# Viral life cycle

Viral replication terminology

- Plaque forming unit (pfu): measure of the number of particles capable of forming plaques per unit volume, such as virus particles
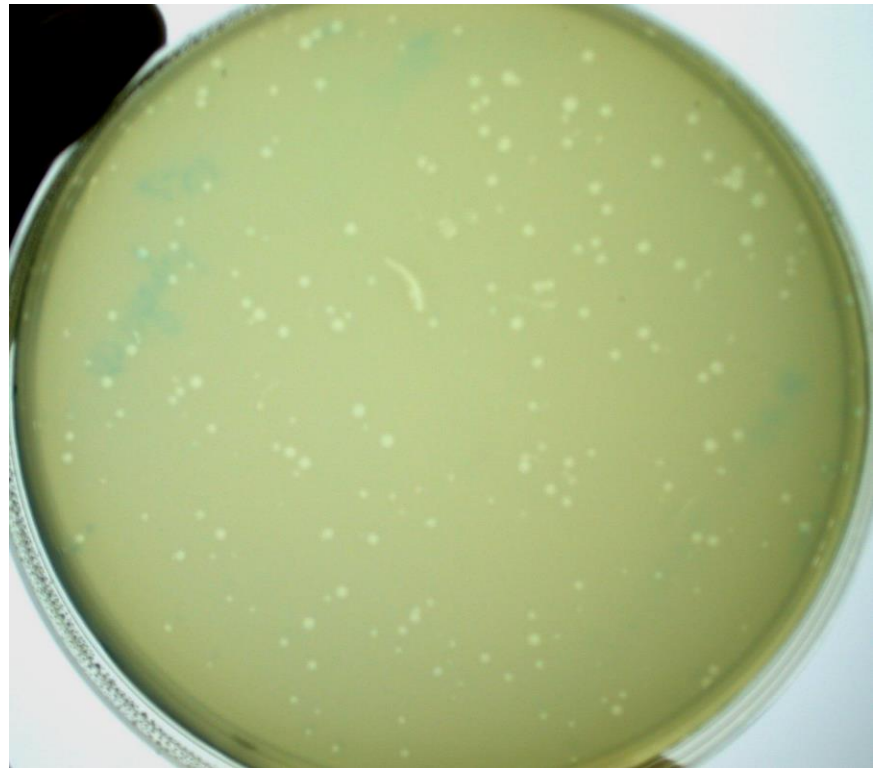

Baculovirus plaques

Zones of clearing (plaques) are generated by infection of insect (Sf9) cells with individual baculovirus particles. Uninfected Sf9 cells surrounding the plaque are stained pink with neutral red.
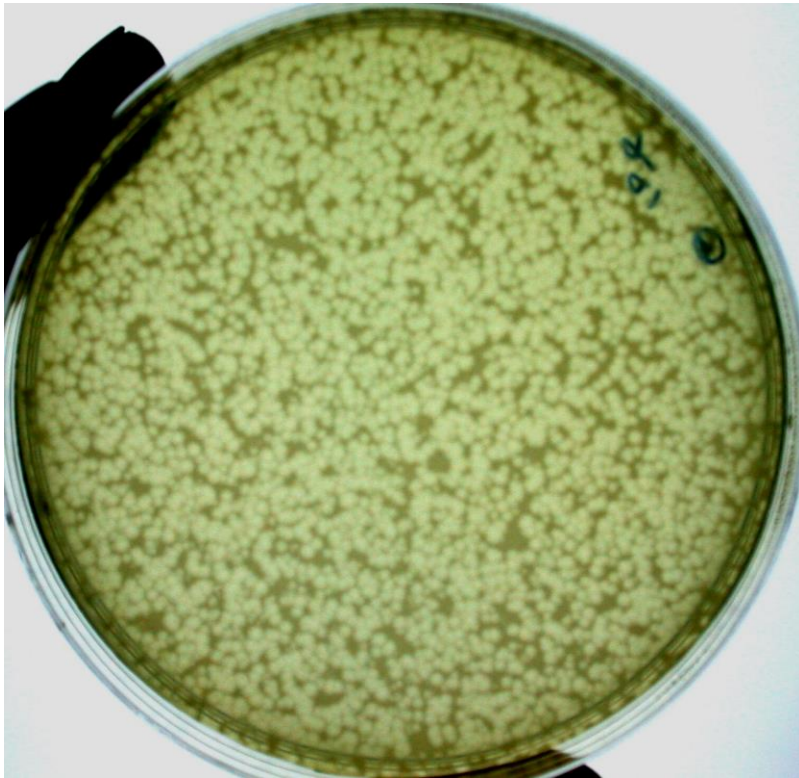
10^{-6} 10^{-7}

PFU: plaque forming unit   211 plaques

1 PFU = 1 plaque = 1 bacterial phage

Count : plaque # x Dilution x volume (ml) = PFU/ml

Ex: 211 x 10^7 x 1 = 2.11 x 10^9 PFU/ml

Viral replication terminology

- Multiplicity of infection (MOI): ratio of infectious agents (e.g. phage or virus) to infection targets

- Eclipse phase: period during which the input virus becomes uncoated; 10-12h

- Synthetic phase: time during which new virus particles are assembled; 4-6h

- Latent period: no extracellular virus can be detected

- Burst size: amount of infectious virus produced, per infected cell ; 10-10,000
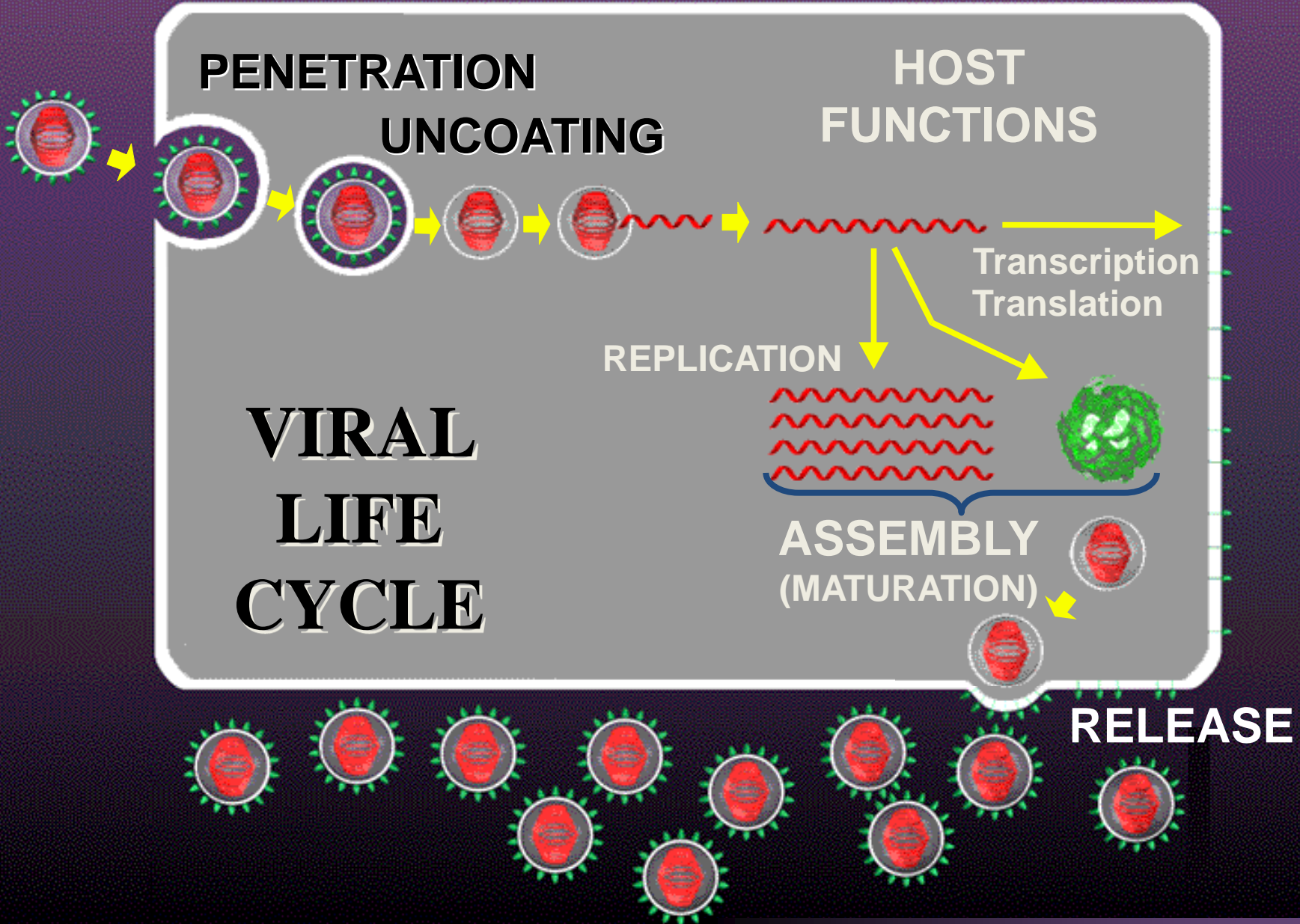
One-step virus growth curve
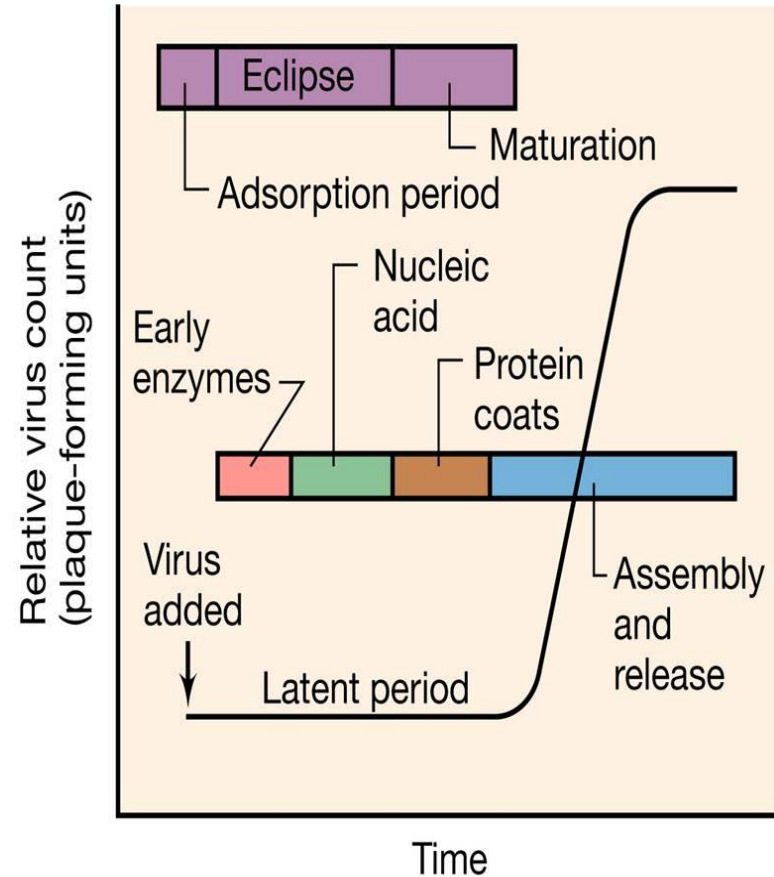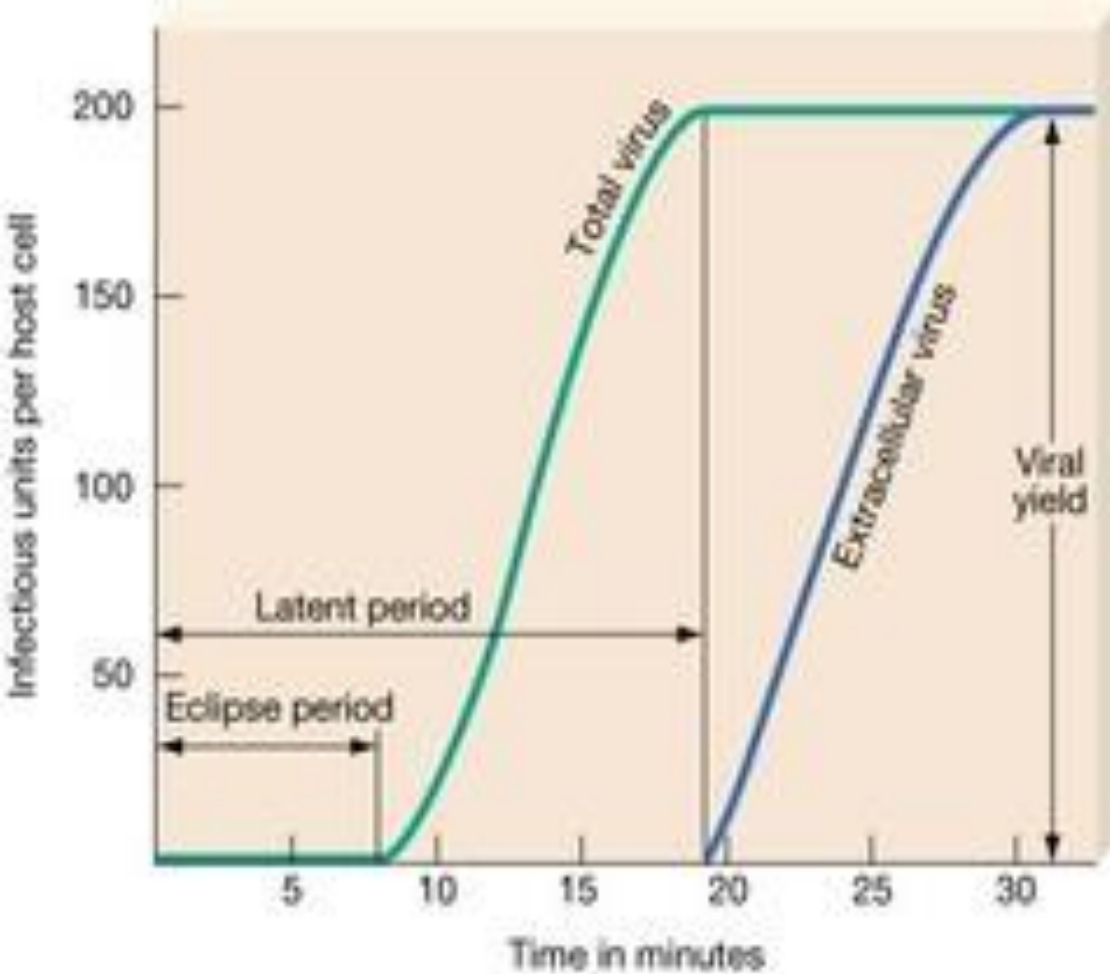
The Replication Cycle

- Virus replication can be divided into eight arbitrary stages.

- Regardless of their hosts, all viruses must undergo each of these stages in some form to complete their replication cycle.

- Not all the steps described here are detectable as distinct stages for all viruses.
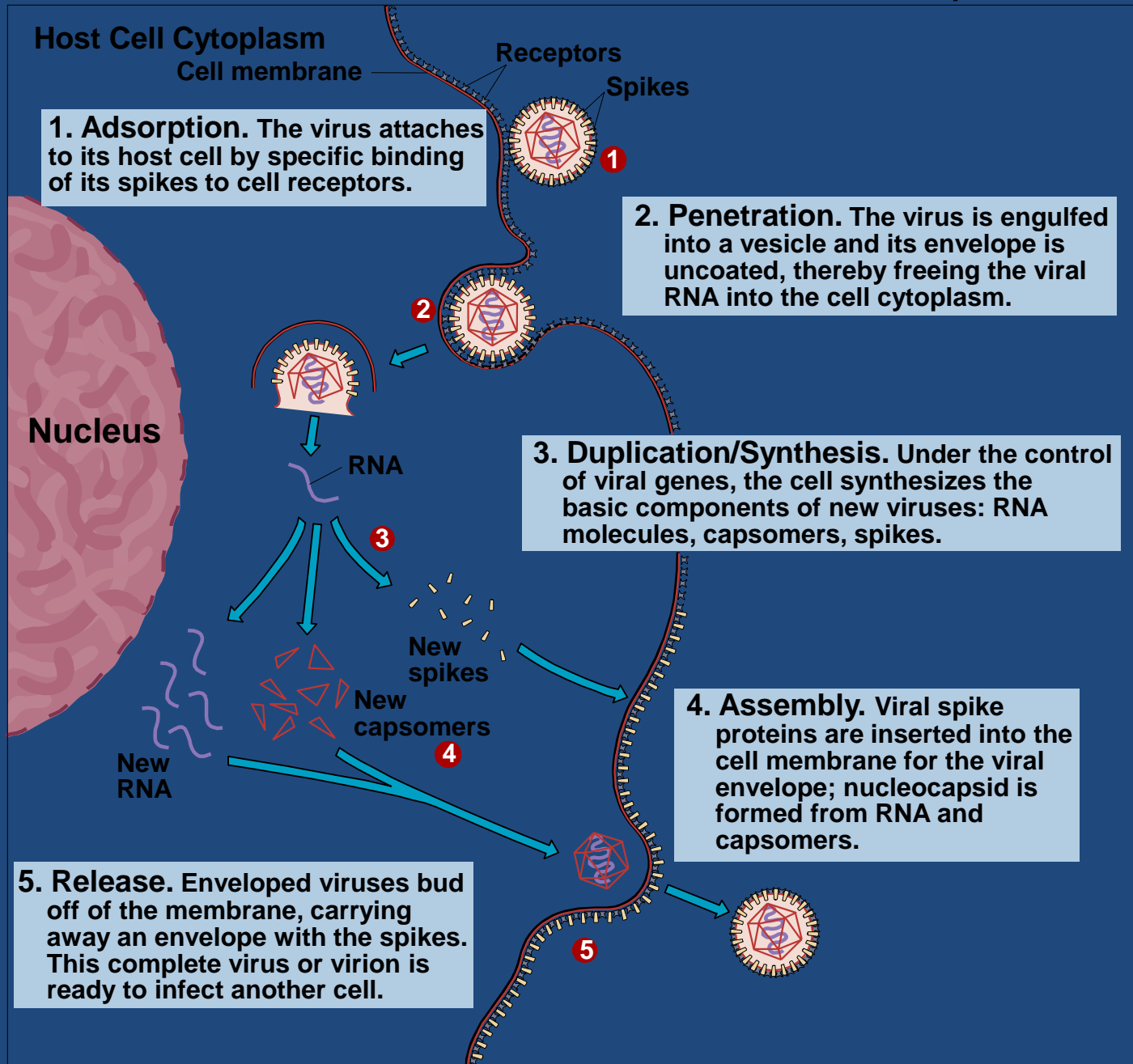
Host Cell Cytoplasm
Cell membrane — **Receptors**
Spikes

1. Adsorption. The virus attaches to its host cell by specific binding of its spikes to cell receptors.

2. Penetration. The virus is engulfed into a vesicle and its envelope is uncoated, thereby freeing the viral RNA into the cell cytoplasm.

Nucleus

RNA

3. Duplication/Synthesis. Under the control of viral genes, the cell synthesizes the basic components of new viruses: RNA molecules, capsomers, spikes.

New spikes

New capsomers

New RNA

4. Assembly. Viral spike proteins are inserted into the cell membrane for the viral envelope; nucleocapsid is formed from RNA and capsomers.

5. Release. Enveloped viruses bud off of the membrane, carrying away an envelope with the spikes. This complete virus or virion is ready to infect another cell.

Attachment

- Virus attachment consists of specific binding of a virus-attachment protein (or 'antireceptor') to a cellular receptor molecule.

- Target receptor molecules on cell surfaces may be proteins (usually glycoproteins), or the carbohydrate residues present on glycoproteins or glycolipids.

- Some complex viruses (e.g. poxviruses, herpesviruses) use more than one receptor and have alternative routes of uptake into cells.
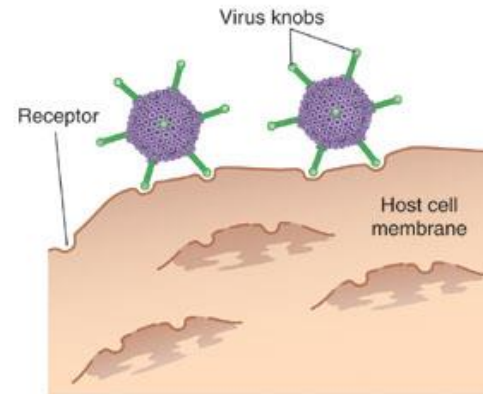
Adsorption

Enveloped
With prominent spikes
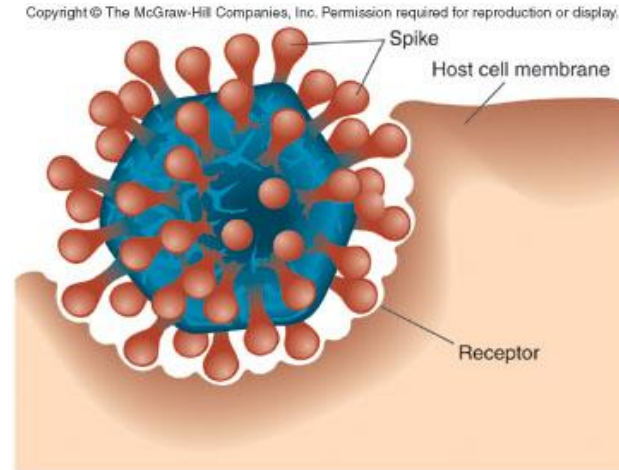
Naked; with capsid spikes

• Host range: the collection of hosts that an organism can utilize as a partner
• Cellular (tissue) tropism: the cells and tissues of a host which support growth of a particular virus

TABLE 6-6. Examples of Viral Receptors

Virus	Target Cell	Receptor*
Epstein-Barr virus	B cell	C3d complement receptor CR2 (CD21)
Human immuno-deficiency virus	Helper T cell	CD4 molecule and chemokine co-receptor
Rhinovirus	Epithelial cells	ICAM-1 (immunoglobulin superfamily protein)
Poliovirus	Epithelial cells	Immunoglobulin superfamily protein
Herpes simplex virus	Many cells	Immunoglobulin superfamily protein
Rabies virus	Neuron	Acetylcholine receptor
Influenza A virus	Epithelial cells	Sialic acid
B19 parvovirus	Erythroid precursors	Erythrocyte P antigen (globoside)

* Other receptors for these viruses may also exist.
ICAM-1 = Intercellular adhesion molecule.

Coreceptor: CCR5
CRCX4

Virus Receptors

Many examples of virus receptors are now known.

Schematic representation of some virus receptors
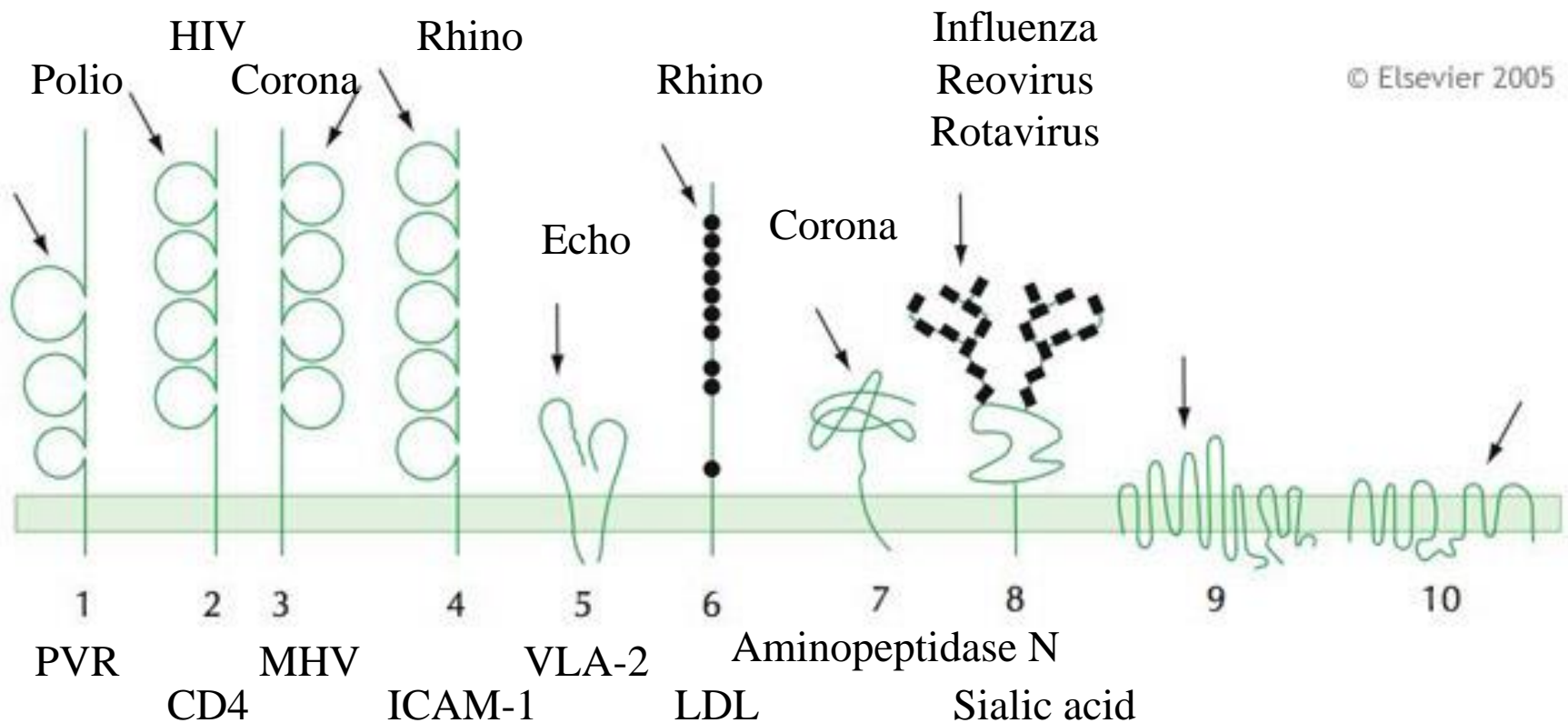
- arrows indicate virus attachment site:

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TABLE 6–5. Examples of Viral Attachment Proteins

| Virus Family	Virus	VAP
Picornaviridae	Rhinovirus	VP1-VP2-VP3 complex
Adenoviridae	Adenovirus	Fiber protein
Reoviridae	Reovirus	σ-1
	Rotavirus	VP7
Togaviridae	Semliki Forest virus	E1-E2-E3 complex
Rhabdoviridae	Rabies virus	G Protein
Orthomyxoviridae	Influenza A virus	HA
Paramyxoviridae	Measles virus	HA
Herpesviridae	Epstein-Barr virus	gp350 and gp220
Retroviridae	Murine leukemia virus	gp70
	Human immunodeficiency virus	gp120

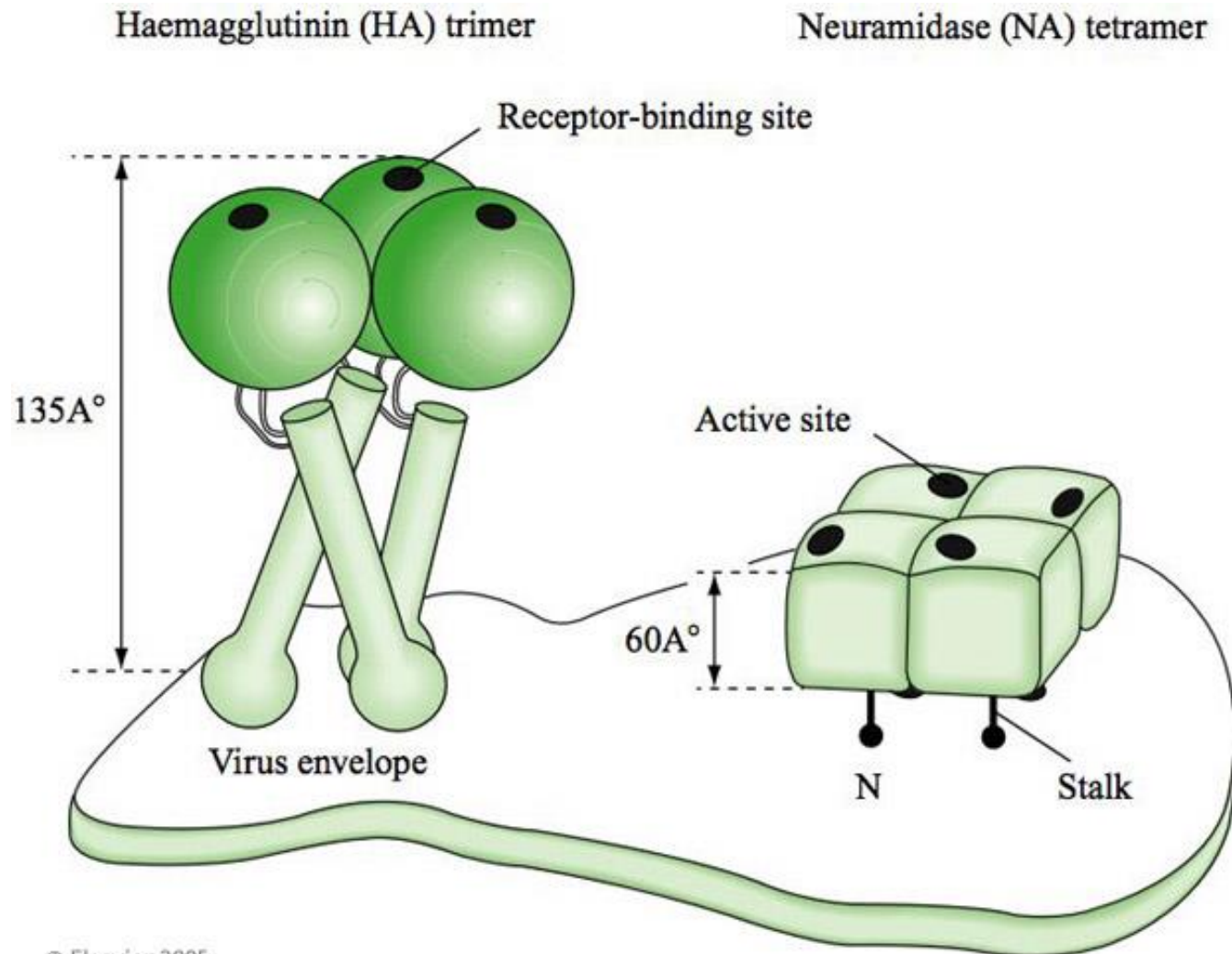gp = glycoprotein; VAP = viral attachment proteins.

HA: Hemagglutinine
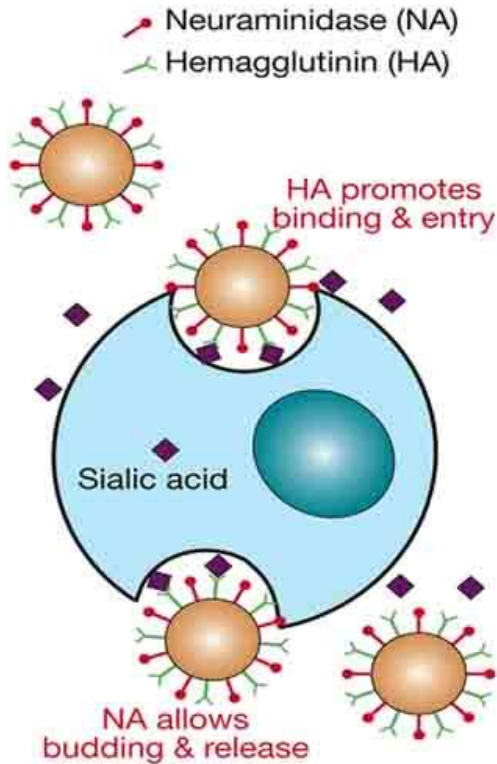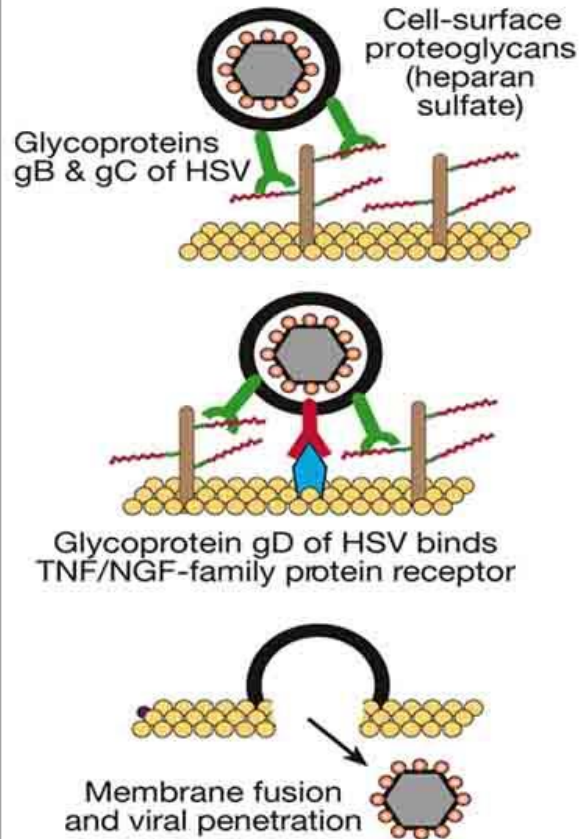
How does an animal virus infect its host?

Examples of Animal Virus Entry

Influenza Virus Receptor Binding

- The influenza haemagglutinin protein is one of two types of glycoprotein spike on the surface of influenza virus particles, the other type being the neuraminidase protein.

- Each haemagglutinin spike is composed of a trimer of three molecules, while the neuraminidase spike consists of a tetramer.

- The haemagglutinin spikes are responsible for binding the influenza virus receptor, which is sialic acid (N-acetyl neuraminic acid).

- As a result, there is little cell-type specificity imposed by this receptor interaction and therefore influenza viruses bind to a wide variety of different cell types.

Influenza Virus Receptor Binding

Haemagglutinin (HA) trimer

Neuramidase (NA) tetramer

Receptor-binding site

Active site

135A°

60A°

Virus envelope

N

Stalk

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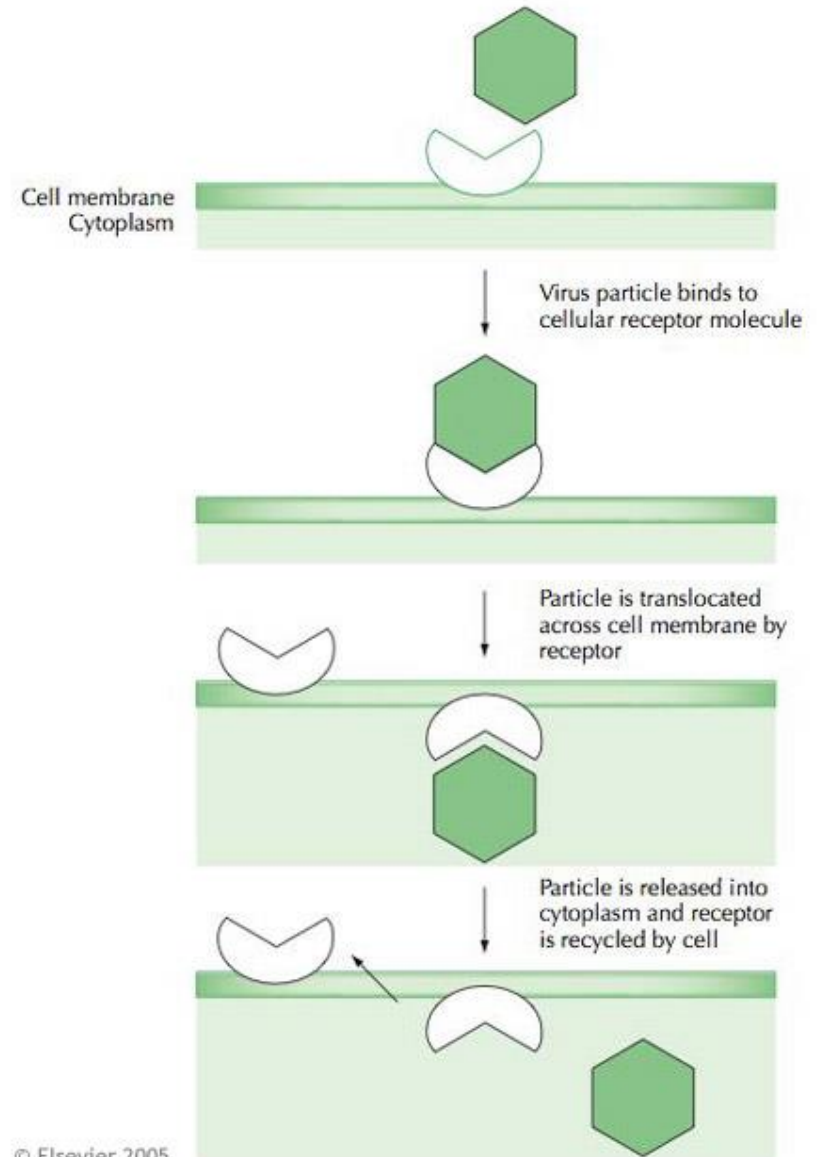
Multiple Receptors

- In some cases, interactions with more than one protein are required for virus entry - neither protein alone is a functional receptor.
- Adenovirus receptor-binding is a two stage process involving an initial interaction of the virion fibre protein with a range of cellular receptors, including MHC class I molecule and the coxsackievirus-adenovirus receptor (CAR).
- Another virion protein, the penton base, then binds to the integrin family of cell surface heterodimers allowing internalization of the particle via receptor-mediated endocytosis.

- The primary receptor for HIV is the T cell antigen, CD4.
- These are Several members of a family of proteins known as b-chemokine receptors play a role in the entry of HIV into cells, and their distribution may be the primary control for the tropism of HIV for different cell types (lymphocytes, macrophages, etc).

Penetration

- Penetration of the target cell normally occurs a very short time after attachment of the virus to its receptor in the cell membrane.

- Unlike attachment, cell penetration is generally an energy-dependent process, i.e. the cell must be metabolically active for this to occur.

- Three main mechanisms are involved.

Translocation

1) Translocation of the entire virus particle across the cytoplasmic membrane of the cell.

- This process is relatively rare among viruses and is poorly understood.

- It is mediated by proteins in the virus capsid and specific membrane receptors.
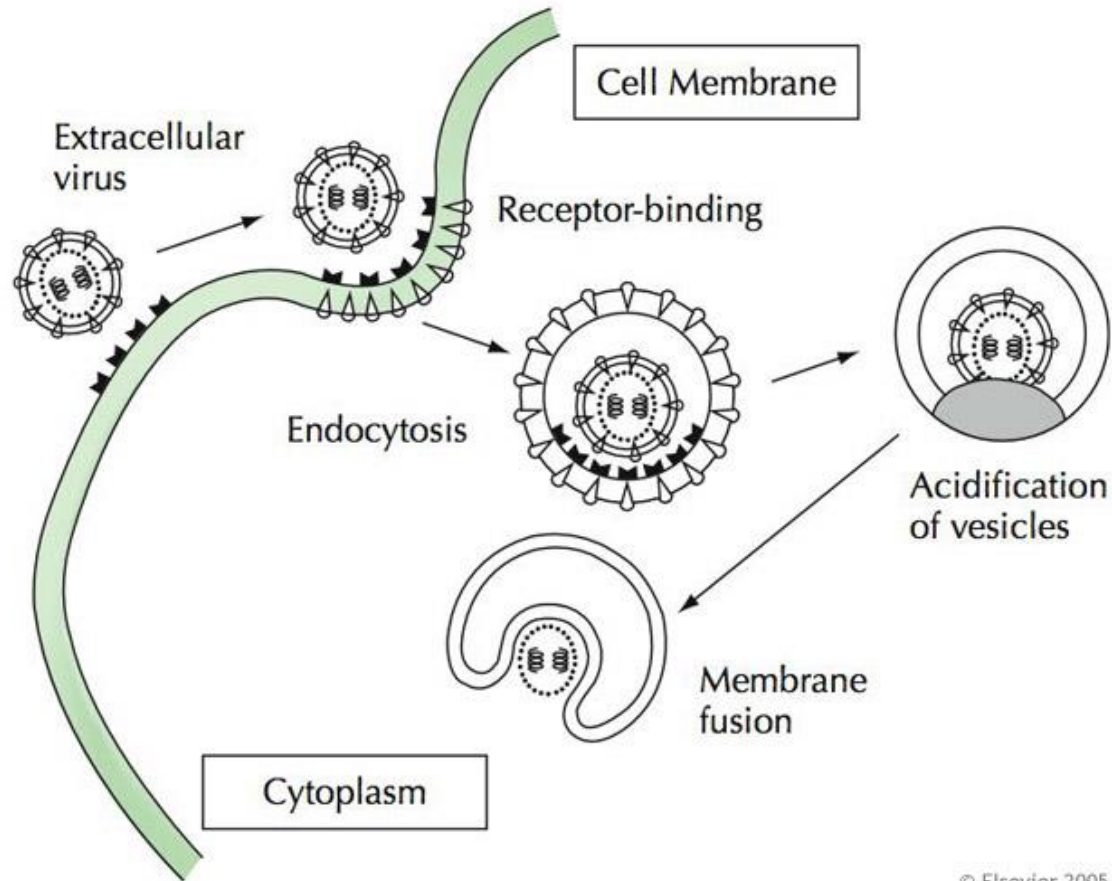
Cell membrane
Cytoplasm

Virus particle binds to cellular receptor molecule

Particle is translocated across cell membrane by receptor

Particle is released into cytoplasm and receptor is recycled by cell

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Endocytosis

2) Endocytosis of the virus into intracellular vacuoles is probably the most common mechanism.

• Does not require any specific virus proteins (other than those utilized for receptor binding) but relies on the formation and internalization of coated pits at the cell membrane.

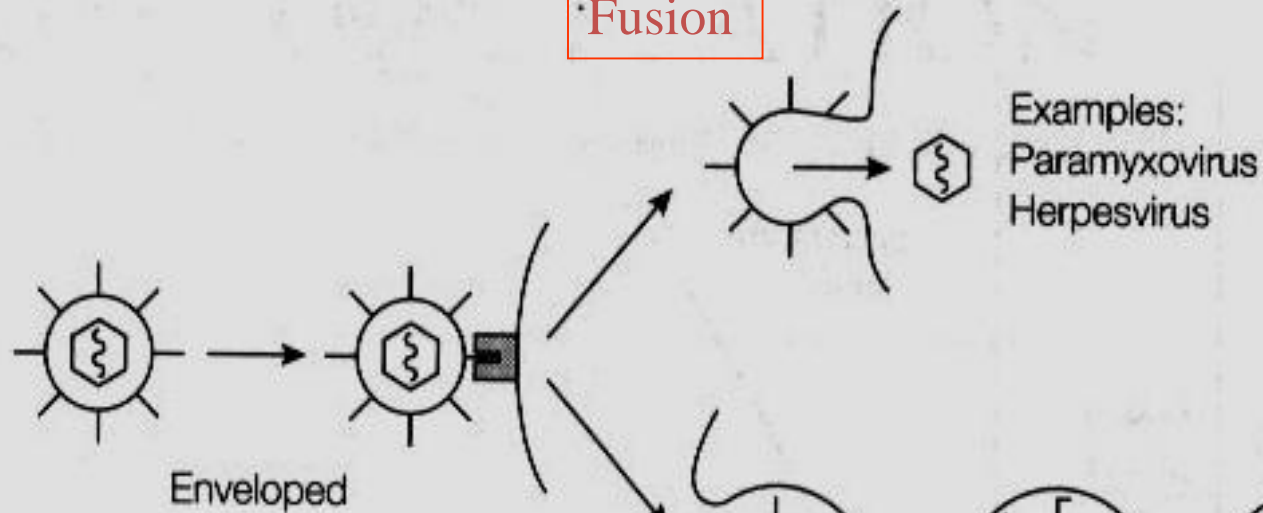• Receptor-mediated endocytosis is an efficient process for taking up and concentrating extracellular macromolecules.
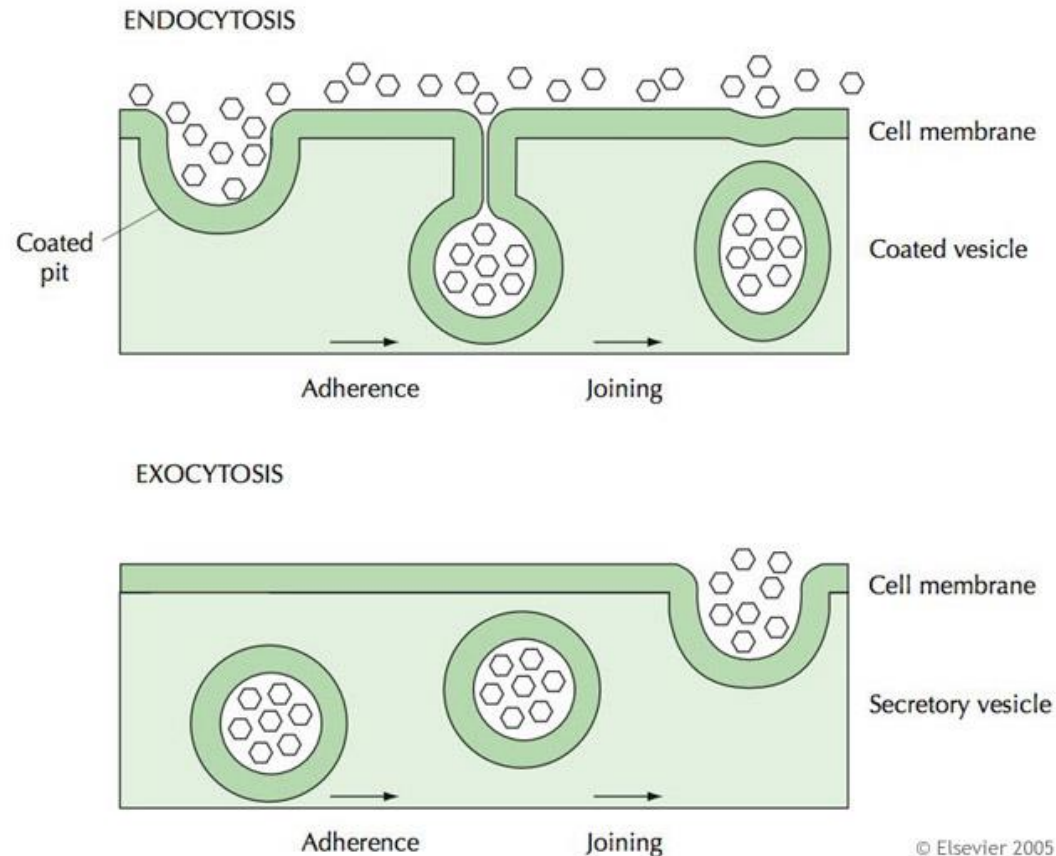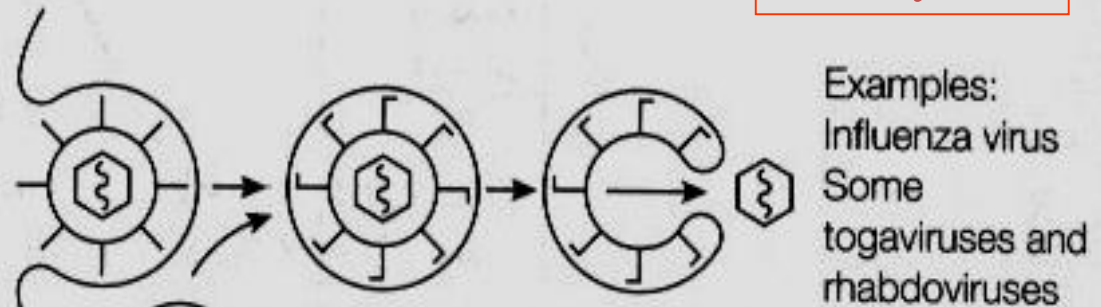
Fusion

3) Fusion of the virus envelope with the cell membrane, either directly at the cell surface or in a cytoplasmic vesicle.

- Fusion requires the presence of a fusion protein in the virus envelope which promotes joining of the cell and virus membranes, resulting in the nucleocapsid being deposited directly in the cytoplasm.

- There are two types of virus-driven membrane fusion: pH-dependent and pH-independent.

Extracellular virus

Cell Membrane

Receptor-binding

Endocytosis

Acidification of vesicles

Membrane fusion

Cytoplasm

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Fusion

Endocytosis

Pinocytosis (Viropexis)

Enveloped

Examples:
Paramyxovirus
Herpesvirus

Examples:
Influenza virus
Some
togaviruses and
rhabdoviruses

Digestive
endosome

Non-enveloped

Examples:
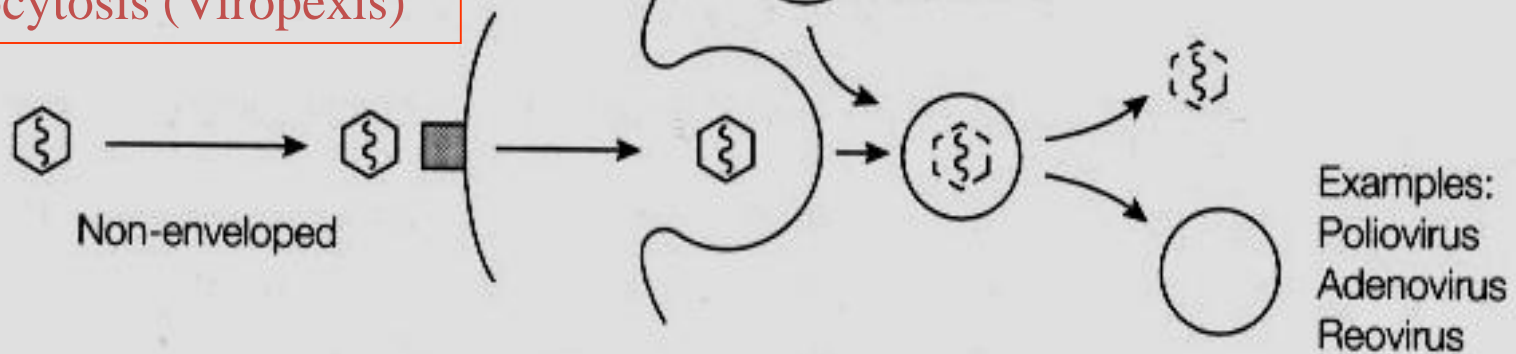Poliovirus
Adenovirus
Reovirus

Fig. 2.   Methods of virus entry. From Harper, D., Molecular Virology, 2nd edn, © BIOS Scientific Publishers Limited, 1998.

Uncoating

- Uncoating is a general term for the events which occur after penetration.

- Uncoating is one of the stages of virus replication that has been least studied and is relatively poorly understood.

- The product of uncoating depends on the structure of the virus nucleocapsid.

- The structure and chemistry of the nucleocapsid determines the subsequent steps in replication.

BOX 6-6. Steps in Viral Replication

1. Recognition of the target cell
2. Attachment
3. Penetration
4. Uncoating
5. Macromolecular synthesis
   a. Early mRNA and nonstructural protein synthesis: genes for enzymes and nucleic acid–binding proteins
   b. Replication of genome
   c. Late mRNA and structural protein synthesis
   d. Post-translational modification of protein
6. Assembly of virus
7. Budding of enveloped viruses
8. Release of virus

Genome Replication and Gene Expression

- All viruses can be divided into seven groups - a scheme was first proposed by David Baltimore in 1971.

- Originally, this classification included only six groups, but it has since been extended to include the hepadnaviruses and caulimoviruses.

- For viruses with RNA genomes in particular, genome replication and the expression of genetic information are inextricably linked, so both are taken into account.

The genomes

- **I: Double-stranded DNA. Examples: Adenoviruses, Herpesviruses, Papillomaviruses, Poxiviruses, T4 bacteriophage**
  Some replicate in the nucleus e.g adenoviruses using cellular proteins. Poxviruses replicate in the cytoplasm
- **II: Single-stranded (+)sense DNA. Examples: phage M13, chicken anaemia virus, maize streak virus**
  Replication occurs in the nucleus, involving the formation of a (-)sense strand, which serves as a template for (+)strand RNA and DNA synthesis.
- **III: Double-stranded RNA. Examples: Reoviruses, Rotavirues**
  These viruses have segmented genomes. Each genome segment is transcribed separately to produce monocistronic mRNAs.
- **IV: Single-stranded (+)sense RNA Examples: Hepatitis A and C, Small RNA phages, common cold viruses, SARS**
  a) Polycistronic mRNA e.g. Picornaviruses; Hepatitis A. Genome RNA = mRNA. Means naked RNA is infectious, no virion particle associated polymerase. Translation results in the formation of a polyprotein product, which is subsequently cleaved to form the mature proteins.
  b) Complex Transcription e.g. Togaviruses. Two or more rounds of translation are necessary to produce the genomic RNA.

- **V: Single-stranded (-)sense RNA. Examples: Influenza viruses, Hantaviruses**
  Must have a virion particle, containing RNA directed RNA polymerase.
  a) Segmented e.g. Orthomyxoviruses. First step in replication is transcription of the (-)sense RNA genome by the virion RNA-dependent RNA polymerase to produce monocistronic mRNAs, which also serve as the template for genome replication.
  b) Non-segmented e.g. Rhabdoviruses. Replication occurs as above and monocistronic mRNAs are produced.

- **VI: Single-stranded (+)sense RNA with DNA intermediate in life-cycle (Retroviruses). Examples: HIV, Avian leukosis virus**
  Genome is (+)sense but unique among viruses in that it is **DIPLOID**, and does not serve as mRNA, but as a template for reverse transcription.

- **VII: Partial double-stranded (gapped) DNA with RNA intermediate (Hepadnaviruses) Example: Hepatitis B** This group of viruses also relies on reverse transcription, but unlike the Retroviruses, this occurs inside the virus particle on maturation. On infection of a new cell, the first event to occur is repair of the gapped genome, followed by transcription.

The monocistronic mRNA problem

mRNA

RIBOSOMES

PROTEIN

AAAAAAAA

AAAAAAAA
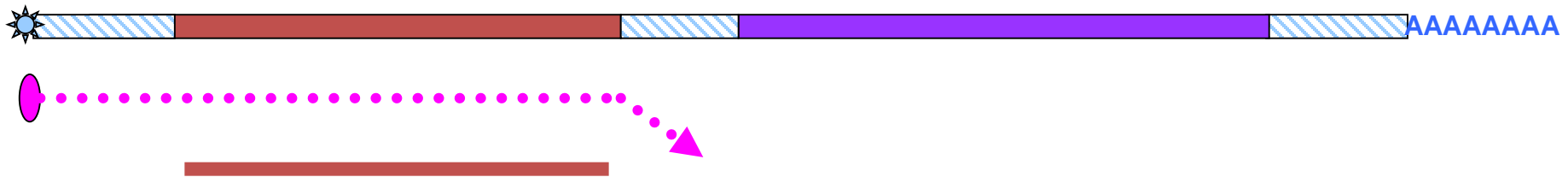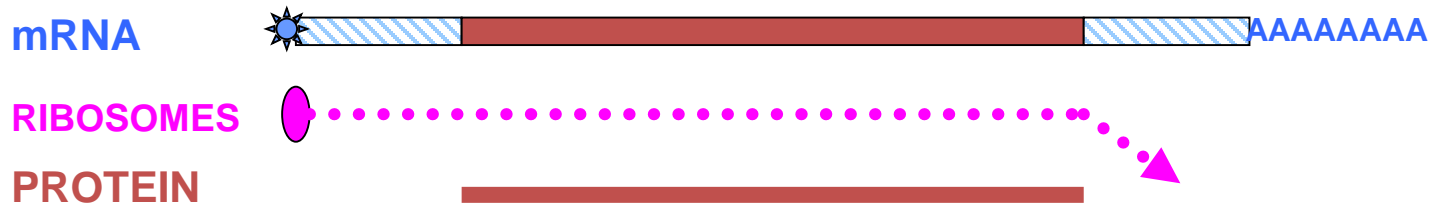
- Make one monocistronic mRNA per protein
- Make a primary transcript and use alternative splicing
- Make a large protein and then cut it into smaller proteins
- Include special features in the mRNA which enable ribosomes to bind internally

28

Class I: Double-stranded DNA

This class can be subdivided into two further groups:

A) Replication is exclusively nuclear. The replication of these viruses is relatively dependent on cellular factors.

B) Replication occurs in cytoplasm (*Poxviridae*). These viruses have evolved (or acquired) all the necessary factors for transcription and replication of their genomes and are therefore largely independent of the cellular machinery.
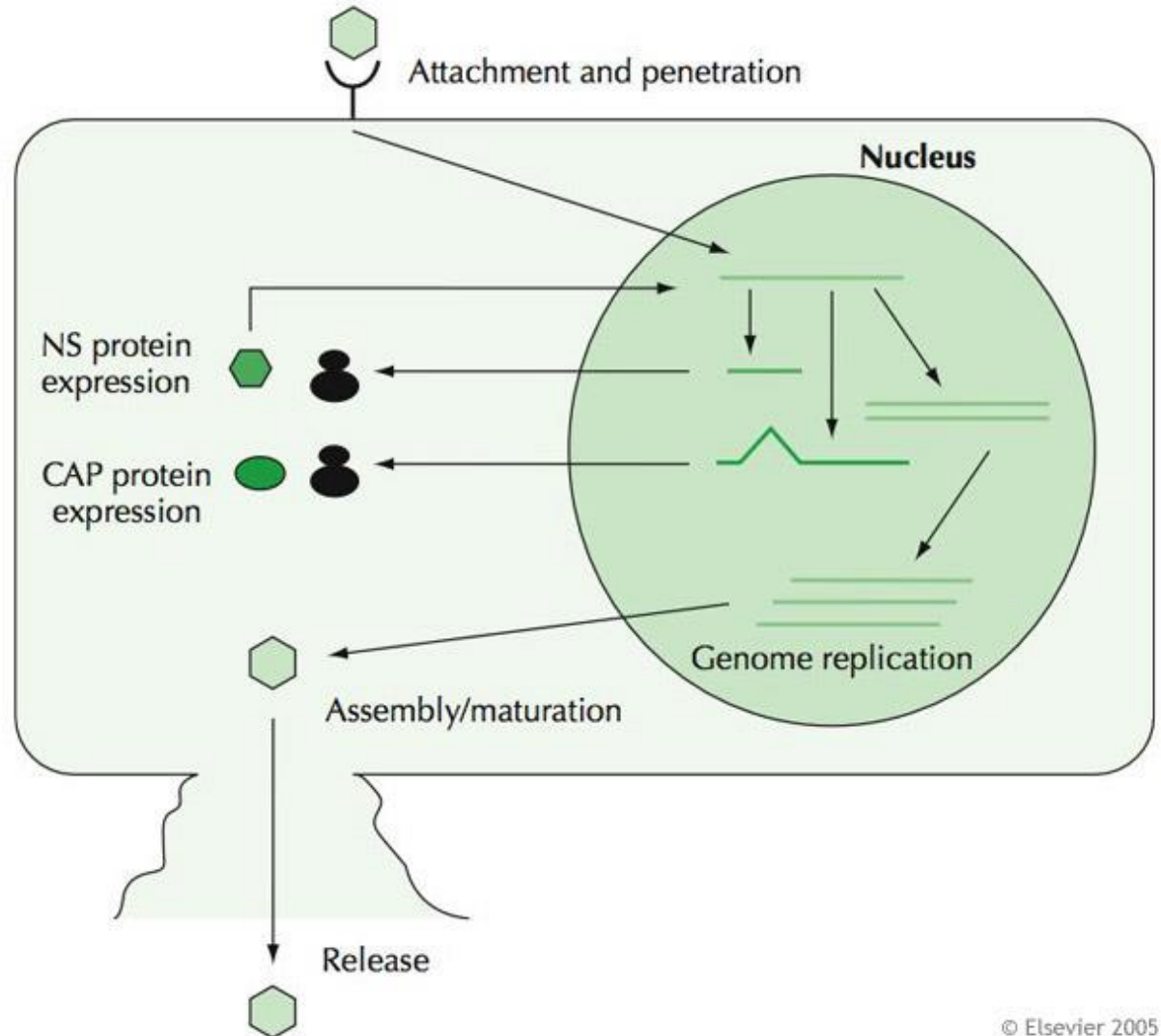
Class I: Double-stranded DNA
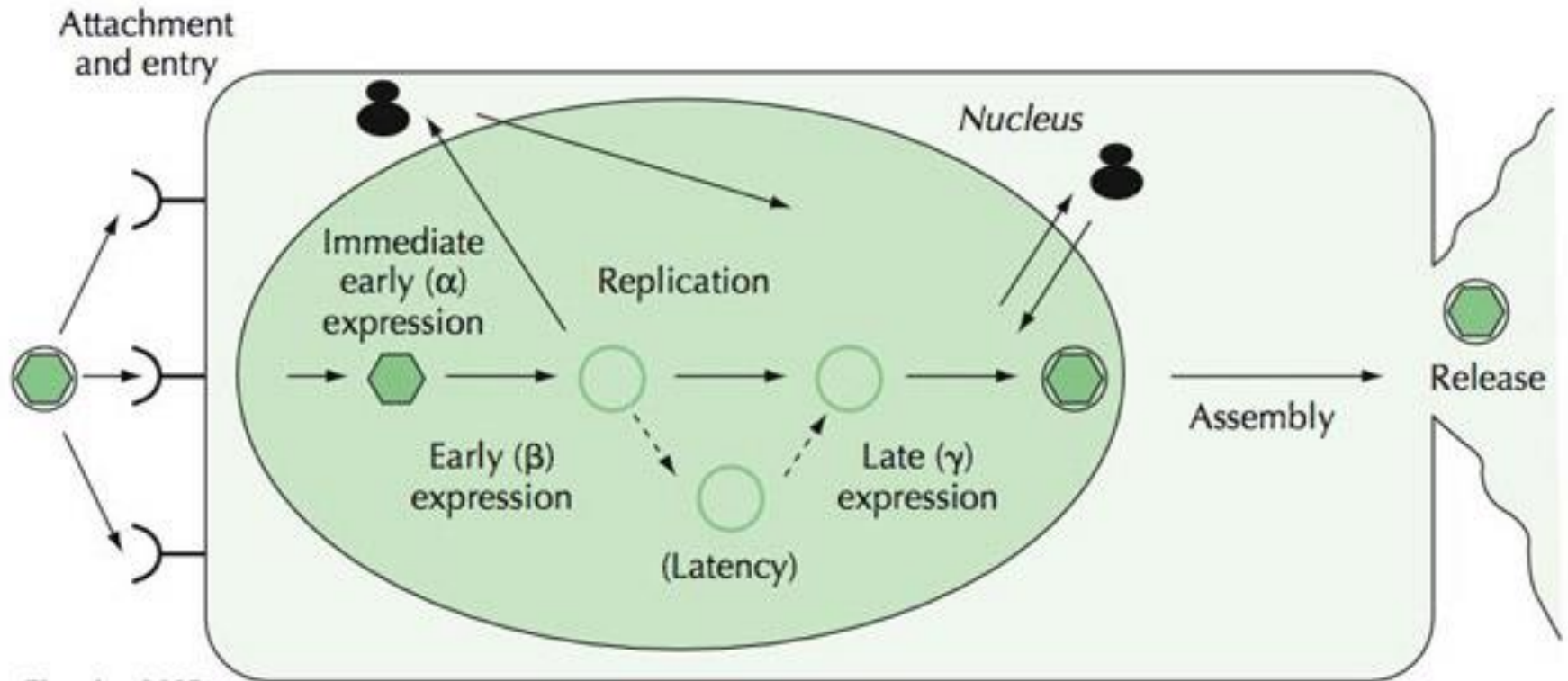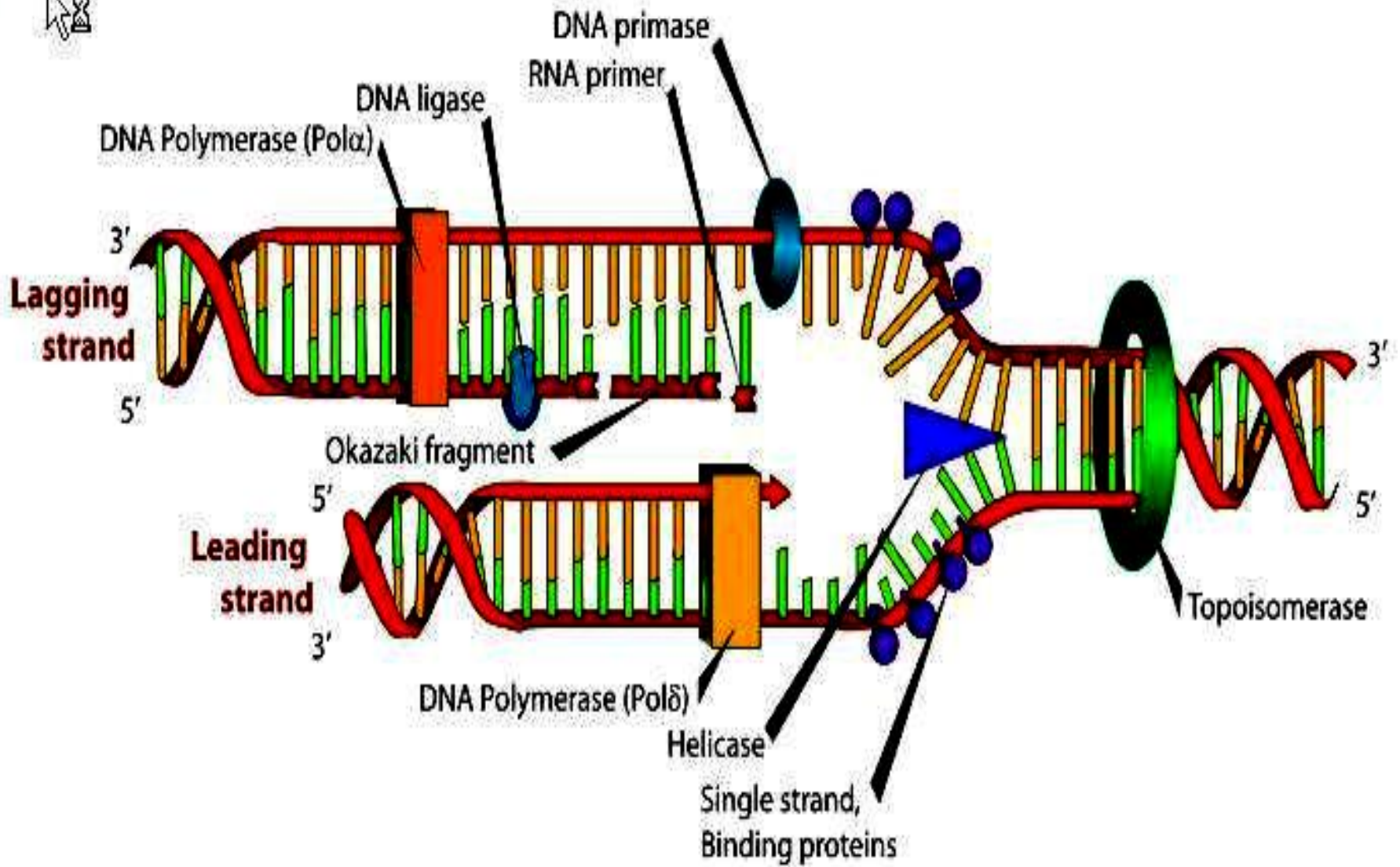
Class II: Single-stranded DNA

- Replication occurs in the nucleus, involving the formation of a double-stranded intermediate which serves as a template for the synthesis of single-stranded progeny DNA.

Attachment and penetration

Nucleus

NS protein expression

CAP protein expression

Genome replication

Assembly/maturation

Release

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Class III: Double-stranded RNA

- These viruses have segmented genomes.

- Each segment is transcribed separately to produce individual monocistronic mRNAs.

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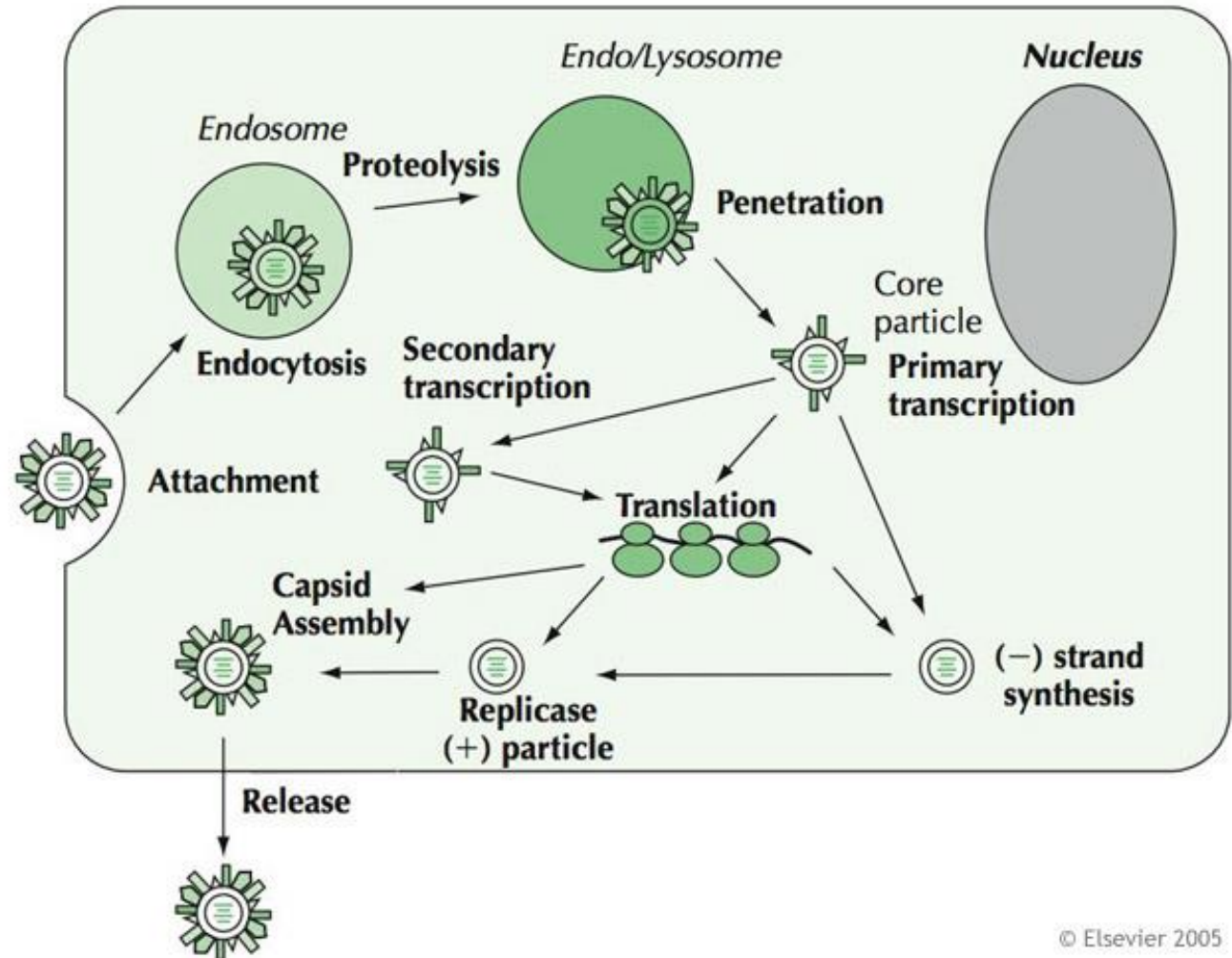
Class IV: Single-stranded (+)sense RNA

- These can be subdivided into two groups:

  - Viruses with polycistronic mRNA. As with all the viruses in this class, the genome RNA forms the mRNA. This is translated to form a polyprotein product, which is subsequently cleaved to form the mature proteins.

  - Viruses with complex transcription. Two rounds of translation (e.g. *Togavirus*) or subgenomic RNAs (e.g. *Tobamovirus*) are necessary to produce the genomic RNA.
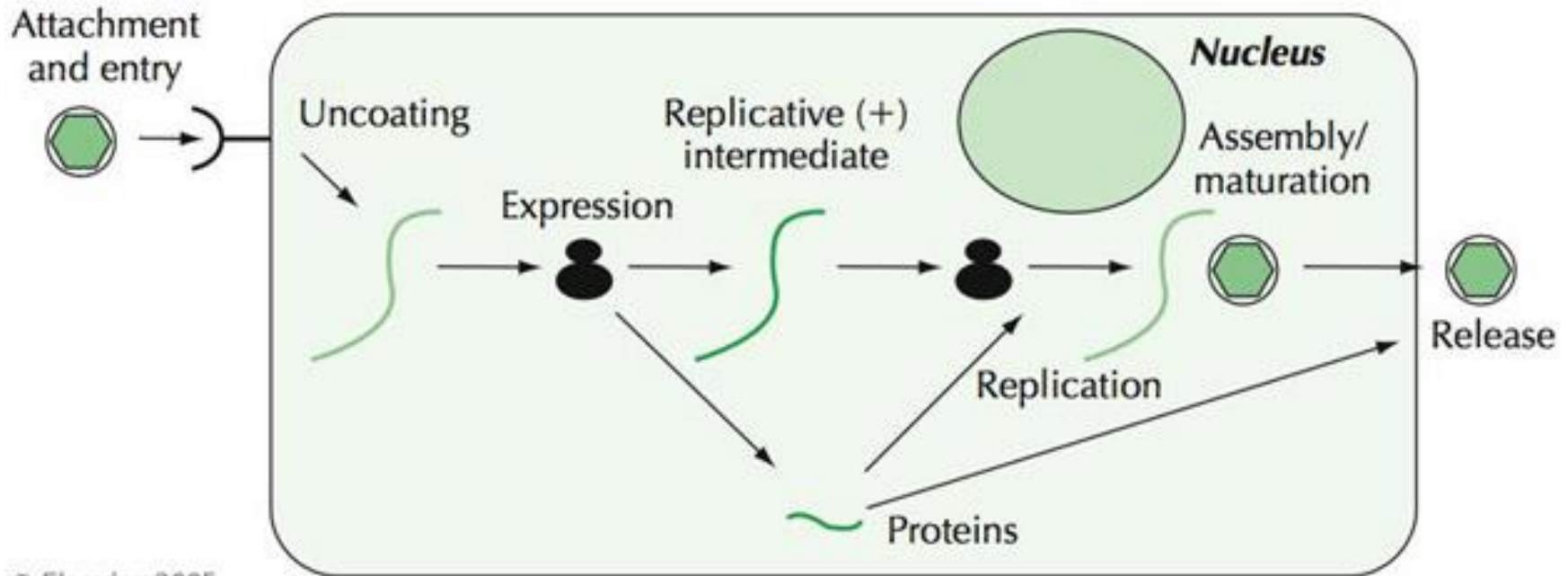
Class IV: Single-stranded (+)sense RNA
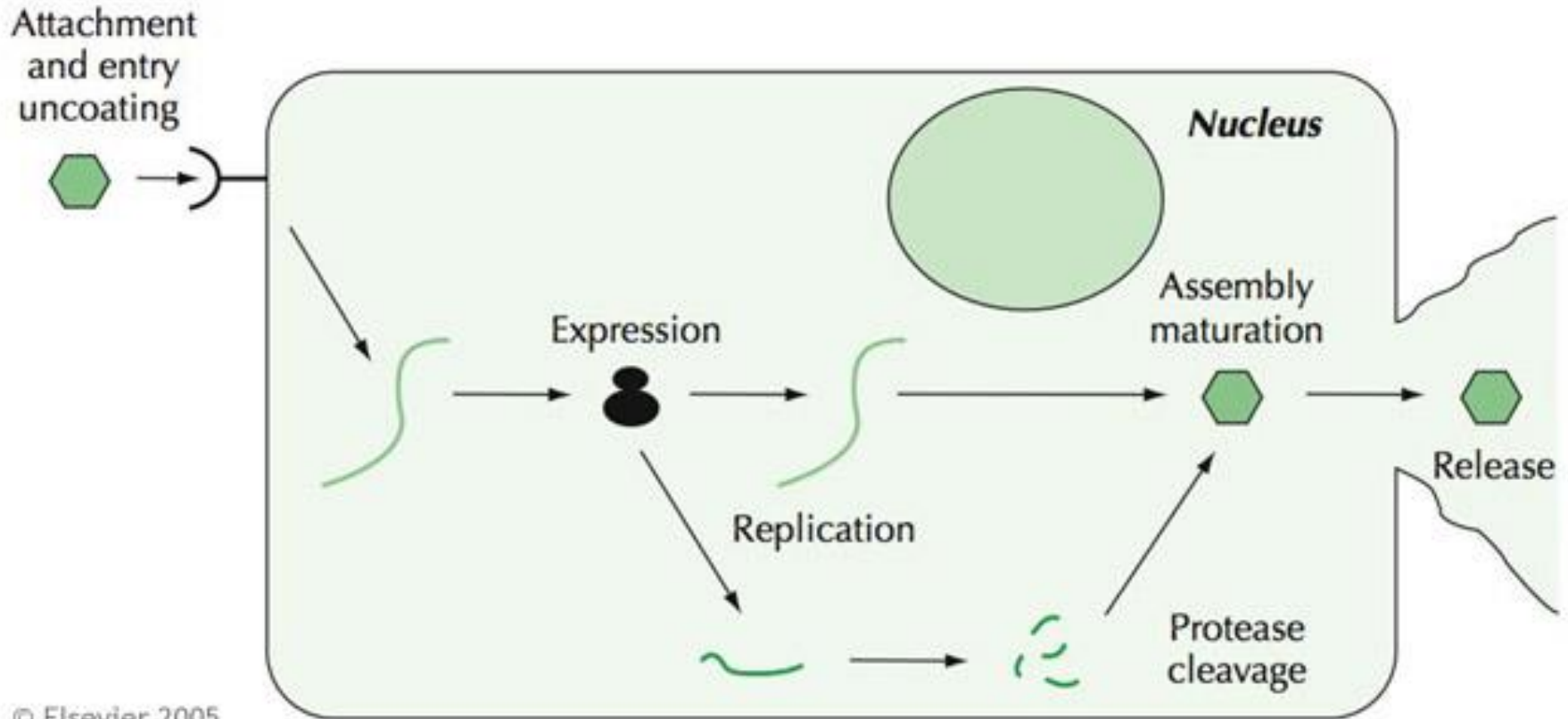
Class V: Single-stranded (–)sense RNA

- The genomes of these viruses can be divided into two types:

  – Segmented genomes

    - First step in replication is transcription of the (-)sense RNA genome by the virion RNA-dependent RNA polymerase to produce monocistronic mRNAs, which also serve as the template for genome replication.

  – Non-segmented genomes

Class V: Single-stranded (–)sense RNA

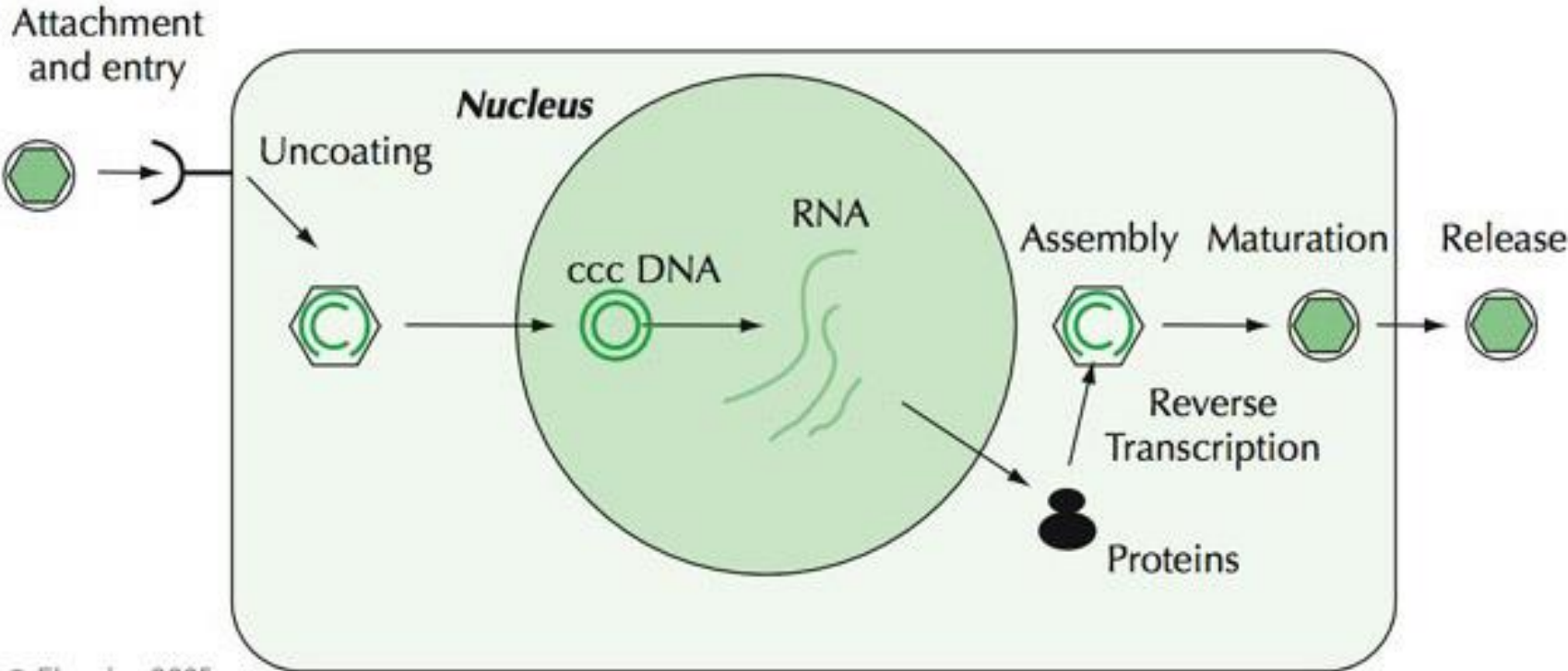# Class VI: Single-stranded (+)sense RNA with a DNA Intermediate

- Retrovirus genomes are (+)sense RNA but unique in that they are diploid, and do not serve directly as mRNA, but as a template for reverse transcription into DNA.

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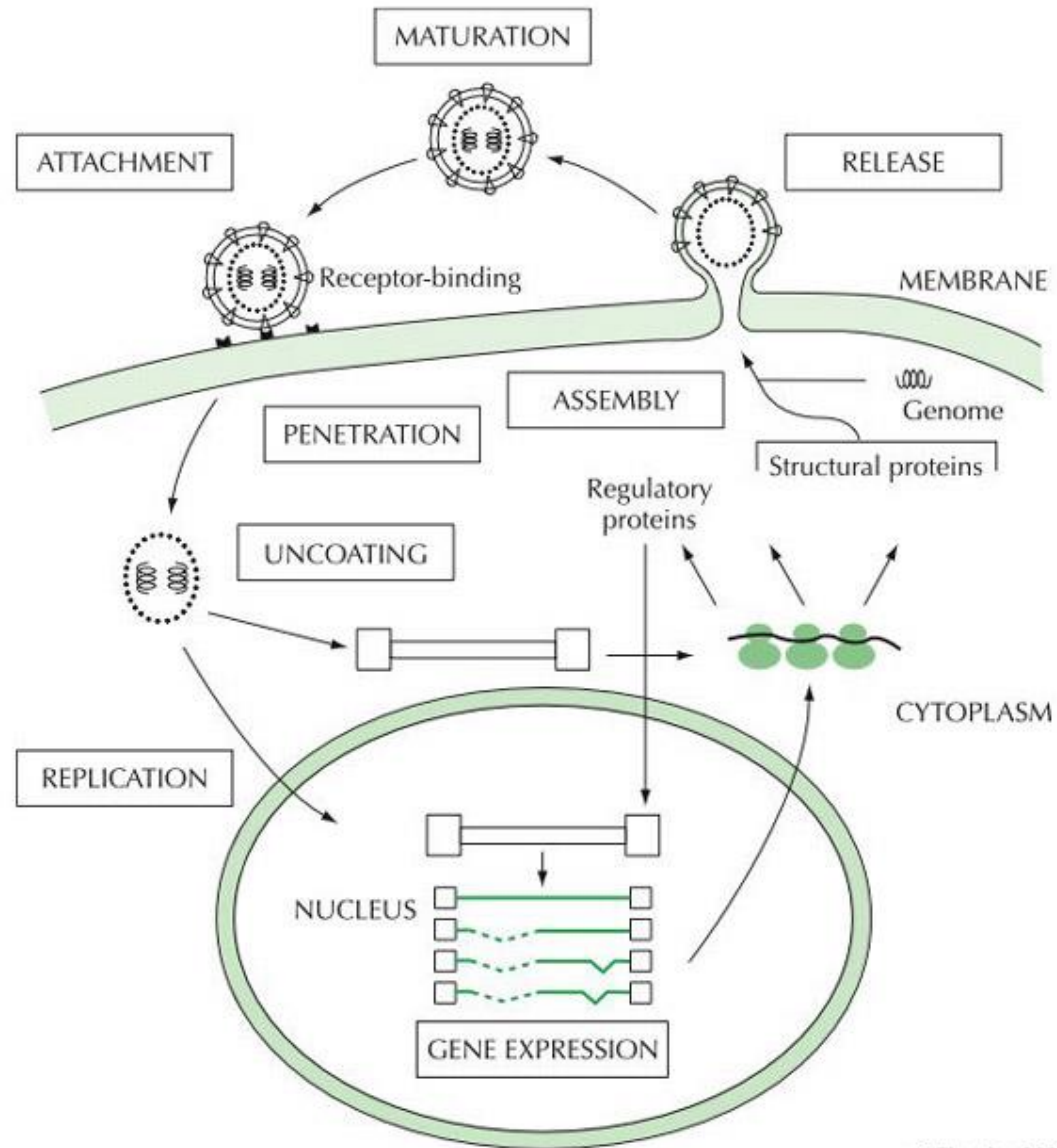
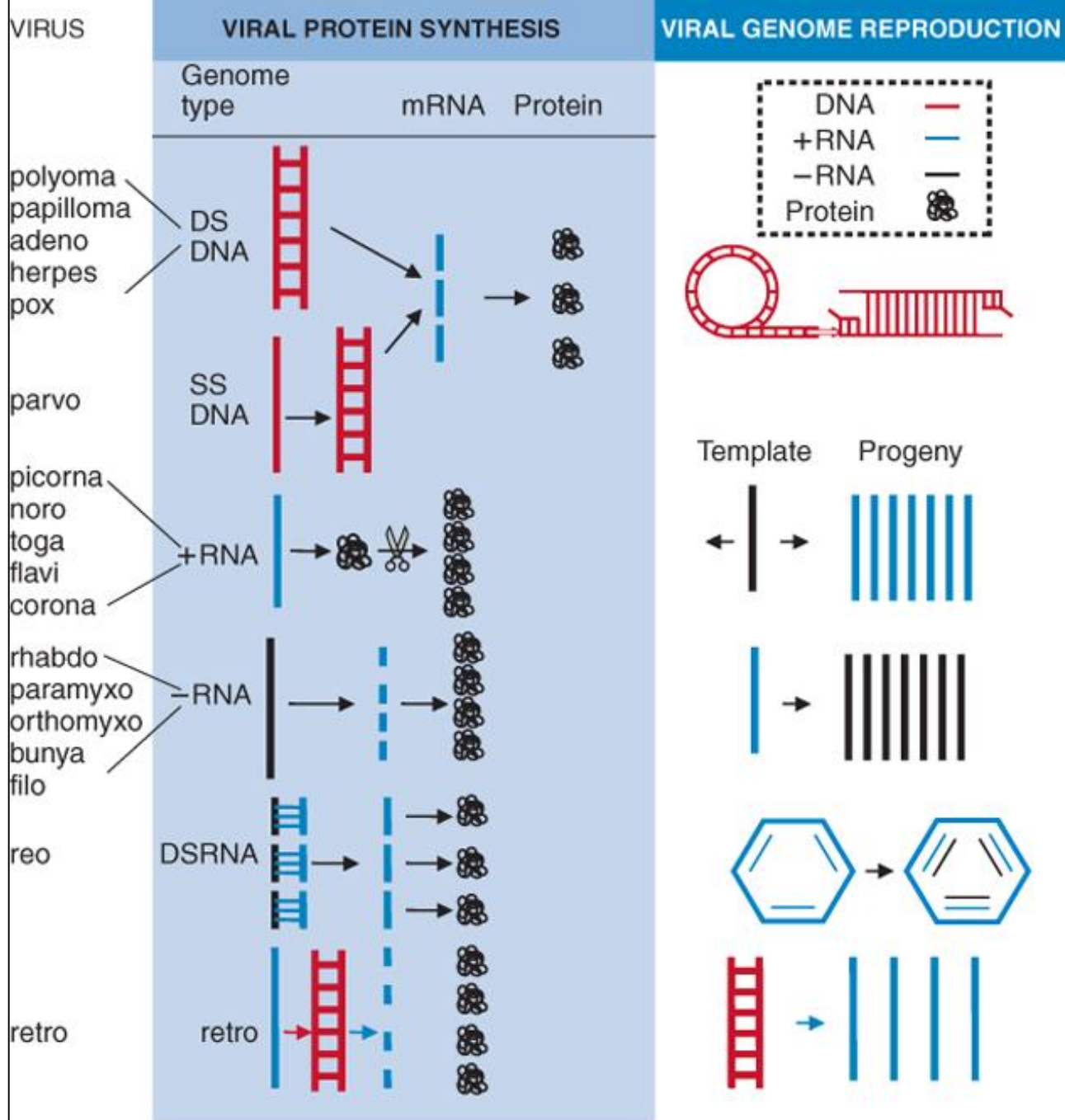# Class VII: Double-stranded DNA with RNA Intermediate

- This group of viruses also relies on reverse transcription.

- Unlike the retroviruses (class VI), this occurs inside the virus particle during maturation.

- On infection of a new cell, the first event to occur is repair of the gapped genome, followed by transcription.

Class VII: Double-stranded DNA with RNA Intermediate

VIRUS | VIRAL PROTEIN SYNTHESIS | VIRAL GENOME REPRODUCTION

Genome type — mRNA — Protein

polyoma, papilloma, adeno, herpes, pox — DS DNA

parvo — SS DNA

picorna, noro, toga, flavi, corona — +RNA

rhabdo, paramyxo, orthomyxo, bunya, filo — −RNA

reo — DSRNA

retro — retro

Legend: DNA, +RNA, −RNA, Protein

Template — Progeny
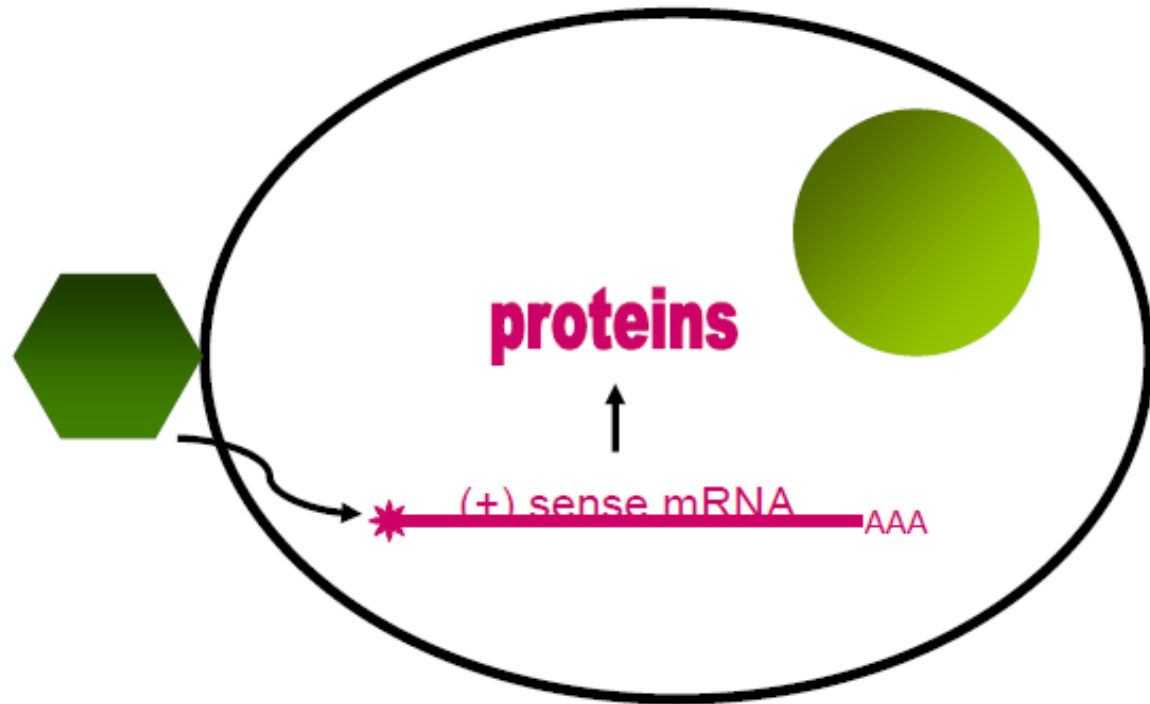
All animal RNA viruses code for a Polymerase

• Positive/negative/double-stranded RNA virus genomes all encode a RNA-depend RNA polymerase.

• RNA-depend RNA polymerase is associated with negative RNA viruses.

• Reverse transcriptase is associated with retroviruses.

PLUS (POSITIVE) sense RNA GENOMES (+RNA)

proteins

(+) sense mRNA AAA

Single-strand positive-sense RNA- the virus genome is the virus mRNA.

Minus (negative) sense RNA genomes:

proteins

(+) sense mRNA AAA
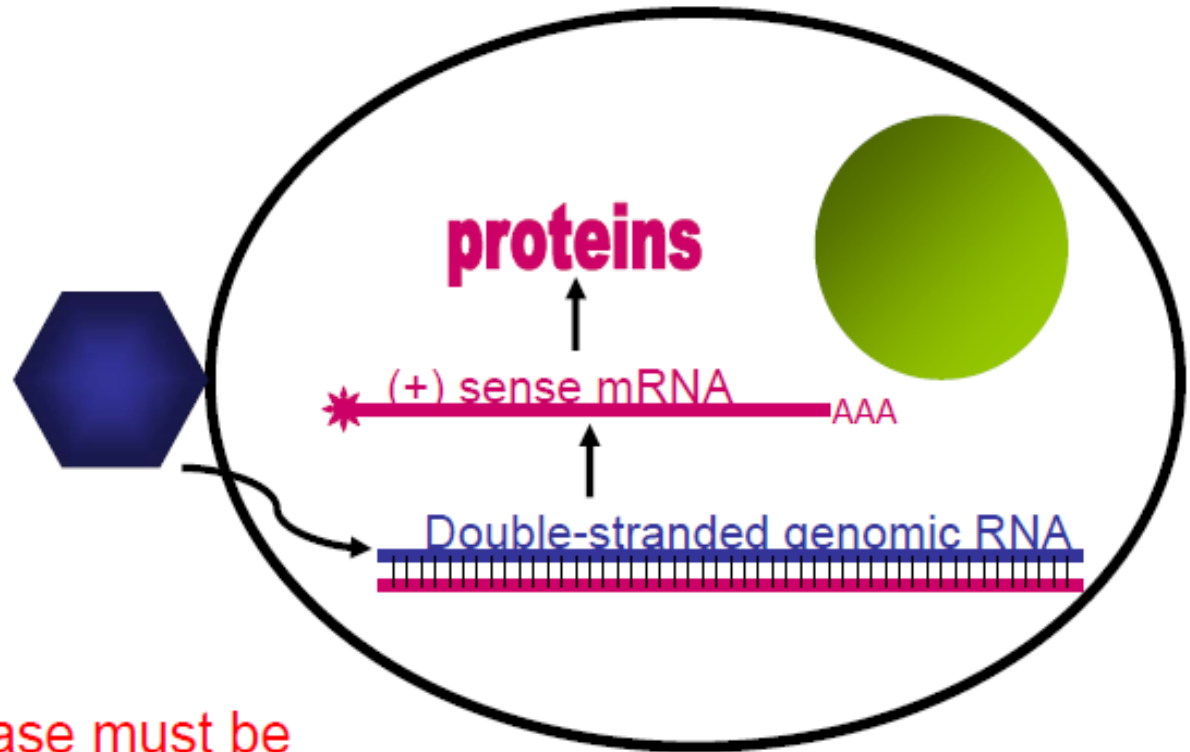
(-) sense genomic RNA
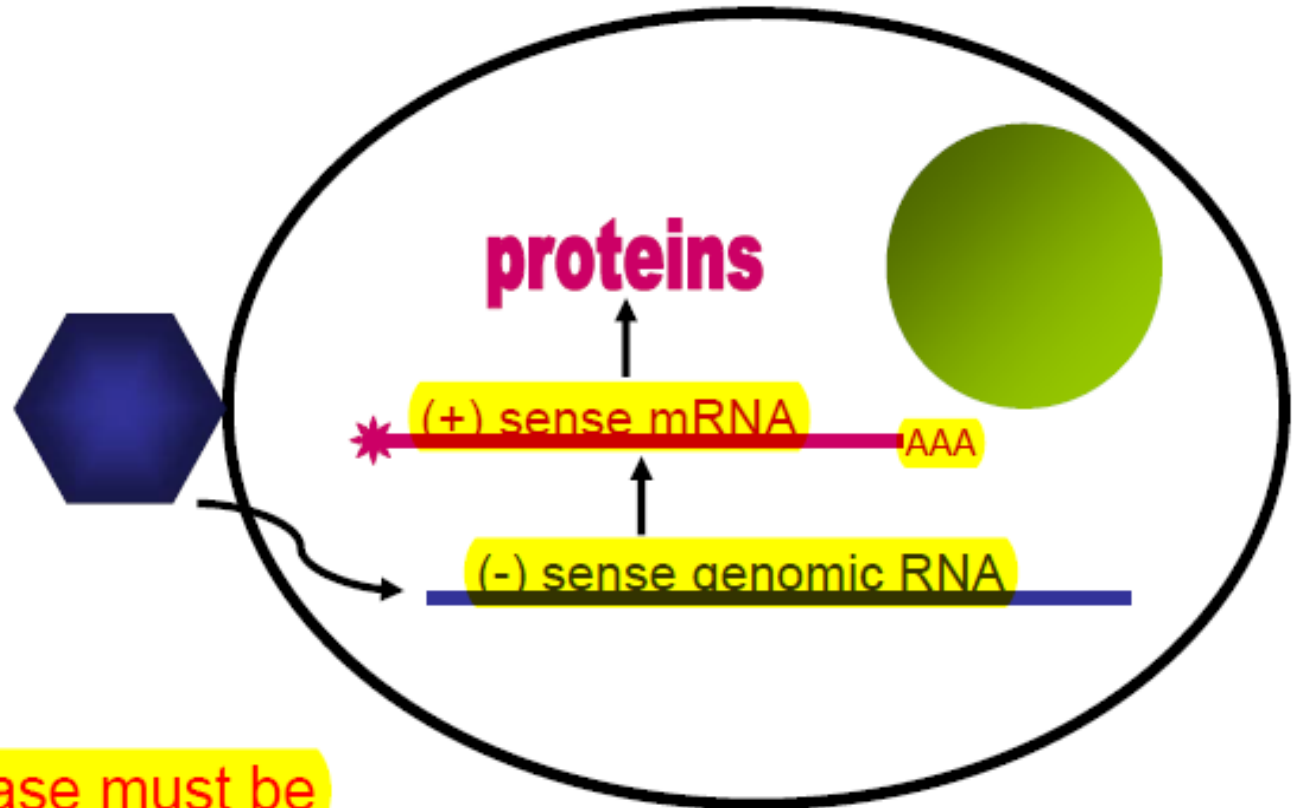
RNA polymerase must be packaged in virion.

Single-strand negative-sense RNA- virus mRNA is transcribed from the parental genome.

Double-stranded RNA genomes:

proteins

(+) sense mRNA   AAA
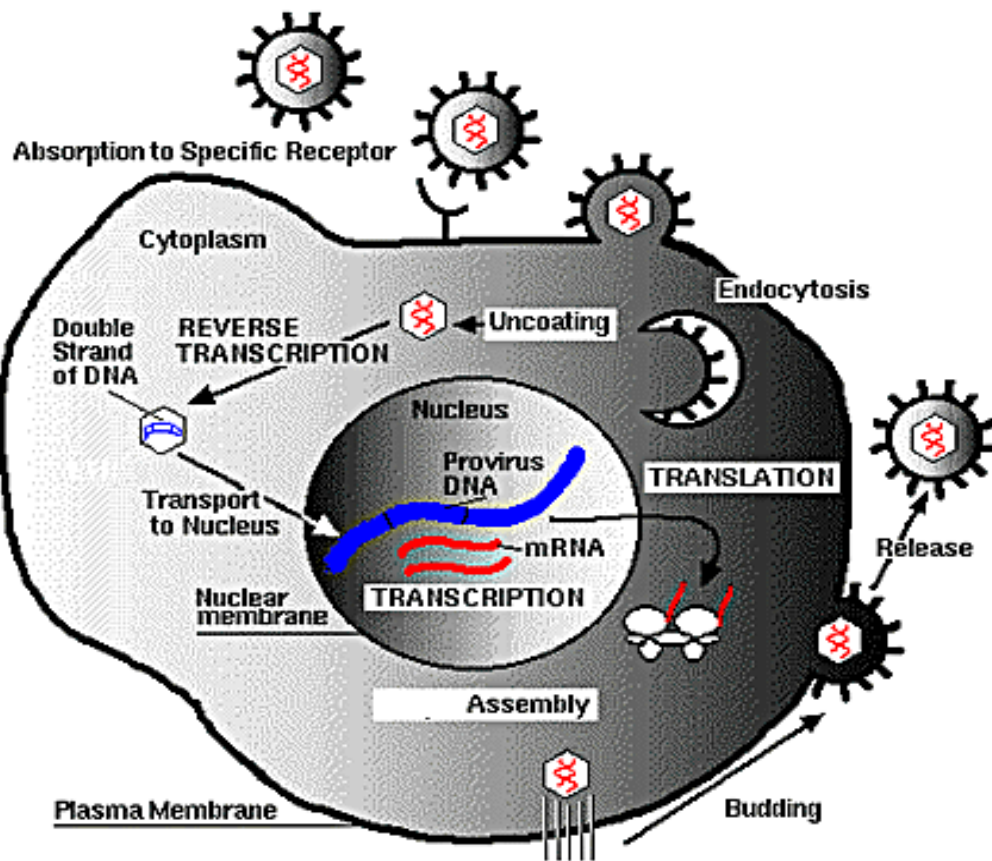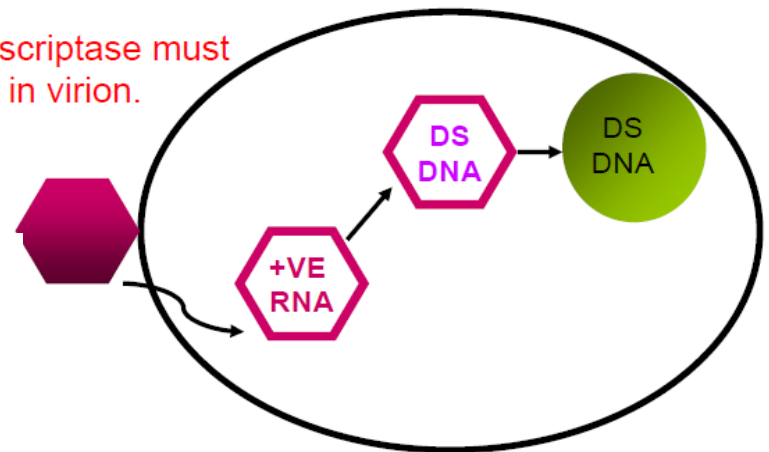
Double-stranded genomic RNA

RNA polymerase must be
packaged in virion.

Double- stranded segmented RNA- individual
virus mRNAs are transcribed separately off the
parental RNA segments using a transcriptase
associated with each segment

RETROVIRUSES

Reverse transcriptase must be packaged in virion.

DS DNA → DS DNA

+VE RNA

Absorption to Specific Receptor

Cytoplasm

Endocytosis

Double Strand of DNA

REVERSE TRANSCRIPTION

Uncoating

Nucleus

Provirus DNA

TRANSLATION

Transport to Nucleus

mRNA

Nuclear membrane

TRANSCRIPTION

Release

Assembly

Plasma Membrane

Budding

Retrovirus replication

Replication Challenges for DNAViruses

- Access to nucleus

- Competing for nucleotides

- Cell cycle control in eucaryotes - S phase dependent materials for some Viruses (Parvo)
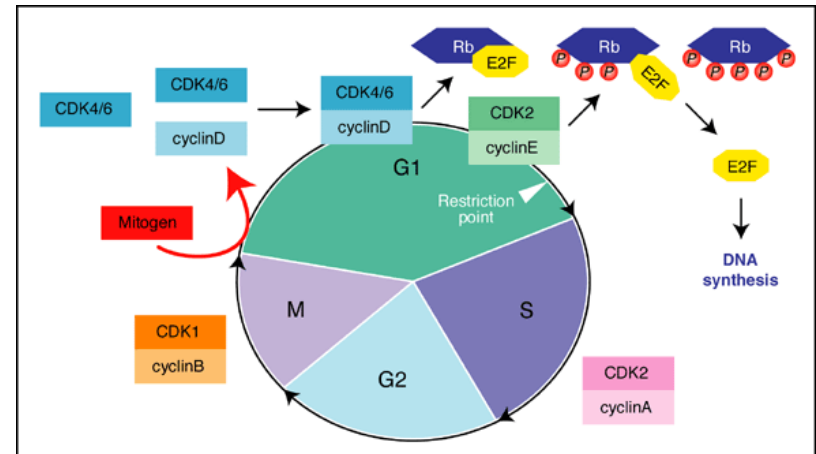
Assembly

- Assembly involves the collection of all the components necessary for the formation of the mature virion at a particular site in the cell.

- During assembly, the basic structure of the virus particle is formed.

- The site of assembly depends on the site of replication within the cell and on the mechanism by which the virus is eventually released.
  - in picornaviruses, poxviruses and reoviruses assembly occurs in the cytoplasm
  - in adenoviruses, polyomaviruses and parvoviruses it occurs in the nucleus
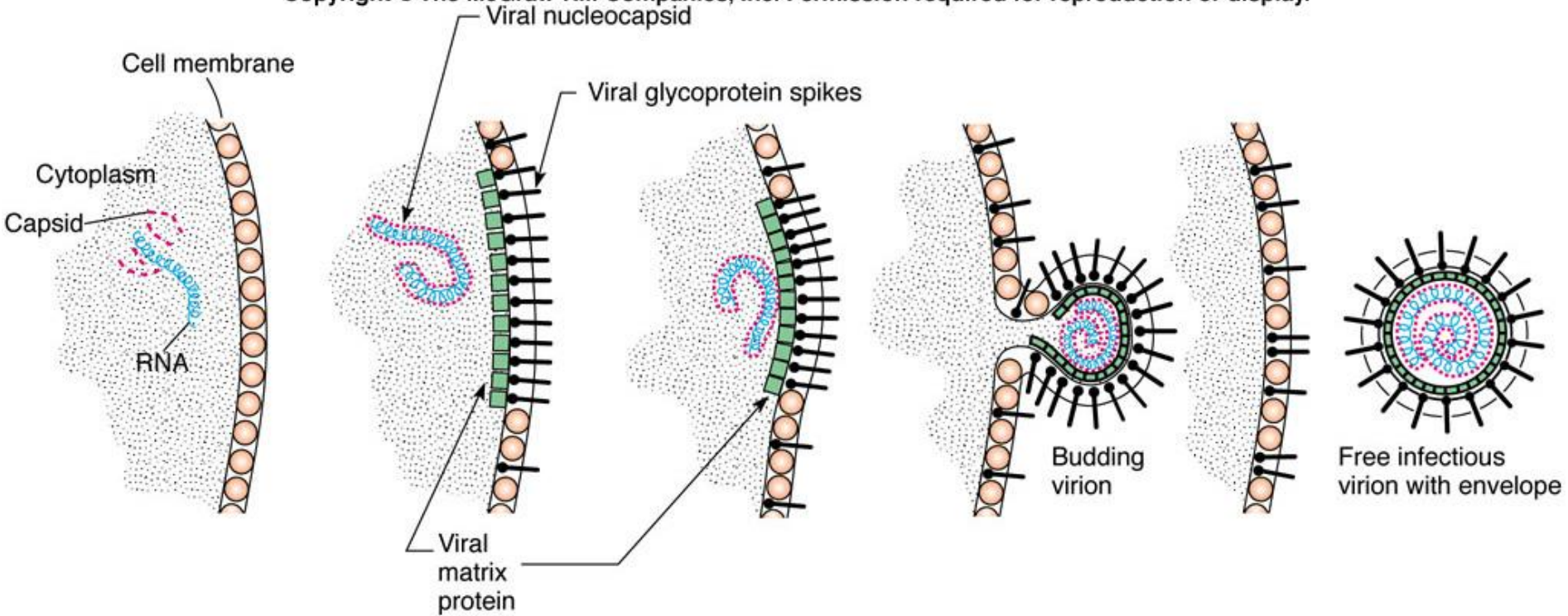
Maturation

- Maturation is the stage of the replication-cycle at which the virus becomes infectious.

- Maturation usually involves structural changes in the virus particle which may result from specific cleavages of capsid proteins conformational changes in proteins.

- Virus proteases are frequently involved in maturation, although cellular enzymes or a mixture of virus and cellular enzymes are used in some cases.

Release

- Apart from plant viruses which have evolved particular strategies to overcome the structure of plant cell walls, all other viruses escape the cell by one of two mechanisms:

- For lytic viruses (most non-enveloped viruses), release is a simple process - the infected cell breaks open and releases the virus.

- Enveloped viruses acquire their lipid membrane as the virus buds out of the cell through the cell membrane or into an intracellular vesicle prior to subsequent release. Virion envelope proteins are picked up during this process as the virus particle is extruded - this process is known as budding.

Release by budding

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Viral nucleocapsid

Viral glycoprotein spikes

Cell membrane

Cytoplasm

Capsid

RNA

Viral matrix protein

Budding virion

Free infectious virion with envelope

Possible consequences to a cell that is infected by a virus:

- **Lytic infections** result in the destruction of the host cell; are caused by virulent viruses, which inherently bring about the death of the cells that they infect.

- When enveloped viruses are formed by budding, the release of the viral particles may be slow and the host cell may not be lysed. Such infections may occur over relatively long periods of time and are thus referred to as **persistent infections**.

- Viruses may also cause **latent infections**. The effect of a latent infection is that there is a delay between the infection by the virus and the appearance of symptoms.

- Some animal viruses have the potential to change a cell from a normal cell into a tumor cell, the hallmark of which is to grow without restraint. This process is called **transformation**.