The structure of a virus particle determines how it is formed.
All virions complete a common set of assembly reactions

- Formation of individual structural units of the protein shell from one or several viral proteins
- Assembly of the protein shell by appropriate, and sometimes variable, interactions among structural units
- Selective packaging of the nucleic acid genome and other essential virion components
- Acquisition of an envelope
- Release from host cell
- Maturation of virus particles

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

**Short distance**
- Energy-dependent-protein channels

**Long distance**
- Energy-dependent-motor proteins on cytoskeletal tracks

**Sites and Membrane**
- Angstroms to nanometers
- Membrane (plasma, nuclear, ER, Golgi)

**Movement**
- Micrometers to meters
- Site of replication, protein synthesis, or assembly

[Image of cellular structures and trafficking pathways]
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
- Mitochondrion
- Influenza virus NP
- Cytoskeleton:
  - Intermediate filament
  - Microtubule
  - Actin filament bundle
- Extracellular matrix
Localization of viral proteins to plasma membrane

- Other viral proteins
- Viral RNA
- Nascent viral protein
- Cell surface viral protein
- Fusion
- Transport vesicle
- Microtubule
- Golgi
- ER
- Nucleus
- Mitochondrion
- Cytoskeleton
- Extracellular matrix
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
   - Simian virus 40
   - SV40 pentamer
   - VP1
   - VP2/VP3

B. Assembly from a polyprotein precursor
   - Poliovirus
   - Folded P1
   - P1
   - 5S structural unit
   - VP1
   - VP2
   - VP3
   - VP4

C. Chaperone-assisted assembly
   - Adenovirus type 2
   - Protein II
   - Ad2 hexon trimer
   - L4 100-kDa protein
Assembly reactions assisted by cellular chaperones

A. T4 gp31 co-chaperone + gp23

B. Hsc70 + ATP → ADP + P₁

C. TriC + Nascent Gag

D. Hsc70 + LT + ATP → ADP + P₁
Sequential capsid assembly: poliovirus
Sequential capsid assembly: herpesvirus

Viral scaffolding proteins
- establish transient intermediate structures
- viral proteases packaged in these intermediate structures become activated to finalize structure
Concerted assembly: Influenza virus

Concerted Assembly
Virus particles assemble only in association with viral genome
Influenza virus particles form by budding
Neuraminidase (NA)
Hemagglutinin (HA)
RNA polymerase (PB1, PB2, PA)
Ion channel (M2)
Matrix protein (M1)
Lipid bilayer
Segmented (-) strand RNA coated with nucleocapsid protein (NP)

Signal peptidase
Signal sequence
Oligosaccharides
Fusion peptide
Transmembrane
COOH

Globular head
Hinge
Stem

Extracellular
Cytoplasmic

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Maturation of influenza HA0

- Oligomerization
- Oligosaccharide trimming
- Addition of GlcNAc
- Addition of galactose
- Addition of sialic acid
- Cleavage of HA0
Go to:

b.socrative.com/login/student
room number: virus

**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
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
**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 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
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

• Bacteriophage $\phi 6$ - 3 dsRNA segments S, M, L
• Serial dependence of packaging: S-M-L
• Particle: pfu ratio $\sim 1$
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)

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  
N  
Hydrophobic regions  
RKLKR  
NES  
NLS  
Lipid binding  
Binding to RNP  
Binding to RNP  
Inhibition of replication  
~252  
C

B  VSV M

1+++  
N  
Hydrophobic region  
Membrane binding  
Binding to RNPs  
229  
C
Retrovirus budding

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
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

Cytoplasm

Nucleocapsid
UL34
UL31
US3

Endoplasmic reticulum

US3, UL51

Trans Golgi network

Membrane associated tegument proteins

Nucleocapsid associated tegument proteins

gE, gl, gM, gD

exocytosis
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
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

Double-membrane vesicles formed by apoptosis