RETROVIRUS (HIV) LIFE CYCLE

Presented By
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Retroviruses and Retrotransposons

❖**Retroviruses and related transposable elements utilize the enzyme reverse transcriptase to copy RNA into DNA. The DNA copies of these entities are subsequently inserted at different positions in genomic DNA.**

❖In addition to cut-and-paste transposons such as *Ac*, *P* and *mariner,* **eukaryotic genomes contain transposable elements whose movement depends on the reverse transcription of RNA into DNA by the help of the enzyme reverse transcriptase. These elements are called "Retrotransposons", from a Latin prefix meaning "backward".**

❖Reverse transcription also plays a crucial role in the life cycle of some viruses. The genome of this viruses are composed of single-stranded RNA. **When one of these viruses infects a cell, its RNA is copied into double-stranded DNA. Because the genetic information moves from RNA to DNA, these viruses are called retroviruses.**

Retrovirus

➢The retroviruses were discovered by studying the causes of certain types of tumors in chickens, cats and mice.

➢An important advance in understanding their life cycle came in **1970 when David Baltimore, Howard Temin and Satoshi Mizutani discovered an RNA-dependent DNA polymerase- that is, a reverse transcriptase, which allows these viruses to copy RNA into DNA.**

➢**Human Immunodeficiency Virus (HIV), which causes Acquired Immune Deficiency Syndrome or AIDS, a disease of the immune system . As it progresses, a person loses the ability to fight off infections by an assortment of pathogens, including organisms that are normally benign. Without treatment, infected individuals succumb to these infections, and eventually die.**

➢ **How AIDS transmitted?**

❑ AIDS is transmitted from one individual to another through **bodily fluids such as blood or semen** that have been contaminated with HIV.
❑ AIDS is a major cause of death among subpopulations in many countries- for example, among **intravenous drug users and sex industry workers.**

➢ **What are the Symptoms?**

❑The initial **symptoms of the disease of the disease are flu-like. Infected individuals experiences aches, fever and fatigue**. After a few weeks, these symptoms abate and health is seemingly restored. This asymptomatic state may last several years.

❑ **However the virus continues to multiply and spread through the body, targeting specialized cells (Helper T lymphocyte, T_H) that play important role in the immune system**.

❑Eventually, these cells are so depleted by the killing action of the virus that the immune system fails and **opportunistic pathogens assert themselves. Many types of illnesses, such as pneumonia, may ensue.**

➢ Structure of HIV:

1) **HIV are approximately 100 nm in diameter**. It has a **lipid envelope, in which are embedded the trimeric transmembrane glycoprotein gp41 to which the surface glycoprotein gp120 is attached.** These two viral proteins are responsible for attachment to the host cell and are encoded by the **'*env*' gene** of the viral RNA genome.
2) Beneath the envelope, is the **matrix protein p17, the core proteins p24 and p6 and the nucleocapsid protein p7** (bound to the RNA) all encoded by the viral **'*gag*' gene**
3) Within the viral core, lies **2 copies of the ~ 10 kilobase (kb) positive-sense, viral RNA genome** together with the **protease, integrase (catalyses the integration of HIV genome into host chromosome) and reverse transcriptase (helps in the convertion HIV RNA into double-stranded DNA) enzymes. These three enzymes are encoded by the viral '*pol*' gene.**
4) There are several other proteins with **various regulatory or immuno-modulatory functions**, including ***vif*** (viral infectivity protein), ***vpr*** (viral protein R), ***tat*** (transactivator of transcription), ***rev*** (regulator of viral protein expression), and ***nef*** (negative regulatory factor). An additional protein found in HIV-1 but not HIV-2 is ***vpu*** (viral protein U), similarly ***vpx*** (viral protein X) is found in HIV-2 and not HIV-1.

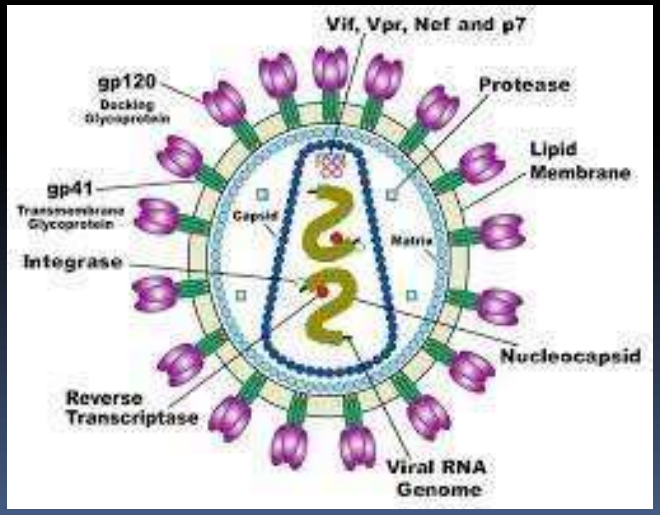


Fig 1: The Structure of HIV

➢ The Life Cycle of HIV:

✓The roughly spherical virus enters a host cell by interacting with specific receptor protein, called **CD4 receptors, present on the CD4 positive T (helper) lymphocyte, macrophages and microglial cells.**

✓ The viral **gp120 (docking protein) binds initially to this CD4 molecule**, which then triggers **a conformational change in the host-cell envelope that allows binding of the co-receptor (either CCR5 or CXCR4) which is required for fusion between virus envelope and cell membrane.**

✓ Inside the cell, the lipid membrane and the protein coat that surround the virus particle are removed, and materials within the virus's core are released into the cell's cytoplasm.

✓**HIV's reverse transcriptase converts single-stranded RNA into double-stranded DNA**, which are then inserted at random positions in the chromosomes of the infected cell, in effect populating the cell's genome with many copies of the viral genome.

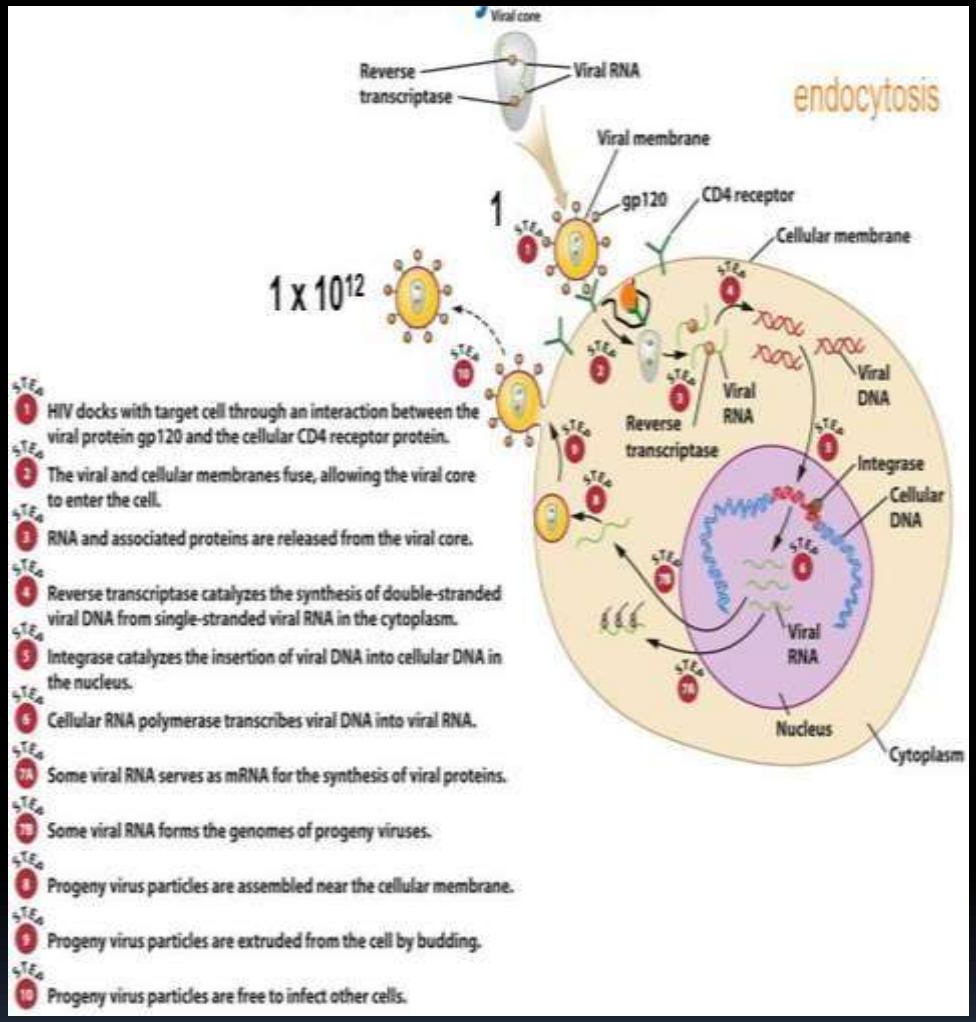


Fig 2: The Life Cycle of HIV

✓These copies can then be transcribed by the cell's ordinary RNA polymerases to produce a large amount of viral RNA, which serves to direct the synthesis of viral proteins and also provides genomic RNA for the assembly of of new viral particles. These particles are extruded from the cell by a process of budding, which further can infect other cells by interacting with the CD4 receptors on their surfaces.

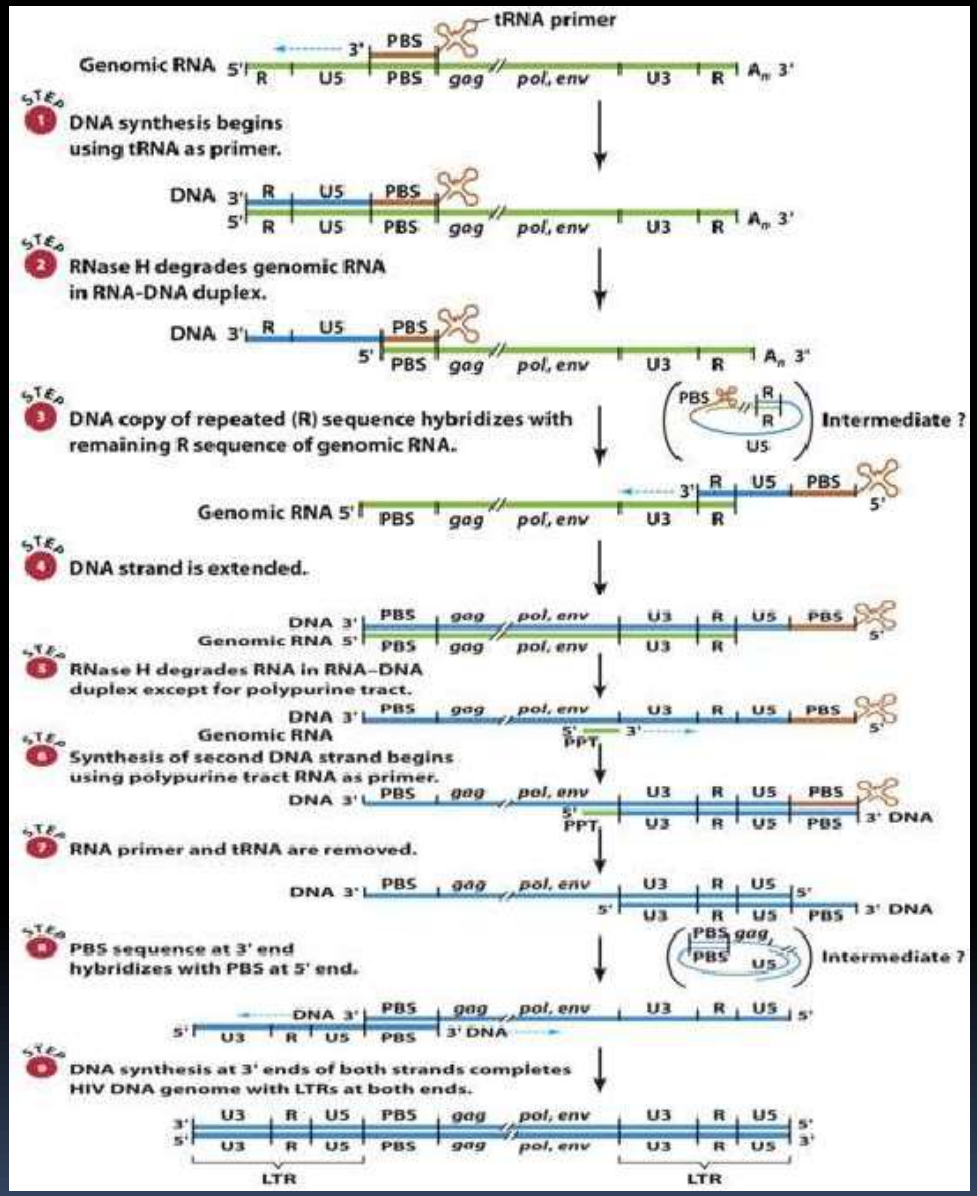


Fig 3: Conversion of HIV genomic RNA into double-stranded DNA. R, repeated sequences; U5, *u*nique sequence near 5′ terminus; U3, *u*nique sequence near 3′ terminus; PBS, *p*rimer *b*inding *s*ite; A_n, poly (A) tail; *gag*, *pol* and *env*, sequences encoding HIV proteins; PPT, polypurine tract rich in adenine and guanine; LTR, *l*ong *t*erminal *r*epeat.

➢ Conversion of HIV genomic RNA into double-stranded DNA:

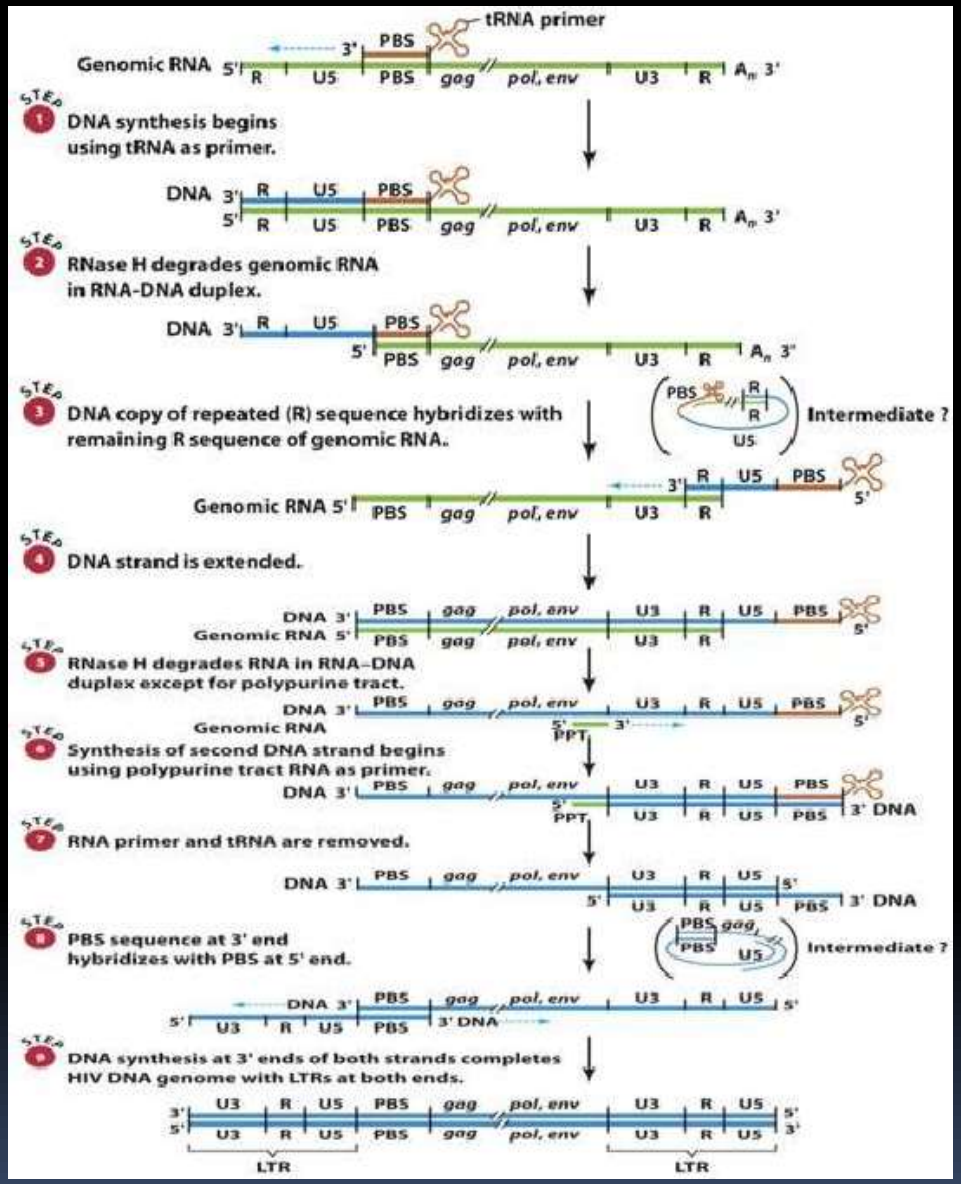
❖This process, catalysed by **reverse transcriptase, begins with the synthesis of a single DNA strand complementary to the single-stranded RNA of the viral genome.**

❖**In step 1**, it is primed by a tRNA that is complementary to a sequence called PBS (primer binding site) situated to the left of the center in the HIV RNA. This tRNA is packaged prehybridized to the PBS in the HIV core.

❖**In step 2**, after reverse transcriptase catalyzes the synthesis of the 3′ end of the viral DNA, ribonuclease H (RNase H) degrades the genomic RNA in the RNA-DNA duplex. This degradation leaves the repeated (R) sequence of the nascent DNA strand free to hybridize with the R sequence at the 3′ end of the HIV RNA.

❖**In step 3**, R region of the nascent DNA strand "jumps" from the 5′ end of the HIV RNA to the 3′ end of the HIV RNA.

❖ **In step 4**, reverse transcriptase next extends the DNA copy by using the 5′ region of the HIV RNA as template.



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❖**In step 5**, RNase H degrades all the RNA in the RNA-DNA duplex except a small region, the polypurine tract (PPT), which is composed of the purines adenine and guanine.

❖**In step 6**, this polypurine tract is used to prime second-strand DNA synthesis of the 3′ half of the HIV genome.

❖ **In step 7**, after that tRNA and the genomic RNA present in the RNA-DNA duplex are removed.

❖**In step 8**, a second DNA "jump" occurs during which the PBS at the 5′ end of the second DNA strand hybridizes with the complementary PBS at the 5′ end of the first DNA strand.

❖**In step 9**, the 3′ -hydroxyl termini of the two DNA strands are then used to prime DNA synthesis to complete the synthesis of double-stranded HIV DNA.

Note that the conversion of the viral RNA to viral DNA produces signature sequences at both ends of the DNA molecule. These sequences , called long terminal repeats (LTRs) which are required for integration of the viral genome into the DNA of the host cell.

➢ **Integration of the HIV double-stranded DNA into the host chromosome:**

❖ **Integration of the viral DNA is catalysed by the enzyme integrase, which has endonuclease activity.**

❖**In step 1**, Integrase first produces recessed 3′ ends in the HIV DNA by making single stranded cuts near the ends of the both LTRs.

❖**In step 2**, these recessed ends are next used for integrase-catalysed attacks on phosphodiester bonds in a target sequence in the DNA of the host cell. This process results in the formation of new phosphodiester linkages between the 3′ ends of the HIV DNA and 5′ phosphates in the host DNA.

❖**In step 3 or in the final stage of integration**, DNA repair enzymes of the host cell fill in the single-stranded gaps to produce an HIV DNA genome covalently inserted into the chromosomal DNA of the host cell.

Notice that the target sequence at the site of integration is duplicated in the process. The integrated HIV genome thereafter becomes a permanent part of the host cell genome, replicating just like any other segment of the host DNA.

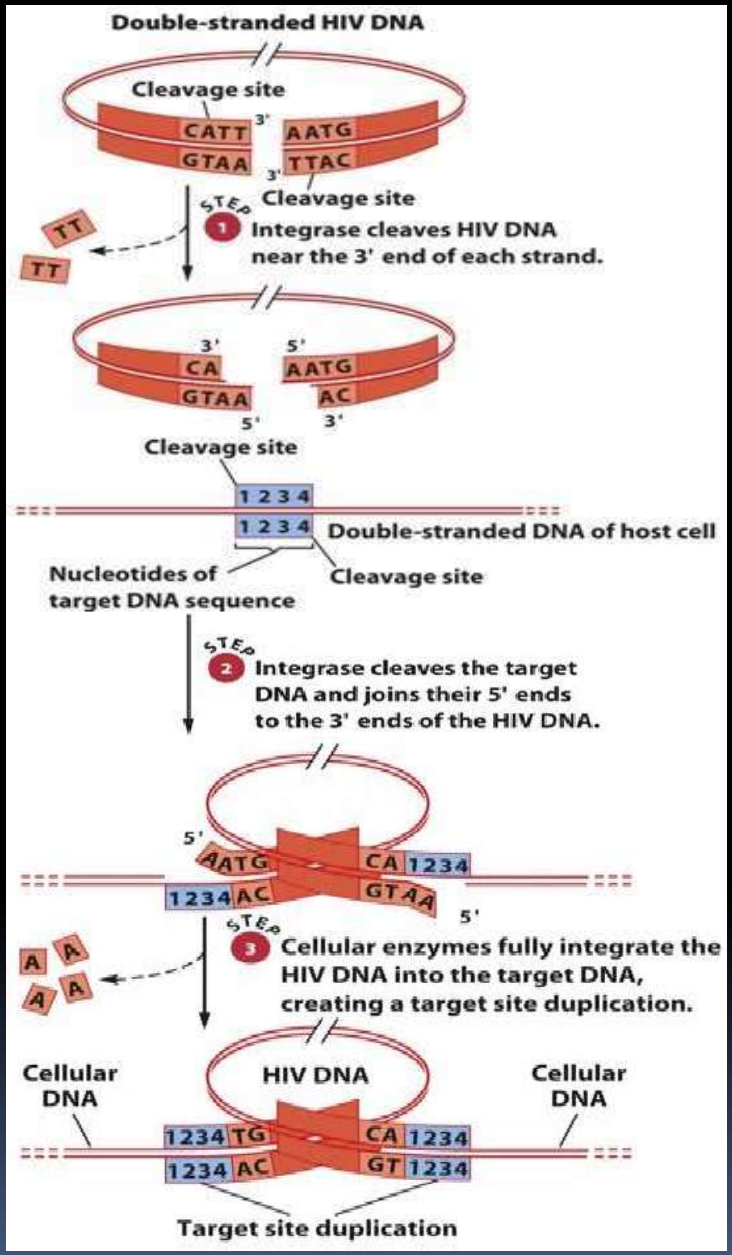

Fig 4: Integration of the HIV double-stranded DNA into the chromosomal DNA of the host cell.