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 (cul1 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 ‘Cula’, ‘Culβ’ and ‘Culy’, 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 (Cula), whereas the Cul3 and Cul4A/4B genes evolved from two distinct ancestors, the Culβ and Culy gene, respectively [4]. Notably, Cul7 and Parc, found only in chordates, are highly similar in
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 [94]. A substrate protein can be conjugated with just one ubiquitin (monoubiquitination), one ubiquitin molecule at different lysines residues (multoubiquitination) 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 Saccharomyces 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 Saccharomyces 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
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/

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

| Gene | Cul1 | Cul2 | Cul3 | Cul3a | Cul3b | Cul4 | Cul4a | Cul4b | Cul5 | Cul6 | Cul7 | Cul8 | Parc |
|------|------|------|------|-------|-------|------|-------|-------|------|------|------|------|------|
| Human | Gene ID | 8454 | 8453 | 8452 | NP | NP | 8451 | 8450 | 8065 | NP | 9820 | NP | 23113 |
| | Chromosomal localization | 7q36.1 | 10p11.21 | 2q36.2 | NP | NP | 13q34 | Xq23 | 11q22-q23 | NP | 6p21.1 | NP | 6p21.1 |
| | Introns | 21 | 20 | 15 | NP | NP | 19 | 19/21 | 18 | NP | 25 | NP | 40 |
| | Isoforms | 1 | 1 | 1 | NP | NP | 2 | 2 | 1 | NP | 2 | NP | 1 |
| Mouse | Gene ID | 26965 | 71745 | 26554 | NP | NP | 99375 | 72584 | 75717 | NP | 66515 | NP | 78309 |
| | Chromosomal localization | 6;B3 | 18;A1 | 1;C4 | NP | NP | 8;A1.1 | X;A2 | 9;C | NP | 17;C | NP | 17;C |
| | Introns | 21 | 20 | 15 | NP | NP | 19 | 22 | 18/19 | NP | 25 | NP | 41 |
| | Isoforms | 1 | 1 | 1 | NP | NP | 1 | 1 | 2 | NP | 1 | NP | 1 |
| Rat | Gene ID | 362356 | 361258 | 301555 | NP | NP | 361181 | 302502 | 64624 | NP | 363191 | NP | 316228 |
| | Chromosomal localization | 4q24 | 17q12.1 | 9q34 | NP | NP | 16q12.5 | Xq11 | 8q24 | NP | 9q12 | NP | 9q12 |
| C. elegans | Gene ID | 176466 | 176806 | 178547 | NP | NP | 174198 | NP | 179413 | NP | 178214 | NP | NP |
| | Chromosomal localization | III | III | V | NP | NP | II | NP | V | IV | NP | NP |
| Drosophila | Gene ID | (lin19) | 35420 | 34896 | NP | NP | 35780 | NP | 43434 | NP | NP | NP | NP |
| | Chromosomal localization | 2R; 43F1-43F2 | 2L; 39E3-39E6 | 2L; 35C5-35C6 | NP | NP | 2R; 44A4-44A4 | NP | 3R; 98F6-98F6 | NP | NP | NP | NP |
| A. thaliana | Gene ID | (ATCUL1) 839415 | 839415 | NP | (ATCUL3) 843303 | 839226 | 843463 | NP | NP | NP | NP | NP | NP |
| | Chromosomal localization | 4 | 1 | NP | 1 | 1 | 5 | NP | NP | NP | NP | NP | NP |
| S. cerevisiae | Gene ID | (Cdc53) 851424 | 851424 | NP | 852886 | NP | NP | NP | NP | NP | NP | NP | NP |
| | Chromosomal localization | V | NP | VII | NP | NP | NP | NP | NP | NP | X | NP |
| S. pombe | Gene ID | 2542393 | 2542637 | NP | (pcu4) 2543116 | NP | NP | NP | NP | NP | NP | NP | NP |
| | Chromosomal localization | I | NP | I | 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.
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 deetiolated 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<sup>Skp2</sup> 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.
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)$_{17-18}$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, and transcriptional regulation [18]. Cullins have been implicated in a wide range of cellular processes, including mitotic checkpoint, DNA damage response, and cell proliferation [19]. The ubiquitination and degradation of specific substrates by cullins play a crucial role in the regulation of cell growth and survival.

| Cullin | Length | Number of Cullin Repeats |
|--------|--------|--------------------------|
| CUL1   | 776aa  | 3                        |
| CUL2   | 745aa  | 3                        |
| CUL3   | 768aa  | 3                        |
| CUL4A  | 759/659aa | 3                       |
| CUL4B  | 913/895aa | 3                       |
| CUL5   | 780aa  | 3                        |
| CUL7   | 1782/1698aa | 3                       |
| PARC   | 2517aa | 3                        |

Figure 2. Cullin protein domain organization in humans. Cullin repeat 1 (CR1) anchors the cognate adaptor proteins, and the cullin homology domain (CH) at the carboxyl terminus is critical for binding of the RING-finger protein. The red line indicates the position of the neddylation site. For CUL7 and PARC the neddylation site is based on consensus sequence alignment without experimental verification. Size and location of the individual domains are schematic representations and do not depict the exact proportions. Abbreviations: aa, amino acids; CH, cullin homology domain; CPH, conserved domain in CUL7, PARC and HERC2; CR, cullin repeat; DOC, a domain similar to the DOC1 domain of the anaphase-promoting complex/cyclosome but of unknown function; IBR, in between RING; RING, really interesting new gene.

**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].
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

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

| Protein | Adaptor | Substrate recognition | RING | Structures solved* | Key recognition fold on adaptor | Key determinants on cullin NTD |
|---------|---------|-----------------------|------|--------------------|-------------------------------|--------------------------------|
| CUL1    | Skp1    | F-box protein         | ROC1/Rbx1 | CUL1-Rbx1-Skp1-F boxRbc1 [6]; Skp1-Skp2 [60] | Skp1/CTBP/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/CTBP/POZ fold in EloC [6,61] | CUL2 H2 and H5 helices [14] |
| CUL3    | BTB (adaptor-targeting) | BTB (adaptor-targeting) | ROC1/Rbx1 | SPOP BTB-5B [13] | BTB [13], 3-box [13] | CUL3 H2 and H5 helices [62] |
| CUL4A   | DDB1    | DCAD                 | 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 | CUL5SOCS1-Rbx1 [57]; SOCS2-eloC-eloB [63]; HIV-FIV-eloC-eloB [16] | 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/CTBP/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.
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 CRL4Cdt2) 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/^VHL^ 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/VHL-mediated HIF-1α 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.
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 enable 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 photosynthesis [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-neddylated 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 $CUL14$ 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 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 myeloma or mantle cell lymphoma (reviewed by [49]).

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