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Review

Novel targets for HIV therapy

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ABSTRACT

There are currently 25 drugs belonging to 6 different inhibitor classes approved for the treatment of human immunodeficiency virus (HIV) infection. However, new anti-HIV agents are still needed to confront the emergence of drug resistance and various adverse effects associated with long-term use of antiretroviral therapy. The 21st International Conference on Antiviral Research, held in April 2008 in Montreal, Canada, therefore featured a special session focused on novel targets for HIV therapy. The session included presentations by world-renowned experts in HIV virology and covered a diverse array of potential targets for the development of new classes of HIV therapies. This review contains concise summaries of discussed topics that included Vif-APOBEC3G, LEDGF/p75, TRIM 5α, virus assembly and maturation, and Vpu. The described viral and host factors represent some of the most noted examples of recent scientific breakthroughs that are opening unexplored avenues to novel anti-HIV target discovery and validation, and should feed the antiretroviral drug development pipeline in the near future.

Contents

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1. Introduction (José A. Esté)

Twenty-five years after the first isolation of the human immunodeficiency virus (HIV), antiretroviral therapy has moved from fast-track licensing of the first effective drug against HIV, zidovudine (AZT) in 1987 to combination therapy with 25 approved drugs belonging to 6 different classes (Table 1). As disease progression is associated with higher HIV RNA levels in blood (viral load), an important objective of antiretroviral therapy is to reduce viral loads below the limit of detection of approved assays. All but one anti-HIV drugs target viral enzymes and proteins that are indispensable for the virus to complete its replication cycle (Fig. 1), but none alone is able to achieve an undetectable viral load for sustained periods of time. Experience has shown that combinations of three or more active drugs, including at least two classes of antiretrovirals, may achieve maximal suppression of plasma viral load and delay the selection of drug resistance mutations.

Table 1
Approved antiretroviral drugs for the treatment of HIV infection

	Approval date
Entry inhibitors	
Maraviroc (UK-427,857, Selzentry®)	06 August 2007
Fusion inhibitors	
Enfuvirtide (T20, Fuzeon®)	13 March 2003
Integrase inhibitors	
Raltegravir (MK-0518, Isentress®)	12 October 2007
Reverse transcriptase inhibitors	
Nucleoside/nucleotide analogues	
Abacavir (ABC, Ziagen®)	17 December 1998
Didanonise (ddI, Videx®)	09 October 1991
Emtricitabine (FTC, Emtriva®)	02 July 2003
Stavudine (d4T, Zerit®)	24 June 1994
Lamivudine (3TC, Epivir®)	17 November 1995
Tenofovir (DF, Viread®)	26 October 2001
Zalcitabine (ddC, Hivid®)	19 June 1992
Zidovudine (AZT, Retrovir®)	19 March 1987
Non-nucleoside inhibitors	
Delavirdine (DLV, Rescriptor®)	4 April 1997
Efavirenz (EFV, Sustiva®)	17 September 1998
Etravirine (TMC125, Intelence®)	18 January 2008
Nevirapine (NVP, Viramune®)	21 June 1996
Protease inhibitors	
Amprenavir (AMP, Agenerase®)	15 April 1999
Atazanavir (ATZ, Reyataz®)	20 June 2003
Darunavir (TMC-114, Prezista®)	23 June 2006
Fosamprenavir (GW-433908, Lexiva®)	20 October 2003
Indinavir (IDV, Crixivan®)	13 March 1996
Lopinavir (ABT-378, Kaletra® (trade name in combination with RTV))	15 September 2000
Nelfinavir (NFV, Viracept®)	14 March 1997
Ritonavir (RTV, Norvir®)	01 March 1996
Saquinavir (SQV, Fortovase®, Invirase®)	07 November 1997
Tipranavir (TPV, Aptivus®)	22 June 2005

A number of factors may influence the safety and efficacy of antiretroviral therapy in individual patients, including non-adherence to therapy, adverse drug reactions, drug–drug interactions, and development of drug resistance. Long-term management of HIV patients is complex and multifactorial; with time, potent drugs may fail because chronic adverse effects may outweigh the initial benefits. With patients failing current antiretroviral drug regimens, the emergence and transmission of drug-resistant variants increase and become a public-health concern. It is therefore essential that new antiretroviral agents become available.

Antiviral drug development has not waned. On the contrary, the pharmaceutical industry has shown a continued interest in further exploiting existing drug targets, reaching proof of concept for new ones and initiating new drug development programs. In recent years, we have witnessed the advent of two HIV-1 protease inhibitors with improved resistance profiles (tipranavir and darunavir) (Clotet et al., 2007; Hicks et al., 2006), and a new non-nucleoside reverse transcriptase inhibitor (NNRTI) etravirine (TMC125) (Lazzarin et al., 2007) with antiviral activity in treatment-experienced patients showing resistance to existing NNRTIs. Furthermore, two additional drugs have been approved, each representing a new class of antiretroviral inhibitors: raltegravir, targeting the viral integrase enzyme (Grinsztejn et al., 2007) and maraviroc, targeting the cellular HIV-1 entry cofactor CCR5 (Fatkenheuer et al., 2005).

Complementary to industry, academic research labs are providing an overwhelming amount of new data. Basic research aimed at understanding the biology and pathogenesis of HIV is also revealing new targets for antiretroviral therapy. Protein–protein interaction technology and RNA interference gain wider use to identify host proteins required for HIV-1 infection (Brass et al., 2008) and a high through-put genomic analysis is now used to understand host genetic control of virus infections such as HIV-1 (Fellay et al., 2007).

G-protein coupled receptors (GPCR) are preferred pharmacological targets for many diseases. Moreover, the discovery of chemokine GPCR, not as mere cofactors, but primordial virus receptors, and the observation that host defects in CCR5 expression

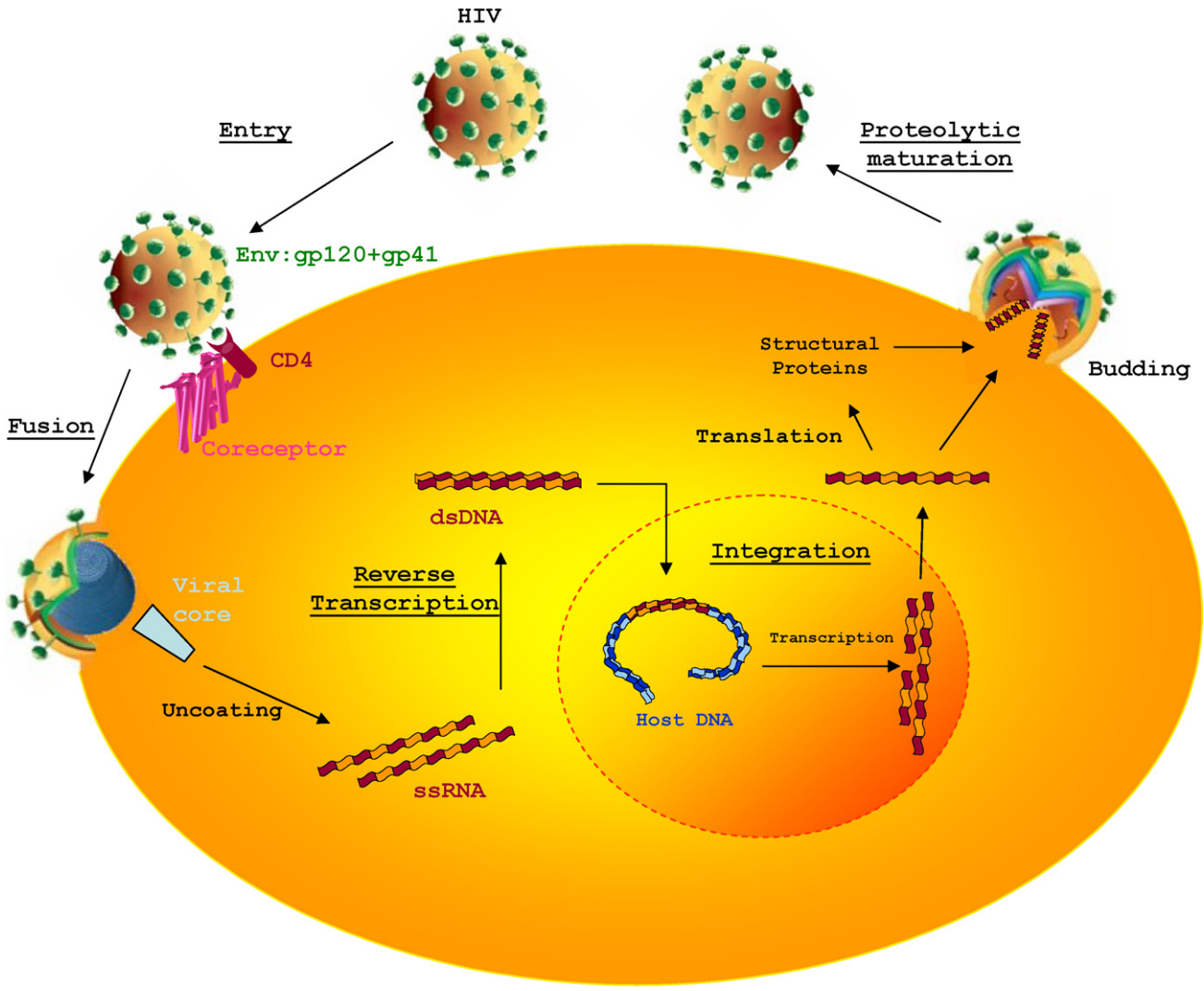

Fig. 1. HIV replication cycle. Entry begins when the gp120 of the viral envelope spikes (in green) binds to the CD4 receptor on the surface of susceptible cells. Changes in gp120 lead to coreceptor (CCR5 or CXCR4) engagement and fusion mediated by the envelope gp41, followed by internalization of the viral core. After uncoating, reverse transcription leads to the generation of double-stranded DNA (dsDNA) from the single-stranded viral RNA genome (ssRNA). The provirus DNA is transported to the nucleus and integrated into the host chromosomal DNA (host DNA). Then DNA is transcribed by cellular polymerases to generate full-length and spliced messenger RNA, as well as full-length progeny virion RNA. Viral messages are translated in the cytoplasm and the newly produced viral proteins, together with genomic RNA, assemble into immature virions. New viral particles are released (budding) and become infectious after proteolytic maturation by the HIV protease enzyme. The targets of approved antiretroviral agents are underlined, namely: virus entry, fusion, reverse transcription, integration and proteolytic maturation.

severely limit acute infection or disease progression, have provided the rationale for the development of maraviroc and vicriviroc (Gulick et al., 2007) (reviewed in Este and Telenti, 2007). It also proves that antiretroviral efficacy can be achieved by targeting cellular instead of viral proteins.

Once HIV has entered the cell, it must disarm and highjack the intracellular machinery for its own benefit. The viral infectivity factor (Vif) directly binds to and inactivates cellular deoxycytidine deaminase APOBEC3G (Sheehy et al., 2002; Greene, 2008), a natural antiviral factor that promotes G-to-A hypermutation of viral DNA during reverse transcription. Viral protein U (Vpu), shown to down-regulate the CD4 receptor, is also required for effective release of newly formed viral particles. Vpu promotes virus release by antagonizing or sequestering the host cell membrane protein CD317 (BST2, HM1.24, tetherin), which is thought to tether nascent virus particles to the cell surface (Neil et al., 2008; Van Damme et al., 2008; Stephens, 2008). Virus restriction factors such as TRIM5α have been identified in non-permissive rhesus monkey cells (Stremlau et al., 2004, 2006; Ikeda, 2008). Following the completion of reverse transcription, a preintegration complex is formed between the viral integrase and a number of cellular proteins including the lens epithelium-derived growth factor or transcriptional co-activator p75 (LEDGF/p75). Integrase binds to LEDGF/p75 and this interaction appears to be essential for viral DNA integration (Vandekerckhove et al., 2006; Debyser, 2008).

During virus assembly, the viral Gag polyprotein must be effectively processed and transported to the cell membrane. (Freed, 2008). Cofactors such as the phospholipid phosphatidylinositol (4,5) bisphosphate [PI(4,5)P_2] (Ono et al., 2004), the ADP ribosylation factor (Arf) binding proteins (Joshi et al., 2008) or tumor susceptibility gene 101 (Tsg101) (Garrus et al., 2001) are required for the intracellular transport and budding of HIV particles. While these are just a few examples of virus–host cell interactions, each one represents a potential new target under rigorous research with their validation being actively pursued. For example, the overexpression of LEDGF/p75 integrase-binding domain blocked HIV-1 replication and led to the emergence of IBD-resistant virus, representing the most relevant proof of concept for this particular target

(Hombrouck et al., 2007). Freed et al. have shown that modified forms of Tsg101 protein can act as potent and specific inhibitors of HIV-1 replication by blocking virus budding (Demirov et al., 2002). Furthermore, dimethylsuccinyl betulinic acid (PA-457 or bevirimat) potently inhibits HIV-1 infectivity by targeting Gag processing in immature viral particle (Li et al., 2003; Zhou et al., 2005).

As a part of the 21st International Conference on Antiviral Research (ICAR), a minisymposium was organized to highlight fundamental research aimed at the discovery and validation of new anti-HIV targets and agents. Due to the scope of the minisymposium, many new targets for drug discovery could not be discussed. However, invited speakers have been pioneers and made groundbreaking discoveries that will surely feed the drug development pipeline in the future. In this review, the most relevant aspects of each presentation have been summarized by respective authors with the hope that readers will gain a first-hand description of the event that took place and the future that lies ahead.

2. The APOBEC3G-Vif axis: a target for antiviral drug discovery? (Warner C. Greene and Wes Yonemoto)

2.1. Background

In addition to essential viral enzymes that have been extensively explored as targets for antiretroviral drugs, HIV-1 encodes several accessory proteins including Vif (viral infectivity factor), a basic 23-kDa phosphoprotein that is expressed late in the viral life cycle. Viruses lacking a functional Vif gene (ΔVif) do not replicate in "non-permissive" cells that include biologically relevant primary CD4+ T-cells and macrophages. Conversely, many "permissive" laboratory T-cell lines and non-hematopoietic cell lines fully support HIV-1 replication in the absence of Vif (Gabuzda et al., 1992). Heterokaryons formed between permissive and non-permissive cells revealed the dominance of the non-permissive phenotype, indicating that non-permissive cells encode an anti-HIV factor whose action is somehow circumvented by Vif. In 2002, Sheehy et al. (2002) identified this antiviral factor as a single-strand DNA deoxycytidine deaminase, APOBEC3G (A3G).

2.2. Antiretroviral activity of APOBEC3G

When non-permissive cells are infected with HIVΔVif viruses, A3G is effectively incorporated into budding HIV-1 virions (Mariani et al., 2003; Stopak et al., 2003) (Fig. 2). This process involves the interaction of A3G with the nucleocapsid region of the Gag polyprotein (Alce and Popik, 2004; Cen et al., 2004; Luo et al., 2004) and is strengthened by concommitant binding to viral RNA (Schafer et al., 2004; Svarovskaia et al., 2004; Zennou et al., 2004). Incorporation of less than 10 molecules of A3G into HIV-1 virions appears sufficient to inhibit HIV-1 replication during the next round of infection (Xu et al., 2007).

Following viral minus-strand DNA synthesis in newly infected cells, A3G deaminates dC residues to dU (Zhang et al., 2003; Suspene et al., 2004; Yu et al., 2004a), promoting both partial minus-strand degradation and dA misincorporation in the subsequently synthesized viral plus strand. The resultant dG-to-dA mutations can alter viral open reading frames and introduce new translation termination codons (Harris et al., 2003; Lecossier et al., 2003; Mangeat et al., 2003) (Fig. 2). Independently, A3G binding to viral RNA may also sterically interfere with priming and/or progression of reverse transcription. The question of whether the deaminase activity is necessary for the antiviral activity of A3G remains a subject of controversy, although recent studies have shown that the catalytically inactive mutant form of A3G retains only a fraction of antiviral activity compared to A3G with intact deaminase activity (Newman et al., 2005). It seems likely that both enzyme-dependent and -independent effects contribute to the overall antiviral activity of A3G.

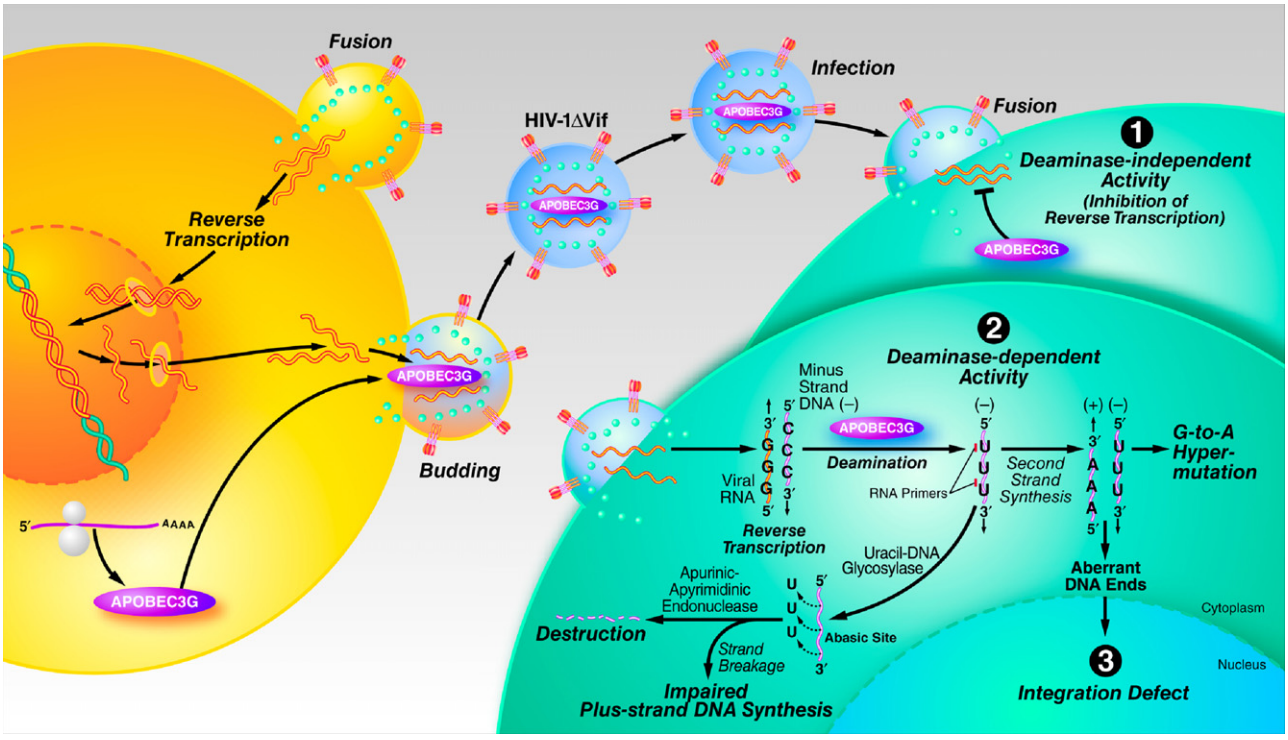

Fig. 2. Antiretroviral activity of APOBEC3G. In the absence of functional Vif, APOBEC3G is effectively packaged into newly formed virions. During the following cycle of infection, APOBEC3G adversely affects the efficiency of reverse transcription (1), possibly by a steric effect caused by its binding to RNA and ssDNA templates. The deaminase activity of APOBEC3G induces G-to-A mutations in proviral DNA (2), resulting in nonfunctional viral genes and integration defect (3).

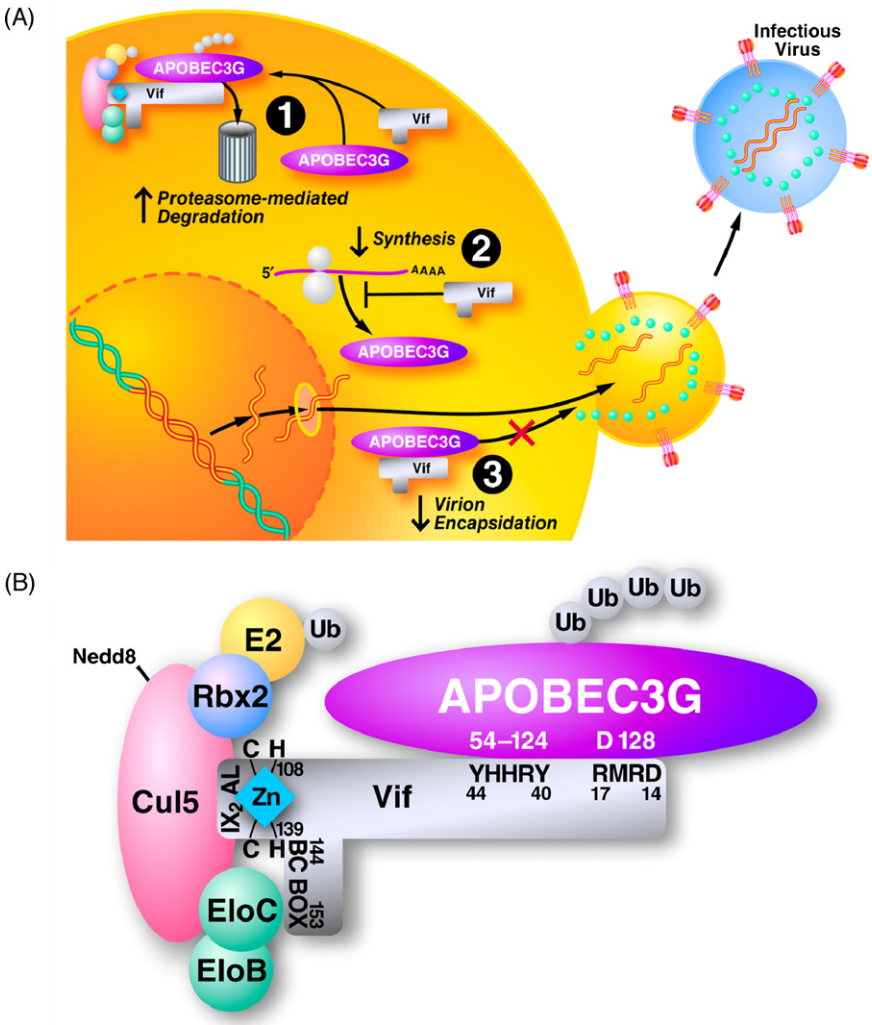

Fig. 3. Function of Vif in counteracting APOBEC3G. (A) Vif interacts with APOBEC3G and promotes its degradation by the 26S proteasome via an ubiquitin-dependent pathway (1). In addition, Vif may inhibit the translation of APOBEC3G (2), while the binding of Vif to APOBEC3G may also directly reduce its encapsidation into newly formed virions (3). (B) Interaction of Vif with the APOBEC3G ubiquitin ligase complex leads to efficient ubiquitination and subsequent degradation of APOBEC3G. Functional motifs of Vif that are important for its interaction with APOBEC3G and the components of Cul5 ubiqiutin ligase are highlighted.

2.3. Neutralization of APOBEC3G by Vif

The primary mechanism of A3G neutralization in HIV-1 infected cells involves direct binding of Vif to A3G, leading to its polyubiquitination and subsequent degradation by the 26S proteasome (Fig. 3A) (Conticello et al., 2003; Marin et al., 2003; Sheehy et al., 2003; Stopak et al., 2003). These effects reflect the ability of Vif to recruit an active ubiquitin ligase complex composed of Elongin B/C, Cullin5, Nedd8, and Rbx1 (Yu et al., 2003), which mediates polyubiquitylation of A3G (Conticello et al., 2003; Marin et al., 2003; Sheehy et al., 2003) (Fig. 3B). In this process, the N-terminal region of Vif binds to the N-terminal region of A3G (Conticello et al., 2003; Simon et al., 2005; Wichroski et al., 2005) and the SLQ(Y/F)LA motif (amino acids 144–150) in the C-terminal region of Vif binds to Elongin C (Marin et al., 2003; Mehle et al., 2004; Yu et al., 2004b). Finally, a Zn-binding motif (amino acids 108–139) interacts with Cullin5 (Mehle et al., 2006; Xiao et al., 2006). Specific mutations in the SLQ or the Zn-binding motif of Vif block A3G polyubiquitylation and preserve the antiviral activity of A3G (Yu et al., 2003; Mehle et al., 2004; Mehle et al., 2006, Xiao et al., 2006).

Two additional Vif domains, the central hydrophilic EWRKKR domain (amino acids 88–93) and the proline-rich PPLP domain (amino acids 161–164) are important, respectively for enhancing steady-state levels of Vif and for interaction with tyrosine kinases (Fujita et al., 2003; Douaisi et al., 2005). Mutation of these domains also compromises Vif activity, but the underlying mechanisms are not well understood. In addition to recruiting A3G to the E3 ubiquitin ligase complex, Vif partially impairs the translation of A3G mRNA (Stopak et al., 2003), but again the mechanism remains undefined. As a result of these Vif-mediated activities, A3G is effectively depleted from HIV-1-infected cells and its incorporation into newly formed virions is thus circumvented, ensuring full infectivity of viral progeny.

2.4. What is known about structures of Vif and APOBEC3G

Structural information about Vif is virtually absent, mainly because of the challenging production of recombinant protein in a soluble and properly folded form. Several attempts were recently published to model Vif structure based on information about other proteins that interact with Elongin B/C (Balaji et al., 2006; Lv et al., 2007). Independent biophysical studies employed proteolytic fragmentation of crosslinked Vif followed by mass spectrometry to characterize Vif in its monomeric and oligomeric forms. These

results indicated that the C-terminal domain of monomeric Vif is somewhat disordered, but assumes a more ordered conformation upon oligomerization (Auclair et al., 2007). It is presently unclear whether this transition detected with recombinant protein is physiologically relevant and if it influences the ability of Vif to bind A3G.

Initial attempts to understand the structure of A3G relied on computational homology modeling based on APOBEC2 single-domain deaminase, which is the closest known paralog of A3G for which a high-resolution X-ray crystallographic structure is available (Prochnow et al., 2007). The APOBEC2 dimer appears to be analogous to a monomer of A3G, which contains two Zn^{2+}-coordination domains. X-ray scattering analysis of A3G provided supporting evidence for such an elongated two-domain symmetric structure (Wedekind et al., 2006) that may be important for the ability of A3G to bind long polynucleotide substrates and to form RNA-associated high molecular mass complexes (Chiu et al., 2005). Of note, multiple APOBEC3 proteins active on polynucleotide substrates, including A3G, A3F and A3DE, have been reported to form homodimers and heterodimers in cells (Wiegand et al., 2004; Dang et al., 2006). Recently, a solution structure of the recombinant A3G C-terminal catalytic domain was determined by NMR (Chen et al., 2008). This analysis identified multiple differences between the structure of APOBEC2 and the catalytic domain of A3G and has extended our understanding of the nature of interactions between A3G and its DNA substrate.

Mapping of functional and evolutionarily conserved amino acids in A3G demonstrates that critical residues cluster in well-defined patches at the surface of the model. Functional evaluation of A3G mutants further indicates that amino acids 128–130 (DPD motif) are important for its interaction with Vif (Bogerd et al., 2004; Mangeat et al., 2004; Schrofelbauer et al., 2004; Huthoff and Malim, 2007). For example, A3G mutants D128K, P129A/G, or D130K are impaired in their ability to bind Vif and to potently inhibit wild type HIV-1 (Huthoff and Malim, 2007). In addition, A3G residues R122 and W127 are required for its efficient encapsidation. Simultaneous functional mutagenesis of Vif identified residues 40 to 44 (YRHHY domain) as critical for the interaction with A3G (Russell and Pathak, 2007). Single and double alanine substitutions in this region impair Vif interaction with A3G and render the virus fully sensitive to A3G (Russell and Pathak, 2007; Mehle et al., 2007). These findings suggest that distinct domains in N-terminal regions of A3G and Vif are involved in their interaction.

2.5. How can small molecules interfere with Vif function?

Many in the HIV field regard the Vif-A3G axis as a compelling new drug target. The first, and perhaps the preferred approach would be to identify small-molecule inhibitors that selectively disrupt the binding of HIV-1 Vif to A3G. Such inhibitors would prevent Vif-mediated ubiquitination of A3G and subsequent degradation by proteasome, thereby leading to the effective encapsidation of A3G into budding virions. While these inhibitors would have to interfere with a protein–protein interface, the fact that specific mutations, in either A3G or Vif, can block binding of the two proteins is encouraging. An alternative drug target might include blocking Vif recruitment of the E3 ligase complex by interfering with the binding of Cullin 5 or Elongin B/C to Vif. However, a potential drawback to this approach is that Vif would still bind to A3G and might be co-encapsidated, with uncertain effects on A3G antiviral activity. In order to facilitate the rational design of such inhibitors, further investigation is required to determine the structural aspects of Vif function, as the key component of the E3 ubiquitin ligase complex that induces the effective elimination of A3G.

3. LEDGF/p75 as a co-factor of HIV-1 integrase and a novel antiviral target (Zeger Debyser)

3.1. The discovery of LEDGF/p75

Upon analysis of protein complexes derived from the nuclei of a cell line stably over-expressing flag-tagged HIV-1 integrase from a synthetic gene, a high-affinity binding partner was identified by co-immunoprecipitation (Cherepanov et al., 2003). This protein was identical to the previously reported lens epithelium-derived growth factor or transcriptional co-activator p75 (LEDGF/p75) (Ge et al., 1998; Singh et al., 2000). This initial identification in 2002 was later confirmed by yeast two-hybrid screening (Emiliani et al., 2005) and co-immunoprecipitation (Turlure et al., 2004). LEDGF/p75 plays a pleiotropic role both in cell survival and in apoptosis-mediated cell death. Although RNAi-mediated knock-down of LEDGF/p75 in laboratory cell lines does not affect cell viability (Vandekerckhove et al., 2006), survival of LEDGF (−/−) mice is severely affected, probably by interference with homeodomain proteins during embryogenesis (Sutherland et al., 2006).

3.2. Structural biology of LEDGF/p75

Although a crystal structure of full-length integrase or full-length LEDGF/p75 is not available, a co-crystal of the interacting domains of integrase and LEDGF/p75 (Cherepanov et al., 2005) provides the required structural information to embark on drug design. LEDGF/p75, a 533 amino acid protein, shares the first 325 amino acids with its alternative splice variant p52. Both proteins have a unique C-terminus, which is eight amino acids in the case of p52 and 205 for LEDGF/p75. In accord with its ability to interact with HIV-1 integrase, an evolutionary highly conserved integrase-binding domain (IBD) is present in the C-terminus (amino acids 347–429). In the co-crystal, two monomers of IBD interact with a dimer of the catalytic core domain (CCD) of integrase (Fig. 4).

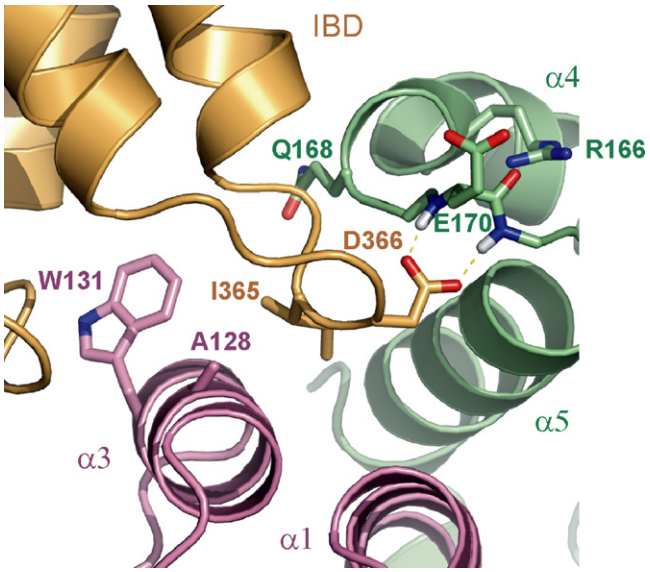

Fig. 4. Structure of the HIV-1 integrase CCD-IBD interface. Close-up view of the interface of an integrase dimer interacting with the LEDGF/p75 IBD. The figure was drawn with PyMOL using Protein Data Bank crystal structure file 2BJ4. The integrase CCD monomers are colored purple and green and the IBD subunit is in orange. The selected key residues are shown as sticks, and hydrogen bonds are indicated by dotted lines.

An interhelical loop of the IBD binds to a defined pocket at the interface of the two CCDs; the most critical interacting residues of the IBD are I365, D366 and F406 (Cherepanov et al., 2005). Although all lentiviral integrases can interact with LEDGF/p75, the key contacts in HIV-1 integrase such as A128, W131 and Q168 are poorly conserved between primate and non-primate lentiviral INs.

3.3. Validation of LEDGF/p75 as an important cofactor for viral replication

After initial identification of LEDGF/p75 as an HIV-1 integrase binding partner, a series of cellular and biochemical experiments was performed to corroborate the direct interaction between the two proteins. Using a series of fluorescently labelled proteins, clear colocalization of HIV-1 integrase and LEDGF/p75 was shown in cells (Maertens et al., 2003; Llano et al., 2004). Furthermore, RNA interference-mediated knock-down of endogenous LEDGF/p75 expression abolished the nuclear/chromosomal localization of integrase. *In vitro*, the specific interaction was confirmed by a pull-down assay with recombinant proteins (Maertens et al., 2003). An important role for LEDGF/p75 in HIV-1 replication was evidenced through various RNAi studies (Vandekerckhove et al., 2006; Llano et al., 2004, 2006). Inhibition of HIV-1 replication in monocyte derived macrophages by silencing LEDGF/p75 has also been observed (Zielske and Stevenson, 2006). Significantly reduced levels of HIV-1 integration were found in embryonic fibroblasts derived from a conditional mouse knock-out (KO) for *LEDGF* (Shun et al., 2007). Reduction of HIV-1 integration in homozygous *LEDGF* disrupted murine cells was also seen by Marshall et al. (2007). These findings all argue for a crucial role of LEDGF/p75 in the integration process.

More proof for LEDGF/p75 as an important integrase cofactor came from different mutagenesis studies (Emiliani et al., 2005; Busschots et al., 2007). Substitution of integrase amino acids in the two LEDGF/p75-interacting regions with alanine diminished interaction with LEDGF/p75, although the mutants (including W131A and Q168A) displayed normal enzymatic activity *in vitro*.

A separate approach was undertaken by stably overexpressing the integrase binding domain (IBD) of LEDGF/p75 fused to enhanced green fluorescent protein (eGFP) in HeLaP4 and MT-4 cells (De Rijck et al., 2006). They showed that HIV-1 replication in these cells was severely blocked at the step of integration. In fact, the strongest phenotype in inhibiting HIV-1 replication was obtained with this approach. This result not only validates LEDGF/p75 as an important cofactor for HIV-1 replication but provides also proof-of-concept for LEDGF/p75-integrase interaction as a novel target for antiviral therapy. By repeatedly passaging HIV-1 in cell lines overexpressing IBD, strains that could overcome the inhibition were selected (Hombrouck et al., 2007). Although resistance developed, the resistant virus was severely crippled in its replication capacity in human primary cells. Analysis of the integrase gene revealed two amino acid mutations: A128T and E170G. These residues are located exactly at the described interface between the integrase CCD dimer and the IBD, with one residue in each chain of the dimer. Although LEDGF/p75 could still interact with the mutant integrase, this interaction occurred with a much lower affinity. Moreover, replication of the IBD-resistant virus was 10-fold more sensitive to depletion of LEDGF/p75 than WT virus, indicating that the resistant virus remained dependent on LEDGF/p75 for its replication. Impaired replication kinetics upon resistance selection and the exclusive role of LEDGF/p75 during HIV-1 integration both support the validity of LEDGF/p75 as an antiviral target. In the same study, a previously described diketo-acid-resistant strain (integrase inhibitor L-708,906) remained fully susceptible to inhibition by IBD. These data suggest that the strategy of integrase-LEDGF/p75 interruption should also work in patients who harbor strains resistant to integrase strand transfer inhibitors.

3.4. Is the integrase-LEDGF/p75 interaction a genuine target for drug discovery?

What makes this protein–protein interaction (PPI) a potential target for discovery and development of small molecules? The following points must be addressed: (i) the target has to be validated as important for HIV-1 replication; (ii) inhibition of the specific PPI should not be associated with toxicity; (iii) structural information on the PPI should be available; and (iv) identification of genuine inhibitors would provide ultimate proof-of-concept.

Most importantly, the virus–host interaction between integrase and LEDGF/p75 is crucial for viral replication. Although the requirement of a relatively high MOI to achieve infection in mouse cells somewhat confounds the interpretation, depletion of LEDGF/p75 resulted in a 2-log reduction of HIV-1 replication in mouse KO cells. Moreover, overexpression of IBD resulted in a complete block of HIV-1 replication in some cell lines and incomplete suppression was associated with emergence of resistant strains. Although an essential role of the cofactor thus has not been demonstrated *strictu sensu*, both latter experiments validate IN-LEDGF/p75 interaction as a genuine antiviral target.

When targeting a cellular protein, one should be concerned about cellular toxicity. So far, in all experiments that have used RNAi to knockdown cellular LEDGF/p75, the knockdown has not been associated with severe toxicity in human cell lines. LEDGF appears to be important during embryonic development, since the majority of homozygous LEDGF KO mice die perinatally, and those that survive display a range of abnormal phenotypes, compatible with defects in homeodomain proteins (Sutherland et al., 2006). However, both p75 and p52 splice variants are depleted in the KO mice, whereas an HIV therapeutic strategy would only target p75. In fact, HIV drug discovery aims at inhibiting protein–protein interaction with HIV-1 integrase without affecting the cellular function of LEDGF/p75. In any case, future lead compounds will have to be carefully evaluated for teratogenicity and toxicity.

Valuable information in this respect will come from cell biology studies on LEDGF/p75. An ongoing effort attempts to identify the cellular binding partners. Two groups independently identified JPO2 as a first cellular binding partner of the C-terminal part (Maertens et al., 2006; Bartholomeeusen et al., 2007). Competition assays using recombinant proteins showed a mutually exclusive binding of either JPO2 or HIV-1 integrase to LEDGF/p75. However, while the binding regions overlap, differential interaction was proposed since JPO2 still interacts with LEDGF/p75 mutants (I365A, D366A, and F406A) that are totally defective for interaction with HIV-1 integrase (Bartholomeeusen et al., 2007). The finding of differential interaction between integrase, LEDGF/75 and JPO2 suggests the feasibility of developing inhibitors specifically targeting the interaction between LEDGF/p75 and HIV-1 integrase.

Next, structural biology data on the interaction are needed. Mutagenesis and structural analysis have generated a good knowledge about the amino acids involved in the interaction. The cocrystal of integrase with the IBD of LEDGF/p75 revealed the IBD to be inserted into a relatively small and deep cleft at the integrase dimer interface. Ideally one could thus look for small molecules that enter this binding pocket in integrase, thereby preventing interaction with the cofactor. Finally, identification of the first small molecules that interfere with integrase-LEDGF/p75 interaction and block HIV-1 replication will provide an enormous impetus to this field.

4. Potential applications of TRIM5α for anti-HIV therapy (Yasuhiro Ikeda)

4.1. Background

Rhesus monkey TRIM5α (TRIM5αrh) was first identified as a cellular factor that restricts HIV-1 infection (Stremlau et al., 2004). TRIM5α is a member of the vast TRIpartite Motif (TRIM) family of proteins. It comprises an RBCC (RING, B-box and coiled-coil motifs) domain and a B30.2 (PRYSPRY) domain. TRIM5αrh recognizes the incoming viral capsid core structure and blocks HIV-1 infection at a post-entry, pre-integration stage in the viral life cycle (Fig. 5) (Stremlau et al., 2004; Stremlau et al., 2006). Sequences in the B30.2 domain determine the potency and specificity for this post-entry restriction (Perez-Caballero et al., 2005; Stremlau et al., 2006; Yap et al., 2005).

Recently, we found that TRIM5αrh also blocks the late phase of HIV-1 replication (Sakuma et al., 2007c), although the late restriction activity of endogenous TRIM5αrh remains controversial (Zhang et al., 2008a). Our data suggest that TRIM5αrh interacts with HIV-1 Gag polyprotein during or before Gag assembly through a mechanism distinct from the post-entry restriction (Sakuma et al., 2007c). The specificity determinant for this late restriction lies in the B-box and coiled-coil motifs of TRIM5α (Sakuma et al., 2007c). Intriguingly, evolutionary analysis has revealed localized, positively selected residues in the B30.2 (Ortiz et al., 2006; Sawyer et al., 2005), B-box and coiled-coil motifs (Ortiz et al., 2006) of TRIM5 protein, suggesting that TRIM5α evolution has been driven by antagonistic interactions with a wide variety of pathogens.

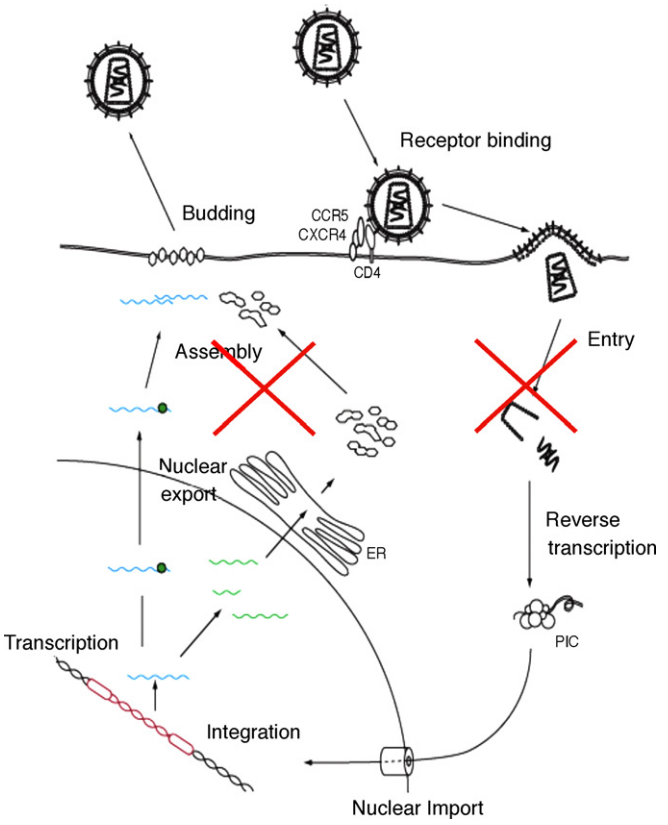

Fig. 5. The model of rhesus TRIM5α-mediated early and late restrictions. TRIM5α blocks early and late phases of the HIV-1 life cycle. In early restriction, TRIM5α recognizes the incoming HIV-1 core structure and accelerates its premature disassembly, while in late restriction it blocks HIV production by targeting HIV-1 Gag polyproteins before or during viral assembly.

4.2. TRIM5α as a therapeutic sequence for AIDS gene therapy

TRIM5α-mediated innate antiviral activities provide novel strategies toward anti-HIV-1 therapeutics. One is to use TRIM5αrh sequences for AIDS gene therapy (Anderson and Akkina, 2008; Sakuma et al., 2007b). We found that introduction of simian TRIM5α cDNA into human lymphocytes significantly delays HIV-1 replication in the cells (Sakuma et al., 2007b). Importantly, TRIM5αrh could block infection of HIV-1 with divergent Gag sequences from different subtypes (Sakuma et al., 2007b). This is a favorable feature as a therapeutic sequence for HIV-1 gene therapy, because it may not allow HIV-1 to escape easily from the restriction. It is also possible to generate transgenic macrophages that are highly resistant to HIV-1 infection by introducing TRIM5αrh into hematopoietic stem cells (Anderson and Akkina, 2005). Thus, TRIM5αrh-mediated AIDS gene therapy is an attractive and straightforward application of the antiviral activities of TRIM5α. Nevertheless, AIDS gene therapy is not a realistic option to treat HIV-1 infection in developing countries, where HIV-1 prevalence is high and public resources are limited.

4.3. Potential applications of TRIM5α restriction

For cost-effective HIV-1 therapy based on TRIM5α-mediated antiviral activities, the development of small molecules becomes essential. There are at least three possible strategies. The first is to map the minimum requirements of TRIM5αrh domains for antiviral activity and design a mini-TRIM5αrh protein as a small molecule. Recent studies with a series of TRIM5α mutants, however, have demonstrated complex, context-dependent requirement of TRIM5α motifs for antiviral activities (Diaz-Griffero et al., 2006; Javanbakht et al., 2005; Perez-Caballero et al., 2005; Sakuma et al., 2007c; Yap et al., 2007), suggesting the difficulty in designing short peptides that mimic the antiviral activity of TRIM5α. This implies that efficient intracellular delivery of a mini-TRIM5α in vivo would be a formidable task.

The second strategy is to induce or increase in vivo endogenous TRIM5α expression by using a small molecule. Because many TRIM family proteins, including TRIM5α, are interferon-responsive (Asaoka et al., 2005; Sakuma et al., 2007a), it is likely that systemic administration of interferon or interferon-like small molecules can enhance endogenous TRIM5α expression in vivo. Unfortunately, this presents a major problem in that TRIM5αhu does not exhibit strong anti-HIV-1 activity (Sakuma et al., 2007c; Stremlau et al., 2004). Thus, simple enhancement of endogenous TRIM5αhu expression may not be sufficient to induce potent anti-HIV-1 activities in vivo. Interferon-associated toxicity is an additional barrier for this approach.

The third strategy is to design a small molecule that modifies the conformation of TRIM5αhu to mimic TRIM5αrh. It is notable that alteration of arginine 332 in the TRIM5αhu B30.2 domain to proline, the residue found in TRIM5αrh, can confer the ability to restrict HIV-1 infection (Li et al., 2006; Yap et al., 2005). This observation suggests that small conformational changes can transform TRIM5αhu into a potent restriction factor. Although the approach of modifying TRIM5αhu by a small molecule is promising, there are also challenges in designing such molecules.

First, we have to find a lead compound that enhances the interaction between the two proteins, TRIM5αhu and HIV-1 Gag. This is an unusual task for identifying a small molecule, as most lead compounds are found in high-throughput screening to block the interaction between two proteins. Although interaction between TRIM5α and HIV-1 Gag was demonstrated by the association between TRIM5αrh and in vitro-assembled CA–NC complex or by incorporation of TRIM5αrh into HIV-1 virus-like particles (Sakuma

et al., 2007c; Stremlau et al., 2006), no simple biochemical method amenable for high-throughput screening is currently available to detect the interaction. Determination of the crystal structure of TRIM5α would help to improve our understanding of this interaction and to establish a simple TRIM5α-HIV-1 Gag binding assay which could be used to screen small molecules (James et al., 2007). Further understanding of the precise mechanisms of TRIM5α-mediated antiviral activities is critical to extend these approaches. It would be a powerful therapy against HIV-1 if we can treat patients with two small molecules, one of which modifies the TRIM5αhu to behave like TRIM5αrh, while the other enhances the levels of endogenous TRIM5αhu in vivo. Since recent studies have shown the anti-HIV-1 activity of other TRIM family proteins (Barr et al., 2008; Tissot and Mechti, 1995; Turelli et al., 2001; Uchil et al., 2008), we may also be able to apply these TRIM proteins for future HIV-1 therapy.

5. Late stages of the HIV-1 replication cycle as targets for novel antiviral agents (Eric O. Freed)

5.1. Background

Retroviral Gag proteins are synthesized in the cytoplasm of the infected cell and assemble into virus particles that typically bud from the plasma membrane (Fig. 6). Expression of Gag proteins alone is sufficient for the assembly and release of noninfectious virus-like particles (VLPs). The mature HIV-1 Gag proteins [matrix (MA), capsid (CA), nucleocapsid (NC), and p6] are generated concomitant with virus release upon cleavage of the Gag precursor by the viral protease (PR). HIV-1 release from the cell is mediated by an interaction between the "late" domain in the p6 domain of Gag and the cellular endosomal sorting factor Tsg101. After virus release, PR-mediated Gag processing leads to virus maturation, a morphological transition essential for virus infectivity. Cholesterol-enriched plasma membrane microdomains known as lipid rafts appear to be favored sites for particle assembly, and phosphatidylinositol (4,5) bisphosphate [$PI(4,5)P_2$] and the ADP ribosylation factor (Arf) proteins are important cellular cofactors in directing Gag to the cell surface. Rapid progress in understanding the late stages of the HIV-1 replication cycle is suggesting a variety of novel targets for the development of antiretroviral inhibitors that disrupt these late steps.

5.2. HIV-1 Gag trafficking

After Gag synthesis, the MA domain directs $Pr55^{Gag}$ to the plasma membrane (Fig. 6A and B) (Freed, 1998). The affinity of the MA domain for membrane is provided in part by a myristic acid moiety covalently attached to the N-terminal Gly of MA. Sequences in MA downstream of the myristate also contribute to membrane binding, in particular a highly basic patch of amino acid residues (Fig. 6A). Structural analysis of myristylated MA has indicated that the myristate moiety adopts both an exposed and a sequestered conformation (Tang et al., 2004).

Although the cellular determinants that determine the site of HIV-1 assembly remain to be fully defined, several studies have begun to identify host factors involved in Gag localization. We observed that depleting $PI(4,5)P_2$ from the plasma membrane leads to the retargeting of HIV-1 assembly to multivesicular bodies (MVBs) and severely disrupts virus particle production (Ono et al., 2004). Structural studies have suggested a direct interaction between the MA domain of HIV-1 Gag and $PI(4,5)P_2$ (Saad et al., 2006; Shkriabai et al., 2006). Interestingly, binding of MA to $PI(4,5)P_2$ appears to stabilize Gag-membrane binding, not only by providing electrostatic interactions, but also by triggering the exposed conformation of the N-terminal myristate (Saad et al., 2006). Our recent studies have identified two families of cellular proteins that play novel roles in retroviral Gag trafficking and virus release. Golgi-localized, gamma-ear containing, Arf-binding (GGA) proteins modulate HIV-1 budding, and the Arf proteins promote trafficking of retroviral Gag proteins to the plasma membrane (Joshi et al., 2008).

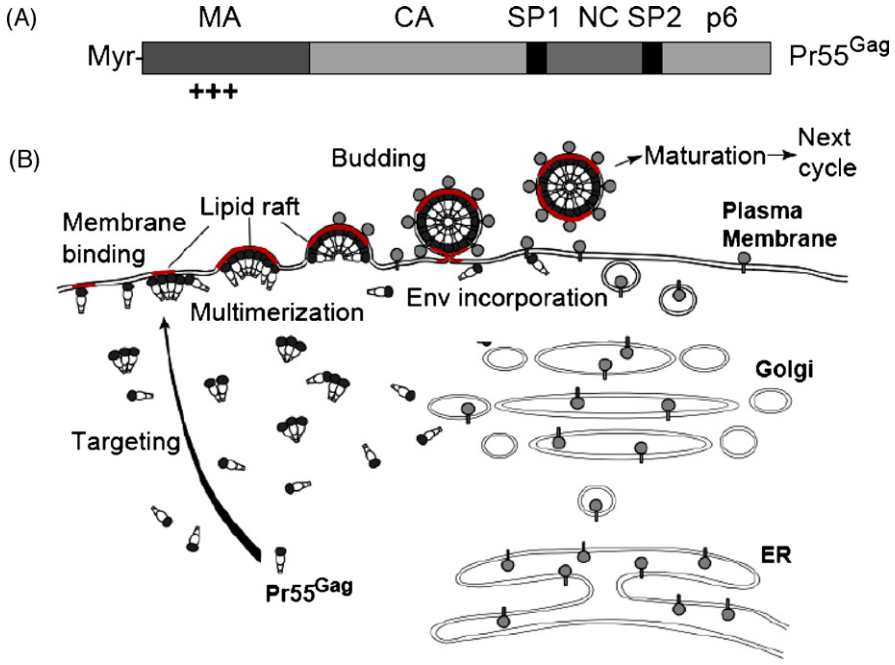

Fig. 6. HIV-1 Gag and virus assembly. (A) Domain organization of HIV-1 Gag. Matrix (MA), capsid (CA), nucleocapsid (NC) and p6 domains, and spacer peptides SP1 and SP2 are shown. The N-terminal myristate and the MA highly basic domain are denoted by Myr and +++, respectively. (B) Schematic representation of the HIV-1 assembly, release, and maturation pathway. The major steps are indicated. Lipid raft microdomains in the plasma membrane and the virion are shown in red. Adapted from Waheed et al. (2008), with permission, copyright Humana Press.

We have developed fluorescent imaging methods for visualizing the movement of fully replication-competent HIV-1 Gag in living cells. We applied this technology to follow the fate of Gag in primary monocyte-derived macrophages. We observe the accumulation of Gag in apparently internal compartments that bear tetraspanin markers. Significantly, this apparently internal Gag moves rapidly to the junction between infected macrophages and uninfected T cells upon formation of a stable macrophage/T-cell synapse (Gousset et al., 2008). These findings suggest that macrophages sequester virus particles internally until productive cell–cell contacts form, at which time the virus moves to the synapse and is transferred to the uninfected target cell.

5.3. Role of lipid rafts in HIV-1 replication

It is now well established that the plasma membrane is composed of various types of microdomains containing unique sets of proteins and lipids. Cholesterol-enriched "lipid rafts" have been associated with many cellular processes and have been implicated in the replication of a number of viruses, including HIV-1 (Ono and Freed, 2005). By using cholesterol-depleting agents, we recently demonstrated that plasma membrane cholesterol is important for efficient binding of Gag to the plasma membrane and for higher-order Gag multimerization (Ono et al., 2007). Because cholesterol-depleting agents like the cyclodextrins are highly cytotoxic, we have investigated the effect of a relatively non-cytotoxic cholesterol-binding compound, amphotericin B methyl ester (AME), on HIV-1 replication. We observe that AME inhibits HIV-1 replication at multiple stages: it potently blocks virus entry and also disrupts virus assembly and release (Waheed et al., 2006).

Interestingly, the ability of AME to block virus infectivity is reversed in virions bearing heterologous Env glycoproteins (e.g., those of murine leukemia virus or vesicular stomatitis virus) or HIV-1 Env glycoproteins with large truncations in the cytoplasmic tail of the transmembrane Env glycoprotein gp41 (Waheed et al., 2006). We were able to select for HIV-1 variants that are resistant to AME, and identified single amino acid mutations in the cytoplasmic tail of gp41 that confer resistance. Remarkably, mechanistic studies with these AME-resistant mutants revealed that the gp41 cytoplasmic tail mutations created novel sites for Env cleavage by PR. Thus, HIV-1 develops resistance to AME by acquiring mutations in gp41 that lead to PR-mediated cleavage of the gp41 cytoplasmic tail after the Env complex has been incorporated into virions (Waheed et al., 2007). Ongoing studies are characterizing the mechanism by which AME disrupts HIV-1 particle production.

5.4. Inhibition of virion maturation

PR-mediated cleavage of the Gag and Gag-Pol precursors leads to a dramatic change in virion morphology, a process known as maturation. The highly ordered nature of Gag processing and the strict dependence on complete processing for proper virion maturation make the Gag processing cascade an attractive target for drug development. Indeed, the betulinic acid derivative dimethylsuccinyl betulinic acid (PA-457 or bevirimat) potently inhibits HIV-1 infectivity by targeting a late Gag processing event: the cleavage of the CA-SP1 processing intermediate to mature CA (Fig. 6A) (Li et al., 2003; Zhou et al., 2005). By specifically disrupting this step in Gag processing, bevirimat treatment leads to the formation of noninfectious viral particles with aberrantly condensed cores.

We have selected, identified, and characterized a panel of mutations in the vicinity of the CA-SP1 cleavage site that confer resistance to bevirimat (Adamson et al., 2006). As bevirimat is currently undergoing phase II clinical trials, our current efforts are aimed at trying to anticipate the likelihood of bevirimat resistance arising in vivo. Specifically, we are investigating the interplay between bevirimat resistance and mutations in the viral protease that confer resistance to PR inhibitors.

In summary, future progress in understanding the late stages of HIV-1 replication (assembly, release, and maturation) and the identification of host factors that participate in these late steps is likely to lead to new antiretroviral strategies that target both viral and cellular factors. The discovery of additional anti-HIV drug candidates will greatly benefit HIV-infected patients, particularly in light of the rapid emergence of resistance to currently available antiretroviral therapies.

6. Role of Vpu protein in HIV-1 pathogenesis (Edward Stephens)

6.1. HIV-1 Vpu and its functions

HIV-1 encodes a small transmembrane protein known as Vpu, which is translated from the same mRNA that encodes the envelope glycoprotein (Cohen et al., 1988; Hout et al., 2004; Strebel et al., 1988). Unlike HIV-1, HIV-2 and most simian immunodeficiency viruses (SIV) lack a *vpu* gene. Exceptions include the SIVs isolated from chimpanzees (SIV_{cpz}) and some monkeys of the *Cercopithecus* genus (Barlow et al., 2003; Courgnaud et al., 2002, 2003; Huet et al., 1990; Peeters et al., 1989, 1992).

The Vpu protein has a short amino terminal domain, a transmembrane domain and longer cytoplasmic domain (Fig. 7). Studies on the Vpu protein from a laboratory-adapted subtype B HIV-1 have revealed two important functions in the virus replication cycle. Vpu is known to interact with the CD4 receptor in the rough endoplasmic reticulum (RER) and shunt it to the proteasome for degradation (Fujita et al., 1997; Schubert et al., 1998). Studies have shown that the highly conserved hinge region of the cytoplasmic domain of Vpu contains two casein kinase II sites which are required for the CD4 degradation (Paul and Jabbar, 1997; Schubert et al., 1994: Willey et al., 1992). Other studies have shown that the two predicted α-helical domains within the cytoplasmic domain and sequences within the transmembrane (TM) domain of the subtype B Vpu are also required for efficient degradation of CD4 (Hout et al., 2006a; Tiganos et al., 1998).

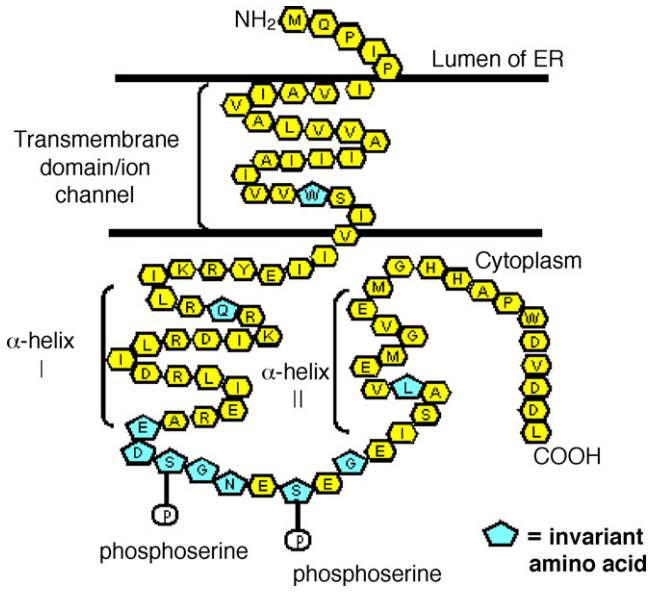

Fig. 7. HIV-1 Vpu. Schematic diagram of the Vpu protein (HXB2 corrected) and its orientation within the cellular membrane.

The other major function of the Vpu protein is the enhancement of virion release from cells, which has been associated with the transmembrane (TM) domain and its ion channel properties (Cordes et al., 2001; Ewart et al., 1996; Grice et al., 1997; Klimkait et al., 1990; Schubert et al., 1996a,b). Examination of cells infected with Δvpu HIV-1 viruses by electron microscopy reveal an altered different pattern of virus maturation that is characterized by viral particles "tethered" together at the cell surface and particles within intracellular vesicles (Klimkait et al., 1990). HIV-2, which lacks a *vpu* gene, has evolved a mechanism to mediate virus release through its Env protein (Bour and Strebel, 1996).

6.2. Simian–human immunodeficiency virus (SHIV) macaque model to study pathogenesis

Since SIVmac strains commonly used in pathogenicity studies do not encode for the Vpu protein, we have used the pathogenic simian human immunodeficiency virus (SHIV), which has the *tat*, *rev*, *vpu* and *env* genes of HIV-1 in a genetic background of the $SIV_{mac}239$, to analyze the role of Vpu in pathogenicity. Infection of macaques with these pathogenic X4 SHIVs results in high viral loads, a rapid loss of $CD4^+$ T cells within 1 month of infection, and severe depletion within lymphoid organs such as the thymus, lymph nodes, and spleen.

Using pathogenic molecular clones that express altered Vpu proteins, we showed that both the transmembrane domain and cytoplasmic domains of the Vpu protein contribute to the severe $CD4^+$ T cell loss in these macaques (Hout et al., 2005; Singh et al., 2003). These studies demonstrated that Vpu enhances the pathogenicity of SHIV and the domains previously shown to be important in cell culture studies are also important in disease progression. In addition, we recently showed that subtype C SHIV in which the *vpu* is exchanged with the *vpu* from a clinical isolate of subtype C HIV-1 ($SHIV_{SCVpu}$) had a decreased rate of $CD4^+$ T cell loss compared with the parental $SHIV_{KU\text{-}1bMC33}$ (Hill et al., 2008). These results suggest that different Vpu proteins can influence the rate of $CD4^+$ T cell loss in the SHIV/macaque model.

6.3. The "ion channel" activities of Vpu

As discussed above, previous studies have shown that the Vpu protein can form an ion channel and thus belongs to a group of viral proteins known as "viroporins." The best-studied viroporin has been the M2 protein of influenza, which has a similar overall structure to Vpu (Gonzalez and Carrasco, 2003). To determine if one viroporin could be substituted for another, we constructed a SHIV in which the TM domain of Vpu was replaced by the TM domain of M2 protein. This SHIV, known as $SHIV_{M2}$, was sensitive to the M2 channel blocking drug rimantadine, while retaining pathogenicity in macaques (Hout et al., 2006b). We further showed that a single amino acid substitution (alanine to histidine) within the TM domain of Vpu, which resulted in the formation of an M2 ion channel gate (His-X-X-X-Trp), was sufficient to introduce rimantadine sensitivity (Hout et al., 2006a). The TM domain containing the alanine-to-histidine substitution has also been examined by NMR spectroscopy, revealing that the alpha-helix was longer and had a greater tilt angle in bicelles when compared to the unmodified Vpu TM (Park et al., 2003; Park and Opella, 2007). Taken together, these results provided additional evidence that the TM domain of Vpu may form an ion channel, which could possibly be used as a target for novel antiviral drugs.

6.4. Identification of host cell targets of Vpu

Recent studies have identified host proteins that Vpu targets to ease virion release. The first protein identified was an interferon-inducible protein, BST-2/HM1.24/CD317/tetherin (Neil et al., 2008; Van Damme et al., 2008). Both groups have shown that exogenous expression of BST-2 inhibits the release of Δvpu HIV-1 and that suppression of BST-2 expression relieved the requirement for Vpu. Both groups showed that the transmembrane domain of Vpu was important in BST-2 mediated inhibition of virion release. It has been suggested that the unusual structure of BST-2, which is an integral membrane protein with a carboxyl terminal GPI anchor, may permit it to retain nascent enveloped virions on cellular membranes. Nevertheless, important questions remain about BST-2 and these include whether the virion contains a sufficient number of molecules to mediate the "tethering" phenotype observed in cells inoculated with a ΔvpuHIV-1 and whether BST-2 directly interacts with Vpu or acts through another molecule.

More recently, the protein calcium modulating cyclophilin ligand (CAML) was identified as a human host restriction factor that acts at the late budding step and is counteracted by Vpu (Varthakavi et al., 2008). Similar to the BST-2 studies, this group of investigators found that expression of human CAML in a non-restrictive cell line (AGM cells) resulted in strong restriction of virus release and that suppression of CAML expression in restrictive human cells eliminated the need for Vpu. They also showed that the hydrophilic N-terminal of CAML was required for the interaction with Vpu. The recent discoveries of these proteins as targets of Vpu and the elucidation of how they mechanistically involve its ion channel activity are clearly of interest, as they may serve as targets for novel anti-HIV-1 drugs.

7. Challenges for pursuing new HIV targets (Tomas Cihlar)

The following section summarizes some of the issues addressed by a panel discussion held as a part of the mini-symposium and further explores challenges likely to be encountered during the design and development of new classes of antiretroviral inhibitors acting on some of the discussed novel targets.

Most antiretroviral therapeutics currently in clinical development belong to existing clinically established classes of inhibitors. It can be argued that pursuing novel therapeutic targets is likely to be more challenging than further improving existing inhibitors against established targets, mainly because only very limited information is often available on naïve targets, and no established small-molecule inhibitors exist that can be used as templates for de novo drug design. Although high content screening, high through-put X-ray crystallography and structural analysis, computational modeling, more effective synthetic methods, and other technologies have been introduced into the drug discovery process over the past years, all of which should increase the chances of finding effective inhibitors of novel targets, the outcome still remains unpredictable.

Information about target structure and how it relates to its function is a very important aspect in the target selection process, as it can shed light on the "drugability" of the target of interest. The interference with target functions via a small molecule binding can be particularly challenging for protein–protein interactions that represent the basis of the vast majority of novel antiretroviral targets. In this regard, the high resolution X-ray structure of the complex of integrase catalytic core domain interacting with LEDGF/p75 integrase binding domain is a good example of the type of useful information that can be directly applied in the inhibitor design process. However, solving analogous structures for other targets and/or complexes with their respective binding partners will likely be a substantial challenge. Vif-APOBEC3G, TRIM5α-capsid, Vpu-tetherin and the oligomeric complex of immature Gag represent some of the most attractive protein complexes for which solving the structure would mean a major progress in further understanding their potential as antiretroviral targets. Applying advanced tech-

niques such as the analysis of two-dimensional crystals or electron cryotomography combined with high resolution X-ray crystallography or nuclear magnetic resonance have recently provided new information about the structure and conformation of key HIV proteins (Wright et al., 2007; Ganser-Pornillos et al., 2007) hence increasing the attractiveness of their targeting via rational drug design.

Another major challenge is the development of relevant screening assays that reliably model the physiological functions of the target of interest. Although this seems fairly obvious, it is not always fully appreciated. One example is the HIV-1 protease, one of the most explored antiviral targets ever. Many early biochemical screening assays with HIV-1 protease were established and used at pH <5.0 because of the highest enzymatic activity, yet it is unlikely that this would represent the native condition for virion maturation. One can argue that the protease inhibitor design was highly successful, as it led to multiple approved drugs, but it should be realized that most protease inhibitors have not been identified through a naïve high through-put screening under physiologically relevant conditions, but rather by a rational substrate derivatization using peptidomimetics, which is not a suitable strategy for most of the novel HIV targets. Hence, close attention should be paid to the functional validation of newly developed screening assays. In the absence of established small molecule inhibitors, functional assay validation can only be performed by using characterized mutant variants that either reduce or eliminate the target function in the context of viral infection. These mutants should behave the same way in the target screening assay. Alternatively, if available, biologically active peptides known to interact specifically with the target of interest can be used to validate screening assays. One such example relevant for novel HIV targets is a peptide CAI-1 that effectively inhibits the assembly of HIV-1 capsid in an in vitro biochemical assay (Sticht et al., 2005). Based on high-resolution X-ray structure, CAI-1 specifically binds to the C-terminal domain of HIV-1 capsid protein (Ternois et al., 2005) and its modified analog shows antiviral activity in HIV-1-infected cells (Zhang et al., 2008b). However, this is a rather rare example among the considered novel HIV targets.

A number of host factors that either support or restrict HIV replication have been discussed in this review, and the list is likely to grow longer, as suggested by results from recent RNA interference studies that have tentatively identified an array of additional new host factors important for HIV replication (Brass et al., 2008). Although sorting out the wealth of initial information remains to be completed, it will inevitably stimulate further exploration of host factors as potential targets for antiretroviral therapy. These approaches, however, will bring a new set of challenges in terms of potential adverse effects associated with targeting host proteins. In the ideal case, the structural determinants for native functions of targeted host factors should not overlap with those important for supporting viral replication. One example might be APOBEC3G, in which the cytidine deaminase active site is located in the C-terminal domain (Chen et al., 2008), whereas the binding region interacting with Vif appears to be localized to a distinct N-terminal domain (Schrofelbauer et al., 2004; Huthoff and Malim, 2007). In contrast, cyclophilin A (CypA) is an example of a host factor with relatively high liability for potential toxicity. CypA is a cellular prolyl-isomerase that binds to the HIV-1 capsid and either stabilizes and protects it against premature disassembly or facilitates the uncoating process (Luban, 2007; Li et al., 2007). However, the interaction of CypA with HIV-1 capsid involves its prolyl-isomerase active site and although a new non-immunosuppressive cyclosporine analog Debio-025 has shown initial antiviral effects in HIV/HCV co-infected patients (Flisiak et al., 2008), it remains to be seen how well the chronic drug treatment will be tolerated.

The goal of the ICAR mini-symposium and of this review was not to exhaustively cover all potential novel HIV targets, but rather to provide examples of some well-characterized essential steps in the HIV replication cycle that may be suitable for interference by novel types of inhibitors. After almost exhaustive exploration of HIV-encoded enzymes as therapeutic targets, we are now at the threshold of a new and challenging era in the discovery of novel HIV therapeutics that will require the systematic exploration of functionally and structurally more complex steps in viral replication. To succeed in further expanding the existing arsenal of antiretroviral drugs by focusing on novel targets, close interdisciplinary collaborative effort and concerted interaction between academia and industry will be needed more than ever.

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References

Adamson, C.S., Ablan, S.D., Boeras, I., Goila-Gaur, R., Soheilian, F., Nagashima, K., Li, F., Salzwedel, K., Sakalian, M., Wild, C.T., Freed, E.O., 2006. In vitro resistance to the human immunodeficiency virus type 1 maturation inhibitor PA-457 (Bevirimat). J. Virol. 80, 10957–10971.

Alce, T.M., Popik, W., 2004. APOBEC3G is incorporated into virus-like particles by a direct interaction with HIV-1 Gag nucleocapsid protein. J. Biol. Chem. 279, 34083–34086.

Anderson, J., Akkina, R., 2008. Human immunodeficiency virus type 1 restriction by human-rhesus chimeric tripartite motif 5alpha (TRIM 5alpha) in CD34(+) cell-derived macrophages in vitro and in T cells in vivo in severe combined immunodeficient (SCID-hu) mice transplanted with human fetal tissue. Hum. Gene Ther. 19, 217–228.

Anderson, J., Akkina, R., 2005. TRIM5alpharh expression restricts HIV-1 infection in lentiviral vector-transduced CD34+-cell-derived macrophages. Mol. Ther. 12, 687–696.

Asaoka, K., Ikeda, K., Hishinuma, T., Horie-Inoue, K., Takeda, S., Inoue, S., 2005. A retrovirus restriction factor TRIM5alpha is transcriptionally regulated by interferons. Biochem. Biophys. Res. Commun. 338, 1950–1956.

Auclair, J.R., Green, K.M., Shandilya, S., Evans, J.E., Somasundaran, M., Schiffer, C.A., 2007. Mass spectrometry analysis of HIV-1 Vif reveals an increase in ordered structure upon oligomerization in regions necessary for viral infectivity. Proteins 69, 270–284.

Balaji, S., Kalpana, R., Shapshak, P., 2006. Paradigm development: comparative and predictive 3D modeling of HIV-1 virion infectivity factor (Vif). Bioinformation 1, 290–309.

Barlow, K.L., Ajao, A.O., Clewley, J.P., 2003. Characterization of a novel simian immunodeficiency virus (SIVmonNG1) genome sequence from a mona monkey (*Cercopithecus mona*). J. Virol. 77, 6879–6888.

Barr, S.D., Smiley, J.R., Bushman, F.D., 2008. The interferon response inhibits HIV particle production by induction of TRIM22. PLoS Pathog. 4, e1000007.

Bartholomeeusen, K., De Rijck, J., Busschots, K., Desender, L., Gijsbers, R., Emiliani, S., Benarous, R., Debyser, Z., Christ, F., 2007. Differential interaction of HIV-1 integrase and JPO2 with the C terminus of LEDGF/p75. J. Mol. Biol. 372, 407–421.

Bogerd, H.P., Doehle, B.P., Wiegand, H.L., Cullen, B.R., 2004. A single amino acid difference in the host APOBEC3G protein controls the primate species specificity of HIV type 1 virion infectivity factor. Proc. Natl. Acad. Sci. U.S.A. 101, 3770–3774.

Bour, S., Strebel, K., 1996. The human immunodeficiency virus (HIV) type 2 envelope is the functional complement to HIV-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., Elledge, S.J., 2008. Identification of host proteins required for HIV infection through a functional genomic screen. Science 319, 921–926.

Busschots, K., Voet, A., De Maeyer, M., Rain, J.C., Emiliani, S., Benarous, R., Desender, L., Debyser, Z., Christ, F., 2007. Identification of the LEDGF/p75 binding site in HIV-1 integrase. J. Mol. Biol. 365, 1480–1492.

Cen, S., Guo, F., Niu, M., Saadatmand, J., Deflassieux, J., Kleiman, L., 2004. The interaction between HIV-1 Gag and APOBEC3G. J. Biol. Chem. 279, 33177–33184.

Chen, K.M., Harjes, E., Gross, P.J., Fahmey, A., Lu, Y., Shindo, K., Harris, R.S., Matsuo, H., 2008. Structure of the DNA deaminase domain of HIV-1 restriction factor APOBEC3G. Nature 452, 116–119.

Cherepanov, P., Ambrosio, A.L., Rahman, S., Ellenberger, T., Engelman, A., 2005. Structural basis for the recognition between HIV-1 integrase and transcriptional coactivator p75. Proc. Natl. Acad. Sci. U.S.A. 102, 17308–17313.

Cherepanov, P., Maertens, G., Proost, P., Devreese, B., Van Beeumen, J., Engelborghs, Y., De Clercq, E., Debyser, Z., 2003. HIV-1 integrase forms stable tetramers and associates with LEDGF/p75 protein in human cells. J. Biol. Chem. 278, 372–381.

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

Clotet, B., Bellos, N., Molina, J.M., Cooper, D., Goffard, J.C., Lazzarin, A., Wohrmann, A., Katlama, C., Wilkin, T., Haubrich, R., Cohen, C., Farthing, C., Jayaweera, D., Markowitz, M., Ruane, P., Spinosa-Guzman, S., Lefebvre, E., 2007. Efficacy and safety of darunavir–ritonavir at week 48 in treatment-experienced patients with HIV-1 infection in POWER 1 and 2: a pooled subgroup analysis of data from two randomised trials. Lancet 369, 1169–1178.

Cohen, E.A., Terwilliger, E.F., Sodroski, J.G., Haseltine, W.A., 1988. Identification of a protein encoded by the vpu gene of HIV-1. Nature 334, 532–534.

Conticello, S.G., Harris, R.S., Neuberger, M.S., 2003. The Vif protein of HIV triggers degradation of the human antiretroviral DNA deaminase APOBEC3G. Curr. Biol. 13, 2009–2013.

Cordes, F.S., Kukol, A., Forrest, L.R., Arkin, I.T., Sansom, M.S., Fischer, W.B., 2001. The structure of the HIV-1 Vpu ion channel: modelling and simulation studies. Biochim. Biophys. Acta 1512, 291–298.

Courgnaud, V., Abela, B., Pourrut, X., Mpoudi-Ngole, E., Loul, S., Delaporte, E., Peeters, M., 2003. Identification of a new simian immunodeficiency virus lineage with a vpu gene present among different cercopithecus monkeys (*C. mona*, *C. cephus*, and *C. nictitans*) from Cameroon. J. Virol. 77, 12523–12534.

Courgnaud, V., Salemi, M., Pourrut, X., Mpoudi-Ngole, E., Abela, B., Auzel, P., Bibollet-Ruche, F., Hahn, B., Vandamme, A.M., Delaporte, E., Peeters, M., 2002. Characterization of a novel simian immunodeficiency virus with a vpu gene from greater spot-nosed monkeys (*Cercopithecus nictitans*) provides new insights into simian/human immunodeficiency virus phylogeny. J. Virol. 76, 8298–8309.

Dang, Y., Wang, X., Esselman, W.J., Zheng, Y.H., 2006. Identification of APOBEC3DE as another antiretroviral factor from the human APOBEC family. J. Virol. 80, 10522–10533.

Debyser, Z., 2008. LEDGF/P75 as a co-factor of HIV-1 integrase and as a new antiviral target. Antiviral Res. 78, A18.

Demirov, D.G., Ono, A., Orenstein, J.M., Freed, E.O., 2002. Overexpression of the N-terminal domain of TSG101 inhibits HIV-1 budding by blocking late domain function. Proc. Natl. Acad. Sci. U.S.A. 99, 955–960.

De Rijck, J., Vandekerckhove, L., Gijsbers, R., Hombrouck, A., Hendrix, J., Vercammen, J., Engelborghs, Y., Christ, F., Debyser, Z., 2006. Overexpression of the lens epithelium-derived growth factor/p75 integrase binding domain inhibits human immunodeficiency virus replication. J. Virol. 80, 11498–11509.

Diaz-Griffero, F., Vandegraaff, N., Li, Y., McGee-Estrada, K., Stremlau, M., Welikala, S., Si, Z., Engelman, A., Sodroski, J., 2006. Requirements for capsid-binding and an effector function in TRIMCyp-mediated restriction of HIV-1. Virology.

Douaisi, M., Dussart, S., Courcoul, M., Bessou, G., Lerner, E.C., Decroly, E., Vigne, R., 2005. The tyrosine kinases Fyn and Hck favor the recruitment of tyrosine-phosphorylated APOBEC3G into vif-defective HIV-1 particles. Biochem. Biophys. Res. Commun. 329, 917–924.

Emiliani, S., Mousnier, A., Busschots, K., Maroun, M., Van Maele, B., Tempe, D., Vandekerckhove, L., Moisant, F., Ben-Slama, L., Witvrouw, M., Christ, F., Rain, J.C., Dargemont, C., Debyser, Z., Benarous, R., 2005. Integrase mutants defective for interaction with LEDGF/p75 are impaired in chromosome tethering and HIV-1 replication. J. Biol. Chem. 280, 25517–25523.

Este, J.A., Telenti, A., 2007. HIV entry inhibitors. Lancet 370, 81–88.

Ewart, G.D., Sutherland, T., Gage, P.W., Cox, G.B., 1996. The Vpu protein of human immunodeficiency virus type 1 forms cation-selective ion channels. J. Virol. 70, 7108–7115.

Fatkenheuer, G., Pozniak, A.L., Johnson, M.A., Plettenberg, A., Staszewski, S., Hoepelman, A.I., Saag, M.S., Goebel, F.D., Rockstroh, J.K., Dezube, B.J., Jenkins, T.M., Medhurst, C., Sullivan, J.F., Ridgway, C., Abel, S., James, I.T., Youle, M., van der Ryst, E., 2005. Efficacy of short-term monotherapy with maraviroc, a new CCR5 antagonist, in patients infected with HIV-1. Nat. Med. 11, 1170–1172.

Fellay, J., Shianna, K.V., Ge, D., Colombo, S., Ledergerber, B., Weale, M., Zhang, K., Gumbs, C., Castagna, A., Cossarizza, A., Cozzi-Lepri, A., De Luca, A., Easterbrook, P., Francioli, P., Mallal, S., Martinez-Picado, J., Miro, J.M., Obel, N., Smith, J.P., Wyniger, J., Descombes, P., Antonarakis, S.E., Letvin, N.L., McMichael, A.J., Haynes, B.F., Telenti, A., Goldstein, D.B., 2007. A whole-genome association study of major determinants for host control of HIV-1. Science 317, 944–947.

Flisiak, R., Horban, A., Gallay, P., Bobardt, M., Selvarajah, S., Wiercinska-Drapalo, A., Siwak, E., Cielniak, I., Higersberger, J., Kierkus, J., Aeschlimann, C., Grosgurin, P., Nicolas-Métral, V., Dumont, J.M., Porchet, H., Crabbé, R., Scalfaro, P., 2008. The cyclophilin inhibitor Debio-025 shows potent anti-hepatitis C effect in patients coinfected with hepatitis C and human immunodeficiency virus. Hepatology 47, 817–826.

Freed, E.O., 1998. HIV-1 Gag proteins: diverse functions in the virus life cycle. Virology 251, 1–15.

Freed, E., 2008. Late stages of the HIV-1 replication cycle as targets for novel antiviral agents. Antiviral Res. 78, A18.

Fujita, M., Sakurai, A., Yoshida, A., Miyaura, M., Koyama, A.H., Sakai, K., Adachi, A., 2003. Amino acid residues 88 and 89 in the central hydrophilic region of human immunodeficiency virus type 1 Vif are critical for viral infectivity by enhancing the steady-state expression of Vif. J. Virol. 77, 1626–1632.

Fujita, K., Omura, S., Silver, J., 1997. Rapid degradation of CD4 in cells expressing human immunodeficiency virus type 1 Env and Vpu is blocked by proteasome inhibitors. J. Gen. Virol. 78, 619–625.

Gabuzda, D., Lawrence, K., Langhoff, E., Terwilliger, E., Dorfman, T., Haseltine, W.A., Sodroski, J., 1992. Role of vif in replication of human immunodeficiency virus type 1 in CD4+ T lymphocytes. J. Virol. 66, 6489–6495.

Ganser-Pornillos, B.K., Cheng, A., Yeager, M., 2007. Structure of full-length HIV-1 CA: a model for the mature capsid lattice. Cell 131, 70–79.

Garrus, J.E., von Schwedler, U.K., Pornillos, O.W., Morham, S.G., Zavitz, K.H., Wang, H.E., Wettstein, D.A., Stray, K.M., Cote, M., Rich, R.L., Myszka, D.G., Sundquist, W.I., 2001. Tsg101 and the vacuolar protein sorting pathway are essential for HIV-1 budding. Cell 107, 55–65.

Ge, H., Si, Y., Roeder, R.G., 1998. Isolation of cDNAs encoding novel transcription coactivators p52 and p75 reveals an alternate regulatory mechanism of transcriptional activation. EMBO J. 17, 6723–6729.

Gonzalez, M.E., Carrasco, L., 2003. Viroporins. FEBS Lett. 552, 28–34.

Gousset, K., Ablan, S.D., Coren, L.V., Ono, A., Soheilian, F., Nagashima, K., Ott, D.E., Freed, E.O., 2008. Real-time visualization of HIV-1 GAG trafficking in infected macrophages. PLoS Pathog. 4, e1000015.

Greene, W., 2008. APOBEC 3G: innate defense against retroviruses and retroelements. Antiviral Res. 78, A18.

Grice, A.L., Kerr, I.D., Sansom, M.S., 1997. Ion channels formed by HIV-1 Vpu: a modelling and simulation study. FEBS Lett. 405, 299–304.

Grinsztejn, B., Nguyen, B.Y., Katlama, C., Gatell, J.M., Lazzarin, A., Vittecoq, D., Gonzalez, C.J., Chen, J., Harvey, C.M., Isaacs, R.D., 2007. Safety and efficacy of the HIV-1 integrase inhibitor raltegravir (MK-0518) in treatment-experienced patients with multidrug-resistant virus: a phase II randomised controlled trial. Lancet 369, 1261–1269.

Gulick, R.M., Su, Z., Flexner, C., Hughes, M.D., Skolnik, P.R., Wilkin, T.J., Gross, R., Krambrink, A., Coakley, E., Greaves, W.L., Zolopa, A., Reichman, R., Godfrey, C., Hirsch, M., Kuritzkes, D.R., 2007. Phase 2 study of the safety and efficacy of vicriviroc, a CCR5 inhibitor, in HIV-1-infected, treatment-experienced patients: AIDS clinical trials group 5211. J. Infect. Dis. 196, 304–312.

Harris, R.S., Bishop, K.N., Sheehy, A.M., Craig, H.M., Petersen-Mahrt, S.K., Watt, I.N., Neuberger, M.S., Malim, M.H., 2003. DNA deamination mediates innate immunity to retroviral infection. Cell 113, 803–809.

Hicks, C.B., Cahn, P., Cooper, D.A., Walmsley, S.L., Katlama, C., Clotet, B., Lazzarin, A., Johnson, M.A., Neubacher, D., Mayers, D., Valdez, H., 2006. Durable efficacy of tipranavir-ritonavir in combination with an optimised background regimen of antiretroviral drugs for treatment-experienced HIV-1-infected patients at 48 weeks in the randomized evaluation of strategic intervention in multi-drug resistant patients with tipranavir (RESIST) studies: an analysis of combined data from two randomised open-label trials. Lancet 368, 466–475.

Hill, M.S., Ruiz, A., Pacyniak, E., Pinson, D.M., Culley, N., Yen, B., Wong, S.W., Stephens, E.B., 2008. Modulation of the severe $CD4^+$ T cell loss caused by a pathogenic simian–human immunodeficiency virus by replacement of the subtype B vpu with the vpu from a subtype C HIV-1 isolate. Virology 371, 86–97.

Hombrouck, A., De Rijck, J., Hendrix, J., Vandekerckhove, L., Voet, A., Maeyer, M.D., Witvrouw, M., Engelborghs, Y., Christ, F., Gijsbers, R., Debyser, Z., 2007. Virus evolution reveals an exclusive role for LEDGF/p75 in chromosomal tethering of HIV. PLoS Pathog. 3, e47.

Hout, D.R., Gomez, M.L., Pacyniak, E., Gomez, L.M., Fegley, B., Mulcahy, E.R., Hill, M.S., Culley, N., Pinson, D.M., Nothnick, W., Powers, M.F., Wong, S.W., Stephens, E.B., 2006a. Substitution of the transmembrane domain of Vpu in simian–human immunodeficiency virus ($SHIV_{KU1bMC33}$) with that of M2 of influenza A results in a virus that is sensitive to inhibitors of the M2 ion channel and is pathogenic for pig-tailed macaques. Virology 344, 541–559.

Hout, D.R., Gomez, L.M., Pacyniak, E., Miller, J.M., Hill, M.S., Stephens, E.B., 2006b. A single amino acid substitution within the transmembrane domain of the human immunodeficiency virus type 1 Vpu protein renders simian–human immunodeficiency virus ($SHIV_{KU-1bMC33}$) susceptible to rimantadine. Virology 348, 449–461.

Hout, D.R., Gomez, M.L., Pacyniak, E., Gomez, L.M., Inbody, S.H., Mulcahy, E.R., Culley, N., Pinson, D.M., Powers, M.F., Wong, S.W., Stephens, E.B., 2005. Scrambling of the amino acids within the transmembrane domain of Vpu results in a simian–human immunodeficiency virus ($SHIV_{TM}$) that is less pathogenic for pig-tailed macaques. Virology 339, 56–69.

Hout, D.R., Gomez, M.L., Pacyniak, E., Mulcahy, E.R., Gomez, L.M., Jackson, M., Flick, M., Fegley, B., McCormick, C., Wisdom, B.J., Culley, N., Pinson, D.M., Powers, M., Wong, S.W., Stephens, E.B., 2004. Fusion of the upstream vpu sequences to the env of simian human immunodeficiency virus ($SHIV_{KU-1bMC33}$) results in the synthesis of two envelope precursor proteins, increased numbers of virus particles associated with the cell surface and is pathogenic for pig-tailed macaques. Virology 323, 91–107.

Huet, T., Cheynier, R., Meyerhans, A., Roelants, G., Wain-Hobson, S., 1990. Genetic organization of a chimpanzee lentivirus related to HIV-1. Nature 345, 356–359.

Huthoff, H., Malim, M., 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.

Ikeda, Y., 2008. Trim 5 alpha-mediated late restriction on HIV-1 production. Antiviral Res. 78, A18.

James, L.C., Keeble, A.H., Khan, Z., Rhodes, D.A., Trowsdale, J., 2007. Structural basis for PRYSPRY-mediated tripartite motif (TRIM) protein function. Proc. Natl. Acad. Sci. U.S.A. 104, 6200–6205.

Javanbakht, H., Diaz-Griffero, F., Stremlau, M., Si, Z., Sodroski, J., 2005. The contribution of RING and B-box 2 domains to retroviral restriction mediated by monkey TRIM5alpha. J. Biol. Chem. 280, 26933–26940.

Joshi, A., Garg, H., Nagashima, K., Bonifacino, J.S., Freed, E.O., 2008. GGA and Arf proteins modulate retrovirus assembly and release. Mol. Cell 30, 227–238.

Klimkait, T., Strebel, K., Hoggan, M.D., Martin, M.A., Orenstein, J.M., 1990. The human immunodeficiency virus type 1-specific protein Vpu is required for efficient virus maturation and release. J. Virol. 64, 621–629.

Lazzarin, A., Campbell, T., Clotet, B., Johnson, M., Katlama, C., Moll, A., Towner, W., Trottier, B., Peeters, M., Vingerhoets, J., de Smedt, G., Baeten, B., Beets, G., Sinha, R., Woodfall, B., 2007. Efficacy and safety of TMC125 (etravirine) in treatment-experienced HIV-1-infected patients in DUET-2: 24-week results from a randomised, double-blind, placebo-controlled trial. Lancet 370, 39–48.

Lecossier, D., Bouchonnet, F., Clavel, F., Hance, A.J., 2003. Hypermutation of HIV-1 DNA in the absence of the Vif protein. Science 300, 1112.

Li, F., Goila-Gaur, R., Salzwedel, K., Kilgore, N.R., Reddick, M., Matallana, C., Castillo, A., Zoumplis, D., Martin, D.E., Orenstein, J.M., Allaway, G.P., Freed, E.O., Wild, C.T., 2003. PA-457: a potent HIV inhibitor that disrupts core condensation by targeting a late step in Gag processing. Proc. Natl. Acad. Sci. U.S.A. 100, 13555–13560.

Li, J., Tang, S., Hewlett, I., Yang, M., 2007. HIV-1 capsid protein and cyclophilin a as new targets for anti-AIDS therapeutic agents. Infect. Disord. Drug Targets 7, 238–244.

Li, Y., Li, X., Stremlau, M., Lee, M., Sodroski, J., 2006. Removal of arginine 332 allows human TRIM5alpha to bind human immunodeficiency virus capsids and to restrict infection. J. Virol. 80, 6738–6744.

Llano, M., Saenz, D.T., Meehan, A., Wongthida, P., Peretz, M., Walker, W.H., Teo, W., Poeschla, E.M., 2006. An essential role for LEDGF/p75 in HIV integration. Science 314, 461–464.

Llano, M., Vanegas, M., Fregoso, O., Saenz, D., Chung, S., Peretz, M., Poeschla, E.M., 2004. LEDGF/p75 determines cellular trafficking of diverse lentiviral but not murine oncoretroviral integrase proteins and is a component of functional lentiviral preintegration complexes. J. Virol. 78, 9524–9537.

Luban, J., 2007. Cyclophilin A, TRIM5, and resistance to human immunodeficiency virus type 1 infection. J. Virol. 81, 1054–1061, 2007.

Luo, K., Liu, B., Xiao, Z., Yu, Y., Yu, X., Gorelick, R., Yu, X.F., 2004. Amino-terminal region of the human immunodeficiency virus type 1 nucleocapsid is required for human APOBEC3G packaging. J. Virol. 78, 11841–11852.

Lv, W., Liu, Z., Jin, H., Yu, X., Zhang, L., Zhang, L., 2007. Three-dimensional structure of HIV-1 VIF constructed by comparative modeling and the function characterization analyzed by molecular dynamics simulation. Org. Biomol. Chem. 5, 617–626, 2007.

Maertens, G.N., Cherepanov, P., Engelman, A., 2006. Transcriptional co-activator p75 binds and tethers the Myc-interacting protein JPO2 to chromatin. J. Cell Sci. 119, 2563–2571.

Maertens, G., Cherepanov, P., Pluymers, W., Busschots, K., De Clercq, E., Debyser, Z., Engelborghs, Y., 2003. LEDGF/p75 is essential for nuclear and chromosomal targeting of HIV-1 integrase in human cells. J. Biol. Chem. 278, 33528–33539.

Mangeat, B., Turelli, P., Caron, G., Friedli, M., Perrin, L., Trono, D., 2003. Broad antiretroviral defence by human APOBEC3G through lethal editing of nascent reverse transcripts. Nature 424, 99–103.

Mangeat, B., Turelli, P., Liao, S., Trono, D., 2004. A single amino acid determinant governs the species-specific sensitivity of APOBEC3G to Vif action. J. Biol. Chem. 279, 14481–14483.

Mariani, R., Chen, D., Schrofelbauer, B., Navarro, F., Konig, R., Bollman, B., Munk, C., Nymark-McMahon, H., Landau, N.R., 2003. Species-specific exclusion of APOBEC3G from HIV-1 virions by Vif. Cell 114, 21–31.

Marin, M., Rose, K.M., Kozak, S.L., Kabat, D., 2003. HIV-1 Vif protein binds the editing enzyme APOBEC3G and induces its degradation. Nat. Med. 9, 1398–1403.

Marshall, H.M., Ronen, K., Berry, C., Llano, M., Sutherland, H., Saenz, D., Bickmore, W., Poeschla, E., Bushman, F.D., 2007. Role of PSIP1/LEDGF/p75 in lentiviral infectivity and integration targeting. PLoS ONE 2, e1340.

Mehle, A., Goncalves, J., Santa-Marta, M., McPike, M., 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., Gabuzda, D., 2006. A zinc-binding region in Vif binds Cul5 and determines cullin selection. J. Biol. Chem. 281, 17259–17265.

Mehle, A., Wilson, H., Zhang, C., Brzier, A.J., McPike, M., Pery, E., Gabuzda, D., 2007. Identification of an APOBEC3G binding site in human immunodeficiency type 1 Vif and inhibitors of Vif-APOBEC3G binding. J. Virol. 81, 13235–13241.

Neil, S.J., Zang, T., 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., Sheehy, A.M., 2005. Antiviral function of APOBEC3G can be dissociated from cytidine deaminase activity. Curr. Biol. 15, 166–170.

Ono, A., Ablan, S.D., Lockett, S.J., Nagashima, K., Freed, E.O., 2004. Phosphatidylinositol (4,5) bisphosphate regulates HIV-1 Gag targeting to the plasma membrane. Proc. Natl. Acad. Sci. U.S.A. 101, 14889–14894.

Ono, A., Freed, E.O., 2005. Role of lipid rafts in virus replication. Adv. Virus Res. 64, 311–358.

Ono, A., Waheed, A.A., Freed, E.O., 2007. Depletion of cellular cholesterol inhibits membrane binding and higher-order multimerization of human immunodeficiency virus type 1 Gag. Virology 360, 27–35.

Ortiz, M., Bleiber, G., Martinez, R., Kaessmann, H., Telenti, A., 2006. Patterns of evolution of host proteins involved in retroviral pathogenesis. Retrovirology 3, 11.

Park, S.H., Mrse, A.A., Nevzorov, A.A., Mesleh, M.F., Oblatt-Montal, M., Montal, M., Opella, S.J., 2003. Three-dimensional structure of the channel-forming transmembrane domain of virus protein "u" (Vpu) from HIV-1. J. Mol. Biol. 333, 409–424.

Park, S.H., Opella, S.J., 2007. Conformational changes induced by a single amino acid substitution in the trans-membrane domain of Vpu: implications for HIV-1 susceptibility to channel blocking drugs. Protein Sci. 16, 2205–2215.

Paul, M., Jabbar, M.A., 1997. Phosphorylation of both phosphoacceptor sites in the HIV-1 Vpu cytoplasmic domain is essential for Vpu-mediated ER degradation of CD4. Virology 232, 207–216.

Peeters, M., Fransen, K., Delaporte, E., Van den, H.M., Gershy-Damet, G.M., Kestens, L., van der, G.G., Piot, P., 1992. Isolation and characterization of a new chimpanzee lentivirus (simian immunodeficiency virus isolate cpz-ant) from a wild-captured chimpanzee. AIDS 6, 447–451.

Peeters, M., Honore, C., Huet, T., Bedjabaga, L., Ossari, S., Bussi, P., Cooper, R.W., Delaporte, E., 1989. Isolation and partial characterization of an HIV-related virus occurring naturally in chimpanzees in Gabon. AIDS 3, 625–630.

Perez-Caballero, D., Hatziioannou, T., Yang, A., Cowan, S., Bieniasz, P.D., 2005. Human tripartite motif 5alpha domains responsible for retrovirus restriction activity and specificity. J. Virol. 79, 8969–8978.

Prochnow, C., Bransteitter, R., Klein, M.G., Goodman, M.F., Chen, X.S., 2007. The APOBEC-2 crystal structure and functional implications for the deaminase AID. Nature 445, 447–451.

Russell, R., 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.

Saad, J.S., Miller, J., Tai, J., Kim, A., Ghanam, R.H., Summers, M.F., 2006. Structural basis for targeting HIV-1 Gag proteins to the plasma membrane for virus assembly. Proc. Natl. Acad. Sci. U.S.A. 103, 11364–11369.

Sakuma, R., Mael, A.A., Ikeda, Y., 2007a. Alpha interferon enhances TRIM5alpha-mediated antiviral activities in human and rhesus monkey cells. J. Virol. 81, 10201–10206.

Sakuma, R., Noser, J.A., Ohmine, S., Ikeda, Y., 2007b. Inhibition of HIV-1 replication by simian restriction factors, TRIM5alpha and APOBEC3G. Gene Ther. 14, 185–189.

Sakuma, R., Noser, J.A., Ohmine, S., Ikeda, Y., 2007c. Rhesus monkey TRIM5alpha restricts HIV-1 production through rapid degradation of viral Gag polyproteins. Nat. Med. 13, 631–635.

Sawyer, S.L., Wu, L.I., Emerman, M., Malik, H.S., 2005. Positive selection of primate TRIM5alpha identifies a critical species-specific retroviral restriction domain. Proc. Natl. Acad. Sci. U.S.A. 102, 2832–2837.

Schafer, A., Bogerd, H.P., Cullen, B.R., 2004. Specific packaging of APOBEC3G into HIV-1 virions is mediated by the nucleocapsid domain of the gag polyprotein precursor. Virology 328, 163–168.

Schrofelbauer, B., Chen, D., Landau, N.R., 2004. A single amino acid of APOBEC3G controls its species-specific interaction with virion infectivity factor (Vif). Proc. Natl. Acad. Sci. U.S.A. 101, 3927–3932.

Schubert, U., Anton, L.C., Bacik, I., Cox, J.H., Bour, S., Bennink, J.R., Orlowski, M., Strebel, K., Yewdell, J.W., 1998. CD4 glycoprotein degradation induced by human immunodeficiency virus type 1 Vpu protein requires the function of proteasomes and the ubiquitin-conjugating pathway. J. Virol. 72, 2280–2288.

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

Schubert, U., Ferrer-Montiel, A.V., Oblatt-Montal, M., Henklein, P., Strebel, K., Montal, M., 1996b. Identification of an ion channel activity of the Vpu transmembrane domain and its involvement in the regulation of virus release from HIV-1-infected cells. FEBS Lett. 398, 12–18.

Schubert, U., Henklein, P., Boldyreff, B., Wingender, E., Strebel, K., Porstmann, T., 1994. The human immunodeficiency virus type 1 encoded Vpu protein is phosphorylated by casein kinase-2 (CK-2) at positions Ser52 and Ser56 within a predicted alpha-helix-turn–alpha-helix-motif. J. Mol. Biol. 236, 16–25.

Sheehy, A.M., Gaddis, N.C., 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.

Sheehy, A.M., Gaddis, N.C., Malim, M.H., 2003. The antiretroviral enzyme APOBEC3G is degraded by the proteasome in response to HIV-1 Vif. Nat. Med. 9, 1404–1407.

Shkriabai, N., Datta, S.A., Zhao, Z., Hess, S., Rein, A., Kvaratskhelia, M., 2006. Interactions of HIV-1 Gag with assembly cofactors. Biochemistry 45, 4077–4083.

Shun, M.C., Raghavendra, N.K., Vandegraaff, N., Daigle, J.E., Hughes, S., Kellam, P., Cherepanov, P., Engelman, A., 2007. LEDGF/p75 functions downstream from preintegration complex formation to effect gene-specific HIV-1 integration. Genes Dev. 21, 1767–1778.

Simon, V., Zennou, V., Murray, D., Huang, Y., Ho, D.D., 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.

Singh, D.K., Griffin, D.M., Pacyniak, E., Jackson, M., Werle, M.J., Wisdom, B., Sun, F., Hout, D.R., Pinson, D.M., Gunderson, R.S., Powers, M.F., Wong, S.W., Stephens, E.B., 2003. The presence of the casein kinase II phosphorylation sites of Vpu enhances the $CD4^+$ T cell loss caused by the simian–human immunodeficiency virus $SHIV_{KU\text{-}IbMC33}$ in pig-tailed macaques. Virology 313, 435–451.

Singh, D.P., Ohguro, N., Kikuchi, T., Sueno, T., Reddy, V.N., Yuge, K., Chylack Jr., L.T., Shinohara, T., 2000. Lens epithelium-derived growth factor: effects on growth and survival of lens epithelial cells, keratinocytes, and fibroblasts. Biochem. Biophys. Res. Commun. 267, 373–381.

Stephens, E., 2008. The role of Vpu protein in HIV-1 pathogenesis. Antiviral Res. 78, A18.

Sticht, J., Humbert, M., Findlow, S., Bodem, J., Müller, B., Dietrich, U., Werner, J., Kräusslich, H.G., 2005. A peptide inhibitor of HIV-1 assembly in vitro. Nat. Struct. Mol. Biol. 12, 671–677.

Stopak, K., de Noronha, C., Yonemoto, W., Greene, W.C., 2003. HIV-1 Vif blocks the antiviral activity of APOBEC3G by impairing both its translation and intracellular stability. Mol. Cell 12, 591–601.
Strebel, K., Klimkait, T., Martin, M.A., 1988. A novel gene of HIV-1, vpu, and its 16-kilodalton product. Science 241, 1221–1223.
Stremlau, M., Owens, C.M., Perron, M.J., Kiessling, M., Autissier, P., Sodroski, J., 2004. The cytoplasmic body component TRIM5alpha restricts HIV-1 infection in Old World monkeys. Nature 427, 848–853.
Stremlau, M., Perron, M., Lee, M., Li, Y., Song, B., Javanbakht, H., Diaz-Griffero, F., Anderson, D.J., Sundquist, W.I., Sodroski, J., 2006. Specific recognition and accelerated uncoating of retroviral capsids by the TRIM5alpha restriction factor. Proc. Natl. Acad. Sci. U.S.A. 103, 5514–5519.
Suspene, R., Sommer, P., Henry, M., Ferris, S., Guetard, D., Pochet, S., Chester, A., Navaratnam, N., Wain-Hobson, S., Vartanian, J.P., 2004. APOBEC3G is a single-stranded DNA cytidine deaminase and functions independently of HIV reverse transcriptase. Nucleic Acids Res. 32, 2421–2429.
Sutherland, H.G., Newton, K., Brownstein, D.G., Holmes, M.C., Kress, C., Semple, C.A., Bickmore, W.A., 2006. Disruption of Ledgf/Psip1 results in perinatal mortality and homeotic skeletal transformations. Mol. Cell Biol. 26, 7201–7210.
Svarovskaia, E.S., Xu, H., Mbisa, J.L., Barr, R., Gorelick, R.J., Ono, A., Freed, E.O., Hu, W.S., Pathak, V.K., 2004. Human apolipoprotein B mRNA-editing enzyme-catalytic polypeptide-like 3G (APOBEC3G) is incorporated into HIV-1 virions through interactions with viral and nonviral RNAs. J. Biol. Chem. 279, 35822–35828.
Tang, C., Loeliger, E., Luncsford, P., Kinde, I., Beckett, D., Summers, M.F., 2004. Entropic switch regulates myristate exposure in the HIV-1 matrix protein. Proc. Natl. Acad. Sci. U.S.A. 101, 517–522.
Ternois, F., Sticht, J., Duquerroy, S., Kräusslich, H.G., Rey, F.A., 2005. The HIV-1 capsid protein C-terminal domain in complex with a virus assembly inhibitor. Nat. Struct. Mol. Biol. 12, 678–682.
Tiganos, E., Friborg, J., Allain, B., Daniel, N.G., Yao, X.J., Cohen, E.A., 1998. Structural and functional analysis of the membrane-spanning domain of the human immunodeficiency virus type 1 Vpu protein. Virology 251, 96–107.
Tissot, C., Mechti, N., 1995. Molecular cloning of a new interferon-induced factor that represses human immunodeficiency virus type 1 long terminal repeat expression. J. Biol. Chem. 270, 14891–14898.
Turelli, P., Doucas, V., Craig, E., Mangeat, B., Klages, N., Evans, R., Kalpana, G., Trono, D., 2001. Cytoplasmic recruitment of INI1 and PML on incoming HIV preintegration complexes: interference with early steps of viral replication. Mol. Cell 7, 1245–1254.
Turlure, F., Devroe, E., Silver, P.A., Engelman, A., 2004. Human cell proteins and human immunodeficiency virus DNA integration. Front. Biosci. 9, 3187–3208.
Uchil, P.D., Quinlan, B.D., Chan, W.T., Luna, J.M., Mothes, W., 2008. TRIM E3 ligases interfere with early and late stages of the retroviral life cycle. PLoS Pathog. 4, e16.
Van Damme, N., Goff, D., Katsura, C., Jorgenson, R.L., Mitchell, R., Johnson, M.C., Stephens, E.B., Guatelli, J., 2008. The interferon-induced protein BST-2 restricts HIV-1 release and is downregulated from the cell surface by the viral Vpu protein. Host Cell Microbe 3, 242–252.
Vandekerckhove, L., Christ, F., Van Maele, B., De Rijck, J., Gijsbers, R., Van den Haute, C., Witvrouw, M., Debyser, Z., 2006. Transient and stable knockdown of the integrase cofactor LEDGF/p75 reveals its role in the replication cycle of human immunodeficiency virus. J. Virol. 80, 1886–1896.
Varthakavi, V., Heimann-Nichols, E., Smith, R.M., Sun, Y., Bram, R.J., Ali, S., Rose, J., Ding, L., Spearman, P., 2008. Identification of calcium-modulating cyclophilin ligand as a human host restriction to HIV-1 release overcome by Vpu. Nat. Med. 14, 641–647.
Waheed, A.A., Ablan, S.D., Mankowski, M.K., Cummins, J.E., Ptak, R.G., Schaffner, C.P., Freed, E.O., 2006. Inhibition of HIV-1 replication by amphotericin B methyl ester: selection for resistant variants. J. Biol. Chem. 281, 28699–28711.
Waheed, A.A., Ablan, S.D., Roser, J.D., Sowder, R.C., Schaffner, C.P., Chertova, E., Freed, E.O., 2007. HIV-1 escape from the entry-inhibiting effects of a cholesterol-binding compound via cleavage of gp41 by the viral protease. Proc. Natl. Acad. Sci. U.S.A. 104, 8467–8471.
Waheed, A.A., Ono, A., Freed, E.O., 2008. Methods for the study of HIV-1 assembly. In: Prasad, V.R., Kalpana, G.V. (Eds.), HIV Protocols, 2nd edition. Humana Press.
Wedekind, J.E., Gillilan, R., Janda, A., Krucinska, J., Salter, J.D., Bennett, R.P., Raina, J., Smith, H.C., 2006. Nanostructures of APOBEC3G support a hierarchical assembly model of high molecular mass ribonucleoprotein particles from dimeric subunits. J. Biol. Chem. 281, 38122–38126.
Wichroski, M.J., Ichiyama, K., Rana, T.M., 2005. Analysis of HIV-1 viral infectivity factor-mediated proteasome-dependent depletion of APOBEC3G: correlating function and subcellular localization. J. Biol. Chem. 280, 8387–8396.
Wiegand, H.L., Doehle, B.P., Bogerd, H.P., Cullen, B.R., 2004. A second human antiretroviral factor, APOBEC3F, is suppressed by the HIV-1 and HIV-2 Vif proteins. EMBO J. 23, 2451–2458.
Willey, R.L., Maldarelli, F., Martin, M.A., Strebel, K., 1992. Human immunodeficiency virus type 1 Vpu protein regulates the formation of intracellular gp160-CD4 complexes. J. Virol. 66, 226–234.
Wright, E.R., Schooler, J.B., Ding, H.J., Kieffer, C., Fillmore, C., Sundquist, W.I., Jensen, G.J., 2007. Electron cryotomography of immature HIV-1 virions reveals the structure of the CA and SP1 Gag shells. EMBO J. 26, 2218–2226.
Xiao, Z., Ehrlich, E., Yu, Y., Luo, K., Wang, T., Tian, C., Yu, X.F., 2006. Assembly of HIV-1 Vif-Cul5 E3 ubiquitin ligase through a novel zinc-binding domain-stabilized hydrophobic interface in Vif. Virology 349, 290–299.
Xu, H., Chertova, E., Chen, J., Ott, D.E., Roser, J.D., Hu, W.S., Pathak, V.K., 2007. Stoichiometry of the antiviral protein APOBEC3G in HIV-1 virions. Virology 360, 247–256.
Yap, M.W., Mortuza, G.B., Taylor, I.A., Stoye, J.P., 2007. The design of artificial retroviral restriction factors. Virology 365, 302–314.
Yap, M.W., Nisole, S., Stoye, J.P., 2005. A single amino acid change in the SPRY domain of human TRIM5alpha leads to HIV-1 restriction. Curr. Biol. 15, 73–78.
Yu, Q., Konig, R., Pillai, S., Chiles, K., Kearney, M., Palmer, S., Richman, D., Coffin, J.M., Landau, N.R., 2004a. Single-strand specificity of APOBEC3G accounts for minus-strand deamination of the HIV genome. Nat. Struct. Mol. Biol. 11, 435–442.
Yu, X., Yu, Y., Liu, B., Luo, K., Kong, W., Mao, P., 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., Yu, X.F., 2004b. 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.
Zennou, V., Perez-Caballero, D., Gottlinger, H., Bieniasz, P.D., 2004. APOBEC3G incorporation into human immunodeficiency virus type 1 particles. J. Virol. 78, 12058–12061.
Zhang, H., Yang, B., Pomerantz, R.J., Zhang, C., Arunachalam, S.C., Gao, L., 2003. The cytidine deaminase CEM15 induces hypermutation in newly synthesized HIV-1 DNA. Nature 424, 94–98.
Zhang, F., Perez-Caballero, D., Hatziioannou, T., Bieniasz, P.D., 2008a. No effect of endogenous TRIM5alpha on HIV-1 production. Nat. Med. 14, 235–236, author reply 236–238.
Zhang, H., Zhao, Q., Bhattacharya, S., Waheed, A.A., Tong, X., Hong, A., Heck, S., Curreli, F., Goger, M., Cowburn, D., Freed, E.O., Debnath, A.K., 2008b. A cell-penetrating helical peptide as a potential HIV-1 inhibitor. J. Mol. Biol. 378, 565–580.
Zhou, J., Huang, L., Hachey, D.L., Chen, C.H., Aiken, C., 2005. Inhibition of HIV-1 maturation via drug association with the viral Gag protein in immature HIV-1 particles. J. Biol. Chem. 280, 42149–42155.
Zielske, S.P., Stevenson, M., 2006. Modest but reproducible inhibition of human immunodeficiency virus type 1 infection in macrophages following LEDGFp75 silencing. J. Virol. 80, 7275–7280.