Viral Manipulations of the Cullin-RING Ubiquitin Ligases

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Abstract

Cullin-RING ubiquitin ligases (CRLs) are efficient and diverse toolsets of the cells to regulate almost every biological process. However, these characteristics have also been usurped by many viruses to optimize for their replication. CRLs are often at the forefront of the arms races in the coevolution of viruses and hosts. Here we review the modes of actions and functional consequences of viral manipulations of host cell CRLs. We also discuss the therapeutic applications to target these viral manipulations for treating viral infections.

Keywords

Cullins · Virus infection · CRL · Degradation · Ubiquitination

Abbreviations

CRL	Cullin-RING ubiquitin ligases
Cul1-5	Cullin 1-5
DCAF	DDB1-Cul4-associated factors
HBV	Hepatitis B virus
HIV	Human immunodeficiency virus
KSHV	Kaposi's sarcoma-associated herpesvirus
RSV	Respiratory syncytial virus
SCF	SKP1-Cullin1-Fbox E3 ligase
SR	Substrate receptor

Viruses rely on the host cell machinery to infect and replicate. It is remarkable that viruses can use their small repertoire of proteins to control the host cells and redirect almost every cellular apparatus toward massive production of viral particles. The protein degradation machinery is not spared by the virus. In fact, viruses heavily manipulate the ubiquitin proteasome system to optimize for viral replication (Hershko and Ciechanover 1998; Schwartz and Ciechanover 1999). Cullin-RING ubiquitin ligases, the largest family of eukaryote ubiquitin ligases, are hijacked by viruses that are very different taxonomically to evade different human defense mechanisms (Mahon et al. 2014; Barry and Fruh 2006). For example, paramyxovirus, which belongs to the *Paramyxoviridae*, and Hepatitis B virus (HBV), which belongs to the *Hepadnaviridae*, both use a small protein (V protein for paramyxovirus and HBx for HBV) to hijack the Cul4-DDB1 (CRL4) E3 ligase to target multiple host proteins for ubiquitination and degradation (Li et al. 2006; Decorsiere et al. 2016). As another example of the widespread viral hijacking of Cullin-RING, HIV use multiple accessory proteins, Vpu, Vpr, Vif, and Vpx (Vpx

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is HIV-2 specific), to hijack multiple Cul1-, Cul4-, and Cul5-based CRLs (Sauter and Kirchhoff 2018; Malim and Bieniasz 2012). These viral proteins usually function as adaptors between the CRLs and the protein targets, which are often involved in host antiviral responses. The accessory proteins possess multiple protein binding sites that can interact with both the CRL substrate receptor and the target proteins, thereby recruiting the target proteins to the CRLs for ubiquitination and subsequent degradation.

Evidence is accumulating to support that a large number of proteins are affected by the viral hijacking. For example, a recent study showed that hundreds of proteins might be downregulated by HIV Vpr, including proteins from a variety of function categories such as RNA-binding proteins and DNA-binding proteins (Greenwood et al. 2019). Our own study quantitated the proteome of HIV-1-infected primary CD4+ T cells and uncovered protein level changes of hundreds of human proteins. Importantly, these protein changes are not associated with mRNA level changes determined by RNA-seq in the same study, supporting widespread alterations of protein stability by the viral proteins (Liu et al. 2019). It is expected that proteins targeted by the virus-hijacked ubiquitin ligases would be ubiquitinated and degraded, explaining the downregulated protein levels. But what is the mechanism of the upregulation of protein levels? An analysis of a published list of physiological substrates of CRL5 E3s shows that most of them are upregulated by HIV infection (Liu et al. 2019). This phenomenon is likely explained by HIV Vif's hijacking of CRL5, which competes away the physiological substrates from the E3s. The breadth and complexity of the cellular proteins destabilized/stabilized by viral hijacking of the host ubiquitin ligases highlight the importance of this hijacking to the viral replication and pathogenesis. The importance of the viral hijacking is also reflected in the positive selection of the amino acid sequences involved in the binding sites between the viral protein and the host target proteins (Daugherty and Malik 2012). Positive selection in amino acid sequences is a hallmark of the evolutionary arms race between a host protein and its viral antagonist protein, providing strong evidence that the interaction is critical to the survival of both the virus and the host during the coevolution of the two parties (Daugherty and Malik 2012).

The widespread viral hijacking of CRLs is not accidental. Ubiquitination-mediated protein degradation is involved in almost every biological process, regulating important protein turnover that often controls the progression and magnitude of the process in a rapid fashion (Deshaies and Joazeiro 2009; Zheng and Shabek 2017). The target proteins can be massively degraded within a few hours, which are critical for viruses to evade the cellular defense system to expedite their replication (Weekes et al. 2014; Matheson et al. 2015). In addition to speed, the catalytic nature of ubiquitination and proteasomal degradation ensures that regulation of the ubiquitin ligases can have a magnifying effect due to the low reaction stoichiometry, i.e., one ligase molecule can ubiquitinate many substrate molecules (Harper and Tan 2012). When hijacked by a virus, this stoichiometry ratio can maximize the impact of the hijacked ligase. A third attribute of CRLs is that they are highly modular, thereby making it straightforward for the viral protein to exploit or interfere (Deshaies and Joazeiro 2009; Zheng and Shabek 2017). Usually, substrate specificity of CRLs are solely determined by individual substrate receptor (SR) proteins. In addition, the catalytic mechanism of the ubiquitination reaction is through induced proximity (Deshaies and Joazeiro 2009). These properties facilitate viral hijacking by simply introducing new protein-protein interaction between SR and a host defense protein. The viral protein can bind both the SR and the host defense protein and functions as a linker between the two (Mahon et al. 2014). The formation of this complex is sufficient to enable the ubiquitination of the host defense protein. The high modularity and proximity-based catalysis of CRLs provide great versatility to evolve new functionality in host evolution, which likely account for the great diversity of the current CRL family members. However, this property has also been taken advantaged by a variety of viruses to work against the hosts. On the other hand, the

widespread viral hijacking of CRLs and the importance of the hijacking to viral replication indicate that targeting these hijacking events might provide opportunities to discover effective antiviral drugs (Huang and Dixit 2016; Votteler and Schubert 2008).

In addition to viral hijacking of the CRL ligases, viral proteins can affect ligase activities in many other ways. For example, they can affect phosphorylation of the substrate proteins to promote their ubiquitination, as in the case of KSHV V-cyclin's phosphorylation of p27, which leads to p27 ubiquitination by CRL1-SKP2 ligase and subsequent degradation (Ashizawa et al. 2012; Ellis et al. 1999; Liu et al. 2007). As another sample, Epstein-Barr virus (EBV) encodes a protease named BPLF1, which can function as a deneddylase to remove Nedd8 protein from Cullins and inhibit CRL activity (Gastaldello et al. 2010). Below, we will discuss in detail a number of examples of viral manipulations of the different family of CRLs and the therapeutic implications of these molecular events. We will emphasize on HIV-1 given its many well-studied examples of hijacking multiple CRLs.

7.1 Cullin 1-RING Ubiquitin Ligases (CRL1)

CRL1 E3 ligases are the prototype of the whole CRL family. The invariable components of CRL1 E3s are Cullin 1, Rbx1 (also named Roc1), and the adaptor protein SKP1. SKP1 recognizes different SRs through binding to the highly conserved F-box domain of the SRs (Zheng and Shabek 2017; Bai et al. 1996; Skowyra et al. 1997; Feldman et al. 1997). Therefore, CRL1s have traditionally been termed as SCF E3 ligases (Skp1-Cullin1-F-box). There are about 70 F-box proteins in the human genome, targeting a large number of proteins in the cells given that each F-box can target multiple proteins for ubiquitination (Jin et al. 2004). The functions of different CRL1s are widely involved in cell cycle regulation; cell growth; cell death, development, and differentiation etc (Skaar et al. 2013). The diversity of the CRL1 family also makes them facile targets to be hijacked by many viruses, including herpesvirus, rotavirus, HIV-1, Hepatitis E virus, etc. (Ashizawa et al. 2012; Liu et al. 2007; Rodrigues et al. 2009; Graff et al. 2009; Surjit et al. 2012; Collins and Collins 2014). We will describe several well-studied examples below (Fig. 7.1).

HIV-1 Vpu had been well known to promote virus release from infected cells. However, it took a 20-year long research to unveil the underlying mechanism. A human membrane protein named tetherin was identified to be an HIV restriction factor that is counteracted by Vpu (Neil et al. 2008; Van Damme et al. 2008). Tetherin, as its name indicates, can function as a tether between nascent virions and infected cells, as well as between nascent virions, the net effect of which is to block the release of the nascent virions. Tetherin accomplishes this interesting function through its two membrane anchoring domains, one at its N-terminus and one at the C-terminus (Perez-Caballero 2009). The expression level of tetherin is induced by interferon (Neil et al. 2008; Van Damme et al. 2008). Therefore, it is an effector of the interferon-mediated antiviral response, and it was later shown to be a general mechanism for defense against many different viruses (Evans et al. 2010). Vpu is also a transmembrane protein that interacts with tetherin through its transmembrane domain (Vigan and Neil 2010). In addition, the intracellular domain of Vpu interacts with the F-box protein β-TrCP, which is the SR for the E3 $SCF^{\beta\text{-}TrCP}$ (Margottin

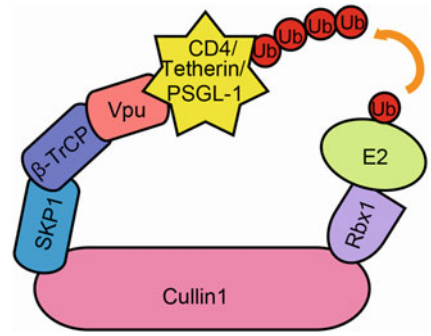

Fig. 7.1 Examples of viral hijacking of CRL1. HIV-1 Vpu protein recruits CD4 or tetherin or PSGL-1 to SCF-β-TrCP E3 ligases for ubiquitination

et al. 1998). This interaction links tetherin to an E3 ligase, which results in its ubiquitination and degradation from the cell surface due to ubiquitination-induced endosomal degradation (Roy et al. 2014). Vpu is not present in the incoming viruses and is only produced late in the HIV-1 life cycle, which suits the timing to antagonize tetherin and promote virus release (Neil et al. 2008).

Another target of $SCF^{\beta\text{-}TrCP}$-Vpu E3 ligase is the CD4 molecule on the surface of T cells. Distinct from antagonism of tetherin, Vpu targets CD4 molecule using its intracellular domain, and the degradation of CD4 after ubiquitination is through the proteasomal pathway (Roy et al. 2014; Dube et al. 2010). The physiological significance of Vpu-induced CD4 degradation is thought to avoid the re-adsorption of the nascent virions on the surface due to the contact of envelop protein and CD4. But the exact significance of this degradation remains to be demonstrated since CD4 is also the receptor for HIV, which complicates genetic studies to confirm the function of the degradation (Dube et al. 2010; Chaudhuri et al. 2007).

A comprehensive study to quantitatively measure membrane protein abundance during HIV infection in CD4+ T cells has revealed more potential targets of Vpu (Matheson et al. 2015). By comparing the differences in proteomic changes in infections with wild-type HIV or Vpu-deficient HIV, this study identified over 100 potential protein targets of Vpu-mediated degradation. From this list, Matheson et al. found an amino acid transporter protein named SNAT1, which was subsequently shown to be ubiquitinated by $SCF^{\beta\text{-}TrCP}$-Vpu and degraded by the endosomal pathway, rather than the proteasomal pathway (Matheson et al. 2015). Why does Vpu target SNAT1, given that this protein does not seem to affect HIV-1 infection *in vitro*? The authors of the study found that SNAT1 is required for T cell activation, which might be linked to the establishment of latent reservoir of the viruses (Sugden and Cohen 2015). But the exact mechanistic explanation of the Vpu-SNAT1 antagonism requires further study.

Our group recently identified another HIV restriction factor and target of $SCF^{\beta\text{-}TrCP}$-Vpu E3 ligase: PSGL-1, from a genome-wide proteomic profiling in human primary CD4+ T cells during HIV-1 infection (Liu et al. 2019). PSGL-1 is significantly downregulated during HIV-1 infection in Vpu-dependent fashion. PSGL-1 is a transmembrane protein and exerts anti-HIV-1 function mainly by associating with the nascent virions and potently inhibiting their infectivity. Vpu can partially relieve the restriction by inducing the ubiquitination and proteasomal degradation of PSGL-1 via $SCF^{\beta\text{-}TrCP2}$. Interestingly, only β-TrCP2, but not β-TrCP1, is responsible for the ubiquitination, which is different from the cases of CD4 and tetherin where both β-TrCP1 and β-TrCP2 can mediate Vpu-dependent CD4 and tetherin ubiquitination (Liu et al. 2019). Also different from tetherin, PSGL-1 is specifically expressed in T cells and monocytes and is induced by interferon γ, but not interferon α. Importantly, PSGL-1 is a key mediator of interferon γ's anti-HIV effects in human CD4+ T cells (Liu et al. 2019). Therefore, Vpu's hijacking of $SCF^{\beta\text{-}TrCP2}$ is key to resist interferon γ's anti-HIV effects.

Remarkably, Vpu, a small transmembrane protein with a size smaller than 10 kilodalton, can perform such a diversity of functions to recruit very different membrane proteins for ubiquitination (Sauter and Kirchhoff 2018). This is a great example demonstrating the versatility and modularity CRLs and the diverse functions of viral hijacking of CRLs in viral replication.

7.2 Cullin 2-RING Ubiquitin Ligases (CRL2)

Cullin 2- and Cullin 5-based CRLs share the same adaptor module: a protein complex consisting of a pair of small proteins—elongin B and elongin C (EloB/EloC). However, the substrate receptor of CRL2s and CRL5s has so-called VHL box and SOCS box sequence motifs, respectively, that differentiate between Cullin 2 and Cullin 5, conferring specificity to these two CRL families (Sarikas et al. 2011). CRL2 prototype is the Cul2-Rbx1-EloB/EloC-VHL E3 ligase that

targets the hypoxia-induced factor 1α (HIF-1α), which is a key transcription factor in regulation of cellular response to hypoxia (Ke and Costa 2006). VHL binds to EloB/EloC using its so-called BC box motif, which can also recognize Cul2. VHL can also bind to HIF-1α and promote the ubiquitination and degradation of HIF-1α under normal oxygen conditions. HIF-1α binding to VHL is dependent on the hydroxylation of two proline residues of HIF-1α, but the hydroxyl groups are removed under hypoxia conditions; therefore HIF-1α degradation is abolished, and HIF-1α is released into the nucleus to promote the transcription of hypoxia response genes (Kaelin 2002). Mutations in VHL can lead to von Hipple-Lindau disease, which is characterized by visceral cysts and benign tumors with potential for malignant transformation (Kaelin 2002).

Respiratory syncytial virus (RSV) is a common respiratory virus that causes cold-like symptoms especially in children below age of 2 years old. It has been reported that the nonstructural protein NS1 of RSV can hijack CRL2 to target human STAT2 for ubiquitination and degradation (Elliott et al. 2007). STAT2, together with STAT1 and IRF-9, form a key transcription factor mediating the signaling transduction of type I interferon pathway, which is a master regulator of the antiviral innate immune responses (Schneider et al. 2014). By degrading STAT2, RSV shuts down the whole type I interferon responses to evade from the human innate defense system. The NS1 protein binds to elongin C and Cul2 to form the CRL2-NS1 E3 ligase to recruit STAT2 for ubiquitination (Elliott et al. 2007) (Fig. 7.2). Inhibition of the functions of STAT1 or STAT2 proteins has been a well-used strategy for viral evasion of the interferon-mediated antiviral responses. For example, HCV nonstructural protein 5A (NS5A) can inhibit STAT1 phosphorylation, which is required for the interaction between STAT1 and STAT2 and subsequent transcription activation (Lan et al. 2007). Influenza virus can also use its NS1 protein to inhibit the phosphorylation of STAT1 as well as STAT2 to block both the type I and type II interferon pathway (Jia et al. 2010). In contrast,

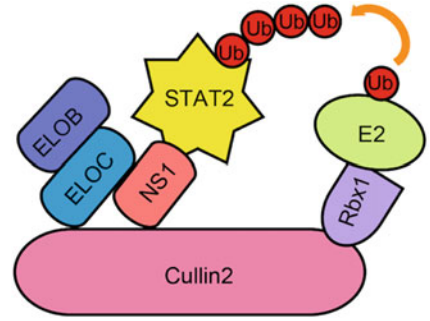

Fig. 7.2 An example of viral hijacking of CRL2. RSV NS1 protein recruits host cell STAT2 protein to CRL2 ligase for ubiquitination

RSV's degradation of STAT2 would be a thorough strategy to block the interferon pathway since it is permanent removal of the protein. Very interestingly, it has also been reported that RSV can stabilize the protein level of HIF-1α, which was attributed to the release of nitric oxide (Kilani et al. 2004). RSV's hijack of Cul2-EloB/EloC complex might be another contributing factor to the stabilization, since the hijack might exclude HIF-1α from ubiquitination and subsequent proteasomal degradation.

7.3 Cullin 3-RING Ubiquitin Ligases (CRL3)

CRL3s usually use one single protein, a member of the so-called BTB family proteins, to perform the functions of adaptor and substrate receptor, which is unique among CRLs (Zhuang et al. 2009). Human BTB family has close to 200 members, and they usually share a BTB domain that binds to Cul3 and a protein-protein interaction domain such as Kelch-like domain or MATH domain that binds to ubiquitination substrates (Stogios et al. 2005). There are relatively fewer known examples of CRL3s involved in viral infection.

One of the examples is that rotavirus uses its nonstructural protein NSP1 to recruit Cul3-Rbx1 to target β-TrCP for ubiquitination and degradation (Fig. 7.3) (Lutz et al. 2016; Davis and Patton 2017). As mentioned above, β-TrCP is the SR for

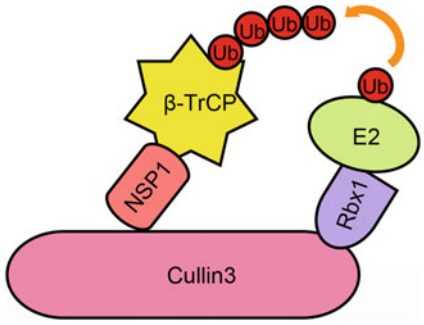

Fig. 7.3 An example of viral hijacking of CRL3: Rotavirus NS1 protein recruits host cell β-TrCP protein to CRL3 ligase for ubiquitination

CRL1, and one of its ubiquitination targets is IκB (Frescas and Pagano 2008). Ubiquitination of IκB is required for the activation of the NF-κB pathway, a key pathway for stimulating cytokine and interferon production to elicit host defenses (Chen 2005). Therefore, rotavirus NSP1's ubiquitination and consequent degradation of β-TrCP lead to inactivation of NF-κB to suppress host antiviral responses. Interestingly, in this case, there is no requirement for a BTB protein since Cul3 mutations that abolish BTB binding still can mediate this interaction. NSP1 binds Cul3 directly to recruit β-TrCP for ubiquitination (Davis and Patton 2017). Another interesting aspect of this targeting is that the ubiquitination is not only dependent on Cul3 but also dependent on Cul1, demonstrating a cross talk between different CRL families (Davis et al. 2017). A third unique aspect of NSP1 as an E3 ligase is that the viral protein itself possesses a RING domain so it can function as an E3 ligase on its own (Davis and Patton 2017). NSP1 from simian and murine rotavirus strains can directly bind IRF3, a key transcription factor for innate immune responses, and promote IRF3 ubiquitination and degradation independent of Cul3 (Sen et al. 2009) . The functional diversity of NSP1 as an E3 ligase highlights the versatility of viral hijacking of the ubiquitin-proteasome pathway.

Another example of viral hijacking of CRL3 is illustrated by human papilloma virus (HPV), one of the most common sexually transmitted viruses that can cause warts and precancerous lesions in persistently infected patients. Among the 150 types of HPV, about a dozen types are known to be associated with different human cancers (Nowinska et al. 2017). HPV encodes E6 and E7 proteins that are potent agents capable of transforming cells into immortalized cells. Their transformation abilities are associated with their hijacking of the human E3 ligases to degrade two key tumor suppressors: p53 and RB, respectively (Ajay et al. 2012). E6 associates with the HECT-type E3 ligase E6AP to recruit p53 for ubiquitination, whereas E7 of the HPV type 16 has been known to associate with Cul2-EloB/EloC E3 ligase to target RB for ubiquitination and proteasomal degradation (Yim and Park 2005). More recent protein-protein interaction profiling studies have revealed that E7 proteins from many different HPV types are associated with Cul3 instead of Cul2 (White et al. 2012). It is hypothesized that CRL3s might be involved in RB degradation in those HPV types. E7 proteins from different HPV types hijack different CRLs, again underscoring the versatility of the hijacking mechanisms.

7.4 Cullin 4-RING Ubiquitin Ligases (CRL4)

CRL4s employ a large, 125-kilodalton protein-DDB1 as the adaptor, which recruits a family of so called DCAF (DDB1-Cul4 -associated factors) proteins as substrate receptors (Zheng and Shabek 2017). CRL4s are widely hijacked by different viral proteins to promote viral infections. Viral proteins can either bind to DDB1 and directly recruit their target proteins or interact with DCAF proteins to change their substrate specificity. Simian virus 5, belonging to the paramyxovirus family, encodes a V protein to bind to DDB1 and recruit human STAT1 protein for ubiquitination and proteasomal degradation (Li et al. 2006). This hijacking leads to the dampening of the interferon responses to viral infection. Similarly, hepatitis B virus encodes a small protein called HBx to bind to DDB1 and recruit a DNA-binding protein complex Smc5-Smc6 for ubiquitination and degradation (Fig. 7.4) (Decorsiere et al. 2016; Murphy et al.

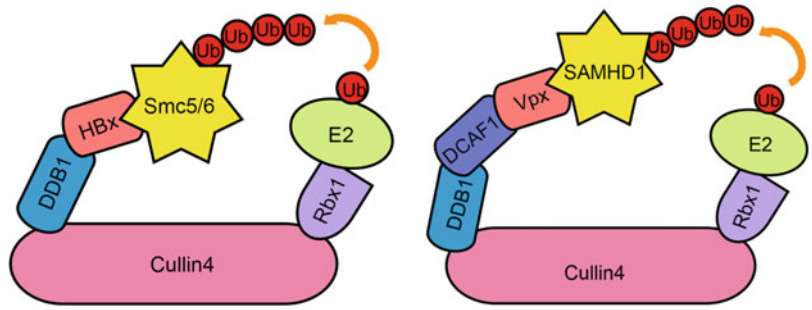

Fig. 7.4 Examples of viral hijacking of CRL4. HIV-2 or SIV Vpx protein recruits host cell SAMHD1 protein to CRL4 ligase for ubiquitination. HBV HBx protein recruits host cell Smc5-Smc6 proteins to CRLS4 ligase for ubiquitination

2016). HBx has long been known to be important for the transcription of HBV genome, but the mechanism was elusive. It had also been known that HBx associates with CRL4 complex, but the substrate protein was unknown until 2016. In that year, two papers reported the identification of Smc5-Smc6 complex as a target of HBx using immunoprecipitation-coupled mass spectrometry and showed that HBx overcomes Smc5-Smc6 inhibition of HBV transcription (Decorsiere et al. 2016; Murphy et al. 2016).

Another way for the viral protein to hijack CRL4 is to bind to one of the DCAFs and change its substrate specificity. A well-known example is the association between HIV-1 accessory protein Vpr and DCAF1 (also called VprBP). The function of Vpr in HIV-1 replication has been enigmatic, but its association with CRL4-DCAF1 provided a clue. Another well-known phenomenon of Vpr is that it can induce cell cycle arrest at the G2/M phase (He et al. 1995). A recent proteomic profiling has revealed that hundreds of proteins are downregulated by Vpr, directly or indirectly (Greenwood et al. 2019). A number of these proteins have been validated to contribute to the cell cycle arrest induced by Vpr, supporting the promiscuous substrate recruitment of Vpr. However, the functional significance of the cell cycle arrest induced by Vpr is still not clear. In comparison, a Vpr homolog protein called Vpx, which is encoded by Simian immunodeficiency virus (SIV) and HIV-2 but not HIV-1, has yielded its secret. Vpx, like Vpr, hijacks CRLs but they target a very different set of targets. One of the targets is SAMHD1, a dNTP hydrolase that is highly expressed in myeloid cells and resting CD4+ T cells (Laguette et al. 2011; Hrecka et al. 2011). SAMHD1 has been shown to potently inhibit lentivirus reverse transcription by reducing the concentration of dNTPs, the building blocks of lentiviral DNA (Lahouassa et al. 2012). Vpx recruits CRL4-VprBP to ubiquitinate SAMHD1 to alleviate this inhibition (Fig. 7.4) (Laguette et al. 2011; Hrecka et al. 2011; Ahn et al. 2012). It is interesting that HIV-1 does not have such a counteracting mechanism. It is speculated that HIV-1 thus avoids infection of myeloid cells such as dendritic cells to evade detection by these cells (Lim and Emerman 2011). The high homology of Vpr and Vpx showcases how evolution can reprogram a viral protein to target different substrate proteins for ubiquitination.

7.5 Cullin 5-RING Ubiquitin Ligases (CRL5)

As described above, CRL5 and CRL2 share the adaptor protein complex, EloB/EloC. Different from CRL2, CRL5 employs the so-called SOCS box protein as SR to recruit substrate proteins. In addition, CRL5 is unique among all CRLs by preferentially using Rbx2 (Roc2/Sag) rather than Rbx1(Roc1) as the catalytic module.

HIV-1 Vif protein hijacks CRL5 to target an HIV restriction factor APOBEC3G for ubiquitination and degradation (Fig. 7.5), which is the first example of HIV-1 accessory protein targeting a restriction factor to an E3 (Yu et al. 2003). APOBEC3G was identified from a comparison of the cDNAs of two closely related cell

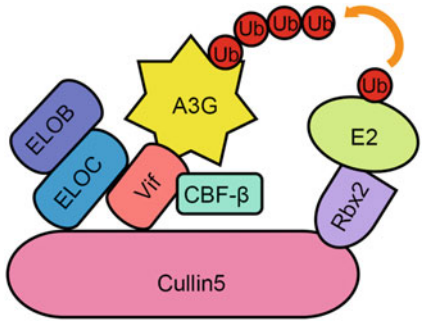

Fig. 7.5 An example of viral hijacking of CRL5. HIV Vif protein, with the host cell cofactor CBFβ, recruits host cell APOBEC3G (A3G) protein to CRL5 ligase for ubiquitination

lines, one susceptible to both Vif-deficient and wild-type HIV-1 and the other only susceptible to the wild-type virus. APOBEC3G, originally named CEM15, is specifically expressed in the cell line that is resistant to Vif-deficient virus (Sheehy et al. 2002). APOBEC3G is a DNA-editing enzyme that associates with the HIV-1 reverse transcriptase to be packaged into nascent virions (Malim and Bieniasz 2012). During the next round of infection, APOBEC3G will extensively mutate the newly synthesized viral DNA to block viral infection (Zhang et al. 2003; Lecossier et al. 2003; Mangeat et al. 2003; Harris et al. 2003). Biochemical work has identified that Vif hijacks CRL5 to recruit APOBEC3G for ubiquitination and proteasomal degradation (Yu et al. 2003). Interestingly, later work uncovered another cofactor CBFβ that is required for Vif's hijacking of CRL5 (Jager et al. 2012; Zhang et al. 2011). Structural studies have revealed that Vif impressively organizes the formation of this E3 ligase complex by simultaneously interacting with four proteins: Cul5, EloC, CBFβ, and APOBEC3G (Guo et al. 2014). Interestingly, CBFβ's physiological function is to bind to the transcription factor RUNX to facilitate the transcription of target genes of RUNX (Kim et al. 2013). Vif's hijacking of CBFβ excludes RUNX from binding to CBFβ and inhibits RUNX-mediated gene transcription, which further benefits the viral replication since many of these target genes are involved in immune responses (Kim et al. 2013). Therefore, this hijacking has dual advantages for the virus. More recently, proteomic profiling has identified another regulator of the CRL5 ligases. ARIH2, a RING-Between-RING (RBR) E3 ligase, has been found to be required for the efficient ubiquitination by CRL5 ligases, including Vif-mediated ubiquitination of APOBEC3. ARIH2 promotes the initial ubiquitination of the substrate, which facilitates the following ubiquitination chain elongation mediated by CRL5s (Huttenhain et al. 2019). ARIH2 is yet another Vif cofactor in promoting APOBEC3 ubiquitination. This intricate structural organization by Vif underlines the amazing capability of viral proteins to evolve complex functions within a compact genome.

7.6 Therapeutic Targeting of the Viral Hijacking of CRLs

Many lines of functional evidence suggest that we might be able to develop new antiviral therapies by blocking the viral hijacking of CRLs. Evolution history has also proven that these hijacking events are vital to the replication of the viruses. Targeting the ubiquitin ligases for drug development has not been as straightforward as targeting other enzymes, since E3 ligases do not have natural binding sites for small molecules. Instead, E3 ligases function mostly through protein-protein interaction, which is traditionally thought to be challenging to target. Nevertheless, there are more and more examples to show that protein-protein interaction might not be as "undruggable" as previously thought. Vif-APOBEC3G, the first pair of HIV accessory protein and its cognizant restriction factor, has been intensively targeted for drug screening, and several studies have identified specific inhibitors. RN-18 was the first reported Vif inhibitor that was identified from a cell-based screen to search for small molecules that stabilizes APOBEC3G in the presence of Vif (Nathans et al. 2008). RN-18 downregulated Vif protein level and increased APOBEC3G protein level. Several RN-18 analogs were developed to optimize its potency and water solubility (Zhou et al. 2017; Bennett et al. 2018). Recently, the same group

have identified a single mutation on Vif that confers resistance to these inhibitors, further proving that the inhibitors indeed target Vif in a specific manner (Sharkey et al. 2019). A different class of Vif inhibitor were identified using a similar method, but these compounds more likely target APOBEC3G based on binding assay (Cen et al. 2010). VEC-5, a compound discovered based on structural model of the Vif-EloB/EloC complex, has been shown to stabilize APOBEC3G (Zuo et al. 2012). More recently, a compound named N.41 was identified from a screen using a FRET-based biochemical assay to search for inhibitor of Vif-APOBEC3G interaction (Pery et al. 2015). N.41 has been shown to possess strong anti-HIV activity in PBMCs in an APOBEC3G-dependent fashion. In general, the multiple protein-protein interfaces in the complex of CRL5-Vif-CBFβ-APOBEC3G provide many potential small-molecule target sites, some of which could be very unpredictable. Additional potential allosteric binding sites in the complex could also be targeted by small molecules. These published Vif inhibitors provided proofs of concept for therapeutic targeting of viral hijacking of CRL E3 ligases. Future development of therapeutics targeting the viral hijacking of E3 ligases should present many new opportunities to treat a variety of pathogenic viruses.

7.7 Perspectives on Future Research

For the past two decades, research on virus-host interaction has revealed remarkable roles of the CRLs E3 ligases in viral infection and pathogenesis. These results were built on the advancement of our understanding of the functions and mechanisms of the ubiquitin-proteasome pathway in general but also significantly enrich and inform about the pathway. Currently knowledge on this topic is only the tip of the iceberg as recent systematic approaches such as mass spectrometry profiling and genome-wide functional genomic screening are suggesting a much larger number of genes involved in the interaction between viruses and hosts (McDougall et al. 2018; Shah et al. 2015). These systematic approaches would continue to reveal new genes, protein complexes, and signaling networks regulating protein degradation in the contexts of viral infections and immune responses. From the application point of view, following the great stride in targeting the CRLs for cancer drug development such as the PROTAC approach, targeting the CRLs for antiviral drug development is expected to reap the benefit of a better mechanistic understanding of the functions and mechanisms of CRLs in viral infections.

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