Viruses

Characteristics

- Physical:
  - Enclosed by protein coat called **capsid** that is made up of **capsomeres**
  - Very small with sizes ranging from 10nm to 300nm
- Non-living:
  - Acellular as no organelles, cytoplasm and are not enclosed by a plasma membrane
  - Geometric shape and may exist as crystalline forms outside the host cell
  - Outside of host cell, do not carry out metabolic processes such as respiration
- Living:
  - Possess **genetic material** in the form of either DNA or RNA but not both
  - Inside the host cell, can reproduce and replicate many copies of the viral genome using host cell machinery
  - Use host cell's enzymes such as RNA polymerase for transcription and ribosomes for translation to produce viral proteins and use host resources such as tRNAs, nucleotides, amino acids and ATP to replicate
- Obligate parasites
  - Require host cell to complete their life cycle and reproduce

Structure

- Size: 10-300nm
- Genome
  - Nucleic acid that codes for viral components and enzymes for viral replication and assembly
- Capsid
  - Protein coat composed of protein subunits called capsomeres
  - Serves to protect, attach and introduce genome into host cells
  - Nucleic acid + Capsid = Nucleocapsid
- Envelope
  - Phospholipid bilayer surrounding the nucleocapsid
  - Derived from host cell membrane
  - Embedded with viral glycoprotein spikes involved in host cell recognition

Bacteriophages

Structure

T4:

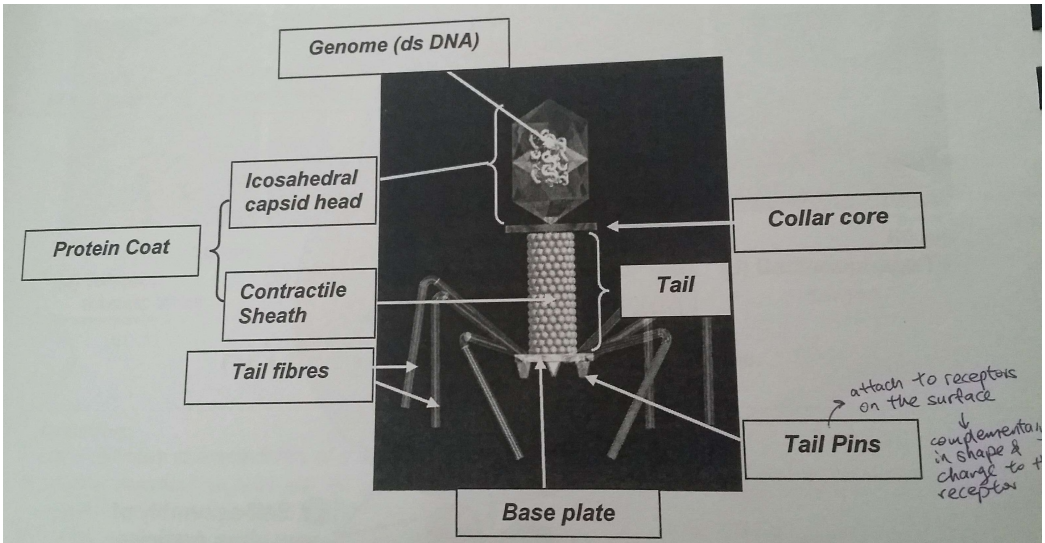

Fig. 5. Structure of T4 bacteriophage

Lambda:

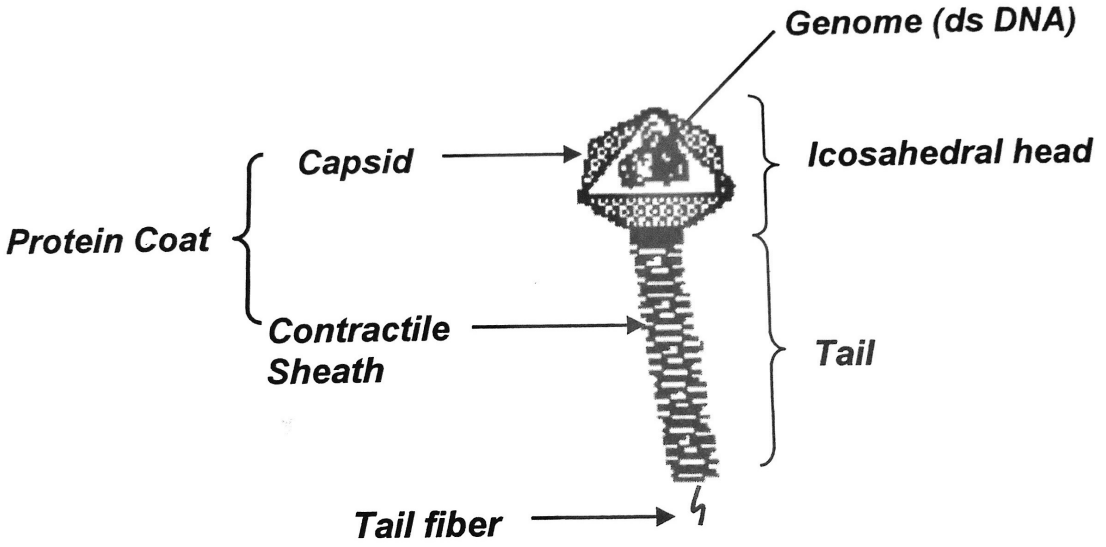

Fig. 7. Structure of lambda bacteriophage

Life Cycle

1. Attachment
    - Attachment sites on tail fibres adsorb to complementary receptor sites on bacterial surface

2. Penetration
    - Tail releases **phage lysozyme** which digests bacterial cell wall
    - Releases molecules from bacterium triggering a change in the shape of proteins in the base plate
    - Causes tail sheath to contract, thrusting the hollow core tube through the cell **wall**
    - When hollow core tube reaches plasma membrane, phage DNA is **injected** into bacterial cell while empty capsid remains outside
3. Lysogenic Cycle (e.g. Lambda Bacteriophage)
    - Linear phage DNA circularises and inserted into host cell genome by **integrase → Prophage**
    - Prophage metabolically silent and remains latent
    - Expression of prophage genes repressed by repressor proteins, hence new phages not synthesised
    - Prophage replicates together with bacterial chromosome
    - Upon spontaneous induction, cellular **proteases** are activated, destroying repressor proteins
    - Prophage is excised and undergoes lytic cycle
4. Lytic Cycle (e.g. T4 Bacteriophage/Lambda Bacteriophage upon activation)
    - Host cell macromolecular machinery used to synthesise phage proteins
    - Early phage proteins produced degrade host DNA and take control of host cell machinery for its own use
    - Phage uses host cell nucleotides and its own early proteins (enzymes) to synthesise phage DNA
    - Late phage proteins synthesised are phage enzymes and structural components
5. Maturation
    - Phage DNA + Capsid = DNA-fillled head
    - Tail fibres + Tail assembled independently and joined
    - Head + Tail to form complete virus6
6. Release
    - Lysozyme coded by phage gene is synthesised and causes bacterial cell wall to break down
    - Bacterial cell membrane lyses and virions are released from host cell and go on to infect other cells

Defense mechanisms of bacteria

- Mutate to code for receptor sites that are no longer complementary to phage attachment sites
- Synthesise restriction enzymes that recognise phage DNA and cleave it
- Develop lysogenic relationship with phage

T4 Bacteriophage	Lambda Bacteriophage

Enzymes coded for by phage genome terminates bacterium's macromolecular synthesis	Phage does not terminate bacterium's macromolecular synthesis
Phage does not incorporate genome into host cell genome	Phage incorporates its genome into the host cell DNA where it is now called a prophage
No repressor protein encoded or synthesised	Prophage gene expresses 2 repressor proteins which block expression of other phage genes involved in phage replication
Phage synthesises enzymes and phage components immediately after infection	Phage undergoes a latent stage where the phage DNA is not expressed and the phage does not undergo replication

Animal Viruses
Influenza - myxovirus
Structure

- Spherical or ovoid in shape
- Genome made up of **8 different** segments of ssRNA associated with nucleoproteins
    - -ve strand RNA (i.e. complementary to mRNA)
    - Each segment packed with 3 polymerase proteins which form RNA-dependent RNA polymerase (necessary to start transcription of -ve strand RNA which code for RNA-dependent RNA polymerase to produce more copies of it)
    - 3 segments code for 3 different polymerases which form RNA-dependent RNA polymerase
    - Other 5 segments code for haemagglutinin, neuraminidase and other proteins/enzymes
- Capsid present
- Envelope derived from the host cell plasma membrane and is studded with haemagglutinin (T-shaped) and neuraminidase (pointed)

Target: Epithelial cells of respiratory tract
Symptoms: Flu symptoms (e.g. fever, runny nose, headache, chills)
Disease:

- Neuraminidase helps to penetrate mucoproteins in mucus layer
- Haemagglutinin binds to receptors on cell membrane of epithelial cells
- Influenza replicates within epithelial cells

- *Infected epithelial cells eventually lyse when influenza buds off
- *Build up of dead epithelial cells leads to inflammation, runny nose and scratchy throat
- *Epithelial layer weakens and more susceptible to secondary bacterial infections of respiratory tract

Treatment:

- Antiviral drugs
    - Neuraminidase inhibitors
        - Halt spread of virus
    - Viral ion channel blocker (M2 protein)
        - Prevent virus from infecting cells
- Antibiotics for secondary infections

Life Cycle

1. Attachment
    - **Haemagglutinin** on influenza virus binds to complementary **sialic acid receptors** on epithelial cells in respiratory tract
2. Penetration and Uncoating
    - Virus enters by **endocytosis** where host cell membrane **invaginates** and pinches off, placing the virus into an **endocytotic vesicle**
    - Endocytotic vesicle fuses with **lysosome** ⇒ pH drops ⇒ Viral envelope fuses with endocytotic vesicle membrane
    - Nucleocapsid is released into cytosol
    - Capsid degraded by cellular enzymes to release viral genome
    - Helical nucleoproteins containing the 8 viral RNA segments enter the nucleus of cell
3. Replication
    - Viral **RNA-dependent RNA polymerase** catalyses transcription of viral RNA
    - Viral genome acts as a template to synthesise complementary mRNA strand
    - mRNA enters cytosol where it is translated into viral structural components in the cytoplasm or at the Rough ER
        - Rough ER → Envelope glycoproteins haemagglutinin and neuraminidase are produced and transported in vesicles to the plasma membrane where they will be embedded
        - Cytosol → Capsid proteins produced
        - Nucleus → **Template** for synthesis of new viral RNA genome
4. Maturation
    - Capsid proteins + Viral glycoproteins at host cell membrane
    - Viral RNA genome + nucleoproteins = helical nucleoprotein
    - Helical nucleoproteins + capsid protein associated with host cell membrane ⇒ Initiates budding process
5. Release (by budding)
    - Virus buds off from cell by **evagination**, acquiring its host membrane embedded with viral glycoproteins

- Release of virions facilitated by **neuraminidase** by cleaving sialic acid from host cell receptor

HIV - retrovirus
Structure

- Spherical in shape
- Genome made up of **2 identical** copies of ssRNA
  - +ve strand RNA (i.e. same sequence as viral mRNA)
  - Tightly bound to **nucleocapsid proteins**
    - Proteins form a tube around RNA strand
- Conical-shaped capsid
  - Contains RNA genome, reverse transcriptase, integrase and protease
- Phospholipid envelope embedded with viral glycoproteins gp41 and gp120 (attached to gp41)

Target: Immune system (Primarily macrophages and T helper cells)
Disease:

- gp120 binds to CD4 on T helper cells
- HIV genome replicates with T helper cells
- HIV eventually bud off, causing T helper cells to lyse
- Macrophages survive infection and act as reservoirs
- Loss of T helper cells leads to impaired immune response ⇒ Increasingly susceptible to opportunistic diseases ⇒ AIDS ⇒ Death
- Avoids detection by immune system as it has high rate of mutation during replication ⇒ Modified surface antigens ⇒ Prevents recognition and elimination by immune system (antigenic drift)
  - Due to lack of proofreading mechanism of reverse transcriptase

Treatment:

- Currently uses combinatorial treatment
- Anti-retroviral drugs
  - Reverse transcriptase, integrase and protease inhibitors
  - Entry inhibitors (target gp120)

Life Cycle

1. Attachment
   - **gp120** binds to **CD4** receptor on cell membrane of T helper cells with the help of a co-receptor
2. Penetration and Uncoating
   - **gp41** helps viral envelope fuse with host cell membrane to release the

nucleocapsid into cell
    - Capsid and nucleocapsid protein degraded by cellular enzymes, releasing **viral enzymes** and the **2 viral RNA strands** into cytosol
3. Replication
    - **Reverse transcriptase** catalyses synthesis of double-stranded DNA strand
        - Viral ssRNA acts as template for the synthesis of cDNA, forming a RNA-DNA hybrid
        - RNA strand then degraded and second DNA strand complementary to cDNA strand is synthesised using first cDNA strand as a template, forming a dsDNA molecule
    - Viral DNA enters nucleus and integrates into host cell genome by **integrase** where it is known as a **provirus** which persists in latent state
    - Upon activation, provirus transcribed into viral mRNA and exits nucleus
        - Serve as viral genomes for new generation of virions
        - Cytosol → Translated into **viral polyproteins**
        - Rough ER → Translated into viral **glycoproteins** gp120 and gp41 and transported to cell membrane by vesicles where they are embedded
4. Maturation
    - Capsid assembles around viral genome and polyprotein at cell membrane where viral glycoproteins have been inserted
    - Completed **after** release of virus
5. Release
    - Virus buds off from cell by **evagination**, acquiring the **host cell membrane** embedded with viral envelope glycoproteins
    - **Polyproteins** cleaved into viral proteins and enzymes by **protease**
    - Viral genome, proteins and enzymes encapsulated by a protein coat to form a capsid
    - Mature HIV virion now able to infect neighbouring cells

Point of Comparison	Influenza	HIV
Attachment	Glycoprotein haemagglutinin on virus binds to sialic acid receptor on cells	Viral glycoprotein, gp120, binds to CD4 receptor on cell
Penetration	Enters by endocytosis	Enters by fusion of viral envelope with host cell membrane
Enzymes	RNA-dependent RNA polymerase Neuraminidase	Reverse transcriptase Integrase Protease

Proteins synthesised in cytoplasm	Capsid protein	Polyprotein

Antigenic Drift & Antigenic Shift

- Process by which viral genomes constantly mutate

Antigenic Drift

- Mutations occur frequently due to poor proofreading mechanism of viral RNA-dependent RNA polymerase
- Accumulation of mutations in viral genomes
- Sometimes mutations in genes encoding for modified surface antigens of virus (e.g. haemagglutinin and neuraminidase)
    - Mutation changed the structure of haemagglutinin such that it has higher affinity for binding to receptor residues on surface of human cells
    - Mutation changed the structure of neuraminidase such that it has a more precise active site to better cleave sialic acid which facilitates the release of virions
- Results in a different conformation of surface antigens which cannot be recognised by antibodies against previous strains OR has a more precise active site ⇒ Host cell susceptible to the virus
- Both influenza A and B

Antigenic Shift

- 2 or more strains of a virus (from different hosts) infect the same cell of an intermediate host
- **Genetic reassortment** may occur → RNA segments from the 2 different viruses may be packaged into the same virion giving rise to new combinations of RNA segments
- May confer phenotypic change where viruses have new surface antigens (e.g. haemagglutinin and neuraminidase)
- Original hosts may not have antibodies that recognise the new antigens and hence are susceptible to the virus
- Mainly in influenza A