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 partners
• 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

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 $\Psi$ site (Fig. 13.11) + the NC packaging protein (Fig. 13.12)

$\Psi$: 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 $\Psi$, i.e. specifically binds with $\Psi$.
- The interaction between $\Psi$ 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)

NC (nucleocapsid) binding site
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.

- **I**: Nucleocapsid
  - Env and capsid essential.
  - E.g. sindbis virus

- **II**: Matrix
  - Only capsid is essential
  - E.g. retrovirus

- **III**: Driven solely by env proteins
  - E.g. coronavirus

- **IV**: 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

- DNA
- Immature virion
- Intracellular mature virion
- Viral particle
- Post-Golgi or early endosome
- Cytoplasm
- Actin tail
- Extracellular enveloped virion
- Plasma membrane
- Cell-associated enveloped virion

Viral particle
Actin tail
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.