CBFß and HIV Infection

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Abstract

In order to achieve a persistent infection, viruses must overcome the host immune system. Host restriction factors dominantly block virus transmission, but are subject to down regulation by viral accessory proteins. HIV encodes several accessory factors that overcome different cellular restriction factors. For example, the HIV-1 protein Vif down regulates the human APOBEC3 family of restriction factors by targeting them for proteolysis by the ubiquitin-proteasome pathway. Recently, this function was shown to require the transcription cofactor CBFβ, which acts as a template to assist in Vif folding and allow for assembly of an APOBEC3-targeting E3 ligase complex. In uninfected cells, CBFβ is an essential binding partner of RUNX transcription factors. By binding CBFβ, Vif has also been shown to perturb transcription of genes regulated by the RUNX proteins, including restrictive APOBEC3 family members. Here we review how the link between CBFβ and Vif supports transcriptional and post-transcriptional repression of innate immunity. The ability of a single viral protein to coopt multiple host pathways is an economical strategy for a pathogen with limited protein coding capacity to achieve a productive infection.

Keywords

HIV • Transcription • RUNX • Restriction factors • Innate immunity • APOBEC3 • Vif

25.1 Introduction

Many viral pathogens have co-evolved with their host organism, often times resulting in a molecular arms race against host immune defenses (Daugherty and Malik 2012). Research designed to elucidate the intermolecular interactions at the

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viral-host interface will therefore be critical to our understanding of both host immunity and viral pathogenesis. As our knowledge of the viral-host interface improves so will our efforts towards the development of new and innovative therapeutic agents to counter viral infections. In this chapter we will focus on HIV related research and how it has informed aspects of host innate immunity- specifically the identification of APOBEC3 host restriction factors and novel roles for CBFβ in viral restriction.

Nearly 25 years ago, binding sites for the core binding factor (CBF) were identified in the enhancer of the Moloney murine leukemia retrovirus (MoMLV) (S. Wang et al. 1993). Parallel studies identified key CBF binding sites in the polyoma virus enhancer (reviewed by Ito) (Ito 2008). These early investigations in viral systems paved the way for what we now know: that CBF is a heterodimeric complex consisting of a DNA binding subunit (RUNX 1, 2 or 3) and a non-DNA binding subunit termed CBFβ (de Bruijn and Speck 2004). CBFβ is required for all RUNX protein function, probably by allosterically enhancing their interactions with DNA and protecting RUNX proteins from degradation (Ogawa et al. 1993; Gu et al. 2000; Tang et al. 2000; Yan et al. 2004; Tahirov et al. 2001; Q. Wang et al. 1996; G. Huang et al. 2001). The diverse biological roles of RUNX proteins include hematopoiesis, neurogenesis and osteogenesis, which are reviewed in other chapters of this book. It is well appreciated that RUNX1 and 3 are important in T-cell differentiation, and have broad roles in immunity (de Bruijn and Speck 2004; S. Wang et al. 1993; Voon et al. 2015). Just like early work, where viruses were used to probe function of CBFβ during development, recent studies with HIV-1 and related lentiviruses reveal an emerging role of RUNX/CBFβ in the regulation of innate immunity (Ito 2008; Jager et al. 2012; W. Zhang et al. 2012; Hultquist et al. 2012; Anderson and Harris 2015).

HIV-1, the pathogen that causes AIDS, causes severe immunodeficiency by the depletion of CD4+ T-cells (Muro-Cacho et al. 1995; Finkel et al. 1995; Doitsh et al. 2015). In order to achieve a productive infection, HIV-1 must counteract several human restriction factors, which act as dominant blocks to viral replication in the absence of accessory proteins (Harris et al. 2012; Malim and Emerman 2008). The APOBEC3 family of restriction factors block the replication of retroviruses and retroelements by binding and enzymatically hypermutating newly transcribed cDNA prior to integration (Harris and Liddament 2004). In order to counteract the restrictive potential of APOBEC3 proteins, lentiviruses encode for the viral infectivity factor (Vif) accessory protein that promotes degradation of APOBEC3 proteins by hijacking the ubiquitin proteasome pathway (Yu et al. 2003). Several years ago, it was reported that CBFβ was required for this effect (Ogawa et al. 1993; W. Zhang et al. 2012; Gu et al. 2000; Jager et al. 2012; Tang et al. 2000; Yan et al. 2004; Tahirov et al. 2001; Q. Wang et al. 1996; G. Huang et al. 2001). This was surprising given that CBFβ has not been documented to play a direct role in the ubiquitin proteasome pathway. Here we review studies linking CBFβ to Vif function, including recent observations that Vif can perturb RUNX mediated transcription and the structural basis for these effects.

25.2 HIV-1 Vif Inhibits Human APOBEC3 to Promote Viral Replication

It has long been appreciated that the Vif protein is essential for viral replication in primary T-cells and natural infection (Fisher et al. 1987). Initially it was unclear what role Vif played in viral replication as it was only required in certain CD4+ T cell lines and not in others. CD4+ T cell lines that are able to support growth of Vif-deficient viruses are termed permissive cells (e.g. SupT1, CEM-SS and Jurkat cells), whereas those that do not support Vif-deficient viruses are termed non-permissive cells (e.g. HuT78, H9 and peripheral blood lymphocytes) (Fig. 25.1). Similar to non-permissive cells, transient heterokaryons formed by the fusion of non-permissive and permissive cells also restrict the spread of Vif-deficient HIV but allow the spread of wild-type HIV-1 (Simon et al. 1998). Characterization of permissive and

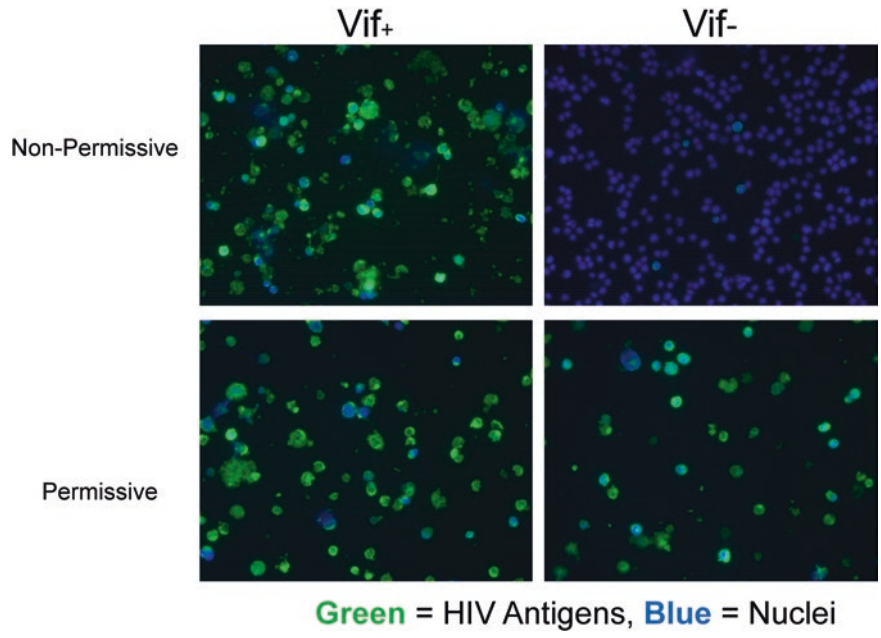

Fig. 25.1 Vif is required for the spread of HIV-1 in non-permissive and primary CD4+ T lymphocytes. Immunofluorescence showing HIV (*green*) and cell nuclei (*blue*). *Top row*, HIV spread in a non-permissive CD4+ T-lymphocyte cell line requires Vif. *Bottom row*, Vif is dispensable for spread in a permissive cell line. APOBEC3 family members are either expressed or not expressed in nonpermissive or permissive cell lines respectively

non-permissive cell lines, as well as cell fusion experiments, suggested that there was a host factor found in non-permissive cells that protected them against HIV infection in the absence of Vif (Simon et al. 1998), and that the primary role of Vif was to counteract this innate antiviral activity of non-permissive cells (Muro-Cacho et al. 1995; Fisher et al. 1987; Finkel et al. 1995; Gabuzda et al. 1992; Doitsh et al. 2015; Sakai et al. 1993; Sova and Volsky 1993; von Schwedler et al. 1993; Bouyac et al. 1997). By comparing the pattern of mRNA expression between permissive and non-permissive cells, APOBEC3G (A3G) was first identified as the antiviral factor that restricts the spread of Vif-deficient HIV-1 in non-permissive cells (Harris et al. 2012; Sheehy et al. 2002; Malim and Emerman 2008). Indeed, transient expression of A3G in permissive cells confers non-permissive phenotype (Harris and Liddament 2004; Sheehy et al. 2002).

In humans, seven members of the A3 protein family- A3A, A3B, A3C, A3D, A3F, A3G and A3H- are encoded in a tandem array on chromosome 22 and their expression levels vary in different tissues and cell types (Yu et al. 2003; Jarmuz et al. 2002; Koning et al. 2009). Although A3G displays the most potent antiviral activities against Vif-deficient HIV-1, other A3 proteins (A3D, A3F and A3H) are also expressed in non-permissive cells and contribute to the restriction of HIV-1 when the *Vif* gene is absent (Dang et al. 2006; Zheng et al. 2004; Mulder et al. 2010; Chaipan et al. 2013) and reviewed in (Desimmie et al. 2014).

Further support indicating the viruses need to counteract A3 proteins stems from the fact that the *vif* gene is found in all known lentiviruses except EIAV (Equine infectious anemia virus). These include HIV-2, simian immunodeficiency virus (SIV) and non-primate lentiviruses such as BIV, MVV, CAEV and FIV. In the absence of Vif, these viruses are all restricted by their respective host APOBEC3 family members. In addition to inhibiting lentiviruses, A3 proteins have also been reported to inhibit the infectivity of diverse retroviruses (HTLV-1, MLV and EIAV),

retro-transposons and other viruses such as HBV and AAV (Holmes et al. 2007). Due to space limitations our review will be focused on HIV-1, the most extensively studied primate lentivirus.

25.3 Molecular Mechanisms of APOBEC3 Family Members and Primate HIV-1 Vif

The proteins of the A3 family contain either one (A3A, A3C and A3H) or two cytidine deaminase domains (A3B, A3D, A3F and A3G), suggesting that A3 proteins are able to restrict viral spread through DNA editing. Indeed, A3G mutates deoxy-cytidine to deoxy-uridine in the minus strand of HIV-1 DNA synthesized by reverse transcription of viral RNA genome. This enzymatic mutation results in the accumulation of non-functional proviruses by G-to-A hypermutation in the viral DNA (Harris et al. 2003; Lecossier et al. 2003; Mangeat et al. 2003; H. Zhang et al. 2003). However, catalytically inactive A3G can also display significant antiretroviral activity when overexpressed, indicating that the deaminase-independent activity of A3G can contribute to HIV-1 inhibition. In this non-editing mode, A3G appears to inhibit the synthesis of viral DNA and its integration into human genome (Newman et al. 2005; F. Guo et al. 2006, 2007; Iwatani et al. 2007; X. Y. Li et al. 2007; Luo et al. 2007; Mbisa et al. 2007; X. Wang et al. 2012). In either case, it is widely accepted that in order for APOBEC3 family members to restrict retroviruses, they must be packed into the viral core to have access to the viral genetic material during reverse transcription in the target cells (Mangeat et al. 2003). The requirement for viral packaging of the APOBEC3 family of restriction factors is referred to as the Trojan Horse model (Fig. 25.2).

HIV-1 Vif plays a critical role in counteracting the APOBEC3 proteins. To this end, Vif reduces the steady-state level of A3G, A3C, A3D, A3F and A3H haplotype II in producer cells, targeting them for degradation by the ubiquitin-proteasome system and preventing their packaging into virions (Conticello et al. 2003; Mariani et al. 2003; Marin et al. 2003; Sheehy et al. 2003; Stopak et al. 2003; Yu et al. 2003). Cytoplasmic localization of HIV-1 Vif is required for its ability to neutralize APOBEC3G and probably all Vif susceptible APOBEC3 family members (Farrow et al. 2005; Goncalves et al. 1994; Wichroski 2004). In order to target A3 proteins for degradation, Vif hijacks a cellular E3 ubiquitin ligase of the Cullin-RING super family (CRL5), comprised of CUL5/RBX2 and the adaptor subunit ELOB/ELOC (Yu et al. 2003; D. J. Stanley et al. 2012; Kamura 2004). Within the context of the E3 ligase, Vif functions as the substrate receptor and directly binds APOBEC3 proteins, thus recruiting them to CRL5 for polyubiquitination (Fig. 25.2) (Yu et al. 2003).

25.4 CBFβ Acts as a Chaperone for HIV-1 Vif

Even though the functional roles of Vif in HIV-1 infectivity have been well-described, the purification of homogeneous recombinant Vif protein has been extremely difficult and for a long while limited research efforts in biochemistry and structure biology. Using novel proteomics approaches, CBFβ was discovered as a potential HIV-1 Vif-binding factor (Jager et al. 2012; W. Zhang et al. 2012). These studies employed affinity chromatography with mass spectrometry and found that over-expressed HIV-1 Vif in human cell lines was co-eluted with several cellular factors including CRL5 and CBFβ. The identification of CBFβ represented a major breakthrough in Vif biochemical studies, finally allowing researchers to reconstitute an active CRL5-Vif-CBFβ ubiquitin E3 ligase from recombinant purified components. This complex can polyubiquitinate A3G *in vitro* and recapitulates the known APOBEC3 substrate specificity in cells (Jager et al. 2012; D. Y. Kim et al. 2013). The role of CBFβ was further validated in cellular assays demonstrating that CBFβ is required for HIV-1 Vif to degrade all Vif-sensitive APOBEC3 family members (Hultquist et al. 2012). Specifically, knockdown of CBFβ reduces polyubiquitination of tran-

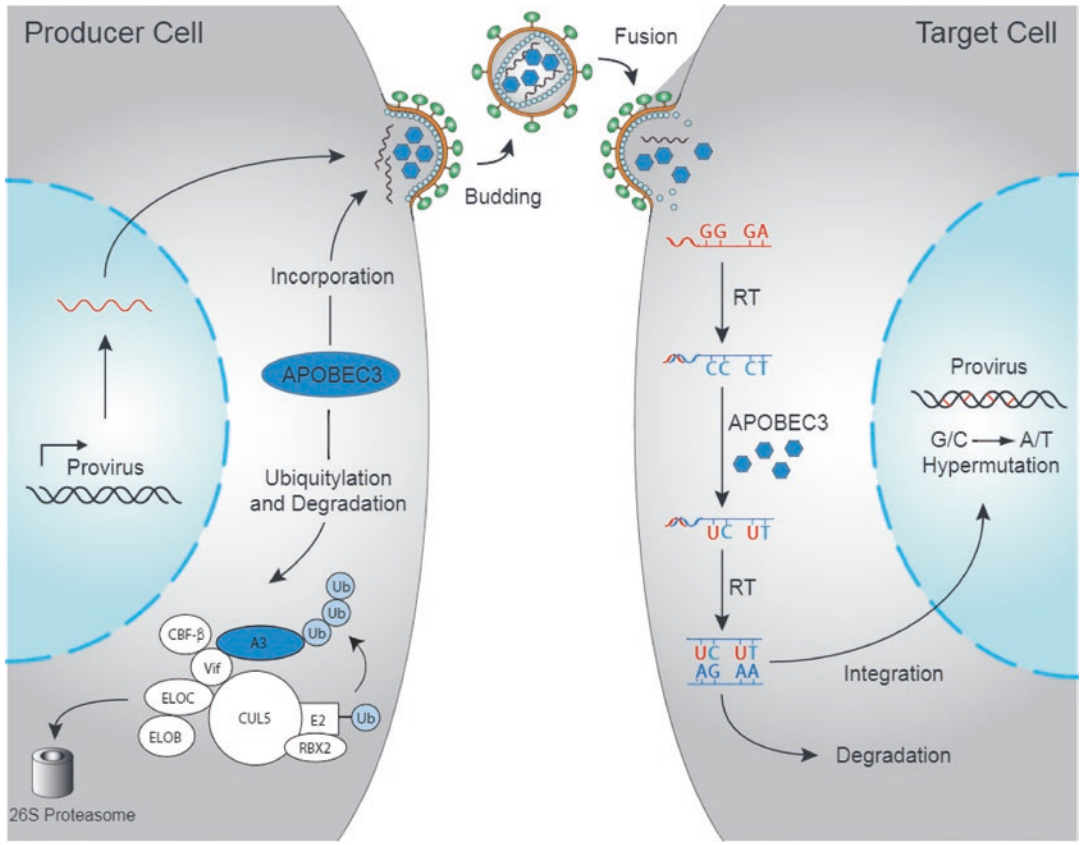

Fig. 25.2 Overview of restriction by APOBEC3 and suppression by HIV Vif. In the absence of Vif, APOBEC3 family members -A3D, A3F, A3G and A3H-are packed into budding virions and restrict HIV by acting as cytidine deaminases resulting in hypermutation, which leads to genetic catastrophe for the virus. Vif promotes infectivity by targeting A3 family members for degradation by the 26S proteasome. To do so, Vif hijacks a cellular E3 ligase (CRL5, or Cul5/RBX2, EloBC) and the transcription cofactor CBFß (Figure adapted from reference Harris et al. 2012)

siently expressed APOBEC3G and subsequently inhibits infectivity of HIV-1(Jager et al. 2012; Miyagi et al. 2014). These studies also showed that a reduction in CBFβ protein levels correlates with a decrease in steady-state levels of Vif protein in a variety of cell types, including CD4+ T-cell lines (Jager et al. 2012; W. Zhang et al. 2012; Miyagi et al. 2014; Han et al. 2014; Anderson and Harris 2015). With this in mind, reduced levels of Vif were compensated for by overexpression, however this does not restore the A3G degradation defect when CBFβ is knocked down (Miyagi et al. 2014). Knockdown of CBFβ by RNAi correlates with a loss of Vif binding to CUL5 in cells (W. Zhang et al. 2012; Han et al. 2014). These studies, together with the biochemical and structural data, indicate that CBFβ acts to 'chaperone' Vif by stabilizing its fold so that it can specifically engage CRL5 and A3 substrates to promote ubiquitination (*vide infra*). This function of CBFβ is likely conserved in all primate lentiviruses, based on sequence similarity and the fact that CBFβ is required for SIV Vif to degrade Vif-sensitive Rhesus Macaque APOBEC3 proteins (Hultquist et al. 2012). In contrast, CBFβ is dispensable for Vif function in several non-primate lentiviruses, consistent with the fact that the sequence of non-primate lentiviral Vif proteins diverges considerably from their primate counterparts (W. Zhang et al. 2014; Ai et al. 2014; Kane et al. 2015).

25.5 HIV-1 Vif Binding to CBFβ Perturbs RUNX Mediated Transcription

It is well established that CBFβ is required for RUNX mediated transcription (Q. Wang et al. 1996). It forms direct interactions with the conserved DNA binding domain of RUNX family members (the Runt domain), enhances RUNX DNA binding activity through an allosteric mechanism, and protects RUNX1 from degradation by the ubiquitin-proteasome system (Ogawa et al. 1993; Gu et al. 2000; Tang et al. 2000; Yan et al. 2004; Tahirov et al. 2001; G. Huang et al. 2001). Several lines of evidence suggest Vif has the capacity to perturb RUNX mediated transcription by binding to CBFβ. First, Vif reduces transcription of a RUNX reporter gene transiently transfected into HEK293T cells. Second, DNA microarray analysis in permissive Jurkat T-cells reveal a large number of differentially expressed genes in cells stably expressing Vif (Fig. 25.3a, b) (D. Y. Kim et al. 2013). Differentially expressed genes were statistically enriched with RUNX1 binding sites, as determined by ChIP-Seq studies using a RUNX1 antibody (D. Y. Kim et al. 2013). RT-qPCR of candidate genes confirmed that Vif had the capacity to repress transcription of known RUNX sensitive genes. For example, *Tbx21* (also known as *Tbet*) was identified in the ChIP-Seq and microarray analysis (D. Y. Kim et al. 2013). Consistent with prior studies, reduction in *Tbx21* expression correlated with enhanced production of IL-2(Szabo et al. 2000). Likewise, infection of a permissive CD4+ T-cell line with HIV-1 reduces expression of *Tbx21* in a Vif-dependent manner (D. Y. Kim et al. 2013). Third, co-IP, *in vitro* binding, and mutagenesis studies suggest CBFβ binds Vif in a manner that is mutually exclusive with RUNX transcription factors (D. Y. Kim et al. 2013). This notion is further supported by a high-resolution crystal structure of Vif-bound to CBFβ (*vide infra*) (Y. Guo et al. 2014). A parsimonious explanation for the ability of Vif to perturb transcription is that it can scavenge CBFβ so that it cannot be incorporated into transcription complexes with RUNX proteins, though this model has not been directly tested.

While the aforementioned studies show Vif has the capacity to affect numerous genes in permissive cells, a clear biological function for these phenomena in APOBEC3 expressing non-permissive CD4+ T-cell cells was not evaluated. A recent study indicates that Vif-susceptible APOBEC3 genes are positively regulated by CBFβ in primary and non-permissive H9 CD4+ T-cells (Anderson and Harris 2015). Reduction or ablation of CBFβ mRNA by RNAi or CRISPR reduces the expression of A3C, A3D, A3F, A3G and A3H mRNA as detected by RT-qPCR (Anderson and Harris 2015). Steady-state levels of A3G and A3F protein were severely diminished when CBFβ was knocked down or knocked out (Anderson and Harris 2015). RNAi resistant CBFβ was able to compliment the CBFβ knockdown by increasing A3G protein expression levels, and this effect required interaction with the Runt domain of RUNX family members (Anderson and Harris 2015). ChIP-Seq studies indicate there are numerous RUNX3 binding sites throughout the entire APOBEC3 locus (Fig. 25.3c). Strikingly, knockout or knockdown of CBFβ rendered non-permissive H9 cells permissive for infection with Vif-deficient virus: the restrictive potential of these cells provided by the APOBEC3 repertoire was nearly completely suppressed (Anderson and Harris 2015). These findings provide a compelling explanation for why HIV-1 Vif hijacks CBFβ: it allows Vif to interfere with RUNX mediated transcription of APOBEC3 family members. It has been suggested that the expanded APOBEC3 repertoire of primates compared to ancestral placental mammals has driven the evolution of primate lentiviral Vif to acquire CBFβ as a binding partner, endowing the primate lentiviruses with the ability to downregulate A3 transcriptionally, in addition to the well established post-transcriptional mechanism of ubiquitin mediated proteolysis (Anderson and Harris 2015).

It is worth mentioning that studies in non-permissive and permissive cells have allowed the unambiguous separation of function of CBFβ as it pertains to viral infectivity at the transcriptional

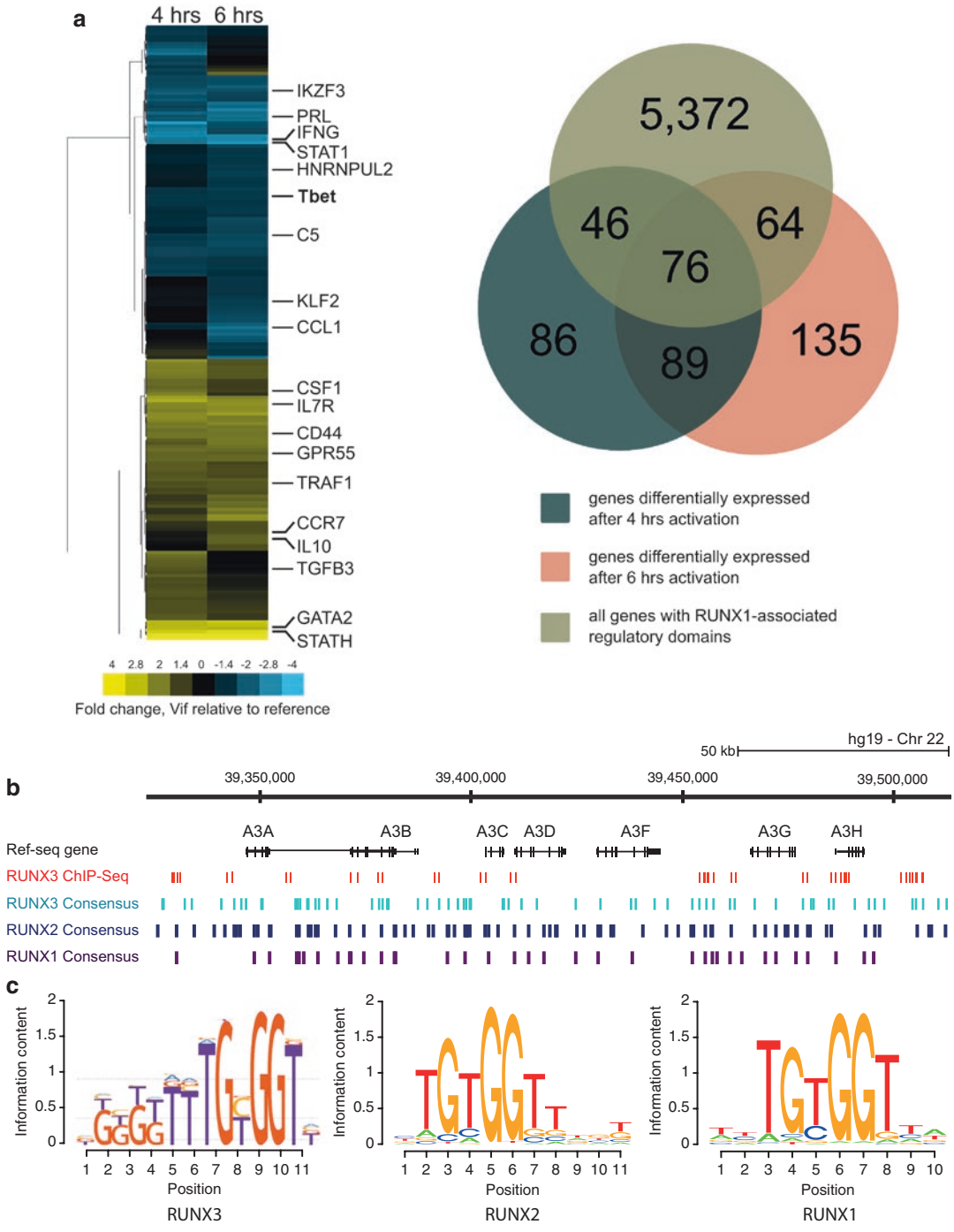

Fig. 25.3 Vif has the capacity to perturb transcription of RUNX genes in host cells. (**a**) Stable expression of Vif in permissive Jurkat T-cell lines perturbs gene expression. Shown is the differential expression of genes 4 and 6 h after activation with the phorbol ester PMA and the lectin PHA in cells either lacking or containing stably expressed Vif. In this study, there was a statistically significant enrichment of RUNX1 sites associated with differentially expressed genes. (**b**) Non-permissive T-cells have RUNX sites associated with APOBEC3 loci. Results from experimental ChIP-Seq data are shown for RUNX3-binding sites demonstrated by ENCODE ChIP-sequencing of the lymphoblastoid cell line GM12878 (ENCSR000BRI) (Consortium et al. 2013). (**c**) Motif for RUNX3 DNA binding site

and post-transcriptional levels. Early work where CBFβ was knocked down in permissive HEK293T or HeLa cells showed that degradation of heterologous expressed A3 family members by Vif required CBFβ, likely due to its chaperone function. In contrast, in non-permissive CD4+ T-cells, ablation of CBFβ nearly bypasses the requirement of the post-transcriptional degradative step, since steady state mRNA and protein levels of APOBEC3 are reduced from what is apparently a transcriptional defect, obviating the need for a functional Vif E3 ligase. The regulation of APOBEC3 expression at transcriptional and post-transcriptional steps by interactions between HIV-1 Vif and CBFβ is a molecular two-step that ensures viral escape from the innate immunity provided by APOBEC3 family of restriction factors (Anderson and Harris 2015).

Some studies indicate CBFβ could also regulate retroviral transcription. For example, this was initially observed in the retrovirus MoMLV, where CBF binding sites in the long-terminal repeat (LTR) were discovered as an enhancer of viral replication (S. W. Wang and Speck 1992). It is interesting to note that CBF binding sites have also been identified in the LTR of SIVmac and HIV-1(S. W. Wang and Speck 1992). Recently, CBFβ/RUNX1 was reported to repress HIV-1 transcription and suggested to be important for viral latency (Klase et al. 2014). Though combination therapy for HIV-1 infection can reduce plasma levels of virus to undetectable levels, HIV-1 can persist in a latent form in resting memory CD4+ T-cells (Finzi et al. 1997, 1999). In these cells, there is minimal transcription from the LTR because of the absence of necessary host factors which are present only in activated T-cells and the additional presence of a putative restriction factor that blocks viral reverse transcription (Nabel and Baltimore 1990; Baldauf et al. 2012; Laguette et al. 2011; Hrecka et al. 2011). Knockdown of CBFβ or RUNX1 in cell culture models of latency results in reactivation of virus (Klase et al. 2014). This observation is consistent with ChIP-qPCR showing RUNX and CBFβ associated with the HIV-1 LTR in latently infected cells (Klase et al. 2014). Likewise, a pharmacologic inhibitor of RUNX1/CBFβ (Ro5–3335) was also capable of reactivating latent cells (Klase et al. 2014; Cunningham et al. 2012). Synergy between Ro5–3335 and the HDAC inhibitor SAHA was observed in HIV-1 reactivation in cell-culture and in PBMCs isolated from patients infected with HIV-1. The available data suggest RUNX1/CBFβ is a potential target to reactive latent reservoirs, a strategy currently being investigated for curative treatments (Klase et al. 2014; Cunningham et al. 2012). In addition to Ro5–3335, other inhibitors RUNX1/CBFβ await testing in this regard (Gorczynski et al. 2007). RUNX1 expression in CD4+ memory T-cells of viremic HIV-1 patients correlates negatively with viral load and positively with CD4+ T-cell count, suggesting RUNX1 may be associated with progression of HIV-1 in the clinic (Klase et al. 2014).

Additional support for a role of RUNX1 and CBFβ in HIV-1 transcription is provided by the following observations (Klase et al. 2014). There are several conserved RUNX1 sites in the group B HIV-1 LTR. Overexpression of RUNX1 and CBFβ can repress transcription off integrated and unintegrated LTRs in cell culture. This effect is abrogated if the last two nucleotides of the RUNX1 consensus-binding motif (TGYGGT) are mutated within one of the predicted RUNX1 binding sites. Over expression of Vif partially restores transcription of the viral LTR and redistributes CBFβ from cell wide to cytoplasmic localization, a result that is consistent with Vif sequestering CBFβ in the cytoplasm. Therefore, CBFβ/RUNX has the capacity to repress transcription off the viral UTR in addition to promoting transcription of APOBEC3 genes, both of which are beneficial to the host.

25.6 The Structure of the Vif-CBFβ-ELOBC-CUL5 Complex

Insights into the multifunctional nature of HIV-1 Vif-CBFβ interactions are provided by the recent crystal structure of the HIV-1 Vif, CBFβ, ELOB and ELOC in complex with the CUL5 N-terminal domain (CUL5NTD) (Y. Guo et al. 2014) (Fig. 25.4). This structure sheds light on how Vif

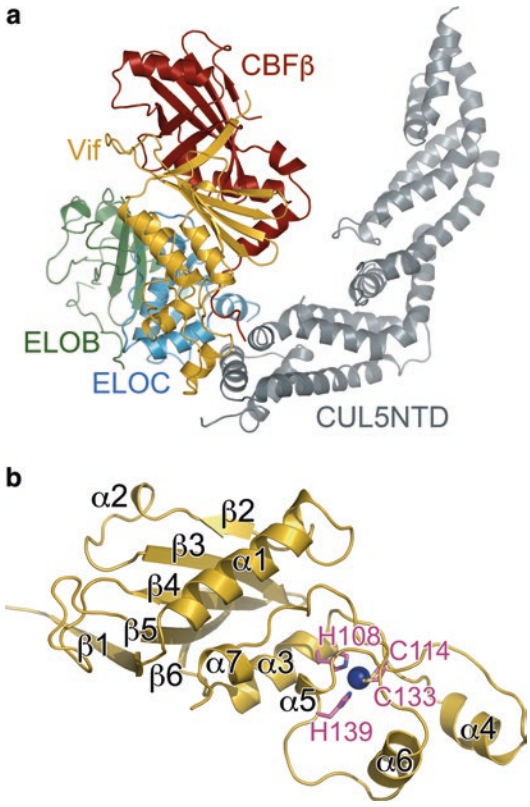

Fig. 25.4 Overview of Vif structure (**a**) Crystal structure of Vif-CBFß-ELOBC in complex with the N-terminal domain of CUL5 (CUL5NTD). The crystal contains 12 pentameric complexes in asymmetrical units, one of which is drawn as ribbon diagram (PDB id 4N9F; chain C, D, E, F and G). CUL5NTD, Vif, ELOB, ELOC and CBFβ are labeled and colored *grey*, *yellow*, *green*, *cyan* and maroon, respectively. Vif mediates direct interactions with CBFβ, CUL5 and ELOC. (**b**) The structure of the Vif monomer in the pentameric complex. Vif structure is drawn as a ribbon diagram and the secondary structure is labeled. The residues that coordinate a zinc atom, termed the HCCH motif, are depicted as sticks and the residue numbers are labeled. The α-helices (α1-α7) and β-strands (β1-β5) are also indicated

folding is promoted by host factors resulting in the formation of a functional CRL5-Vif-CBFβ holoenzyme, how Vif binding to CBFβ could perturb RUNX-mediated transcription, and how Vif recognizes A3 substrates. Below we will discuss each of these functions from a structural perspective.

The Vif subunit (residues 3–171) in the complex maintains a conical shape formed by the arrangement of two globular folds, a large α/β fold and small α fold (Fig. 25.4b). The large α/β fold consists of an antiparallel beta-sheet (β1–β5) and four alpha-helices (α1–α3 and α7) that are aligned along the convex side of the beta-sheet. The α fold (residues 112–161) inserted between α3 and α7 in the α/β fold is composed of three helices (α4, α5 and α6). Additionally, the HCCH motif in Vif coordinates a zinc atom between α/β and α folds. The coordination of the zinc atom is mediated by H108 in the α/β fold and by C114, C133 and H139 in the α fold and appears to stabilize the tertiary structure by reducing the flexibility between two globular folds of Vif (Fig. 25.4b). In the surface structure of Vif, most of the charged surface is exposed to solvent even in the pentameric complex. This indicates that the Vif surface for the binding of CUL5, ELOC and CBFβ is composed of mainly hydrophobic patches (Y. Guo et al. 2014).

The complete structure reveals that Vif mediates the formation of hetero-pentameric Vif-CBFβ-ELOBC-CUL5NTD complex by directly interacting with all affiliated subunits (Fig. 25.4a) (Y. Guo et al. 2014). As a central part of the assembly of CRL5-Vif E3 ligase, α3 in HIV-1 Vif (residues 121–127) interacts with the LWDD motif in CUL5 (residues 52–55), which is not conserved among other Cullin family proteins, indicating that the LWDD motif is required for the selective recruitment of CUL5 to HIV-1 Vif (Y. Guo et al. 2014). This mode of interaction between Vif and CUL5 is quite different than the binding in cellular SOCS2-CUL5, wherein the CUL box in SOCS2 (residues 182–186) interacts with residues L52, W53, Q113 and I116 in CUL5(Y. K. Kim et al. 2013; Y. Guo et al. 2014). For the binding of the ELOB/ELOC heterodimer, the BC box in HIV-1 Vif (residues 141–153 of α4) interacts with the surface residues distributed at the C-terminus of ELOC (B. J. Stanley et al. 2008). Vif seems to mimic the BC box in cellular SOCS proteins for ELOC binding (Kamura et al. 1998; Yu et al. 2003; Mehle 2004). As explained above, the BC box contains the one motif conserved among all lentiviral Vif proteins, the SLQxLA motif (Kane et al. 2015). Thus, Vif homologues may recruit ELOB/ELOC for the CRL-Vif assembly in a common manner.

25.7 The Binding Mode of CBFβ with HIV-1 Vif and Its Implications for Transcription

In contrast to the recruitment of CUL5 and ELOBC into CRL5-Vif, which is mediated by interactions between small binding motifs, the interaction between Vif and CBFβ is mediated by a large surface area, burying a total of 4800 Å^2 (Fig. 25.5) (Y. Guo et al. 2014). The binding interface is widely dispersed through residues 1–120 in HIV-1 Vif and encompasses the entire length of CBFβ. For example, a N-terminal beta strand in HIV-1 Vif (residues 2–11) pairs with a beta strand in CBFβ (residues 63–69) forming an intermolecular beta sheet through a network of hydrogen bonds whereas the C-terminal tail of CBFβ (residues 135–157), containing an alpha helix and a flexible loop, binds to a surface crevice near a zinc atom coordinated by the HCCH motif of Vif. Both interactions appear to support tight binding between HIV-1 Vif and CBFβ. The extensive buried surface area lining the interface between CBFβ and Vif may explain the susceptibility of Vif to proteasomal degradation when CBFβ is knocked down (Jager et al. 2012; W. Zhang et al. 2012; Miyagi et al. 2014; Anderson and Harris 2015). This effect is mirrored by an increase to protease susceptibility *in vitro* when CBFβ is absent from purification (D. Y. Kim et al. 2013). Therefore, CBFβ templates the folding of Vif to allow it to function as a substrate receptor for the CRL5-Vif-CBFβ E3 ligase. In this way, it functions to "chaperone" Vif into a conformation that allows proper folding and function.

CBFβ regulates transcriptional activities of RUNX proteins in human cells by binding to Runt domains conserved among the RUNX proteins and enhancing the association of the Runt domain with DNA (Ogawa et al. 1993; Gu et al. 2000; Tang et al. 2000; Tahirov et al. 2001; Q. Wang et al. 1996). It also protects RUNX family members from ubiquitination and proteasomal degradation (G. Huang et al. 2001). Even though there is no structural similarity between HIV-1 Vif and the Runt domain, available structures of both

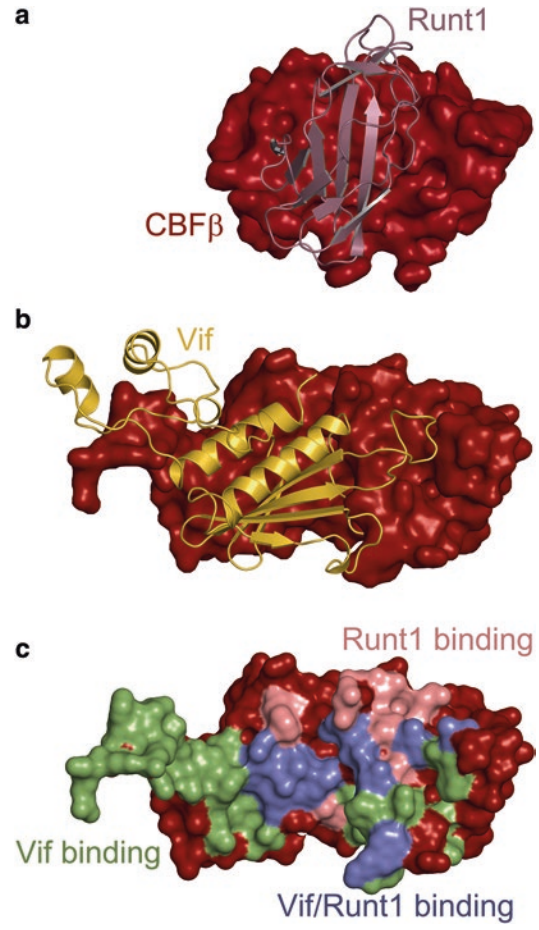

Fig. 25.5 Mutually exclusive interaction of Vif and RUNX proteins with CBFβ. (**a**) Binding interface between the Runt domain of RUNX1 (Runt1) and CBFβ. Complex structure of Runt1 and CBFβ is drawn as a ribbon diagram and surface model (PDB id 1E50; Chain G and H). The structure contains residues 2–135 in CBFβ and residues 57–175 in Runt1. (**b**) Binding interface between Vif (residues 3–172) and CBFβ (residues 3–156). (**c**) Surface patches on CBFβ for Vif and Runt1 binding. The residues specific for Vif and Runt1 binding are colored on the CBFβ surface model. Green, pink and blue indicate the residues for the binding of Vif, CBFβ and both, respectively

Runt1-CBFβ and Vif-CBFβ reveal insight into how each of these proteins engage CBFβ (Y. Guo et al. 2014; Tahirov et al. 2001; X. Huang et al. 1999). Based on the structural data it is apparent that Runt1 and Vif bind CBFβ with overlapping, but not identical, binding sites (Fig. 25.5). Therefore, it is not surprising that Vif binding to CBFβ partially occludes Runt1 binding (Warren

et al. 2000; Y. Guo et al. 2014). Despite having overlapping binding site on CBFβ, the CBFβ binding modes for Vif and Runt1 are different (D. Y. Kim et al. 2013; Y. Guo et al. 2014). For example, HIV-1 Vif binds residues 63–69 on CBFβ to form a continuous beta sheet that is mediated by hydrogen bonds, whereas Runt1 domain binds the residues on CBFβ mainly through hydrophobic interactions. Moreover, an additional HIV-1 Vif binding motif on CBFβ (residues 135–157) reinforces the Vif-CBFβ interaction. Together these data establish that Vif and RUNX bind CBFβ in a mutually exclusive manner, and supports the hypothesis that HIV-1 Vif perturbs RUNX transcription activities by scavenging CBFβ from RUNX proteins.

25.8 Recognition of A3 Family Members by Vif

While there are no structures of a Vif-A3 complex, functional studies of mutants combined with structural studies of single domain A3C or the individual Vif interaction domains of A3F and A3G have provided insights into critical hotspots for Vif recognition (Kitamura et al. 2012; Bohn et al. 2013; Siu et al. n.d.; Nakashima et al. 2016; Kouno et al. 2015). This information is covered in several recent reviews, so only the key features will be summarized (Aydin et al. 2014; Chelico 2014; Desimmie et al. 2014). These experiments have revealed that the interaction modes between HIV-1 Vif and A3 family members differ even though the A3 proteins share a highly conserved tertiary structure. Binding of A3 family to Vif can be classified by different interaction surfaces. For example A3C, A3F and A3D form interactions with Vif using a shallow hydrophobic pocket and surface exposed acidic residues distributed across α-helices 2, 3 and 4, which form one face of the cytosine deaminase fold (Russell et al. 2009; Smith and Pathak 2010; Kitamura et al. 2012; Bohn et al. 2013; Siu et al. n.d.; Nakashima et al. 2016). In contrast, A3G forms interactions with Vif using hydrophobic and acidic residues within a loop between β4 and α4 (also known as the L7 loop). These include F126 and 128-DPD-130 (Bogerd et al. 2004; Mangeat et al. 2004; Schröfelbauer et al. 2004; Huthoff and Malim 2007; Russell et al. 2009; W. Zhang et al. 2008; Letko et al. 2015; Kouno et al. 2015). Interactions of Vif with A3HhapII are less well understood. Based on homology modeling, a residue (D121) on helix α4 is implicated in binding Vif and the charged character of the surface formed by α-helices 2, 3 and 4 is different from the A3C/D/F surface suggesting a different mode of recognition (M. M. H. Li et al. 2009; Zhen et al. 2010). Together these data suggest that A3C, A3F and A3D share a common Vif binding mode, which is distinct from that of A3G and A3HhapII.

Further support for different binding modes of A3 family members is provided by the crystal structure of the HIV-1 Vif-CBFβ-ELOBC-CUL5NTD complex and prior functional studies of mutants. Critical interaction residues have been mapped onto the crystal structure of Vif-CBFβ-ELOBC-CUL5NTD (Fig. 25.6) (Y. Guo et al. 2014). The A3HhapII binding motif (F39 and H48) (Binka et al. 2011; Ooms et al. 2013a, b), the A3G binding motif (40-YRHHY-44) (Russell and Pathak 2007; Yamashita et al. 2008), the A3F binding motifs (14-DRMR-17, 74-TGERxW-79 and 171-EDRW-174) (Russell and Pathak 2007; Z. He et al. 2008; Dang et al. 2010) and the shared A3F/A3G binding motifs (21-WxSLVK-26 and 55-VxIPLx$_4$L-64) (Chen et al. 2009; Dang et al. 2009) are surface-exposed Vif residues, indicating that these motifs may mediate direct binding between Vif and A3 proteins (Fig. 25.6). In addition, viral adaptation experiments and functional assays with patient derived Vif variants have allowed researchers to build models Vif-A3 domain complexes (Letko et al. 2015; Richards et al. 2015). While these models are informative, ultimately a structure of the Vif-A3 interface will be instrumental in improving our understanding of Vif-A3 interactions and the molecular details that drive specific Vif-A3 binding.

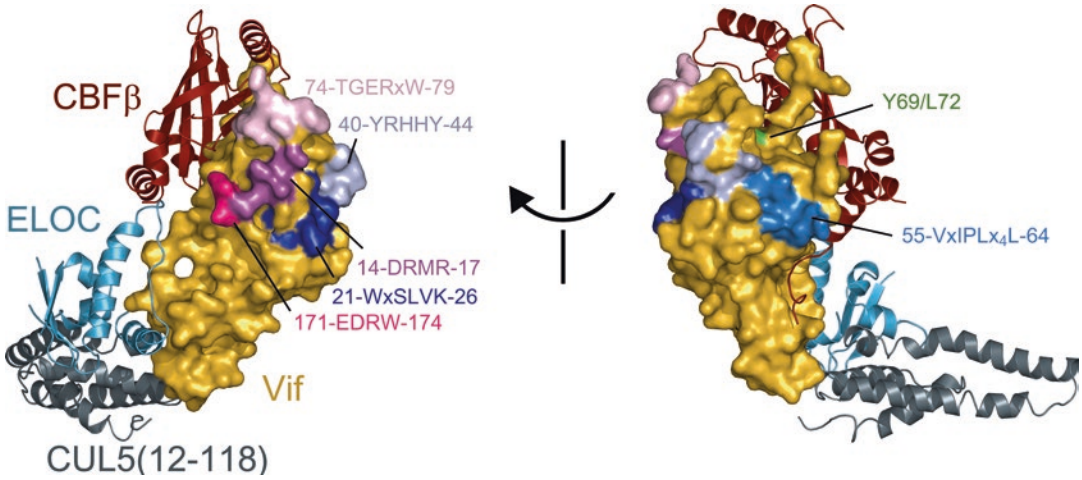

Fig. 25.6 Residues of Vif required for A3G and A3F neutralization. Clusters of surface exposed residues required for neutralization of A3F, A3G or both are indicated on a surface representation of Vif (*yellow*). Residues required for A3F, but not A3G, neutralization reside within the 14–17, 74–79 and 171–174 motifs colored *purple*, *fuchsia* and *light pink*. Residues required for A3G, but not A3F, neutralization are contained within the 40–44 motif colored light blue. Residues required for neutralization of both A3F and A3G reside within the 21–26 and 55–64 motif and are colored *blue*. Residues 69 and 72 are required for A3F and A3G neutralization (Pery et al. 2009), but probably because they are buried by CBFβ and stabilize the Vif fold (Y. Guo et al. 2014)

25.9 Outlook and Conclusion

The discovery of CBFβ as a Vif interaction partner is just one example of how systematic, unbiased studies of virus-host interactions have revealed new connections of how viruses co-opt host-cell biology (N. He et al. 2010). For the case of Vif, biochemical, structural and cell biological studies are consistent with the notion that CBFβ, along with other factors in the CRL5 E3, act to template Vif folding so that is can promote A3 polyubiquitination and degradation (Jager et al. 2012; D. Y. Kim et al. 2013). At the same time, Vif interacts with CBFβ in a manner that is competitive with RUNX binding, preventing formation of the CBFβ/RUNX heterodimer and perturbing RUNX transcriptional regulation (D. Y. Kim et al. 2013). CBFβ positively regulates expression of the full armament of A3 restriction factors, and Vif may repress transcription of these genes through interaction with CBFβ (Anderson and Harris 2015). Therefore, the interaction between Vif and CBFβ illustrates an economical strategy for a virus with limited protein coding capacity to perturb multiple host pathways, specifically by reducing the steady-state levels of A3 restriction factors by perturbing transcriptional and post-transcriptional steps of gene expression (Fig. 25.7). This phenomenon of dual hijacking is probably conserved in all primate lentiviruses, since CBFβ -Vif interactions are similarly conserved (Hultquist et al. 2012). It seems likely that other viruses with limited protein coding capacity may also use dual hijack mechanisms, but prevalence of this phenomenon is unclear as systematic, unbiased studies of virus-host interactions are in their infancy.

There are many remaining questions about the interaction of primate lentiviral Vif with CBFβ. First, though the simplest explanation for the Vif- CBFβ interaction is that it has evolved to reduce A3 gene expression, the effects on other host genes and their biological significance is unknown (Anderson and Harris 2015). It is tempting to speculate that the misregulation of additional host genes could be important for chronic development of infection in an animal model but this has not been tested. In this regard,

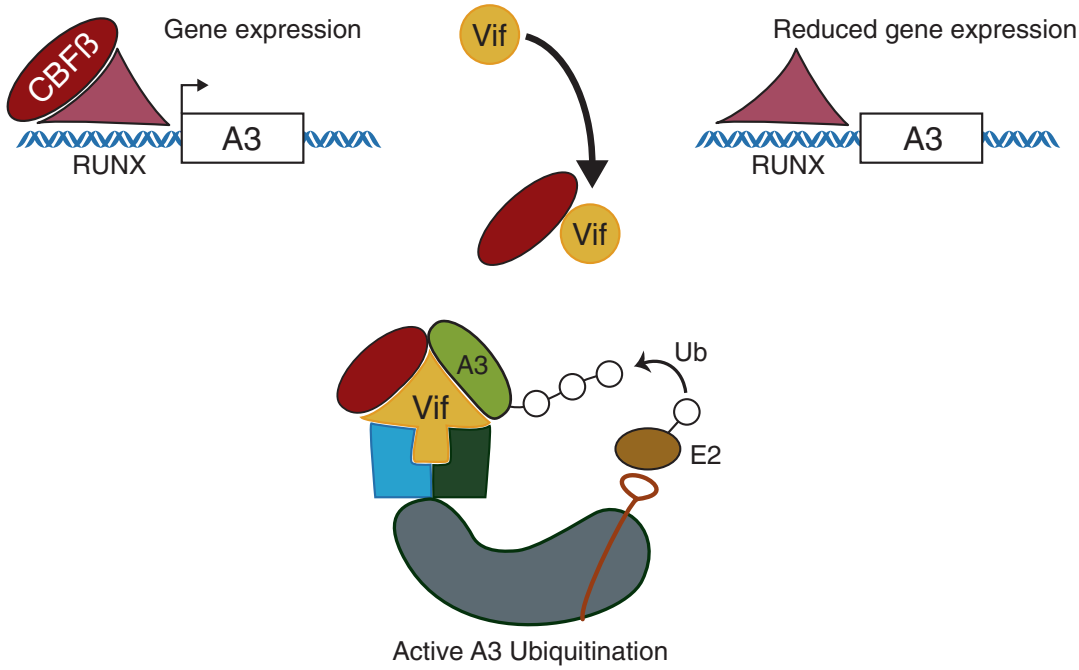

Fig. 25.7 Dual hijack model for Vif neutralization of APOBEC3. Vif binds CBFβ in a manner that is mutually exclusive with RUNX proteins, effectively preventing CBFβ from activating transcription of genes such as APOBEC3. In addition, Vif promotes the polyubiquitination and degradation of A3 family members by the 26S proteasome. This activity requires CBFβ, which promotes Vif folding. Thus, a single viral protein can perturb multiple host pathways by recruiting host factors to a common complex (Figure adapted from reference D. Y. Kim et al. 2013)

Vif could be used as a tool to study RUNX/CBFβ transcription in mouse models, since the murine CBFβ can complement the knockdown of the human counterpart (Han et al. 2014). Second, for Vif to effectively sequester CBFβ, its cellular concentrations during infection have to higher than CBFβ, which is relatively abundant in CD4+ T cells (GCID16P067063). Are Vif levels in infected cells high enough to sequester CBFβ and shutdown RUNX transcription, or might a catalytic mechanism be employed to promote CBFβ cytoplasmic retention? Third, binding of CBFβ to Vif limits its surface area for interaction with APOBEC3 family members, so does CBFβ/Vif form a composite surface for APOBEC3 binding? Models based on mutational analysis and viral adaptation have provided insights into how A3G and A3F are bound to Vif, but high-resolution structures of Vif bound to A3 enzymes will be required to address this question (Letko et al. 2015; Richards et al. 2015). Fourth, could small molecule inhibitors of the Vif- CBFβ interaction be discovered in order to unleash the restriction potential of APOBEC3 enzymes? The Vif-CBFβ interface is large, so finding a potent inhibitor of this protein-protein interaction could be challenging (Y. Guo et al. 2014). Allosteric inhibitors of Runt/CBFβ have been described, suggesting it may be feasible to inhibit Vif-CBFβ in a similar manner (Gorczynski et al. 2007). In sum, there are many exciting directions to explore between CBFβ, immunity and HIV infection.

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