

PROTEIN FAMILY REVIEW

The cullin protein family

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Summary

Cullin proteins are molecular scaffolds that have crucial roles in the post-translational modification of cellular proteins involving ubiquitin. The mammalian cullin protein family comprises eight members (CUL1 to CUL7 and PARC), which are characterized by a cullin homology domain. CUL1 to CUL7 assemble multi-subunit Cullin-RING E3 ubiquitin ligase (CRL) complexes, the largest family of E3 ligases with more than 200 members. Although CUL7 and PARC are present only in chordates, other members of the cullin protein family are found in *Drosophila melanogaster*, *Caenorhabditis elegans*, *Arabidopsis thaliana* and yeast. A cullin protein tethers both a substrate-targeting unit, often through an adaptor protein, and the RING finger component in a CRL. The cullin-organized CRL thus positions a substrate close to the RING-bound E2 ubiquitin-conjugating enzyme, which catalyzes the transfer of ubiquitin to the substrate. In addition, conjugation of cullins with the ubiquitin-like molecule Nedd8 modulates activation of the corresponding CRL complex, probably through conformational regulation of the interactions between cullin's carboxy-terminal tail and CRL's RING subunit. Genetic studies in several model organisms have helped to unravel a multitude of physiological functions associated with cullin proteins and their respective CRLs. CRLs target numerous substrates and thus have an impact on a range of biological processes, including cell growth, development, signal transduction, transcriptional control, genomic integrity and tumor suppression. Moreover, mutations in *CUL7* and *CUL4B* genes have been linked to hereditary human diseases.

Gene organization and evolutionary history

Cullins, containing an evolutionarily conserved cullin homology domain, are a family of structurally related

proteins required for ubiquitin-dependent protein degradation (Box 1). Two groups have made important contributions to the discovery of this protein family. Kipreos *et al.* [1] identified cullins as a novel gene family involved in cell cycle regulation in nematodes. Independently, Mathias *et al.* [2] isolated Cdc53 (cell division control protein 53), a cullin homolog in budding yeast, and elucidated its role in ubiquitin-dependent proteolysis of cell-cycle regulators. Thus, cullins are named after their role in 'culling', sorting or 'selecting' cellular proteins for ubiquitin-mediated proteasomal degradation (E Kipreos, personal communication).

The cullin gene family is evolutionarily conserved. Table 1 presents a list of the cullin family genes from a range of representative species with respect to their gene organization and expression. There are seven cullins in mammals (CUL1 to CUL3, CUL4a, CUL4b, CUL5, CUL7 and the closely related p53-associated parkin-like cytoplasmic protein (Parc) in *Homo sapiens*, *Mus musculus* and *Rattus norvegicus*), six in *C. elegans* (cul-1 to cul-6) and five in *Drosophila* (CUL1 to CUL5). *Arabidopsis* has five cullins (CUL1, CUL2, CUL3A, CUL4 and CUL5), and yeast genomes encode three cullin proteins (cul1, cul3, cul8 in *Saccharomyces cerevisiae*; cul1, cul3 and cul4 in *Schizosaccharomyces pombe*). In total, 490 cullin domains in 490 proteins are described in the SMART nrdb database [3], which estimates 60% conservation in the cullin homology domains.

Figure 1 summarizes the phylogenetic relationships among the cullins based on sequence alignment. The presence of the *Cul1* to *Cul5* genes in the early-branching metazoans *Trichoplax adhaerens* and *Nematostella vectensis* indicates that cullin genes are ancient and originated before the separation of the different animal lineages. An extensive genome-wide analysis of the cullin family has suggested that three ancestral cullin genes, termed '*Cul α* ', '*Cul β* ' and '*Cul γ* ', appeared in early eukaryotic evolution, from which the cullin genes evolved after the split of the unikonts (which include animals and fungi) and the bikonts (which include plants). In this model, the human *CUL1*, *CUL2*, *CUL5*, *CUL7* and *PARC* genes were derived from one common ancestral gene (*Cul α*), whereas the *Cul3* and *Cul4a/4b* genes evolved from two distinct ancestors, the *Cul β* and *Cul γ* gene, respectively [4]. Notably, *Cul7* and *Parc*, found only in chordates, are highly similar in

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Box 1. The ubiquitin-proteasome system

The ubiquitin-proteasome system is a selective protein degradation pathway in which a substrate is first tagged with a chain of ubiquitin and the resulting modified protein is then recognized by the 26S proteasome, where proteolysis of the substrate takes place. The process of ubiquitination involves a three-tiered enzymatic cascade. First, the chemically inert ubiquitin molecule is activated in an ATP-dependent reaction by forming a thioester bond between the carboxy-terminal carboxyl group of ubiquitin and the catalytic cysteine of an E1 activating enzyme. Second, in a trans(thio)esterification reaction ubiquitin is transferred to the active site cysteine of an E2 ubiquitin-conjugating enzyme. In the last step, an E3 ubiquitin ligase functions to orchestrate the transfer of ubiquitin to a substrate protein, forming an isopeptide bond between the ubiquitin carboxy-terminal glycine residue and substrate lysine ϵ -amino group. The action of an E3 typically involves recognition of a specific degradation motif (degron) on the substrate. The human genome encodes two E1 activating enzymes, 37 E2 conjugating enzymes and more than 500 E3 ubiquitin ligases [54]. A substrate protein can be conjugated with just one ubiquitin (monoubiquitination), one ubiquitin molecule at different lysines residues (multiubiquitination) or by a chain of several ubiquitin moieties (polyubiquitination). Ubiquitin contains seven lysine residues (Lys6, Lys11, Lys27, Lys29, Lys33, Lys48 and Lys63) that can act as acceptors and result in entirely different chain conformations. Polyubiquitination has multifaceted outcomes that depend on the respective chain structure [54]. For instance, although Lys48-linked chains are the canonical recognition motif for the proteasome, Lys63-linked chains have important non-degradative roles in cell signaling, DNA-damage response and endocytosis [55]. Monoubiquitination typically has non-proteolytic functions, such as the internalization of cell-surface receptors [56].

sequence and both contain a CPH (conserved in CUL7, PARC and HERC2) domain and a DOC domain (similar to the DOC1 of the anaphase-promoting complex/cyclosome) of unknown functions (Figure 2). In human and mouse, *Cul7* and *Parc* are located on the same chromosome in close proximity (260 kb apart). Based on these findings, Marin *et al.* [5] suggested that *Parc* originated from a gene fusion of a duplicate of *Cul7* and an *ariadne* gene, which encodes a putative E3 ubiquitin ligase sharing structural similarity with Parkin. Fungal species contain only three cullin genes: *Cdc53*, *Cul3* and *Cul8* (also known as *RTT101*) in *S. cerevisiae* and *Cul1*, *Cul3* and *Cul4* in *S. pombe* [4]. However, *Cul8/RTT101* in *Saccharomycotina* differs significantly from the *Cul4*-like genes of other fungi (such as ascomycetes and basidiomycetes) [4]. It was postulated that *Cul8/RTT101* originated from the *Cul4* gene that underwent an accelerated evolution, or that *Saccharomycotina* has lost their *Cul4*-like gene and the *Cul8/RTT101* gene arose in parallel as a result of gene duplication.

Characteristic structural features

RING (really interesting new gene)-type E3 ubiquitin ligases orchestrate ubiquitination by simultaneously binding to a protein substrate and anchoring an E2 ubiquitin-conjugating enzyme through the RING domain (for detailed description of the actions of E2 and E3 in ubiquitination, see Boxes 1 and 2 and Figure 3). Cullins are molecular scaffolds that organize the largest class of RING E3 ligases, known as the cullin-RING ligase complexes (CRLs). Below we discuss how cullins use their unique structural properties to assemble their cognate CRLs.

The structural properties of cullins have been revealed in the context of CRLs by high-resolution structural studies and biochemical reconstitution experiments (Table 2). Although all the cullin (and CRL) structures that have been solved so far have been mammalian proteins (Table 2), the interactions between cullins and their CRL components have been analyzed using biochemical assays in systems from yeast to humans, highlighting an extraordinary conservation regarding the scaffolding functions of cullins. Thus, the cullin and CRL structural models discussed in this section are generally applicable to counterparts of all origins.

CUL1 to CUL5 have a long stalk-like amino-terminal domain (NTD), consisting of three cullin repeats (CR1 to CR3), and a globular carboxy-terminal domain (CTD), which harbors a signature cullin homology domain (CH), a highly conserved stretch of about 200 amino acids (Figure 2).

The cullin CTD binds to its RING partner, Regulator of cullins 1 (ROC1) or ROC2 (also called RING box protein (Rbx)1 and Rbx2, respectively), which recruits the ubiquitin-loaded E2 enzymes for catalysis. The CUL1-ROC1 association is established by multiple interface interactions, primarily involving CUL1's α/β domain and the amino-terminal S1 β -strand of ROC1, which enable the formation of an intermolecular α/β hydrophobic core that essentially renders CUL1-ROC1 physically inseparable [6]. The cullin-RING interaction creates a catalytic core and is the most characteristic structural feature that defines CRLs [7].

On the basis of structural studies of the human S-phase kinase-associated protein 1 (Skp1)-CUL1-F-box (SCF) complex [6] and the CUL4A RING (CRL4A) complex [8,9], cullins organize CRLs by forming two distinct modules: a substrate-targeting unit, composed of a substrate-recognition protein and an adaptor protein that links the module to the cullin, and the RING component that is active in recruiting an E2 ubiquitin-conjugating enzyme (Figure 4). For instance, SCF contains a substrate recognition subunit known as the F-box protein, which is characterized by a 40-amino-acid F-box domain [10]. The Skp1 adaptor protein mediates binding of the F-box

Table 1. Chromosomal localization of the cullin genes from several representative species

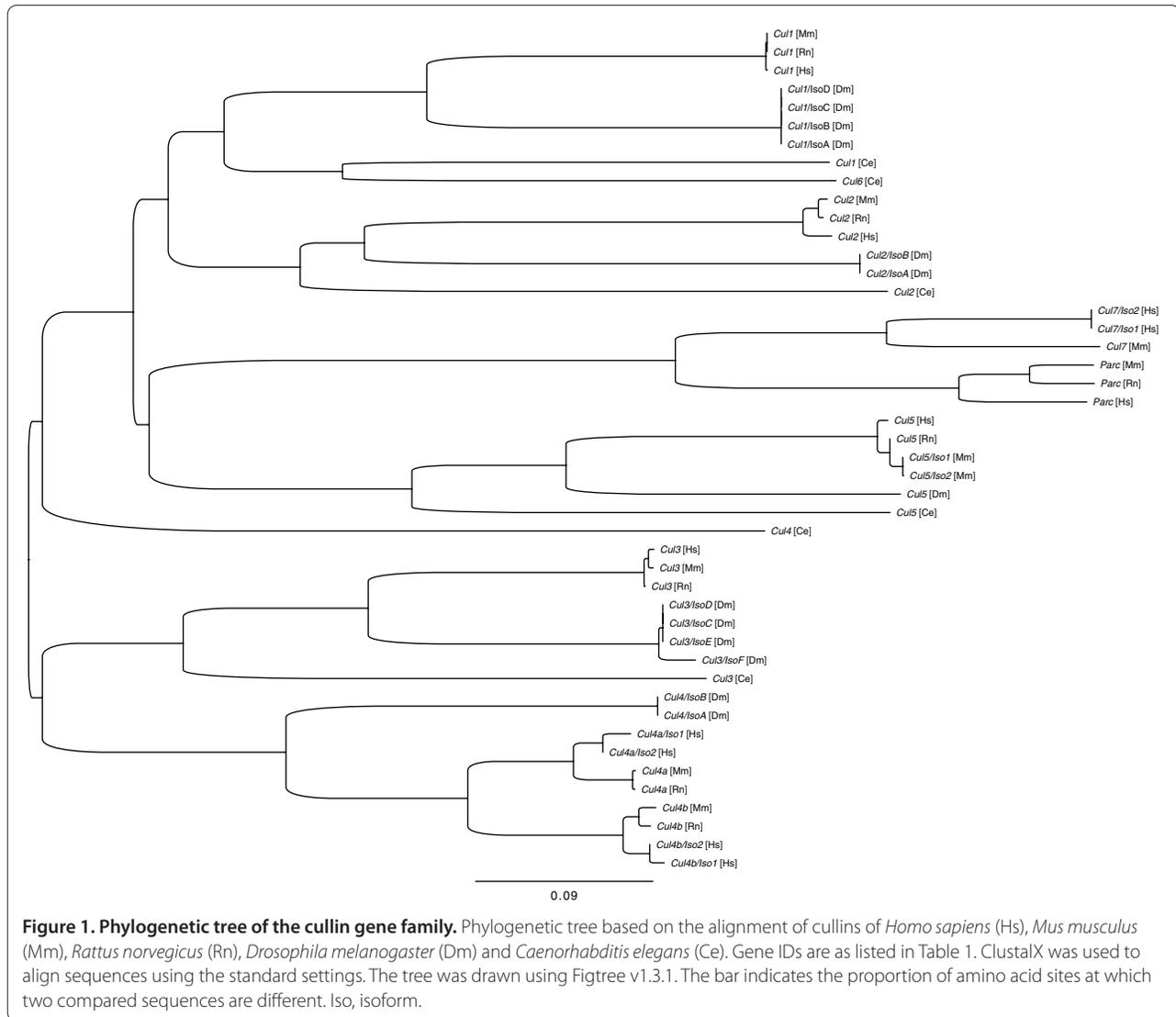
	Gene												
	<i>Cul1</i>	<i>Cul2</i>	<i>Cul3</i>	<i>Cul3a</i>	<i>Cul3b</i>	<i>Cul4</i>	<i>Cul4a</i>	<i>Cul4b</i>	<i>Cul5</i>	<i>Cul6</i>	<i>Cul7</i>	<i>Cul8</i>	<i>Parc</i>
Human													
Gene ID	8454	8453	8452	NP	NP	NP	8451	8450	8065	NP	9820	NP	23113
Chromosomal localization	7q36.1	10p11.21	2q36.2	NP	NP	NP	13q34	Xq23	11q22-q23	NP	6p21.1	NP	6p21.1
Introns	21	20	15	NP	NP	NP	19	19/21	18	NP	25	NP	40
Isoforms	1	1	1	NP	NP	NP	2	2	1	NP	2	NP	1
Mouse													
Gene ID	26965	71745	26554	NP	NP	NP	99375	72584	75717	NP	66515	NP	78309
Chromosomal localization	6; B3	18; A1	1; C4	NP	NP	NP	8; A1.1	X; A2	9; C	NP	17; C	NP	17; C
Introns	21	20	15	NP	NP	NP	19	22	18/19	NP	25	NP	41
Isoforms	1	1	1	NP	NP	NP	1	1	2	NP	1	NP	1
Rat													
Gene ID	362356	361258	301555	NP	NP	NP	361181	302502	64624	NP	363191	NP	316228
Chromosomal localization	4q24	17q12.1	9q34	NP	NP	NP	16q12.5	Xq11	8q24	NP	9q12	NP	9q12
<i>C. elegans</i>													
Gene ID	176466	176806	178547	NP	NP	174198	NP	NP	179413	178214	NP	NP	NP
Chromosomal localization	III	III	V	NP	NP	II	NP	NP	V	IV	NP	NP	NP
<i>Drosophila</i>													
Gene ID	(lin19) 35742	35420	34896	NP	NP	35780	NP	NP	43434	NP	NP	NP	NP
Chromosomal localization	2R; 43F1- 43F2	2L; 39E3- 39E6	2L; 35C5- 35C5	NP	NP	2R; 44A4- 44A4	NP	NP	3R; 98F6- 98F6	NP	NP	NP	NP
<i>A. thaliana</i>													
Gene ID	(ATCUL1) 825648	839415	NP	(ATCUL3) 839226	843303	834663	NP	NP	NP	NP	NP	NP	NP
Chromosomal localization	4	1	NP	1	1	5	NP	NP	NP	NP	NP	NP	NP
<i>S. cerevisiae</i>													
Gene ID	(Cdc53) 851424	NP	852886	NP	NP	NP	NP	NP	NP	NP	NP	(RTT101) 853400	NP
Chromosomal localization	V	NP	VII	NP	NP	NP	NP	NP	NP	NP	NP	X	NP
<i>S. pombe</i>													
Gene ID	2542393	NP	2542637	NP	NP	(pcu4) 2543116	NP	NP	NP	NP	NP	NP	NP
Chromosomal localization	I	NP	I	NP	NP	I	NP	NP	NP	NP	NP	NP	NP

Gene IDs are as listed in Entrez Gene [59]. The intron number and transcript information are indicated for the cullin genes in human and mouse. Only the variants in Entrez Gene are indicated and other variants may exist. NP, not present.

protein to the amino terminus of CUL1. In CRL4A, the substrate-targeting unit is composed of damage-specific DNA binding protein 1 (DDB1) as the adaptor protein, and a member of the DDB1 and CUL4 associated factor (DCAF) family that recognizes a substrate. However, CRL3 does not contain a separate adaptor subunit. Instead, it incorporates BTB (Bric-a-brac, Tramtrack, Broad-complex), a dual function molecule capable of binding to CUL3 and targeting a substrate (Figure 4). (SCF, also called CRL1, is historically the prototype of all

CRLs and thus the name SCF remains commonly used in the current literature.)

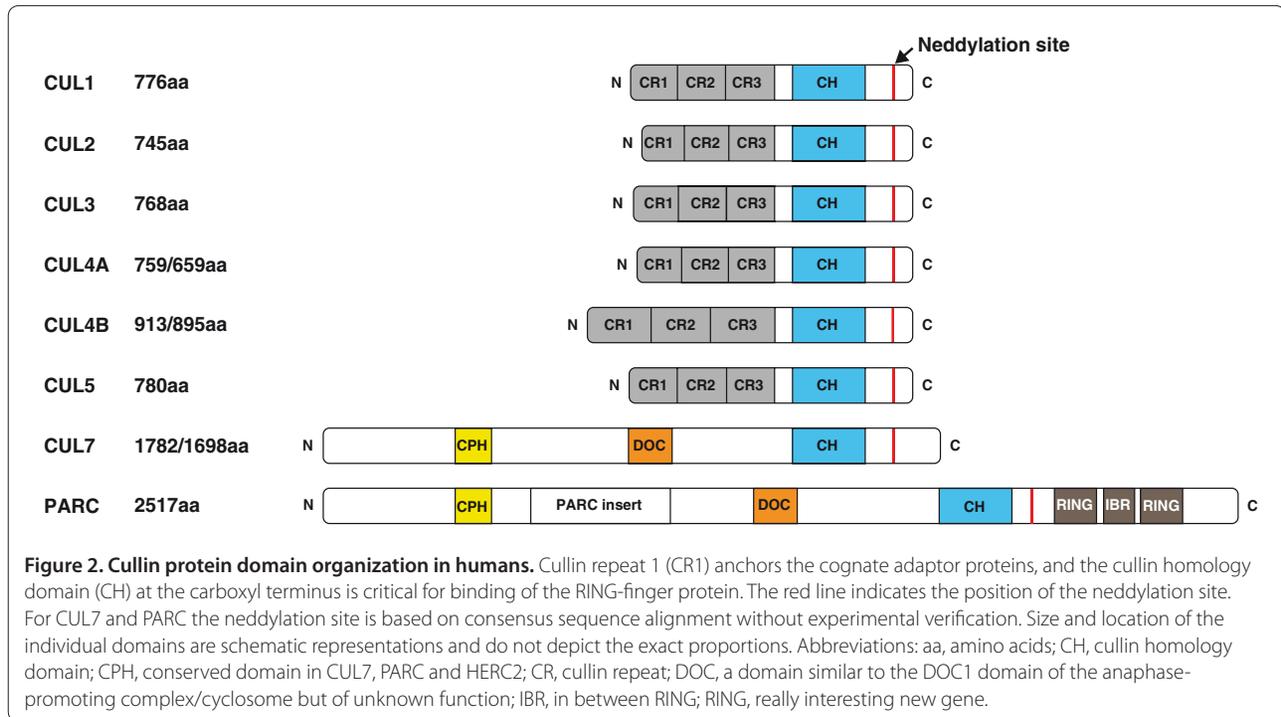
Accumulating evidence points to a common mechanism by which CUL1 to CUL5 build a substrate-targeting unit. CUL1 to CUL5 use amino-terminal helices H2 and H5 of CR1 to anchor their cognate adaptors. There are two distinct types of recognition fold in the adaptor (Table 2). In the SCF, CRL2, CRL3 and CRL5 E3s, different adaptors (Skp1, Elongin C (EloC) or BTB) share a similar structural motif termed the Skp1/



BTB/Pox virus and zinc finger (POZ) fold, which is a primary determinant for affinity interactions with the cullin amino terminus. By contrast, the DDB1 adaptor of CRL4 lacks the Skp1/BTB/POZ fold and instead uses its BPB domain to interact with the CUL4A H2 and H5 helices, as well as the amino-terminal extension [9]. In this regard, it is worth commenting on the enormous structural complexity of DDB1, a 127 kDa protein containing three large propeller folds [8], which potentially enable multiple interactions with cellular proteins. Indeed, DDB1 was found to form a complex with de-tiolated 1 (DET1), DDB1 associated 1 (DDA1) and the E2 ubiquitin-conjugating enzyme UBE2E [11]. Initial efforts to isolate the human CUL4A-containing complexes resulted in very large complexes that contained the constitutive photomorphogenesis 9 (COP9) signalosome [12]. It remains to be determined whether DDB1 enhances the association between CRL4 and COP9. The

role of the COP9 signalosome in CRL function is discussed in the next section.

Structural and biochemical analyses have revealed additional protein-protein interactions that contribute to the cullin-mediated CRL assembly. In addition to the Skp1-CUL1 interactions, Skp2's F-box domain also binds to CUL1, thus contributing to the assembly of the SCF^{Skp2} complex [6]. Although CUL3 mediates interactions to BTB proteins through the Skp1/BTB/POZ fold, it binds to a conserved helical structure carboxy-terminal of the BTB domain, which was named '3-box' for CUL3-interacting box [13] (Table 2). The CUL3-3-box association strengthens the CUL3-BTB protein interactions. Despite sharing the identical adaptor protein Elongin C (EloC), CUL2 and CUL5 direct the assembly of distinct E3 complexes: CRL2 with von Hippel-Lindau (VHL) or related BC box proteins, and CRL5 containing suppressors of cytokine signaling (SOCS)-box proteins



Box 2. E3 ubiquitin ligases

E3 ubiquitin ligases are a diverse group of enzymes that recognize both a substrate protein and an E2 ubiquitin-conjugating enzyme. E3 ubiquitin ligases can be subdivided into two major classes [57]: HECT-type and RING-type E3 ligases.

The single-molecule HECT-type E3 ligases are characterized by a Homologous to the E6-AP carboxyl terminus (HECT) domain that forms a thioester intermediate with ubiquitin as a prerequisite for ubiquitin transfer to the substrate protein.

In contrast, RING-type E3 ligases use RING (really interesting new gene)-zinc finger domains to recruit and allosterically activate an ubiquitin-charged E2 enzyme for direct ubiquitin transfer to the substrate. RING finger domains have a characteristic architecture of three β strands, one α -helical domain and two free loops that are arranged by Zn^{2+} ions. The loops are stabilized by a cluster of cysteine residues and up to two histidines [57]. U-box E3 ligases are a subgroup of the RING-type E3s and contain a structurally modified RING-motif (the U-box) that lacks the ability to chelate Zn^{2+} ions [58].

Of about 300 RING proteins expressed in human cells, the multi-subunit cullin-RING Ligase (CRL) complexes constitute the major group and are characterized by two signature components: a cullin (CUL) scaffold protein and the RING-finger protein ROC1 or ROC2 (also known as Rbx1/Hrt1 or Rbx2, respectively) [7].

(Figure 4). However, it is unclear whether CUL2 and CUL5 recognize specific determinants within VHL and SOCS-box proteins, respectively [14,15]. A recent study provided some insight into how CUL5 assembles into a

CRL5 complex with the HIV protein Virion infectivity factor (Vif; a SOCS-box protein), thereby yielding an E3 ligase that targets the human antiviral protein APOBEC3G for proteasomal degradation [16]. It seems that loops 2 and 5 of CUL5 are engaged in interactions with Vif's SOCS-box and zinc finger motif (H-(X)₅-C-(X)₁₇₋₁₈-C-(X)₃₋₅-H), respectively.

In summary, it seems that to assemble CRLs, cullins not only bind to a common recognition fold in the adaptor, such as the Skp1/BTB/POZ motif (Table 2), but also form interface interactions with structural determinants within the substrate-recognition molecules (SRMs) that include F-box, BTB, VHL and SOCS proteins. However, future structural and biochemical studies, using a larger set of substrates, are required to more rigorously define the 'SRM determinants.' To understand the differential ability of CUL2 and CUL5 to assemble CRL2 and CRL5, respectively, it is critical to solve their structures, especially the amino terminus.

There are no structures available that reveal the three-dimensional organization of either CUL7 or PARC. Although CUL7 resembles CUL1 in using the Skp1 adaptor [17], it remains to be determined how CUL7 selects F-box and WD-repeat-domain-containing protein 8 (Fbw8). At present, it is also unclear whether PARC forms a multi-subunit complex.

Localization and function

Cullin family proteins are involved in a diverse array of functions, including cell-cycle control, DNA replication

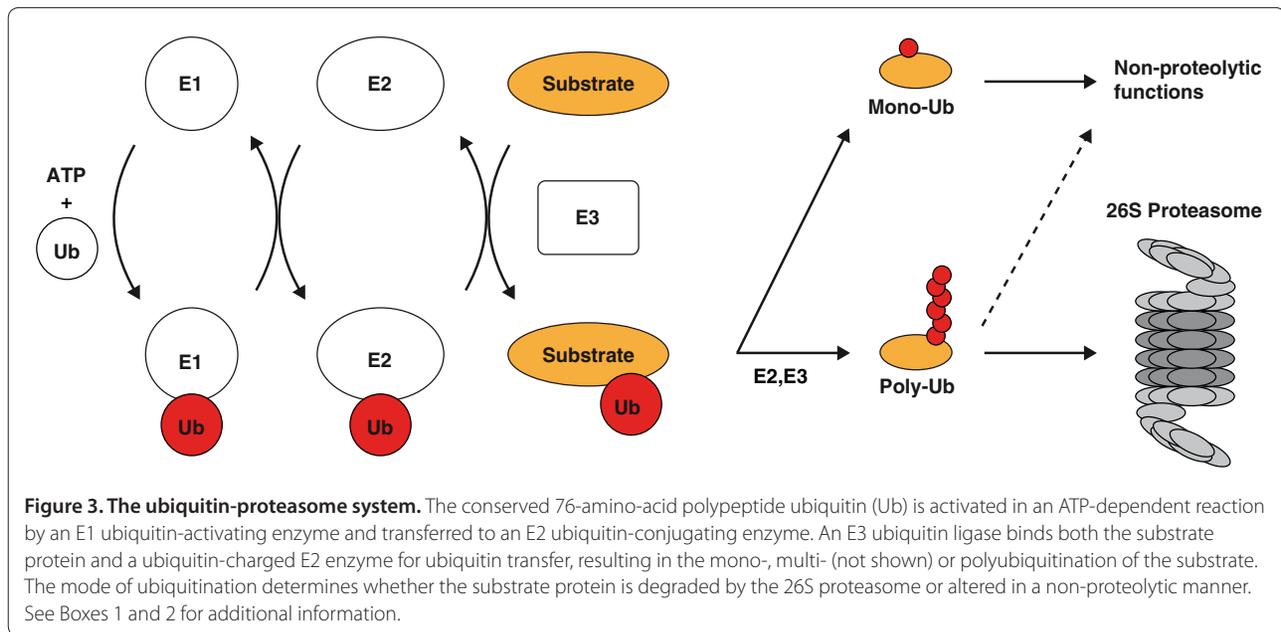


Table 2. Cullin structures and cullin-RING E3 complex assembly

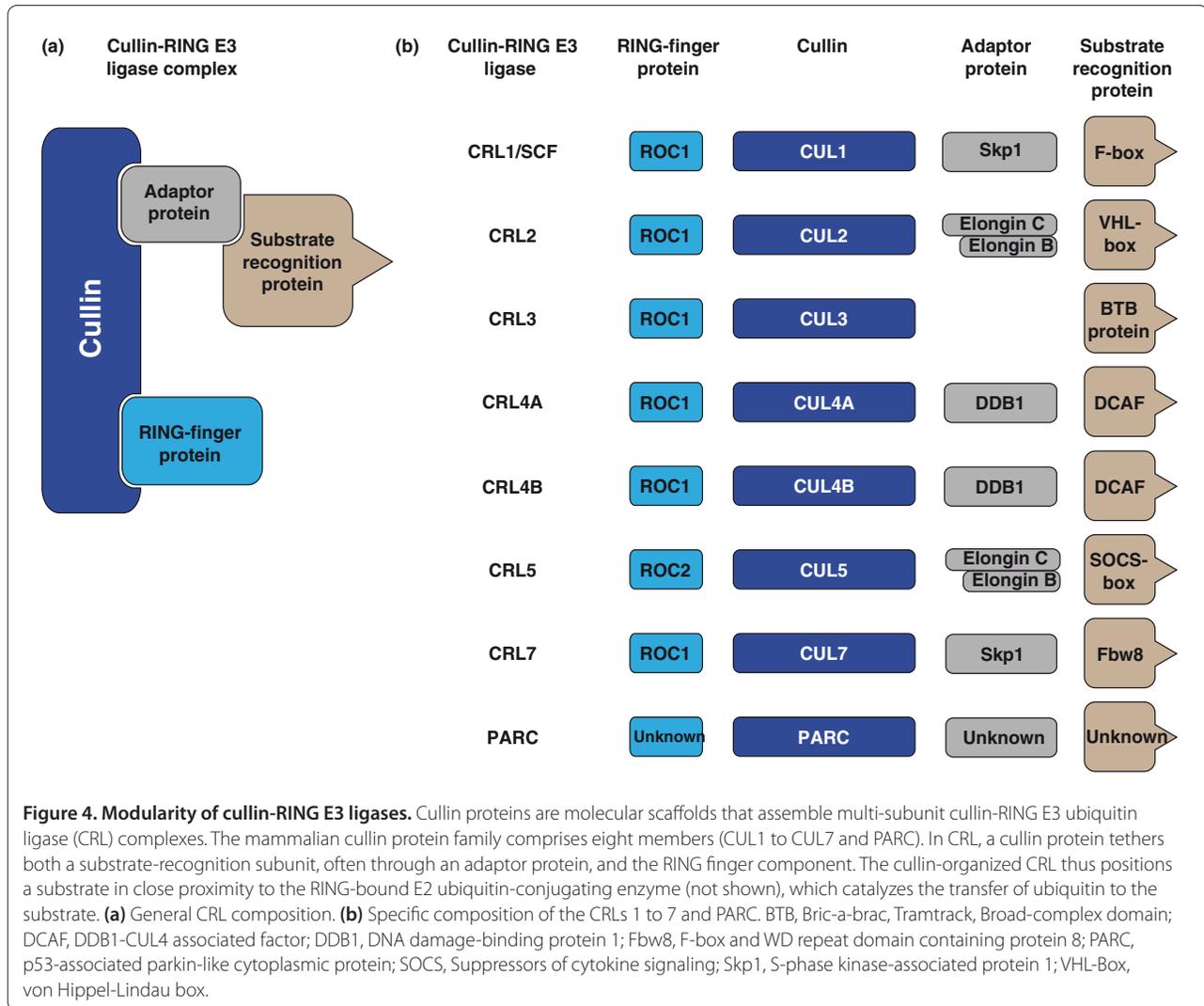
Protein	Adaptor	E3 components			Key recognition fold on adaptor	Key determinants on cullin NTD
		Substrate recognition	RING	Structures solved*		
CUL1	Skp1	F-box protein	ROC1/Rbx1	CUL1-Rbx1-Skp1-F box ^{Skp2} [6]; Skp1-Skp2 [60]	Skp1/BTB/POZ fold in Skp1 [6]	CUL1 H2 and H5 helices [6]
CUL2	EloC/EloB	VHL	ROC1/Rbx1	No CUL2 structure; VHL-EloC-EloB [60]	Skp1/BTB/POZ fold in EloC [6,61]	CUL2 H2 and H5 helices [14]
CUL3	BTB (adaptor-targeting)	BTB (adaptor-targeting)	ROC1/Rbx1	SPOP BTB-SBC [13]	BTB [13]; 3-box [13]	CUL3 H2 and H5 helices [62]
CUL4A	DDB1	DCAF	ROC1/Rbx1	DDB1-CUL4A-ROC1 [9]; DDB1-V protein [8]	BPB in DDB1 [9]	CUL4A H2 and H5 helices [9]; CUL4A amino-terminal extension [9]
CUL4B	DDB1	DCAF	ROC1/Rbx1	Not available	BPB in DDB1 (predicted)	CUL4B H2/H5 (predicted)
CUL5	EloC/EloB	SOCS protein	ROC2/Rbx2	CUL5 ^{CTD} -Rbx1 [37]; SOCS2-EloC-EloB [63]; HIV Vif-EloC-EloB [16]	Skp1/BTB/POZ fold in EloC [6,61]	CUL5 H2 and H5 helices [64]; CUL5 loop 2 (amino acids 51 to 60) [16]; CUL5 loop 5 (amino acids 118 to 134) [16]
CUL7	Skp1	Fbw8	ROC1/Rbx1	CUL7 CPH-p53 TD [65]	Skp1/BTB/POZ fold in Skp1 (predicted)	Unknown
PARC	Unknown	Unknown	Unknown	Not available	Unknown	Unknown

*Crystal structures of CRL complexes were solved with recombinant human proteins.

and development. The major physiological functions of the cullin family proteins have been revealed by genetic ablation experiments in a variety of metazoan model organisms, including mouse, *C. elegans* and *Drosophila* (Table 3). In *Arabidopsis*, CRLs regulate hormonal signaling, light responses, circadian rhythms and photomorphogenesis (for a recent review, see [18]). The cullin family proteins seem to be widely expressed and to locate both to the nucleus and cytoplasm, but there are no compelling data suggesting that cullin activity is

controlled by subcellular localization or by differential expression in a tissue-specific manner.

Cul1 and *Cul3* mouse knockout experiments have revealed their indispensable roles in cell cycle progression and early embryogenesis (Table 3). The role of CUL1 in cell cycle control is understood in considerable detail. It was the pioneering work using the budding and fission yeast systems that led to the discovery of cullins and other CRL components and their role in cell cycle control (reviewed in [19]). Work in *C. elegans* and *Drosophila* has



demonstrated the requirement of CUL1 for cell cycle progression (Table 3). Mechanistically, it is believed that the CUL1-based SCF regulates the mammalian cell cycle, at least in part, by using the Skp2 F-box protein, which directs the ubiquitin-dependent degradation of p27 and p21 (inhibitors of cyclin-dependent kinases), thereby activating cyclin-dependent kinases [20]. SCF malfunction has been linked to malignancy, as mutations in the Fbw7 F-box protein are frequently found in a variety of human cancers [21].

Studies with *Cul4* deletion in *C. elegans* have established a crucial role for CUL4 in DNA replication (Table 3). It is well accepted that the CRL4 complex with the DCAF protein Cdt2 as substrate-recognition molecule (referred to as CRL4^{Cdt2}) targets the replication initiation factor Cdt1 for degradation, thereby preventing DNA re-replication [22]. In mammals, CUL4A and CUL4B are believed to be functionally redundant, as deletion of *Cul4a* in mice resulted in viable animals and

relatively subtle phenotypes (Table 3). Clearly, complete understanding of the physiological functions of *Cul4* in mouse development requires future studies with animals lacking *Cul4b* and *Cul4a/Cul4b*.

There have been no mouse models for either *Cul2* or *Cul5*. However, CRL2^{pVHL} has a critical role in control of oxygen homeostasis, acting by targeting the α subunit of hypoxia-inducing transcription factor (HIF) for degradation (reviewed in [23]). Tissue-specific gene targeting of VHL in mice has demonstrated that efficient execution of CRL2^{pVHL}-mediated HIF-1 α proteolysis under normal levels of oxygen is fundamentally important for survival, proliferation, differentiation and normal physiology of many cell types (reviewed in [23]). These studies have explained the tumor suppressor function of VHL, whose germline mutations inactivate its ability to form the CRL2 complex or bind to HIF- α , leading to the formation of highly vascularized tumors such as renal clear-cell carcinomas.

Table 3. Major physiological roles of cullins revealed by deletion studies with model organisms

Protein	Mouse	<i>C. elegans</i>	<i>Drosophila</i>
CUL1	Cell cycle and embryogenesis [66,67], with KO phenotypes, including: embryonic lethality E5.5 to E6.5; accumulation of cyclin E; increased apoptosis in the ectoderm; large trophoblast giant cells in blastocysts	Cell cycle [1]; germline apoptosis [68]; sex determination [69]	Cell cycle [70,71]; apoptosis [72]; eye development [73]
CUL2	No mouse model	G1-to-S transition [74]; mitosis [74,75]; germline lineage [76]; meiosis [77-79]; polarity [77-79]; oogenesis [80]; MPK1 activation [80]; hypoxic response, aging [81,82]	
CUL3	Cell cycle and embryogenesis [83], with KO phenotypes including embryonic lethality <E7.5; accumulation of cyclin E; impaired S-phase entry; failure to endocycle in trophoblasts	Meiosis/mitosis transition [84]; mitosis [85]; meiosis [86]	Eye development [72]; sensory organ [87]; neurons [88]; hedgehog signaling [89,90]; actin cytoskeleton and cell movement [91,92]
CUL4	Deletion of <i>Cul4a</i> alone yields no major defects in development as mice lacking <i>Cul4a</i> exons 17 to 19 are viable and normal [93], and mice lacking <i>Cul4a</i> exons 4 to 8 are viable, showing mild decrease in mouse embryonic fibroblast proliferation [33]; a role in DNA repair as skin-specific CUL4A KO show increased resistance to UV-induced skin carcinogenesis [93]	DNA replication [22]	Cell cycle [94]; DNA damage response [95]
CUL5	No mouse model	Oogenesis [80]; MPK1 activation [80]	Cell fate specification [96]; synapse formation [96]; oogenesis [97]
CUL7	Embryonic development, as KO showed neonatal death [98]; required for growth in embryo and placenta [98]; formation of vascular structure [98]		
PARC	Not essential for development, as KO is viable and normal [99]		

E, embryonic day; KO, knockout.

Two hereditary human diseases have been linked to genes encoding members of the cullin protein family. Mutations in the *CUL7* gene were linked to 3-M syndrome (Online Mendelian Inheritance in Man (OMIM) ID 273750), an autosomal-recessive disorder characterized by pre- and postnatal growth retardation (final height 3 to 4 standard deviations below the mean for the population), facial dysmorphism, large head circumference, normal intelligence, and skeletal anomalies that include long slender tubular bones and tall vertebral bodies [24,25]. Huber *et al.* [24,25] identified *CUL7* gene mutations in 52 out of 62 cases (84%), arguing for *CUL7* as the major disease gene of 3-M syndrome. The mutations were located throughout the *CUL7* gene and most are predicted to cause premature termination of translation. Reverse transcriptase (RT)-PCR analysis of patient fibroblast mRNA detected a *CUL7*-specific transcript, but at reduced levels, arguing that *CUL7* mRNA is expressed at least in a subset of 3-M syndrome patients. Approximately 50% of the mutations identified by Huber *et al.* [24,25] are located within the cullin homology domain (exons 19 to 24), which is responsible for ROC1 binding. Biochemical characterization of the *CUL7* nonsense and missense mutations Arg1445X (where X indicates a stop codon) and His1464Pro, respectively, were shown to render *CUL7* deficient in recruiting ROC1. Arg1445X was predicted to yield a truncated *CUL7*

polypeptide (lacking the 254 carboxy-terminal amino acids), and His1464Pro was predicted to introduce a structural alteration in the cullin homology domain [24].

A study by Maksimova *et al.* [26] identified 43 patients from 37 Yakut families, a geographically isolated ethnic group in Russia, with a short stature syndrome similar to 3-M syndrome. A common mutation in the *CUL7* gene, insertion T at position 4582 in exon 25, was identified that is predicted to cause a frameshift and subsequent premature stop codon at position 1553 (Q1553X).

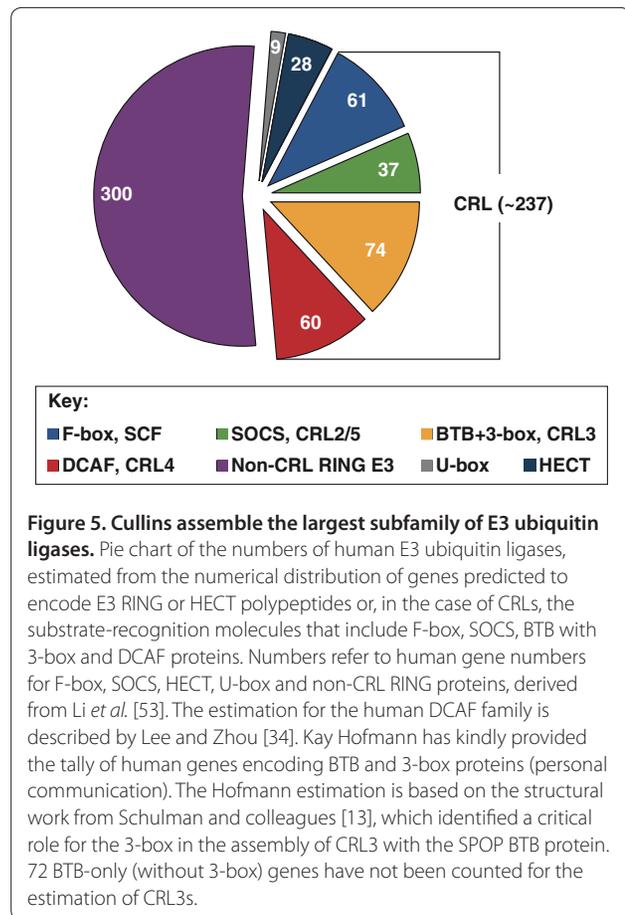
Given that cyclin D1 [27] and insulin receptor substrate 1 (IRS-1) [28] are potential proteolytic targets of the *CUL7* E3 ligase, it is tempting to speculate that either disturbed cyclin D1-dependent mechanisms or dysregulated IRS-1-mediated signaling pathways might contribute to the pathomechanism of 3-M syndrome. Altogether, studies with 3-M and Yakut patients, combined with proliferative defects observed in *Cul7* knockout mice (Table 3), have strongly suggested a prominent role for *CUL7* in growth regulation (reviewed by [29]).

Several familial mutations in the *CUL4B* gene were associated with X-linked mental retardation syndrome (XLMR; OMIM 300639) [30]. The authors [30] reported three truncating, two splice-site and three missense variants at conserved amino acids in the *CUL4B* gene on Xq24 in 8 of 250 families (3%) with XLMR. During adolescence of these patients a syndrome emerged with

delayed puberty, hypogonadism, growth retardation, foot abnormalities, relative macrocephaly, central obesity, aggressive outbursts and seizures. The complex phenotype of patients with *CUL4B* mutations argues for pleiotropic roles of *CUL4B* that remain to be determined.

The cullin-based CRLs function through their cognate substrate-recognition molecules, such as the F-box, SOCS, BTB and DCAF proteins (Figure 4 and Table 2). The F-box, SOCS, BTB and DCAF protein families each contain a distinct motif that is recognized by an adaptor molecule, which is linked to a cognate cullin (Figure 4). For example, humans contain about 61 F-box proteins, all of which can bind to Skp1 through the F-box domain. Through Skp1, which binds to CUL1 (Table 2), most of the F-box proteins can thus be assembled into the SCF E3 complex (Figure 4). Bioinformatics studies have identified hundreds of human genes that are predicted to encode F-box, SOCS, BTB and DCAF proteins, thereby potentially forming over 200 CRLs (Figure 5). Although the extent of the CRL family requires experimental verification, it is nonetheless reasonable to assume that CRLs target numerous cellular protein substrates and hence have an impact on all biological processes. In addition, studies with F-box proteins, including β -TrCP, Skp2 and Fbw7, demonstrate an ability of one substrate recognition protein to bind multiple substrates, thereby expanding the functional range of CRLs. For recent reviews on the diverse targeting functions of these proteins, see [10,31] (F-box family), [32] (BTB family) and [33,34] (DCAF family).

Most, if not all, cullins are found covalently conjugated with an ubiquitin-like molecule, Nedd8. This modification, termed neddylation, activates the E3 ligase activity of CRLs by promoting substrate polyubiquitination (reviewed by [35]). Recent studies have suggested conformation-based mechanisms that explain the activating role of neddylation. *In vitro* mutagenesis experiments have suggested that the interactions between human ROC1 and CUL1's carboxy-terminal tail in the unmodified state render SCF inactive [36]. It was proposed that the conjugation of Nedd8 to the residue K720 of CUL1 induces drastic conformational changes in CUL1 that liberate ROC1, thereby driving SCF into an active state. This hypothesis was supported by recent structural studies by Schulman and colleagues [37] that revealed extensive conformational changes in CUL5 when conjugated with Nedd8. Another activating mechanism, proposed by Schulman and colleagues [37], suggests that the neddylation-mediated conformational changes in cullin enabled the repositioning of the RING-tethered ubiquitin-loaded E2 to a bound substrate for catalysis. In support of this model, *in vitro* cross-linking experiments have revealed that neddylation brought a SCF substrate into a close proximity to an E2 ubiquitin-conjugating enzyme [38].



Neddylation is reversed by the COP9 signalosome, which enzymatically removes Nedd8 from a cullin molecule [39]. COP9 is an eight-subunit complex that was originally identified as a suppressor of plant photomorphogenesis [40]. It was shown that COP9's Jab1/Csn5 subunit contains a Jab1/MPN domain metalloenzyme (JAMM) motif critical for COP9's Nedd8 isopeptidase activity [39]. It is thus believed that CRL activities are dynamically controlled by cullin neddylation-deneddylation cycles. It was observed that an SCF complex bound to a substrate contained higher levels of neddylated CUL1, suggesting that substrate-E3 interactions may trigger neddylation [41]. The detailed mechanism, however, remains elusive.

CRL is also regulated by Cullin-associated and neddylation-dissociated-1 (CAND1), which inhibits the E3 ligase activity of CRLs by binding to all cullins in their un-neddyated forms [42,43]. The CUL1-CAND1 interaction is understood at the structural level, as the human CUL1-Rbx1-CAND1 complex showed that CAND1 binds both the CUL1 amino and carboxyl termini [44]. However, a recent study [45] has revealed that only a small fraction of cullin is bound to CAND1 in

human cells. Future studies are required to determine the precise role of CAND1 in regulating CRLs.

Frontiers

By organizing CRLs that presumably direct numerous substrates to ubiquitin-dependent degradation, the cullin family proteins build a cellular regulatory network of fundamental importance in controlling protein homeostasis, thereby altering a wide range of biological processes, from cell cycle regulation to signal transduction.

However, there are several areas that need attention. The development of cutting edge technology for the identification of CRL substrates is crucial. Although bioinformatics predicts a large number of CRLs (Figure 5), we still have knowledge on only a handful of substrates. Proteomics-based approaches and newly developed global protein stability profiling technology [46] have proven effective in the identification of novel substrates. However, it remains to be seen whether these methods, coupled with agents that affect cell signaling, could lead to the isolation of substrates, whose turnover rates are dictated by often transient post-translational modifications, such as phosphorylation [10], prolyl hydroxylation [23] and glycosylation [47].

Although the assembly of a majority of CRLs is understood in considerable detail (Table 2 and Figure 4), little is known about the control of the assembly in cells. It has long been suggested that the substrate-CRL interactions dictate the cullin neddylation-deneddylation cycle, which turns on and off the CRL's E3 ubiquitin ligase activity. However, the mechanism by which this is achieved is elusive.

Given the intricate role of cullins and CRLs in a multitude of biological processes, it is likely that cullin dysfunction will emerge as a pathogenetic factor in diseases. Indeed, *CUL7* and *CUL4B* mutations have been identified in human disorders, but further studies are required to determine the underlying pathomechanisms.

Genetic studies in organisms from yeast to mouse have revealed a prominent role for cullins and CRLs in cell-cycle progression (Table 3). Dysfunction of CRL activities has been associated with oncogenic transformation (reviewed by [48]). Thus, targeting CRLs is an emerging frontier in rational drug design. Recent advances have validated efforts in drug-targeting the ubiquitin-proteasome system, with the proteasome inhibitor bortezomib now approved for the treatment of patients with multiple myeloma or mantle cell lymphoma (reviewed by [49]). A small molecule inhibitor (MLN4924) suppressing the Nedd8 activating pathway is currently in clinical trials, having demonstrated success in tumor suppression in animal model studies [50], and two small molecule inhibitors have been identified recently to inhibit SCF activities by different mechanisms [51,52].

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