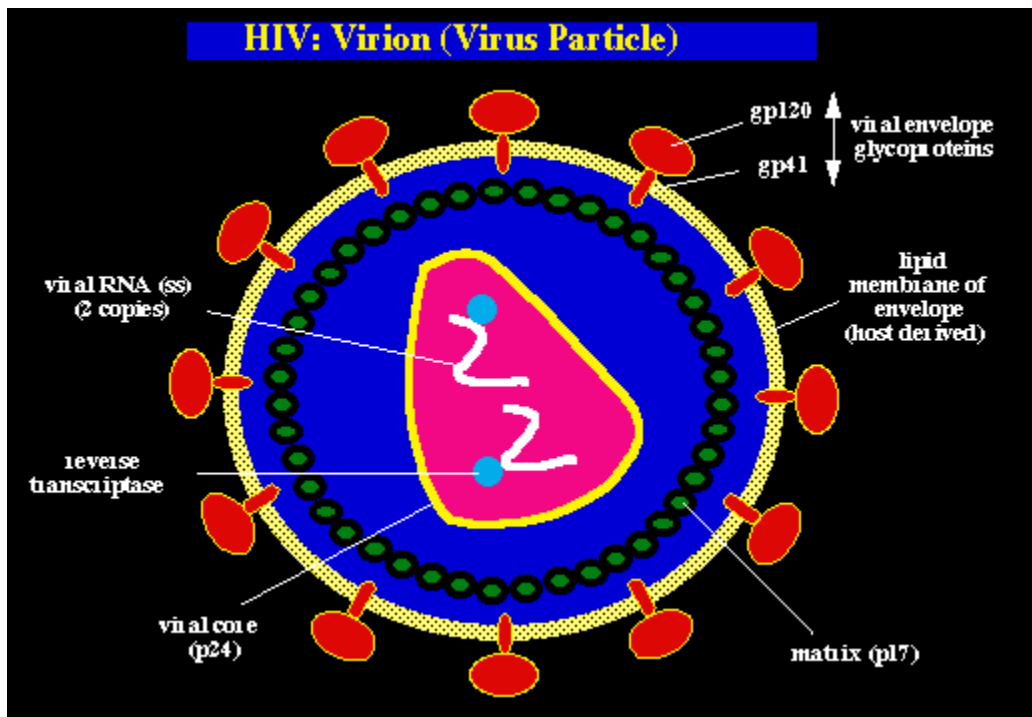
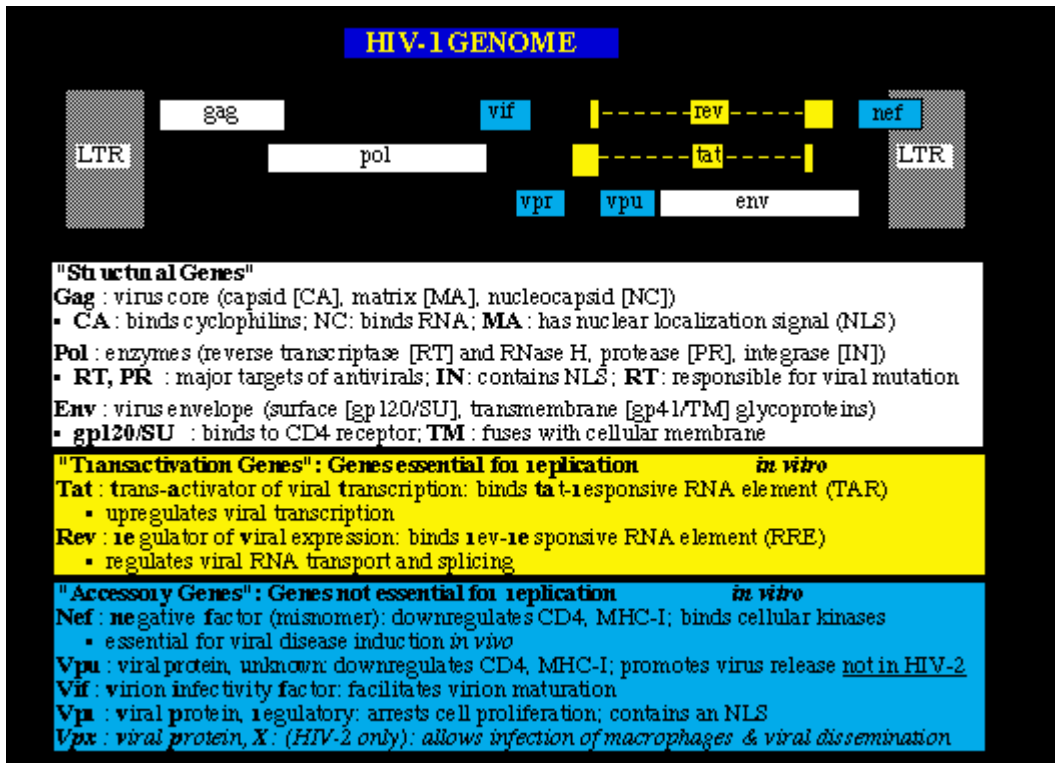


# Lentiviruses II (HIV-1, Molecular Biology)

Trono, D. *Nature Medicine* 4:1368, 1998 & Cullen, Cullen, B. *Cell* 93:685, 1998  
Littman, D.R. *Cell* 93:677, 1998 & Stewart, G. *Nature Med.* 4:275, 1998



**Virion Structure:** This above cartoon shows the HIV virion. The central nucleocapsid core contains two copies of the viral genome (ssRNA), plus core proteins and reverse transcriptase. Surrounding this is the viral envelope, which is comprised of cellular lipids interspersed with viral envelope proteins.



## Key genes/features of HIV-1 genome

1. **CD4 downregulation.** Much of the genetic information is devoted to downregulation of CD4 (nef, env, vpu), suggesting that this must be a very important biological property. CD4 downregulation is probably important to promote virion release from infected cells.
2. **Nuclear import.** The “central DNA flap” (a triple-stranded HIV cDNA intermediate of reverse transcription) enhances nuclear import of the viral preintegration complex, as do nuclear localization signals found in three viral gene products (Vpr, IN and MA). These elements allow the HIV-1 preintegration complex to enter the nucleus of non-dividing cells (such as macrophages).
3. **Nef.** The *nef* gene is critical for disease induction.
4. **T-cell activation.** HIV-1 gp120 (which binds to CD4), Tat and Nef (which interacts with cellular kinases) can all activate T-cells to some degree, which may help to promote HIV-1 replication (HIV-1 replicates most efficiently in activated T-cells, which contain high levels of transcription factors that enhance viral replication -- such as NFκB).
5. **Inhibition of MHC class I.** Vpu and Nef can downregulate cell surface expression of MHC class I molecules. MHC class I is essential for immune

recognition and attack by cytotoxic T cells. *HIV also evades host immunity via genetic mutation, glycosylation of Env & other means.*

## Virion Proteins

**Capsid (Core) proteins.** Capsid (core) proteins are products of the *gag* gene. **Gag** products include the Matrix (MA), Capsid (CA) and Nucleocapsid (NC) proteins, which are derived by the proteolytic cleavage of the Gag polyprotein, Pr55<sup>gag</sup>. This cleavage is mediated by the viral protease enzyme (PR), which is the target of a number of antiviral drugs.

**MA (matrix protein).** MA is believed to interact with the viral transmembrane envelope protein (TM) -- an interaction which may help to recruit the envelope protein to core particles, during virus assembly.

**CA (capsid protein).** CA plays a crucial role in the assembly of viral nucleocapsids. CA (and the Gag precursor Pr55<sup>gag</sup>) also interacts with the cellular protein, **cyclophilin A** (CyA), which is incorporated into HIV-1 virions and which is required for viral infectivity. CyA is believed to facilitate the uncoating of the HIV-1 capsid during the early stages of viral infection, probably by destabilizing the CA protein from the viral nucleoprotein complex (CyA is a molecular chaperone which can alter protein folding).

**NC (nucleocapsid protein).** NC is a basic protein that binds (nonspecifically) to viral RNA. It may help to condense the viral RNA for packaging into the nucleocapsid. NC also binds specifically to the major packaging signal on the HIV-1 genome (the psi or  $\psi$  signal).

## Viral Enzymes

Viral enzymes are products of the *pol* gene. Pol encodes the enzymes protease (PR), reverse transcriptase (RT) and RNase H, and integrase (IN). As with the Gag gene products, these are produced by cleavage of a larger precursor polyprotein. In this case, the protein is a Gag-Pol polyprotein, Pr160<sup>gag-pol</sup>, which is made as the result of a site-specific frame-shifting event during translation of the viral mRNA (Gag and Pol are encoded in different reading frames on the HIV-1 genome, and the two open reading frames overlap). *Note that Pr160<sup>gag-pol</sup> is a self-cleaving protein.*

**PR (protease).** This is a dimeric enzyme which cleaves the HIV-1 Gag-Pol polyprotein. Its crystal structure and site-specificity have been defined, allowing the design of a variety of enzyme inhibitors.

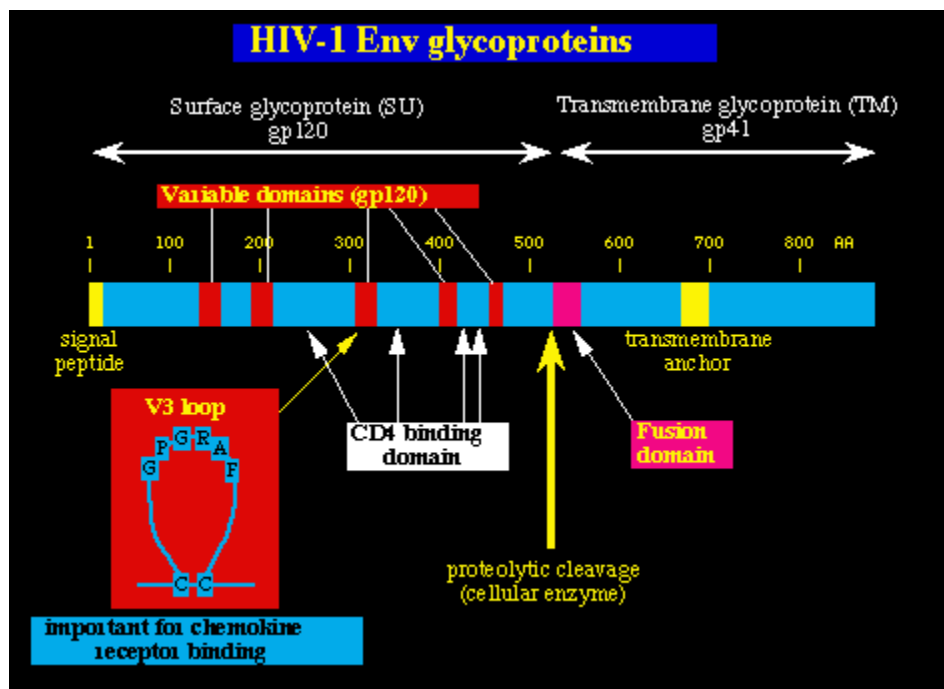
**RT (reverse transcriptase) and RNase H.** RT is an RNA-dependent DNA polymerase

which synthesizes DNA from an RNA template. It also has RNase H activity, which allows it to degrade RNA from RNA:DNA hybrids (this is a necessary step during reverse transcription). The crystal structure of RT has been defined and many specific inhibitors of this enzyme have been derived. One of the most important properties of RT is that it lacks proofreading activity. As a result, RT has a high error rate and generates a large number of **mutations**.

**IN (integrase)**. IN catalyzes the insertion of linear double-stranded HIV-1 DNA into the host cell chromosome. This is an obligatory step in the HIV-1 life cycle.

## Viral Envelope glycoproteins

The HIV-1 envelope glycoproteins are the product of the Env gene. **Env** products include the surface envelope glycoprotein (SU or gp120) and the transmembrane glycoprotein (TM or gp41). As with the Gag and Pol, these are produced by cleavage of a larger precursor polyprotein (gp160). This cleavage is mediated by a cellular protease.



**SU or gp120 (surface glycoprotein)**. This is a highly glycosylated protein which is located on the outside of the viral particle. It has no transmembrane domain, and is attached non-covalently (ie, weakly) to the TM/gp41 glycoprotein. Both gp120 and gp41 are found as oligomers in the viral particle (ie, gp120 interacts both with gp41 and with other gp120 molecules, while gp41 has similar properties).

Overall, Gp120 encoding sequences from different HIV-1 strains are **highly variable**.

This variation can be mapped to 5 variable domains, of which the V3 loop is particularly important. This loop of roughly 30 amino acids is anchored by two cysteine residues (which are covalently linked by a disulfide bond), and contains conserved amino acids (GPGRAF) at its “crown”. The rest of the loop is more variable, and it has been shown that variation in the V3 loop alters the host cell range of HIV-1, due to its effects on the interaction between gp120 and cellular chemokine receptors.

Most important, **gp120 binds with high affinity to the cellular CD4 protein.** Following CD4 binding, **gp120 can also bind to cellular chemokine receptors** (notably, CCR5). The CD4 and chemokine receptor binding domains of gp120 are generally well conserved, and are comprised of multiple discontinuous regions which are brought together as a result of protein folding.

**TM or gp41 (transmembrane glycoprotein).** Gp41 contains a short stretch of highly hydrophobic residues at its N terminus (this is the **fusion domain**). The fusion domain is important since it allows the virion membrane to fuse with the plasma membrane during virus entry into the host cell. In addition to the fusion domain, there is a second hydrophobic domain in the middle of gp41, which serves to **anchor** the protein in the lipid bilayer of the viral envelope.

## Viral accessory genes

These are genes that are non-essential *in vitro*

**Vif. Virion Infectivity Factor.**

**Important for virion maturation;** appears to be needed to counteract a cellular factor that inhibits HIV-1.

**Vpu. Viral Protein, Unknown.**

Present in HIV-1, but absent in HIV-2. **Downregulates CD4 expression and promotes virus budding.** Vpu also downregulates MHC class-I. Finally, Vpu has structural and functional similarities to the influenza A virus M<sub>2</sub> protein, which acts as an ion channel.

**Vpr. Viral Protein, Regulatory.**

**Arrests cellular proliferation** in the G<sub>2</sub> phase of the cell cycle, promotes cellular differentiation, and interacts with cellular proteins involved in DNA repair. One consequence of Vpr-mediated G<sub>2</sub> cell cycle arrest is that it results in enhanced viral replication, since the viral LTR is most active in the G<sub>2</sub> phase of the cell cycle. Vpr also causes cellular **apoptosis**, and it is a **weak transactivator** of viral transcription. Finally, Vpr also **contains a nuclear localization signal (NLS)**, which is important for nuclear import of the HIV pre-integration complex. *In SIV, Vpx carries out the nuclear import function of Vpr, and is required for infection of macrophages. Vpx is also needed for*

*viral dissemination following infection in vivo, suggesting that macrophages may be important for early replication and spread of the virus (Hirsch et al. Nature Med. 4:1401, 1998).*

**Nef.** Negative factor (a misnomer!).

The function of the Nef gene product was mysterious for many years, since it could be deleted from HIV-1 clones with little obvious effect on viral activity *in vitro*.

Subsequently, Nef was shown to be localized to cellular membranes (due to the addition of a long-chain lipid moiety to the protein, via a process known as **myristoylation**). In addition, Nef was found to have a number of interesting and potentially important properties, including the ability to **downregulate CD4 and MHC class-I**, and the ability to upregulate cellular expression of Fas ligand (FasL). **FasL upregulation** may result in the killing of virus-reactive cytotoxic T cells as they try to kill virally-infected targets. Finally, Nef has also been shown to **activate cellular protein kinases** -- thereby interfering with cell signaling processes. Specifically, Nef contains a **PxxP peptide motif**, which allows Nef to bind to the *src*-homology region-3 (**SH3**) domain of cellular tyrosine kinases which are part of the *src* family.

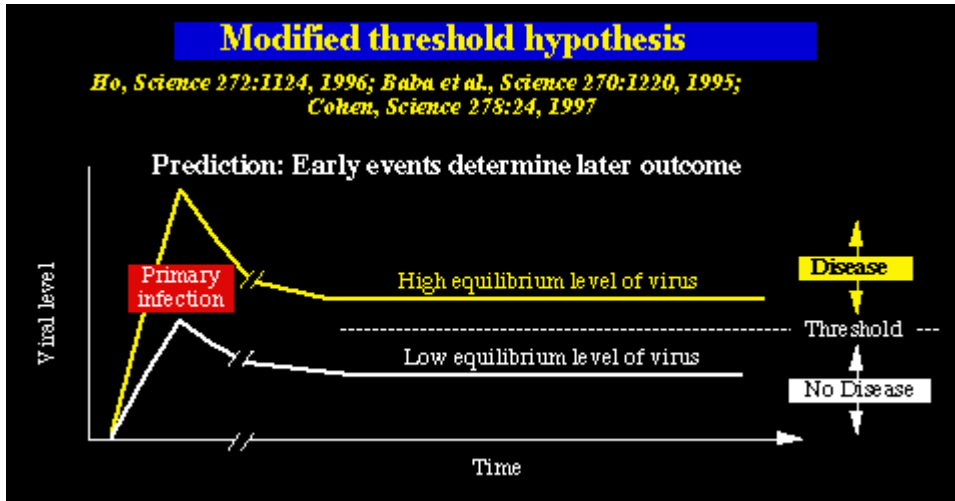
The most dramatic property of Nef was revealed by a landmark experiment by Ronald Desrosiers and colleagues in 1991 (*Kestler et al. Cell 65:651, 1991*). Briefly, deletion of the Nef gene from a pathogenic molecular clone of SIV rendered the virus non-pathogenic in adult macaque monkeys -- thereby establishing that **Nef is crucial for the ability of SIV to induce disease *in vivo***

Studies on SIV *nef* have been supported by analyses of the "Sidney Blood Bank Cohort" (SBCC) in Australia. This group of six HIV-1 infected individuals were all recipients of blood or blood products from a single HIV-1+ donor, and all of these persons remained healthy for over 10 years. Sequence analysis revealed that the HIV-1 strain which had infected these individuals contained a large deletion in *nef* (*Deacon et al. Science 270:988, 1995*). These data, together with Desrosiers's results, suggest that **Nef-deleted strains of HIV-1 may be attenuated**. Notably, however, the SBCC cohort has recently shown signs of disease, including measurable declines in circulating CD4+ cell levels; thus, attenuation may be incomplete.

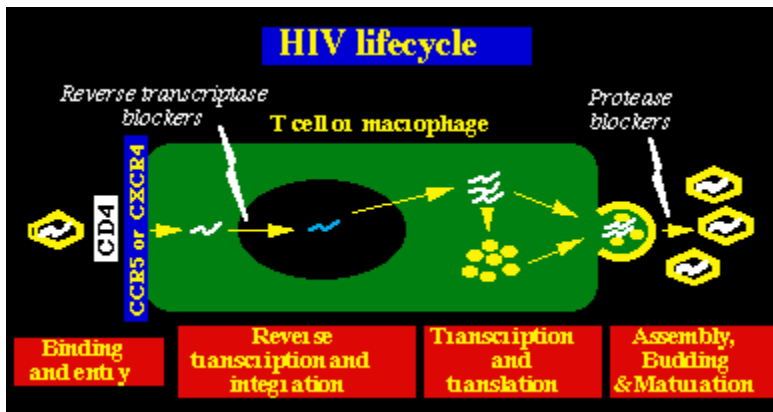
Desrosiers' group has gone on to show that infection of macaques with Nef-deleted SIV strains can protect the animals against subsequent infection by wild-type strains of SIV -- raising the possibility that it may be possible to design a live, attenuated, HIV vaccine. Improved vaccine strains, bearing multiple gene deletions that allow for the optimum balance of immunogenicity and safety are being developed, and a human trial of a Nef-, Vpr- & Vpu- deleted vaccine strain is being planned (<http://www.iapac.org/>).

It is clear, however, that HIV is still capable of causing disease even in the absence of *nef*. A few of the animals inoculated with either SIV $\Delta$ Nef, or with a triply-deleted variant (SIV $\Delta$ 3) have developed immunodeficiency, and some neonatal monkeys injected with a high dose of SIV $\Delta$ Nef have also developed AIDS. In general, the ability to cause disease

was correlated with the ability of the virus to replicate to high levels *in vivo*. This has led to the **threshold hypothesis**, which essentially states that the early level of viral replication determines whether or not viral infection will be pathogenic in any given host or individual.



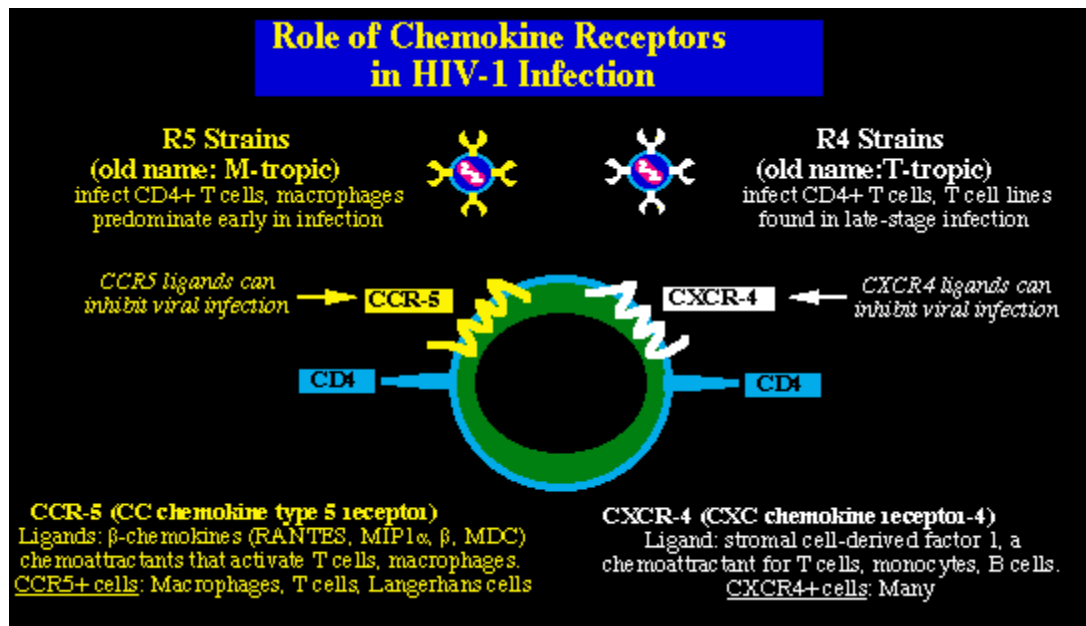
## Virion binding and entry.



The initial step in HIV-1 infection is **binding of gp120 to the cellular CD4 molecule**, which is required for HIV-1 infection of T-cells and macrophages. This interaction results in a conformational change in gp120 that enables it to bind to the chemokine receptor(s) which serve as viral entry co-receptors (see below). The combined effect of CD4 binding and coreceptor binding is that a conformational change occurs in gp41, resulting in the formation of a coiled-coil structure that exposes the **hydrophobic fusion domain** at the N-terminus of gp41. This in turn allows fusion between the viral and cell membranes. *Note that this conformational change in gp41 can be inhibited by an antiviral peptide, T-20 (Nature Med. 4:1302, 1998).*

**Entry cofactors:** Although CD4 is *necessary* for HIV-1 infection, it is not by itself *sufficient* to make a cell susceptible to HIV-1 (for example, human glioma cells can be engineered to express CD4, but cannot be infected with HIV-1). Thus, HIV-1 requires an additional entry cofactor. Ed Berger and colleagues cloned the first of these cofactors in 1996, and subsequent work from several labs resulted in identification of two major HIV-1 entry cofactors, both of which correspond to cellular **chemokine receptors**:

- **CCR5.** This is used by most primary isolates of HIV-1, including the major sexually-transmitted HIV-1 strains. This co-receptor allows the virus to infect CD4+ T cells as well as macrophages and Langerhans cells (which are found in the mucosal epithelium). The natural ligands of CCR5 are  $\beta$ -chemokines (RANTES, MIP-1- $\alpha$ , MIP-1- $\beta$  and macrophage-derived chemokine or MDC). These molecules, and synthetic derivatives thereof, can block infection by R5 strains of HIV-1 (below).
- **CXCR4.** Used by R4 strains of HIV-1 (T-cell line-adapted HIV-1 isolates and late-stage HIV-1 isolates). This receptor is widely expressed *in vivo*.



The ability of HIV to access its coreceptor may have important **therapeutic implications**, in terms of the design of new antiviral drugs. In addition, **naturally occurring mutations** in chemokine receptor genes and in chemokine genes have been shown to influence the (1) susceptibility to HIV-1 infection and (2) the progression of virally-induced disease in infected persons. Notable examples of such mutations include:

1. A promoter variant of CCR5, CCR5-Δ32. The homozygous genotype, *CCR5-Δ32/Δ32* predisposes to the acceleration of AIDS progression. (*Martin et al. Science 282:1907, 1998*).
2. A mutant CCR5 allele (CCR5-Δ32). This leads to a 32-nucleotide deletion within the *CCR5*, that results in a premature stop codon and the complete lack of CCR5 protein. The absence of CCR5, due to the homozygous mutation, very strongly protects against HIV-1 transmission. Also, heterozygosity for the mutant allele delays disease progression. The CCR5-Δ32 allele is common in caucasians of European descent (11% are heterozygotes and ~1% are homozygotes) (*Dean et al. Science 273:1186, 1996*).
3. Other genes which influence disease progression include the major histocompatibility complex [human leukocyte antigen (*HLA*) class I and II] genes; these influence host immune responses. For example, persons with full heterozygosity at *HLA* class I loci have a slower rate of disease progression; this is presumably because the greater diversity of available class I molecules allows these individuals to respond to an increased number of viral antigenic epitopes (*Carrington et al. Science 283:1748, 1999*)

## Reverse transcription and integration

Following entry of HIV-1 into the host cell, the viral core particle is released and the viral RNA is converted into double-stranded linear DNA by the viral **reverse transcriptase**.

The core-DNA complex (or *preintegration complex*) is then **imported into the nucleus** of the host cell. This occurs via an active process that depends on the (1) presence of nuclear localization signals (NLS) within several virion proteins, and (2) the presence of a triple-stranded HIV cDNA intermediate of reverse transcription (the “central DNA flap”). As a result, lentiviruses are capable of infecting non-dividing cells. *In contrast, oncoretroviruses cannot infect non-dividing cells since their preintegration complexes can enter the nucleus only during mitosis, after the nuclear membrane has dissolved.*

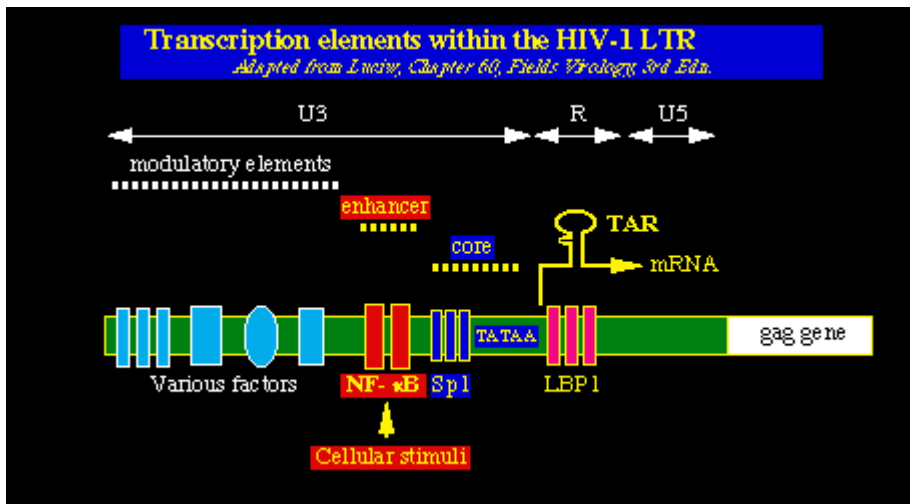
Following nuclear import of the preintegration complex, the linear HIV-1 DNA becomes integrated into the host cell chromosome, through the action of the viral **integrase** enzyme.

## Transcription and translation

Control of HIV-1 gene expression is regulated by both cellular and viral factors. Viral RNA is synthesized in the nucleus, from the integrated HIV-1 provirus and expression is

driven by the transcriptional control elements present in the U3 domain of the viral long terminal repeat (LTR). Control elements include:

1. A **core promoter**, which includes a TATAA box that recruits cellular RNA polymerase II.
2. **Enhancer elements**. These include binding sites for the cellular transcription factors NFAT and NF- $\kappa$ B, which act to upregulate HIV-1 gene expression in response to cellular stimulation by a variety of pathways, including exogenous cytokines and T cell activation. The ability of HIV-1 to respond to cellular activation is important since the great majority of virally-infected T cells are normally quiescent. Conditions which provoke immune activation enhance viral replication. **This may explain why concurrent infections with other pathogens can hasten disease progression in HIV-1 infected persons, and why routine immunizations result in transient increases in HIV-1 replication in HIV-1+ individuals.**
3. **Modulatory elements**. These include binding sites for a range of cellular factors.

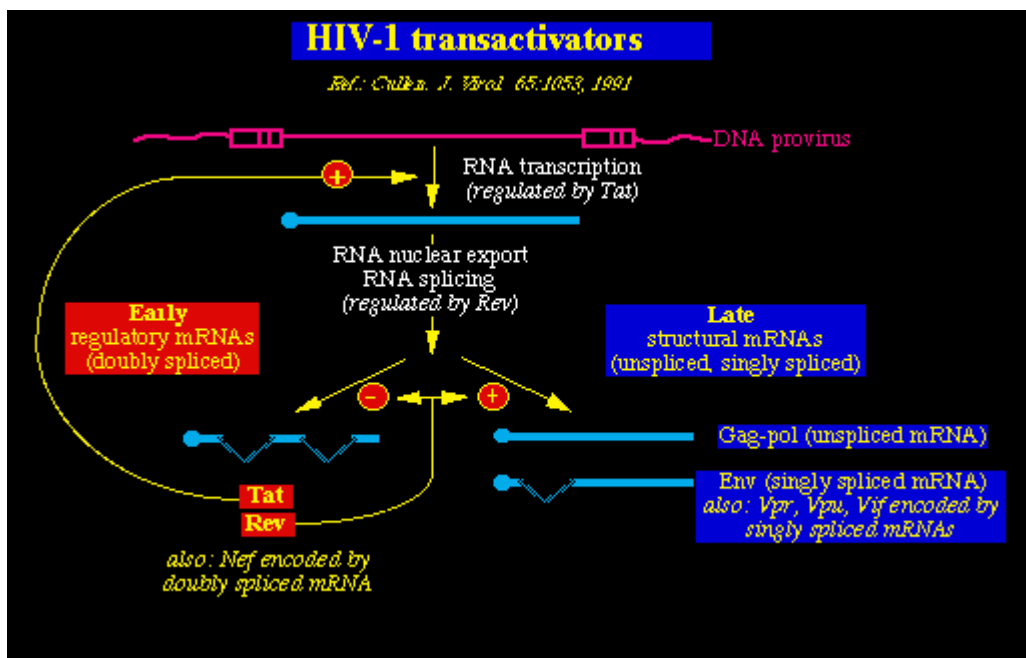


## Viral transactivators

Lentiviruses, in contrast to oncoretroviruses, possess essential viral transactivators which fall into two classes -- **Tat-like** and **Rev-like** gene products (*note that HTLV-I and -II also encode analogous proteins -- Tax and Rex*). The combined action of these additional regulatory proteins divides the lentivirus lifecycle into **two temporal phases -- early and late**.

HIV-1 Tat and Rev have the following properties:

- Tat and Rev are **encoded by multiply spliced mRNAs**. In contrast, “simple” oncoretroviruses encode only unspliced and singly-spliced mRNA species.
- **Tat** is a **sequence-specific *trans*-activator of viral transcription**. It is nuclear acting and it binds to a specific RNA stem-loop structure known as **TAR**). Tat **interacts with cellular kinases to enhance the processivity of RNA polymerase II at an early elongation step**.
- **Rev** is a **regulator of viral RNA transport & splicing**. Rev shuttles between the nucleus and cytoplasm, and it too binds to viral RNA (Rev binds a structure known as the **RRE**). The net effect of Rev production is to favor production of viral structural proteins (encoded by unspliced and singly spliced mRNAs), while inhibiting the expression of the regulatory transactivators (encoded by multiply spliced mRNAs). This occurs because Rev shuttles unstable HIV-1 RNAs (unspliced and singly spliced mRNAs) out of the nucleus and into the cytoplasm, where they can be translated.



## Action of Tat

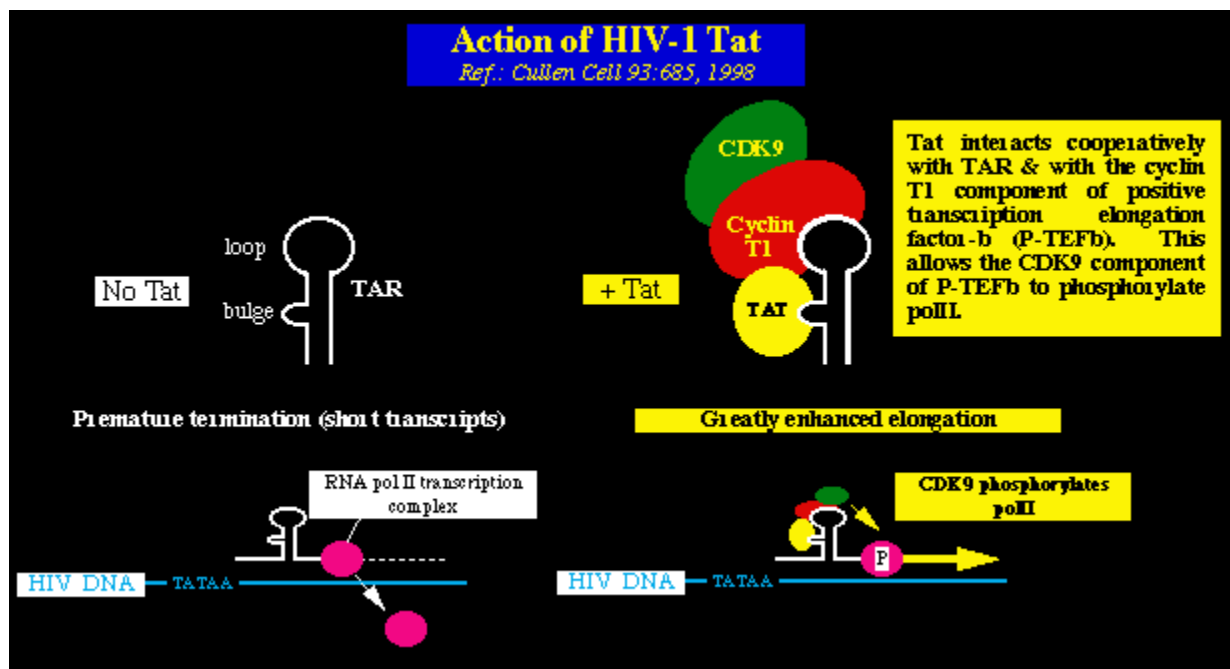
Tat (**trans-activator of transcription**) is essential for viral replication and is a short protein (approx. 100 amino acids). Tat acts through a stem-loop RNA element that is found in the R region of the viral LTR (very close to the site where viral transcription starts). This element is known as **TAR** (the **Tat-responsive element**), and Tat binds to a short bulge within this structure (cellular proteins also bind to the loop domain of TAR). Overall, Tat is made up of two major domains:

1. An N-terminal **cofactor-binding** domain. This domain resembles the acidic activation domains of transcription factors.
2. A central **arginine-rich RNA-binding motif (ARM)**. This domain also acts as a **nuclear localization signal**, which allows Tat to enter the nucleus (where Tat acts).

The major effect of Tat binding to TAR is to greatly increase the efficiency of **transcription elongation** (i.e., Tat interacts with the RNA polymerase II complex and increases its processivity). This effect is important because most HIV-1 mRNA transcripts terminate prematurely in the absence of Tat. Thus, Tat can be viewed as a transcriptional anti-terminator (rather like the N protein of bacteriophage lambda)

### How does Tat increase transcriptional processivity?

Tat acts to target cellular kinases that enhance the processivity of RNA polymerase II at early elongation steps. To do this, Tat interacts cooperatively with the TAR RNA sequence and with the human cyclin T1 (**hCycT1**) component of positive transcription elongation factor b (**P-TEFb**). Recruitment of hCycT1/P-TEFb to TAR is thought to allow the **CDK9** component of P-TEFb to phosphorylate the C-terminal domain of RNA polymerase II in such a way as to enhance the processivity of the enzyme.



### Action of Rev

Rev (**regulator of viral expression**) is, like Tat, a small protein (approx. 116 amino acids) that is essential for viral replication. Also, like Tat, it is made up of distinct domains, including:

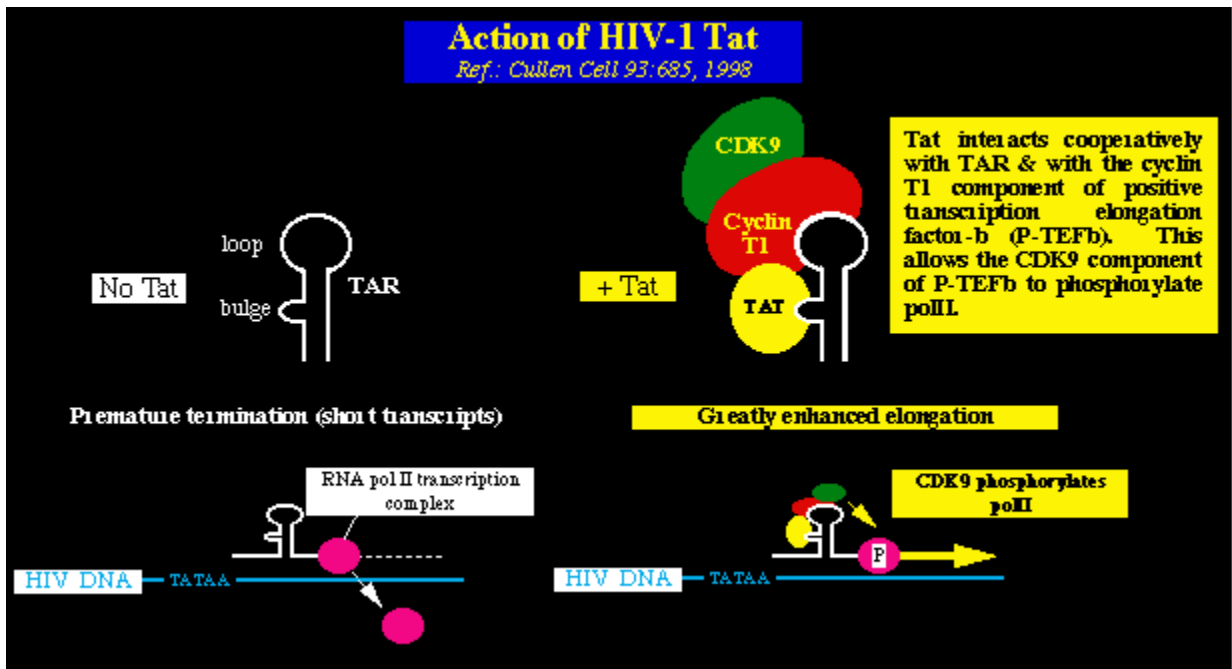
1. An N-terminal **arginine-rich RNA-binding motif (ARM)**. This domain also acts as a **nuclear localization signal**, which allows Tat to enter the nucleus (where Tat acts). The ARM is flanked by sequences which allow Rev to assemble into multimeric complexes. *Note that the Rev ARM can bind to its target sequence without the need for cellular cofactors, unlike its Tat counterpart.*
2. A central leucine-rich domain that functions as a **nuclear export signal (NES)**, and which binds to specific cellular cofactors that are involved in the nuclear export of HIV-1 (and cellular) RNA molecules. The Rev NES and NLS motifs have the combined effect of allowing Rev to shuttle back-and-forth between the cytoplasm and the nucleus.

**Rev target sequence.** Rev acts through a complex RNA structure called the **RRE** (the **Rev-responsive element**), which is found in the *env* gene of HIV-1. Rev binding to RRE is required for the nuclear export and expression of late HIV-1 mRNAs (ie, the unspliced and singly-spliced mRNAs which encode viral structural proteins). As with Tat and TAR, cellular factors also bind to Rev and to the RRE.

**How does Rev work?** (*see Cullen, B. Cell 93:685, 1998*)

1. Rev is made in the cytoplasm.
2. The **Rev NLS** interacts with **importin  $\beta$  (IMP  $\beta$ )**, and the IMP  $\beta$ -Rev NLS complex is brought into the nucleus (IMP  $\beta$  is a cellular protein that interacts directly with nucleoporins, to import proteins into the nucleus).
3. Once in the nucleus, the IMP  $\beta$  component of the IMP  $\beta$ -Rev NLS complex forms an interaction with the GTP-bound form of the G-protein Ran (note that Ran-GTP is present at high levels in the nucleus). This triggers the release of Rev.
4. Rev then binds to, and multimerizes on, its target RNA (via the RRE motif).
5. This results in the recruitment of **Crm1** to the **Rev NES** motif, and the Crm1-Rev NES complex is transported into the cytoplasm (Crm1 is also a member of the IMP  $\beta$  protein family, but is involved in protein export from the nucleus; in contrast to IMP  $\beta$ , it is able to mediate its function only when complexed to Ran-GTP).
6. Once the HIV-1 RNA cargo reaches the cytoplasm, the Crm1-bound Ran-GTP molecules undergo hydrolysis to Ran-GDP. This results in the release of Crm1

from the Rev NES. Rev then detaches from its target RNA by an unknown mechanism, freeing the HIV-1 RNA for translation.



### Overall Effect of Rev on the HIV-1 Life Cycle:

- Rev binding to RRE results in the **nuclear export of late HIV-1 mRNAs**. These late HIV-1 mRNAs are incompletely spliced (ie, singly spliced or unspliced) and as a result, they contain the RRE element (the RRE is removed from early HIV-1 mRNAs, which are doubly-spliced). The effect of Rev is important because late HIV-1 mRNAs do not exit the nucleus in the absence of Rev.
- Rev **inhibits the splicing of late HIV-1 mRNAs**. Rev binding to the RRE may inhibit the formation of the spliceosome complex on the late HIV-1 mRNAs.

### Tat and Rev: common themes

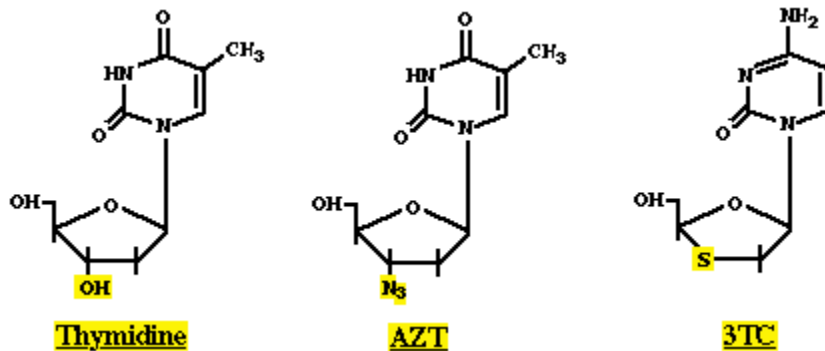
- Act together to regulate HIV-1 gene expression
- Bind to specific RNA target sequences  
Require cellular cofactors for their activity
- Nuclear acting
- Made up of modular domains with specific functions

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## Drugs active against HIV-1

### Nucleoside Reverse Transcriptase Inhibitors (NRTIs)

**Zidovudine (AZT):** Mechanism of action of zidovudine (3'-azido-3'-deoxythymidine) on HIV replication. It is a synthetic pyrimidine analogue and inhibits animal and mammal retroviruses (HTLV-1 and HIV). AZT is phosphorylated to the triphosphate form by host cell enzymes and then the AZT-TP is incorporated into the new DNA chain and inhibits further elongation (since it lacks the -OH group at the 3' position). Therefore, the target is the reverse transcriptase, which prefers AZT-TP to the normal thymidine triphosphate. HIV can become resistant to the effects of AZT, due to mutations which affect the structure of the reverse transcriptase enzyme (including changes in the enzyme active site).



#### Other NRTIs:

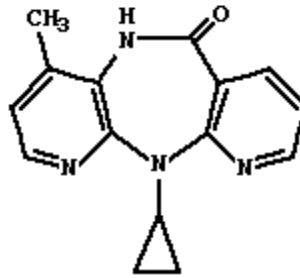
**Lamivudine (3TC), Didanosine(ddI), Zalcitabine (ddC), Stavudine (d4T), Abacavir (ABC):** Similar mechanism of action to AZT. **3TC:** 2'-deoxy-3'-thiacytidine; **ddI:** 2',3'-dideoxyinosine; **ddC:** 2',3'-dideoxycytidine; **d4T:** 2',3'-didehydro-3'-deoxythymidine. These are dideoxynucleoside analogs of dC, dA, dC and dT, respectively. Abacavir is a dideoxy synthetic analog of dGTP. **3TC has also been approved for treatment of Hepatitis B.**

### Non-Nucleoside Reverse Transcriptase Inhibitors

Unlike nucleoside analogs, these drugs do not need to be phosphorylated to be active; rather they bind to RT and inactivate the enzyme in a non-competitive fashion.

### Nevirapine

**Nevirapine:** This was the first non-nucleoside inhibitor of HIV-1 reverse transcriptase (RT). Nevirapine has been shown to bind directly to a 3-dimensional pocket near to the active site within the RT enzyme molecule. A short course of nevirapine therapy has been shown (1999) to prevent perinatal HIV infection, at very low cost (~\$4/person).



**Delavirdine, Efavirenz:** Additional NNRTIs. Efavirenz (approved 1998) was the first anti-HIV drug with once-daily dosing.

### HIV protease inhibitors.

HIV protease (HIV PR) is a symmetric dimer, which contains paired aspartic acid residues at its active site. The enzyme-substrate complex also contains a key structural water molecule, which is unique for retroviral proteases, as compared to other aspartic acid proteases. Most enzyme inhibitors are peptide-mimicking compounds that are *transition state analogs*. These compounds therefore resemble the transition state configuration of the natural substrate of the HIV PR. As a result, the inhibitors bind the enzyme much more tightly than the natural substrate (since the substrate must be distorted in order to assume its transition state configuration). These compounds are therefore competitive enzyme inhibitors.

Drugs in this category include the following: **Saquinavir** (the first such drug to be licensed), **Indinavir**, **Ritonavir**, **Nelfinavir** and **Amprenavir** (licensed in 1999).

### New Antivirals

Several new classes of antiviral drugs are being developed, including inhibitors of the HIV-1 integrase, as well as compounds targeted against the highly conserved HIV-1 nucleocapsid protein zinc fingers involved in genome packaging and virus assembly. Compounds that block chemokine receptors are also being actively pursued; these include small molecules such as TAK-779 for CCR5, and T22, AMD3100 or ALX40-4C for CXCR4. Other molecules which inhibit virus entry may also prove to be effective antivirals. For example, T-20 (previously known as DP178) is a synthetic peptide corresponding to a region of the transmembrane subunit of the HIV-1 envelope glycoprotein (gp41), that has been shown to block virus fusion and entry *in vitro* at

nanomolar concentrations.

## Antiviral Drug Resistance

This is a major problem for all drug monotherapies for HIV-1, since the virus generates an enormous level of genetic diversity. It is less of a problem for combination therapies. Even so, there is evidence that the virus continues to replicate in the face of combination therapy – although this appears to be due in large part to the replication of wild-type (non-drug-resistant) virus, presumably because of limited biodistribution of some or all of the antiviral drugs (i.e., there may be cells or tissues which the drugs fail to penetrate adequately).

## Combination Therapy (aka: Highly Active Anti-Retroviral Therapy; HAART).

Combination therapy, or the simultaneous use of multiple anti-HIV drugs, is the most effective means of controlling HIV-1 infection. Typically, one combines one protease inhibitor with two reverse transcriptase inhibitors (so as to simultaneously target two different HIV-1 gene products). An updated list of approved drug combinations for HIV therapy is maintained online at <http://www.hivatis.org>

Much current research is focused on whether HIV-1 positive persons need to continue to take antiviral drugs lifelong (with the attendant costs, side-effects and inconvenience), or whether it might be possible to withdraw drug treatment after a time. Most evidence suggests that even a 2 to 3 year course of HAART is insufficient to eliminate all of the virus in the body, and that the virus will “rebound” immediately following the discontinuation of the drug.

One area of hope, however, involves boosting host immune responses prior to discontinuation of drug therapy. There is evidence to show that the low virus loads that result following HAART can lead to a decrease in host immune responses to HIV-1 (presumably because the antigenic stimulus has declined). If one can stimulate the antiviral immune response in persons receiving HAART, it may be possible to then discontinue HAART without necessarily triggering an immediate and rapid “rebound” in virus replication (because the host immune response should be able to contain the virus). Efforts to pursue this strategy are presently in progress, and they include the use of vaccination strategies as well as the deployment of short, structured treatment interruptions (to allow for brief periods of virus replication, with resultant stimulation of the host immune response).