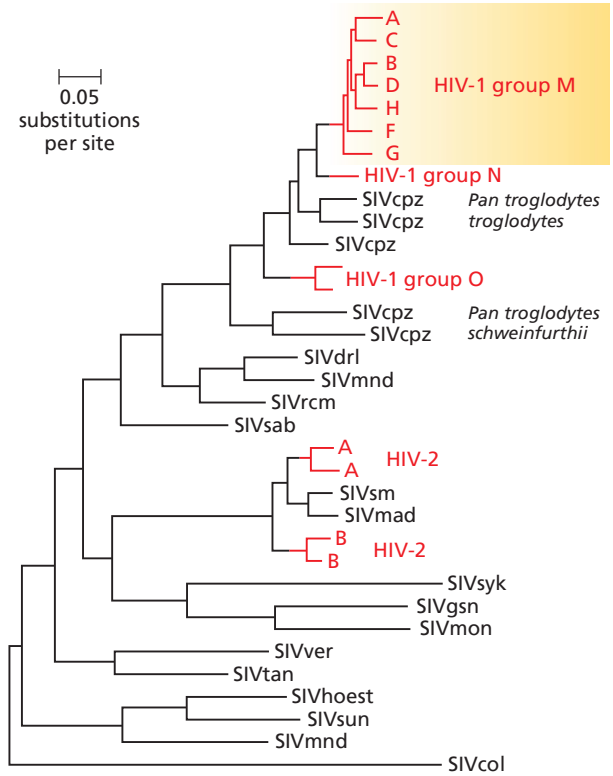
Table 7.1 Lentiviruses[a]

Virus	Host infected	Primary cell type infected	Clinical disorder(s)
Equine infectious anemia virus	Horse	Macrophages	Cyclical infection in the first year, autoimmune hemolytic anemia, sometimes encephalopathy
Visna/maedi virus	Sheep	Macrophages	Encephalopathy/pneumonitis
Caprine arthritis-encephalitis virus	Goat	Macrophages	Immune deficiency, arthritis, encephalopathy
Bovine immunodeficiency virus	Cow	Macrophages	Lymphadenopathy, lymphocytosis
Feline immunodeficiency virus	Cat	T lymphocytes	Immune deficiency
Simian immunodeficiency virus	Primate	T lymphocytes	Immune deficiency and encephalopathy
Human immunodeficiency virus	Human	T lymphocytes	Immune deficiency and encephalopathy

[a]Adapted from Table 1.1 (p. 2) of J. A. Levy, *HIV and the Pathogenesis of AIDS*, 3rd ed. (ASM Press, Washington, DC, 2007).

Figure 7.2 Phylogenetic relationships among primate lentiviruses. The tree was reconstructed by computational methods and alignment of 34 published nucleotide sequences of the retroviral *pol* genes, taken from GenBank. SIVcol signifies black and white colobus; SIVdrl, drill; SIVgsn, greater spot-nosed monkey; SIVlhoest, L'Hoest monkey; SIVmac, macaque; SIVmnd, mandrill; SIVmon, Campbell's mona monkey; SIVrcm, red-capped monkey; SIVsab, Sabaeus monkey; SIVsun, sun-tailed monkey; SIVsyk, Sykes' monkey; SIVtan, tantalus monkey; SIVver, vervet monkey; SIVcpz, chimpanzee. The species of transmission from chimps to humans, giving rise to HIV-1 groups M, N, and O, are identified. For clarity, only some subtypes of HIV-1 and HIV-2 are shown. From Figure 2 of A. Rambaut, D. Posada, K. A. Crandall, and C. Holmes, The causes and consequences of HIV evolution. *Nat Rev Genet* **5**:52–56, 2004, with permission.



Distinctive Features of the HIV Reproduction Cycle and the Functions of Auxiliary Proteins

Much of what we know about the function of the auxiliary proteins of HIV comes from studies of their effects on cells in culture, often produced transiently from plasmid expression vectors in the absence of other viral components (Volume I, Box 8.8). Although these methods are simple and sensitive, they do not necessarily reproduce the conditions of viral infection. Preparation and analysis of viral mutants have also been used to investigate the functions of these proteins in cell culture. However, as the hosts for these viruses are humans, it is difficult to evaluate the significance of many of the functions deduced from cell culture to pathology in the whole organism.

The Regulatory Proteins Tat and Rev

Tat (for transactivator of transcription) stimulates processive transcription. As in all retroviruses, expression of integrated HIV DNA is regulated by sequences in the transcriptional control region of the viral long terminal repeat (LTR), which are recognized by the cellular transcriptional machinery. The HIV LTR functions as a promoter in a variety of cell types, but its basal level is very low. As described in Volume I, Chapter 8 (Fig. 8.10), the LTR of HIV includes an enhancer sequence that binds a number of cell type-specific transcriptional activators, among them Nf-κb (Volume I, Fig. 8.11). The fact that Nf-κb enters the nucleus to promote T cell activation may explain why HIV reproduction requires T cell stimulation.

Just downstream of the site of initiation of transcription in the HIV LTR is a unique viral regulatory sequence, the *trans*-activating response element TAR (Fig. 7.4). TAR RNA forms a stable, bulged stem-loop structure that binds Tat together with a number of host proteins (Volume 1, Chapter 8, Fig. 8.13). The principal role of Tat is to stimulate processivity of transcription and thereby facilitate the elongation of viral RNA.

BOX 7.2

BACKGROUND

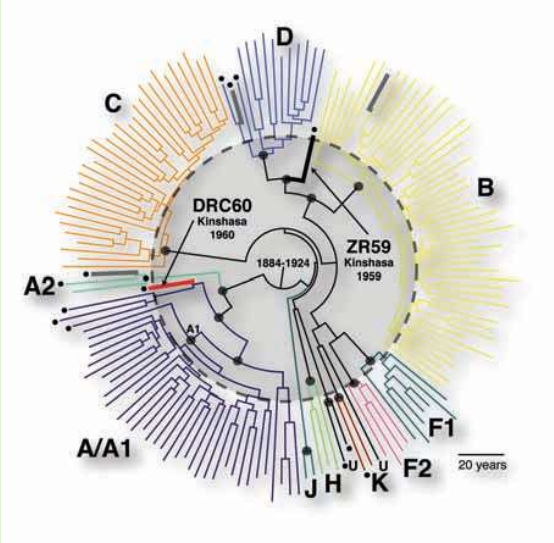
The earliest record of HIV-1 infection

For some time, the earliest record of HIV-1 infection came from a serum sample obtained in 1959 from a Bantu male in the city of Leopoldville, now known as Kinshasa, in the Democratic Republic of Congo (DRC). Phylogenetic analyses placed this viral sequence (ZR59) near the ancestral node of clades B and D. As this is not at the base of the M group, this group must have originated earlier, and back calculations suggested that the M group of viruses arose via cross-species transmission from a chimpanzee into the African population around 1930. Subsequent characterization of viral sequences in a paraffin-embedded lymph node biopsy specimen prepared in 1960 from another individual in Kinshasa (DRC60) led to a revision of that estimate. The ZR59 and DRC60 sequences differ by a degree (12%) seen in the most divergent strains within subtypes. Results from a variety of statistical analyses with these and additional archived samples indicate that the epidemic was well established by 1959/1960 and that the common ancestor was probably circulating as early as 1910. The initial transmission event could have occurred even earlier. Because the human strains shared a common ancestor with the chimp strains in about 1850, the period between ~1850 and ~1910 is the most likely window for the fateful first jump of what became the pandemic HIV/AIDS lineage. As the greatest diversity of group M subtypes has been found in Kinshasa, it seems likely that all of the early diversification of HIV-1 group M viruses occurred in the Leopoldville area, which was one of the largest urban centers at the time.

Sharp PM, Hahn BH. 2008. Prehistory of HIV. *Nature* **455:**605–606. (A personal account of the efforts to determine the routes of transmission from primates to humans can be found in an interview with Dr. Beatrice Hahn: http://bit.ly/Virology_Hahn)

Worobey M, Gemmel M, Teuwen DE, Haselkorn T, Kunstman K, Bunce M, Muyembe J-J, Kabongo J-MM, Kalengayi RM, Van Marck E, Gilbert MTP, Wolinsky SM. 2008. Direct evidence of extensive diversity of HIV-1 in Kinshasa by 1960. *Nature* **455:**661–665.

Branch lengths are depicted in unit time (years) and represent the median of those nodes that were present in at least 50% of the sampled trees. DRC60 (red), ZR59 (black), and the three control sequences from paraffin-embedded specimens from known AIDS patients (gray) are depicted in bold. Sequences sampled in the DRC are highlighted with a bullet at the tip. Nodes (sub-subtype and deeper) are marked with gray circles. DRC60 and the two control sequences from the DRC each form monophyletic clades with previously published sequences from the DRC, whereas the Canadian control sequence clusters, as expected, with subtype B sequences. Unclassified strains are labeled U. The dashed circle and shaded area show the extensive HIV-1 diversity in Kinshasa in the 1950s. From Fig. 2 of M. Worobey et al. *Nature* **455:**661–664, 2008, with permission.



Tat is released by infected cells; it can then be taken up by other cells and influence their function. Tat can act as a chemo-attractant for monocytes, basophils, and mast cells. It also induces synthesis of a variety of important proteins in the cells that it enters, and some of these can have a profound effect on virus spread and immune cell function. For example, in transient-expression assays Tat can stimulate the expression of genes encoding the CXCr4 and CCr5 coreceptors in target cells and also enhance the synthesis of a number of chemokines. The Tat protein is cytotoxic to some cultured cells and is neurotoxic when inoculated intracerebrally into mice.

Multiple splice sites and the function of Rev. In contrast to the oncogenic retroviruses with simpler genomes, the full-length HIV transcript contains numerous 5′ and 3′ splice sites. The regulatory proteins Tat and Rev (for regulator of expression of virion proteins) and the accessory protein Nef are synthesized early in infection from multiply spliced mRNAs (Volume 1, Appendix Fig. 29). As Tat then stimulates transcription, these mRNAs are found in abundance at this early time. However, accumulation of the Rev protein brings about a change in the pattern of mRNAs, leading to a temporal shift in viral gene expression.

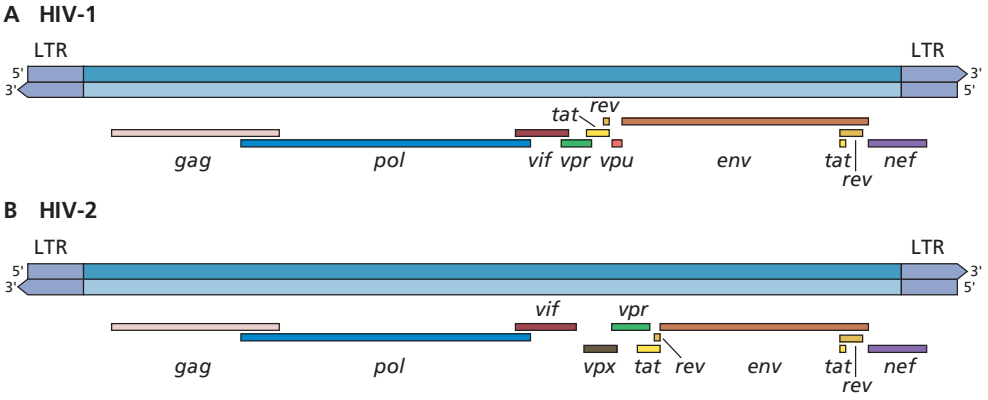
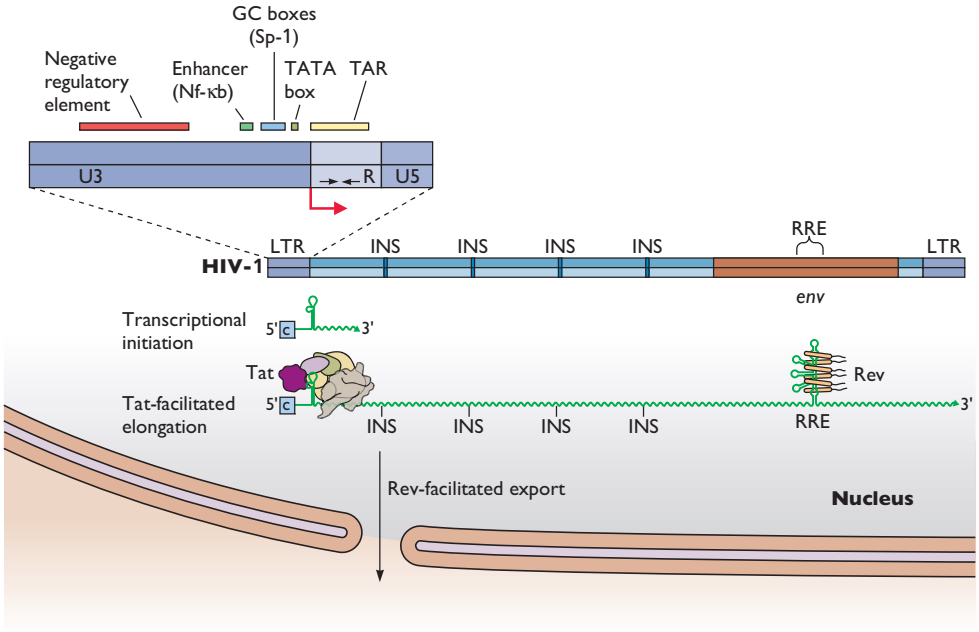


Figure 7.3 Organization of HIV-1 (A) and HIV-2 proviral DNA (B). Vertical positions of the colored bars denote each of the three different reading frames that encode viral proteins. The LTRs contain sequences necessary for transcriptional initiation and termination, reverse transcription, and integration.

Rev is an RNA-binding protein that recognizes a specific sequence within a structural element in the *env* region of the elongated transcript, called the **Rev-responsive element (RRE)** (Fig. 7.4). Rev mediates the nuclear export of any RRE-containing RNA by a mechanism discussed more fully in Volume I, Chapter 10 (Figs. 10.14 to 10.16). As the concentration of Rev increases, unspliced or singly spliced transcripts containing the RRE are exported from the nucleus. In this way, Rev promotes synthesis of the viral structural proteins and enzymes and ensures the availability of full-length

Figure 7.4 Mechanisms of Tat and Rev function. Some regulatory sequences in the HIV LTR are depicted in the expanded section at the top. The numbers refer to positions relative to the site of initiation of transcription. The opposing arrows in R represent a palindromic sequence that folds into a stem-loop structure (TAR) in the transcribed mRNA to which Tat binds (center). Tat recruits cellular proteins that are required for efficient elongation during HIV-1 RNA synthesis. The position of the RRE in the *env* transcript (with bound Rev dimers) and the *cis*-acting repressive sequences (instability sequences, INS) in the unspliced or singly spliced transcripts are also illustrated. Mutations in the A+U-rich INS increase the stability, nuclear export, and translation efficiency of the transcripts in the absence of Rev. Response to INS appears to be cell type-dependent, but the mechanisms by which they act, and exactly how Rev counteracts their effects, are not understood.



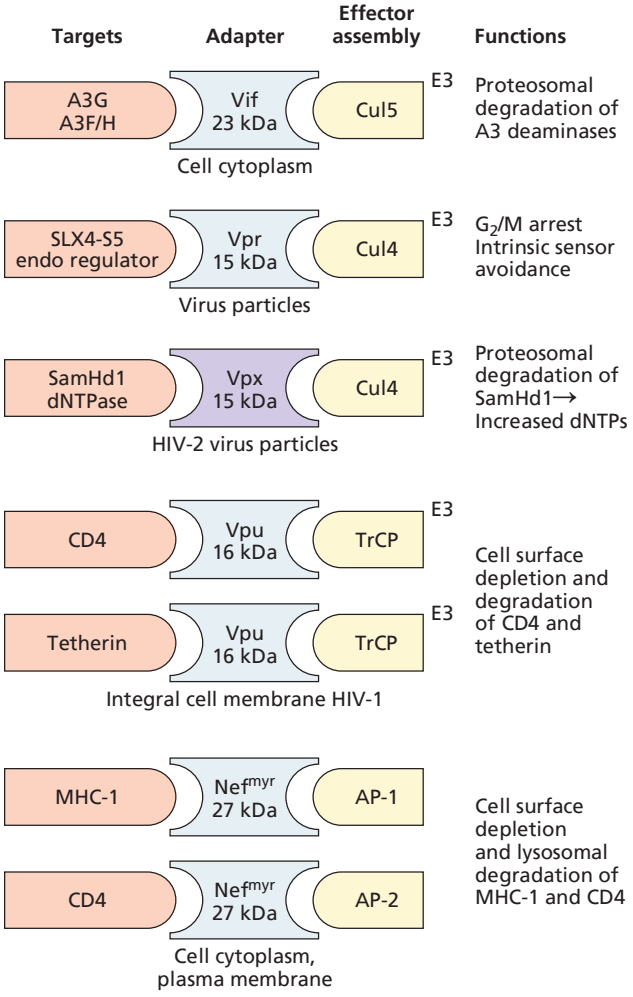


Figure 7.5 Adapter functions of HIV-1 accessory proteins. The major targets for the HIV accessory proteins, their locations, and the effector assemblies with which they interact are noted. Most effector assemblies function by mediating destruction of the targets via reactions in which the viral proteins are recycled: In a reaction that requires binding of the transcription regulator, cellular core binding factor beta (Cbfβ), **Vif** assembles with additional cellular proteins (Cul5, Elongins B and C, and Rbx1) in an E3 ubiquitin ligase that then targets Apobec proteins (A3G, F, and H) for ubiquitination and proteosomal degradation. While less potent than A3G, A3F and A3H have antiviral activities and are produced in abundance in lymphoid cells (Chapter 3). **Vpr** functions in a similar way, but interacts with a different member of the cullin family (Cul 4), in a damaged DNA-binding protein (Ddb1) and Cul-associated factor 1 (Dcaf1)-E3-ligase assembly. This interaction leads to dysregulation of cellular endonuclease activities, triggering a DNA damage response and cell cycle arrest. The **Vpx** protein of HIV-2 engages the same Cul 4-E3 complex as Vpr, but their targets are quite distinct: Vpx targets the dGTP-dependent deoxynucleoside triphosphohydrolase, SamHd1, for ubiquitination and proteosomal degradation. Phosphorylation of **Vpu** leads to recruitment of another multiprotein E3 ubiquitin ligase, Scf (which includes Cul1, Skp1, and Roc1) via interaction with the adapter, β-transducin repeat-containing protein (β-TrCP), and targets both CD4 and tetherin degradation. **Nef** binds to the cytoplasmic tail of CD4 and links this receptor to components of a clathrin-dependent trafficking pathway at the plasma membrane, leading to its internalization and delivery to lysosomes for degradation. Nef decreases cell surface expression of MHC class I molecules by a different pathway, mediating an interaction between the cytoplasmic domain of the MHC class I molecules and the clathrin adapter protein complex (AP-1).

genomic RNA for incorporation into progeny virus particles. The accessory proteins Vif, Vpr, and Vpu (for HIV-1) or Vif, Vpr, and Vpx (for HIV-2) are also produced later in infection from singly spliced mRNAs that are dependent on Rev for export to the cytoplasm (Volume I, Appendix Fig. 29).

The Accessory Proteins

While a very large number of seemingly disparate activities have been attributed to the HIV accessory proteins, recent studies have revealed a common mechanism for their action: all are **adapter proteins** that disrupt the normal trafficking of particular cellular proteins and, in most cases, lead to their destruction (Fig. 7.5). In this way, HIV accessory proteins function as antagonists of cellular intrinsic defense mechanisms that detect infection and counteract virus reproduction.

Vif protein. Vif (viral infectivity factor) is a 23-kDa protein that accumulates in the cytoplasm and at the plasma membrane of infected cells. Early studies showed that mutant virus particles lacking the *vif* gene were approximately 1,000 times less infectious than the wild type in certain $CD4^+$ T cell lines and peripheral blood lymphocytes and macrophages. It was discovered that production of Vif from a plasmid vector in susceptible host cells did not compensate for its absence in the cell that produced virus particles. Rather, Vif was needed at the time of virus assembly.

Vif is an RNA-binding protein and small quantities can be detected in HIV particles. Virus particles produced from *vif*-defective HIV genomes contain the normal complement of progeny RNA, and they are able to enter susceptible cells and to initiate reverse transcription, but full-length double-stranded viral DNA is not detected. These observations demonstrated that Vif is required in a step that is essential for completion of reverse transcription. The requirement for Vif is strikingly cell type-dependent, and experiments in which cells that are permissive for *vif* mutants were fused with cells that are nonpermissive established that the nonpermissive phenotype is dominant. The infectivity of virus particles produced in such heterokaryons was enhanced by Vif production. This observation suggested that Vif may suppress a host cell function that otherwise inhibits progeny virus infectivity.

All of these seemingly unusual properties were demystified with the discovery that Vif blocks the antiviral action of members of an RNA-binding family of cellular cytidine deaminases, called apolipoprotein B mRNA editing enzyme catalytic peptides 3 (Apobec3). In nonpermissive cells, these enzymes are incorporated into progeny virus particles via interactions with

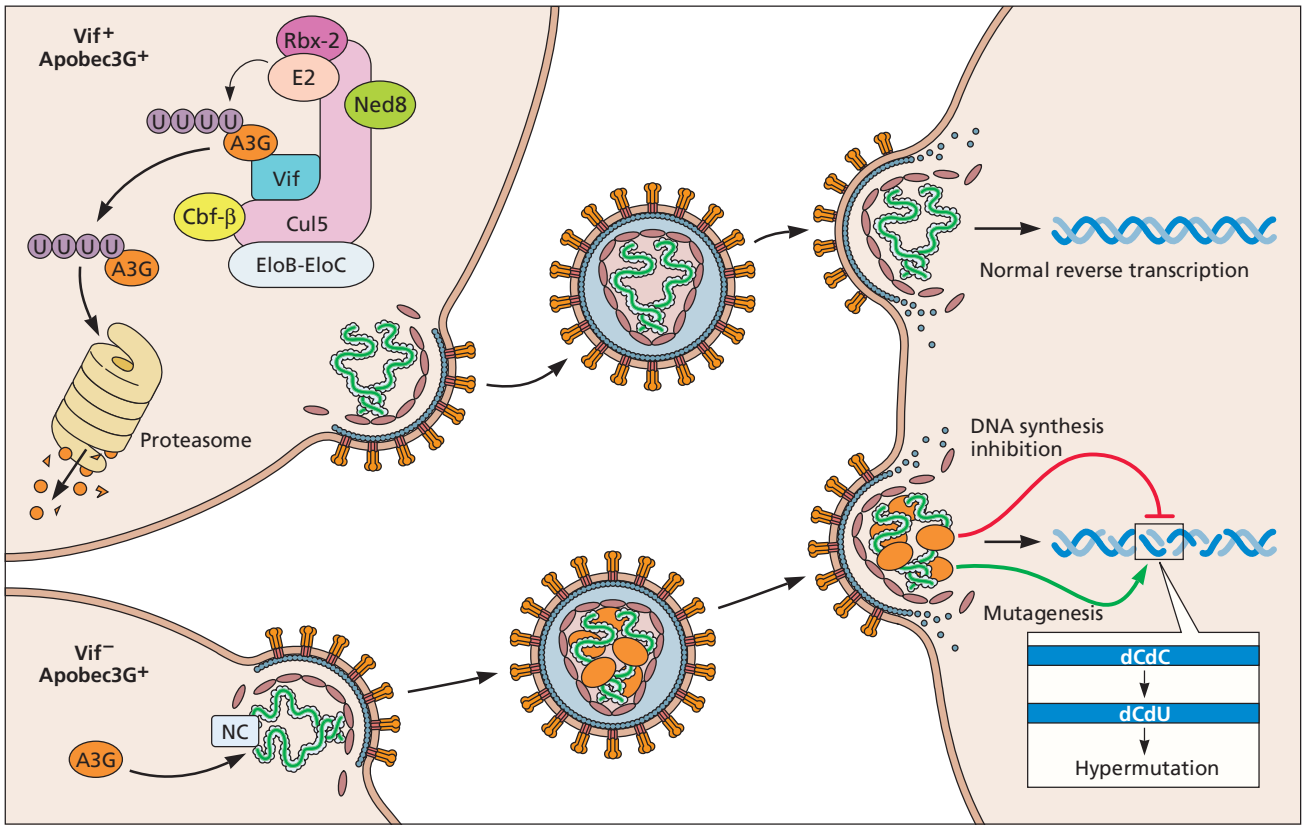


Figure 7.6 Mechanisms of action of Vif and Apobec3G. **(Top)** Vif counteracts the antiviral effects of Apobec3G (A3G) by mediating its polyubiquitination, which leads to proteosomal degradation. **(Bottom)** In the absence of Vif, A3G is incorporated into newly formed virus particles through interaction between viral RNA and NC protein. In the newly infected cell, reverse transcription is inhibited by A3G, and cytosines in the newly synthesized DNA are converted to uracil, causing hypermutation through eventual C to A transversions. Adapted from B. Cullen, *J Virol* **80:**1067–1076, 2006, with permission.

the viral RNA and possibly NC protein (Fig. 7.6). Apobec3G was the first family member to be identified as a Vif target. It was subsequently shown that Vif prevents its incorporation into virus particles by binding to the protein and inducing its depletion. In a reaction that requires binding of a transcriptional regulator, Vif assembles with additional cellular proteins to form an E3 ubiquitin ligase that recognizes Apobec3G as a substrate for polyubiquitination, a signal for its subsequent degradation in proteasomes (Fig. 7.7).

Apobec3G appears to inhibit virus reproduction in a number of ways. It has been proposed that binding to viral RNA may account, in part, for the deaminase-independent inhibition of reverse transcription in newly infected cells. The deaminase activity of Apobec3G leads to formation of deoxyuridine (dU), most frequently at preferred deoxycytidine (dC) sites in the first (−) strand of viral DNA to be synthesized by reverse transcriptase. Consequently, the (+) strand complement of the deaminated (−) strand will contain deoxyadenosine in place of the normal deoxyguanosine at such sites (Fig.7.6). Indeed, the frequency of G→A transitions is abnormally high in the genomes of *vif*-defective particles produced in nonpermissive cells, and incomplete protection from Apobec3 proteins by Vif may explain why such transitions are the most frequent point mutations in HIV genomes. It has been suggested that the Apobec3 proteins represent an ancient intrinsic cellular defense against retroviruses (see Chapter 3).

Vpr protein. The 15-kDa viral protein R, Vpr, derives its name from the early observation that it affects the **rapidity** with which the virus reproduces in, and destroys, T cells. Most T cell-adapted strains of HIV-1 carry mutations in *vpr*. The SIV and HIV-2 genomes include a second, related gene *vpx,* which is discussed below and appears to have arisen as a duplication of *vpr*. The other lentiviruses do not contain sequences related to *vpr* but do include small open reading frames that might encode proteins with similar functions.

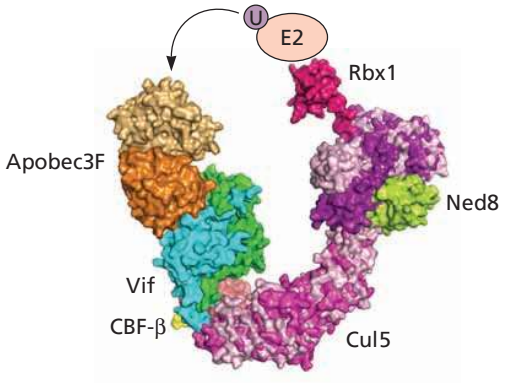


Figure 7.7. Molecular model for Apobec3F degradation by the Vif/Cbf-β ubiquitin ligase. Existing crystal structures of analogous proteins and complexes were used to model the Apobec3F degradation complex. In the model, Vif (green and blue) functions as a scaffold to assemble the E3 ligase by binding to Cbf-β (yellow and pink) and to Elo C and Cul5 (N-terminal domain). Activation of the E3 ligase is thought to occur by neddylation by Ned8 (lime green) of a lysine on the Cul5 (C-terminal domain). Subsequently, Rbx1 (magenta) adopts a conformation that facilitates polyubiquitination of Vif-bound Apobec3F (N-terminal domain, light orange and C-terminal domain, dark orange) via a cellular E2 ubiquitination enzyme. The model and Movie 7.1 was created by Drs. Nadine Shaban and Reuben Harris, University of Minnesota. For more details see J. S. Albin et al., A structural model of the APOBEC3F-Vif interaction informed by biological and computational studies. In preparation.

Vpr is incorporated into HIV-1 particles via specific interactions with a proline-rich domain at the C terminus of the Gag polyprotein. The host's uracil DNA glycosylase, Ung2, is then incorporated into particles by binding to Vpr. A substantial quantity, about 100 to 200 molecules of Vpr, is present in capsids. Its presence in virus particles is consistent with the observation that Vpr function is required at an early stage in the virus reproduction cycle.

Like Vif, Vpr functions as an adapter protein in an E3-ligase, but via interaction with a different member of the cullin family (Cul4, Fig. 7.5). Proteomic and biochemical studies have demonstrated that Vpr in this protein assembly facilitates recruitment of the cellular Slx4 structure-specific endonuclease regulator and untimely activation of the endonuclease activities that it controls. Such unleashed endonuclease activity could lead to the degradation of excess viral DNA, and it has been proposed that this feature may prevent detection by the intrinsic immune system (Chapter 3) and limit a defensive interferon response at early times after infection. Loss of endonuclease regulation at replication forks in the host DNA induces a damage response that may explain the G2/M arrest and apoptosis responses that are known to be triggered by Vpr. The possible advantage of preventing infected cells from entering mitosis is not apparent, especially as the requirement for Vpr function is most evident in HIV infection of macrophages, cells that do not divide. One idea is that the increased activity of the LTR promoters in the G_2 phase of the cell cycle may lead to enhanced virus production in the presence of Vpr.

In addition to cell cycle arrest and apoptosis, numerous other functions and interactions have been ascribed to this tiny protein, including modulation of the transcription of host and viral genes, maintenance of reverse transcriptase fidelity, recruitment of Ung2, and nuclear import of preintegration complexes in nondividing cells. Vpr has been shown to bind to nuclear pore proteins. Although these interactions are not essential for nuclear import, they may facilitate docking of the HIV-1 preintegration complex at the nuclear pore in preparation for import (Volume I, Chapter 5).

Vpx protein. Vpx is also packaged specifically via interaction with the Gag polyprotein. Vpx functions as an adapter that engages the same Cul4-E3 ubiquitin ligase as Vpr, but targets quite distinct proteins: Vpx recruits the dGTP-dependent deoxynucleoside triphosphohydrolase, SamHd1, for ubiquitination and proteosomal degradation (Fig. 7.5). SamHd1 blocks lentiviral DNA synthesis in myeloid cells by hydrolyzing cellular deoxynucleotide triphosphates to reduce concentrations to below those required for reverse transcription. The finding that Vpx can mediate degradation of this enzyme helped to explain why HIV-2 but not HIV-1 can propagate efficiently in macrophages or dendritic cells. Indeed, ectopic production of Vpx enhances HIV-1 infection in myeloid and $CD4^+$ T cells, as does RNA interference-mediated knockdown of SamHd1.

Vpu protein. This small, 16-kDa viral protein is unique to HIV-1 and the related SIV_{cpz} (Fig. 7.3), hence the name viral protein U. The predicted sequence of Vpu includes an N-terminal stretch of 27 hydrophobic amino acids comprising a membrane-spanning domain and a cytoplasmic domain containing two α-helices. Biochemical studies show that Vpu is an integral membrane protein that self-associates to form oligomers. In infected cells, the protein is located on all major membranes, but is concentrated mainly in the endoplasmic reticulum, the *trans*-Golgi network, and endosomes.

Synthesis of Vpu is required for the proper maturation and targeting of progeny virus particles and for their efficient release. In its absence, particles containing multiple cores are produced, and budding is targeted to multivesicular bodies rather than to the plasma membrane (Fig 7.8). Vpu also reduces the syncytium-mediated cytopathogenicity of HIV-1, perhaps because the inefficient release of virus particles prevents the accumulation of sufficient Env protein at the cell surface to promote cell fusion.

A major function of Vpu in the pandemic group M strains of HIV-1 is to block the activity of a cellular membrane protein initially called bone stromal antigen 2 (Bst-2), but now more commonly known by the more descriptive name, tetherin. Tetherin is a dimeric type II membrane protein with an N-terminal cytoplasmic tail, a transmembrane region, and a C-terminal glycophosphatidyl inositol membrane anchor.

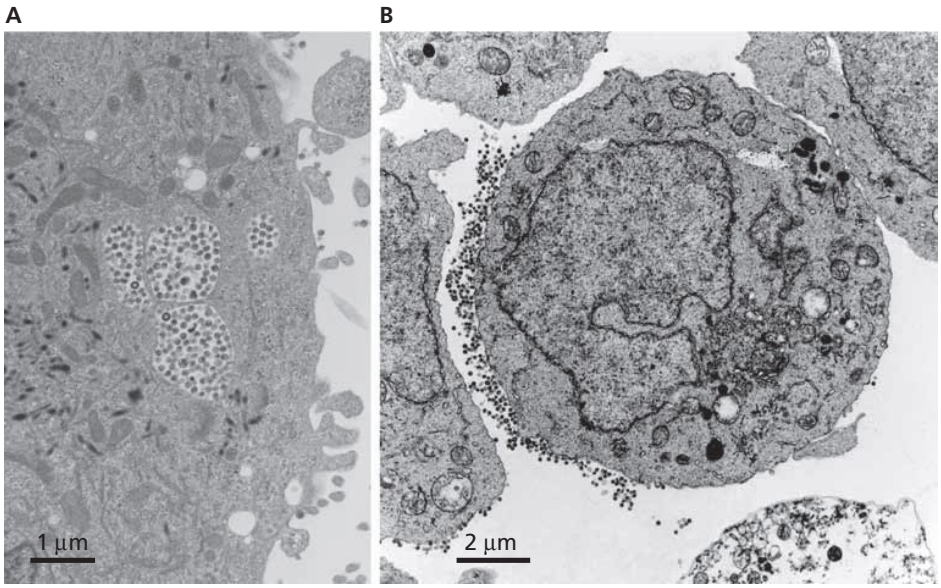


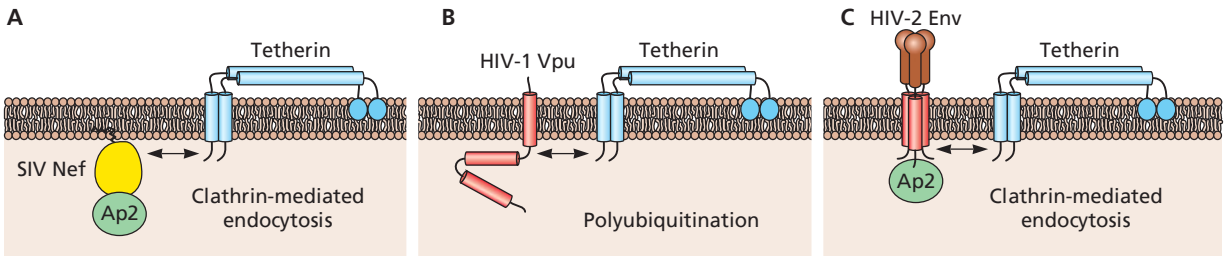
Figure 7.8 Human cells infected with an HIV-1 virus lacking Vpu. **(A)** Electron microscope image of a human macrophage showing intracellular accumulation of virus particles. **(B)** Electron microscope image of an infected H9 T cell showing accumulation of virus particles at the cell surface. Images courtesy of Drs. Jaang-Jiun Wang and Paul Spearman.

An interferon-inducible protein, tetherin restricts the propagation of enveloped viruses by inhibiting their release from infected cells (Fig. 7.9) and has been shown to act as a pattern-recognition receptor that induces Nf-κb-dependent proinflammatory gene expression in infected cells (Chapter 3).

Several residues in the transmembrane domain of Vpu interact directly with the transmembrane domain of tetherin. Vpu binds tetherin in the *trans*-Golgi network, inhibiting the transport of tetherin to the plasma membrane. Phosphorylation of the Vpr then leads to recruitment of another multiprotein E3 ubiquitin ligase, Scf, and the subsequent ubiquitinylation and degradation of tetherin in an endolysosomal pathway (Fig. 7.5). Vpu also facilitates the degradation of CD4: the viral protein traps newly formed CD4 receptor molecules in the endoplasmic reticulum, mediates ubiquitinylation by the Scf ubiquitin ligase, and entry of CD4 into the endoplasmic reticulum-associated proteasome degradation pathway. Reducing the quantity of CD4 at the cell surface limits superinfection by HIV-1. It also reduces loss of Env protein via CD4 binding, thereby enhancing production of infectious particles.

Oligomerization of the membrane-spanning domains of Vpu is the basis of yet another property of Vpu, namely formation of an ion-conducting channel known as a **viroporin**, similar to that of the influenza A virus protein M2. While cell membrane integrity is disrupted and permeability to small molecules is increased when Vpr is produced in *Escherichia coli* or cultured mammalian cells, the relevance of Vpr viroporin activity to HIV pathogenesis is still unclear. Such activity could certainly affect the function of internal membranes, which are the major sites of Vpr accumulation. It has also been proposed that virus particle release may be enhanced by changes in the membrane potential across the budding plasma membrane.

Figure 7.9 Antagonism by viral proteins. Illustration of ways in which simian immunodeficiency virus (SIV) Nef protein **(A)**, HIV-1 Vpu **(B)**, and HIV-2 Env **(C)** target the cellular protein tetherin. Protein interactions are indicated by the double-headed arrows; Ap2, clathrin adapter protein complex-2.



Genetic studies and experiments with a viroporin-specific inhibitor (BIT225) indicate that the ion channel and tetherin-antagonizing activities of the membrane-spanning domain of Vpu are independent of one another.

One might wonder: if tetherin antagonism is so important to HIV reproduction, why is Vpu only found in HIV-1? The answer is that different retroviral proteins have assumed this function during evolution (Fig. 7.9). The envelope protein of HIV-2 has evolved to include this function and the Nef proteins of several primate viruses are antagonists of the tetherin orthologs in their host species

Nef protein. Most laboratory strains of HIV-1, which have been adapted to grow well in T cell lines, contain deletions or other mutations in the *nef* gene. Restoration of *nef* reduces the efficiency of virus reproduction in these cells, hence the name "negative factor." Multiple functions have been attributed to Nef, and it is now clear that its importance may vary in different cell types.

Nef is translated from multiply spliced early transcripts. The 5′ end of Nef mRNA includes two initiation codons and, as both are utilized, two forms of Nef are produced in infected cells. The apparent size of these proteins can vary because of differences in posttranslational modification. Like Vpr, Nef is incorporated into virus particles via interaction with the Gag polyprotein. Nef molecules in virus particles appear to contribute to capsid disassembly following infection and may also enhance reverse transcription. The protein is myristoylated posttranslationally at its N terminus and thereby anchored to the inner surface of the plasma membrane.

Nef includes a protein-protein interaction domain (SH3) that mediates binding to components of intracellular signaling pathways, eliciting a program of gene expression similar to that observed after T cell activation. Such gene expression is thought to provide an optimal environment for viral reproduction. Among the best-studied, and clearly physiologically relevant activities of Nef is its downregulation of surface concentrations of CD4 and major histocompatibility complex (MHC) class I molecules (Fig. 7.5). The former activity is shared with Vpu. At the plasma membrane Nef binds to the cytoplasmic tail of CD4 and components of a clathrin-dependent trafficking pathway, leading to its internalization and delivery to lysosomes for degradation (Fig. 7.10, left). As with Vpu, the ensuing reduction in the number of CD4 molecules at the cell surface facilitates virus particle release and limits reinfection.

Nef decreases cell surface MHC class I molecules by a different pathway. It mediates interaction between the cytoplasmic domain of the MHC class I molecules and the clathrin adapter protein complex (AP-1) in the *trans*-Golgi network, prior to their transport to the cell surface (Fig. 7.10, right). The Nef-induced complex is retained in this Golgi compartment and MHC class I molecules are subsequently diverted to lysosomes for degradation. As a strong cytotoxic T lymphocyte (CTL) response against viral infection requires

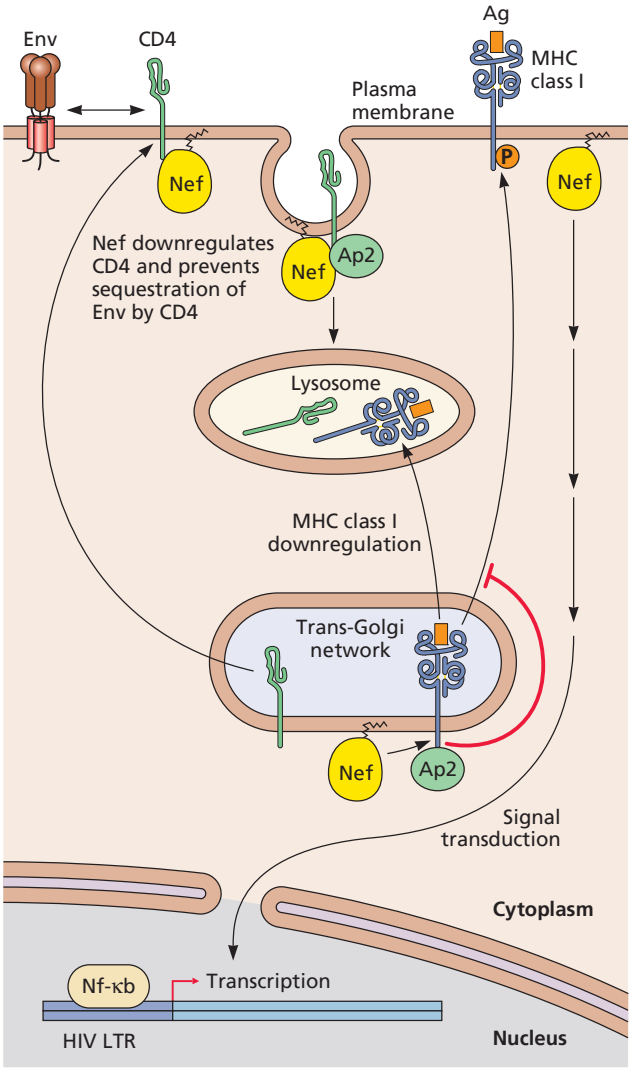


Figure 7.10 Intracellular functions attributed to Nef. Nef is myristoylated posttranslationally; the jagged protrusion represents myristic acid covalently linked to the glycine residue at position 2. Myristoylation enables Nef to attach to cell membranes, where it can interact with membrane-bound cellular proteins. Nef reduces the cell surface expression of CD4 by binding to sequences in the cytoplasmic domain of this receptor and enhancing clathrin-dependent endocytosis and the subsequent degradation of CD4 within lysosomes; Ap2, clathrin adapter protein complex-2. This activity reduces interaction of CD4 with surface Env proteins, thereby enhancing Env incorporation into budding virus particles. In contrast, MHC class I expression on the cell surface is reduced by Nef binding in the membrane of the *trans*-Golgi network. This interaction interferes with the normal vesicular sorting required for passage of the receptor to the cell surface, and MHC class I is directed to the lysosome for degradation. Nef also affects signal transduction by increasing the activity of the cellular transcriptional activator Nf-κb and perhaps other cellular transcription proteins.

recognition of viral epitopes presented by MHC class I molecules, this inhibitory activity of Nef allows infected cells to escape lysis by CTLs and is probably a major factor contributing to HIV-1 pathogenesis. Nef induces decreased concentrations of a number of other cell surface molecules including a component of the T cell receptor complex (CD3), the lymphocyte-specific protein tyrosine kinase (Lck), and the costimulatory molecule for T cell activation (CD28). These activities of Nef contribute to T cell activation and recognition of infected cells by cells of the immune system (Chapter 4).

Other activities ascribed to Nef also seem likely to contribute to pathogenesis in important ways. One example is Nef-mediated inhibition of endocytosis of the type II transmembrane lectin, DC-Sign (dendritic-cell-specific, Icam-3-grabbing nonintegrin), which binds to the HIV-1 envelope protein with high affinity. Such inhibition by Nef leads to **increased** concentration of this lectin on the surfaces of immature and mature dendritic cells. DC-Sign facilitates dendritic cell transmigration through the vascular and lymphoid endothelium, as well as the adhesion of these cells to T cells during antigen presentation. Studies with dendritic cells infected with HIV-1 have shown that such cells form many more clusters with activated primary T cells than dendritic cells infected with a *nef*− HIV-1 mutant. As dendritic cells can retain attached infectious virus particles for several days, the increase in surface accumulation of DC-Sign cell surface concentration induced by Nef may be important for both viral spread and transmission to T cells.

Although the initial cell culture experiments suggested a negative effect on virus production, subsequent experiments with animals showed that Nef augments HIV pathogenesis quite significantly. Rhesus macaques inoculated with a Nef-defective mutant of SIV had low virus titers in their blood during early stages of infection, and the later appearance of high titers was associated with reversion of the mutation. More importantly, adult macaques inoculated with a virus strain containing a deletion of *nef* did not progress to clinical disease and were, in fact, immune to subsequent challenge with wild-type virus. The observation that *nef* had been deleted in HIV-1 isolates from some individuals who remained asymptomatic for long periods also suggests that this viral protein can contribute to pathogenesis. Initial hopes that intentional deletion of *nef* might facilitate the development of a vaccine strain for humans were dashed when it was discovered that the humans infected with *nef* deletion mutants eventually developed AIDS.

The Viral Capsid Counters Intrinsic Defense Mechanisms

Following entry of HIV-1, capsid proteins remain associated with the reverse transcription machinery (Volume I, Chapter 7). This subviral structure moves through the cytoplasm to the nuclear pore via interaction with the host cell cytoskeletal fibers, as viral DNA is synthesized and an integration-competent nucleoprotein assembly is formed. Genetic and biochemical studies have identified two host proteins that bind to the HIV-1 capsid protein, Cpsf 6 (a cleavage and polyadenylation factor) and CypA (the peptidyl-prolyl isomerase cyclophilin A). Such binding imparts stability to the capsid structure and helps to suppress its premature disassembly by host proteins such as Trim5α and TrimCypA. The stabilized capsid structure also shields viral nucleic acids from detection by intrinsic immune sensors in the cytoplasm, such as double-stranded RNA helicase, Rig-I (viral RNA), and the cyclic GMP-AMP synthase, cGAs (viral DNA) (Chapter 3). The biological importance of these capsid protein interactions early in infection is emphasized by the finding that HIV-1 mutants with capsids that are either fragile, or abnormally stable, are replication defective.

Capsid protection of viral nucleic acids may account for the observation that there is little or no interferon response at early times after HIV infection. At late times, however, when large quantities of viral RNAs are produced, there is a robust interferon response. One interferon-induced human protein, Mx2, was found to be a potent inhibitor of HIV-1 reproduction in certain cell types, particularly macrophages. Mx2 also targets the HIV-1 capsid, inhibiting nuclear import of viral DNA by a mechanism yet to be discovered. Mx2 derives its name from its close sequence relationship to the myxovirus resistance 1 protein (Mx1), which is a broadly acting inhibitor of RNA and DNA viruses, including the orthomyxovirus influenza A virus (Chapter 3). However, these proteins have distinct activities as Mx1 is not an inhibitor of HIV-1, and Mx2 is ineffective against influenza A virus.

Cellular Targets

Attachment and entry into host cells depend on the interaction between viral proteins and cellular receptors (Volume I, Chapter 5). While the major receptor for the HIV envelope protein is the cell surface CD4 molecule, the envelope protein must also interact with a coreceptor to trigger fusion of the viral and cellular membranes and gain entry into the cytoplasm. The two major coreceptors for HIV-1 are the α- and β-chemokine receptors, CXCr4 and CCr5. Strains of HIV that bind to CXCr4 or CCr5 are commonly referred to as X4 and R5 strains, respectively (Fig. 7.11). For reasons that are still not completely understood, R5 viruses are transmitted preferentially during infection. X4 viruses predominate in the late stages, following extensive evolution of the virus population within an infected individual and concomitant with immune system breakdown (Box 10.5). The importance of these two chemokine receptors to HIV pathogenesis is demonstrated by two findings. People who carry a particular mutation in the gene encoding CCr5 produce a defective receptor protein and are resistant to HIV-1 infection. So too are individuals who carry a mutation in the gene for the ligand of CXCr4

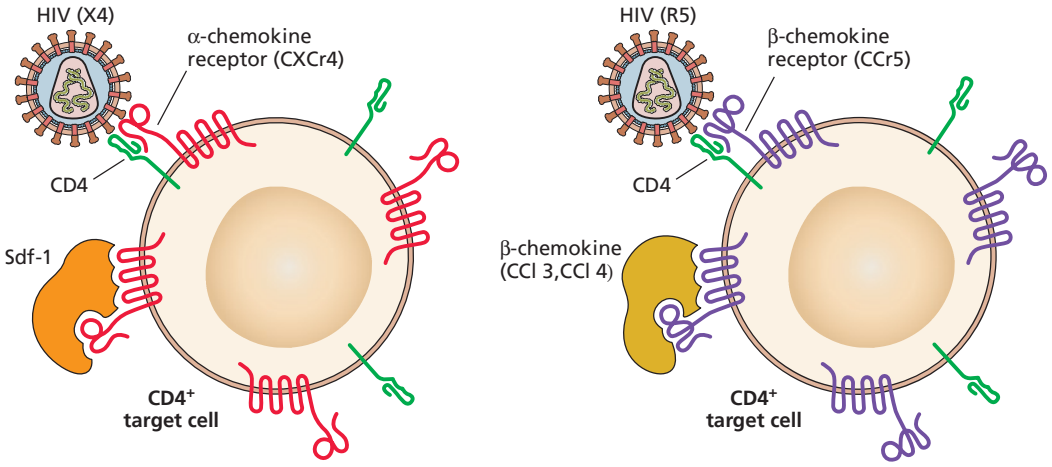


Figure 7.11 Coreceptors for HIV-1. CxCr4 is the coreceptor for HIV-1 variants that predominate during the late phase of infection; entry of such strains (denoted X4) is inhibited by the coreceptor's natural ligand, Sdf-1. CCr5 is the coreceptor for HIV-1 variants that predominate early in infection (denoted R5); their entry is inhibited by the coreceptor's natural ligands, CCl5 (Rantes), and the macrophage inflammatory proteins CCl3 and CCl4 (Mip-1α and Mip-1β). Primary T cells and monocytes can produce both coreceptors. Adapted from Fig. 3 of A. S. Fauci, *Nature* **384:**529–533, 1996, with permission.

(Table 3.7). The latter mutation may lead to increased availability of the ligand, which then blocks virus entry by competing for coreceptor binding. This idea is consistent with earlier observations that chemokine binding to the receptors inhibits the infectivity of specific strains of HIV in cell culture (Fig. 7.11). Cells of the hematopoietic lineage that bear CD4 and one or more of these chemokine receptors are the main targets of infection, and they produce the highest titers of progeny virus particles.

Several additional chemokine receptors have been identified as coreceptors for HIV and SIV in cell culture experiments, but their roles in natural infection remain to be determined. These additional coreceptors may allow the virus to enter a broader range of cells than first appreciated. Some are found on cells of the thymus gland and the brain, and some could play a role in infection in infancy or of cells in the central nervous system. It has also been proposed that binding to these additional coreceptors may trigger signals that affect virus reproduction in target cells, or that harm nonpermissive cells, producing a "bystander" effect.

Experiments in cell cultures have identified additional mechanisms by which HIV may enter cells. For example, the virus can be transmitted very efficiently through direct cell contact. In addition, cells may be infected by virus particles that are endocytosed after binding to cell surface galactosyl ceramide or to Fc receptors (as antibody-virus complexes). HIV can infect many different types of human cells in culture and has been found in small quantities in several tissues of the body. As discussed below, infection of these cells and tissues is likely to be relevant to HIV-1 pathogenesis.

Routes of Transmission

Even before HIV-1 was identified, epidemiologists had established the most likely routes of the agent's transmission to be sexual contact, blood exchange, and from mother to child. As might be anticipated, the efficiency of transmission is influenced greatly by the concentration of the virus particles in the body fluid to which an individual is exposed. Estimates of the percentage of infected cells and the concentration of HIV-1 in different body fluids indicate that highest quantities are observed in peripheral blood monocytes, in blood plasma, and in cerebrospinal fluid (Table 7.2), but semen and female genital secretions also appear to be important sources of the virus.

Other routes of transmission are relatively unimportant or nonexistent. Among these are nonsexual physical contact, exposure to saliva or urine from infected individuals, and exposure to blood-sucking insects. Fortunately, HIV-1 infectivity is reduced upon air-drying (by 90 to 99% within 24 h), by heating (56 to 60°C for 30 min), by exposure to standard germicides (such as 10% bleach or 70% alcohol), or by exposure to pH extremes (e.g., <6 or >10 for 10 min). This information and results from epidemiology studies have been used to establish safety regulations to prevent transmission in the public sector and the health care setting.

Modes of Transmission

Modes of HIV transmission vary in different geographic locations and among different populations within the same locations (Fig. 7.12). In the United States, the major overall route is via homosexual contact, specifically among men who have sex with men. Heterosexual contact is the predominant manner in