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HIV-1 Regulatory/Accessory Genes: Keys to Unraveling Viral and Host Cell Biology

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Human immunodeficiency virus type–1 (HIV-1) manipulates fundamental host cell processes in sophisticated ways to achieve optimum replicative efficiency. Recent studies have provided new details on the molecular interactions of HIV-1 with its host cell. For example, HIV-1 encodes a protein that regulates transcriptional elongation by interacting with a cellular cyclin-dependent kinase, another that activates the specific nuclear export of viral RNA, and several others that affect the intracellular trafficking of viral and host cell proteins. Detailed analysis of the interplay between these viral proteins and normal cellular activities has provided new insights into central questions of virology and host cell biology.

HIV-1 is a member of one of the five major primate lineages of the lentivirus family of retroviruses (*1*). Although the basic steps of the HIV-1 life cycle are the same as for other retroviruses, six virally encoded regulatory/accessory proteins (Tat, Rev, Vif, Vpr, Vpu, and Nef) that are not found in other classes of retroviruses impart novel levels of complexity to lentiviral replication (*2*). Here we review some of the most recent progress in our understanding of the interactions between these gene products and host cell factors and discuss possible selective pressures that have imposed the need for these specialized viral proteins.

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Transcriptional Control by Manipulation of Host Cell Factors

High-level HIV-1 transcription from the integrated DNA form of the virus (the provirus) is regulated by an ~14-kD viral protein called Tat. The domain structure of Tat is typical of many transcriptional activators and includes an activation domain and a nucleic acid (in this case, RNA) binding domain. Tat function is dependent on a bulged RNA stem-loop structure, TAR (Tat activation region), that is present at the 5′-terminus of all viral mRNAs (Fig. 1). Although HIV-1 transcription is mediated by cellular RNA polymerase II, Tat acts mostly at the level of transcriptional elongation rather than at initiation itself. Because of this apparently novel mode of transcriptional regulation, there has been a prolonged effort to identify cellular Tat cofactors (*3*). It was anticipated that definition of a cellular cofactor or cofactors could explain several intriguing observations about Tat trans activation: First, binding of recombinant Tat to TAR in vitro does not require the loop sequences known to be necessary in vivo for function; second, preincubation of nuclear extracts with the activation domain of recombinant Tat depletes a factor necessary for Tat-mediated transcription in vitro; and finally, Tat functions poorly in rodent cells unless complemented by a factor or factors that can be supplied in trans by human chromosome 12. A cellular protein complex whose attributes explain these diverse phenomena has now been found.

A cellular protein kinase complex called TAK (Tat-associated kinase) was identified that specifically binds to the activation domain of Tat and can phosphorylate the COOH-terminal domain (CTD) of RNA polymerase II (*4*)—a step that had already been implicated in regulation of transcriptional elongation (*5*). The kinase component of TAK was then shown to be the same as a previously identified kinase named PITALRE that was also implicated in transcriptional elongation. The kinase activity of the PITALRE complex is disrupted by compounds that were identified during an in vitro drug screen as inhibitors of Tat activity (*6*). PITALRE has since been renamed Cdk9 because it is related to the family of cyclin-dependent kinases (Cdks).

By analogy with other Cdks, it was assumed that Cdk9 would have a cyclin-related partner that would confer substrate specificity on the kinase. This protein has been identified and is called cyclin T (CycT) (*7*). CycT binds the activation domain of Tat both on its own and in the context of a Cdk9-CycT complex (Cdk9 does not bind Tat on its own) (Fig. 1). CycT increases the affinity of Tat for TAR, increases the specificity of Tat for residues of TAR known to be important for activity (the loop and bulge residues), and is necessary for transcriptional

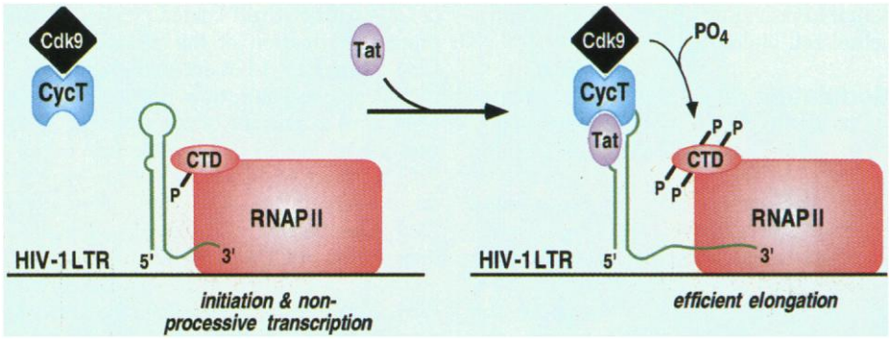

Fig. 1. Simplified model of Tat action. (**Left**) Transcription initiates on the HIV-1 LTR with the RNA polymerase II holoenzyme complex (RNAP II, red); in the absence of Tat, transcription elongation is inefficient because of hypophosphorylation of the CTD of RNAP II. Early elongation synthesizes the 5′ end of the HIV-1 mRNA (green); this forms a bulged stem-loop structure called TAR. (**Right**) The viral Tat protein in a complex with CycT and Cdk9 binds to TAR near the stalled RNAP II. Cdk9 then hyperphosphorylates the CTD, which stimulates efficient transcriptional elongation of the nascent viral mRNA.

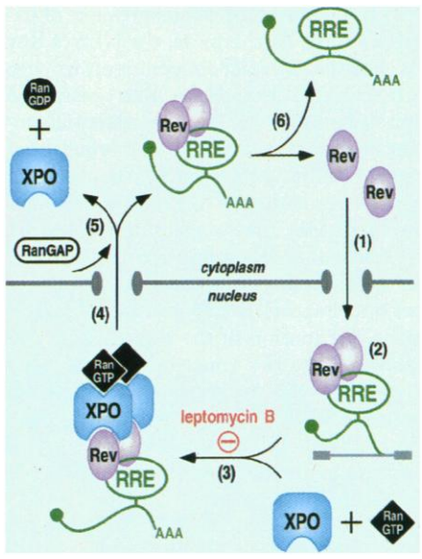

Fig. 2. Model of the Rev nuclear transport cycle. Upon entry into the nucleus (**1**), Rev binds and multimerizes on the RRE of nascent unspliced mRNAs (green) (**2**). Exportin-1 (XPO) and Ran GTP (black diamond) bind cooperatively to Rev-RRE complexes (in an LMB-sensitive reaction) (**3**) and target them for nuclear export (**4**). In the cytoplasm, Ran GTPase–activating protein (RanGAP) stimulates GTP hydrolysis and triggers the dissociation of exportin-1 and Ran GDP (black circle) from Rev (**5**). Finally, Rev shuttles back to the nucleus (1), and the exported RNA is either translated or packaged into virions (**6**).

elongation in vitro. Finally, as predicted for the bona fide human host cell factor for Tat function that is missing in murine cells, the human *CycT* gene maps to human chromosome 12 and potentiates Tat trans activation by 50- to 100-fold when introduced into murine cells (*7*). Thus, although other Tat and TAR interacting factors are also thought to contribute to HIV-1 transcription (*8*), the interaction of Tat and TAR with Cdk9-CycT on stalled RNA polymerase II holoenzyme complexes and the ensuing phosphorylation of the CTD (Fig. 1) appear to be the critical determinants of HIV-1 transcriptional processivity.

Why does HIV-1 require this complicated mechanism of transcriptional regulation? There are at least two plausible explanations. First, Tat cofactors may be sensitive to the presence or absence of cellular activation signals, and therefore in certain cellular conditions Tat may fail to function, leading to the repression of viral transcription and subsequent entrance into viral latency. Although latency is rare (9), its dynamics may be important for viral escape from immune recognition. Second, it may simply be that the potent action of Tat in activated T cells is the only means by which viral transcription can be vigorous enough (*10*) to sustain persistent infections in vivo. In particular, because the half-life of infected cells is very short [less than 2 days (*11*)], the virus must be under tremendous selective pressure to replicate rapidly. Tat may therefore provide the virus with the means to overcome a rate-limiting step in transcription.

The need to maximize virus production during the short survival time of infected cells in vivo might also explain the enigmatic effect of the HIV-1 accessory/regulatory protein Vpr. This ~14-kD viral protein prevents infected cells from proliferating by causing them to delay for extended periods of time in the G_2 phase of the cell cycle (*12*) (an independent function of Vpr in facilitating nuclear import will be discussed later). As the viral long terminal repeat (LTR) is more active in the G_2 phase of the cell cycle than during other phases of the cell cycle, virus production is actually increased in cells that are delayed in G_2 by Vpr. Hence, given the short half life of infected cells, the ability of HIV-1 to maximize its own transcription at the expense of cell division may confer an important selective advantage (*13*).

Rev Activates Export of Unspliced RNA

HIV-1 uses alternative splicing of its full-length transcript to generate the array of mRNAs required for efficient expression of all viral genes. In addition, the full-length transcript also serves both as a source of new viral genomes and as a translational template. An inevitable corollary of this expression strategy is that HIV-1 RNAs that contain functional introns must be selectively exported from the nucleus. In contrast, the majority of unspliced cellular RNAs are ordinarily retained in the nucleus and either spliced to completion or degraded. To circumvent this problem, HIV encodes a protein called Rev that binds to a cis-acting RNA target [the Rev response element (RRE)] present in all unspliced viral transcripts and targets them for nuclear export (*14*).

The ~18-kD Rev protein consists of an NH_2-terminal domain that mediates RRE-binding, Rev-Rev multimerization as well as nuclear localization, and a COOH-terminal leucine-rich domain that contains a nuclear export signal (NES). There has been much interest in identifying cellular NES-binding partners, because it was proposed that such proteins might represent hitherto elusive nuclear export receptors. Recent analyses of a protein, now called exportin 1, appear to have borne out this prediction (Fig. 2).

Exportin 1 was originally identified in *schizosaccharomyces pombe* as a protein involved in chromosome structure (hence its earlier name of CRM1, chromosome region maintenance gene 1). However, the convergence of several lines of evidence suggested that exportin 1 might participate in Rev function (or more broadly in leucine-rich NES-mediated nuclear export). The first link between Rev and exportin 1/Crm1 was made when the antibiotic leptomycin B (LMB) was shown to inhibit Rev-dependent mRNA export in cultured cells (*15*). This observation was revealing because one class of LMB-resistant *S. pombe* mutants had previously been mapped to the *Crm1* gene (*16*). Finally, the primary sequence of exportin 1/Crm1 showed that it is a member of the importin-β (karyopherin-β) superfamily of shuttling nuclear transport receptors (*17*).

Several groups then demonstrated that leucine-rich NES activity could be disrupted by mutant forms of exportin 1 and that the inhibition of Rev (or NES) export by

LMB is suppressed by overexpression of exportin 1 (*18*). Furthermore, the NES of Rev was shown to interact cooperatively in vitro with exportin 1 and the Ran guanosine triphosphatase (GTPase) (an essential nuclear transport factor) in a manner that requires Ran to be in its GTP-bound form (Fig. 2). Formation of this complex is abrogated by LMB, thus explaining the inhibitory effect of LMB on Rev activity. Because the directionality of nuclear transport (export and import) is imposed by the asymmetric distribution of the Ran GDP (cytoplasm)—Ran GTP (nucleus) gradient that exists across the nuclear envelope, the dependence of the Rev–exportin 1 interaction on Ran GTP presumably ensures that it occurs in the nucleus and is reversed in the cytoplasm after export and RanGAP-induced GTP hydrolysis (Fig. 2). Although issues such as the mechanisms of exportin 1–mediated transit to and through nuclear pore complexes, the roles of other Rev cofactors (*19*), and the essential contribution made by Rev multimerization still await detailed elucidation, the continued analysis of how HIV-1 Rev coopts a cellular mechanism for nuclear export has been instrumental in providing insight into this fundamental cell biological process.

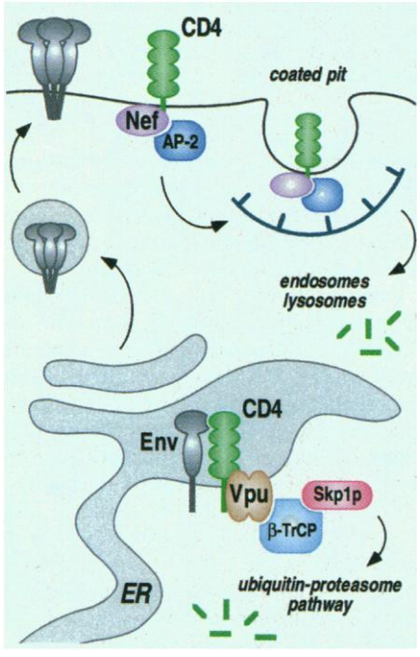

Fig. 3. Vpu and Nef prevent cell surface expression of CD4 by different mechanisms. The viral glycoprotein Env (gray) binds to the cellular receptor CD4 (green) during transport in the ER. Vpu also binds to CD4 in the ER and targets it for degradation (green fragments) by recruitment to the ubiquitin-proteasome pathway through interactions with β-TrCP and Skp1p. Nef removes preexisting CD4 from the cell surface by recruiting CD4 into clathrin-coated pits, and ultimately into degradative lysosomes, through interactions with the AP-2 adaptor complex.

Modulation of Cellular Membrane Proteins: The Complementary Roles of Vpu and Nef

HIV-1 appears to go to extraordinary lengths to down-regulate the surface expression of its primary receptor, CD4. The viral envelope glycoprotein Env binds to CD4 not only on the cell surface but also in the endoplasmic reticulum (ER) before translocation to the plasma membrane. In addition, HIV-1 encodes two regulatory/accessory proteins, Vpu and Nef, that further affect the intracellular trafficking of CD4.

Vpu is an ~16-kD viral membrane-spanning protein that binds CD4 in the ER and targets it for proteolysis by recruitment into the cytosolic ubiquitin-proteasome pathway (*20*). Degradation of CD4 in the presence of Vpu is disrupted by specific inhibitors of proteasome-mediated proteolysis and also by dominant negative mutants in the ubiquitin pathway. Moreover, degradation of CD4 in the presence of Vpu is inhibited by the removal of lysine residues (the target of ubiquitination) from the cytoplasmic tail of CD4. A direct connection between Vpu and the proteasome was established by the demonstration that Vpu binds to a protein termed β-TrCP, which in turn binds to the proteasome targeting factor Skp1p (Fig. 3). The importance of these interactions was confirmed by the isolation of CD4—Vpu–β-TrCP ternary complexes in vivo and by the demonstration that Vpu-induced degradation of CD4 was inhibited by a mutant form of β-TrCP that had lost the ability to bind Skp1p (*21*). During viral infection, it is presumed that the binding of Env to CD4 in the ER (*22*) will retard its transit to the plasma membrane and consequently enhance Vpu-CD4 interactions and subsequent CD4 degradation (Fig. 3).

In contrast to the effects of Env and Vpu on CD4 en route to the plasma membrane, Nef acts to remove CD4 that is already on the cell surface by accelerating endocytosis through clathrin-coated pits. Nef-mediated targeting of CD4 to the endocytic pathway is critically dependent on a dileucine motif within the cytoplasmic tail of CD4 and is thought to be initiated by the interaction of Nef with CD4 at the plasma membrane. New evidence suggests that endocytosis occurs through interactions between Nef and a protein complex, the AP-2 adapter complex, that recruits transmembrane proteins to clathrin-coated pits (Fig. 3). Specifically, Nef was found to colocalize with the AP-2 complex at the plasma membrane and to bind one of its subunits directly (*23*). This serves not only to increase the association of CD4 with clathrin-coated pits but also to promote formation of the pits themselves (*24*). Thus, HIV-1 has evolved ways both to remove its receptor from the cell surface (Nef) and to prevent newly synthesized receptor from reaching the cell surface (Vpu).

The selective advantage for the virus in down-regulating CD4 in vivo remains unclear. One possibility is that Vpu-mediated degradation of CD4 in the ER increases the ability of Env to transit to the cell surface (*25*). Likewise, receptor-envelope interactions on the cell surface could either reduce virion release or inhibit the incorporation of Env into virions, and therefore it would be a selective advantage for HIV-1 to encode a protein such as Nef that removes CD4 from the cell surface. Both Vpu and Nef have also been reported to down-regulate expression of the major histocompatibility complex I (albeit to lesser extents) (*26*). Thus, HIV-1 appears to have evolved diverse mechanisms to perturb the intracellular trafficking of host proteins for the purposes of enhancing particle infectivity and avoiding immune surveillance.

Events Early After Virus Entry: The Next Frontier

Perhaps the least understood aspects of the HIV-1 life cycle are the events that occur immediately after entry of the virus into the cell by membrane fusion. Because the process of viral assembly is intimately linked to its subsequent reversal (disassembly), it is not unexpected that interactions that occur between viral proteins and cellular factors in the cells that produce virus frequently influence early post-entry events at the next round of infection. For example, studies of HIV-1 structural protein interactions with cellular proteins have revealed that productive infection depends on a cellular protein, cyclophilin A (CyPA). Inhibition of CyPA incorporation into virions results in a profound post-entry block when that virus is used to infect new cells (*27*). Mutational and structural studies have mapped the binding site of CyPA to an exposed proline loop within the viral capsid (CA) protein (*28*). Because CA is a major structural protein of the virion core but is removed from the particle soon after entry, it has been suggested that CyPA promotes core disassembly by destabilizing CA-CA interactions (*29*). Another cellular protein that is incorporated into HIV-1 virions is a mitogen-activated protein kinase (MAPK). Inhibition of the kinase reduces infectivity, and it is thought that specific phosphorylation of viral (and perhaps cellular) targets contributes to virus disassembly (*30*).

Similarly, at least three of the HIV-1 regulatory/accessory proteins appear to act

in early post-entry events. For example, the HIV-1 Vif protein, like CyPA and MAPK, must be present in the cells that produce virus, and the absence of this protein results in a block of infection soon after viral entry into subsequent target cells. A substantial fraction of Vif is present at the site of virus assembly near the plasma membrane, and it has been suggested that this enables Vif to modulate virion assembly in a manner that facilitates disassembly or other early events (*31*). Likewise, early post-entry events in the virus life cycle are also affected by the HIV-1 Nef gene product (*32*). Virus produced in the absence of Nef is less infectious than virus produced in the presence of Nef. However, if the virus is artificially made to enter the cytosol via late endosomes rather than at the plasma membrane, the need for Nef is obviated (*33*). This suggests either that the endosomes can facilitate uncoating of the virus particle or, alternatively, that Nef may modify the virion so that it can avoid an interaction with a cellular factor that would ordinarily limit infection.

Indeed, there are precedents for host cell proteins blocking retroviral infection at an early post-entry stage. For example, the *Fv-1* locus of mice can dominantly inhibit productive infection by some murine retroviruses (*34*), and the cellular proteasome can partially protect cells from HIV-1 infection by degrading incoming virions (*35*). Moreover, post-entry blocks to productive infection by HIV-1 or SIV (simian immunodeficiency virus) can help determine viral tropism (*36*). Thus, perhaps the early stages of viral infection are when the virus is most vulnerable and, therefore, cells may have targeted this stage of the life cycle to develop systems of resistance. The primate lentiviruses may have responded by developing a number of strategies to counter these natural antiviral effects.

One aspect of post-entry HIV-1 biology that is becoming better understood is how the uncoated nucleoprotein complexes [often termed preintegration complexes (PICs)] enter the nucleus of the infected cell. In contrast to some other retroviruses that require mitotic nuclear envelope dissolution to gain access to the nuclear interior, the HIV-1 PIC is imported into the nucleus during interphase (*37*). Consistent with the general principles of nuclear transport, PIC import occurs by an energy-dependent process that requires cis-acting nuclear localization signals (NLSs) provided by components of the PIC. Although the NLSs that are critical for PIC import await complete definition, recruitment of the cellular import factor importin-α to these complexes has been demonstrated (*38*). In addition to the inferred engagement of the classical nuclear import pathway (which utilizes importin-α and importin-β), it appears that Vpr acts in a complementary (but mechanistically distinct) fashion to augment PIC import. In particular, Vpr does not contain a classical NLS but is able to interact with importin-α as well as with nucleoporins (*39*), and may therefore serve to directly connect the PICs to the nuclear pore.

Selection for efficient signal-mediated mechanisms for PIC nuclear import may be driven by the fact that terminally differentiated tissue macrophages (which pass through mitosis infrequently) are critical in vivo targets for HIV-1 infection. Although few macrophages may actually be infected with HIV-1, cell-cell contact between infected macrophages and activated T cells may spread infectious virus more efficiently than does the very labile cell-free virus. In addition, PIC import would also maximize virus production in short-lived T cells by reducing the time interval between the initial infection and the active production of new virions.

Conclusion: The Virus and Its Host

Unlike some other classes of retroviruses, the primate lentiviruses are not transmitted through the germ line, and no endogenous copies exist in the genome of susceptible species. Nonetheless, these viruses have evolved over long periods of time with their natural hosts. For example, the SIV strains isolated from African green monkeys (SIV_{AGM}) are unique for each geographically distinct species of monkey in this superspecies (*40*). On the other hand, phylogenetic analysis of the primate lentiviruses has revealed that multiple cross-species transmissions must have led to the introduction of these viruses into new host species, including humans (*1*). Because these viruses depend on critical interactions with host cell factors, one might expect viral proteins to evolve and adapt to new hosts. Indeed, the Vpr and Vif proteins of SIV_{AGM}, although active in African green monkey cells, are nonfunctional in human cells (*41*). In contrast, the equivalent gene products from SIV_{SMM} (the primate lentiviruses isolated from sooty mangabey monkeys) do function in human cells. As SIV_{SMM}, but not the more widely distributed SIV_{AGM}, appears to have successfully entered human populations (*1*), the ability of a viral gene product to interact appropriately with a human host cell protein may determine cross-species transmission and possibly the generation of new viral pathogens. Ultimately, the elucidation of the interactions between HIV-1 proteins and host cells will lead to improved understanding of viral replication and host cell biology, as well as suggesting additional targets for antiviral strategies.

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42. We apologize to those whose work was not discussed or cited in this limited review. Interested readers are referred to A. D. Frankel and J. T. Young, *Annu. Rev. Biochem.* **67**, in press, for a more comprehensive review of HIV molecular biology. We thank M. Bartolomei, P. Bates, S. Bartz, R. Fouchier, R. Gummuluru, J. Simon, G. Stivahtis, and M. Vodicka for comments on the manuscript and numerous colleagues who forwarded preprints of manuscripts to us.

The HIV-1 Envelope Glycoproteins: Fusogens, Antigens, and Immunogens

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The human immunodeficiency virus–type 1 (HIV-1) envelope glycoproteins interact with receptors on the target cell and mediate virus entry by fusing the viral and cell membranes. The structure of the envelope glycoproteins has evolved to fulfill these functions while evading the neutralizing antibody response. An understanding of the viral strategies for immune evasion should guide attempts to improve the immunogenicity of the HIV-1 envelope glycoproteins and, ultimately, aid in HIV-1 vaccine development.

The human immunodeficiency viruses (HIV-1 and HIV-2) and simian immunodeficiency viruses (SIVs) are the etiologic agents of acquired immunodeficiency syndrome (AIDS) in their respective human and simian hosts (*1*). Typically, infection with primate immunodeficiency viruses is characterized by an initial phase of high-level viremia, followed by a long period of persistent virus replication at a lower level (*2*). Viral persistence occurs despite specific antiviral immune responses, which include the generation of neutralizing antibodies.

The primate immunodeficiency viruses, like all retroviruses, are surrounded by an envelope consisting of a host cell–derived lipid bilayer and virus-encoded envelope glycoproteins (*3*). For the virus to enter target cells, the viral membrane must be fused with the plasma membrane of the cell, a process mediated by the envelope glycoproteins. The exposed location of these proteins on the virus allows them to carry out their function but also renders them uniquely accessible to neutralizing antibodies. Thus, dual selective forces—virus replication and immune pressure—have shaped the evolution of the envelope glycoproteins and continue to do so within each infected host. Here, we summarize our current understanding of the functional features of these proteins.

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Synthesis and Assembly

In the infected cell, the envelope glycoproteins are synthesized as an approximately 845– to 870–amino acid precursor in the rough endoplasmic reticulum. Asparagine-linked, high-mannose sugar chains are added to form the gp160 glycoprotein, which assembles into oligomers (*4*–*6*). The preponderance of evidence suggests that these oligomeric complexes are trimers (*4*, *5*). The gp160 trimers are transported to the Golgi apparatus, where cleavage by a cellular protease generates the mature envelope glycoproteins: gp120, the exterior envelope glycoprotein, and gp41, the transmembrane glycoprotein (*3*). The gp41 glycoprotein has an ectodomain that is largely responsible for trimerization (*7*), a membrane-spanning anchor, and a long cytoplasmic tail. Most of the surface-exposed elements of the mature, oligomeric envelope glycoprotein complex are contained on the gp120 glycoprotein. Selected, presumably well-exposed, carbohydrates on the gp120 glycoprotein are modified in the Golgi apparatus by the addition of complex sugars (*6*). The gp120 and gp41 glycoproteins are maintained in the assembled trimer by noncovalent, somewhat labile, interactions between the gp41 ectodomain and discontinuous structures composed of NH_2- and COOH-terminal gp120 sequences (*8*). When they reach the infected cell surface, a fraction of these envelope glycoprotein complexes are incorporated into budding virus particles. A large number of the complexes disassemble, releasing gp120 and exposing the previously buried gp41 ectodomain. These events contribute to the formation of defective virions, which predominate in any retroviral preparation (*9*).

Binding to the CD4 Receptor

Many cell surface proteins, including adhesion molecules, are incorporated into HIV-1 virions along with the envelope glycoprotein complexes (*10*). These host cell–derived molecules can assist the attachment of viruses to potential target cells. Virus attachment also involves the interaction of the gp120 envelope glycoproteins with specific receptors—the CD4 glycoprotein (*11*) and members of the chemokine receptor family (*12*, *13*) (Fig. 1). The CD4 glycoprotein is expressed on the surface of T lymphocytes, monocytes, dendritic cells, and brain microglia, the main target cells for primate immunodeficiency viruses in vivo. The requirement for CD4 binding exhibited by most primate immunodeficiency viruses for efficient entry is consistent with this observed in vivo tropism.

A major function of CD4 binding is to induce conformational changes in the gp120 glycoprotein that contribute to the formation or exposure of the binding site for