Structural Biology of CRL Ubiquitin Ligases

2

Domnița-Valeria Rusnac and Ning Zheng

Abstract

Cullin-RING ubiquitin ligases (CRLs) represent the largest superfamily of multi-subunit E3s conserved in all eukaryotes. Soon after the discovery of these important ubiquitin ligase machineries, structural studies have made tremendous contributions to our understanding of their functions. Identification of the key components of CRLs by early studies raised immediate questions as to how these multi-subunit complexes assemble to promote the polyubiquitination of substrates. Specifically, how do the CRL subunits interact with each other to form a versatile E3 platform? How do they recognize specific substrates? How are the CRL-substrate interactions regulated in response to upstream signals? How are the CRL E3s themselves activated and deactivated, and how are substrate receptor subunits of CRLs exchanged in the cell? Even though we might not yet have complete answers to these questions, extensive structural analyses of CRL complexes in the past two decades have begun to unveil the themes and variations of CRL biology. In this chapter we will discuss both classic and emerging structures that help elucidate the overall architecture of CRLs, their substrate recognition modes, and regulatory mechanism of CRLs by NEDD8 modification.

Keywords

Cullin-RING ubiquitin ligases · Structural biology · Ubiquitination · Neddylation · DCN1

Abbreviations

APOBEC3	Apolipoprotein B mRNA editing enzyme catalytic polypeptide-like 3
ARIH1	Ariadne-1 homolog
ASK1	Apoptosis signal-regulating kinase 1
AUX/IAA	Auxin/indole-3-acetic acid
BPA	Beta-propeller A
BPB	Beta-propeller B
BPC	Beta-propeller C
BTB	Broad-complex, tramtrack, and bric-a-brac
CAND1	Cullin-associated NEDD8-dissociated protein 1
CBF-β	Core-binding factor beta
CDC34	Cell division cycle 34/ubiquitin-conjugating enzyme E3 R1

D.-V. Rusnac
Department of Pharmacology, University of Washington, Seattle, WA, USA

N. Zheng (✉)
Department of Pharmacology, University of Washington, Seattle, WA, USA

Howard Hughes Medical Institute, University of Washington, Seattle, WA, USA
e-mail: nzheng@uw.edu

Y. Sun et al. (eds.), *Cullin-RING Ligases and Protein Neddylation*, Advances in Experimental Medicine and Biology 1217, https://doi.org/10.1007/978-981-15-1025-0_2

CDK2	Cyclin-dependent kinase 2
CK1α	Casein kinase 1
CKS1	Cyclin-dependent kinases regulatory subunit 1
COI1	Coronatine-insensitive protein 1
COP9	Constitutive photomorphogenesis 9
CRBN	Cereblon
CRL	Cullin-RING ubiquitin ligases
CRY1/2	Cryptochrome circadian regulator 1/2
CSN	COP9 signalosome
CTD	C-terminal domain
CUL	Cullin
DCAFs	DDB1-CUL4A-associated factors
DCN1	Defective in cullin neddylation protein 1-like protein 1
DDB1/2	DNA damage-binding protein 1/2
EB	Elongin B
EC	Elongin C
FBXL	F-box and leucine-rich repeat proteins
FBXO	F-box and other repeat-containing proteins
FBXW	F-box and WD repeat-containing proteins
GLMN	Glomulin
GSPT1	G1 to S phase transition 1
HHARI/ ARIH1	Ariadne-1 homolog
HIF1α	Hypoxia-inducible factor 1 subunit alpha
IKZF1/3	IKAROS family zinc finger 1/3
IP6	Inositol hexakisphosphate
JA	Jasmonic acid
JAZ	Jasmonate ZIM domain
KEAP1	Kelch-like ECH-associated protein 1
KLHDC2	Kelch domain-containing protein 2
LRR	Leucine-rich repeat
MATH	Meprin and TRAF-C homology
MEIS2	Myeloid ecotropic viral integration site 1 homolog 2
NEDD8	Neural precursor cell-expressed developmentally downregulated protein 8
NRF2	Nuclear factor erythroid 2-related factor 2
NTD	N-terminal domain
ODD	Oxygen-dependent degradation
PTM	Post-translational modification
RBX1/2	RING box protein 1/2
RING	Really interesting new genes
RING-IBR-RING RBR	RING – in between ring – RING
SALL4	Sal-like protein 4
SCF	SKP1, CUL1, and F-box proteins
SELK	Selenoprotein K
SELS	Selenoprotein S
SKP1/2	S-phase kinase-associated protein 1/2
SPOP	Speckle-type POZ protein
STAT	Signal transducer and activator of transcription
SV5-V	Simian virus 5 V protein
TIR1	Transport inhibitor response 1
UBC12/ UBE2M	Ubiquitin-conjugating enzyme E2 M
USP1	Ubiquitin specific peptidase 1
VHL	von Hippel-Lindau tumor suppressor
Vif	Viral infectivity factor
WHB	Winged-helix B
Wnt	Wingless
β-TrCP	Beta-transducin repeat-containing Protein

2.1 Overall Architecture of CRLs

2.1.1 Introduction

CRLs are modular E3 ligases, which utilize interchangeable substrate receptors to recruit a variety of substrates onto a common catalytic scaffold (Fig. 2.1a). All CRLs contain a scaffolding cullin protein, namely, CUL1, CUL2, CUL3, CUL4A, CUL4B, or CUL5. Most of these cullin proteins bind a specific adaptor polypeptide through their N-terminal regions, which helps engage interchangeable substrate receptors to dock their cognate substrates to the E3. The ubiquitination reaction involves the transfer of ubiquitin from an E2 ubiquitin-conjugating enzyme to the

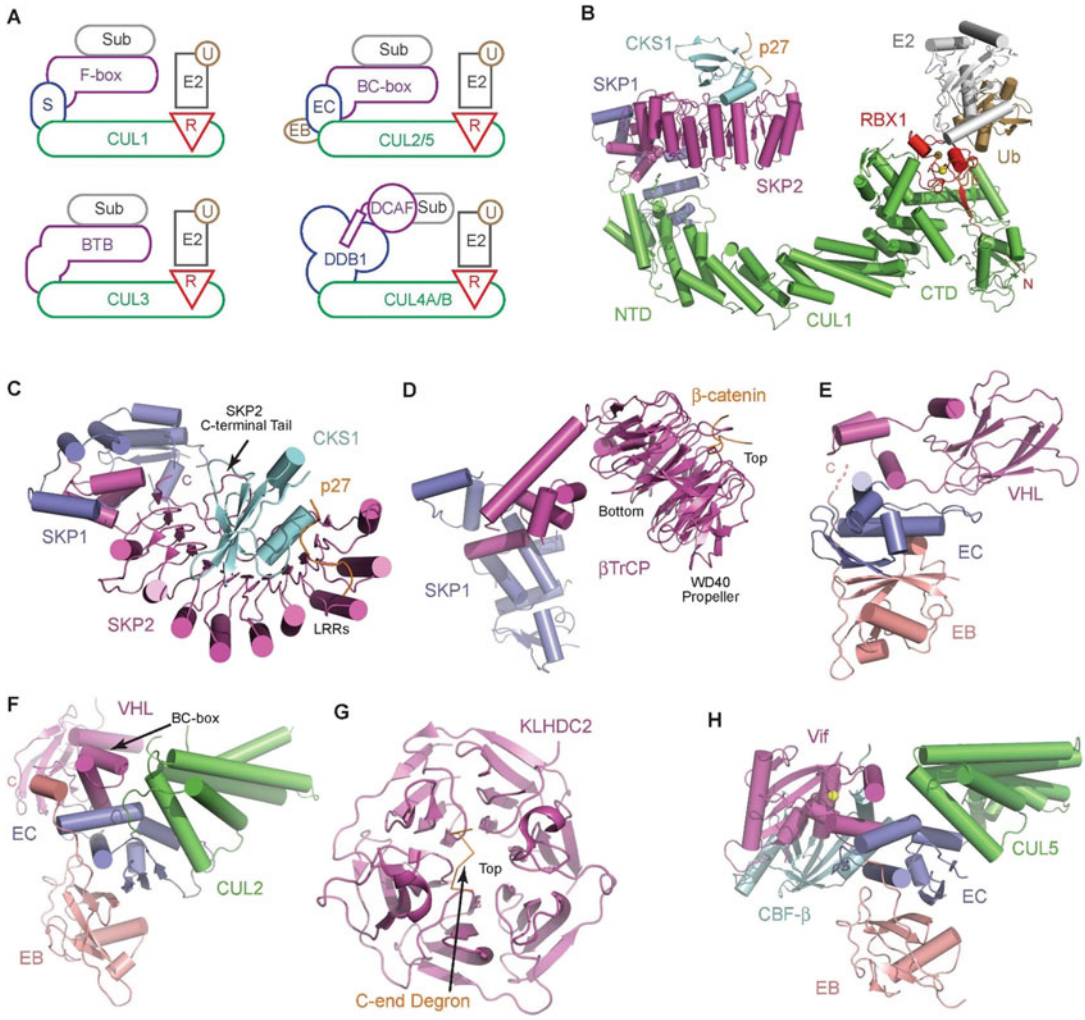

Fig. 2.1 Overall architecture of CRLs and structural components of CRL1 and CRL2/5. (**a**) Schematic drawing of all CRLs. S, SKP1; R, RBX1; EB, Elongin B; EC, Elongin C; U, ubiquitin; Sub, substrate. (**b**) A model of CRL1(SCF)$^{SKP2\text{-}CKS1}$ in complex with substrate p27 and a ubiquitin-charged E2. (**c**) An orthogonal view of the SKP1-SKP2-CKS1-p27 complex (PDB: 1LDK, 2AST). (**d**) Crystal structure of the SKP1 in complex with the WD40-repeat domain containing F-box protein, β-TrCP, which interacts with the degron of the substrate protein, β-catenin (PDB: 1P22). (**e**) Crystal structure of the BC-box protein, VHL, in complex with Elongin B and Elongin C (PDB: 1VCB). (**f**) A different view of VHL-EB-EC complex bound to CUL2 with the stabilized C-terminus of EB labeled with "C" (PDB: 4WQO). (**g**) The substrate-binding kelch repeat domain of the BC-box protein KLHDC2 (PDB: 6DO3). (**h**) A crystal structure of the HIV accessory protein Vif in complex with EB, EC, CUL5, and a cellular factor, CBF-β (PDB: 4N9F)

substrate. The cullin scaffold facilitates this process by using its C-terminus to house the catalytic subunit, RBX1 or RBX2, which directly interacts with the ubiquitin-conjugated E2 enzyme.

The first glimpse of the architecture of a CRL complex came from the X-ray crystal structure of human CUL1-RBX1 (Fig. 2.1b) (Zheng et al. 2002b). In the structure, CUL1 adopts a highly elongated shape with two functional domains. The stalk-like N-terminal domain of CUL1 is made of helical repeats that interact with the CRL1 adaptor protein, SKP1. The C-terminal domain of the scaffold has a more globular fold, which harbors RBX1. The N-terminal and

C-terminal domains are coupled through a hydrophobic interface, which structurally affixes the two functional portions of the E3 machinery. Mutational work that introduced flexibility to CUL1 abolished its ability to promote substrate ubiquitination, but not substrate binding, reinforcing the idea that its rigidity is necessary for its E3 activity. The inflexible CUL1-RBX1 ubiquitin ligase scaffold spans more than 100 Å, allowing the E3 to accommodate substrates of various shapes and sizes. While this distance is beneficial for the docking and ubiquitination of large substrate proteins, it seems to also present a challenge for small substrates to be ubiquitinated by the E2 that is tens of ångström away, unless flexibility is introduced somewhere (see below).

The globular domain of CUL1 not only fosters RBX1, but also intercalates the catalytic subunit. RBX1 is a RING-type zinc finger, which consists of a N-terminal β-strand and a C-terminal core domain that coordinates three zinc ions. The RBX1 β-strand inserts itself between five β-strands of CUL1's globular domain to create a stable intermolecular β-sheet. At the same time, the RING domain of RBX1 is hosted by the rest of CUL1 C-terminal region through a seemingly loose interface. Overall, the two proteins appear to exist in a "symbiotic" relationship, where they stay together throughout their life cycles. This bimodal interaction between CUL1 and RBX1, which involves an anchored end and a relaxed interface, allows for the RING domain of RBX1 to pop out upon the post-translational modification (PTM) of CUL1 by NEDD8, a ubiquitin-like molecule. Such an intermolecular topological change provides flexibility to CRL1 that helps reposition the ubiquitin-conjugated E2 to potentially approach the substrate. Further discussion about the regulation of CRLs by neddylation will be in Sect. 2.3.1.

CUL1 uses two α-helices to bind SKP1. The majority of the residues clustered at this interface are strictly conserved between CUL1 orthologues. Interestingly, these residues are not conserved across CUL1 paralogues, i.e., CUL1–CUL5. But they are conserved within orthologues of each cullin family member (Zheng et al. 2002b), suggesting that the binding mode between CUL1 and SKP1 is not unique to CRL1, but common to all cullins and their adaptors. This has been confirmed by follow-up structural studies of other CRLs. Sharing sequence homology with SKP1, Elongin C (EC) serves as the adaptor for CUL2 and CUL5 together with Elongin B (EB) to recruit BC-box substrate receptors. Despite the lack of sequence homology with SKP1, the CUL3 adaptors/substrate receptors, BTB proteins, share structure homology with SKP1 within their BTB domains. On one hand, it is not unexpected that the same two N-terminal helices of CUL1, CUL2, and CUL3 are used for the recruitment of their adaptor proteins. On the other hand, it is surprising that CUL4 uses the exact same region as CUL1–CUL3 to interact with its adaptor, DDB1, which is neither structurally nor sequence-wise similar to SKP1, Elongin C, or the BTB domain.

2.1.2 CRL1 Adaptor and Substrate Receptors

CRL1, the prototype of CRL E3s, is also named as SCF, which stands for SKP1, CUL1, and F-box proteins. The substrate recruitment function of CRL1 is conducted by a family of proteins, which share an N-terminal ~40 residues F-box motif that constitutively interacts with SKP1 (Skowyra et al. 1997). These F-box proteins feature characteristic C-terminal protein-protein interaction domains, which are responsible for binding specific substrates. Based on their predicted tertiary structures, the 69 human F-box proteins have been classified in three groups: FBXL for leucine-rich repeat (LRR)-containing proteins, FBXW for the ones containing WD40 repeats, and FBXO for F-box protein with other folds (Jin et al. 2004). The diverse protein-protein interaction domains used by F-box proteins enable them to recognize different substrates with high specificity and optimally orient these substrates to receive ubiquitin from the RBX1-bound E2 enzyme. In most, if not all, structures of F-box proteins in complex with SKP1, the N-terminal domain of the substrate receptors appears to be structurally coupled to

the SKP1-F-box module, which displays little structural variation. Together with the stable SKP1-CUL1 interface, the entire CUL1-SKP1-F-box protein complex has been postulated to play a role in spatially positioning the bound substrate relative to the catalytic end of the E3 platform (Zheng et al. 2002b).

The first structure of an F-box protein, SKP2, was determined almost 20 years ago, which revealed the binding mode of the F-box motif to SKP1 (Fig. 2.1b, c) (Schulman et al. 2000). $CRL1^{SKP2}$ is a key regulator of mammalian cell cycle progression. The overall structure of the SKP1-SKP2 complex resembles a sickle. The handle of the sickle is made of the SKP1-F-box structural module, while the variable linker and leucine-rich repeats of SKP2 constitute the blade. SKP1 and the F-box motif of SKP2 interact via an extensive interdigitated interface that consists of four alternating layers from each protein. Although part of the interface is mediated by residues that are not strictly conserved among all F-box motifs, it is generally believed, and subsequently validated, that all F-box proteins engage SKP1 in a similar binding mode.

The C-terminal domain of SKP2 is composed of ten LRRs, each of which is made of an α-helix and a β-strand. These LRRs pack in tandem and give rise to an arc-shaped structure, which is characterized by a concave side formed by parallel β-strands and a convex surface presented by α-helices. In many known non-E3 LRR proteins, the concave surface is involved in protein-protein interaction. The FBXLs, including SKP2, are no exception. Remarkably, the FBXL subfamily of F-box proteins have evolved different numbers of LRRs, which give rise to curved structures with different diameters, arc lengths, and pitches. These features presumably allow the FBXLs to hold diverse substrates. Interestingly, SKP2 also features a long C-terminal tail, which wraps back into the concave surface of the LRR domain and provides an additional structural element for substrate recruitment.

Unlike many F-box proteins, which directly recruit CRL1 substrates, SKP2 requires yet another adaptor protein, CKS1, to bind and ubiquitinate its substrate $p27^{Kip1}$. The crystal structure of the SKP1-SKP2-CKS1-p27 complex shows that CKS1 is anchored to the concave surface of SKP2 LRR domain and is supported underneath by the SKP2 C-terminal tail (Fig. 2.1c) (Hao et al. 2005). The residues that are involved in the CKS1-SKP2 interaction are conserved in animal orthologues on both sides of the interface, underlying the functional importance of CKS1. Furthermore, CKS1 has been found to form a stable complex with CDK2-cyclin A, which might contribute to the binding of $p27^{Kip1}$ to the CRL1 machinery.

The crystal structure of SKP1-β-TrCP offered the first sight of an FBXW-type F-box protein, which plays a major role in the Wnt signaling pathway by catalyzing the polyubiquitination and degradation of β-catenin (Fig. 2.1d) (Wu et al. 2003). The SKP1-β-TrCP complex adopts a bi-lobal structure, displaying the substrate at the opposite side of SKP1. The F-box motif of β-TrCP interacts with SKP1 in a similar fashion as SKP2. The substrate-binding function of the F-box protein is performed by its C-terminal WD40-repeat domain, whose name is derived from the ~40 amino acids sequence repeat that contains structurally essential tryptophan (W) and aspartic acid (D) amino acids. WD40 repeats are known to fold into a β-propeller structure, which is usually made of seven β-sheets arranged in a circular manner around a central narrow channel. In β-TrCP and other FBXW proteins with known structures, the amino acids located on the "top" surface are involved in substrate recognition. Through a rigid linker helix connecting to the SKP1-F-box module, β-TrCP is thought to position its substrate toward the E2 for ubiquitin transfer.

2.1.3 CRL2 and CRL5

CRL2 is organized in a similar manner as CRL1, with CUL2 serving as the scaffolding protein, EB-EC assisting as adaptor proteins, and members from the BC-box family of proteins acting as substrate receptors (Mahrour et al. 2008). CUL2 shares 38% of sequence identity with CUL1.

As predicted by homology models and subsequently confirmed by crystal structure, CUL2 adopts a similar elongated structure as CUL1 (Cardote et al. 2017). As the adaptor protein of CUL2, EC shares about 30% sequence identity with SKP1 and has a BTB core fold. Unlike SKP1, EC does not act alone to bridge the substrate receptor BC-box proteins to CUL2 and mandates its association with the ubiquitin-like molecule EB. It is not clear yet if EB with the ubiquitin-like fold plays any particular role in CRL2 besides stabilizing EC. In comparison with the F-box proteins, the BC-box proteins contain structural elements that do not only interact with the adaptor proteins, but also make direct contacts with their cognate cullin scaffolds. Besides a short BC-box motif, some BC-box proteins feature a CUL2-box motif that specifically recognizes CUL2. Interestingly, these two motifs are always consecutively localized in the BC-box protein sequences but could be found at different positions relative to the substrate-binding domain in the polypeptide. The best studied member from this family of proteins is the VHL tumor suppressor protein that promotes the ubiquitination of HIF1α under normoxia (Kaelin 2005). When its substrate-binding domain or BC-box motif is mutated, VHL fails to target HIF1α for ubiquitination and degradation, which leads to von Hippel-Lindau disorder, a hereditary predisposition to develop tumors in a variety of organs.

The structure of VHL-EB-EC unveils the portion of VHL that is involved in substrate recognition as a beta-sandwich (Fig. 2.1e) (Stebbins et al. 1999). The BC-box and CUL2-binding motifs of VHL together form a three α-helices structural module that is very similar to the structure of the F-box motif. Moreover, EC can be superimposed almost perfectly on part of SKP1. As predicted, the other adaptor protein, EB, adopts a ubiquitin-like α-/β-roll structure. In this crystal structure, the C-terminal tail of EB interacts with EC and points toward VHL with the last 20 amino acids disordered. Snapshots of VHL-EB-EC in complex with the first N-terminal repeat of CUL2 or the full-length protein reveal that the C-terminal tail of EB adopts an ordered structure interacting with VHL (Nguyen et al. 2015; Cardote et al. 2017). In addition, an internal loop of EC that is disordered in the VHL-EB-EC complex becomes structured upon its interaction with CUL2 (Fig. 2.1f). Remarkably, the CUL2-box of VHL makes polar interactions with part of the cullin scaffold, which hints at how different BC-box proteins could differentiate between CUL2 and CUL5, even if they use the same adaptor proteins.

A myriad of BC-box proteins with various folds have been identified by biochemical and bioinformatical approaches (Mahrour et al. 2008). A variety of substrate-binding domains have been mapped for the ones that bind CUL2, such as leucine-rich repeats, ankyrin repeats, tetratricopeptide repeats, armadillo repeats, kelch repeats, or SWIM zinc fingers. Recently, the structure of a kelch repeat BC-box protein, KLHDC2, has been determined (Fig. 2.1g) (Rusnac et al. 2018). It adopts a β-propeller fold composed of six β-sheets that are arranged in a circular fashion around a central axis. As observed for most kelch repeat domains, the "top" surface of KLHDC2 sports long variable loops that give rise to a deep binding pocket, which recognizes the degron of its substrates. The "bottom" part of the repeat is predicted to neighbor the BC-box and CUL2-box motifs, which have been removed for ease of crystallization.

Similar to CUL2, CUL5 employs the same adaptor proteins EB and EC to associate with a distinct subfamily of the BC-box proteins (Mahrour et al. 2008). The substrate receptors that bind CUL5 can have one of the following folds: Src homology 2 phosphotyrosine-binding domain, ankyrin repeats, SP1a and ryanodine receptor domain, WD40 repeats, Rab-like GTPase domain, protein-L-isoaspartate carboxymethyltransferase, and transcription factor SII-like domain. Unexpectedly, not only cellular BC-box proteins have been found to bind CUL5-EB-EC. A very interesting study has demonstrated that HIV-1 virion is able to hijack CRL5 via an accessory protein, Vif, to promote the degradation of APOBEC3, the host restriction factor that blocks the replication of the virus (Yu et al. 2003). Recent studies showed that this

function of Vif entails yet another host protein, the transcription factor CBF-β that plays a role in APOBEC3 expression (Kim et al. 2013). The structure of the Vif-CBF-β-$CUL5_N$-EB-EC complex reveals an overall U shape, with Vif-CBF-β and $CUL5_N$ making up the arms and EB-EC sticking out at the bottom (Fig. 2.1h) (Guo et al. 2014). Akin to cellular BC-box proteins, Vif uses its BC-box and CUL5-box to bridge EC and CUL5, mimicking the positioning of SKP1 and SKP2 in the context of CRL1. This structure provides further evidence supporting the notion that there is a conserved assembly mode for most CRLs.

2.1.4 CRL3

CUL1, CUL2, CUL4, and CUL5 all use adaptor proteins that recruit specific substrate receptors. CUL3, on the other hand, recruits BTB domain-containing proteins that combine the adaptor and substrate receptor functions into a single polypeptide (Pintard et al. 2003; Xu et al. 2003; Geyer et al. 2003). Without sharing sequence homology to SKP1 or EC, the BTB domain adopts a structural fold analogous to these two CRL adaptors and anchors to CUL3 in a similar manner. In addition, the BTB domain is known to dimerize, which facilitates the formation of homodimeric CRL3 complexes with two copies of each components of the E3 machinery. This property is reminiscent of select F-box proteins, such as FBXW7 and β-TrCP, which also contain a dimerization domain on the N-terminal side of the F-box motif. Similar to the CRL1 and CRL2/5 substrate receptors, the BTB family of proteins features different protein-protein interaction domains to recruit substrates. These include, but are not limited to, the well-characterized MATH and kelch repeats domains that are present in SPOP and KEAP1, respectively.

KEAP1 is one of the best studied CUL3 substrate-specific adaptors, which controls the degradation of NRF2 transcription factor involved in oxidative stress response pathway (Yamamoto et al. 2018). KEAP1 consists of four functional domains: BTB, intervening region (IVR), double glycine repeats (DGR, aka kelch repeats), and C-terminal region (CTR). While the DGR and CTR give rise to the kelch repeat β-propeller, the IVR domain contains two reactive cysteine residues that can be modified by toxic electrophiles and subsequently alter the spatial configuration of other domains of the BTB protein. Despite extensive studies, it is still unclear how these chemical-induced structural changes affect the overall architecture of the KEAP1 dimer, thereby affecting NRF2 polyubiquitination and degradation.

SPOP is a BTB-domain protein that is frequently mutated in human cancer. The dimerization and substrate-binding mode of SPOP have been determined in its near full-length form (Fig. 2.2a) (Zhuang et al. 2009). The MATH domain of SPOP forms an antiparallel β-sandwich, with a central shallow grove that is used by substrates to dock. Dimerization of the CRL3 substrate receptor takes place through a hydrophobic interface between the BTB domains. Mutational analysis indicates that BTB domain dimerization is not necessary for CUL3 binding, which is consistent with its spatial separation from the BTB-CUL3 interface (Fig. 2.2b). Nonetheless, defects in BTB dimerization negatively impact the polyubiquitination of the SPOP substrates, suggesting that the dimeric architecture of the CRL3 complex is critical for productive substrate ubiquitination. Interestingly, the two MATH domains in the crystal structure are asymmetrically arranged with one MATH domain cradled by the grove between the BTB domains and the other one pushed away from its BTB. Based on different crystal structures obtained for the same complex, slight topological differences have been observed, suggesting that the linker between the two functional domains of SPOP confers structural flexibility to the E3 complex. This structural plasticity has not been previously observed for other CRL family members. The MATH domain of SPOP can recognize a host of substrate degron motifs. It is possible that MATH domain-containing BTB proteins, including SPOP, have evolved a mechanism of engaging substrates with high affinity by simultaneously recognizing two low-affinity degron motifs.

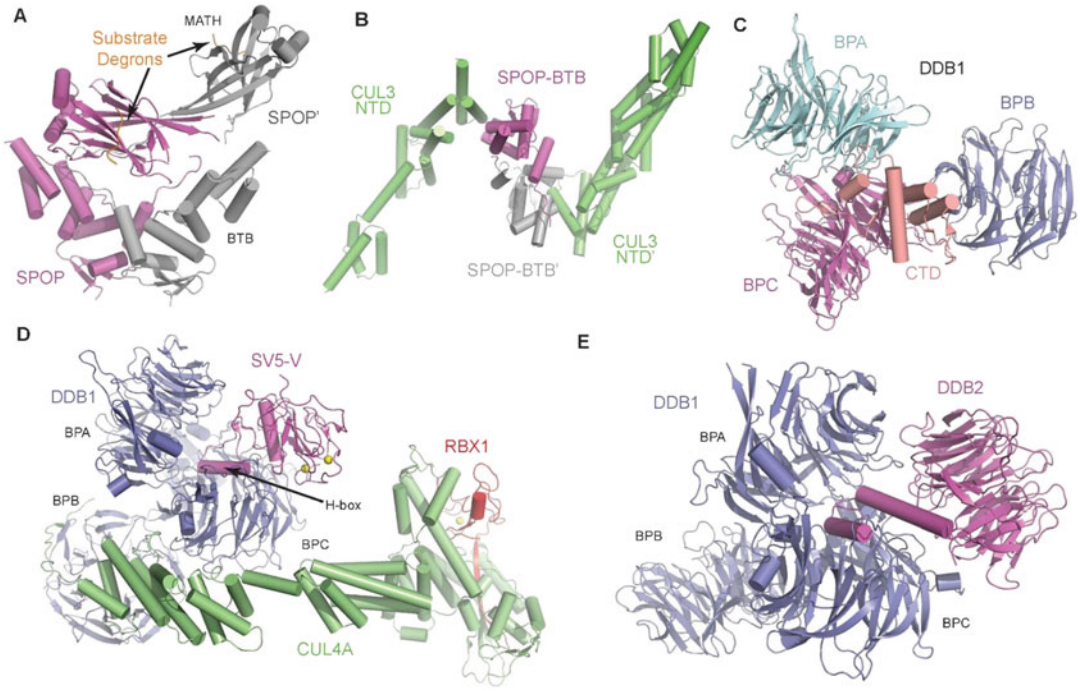

Fig. 2.2 Overall architecture and structural components of CRL3 and CRL4. (**a**) An asymmetric dimeric structure of the BTB-domain protein, SPOP, in complex with two substrate degron peptides (PDB: 3HQI). (**b**) The dimeric SPOP BTB domain in complex with CUL3-NTD (PDB: 4EOZ). (**c**) Domain architecture of DDB1 with three propellers (BPA, BPB, and BPC) and a helical CTD (PDB: 2B5M). (**d**) Complex structure of CUL4A-RBX1-DDB1-SV5-V. The H-box motif of SV5-V responsible for binding DDB1 is labeled (2HYE). (**e**) Crystal structure of DDB1 in complex with the DCAF protein, DDB2 (PDB: 3EI3)

Although the BTB domains of KEAP1 and SPOP are responsible for CUL3 binding, not all BTB domain-containing proteins function as CRL3 substrate adaptors. An interesting finding from the SPOP structure is a pair of C-terminal helices that have structural equivalence in the F-box or CUL2-box. Because the function of the BTB domain was thought to bridge CUL3 and the MATH domain of SPOP, it seemed unnecessary to have such a vestigial element. Surprisingly, this helix pair of the SPOP BTB domain has been proven to be crucial for CUL3 interaction. Because this structural element, named 3-box, is found in some, but not all, BTB proteins, it could be the structural determinant that allows a subset of BTB proteins to function as CRL3 substrate adaptors.

2.1.5 CRL4

While most CRL adaptors adopt a BTB or BTB-like fold, CRL4 employs a 127 kDa protein, DDB1, to dock substrate receptors. DDB1 is a multi-domain protein, made of three β-propellers, labeled as BPA, BPB, and BPC, and a C-terminal helical domain (CTD) (Fig. 2.2c) (Li et al. 2006). Remarkably, the three β-propellers are not folded in a linear fashion within the polypeptide. Instead, two of the β-propellers, BPA and BPB, are inserted into two internal loops of BPC. Together, the three propellers adopt a compact tri-star structure with the CTD housed in the middle. The BPB propeller binds the N-terminal domain (NTD) of CUL4 via two interfaces, one resembling SKP1 binding to CUL1 and the other involving an

N-terminal conserved sequence of CUL4 cradling DDB1 (Fig. 2.2d) (Angers et al. 2006). The BPA and BPC propellers, on the other hand, pack against each other to create an open clam-shaped structure, which is responsible for holding CRL4 substrate receptors.

To date, a number of DDB1 structures have been documented. Strikingly, the linker between the BPB domain and the BPA-BPC double propeller appears to have a large degree of plasticity, which enables the two functional modules of DDB1 to adopt different orientations relative to each other (Fig. 2.2d, e). This feature might allow CRL4 to accommodate and polyubiquitinate substrates of different shapes and sizes. In addition, the structural flexibility of DDB1 enables CUL4 to rotate up to 150° around the substrate receptor, thereby creating a ubiquitination zone that could help detect various lysines on a substrate and promote their ubiquitination. The precise role of the structural flexibility within CRL4 remains to be elucidated.

Similar to CRL5, CRL4 is also known to be hijacked by viruses. The SV5-V protein encoded by paramyxovirus has been shown to functionally mimic the CRL4 substrate receptors to mediate the polyubiquitination and degradation of the otherwise stable STAT proteins in the interferon pathway (Horvath 2004). SV5-V anchors itself to DDB1 by inserting its N-terminal helix into the opening of the BPA-BPC double propeller, interacting predominantly with the "top" surface of BPC (Fig. 2.2d) (Li et al. 2006). The C-terminal region of SV5-V folds into unique globular structure featuring a bowl-shaped depression with many conserved hydrophobic and nonpolar residues. This surface region of the viral protein is critical for the recruitment of STATs. Perhaps due to its rich structural features, DDB1 appears to be a frequent target for viral hijacking. Besides SV5-V, hepatitis B virus X protein (HBx) and woodchuck hepatitis virus X protein (WHx) have also been reported to reprogram the CRL4 adaptor to degrade host factors (Decorsière et al. 2016). Peptide motifs from these proteins have been mapped and crystalized in complex with DDB1. Despite their divergent sequences, these motifs form a common three-turn α-helix (termed H-box) that anchors to the "top" surface of BPC in a similar fashion as the SV5-V N-terminal helix (Fig. 2.2d) (Li et al. 2010).

The structural insights obtained from the DDB1-viral hijacker complexes prompted multiple proteomics studies that were aimed at identifying possible cellular CRL4 substrate receptors (Angers et al. 2006; Jin et al. 2006). Multiple DDB1-CUL4A-associated factors (DCAFs), most of which contain a WD40-repeat domain, have been classified as subunits of CRL4 E3 complexes for recruiting substrates. Interestingly, DDB2, which was originally identified together with DDB1 as a UV-damaged DNA-binding protein, anchors to DDB1, like a canonical DCAF protein, but functions to recognize DNA abduct in the nuclear excision repair pathway (Fig. 2.2e) (Fischer et al. 2011). With the structural knowledge gathered from the DDB1-hijacking viral proteins, the H-box motif has been found in a number of DCAFs and validated by crystallography. A natural question arises as to whether the H-box motif exists in other DCAFs. However, the lack of an obvious consensus sequence for the H-box motif has made it challenging to find the answer to this question.

Distinct from most DCAFs, cereblon (CRBN) does not contain a WD40-repeat domain. Instead, it consists of a seven-stranded β-sheet NTD, a bundle domain composed of seven α-helices (HBD), and an eight-stranded β-sheet CTD (Petzold et al. 2016; Matyskiela et al. 2016). Unlike DCAFs, CRBN uses its HBD with its α-helices to bind DDB1 in the cavity between BPA and BPC. Its CTD domain is involved in the recruitment of native substrates like MEIS2 via a conserved binding pocket. Among all DCAFs, CRBN stands out by being the target of thalidomide, which has a notorious history in biomedicine, but is now repurposed to treat multiple myeloma (see Sect. 2.2.4).

The combination of structural, biochemical, and proteomic approaches has helped delineate the composition and architecture of the CRL E3 superfamily in great details. Although there might be outliers unknown to us, most CRL complexes are expected to assemble following the structural

principles described above. In contrast to the common architecture shared among different CRL E3s, the mechanisms by which these ubiquitin ligases recognize their specific substrates in response to different cellular cues are incredibly diverse.

2.2 Substrate Recognition by CRLs

To achieve spatially and temporally controlled degradation, a substrate needs to be recognized by its cognate E3 with high specificity. This interaction is often mediated by a short linear sequence motif, termed degron, present on the substrate. It has been challenging to identify substrates for different E3 ligases and to fully understand how their recognition is regulated. To date, some substrates have been found to have unmodified degrons that are recognized by the E3 either directly or with the help of a small molecule, while others utilize post-translationally modified degrons to engage their E3 ligases. In some emerging cases, the whole globular domain of a substrate protein contributes to the specific interaction. It is expected that more regulatory mechanisms will be revealed in the future.

2.2.1 PTM-Dependent Substrate Degron Recognition

Protein phosphorylation is one of the most common forms of post-translational modification and is involved in regulating essentially all cellular processes. Many degrons are under the control of this PTM. One classic example is the cell cycle regulatory protein, cyclin E, which has not just one, but two phosphorylated degrons. These phospho-degrons are specifically recognized by FBXW7, a substrate receptor for CRL1 that is responsible for the degradation of cyclin E (Welcker and Clurman 2008). The FBXW7 F-box protein contains a D-box dimerization domain N-terminal to its F-box motif and a C-terminal eight β-sheets WD40 propeller. The FBXW7 D-box promotes the dimerization of the F-box protein, which enables simultaneous binding of two different phosphorylated degrons that independently dock to the "top" surface of the β-propeller in an extended conformation (Fig. 2.3a).

The two cyclin E degrons share similarities but also differ in certain aspects. Both phosphorylated degrons have been co-crystalized with FBXW7 (Hao et al. 2007). The degron located at the C-terminus of cyclin E has three phosphorylated residues and is characterized as a strong degron due to its nanomolar affinity to FBXW7. This tight interaction can be easily explained by the numerous contacts that the degron makes with the substrate receptor, including both polar interactions and van der Waals packings. It is notable that two out of the three phosphate groups present on the degron are recognized by three arginine residues that are strictly conserved among FBXW7 orthologues. By contrast, the N-terminal degron of cyclin E features only one phosphorylated threonine residue and makes fewer interactions with FBXW7, which leads to weaker binding. Interestingly, the three conserved FBXW7 arginine residues are frequently mutated in human cancers, rendering the E3 incapable of promoting the degradation of cyclin E and possibly other substrates. Despite the well-elucidated mechanism of the FBXW7-cyclin E interaction, the necessity of having two degrons on cyclin E for its productive degradation remains unclear. Cell-based study suggests that the strong degron alone is sufficient for substrate degradation (Welcker and Clurman 2008).

Proline hydroxylation is another well-known, albeit less common, form of PTM that is involved in degron regulation. Hypoxia-inducible factor 1-alpha (HIF1α) contains a critical proline in its oxygen-dependent degradation domain (ODD) that gets hydroxylated in the presence of oxygen (Ivan et al. 2001). This normoxia-associated modification allows VHL, a BC-box protein, to bind, ubiquitinate, and target HIF1α for degradation. Under low oxygen conditions, HIF1α is spared from degradation and functions as a transcription factor activating angiogenic gene expression. The crystal structure of VHL-EB-EC in complex with a partial ODD peptide from HIF1α elucidated how the post-translational modification dictates

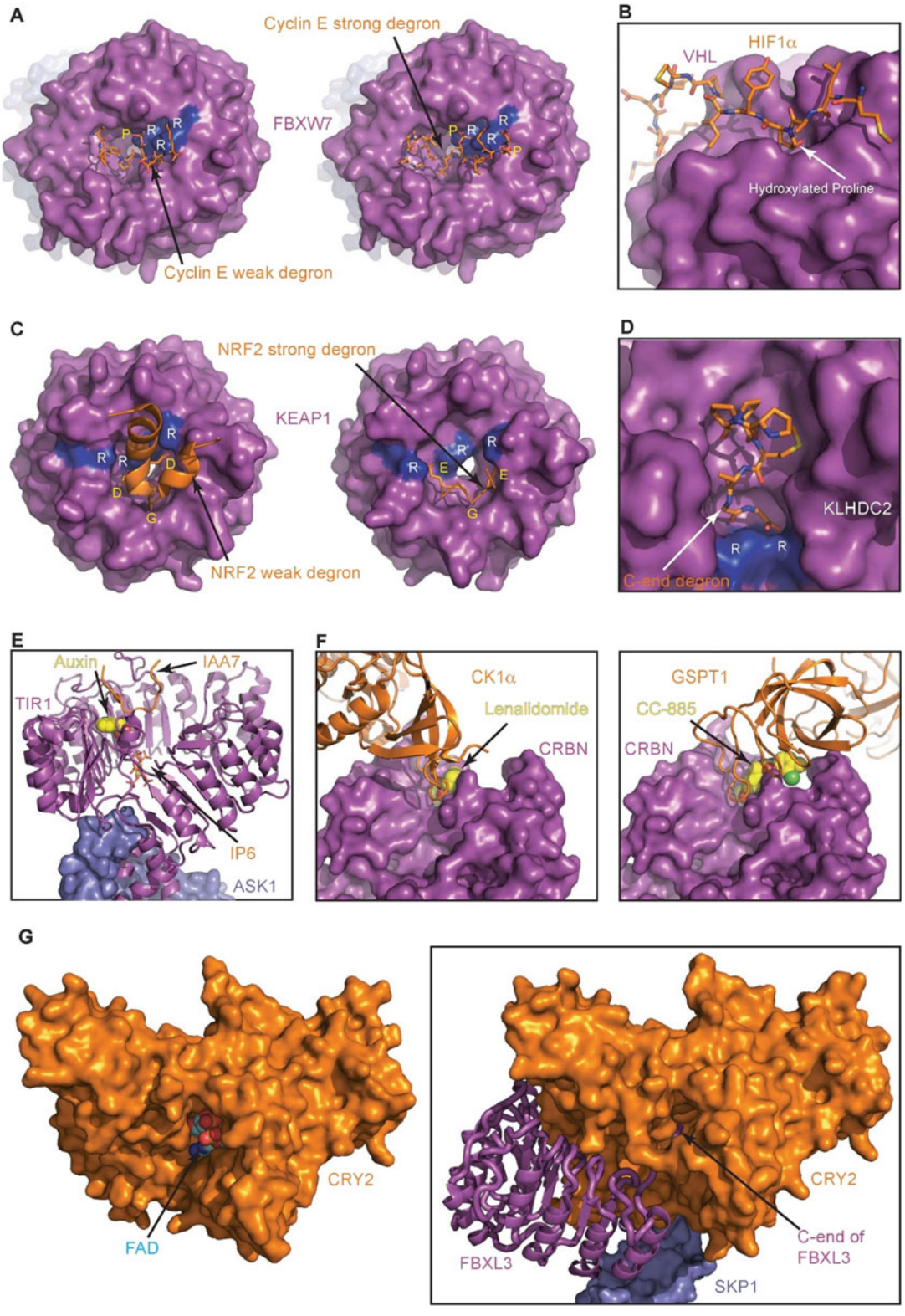

Fig. 2.3 Structural mechanisms of substrate-CRL interactions. (**a**) Binding modes of the strong and weak degrons of cyclin E to FBXW7. R, Arg; P, phosphate. Phosphate-binding arginine residues of FBXW7 are

interaction (Fig. 2.3b) (Min et al. 2002). The elongated HIF1α peptide adopts a β-strand-like conformation and interacts with the β-domain of VHL in a bipartite manner. Within the N-terminal segment of the HIF1α peptide, the hydroxyproline is deeply embedded at the interface, forming multiple van der Waals contacts and hydrogen bonds with highly conserved residues of the BC-box protein. This interaction is substantiated by the backbones of the amino acids neighboring the proline, which interact with VHL via additional hydrogen bonds. At the C-terminal segment, there are a few interactions that do not seem essential for binding to VHL. The importance of hydroxyproline interface is highlighted by the clustering of cancer inducing mutations involved in von Hippel-Lindau disorder around this site.

2.2.2 Native Substrate Degron Recognition

Although many degrons are post-translationally modified, substrate-E3 interaction can be regulated not only on the substrate side but also on the E3 side. Regulation on the E3 side is best exemplified by KEAP1, a CUL3 substrate receptor (Yamamoto et al. 2018). Under normal cellular conditions, KEAP1 seizes the native transcription factor NRF2 in the cytoplasm and mediates its constitutive ubiquitination and degradation without PTM. When the cell goes through oxidative stress, two reactive cysteines on the IVR domain of KEAP1 are modified, which alters the topological configuration of the substrate receptor domains. The resulting E3 is inactivated and can no longer bind and ubiquitinate NRF2. This allows the transcription factor to translocate into the nucleus and upregulate the expression of cytoprotective genes in response to oxidative insults.

The proper positioning of the two kelch repeat domains from the KEAP1 dimer is essential for productive binding of NRF2, which holds two degron motifs, one with a low affinity and the other with a high affinity to the E3. The structure of the KEAP1 kelch repeat domain has been determined in complexes with both degrons (Fig. 2.3c) (Padmanabhan et al. 2006; Fukutomi et al. 2014). As expected, the two degrons bind to the same "top" surface of KEAP1 kelch repeat propeller, although they seem to adopt different conformations. The weak degron contains 35 amino acids and forms a three-helix structure, whereas the strong degron is only 9 amino acids long and folds into a β-hairpin upon binding to the E3. The only common feature shared by both degrons is a central glycine residue preceded by a negatively charged amino acid. These two residues are located at the tip of both degron structures, anchoring themselves deep into the substrate-binding pocket of the E3. Importantly, KEAP1 employs multiple conserved arginine residues to stabilize the two degrons, two of which are dedicated to interact with the negatively charged amino acid preceding the central glycine residue.

Multiple cancer-related loss-of-function mutations have been found in NRF2, many of which are localized within the low-affinity degron. These mutations correlate with either disruption of the three-helix structure or steric hindrance introduced by a bulky side chain. It is still

Fig. 2.3 (continued) colored in blue (PDB: 2OVR, 2OVQ). (**b**) Recognition of HIF1α degron with a hydroxylated proline by VHL (PDB: 1LM8). (**c**) Binding modes of the strong and weak degrons of NRF2 to KEAP1. R, Arg; D, Asp; E, Glu; G, Gly. Arginine residues of KEAP1 responsible for binding negatively charged NRF2 degron residues are colored in blue (PDB: 3WN7, 1X2R). (**d**) Recognition of SELK C-end degron by KLHDC2. R: Arg (PDB: 6DO3). (**e**) Auxin-facilitated IAA7 degron recognition by TIR1. Inositol hexakisphosphate (IP6) serves as a cofactor of the ubiquitin ligase-based receptor (PDB: 2P1Q). (**f**) The recognition of CK1α by CRBN is promoted by lenalidomide (PDB: 5FQD). Structure of CRBN in complex with CC-885 and substrate protein GSPT1 (PDB: 5HXB). (**g**) FAD binding to human CRY2 and the complex structure of SKP1-FBXL3-CRY2 (PDB: 4I6G, 4I6J)

unclear how the full-length NRF2 substrate interacts with KEAP1 dimer at the structural level. Depicting how NRF2 is positioned relative to the E3 and determining which lysine residues are ubiquitinated would clarify a significant mystery in the field.

Besides internal short linear motifs, a new class of unmodified degrons has recently been identified at the C-terminus of various early terminated proteins. These so-called C-end degrons feature key glycine, arginine, or a combination of both at critical positions (Lin et al. 2015, 2018; Koren et al. 2018). One such degron was found in the early terminated selenoproteins, SELK and SELS, as well as the N-terminal fragment of USP1, which cleaves itself at an internal site. The common characteristic of these three polypeptides is a degenerate C-terminal sequence that ends in a diglycine motif. Recent studies have revealed that this diglycine-containing C-end degron is specifically recognized by the BC-box protein KLHDC2. To understand the structural mechanism by which KLHDC2 recognizes such a simple degron, the crystal structure of the kelch repeat domain of the BC-box protein in complexes with the degron peptide of each of the three polypeptides has been determined (Fig. 2.3d) (Rusnac et al. 2018). The "top" surface of the KLHDC2 β-propeller engages the degrons that adopt a common helical fold. The C-terminus of the degrons inserts itself into a deep binding pocket, while the N-terminus is more solvent exposed. The extreme C-terminal carboxyl group of the degron is recognized by two strictly conserved arginine residues and a highly conserved serine residue. The penultimate glycine adopts a phi/psi angle that is in the generously allowed region of the Ramachandran plot, suggesting that only a glycine would be accommodated at this position. Strikingly, the remainder of the peptides interacts with KLHDC2 exclusively via its backbone. The lack of interactions between the E3 and the side chains of the degrons explains how these degenerate degradation motifs can be contained in the binding pocket in an identical manner. An unexpected finding from these studies is the single-digit nanomolar affinity of these seemingly simple C-end degrons, which is in stark contrast to the N-end degrons previously documented (Choi et al. 2010; Matta-Camacho et al. 2010; Chen et al. 2017; Dong et al. 2018). Such a tight interaction suggests that the substrates of KLHDC2 are either in low abundance or highly toxic to the cell.

2.2.3 Compound-Dependent Substrate Degron Recognition

Besides PTMs, cellular signals such as hormones and secondary metabolites can directly participate in degron recognition by E3 ligases. In the green kingdom of life, two plant hormones, auxin and jasmonate (JA), have been shown to serve as molecular glue bridging the CRL1 F-box proteins TIR1 and COI1 to their specific substrates, respectively (Shabek and Zheng 2014). These are the first known cases where E3s function in hormone perception and as a result regulate gene expression. The transcriptional regulation is achieved in the auxin and JA signaling pathways through the ubiquitination and degradation of AUX/IAA and JAZ transcription repressor proteins, respectively.

The crystal structure of the F-box protein, TIR1, has been determined in complex with ASK1, one of the plant homologues for SKP1 (Fig. 2.3e) (Tan et al. 2007). The complex adopts an overall mushroom-shaped structure, where the cap is composed of the TIR1 LRR domain and the stem contains the TIR1 F-box motif and ASK1. TIR1 folds into a twisted horseshoe-shaped solenoid and provides its top surface pocket for auxin-mediated degron binding. The crystal structure of TIR1 in complex with auxin and an AUX/IAA degron reveals that the hormone fills up a gap at the protein-protein interaction interface without inducing any detectable conformational changes of the F-box protein. Its unique mechanism of action helps raise the concept of molecular glue. By serendipity, an inositol hexakisphosphate (IP6) was discovered in the middle of the TIR1 LRR domain underneath the hormone-binding pocket. Its strategic location and its conserved binding site strongly argue for a cofactor role in

stabilizing hormone binding. Remarkably, JA-isoleucine, the active form of JA, acts through the same mechanism as auxin, mediating the interaction between the F-box protein COI1 and its substrate JAZ proteins (Sheard et al. 2010). Surprisingly, instead of IP6, COI1 uses a specific inositol pentakisphosphate as its cofactor, which is essential for its hormone-sensing function. The TIR1 and COI1 F-box proteins, therefore, are each regulated by two naturally occurring small molecules, a molecular glue hormonal signal and an inositol polyphosphate cofactor, which might function as a proxy signal for phosphate abundance (Wild et al. 2016).

Previously, we briefly mentioned CRBN as the target of the anticancer drug, thalidomide. Recent studies have shown that thalidomide and its derivatives, lenalidomide and pomalidomide, also work as molecular glues, rewiring CRBN to bind and degrade several clinically relevant substrates, including IKZF1, IKZF3, CK1α, and SALL4 (Krönke et al. 2014, 2015; Lu et al. 2014; Matyskiela et al. 2018; Donovan et al. 2018). These substrates do not normally interact with CRBN in the absence of the small molecules. Therefore, they have been referred to as neo-substrates. The structure of DDB-CRBN-lenalidomide-CK1α complex has elucidated the detailed mechanism of interaction among all the components (Fig. 2.3f). The small-molecule drug targets a tryptophan cage surface pocket of CRBN and is stabilized by a combination of hydrogen bonds and hydrophobic interactions. Upon binding to the E3, lenalidomide offers a novel binding surface for the neo-substrate to exploit. CK1α uses a β-hairpin loop to dock into the newly formed hydrophobic pocket, contacting both the drug and the E3 substrate receptor.

CC-885 is a lenalidomide derivative that reprograms CRBN to ubiquitinate yet another neo-substrate, GSPT1 (Matyskiela et al. 2016). This compound shares part of its structure with lenalidomide and interacts with CRBN in an almost identical manner (Fig. 2.3f). The additional moiety of CC-885 that differs from lenalidomide participates in the formation of additional hydrogen bonds with the E3 and presents a new hydrophobic interface and hydrogen bonding for GSPT1 docking. Despite adopting a completely different fold and packing against CRBN from a different direction, GSPT1 interacts with the compound-reshaped E3 pocket using a β-hairpin motif that is superimposable to the one found in CK1α. Based on structural and mutational analyses of the CRBN-substrate complexes, a key glycine residue within the β-hairpin shared among all neo-substrates has emerged. Although the target of thalidomide and its derivatives was elucidated post hoc, their mechanism of action closely resembles that of the naturally occurring plant hormones, auxin and JA. Together, these E3-reshaping molecular-glue small molecules inspire the discovery of novel compounds with therapeutic potentials through targeted protein degradation.

2.2.4 Globular Substrate Protein Recognition

Although degron-mediated substrate-E3 interaction has become a widely accepted dogma, an increasing number of studies have revealed an alternative strategy for certain CRL E3s to recognize their cognate substrates with high specificity. Perhaps the best example comes from the mammalian cryptochrome proteins, CRY1 and CRY2, which are central components of the circadian clock in mammals (Takahashi 2017). The mammalian circadian rhythm is an internal timing system that synchronizes physiological processes to the ~24 h solar day. In all mammalian cells, the circadian clock is driven by a transcription-translation negative feedback loop, in which the CRY1/2 and PERIOD proteins heterodimerize and suppress their own gene expression. Protein degradation plays an important role in oscillating the clock by periodically removing both proteins, thereby alleviating their inhibitory effects. While the PERIOD proteins are polyubiquitinated by $CRL1^{\beta\text{-}TrCP}$, which recognizes their phosphorylated degrons, the CRY1/2 protein is destabilized by $CRL1^{FBXL3}$ without an obvious degron (Shirogane et al. 2005; Busino et al. 2007; Siepka et al. 2007; Godinho et al. 2007). Similar to their orthologues in insects and plants,

mammalian CRY1/2 adopts a large globular fold with a deep binding pocket for flavin adenine dinucleotide (FAD) (Xing et al. 2013). The structure of the mammalian CRY2 in complex with FAD shows a partially solvent-exposed pocket, which differs from the closed pocket seen in its plant and insect orthologues (Fig. 2.3g). In the crystal structure of the CRY2-FBXL3-SKP1 complex, the LRR domain of FBXL3 adopts an expected arc-shaped structure, whose concave surface wraps around the CRY2 globular domain, burying many residues that are only spatially but not sequence-wise connected. A surprising and crucial element of the FBXL3-CRY2 interaction involves the C-terminal tail of the F-box protein, which inserts into the FAD-binding pocket of CRY2. This unexpected interface strongly suggests that FAD might be able to compete with the ubiquitin ligase and protect CRY2 from polyubiquitination. Moreover, the surface area of CRY2 involved in binding FBXL3 overlaps with PERIOD2-binding interface, indicating that the PERIOD proteins might also play an antagonistic role in keeping the E3 ligase in check (Nangle et al. 2013; Schmalen et al. 2014). Because the cellular circadian clock can be entrained by many signals, such as metabolism and hormones, the complex binding mode of FBXL3-CRY2 might have been evolved to allow the single substrate-E3 interacting pair to be regulated through multi-faceted mechanisms. As more substrate-E3 interactions are mechanistically interrogated, it is expected that a wider variety of regulatory and structural factors will be revealed beyond the simple degron.

2.3 Regulation of CRLs by NEDD8 Modification

As the central ubiquitin ligase machineries regulating diverse cellular pathways, CRLs rely on a multitude of substrate receptors to recognize and recruit their specific substrates. How do these interchangeable substrate receptor subunits share the common cullin scaffolds without interfering with each other's function? How is the ubiquitin ligase activity of the resulting E3 complexes modulated in the cell? Since CRLs were discovered, a battery of cullin-interacting proteins has been identified as important cellular factors that coordinate CRL complex assembly and control their ubiquitin ligase functions. The structural biology approach has not only helped establish the structural framework for investigating the regulation of CRL E3s but also revealed the detailed mechanisms for several key steps.

2.3.1 NEDD8-Modified CRLs

Many cellular enzymes catalyzing a form of post-translational modification are themselves subject to the same modification. For example, protein kinases are often activated by phosphorylation. CRL E3s follow this trend with a slight variation. All cullins can be modified by the ubiquitin-like molecule, NEDD8, at a specific lysine residue in their C-terminal WHB domain, which is close to the RBX1/2 binding site (Hori et al. 1999). This form of cullin modification, often referred to as neddylation, is conserved from fungi to humans and plays a role in stimulating the E3 activity of CRLs. Although cullin neddylation is not essential in budding yeast, it has been shown to alleviate the autoinhibition of CRLs through augmenting CRL-E2 interaction, closing the gap between the CRL-bound substrate and RBX1-bound E2, and promoting the amide bond formation at the E2 active site (Saha and Deshaies 2008; Yamoah et al. 2008).

A major breakthrough in our understanding of the effect of cullin neddylation came from the crystal structure of a NEDD8-modified CUL5-CTD-RBX1 complex (Fig. 2.4a, b) (Duda et al. 2008). Upon NEDD8 conjugation, the C-terminal portion of the CUL5-CTD undergoes a large degree of rotation, which reorients the WHB domain relative to the rest of the cullin scaffold. Because the WHB domain and its preceding long α-helix are responsible for cradling and stabilizing the globular RING domain of RBX1 in the unmodified form of cullin, this neddylation-induced conformational change releases the RING domain of the catalytic subunit from the cullin CTD. Due to the stable intermolecular

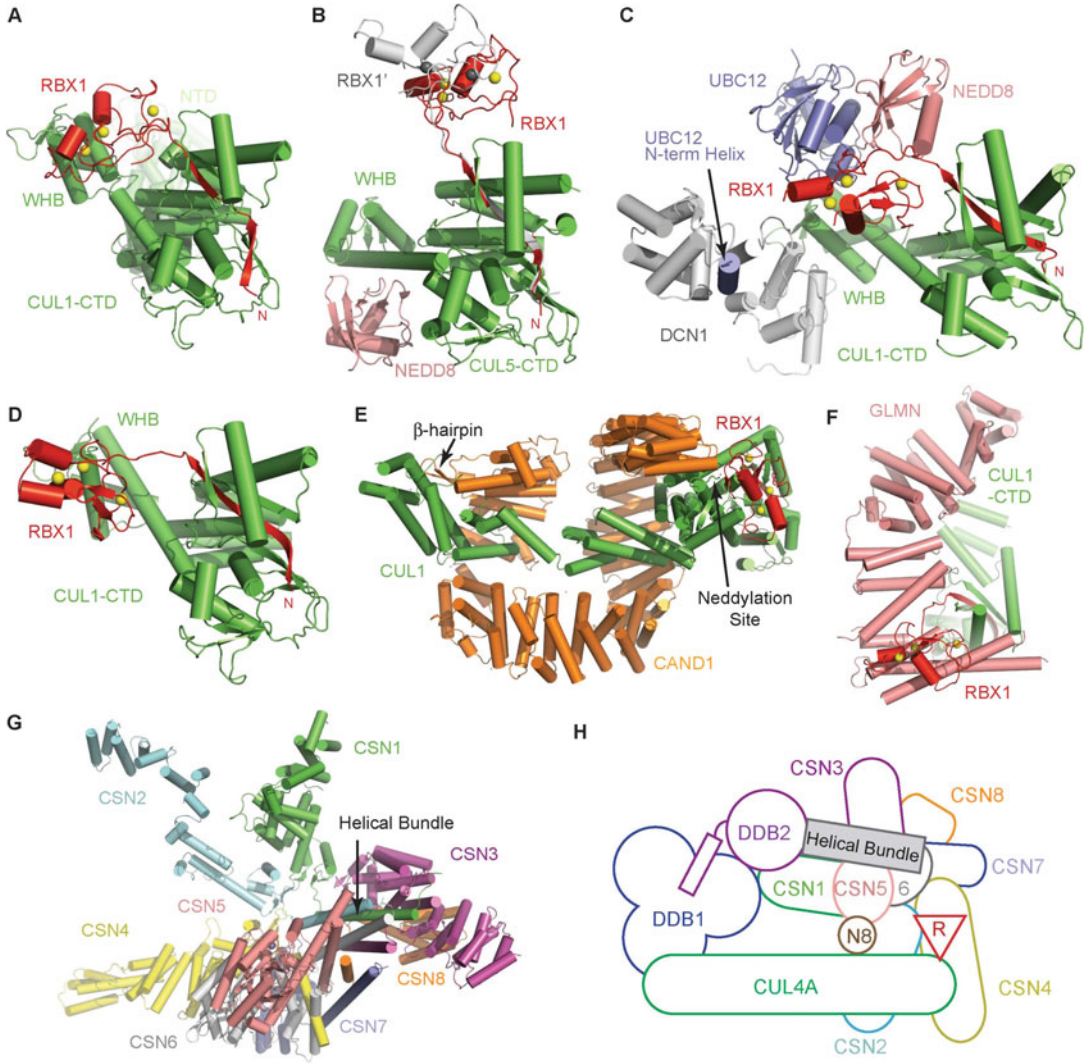

Fig. 2.4 Regulatory mechanisms of CRL assembly and function. (**a**) Interaction between CUL1-CTD and RBX1 viewed 90 degrees from Fig. 2.1b (PDB: 1LDJ). The N-terminus of RBX1 is indicated by "N." Yellow spheres represent zinc ions. (**b**) Dislodging of RBX1 RING domain from CUL5-CTD pocket upon cullin neddylation (PDB: 3DQV). Two orientations of the RBX1 RING domain captured in the crystal structure are shown. (**c**) Complex structure of CUL1-CTD-RBX1 bound to NEDD8-charged UBC12 and DCN1 (PDB: 4P5O). The linker between the N-terminal helix and catalytic domain of UBC12 is disordered. (**d**) A new position of RBX1 RING domain revealed by a CUL1-CTD-RBX1 complex structure (PDB: 3RTR). (**e**) CAND1 wraps around CUL1-RBX1, burying the cullin neddylation site lysine residue and blocking SKP1-binding site with a β-hairpin (PDB: 1U6G). (**f**) GLMN binds and blocks the E2-binding surface of RBX1 RING domain, which is flexibly tethered to the CUL1-CTD via an N-terminal β-strand (PDB: 4F52). (**g**) The overall architecture of COP9 signalosome (PDB: 4D10). (**h**) A schematic drawing of NEDD8-modified CUL4A-RBX1 in complex with DDB1-DDB2 and COP9 signalosome. R: RBX1. 6: CSN6. N8: NEDD8

β-sheet formed between the N-terminal β-strand of RBX1 and the α/β sub-domain of CUL5-CTD, RBX1 remains bound to the cullin scaffold with its RING domain gaining a significant degree of freedom to move around. This topological change of the cullin-RBX1 complex is thought to help bring the RBX1-bound E2 closer to the substrate anchored on the substrate receptor.

Just like protein ubiquitination, cullin neddylation requires the actions of the NEDD8-specific E1, E2 (UBC12), and E3 (DCN1 and DCN1 paralogues) enzymes (Liakopoulos et al. 1998; Osaka et al. 1998; Kurz et al. 2005). Similar to all ubiquitin-specific E2s, UBC12 (aka UBE2M) features a canonical E2 catalytic core domain, harboring an active site cysteine residue that can form a thioester bond with NEDD8 after it is activated by the E1 enzyme. Distinct from most ubiquitin-specific E2s, however, UBC12 contains an N-terminal extension sequence, whose extreme N-terminus has been shown to be acetylated. Remarkably, the acetylated UBC12 N-terminal extension adopts an α-helical conformation and specifically interacts with the neddylation E3 protein, DCN1 (Scott et al. 2011). With two EF hand-like sub-domains juxtaposed together, DCN1 is a compact all-helical protein with a slightly elongated shape (Fig. 2.4c). At the center of the protein is a hydrophobic pocket, which can specifically recognize the acetyl group of UBC12 N-terminus and the first methionine residue. Separate from this pocket, DCN1 also features a surface area that is able to engage cullin C-terminal WHB domain. Together, these interactions represent major interfaces through which DCN1 recruits the NEDD8-specific E2, UBC12, to catalyze the NEDD8 transfer reaction.

Strictly speaking, the NEDD8 E3 ligase function is performed by DCN1 in conjugation with RBX1, which plays a critical role in docking and activating the NEDD8-charged UBC12 catalytic core for cullin neddylation. Given the structural similarity of the catalytic domain between UBC12 and ubiquitin-specific E2s, the NEDD8 E2 is expected to interact with RBX1 RING domain in a similar fashion as ubiquitin-specific E2s to RING E3s. A simple modeling of a UBC12-RBX1 complex in the context of the unmodified cullin-RBX1 structures, however, readily reveals a long distance between the catalytic cysteine residue of UBC12 and the cullin neddylation site. For the cullin's lysine residue to attack the thioester bond formed between the UBC12 active site cysteine and the carboxyl terminus of NEDD8, the two residues have to be close to each other. This geometrical requirement strongly suggests that the RBX1 RING domain has to be reoriented before cullin neddylation can take place. Indeed, the crystal structure of an isolated CUL1-CTD-RBX1 complex revealed that the RING domain of RBX1 can be disengaged from its binding site on the CUL1-CTD in the absence of neddylation (Fig. 2.4d) (Calabrese et al. 2011). When UBC12 was modeled onto RBX1 in this structure, the gap between the UBC12 catalytic site and the cullin neddylation site is mostly closed.

The final picture of cullin neddylation has been depicted by the crystal structure of CUL1-CTD-RBX1 in complex with DCN1 and NEDD8-charged UBC12 (Fig. 2.4d) (Scott et al. 2014). In this structure, the CUL1 C-terminal WHB domain and its preceding long α-helix are shifted away from the rest of the CUL1-CTD, which allows the RING domain of RBX1 to adopt yet a new orientation. Resembling the previously reported docking model of ubiquitin-charged E2 to RING E3s, NEDD8-charged UBC12 is anchored to the RBX1 RING domain, and their compact structure is stabilized by a "linchpin" arginine residue unique to RBX1. Importantly, the NEDD8 molecule conjugated to the E2 also makes contacts with the linker sequence that connects the N-terminal β-strand of RBX1 to its RING domain, thereby optimally positioning the NEDD8 transfer module so that the catalytic site of the NEDD8 E2 is placed right next to the CUL1 neddylation site. Consistent with this notion, the interface between UBC12 catalytic domain and CUL1-WHB, which harbors the neddylation site, is kept minimal. Although DCN1 is also co-crystallized with the complex, it does not make direct interactions with the UBC12 catalytic domain (Fig. 2.4c). A flexible linker between the catalytic domain of the NEDD8 E2 and its N-terminal extension, which stably binds DCN1, is thought to accommodate the movement of the NEDD8 transfer module formed between RBX1 and NEDD8-charged UBC12 catalytic domain relative to the cullin scaffold.

2.3.2 CAND1 and Cullin Cycle

Cullin-associated and neddylation-dissociated protein 1 (CAND1) was the first cullin-binding protein identified that does not belong to the basal subunits of CRLs (Zheng et al. 2002a; Liu et al. 2002). It is a 120 kDa HEAT-repeat proteins that can form a stable complex with native, but not neddylated, cullin-RBX1 catalytic core. Interestingly, CAND1 binding seems to inhibit CUL1 from binding SKP1 and the substrate receptor F-box proteins, suggesting that CAND1 and SKP1-F-box proteins are mutually exclusive on the CRL1 scaffold. The crystal structure of a CAND1-CUL1-RBX1 complex unveiled the structural basis of all these biochemical activities of CAND1 (Fig. 2.4e) (Goldenberg et al. 2004). The 120 kDa protein adopts a superhelical structure with 27 consecutively stacked HEAT repeats that together form a long but highly sinuous fold. By curving around the entire CUL1-RBX1 structure, CAND1 grasps onto CUL1 like a two-pronged clamp. Importantly, CAND1 sports a β-hairpin projecting out of one of its HEAT repeats and reaching to the SKP1-binding site of the cullin scaffold. In doing so, CAND1 is able to compete with SKP1 for binding to the N-terminal end of CUL1. At the opposite end, the first two HEAT repeats of CAND1 closely pack against the WHB domain of CUL1, burying the neddylation site lysine residue. This suggests that CUL1 neddylation would sterically block CAND1 from binding.

Because SKP1-F-box proteins are responsible for recruiting substrates and CUL1 neddylation is thought to activate the E3 complex, the binding mode and biochemical properties of CAND1 seem to suggest that it acts as an inhibitor of CRL1. However, genetic studies indicate that CAND1 plays a positive role in regulating substrate ubiquitination and degradation by the E3 machinery. A growing body of evidence has helped raise an interesting model designating CAND1 as an exchange factor of CRL-substrate receptors (Pierce et al. 2013; Reitsma et al. 2017; Liu et al. 2018; Wu et al. 2013; Zemla et al. 2013). In this model, CAND1 can promote the disassembly of a SKP1-F-box protein complex from CUL1-RBX1, thereby allowing another SKP1-F-box protein complex to engage the cullin scaffold. While details of this model are discussed in Chap. 3 of this book, the structure of the CAND1-CUL1-RBX1 complex supports the notion that NEDD8 conjugation not only stimulates the activity of the E3 complex but also prevents CAND1 from dislodging an existing SKP1-F-box protein on the cullin scaffold.

In addition to CAND1, two other cellular factors have been documented to regulate a subset of CRLs, an α-helical protein known as Glomulin (GLMN) and a RING-IBR-RING protein, HHARI (aka ARIH1). The gene encoding GLMN is mutated in the hereditary disease glomuvenous malformations, which are characterized by venous lesions involving glomus cells. GLMN was initially identified as a protein that binds the C-terminus of CUL7, a distinct family member of CRLs (Arai et al. 2003). It was later shown to directly interact with the RBX1 RING domain and block its E3 ubiquitin ligase activity (Tron et al. 2012). The crystal structure of GLMN in complex with RBX1 bound to a fragment of CUL1-CTD revealed that GLMN contains two HEAT repeat-like sub-domains, which show structural similarity to each other (Fig. 2.4f) (Duda et al. 2012). One side of the GLMN C-terminal domain forms an extensive interface with the RING domain of RBX1, masking its E2-binding site. Although the CUL1-CTD fragment was present in the crystal and makes contact with GLMN C-terminal domain, it plays minimal role in stabilizing the complex formation. Owing to the orientational flexibility of RBX1 RING domain relative to the rest of the CRL1, GLMN binding is compatible with CRL assembly both at the RBX1-cullin interface and the cullin-adaptor-substrate receptor site. Even cullin neddylation showed no effect to GLMN-RBX1 interaction. Overall, GLMN appears to be an RBX1-specific inhibitor. Nonetheless, GLMN only binds a small subset of CRLs in human cells, suggesting that an unknown mechanism is involved in selectively controlling GLMN-RBX1 interaction in the context of the CRL functions.

RING-IBR-RING E3s represent a distinct class of ubiquitin ligases, which is a hybrid of the canonical RING-type and the HECT-type E3s (Zheng and Shabek 2017). RING-IBR-RINGSs are characterized by multiple RING domains and thioester intermediates they form with ubiquitin before the modifier is transferred to a substrate. ARH1, a member of the RING-IBR-RING E3s, has recently been identified to be preferentially associated with NEDD8-modified CRL1-CRL3 (but not CRL4) (Scott et al. 2016). Interestingly, it catalyzes mono-ubiquitination of representative substrates of these CRL E3s, which can be further polyubiquitinated by CDC34, the cognate E2 for RBX1. The precise mechanism by which ARH1 coordinates with neddylated CRLs to mediate the ubiquitin transfer reaction awaits future structural studies.

2.3.3 COP9 Signalosome-CRL Interactions

Just like protein ubiquitination, cullin neddylation is reversible. Deconjugation of NEDD8 from cullins is catalyzed by an evolutionarily conserved eight-subunit protein complex, known as the COP9 signalosome (CSN). CSN was first identified in plants based on mutants that showed a *constitutive photomorphogenesis* (*COP*) phenotype (Wei et al. 1994). These plant mutants turned out to carry mutations in eight genes, whose protein products form a stable complex with each subunit sharing sequence homology with one component of the eight-subunit lid complex of the 19S proteasome (Chamovitz et al. 1996; Wei et al. 1994). Among the eight CSN subunits, CSN5 is a zinc-containing metalloprotease that is responsible for cleaving the iso-peptide bond between cullins and NEDD8 (Cope et al. 2002). The assembly mechanism of the COP9 signalosome was first revealed in the crystal structure of the human CSN complex (Fig. 2.4g) (Lingaraju et al. 2014). Each of the CSN subunits employs one or two α-helices to build a superhelical bundle, which contributes to the stable assembly of the deneddylase complex. Meanwhile, six of the CSN subunits encircle a horseshoe-shaped ring structure with elongated α-helical PCI (proteasome lid-CSN-initiation factor 3) domains projecting away from the center. CSN5 and CSN6 share a common MPN (MPR1/PAD1) domain with a metalloprotease fold and together form a heterodimer. With their C-terminal regions integrated into the superhelical bundle, these two subunits anchor themselves onto one side of the CSN ring structure. Interestingly, the catalytic site of CSN5 in the CSN holoenzyme was found in an autoinhibited state, suggesting that CSN has to be activated upon binding to its substrate.

Recent advances in cryo-EM technology have enabled several studies that have shed light on how CSN interacts with different CRL complexes. Despite limited resolution, single particle analysis of CSN bound to neddylated CUL1-RBX1 in complexes with SKP1-SKP2-CKS1 and monomeric SKP1-FBXW7 offered the first glimpse of the CSN-CRL interaction (Enchev et al. 2012). In the structural models derived from the EM density maps, CSN2 appears to make major contacts with CUL1-CTD, whereas the distal end of the two F-box proteins is located close to CSN1 and CSN3. Structural modeling and biochemical analysis indicated that CSN competes with both substrates and E2 for binding the E3 platform, thereby raising the possibility that substrate-loaded CRL1 might protect neddylated CUL1 by preventing CSN from accessing the E3. This notion was subsequently supported by the cryo-EM structure of CSN bound to the neddylated CUL4A-RBX1-DDB1-DDB2 complex (Fig. 2.4h) (Cavadini et al. 2016). In this super-assembly, CSN2 not only interacts with CUL4A-CTD but also sandwiches RBX1 RING domain together with CSN4, thereby preventing the E3 scaffold from recruiting an E2 molecule. While CSN1 makes specific interactions with DDB1, DDB2 is snugly situated in between DDB1 and the CSN helical bundle. In comparison with the CSN holoenzyme structure, the CSN helical bundle is repositioned to accommodate the CUL4A-DDB1 substrate receptor. Interestingly, despite its topological flexibility, the CSN helical bundle cannot accommodate additional cellular factors that interact with DDB2, corroborating the idea that substrate binding to CRL4 will introduce steric hindrance for

CSN binding. Nevertheless, question remains as to whether CSN has the ability to differentiate variations of CRL-substrate receptor in size and shape from the binding of a small degron as part of a flexible substrate polypeptide.

Upon binding to CRL4, CSN undergoes multiple conformational changes to not only adapt to the landscape of its substrate but also alleviate its autoinhibition. These changes include movements of the PCI domains of CSN2 and CSN4 for clamping down to CUL4A-CTD and RBX1 and the translocation of the CSN5-CSN6 dimer to approach NEDD8. Although CRL4 is significantly different from other CRLs, similar structural changes have also been observed in the EM structure of CSN in complex with neddylated CUL1 with SKP1-SKP2-CKS1 bound. Although biochemical analyses have helped identify several structural elements that relay these structural changes to the alleviation of CSN autoinhibition, the detailed structural mechanism underlying CSN5 activation requires structural analysis at a higher resolution (Cavadini et al. 2016; Mosadeghi et al. 2016).

Acknowledgments We thank all members of the Zheng laboratory for their support and help. We apologize to all colleagues whose work could not be cited due to space constraints and the timing of manuscript preparation. Both D-V.R. and N.Z. are supported by Howard Hughes Medical Institute. N.Z. is a Howard Hughes Medical Institute Investigator.

References

Angers S, Li T, Yi X, Maccoss MJ, Moon RT, Zheng N (2006) Molecular architecture and assembly of the DDB1-CUL4A ubiquitin ligase machinery. Nature 443:590–593

Arai T, Kasper JS, Skaar JR, Ali SH, Takahashi C, Decaprio JA (2003) Targeted disruption of p185/Cul7 gene results in abnormal vascular morphogenesis. Proc Natl Acad Sci U S A 100:9855–9860

Busino L, Bassermann F, Maiolica A, Lee C, Nolan PM, Godinho SI, Draetta GF, Pagano M (2007) SCFFbxl3 controls the oscillation of the circadian clock by directing the degradation of cryptochrome proteins. Science 316:900–904

Calabrese MF, Scott DC, Duda DM, Grace CR, Kurinov I, Kriwacki RW, Schulman BA (2011) A RING E3-substrate complex poised for ubiquitin-like protein transfer: structural insights into cullin-RING ligases. Nat Struct Mol Biol 18:947–949

Cardote TAF, Gadd MS, Ciulli A (2017) Crystal structure of the Cul2-Rbx1-EloBC-VHL ubiquitin ligase complex. Structure 25:901–911.e3

Cavadini S, Fischer ES, Bunker RD, Potenza A, Lingaraju GM, Goldie KN, Mohamed WI, Faty M, Petzold G, Beckwith RE, Tichkule RB, Hassiepen U, Abdulrahman W, Pantelic RS, Matsumoto S, Sugasawa K, Stahlberg H, Thomä NH (2016) Cullin-RING ubiquitin E3 ligase regulation by the COP9 signalosome. Nature 531:598–603

Chamovitz DA, Wei N, Osterlund MT, Von Arnim AG, Staub JM, Matsui M, Deng XW (1996) The COP9 complex, a novel multisubunit nuclear regulator involved in light control of a plant developmental switch. Cell 86:115–121

Chen SJ, Wu X, Wadas B, Oh JH, Varshavsky A (2017) An N-end rule pathway that recognizes proline and destroys gluconeogenic enzymes. Science 355

Choi WS, Jeong BC, Joo YJ, Lee MR, Kim J, Eck MJ, Song HK (2010) Structural basis for the recognition of N-end rule substrates by the UBR box of ubiquitin ligases. Nat Struct Mol Biol 17:1175–1181

Cope GA, Suh GS, Aravind L, Schwarz SE, Zipursky SL, Koonin EV, Deshaies RJ (2002) Role of predicted metalloprotease motif of Jab1/Csn5 in cleavage of Nedd8 from Cul1. Science 298:608–611

Decorsière A, Mueller H, Van Breugel PC, Abdul F, Gerossier L, Beran RK, Livingston CM, Niu C, Fletcher SP, Hantz O, Strubin M (2016) Hepatitis B virus X protein identifies the Smc5/6 complex as a host restriction factor. Nature 531:386–389

Dong C, Zhang H, Li L, Tempel W, Loppnau P, Min J (2018) Molecular basis of GID4-mediated recognition of degrons for the Pro/N-end rule pathway. Nat Chem Biol 14:466–473

Donovan KA, An J, Nowak RP, Yuan JC, Fink EC, Berry BC, Ebert BL, Fischer ES (2018) Thalidomide promotes degradation of SALL4, a transcription factor implicated in Duane Radial Ray syndrome. Elife 7

Duda DM, Borg LA, Scott DC, Hunt HW, Hammel M, Schulman BA (2008) Structural insights into NEDD8 activation of cullin-RING ligases: conformational control of conjugation. Cell 134:995–1006

Duda DM, Olszewski JL, Tron AE, Hammel M, Lambert LJ, Waddell MB, Mittag T, Decaprio JA, Schulman BA (2012) Structure of a glomulin-RBX1-CUL1 complex: inhibition of a RING E3 ligase through masking of its E2-binding surface. Mol Cell 47:371–382

Enchev RI, Scott DC, da Fonseca PC, Schreiber A, Monda JK, Schulman BA, Peter M, Morris EP (2012) Structural basis for a reciprocal regulation between SCF and CSN. Cell Rep 2:616–627

Fischer ES, Scrima A, Böhm K, Matsumoto S, Lingaraju GM, Faty M, Yasuda T, Cavadini S, Wakasugi M, Hanaoka F, Iwai S, Gut H, Sugasawa K, Thomä NH (2011) The molecular basis of CRL4DDB2/CSA ubiquitin ligase architecture, targeting, and activation. Cell 147:1024–1039

Fukutomi T, Takagi K, Mizushima T, Ohuchi N, Yamamoto M (2014) Kinetic, thermodynamic, and structural characterizations of the association between Nrf2-DLGex degron and Keap1. Mol Cell Biol 34:832–846

Geyer R, Wee S, Anderson S, Yates J, Wolf DA (2003) BTB/POZ domain proteins are putative substrate adaptors for cullin 3 ubiquitin ligases. Mol Cell 12:783–790

Godinho SI, Maywood ES, Shaw L, Tucci V, Barnard AR, Busino L, Pagano M, Kendall R, Quwailid MM, Romero MR, O'Neill J, Chesham JE, Brooker D, Lalanne Z, Hastings MH, Nolan PM (2007) The after-hours mutant reveals a role for Fbxl3 in determining mammalian circadian period. Science 316:897–900

Goldenberg SJ, Cascio TC, Shumway SD, Garbutt KC, Liu J, Xiong Y, Zheng N (2004) Structure of the Cand1-Cul1-Roc1 complex reveals regulatory mechanisms for the assembly of the multisubunit cullin-dependent ubiquitin ligases. Cell 119:517–528

Guo Y, Dong L, Qiu X, Wang Y, Zhang B, Liu H, Yu Y, Zang Y, Yang M, Huang Z (2014) Structural basis for hijacking CBF-β and CUL5 E3 ligase complex by HIV-1 Vif. Nature 505:229–233

Hao B, Zheng N, Schulman BA, Wu G, Miller JJ, Pagano M, Pavletich NP (2005) Structural basis of the Cks1-dependent recognition of p27(Kip1) by the SCF(Skp2) ubiquitin ligase. Mol Cell 20:9–19

Hao B, Oehlmann S, Sowa ME, Harper JW, Pavletich NP (2007) Structure of a Fbw7-Skp1-cyclin E complex: multisite-phosphorylated substrate recognition by SCF ubiquitin ligases. Mol Cell 26:131–143

Hori T, Osaka F, Chiba T, Miyamoto C, Okabayashi K, Shimbara N, Kato S, Tanaka K (1999) Covalent modification of all members of human cullin family proteins by NEDD8. Oncogene 18:6829–6834

Horvath CM (2004) Weapons of STAT destruction. Interferon evasion by paramyxovirus V protein. Eur J Biochem 271:4621–4628

Ivan M, Kondo K, Yang H, Kim W, Valiando J, Ohh M, Salic A, Asara JM, Lane WS, Kaelin WG (2001) HIFalpha targeted for VHL-mediated destruction by proline hydroxylation: implications for O2 sensing. Science 292:464–468

Jin J, Cardozo T, Lovering RC, Elledge SJ, Pagano M, Harper JW (2004) Systematic analysis and nomenclature of mammalian F-box proteins. Genes Dev 18:2573–2580

Jin J, Arias EE, Chen J, Harper JW, Walter JC (2006) A family of diverse Cul4-Ddb1-interacting proteins includes Cdt2, which is required for S phase destruction of the replication factor Cdt1. Mol Cell 23:709–721

Kaelin WG (2005) Proline hydroxylation and gene expression. Annu Rev Biochem 74:115–128

Kim DY, Kwon E, Hartley PD, Crosby DC, Mann S, Krogan NJ, Gross JD (2013) CBFβ stabilizes HIV Vif to counteract APOBEC3 at the expense of RUNX1 target gene expression. Mol Cell 49:632–644

Koren I, Timms RT, Kula T, Xu Q, Li MZ, Elledge SJ (2018) The eukaryotic proteome is shaped by E3 ubiquitin ligases targeting C-terminal degrons. Cell

Krönke J, Udeshi ND, Narla A, Grauman P, Hurst SN, McConkey M, Svinkina T, Heckl D, Comer E, Li X, Ciarlo C, Hartman E, Munshi N, Schenone M, Schreiber SL, Carr SA, Ebert BL (2014) Lenalidomide causes selective degradation of IKZF1 and IKZF3 in multiple myeloma cells. Science 343:301–305

Krönke J, Fink EC, Hollenbach PW, Macbeth KJ, Hurst SN, Udeshi ND, Chamberlain PP, Mani DR, Man HW, Gandhi AK, Svinkina T, Schneider RK, Mcconkey M, Järås M, Griffiths E, Wetzler M, Bullinger L, Cathers BE, Carr SA, Chopra R, Ebert BL (2015) Lenalidomide induces ubiquitination and degradation of CK1α in del(5q) MDS. Nature 523:183–188

Kurz T, Ozlü N, Rudolf F, O'Rourke SM, Luke B, Hofmann K, Hyman AA, Bowerman B, Peter M (2005) The conserved protein DCN-1/Dcn1p is required for cullin neddylation in C. elegans and S. cerevisiae. Nature 435:1257–1261

Li T, Chen X, Garbutt KC, Zhou P, Zheng N (2006) Structure of DDB1 in complex with a paramyxovirus V protein: viral hijack of a propeller cluster in ubiquitin ligase. Cell 124:105–117

Li T, Robert EI, Van Breugel PC, Strubin M, Zheng N (2010) A promiscuous alpha-helical motif anchors viral hijackers and substrate receptors to the CUL4-DDB1 ubiquitin ligase machinery. Nat Struct Mol Biol 17:105–111

Liakopoulos D, Doenges G, Matuschewski K, Jentsch S (1998) A novel protein modification pathway related to the ubiquitin system. EMBO J 17:2208–2214

Lin HC, Ho SC, Chen YY, Khoo KH, Hsu PH, Yen HC (2015) SELENOPROTEINS. CRL2 aids elimination of truncated selenoproteins produced by failed UGA/Sec decoding. Science 349:91–95

Lin HC, Yeh CW, Chen YF, Lee TT, Hsieh PY, Rusnac DV, Lin SY, Elledge SJ, Zheng N, Yen HS (2018) C-terminal end-directed protein elimination by CRL2 ubiquitin ligases. Mol Cell 70:602–613.e3

Lingaraju GM, Bunker RD, Cavadini S, Hess D, Hassiepen U, Renatus M, Fischer ES, Thomä NH (2014) Crystal structure of the human COP9 signalosome. Nature 512:161–165

Liu J, Furukawa M, Matsumoto T, Xiong Y (2002) NEDD8 modification of CUL1 dissociates p120 (CAND1), an inhibitor of CUL1-SKP1 binding and SCF ligases. Mol Cell 10:1511–1518

Liu X, Reitsma JM, Mamrosh JL, Zhang Y, Straube R, Deshaies RJ (2018) Cand1-mediated adaptive exchange mechanism enables variation in F-box protein expression. Mol Cell 69:773–786.e6

Lu G, Middleton RE, Sun H, Naniong M, Ott CJ, Mitsiades CS, Wong KK, Bradner JE, Kaelin WG (2014) The myeloma drug lenalidomide promotes the cereblon-dependent destruction of Ikaros proteins. Science 343:305–309

Mahrour N, Redwine WB, Florens L, Swanson SK, Martin-Brown S, Bradford WD, Staehling-Hampton K, Washburn MP, Conaway RC, Conaway JW (2008) Characterization of Cullin-box sequences that direct recruitment of Cul2-Rbx1 and Cul5-Rbx2 modules to Elongin BC-based ubiquitin ligases. J Biol Chem 283:8005–8013

Matta-Camacho E, Kozlov G, Li FF, Gehring K (2010) Structural basis of substrate recognition and specificity in the N-end rule pathway. Nat Struct Mol Biol 17:1182–1187

Matyskiela ME, Lu G, Ito T, Pagarigan B, Lu CC, Miller K, Fang W, Wang NY, Nguyen D, Houston J, Carmel G, Tran T, Riley M, Nosaka L, Lander GC, Gaidarova S, Xu S, Ruchelman AL, Handa H, Carmichael J, Daniel TO, Cathers BE, Lopez-Girona A, Chamberlain PP (2016) A novel cereblon modulator recruits GSPT1 to the CRL4(CRBN) ubiquitin ligase. Nature 535:252–257

Matyskiela ME, Couto S, Zheng X, Lu G, Hui J, Stamp K, Drew C, Ren Y, Wang M, Carpenter A, Lee CW, Clayton T, Fang W, Lu CC, Riley M, Abdubek P, Blease K, Hartke J, Kumar G, Vessey R, Rolfe M, Hamann LG, Chamberlain PP (2018) SALL4 mediates teratogenicity as a thalidomide-dependent cereblon substrate. Nat Chem Biol 14:981–987

Min JH, Yang H, Ivan M, Gertler F, Kaelin WG, Pavletich NP (2002) Structure of an HIF-1alpha -pVHL complex: hydroxyproline recognition in signaling. Science 296:1886–1889

Mosadeghi R, Reichermeier KM, Winkler M, Schreiber A, Reitsma JM, Zhang Y, Stengel F, Cao J, Kim M, Sweredoski MJ, Hess S, Leitner A, Aebersold R, Peter M, Deshaies RJ, Enchev RI (2016) Structural and kinetic analysis of the COP9-Signalosome activation and the cullin-RING ubiquitin ligase deneddylation cycle. Elife 5

Nangle S, Xing W, Zheng N (2013) Crystal structure of mammalian cryptochrome in complex with a small molecule competitor of its ubiquitin ligase. Cell Res 23:1417–1419

Nguyen HC, Yang H, Fribourgh JL, Wolfe LS, Xiong Y (2015) Insights into Cullin-RING E3 ubiquitin ligase recruitment: structure of the VHL-EloBC-Cul2 complex. Structure 23:441–449

Osaka F, Kawasaki H, Aida N, Saeki M, Chiba T, Kawashima S, Tanaka K, Kato S (1998) A new NEDD8-ligating system for cullin-4A. Genes Dev 12:2263–2268

Padmanabhan B, Tong KI, Ohta T, Nakamura Y, Scharlock M, Ohtsuji M, Kang MI, Kobayashi A, Yokoyama S, Yamamoto M (2006) Structural basis for defects of Keap1 activity provoked by its point mutations in lung cancer. Mol Cell 21:689–700

Petzold G, Fischer ES, Thomä NH (2016) Structural basis of lenalidomide-induced CK1α degradation by the CRL4(CRBN) ubiquitin ligase. Nature 532:127–130

Pierce NW, Lee JE, Liu X, Sweredoski MJ, Graham RL, Larimore EA, Rome M, Zheng N, Clurman BE, Hess S, Shan SO, Deshaies RJ (2013) Cand1 promotes assembly of new SCF complexes through dynamic exchange of F box proteins. Cell 153:206–215

Pintard L, Willis JH, Willems A, Johnson JL, Srayko M, Kurz T, Glaser S, Mains PE, Tyers M, Bowerman B, Peter M (2003) The BTB protein MEL-26 is a substrate-specific adaptor of the CUL-3 ubiquitin-ligase. Nature 425:311–316

Reitsma JM, Liu X, Reichermeier KM, Moradian A, Sweredoski MJ, Hess S, Deshaies RJ (2017) Composition and regulation of the cellular repertoire of SCF ubiquitin ligases. Cell 171:1326–1339.e14

Rusnac DV, Lin HC, Canzani D, Tien KX, Hinds TR, Tsue AF, Bush MF, Yen HS, Zheng N (2018) Recognition of the diglycine C-end degron by CRL2. Mol Cell 72:813–822.e4

Saha A, Deshaies RJ (2008) Multimodal activation of the ubiquitin ligase SCF by Nedd8 conjugation. Mol Cell 32:21–31

Schmalen I, Reischl S, Wallach T, Klemz R, Grudziecki A, Prabu JR, Benda C, Kramer A, Wolf E (2014) Interaction of circadian clock proteins CRY1 and PER2 is modulated by zinc binding and disulfide bond formation. Cell 157:1203–1215

Schulman BA, Carrano AC, Jeffrey PD, Bowen Z, Kinnucan ER, Finnin MS, Elledge SJ, Harper JW, Pagano M, Pavletich NP (2000) Insights into SCF ubiquitin ligases from the structure of the Skp1-Skp2 complex. Nature 408:381–386

Scott DC, Monda JK, Bennett EJ, Harper JW, Schulman BA (2011) N-terminal acetylation acts as an avidity enhancer within an interconnected multiprotein complex. Science 334:674–678

Scott DC, Sviderskiy VO, Monda JK, Lydeard JR, Cho SE, Harper JW, Schulman BA (2014) Structure of a RING E3 trapped in action reveals ligation mechanism for the ubiquitin-like protein NEDD8. Cell 157:1671–1684

Scott DC, Rhee DY, Duda DM, Kelsall IR, Olszewski JL, Paulo JA, de Jong A, Ovaa H, Alpi AF, Harper JW, Schulman BA (2016) Two distinct types of E3 ligases work in unison to regulate substrate ubiquitylation. Cell 166:1198–1214.e24

Shabek N, Zheng N (2014) Plant ubiquitin ligases as signaling hubs. Nat Struct Mol Biol 21:293–296

Sheard LB, Tan X, Mao H, Withers J, Ben-Nissan G, Hinds TR, Kobayashi Y, Hsu FF, Sharon M, Browse J, He SY, Rizo J, Howe GA, Zheng N (2010) Jasmonate perception by inositol-phosphate-potentiated COI1-JAZ co-receptor. Nature 468:400–405

Shirogane T, Jin J, Ang XL, Harper JW (2005) SCFbeta-TRCP controls clock-dependent transcription via casein kinase 1-dependent degradation of the mammalian period-1 (Per1) protein. J Biol Chem 280:26863–26872

Siepka SM, Yoo SH, Park J, Song W, Kumar V, Hu Y, Lee C, Takahashi JS (2007) Circadian mutant overtime reveals F-box protein FBXL3 regulation of

cryptochrome and period gene expression. Cell 129:1011–1023

Skowyra D, Craig KL, Tyers M, Elledge SJ, Harper JW (1997) F-box proteins are receptors that recruit phosphorylated substrates to the SCF ubiquitin-ligase complex. Cell 91:209–219

Stebbins CE, Kaelin WG, Pavletich NP (1999) Structure of the VHL-Elongin C-Elongin B complex: implications for VHL tumor suppressor function. Science 284:455–461

Takahashi JS (2017) Transcriptional architecture of the mammalian circadian clock. Nat Rev Genet 18:164–179

Tan X, Calderon-Villalobos LI, Sharon M, Zheng C, Robinson CV, Estelle M, Zheng N (2007) Mechanism of auxin perception by the TIR1 ubiquitin ligase. Nature 446:640–645

Tron AE, Arai T, Duda DM, Kuwabara H, Olszewski JL, Fujiwara Y, Bahamon BN, Signoretti S, Schulman BA, Decaprio JA (2012) The glomuvenous malformation protein Glomulin binds Rbx1 and regulates cullin RING ligase-mediated turnover of Fbw7. Mol Cell 46:67–78

Wei N, Chamovitz DA, Deng XW (1994) Arabidopsis COP9 is a component of a novel signaling complex mediating light control of development. Cell 78:117–124

Welcker M, Clurman BE (2008) FBW7 ubiquitin ligase: a tumour suppressor at the crossroads of cell division, growth and differentiation. Nat Rev Cancer 8:83–93

Wild R, Gerasimaite R, Jung JY, Truffault V, Pavlovic I, Schmidt A, Saiardi A, Jessen HJ, Poirier Y, Hothorn M, Mayer A (2016) Control of eukaryotic phosphate homeostasis by inositol polyphosphate sensor domains. Science 352:986–990

Wu G, Xu G, Schulman BA, Jeffrey PD, Harper JW, Pavletich NP (2003) Structure of a beta-TrCP1-Skp1-beta-catenin complex: destruction motif binding and lysine specificity of the SCF(beta-TrCP1) ubiquitin ligase. Mol Cell 11:1445–1456

Wu S, Zhu W, Nhan T, Toth JI, Petroski MD, Wolf DA (2013) CAND1 controls in vivo dynamics of the cullin 1-RING ubiquitin ligase repertoire. Nat Commun 4:1642

Xing W, Busino L, Hinds TR, Marionni ST, Saifee NH, Bush MF, Pagano M, Zheng N (2013) SCF(FBXL3) ubiquitin ligase targets cryptochromes at their cofactor pocket. Nature 496:64–68

Xu L, Wei Y, Reboul J, Vaglio P, Shin TH, Vidal M, Elledge SJ, Harper JW (2003) BTB proteins are substrate-specific adaptors in an SCF-like modular ubiquitin ligase containing CUL-3. Nature 425:316–321

Yamamoto M, Kensler TW, Motohashi H (2018) The KEAP1-NRF2 system: a thiol-based sensor-effector apparatus for maintaining redox homeostasis. Physiol Rev 98:1169–1203

Yamoah K, Oashi T, Sarikas A, Gazdoiu S, Osman R, Pan ZQ (2008) Autoinhibitory regulation of SCF-mediated ubiquitination by human cullin 1's C-terminal tail. Proc Natl Acad Sci U S A 105:12230–12235

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

Zemla A, Thomas Y, Kedziora S, Knebel A, Wood NT, Rabut G, Kurz T (2013) CSN- and CAND1-dependent remodelling of the budding yeast SCF complex. Nat Commun 4:1641

Zheng N, Shabek N (2017) Ubiquitin ligases: structure, function, and regulation. Annu Rev Biochem 86:129–157

Zheng J, Yang X, Harrell JM, Ryzhikov S, Shim EH, Lykke-Andersen K, Wei N, Sun H, Kobayashi R, Zhang H (2002a) CAND1 binds to unneddylated CUL1 and regulates the formation of SCF ubiquitin E3 ligase complex. Mol Cell 10:1519–1526

Zheng N, Schulman BA, Song L, Miller JJ, Jeffrey PD, Wang P, Chu C, Koepp DM, Elledge SJ, Pagano M, Conaway RC, Conaway JW, Harper JW, Pavletich NP (2002b) Structure of the Cul1-Rbx1-Skp1-F boxSkp2 SCF ubiquitin ligase complex. Nature 416:703–709

Zhuang M, Calabrese MF, Liu J, Waddell MB, Nourse A, Hammel M, Miller DJ, Walden H, Duda DM, Seyedin SN, Hoggard T, Harper JW, White KP, Schulman BA (2009) Structures of SPOP-substrate complexes: insights into molecular architectures of BTB-Cul3 ubiquitin ligases. Mol Cell 36:39–50