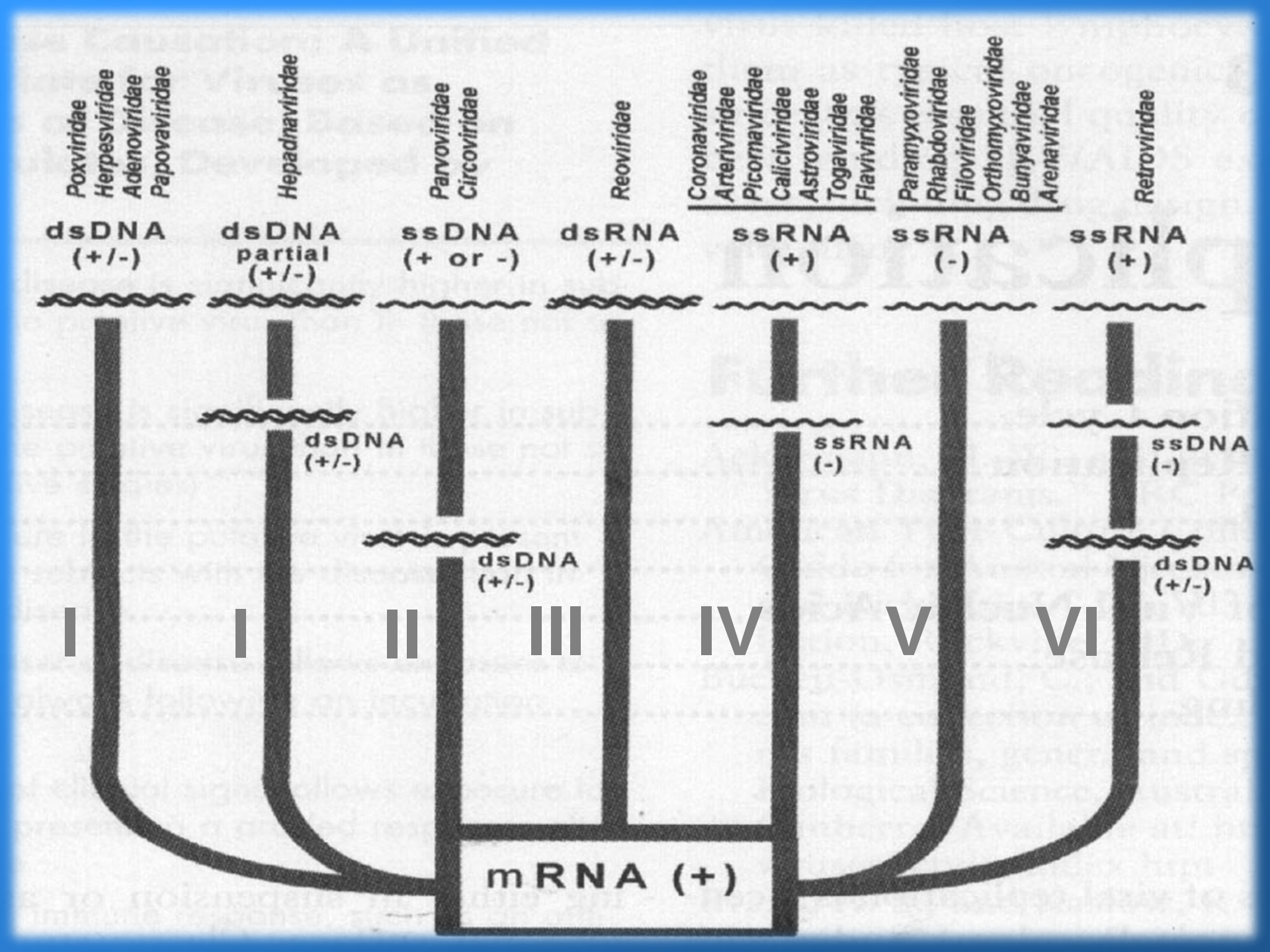
Main Topics of the lecture

- Introduction.
- One-step growth curve.
- Steps of multiplication cycle.
- Attachment.
- Penetration (entry).
- Uncoating.

Introduction

- Studies with the bacteriophages (at 1940/50s) and mammalian cell culture have revolutionized the progress of understanding virus replication.
- Every virus family employs a unique strategy for replication.
- One important concept to unify and simply the replication process was proposed by David Baltimore at 1978, to assign viruses to one of six classes based on their genome structure and the pathways they use to produce their mRNAs.


Poxviridae
Herpesviridae
Adenoviridae
Papovaviridae
Hepadnaviridae
Parvoviridae
Circoviridae
Reoviridae
Coronaviridae
Arteriviridae
Picornaviridae
Caliciviridae
Astroviridae
Togaviridae
Flaviviridae
Paramyxoviridae
Rhabdoviridae
Filoviridae
Orthomyxoviridae
Bunyaviridae
Arenaviridae
Retroviridae
dsDNA (+/-)
dsDNA partial (+/-)
ssDNA (+ or -)
dsRNA (+/-)
ssRNA (+)
ssRNA (-)
ssRNA (+)
dsDNA (+/-)
ssRNA (-)
ssDNA (-)
dsDNA (+/-)
dsDNA (+/-)
I
I
II
III
IV
V
VI
mRNA (+)

Steps of Virus Replication

- Attachment to target cell.
- Penetration (entry) from cell membrane.
- Uncoating.
- Expression (transcription and translation) of viral proteins.
- Replication of the viral nucleic acid.
- Virus assembly
- Maturation and release.

Virus Replication

Virus Entry and Uncoating

Steps in Replication cycle of virus

خطوات دورة تكاثر الفيروس

Attachment

الأتصال

I - Through receptors

من خلال مستقبلات

CD4 —T- cell→ HIV

Ig → Poliovirus

Heparan sulfate → HSV, Adenovirus

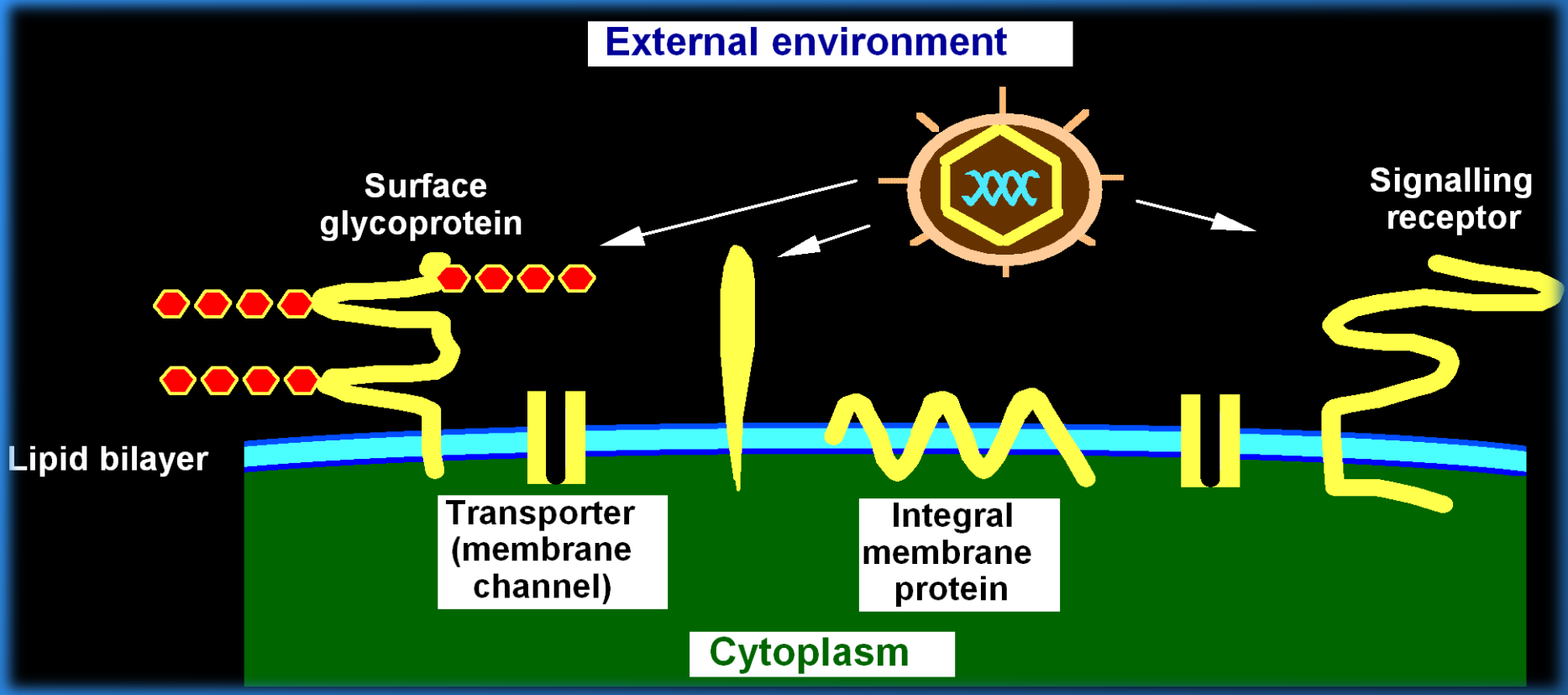
II - Random collision

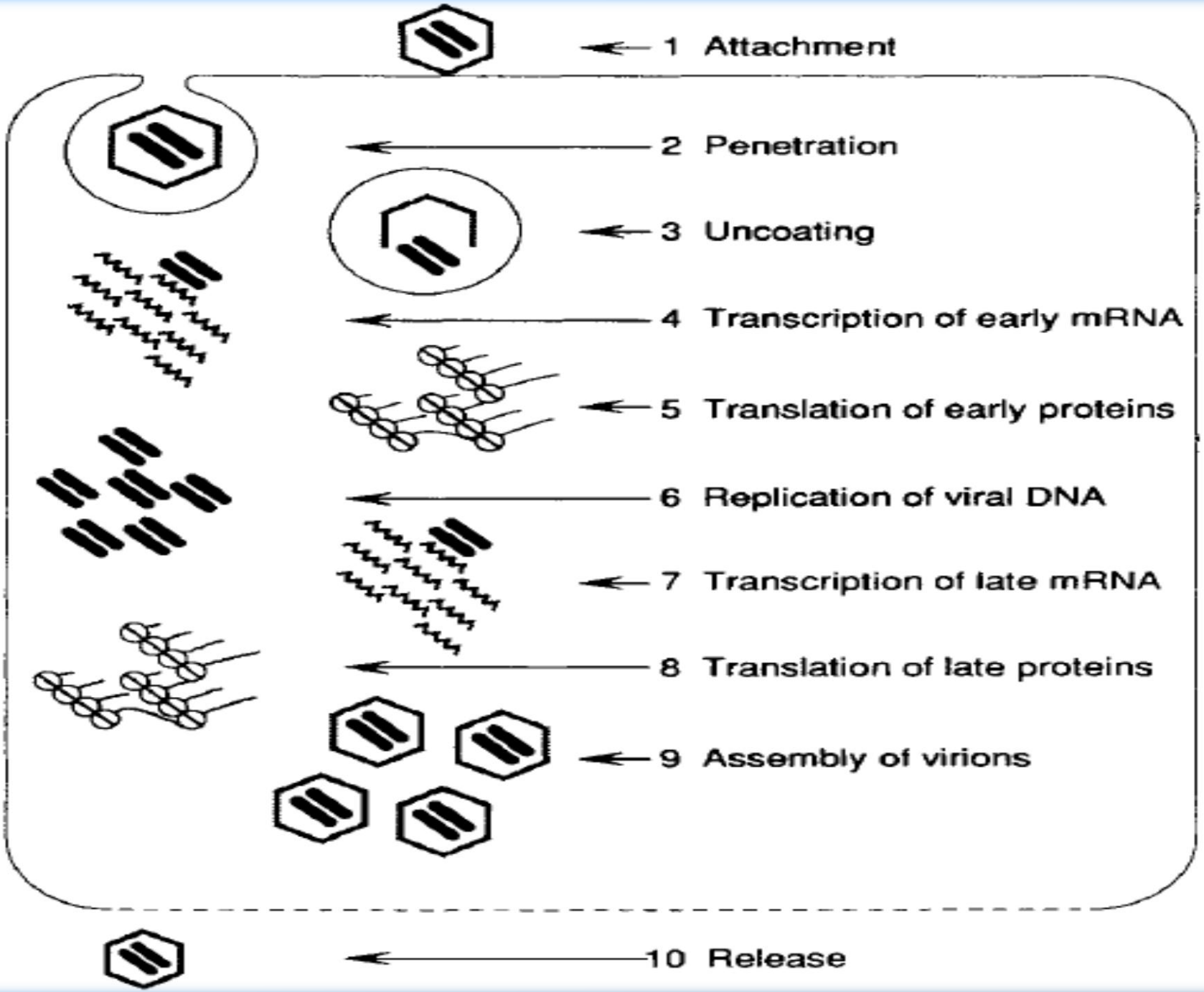
التصادم العشوائي

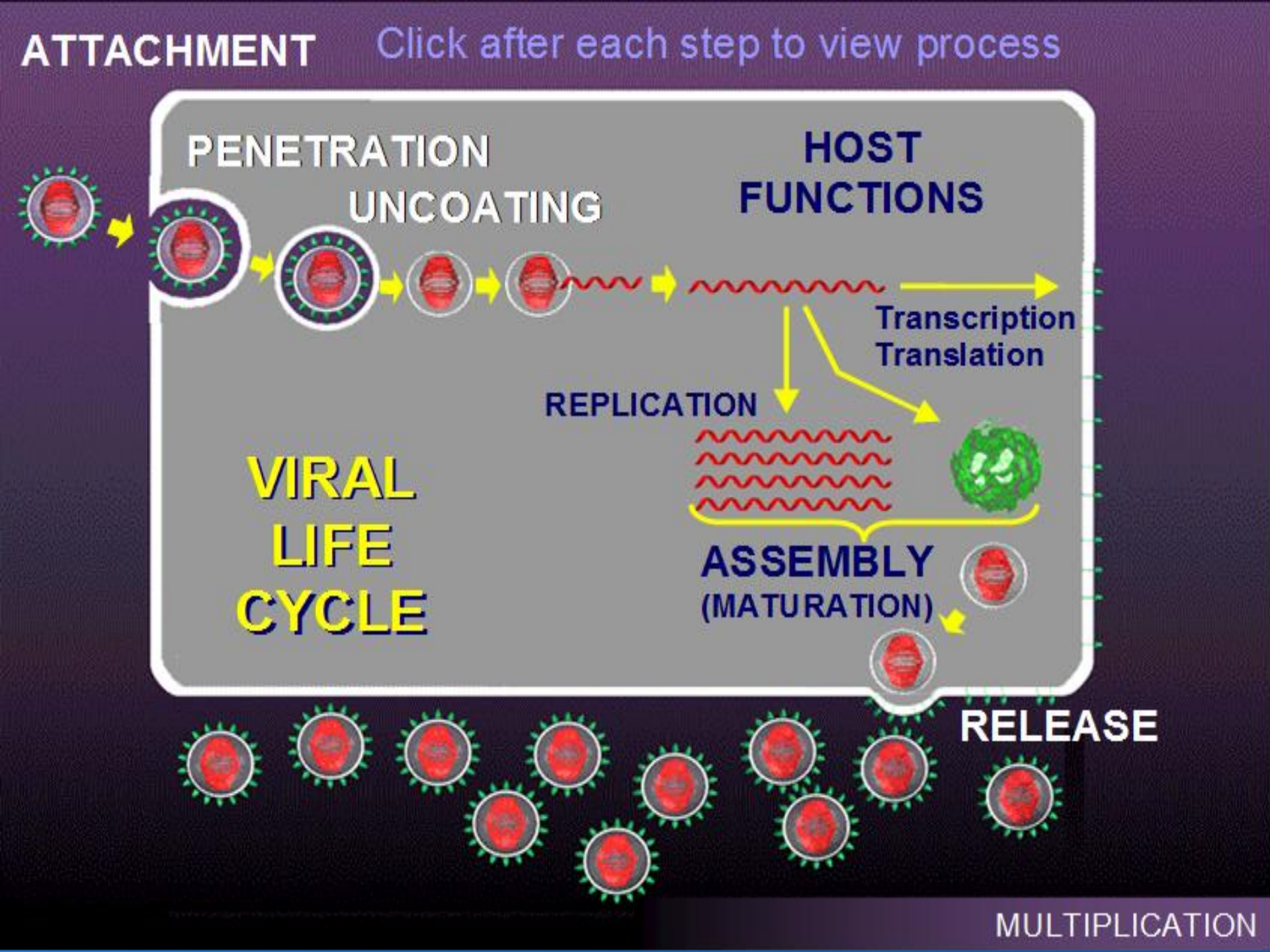
Attachment

- To initiate infection, the virus must be able to bind to target cell.
- Binding occurs between:
  - Ligands on the virus surface
    (viral attachment proteins)
  - Receptors on the plasma membrane of cell.
- Although there is a degree of specificity, quite different viruses may utilize the same receptor and, conversely, viruses in the same family or genus may use different receptors

Virus attachment




1 Attachment
2 Penetration
3 Uncoating
4 Transcription of early mRNA
5 Translation of early proteins
6 Replication of viral DNA
7 Transcription of late mRNA
8 Translation of late proteins
9 Assembly of virions
10 Release


ATTACHMENT
Click after each step to view process
PENETRATION
UNCOATING
HOST
FUNCTIONS
Transcription
Translation
REPLICATION
VIRAL
LIFE
CYCLE
ASSEMBLY
(MATURATION)
RELEASE
MULTIPLICATION

Examples:

- **Influenza viruses:**
  Viral Haemagglutinin (HA) peplomer

  Sialic acid containing cell receptor

- **HIV:**
  Viral surface glycoprotein gp120 subunit SU binds to cellular CD4 receptor.

  Then the complex binds to a second cell receptor 'fusin' which displaces SU and brings TM subunit into contact with cell membrane.

TABLE 1. *Protein viral receptors and coreceptors*

Virus	Family	Receptor	Function
G-protein-coupled receptors			
HIV	*Retroviridae*	CXCR4	Chemokine receptor
HIV	*Retroviridae*	CCR3	Chemokine receptor
HIV	*Retroviridae*	CCR2b	Chemokine receptor
HIV	*Retroviridae*	CCR8	Chemokine receptor
HIV/SIV	*Retroviridae*	CCR5	Chemokine receptor
HIV/SIV	*Retroviridae*	Bonzo/STRL-33/TYMSTR	Chemokine receptor
HIV/SIV	*Retroviridae*	BOB/GPR15	Chemokine receptor
SIV	*Retroviridae*	GPR1	Chemokine receptor
Proteins with multiple membrane-spanning domains			
GALV/FeLV-B/SSAV	*Retroviridae*	PiT-1	Phosphate transport
MLV-E	*Retroviridae*	MCAT-1	Cationic amino acid transport
MLV-A	*Retroviridae*	PiT-2	Phosphate transport
MLV-X/MLV-P	*Retroviridae*	XPR1/Rmc1/SYG1	Transporter
Immunoglobulin-related proteins			
Poliovirus	*Picornaviridae*	PVR	Unknown
PRV/BHV-1	*Herpesviridae*	PVR	Unknown
HSV-1/HSV-2/PRV	*Herpesviridae*	Prr2/HveB	Unknown
HSV-1/HSV-2/ BHV-1/PRV	*Herpesviridae*	Prr1/HveC	Unknown
Coxsackie B	*Picornaviridae*	CAR	Unknown
Ad-2/Ad-5	*Adenoviridae*	CAR	Unknown
MHV-A59	*Coronaviridae*	MHVR/Bgp1 (a)	Biliary glycoprotein
Major rhinoviruses	*Picornaviridae*	ICAM-1	Cell adhesion/signaling
HIV/SIV	*Retroviridae*	CD4	T-cell signaling
HHV-7	*Herpesviridae*	CD4	T-cell signaling
Low-density lipoprotein receptor–related proteins			
ALV-A	*Retroviridae*	TVA	Unknown
Minor rhinoviruses	*Picornaviridae*	LDLR/α2MR/LRP	Lipoprotein receptors
Integrins			
Adenovirus	*Adenoviridae*	αvβ3	Vitronectin binding
Coxsackie A9	*Picornaviridae*	αvβ3	Vitronectin binding
Adenovirus	*Adenoviridae*	αvβ5	Vitronectin binding
Echoviruses-1/-8	*Picornaviridae*	α2β1	Collagen/laminin binding
Tumor necrosis factor receptor–related proteins			
ALV-B/D/E	*Retroviridae*	TVB	Apoptosis-inducing receptor
HSV-1	*Herpesviridae*	HveA	LIGHT receptor
Small consensus repeat–containing proteins			
EBV	*Herpesviridae*	CR2	C3d/C3dg/iC3b binding
Measles	*Paramyxoviridae*	CD46	Complement inhibition
Echoviruses	*Picornaviridae*	CD55	Complement inhibition
Coxsackie B-1/-3/-5	*Picornaviridae*	CD55	Complement inhibition
Miscellaneous			
BLV	*Retroviridae*	BLVRcp1	Unknown
Coronavirus-229E/TGEV	*Coronaviridae*	Aminopeptidase-N	Metalloproteinase
LCMV/lassa fever virus	*Arenaviridae*	α-Dystroglycan	Laminin/agrin binding
Sindbis	*Togaviridae*	Laminin receptor	Laminin binding

TABLE 2. *Carbohydrate viral receptors*

Virus	Family	Receptor
Influenza A	*Orthomyxoviridae*	Sialic acid–containing oligosaccharides
Sendai	*Paramyxoviridae*	Sialic acid–containing oligosaccharides
Reovirus-3	*Reoviridae*	Sialic acid–containing oligosaccharides
Murine polyomavirus	*Papovaviridae*	Sialic acid–containing oligosaccharides
Canine parvovirus	*Parvoviridae*	Sialic acid–containing oligosaccharides
Influenza C	*Orthomyxoviridae*	9-O-acetylsialic acid
Human/bovine coronaviruses	*Coronaviridae*	N-acetyl-9-O-acetylsialic acid
HIV	*Retroviridae*	Galactosyl ceramide
HSV	*Herpesviridae*	Heparan sulfate
Human CMV	*Herpesviridae*	Heparan sulfate

HIV, human immunodeficiency virus; HSV, herpes simplex virus; CMV, cytomegalovirus.

Penetration (Entry)

- Following attachment, the virus enters the cell by one of two means:

1- Endocytosis; Receptor-mediated Endocytosis

A- Fusion in Endosome ex: Influenzavirus

B- Lysis of Endosome ex: Adenovirus

2- Fusion; Membrane fusion

Ex: HIV

Penetration (Entry)

- Following attachment, the virus enters the cell by one of two means:

1- Endocytosis:

- Receptor mediated endocytosis is a normal cell mechanism for the uptake of macromolecules.
- Many enveloped and nonenveloped viruses use this essential cell function to initiate infection.

Steps of Endocytosis:

1- Virion attachment to receptors, which cluster at clathrin-coated pits.

2- Endocytosis into clathrin-coated vesicles.

3- Vesicles enter the cytoplasm

4- After removal of the clathrin coat, vesicle fused with the endosome (acidic prelysosomal vacuoles).

5- Acidification within the vesicle triggers changes in virion proteins and surface structures.

6- These changes lead to the release of virus in the cytoplasm (e.g. fusion with the endonsomal membrane in influenza).

Entry

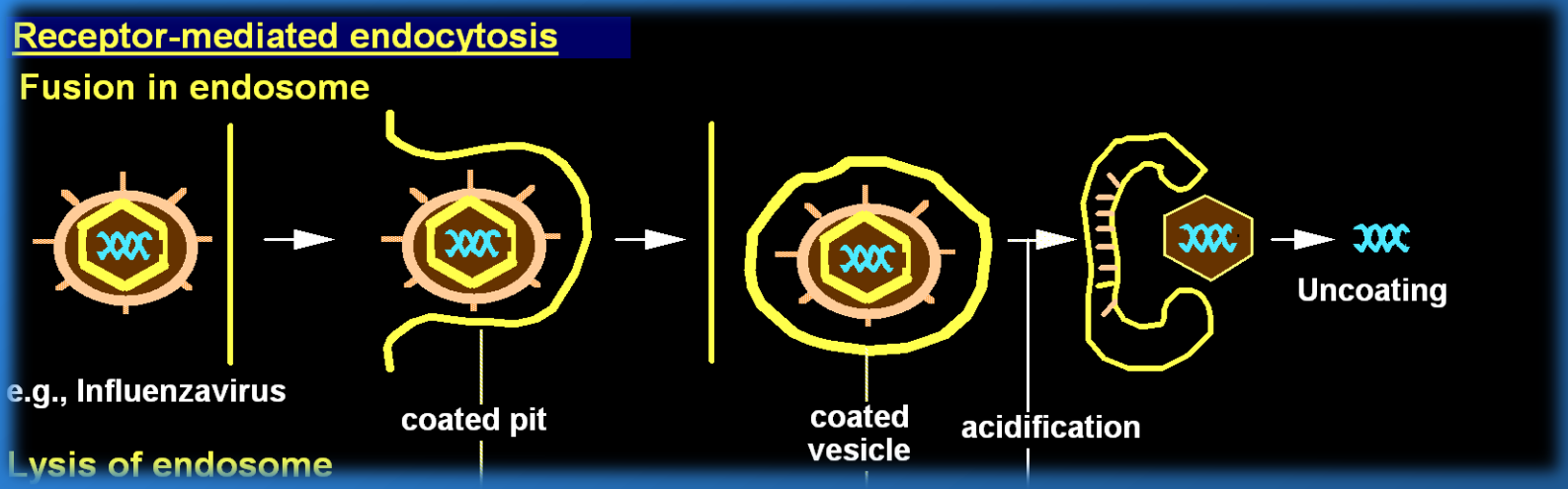
الدخول

Receptor-mediated Endocytosis

التحوصل من خلال مستقبلات

Fusion in Endosome
Influenza virus

الاندماج في الأ ندوسوم



Entry

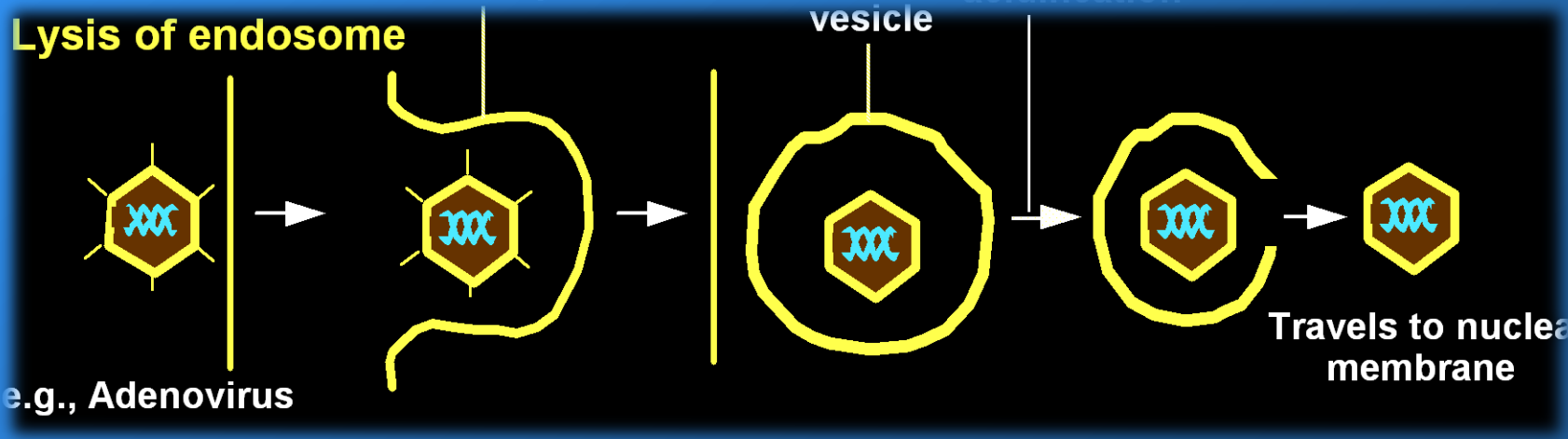
الدخول

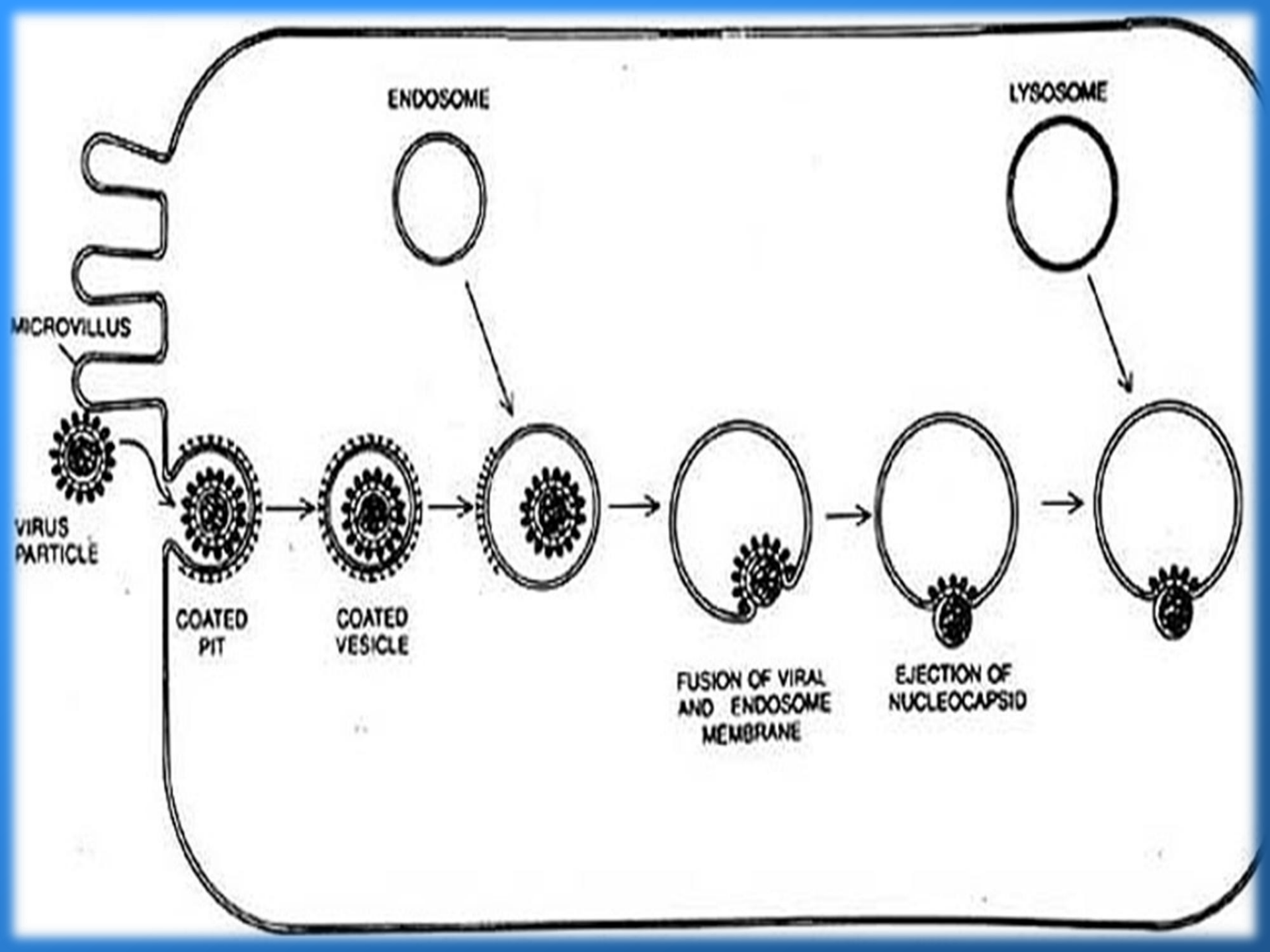
Receptor-mediated Endocytosis

التحوصل من خلال مستقبلات

Lysis of Endosome Adenovirus

تحلل الأ ندوسوم




ENDOSOME
LYSOSOME
MICROVILLUS
VIRUS PARTICLE
COATED PIT
COATED VESICLE
FUSION OF VIRAL AND ENDOSOME MEMBRANE
EJECTION OF NUCLEOCAPSID

2- Fusion:

- F (fusion) glycoprotein present in some viruses causes the envelope of these viruses to fuse directly with the plasma membrane of the cell.
- This allows the nucleocapsid to be released directly into the cytoplasm.
- e.g. Paramyxoviruses and some other enveloped viruses

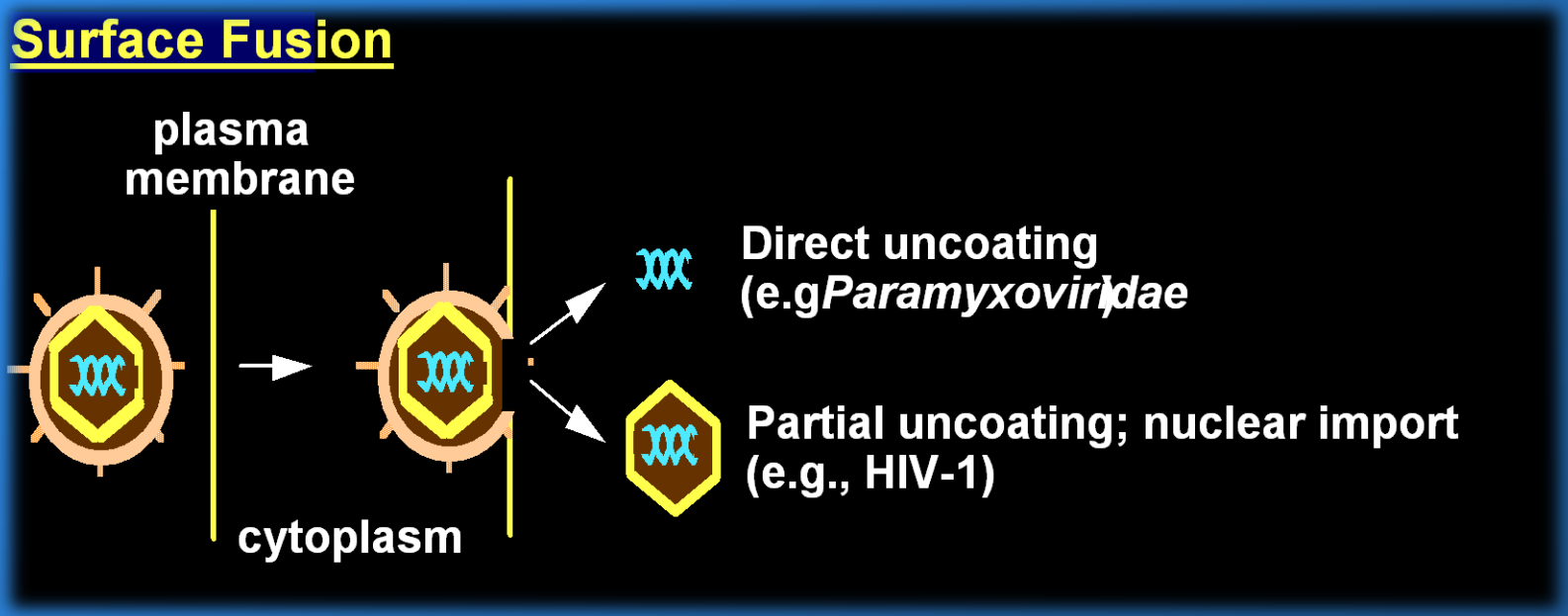
Entry

الدخول

Membrane Fusion

الاندماج الغشائي

Paramyxovirus + HIV



3- Uncoating

Aim:

To expose viral genes for transcription and replication

1- Complete uncoating:

- Occurs in **enveloped viruses** that enter the cell by fusion with plasma or endosomal membrane.
- The nucleocapsid is discharged directly into the cytoplasm and transcription occurs directly.

2- Partial uncoating:

- Occurs with some **naked viruses**.
- Only certain capsid proteins are removed and the viral genome expresses all its functions without ever being released from the virion core.

Uncoating

3. التقشر

- Direct or Partial uncoating

مباشر أو جزئي

Paramyxovirus + HIV

Endosome …. Conformational changes

تغيرات شكلية

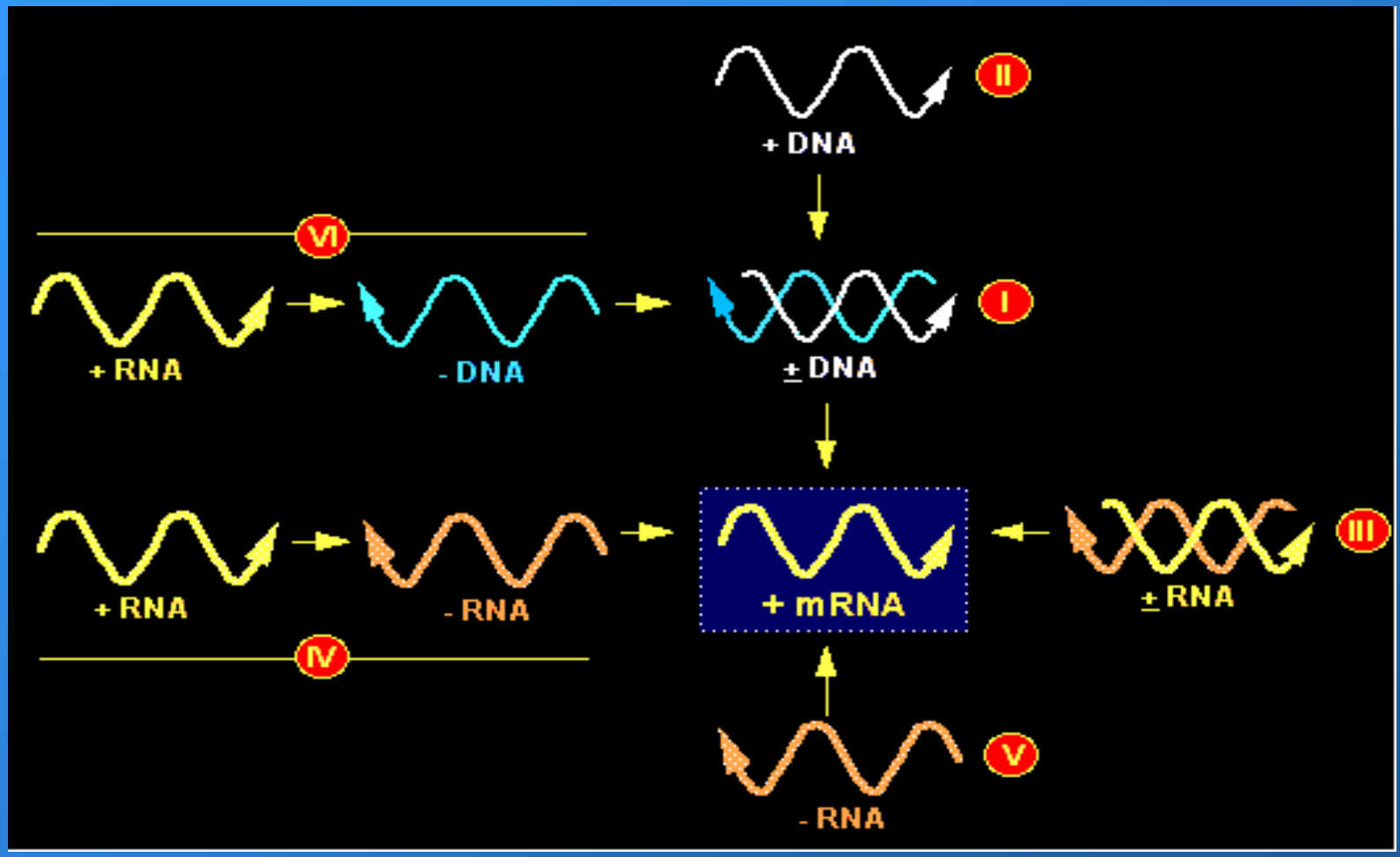
Influenza + Adenovirus

4. التكاثر وإنتاج بروتينات الفيروس

Replication and viral protein production

- **Replication:** Copy (RNA or DNA); duplication.
- **Synthesis:** Translation of mRNA into Viral proteins.

Baltimore Classification



All viruses must generate positive strand mRNAs from their genomes, in order to produce proteins and replicate themselves.

DNA Viruses

DNA viruses Replicate in the Nucleus

Except: Poxvirus

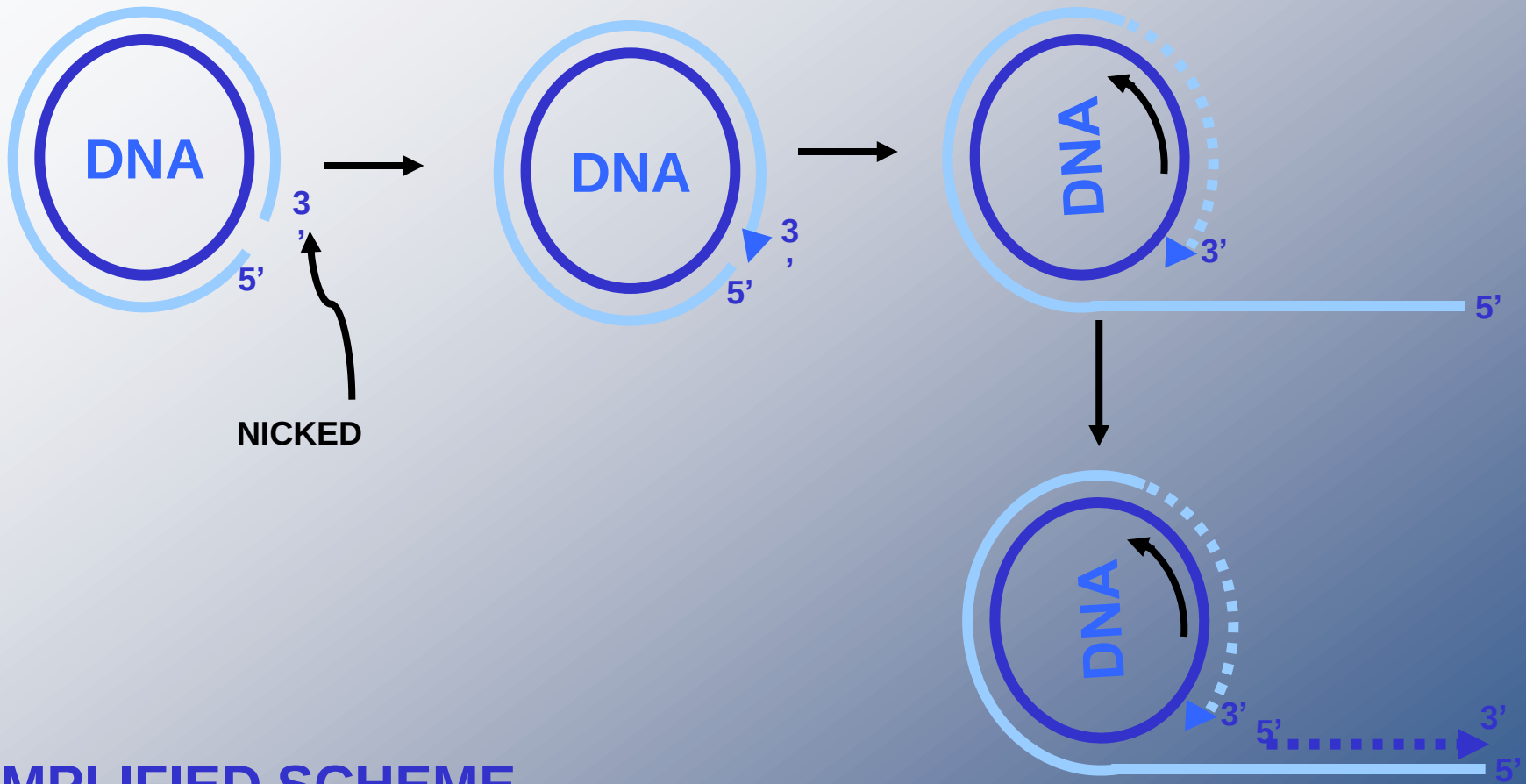
Most DNA viruses encode DNA polymerase

(Except small DNA viruses; parvoviruses and papillomaviruses)

DNA viruses replicate their genetic material by:

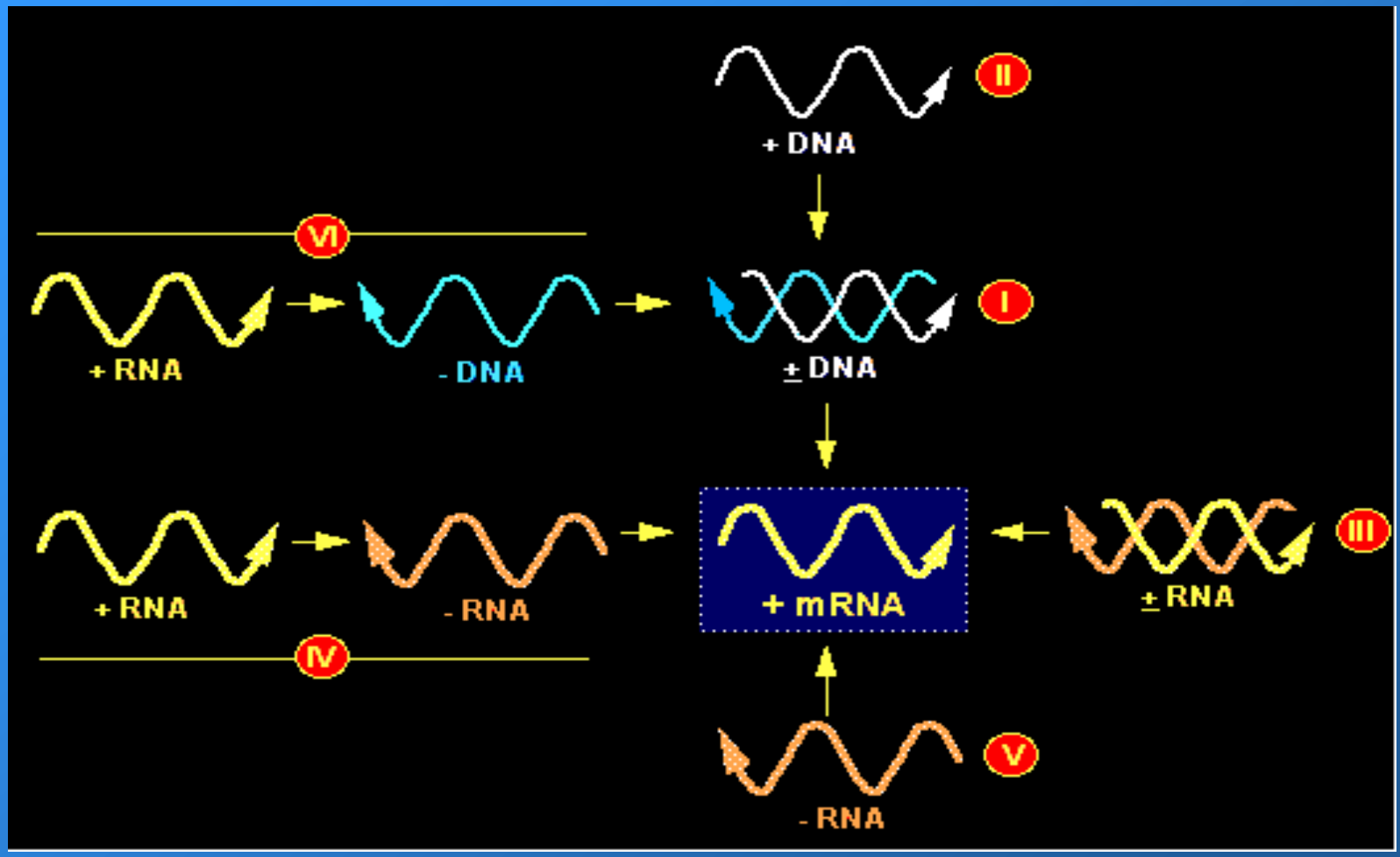
- **Bidirectional replication from a circular substrate**
  - The process include:
  - "theta-form" intermediate (eg, papillomaviruses),
  - "rolling circle" mechanism
- **Replication from a linear substrate**
  - Examples include adenoviruses
- **Replication via an RNA intermediate**
  - Hepadnaviruses (hepatitis B virus) are unique since they contain a partially dsDNA genome that must be converted into an RNA form by the viral reverse transcriptase (RT) during the virus life cycle .

DNA REPLICATION



SIMPLIFIED SCHEME

Baltimore Classification



All viruses must generate positive strand mRNAs from their genomes, in order to produce proteins and replicate themselves.

I- Double stranded DNA

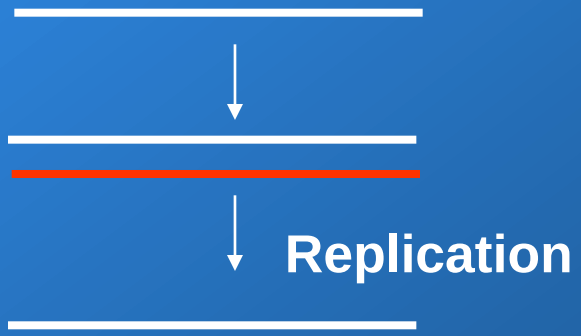
A- Nucleus ex: Adenovirus and Herpesvirus

B- Cytoplasm ex: Poxvirus

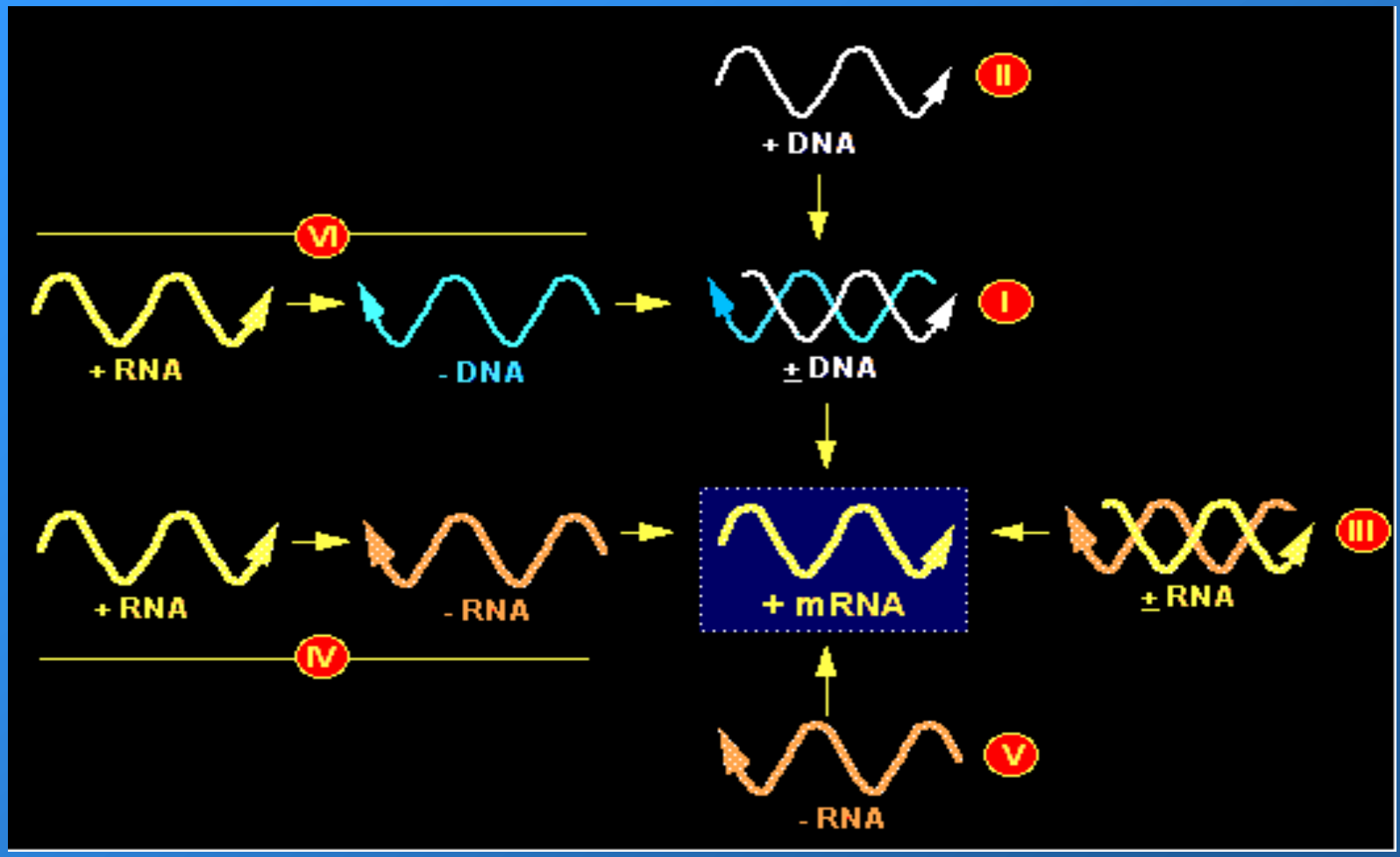
II- Single stranded DNA

Nucleus ex: Parvovirus

Template D.STD. intermediate



Baltimore Classification



All viruses must generate positive strand mRNAs from their genomes, in order to produce proteins and replicate themselves.

RNA Viruses

All RNA viruses Replicate in the Cytoplasm

Except: Orthomyxoviruses (influenza A & B)
Borna disease virus,
Hepatitis delta virus
Retrovirses

All RNA viruses encode RNA dependent RNA polymerase

Except; Retrovirus

RNA Genome:

1- Copy RNA

2- Translate mRNA

RNA Genome (-) and (-,+);

The virus carry the Enzyme

Noninfectious

RNA Genome (+);

The virus produces the Enzyme

Infectious

Enzyme: RNA dependent RNA polymerase

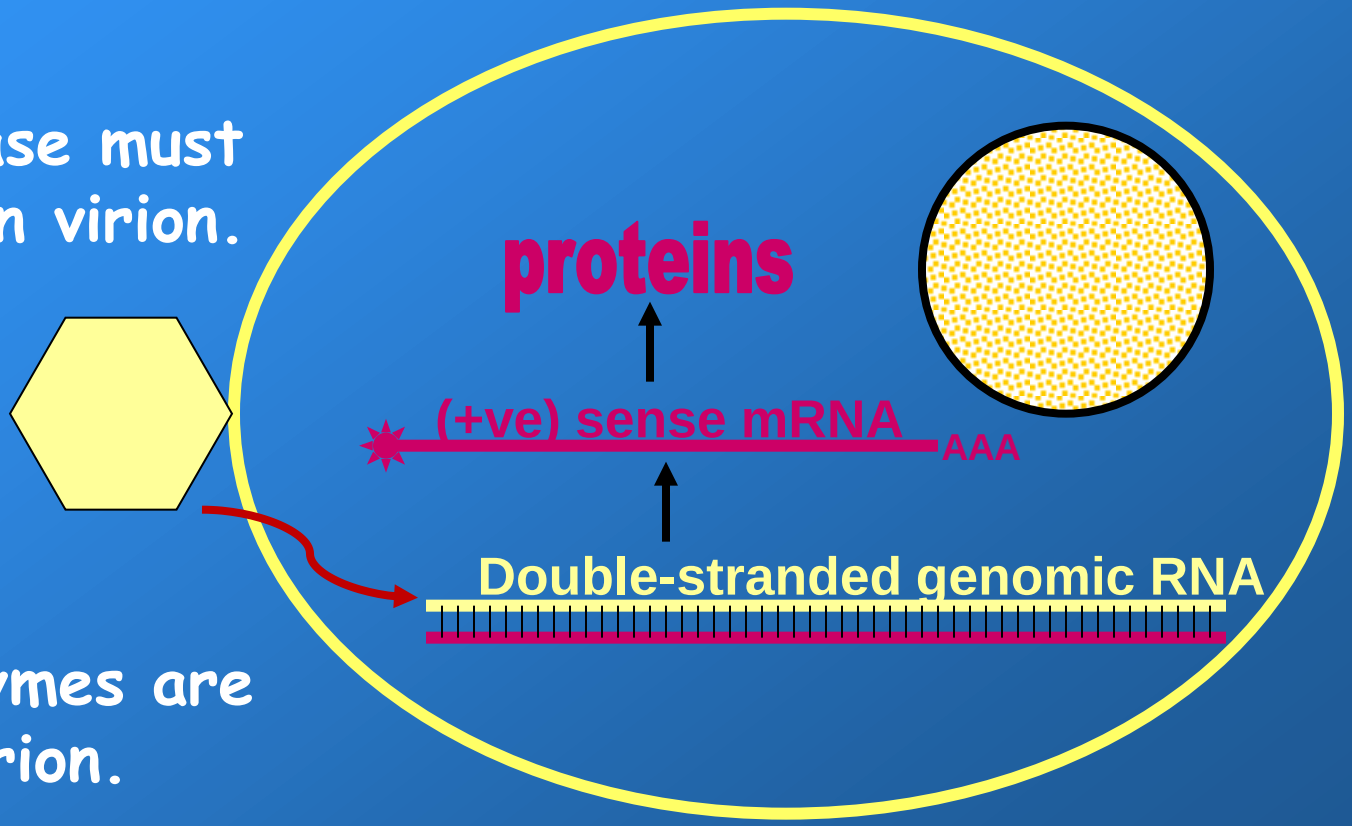
III- Double stranded RNA

Cytoplasm ex: Reovirus

Segmented

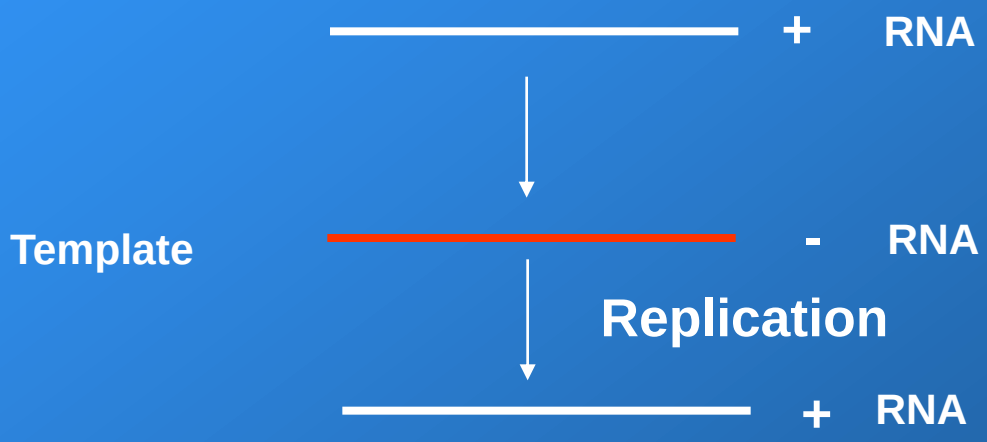
DOUBLE-STRANDED RNA GENOMES

RNA polymerase must be packaged in virion.

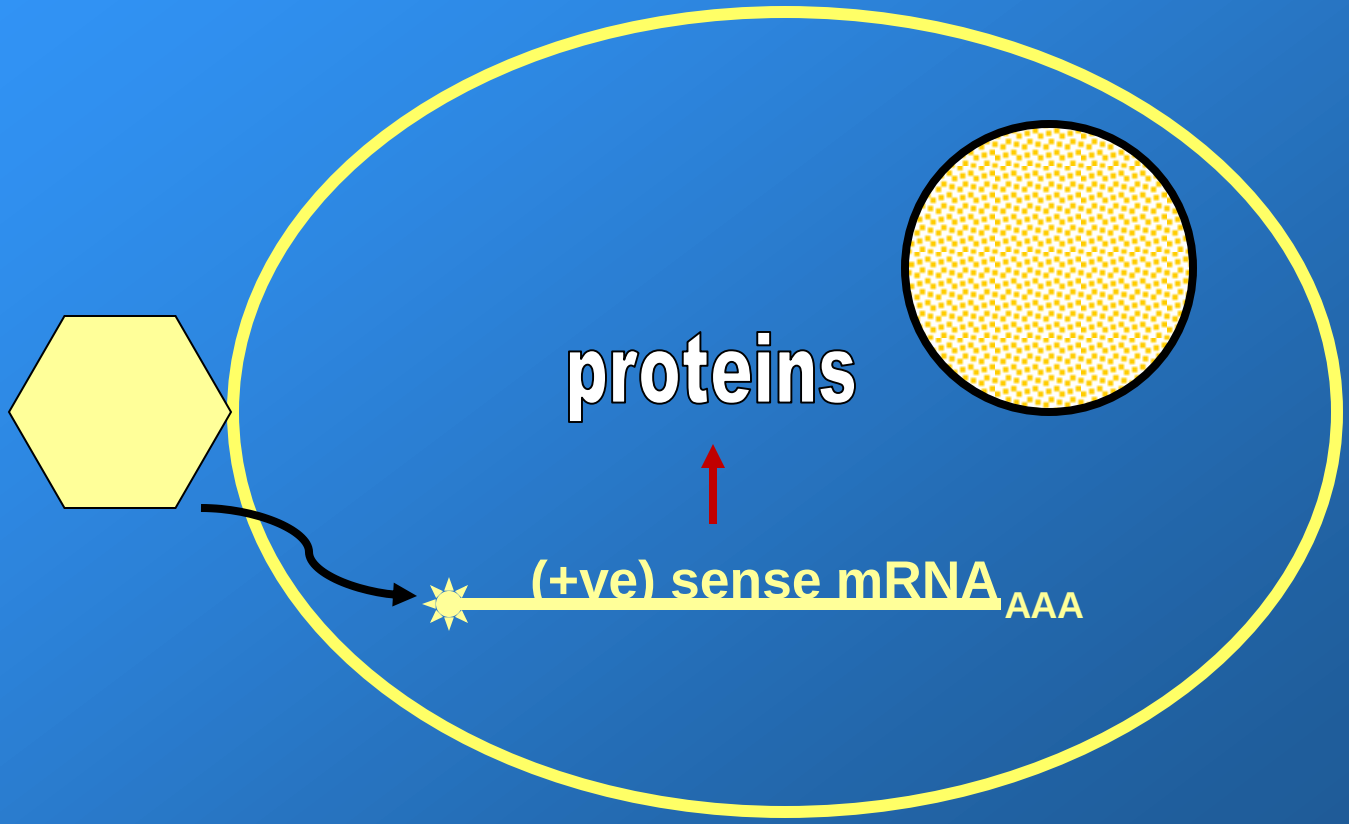


If used, RNA modifying enzymes are packaged in virion.

IV- Single stranded RNA (+) Sense



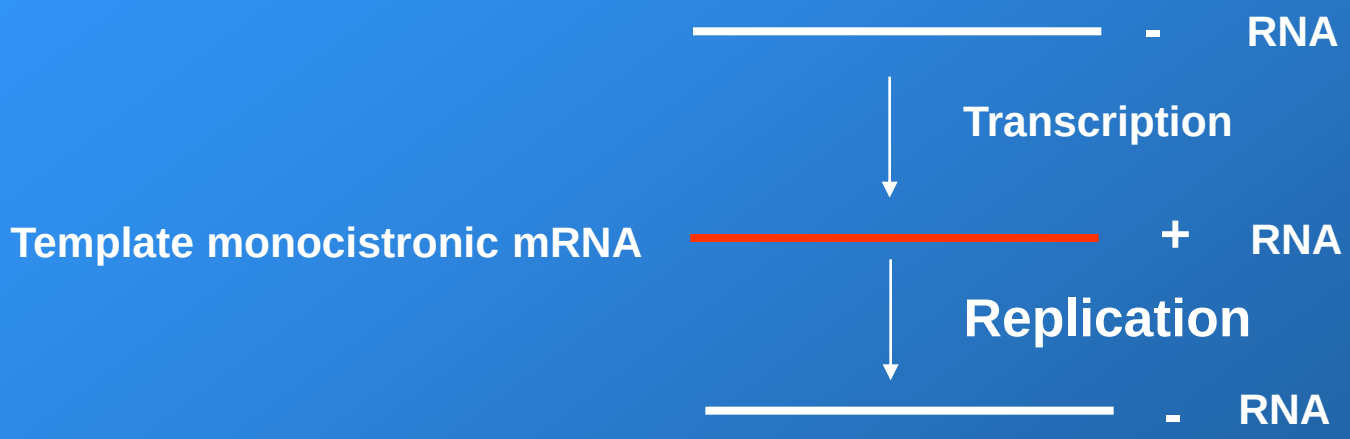
PLUS (POSITIVE) SENSE RNA GENOMES



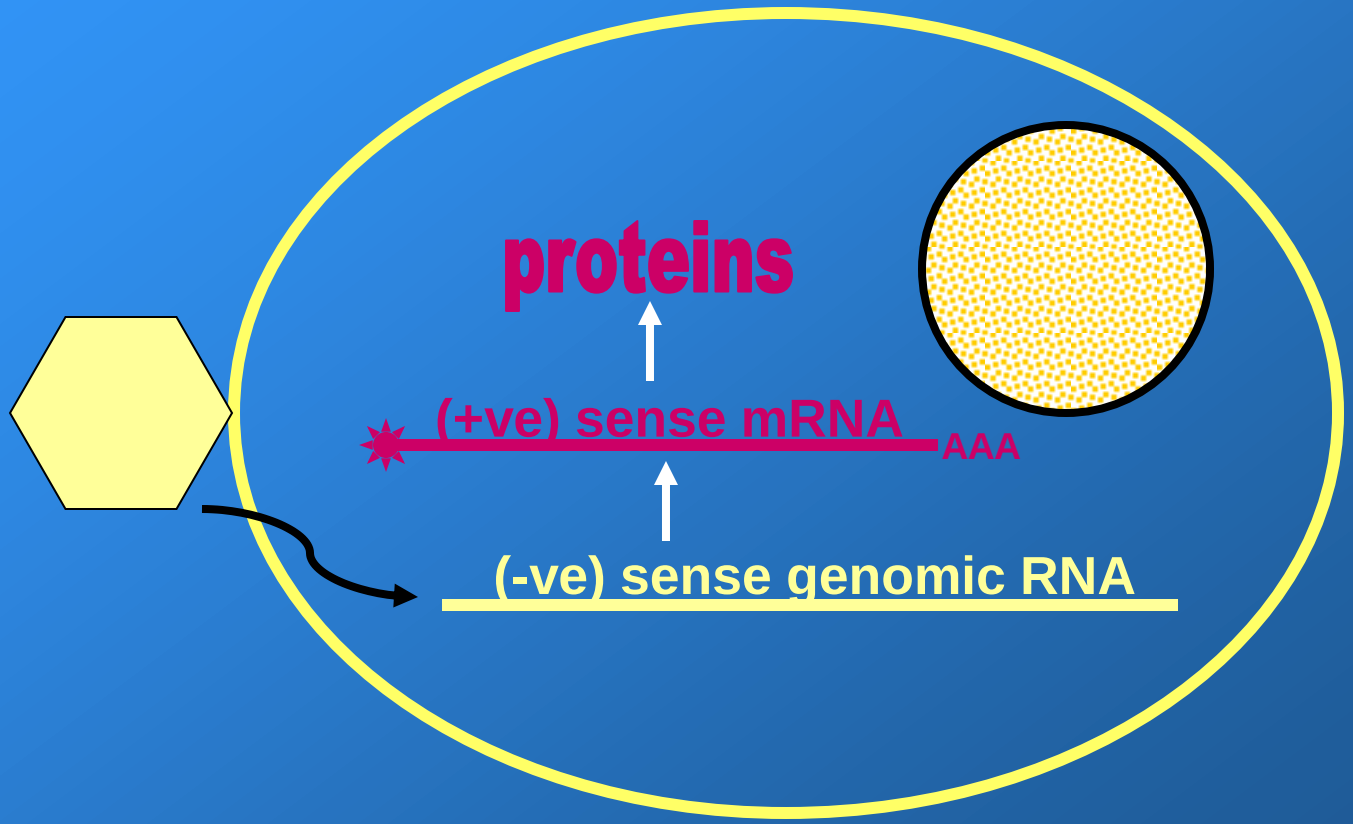
V- Single stranded RNA (-) Sense

Cytoplasm ex: Paramyxovirus

Non-Segmented

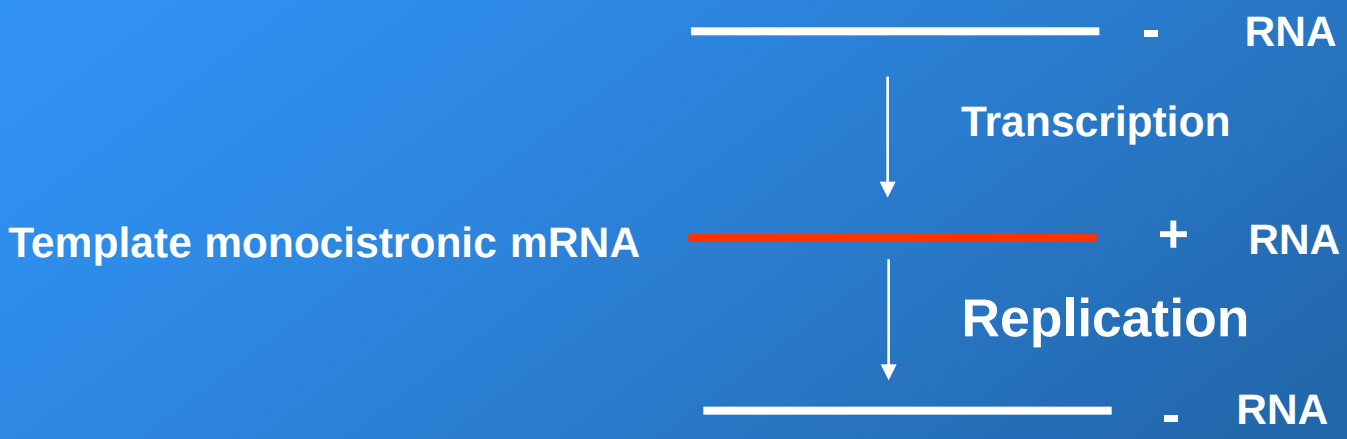


MINUS (NEGATIVE) SENSE RNA GENOMES



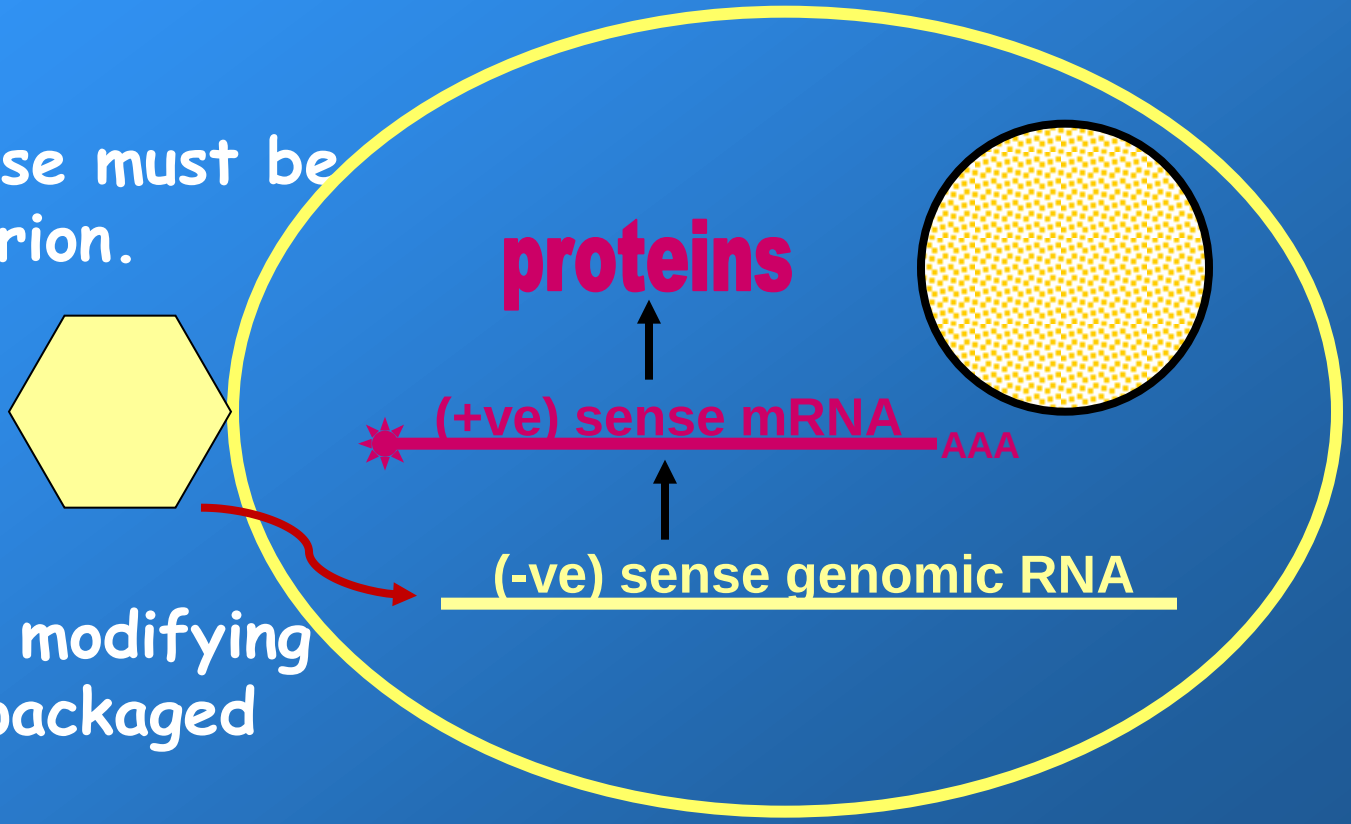
V- Single stranded RNA (-) Sense

- Nucleus ex: Orthmyxovirus
- Segmented



MINUS (NEGATIVE) SENSE RNA GENOMES

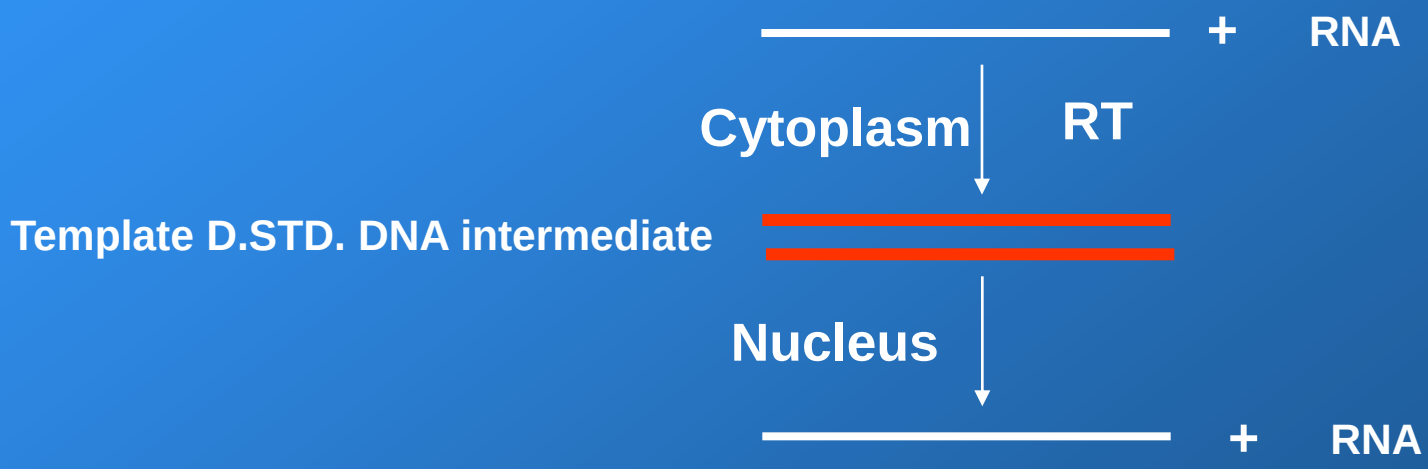
RNA polymerase must be packaged in virion.



If used, RNA modifying enzymes are packaged in virion.

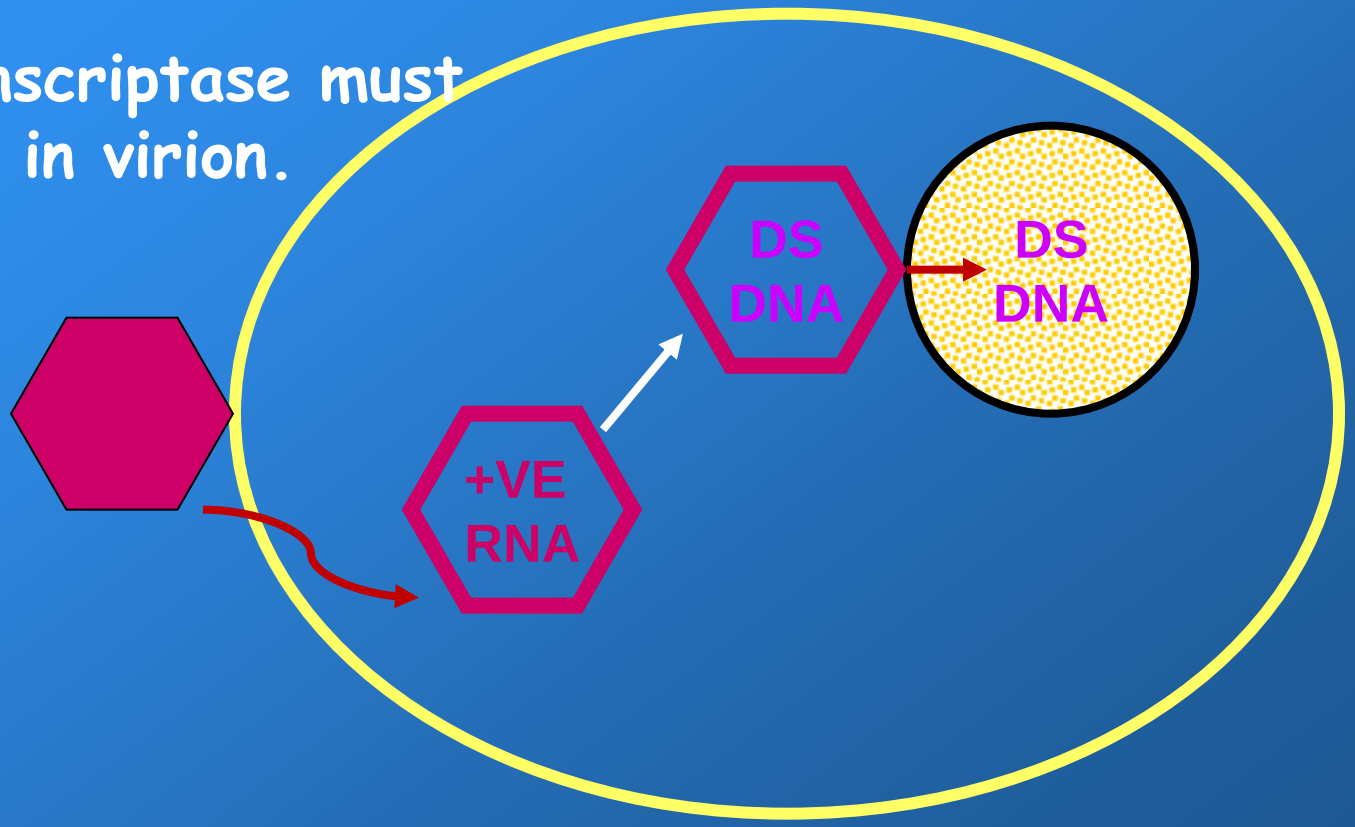
VI- Single stranded RNA (+) Sense With DNA Intermediate

Retrovirus



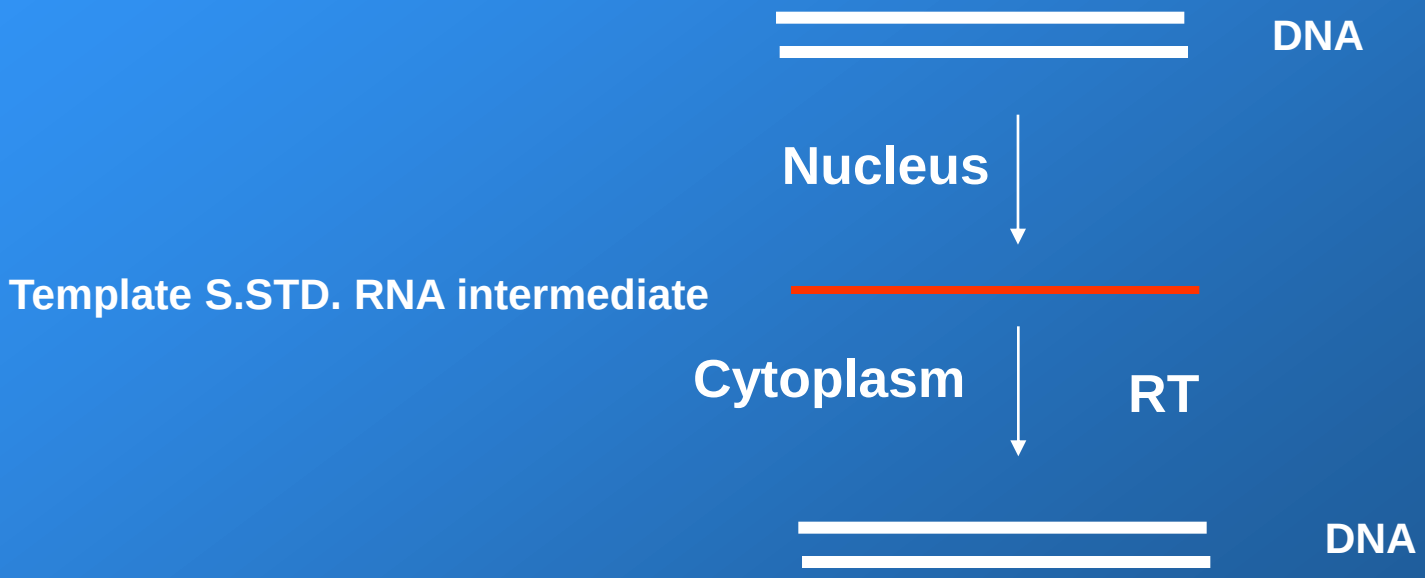
RETROVIRUSES

Reverse transcriptase must be packaged in virion.



VII- Double stranded DNA With RNA Intermediate

Hepadnavirus



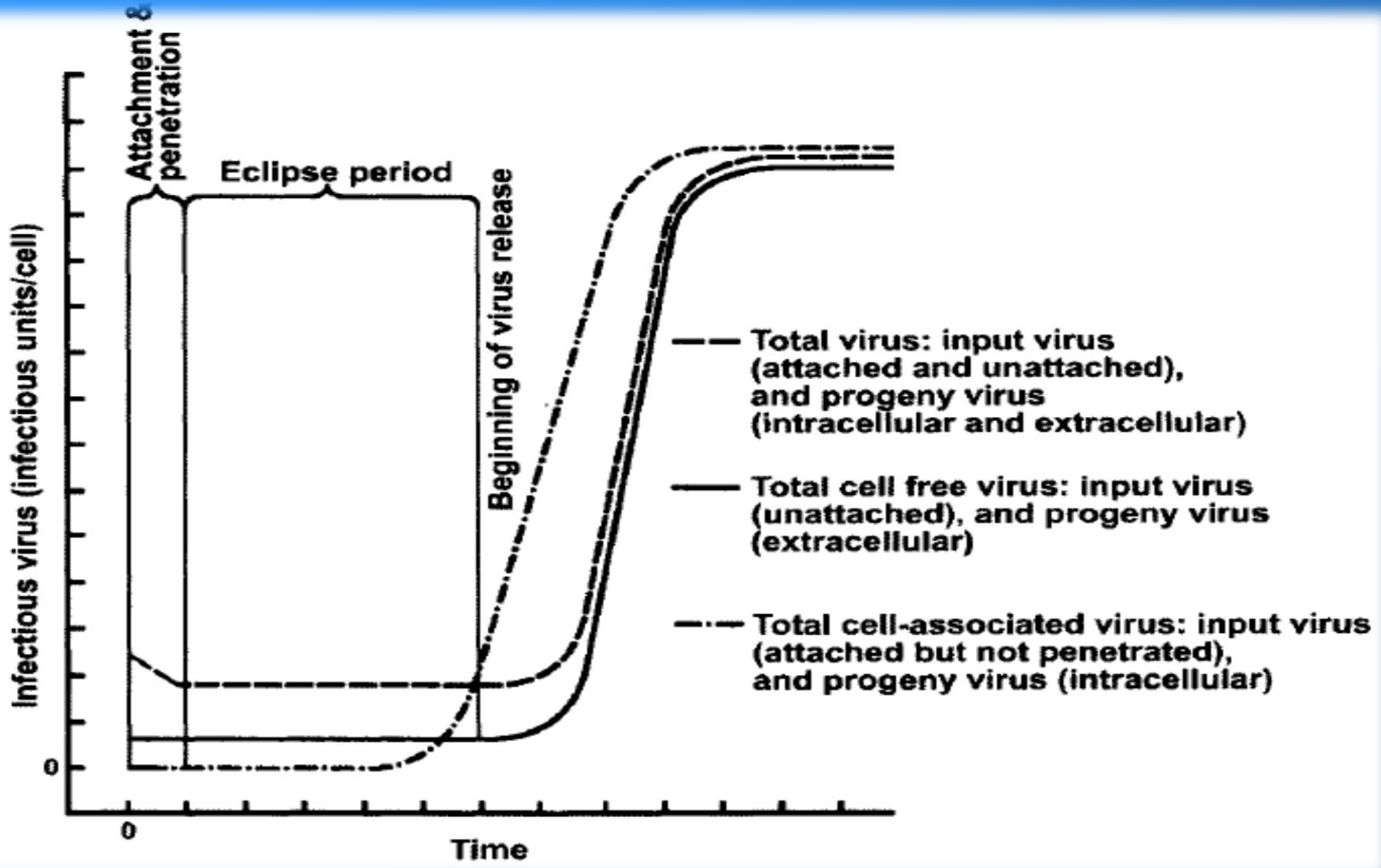
One-step Growth Curve

- Defined by classical studies on cell culture, in which multiple cultures are infected simultaneously.
- The increase in infectious virus over time is followed by sequential sampling and titration.

Cell-free and Cell-associated

- Shortly after infection, the inoculated 'Cell-free' virus disappears (extra- and intra-cellular).
- This period extends for 2-12 hours, until first progeny viruses become detectable (Eclipse Period)

One-step Growth Curve



One - Step Growth Cycle:

Eclipse period

(11H) Post-infection (PI)

Genome Free inside cell

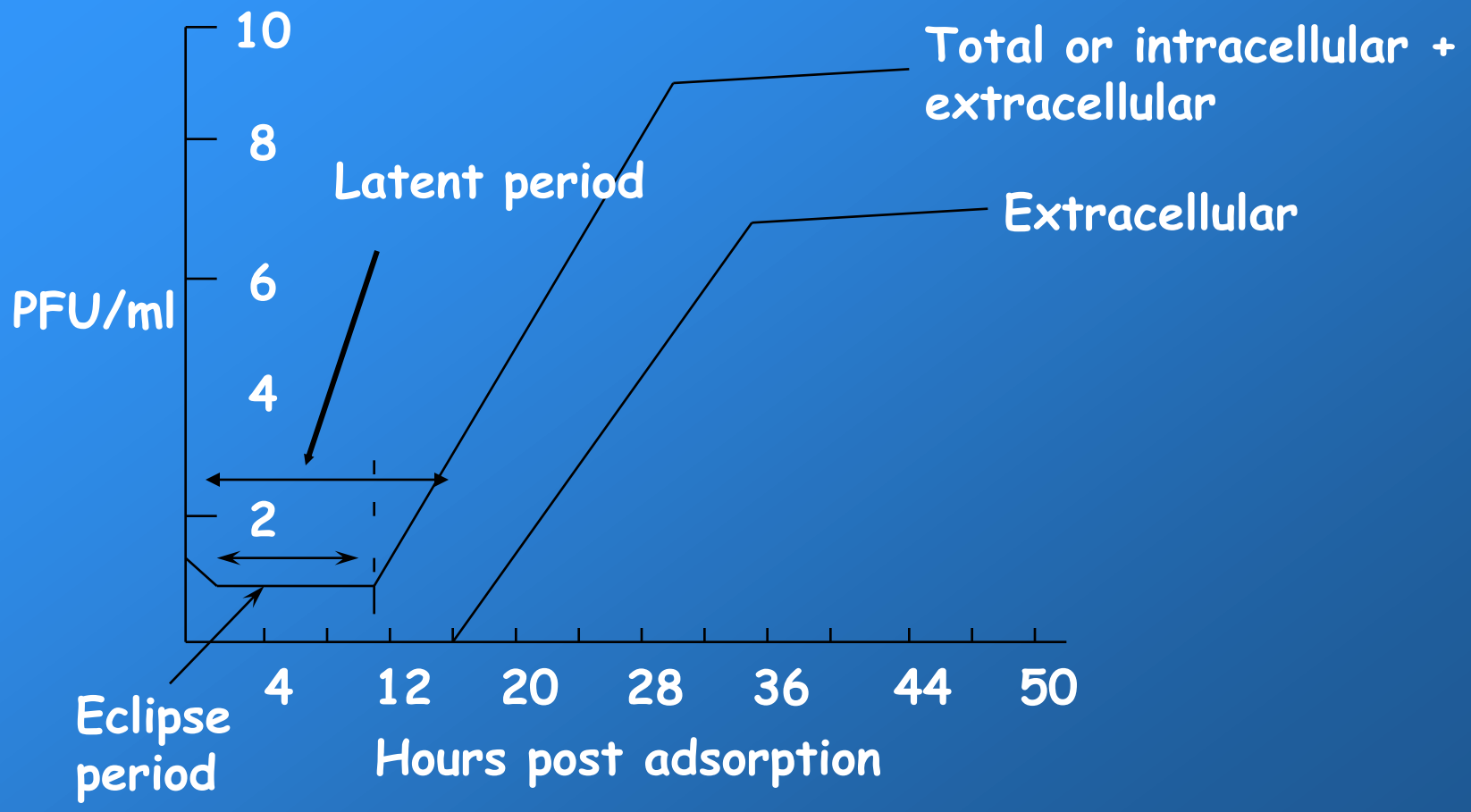
No infectious Particles

Synthetic phase

(12 H) (PI)

Infections particles

Typical Growth Curve:



One - Step Growth Cycle:

Eclipse period

Synthetic phase

Latent period (18 H)

P.I.

No extracellular virus

5. التجمع والتحرر Assembly + Release

- البروتينات التركيبية - الأحماض النووية
- الأنزيمات الفيروسية

- موقع التجمع يعتمد على موقع التكاثر

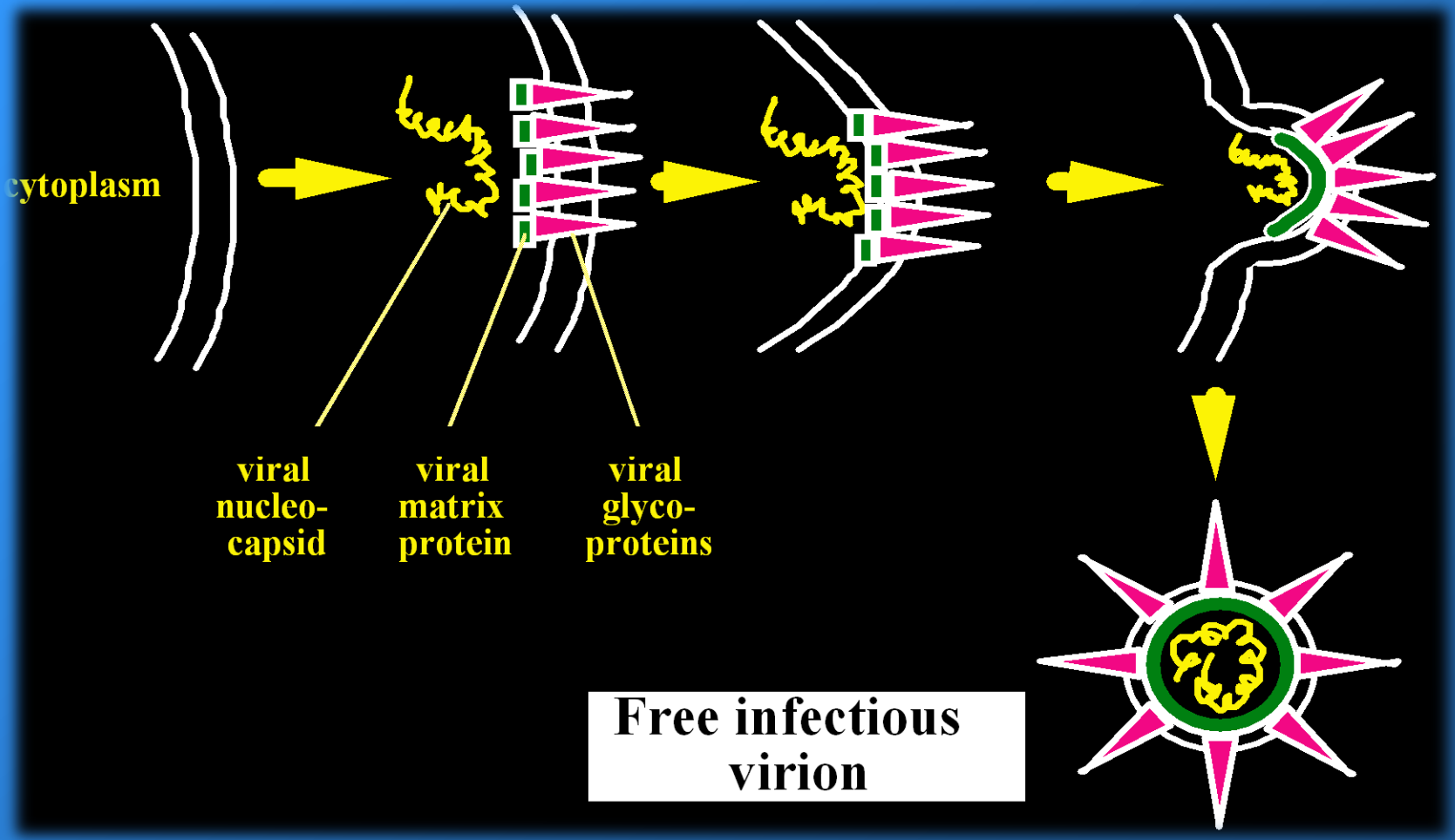
Picornavirus , Poxvirus , Reovirus…etc

Cytoplasm

Adenovirus , Polyomavirus …etc

Nucleus

Virus Assembly



Assembly + Release

5. التجمع والتحرر

Maturation

-النضج

- Infectious
- Conformational changes

Fo $\xrightarrow[\text{Proteases}]{\text{Cleavage}}$ F1 + F2 paramyxovirus

Fusion protein

Release

التحرر

I - التحلل

Lysis

Non - enveloped

Lytic viruses

II - التبرعم

Budding

Enveloped

I + II ⟶ death of cell (CPE)

Vaccines and antiviral drugs

-التحصين النشط Active immunization

- اللقاحات Vaccines

- انتجينات الفيروس Ag

- الجلد ، داخل الجلد ، الأنف ، العضل ، الوريد، الفم

- حث جهاز المناعة لأنتاج أجسام مضادة ضد Ag

أجسام مضادة Antibodies / نوعية متخصصة

Immunoglobulins (Ig)

(Adaptive) Acquired immunity

Live Vaccine

Killed Vaccine

شروط فعالية اللقاحات

1. لا يسبب مرضاً

-Live attenuated Vaccine

2.استحثاث مقامه طويل لإمد مكتسبة

Pox – Yello – Polio – Measles

3. يجب أن يكون مامونـاً

4. يجب أن يكون ثابتاً

Passive immunization التحصين السالب

Natural Passive immunity المناعة السالبة الطبيعية

Maternal immunity مناعة الأم المنقولة عبر المشيمة

المناعة السالبة الصناعية

HBV - (HBIG)

Antiviral drugs المضادات الفيروسية

Acyclovir اسايكلوفير

Herpesviruse

Viral DNA synthesis

Amantadine / Rimantadine

Influenza virus
Type-A

Pentration

Zidovudine (AZT) زيدوفيودين (HIV)

Ribavirin

RSV

HCV

(Guanine)

RNA synthesis