Cell PRESS
Cell Host & Microbe
Review

HIV-1 Accessory Proteins—Ensuring Viral Survival in a Hostile Environment

Michael H. Malim[1,*] and Michael Emerman[2,*]
[1]Department of Infectious Diseases, King's College London School of Medicine, 2nd Floor, Borough Wing, Guy's Hospital, London Bridge, London, SE1 9RT, UK
[2]Division of Human Biology, Fred Hutchinson Cancer Research Center, Room C2-023, P.O. Box 19024, 1100 Fairview Avenue North, Seattle, WA 98109-1024, USA
*Correspondence: michael.malim@kcl.ac.uk (M.H.M.), memerman@fhcrc.org (M.E.)
DOI 10.1016/j.chom.2008.04.008

One of the features of primate immunodeficiency viruses (HIVs and SIVs) that distinguishes them from other retroviruses is the array of "accessory" proteins they encode. Here, we discuss recent advances in understanding the interactions of the HIV-1 Nef, Vif, Vpu, and Vpr proteins with factors and pathways expressed in cells of the immune system. In at least three instances, the principal activity of the accessory proteins appears to be evasion from various forms of cell-mediated (or intrinsic), antiviral resistance. Broadly speaking, the HIV-1 accessory proteins modify the local environment within infected cells to ensure viral persistence, replication, dissemination, and transmission.

Introduction

The retrovirus that causes AIDS, human immunodeficiency virus type-1 (HIV-1), not only persists by populating sanctuary sites throughout the body but also employs multiple genetic strategies that, together, contribute to lifelong infection after successful transmission. First, by irreversibly integrating its viral DNA into the host cell genome to establish the provirus, HIV-1 safeguards its survival for the lifetime of the infected cell. Second, viral sequence diversification during spreading infection allows the virus to escape or tolerate adaptive immune responses. And, third, despite its compact genome comprising just nine genes, four of these (*nef*, *vif*, *vpu*, and *vpr*) now appear to be dedicated to various aspects of evasion from (and manipulation of) adaptive and innate immunity. Indeed, as has been recognized for other viruses (e.g., poxviruses and herpes viruses), these viral immunomodulatory genes are frequently seen as dispensable in many in vitro cell culture systems—leading to their loss during long-term propagation—yet are strongly maintained in the context of natural infections in vivo.

The reliance of HIV-1 upon numerous cellular host factors for nearly every step of viral replication is well appreciated (Brass et al., 2008; Swanson and Malim, 2008). In many cases, the roles of viral proteins are to recruit already assembled cellular machinery to perform essential roles in the virus life cycle. For example, one of the Gag proteins, p6, interacts with ESCRT complexes during virus assembly to facilitate viral budding, and the Rev protein recruits nuclear export factors to allow the nucleocytoplasmic transport of unspliced viral RNA. In contrast, a more recently appreciated phenomenon is the role of HIV-1 proteins in antagonizing host proteins that have evolved to defend against retroviral infections via diverse, cell-autonomous mechanisms: these have variously been called either intrinsic immunity factors or restriction factors. Here, we review how the Vif and Vpu (and probably Vpr) proteins each suppress the antiviral activity of specific restriction factors, whereas the multifunctional Nef protein contributes, *inter alia*, to partial evasion from adaptive, cell-mediated immunity. In addition to describing recent advances in our mechanistic understanding of these host-pathogen interactions, we will speculate on the importance of maintaining a balance between host and viral functions, discuss potential implications for viral zoonoses, and highlight some important questions for the future.

Proteasome-Mediated Degradation: A Frequent Viral Target

A recurring theme throughout this review is the use of protein degradation, and in particular cullin-RING finger ubiquitin ligases (Petroski and Deshaies, 2005), to avert the action of host proteins that interfere with HIV-1 replication. More specifically, Vif, Vpu, and Vpr all link to members of this superfamily of modular ubiquitin ligases to induce the polyubiquitylation and proteasomal degradation of their cellular targets (Table 1). At the heart of each cullin-RING ubiquitin ligase is a cullin—of which there are seven in vertebrates—that serves as a central scaffold. The C-terminal region of cullins binds an Rbx/Roc RING finger protein and recruits an E2 conjugating enzyme to form the catalytic core of the enzyme. The N terminus binds to an adaptor protein (e.g., Skp1), which, in turn, recruits a further subunit that functions as the receptor for substrate binding (e.g., β-TrCP). As substrates (e.g., Vpu) engage their receptors, they are brought into the proximity of the E2 subunit such that they and/or additional interacting proteins (e.g., CD4, the cell surface receptor for HIV-1) are ubiquitylated by a poorly understood process that also involves the cullin-RING finger region. By mixing and matching the wide variety of different ligase subunits, it is easy to see how one regulatory pathway (ubiquitylation and degradation) can be coupled to a tremendous diversity of protein interaction sites. This feature has been exploited by (at least) three HIV-1 accessory proteins to ensure that an otherwise hostile intracellular environment becomes conducive to effective viral replication and spread.

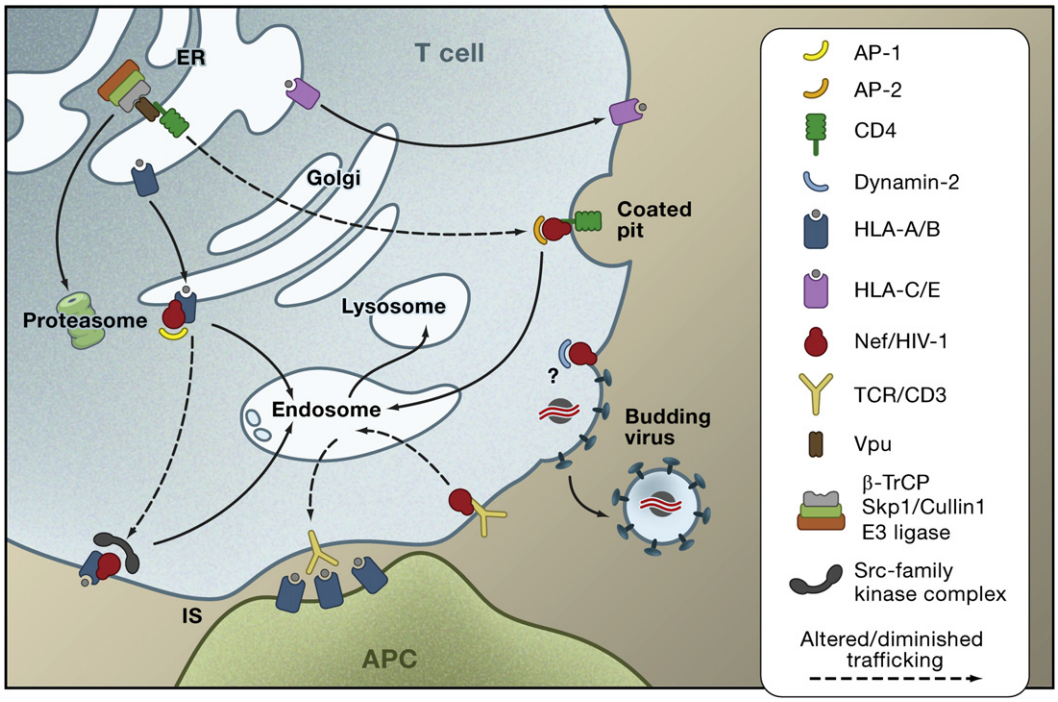

Figure 1. HIV-1 Nef and Vpu Regulate the Surface Expression and Localization of Host Cell Membrane Proteins
The effects of Nef on MHC class I are specific for HLA-A/B; the -C/E allotypes are not affected as their presence on the cell surface is important for protection from NK-mediated killing. ER, endoplasmic reticulum; IS, immunological synapse; APC, antigen presenting cell.

Modulation of Host Cell Surface Molecules: Nef and Vpu

Cell surface interactions are crucial for the life cycle and survival of any intracellular parasite. In the case of HIV-1, the virus enters target cells at their surface using the entry receptor CD4, and the coreceptors CCR5 or CXCR4 and newly synthesized viral particles bud from and are released from the surface of infected cells. However, the cell surface is also where the adaptive immune response recognizes virally infected cells in the context of viral epitopes presented by major histocompatibility class I complexes (MHC class I). Thus, viral accessory proteins that modulate the cell surface both aid certain virus replication steps that occur there, and also help the virus evade immune recognition. Such is the case for the Nef and Vpu proteins of HIV-1, which regulate the activity, localization, and abundance of surface membrane proteins in ways that profoundly influence viral replication, dissemination, and persistence.

Nef is a ~27 kD myristoylated protein that is associated with the cytoplasmic face of cellular membranes. It is one of the first viral proteins to be expressed following infection, implying perhaps that it plays an important role in helping set the tempo or magnitude of infectious virus propagation. Despite its seemingly erroneous name (negative factor), the importance of Nef as a critical determinant of pathogenicity has been established through the observed long-term survival of humans or rhesus macaques infected with HIV-1 or SIV (simian immunodeficiency virus) strains lacking intact *nef* genes (Deacon et al., 1995), a view that is further supported by recent work indicating that Nef may help dictate pathogenic outcome in natural infections of different species of primates (Schindler et al., 2006).

The intracellular trafficking of a number of cell surface proteins of helper T cells and macrophages (the targets of HIV-1 infection) with central roles in immunity and the virus life cycle is regulated by Nef (Roeth and Collins, 2006). Prominent among these is CD4, the primary entry receptor for this virus. It has long been known that the endocytosis of CD4 from the surface of infected cells is accelerated in response to Nef. This occurs through the interaction of Nef with the cytoplasmic tail of CD4, the recruitment of AP2 (clathrin adaptor protein complex 2), internalization through clathrin coated pits, and subsequent transport to endosomes and then lysozomes for degradation (Figure 1) (Chaudhuri et al., 2007). The involvement of a number of other participants in vesicular trafficking has been documented (Roeth and Collins, 2006), but a complete understanding of their respective roles in CD4 regulation is awaited.

Importantly, HIV-1 also employs another accessory protein, Vpu, to reduce the surface expression of CD4 (Figure 1) (a second major activity of Vpu is discussed in the following section). Vpu is an 81 amino acid dimeric integral membrane protein that induces the turnover of CD4 by recruiting a cullin1-Skp1 ubiquitin ligase complex to the cytoplasmic tail of CD4 (Table 1) (Margottin et al., 1998). Like Nef, Vpu interacts with the cytoplasmic tail of CD4, but in this case the interaction is with CD4 that has been retained in the endoplasmic reticulum (ER) by binding to the nascently synthesized viral Env glycoprotein, gp160, that would otherwise traffic to the cell surface for incorporation into assembling virus particles. As noted above, Vpu also binds to the Skp1-binding receptor protein, β-TrCP, thereby connecting the ligase complex to the cytoplasmic tail of CD4 and triggering polyubiquitylation and proteasomal degradation. The mechanism

Table 1. HIV-1 Accessory Proteins Recruit Cullin-RING Finger Ubiquitin Ligases to Eliminate Host Cell Proteins that Impede Replication

Accessory Protein	Cellular Substrate(s)	Receptor Protein	Adaptor Protein(s)	Cullin Scaffold	Benefit to Virus
Vif	APOBEC3G; APOBEC3F	none	elongin B/C	cullin5	protection from APOBEC-mediated inhibition and G-to-A hypermutation
Vpu	CD4	β-TrCP	Skp1	cullin1	efficient release of virus particles from infected cells
Vpr (and Vpx)	unknown	DCAF1(VprBP)	DDB1	cullin4A	G2/M arrest; suppression of postentry "restriction"

for extracting CD4 from the ER membrane is not yet fully understood, with evidence for and against a dependence upon ER-associated protein degradation (ERAD) components having been described (Binette et al., 2007; Meusser and Sommer, 2004). Ultimately, because two different HIV-1 accessory proteins contribute to the downregulation of CD4 from the cell surface, it is logical to infer that this must be important for viral propagation: for instance, cultured cell assays where surface interactions between CD4 and Env have been prevented show enhancements in virus release and Env incorporation (Lama et al., 1999; Ross et al., 1999). Consistent with this, experimental challenges of rhesus macaques with SIV carrying *nef* mutations that prevent Nef-AP2 interactions result in less virulent infections with lower viral loads (Brenner et al., 2006). However, these effects are difficult to assign to specific molecular defects, as other attributes of Nef such as effects on other cell surface proteins and/or viral infectivity (see below) were also influenced by these mutations.

MHC class I and specifically the HLA-A and -B allotypes, are also downregulated from the surface of HIV-1 infected cells by Nef (Roeth and Collins, 2006). Two alternative mechanisms have been proposed for this (Figure 1): first, Nef interacts with the cytoplasmic tail of HLA-A/B and recruits AP1 complexes to mis-route MHC class I from the trans-Golgi network to endosomes (rather than to the cell surface) (Lubben et al., 2007; Noviello et al., 2008; Roeth et al., 2004); or, second, Nef assembles a multicomponent Src-family kinase containing cascade to induce the endocytosis of MHC class I from the cell surface (Atkins et al., 2008). Irrespective of the precise mechanism, it seems plausible that it would be a selective advantage for the virus to suppress MHC class I function in order to blunt cytotoxic T cell (CTL) recognition of infected cells. This is supported by data from the rhesus macaque/SIV model showing that mutations in *nef* that prevent (solely) MHC class I downregulation are associated with heightened SIV-specific CTL responses or by mutations in *nef* that restore downregulation (Swigut et al., 2004). However, paradoxically, HIV-1 and SIV mutations driven by escape from CTL recognition are well described in the HIV-1 literature (Goulder and Watkins, 2004), implying that the presentation of viral epitopes by MHC class I continues throughout the course of infection. Thus, although the ability of Nef to downregulate MHC class I is well conserved during natural infection and across multiple lineages of primate lentiviruses (Lewis et al., 2008; Specht et al., 2008), in reality this effect can only be partial in vivo, thus making mutation-assisted escape from CTL recognition a critical driver of immune evasion.

A third cell surface complex that has emerged as a target for Nef regulation is the T cell receptor (TCR-CD3). TCR-CD3 is a critical component of the "immunological synapse" that forms between antigen-presenting cells (APCs) and T cells for antigen recognition and sustained T cell activation. In mixed cell culture systems in which antigen-pulsed APCs contact HIV-1 infected T cells and form immunological synapses, the endocytosis of TCR-CD3 from the cell surface and its transport through recycling endosomes were retarded by Nef (Thoulouze et al., 2006). This results not only in reduced clustering of TCR-CD3 at the immunological synapse, but also in inefficient synapse formation (Figure 1). Comparatively less is known about the molecular interactions that govern TCR-CD3 regulation by Nef, though Nef has been shown to: interact with the ζ chain of CD3 (Howe et al., 1998); induce the accumulation of Lck, a kinase important for sustained signaling from the immunological synapse, in the recycling endosome (Thoulouze et al., 2006); and inhibit the activity of N-WASP, a positive regulator of actin polymerization and critical mediator of T cell activation (Haller et al., 2006).

It has been proposed that differences in the capacity of Nef proteins from different primate lentiviruses (which comprise HIV-1, HIV-2, and many SIVs) to downregulate TCR-CD3 may play a central role in dictating pathogenic outcome (Schindler et al., 2006). The SIVs are generally not pathogenic in their natural hosts, but, like HIV-1 in humans, can be pathogenic when transferred to a novel host species. Indeed, for the majority of SIV/HIV Nef proteins tested, the capacity to downregulate TCR-CD3 in human T cells correlated with a lack of both T cell activation and pathogenicity during infection of the natural host with the corresponding virus. For instance, Nef proteins derived from nonpathogenic SIVs such as the virus found in African green monkeys (SIV-AGM) are very effective at removing TCR-CD3 from the cell surface. In contrast, the action of HIV-1 Nef appears to be more subtle: while it appears to negatively regulate the movement of TCR-CD3 to the synapse (Figure 1), it does not inhibit the overall cell surface expression of TCR-CD3. By perturbing TCR-CD3 trafficking in this manner, the capacity of T cells to respond to activation cues from APCs is diminished (Thoulouze et al., 2006). Why would dampening T cell activation, a process that stimulates virus replication, be advantageous for the virus? One possibility is that by extending the survival of infected cells, and perhaps inhibiting homeostatic processes such as apoptosis (Geleziunas et al., 2001), Nef may balance T cell activation to limit the deleterious effects of infection on host immunity, while helping ensure prolonged (albeit less rampant) viral production and long-term persistence. Nonetheless, it should be pointed out that either through differences in experimental systems, or perhaps variations in the phenotypes of different HIV-1 *nef* alleles, some apparently conflicting ideas regarding the functional consequences (impaired or enhanced T cell activation) (Fenard et al., 2005; Haller et al., 2006; Thoulouze

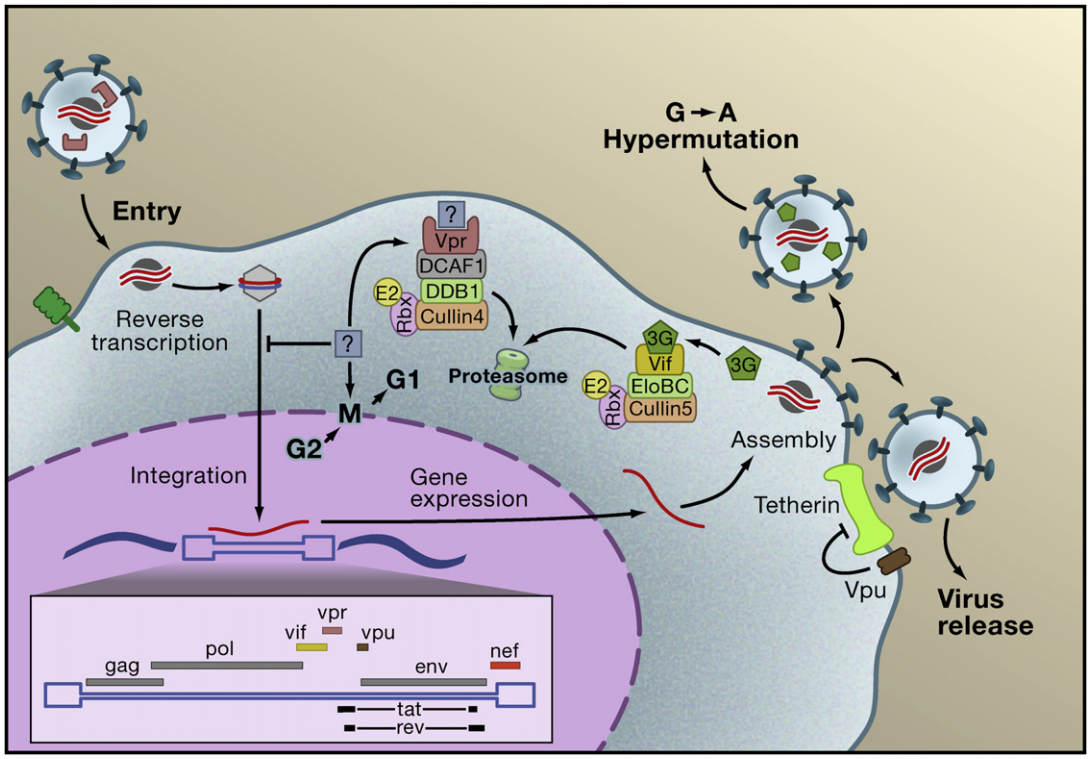

Figure 2. Vif, Vpu, and Vpr Inhibit Host Cell Restriction Factors to Promote Efficient Virus Replication
The inset depicts the genomic organization of HIV-1 and highlights the presence of accessory genes.

et al., 2006) of the influence of Nef on TCR-CD3 remain. In addition, a positive correlation between Nef and a significant defect in T cell responses to viral epitopes presented by APCs in vivo remains to be demonstrated.

In addition to Env/CD4-associated effects that modulate HIV-1 infectivity, Nef further enhances HIV-1 infectivity by facilitating viral core penetration of the cortical actin network during the initial phases of infection. Recent new insight into this enigmatic phenotype comes from the finding that dynamin 2, a GTPase that is required for clathrin-mediated endocytosis, interacts with Nef and is essential for Nef's effect on infectivity (Figure 1) (Pizzato et al., 2007). While the molecular basis for this is obscure, it has been suggested that the recruitment of dynamin 2 by Nef to certain membranes, and perhaps sites of viral assembly and budding, may manipulate the local composition of such membranes and, hence, of progeny virus particles themselves (Pizzato et al., 2007). Taken together, it is evident that Nef manipulates the composition of the infected cell surface in a variety of ways that may benefit viral propagation. Major challenges for the future include defining which of these (as well as other) demonstrable—and in some cases seemingly opposing—phenotypes are important in the context of natural infection, and determining how they integrate with each other with respect to various pathogenic outcomes, immune evasion, and viral replication.

Modulation of Antiviral Activities at the Cell Surface: Vpu

Type 1 interferons induce a large number of host genes with diverse antiviral functions. The effects of such activities on HIV-1 include the inhibition of late stages of replication and, in the case of interferon-α, the dramatic attachment of fully assembled HIV-1 particles to the surface of infected cells (Neil et al., 2007). Experimentally, particles retained in this manner can be liberated by adding a protease, suggesting that a protein linkage "tethers" them to the cell surface (Neil et al., 2006). Significantly, this restriction to the release of viruses that have completed membrane fission can be completely overcome by the HIV-1 Vpu protein (Figure 2).

The influence of Vpu is cell-type specific: some cells require Vpu for virus release, while others do not. By employing classical cell fusions between cells in which efficient release is Vpu dependent or Vpu independent, it was shown that the Vpu-dependency phenotype is dominant, indicating that such cells express Vpu-sensitive cellular factor(s) that prevent HIV-1 release (Varthakavi et al., 2003). The interferon-induced restriction factor that prevents retrovirus release from the plasma membrane, and is counteracted by Vpu, was recently identified (Neil et al., 2008; Van Damme et al., 2008) as a cellular protein of previously unknown function called B cell stromal factor 2 (BST-2) or CD317: it has also been called "tetherin" to reflect its antiviral activity (Neil et al., 2008).

Tetherin is a heavily glycosylated membrane protein that is anchored to lipid bilayers both by an N-terminal transmembrane region, and by a C-terminal glycosylphosphatidylinositol (GPI) linker; thus, its N terminus is predicted to be cytosolic with the remainder of the protein positioned on the outside of the membrane (Kupzig et al., 2003). Its cellular localization is also known to be highly dynamic, with a plasma membrane pool that is associated with lipid rafts but continuously internalized

to the trans-Golgi network (Rollason et al., 2007). Viral particles made in the absence of Vpu in tetherin-expressing cells still form at the plasma membrane with similar kinetics as virions made in the presence of Vpu. However, the failure of these particles to be released results not only in their striking accumulation at the exterior of the cell, but also in subsequent transport to endosomes (Neil et al., 2006).

It is not yet clear how tetherin prevents virus release; however, the mechanism must be relatively nonspecific since tetherin affects the release of very distinct classes of virus. For example, tetherin blocks the release of diverse retroviruses as well as Ebola virus-like particles (Gottlinger et al., 1993; Neil et al., 2007), and it is likely that it affects the Kaposi's sarcoma-associated herpesvirus (KSHV) since this virus encodes a protein, K5, that induces the degradation of tetherin (Bartee et al., 2006). It therefore seems unlikely that there is specific recognition between tetherin and viral structural proteins. One especially intriguing hypothesis derives from the notion that tetherin serves to link cholesterol-rich lipid rafts together on the plasma membrane (Kupzig et al., 2003): Accordingly, since many enveloped viruses, and HIV-1 in particular, are known to accumulate and bud from lipid raft-rich regions of the plasma membrane, tetherin may form connections between lipid rafts on plasma and viral membranes and thereby physically prevent virus egress.

It is also not yet evident how Vpu counteracts tetherin function. Given that Vpu induces the proteasomal degradation of CD4 and the KSHV K5 protein is a RING-type ubiquitin ligase, it made sense that Vpu might also target tetherin for degradation. However, although overexpressed Vpu has been reported to reduce tetherin expression levels (Bartee et al., 2006), the physiological pertinence of this remains questionable since similar effects have not been observed in the context of HIV-1 infection (Neil et al., 2008). Moreover, the CD4 degradation function of Vpu (described above) is dispensable for the ability of Vpu to enhance virus release (Schubert et al., 1996). What currently seems more likely is that Vpu either interferes with tetherin function directly and/or alters the trafficking of tetherin between different cytoplasmic sites. By colocalizing closely with tetherin at various cellular membranes (Neil et al., 2008; Van Damme et al., 2008), Vpu appears to be appropriately placed to interact with tetherin (either directly or indirectly) and influence activity or localization. In keeping with the second possibility, Vpu significantly reduces the surface expression of tetherin (Van Damme et al., 2008) as well as its colocalization with the HIV-1 Gag protein (Neil et al., 2008), and disruption of protein sorting through early/recycling endosomes has been shown to prevent Vpu-induced virus release (Varthakavi et al., 2006). Thus, Vpu can be considered to be somewhat analogous to Nef in that it may modulate the subcellular compartmentalization of a host membrane protein to help promote viral dissemination and replication. Important next steps in this area are to elucidate tetherin function in molecular terms, determine whether Vpu influences this directly, and identify the trafficking pathways and connecting factors that underlie the ability of Vpu to overcome tetherin. It will also be interesting to see if the capacity of Vpu function as an ion channel (a "viroporin") is important for the suppression of tetherin since a number of other viruses also encode viroporins that play roles in virus release and/or protein trafficking (Gonzalez and Carrasco, 2003).

Since tetherin is induced by interferon-α, it is almost certainly part of a broad antiviral defense system that retains budding viruses on the surfaces of cells. Such a system might play a direct role in increasing the likelihood that viral antigens will be better presented to the adaptive immune system, or its existence may reflect an evolutionary selection to prevent long-range virus transmission between hosts (although under certain experimental selective pressures, retention of virus particles on the cell surface actually increases cell-to-cell transmission [Gummuluru et al., 2000]). Thus, one might anticipate that the ability of HIV-1 Vpu to counteract tetherin is not unique: indeed, as well as the KHSV K5 protein described above, the HIV-2 Env protein also stimulates virus release (Bour and Strebel, 1996). In addition, because cells from AGMs encode an interferon-inducible factor (presumably the AGM version of tetherin) that is effective against HIV-1, yet is not overcome by Vpu (Neil et al., 2007), it is most likely that this form of cell-mediated viral inhibition is an ancient one and that the evolution of Vpu is one of the critical factors that allowed the SIVs that are direct ancestors of HIV-1 to become established in chimpanzees and gorillas, and then, ultimately, in humans.

Inhibition of Cytoplasmic Defenses: Vif

The HIV-1 Vif protein (virion infectivity factor) is 192 amino acid cytoplasmic protein whose essential role in replication in primary T cells and during natural infection has long been established. Though certain cultured cell lines are able to support growth of *vif*-deficient viruses, cell fusion experiments indicated that such cells lack expression of inhibitory factor(s) that naturally block viral replication when Vif is absent. By comparing mRNA expression profiles in cells where Vif is, or is not, required for HIV-1 replication, the human gene *APOBEC3G* (*A3G*) was identified as being fully sufficient to prevent productive infection in the absence of Vif (Sheehy et al., 2002).

The rather cumbersome name for this protein, apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like 3G, provided an immediate clue regarding the possible mechanism for viral suppression. Specifically, A3G is a member of the APOBEC family of editing enzymes, many of which can mutate polynucleotides by deaminating cytidine (C) to uridine (U) (Conticello et al., 2005; Harris and Liddament, 2004; Holmes et al., 2007b). Indeed, in the absence of Vif, newly synthesized A3G is packaged into budding viral particles through a combination of A3G-RNA and A3G-Gag interactions (Bogerd and Cullen, 2008; Soros et al., 2007) and consequently carried forward to newly infected cells where it deaminates C residues to U residues in nascent minus (first) strand reverse transcripts (Conticello et al., 2005; Harris and Liddament, 2004; Holmes et al., 2007b). Should these changes become fixed, they register as guanosine (G)-to-adenosine (A) transitions in plus strand sequence: since ~10% of G residues can be mutated, this phenomenon is called G-to-A hypermutation and, by itself, is sufficient to stop further viral spread through the gross loss of genetic integrity (a form of error catastrophe). Indeed, evidence from examining the fossil record of endogenous retroviruses in the genome of mice and humans indicates that some "ancient" retroviral infections were inactivated by APOBEC3-mediated mutations (Esnault et al., 2005; Jern et al., 2007; Y.N. Lee, M.H.M., and P.D. Bieniasz, unpublished data).

In the majority of wild-type viral infections, Vif effectively antagonizes the antiviral effects of A3G through the recruitment

of a cullin-RING ubiquitin ligase and the induction of polyubiquitylation (of A3G and Vif itself) and degradation (Table 1; Figure 2) (Mehle et al., 2004; Yu et al., 2003). In the case of A3G, Vif simultaneously binds to a specific region of A3G centered around an aspartic acid residue at position 128, as well as to the elonginC and cullin5 components of the cullin5-elonginB/C complex, via its BC box (a peptide motif that binds elonginB/C) and a zinc coordinating motif, respectively (Huthoff and Malim, 2007; Mehle et al., 2006; Yu et al., 2004). By eliminating A3G from virus-producing cells, and perhaps by impeding packaging via a more direct mechanism, Vif therefore allows progeny particles to be produced that are free of A3G. Interestingly, phosphorylation of a conserved serine in Vif's BC box inhibits binding to elonginC (Mehle et al., 2004), suggesting that the degradation of A3G in infected cells may also be regulated by signaling pathways that have yet to be explored in detail.

Like aspects of Nef biology described above (Schindler et al., 2006), Vif function is also species specific. Vif proteins from HIVs and SIVs whose ancestors have established infections in humans efficiently inhibit human-A3G, whereas Vifs from SIVs whose ancestors have not been transmitted to humans do not (Gaddis et al., 2004). For A3G, the molecular basis for specificity corresponds to Vif binding (Holmes et al., 2007b). For example, HIV-1 Vif binds human-A3G but not A3G from AGMs; conversely, SIV-AGM Vif binds AGM-A3G but not human A3G. Thus, the ability to overcome the APOBEC proteins of a recipient species correlates with transmission potential, thus marking Vif as an important determinant of lentivirus transmission. Importantly, *A3G* is only one of a set of seven cytidine deaminase genes encoded by a locus on human chromosome 22 (*APOBEC3A-H*), and the anti-HIV-1 phenotypes of these other APOBEC proteins have been extensively cataloged using cultured cell assays (Holmes et al., 2007b). In sum, A3G has the most potent inhibitory effect and A3F is also very active, while A3B is much less so. Of these, A3B is not regulated by Vif and is barely expressed in T cells, which suggests a lack of relevance in vivo for HIV-1 infections. A3F, like A3G, is also linked to the cullin5-elonginB/C ligase by Vif and correspondingly degraded (Table 1) (Liu et al., 2005). Interestingly, mutations have been described in Vif that segregate the ability to downregulate A3G versus A3F (Russell and Pathak, 2007; Simon et al., 2005; Tian et al., 2006), implying that the adaptation of HIV-1 to humans necessitated that Vif maintain at least two distinguishable APOBEC3 binding interfaces.

Though A3G and A3F are each suppressed by Vif, there is persuasive evidence to support their functional interaction with HIV-1 during natural infections. Specifically, analyses of HIV-1 sequences from infected persons frequently reveal subsets of sequences that are distinguished by excessive G-to-A hypermutation (Janini et al., 2001). The local nucleotide sequence preferences for such mutations have been calculated and match those determined for A3G (predominantly) and A3F in transfection-based experiments (Holmes et al., 2007b), suggesting that these APOBEC proteins are the most significant for driving HIV-1 hypermutation in vivo. Presumably, such sequences arise when A3G/F occasionally escape Vif-mediated inhibition and become encapsidated into viral particles.

Hypermutation, while clearly central to the profound impact of A3G on HIV-1 infection, is not the only mechanism through which antiviral effects are exerted. First, and noted originally by examining deaminase-deficient proteins, infectivity can be reduced in the absence of DNA editing (Newman et al., 2005). While not yet entirely explained at the molecular level, these effects are associated with diminished reverse transcription (Holmes et al., 2007a; Iwatani et al., 2007; Mbisa et al., 2007). Second, and perhaps mechanistically related, A3G residing in target cells (i.e., not present in virions) has been reported to impede the synthesis of viral DNA by incoming viral particles in quiescent T cells without inducing hypermutation (Chiu et al., 2005). However, the relative contributions of deamination-dependent versus -independent effects of the APOBEC3 proteins during physiologic infections remains unresolved and may well differ among different family members (Holmes et al., 2007a; Miyagi et al., 2007; Schumacher et al., 2008). A further area of uncertainty is the fate of the uridines generated by deamination: it had been proposed that U residues would be recognized by cellular DNA repair enzymes, perhaps initiating viral DNA degradation, but the inhibition of such enzymes neither ameliorates the A3G antiviral phenotype nor promotes the accumulation of viral DNA (Kaiser and Emerman, 2006; Langlois and Neuberger, 2008).

Different retroviruses have adopted different strategies to evade suppression by APOBEC proteins. Human T cell leukemia virus type 1, like HIV-1, replicates in CD4 T cells but averts significant inhibition by not packaging A3G into virions through the action of sequences in the nucleocapsid (NC) region of Gag, thus avoiding the need for a Vif-like factor (Derse et al., 2007). In contrast, the HIVs and SIVs have adopted the more elaborate tactic of acquiring an additional regulatory protein, namely Vif. Because a regulatory interaction has the potential to be variable in its extent, sporadic partial inhibition of A3G/F by Vif (e.g., through variation in either A3G/F expression or Vif sequences [Simon et al., 2005]) may allow sufficient levels of these proteins to survive and confer low levels of editing. Rather than being detrimental for the virus in the way that hypermutation is, this has the potential to afford the virus an additional mechanism for promoting beneficial sequence diversification that could, for instance, facilitate escape from adaptive immunity or help drive phenotypic changes in the virus: indeed, detailed analyses of recently transmitted viruses indicate that A3G/F can contribute to single-nucleotide sequence variation (Keele et al., 2008). Population level studies of HIV-1 infected cohorts also support the notion that the balance between APOBEC proteins and their downregulation by Vif is dynamic and subject to variation: specifically, there is evidence that genetic polymorphisms in *A3G* or *cullin5* are associated with differences in the rates of disease progression (An et al., 2007).

As potential DNA mutagens, especially those that accumulate in the nucleus (A3A, A3B, and A3C), it seems intuitive that APOBEC3 protein function would be negatively regulated in some manner in the absence of viral infection to protect against deleterious mutation of cellular genomic DNA. Conversely, some level of constitutive expression may be necessary to control the movement of the many endogenous retroviruses and retrotransposons (LTR and non-LTR types such as LINE-1 and Alu elements) that have been shown to be inhibited to various degrees (and in different ways) by many APOBEC3 proteins (Esnault et al., 2005; Holmes et al., 2007b). Such questions of fine-tuning are starting to receive attention: enzymatic measurements have shown that T cells tightly control A3G mediated deamination

(Thielen et al., 2007), and biochemical analyses have shown that the association of A3G with an array of cytoplasmic ribonucleoprotein complexes that localize to important sites of RNA function, storage, and metabolism can also modulate enzymatic activity (Chiu et al., 2005, 2006; Gallois-Montbrun et al., 2007; Kozak et al., 2006; Wichroski et al., 2006). Among A3G's (and A3F's) interactions with cellular proteins, those with the Argonaute proteins, the effector components of RNA-induced silencing complexes, are notable (Gallois-Montbrun et al., 2007, 2008; Wichroski et al., 2006). Whether APOBEC proteins are therefore able to modulate RNA silencing pathways and translational regulation (Huang et al., 2007), and what possible connections there may be to cellular function and/or HIV-1 replication, remains largely unexplored (readers should refer to the accompanying Review by Gottwein and Cullen on page 375 that discusses interactions between viruses, microRNAs, and RNA silencing).

Primates have a total of eleven *APOBEC* genes, yet birds and fish have only two each, which suggests that evolutionary pressures have driven the expansion of this gene family (Conticello et al., 2005; Harris and Liddament, 2004). Moreover, comparisons among the *A3G* sequences of a large panel of diverse primates revealed that these genes have been subject to nearly constant severe positive selection throughout the past 33 million years of primate evolution (Sawyer et al., 2004) and comparison of the entire cluster of *APOBEC3* genes indicates that most of them have rapidly evolved since human-chimpanzee speciation (Sawyer et al., 2004). What could have applied such pressures? Since many retroviruses and retrotransposons, as well as hepatitis B virus, can be inhibited by APOBEC3 family members (Holmes et al., 2007b), and the human genome contains active LINE-1 and Alu elements, it is likely that the *APOBEC3* genes have evolved to defend against genomic assaults by a broad spectrum of retrovirus-like parasites. Given that many of the APOBEC3 proteins are expressed in the testes and/or ovaries (e.g., Jarmuz et al., 2002), this appears, perhaps not unexpectedly, to be of particular importance for the protection of germline. Recent results showing that two very different DNA viruses, adeno-associated virus and human papillomavirus, can be inhibited or subjected to G-to-A editing, respectively, by APOBEC3 proteins reveals that the range of substrates for these enzymes extends beyond those requiring reverse transcription (Chen et al., 2006; Vartanian et al., 2008). It will be fascinating to see how many additional classes of virus are also targeted by APOBEC proteins, either as inhibitors of replication or as sources for sequence variation, and to determine whether such effects are subjected to regulation by proteins analogous to Vif.

Modulation of the Intracellular Environment: Vpr

Vpr is a 96 amino acid protein that is packaged into mature virions, but whose function has been difficult to elucidate. Vpr is cytopathic to cells, although there has been some debate as to whether or not the cell death is apoptotic (Muthumani et al., 2005) or necrotic (Sakai et al., 2006). However, the whole question of whether or not HIV-1-induced cyopathicity is important for its pathogenesis has been called into question with recent findings made during nonpathogenic infections of natural sooty mangabey (SM) or AGM hosts with SIV-SM or SIV-AGM, respectively, where the turnover time of infected cells was found to be just as short as seen in HIV-1 infection of humans (Gordon et al., 2008; Pandrea et al., 2008). Nevertheless, one unambiguous attribute of Vpr expression is its ability to delay or arrest cells in the G2 phase of the cell cycle. The bulk of the data indicates that the cell death phenotype induced by Vpr is linked to the pathway leading to G2 arrest (Andersen et al., 2006), but in some culture systems those two phenotypes might be independent (Bolton and Lenardo, 2007). These differences notwithstanding, the presumed relevance of the ability of Vpr to cause a G2 cell-cycle arrest is illustrated not only by its conservation among the HIV and SIV Vpr proteins, but also by the observation that infected cells in HIV-1 infected people appear to be enriched for cells in G2 (Zimmerman et al., 2006).

The distal events that lead to G2 arrest by Vpr appear to mimic a DNA stress/damage checkpoint arrest by involving the DNA damage-sensing kinase ATR (Zimmerman et al., 2004). However, since Vpr does not appear to cause DNA damage directly (Lai et al., 2005), it was not immediately clear how the pathway became activated in the first place. A flurry of recent papers has provided some important clues. Mass spectrometry and protein-protein interaction assays revealed that Vpr, like Vpu and Vif, engages a cullin-RING ubiquitin ligase (Table 1; Figure 2). In this case, it is the cullin4A-DDB1 complex, with Vpr making contact via a receptor called DCAF1 (originally VprBP) that links it to DDB1 (Belzile et al., 2007; DeHart et al., 2007; Hrecka et al., 2007; Le Rouzic et al., 2007; Schrofelbauer et al., 2007; Tan et al., 2007; Wen et al., 2007). Experiments showing that preventing Vpr's interaction with DCAF1 or inhibiting DCAF1 expression using RNAi both block Vpr-induced G2 arrest attest to the significance of the cullin4A-DDB1-DCAF1-Vpr complex (Dehart and Planelles, 2008).

Interestingly, DCAF1 was also identified as an HIV-1-dependency factor (i.e., a protein whose expression promotes replication) in a large-scale siRNA screen using a viral strain that was deficient for Vpr (Brass et al., 2008). This suggests that the cullin4A-DDB1-DCAF1 complex is involved in an important step of the viral life cycle, and that the role of Vpr might be to stabilize the normal activity of the complex in a way that benefits the virus. Supporting this, recent data show that the binding of Vpr to cullin4A-DDB1-DCAF1 augments its activity and is associated with an increase in cullin4A neddylation—a posttranslational modification with the ubiquitin-like protein Nedd8 that stimulates cullin ligase function by facilitating E2 recruitment (Hrecka et al., 2007). If this hypothesis is correct, then it becomes very important to identify the relevant substrates of the cullin4A-DDB1-DCAF1 complex, to determine how their (presumed) degradation affects HIV-1, and to resolve whether Vpr acts simply to improve the efficiency of this process.

Rather than increasing the activity of the cullin4A-DDB1-DCAF1 complex toward normal substrates, an alternative model is that Vpr recruits a novel substrate (whose expression is necessary for cell-cycle progression) for ubiquitylation (and degradation). Evidence supporting this view comes from the finding that some mutations in the C terminus of Vpr do not affect binding to DCAF1, but abolish Vpr-triggered G2 arrest. Given that such proteins are dominant inhibitors of cell-cycle arrest induced by wild-type Vpr (DeHart et al., 2007; Le Rouzic et al., 2007), the simplest explanation is that the C terminus of Vpr recruits a novel substrate to the cullin4A-DDB1-DCAF1 for ubiquitylation (Figure 2). It is not known what this substrate could be; determining

whether it is among the many previous binding partners described for Vpr, or awaits identification, will be central to understanding the effect of Vpr on the cell cycle.

In addition to G2 arrest, HIV-1 Vpr also facilitates infection of macrophages. Earlier data attributed this effect to the stimulation of viral nuclear import, but this model has not held up (Yamashita and Emerman, 2005). Viruses of the HIV-2/SIV-SM phylogenetic lineage encode both Vpr and a paralog, Vpx, whereas HIV-1 and the SIVs of the remaining lineages encode just Vpr. The two main functions of HIV-1 Vpr are segregated in HIV-2/SIV-SM with Vpr causing cell-cycle arrest and Vpx being important for the infection of macrophages, though HIV-2/SIV-SM Vpx seems to be much more important for macrophage infection than HIV-1 Vpr. Indeed, recent studies on Vpx may have provided key insight into the ultimate role of Vpr in the viral life cycle: specifically, HIV-2/SIV-SM vectors can only infect macrophages or monocyte-derived dendritic cells efficiently if the *vpx* gene is intact (Goujon et al., 2007; Sharova et al., 2008). Moreover, the Vpx protein can be supplied in *trans* to allow macrophage or dendritic cell infection and, in fact, can be added to allow HIV-1, as well as more distantly related retroviruses, to infect these cells (Goujon et al., 2007; Sharova et al., 2008). In this respect, Vpx appears to act in a somewhat analogous way that excess viral capsids can be added to cells to saturate the host restriction factor, TRIM5α (Towers, 2007).

Importantly, Vpx, like Vpr, binds to DCAF1 (Le Rouzic et al., 2007; Srivastava et al., 2008), and the ability of Vpx to interact with the same cullin4A-DDB1-DCAF1 complex as Vpr is essential for promoting SIV-SM infection of macrophages (Sharova et al., 2008; Srivastava et al., 2008). Thus, a plausible explanation for the effect of Vpx on the early stages of macrophage infection is that it is introduced into cells by virtue of its association with incoming viral cores and then interacts with a host defense protein to target that protein to cullin4A-DDB1-DCAF1 for elimination (Figure 2). This notion is further supported by cell-fusion experiments in which macrophages were shown to harbor a dominantly acting, but Vpx-sensitive, repressor of SIV-SM infection (Sharova et al., 2008).

Whether HIV-1 Vpr also interacts with this putative restriction factor, and whether this factor plays a role in Vpr-induced G2 arrest, will only be answered once its identity is resolved. While G2 arrest by Vpr can be rationalized by the finding that HIV-1 transcription is more active in G2 (and therefore more virus is produced) (Goh et al., 1998), it is also possible that G2 arrest is an unavoidable by-product of the destruction of a host protein that plays one role in cell-cycle progression and another in host-mediated protection against viruses. Indeed, evolutionary analysis suggests that such a class of host genes that both act in the DNA-repair pathway in nonhomologous end joining and also show evolutionary signs of pathogen defense genes (because of they are under positive selection) does exist in yeast (Sawyer and Malik, 2006). Moreover, there is precedence with the V protein of paramyxoviruses, which also binds to DDB1, and although the mechanism is entirely different, also causes both cell-cycle arrest and is involved in abrogation of host defense through interference with interferon signaling (Horvath, 2004). Thus, while speculative at this time, it is possible that certain proteins that are involved in DNA repair or cell-cycle progression may also serve a dual purpose in host antiviral defense, and that these are targeted by Vpr for degradation. If true, then this implies that Vpr, like Vif and Vpu, is fundamentally a viral countermeasure to host-mediated restriction.

Conclusion

While HIV-1 is a relatively new human infection, human ancestors have been infected with retroviruses for many millions of years. We know this because nearly 8% of the human genome is comprised of endogenous retroviruses (Bannert and Kurth, 2004), of which at least some of each family must have been the result of an exogenous retroviral infection that entered the germline and then became fixed within the species. Many of these infections are quite old (over 25 million years), but episodic infections have continued even since human/chimpanzee speciation (Bannert and Kurth, 2004). Thus, host defenses that are operative against HIV-1 were not selected to inhibit this virus. Rather, they were likely selected to work against much more ancient viral infections. HIV-1, on the other hand, must have adapted to humans (or more likely to hominoids since HIV-1 itself is a direct descendant of similar chimpanzee and gorilla viruses) through the evolution of viral genes that allowed it to replicate in the face of ancient antiviral defense mechanisms. As these defenses are still active, finding ways to stabilize or derepress them in the presence of the HIV-1 accessory proteins is a potential strategy for antiviral drug development that would turn the naturally hostile environment of human cells into a more effective one for viral control.

ACKNOWLEDGMENTS

Owing to space limitations, we have predominantly cited recent publications and therefore apologize to those whose original contributions have not been specifically referenced. We are indebted to Stuart Neil, Vincente Planelles, and J. Victor Garcia-Martinez for many helpful suggestions and discussions. M.H.M. is an Elizabeth Glaser Scientist.

REFERENCES

An, P., Duggal, P., Wang, L.H., O'Brien, S.J., Donfield, S., Goedert, J.J., Phair, J., Buchbinder, S., Kirk, G.D., and Winkler, C.A. (2007). Polymorphisms of CUL5 are associated with CD4+ T cell loss in HIV-1 infected individuals. PLoS Genet. *3*, e19.

Andersen, J.L., DeHart, J.L., Zimmerman, E.S., Ardon, O., Kim, B., Jacquot, G., Benichou, S., and Planelles, V. (2006). HIV-1 Vpr-induced apoptosis is cell cycle dependent and requires Bax but not ANT. PLoS Pathog. *2*, e127.

Atkins, K.M., Thomas, L., Youker, R.T., Harriff, M.J., Pissani, F., You, H., and Thomas, G. (2008). HIV-1 Nef binds PACS-2 to assemble a multi-kinase cascade that triggers MHC-I downregulation: Analysis using siRNA and knockout mice. J. Biol. Chem. *283*, 11,772–11,784.

Bannert, N., and Kurth, R. (2004). Retroelements and the human genome: new perspectives on an old relation. Proc. Natl. Acad. Sci. USA *101* (*Suppl 2*), 14572–14579.

Bartee, E., McCormack, A., and Fruh, K. (2006). Quantitative membrane proteomics reveals new cellular targets of viral immune modulators. PLoS Pathog. *2*, e107.

Belzile, J.P., Duisit, G., Rougeau, N., Mercier, J., Finzi, A., and Cohen, E.A. (2007). HIV-1 Vpr-Mediated G2 Arrest Involves the DDB1–CUL4A(VPRBP) E3 Ubiquitin Ligase. PLoS Pathog. *3*, e85.

Binette, J., Dube, M., Mercier, J., Halawani, D., Latterich, M., and Cohen, E.A. (2007). Requirements for the selective degradation of CD4 receptor molecules by the human immunodeficiency virus type 1 Vpu protein in the endoplasmic reticulum. Retrovirology *4*, 75.

Bogerd, H.P., and Cullen, B.R. (2008). Single-stranded RNA facilitates nucelocapsid:APOBEC3G complex formation. RNA *14*, 1228–1236.

Bolton, D.L., and Lenardo, M.J. (2007). Vpr cytopathicity independent of G2/M cell cycle arrest in human immunodeficiency virus type 1-infected CD4+ T cells. J. Virol. *81*, 8878–8890.

Bour, S., and Strebel, K. (1996). The human immunodeficiency virus (HIV) type 2 envelope protein is a functional complement to HIV type 1 Vpu that enhances particle release of heterologous retroviruses. J. Virol. *70*, 8285–8300.

Brass, A.L., Dykxhoorn, D.M., Benita, Y., Yan, N., Engelman, A., Xavier, R.J., Lieberman, J., and Elledge, S.J. (2008). Identification of host proteins required for HIV infection through a functional genomic screen. Science *319*, 921–926.

Brenner, M., Munch, J., Schindler, M., Wildum, S., Stolte, N., Stahl-Hennig, C., Fuchs, D., Matz-Rensing, K., Franz, M., Heeney, J., et al. (2006). Importance of the N-distal AP-2 binding element in Nef for simian immunodeficiency virus replication and pathogenicity in rhesus macaques. J. Virol. *80*, 4469–4481.

Chaudhuri, R., Lindwasser, O.W., Smith, W.J., Hurley, J.H., and Bonifacino, J.S. (2007). Downregulation of CD4 by human immunodeficiency virus type 1 Nef is dependent on clathrin and involves direct interaction of Nef with the AP2 clathrin adaptor. J. Virol. *81*, 3877–3890.

Chen, H., Lilley, C.E., Yu, Q., Lee, D.V., Chou, J., Narvaiza, I., Landau, N.R., and Weitzman, M.D. (2006). APOBEC3A is a potent inhibitor of adeno-associated virus and retrotransposons. Curr. Biol. *16*, 480–485.

Chiu, Y.L., Soros, V.B., Kreisberg, J.F., Stopak, K., Yonemoto, W., and Greene, W.C. (2005). Cellular APOBEC3G restricts HIV-1 infection in resting CD4+ T cells. Nature *435*, 108–114.

Chiu, Y.L., Witkowska, H.E., Hall, S.C., Santiago, M., Soros, V.B., Esnault, C., Heidmann, T., and Greene, W.C. (2006). High-molecular-mass APOBEC3G complexes restrict Alu retrotransposition. Proc. Natl. Acad. Sci. USA *103*, 15588–15593.

Conticello, S.G., Thomas, C.J., Petersen-Mahrt, S.K., and Neuberger, M.S. (2005). Evolution of the AID/APOBEC family of polynucleotide (deoxy)cytidine deaminases. Mol. Biol. Evol. *22*, 367–377.

Deacon, N.J., Tsykin, A., Solomon, A., Smith, K., Ludford-Menting, M., Hooker, D.J., McPhee, D.A., Greenway, A.L., Ellett, A., Chatfield, C., et al. (1995). Genomic structure of an attenuated quasi species of HIV-1 from a blood transfusion donor and recipients. Science *270*, 988–991.

Dehart, J.L., and Planelles, V. (2008). Human immunodeficiency virus type 1 Vpr links proteasomal degradation and checkpoint activation. J. Virol. *82*, 1066–1072.

DeHart, J.L., Zimmerman, E.S., Ardon, O., Monteiro-Filho, C.M., Arganaraz, E.R., and Planelles, V. (2007). HIV-1 Vpr activates the G2 checkpoint through manipulation of the ubiquitin proteasome system. Virol. J. *4*, 57.

Derse, D., Hill, S.A., Princler, G., Lloyd, P., and Heidecker, G. (2007). Resistance of human T cell leukemia virus type 1 to APOBEC3G restriction is mediated by elements in nucleocapsid. Proc. Natl. Acad. Sci. USA *104*, 2915–2920.

Esnault, C., Heidmann, O., Delebecque, F., Dewannieux, M., Ribet, D., Hance, A.J., Heidmann, T., and Schwartz, O. (2005). APOBEC3G cytidine deaminase inhibits retrotransposition of endogenous retroviruses. Nature *433*, 430–433.

Fenard, D., Yonemoto, W., de Noronha, C., Cavrois, M., Williams, S.A., and Greene, W.C. (2005). Nef is physically recruited into the immunological synapse and potentiates T cell activation early after TCR engagement. J. Immunol. *175*, 6050–6057.

Gaddis, N.C., Sheehy, A.M., Ahmad, K.M., Swanson, C.M., Bishop, K.N., Beer, B.E., Marx, P.A., Gao, F., Bibollet-Ruche, F., Hahn, B.H., et al. (2004). Further investigation of simian immunodeficiency virus Vif function in human cells. J. Virol. *78*, 12041–12046.

Gallois-Montbrun, S., Holmes, R.K., Swanson, C.M., Fernández-Ocaña, M., Byers, H.L., Ward, M.A., and Malim, M.H. (2008). Comparison of cellular ribonucleoprotein complexes associated with the APOBEC3F and APOBEC3G antiviral proteins. J. Virol. *82*, 5636–5642.

Gallois-Montbrun, S., Kramer, B., Swanson, C.M., Byers, H., Lynham, S., Ward, M., and Malim, M.H. (2007). Antiviral protein APOBEC3G localizes to ribonucleoprotein complexes found in P bodies and stress granules. J. Virol. *81*, 2165–2178.

Geleziunas, R., Xu, W., Takeda, K., Ichijo, H., and Greene, W.C. (2001). HIV-1 Nef inhibits ASK1-dependent death signalling providing a potential mechanism for protecting the infected host cell. Nature *410*, 834–838.

Goh, W.C., Rogel, M.E., Kinsey, C.M., Michael, S.F., Fultz, P.N., Nowak, M.A., Hahn, B.H., and Emerman, M. (1998). HIV-1 Vpr increases viral expression by manipulation of the cell cycle: a mechanism for selection of Vpr in vivo. Nat. Med. *4*, 65–71.

Gonzalez, M.E., and Carrasco, L. (2003). Viroporins. FEBS Lett. *552*, 28–34.

Gordon, S.N., Dunham, R.M., Engram, J.C., Estes, J., Wang, Z., Klatt, N.R., Paiardini, M., Pandrea, I.V., Apetrei, C., Sodora, D.L., et al. (2008). Short-lived infected cells support virus replication in naturally SIV-infected sooty mangabeys: implications for AIDS pathogenesis. J. Virol. *82*, 3725–3735.

Gottlinger, H.G., Dorfman, T., Cohen, E.A., and Haseltine, W.A. (1993). Vpu protein of human immunodeficiency virus type 1 enhances the release of capsids produced by gag gene constructs of widely divergent retroviruses. Proc. Natl. Acad. Sci. USA *90*, 7381–7385.

Goujon, C., Riviere, L., Jarrosson-Wuilleme, L., Bernaud, J., Rigal, D., Darlix, J.L., and Cimarelli, A. (2007). SIVSM/HIV-2 Vpx proteins promote retroviral escape from a proteasome-dependent restriction pathway present in human dendritic cells. Retrovirology *4*, 2.

Goulder, P.J., and Watkins, D.I. (2004). HIV and SIV CTL escape: implications for vaccine design. Nat. Rev. Immunol. *4*, 630–640.

Gummuluru, S., Kinsey, C.M., and Emerman, M. (2000). An in vitro rapid-turnover assay for human immunodeficiency virus type 1 replication selects for cell-to-cell spread of virus. J. Virol. *74*, 10882–10891.

Haller, C., Rauch, S., Michel, N., Hannemann, S., Lehmann, M.J., Keppler, O.T., and Fackler, O.T. (2006). The HIV-1 pathogenicity factor Nef interferes with maturation of stimulatory T-lymphocyte contacts by modulation of N-Wasp activity. J. Biol. Chem. *281*, 19618–19630.

Harris, R.S., and Liddament, M.T. (2004). Retroviral restriction by APOBEC proteins. Nat. Rev. Immunol. *4*,, 868–877.

Holmes, R.K., Koning, F.A., Bishop, K.N., and Malim, M.H. (2007a). APOBEC3F can inhibit the accumulation of HIV-1 reverse transcription products in the absence of hypermutation. Comparisons with APOBEC3G. J. Biol. Chem. *282*, 2587–2595.

Holmes, R.K., Malim, M.H., and Bishop, K.N. (2007b). APOBEC-mediated viral restriction: not simply editing? Trends Biochem. Sci. *32*, 118–128.

Horvath, C.M. (2004). Weapons of STAT destruction. Interferon evasion by paramyxovirus V protein. Eur. J. Biochem. *271*, 4621–4628.

Howe, A.Y., Jung, J.U., and Desrosiers, R.C. (1998). Zeta chain of the T-cell receptor interacts with nef of simian immunodeficiency virus and human immunodeficiency virus type 2. J. Virol. *72*, 9827–9834.

Hrecka, K., Gierszewska, M., Srivastava, S., Kozaczkiewicz, L., Swanson, S.K., Florens, L., Washburn, M.P., and Skowronski, J. (2007). Lentiviral Vpr usurps Cul4–DDB1[VprBP] E3 ubiquitin ligase to modulate cell cycle. Proc. Natl. Acad. Sci. USA *104*, 11778–11783.

Huang, J., Liang, Z., Yang, B., Tian, H., Ma, J., and Zhang, H. (2007). Derepression of microRNA-mediated protein translation inhibition by apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like 3G (APOBEC3G) and its family members. J. Biol. Chem. *282*, 33632–33640.

Huthoff, H., and Malim, M.H. (2007). Identification of amino acid residues in APOBEC3G required for regulation by human immunodeficiency virus type 1 Vif and Virion encapsidation. J. Virol. *81*, 3807–3815.

Iwatani, Y., Chan, D.S., Wang, F., Maynard, K.S., Sugiura, W., Gronenborn, A.M., Rouzina, I., Williams, M.C., Musier-Forsyth, K., and Levin, J.G. (2007). Deaminase-independent inhibition of HIV-1 reverse transcription by APOBEC3G. Nucleic Acids Res. *35*, 7096–7108.

Janini, M., Rogers, M., Birx, D.R., and McCutchan, F.E. (2001). Human immunodeficiency virus type 1 DNA sequences genetically damaged by hypermutation are often abundant in patient peripheral blood mononuclear cells and may be generated during near-simultaneous infection and activation of CD4(+) T cells. J. Virol. *75*, 7973–7986.

Jarmuz, A., Chester, A., Bayliss, J., Gisbourne, J., Dunham, I., Scott, J., and Navaratnam, N. (2002). An anthropoid-specific locus of orphan C to U RNA-editing enzymes on chromosome 22. Genomics *79*, 285–296.

Jern, P., Stoye, J.P., and Coffin, J.M. (2007). Role of APOBEC3 in genetic diversity among endogenous murine leukemia viruses. PLoS Genet. *3*, 2014–2022.

Kaiser, S.M., and Emerman, M. (2006). Uracil DNA glycosylase is dispensable for human immunodeficiency virus type 1 replication and does not contribute to the antiviral effects of the cytidine deaminase Apobec3G. J. Virol. *80*, 875–882.

Keele, B.F., Giorgi, E.E., Salazar-Gonzalez, J.F., Decker, J.M., Pham, K.T., Salazar, M.G., Sun, C., Grayson, T., Wang, S., Li, H., et al. (2008). Identification and Characterization of Transmitted and Early Founder Virus Envelopes in Primary HIV-1 Infection. Proc. Natl. Acad. Sci. USA, in press. Published online May 19, 2008. 10.1073/pnas.0802203105.

Kozak, S.L., Marin, M., Rose, K.M., Bystrom, C., and Kabat, D. (2006). The anti-HIV-1 editing enzyme APOBEC3G binds HIV-1 RNA and messenger RNAs that shuttle between polysomes and stress granules. J. Biol. Chem. *281*, 29105–29119.

Kupzig, S., Korolchuk, V., Rollason, R., Sugden, A., Wilde, A., and Banting, G. (2003). Bst-2/HM1.24 is a raft-associated apical membrane protein with an unusual topology. Traffic *4*, 694–709.

Lai, M., Zimmerman, E.S., Planelles, V., and Chen, J. (2005). Activation of the ATR pathway by human immunodeficiency virus type 1 Vpr involves its direct binding to chromatin in vivo. J. Virol. *79*, 15443–15451.

Lama, J., Mangasarian, A., and Trono, D. (1999). Cell-surface expression of CD4 reduces HIV-1 infectivity by blocking Env incorporation in a Nef- and Vpu-inhibitable manner. Curr. Biol. *9*, 622–631.

Langlois, M.A., and Neuberger, M.S. (2008). Human APOBEC3G can restrict retroviral infection in avian cells and acts independently of both UNG and SMUG1. J. Virol. *82*, 4660–4664.

Le Rouzic, E., Belaidouni, N., Estrabaud, E., Morel, M., Rain, J.C., Transy, C., and Margottin-Goguet, F. (2007). HIV1 Vpr arrests the cell cycle by recruiting DCAF1/VprBP, a receptor of the Cul4–DDB1 ubiquitin ligase. Cell Cycle *6*, 182–188.

Lewis, M.J., Balamurugan, A., Ohno, A., Kilpatrick, S., Ng, H.L., and Yang, O.O. (2008). Functional Adaptation of Nef to the Immune Milieu of HIV-1 Infection In Vivo. J. Immunol. *180*, 4075–4081.

Liu, B., Sarkis, P.T., Luo, K., Yu, Y., and Yu, X.F. (2005). Regulation of Apobec3F and human immunodeficiency virus type 1 Vif by Vif-Cul5-ElonB/C E3 ubiquitin ligase. J. Virol. *79*, 9579–9587.

Lubben, N.B., Sahlender, D.A., Motley, A.M., Lehner, P.J., Benaroch, P., and Robinson, M.S. (2007). HIV-1 Nef-induced down-regulation of MHC class I requires AP-1 and clathrin but not PACS-1 and is impeded by AP-2. Mol. Biol. Cell *18*, 3351–3365.

Margottin, F., Bour, S.P., Durand, H., Selig, L., Benichou, S., Richard, V., Thomas, D., Strebel, K., and Benarous, R. (1998). A novel human WD protein, h-beta TrCp, that interacts with HIV-1 Vpu connects CD4 to the ER degradation pathway through an F-box motif. Mol. Cell *1*, 565–574.

Mbisa, J.L., Barr, R., Thomas, J.A., Vandegraaff, N., Dorweiler, I.J., Svarovskaia, E.S., Brown, W.L., Mansky, L.M., Gorelick, R.J., Harris, R.S., et al. (2007). Human immunodeficiency virus type 1 cDNAs produced in the presence of APOBEC3G exhibit defects in plus-strand DNA transfer and integration. J. Virol. *81*, 7099–7110.

Mehle, A., Goncalves, J., Santa-Marta, M., McPike, M., and Gabuzda, D. (2004). Phosphorylation of a novel SOCS-box regulates assembly of the HIV-1 Vif-Cul5 complex that promotes APOBEC3G degradation. Genes Dev. *18*, 2861–2866.

Mehle, A., Thomas, E.R., Rajendran, K.S., and Gabuzda, D. (2006). A zinc-binding region in Vif binds Cul5 and determines cullin selection. J. Biol. Chem. *281*, 17259–17265.

Meusser, B., and Sommer, T. (2004). Vpu-mediated degradation of CD4 reconstituted in yeast reveals mechanistic differences to cellular ER-associated protein degradation. Mol. Cell *14*, 247–258.

Miyagi, E., Opi, S., Takeuchi, H., Khan, M., Goila-Gaur, R., Kao, S., and Strebel, K. (2007). Enzymatically active APOBEC3G is required for efficient inhibition of human immunodeficiency virus type 1. J. Virol. *81*, 13346–13353.

Muthumani, K., Choo, A.Y., Premkumar, A., Hwang, D.S., Thieu, K.P., Desai, B.M., and Weiner, D.B. (2005). Human immunodeficiency virus type 1 (HIV-1) Vpr-regulated cell death: insights into mechanism. Cell Death Differ. *12* (*Suppl 1*), 962–970.

Neil, S.J., Eastman, S.W., Jouvenet, N., and Bieniasz, P.D. (2006). HIV-1 Vpu promotes release and prevents endocytosis of nascent retrovirus particles from the plasma membrane. PLoS Pathog. *2*, e39.

Neil, S.J., Sandrin, V., Sundquist, W.I., and Bieniasz, P.D. (2007). An interferon-alpha-induced tethering mechanism inhibits HIV-1 and Ebola virus particle release but is counteracted by the HIV-1 Vpu protein. Cell Host Microbe *2*, 193–203.

Neil, S.J., Zang, T., and Bieniasz, P.D. (2008). Tetherin inhibits retrovirus release and is antagonized by HIV-1 Vpu. Nature *451*, 425–430.

Newman, E.N., Holmes, R.K., Craig, H.M., Klein, K.C., Lingappa, J.R., Malim, M.H., and Sheehy, A.M. (2005). Antiviral function of APOBEC3G can be dissociated from cytidine deaminase activity. Curr. Biol. *15*, 166–170.

Noviello, C.M., Benichou, S., and Guatelli, J.C. (2008). Cooperative binding of the class I major histocompatibility complex cytoplasmic domain and human immunodeficiency virus type 1 Nef to the endosomal AP-1 complex via its mu subunit. J. Virol. *82*, 1249–1258.

Pandrea, I., Ribeiro, R.M., Gautam, R., Gaufin, T., Pattison, M., Barnes, M., Monjure, C., Stoulig, C., Dufour, J., Cyprian, W., et al. (2008). Simian Immunodeficiency Virus SIVagm Dynamics in African Green Monkeys. J. Virol. *82*, 3713–3724.

Petroski, M.D., and Deshaies, R.J. (2005). Function and regulation of cullin-RING ubiquitin ligases. Nat. Rev. Mol. Cell Biol. *6*, 9–20.

Pizzato, M., Helander, A., Popova, E., Calistri, A., Zamborlini, A., Palu, G., and Gottlinger, H.G. (2007). Dynamin 2 is required for the enhancement of HIV-1 infectivity by Nef. Proc. Natl. Acad. Sci. USA *104*, 6812–6817.

Roeth, J.F., and Collins, K.L. (2006). Human immunodeficiency virus type 1 Nef: adapting to intracellular trafficking pathways. Microbiol. Mol. Biol. Rev. *70*, 548–563.

Roeth, J.F., Williams, M., Kasper, M.R., Filzen, T.M., and Collins, K.L. (2004). HIV-1 Nef disrupts MHC-I trafficking by recruiting AP-1 to the MHC-I cytoplasmic tail. J. Cell Biol. *167*, 903–913.

Rollason, R., Korolchuk, V., Hamilton, C., Schu, P., and Banting, G. (2007). Clathrin-mediated endocytosis of a lipid-raft-associated protein is mediated through a dual tyrosine motif. J. Cell Sci. *120*, 3850–3858.

Ross, T.M., Oran, A.E., and Cullen, B.R. (1999). Inhibition of HIV-1 progeny virion release by cell-surface CD4 is relieved by expression of the viral Nef protein. Curr. Biol. *9*, 613–621.

Russell, R.A., and Pathak, V.K. (2007). Identification of two distinct human immunodeficiency virus type 1 Vif determinants critical for interactions with human APOBEC3G and APOBEC3F. J. Virol. *81*, 8201–8210.

Sakai, K., Dimas, J., and Lenardo, M.J. (2006). The Vif and Vpr accessory proteins independently cause HIV-1-induced T cell cytopathicity and cell cycle arrest. Proc. Natl. Acad. Sci. USA *103*, 3369–3374.

Sawyer, S.L., Emerman, M., and Malik, H.S. (2004). Ancient adaptive evolution of the primate antiviral DNA-editing enzyme APOBEC3G. PLoS Biol. *2*, E275.

Sawyer, S.L., and Malik, H.S. (2006). Positive selection of yeast nonhomologous end-joining genes and a retrotransposon conflict hypothesis. Proc. Natl. Acad. Sci. USA *103*, 17614–17619.

Schindler, M., Munch, J., Kutsch, O., Li, H., Santiago, M.L., Bibollet-Ruche, F., Muller-Trutwin, M.C., Novembre, F.J., Peeters, M., Courgnaud, V., et al. (2006). Nef-mediated suppression of T cell activation was lost in a lentiviral lineage that gave rise to HIV-1. Cell *125*, 1055–1067.

Schrofelbauer, B., Hakata, Y., and Landau, N.R. (2007). HIV-1 Vpr function is mediated by interaction with the damage-specific DNA-binding protein DDB1. Proc. Natl. Acad. Sci. USA *104*, 4130–4135.

Schubert, U., Bour, S., Ferrer-Montiel, A.V., Montal, M., Maldarell, F., and Strebel, K. (1996). The two biological activities of human immunodeficiency virus type 1 Vpu protein involve two separable structural domains. J. Virol. *70*, 809–819.

Schumacher, A.J., Hache, G., Macduff, D.A., Brown, W.L., and Harris, R.S. (2008). The DNA deaminase activity of human APOBEC3G is required for Ty1, MusD, and human immunodeficiency virus type 1 restriction. J. Virol. *82*, 2652–2660.

Sharova, N., Wu, Y., Zhu, X., Stranska, R., Kaushik, R., Sharkey, M., and Stevenson, M. (2008). Primate lentiviral Vpx commandeers DDB1 to counteract a macrophage restriction. PLoS Pathog. *4*, e1000057.

Sheehy, A.M., Gaddis, N.C., Choi, J.D., and Malim, M.H. (2002). Isolation of a human gene that inhibits HIV-1 infection and is suppressed by the viral Vif protein. Nature *418*, 646–650.

Simon, V., Zennou, V., Murray, D., Huang, Y., Ho, D.D., and Bieniasz, P.D. (2005). Natural variation in Vif: differential impact on APOBEC3G/3F and a potential role in HIV-1 diversification. PLoS Pathog. *1*, e6.

Soros, V.B., Yonemoto, W., and Greene, W.C. (2007). Newly synthesized APOBEC3G is incorporated into HIV virions, inhibited by HIV RNA, and subsequently activated by RNase H. PLoS Pathog. *3*, e15.

Specht, A., Degottardi, M.Q., Schindler, M., Hahn, B., Evans, D.T., and Kirchhoff, F. (2008). Selective downmodulation of HLA-A and -B by Nef alleles from different groups of primate lentiviruses. Virology *373*, 229–237.

Srivastava, S., Swanson, S.K., Manel, N., Florens, L., Washburn, M.P., and Skowronski, J. (2008). Lentiviral Vpx accessory factor targets VprBP/DCAF1 substrate adaptor for Cullin 4 E3 ubiquitin ligase to enable macrophage infection. PLoS Pathog. *4*, e1000059.

Swanson, C.M., and Malim, M.H. (2008). SnapShot: HIV-1 Proteins. Cell *133*, 742.

Swigut, T., Alexander, L., Morgan, J., Lifson, J., Mansfield, K.G., Lang, S., Johnson, R.P., Skowronski, J., and Desrosiers, R. (2004). Impact of Nef-mediated downregulation of major histocompatibility complex class I on immune response to simian immunodeficiency virus. J. Virol. *78*, 13335–13344.

Tan, L., Ehrlich, E., and Yu, X.F. (2007). DDB1 and Cul4A are required for human immunodeficiency virus type 1 Vpr-induced G2 arrest. J. Virol. *81*, 10822–10830.

Thielen, B.K., Klein, K.C., Walker, L.W., Rieck, M., Buckner, J.H., Tomblingson, G.W., and Lingappa, J.R. (2007). T cells contain an RNase-insensitive inhibitor of APOBEC3G deaminase activity. PLoS Pathog. *3*, 1320–1334.

Thoulouze, M.I., Sol-Foulon, N., Blanchet, F., Dautry-Varsat, A., Schwartz, O., and Alcover, A. (2006). Human immunodeficiency virus type-1 infection impairs the formation of the immunological synapse. Immunity *24*, 547–561.

Tian, C., Yu, X., Zhang, W., Wang, T., Xu, R., and Yu, X.F. (2006). Differential requirement for conserved tryptophans in human immunodeficiency virus type 1 Vif for the selective suppression of APOBEC3G and APOBEC3F. J. Virol. *80*, 3112–3115.

Towers, G.J. (2007). The control of viral infection by tripartite motif proteins and cyclophilin A. Retrovirology *4*, 40.

Van Damme, N., Goff, D., Katsura, C., Jorgenson, R.L., Mitchell, R., Johnson, M.C., Stephens, E.B., and Guatelli, J.C. (2008). The interferon-induced protein BST-2 restricts HIV-1 release and Is downregulated from the cell surface by the viral Vpu protein. Cell Host Microbe *3*, 1–8.

Vartanian, J.-P., Guétard, D., Henry, M., and Wain-Hobson, S. (2008). Evidence for editing of human papillomavirus DNA by APOBEC3 in benign and precancerous lesions. Science *320*, 230–233.

Varthakavi, V., Smith, R.M., Bour, S.P., Strebel, K., and Spearman, P. (2003). Viral protein U counteracts a human host cell restriction that inhibits HIV-1 particle production. Proc. Natl. Acad. Sci. USA *100*, 15154–15159.

Varthakavi, V., Smith, R.M., Martin, K.L., Derdowski, A., Lapierre, L.A., Goldenring, J.R., and Spearman, P. (2006). The pericentriolar recycling endosome plays a key role in Vpu-mediated enhancement of HIV-1 particle release. Traffic *7*, 298–307.

Wen, X., Duus, K.M., Friedrich, T.D., and de Noronha, C.M. (2007). The HIV1 protein Vpr acts to promote G2 cell cycle arrest by engaging a DDB1 and Cullin4A-containing ubiquitin ligase complex using VprBP/DCAF1 as an adaptor. J. Biol. Chem. *282*, 27046–27057.

Wichroski, M.J., Robb, G.B., and Rana, T.M. (2006). Human retroviral host restriction factors APOBEC3G and APOBEC3F localize to mRNA processing bodies. PLoS Pathog. *2*, e41.

Yamashita, M., and Emerman, M. (2005). The cell cycle independence of HIV infections is not determined by known karyophilic viral elements. PLoS Pathog. *1*, e18.

Yu, X., Yu, Y., Liu, B., Luo, K., Kong, W., Mao, P., and Yu, X.F. (2003). Induction of APOBEC3G ubiquitination and degradation by an HIV-1 Vif-Cul5-SCF complex. Science *302*, 1056–1060.

Yu, Y., Xiao, Z., Ehrlich, E.S., Yu, X., and Yu, X.F. (2004). Selective assembly of HIV-1 Vif-Cul5-ElonginB-ElonginC E3 ubiquitin ligase complex through a novel SOCS box and upstream cysteines. Genes Dev. *18*, 2867–2872.

Zimmerman, E.S., Chen, J., Andersen, J.L., Ardon, O., Dehart, J.L., Blackett, J., Choudhary, S.K., Camerini, D., Nghiem, P., and Planelles, V. (2004). Human immunodeficiency virus type 1 Vpr-mediated G2 arrest requires Rad17 and Hus1 and induces nuclear BRCA1 and gamma-H2AX focus formation. Mol. Cell. Biol. *24*, 9286–9294.

Zimmerman, E.S., Sherman, M.P., Blackett, J.L., Neidleman, J.A., Kreis, C., Mundt, P., Williams, S.A., Warmerdam, M., Kahn, J., Hecht, F.M., et al. (2006). Human immunodeficiency virus type 1 Vpr induces DNA replication stress in vitro and in vivo. J. Virol. *80*, 10407–10418.