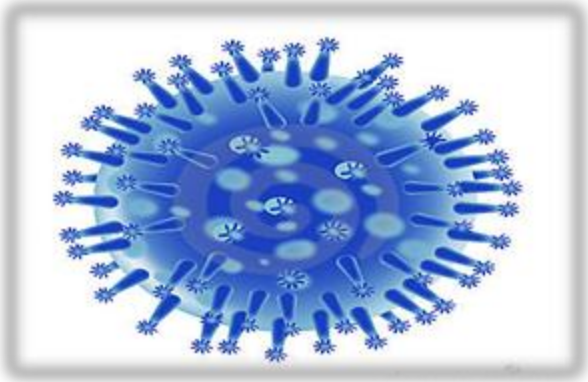

General Microbiology
2023-2024

Viral replication

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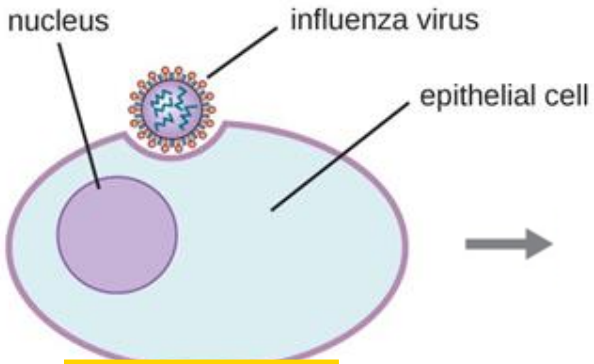
Introduction

- Virus (Latin, poison)
- Viruses are non-living, infectious entities which only become part of a living system when they have infected host cells, a form of borrowed life.
- They need the help of a host cell for their replication.
- All viruses have to penetrate, replicate & come out of a cell.

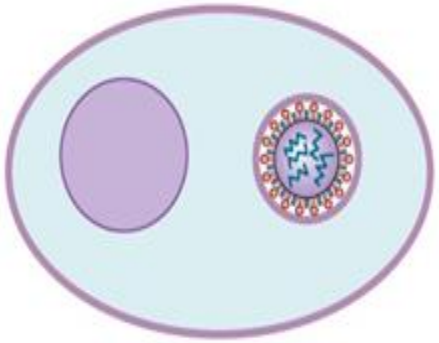
Basic steps in viral life cycle

Phase I

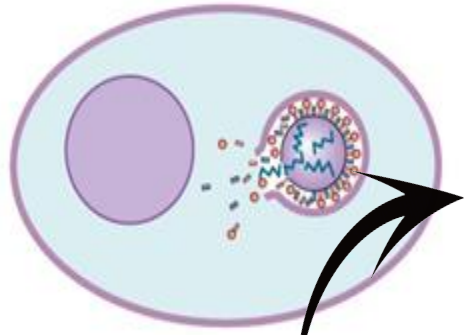


By the spike proteins

1 **Attachment**
Influenza virus becomes attached to a target epithelial cell.



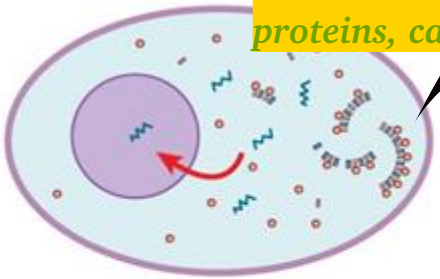
2 **Penetration**
The cell engulfs the virus by endocytosis.



3 **Uncoating**
Viral contents are released.

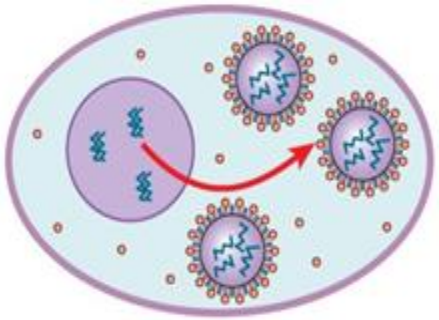
To expose its genetic material

associated viral genomic polymerase
Start synthesizing:
For the viral genome it self
For viral components such as (spike proteins, capsid etc.)

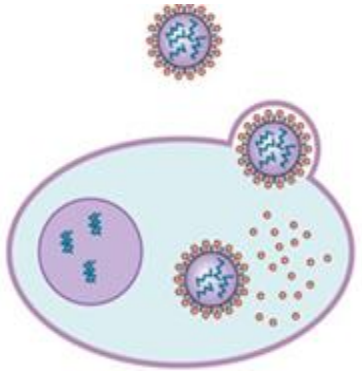


4 **Biosynthesis**
Viral RNA enters the nucleus, where it is replicated by the viral RNA polymerase.

Phase II



5 **Assembly**
New phage particles are assembled.



6 **Release**
New viral particles are made and released into the extracellular fluid. The cell, which is not killed in the process, continues to make

Phase III

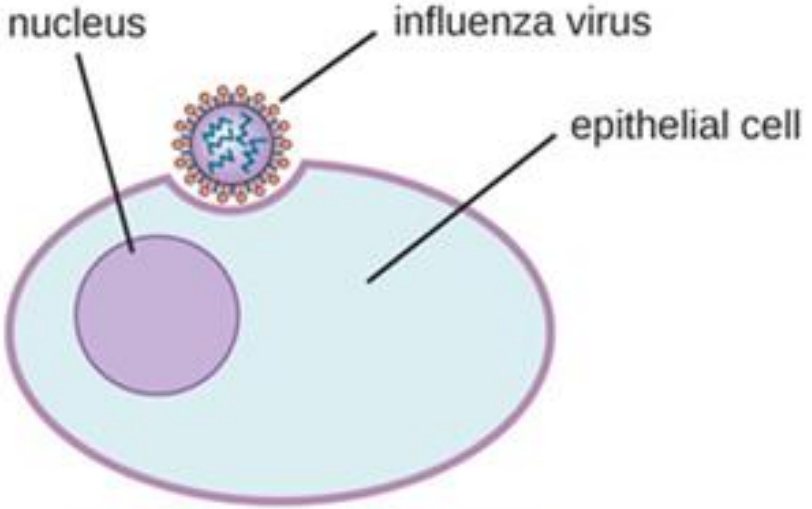
Stages of virus replication

- **Phase – I Initiation:** This stage is characterized by introduction of genetic material of the virus into the cell
  - Attachment
  - Penetration
  - Uncoating
- **Phase – II Biosynthesis:** This stage is characterized by:
  - Genome synthesis
  - RNA production
  - Protein synthesis
- **Phase – III Assembly, Release, Maturation.**

Stages of virus replication

Phase I - Initiation

1. **Attachment: Virus attaches to the cell surface.**
   - Attachment is via ionic interactions.
   - Viral attachment proteins referred as ligands are present on the surface of viruses, which recognizes specific receptors on the cell surface.



1 **Attachment**
Influenza virus becomes attached to a target epithelial cell.

The ligands in viruses are usually the fibers and spikes in the virus structures.

It's like the fibers & spike proteins are the ligands for a specific Receptor on the surface of the host cell : ex influenza virus spikes, and fiber (specific ligand) has specific receptors expressed on the epithelial cells
It is called **Tropism (Affinity)**

Stages of virus replication

- The receptors on cells are protein or carbohydrate or lipid components of the cell surface.

- Cells without the appropriate receptors are not susceptible to the virus.

Examples:

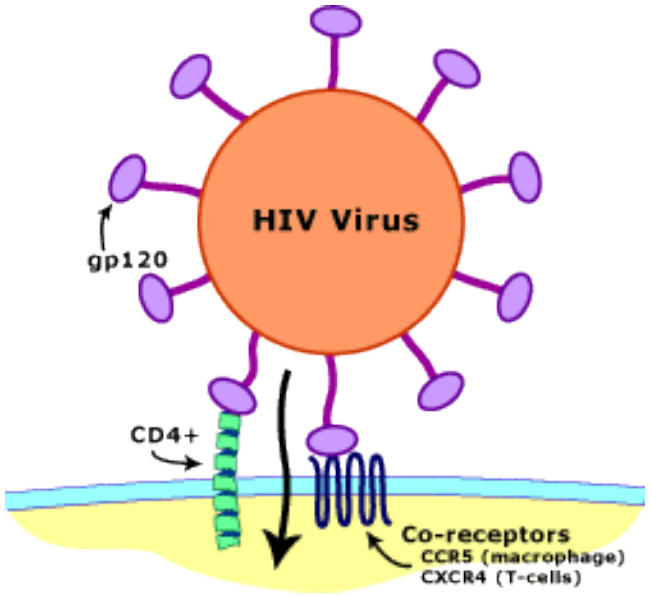
I. **Influenzas virus**

II. **HIV**: The joining ligand of HIV is gp120 which binds to the most common cellular receptors glycoproteins (CD4).

III. **COVID-19**

T-cell helper 2

HIV

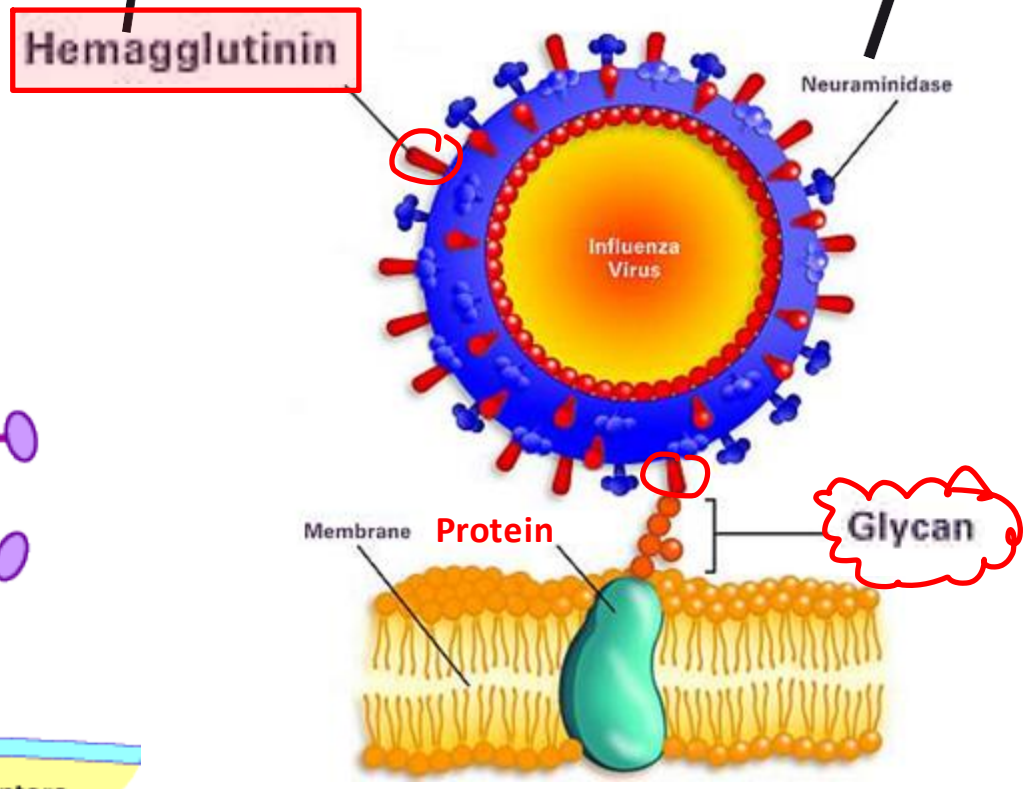


influenza virus has spike proteins or Fibers
: Hemagglutinin (H1)
: Neuraminidase (N1)
These can deferent according to structure: (H2 , N2 , H3 ...)
Vaccines are made against : Hemagglutinin (H1)

Attachment & entry

For release

Influenzas virus

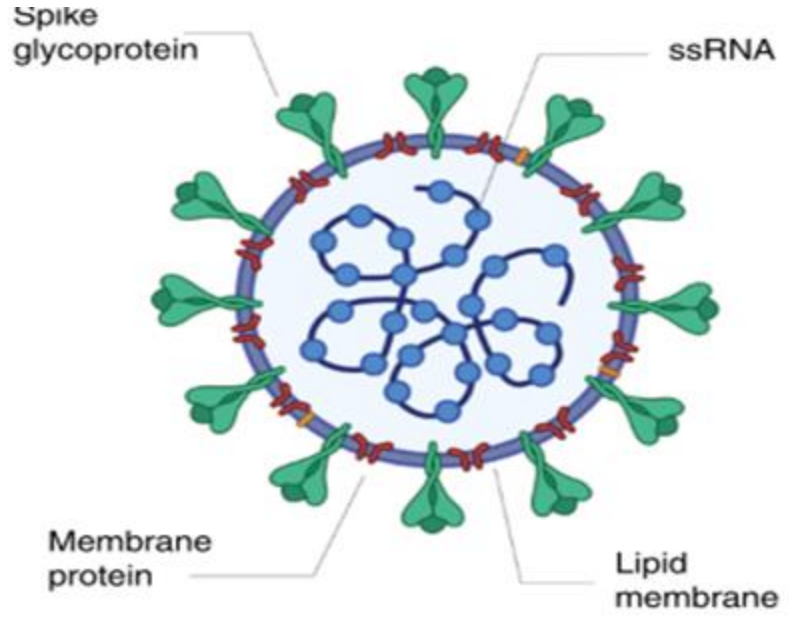


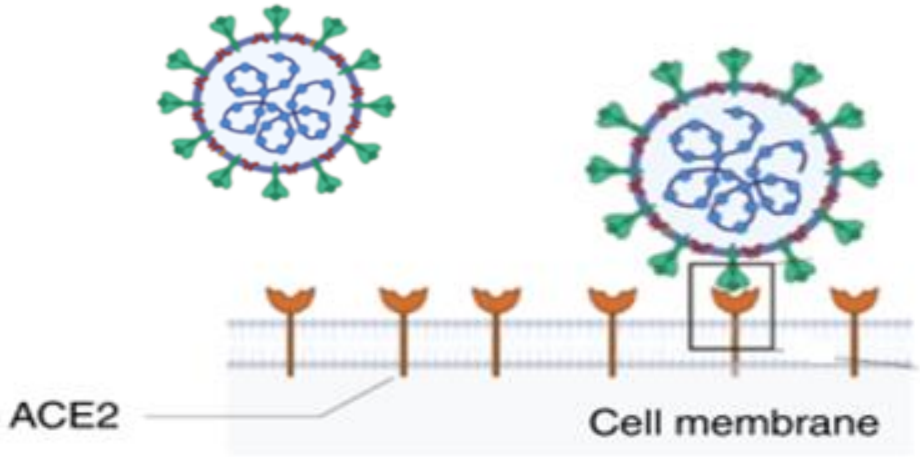
Chains of sugars called glycans sit on the surface of our cells and control the gates through which different molecules enter. For a virus to gain access to a cell, proteins on the virus's surface must bind to certain glycans.

Stages of virus replication

COVID-19

- The COVID-19 entry into host cells is mediated by its spike glycoprotein (S-glycoprotein), and the angiotensin-converting enzyme 2 (ACE2) has been identified as a cellular receptor.
- ACE2 is expressed in nearly all human organs in varying degrees. In the respiratory system ACE2 is mainly expressed on type II alveolar epithelial cells

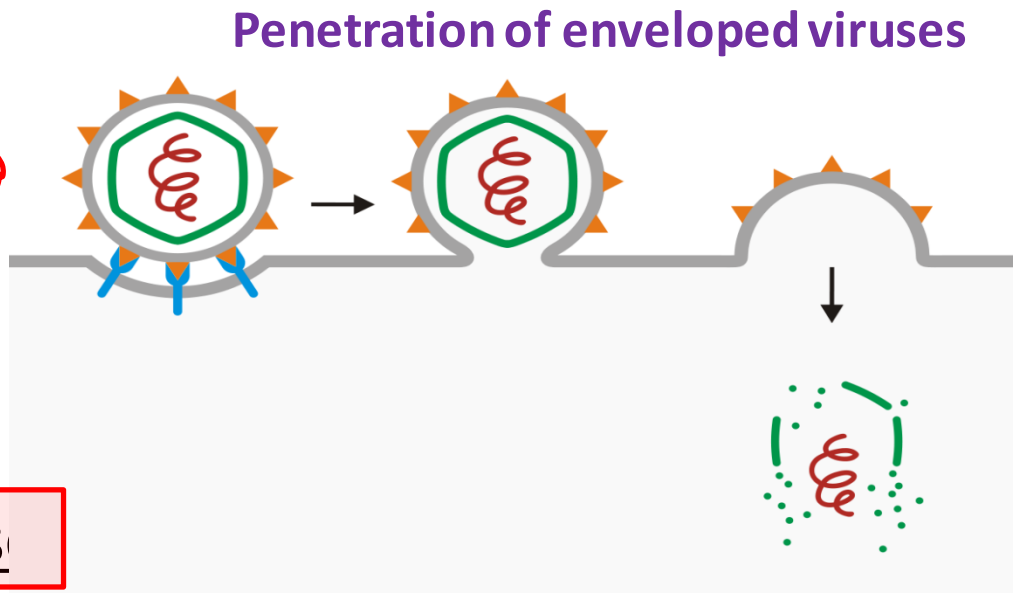




Stages of virus replication

2. Penetration:

- It is a process by which a virus enters into the cell.
- It is an energy dependant reaction and occurs quickly.
- Methods of penetration:
  - fusion
  - endocytosis
- Two methods of Penetration of enveloped virus



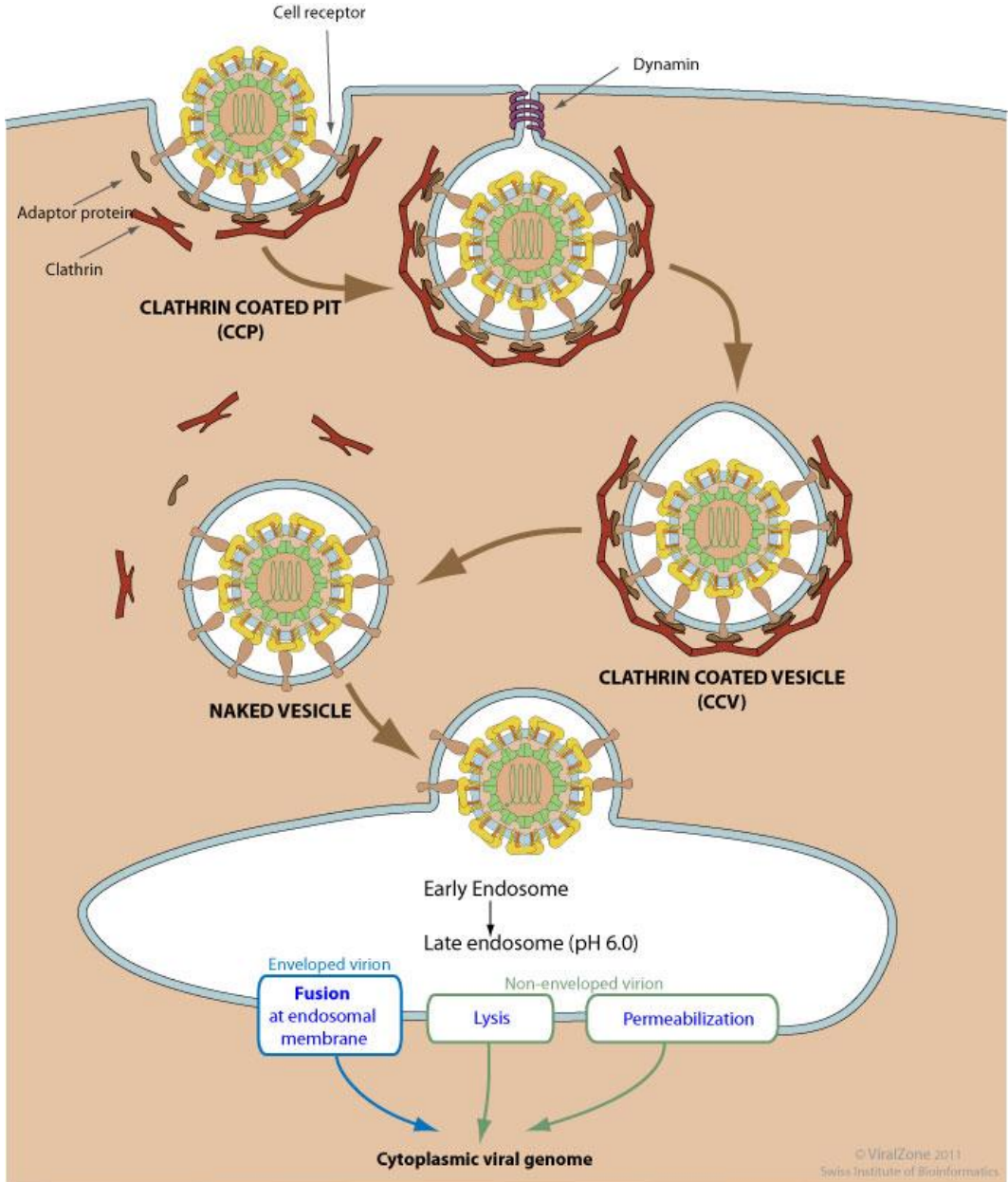
A. Entry by fusing with the plasma membrane : Some enveloped viruses fuse directly with the plasma membrane. Thus, the internal components of the virion are immediately delivered to the cytoplasm of the cell.

Stages of virus replication

Ligands bind with adapter proteins
Which induces the clathrin-coated pits

B. Entry via clathrin-coated pits at the cell surface:

- Some enveloped viruses are unable to fuse directly with the plasma membrane.
- These viruses are taken up by the invagination of clathrin-coated pits into endosomes.
- As the endosomes become acidified, the fusion activity of the virus proteins becomes activated by the fall in pH, and the virion membrane fuses with the endosome membrane.
- This results in the delivery of the internal components of the virus to the cytoplasm of the cell.
- This endocytosis is also called viropexis (where the virus membrane does not become part of the vesicle membrane).



Stages of virus replication

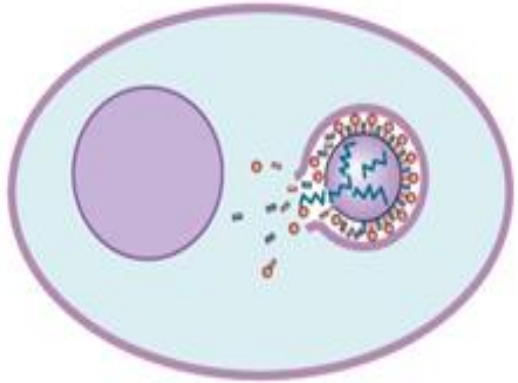
- **Two methods of penetration of non-enveloped viruses:**
  A. Direct endocytosis.
  B. or may be taken up via clathrin-coated pits into endosomes.
- They then cross the endosomal membrane

3. Uncoating:

For viruses that can't do fusion —> clathrin-coated pits
Endocytosis

- This is the general term applied to events after penetration, which allow the virus to express its genome.
- For successful viral infection, nucleic acid has to be sufficiently uncoated.
- The lysosomal enzymes play a major role in uncoating

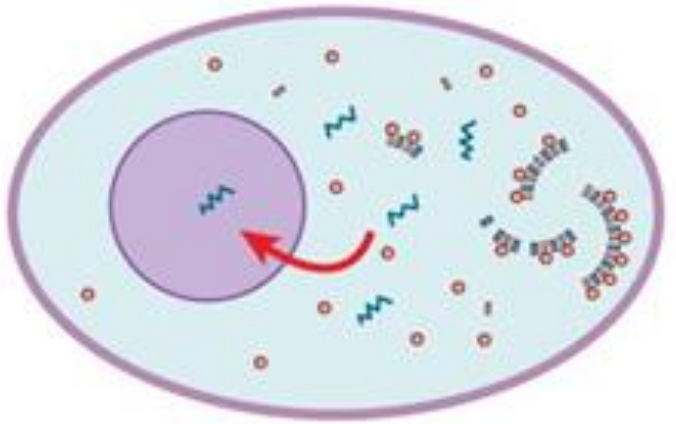
Uncoating



3 **Uncoating**
Viral contents are released.

Stages of virus replication

- **Phase II: Replication of viral nucleic acid and protein synthesis**
- Once uncoating has taken place, synthesis of viral nucleic acid starts.
- The site of production of nucleic acid also varies between viruses.
  - Most of the DNA viruses except Pox and Herpes replicate in nucleus.
  - All RNA viruses replicate in cytoplasm except Orthomyxoviruses and Retroviruses, which for certain stages of replication get into the nucleus of the cell



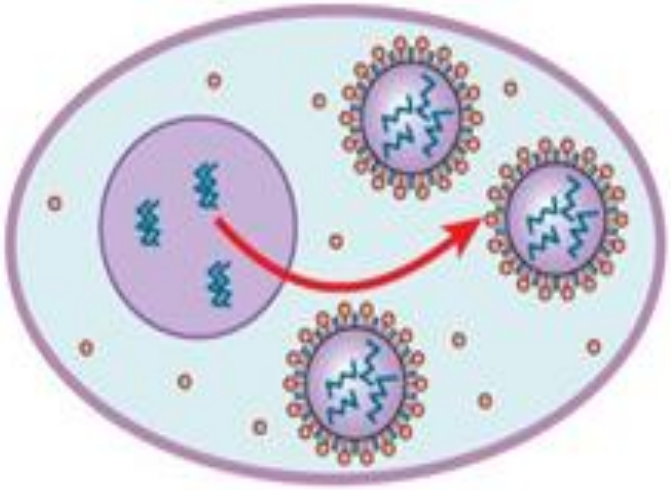
4 **Biosynthesis**
Viral RNA enters the nucleus, where it is replicated by the viral RNA polymerase.

Stages of virus replication

Phase III: Assembly, Release, Maturation.

Assembly

- Assembly: This stage involves the assembly of all the components necessary for the formation of the mature virion at a particular site in the cell.
- During this process, the basic structure of the virus is formed.
- The site of assembly varies for different viruses, e.g: Picornaviruses, Poxviruses, Reoviruses - In the cytoplasm. Adenoviruses, povaviruses, Parvoviruses - In the nucleus.



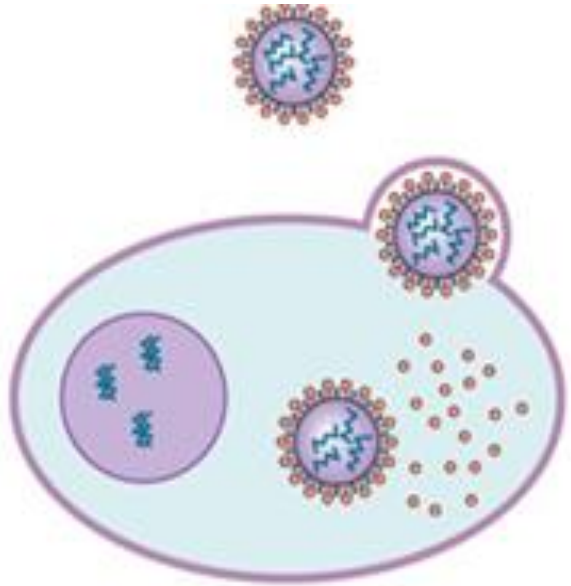
5 **Assembly**
New phage particles are assembled.

Stages of virus replication

- **Phase III: Assembly, Release, Maturation.**

Release

- Release is a simple process - the cell breaks open and releases the virus.
- Enveloped viruses acquire the lipid membrane as the virus buds out through the cell membrane.



6 **Release**
New viral particles are made and released into the extracellular fluid. The cell, which is not killed in the process, continues to make

Might be enveloped or non-enveloped

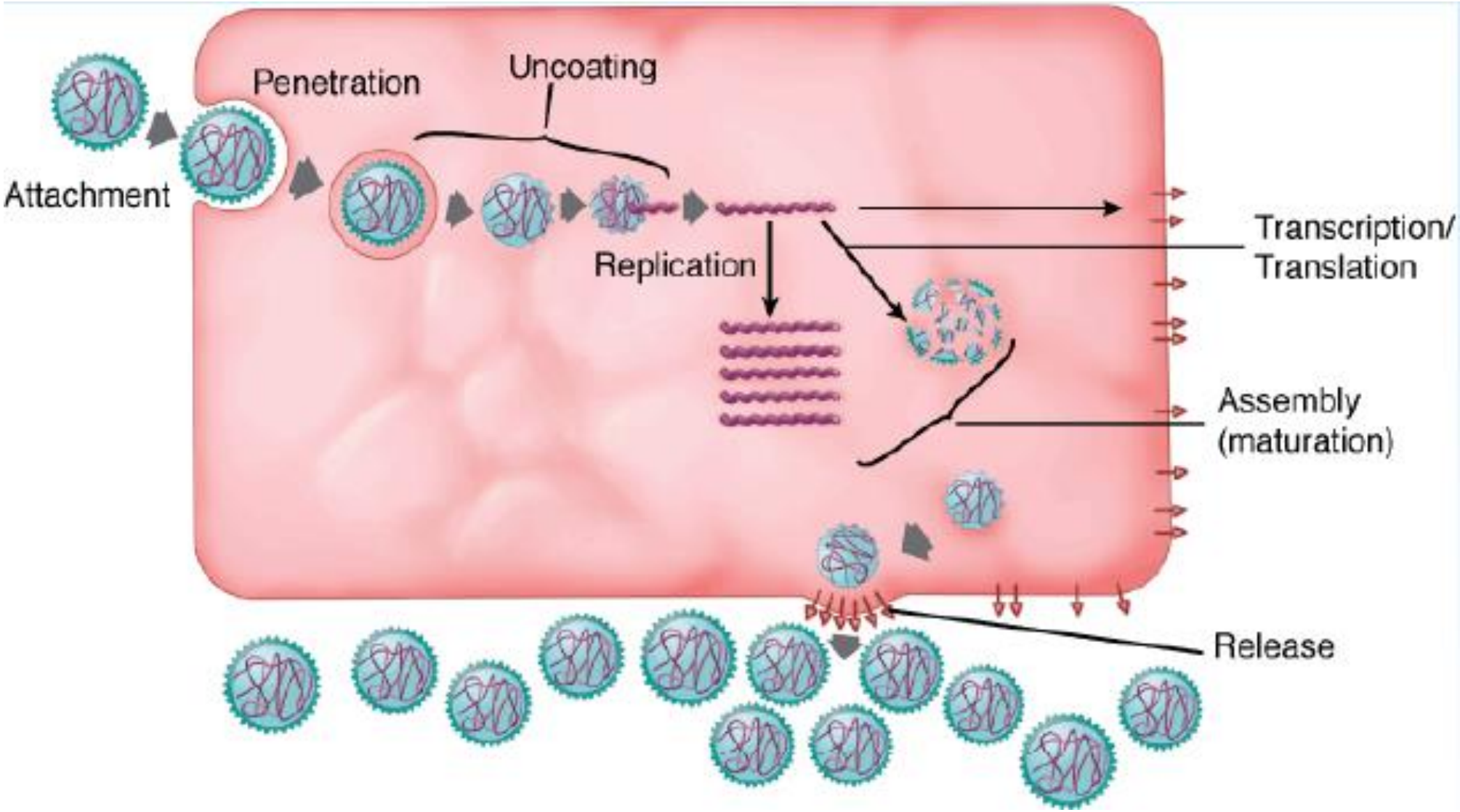
Stages of virus replication

- **Phase III: Assembly, Release, Maturation.**

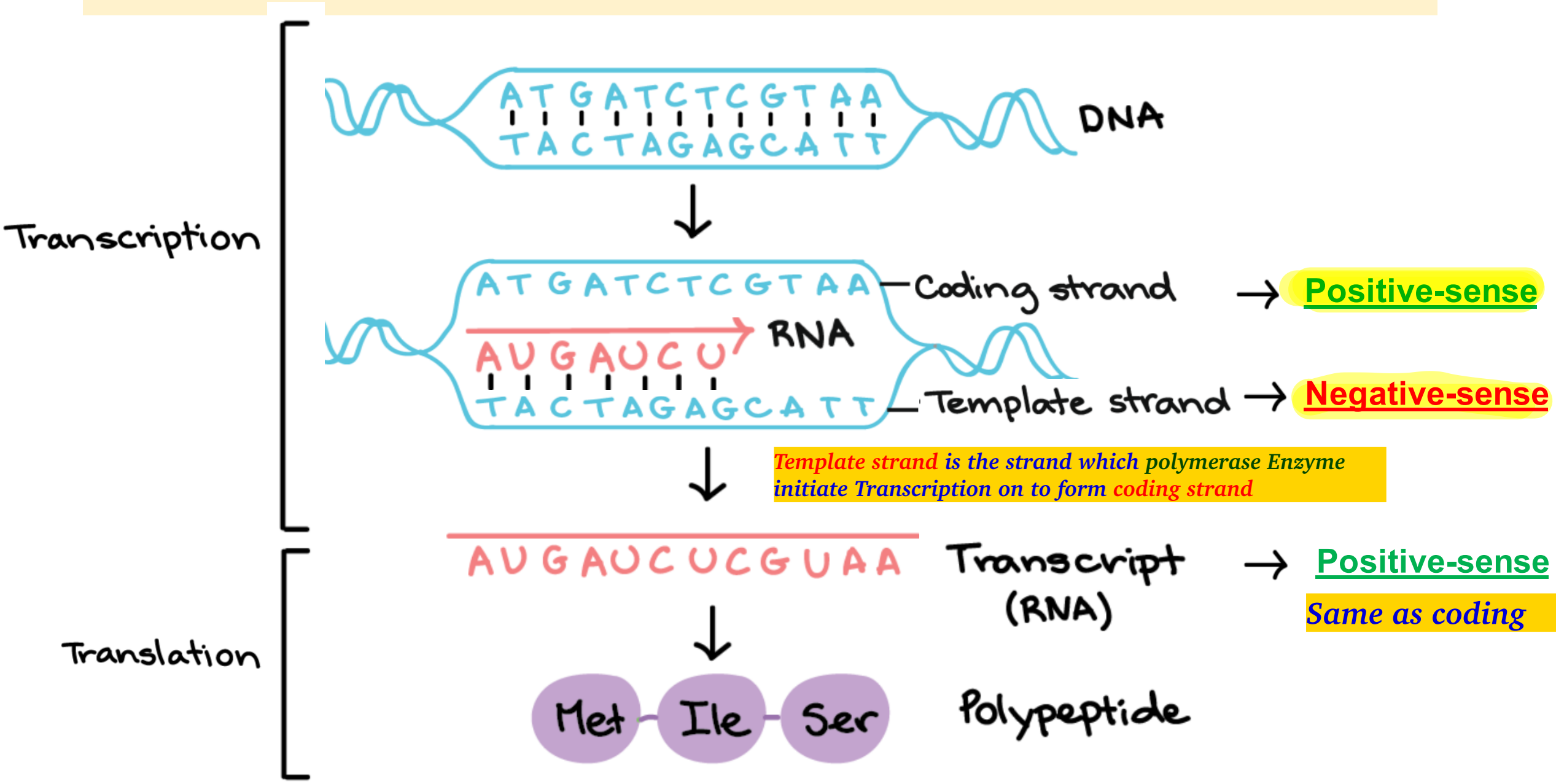
Maturation:

- At this stage of the lifecycle normally the virus becomes infećtious.
- Usually it involves structural changes in the particle, often resulting from specific cleavage of capsid proteins to form the mature products, which frequently leads to a conformational change in the capsid *Spike proteins*

Generalized Model of Viral Replication Cycle



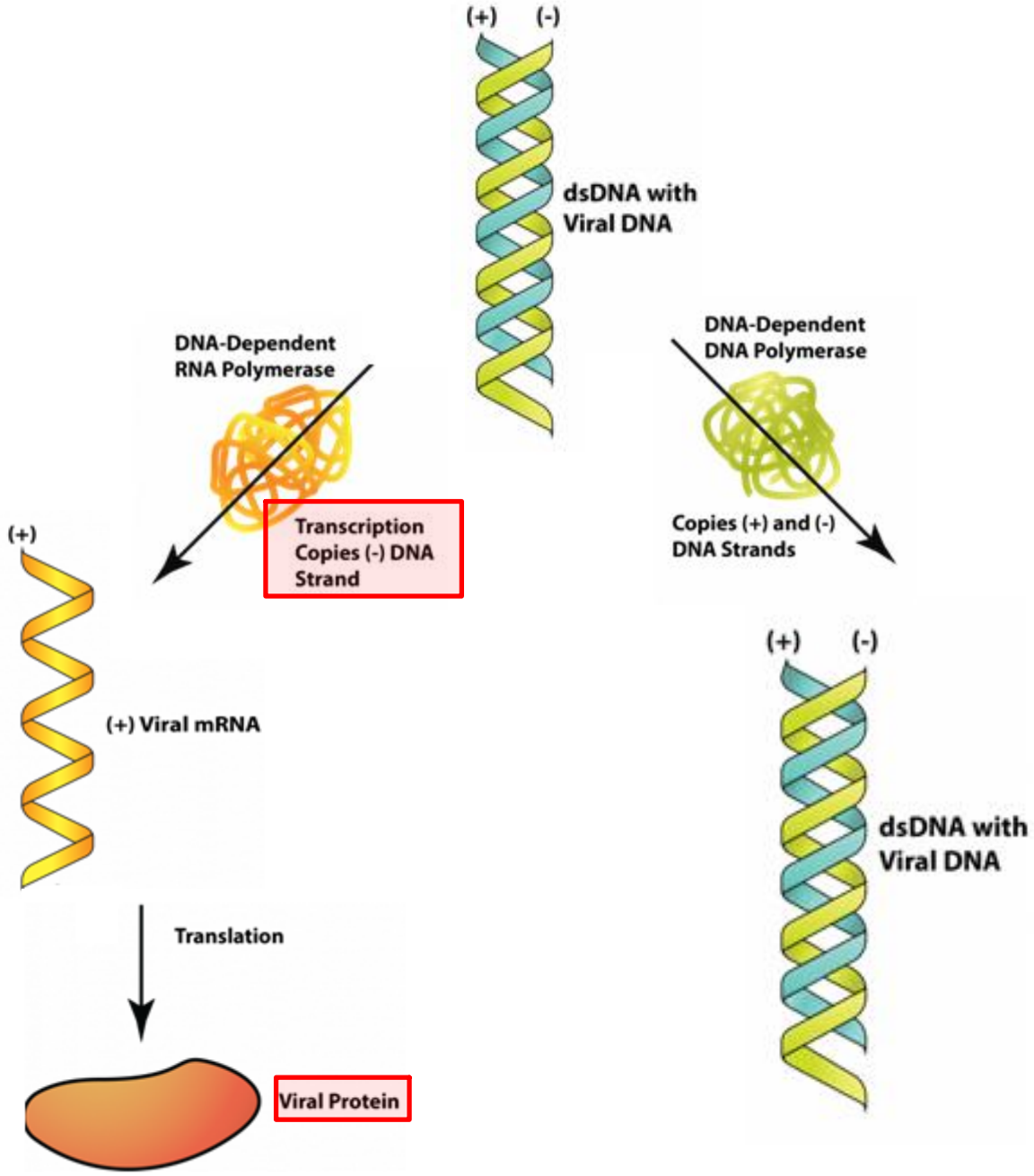
Negative vs. Positive Sense Strand of DNA and RNA



Replication of dsDNA Virus

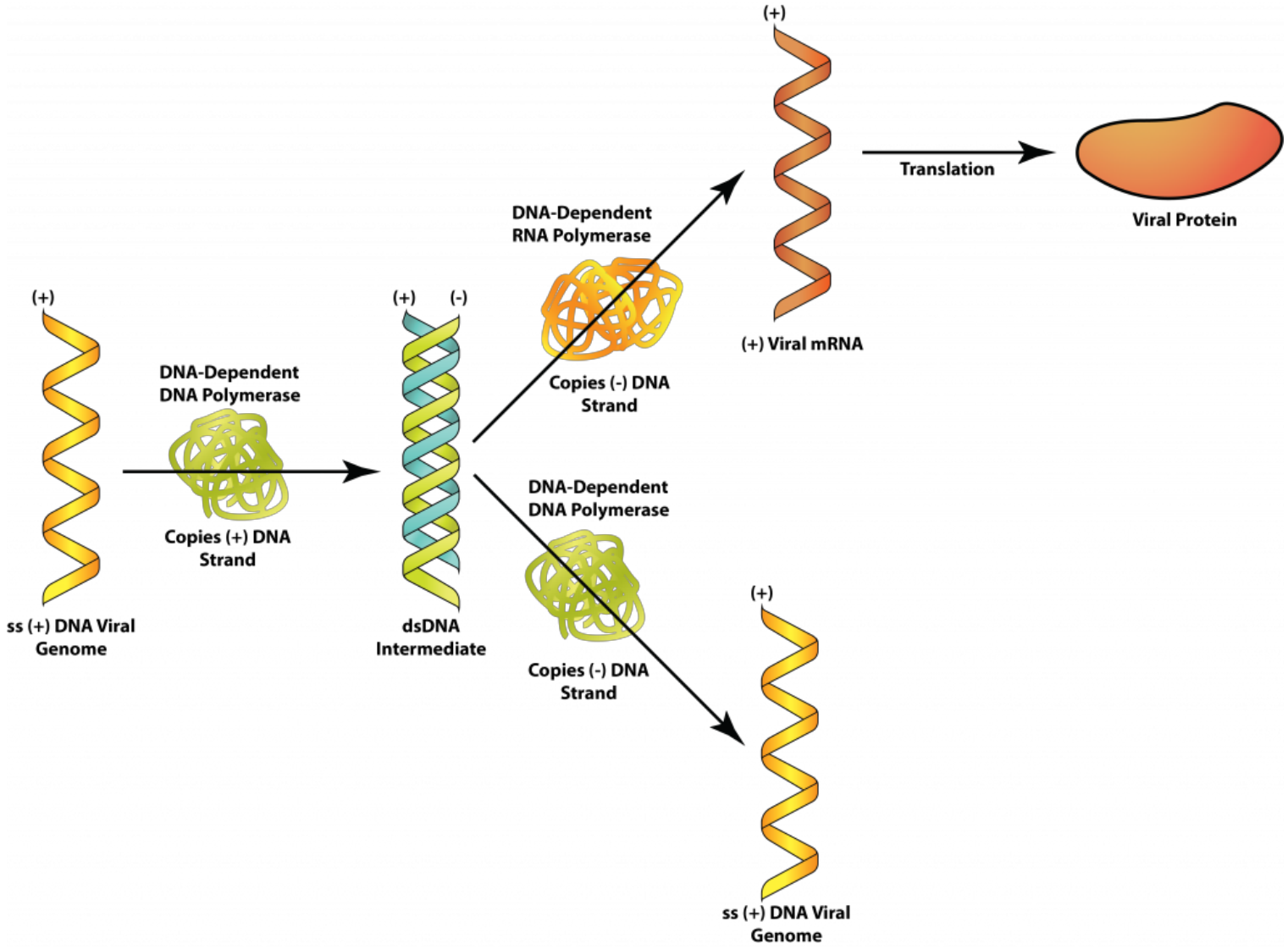
- The replication of dsDNA viruses is a straight-forward.
- They use the cell's replication machinery to transcribe their genome into mRNA immediately.
- Host enzymes for mRNA synthesis and DNA replication are available in nucleus hence, it needs to enter the nucleus.

Example: papillomaviruses, polyomaviruses, adenoviruses and herpesviruses.



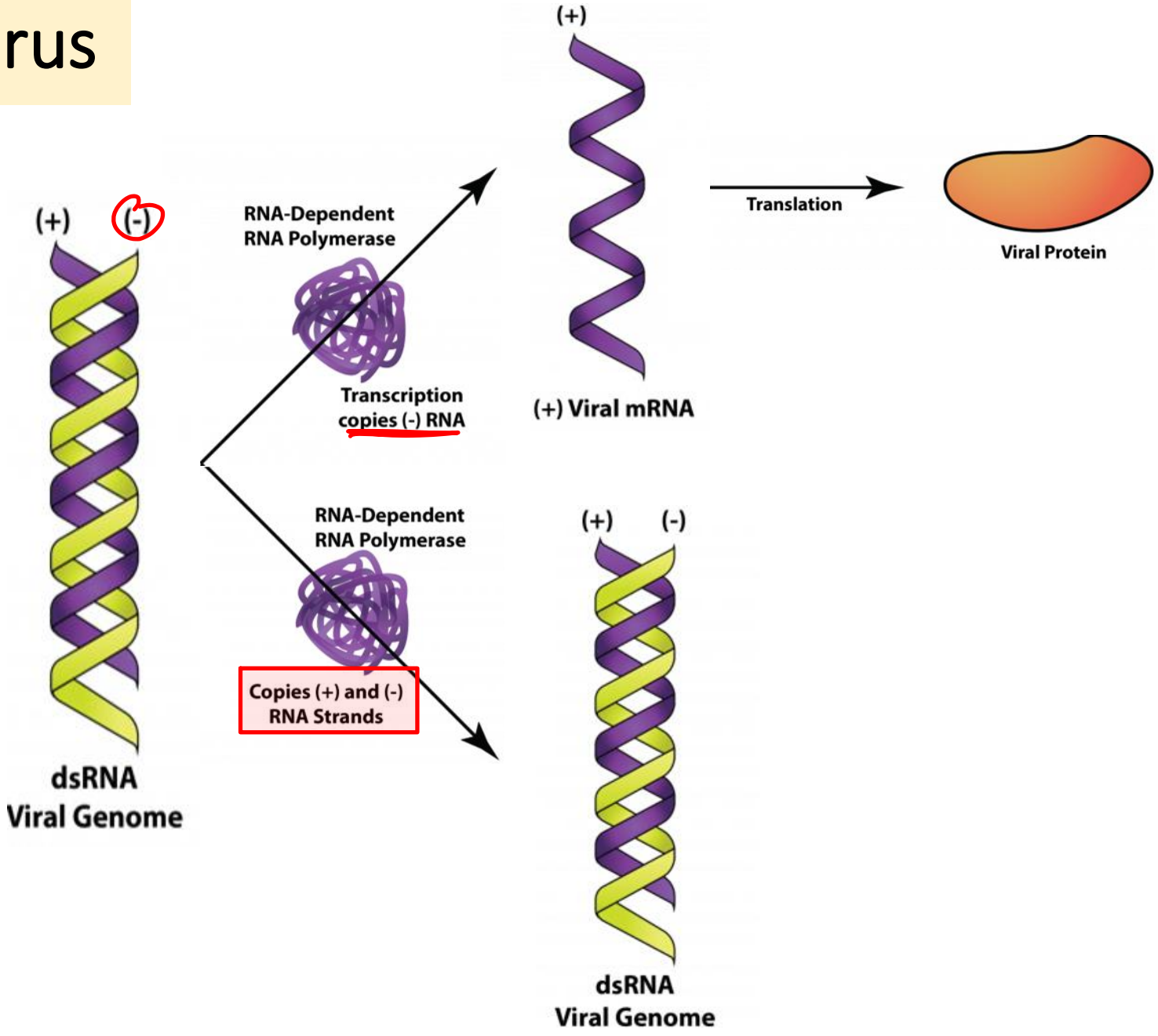
Replication of +ve and -ve ssDNA Virus

- This can be used to both manufacture viral proteins and as a template for viral genome copies.
- For the minus-strand DNA viruses, the genome can be used directly to produce mRNA but a complementary copy will still need to be made, to serve as a template for viral genome copies.



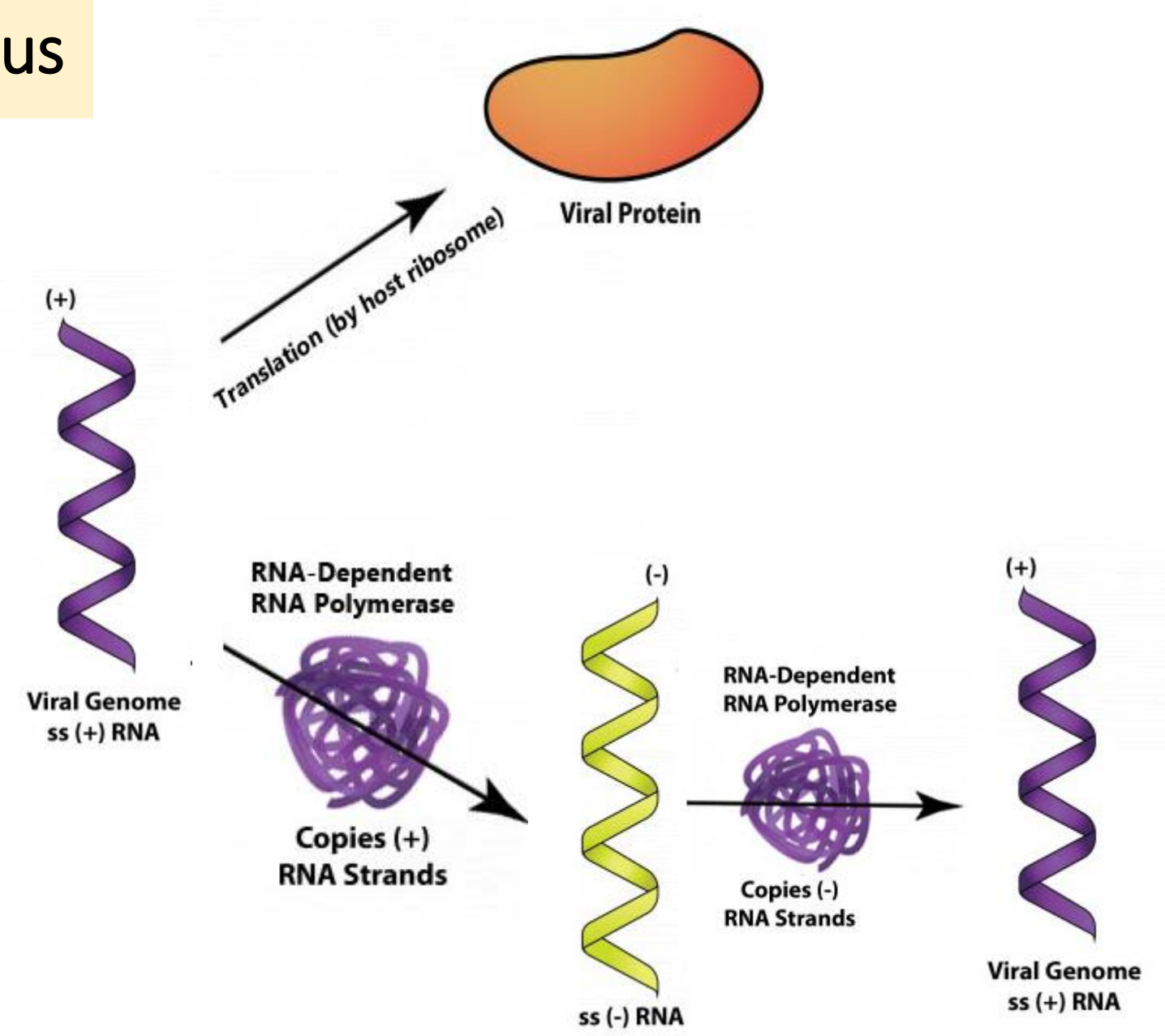
Replication of dsRNA Virus

- Double-stranded RNA viruses infect bacteria, fungi, plants, and animals, such as the rotavirus that causes diarrheal illness in humans.
- The viral RNA-dependent RNA polymerase acts as both a **transcriptase** to transcribe mRNA, as well as a **replicase** to replicate the RNA genome.
- Prokaryotic and eukaryotic cells do not carry **RdRp**.



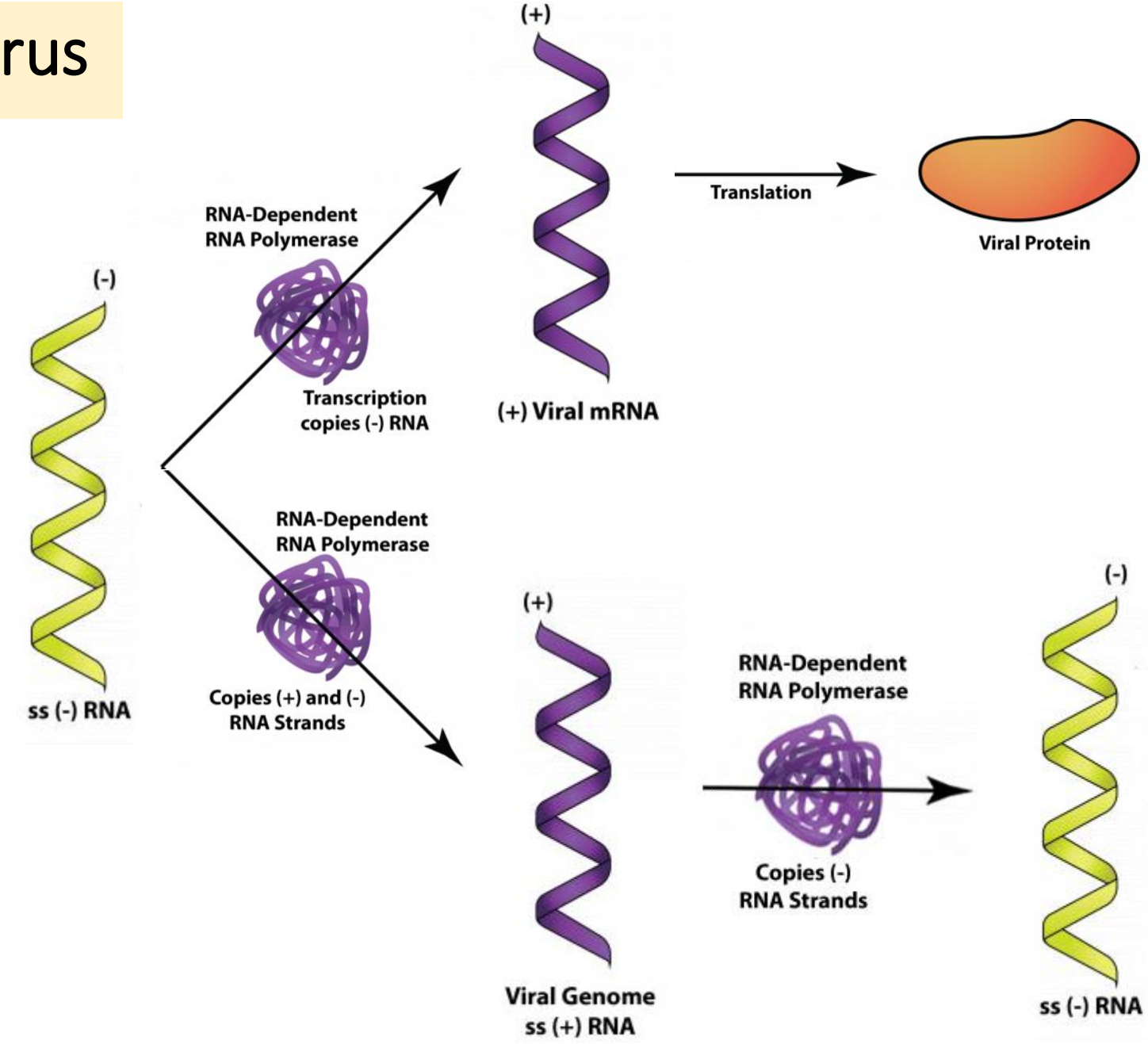
Replication of (+) ssRNA Virus

- Viruses with plus-strand RNA, such as poliovirus, can use their genome directly as mRNA with translation by the host ribosome occurring as soon as the unsegmented viral genome gains entry into the cell.
- One of the viral genes expressed yields an RNA-dependent RNA-polymerase (or RNA replicase), which creates minus-strand RNA from the plus-strand genome.
- The minus-strand RNA can be used as a template for more plus-strand RNA, which can be used as mRNA or as genomes for the newly forming viruses.

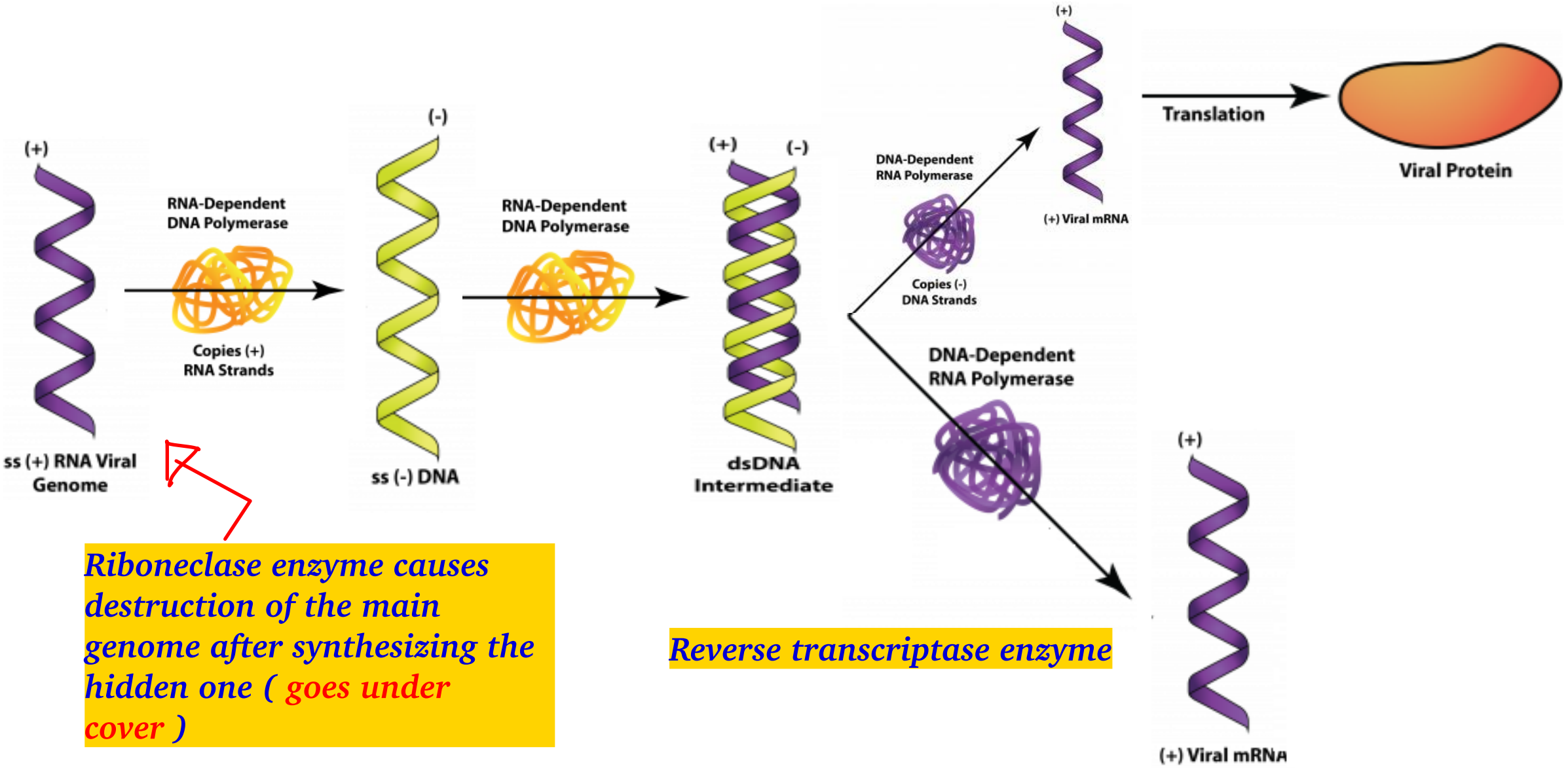


Replication of (-) ssRNA Virus

- Minus-strand RNA viruses include many members notable for humans, such as influenza virus, rabies virus, and Ebola virus.
- Since the genome of minus-strand RNA viruses cannot be used directly as mRNA, the virus must carry an RNA-dependent RNA-polymerase within its capsid.
- Upon entrance into the host cell, the plus-strand RNAs generated by the polymerase are used as mRNA for protein production.
- When viral genomes are needed the plus-strand RNAs are used as templates to make minus-strand RNA.



Replication of Retrovirus



Riboneclase enzyme causes destruction of the main genome after synthesizing the hidden one (goes under cover)

Reverse transcriptase enzyme

Replication of Retrovirus

Despite the fact that the retroviral genome is composed of +ssRNA, it is not used as mRNA. Instead, the virus uses its reverse transcriptase to synthesize a piece of ssDNA complementary to the viral genome. The reverse transcriptase also possesses **ribonuclease** activity, which is used to degrade the RNA strand of the RNA-DNA hybrid. Lastly, the reverse transcriptase is used as a DNA polymerase to make a complimentary copy to the ssDNA, yielding a dsDNA molecule. This allows the virus to insert its genome, in a dsDNA form, into the host chromosome, forming a **provirus**. Unlike a prophage, a provirus can remain latent indefinitely or cause the expression of viral genes, leading to the production of new viruses. Excision of the provirus does not occur for gene expression.