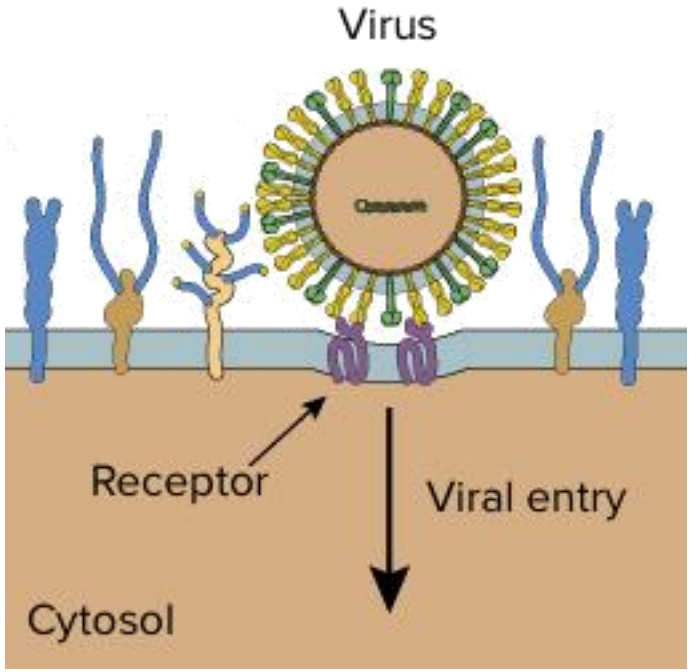
VIRAL REPLICATION

- The virus absolutely looks for cells that are sensitive to itself in order to replicate. This situation is determined by the epitope-paratope (receptor-antireceptor) relationship between the virus and the host cell, and this is called the host spectrum.



https://www.khanacademy.org/science/biology/biology-of-viruses/virus-biology/a/intro-to-viruses

It consists of 3 basic steps;

✓Preparation for replication

✓Replication and virus genome expression

✓Assembly and release of mature virus particle

Replication Cycle

- It is the name given to the time and chain of events from a mature virus particle (Parental virus) to the formation of new infective viruses (Progeny virus).

✓Attachment (Adsorption)

✓Penetration

✓Uncoating (Removal from the capsid)

✓Replication (Genome replication and gene expression)

Eclipse

✓Assembly

✓Maturation

✓Release

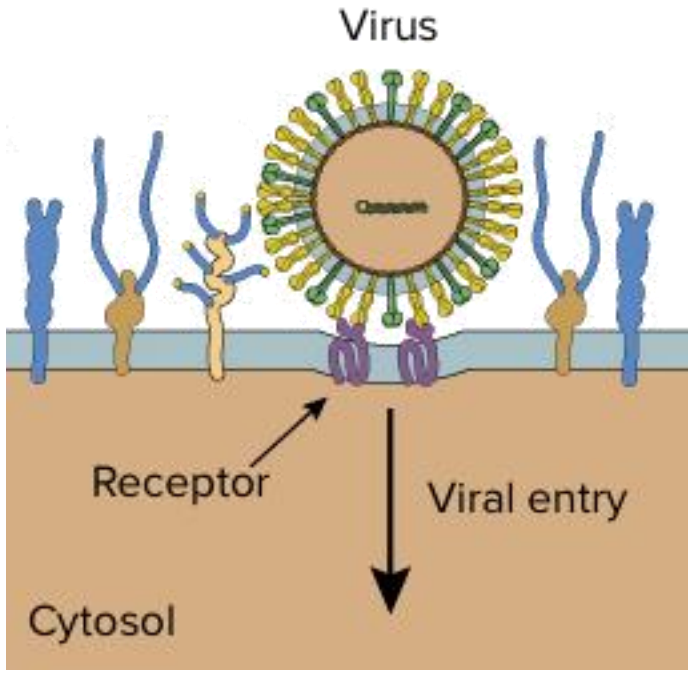
A PURple Apple Might Redden

- ✓Attachment
- ✓Penetration
- ✓Uncoating
- ✓Replication
- ✓Assembly
- ✓Maturation
- ✓Release

Louten J Virus Replication, Essential Human Virology. 2016 : 49–70.
doi: 10.1016/B978-0-12-800947-5.00004-1

1. Attachment

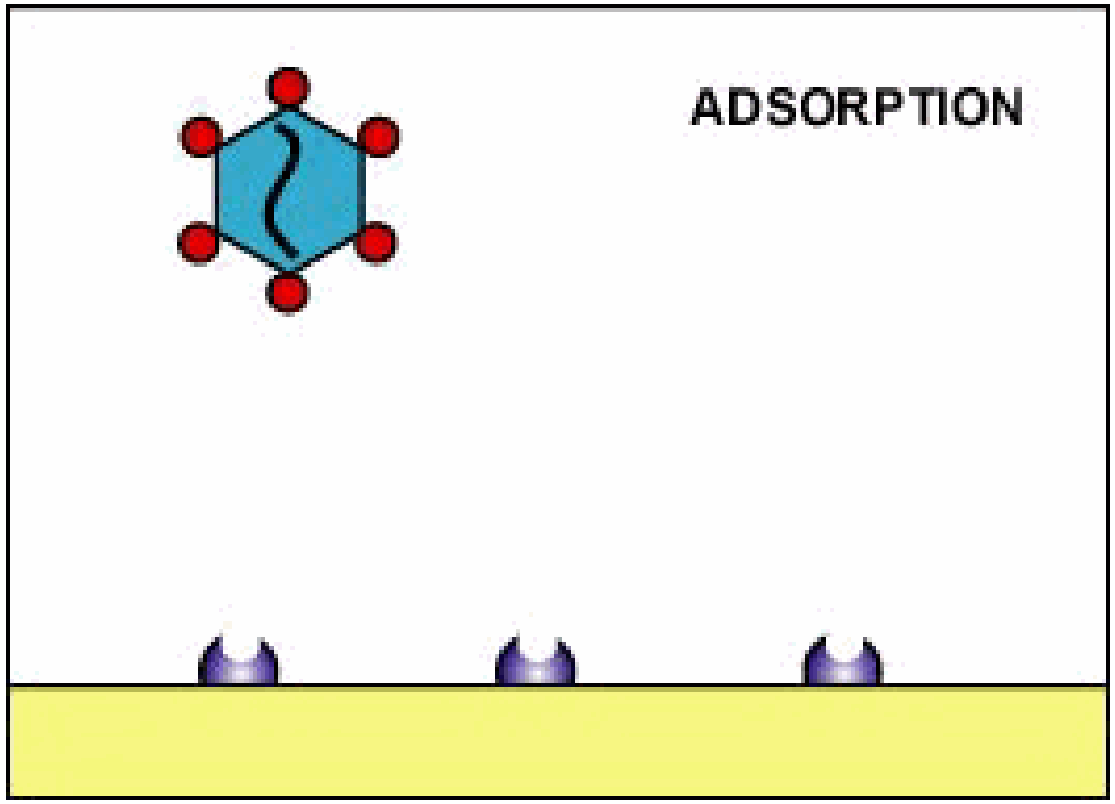
- It is a reversible event that occurs between receptors (paratope) on the cell surface and virus attachment proteins (epitope), and is directly affected by the environment, salt concentration, pH and temperature.
- While changes that may occur in these conditions may disrupt attachment, attachment may not occur under inappropriate conditions.
- The presence or absence of receptors on the cell surface determines TROPISM.
- Viral proteins responsible for attachment are located on the envelope in enveloped viruses and on the capsid in non-enveloped viruses.



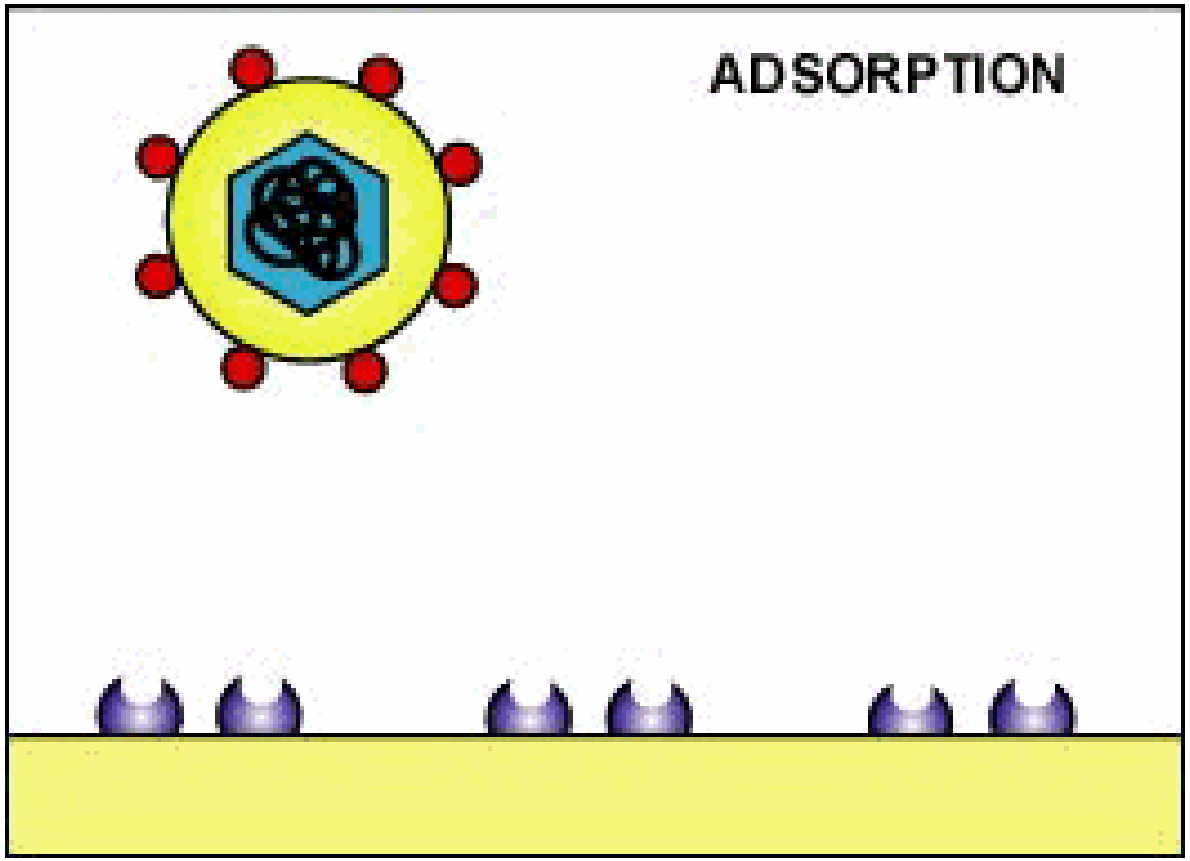
https://www.khanacademy.org/science/biology/biology-of-viruses/virus-biology/a/intro-to-viruses

Virus	Cell receptor	Virus protein(s) involved in	
		attachment to receptor	fusion
Naked viruses			
Approx. 90% of human rhinoviruses	Intercellular adhesion molecule-1 (ICAM-1)	VP1 + VP3	
Approx. 10% of human rhinoviruses	Low-density lipoprotein receptors	VP1	
Poliovirus	CD155	VP1	
Enveloped viruses			
Murine leukemia viruses	Mouse cationic amino acid transporter	SU (surface glycoprotein)	TM (transmembrane glycoprotein)
HIV-1	CD4	gp120	gp41
Influenza viruses A and B	Sialic-acid-containing glycoproteins	Hemagglutinin	Hemagglutinin
Measles virus	Signaling lymphocyte activation molecule (CD150)	Hemagglutinin	Fusion

Adsorption in Non-Enveloped Viruses



Adsorption in Enveloped Viruses



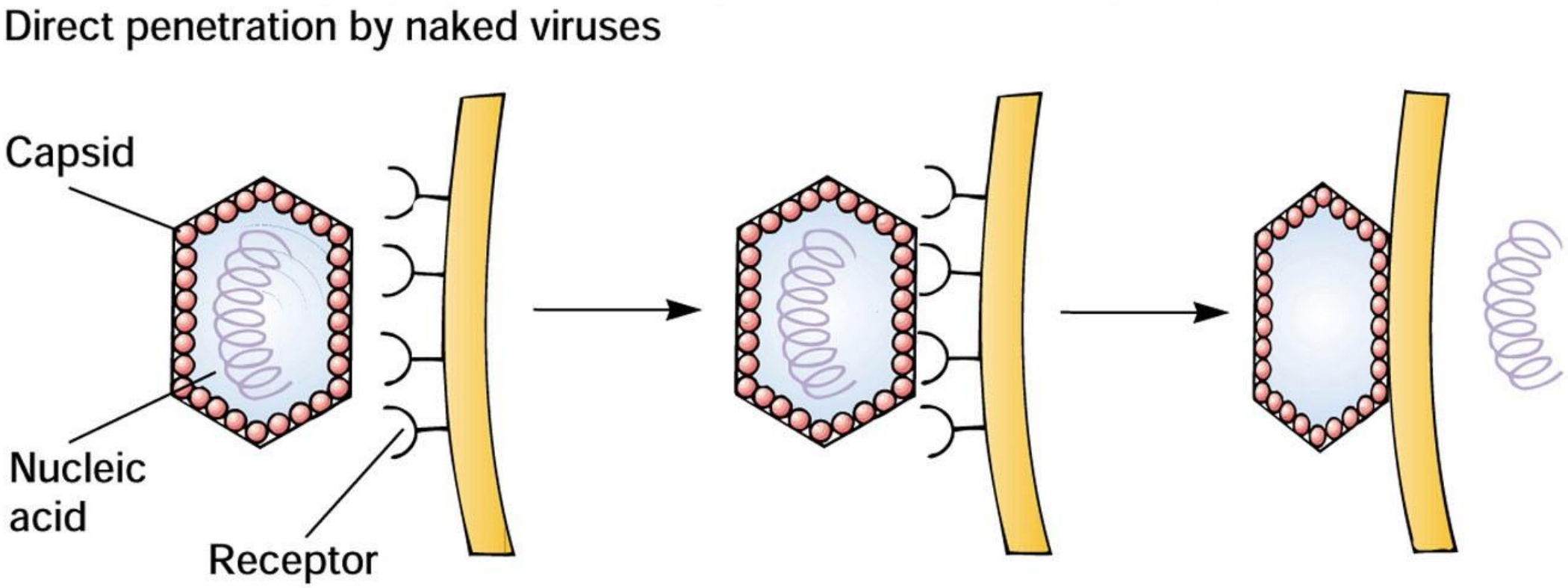
2. Penetration

- It describes the virus that attaches to the cell surface and is taken into the cell.
- Penetration is an energy-dependent process
- This event occurs in a very short time and with three basic mechanisms.

- Direct (Translocation)
- Endocytosis (Pinocytosis)
- Fusion

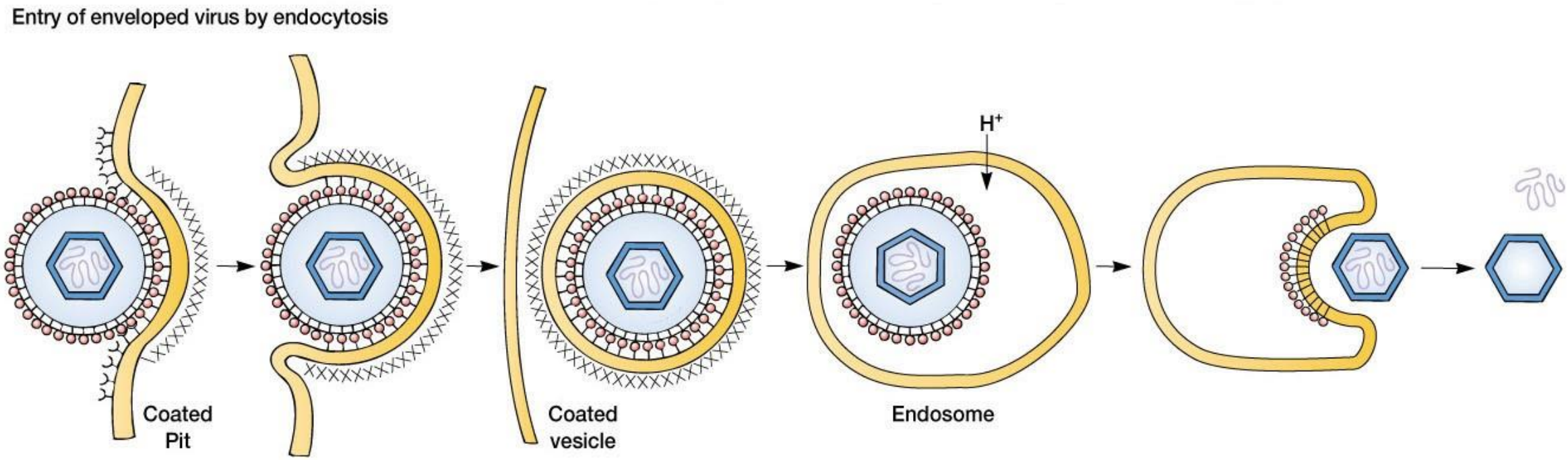
Direct Penetration

- Mostly seen in non-enveloped viruses. The genetic material of the nucleocapsid, which is attached on the cell surface, is taken directly into the cell.



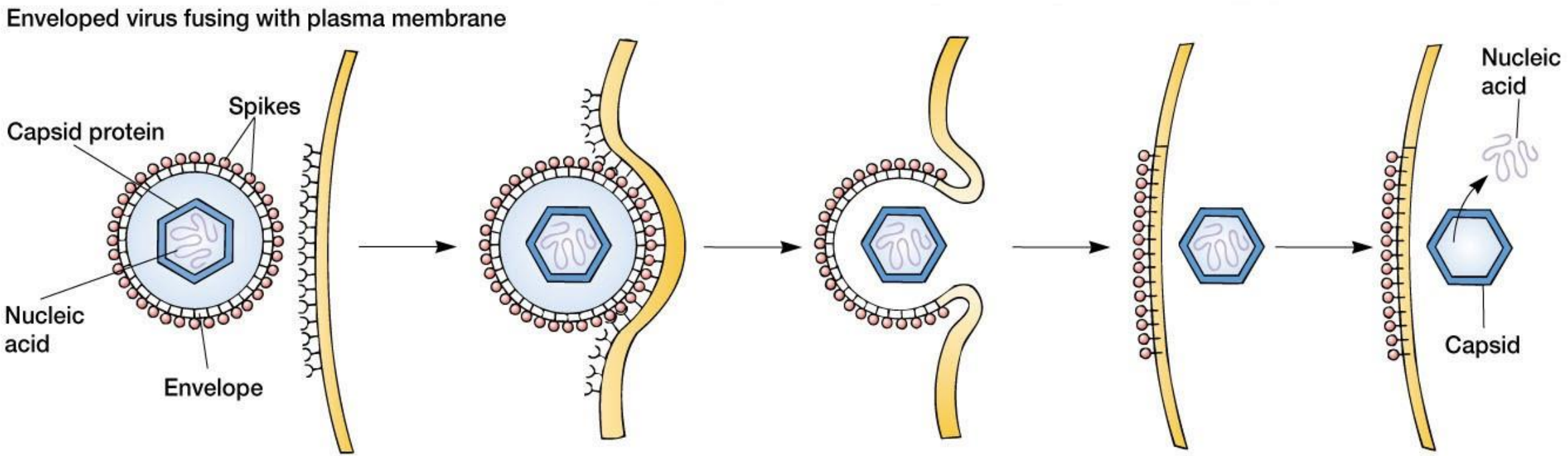
Endocytosis

- It is observed in both enveloped and non-enveloped viruses. The virus attached to the cell surface is taken into the cytoplasm by invagination of the cell membrane.
- It is degraded by acidic vesicles and the nucleocapsid is released.



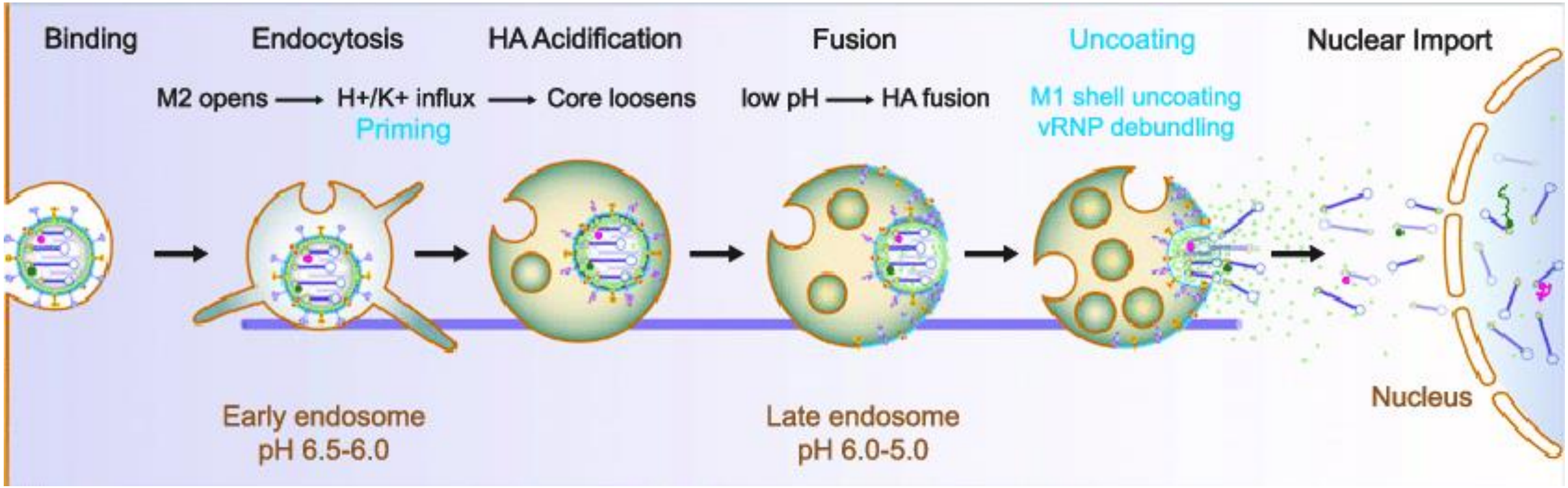
Fusion

- It is the penetration mechanism used by enveloped viruses.
- The virus membrane adsorbed to the cell surface fuses with the cell membrane and the nucleocapsid is taken directly into the cell.



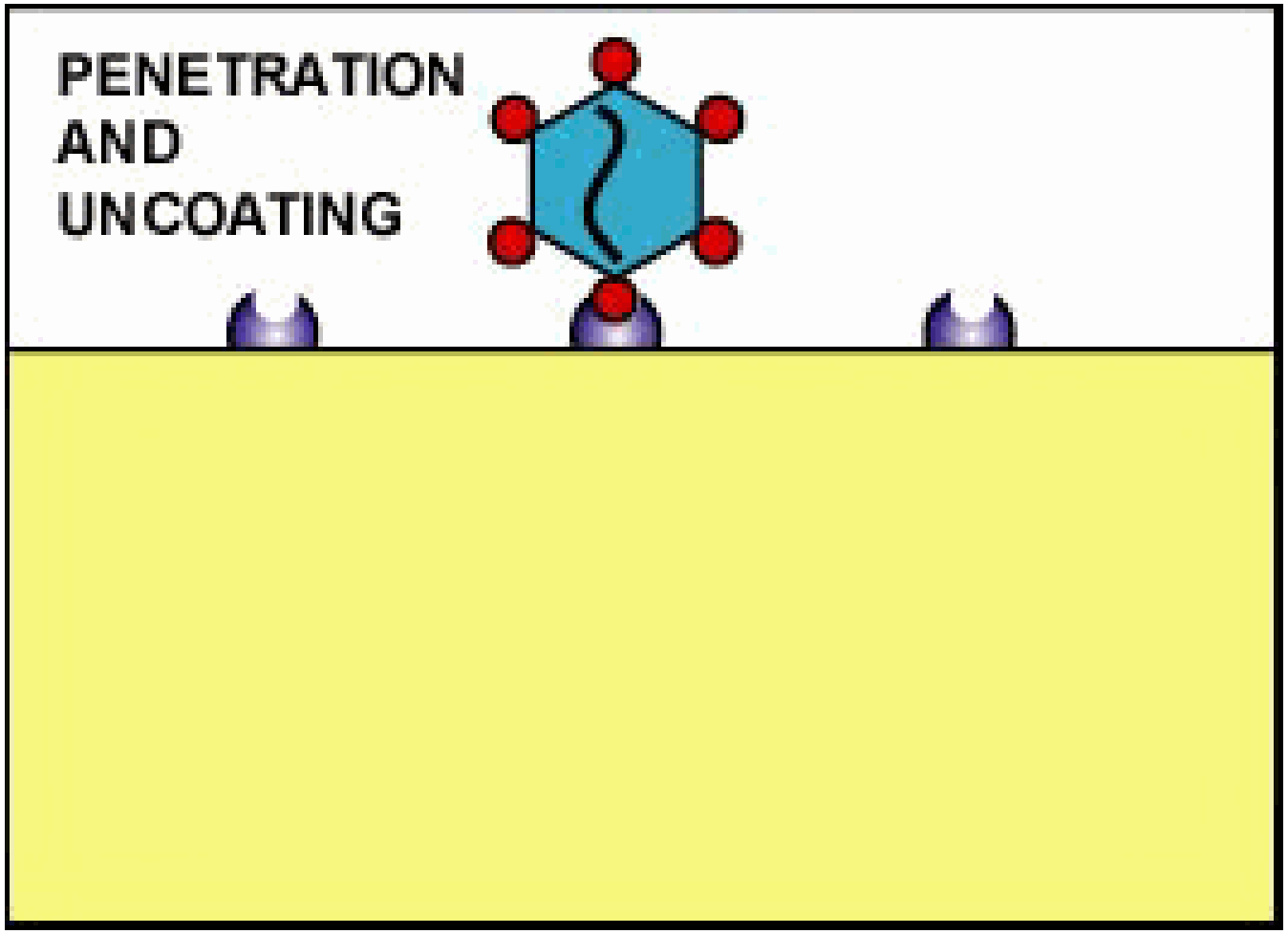
3. Uncoating

- Although partial detachment from the capsid occurs due to the fusion event that occurs in enveloped viruses, complete decapsidation occurs within endosomes.
- Enzyme-containing vesicles acidify the virus in the endosome or cytoplasm with the help of low pH.
- A key step in uncoating is the acidification of the content of the endosome to a pH of about 5.
- In this acidic environment, the capsid partially or completely disintegrates, allowing the nucleic acid to be released.
- Makes viral nucleic acid available for transcription to permit multiplication to proceed.
- The next period is the ECLIPSE period.

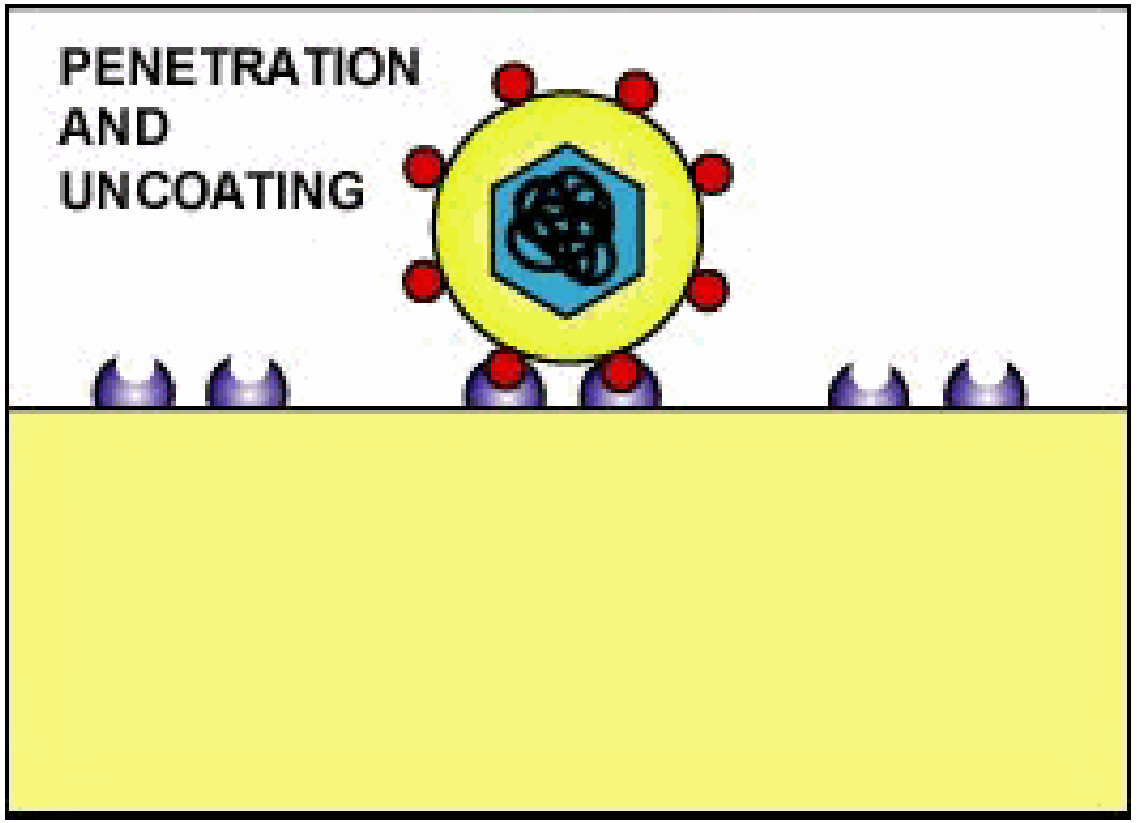


Yamauchi, Y. (2019). Quantum dots crack the influenza uncoating puzzle. Proceedings of the National Academy of Sciences, 116(7), 2404-2406.

In Non-Enveloped Viruses



In Enveloped Viruses



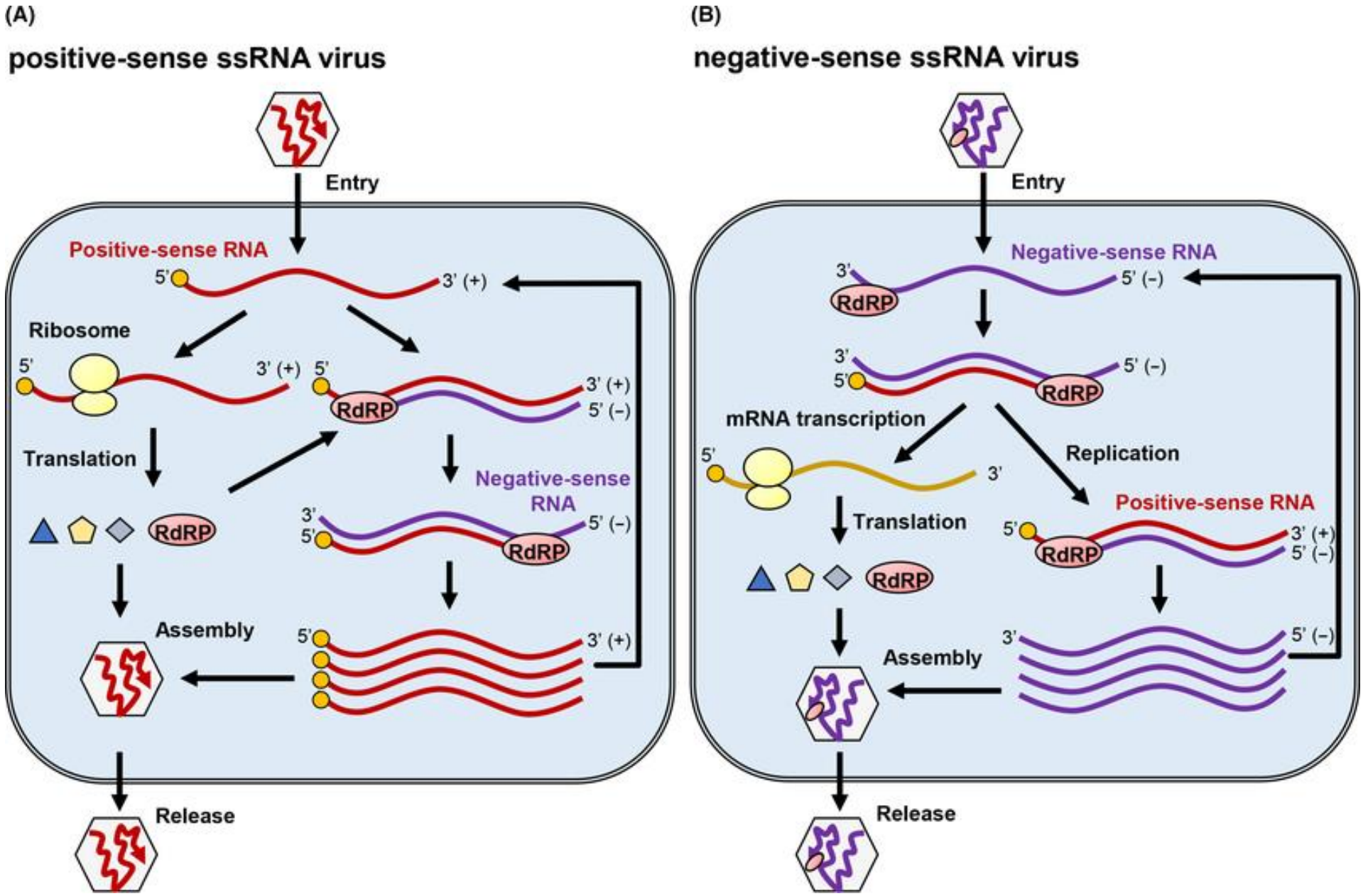
4. Replication

- Genome replication and translation processes differ according to the genomic characteristics of the virus.
- After mRNA translation - transcription - is completed, translation of early and late proteins takes place.
  - Early proteins are involved in genome replication.
  - Late proteins are virus structural proteins.
- Finally, PROGENY virus nucleic acid are synthesized.

The replication strategy of the virus depends on the nature of its genome.

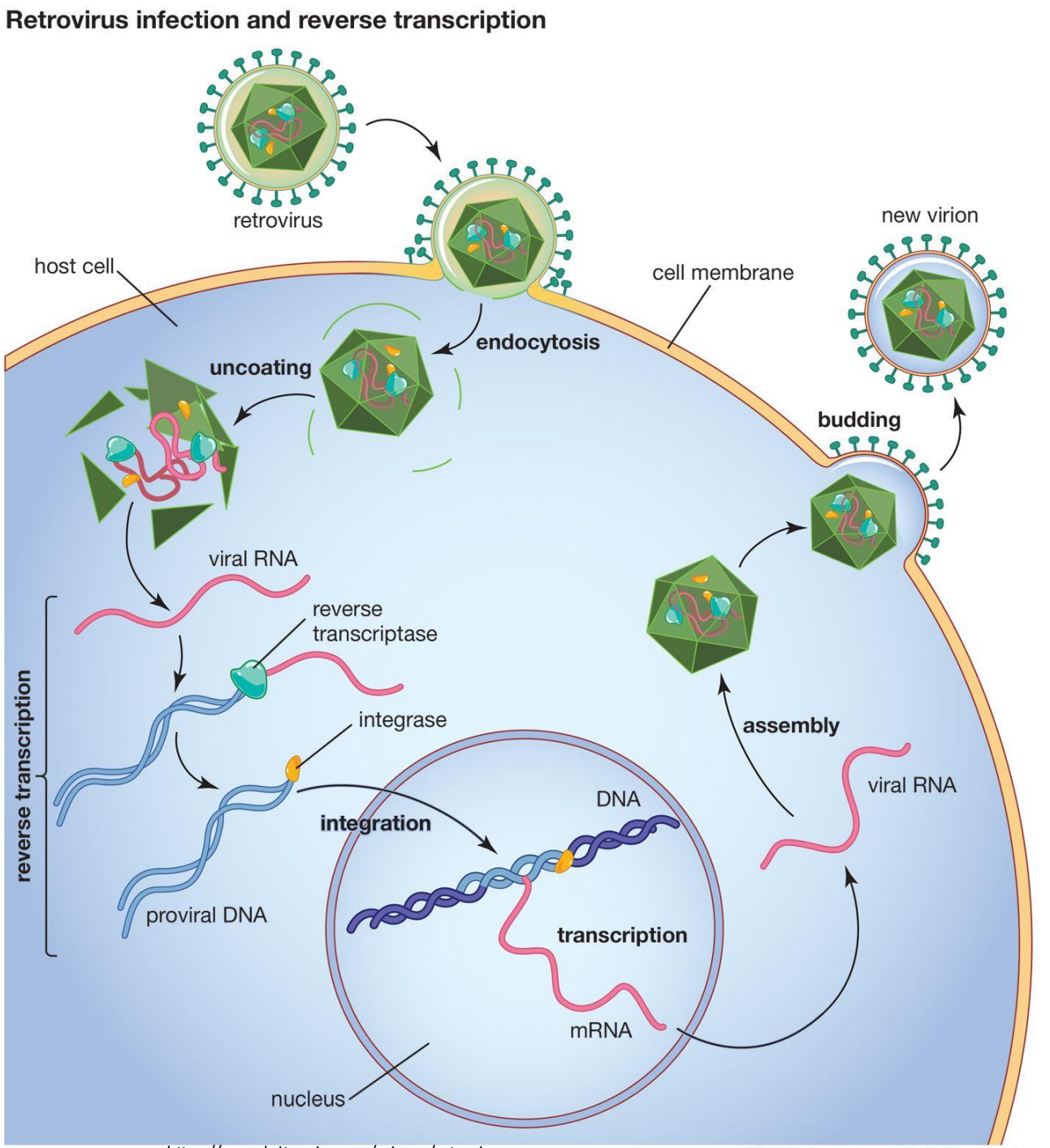
- ***I***: Double-stranded DNA (Adenoviruses; Herpesviruses; Poxviruses, etc)
- ***II***: Single-stranded (+) sense DNA (Parvoviruses)
- ***III***: Double-stranded RNA (Reoviruses; Birnaviruses)
- ***IV***: Single-stranded (+) sense RNA (Picornaviruses; Togaviruses, etc)
- ***V***: Single-stranded (-) sense RNA (Orthomyxoviruses)
- ***VI***: Single-stranded (+) sense RNA with DNA intermediate in life-cycle (Retroviruses)
- ***VII***: Double-stranded DNA with RNA intermediate (Hepadnaviruses)

- (+) RNA to Proteins
- (-) RNA to (+) RNA to Proteins

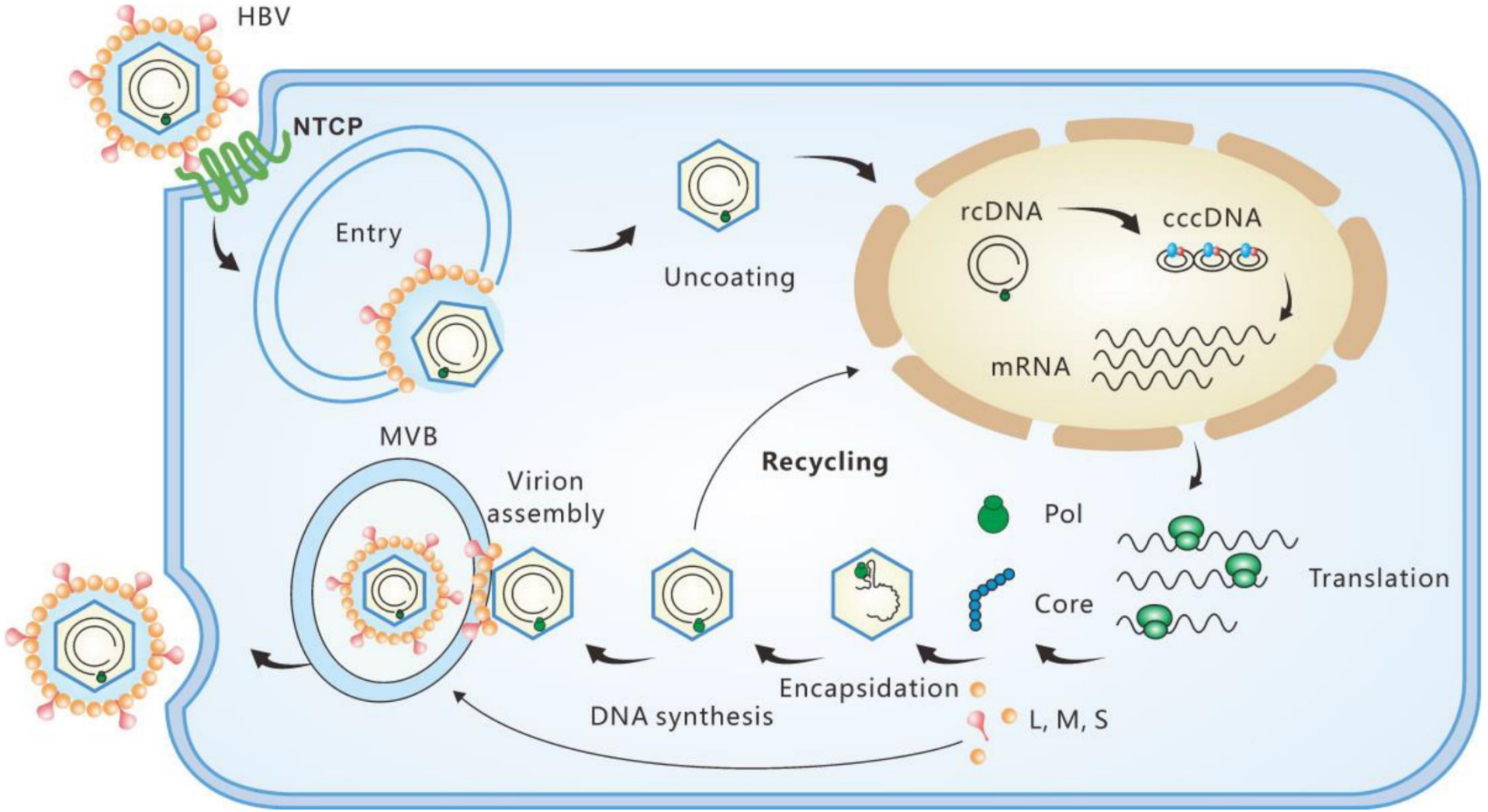


Machitani, M., Yasukawa, M., Nakashima, J., Furuichi, Y., & Masutomi, K. (2020). RNA-dependent RNA polymerase, RdRP, a promising therapeutic target for cancer and potentially COVID-19. *Cancer Science*, *111*(11), 3976-3984.

- RNA to DNA to RNA to Proteins



- DNA to RNA to Proteins



Hu J-L, Huang A-L. Dynamics of Hepatitis B Virus Covalently Closed Circular DNA: A Mini-Review. *Microorganisms*. 2023; 11(3):600. https://doi.org/10.3390/microorganisms11030600

5. Assembly

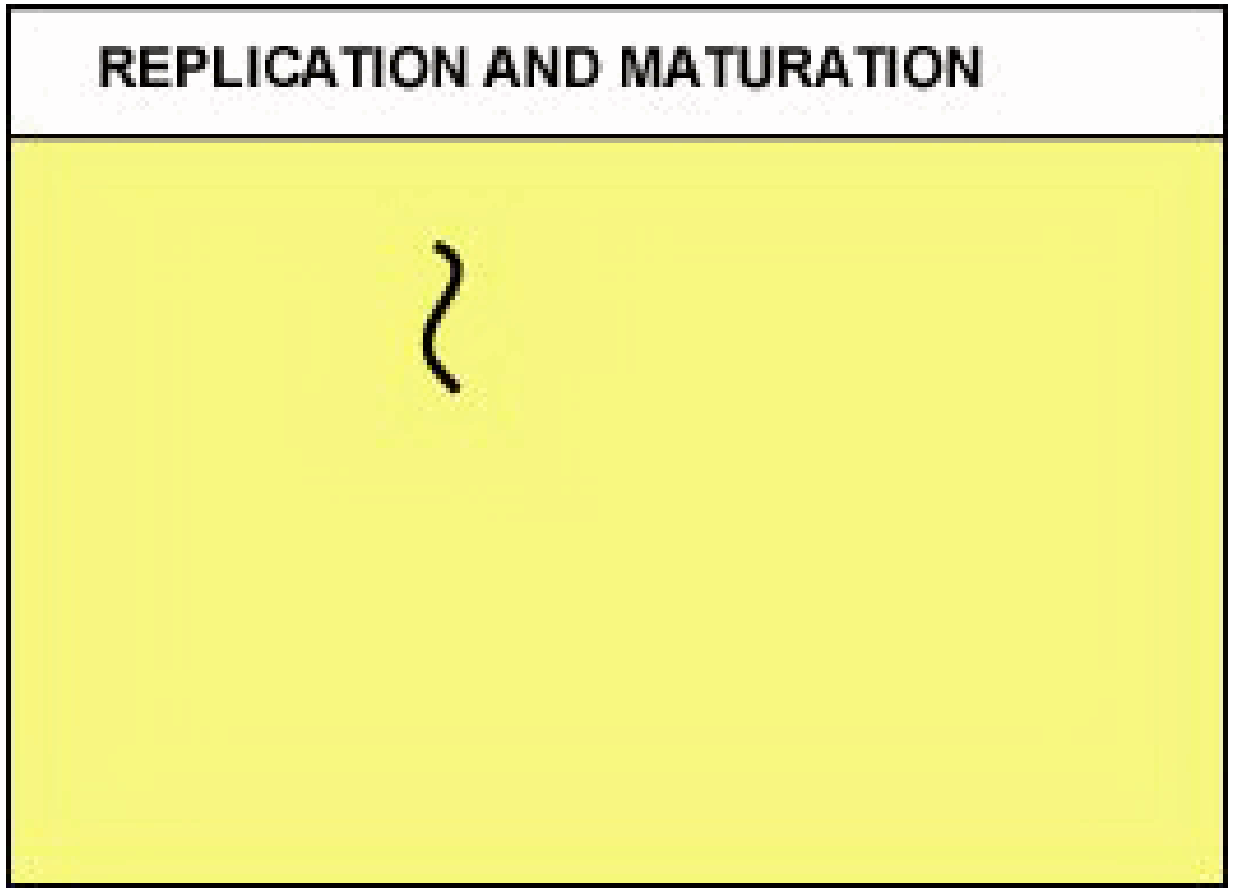
- It is the assembly of newly synthesized viral proteins in accordance with their symmetry by containing the nucleic acid in the middle.
  - Assembly of all the components necessary for the formation of the mature virion.
- Process involves bringing together newly formed genomic nucleic acid and structural proteins to form the nucleocapsid of the virus.

6. Maturation

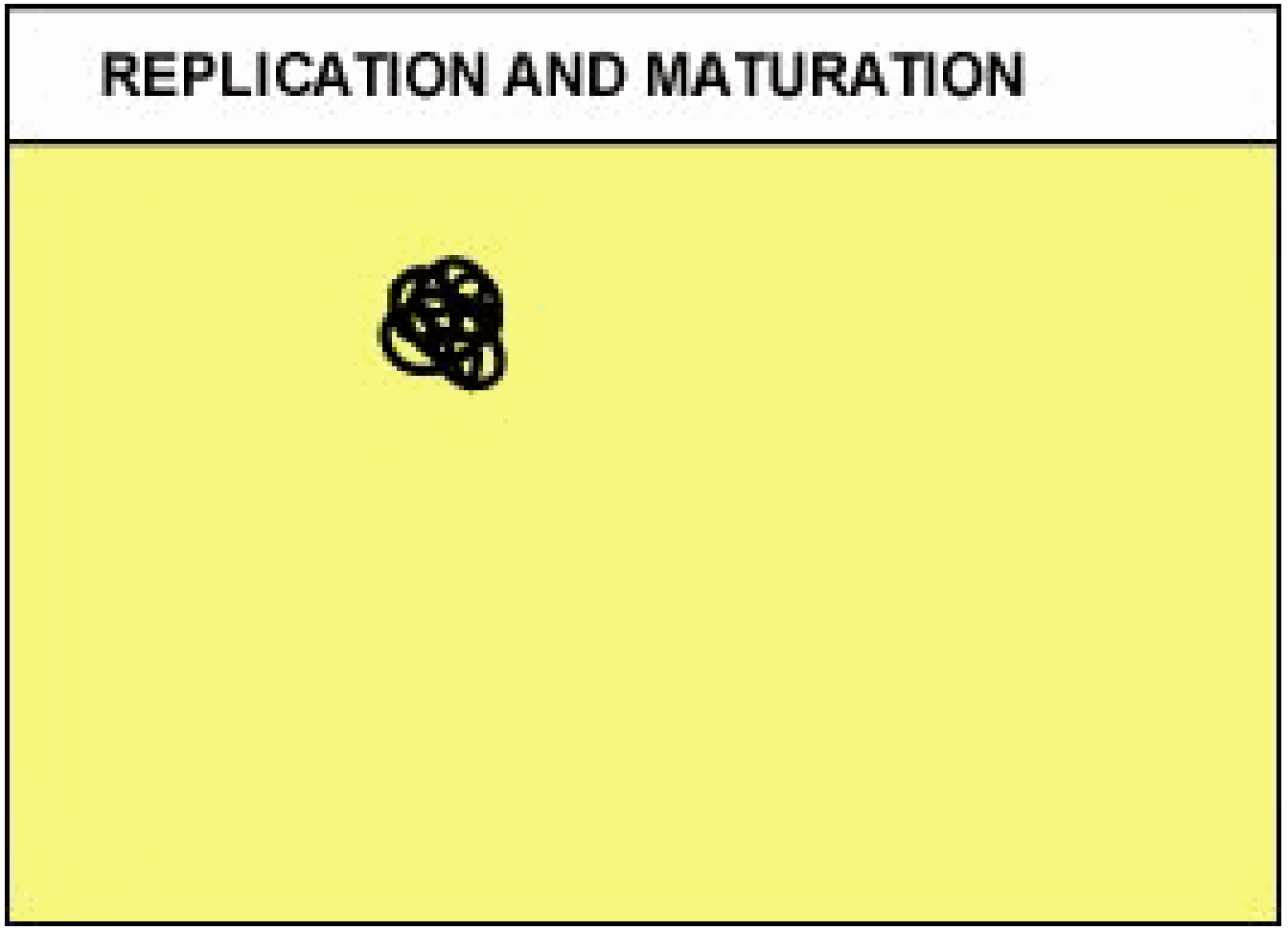
This is the period when the virus particle acquires its infectious feature.

- During this period:
  - The final arrangements between the capsid proteins
  - the functioning of viral proteases
  - new structures on the genome
- The process involves structural changes in the particle, cleavage of capsid proteins to form the mature products, which frequently leads to a conformational change in the capsid.

Replication and Maturation in Non-Enveloped Viruses



Replication and Maturation in Enveloped Viruses



7. Release

The shedding of mature viruses from the cell.

- Several different ways:

➢Non-enveloped viruses are considered lytic as they cause the cell they infect to disintegrate and are then expelled en masse.

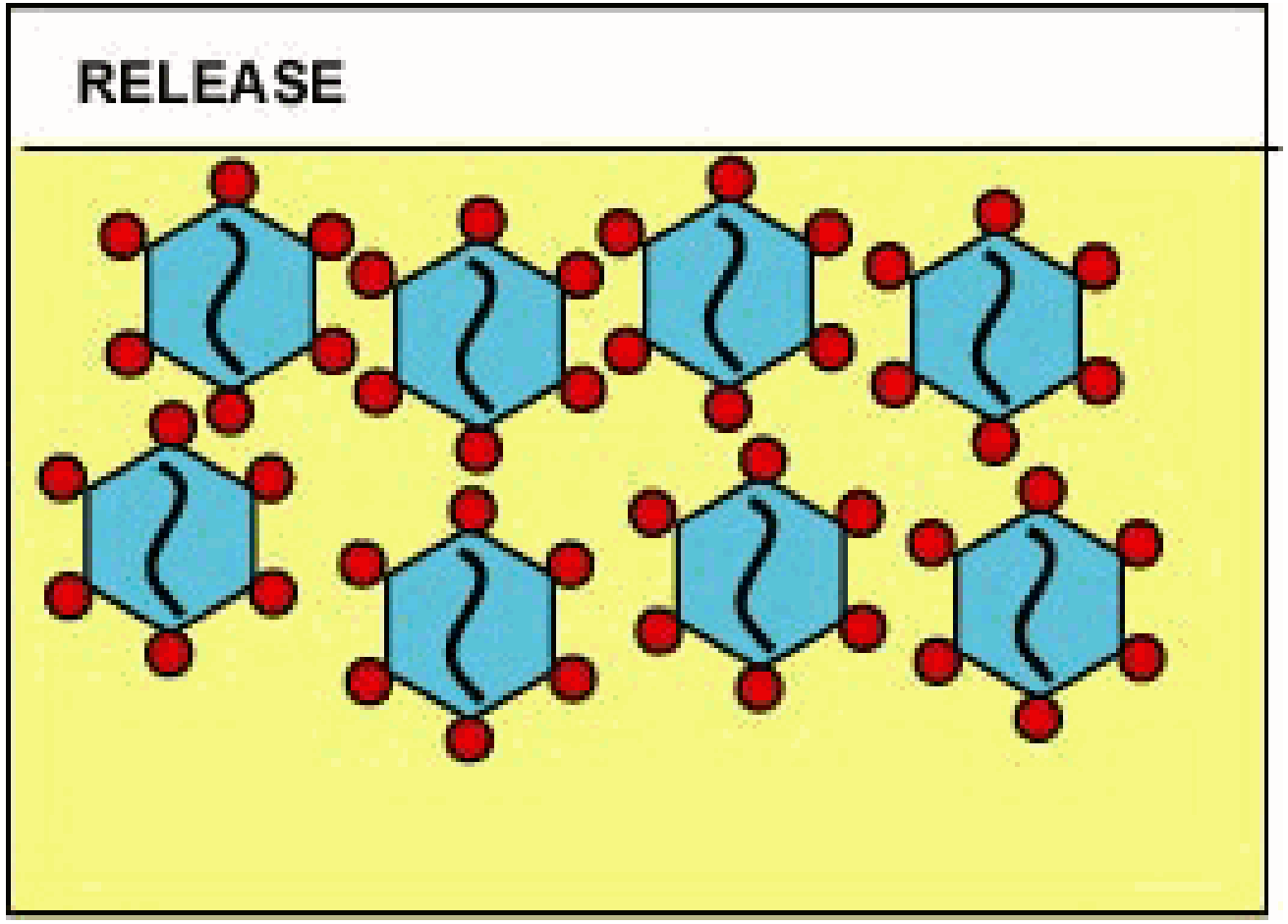
  ➢ lytic viruses (non-enveloped viruses), breaks cell; releases the virus

➢Enveloped viruses acquire the lipid membran. Enveloped viruses can leave the cell by BUDDING the cytoplasm or nucleus membrane of the cell in which they are present.

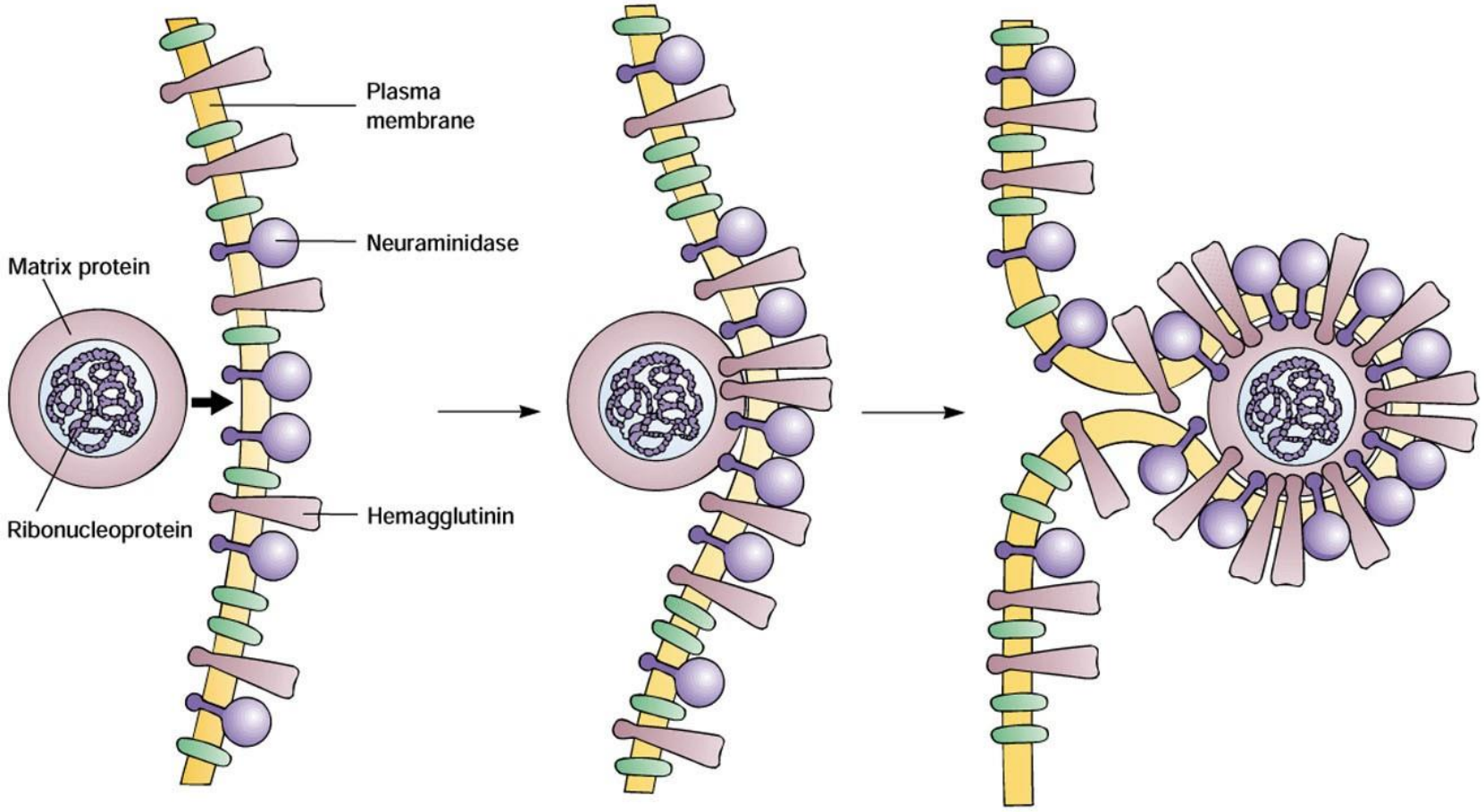
- Budding viruses do not necessarily kill the cell. Thus, some budding viruses may be able to set up persistent infections.

➢Some enveloped and non-enveloped viruses leave the cell by exocytosis.

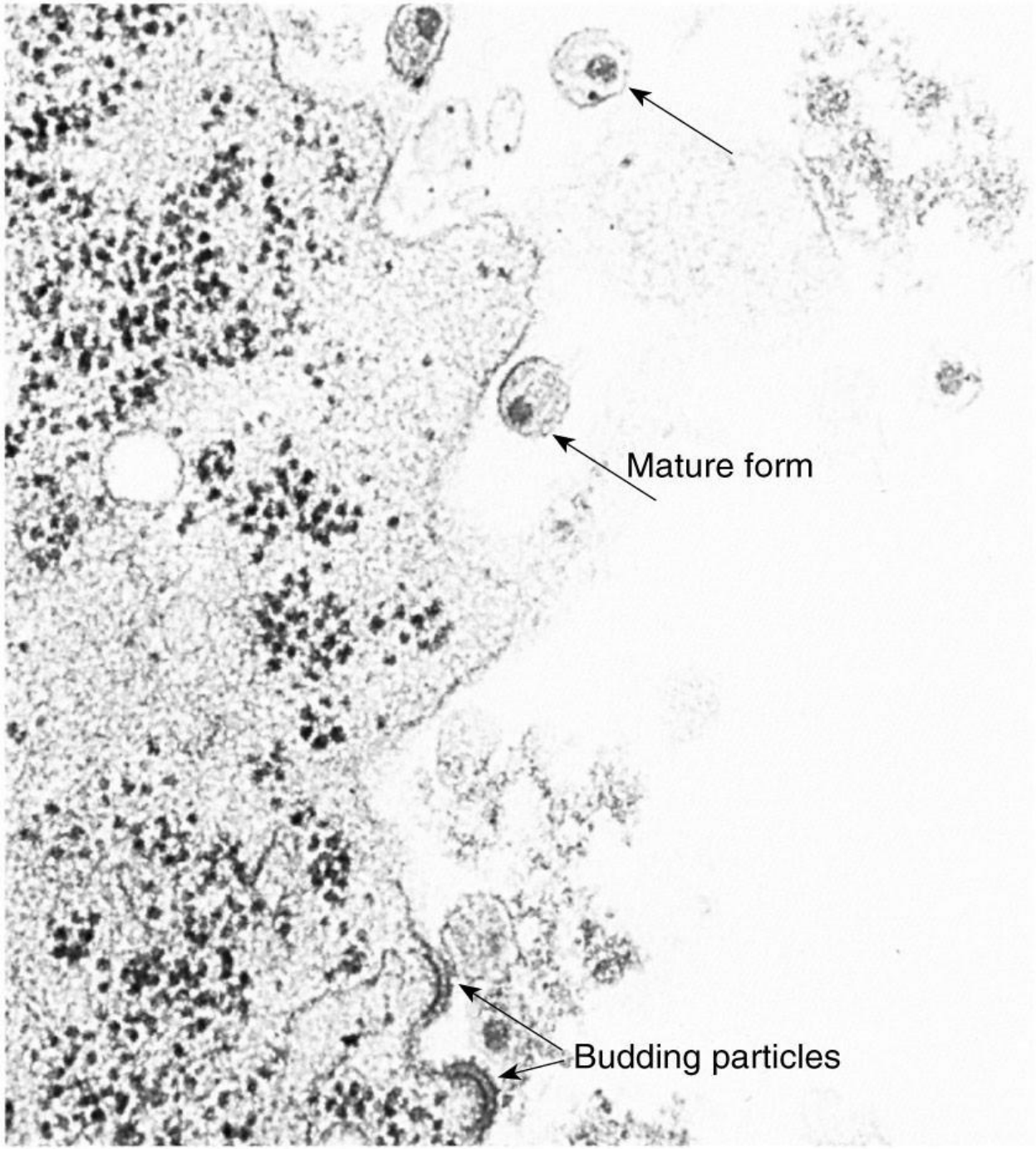
Releasing in Non-Enveloped Viruses



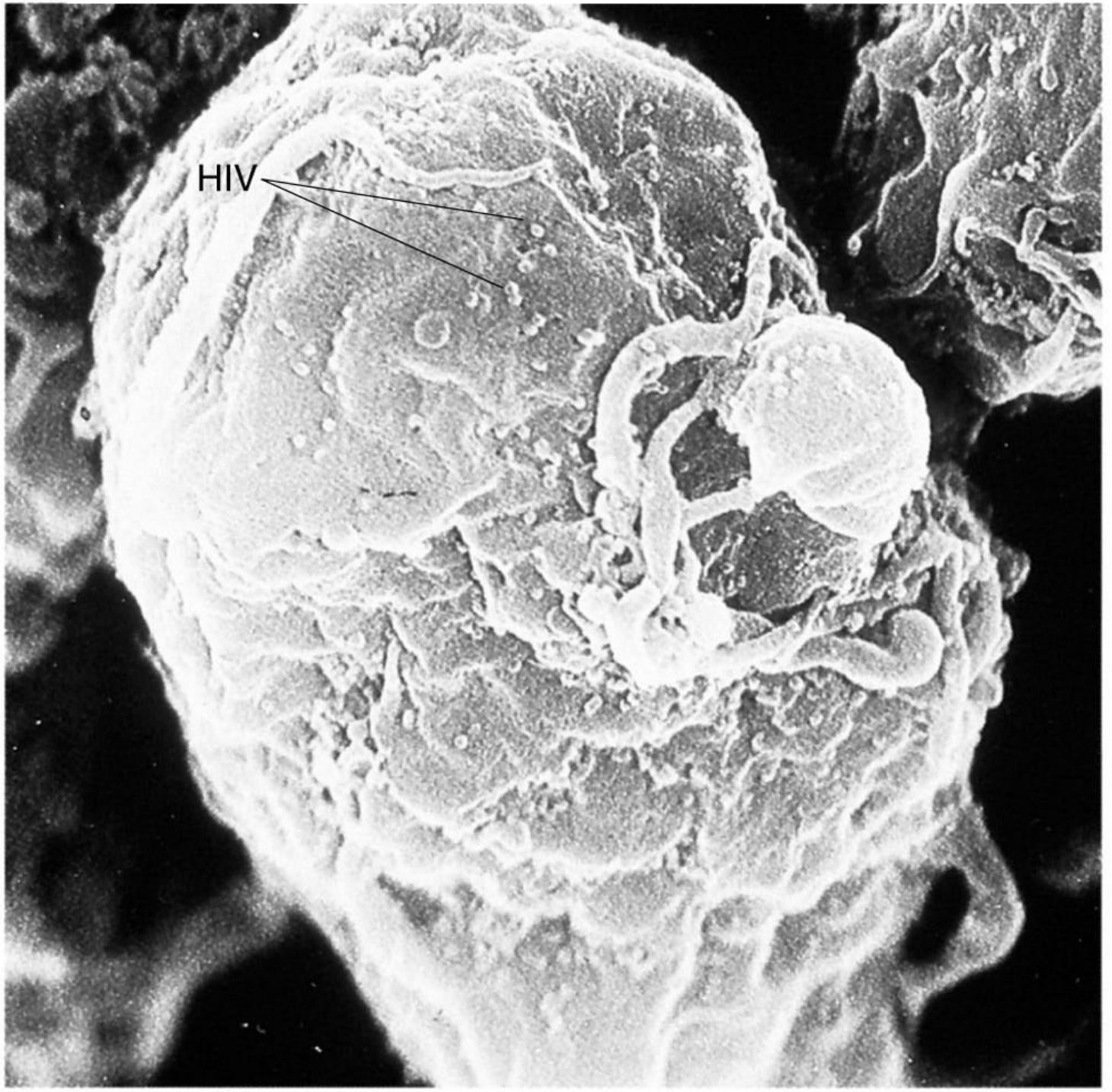
Releasing in Enveloped Viruses (BUDDING)



Budding Enveloped Virus



Budding Enveloped Virus

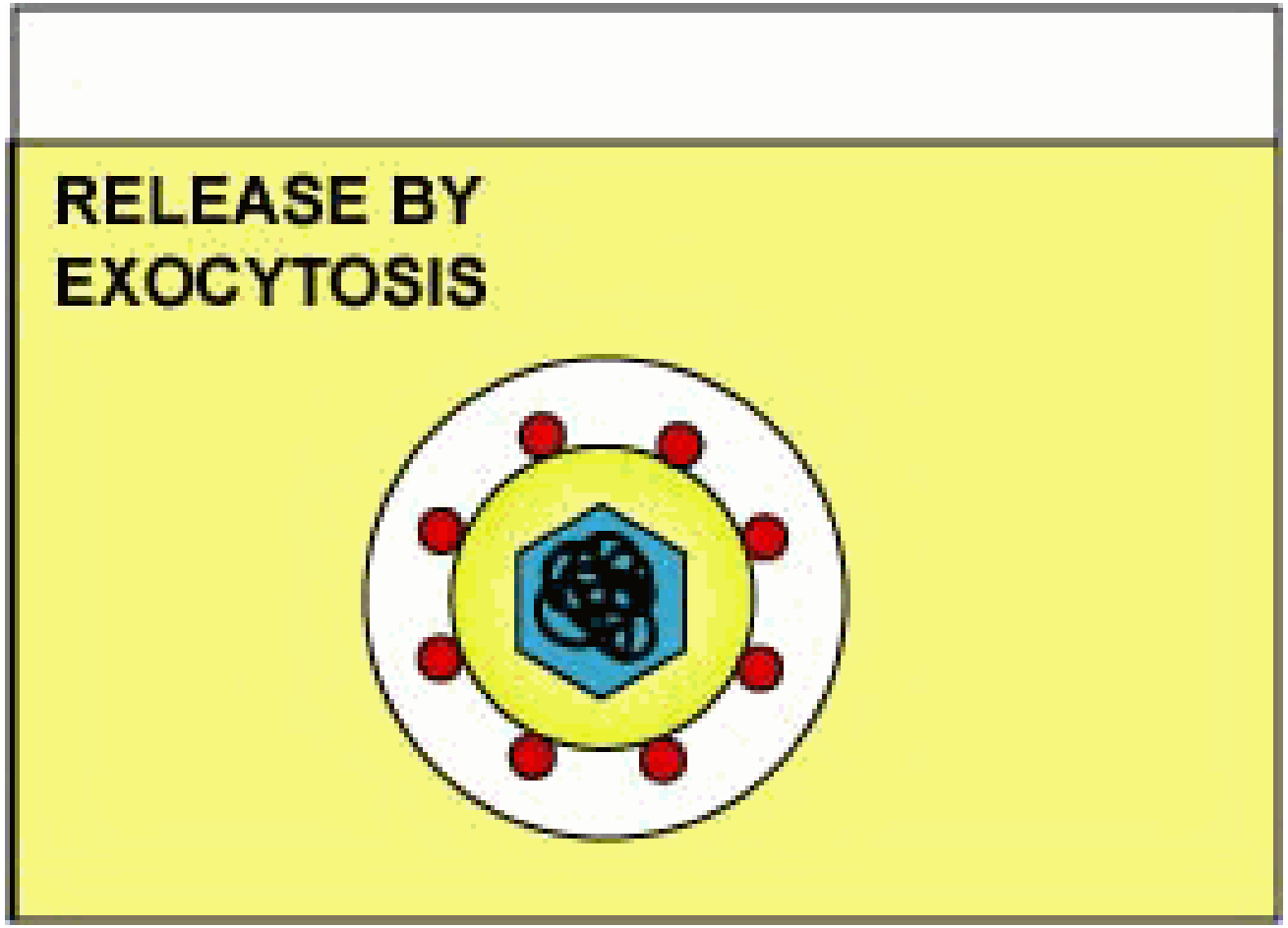


Envelope Origin in Enveloped Viruses

Table 18.2 Intracellular Sites of Animal Virus Reproduction

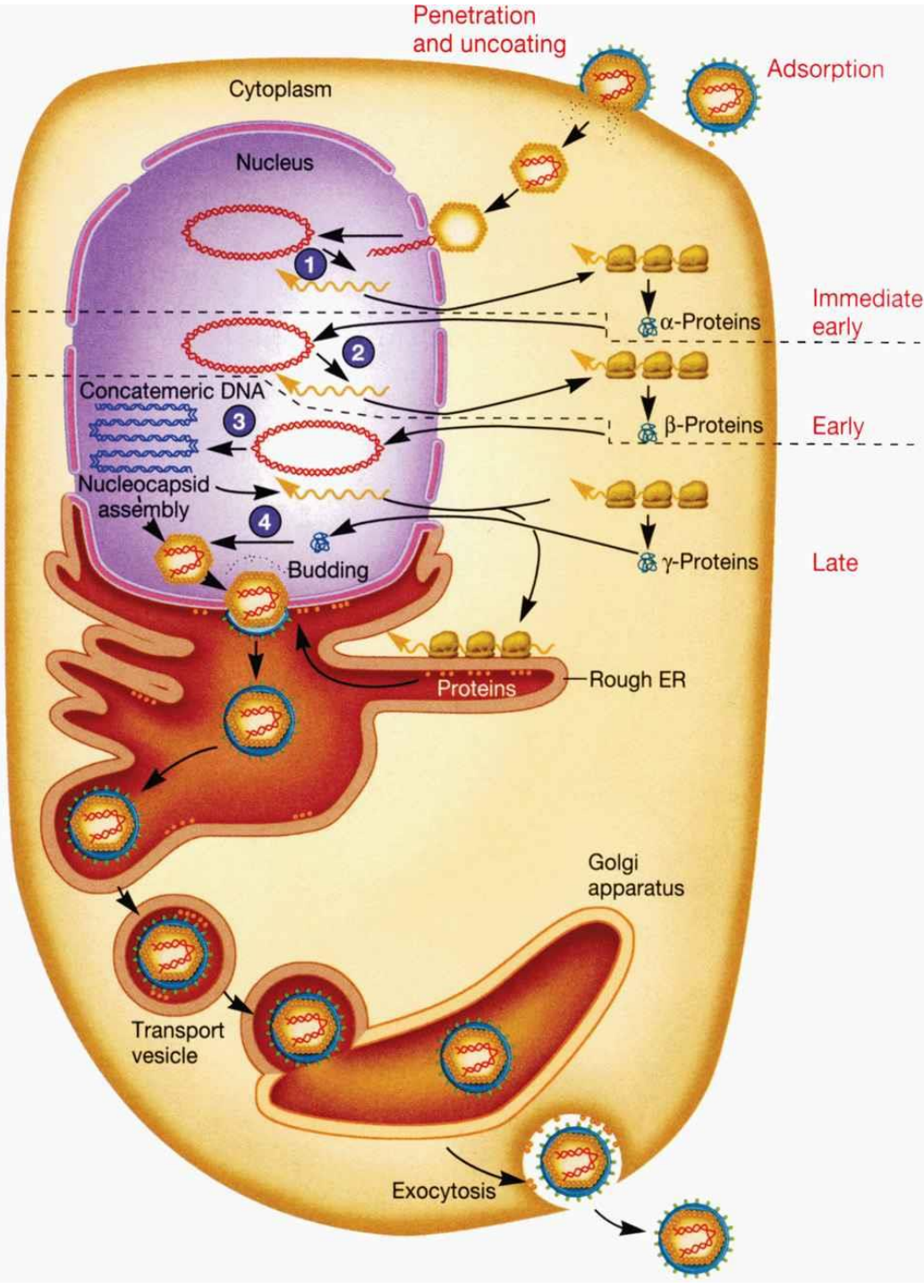
Virus	Nucleic Acid Replication	Capsid Assembly	Membrane Used in Budding
DNA Viruses			
Adenoviruses	Nucleus	Nucleus	
Hepadnaviruses	Cytoplasm	Cytoplasm	Endoplasmic reticulum
Herpesviruses	Nucleus	At nuclear membrane	Nucleus
Papillomaviruses	Nucleus	Nucleus	
Parvoviruses	Nucleus	Nucleus	
Polyomaviruses	Nucleus	Nucleus	
Poxviruses	Cytoplasm	Cytoplasm	
RNA Viruses			
Coronaviruses	Cytoplasm	Cytoplasm	Golgi apparatus and endoplasmic reticulum
Orthomyxoviruses	Nucleus	Cytoplasm	Plasma membrane
Paramyxoviruses	Cytoplasm	Cytoplasm	Plasma membrane
Picornaviruses	Cytoplasm	Cytoplasm	
Reoviruses	Cytoplasm	Cytoplasm	
Retroviruses	Cytoplasm and nucleus	At plasma membrane	Plasma membrane
Rhabdoviruses	Cytoplasm	Cytoplasm	Plasma membrane, intracytoplasmic membranes
Togaviruses	Cytoplasm	Cytoplasm	Plasma membrane, intracytoplasmic membranes

Releasing via Exocytosis



HERPESVIRUS REPLICATION

1. Circularization of genome and transcription of immediate early genes
2. α-Proteins, products of immediate early genes, stimulate transcription of early genes.
3. β-Proteins, products of early genes, function in DNA replication, yielding concatemeric DNA. Late genes are transcribed.
4. γ-Proteins, products of late genes, participate in virion assembly.

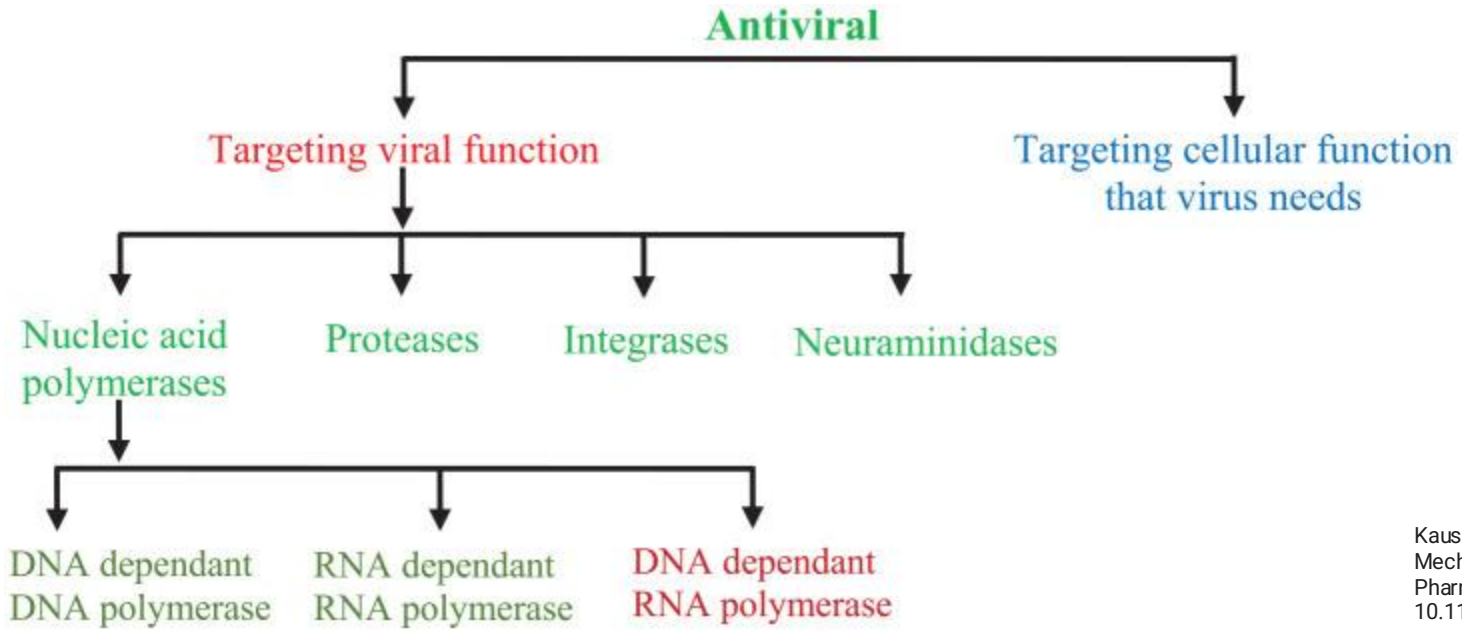


(Reproduced with permission from Willey JM: *Prescott, Harley, & Klein's Microbiology*, 7th edition. McGraw-Hill, 2008.)

ANTIVIRAL THERAPY

Prevention of virus replication

- The use of antiviral drugs requires great care, as their systemic use can cause many problems.
- Preventing virus replication is basically possible by targeting the following periods.
  - ➢Adsorption and penetration stage
  - ➢During the eclipse period
  - ➢During the period of releasing from the cell



Kausar S, Said Khan F, Ishaq Mujeeb Ur Rehman M, et al,. A review: Mechanism of action of antiviral drugs. Int J Immunopathol Pharmacol. 2021 Jan-Dec;35:20587384211002621. doi: 10.1177/20587384211002621

➢ Substances that prevent virus absorption

- Sulfated polysaccharides (Agar extract, mucopolysac., polyvinyl sulfate)
- Detergents
- Antibodies

➢Antiviral substances that are effective during the eclipse period are examined in 3 basic subgroups;

1. Nucleotide Analogs ⟹ Antiherpetics

- These substances terminate DNA chain elongation by being used instead of regular nucleotides during DNA or RNA synthesis.
  e.g. Acyclovir, Vidarabine, Gancyclovir, Ribavirin (purine RNA nucleotide, RSV, HCV, CCHFV).

2. Antivirals that prevent acidification

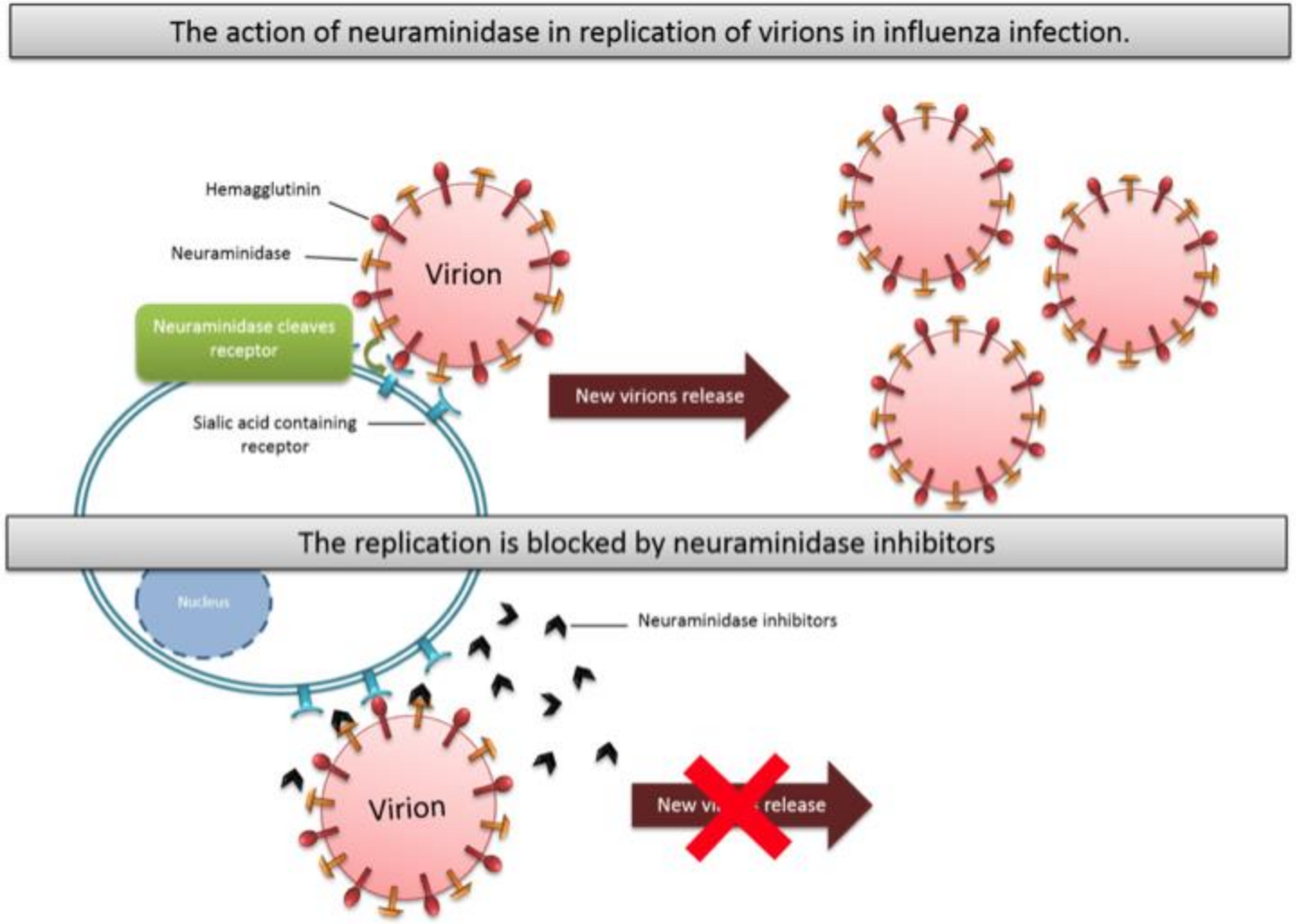
- These substances are used especially during Paramyxo- and Orthomyxovirus infections. They prevent the penetrating nucleocapsid from being disintegrated by acid vesicles.
  E.g. Amantadine

3. Antivirals, which block transcription

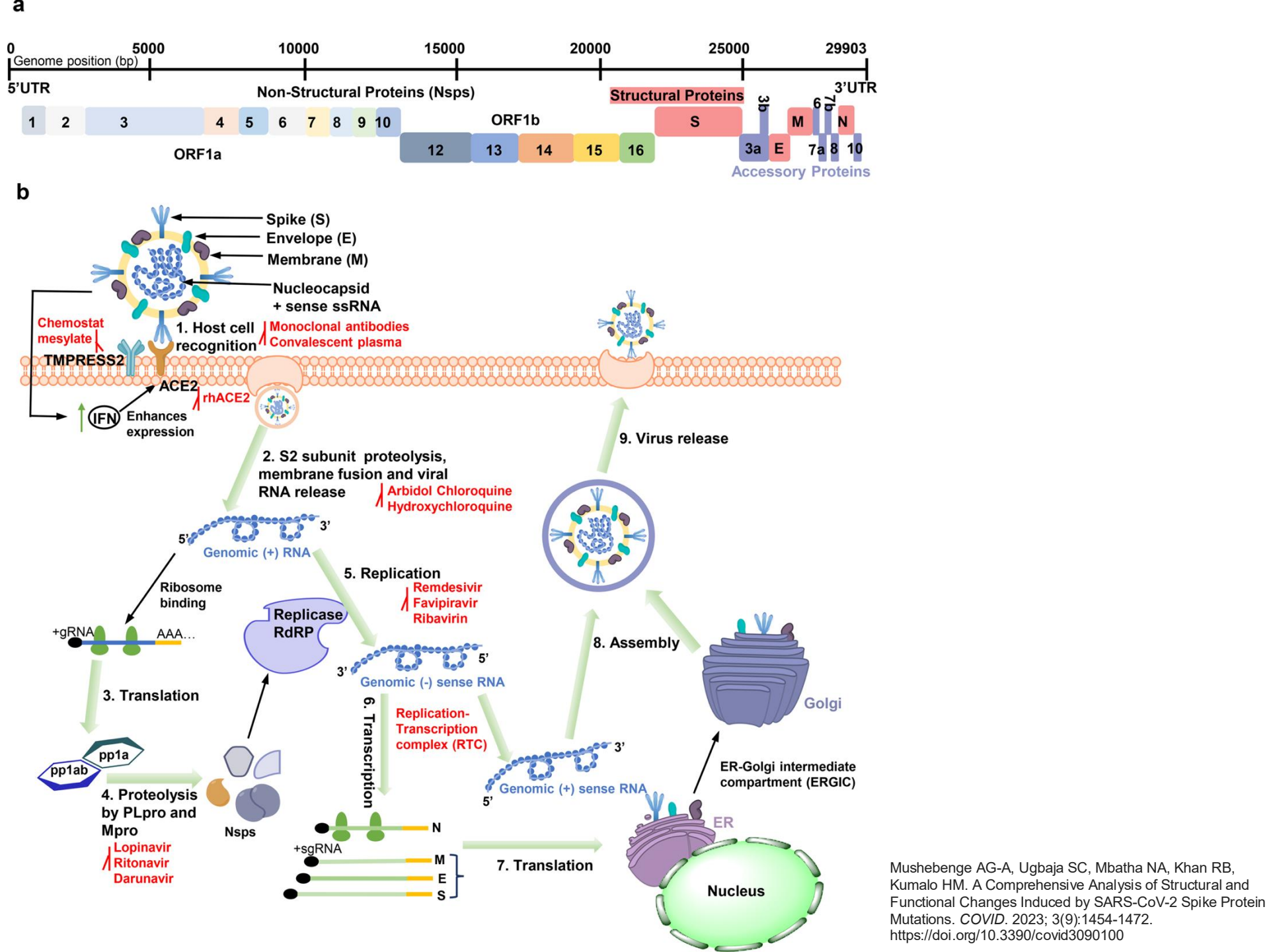
- They inhibit transcription of released viral nucleic acid, reverse transcription, and mRNA expression. They are used in smallpox infections, retrovirus infections and hepatitis.
  E.g. Azidovidin (AZT), Interferon.

➢ Substances that prevent exit from the cell

- Oseltamivir is a competitive inhibitor of influenza's neuraminidase enzyme, which cleaves the sialic acid found on glycoproteins on the surface of human cells, helping new virions to exit the cell.
- By inhibiting this process, oseltamivir prevents the release of new viral particles.
- Oseltamivir (Tamiflu) It is a substance that acts as a Neuraminidase inhibitor in Orthomyxoviruses. In flu treatment.

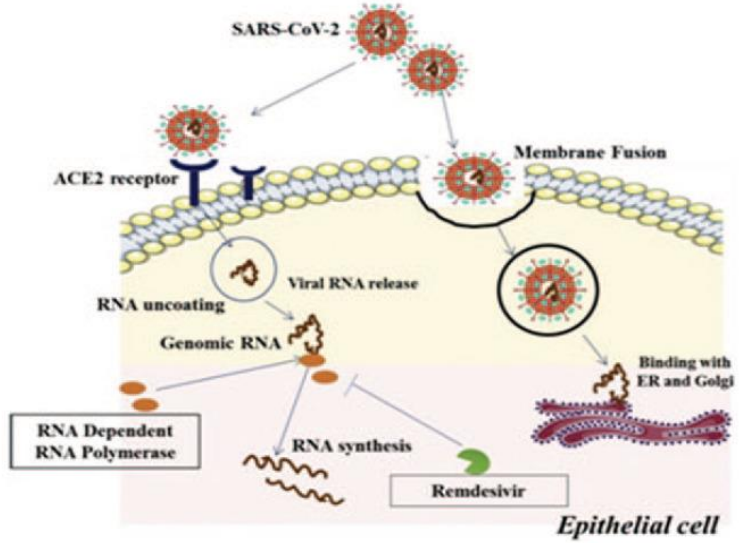


https://en.wikipedia.org/wiki/Oseltamivir#cite_note-Tamiflu_Label-25
https://tmedweb.tulane.edu/pharmwiki/lib/exe/fetch.php/tamiflu.png?cache=

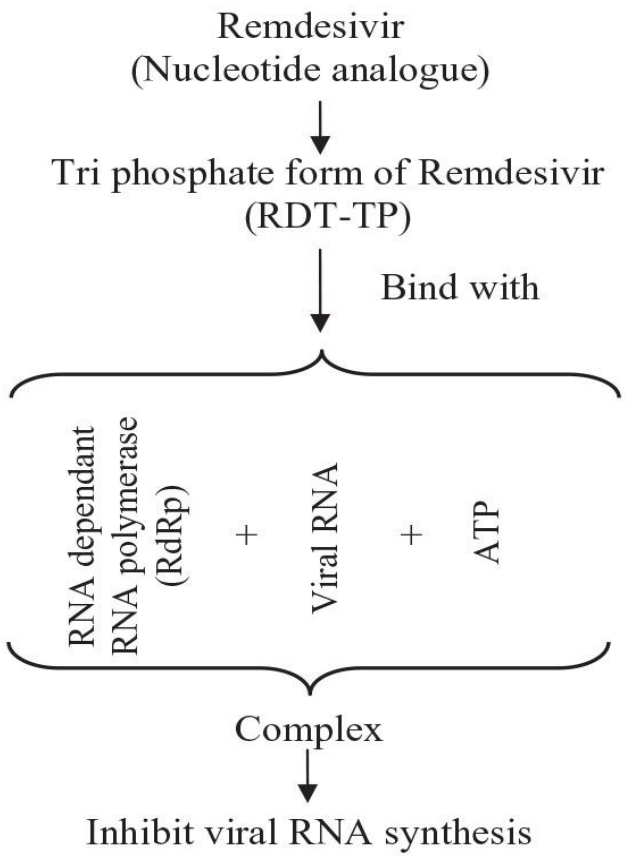


Mushebenge AG-A, Ugbaja SC, Mbatha NA, Khan RB, Kumalo HM. A Comprehensive Analysis of Structural and Functional Changes Induced by SARS-CoV-2 Spike Protein Mutations. *COVID*. 2023; 3(9):1454-1472. https://doi.org/10.3390/covid3090100

(a)



(b)

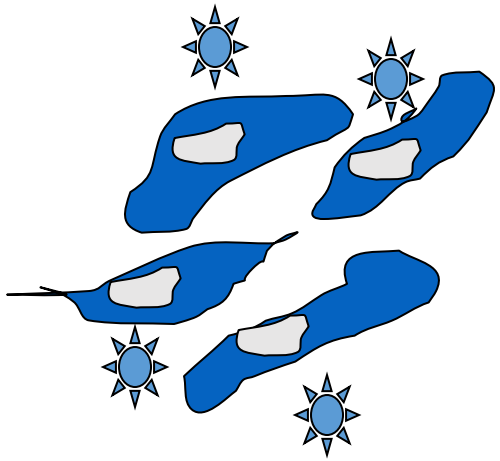


Kausar S, Said Khan F, Ishaq Mujeeb Ur Rehman M, et al,. A review: Mechanism of action of antiviral drugs. Int J Immunopathol Pharmacol. 2021 Jan-Dec;35:20587384211002621. doi: 10.1177/20587384211002621

Interferon and Interference

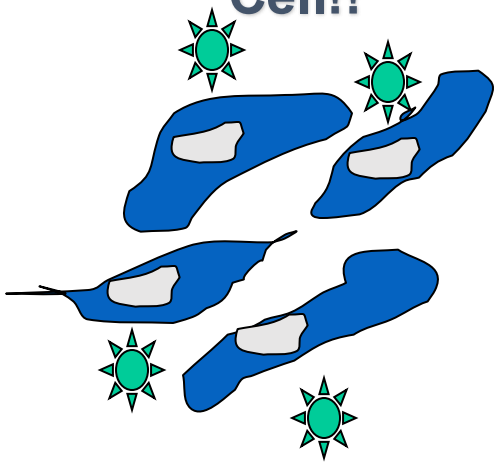
The Discovery of Issac and Linderman (1957)

Cell + Heat inactivated influenza virus



Incubate Overnight

Discard Cell, reserve the supernatant and add it on New Cell!!



Incubate Overnight and Add live virus

NO INFECTION!!

They found that virus-infected cells contain a soluble agent that can prevent the growth of other viruses, which was later identified as interferon.

Interferon

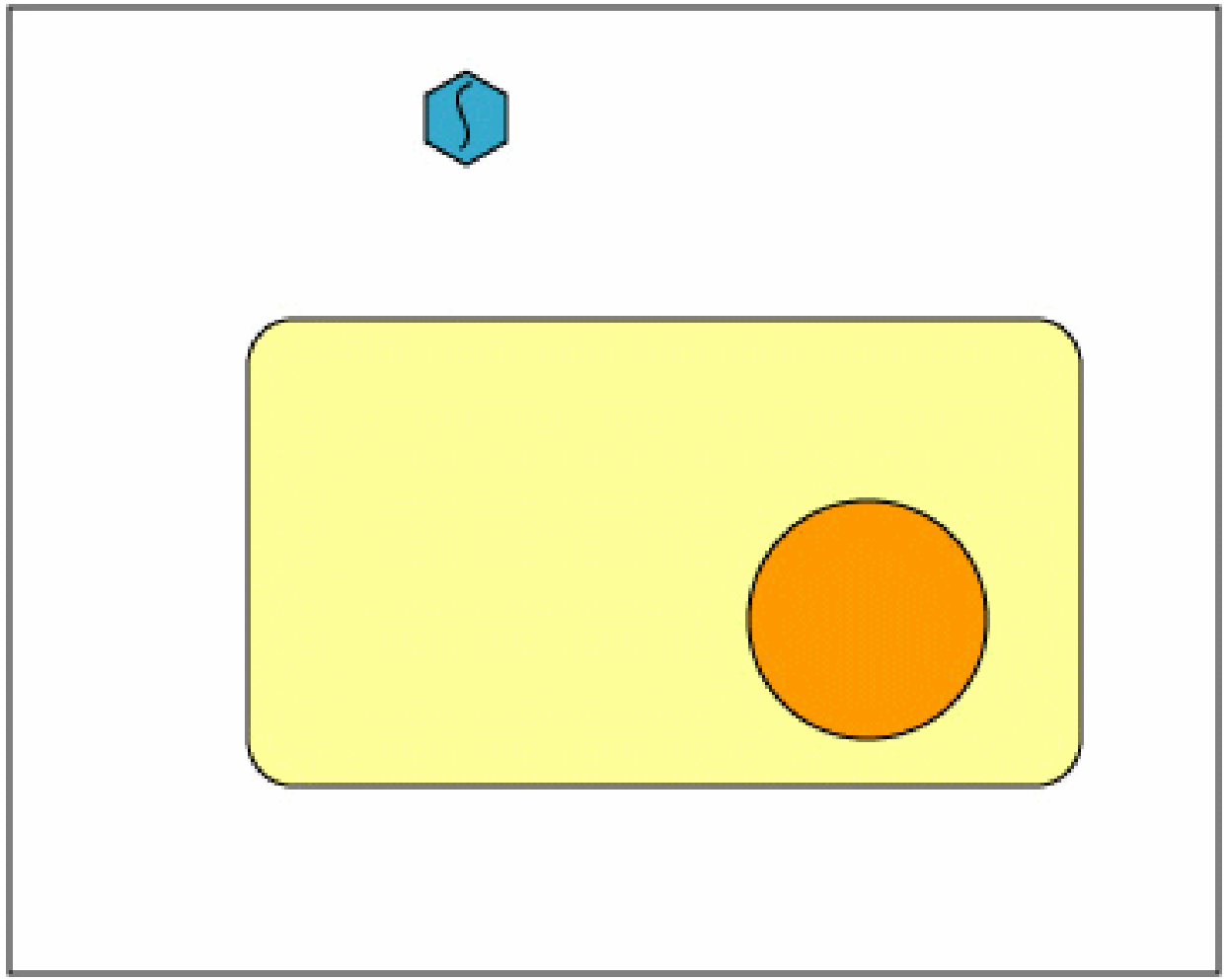
“Interferons are proteins that are synthesized and secreted by the cell in the presence of different stimulants, protecting other cells from active virus infection.”

DeSomer and Cocito 1968

Interferon vs. Interference

- Interference is defined when a virus infecting a cell partially or completely prevents that cell from being re-infected with a second virus of the same or different type. So interference is an event.
- Here, the virus that causes the first infection is called "interfering virus", and the second virus whose infection is prevented is called "challenge virus".
- Interferon is a biological product in protein structure secreted by virus-infected cells. There are basically three types of interferon: alpha (IFN-α), beta (IFN-β) and gamma (IFN-γ) interferon.

Interferon vs. Interference



What Induces Interferon (IFN)?

Interferon release can be stimulated by active or inactive viruses as well as naked viral RNAs, endotoxin and some chemical agents (mitogens).

- Viruses
  - Influenza virus – Heat and UV treated
  - DNA viruses –

    While DNA viruses must replicate, there is no such requirement for RNA viruses!!!
- Nucleic acids, especially dsRNA

dsRNA is the best IFN gene activator!!

IFN is also induced by other agents

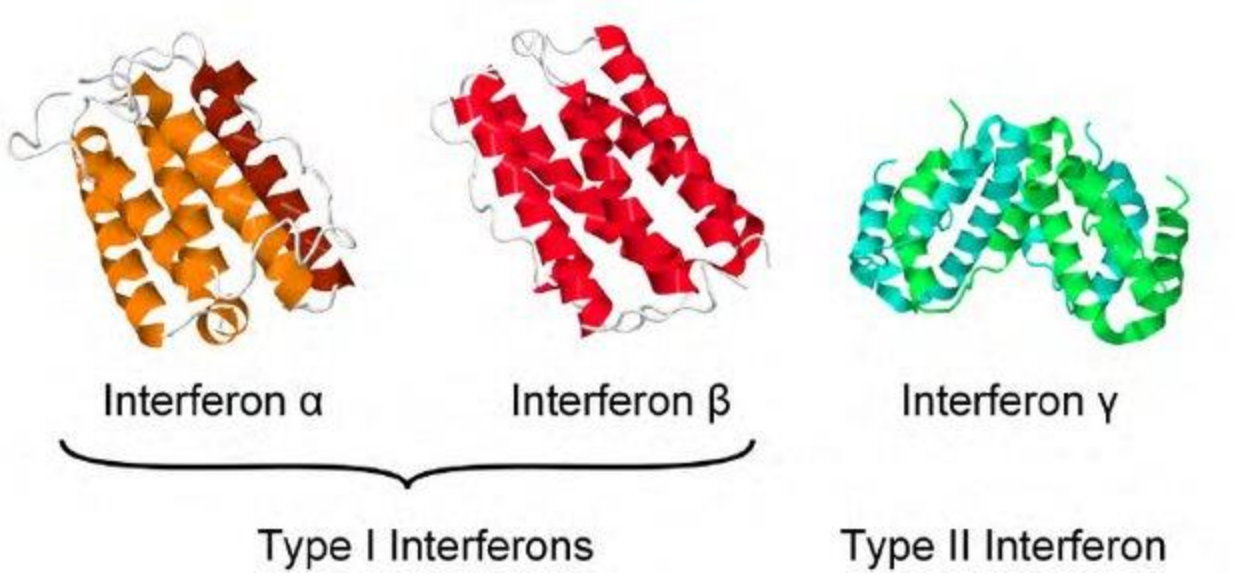
- Rickettsia
- bacteria (esp. gram-negative)
- live/inactive mycoplasma
- Protozoa

- Secreted interferon mixes with culture media and body fluids. Thus, it can be effective in areas far from where it is secreted.
- It is possible to collect, store and use secreted interferons for antiviral purposes.
- Interferons have anti-tumor activities as well as antiviral activity.
- Interferon secretion is a temporary event; interferon secretion ends when the cause is eliminated.

- Following the virus infection of the cell, interferon secretion begins in a short time. Interferon production can be detected in the body within a few hours following virus infection.
- Interferon secretion in the early stages of infection helps prevent the spread of the virus to nearby and distant cells. This limits the infection to the tissue level until the immune response develops.
- Interferon secreted against one type of virus generally shows antiviral activity against other viruses.
- However, interferon produced in one living species is not effective in another living species.

Three basic mechanisms in interferon activity;

- Stopping viral RNA synthesis,
- Degradation of viral RNAs
- Inhibition of protein synthesis from viral mRNAs



- Divided into three classes:
  - IFN-α
  - IFN-β
  - IFN-γ
- They have 2 receptors (one for α/β and one for γ)

Daniel N Clark, Brian D Poole 2012. Interferon and Apoptosis in Systemic Lupus Erythematosus In book: Systemic Lupus Erythematosus DOI: 10.5772/25734

IFN-α

- antiviral activity,
- secreted from leukocytes and other body cells
- can be synthesized both in vivo and in cell cultures following virus infections.
- is induced by the virus
- prevents virus replication and cell proliferation

IFN-β

- antiviral activity,
- secreted from fibroblasts and epithelial cells
- can be synthesized both in vivo and in cell cultures following virus infections.
- is induced by the virus
- prevents virus replication

IFN-γ

- plays a role in regulating the immune system
- secreted from T lymphocytes and natural killer (NK) cells.
- antiviral, immunoregulatory, and anti-tumor properties

Cultivation of Viruses

The Aim

- To isolate and identify the viruses,
- to establish inter-vital relationships,
- To prepare viruses for vaccines
- To use in serological tests,
- to obtain hyperimmune or type-specific serum,
- to demonstrate epidemiological and pathogenetic properties,

- Viruses are produced only in live systems. This system is called host.
- Host systems are examined in two basic groups, in vivo or in vitro.

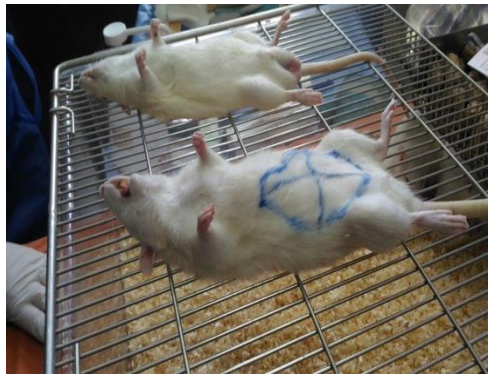
- In vivo system: Laboratory animals, Embryonated Chicken Egg (ECE)
- In vitro system: Cell culture

In vivo Systems -1
Animals

- Animals are used for studying viruses which do not grow in cell cultures or eggs, and for testing vaccines

1. Conventional Animals:

These are animals subjected to standard rearing conditions and without any microbiological control. Naturally, they can contain many saprophytic or pathogenic microorganisms. They can be used in some routine experiments and to obtain certain biological products (complement, negative or positive serum).



http://denhay.aku.edu.tr/genel-bilgiler/

2. Spesific Pathogen Free (SPF) animals:

- Although they contain different microorganisms in their bodies, they do not contain pathogenic microorganisms.
- They are kept under constant control and are subject to special care.
- Their water and feed are subjected to microbiological control, and filtered air is supplied to their living environment.
- The results of the experiments with these animals; It is more specific and safer than conventional animal experiments.

http://www.nzavs.org.nz/articles/what-is-the-extent-of-animal-use-for-vivisection-in-new-zealand/

3. Germ Free (GF) animals:

- They are special animals that do not contain any microorganisms and do not have any antibodies against them in their bodies.
- They are taken from healthy mothers by cesarean section under sterile conditions and raised in an environment where all environmental conditions are controlled.
- Their feed and water are sterilized, and filtered air is supplied to their living environment. Stool and blood samples are subjected to regular microbiological control.
- They are used in special pathogenesis studies. It is expensive and costly but extremely reliable.

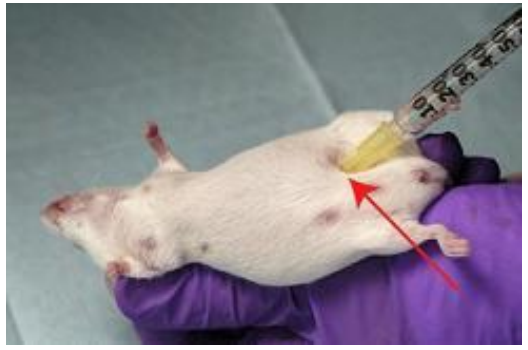
http://www.narlabs.org.tw/en/news/news.php?news_id=387

http://microbe.med.umich.edu/services/germ-free-gnotobiotic-mouse-facilities

Inoculation of Virus in Animals

- Intranasal
- Intratracheal
- Oral
- Corneal
- Subkutan
- Intradermal
- Intravenous
- Intraperitoneal
- Intracerebral
- Intramuskuler
- Etc.

- After the animal is inoculated with the virus suspension, the animal is:
  - observed for signs of disease
  - visible lesions
  - or is killed so that infected tissues can be examined for virus



https://norecopa.no/norina/procedures-with-care
http://www.nobivacbb.com/catteries/