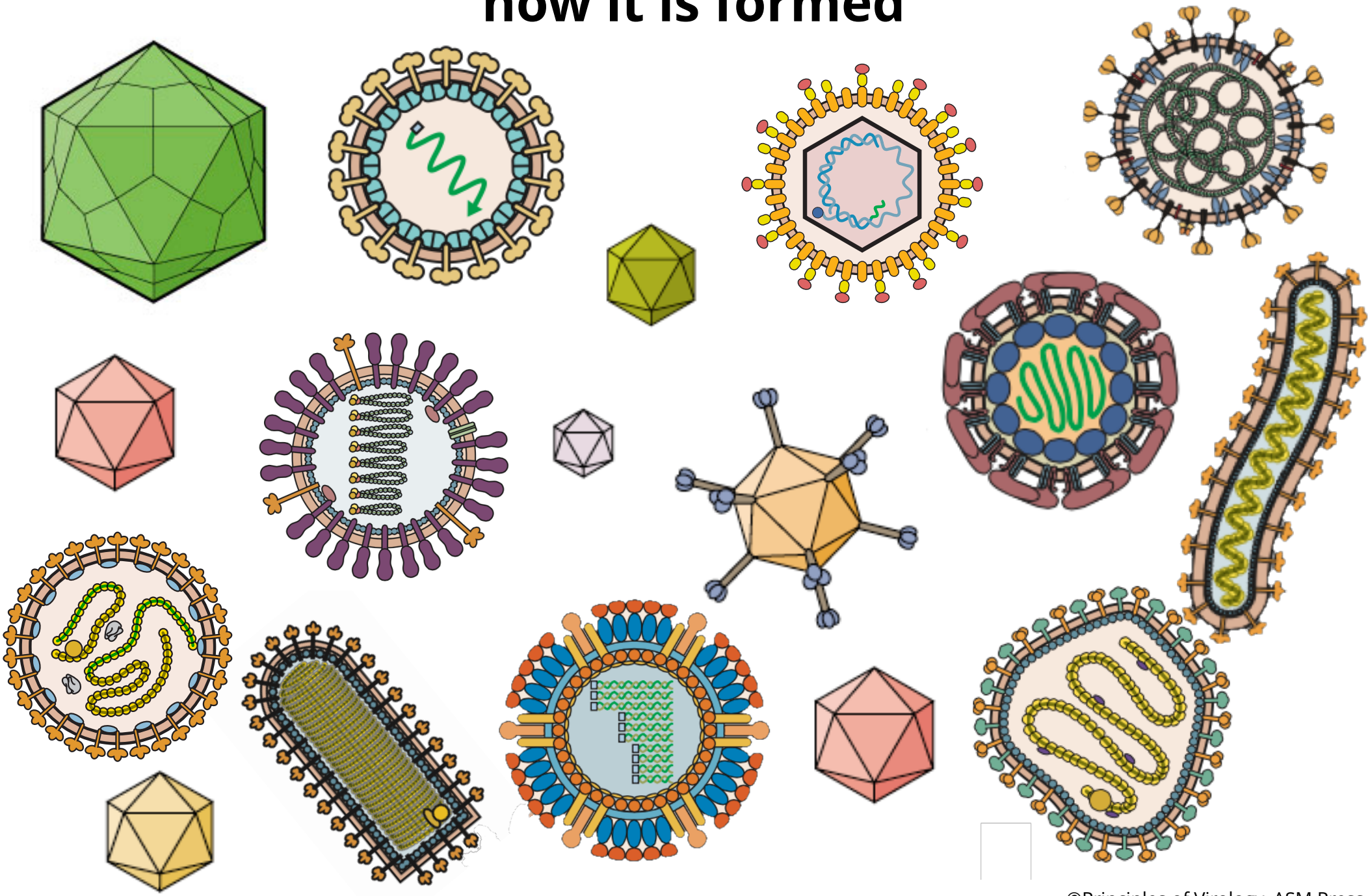
Assembly

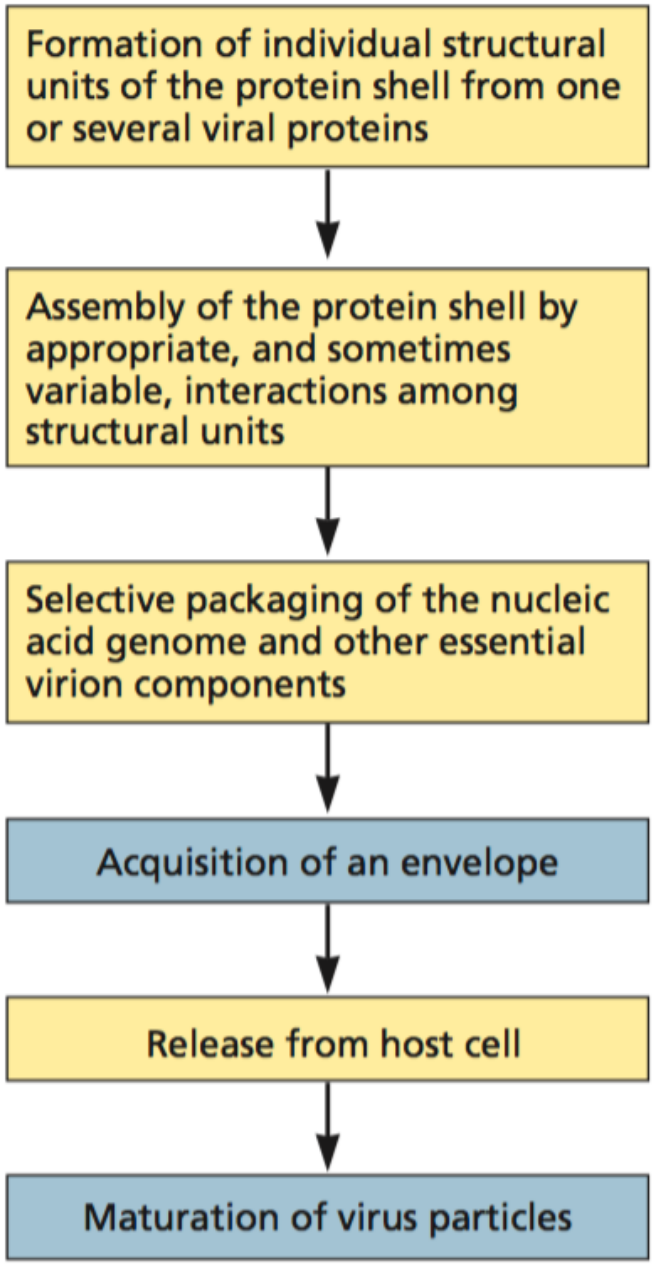
Lecture 11
Biology W3310/4310
Virology
Spring 2016

"Anatomy is destiny."
--SIGMUND FREUD

The structure of a virus particle determines how it is formed

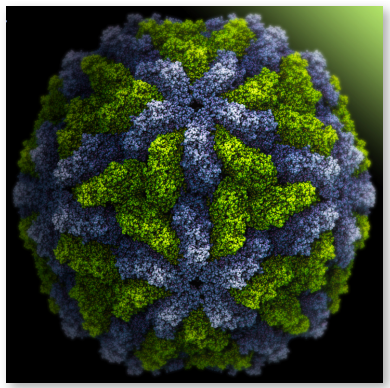


All virions complete a common set of assembly reactions



common to all viruses

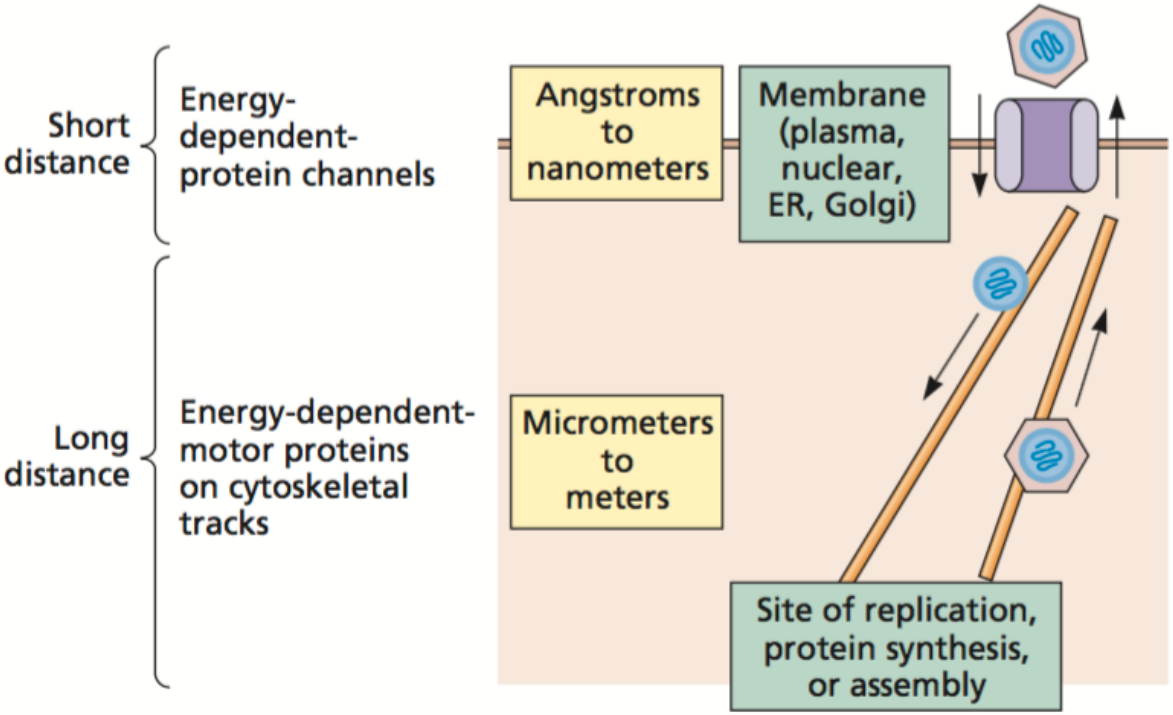
common to many viruses

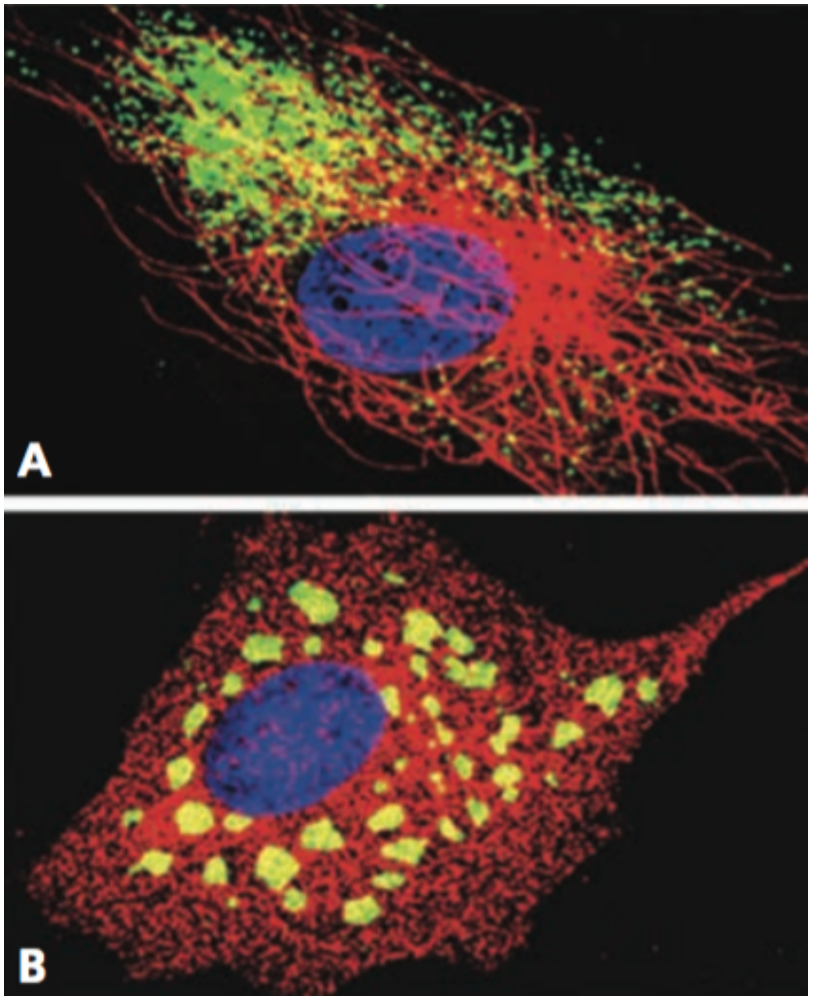


Assembly is dependent on host cell machinery

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

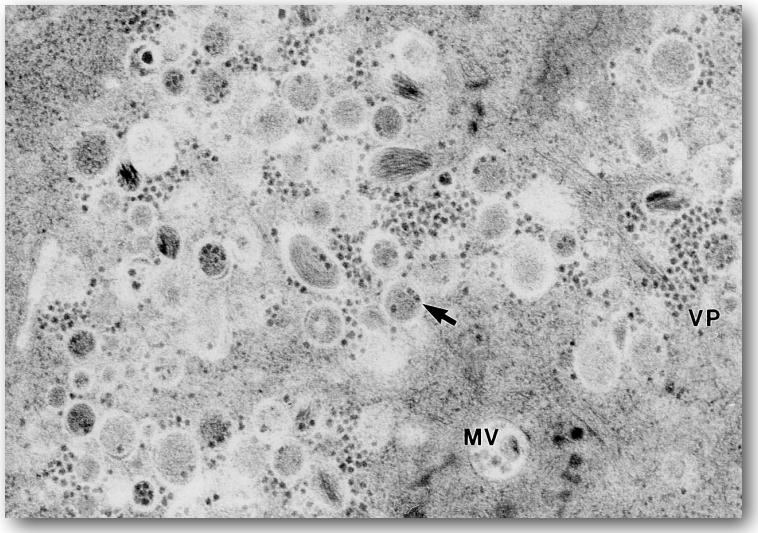
Moving in heavy traffic

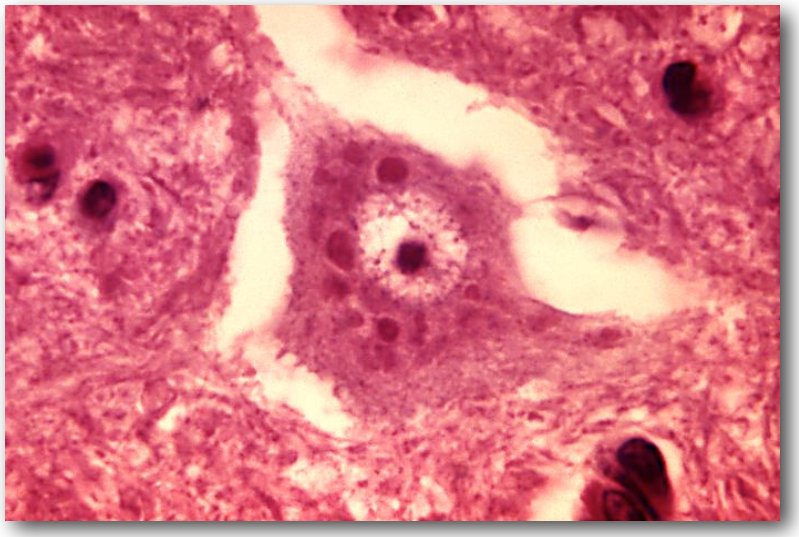




Nothing happens fast in dilute solutions

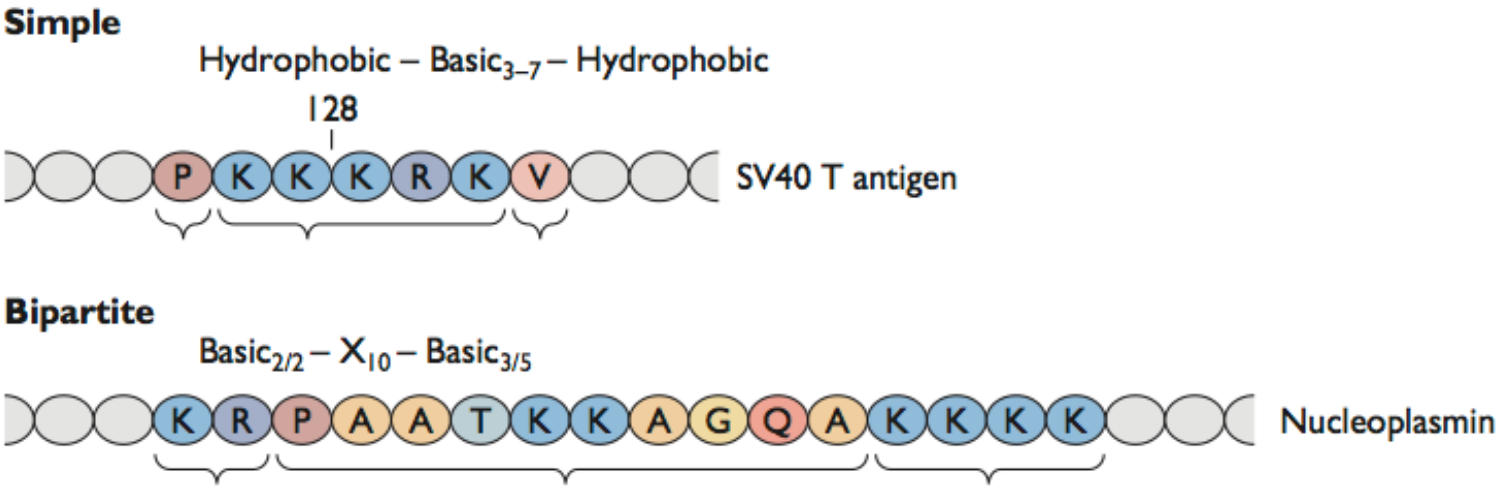
- Viral components often visible by light microscopy (‘factories’ or ‘inclusions’)
- Concentrate proteins on internal membranes *(poliovirus)*
- Negri bodies *(rabies virus)*



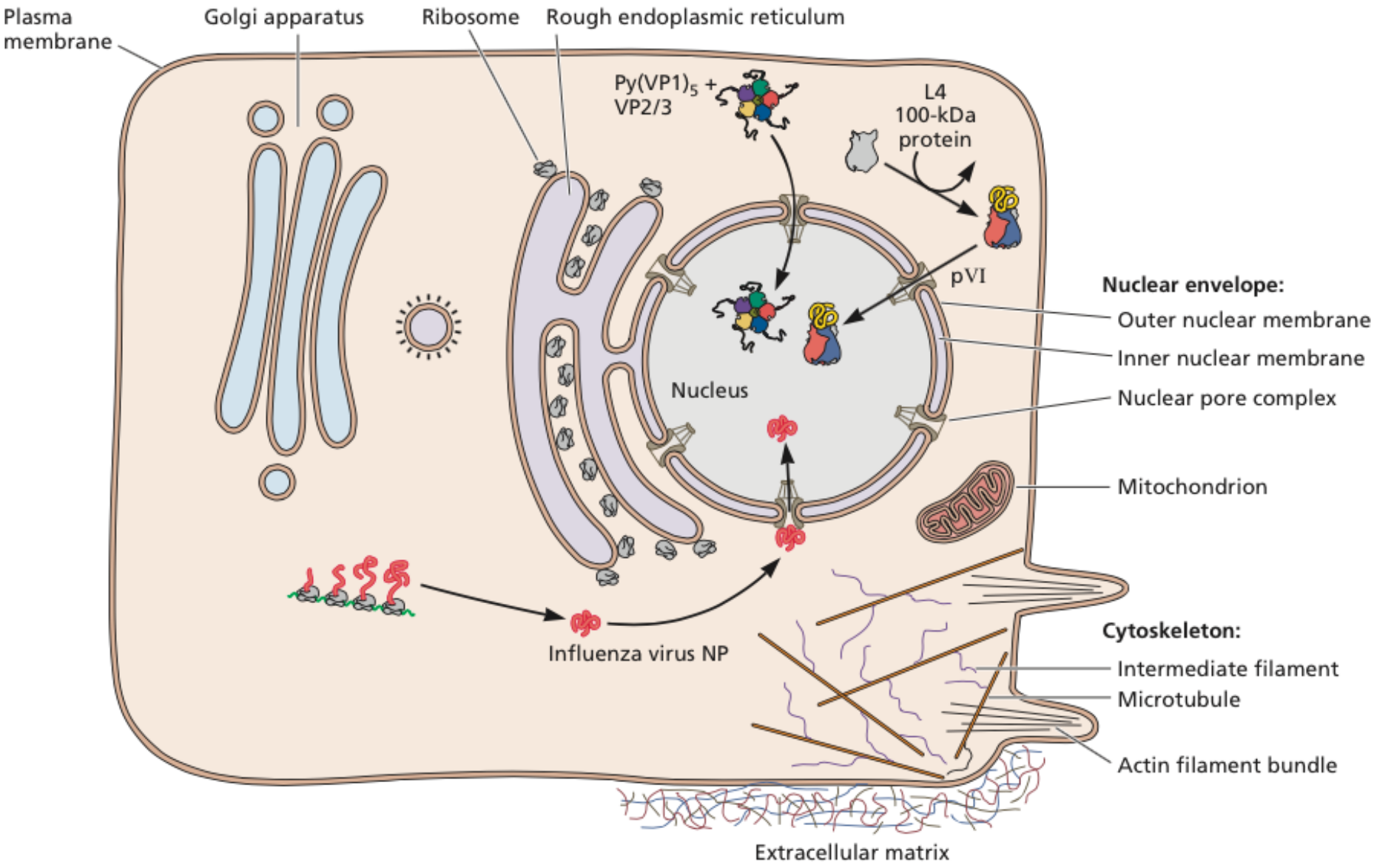


Viral proteins have 'addresses'

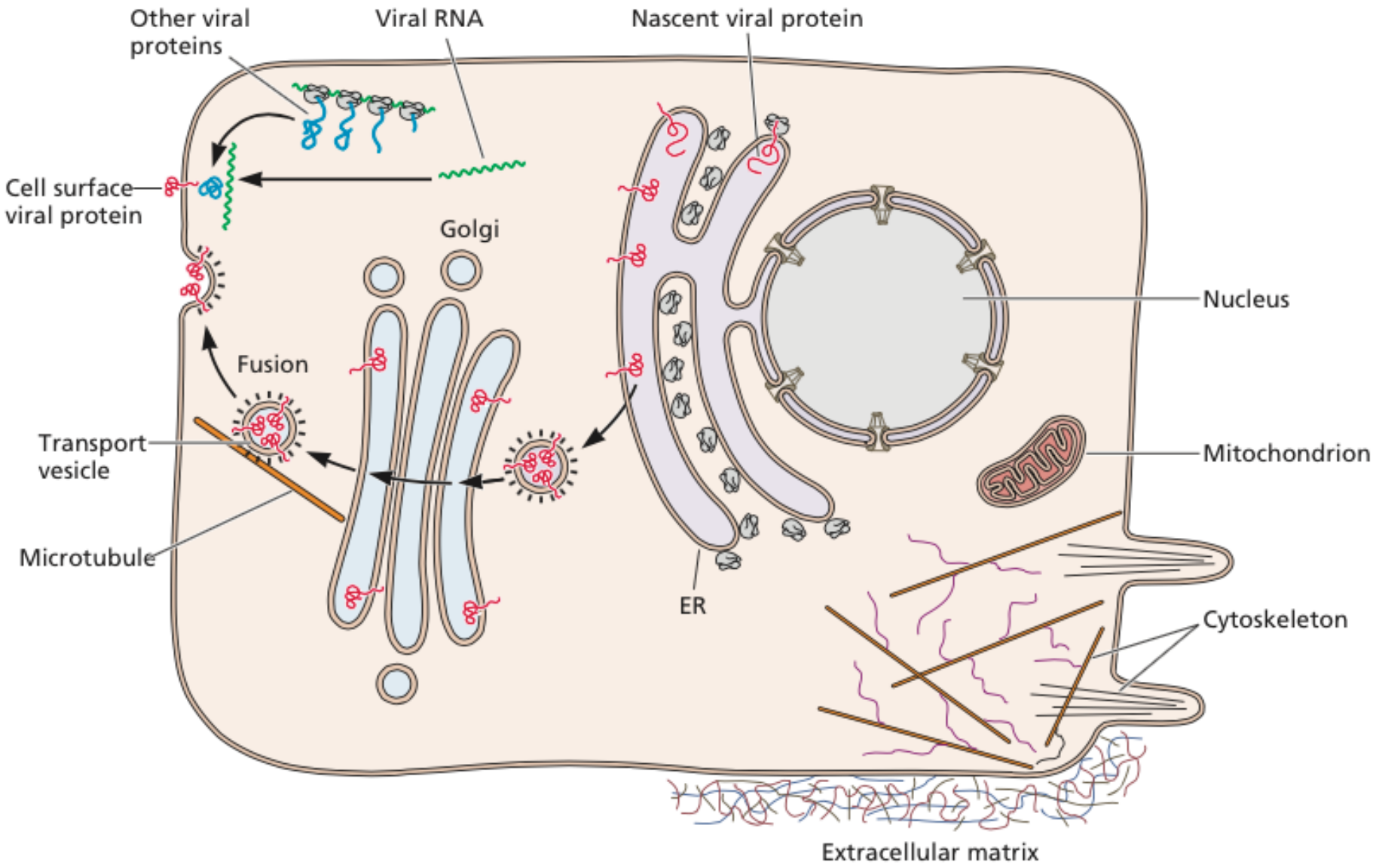
- Membrane targeting: Signal sequences, fatty acid modifications
- Membrane retention signals
- Nuclear localization sequences (NLS)
- Nuclear export signals



Localization of viral proteins to nucleus

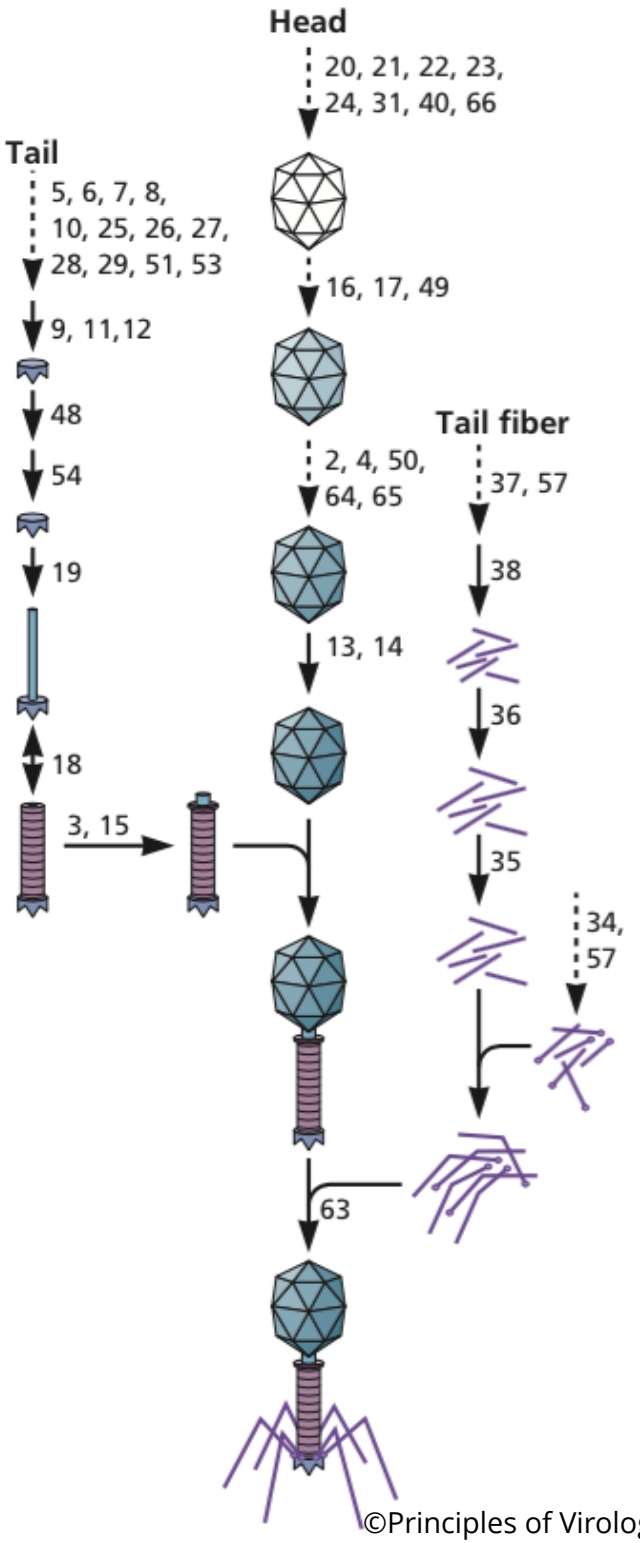


Localization of viral proteins to plasma membrane

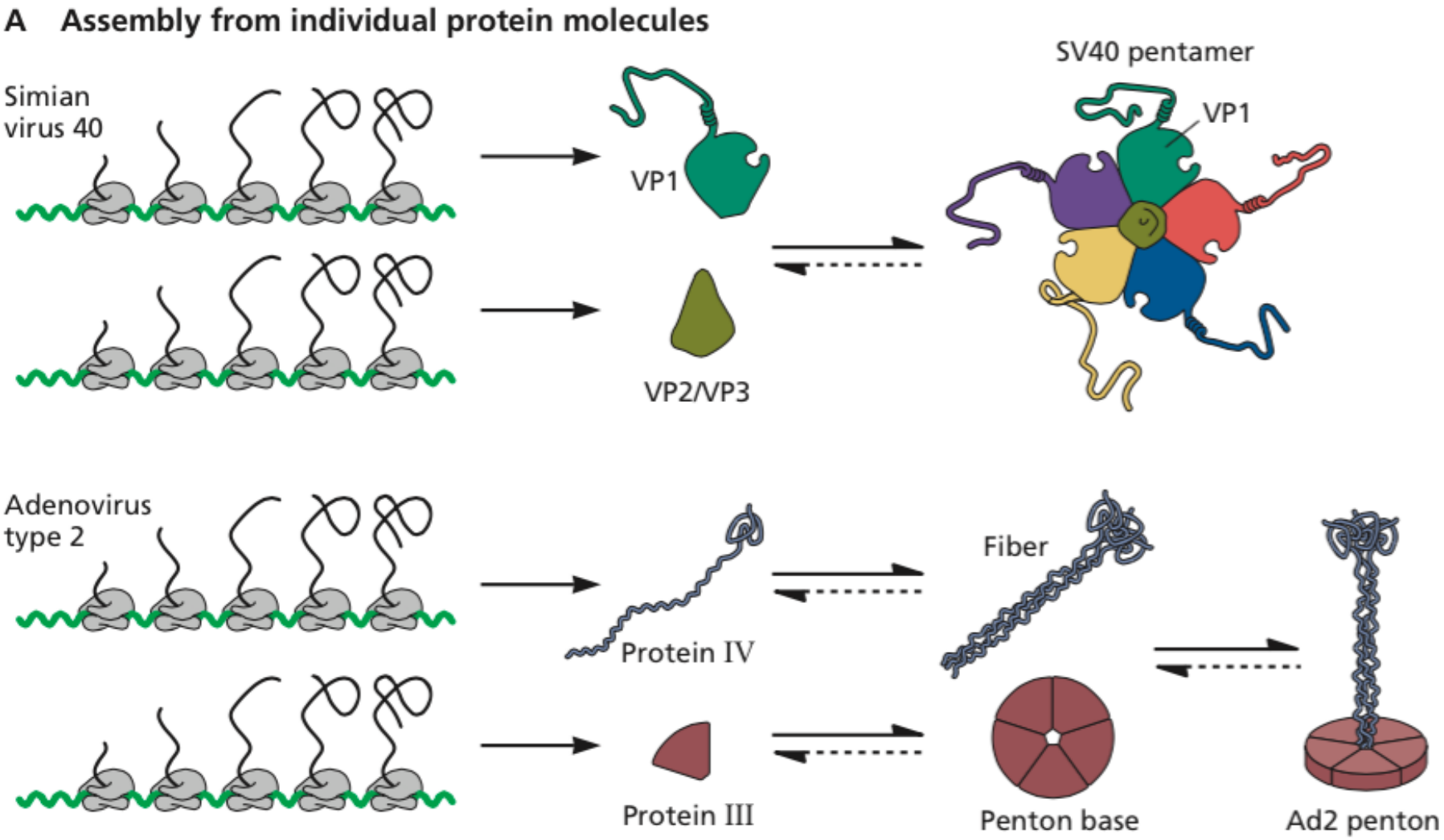


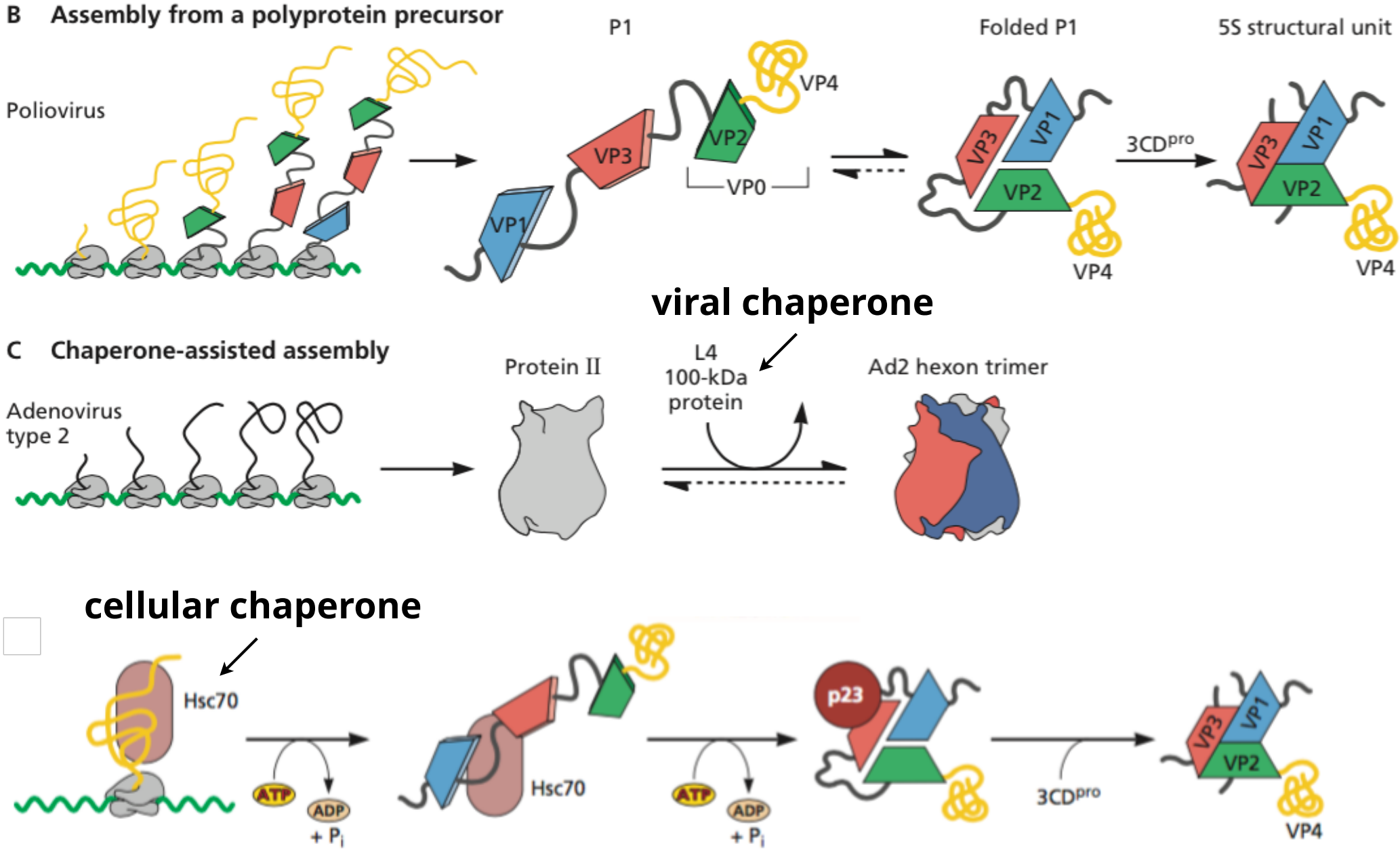
Sub-assemblies

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

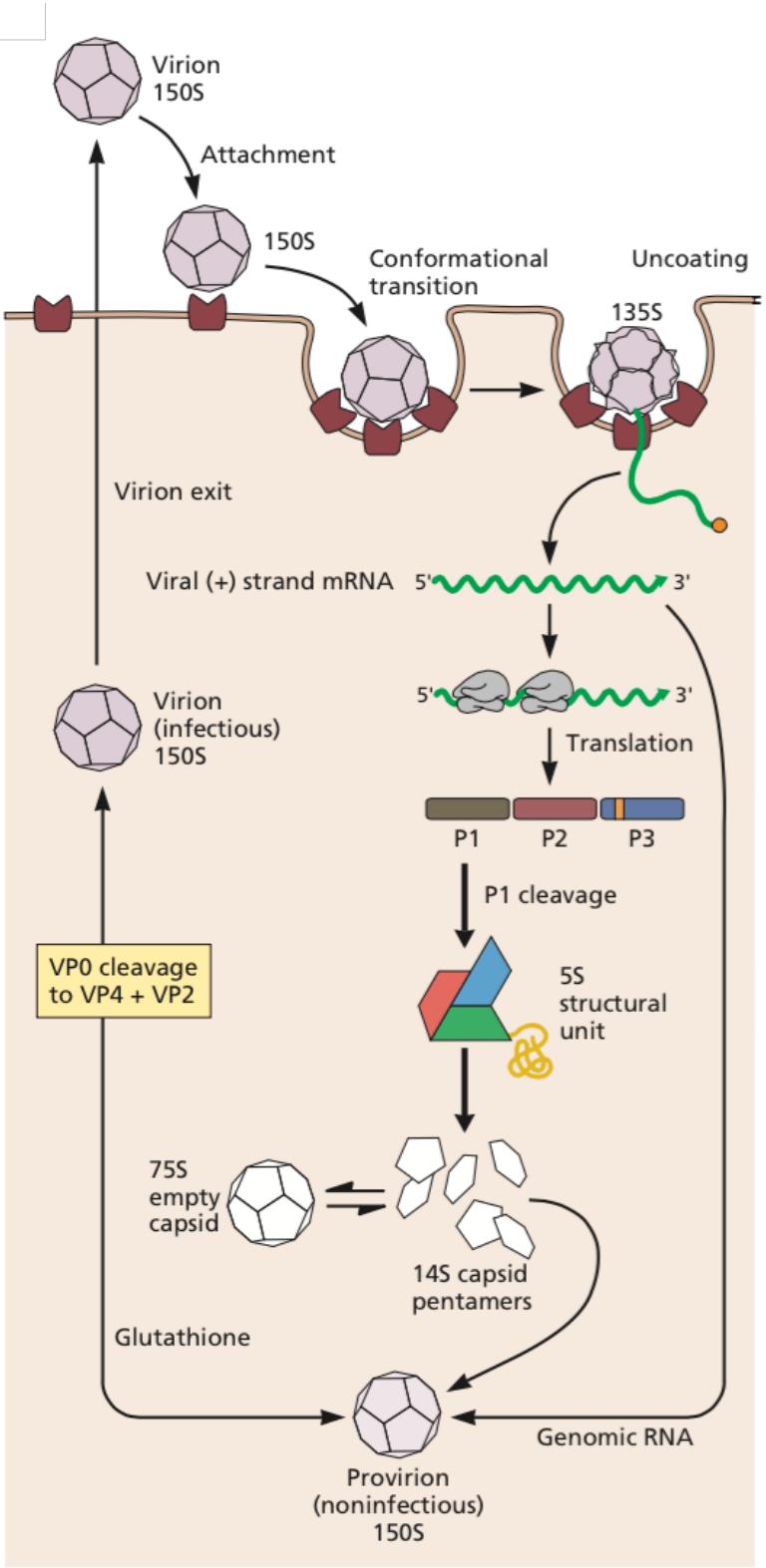


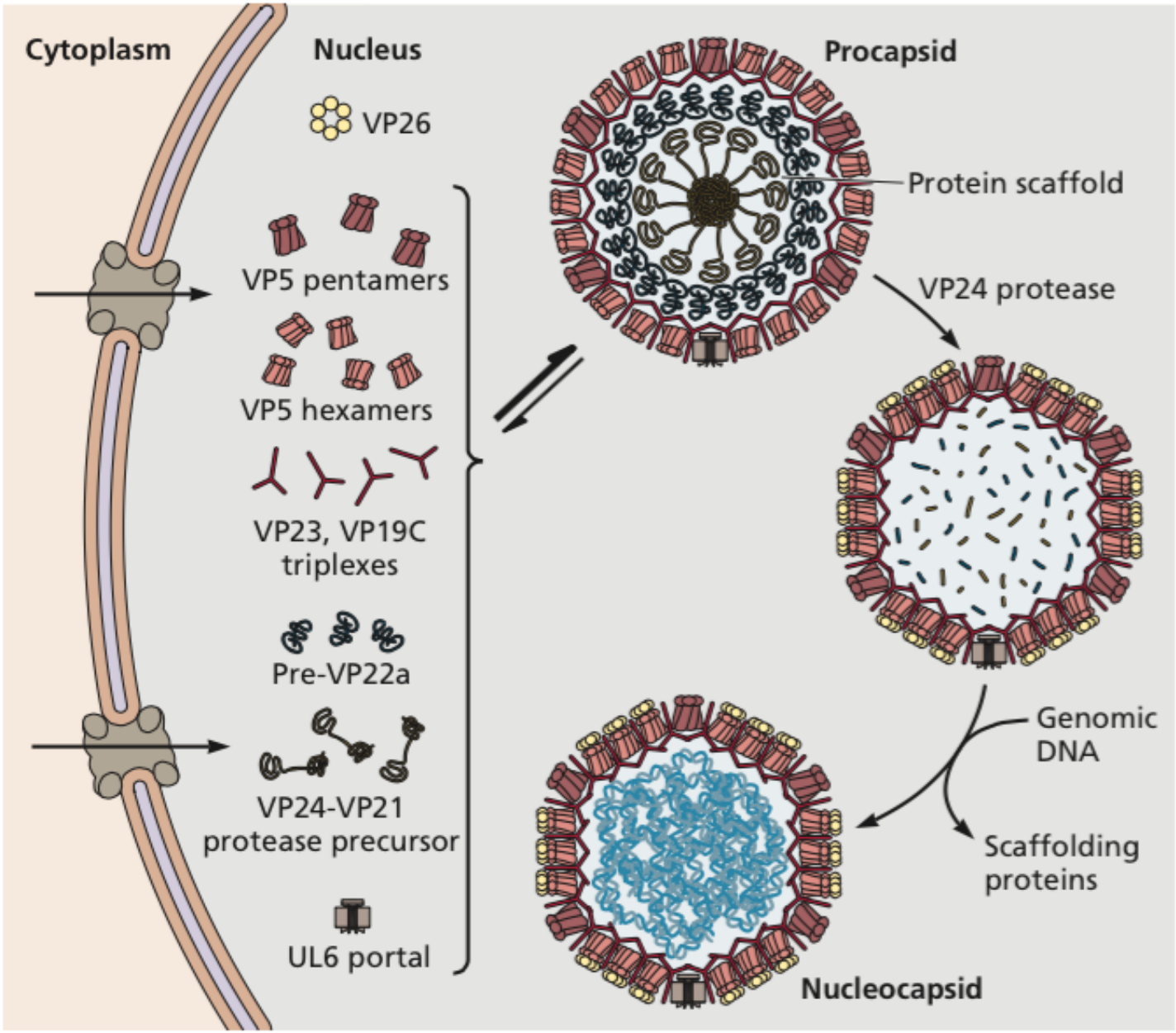
Three strategies for making sub-assemblies





Sequential capsid assembly: poliovirus

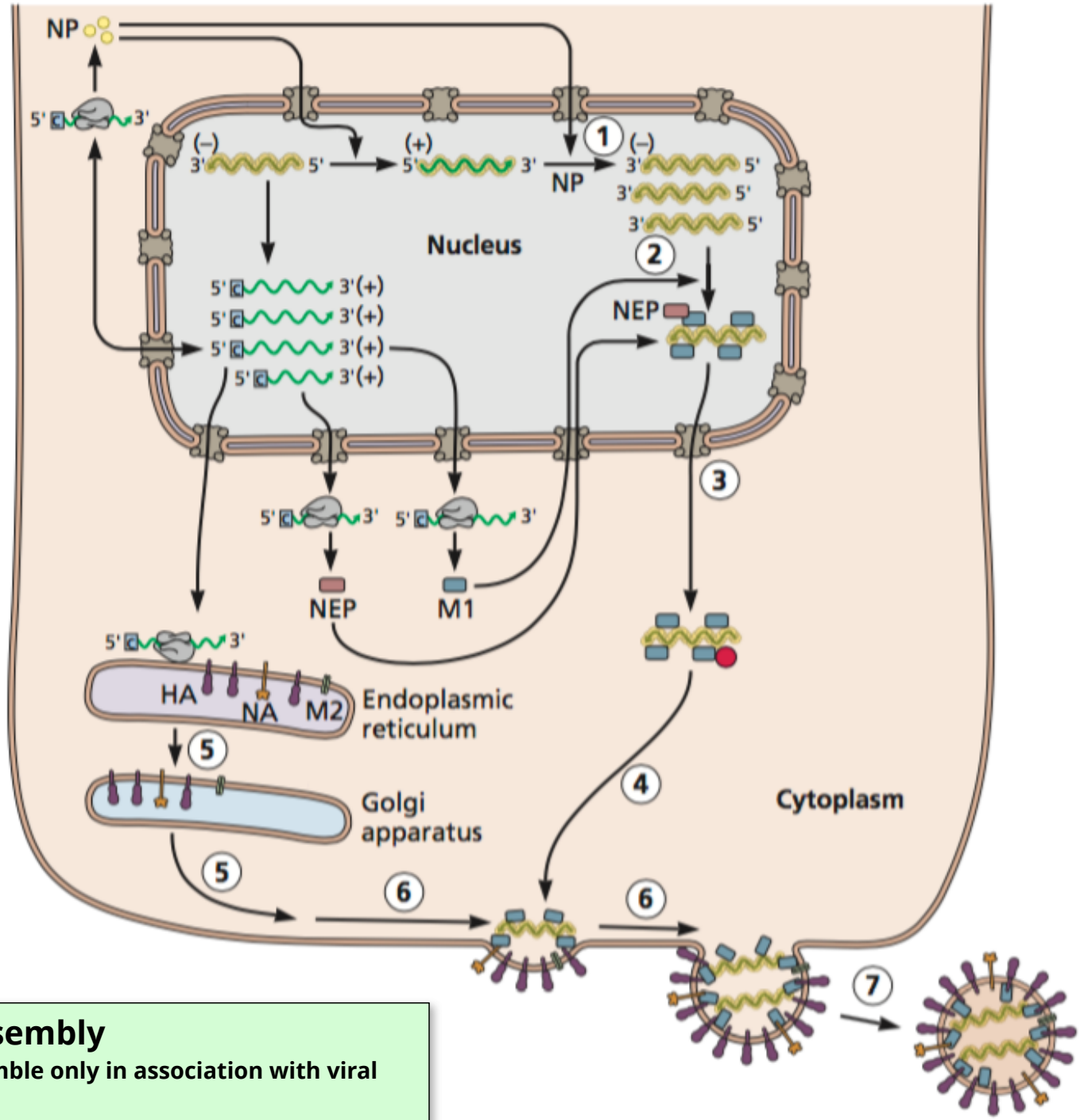




Viral scaffolding proteins

- establish transient intermediate structures
- viral proteases packaged in these intermediate structures become activated to finalize structure

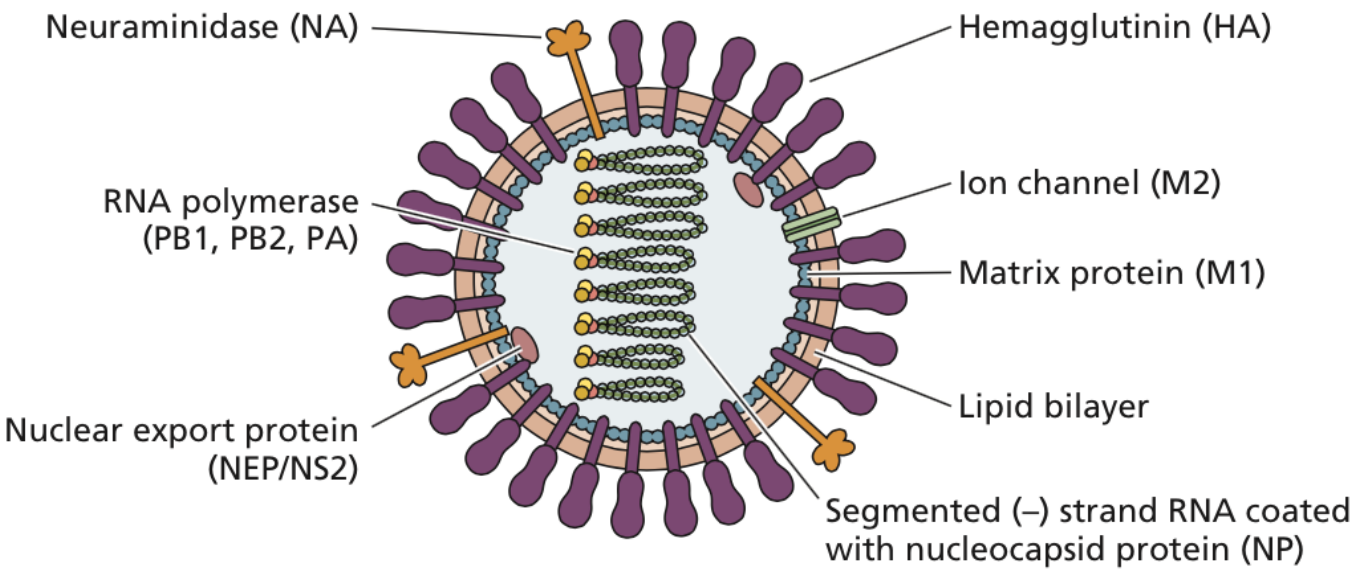
{sequential}

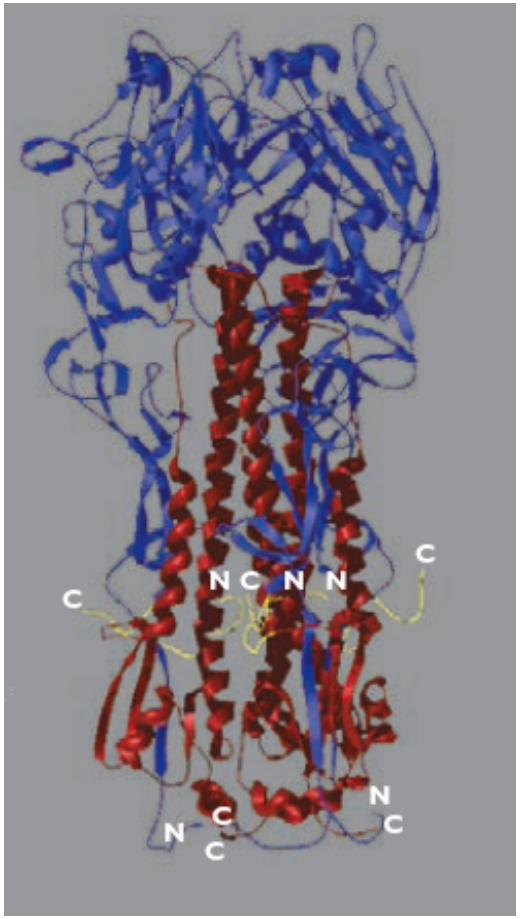


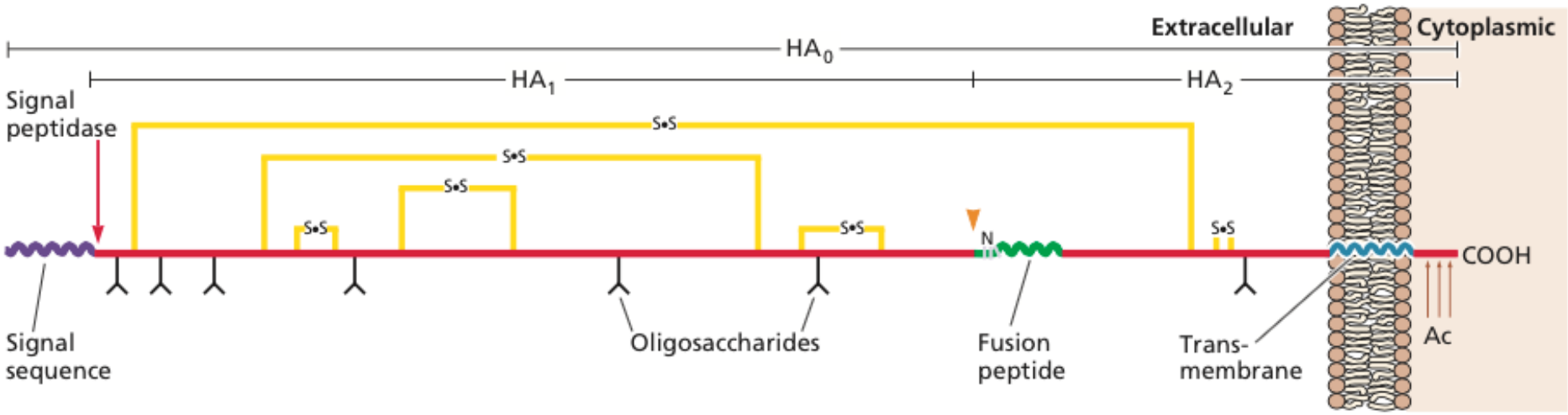
Concerted Assembly

Virus particles assemble only in association with viral genome

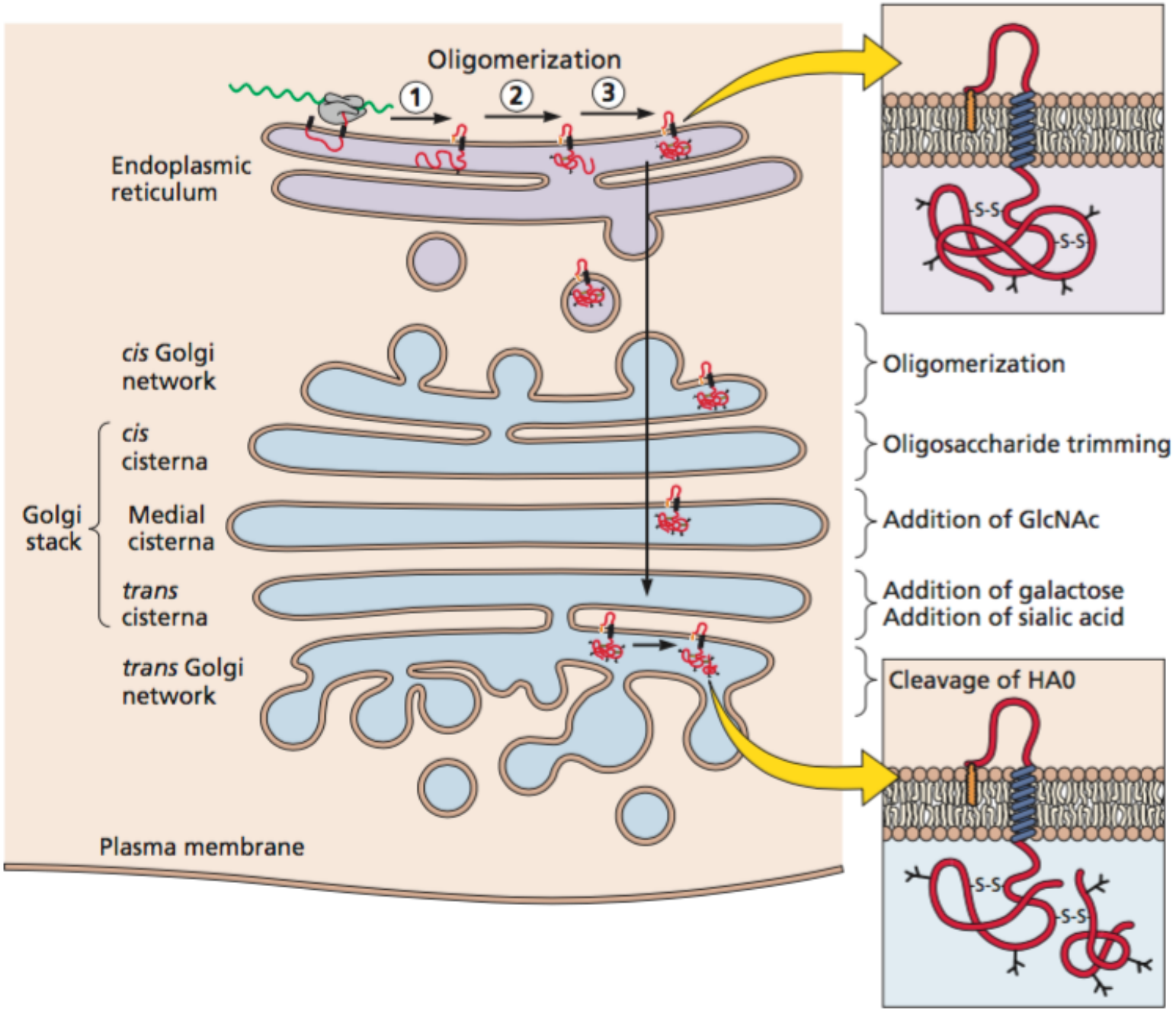
Influenza virus particles form by budding







Maturation of influenza HA0



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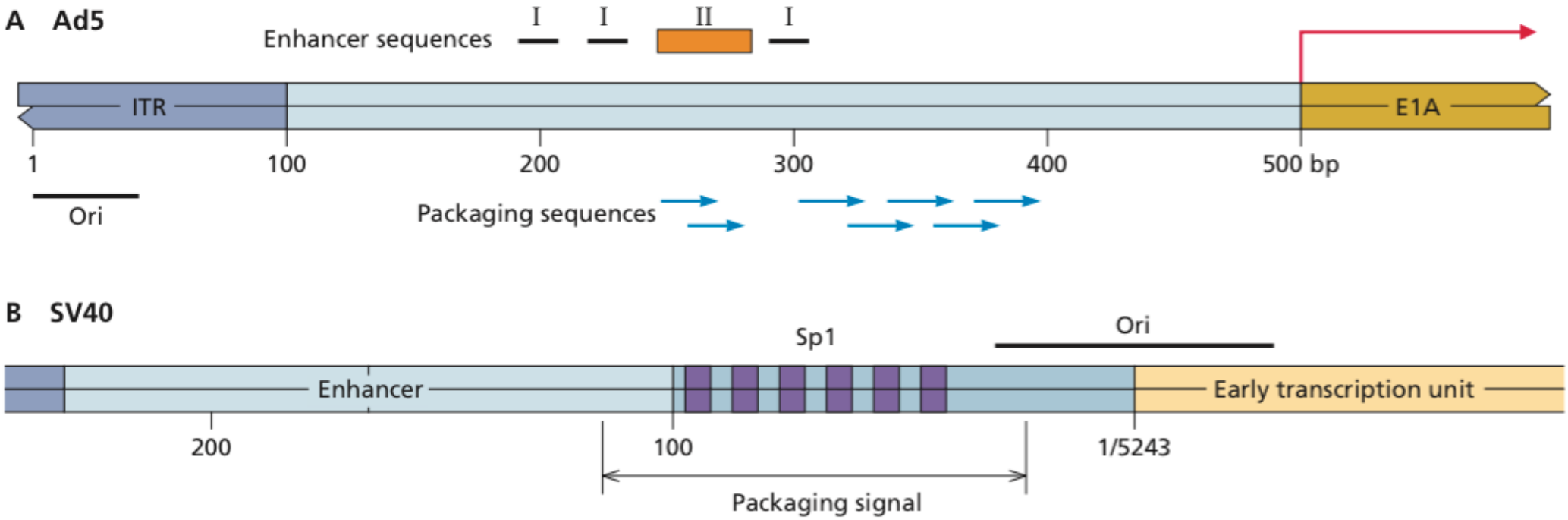
Subassemblies are involved in which of the following types of virus particle production?

1. Concerted assembly
2. Sequential assembly
3. Assembly lines
4. Chaperone-assisted assembly
5. All of the above

Genome packaging

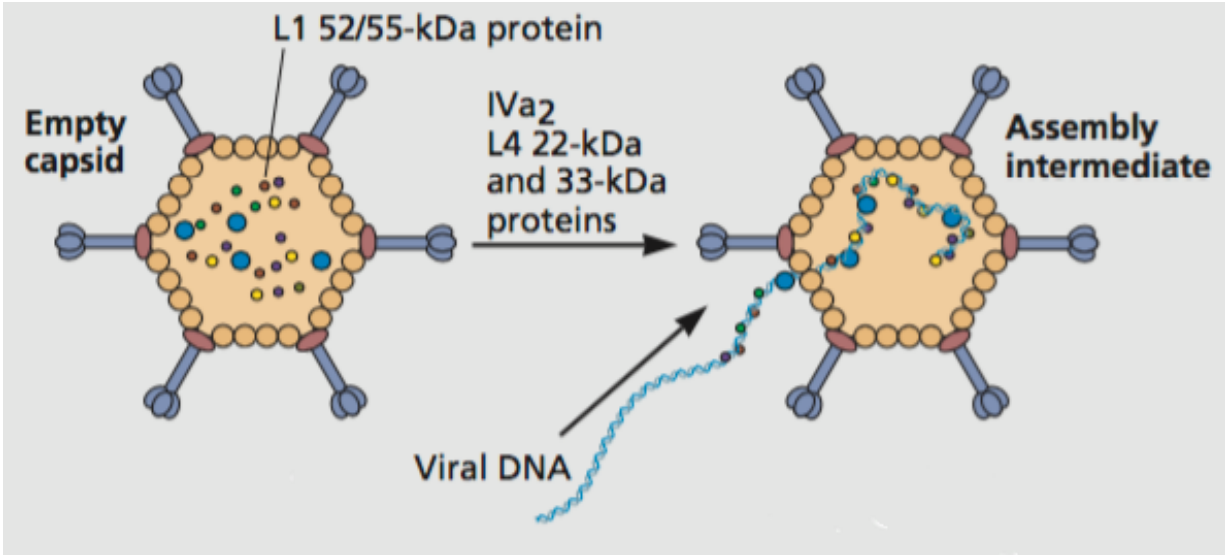
- Problem: Viral genomes must be distinguished from cellular DNA or RNA molecules where assembly takes place
- Solution: **Packaging signals** in the viral genome

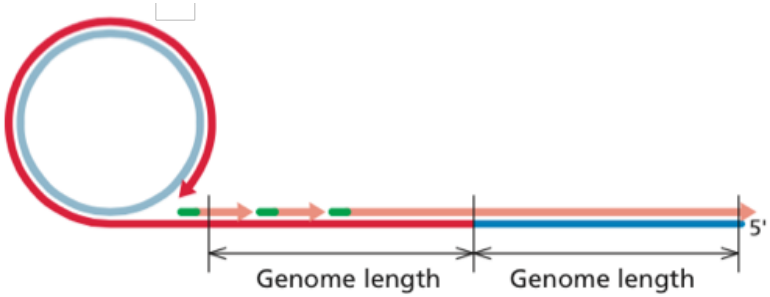
Packaging signals - DNA genomes

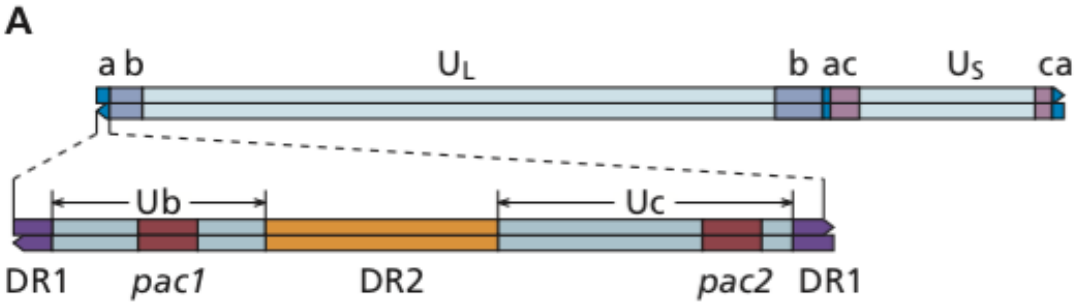


Adenovirus

- 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 IV2a

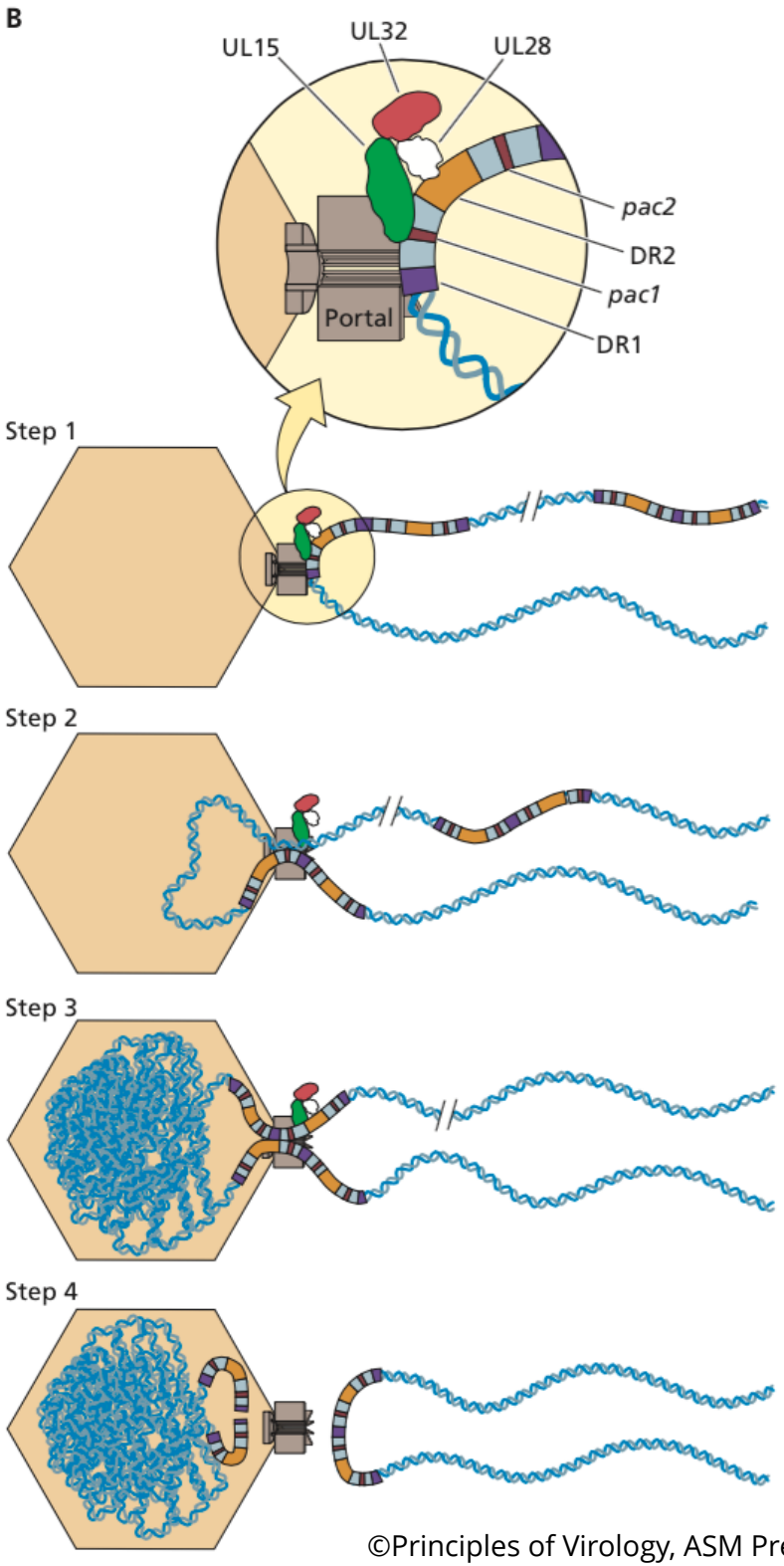




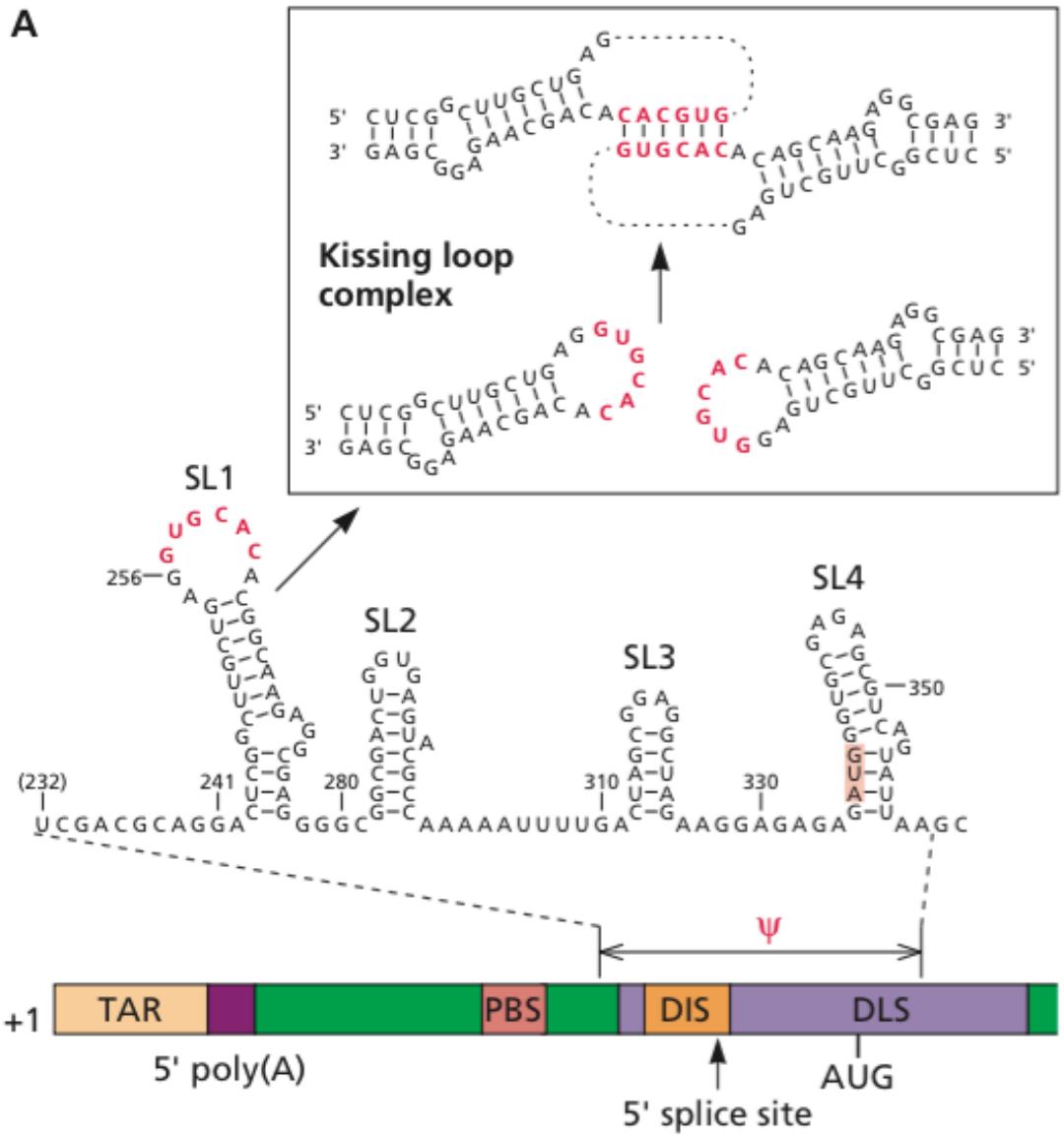


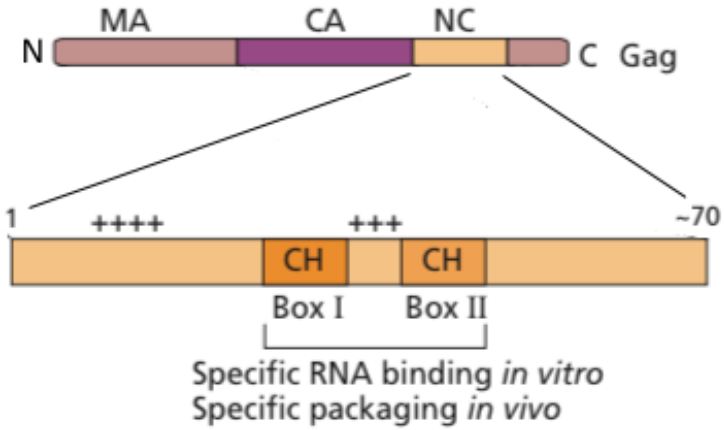
- Herpesvirus genome replication produces concatemers with head-to-tail copies of viral genome

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



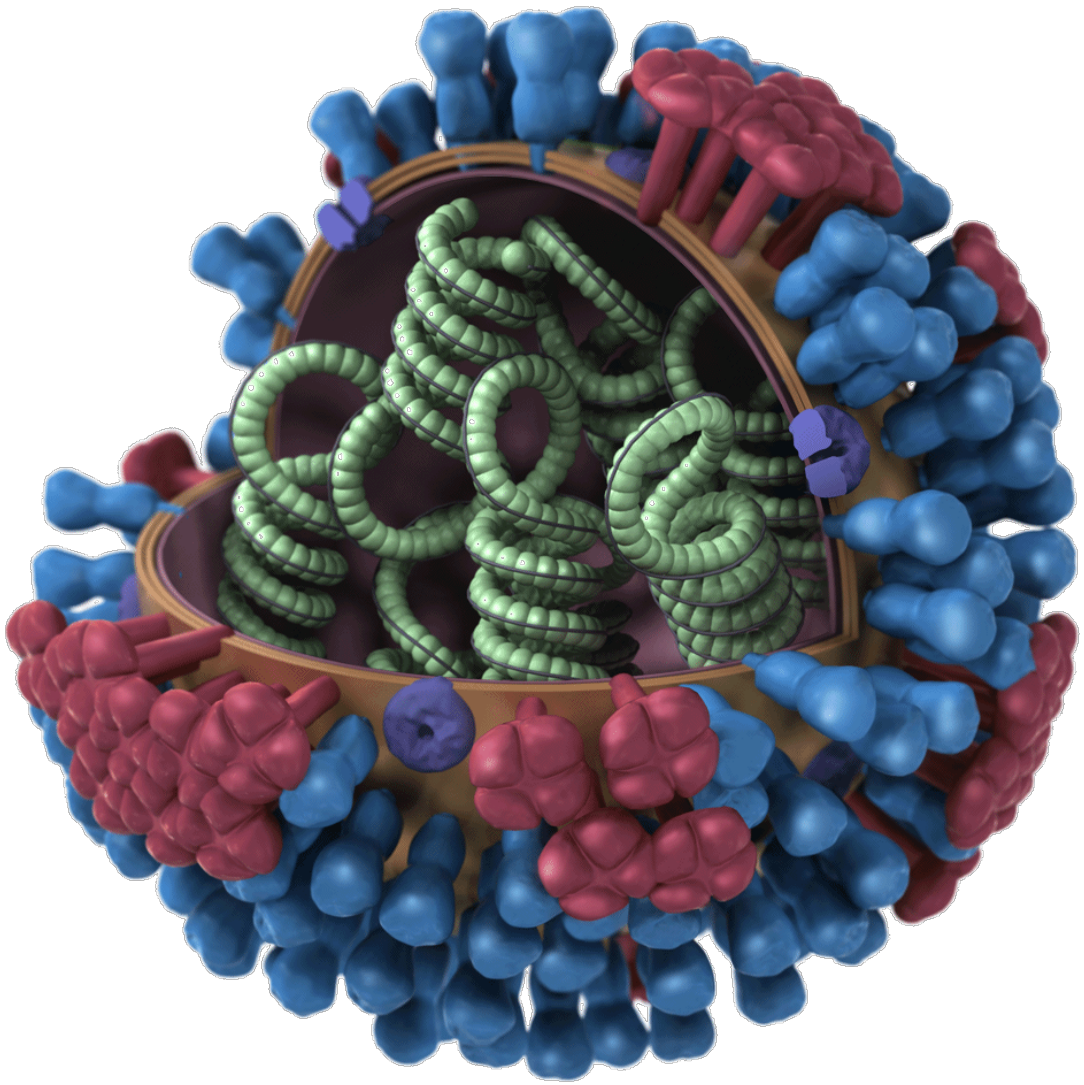
Packaging signals - RNA genomes





Necessary but not sufficient for HIV-1 genome packaging

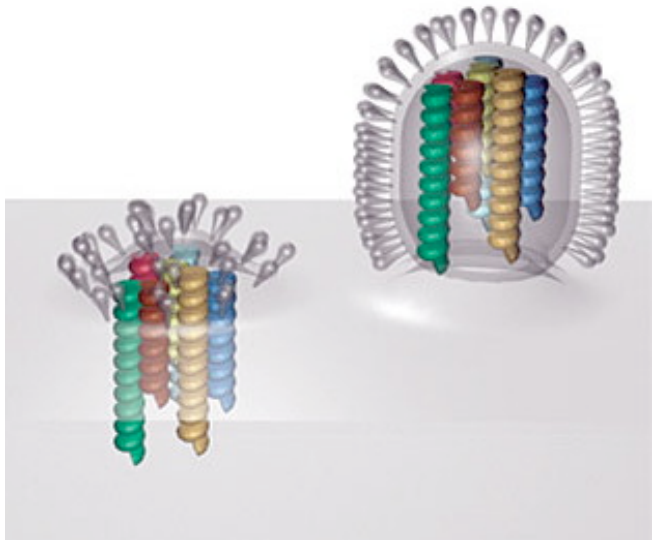
Packaging of segmented genomes

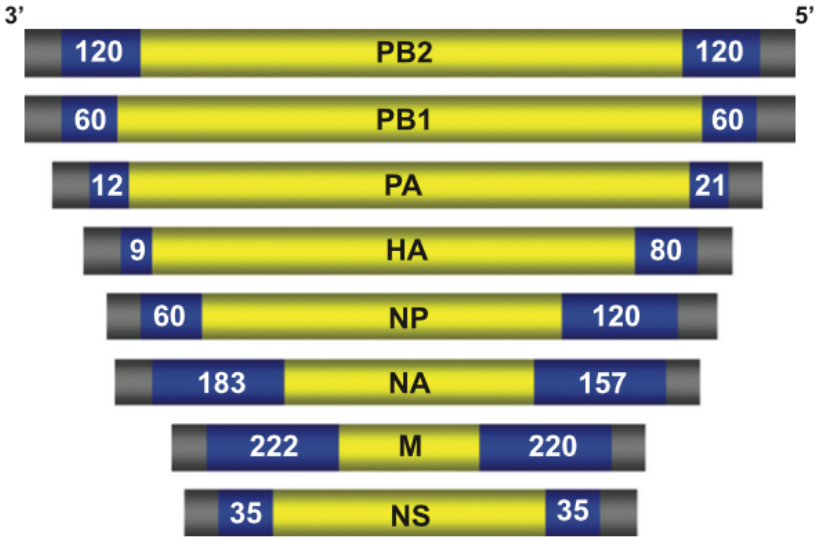


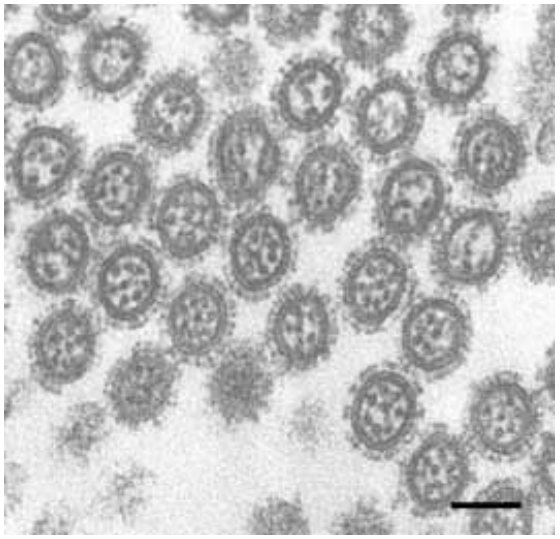
- *Random* mechanism would yield 1 infectious particle per 400 assembled - within known particle:pfu ratio

- Evidence for *specific* packaging sequence on each RNA segment

Influenza virus RNA packaging



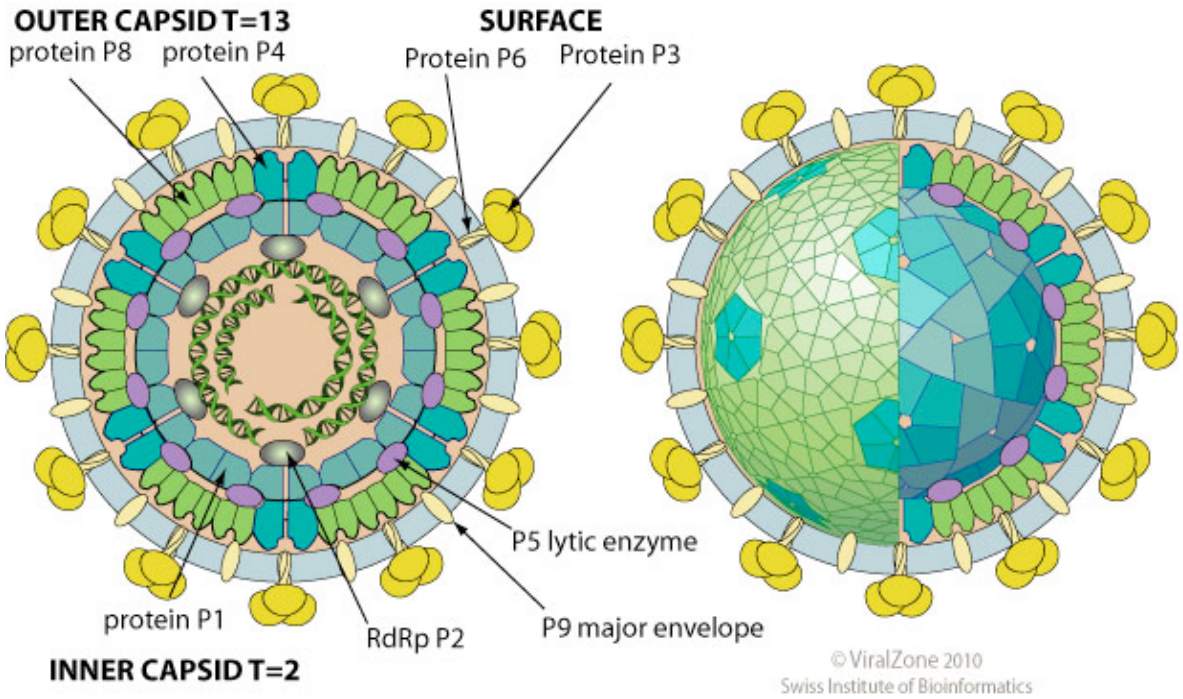




- Always 8 RNA segments
- Segments oriented perpendicular to budding tip
- HA, NS signals swapped
- RNA-RNA or RNA-protein interactions

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
- Rotavirus

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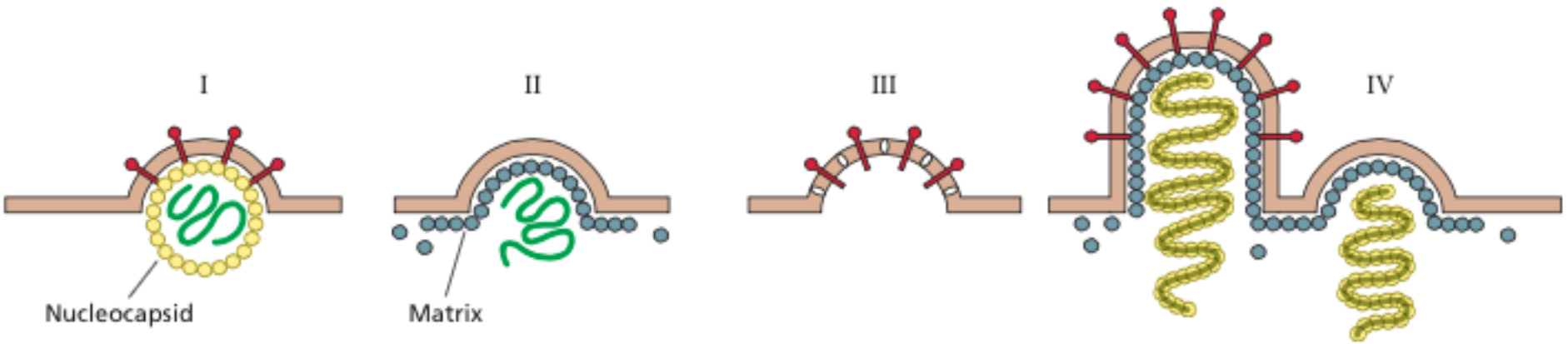
Packaging signals on viral _____ interact with viral ____ during virus assembly.

1. Lipids, proteins
2. Proteins, subassemblies
3. Genomes, proteins
4. Proteases, membranes
5. Proteins, genomes

Acquisition of an envelope

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



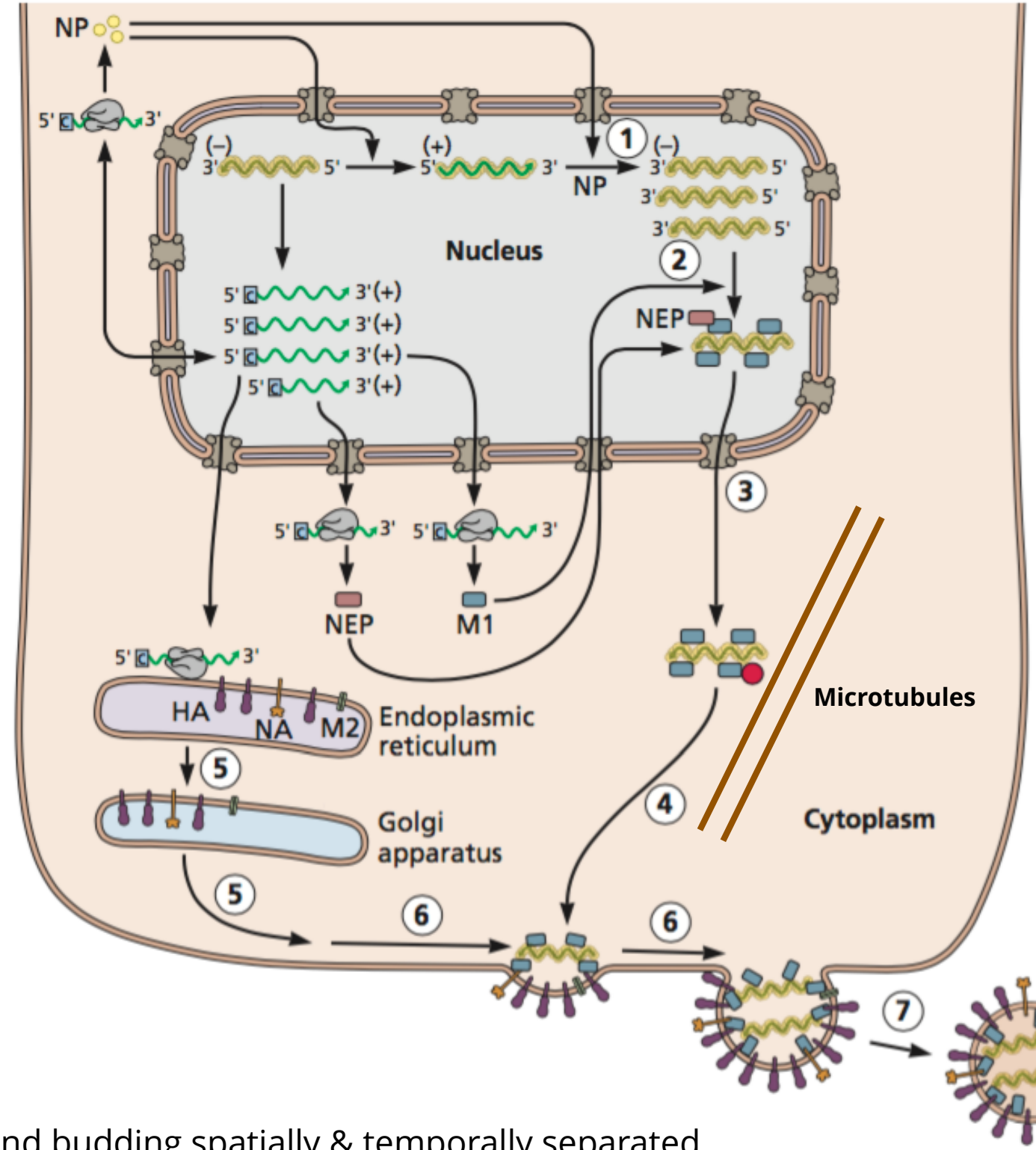
Envelope glycoproteins and capsid essential for budding - alphaviruses

Internal matrix or capsid proteins drive budding - retroviruses

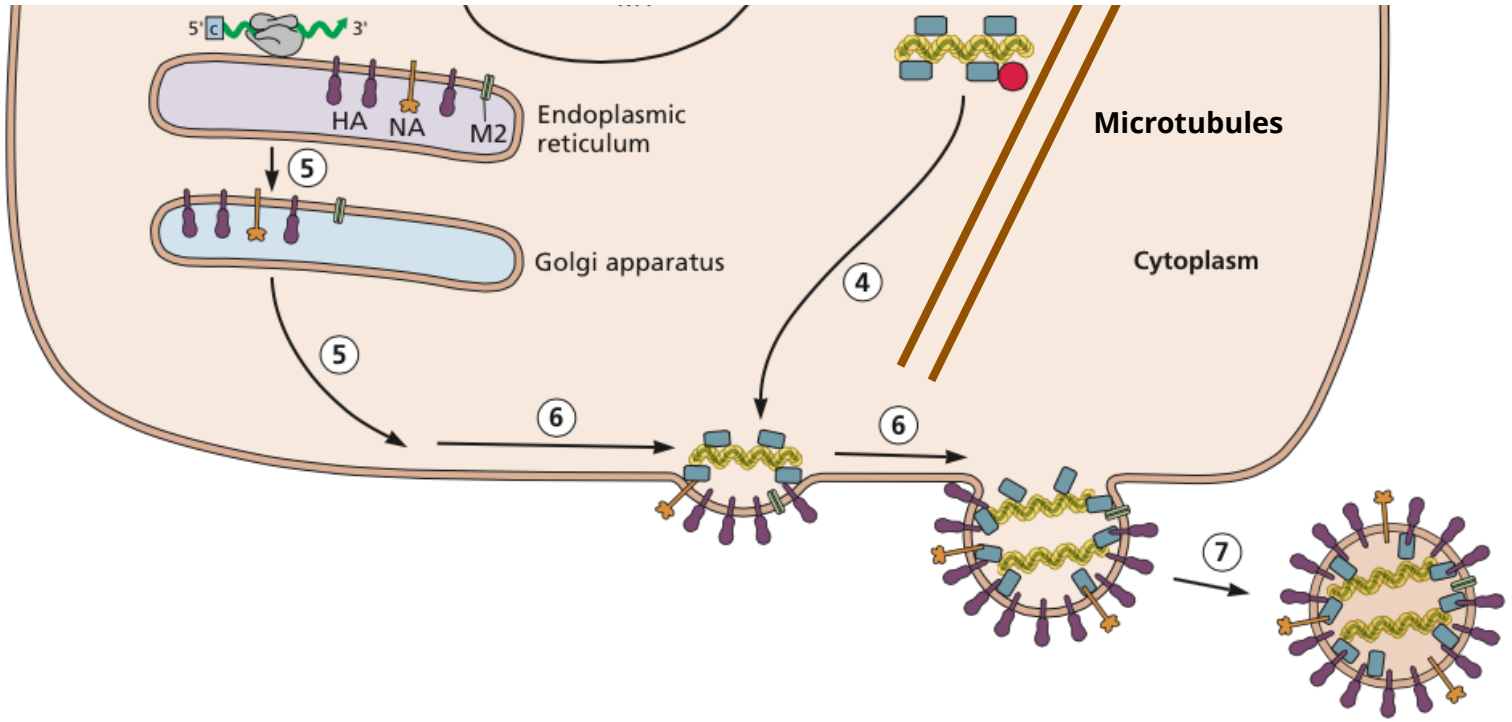
Envelope proteins drive budding - influenza virus, coronavirus

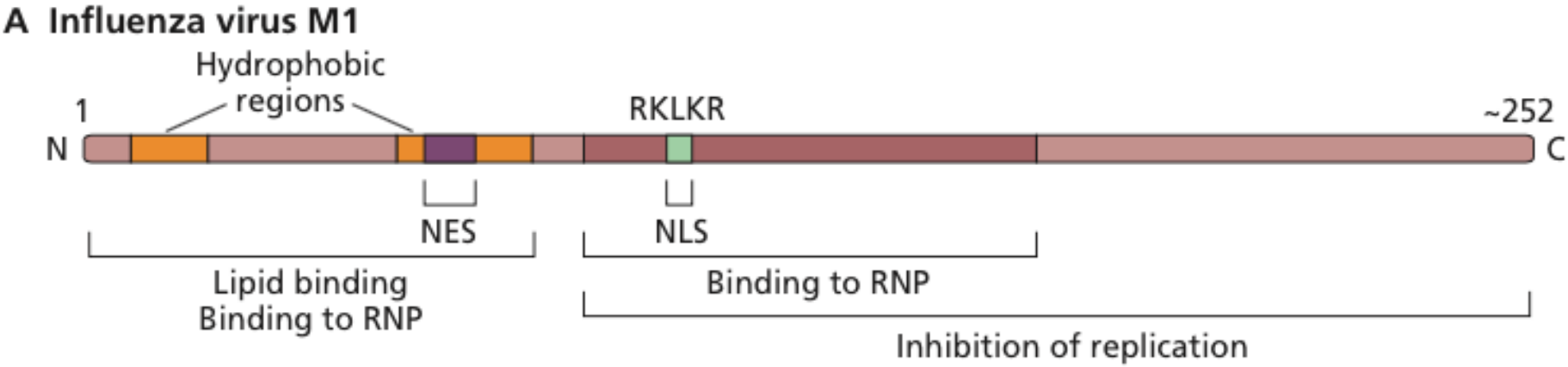
Matrix proteins drive budding, but additional components (glycoproteins, RNP) needed for efficiency or accuracy

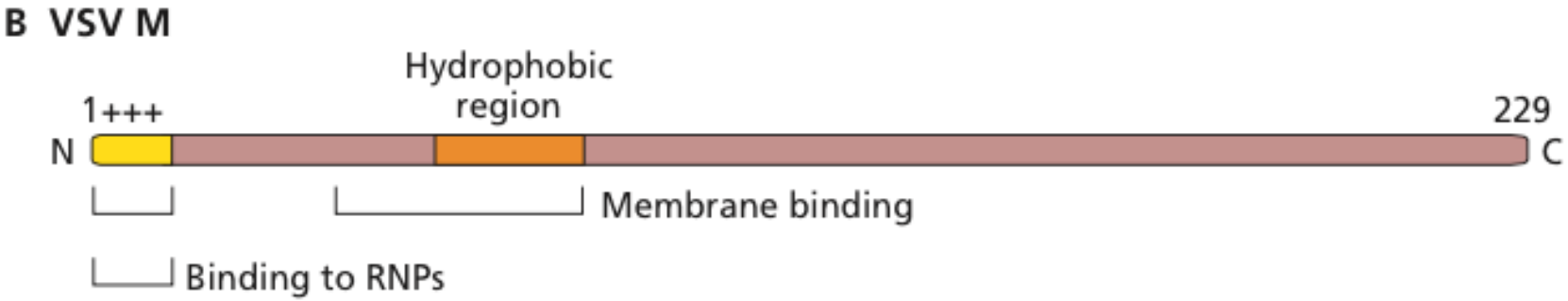
Influenza virus budding



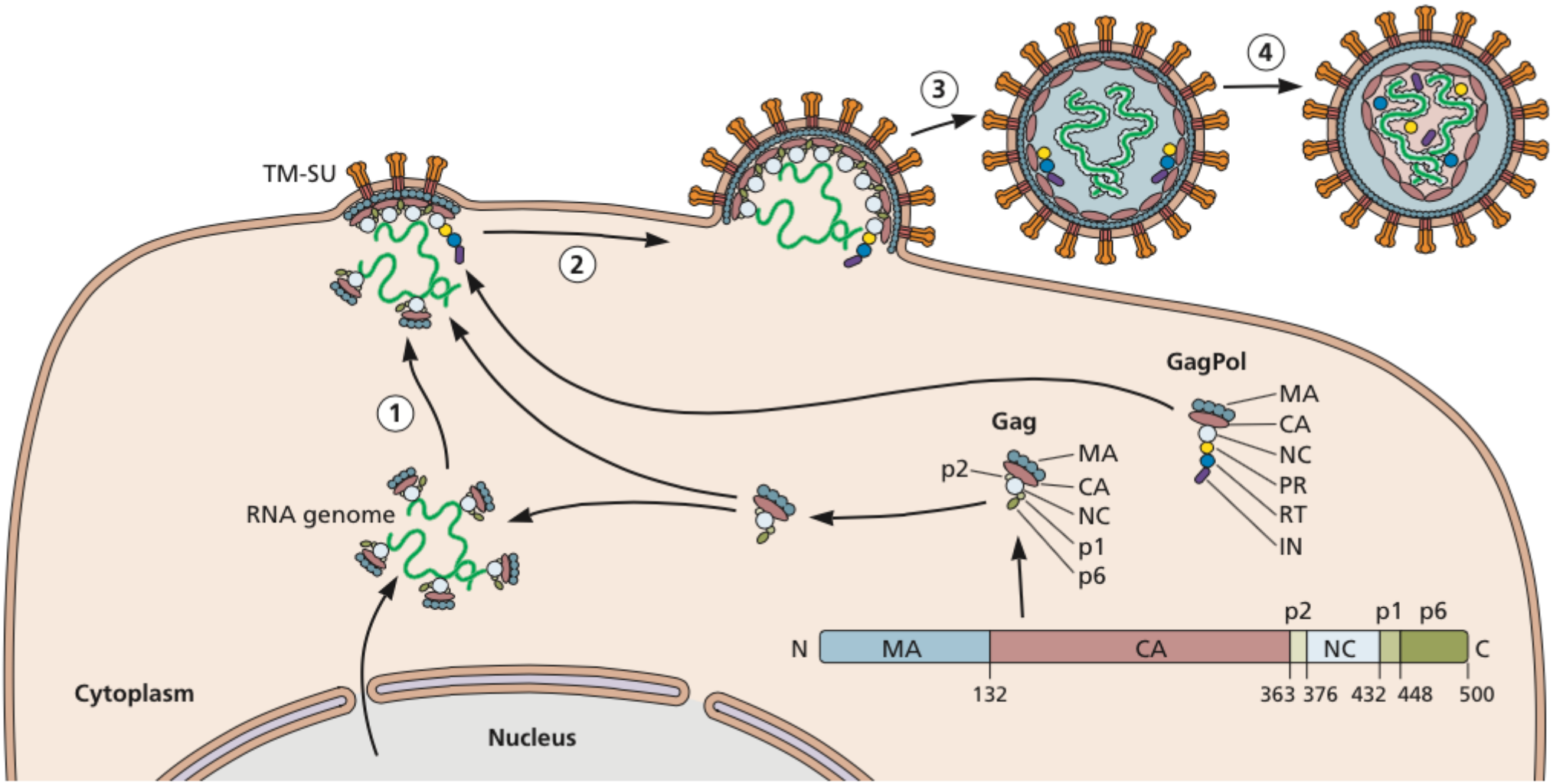
Internal structure assembly and budding spatially & temporally separated



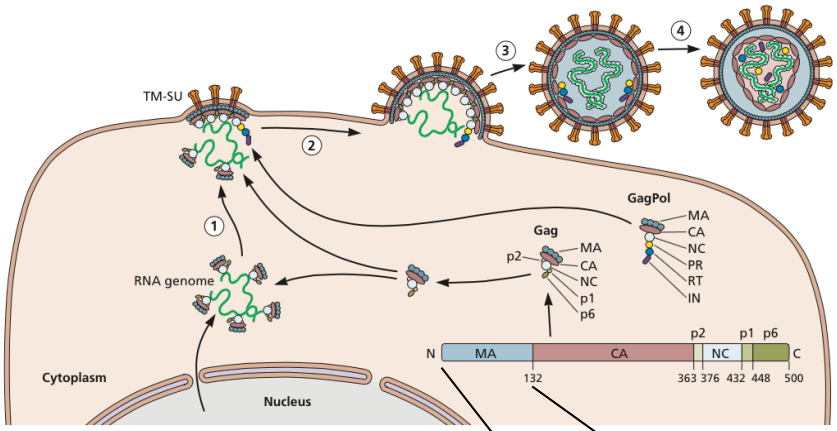


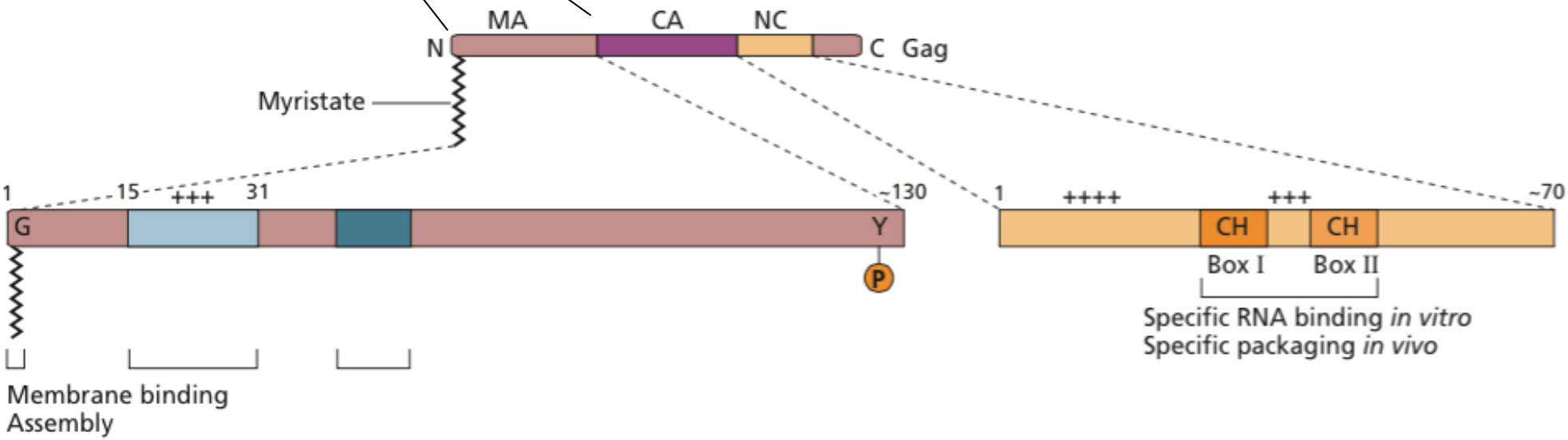


Retrovirus budding

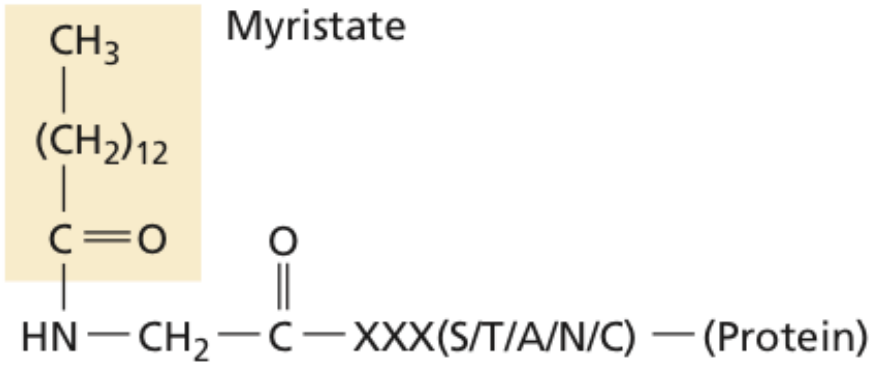


Gag alone produces virus-like particles



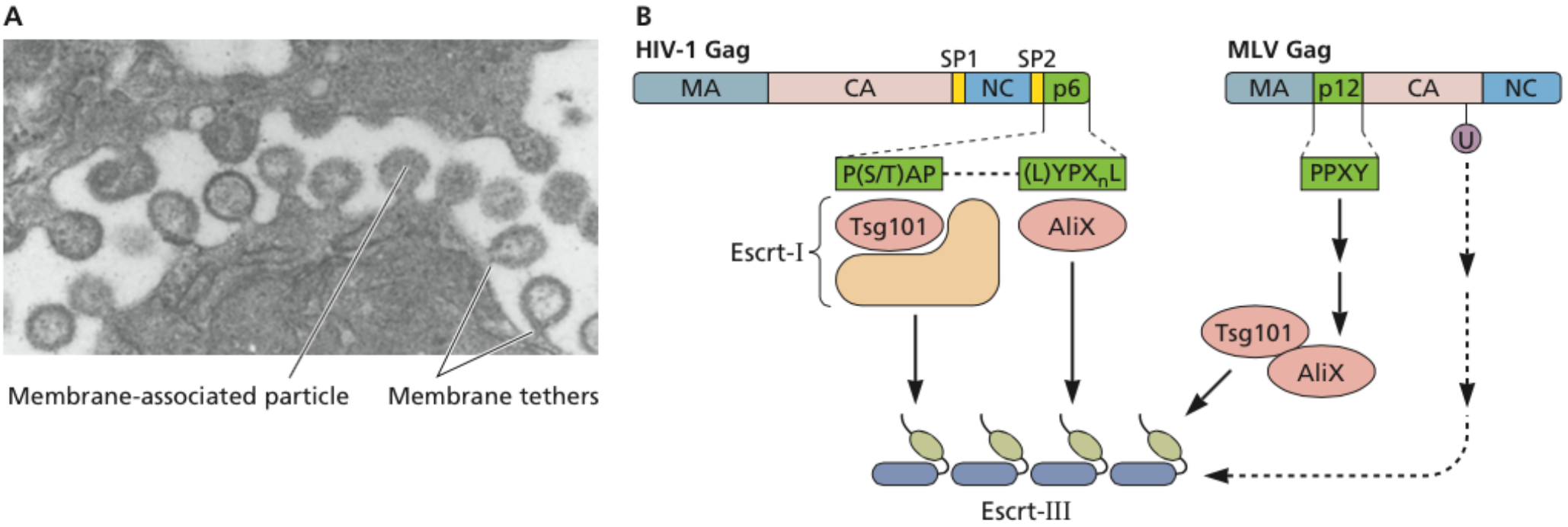


- Changes at myristoylation sequence prevent interaction of Gag with the cytoplasmic face of the plasma membrane
- Virus assembly and budding are inhibited



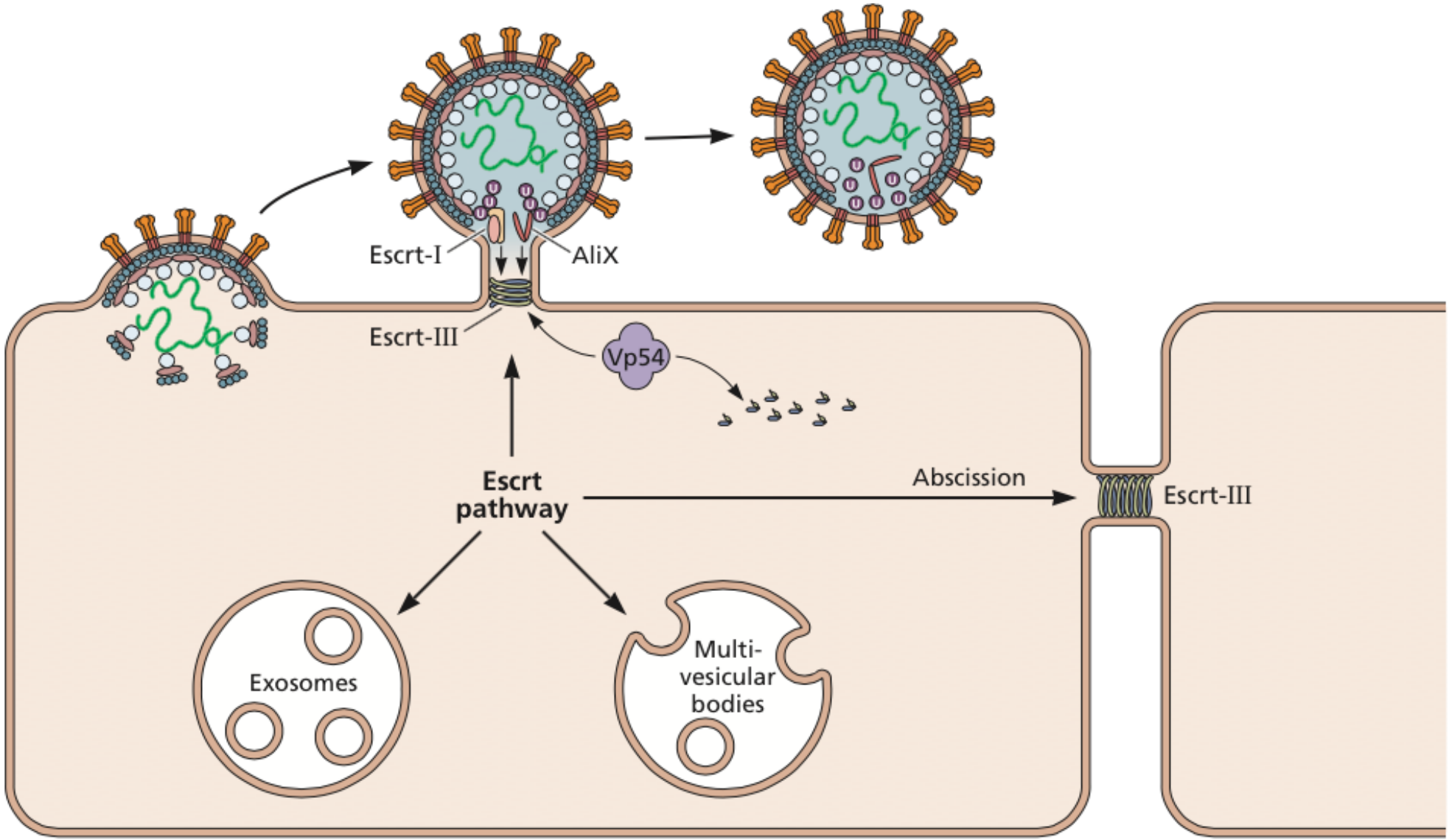
- Addition of lipid to viral proteins allows targeting to membranes independent of signal sequence

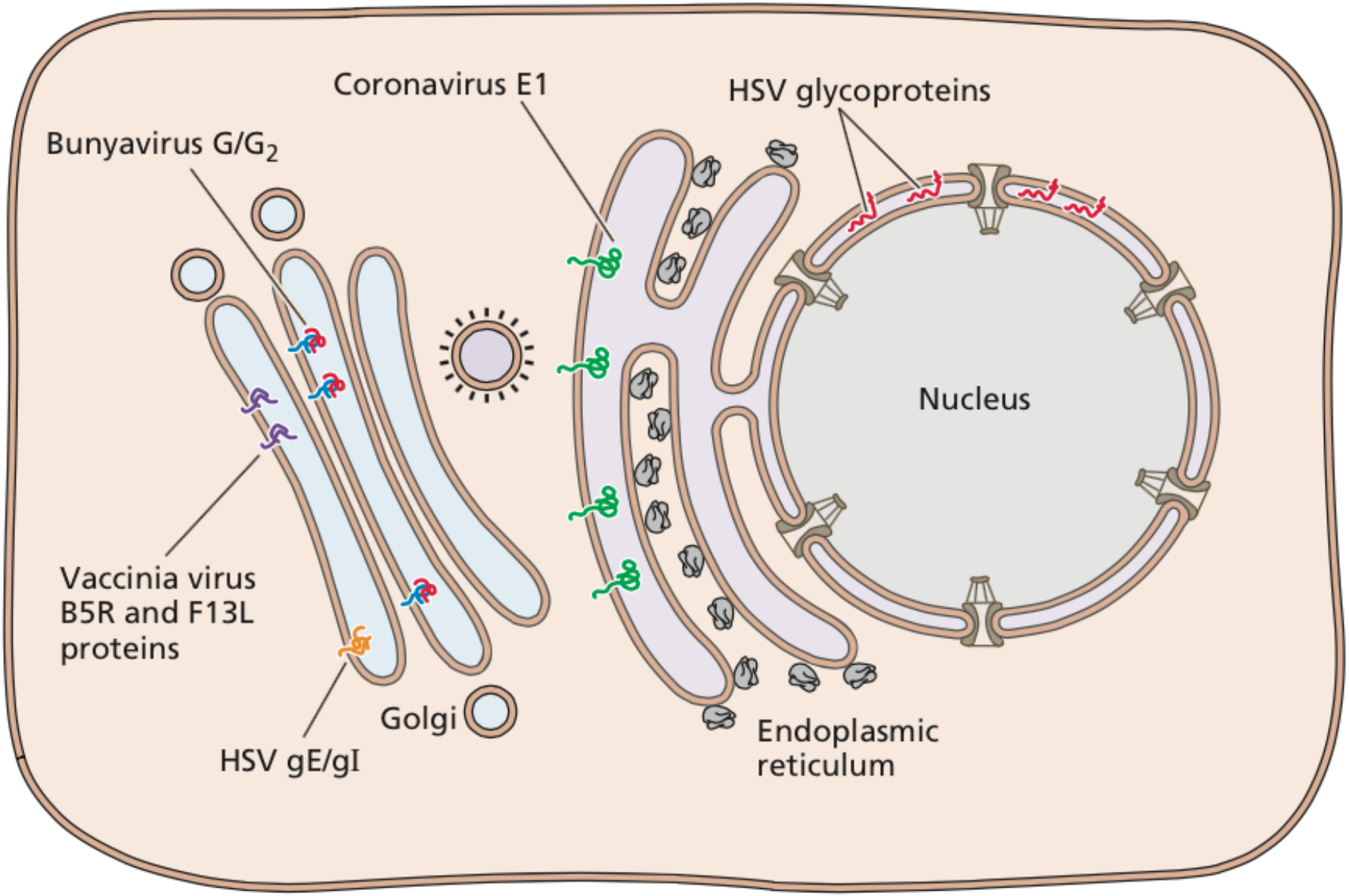
- Viral proteins are synthesized in the cytoplasm, and modified with lipids post-translationally



- Amino acid change in Gag cause arrest of budding at a 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

Endosomal sorting complexes required for transport (ESCRT) machinery





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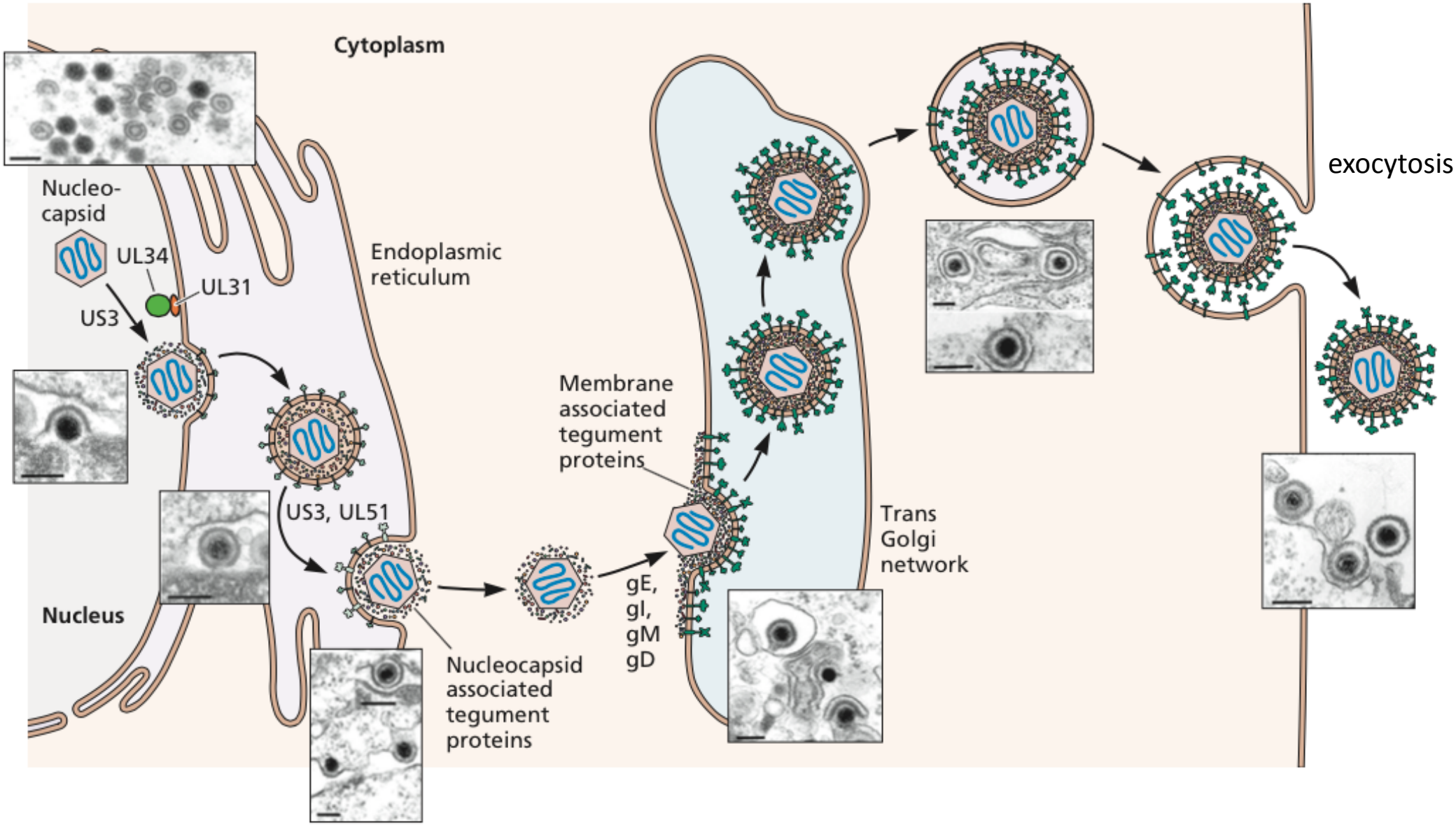
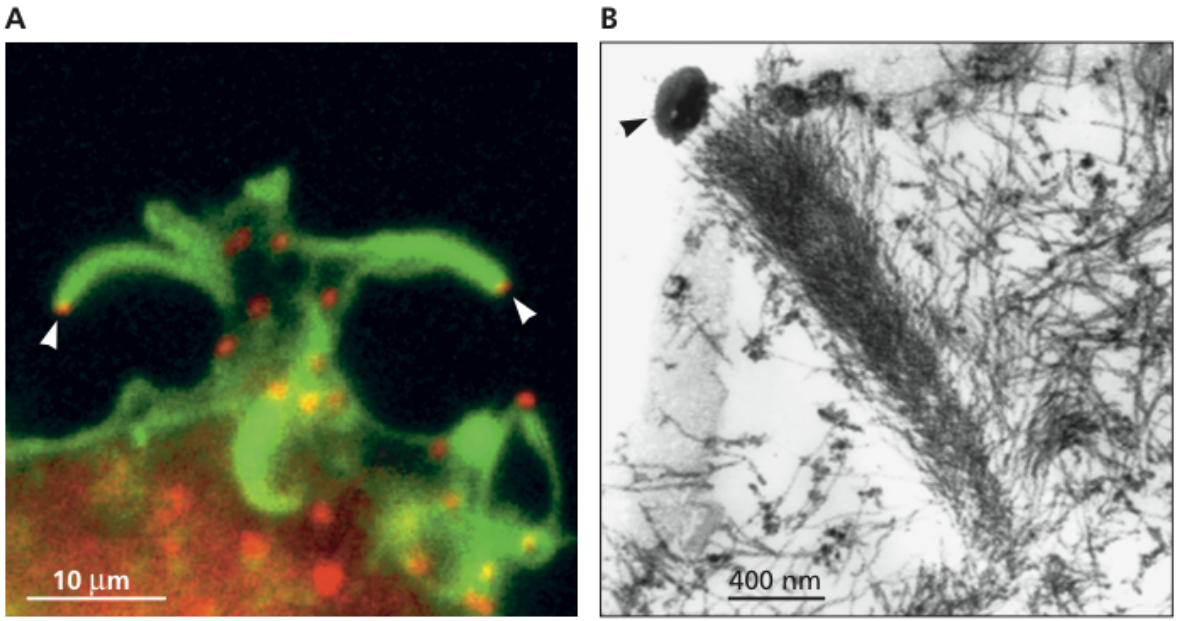
Which statement about viral budding is incorrect?

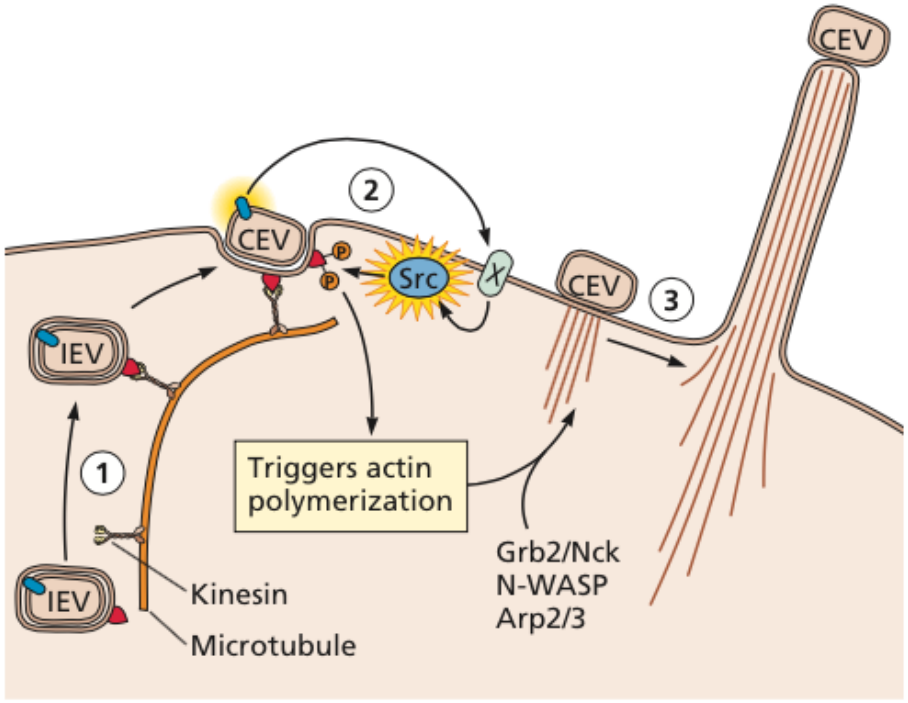
1. The envelope can be acquired before or simultaneous with assembly of internal components
2. The viral spike glycoprotein can drive budding
3. No host proteins are involved in the budding process
4. Lipids assist structural proteins to interact with the membrane
5. Budding can occur from the nucleus,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

Herpesvirus assembly and egress

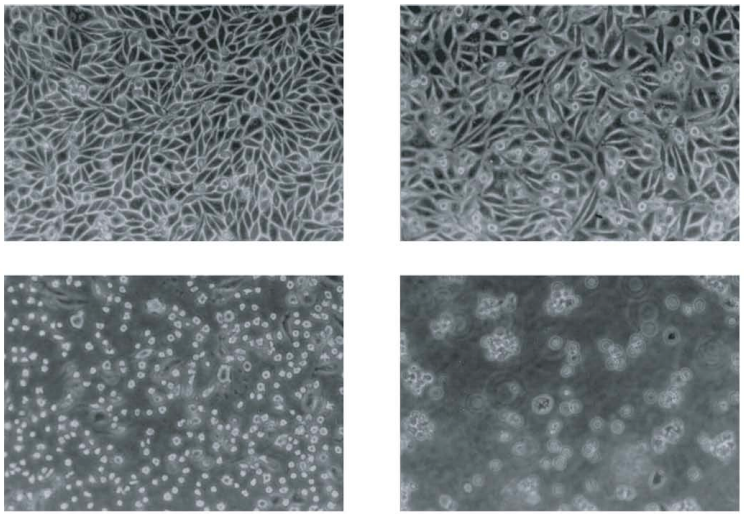


Propulsion of vaccinia virus on actin tails



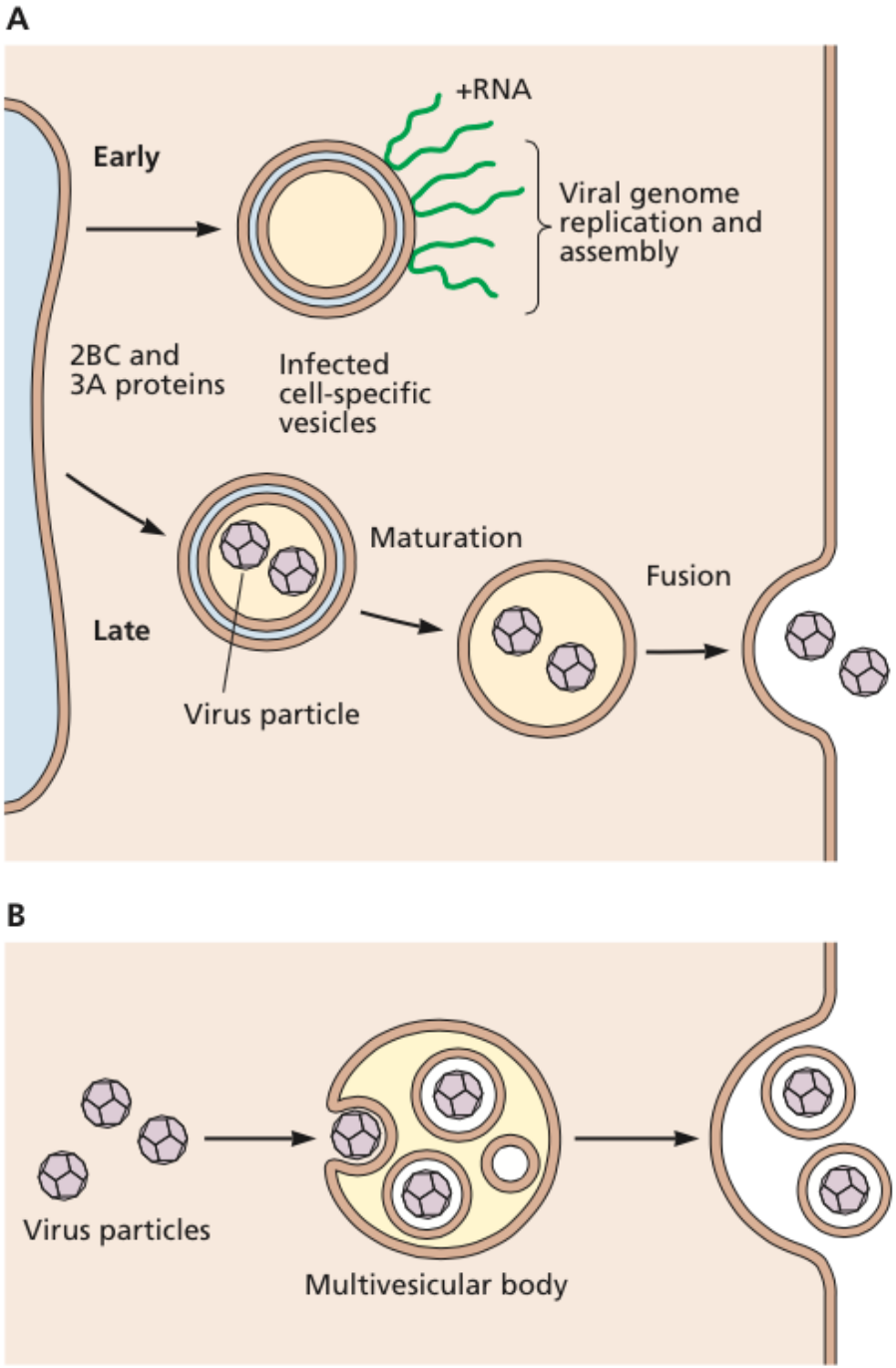


Release of nonenveloped viruses



- 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

Non-lytic release of nonenveloped viruses



Exosomes