Dual Functionality of HIV-1 Vif in APOBEC3 Counteraction and Cell Cycle Arrest

Daniel J. Salamango1,2,3,4* and Reuben S. Harris1,2,3,4*

1 Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, Minneapolis, MN, United States, 2 Masonic Cancer Center, University of Minnesota, Minneapolis, MN, United States, 3 Institute for Molecular Virology, University of Minnesota, Minneapolis, MN, United States, 4 Howard Hughes Medical Institute, University of Minnesota, Minneapolis, MN, United States

Accessory proteins are a key feature that distinguishes primate immunodeficiency viruses such as human immunodeficiency virus type I (HIV-1) from other retroviruses. A prime example is the virion infectivity factor, Vif, which hijacks a cellular co-transcription factor (CBF-β) to recruit a ubiquitin ligase complex (CRL5) to bind and degrade antiviral APOBEC3 enzymes including APOBEC3D (A3D), APOBEC3F (A3F), APOBEC3G (A3G), and APOBEC3H (A3H). Although APOBEC3 antagonism is essential for viral pathogenesis, and a more than sufficient functional justification for Vif’s evolution, most viral proteins have evolved multiple functions. Indeed, Vif has long been known to trigger cell cycle arrest and recent studies have shed light on the underlying molecular mechanism. Vif accomplishes this function using the same CBF-β/CRL5 ubiquitin ligase complex to degrade a family of PPP2R5 phospho-regulatory proteins. These advances have helped usher in a new era of accessory protein research and fresh opportunities for drug development.

Keywords: APOBEC3, APOBEC3G, cell cycle arrest, PPP2R5, Vif

INTRODUCTION

It has been nearly 40 years since the discovery that acquired immune deficiency syndrome (AIDS) is caused by a retrovirus named human immunodeficiency virus type I (HIV-1) (Barré-Sinoussi et al., 1983; Gallo et al., 1984; Popovic et al., 1984). AIDS arises from a profound state of immune suppression brought on by a massive depletion of CD4+ T lymphocytes following HIV-1 infection, which results in patients succumbing to opportunistic infections and rare malignancies (Klatzmann et al., 1984; Masur et al., 1989; Ho et al., 1995). Soon after its discovery, the HIV-1 genome was sequenced and found to contain nine open reading frames that encode over a dozen viral proteins essential for pathogenicity (Emerman and Malim, 1998; Frankel and Young, 1998; Malim and Emerman, 2008).

All retroviruses encode Gag, Pol, and Env polyproteins that are processed by viral and cellular proteases to release individual proteins during nascent particle assembly. Gag encodes structural proteins matrix (MA), capsid (CA), and nucleocapsid (NC), Pol encodes the viral enzymes protease (PR), reverse-transcriptase (RT), and integrase (IN), and Env encodes the surface glycoprotein GP160, which is processed into the receptor-binding GP120 and transmembrane GP41 domains by a cellular furin-like protease (Figure 1A). Lentiviruses encode additional proteins, such as
the regulatory proteins Tat and Rev and several accessory proteins (Vif, Vpr, Vpu, and Nef) that perform various functions to enhance HIV-1 pathogenicity (Emerman and Malim, 1998; Malim and Emerman, 2008; Figures 1A,B). In at least three instances, the major function of these accessory proteins is to promote HIV-1 evasion of cell-intrinsic antiviral resistance by either re-localizing antiviral factors from their sites of virus restriction (Vpu-TETHERIN), targeting them for proteasomal degradation (Vif-APOBEC3), or a combination of both activities (Nef-SERINC3/5) (Madani and Kabat, 1998; Simon et al., 1998a; Sheehy et al., 2002; Neil et al., 2008; Van Damme et al., 2008; Rosa et al., 2015; Usami et al., 2015; Figure 1B). In the case of SERINC3/5 counteraction, Nef re-targets these proteins from the plasma membrane to endosomal/lysosomal compartments for degradation (Usami et al., 2015; Rosa et al., 2015; Shi et al., 2018). For Vpr, its role in HIV-1 pathogenesis remains enigmatic, with reported activities ranging from manipulation of the DNA-damage response (Hrecka et al., 2016; Wu et al., 2016) and induction of G2/M cell cycle arrest (Rart et al., 1996; Sakai et al., 2006), to facilitating nuclear import of the HIV-1 genome (Heinzinger et al., 1994; Jenkins et al., 1998) and enhancing viral gene expression (Thierry et al., 2004; Zhang and Bieniasz, 2020; Figure 1B).

As is true for other viruses (e.g., herpesviruses and poxviruses), immunomodulatory accessory genes are frequently deleted, or dispensable, for viral replication in vitro; however, in vivo they are strongly maintained during natural infections. For example, in vitro, Vif, Vpr, Vpu, and Nef exert functions in a cell-type dependent manner, where these genes are dispensable for producing infectious viral particles in some T cell and myeloid cell lines but not others (i.e., permissive vs. non-permissive cells). However, in physiologically relevant primary CD4+ T cells and monocytes, loss of these genes results in severe impairment of HIV-1 replication (Deacon et al., 1995; Gaddis et al., 2004; Schindler et al., 2006). To further emphasize this point, HIV-1 deficient in the vif gene produces particles that are roughly 1000 times less infectious compared to wild-type virus in primary and non-permissive CD4+ T cells (Fisher et al., 1987; Strebel et al., 1987; Gabuzda et al., 1992).

**DISCOVERY OF Vif FUNCTION IN APOBEC3 DEGRADATION**

The underlying mechanism of how Vif enhanced HIV-1 pathogenesis remained elusive for many years following its identification. The key to uncovering Vif’s function originated from observations that in some adherent (HeLa and HEK293T) and T cell lines (CEM-SS and SupT1) Vif is dispensable for producing infectious virus. In contrast, loss of Vif in
physiologically relevant primary CD4+ T cells and macrophages, and some immortalized T cell lines (CEM and H9), results in the production of almost no infectious virus (Gabuzda et al., 1992; Sakai et al., 1993; Simon et al., 1998b). Comprehensive interrogation of these cell lines using heterokaryon experiments in parallel with subtraction cloning methods indicated the presence of a dominant factor, rather than the lack of a recessive gene product, and led to the identification of the host factor CEM15, now known as APOBEC3G (Madani and Kabat, 1998; Simon et al., 1998a; Sheehy et al., 2002).

APOBEC3G (A3G) belongs to a family of cytosine deaminase enzymes that converts cytosine to uracil (C-to-U) in single-stranded DNA (Harris et al., 2002; Petersen-Mahrt et al., 2002; Figures 2A,B). DNA C-to-U deamination is catalyzed by a zinc-mediated hydrolysis reaction driven by a highly conserved glutamic acid residue located within the substrate-binding pocket. APOBEC3 enzymes exhibit an intrinsic dinucleotide preference with the cytosine base being preceded by either a thymine (TC) or another cytosine (CC) (Carpenter et al., 2010; Kohli et al., 2010; Rathore et al., 2013; Figure 2B). Because of its enzymatic classification, researchers postulated that A3G was able to restrict HIV-1 replication through deamination of the viral RNA during reverse-transcription, when the genome is converted to single-stranded DNA (Harris et al., 2003; Mangeat et al., 2003; Zhang et al., 2003; Di Noia and Neuberger, 2007). In fact, extensive biochemical characterization of A3G and related family members revealed that these enzymes could package into nascent HIV-1 particles and inflict catastrophic levels of C-to-T mutations in the viral genome during its conversion to single-stranded DNA (accounts for G-to-A mutations observed on the genomic plus strand) (Bishop et al., 2004; Desimmie et al., 2010; Feng et al., 2014; Figures 2A,B). In addition, several APOBEC3 enzymes also exert deaminase-independent antiviral activities and hinder reverse transcription, likely through high affinities for RNA and single-stranded DNA viral replication intermediates (Newman et al., 2005; Holmes et al., 2007; Shaban et al., 2018).

Follow-up studies revealed that Vif counters the mutagenic potential of the APOBEC3s by nucleating the formation of an E3-ubiquitin ligase complex that targets A3s for proteasomal degradation prior to virion encapsidation (Marin et al., 2003; Sheehy et al., 2003; Mehle et al., 2004). Proteomic and genetic studies determined that this E3-ubiquitin ligase complex is comprised of Cullin 5 (CUL5), Elongin B and C (ELOB/C), Ring-Box 2 (RBX2), and CBF-β (Yu et al., 2003; Jäger et al., 2011; Zhang et al., 2011; Figure 2C). Interestingly, CBF-β was not part of the original proteomics discovery, but instead, came as evidenced by a high-resolution structure (Guo et al., 2014; Figure 2C).

**DYNAMIC NATURE OF VIF-SUBSTRATE PROTEIN–PROTEIN INTERFACES**

One of the major questions following the discovery of the Vif-APOBEC3 interaction was how can a relatively small viral protein (~20 kDa) interact with four restrictive APOBEC3 enzymes (five in total), recruit CBF-β, nucleate the formation of an E3-ubiquitin ligase complex, and induce G2/M cell cycle arrest (discussed in further detail below)? Remarkably, it has become apparent that each Vif-substrate pairwise combination utilizes largely distinct, and genetically separable, interaction surfaces (Figure 2D). Importantly, these biochemical constraints suggest that a single Vif/E3-complex can only degrade one substrate at a time, making Vif’s ability to target so many distinct substrates even more impressive.

Extensive mutagenesis studies have identified several Vif amino acid substitutions that can disrupt individual pairwise interactions and leave others intact. Distinct clusters of separation-of-function mutants have been identified that disrupt degradation of A3D/F, A3G, or A3H. The Vif surface utilized for A3G recognition is clustered near the N-terminus, encompassing three distinct sets of amino acid residues, with the 40YRHHY44 segment being unique to A3G recognition (Russell and Pathak, 2007; He et al., 2008; Yamashita et al., 2008; Chen et al., 2009; Deng et al., 2009; Pery et al., 2009; Letko et al., 2015; Figure 2D). While the A3D/F interface partially overlaps with A3G, it also extends to residues 71WQxDRMR17 and 74TGERxW17, which imparts specificity (Russell and Pathak, 2007; He et al., 2008; Zhang et al., 2008; Figure 2D). Interestingly, the surface residues used to recognize A3H are clustered at the top of the β-fold and are completely distinct from those used to recognize the other APOBEC3s (Zhen et al., 2012; Ooms et al., 2013, 2017; Nakashima et al., 2017; Wang et al., 2019). Likewise, the same is true for the Vif surfaces that maintain interactions with CBF-β (residues located on β-strand 1 and 6) (Zhou et al., 2012, 2014) and the E3-ubiquitin ligase complex (residues located on α-helix 3 and 4) (Kamura et al., 1998; Yu et al., 2004; Bergeron et al., 2010; Wolfe et al., 2010; Figure 2D).

Previous analyses of available Vif sequences have highlighted evolutionary conservation for some Vif-substrate surfaces but not others (Azimi and Lee, 2020). As one would predict, residues involved in binding CBF-β and the E3-ubiquitin ligase complex are highly conserved; however, the same is not true for residues involved in APOBEC3 recognition. The conservation of interface residues diminishes with A3F > A3G >> A3H, which is consistent with the current model that A3H may only be a threat to the virus in certain regions of the globe (Ebrahimi et al., 2018). This observation is also consistent with the “wobble model” which provides an evolutionary paradigm explaining the biochemical drift between Vif-APOBEC3 interactions (Richards et al., 2015; Harris and Anderson, 2016). This model is predicated on the concept...
that an ancestral lentivirus was established in a non-primate mammal with a relatively modest APOBEC3 gene-set prior to transmitting into a primate with an expanded APOBEC3 repertoire. In this hypothetical scenario, the strong interaction between Vif and the ancestral APOBEC3 is attenuated following zoonosis. The Vif-APOBEC3 interaction(s) are then restored following compensatory mutations encoding residues at, or near, the periphery of the ancestral Vif-APOBEC3 interaction surface. Importantly, these adaptations would have occurred independently for each novel APOBEC3 restriction factor, giving
rise to the distinct interaction surfaces observed on present-day Vif (Figure 2D).

**Vif INDUCES G2/M CELL CYCLE ARREST THROUGH DEGRADATION OF PPP2R5s**

Because counteraction of the APOBEC3s is critical for HIV-1 replication, this was thought to be Vif’s only function for many years. However, in the early 2000s it was discovered that Vif could also induce G2/M cell cycle arrest in a variety of cell lines (Sakai et al., 2006; Wang et al., 2007). Genetic studies established that Vif-induced G2/M arrest requires both CBF-β and the same E3-ubiquitin ligase complex utilized for APOBEC3 degradation (DeHart et al., 2008; Du et al., 2019). Additionally, fine-mapping of the Vif residues required for inducing G2/M arrest revealed that this interface is mostly distinct from those of the APOBEC3s (Izumi et al., 2010; Zhao et al., 2015; Salamango et al., 2019, 2020; Marelli et al., 2020; Nagata et al., 2020; Figure 2D). While these observations collectively pointed toward Vif degrading a cellular factor to induce arrest, the identity of this factor remained elusive for nearly 10 years following the discovery of this activity.

A major breakthrough regarding Vif-induced G2/M arrest came from recent quantitative proteomics studies that revealed Vif-dependent remodeling of the host phosphoproteome (Greenwood et al., 2016; Naamati et al., 2019). These studies discovered that Vif could efficiently deplete multiple members of the PPP2R5 family of protein phosphatase 2A (PP2A) regulators in several cell lines, including primary and immortalized CD4+ T cells (Greenwood et al., 2016; Naamati et al., 2019). PP2As account for a majority of the phosphatase activity in eukaryotic cells and function as heterotrimeric complexes comprised of a phosphatase enzyme (PP2Cα), a scaffolding protein (PPP2R1α or PPP2R1β), and a regulatory subunit (B55, PPP2R5/B56, or PR72/130) (Thompson and Williams, 2018; Nilsson, 2019). The regulatory subunit can be from one of three distinct protein families, which regulate subcellular localization and substrate recognition of PP2A holoenzyme complexes (McCright et al., 1996; Wang J. et al., 2016). Importantly, PP2A/PPP2R5 holoenzymes have been shown to regulate multiple aspects of the G2-to-M phase transition (Moura and Conde, 2019; Nilsson, 2019).

The connection between PP2A/PPP2R5 complexes and regulation of G2/M progression prompted our group, and others, to investigate if these were the factors antagonized by Vif to induce G2/M arrest. Comprehensive mutagenesis using both single amino acid substitutions and large-scale mutagenic libraries revealed that Vif residues required for degradation of PPP2R5 substrates overlap with those required for inducing G2/M arrest (Evans et al., 2018; Salamango et al., 2019, 2020; Marelli et al., 2020; Nagata et al., 2020). These studies also showed that siRNA-mediated knock-down of specific combinations of PPP2R5 transcripts could induce a robust G2/M arrest phenotype in the absence of Vif; however, strong knock-down of single PPP2R5 family members had no impact on cell cycle progression (Salamango et al., 2019; Marelli et al., 2020).

While subtle differences were observed for which combinations of PPP2R5 knock-downs were most impactful, the overall conclusions were complementary in that at least two family members need to be simultaneously depleted to induce arrest in the absence of Vif (Salamango et al., 2019; Marelli et al., 2020). Additionally, chemical inhibition of PP2A activity using okadaic acid induced a robust G2/M arrest phenotype, further supporting a direct relationship between Vif-induced PPP2R5 degradation and subsequent G2/M arrest (Salamango et al., 2019; Marelli et al., 2020).

**Vif BINDS THE SAME SURFACE AS PHYSIOLOGIC PPP2R5 SUBSTRATES**

Characterization of Vif separation-of-function mutants suggested that PPP2R5 recognition occurs through electrostatic interactions, as a majority of residues required for degradation are positively charged (8 out of 12 residues) (Salamango et al., 2019, 2020; Marelli et al., 2020; Figure 2D). Using high-resolution crystal structures and homology modeling, it is evident that all Vif-substrate interaction surfaces are largely electrostatic in nature (Kitamura et al., 2012; Li et al., 2012; Bohn et al., 2013; Byeon et al., 2013; Siu et al., 2013; Wang J. et al., 2016; Kouno et al., 2017; Shi et al., 2017; Ito et al., 2018; Shaban et al., 2018; Figure 3A). The PPP2R5 surface recognized by Vif is extremely electronegative and supports a model in which Vif maintains this interaction through a favorable network of electrostatics. Importantly, all of the residues required for Vif recognition are conserved among all five family members, which clarifies how Vif can recognize and degrade five new cellular substrates and explains genetic evidence that loss of at least two family members is required for inducing G2/M arrest.

Interestingly, the PPP2R5 surface recognized by Vif overlaps with the substrate binding pocket used by PPP2R5s to bind cellular substrates (Hertz et al., 2016; Wang J. et al., 2016; Wang X. et al., 2016; Figure 3B). Crystallographic and proteomics studies have combined to identify the substrate binding motif recognized by PPP2R5 proteins and the mode in which binding occurs. PPP2R5s recognize substrates through a conserved LxxIxE motif that directs the PP2A holoenzyme complex to cellular targets for dephosphorylation (Hertz et al., 2016; Wang J. et al., 2016; Wang X. et al., 2016). The LxxIxE motif is stabilized by two distinct substrate binding pockets on the surface of PPP2R5 proteins. The “Leu pocket,” comprised of residues K208, T184, H187, R188, E251, and I227, and the “Ile pocket,” which contains residues H187, Y215, I227, and I256. In addition, a recent study demonstrated that PPP2R5 substrate recognition is enhanced by electrostatic interactions that occur adjacent to the peptide binding cleft, encompassing residues E335 and D338 (Wang X. et al., 2020). These residues are of interest because E251, E335, and D338 are required for Vif-induced degradation of PPP2R5s (Salamango et al., 2019; Figure 3B). Furthermore, residues S255 and N258, which are located within the “Ile pocket,” are also required for Vif-induced degradation (Figure 3B).
The close proximity and partial overlap between these interfaces raised the question of whether Vif and cellular substrates compete for binding. To test this model, we utilized a well-characterized peptide inhibitor that contains the LxxIxE motif recognized by PPP2R5s to determine if Vif-induced degradation could be blocked. Co-transfection of a plasmid expressing four tandem copies of this LxxIxE peptide caused a dose-dependent inhibition of Vif-mediated degradation of PPP2R5A (Salamango et al., 2020). However, a control plasmid containing an AxxAxA motif had no effect on Vif-induced degradation of either PPP2R5A or APOBEC3G (Salamango et al., 2020). Taken together, these observations support a competitive binding model in which Vif directly interacts with the surface of PPP2R5 proteins and occludes the binding of cellular substrates.

MECHANISTIC MODEL FOR Vif-INDUCED G2/M ARREST

The discovery of PPP2R5 substrates was a major step forward in understanding Vif-induced cell cycle arrest. However,
the regulatory checkpoints altered downstream of PPP2R5 degradation remain unknown. PP2A/PPP2R5 complexes have been shown to regulate entrance and exit of the G2-to-M phase transition at multiple different checkpoints. Below, we discuss these checkpoints in the context of previous observations regarding Vif-induced G2/M arrest and postulate a model in which antagonism of discrete PP2A/PPP2R5 complexes would lead to simultaneous inhibition of multiple checkpoints.

First, arguably the most critical event for mitotic entry is CDC25-mediated activation of CDK1, which allows for nuclear translocation of the CDK1-CyclinB complex (Lee and Kirschner, 1996; Timofeev et al., 2010; Figure 4, top). Activation of CDC25 requires PP2A-mediated dephosphorylation of specific residues to release inhibitory 14-3-3 proteins (Margolis et al., 2006; Figure 4, top). Therefore, Vif-induced antagonism of PP2A/PPP2R5 complexes would render CDC25 inactive and inhibit nuclear translocation of CDK1-CyclinB (Figure 4, bottom). In support of this mechanism, a previous study reported that infection of Jurkat T cells with arrest-proficient HIV-1 resulted in constitutive phosphorylation of Tyr15 on CDK1, and subsequent inhibition of CDK1-CyclinB nuclear translocation (Sakai et al., 2011). Additionally, quantitative proteomics and immunoblot analyses revealed elevated levels of CyclinB in CEM T4 cells infected with HIV-1 expressing arrest-proficient Vif (Marelli et al., 2020).

Second, PP2A/PPP2R5 complexes also control G2-to-M progression through spatiotemporal regulation of the Aurora kinases. Aurora kinases are involved in numerous G2-to-M

---

**Figure 4** | Diagram of potential Vif-induced G2/M arrest mechanisms. Depiction of normal and aberrant regulation of the G2-to-M phase transition in the absence (top) and presence (bottom) of Vif. Key cell cycle checkpoints regulated by PP2A/PPP2R5 complexes are color-coded in the absence or presence of Vif. See text for additional details.
However, at least three key observations have been made that demonstrate the importance of PPP2R5 degradation remains to be established. (Horn et al., 2007; Jeong and Yang, 2013; Lindon et al., 2015; Figure 4, top). Recent studies have demonstrated striking activation of Aurora kinase A and B following infection of CEM T4 cells with HIV-1 expressing arrest-proficient Vif, supporting a model in which Vif simultaneously antagonizes PP2A/PPP2R5 complexes to inactivate the APC system and stall mitotic exit (Figure 4, bottom).

Lastly, PP2A/PPP2R5 holoenzymes have been shown to serve as scaffolds for multiple distinct complexes to initiate exit from mitosis. For example, Shugoshin (SGO1) directly binds to a PP2A/PPP2R5 holoenzyme to form a complex that regulates accurate chromosome segregation during mitosis (Tang et al., 2006; Xu et al., 2009; Figure 4, top). Without the formation of this complex, centromeric cohesion is prematurely cleaved which results in untimely centromere disassociation and chromosome mis-segregation (Tang et al., 2006; Xu et al., 2009; Figure 4, bottom). Additionally, PP2A/PPP2R5 holoenzymes directly bind to BUBR1 and regulate the spindle assembly checkpoint and mitotic progression by counteracting Aurora kinase activity at improperly attached kinetochores (Cheeseman et al., 2006; DeLuca et al., 2006; Kruse et al., 2013). Therefore, disruption of BUBR1 regulatory activity and hyper-activation of Aurora kinases through Vif-mediated antagonism of PP2A/PPP2R5 complexes would result in kinetochore dysfunction and a failure to exit mitosis (Figure 4, bottom).

In addition to manipulation of the PP2A/PPP2R5 axis, it has been suggested that Vif can induce G2/M arrest by blocking MDM2-mediated ubiquitination and nuclear export of TP53 (Izumi et al., 2010). This study observed that Vif binding to TP53 could block MDM2 recognition and subsequent turnover of TP53, which is required for proper cell cycle progression. Although not addressed directly, it is likely that the Vif/TP53 interaction occurs in the nuclear compartment, thus directly shielding TP53 from MDM2 recognition and subsequent nuclear export. If this is indeed the case, it is plausible that there are two pools of Vif that act in concert to stall cell cycle progression. Nuclear localized Vif protects TP53 from MDM2 and antagonizes nuclear PPP2R5C/D substrates, whereas cytoplasmic Vif antagonizes the APOBEC3s and cytoplasmic PPP2R5A/B/E. Additional work will be required to determine if these mechanisms are separate or connected through shared components.

CONCLUDING REMARKS

Now, Vif clearly has two distinct sets of cellular substrates, APOBEC3 enzymes and PPP2R5 phospho-regulators (10 proteins total). While APOBEC3 counteraction has been shown to be essential for viral infectivity and pathogenesis in vivo, the importance of PPP2R5 degradation remains to be established. However, at least three key observations have been made that imply that alteration of the cell cycle may be beneficial for HIV-1 pathogenesis.

First, HIV-1 Vpr also potently induces G2/M arrest through a mechanism that is clearly distinct from that of Vif. Vpr activates the ATR DNA damage response pathway by inducing proteasomal degradation of diverse cellular substrates using a Cullin 4 (CUL4), DNA damage-binding protein 1 (DBB), and DDB1-CUL4-associated factor (DCAF1) E3-ubiquitin ligase complex (Hrecka et al., 2007; Andersen et al., 2008; Greenwood et al., 2019). While Vif degrades a smaller set of cellular proteins, Vpr has been shown to be much more promiscuous. A recent study demonstrated that Vpr induces the degradation of roughly 40 cellular proteins causing a systems-level remodeling of the cellular proteome, which may cumulatively lead to G2/M arrest (Greenwood et al., 2019). Collectively, over half a dozen cellular proteins have been directly implicated as being essential for Vpr-induced arrest [e.g., MCM10, SMN1, CDCA2, ZNF267, MUS81, CCDC137, etc. (Laguet et al., 2014; Romani et al., 2015; Greenwood et al., 2019; Zhang and Bieniasz, 2020)].

At least two phenotypes have been associated with Vpr-induced arrest that may enhance HIV-1 pathogenicity. First, degradation of MUS81 and EME1 by Vpr leads to G2/M arrest and prematurely activates the SLX4 complex, which suppresses spontaneous and HIV-1-mediated induction of type I interferon responses and may contribute to immune evasion (Laguet et al., 2014). Second, depletion of CCDC137 by Vpr has been shown to induce G2/M arrest and enhance HIV-1 gene expression (Zhang and Bieniasz, 2020). It is worth emphasizing that during the G2/M phase of the cell cycle, cellular translation and transcription are significantly diminished (Parsons and Spencer, 1997; Kronja and Orr-Weaver, 2011; Tanenbaum et al., 2015; Strayz, 2017). Therefore, Vpr's ability to simultaneously induce arrest and enhance HIV-1 gene expression would lead to an enrichment of viral transcripts during G2/M. Interestingly, PP2A/PPP2R5 complexes have been shown to regulate protein translation kinetics through mTOR, S6 kinase, and 4E-BP1 (Hahn et al., 2010; Gardner et al., 2015). Therefore, it is possible that Vif antagonism of PP2A holoenzymes leads to increased protein translation during G2/M when host cell translation is normally stalled. Taken together, it is possible that Vif and Vpr act in concert to induce G2/M arrest and boost transcription and translation of HIV-1 genes.

Second, arrest-proficient Vif variants are prominent in global populations and in patient derived Vif isolates. Bioinformatic analyses of sequences obtained from the Los Alamos database have drawn correlations between predicted arrest-proficient Vif sequences and the most abundant subtypes in global circulation (Salamango et al., 2019; Marelli et al., 2020). Additionally, a previous study suggested that Vif-induced arrest leads to increased production of HIV-1 particles from both primary and immortalized CD4+ T cells (Izumi et al., 2010). Although this is the only study to-date that demonstrates this phenotype, these findings could explain why arrest-associated Vif variants are prevalent in circulating populations. Furthermore, live cell imaging studies assessing degradation kinetics of APOBEC3G and PPP2R5A in the same cell established that the half-life of PPP2R5A is only ~2 h slower than that of APOBEC3G.
following infection, which is striking given that APOBEC3G degradation is essential for HIV-1 infectivity and pathogenesis (Salamango et al., 2020).

Third, subversion of the cell cycle is a mechanism used by diverse viral pathogens to create a favorable environment for replication. Oncogenic viruses, RNA viruses, and DNA viruses have all been shown to hijack the host cell cycle during the course of an infection, many of which arrest cells in the G2/M phase (e.g., human T-lymphotropic virus (HTLV), human polyoma virus, infectious bronchitis virus, simian virus 40, and adenovirus (Zhao and Elder, 2005; Dove et al., 2006; Fan et al., 2018)). Interestingly, P2PA appears to be a conserved target as well, given that simian virus 40, polyoma virus, HTLV, adenovirus, and HIV-1 affect the enzymatic activity of at least a subset of P2PA complexes (Roopchand et al., 2001; Haoudi et al., 2003; Zhao and Elder, 2005). Importantly, P2P25 proteins have also been shown to be hijacked by other viral pathogens.

Ebola virus nucleoprotein recruits P2P25 proteins through a highly conserved LxxIxE motif to stimulate dephosphorylation of VP30, which initiates transcription of the viral genome and subsequent infection (Kruse et al., 2018). Additionally, HTLV requires P2P25 for efficient strand-transfer activity and target integration (Maertens, 2016). HTLV integrase uses a LxxIxE motif to recruit P2P25 to the viral integration complex (Bhatt et al., 2020). Taken together, these observations support a model in which P2P25 antagonism, and global changes in the cellular phospho-proteome, are likely to be advantageous for the pathogenesis of HIV-1 as well as other prominent viruses. Furthermore, continuing to unravel the complex molecular mechanisms HIV-1 has evolved to subvert cellular processes and enhance pathogenicity may provide major clues for the development of innovative therapeutics that lead to virus eradication.

**AUTHOR CONTRIBUTIONS**

DS drafted the manuscript. DS and RH edited the manuscript. Both authors contributed to the article and approved the submitted version.

**FUNDING**

This work was supported by NIAID R37-AI064046 (to RH). DS received salary support from an NIAID K99/R00 transition award (K99-AI147811). RH is the Margaret Harvey Schering Land Grant Chair for Cancer Research, a Distinguished University McKnight Professor, and an Investigator of the Howard Hughes Medical Institute.

**REFERENCES**

Andersen, J. L., Le Rouzic, E., and Panelles, V. (2008). HIV-1 Vpr: mechanisms of G2 arrest and apoptosis. Exp. Mol. Pathol. 85, 2–10. doi: 10.1016/j.yexmp.2008.03.015

Azimi, F. C., and Lee, J. E. (2020). Structural perspectives on HIV-1 Vif and APOBEC3 restriction factor interactions. Protein Sci. 29, 391–406. doi: 10.1002/pro.3729

Barré-Sinoussi, F., Chermann, J. C., Rey, F., Nugeyre, M. T., Chamaret, S., Gruest, J., et al. (1983). Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science 220, 868–871. doi: 10.1126/science.6189183

Bartz, S. R., Rogel, M. E., and Emerman, M. (1996). Human immunodeficiency virus type 1 cell cycle control: Vpr is cytosstatic and mediates G2 accumulation by a mechanism which differs from DNA damage checkpoint control. J. Virol. 70, 2324–2331. doi: 10.1128/jvi.70.7.2324-2331.1996

Bergeron, J. R., Huthoff, H., Veselkov, D. A., Beavil, R. L., Simpson, P. J., Matthews, S. J., et al. (2010). The SOCS-box of HIV-1 Vif interacts with ELONGINBC by induced-folding to recruit its Cul5-containing ubiquitin ligase complex. PLoS Pathog. 6:e1000925. doi: 10.1371/journal.ppat.1000925

Bhatt, V., Shi, K., Salamango, D. J., Moeller, N. H., Pandey, K., Bera, S., et al. (2020). Structural basis of host protein hijacking in human T-cell leukemia virus integration. Nat. Commun. 11:3121.

Bishop, K. N., Holmes, R. K., Sheehy, A. M., Davidson, N. O., Cho, S. J., and Malim, M. H. (2004). Cytidine deamination of retroviral DNA by diverse APOBEC proteins. Curr. Biol. 14, 1392–1396. doi: 10.1016/j.cub.2004.06.057

Bohn, M. F., Shandilya, S. M., Albon, S. J., Kouno, T., Anderson, B. D., McDougle, R. M., et al. (2013). Crystal structure of the DNA cytosine deaminase APOBEC3F: the catalytically active and HIV-1 Vif-binding domain. Structure 21, 1042–1050. doi: 10.1016/j.str.2013.04.010

Byeon, I. J., Ahn, J., Mitra, M., Byeon, C. H., Hercik, K., Hritz, J., et al. (2013). NMR structure of human restriction factor APOBEC3A reveals substrate binding and enzyme specificity. Nat. Commun. 4:1890. doi: 10.1038/ncomms2883

Carpenter, M. A., Rajagurubandara, E., Wijesinghe, P., and Bhagwat, A. S. (2010). Determinants of sequence-specificity within human AID and APOBEC3G. DNA Repair. 9, 579–587. doi: 10.1016/j.dnarep.2010.02.010

Cheeseman, I. M., Chappie, J. S., Wilson-Kubalek, E. M., and Desai, A. (2006). The conserved KMN network constitutes the core microtubule-binding site of the kinetochore. Cell 127, 983–997. doi: 10.1016/j.cell.2006.09.039

Chen, G., He, Z., Wang, T., Xu, R., and Yu, X. F. (2009). A patch of positively charged amino acids surrounding the human immunodeficiency virus type 1 Vif SLVx4Yx9Y motif influences its interaction with APOBEC3G. J. Virol. 83, 8674–8682. doi: 10.1128/JVI.00653-09

Dang, Y., Wang, X., Zhou, T., York, I. A., and Zheng, Y. H. (2009). Identification of a novel WxSLVK motif in the N terminus of human immunodeficiency virus and simian immunodeficiency virus Vif that is critical for APOBEC3G and APOBEC3F neutralization. J. Virol. 83, 8544–8552. doi: 10.1128/JVI.00156-09

Deacon, N. J., Tsykin, A., Solomon, A., Smith, K., Ludford-Menting, M., Hooker, D. J., et al. (1995). Genomic structure of an attenuated quasi species of HIV-1 from a blood transfusion donor and recipients. Science 270, 988–991. doi: 10.1126/science.270.5238.988

DeHart, J. L., Bosque, A., Harris, R. S., and Panelles, V. (2008). Human immunodeficiency virus type 1 Vif induces cell cycle delay via recruitment of the same E3 ubiquitin ligase complex that targets APOBEC3 proteins for degradation. J. Virol. 82, 9265–9272. doi: 10.1128/JVI.00103-07

DeLuca, J. G., Gall, W. E., Ciferri, C., Cimini, D., Musacchio, A., and Salmon, E. D. (2006). Kinetochore microtubule dynamics and attachment stability are regulated by Hec1. Cell 127, 969–982. doi: 10.1016/j.cell.2006.09.047

Desimme, B. A., Delviks-Frankenberg, K. A., Burdick, R. C., Qi, D., Izuami, T., and Pathak, V. V. (2014). Multiple APOBEC3 restriction factors for HIV-1 and one Vif to rule them all. J. Mol. Biol. 426, 1220–1245. doi: 10.1016/j.jmb.2013.10.033

Di Noia, J. M., and Neuberger, M. S. (2007). Molecular mechanisms of antibody somatic hypermutation. Annu. Rev. Biochem. 76, 1–22. doi: 10.1146/annurev.biochem.76.061705.090740

Dove, B., Brooks, G., Bicknell, K., Wurm, T., and Hiscox, J. A. (2006). Cell cycle perturbations induced by infection with the coronavirus infectious bronchitis virus and their effect on virus replication. J. Virol. 80, 4147–4156. doi: 10.1128/JVI.80.8.4147-4156.2006
Du, J., Rui, Y., Zheng, W., Li, P., Kang, J., Zhao, K., et al. (2019). Vif-CREB interaction is essential for Vif-induced cell cycle arrest. *Biochem. Biophys. Res. Commun.* 511, 910–915. doi: 10.1016/j.bbrc.2019.02.136

Ebrahim, D., Richards, C. M., Carpenter, M. A., Wang, J., Ikeda, T., Cheng, A. Z., et al. (2016). Genetic and mechanistic basis for APOBEC3 alternative splicing, differential retrovirus restriction, and counteraction by HIV-1 protease. *Nat. Commun.* 9:4317.

Emerman, M., and Malim, M. H. (1998). HIV-1 regulatory/accessory genes: keys to unraveling viral and host cell biology. *Science* 280, 1888–1884. doi: 10.1126/science.280.5371.1880

Evans, E. L., Becker, J. T., Fricke, S. L., Patel, K., and Sherrer, N. M. (2018). HIV-1 Vif’s capacity to manipulate the cell cycle is species specific. *J. Virol.* 92:e02102-17. doi: 10.1128/JVI.02102-17

Fan, Y., Sanyal, S., and Bruzzone, R. (2018). Breaking bad: how viruses subvert the cell cycle. *Front. Cell Infect. Microbiol.* 8:396. doi: 10.3389/fmicb.2018.00396

Feng, Y., Baig, T. T., Love, R. P., and Chelico, L. (2014). Suppression of APOBEC3-mediated restriction of HIV-1 by Vif. *Front. Microbiol.* 5:450. doi: 10.3389/fmicb.2014.00450

Fishier, A. G., Enolli, B., Ivanoff, L., Chamberlain, M., Petteway, S., Ratner, L., et al. (1987). The sor gene of HIV-1 is required for efficient virus transmission in vitro. *Science* 237, 888–893. doi: 10.1126/science.3497453

Frankel, A. D., and Young, J. A. (1998). HIV-1: fifteen proteins and an RNA. *Annu. Rev. Biochem.* 67, 1–25. doi: 10.1146/annurev.biochem.67.1.1

Fu, J., Bian, M., Jiang, Q., and Zhang, C. (2007). Roles of aurora kinases in mitosis and tumorigenesis. *Mol. Cancer Res.* 5, 1–10. doi: 10.1158/1541-7786.MCR-06-0208

Gabuzda, D. H., Lawrence, K., Langhoff, E., Terwilliger, E., Dorfman, T., Heslin, W. A., et al. (1992). Role of vif in replication of human immunodeficiency virus type 1 in CD4+ T lymphocytes. *J. Virol.* 66, 6489–6495. doi: 10.1128/jvi.66.11.6489-6495.1992

Gaddis, N. C., Sheehy, A. M., Ahmad, K. M., Swanson, C. M., Bishop, K. N., Beer, B. E., et al. (2004). Further investigation of simian immunodeficiency virus Vif function in human cells. *J. Virol.* 78, 12041–12046. doi: 10.1128/JVI.78.21.12041-12046.2004

Gallo, R. C., Salahuddin, S. Z., Popovic, M., Shearer, G. M., Kaplan, M., Haynes, E. J., et al. (1984). Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. *Proc. Natl. Acad. Sci. U.S.A.* 81, 7311–7315. doi: 10.1073/pnas.81.15.7311

Hertz, E. P., Kruse, T., Davey, N. E., López-Méndez, B., Sigurjónsson, J. O., Montoya, G., et al. (2016). A conserved motif provides binding specificity to the PP2A-B56 phosphatase. *Mol. Cell* 63, 686–695. doi: 10.1016/j.molcel.2016.06.024

Hoj, D. D., Neumann, A. U., Perelson, A. S., Chen, W., Leonard, J. M., and Markowitz, M. V. (1995). Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. *Nature* 373, 123–126. doi: 10.1038/373123a0

Holmes, R. K., Koning, F. A., Bishop, K. N., and Malim, M. H. (2007). APOBEC3F cannot inhibit the accumulation of HIV-1 reverse transcription products in the absence of hypermutation. Comparisons with APOBEC3G. *J. Biol. Chem.* 282, 2587–2595. doi: 10.1074/jbc.M607298200

Ho, R. S., Thelma, J., Garcia, A., Albiges-Rizo, C., Block, M. R., and Viallet, J. (2007). Functional interaction of Aurora-A and PP2A during mitosis. *Mol. Biol. Cell* 18, 1233–1241. doi: 10.1091/mbc.e06-12-1152

Hrecka, K., Gierszewska, M., Sriwastava, S., Kozačkiewicz, L., Swanson, S. K., and Flores, L., et al. (2007). Lentiviral Vpr usurps Cul4-DDB1[VprBP] E3 ubiquitin ligase to modulate cell cycle. *Proc. Natl. Acad. Sci. U.S.A.* 104, 11778–11783. doi: 10.1073/pnas.0702102104

Hrecka, K., Hao, C., Shun, M. C., Kaur, S., Swanson, S. K., Flores, L., et al. (2016). HIV-1 and HIV-2 exhibit divergent interactions with HLTF and UNG2 DNA repair proteins. *Proc. Natl. Acad. Sci. U.S.A.* 113, E9321–E9330. doi: 10.1073/pnas.1605023113

Ito, F., Yang, H., Xiao, X., Li, S. X., Wolfe, A., Zirkle, B., et al. (2018). Understanding the structure, multimerization, subcellular localization and mC selectivity of a genotoxic mutator and Anti-HIV factor APOBEC3H. *Sci. Rep.* 8:3763. doi: 10.1038/s41598-018-21955-0

Izumi, T., Io, K., Matsui, M., Shirakawa, K., Shinohara, M., Nagai, Y., et al. (2010). HIV-1 viral infectivity factor interacts with TP53 to induce G2 cell cycle arrest and positively regulate viral replication. *Proc. Natl. Acad. Sci. U.S.A.* 107, 20798–20803. doi: 10.1073/pnas.1008076107

Jäger, S., Kim, D. Y., Hultquist, J. F., Shindo, K., LaRue, R. S., Kwon, E., et al. (2011). Vif hijacks β-CFβ to degrade APOBEC3G and promote HIV-1 infection. *Nature* 481, 371–375. doi: 10.1038/nature10693

Jenkins, Y., McEntire, M., Weiss, K., and Greene, W. C. (1998). Characterization of HIV-1 vpr nuclear import: analysis of signals and pathways. *J. Cell Biol.* 143, 875–885. doi: 10.1083/jcb.143.4.875

Jeong, A. L., and Yang, Y. (2013). PP2A function toward mitotic kinases and substrates during the cell cycle. *BMB Rep.* 46, 289–294. doi: 10.5483/bmbrep.2013.46.6.041

Kamura, T., Sato, S., Haque, D., Liu, L., Kaelin, W. G., Conaway, R. C., and He, Z., Zhang, W., Chen, G., Xu, R., and Yu, X. F. (2008). Characterization of the structure, multimerization, subcellular localization and mC selectivity of a genotoxic mutator and Anti-HIV factor APOBEC3H. *Sci. Rep.* 8:3763. doi: 10.1038/s41598-018-21955-0

Kohli, R. M., Maul, R. W., Guminski, A. F., McClure, R. L., Gajewski, K. S., Saribasak, H., et al. (2010). Local sequence targeting in the AID/APOBEC family differentially impacts retroviral restriction and antibody diversification. *J. Biol. Chem.* 285, 40956–40964. doi: 10.1074/jbc.M110.177402

Kouno, T., Silva, T. V., Hilbert, B. J., Shandilya, S. M. D., Bohn, M. F., Kelch, B. A., et al. (2017). Crystal structure of APOBEC3G bound to single-stranded DNA reveals structural basis for cytidine deamination and specificity. *Nat. Commun.* 8:15024. doi: 10.1038/ncomms15024
Maertens, G. N. (2016). An endogenous inhibitor of human CD4+ T cells.

Madani, N., and Kabat, D. (1998). An endogenous inhibitor of human immunodeficiency virus in human lymphocytes is overcome by the viral Vif protein. J. Virol. 72, 10251–10255. doi: 10.1128/JVI.72.12.10251-10255.1998

Letko, M., Booiman, T., Kootstra, N., Simon, V., and Ooms, M. (2015). Ubiquitin-mediated degradation of cytidine deaminase APOBEC3G. Cell Host Microb. 13, 1789–1799. doi: 10.1016/j.chom.2013.12.011

Li, M., Shandilya, S. M., Carpenter, M. A., Rathore, A., Brown, W. L., Perkins, A. L., et al. (2012). Direct binding between BubR1 and B56-PP2A phosphatase complexes regulate mitotic progression. J. Cell Sci. 126(PT 5), 1086–1092. doi: 10.1242/jcs.122481

Lagouette, N., Brégnard, C., Hue, P., Basbous, J., Yatim, A., Larroque, M., et al. (2015). Translational regulation of the cell cycle: when, where, how and why? Philos. Trans. R. Soc. Lond. B Biol. Sci. 366, 3638–3652. doi: 10.1098/rstl.2011.0084

Krone, I., and Orr-Weaver, T. L. (2011). Critical role of PP2A-B56 family protein degradation in HIV-1 Vif mediated G2 cell cycle arrest. Biochem. Biophys. Res. Commun. 527, 257–263. doi: 10.1016/j.brc.2020.04.123

Naamati, A., Williamson, J. C., Greenwood, E. J., Marelli, S., Lehner, P. J., and Matheson, N. J. (2019). Functional proteomic atlas of HIV infection in primary human CD4+ T cells. eLife 8:e41431. doi: 10.7554/eLife.41431

Nagata, K., Shindo, K., Matsui, Y., Shirakawa, K., and Takatori-Kondo, A. (2020). Critical role of PP2A-B56 family protein degradation in HIV-1 Vif mediated G2 cell cycle arrest. Biochem. Biophys. Res. Commun. 527, 257–263. doi: 10.1016/j.brc.2020.04.123

Nakashima, M., Tsuzuki, S., Awaiz, H., Hamano, A., Okada, A., Ode, H., et al. (2017). Mapping region of human restriction factor APOBEC3H critical for interaction with HIV-1 Vif. J. Mol. Biol. 429, 1262–1276. doi: 10.1016/j.jmb.2017.03.019

Neil, S. J., Zang, T., and Bieniasz, P. D. (2008). Tetherin inhibits retrovirus release and is antagonized by HIV-1 Vpu. Nature 451, 425–430. doi: 10.1038/nature06553

Newman, E. N., Holmes, R. K., Craig, H. M., Klein, K. C., Lingappa, J. R., Malim, M. H., et al. (2005). Antiviral function of APOBEC3G can be dissociated from cytidine deaminase activity. J. Biol. Chem. 15, 166–170. doi: 10.1016/j.jcb.2004.12.068

Nilsson, J. (2019). Protein phosphatases in the regulation of mitosis. J. Cell Biol. 218, 395–409. doi: 10.1083/jcb.201809138

Ooms, M., Brayton, B., Letko, M., Maio, S. M., Pilcher, C. D., Hecht, F. M., et al. (2013). HIV-1 Vif adaptation to human APOBEC3H haplotypes. Cell Host Microb. 14, 411–421. doi: 10.1016/j.chom.2013.09.006

Ooms, M., Letko, M., and Simon, V. (2017). The structural interface between HIV-1 Vif and human APOBEC3H. J. Virol. 91:22289-16. doi: 10.1128/JVI.02289-16

Parsons, G. G., and Spencer, C. A. (1997). Mitotic repression of RNA polymerase II transcription is accompanied by release of transcription elongation complexes. Mol. Cell. Biol. 17, 5791–5802. doi: 10.1128/mcb.17.10.5791

Peru, E., Rajendran, K. S., Brazier, A. J., and Gabuzda, D. (2009). Regulation of APOBEC3 proteins by a novel YXXL motif in human immunodeficiency virus type 1 Vif and simian immunodeficiency virus SIVagm Vif. J. Virol. 83, 2374–2381. doi: 10.1128/JVI.01898-08

Peterson-Mahrt, S. K., Harris, R. S., and Neuberger, M. S. (2002). AID mutants E. coli suggesting a DNA deamination mechanism for antibody diversification. Nature 418, 99–103. doi: 10.1038/nature00862

Popovich, M., Sarragdharan, M. G., Read, E., and Gallo, R. C. (1984). Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. Science 224, 497–500. doi: 10.1126/science.6200935

Rathore, A., Carpenter, M. A., Demir, O, Ikeda, T., Li, M., Shaban, N. M., et al. (2013). The local dinucleotide preference of APOBEC3G can be altered from 5′-CC to 5′-TC by a single amino acid substitution. J. Mol. Biol. 425, 4442–4445. doi: 10.1016/j.jmb.2013.07.040

Richards, C., Albin, J. S., Demir, O, Shaban, N. M., Luengas, E. L., Land, A. M., et al. (2015). The binding interface between human APOBEC3F and HIV-1 Vif elucidated by genetic and computational approaches. Cell Rep. 13, 1781–1788. doi: 10.1016/j.celrep.2015.02.088

Romani, B., Shaykh Bayglooo, N., Aghasadeghi, M. R., and Allahbakhshi, E. (2015). HIV-1 Vpr protein enhances proapoptotic degradation of MCM10 DNA replication factor through the Cul4-DDB1[VprBP] E3 Ubiquitin ligase to induce G2/M cell cycle arrest. J. Biol. Chem. 290, 17380–17389. doi: 10.1074/jbc.M115.641522

Roochand, D. E., Lee, J. M., Shahinian, S., Paquette, D., Bussey, H., and Branton, P. E. (2001). Toxicity of human adenovirus E4orf4 protein in Saccharomyces cerevisiae results from interactions with the Cdc55 regulatory B subunit of PP2A. Oncogene 20, 5279–5290. doi: 10.1038/sj.onc.1204693

Rosa, A., Chande, A., Zuligio, S., De Sanctis, V., Bertorelli, R., Goh, S. L., et al. (2015). HIV-1 Nef promotes infection by excluding SERINC5 from virion incorporation. Nature 526, 212–217. doi: 10.1038/nature15399

Russell, R. A., and Pathak, V. K. (2007). Identification of two distinct human immunodeficiency virus type 1 Vif determinants critical for interactions with human APOBEC3G and APOBEC3F. J. Virol. 81, 8201–8210. doi: 10.1128/JVI.00395-07

Sakai, H., Shibata, R., Sakuragi, J., Sakuragi, S., Kawamura, M., and Adachi, A. (1993). Cell-dependent requirement of human immunodeficiency virus type 1 Vif protein for maturation of virus particles. J. Virol. 67, 1663–1666. doi: 10.1128/JVI.67.3.1663-1666.1993

Sakai, K., Barnitz, R. A., Chaigne-Delalande, B., Bide, N., and Lenardo, M. J. (2011). Human immunodeficiency virus type 1 Vif causes dysfunction of Cdk1
Salamango and Harris

Dual Function of HIV-1 Vif

Thierry, S., Marechal, V., Rosenzwajg, M., Sabbah, M., Redeuilh, G., Nicolas, J. C., et al. (2004). Cell cycle arrest in G2 induces human immunodeficiency virus type 1 transcriptional activation through histone acetylation and recruitment of CBP/ NF-kappaB, and c-Jun to the long terminal repeat promoter. J. Virol. 78, 12196–12206. doi: 10.1128/JVI.78.22.12196-12206.2004

Thompson, J. J., and Williams, C. S. (2018). Protein phosphatase 2A in the regulation of mtDNA signaling, stem cells, and cancer. Genes 9:121. doi: 10.3390/genes9030121

Timofeev, O., Cizmecioglu, O., Settele, F., Kempf, T., and Hoffmann, I. (2010). Cdc25 phosphatases are required for timely assembly of CDK1-cyclin B at the G2/M transition. J. Biol. Chem. 285, 16978–16990. doi: 10.1074/jbc.M109.096552

Usami, T., Wu, Y., and Götting, H. G. (2015). SERINC3 and SERINC5 restrict HIV-1 infectivity and are counteracted by Nef. Nature 526, 218–223. doi: 10.1038/nature15400

Van Damme, N., Goff, D., Katsura, C., Jorgenson, R. L., Mitchell, R., Johnson, M. C., et al. (2008). The interferon-induced protein BST-2 restricts HIV-1 release and is downregulated from the cell surface by the viral Vpu protein. Cell Host Microbe 3, 245–252. doi: 10.1016/j.chom.2008.03.001

Wang, J., Becker, J. T., Shi, K., Lauer, K. V., Salamango, D. J., Ahaira, H., et al. (2019). The role of RNA in HIV-1 Vif-mediated degradation of APOBC3. J. Mol. Biol. 431, 5019–5031. doi: 10.1016/j.jmb.2019.09.014

Wang, J., Shackelford, J. M., Casella, C. R., Shivers, D. K., Rapaport, E. L., Liu, B., et al. (2007). The Vif accessory protein alters the cell cycle of human immunodeficiency virus type 1 infected cells. Virology 359, 243–252. doi: 10.1016/j.virol.2006.09.026

Wang, J., Wang, Z., Yu, T., Yang, H., Hirsh, D. M., Kops, G. J., et al. (2016). Structural crystal structure of a PP2A B56-BubR1 complex and its implications for PP2A substrate recruitment and localization. Protein Cell 7, 516–526. doi: 10.1007/s13238-016-0283-4

Wang, X., Bajaj, R., Bollen, M., Peti, W., and Page, R. (2016). Expanding the PP2A interactome by defining a B56-Specific SLiM. Structure 24, 2174–2181. doi: 10.1016/j.str.2016.09.010

Wang, X., Garvasova, D. H., Nasa, I., Ueki, Y., Zhang, G., Kettenbach, A. N., et al. (2020). A dynamic charge-charge interaction modulates PP2A:B56 substrate recruitment. eLife 9:e55966. doi: 10.7554/eLife.55966

Wolfle, L. S., Stanley, B. J., Liu, C., Eliason, W. K., and Xiong, Y. (2010). Dissection of the HIV-1 Vif virus-human interaction with E3 ubiquitin ligase. J. Virol. 84, 7135–7139. doi: 10.1128/JVI.00031-10

Wu, Y., Zhou, X., Barnes, C. O., Delucia, M., Cohen, A. E., Gronenborn, A. M., et al. (2016). The DDB1-DCAF1-Vpr-UNG2 crystal structure reveals how HIV-1 Vpr steers human UNG2 toward destruction. Nat. Struct. Mol. Biol. 23, 933–940. doi: 10.1038/nsmb.3284

Xu, Z., Cetin, B., Anger, M., Cho, U. S., Helmhart, W., Nasmyth, K., et al. (2009). Structure and function of the PP2A-shugoshin interaction. Mol. Cell 35, 426–441. doi: 10.1016/j.molcel.2009.06.031

Yamashita, T., Kamada, K., Hatcho, K., Adachi, A., and Nomaguchi, M. (2008). Identification of amino acid residues in HIV-1 Vif critical for binding and exclusion of APOBC3/F. Microb. Infect. 10, 1142–1149. doi: 10.1016/j.micinf.2008.06.003

Yu, X., Yu, T., Liu, B., Luo, K., Kong, W., Mao, P., et al. (2003). Induction of APOBC3 ubiquitination and degradation by an HIV-1 Vif-Cu5-SCF complex. Science 302, 1056–1060. doi: 10.1126/science.108991

Yu, Y., Xiao, Z., Ehrlich, E. S., Yu, X., and Yu, X. F. (2004). Selective assembly of HIV-1 Vif-Cu5-ElonginB-ElonginC E3 ubiquitin ligase complex through a novel SOCS box and upstream cytoplasm. Genes Dev. 18, 2867–2872. doi: 10.1101/gad.125024

Zhang, F., and Bieniasz, P. D. (2020). HIV-1 Vpr induces cell cycle arrest and enhances viral gene expression by depleting CCDC137. eLife 9:e055806. doi: 10.7554/eLife.55806

Zhang, H., Yang, B., Pomerantz, R. J., Zhang, C., Arunachalam, S. C., and Gao, L. (2003). The cytidine deaminase CEM15 induces hypermutation in newly synthesized HIV-1 DNA. Nature 424, 94–98. doi: 10.1038/nature01707

Zhang, W., Du, J., Evans, S. L., Yu, Y., and Yu, X. F. (2011). T-cell differentiation factor CBF-β regulates HIV-1 Vif-mediated evasion of host restriction. Nature 481, 376–379. doi: 10.1038/nature10718

Zhang, W., Huang, M., Wang, T., Tan, L., Tian, C., Yu, X., et al. (2008). Conserved and non-conserved features of HIV-1 and SIVagm Vif mediated suppression of APOBC3 cytidine deaminases. Cell Microbiol. 10, 1662–1675. doi: 10.1111/j.1462-5822.2008.01157.x

Zhao, K., Du, J., Rui, Y., Zheng, W., Kang, J., Hou, J., et al. (2015). Evolutionarily conserved pressure for the existence of distinct G2/M cell cycle arrest and A3H inactivation functions in HIV-1 Vif. Cell Cycle 14, 838–847. doi: 10.1080/15384101.2014.1000212
Zhao, R. Y., and Elder, R. T. (2005). Viral infections and cell cycle G2/M regulation. *Cell Res.* 15, 143–149. doi: 10.1038/sj.cr.7290279

Zhen, A., Du, J., Zhou, X., Xiong, Y., and Yu, X. F. (2012). Reduced APOBEC3H variant anti-viral activities are associated with altered RNA binding activities. *PLoS One* 7:e38771. doi: 10.1371/journal.pone.0038771

Zhou, X., Evans, S. L., Han, X., Liu, Y., and Yu, X. F. (2012). Characterization of the interaction of full-length HIV-1 Vif protein with its key regulator CBFβ and CRL5 E3 ubiquitin ligase components. *PLoS One* 7:e33495. doi: 10.1371/journal.pone.0033495

Zhou, X., Han, X., Zhao, K., Du, J., Evans, S. L., Wang, H., et al. (2014). Dispersed and conserved hydrophobic residues of HIV-1 Vif are essential for CBFβ recruitment and A3G suppression. *J. Virol.* 88, 2555–2563. doi: 10.1128/JVI.03604-13

**Conflict of Interest:** RH is a co-founder, shareholder, and consultant of ApoGen Biotechnologies Inc.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

_Copyright © 2021 Salamango and Harris. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms._