Genetics of Bacteria and Viruses- Viruses

1. Overview of Topic

Viruses are biological entities which defy the definition of life. Outside of a host cell, a virus particle is nothing more than a speck of dust. However, once a virus infects a host cell, it has the capability of reproducing at the expense of the host itself. The Spanish flu which surfaced in 1918 lead to a pandemic which claimed more lives than those lost during the 1st World War. Despite being one of the most microscopic entities in the biological world, viruses can threaten the existence of human life itself on earth. In this chapter, we will learn about the different types of viruses (Bacteriophages & Animal Viruses), their reproduction cycles as well as how they undergo evolution.

2. Learning Outcomes

a. Discuss how viruses challenge the cell theory and concepts of what is considered living.
b. Describe the structural components of viruses, including enveloped viruses and bacteriophages, and interpret drawings and photographs of them.
c. Describe the structure and organisation of viral, prokaryotic and eukaryotic genomes (including DNA/RNA, single-/double-stranded, number of nucleotides, packing of DNA, linearity/circularity and presence/absence of introns).
d. Describe how the genomes of viruses are inherited through outlining the reproductive cycles of:
   i. bacteriophages that reproduce via lytic cycle only, eg. T4 phage;
   ii. Bacteriophages that reproduce via lytic and lysogenic cycles, eg. Lambda phage;
   iii. enveloped viruses, eg. influenza
   iv. retroviruses, eg. HIV.
e. Describe how variation in viral genomes arises, including antigenic shift and antigenic drift.

3. References

- Biology, 9th Edition, by Campbell, N.A. and Reece J.B. (2011).
- Microbiology – An Introduction, 9th Edition by Tortora, Funke and Case. (2007).
- Molecular Biology of the Cell, 3rd Edition, by Alberts, Bray and Lewis. (2008).

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Contents

4. Introduction

A Brief History of Virology:

1796:	Edward Jenner used cowpox to vaccinate against smallpox. Jenner was the first person to deliberately vaccinate against any infectious disease. Although Jenner is commonly given the credit for vaccination, variolation, the practice of deliberately infecting people with smallpox to protect them from the worst type of the disease, had been practiced in China at least two thousand years previously.
1885:	Louis Pasteur experimented with rabies vaccination, using the term "virus" (Latin, poison) to describe the agent. Although Pasteur did not discriminate between viruses and other infectious agents, he originated the terms "virus" and "vaccination" (in honour of Jenner) and developed the scientific basis for Jenner's experimental approach to vaccination.
1892:	Dimitri Iwanowski described the first "filterable" infectious agent - tobacco mosaic virus (TMV) - smaller than any known bacteria. Iwanowski was the first person to discriminate between viruses and other infectious agents, although he was not fully aware of the significance of this finding.
1898:	Martinus Beijerinick extended Iwanowski's work with TMV and formed the first clear concept of the virus "contagium vivum fluidum" - soluble living germ. Freidrich Loeffler and Paul Frosch demonstrated that foot and mouth disease is caused by such "filterable" agents. Loeffler and Frosch were the first to prove that viruses could infect animals as well as plants.
1915:	Frederick Twort discovered viruses infecting bacteria.
1917:	Felix d'Herelle independently discovered viruses of bacteria and coins the term bacteriophage. The discovery of bacteriophages provided an invaluable opportunity to study virus replication at a time prior to the development of tissue culture when the only way to study viruses was by infecting whole organisms.
1935:	Wendell Stanley crystallized TMV and showed that it remained infectious (Nobel Prize, 1946). Stanley's work was the first step towards describing the molecular structure of any virus and helped to further illuminate the nature of viruses.
1939:	Emory Ellis and Max Delbruck established the concept of the "one step virus growth cycle" essential to the understanding of virus replication (Nobel Prize, 1969). This work laid the basis for the understanding of virus replication - that viral particles do not "grow" but are instead assembled from preformed components.
1940:	Helmuth Ruska used an electron microscope to take the first pictures of viral particles. Along with other physical studies of viruses, direct visualization of virions was an important advance in understanding virus structure.
1961:	Sydney Brenner, Francois Jacob, and Matthew Meselson demonstrated that bacteriophage T4 uses host cell ribosomes to direct virus protein synthesis. This discovery revealed the fundamental molecular mechanism of protein translation.
1970:	Howard Temin and David Baltimore independently discovered reverse transcriptase in retroviruses (Nobel Prize, 1975). The discovery of reverse transcription established a pathway for genetic information flow from RNA to DNA, refuting the so-call "central dogma" of molecular biology.
1970s:	Advances in molecular biology techniques have led to the discovery of many new viruses.
1979:	Smallpox was officially declared to be eradicated by the World Health Organization (WHO). The last naturally occurring case of smallpox was seen in Somalia in 1977.
1983:	Luc Montaigner and Robert Gallo announced the discovery of human immunodeficiency virus (HIV), the causative agent of AIDS.
1990:	First (approved) human gene therapy procedure was carried out on a child with severe combined immune deficiency (SCID), using a retrovirus vector.
1999:	Number of confirmed cases of people living with HIV/AIDS worldwide reaches 33 million. The AIDS pandemic continues to grow. Nucleotide sequence of the largest known virus genome completed: *Paramecium bursaria* Chlorella virus 1. This 330,742 bp sequence represents the technical advances in sequencing which have occurred since the first genome sequence was completed in 1977.

2002-2004:	Outbreak of severe acute respiratory syndrome (SARS) caused by the SARS coronavirus.
2006:	Two vaccines protecting against several cervical cancer-causing strains of human papillomavirus (HPV) were released.
2009 - 2010:	Outbreak of H1N1 influenza pandemic. Two babies from Mexico die in the outbreak. By end of May 2010, steep decline in number of reported cases. WHO statistics report more than 18 000 cases of death from H1N1.
2013 - present:	Outbreak of Ebola epidemic. Started in West Africa. At present, 24 000 confirmed cases and the number of deaths have exceeded 9800.

5. Viruses challenge the cell theory and the definition of an living organism

1. Are Viruses Living or Non-living?

Unlike bacteria, viruses lack the ability to replicate on their own. They absolutely require a living host, or a cell to support their metabolism and replication. They are able to enter a cell and then take over that cell, directing it to make more viral particles. In this sense, viruses are said to be obligate parasites.

Question:

What is an obligate parasite?

An obligate parasite is an organism that cannot live independently of its host and depends on its host to complete its life cycle.

Characteristics of living organisms based on the cell theory:

1. Have a cellular organization

Regardless of whether they are unicellular or multi-cellular organisms, the smallest level of organization of living organisms is the cell.

2. Show metabolic activity

All organisms need to acquire and use energy in order to maintain metabolic processes for survival.

3. Grow and develop

Growth involves both the increase in size and number of cells. When organisms grow, they undergo changes known as development.

4. Reproduce

Organisms produce offspring like themselves through sexual or asexual means.

5. Have a common hereditary molecule

All living organisms have a common molecular inheritance based on the nucleic acid, which contains instructions for the structure and function of cells.

6. Respond to stimuli

Organisms have specialised receptors that detect environmental stimuli to allow their cells to adjust metabolism in response.

7. Adapt to the environment

Adaptation is the accommodation of a living organism to its environment, which is fundamental to the process of evolution.

Question:

Which of the above characteristics do viruses show in isolation (without their hosts)? 5

There is great contention amongst scientists as to whether viruses should be classified as being living or non-living. They have been described as "organisms at the edge of life",

Q1 Explain why viruses may be regarded as living organisms

1) Viruses possess **genetic material** and are capable of propagating their genetic information.
2) Once inside the host cell it uses/directs host enzymes to carry out metabolic processes.
3) Once **inside the host cell**, and directs its own self-replication. They **reproduce by creating multiple copies** of themselves through self-assembly.
4) Some viruses undergo mutation and reassortment of their genetic material during their replication giving rise to new viral strains.
5) Virus can react to its environment. They are able to respond to stimuli such as heat, radiation and chemical when inside the host cell.
6) They are able to evolve to adapt to new environment

Q2 Explain why viruses may be regarded as non-living organisms

1) They are **acellular** and **lack cellular organelles**
2) Viruses **do not carry out metabolism** (e.g. respiration)
3) They **lack the ability to reproduce on their own independently** and can only undergo replication in living cell
4) They **do not grow** and undergo developmental changes and require a host cell to make new products such as coat protein and nucleic acids
5) They **do not respond to stimuli** when outside the host cell.
6) They can **only evolve** by natural selection **within a host cell**.

Conclusion:

Viruses are ***obligate parasites*** which requires a living host to support many of their functions. Viruses can be crystallized like an ordinary chemical the capsid coat is very regular.

6. Structure of Viruses

The sizes of viruses ranged from **10 to 300nm**. An intact **infectious** viral particle is called a **virion.** A virion is a complete, fully developed viral particle composed of the **genome** and surrounded by a protein coat, the **capsid**.

Some types of viruses have an additional outer layer, an **envelope** in which viral **glycoproteins** are embedded in.

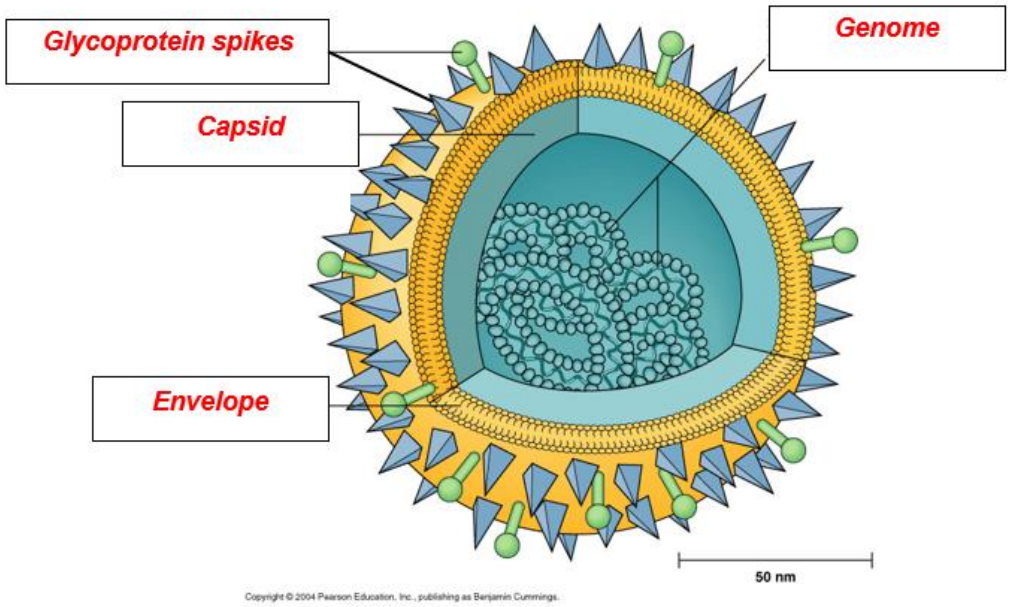


Fig. 1 Diagram of a generalized virus

a. Genome

The viral genome is **single** or several/**segmented**, **circular** or **linear** molecules of nucleic acid that functions as the genetic material of the virus.

In contrast to prokaryotic and eukaryotic cells in which DNA is usually the primary genetic material, a virus can have **either DNA** or **RNA** but **never both**.

The nucleic acid can be **single-stranded** or **double-stranded**. Thus, in addition to the familiar double-stranded DNA, there are viruses with single-stranded DNA, double stranded RNA, or single-stranded RNA.

The genome codes for the synthesis of **viral components** and **viral enzymes** for **replication** and **assembly** of a virion.

The genes are few in number ranging from 3 – 100 depending on the class of virus. The number of genes present in the virus determines the degree of complexity displayed by the virus.

Table 19.1 Classes of Animal Viruses

Class/Family	Envelope	Examples That Cause Human Diseases
I. Double-Stranded DNA (dsDNA)		
Adenovirus (see Figure 19.3b)	No	Respiratory viruses; tumor-causing viruses
Papovavirus	No	Papillomavirus (warts, cervical cancer); polyomavirus (tumors)
Herpesvirus	Yes	Herpes simplex I and II (cold sores, genital sores); varicella zoster (shingles, chicken pox); Epstein-Barr virus (mononucleosis, Burkitt's lymphoma)
Poxvirus	Yes	Smallpox virus; cowpox virus
II. Single-Stranded DNA (ssDNA)		
Parvovirus	No	B19 parvovirus (mild rash)
III. Double-Stranded RNA (dsRNA)		
Reovirus	No	Rotavirus (diarrhea); Colorado tick fever virus

Table 19.1 Classes of Animal Viruses (continued)

Class/Family	Envelope	Examples That Cause Human Diseases
IV. Single-Stranded RNA (ssRNA); Serves as mRNA		
Picornavirus	No	Rhinovirus (common cold); poliovirus; hepatitis A virus; other enteric (intestinal) viruses
Coronavirus	Yes	Severe acute respiratory syndrome (SARS)
Flavivirus	Yes	Yellow fever virus; West Nile virus; hepatitis C virus
Togavirus	Yes	Rubella virus; equine encephalitis viruses
V. ssRNA; Template for mRNA Synthesis		
Filovirus	Yes	Ebola virus (hemorrhagic fever)
Orthomyxovirus (see Figures 19.3c and 19.9a)	Yes	Influenza virus
Paramyxovirus	Yes	Measles virus; mumps virus
Rhabdovirus	Yes	Rabies virus
VI. ssRNA; Template for DNA Synthesis		
Retrovirus (see Figure 19.8)	Yes	Human immunodeficiency virus (HIV/AIDS); RNA tumor viruses (leukemia)

Table 1: Classification of animal viruses based on types of genome

b. Capsid

The genome is surrounded by a protein coat called a **capsid**. The structure of the **capsid** is ultimately determined by the viral genome and accounts for most of the mass of a virus, especially of the small ones.

Each **capsid** is composed of protein subunits called **capsomeres.**

The **capsid** serves to **protect, attach** and **introduce the genome into host cells**.

Together, the **capsid** and the viral nucleic acid form the **nucleocapsid.** An infectious viral particle, a virion, will contain at minimum a nucleocapsid.

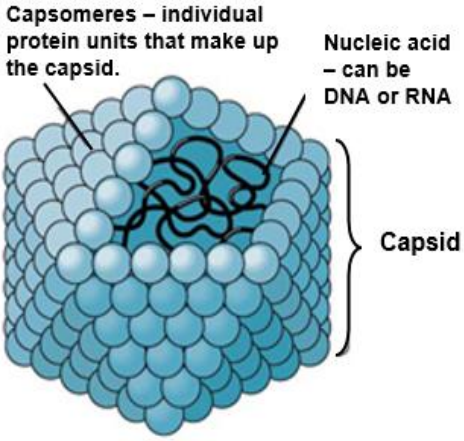


Fig. 2 Diagram of a viral capsid surrounding its genome (nucleocapsid).

c. Envelope

Some types of viruses have an additional outer layer, an **envelope** that surrounds the nucleocapsid.

The envelope is composed of **phospholipids** and **glycoproteins** that are arranged to form a **lipid bilayer**. For most viruses, it is derived from the host cell membranes by a process called **budding.** The envelope comes mostly from the host cell's plasma membranes.

Although the envelope is usually of host cell origin, the virus does incorporate viral proteins of its own, often appearing as **glycoprotein spikes**, into the envelope.

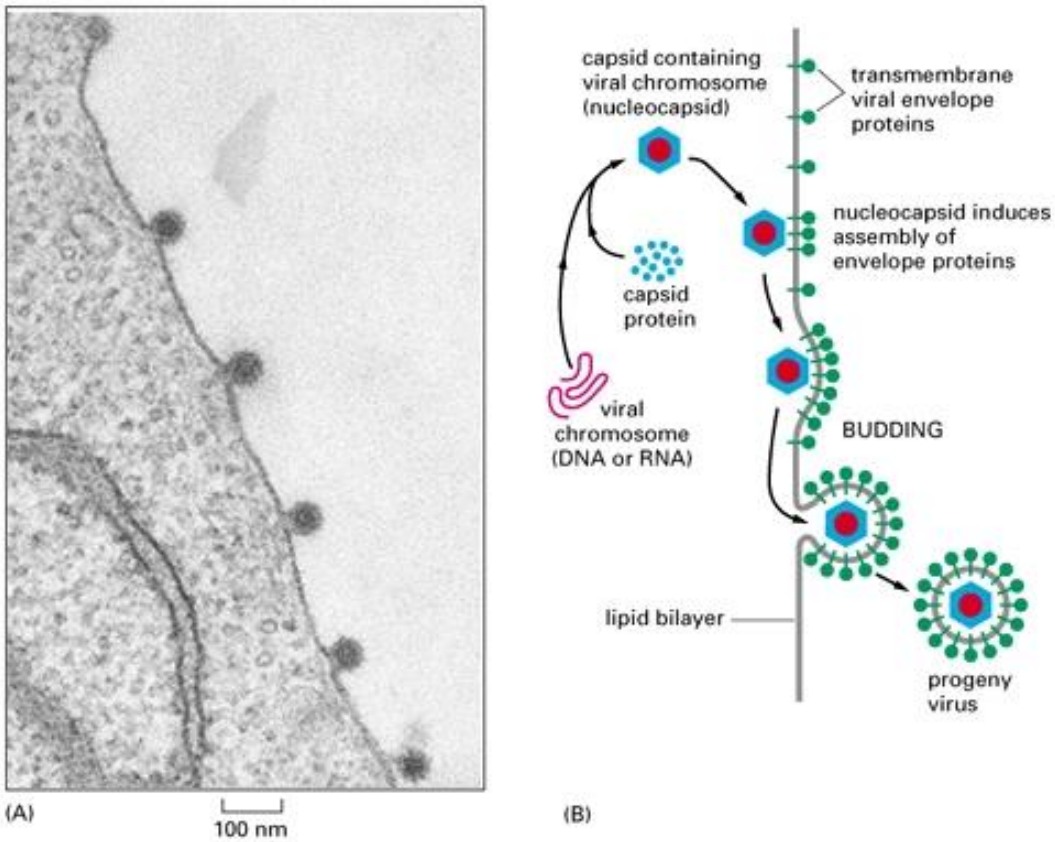


Fig. 3(A) Newly replicated viruses budding off from host cell.

Fig. 3(B) Assembly of viral components at the cell surface membrane of the host cell before budding off.

Most animal viruses have an envelope surrounding their nucleocapsid.

Viruses that are composed of just the nucleocapsid are called **naked viruses** or **non-enveloped viruses** whilst those viruses whose nucleocapsids are covered by an envelope are termed **enveloped viruses**.

d. General Morphology

Viruses may be classified into several different types based on the:

- shape of the viruses
- type and structure of the genome,
- the presence or absence of a viral envelope and
- mode of replication.

In general, four main morphological virus types can be identified: **Helical, Icosahedral, Enveloped & Complex viruses.**

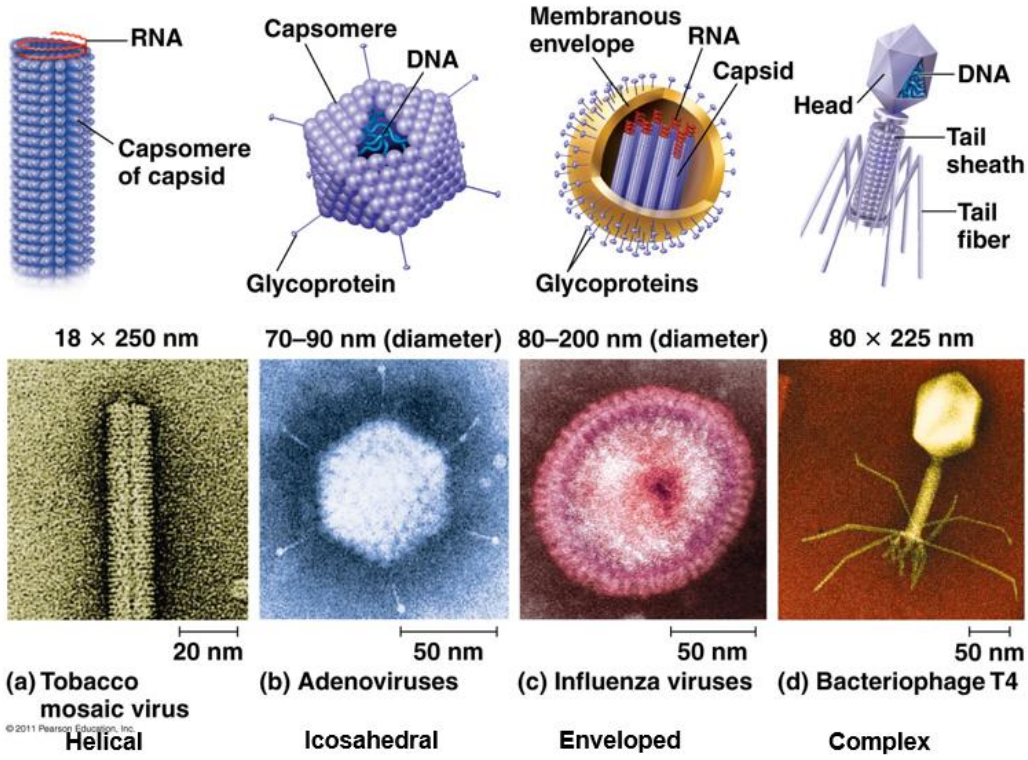


Fig. 4. Different morphological structures of viruses

Question:

List 3 definitive characteristics of a virus.

1. *As obligate parasites, they are totally dependent on a host cell for replication.*
2. *The genome is made up of only 1 type of nucleic acid: DNA or RNA but never both.*
3. *Viral components must assemble into complete viruses (virions) to be able to infect another cell.*

7. Viral Replication

How does a virus replicate?

- Viral replication begins with the virus invading the host cell and taking over the host's metabolic machinery.
- The virus has a specific host that it infects and recognises its host cell via host cell antigen e.g. glycoprotein at cell surface membrane.
- Viral genetic material is injected into the host cell or the entire virus may enter and disassemble inside the host cell to free the genetic material
- The virus uses the host metabolism and machinery to synthesise its own nucleic acid e.g. a DNA virus hydrolyses the DNA of host cell and uses the host's DNA polymerase and nucleotides to replicate its own DNA.
- The virus uses the host cell RNA polymerase to transcribe its genes and ribosome to translate its mRNA to viral coat proteins and enzymes. Also supplied by the host cells are tRNA, nucleotides, amino acids and ATP.
- The viral genome contains only a few genes which code for the viral structural components like the capsid proteins and viral enzymes that are involved in the viral life cycle.
- Once all the viral components are synthesised, they will self-assemble to form new viral particles or virion
- and exit the cell via **budding, exocytosis** or through **lysis** of the host cell.

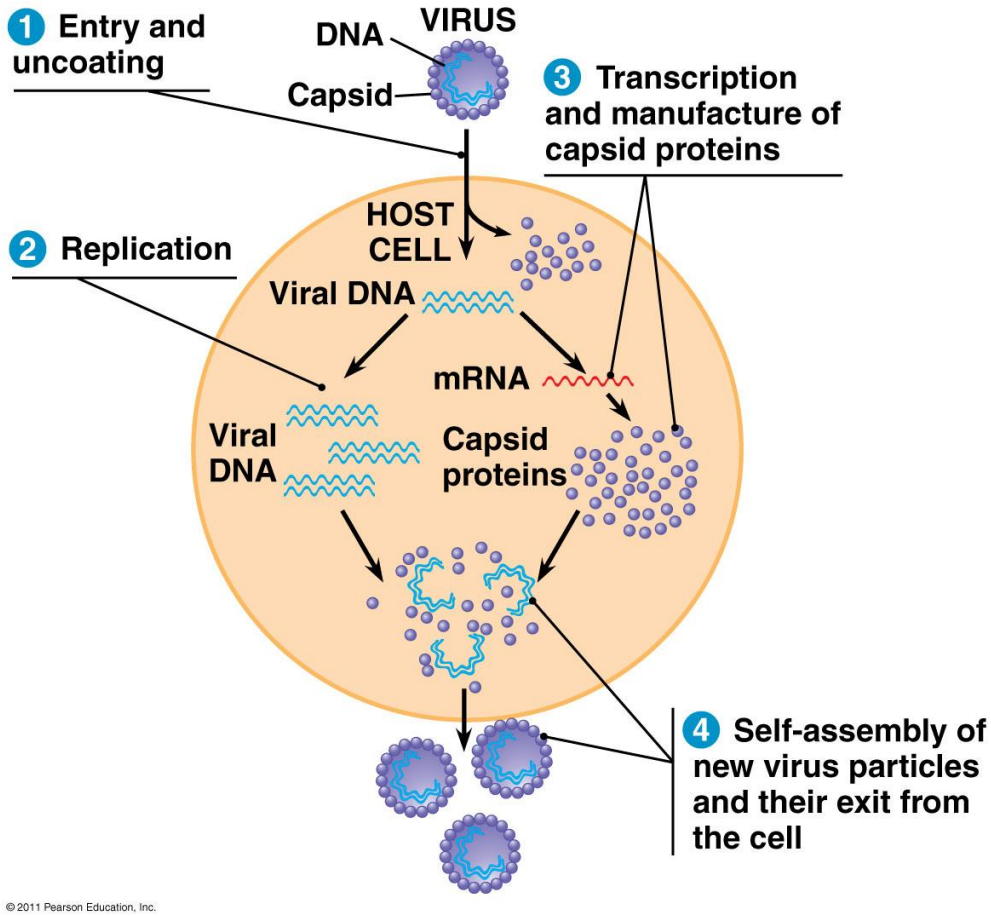


Fig. 5. A simplified viral reproductive cycle

8. Bacteriophages

Notes to self

Bacteriophages are **viruses that only infect bacteria**.

There are two primary types of bacteriophages:
a. **Lytic bacteriophages**; and
b. **Temperate bacteriophages**.

Bacteriophages that replicate through the **lytic life cycle** are called **lytic bacteriophages**. They lyse (*break up or disintegrate*) the host bacterium as a normal part of their life cycle.

Bacteriophages capable of a **lysogenic life cycle** are termed **temperate phages**. When a temperate phage infects a bacterium it can either replicate by means of the lytic life cycle (and cause **lysis** of the host bacterium) or it can incorporate its DNA into the bacterium's DNA and become a **prophage**.

i. Bacteriophages which undergo the lytic cycle – T4 bacteriophage

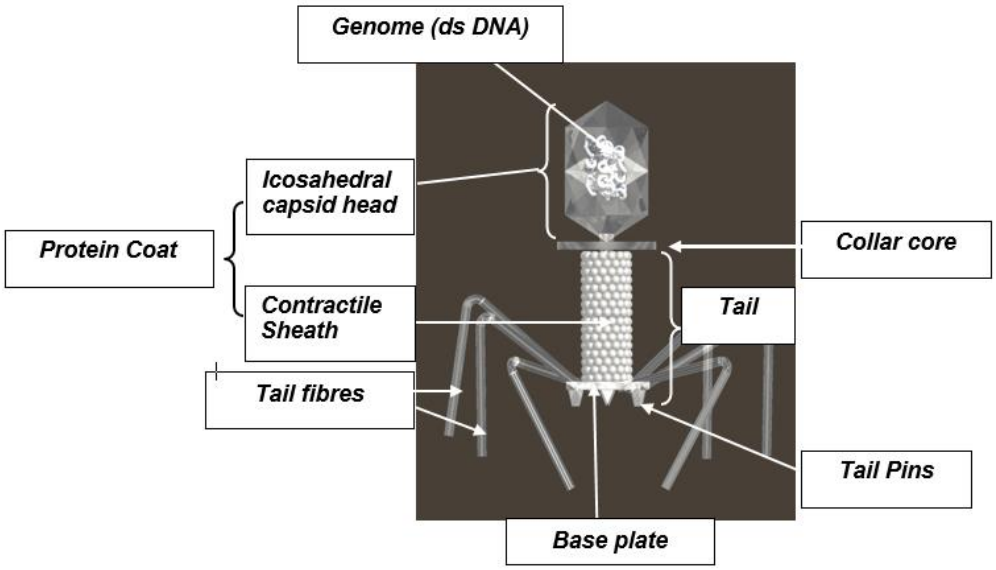


Fig. 6. Structure of T4 bacteriophage

Lytic Cycle:

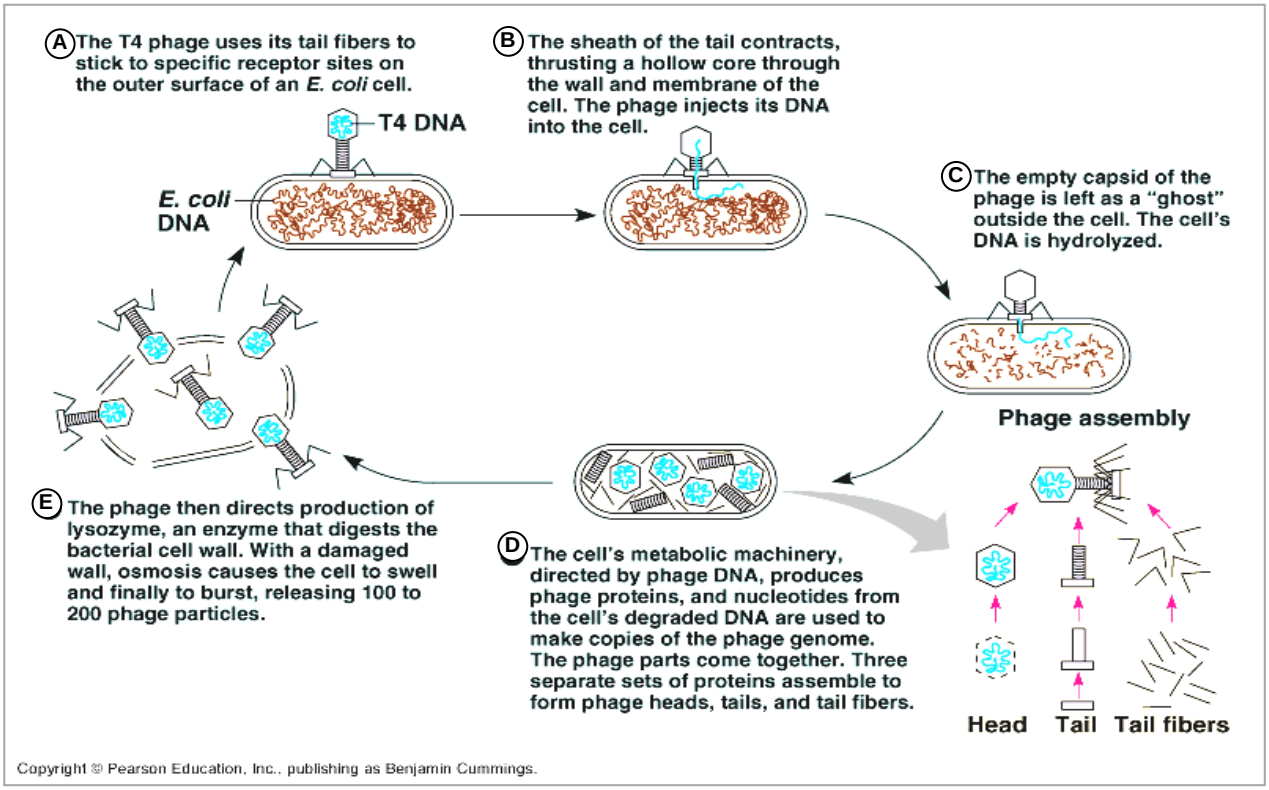
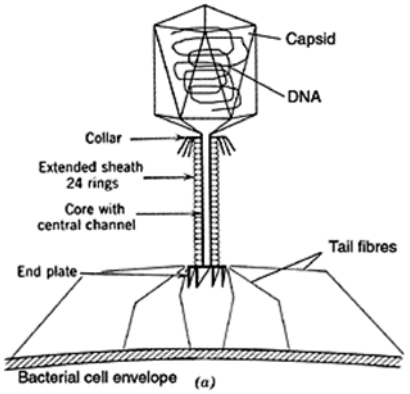


Fig. 7. Lytic life cycle of T4 bacteriophage

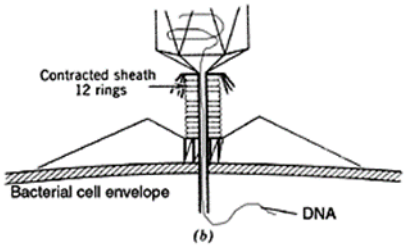
A. Attachment

- Attachment sites on the tail fibres recognise and attach or adsorb to complementary receptor sites on the bacterial surface. This attachment is a chemical interaction in which weak bonds are formed between the attachment and receptor sites.
- Specific strains of bacteriophages can only adsorb to specific strains of host bacteria. This is known as **viral specificity**.
- Although most bacteriophages attach to the bacterial cell wall, some are able to attach to the flagella or pili.



B. Penetration

- The bacteriophage tail releases an enzyme, phage **lysozyme**, which digest the bacterial cell wall, allowing molecules to be released. When these molecules reach the virus, they trigger a change in the shape of the base plate. This initiates a contraction of the bacteriophage tail sheath, thrusting the hollow core tube through the cell wall.
- When the tip of the core reaches the plasma membrane, DNA from the bacteriophage is injected into the bacterial cell. The empty **capsid** remains outside the cell.



C. Replication

- Inside the cell, the bacteriophage DNA is **immediately transcribed** to synthesise messenger RNA using the host RNA polymerase.

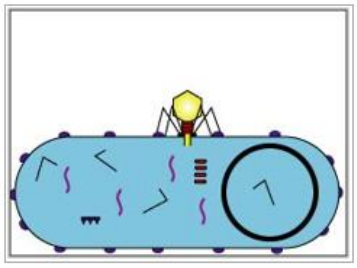
- Phages that are highly virulent produce early proteins that completely take control from the host cell. For example, host cell DNA is degraded within minutes into nucleotides that are later reused to synthesise viral DNA. Viral DNA escapes degradation because of methylation of its DNA.

- Enzymes coded by the phage genome takes over the bacterium's macromolecular (protein, RNA, DNA) synthesising machinery for its own use.

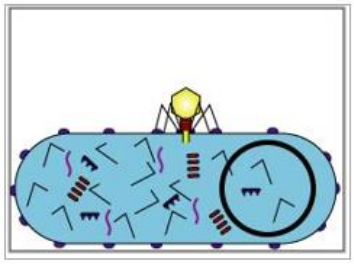
- The phage uses the host cell's nucleotides and several of its own enzymes to synthesise many copies of phage DNA.

- Soon after, biosynthesis of viral proteins begins. It uses the bacterium's metabolic machinery to synthesise phage enzymes and phage structural components.

- For several minutes following infection, complete phages cannot be found in the host cell. Only separate components like the DNA and protein are present. This period, when complete, infective virions are not yet present, is known as the **eclipse period**.



Early Replication
The bacteriophage genome replicates and bacteriophage components begin to be produced by way of the host bacterium's metabolic machinery.



Late Replication
The production of bacteriophage components and enzymes progresses.

D. Maturation

- Bacteriophage DNA and **capsid** are assembled into a DNA-filled head. The head, tail and tail fibres are then assembled independently and joined with each other in a specific sequence. First, the tail fibres join with the tail, then a DNA-filled head attaches to the tail.

E. Release

- The final stage of viral multiplication is the release of virions from the host cell. The term lysis is generally used for this stage in the multiplication of T4 bacteriophages because the plasma membrane of the host cell actually breaks open (**lyses**).

- Lysozyme, which is coded for by a phage gene, is synthesised within the cell. This enzyme causes the bacterial cell wall to break down.

- The newly produced bacteriophages are released from the host cell. The mature phage particles will infect other susceptible cells in the vicinity and the viral multiplication cycle is repeated within those cells.

ii. Bacteriophages which undergo the lysogenic cycle – lambda bacteriophage

Lambda bacteriophage (λ bacteriophage)

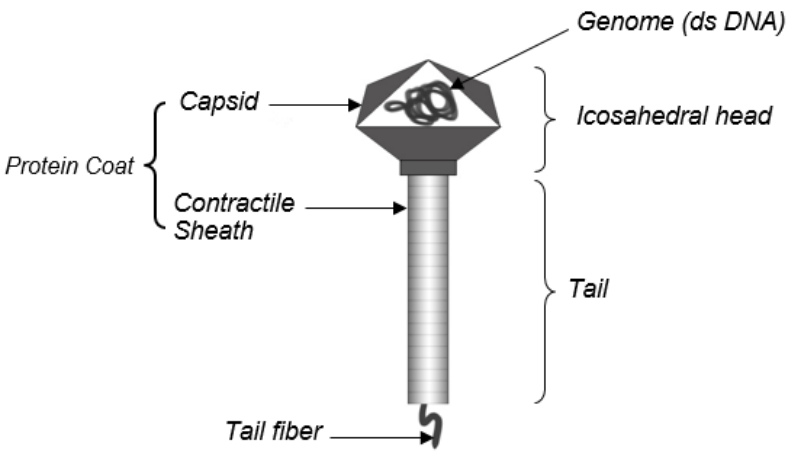


Fig. 8. Structure of lambda bacteriophage

The Lytic and Lysogenic Life Cycle of Lambda bacteriophage

Bacteriophages capable of a lysogenic life cycle are termed **temperate phages**. When a temperate phage infects a bacterium, it can either:

- **replicate by means of the lytic life cycle** and cause lysis of the host bacterium, or,
- it can **incorporate its DNA into the bacterium's DNA** and become a non-infectious **prophage.**

Lysogenic Life Cycle

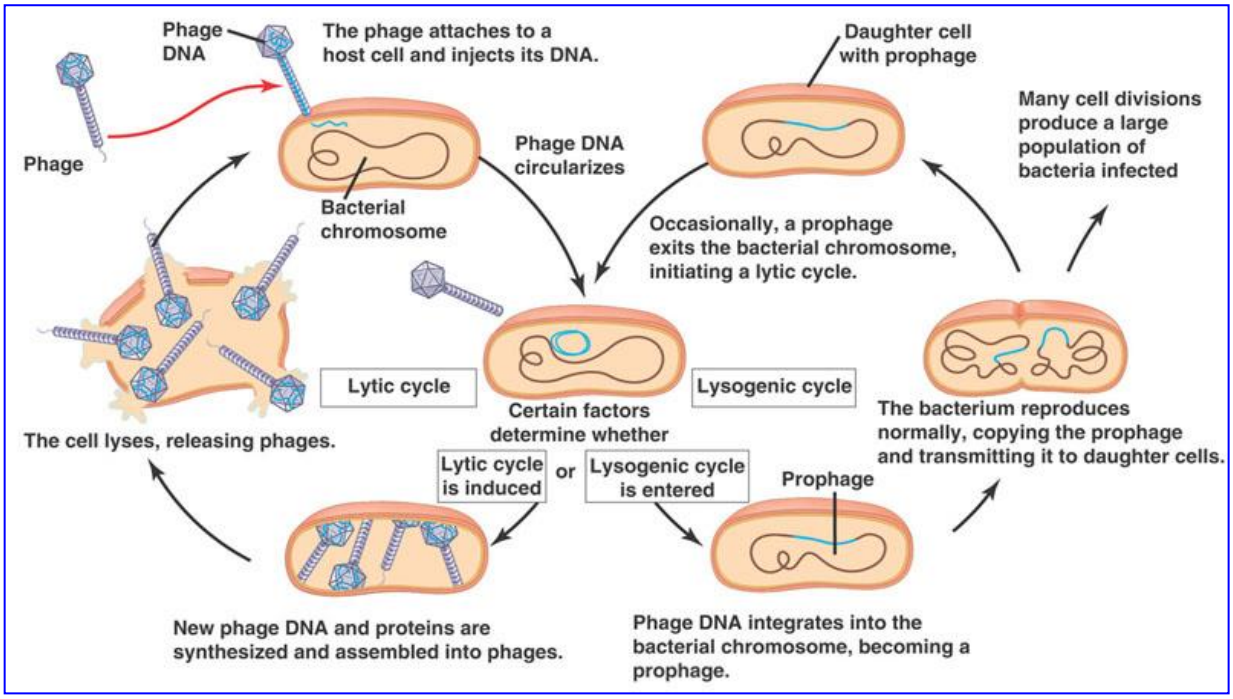


Fig. 9. Lysogenic life cycle of lambda bacteriophage

A. Attachment

- Tail fiber adsorb to complementary receptor site on host bacterium cell wall *(same as T4 phages)*.

B. Penetration

- Sheath of tail contracts and drives a hollow tube through the bacteria cell wall.
- Phage genome enters the bacterium *(same as T4 phages)*.

C. Replication

- Upon penetration into the bacteria cell, the originally linear phage DNA forms a circle. (Fig.10)
- This circular DNA can multiply and be transcribed leading to the production of new phage and to cell lysis (via the lytic cycle).
- Alternatively, the circular DNA can integrate into and become part of the circular bacterial DNA (the lysogenic cycle).
- The inserted phage DNA is now called a **prophage**.
- Most of the prophage genes are repressed by repressor proteins that are the products of phage genes. These repressors stop transcription of all the other phage genes. Thus, the phage genes that would otherwise direct the synthesis and release of new virions are turned off.

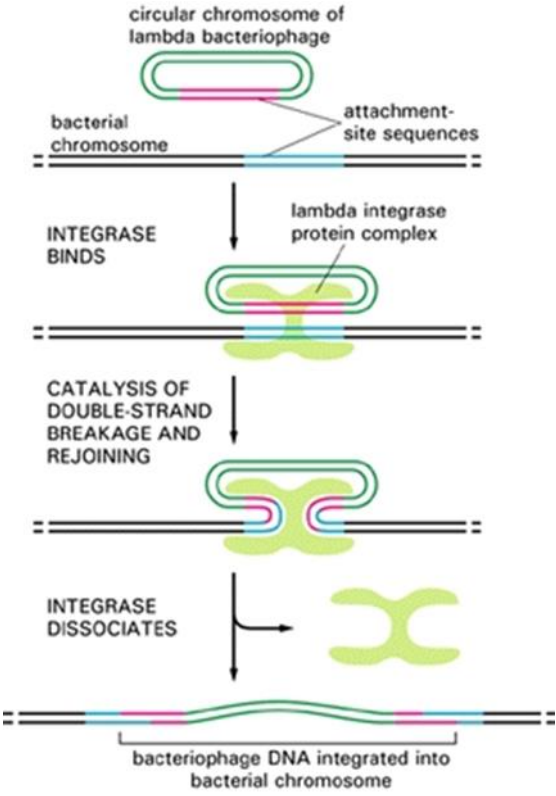


Fig. 10. Integration of lambda genome into host cell genome.

- Every time the host cell's machinery replicates the bacterial chromosome, it also replicates the prophage DNA. The prophage will be found in all progeny cells, where it remains **latent.**

D. Spontaneous induction

- Occurs in one of every million to every billion bacteria containing a prophage.
- Induction occurs spontaneously but its frequency is enhanced by irradiation with ultraviolet light or exposure to agents that damage DNA. It activates the cellular proteases.
- Under these conditions, the repressor protein is destroyed by increased protease activity.
- The prophage is no longer repressed but is instead excised and **enters the lytic cycle**.

E. Maturation

- Since the phage genome is no longer repressed, phage components are produced using the host bacterium's metabolic machinery.
- More copies of viral genome are produced by DNA replication using host cell machinery.
- The bacteriophage components then assemble into complete virions. *(same as T4 phages)*

F. Release

- The complete virions are then released from the host cell in the same manner as the lytic cycle. *(same as T4 phages).*

***Question**:*
What are the possible defense mechanisms of bacteria against phages?

*- Mutant bacteria with **receptor sites** that are **no longer complementary** to the phage attachment sites.*

*- Develop **restriction enzymes** (endonucleases) which recognise foreign phage DNA & cleave them. Modify bacteria's own DNA to prevent attack by restriction enzymes*

- (Develop a lysogenic relationship with the phage)

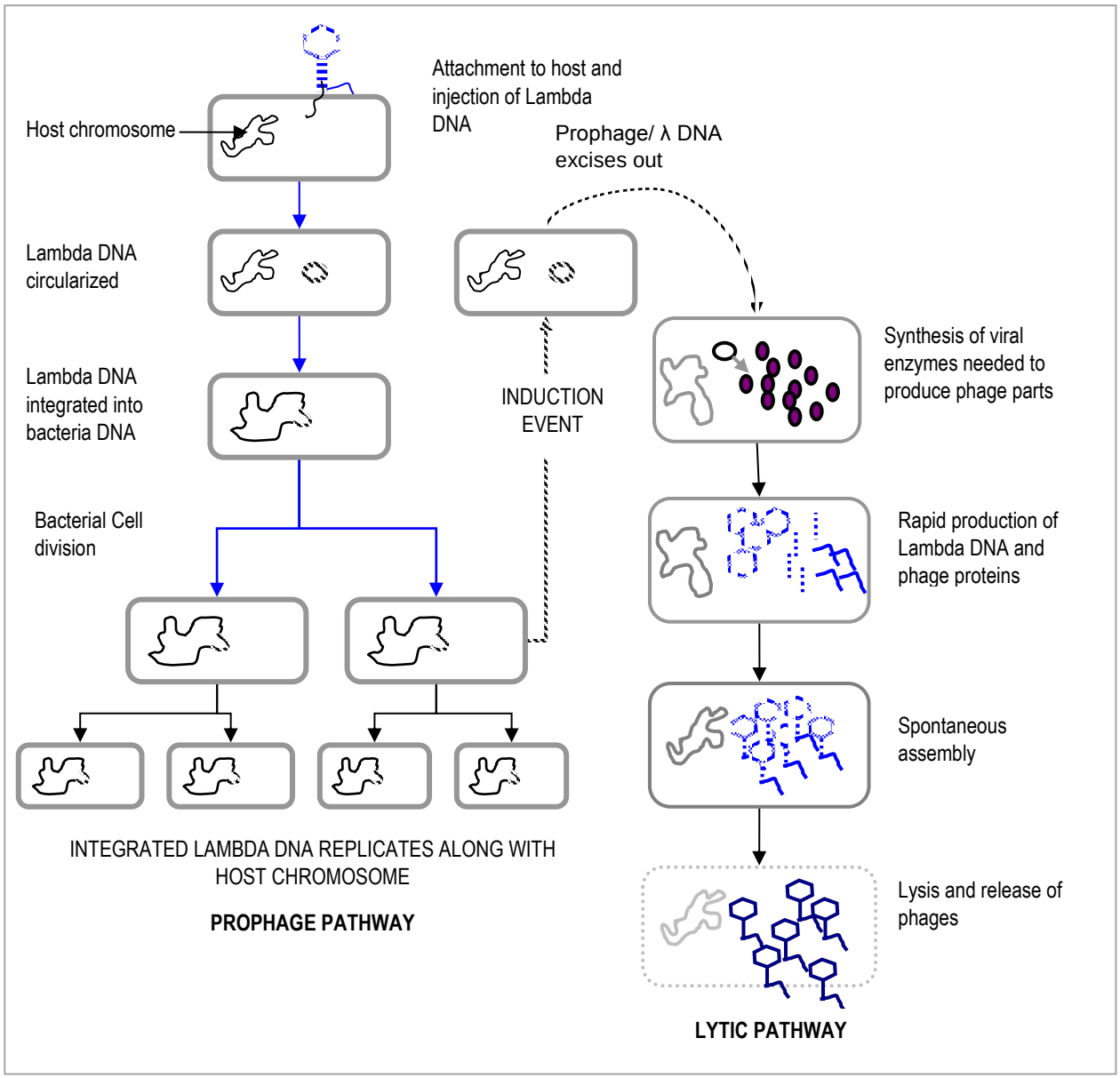


Fig. 11. Summary of the lambda bacteriophage reproductive pathway

Lysogenic host cells are immune to re-infection by the same bacteriophage. However, other types of bacteriophage may still infect the host cell.

Lysogenic bacterial cells may exhibit phage conversion. That is, the host cell may exhibit new properties following integration of the **prophage** into the host genome. For example, the bacterium *Corynebacterium diphtheriae*, which causes diphtheria by means of a toxin it produces can only produce this toxin when it is carrying a lysogenic phage because the **prophage** carries the gene coding for the toxin.

Lysogenic bacterial cells are capable of specialised transduction in which the lysogenic phage packages adjacent genes of bacterial DNA along with its own viral DNA in the same **capsid**. These genes are then transferred to a new bacterial cell along with the **prophage** when the virion infects a new cell. *(To be covered under Genetics of Bacteria and Viruses – Bacteria).*

9. Animal Viruses

a. Influenza

Structure

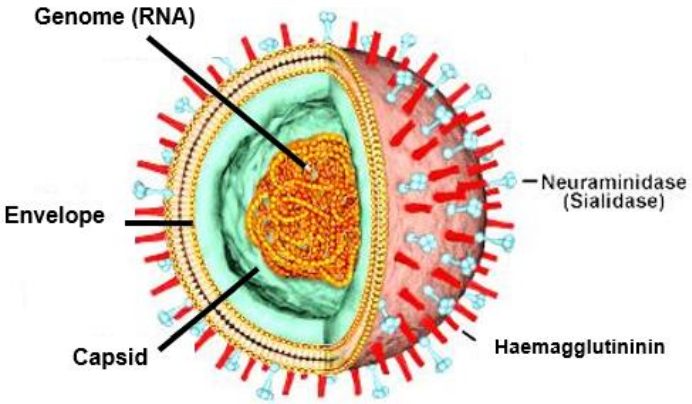


Fig. 12. Structure of the influenza virus

The structure of the influenza virus is somewhat variable, but the virion particles are usually spherical or ovoid in shape and 80 to 120 nm in diameter. Sometimes filamentous forms of the virus occur as well, and are more common among some influenza strains than others.

Genome

- The influenza genome is organised into **eight segments** of **single-stranded RNA.**
- The RNA genome is a **negative (- ve) strand**, i.e. the sequence of the viral RNA genome is complementary to the sequence of the viral mRNA.
- The RNA is packaged with protein into a **helical nucleoprotein** form, with **three of the 8 RNA segments** coding for **three different polymerases**. The three polymerases form an enzyme complex**, RNA-dependent RNA polymerase** or **RNA replicase,** which functions in both **replication** and **transcription** of the viral genome.
- The other **five RNA segments** code for other viral proteins such as **haemagglutinin, neuraminidase**, nucleoprotein, matrix protein M1 and non-structural proteins.

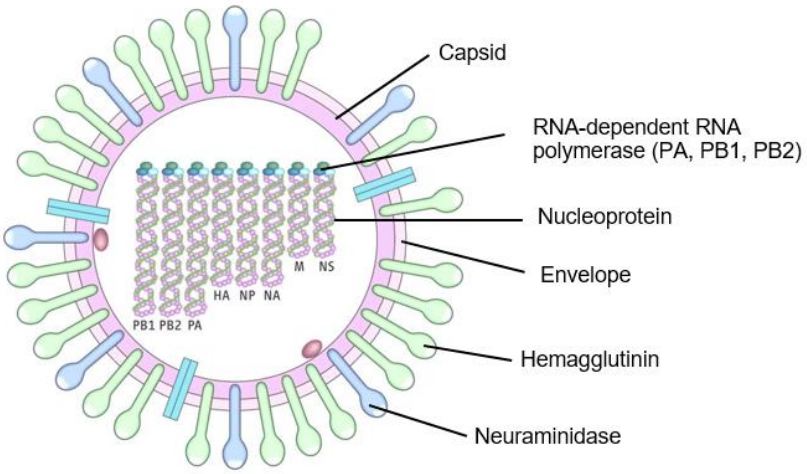


Fig. 13. Structure of influenza virus with RNA-dependent RNA polymerase complex attached to each segment of the RNA genome.

Capsid

- The **capsid** is an antigenic protein lining on the inner side of the envelope.

Envelope

- The influenza virion is an enveloped virus that derives its lipid bilayer from the plasma membrane of a host cell.

- **Haemagglutinin (a glycoprotein)** and **neuraminidase (an enzyme)**, are embedded in the envelope. Different types of **haemagglutinin** and **neuraminidase** glycoproteins give rise to different strains of influenza virus.

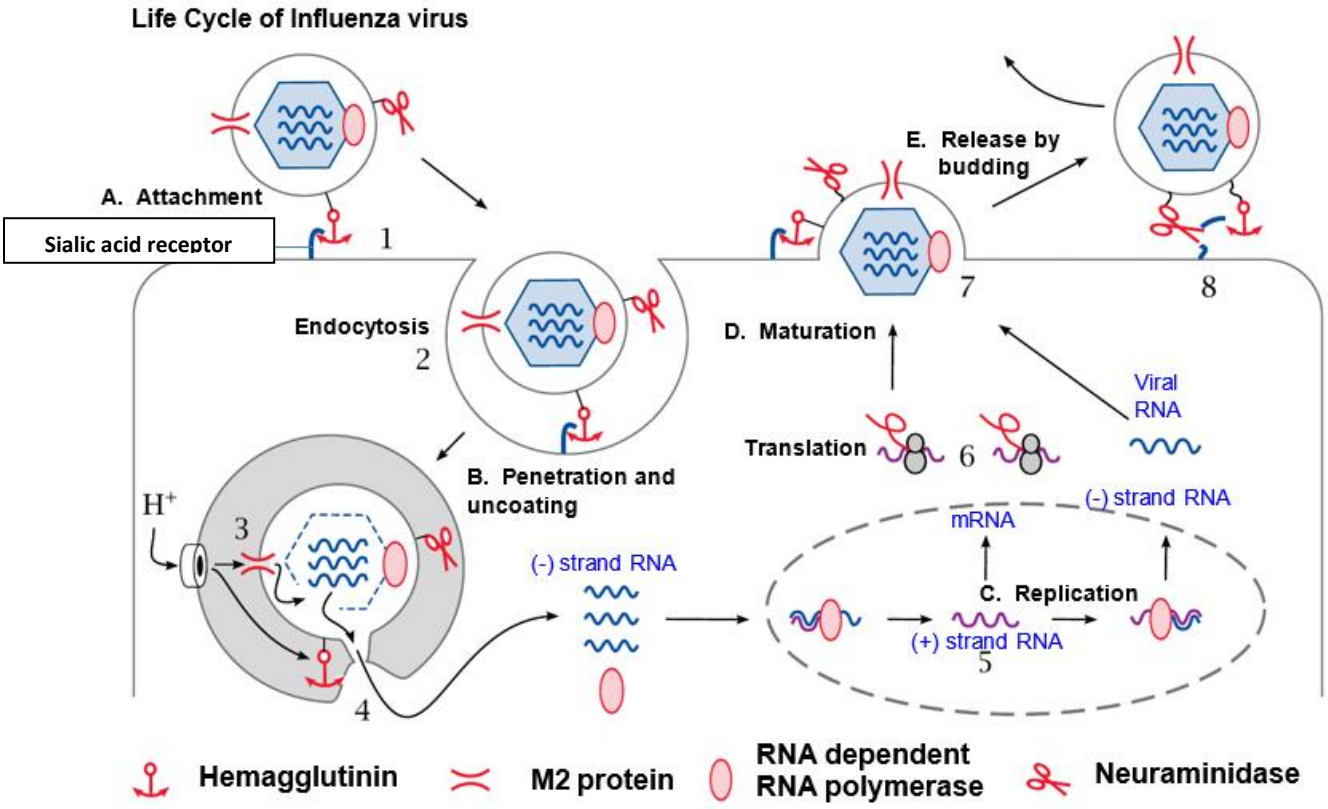


Fig. 14. Life cycle of an influenza virus

A. Attachment

- Protruding glycoproteins bind to specific receptor molecules on surface of host cell. In humans, **haemagglutinin** on the influenza virus binds to the **sialic acid receptor** on the host cell membrane.

B. Penetration and Uncoating

- The virus usually enters by **endocytosis**. The host plasma membrane invaginates and pinches off, placing the virus in an endocytic vesicle/endosome.

- The vesicle will then fuse with a lysosome causing its pH to drop. Within the vesicle, the low pH environment will stimulate the viral envelope to fuse with lipid bilayer of the vesicle membrane and nucleocapsid is released into the cytoplasm.

- The **capsid** is then degraded by cellular enzymes leaving behind the helical nucleoprotein. The helical nucleoprotein then enters the nucleus of the cell.

Notes to self

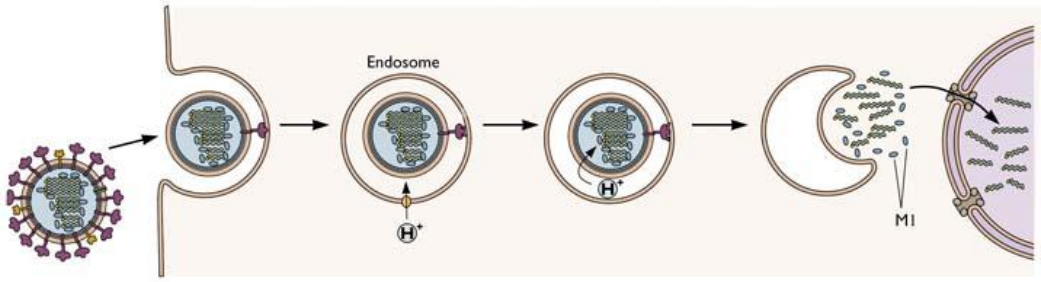


Fig. 15. Entry and uncoating of influenza virus in the host cell

C. Replication

- The viral genome is used as a template to synthesise the viral mRNA/(+) strand RNA catalysed by the viral **RNA-dependent RNA polymerase.** The mRNA produced in turn acts as a template for the synthesis of new viral RNA genome.

- The mRNA strands then exit the nucleus to the cytosol and RER where they are translated into viral structural components such as the glycoproteins to be incorporated into the viral envelope (at the ER) and capsid proteins (in the cytosol).

D. Maturation

- Viral glycoproteins are transported by the vesicles from the ER. They are incorporated into the plasma membrane.

- Capsid proteins then associate with these glycoproteins at the plasma membrane.

- The viral genome associates with proteins to form the helical nucleoprotein which then interacts with the capsid proteins at the plasma membrane of the host cell.

- Interaction of the **capsid** with the nucleoprotein will initiate the budding process.

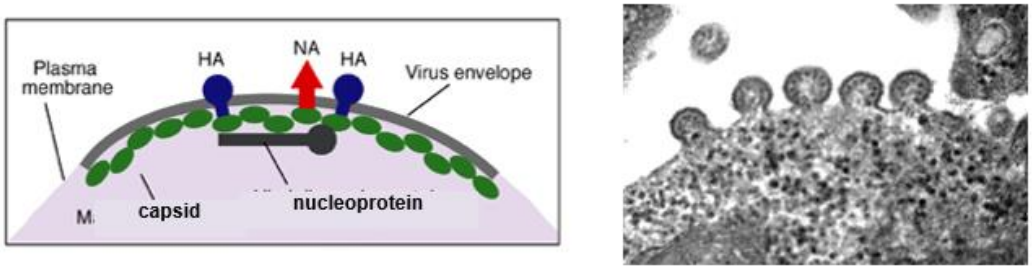


Fig. 16. Budding of influenza virus

E. Release by budding

- Each new virus **buds** from the cell (**evagination**).
- It will acquire the host membrane with viral glycoproteins (**haemagglutinin & neuraminidase)** embedded.
- With enveloped viruses, host cells may or may not be lysed
- The release is facilitated by neuraminidase. Neuraminidase cleaves sialic acid from cell surface and progeny virions facilitating virus release from infected cells.

b. Retrovirus – Human Immunodeficiency Virus (HIV)

Structure

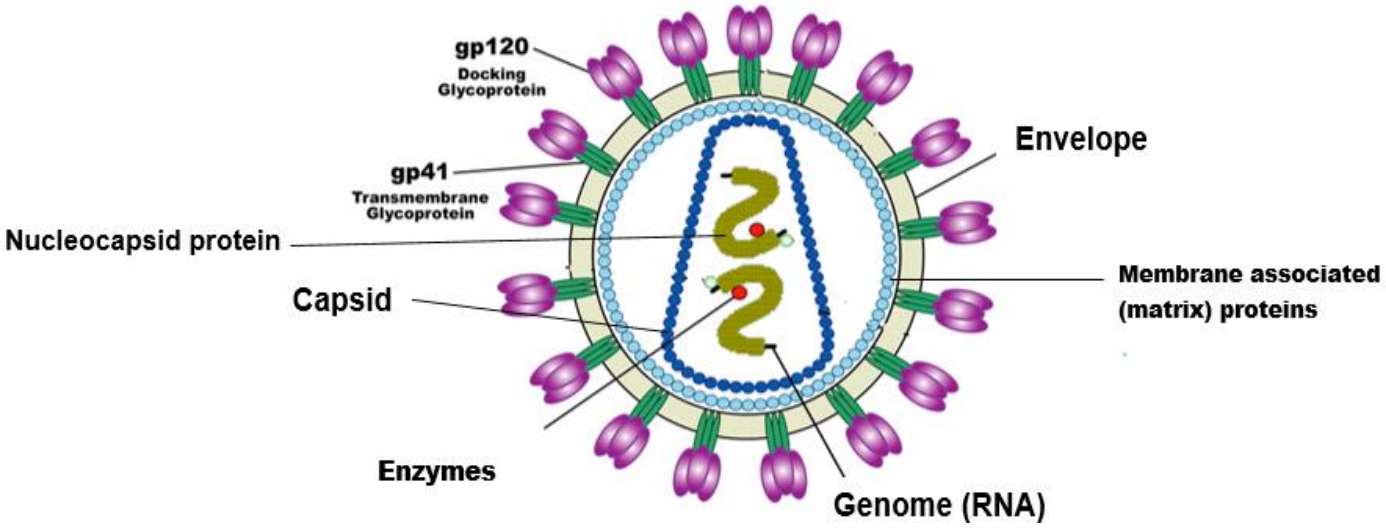


Fig. 17 Structure of HIV

The HIV virus is around 120 nm in diameter and roughly spherical.

A. Genome

- HIV-1 has **two copies** of **single-stranded RNA.**
- The two copies of single-stranded RNA are **positive strands**, i.e. the viral genome has same sequence as the viral mRNA.
- RNA is tightly bound to proteins, known as the nucleocapsid proteins. (Please note that the nucleoprotein in HIV is known as the nucleocapsid protein)

- The HIV genome contains three major genes, 5'gag-pol-env-3', encoding major structural proteins as well as essential enzymes.

These are synthesized as polyproteins which produce proteins for the virion:

- *Gag* codes for structural proteins (capsid, matrix and nucleocapsid protein)
- *Pol* codes for the viral enzymes (reverse transcriptase, integrase and HIV protease)
- *Env codes for* the glycoproteins gp120 and gp41

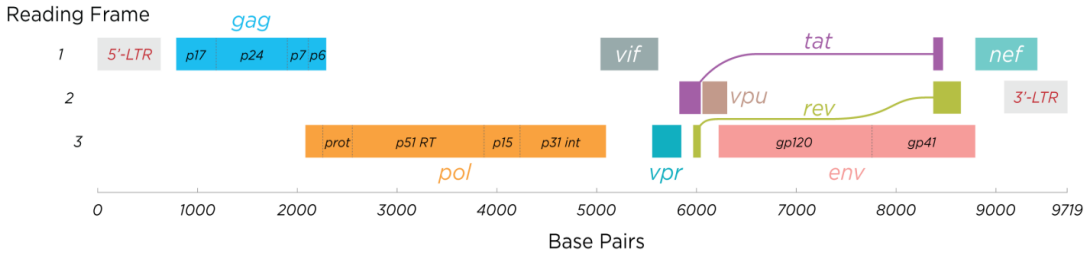


Fig. 18. Structure of the RNA genome in HIV

B. Capsid

- The **capsid** is usually **conical-shaped** and made of another type of proteins different from the nucleocapsid proteins.

- Within the **capsid** are two molecules of enzyme **reverse transcriptase.** The reverse transcriptase transcribes RNA (as template) into DNA. Two other enzymes contained within the **capsid** are **integrase** and **protease.**

- The **capsid** together with the viral genome forms the virus **core**.

C. Envelope

- The **capsid** is in turn surrounded by an envelope that is formed from part of the host cell plasma membrane.

- Through the envelope, glycoproteins protrude. These glycoproteins, **gp120 and gp41**, have a specific conformation that allows the virus to bind to certain receptors on T_4 helper cells.

Life Cycle of HIV

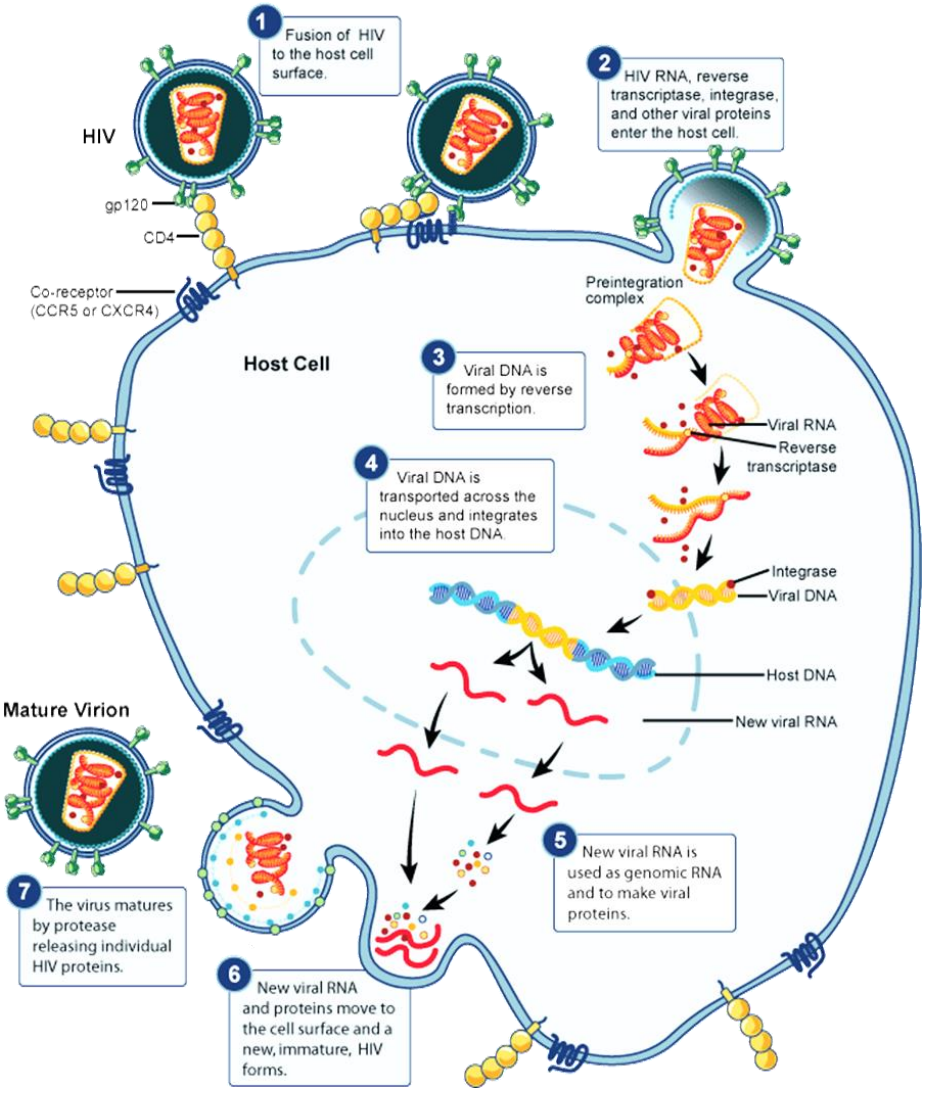


Fig. 19. Life cycle of HIV

A. Attachment

- The process typically begins when a viral particle comes into contact with a cell that carries on its surface a special protein called CD4. The glycoprotein, gp120 on the surface of the viral particle interacts with the CD4 on the target cell (T lymphocytes e.g. T_4 helper cells, macrophages), with the help of a co-receptor. (**Step 1**).

B. Penetration and Uncoating

- (**Step 2**) With the help of gp41, the viral envelope will fuse with the host cell membrane and the **capsid** is then released into the cell, leaving the envelope behind.

- The **capsid** and the **nucleocapsid protein** are then degraded, releasing viral enzymes and the RNA into the cytoplasm.

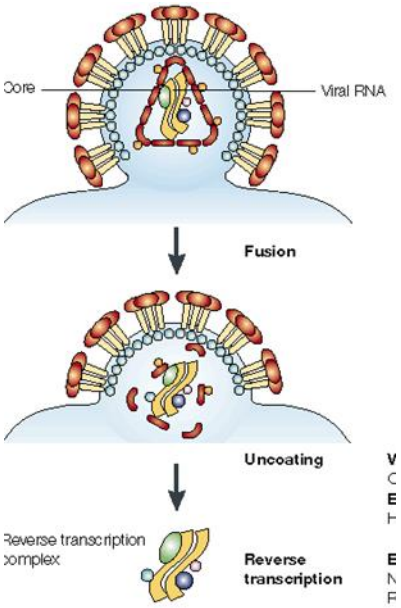


Fig. 19. Entry of HIV by fusion with host cell membrane followed by uncoating of the capsid and nucleocapsid to release the RNA genome

C. Replication

- (**Step 3**) The viral reverse transcriptase enzyme will then catalyse the conversion of the viral RNA into DNA. Reverse transcriptase will first catalyse synthesis of a DNA strand complementary to the viral RNA strand → form RNA-DNA hybrid.

- The RNA strand is degraded and a second DNA strand complementary to the first is synthesised to form a double-stranded DNA molecule.

- (**Step 4**) Viral DNA enters the host cell nucleus where it is integrated into the genetic material of the host. It is now known as a **provirus**. The enzyme integrase catalyses this process. Once viral DNA is integrated into the host genetic material, it may persist in its **latent** state for many years.

- Activation of the host cell will result in transcription of viral DNA into viral RNA which serves as the mRNA.

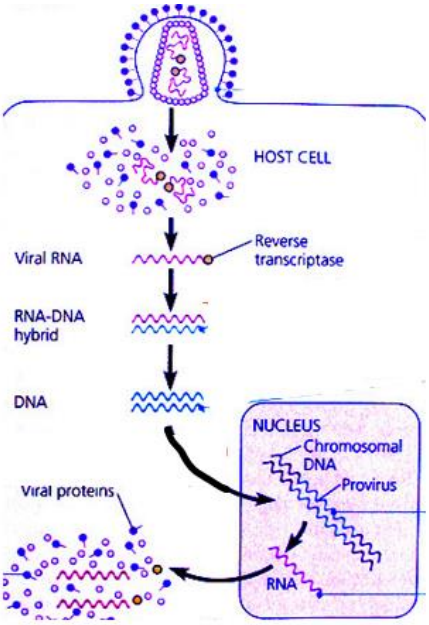


Fig. 20. Reverse transcription and integration into host genome by HIV virus.

- (**Step 5**) The mRNA exits the nucleus into the cytoplasm where it is translated into viral **polyproteins**.

- The envelope glycoproteins gp 120 and gp 41 are made in the ER and vesicles will transport them to the cell membrane. (The *env* polyprotein is cleaved by host cell protease in the ER)

- The viral RNA also forms the genetic material for the next generation of viruses.

D. Maturation

- (**Step 6**) Polyproteins and HIV genomic RNA assemble at the inner surface of the plasma membrane of the host cell.

E. Release

- After assembly at the plasma membrane, the virus **buds off/evaginates** from the cell is released.
- The viral envelope is derived from the host cell membrane containing gp41 and gp120.
- Polyproteins will be cleaved into the functional proteins by **HIV protease.**
- The functional proteins include structural proteins (matrix, capsid, nucleocapsid proteins) and viral enzymes (reverse transcriptase, integrase, HIV protease).
- The virion is now considered mature and ready to infect another cell.

Different modes of penetration for different enveloped viruses.

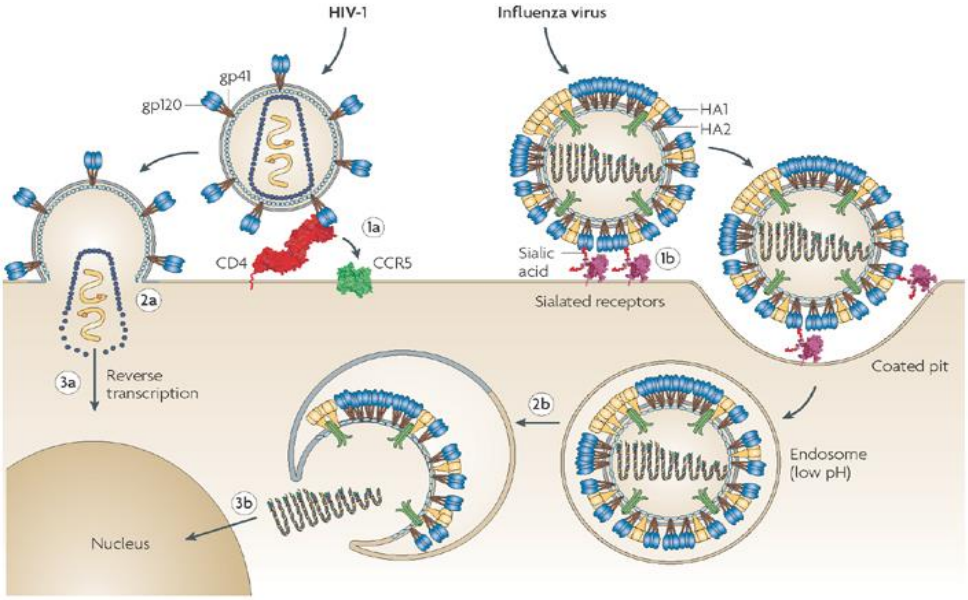


Fig. 21. Diagram showing entry of HIV virus via fusion of lipid membranes and entry of influenza virus via endocytosis.

Question:

What are the differences between multiplication of animal viruses and that of bacterial viruses?

Stage	***Bacteriophage***	***Animal Viruses***
Adsorption	Tail fibres attach to cell wall proteins	Attachment sites are plasma membrane proteins and glycoproteins.
Penetration	Viral DNA injected into host cell	**Capsid** enters by **endocytosis** or fusion
Uncoating	Not required	Enzymatic removal of capsid proteins
Genome replication	In cytoplasm	In nucleus (DNA viruses) or cytoplasm (R NA viruses with the exception of HIV and Influenza)
Release	Host cell lysed	Enveloped viruses **bud off;** non-enveloped viruses rupture the plasma membrane.

10. Variation in Viral Genomes by Antigenic Shift & Antigenic Drift

Antigenic Drift and Antigenic Shift

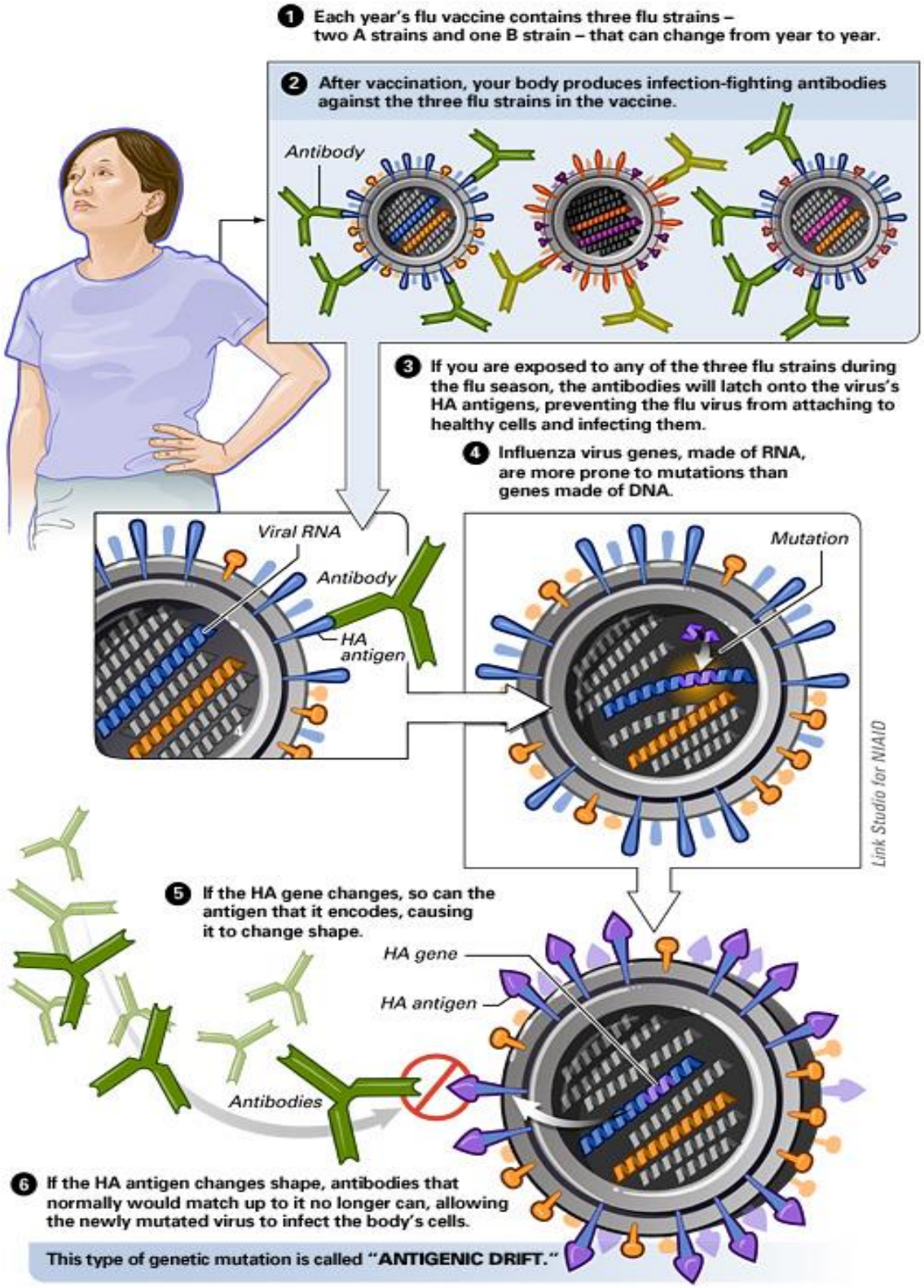
- Antigens are the specific molecular structures that antibodies and other receptors in our immune systems recognize.
- The immune system recognizes viruses when antigens on the surfaces of virus particles bind to immune receptors that are specific for these antigens.
- After an infection, the body produces many more of these virus-specific immune receptors, which prevent re-infection by this particular strain of the virus and produce acquired immunity.
- Similarly, a vaccine against a virus works by enabling the immune system to recognize the antigens exhibited by this virus.
- However, viral genomes are constantly mutating, producing new forms of these antigens. If one of these new forms of an antigen is different from the old antigen, it will no longer bind to the receptors. Viruses with these new antigens can evade immunity to the original strain of the virus. When such a change occurs, people who have had the infection in the past do not have immunity to the new virus and vaccines against the original virus will also become less effective.
- Two processes drive the antigens to change: **antigenic drift** and **antigenic shift**, antigenic drift being the more common.

Antigenic drift

- A mechanism of variation by viruses that involves the accumulation of **mutations** in the genes encoding the surface glycoproteins of the virus. The resulting viruses **have surface antigens or glycoproteins** that have a **different conformation** to the previous virus strain.
- Hence, the new virus strain cannot be recognised by antibodies against previous strains making it easier for them to infect the host and spread throughout a partially immune population.
- Antigenic drift occurs in both influenza A and influenza B viruses. In the influenza virus, the two relevant antigens are the surface proteins, **haemagglutinin** and neuraminidase.
- Sites recognized on the **haemagglutinin** and neuraminidase proteins by host immune systems are under constant selective pressure. Antigenic drift allows for evasion of these host immune systems by small mutations in the **haemagglutinin** and **neuraminidase** genes that make the protein unrecognizable to pre-existing host immunity.
- Antigenic drift is this continuous process of genetic and antigenic change among influenza strains as a result of the **lack of proof reading** ability of **RNA-dependent RNA polymerase** and the **fast/high rate of replication** of the virus.

From: http://en.wikipedia.org/wiki/Antigenic_drift,

Fig. 22. Antigenic Drift



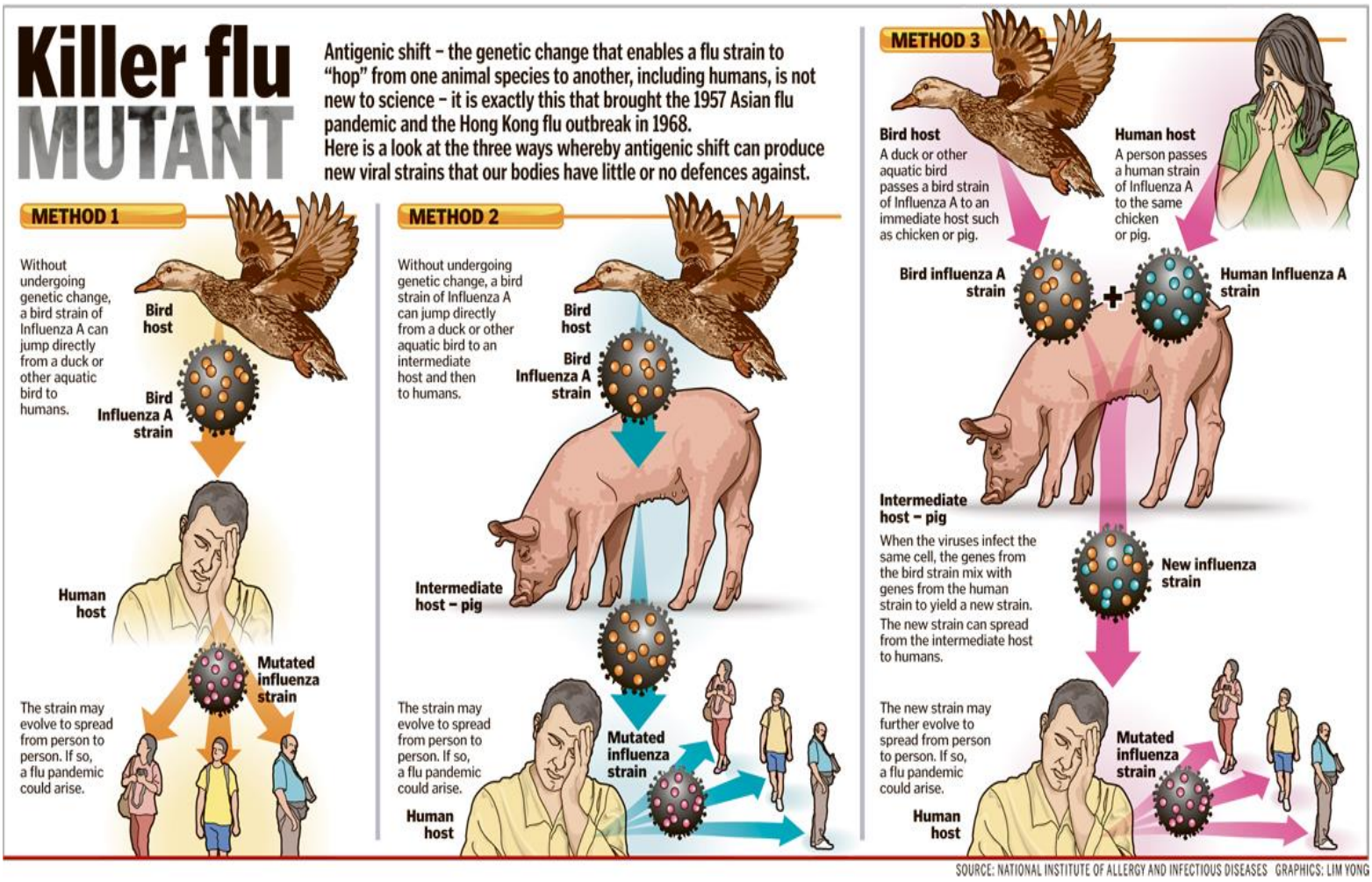
Antigenic shift

- A process whereby there is a sudden and **major change** in the **surface antigens** of a virus. The genetic change that enables a flu strain to jump from one animal species to another, including humans, is called antigenic shift.
- This occurs when **two or more different strains** of a virus, or strains of two or more different viruses, combine to form a **new subtype** having a mixture of the surface antigens of the two or more original strains (refer to method 3 in Fig. 23)
- Antigenic shift is a specific case of **genetic reassortment** that confers a phenotypic change.
- The term is often applied specifically to influenza, as that is the best-known example. Antigenic shift occurs mainly in influenza A. This is because influenza A viruses are found in many different animals, including ducks, chickens, pigs, humans, whales, horses, and seals.
- Antigenic shift occurs because the genome of the virus is segmented, allowing for major genetic changes of type by re-assortment of its segmented RNA genome.
- Flu strains are named after their types of **haemagglutinin** and neuraminidase surface proteins, so they will be called, for example, H3N2 for type-3 **haemagglutinin** and type-2 neuraminidase.
- When two different strains of influenza infect the **same cell** simultaneously, their protein capsids and lipid envelopes are removed, exposing their RNA, which is then transcribed to mRNA. The host cell then forms new viruses that combine their antigens; for example, H3N2 and H5N1 can form H5N2 this way.
- Because the human immune system has difficulty recognizing the new influenza strain, most people do not have pre-existing antibody protection to these novel viruses.
- The new strain may further **evolve** to spread from person to person. This could cause the formation of a highly virulent virus. If so, a flu pandemic could arise.

  Note:
  1. Refer to Fig. 23, Method 1 and 2 where infection jump from one species to another are also considered as antigenic shift.
  2. Influenza viruses which have undergone antigenic shift have caused the Asian Flu pandemic of 1957, the Hong Kong Flu pandemic of 1968, and the Swine Flu scare of 1976.

From: http://en.wikipedia.org/wiki/Antigenic_shift

Fig. 23. Antigenic Shift



Question:

What are the differences between antigenic shift and antigenic drift?

Feature	***Antigenic shift***	***Antigenic drift***
Change in antigen	Major Antigenic Change An antigenic change which results in drastic or dramatic alternation in HA (hemagglutinin) or NA (neuraminidase) subtypes.	Minor Antigenic Change An antigenic change can alter antigenic sites on the molecule such that a virion can escape recognition by the host's immune system.
New strain or subtype	Forming new sub-type (Subtype A + Subtype B –> New Subtype)	Forming new strain of virus
No. of type of virus involved	One or Two Viruses are Involved	Only one virus is involve
Host species	May jump from one species to another (animal-human)	Infect animals of the same species
Change in genome	Large change in nucleotides of RNA	Small mutation of RNA
Process that leads to change in genome	Occurs as a results of genome reassortment between difference subtypes	Occurs as a result of the accumulation of point mutations in the gene.
Frequency of occurrence	Occurs once in a time	Occurs frequently
Consequences	Give rise to pandemics, which occurs irregularly and unpredictably.	Usually responsible for epidemics in between pandemics.

11. Pathogenicity of Animal Viruses

(Will be revisited in topic of Infectious disease)

Pathogenicity of a virus refers to its ability to cause disease.

Most pathogenic viruses produce acute or asymptomatic infections that rapidly run their course and stimulate permanent immunity in survivors. This is due to the production of immune cells and antibodies that specifically recognise and inhibit subsequent infection by the same types of viruses. E.g. chickenpox, measles and mumps.

On the other hand, common colds may be caused by more than 100 distinct strains of the *rhinoviruses* and because immunity is specific, infection with one strain fails to induce host immunity to the other strains.

Some viruses escape elimination by the immune response by establishing **latent** (hidden) **infection**. These viruses remain in the host even after disease symptoms disappear and are generally undetectable during the latent periods. The disease may be periodically reactivated by various stimuli.

a. Influenza

- ***Pathogen:***
  - A **myxovirus**

- ***Target organ:***
  - Epithelial cells of the respiratory tract; virus binds to the sialic acid receptor found on the cell membrane.

- ***Symptoms:***
  - Body aches, headache, chills and fever, running nose. In more serious cases, influenza can cause pneumonia which can be fatal especially in young children and the elderly.

- ***The disease:***
  - Influenza is a respiratory disease in humans, with the infection usually localised in the respiratory tract.

  - Once the virus settles on the mucous membrane lining the nose, pharynx, trachea and bronchi, the neuraminidase enzyme on the surface of the viruses helps them to penetrate the mucoproteins in the mucus layer. The mucoproteins are glycoproteins in nature.

  - The haemagglutinin, a glycoprotein on the viral envelope, then helps the virus bind to specific receptors on cell membrane of the epithelial cell lining the respiratory tract. Eventually, the virus penetrates into these host cells. Once inside, the virus replicates within them.

  - The incubation period is around 24 to 48 hours, after which the infected epithelial cells are destroyed. These lead to inflammation and the buildup of dead epithelial cells in the airways cause the symptoms of influenza like running nose and scratchy throat to appear.

- Weakening of the epithelial layer caused by viral replication can make the respiratory passage more susceptible to secondary bacterial infections leading to diseases like pneumonia which can be fatal.

- ***Mode of transmission:***
  - Droplets of moisture from lungs of infected persons; from infected bird droppings

- ***Treatment***
  - There is no treatment for most people who develop influenza. However, bed rest, and perhaps the administration of aspirin or paracetamol to alleviate headaches and fever; is the only helpful means towards recovery.

  - Antibiotics are administered to prevent secondary bacterial infection like pneumonia.

  - Vaccinations against influenza are also sometimes administered. The influenza vaccine contains purified and inactivated material from the three common influenza viral strains.

  - Antiviral drugs such as oseltamivir (trade name Tamiflu) and zanamivir (trade name Relenza) are neuraminidase inhibitors that are designed to halt the spread of the virus in the body. However, these drugs are more suited towards the Influenza A and Influenza B strains. The antiviral drugs amantadine and rimantadine are designed to block a viral ion channel (M2 protein) and prevent the virus from infecting cells. These drugs are effective against Influenza A but not against Influenza B.

b. HIV

- ***Pathogen :***
  - Human immunodeficiency virus (HIV), which is a **retrovirus**

- ***Target organ :***
  - The immune system

- ***Mode of transmission***:
  - Transmitted primarily through unprotected sexual contact or by exposure to infected blood and blood products.

  - It can also be transmitted from mother to child either via the placenta, childbirth or during breastfeeding.

- ***The disease***
  - Once the virus enters the blood stream, its primary targets are macrophages (a large phagocytic cell) and T helper cells (a type of lymphocyte responsible for the coordination of an immune response to infection).

  - The HIV virus has a very strong affinity for and binds to CD4, a surface protein of T helper cells. As the HIV infects more and more T helper cells, levels of T helper cells lower as the infected cells are destroyed.

- The macrophages may survive HIV infection (because they are not lysed by the virus) and may thus act as reservoirs.

- HIV may be passed from cell to cell in an infected individual, or it may be transmitted via body fluids to another person, while still remaining undetected.

- The virus mutates at a very high rate during replication resulting in altered proteins on the surface of the virus. In this way, the virus prevents recognition and elimination by the immune system allowing it to evolve rapidly within the body.

- The increasing loss of T helper cells leads to impaired immune responses in the affected individual who then becomes increasingly susceptible to opportunistic diseases. 'Full-blown AIDS' occurs when the infections become unmanageable. The immuno-suppression becomes worse, usually with fatal results. Death usually results from secondary infections.

- ***Treatment***
  - Due to the high rates of virus productions and mutation rate of the virus, treatment of HIV infection generally includes administration of 3 agents in combination. Sustained treatment results in suppression of viral replication, dramatically increasing life expectancy of HIV-infected individuals.

  - Currently, there are 24 approved retroviral drugs which can be used to treat HIV infection. They include reverse transcriptase inhibitors, protease inhibitors, integrase inhibitors and entry inhibitors. The first three enzyme inhibitors work by inhibiting their respective enzymes. Entry inhibitors work by blocking interaction between the HIV envelope and CD4 or the co-receptor, or by preventing fusion of the viral and host cell membranes, thus blocking entry of HIV into the cells.

c. Pathogenesis of other viruses

Infection of cells by a virus can alter the host cell in various ways resulting in disease in the affected individual. Here are some of the ways in which viruses cause disease:

1. Death of host cell

When a virus enters a cell, it can incur temporary or permanent changes to the host cell. In bacteria viruses, it usually ends with lysis of the bacterium. Cell respond to the presence of viral DNA in animal cells may result in apoptosis of the cell.

2. Production of toxic substances

During the course of virus replication, many viral components as well as by-products of viral replication accumulate in the cell. These are often cytotoxic. The molecular mechanism of these toxins is not known in most cases.

Some examples of toxin effects include:

i. the Herpesvirus components produce syncytia – a multi-nucleated protoplasmic mass that is not viable and eventually dies.
ii. Cytotoxicity of preformed viral parts. e.g., Sendoi virus, Newcastle disease virus, measles virus and SV5 produce rapid polykaryocytosis (fusion of chromosomes).
iii. The antigen of the adenovirus **capsid** inhibits RNA, DNA and protein synthesis.

3. Cell transformation

Certain viruses have the ability to enter a cell and follow one of two alternative courses. They either multiply in a normal manner and are eventually released from the cell, or they may be dormant in the cell and eventually transform the cell into a cancerous cell. The growth and division of normal cells is regulated by proto-oncogenes and tumour suppressor genes. Changes in expression of either gene will lead to the uncontrolled cell division and growth causing tumour formation. Tumours can be either benign or malignant. Malignant tumours are able to travel to other parts of the body and invade tissues and organs. Viruses usually cause genetic changes which affect the proto-oncogenes or the tumour suppressor genes resulting in cancer. For example, some retroviruses activate the proto-oncogenes to oncogenes (a gene that causes tumour formation).

4. Suppression of immune mechanisms

Since many viruses are known to replicate in cells of the lymphatic system, it is possible that these viruses can affect the immune system. The nature and extent of the immunologic alteration depends on the organ or cell type infected and the species of virus causing the infection. Example, HIV.

5. Induction of non-normal host-specified products

Virus-infected cells, at times, will produce compounds coded for by the host DNA, but which are not normally produced by the host. These are often cytotoxic at relatively high concentrations. Other host compounds which are normally found in low concentration may be produced in higher concentration during a virus infection. Again, this high concentration may be cytotoxic. Some virus-induced products release autolytic enzymes from the cells own lysosomes.

6. Induction of structural alterations to the host cell

Viruses can induce structural alterations in the host cell's cytoplasm and nucleus.

1. Cytoplasmic changes

(a) Small non-enveloped RNA viruses produce a large eosinophilic mass which displaces the nucleus. There is a generalized increase in basophilia. The cytoplasm appears to bubble at the cell periphery.

(b) Herpesvirus causes vacuolization.

2. Nuclear changes

(a) Nuclear inclusion (bodies in the nucleus); e.g., herpesvirus, adenovirus.
(b) Margination and coarsening of chromatin; e.g. herpesvirus, poxvirus.
(c) Formation of chromosomal breaks.

3. Membrane changes

(a) The human cell membrane is a dynamic structure continually changing in lipid and protein content during normal cellular growth and division. Viral infection of the cell often results in viral protein being incorporated into this membrane. These changes can lead to production of antibodies against the cell membrane and lysis of this membrane.

In summary, we get sick from viral infections because:

1) The infected cells die as a direct or indirect result of the virus,
2) The viral products or components can cause an immune response e.g. fever, allergy, etc.
3) The virus can suppress the immune system increasing the susceptibility to secondary infections.
4) Viruses can cause cancer.

Antiviral Drugs

- Chemically resembles nucleosides (e.g. AZT)
- Interferes with the viral nucleic acid synthesis
- E.g. acyclovir – inhibits viral polymerase (disrupts viral DNA synthesis) in herpes virus
- azidothymidine (AZT) inhibits the enzyme reverse transcriptase in HIV, preventing the production of a DNA copy of the virus RNA genome.

12. Links

Topics	Link to
T4 life cycle lambda bacteriophage life cycle	Transduction in Bacteria
Retrovirus or other virus that integrate into host cell genome	Cancer
HIV, influenza	Infectious diseases
Antigenic shift and drift	Evolution, Mutation
Enveloped virus glycoprotein synthesis	Protein synthesis through endomembrane system
Viral dengue disease	Impact of Climate Change

13. Glossary

Antigen – any substance capable of inducing a specific immune response and of reacting with the products of that response, i.e., with specific antibody or specifically sensitized T lymphocytes, or both

Antigenic drift – Minor changes in the structure and immunogenicity of antigens, specifically surface proteins of influenza virus, caused by mutation.

Antigenic shift – major changes in the structure and immunogenicity of the surface proteins of influenza virus caused by gene exchange with related viruses.

Bacteriophage - A virus that parasitizes a bacterium by infecting it and reproducing inside it.

Budding - is a form of **viral** shedding by which enveloped **viruses** acquire their external envelope from the host cell membrane, which bulges outwards and encloses the virion

Capsid - the protein coat or shell of a viral particle, surrounding the nucleic acid or nucleoprotein core

Capsomeres – the protein components of **capsid**

Genome - the total genetic content contained in a haploid set of chromosomes in eukaryotes, in a single chromosome in bacteria, or in the DNA or RNA of viruses

Integrase - a viral enzyme that enables the integration of viral genetic material into a host cell's DNA. Usually refer to retroviral integrase (IN) in HIV but should not confuse with phage integrases, such as λ phage integrase (Int).

Lysogenic cycle – a type of phage replicative cycle in which the viral genome becomes incorporated into the bacterial host chromosome as a **prophage**, is replicated along the chromosome, and dose not kill the host.

Lytic cycle - a type of phage replicative cycle resulting in the release of new phages by lysis of the host cell.

Nucleocapsid – nucleic acid plus capsid. A complex of proteins and the viral genomic nucleic acid.

Nucleoprotein - A complex consisting of a nucleic acid bonded to a protein

Prophage - The genetic material of a bacteriophage, incorporated into the genome of a bacterium and able to produce phages if specifically activated

Provirus - The viral genome that is incorporated into, and able to replicate with, the genome of a host cell

Retrovirus - A retrovirus is an RNA virus that is duplicated in a host cell using the reverse transcriptase enzyme to produce DNA from its RNA genome

Reverse transcription - DNA synthesis from RNA templates, catalysed by the enzyme reverse transcriptase; the opposite of transcription. Occurs naturally in retroviruses.

Reverse transcriptase - a **reverse transcriptase**, also known as **RNA-dependent DNA polymerase**, is a DNA polymerase enzyme that transcribes single-stranded RNA into single-stranded DNA**.**

Virion - The complete, infective form of a virus outside a host cell, with a core of RNA or DNA and a capsid

Vaccine – a harmless variant or derivative of a pathogen that stimulates the host's immune system to mount defenses against the pathogen**.**