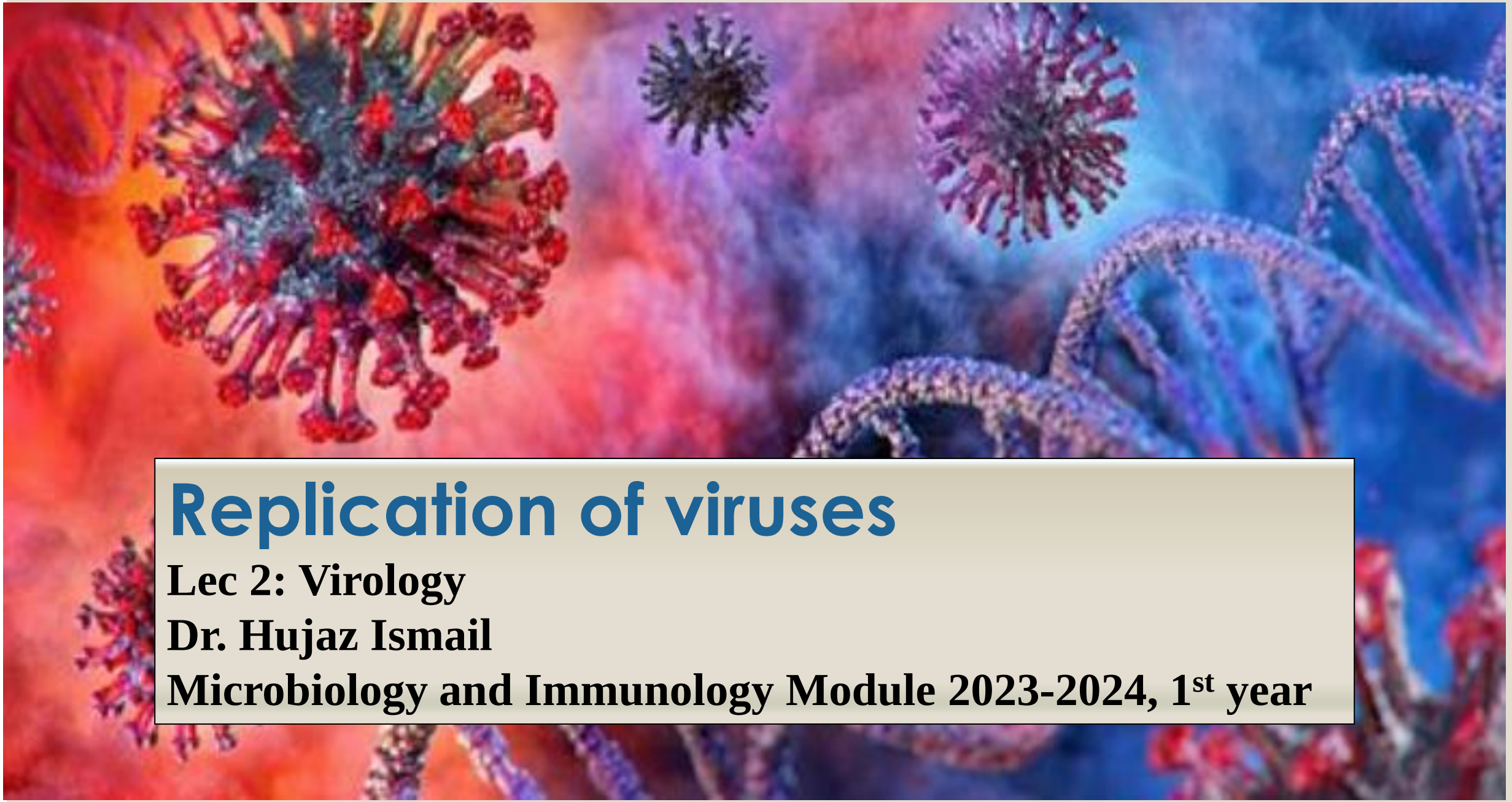

Replication of viruses

Lec 2: Virology

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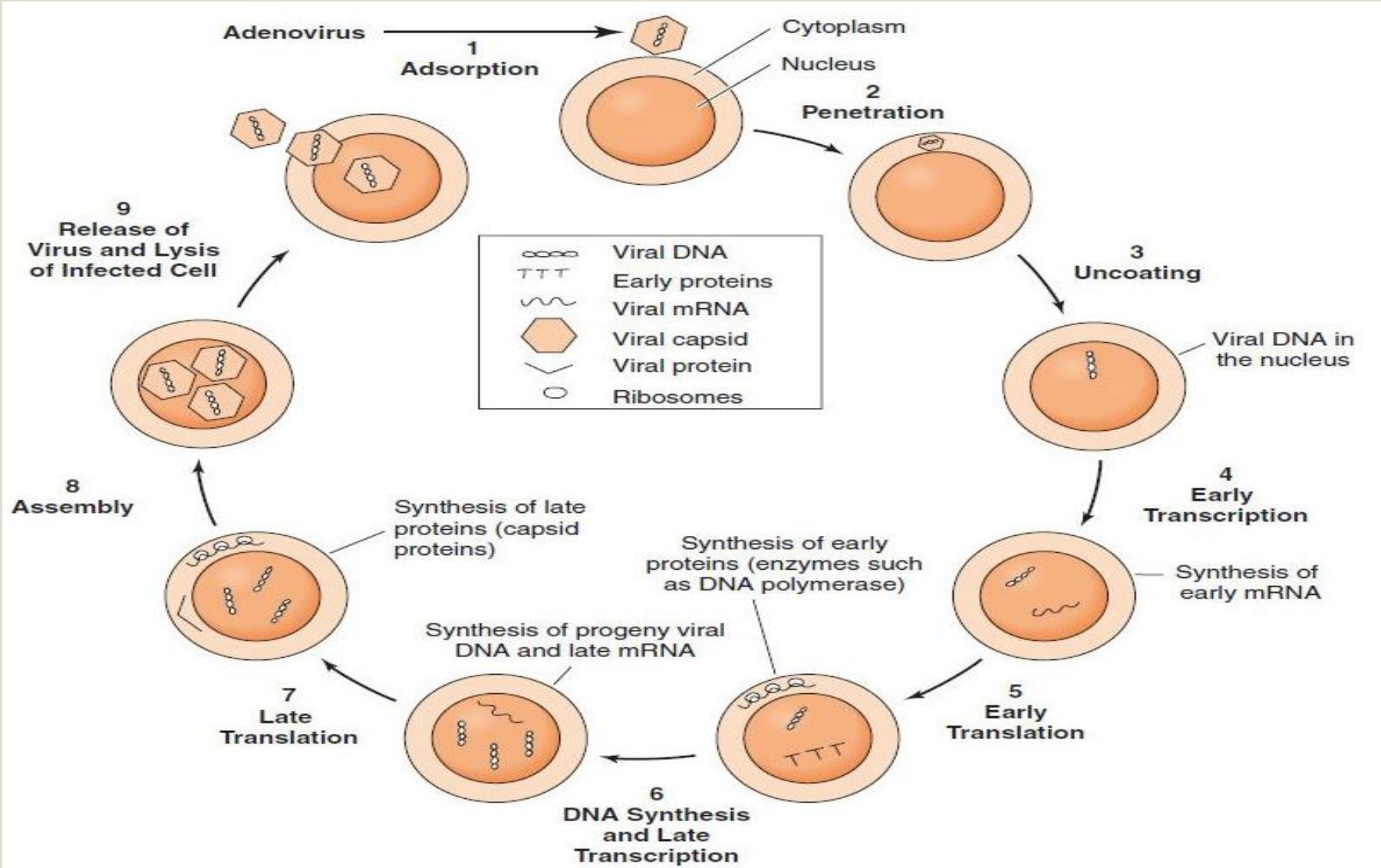
Microbiology and Immunology Module 2023-2024, 1st year

Replication of viruses

- Viruses multiply **only in living cells**.
- The host cell provides the energy and synthetic machinery and the low-molecular-weight precursors for the synthesis of viral proteins and nucleic acids. The viral nucleic acid carries the genetic specificity to code for all of the virus-specific macromolecules in a highly organized fashion.
- For a virus to replicate, **viral proteins must be synthesized by the host cell protein-synthesizing machinery**. Therefore, the virus genome must be able to produce a functional mRNA.
- Various mechanisms have been identified that allow viral RNAs to compete successfully with cellular mRNAs to produce adequate amounts of viral proteins.

General Steps in Viral Replication Cycles

- **A. Adsorption**
- **B. Penetration**
- **C. Uncoating**
- **D. Gene Expression & Genome Replication**
- **E. Assembly and release of progeny viruses**


Adenovirus
1
Adsorption
Cytoplasm
Nucleus
2
Penetration
3
Uncoating
Viral DNA in the nucleus
4
Early
Transcription
Synthesis of early mRNA
5
Early
Translation
6
DNA Synthesis
and Late
Transcription
Synthesis of early proteins (enzymes such as DNA polymerase)
Synthesis of progeny viral DNA and late mRNA
7
Late
Translation
Synthesis of late proteins (capsid proteins)
8
Assembly
9
Release of
Virus and Lysis
of Infected Cell
Viral DNA
Early proteins
Viral mRNA
Viral capsid
Viral protein
Ribosomes

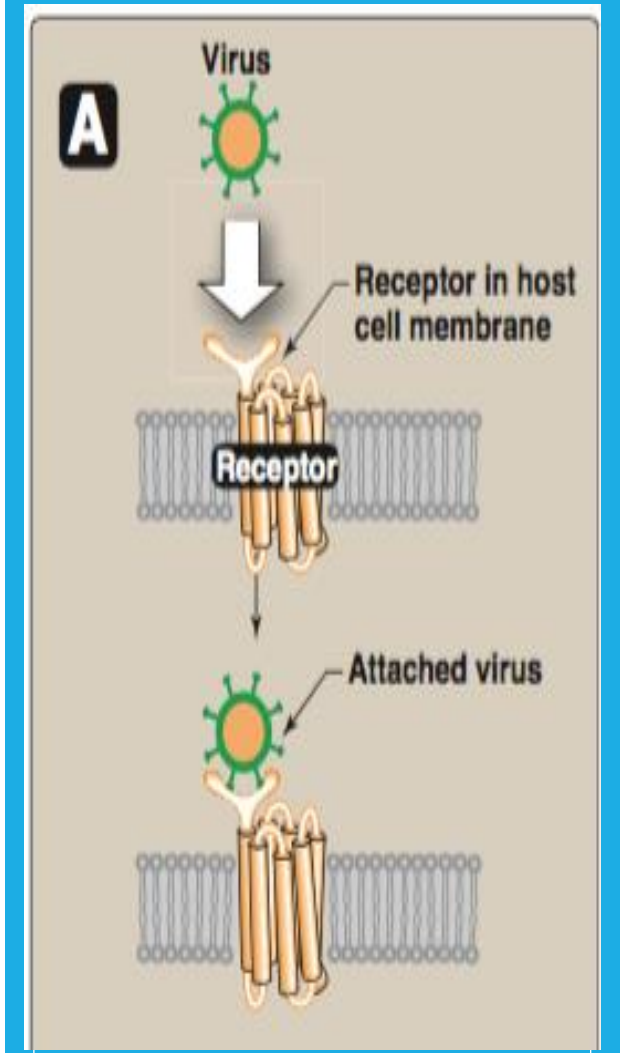
Adsorption

The initial attachment of a virus particle to a host cell involves an interaction between **specific molecular structures on the virion surface** and **receptor molecules in the host cell membrane** that recognize these viral structures.

Adsorption

1. Attachment sites on the viral surface: Some viruses have specialized attachment structures, such as the **glycoprotein spikes found in viral envelopes** whereas for others, the **unique folding of the capsid proteins forms the attachment sites**. In both cases, multiple copies of these molecular attachment structures are distributed around the surface of the virion.

2. Host cell receptor molecules: The receptor molecules on the host cell membrane are specific for each virus family. These receptors have been found to be molecular structures that usually carry out normal cell functions. For example, cellular membrane receptors for compounds such as growth factors may also inadvertently serve as receptors for a particular virus. Many of the compounds that serve as virus receptors are present only on specifically differentiated cells or are unique for one animal species. Therefore, the presence or absence of host cell receptors is one important determinant of tissue specificity within a susceptible host species, and also for the susceptibility or resistance of a species to a given virus.



Attachment of virus to receptor on host cell membrane

Penetration

Penetration is the passage of the virion from the surface of the cell, across the cell membrane and into the cytoplasm. There are two principal mechanisms by which viruses enter animal cells:

Receptor- mediated endocytosis and **direct membrane fusion.**

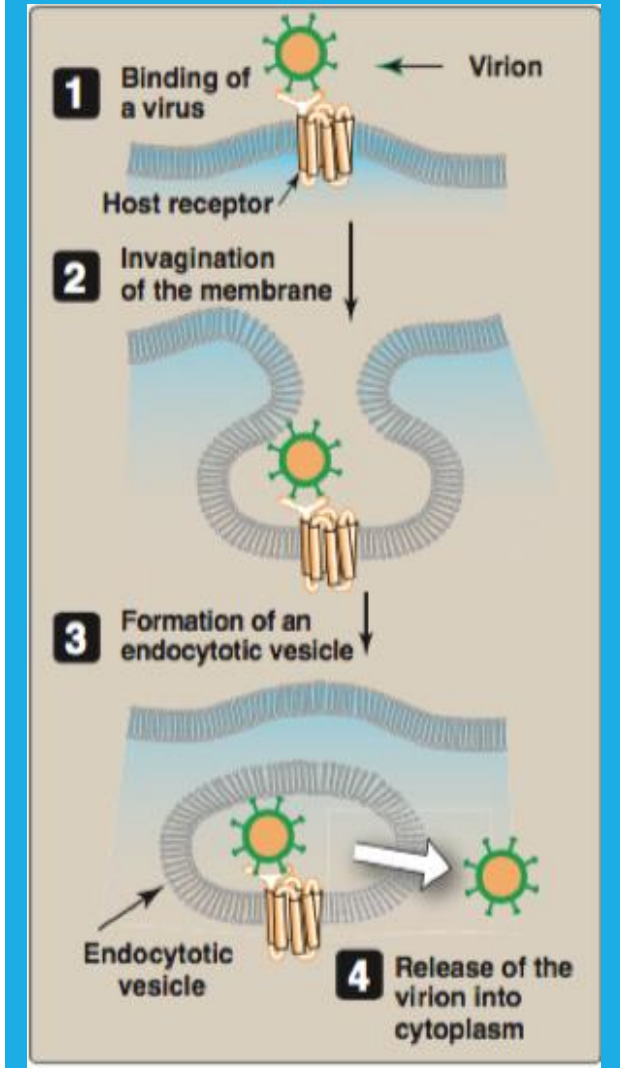
Receptor-mediated endocytosis:

This is basically the same process by which the cell internalizes compounds such as growth regulatory molecules and serum lipoproteins, except the infecting virus particle is bound to the host cell surface receptor in place of the normal ligand.

The cell membrane invaginates, enclosing the virion in an endocytotic vesicle (endosome).

Release of the virion into the cytoplasm occurs by various routes, depending on the virus but, in general, it is facilitated by one or more viral molecules.

In the case of an enveloped virus, its membrane may fuse with the membrane of the endosome, resulting in the release of the nucleocapsid into the cytoplasm.

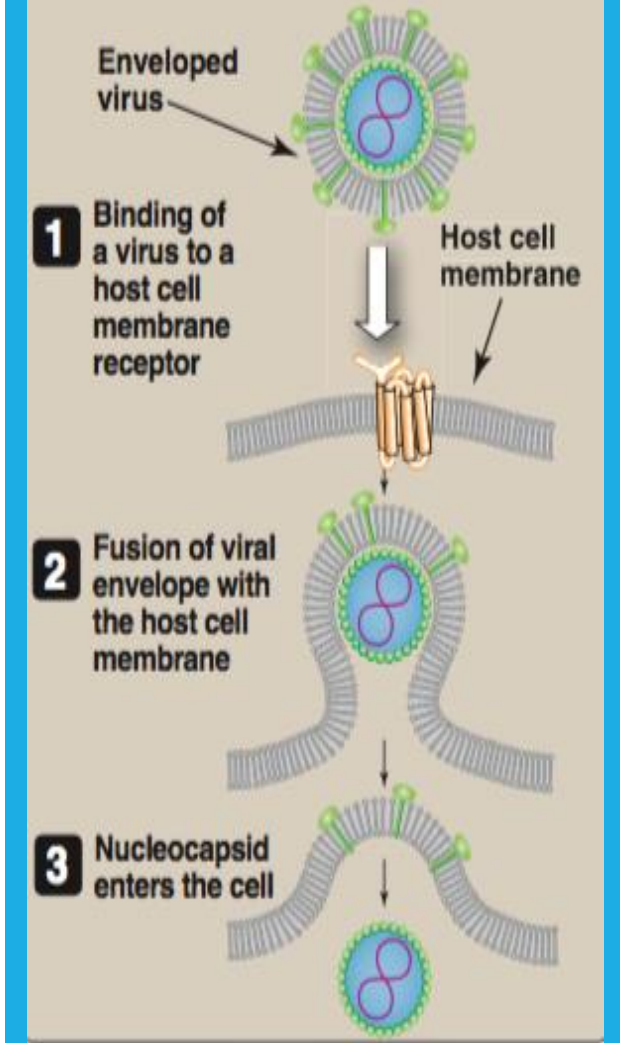


Receptor-mediated endocytosis of virus particle.

Membrane fusion: Some enveloped viruses (for example, human immunodeficiency virus) enter a host cell by fusion of their envelope with the plasma membrane of the cell.

One or more of the glycoproteins in the envelope of these viruses promotes the fusion.

The end result of this process is that the nucleocapsid is free in the cytoplasm, whereas the viral membrane remains associated with the plasma membrane of the host cell.



Fusion of viral envelope with membrane of host cell.

Uncoating

“Uncoating” refers to the stepwise process of disassembly of the virion that enables the expression of the viral genes that carry out replication.

For enveloped viruses, the penetration process itself is the first step in uncoating.

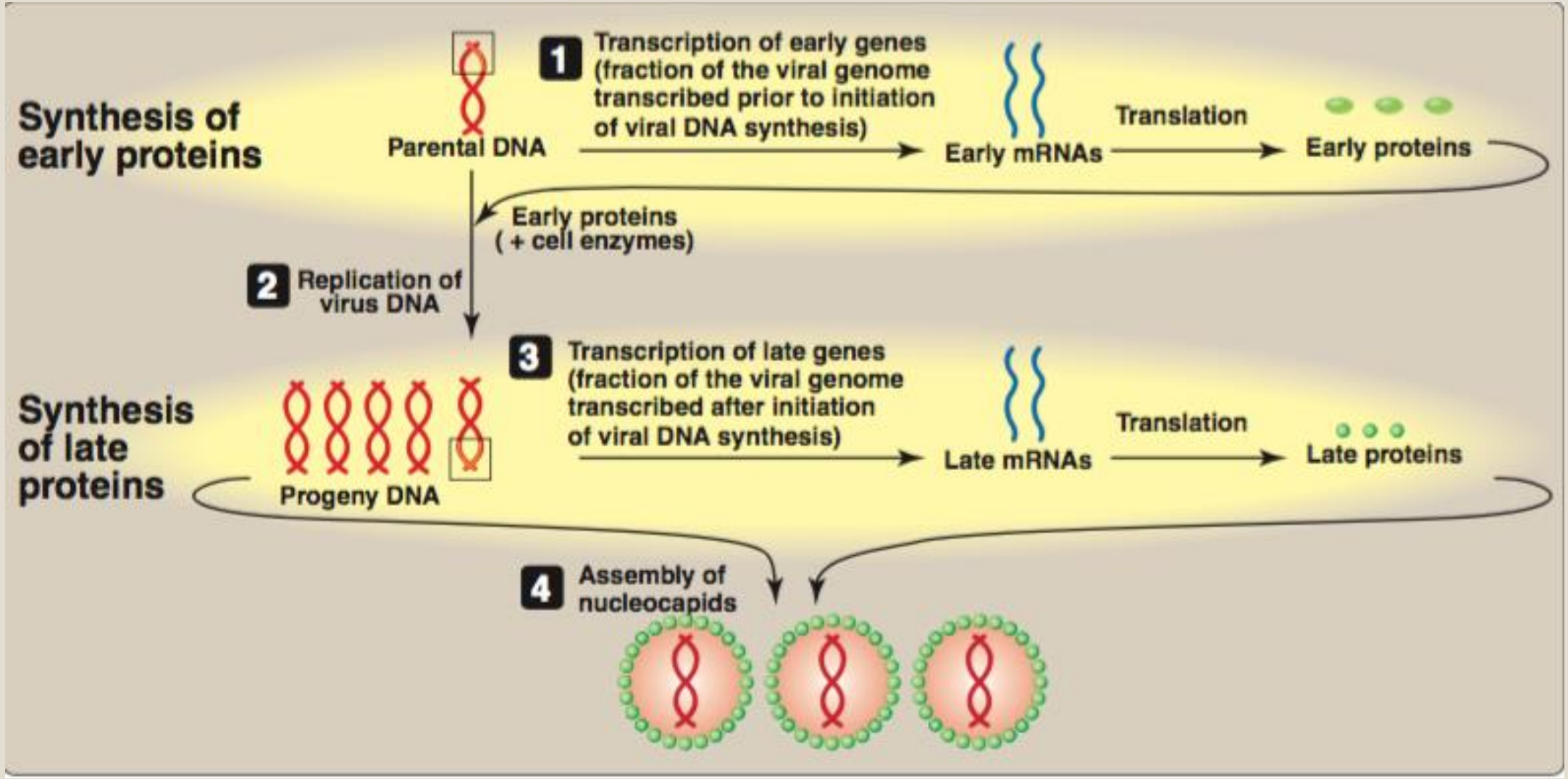
In general, most steps of the uncoating process occur within the cell and depend on cellular enzymes; however in some of the more complex viruses, newly synthesized viral proteins are required to complete the process.

Gene Expression & Genome Replication

- The first step in viral gene expression is **mRNA synthesis**. It is at this point that viruses follow different pathways depending on the nature of their nucleic acid and the part of the cell in which they replicate.

➢ **DNA viruses,** with one exception, **replicate in the nucleus** and use the **host cell DNA-dependent RNA polymerase** to synthesize their mRNA.

- The poxviruses are the exception because they replicate in the cytoplasm, where they do not have access to the host cell RNA polymerase. They therefore carry their own polymerase within the virus particle. **The genome of all DNA viruses consists of double- stranded DNA, except for the parvoviruses, which have a single-stranded DNA genome**



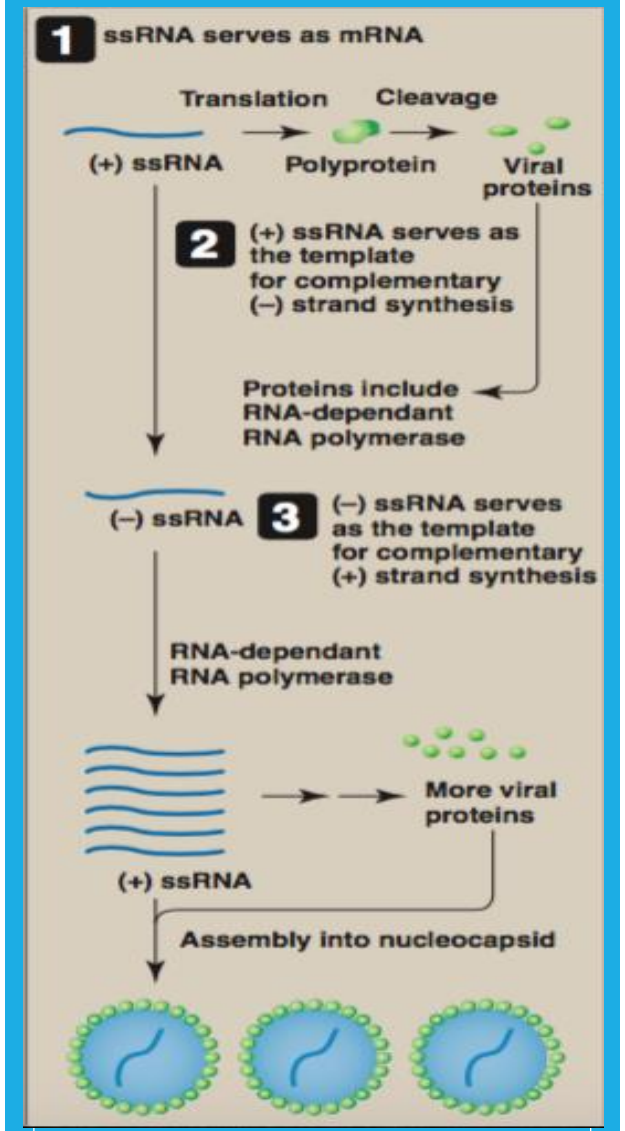
Replication of DNA viruses

➢RNA viruses

- **Most RNA viruses undergo their entire replicative cycle in the cytoplasm**. The two principal exceptions are **retroviruses and influenza viruses**, both of which have an important replicative step in the nucleus. Retroviruses integrate a DNA copy of their genome into the host cell DNA, and influenza viruses synthesize their progeny genomes in the nucleus. In addition, the mRNA of hepatitis delta virus is also synthesized in the nucleus of hepatocytes.
- **The genome of all RNA viruses consists of single- stranded RNA, except for members of the reovirus family, which have a double-stranded RNA genome**. Rotavirus is the important human pathogen in the reovirus family.
- RNA viruses fall into four groups with quite different strategies for synthesizing mRNA

(1) The simplest strategy is illustrated by poliovirus, which has **single-stranded RNA** of **positive polarity** as its genetic material. These viruses use their RNA genome directly as mRNA.

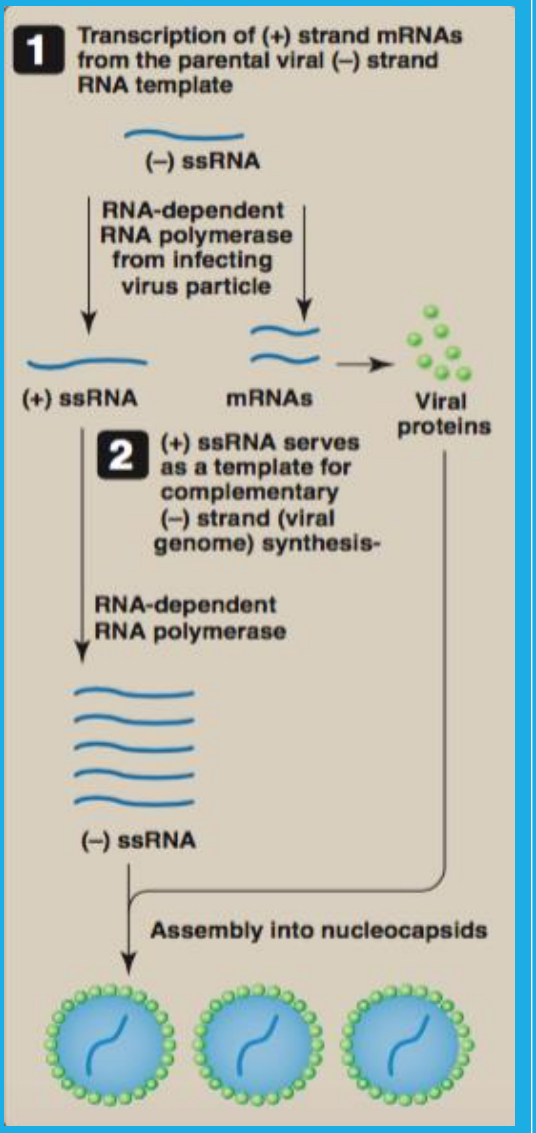
Because the parental RNA genome is of (+), or messenger, polarity, it can be translated directly upon uncoating and associating with cellular ribosomes. The product is usually a single polyprotein from which individual polypeptides, such as **RNA-dependent RNA polymerase and various proteins of the virion, are cleaved by a series of proteolytic processing events carried out by a protease domain of the polyprotein.**



Virus with a ssRNA genome of (+) polarity replicates via a complementary (–) strand intermediate.

(2) The second group has **single-stranded RNA** of **negative polarity** as its genetic material. An mRNA must be transcribed by using the negative strand as a template. Because the cell does not have an RNA polymerase capable of using RNA as a template, the virus carries its own **RNA- dependent RNA polymerase**. There are two subcategories of negative-polarity RNA viruses: those that have a single piece of RNA (e.g., measles virus [a paramyxovirus] or rabies virus [a rhabdovirus]) and those that have multiple pieces of RNA (e.g., influenza virus [a myxovirus]).

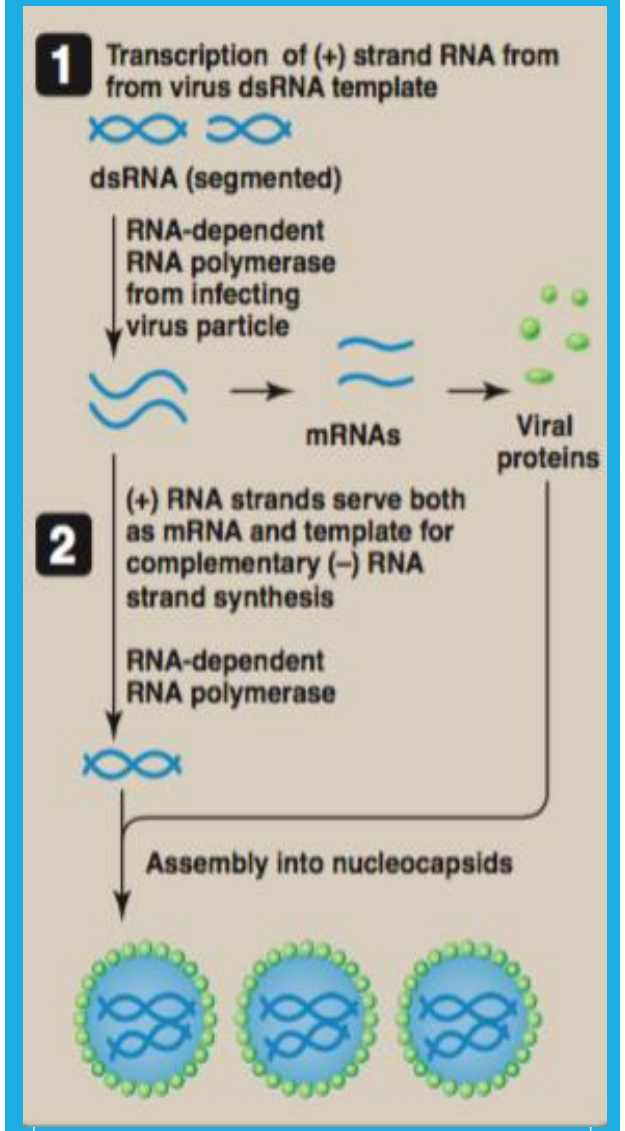
Certain viruses, such as arenaviruses and some bunyaviruses, have a segmented RNA genome, most of which is negative stranded, but there are some positive strand regions as well. RNA segments that contain both positive polarity and negative polarity regions are called **"ambisense."**



Virus with an ssRNA genome of (–) polarity that replicates via a complementary (+) strand intermediate.

3) The third group has **double-stranded RNA** as its genetic material. Because the cell has no enzyme capable of transcribing this RNA into mRNA, **the virus carries its own polymerase.**

Note that plus strand in double-stranded RNA cannot be used as mRNA because it is hydrogen-bonded to the negative strand. Rotavirus, an important cause of diarrhea in children, has 11 segments of double-stranded RNA.

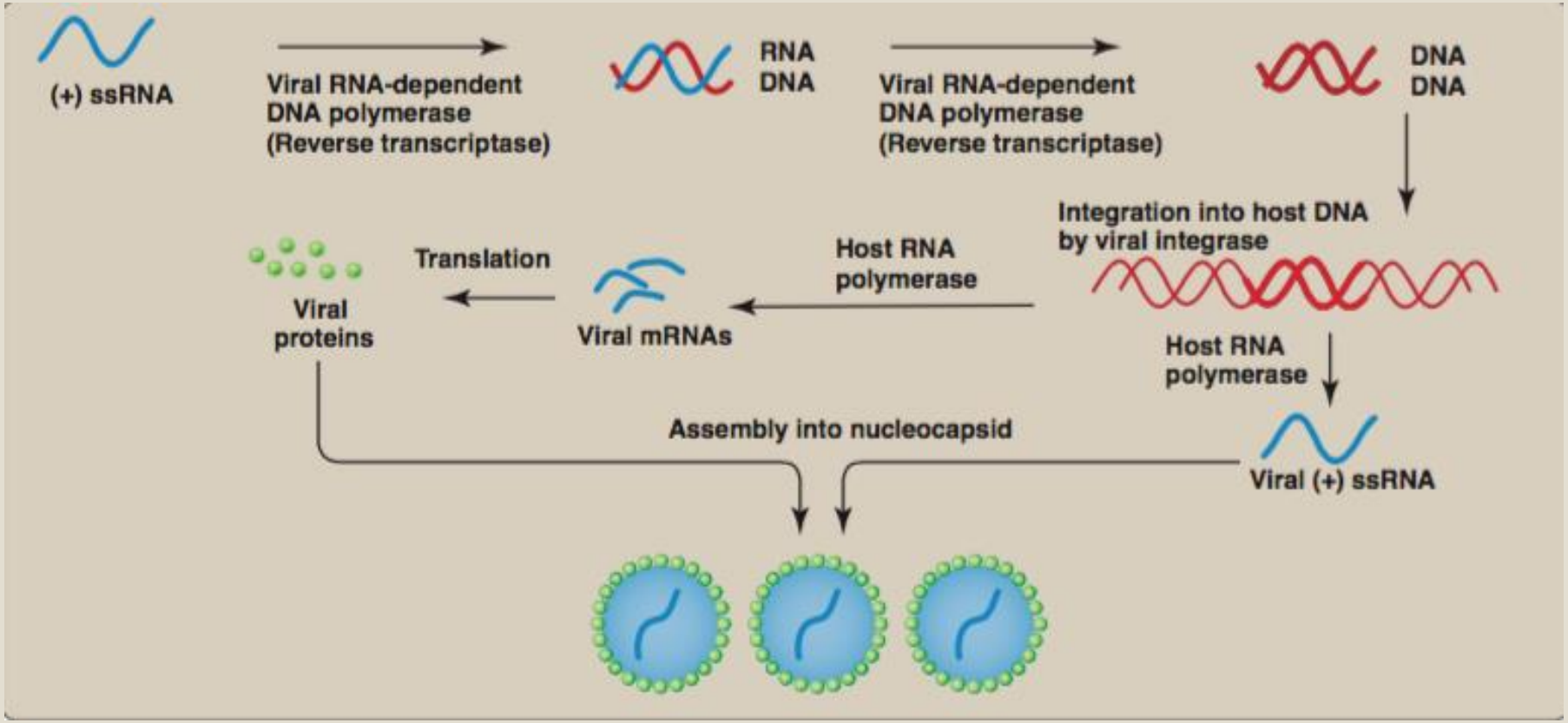


Virus with a dsRNA genome

(4) The fourth group, exemplified by retroviruses, has single-stranded RNA of positive polarity that is transcribed into double-stranded DNA by **the RNA-dependent DNA polymerase (reverse transcriptase) carried by the virus**. This DNA copy is then transcribed into viral mRNA by the regular **host cell RNA polymerase (polymerase II).**

Retroviruses are the only family of viruses that are **diploid** (i.e., that have two copies of their genome RNA).

The conversion of a (+) strand RNA to a double-stranded DNA is accomplished by an RNA- dependent DNA polymerase, commonly referred to as a "reverse transcriptase," that is contained in the virion. The resulting dsDNA becomes integrated into the cell genome by the action of a viral "integrase." Viral mRNAs and progeny (+) strand RNA genomes are transcribed from this integrated DNA by the host cell RNA polymerase



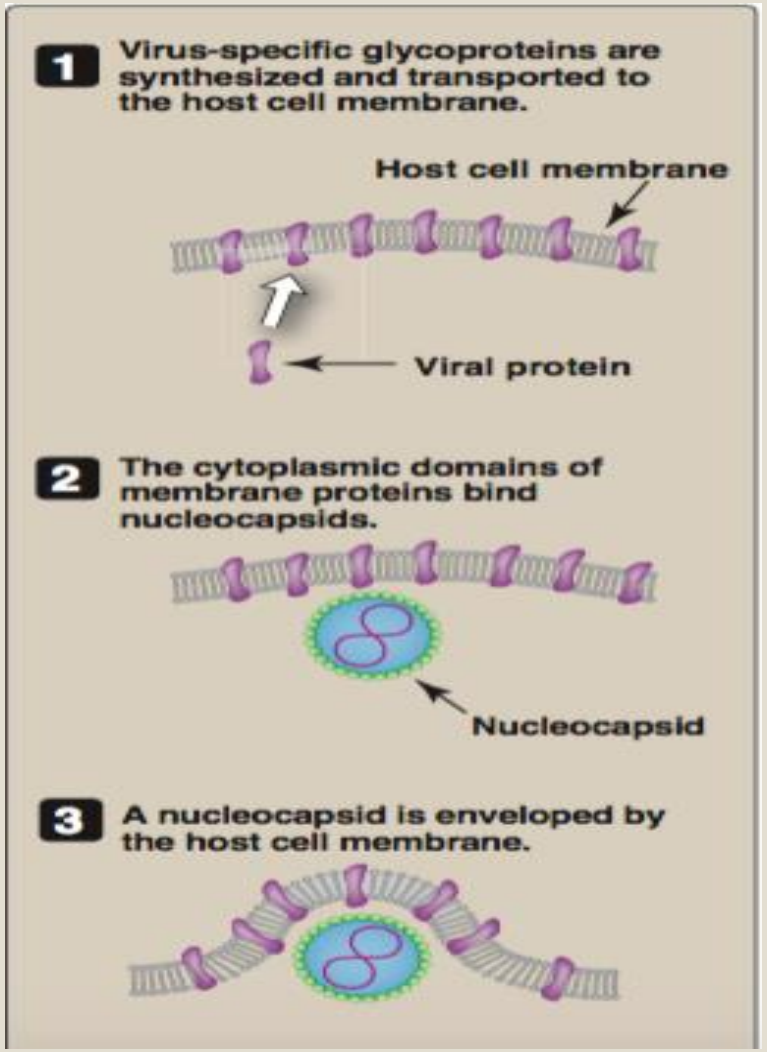
Virus with a ssRNA genome of (+) polarity that replicates via a DNA intermediate.

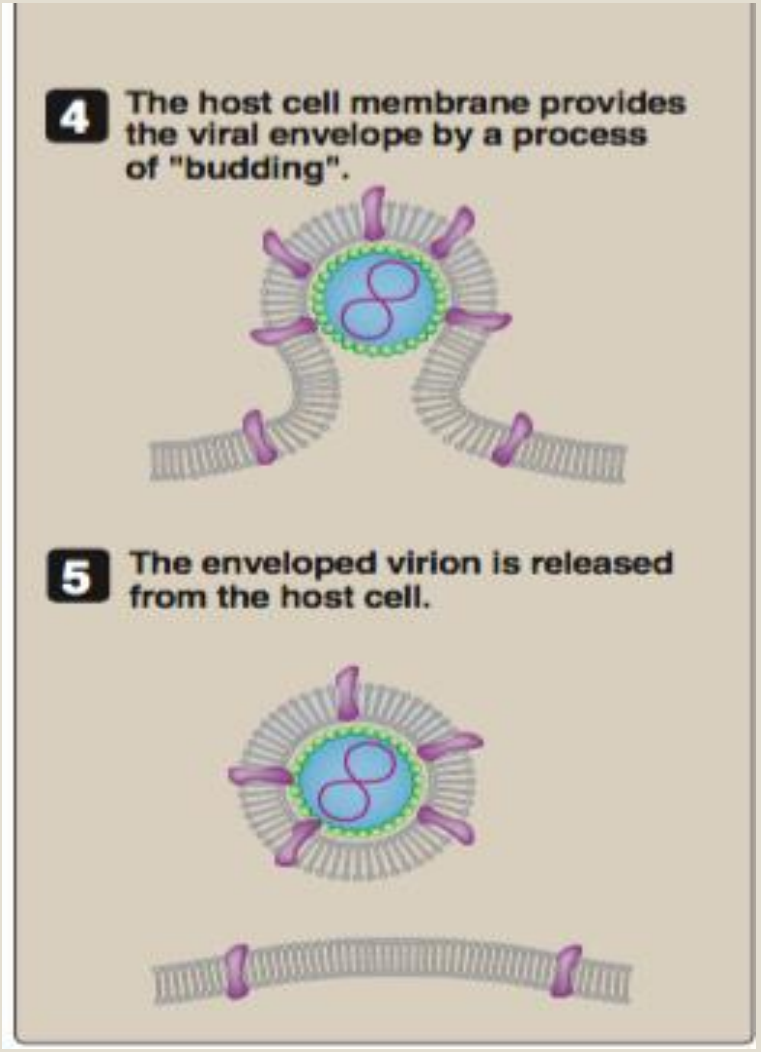
Assembly and release of progeny viruses

- Assembly of nucleocapsids generally takes place in the host cell compartment where the viral nucleic acid replication occurs **(in the cytoplasm for most RNA viruses** and **in the nucleus for most DNA viruses).**
- For DNA viruses, this requires that capsid proteins be transported from their site of synthesis (cytoplasm) to the nucleus. The various capsid components begin to self-assemble, eventually associating with the nucleic acid to complete the nucleocapsid.

- **Naked viruses:** In naked (unenveloped) viruses, the virion is complete at this point. Release of progeny is usually a passive event resulting from the disintegration of the dying cell and, therefore, may be at a relatively late time after infection.

- **Enveloped viruses:** In enveloped viruses, virus-specific glycoproteins are synthesized and transported to the host cell membrane in the same manner as cellular membrane proteins. When inserted into the membrane, they displace the cellular glycoproteins, resulting in patches on the cell surface that have viral antigenic specificity. The cytoplasmic domains of these proteins associate specifically with one or more additional viral proteins (matrix proteins) to which the nucleocapsids bind.
- Final maturation then involves envelopment of the nucleocapsid by a process of **"budding".**
- A consequence of this mechanism of viral replication is that progeny virus are released continuously while replication is proceeding within the cell and **ends when the cell loses its ability to maintain the integrity of the plasma membrane**. A second consequence is that with most enveloped viruses, all infectious progeny are extracellular. The exceptions are those viruses that acquire their envelopes by budding through internal cell membranes, such as those of the endoplasmic reticulum or nucleus.





Release of enveloped virus from a host cell by the process of “budding.”


thank you!