INTRODUCTORY BIOLOGY AND MICROBIOLOGY

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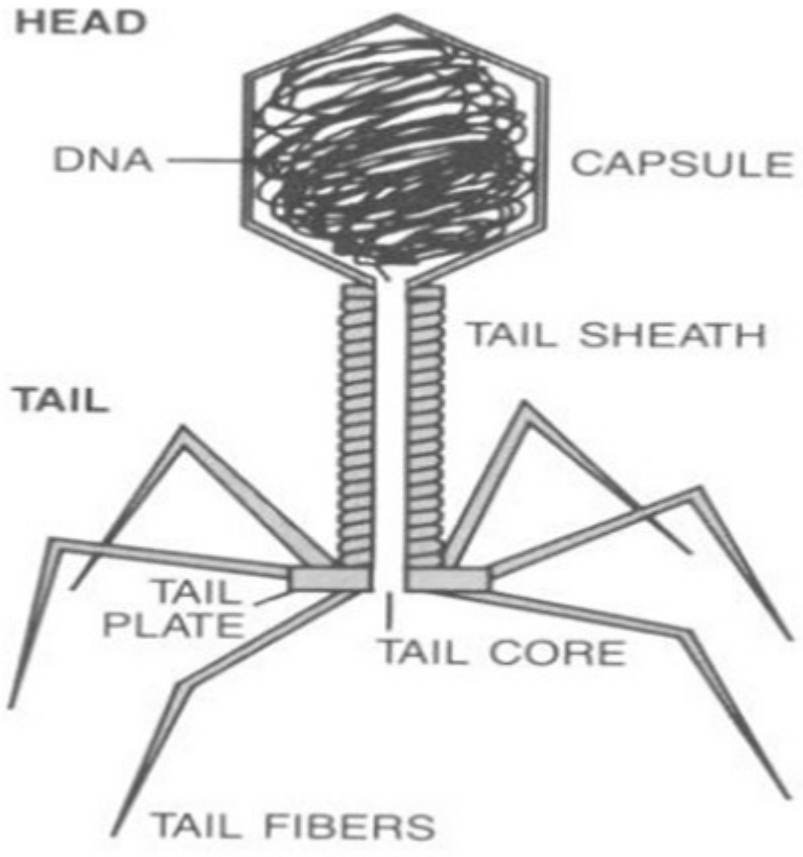
Bacteriophages and HIV

Bacteriophages

- These are viruses that infect bacteria. They are tailed dsDNA phages that are commonly found in environmental samples such as sewage water.
- They were first observed by Twort and d'Herelle in 1915 and 1917 respectively.
- They observed that broth cultures of certain intestinal bacteria could be dissolved by addition of a bacteria-free filtrate obtained from sewage.

Bacteriophage Structure



- Each bacteriophage is specific to one. Like most viruses, bacteriophages typically carry only genetic information needed for replication of nucleic acid and synthesis of their protein coats.
- They require precursors, energy generation and ribosomes supplied by their bacterial host cell.

Classification of Bacteriophages

- Classification is according to the Baltimore System of Classification
- Bacteriophages can be classified as:
  - Double stranded DNA phages
  - Single stranded DNA phages
  - Single stranded RNA phages and
  - Double stranded RNA phages

Double stranded DNA phages

- Are found in the families:
  - Myoviridea e.g. T-even phage of *E. Coli*
- These viruses infect, reproduce within the host cell and are released when the cell is destroyed by lysis.
- A phage life cycle that culminates with a host cell bursting and releasing virions is called a lytic cycle.
- Such viruses are virulent

Single stranded DNA phages

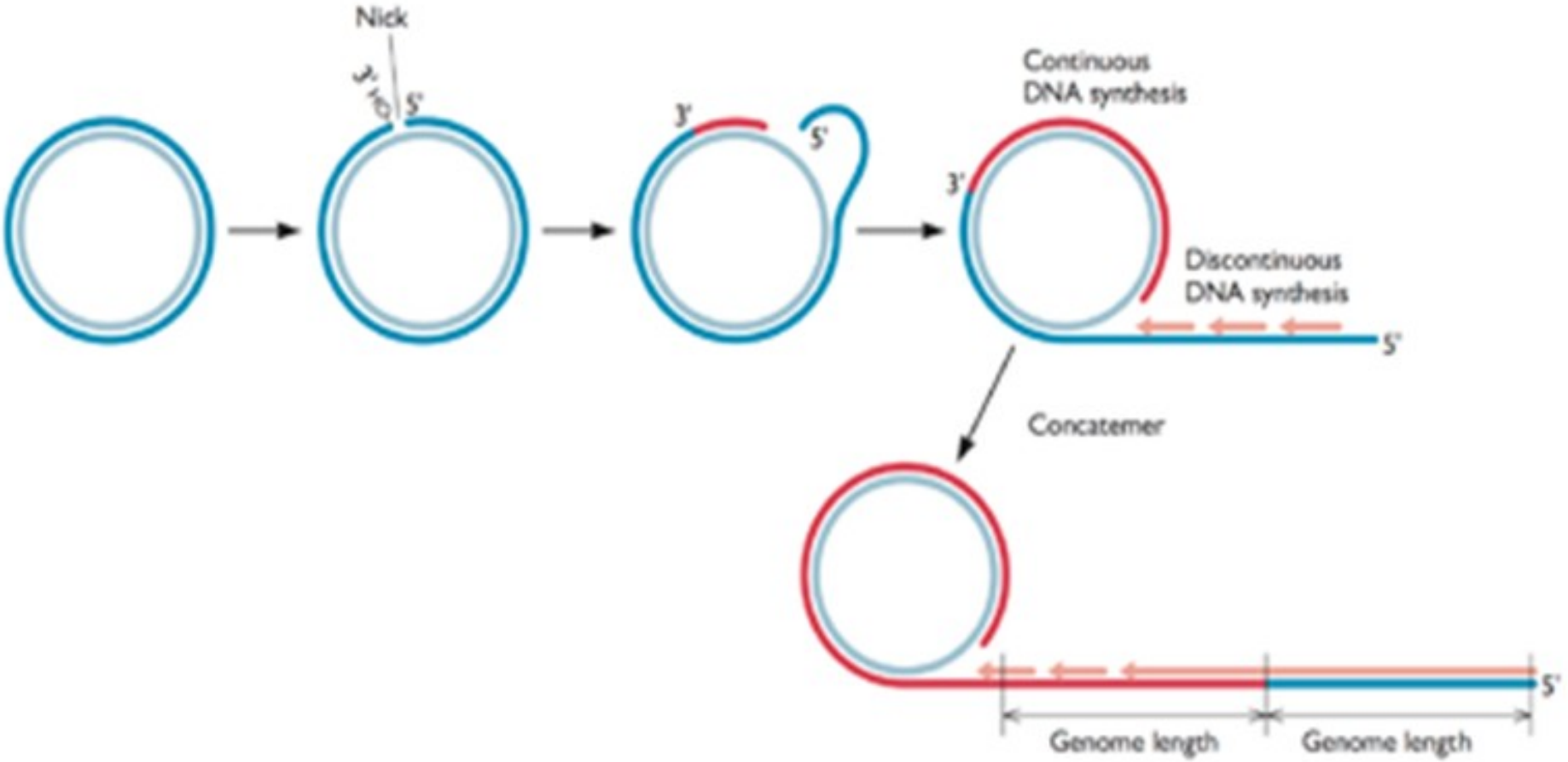
- The two ssDNA phages that have been extensively studied are:
  - φ (phi)X174
    - an icosahedral phage, which belongs to the Family *Microviridae*
    - It has a circular ssDNA genome
  - fd phage
    - a filamentous phage
    - belong to the Family *Inoviridae*.

φX174 Lytic Life cycle

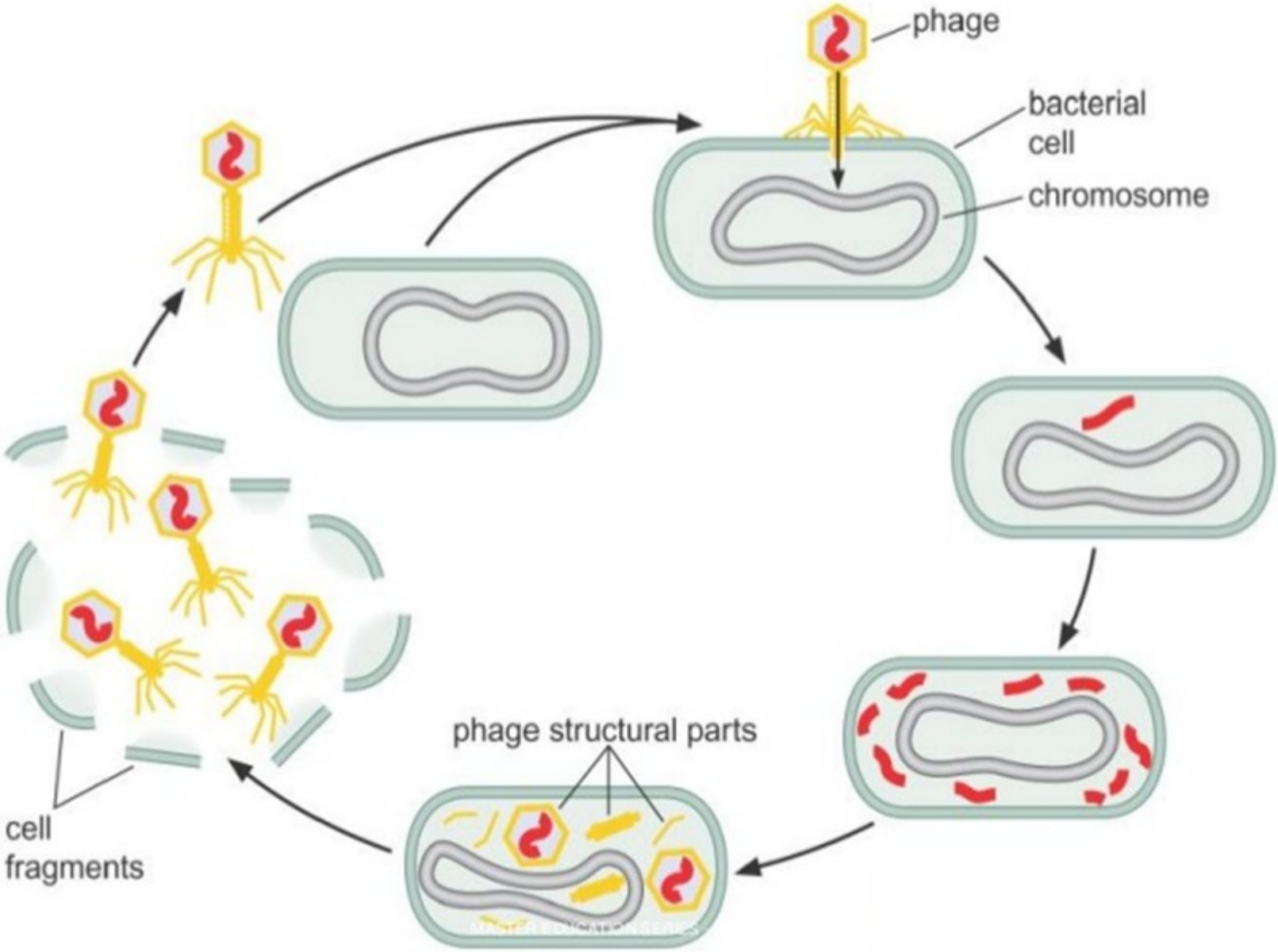
- Its life cycle starts with its attachment to host's cell wall
- The ssDNA is then injected into the cell but the protein capsid remains outside of the cell
- The genome has similar sequence as that of viral mRNA and so is said to be plus-strand DNA.
- Before transcription or replication, the phage DNA is converted to dsDNA form, which is called the **replicative form** (**RF**).

- This is aided by the bacterial DNA polymerase. The RF directs synthesis of more RF copies and the plus-strand DNA by rolling-circle replication process.

Rolling circle replication



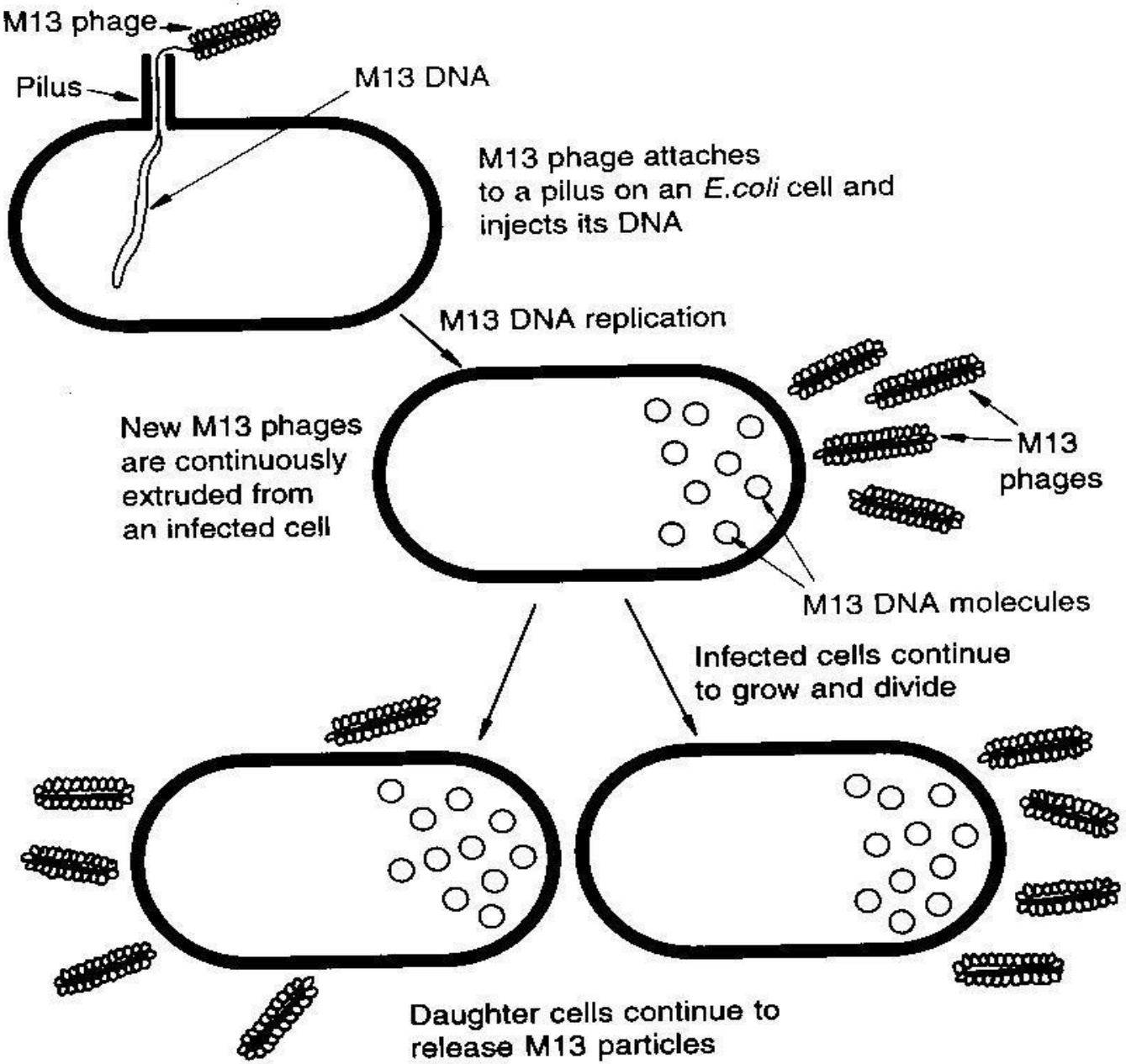
- The phage virions are then assembled. After assembly of the virions, φX174 releases an enzyme (enzyme E) that blocks bacterial cell wall synthesis.
- This weakens the bacterial cell wall, causing the cell to lyse and progeny virions are released.


phage
bacterial cell
chromosome
phage structural parts
cell fragments

fd phage and its Life Cycle

- It has a circular positive strand ssDNA genome
- It is shaped like a long fiber that is about 6 nm in diameter by 900 to 1,900 nm in length.
- The ssDNA lies in the centre of the filament and has tube like helical coat protein around it.
- The phage infects *E. coli* cells by attaching to the tip of the pilus.

- DNA then penetrates the bacterial cell through an F factor encoded sex pilus
- These phages do not kill their host cell but new virions are released by a secretory process.
- This happens by the phage coat protein attaching onto the host cell membrane.
- The coat then assembles around viral DNA before the virus is secreted through the host plasma membrane.
- The process slows down the host cell growth and division.

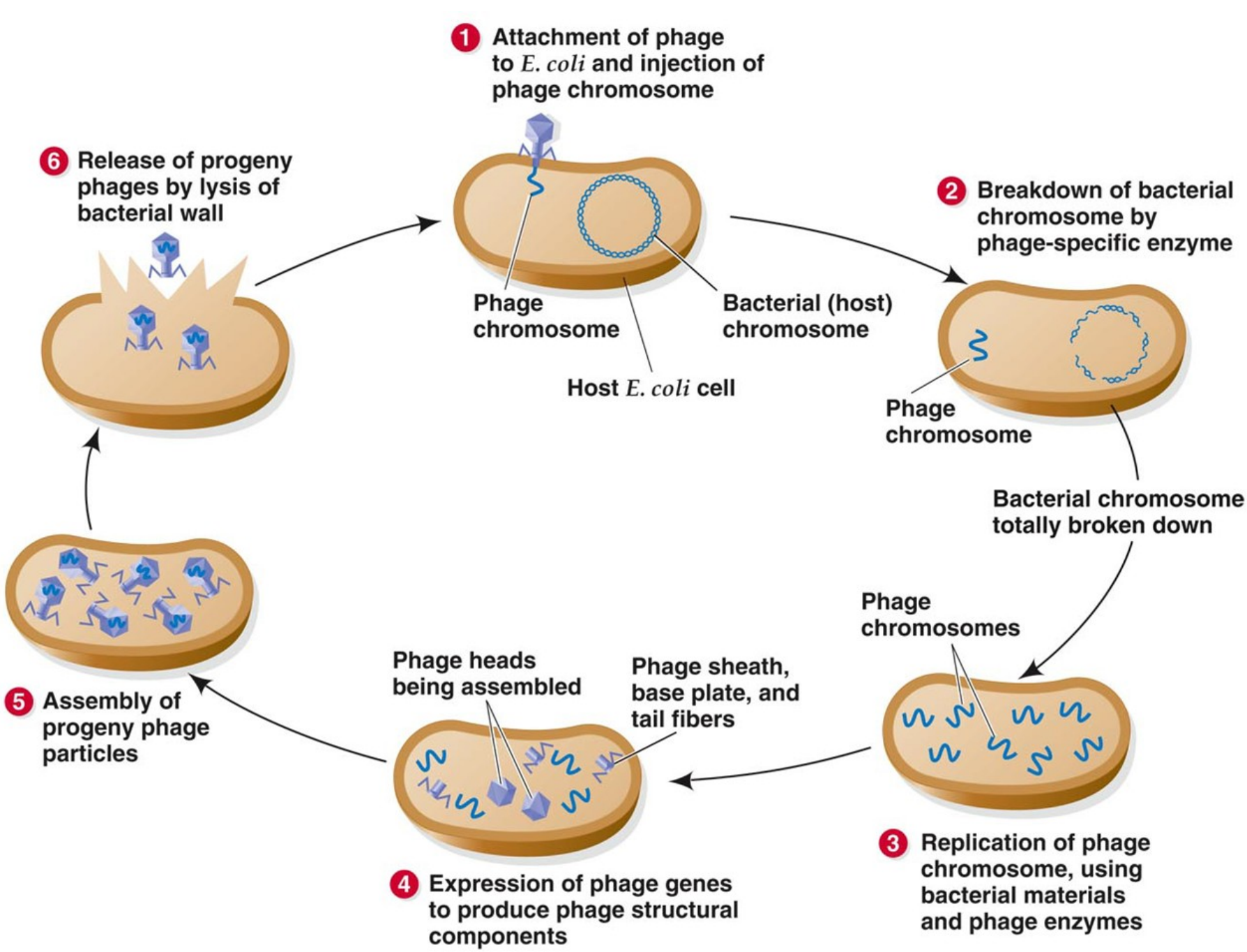

M13 phage
Pilus
M13 DNA
M13 phage attaches to a pilus on an *E.coli* cell and injects its DNA
M13 DNA replication
New M13 phages are continuously extruded from an infected cell
M13 phages
M13 DNA molecules
Infected cells continue to grow and divide
Daughter cells continue to release M13 particles

RNA Phages and Lytic Life Cycle

- Most of them are ssRNA viruses
- Examples include:
  - Family Leviviridea
  - They are small, tailless, icosahedral structure and +RNA
- They attach to the side of the F-pilus of *E. coli*.
- Retraction of the pilus enables the phage to attach to the host membrane and eventually gain entry.

- Only the RNA genome enters the host cell while the capsids remains outside.
- The RNA then acts as mRNA and directs synthesis of viral proteins.
- First RNA replicase, which is an RNA-dependent RNA polymerase, copies the +strand to produce a double stranded intermediate (±RNA), which is analogous to (±DNA).

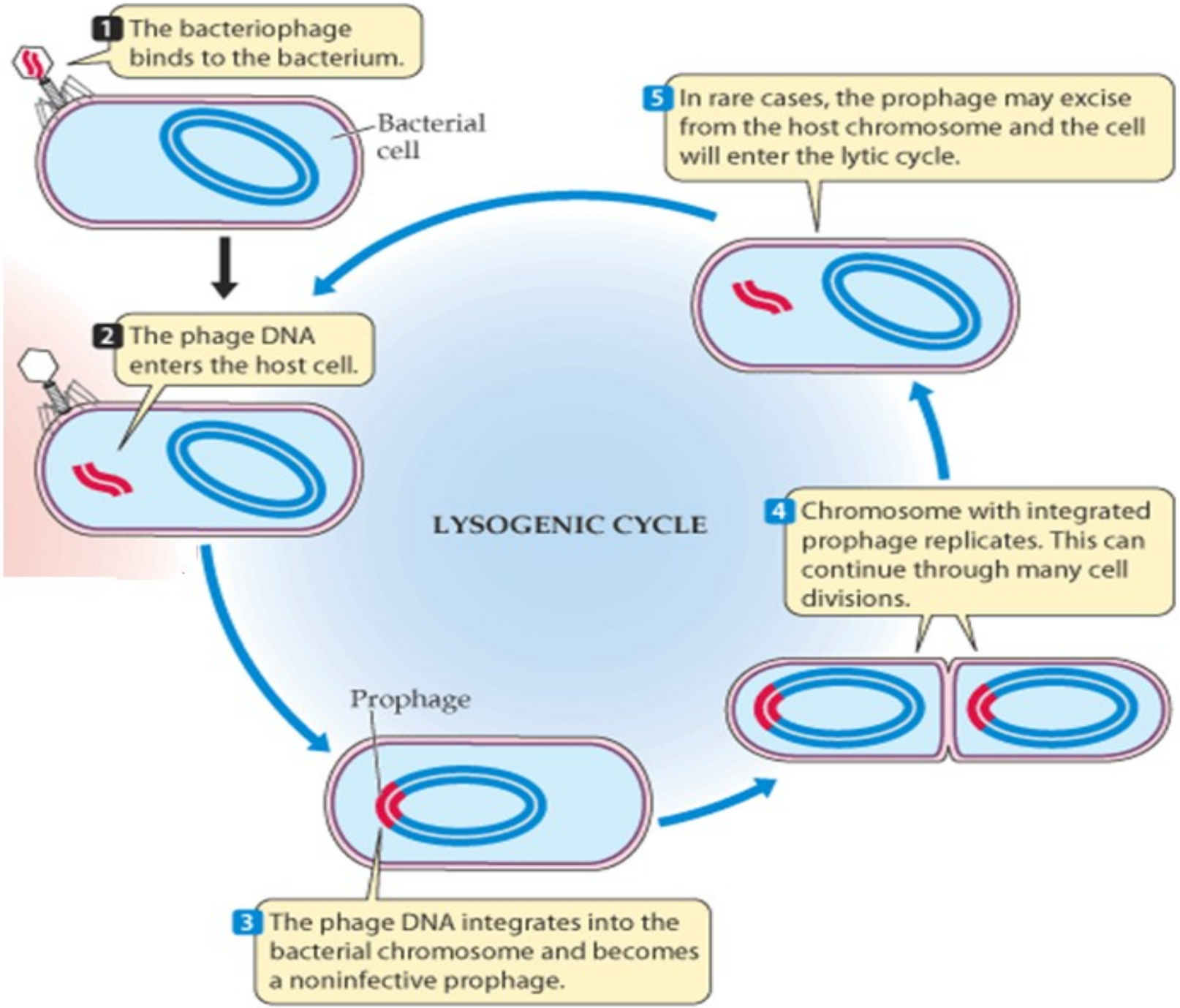
- The resultant (±RNA) serves as a replicative form for the synthesis of more copies of +RNA.
- Other +RNA strands act as mRNA for the synthesis of phage proteins. Mature virions are then released by lysis.


1 Attachment of phage to E. coli and injection of phage chromosome
Phage chromosome
Bacterial (host) chromosome
Host E. coli cell
2 Breakdown of bacterial chromosome by phage-specific enzyme
Phage chromosome
Bacterial chromosome totally broken down
Phage chromosomes
3 Replication of phage chromosome, using bacterial materials and phage enzymes
Phage heads being assembled
Phage sheath, base plate, and tail fibers
4 Expression of phage genes to produce phage structural components
5 Assembly of progeny phage particles
6 Release of progeny phages by lysis of bacterial wall

Temperate Bacteriophages and Lysogenic Cycle

- Most DNA phages are temperate bacteriophages
- They have two reproductive options:
  - Reproduce by lysis of their host cell as virulent phages do OR
  - They remain within host cell without destroying the host.
- This is accomplished by integration of viral genome into the host cell's chromosome.

- Relationship between a temperate phage and its host is called lysogeny.
- Viruses that remain within the host are called prophage and the infected bacteria are called lysogens or lysogenic bacteria.


1 The bacteriophage binds to the bacterium.
Bacterial cell
2 The phage DNA enters the host cell.
LYSOGENIC CYCLE
3 The phage DNA integrates into the bacterial chromosome and becomes a noninfective prophage.
Prophage
4 Chromosome with integrated prophage replicates. This can continue through many cell divisions.
5 In rare cases, the prophage may excise from the host chromosome and the cell will enter the lytic cycle.

- Lysogenic bacteria are:
  - Can not be re-infected by the same virus i.e. Posses immunity to superinfection
  - Under certain conditions they can lyse and release phage particles.
  - This happens when conditions within the cell forces prophage to syntheisze proteins and assemble new virions, a process called induction.

- A temperate phage can also induce a change in the phenotype (change in surface characteristics) of its host, a process called lysogenic conversion.
- Lysogenic conversions can confer pathogenic properties to the host.
- This has been seen in epsilon phage infection of *Salmonella* and phage β infection of *Corynebacterium diptheriae.*

Importance of Bacteriophages

- Phages are important in industry and medicine i.e. Many phages destroy gram-positive lactic acid bacteria that are critical in to the production of fermented milk products e.g. Yogurt and cheese.
- Bacteriophages can also carry virulent factors that convert their bacterial hosts to pathogens e.g. *Streptococcus pyogenes; Staph. Aureus; Corrynebacterium diptheria; Vibrio cholerae, E. Coli O157:H7 and Salmonella enterica.*

HIV Biology and Replication Cycle

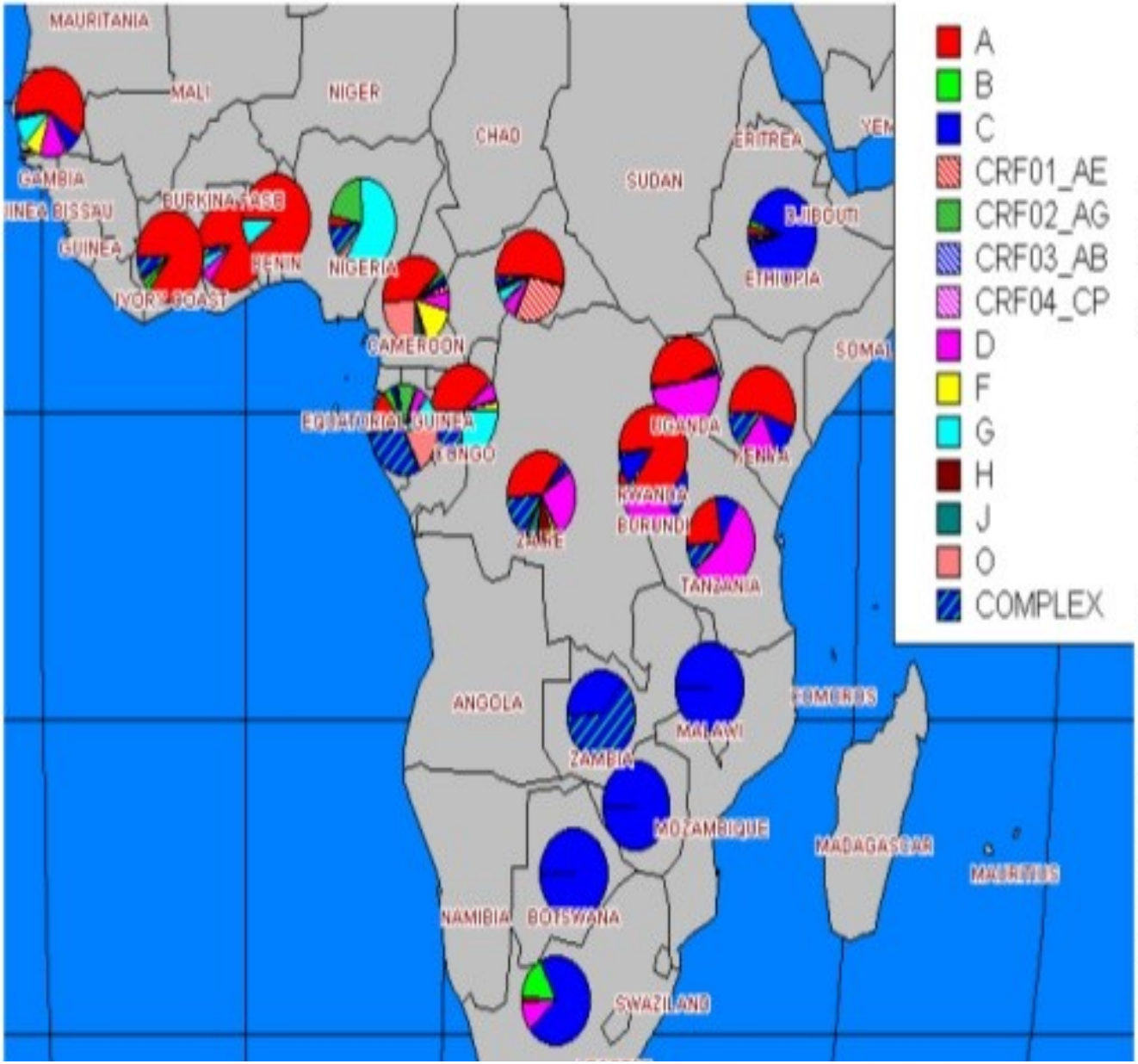
- HIV is from the Lentivirus family. Genetic material consists of single-stranded ribonucleic acid (RNA).
- Viral particle is spherical in shape with a diameter of 80 – 100 nanometers (nm).

Biology of HIV

- There are two types of HIV
- HIV -1
  - Is found worldwide and is the main cause of world pandemic. Pandemic is a widespread epidemic distributed or occurring widely throughout a region, country, continent, or globally.

- HIV – 2
  - Is mainly found in West Africa, Mozambique and Angola.
  - Causes a similar illness to HIV – 1.
  - It is less efficiently transmissible rarely causing vertical transmission.
  - Less aggressive with slower disease progression.

HIV-1 Subtype Distribution in Africa



East and Central Africa has mainly subtype A and D.

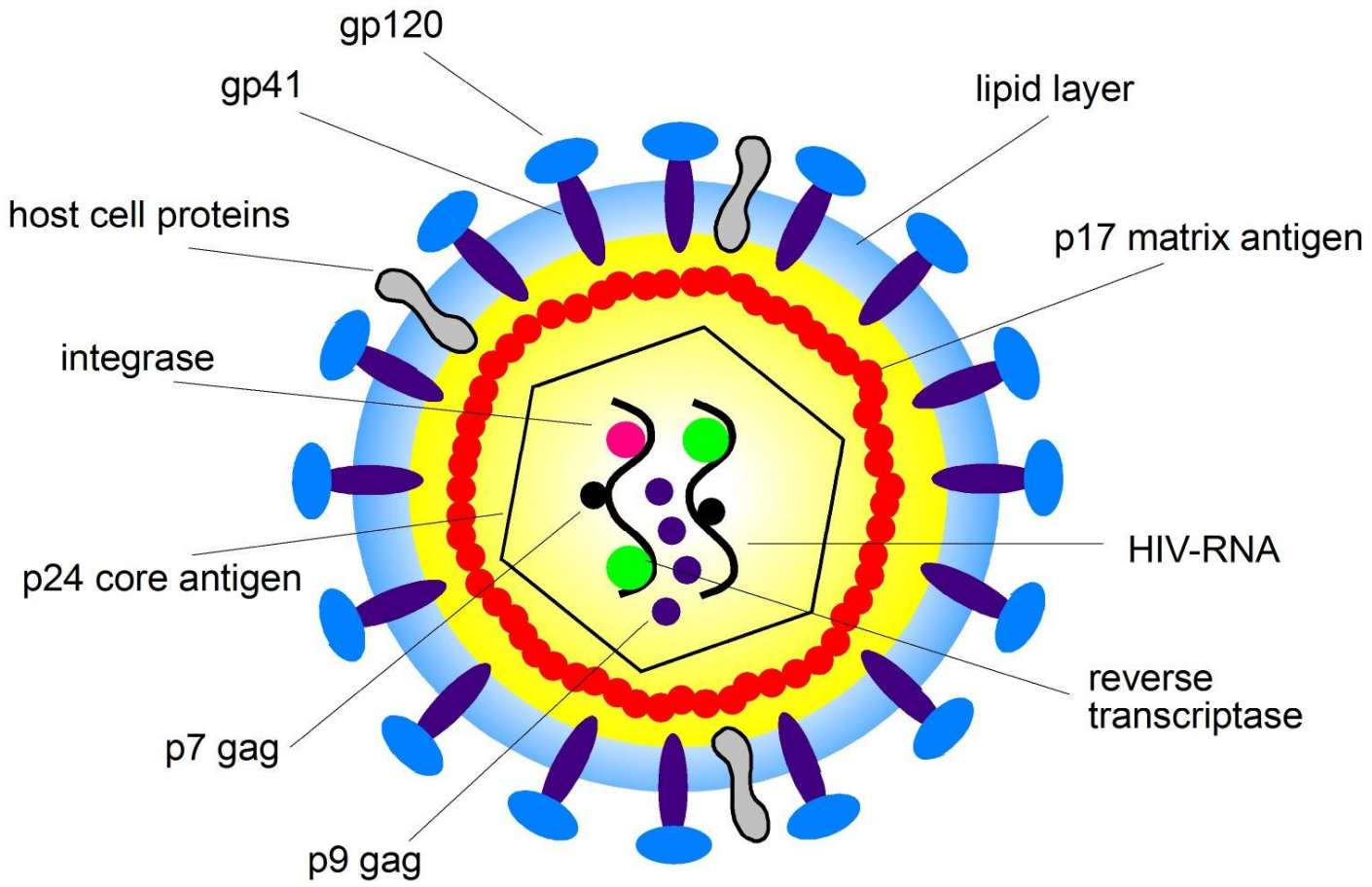
Southern Africa mainly subtype C.

West Africa mainly A

Different subtypes can combine to form diverse recombinants.

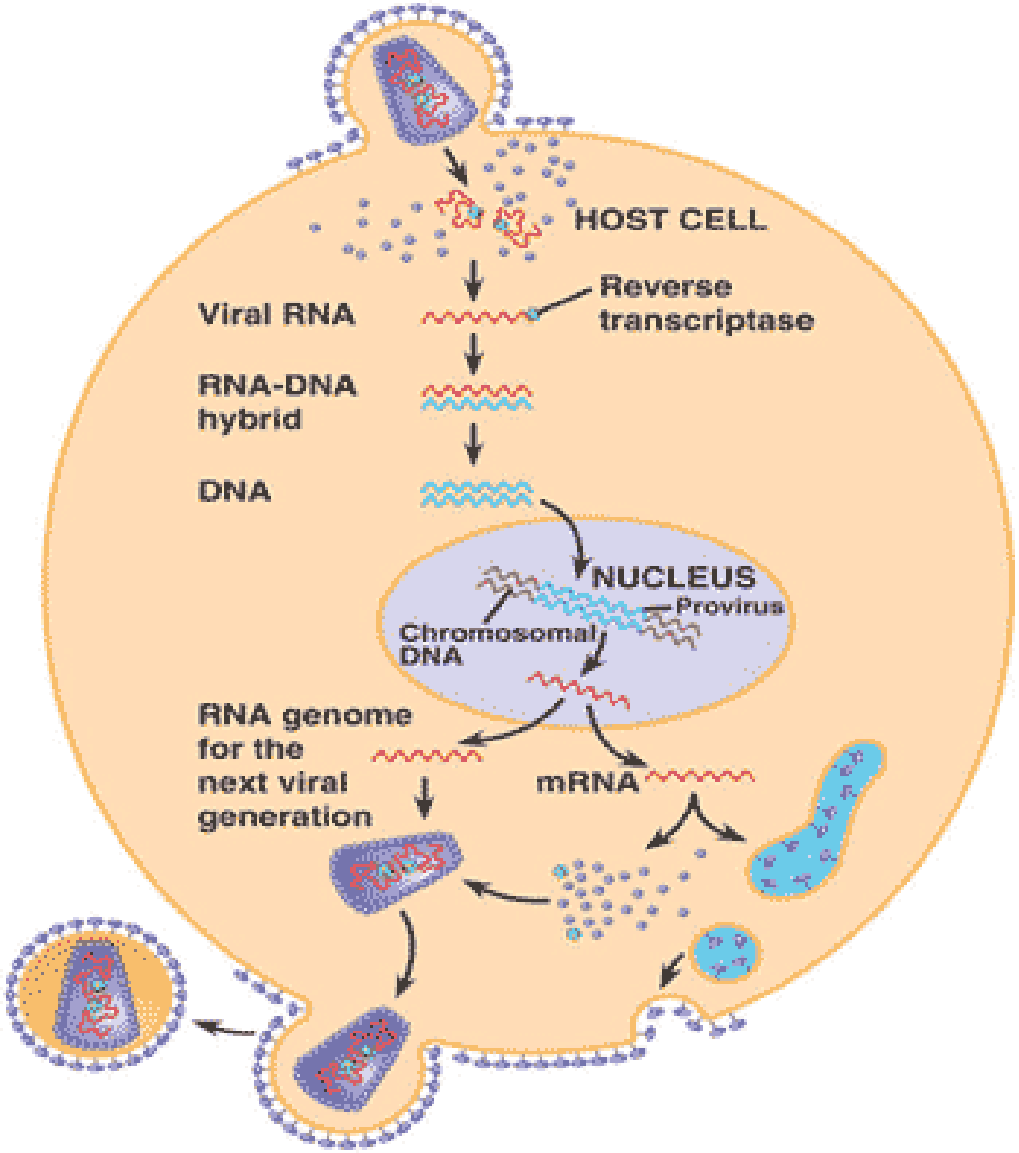
Structure of HIV

- It has an outer double lipid membrane, derived from the host membrane.
- The lipid membrane is lined by a matrix protein.
- The lipid membrane is studded with the surface glycoprotein (gp 120) and the transmembrane protein gp41.
- These glycoprotein spikes surround the cone-shaped protein core.


gp120
gp41
lipid layer
host cell proteins
p17 matrix antigen
integrase
HIV-RNA
p24 core antigen
reverse
transcriptase
p7 gag
p9 gag

- HIV glycoproteins
  - The gp120 and gp41 mediate the entry of virus into the host cells.
- The core (capsid) is made up of several proteins:
  - P24 the main protein
  - Within the capsid are:
    - two identical single RNA – the viral genetic material.
    - Viral enzymes

- Viral enzymes
- Most important are:
  - Reverse Transcriptase (RT),
  - Protease and
  - Integrase.
- RT converts viral single stranded RNA into double stranded deoxyribonulceic acid (DNA).
- DNA is incorporated into the host nucleus as the proviral DNA (Initaail viral DNA).



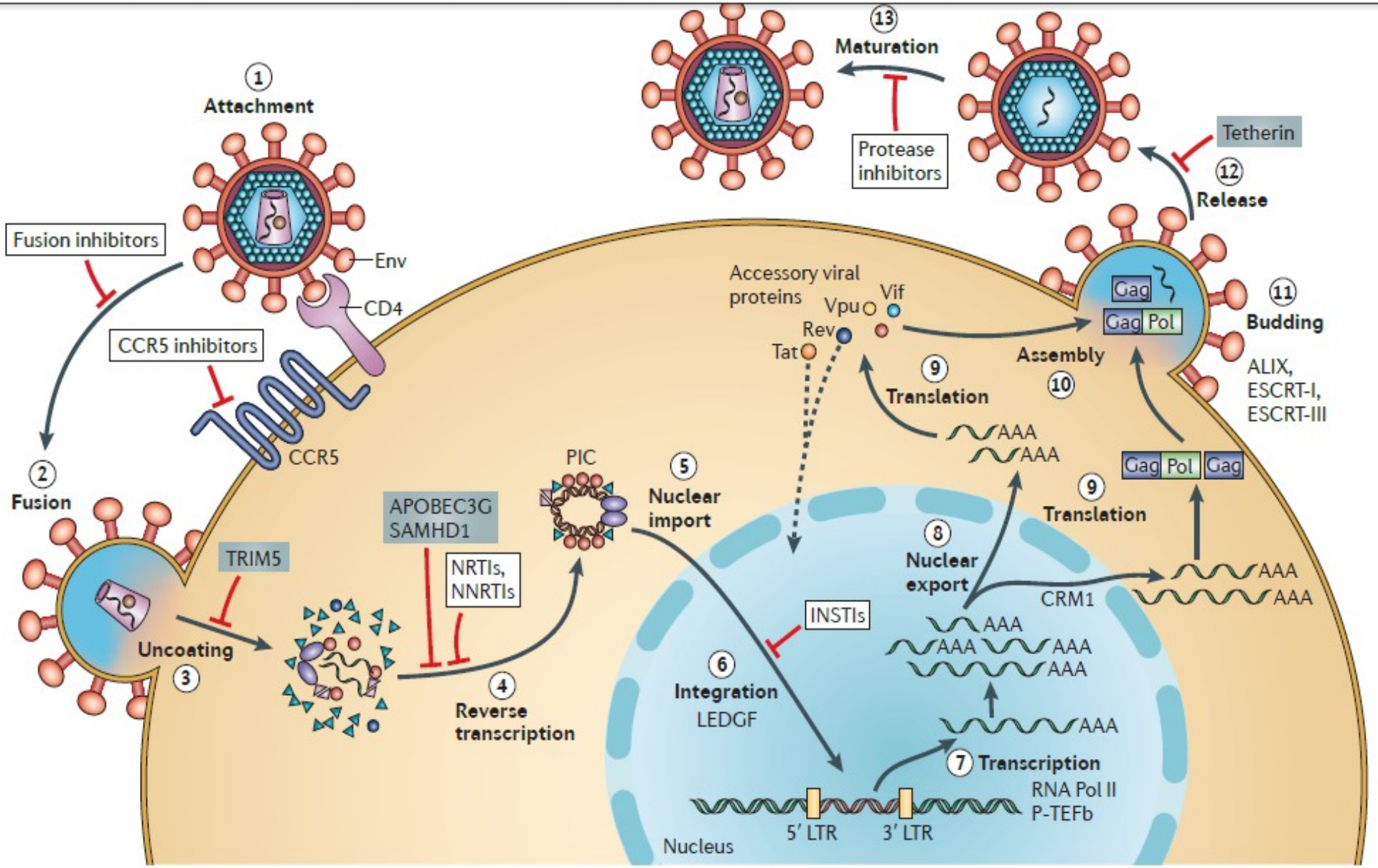
Conversion of Viral RNA into double stranded DNA.

- Integrase facilitates integration of the DNA into the host’s chromosomal DNA.
- Protease enzyme splits generated macro-proteins into smaller viral proteins such as core, envelope and regulatory proteins and enzymes, which go into forming new viral particles.

HIV Life cycle/Replication: The infection process

- Hi life cycle can be summarized into:
  - Binding, fusion and Entry
  - Transcription
  - Integration and Replication
  - Budding
  - Maturation

Schematic overview of HIV-1 replication cycle.

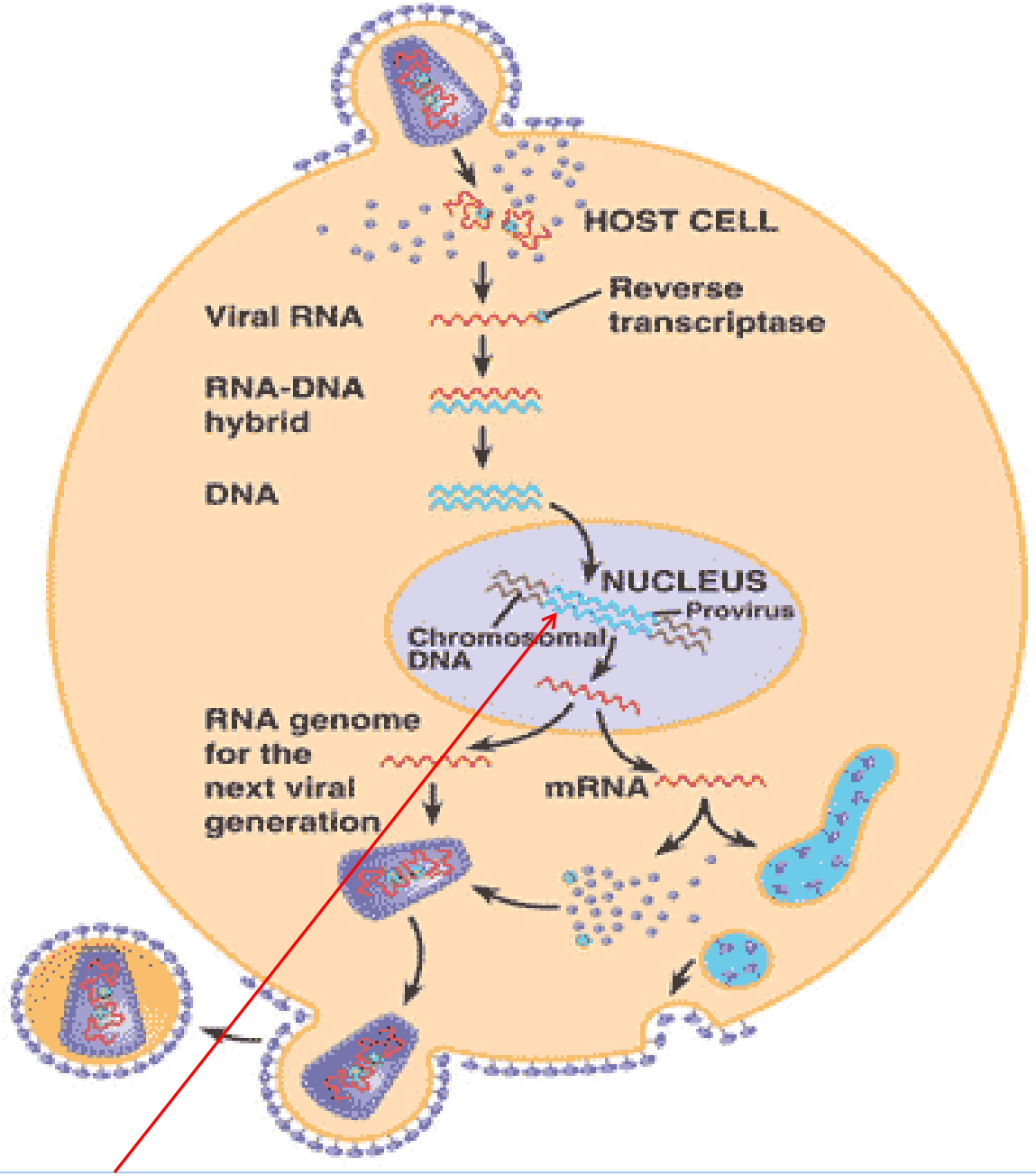


HIV-1 Replication Cycle: The infection

- **Binding**
  - The infection begins when the envelope (Env) glycoprotein spikes engage the receptor CD4 and the membrane-spanning co-receptor CC-chemokine receptor 5 (CCR5/CXCR4).
- **Fusion**
  - Involves fusion of the viral and cellular membranes and entry of the viral particle into the host cell. Fusion is mediated by gp41.

- Here there is partial core shell uncoating., allows entry of viral core into host cytoplasm. Core protein is dissolved by the host enzymes releasing viral RNA and enzymes.

- **Integration**
  - Reverse Transcriptase converts the viral RNA into a DNA molecule.
  - The DNA enters the host cell nucleus.
  - Integrase catalyses the process of integration of the viral DNA into the host cell's DNA to form a provirus.



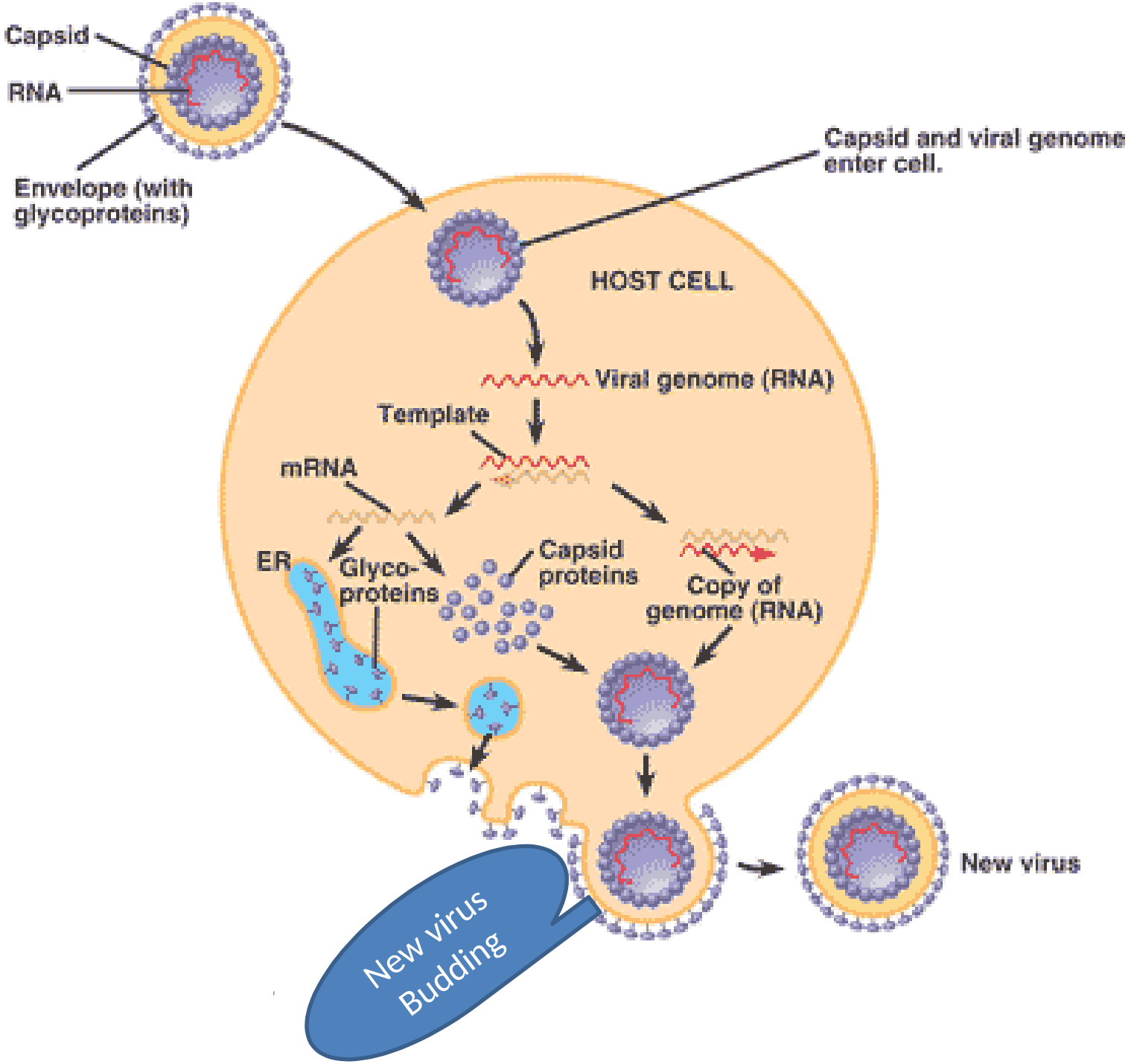
Integration of the viral DNA into the host cell's DNA

- **Replication**
  - Integrated viral DNA turns the host cell into a factory" for manufacturing more virus particles.
  - Viral proteins are produced as a single multi-protein molecule.
  - Viral proteins are then cleaved (cut) by protease enzyme.

- Large sized mRNAs which require energy-dependent export mechanism are exported to leave the nucleus via host protein CRM1 .
- The mRNAs serve as templates for protein production such as Tat, Rev, vpu and vif.
- Genome-length RNA is incorporated into assembled viral particles with protein components such as Gag and Pol.

- **Budding and Maturation**
  - Viral proteins together with RNA gather at the membrane of the CD4+ cells
  - Viral particles are formed which bud off the cell and enter the blood stream.
  - The CD4+ cells are often destroyed by HIV virus infection and replication resulting in profound immunodeficiency.

- The already budding viral particles are released from the cell, a process mediated by endosomal sorting complex required for transport (ESCRT) complexes and is accompanied or soon followed by protease-mediated maturation to create an infectious viral particle.


Capsid
RNA
Envelope (with glycoproteins)
Capsid and viral genome enter cell.
HOST CELL
Viral genome (RNA)
Template
mRNA
ER
Glyco-proteins
Capsid proteins
Copy of genome (RNA)
New virus
New virus Budding

HIV life cycle and Sections Targeted by ARVs

- Nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) target the reverse transcription step that converts the viral genomic RNA into linear double-stranded DNA.
- Protease inhibitors inhibit the protease activity that is critical for the maturation of viral particles which bud from infected cells.

- Two different inhibitors can block entry of the virus into new target cells by thwarting either the interaction between the viral envelope glycoprotein gp120 and the co-receptor CC-chemokine receptor 5 (CCR5). For instance maraviroc does this kind of inhibition.
- The formation of the six-helix bundle of transmembrane glycoprotein gp41 leads to blocking of fusion between the viral and cellular membranes. Enfuvirtide does this kind of inhibition.

- The sole integrase strand transfer inhibitor (INSTI), raltegravir, blocks the strand transfer activity of integrase, which is required for insertion of viral DNA into a host cell chromosome.
- Highly active antiretroviral therapy (HAART) routinely prescribes nucleoside reverse transcriptase inhibitor (NRTI), non-nucleoside reverse transcriptase inhibitor (NNRTI) and a protease inhibitor as a single pill or in various pill combinations.

- This combinatorial approach to drug treatment significantly suppresses the probability of selection for, and resulting outgrowth of, resistant HIV-1 strains that quickly arise during monotherapy.

- Assignment:
  - Discuss the economic importance of viruses

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