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SIV Envelope Acquires a Nefarious Habit

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Deletion of the *nef* gene from macaque simian immunodeficiency virus (SIVmac) attenuates its ability to cause disease. Pathogenic viruses occasionally emerge in macaques infected with Nef-deleted SIVmac, with some genetic determinants mapping to the envelope (*env*) gene. An intriguing new study shows that these changes endow Env with a Nef-like ability to counteract tetherin/BST2 (Serra-Moreno et al., 2011).

Tetherin (bone marrow stromal cell antigen 2, BST2/CD317) is an interferon-induced membrane protein that inhibits the release of diverse mammalian enveloped virus particles by mediating their retention on the surface of infected cells (Evans et al., 2010). Tetherin dimers are proposed to partition into assembling virions and crosslink the nascent particle to the plasma membrane. The acquisition of countermeasures encoded by various mammalian viruses that target its function highlights the potential importance of tetherin in innate antiviral immunity.

The ability to counteract tetherin is conserved among primate immunodeficiency viruses (Figure 1) (Sauter et al., 2010). While HIV-1 employs its accessory protein Vpu to target human tetherin, many SIVs that do not encode a *vpu* gene harbor an analogous activity in their Nef protein. Nef, a multifunctional adaptor protein that mediates the downregulation of a host of cell surface immunoregulatory proteins, is critical for SIVs to replicate efficiently in vivo. Nef-deleted macaque simian immunodeficiency virus (SIVmac Δ*nef*) strains are attenuated in macaques but maintain a persistent low-level replication. Occasionally, however, SIVmacΔ*nef*-infected macaques progress to disease, and the reisolated virus is pathogenic (Alexander et al., 2003). The genetic changes associated with *pathogenic* SIVmac Δ*nef (ΔNefP)* include amino acid substitutions in the cytoplasmic tail of the gp41 subunit of the Env glycoprotein.

Tetherin antagonism by SIV Nef requires a G/DDIWK motif present in the cytoplasmic tails of primate tetherins (Sauter et al., 2010). This motif has been deleted in humans, and thus human tetherin is resistant to SIV/HIV Nef proteins. HIV-2, like SIVmac, is derived from the sooty mangabey SIV (SIVsm) that does not encode a *vpu* gene. Remarkably, in some laboratory isolates of HIV-2 and one human $CD4^+$ T cell-line passaged tantalus SIV (SIVtan), the envelope glycoprotein can antagonize human and primate tetherins (Gupta et al., 2009; Hauser et al., 2010; Le Tortorec and Neil, 2009). Like Vpu, HIV-2 Env downregulates surface tetherin levels and induces its accumulation in intracellular compartments, away from viral assembly sites on the plasma membrane. Env interacts with tetherin, determined by the extracellular domains of both proteins. However, this interaction is not sufficient; tetherin antagonism by HIV-2 Env is dependent both on proteolytic Env maturation to its mature subunits (gp105 and gp41) and a tyrosine-based sorting signal (GYXXΦ) that binds the clathrin adaptor AP2—and which is conserved in the cytoplasmic tails of HIV/SIV Envs. It is unclear how widespread tetherin antagonism is among primary HIV-2 isolates and whether the development of tetherin antagonism in Env was essential for the virus to adapt to its new host after cross-species transmission. Now, Serra-Moreno and coworkers demonstrate that in macaques there is evidence that tetherin *can* exert such a selective pressure on the closely related SIVmac Env and that this correlates with enhanced viral replication and pathogenesis in vivo (Serra-Moreno et al., 2011).

The genetic changes acquired by the SIVmac Δ*nefP* isolate are sufficient to confer the ability to antagonize rhesus tetherin to the virus (Serra-Moreno et al., 2011). Moreover, SIVmac Δ*nef* replication in macaque $CD4^+$ T cells displays greater sensitivity to type-1 interferon than wild-type SIVmac, and the Δ*NefP* adaptions reverse this. Since the *nef* gene is not reconstituted in Δ*NefP*, the authors go on to show that the resistance to rhesus tetherin was attributable to five amino acid changes in the cytoplasmic tail of Δ*NefP* Env. Δ*NefP* Env had an enhanced ability to interact with rhesus tetherin in coimmunoprecipitations, and like HIV-2 Env, antagonism of tetherin required the membrane-proximal GYXXΦ sorting signal. Δ*NefP* Env colocalized with tetherin in intracellular compartments, although surface downregulation in infected cells was minor, perhaps suggesting tetherin removal from the surface is not strictly required.

There are some key differences between Δ*NefP* Env-mediated tetherin antagonism and that by HIV-2 Env, implying that distinct adaptions in different parts of the Env molecule result in similar mechanisms. First, aside from the membrane-proximal amino acids that include the GYXXΦ motif, the gp41 cytoplasmic tail is dispensable for HIV-2; here, it is the fundamental determinant of tetherin antagonism by Δ*NefP* Env. Remarkably, grafting the Δ*NefP* gp41 tail onto a heterologous membrane protein (CD4) endows the chimera with the ability to bind tetherin and counteract restriction. Second, unlike HIV-2 and SIVtan Envs, which target human *and* primate tetherins through a determinant in their extracellular coiled-coil domains (Gupta et al., 2009; Hauser et al., 2010; Le Tortorec and Neil, 2009), Δ*NefP* Env is specific for rhesus tetherin. While Δ*NefP* Env does not require the G/DDIWK patch in the cytoplasmic tail of tetherin that is deleted in humans, it does depend on other primate species-specific residues flanking the tetherin's dual tyrosine endocytic motif. Rhesus tetherin is polymorphic, and recent analysis of primate tetherins suggests that the cytoplasmic tail has been under heavy positive

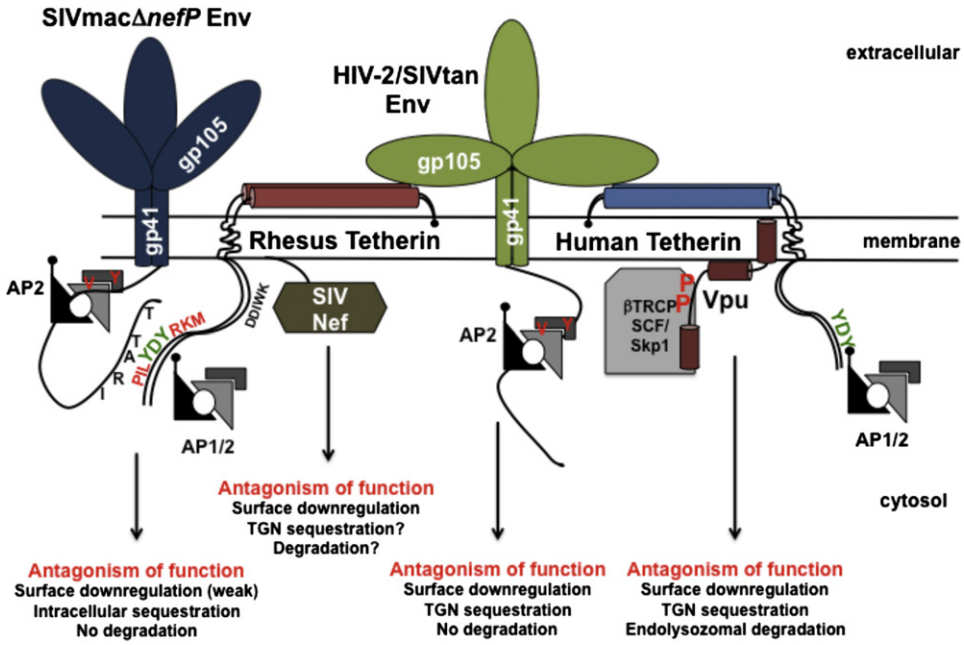

Figure 1. Multiple Mechanisms for Targeting Tetherin in Primate Immunodeficiency Viruses
The ability to counteract tetherin-mediated restriction of viral particle release is conserved among primate immunodeficiency viruses. Tetherin dimers cycle between the cell surface, trans-Golgi Network (TGN), and the endosomal network via clathrin adaptors AP1 and AP2. The majority of SIVs target their host species' tetherin through the accessory protein Nef, determined by a conserved G/DDIWK motif in primate tetherin cytoplasmic tails. This motif is absent in human tetherin, and it is thus resistant to all SIV Nefs. The HIV-1 Vpu has adapted to specifically target human tetherin through direct interaction between their respective transmembrane domains and mediates its TGN sequestration. Phosphorylation-dependent recruitment of a βTRCP2-SCF-Skp1 ubiquitin ligase complex to Vpu induces ubiquitylation of the tetherin cytoplasmic tail and its subsequent degradation. The HIV-2 Env interacts with primate and human tetherins through an undefined extracellular determinant and sequesters it in the TGN-dependent on an AP2-binding sorting signal in the gp41 cytoplasmic tail. Serra-Moreno et al. show that in SIVmac Δ*Nef* pathogenic revertant isolates, adaptive changes in Env gp41 cytoplasmic tail allow it to interact with rhesus tetherin dependent on species-specific residues (PIL and RKM motifs) flanking its YDY sorting signal. Downregulation and sequestration of tetherin away from viral assembly sites requires both an AP2-dependent sorting signal in Env and the AP1/2-dependent sorting signal in rhesus tetherin.

selection—potentially by viral tetherin countermeasures (Lim et al., 2010).

This study raises intriguing questions about the role tetherin plays in SIV/HIV pathogenesis and transmission. It is unlikely that adaption to tetherin by Δ*NefP* Env is itself the reason that this virus reverted to pathogenicity. Many SIVs, whose Nef proteins target tetherin, do not cause progressive disease in their natural hosts, and Δ*NefP* Env alone is insufficient to restore pathogenicity to parental SIVmacΔ*Nef* (Alexander et al., 2003). However, the potential growth advantage that adapting Env gave to the Δ*NefP* virus is likely to be an important contributing factor. Importantly, these data add significantly to the evidence that HIVs and SIVs are under a strong selective pressure to maintain a tetherin countermeasure. Is this simply due to tetherin inhibiting cell-free virion production, or is there a further underlying reason? Important questions to answer now revolve around how widespread is HIV-2/SIV Env-mediated counteraction of tetherin in infected individuals. HIV-2 infection displays a high incidence of long-term nonprogression as well as a limited geographical distribution (de Silva et al., 2008). Does co-opting Env for tetherin antagonism due to a lack of Nef function (either through its deletion or, as in HIV-2, the lack of a target sequence in human tetherin) interfere with its "normal" essential function in virus replication? And can similar adaptions occur in the HIV-1 Env when Vpu cannot counteract tetherin? Because Vpu-mediated antagonism of human tetherin is efficient in the pandemic HIV-1 group M but weaker or nonexistent in the nonpandemic groups N and O, respectively, some speculate that Vpu adaption to tetherin is associated with efficient human-to-human spread (Sauter et al., 2010). However, is there evidence of Env adaption in HIV-1 groups N or O that may compensate for lack of Vpu function? One laboratory isolate of HIV-1 group M (AD8) has been reported to have a Vpu-like activity associated with Env (Schubert et al., 1999). Whether this is a true tetherin antagonist remains to be determined.

The interactions of primate immunodeficiency viruses with mammalian antiviral factors, highlighted here by tetherin, continue to provide fascinating insights into the evolutionary fight for supremacy between pathogen and host, with direct relevance to human disease.

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RNA Virus Harnesses MicroRNAs to Seize Host Translation Control

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Picornaviruses have evolved elaborate strategies to subvert host translation. In this issue of *Cell Host and Microbe*, Ho et al. (2011) report that enterovirus infection induces the synthesis of a transcription factor that enhances the synthesis of microRNA-141, which suppresses translation of the cap-binding protein, eIF4E, mRNA to inhibit cap-dependent translation.

Viruses are unable to carry the huge amount of genetic information needed to synthesize ribosomal proteins and RNA. Thus, viruses have developed numerous ways to compete for the host translation machinery. All host mRNAs contain 5′ terminal cap structures. These terminal cap structures bind the cap-binding protein, eIF4E, which is part of a larger complex, termed eIF4F, which includes the RNA helicase eIF4A and the multisubunit complex eIF4G (Figure 1A). The eIF4F complex is thought to recruit the 40S ribosomal subunit to the mRNA. The 40S subunit scans the mRNA in a 5′-to-3′ direction until an appropriate start codon is encountered, at which point the 60S subunit joins and polypeptide synthesis begins (Sonenberg and Hinnebusch, 2009). Picornaviral mRNAs, on the other hand, do not contain 5′ cap structures. A large RNA structure in the viral 5′ noncoding region, termed internal ribosome entry site (IRES), recruits the 40S subunit. Consequently, IRES-mediated association of 40S subunits does not require eIF4E in most, but not all, cases (Belsham and Sonenberg, 1996). To effectively compete for host ribosomal subunits, picornaviruses employ several major strategies (Figure 1A). (1) For example, poliovirus infection induces the dephosphorylation of eIF4E binding proteins (4E-BPs) that sequester eIF4E, resulting in the inhibition of cap-dependent translation (Gingras et al., 1996). (2) Poliovirus-encoded protease 2A cleaves eIF4G. The proteolysis of eIF4G does not cause but enhances the inhibition of cap-dependent translation of host mRNAs (Gradi et al., 1998). (3) Both poliovirus-encoded 2A and 3C proteases cleave the polyadenosine binding protein (PABP), leading to reduced translation of both host and viral mRNAs late in infected cells (Kuyumcu-Martinez et al., 2004). (4) 2A and 3C proteases cleave host transcription factors, limiting newly transcribed mRNA species that are preferentially translated (Yalamanchili et al., 1997). Thus, it has been assumed that picornavirus-induced cytopathic effects can be explained by the inhibition of transcription and translation of host genes.

In this issue, Ho and colleagues challenge this view by presenting the remarkable finding that enterovirus 71 (EV71) infection results in the transcriptional induction of the early growth response 1 (EGR1) gene, a host transcription factor. EGR1 protein activates the transcription of a microRNA that represses eIF4E mRNA translation, leading to decreased abundance of eIF4E protein (Figure 1B).

The study by Ho et al. started with a straightforward question: Do microRNA abundances change during EV71 infection of human rhabdomyosarcoma cells? Because enterovirus infection inhibits host-cell transcription, one would expect downregulation of microRNAs or unaltered change in abundance of long-lived microRNAs in infected cells. While 248 microRNAs followed that predicted pattern, two microRNAs, miR-141 and miR-146a, were upregulated more than 15-fold in EV71-infected cells compared to uninfected cells (Ho et al., 2011).

Aided by target prediction programs and verified in reporter-expression assays, the authors showed that miR-141 targets eIF4E mRNA, leading to decreased abundance of eIF4E. Sequestration of miR-141 by anti-sense RNAs, so-called antagomirs, effectively rescued eIF4E protein abundance. This phenotype was completely reversed by siRNA-mediated depletion of eIF4E mRNA, suggesting that miR-141 directly regulates eIF4E mRNA expression (Ho et al., 2011). Interestingly, treatment of infected cells with miR-141 antagomir leads to a 1000-fold decrease in virus production at a time when eIF4G was completely cleaved