# TU 02 : Basic knowledge in Virology

université PARIS-SACLAY | FACULTÉ DE PHARMACIE

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# Plan

- Definition
- Virus Structure
  - Genome
  - Capside
  - Envelope
- Classification
- Multiplication des virus (within a cell)
  - Initiation
  - Replication/ Transcription
  - maturation

# Viral-associated Pathologies

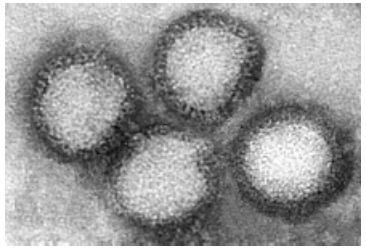
- Respiratory infections
  - Rhino pharyngitis, laryngitis, otitis…
  - Influenza
  - Bronchiolitis in infants
  - COVID19 (SARS-CoV2), SRAS (SARS-CoV1)
- Infections of the gastro intestinal tract
  - Viral gastroenteritis, rotavirus
- Poliomyelitis
- Viral hepatitis
  - Hepatitis A
  - Hepatitis B
  - Hepatitis C
  - Hepatitis D
  - Hepatitis E
- AIDS
  - HIV
  - Opportunistic infections

- Arboviruses (transmitted by arthropods)
  - Dengue
  - Yellow fever
  - Chikungunya, Zika…
- Hemorrhagic fevers
  - Ebola, Marburg
- Eruptive diseases
  - Measles
  - Rubella
  - Mumps
  - Herpes, chicken pox
  - Papillomavirus…
- Zoonosis….
  - Rabies
  - Mpox

A virus
A virosis

# Definition of a virus

- 4 essential criterions :



1) Only one type of nucleic acid

   either DNA or RNA → viral genome, carries all the genetic information of the virus

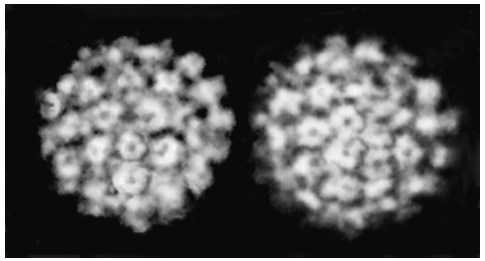
2) Replication form genetic material: a virus cannot growth or divide

3) Obligate intracellular Parasitism

   . Lack of enzymatic or energetic systems → hijacking of the cellular machinery

   . Isolation of viruses by cell culture

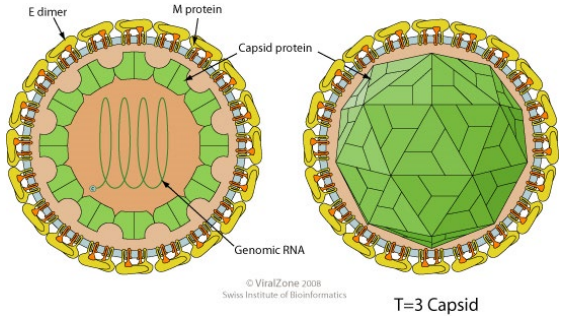
   . Difficult to find an antiviral chemotherapy



4) A define structure

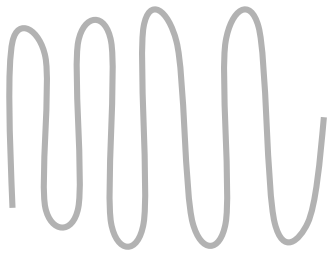
   . different from the one of cells or bacteria

   . a characteristic type of symmetry

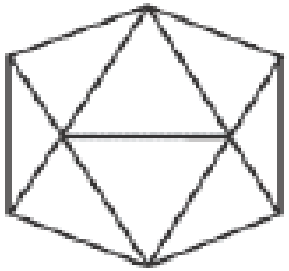



- virion : mature, infectious, extracellular viral particle
- virus : the infectious agent at every step of the viral cycle (intra and extra cellular)

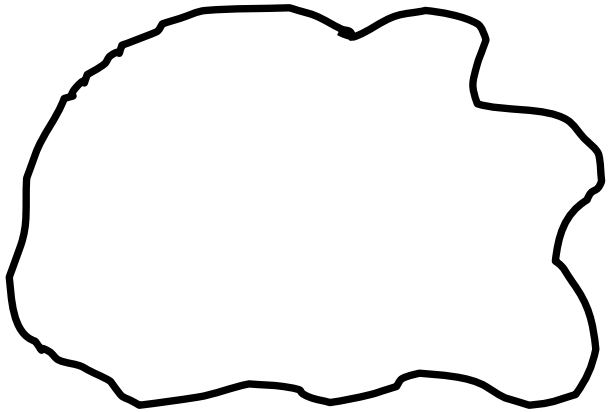
# Virion Structure



Genome

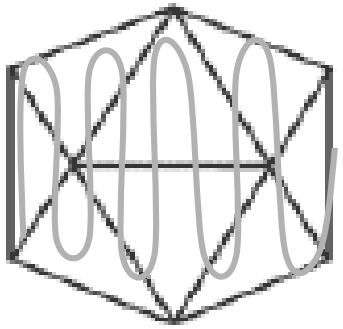


Capsid

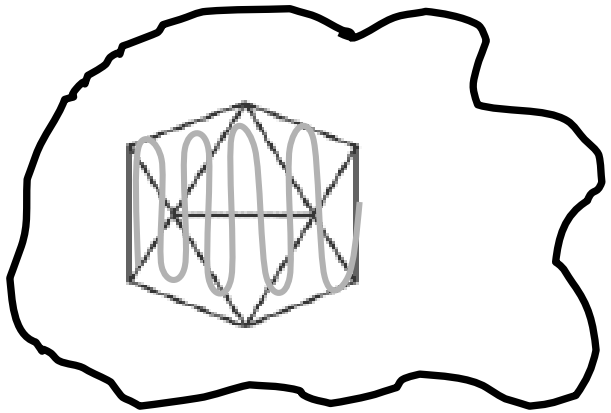


Envelope

The nucleic acid and capsid constitute the nucleocapsid



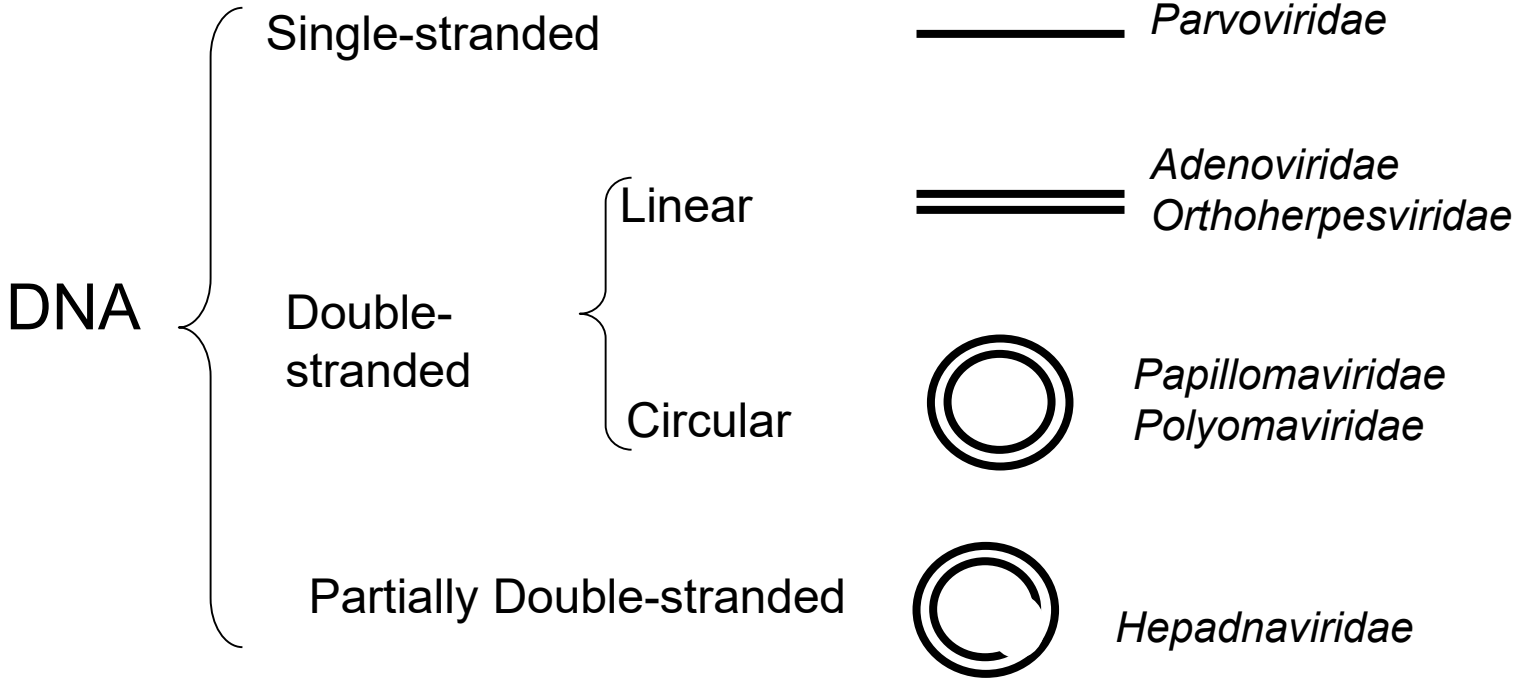
Naked Virus



Enveloped Virus

# DNA genomes

- Variable genome size (in base or base pair)
  - 3,2 kbp for Hepatitis B Virus (HBV)
  - 235 kbp for human cytomegalovirus
  - 1-5 Mb for bacteria and 3400 Mb for human genome
- Most often double-stranded and linear DNA
- Classical three-dimensional double helix structure

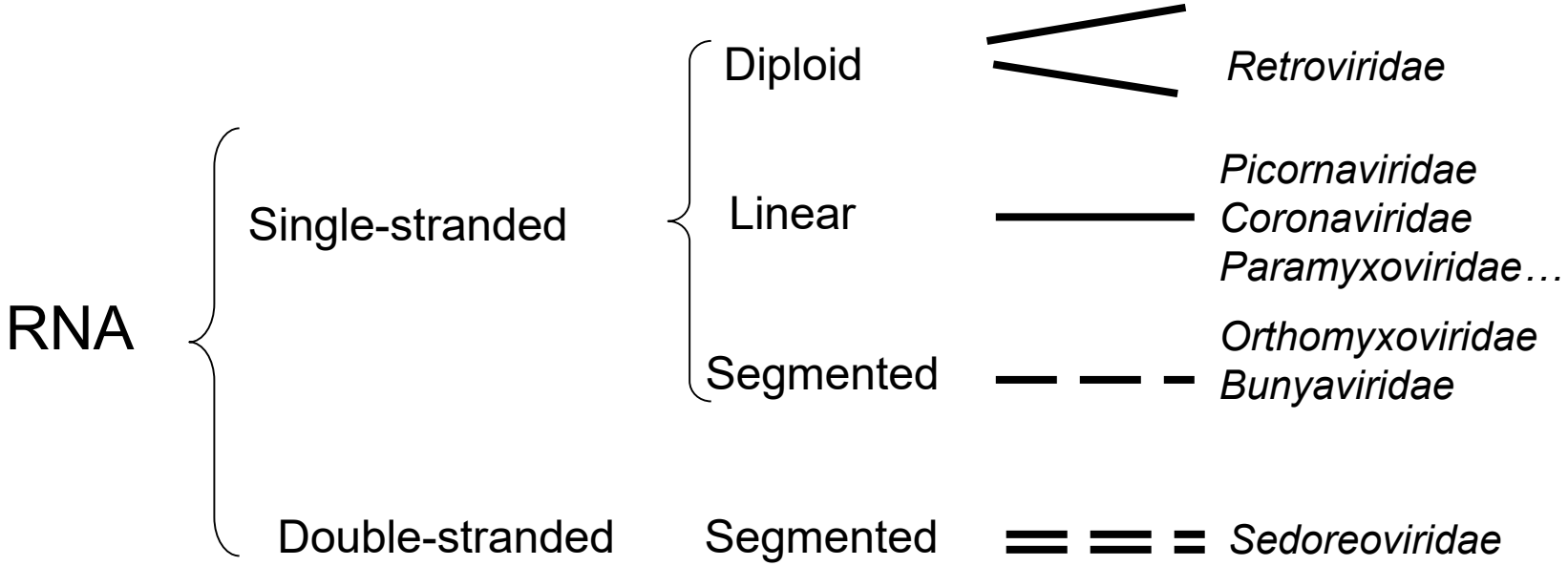


# RNA genomes

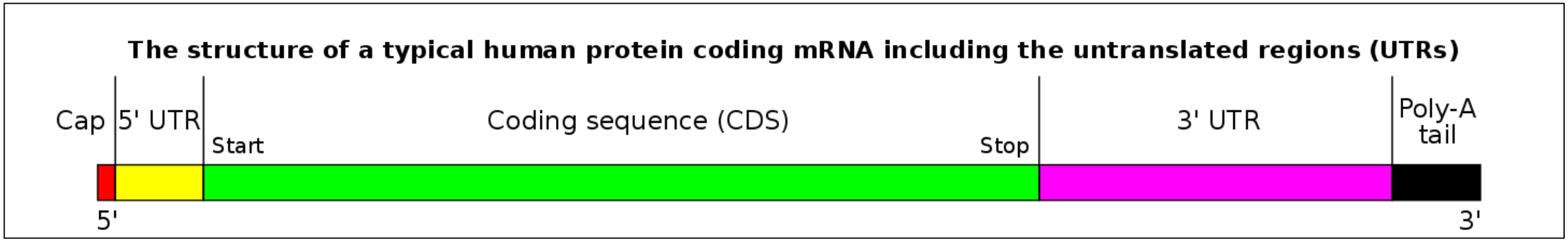
- Size (in bases)

From 7000 nucleotides for the smallest (enterovirus) to 30 kb for the biggest (*Coronaviridae*)

- Needs a viral RNA dependent RNA polymerase to replicate
- Most often single-stranded and linear RNA
- Some genomes are double-stranded (*Sedoreoviridae*)
- Some genomes (segmented genomes) are organized into distinct subgenomic fragments (10 to 11 segments for *Sedoreoviridae* and 7 to 8 segments for influenza viruses)



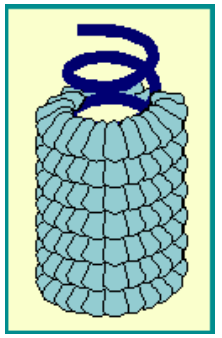
# RNA genomes



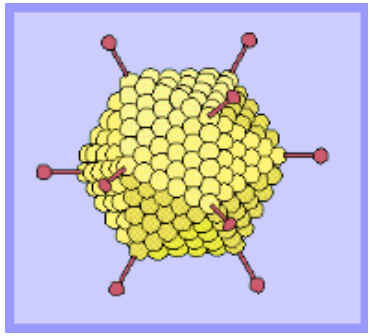

- Positive-strand RNA genomes can act as messenger RNAs (mRNAs), are able to be immediately translated into proteins
- Negative-strand RNA genomes have to be transcribed first
- Negative-strand RNA
  - Some viral polymerase proteins associated with the genome inside the virion
- Positive-strand or positive-sense RNA => Special characteristics
  - A cap at the 5’ terminal end
  - A poly-A Sequence at the 3’ terminal end
  - IRES *internal ribosome entry site…*

# capsids

# Viral capsid



- Three roles :
  - Protect the genome from the environment
  - For naked viruses, involved in the attachment of the virus the host cell
  - For DNA viruses and Retroviruses, transport of the viral genome inside the cell
- Resistant and very stable structure
- Limited viral genome coding capacity
- ➡ Capsid is formed by polymerization of one or a small number of proteins
- Principe of self-assembly
- Two main shapes
  - Tubular capsid with helical symmetry
  - Icosahedral capsid with cubic symmetry



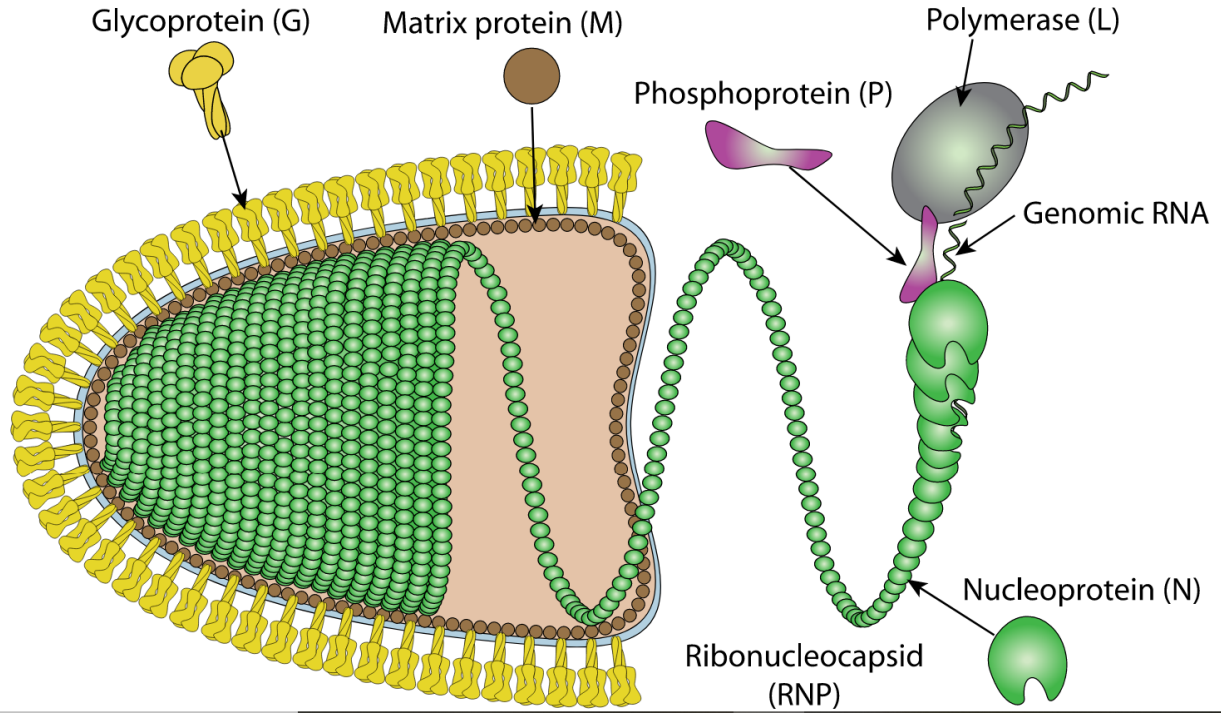
- Nature of the capsid constitutes a criterion for the virus classification

# Helical capsids structure

- Animal viruses with helical capsid are always enveloped
- A flexible nucleocapsid folded inside the viral envelope
- Most of them are negative-sense RNA virus

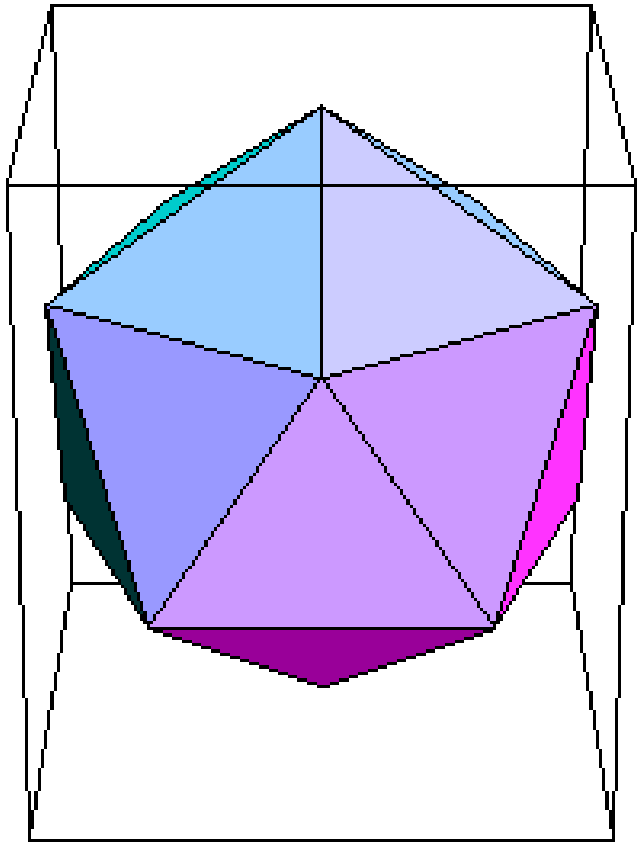
➡ Some viral proteins which function as a RNA polymerase have to be inside the capsid

- +RNA viruses
  - Coronavirus
- -RNA viruses
  - Rabies Virus
  - Mumps Virus
  - Measles Virus
  - Influenza Virus




# Icosahedral capsids with cubic symmetry

An icosahedron is a geometric shape with 20 sides, each composed of an equilateral triangle, 12 vertices and 30 edges

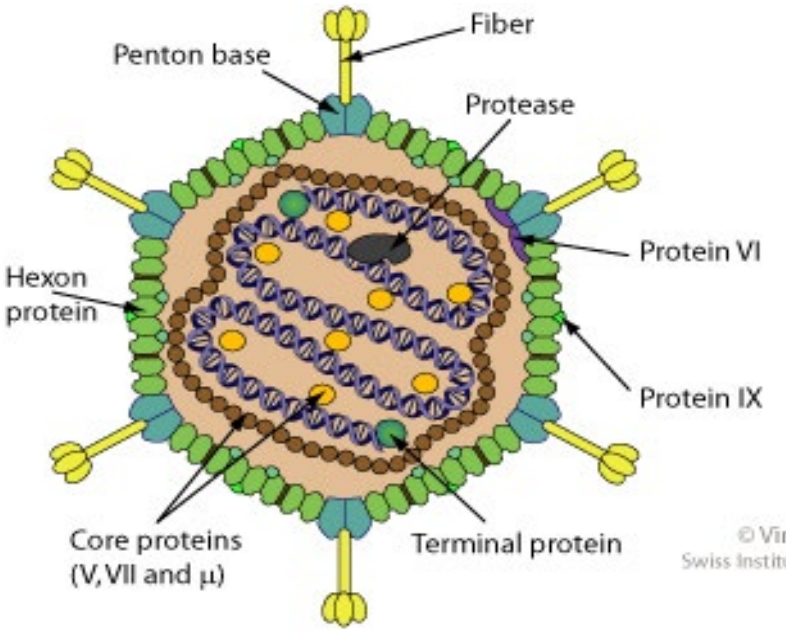


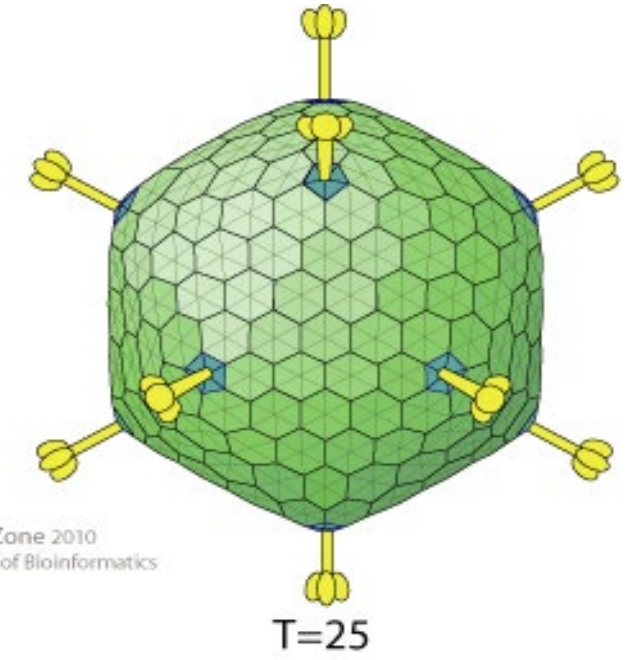
- Capsomeres = morphological units
  - Pentons (containing 5 structural units)
  - Hexons (containing 6 structural units)
- Every icosahedral virus has 12 pentons and a variable number of hexons

# Icosahedral capsids with cubic symmetry

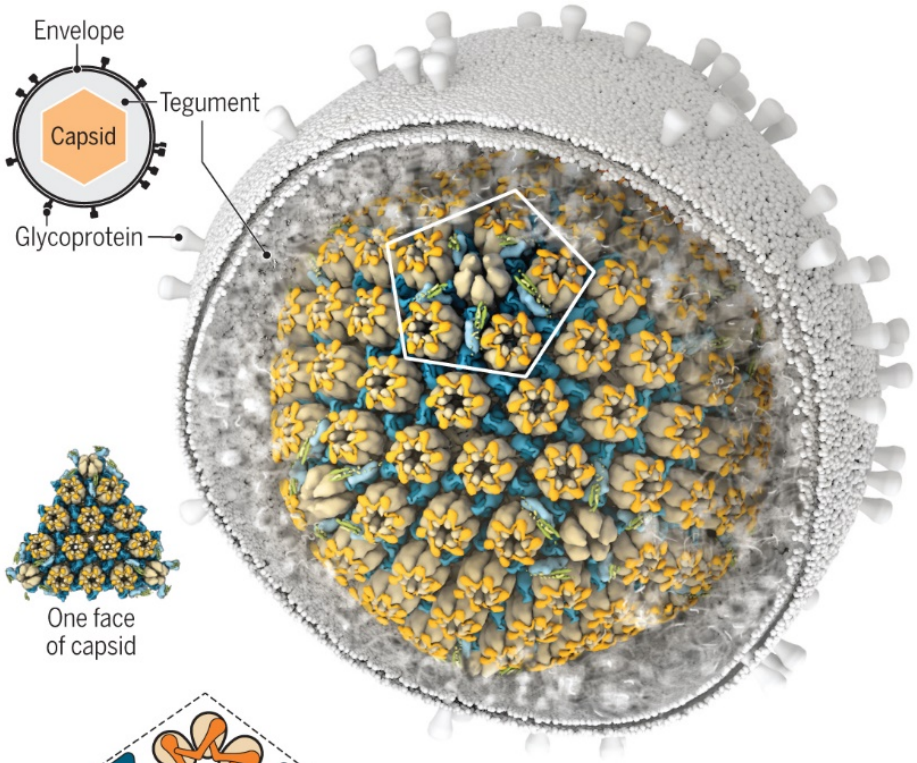
Examples

## Adenovirus




## Herpesvirus




**Local structure**
Pentons are surrounded by hexons and are glued together by triplexes. Complexes of UL17, UL25, and UL36C decorate the pentons.

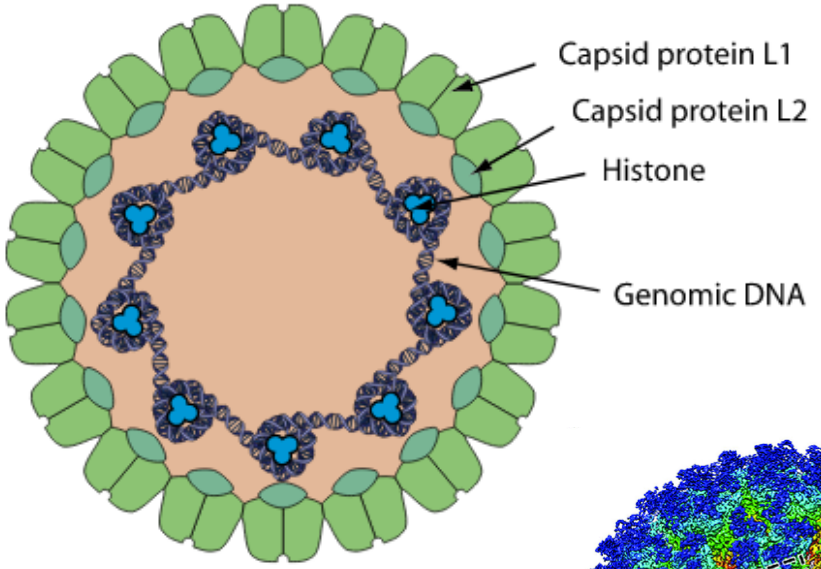
VP5 · VP23 · UL17, UL25, UL36C
VP26 · VP19C · Tegument proteins

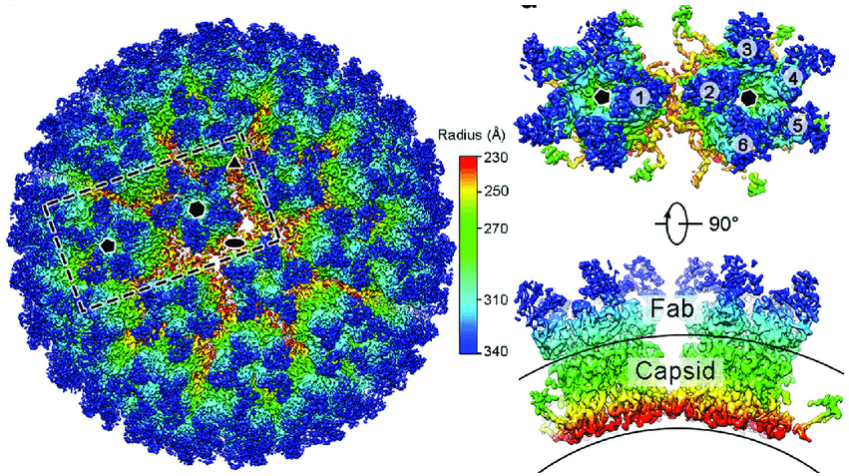
Two types of capsomeres
Hexons
Pentons

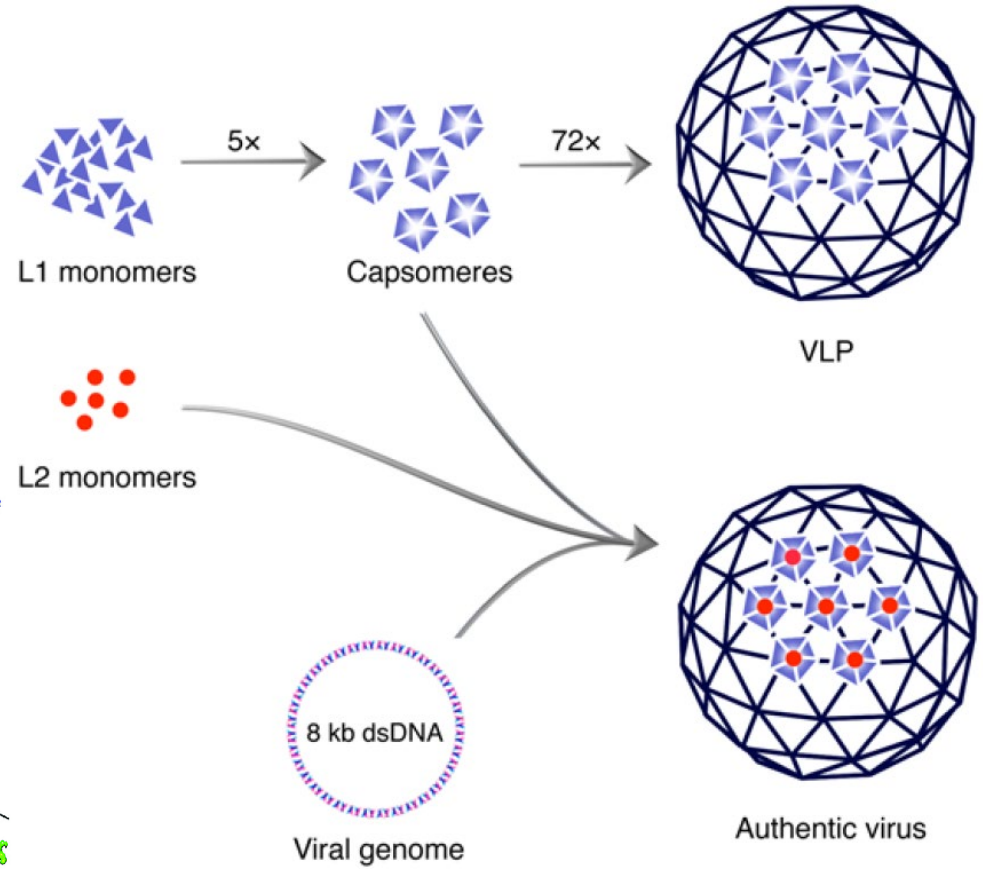
# Icosahedral capsids with cubic symmetry

Examples

Papillomavirus



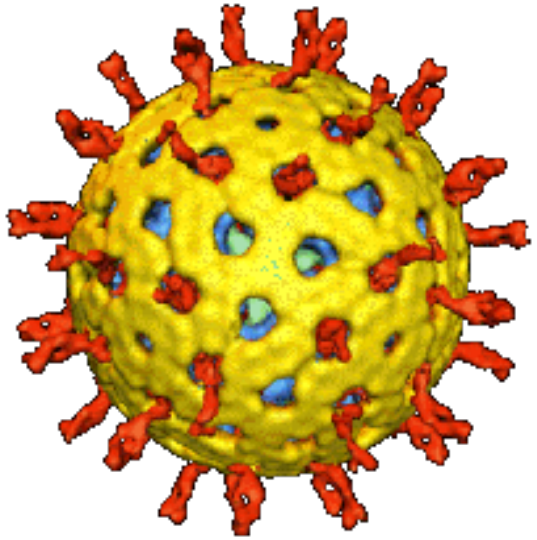



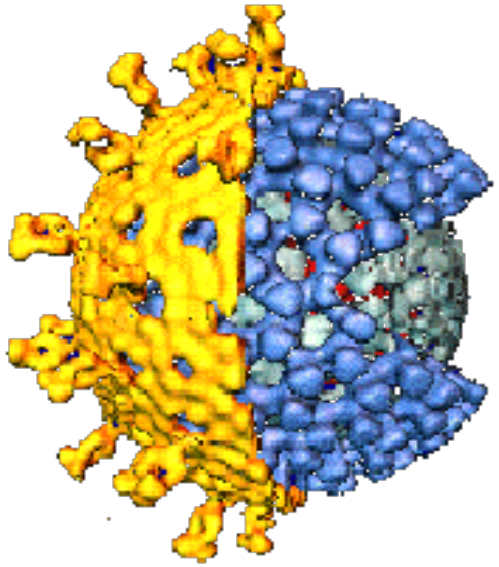

Two proteins form the capsid : L1 and L2

Viralzone
Lowy, JCI, 2016
Huang et al, Vaccines, 2020

# Sedoreoviridae

Virus with a triple capsid (double icosahedral capsid)





Rotavirus

- VP2
- VP6
- VP7
- VP4

- VP2
- VP6
- VP7
- ARN et Protéines Virales

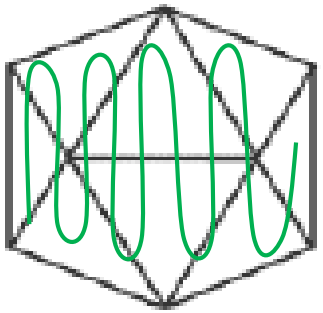
From Prasad et coll. 1994. Curr. Top. Microbiol. Immunol. 185: 9-29

# The viral envelope

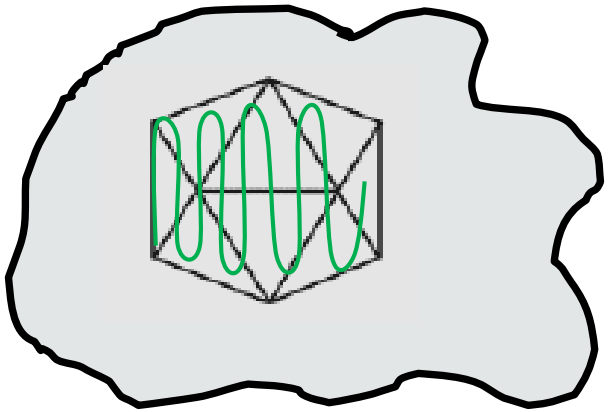
- Some but not all viruses have an envelope surrounding the capsid
- All helical animal viruses are enveloped
- Complex composition with lipids, glycoproteins
  - Due to their dual viral and cellular origin
- Very sensitive to physico-chemical actions, thermo-sensitive
- More susceptible to environmental conditions

➡ Consequences for epidemiology and diagnosis

Naked virus

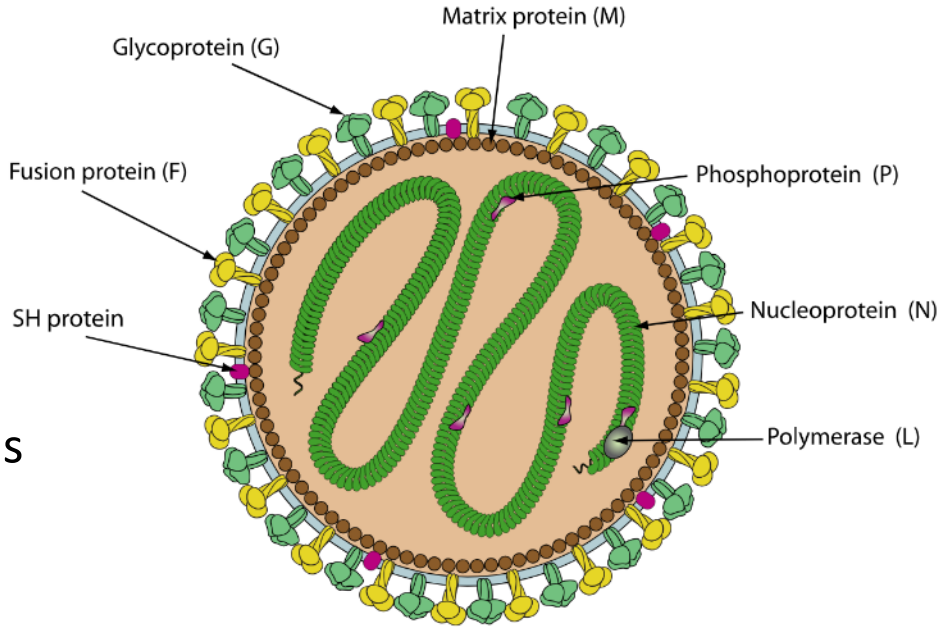


Enveloped virus

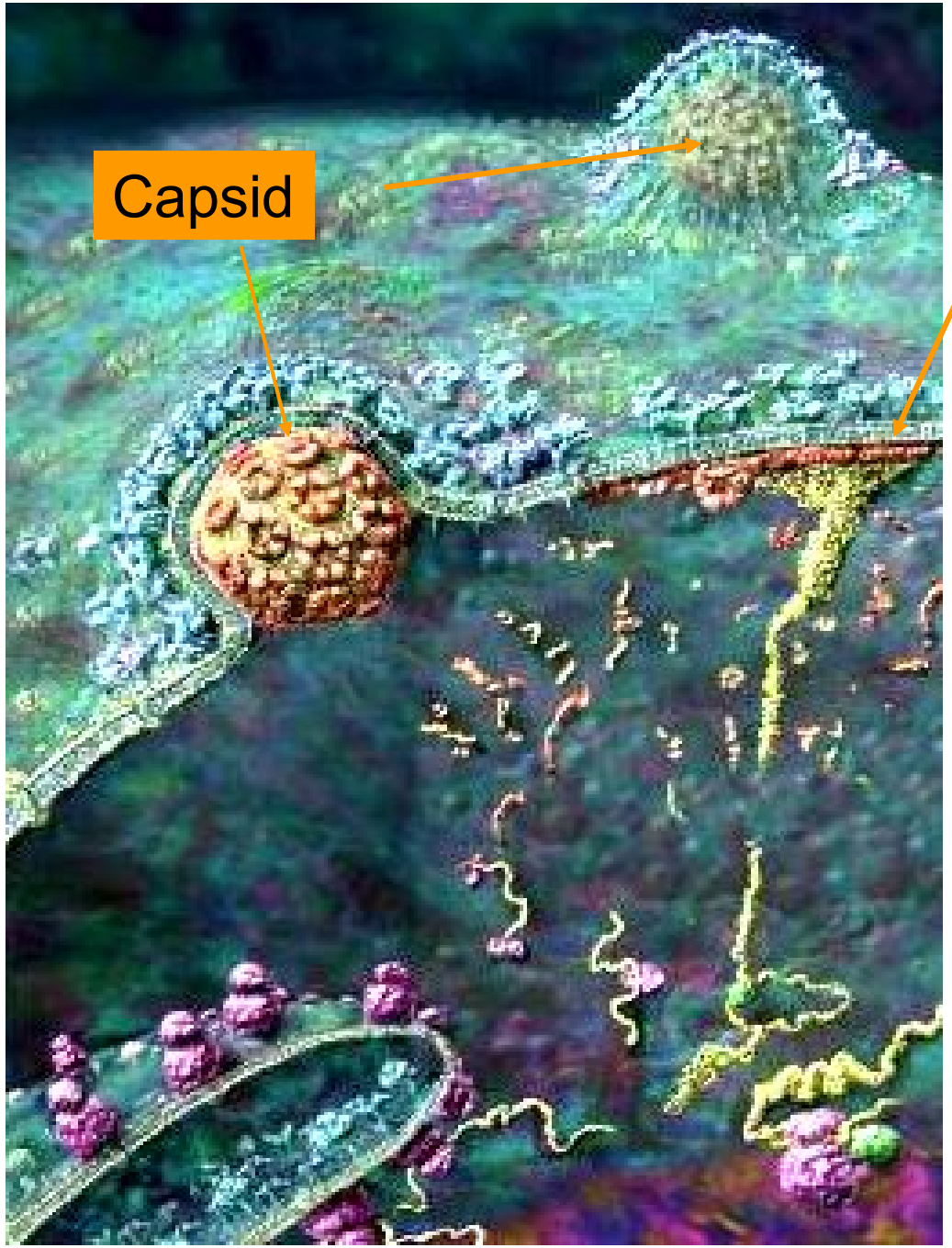


# The viral envelope

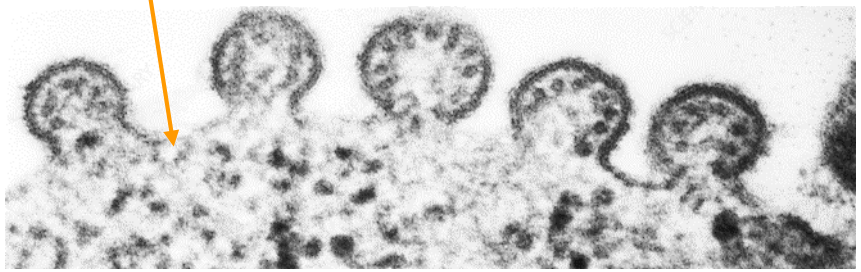
- Enveloped viruses acquire their envelope by budding through a cellular membrane
  - Either through the plasma membrane (influenza virus, HIV, rabies virus)
  - or through intra-cytoplasmic membranes, Golgi apparatus, endoplasmic reticulum, intracellular vesicles…
  - Transient envelopment of Herpes virus in the nuclear membrane
- Viral glycoproteins
  - Specific biological activities
  - Projections perpendicular to the surface : spikes




Respiratory Syncytial Virus
*Pneumoviridae*



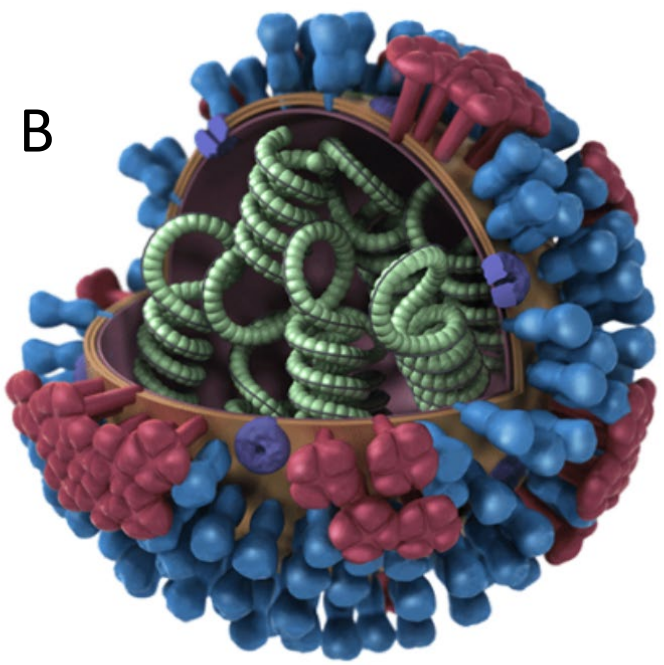

Plasma membrane

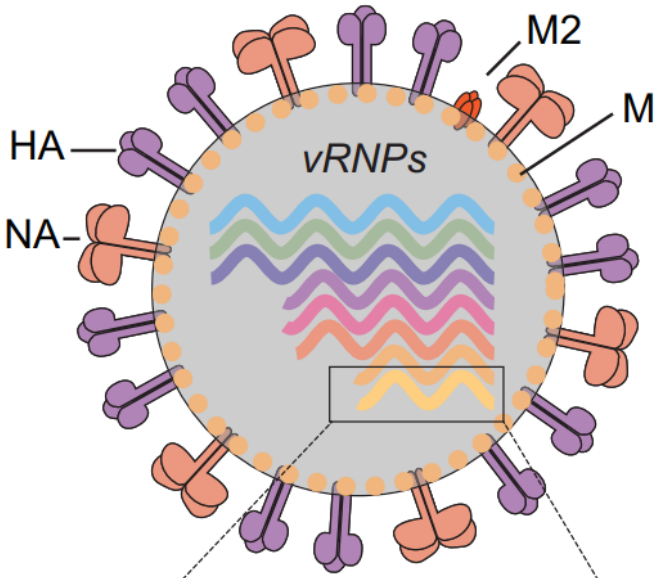


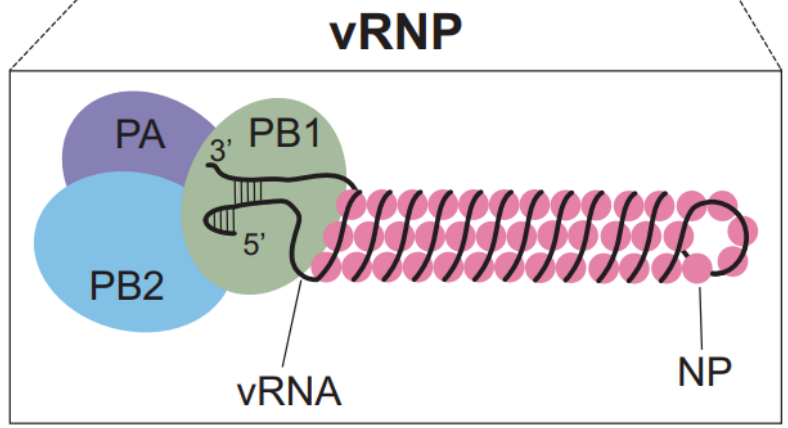
Formation of the viral envelope by budding at the plasma membrane

# Influenza virus

- Envelope + spikes hemagglutinin and neuraminidase
- Under the envelope the matrix (M1)
- Capsid with helical symmetry
- Single-stranded negative-sense linear RNA associated with polymerase PA/PB1/PB2
- Segmented genome
- => 8 fragments for A and B

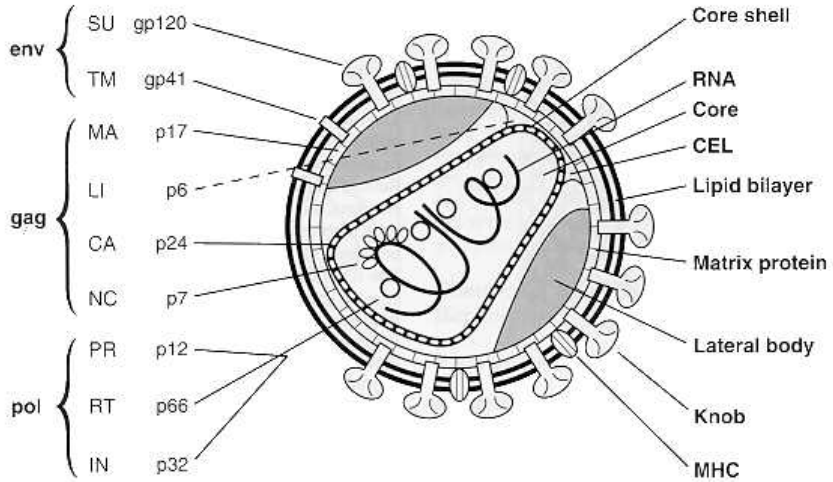


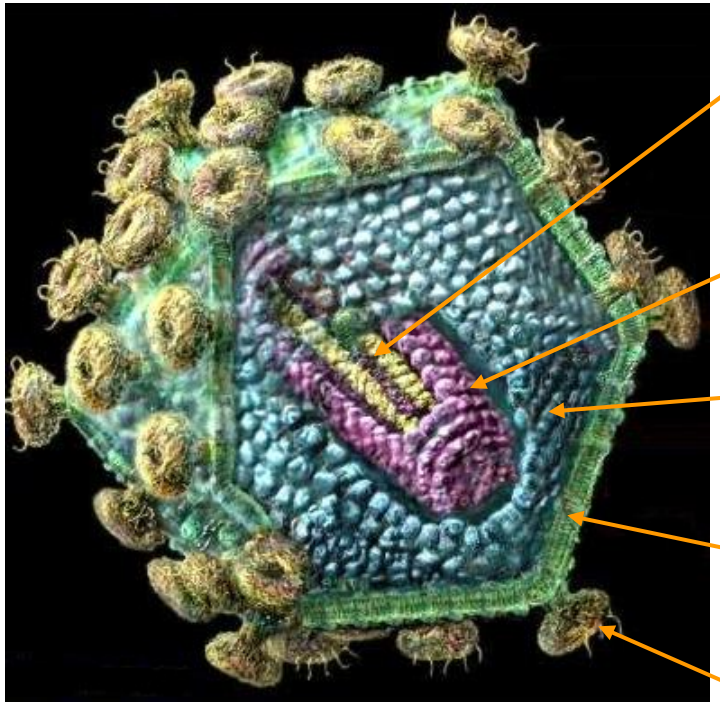

Dou et al, Frontiers Immunol, 2018

Lancaster University



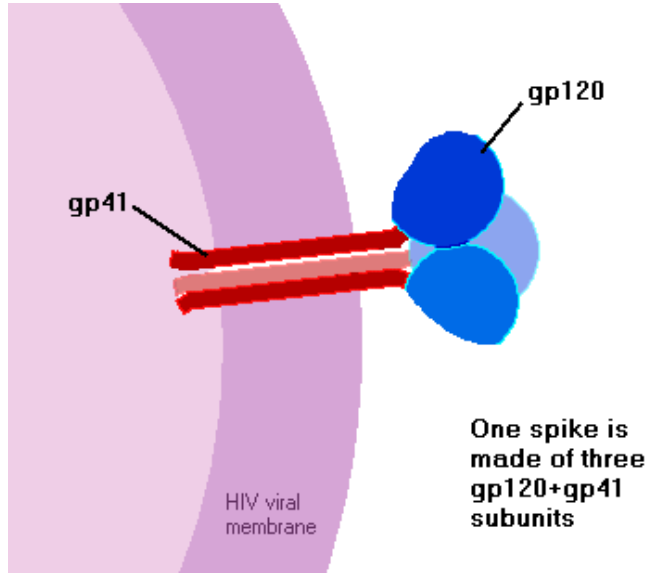

# HIV-1

human immunodeficiency virus type 1

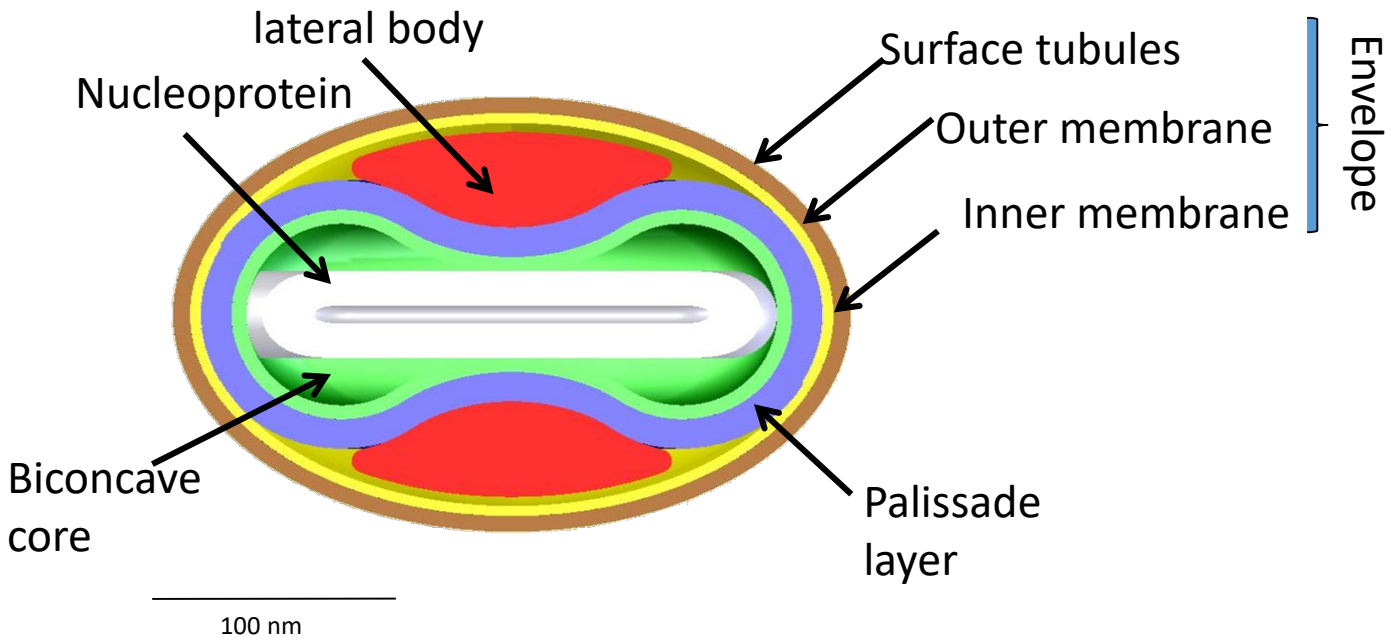


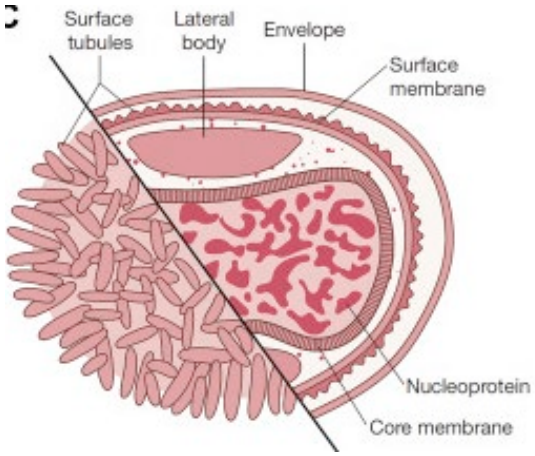
- **Diploid RNA genome**
- **Cone-shaped Capsid**
- **Matrix**
- **Envelope**
- **Envelope (Env) spike**

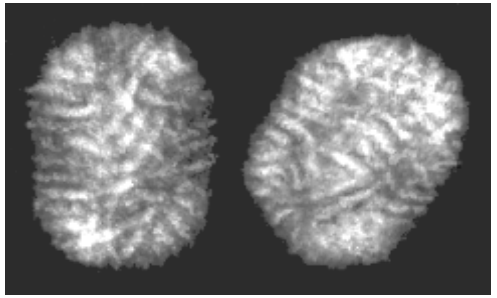
HIV1 spike : a trimer of gp120 and gp41



# Poxvirus



Molluscum contagiosum

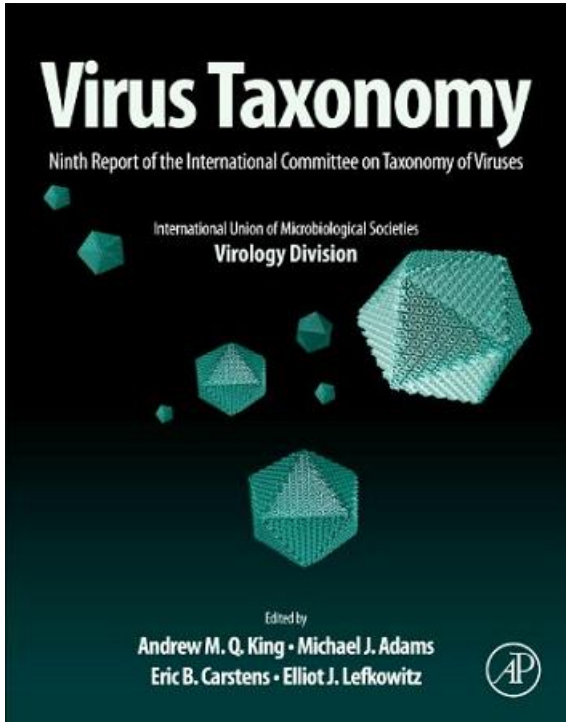
- Big size
- Brick-shaped
- Complexe internal structure
- Lateral bodies
- A complex envelope


Orthopoxvirus : Mpox

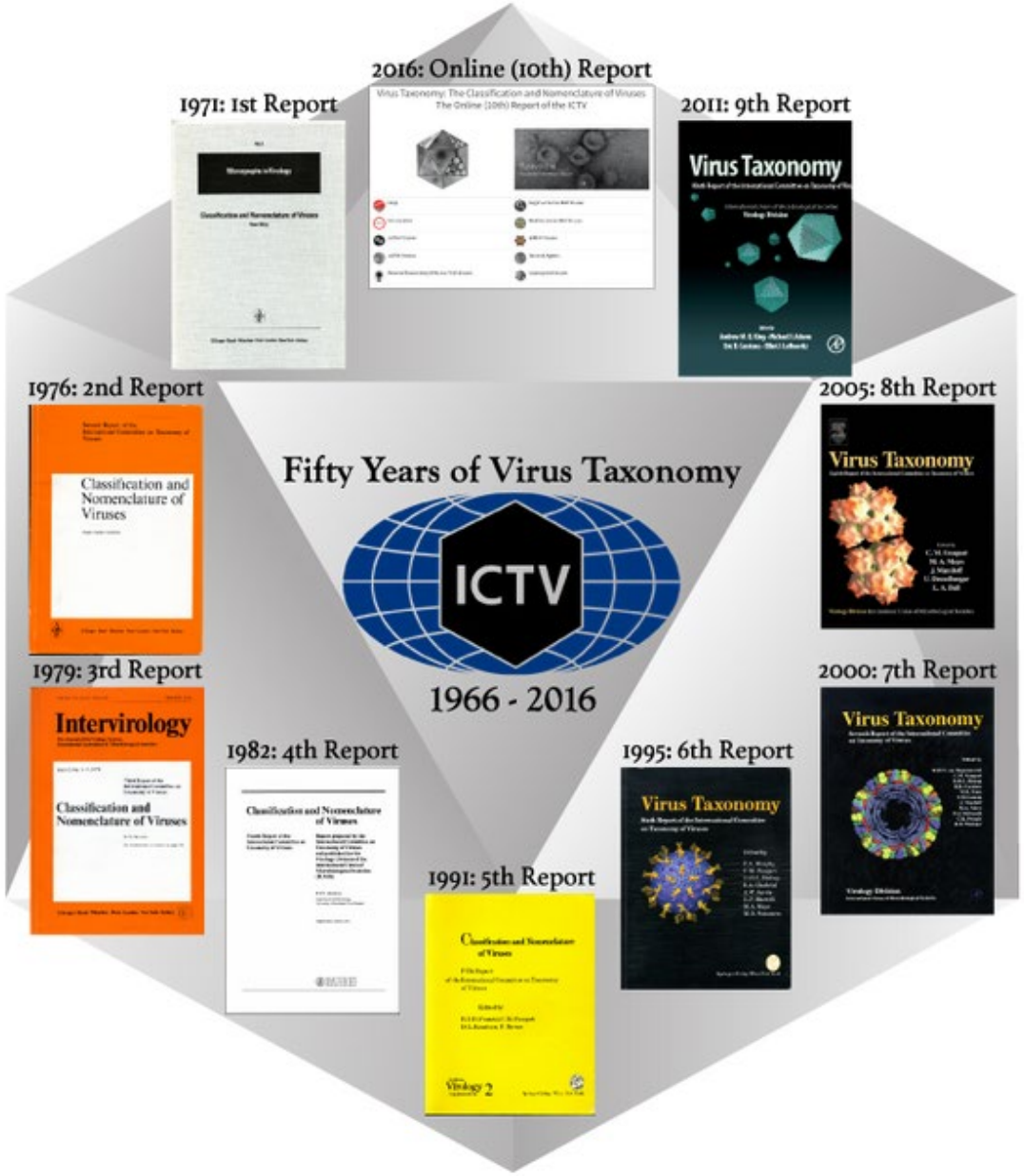
# VIRUS CLASSIFICATION

- Classification determined by the International Committee on Taxonomy of Viruses (ICTV)
- Helps to define the evolutionary relationships between viruses and understand the consequences of virus diversity
- website ictvonline.org
- Based on

  1. **type and organization of the viral genome**
  2. **viral replication strategy**
  3. virion **structure**
     (virion size, capsid shape, envelope or not)

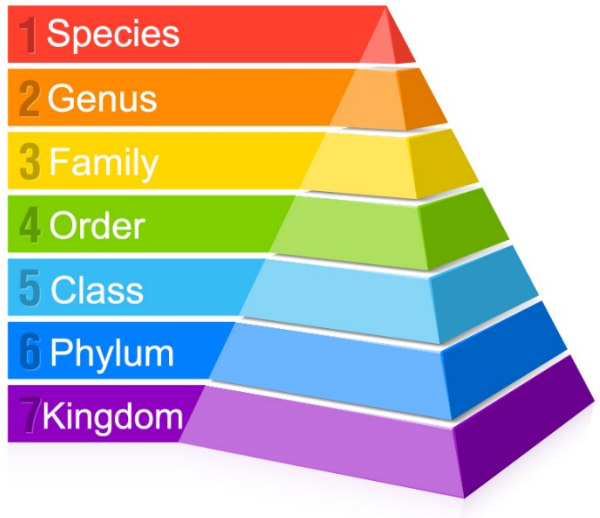


# VIRUS CLASSIFICATION




- **Realm:** *Duplodnaviria*
  - **Kingdom:** *Heunggongvirae* Realm: *Duplodnaviria*
    - **Phylum:** *Peploviricota* Kingdom: *Heunggongvirae*
      - **Class:** *Herviviricetes* Phylum: *Peploviricota*
        - **Order:** *Herpesvirales* Class: *Herviviricetes*
          - + **Family:** *Alloherpesviridae* Order: *Herpesvirales*
          - + **Family:** *Malacoherpesviridae* Order: *Herpesvirales*
          - − **Family:** *Orthoherpesviridae* Order: *Herpesvirales*
            - − **Subfamily:** *Alphaherpesvirinae* Family: *Orthoherpesviridae*
              - + **Genus:** *Iltovirus* Subfamily: *Alphaherpesvirinae*
              - + **Genus:** *Mardivirus* Subfamily: *Alphaherpesvirinae*
              - + **Genus:** *Scutavirus* Subfamily: *Alphaherpesvirinae*
              - + **Genus:** *Simplexvirus* Subfamily: *Alphaherpesvirinae*
              - − **Genus:** *Varicellovirus* Subfamily: *Alphaherpesvirinae*
                - **Species:** *Varicellovirus humanalpha3* Genus: *Varicellovirus*

3 families, 3 subfamilies, 23 genera, 133 species



# Classification

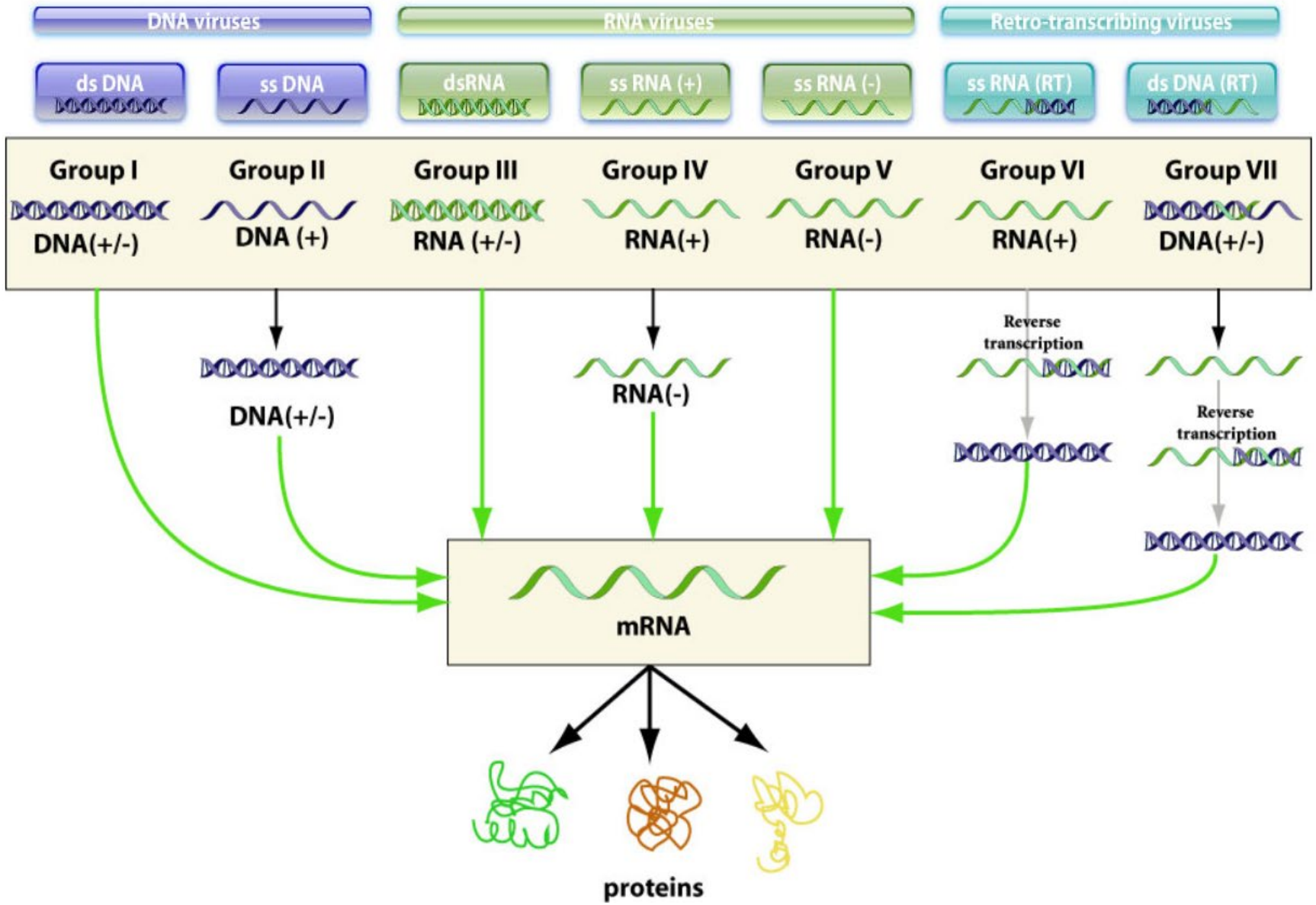
- the same taxonomical hierarchy than other organisms
- Viruses are classified using domain, kingdom, phylum, class, order, family, genus, and species **taxa** (singular: **taxon**)
- Open access up-date available online

| Taxon | Suffix | Examples |
|---|---|---|
| order | virales | Mononegavirales |
| family | viridae | *Paramyxoviridae* |
| Sub-family | virinae | *Paramyxovirinae* |
| genus | virus | Morbilivirus |
| species | (virus name) | Measles Virus |

constantly evolving

In 2024, 81 orders, 314 families, 200 subfamilies, 3522 genus and 14690 species

# Baltimore classification



# DNA viruses

## ds DNA

### Human

- Adenoviridae
- Herpesviridae
- Papillomaviridae
- Polyomaviridae
- Poxviridae

## ss DNA

### Vertebrate

- Circoviridae
- Parvoviridae

### Human

- Anelloviridae

# RNA viruses

## dsRNA

### Human

- Reoviridae

## ss RNA (+)

### Human

- Astroviridae
- Caliciviridae
- Coronaviridae
- Flaviviridae
- Hepeviridae
- Picornaviridae
- Togaviridae

## ss RNA (-)

### Human

- Arenaviridae
- Filoviridae
- Hantaviridae
- Nairoviridae
- Orthomyxoviridae
- Paramyxoviridae
- Peribunyaviridae
- Phenuiviridae
- Rhabdoviridae

# Retro-transcribing viruses

## ss RNA (RT)

### Human

- Hepadnaviridae

## ds DNA (RT)

### Human

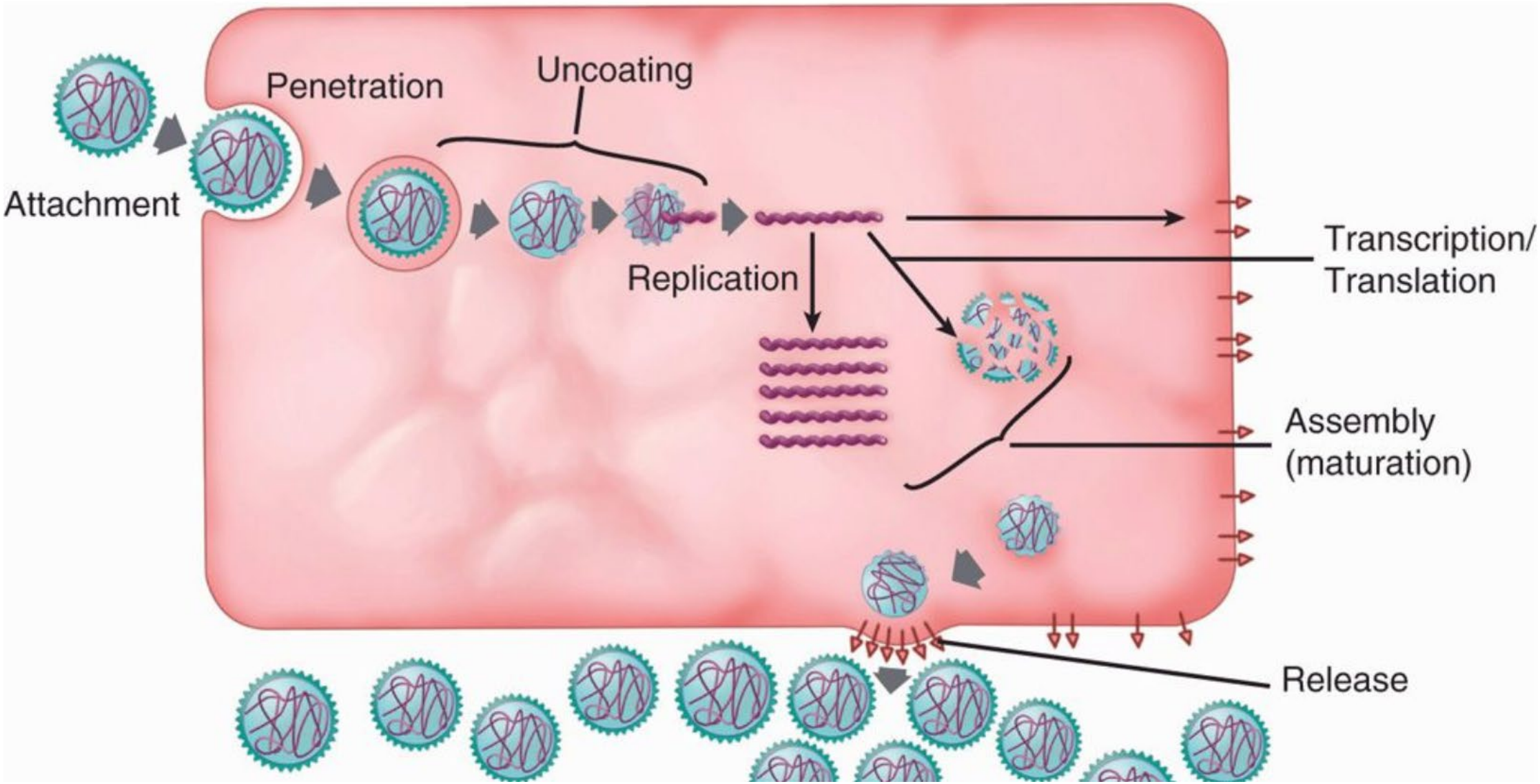
- Gammaretrovirus
- Deltaretrovirus
- Lentivirus
- Spumavirus

# MULTIPLICATION

# OF VIRUSES

# Different steps of the viral replication cycle

general scheme



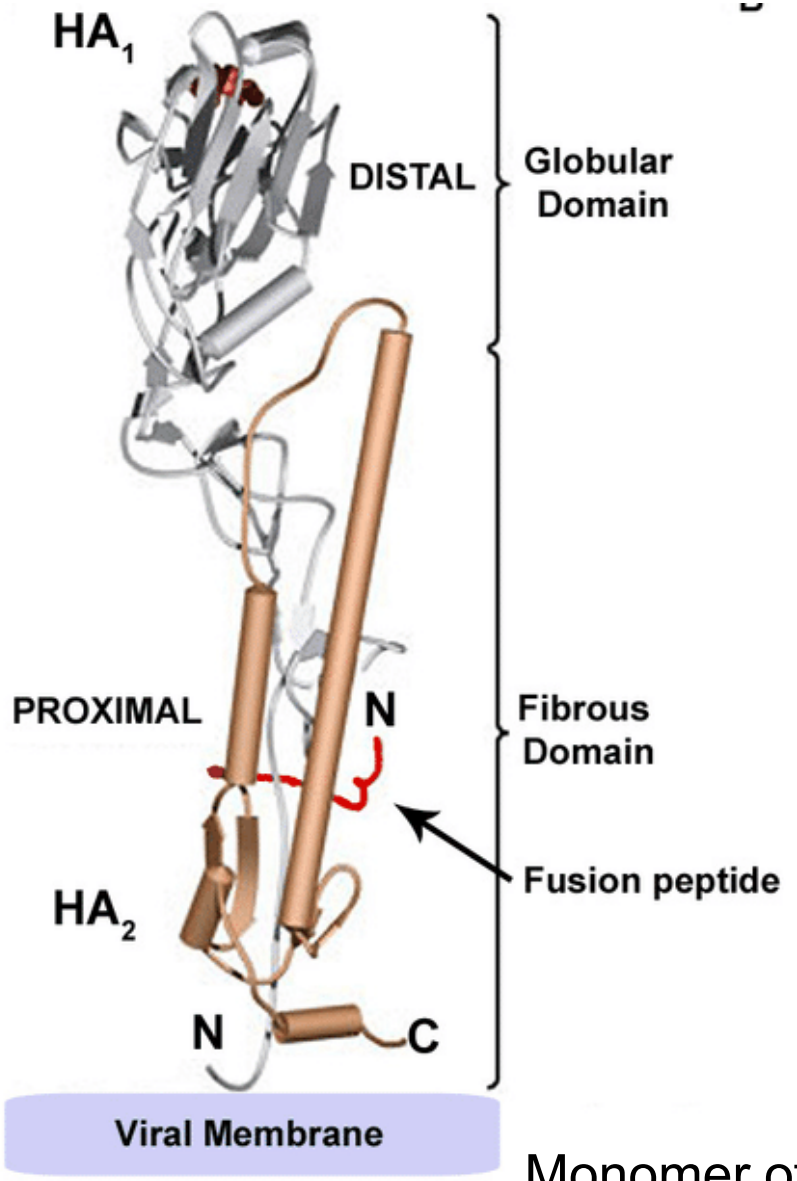
# Viral attachment

- First step of the infection, mandatory
- Interactions between viral glycoproteins, proteins and cellular receptors
- Viral proteins are called « ligands » or receptor-binding protein
- Integrity of ligand is essential for attachment of the virus (mutations, neutralizing antibodies)

## Viral ligands

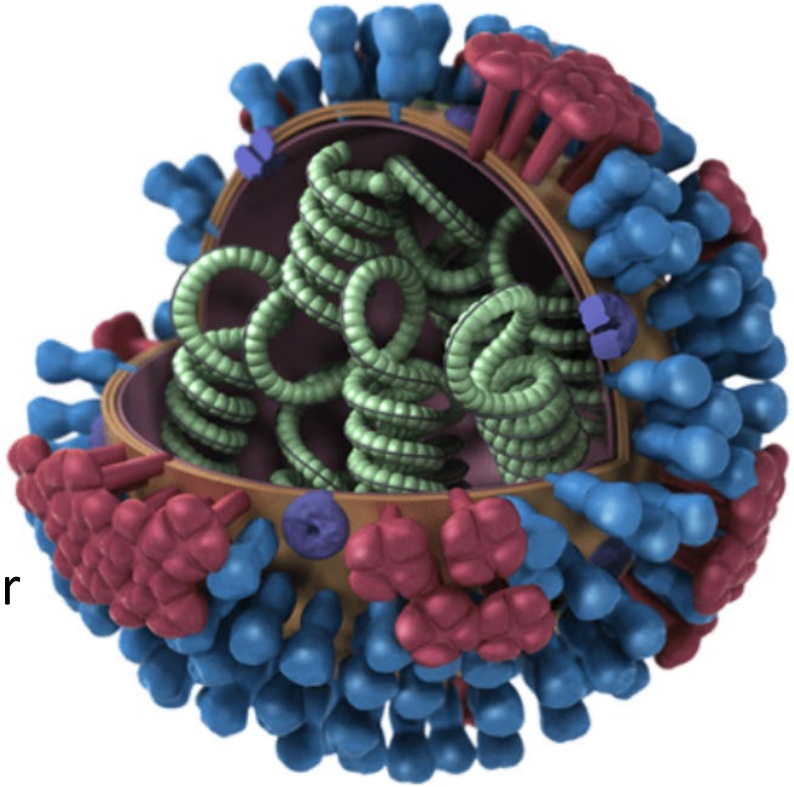
- Enveloped viruses : glycoproteins
- Naked viruses : proteins

# Ligands of enveloped virus




## Influenza virus

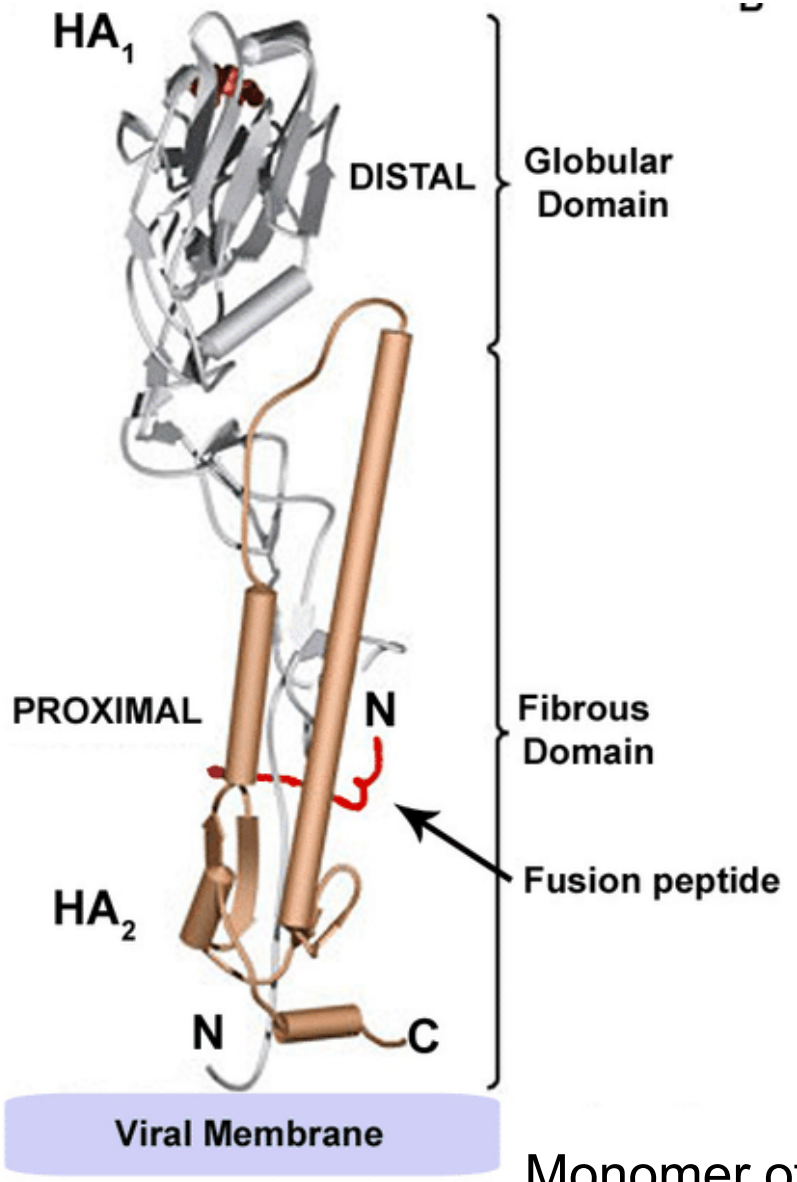
- Trimer
- composed of 2 subunits HA1 and HA2
- membrane-anchored HA2
- linked to HA1 by a disulfure bond
- HA1 contains the receptor site to sialic acid
- receptor site = conserved AA in different HA sub-types




Monomer of hemagglutinin

Lancaster University

# Ligands of enveloped virus

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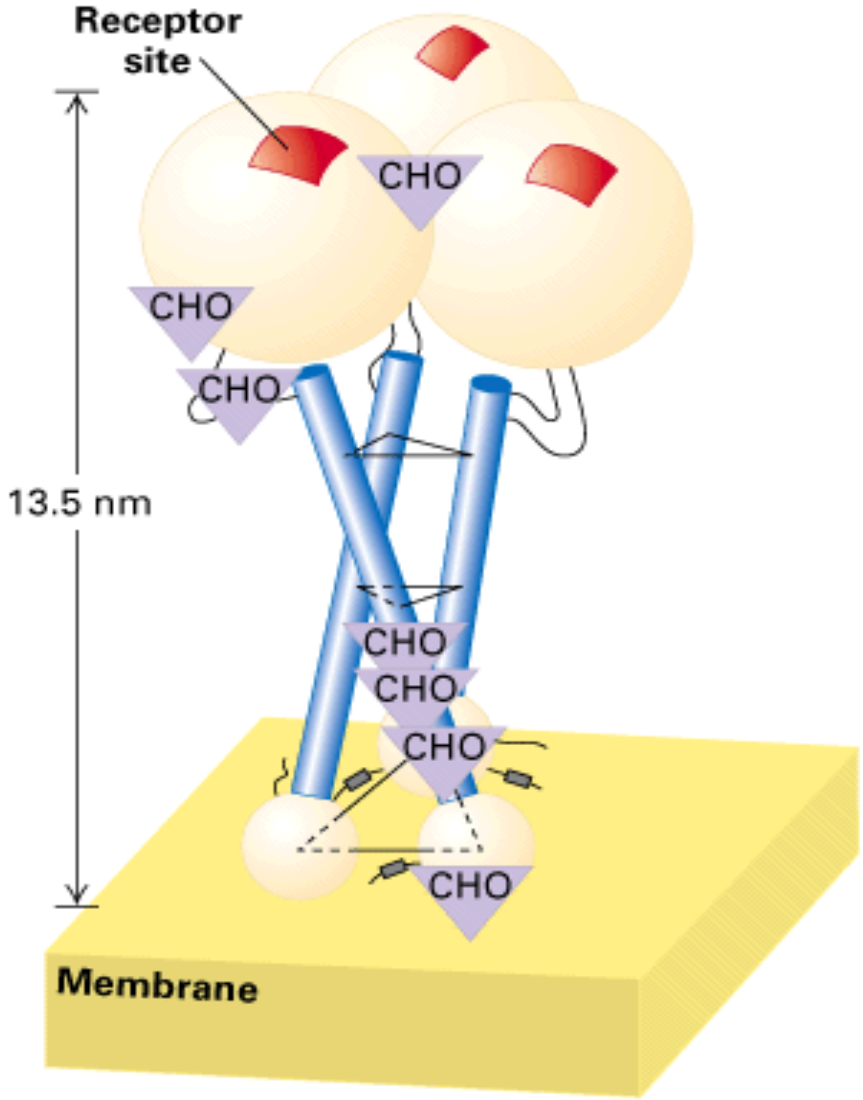
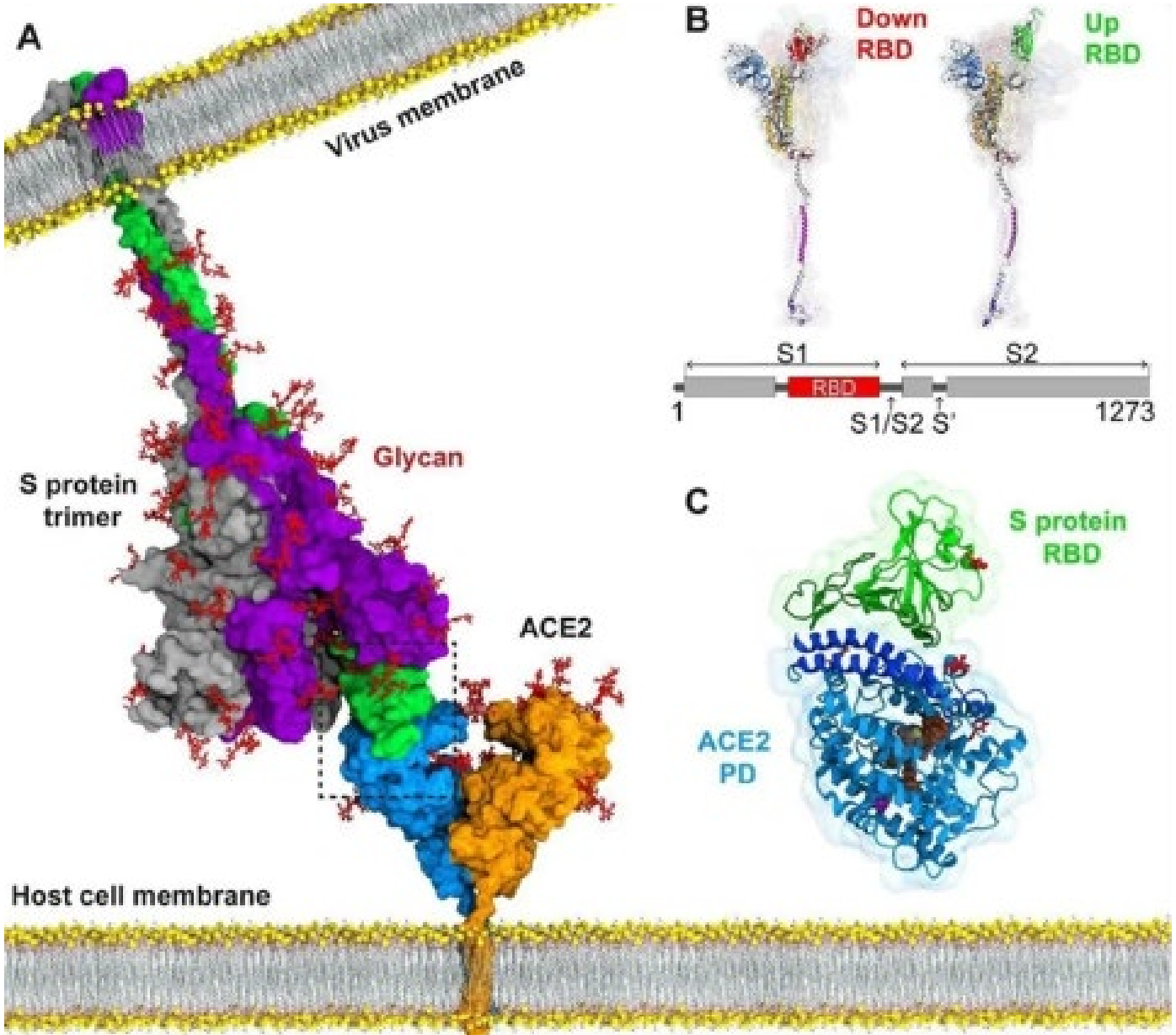



Monomer of hemagglutinin

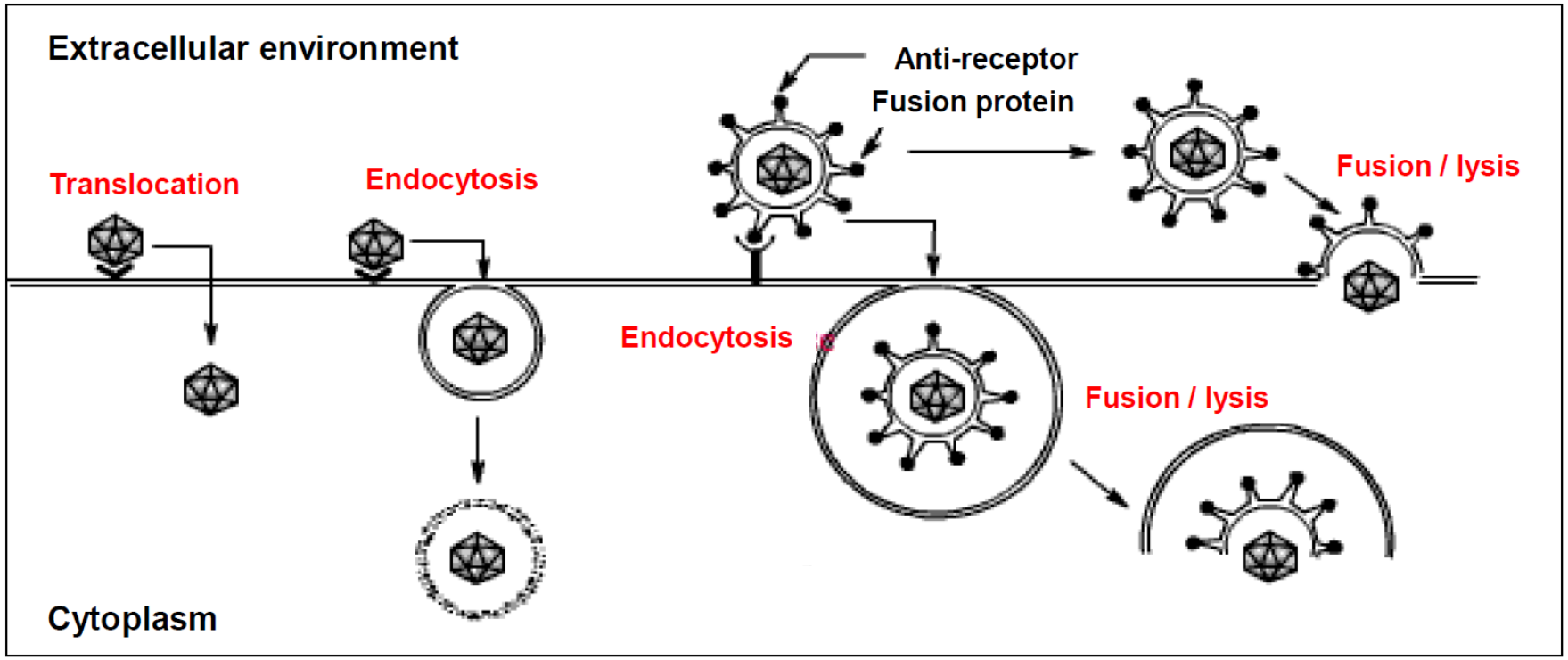
# Ligands of enveloped virus



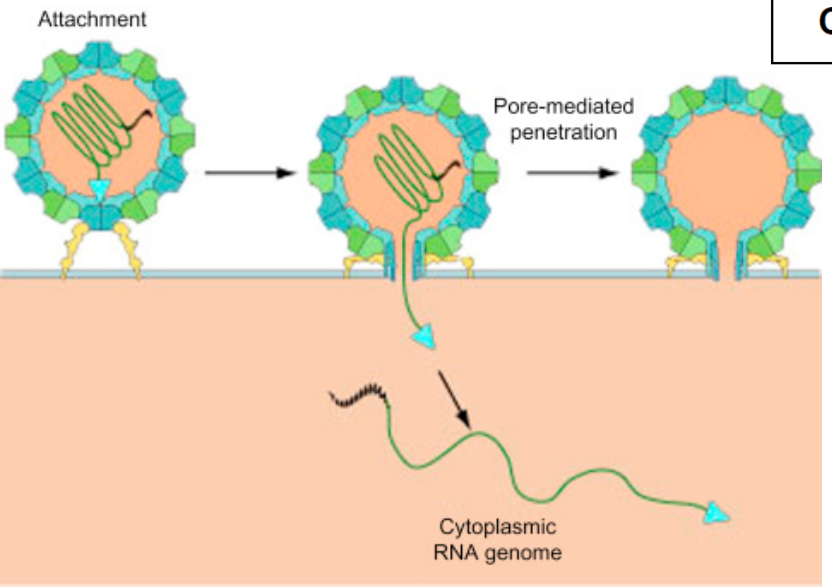

SARS-CoV-2

- Spike (S) Glycoprotein trimer of S (S1 and S2)
- binds on ACE2 (angiotensin I-converting enzyme-2)
- RBD = receptor binding domain
- Triggers the entry of the virus

# Penetration

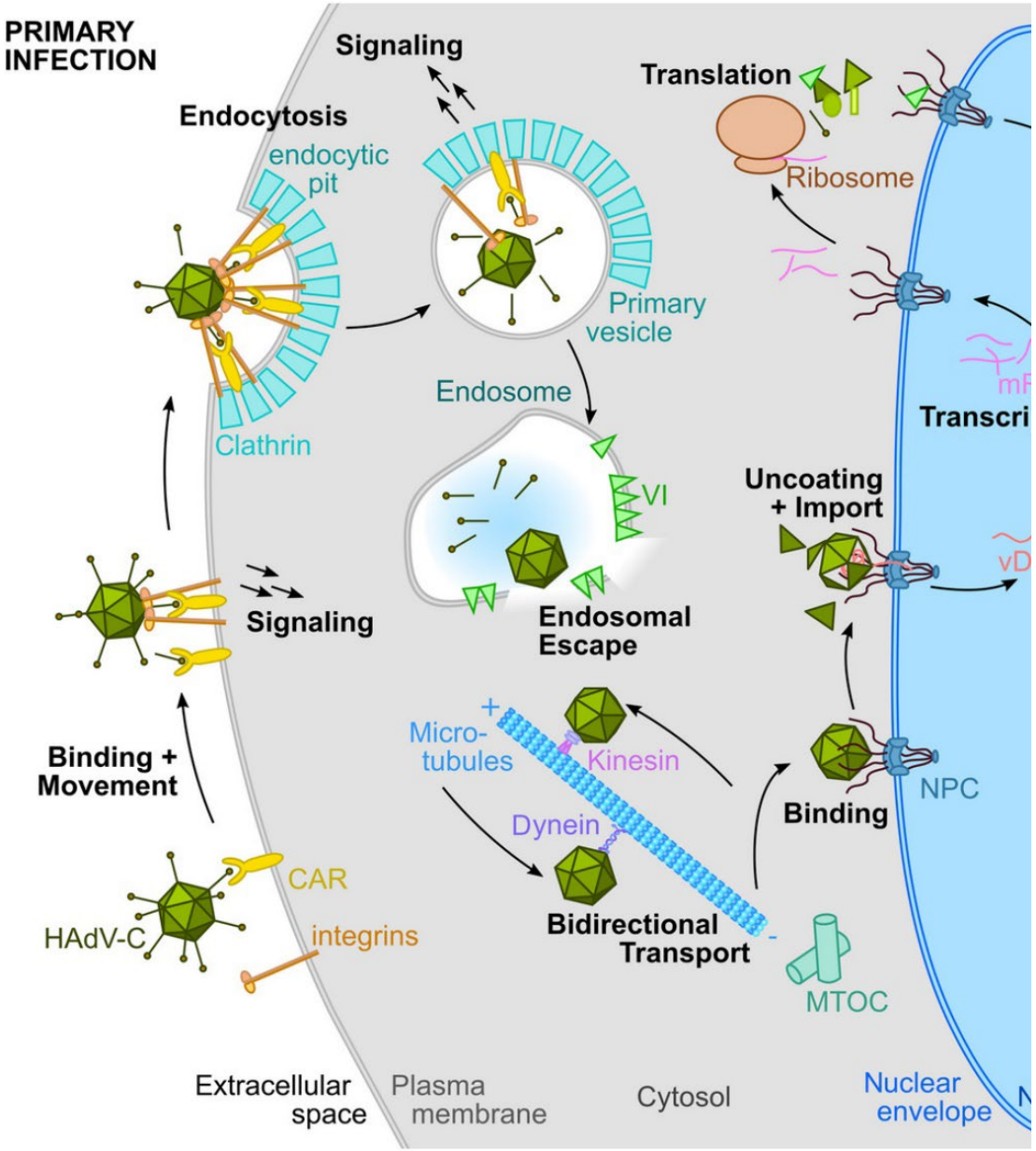



Translocation




- **Different entry strategies**
  - Endocytosis : naked or enveloped viruses
  - Fusion : only enveloped viruses
  - Translocation : poliovirus

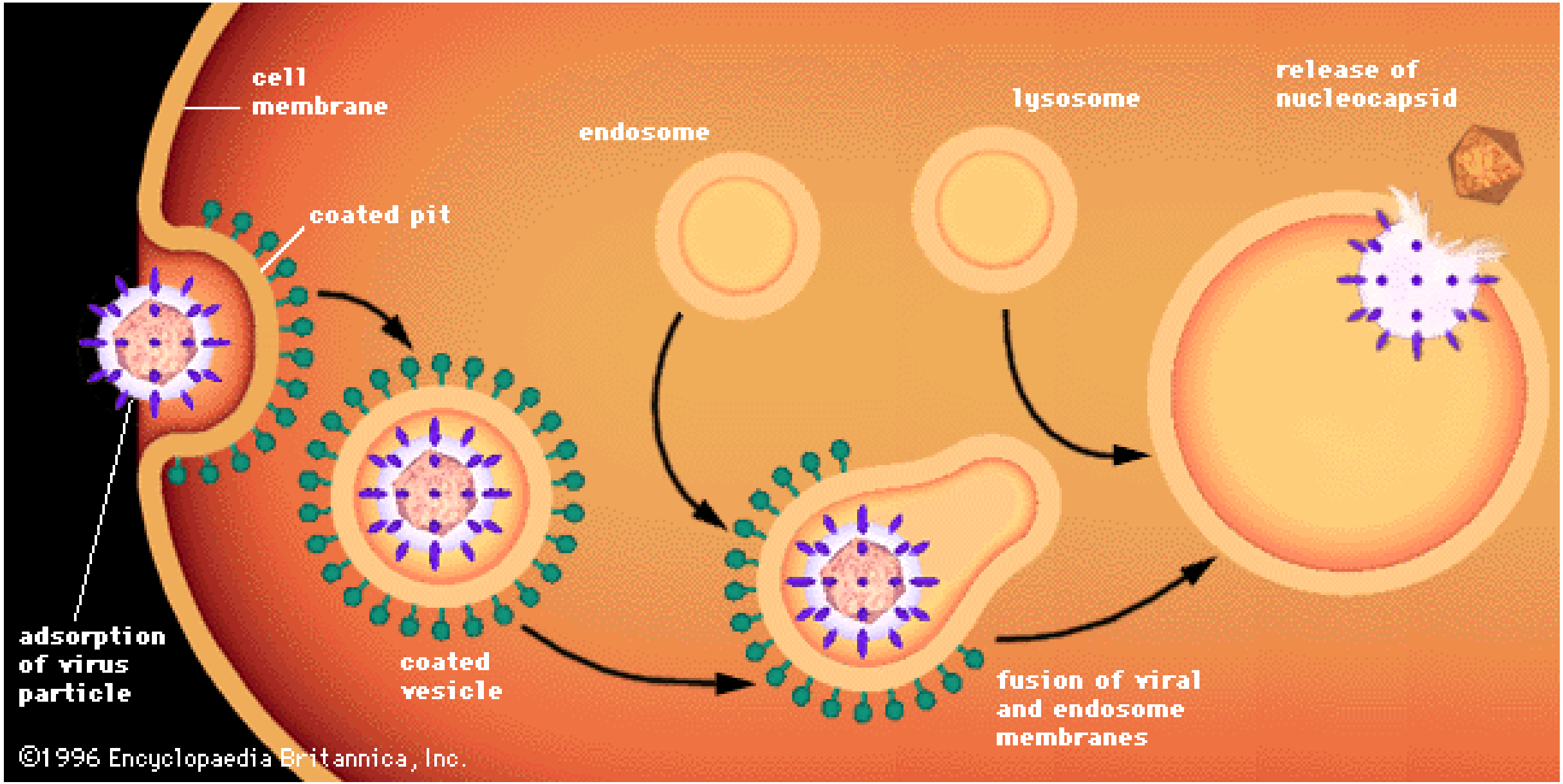
# Adenovirus enters into the cell by endocytosis




Virus attaches to **CAR** (Coxsackie adenovirus receptor) via fiber tip (terminal button)
Penton base interacts with integrins: triggers entry by endocytosis
**Acidification** of the endosome: partial degradation of the capsid and lysis of the endocytosis vesicle, releasing the capsid into the cytoplasm
**Decapsidation:** the capsid is delivered to the nucleus, and the genome penetrates the nuclear pores.

FEBS Letters, Volume: 594, Issue: 12, Pages: 1861-1878, First published: 30 May 2020, DOI: (10.1002/1873-3468.13848)

# Entry of an enveloped virus by endocytosis

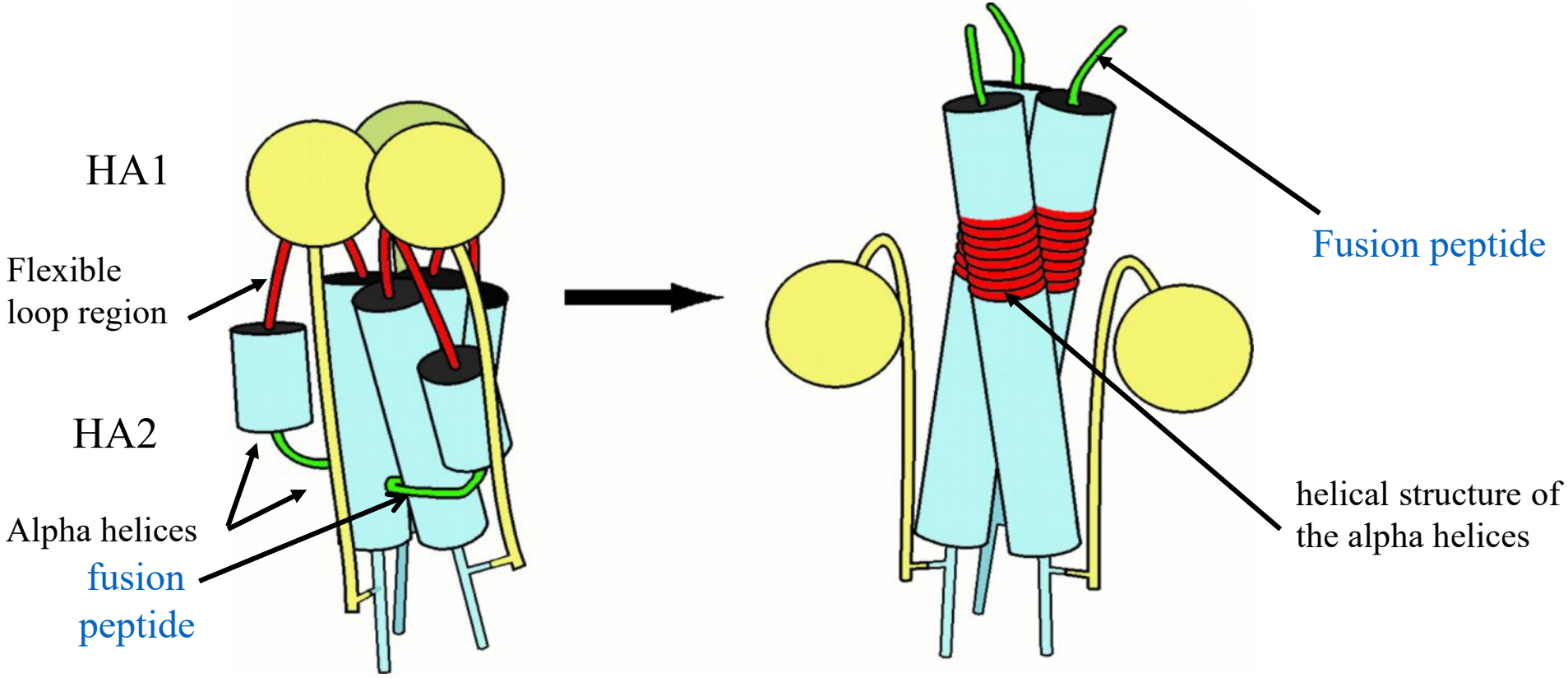



Myxovirus, Rhabdovirus, Bunyavirus, Togavirus, Flavivirus, Coronavirus

# Enveloped virus

## Influenza virus

- structural alterations at low pH
- hydrophobic fusion peptide released and projected
- Anchoring in the endosomal membrane



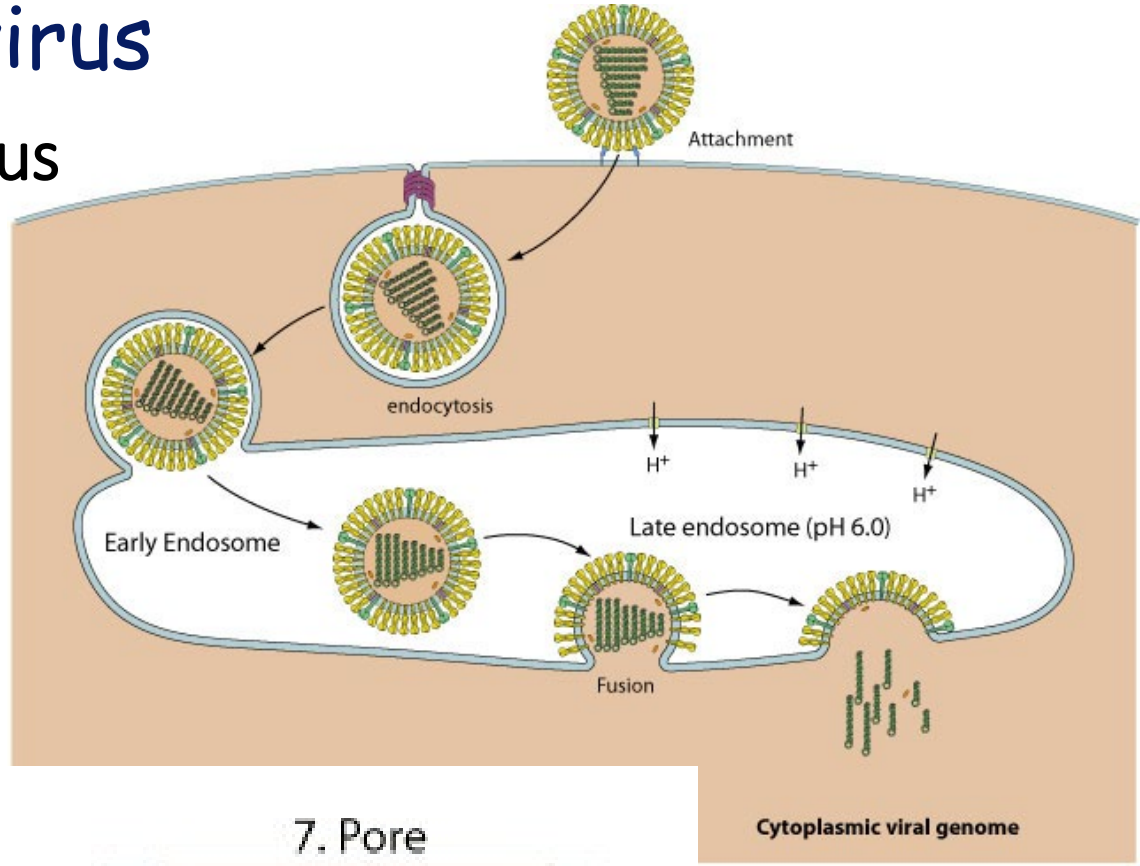

native form at neutral pH  post fusion structure at acid pH

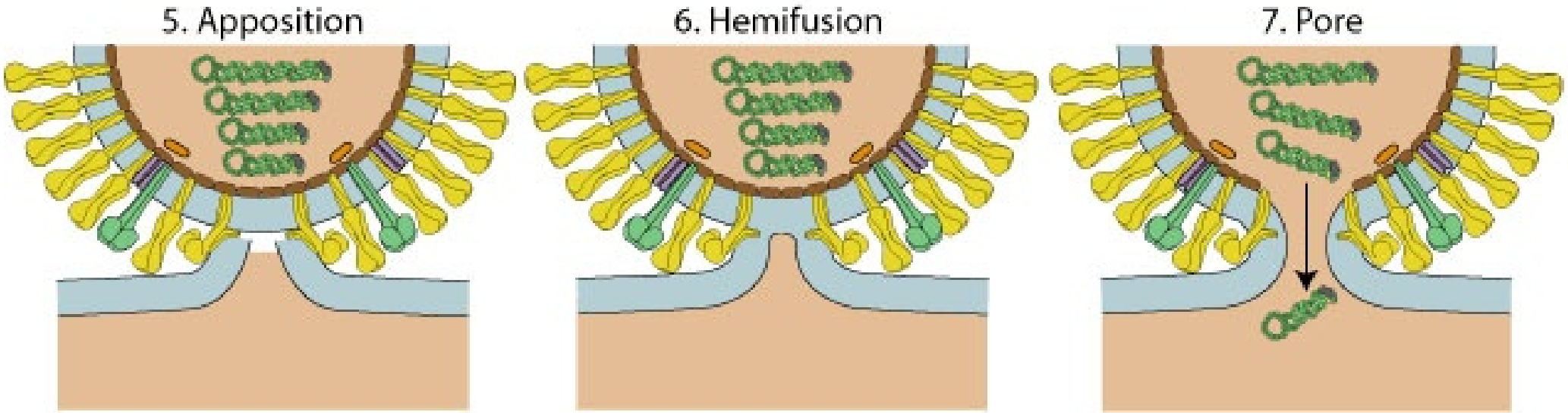
# Endocytosis

## Enveloped virus

### Influenza virus

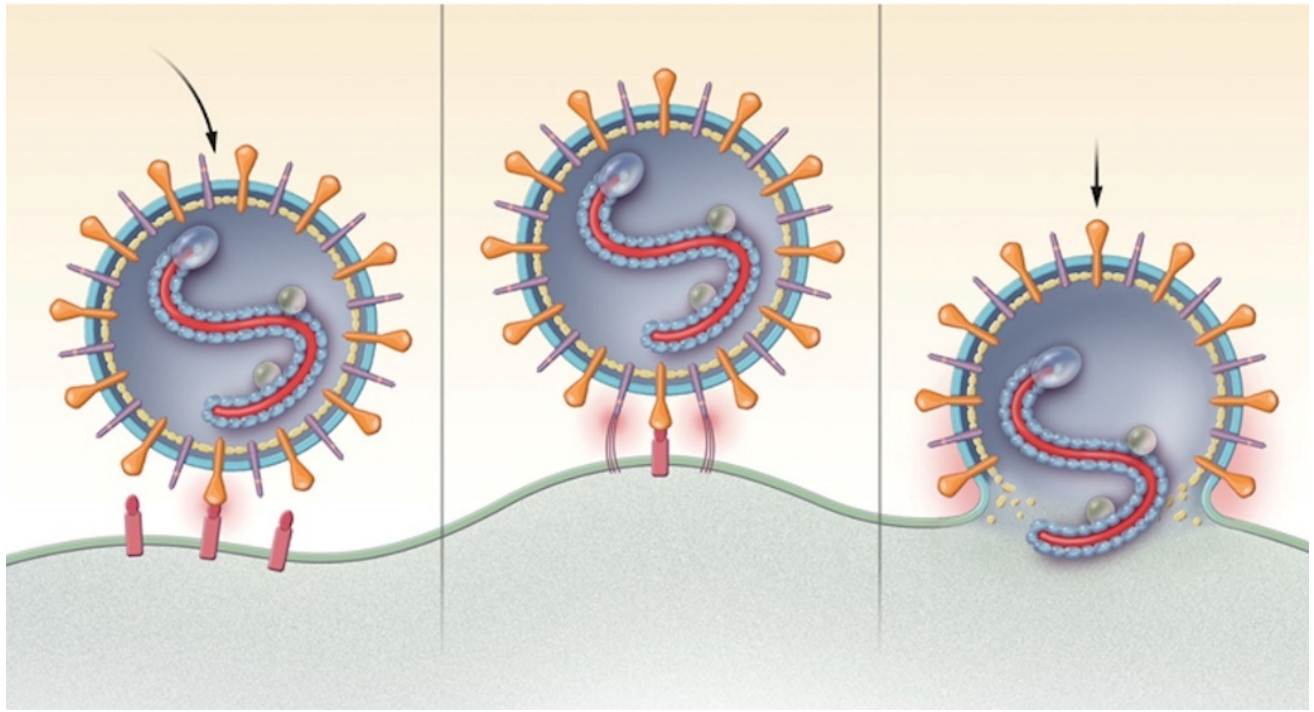
- structural alterations at low pH
- hydrophobic fusion peptide released and projected
- Anchoring in the endosomal membrane
- Hemifusion
- Formation of a pore



# Fusion

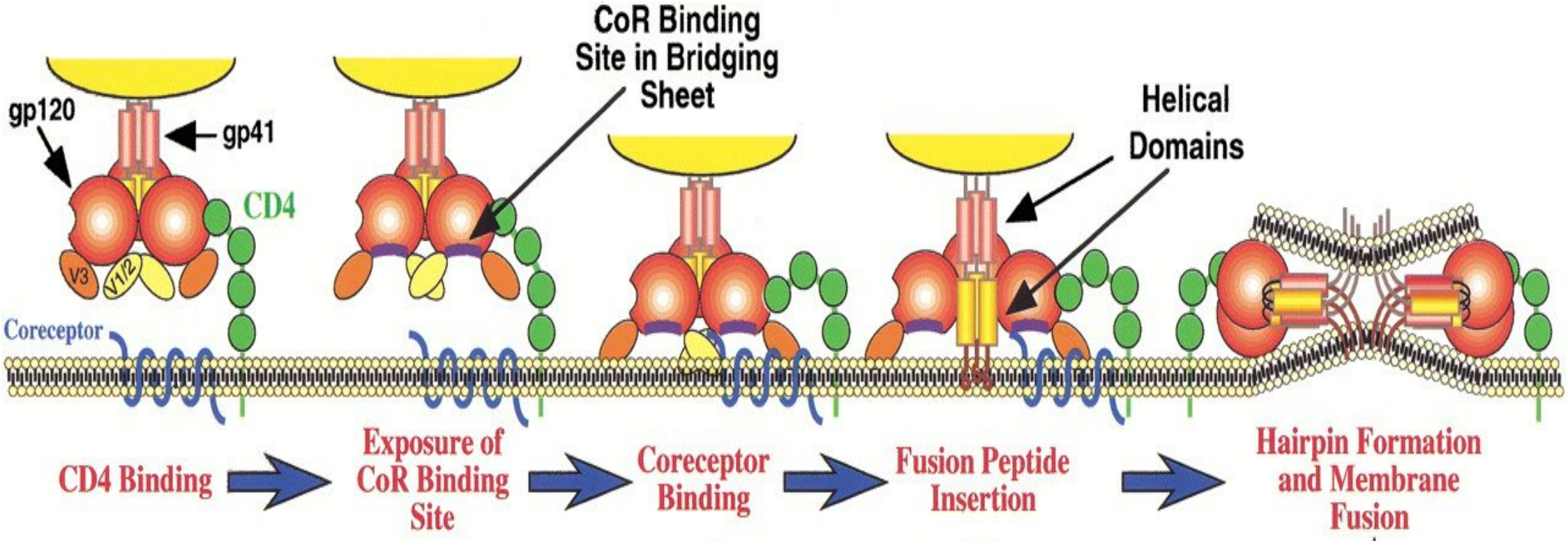
- **Only for enveloped virus**
- Fusion of the viral envelope with the plasma membrane
  → penetration of the nucleocapsid into the cytoplasm
- Fusion peptide is active at physiological pH
- Some examples :
  - HIV
  - Paramyxovirus
  - Herpesvirus



# Attachment / fusion

## HIV entry

- Interaction between gp120 and CD4, the HIV receptor
- Binding of gp120 to a coreceptor (CCR5 or CXCR4)
- Anchoring of the fusion peptide (gp41) in the membrane
- Hairpin structure of gp41 and membrane fusion, formation of a pore

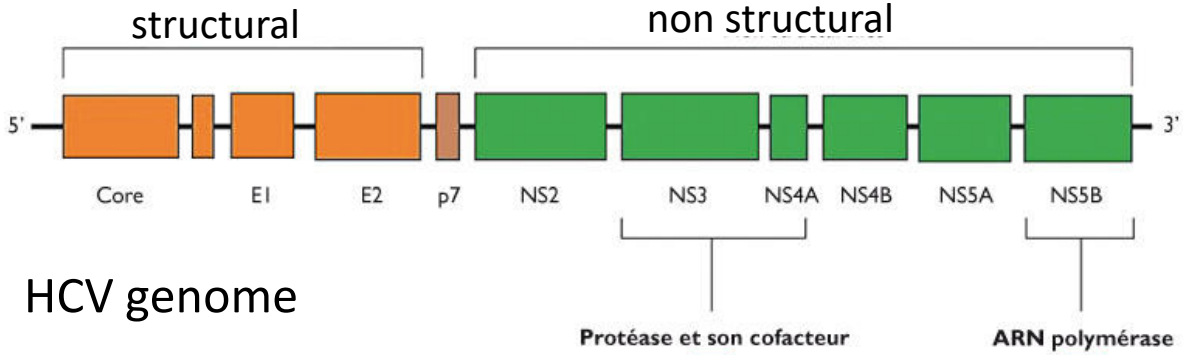


# Expression and replication of the viral genome

- Synthesis of the viral proteins and replication of the viral genome
  - ✓Transcription (mRNA)
  - ✓Translation

    -non structural proteins (present in the infected cell, absente in viral particles) : enzymes, proteins which regulate the virus and the cellular metabolism

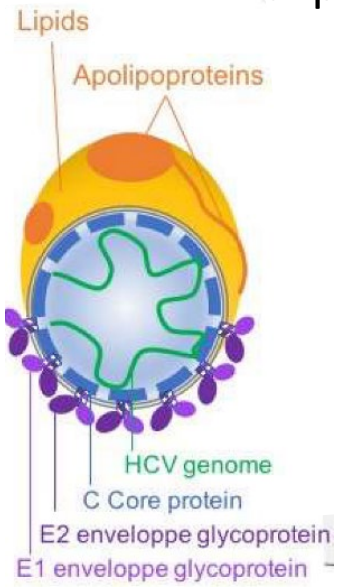
    - structural proteins : capsid, envelope, enzymes
  - ✓Replication of the genome




HCV genome

Hepatitis C virus

Viral particle

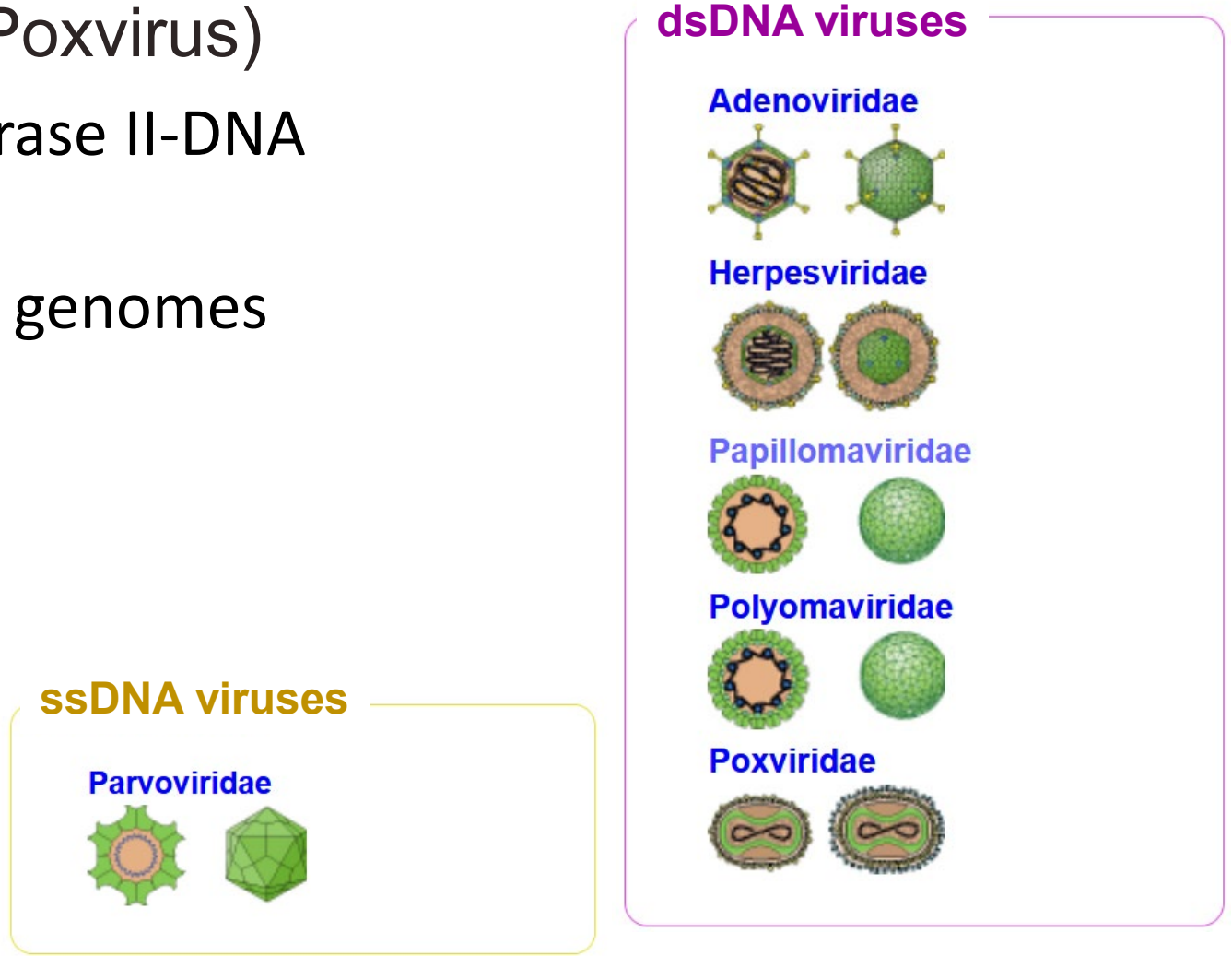


# DNA VIRUSES REPLICATION

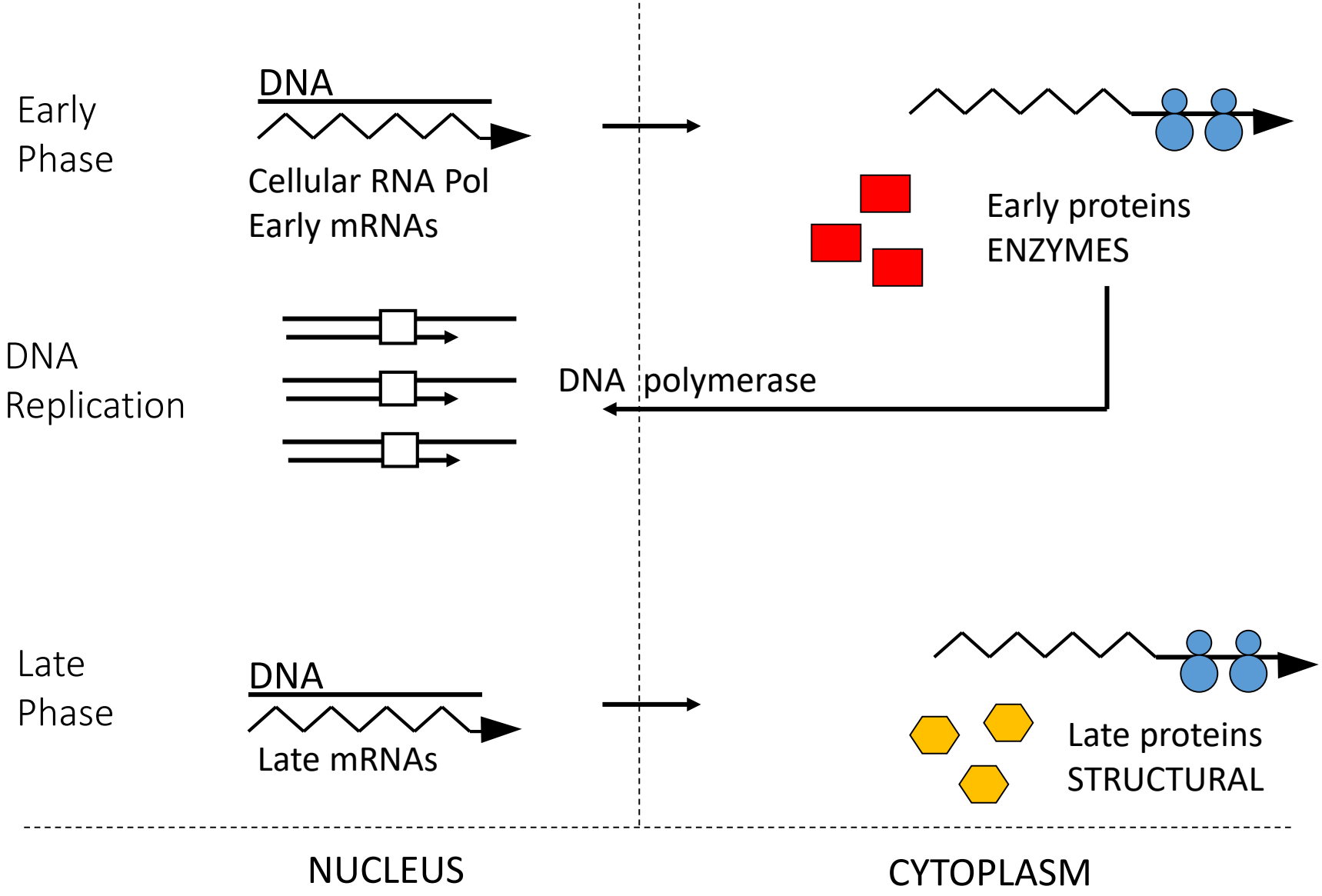
# DNA viruses

Parvovirus, Adenovirus, Herpesvirus, Papillomavirus…

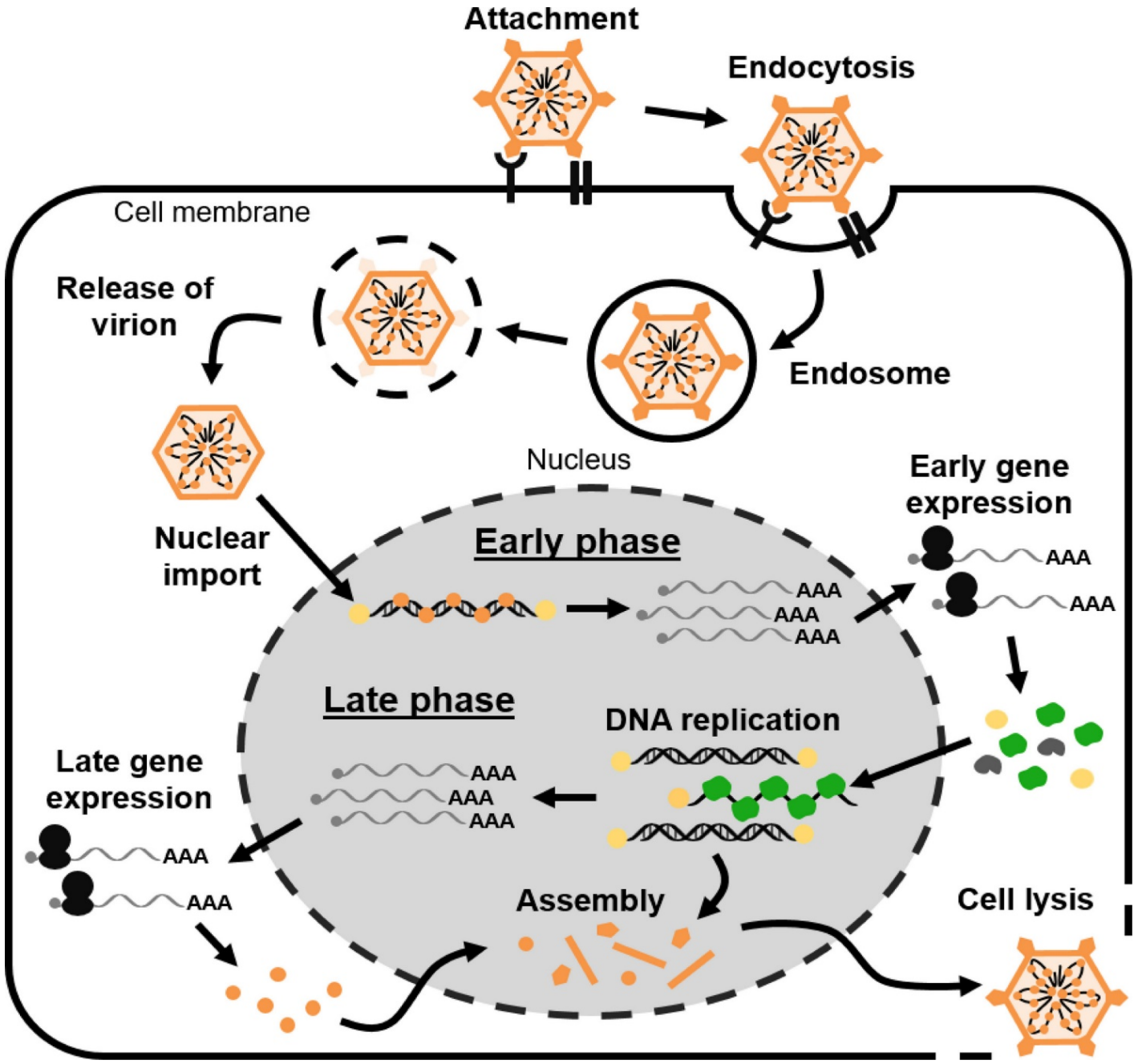
- Intranuclear replication (except Poxvirus)
- Transcription : cellular RNA polymerase II-DNA dependent
- single-stranded or double-stranded genomes
-



# Replication of DNA viruses



# Adenovirus DNA replication




FEBS Letters, Volume: 593, Issue: 24, Pages: 3531-3550, First published: 25 November 2019, DOI: (10.1002/1873-3468.13695)

# RNA VIRUSES REPLICATION

# Replication of RNA viruses

- Intracytoplasmic multiplication (except nuclear phase of Orthomyxovirus)
- need to code a viral RNA-dependent RNA polymerase
- They have a single-stranded genome (except Reovirus*)*
- Positive or negative sense ssRNA
  - **+ssRNA**, same sense as cellular mRNA:

    cap in 5', polyA tails in 3'

    (Picornavirus, Coronavirus,Togavirus)
  - **- ssRNA**, have to be transcribed in mRNA

    (Orthomyxovirus, Paramyxovirus, Rhabdovirus)
  - **Retrovirus,** constitutive reverse transcriptase

    RNA+ → DNA, DNA integration

# +ssRNA viruses

- Two roles for genomic RNA:

  - mRNA, directly translated

  - template for the synthesis of a complementary strand -RNA → +RNA

- Viral RNA polymerase is synthetized
- Two roles for neosynthesized RNA:

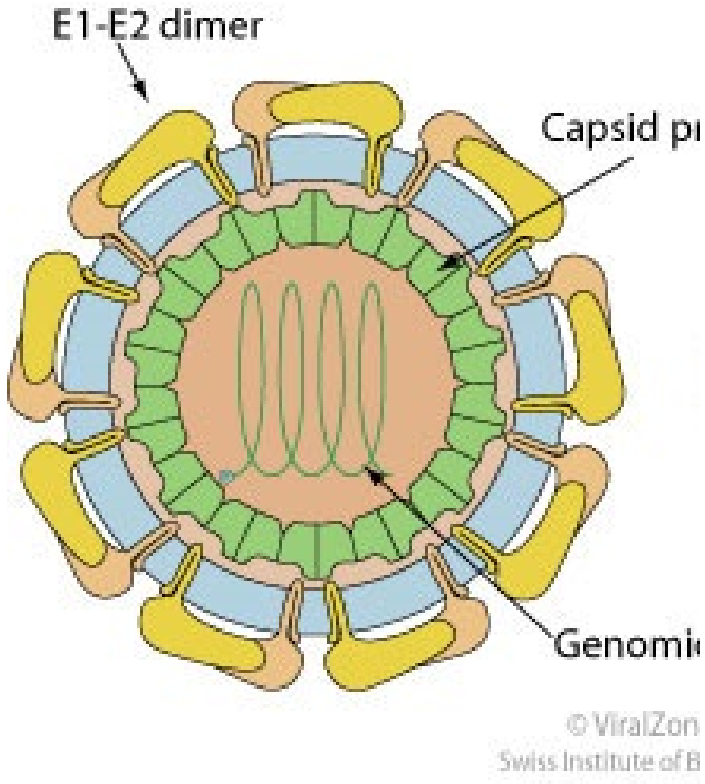
  - mRNA, which will be translated

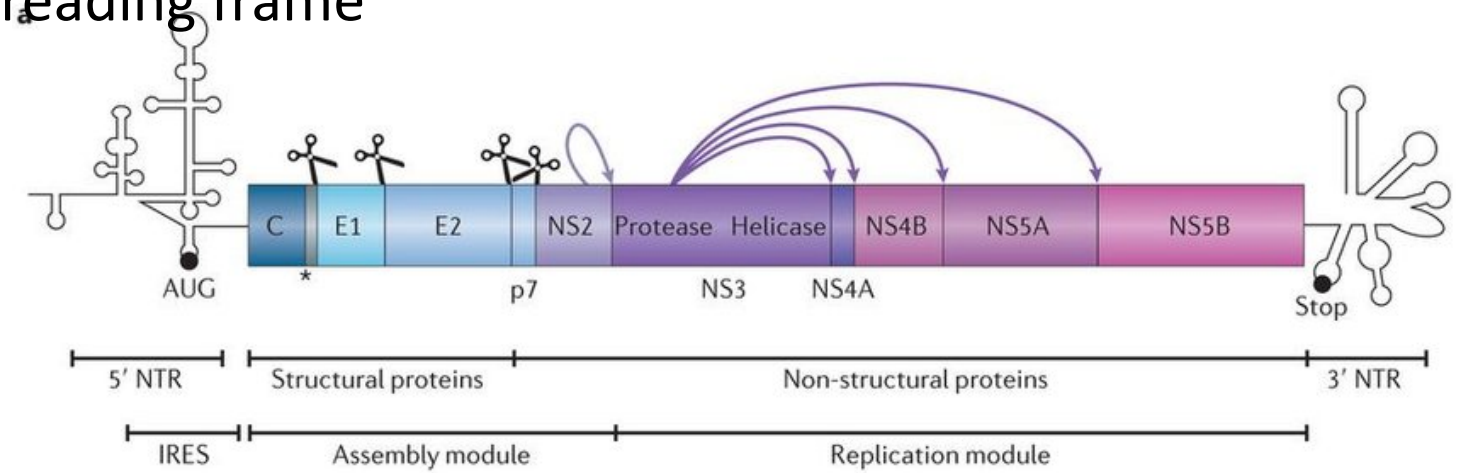
  - genomic RNA, which will be incorporated in the capsid to form new virions

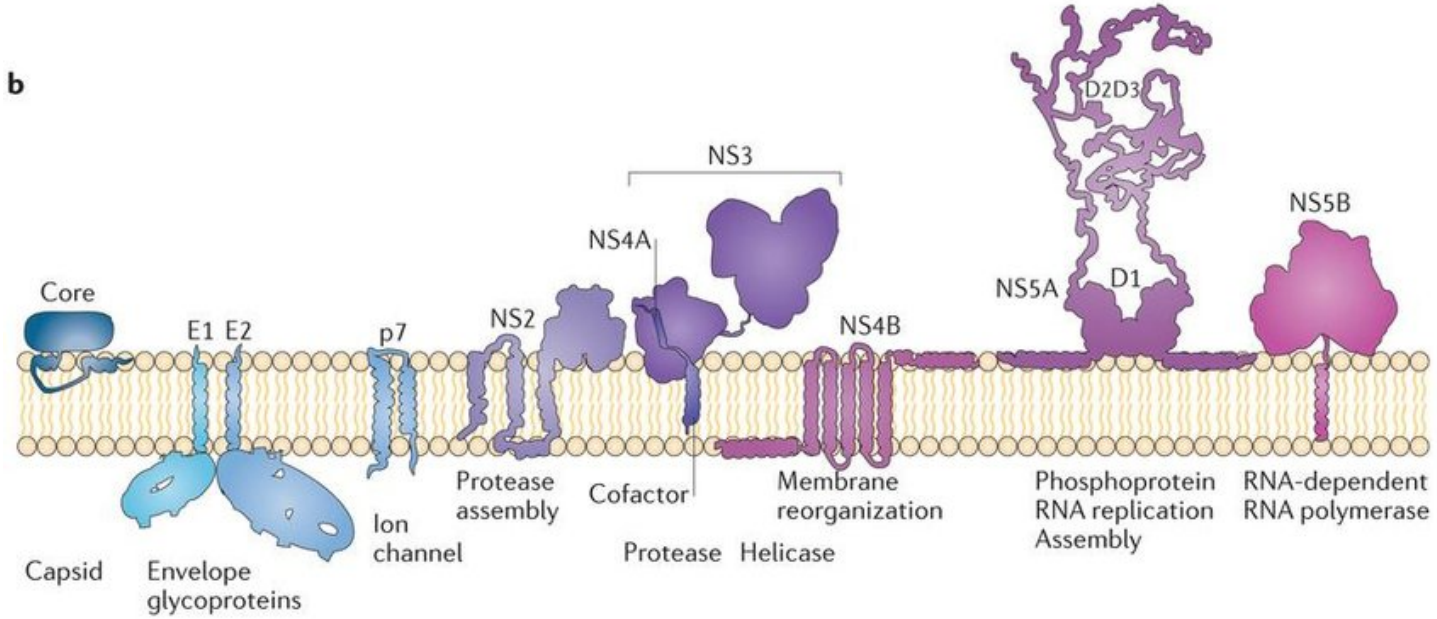
# +ssRNA virus: *Flaviviridae* - Hepatitis C virus

+ssRNA containing only one open reading frame

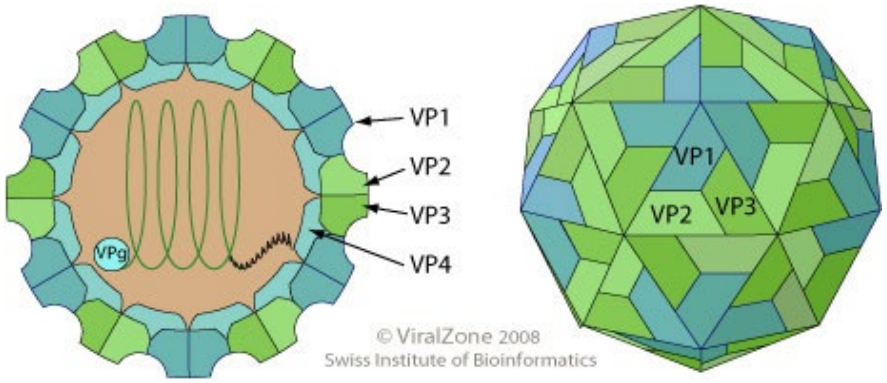
No cap

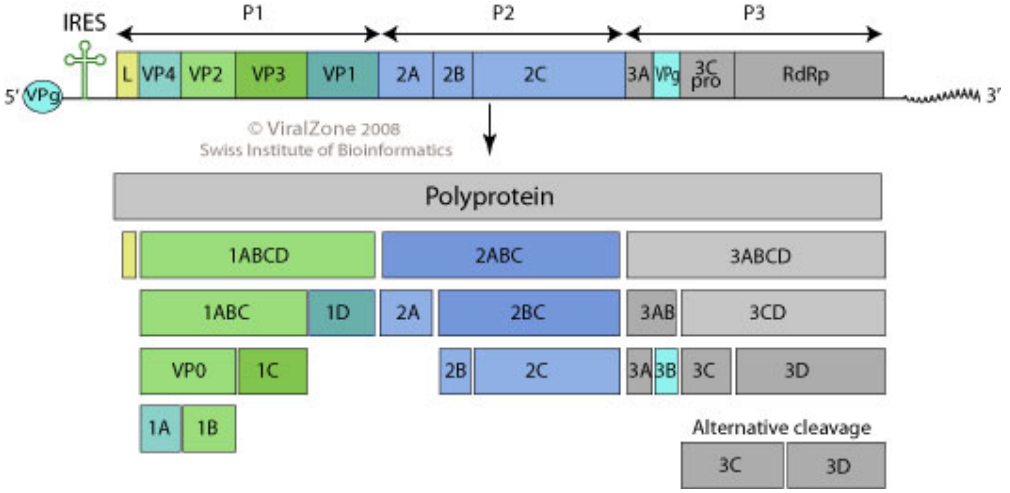


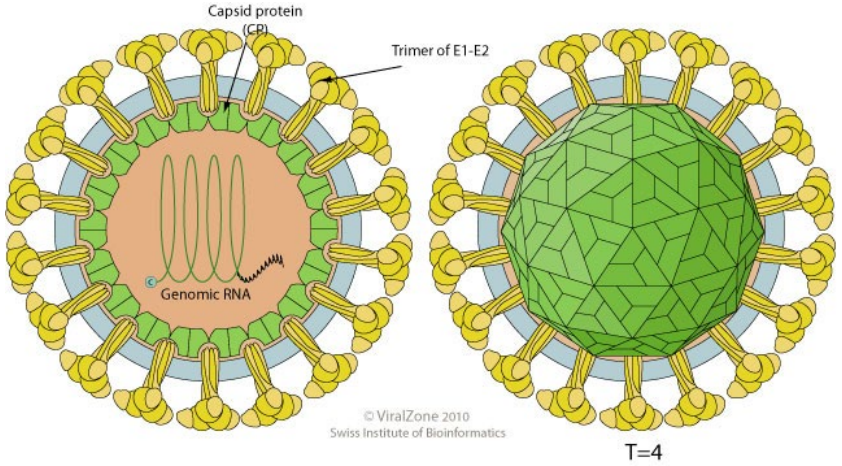
# *Picornaviridae*

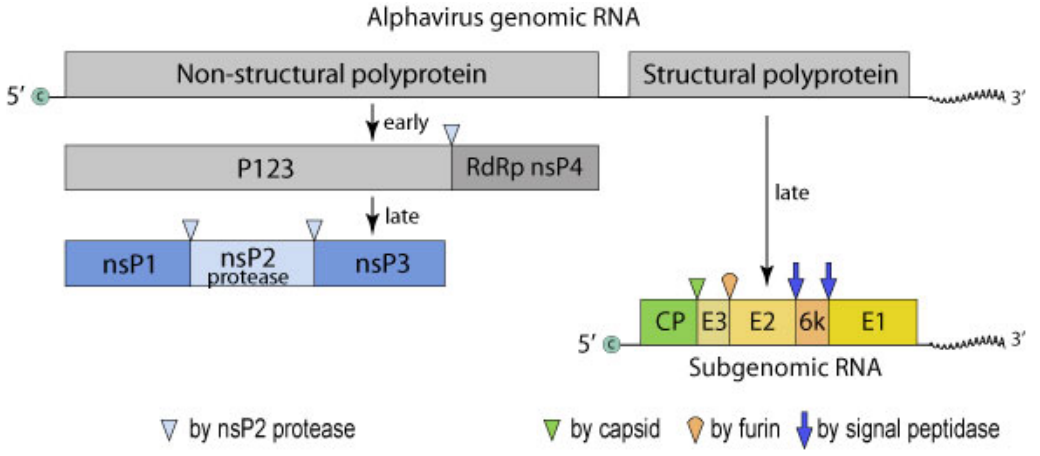



Ex: Poliovirus, Hepatitis A virus

# *Togaviridae*




Ex: Rubella virus, Chikungunya virus

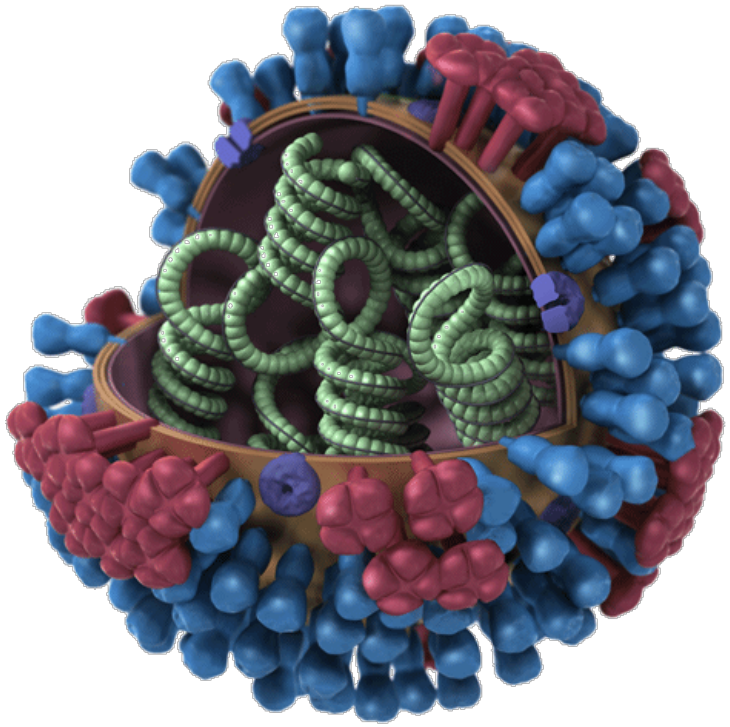
# -ssRNA and dsRNA VIRUSES

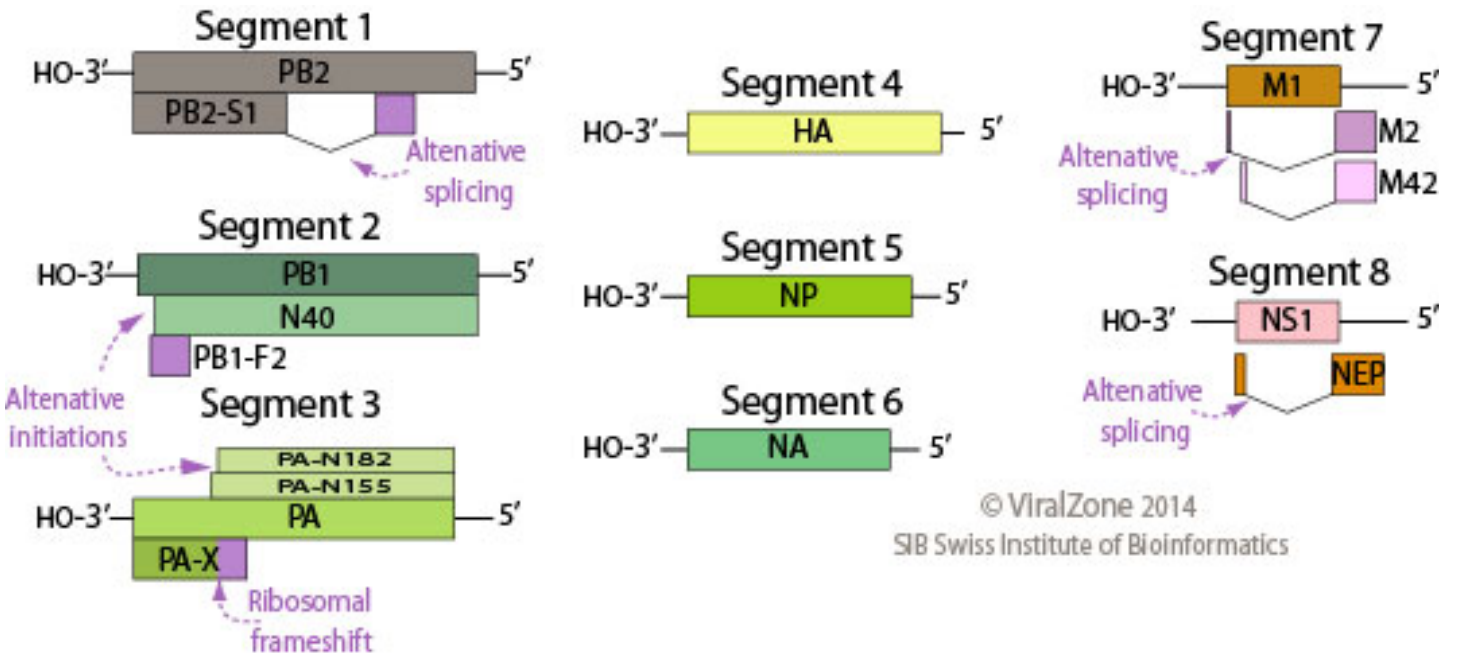
- Non messenger genomic RNA

→ needs to be transcribed in mRNA

- Constitutive viral RNA polymerase (transcriptase)
- Two roles for neosynthetized RNA:
  - mRNA
  - Template for synthesis of complementary genomic -RNA, incorporated in the capsid

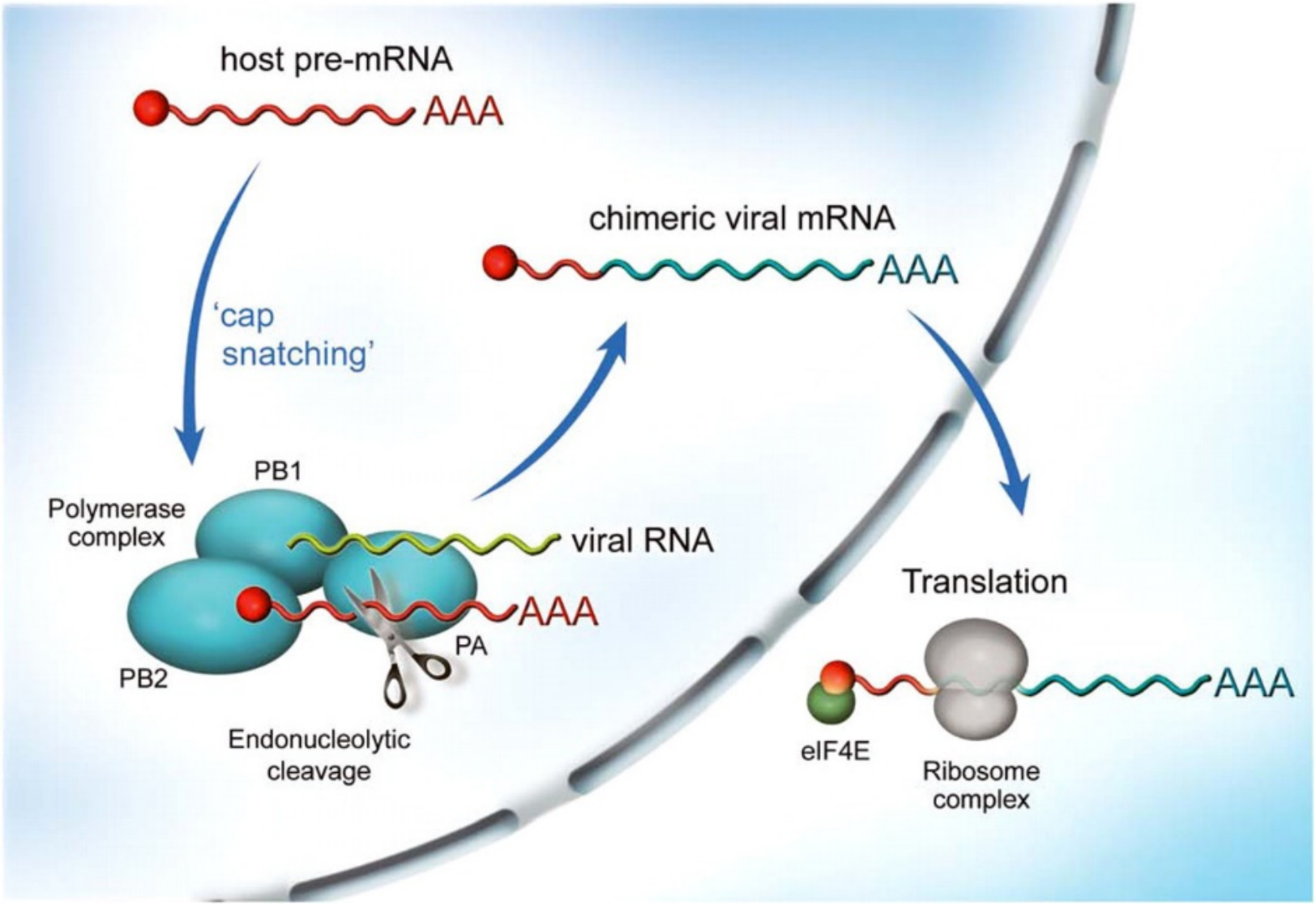
# *Orthomyxoviridae*



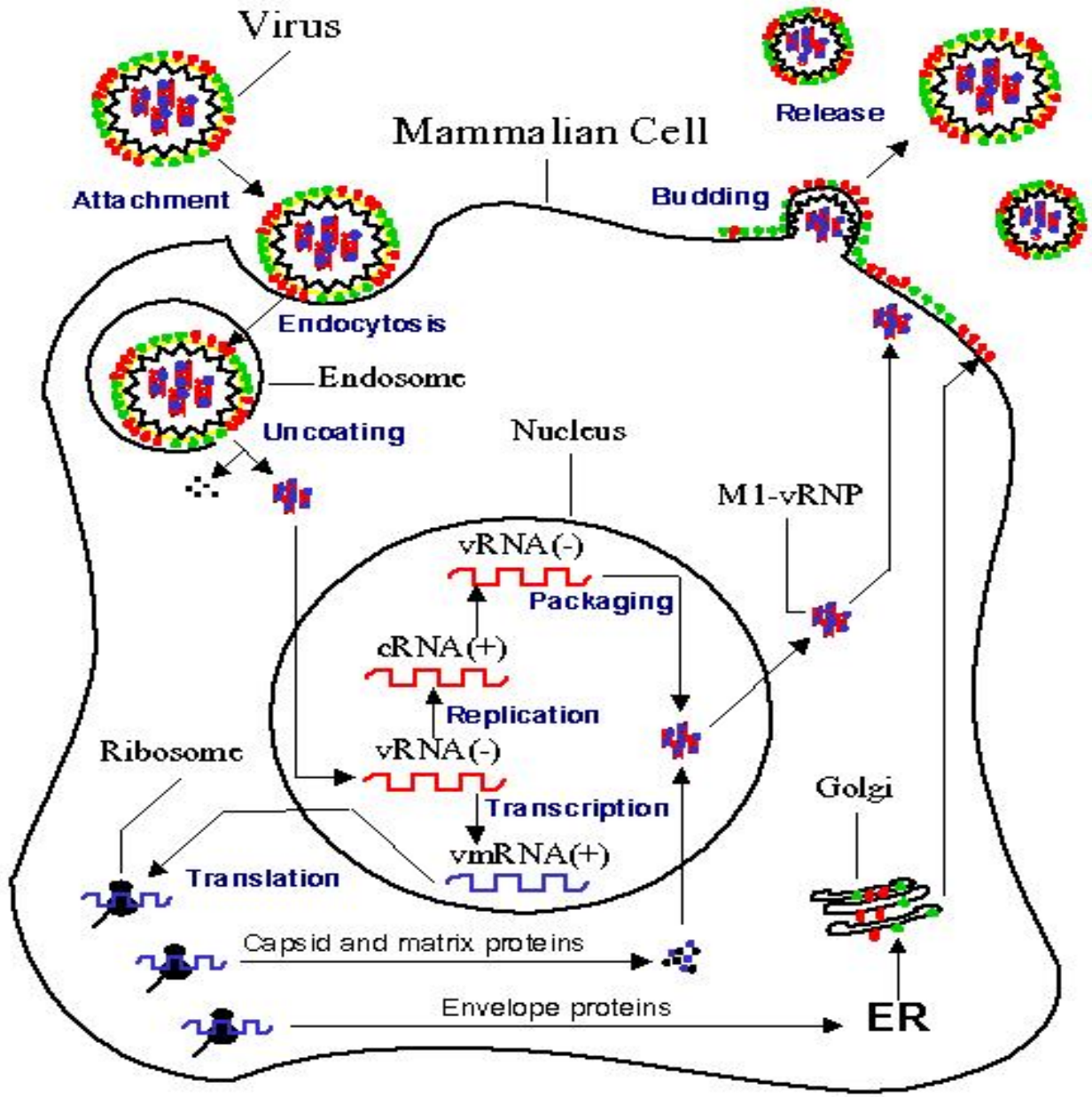




Ex: Influenza Virus

# Cap snatching *Orthomyxoviridae* (influenza virus)



# Influenza virus Cycle

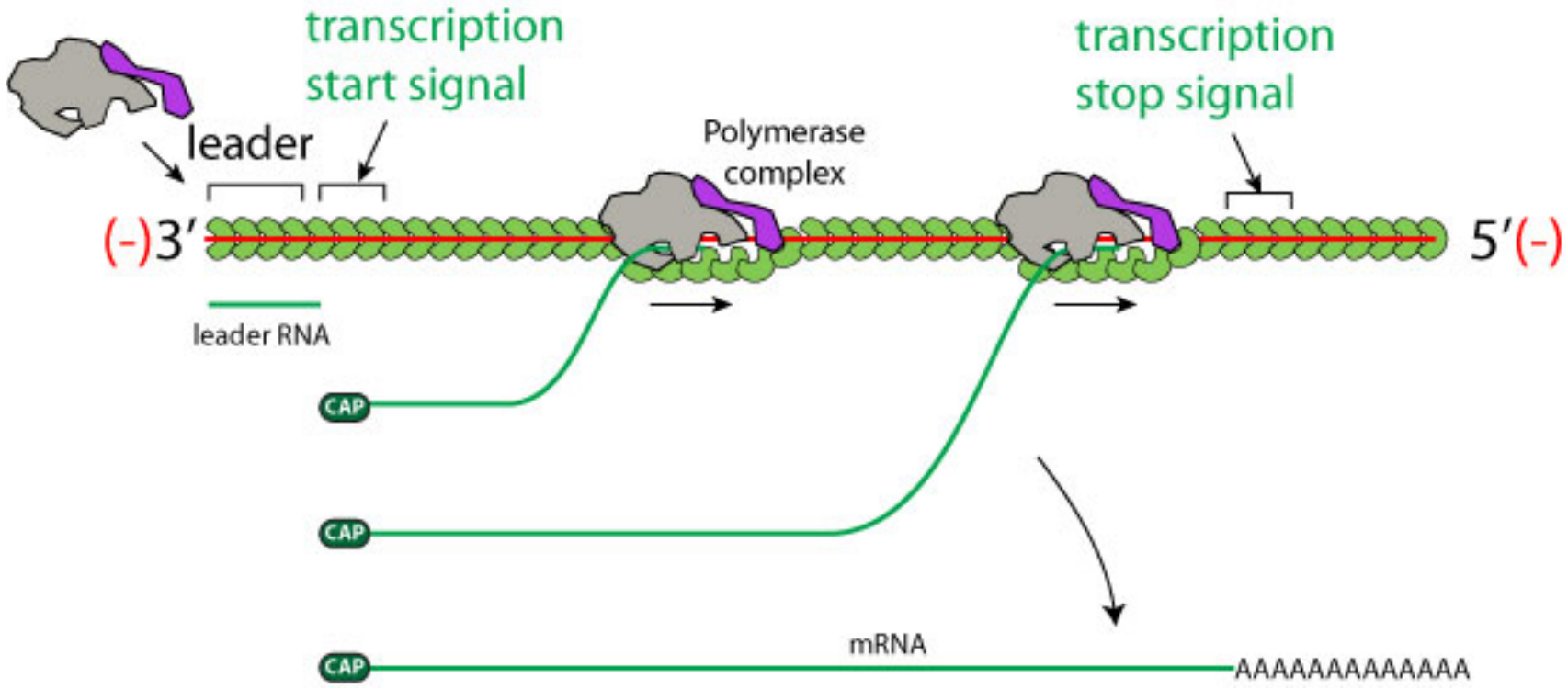


# Example of a Negative RNA Virus : Measles virus

Non segmented genome

*Filoviridae, Paramyxoviridae, Rhabdoviridae*

- Transcription and replication by the viral RNA polymerase
- Each gene is framed by a starting sequence and a stop sequence



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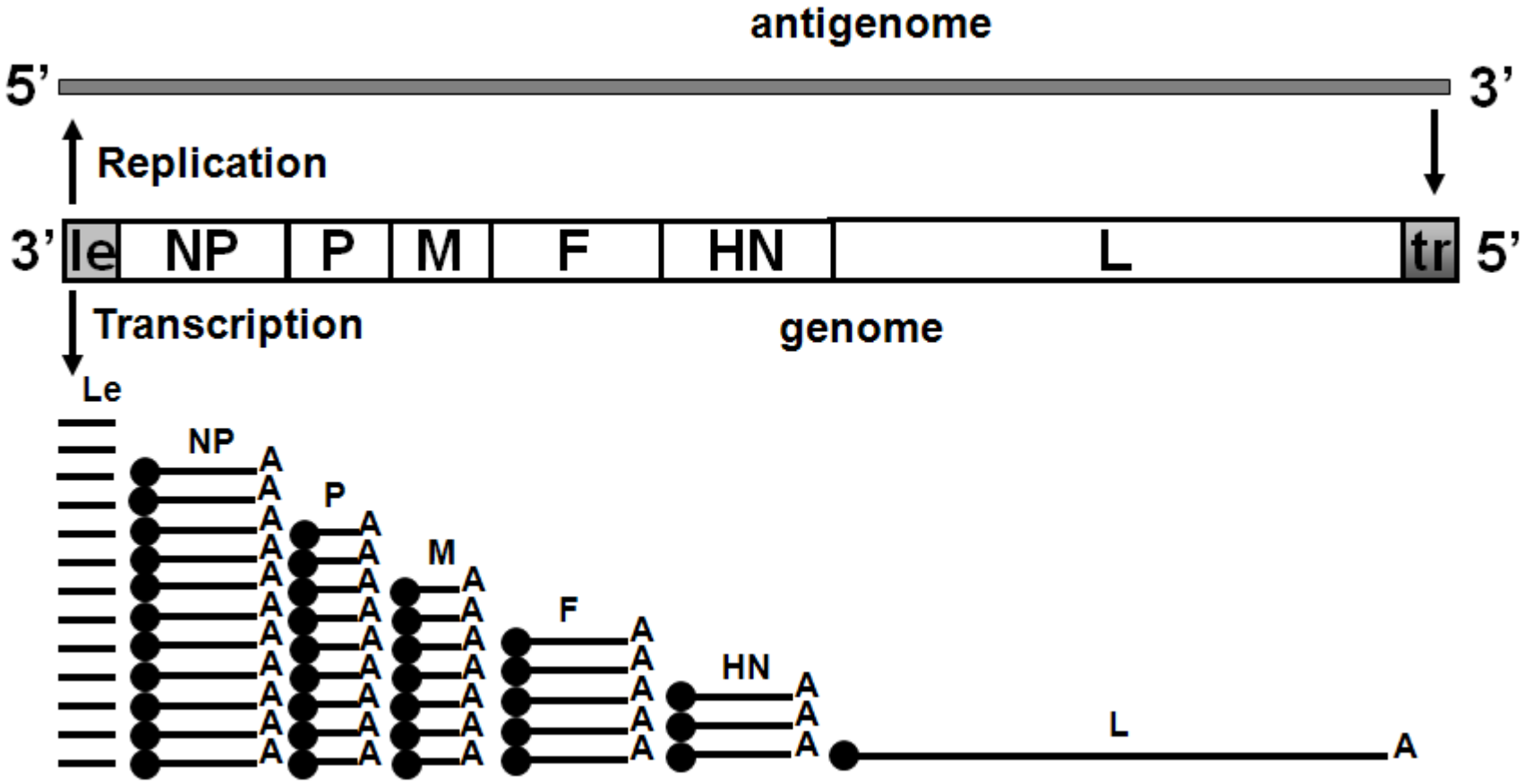
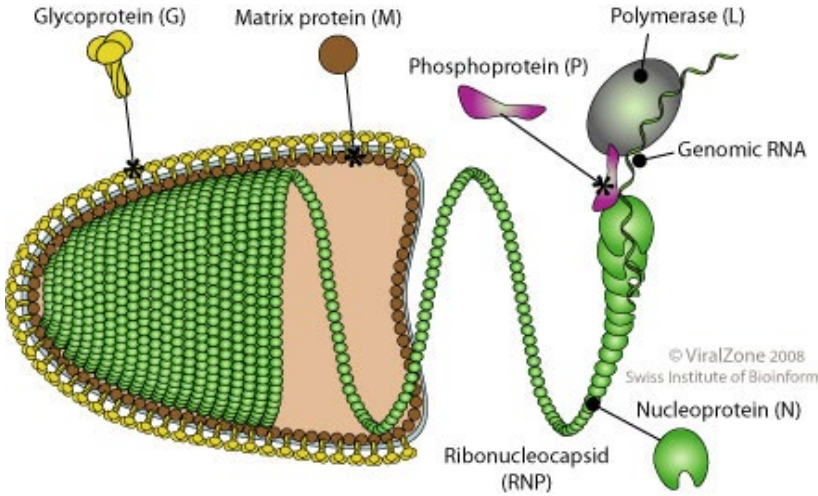
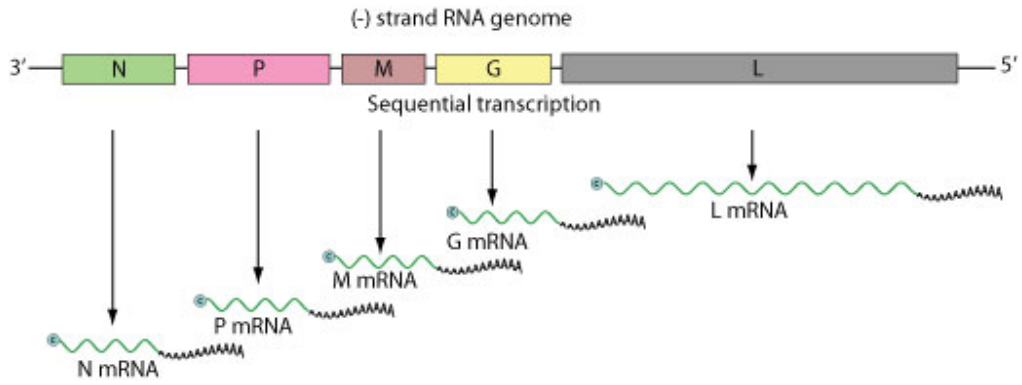



The place of the gene in the genome influences the quantity of transcript formed

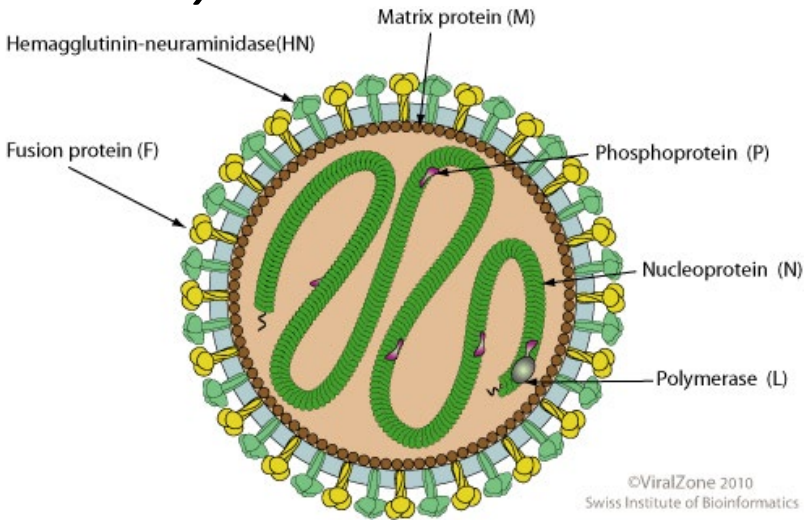
# *Rhabdoviridae*

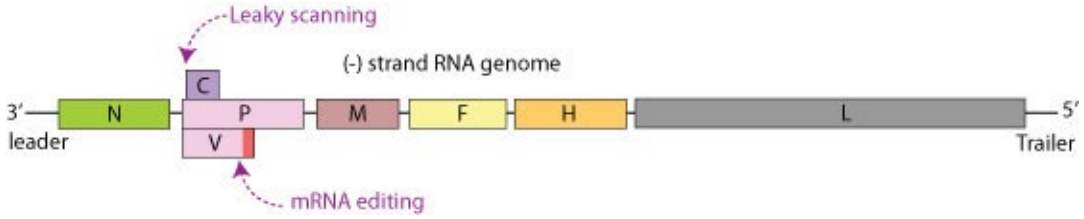



Ex: Rabies virus

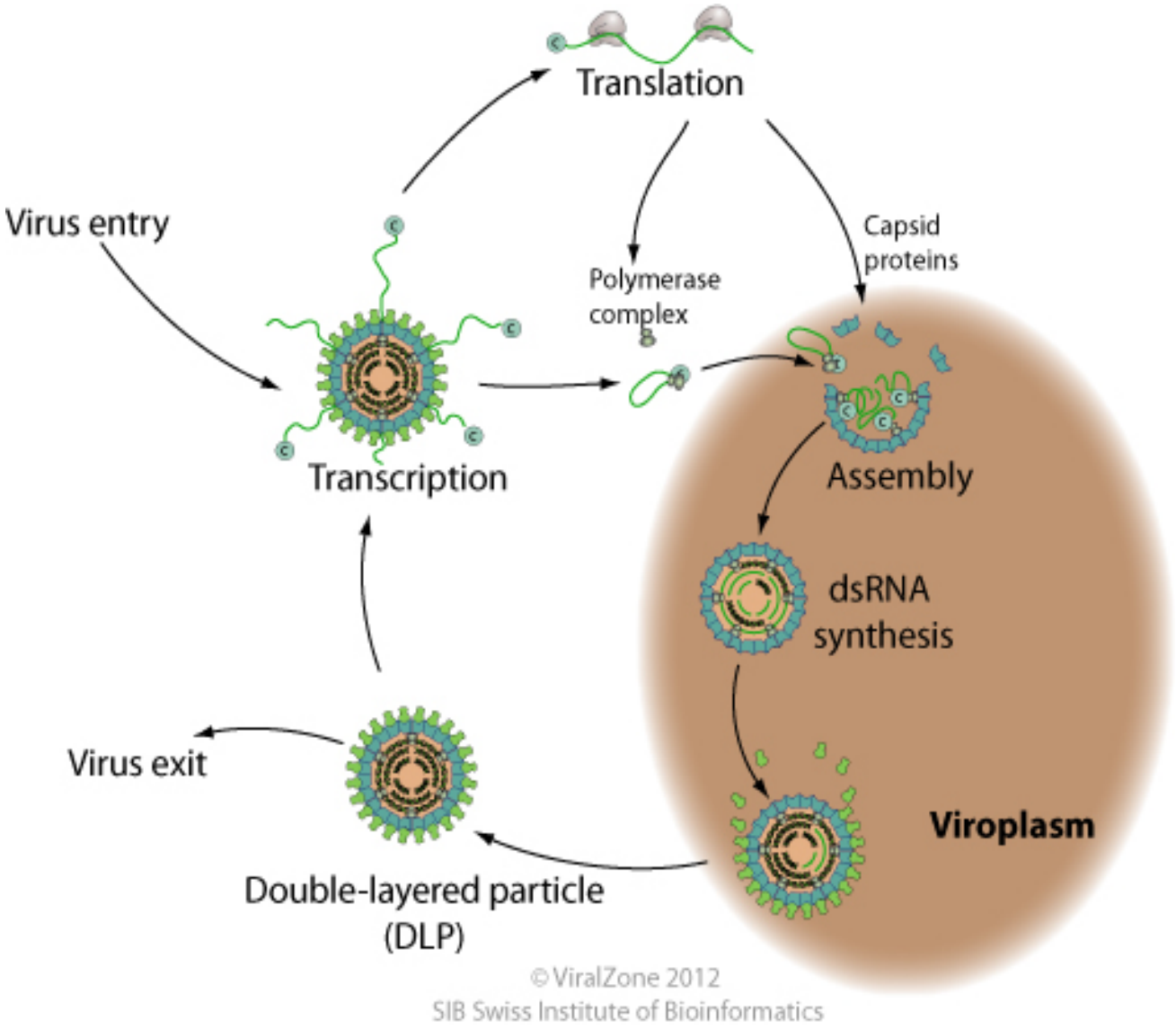
# *Paramyxoviridae*




Ex: Measles Virus, Mumps Virus

# Reoviridae multiplication




- innate immunity dsRNA sensors
- Transcription begins in a partially decapsidated particle
- Synthesis of the –RNA strand inside the capsid
- Neither dsRNA nor -RNA in the cytoplasm

# Replication of viruses involving a reverse transcriptase

- Reverse transcriptase (RT) : production of DNA from +RNA
- inverse of cellular transcription
- Retrovirus: genome

+RNA $\xrightarrow[RT]{}$ DNA

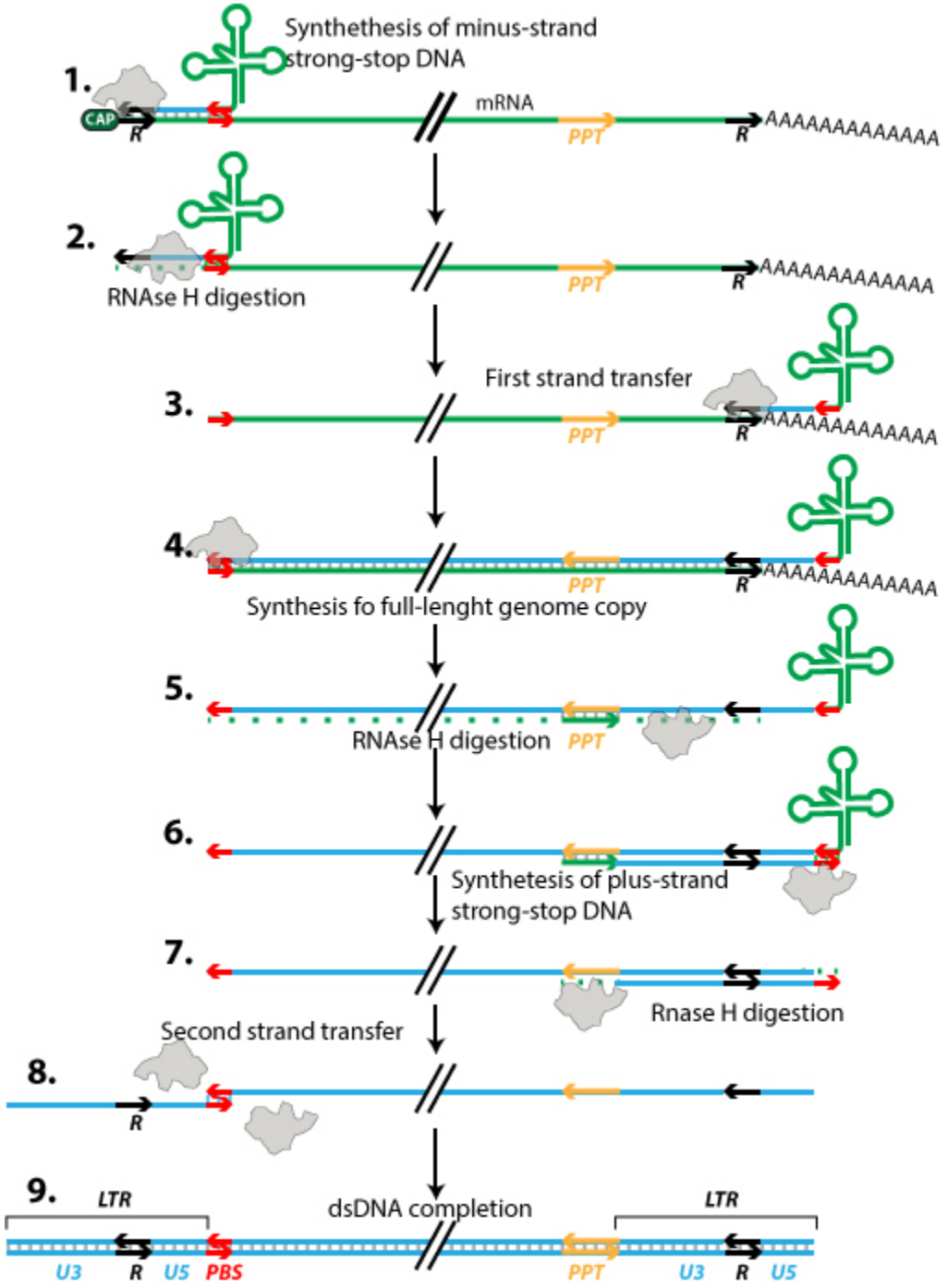
- Hepatitis B virus: replication intermediate

+RNA $\xrightarrow[RT]{}$ DNA

# Reverse transcription RNA-DNA

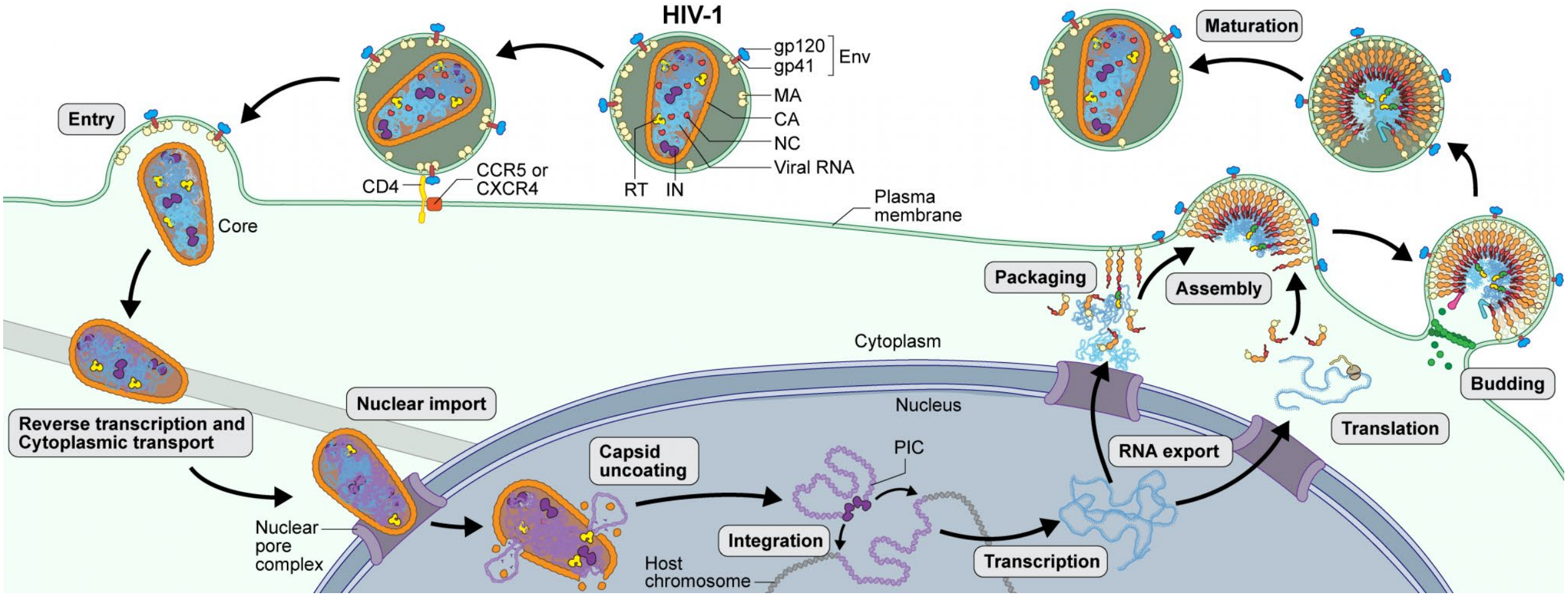
3 activities of the RT, carried by distinct sites

- RNA-dependent DNA polymerase
- RNAse H
- DNA-dependent DNA polymerase



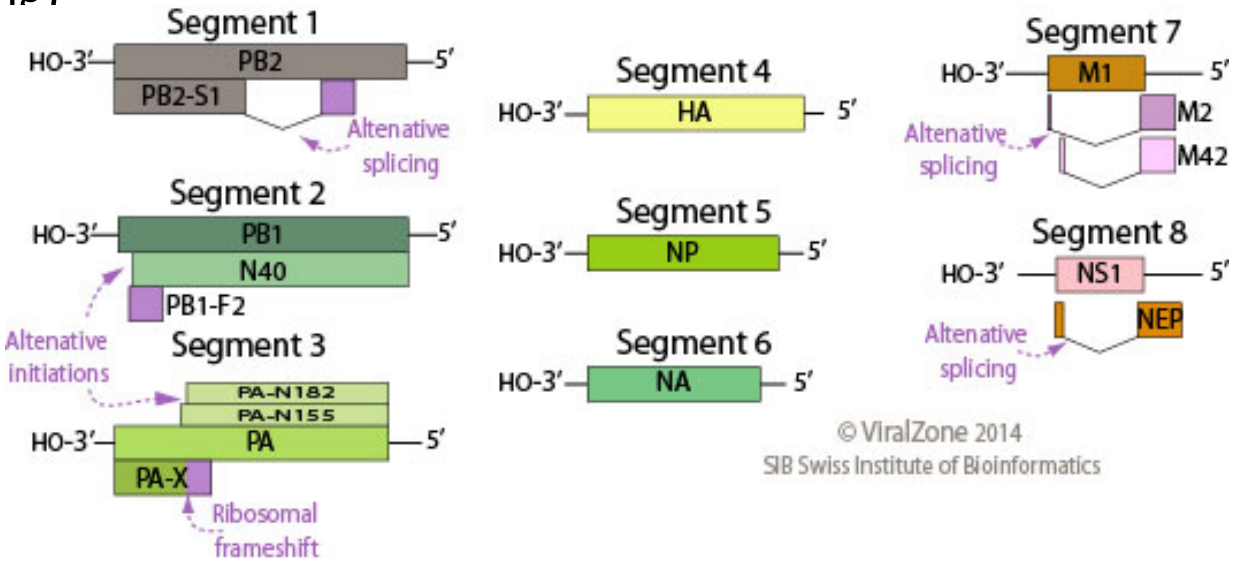

ViralZone

# Multiplication cycle of HIV-1



# Strategies of viral protein synthesis in RNA viruses

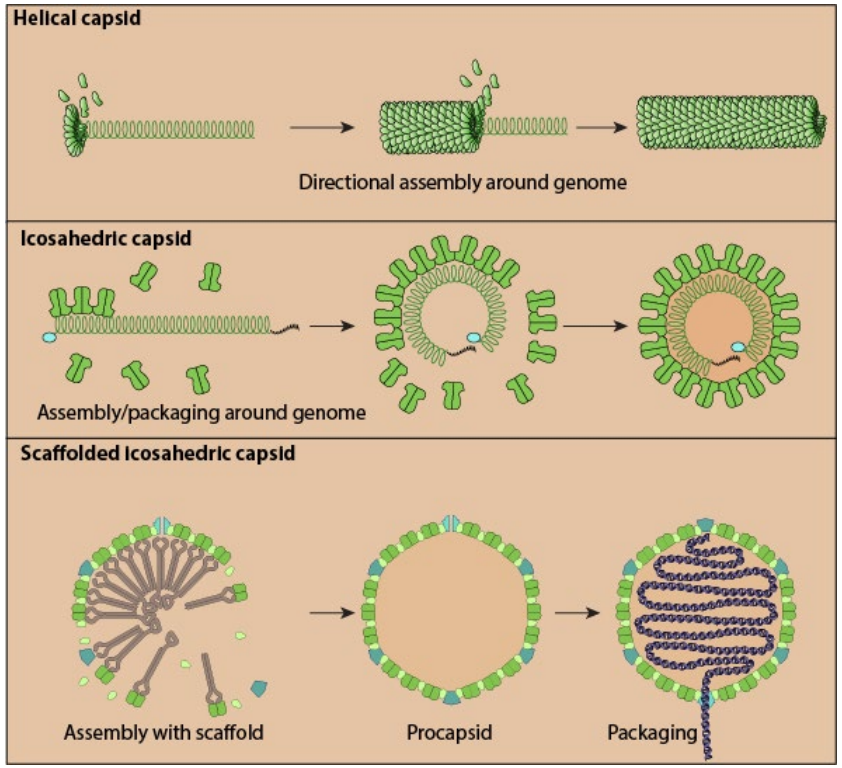
- Translation in eukaryotic cells
  - 1 mRNA = 1 protein
- Several strategies for RNA viruses
  - One polyprotein, cleaved to give all the necessary proteins (picornavirus, flavivirus)
  - Segmented genome 1 segment = 1 mRNA
  - Initiation of the mRNA synthesis at the level of de internal sites by RNA-dep RNA pol
  - Alternative splicing
  - Translation by itself (ribosomal frameshifting)
- Combination



# Viral cycle ends by assembly, maturation and exit of the virus

- Capsid assembly occurs at different levels:
  - DNA viruses → nucleus (except Poxvirus)
  - RNA viruses → cytoplasm (except influenza virus)

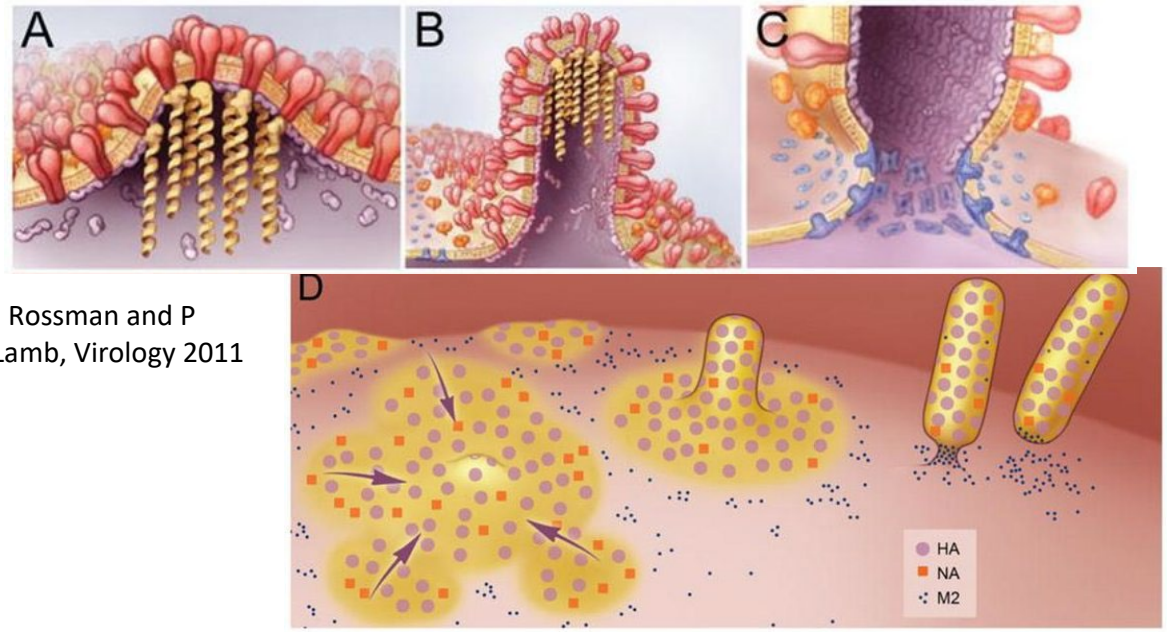
- Self assembly of neosynthesized proteins and nucleic acids - Procapsid
- Post translational modifications of viral proteins (cleavages, disulfide bridges, glycosylation …)
- Oligomerization of envelope glycoproteins



# Virus release

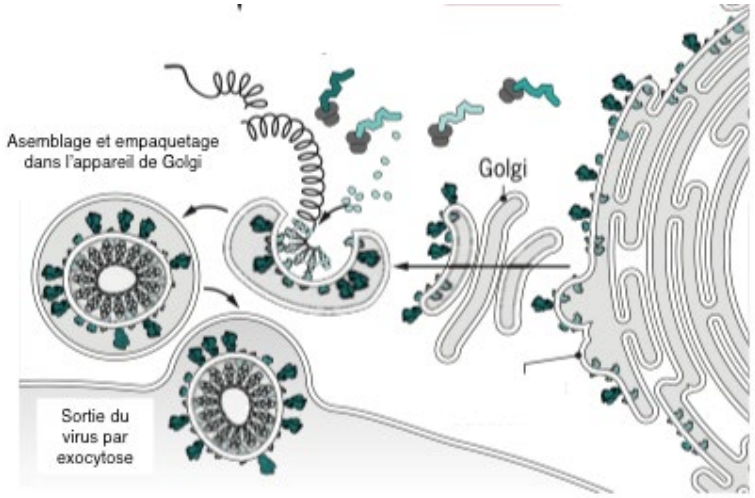
- **Naked viruses**: cell lysis (ex : Adenovirus)
- **Enveloped viruses**:
  - Budding of the nucleocapsid through cell membranes (nuclear, ER, Golgi, plasma membranes) modified by insertion of viral glycoproteins
  - Release by budding (plasma membrane) or by exocytosis (internal membranes)
  - This maturation step is absolutely required for infectivity
  - Viral release possible without cell lysis, contrary to naked viruses

Release by budding (plasma membrane)

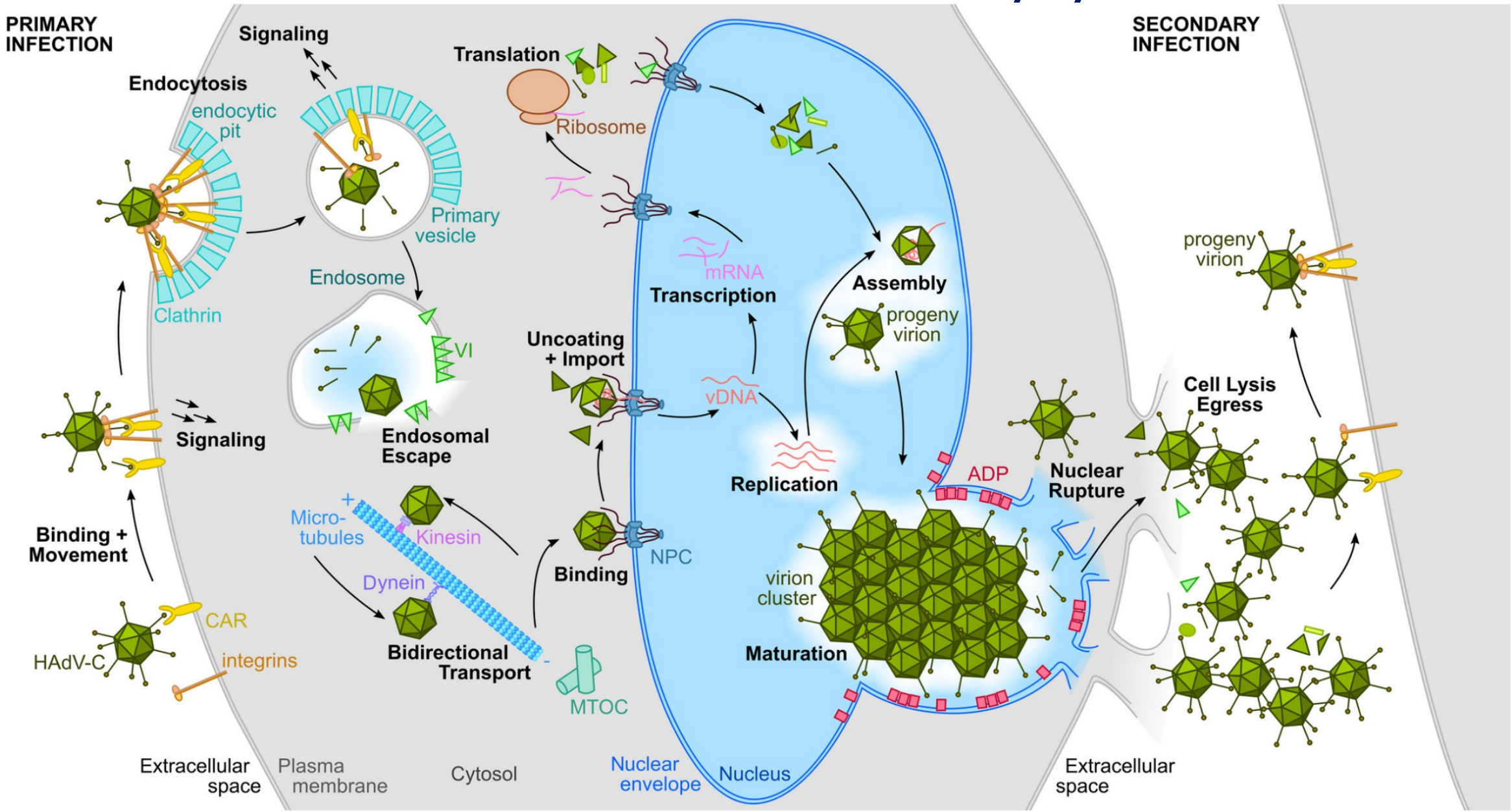



J Rossman and P Lamb, Virology 2011

Release by exocytosis (Golgi apparatus)



# Adenovirus exits the cell by lysis

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