Viruses and Other Acellular Infectious Agents
Acellular Agents

- Viruses – protein and nucleic acid
- Viroids – only RNA
- Satellites – only nucleic acids
- Prions – proteins only
Viruses

• Major cause of disease
  – also importance as a new source of therapy
  – new viruses are emerging
• Important members of aquatic world
  – move organic matter from particulate to dissolved
• Important in evolution
  – transfer genes between bacteria, others
• Important model systems in molecular biology
• Currently being used to control certain bacterial pathogens
General Properties of Viruses

• Virion
  – complete virus particle
  – consists of ≥1 molecule of DNA or RNA enclosed in coat of protein
  – may have additional layers
  – cannot reproduce independent of living cells nor carry out cell division
  • but can exist extracellularly
Virions Infect All Cell Types

• Bacterial viruses called bacteriophages (phages)
• Few archaeal viruses
• Most are eukaryotic viruses
  – plants, animals, protists, and fungi
• Classified into families based on
  – genome structure, life cycle, morphology, genetic relatedness
The Structure of Viruses

- Virion size range is ~10–400 nm in diameter and most viruses must be viewed with an electron microscope.

- All virions contain a nucleocapsid which is composed of nucleic acid (DNA or RNA) and a protein coat (capsid).
  - Some viruses consist only of a nucleocapsid, others have additional components.

- Envelopes

(a) Nonenveloped virus
(b) Enveloped virus
(a) Vaccinia virus
(b) Herpesvirus
(c) Rhabdovirus
(d) T-even coliphage

(e) A flexuous-tailed phage
(f) Human papillomavirus

(g) φX174 phage
(h) Tobacco mosaic virus
(i) Mimivirus
Capsids

- Large macromolecular structures which serve as protein coat of virus
- Protect viral genetic material and aids in its transfer between host cells
- Made of protein subunits called capsomers/protomers
- Capsids are helical, icosahedral, or complex
Helical Capsids

- Shaped like hollow tubes with protein walls
- Protomers self assemble
- Size of capsid is a function of nucleic acid
Influenza Virus

CDC/Dr. F.A. Murphy
Icosahedral Capsids

- An icosahedron is a regular polyhedron with 20 equilateral faces and 12 vertices

- Capsomeres
  - ring or knob-shaped units made of 5 or 6 protomers
  - pentamers (pentons) – 5 subunit capsomers
  - hexamers (hexons) – 6 subunit capsomers
Capsids of Complex Symmetry

- Some viruses do not fit into the category of having helical or icosahedral capsids
  - poxviruses – largest animal virus
  - large bacteriophages – bival symmetry
- head resembles icosahedral, tail is helical

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Bacteriophage
T4 Coliphage
Viral Envelopes and Enzymes

• Many viruses are bound by an outer, flexible, membranous layer called the envelope

• Animal virus envelopes (lipids and carbohydrates) usually arise from host cell plasma or nuclear membranes
Viral Envelope Proteins

• Envelope proteins, which are viral encoded, may project from the envelope surface as spikes or peplomers
  – involved in viral attachment to host cell
    • e.g., hemagglutinin of influenza virus
  – used for identification of virus
  – may have enzymatic or other activity
    • e.g., neuraminidase of influenza virus
  – may play a role in nucleic acid replication
Virion Enzymes

- It was first erroneously thought that all virions lacked enzymes
- Now accepted that a variety of virions have enzymes
  - some are associated with the envelope or capsid but most are within the capsid
Viral Genome

• Diverse nature of genomes
• A virus may have single or double stranded DNA or RNA
• The length of the nucleic acid also varies from virus to virus
• Genomes can be segmented or circular
Viral Multiplication

- Mechanism used depends on viral structure and genome
- Steps are similar
  - attachment to host cell
  - entry
  - uncoating of genome
  - synthesis
  - assembly
  - Release
- This represents “Lytic Cycle”
Attachment (Adsorption)

• Specific receptor attachment
• Receptor determines host preference
  – may be specific tissue (tropism)
  – may be more than one host
  – may be more than one receptor
  – may be in lipid rafts providing entry of virus
Viral Entry and Uncoating

• Entire genome or nucleocapsid
• Varies between naked or enveloped virus
• Three methods used
  – fusion of the viral envelope with host membrane; nucleocapsid enters
  – endocytosis in vesicle; endosome aids in viral uncoating
  – injection of nucleic acid
(a) Entry of enveloped virus by fusing with plasma membrane

Viral envelope spikes bind to receptors on the cell's surface. Lipid bilayer of viral envelope fuses with host cell membrane. Nucleocapsid is released into the cytoplasm.

(b) Entry of enveloped virus by endocytosis

Capsid proteins bind to receptors on cell surface and trigger receptor-mediated endocytosis. Nucleic acid is extruded from the endosome into the cytoplasm.

(c) Entry of nonenveloped virus by endocytosis
There are two mechanisms by which enveloped viruses enter host cells. In one of the mechanisms, the virion attaches to host cell receptors by specific proteins on its surface, called spikes.
Virion Release

• Nonenveloped viruses lyse the host cell
  – viral proteins may attack peptidoglycan or membrane
• Enveloped viruses use budding
  – viral proteins are placed into host membrane
  – nucleocapsid may bind to viral proteins
  – envelope derived from host cell membrane, but may be Golgi, ER, or other
  – virus may use host actin tails to propel through host membrane
Enveloped viruses are usually released from the host cell by a budding mechanism.
Bacterial and Archaeal Viral Infections

- Virulent phage – one reproductive choice
  - multiplies immediately upon entry
  - lyses bacterial host cell
- Temperate phages have two reproductive options
  - reproduce lytically as virulent phages do
  - remain within host cell without destroying it
  - many temperate phages integrate their genome into host genome (becoming a ‘prophage’ in a ‘lysogenic bacterium’) in a relationship called lysogeny
The first step in the replication of virulent phage such as T4 is attachment. Protein fibers at the end of the phage tail attach to specific receptors on the bacterial cell wall.
Phage DNA

New phages can bind to bacterial cells.

Bacterial chromosome

Phage injects its DNA into cytoplasm.

Lytic cycle

Phage DNA integrates into host chromosome.

Lysogenic cycle

Exposure to stress such as UV light triggers excision from host chromosome.

Prophage DNA is copied when cell divides.

Prophage

Cell lyses and releases the new phages.

Phage DNA directs the synthesis of many new phages.
Lysogenic Conversion

• Temperate phage changes phenotype of its host
  – bacteria become immune to superinfection
  – phage may express pathogenic toxin or enzyme

• Two advantages to lysogeny for virus
  – phage remains viable but may not replicate
  – multiplicity of infection ensures survival of host cell

• Under appropriate conditions infected bacteria will lyse and release phage particles
  – occurs when conditions in the cell cause the prophage to initiate synthesis of new phage particles, a process called induction
When phage Lambda infects E. coli, either the lytic or the lysogenic cycle may be followed. In both cases, the first step involves the phage attaching to the host cell and injecting its DNA into the host cell.
Archaeal Viruses

- May be lytic or temperate
- Most discovered so far are temperate by unknown mechanisms
Infection in Eukaryotic Cells

- Cytocidal infection results in cell death through lysis
- Persistent infections may last years
- Cytopathic effects (CPEs)
  - degenerative changes
  - abnormalities
- Transformation to malignant cell
Examples of Viral Disease: Influenza (Flu)

- Respiratory system disease caused by influenza virus
  - Enveloped RNA virus; segmented genome
  - Four groups A, B, C, and Thorgoto
  - Numerous animal reservoirs
  - Acquired by inhalation or ingestion of respiratory secretions

- 31 possible pandemics
- Worst pandemic in 1918 killed ~50 million people
Influenza (Flu)

- An important feature of the influenza viruses is the frequency with which changes in antigenicity occur
  - antigenic drift – due to accumulation of mutations in a strain within a geographic area
  - antigenic shift – due to reassortment of genomes when two different strains of flu viruses (from humans and animals) infect the same cell and are incorporated into a single new capsid
Influenza Antigenic Shift

- **H5N1** subtype (known as bird flu)
  - severe disease and death in humans but low infection in humans
  - Not spread person to person
  - 600 cases in 15 countries since 2003 with 60% fatality rate

- **H1N1** (swine flu) is current pandemic
Influenza Infection:

• Clinical manifestations
  – chills, fever, headache, malaise, and general muscular aches and pains
  – usually significantly more severe than common cold
  – recovery usually within 3 to 7 days
  – often leads to secondary infections by bacteria

• Treatment, prevention, and control
  – rapid immunologic tests
  – symptomatic/supportive therapy
  – inactivated virus vaccine
West Nile Fever (Encephalitis)

- Caused by a flavivirus, RNA virus
- Appeared in New York in 1999 causing human and animal deaths
- By 2006 found in all continental U.S. states, infecting humans or animals
- Transmitted to humans by *Culex* spp., mosquitoes that feed on sparrows and crows
- Only one antigen type exists and immunity is presumed to be permanent
- Human to human spread has occurred through blood and organ donation
West Nile Symptoms:

- Clinical manifestations
  - fever, lymphadenopathy, rash
  - >1/100 patients develop serious complications (e.g., encephalitis)

- Treatment, prevention, and control
  - serology tests
  - supportive treatment
  - mosquito control measures
Gastroenteritis (Viral)

• Acute viral gastroenteritis
  – inflammation of stomach or intestines
  – important disease of infants and children
  – leading cause of childhood death in developing countries
  – spread by fecal-oral route

• Caused by four major groups of viruses
Gastroenteritis (Viral)

- Rotavirus, adenovirus, and astrovirus
  - ~5–10 million deaths/year worldwide
  - viral diarrhea transmitted by fecal oral route
  - rotavirus live oral vaccines

- Norovirus
  - ~23 million cases of acute gastroenteritis per year
  - usually self-limited disease
  - symptomatic/supportive therapy
### Table 38.3  Medically Important Gastroenteritis Viruses

<table>
<thead>
<tr>
<th>Virus</th>
<th>Epidemiological Characteristics</th>
<th>Clinical Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotaviruses</td>
<td>Endemic diarrhea in infants worldwide</td>
<td>Dehydrating diarrhea for 5–7 days; fever, abdominal cramps, nausea, and vomiting common</td>
</tr>
<tr>
<td>Group A</td>
<td></td>
<td></td>
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<tr>
<td>Group B</td>
<td>Large outbreaks in adults and children in China</td>
<td>Severe watery diarrhea for 3–5 days</td>
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<tr>
<td>Group C</td>
<td>Sporadic cases in children in Japan</td>
<td>Similar to group A</td>
</tr>
<tr>
<td>Noroviruses</td>
<td>Epidemics of vomiting and diarrhea in older children and adults; occur in families, communities, and nursing homes; often associated with shellfish, other food, or water and infected food handlers, cruise ship occurrences</td>
<td>Acute vomiting, fever, myalgia, and headache lasting 1–2 days, diarrhea</td>
</tr>
<tr>
<td>Sapoviruses</td>
<td>Pediatric diarrhea; also associated with shellfish and other foods in adults</td>
<td>Rotavirus-like illness in children; norovirus-like illness in adults</td>
</tr>
<tr>
<td>Astroviruses</td>
<td>Pediatric diarrhea; also reported in nursing homes</td>
<td>Watery diarrhea for 1–3 days</td>
</tr>
<tr>
<td>Adenoviruses</td>
<td>Pediatric diarrhea; also reported on military bases</td>
<td>Gastroenteritis, more severe in immunocompromised adults</td>
</tr>
</tbody>
</table>
Rabies

- Caused by different strains of rabies virus
  - negative strand, bullet shaped, RNA virus
  - highly neurotropic
- Transmitted by:
  - bites of infected animals
  - aerosols in caves where bats roost
  - contamination of scratches, abrasions, open wounds, or mucous membranes with saliva of infected animals
- Fewer than 10 cases/yr in U.S. but 55,000 deaths/yr worldwide
Rabies Symptoms:

- Enters host cell and multiplies
- Enters nervous system, spreads to spinal cord
  - first specific symptoms (pain or paresthesia at wound) may occur
- Rapidly progressive encephalitis develops as virus spreads through central nervous system
- Then spreads through body along peripheral nerves, including salivary glands, where it is shed in saliva
Rabies Symptoms cont.

• Clinical manifestations
  – begins 2 to 16 weeks after exposure
  – pain or paresthesia at wound site, anxiety, irritability, depression, fatigue, loss of appetite, fever, and sensitivity to light and sound
  – quickly progresses to paralysis
  – death results from destruction of regions of the brain that regulate breathing
Rabies continued:

- Diagnosis
  - previously used microscopic observation of Negri bodies, masses of viruses, currently use direct immunofluorescent antibody (dIFA) of brain tissue, virus isolation, Negri body detection and a rapid rabies enzyme-mediated immunodiagnosis test

- Treatment, prevention, and control
  - postexposure vaccination
  - preexposure vaccination of individuals with high risk of exposure, dogs, and cats

- Milwaukee Protocol – possibly life-saving procedure
Growth of Viruses: Hosts for Bacterial and Archaeal Viruses

• Usually cultivated in broth or agar cultures of suitable, young, actively growing bacteria
  (Early Log Phase *E. coli* B from lab)

• Broth cultures lose turbidity as viruses reproduce
  (Clearing exercise from lab)

• Plaques observed on agar cultures
  (Bacteriophages Plaque Assay from lab)
Hosts for Animal Viruses

• Tissue (cell) cultures
  – cells are infected with virus (phage)
  – viral plaques
    • localized area of cellular destruction and lysis that enlarge as the virus replicates

• Embryonated eggs
Hosts for Plant Viruses

- Plant tissue cultures
- Plant protoplast cultures
- Suitable whole plants
  – may cause localized necrotic lesions or generalized symptoms of infection
Quantification of Virus

- Direct counting – count viral particles
- Indirect counting by an observable of the virus
  - hemagglutination assay
  - plaque assays
Measuring Concentration of Infectious Units

• Plaque assays
  – dilutions of virus preparation made and plated on lawn of host cells
  – number of plaques counted
  – results expressed as plaque-forming units (PFU) – plaque forming units (PFU)
  • PFU/ml = number of plaques/sample dilution
Other Acellular Agents:

• Viroids
  – RNA only
  – Known to cause over 20 plant disease

• Satellites
  – Nucleic acid only, but need a “helper virus” to be infective

• Prions
  – Protein only (no nucleic acids)
Viroids

- Infectious agents composed of closed, circular ssRNAs
- Requires host cell DNA-dependent RNA polymerase to replicate
- Cause plant diseases
Satellites

• Infectious nucleic acids (DNA or RNA)

• There are 3 types:
  – Satellite Viruses (can encode their own capsid proteins with the helper virus), Satellite DNAs, Satellite RNAs

• Require a helper virus for replication
  – human hepatitis D virus is satellite
  – requires human hepatitis B virus
Prions – Proteinaceous Infectious Particle

- Cause a variety of degenerative diseases in humans and animals
  - scrapie in sheep
  - bovine spongiform encephalopathy (BSE) or mad cow disease
  - Creutzfeldt-Jakob disease (CJD) and variant CJD (vCJD) in humans
  - kuru in humans
Prion Diseases

• Transmissible spongiform encephalopathies
  – fatal neurogenerative disorders caused by prions
  – remains clinically silent for months or years
  – ends in profound disability or death

• Diseases include
  – Kuru, Creutzfeldt-Jacob (CJD) disease, and variant CJD, bovine spongiform encephalopathy (BSE)

• Transmission
  – medical procedures, genetic, food-borne
Prion Diseases

- Prions (protein infectious particles) consist of abnormally folded proteins (PrPSc) which can induce normal forms of protein PrPc to abnormally fold
- BSE agent survives gastrointestinal tract passage, and is neurotropic, both serve as source of agent
Prion Disease Symptoms:

- Dementia is primary symptom
- Usually accompanied by motor dysfunction
- Symptoms appear after prolonged incubation and last from months to years prior to death
- Produce characteristic spongiform degeneration of brain and deposition of amyloid plaques
- Share many characteristics with Alzheimer’s disease
New-Variant CJD

- Transmitted from cattle with bovine spongiform encephalopathy (BSE) or mad cow disease
- Cattle experimentally infected with BSE test positive for prion agent
  - evidence suggests human vCJD may be acquired by eating meat products (brain and spinal cord tissue) from infected cattle
Current Model of Disease Production by Prions

- **PrP<sup>C</sup>** (prion protein) is present in “normal” form (abnormal form of prion protein is **PrP<sup>Sc</sup>**)
- **PrP<sup>Sc</sup>** causes **PrP<sup>C</sup>** protein to change its conformation to abnormal form
- newly produced **PrP<sup>Sc</sup>** molecules convert more normal molecules to the abnormal form through unknown mechanism
Neural Loss

• Evidence suggests that PrP$^C$ must be present for neural degeneration to occur

• Interaction of PrP$^{Sc}$ with PrP$^C$ may cause PrP$^C$ to crosslink and trigger apoptosis

• PrP$^C$ conversion causes neuron loss, PrP$^{Sc}$ is the infectious agent

• All prion caused diseases
  – have no effective treatment
  – result in progressive degeneration of the brain and eventual death