CORE IDEA 2: GENETICS AND INHERITANCE
TOPIC 2.10: Genetics of Virus

H2

Learning Outcomes:

You should be able to:

Core Idea 1

(e) describe the structural components of viruses, including enveloped viruses and bacteriophages, and interpret drawings and photographs of them

(f) discuss how viruses challenge the cell theory and concepts of what is considered living

Core Idea 2

(d) [modifed] describe the structure and organisation of viral genomes (including DNA/RNA, single-/double-stranded, number of nucleotides, packing of DNA, linearity/circularity and presence/absence of introns)

(e) describe how the genomes of viruses are inherited through outlining the reproductive cycles of:
- i. bacteriophages that reproduce via a lytic cycle only, including T4 phage
- ii. bacteriophages that reproduce via lytic and lysogenic cycles, including lambda phage
- iii. enveloped viruses, including influenza virus
- iv. retroviruses, including HIV

(f) describe how variation in viral genomes arises, including antigenic shift and antigenic drift

LECTURE OUTLINE

Learning Outcome 1(f):

discuss how viruses challenge the cell theory and concepts of what is considered living

1 Nature of a virus particle

1.1 Are viruses alive?

- Viruses are small infectious particles that can only replicate in their host cells.
- These particles have a simple acellular organisation, consisting of viral genetic material (either DNA or RNA) enclosed in a protein coat called capsid (and a membranous envelope in some viruses).
- Viruses are considered to exist at the border between chemistry and life, because they posses both living and non-living characteristics.
- They display properties of living organisms only inside their host cells (intracellular phase) and are non-living outside their host cells (extracellular phase).
    - **Living characteristics of viruses:**
        - They are able to **reproduce** (though not independently).
        - They **possess nucleic acids** e.g. DNA or RNA that encode information for the reproduction of new viruses with the same characteristics as themselves.
        - They are able to **obtain and use energy** from host, but cannot generate or store energy in the form of ATP.
        - They are able to **respond to the environment** such as radiation, chemicals and heat when inside the host cell.
        - They are able to **evolve and mutate**. It was deduced that since viruses can reproduce only within cells, they probably evolved as bits of cellular nucleic acid.
    - **Non-living characteristics of viruses:**
        - Viruses are **acellular**. They lack cytoplasm, all metabolic machinery (e.g enzymes required for metabolism) and cellular organelles (eg. nucleus, mitochondria, ribosomes, etc.) hence
        - **cannot undergo metabolism** (eg. cellular respiration, synthesis of organic compounds).
        - **cannot replicate independently.** Viruses are dependent on a compatible host cell's metabolic machinery to synthesise new viral components for replication.

1.2 Viruses are obligate intracellular parasites

A virus is a sub-microscopic infectious agent that cannot replicate independently of a living cell, which is called a host cell.

Viruses are therefore **obligate intracellular parasites** that must rely on entering a suitable living cell i.e. infecting a host cell, to carry out their replication cycle and thus multiply.

- Upon infecting a host cell, the virus exploits the energy resources, raw materials and metabolic machinery of the host cell.
- The virus redirects host metabolic functions towards supporting its replication cycle via the assembly of new viral particles.
- Eventually, the new viral particles are released, and the process can repeat itself as the infection spreads.

Although viruses can only replicate after infecting a host cell, they have an extracellular form, known as the viral particle or virion that enables them to exist outside the host for long periods, and that facilitates their transmission from one host cell to another.

Viral particles can be passed from host to host either through direct contact (e.g. body fluids) or through a vector or carrier (e.g. dengue is transmitted by Aedes mosquito)

1.3 Are viruses cells?

Viruses were neither considered cells nor organisms as many characteristics of viruses are in contradiction with the cell theory.

Characteristics of viruses that defy the cell theory

Cell theory	How viruses defy the cell theory
1. Cells are the smallest unit of life.	Viruses are much smaller than cells and once thought to be the most basic unit of life. However, whether viruses can be considered living is questionable, as they do not fulfil ALL characteristics of life.
2. All cells come from pre-existing cells.	Viruses do not originate from pre-existing viruses; they cannot replicate independently outside a host cell.
3. All living organisms are composed of cells.	The basic structure of viruses consists of the viral genome enclosed in a capsid. Viruses do not possess metabolic machinery or cellular organelles, hence are considered acellular.

1.4 Conclusion on whether viruses are living

The general consensus of the scientific community is that viruses are **non-living**, best described as **obligate intracellular parasites**.

Viruses exhibit **host specificity**. They infect specific host cells by a "lock and key" fit between viral surface proteins and specific receptor molecules on the host cell surface.

They can be classified based on the hosts they infect.

- Viruses that infect only bacteria are called **bacteriophages**.
- Viruses that infect only animals are called **animal viruses**

Learning Outcome 1(e):

describe the structural components of viruses, including enveloped viruses and bacteriophages, and interpret drawings and photographs of them

2 Structure of a Virus particle

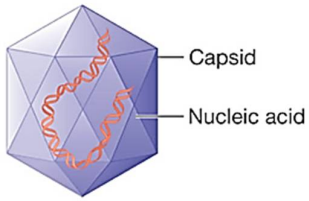
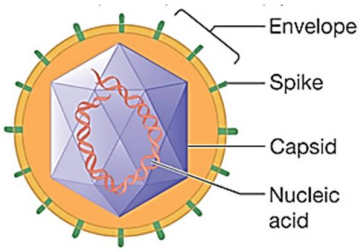
All viruses contain the following two components:

i) **a nucleic acid genome** and

ii) a protein coat called a **capsid** that encloses the genome.

Nucleocapsid compromises of both the viral genome and capsid. An infectious viral particle, a **virion**, will contain a nucleocapsid at least.

There are two main classifications of viruses

Naked Virus / Non-enveloped viruses	Enveloped Viruses
Viruses without an envelope are known as **naked viruses** or **non-enveloped viruses**.	Viruses with a membranous envelope surrounding the nucleocapsid are known as **enveloped viruses.**
Viral genome + Capsid = Nucleocapsid = Naked viruses	Viral genome + Capsid + Envelope = Enveloped viruses
 **(a)** Naked virus	 **(b)** Enveloped virus

Some viruses may also contain **viral enzymes**.

The structure and composition of these components can vary widely among different viruses.

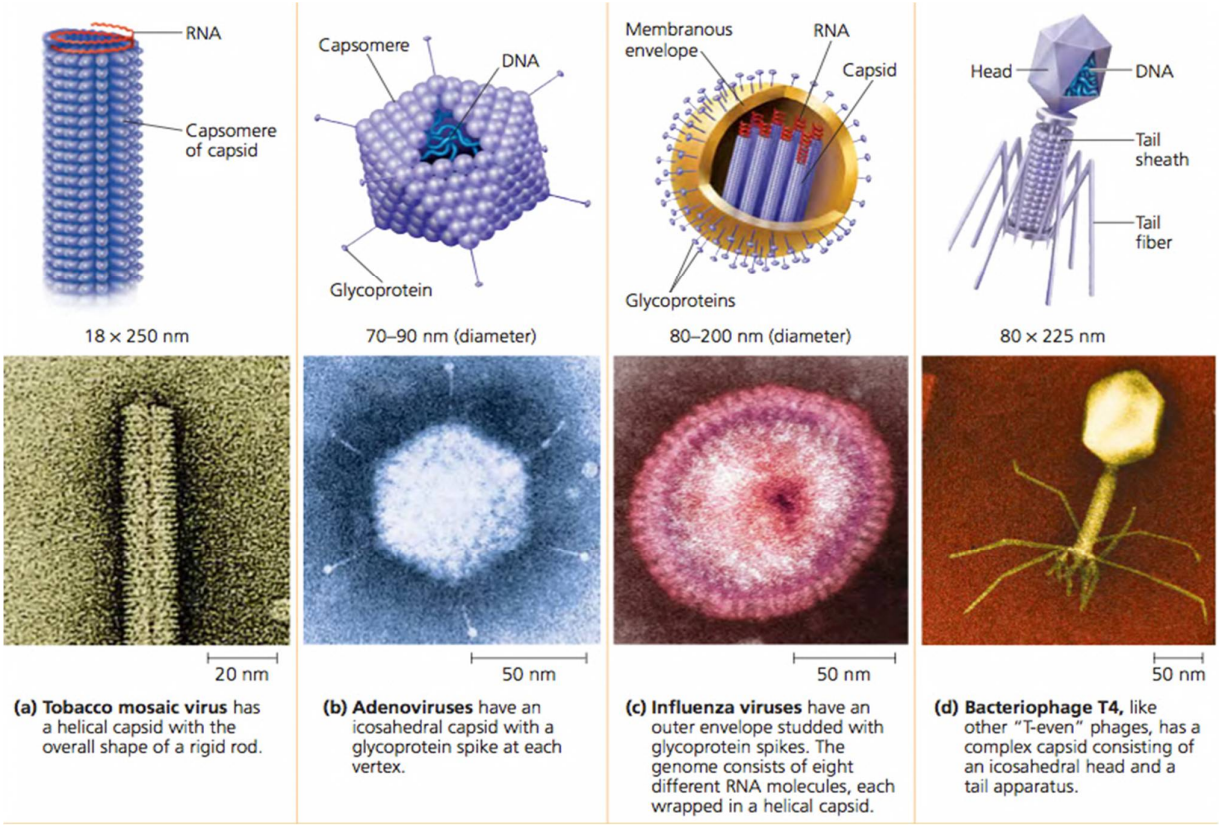


Fig. 2: Diverse structures of viruses

Learning Outcome 2(d) modified:

Describe the structure and organisation of viral genomes
(including DNA/RNA, single- / double-stranded, number of nucleotides, packing of DNA, linearity/circularity and presence/absence of introns)

2.1 Genome

Viral genomes vary among <u>different</u> viruses and may be:

1. **DNA** or **RNA** but never at the same time. Hence, viruses can be classified as **<u>DNA viruses</u>** or **<u>RNA viruses</u>** according to the type of nucleic acid that makes up its genome.
2. **Single-stranded (ss)** or **double-stranded (ds)**
3. **Linear** or **circular**
4. Made up of a **single** molecule or **segmented** into multiple nucleic acid molecules
5. **RNA viral genomes** can be either **positive-sense (+)** or **negative-sense (-)**
    - **(+) RNA strand** serve as viral mRNA and <u>can be translated directly into viral proteins</u> within the infected host cell. (identical to viral mRNA)
    - **(-) RNA strand** <u>cannot be translated directly into viral proteins</u> within the infected host cell. The (-) RNA strand must be <u>first be transcribed into (+) RNA</u> strand prior to translation. (complementary to viral mRNA)

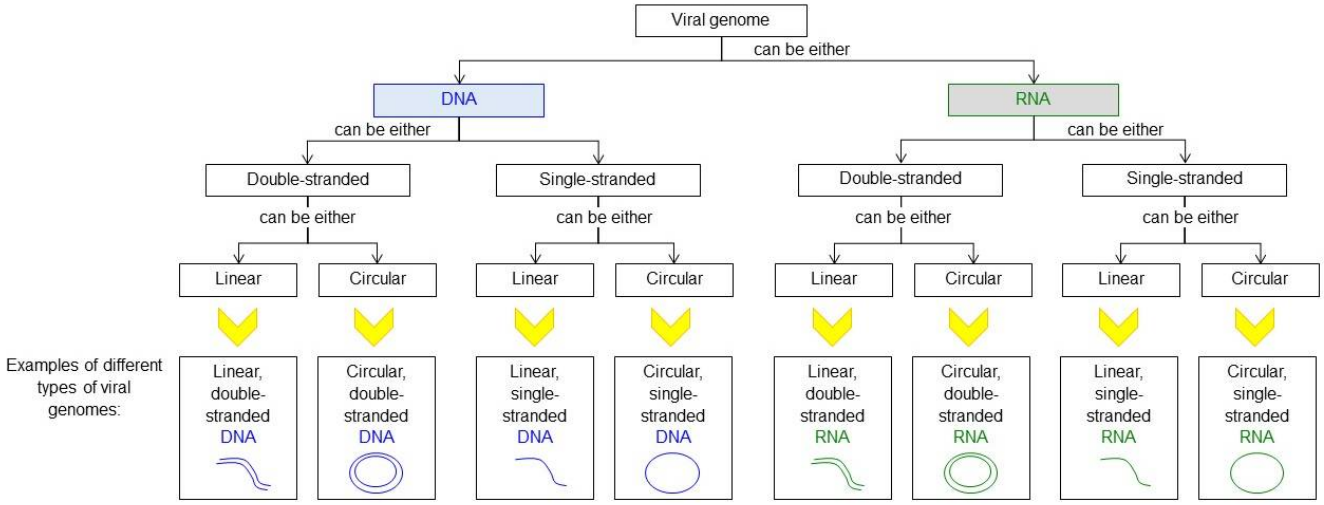


Fig. 2.1.1: Types of viral genomes

6. Genes in the viruses may be organised into coding regions (exons) and non-coding regions (introns).

<u>Function of the viral genome:</u>

The genome codes for the synthesis of <u>viral proteins and enzymes</u> during viral replication.

2.2 Capsid

- **Capsid** is the protein coat that encloses the nucleic acid genome of a virus.
- Capsids are comprised of protein subunits called **capsomeres**. The structure of capsomeres depends on the viral genome that encodes them.
- Depending on how capsomeres are arranged, the capsid can vary in shape. The two most common kinds of capsid symmetry are:
    - **Helical**: A hollow cylinder made up of capsomeres, forming a coil around the nucleic acid (e.g. tobacco mosaic virus).
    - **Icosahedral**: A regular polyhedron with 20 triangular facets, known as an icosahedron (e.g. adenovirus).

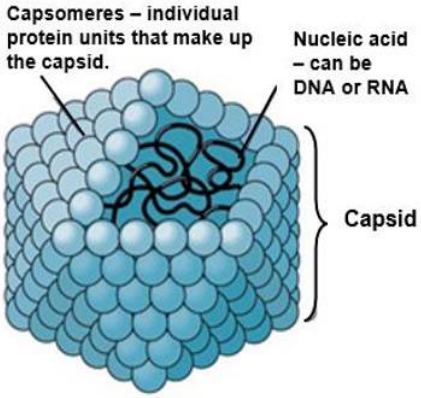


Fig. 2.2.1 (Far left): Diagram of a viral capsid surrounding its genome forming a nucleocapsid

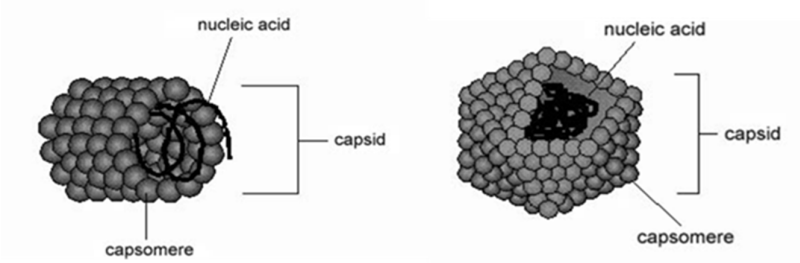


Fig. 2.2.2: Capsid symmetry - helical capsid (left) and icosahedral capsid (right)

Functions of capsid:

- **Protection**: The capsid protects the viral genome from degradation by nucleases.
- **Attachment**: For naked viruses, the capsid contains special sites on its surface that allow the virus to attach to a host cell.
- **Penetration**: The capsid may contain proteins that enable the virus to penetrate the host cell surface membrane.

2.3 Envelope

- Some viruses have an envelope that surrounds the nucleocapsid and are thus classified as **enveloped viruses**.
- The **viral envelope** is composed of phospholipids and glycoproteins that are arranged to form a lipid bilayer. The viral envelope is usually derived from the host cell surface membrane by a process called **budding**.
- Although the envelope is usually of host cell origin, viral proteins are often incorporated into the envelope, appearing as glycoprotein spikes.

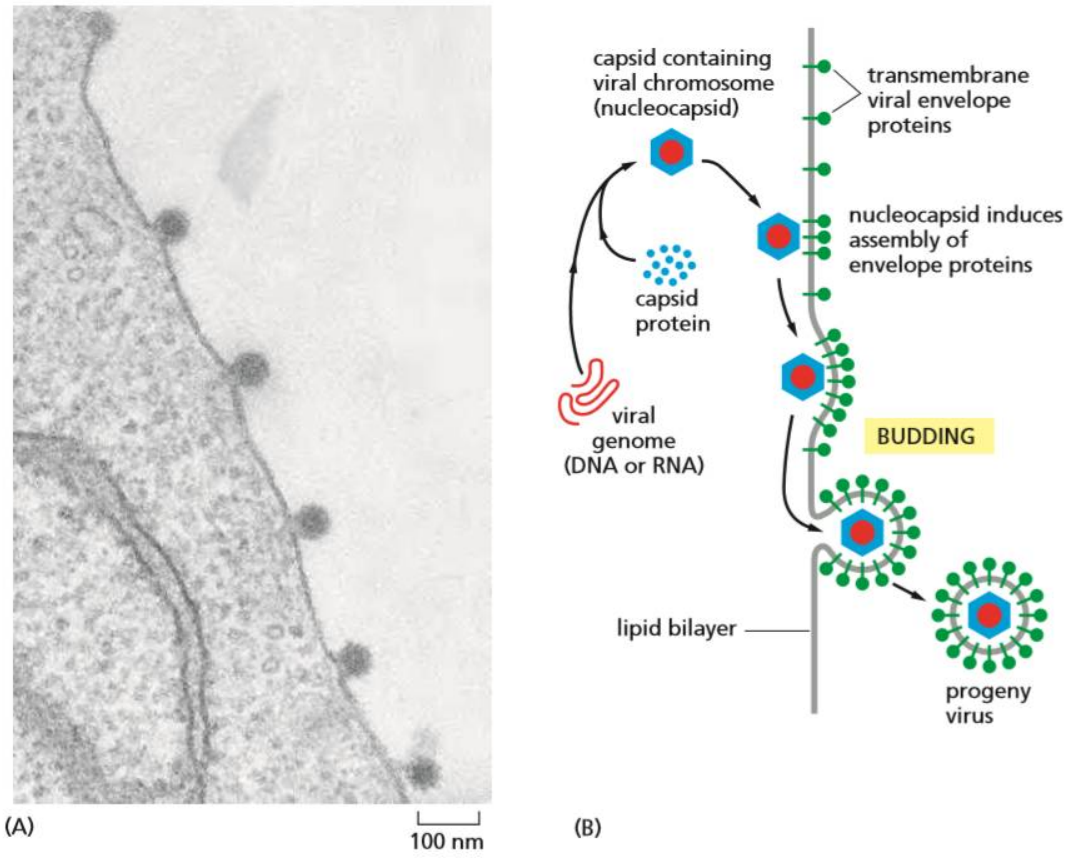


Fig. 2.3.1: (A) Newly replicated viruses budding off from host cell. (B) Assembly of viral components at the host cell surface membrane before budding.

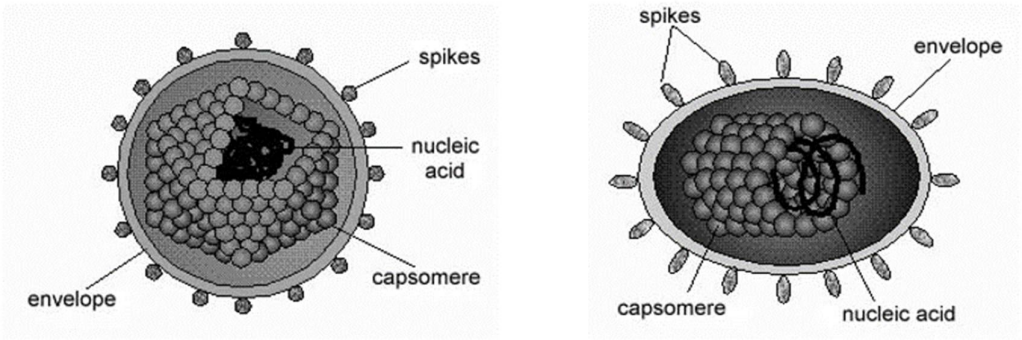


Fig 2.3.2: Enveloped viruses with icosahedral (left) and helical (right) capsid symmetry.

<u>Function of envelope:</u>

Attachment: For enveloped viruses, the glycoprotein spikes bind to specific receptors on the host cell surface membrane, allowing attachment of the virus to its host.

Release: Release of new viral particles from the host cell after replication

2.4 Viral Enzymes

- In some viruses, viral-specific enzymes may be found inside the capsid. These enzymes are coded for by the viral genome.
- Some examples of viral enzymes and their corresponding functions are shown below:

Virus	Enzyme	Function
Bacteriophages (e.g. T4 bacteriophage)	Lysozyme	Breaks down peptidoglycan cell wall of bacteria to facilitate viral entry and exit. ***(KIV: Lytic cycle of T4 phage)***
RNA viruses (e.g. influenza virus)	RNA-dependent RNA polymerase	Synthesises a complementary **(+) RNA strand** from the **(-) RNA viral genome**. ***(KIV: Influenza reproductive cycle)***
Retroviruses (e.g. HIV)	Reverse transcriptase	**Transcribes** viral RNA to form a complementary DNA strand. ***(KIV: HIV reproductive cycle)***

Function of viral enzymes:

- They play important roles during the life cycle of viruses.

CHECKPOINT (1) – Structure of Viruses

Match the following viral structure to its respective description.

Receptor	•	• Structure comprising viral genetic material surrounded by a protein coat
Capsid	•	• A lipid bilayer studded with glycoproteins which may help viral entry into host cell
Glycoprotein spikes	•	• Highly specific molecules located on surface of host cell
Envelope	•	• Genetic material encoding viral proteins needed for viral replication and infection
Nucleocapsid	•	• Recognise and attach to specific receptor on host cell
Genome	•	• Made up of protein subunits called capsomeres, and surrounds the viral genome

3 General features of viral reproductive cycle

For a virus to replicate, it must induce a living host cell to synthesis all the essential components needed to make more viral particles. These components must then be assembled into new viral particles that escape from the cell.

1	Attachment	Attachment of viral particle to a specific host cell
2	Penetration	Penetration of viral particle or its nucleic acid into the cell
3	Synthesis of viral nucleic acid and protein	Synthesis of viral nucleic acid and protein by host cell machinery as directed by the virus. The virus uses host cell's nucleotides, enzymes, ribosomes, tRNAs, amino acids, ATP and other components for such synthesis.
4	Maturation	Maturation invovles the assembly of capsids and packaging of viral genomes into new viral particles.
5	Release	Lysis of cell allows for the release of mature viral particles from the cell

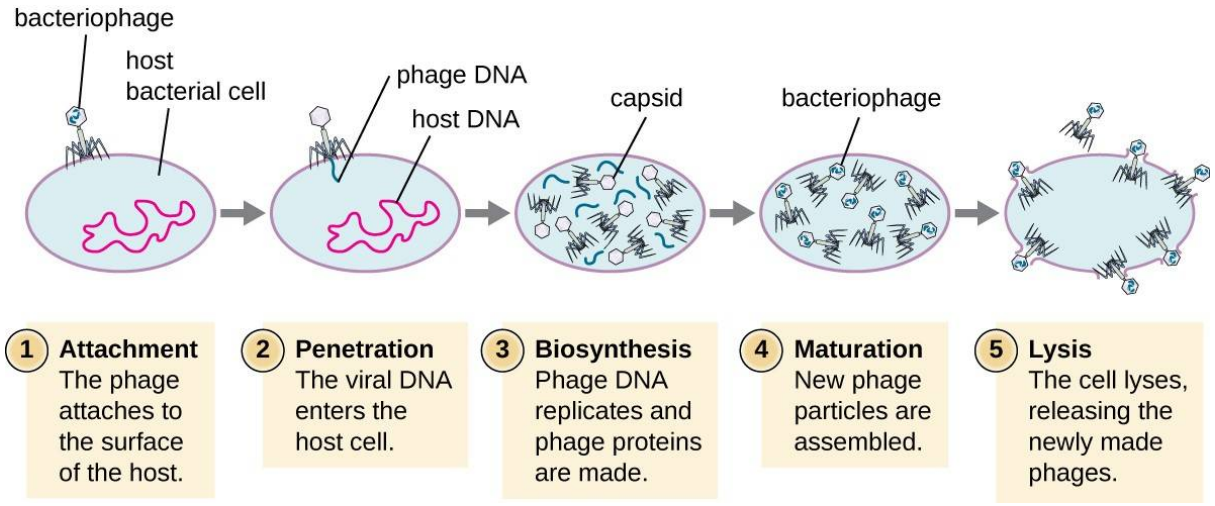


Fig. 3.1 General viral reproductive cycle of naked virus

Learning Outcome 2(e)(i):

Describe how the genomes of viruses are inherited through outlining the reproductive cycles of bacteriophages that reproduce via a lytic cycle only, including T4 phage.

4 Structure and Reproductive cycle of virulent bacteriophages e.g. T4 phage

Virus	T4 bacteriophage
Host	*Escherichia coli* (*E. coli*).
Capsid	Complex capsid with an elongated icosahedral head
Genome	one linear double-stranded DNA
Structure	Attached to the head is a tail apparatus that facilitates attachment to and penetration of a host bacterial cell. The tail apparatus comprises a tail sheath, baseplate (with baseplate pins) and tail fibres.
Reproductive cycle	**Lytic cycle** • This culminates in the lysis of the host bacterium during the release of new phages, leading to death of the host cell. • A phage that can reproduce only by a lytic cycle is known as a lytic/ virulent phage

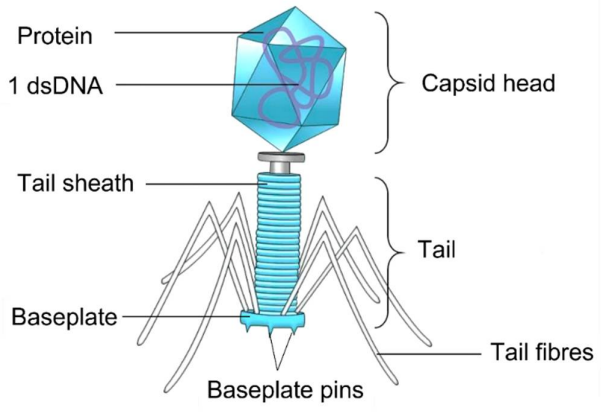


Fig. 4 (a): Structure of a T4 phage

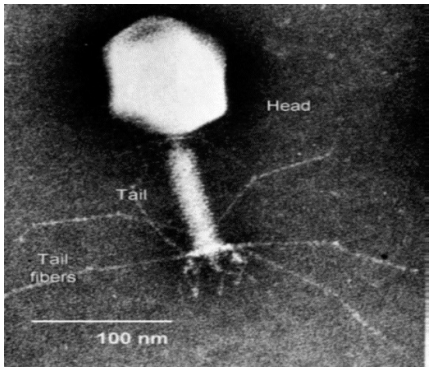
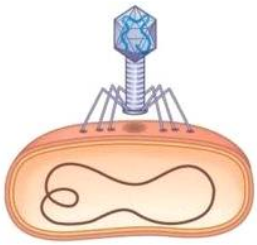
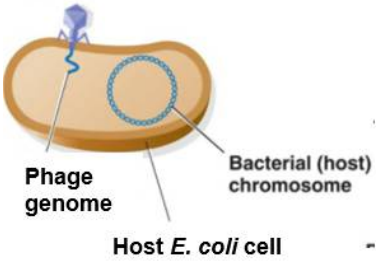
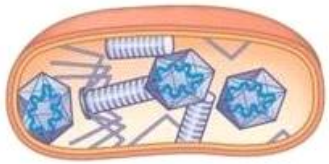
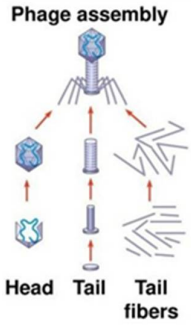
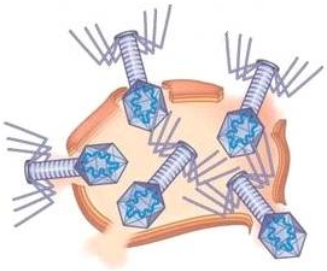


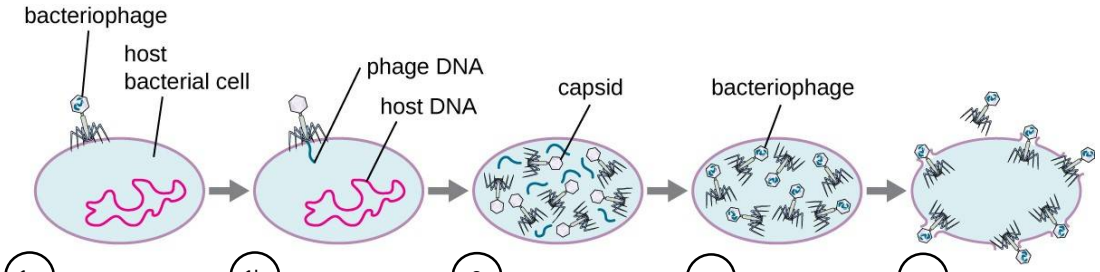
Fig. 4 (b): Electron micrograph of a T4 phage

4.1 The Lytic Reproductive Cycle

	Stage	Description
1	**Attachment** 	• The phage tail fibres bind to specific receptors on host bacterium outer cell surface membrane.
2	**Penetration** 	• The bacteriophage **injects** its DNA genome into the host cell, leaving an empty capsid outside.
3	**Biosynthesis of viral genomes and proteins** 	• The T4 phage DNA directs the metabolic machinery (e.g. ribosomes, DNA polymerase, RNA polymerase) and raw materials (e.g. amino acids, ATP, nucleotides) of the host cell towards o Synthesising phage proteins (e.g. lysozyme) o Making new copies of phage genome
4	**Maturation** 	• Phage components self-assemble into complete viruses • Phage DNA is packaged inside the capsid as the head forms, forming the nucleocapsid. 
4	**Release** 	• Phage lysozyme breaks down the peptidoglycan cell wall, causing fluid to enter the bacterial cell. • The cell thus undergoes osmotic lysis, whereby it swells and bursts. • Newly synthesised T4 phages are released into the extracellular environment, and each can now infect a new host cell.

CHECKPOINT (2) – Reproductive Cycle of T4 Bacteriophage

Fill in the names of the stages in the lytic cycle



1a The phage attaches to the surface of the host.

1b The viral DNA enters the host cell.

2 Phage DNA replicates and phage proteins are made.

3 New phage particles are assembled.

4 The cell lyses, releasing the newly made phages.

Learning Outcome 2(e)(ii):

describe how the genomes of viruses are inherited through outlining the reproductive cycles of bacteriophages that reproduce via lytic and lysogenic cycles, including lambda phage

5 Structure & Reproductive cycle of temperate bacteriophages e.g. Lambda phage

Virus	Lambda(λ) bacteriophage
Host	*Escherichia coli* (*E. coli*).
Capsid	Complex capsid with an elongated icosahedral head
Genome	one linear double-stranded DNA
Structure	Resembles the T4 phage, but its tail contain only one short tail fibre
Reproductive cycle	**Lysogenic** or Lytic Cycle • Unlike the lytic cycle that kills the host cell, the lysogenic cycle replicates the phage genome without destroying the host cell. • In the lysogenic cycle, the phage genome becomes integrated into the host chromosome and is called a **prophage**. • A phage undergoes either the lytic or lysogenic cycle depending on various factors (e.g. host cell's physiological status). • A phage which undergo both the lytic and the lysogenic cycles is known as a lysogenic/ temperate phage.

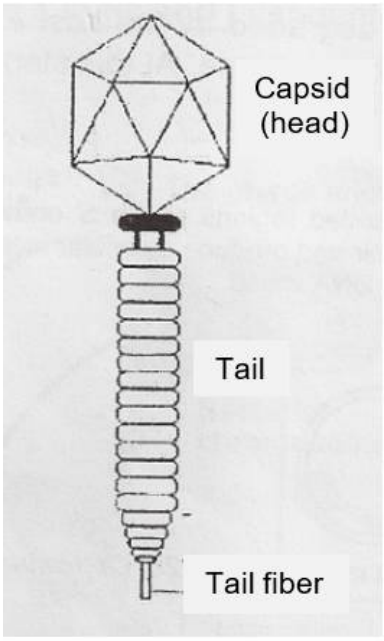


Fig. 5 (a): Structure of a λ phage

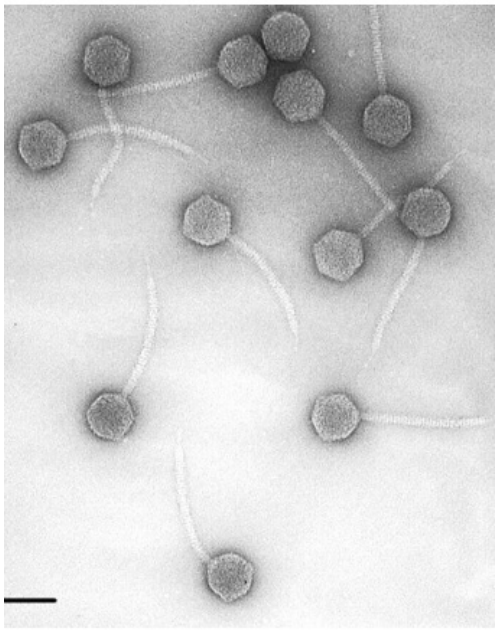
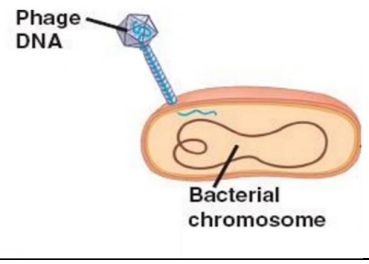
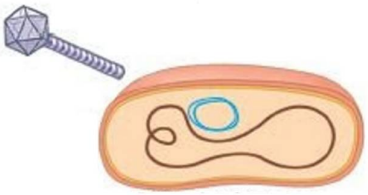
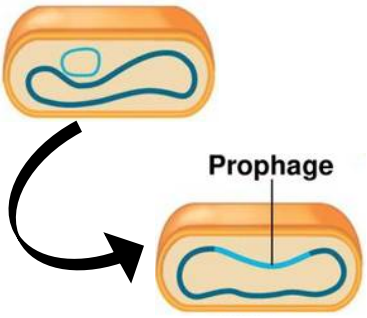
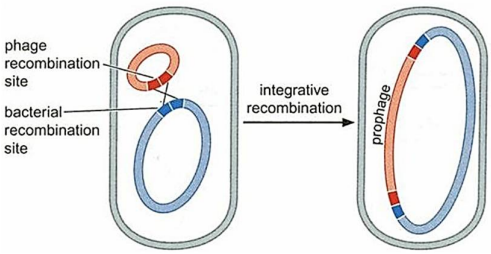
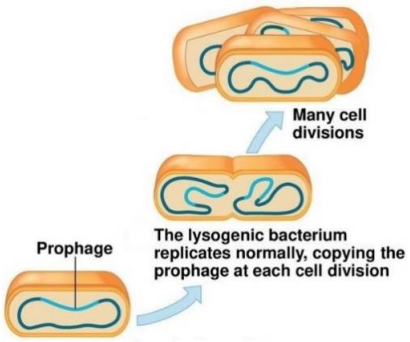
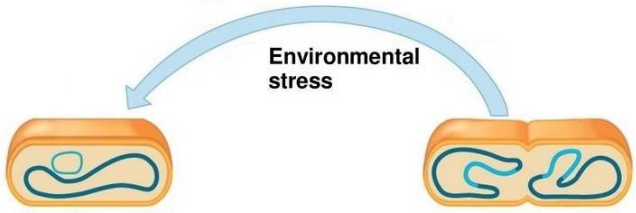


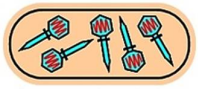
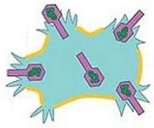
Fig. 5 (b): Electron micrograph of a λ phage

5.1 The Lysogenic Reproductive Cycle

	Stage	Description
1	**Attachment** 	• The phage's single short tail fibre bind to specific receptors on host bacterium outer cell surface membrane.
2	**Penetration** 	• Once attached, λ phage ejects its DNA into the bacteial cell, leaving the empty capsid outside. • The λ phage DNA circularises inside the bacterium to prevent the host exonucleases from degrading it.
3	**Integration of phage DNA into host chromosome to form a prophage** 	• An enzyme will integrate the λ phage DNA into a specific recombination site on the bacterial chromosome. • The integrated λ phage DNA is now known as a **prophage**.  • A particular prophage gene coding for a repressor protein called the **lambda repressor** is expressed, resulting in the synthesis of the lambda repressor. • The lambda repressor blocks transcription of most other prophage genes, as a result, the rest of the prophage gene remains silent (transcriptionally inactive) (Note: Host cell machinery and resources thus not hijacked, and host DNA is not hydrolysed, no biosynthesis of viral genome and proteins, no new phages are formed.)

4	**Prophage Replication** 	• Each time the host cell divides, the **prophage is replicated along with the host chromosome** and is passed to the daughter bacterial cell. • This allows a single infected cell to quickly give rise to a **large population** of bacteria carrying the prophage.
5	**Spontaneous induction of prophage** **(Switch from lysogenic to lytic cycle)**	• Upon detection of host cell damage or stress (due to factors such as starvation, radiation, and presence of poisons such as antibiotics), **lambda repressor is broken down**, and phage genes are expressed. • The λ phage DNA genome of the activated phage is excised from the bacterial chromosome and enters the lytic cycle.
		

5.2 The Lytic Reproductive Cycle (environment stressors present / after lysogenic cycle)

6	**Biosynthesis of viral DNA and proteins** 	• The λ phage DNA directs the metabolic machinery (e.g. ribosomes, DNA polymerase, RNA polymerase) and raw materials (e.g. amino acids, ATP, nucleotides) of the host cell towards o Synthesising phage proteins (e.g. lysozyme) o Making new copies of phage genome
7	**Maturation** 	• Phage components **self-assemble** into complete virions.
8	**Release** 	• Phage lysozyme breaks down the peptidoglycan cell wall, causing fluid to enter the bacterial cell. • The cell thus undergoes osmotic lysis, whereby it swells and bursts. • Newly synthesised λ phages are released into the extracellular environment, and each can now infect a new host cell.

*Similar to the lytic cycle of the T4 bacteriophage

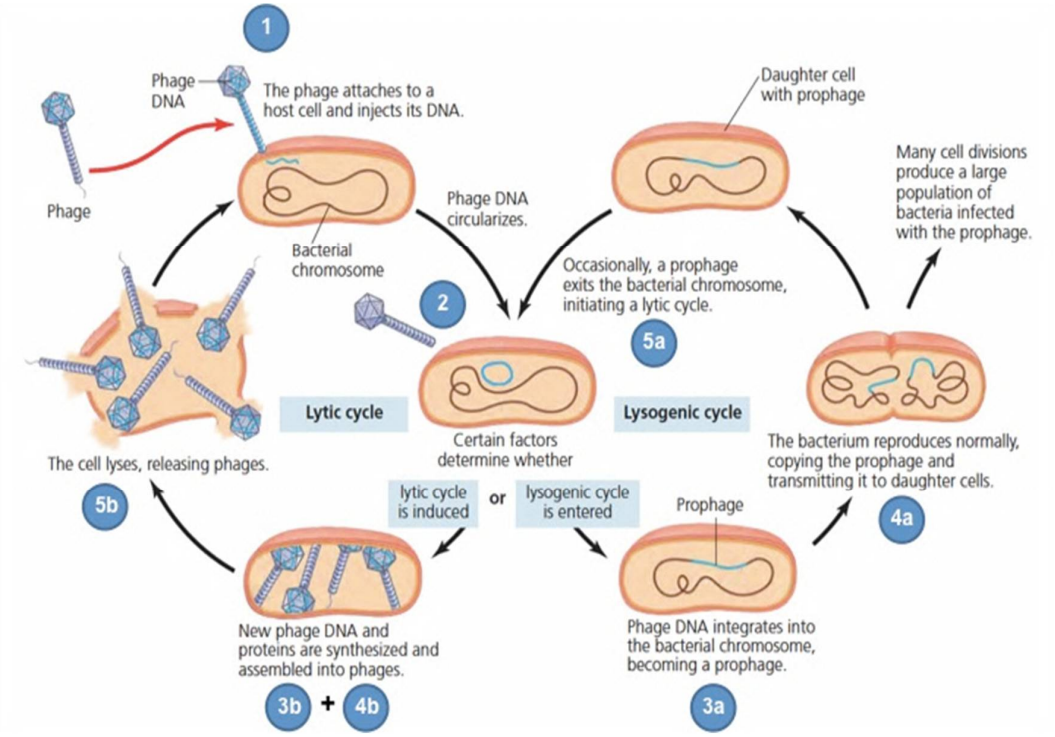


Fig 5.2: Lytic and lysogenic cycles of a lambda phage

CHECKPOINT (3) – Lytic vs Lysogenic Cycles

Put a tick (√) in the correct boxes.

S/N	Statement	Lytic cycle	Lysogenic cycle
a.	Bacteriophage genes are expressed immediately after the bacteriophage infects the host cell.		
b.	Host chromosome is degraded upon entry of bacteriophage DNA.		
c.	Bacteriophage DNA is integrated into the host cell chromosome.		
d.	Bacteriophage DNA is replicated along with the bacteria chromosome.		
e.	Bacteriophages are released when the bacteria lyse.		
f.	Bacteriophages undergoing this type of life cycle allow generalised transduction to occur between bacterial cells.		
g.	Bacteriophages undergoing this type of life cycle allow specialised transduction to occur between bacterial cells.		

6 Structure & Reproductive cycle of enveloped viruses e.g. Influenza virus

Virus	Influenza virus
Host	Birds, pigs and mammals – epithelial cells lining mucous membranes of respiratory tracts
Capsid	Helical capsid
Genome	• Eight negative-sense single-stranded RNA segments. • ssRNA genome is negative configuration (i.e. complementary to viral mRNA)
Enzyme in Capsid	• **Viral RNA-dependent RNA polymerase** found in the viral particle uses the ssRNA (-) genome as a template for mRNA production. (Note: The host cell's RNA polymerases cannot be used to synthesise mRNA from the viral RNA genome since they use a DNA template)
Viral Envelope	• Each virus particle is surrounded by a host-derived phospholipid envelope. • Two types of viral glycoproteins are embedded in the envelope. o **Haemagglutinin (HA)** – a glycoprotein that facilitates attachment of viral particle to host cell surface membrane by binding to specific receptors on a variety of cells (by binding to sialic acid-containing residues found in glycoproteins or glycolipids on host cell surface) o **Neuraminidase (NA)** – an enzyme that facilitates the release of newly formed viral particles from the infected host cell (by cleaving sialic acid found in glycoproteins or glycolipids on host cell surface)

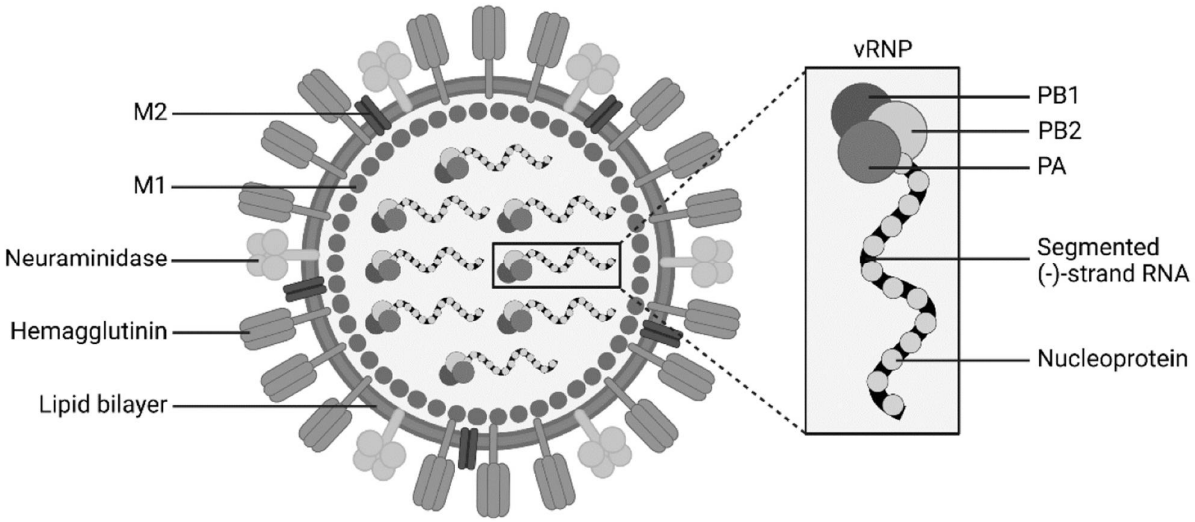
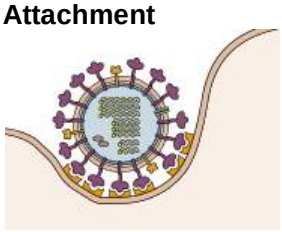
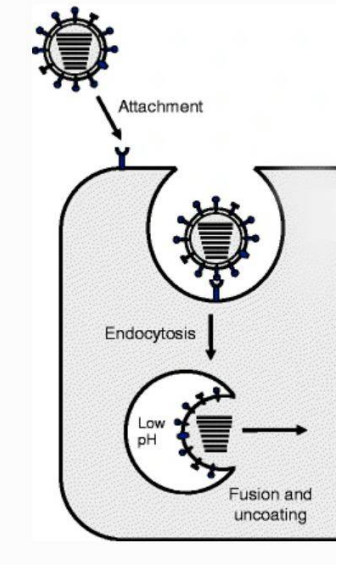
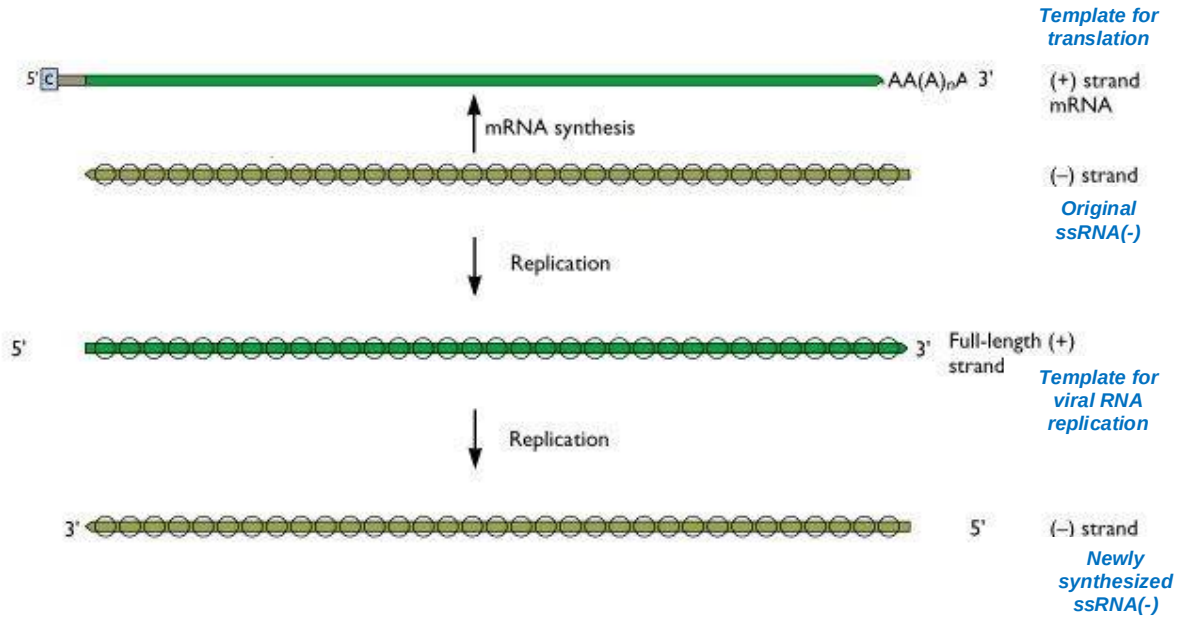


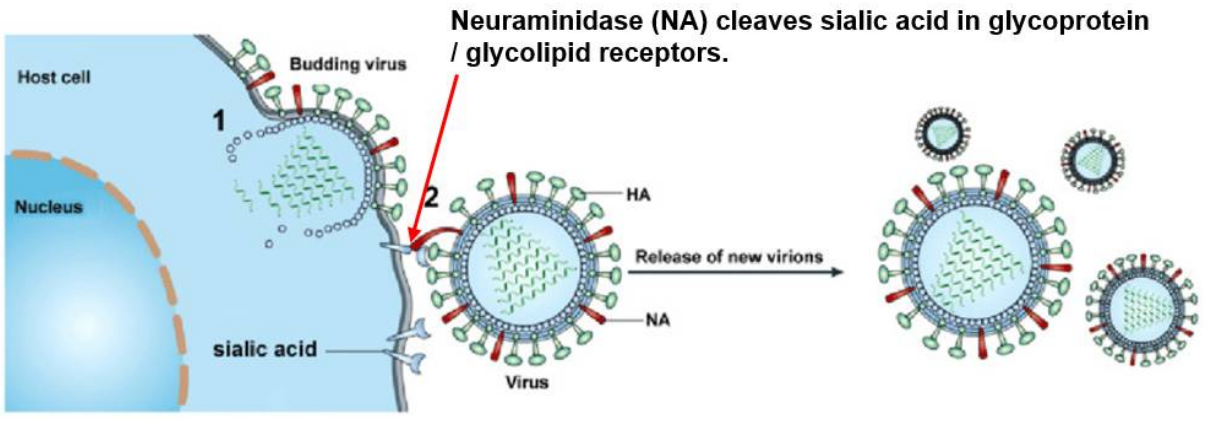
Fig. 6.1 Structure of influenza virus

Key: subunits (PA, PB1, PB2) of RNA-dependent RNA polymerase (RdRp) complex

No.	Stage	Description
1	**Attachment** 	• Haemagglutinin attaches to sialic acid receptor on the epithelial cell surface membrane.
2	**Penetration & Uncoating** 	• The virus enters the host cell by receptor-mediated endocytosis, as the host cell surface membrane invaginates and pinches off, enclosing the viral particle in a coated vesicle. • Acidification of the endosome occurs to trigger the fusion of the viral envelope with the endosomal membrane. • The capsids are degraded by cellular enzymes, releasing the ssRNA (-) genome segments and viral RNA dependent RNA polymerase into the cytoplasm. • The viral RNA polymerase and ssRNA(-) are transported into the nucleus.
3	**Biosynthesis** • In the nucleus, the viral RNA-dependent RNA polymerase uses the ssRNA(-) as a template to transcribe complementary ssRNA(+). • These complementary ssRNA(+) strands serve as 1. template for synthesis of new negative-sense viral RNA genome 2. mRNA, which will be transported to the cytoplasm and translated to viral proteins • The host cell's transcription and translation machinery are used for the synthesis of viral RNA and proteins. • Viral mRNA is exported from the nucleus to be translated into viral proteins. o i.e. viral RNA-dependent RNA polymerase, capsid proteins, haemagglutinin, neuraminidase o • Newly synthesised viral proteins are: o Transported back into the nucleus (i.e. capsid protein and RNA-dependent RNA polymerase) o Incorporated into the cell surface membrane (i.e. haemagglutinin, neuraminidase)	



4	**Maturation** • In the nucleus, capsid proteins <u>assemble</u> and the ssRNA(-) strands and RNA-dependent RNA polymerase are <u>packaged</u> to form new viral particles.
5	**Release** • Viral particles migrate to a site near the host cell surface membrane where haemagglutinin and neuraminidase have been embedded. • Mature virions are formed and released by **budding**, <u>acquiring their envelope</u> from portions of the host cell surface membrane with <u>haemagglutinin and neuraminidase embedded.</u> • **Neuraminidase** facilitates the release of new virions from the host cell surface membrane by cleaving sialic acid from the host cell receptors. • Released <u>viral particles are ready to infect other cells</u>



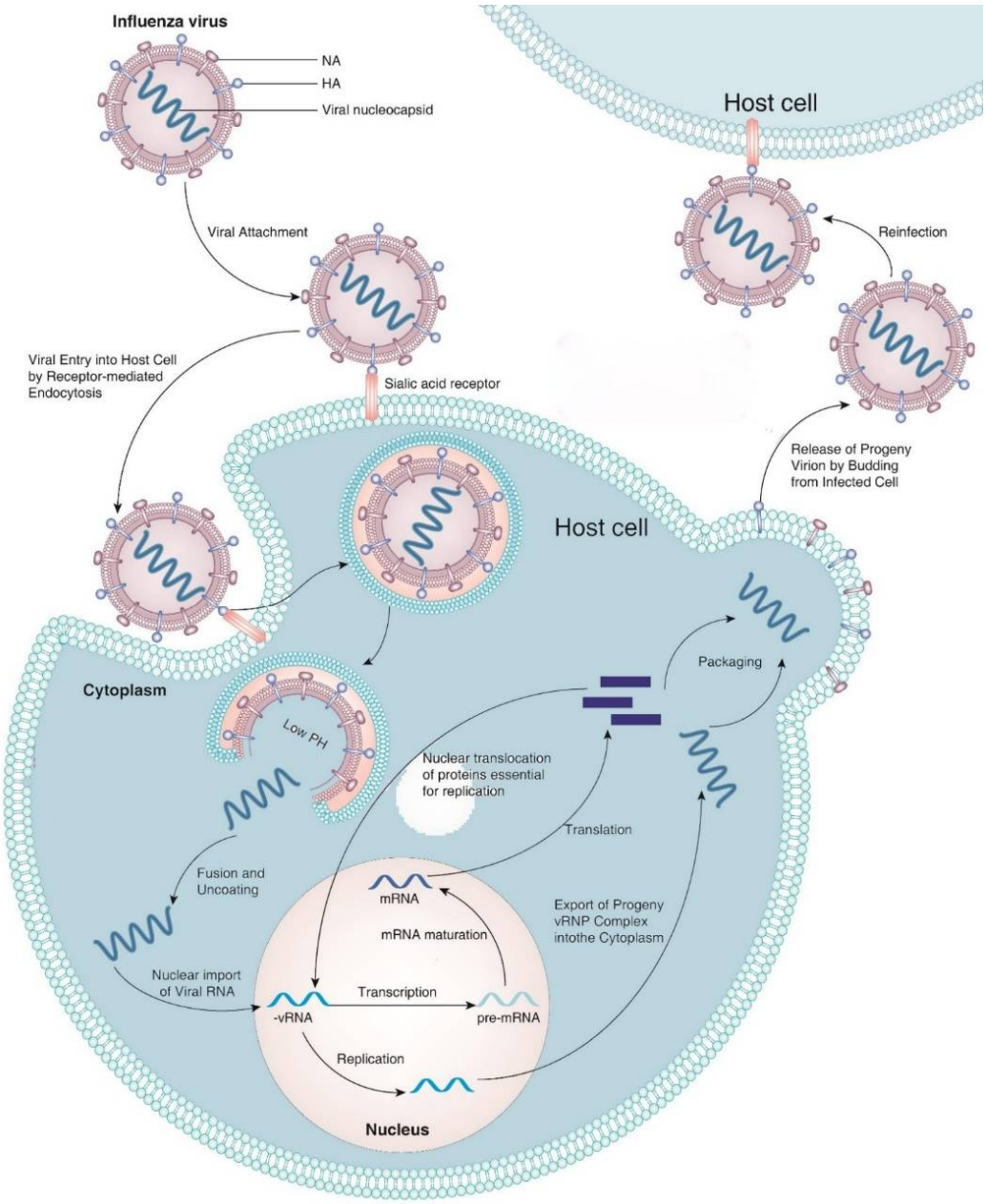


Fig. 6.2: Influenza virus reproductive cycle

7 Structure & Reproductive cycle of retroviruses e.g. Human Immunodeficiency virus (HIV)

Virus	Human Immunodeficiency virus (HIV), a retrovirus that is enveloped
Host	Human immune cells that express the CD4 cell surface protein, especially T helper cells, a type of lyphocyte
Capsid	Conical capsid
Genome	2 identical copies of ssRNA(+) enclosed in a conical capsid
Enzyme in Capsid	o **<u>Reverse transcriptase</u>**, which is an RNA-dependent DNA polymerase that uses positive-sense single stranded RNA (ssRNA) as a template to synthesise **double stranded DNA (dsDNA)**. o **Integrase**, which is required for insertion of the viral genome (after it has been reverse transcribed into dsDNA) into host cell's DNA. o **HIV protease**, which cleaves inactive viral polyproteins into functional structural and enzymatic proteins.
Viral Envelope	• The **nucleocapsid** is surrounded by an **envelope** derived from the host cell surface membrane. • **Glycoproteins gp120 and gp41** are embedded in the envelope. o **<u>Glycoprotein 120 (gp120)</u>** is a **glycoprotein** that binds to CD4 receptors on the host cell surface (e.g. T-helper cells and macrophages). o **<u>Glycoprotein 41 (gp41)</u>** is a **transmembrane glycoprotein** that facilitates fusion of the viral envelope by binding to the coreceptor (CXCR4 / CCR5) on the host cell surface membrane.

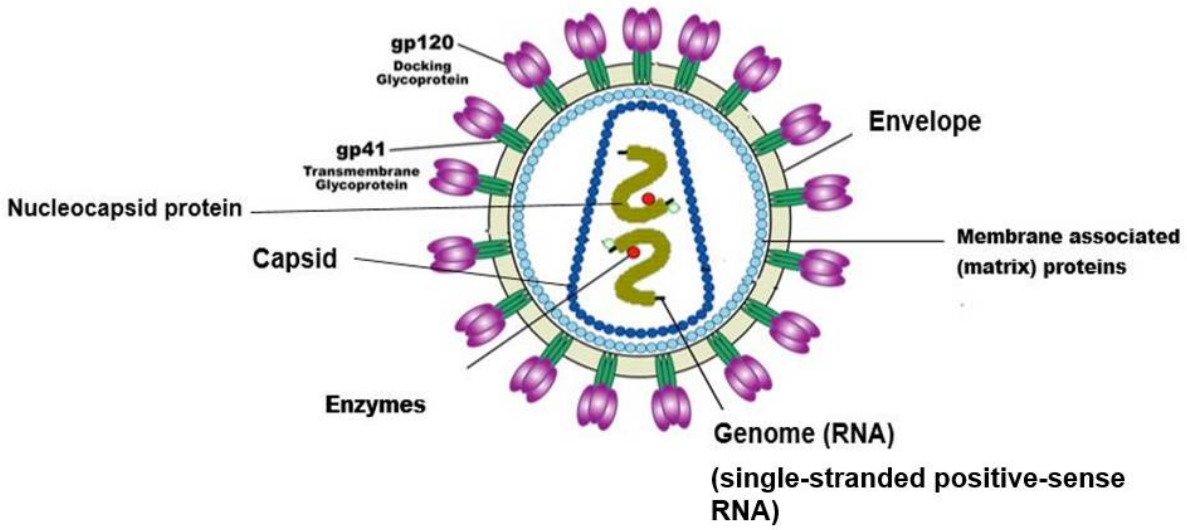
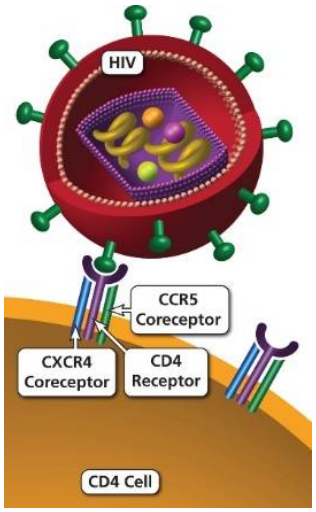
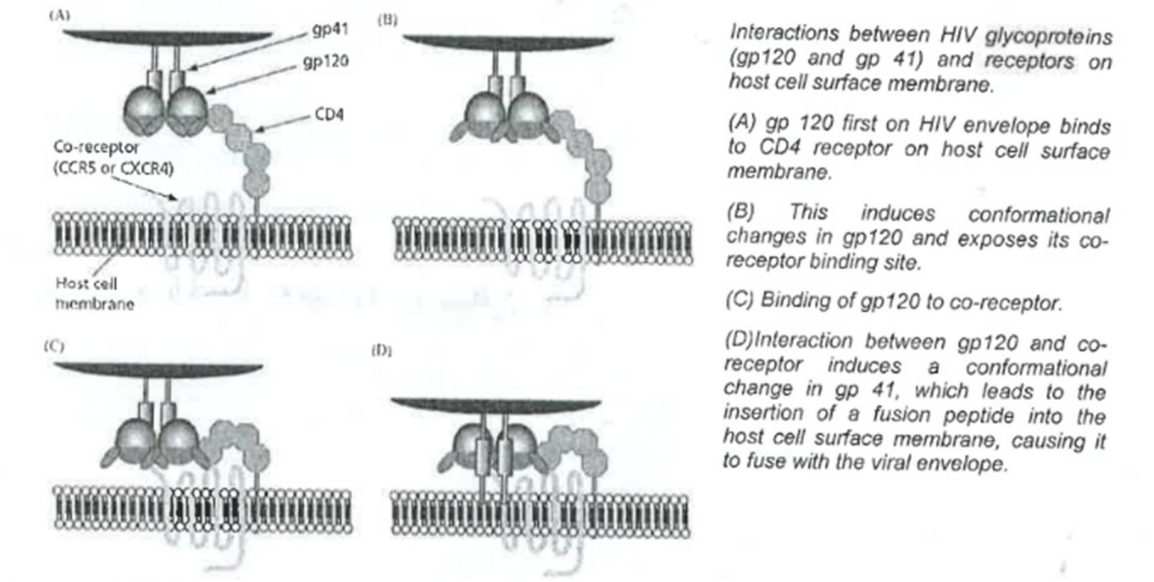
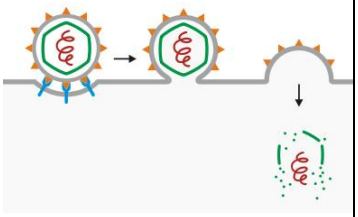
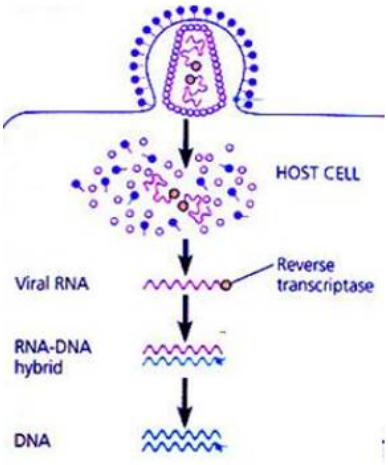


Fig 7.1: Structure of HIV

	Stage	Description
1	**Attachment** 	Glycoprotein 120 (gp120) on HIV envelope binds specifically to both a CD4 receptor and co- receptor on the cell surface membrane of T helper cells in the following sequence of events: • gp120 binds first to a CD4 receptor on the host cell surface membrane. • The interaction between gp120 and CD4 receptor induces a conformational change in gp120, enabling binding of gp120 to host cell's co-receptor (Le. CXCR4 on T helper cell or CCR5 on macrophage). • The interaction between gp120 and the co-receptor brings about a conformational change in gp41 on the HIV viral particle, which leads to fusion of the HIV envelope to the host cell surface membrane.
	 **Fig 7.2: Interactions between HIV glycoproteins (gp120 and gp41) and receptors on host cell surface membrane**	
2	**Penetration & Uncoating** 	• Fusion of the HIV envelope with the host cell surface membrane releases the capsid into the cytoplasm of the host cell. • The capsid is subsequently degraded by cellular enzymes, thus releasing the viral genome and enzymes (reverse transcriptase, integrase, HIV protease) into the host cell. • This process is known as uncoating. *uncoating ≠ receptor mediated endocytosis as a vesicle is not formed.

3.	**Synthesis of viral genome and proteins** **(a) Reverse Transcription** • The RNA genome is reverse transcribed into double-stranded DNA by viral **reverse transcriptase**. o **Reverse transcriptase** catalyses the synthesis of a DNA strand complementary to the viral RNA, forming a RNA-DNA hybrid. o The RNA strand is degraded and a second DNA strand complementary to the first is synthesised to form a double-stranded DNA (dsDNA) molecule known as **complementary DNA (cDNA).**  **(b) Integration of Viral DNA** • The viral dsDNA enters the host cell nucleus. • Viral **integrase** catalyses the integration of viral DNA into the host cell genome. • The incorporated viral DNA is now known as a provirus. • Once viral DNA is integrated, it may persist in its latent state for many years. **(c) Provirus activation and Biosynthesis** • Upon **activation**, the host cell's RNA polymerase **transcribes the proviral DNA into viral RNA**. Viral RNA molecules serve two functions: 1. New ssRNA(+) genomes for the next viral generation 2. mRNA for synthesis of viral proteins (e.g. gp120, gp4, immature viral polyproteins) • The host cell's transcription and translation machinery are used for the synthesis of viral RNA and proteins.
4	**Maturation** • Viral glycoproteins gp41 and gp120 are transported via vesicles and embedded into the host cell surface membrane. • The viral RNA genome and immature viral polyproteins assemble at the cell surface membrane where gp41 and gp120 have been inserted.
5	**Release** • Immature viruses bud off, acquiring host cell surface membrane as their envelope. HIV protease immature polyproteins into individual functional HIV proteins, giving rise to functional capsid proteins, reverse transcriptase and integrase. • The viral RNA genome and enzymes (reverse transcriptase, integrase and HIV protease) are then enclosed by a capsid, completing the maturation process. • The mature HIV viruses are now able to infect another cell.

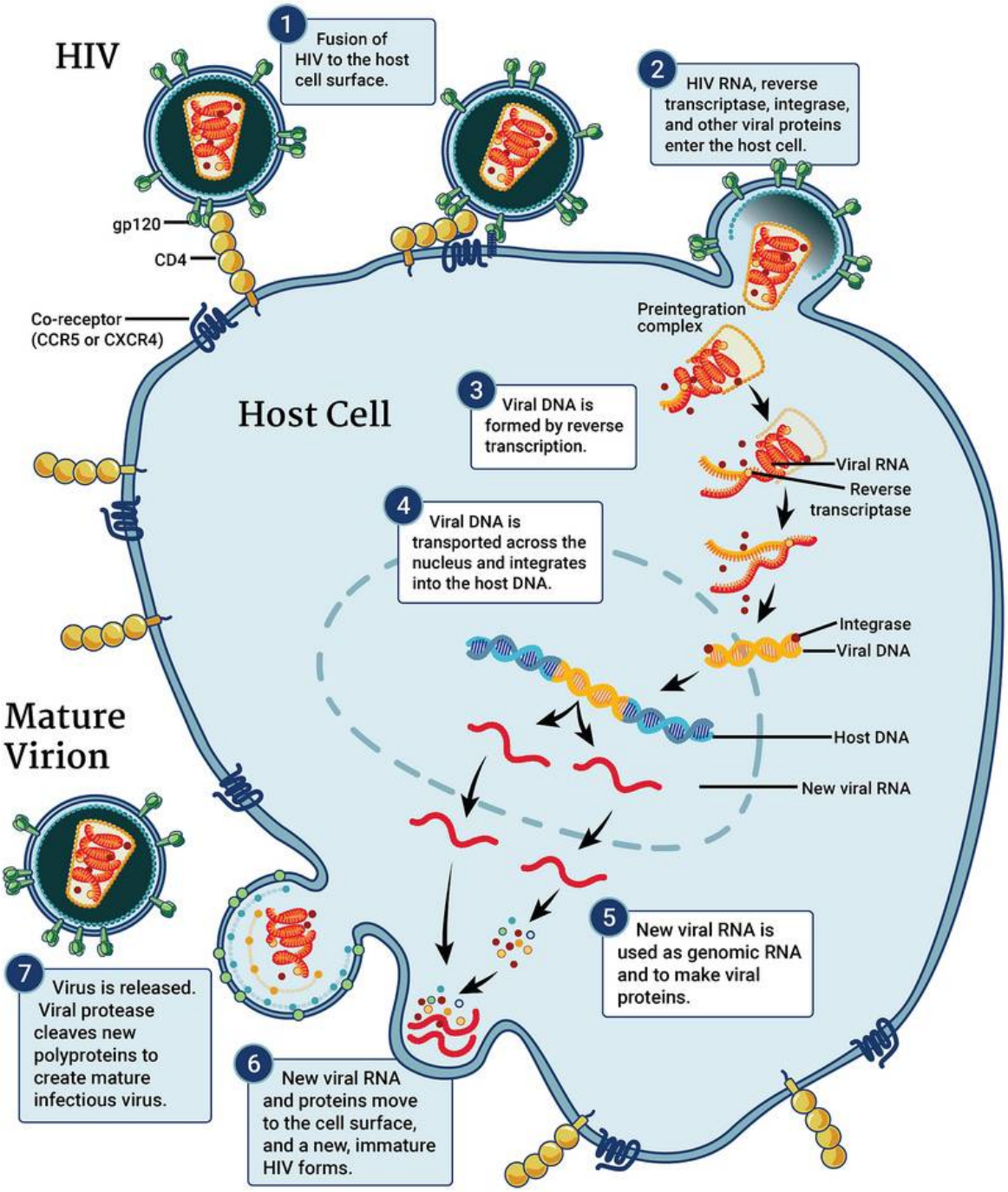
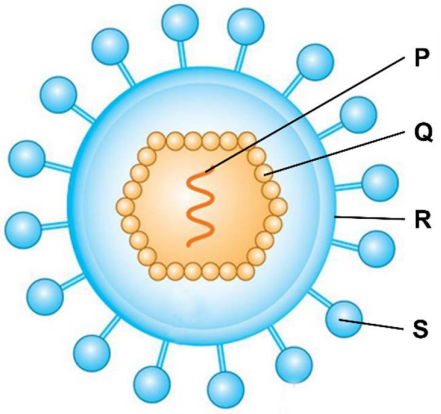


Fig 7.3: HIV reproductive cycle

CHECKPONT (4) – Features of Influenza virus and HIV

Choose the correct answer.

1) The diagram shows the structure of an enveloped virus.



Which of the following statements are true?

1 **P** determines the structure of **Q** and **S**.
2 **Q** assists in viral entry into the host cell.
3 **R** and **S** are required for viral entry into the host cell.
4 **Q** and **R** are made of the same components.

A. 1 and 2 only
B. 1 and 3 only
C. 2 and 3 only
D. 2 and 4 only ( )

2) Which of the following identifies the genome of an influenza virus?

	percentage of nucleotides needed with particular base				
	adenine	cytosine	guanine	thymine	uracil
A	15	20	25	0	40
B	40	10	10	40	0
C	30	15	15	0	30
D	10	35	35	10	10

( )

3) The neuraminidase of influenza virus exhibits all the following properties **except**:

A. facilitating release of virus particles from infected cells.
B. carrying out enzymatic acitivity.
C. embedding in the outer surface of the viral envelope.
D. attaching with sialic acid receptors present in the respiratory tract. ( )

4) The HIV protein that is involved in attachment of the virus to host cells is known as

A. CD4
B. gp120
C. Integrase
D. Reverse transcriptase ( )

5) Which of the following best describes the flow of genetic information in the HIV reproductive cycle?

A. DNA – RNA – Protein
B. DNA – Protein – RNA
C. RNA – DNA – RNA – Protein
D. RNA – RNA – DNA – Protein ( )

6) The provirus state exists when _____ is integrated into the host genome.

A. single stranded viral DNA
B. single stranded viral RNA
C. double stranded viral DNA
D. double stranded viral RNA ( )

7) Which of the following statements about the HIV is incorrect?

A. HIV enters its host cell with the help of its envelope glycoproteins, gp120 and gp41.
B. HIV targets all cells of the immune system, resulting in a immunodeficient individual.
C. The HIV genome comprises two positive-sense single-stranded RNA.
D. The envelope of the HIV is derived from the cell surface membrane of its host cell. ( )

8) Which of the following statements are true of both HIV and influenza virus?

1 Carries negative-sense single stranded RNA
2 Enters host cell via receptor-mediated endocytosis
3 Carries specific enzymes not found in the host cell
4 Genetic variation may arise due to errors in replication
5 Exits host cell via budding

A. 1, 3 and 5 only
B. 2, 3 and 4 only
C. 2, 4 and 5 only
D. 3, 4 and 5 only ( )

Learning Outcome 2(f)
Describe how variation in viral genomes arises, including antigenic shift and antigenic drift.

8 Mechanisms for Variation in Viral Genomes

Viruses are continuously evolving as a result of genetic selection. They can undergo subtle changes through mutation (**antigenic drift**), or major changes through genetic reassortment (**antigenic shift**).

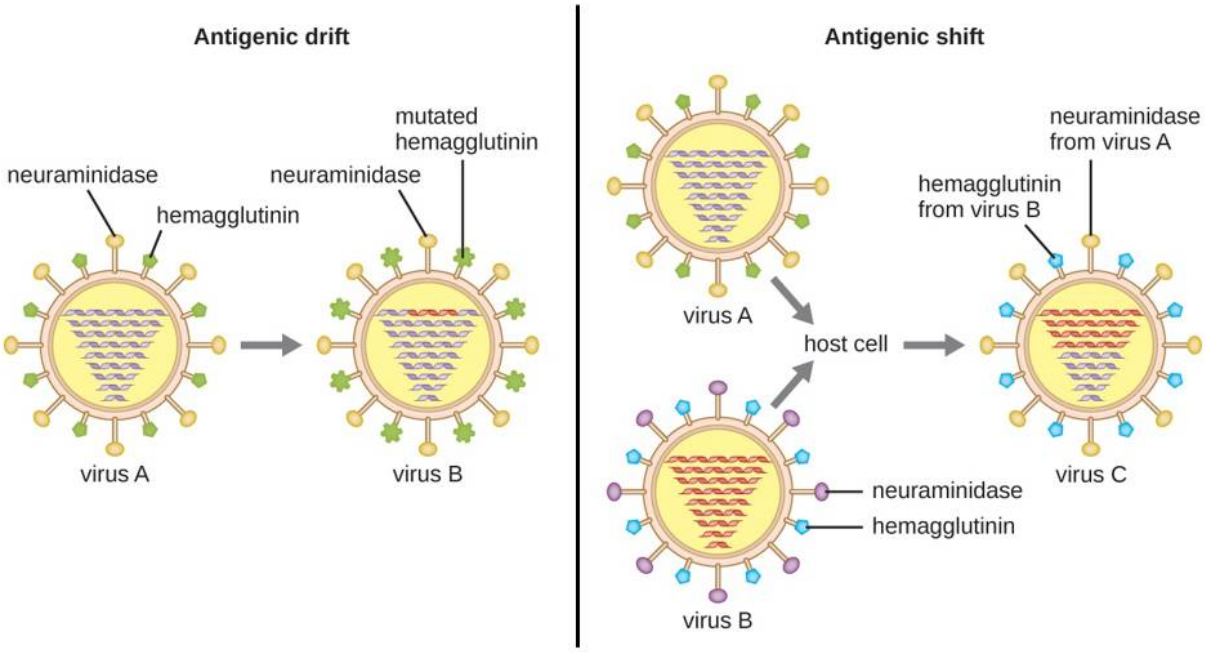


Fig. 8 Antigenic drift and antigenic shift

8.1 Antigenic Drift

- **Antigenic drift** refers to the **accumulation of mutations** in the viral genome that results in a **gradual change** to the structures of haemagglutinin (HA) and neuraminidase (NA) on the viral envelope.
- This is possibly due to the **lack of proofreading mechanisms by RNA polymerases** during the replication of viral genome.
- Since the new virus strain cannot be recognised by/ appear antigenically different to antibodies which targeted previous strains of the virus, the new virus strain can evade host immune defence mechanisms and infect the host.
- Antigenic drift occurs in influenza viruses. The constant evolution of influenza viral genome is the reason why vaccines have to be periodically updated to provide the population with protection against the virus.

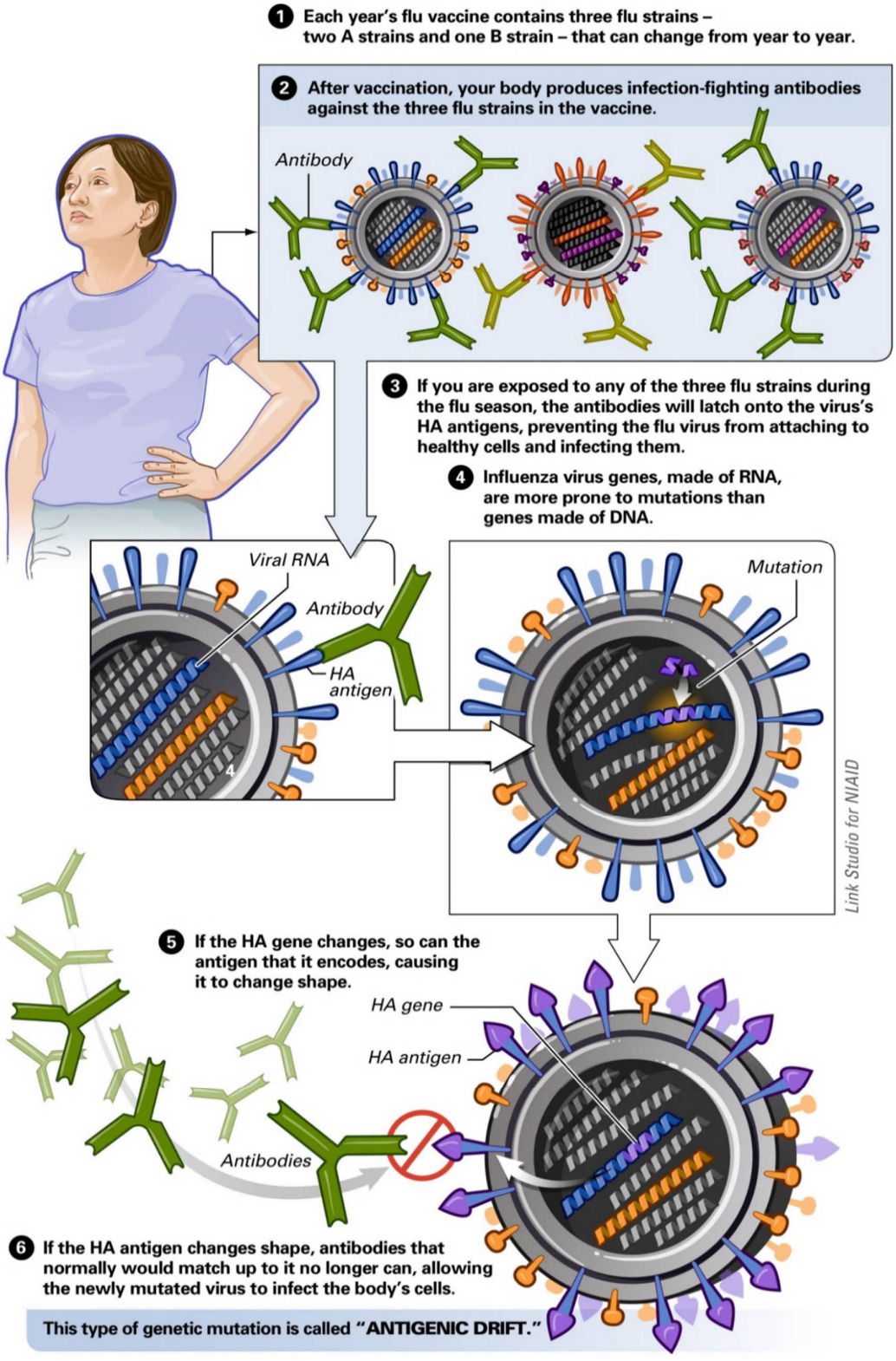


Fig 8.1 Antigenic drift

8.2 Antigenic Shift

- **Antigenic shift** refers to the process whereby **two or more strains** of the **influenza virus co-infect a single cell,** resulting in a **novel combination of antigens** because **genetic reassortment of RNA segments** from different viral strains occurred during viral assembly.
- The resultant virus thus contains a novel combination of RNA segments, which in turn results in a novel combination of glycoproteins (HA and NA) on the viral envelope.
- This **major change** in the viral antigens gives rise to a novel viral strain which the human immune system may not have encountered before. The new virus strain can thus evade host immune defence mechanisms and infect the host.
- This novel viral strain may evolve to spread from person to person, resulting in the formation of a highly virulent virus which could cause a flu pandemic.

CHECKPONT (5) – Antigenic Drift vs Antigenic Shift

Put a tick (√) in the correct boxes.

Statement	Antigenic Drift	Antigenic Shift
Occurs due to RNA polymerases lacking proofreading ability.		
The 3D structure of HA and NA remains unchanged, however new combinations of HA and NA are found on the viral envelope		
Mutations in the coding sequences in HA resulted in changes to the 3D structure of HA.		
RNA segments from more than one influenza virus are incorporated into the new virus particle.		
Results in the occurrence of influenza pandemics due to total absence of host immunity.		
Accounts for the need for annual influenza vaccinations in order to be protected against the influenza virus.		

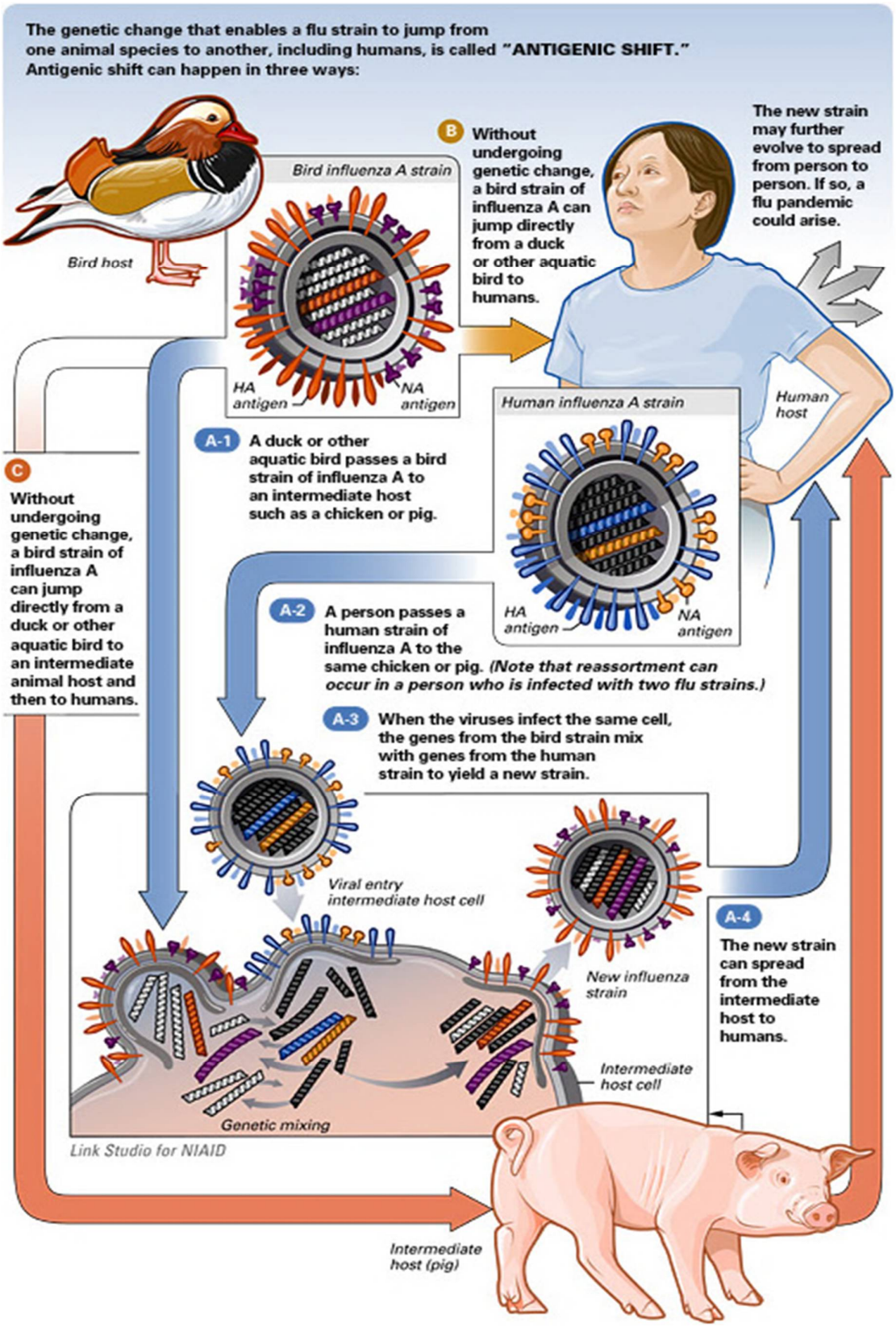


Fig 8.2 Antigenic shift

Annex: DISCOVERY OF VIRUSES

Viruses were detected indirectly long before they were actually seen. The discovery of viruses first started in 1883 with Adolf Mayer's research on the cause of tobacco mosaic disease, a disease which stunts tobacco plant growth and mottled plant leaves (Fig. 1.1). It was later discovered that this disease is caused by the tobacco mosaic virus (TMV), a virus that infects plants.

The timeline of events leading to the discovery of the first virus, TMV is shown below:

Year	Key Events
1883	Adolf Mayer found out that the tobacco mosaic disease can be transmitted from plant to plant by rubbing sap extracted from diseased leaves onto healthy plants. After an unsuccessful search for an infectious microbe in the sap, he concluded that the disease was caused by an unusually small bacterium that was invisible under a light microscope.
1892	Dimitri Ivanowsky showed that the sap was still infectious even after it was passed through a special filter that can remove bacteria (Fig. 1.2)
1897	Martinus Beijerinck showed that the infectious agent in the filtered sap: • could replicate, only within the host it infected • but could not be cultivated in nutrient media in test-tubes or petri dishes like bacteria Beijerinck coined the term 'virus' and proposed that it was an infectious, reproducing particle that was much smaller and simpler than a bacterium.
1935	Wendell Stanley confirmed Beijerinck's hypothesis by crystallising the particle, now known as the tobacco mosaic virus (TMV).

Fig 1.1: Mosaic pattern seen in diseased tobacco leaf (right) compared to normal leaf (left).

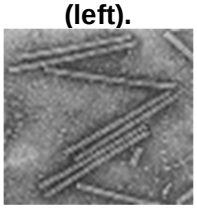


Fig 1.2: Electron micrograph of Tobacco Mosaic Virus (TMV).

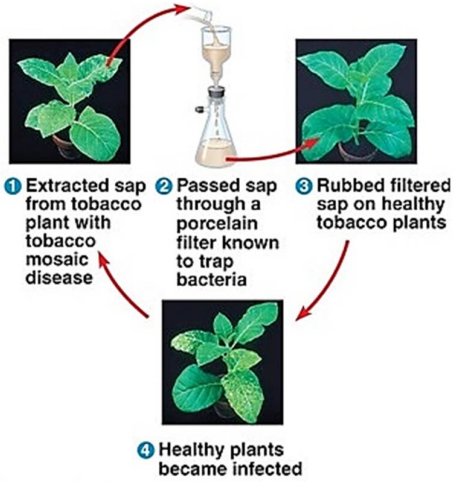


Fig 1.3: Experiment showing that sap was still infectious after passing through special filter that can remove bacteria.

Most viruses are too small to be seen under the light microscope. However, they can be viewed using electron microscopy.

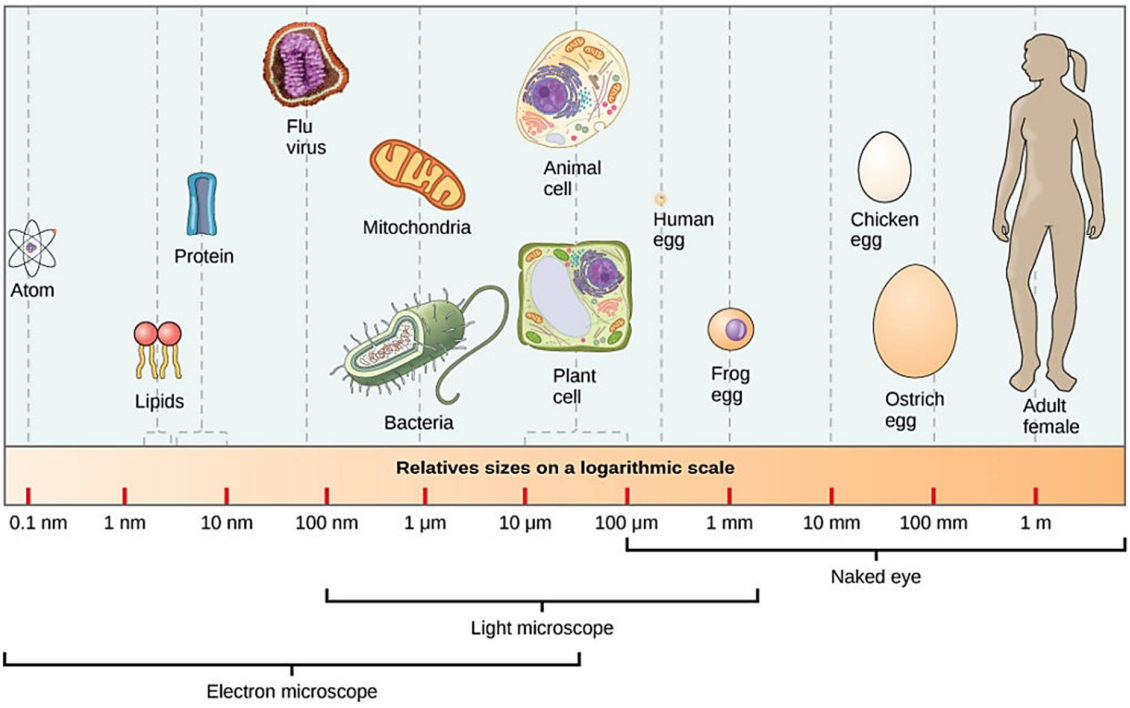


Fig. 1.4: Relative size of organisms

Annex: CHARACTERISTICS OF LIFE

To understand what is defined as life, scientists refer to the characteristics of life, as listed below:

Characteristic of life	Feature
Organisation	Being structurally composed of one or more cells.
Acquiring energy	Energy is the capacity to do work. To maintain their organisation and carry out activities essential to life, living organisms need to acquire energy from food through a series of chemical reactions. The sum of all chemical reactions that occur in a cell is termed metabolism.
Growth	A permanent increase in size of all parts of an organism.
Adaptation	The ability to change over a period in response to the environment to increase the survival rate.
Homeostasis	The ability to monitor internal conditions and make necessary adjustments to maintain a state of biological balance.
Response to environment	The interaction between a living organism with its environment and other living organisms. A response can take on many forms, from contractions in a unicellular organism to complex reactions involving senses in higher animals.
Reproduction	The ability to produce new organisms of its kind.

Annex: TYPES OF INFLUENZA VIRUSES

There are four types of influenza viruses: A, B, C, and D.

Influenza A and B viruses cause seasonal epidemics of disease in people (known as flu season) almost every winter in the United States.

Influenza A viruses are the only influenza viruses known to cause flu pandemics (i.e., global epidemics of flu disease). A pandemic can occur when a new and different influenza A virus emerges that infects people, has the ability to spread efficiently among people, and against which people have little or no immunity.

Influenza C virus infections generally cause mild illness and are not thought to cause human epidemics.

Influenza D viruses primarily affect cattle with spillover to other animals but are not known to infect people to cause illness.

Annex: ANTIVIRALS FOR INFLUENZA

Did you know?

To treat influenza, antivirals have been discovered based on knowledge of the influenza virus life cycle. One class of antivirals is the neuraminidase inhibitors, which prevent the release of newly formed virions from the host cell by inhibiting viral neuraminidase activity. An example of a neuraminidase inhibitor is oseltamivir, a medicine also known by the brand of Tamiflu®.

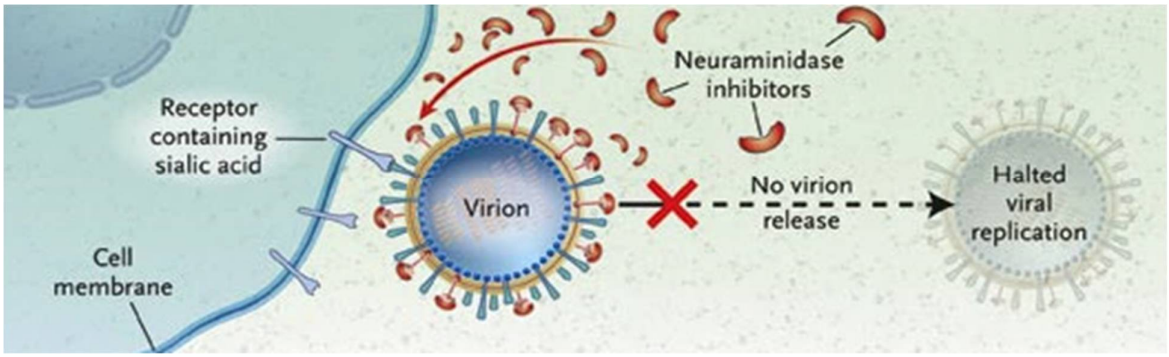


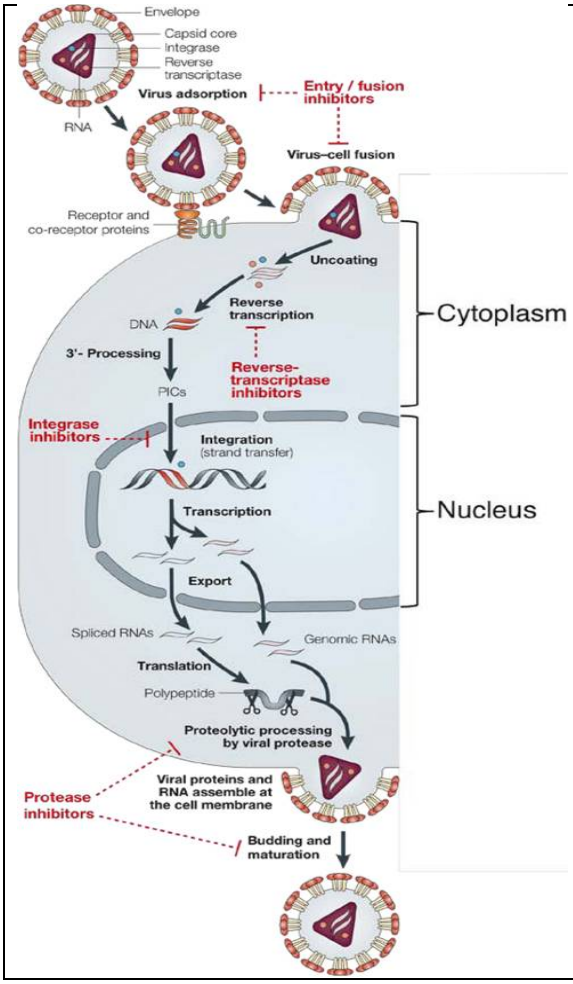
Fig. 6.3 Action of neuraminidase inhibitors on influenza virus reproductive cycle

List of drugs commonly prescribed by doctors for influenza

Drug name	Targeted step	Drug action
Amantadine	Uncoating	Inhibit the M2 proteins (proton pumps) therefore preventing the acidification of endosome and subsequent fusion of viral envelope with membrane of endosome
Ribavirin	Synthesis (transcription)	Inhibit viral RdRp therefore preventing the synthesis of (+)ssRNA from (-)ssRNA genome
Zanamivir (Relenza®) Oseltamivir (Tamiflu®)	Release	Inhibit neuraminidase (NA) therefore preventing the release of newly assembled virus particles

Annex: ANTI-VIRALS FOR HIV

Did you know?



Antivirals used to treat HIV infections targets multiple processes in the viral life cycle. These antivirals can be classified into the following categories:

(a) Fusion / entry inhibitors
(b) Nucleoside reverse transcriptase inhibitors
(c) Integrase inhibitors
(d) Protease inhibitors

Drug class	Targeted step	Drug action
Fusion / entry inhibitors	Attachment Penetration	Block attachment sites of host cell receptors such as CCR5 to prevent binding of gp120 to co-receptors Inhibit gp41 to prevent membrane fusion and penetration
Nucleoside reverse transcriptase inhibitors (NRTI)	Reverse transcription	Inhibit reverse transcriptase enzyme therefore preventing reverse transcription of RNA virus genome into DNA
Integrase inhibitors	Integration	Inhibit integrase enzyme therefore preventing dsDNA insertion into host cell DNA
Protease inhibitors	Assembly	Inhibit protease enzyme therefore preventing cleavage of polyprotein thus virus maturation is blocked