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# The role of BST2/tetherin in infection with the feline retroviruses

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## Abstract

The recently identified host restriction factor tetherin (BST-2, CD317) potently inhibits the release of nascent retrovirus particles from infected cells. Recently, we reported the identification and characterization of tetherin as a novel feline retroviral restriction factor. Based on homology to human tetherin we identified a putative tetherin gene in the genome of the domestic cat (*Felis catus*) which was found to be expressed in different feline cell lines both prior to and post treatment with either type I or type II interferon (IFN). The predicted structure of feline tetherin (feTHN) was that of a type II single-pass transmembrane protein encoding an N-terminal transmembrane anchor, central predicted coiled-coil bearing extracellular domain to promote dimerization, and a C-terminal GPI-anchor, consistent with conservation of structure between human and feline tetherin. FeTHN displayed potent inhibition of feline immunodeficiency virus (FIV) and human immunodeficiency virus type 1 (HIV-1) particle release in single-cycle replication assays. Notably, feTHN activity was resistant to antagonism by HIV-1 Vpu. However, stable ectopic expression of feTHN mRNA in different feline cell lines had no inhibitory effect on the growth of diverse primary or cell culture-adapted strains of FIV. Hence, whereas feline tetherin efficiently blocks viral particle release in single-cycle replication assays, it might not prevent dissemination of feline retroviruses *in vivo*.

## 1. Introduction

Feline immunodeficiency virus (FIV) is an important global lentiviral pathogen that infects both domestic and nondomestic felids (Brown et al., 1993, 1994; Carpenter et al., 1996; Hofmann-Lehmann et al., 1996; Troyer et al., 2004, 2005). FIV infection of domestic cats (*Felis catus*) results in a fatal immunodeficiency syndrome similar to AIDS in humans infected with human immunodeficiency virus (HIV) (Pedersen et al., 1987, 1989; Yamamoto et al., 1988; Pedersen, 1993; Bendinelli et al., 1995). The virus-induced gradual immunological deterioration leads to common clinical signs such as recurrent gingivitis and stomatitis, lymphoma, loss of condition (cachexia/wasting), neurological disorders and high

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mortality in infected cats (Pedersen et al., 1987; Hosie et al., 1989; Sparger et al., 1989; Yamamoto et al., 1989; Ackley et al, 1990; Torten et al., 1991; Callanan et al., 1992, 1996; Pedersen, 1993). Because of the high degree of similarity between the genomic organization, the mode of transmission and the pathology of HIV and FIV infections, the domestic cat has been established as the smallest natural animal model for studying the development of AIDS in humans and for evaluating potential intervention strategies (Willett et al,, 1997; Miller et al., 2000; Troyer et al., 2004).

The ability of retroviruses to initiate a complex array of interactions with host cell proteins and other factors is a critical determinant of cell tropism, successful replication and persistence within the host. The majority of these host-virus interactions are beneficial for the virus (Malim, 2009). In recent years, however, a group of intracellular proteins has been identified that specifically evolved to interfere with viral replication. These proteins are collectively called restriction factors and form a separate branch of the innate immunity termed intrinsic immunity (Bieniasz, 2004; Goff, 2004). Restriction factors affect almost all stages of the viral lifecycle (Bieniasz, 2004), such as uncoating, reverse transcription, nuclear entry and egress, and their cell-type and species-specific expression and activity control the viral host spectrum and may impose a barrier to cross-species transmission events (Troyer et al., 2008). In order to efficiently replicate and to evade immune surveillance, retroviruses have to overcome this line of defense and, thus, have evolved proteins that antagonize the actions of restriction factors or mechanisms to avoid them.

A better understanding of the interactions between host restriction factors and their viral antagonists will help to improve animal models for infection and to facilitate the identification of potential targets for antiviral therapies as well as retroviral gene delivery.

## 2. Restriction factors to retroviral replication

The longest (alpha) isoform of TRIM5, a member of the tripartite interaction motif family of proteins (Reymond et al., 2001, Stremlau et al., 2004), and APOBEC3 (apolipoprotein B mRNA-editing catalytic polypeptide 3) proteins, a family of cellular polynucleotide cysteine deaminases (Teng et al., 1993; Sheehy et al., 2002; Mangeat et al., 2003; Zhang et al., 2003), constitute the so-called early post-entry blocks to retroviral infection and have been well characterized in humans, non-human primates and domestic cats.

TRIM5α binds to the incoming retroviral capsid (CA) in the cytoplasm via its C-terminal PRY/SPRY (B30.2) domain (Mische et al., 2005; Sebastian and Luban, 2005; Stremlau et al., 2006; Langelier et al., 2008) and the resulting capsid/TRIM5α complex is incapable of completing reverse transcription (Keckesova et al., 2004; Stremlau et al., 2004). Instead, the N-terminal RBCC (RING, B-box and coiled coil) domain of TRIM5α possesses E3 ubiquitin ligase activity (RING) (Yamauchi et al., 2008) and ubiquination of the complex targets it for proteosome-mediated degradation (Diaz-Griffero et al., 2006; Towers, 2007). It has been proposed that TRIM5α may accelerate or abrogate viral uncoating (Stremlau et al., 2006) which not only inhibits reverse transcription but also nuclear import of viral cDNA (Berthoux et al., 2004; Wu et al, 2006). Previously, we reported that the TRIM5 transcript in

cat cells possesses a truncation in the B30.2 capsid binding domain, which ablates its restrictive function (McEwan et al., 2009).

The antiviral activity of APOBEC3 proteins was discovered through the study of the HIV-1 accessory protein Vif (viral infectivity factor) (Wolf and Goff, 2008) which was shown to be dispensable for viral replication in certain permissive cell lines such as CEM-SS and SupT1, but absolutely required in non-permissive cells such as primary CD4+ T cells, monocyte-derived macrophages, and some T cell leukemia lines such as CEM (Fisher et al., 1987; Strebel et al., 1987; Gabuzda et al., 1992; Sakai et al., 1993; Sova and Volsky, 1993). The human APOBEC3G protein (A3G; initially called CEM-15) was identified as the responsible cellular factor whose expression renders human cells non-permissive for infection by HIV-1 strains devoid of the Vif gene, but not by Vif-proficient HIV-1 strains (Sheehy et al., 2002). A3G belongs to a large family of cytosine deaminases (reviewed in Harris and Liddament, 2004; Conticello et al., 2007; Holmes et al., 2007; Aguiar and Peterlin, 2008; Conticello, 2008; Goila-Gaur and Strebel, 2008) that catalyze the hydrolysis of cytosines to uracils. In order to carry out its anti-viral activity, A3G has to be packaged into Vif-deficient virions as they are formed in producer cells (Sheehy et al., 2002; Harris et al., 2003; Lecossier et al., 2003; Mangeat et al., 2003; Zhang et al., 2003). A3G is then carried to the target cell, where it, upon initiation of reverse transcription, deaminates cytosine residues in nascent retroviral minus-strand cDNA to uracils. Subsequently, the uracils function as a template for the incorporation of plus-strand adenines resulting in guanine to adenine hypermutations in the viral genome that critically affect viability and infectivity of the virus (Harris et al., 2003; Mangeat et al., 2003; Zhang et al., 2003; Bishop et al., 2004; Liddament et al., 2004; Zheng et al., 2004). Recent studies propose that, in addition to deamination, deamination-independent mechanisms of A3G to inhibit viral replication exist (Shindo et al., 2003; Newman et al., 2005; Guo et al., 2006, 2007; Iwatani et al., 2006, 2007; Opi et al., 2006; Bishop et al., 2006; Holmes et al., 2007; Li et al., 2007; Yang et al., 2007). These affect multiple stages of the reverse transcription and collectively impair the accumulation of reverse transcription products (Mangeat et al., 2003, Guo et al., 2006, 2007; Iwatani et al., 2007; Li et al., 2007; Luo et al., 2007; Mbisa et al., 2007).

The primary role of Vif is to prevent A3G incorporation into virions. It targets A3G for proteasome-mediated degradation (Conticello et al., 2003; Marin et al., 2003; Sheehy et al., 2003; Stopak et al., 2003; Liu et al., 2004, 2005; Mehle et al., 2004a, 2004b) by bridging an interaction between A3G and a ubiquitin E3 ligase complex consisting of elongins B and C, cullin 5 and ring-box-1 (Yu et al., 2003; Yu et al., 2004; Mehle et al., 2004b, Bergeron, 2010). The interaction between A3G and Vif is species-specific and partly determines the host range of a virus (Hatziioannou et al., 2006).

Several APOBEC3 genes have recently been identified and characterized in the genome of domestic cats (Münk et al., 2008). The A3 gene locus encodes three highly similar A3C (A3Z2) genes and an A3H (A3Z3) gene. Additionally, a fifth transcript, which is generated by read-through alternative splicing, encodes the protein A3CH (A3Z2-Z3) (Münk et al., 2008; Zielonka et al., 2010). The feline A3 proteins display different degrees of activity against feline retroviruses. Feline A3C proteins inhibit the replication of Bet-deficient feline foamy virus (FeFV) but do not restrict Vif-deficient FIV or feline leukemia virus (FeLV). In

contrast, feline A3H and A3CH proteins are active against Vifdeficient FIV as well as FeLV but not against Bet-deficient FeFV (Löchelt et al., 2005; Münk et al., 2008). Feline A3 proteins are overcome by the FIV Vif and the FeFV Bet protein (Löchelt et al., 2005; Münk et al., 2008; Stern et al., 2010; Zielonka et al., 2010).

In addition to the early post-entry blocks, restriction factors such as tetherin contribute to a late block to retroviral replication in that they prevent the release of mature enveloped viral particles from the membranes of infected cells. Tetherin (also called HM1.24/BST-2/CD317) was originally identified as a bone marrow stromal cell surface antigen selectively expressed on terminally differentiated normal and neoplastic human B cells and corresponding cell lines (Goto et al., 1994, Ishikawa et al., 1995). Several studies have shown that tetherins are novel type II transmembrane proteins with a molecular weight of 30-36 kDa (Ishikawa et al., 1995; Ohtomo et al., 1999, Kupzig et al., 2003). They harbour an N-terminal cytoplasmic tail, followed by a transmembrane domain, an extracellular parallel, dimeric, alpha-helical coiled coil domain and a C-terminal glycosyl-phosphatidylinositol (GPI) anchor (Ishikawa et al., 1995; Ohtomo et al., 1999; Kupzig et al., 2003, Rollason et al., 2007; Hinz et al., 2010). Two potential N-linked glycosylation sites and three conserved cysteine residues are present in the extracellular domain (Ishikawa et al., 1995; Ohtomo et al., 1999; Kupzig et al., 2003). Heterogeneous glycosylation of tetherin has been shown to be essential for efficient secretion and folding (Andrew et al., 2009; Goffinet et al., 2009; Kaletsky et al., 2009; McNatt et al., 2009; Miyagi et al., 2009; Perez-Caballero et al., 2009). The cysteines take part in intra- and intermolecular disulfide bond formation and enable the homodimerization of tetherins (Ohtomo et al., 1999, Kupzig et al., 2003; Perez-Caballero et al., 2009). The GPI-modification causes tetherin to partition into and cross-link cholesterol- and sphingolipid-rich microdomains in the plasma membrane (Simons and Ikonen, 2000; Simons and Toomre, 2000, Kupzig et al., 2003). Tetherin cycles between the lipid rafts on the cell surface and an intracellular pool where it localizes predominantly to the Golgi apparatus, the trans-Golgi network (TGN) and recycling endosomes (Kupzig et al., 2003). Internalization from the plasma membrane is mediated by clathrin-dependent endocytosis (Rollason et al., 2007; Masuyama et al., 2009).

The antiviral activity of tetherin was not discovered until 2008, when it was noted that its cell-type specific expression matched closely the dependency of HIV-1 on the accessory protein Vpu (viral protein U) for virus release from certain human cell lines (Strebel et al., 1989; Terwilliger et al., 1989; Klimkait et al., 1990; Varthakavi et al., 2003; Neil et al., 2008; Van Damme et al., 2008). Tetherin is constitutively expressed in human cell lines such HeLa cells (Gottinger et al., 1993), several cancer cell lines (Ohtomo et al., 1999), B cells, T cells, monocytes, macrophages and plasmacytoid dendritic cells (Vidal-Laliena et al., 2005; Blasius et al., 2006; Miyagi et al., 2009) and its expression is induced or enhanced by type I and type II interferons (IFN) in cell lines such as HOS, 293T, HT1080 cells (Neil et al., 2006, 2007, 2008; Van Damme et al., 2008; Miyagi et al., 2009). Interferon treatment renders cell lines that do not normally require Vpu for efficient virus release Vpu-dependent (Neil et al., 2007).

Tetherin causes the retention of fully formed, mature virions on the surface of cells infected with Vpu-deficient HIV-1 (Neil et al., 2008; Van Damme et al., 2008). At the expense of

particle release, virions accumulate at the cell surface and a fraction of them are endocytosed via a clathrin-dependent mechanism and degraded (Neil et al., 2006, 2007, Miyakawa et al., 2009). Current models predict that tetherin is present at sites of particle assembly in the cell membrane and is incorporated into virions (Perez-Caballero et al., 2009; Fitzpatrick et al., 2010). Presumably, one end of tetherin embeds in the lipid bilayer of the cell and the other in that of the virion, so that cell-surface tetherin homodimerizes with virion-associated tetherin via disulfide bonds or via coiled-coil regions in the extracellular domain (Fitzpatrick et al., 2010). Thus, virions remain bound to the cell surface and are cross-linked to each other by tetherin.

HIV-1 Vpu is an integral class I membrane phosphoprotein (Cohen et al., 1988) that promotes virion release from HIV-1 infected human cells that express tetherin (Klimkait et al., 1990; Neil et al., 2006; Neil et al., 2008; Van Damme et al., 2008). It has been shown to colocalize with tetherin (Neil et al., 2008; Van Damme et al., 2008) and to reduce its cell-surface expression by targeting it for degradation (Van Damme et al., 2008; Miyagi et al., 2009; Douglas et al., 2009; Goffinet et al., 2009; Mitchell et al., 2009). A well-studied role of Vpu is to mediate the proteasomal degradation of the HIV-1 receptor CD4 in the ER through the recruitment of the β-transducin repeat-containing protein (βTrcP) subunit of the Skp1-cullin1-F-box (SCF) ubiquitin ligase complex (Bour et al., 1995; Margottin et al., 1998; Willey et al., 1992). βTrCP is also involved in the antagonism of tetherin because disruption of the interaction between βTrCP and the βTrCP binding motif in the cytoplasmic domain of Vpu reduces the capacity of Vpu to promote virus release (Mitchell et al., 2009; Mangeat et al., 2009; Douglas et al., 2009). Vpu serves as an adapter between βTrCP and tetherin. Tetherin and Vpu bind to each other through their transmembrane domains (Rong et al., 2009; Iwabu et al, 2009). It seems that Vpu sequesters tetherin within the endolysosomal system either within the TGN after it has been synthesized or within recycling endosomes after natural endocytosis of tetherin from the cell surface has occurred (Mitchell et al., 2009; Dube et al., 2010). This intracellular sequestration is followed by partial lysosomal degradation of both tetherin and Vpu.

Vpu is only encoded by a unique lineage of primate lentiviruses that include HIV-1 and the simian immunodeficiency viruses (SIVs) of chimpanzees (*Pan troglodytes*) (Cohen et al., 1988), Mona monkeys (*Cercopithecus mona*), Mustached monkeys (*C. cephus*) and greater spot-nosed monkeys (*C. nictitans*), $SIV_{cpz}$, $SIV_{mon}$, $SIV_{mus}$ and $SIV_{gsn}$, respectively (Courgnaud et al., 2003). $SIV_{mon}$, $SIV_{mus}$ and $SIV_{gsn}$ Vpu counteract tetherins of their respective host species as well as macaque tetherins, but, with the exception of $SIV_{gsn}$, not human tetherin (huTHN). Accordingly, non-human, non-chimpanzee tetherins are usually insensitive to antagonism by HIV-1 Vpu (Goffinet et al., 2009; Gupta et al., 2009b; Jia et al., 2009; McNatt et al., 2009; Sauter et al., 2009; Zhang et al., 2009). $SIV_{cpz}$ is the immediate precursor of HIV-1 and its Vpu shares a common ancestry with $SIV_{mon/mus/gsn}$ Vpu (Sauter et al., 2009). However, $SIV_{cpz}$ Vpu is non-functional against both chimpanzee tetherin (cpzTHN) and huTHN. Instead, in $SIV_{cpz}$ the accessory protein Nef has adopted a Vpu-like function. It is likely that, after cross-species transmission from chimpanzees to humans, HIV-1 Vpu has adapted to counteract huTHN, because huTHN is resistant to Nef due to a deletion in the cytoplasmic tail of huTHN (Sauter et al., 2009; Zhang et al., 2009). Species-

specific tetherin antagonism by Nef is also conserved in SIVs of sooty mangabeys/rhesus macaques and African green monkeys, $SIV_{smm/mac}$ and $SIV_{agm}$, respectively. Like Vpu, Nef also induces cell-surface downregulation of monkey tetherins (Jia et al., 2009). Additionally to Vpu and Nef, the HIV-2 and $SIV_{agm}$Tan ($SIV_{agm}$ of the Tantalus monkey, *Chlorocebus tantalus*) envelope glycoproteins (Envs) possess anti-tetherin activities (Abada et al., 2005; Gupta et al., 2009a; Le Tortorec, 2009).

Interestingly, in addition to lentiviruses, tetherin blocks the virion release from members of the alpha-, beta-, deltaretrovirus, spumaretrovirus, arenavirus (Lassa) and filovirus (Ebola, Marburg) families (Sakuma et al., 2009; Jouvenet et al., 2009; Kaletsky et al., 2009).

## 3. Significance of tetherin in felids

Retroviruses have invaded members of the *Felidae* on multiple occasions. Of the 37 known species of felids, 21 species such as the African lion (*Panthera leo*), the North American puma (*Puma concolor*) or the domestic cat have been shown to harbour antibodies reactive to FIV and many of these species harbour viral sequences consistent with species-specific strains (VandeWoude and Apetrei, 2006; Troyer et al., 2008). In addition to FIV, domestic cats harbour gamma retroviruses such as exogenous and endogenous feline leukemia viruses (FeLVs) or RD114 and the spumaretrovirus FeFV (Reeves and O'Brien, 1984). In contrast to the high prevalence of FIV in different felid species, gamma retroviruses are, with the exception of sporadic cross-species transmission events, restricted to domestic cats (Benveniste and Todaro, 1975; Reeves and O'Brien, 1984), which suggests that they entered the domestic cat lineage after it had evolved 10,000 years ago (Vigne et al., 2004). The abundance of different retroviruses in cats necessitates the presence of potent and broadly specific host restriction factors. However, as mentioned above, cats express a truncated and non-functional TRIM5 protein (McEwan et al., 2009) and their A3 proteins are counteracted by wild-type FIV and FeFV (Löchelt et al., 2005; Münk et al., 2008; Stern et al., 2010; Zielonka et al., 2010). Therefore, their ability to suppress retroviral replication may critically depend on the activity of a feline homologue of tetherin.

## 4. Identification of a feline homologue of BST-2/tetherin

Blast searches of the feline genome using known primate, rodent and canine tetherin sequences identified a candidate gene for a feline homologue of tetherin. The transcript was amplified from interferon-ω stimulated feline IL2-dependent T cell (MYA-1) cDNA. The nucleotide sequence (Genbank accession HM461970) was analyzed and revealed 59% nucleic acid and 44% amino acid identity between cat tetherin (hereafter referred to as feTHN) and its human homologue and 77% nucleic acid and 60% amino acid identity to canine tetherin, transcript variant 2 (XM860510) (Figure 1). Tetherin configuration rather than its amino acid sequence has been shown to be critical for its antiviral activity (Perez-Caballero et al., 2009). Thus, we asked whether feTHN would adopt the same typical protein topology described for other tetherins (Ishikawa et al., 1995; Ohtomo et al., 1999, Kupzig et al., 2003). A hydropathy plot and secondary structure predictions of the feTHN amino acid sequence confirmed the presence of an N-terminal transmembrane domain, which is followed by an alpha-helical region and a coiled-coil domain (Figure 1). The alpha-

helical region contains three conserved cysteines (C59, C69, C97). Additionally, feTHN was predicted to contain a C-terminal GPI anchor signal sequence and the potential GPI anchor attachment site has been mapped to S161. Thus, both amino acid sequence and topology described for different tetherins are conserved in feTHN.

The expression levels of feTHN in feline T cell (MYA-1), fibroblast (AH927), kidney epithelioid (CrFK) and fetal embryo fibroblast-like (FEA) cell lines and the effect of treatment with type I interferons and IFN-γ (1000 U/ml) on its expression were examined by qRT-PCR. All cell lines showed a basal feTHN expression with FEA cells expressing approximately 10-fold lower levels compared to the other cell lines tested. Tetherin expression was inducible by type I IFN (α, ω) in all four cell lines, whereas treatment with IFN-γ had little effect on tetherin expression in MYA cells but up-regulated tetherin expression markedly in AH927, CrFK and FEA cells. In conclusion, feTHN shares the expression profile of huTHN.

## 5. Antiviral activity of feline tetherin

In order to assess the potency of feline tetherin to inhibit viral release, single-cycle viral replication assays were performed. FIV(VSV-G)-GFP pseudotypes were produced by transfecting 293T cells with the FIV-based vectors FP93 (Gagpol) and pGinSin (GFP) (Poeschla et al., 1998) and the vesicular stomatitis virus G glycoprotein (VSV-G)-encoding vector pMDG (Yee et al., 1994) in the presence or absence of feTHN. The pseudotypes were used to transduce CrFK cells and the viral titre was determined by flow cytometry. FeTHN caused a marked and dose-dependent reduction of the FIV(VSV-G)-GFP titer (Figure 2A). Inhibition of viral release was confirmed by immunoblotting against viral p24 in the culture supernatants (Figure 2D). In contrast to viral release, virus production was unaffected by the expression of feTHN. HIV-1 wild-type pseudotypes were produced as described above using the HIV-1-derived vector p8.2 (Gagpol) ans CSGW (GFP) (Naldini et al., 1996) and pMDG. Pseudotypes of Vpu-deficient HIV-1 (HIV-1 Δ Vpu) were generated using p8.91 (Gagpol) (Naldini et al., 1996), CSGW and pMDG. Feline tetherin was equally effective in blocking HIV-1 ΔVpu and HIV-1 wild-type viral release (Figures 2B,C and 2D), suggesting that its activity was not counteracted by HIV-1 Vpu. This finding underlines the concept of species-specificity of the tetherin-Vpu interaction (Yang et al., 2010).

In contrast to the well-defined role of tetherin in preventing viral release, information on its potency to block viral replication and spread is sparse. To this end, CrFK cells were stably transduced with a feTHN expression construct and infected with low or high inputs of CrFK-tropic strains of FIV-Pco (CoLV) or FIV-Fca (Petaluma F14) and virus production monitored by RT assay. Surprisingly and in contrast to the marked inhibitory effect of tetherin on lentiviral pseudotype production, ectopic expression of tetherin did not inhibit virus production from FIV-infected CrFK cells. Instead, syncytium formation was enhanced in the tetherin-expressing cells compared with control cells as virions are trapped at the cell surface promoting cell-cell fusion. As FIV-Pco and FIV-Fca (Petaluma F14) are cell culture-adapted viral strains, we generated CrFK cells and CrFK-feTHN cells stably expressing the viral primary receptor CD134 (Shimojima et al., 2004) and studied the effect of feTHN on replication of the primary strains of FIV, GL8 and PPR. Again, feTHN did not influence the

viral growth rate. In summary, these findings suggest that feTHN is unable to prevent replication of cell-culture adapted and primary strains of FIV.

## 6. Conclusion and future directions

Overall, feline tetherin resembles human tetherin in amino acid sequence, protein topology and anti-viral activity. It is expressed in different feline cells in to a basic level and its expression can be significantly enhanced by treatment with type I or type II IFN. FeTHN exhibited a potent, dose-dependent block to retroviral particle release, which was not relieved by the HIV-1 accessory protein Vpu. In stark contrast to particle release, stable expression of feTHN had no effect on FIV replication and even increased the likelihood of cell-cell fusion events thus possibly promoting viral cell-to-cell spread. Given the fact that feTHN was expressed from a CMV promoter in both the transiently and stably transfected cells, these findings suggest that the number of tetherin molecules on the cell surface might be limited and that feTHN therefore has only a saturable capacity to prevent viral particle release from productively infected cells. In single-cycle replication assays, however, the amount of virus particles to be retained at the cell surface might be lower so that virus release can be controlled by tetherin.

Indeed, there is evidence that Vpu-deficient HIV-1 can replicate in tissue culture with the same kinetics as wild-type virus (Strebel et al., 1988; Terwilliger et al., 1989; Klimkait et al., 1990) by shifting from a cell-free to a cell-to-cell mode of replication. As a consequence of this shift, viral replication was, in contrast to viral release, not inhibited. Further, it was recently shown that in T cells infected with Vpu-defective HIV-1, but not wild-type HIV-1, virus envelope proteins accumulated on the cell surface due to the action of tetherin, which promoted formation of virological synapses (VS) and direct cell-to-cell spread of virions (Jolly et al., 2010).

Future research should focus on the role of tetherin as a regulator of innate immunity. Tetherin has been shown to be a specific marker of type I IFN-producing cells (IPCs) or plasmacytoid dendritic cells (pDCs) (Blasius et al., 2006). These cells circulate through the blood and infiltrate lymph nodes that drain sites of infection. Viruses trigger Toll-like receptor (TLR) 7/9-induced production of large amounts of type I IFN and proinflammatory cytokines that activate anti-viral intrinsic, innate and adaptive immune responses (Colonna et al., 2004; Liu, 2005). A chronic activation of pDCs and continuous IFN production caused by lentivirus infection leads to immune dysregulation, T cell anergy and apoptosis (Tompkins and Tompkins, 2008). Tetherin has been shown to interact with the orphan receptor immunoglobin-like transcript (ILT7), which is expressed exclusively on pDCs (Cao et al., 2006). This interaction induces a negative feedback loop on the production of type I IFN and proinflammatory cytokine production and adjusts the magnitude of immune activation upon viral infection. Additionally, tetherin incorporation into the lipid envelopes of viral particles could enhance their uptake into professional antigen presenting cells (APCs).




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The elucidation of the role of feline tetherin in controlling replication of feline retroviruses in vivo by balancing immune responses will help to develop promising new approaches for the prevention and treatment of infections.

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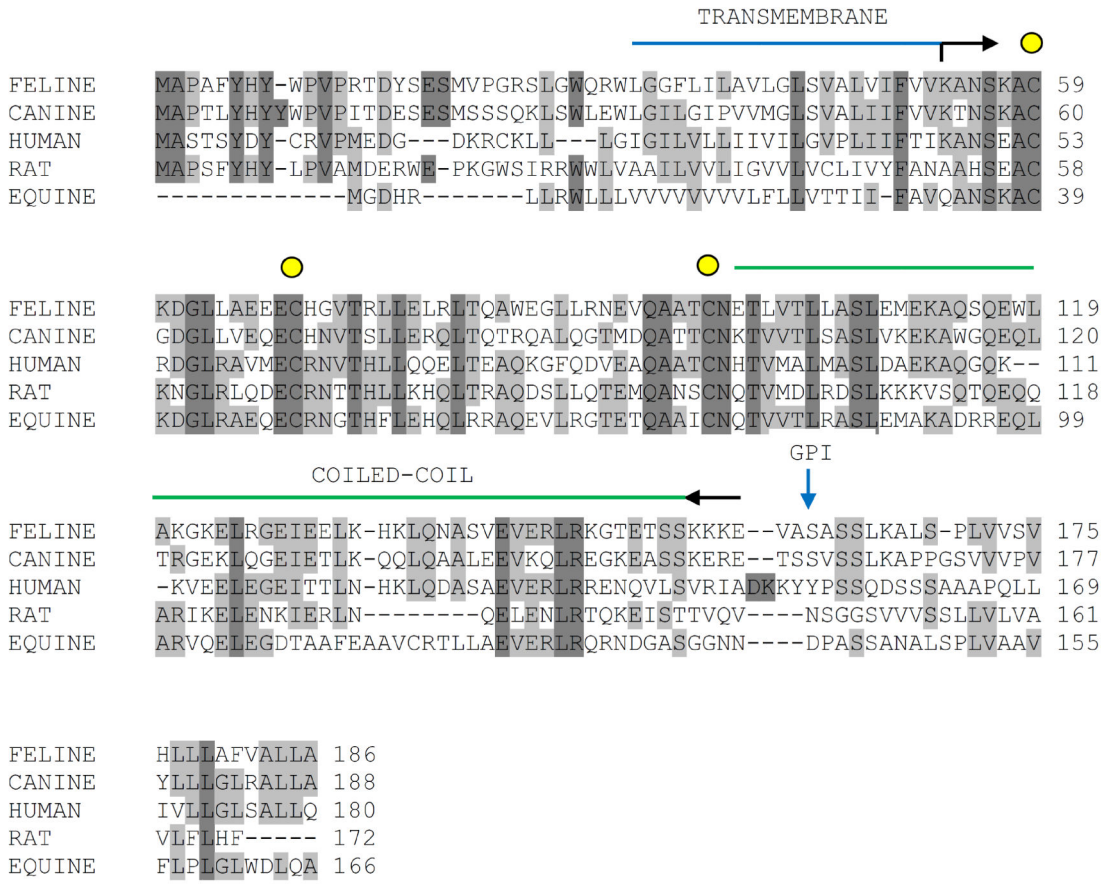


**Figure 1.**

Amino acid sequence alignment of tetherins. The amino acid sequences of feline, canine (transcript variant 2), human, rat and horse tetherin are compared. Amino acids conserved between all tetherin orthologs are highlighted in dark grey, those conserved between at least three sequences in light grey. The positions of predicted protein domains are indicated. The position of the transmembrane domain is marked by a blue bar and the position of the coiled-coil domain, which contains the three conserved cysteine residues, by a green bar. The length of the extracellular domain is indicated by black arrows. The position of the potential GPI anchor attachment site (ω-site) is marked by a blue arrow.

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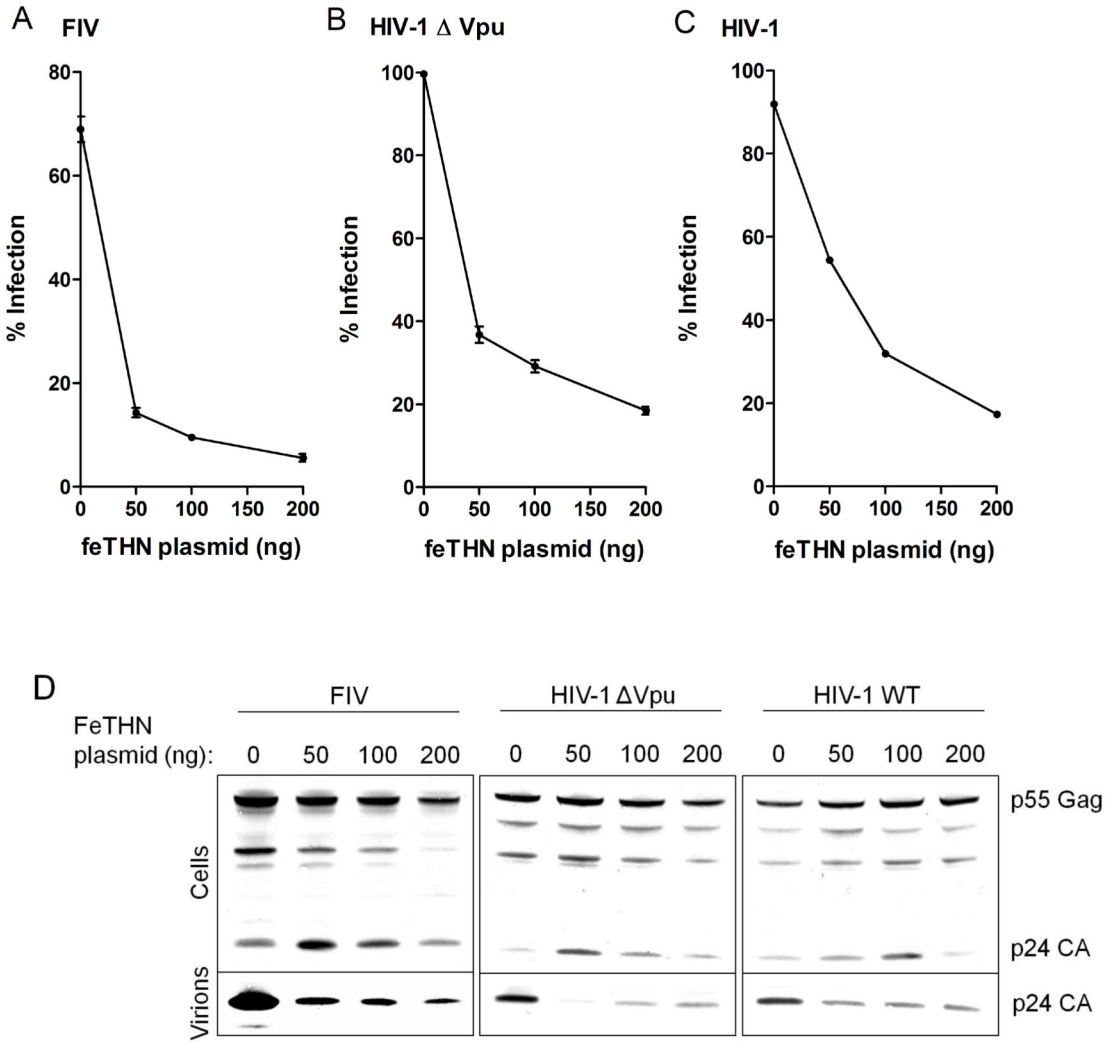


**Figure 2.**

Feline tetherin restricts FIV and HIV-1 particle release and is not overcome by the HIV-1 accessory protein Vpu. (A) 293T cells were co-transfected with the FIV expression plasmids FP93 (Gagpol), pGinSin (GFP), pMDG (VSV-G) and indicated amounts of feline tetherin (feTHN) plasmid DNA. Infectious virus yield (expressed as percentage of infection) was determined by transducing CrFK cells with the pseudotype-containing culture supernatants of the producer cells and by quantifying the percentage of GFP-expressing cells using flow cytometry (± s.d., n=3). (B) 293T cells were co-transfected with the HIV-1 ΔVpu expression plasmids p8.91 (Gagpol), CSGW (GFP) and pMDG and indicated amounts of feTHN plasmid DNA. The infectious virus yield was determined as described for (A). (C) 293T cells were co-transfected with the HIV-1 wild-type expression plasmids p8.2 (Gagpol), CSGW (GFP) and pMDG and indicated amounts of feTHN plasmid DNA. The infectious virus yield was determined as described for (A). (D) Western blot analysis (anti-p24 capsid) of 293T cell lysates and virions after co-transfection of FIV, HIV-1 ΔVpu or HIV-1 wild-type expression plasmids and varying amounts of feTHN plasmid DNA.