Assembly

Lecture 11
Biology W3310/4310
Virology
Spring 2014

“Anatomy is destiny.”
--SIGMUND FREUD
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. Acquisition of an envelope
5. Release from host cell
6. Virion maturation

*common to all viruses

*common to many viruses

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The structure of a virus particle determines how it is formed.
Assembly is dependent on host cell machinery

- Cellular chaperones
- Transport systems
- Secretory pathway
- Nuclear import and export machinery
Concentrating components for 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’ built into their structure

- Membrane targeting: Signal sequences, fatty acid modifications
- Membrane retention signals
- Nuclear localization sequences (NLS)
- Nuclear export signals
polyomaviruses, must enter the nucleus despite continual transport of cellular proteins. Whether import of viral proteins is favored in such circumstances, for example, by the presence of particularly effective nuclear localization signals, is not known.

Many viral structural proteins that enter infected cell nuclei form multimeric capsid components. In some cases, structural units of the virion are assembled in the cytoplasm prior to import into the nucleus. Pentamers of the major capsid protein (VP1) of simian virus 40 and polyomavirus specifically bind a monomer of either VP2 or VP3, the minor virion proteins, which share C-terminal sequences (Appendix, Fig. 15B). Such heteromeric assemblies are the substrates for import into the nucleus. Indeed, efficient nuclear localization of polyomavirus VP2 and VP3 proteins occurs only in cells in which VP1 is also made. Assembly of the heteromeric complex facilitates import of the minor structural proteins, even though each contains a nuclear localization signal. The increased density of these signals may allow more effective competition for essential components of the import pathway, or the nuclear localization signals may be more accessible in the complex.

Despite such potential advantages as increased efficiency of import of viral proteins and transport of the structural proteins in the appropriate stoichiometry, import of preassembled capsid components is not universal. For example, adenoviral hexons, which are trimers of viral protein II, are found only in the nucleus of the infected cell. Association of newly synthesized hexon monomers with a late, nonstructural protein, the L4 100-kDa protein, is essential for both the entry of hexon subunits into the nucleus and their assembly into trimers.

Figure 12.1 Localization of viral proteins to the nucleus.

The nucleus and major membrane-bound compartments of the cytoplasm, as well as components of the cytoskeleton, are illustrated schematically and not to scale. Viral proteins destined for the nucleus are synthesized by cytoplasmic polyribosomes, as illustrated for the influenza virus NP protein. They engage with the cytoplasmic face of the nuclear pore complex and are translocated into the nucleus by the protein import machinery of the host cell. Some viral structural proteins enter the nucleus as preassembled structural units (polyomaviral [Py] VP1 pentamers associated with one molecule of either VP2 or VP3) or in association with a viral chaperone (adenoviral [Ad] hexon monomers bound to the L4 100-kDa protein).
Intracellular Trafficking

Hexon-L4 protein complex must be the import substrate. The viral L4 100-kDa protein might supply the nuclear localization signal for hexon import, or promote nuclear retention, by facilitating assembly of hexon trimers within the nucleus.

Assembly at the Plasma Membrane

Assembly of enveloped viruses frequently takes place at the plasma membrane of infected cells (Table 12.1). Before such virions can form, viral integral membrane proteins must be transported to this cellular membrane (Fig. 12.2). The first stages of the pathway by which viral and cellular proteins are delivered to the plasma membrane were identified more than 30 years ago, and the process is now quite well understood. Viruses with envelopes derived from the plasma membrane also contain internal proteins, which may be membrane associated, and, of course, nucleic acid genomes. These internal components must also be sorted to appropriate plasma membrane sites for assembly (Fig. 12.2).

Transport of Viral Membrane Proteins to the Plasma Membrane

Viral membrane proteins reach their destinations by the highly conserved, cellular secretory pathway. Many of the steps in the pathway have been studied by using viral membrane glycoproteins, such as the vesicular stomatitis virus G and influenza virus hemagglutinin (HA) proteins. These viral proteins offer several experimental advantages: they are frequently synthesized in large quantities, their synthesis is initiated in a controlled fashion following infection, and their transport can be studied readily by genetic, biochemical, and imaging methods.

Entry into the first staging post of the secretory pathway, the endoplasmic reticulum (ER), is accompanied by membrane insertion of integral membrane proteins. Viral envelope proteins generally span the cellular membrane into which they are inserted only once, and therefore contain a single transmembrane domain. In viral proteins, transmembrane segments (described in Chapter 5) usually

Figure 12.2 Localization of viral proteins to the plasma membrane.

Viral envelope glycoproteins (red) are cotranslationally translocated into the ER lumen and folded and assembled within that compartment. They travel via transport vesicles to and through the Golgi apparatus and from the Golgi apparatus to the plasma membrane. The internal proteins of the particle (purple) and the genome (green) are also directed to plasma membrane sites of assembly.
Three strategies for making sub-assemblies

A  Assembly from individual protein molecules

Simian virus 40

SV40 pentamer

Adenovirus type 2

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**B  Assembly from a polyprotein precursor**

Poliovirus

**C  Chaperone-assisted assembly**

Adenovirus type 2

Protein II

Ad2 hexon trimer

viral chaperone
Sequential capsid assembly: Poliovirus

Virion 150S
Attachment
150S
Conformational transition
Uncoating
135S

Virion exit
Viral (+) strand mRNA
5'→ 3'
Translation
5'→ 3'
P1 cleavage

VP0 cleavage to VP4 + VP2

75S empty capsid
14S capsid pentamers

Provision (noninfectious) 150S
Genomic RNA

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Assembly intermediates and the assembly-line concept

- Ensure orderly formation of viral particles and virion subunits
- Formation of discrete intermediate structures
- Can’t proceed unless previous structure is formed: *quality control*
Viral scaffolding proteins
- establish transient intermediate structures
- viral proteases packaged in these intermediate structures become activated to finalize structure
Sequential Assembly

Adenovirus genome packaged into a preformed shell in the nucleus
Self-assembly vs assisted assembly reactions

- **Self-assembly**
  - Retrovirus gag, influenza HA, HBV surface form VLPs

- **Assisted assembly**
  - Can’t assemble alone
  - Proteins and genomes required as scaffolds or chaperones to form structure
Concerted Assembly
Influenza virus particles form by budding
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 (also a transcriptional activator)
• 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

Necessary but not sufficient for HIV-1 genome packaging
Packaging signals - RNA genomes

MoMLV

350 nt

ALV

env mRNAs not packaged
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

Selective packaging

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

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

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

II
Matrix
Internal matrix or capsid proteins drive budding - retroviruses

III
Envelope proteins drive budding - coronavirus

IV
Matrix proteins drive budding, but additional components (glycoproteins, RNP) needed for efficiency or accuracy
Internal structure assembly and budding spatially & temporally separated.
A  Influenza virus M1

1. Hydrophobic regions
2. RKLR
3. Zn finger motif
4. Inhibition of replication

Lipid binding
Binding to RNP
NLS
Binding to RNA

B  VSV M

1++. Hydrophobic region

Membrane binding
Binding to RNPs
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
L domain motifs

Retroviruses

HIV-1 Gag

HTLV-1 Gag

RSV Gag

MPMV Gag

MMuLV Gag

EIAV Gag

Rhabdoviruses

VSV M

Rabies M

Filoviruses

Ebola VP40

Marburg VP40

Arenaviruses

LCMV Z

Lassa Z

Tacaribe Z

: P(T/S)AP

: PPxY

: YP(x)nL

: 50 residues
Involvement of the ESCRT machinery in three topologically equivalent types of membrane abscission
Herpesvirus assembly and egress
How do viruses leave an infected cell?

- Release from the cell by budding or lysis
- Movement from cell to cell
Polarized release of HIV from infected T-cell.
Why do infected cells lyse?

- Inhibition of cell macromolecular processes and transport
- Apoptosis, pyroptosis, necrosis, autophagy