

Vif Proteins of Human and Simian Immunodeficiency Viruses Require Cellular CBF β To Degrade APOBEC3 Restriction Factors

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HIV-1 requires the cellular transcription factor CBF β to stabilize its accessory protein Vif and promote APOBEC3G degradation. Here, we demonstrate that both isoforms of CBF β allow for increased steady-state levels of Vif, enhanced APOBEC3G degradation, and increased viral infectivity. This conserved functional interaction enhances the steady-state levels of Vif proteins from multiple HIV-1 subtypes and is required for the degradation of all human and rhesus Vif-sensitive APOBEC3 proteins by their respective lentiviral Vif proteins.

Human immunodeficiency virus type 1 (HIV-1) and related lentiviruses require the viral accessory protein Vif to neutralize members of the APOBEC3 family of retrovirus restriction factors and render host cells permissive for productive viral replication. HIV-1 Vif neutralizes the APOBEC3 proteins by recruitment of an E3 ubiquitin ligase complex that polyubiquitinates APOBEC3 proteins and targets them for proteasomal degradation (13; reviewed in references 1, 9, and 12). Recently, the cellular transcription factor CBF β was found to be associated with this complex and to allow for its reconstitution *in vitro* (6). Furthermore, CBF β was found to be required for the stability of HIV-1_{IIIIB} Vif *in vivo*, allowing for efficient degradation of APOBEC3G (A3G) and increased viral infectivity (6). The current model is that HIV-1 Vif hijacks cellular CBF β to facilitate Vif folding and/or stability, as well as nucleation of the E3 ubiquitin ligase complex. While it has been shown that rhesus macaque simian immunodeficiency virus molecular clone 239 (SIV_{mac239}) Vif also requires CBF β to degrade rhesus A3G (6), the generality of the CBF β /Vif/APOBEC3 functional interplay remains to be determined. The goal of the current study was to determine which isoforms of CBF β contribute to Vif stabilization, whether CBF β is required to stabilize Vif proteins of multiple different HIV subtypes, and finally, if CBF β is required by Vif to neutralize the entire repertoire of Vif-sensitive APOBEC3 proteins.

Alternative splicing generates at least two isoforms of CBF β in human cells (GenBank accession numbers NM_022845.2 and NM_001755.2). Though they differ in size and in amino acid sequence at their C-terminal end, these splice variants share 165 N-terminal residues, including the RUNX heterodimerization domain, and a clear functional difference has yet to be delineated. To determine if HIV-1 Vif distinguishes between these CBF β isoforms, a stable CBF β knockdown clone of HEK293T was created using a stably integrated small hairpin RNA (shRNA) that targets both isoforms (6). This line was transiently transfected with a Vif-proficient or Vif-deficient A200C HIV-1_{IIIIB} molecular clone (3) in the presence or absence of human A3G and complemented with either the 187-amino-acid CBF β isoform 1 (cloned from CEM cell cDNA by PCR and standard molecular biology techniques) or the shorter 182-amino-acid CBF β isoform 2 (as used previously [6]). Forty-eight hours after transient transfection, cell lysates and viral particles were collected for immunoblotting and

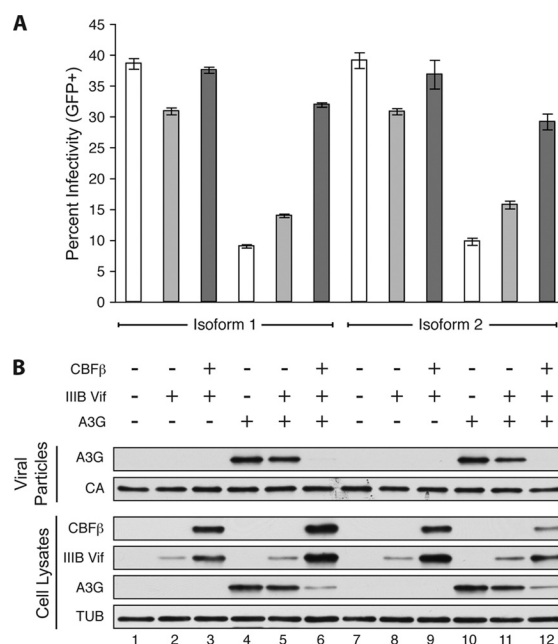


FIG 1 CBF β isoform 1 and isoform 2 stabilize HIV-1 Vif to degrade A3G and increase viral infectivity. (A) Percent infectivity of HIV-1_{IIIIB} measured by duplicate infection of CEM-GFP and flow cytometry, reported as the mean \pm standard deviation of the results for the technical replicate. Constant amounts of Vif-deficient or Vif-proficient A200C HIV-1_{IIIIB} molecular clone (1 μ g) were cotransfected with A3G or empty plasmid (50 ng) in the presence or absence of CBF β complementation vector (25 ng) as indicated. (B) Immunoblots of CBF β , Vif, and hemagglutinin (HA)-tagged human A3G in cell lysates and of A3G in HIV-1 particles produced by those cells. Tubulin (TUB) and p24 (CA) served as cell and viral lysate loading controls.

Received 29 November 2011 Accepted 16 December 2011

Published ahead of print 28 December 2011

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doi:10.1128/JVI.06950-11

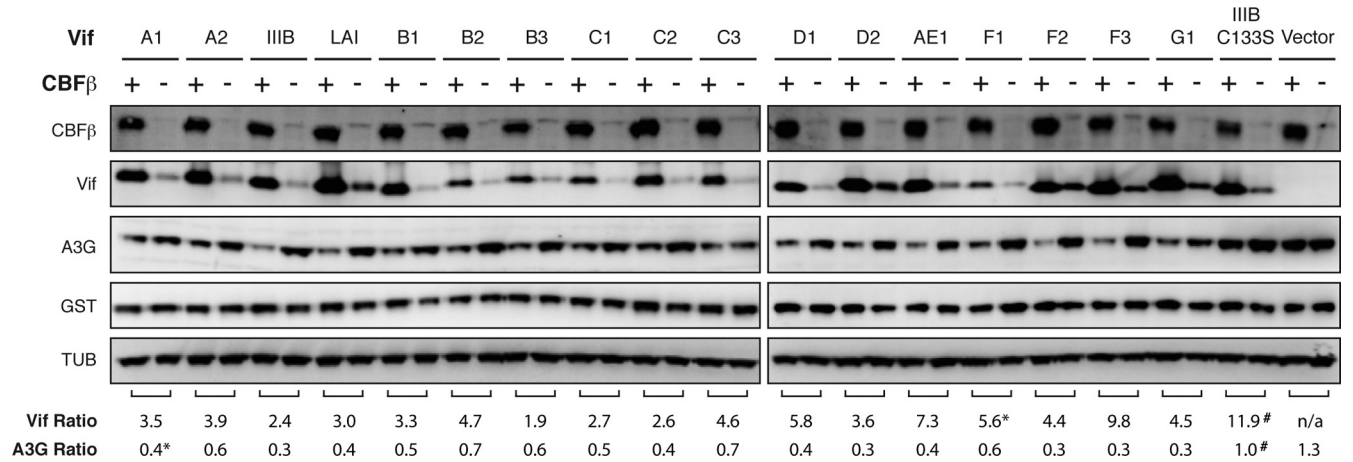


FIG 2 CBF β stabilizes Vif proteins from multiple HIV-1 subtypes. Immunoblots of HA-tagged CBF β , HIV-1 Vif, and FLAG-tagged human A3G in cell lysates. Tubulin (TUB) and V5-tagged GST served as cell lysate loading and transfection controls, respectively. Constant amounts of the indicated Vif variants (pCRV1 expression vector, 50 ng) were cotransfected with A3G (300 ng), GST (200 ng), and either CBF β isoform 2 (100 ng) or empty vector. The untagged Vif variants were detected with a polyclonal rabbit anti-Vif antibody (NIH catalog no. 2221). One representative experiment of three independent transfections is shown. The Vif ratio represents the average ratio of Vif in the presence versus the absence of CBF β (relative to GST) over three experiments unless otherwise noted (*, $n = 2$; #, $n = 6$). The A3G ratio was calculated analogously. Quantification was performed using Image Gauge version 4.0.

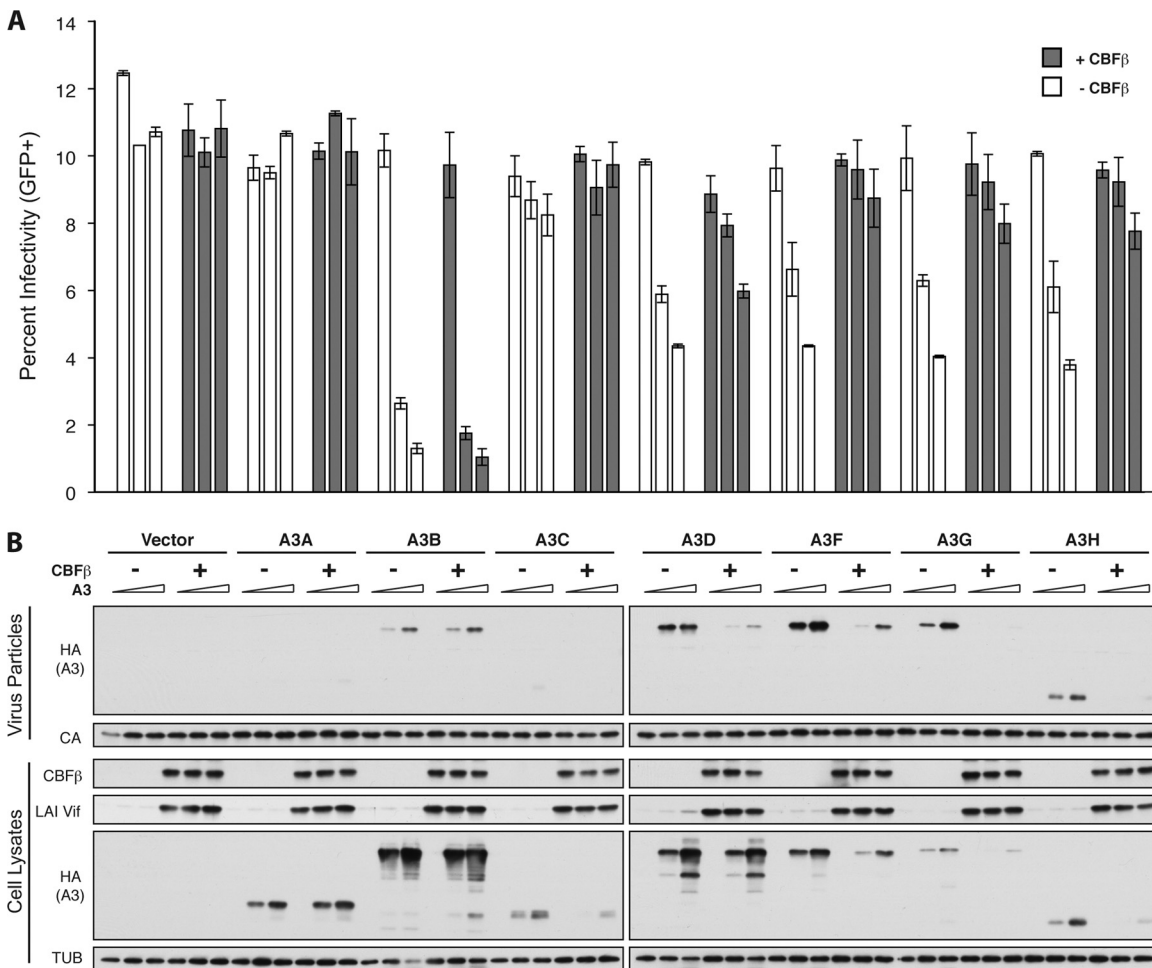


FIG 3 CBF β is required for HIV-1 Vif to degrade all Vif-sensitive human APOBEC3 proteins. (A) Percent infectivity of HIV-1_{LAI} measured by duplicate infection of CEM-GFP cells and flow cytometry, reported as the mean \pm standard deviation of the results for the technical replicate. Constant amounts of Vif-proficient HIV-1_{LAI} proviral construct (1 μ g) were cotransfected with increasing concentrations of each human HA-tagged APOBEC3 protein (0, 50, or 100 ng) in the presence or absence of CBF β isoform 2 complementation vector (25 ng) as indicated. (B) Immunoblots of CBF β , Vif, and the HA-tagged human APOBEC3 proteins in cell lysates and of the APOBEC3 proteins in HIV particles produced by those cells. Tubulin (TUB) and p24 (CA) served as cell and viral lysate loading controls.

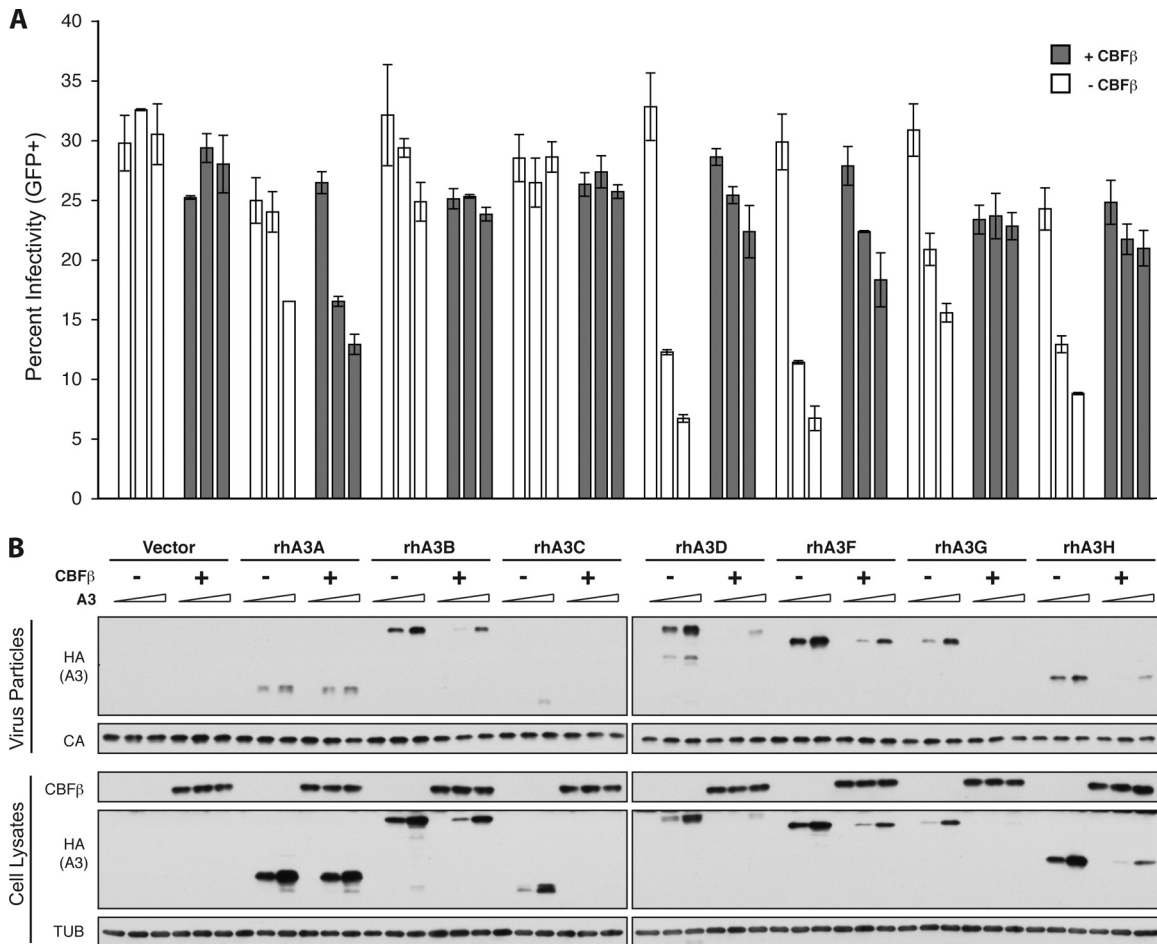


FIG 4 CBF β is required for SIV Vif to degrade all Vif-sensitive rhesus APOBEC3 proteins. (A) Percent infectivity of Vif-deficient HIV-1_{IIIB} supplemented with SIV_{mac239} Vif measured by duplicate infection of CEM-GFP and flow cytometry, reported as the mean \pm standard deviation of the results for the technical replicate. Constant amounts of Vif-deficient A200C HIV-1_{IIIB} proviral construct (1 μ g) were cotransfected with untagged SIV_{mac239} Vif (pVR1012 expression vector, 50 ng) and increasing concentrations of each HA-tagged rhesus (rh) APOBEC3 protein (0, 50, or 100 ng) in the presence or absence of CBF β isoform 2 complementation vector (25 ng) as indicated. (B) Immunoblots of CBF β , Vif, and the HA-tagged rhesus APOBEC3 proteins in cell lysates and of the rhesus APOBEC3 proteins in HIV particles produced by those cells. Tubulin (TUB) and p24 (CA) served as cell and viral lysate loading controls.

viral infectivity was monitored by infection of the reporter cell line CEM-GFP (5). The two isoforms resulted in comparable increases in HIV-1_{IIIB} Vif steady-state levels, enhanced degradation of A3G, and rescue of viral infectivity (Fig. 1). In the absence of A3G, neither CBF β isoform had an impact on viral infectivity.

Most laboratory strains of HIV-1, including HIV-1_{IIIB}, HIV-1_{NL4-3'}, and HIV-1_{LAI}, are subtype B, but over 10 different HIV-1 subtypes are found worldwide, with subtype C being the most prevalent (4). To determine if CBF β can stabilize Vif proteins from multiple subtypes, representative Vif alleles from HIV-1 subtypes A, B, C, D, AE, F, and G (as described previously [2]) were cotransfected into the HEK293T CBF β knockdown cell line with A3G in the presence or absence of CBF β isoform 2 and glutathione S-transferase (GST) as a transfection control. In every case, CBF β increased the steady-state level of the Vif variant and resulted in increased degradation of A3G (Fig. 2). While basal Vif expression levels varied, CBF β increased the steady-state level of each Vif variant by an average of approximately 4-fold. Furthermore, while each variant also differs in its ability to neutralize A3G (2), steady-state levels of A3G were decreased upon CBF β com-

plementation in every case by an average of 2-fold. A3G levels were not affected by CBF β in the absence of Vif or in the presence of HIV-1_{IIIB} Vif C133S, which fails to recruit the E3 ubiquitin ligase complex (7, 8). CBF β did not affect the expression of the GST control. Thus, the dependency of Vif on CBF β is broadly conserved across multiple HIV-1 subtypes.

Human CD4⁺ T cells express six APOBEC3 proteins, of which HIV-1 Vif degrades five: A3C, A3D, A3F, A3G, and A3H (5, 10). To determine if HIV-1 Vif requires CBF β to neutralize not only A3G but the other Vif-sensitive APOBEC3 proteins as well, the HIV-1_{LAI} molecular clone was transfected into HEK293T CBF β knockdown cells with increasing amounts of each human APOBEC3 protein in the presence or absence of CBF β isoform 2. CBF β increased Vif steady-state levels and resulted in decreased cellular levels of all Vif-sensitive APOBEC3 proteins (A3C, A3D, A3F, A3G, and A3H haplotype II) (Fig. 3). In the presence of CBF β , packaging of A3D, A3F, A3G, and A3H was also decreased and viral infectivity increased accordingly. Neither A3A nor A3B are sensitive to HIV-1_{LAI} Vif, and consequently, their expression, packaging, and impact on viral infectivity were not affected by CBF β . Thus,

HIV-1 Vif requires CBF β to neutralize not only A3G but the entire repertoire of Vif-sensitive human APOBEC3 proteins.

Rhesus macaques also encode seven distinct APOBEC3 proteins, of which rhesus A3D, A3F, A3G, and A3H can restrict Vif-deficient HIV-1 and SIV (5, 11). SIV_{mac239} Vif neutralizes all four restrictive rhesus APOBEC3 proteins and also degrades rhesus A3B and rhesus A3C (5). To determine if SIV_{mac239} Vif requires CBF β to neutralize the rhesus APOBEC3 proteins, the Vif-deficient HIV-1_{IIB} molecular clone was transfected into HEK293T CBF β knockdown cells alongside SIV_{mac239} Vif and increasing amounts of each rhesus APOBEC3 protein in the presence or absence of CBF β isoform 2. Human CBF β isoforms 1 and 2 are identical at the amino acid level to rhesus CBF β isoforms 1 and 2, respectively. While there is no antibody for SIV_{mac239} Vif, the addition of CBF β resulted in decreased cellular levels of all Vif-sensitive rhesus APOBEC3 proteins (rhesus A3B, A3C, A3D, A3F, A3G, and A3H) (Fig. 4). In the presence of CBF β , packaging of rhesus A3D, A3F, A3G, and A3H was also decreased and viral infectivity consequently increased. Rhesus A3A is not sensitive to SIV_{mac239} Vif, and so its expression, packaging, and effect on viral infectivity were unaltered by CBF β . Thus, SIV Vif demonstrates a conserved requirement for CBF β to neutralize the rhesus repertoire of APOBEC3 proteins.

HIV-1_{IIB} Vif was previously shown to require CBF β isoform 2 for stable expression and neutralization of A3G (6). Here, we demonstrate that both CBF β isoform 1 and isoform 2 may be hijacked to stabilize HIV-1_{IIB} Vif and degrade A3G. This functional interaction was conserved across all tested HIV-1 subtypes and was required for the neutralization of not only A3G but all Vif-sensitive human APOBEC3 proteins. SIV_{mac239} Vif also required CBF β to neutralize all restrictive rhesus APOBEC3 proteins. Taken together, the CBF β -Vif interaction appears to be broadly conserved and essential for Vif function, implicating this interface as a candidate for disruption by small-molecule therapeutics that would alleviate the repression of multiple restrictive APOBEC3 proteins.

ACKNOWLEDGMENTS

We thank N. Krogan and J. Gross for discussions and data sharing prior to publication and the NIH AIDS Research and Reference Reagent Program for materials.

This research was funded by grants NIH R01 AI064046 and NIH P01 GM091743 to R.S.H. and NIH R01 AI064001 and NIH R01 AI089246 to V.S. J.F.H. was supported by an NSF predoctoral fellowship.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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