Structural Insight into the Human Immunodeficiency Virus Vif SOCS Box and Its Role in Human E3 Ubiquitin Ligase Assembly

Bradford J. Stanley, Elana S. Ehrlich, Leslie Short, Yunkai Yu, Zuoxiang Xiao, Xiao-Fang Yu, and Yong Xiong

Department of Molecular Biophysics and Biochemistry, Yale University, P.O. Box 208114, New Haven, Connecticut 06510, and Department of Molecular Microbiology and Immunology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland 21205

Received 8 April 2008/Accepted 10 June 2008

Human immunodeficiency virus (HIV) virion infectivity factor (Vif) causes the proteasome-mediated destruction of human antiviral protein APOBEC3G by tethering it to a cellular E3 ubiquitin ligase composed of ElonginB, ElonginC, Cullin5, and Rbx2. It has been proposed that HIV Vif hijacks the E3 ligase through two regions within its C-terminal domain: a BC box region that interacts with ElonginC and a novel zinc finger motif that interacts with Cullin5. We have determined the crystal structure of the HIV Vif BC box in complex with human ElonginB and ElonginC. This complex presents direct structural evidence of the recruitment of a human antiviral ligase by a viral BC box protein that mimics the conserved interactions of cellular ubiquitin ligases. We further mutated conserved hydrophobic residues in a region downstream of the BC box and found that mutation of this region inhibits the interaction of Vif with ElonginB and ElonginC and renders the virus ineffective.

Virus-host interactions play critical roles in the conflict between host fitness and viral survival. Host immune systems have evolved a vast repertoire of tactics to suppress viral infections, and viruses, in turn, have developed an equally impressive arsenal of methods to evade these host defenses. A common evasion strategy used by viruses is the destruction of specific host defense proteins by hijacking the host ubiquitin-proteasome pathway (2). Viruses have evolved accessory proteins that mimic the substrate receptor molecule in host ubiquitin ligases to target cellular defense proteins for polyubiquitination, a signal for proteasome-mediated degradation. One such viral protein is the human immunodeficiency virus (HIV) virion infectivity factor (Vif) that targets the human antiviral protein APOBEC3G by mimicking cellular suppressor of cytokine signaling (SOCS) box proteins (8, 24, 33, 35, 42) (Fig. 1). In the absence of Vif, APOBEC3G causes extensive C-to-U mutations in viral reverse transcripts (12, 18, 22–24, 44) and may also physically block reverse transcription (4, 14, 29), rendering the virus ineffective.

A normal cellular elon-ginu-cullin-SOCS-box (ECS) ubiquitin ligase is comprised of ElonginB (EloB), ElonginC (EloC), Cullin5 (Cul5), Rbx2, and a SOCS-box protein (Fig. 1). SOCS box proteins were first identified as negative regulators of cytokine signaling and are characterized by a conserved interaction domain that provides a link between cellular substrates and the E3 ubiquitin ligase. This conserved domain, the SOCS box, includes the BC box, which binds EloB and EloC (EloBC), and the Cullin box that has been proposed to participate in the binding of either Cul5 or Cul2 (16). Since its original discovery, the BC box consensus sequence has expanded to include a more diverse range of sequences, as more EloBC binding proteins have been identified (31, 41, 43). Existing crystal structures show that the SOCS box has a conserved structure composed of a BC box helix, a short 90° turn, a second short helix ending in a proline-rich region, a loop, and a third helix (Fig. 1B) (6, 34, 36). For clarity, we shall refer to the first helix as the BC box and the rest as the Cullin box.

Despite the sequence diversity among different HIV isolates, Vif uses conserved regions to orchestrate the degradation of APOBEC3G. Vif functions as the APOBEC3G substrate receptor molecule by mimicking the SOCS box component of a cellular E3 ubiquitin ligase (Fig. 1). It has been proposed that the Vif N-terminal domain interacts with APOBEC3G (8, 24), while its C-terminal half recruits the E3 ligase through two conserved regions. HIV Vif contains a BC-box region (residues 144 to 155) that interacts with EloC and is essential for recruiting the cellular E3 ubiquitin ligase (26, 42, 43). A second E3 ligase-interacting motif is the conserved H-x5-C-x17-18C-x5-5-H (HCCH) zinc-binding motif that spans Vif residues 108 to 139 and interacts with Cul5 (20, 27, 30, 37, 38). Mutation of the critical His or Cys residues, or the addition of a zinc chelator, abolishes the Vif-Cul5 interaction (37).

The interaction between a third Vif C-terminal region, the Vif Cullin box, and the E3 ubiquitin ligase has not been extensively studied. Although the Vif-Cul5 interaction is confirmed by several independent experiments (17, 26, 27, 42),
there has been no evidence of a direct interaction between the Vif Cullin box and Cul5. Cullin boxes have been shown to mediate interaction with Cul5 or Cul2 in a number of cellular SOCS-box proteins (16); however, the sequence of the Vif Cullin box only partially matches the previously proposed Cul5 box consensus (16) (Fig. 1C). In addition, a proline-rich region in the Vif Cullin box (161-PPLP-164) has been previously implicated in Vif homo-oligomerization and shown to be important to Vif function (39, 40), but its exact functional relevance has not been established.

Despite the rapid accumulation of biochemical and genetic data, the details of how HIV Vif recruits the E3 ubiquitin ligase largely remain unknown because of the lack of any structural data on Vif. We conducted a series of experiments to obtain structural insight into the interactions between the Vif SOCS box and the E3 ligase. We have obtained a crystal structure of the HIV Vif BC-box in complex with human EloBC. This provides structural evidence that a viral BC-box hijacks a host E3 ligase by mimicking the conserved binding interface used by cellular SOCS-box proteins. We further performed a mutational analysis to demonstrate direct interaction between the Vif Cullin box and Cul5. Cullin boxes have been shown to mediate interaction with Cul5 or Cul2 in a number of cellular SOCS-box proteins (16); however, the sequence of the Vif Cullin box only partially matches the previously proposed Cul5 box consensus (16) (Fig. 1C). In addition, a proline-rich region in the Vif Cullin box (161-PPLP-164) has been previously implicated in Vif homo-oligomerization and shown to be important to Vif function (39, 40), but its exact functional relevance has not been established.

Despite the rapid accumulation of biochemical and genetic data, the details of how HIV Vif recruits the E3 ubiquitin ligase largely remain unknown because of the lack of any structural data on Vif. We conducted a series of experiments to obtain structural insight into the interactions between the Vif SOCS box and the E3 ligase. We have obtained a crystal structure of the HIV Vif BC-box in complex with human EloBC. This provides structural evidence that a viral BC-box hijacks a host E3 ligase by mimicking the conserved binding interface used by cellular SOCS-box proteins. We further performed a mutational analysis to demonstrate direct interaction between the Vif Cullin box and Cul5. The structural and mutational data, together with available structures of Cullin complexes, allowed us to propose a homology model of the complex between the Vif SOCS box, EloBC, and Cul5. This model provides further insight into how the HIV Vif viral SOCS box assembles with a cellular ubiquitin ligase to polyubiquitinate APOBEC3G.

**MATERIALS AND METHODS**

Cloning, expression, and purification of Vif/EloB/EloC complex. HIV Vif HXB3 amino acids 139 to 176 were cloned into pETDuet-1 with an N-terminal His6 tag and TEV protease cleavage site. This plasmid was cotransformed into BL21(DE3) cells with pACYCDuet-1 containing EloB and EloC (6). The Vif-EloB-EloC complex was purified on a nickel affinity column, and fractions containing the Vif-EloB-EloC complex were pooled, concentrated, and incubated at 4°C overnight with TEV protease. A second nickel affinity step was used to remove the TEV and cut tag, and then the flowthrough was concentrated and loaded onto a Superdex-75 gel filtration column. Fractions containing the Vif-EloB-EloC complex were pooled and concentrated to approximately 7 mg/ml in 25 mM Tris-HCl (pH 7.5), 150 mM NaCl, and 1 mM dithiothreitol.

Crystallization and structure determination. Crystals were obtained by hanging-drop vapor diffusion in 0.1 M Tris-HCl (pH 7.0) and 40% PEG 350 monomethyl ether. Crystals were frozen in liquid propane with the reservoir solution. Due to the high polyethylene glycol concentration, the reservoir solution made a suitable cryoprotectant. Diffraction data were collected at the Advanced Photon Source NECAT beamline ID24 (Argonne, IL) and the Brookhaven National Laboratory beamline X29 (Upton, NY). The crystals belonged to space group P2₁2₁2₁ and diffracted to 2.4 Å. The structure was resolved by molecular replacement using the coordinates of EloB/EloC from the crystal structure of EloB/EloC/VHL (PDB ID 1vcb) (34). Two copies of the EloB/EloC complex were found in the asymmetric unit of the crystal with the program Phaser (25). After rigid body and restrained refinement in Refmac (28), the Vif BC-box region, which was not included in the model, was clearly visible in the 2Fo-Fc electron density map. The electron density was further improved by twofold multidomain noncrystallographic symmetry (NCS) averaging using the program DM (9). Two NCS-related copies of the Vif BC box (residues 140 to 155) were then built into the electron density using the programs O (15) and Coot (11). A simulated annealing omit map with the Vif BC-box omitted (Fig. 2A) was
TABLE 1. Crystal data and refinement statistics

| Parameter                  | Value* |
|----------------------------|--------|
| Space group                | P2₁,2,2, |
| Unit cell a, b, c (Å)       | 55.51, 66.91, 122.64 |
| Resolution (Å) (range)     | 30–24 (2.49–2.4) |
| Rmerge (%)                 | 9.6 (44.1) |
| Rfree (%)                  | 11.7 (2.5) |
| Completeness (%)           | 98.2 (97.3) |
| Redundancy (%)             | 3.9 (3.3) |
| R/R-free (%)               | 18.6/23.5 (18.9/26.9) |
| Ramachandran angle         |        |
| % Preferred regions        | 94.4 |
| % Allowed regions          | 95.0 |
| % Outliers                 | 0.6 |
| RMSD, bond (Å)             | 0.008 |
| RMSD, angle (°)            | 1.1 |

* Values in parenthesis are for the highest-resolution shells.

The geometry of the final Vif SOCS-box/EloBC/Cul5 complex model was idealized in Refmac.

Plasmids and antibodies. The following HIV-1 Vif expression vectors have been described previously: wild-type Vif-hemagglutinin (HA), wild-type Vif-myc, L145A, L168S, D58Q, L169S, and SIV Vif (20, 42, 43). Vif S144A, L148A, L153A, Q146A, and AALA were constructed by changing S144, L148, L153, Q146, and the prolines at positions 160 to 163 to A, respectively, using site-directed mutagenesis. The following antibodies have been described previously: HA, myc, Cul5, ElnB, and EloC (20, 42, 43).

Cells, transfection, and immunoprecipitation. 293T cells were maintained as suggested by the American Type Culture Collection. Transfections were carried out by using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions. At 48 h posttransfection cells were harvested for immunoprecipitation as described previously (20, 42, 43). Immunoprecipitates were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, followed by immunoblotting against the indicated proteins.

Data deposition. Coordinates and structure factors have been deposited in the Protein Data Bank under accession code 3DCG.

**RESULTS**

Crystal structure of the HIV Vif BC box in complex with EloBC. We have determined the crystal structure of the Vif BC box-EloBC complex at 2.4-Å resolution (Fig. 2A). The complex structure was solved by molecular replacement using the coordinates of EloBC from the VHL-EloBC complex (PDB ID 1vcb) (34). Clear electron density was observed in the 2Fo-Fc map for Vif residues 140 to 155 before their inclusion in the model. These residues correspond to the Vif BC-box helix that includes the consensus sequence of SLQYLA. The C terminus of the Vif construct, containing the Cul5 box, was disordered and not observed in the crystal structure. Mass spectrometry of dissolved crystals confirmed that Vif had not been degraded (data not shown).

The Vif BC box adopts a loop-helix structure, with the helix starting at the second residue (L145) in the consensus SLQYLA sequence (Fig. 2B). Both the loop and the helix form a largely hydrophobic interface that binds EloC. Extensive hydrophobic interactions occur between side chains of the Vif BC box and those of helix 3 and helix 4 of EloC. Vif residues V142, L145, L148, A149, A152, and L153 create the hydrophobic face that interacts with EloC (Fig. 3A and B). Hydrogen bonding between the backbone carbonyl of Vif G143 and EloC Y76 also contributes to the Vif-EloC interaction. Similar interactions are made by cellular BC box-EloC complexes (6, supplemental material). The program Coot was used for the superpositioning.
7, 34, 36), which adopt a conformation like that of the viral Vif BC box (Fig. 2C). A notable difference between the cellular and viral BC boxes is that the highly conserved cysteine and arginine residues in the middle of the cellular BC box are replaced by alanine (A149) and leucine (L150) in Vif (19, 41) (Fig. 1C). The alanine residue in Vif is located at a position that has the closest Cα-Cα distance between the BC-box helix and helix 4 of EloC. The position of the Vif BC box relative to EloC is the same as those in the SOCS2 (6) and SOCS4 (7) structures but slightly shifted from that in the VHL (34) structure by about 1 Å at its C terminus. The two copies of the Vif-EloB/EloC complex in the asymmetric unit are identical when superimposed.

The Vif Cullin box is predicted to form a second hydrophobic interface with EloC. To delineate the role of the Vif Cullin box, we first analyzed the known structures of cellular SOCS box proteins. Although the Cullin-box amino acid sequences of all SOCS box proteins solved to date are different (Fig. 1C), their structures remain essentially the same (Fig. 1B). For example, the SOCS box sequences of SOCS2 (amino acids 161 to 193) and VHL (amino acids 156 to 190) are only 18% identical. However, superimposition of their structures using the program DALI (13) gives a root mean square deviation (RMSD) of 0.7 Å between their backbones and a Z-score of 5.9 (a Z-score higher than 3 is considered significant). Even with a two-amino-acid insertion in VHL (P172-E173), the rest of its Cullin box aligns with SOCS2 nearly identically (Fig. 1B).

Comparison of existing Cullin box structures reveals that the conserved hydrophobic residues in this region (Fig. 1C), mostly leucines, point toward a different face of the same EloC helix (H4) that interacts with the BC box (Fig. 4A and B). Using SOCS2 as an example, I178, L181, L183, L187, and L191 all face inward toward a hydrophobic pocket formed at the EloC–BC-box interface (6) (Fig. 4B). Similarly, L178, I180, L184, and L188 of VHL interact with this same EloC–BC-box interface (Fig. 4A) despite the fact that its sequence does not follow the Cullin box consensus (Fig. 1C). Overall, Vif/VHL share six identical residues in their last 11 Cullin-box residues, including leucine 163/178, valine 166/181, arginine 167/182, leucine 169/184, glutamate 171/186, and aspartate 172/187 (Fig. 1C). This is a higher degree of sequence homology than is observed between the VHL and SOCS2 Cullin boxes, which superimpose almost identically. Vif’s high level of sequence identity to VHL in the Cullin box motif, which has a highly conserved structure, suggests that the Vif Cullin box may form the same structure.

Although the Cullin box in HIV-1 Vif is apparent, HIV-2 and SIV Vif do not have readily distinguishable Cullin boxes. It is still possible that the conserved structure of the Cullin box does form in HIV-2 and SIV Vif even though there may be limited sequence similarity. Also, a recent study of Cullin-box sequences (21) has shown that the spacing between the BC box and the Cullin box is quite variable. Considering the divergence between the Cullin box from HIV Vif and SOCS2/SOCS4/VHL, it is possible that the HIV-2 and SIV Cullin boxes have diverged even further and/or are not necessarily adjacent to the BC box. Alternatively, HIV-2 and SIV Vif may...
have evolved a different, yet functionally equivalent, motif that serves the same purpose as the Cullin box.

The Vif Cullin box-EloC interaction is required for the binding of Cul5 but not EloBC. The formation of a second interface with EloC by the Vif Cullin box raises a question: is this interaction required for Vif to bind EloBC or to position the conserved Cullin-box structure for interaction with Cul5? A series of mutations in the SOCS box of Vif were made to address this question (Fig. 5A). 293T cells were transfected with wild-type or mutant Vif expression vectors. The HA-tagged Vif proteins were then coimmunoprecipitated with wild-type or mutant Vif expression vectors and HA-myc-tagged wild-type Vif expression vectors. HA-tagged proteins were immunoprecipitated with anti-HA conjugated agarose and Western blotted with antibodies against EloB, EloC, Cul5, and Vif. EloB and EloC binding is affected by mutation of critical BC-box residues, and Cul5 binding is lost upon mutation of downstream conserved hydrophobic residues.

FIG. 5. Mutation of conserved residues in the Vif SOCS box. (A) Map of alanine substitutions in the Vif BC box used in immunoprecipitation experiments. (B) Anti-Vif immunoprecipitations were Western blotted with antibodies against EloB, EloC, Cul5, and Vif. EloB and EloC binding is affected by mutation of critical BC-box residues, and Cul5 binding is lost upon mutation of downstream conserved hydrophobic residues. (C) Vif oligomerization is independent of conserved residues in the Vif SOCS box. Cells were cotransfected with myc-tagged wild-type or mutant Vif expression vectors and HA-tagged wild-type Vif expression vectors. HA-tagged proteins were immunoprecipitated with anti-HA conjugated agarose and Western blotted with antibodies against HA- and myc-tagged Vif proteins.

have evolved a different, yet functionally equivalent, motif that serves the same purpose as the Cullin box.

The Vif Cullin box-EloC interaction is required for the binding of Cul5 but not EloBC. The formation of a second interface with EloC by the Vif Cullin box raises a question: is this interaction required for Vif to bind EloBC or to position the conserved Cullin-box structure for interaction with Cul5? A series of mutations in the SOCS box of Vif were made to address this question (Fig. 5A). 293T cells were transfected with wild-type or mutant Vif expression vectors. The HA-tagged Vif proteins were then coimmunoprecipitated with endogenous Cul5, EloB, and EloC and subsequently examined by immunoblotting. In agreement with previous studies (26, 43), the L145A mutation results in loss of binding to EloB, EloC, and Cul5 (Fig. 5B, lane 3). On the other hand, mutations of the highly conserved L163 or L169 had little effect on Vif interaction with EloB and EloC (Fig. 5B, lanes 5 and 6), and interaction with Cul5 was severely inhibited compared to parental Vif. This suggests that the interaction involving the conserved leucines in the Vif Cullin box is critical for Cul5 binding, not EloBC binding.

The conserved amino acids in Vif SOCS box are not required for Vif oligomerization. To assess whether the Vif SOCS-box consensus residues play a role in Vif oligomerization as suggested by previous studies (40), we cotransfected 293T cells with HA-tagged wild-type Vif, along with a series of myc-tagged Vif expression vectors. The myc-tagged Vif expression vectors (Fig. 5A) included wild-type Vif, Vif-AALA (all prolines of the PPLP motif mutated to alanine), Vif-ΔSLQ (the SLQ consensus mutated to AAA), simian immunodeficiency virus Vif (which naturally has no PPLP yet still interacts with Cul5), and control parental vector. Our data show that Vif SOCS-box consensus mutants still interact with wild-type HIV-1 Vif (Fig. 5C), suggesting that other regions of Vif may mediate oligomerization.

Potential structural determinants for Cul5 binding. The crystal structure of the Vif BC box in complex with EloBC and our mutational evidence for the role of the Vif Cullin box in the recruitment of Cul5 enabled us to propose a homology model of Vif-Cul5 binding (Fig. 6). The crystal structure of the Cul1-Rbx1-Skp1-F box SCF ubiquitin ligase (45) was used as a template. Cul5 was modeled onto Cul1 and our Vif SOCS-box EloBC model was then positioned in the SCF complex by aligning EloC with Skp1. This SCF ubiquitin ligase structure (45) serves as a good template for modeling the Vif-EloBC-Cul5 complex for the following reasons. First, as demonstrated by the resemblance of the Cul1 (45) and Cul4A (1) structures (see Fig. S2 in the supplemental material), the cullins, being close paralogues, are likely to have very similar structures. Second, EloC is the Skp1 equivalent for Cul5-Rbx2 ubiquitin ligases, and superimposition of EloC onto Skp1 in DALI (13) results in the alignment of S2 residues with a sequence identity of 34% and a Z-score of 11.2. Lastly, structural superimposition of Skp1 and EloC has also been used by others to investigate potential interfaces between cellular SOCS box proteins and their respective cullins (6, 16).

The homology model of the Vif-EloBC-Cul5 complex reveals two potential Vif-Cul5 interfaces. The first interface is observed between the PPLP downstream region in the Vif Cullin box and Cul5 loop 2 (residues 51 to 60) (Fig. 6). Interaction at this interface is supported by our mutational data and provides an explanation for the loss of Cul5 binding upon mutation of L163 in the PPLP. The second potential interface is found between the Vif HCCH zinc finger motif and Cul5 loop 5 (residues 118 to 134) (Fig. 6). The last histidine (H139) of the HCCH motif is immediately next to the first amino acid (N140) that we see density for in our crystal structure; therefore, we can estimate the position of the zinc finger with respect to Cul5. The position of H139 indicates that this end of the HCCH motif is immediately next to the first amino acid (N140) that we see density for in our crystal structure; therefore, we can estimate the position of the zinc finger with respect to Cul5. The position of H139 indicates that this end of the HCCH motif is more than 20 Å away from any Cul5 residues except for those in loop 5. Loop 5 is variable in length and composition between the different Cullins (see Fig. S2 in the supplemental material) and could provide potential specificity determinants. In addition, loop 5 is likely to be flexible and, depending on its orientation, it could also interact with the last helix in the Vif Cullin box (Fig. 6).

DISCUSSION

HIV Vif makes a number of critical interactions with human ubiquitin ligase components EloB, EloC, and Cul5 to tether the ligase to antiviral APOBEC3G. Assembly of this ubiquitin ligase leads to the polyubiquitination and subsequent protea-
some-dependent destruction of APOBEC3G. Therefore, Vif and the interactions that it makes with the host ubiquitin ligase are of great interest because of their potential as therapeutic targets. However, our understanding of these interactions has been impaired by the lack of structural information. In order to further our understanding of how Vif interacts with components of human E3 ubiquitin ligase, we determined the structure of a Vif SOCS box construct with elongins B and C, used mutational analysis to determine the role of a downstream region that was disordered in our structure, and then proposed a model for the Vif-EloBC-Cul5 interactions.

Our current crystal structure of the Vif BC box-EloBC complex provides direct evidence for the proposed viral hijacking of human E3 ubiquitin ligase components. The viral Vif BC-box conformation and the binding interactions are similar to those observed in structures of cellular SOCS box protein-EloBC complexes (6, 7, 34, 36) (Fig. 2C). This demonstrates that HIV Vif uses a mechanism similar to that of endogenous substrate receptors to recruit the ligase for polyubiquitination of APOBEC3G. The interface between the Vif BC box and EloC is dominated by hydrophobic interactions as in cellular BC boxes, but there are some notable differences. The highly conserved cysteine in cellular BC boxes is replaced by alanine (A149) in Vif. The space between the BC box and EloC helix 4 appears to be able to accommodate an amino acid as large as a cysteine but may exclude any residue larger. This is consistent with the mutational studies at this position (43). In addition, the conserved arginine that follows the cellular BC box cysteine is a leucine (L150) in the Vif sequence. This residue is located opposite the hydrophobic face of Vif that interacts with EloC, so it is unlikely to affect the Vif-EloC interaction.

Our mutational data also suggest that there are two interfaces at which HIV Vif binds to EloC. Mutation of critical residues within the SOCS box indicates that the conserved residues of the BC box and Cul5 box have distinct roles.
Mutation of L145 in the SOCS box abolishes EloBC and Cul5 binding (Fig. 5B). The L145 mutation contributes substantially to the hydrophobic interface between Vif and EloC by fitting into a hydrophobic pocket formed by EloC helices 3 and 4 (Fig. 3B), and therefore the L145A mutant no longer binds EloC. This hydrophobic pocket is lined by EloC residues L110, L102, V73, and V69 (Fig. 3B). Vif Cullin box mutants L163S and L169S lose the ability to bind Cul5 but retain the ability to bind EloBC. A possible explanation for these data is that the EloC-L163/L169 interactions position the Cullin box for interaction with Cul5. This would explain why the Cullin box is not required for binding to EloBC but is required for the recruitment of Cul5, as shown in a previous study in which truncated HIV Vif (residues 90 to 160) still bound EloC but not Cul5 (26). This also explains the loss of Cul5 binding to Vif when the interaction with EloBC is abolished by the L145A mutation. When binding to EloBC is lost, as in L145A, the Cullin box is not positioned properly for interaction with Cul5.

For the two Vif SOCS-box-EloC interfaces we have described, the most extensive interaction occurs between the Vif BC box and EloC. This serves as the primary attachment between Vif and the EloBC component of the ligase. The role of a secondary, less-extensive interaction between the Cullin box and EloC is not for EloBC binding, but rather for positioning the Cullin box, which is critical to binding Cul5. This second EloC-interacting interface is probably weaker than that formed by the BC box, since the distance between helices at the interface is further and the interactions are less extensive. The relatively weak interaction may explain why the Vif Cullin box region is disordered in our crystal structure. It is apparent that the crystallographic symmetry mates occupy part of the expected Cullin-box position. This also could have prevented formation of an ordered Cullin box.

Recent experimental evidence has shown that the Vif HCCH motif interacts with Cul5 (20, 27, 30, 37, 38). Our crystal structure and homology model reveals Cul5 loop 5 as a potential interaction site for the Vif HCCH motif. In our crystal structure, the observed protein density begins at Vif residue N140, which is next to the last histidine (H139) in the HCCH motif. This places the zinc finger adjacent to the N140 observed in our structure, which faces Cul5 loop 5 in our homology model (Fig. 5). The importance of loop 5 in Vif-Cul5 binding has been demonstrated in a series of deletion experiments where only the deletion of loop 5 (amino acids 119 to 139) abolished the Vif-Cul5 interaction without affecting EloBC-Cul5 binding (38). Our model, in which the Vif HCCH motif interacts with Cul5 loop 5 (Fig. 6), provides an explanation that is consistent with the deletion studies. This also is supported by the fact that Vif selectively binds Cul5 over Cul2 even though other Elob containing ligases such as VHL bind Cul2 (34). The lengths and sequences of loop regions in Cul5 and Cul2 have notable differences and could account for this selectivity (see Fig. S2A in the supplemental material).

In the present study, we investigated HIV Vif recruitment of the cellular E3 ubiquitin ligase complex by using crystallography, mutagenesis, and homology modeling. Our crystal structure of the Vif-EloBC complex demonstrates binding of a viral BC box to a cellular ubiquitin ligase; our mutational studies reveal that the Vif Cullin box is critical to Vif-Cul5 interaction, and our homology modeling suggests potential interfaces between Vif and Cul5. These results give structural insight and may provide directions for further experiments to probe the assembly to Vif-E3 ubiquitin ligase complex.

ACKNOWLEDGMENTS

We thank Alex Bullock for providing the ElonginBC plasmid, Bryan Cullen for providing Vif HXB3 DNA, Matthew Calabrese for the mass spectrometry, and the synchrotron staff at NECAT beamline ID24 and NSLS beamline X29 for assistance in data collection. This study is supported in part by a Smith Family New Investigator Award to Y.X. and a grant from the NIH (AI062044) to X.-F.Y.

REFERENCES

1. Angers, S., T. Li, X. Yi, M. J. MacCoss, R. T. Moon, and N. Zheng. 2006. Molecular architecture and assembly of the DDB1-CUL4A ubiquitin ligase machinery. Nature 443:590–593.
2. Barry, M., and K. Fruh. 2006. Viral modulators of cullin RING ubiquitin ligase. Curr. Opin. Cell Biol. 18:280–287.
3. Bates, P. A., L. A. Kelley, R. M. MacCallum, and M. J. Sternberg. 2000. Enhancement of protein modeling by human intervention in applying the automatic programs 3D-HomSS and 3D-PSSM. Proteins 41(Suppl. 5): 38–46.
4. Bishop, K. N., R. K. Holmes, and M. H. Malim. 2006. Antiviral potency of APOBEC proteins does not correlate with cytidine deamination. J. Virol. 80:8540–8548.
5. Brumm, A. T., P. D. Adams, G. M. Clure, W. L. DeLano, P. Gross, R. W. Grosse-Kunstleve, J. S. Jiang, K. Juszkiewicz, M. Nilges, N. S. Pannu, R. J. Read, L. M. Rice, T. Simonsom, and G. L. Warren. 1998. Crystallography and NMR system: a new software suite for macromolecular structure determination. Acta Crystallogr. D Biol. Crystallogr. 54:605–621.
6. Bullock, A. N., J. E. Debrezczeni, A. M. Edwards, M. Sundstrom, and S. Knapp. 2006. Crystal structure of the SOCS2-elongin C-elongin B complex defines a prototypical SOCS box ubiquitin ligase. Proc. Natl. Acad. Sci. USA 103:7637–7642.
7. Bullock, A. N., M. C. Rodriguez, J. E. Debrezczeni, Z. Songyang, and S. Knapp. 2007. Structure of the SOCS4-ElonginB/C complex reveals a distinct SOCS box interface and the molecular basis for SOCS-dependent EGFR degradation. Structure 15:1493–1504.
8. Conticello, S. G., R. S. Harris, and M. S. Neuberger. 2003. The Vif protein of HIV triggers degradation of the human antiretroviral DNA deaminase APOBEC3G. Curr. Biol. 13:2009–2013.
9. Cowtan, K., and P. Main. 1996. Miscellaneous algorithms for density modification. Acta Crystallogr. D Biol. Crystallogr. 52:487–493.
10. Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32:1792–1797.
11. Emsley, P., and W. L. DeLano. 2004. Coot: model-building tools for molecular graphics. Acta Crystallogr. D Biol. Crystallogr. 60:220–232.
12. Harris, R. S., K. N. Bishop, A. M. Sheeby, H. M. Craig, S. K. Petersen-Mahrt, I. N. Watt, M. S. Neuberger, and M. H. Malim. 2003. DNA deamination mediates innate immunity to retroviral infection. Cell 113:803–809.
13. Holm, L., and C. Sander. 1996. Dictionary of recurrent domains in protein structures. Proteins 35:88–96.
14. Holmes, R. K., F. A. Koning, K. N. Bishop, and M. H. Malim. 2007. APOBEC3F can inhibit the accumulation of HIV-1 reverse transcription products in the absence of hypermutation: comparisons with APOBEC3G. J. Biol. Chem. 282:2587–2595.
15. Jones, T. A., J. Y. Zou, S. W. Cowan, and M. Kjeldgaard. 1991. Improved building methods for building protein models in electron density maps and the location of errors in these models. Acta Crystallogr. A 47:Pt. 2E:110–119.
16. Kamura, T., K. Maenaka, S. Kotoshiba, M. Matsumoto, D. Kohda, R. C. Conaway, J. W. Conaway, and K. I. Nakayama. 2004. VHL-box and SOCS-box domains determine binding specificity for Cul2-Rbx1 and Cul5-Rbx2 modules of ubiquitin ligases. Genetics 168:305–3065.
17. Kobayashi, M., A. Takaori-Kondo, Y. Miyachi, K. Iwai, and T. Uchiyama. 2006. The Vif protein of HIV-1 triggers degradation of the human antiretroviral DNA deaminase APOBEC3G. Curr. Biol. 16:1792–1797.
18. Lecossier, D., F. Bouthoughet, F. Clavel, and A. J. Hance. 2003. Hypermutation of HIV-1 DNA in the absence of the Vif protein. Science 300:1112.
19. Luo, K., E. Ehrlich, Z. Xiao, W. Zhang, G. Ketner, and X. F. Yu. 2007. Adenovirus E4orf6 assembles with Cul5 and Cul5-ElonginB-ElonginC E3 ubiquitin ligase through an HIV/SIV-Vif like BC-box to regulate p53.FASEB J. 21:1742–1750.
20. Luo, K., Z. Xiao, E. Ehrlich, Y. Yu, B. Liu, S. Zheng, and X. F. Yu. 2005. Primate lentiviral virion infectivity factors are substrate receptors that assemble with culin 5-E3 ligase through a HCCH motif to suppress APOBEC3G. Proc. Natl. Acad. Sci. USA 102:11444–11449.
21. Mahrour, N., B. W. Redwine, L. Florens, S. K. Swanson, S. Martin-Brown,
VOL. 82, 2008

STRUCTURAL INSIGHT INTO THE HIV Vif SOCS BOX 8663

W. D. Bradford, K.斯塔奇-汉普顿, M. P. Washburn, R. C. Conaway, and J. W. Conaway. 2008. Characterization of Cullin-box sequences that direct recruitment of Cul2-Rbx1 and Cul5-Rbx2 modules to ElonginBC-based ubiquitin ligases. J. Biol. Chem. 283:8005–8013.

Mangat, R., P. Turelli, G. Caron, M. Friedli, L. Perrin, and D. Tron. 2003. Broad antiretroviral defence by human APOBEC3G through lethal editing of nascent reverse transcripts. Nature 424:99–103.

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

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

McGoy, A. J., D. C. Noronha, W. Yonemoto, and W. C. Greene. 2003. HIV-1 Vif blocks the antiviral activity of APOBEC3G by impairing both its translation and intracellular stability. Mol. Cell 12:591–601.

Woo, J., S. J. Imm, C. K. Min, K. J. Kim, S. S. Cha, and B. H. Oh. 2006. Structural and functional insights into the B30.2/SPRY domain. EMBO J. 25:1353–1363.

Xiao, Z., E. Ehrlich, K. Luo, Y. Xiong, and X. F. Yu. 2007. Zinc chelation inhibits HIV Vif activity and liberates antiviral function of the cytidine deaminase APOBEC3G. FASEB J. 21:217–222.

Xiao, Z., E. Ehrlich, Y. Yu, K. Luo, T. Wang, C. Tian, and X. F. Yu. 2006. Assembly of HIV-1 Vif-Cul5 E3 ubiquitin ligase through a novel zinc-binding domain-stabilized hydrophobic interface in Vif. Virology 349:290–299.

Yang, B., L. Gao, L. Li, Z. Lu, X. Fan, C. A. Patel, R. J. Pomerantz, G. C. DuBois, and H. Zhang. 2003. Potent suppression of viral infectivity by the peptides that inhibit multimerization of human immunodeficiency virus type 1 (HIV-1) Vif proteins. J. Biol. Chem. 278:6596–6602.

Yang, S., Y. Sun, and H. Zhang. 2001. The multimerization of human immunodeficiency virus type 1 Vif protein: a requirement for Vif function in the viral life cycle. J. Biol. Chem. 276:4889–4893.

Ving Cheng, C., P. Blanchette, and P. E. Branton. 2007. The adenovirus E4orf6 E3 ubiquitin ligase complex assembles in a novel fashion. Virology 364:36–44.

Yu, X., Y. Yu, B. Liu, K. Luo, W. Kong, P. Mao, and X. F. Yu. 2003. Induction of APOBEC3G ubiquitination and degradation by an HIV-1 Vif-Cul5-SCF complex. Science 302:1056–1060.

Yu, Y., Z. Xiao, E. S. Ehrlich, X. Yu, and X. F. Yu. 2004. Selective assembly of HIV-1 Vif-Cul5-ElonginB-ElonginC E3 ubiquitin ligase complex through a novel SOCS box and upstream cysteines. Genes Dev. 18:2867–2872.

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

Zheng, N., B. A. Schulman, L. Song, J. J. Miller, P. D. Jeffs, P. Wang, C. Chu, D. M. Koepp, S. J. Elledge, M. Pagano, R. C. Conaway, J. W. Conaway, J. W. Harper, and N. P. Pavletich. 2002. Structure of the Cul5-Rbx1-Skp1-F boxSkp2 SCF ubiquitin ligase complex. Nature 416:703–709.

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