REVIEW

The plasma membrane as a combat zone in the HIV battlefield

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In the life of a cell, the plasma membrane fulfills a range of functions that go far beyond the shaping and maintenance of architectural features and the absorption of nutrients. The plasma membrane is a highly sophisticated structure whose phospholipidic backbone is loaded with proteins responsible for channeling the stream of information that continuously flows between a cell and its environment. Although the nucleus can intuitively be viewed as the cell's center of command, the translation of its genetic content is constantly modulated by signals triggered and often integrated at the level of the plasma membrane. Reciprocally, the cell exposes on or releases from its surface a wide variety of molecules that regulate its recognition by other cells and that sometimes influence the homeostasis of the whole organism.

The plasma membrane is also the site where intracellular pathogens first clash with their target and the place from which the immune system is subsequently called to the rescue. Correspondingly, the study of viruses has provided great strides in the comprehension of such fundamental processes as membrane fusion, protein transport, endocytosis, signal transduction, and antigen presentation, all phenomena that are intimately intertwined with the biology of membranes and their associated proteins. Recent progress in the analysis of the HIV, probably by now the most extensively characterized of all human pathogens, provides a good illustration of this paradigm. Just as the composition of the plasma membrane influences viral infectivity, the virus in turn uses components of the plasma membrane that are to its advantage and modifies others to suit its purposes. The interplay between HIV and the plasma membrane has much to offer in terms of understanding viral tropism and pathogenicity and normal cellular functions, and for developing new antiviral approaches.

Fancy break in: viral entry

To infect a cell, a membrane-enveloped virus such as HIV must transfer its genome across both the viral and

cellular membranes—not a trivial task given the inherent stability of biological membranes. Enveloped viruses accomplish this feat by encoding and expressing on their surface integral membrane proteins that, under the right conditions, undergo conformational changes that cause the viral and cellular membranes to fuse with one another, providing a portal of entry (Hernandez et al. 1996). The entry process can be divided into three components: attachment of the virus to the cell surface, involving recognition and binding to specific cell surface receptors; a triggering event that causes the viral fusion protein to undergo conformational changes; and the membrane fusion reaction itself. The presence or absence of molecules on the cell surface necessary for attachment and triggering greatly influences viral tropism: the ability of a given virus to infect only specific cell types.

First contact

The HIV-1 envelope (Env) protein is a type I integral membrane protein that mediates viral attachment and membrane fusion and is also the target for neutralizing antibodies. Synthesized as a single polypeptide precursor that forms trimers, Env is subsequently cleaved by a cellular protease to generate two noncovalently associated subunits, gp120 and gp41. The gp120 binds virus to the cell surface, whereas the membrane-spanning gp41 subunit is largely responsible for membrane fusion (Wyatt and Sodroski 1998). The primary receptor for HIV-1 is CD4, explaining the propensity of this virus to infect certain T cells and macrophages, ultimately leading to immune dysfunction. Although CD4 binding is a prerequisite for HIV-1 entry, attachment of virus per se may be mediated by an impressive list of molecules that may serve to concentrate virus on the cell surface and increase the frequency of Env-receptor interactions. The most striking example of an attachment molecule is DC-SIGN, a type II membrane protein with a mannose-binding, C-type lectin domain found on some types of dendritic cells (DCs) (Geijtenbeek et al. 2000a,b). DC-SIGN captures HIV-1 to the surface of the DC, retaining it in a native, infectious form that can be efficiently presented to permissive CD4-positive T cells, resulting in enhanced infection (Geijtenbeek et al. 2000a). DC-SIGN

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does not appear to mediate virus entry or to influence the dependence of virus infection on CD4, but rather increases the efficiency of the process. This interaction may be particularly important in mucosal transmission, with virus being efficiently captured by DC-SIGN-positive subepithelial DCs and ferried to lymphoid tissue where permissive target cells abound. If so, DC-SIGN itself could be a therapeutic target, and if the structural features underlying its ability to bind Env are clarified, this property could be used to advantage in generating subunit vaccines that might be efficiently captured by DC-SIGN, retained in a native state, and presented to B cells. Whether virus-binding proteins exist on other cell types, such as macrophages, is not clear, but their presence could render cells expressing low levels of receptor more permissive to viral entry.

Coreceptor engagement

Binding of the gp120 subunit to CD4 by itself does not trigger membrane fusion (Maddon et al. 1986; Ashorn et al. 1990; Chesebro et al. 1990; Clapham et al. 1991). However, CD4 binding causes conformational changes in gp120 that enable it to bind to a second receptor, termed a coreceptor, and it is this second receptor binding event that leads to membrane fusion (Lapham et al. 1996; Trkola et al. 1996; Wu et al. 1996). Most primary HIV-1 strains use the chemokine receptor CCR5 in conjunction with CD4 for virus entry (termed R5 virus strains; Berger et al. 1998; Doms and Moore 1998) and in absence of CCR5 because the $\Delta 32$ -ccr5 polymorphism is associated with an impressive degree of resistance to virus infection (Dean et al. 1996; Liu et al. 1996; Samson et al. 1996). In some individuals, viruses evolve to use a related receptor, CXCR4, either in place of (X4 virus strains) or in addition to CCR5 (R5X4 strains). Emergence of X4 virus types is associated with accelerated progression to AIDS (for review, see Miedema et al. 1994). In addition to CCR5 and CXCR4, a host of alternative coreceptors have been identified that enable smaller numbers of HIV strains to infect cells (Berger et al. 1999), but their *in vivo* relevance is for the most part questionable. Potentially, use of receptors other than CCR5 and CXCR4 could enable virus to infect different cell types and could provide an evolutionary escape route for the virus if effective small molecule inhibitors of the major coreceptors are developed.

Identification of the cell surface receptors to which HIV-1 Env binds and of the conformational changes in Env that ensue have provided great explicatory power for understanding viral tropism and pathogenesis and have identified novel viral and cellular targets for antiretroviral agents. Coreceptor choice is largely governed by variable regions within the gp120 subunit, notably the V3-loop and, to a lesser degree, the V1/2 region (Choe et al. 1996; Bieniasz and Cullen 1998; Cho et al. 1998; Hoffman and Doms 1998; Hoffman et al. 1998). However, the fact that so many divergent virus strains use CCR5 argues for the presence of a conserved coreceptor binding region in Env. The recently solved structure of a gp120

core fragment complexed with CD4 reveals the presence of an extraordinarily well conserved region in the bridging sheet, a portion of gp120 that lies between the base of the V3 and V1/2 regions, that is involved in coreceptor binding (Kwong et al. 1998; Rizzuto et al. 1998). That this highly conserved region may be the target for neutralizing antibodies is suggested by the fact that primary SIV (simian immunodeficiency virus) and HIV-2 strains often show at least some degree of CD4-independence, being able to infect cells expressing coreceptor alone (Edinger et al. 1997b; Reeves et al. 1999). The ability of SIV strains to use CCR5 to infect cells independently of CD4 suggests that CCR5 was the primordial receptor for the primate lentiviruses, with the ability to use CD4 evolving later (Edinger et al. 1997b; Martin et al. 1997). It is interesting to note that CD4-independent viruses are invariably neutralization sensitive, perhaps because of constitutive exposure of the coreceptor binding site in gp120 (Hoffman et al. 1999). By acquiring the ability to use CD4, this conserved region can be sequestered until immediately before viral entry, minimizing the time during which it is exposed. This also suggests that genetically triggering Env to become CD4-independent could result in better exposure of conserved neutralizing domains and, perhaps, a more robust humoral response. Indeed, several modified forms of SIV and HIV-1 Env have shown promise as immunogens (Reitter et al. 1998; LaCasse et al. 1999).

Fusion

It is not clear how binding of coreceptor to gp120 transmits information to gp41, causing it to elicit membrane fusion, although the structural changes undergone by gp41 are increasingly well understood. On coreceptor triggering, the hydrophobic amino-terminal fusion peptide of gp41 is exposed and likely interacts with the membrane of the target cell through the formation of a triple-stranded coiled-coil, effectively bridging the two membranes. The coiled-coil structure, composed of one amino-terminal leucine/isoleucine heptad repeat domain from each Env subunit, contains hydrophobic grooves into which the carboxy-terminal heptad repeat regions of each gp41 subunit pack, thus forming a six-helix bundle (Fig. 1; Chan et al. 1997; Weissenhorn et al. 1997). Formation of the six-helix bundle is rate-limiting for fusion, and the change in free energy on its formation is sufficient to form a fusion pore (Melikyan et al. 2000). This fusion mechanism is shared by many other viral fusion proteins, including those from influenza, Ebola virus, and paramyxoviruses, all of which form similar six-helix bundles that bring the fusion peptide (and the cellular membrane) in close proximity to the transmembrane domain (and the viral membrane) (for review, see Chan and Kim 1998; Skehel and Wiley 1998). Similar mechanisms may be used by cellular proteins that mediate intracellular membrane fusion events (Poirier et al. 1998; Sutton et al. 1998).

Formation of the six-helix bundle can be inhibited by addition of peptides based on the gp41 carboxy-terminal

Figure 1. Model for HIV-1 entry. Binding of CD4 to gp120 results in exposure of a conserved coreceptor (CoR) binding site in gp120, perhaps by movement of the V3 and V1/2 loops. Coreceptor binding causes the fusion peptide of gp41 to be exposed and inserted into the membrane of the target cell in a triple-stranded coiled-coil. Formation of a helical hairpin structure in which gp41 folds back on itself is coincident with membrane fusion. The bottom portion of the figure displays gp41 alone. Addition of the T20 peptide blocks membrane fusion by preventing the formation of the hairpin structure.

helical domain (Wild et al. 1994, 1995). These peptides bind to the amino-terminal triple-stranded coiled-coil in gp41, blocking formation of the six-helix bundle with impressive efficiency (Fig. 1; Furuta et al. 1998). One such peptide, termed T20, has been shown to reduce virus loads in vivo by one to two orders of magnitude and is currently in Phase II clinical trials (Kilby et al. 1998). Besides peptides, small molecule inhibitors may also be able to block membrane fusion (Eckert et al. 1999; Ferrer et al. 1999). The triple-stranded coiled-coil contains a hydrophobic pocket near its carboxy-terminal end that has been shown, in principle, to be a target for small MW compounds (Eckert et al. 1999). These studies show how structural intermediates of the fusion process can be effective targets for broadly effective inhibitors.

In addition to identifying new ways to block HIV infection, identification of the viral coreceptors has also shown that virus interactions with the cell surface are highly complex, with viral tropism at the level of entry not being entirely explained by the mere presence of the appropriate cell surface receptors. For example, some X4 viruses can enter macrophages whereas others cannot (Schmidt-Mayerova et al. 1998; Simmons et al., 1998; Yi et al. 1998), just as only a subset of R5 SIV strains are macrophage tropic (Edinger et al. 1997a, 1999b). Other examples of restricted virus entry abound. In addition, the pathogenic potential of well-characterized SHIV (SIV/HIV chimeric viruses) and SIV isolates often maps to Env in ways that are not entirely clear and that cannot be explained solely on the basis of the types of receptors used. However, recent studies have shown that receptor

density, conformation, and Env-receptor affinity may all influence viral tropism and pathogenesis, perhaps making the question of how a virus strain interacts with its receptors and the cell surface as meaningful as assigning R5 or X4 designations.

Membrane fusion is a cooperative process, and it is currently estimated that four to six CCR5 receptors (Kuhmann et al. 2000), multiple CD4 molecules (Layne et al. 1990), and three to six Env trimers are needed to form a fusion pore. It logically follows that virus entry will depend on receptor density and that Env-receptor affinity can impact that rate and efficiency of infection. The fact that *ccr5* promoter polymorphisms and heterozygosity for the $\Delta 32$ -*ccr5* polymorphism are associated with altered disease course argues that relatively modest changes in receptor density can influence viral infectivity in vivo (Dean et al. 1996; McDermott et al. 1998). Studies with cell lines show that infection efficiency decreases as receptor density decreases, although the major coreceptors CCR5 and CXCR4 still support at least some virus infection even at very low levels of expression (<1000 copies per cell; Kozak et al. 1997; Platt et al. 1998; Edinger et al. 1999a; Kuhmann et al. 2000). Receptor density can be influenced by a multitude of factors at both the macro and molecular levels. Cytokines such as interleukin-10 can result in up-regulation of coreceptor expression and enhanced viral entry (Sozzani et al. 1998), just as secretion of their cognate chemokine ligands leads to down-regulation and decreased susceptibility to infection (for review, see Lee and Montaner 1999). Receptor density could potentially be influenced by

changes in microenvironment as well. A number of cell surface receptors can cluster in detergent insoluble, glycolipid-rich domains termed rafts (Brown and Rose 1992). In T cells, such clusters have been referred to as an immunological synapse, an area of close contact between an antigen-presenting cell and a T cell that results from activation of the T-cell receptor (Grakoui et al. 1999). Concentration of receptors within an immunological synapse may stabilize low affinity interactions between the T-cell receptor and its ligands (Grakoui et al. 1999). Whether rafts may serve to concentrate viral receptors in some primary cell types is an intriguing possibility that warrants investigation. CD4 may also associate with CCR5, making virus entry more efficient, and competition between CCR5 and CXCR4 for CD4 association may help account for the preferential use of CCR5 on some cell types that express both coreceptors (Lee et al. 2000).

Just as the cooperative nature of the fusion process predicts that receptor density influences susceptibility of cells to virus infection, it can be posited that Env-receptor affinity can also influence this process. Although all Env proteins seem to bind CD4 with affinities of <10 nM, there is considerable variation in the affinities of Env-coreceptor interactions. Whereas many R5 virus types bind to CCR5 with high affinity (<15 nM; Doranz et al. 1999a), binding of X4 Envs to CXCR4 and R5X4 Envs to either CCR5 or CXCR4 has proven difficult to measure (Baik et al. 1999; Doranz et al. 1999b). The affinity of the prototype X4 virus strain IIIB for CXCR4 is ~400 nM, for example (T.L. Hoffman, unpubl.). Although a broad range of receptor affinities is compatible with infection of cell lines that typically express tens of thousands of receptor molecules, this may not be the case for primary cell types in which receptor expression levels are typically <10,000 per cell (Lee et al. 1999b). Because Env-coreceptor binding is reversible (Doranz et al. 1999a) and multiple Env-receptor interactions are required for fusion (Kuhmann et al. 2000), viruses with low receptor affinity are likely to fuse more slowly and inefficiently than viruses with higher binding constants. Could high affinity result in a greater ability to infect cells with low levels of receptor, enabling virus to infect a greater proportion of CD4-positive cell types? It is interesting to note that alterations in a viral Env protein that increase receptor affinity without affecting the types of receptors used have been associated with markedly increased pathogenicity in a nonhuman primate model of infection (Karlsson et al. 1998).

Finally, other less well understood aspects of HIV-cell surface interactions may impact viral tropism and pathogenesis. Differences in how R5 and X4 Env proteins interact with their coreceptors have been noted (Berger et al. 1999), and in some cases Env binding to coreceptor induces receptor signaling (Davis et al. 1997; Weissman et al. 1997). Although Env-induced receptor signaling is not required for infection of transformed cell lines, it is possible that signaling in primary cells could impact postentry steps of virus replication and cell viability. As

for the other seven transmembrane domain receptors, the chemokine receptors CCR5 and CXCR4 exist in distinct conformational states and are subject to a variety of posttranslational modifications, which in some cases influence virus infection (Farzan et al. 1999; Lee et al. 1999a; Chabot et al. 2000). Whether all conformations function equally well as coreceptors is not known. It is interesting to note that a small molecule inhibitor of CCR5, TAK779 (Baba et al. 1999), binds to a region of CCR5 that has thus far not been directly implicated in receptor-Env interactions (Dragic et al. 2000). Nonetheless, TAK779 blocks gp120-CCR5 binding (Dragic et al. 2000). Perhaps TAK779 prevents virus infection by altering CCR5 conformation rather than by sterically hindering Env binding or by inducing receptor down-regulation. A similar mechanism has been reported for a small molecule inhibitor of the substance P receptor (Gether et al. 1993).

During the siege: perturbations of plasma membrane homeostasis

Once delivered inside the cell, the HIV genome is reverse transcribed and transported to the nucleus, where it integrates in the host-cell chromosome as a provirus. Viral gene expression then proceeds in two stages, first yielding the regulatory proteins Tat, Rev, and Nef, and secondly producing the late gene products Gag, Env, Vif, Vpr and Vpu, or Vpx, all involved in some aspect of virion formation. Through several of these factors, the virus toys with the homeostasis of the plasma membrane, affecting in particular the surface expression of specific receptors, including its own. Our comprehension of these phenomena is still a bit sketchy and pales in comparison with our sophisticated understanding of the mechanisms of viral entry. Nevertheless, this less mature area of HIV research has already yielded a few paradigms, for instance, how a cell can regulate the surface expression of some receptors. The HIV Nef protein plays a prominent role in these events. Abundantly produced during the earliest phase of viral gene expression, Nef is a short cytoplasmic protein recruited to membranes via amino-terminal myristoylation, a modification essential for all of its known functions. Nef impacts remarkably on the replication and survival of HIV in the body, as a subset of so-called long-term nonprogressors of HIV-1 infection harbors *nef*-deleted strains (Deacon et al. 1995), and *nef* inactivation results in viral attenuation in the SIV-rhesus macaque model (Kestler et al. 1991). From *in vitro* studies, it turns out that Nef is packed with functions ranging from the down-regulation of certain receptors, the perturbation of signaling pathways, and the stimulation of virion infectivity. However, determining whether one of these effects plays a predominant role *in vivo* has been difficult, in particular because of the lack of a fully satisfying animal model of HIV-1 infection, the difficulty of creating SIV *nef* point mutants that are defective in only one function, and the rapid rate of reversion of such mutants in monkeys.

MHC-I down-regulation

Cells infected by a virus are normally recognized and eliminated by the immune system, owing in part to the surface presentation of viral peptides by proteins of the class I major histocompatibility complex (MHC-I), which allows their detection and killing by cytotoxic T lymphocytes. In the case of HIV, this process initially functions well, but it achieves only a temporary success. Mutational escape and possible sheltering of the virus in cellular hideouts such as resting memory T lymphocytes and glial cells contribute to this phenomenon, yet emerging evidence suggests that Nef-induced MHC-I down-modulation also plays an important role (Kerkau et al. 1989; Scheppeler et al. 1989; Schwartz et al. 1996; Collins et al. 1998). This is not an unprecedented strategy in the realm of viruses causing chronic infections, because it is also exploited by Epstein-Barr virus, cytomegalovirus, and herpes simplex virus (Ploegh 1998; Brodsky et al. 1999).

MHC-I is the heterodimeric complex of a highly polymorphic, membrane-anchored heavy chain noncovalently associated with β 2-microglobulin (β 2m). The assembly of the heavy chain with β 2m and the loading of antigenic peptides occur in the endoplasmic reticulum (ER), and only fully assembled complexes are transported to the cell surface (Bijlmakers and Ploegh 1993). In the presence of Nef, these steps appear to proceed normally at least up to the Golgi, but MHC-I is then diverted to the endosomal pathway and retrieved to the trans-Golgi network (TGN) before undergoing degradation (Schwartz et al. 1996; Greenberg et al. 1998b; Le Gall et al. 1998; Piguet et al. 2000). The cytoplasmic tail of the HLA-A heavy chain is sufficient to confer Nef responsiveness to a chimeric integral membrane protein, and in this region a tyrosine residue found in HLA-A and B but not in HLA-C plays a crucial role (Le Gall et al. 1998). The corresponding resistance of HLA-C to the effect of Nef may be physiologically relevant, because HLA-C molecules are dominant inhibitory ligands that protect cells against lysis by natural killer (NK) lymphocytes, which normally destroy cells devoid of surface MHC-I (Brutkiewicz and Welsh 1995; Parham et al. 1995; Cohen et al. 1999).

A highly conserved acidic cluster (AC) in the amino-proximal third of Nef (EEEE⁶⁵) binds PACS-1 (phosphofurin acidic cluster sorting protein-1), the first identified member of a new family of coat proteins. PACS-1 governs the endosome-to-Golgi trafficking of furin and mannose phosphate receptor (MPR) by connecting the AC-containing cytoplasmic domain of these molecules with the adaptor protein complex (AP-1) of endosomal clathrin-coated pits (CCPs; Wan et al. 1998). Nef binds PACS-1 in an AC-dependent manner, and this interaction is essential for MHC-I down-regulation and TGN targeting. Furthermore, a chimeric integral membrane protein harboring Nef as its cytoplasmic domain localizes to the TGN after internalization, in an AC- and PACS-1-dependent manner (Piguet et al. 2000). This supports a model in which Nef down-regulates MHC-I by

acting as a connector between the receptor cytoplasmic tail and the PACS-1 sorting pathway (Fig. 2).

An interaction between Nef and MHC-I, however, has not yet been shown, and a number of additional questions remain unanswered. For instance, how does Nef first direct MHC-I to the endosomal compartment? Remarkably, CCP-mediated endocytosis does not seem to be involved (Le Gall et al. 2000). What is the role of other determinants of Nef whose mutation abrogates MHC-I down-modulation (Fig. 3), such as an amino-terminal α -helix and a centrally located SH3-binding proline-based repeat located just downstream from the EEEE⁶⁵ acidic cluster (Greenberg et al. 1998b; Mangasarian et al. 1999)? It is noteworthy that the TGN targeting of a CD4-Nef chimera is not prevented by mutating either one of these two other motifs, indicating that they are probably not involved in PACS-1 binding but rather in some other step necessary for MHC-I modulation (Piguet et al. 2000). Both motifs have been shown to participate in the binding of Nef to protein kinases, and the Nef proline repeat constitutes the docking site for SH3-containing Src family tyrosine kinases (Saksela et al. 1995). It could be that one such Nef-interacting protein serves as a bridge with the MHC-I cytoplasmic tail. Also, why does HIV trigger MHC-I retrieval to the TGN? All the other viruses known to down-modulate MHC-I interfere instead with MHC assembly and transport along the exocytic pathway (Ploegh 1998; Brodsky et al. 1999; Yewdell and Bennink 1999). This originality is probably functionally significant, yet for reasons that remain to be elucidated. Finally, because Nef is expressed in some forms of viral latency (Pomerantz et al. 1990), it could be that its ability to promote immune escape via MHC-I down-regulation is particularly relevant in the reservoir of infected lymphocytes that persists in patients treated with highly active antiretroviral therapies (HAART). Because this cell population represents an obstacle to the eradication of the virus, anti-Nef drugs could represent useful complements to more conventional antiviral therapies.

CD4 down-modulation

Like many enveloped viruses, HIV down-regulates the cell surface expression of its cognate receptor. However, although this effect is achieved by most viruses through simple trapping of Env-receptor complexes in the ER, HIV-1 engages two additional proteins besides Env in CD4 down-modulation: Nef and Vpu (Chen et al. 1996). Why such a rage? Apparently an excess of CD4 molecules on the surface of an infected cell can be fatal to the envelope incorporation and even the release of newly formed particles (Lama et al. 1999; Ross et al. 1999). Of the three HIV-1 proteins affecting CD4, Nef acts the fastest, because it both is an early viral gene product and removes CD4 directly from the cell surface (Aiken et al. 1994; Rhee and Marsh 1994; Chen et al. 1996). In contrast, Env and Vpu are late viral proteins that target the biosynthetic pathway. Despite these nuances, Nef and Vpu both act as connectors that precipitate CD4 into degradation pathways.

Figure 2. Model for Nef-induced MHC-I and CD4 down-modulation. Nef-induced alterations in the trafficking of MHC-I (*left side*) and CD4 (*right side*) are depicted with red arrows. It is possible that, after targeting CD4 to the late endosomes/lysosomes through a COP-I coatamer-mediated mechanism, Nef is retrieved to the Golgi (via PACS-1). (TGN) Trans-Golgi network; (MHC-I) class I major histocompatibility complex; (PACS-1) phosphofurin acidic cluster sorting protein-1; (AP) adaptor protein complex (of clathrin coated pits); (COP-1) coat protein-1; (NBP-1) Nef-binding protein-1 (=v-ATPase: vacuolar ATPase).

Nef-induced CD4 down-regulation is a two-step process that reflects the sequential involvement of a series of Nef-recruited components of the protein trafficking machinery (Fig. 2). At the cell surface (and to a lesser degree in the Golgi), Nef bridges the CD4 cytoplasmic

tail with the adaptor protein complex of CCPs, thereby triggering the formation of CD4-specific endocytic vesicles (Schwartz et al. 1995; Foti et al. 1997; Mangasarian et al. 1997; Bresnahan et al. 1998; Craig et al. 1998; Greenberg et al. 1998a; Piguet et al. 1998; Lock et al.

Figure 3. Nef protein interactions participating in MHC-I and CD4 down-regulation. A putative three-dimensional structure of full-length Nef is drawn, reconstructed from data published by Barnham et al. (1997), Grzesiek et al. (1996a,b, 1997) and Lee et al. (1996). Alpha-helices are painted in red, beta-sheets in blue. Items concerning CD4 down-modulation are in green; those important for MHC-I regulation in brown. The myristoylation signal, essential for both functions, is in maroon.

1999). Another Nef-binding protein, NBP1, which represents the catalytic subunit of a vacuolar ATPase, might consolidate the Nef-AP interaction (Lu et al. 1998). In the early endosomes, Nef then switches to another downstream partner, the COP-I coatomer, which in turn mediates the transport of CD4 to lysosomes where the receptor is degraded (Piguet et al. 1999). An interaction between Nef and the β COP subunit of the coatomer, which seems greatly potentiated by yet unidentified additional factors, correlates with this effect (Benichou et al. 1994; Piguet et al. 1999).

In this function as well, HIV-1 Nef stands as a multi-valent connector, which contains at least four distinct determinants crucial for efficient CD4 down-regulation (Fig. 3): the amino-terminal myristoylation signal for attachment to membrane; a CD4-binding domain centered on amino acids 57 to 59; a dileucine-based endocytosis signal located in a carboxy-terminal flexible loop of the viral protein, responsible for interacting with adaptor complexes perhaps with the help of the nearby V-ATPase-binding site; and another diacidic sequence just upstream of the endocytosis motif for the recruitment of COP-I in endosomes. The proximity of the AP- and COP-I-binding sites of Nef most probably excludes the simultaneous binding of both transporters to the viral protein. This fits well with their sequential involvement in CD4 down-regulation. In contrast, it is difficult to understand how a single molecule of Nef could interact at the same time with a chain of the CCP adaptor complex and with the V-ATPase subunit of the proton pump through determinants that are less than ten amino acids apart, considering the bulkiness of both of the macromolecular structures involved. However, recent evidence suggests that Nef might oligomerize to down-regulate CD4 (Liu et al. 2000; J. Stalder and D. Trono, unpubl.). This would allow for the binding of distinct downstream partners by individual Nef monomers.

Still, one must admit that, so far, the molecular interactions that govern Nef-induced MHC-I and CD4 down-regulation have been investigated mainly through a combination of genetic and functional analyses, with little biochemical and structural data if one excepts the NMR documentation of a Nef-CD4 complex (Grzesiek et al. 1996). Efforts in this direction should be intensified, as they might greatly facilitate the development of inhibitors targeting these functions of Nef.

The connector model also applies to Vpu-induced CD4 degradation, because this other HIV-1 protein targets ER-trapped CD4 molecules to the proteasome by bridging the cytoplasmic tail of the receptor with a protein known as h- β TrCP (Bour et al. 1995; Margottin et al. 1998; Schubert et al. 1998). h- β TrCP contains a WD repeat region, which recognizes Vpu, and an F-box, which recruits Skp1p. Skp1p in turn provides a link with the ubiquitin proteolysis machinery (Margottin et al. 1998). Whether Vpu action involves dislocation of CD4 from the ER into the cytoplasm, direct attack of the cytosolic part of the glycoprotein by the proteasome, or a different, undefined mechanism remains unclear. Nevertheless, mutation of putative ubiquitination sites in the CD4 cy-

toplasmic domain or thermal inactivation of the E1 ubiquitin-conjugating enzyme inhibit Vpu-induced CD4 degradation, supporting a role for the proteasome in this process (Fujita et al. 1997).

Fulfilling the prediction that Nef and Vpu must mimic the mechanisms of action of endogenous molecules responsible for linking specific targets to components of the protein trafficking machinery, several cellular proteins have been found shown to function through similar mechanisms. For instance, β -arrestin acts as a clathrin adaptor that facilitates the endocytosis of the β -2 adrenergic receptor, an event crucial for synaptic desensitization in the sympathetic nervous system (Ferguson et al. 1996). Similarly, the receptor component of the ubiquitin ligase responsible for targeting the NF- κ B inhibitor I κ B α to the proteasome is h- β TrCP, the previously identified Vpu ligand (Yaron et al. 1998).

New perspectives in viral assembly and budding

Just as virus infection requires an assemblage of proteins at the point of entry, so does budding, in which new virions emerge from the plasma membrane wrapped in a lipid bilayer and loaded with surface proteins, including Env. The viral Env and Gag proteins constitute an exceedingly small fraction of the total proteins in a cell, which creates a challenge—how are these components concentrated? HIV-1 Gag associates with the inner leaflet of the plasma membrane via its amino-terminal myristate and a cluster of basic residues near its proximal end, with the help of some more distal determinants (Zhou et al. 1994; Sandefur et al. 1998; Paillart and Göttinger 1999). Gag also interacts with the cytoplasmic tail of gp41, the transmembrane moiety of Env (Cosson 1996). Recent work indicates that HIV may selectively bud from lipid rafts, the glycolipid-rich microdomains into which some types of proteins partition (Nguyen and Hildreth 2000). It is Gag that apparently contains the signals responsible for this targeting, whereas Env seems to be found in both raft and nonraft regions of the membrane. Nevertheless, even though viral assembly and release can occur in the absence of viral envelope, the site of budding is influenced by Env. In polarized cells in the absence of Env, HIV-1 is released from the entire cell surface; the viral glycoprotein restricts this process to the basolateral region (Owens et al. 1991). This targeting depends on the presence of an endocytosis signal in the cytoplasmic tail of gp41, pointing to a complicated set of interactions between Env, Gag, and intracellular trafficking pathways (Lodge et al. 1997; Deschambault et al. 1999). The virus also faces a second conundrum: the terminal step of the budding process is necessarily a membrane fusion reaction. Essentially nothing is known about how HIV mediates this event, because it is independent of Env and receptors. The virus might, however, take advantage of cellular proteins normally involved in endocytosis, a process that mirrors viral budding in that it likewise necessitates membrane fusion events that pinch off small vesicles. In that respect, it is interesting that in the case of equine infectious anemia virus, an-

other lentivirus, a YXXL motif—similar to prototypic endocytosis signals—in the carboxyl terminus of Gag has been shown to be both critical for viral release and responsible for mediating interactions with AP50, a component of the AP-2 complex that associates with clathrin-coated pits and aids in sequestering proteins in these endocytic structures (Puffer et al. 1997, 1998).

As for the steps in virus entry, virus assembly may hold important insights into viral pathogenesis. In murine cells, for example, HIV fails to assemble and bud correctly, suggesting that these cells either lack a cellular factor needed for budding or contain a factor that inhibits this process (Mariani et al. 2000). Clearly, a greater appreciation of the molecular events that describe HIV interactions with the plasma membrane will further our understanding of viral tropism and provide new therapeutic opportunities. Furthermore, it will continue to yield important information on the complicated set of interactions and biochemical processes that allow the plasma membrane to mastermind many aspects of the biology of a cell.

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