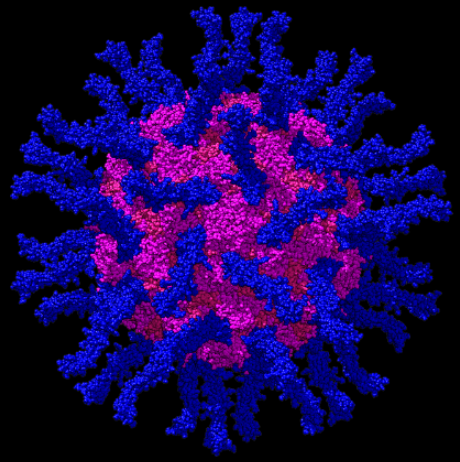


VIROLOGY LIVE

WITH VINCENT RACANIELLO

Assembly of Viruses

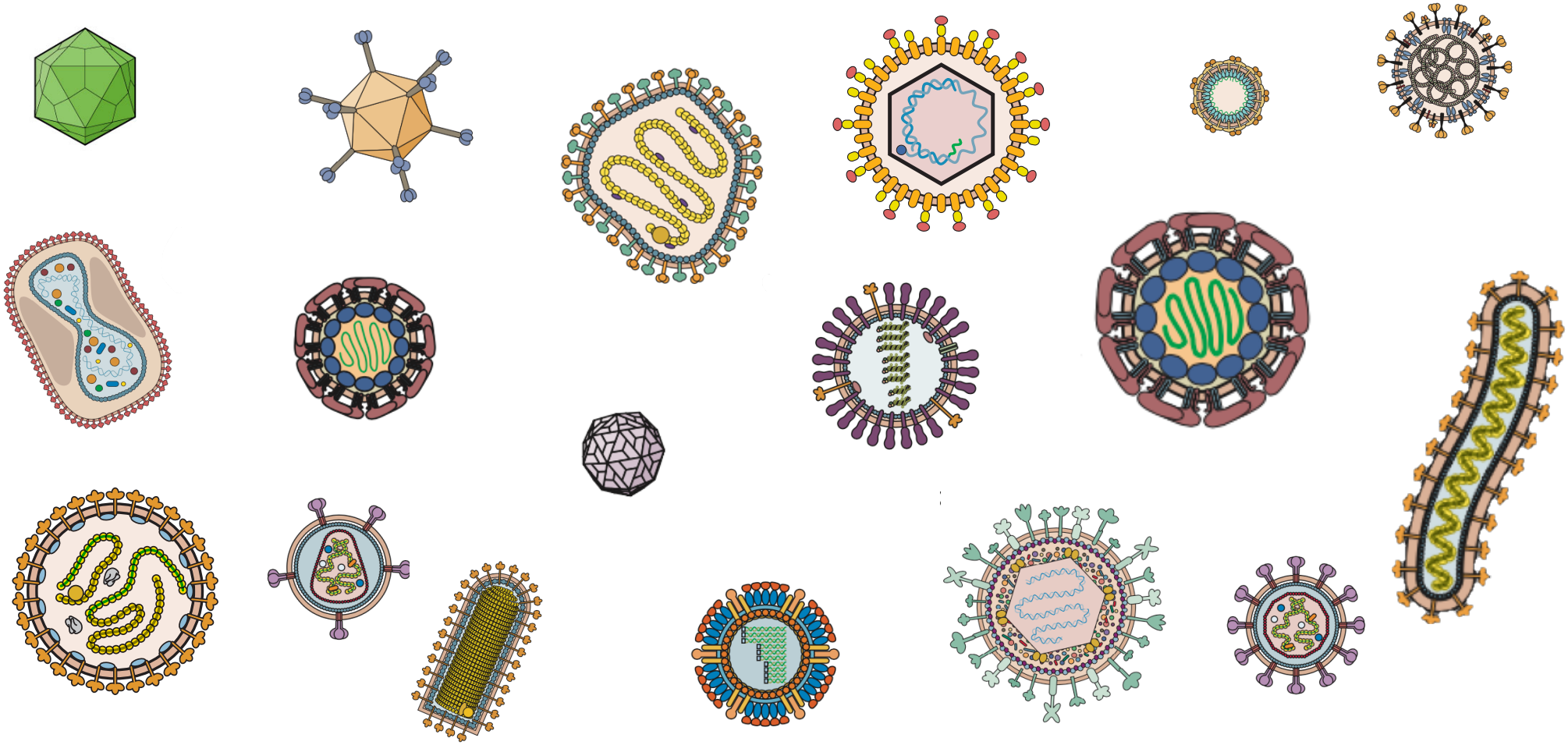
Session 10

Virology Live

Fall 2021

"Anatomy is destiny."
--Sigmund Freud

The structure of a virus particle determines how it is formed



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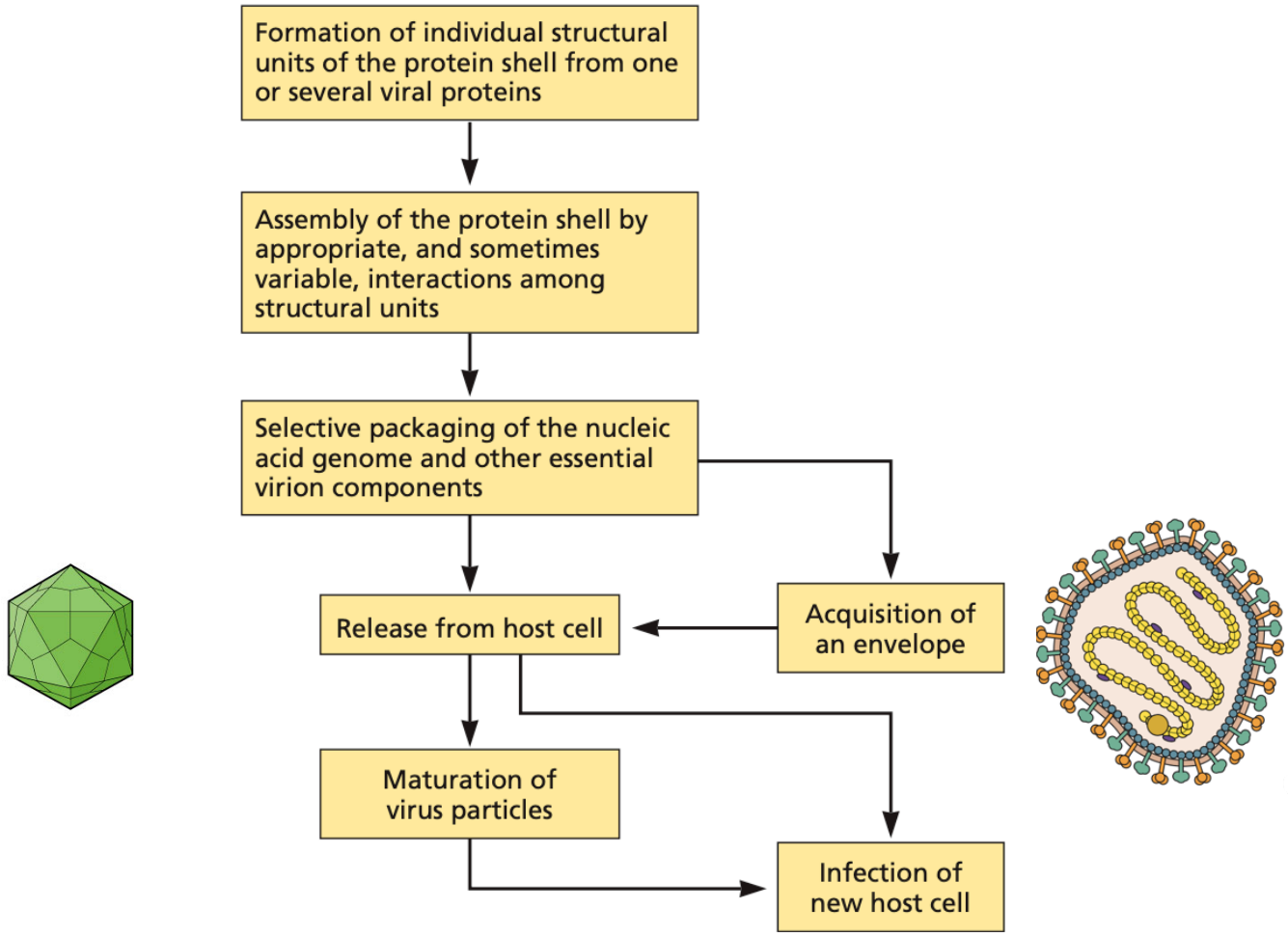
All virions complete a common set of assembly reactions



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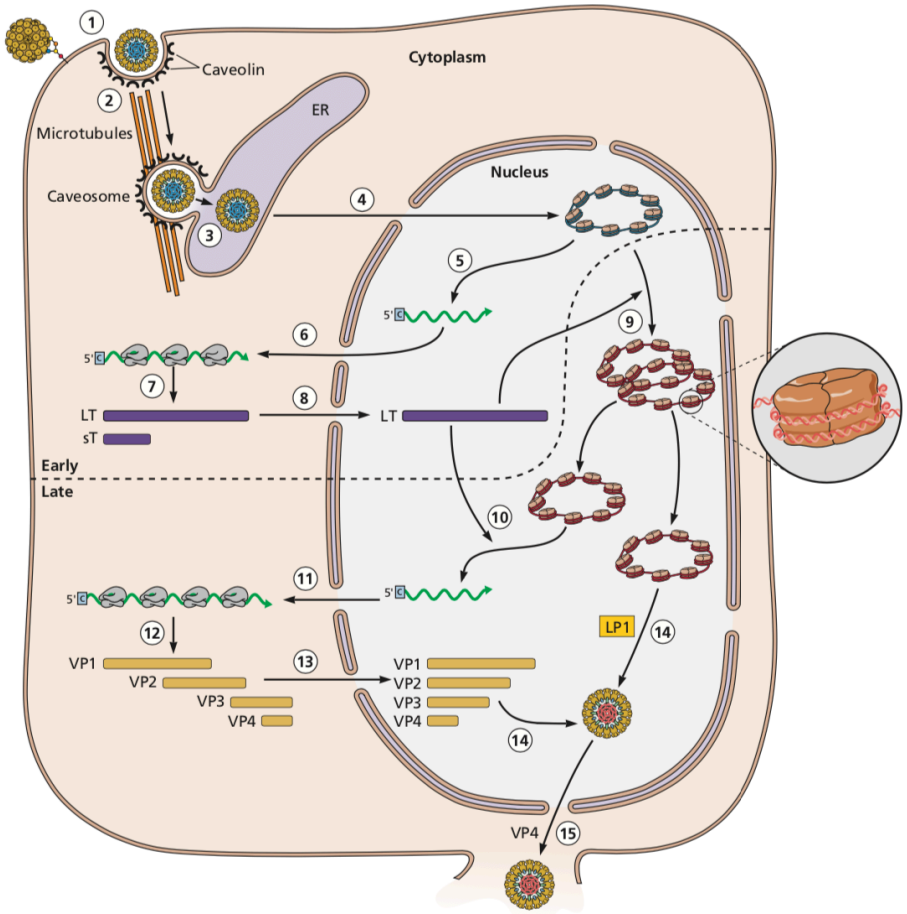
Assembly is dependent on host cell machinery

- Cellular chaperones
- Transport systems
- Secretory pathway
- Nuclear import and export machinery



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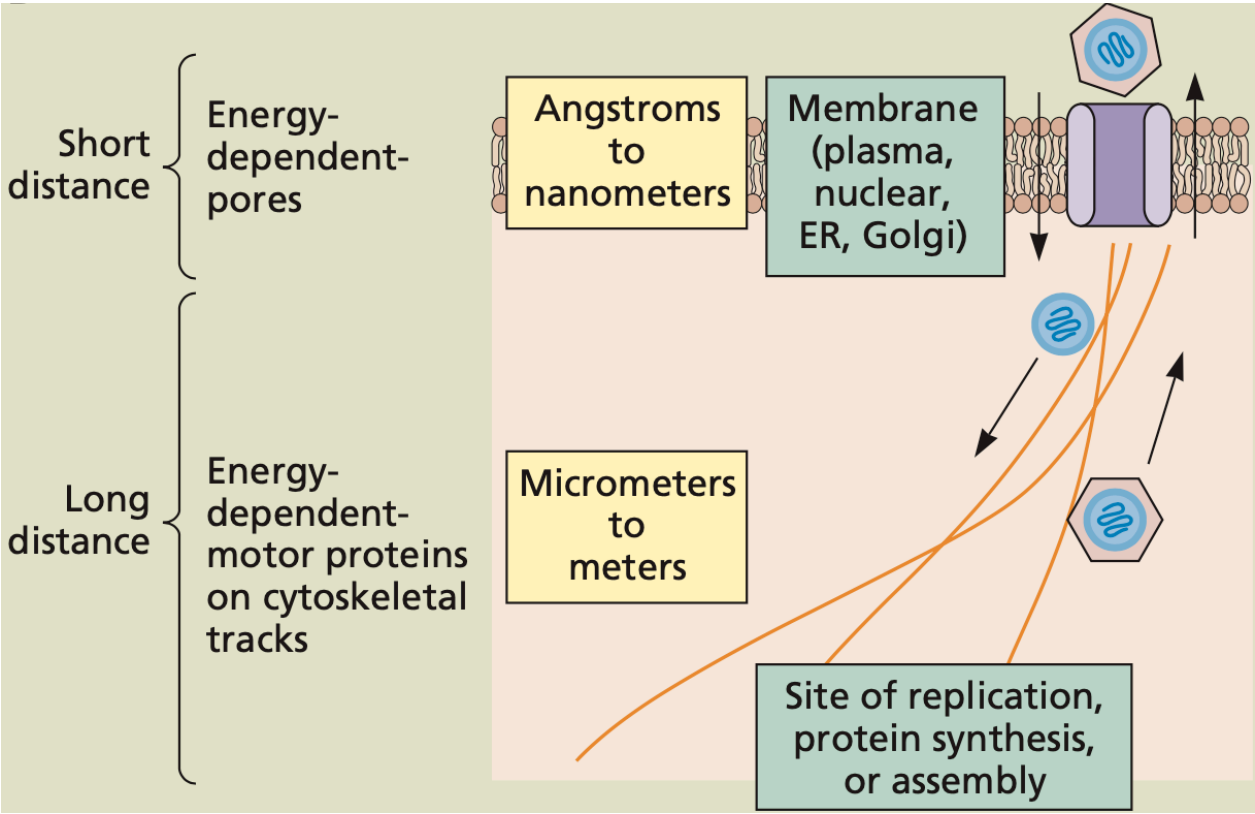
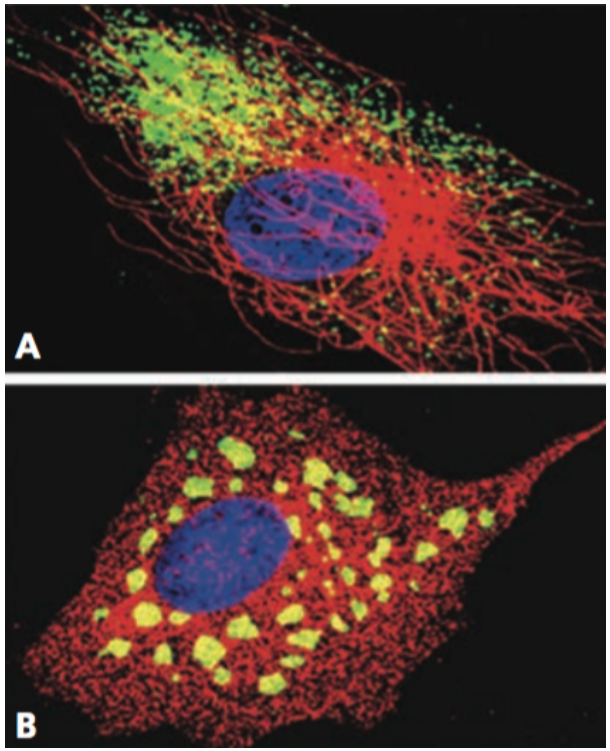
Moving in heavy traffic





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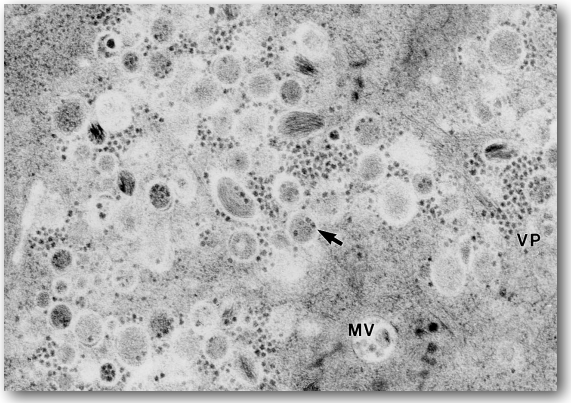
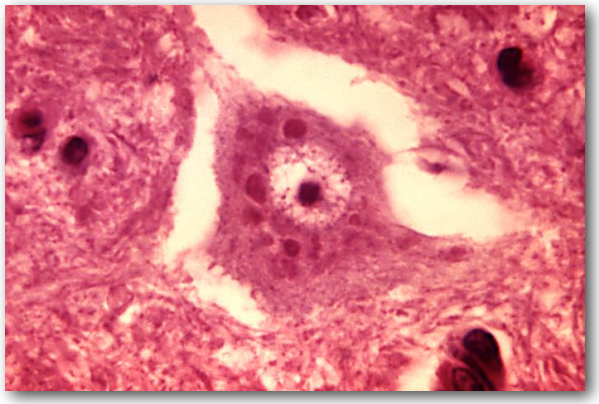
Nothing happens fast in dilute solutions

- Viral components often visible by light microscopy (‘factories’ or ‘inclusions’)
- Concentrate proteins on internal membranes *(poliovirus)*
- Negri bodies *(rabies virus)*





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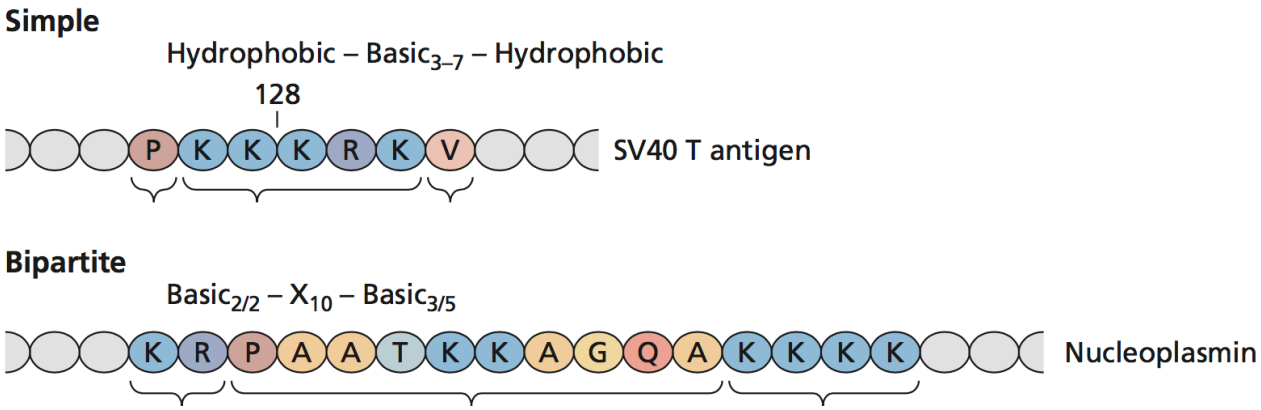
Viral proteins have ‘addresses’

- Membrane targeting: Signal sequences, fatty acid modifications
- Membrane retention signals
- Nuclear localization sequences (NLS)
- Nuclear export signals



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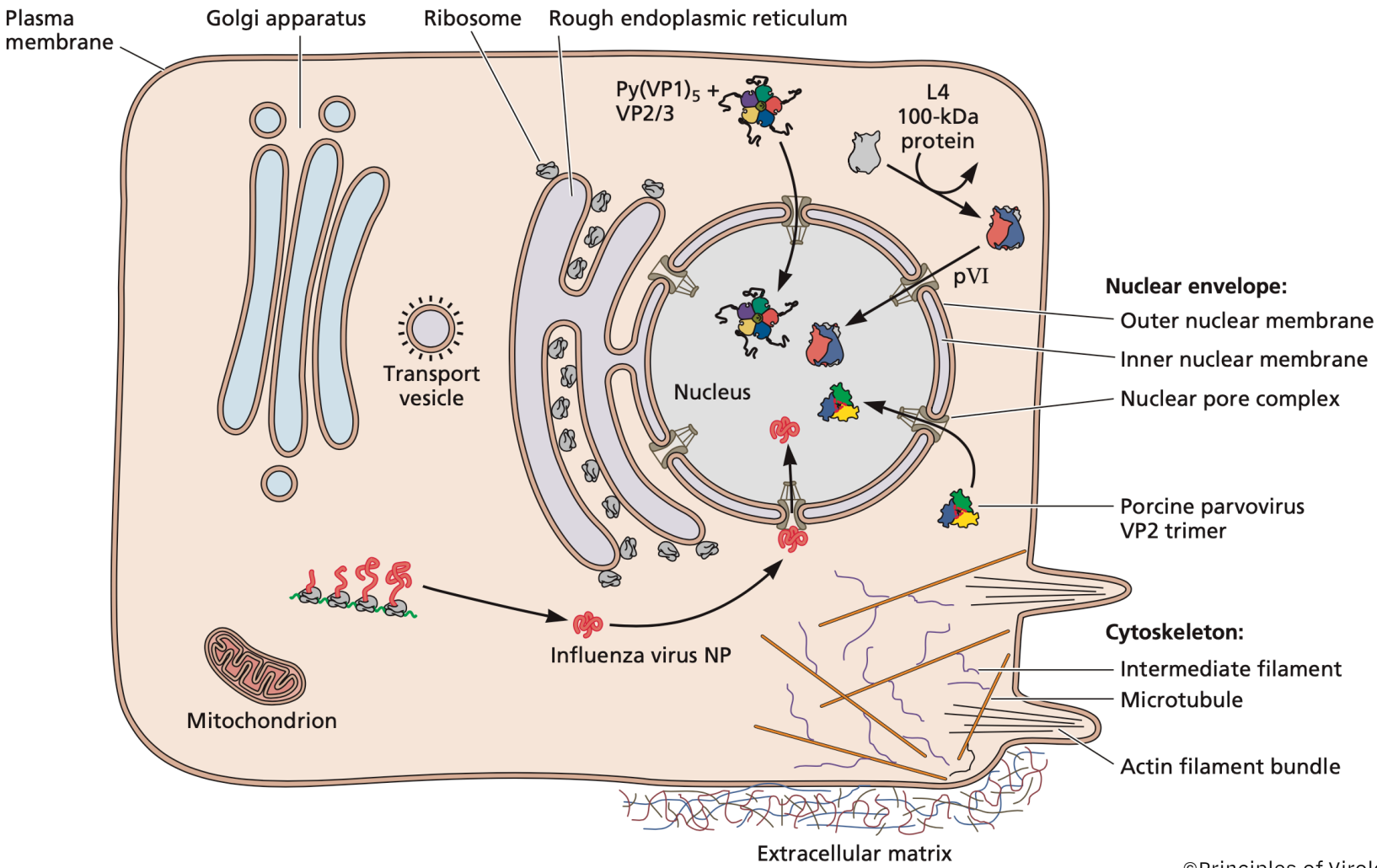
Localization of viral proteins to nucleus



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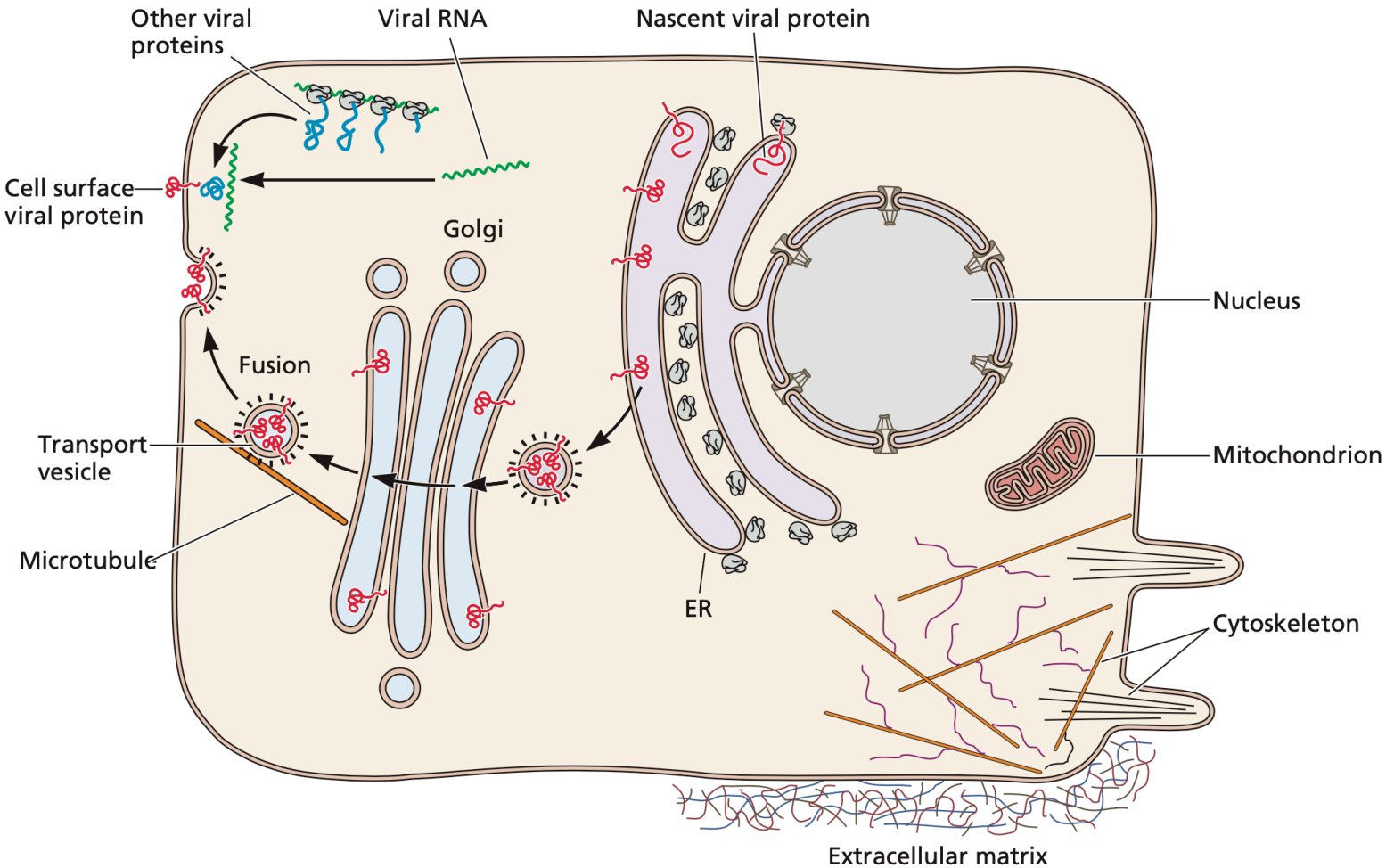
Localization of viral proteins to plasma membrane



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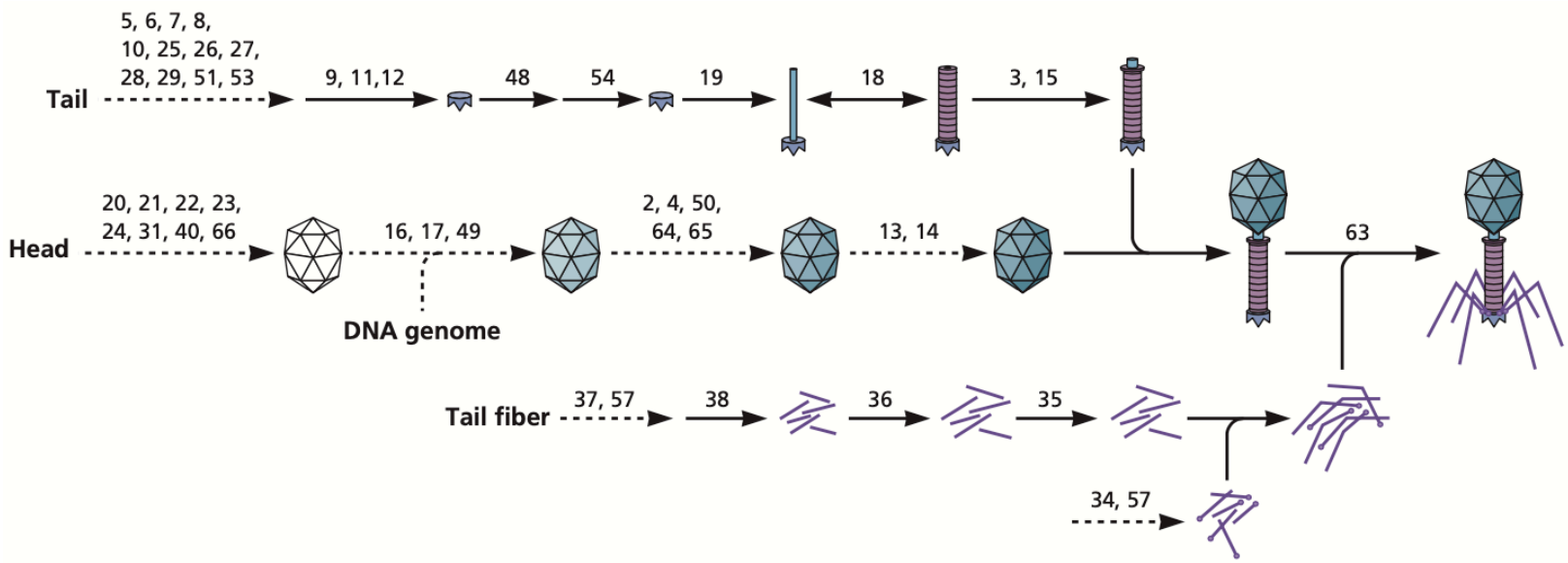
Sub-assemblies



- Formation of discrete intermediate structures
- Ensure orderly formation of viral particles and virion subunits
- Can’t proceed unless previous structure is formed: *quality control*

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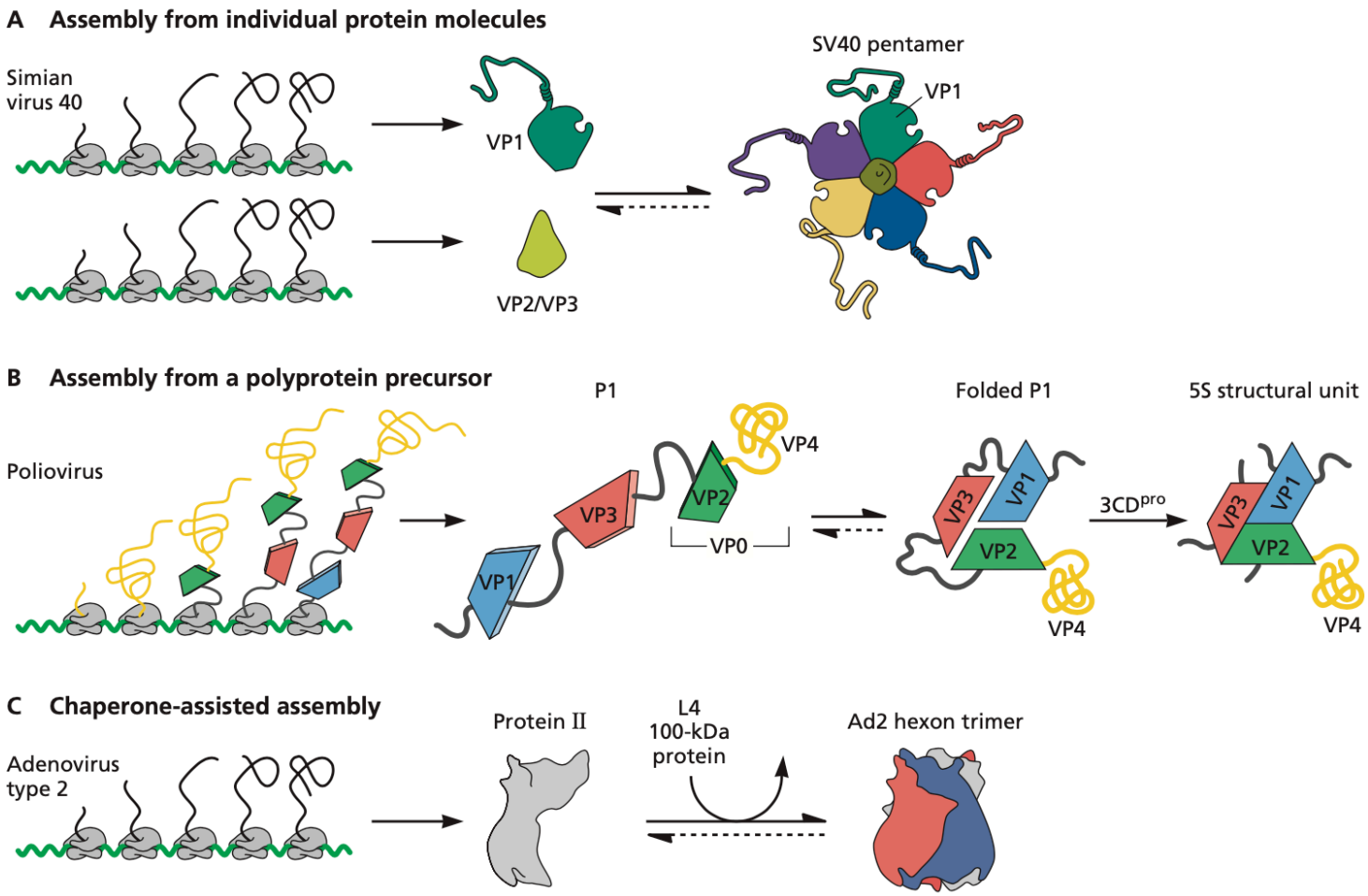
Three strategies for making sub-assemblies



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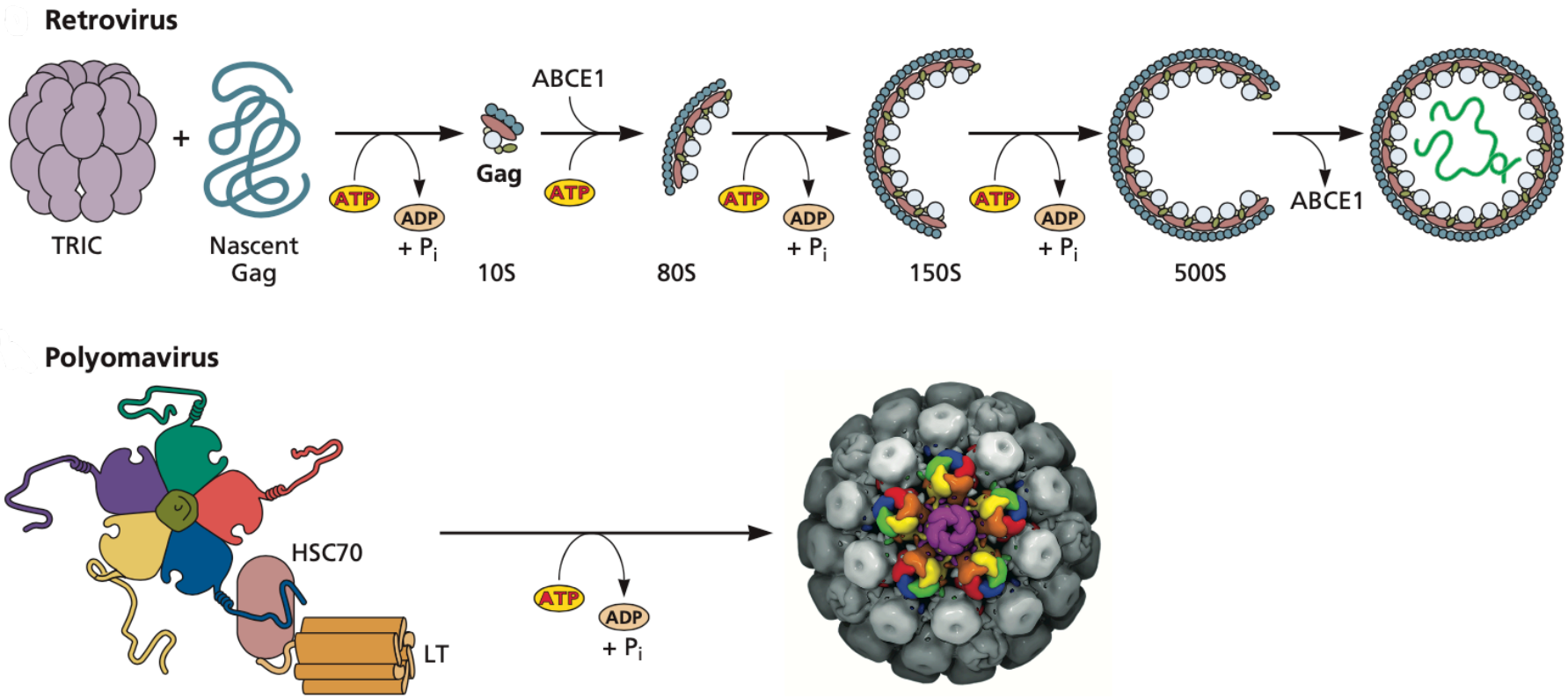
Assembly reactions assisted by cellular chaperones



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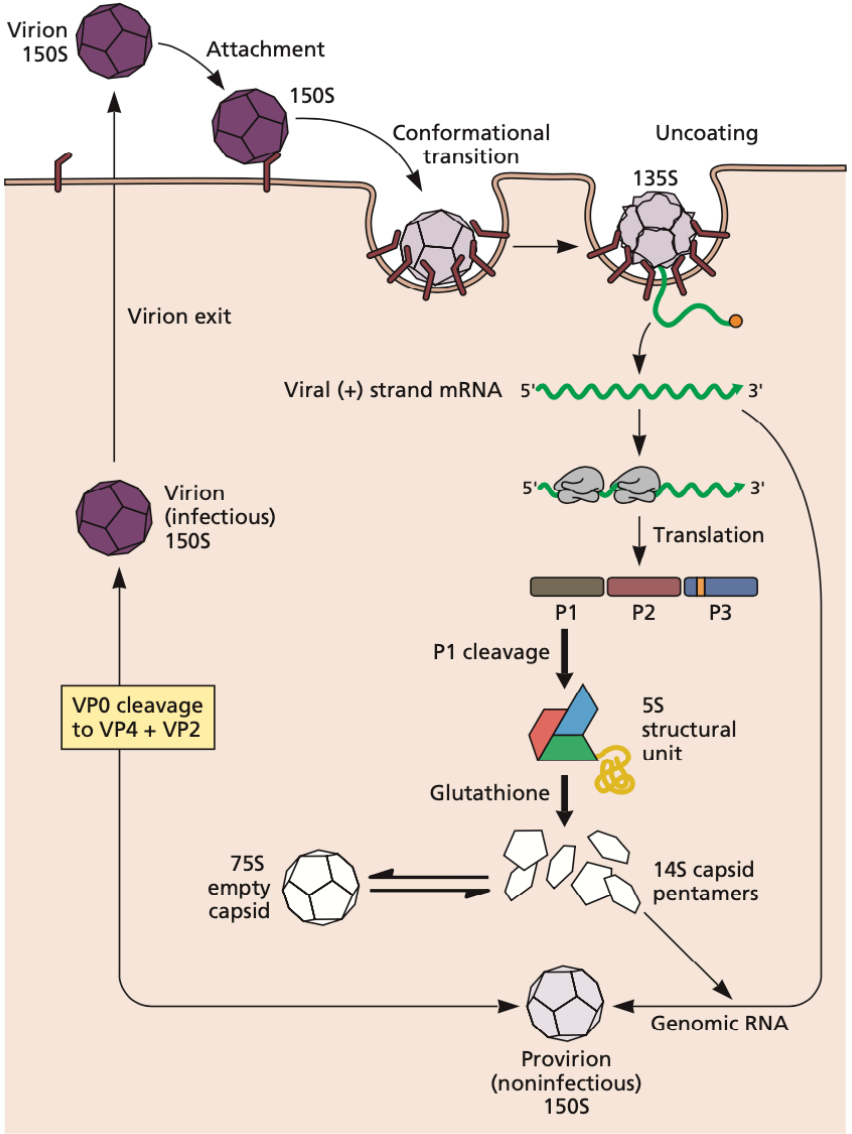
Sequential capsid assembly: poliovirus



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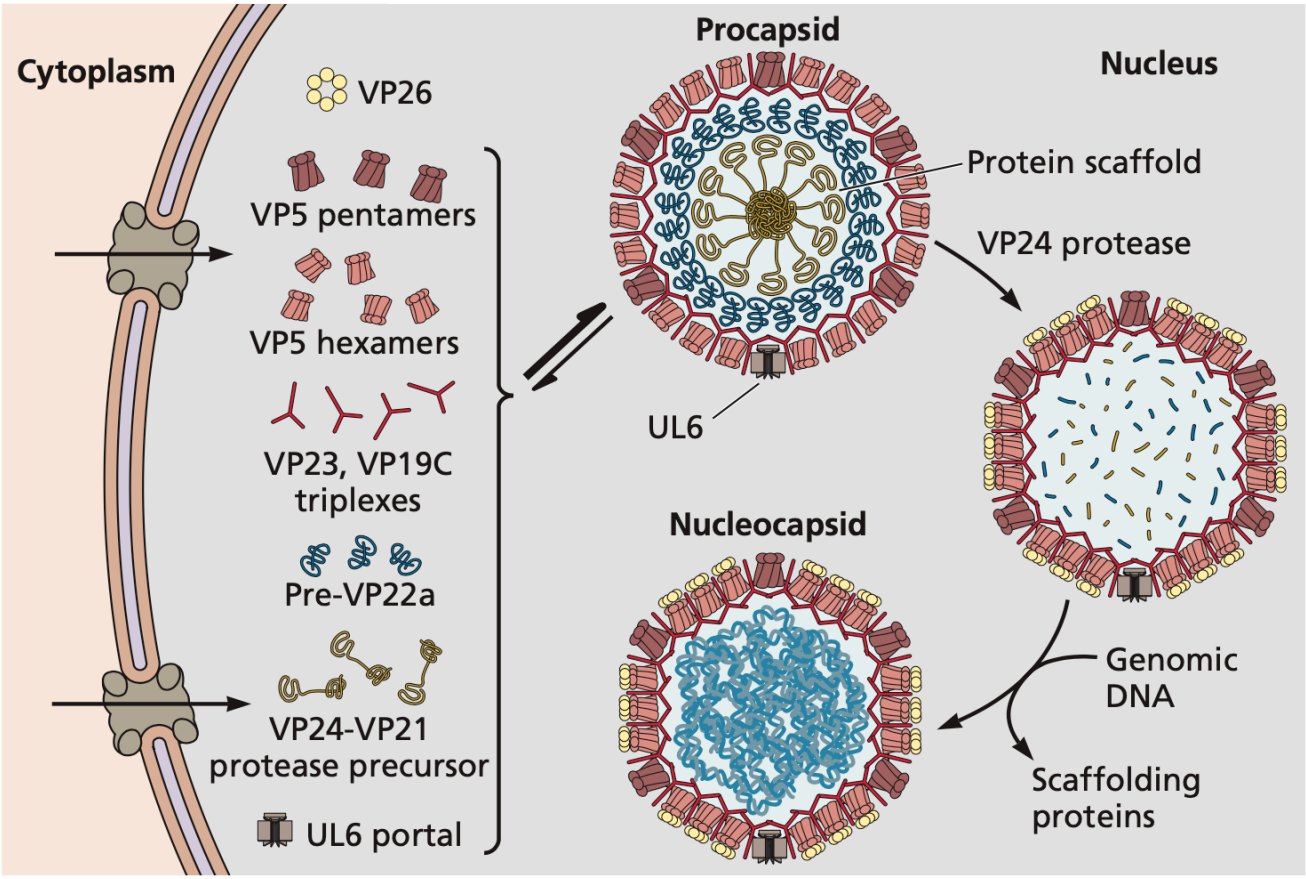
Sequential capsid assembly: herpesvirus



Viral scaffolding proteins

- Establish transient intermediate structures
- Viral proteases packaged in these intermediate structures become activated to finalize structure

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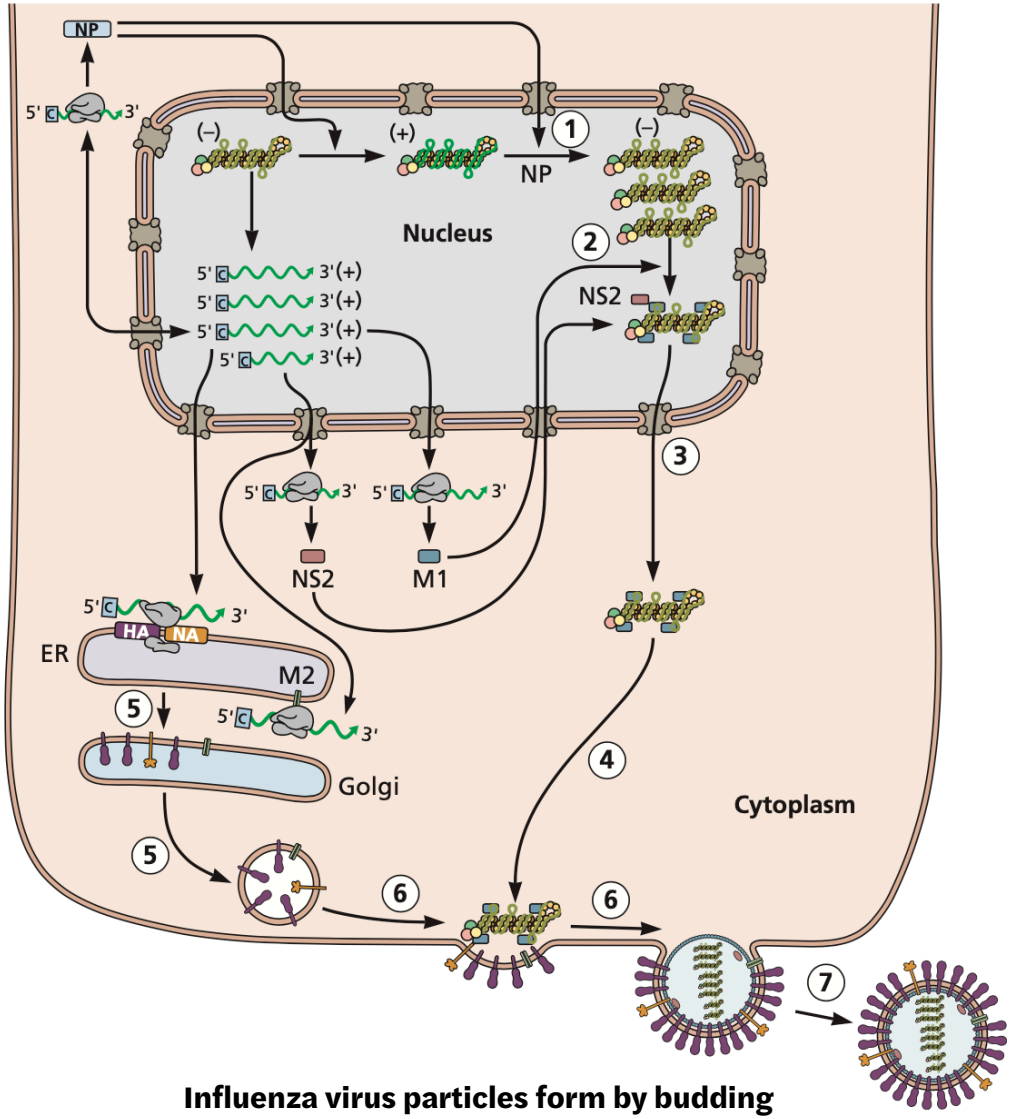
Concerted assembly: Influenza virus

Virus particles assemble only in association with viral genome



Influenza virus particles form by budding

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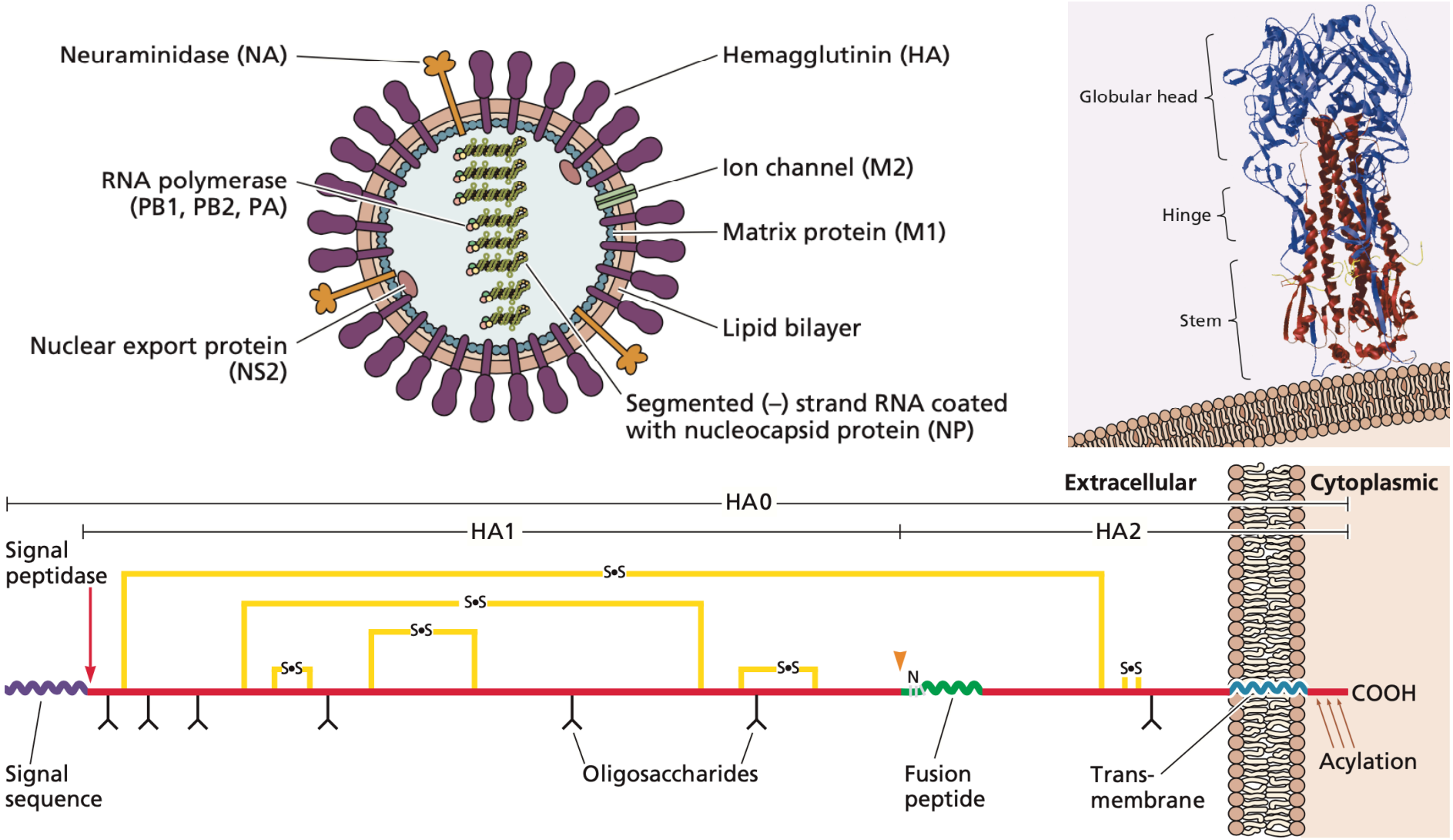

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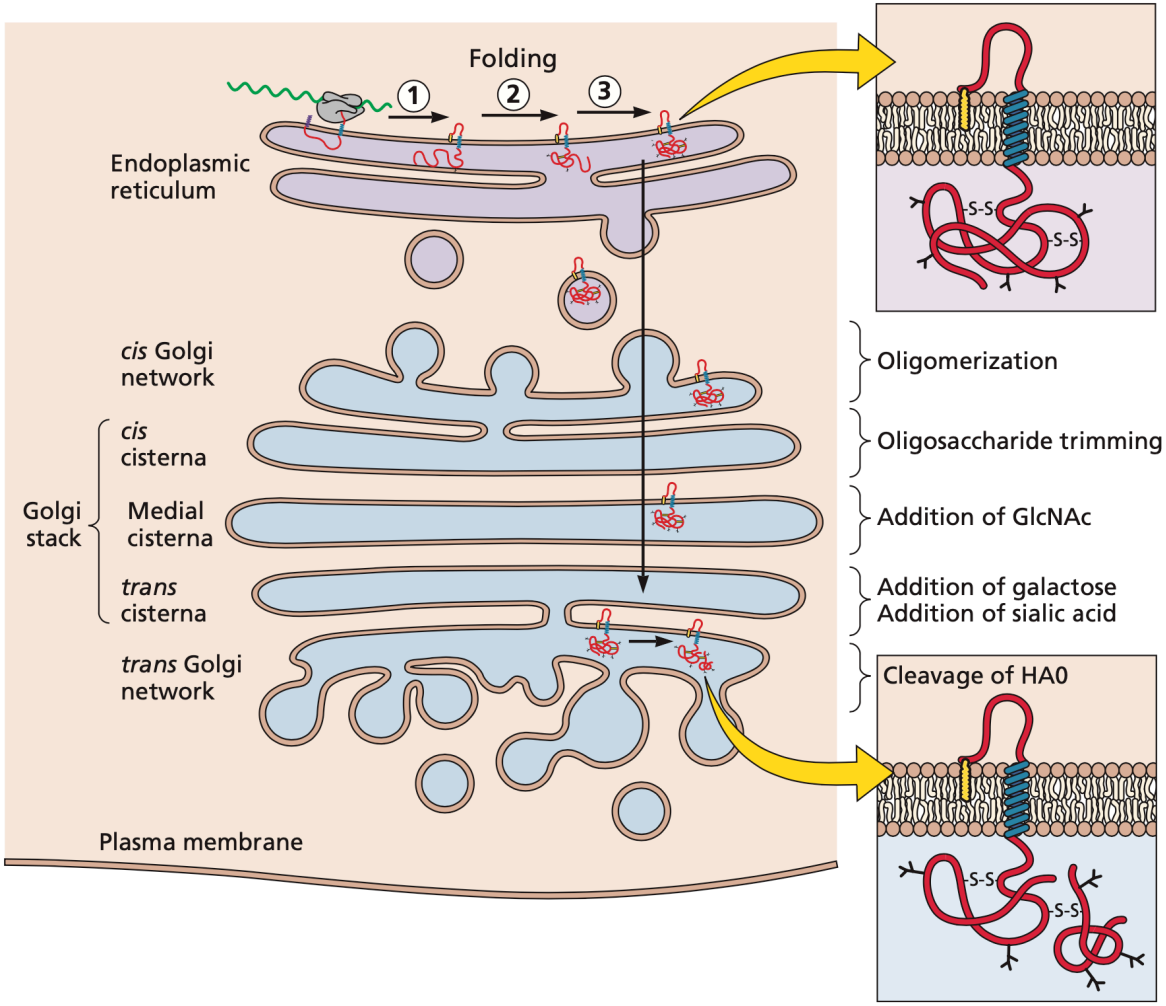
Maturation of influenza HA0



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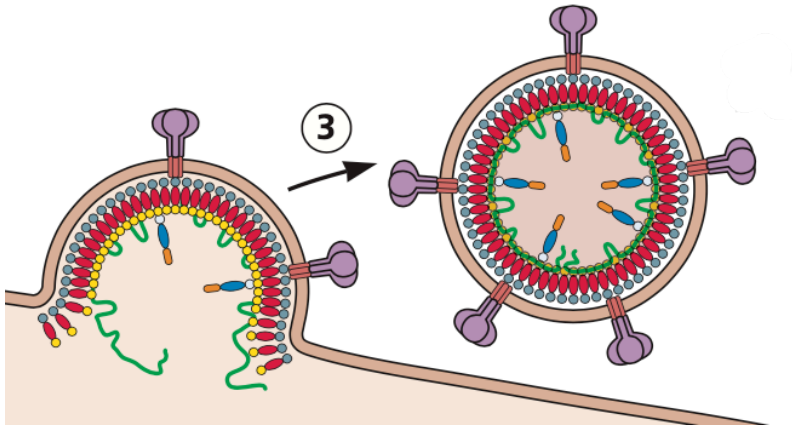
Subassemblies are involved in which of the following types of virus particle production?

A. Concerted assembly
B. Sequential assembly
C. Assembly lines
D. Chaperone-assisted assembly
E. All of the above

Genome packaging



- Problem: Viral genomes must be distinguished from cellular DNA or RNA molecules where assembly takes place
- Solution: **Packaging signals** in the viral genome

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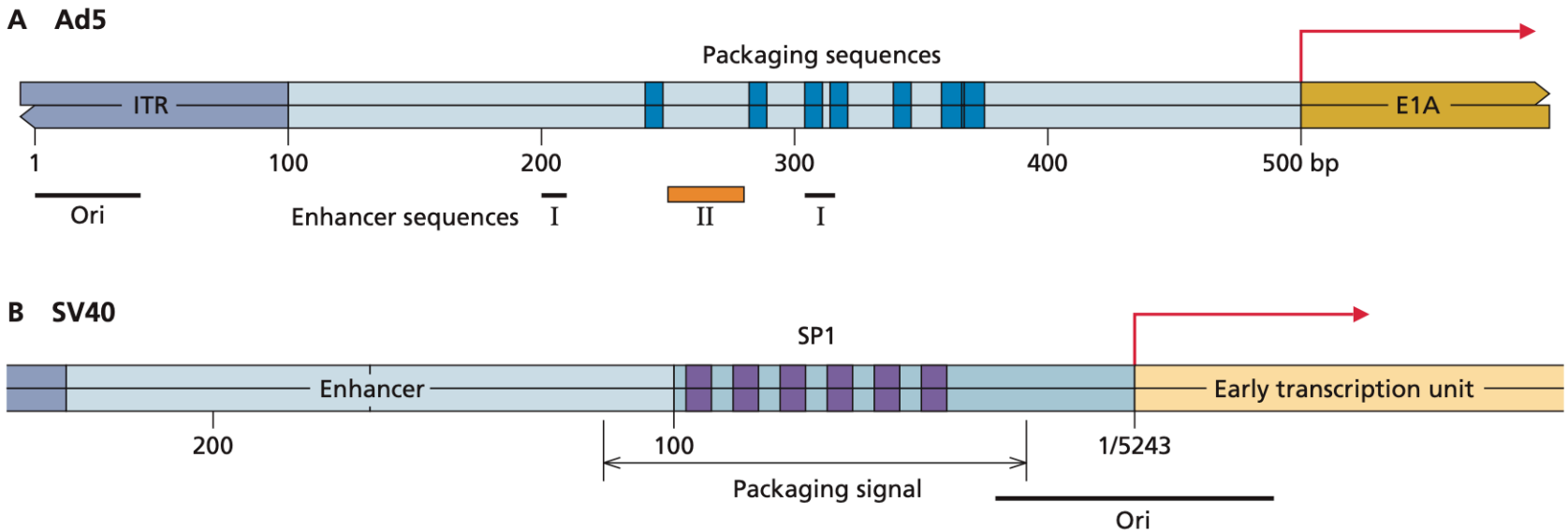
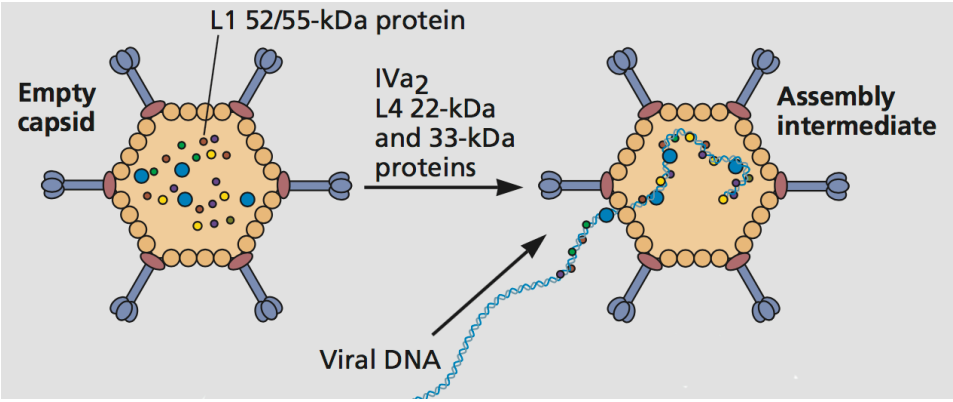
Packaging signals - DNA genomes





Adenovirus

- Packaging signal near left inverted repeat and origin
- Signal is complex: a set of repeated sequences; overlapping with enhancers that stimulate late transcription
- Recognized by viral protein IVa2

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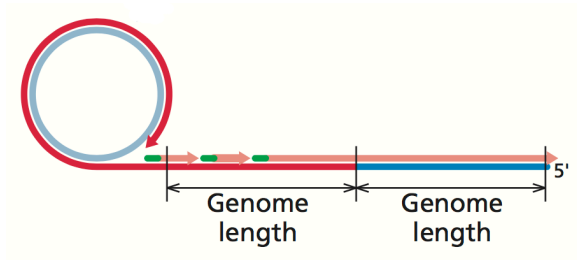

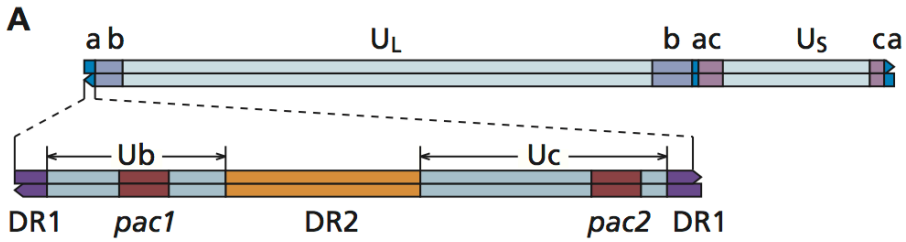

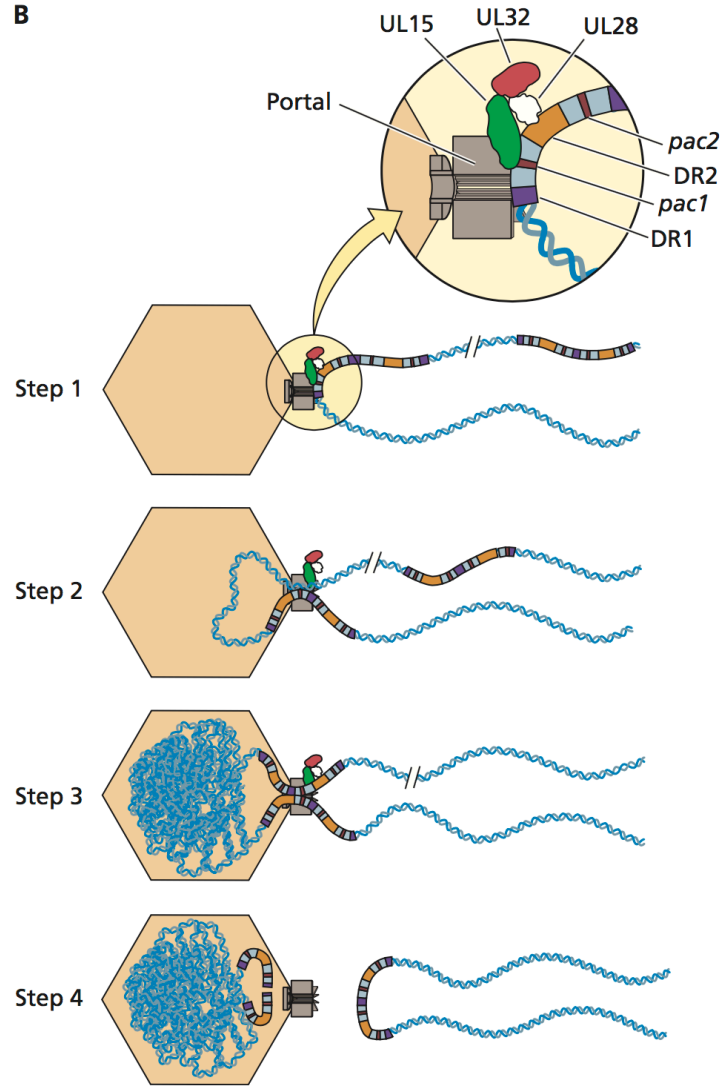

- Herpesvirus genome replication produces concatemers with head-to-tail copies of viral genome

- HSV-1 packaging signals *pac1* and *pac2* needed for recognition of viral DNA and cleavage within DR1

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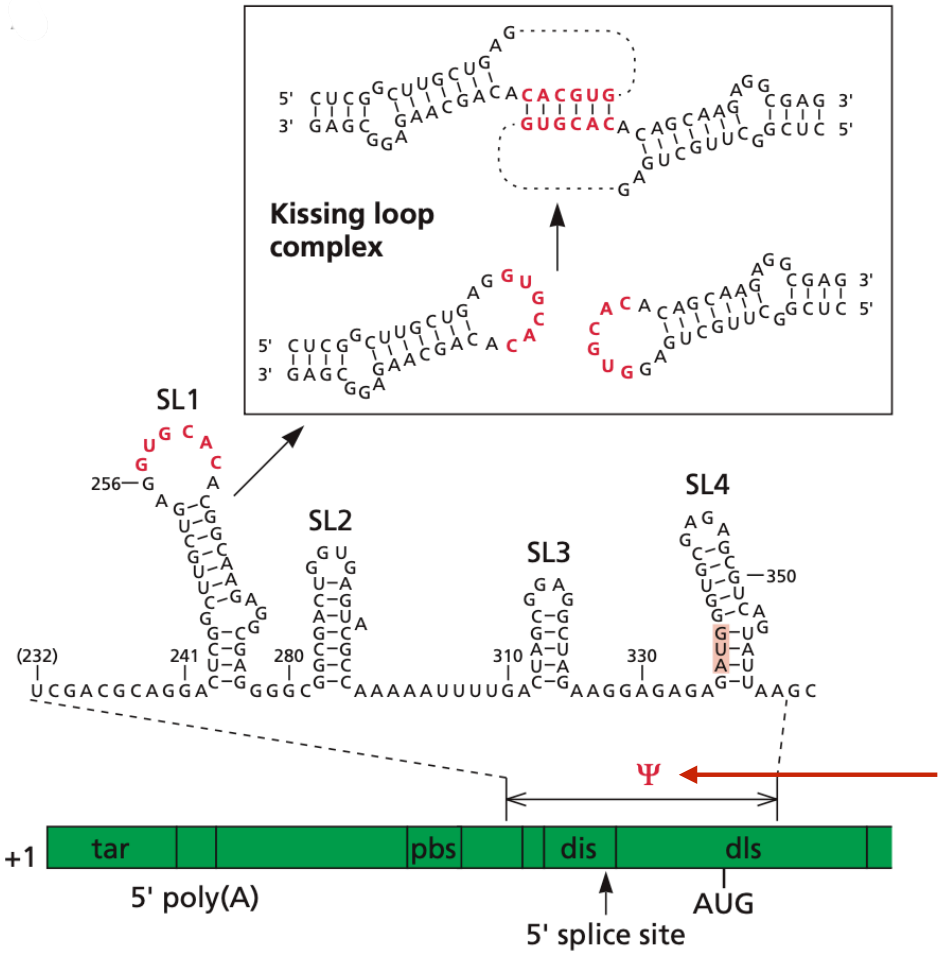
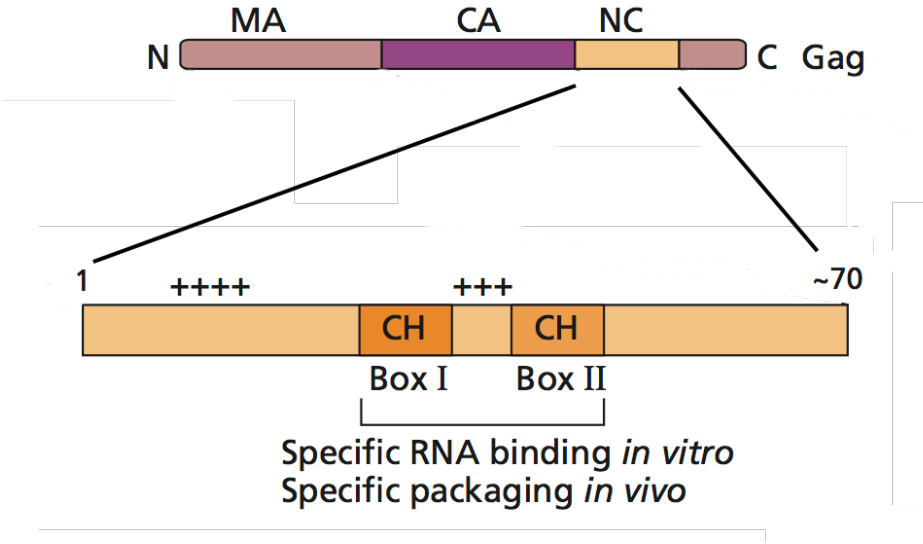
Packaging signals - RNA genomes





Necessary but not sufficient for HIV-1 genome packaging

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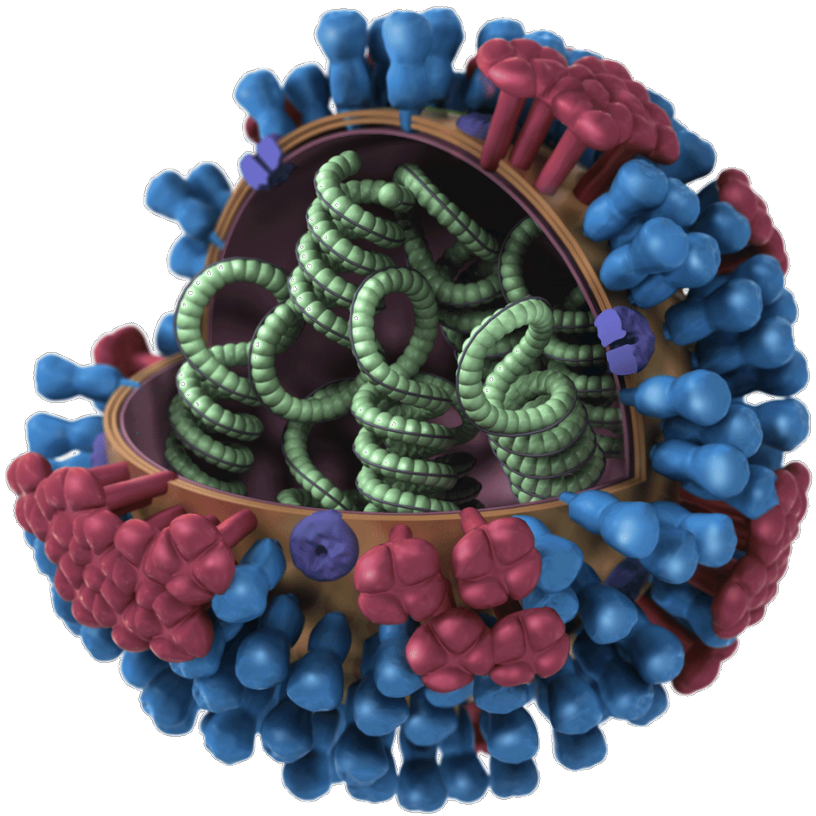
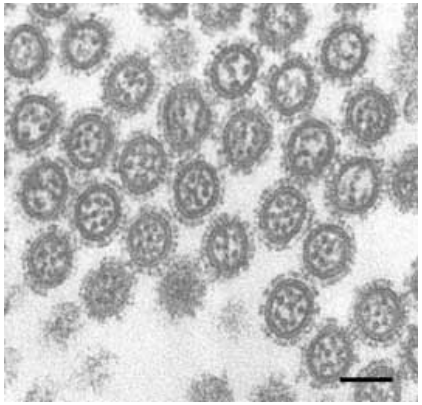
Packaging of segmented genomes

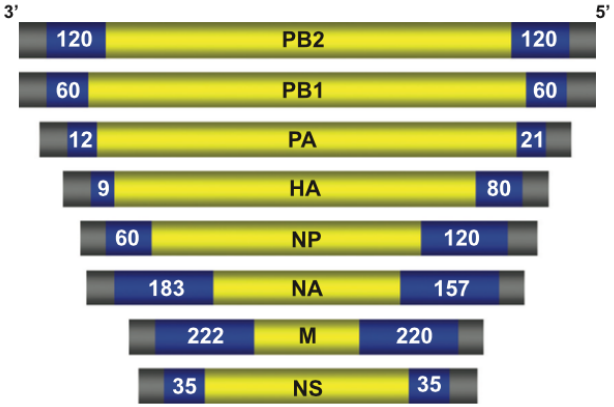


- *Random* mechanism would yield 1 infectious particle per 400 assembled - within known particle:pfu ratio
- Evidence for *specific* packaging sequence on each RNA segment

Influenza virus RNA packaging

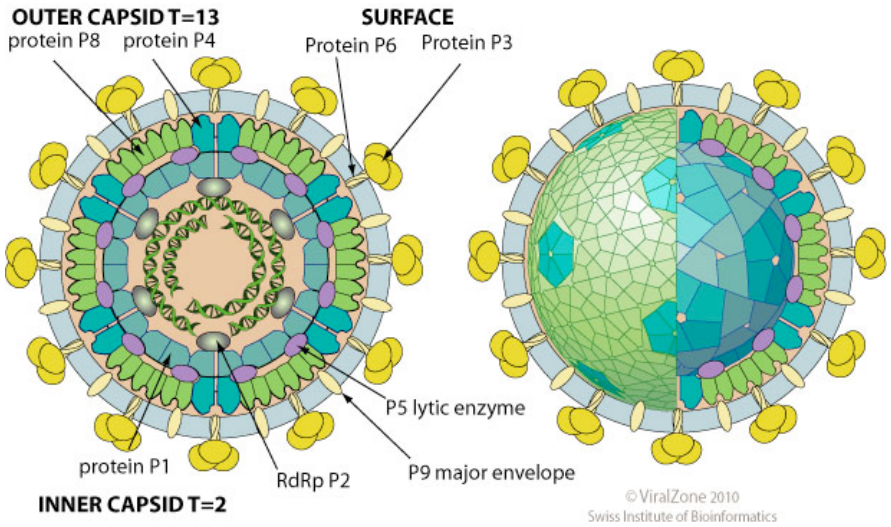
- Always 8 RNA segments
- Segments oriented perpendicular to budding tip
- HA, NS signals swapped
- RNA-RNA or RNA-protein interactions





http://www.virology.ws/2009/06/26/packaging-of-the-segmented-influenza-rna-genome/
http://www.virology.ws/2009/09/15/what-if-influenza-virus-did-not-reassort/

Selective packaging



- Bacteriophage ϕ6 - 3 dsRNA segments S, M, L
- Serial dependence of packaging: S-M-L
- Particle:pfu ratio ~1

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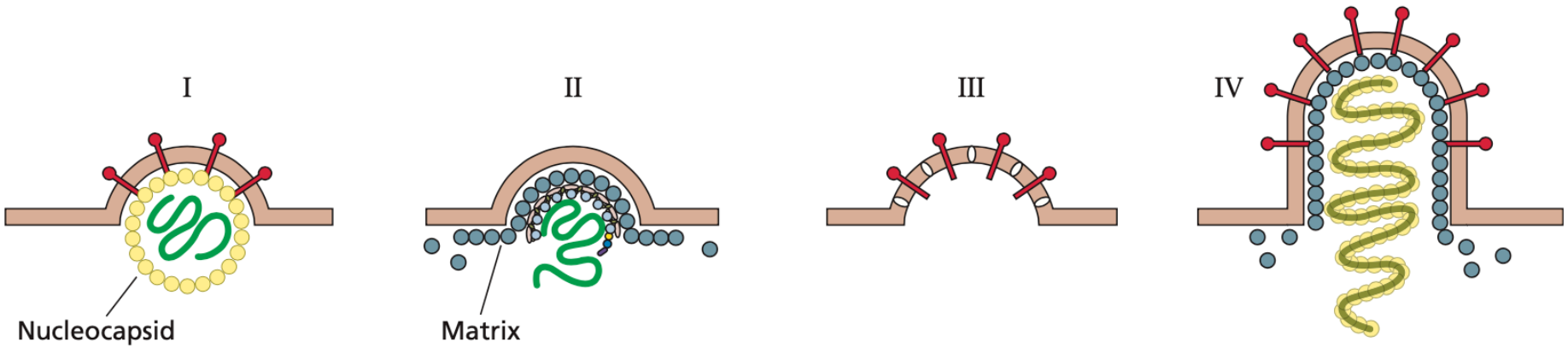
Packaging signals on viral _____ interact with viral _____ during virus assembly.

A. Lipids, proteins
B. Proteins, subassemblies
C. Genomes, proteins
D. Proteases, membranes
E. Proteins, genomes

Acquisition of an envelope

- After assembly of internal structures (most enveloped viruses)
- Simultaneous with assembly of internal structures (retroviruses)



Envelope glycoproteins and capsid essential for budding - alphaviruses

Internal matrix or capsid proteins drive budding - retroviruses

Envelope proteins drive budding - influenza virus, coronavirus

Matrix proteins drive budding, but additional components (glycoproteins, RNP) needed for efficiency or accuracy

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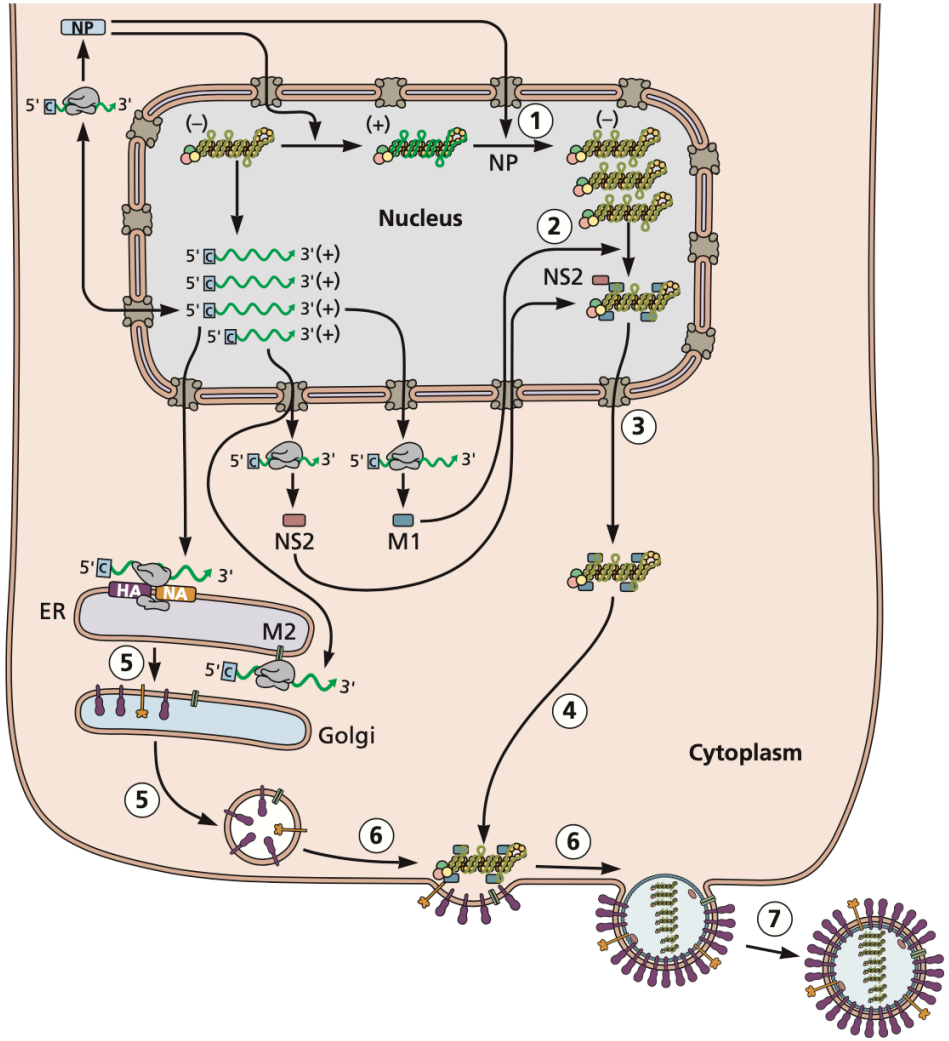
Influenza virus budding

Internal structure assembly and budding spatially & temporally separated



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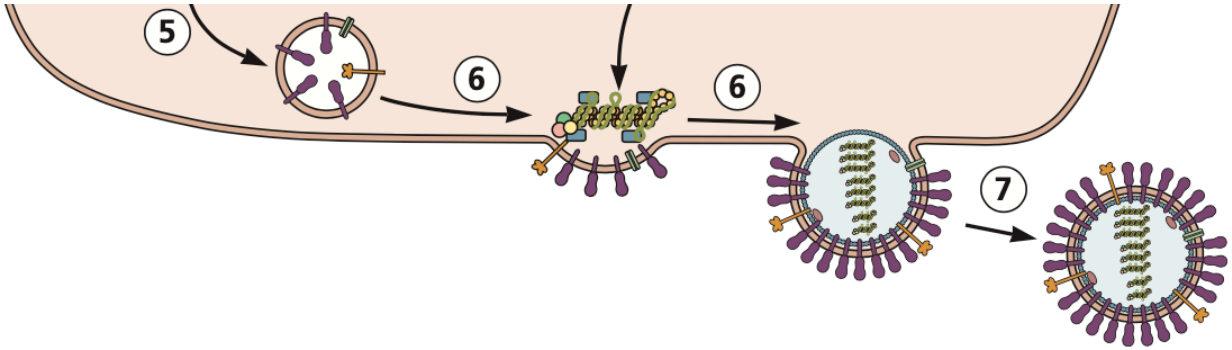
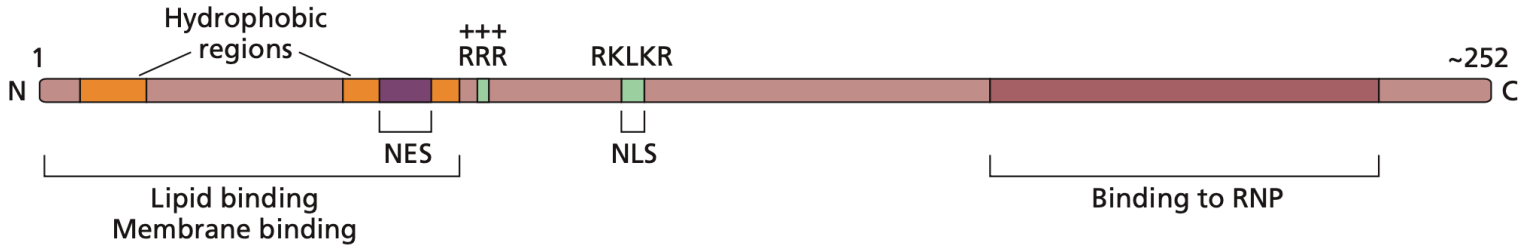
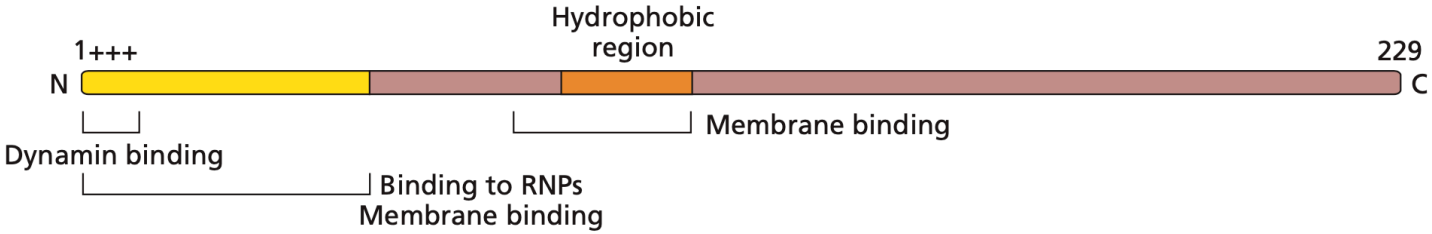
Membrane targeting sequences



A Influenza virus M1



B VSV M



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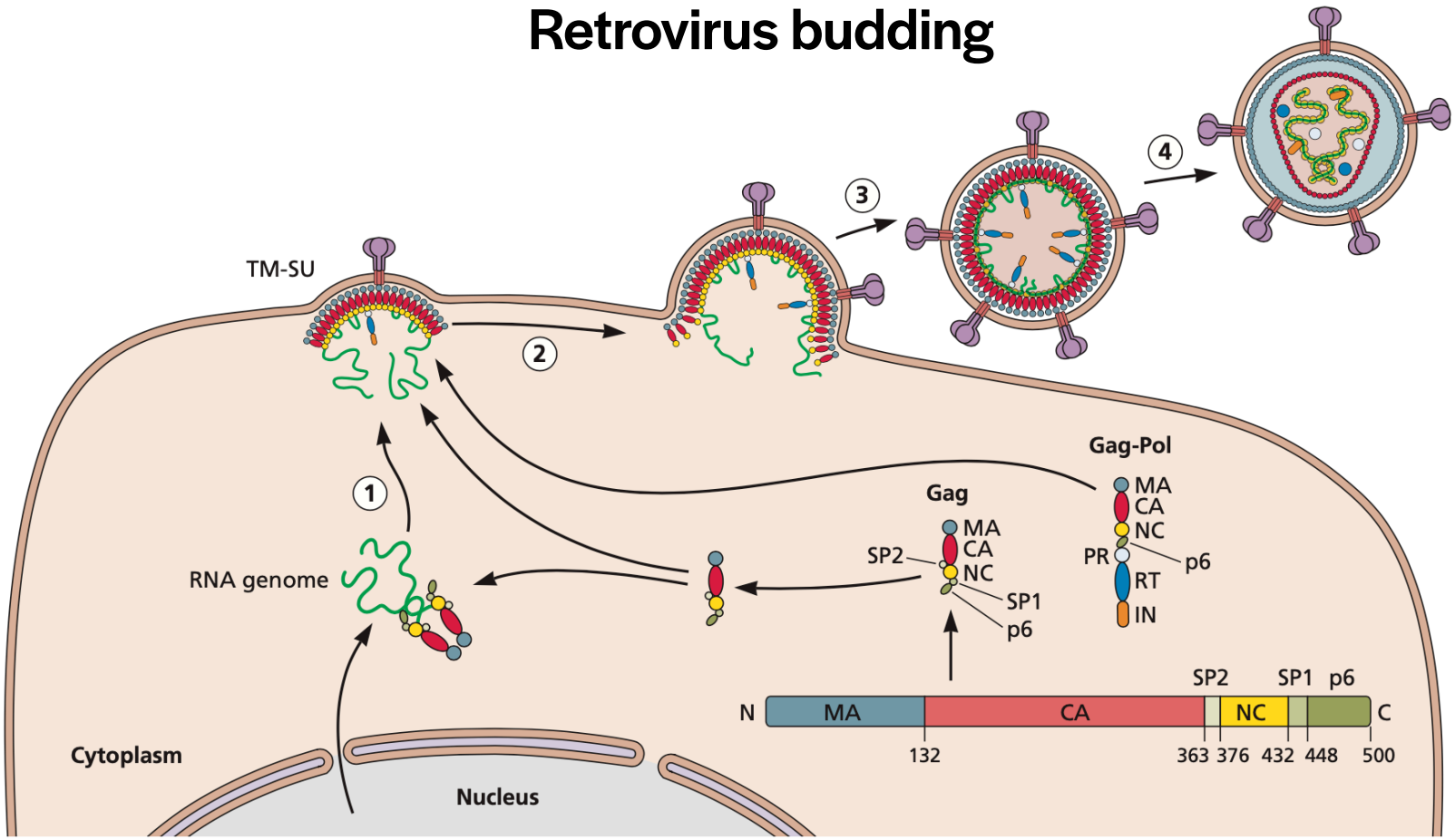
Retrovirus budding



- Gag alone produces virus-like particles
- Internal structure assembly and budding spatially & temporally coincident

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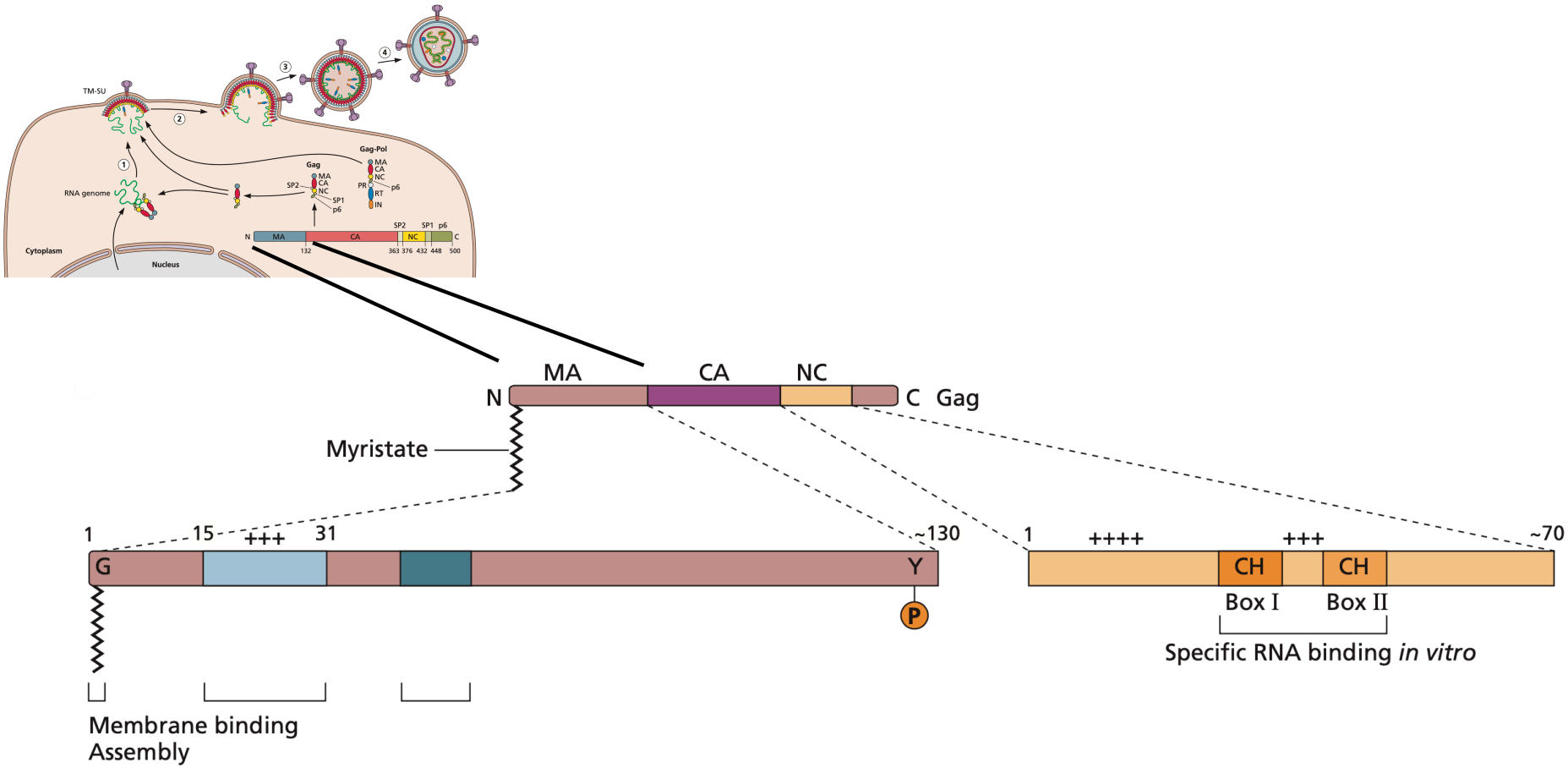

- Changes at myristoylation sequence prevent interaction of Gag with the cytoplasmic face of the plasma membrane
- Virus assembly and budding are inhibited

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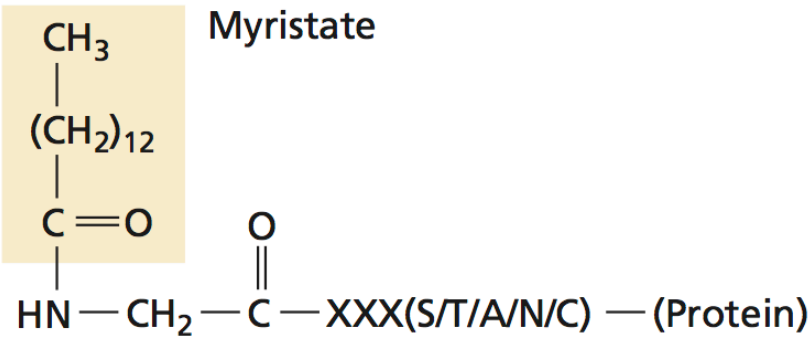

- Addition of lipid to viral proteins allows targeting to membranes independent of signal sequence

- Viral proteins are synthesized in the cytoplasm, and modified with lipids post-translationally

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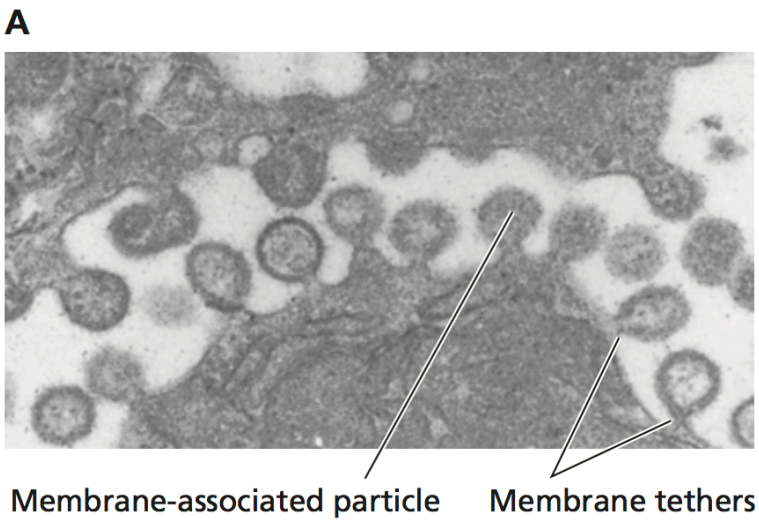

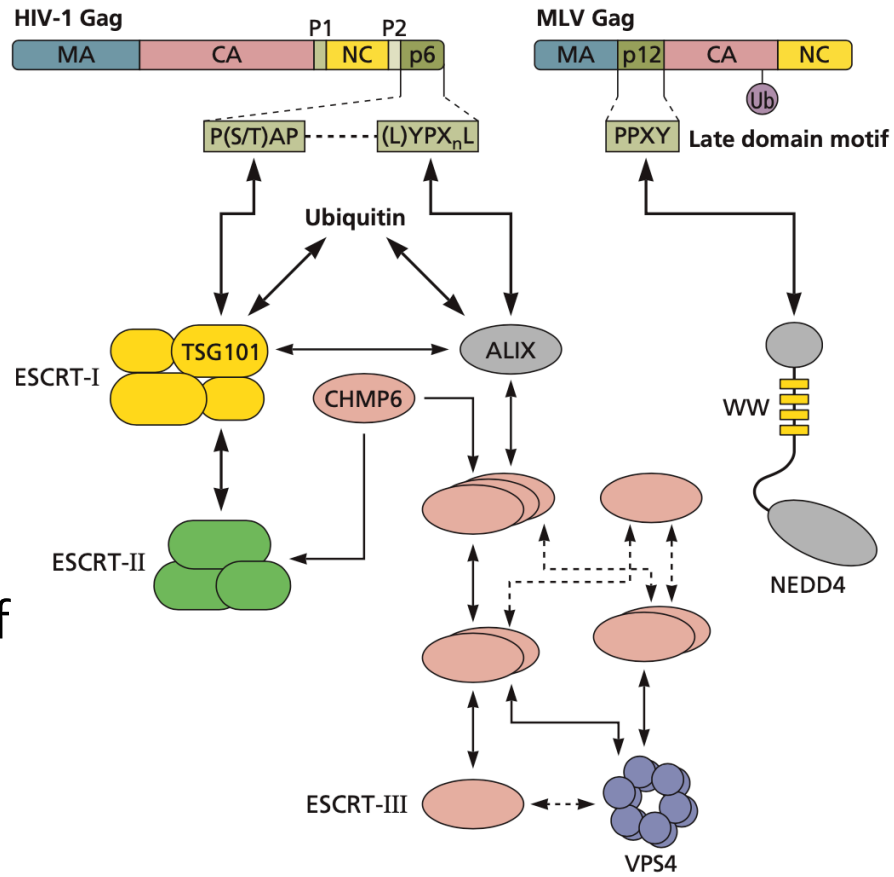

- Amino acid changes in Gag cause arrest of budding at late stage (late or L domains)
- Found in + and - strand enveloped viruses
- L domains bind cell proteins involved in vesicle trafficking, needed for virus release

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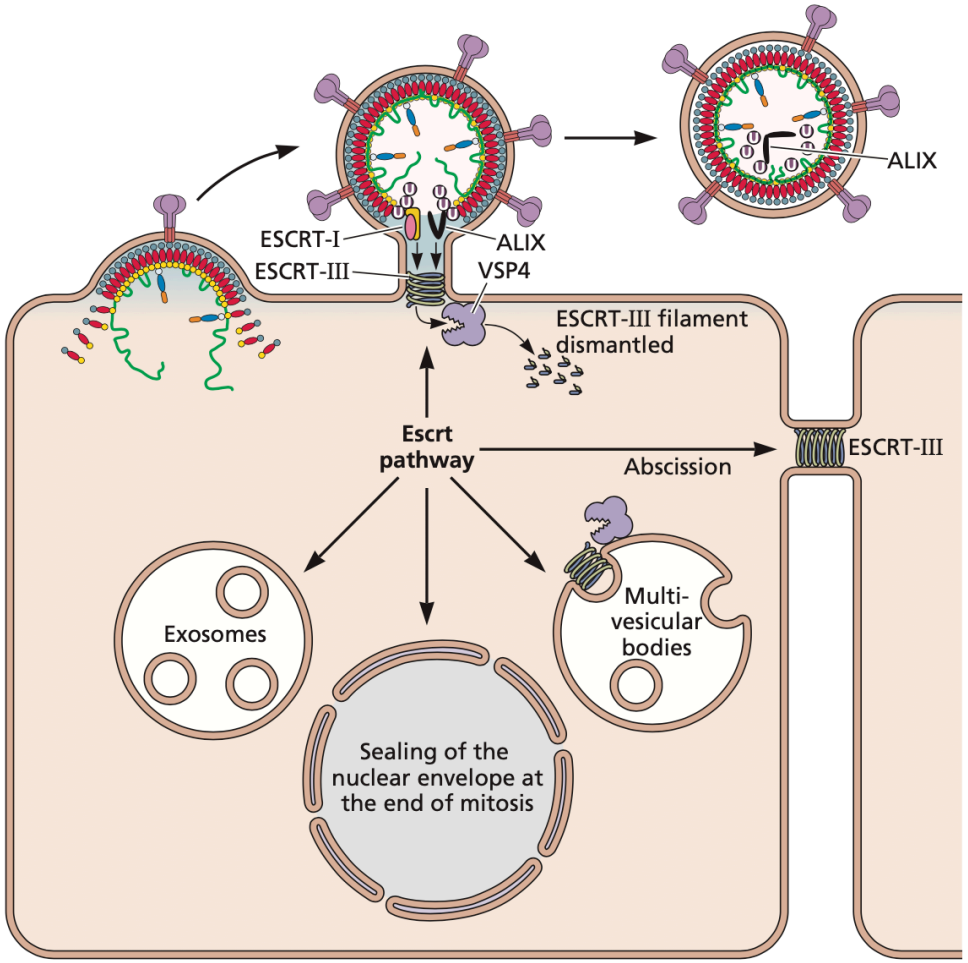
Endosomal sorting complexes required for transport (ESCRT) machinery



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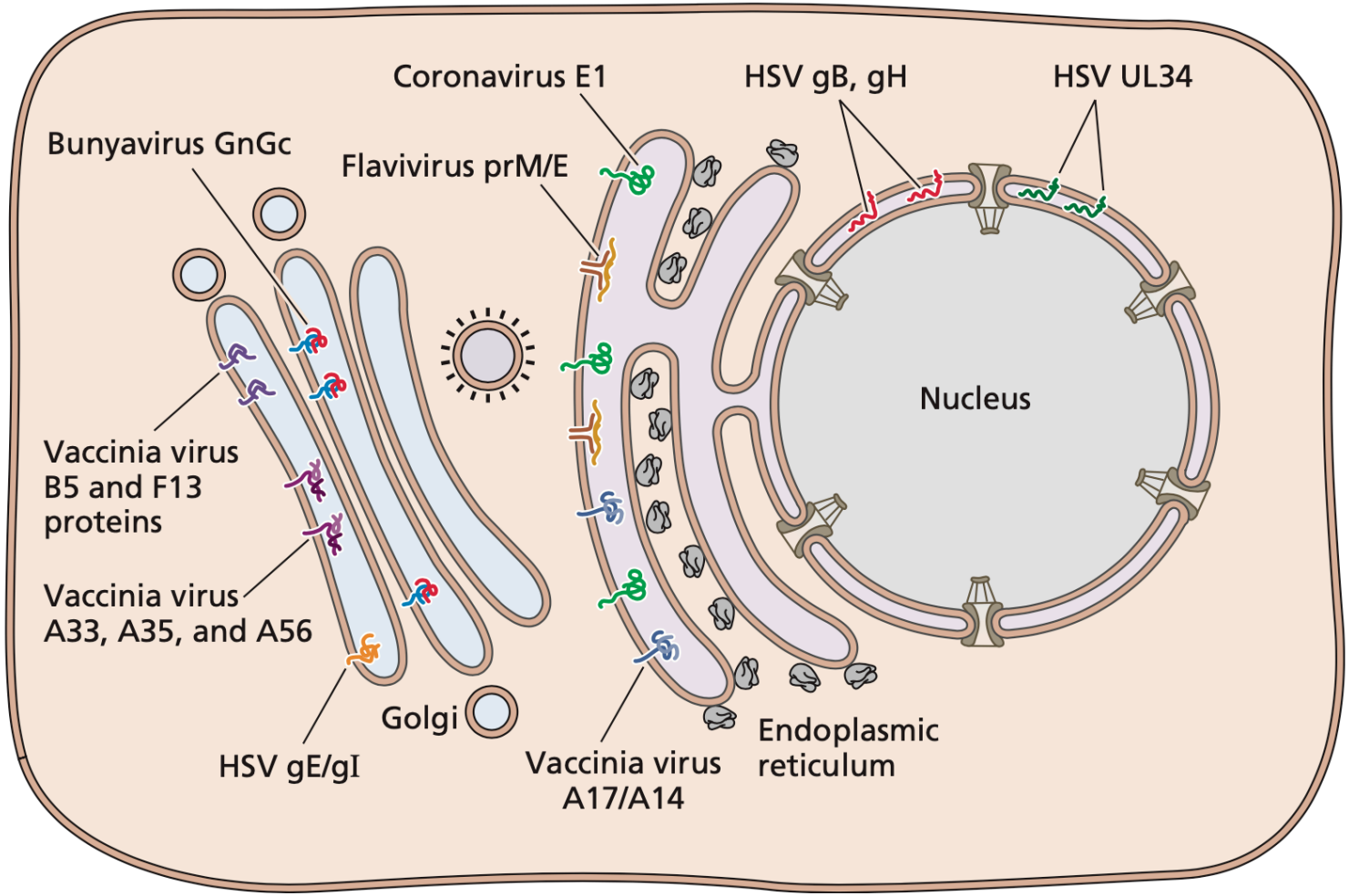
Sorting of viral glycoproteins to internal membranes



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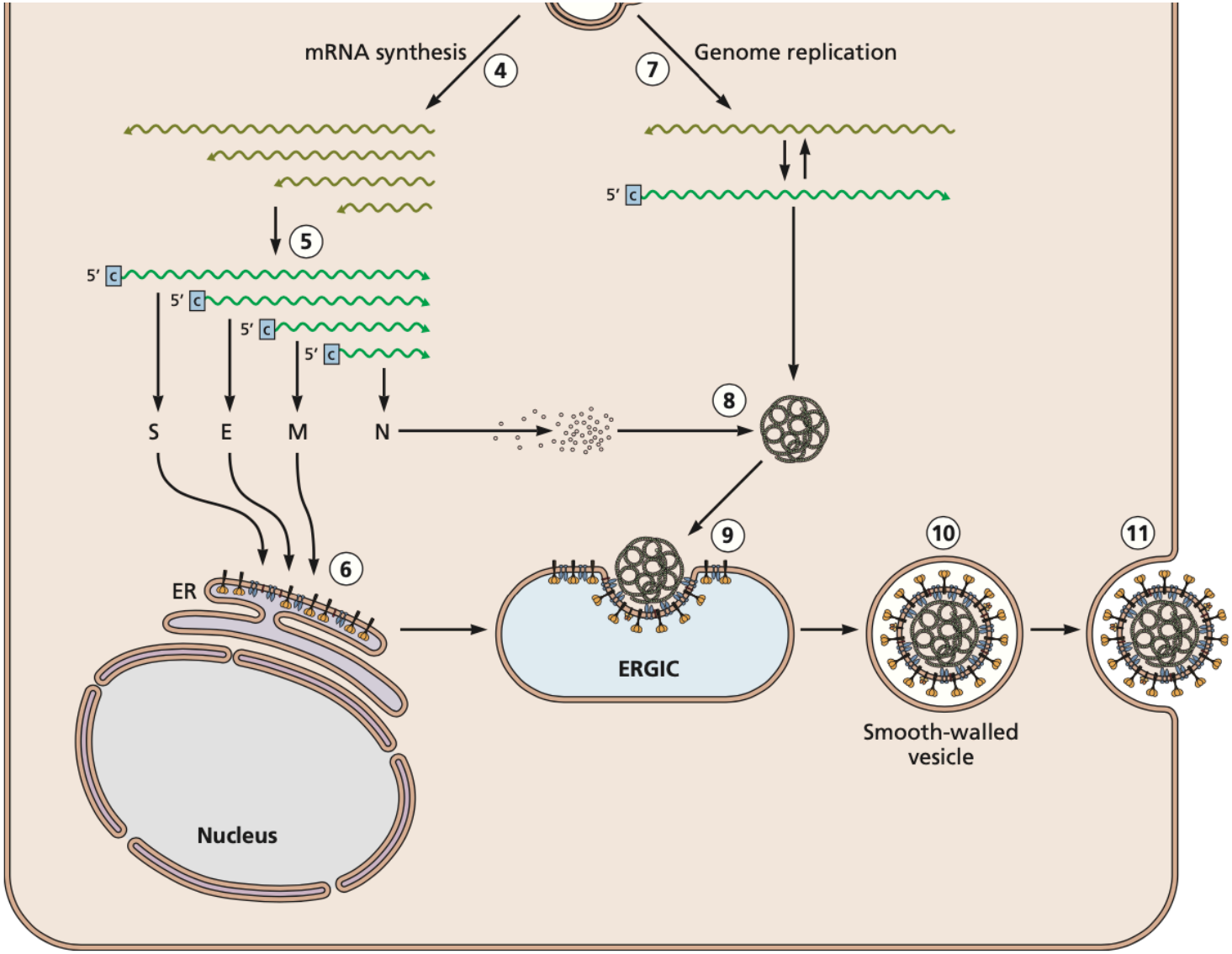

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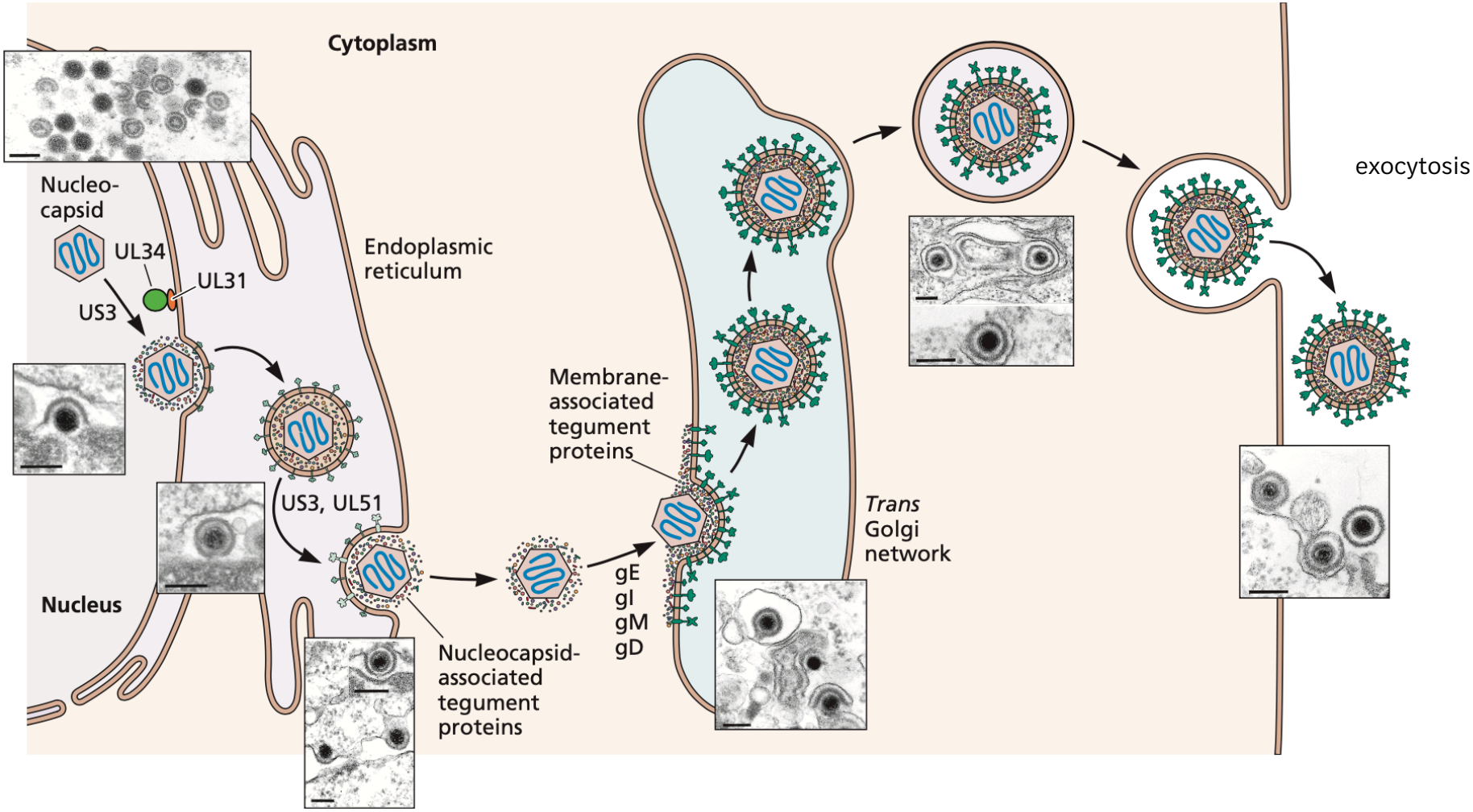
Which statement about viral budding is incorrect?

A. The envelope can be acquired before or simultaneous with assembly of internal components
B. The viral spike glycoprotein can drive budding
C. No host proteins are involved in the budding process
D. Lipids assist structural proteins to interact with the membrane
E. Budding can occur from the nucleus, ER, Golgi, or plasma membrane

Herpesvirus assembly and egress



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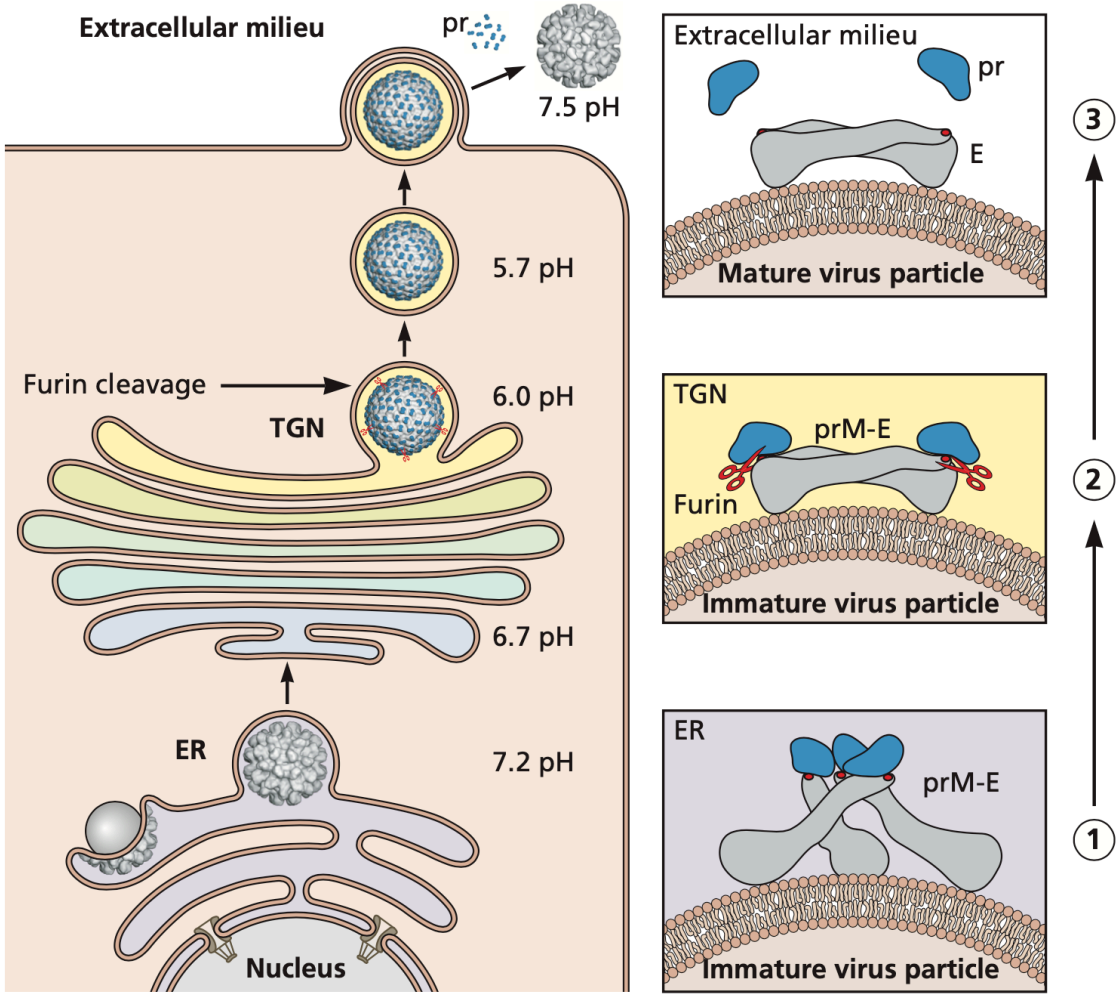
Low pH induced conformational change and maturation

Dengue virus



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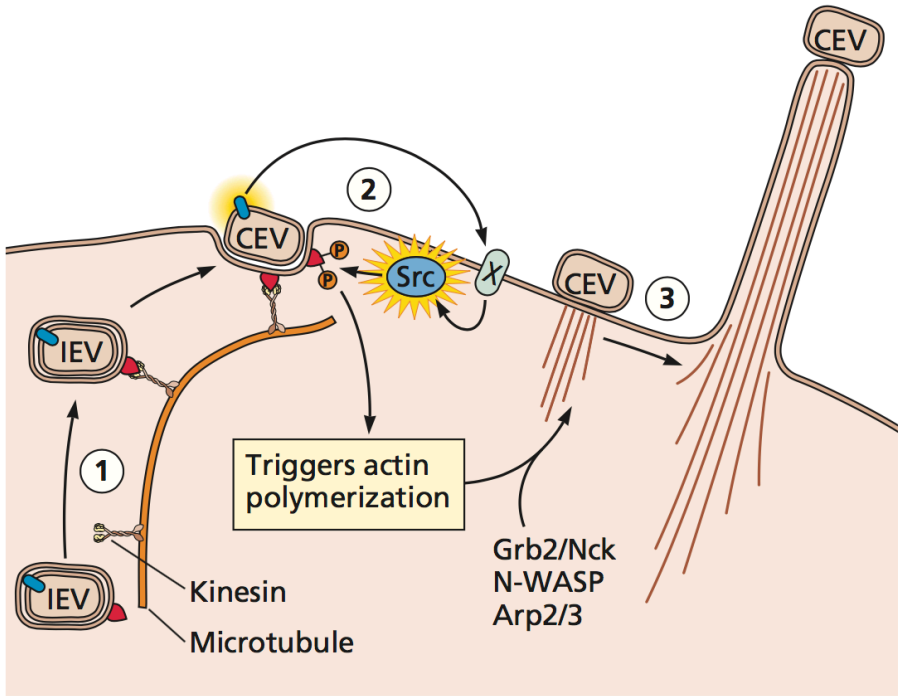
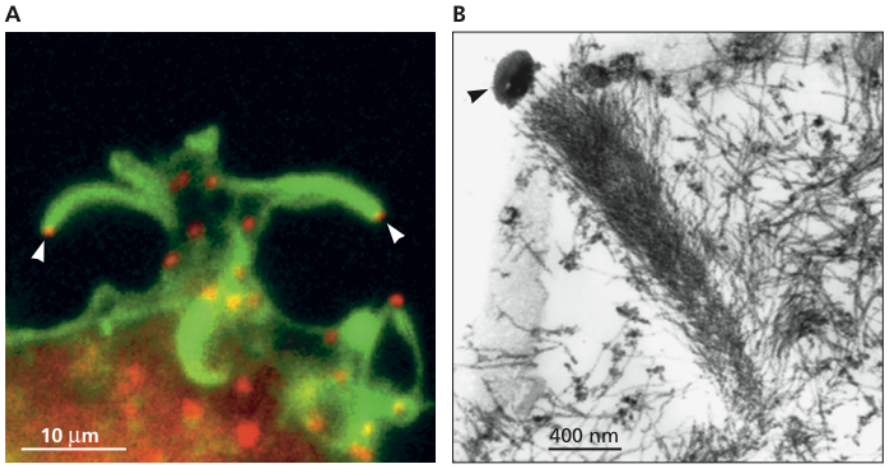
Leaving the cell:
Propulsion of vaccinia virus on actin tails





IEV = intracellular enveloped virion
CEV = cell associated enveloped virion

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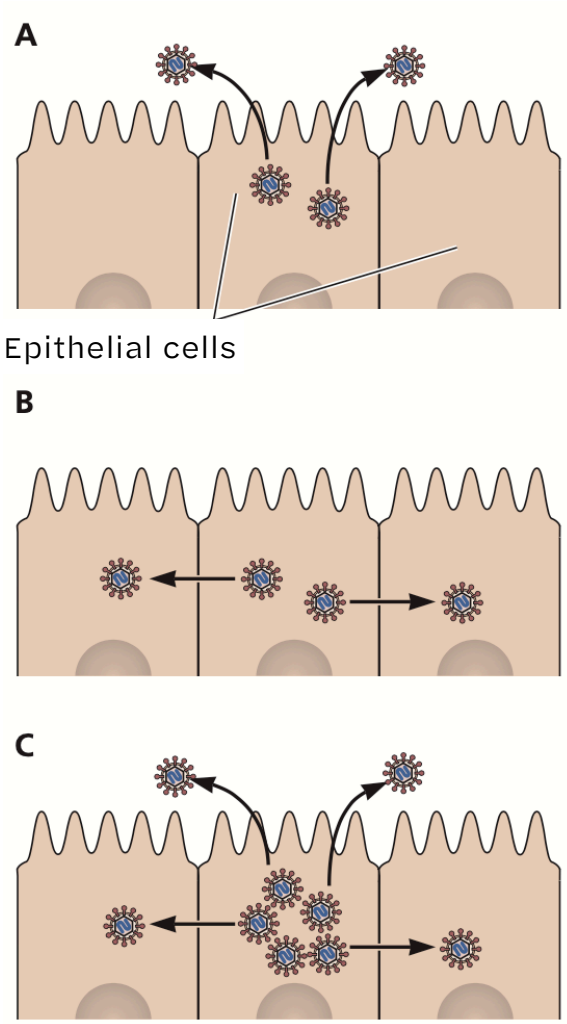
Leaving the cell



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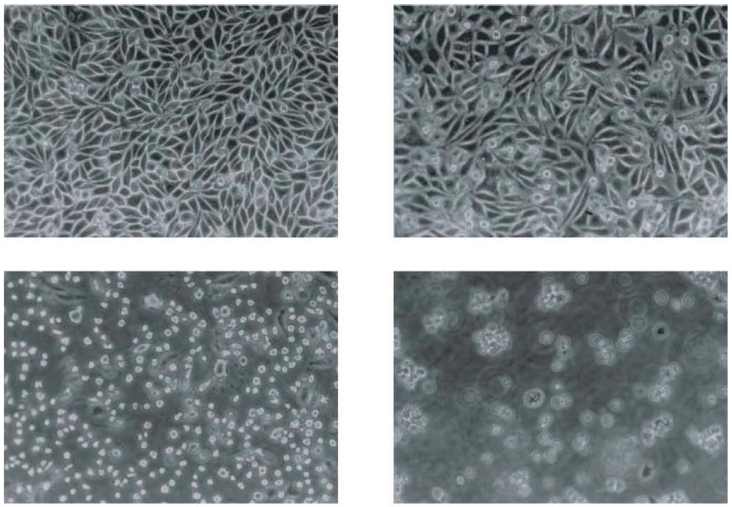
Release of non-enveloped viruses



- Cell lysis: apoptosis, necroptosis
- Viral proteins that induce rupture of cell membranes
  - Viroporins form pores in cell membranes (polyomavirus)
- Loss of membrane integrity with inhibition of protein synthesis

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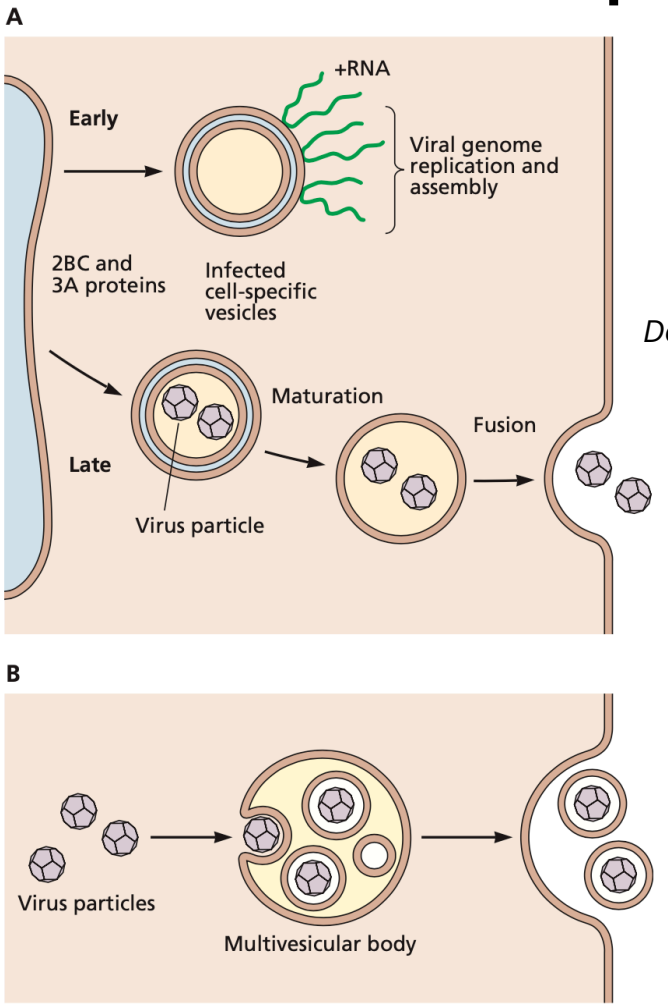
Non-lytic release of nonenveloped viruses



Double-membrane vesicles formed by autophagy

Exosomes

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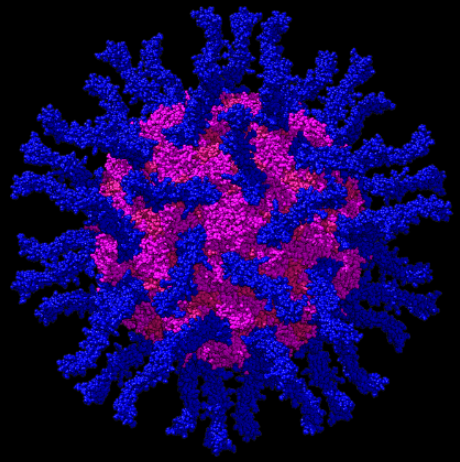

VIROLOGY LIVE

WITH VINCENT RACANIELLO

Next time: The infected cell