Assembly

Lecture 10
Biology 4310
Virology
Spring 2021

“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

1. Formation of individual structural units of the protein shell from one or several viral proteins
2. Assembly of the protein shell by appropriate, and sometimes variable, interactions among structural units
3. Selective packaging of the nucleic acid genome and other essential virion components
4. Maturation of virus particles
5. Release from host cell
6. Acquisition of an envelope
7. Infection of new host cell
Assembly is dependent on host cell machinery

- Cellular chaperones
- Transport systems
- Secretory pathway
- Nuclear import and export machinery
Moving in heavy traffic

- **Short distance**
  - Energy-dependent pores
  - Angstroms to nanometers
  - Membrane (plasma, nuclear, ER, Golgi)
  - Micrometers to meters
- **Long distance**
  - Energy-dependent motor proteins on cytoskeletal tracks
  - Site of replication, protein synthesis, or assembly
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

- Plasma membrane
- Golgi apparatus
- Ribosome
- Rough endoplasmic reticulum
- Py(VP1)$_5$ + VP2/3
- L4 100-kDa protein
- Nuclear envelope:
  - Outer nuclear membrane
  - Inner nuclear membrane
  - Nuclear pore complex
- Porcine parvovirus VP2 trimer
- Cytoskeleton:
  - Intermediate filament
  - Microtubule
  - Actin filament bundle
- Influenza virus NP
- Mitochondrion
- Extracellular matrix
Localization of viral proteins to plasma membrane
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
Three strategies for making sub-assemblies

A. Assembly from individual protein molecules

B. Assembly from a polyprotein precursor

C. Chaperone-assisted assembly
Assembly reactions assisted by cellular chaperones

**Retrovirus**

TRIC + Nascent Gag → Gag → ABCE1 → 10S → 80S → 150S → 500S

**Polyomavirus**

HSC70 + LT → ATP → ADP + Pi
Sequential capsid assembly: poliovirus
Viral scaffolding proteins

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

Influenza virus

Virus particles assemble only in association with viral genome
Segmented (−) strand RNA coated with nucleocapsid protein (NP)
Maturation of influenza HA0

1. Folding
2. Oligomerization
3. Oligosaccharide trimming
4. Addition of GlcNAc
5. Addition of galactose
6. Addition of sialic acid
7. Cleavage of HA0
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
• 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 IVa2
- Herpesvirus genome replication produces concatamers with head-to-tail copies of viral genome

- HSV-1 packaging signals \textit{pac1} and \textit{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

Selective packaging

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

I. Nucleocapsid
   - Envelope glycoproteins and capsid essential for budding - alphaviruses

II. Matrix
   - Internal matrix or capsid proteins drive budding - retroviruses

III. Envelope proteins drive budding - influenza virus, coronavirus

IV. 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
Membrane targeting sequences

A Influenza virus M1

1. Hydrophobic regions
2. RRR
3. RKLR
4. NES
5. NLS

6. Binding to RNP

B VSV M

1. Hydrophobic region

2. Binding to RNPs
3. Membrane binding
- Gag alone produces virus-like particles
- Internal structure assembly and budding spatially & temporally coincident
- 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 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
Endosomal sorting complexes required for transport (ESCRT) machinery
Sorting of viral glycoproteins to internal membranes

- Bunyavirus GnGc
- Flavivirus prM/E
- Coronavirus E1
- HSV gB, gH
- HSV UL34
- Nucleus
- Vaccinia virus B5 and F13 proteins
- Vaccinia virus A33, A35, and A56
- HSV gE/gI
- Vaccinia virus A17/A14
- Endoplasmic reticulum
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
Low pH induced conformational change and maturation

Dengue virus
Leaving the cell:

Propulsion of vaccinia virus on actin tails

IEV = intracellular enveloped virion
CEV = cell associated enveloped virion
Leaving the cell

A

Epithelial cells

B

C
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
Non-lytic release of nonenveloped viruses

**A**
- Early
- +RNA
- Viral genome replication and assembly
- 2BC and 3A proteins
- Infected cell-specific vesicles

**B**
- Late
- Maturation
- Fusion
- Virus particle
- Multivesicular body
- Exosomes

*Double-membrane vesicles formed by autophagy*
Next time: The infected cell