HIV INFECTION: An Overview

UNIVERSITY OF PAPUA NEW GUINEA
SCHOOL OF MEDICINE AND HEALTH SCIENCES
DIVISION OF BASIC MEDICAL SCIENCES
DISCIPLINE OF BIOCHEMISTRY & MOLECULAR BIOLOGY

PBL MBBS II SEMINAR

VJ TEMPLE

What is HIV?

- HIV: Human Immunodeficiency Virus;
- HIV belongs to group of viruses called Retrovirus;
- Re-Tro-Virus:
  - **Re** = Reverse,
  - **Tr** = Transcription (Viruses that reversibly transcribe their genetic material, from **RNA ====➔ DNA**)
- HIV causes AIDS;
- HIV damages the immune system in humans,
- Two types of HIV: **HIV-1** & **HIV-2**; both types of HIV cause AIDS;

What is AIDS?

- **AIDS**: Acquired Immune Deficiency Syndrome;
- **AIDS** is collection of signs and symptoms (Syndrome) resulting from many diseases in a patient with HIV;
- HIV specifically affect the T-Helper Cells,
- HIV breaks down the immune system exposing the patient to “Opportunistic Infections”;

How long after HIV infection does an individual develops AIDS?

- **HIV is a Lentivirus**,
- Patient recently infected with HIV may not develop AIDS immediately;
- In some individuals:
  - Decline in T-cell counts and opportunistic infections that signal AIDS may develop soon after HIV infection;
- Most individuals may not develop symptoms for 10 to 12 years after infection,
- A few individuals may remain free of symptoms for much longer;

How is HIV related to AIDS?

- HIV mainly enters the White blood cells (Immune cells);
- Over time HIV causes progressive damage of T-helper cells (CD4 cells);
  - Reducing ability of the immune system to protect the body from infections;
- Ultimately patient becomes vulnerable to various opportunistic infections and other diseases;

- According to WHO and Centers for Disease Control and Prevention (CDC USA) Clinical diagnosis of AIDS may be made if a patient:
- Tested positive for HIV (using approved confirmatory test methods),
- Meets one or both of the following conditions:
  - Presence of one or more AIDS-related infections or illnesses;
  - CD4 (T-cell) Count fallen below 200 Cells per cubic millimeter of blood (CD4 count ranges from 450 to 1200 in healthy individuals);

IMPORTANT TO NOTE

- Some AIDS related infections or illnesses include:
  - Fungus infection – Candidiasis (Thrush),
  - Skin infections,
  - TB,
  - Herpes Zoster (Shingles),
- Patient may have frequent Diarrhea,

What is the basic structure of HIV?

- **Viral Envelope** (**VE**): lipid bilayer that forms the outer membrane of HIV;
- Embedded in VE are complex HIV proteins **Env** (**Spikes**);
- Each Env is made up of:
  - Glycoprotein 120 (**GP 120**), and
  - Transmembrane Glycoprotein 41 (**GP 41**);
- Within VE is **Matrix** made up of Glycosaminoglycans (**GAG**) and Viral Protein (**P 17**);
- Within Matrix is **Viral Capsid** made up of subunits: **Capsomeres** (Viral Protein, **P 24**);

- Within Capsid are **Two Single Strands (Diploid) HIV RNA (Viral RNA Genome)**,
- Each Viral RNA has complete copy of **HIV genes**;
- Within Capsid are **Three Enzymes**:
  - **Reverse Transcriptase (P 66),**
  - **Integrase (P32),**
  - **Protease (P 11)**;
- Fig. 1: Schematic diagram of basic HIV structure

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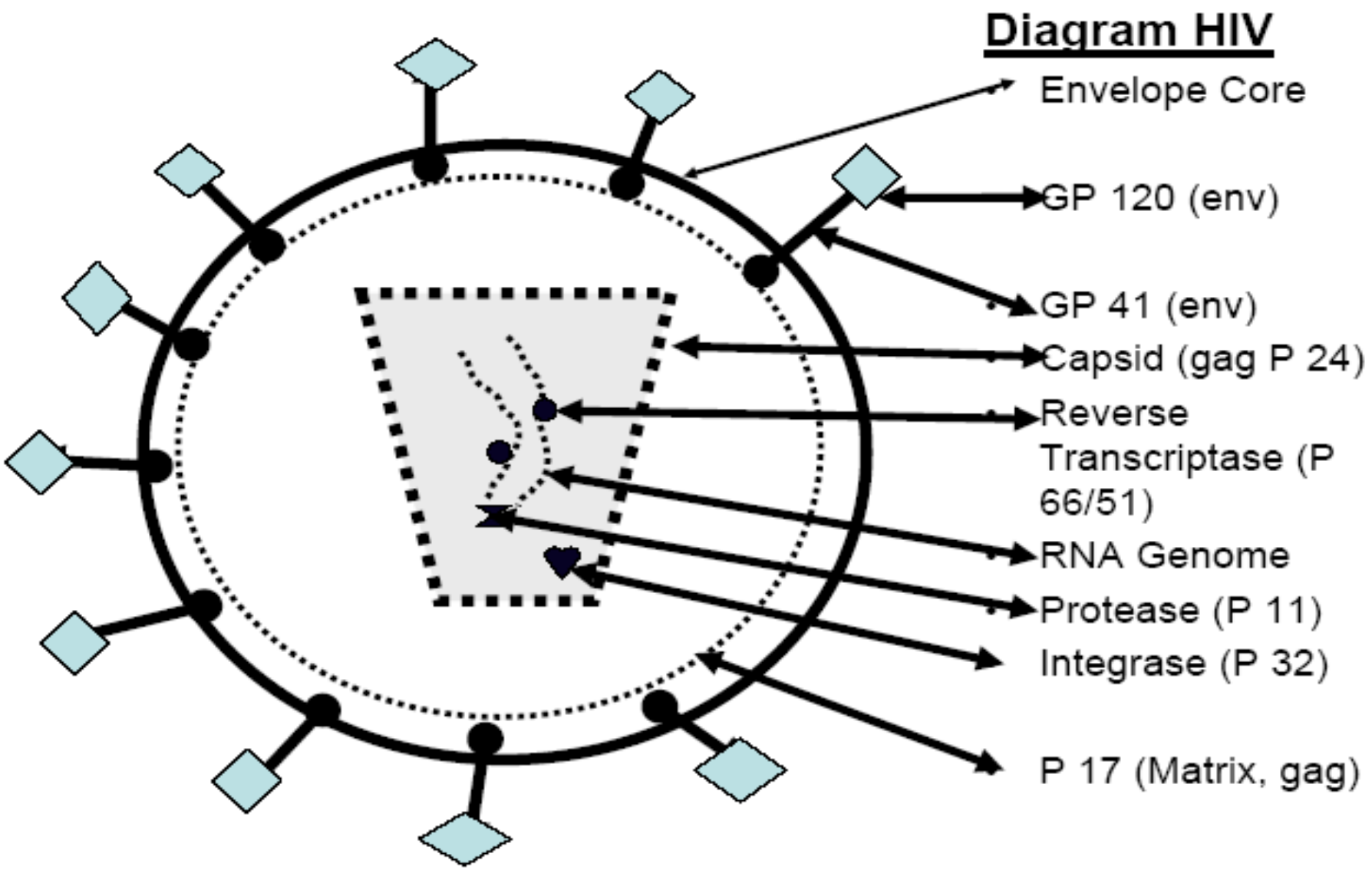


What is the basic structure of the Genome of HIV?

- HIV genome consists of **2** (Diploid) **RNA** Molecules (9.8kilobases in length),
- Genome contains **9 different genes**:
  - **Three Structural Genes,**
  - **Six Regulatory Genes,**
- The **3 Structural Genes** encode for major structural proteins: **Gag, Pol, Env;**
    - **To make structural proteins for new virus particle**
- Example: **Env** gene codes for a large protein **GP 160** that is cleaved by viral enzyme to form **GP120** and **GP41**, which are components of Viral Envelope;

- The **6 Regulatory Genes** encode for:
  - Regulatory Proteins: **Tat and Rev,**
  - Accessory Proteins: **Nef, Vif, Vpr, Vpu**;
- **Nef** (Negative Factor) encoded protein is necessary for HIV to replicate efficiently;
- **Vpu**-encoded protein influences release of new virus particles from infected cells;
- **Vif** encoded Protein interacts with antiviral defense protein in host cells (APOBEC3G),
  - It causes inactivation of antiviral effect, thus enhancing HIV replication;

- Both ends of each Strand of Diploid RNA genome contain RNA sequence: **Long Terminal Repeat (LTR)**
- Specific regions in LTR act as switches to control production of new viruses and can be triggered by proteins from either HIV or host cell;

What receptor on host cells interacts with HIV?

- To infect host, genetic material of HIV must enter cells;
- HIV interacts with **Specific Cell Surface Receptor** and **Co-receptors** on host cells;
- **CD4**: major Specific Cell Surface Receptor on host cells,
  - CD4: Large Glycoprotein located on surface of, Helper T cells, Regulatory T cells, Monocytes, Dendritic cells;
- **CD4** is the receptor that assists T Cell Receptor (TCR) to activate T Cell following interaction with Antigen Presenting Cell (APC);
- **CD4**: **Primary Receptor used by HIV to gain entry into Host T Cells**;
- {CD = Clustered Differentiation}

What co-receptors on host cells interact with HIV?

- **Co-receptors** on host cells are: **CCR5** or **CXCR4,**
  - Proteins on surface of Lymphocytes or Monocytes that bind to **GP120** protein of HIV and with CD4 facilitate entry of diploid Viral RNA and proteins into Host cells,
- **CCR5** binds Macrophage-Tropic, Non-syncytium-inducing (R5) Viruses, associated with Mucosal and Intravenous Transmission of HIV infection;
- **CXCR4** binds T-cell-tropic, Syncytium-inducing (X4) Viruses, which are frequently found during the later stages of AIDS;

IMPORTANT TO NOTE

- Naturally occurring deletion of 32 base pairs in CCR5 gene results in Mutant CCR5 Co-Receptor;
  - Individuals homozygous for this mutation are almost completely resistant to HIV infection;
- It indicates the role of CCR5 in spread of HIV and suggests that small molecules that prevent HIV interaction with CCR5 might form promising new class of antiretroviral drugs;

How does Fusion of the HIV with T-Cells occur?

- **GP 120** on envelope binds **CD4** Receptor on Host Cell;
- Co-receptor (CCR5 or CXCR4) on host cell participates in Fusion of GP 120 with CD4 receptor; (**Fig. 2**)
- Fusion separates GP120 from GP 41, thus releasing Spike on GP 41;
- Spike (GP 41) like a spring-loaded lancet pierces the cell membrane of the Host Cell; (**Fig. 3**)
- {**NOTE**: Fusion Inhibitors (T-20 and T-1249) can prevent Fusion by blocking conformational changes resulting in the release of the spike}

Fig. 2: Interaction of HIV with target cell in Host

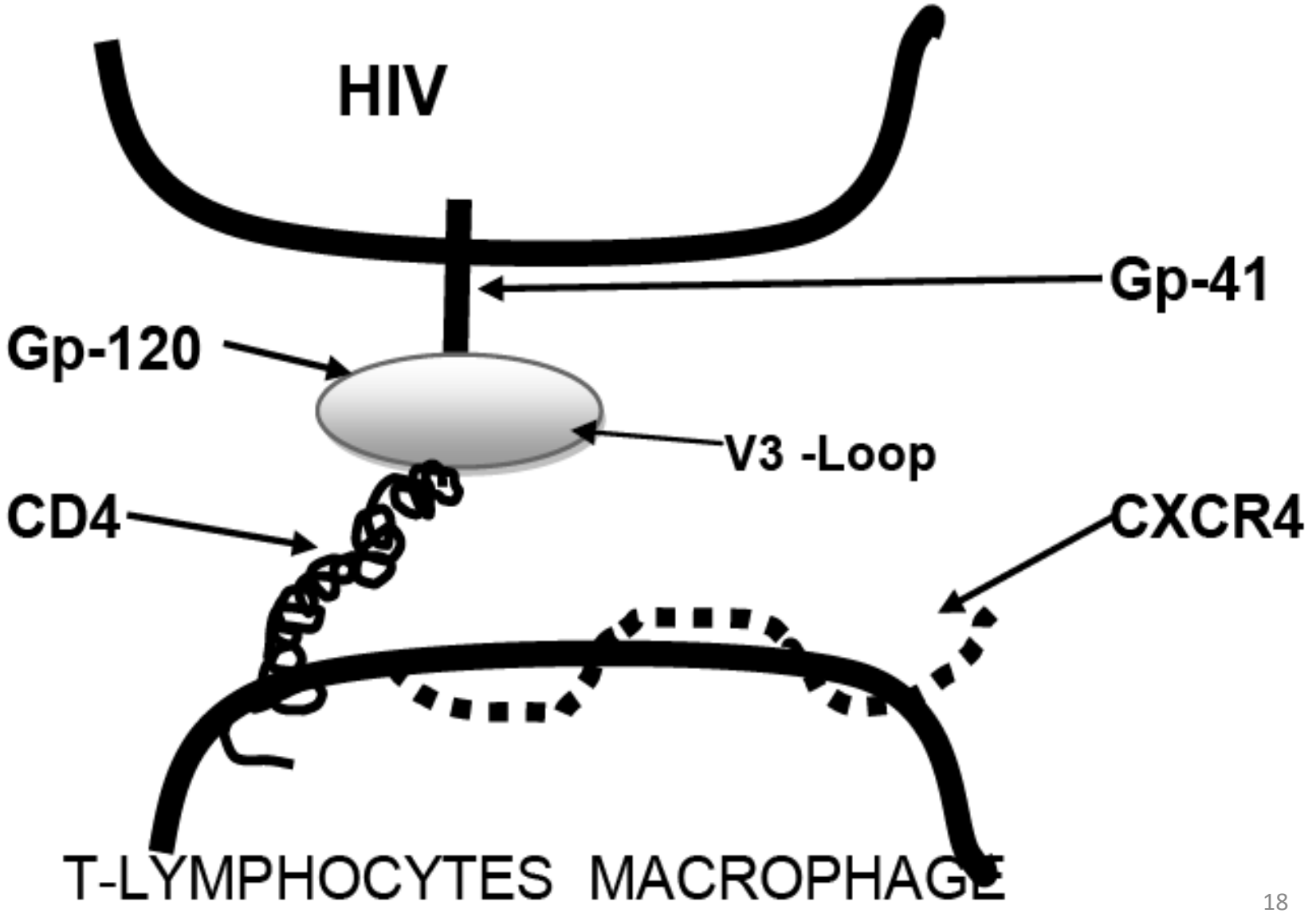
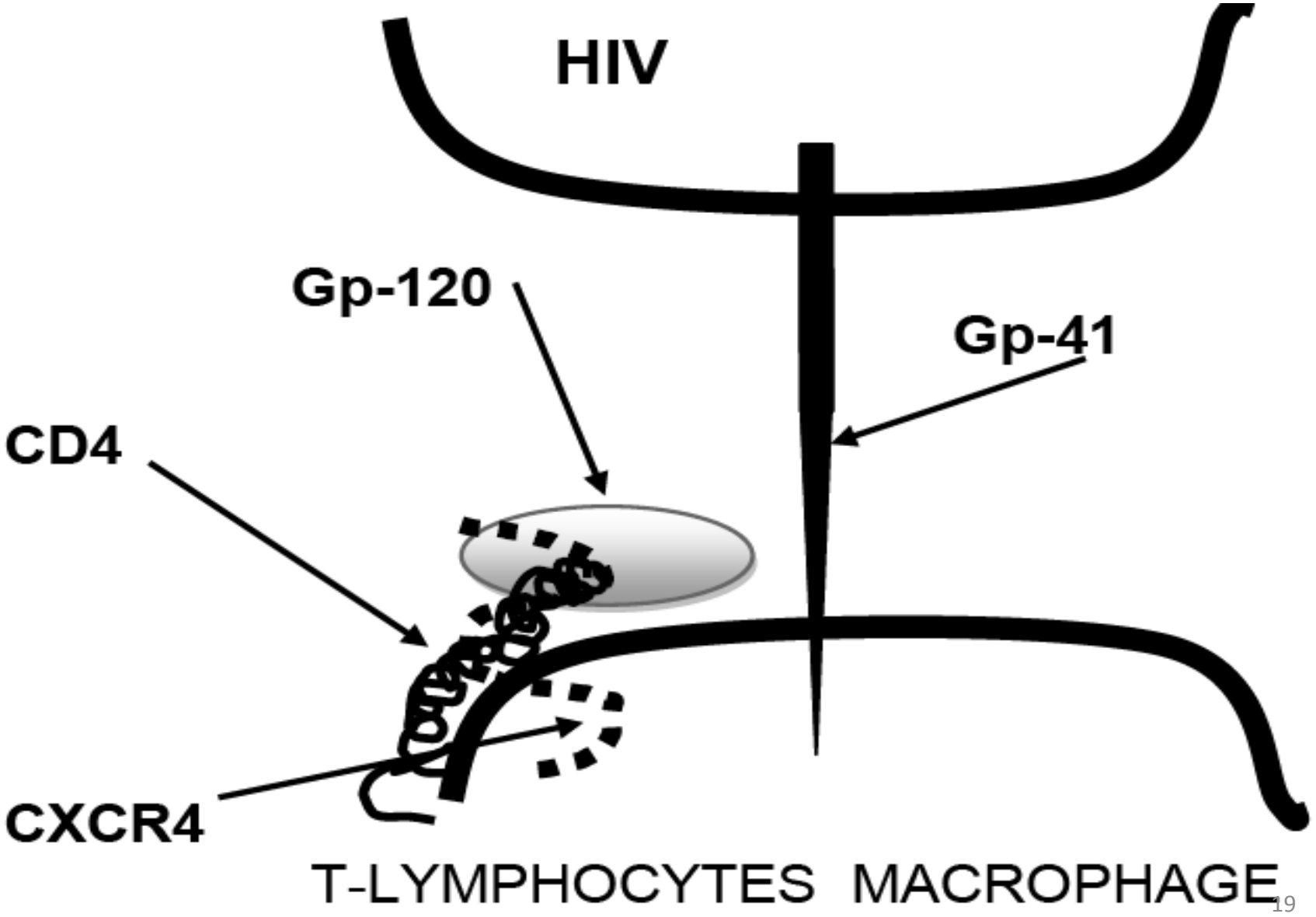


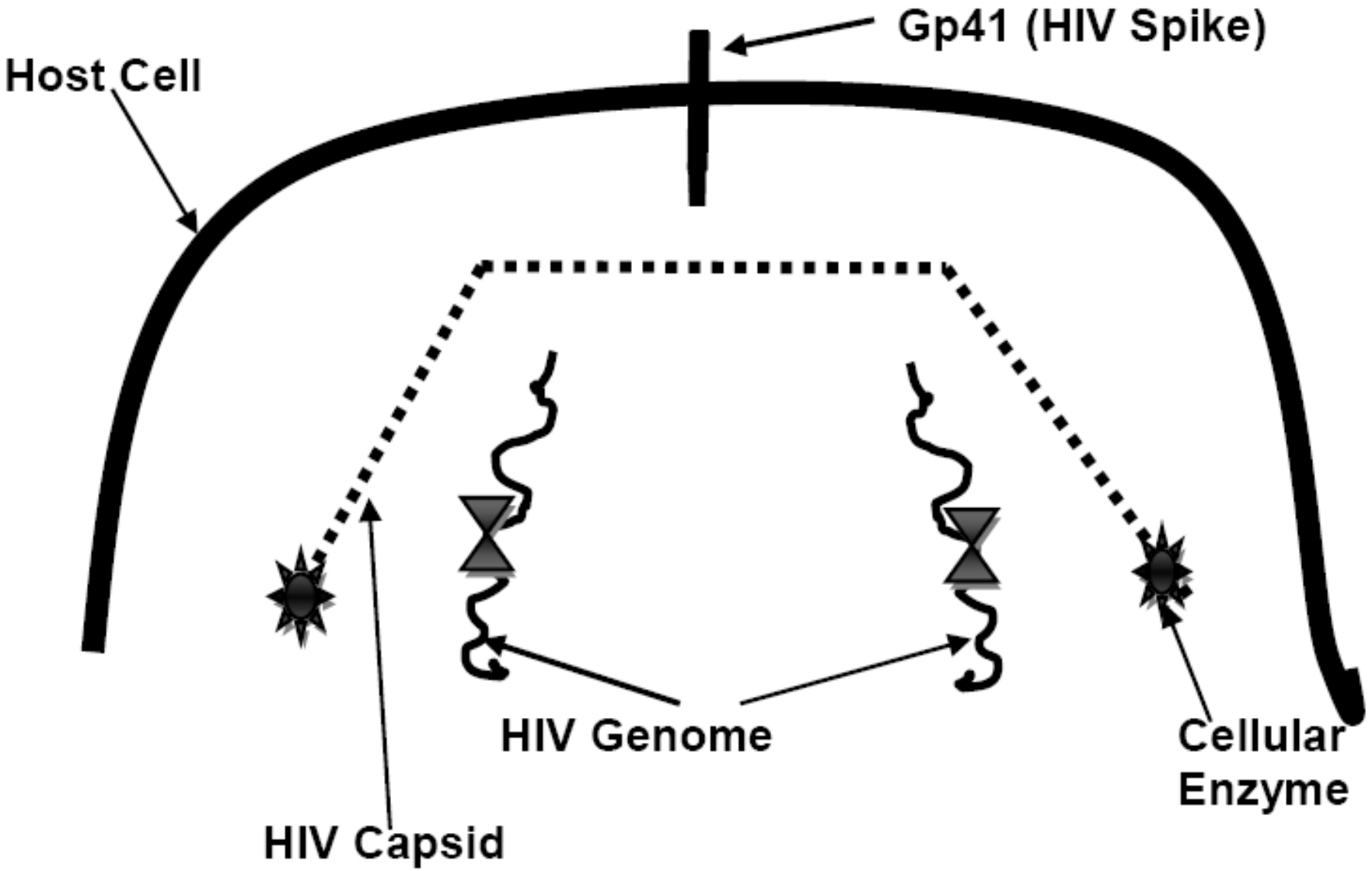
Fig. 3: Fusion and release of HIV Spike



How does the viral genome enters the Host cell?

- Piercing of Host membrane by GP 41 is followed by injection of HIV Capsid into Cytoplasm of Host cell,
- Viral Envelope does not enter the Host cell,
- Host enzymes hydrolyze Viral Capsid, releasing contents:
  - Diploid **Viral RNA genome**,
  - **Lysine Transfer RNA** (tRNA-Lys)
    - Primer for Reverse Transcription,
  - **Viral Reverse Transcriptase (P66),**
  - **Integrase (P32),**
  - **Protease (P11),**
  - **Viral Protein R (Vpr),**
  - **Other Viral proteins**

Fig. 4: Release of HIV Capsid into Host cell



How is HIV genome Reversibly Transcribed?

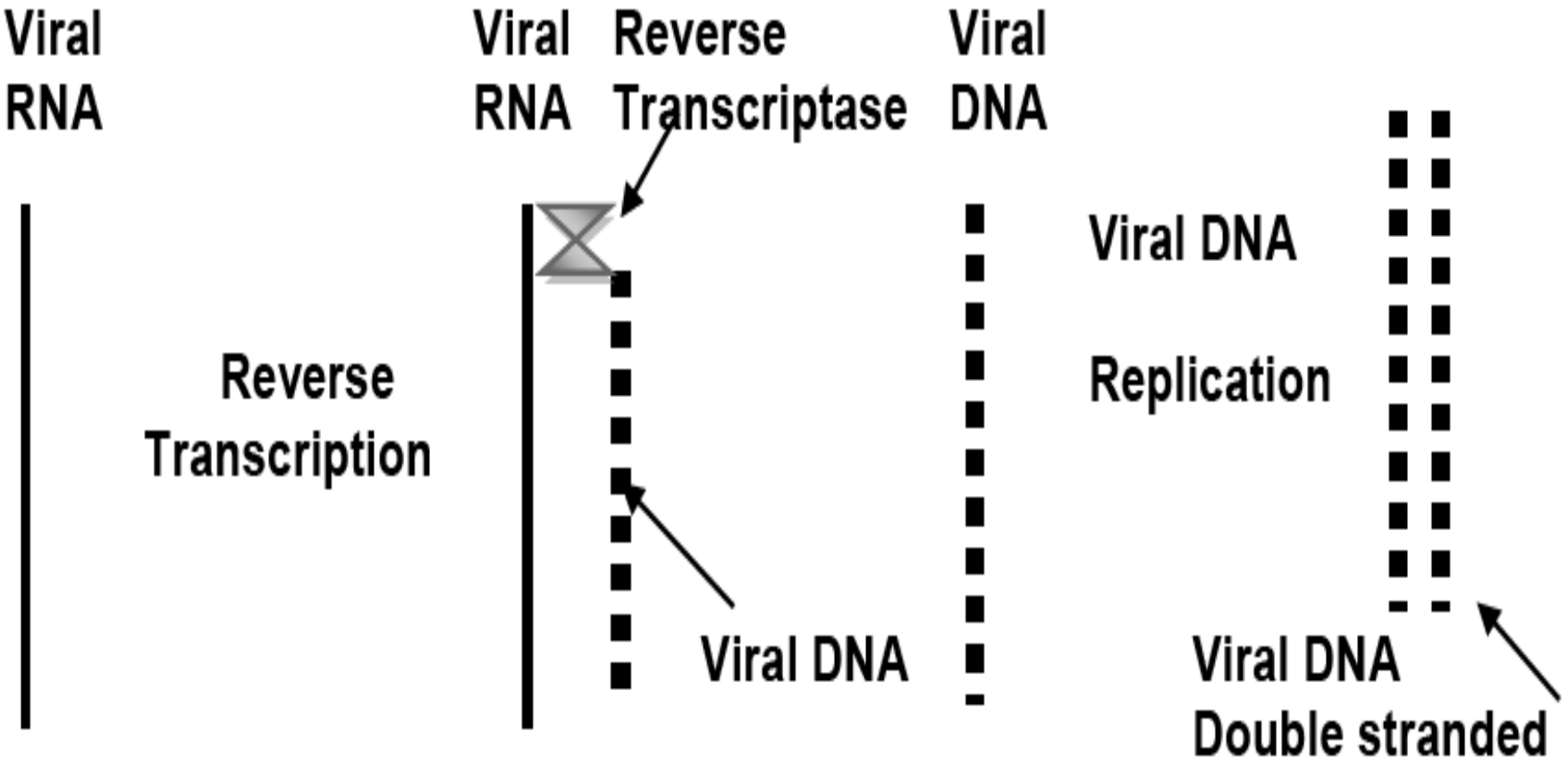
- Reverse Transcription:

  Viral RNA ====➔double stranded viral DNA

- Reverse Transcriptase (**P66**) is a complex viral enzyme that uses one of the Viral RNA strands as template for formation of DNA;
- DNA Polymerase is part of Reverse Transcriptase complex;
- Lysine Transfer RNA (tRNA-Lys) acts as Primer for Reverse Transcription;

- DNA Polymerase uses the Primer to synthesize a Single Stranded DNA copy using the Viral RNA as template,
  - Forming a DNA/RNA hybrid molecule; **(Fig. 5)**
- RNA and DNA are separated;
- RNA template is destroyed by Ribonuclease;
- Single Stranded Viral DNA Replicates forming a Double-Stranded Viral DNA molecule;

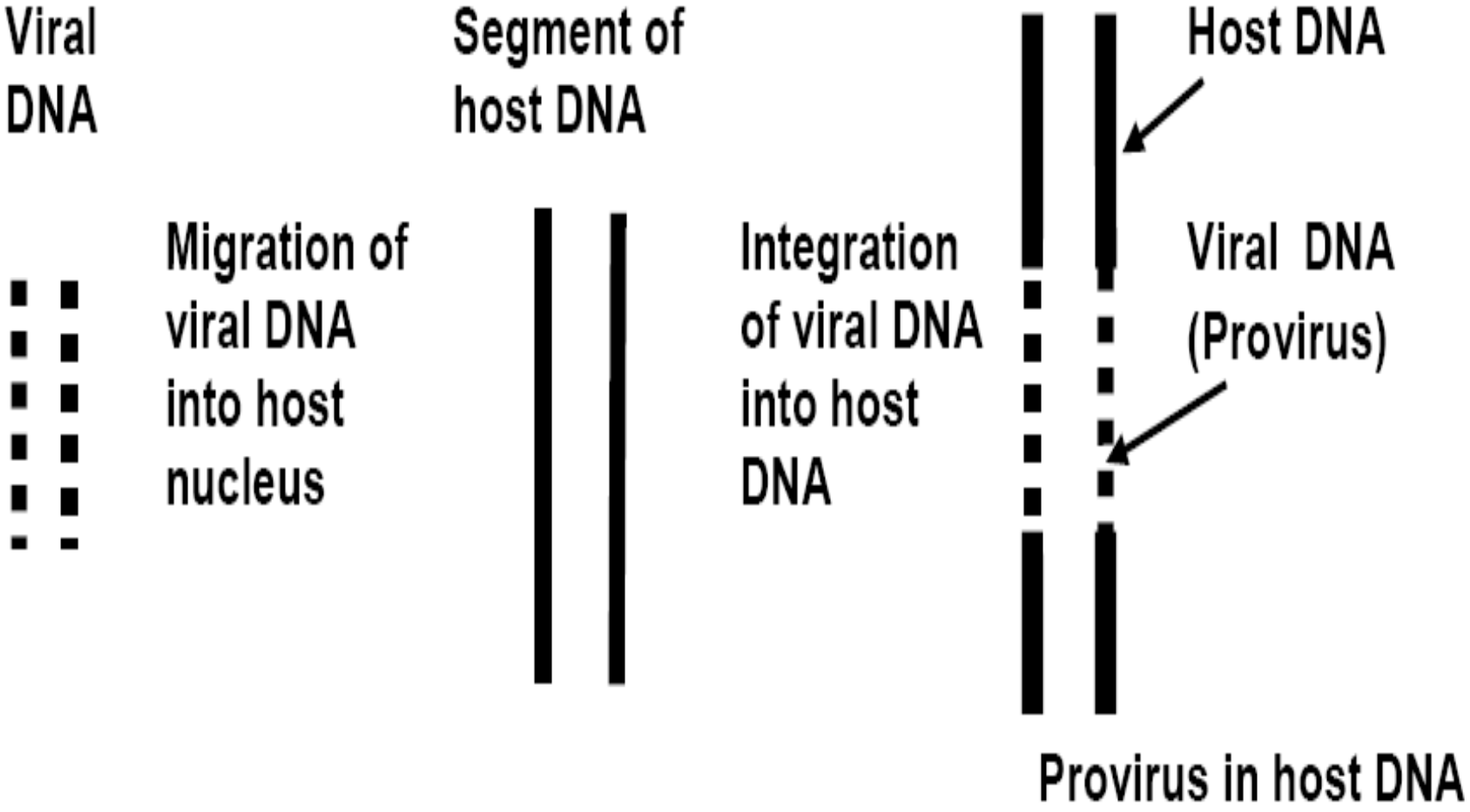
Fig. 5: Reverse Transcription of HIV RNA genome in host



How is Viral DNA Integrated into the Host DNA? (Formation of Provirus)

- Integration of newly formed Double-Stranded Viral DNA into Host DNA is catalyzed by Viral Integrase (**P32**) made up of **three enzymes**;
- Process can be separated thus:
  - Exonuclease removes **2 Nucleotides** from each **3‘-end of the Viral DNA Duplex**;
  - Double-Stranded Endonuclease cleaves Host DNA at integration site;
  - Ligase generates a single covalent linkage at each end of the Proviral DNA, thus forming the HIV Provirus within the Host DNA; (**Fig. 6**)

Fig. 6: Integration of double stranded Viral DNA into host DNA to form Provirus



What is Dormancy period or Transcriptional Latency?

- After formation of Provirus, a relatively long period may occur during which infected individuals show no signs or symptoms and may not be aware of their HIV status;
- **Integration is followed by latent forms of infection or Transcriptional Latency**;
- Transcriptional latency explains inability of antiviral therapies to eradicate HIV from the body;
- **Silent pro-viruses are reservoir for re-emergence of HIV when the body's defenses grow weaker**;
- Understanding latency and developing approaches to target latent virus are essential for control of HIV;

How are new viruses formed from Proviral DNA?

- Activation causes Transcription of Proviral DNA to RNA;
- Multiple copies of Viral RNA are produced and released into the Cytoplasm,
- Viral RNA acting as Messenger RNA attaches to Ribosome in the Host cell for Translation to occur;
- Translation of Viral RNA produces Viral Proteins:
  - Core,
  - Matrix and Envelope proteins,
  - Transmembrane and Control proteins,
  - Reverse Transcriptase,
  - Integrase and Protease;

- Transmembrane Glycoproteins are formed and migrate into host cell membrane;
- Viral particles are assembled via self arrangement of Capsid around Viral RNA and enzymes;
- Each Viral unit buds from host cells, collecting Envelope with GP 120;

How is HIV transmitted from infected person?

- HIV is in body fluids (blood, semen, vaginal secretions, breast milk)
- HIV can be transmitted when these fluids enter the bloodstream of another person;
- Sharing needles or syringes with someone who is HIV infected; (needles or syringes used to inject drugs),
- Other types of needles, such as those used for body piercing and tattoos, can also carry HIV;
- Laboratory studies show that infectious HIV can survive in used syringes for a month or more;
- During childbirth, or breast-feeding;

Laboratory Test for HIV / AIDS

- Laboratory methods can be used for:
  - Screen Blood,
  - Diagnose Infection, and
  - Monitor disease progression
- Tests can be used to:
  - Detect Antibody;
  - Identify Antigen;
  - Detect or Monitor Viral RNA,
  - Estimate T-lymphocyte numbers
    - Cell Phenotyping);

- Tests to detect Antibody to HIV are classified as:
  - **Screening Assays**, designed to detect infected individuals,
  - **Confirmatory** (supplemental) assays, designed to identify individuals who are not infected but who have reactive screening test results;
- **Screening tests** possess a **high degree of Sensitivity**,
- **Confirmatory Assays** have **a high Specificity**;
- Technical errors may occur, and there are biologic factors that can limit the accuracy of HIV tests;
- **Effective QA Programs are needed in all HIV labs**;
- **Lab tests are used to supplement Clinical diagnosis**;

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