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HIV Accessory Proteins: Leading Roles for the Supporting Cast

Review

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Considering that a set of three genes is sufficient for the replication of most retroviruses, the human and simian immunodeficiency viruses (HIV and SIV) exhibit a surprising degree of genomic complexity. HIV-1 contains no less than six open reading frames in addition to the prototypic *gag*, *pol*, and *env* coding sequences (see Figure 1). Two of these supplementary genes, *tat* and *rev*, are absolutely necessary for virus growth: Tat is a major transactivator of the proviral long terminal repeat (LTR), and Rev acts posttranscriptionally to ensure the switch from the early to the late phase of viral gene expression. In contrast, *nef*, *vpu*, *vif*, *vpr*, and the HIV-2 and SIV *vpx* genes encode factors that are dispensable for virus growth in many in vitro systems and are thus commonly called accessory proteins. Nevertheless, the high degree of conservation of these products indicate that they fulfill crucial functions in vivo. Exciting new studies are shedding light on these functions and reveal surprising interactions between HIV and its host cell.

Nef: Negative Only for the Host

Nef is encoded only by primate lentiviruses and is produced at all stages of viral gene expression. A predominantly cytoplasmic protein that associates with the plasma membrane through an N-terminal myristate, Nef was first suspected to repress transcription from the LTR, hence its acronym for "negative factor." However, studies in rhesus monkeys infected with *nef*-deleted strains of SIV demonstrated that this protein is necessary for high titer replication of SIV in vivo and for AIDS induction (Kestler et al., 1991). This can be explained by at least three effects of Nef: the induction of CD4 down-regulation, the alteration of T cell activation pathways, and the enhancement of virus infectivity.

Nef triggers the rapid endocytosis and lysosomal degradation of CD4, the main virus receptor (Aiken et al., 1994). A number of other cell surface molecules are not affected, ruling out a nonspecific stimulation of endocytosis. Instead, when 20 membrane-proximal residues from the CD4 cytoplasmic domain, including a critical dileucine-based sorting signal, are transferred to CD8, the resulting chimera becomes Nef responsive. Several models can thus be proposed. Nef could modify CD4 so that the receptor dileucine motif becomes accessible to clathrin-coated pits, the mediators of CD4 endocytosis. This would be reminiscent of CD4 response to phorbol esters, although the latter process involves phosphorylation of the receptor, a modification not triggered by Nef. Alternatively, Nef might liberate CD4 from an anchor that normally prevents its recruitment into coated pits. The lymphoid-specific p56^{lck} tyrosine kinase bound to the CD4 cytoplasmic tail plays such an inhibitory role. While Lck is released by Nef, this seems to be an epiphenomenon, because Nef-induced CD4 down-modulation is observed in cells that do not express Lck and on truncated CD4 molecules that do not bind the kinase. In yet another model that would be unprecedented, Nef could physically connect CD4 with a component of the internalization pathway. Future experiments should test these various possibilities, in particular by determining conclusively whether Nef, directly or indirectly, interacts with CD4 or with the endocytic apparatus. Irrespective of its mechanism, what advantage does Nef-mediated CD4 down-regulation confer to the virus? Does it rapidly block detrimental superinfection events? Does it prevent virus aggregation on the surface of cells during budding, analogous to the role played by neuraminidase for influenza virus? Or does it indirectly enhance viral gene expression through an alteration of T cell activation pathways, for instance by increasing the levels of free Lck?

T cell activation is perturbed by Nef, but different experimental settings have yielded conflicting results. Defective NF-κB induction and interleukin-2 (IL-2) expression were reported in Jurkat cells stably producing Nef, whereas an elevated signaling was observed in other cellular contexts, including thymocytes from *nef* transgenic mice (Luria et al., 1991; Niederman et al., 1992; Skowronski et al., 1993). In a recent study, expression of a chimeric CD8–Nef molecule in Jurkat cells induced two opposite phenotypes, which depended on the intracellular location of the fusion protein (Baur et al., 1994). In most cells, the chimera accumulated in the cytoplasm, and this was associated with a block in the early events of T cell signaling through the T cell receptor (TCR). In contrast, cells selected for their high surface levels of CD8–Nef showed spontaneous activation, which led to apoptosis. These results could be explained if Nef binds a cellular protein involved in activation. When kept in the cytoplasm, Nef would sequester the putative signaling molecule away from the TCR; when translo-

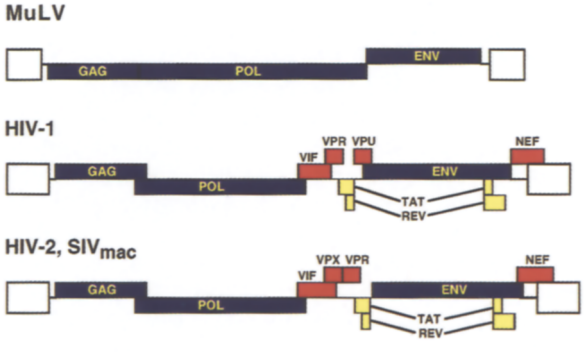

Figure 1. Genomes of the Murine Leukemia and the Human and Simian Immunodeficiency Viruses

cated to the cell surface, Nef and its associated protein would induce activation.

A third demonstrated function of Nef is the so-called enhancement of HIV-1 infectivity (Miller et al., 1994; Spina et al., 1994). Independent of CD4 down-regulation, both functionally and genetically, this effect is exerted at the stage of virus particle formation, yet manifested only after the virus enters target cells. Recent results suggest that it might reflect a stimulation of proviral DNA synthesis (Schwartz et al., 1995). Since only minimal amounts of Nef are detected in particles, the protein must act through an intermediate, for instance a component of the reverse transcription complex. Interestingly, the phenotype of *nef*-defective HIV-1 is best revealed when peripheral blood lymphocytes (PBL) are infected prior to stimulation. Is it because reverse transcription is already inefficient in resting T cells (Zack et al., 1990), or because Nef also influences activation?

Understanding the mechanisms of Nef action will require the identification of its cellular partners. Two distinct families of protein kinases are among the strongest candidates. First, a cellular serine kinase can be immunoprecipitated with Nef, together with proteins that undergo phosphorylation in an in vitro kinase assay (Sawai et al., 1994). The nature of the protein kinase and of its substrate(s), as well as the functional significance of their interaction with Nef, remain to be determined. Second, the Src homology region 3 (SH3) domains of Hck and Lyn show specific binding to a conserved (PxxP) repeat in Nef, corresponding to the minimal consensus site for SH3-mediated protein–protein interactions (Saksela et al., 1995). A functional role for this type of interaction is suggested by the finding that a PxxP-mutated *nef* allele is still competent for CD4 down-regulation, yet fails to enhance HIV-1 replication in PBL infected prior to stimulation. Further characterization of the intricate network connecting Nef with cellular pathways is warranted.

Vpu: Dual Overdrive

A late viral gene product translated from a bicistronic mRNA that also contains the *env* open reading frame, Vpu (viral protein U) is expressed by most HIV-1 isolates but not by HIV-2 or SIV, with the notable exception of SIV_{cpz}, a close relative of HIV-1. Vpu is an integral membrane phosphoprotein that associates mainly with the internal membranes of the cell, apparently in an oligomeric form (Maldarelli et al., 1993). Vpu carries a dual function, down-modulating CD4 and enhancing virion release.

In HIV-infected cells, intracellular complexes between CD4 and the viral envelope form in the endoplasmic reticulum (ER), trapping both proteins in this compartment. Vpu interacts with the cytoplasmic domain of ER-retained CD4 molecules and triggers their accelerated degradation (Willey et al., 1992b; Bour et al., 1995). The mechanism of this process is still obscure, although it likely involves proteolytic pathways utilized by cells to eliminate proteins that fail to fold correctly or to assemble into proper oligomeric complexes, such as observed with the components of the TCR. By analogy, Vpu could induce aggregation or some other structural change of CD4, thus exposing the receptor to the ER degradative pathway. Vpu-induced CD4 down-modulation enhances the intracellular transport and maturation of Env (Willey et al., 1992a). This is predicted to increase the infectivity of HIV-1 virions, perhaps mostly for cells expressing low levels of the virus receptor, such as macrophages. This point remains to be formally demonstrated.

Vpu also stimulates the release of virions from the surface of infected cells. This effect is both functionally and genetically distinguishable from CD4 down-regulation. For instance, the promotion of virus release can occur in the absence of Env and CD4. Also, it does not require Vpu phosphorylation but is blocked when the protein is retained in the ER, whereas the inverse is true of CD4 down-modulation (Schubert and Strebel, 1994). Curiously, Vpu also stimulates the budding of retroviruses that lack a Vpu-like activity, such as HIV-2, visna virus, and murine leukemia virus (Göttlinger et al., 1993). This raises the possibility that Vpu might alter a common intracellular pathway that is followed by different retroviral Gag proteins. The nature of this pathway is unknown.

An intriguing structural homology was noted between Vpu and the M2 protein of influenza, whose capacity as an ion channel seems to regulate the pH of some intracellular compartments. Whether one of the effects of Vpu is associated with a similar type of activity awaits demonstration.

Vif: Essential yet Elusive

Vif (virion infectivity factor) is a late gene product present in most lentiviruses that acts during assembly to allow the formation of particles competent for the early steps of infection (Gabuzda et al., 1992; von Schwedler et al., 1993). This effect is probably indirect, because only traces of the protein are found in virions. *vif*-mutated HIV-1 efficiently produces viral particles, but these exhibit an improperly packed nucleoprotein core (Höglund et al., 1994) and fail to synthesize the proviral DNA in target cells (von Schwedler et al., 1993). Whether this phenotype primarily reflects a decreased stability of the reverse transcription complex, an impaired uncoating of the internalized virion, or an abnormal entry process is still a matter of debate (von Schwedler et al., 1993; Borman et al., 1995). The Vif requirement is strictly cell dependent and, furthermore, determined solely by the cells releasing the virions, not by the targets of the infection. *vif*-mutated HIV-1 is thus functionally impaired only if produced from PBL, macrophages, and some rare T lymphoid cell lines such as H9. The restrictiveness of primary cells corroborates the failure of *vif*-mutated SIV_{mac} to replicate in vivo. Whether the permissiveness of other cells reflects the expression of a cellular homolog of Vif, or the absence of an inhibitor of virus replication normally counteracted by the viral protein, remains to be determined. A complication resulting from the cell specificity of the *vif*-defective phenotype is that it is technically difficult to produce high titer stocks of functionally relevant virus. Identifying conditional mutants of *vif* or using controllable *vif* expression systems could help solve this problem. Future investigations should also aim

at characterizing the cellular or viral proteins that interact with Vif. Finally, one should ask what unique aspect of lentiviral replication explains that the presence of *vif* be limited to this group of retroviruses.

Vpr: Manipulating the Cell

Vpr (viral protein R) is encoded by HIV-1, HIV-2, and most SIV isolates. Vpr is a short basic protein that associates with viral particles through an interaction with the C-terminal region of Gag and accumulates in the nucleus of infected cells (Lu et al., 1993). Long in quest of a function, Vpr has now been recognized as a key to crucial interactions between the virus and its host cell.

Oncoretroviruses depend on cell proliferation for their replication, because the breakdown of the nuclear envelope at mitosis allows the viral preintegration complex to interact with the host cell chromosomes. In contrast, HIV and other lentiviruses can infect nondividing cells, a property essential for spreading in such critical targets as terminally differentiated macrophages. Together with the viral matrix (MA) protein, Vpr plays a fundamental role in this process by connecting the preintegration complex with the cell nuclear import pathway (Heinzinger et al., 1994). HIV-1 strains carrying mutations in both Vpr and the karyophilic determinants of MA are thus unable to infect macrophages or growth-arrested cells, owing to a block in nuclear migration. In this context, the functions of MA and Vpr are redundant. However, the karyophilic motifs of these two proteins differ, suggesting that they govern viral nuclear import through the recognition of distinct cellular receptors. Such versatility would help ensure the completion of a step critical for viral spread in vivo. Vpr/MA double mutants also cannot establish a stable infection intermediate in quiescent T lymphocytes, indicating that this process too depends on the affinity of the preintegration complex for the nuclear transport apparatus (von Schwedler et al., 1994). MA alone suffices to restore this property. Whether Vpr also plays a role in this setting is yet undefined.

A puzzling discovery is that Vpr can perturb the cell cycle. HIV-1 *vpr* expression was first noted to induce growth arrest and differentiation in rhabdomyosarcoma and osteosarcoma cell lines (Levy et al., 1993). Recent experiments have indirectly confirmed these data, revealing that Vpr produces an accumulation of cells in the G2 phase of the cell cycle and, thereby, prevents the establishment of chronic HIV-1 infection in T lymphocytes (Rogel et al., 1995). This is a counterintuitive event, but the loss of chronically infected T cells might not be an issue in vivo, since virus-producing T lymphocytes are rapidly eliminated anyway because of either Env-mediated cytopathicity or immune cytotoxic responses (Ho et al., 1995; Wei et al., 1995). The differentiating effect of Vpr might thus have beneficial consequences before this happens, or in nondividing cells such as macrophages. In that respect, Vpr has been shown to increase the permissiveness of cells to HIV transcription, albeit slightly (Cohen et al., 1990). In one instance, it was reported to act in a paracrine manner, even when cells are exposed to a single dose of recombinant Vpr several days prior to infection (Levy et al., 1995). Confirming this observation and exploring further the mechanism of the Vpr-induced cell cycle arrest could provide important insights on basic events controlling cell growth.

It may seem surprising that *vpr*-defective SIV_{mac} is as pathogenic as wild type in rhesus monkeys (Gibbs et al., 1995). However, SIV and HIV-2 express *vpx*, whose sequence presents striking homologies with that of *vpr*, suggesting a genetic duplication. Like Vpr, Vpx associates with virions through an interaction with the C-terminal region of Gag (Wu et al., 1994). Furthermore, *vpr–vpx* double-mutant SIV replicates poorly in vivo and fails to induce disease (Gibbs et al., 1995). It will be interesting to investigate this possible overlap by testing whether Vpx also carries some of the recently discovered functions of Vpr.

Concluding Remarks

Accessory in name only, Nef, Vpu, Vif, and Vpr–Vpx are crucial determinants of HIV virulence. As such, these factors represent potentially valuable targets for the treatment of HIV-induced disease. Developing methods to interfere with these proteins will require an exquisite understanding of their mechanisms of action, the identification of their interlocutors in the cell, the creation of simple in vitro assays reflecting their activities, and ultimately, a detailed knowledge of their three-dimensional structure. As a bonus, efforts aimed at meeting this challenge will likely be rewarded along the way by further discoveries on basic cellular processes.

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