Assembly, exit and maturation of progeny virions

Lecture 19 Flint et al., Chapter 13

Common problems

- **Must form structural units to protect genome**
- **Assemble coat by interactions among structural units**
  - **Self-self interactions**
  - **Help by ‘chaperones’**
- **Incorporation of nucleic acid genome**
  - **Protein and nucleic acid based ‘packaging signals’**
- **Release newly assembled viral progeny**
  - **Budding**
  - **Lysis**
- **Must be built to Protect genome, yet allow disassembly upon infection**
  - **Covalent modifications at different stages of maturation**

Methods to study virus assembly and exit

- Structural studies, e.g. X-ray crystallography
- Visualization of assembly and exit: EM studies
- Biochemical analyses; identify and characterize interacting parteners
- Genetic methods: make mutants and see what goes wrong.
- Molecular Biology: synthesize pure proteins/nucleic acids.

Making structural units

3 general strategies

1. Assemble from individual protein molecules
2. Assemble from polyprotein precursor
3. Chaperone-assisted assembly

Assembly from individual protein molecules

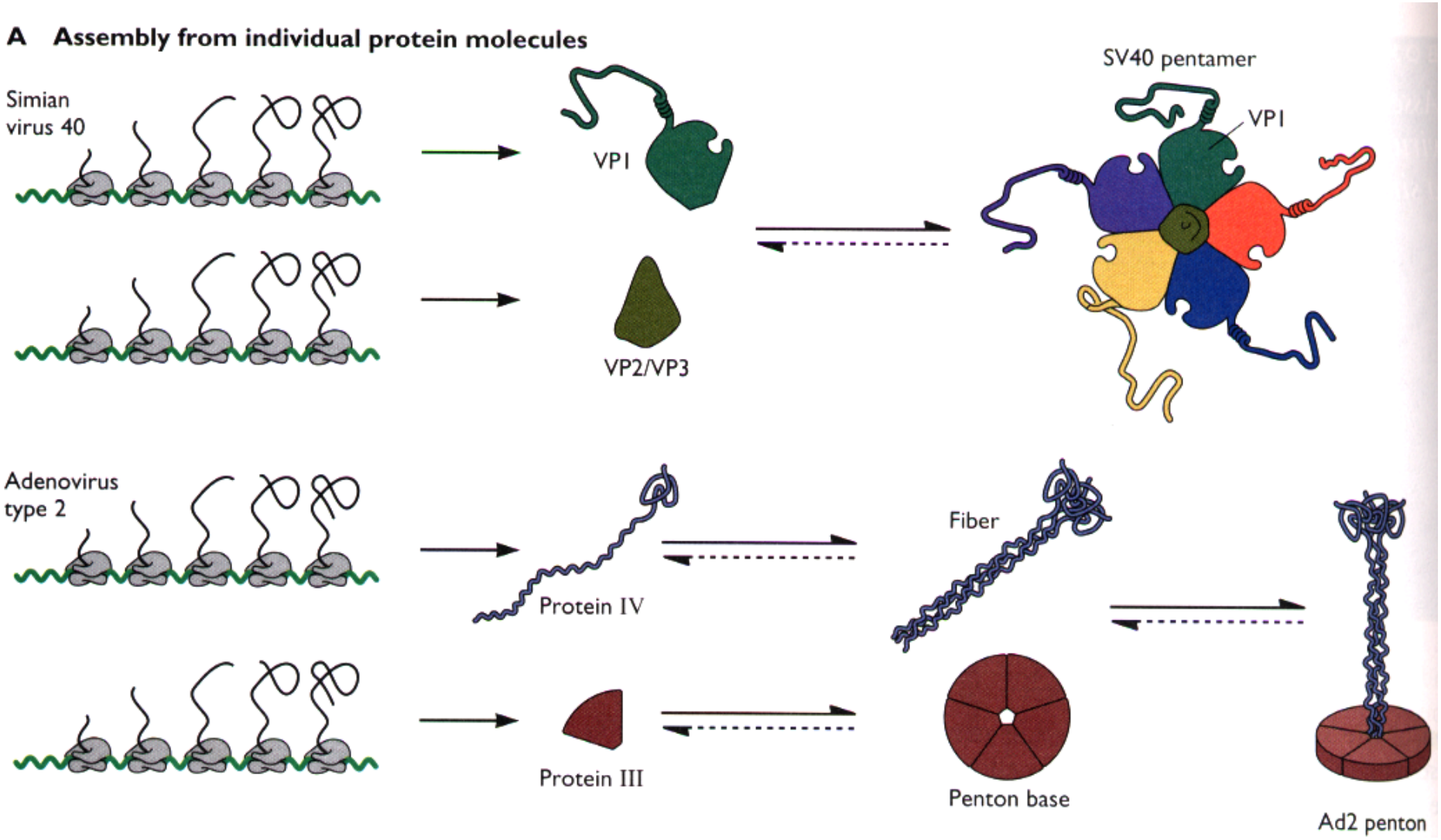


Fig. 13.2A

Assembly from polyprotein precursor

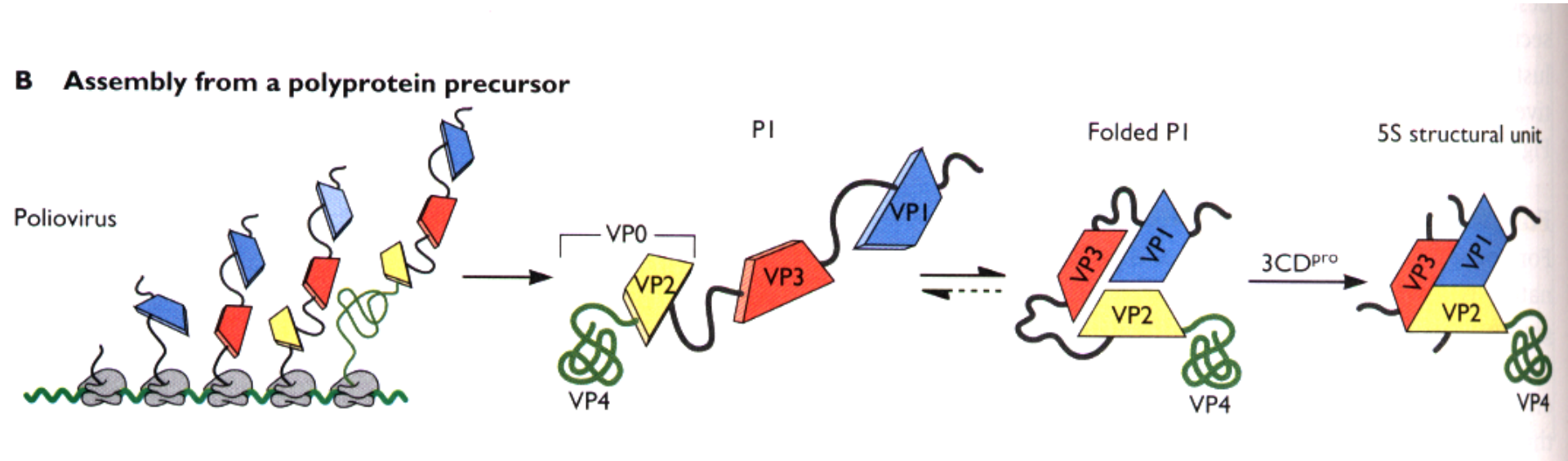


Fig. 13.2B

Chaperone-assisted assembly

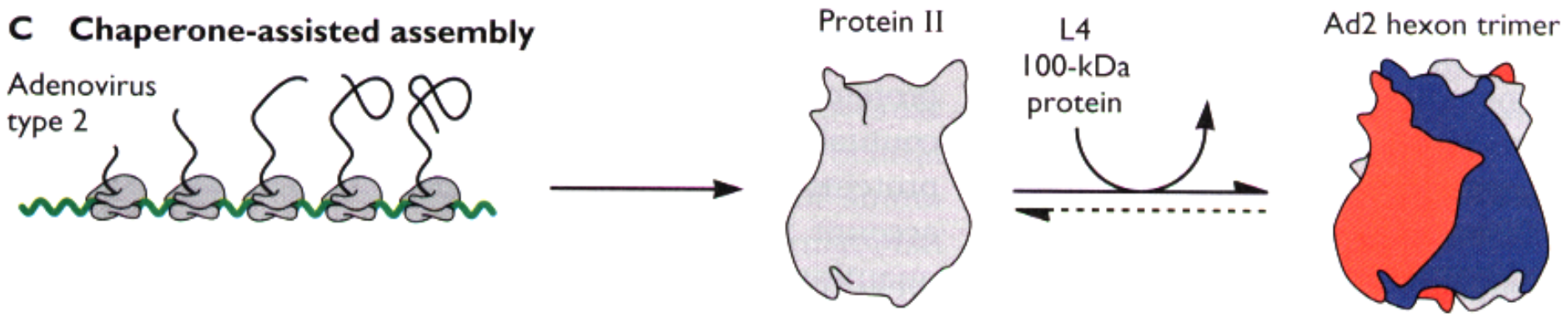
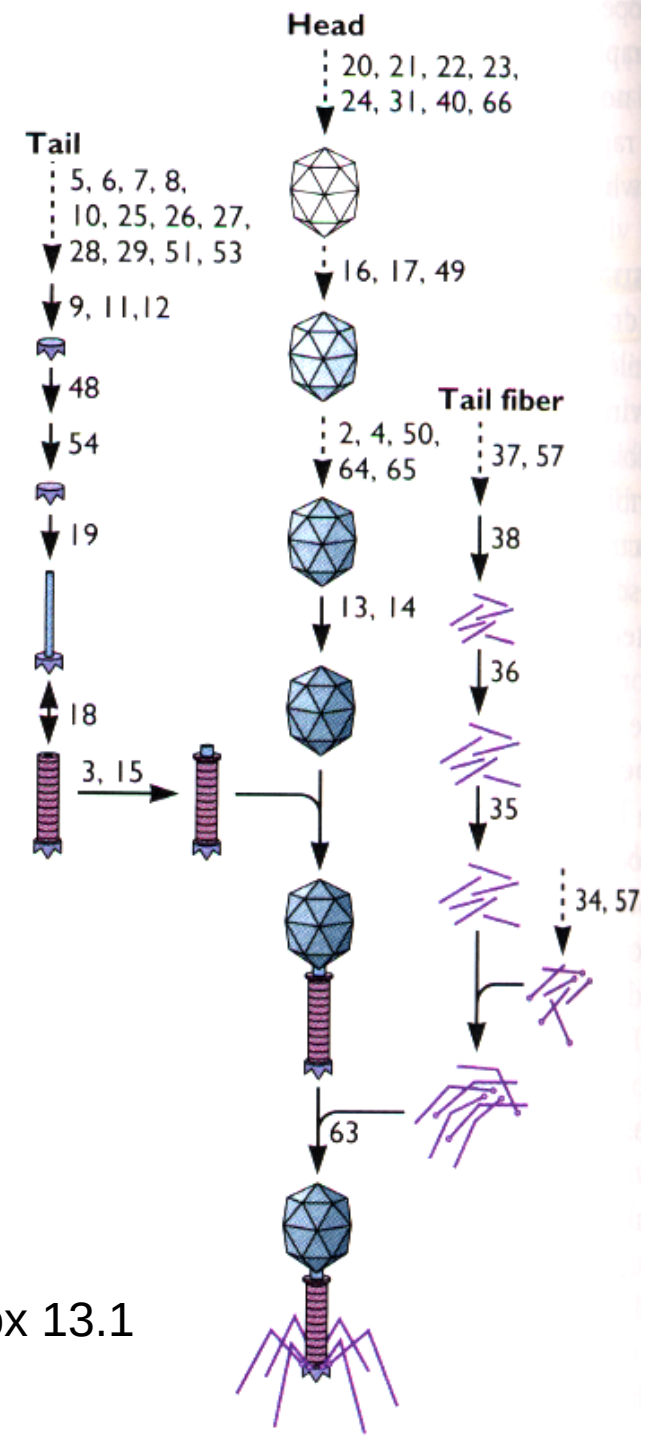


Fig. 13.2C

Assembly intermediates

- Assembly line mechanism ensures orderly formation of virus particles.
- Formation of discrete intermediate structures



T4 phage assembly. Box 13.1

Self- versus assisted-assembly reactions

- Structures associated with virus particles can self assemble
  - Example: Gag proteins of L-A and HIV can form icosahedral structures by themselves
- Assisted assembly
  - Proteins and nucleic acid genomes can assist particle formation as scaffolds/chaperones

Viral scaffolding proteins as templates for assembly

- Important points:
- Viral proteins initially used to establish transient, intermediate structures, and to package genomes.
  - Provirus, procapsids
- Viral proteases used to finalize structures, create metastable structures.

Viral scaffolding proteins as templates for assembly

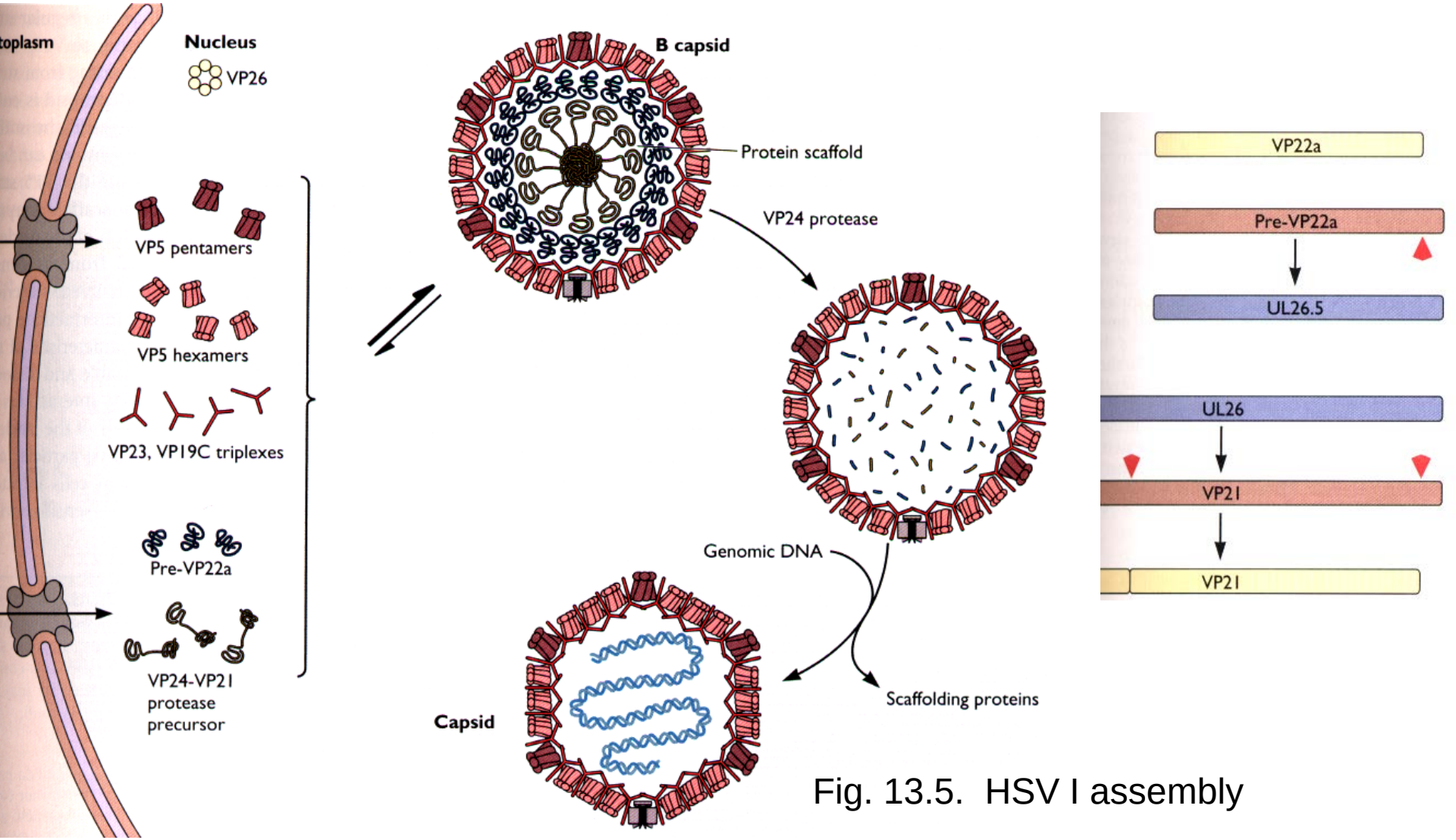


Fig. 13.5. HSV I assembly

Viral scaffolding proteins as templates for assembly

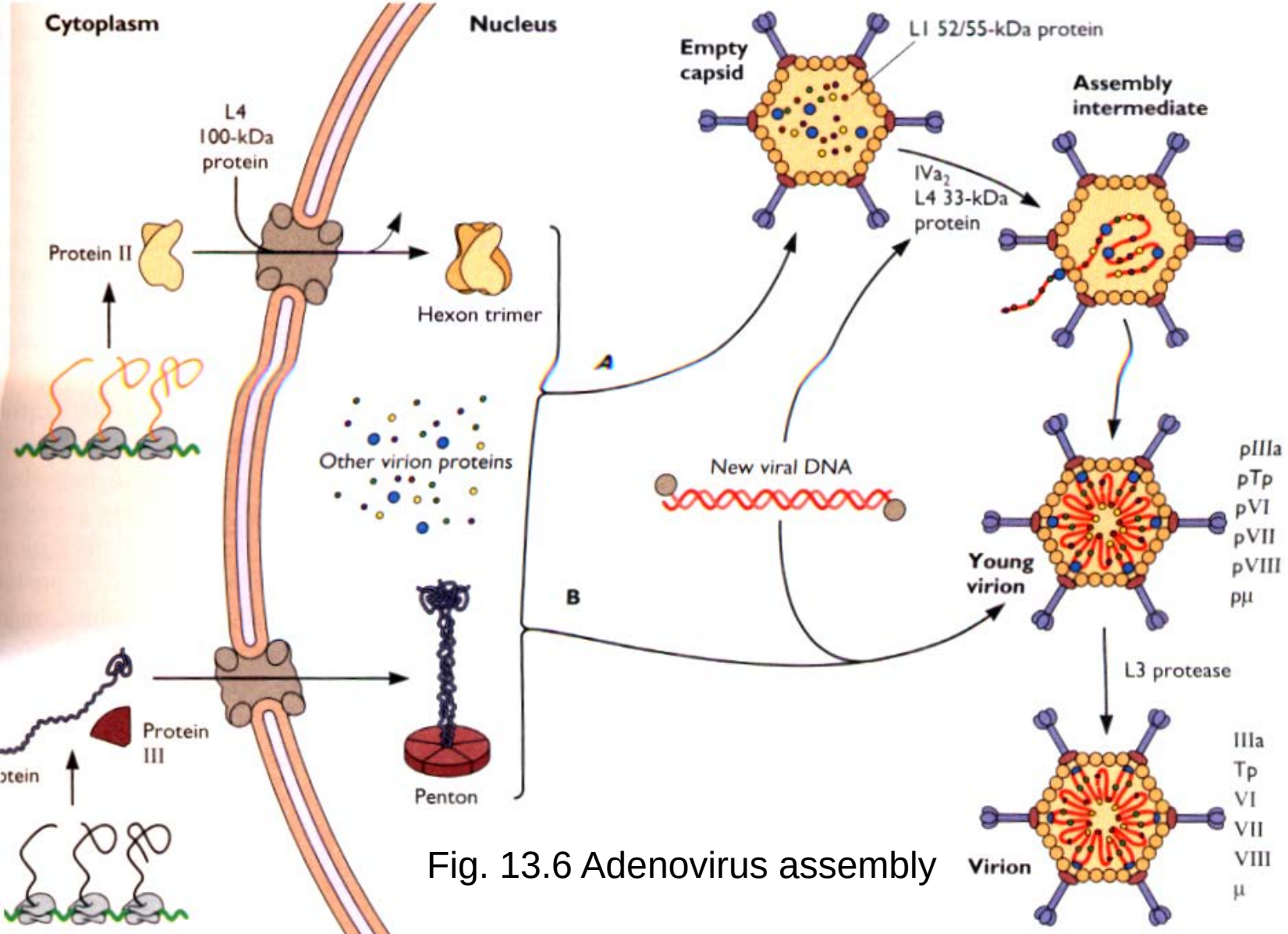
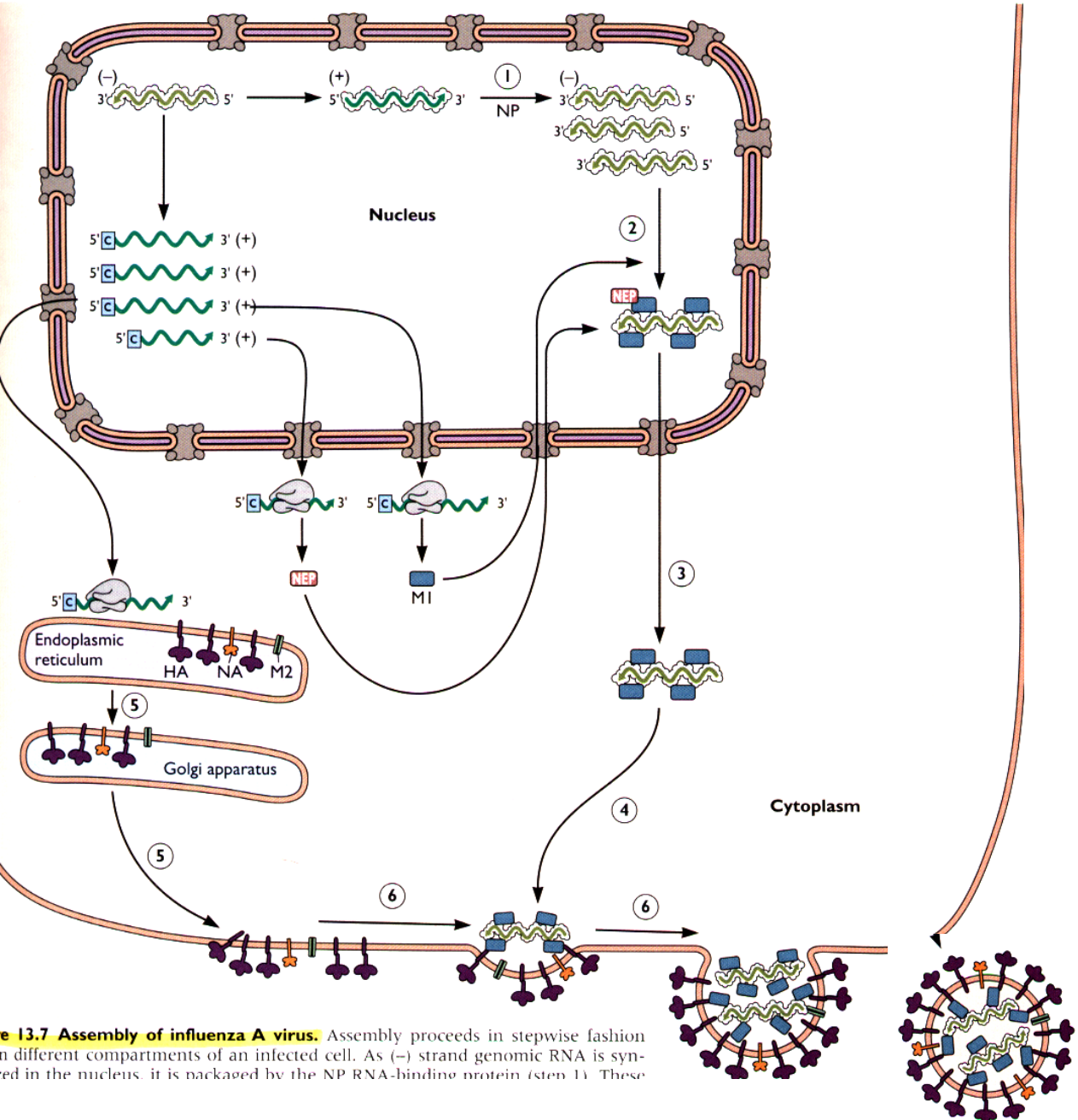


Fig. 13.6 Adenovirus assembly

Packaging

- Viral genomes must be *packaged* inside of nascent viral particles
- Requires interaction between
  - *cis-acting* signals on genomic nucleic acid and
  - *trans-acting* viral factors
- Two modes of assembly:
  - **Concerted assembly:** structural units of capsids shell only assemble productively in association with genomic nucleic acid.
    - Examples: Influenza A (Fig. 13.7), Retroviruses (Fig. 13.8)
  - **Sequential assembly:** genome inserted into preformed shell.
    - Example: Herpesviruses (Fig. 13.5)

Concerted assembly



e 13.7 Assembly of influenza A virus. Assembly proceeds in stepwise fashion n different compartments of an infected cell. As (–) strand genomic RNA is syn- ed in the nucleus, it is packaged by the NP RNA-binding protein (step 1). These

Fig. 13.7: Stepwise assembly of Influenza A virus

Concerted assembly

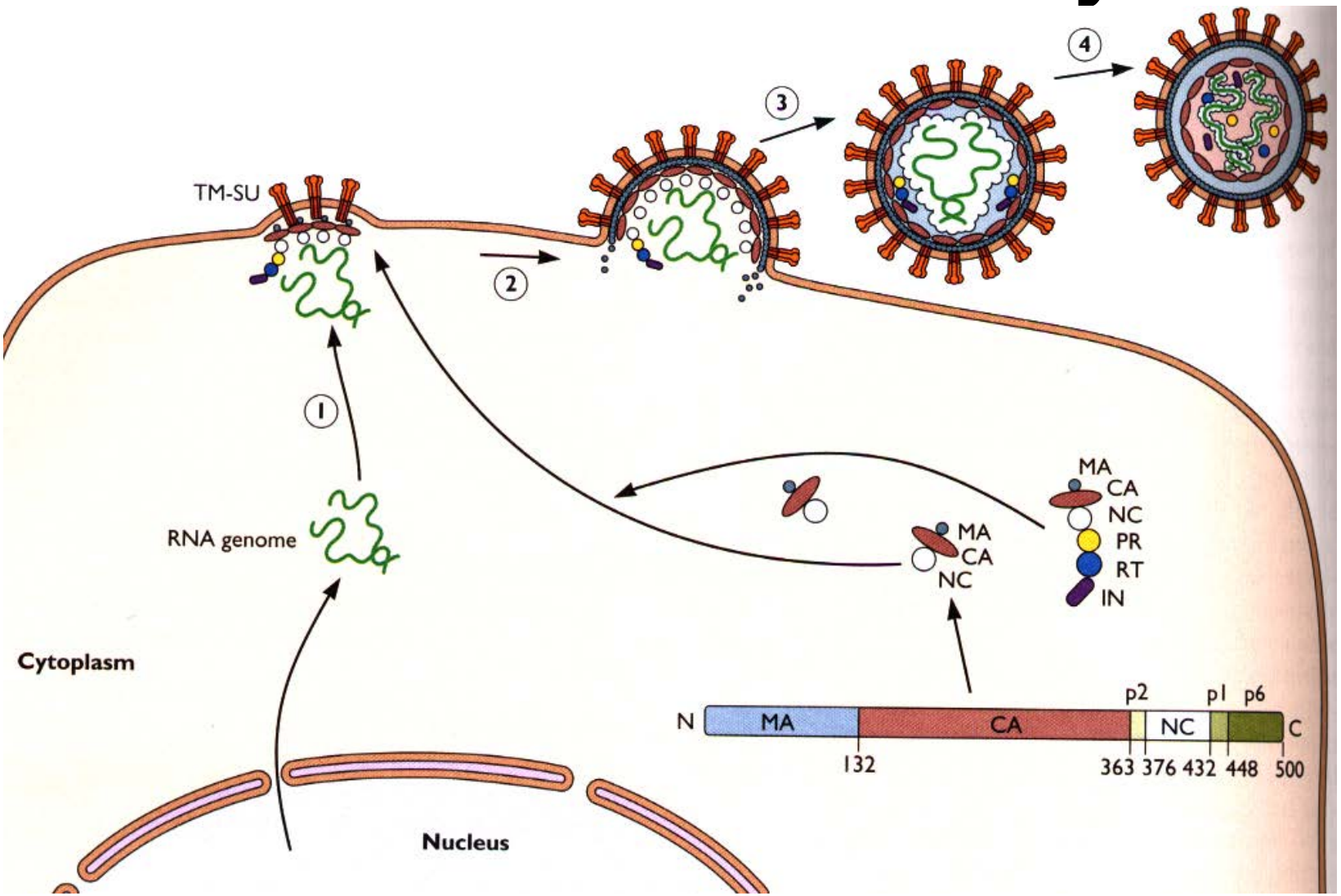


Fig. 13.8. Assembly of retrovirus from polyprotein precursors.

Sequential assembly

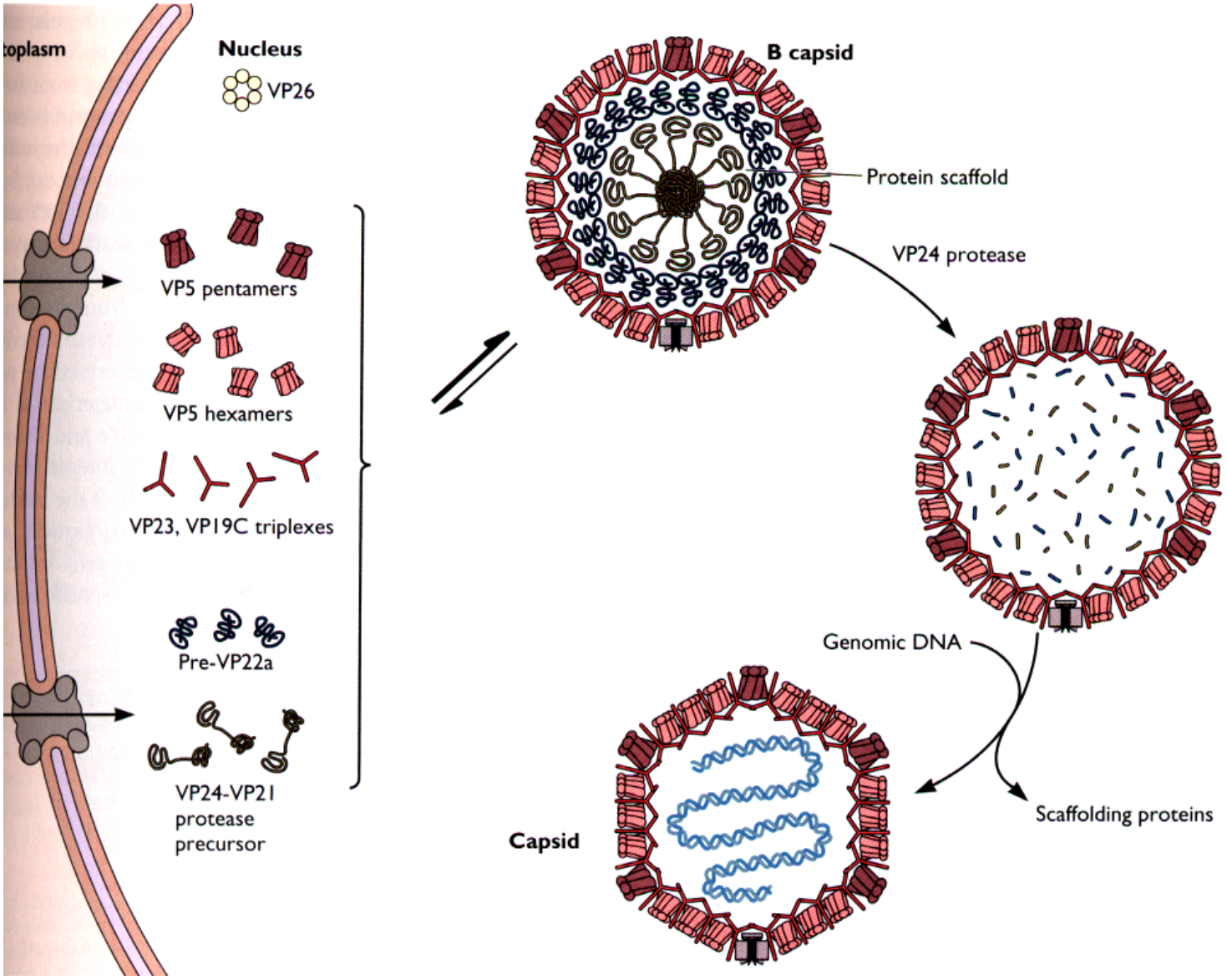


Fig. 13.5. HSV I genome is packaged into preformed shell

Recognition and packaging of nucleic acid genomes

- Without a genome, a viral particle is useless.
- Viral genomes contain *packaging signals*: Nucleic acid sequences and/or structures that physically interact with specific viral proteins.

Recognition and packaging of nucleic acid genomes

Example: the HIV-1 Ψ site (Fig. 13.11) + the NC packaging protein (Fig. 13.12)

Ψ: Only present on full-length (+) RNA.

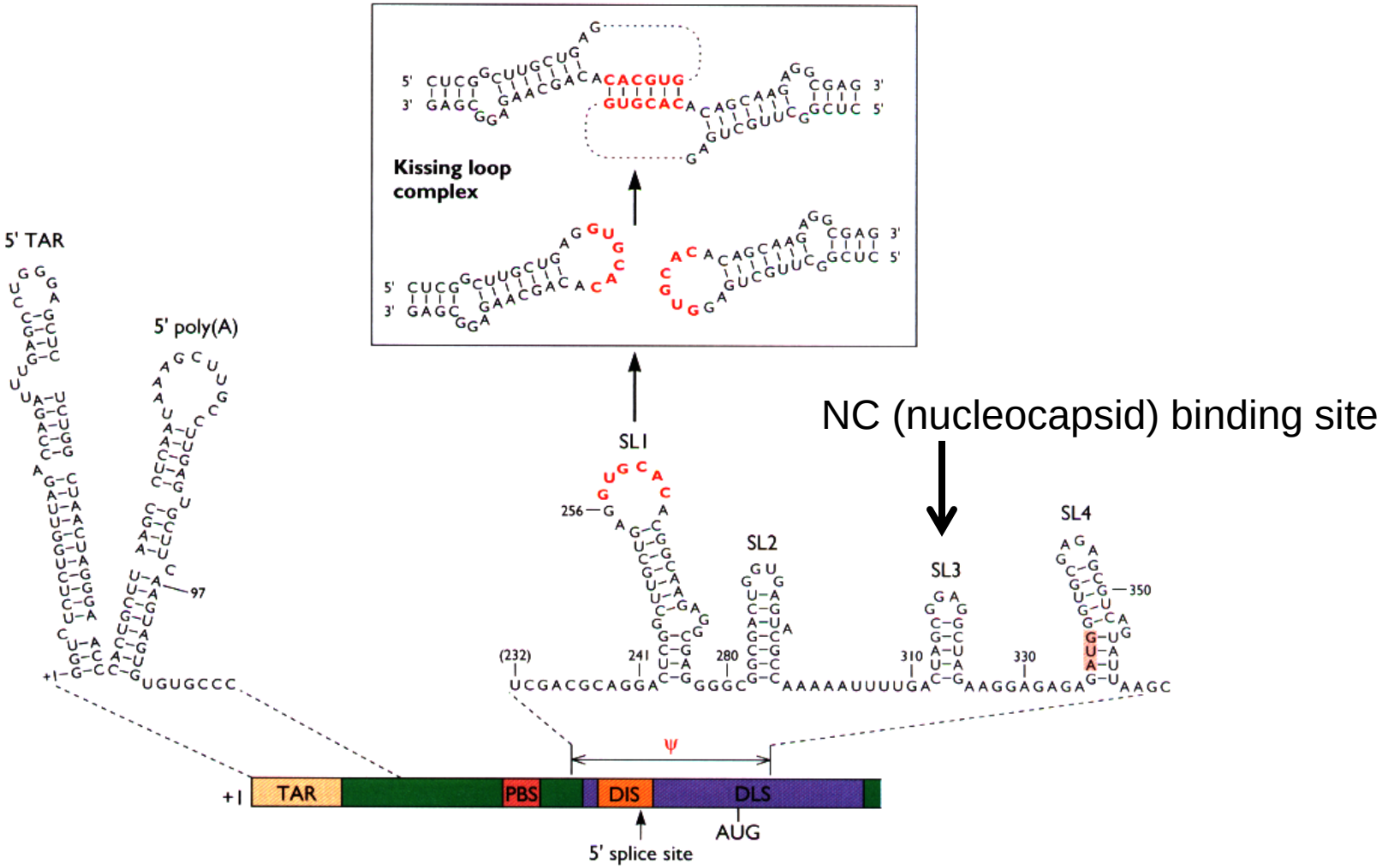
- Spliced out in subgenomic mRNAs…therefore these mRNAs cannot be packaged into viral particles
- Highly structured RNA: forms kissing-loop complex between 2 RNA molecules
- Serves as the cis-acting element on the HIV-1 genomic RNA

NC (Nucleocapsid) protein:

- Formed from the Gag protein precursor
- Part of the nucleocapsid
- Acts as the trans-acting factor for Ψ, i.e. specifically binds with Ψ.
- The interaction between Ψ and NC ensures that the viral genome physically associates with viral particles as they are being assembled.

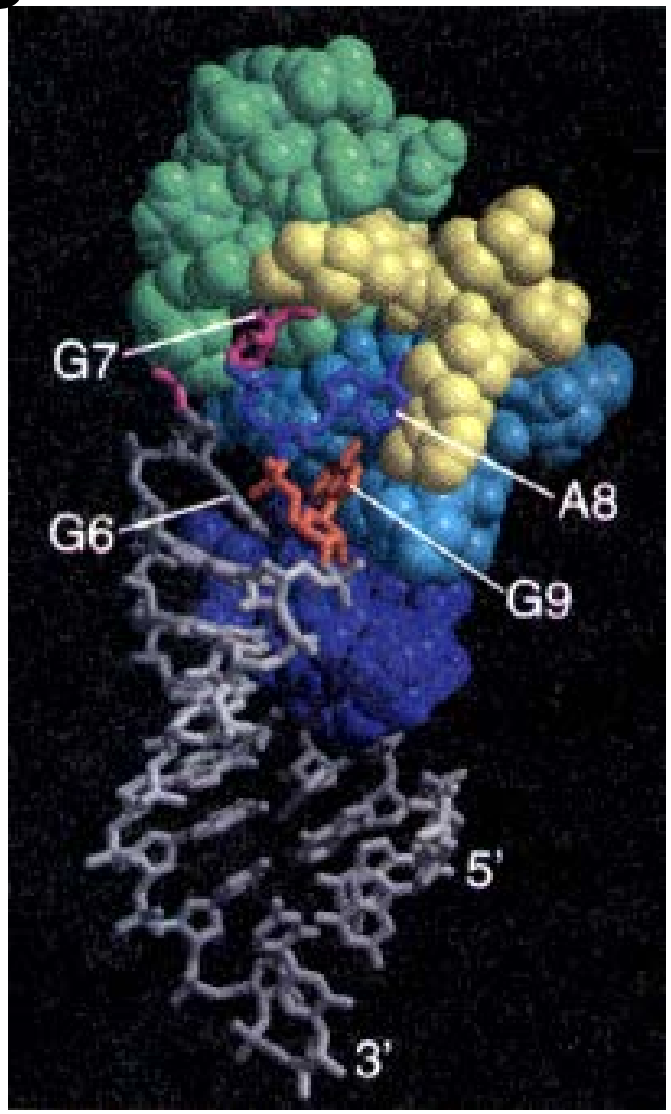
Recognition and packaging of nucleic acid genomes

Example: the HIV-1 Ψ site (Fig. 13.11)



Recognition and packaging of nucleic acid genomes

- SL3 of HIV-1 Ψ site) bound to the NC packaging protein (Fig. 3.12)



Packaging of segmented genomes

- Segmented genomes present a special problem in virus assembly
- For such a virus to be viable, it needs all segments packaged
- Two strategies: Random and selective
- Random packaging
  - Genome segments randomly packaged into viral particles.
    - Upside: no requirement for evolution of a highly complex program
    - Downside: wasteful
- Selective packaging
  - Genome segments packaged in an ordered manner.
  - e.g. Segment 2 cannot be packaged until Segment 1 is, etc.
    - Downside: requires evolution of a complex packaging program with multiple physical/biochemical mechanisms
    - Upside: Highly efficient

Envelope acquisition

- Capsid formation tends to separated from envelope acquisition for most enveloped viruses.
- Typically, capsids structures assemble inside of the cell while envelopes are acquired in association with membranes.
- Exceptions exist where the assembly of internal structures, e.g. nucleocapsids, are coordinated with envelope acquisition.

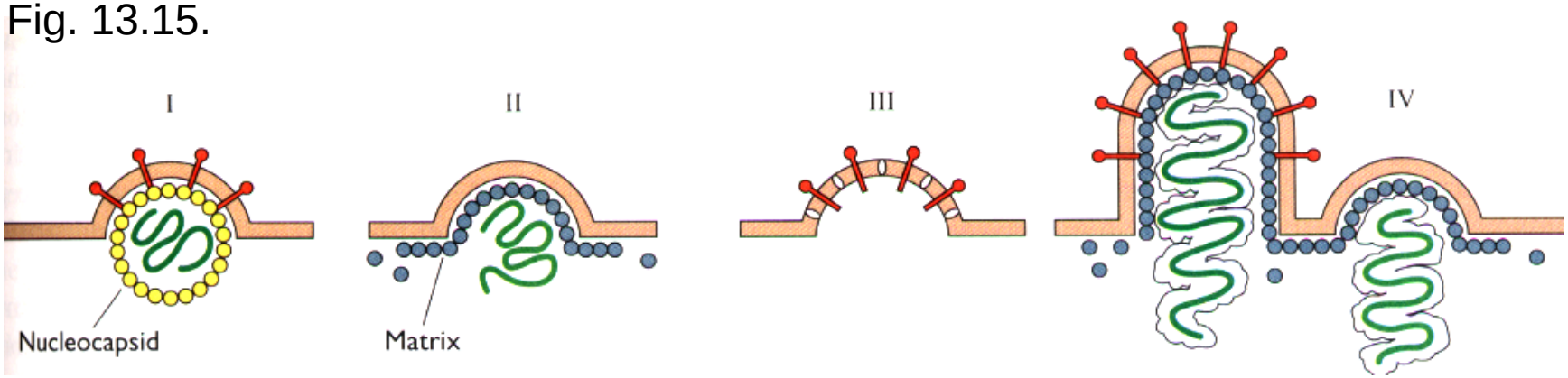
Release of viral particles

- Nonenveloped viruses.
- Lysis is the preferred mechanism
- Just bust out and wreak havoc.

Release of viral particles

- Budding: preferred by enveloped viruses that assembly at the plasma membrane

Fig. 13.15.



Env and capsid essential. E.g. sindbis virus

Only capsid is essential e.g. retrovirus

Driven solely by env proteins e.g. coronavirus

Complex, e.g. rhabdoviruses

Release of viral particles

- Exocytosis: reverse of endocytosis. Preferred by viruses that assembly within vesicular compartments, e.g. in the ER or Golgi.

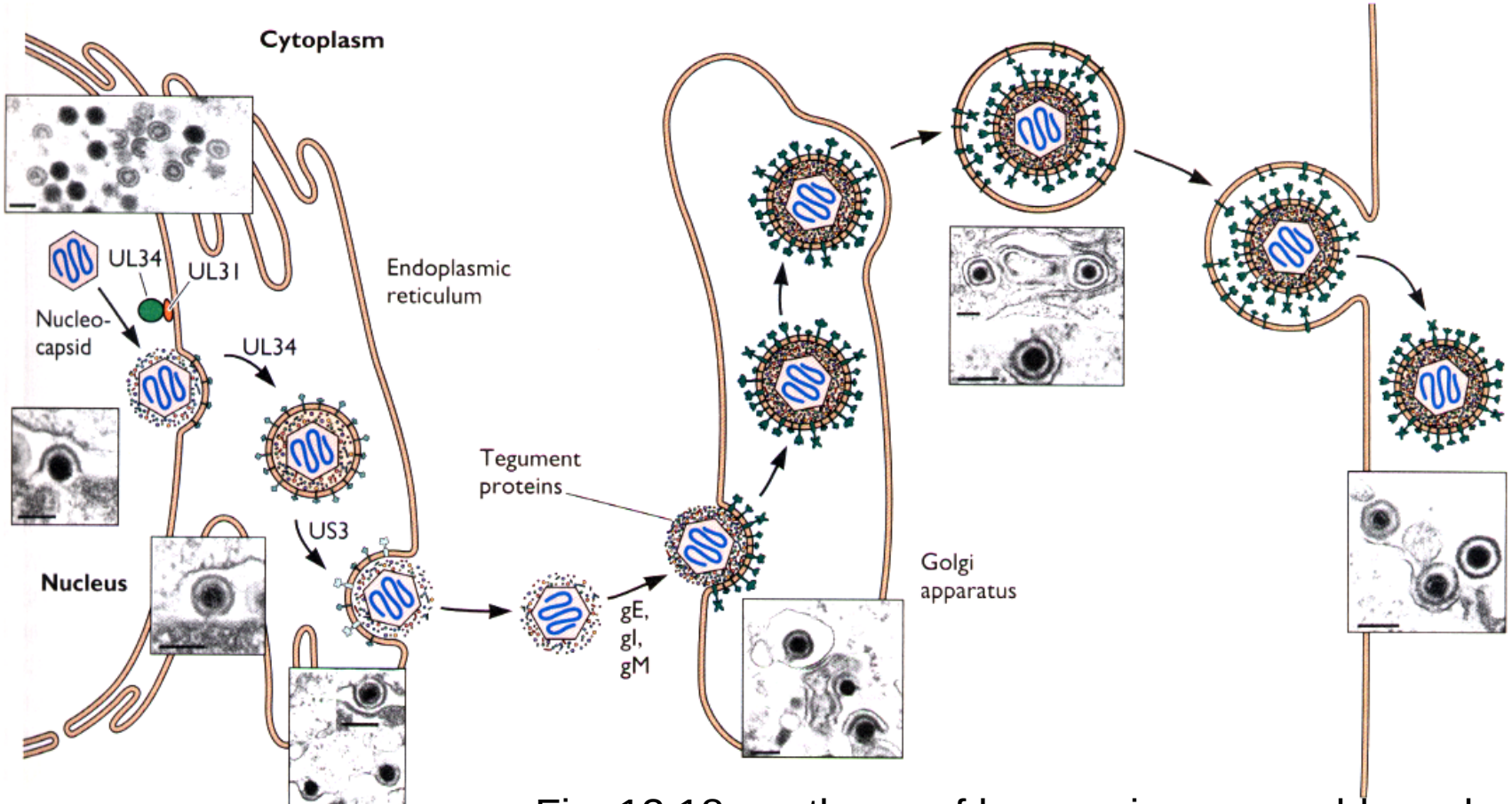
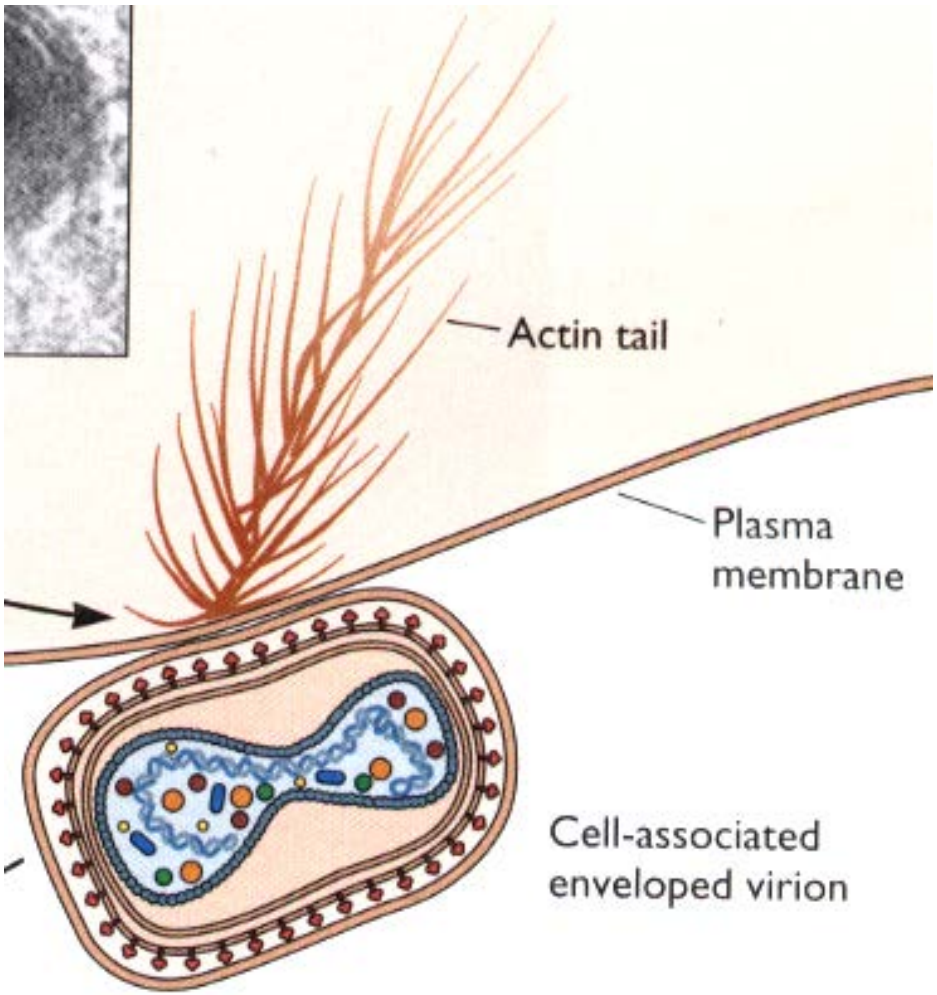


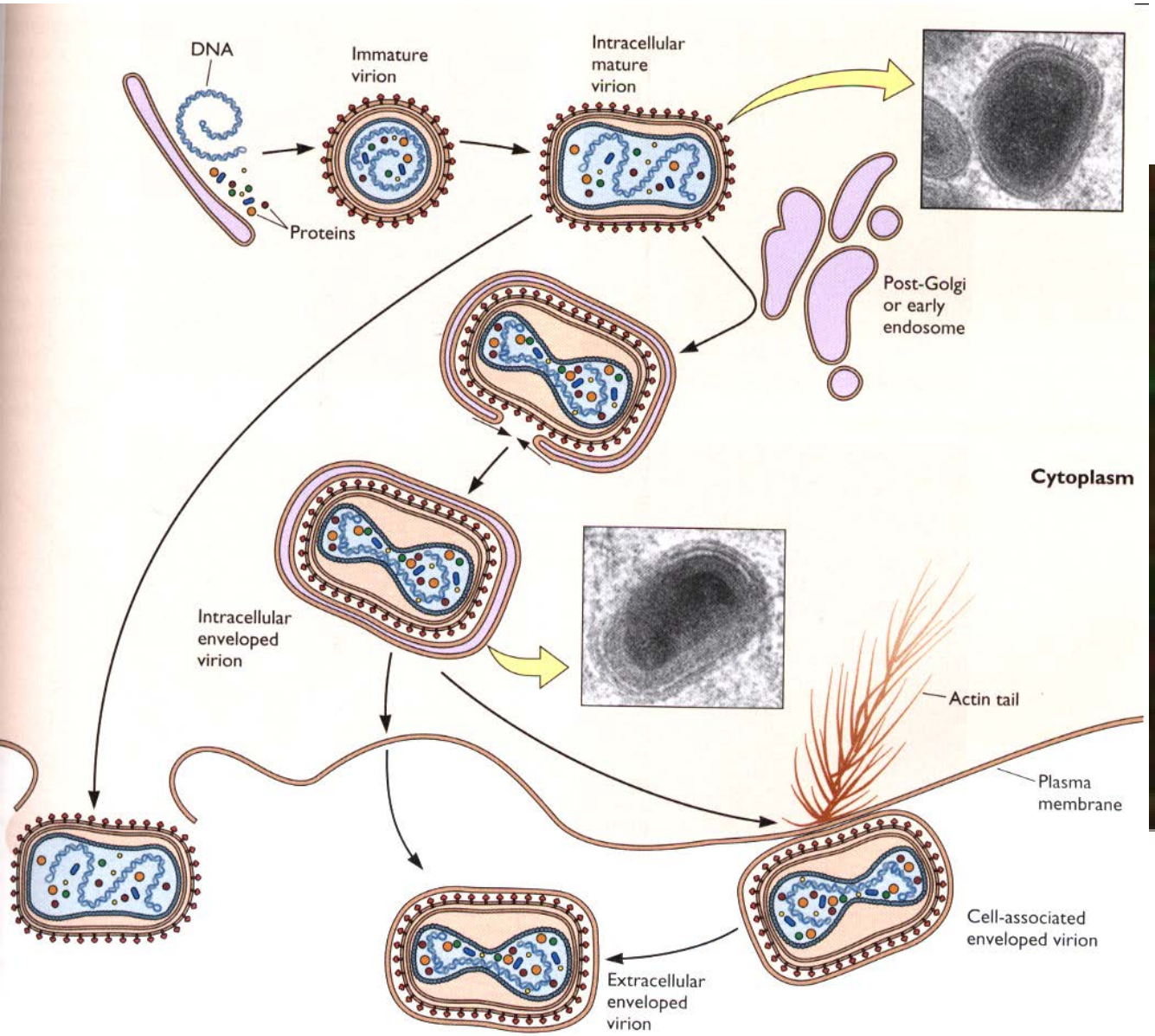
Fig. 13.18. pathway of herpesvirus assembly and exit.

Release of viral particles

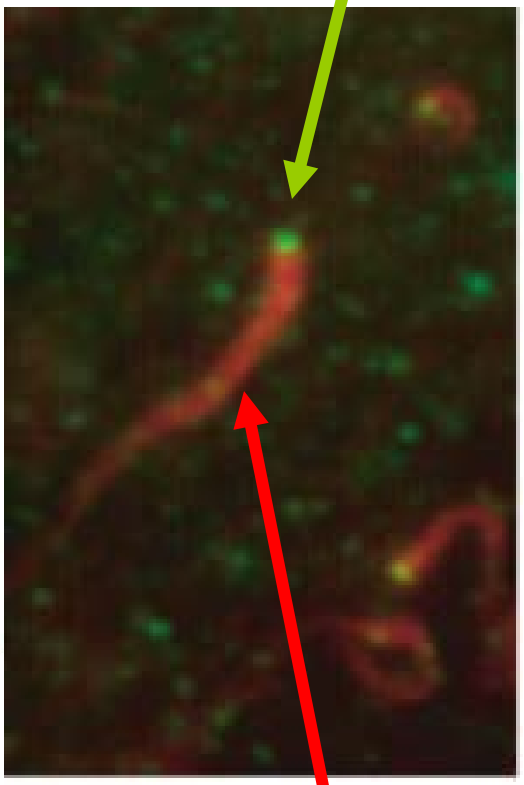
- Expulsion via polymerization of actin tails
- Directionally polymerize actin, propelling them out of cells!



Poxviruses: 3 for 1 exit



gure 13.16 *(continued)*

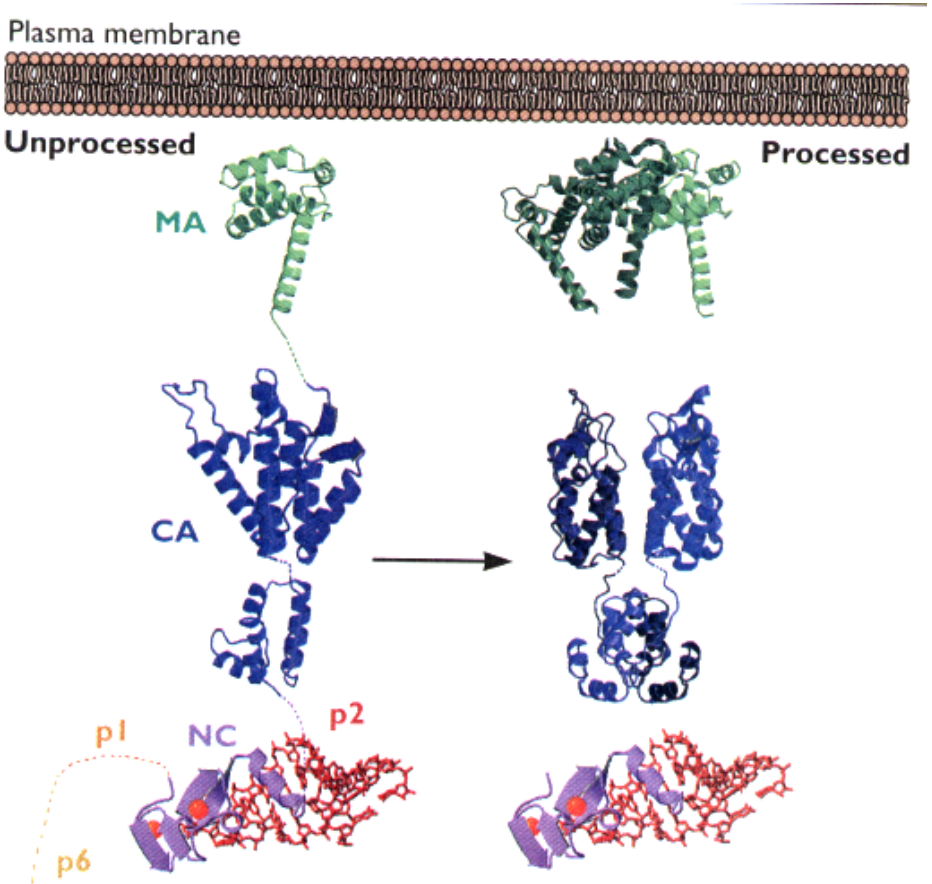


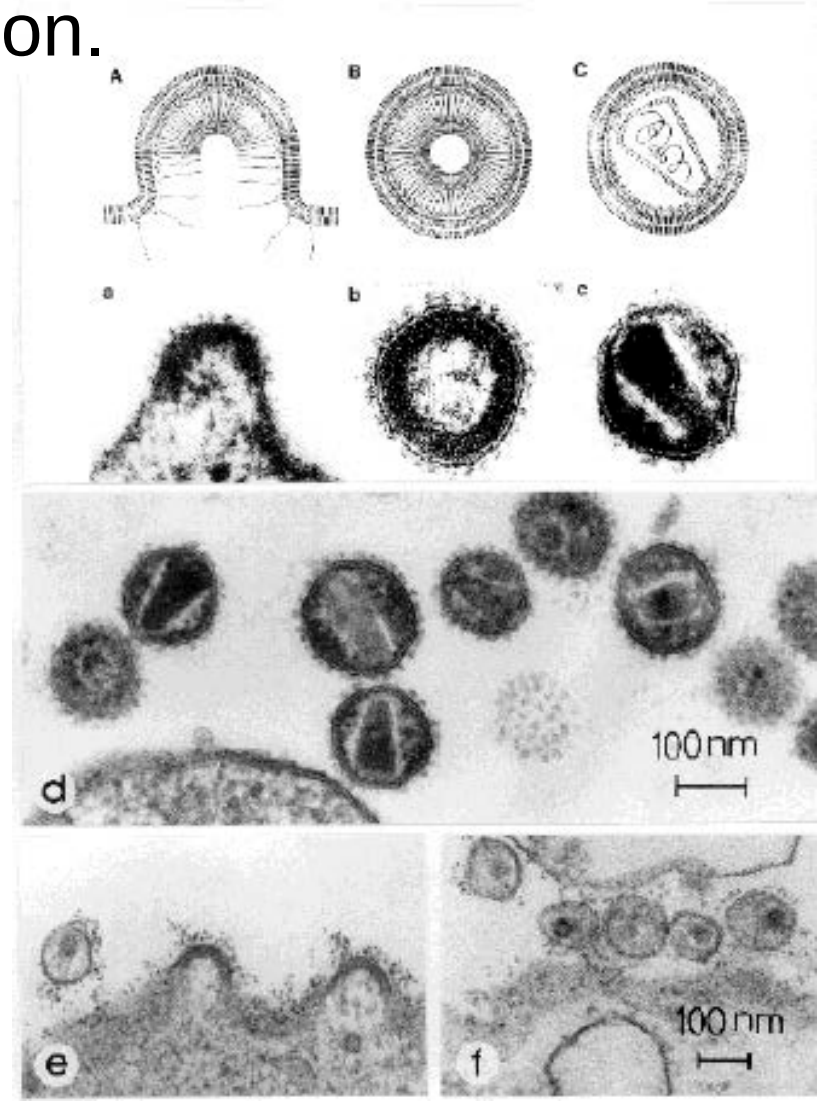
Virion maturation

- For many viruses, the primary viral particle products are not infectious. They need to undergo additional steps of maturation after particle assembly
- Maturation most often accomplished by proteolytic processing inside of the particle.
- Protein cleavage exchanges covalent for non-covalent interactions.
- Creation of additional N- and C-terminal ends creates new sites for interactions.
- Provides a mechanism to resolve contradictory requirements of stable assembly of particle versus the need for the virion to disassemble upon infection.

Viron maturation

- Example: retroviruses. Cleavage of Gag precursor defines final densities and shapes within infections virion.





•Fig. 13.19 and Box 13.5.