Environmental Microbiology

Course 5
Basic Virology

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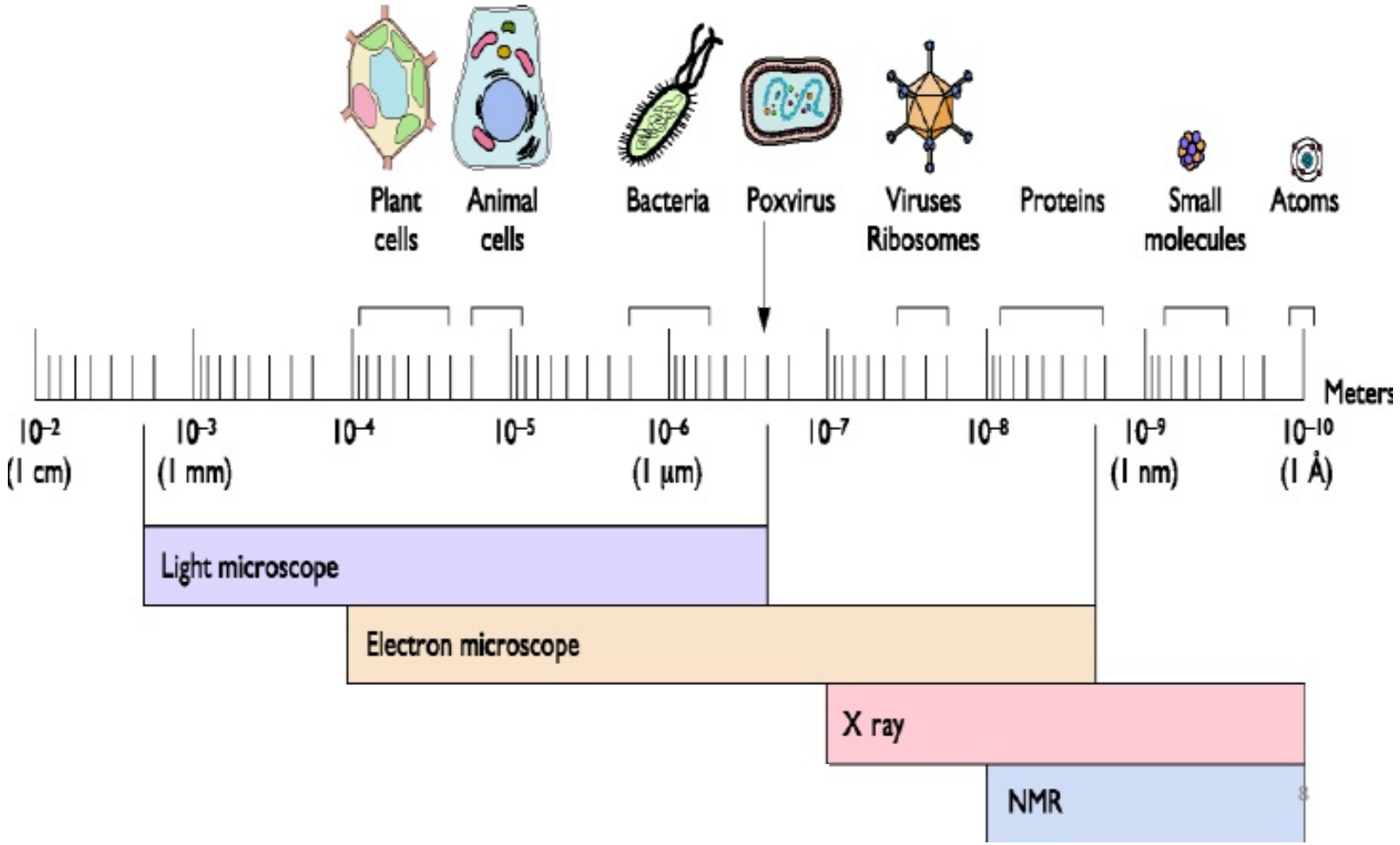
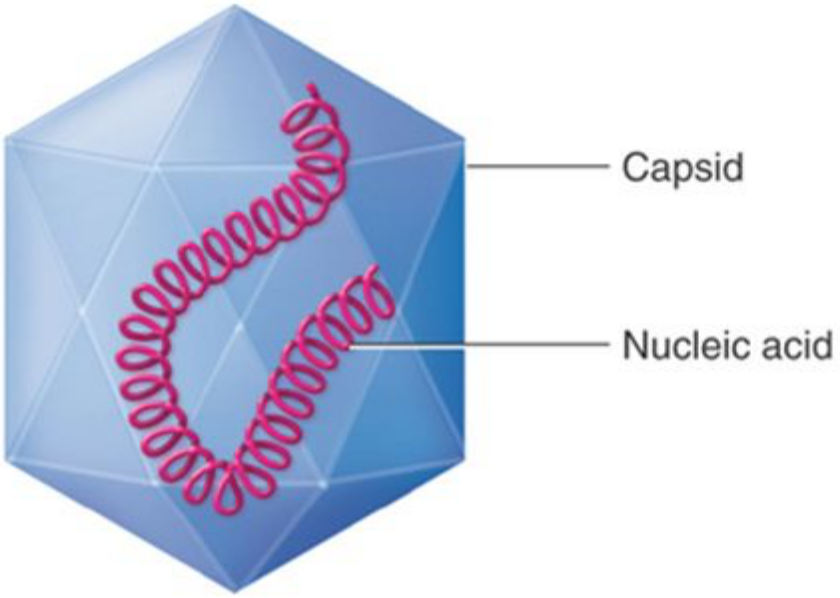
Introduction to Virology

- A virus is an obligate intracellular parasite containing genetic material surrounded by protein
- Virus particles can only be observed by an **electron microscope**

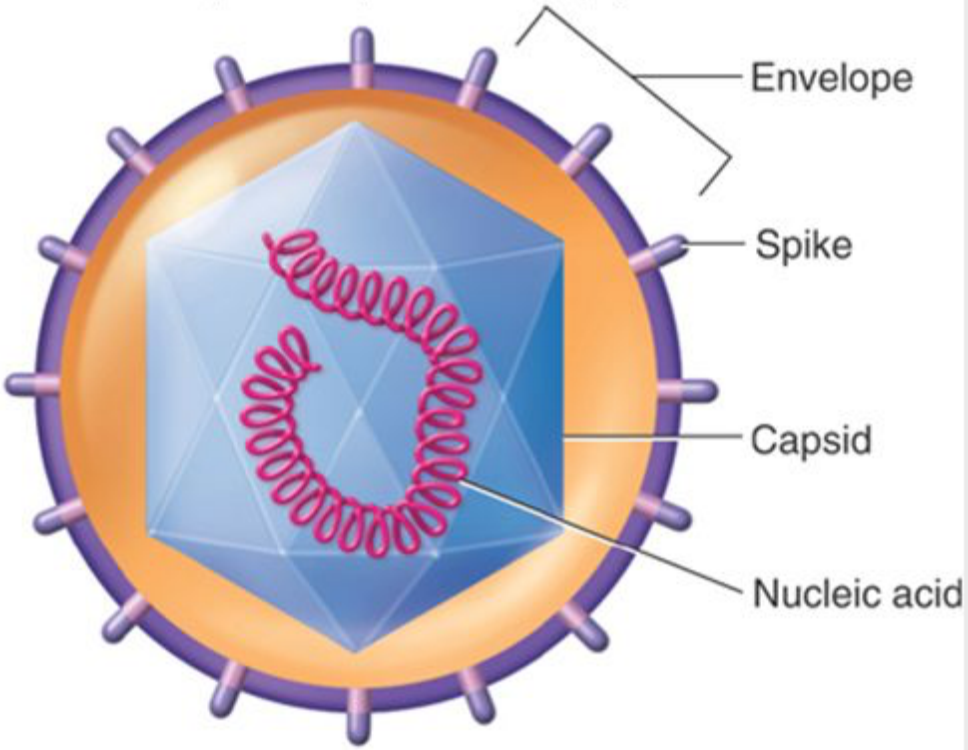
The size of viruses



Structure of Virus



(a) **Naked Nucleocapsid Virus**



(b) **Enveloped Virus**

Viruses are Cells?

- Bacteria, fungi, protozoa or worms are either single-celled or multi-cellular microorganisms.
- These cells can multiply independently, synthesize their own energy and proteins, and can be seen in the light microscope.
- In contrast, viruses are not cells
  - They can not show independent replication
  - They cannot synthesize their own energies and their own proteins,
  - They are too small to be seen in the light microscope.

General Features of Viruses

1. Viruses are DNA or RNA-containing particles, covered with a protective protein sheath.

Some viruses have an outer lipoprotein membrane located outside the sheath called as **the envelope**

Viruses do not have a nucleus, cytoplasm, mitochondria or ribosomes

General Features of Viruses

2. Since viruses do not produce their own energy and do not synthesize their own proteins, they have to reproduce in the host cell.

- These are only obligatory intracellular parasites because they can reproduce in the host cell.

General Features of Viruses

3. Unlike normal cells, viruses do not multiply by division or mitosis. A virus can replicate itself by performing host-related replication.

D 31. Describe the similarities and differences between bacteria and viruses.

	Bacterium	Virus
Living Attributes	Living	Opinions differ on whether a virus is a form of life or organic structure that interacts with living organisms.
Cell Number	Unicellular (one cell)	No cells; not living?
Structures	DNA, RNA, cell wall, cell membrane	DNA or RNA enclosed inside a coat of protein
Ribosomes	Present	Absent
Enzymes	Yes	Yes – in some
Nucleus	No	No
May Cause Disease	Yes	Yes
Treatment	Antibiotics	Vaccines-prevent the spread and antiviral meds help to slow reproduction but cannot stop it completely.
Beneficial	Some beneficial – certain bacteria produce vitamins in gut; used to make yogurt , cheese	Specific viruses may be able to destroy tumors and may be useful in genetic engineering.
Reproduction	Fission –form of asexual reproduction	Invades host cell and takes over the cell causing it to make copies of the viral DNA or RNA. Destroys the host cell releasing new viruses.
Size	Larger (1,000nm)	Smaller (20-400nm)

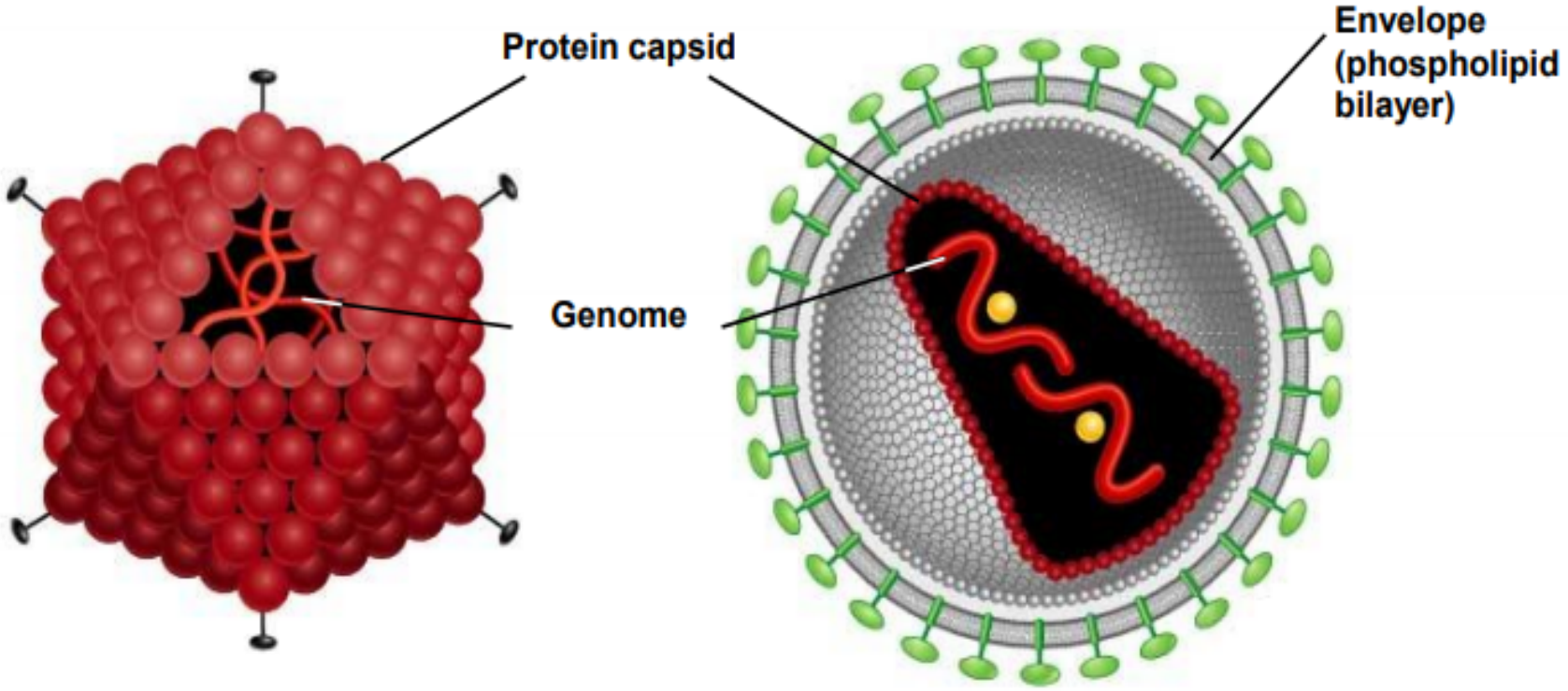
Size and Shape of Viruses

- The diameter of the viruses varies between **20-300 nm.**
- As a shape, they have complex geometric structures such as spheres, rods, bullets or bricks.

Viral Structures

- One virion is consisted of 3 structures:

1. Core
2. Capsid
3. Envelope


Protein capsid
Genome
Envelope
(phospholipid
bilayer)

Viral Core Structure

- Viral core includes;

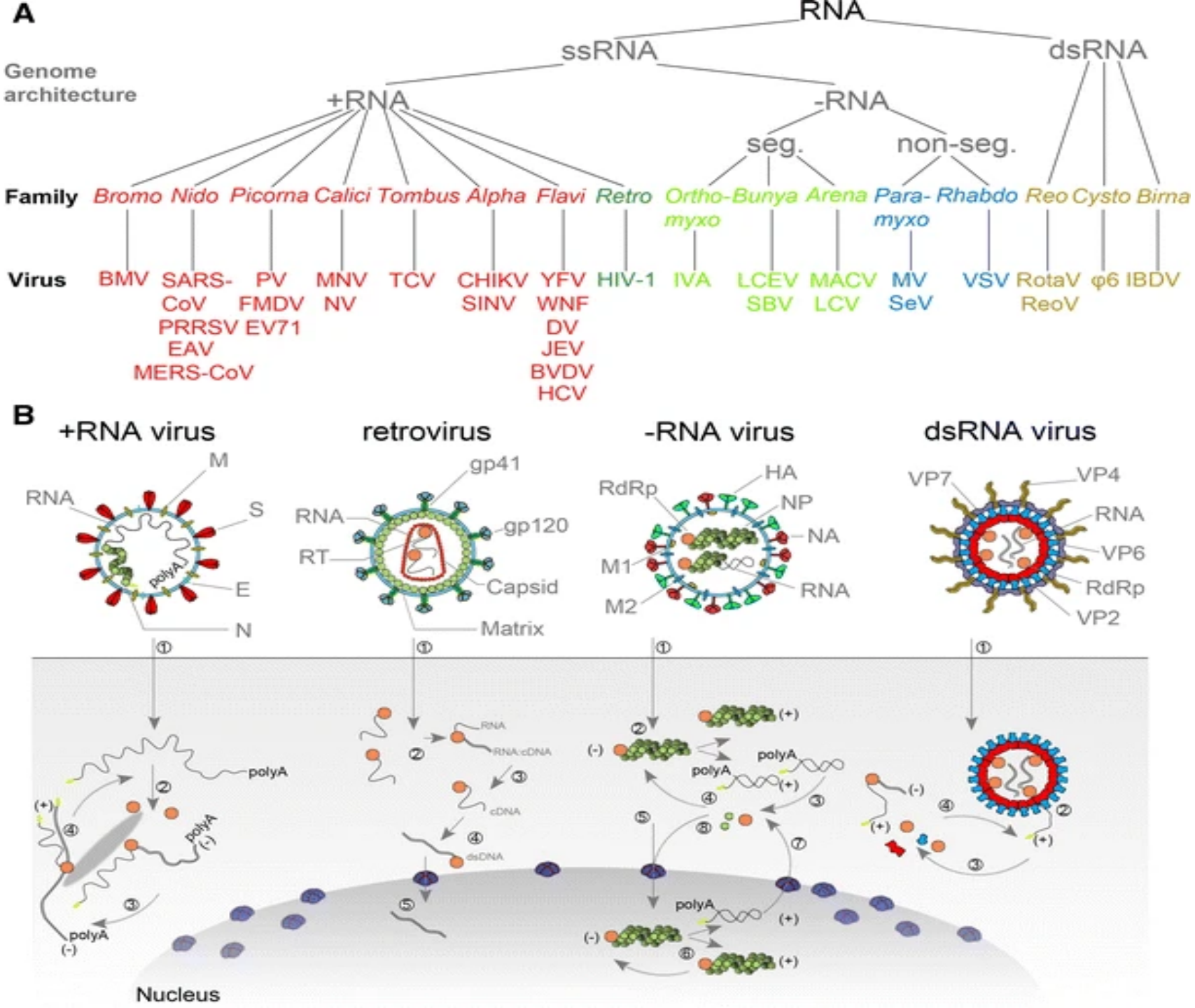
1. Nucleic acids
2. Enzymes
3. Viral inner proteins

Viral Nucleic Acids

- The virus particles can have 4 different genomes:

1. single-stranded DNA
2. double stranded DNA
3. single-stranded RNA or
4. double-stranded RNA

- No organisms other than viruses have genetic material consisting of single-stranded DNA, single-stranded RNA and double-stranded RNA
- Nucleic acids of viruses can be linear or circular


A
RNA
ssRNA
dsRNA
Genome architecture
+RNA
-RNA
seg.
non-seg.
Family
Bromo Nido Picorna Calici Tombus Alpha Flavi Retro Ortho-myxo Bunya Arena Para-myxo Rhabdo Reo Cysto Birna
Virus
BMV SARS-CoV PRRSV EAV MERS-CoV PV FMDV EV71 MNV NV TCV CHIKV SINV YFV WNF DV JEV BVDV HCV HIV-1 IVA LCEV SBV MACV LCV MV SeV VSV RotaV ReoV φ6 IBDV
B
+RNA virus
retrovirus
-RNA virus
dsRNA virus
M
RNA
S
E
N
polyA
gp41
gp120
RT
Capsid
Matrix
HA
RdRp
NP
NA
M1
M2
VP7
VP4
VP6
VP2
Nucleus

- Single stranded RNA viruses can be classified according to the sense or polarity of their RNA into negative-sense and positive-sense.
- Positive-sense viral RNA is similar to mRNA and thus can be immediately translated by the host cell. They do not include their own RNA polymerase enzyme.
- Negative-sense viral RNA is complementary to mRNA and thus must be converted to positive-sense RNA by an RNA polymerase before translation.

Viral Enzymes

RNA Polymerase Enzyme:

- They transcribe viral RNA into mRNA.
- This enzyme is only found in viruses with negative polarity.
- This enzyme is not present in the human or animal cell.

DNA Polymerase Enzyme:

- It is an enzyme that synthesizes DNA from DNA.
- It is found in DNA viruses.

Reverse Transcriptase Enzyme:

- It is an enzyme that directs DNA synthesis from RNA.
- The enzyme that enters the cell through this enzyme creates double-stranded DNA from the RNA to integrate this newly formed DNA with the cell chromosome.
- It is an enzyme found in retroviruses.

Viral Capsid

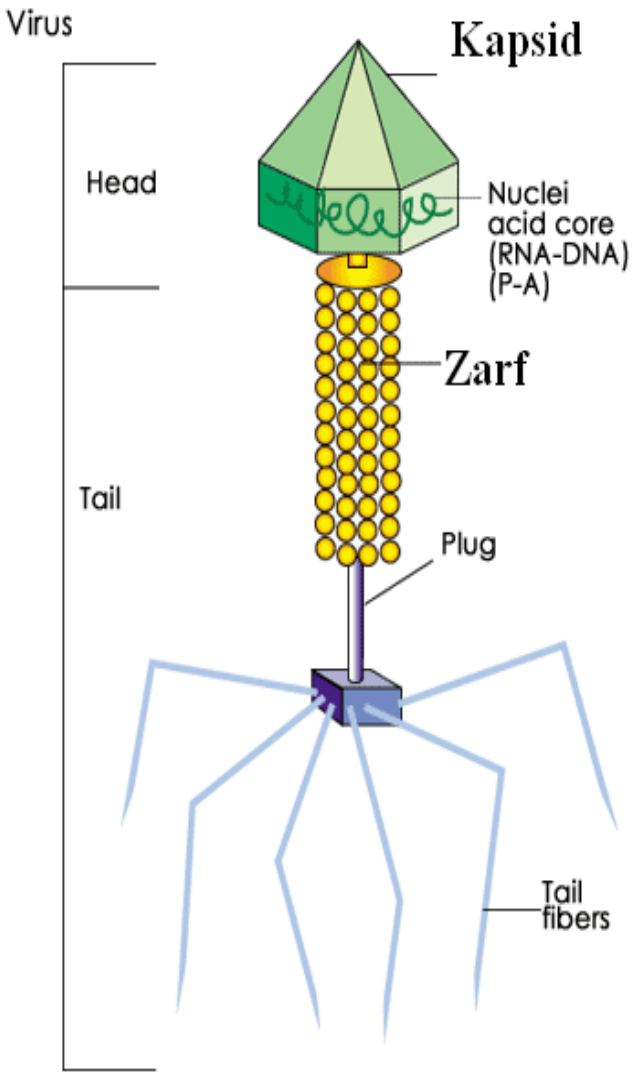
- The nucleic acid is encapsulated by a protein sheath called capsid, which is composed of subunits called capsomer.
- The structure consisting of the nucleic acid genome and capsid proteins is called **nucleocapsid**.
- The sequence of the capsomers gives the geometric shape of the virus

Functions of Viral Capsid

- Gives the morphological character of the virus particle.
- Protects the viral nucleic acid all around and protects it from external influences and nuclease.
- Creates a suitable film for packaging the viral nucleic acid.
- It gives the virus its antigenic property.
- Gives the specificity of the virus to the host cell

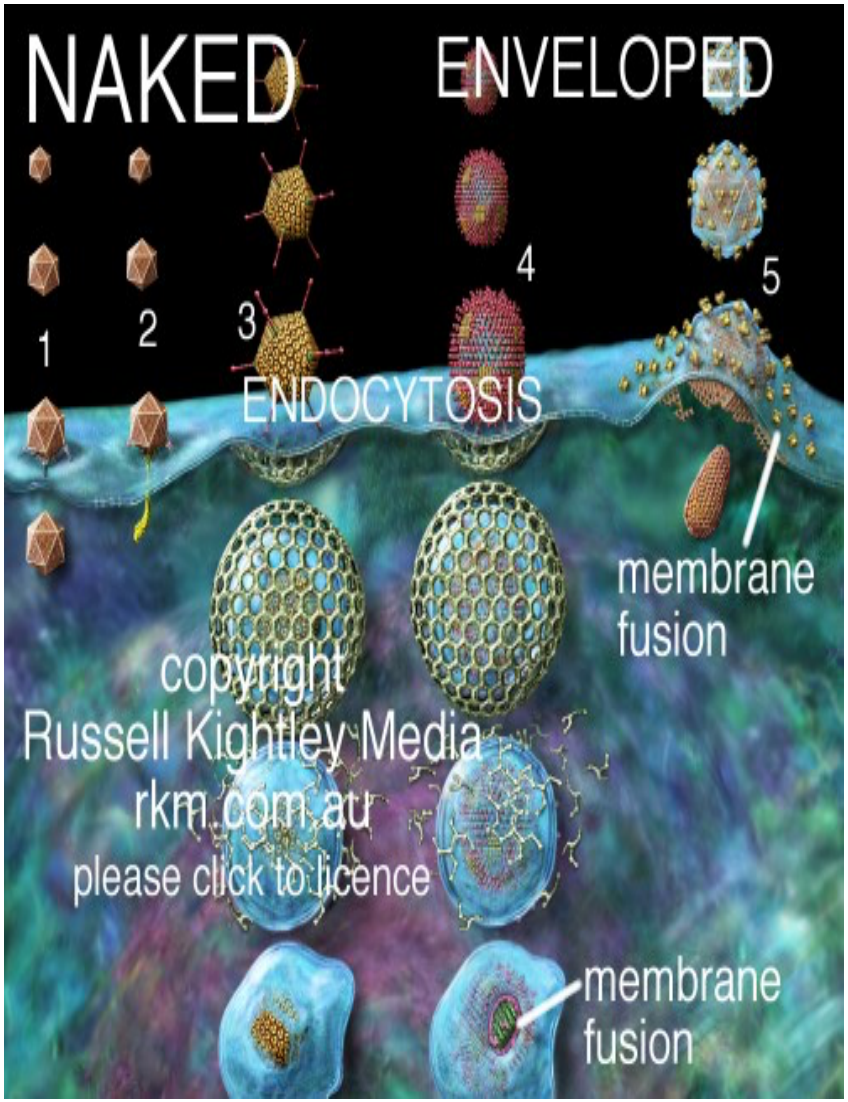
Viral Envelope

- The envelope is a lipoprotein membrane composed of lipid derived from the host cell membrane with virus-specific protein
- Glycoproteins are also present on the envelope surface in the form of protrusions that allow the binding of host cell receptors to the virus during cell entry.



Viral Envelope

- Viral envelope; occurs when the virus leaves the cell with an event called budding.



Viral Envelope

- Envelopes of most viruses are generated from the outer membrane of the cell.
- Only Herpes viruses produce envelopes from the cell's nuclear membrane.
- In general, the presence of the envelope causes virus insensitivity.
- Enveloped viruses are much more susceptible to heat, detergents and lipid solvents such as alcohol and ether compared to non-enveloped (nucleocapsid) viruses consisting only of nucleic acid and capsid proteins

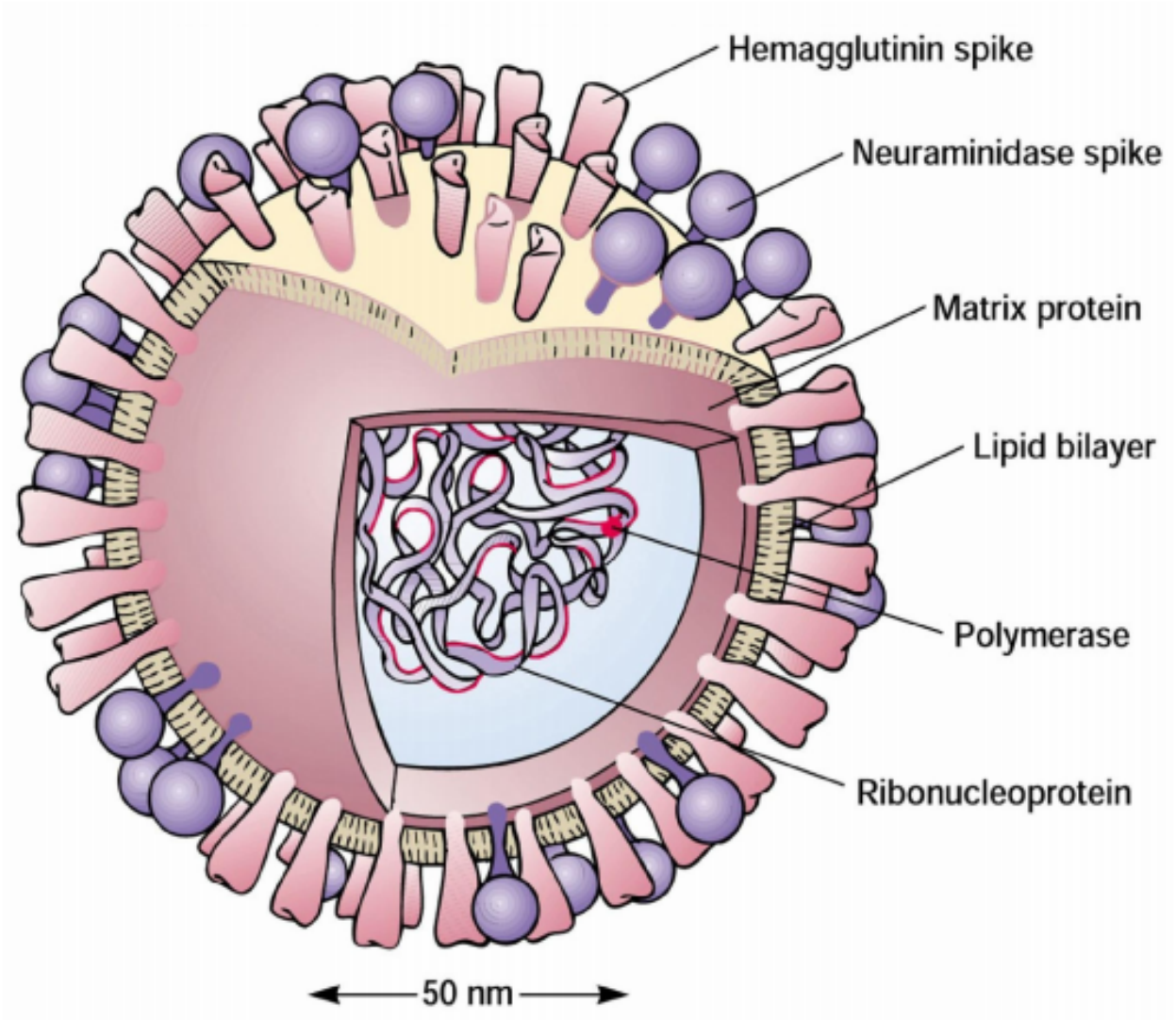
Viral Peplomers

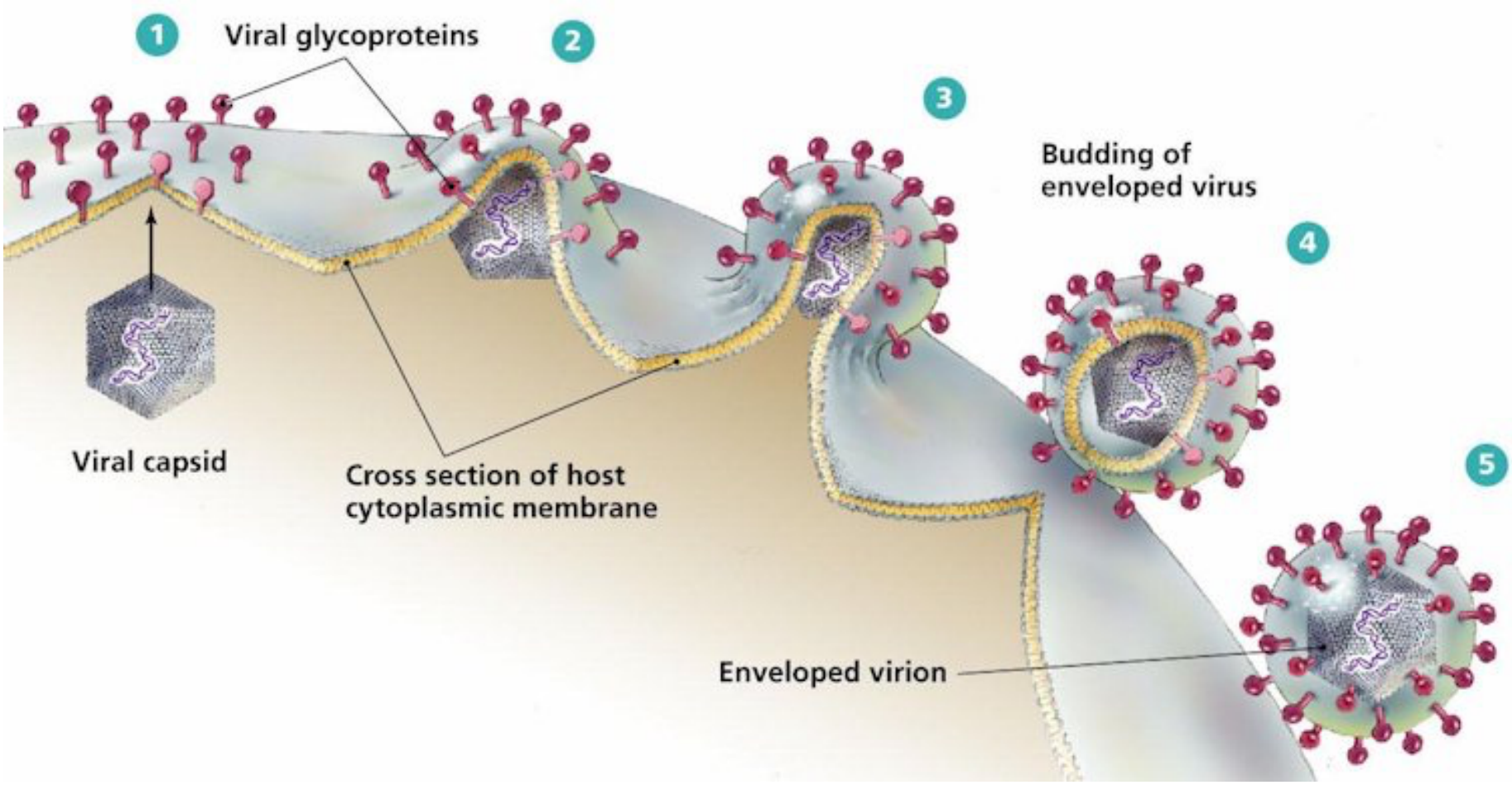
- Peplomers are virus-specific glycoprotein units that compose antigenic structures of viruses
- These glycoproteins are found as spikes at outer layer of envelope
- Hemagglutinin
- Neuroaminidase

Viral Peplomers

- They provide the adsorption of virus onto host cells
- Increase their pathogenicity
- Lead to hemaglutination of mammalian blood cells especially eryhtrocytes
- Cause fusions of cells during measles

Enveloped viruses –Influenza virus




1
Viral glycoproteins
2
3
Budding of
enveloped virus
4
5
Viral capsid
Cross section of host
cytoplasmic membrane
Enveloped virion

Enveloped Viruses

- Enveloped viruses are mostly transmitted by direct contact, such as blood or sexual contact. These are;
- Human immunodeficiency virus (HIV),
- Herpes simplex virus type 2
- Hepatitis B and
- Hepatitis C viruses.
- Other enveloped viruses are transmitted directly with insect bites (yellow fever virus, West Nile virus) or by bite of the animal (rabies)

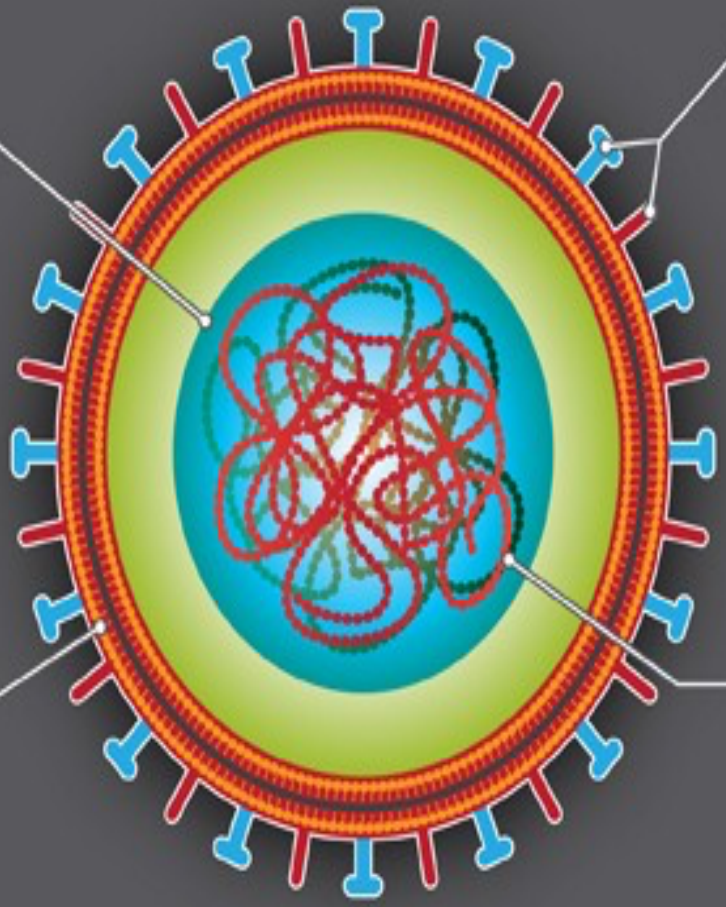
Non-enveloped Viruses

- Almost all non-enveloped viruses transmitted by feces-mouth route. These are:
- Hepatitis A virus,
- Poliovirus
- Coxsakie virus
- Echovirus,
- Norwalk virus and
- Rotavirus.

Viral Proteins

- Viral proteins have many important functions:
- External capsid proteins protect genetic material
- It mediates the binding of the virus to specific receptors on the host cell surface.
- The interaction between viral proteins and the cell receptor is the main determinant of the host type and organ specificity that the virus infects.

VIRUS STRUCTURE



Capsid
The capsid contains the virus' genetic material (DNA or RNA)

Surface proteins
These help the virus recognise and bind to cells in the host organism

Viral envelope
The viral envelope is made from fatty lipid molecules taken from cells in the host

Virus genetic material
(DNA or RNA)
The virus' genetic material contains the instructions for making new copies of the virus

Atypical Virus-like Agents

- There are 4 exceptions that do not comply with the virus definition:

1. Defective Viruses
2. Pseudovirions
3. Viroids
4. Prions

1. Defective Viruses

- They contain viral nucleic acid and protein, but they cannot be replicated without a "helper" virus performing their incomplete functions.
- In defective viruses, there has often been rupture in either part of the mutation or genetic materials.

2. Pseudovirions

- **The capsid carries the DNA of the host cell instead of the viral DNA.**
- During infections caused by some viruses, the host cell DNA is cleaved and some of these fragments are located in the capsid protein. Thus, pseudovirions occur.
- Pseudovirions can infect cells but not replicate

3. Viroids

- **A protein consists of only a single circular RNA molecule without cover or envelope.**
- RNA is relatively small and is not considered to encode any protein.
- However, viroids are replicated by an unknown mechanism.
- Although they cause many plant diseases, they do not cause any disease in humans.

4. Prions

- It is the only protein-generated infective protein particles; there is no specific nucleic acids.
- In humans, it causes Creutzfeldt-Jacob D (encephalopathy-like nervous system disease), while leading to mad cow disease in sheep.
- Since there is no DNA and RNA in the prions, it differs significantly from the viruses.

Prions

- Prions consist of a single glycoprotein having a molecular weight of 27,000-30,000.
- When used as a model of mad veal prions, it has been found that this protein is encoded by a single cellular gene, which is equal to the number of infected and non-infected animal cells.
- Prions are more resistant to ultraviolet radiation and heat inactivation than viruses.
- They are resistant to formaldehyde and nucleases.
- On the other hand, prions are inactivated by hypochloryte, NaOH and autoclaving.

Viruses, Viroids and Prions

Comparison of Viruses, Viroids, and Prions

	Virus	Viroid	Prion
Nucleic acid	+ (ssDNA, dsDNA, ssRNA, or dsRNA)	+ (ssRNA)	−
Presence of capsid or envelope	+	−	−
Presence of protein	+	−	+
Need for helper viruses	+/− (Needed by some of the smaller viruses such as the parvoviruses)		
Viewed by	Electron microscopy	Nucleotide sequence identification	Host cell damage
Affected by heat and protein denaturing agents	+	−	−
Affected by radiation of enzymes that digest DNA or RNA	+	+	−
Host	Bacteria, animals, or plants	Plants	Mammals

Table 10-7 Microbiology, 7/e

❖ Characteristic between **virus**, **viroid** and **prion**

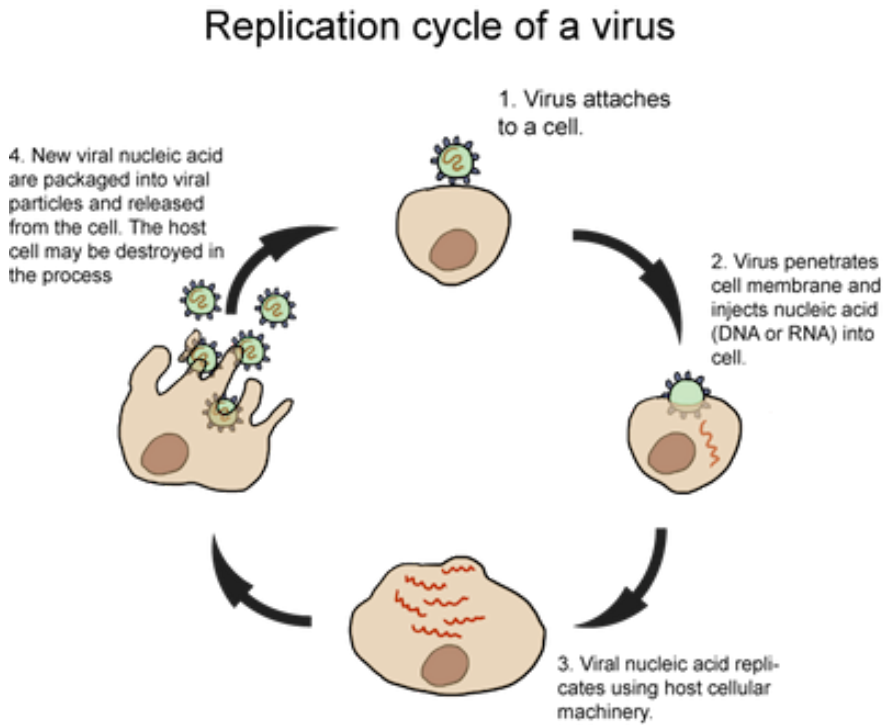
❖**Nucleic acid type**, NA strandedness, **host range** and **structural features**

Replication

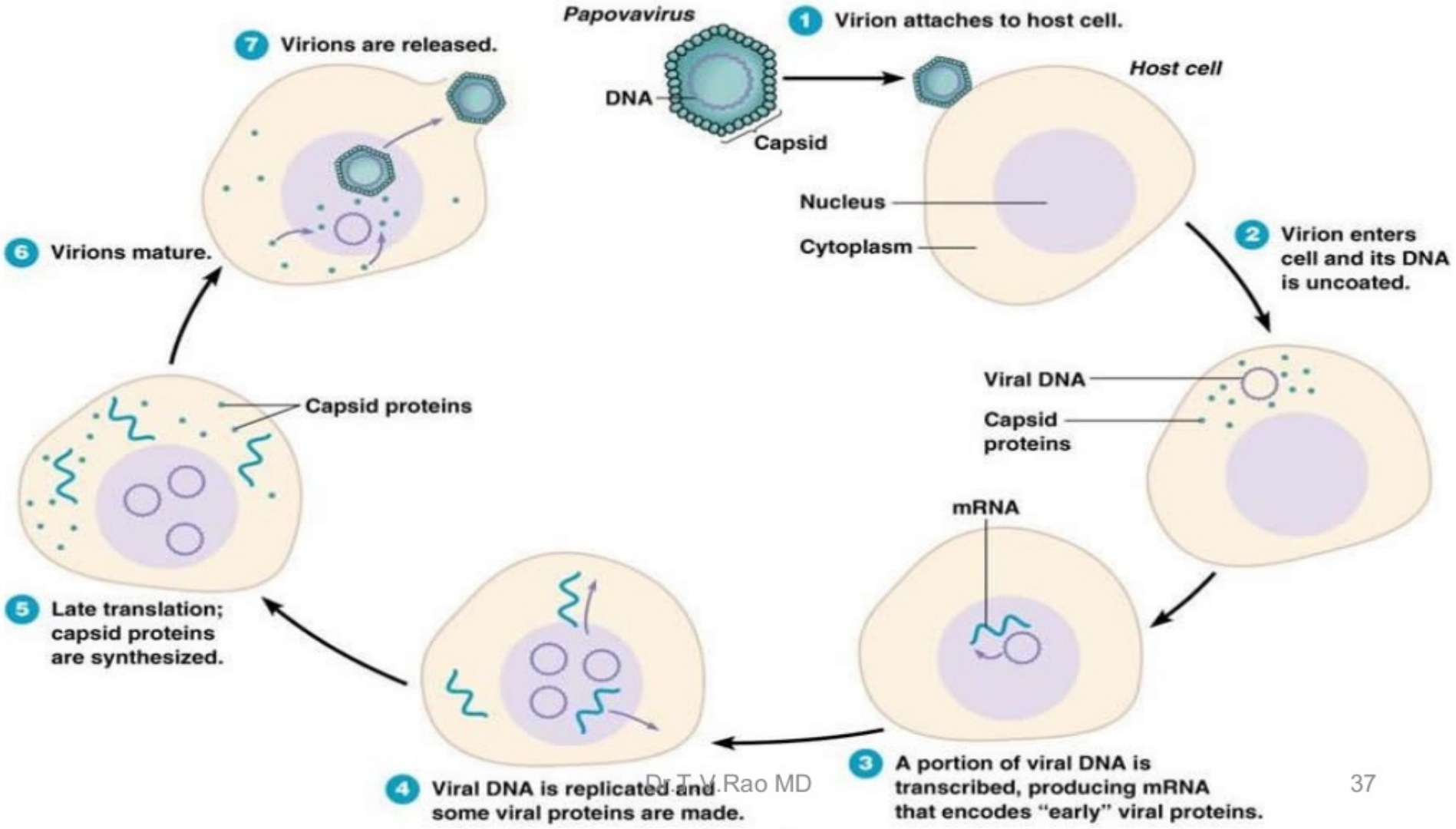
Viral Replication Stages

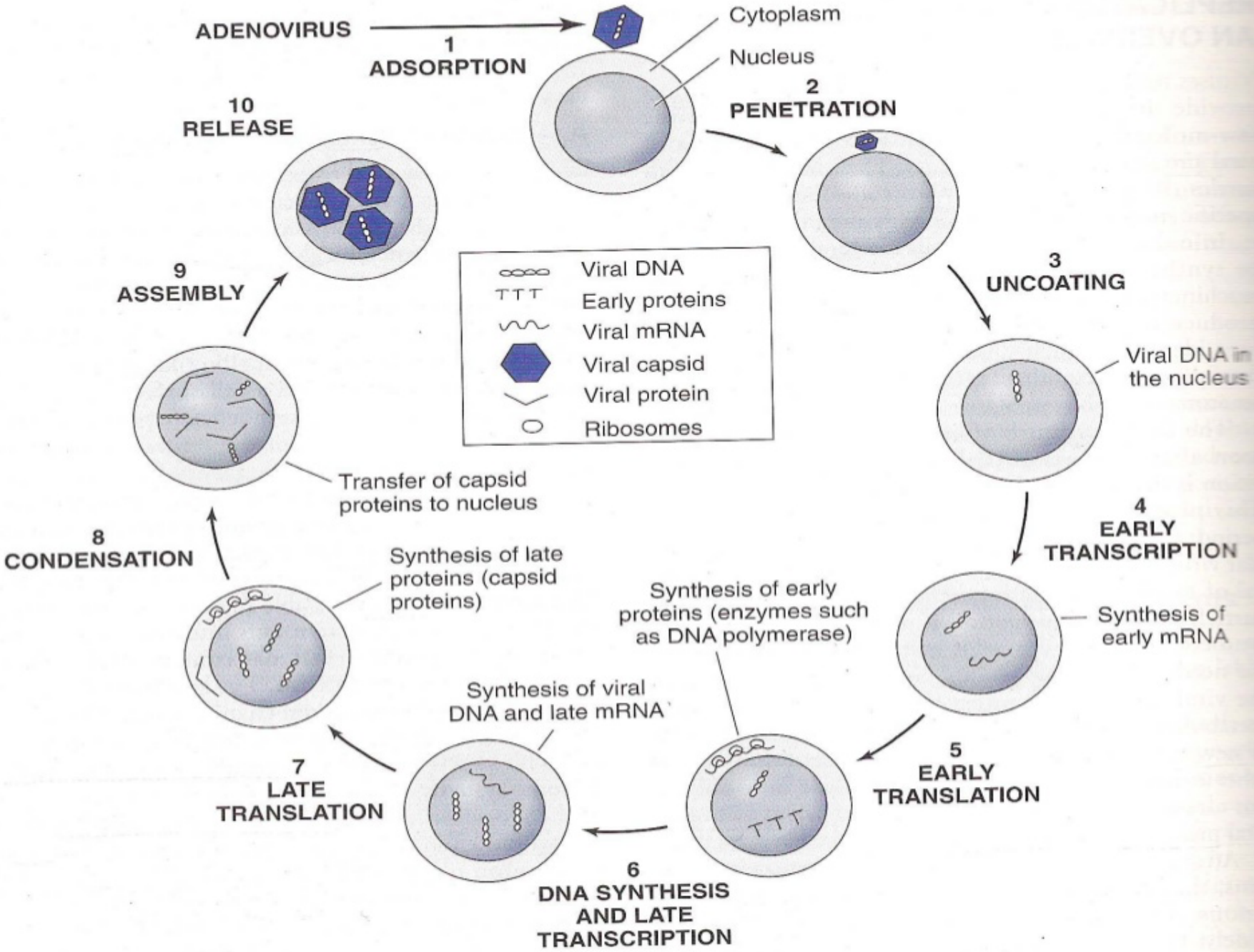
The reproductive cycle of the virus is generally expressed in 3 steps:

1. Early events: adhesion, penetration and uncoating
2. Moderate events: gene expression and genome replication
3. Late events: assembly and release



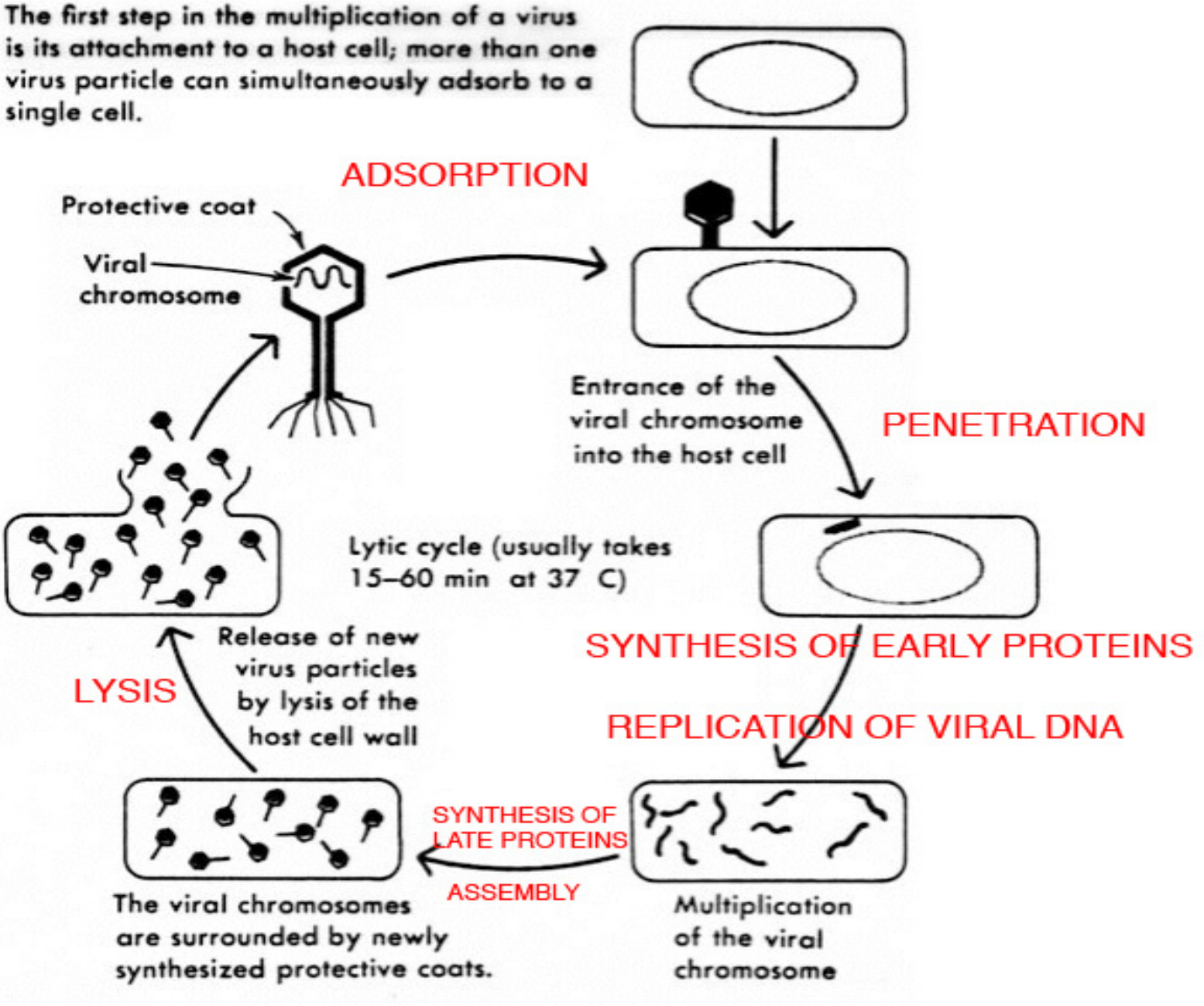
Synthesis of PapovaVirus




ADENOVIRUS
1
ADSORPTION
Cytoplasm
Nucleus
2
PENETRATION
3
UNCOATING
Viral DNA in the nucleus
4
EARLY
TRANSCRIPTION
Synthesis of early mRNA
5
EARLY
TRANSLATION
Synthesis of early proteins (enzymes such as DNA polymerase)
6
DNA SYNTHESIS
AND LATE
TRANSCRIPTION
Synthesis of viral DNA and late mRNA
7
LATE
TRANSLATION
Synthesis of late proteins (capsid proteins)
8
CONDENSATION
Transfer of capsid proteins to nucleus
9
ASSEMBLY
10
RELEASE
Viral DNA
Early proteins
Viral mRNA
Viral capsid
Viral protein
Ribosomes

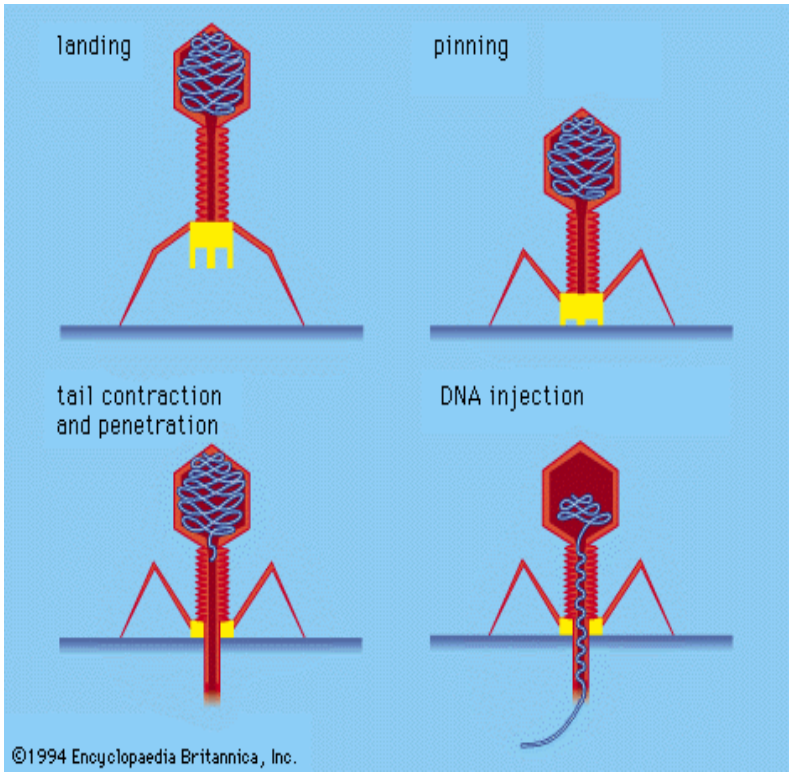
Attachment, Penetration and Uncoating

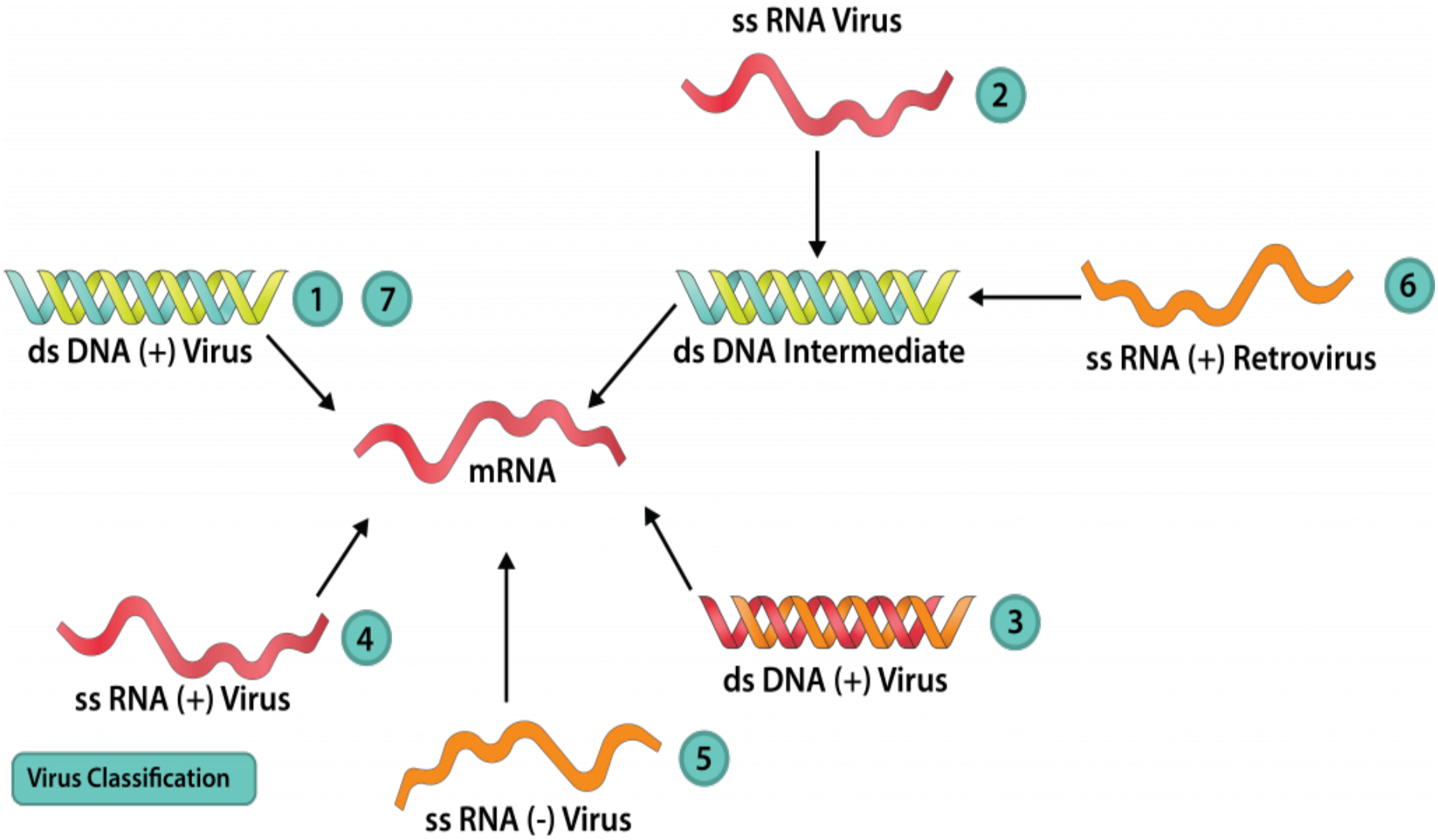
- Virion surface proteins bind to specific receptor proteins on the cell surface with weak bonds.
- The specificity of this binding determines the host diversity of the virus.
- Ex. The rabies virus can enter all mammalian cells while the poliovirus can only penetrate human and other primate cells.
- The organ specificity of the viruses is also governed by receptor interaction


The first step in the multiplication of a virus is its attachment to a host cell; more than one virus particle can simultaneously adsorb to a single cell.
ADSORPTION
Protective coat
Viral chromosome
Entrance of the viral chromosome into the host cell
PENETRATION
Lytic cycle (usually takes 15–60 min at 37 C)
SYNTHESIS OF EARLY PROTEINS
REPLICATION OF VIRAL DNA
Release of new virus particles by lysis of the host cell wall
LYSIS
SYNTHESIS OF LATE PROTEINS
ASSEMBLY
The viral chromosomes are surrounded by newly synthesized protective coats.
Multiplication of the viral chromosome

Bacteriophages

- Some bacterial viruses (bacteriophages) use a special mechanism that does not correspond to human, animal and plant viruses when entering bacteria.
- Some T-group bacteriophages infect Escherichia coli by binding the tail fibrils on the cell surface and eroding a portion of the cell wall with lysozyme in the tail.
- In the meantime, the tail sheath contracted viral DNA enters the cell through this tail so that the capsid proteins remain outside.



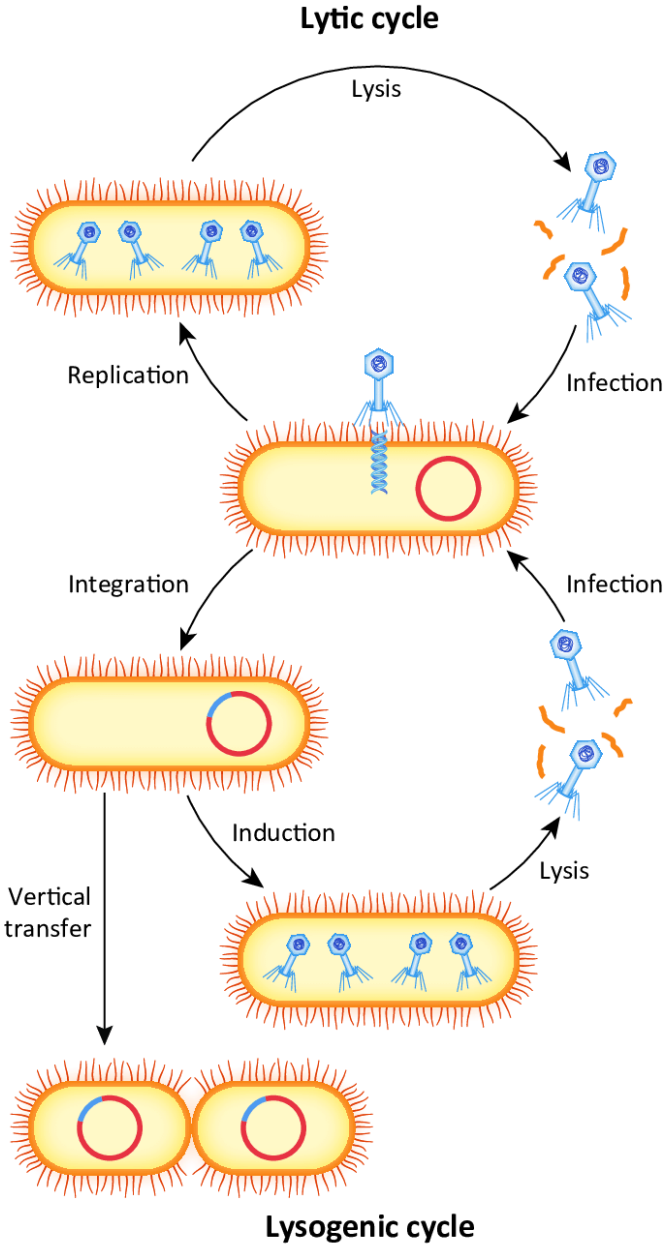

ss RNA Virus
2
1
7
ds DNA (+) Virus
ds DNA Intermediate
6
ss RNA (+) Retrovirus
mRNA
4
ss RNA (+) Virus
3
ds DNA (+) Virus
5
ss RNA (-) Virus
Virus Classification

Life Cycles of Bacteriophages

- A bacteriophage has two different life cycles:
- 1. Lytic Cycle
- 2. Lysogenic Cycle
- Reproduced viruses inside the host in the lytic cycle cause the cell disintegration when leaving the host
- In the lysogenic cycle; viral DNA is incorporated into the host cell chromosome and new viruses from host cells are released out of the cell without leading to any damage

Lysogeny

- In the lytic cycle, replication of the phage is complete without interruption.
- In the lysogenic cycle, the replication of the phage is interrupted and the phage DNA is integrated into the bacterial DNA.
- Integrated DNA is called prophage
- When the bacteria are exposed to the activators such as the ultraviolet light, the prophage DNA is excised from the DNA of the bacteria and the phage enters the lytic cycle.
- Then the progeny phage is produced.



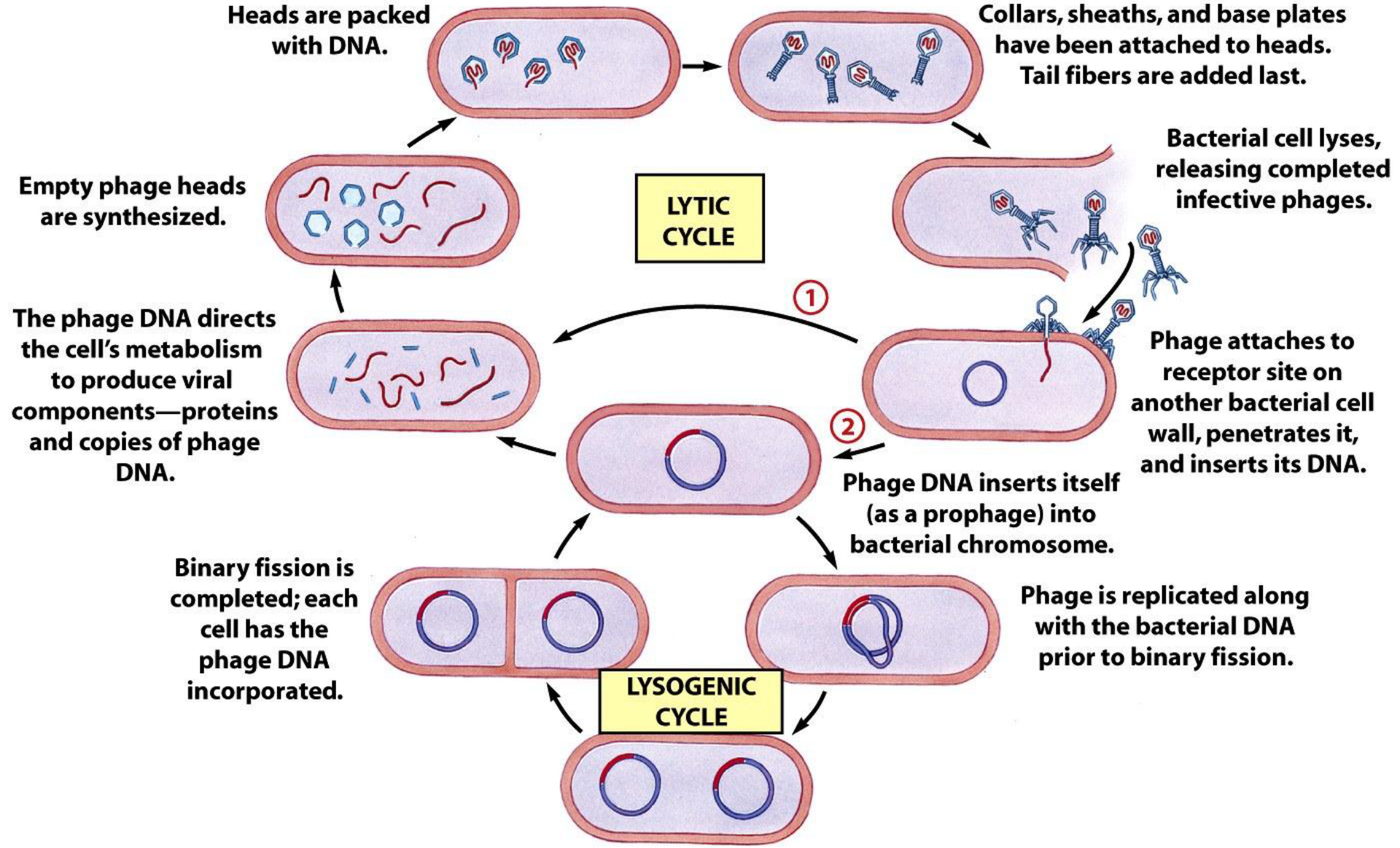


Figure 8-3 Microbiology, 7/e

Events In Infected Cells

Viral infection has 4 main effects in the cell;

- Death
- Cytopathic effects
- Transformation of malignant cells (malignant transformation)
- No morphological or functional changes

1. Death of an Infected Cell

- Cell death usually depends on the inhibition of macromolecule production.
- First inhibits protein synthesis of the host cell
- It then inhibits DNA and RNA synthesis.
- A virus is able to continue the synthesis of its own protein when the synthesis of cellular proteins is inhibited

- The poliovirus inactivates the initiating factor (IF), which is involved in the translation of the mRNA of the infected cell.
- Since their mRNA has a specific ribosome initiation point, viral proteins can be synthesized

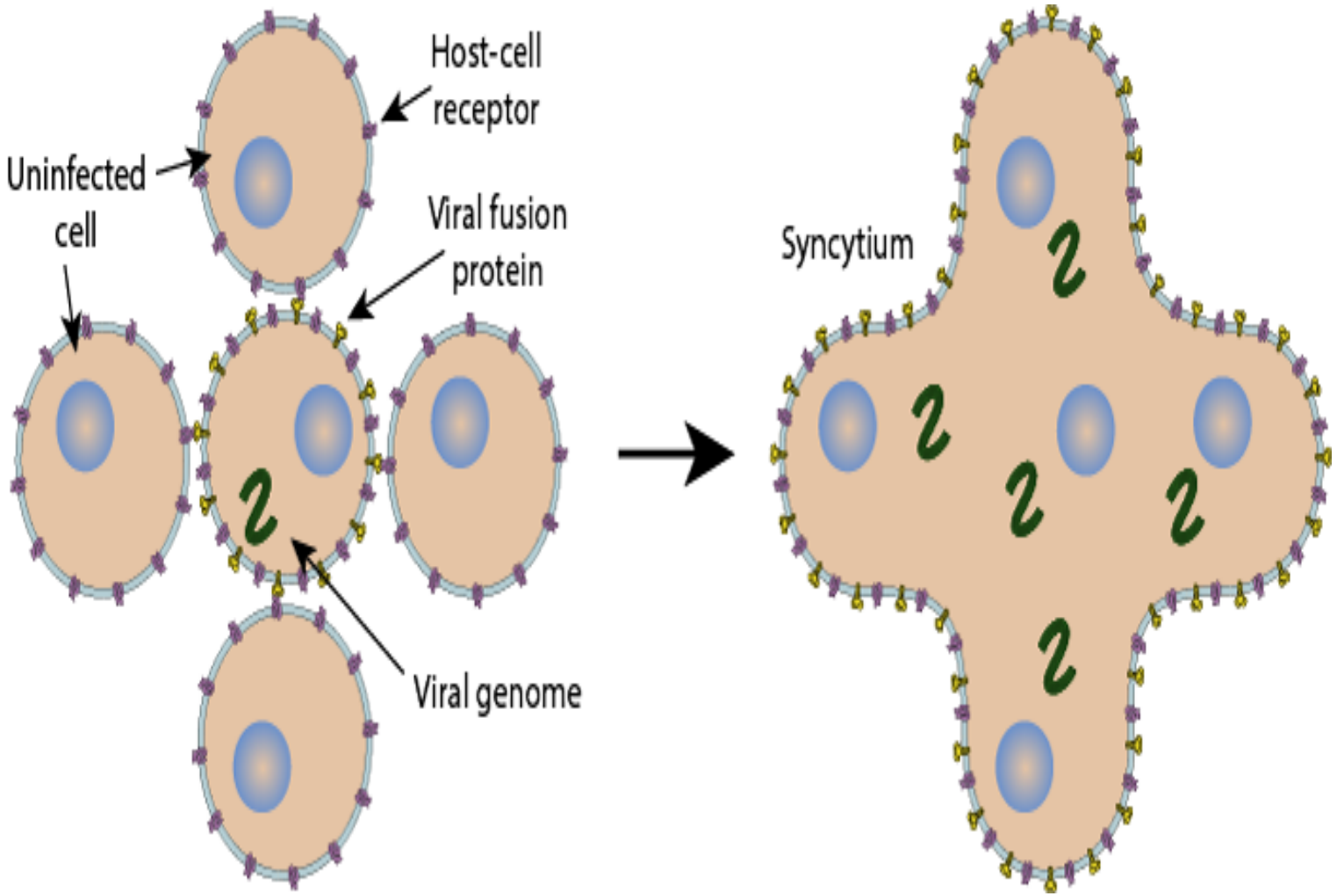
2. Cytopathic Effect

- The most significant evidence of viral infection in the cell is the cytopathic effect (CPE).
- Cytopathic effect can be observed in 4 different ways;
- Cell rounding
- Cell Lysis (disintegration)
- Giant cell formation.
- Inclusion bodies formation

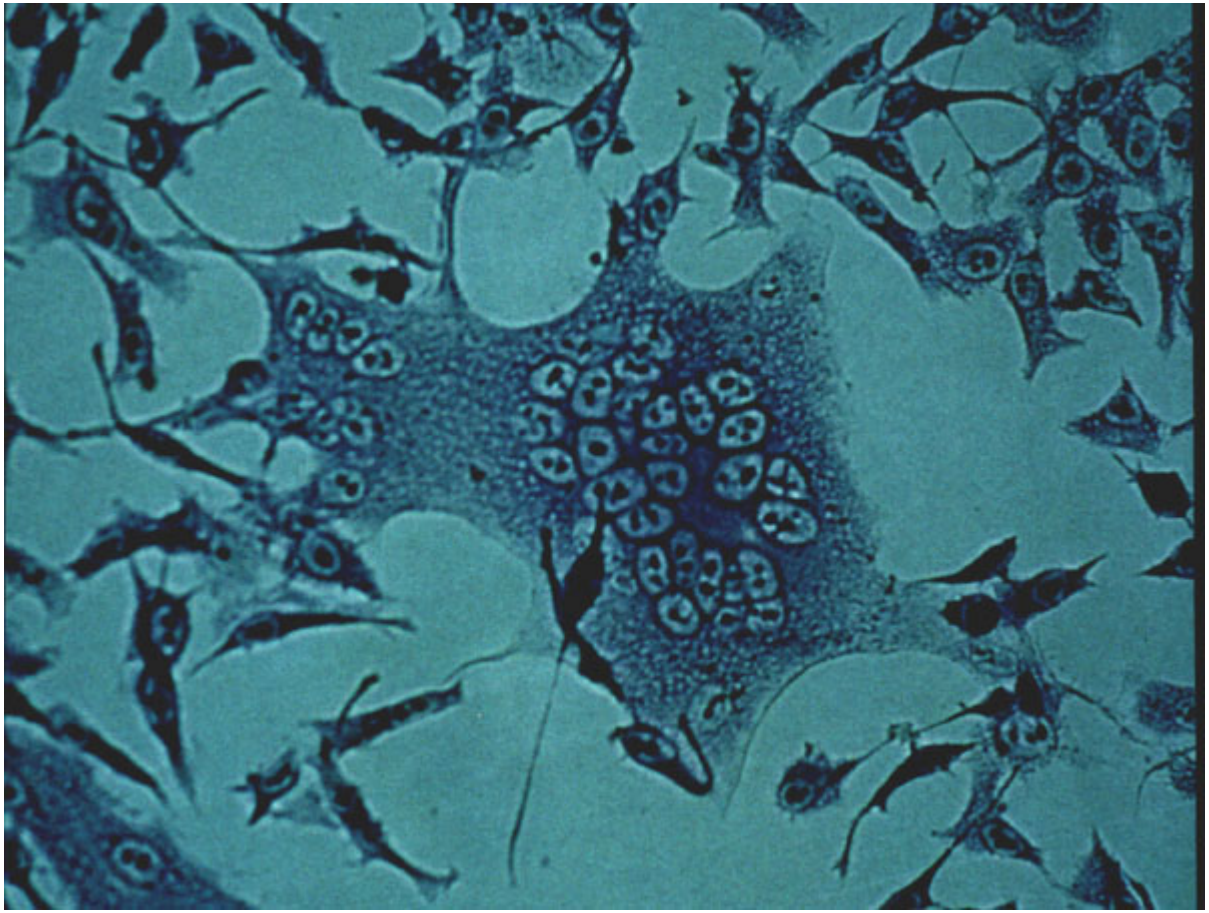
2. Cytopathic Effect

- In some cases (especially after herpesvirus infections), multinucleated giant cells are formed by the fusion of virus infected cells. This is called **syncytium**
- The source of this fusion is alterations in the membrane structure as a result of **viral protein adherence** to the membrane.
- Viral fusion proteins play an important role in syncytium

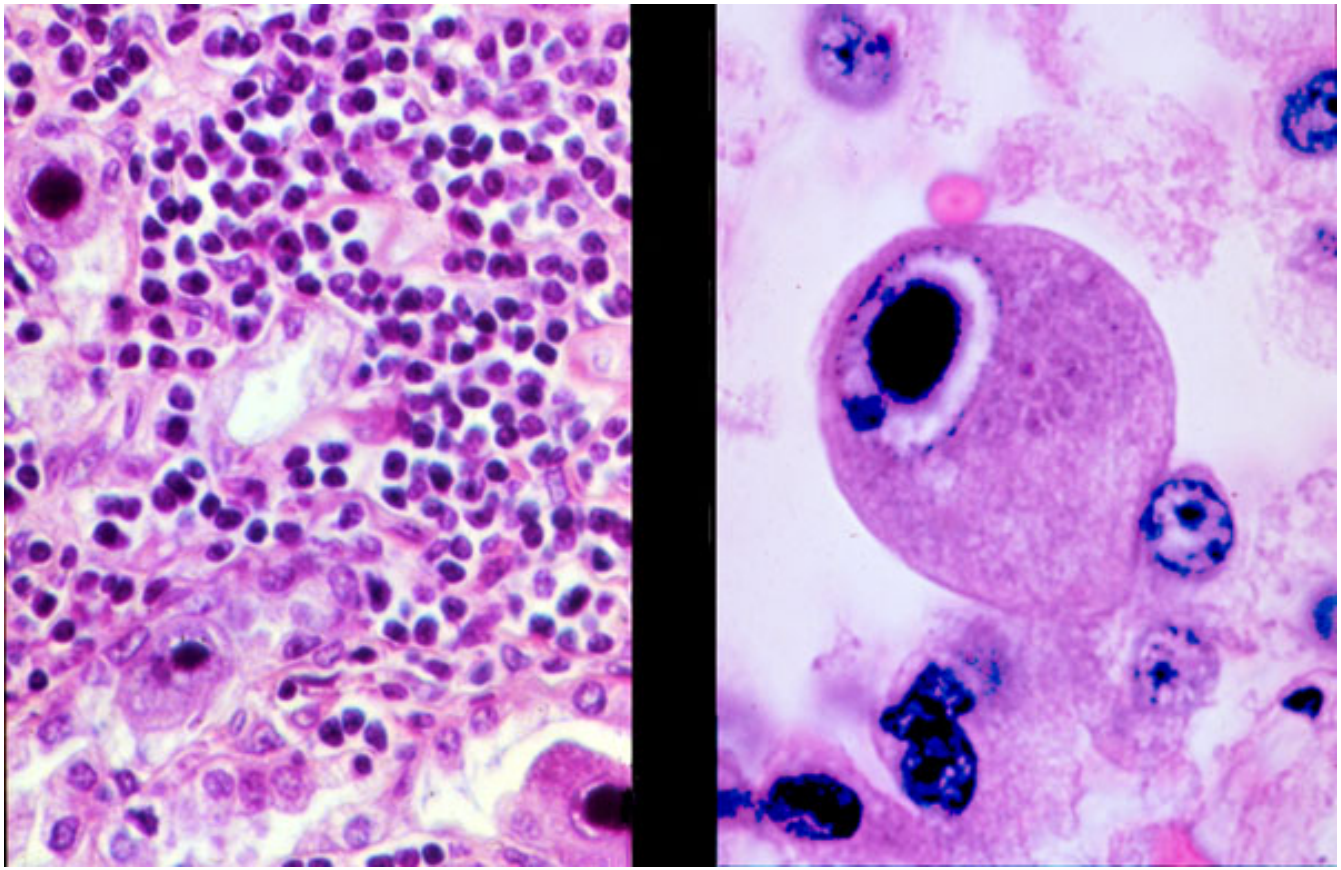
Syncytium

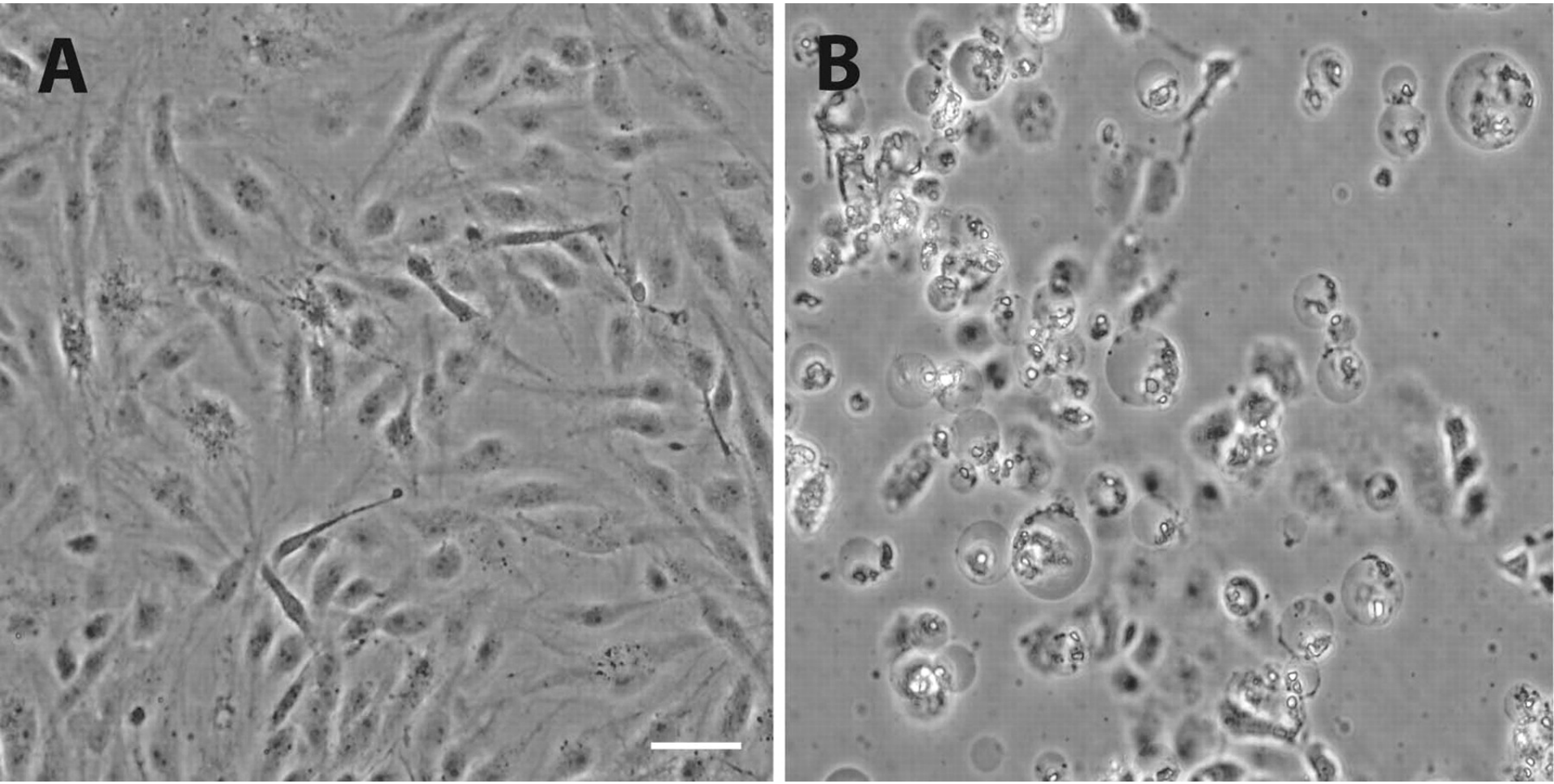

Host-cell
receptor
Uninfected
cell
Viral fusion
protein
Viral genome
Syncytium

Syncytium

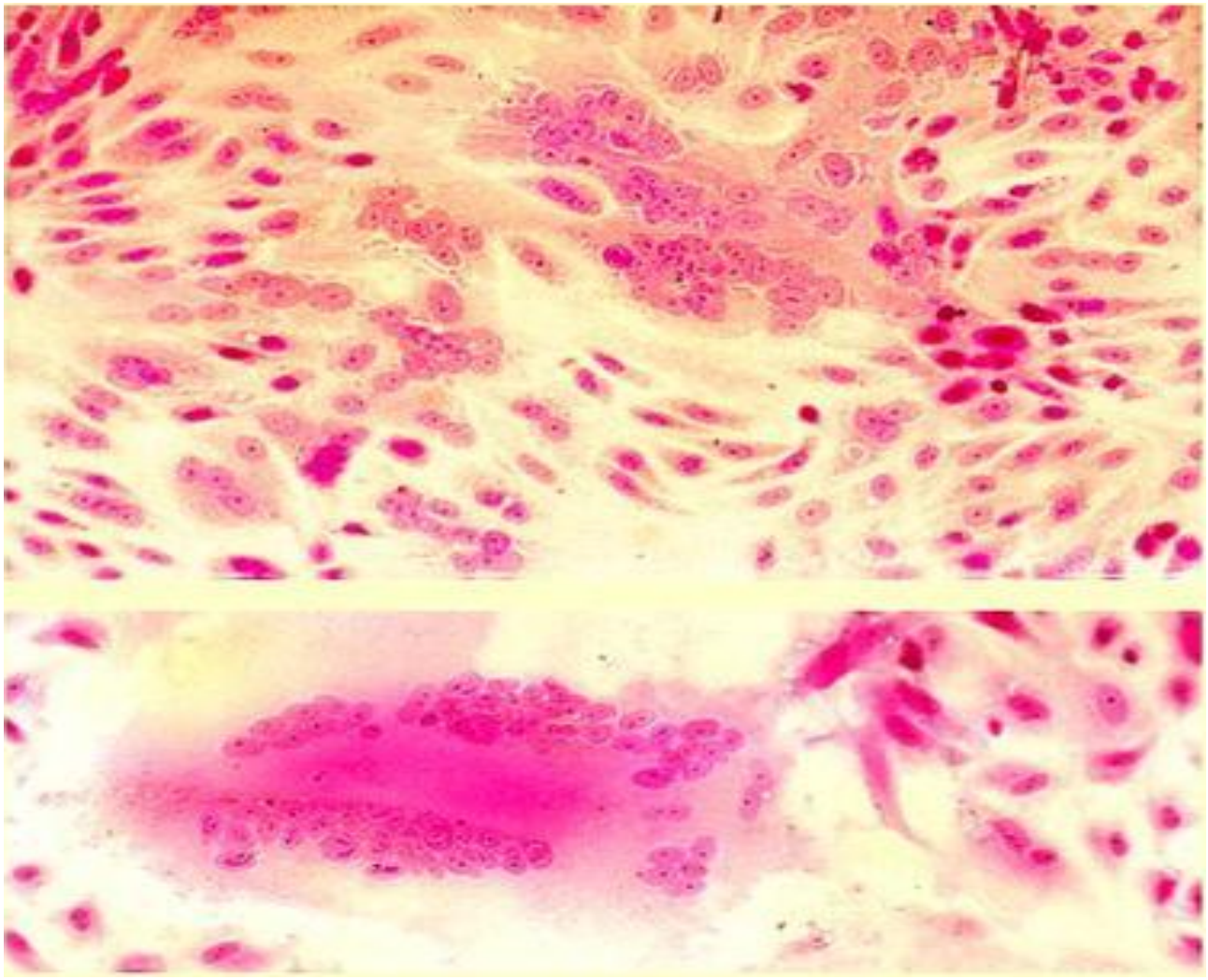


Inclusion bodies formation




A
B

Cytopathic Effect-Lysis



Viral Entry into Host Organism

- As with bacterial infections, the virus may enter into organism in various ways to cause disease as viral infections.

1 - Respiratory tract

2 - Oral Route

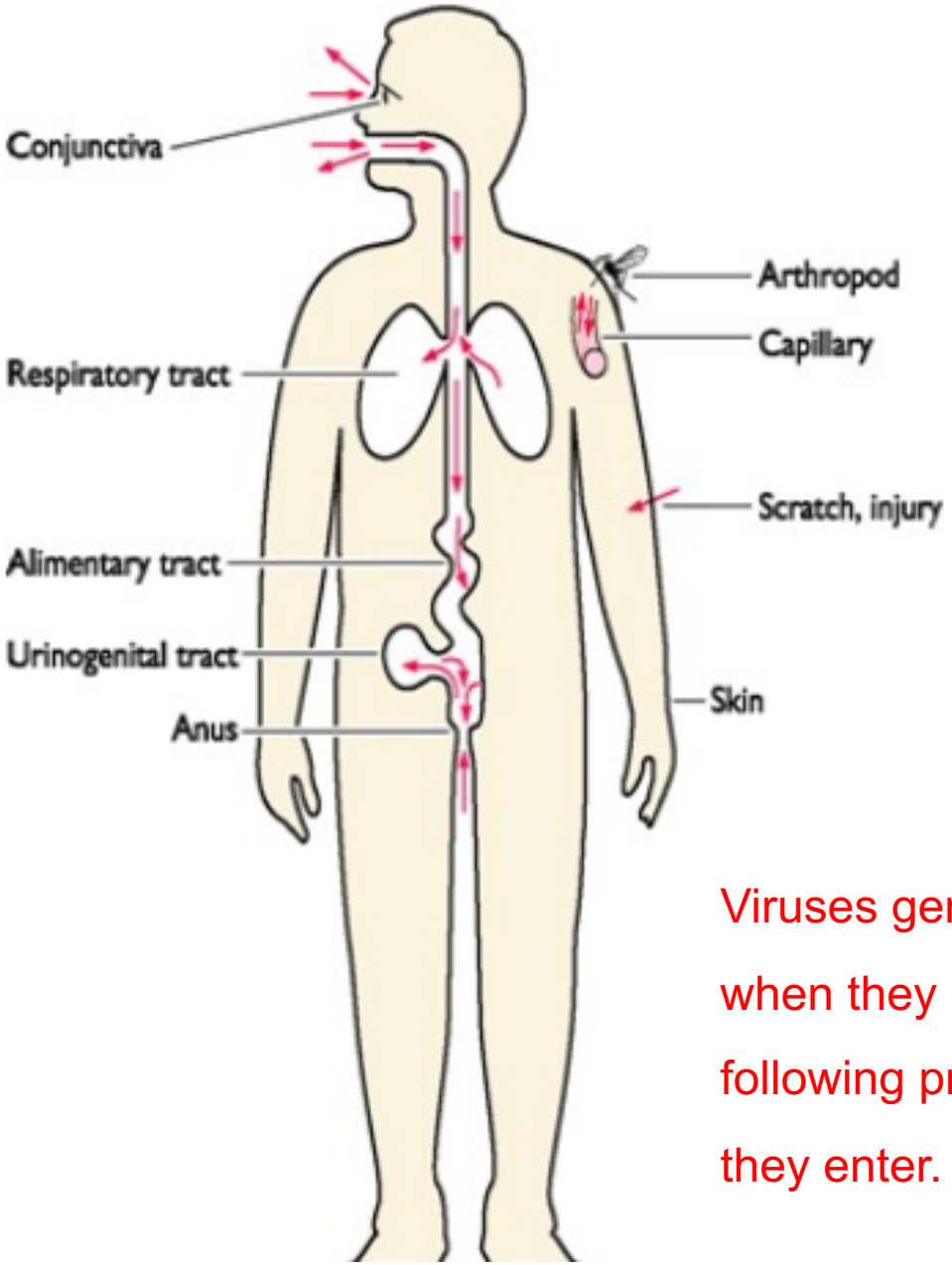
3 - Skin and Mucosa

4 - Injection and Transfusion Path

5 - Genital Path

6 - Placenta Way

Sites of virus entry into the host

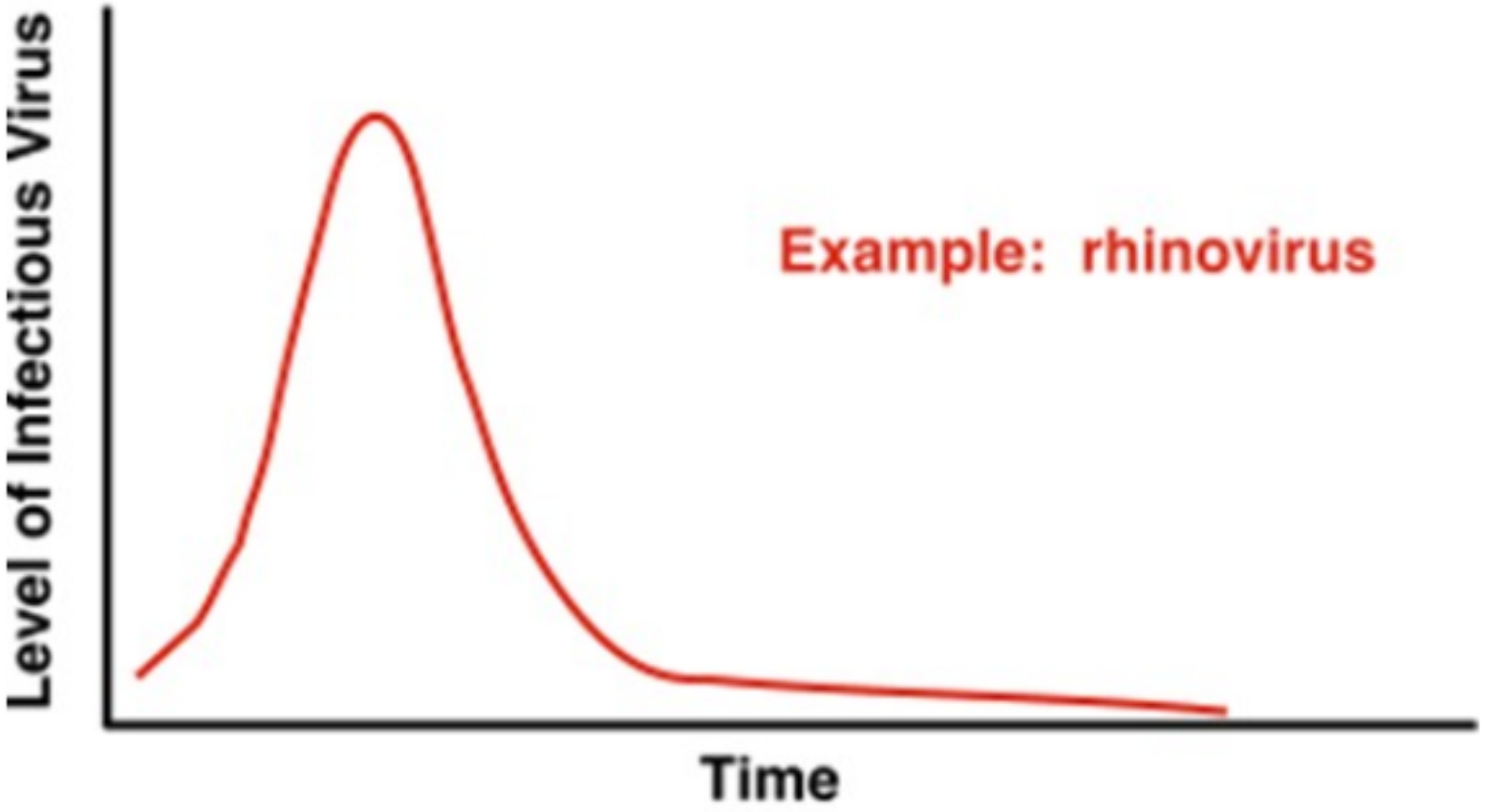


Viruses generate maximum replication when they reach their susceptible cells following primary replication in the area they enter. As a result, symptoms occur.

Viral Infection Stages in Patients

A typical acute viral infection consists of the following stages;

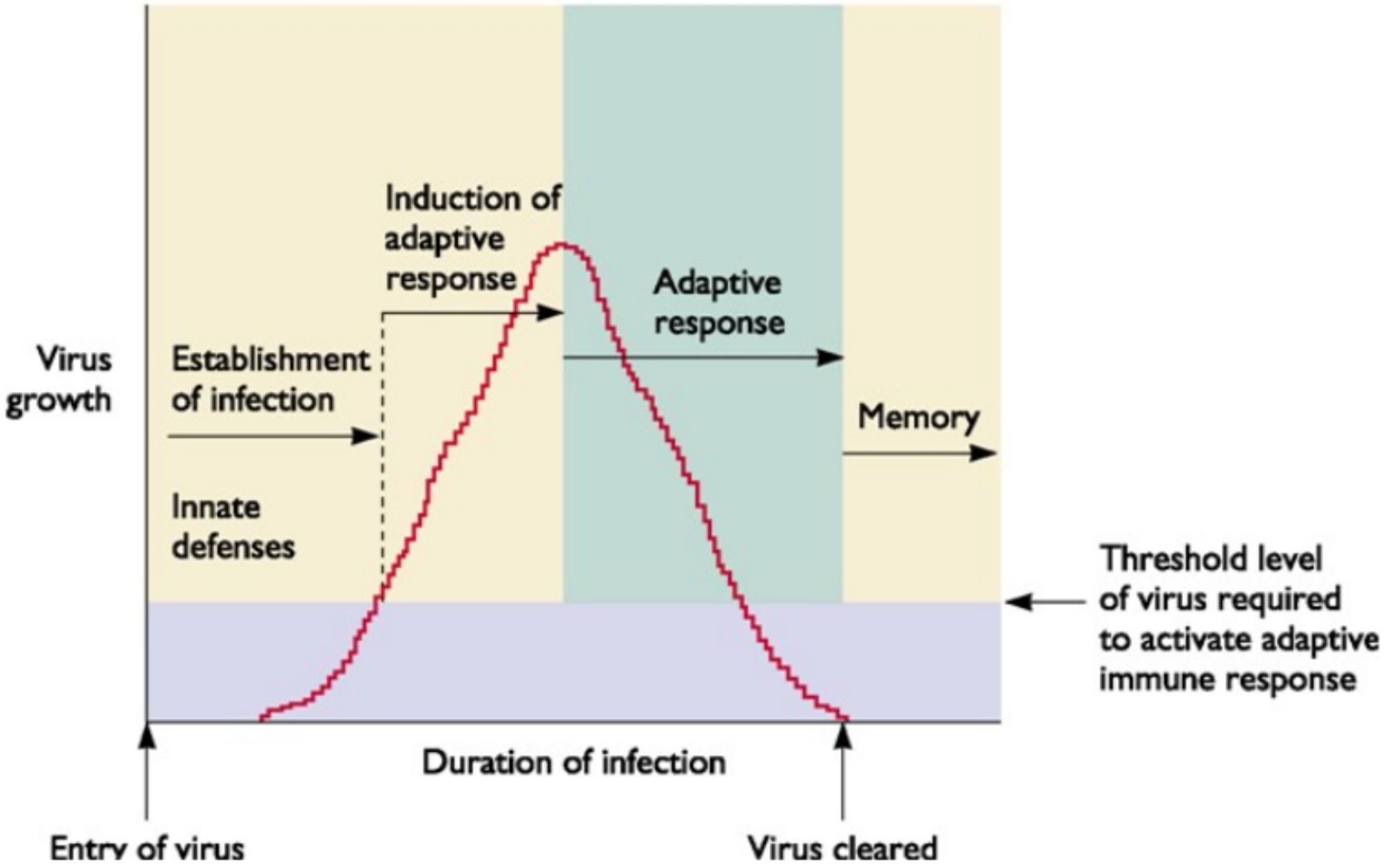
- **Incubation stage** in which the patient has no symptoms
- **A prodromal period** in which non-specific symptoms occur
- **Specific disease period** in which characteristic signs of the disease are observed
- **A recovery period** in which the disease passes and the patient regains its health



Acute infection followed by clearance of virus:

- productive infection
- viremia (circulating virus)
- clearance by immune system
- example: rhinovirus (common cold)

The course of typical acute infection



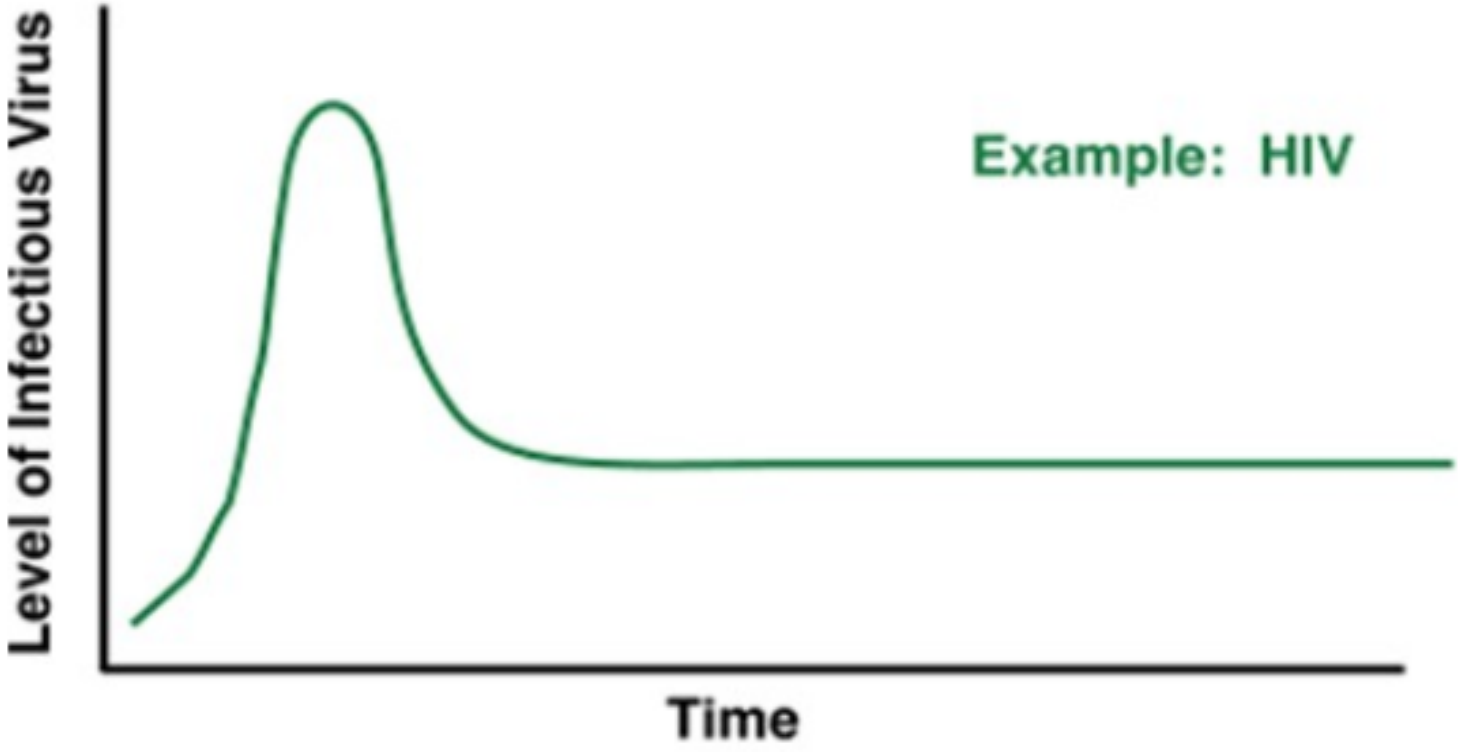
- In most viral infections, the virus does not remain in the body for a long time after clinical recovery.

- However, in some cases, the virus will continue to exist for a long time. Viruses that survive in this way are called persistent viruses.

1. **Chronic Infections**
2. **Latent Infections**
3. **Slow Infections**

Chronic Infections

- They usually develop from acute infections and can last for days to months to a lifetime.
- Recovery from this infection is rare.
- People may be able to transmit the germ to others.
- Serious signs may not appear until as long as 20 years after the infection began.
- For example: **hepatitis C**, which affects the liver is a chronic viral infection.

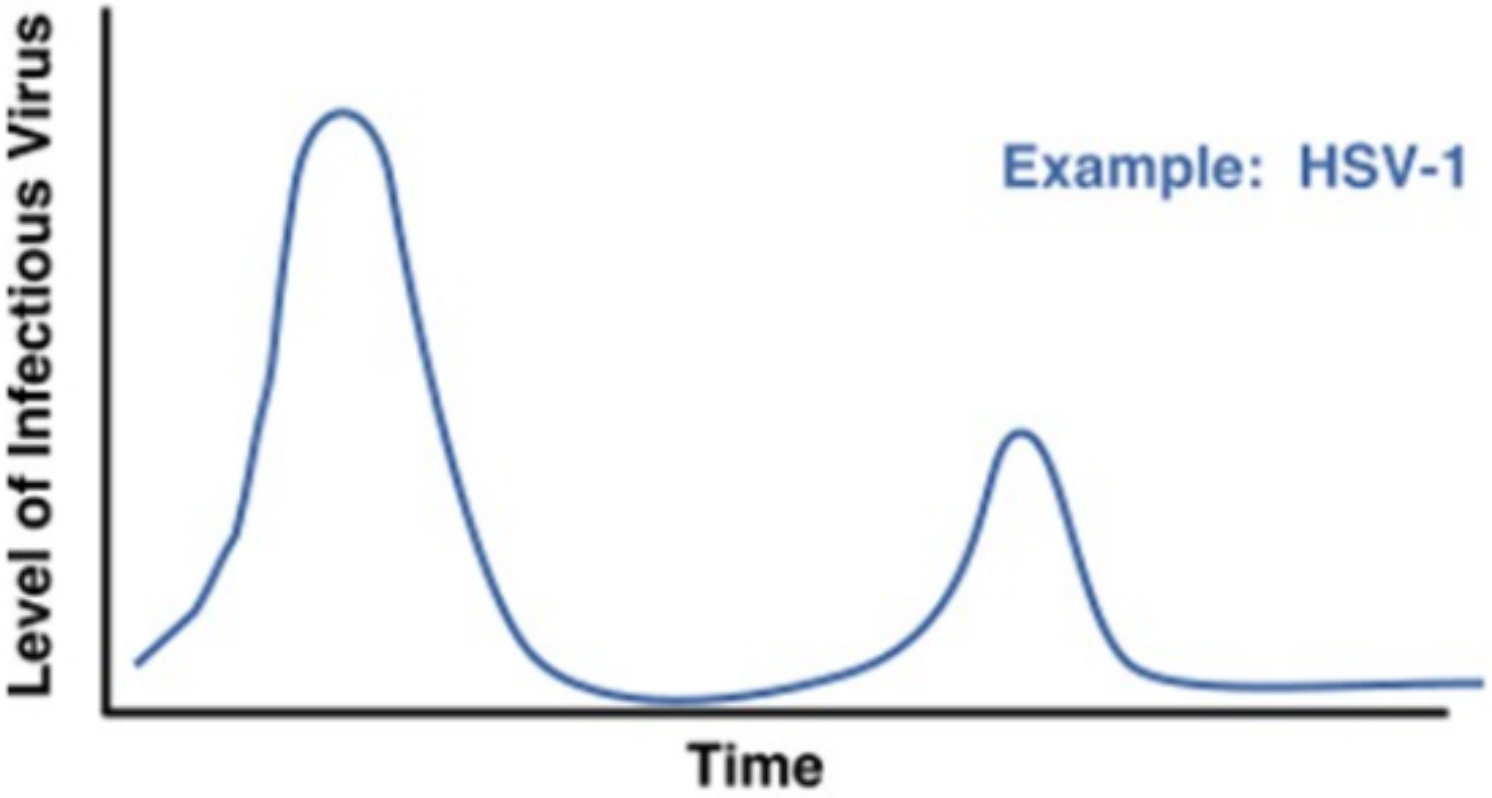


Acute infection followed by chronic infection:

- initial productive infection with viremia
- virus not cleared completely by immune system
- continuous, low-level productive infection
- may be "smoldering" infection (productive infection by small fraction of cells)
- example: human immunodeficiency viruses (HIV)

Latent Infection

- Virus is present in the body, but it remains dormant, not causing any overt symptoms.
- People can pass the virus on to others.
- Latent infections can also be activated, causing symptoms and illness to emerge again.
- Eg: latent infection is **Herpes simplex**, which periodically flares up to cause cold sores before going dormant again.



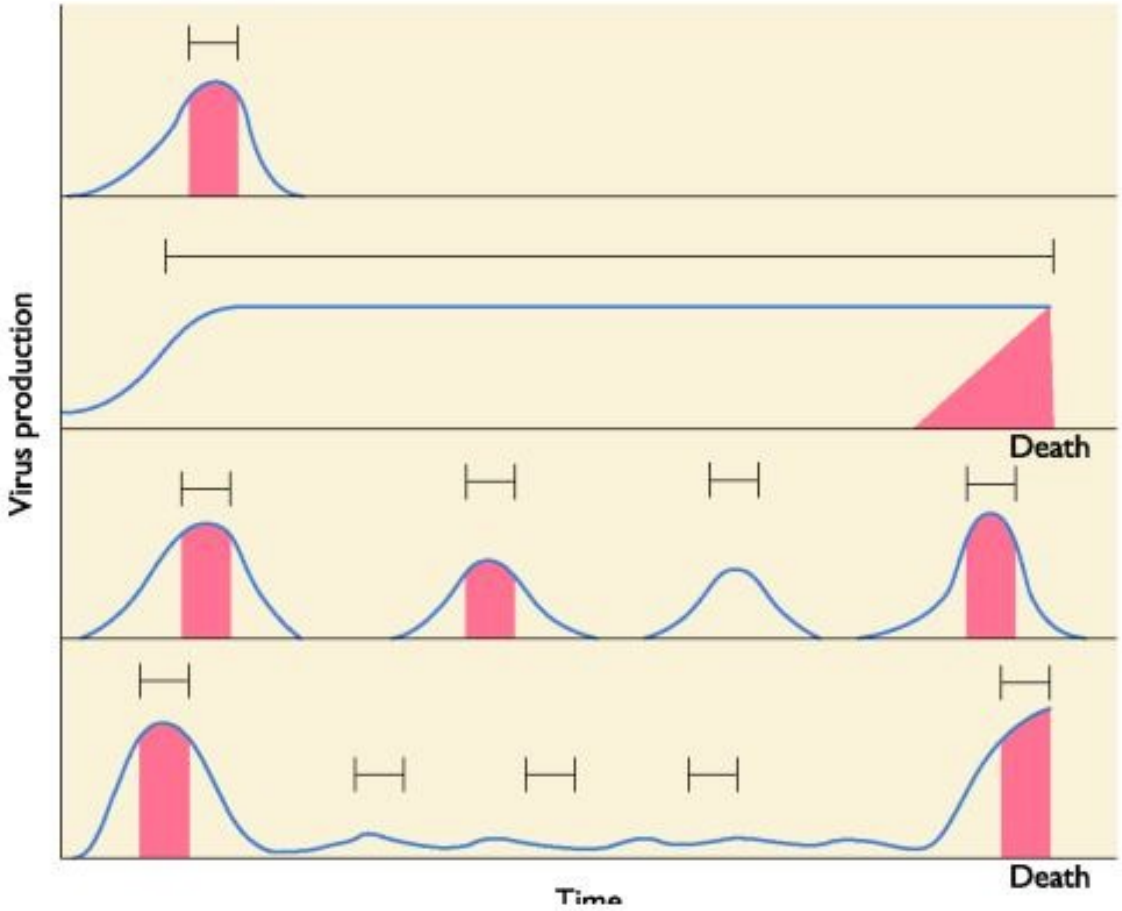
Acute infection followed by latent infection and periodic reactivation:

- initial productive infection with viremia
- viral persistence in non-infectious form
- intermittent reactivation with productive infection
- example: herpes simplex virus type 1 (HSV-1)

Slow infections

- These diseases have a prolonged incubation period (which can be months or years), and a progressive clinical course.
- e.g: Subacute Sclerosing Pan Encephalite (SSPE) occurs after many years in people with measles infection.

General patterns of infection



Acute infection
- Rhinovirus
- Rotavirus
- Influenza virus

Persistent infection
- Lymphocytic choriomeningitis virus

Latent, reactivating infection
- Herpes simplex virus

Slow virus infection
- Measles SSPE
- Human immunodeficiency virus

From Flint et al Principles of Virology

Antiviral Drugs

- Compared to the number of drugs that can be used for the treatment of bacterial diseases, the number of antiviral drugs is quite low.

- The main reason for this difference is **the difficulty of providing selective toxicity against viruses**; the replication of these cells is accompanied by normal synthesis events of the cell.

- Another limiting factor that renders antiviral drugs ineffective is the fact that most of the viral replication cycle takes place during the incubation period in which the patient feels good.

- **When the patient was diagnosed with a systemic viral disease, the virus spread throughout the body and it was too late to prevent it.**

- Another potential limiting factor is the emergence of drug-resistant viral mutants. This phenomenon is of great clinical importance today.

CLASSIFICATION