Objectives:

• Cont-structure of viral capsids
• Enveloped viruses
• Packaging of viral RNA or DNA
• Complex viruses
• Virus maturation, assembly and release
Adenovirus Assembly with Pentons, Hexons and Cement Proteins

T=25
420 subunits
Reoviridae

- Reoviruses have non-enveloped, icosahedral T = 13 capsids composed of double protein shell with a complex structure.
- The structure of the bluetongue virus core was recently reported & represents the largest structure yet determined to atomic resolution (3.5 Å).
- The outer shell of this virus is approximately 80 nm in diameter & the inner shell (core) about 60 nm.
- The double-stranded RNA genome of the virus is packed tightly inside the core surrounding transcription complexes at the apices of the particle. These genome segments maintain their order during transcription.
Bluetongue Virus

T=13
780 subunits
Max. diameter 711Å

Source: Jack Johnson’s “VIPER” site http://mmtsdb.scripps.edu/viper/viper.html
Bluetongue virus
Multishelled Structure of Rotavirus

$T=13$

Inner shell – VP2, 120 copies

Outer shell – VP6, 780 copies
The primary function of the virus particle is to contain & protect the genome before delivering it to the appropriate host cell.

Therefore, the proteins of the capsid must interact with the nucleic acid genome.

In most cases, the linear virus genome when stretched out in solution is at least an order of magnitude longer than the diameter of the capsid.

Merely folding the genome in order to stuff it into such a confined space is complex, but is compounded by the fact that repulsion by the cumulative negative electrostatic charges on the phosphate groups of the nucleotide backbone mean that the genome resists being crammed into a small space.
Viral Genome Packaging

• Viruses package, along with the genome, a number of positively charged molecules to counteract negative charges.
• These include small, positively charged ions (Na, Mg, K, etc.), polyamines & various nucleic acid-binding proteins.
• Some of these proteins are virus-encoded & contain amino acids with basic side-chains such as arginine & lysine which interact with the genome, e.g. retrovirus NC & rhabdovirus N (nucleocapsid) proteins, & influenza virus NP protein (nucleoprotein).
• Many viruses with double-stranded DNA genomes have basic histone-like molecules closely associated with the DNA. Some are virus-encoded, e.g. adenovirus polypeptide VII. Other viruses utilize cellular proteins, e.g. the polyomavirus genome assumes a chromatin-like structure in association with four cellular histone proteins, H2A, H2B, H3 & H4, similar to that of the host cell genome.
Viral Genome Packaging

- Another problem viruses must overcome is how to achieve the specificity required to select & encapsidate the virus genome from the large background of cellular nucleic acids.

- In most cases, by the late stages of virus infection when assembly of virus particles occurs, transcription of cellular genes has been reduced & a large pool of virus genomes have accumulated - overproduction of virus nucleic acids eases but does not eliminate the problem of specific genome packaging.

- Therefore, a specific virus-encoded capsid or nucleocapsid protein is required to achieve this end & many viruses, even those with relatively short, compact genomes such as retroviruses & rhabdoviruses, encode this type of protein.
Viral Genome Packaging

• During particle assembly, viruses frequently make mistakes.

• These can be physically measured by particle:infectivity ratios, i.e. the ratio of the total number of particles in a virus preparation (counted by electron microscopy) to the number of particles able to give rise to infectious progeny (measured by plaque or limiting dilution assays).

• This value is in some cases found to be several thousand particles to each infectious virion & only rarely approaches a ratio of 1:1.
Viral Genome Packaging

• Specific nucleotide sequences in the genome (the packaging signal) permit the virus to select genomic nucleic acids from the cellular background.

• The packaging signal from a number of virus genomes has been identified, e.g. the $\Psi$ ('psi') signal in murine retrovirus genomes & the sequences responsible for packaging the genomes of several DNA virus genomes (some adenoviruses & herpesviruses) have been clearly & unambiguously defined.

• Accurate & efficient genome packaging requires information also from regions of secondary structure formed by the folding of the genomic nucleic acid into complex forms.
Positive-strand RNA Genome Packaging

- Simple ssRNA genomes (plant viruses, picornaviruses, alphaviruses) – no specific overall fold, pack tightly highly structured RNA
- May pack into shallow groove of the capsid
- Recognition – MS2, a dimer of a coat protein binds a hairpin
- None-enveloped eukaryotic coat proteins extend a flexible positively charged arm that recognize RNA (alfalfa virus)
Packaging of MS2 virus (T3)
Packaging of Alfalfa Mosaic Virus
(a) Amino acid sequence of the HIV-1\textsubscript{pNL4-3} nucleocapsid protein showing the zinc binding mode of the CCHC zinc knuckles. (b) Nucleotide sequence and secondary structure of the HIV-1\textsubscript{pNL4-3} Ψ-site. The sequence of the SL2 RNA construct used here is shown in bold letters, and residues of the major splice donor site are denoted by open letters. (c) A representative NC-SL2 structure. The nucleobases of residues G9, U10 and G11 are colored green, orange and purple, respectively. The side-chains of selected basic residues are colored blue, and the zinc atoms are displayed as silver spheres. The N-terminal zinc knuckle packs against A15 of the A5-U14-A15 base triple, and the side-chains of Lys34 and Lys47 are poised to form salt-bridges with phosphodiester groups.

Amarasinghe et al., 2000 *J Mol Biol*, 301: 491-511
Genome Packaging

• Rather less is known about the arrangement of the genome inside virus particles with icosahedral symmetry.
• T = 3 icosahedral RNA virus capsid subunits consist largely of the '8-strand anti-parallel β-barrel' structural motif, discussed earlier.
• In these viruses, positively-charged inward-projecting arms of the capsid proteins interact with the RNA in the center of the particle.
• BPMV (bean pod mottle virus), a T = 3 Comovirus with a bipartite genome. X-ray crystallography showed that the RNA is folded in such a way that it assumes icosahedral symmetry, corresponding to that of the capsid surrounding it.
• The regions which contact the capsid proteins are single-stranded & appear to interact by electrostatic forces rather than covalent bonds. The atomic structure of φX74 also shows that a portion of the DNA genome interacts with arginine residues exposed on the inner surface of the capsid in a manner similar to BPMV.
Genome Packaging in TMV

- TMV, (+)sense RNA helical plant virus
- Only has a single major coat protein, & will spontaneously assemble from its purified RNA & protein components \textit{in vitro}.
- Particle assembly is initiated by association of preformed aggregates of coat protein molecules ('discs') with residues 5444-5518 in the 6.4 kb RNA genome, known as the origin of assembly sequence (OAS).
- The flat discs have 17 subunits per ring, close to the 16.34 subunits per turn found in the mature virus particle.
Genome Packaging in TMV

Disc

Locked washer

RNA binds to disc

3' 5'

3' 5'

Travelling loop

OAS

3' 5'
Genome Packaging in TMV

• The discs have a pronounced polarity. Assembly begins when a disc interacts with the OAS in genomic RNA.

• This converts the discs to a helical 'locked washer' structure, each of which contains 3' coat protein subunits.

• Further discs add to this structure, switching to the 'locked washer' conformation.

• RNA is drawn into the assembling structure in what is known as a 'travelling loop', which gives the common name to this mechanism of particle formation - the vRNA is trapped & subsequently buried in the middle of the disc as the helix grows.

• Extension of the helical structure occurs in both directions but at unequal rates. Growth in the 5' direction is rapid because a disc can add straight to the protein filament & the travelling loop of RNA is drawn up through it. Growth in the 3' direction is slower because the RNA has to be threaded through the disc before it can add to the structure.
dsDNA Genome Packaging

- dsDNA packaging of tailed bacteriophages – DNA insertion into icosahedral head is ATP dependent, driven by a motor
- Herpesvirus – rolling circle replication
- Adenovirus – AT-rich repeat that determines incorporation into
- PRD1 (similar to adenovirus) – unique vertex containing a protein needed for DNA packaging, that also includes ATPase
- Papovaviruses – incorporate cellular histones, to form 20-25 nucleosomes
Genome Packaging in M13

- *Enterobacteria phage M13* is another helical virus where protein-nucleic acid interactions in the virus particle are relatively simple.
- The primary sequence of the g8p molecule determines the orientation of the protein in the capsid.
- The inner surface of the rod-like phage capsid is positively charged & interacts with the negatively charged genome, while the outer surface of the cylindrical capsid is negatively charged.
Genome Packaging in M13

- During replication, the genomic DNA is associated with a non-structural DNA-binding protein, g5p.
- g5p is the most abundant of all virus proteins in the infected *E. coli* cell, & coats the newly replicated single-stranded phage DNA, forming an intracellular rod-like structure similar to the mature phage particle.
- The function of g5p is to protect the genome from host cell nucleases & to interrupt genome replication, sequestering newly formed strands as substrates for encapsidation.
- Newly synthesized coat protein monomers (g8p) are associated with the inner (cytoplasmic) membrane of the cell & it is at this site that assembly of the virus particle occurs.
- The g5p coating is stripped off as the particle passes out through the membrane & is essentially exchanged for the mature g8p coat (plus the accessory proteins).
- The protein-nucleic acid interactions which occur appear to be rather simple & involve opposing electrostatic charges & the stacking of the DNA bases between the planar side-chains of the proteins.
dsRNA Genome Packaging

• Reovirus – 12 RNA segments, selection signal is unclear, dsRNA is tightly packaged

• Bacteriophage φ5 – sequential incorporation of three dsRNA segments. Includes ATPases and assembly clamp

• ATPase could be a unwinding motor to package ssRNA instead of dsRNA

Negative-strand RNA Genome Packaging

• Influenza – eight-segment genome packaged with the help of N protein. Form rods, 300-1,200 angstroms.
Packaging of dsDNA and dsRNA viruses
The Structure of a Herpesvirus

Icosahedral cores  Spikes  Envelope  Tegument
Enveloped viruses

- In an enveloped virus, the capsid is covered by an envelope
  - The envelope is usually made of some combination of lipids, proteins, and carbohydrates
  - Some envelopes contain spikes that allow them to attach to the host
Enveloped Viruses

- Enveloped Viruses

- Influenzavirus

- herpes simplex virus
Enveloped Viruses

• Many viruses have devised strategies to exit from the infected cell without its total destruction.

• All living cells are covered by a membrane composed of a lipid bilayer - the viability of the cell depends on the integrity of this membrane. Viruses leaving the cell must, therefore, allow this membrane to remain intact.

• This is achieved by extrusion (budding) of the particle through the membrane, during which process the particle becomes coated in a lipid envelope derived from the host cell membrane & with a similar composition.
Viral Budding

- **Budding**
  - Host cell proteins
  - Virus glycoproteins
  - Cell membrane

- **Release**
  - Nucleocapsid protein
  - Virus genome
  - Matrix protein

- **Maturation**
Formation of enveloped virus particles

• The structure underlying the envelope may be based on helical or icosahedral symmetry & may be formed before or as the virus leaves the cell.
• In the majority of cases, enveloped viruses use cellular membranes as sites allowing them to direct assembly.
• The formation of the particle inside the cell, maturation & release are in many cases a continuous process.
• The site of assembly varies for different viruses - not all use the cell surface membrane; many use cytoplasmic membranes such as the Golgi apparatus, others, such as herpesviruses, which replicate in the nucleus may utilize the nuclear membrane. In these cases, the virus is usually extruded into some form of vacuole, in which it is transported to the cell surface & subsequently released.
Viral Structure: Envelope Proteins

- Lipid envelope
- Matrix proteins
- Nucleocapsid
- Glycoprotein (trimer "spike")
- Internal "tails" interact with matrix proteins
- Transmembrane protein
- Disulfide bonds
- External glycoprotein
Envelope proteins

• If the virus particle became covered in a smooth, unbroken lipid bilayer, this would be its undoing.

• Such a coating is effectively inert, & although effective in preventing desiccation of or enzymatic damage to the particle, would not permit recognition of receptor molecules on the host cell.

• Therefore, viruses modify their lipid envelopes by the synthesis of several classes of proteins which are associated in one of three ways with the envelope.
Viral Structure

- **Matrix proteins:** are internal virion proteins whose function is effectively to link the internal nucleocapsid assembly to the envelope. Such proteins are not usually glycosylated & are often very abundant, for example, in retroviruses they comprise approximately 30% of the total weight of the virion.

- **Glycoproteins:** are transmembrane proteins anchored to the membrane by a hydrophobic domain, & can be subdivided into two types by their function:
  - **External glycoproteins** are anchored in the envelope by a single transmembrane domain.
  - **Transport channel proteins** contain multiple hydrophobic transmembrane domains, forming a protein-lined channel through the envelope.
Fusion of Bilayer Membrane

A → B → C → D

Hemifusion

Stalk

Transition

Structure

Fusion

pore

Hemifusion

diaphragm
Fusion of Class I Fusion Proteins
Fusion of Class II Fusion Proteins

- Receptor binding
- Dimer dissociates hinge flexion
- Fusion peptide inserts trimerization
- Foldback
- Hemifusion
- Fusion pore
Complex Virus Structures

• However, there are many viruses whose structure is more complex than those with helical symmetry or icosahedral symmetry.

• In these cases, although the general principles of symmetry are often used to build part of the virus shell, the larger & more complex viruses cannot be simply defined by a mathematical equation as can a simple helix or icosahedron.

• Because of the complexity of some of these viruses, they have defied attempts to determine detailed atomic structures.
Poxvirus Particle
Poxviruses

• Example of complex viral structure - *Poxviridae*.

• These viruses have oval or 'brick-shaped' particles 200-400 nm long.

• These particles are so large that they were first observed in using high-resolution optical microscopes in 1886, & thought at that time to be 'the spores of micrococci'.

• The external surface of the virion is ridged in parallel rows, sometimes arranged helically.

• The particles are extremely complex & have been shown to contain more than 100 different proteins.
Poxviruses

- Under the electron microscope, thin sections of poxviruses reveal that the outer surface of the virion is composed of lipid & protein.
- This surrounds the core, which is biconcave (dumbbell-shaped), & two 'lateral bodies' whose function is unknown.
- The core is composed of a tightly compressed nucleoprotein & the double-stranded DNA genome is wound around it.
- Antigenically, poxviruses are very complex, inducing both specific & cross-reacting antibodies - hence the possibility of vaccinating against one disease with another virus (e.g. the use of Vaccinia virus to immunize against smallpox (variola) virus).
- Poxviruses & a number of other complex viruses also emphasize the true complexity of some viruses - there are at least 10 enzymes present in poxvirus particles, mostly involved in nucleic acid metabolism/genome replication.
Poxviruses

• During replication, two forms of poxvirus particle are observed:
  – extracellular forms which contain two membranes
  – intracellular particles which only have an inner membrane

• Poxviruses & other virus with complex structures obtain their membranes in a different way from "simple" enveloped viruses such as retroviruses or influenza.

• Rather than budding at the cell surface or into an intracellular compartment, acquiring a single membrane, these complex viruses are wrapped by the endoplasmic reticulum, acquiring two layers of membrane.
Bacteriophages

- Complex virus
  - Head is polyhedral
  - Tail is helical
  - It is surrounded by a protein coat (capsid)
Caudovirales: Myoviridae, Siphoviridae & Podoviridae

- The tailed phages of enterobacteria have been extensively studied for excellent reasons - easy to propagate in bacterial cells, can be obtained in high titres, & are easily purified, facilitating biochemical & structural studies.
- The head of the particles consists of an icosahedral shell with T = 7 symmetry, attached by a collar to a contractile, helical tail. At the end of the tail is a plate which functions in attachment to the bacterial host & also in penetration of the bacterial cell wall using lysozyme-like enzymes associated with the plate.
- Thin protein fibres attached to the plate & the tail plate itself are involved in binding to the receptor molecules in the wall of the host cell.
The Caudovirales

- There are separate assembly pathways for the head & tail sections of the particle, which come together at a late stage to make up the virion.
- These viruses illustrate how complex particles can be built up from the simple principles outlined before.
Another example is provided by the structure of geminivirus particles, which consist of two twinned T = 1 icosahedra. Each icosahedron has one morphological subunit missing & the icosahedra are joined so the mature particle contains 110 protein monomers arranged in 22 morphological subunits.
Baculoviridae

Ingestion by insect host

Persistence in environment

Peplomers (envelope glycoprotein)

Virion

Polyhedrin

Virus DNA
Capsid proteins
DNA-binding protein

Budded virus particle: Replication and cell-to-cell spread within insect

Occluded virus particle: Produced late in infection; released into environment when insect dies
Baculoviridae

• Baculoviruses contain 12-30 structural proteins, & consist of a rod-like (hence “baculo”) nucleocapsid 30-60 nm diameter & 250-300 nm long which contains the 88-160 kbp double-stranded DNA genome.

• The nucleocapsid is surrounded by an envelope, outside which there may or may not be a crystalline protein matrix. If this outer protein shell is present, the whole assemblage is referred to as an 'occlusion body' & the virus is said to be occluded.

• The function of the occlusion bodies is to confer resistance to adverse environmental conditions, which enables the virus to persist in soil or on plant materials for extended periods of time waiting to be ingested by a new host.
Structural Heterogeneity of HIV Particles

- Mean diameter approximately 120nm.
- Average volume approximately $10^6 \text{ nm}^3$
- Approximate mass per virus particle is 1fg (650 Mda)
- Note that the structures of these viruses are a lot more sloppy than the structures of the icosahedral viruses we discussed earlier.
- Red corresponds to conical, orange to rod shapes and yellow is psycho.

Each picture is 160nm wide.
A Reminder on Cryo Electron Microscopy

By rotating a sample through a number of different orientations it is possible to generate a series of images which with careful analysis can be used to garner a three-dimensional picture of macromolecular complexes (ribosomes, viruses, etc.)
HIV-1 Capsid Structure

- Fascinating structure of the internal capsid of HIV.
- Shape conferred by geometric rules about 5-fold defects.

Sundquist et al.
HIV-1 Capsid
Assembly

• Assembly involves the collection of all the components necessary for the formation of the mature virion at a particular site in the cell.
• During assembly, the basic structure of the virus particle is formed.
• The site of assembly depends on the site of replication within the cell & on the mechanism by which the virus is eventually released from the cell & varies for different viruses:
  • in picornaviruses, poxviruses & reoviruses assembly occurs in the cytoplasm
  • in adenoviruses, polyomaviruses & parvoviruses it occurs in the nucleus
• As with the early stages of replication, it is not always possible to identify the assembly, maturation & release of virus particles as distinct & separate phases.
Maturation

• Maturation is the stage of the replication-cycle at which the virus becomes infectious.

• Maturation usually involves structural changes in the virus particle which may result from specific cleavages of capsid proteins to form the mature products or conformational changes in proteins during assembly.

• Such events frequently lead to substantial structural changes in the capsid which may be detectable by criteria such as differences in the antigenicity of incomplete & mature virus particles or the condensation of nucleoproteins with the virus genome.

• Virus proteases are frequently involved in maturation, although cellular enzymes or a mixture of virus & cellular enzymes are used in some cases.
Release

- Apart from plant viruses which have evolved particular strategies to overcome the structure of plant cell walls, all other viruses escape the cell by one of two mechanisms:

  - For lytic viruses (most non-enveloped viruses), release is a simple process - the infected cell breaks open & releases the virus.

  - Enveloped viruses acquire their lipid membrane as the virus buds out of the cell through the cell membrane or into an intracellular vesicle prior to subsequent release. Virion envelope proteins are picked up during this process as the virus particle is extruded - this process is known as budding.
Maturation: Naked Icosahedral Viruses

- Preassembled capsomeres are joined to form empty capsids (procapsid)

- The assembly of capsomeres to form the procapsid is often accompanied by extensive reorganization, which is revealed by changes in serological specificity and isoelectric point. e.g. picornaviruses and adenoviruses.
Release: Naked Icosahedral Viruses

**Poliovirus** - released, with lysis of infected cells

**Virions of DNA viruses** - mature in the nucleus and tend to accumulate within infected cells over a long period and are released when the cell undergoes autolysis, and in some cases, may be extruded without lysis.
Maturation: Enveloped Viruses

• Viral proteins are first associated with the nucleic acid to form nucleocapsid

• Nucleocapsid is then surrounded by an envelope

• In nucleocapsid formation, the proteins are all synthesized on cytoplasmic polysomes and are rapidly assembled into capsid components

• In envelope assembly, virus-specified envelope proteins go directly to the appropriate cell membrane (the plasma membrane, the ER, the Golgi apparatus), displacing host proteins.
Maturation: Enveloped Viruses

- Viral envelope is made of carbohydrates and lipids produced by the host cell (eg. the plasma membrane for orthomyxoviruses and paramyxoviruses, the nuclear membrane for herpesviruses)

- A given virus will differ in its lipids and carbohydrates when grown in different cells, with consequent differences in physical, biological, and antigenic properties.

- The envelope glycoproteins are synthesized in the following manner: the polypeptide backbone is first formed on polysomes bound to the ER, which then moves via transport vesicles to the Golgi apparatus where it attains its full glycosylation and fatty acid acylation.

- The matrix proteins that are present in viral envelope are usually not glycosylated and stick to the cytoplasmic side of the plasma membrane through hydrophobic domains.
Maturation: HIV-1
Virion Enzyme Activities

- Virions are not inert structures.
- Many virus particles contain one or more enzymatic activities, although in most cases these are not active outside the biochemical environment of the host cell.
- All viruses with negative-sense RNA genomes must carry with them a virus-specific RNA-dependent RNA polymerase because most eukaryotic cells have no mechanism for RNA-dependent RNA polymerization - genome replication could not occur if this enzyme were not included in the virus particle.
- Reverse transcription of retrovirus genomes occurs inside a particulate complex & not free in solution.
- The more complex DNA viruses (e.g. herpesviruses & poxviruses) carry a multiplicity of enzymes, mostly concerned with some aspect of nucleic acid metabolism.
HIV-1 Restriction Factors: APOBEC3G and Cyclophilin A

**Figure 5.29 Model of the role of cyclophilin A in human immunodeficiency virus type I uncoating.** The pathways of infection for mature virus particles containing (A) or lacking (B) cyclophilin A are depicted. After fusion at the plasma membrane, the viral nucleocapsid is released into the cytoplasm. Cyclophilin A (orange) packaged into the virus particle destabilizes the nucleocapsid, leading to release of CA and the initiation of reverse transcription. Adapted from J. L. Luban, Cell 87:1–20, 1996, with permission.
Retroviral Restriction Factor: TRIM5

Ganser-Pornillos et al, PNAS 2011

Pertel et al., Nature 2011
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Lecture Slides:

www.sicklecell.howard.edu/research.htm