Tutorial 2 : Retroviruses and human immunodeficiency virus

Human immunodeficiency virus (HIV) is an enveloped lentivirus. Its RNA is reverse-transcribed into DNA, which can integrate into the host genome. CD4+ T lymphocytes are the central target of HIV, but monocytes/macrophages and some dendritic-cell populations also participate in viral dissemination and persistence. Without effective treatment progressive immune dysfunction can lead to acquired immunodeficiency syndrome (AIDS)

1. History and origins

Origin and Evolution of HIV

HIV originated through zoonotic transmission of simian immunodeficiency viruses (SIVs) from African primates to humans, probably through exposure to infected blood during hunting or butchering.

- HIV-1: Includes four groups (M, N, O, and P). Groups M and N originated from chimpanzees (SIVcpz), while groups O and P are linked to gorillas (SIVgor). Group M is responsible for the global pandemic.
- HIV-2: Originated from sooty mangabeys (SIVsmm) in West Africa. It is generally less transmissible and progresses more slowly than HIV-1, but can still cause AIDS.

These HIV groups resulted from independent cross-species transmission events.

2. Routes of transmission

Relevant fluids include blood, semen and pre-seminal fluid, vaginal and rectal fluids, and breast milk. Transmission requires exposure of a mucous membrane or damaged tissue, or direct inoculation into the bloodstream.

- Sexual exposure: anal and vaginal intercourse can transmit HIV. Oral sex carries little to no risk and should not be grouped with anal or vaginal sex as an equivalent-risk exposure.
- Blood exposure: shared injection equipment, contaminated instruments, unsafe transfusion or occupational needlestick injury. Properly screened blood greatly reduces transfusion risk.
- Vertical transmission: during pregnancy, childbirth or breastfeeding. Casual contact, intact skin contact, shared dishes and insects do not transmit HIV.

Undetectable = Untransmittable (U=U): people who take antiretroviral therapy and maintain an undetectable viral load do not transmit HIV through sex.

3. Classification and genome organization

Family: *Retroviridae*. Subfamily: Orthoretrovirinae. Genus: *Lentivirus*. Retroviruses belong to Baltimore group VI: single-stranded RNA viruses that replicate through a DNA intermediate. HIV particles contain two positive-sense single-stranded RNA genomes, often described as a diploid or pseudodiploid genome. The two copies need not be genetically identical; copackaging of different variants enables recombination during reverse transcription.

HIV-1 causes most infections worldwide. HIV-2 is concentrated in West Africa but also occurs elsewhere. It is generally less transmissible and associated with slower progression, yet untreated infection can still cause AIDS.

Group	Gene	Main products or functions
Structural	gag	Gag precursor: matrix MA p17, capsid CA p24, nucleocapsid NC p7 and p6, plus spacer peptides.
Enzymatic	pol	Protease PR, reverse transcriptase RT with RNase H activity, and integrase IN; produced through the Gag-Pol precursor.
Envelope	env	gp160 precursor, cleaved by host proteases into gp120 and gp41.

Regulatory	tat	Enhances productive transcription from the provirus through TAR and host transcription factors.
	rev	Promotes nuclear export of unspliced and incompletely spliced viral RNAs through the Rev response element.
Accessory	vif	Counteracts antiviral APOBEC3 proteins.
	vpr	Modulates host cell functions, including cell-cycle regulation.
	vpu	Present in HIV-1; promotes CD4 degradation and counteracts tetherin-mediated restriction of virion release.
	nef	Modulates cell signaling and reduces surface CD4 and selected MHC class I molecules.
	vpx	Present in HIV-2, which lacks vpu; counteracts SAMHD1. HIV-2 retains vpr and nef.

4. Structure of the HIV virion

The mature HIV-1 particle has a host-derived lipid envelope, an underlying matrix and a conical capsid enclosing the viral ribonucleoprotein complex and enzymes.

- Lipid envelope: acquired during budding from the host-cell plasma membrane. It contains trimeric Env spikes, each composed of three gp120–gp41 heterodimers.
- gp120: surface subunit that binds CD4 and a suitable coreceptor, usually CCR5 or CXCR4.
- gp41: transmembrane subunit that mediates membrane fusion.
- Matrix: MA p17 lies beneath the viral envelope.
- Capsid: CA p24 forms the mature conical shell. p24 is also an antigen used in HIV diagnostic testing.
- Nucleocapsid and genome: NC p7 associates with two genomic RNAs. Packaged cellular tRNA Lys3 primes reverse transcription.
- Enzymes: RT synthesizes viral DNA and has RNase H activity; IN catalyzes integration; PR processes viral polyproteins during maturation.

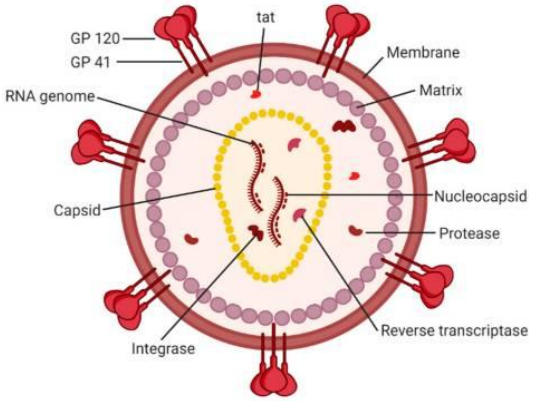


Figure 1. HIV-1 structure

5. Envelope glycoproteins and viral entry

Env is synthesized as gp160 in the secretory pathway, glycosylated and assembled into trimers. Host furin or related proteases cleave it into gp120 and gp41 during secretory processing.

- **gp120 recognition subunit**

gp120 contains relatively conserved regions C1–C5 and variable regions V1–V5. The CD4-binding surface is a three-dimensional, discontinuous site.

- **gp41 fusion subunit**

gp41 contains an N-terminal fusion peptide, heptad-repeat regions HR1 and HR2, a membrane-proximal external region (MPER), a transmembrane segment and a cytoplasmic tail.

6. Replication cycle

a. Entry sequence

1. CD4 engagement. gp120 binds CD4 on a susceptible cell. CD4 T cells are major targets; macrophages and some dendritic-cell populations can also participate in infection or viral transfer.

2. Env opening. Receptor binding shifts Env toward an open conformation, rearranging V1/V2 and exposing the coreceptor-binding surface.

3. Coreceptor engagement: gp120 binds CCR5 or CXCR4 according to viral tropism and receptor availability.

4. Fusion-peptide insertion. Env rearrangement allows the gp41 fusion peptide to insert into the cell membrane.

5. Membrane fusion: Three HR1 and three HR2 helices form a six-helix bundle. Membranes are brought together, pass through lipid-mixing intermediates and form a fusion pore.

6. Core delivery: The viral core enters the cytoplasm.

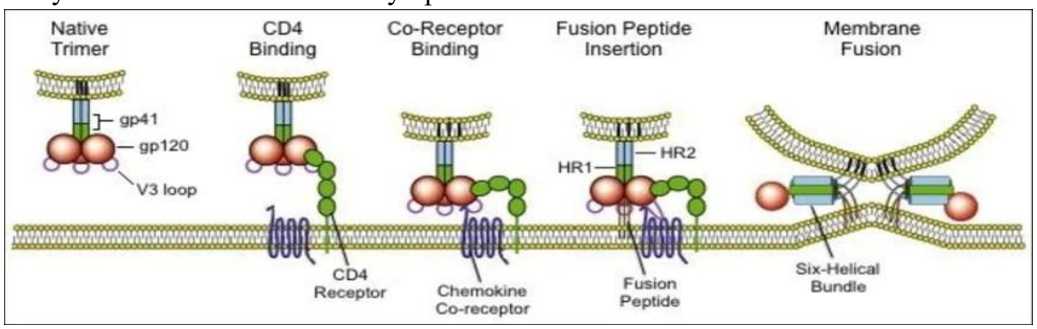


Figure 2. Entry mechanism

b. Reverse transcription and uncoating

After entry, capsid-associated complexes traffic toward and through the nuclear pore. Reverse transcription, nuclear import and capsid remodeling are coordinated and can overlap. Studies show that largely intact capsids can enter the nucleus, with reverse transcription completing and uncoating occurring there.

- The packaged tRNA Lys3 initiates minus-strand DNA synthesis from the RNA template.
- The RNase H domain of RT degrades RNA within RNA–DNA hybrids.
- Template transfers and plus-strand synthesis produce linear double-stranded viral DNA.

c. Integration

Integrase processes the viral DNA ends and catalyzes strand transfer into chromosomal DNA; host repair completes the process. The integrated DNA is called a provirus. It can be transcriptionally active or enter a state of latency.

d. Viral transcription

Host RNA polymerase II transcribes integrated HIV-1 proviral DNA into full-length viral RNA, while Tat enhances transcriptional elongation. Alternative splicing generates three main RNA classes. Unspliced full-length RNA serves both as the genome packaged into new virions and as the messenger RNA for Gag and Gag-Pol synthesis. Incompletely spliced RNAs encode proteins including Env, Vpu, Vif and Vpr, whereas multiply spliced RNAs primarily encode Tat, Rev and Nef, particularly early in viral gene expression. Rev promotes the nuclear export of unspliced and incompletely spliced RNAs, allowing their translation in the cytoplasm or, for full-length RNA, packaging into new viral particles.

e. Translation and precursor processing

Gag is translated from full-length unspliced viral RNA. A programmed −1 ribosomal frameshift allows a minority of ribosomes to produce Gag-Pol, providing the viral.

- Gag processing yields MA p17, CA p24, NC p7 and p6, with spacer peptides removed during maturation.
- Gag-Pol processing releases protease, reverse transcriptase with RNase H activity, and integrase.
- Host proteases cleave Env gp160 into gp120 and gp41.

f. Assembly

Gag drives assembly at the inner face of the plasma membrane, recruits two copies of genomic RNA, and packages Gag-Pol and other viral components. Env is incorporated from the host membrane. Assembly creates an immature protein lattice beneath the viral envelope.

g. Budding and release

The growing particle buds through the plasma membrane and acquires its lipid envelope. Gag recruits host membrane-scission machinery to complete release.

h. Maturation

Viral protease cleaves Gag and Gag-Pol during and around budding and release. Structural rearrangement then generates the mature conical capsid and an infectious virion.

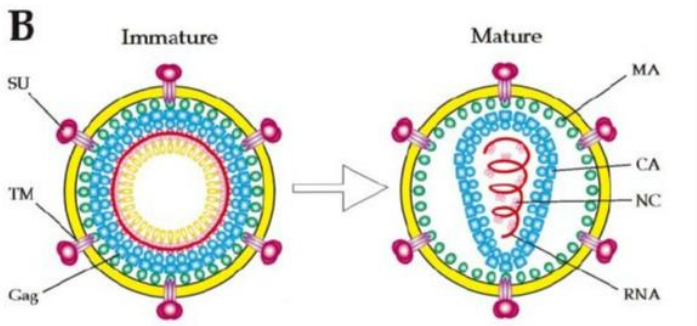


Figure 3. Mature and Immature HIV Virions.

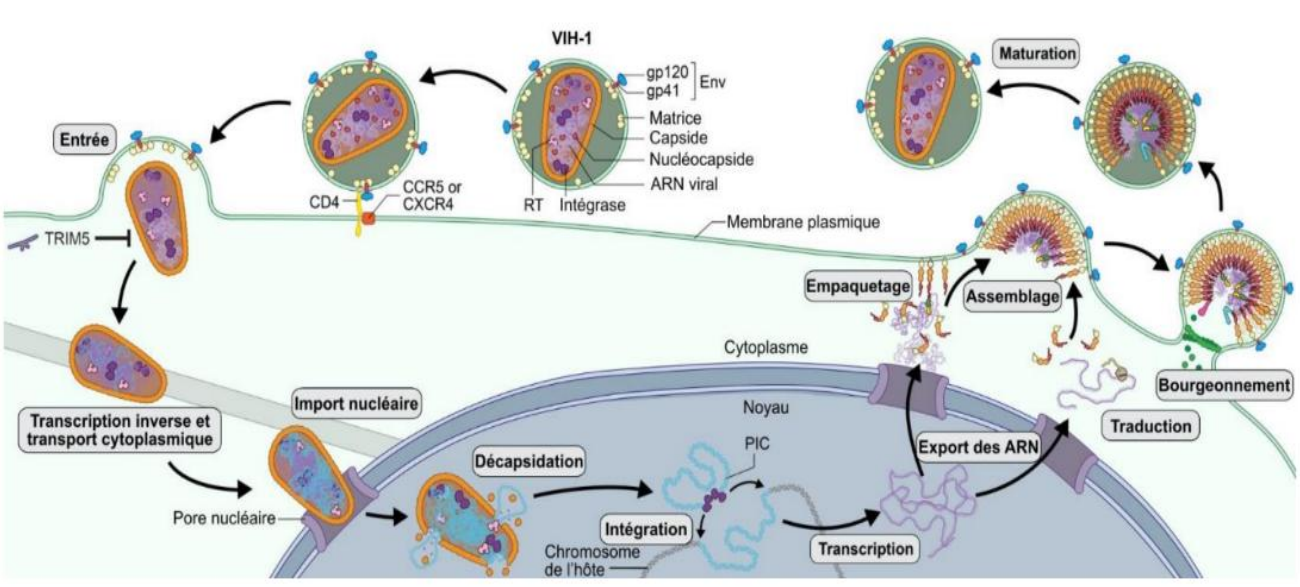


Figure 4. VIH replication cycle

7. Antiretroviral targets

Replication step	Key molecular event	Drug class targeting the step
Attachment / entry	gp120–CD4/coreceptor interaction and gp41 fusion	Attachment/post-attachment inhibitors, CCR5 antagonist, fusion inhibitors
Reverse transcription	RT makes DNA from RNA; RNase H removes RNA template	NRTIs and NNRTIs
Capsid trafficking / uncoating	CA-mediated transport, nuclear entry and controlled disassembly	Capsid inhibitor (lenacapavir)
Integration	Integrase inserts viral DNA into host chromosome	Integrase strand transfer inhibitors (INSTIs)
Maturation	Protease cleaves Gag/Gag-Pol polyproteins	Protease inhibitors

8. Acute infection

After acquisition, HIV disseminates rapidly through lymphoid tissues. Plasma viral load rises sharply, often before antibodies are detectable. A substantial loss of CD4+ T cells can occur in gut-associated lymphoid tissue. Many people develop an acute retroviral syndrome characterized by fever, rash, pharyngitis, lymphadenopathy, myalgia or other nonspecific symptoms. Because symptoms are nonspecific, diagnosis requires testing rather than clinical appearance.

9. Chronic infection and progressive immunodeficiency

The immune response reduces viremia after the acute peak, but does not eliminate the infection. Ongoing replication and long-lived infected cells maintain the virus. Progressive disease reflects a combination of direct cytopathic effects, immune-mediated killing of infected cells, bystander-cell death, lymphoid-tissue fibrosis and persistent immune activation. Without effective ART, CD4 counts typically decline over years

10. Laboratory diagnosis

HIV infection is diagnosed using three main types of tests:

- Antibody tests: Detect anti-HIV antibodies, usually at a later stage.
- Antigen/antibody tests (4th generation): Detect both p24 antigen and HIV antibodies, allowing earlier diagnosis.
- Nucleic acid tests (NATs): Detect HIV RNA and can identify infection at the earliest stage. They are also used for viral load monitoring.