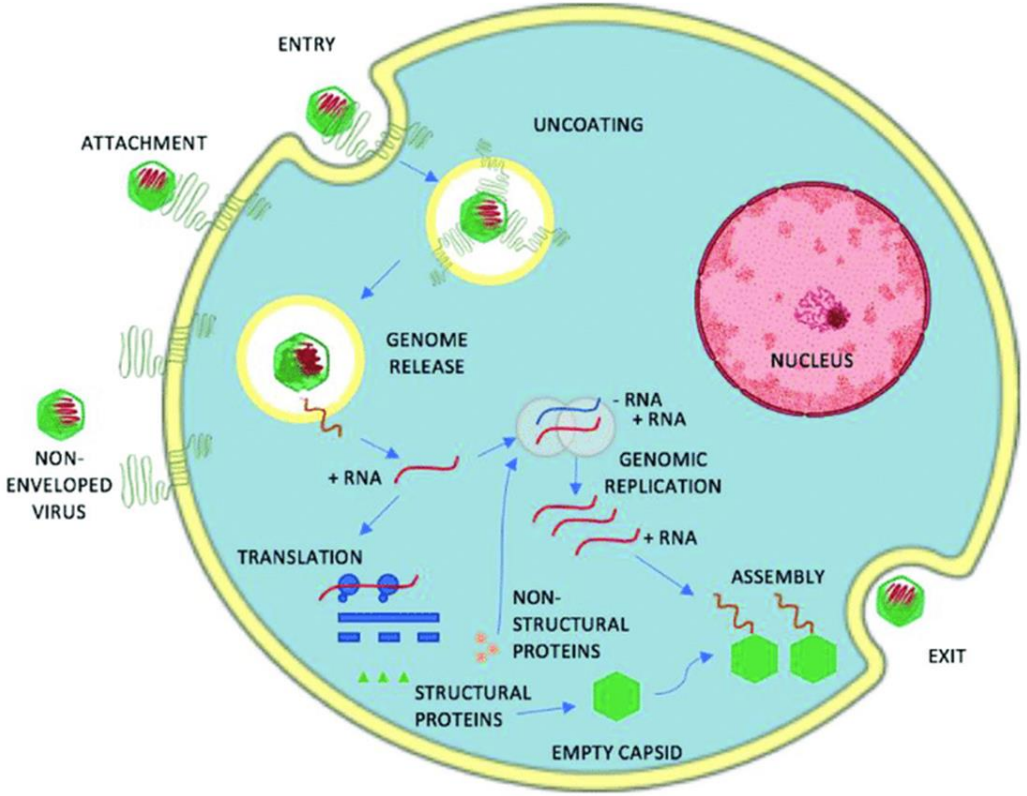
i. Nonenveloped Viruses

All nonenveloped animal viruses have an icosahedral structure. The structural proteins of simple icosahedral viruses associate spontaneously to form capsomers, which self-assemble to form capsids into which viral nucleic acid is packaged.

The mechanism of packaging viral nucleic acid into a preassembled empty procapsid has been elucidated for adenovirus.

- **A particular protein binds to a nucleotide sequence at one end of the viral DNA known as the packaging sequence; this enables the DNA to enter the procapsid bound to basic core proteins, after which so of the capsid proteins are cleaved to make the mature virion.**
- **Most nonenveloped viruses accumulate within the cytoplasm or nucleus and <u>are released</u> <u>only when the cell eventually lyses.</u>**

Non-envelope viruses



DNA

Parvovirus

Adenovirus

Polyomavirus

Papilloma

RNA

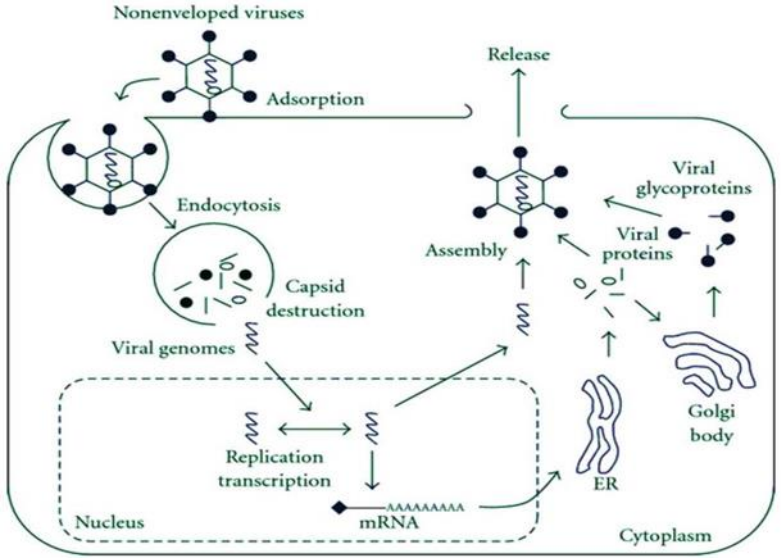
Picornavirus

Astrovirus

Reovirus

Calicivirus

Hepeviridae

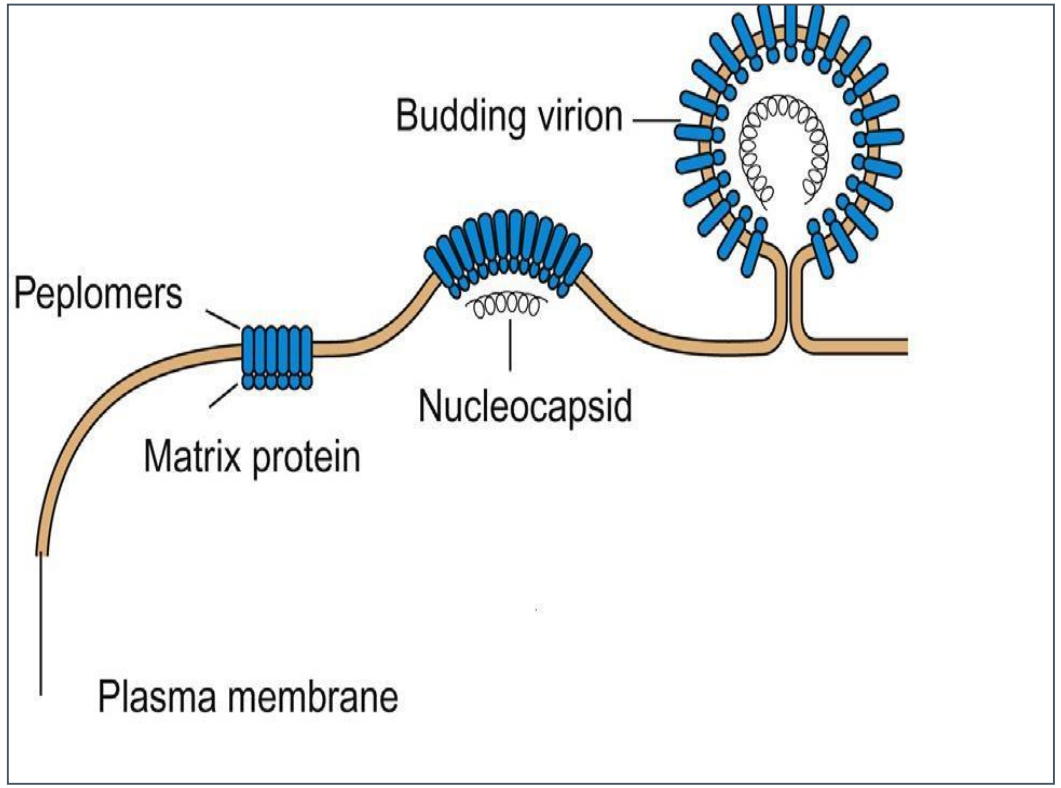


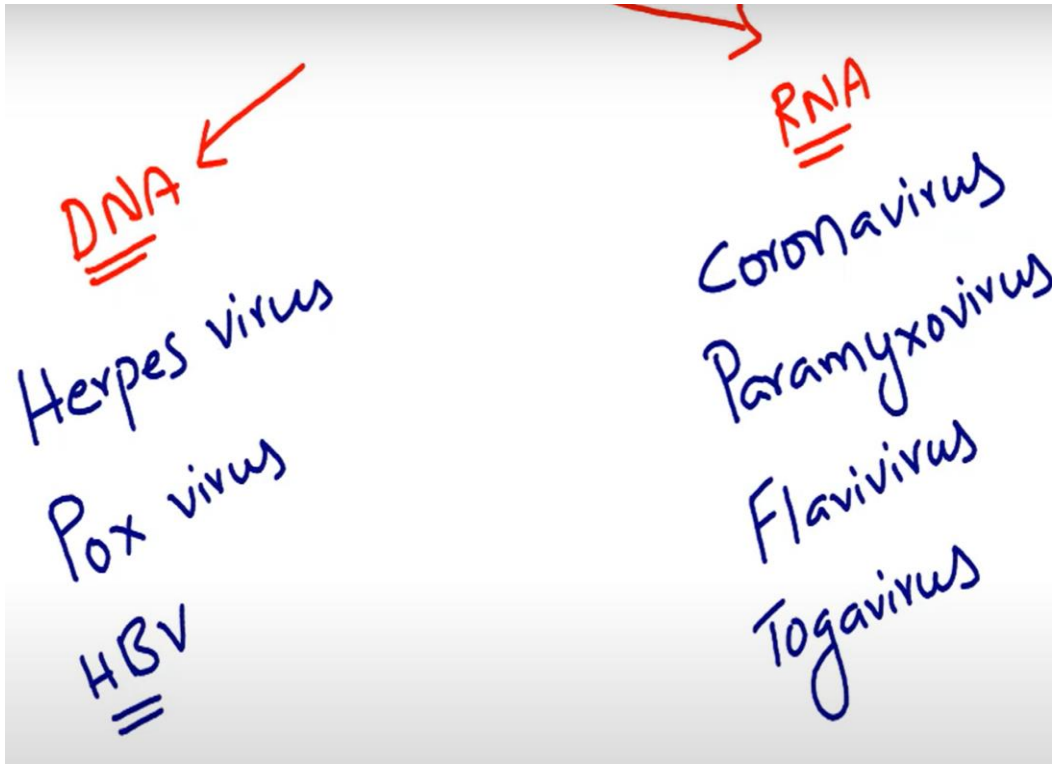
i. Enveloped Viruses

All mammalian viruses with helical nucleocapsids, as well as some with icosahedralnucleocapsids (e.g., herpesviruses, togaviruses, and retroviruses), mature by acquiring an envelope by budding through cellular membranes and Exocytosis.

a. Budding from Cellular Membranes

Enveloped viruses bud from the plasma membrane, from internal cytoplasmic membranes, or from the nuclear membrane. Viruses that gain their envelope within the cell are then transported in vesicles to the cell surface.





a. Exocytosis

Flaviviruses, coronaviruses, arteriviruses, and bunyaviruses mature by budding through membranes of the Golgi complex or rough endoplasmic reticulum; vesicles containing the virus then migrate to the plasma membrane with which they fuse, thereby releasing thevirions by *exocytosis* .