Ubiquitin-mediated pathways in *C. elegans*[*]

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Abstract

Ubiquitin is a highly conserved 76 amino acid polypeptide, which is covalently attached to target proteins to signal their degradation by the 26S proteasome or to modify their function or localization. Regulated protein degradation, which is associated with many dynamic cellular processes, occurs predominantly via the ubiquitin-proteasome system. Ubiquitin is conjugated to target proteins through the sequential actions of a ubiquitin-activating enzyme, ubiquitin-conjugating enzymes, and ubiquitin-protein ligases. The nematode *Caenorhabditis elegans* has one ubiquitin-activating enzyme, twenty putative ubiquitin-conjugating enzymes, and potentially hundreds of ubiquitin-protein ligases. Research in *C. elegans* has focused on the cellular functions of ubiquitin pathway components in the context of organismal development. A combination of forward genetics, reverse genetics, and genome-wide RNAi screens has provided information on the loss-of-function phenotypes for the majority of *C. elegans* ubiquitin pathway components. Additionally, detailed analysis of several classes of ubiquitin-protein ligases has led to the identification of their substrates and the molecular pathways that they regulate. This review presents a

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comprehensive overview of ubiquitin-mediated pathways in *C. elegans* with a description of the known components and their identified molecular, cellular, and developmental functions.

1. Overview of ubiquitin conjugation

Ubiquitin (Ub) is a ubiquitously expressed and highly conserved 76 amino acid polypeptide (Hershko and Ciechanover, 1998; Figure 1A). The covalent tandem attachment of multiple Ub to a target protein to form poly-ubiquitin chains can mark the protein for degradation by the 26S proteasome. Ub is covalently attached to substrate proteins by the concerted actions of three classes of enzymes (Hershko and Ciechanover, 1998). A ubiquitin-activating enzyme (E1) uses one ATP molecule to bind Ub via a thiolester linkage. The activated Ub is transferred to a ubiquitin-conjugating enzyme (E2), also via a thiolester linkage. The E2 is brought to the substrate by binding a ubiquitin-protein ligase (E3) that binds both the E2 and the substrate. Once bound to an E3, the E2 either directly transfers Ub to the substrate or transfers the Ub through a thiolester linkage to the E3, which then transfers the Ub to the substrate. Multiple rounds of E2 interactions with substrate-bound E3 are required to produce a poly-Ub chain on the substrate. In a few cases, the E2-E3 combination is not capable of adding more than a few Ub, and in this situation a ubiquitin chain assembly factor (E4) is required for the conjugation of additional Ub to form a poly-Ub chain (Koegl et al., 1999).

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                     6    11              27 29  33             48              63
C.e. Ub:    MQIFVKTLTGKTITLEVEASDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG  76
H.s. Ub:    MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG  76
S.c. Ub:    MQIFVKTLTGKTITLEVESSDTIDNVKSKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG  76
```

B

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repeat 1:   MQIFVKTLTGKTITLEVEASDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG   76
repeat 2:   MQIFVKTLTGKTITLEVEASDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG  152
repeat 3:   MQIFVKTLTGKTITLEVEASDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG  228
repeat 4:   MQIFVKTLTGKTITLEVEASDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG  304
repeat 5:   MQIFVKTLTGKTITLEVEASDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG  380
repeat 6:   MQIFVKTLIGKTITLEVEASDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG  456
repeat 7:   MQIFVKTLTGKTITLEVEASDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG  532
repeat 8:   MQIFVKTLTGKTITLEVEASDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG  608
repeat 9:   MQIFVKTLTGKTITLEVEASDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG  684
repeat 10:  MQIFVKTLTGKTITLEVEASDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG  760
repeat 11:  MQIFVKTLTGKTITLEVEASDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG  836
            DI                                                                            838
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Figure 1. A) Alignment of individual ubiquitin polypeptides from *C. elegans (C.e.)*, *H. sapiens (H.s.)*, and *S. cerevisiae (S.c.)*. Differences between Ub residues are boxed. Note that *C. elegans* and humans only differ at one position relative to each other and at three positions relative to budding yeast. The locations of the seven lysines in Ub are marked with the residue numbers provided above the alignment. **B)** Translation of the *C. elegans ubq-1* polyubiquitin gene. The amino acid sequence is presented at 76 amino acids per line so that individual ubiquitin repeats are aligned. Note that repeat 6 has a different amino acid at position 9 of the repeat (yellow box, isoleucine rather than threonine). There are two amino acids after the final ubiquitin repeat.

Ub is conjugated to target proteins or other Ub through a bond between the conserved C-terminus of Ub and the ε-amino group of a lysine residue on the target protein or other Ub (Hershko and Ciechanover, 1998). A minimum of four tandemly-attached Ub are required to allow recognition of the target protein by the 26S proteasome, presumably because a tetramer of poly-Ub assumes a higher order structure that is required for recognition (Pickart, 2000). Ub has seven lysine (Lys) residues (Figure 1A) and Ub can be conjugated to several of these Lys residues. Poly-Ub chains created by conjugation through a Lys-48 linkage targets a substrate for degradation by the 26S proteasome. In contrast, poly-Ub formed by Lys-63 conjugation does not lead to proteasome-mediated degradation, but instead is associated with the regulation of endocytosis or changes in target

protein function (Schnell and Hicke, 2003). Similarly, conjugation of a single Ub (mono-ubiquitination) or less than four Ub can affect protein activity, including aspects of transcriptional regulation, protein trafficking, and endocytosis (Schnell and Hicke, 2003). The functions of poly-Ub chains created with Lys-11 and Lys-29 linkages have not been determined (Aguilar and Wendland, 2003).

Ubiquitin-mediated proteolysis is the most important pathway for the degradation of nuclear and cytosolic proteins. Inactivation of the Ub proteolytic pathway inhibits the degradation of the majority of cellular proteins, regardless of whether the proteins have short or long half-lives (Rock et al., 1994). Given the central importance of ubiquitin-mediated protein degradation in a range of cellular processes, it is not surprising that Ub-mediated pathways are important for multiple aspects of *C. elegans* development and cellular physiology.

2. 26S Proteasome

The 26S proteasome is a conserved chambered protease complex that is present in both the cytoplasm and the nucleus (Wojcik and DeMartino, 2003). It consists of a 20S proteasome, a central core containing proteolytic subunits, and two 19S regulatory complexes that bind to ubiquitinated substrates, cleave off ubiquitin, and then unfold and translocate the substrate into the 20S core (Pickart and Cohen, 2004). *C. elegans* has 14 conserved subunits that comprise the 20S core, as well as 18 conserved 19S components (Davy et al., 2001). RNAi depletion of proteasome components during larval stages produces larval arrest and lethality while RNAi depletion in adult hermaphrodites produces progeny that arrest at the one-cell stage with defective meiosis I, indicating the central importance of this pathway (Takahashi et al., 2002; Gonczy et al., 2000).

3. Ubiquitin

There are two loci for ubiquitin (Ub) in *C. elegans*, *ubq-1* and *ubq-2*. *ubq-1* is a polyubiquitin locus (Graham et al., 1989). The predominant splice form of *ubq-1* encodes an 838 amino acid peptide that includes 11 tandem Ub sequences (Figure 1B). The polyubiquitin structure of the locus is common to other eukaryotic species (Schlesinger and Bond, 1987). The polyubiquitin protein is post-translationally cleaved into individual Ub peptides by ubiquitin C-terminal hydrolases (Johnston et al., 1999). The Ub peptides of *ubq-1* are identical with the exception of repeat 6, which substitutes an isoleucine for a highly conserved threonine at position 9 of the repeat (Figure 1B). The functional significance of this altered Ub peptide is not known. In *C. briggsae*, this atypical Ub repeat is not present, instead the orthologous polyubiquitin locus comprises ten Ub repeats that are all identical to the predominant *C. elegans* Ub sequence.

The second Ub locus, *ubq-2*, includes an intact canonical Ub fused to the L40 ribosomal large subunit protein (Jones and Candido, 1993). This fusion gene is broadly conserved in eukaryotes (Schlesinger and Bond, 1987). The *ubq-2* locus contains the only copy of the L40 ribosomal subunit in the *C. elegans* genome. In yeast, the hybrid protein is rapidly cleaved to form Ub and the L40 ribosomal subunit (Finley et al., 1989). The transient presence of Ub in the fusion protein promotes the incorporation of the L40 subunit into ribosomes (Finley et al., 1989). Once cleaved, the Ub is functional for covalent attachment to proteins (Ozkaynak et al., 1987).

RNAi of *ubq-1* or *ubq-2* produces a one-cell stage arrest during the meiotic divisions, similar to inactivation of the proteasome (Gonczy et al., 2000; Piano et al., 2000). The relative importance of *ubq-1* vs *ubq-2* is not known, as RNAi is expected to inactivate both genes due to their extensive homology (Tijsterman et al., 2002).

4. Ubiquitin-activating enzyme (E1)

As in other eukaryotes, there is only a single ubiquitin-activating enzyme in *C. elegans*, UBA-1. Disruption of UBA-1 activity would be expected to completely inactivate the Ub proteolytic pathway. However, while RNAi of *uba-1* produces an embryonic arrest, it is not as penetrant as RNAi for *ubq-1* or particular proteasome components, perhaps because, as an enzyme, it is more resistant to effects of depletion (Maeda et al., 2001; Kamath et al., 2003; Simmer et al., 2003; Piano et al., 2000; Gonczy et al., 2000).

5. Ubiquitin-conjugating enzymes (E2s)

There are 22 proteins with homology to ubiquitin-conjugating enzymes (UBCs) in *C. elegans*, with an additional three ubiquitin E2 variants (UEVs) that lack the critical cysteine residue in the catalytic site (Jones et al., 2002). The *C. elegans* UBCs are numbered *ubc-1-3*, *6-9*, and *12-26*; with numbers *4*, *5*, *10*, and *11* skipped. The

numbering of UBCs in *C. elegans* does not match that of *S. cerevisiae* or humans, and orthologous groupings are presented in Table 1. Note that *ubc-9* and *ubc-12* designate conjugating enzymes for the Ub-like proteins SUMO (SMO-1) and Nedd8 (NED-8), respectively, and do not conjugate Ub (Jones and Candido, 2000; Jones et al., 2002). The functions of the E2 genes have been studied in systematic RNAi screens. Only two of the 20 E2 enzymes that are specific for Ub-conjugation are essential for embryonic viability, *ubc-2*/*let-70* and *ubc-14* (Table 1; Jones et al., 2002). This is surprising given the relatively large number of E3 genes that are associated with embryonic lethal phenotypes (see below). This suggests either that UBC-2 and UBC-14 are the only E2s that function with these essential E3s or that there is significant redundancy of E2 function. In general, very little is known about which *C. elegans* E2s function with particular E3s. Of the remaining Ub-specific E2 genes, the RNAi depletion of four are associated with post-embryonic phenotypes: *ubc-19* RNAi produces unhealthy larvae; *ubc-20* RNAi produces an impenetrant L3 or L4 larval arrest; *ubc*-25 RNAi produces defects in neuromuscular function; and *ubc-18* RNAi produces animals that have slightly slower growth and reduced brood sizes but otherwise appear wild-type (Maeda et al., 2001; Jones et al., 2002; Schulze et al., 2003; Fay et al., 2003; Table 1). Interestingly, *ubc-18* functions redundantly with *lin-35* Rb to promote normal pharyngeal morphogenesis, and the simultaneous inactivation of both genes causes synthetic embryonic lethality (Fay et al., 2003). For the remaining 14 E2s, inhibition by RNAi was not associated with any apparent defects.

Table 1. Ubiquitin-conjugating enzymes in *C. elegans*: homologs and loss-of-function phenotypes.

C. elegans	**Peptide-conjugated**	***S. cerevisiae***	***Drosophila***	**Human**	**Phenotypes**	**References**
ubc-1	Ub	*UBC2*	*UbcD6*	*UBE2A; UBE2B*	WT (RNAi)	Jones et al., 2002; Kamath et al., 2003
ubc-2/let-70	Ub	*UBC4; UBC5*	*effete; Dsi/Ubc1*	*UBE2D1/ UBCH5A; UBE2D2/ UBCH5B; UBE2D3/ UBCH5C*	embryonic arrest at pre-comma stage (RNAi)	Jones et al., 2002
ubc-3	Ub	*UBC3/CDC34*	*CG7656*	*CDC34; FLJ20419*	WT (RNAi)	Jones et al., 2002; Fraser et al., 2000
ubc-6	Ub	*UBC6*	*CG5823*	*NCUBE1*	WT (RNAi)	Jones et al., 2002; Kamath et al., 2003
ubc-7	Ub	*UBC3*	*CG9602*	*UBE2G1*	WT (RNAi)	Jones et al., 2002; Kamath et al., 2003; Gonczy et al., 2000; Maeda et al., 2001
ubc-8	Ub	*UBC8*	*CG2257; CG14739*	*UBE2H*	WT (RNAi)	Jones et al., 2002
ubc-9	SUMO	*UBC9*	*lesswright*	*UBE2I*	embryonic arrest post-gastrulation before muscle movement (RNAi)	Jones et al., 2002
ubc-12	NED-8 (Nedd8)	*UBC12*	*CG7375*	*UBE2M*	embryonic arrest at the comma stage (RNAi)	Jones et al., 2002
ubc-13	Ub	*UBC13*	*bendless;*	*UBE2N;*	WT (RNAi)	Jones et al.,

C. elegans	**Peptide-conjugated**	***S. cerevisiae***	***Drosophila***	**Human**	**Phenotypes**	**References**
			CG3473	*BAA93711*		2002
ubc-14	Ub	*UBC7*	*courtless*	*UBE2G2*	embryonic arrest post-gastrulation before muscle movement (RNAi)	Jones et al., 2002
ubc-15	Ub	*UBC6*	*CG5823*	*NCUBE1*	WT (RNAi)	Jones et al., 2002; Kamath et al., 2003
ubc-16	?	-	*CG7220*	*BAA91954*	WT (RNAi)	Jones et al., 2002; Fraser et al., 2000
ubc-17	?	-	*CG6303*	*BAB14320; BAB14724*	WT (RNAi)	Jones et al., 2002; Kamath et al., 2003
ubc-18	Ub	-	*CG17030; UbcD10; Ubc84D*	*UBE2L1; UBE2L3/ UBCH7; UBE2L6*	reduced growth rate and brood size (mut)	Fay et al., 2003
ubc-19	?	-	-	-	unhealthy larvae (RNAi)	Maeda et al., 2001
ubc-20	Ub	*UBC1*	*UbcD4*	*HIP2*	impenetrant L3 & L4 larval arrest (RNAi)	Jones et al., 2002
ubc-21	Ub	*UBC1*	*UbcD4*	*HIP2*	WT (RNAi)	Jones et al., 2002; Kamath et al., 2003
ubc-22	Ub	-	*CG17030; UbcD10; Ubc84D*	*UBE2L1; UBE2L3/ UBCH7; UBE2L6*	WT (RNAi)	Jones et al., 2002; Kamath et al., 2003
ubc-23	Ub	*UBC1*	*UbcD4*	*HIP2*	WT (RNAi)	Jones et al., 2002; Kamath et al., 2003
ubc-24	?	-	-	-	WT (RNAi)	Jones et al., 2002; Kamath et al., 2003
ubc-25	?	-	*CG2924*	*UBE2Q1; UBE2Q2*	defective postembryonic neuromuscular function (RNAi)	Schulze et al., 2003
ubc-26	Ub	*UBC6*	*CG5823*	*NCUBE1*	-	-

Orthologous groupings are based on published phylogenetic analysis (Jones et al., 2002; Schulze et al., 2003), with updates of other species homolog names. *ubc-26* (*Y110A2AM.3*), was named in this study. The peptide predicted to be conjugated (Ub or Ub-like) is derived from information of homologs in other species when not known in *C. elegans*. Only the more severe phenotypes are listed. Phenotypes derived from RNAi or mutant analysis are denoted (brackets). WT = wild-type phenotype. References for the phenotypes listed are given.

6. Ubiquitin-protein ligases (E3s)

There are four major classes of ubiquitin ligases: HECT-domain proteins; U-box proteins; monomeric RING finger proteins; and multisubunit complexes that contain a RING finger protein (Passmore and Barford, 2004). HECT-domain E3s are unique in that Ub is transferred to a conserved cysteine residue of the E3 in a thiolester linkage, and then the E3 transfers the Ub to the substrate (Passmore and Barford, 2004; Figure 2). This function as a covalent intermediary in the transfer of Ub is not found in other classes of E3 proteins. The RING finger motif (Really Interesting New Gene) comprises eight cysteine or histidine residues that bind two Zn^{2+} ions in a cross-brace structure (Fang et al., 2003). The U-box is structurally similar to the RING finger motif, but it does not bind Zn^{2+}, instead, hydrogen bonds take the place of the Zn^{2+} in the structure (Fang et al., 2003). Monomeric RING finger E3s and U-box E3s bind to both the substrate and the E2 enzyme (Figure 2). In multimeric RING finger complexes, the RING finger protein binds the E2 while other proteins in the complex bind the substrate. These multimeric complexes fall into two classes: cullin-based complexes, and the APC/C (anaphase promoting complex/cyclosome), which contains the cullin-like protein APC2 (Vodermaier, 2004).

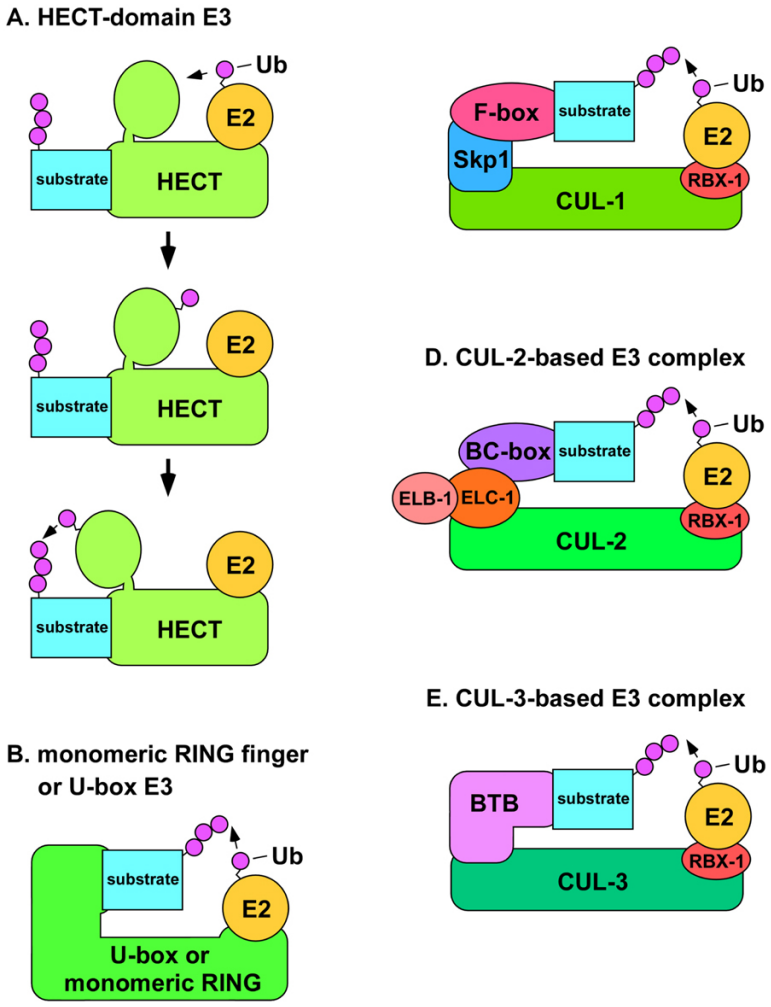

Figure 2. A) Structural model of HECT-domain E3 complex. The mechanism for the conjugation of Ub to a substrate by a HECT-domain E3 is shown. The E2 binds the N-terminal lobe of the HECT-domain E3 (top) and transfers Ub to the C-terminal lobe via a thiolester linkage (middle). The C-terminal lobe swivels on a hinge-loop and catalyzes the transfer of Ub to the substrate protein (bottom). **B)** Model of U-box or monomeric RING finger E3s. The U-box or RING finger domains of the E3 are directly involved in binding the E2. **C)** Model of SCF complexes. The N-terminus of CUL-1 binds the adaptor Skp1 (SKR proteins in *C. elegans*), while the C-terminus binds the RING finger protein Rbx1, which binds the E2. The substrate recognition subunit (SRS) binds to Skp1 through an F-box motif. **D)** Model of CUL-2-based E3 complexes. The N-terminus of CUL-2 binds to the adaptor elongin C (ELC-1), which is in complex with elongin B (ELB-1). The SRS binds to elongin C through a BC-box motif. **E)** Model of CUL-3-based E3 complexes. The N-terminus of CUL-3 binds directly to the SRS, which utilizes a BTB/POZ domain to bind to CUL-3.

6.1. HECT-domain E3s

There are nine genes in *C. elegans* that encode proteins with a HECT domain (Table 2). Of these genes, only two have been studied in detail: *oxi-1* and *wwp-1*. *oxi-1* was cloned as a gene whose expression increases under oxidative stress (growth in high oxygen concentrations; Yanase and Ishi, 1999). There is no observed RNAi phenotype of *oxi-1*; however, the RNAi analysis was not performed under high oxygen conditions when *oxi-1* would be assumed to be active (Table 2). *wwp-1* encodes a conserved protein with HECT and WW domains, and its RNAi depletion causes embryonic lethality with defective morphogenesis after the comma stage (Huang et al., 2000). Large-scale RNAi screens revealed that RNAi depletion of the HECT-domain gene *D2085.4* produces sterility in the P0 (Maeda et al., 2001). The remaining six HECT-domain genes were not associated with reproducible RNAi phenotypes (Table 2).

Table 2. HECT-domain encoding genes in *C. elegans*.

Cosmid designation	Gene name	Phenotype	References
C34D4.14	-	WT (RNAi)	Kamath et al., 2003
D2085.4	-	P0 sterile (RNAi)	Maeda et al., 2001
F36A2.13	-	WT (RNAi)	Jones et al., 2002
F45H7.6	-	WT (RNAi)	Kamath et al., 2003
Y39A1C.2	*oxi-1*	WT (RNAi)	Kamath et al., 2003; Gonczy et al., 2000
Y48G8AL.1	-	WT (3/4 trials in rrf-3 background); sterile (1/4 trials in rrf-3; RNAi)	Simmer et al., 2003
Y65B4BR.4	*wwp-1*	late stage embryonic arrest with defects in morphogenesis	Huang et al., 2000
Y67D8C.5	-	embryonic lethal (10%); WT (90%; RNAi)	Maeda et al., 2001
Y92H12A.2	-	WT (RNAi)	Maeda et al., 2001
Only the more severe phenotypes are listed. Phenotypes derived from RNAi or mutant analysis are denoted (brackets). WT = wild-type phenotype. References for the phenotype listed are given.			

6.2. U-box E3s & E4s

There are four genes in *C. elegans* that encode proteins with a U-box domain (Table 3). The *C. elegans* U-box protein CHN-1 is the homolog of mammalian CHIP. CHIP binds to the chaperones Hsp70 and Hsp90 and functions as an E3 to degrade misfolded proteins (Hatakeyama and Nakayama, 2003). *C. elegans* CHN-1 also binds to the Hsp70 homolog HSP-1, suggesting a similar function (Hoppe et al., 2004). Animals homozygous for a null allele of *chn-1* have slightly lower brood sizes at 20°C, but otherwise appear normal. Consistent with a cellular role with heat shock proteins, *chn-1* homozygotes are sensitive to heat-stress, exhibiting larval arrest and lethality at higher temperatures (Hoppe et al., 2004; Table 3).

Table 3. U-box-domain encoding genes in *C. elegans*.

Cosmid designation	Gene name	Phenotype	References
F59E10.2	*cyp-4/mog-6*	masculinization of the germ line; embryonic arrest (mut)	Graham et al., 1993
T05H10.5	*ufd-2*	WT (RNAi)	Kamath et al., 2003; Piano et al., 2000
T09B4.10	*chn-1*	slightly lower brood size at	Hoppe et al., 2004

Cosmid designation	Gene name	Phenotype	References
		20°; larval arrest at higher temperatures (mut)	
T10F2.4	-	embryonic lethal (RNAi)	Kamath et al., 2003; Simmer et al., 2003; Gonczy et al., 2000
Only the more severe phenotypes are listed. Phenotypes derived from RNAi or mutant analysis are denoted (brackets). WT = wild-type phenotype. References for the phenotype listed are given.			

C. elegans CHN-1 physically interacts with a second U-box protein, UFD-2 (Hoppe et al., 2004). UFD-2 is the ortholog of budding yeast Ufd2, which functions as an E4 (Koegl et al., 1999). As described above, an E4 enzyme catalyzes the elongation of ubiquitin chains on proteins that already have one or a few conjugated Ub (Koegl et al., 1999). Hoppe et al., found that both *C. elegans* CHN-1 and UFD-2 can function independently of each other as E3s for the addition of one to three Ub to UNC-45, a myosin-directed chaperone (Hoppe et al., 2004). However, more extensive poly-ubiquitination of UNC-45 *in vitro* required both CHN-1 and UFD-2, suggesting a novel mechanism in which a combination of E3s can produce E4 activity (Hoppe et al., 2004). The CHN-1-UFD-2 complex was able to function *in vitro* with UBC-2/LET-70, suggesting that UBC-2/LET-70 is the *in vivo* E2 (Hoppe et al., 2004).

The third U-box gene in *C. elegans* is *cyp-4*/*mog-6*, which is a homolog of human cyclophilin-60 (hCyp60/CYC4). In humans, hCyp60 has both peptidyl-prolyl cis/trans isomerase activity associated with its C-terminus and E3 activity associated with the U-box in its N-terminus (Hatakeyama and Nakayama, 2003). *C. elegans* CYP-4 also has both domains and exhibits protein-folding activity indicative of a functional prolyl isomerase (Page et al., 1996). Loss of *cyp-4*/*mog-6* results in a failure of the hermaphrodite germ line to switch from producing sperm to producing oocytes, so that only sperm are produced (Graham et al., 1993). The prolyl isomerase domain of CYP-4/MOG-6 is not required for the sperm/oocyte switch, while the N-terminus, containing the U-box, is required (Belfiore et al., 2004). CYP-4/MOG-6 is also required for embryogenesis (Graham et al., 1993).

The final U-box gene is *T10F2.4*, which is homologous to yeast and human *PRP19*. Budding yeast Prp19 functions in spliceosome assembly, and human PRP19 has been shown to possess E3 activity (Blanton et al., 1992; Hatakeyama and Nakayama, 2003). Large-scale RNAi screens revealed that T10F2.4 is required for embryonic viability (Table 3).

6.3. Monomeric RING finger proteins

There are 152 RING finger proteins in the *C. elegans* genome (Table 4). While a majority of RING finger proteins tested *in vitro* exhibit E3 activity, it is unclear if all RING finger proteins function as E3s *in vivo* (Fang et al., 2003). There are two classes of RING finger motifs, H2 and HC, based on the placement of His or Cys residues in positions 4 and 5 of the motif (Fang et al., 2003). The three RING finger proteins that are known to be integral components of multisubunit complexes (RBX-1, RBX-2, and APC-11) are of the H2 class and are very small proteins of 110-135 amino acids. A recent survey of RING finger proteins in *C. elegans* found more to be of the HC class (90 genes) than the H2 class (13 genes; Moore and Boyd, 2004). The majority of RING finger genes of either class encode proteins that are much larger than the multisubunit E3 RING-H2 proteins, as would be expected for proteins that function as monomeric E3s that bind to both the E2 and the substrate.

Table 4. RING finger encoding genes in *C. elegans*.

Cosmid designation	Gene name	Phenotype	References
B0281.3	-	WT (RNAi)	Kamath et al., 2003
B0281.8	-	WT (RNAi)	Kamath et al., 2003
B0393.6	-	Emb (RNAi)	Kamath et al., 2003; Simmer et al., 2003
B0416.4	-	WT (RNAi)	Kamath et al., 2003
B0432.13	-	WT (RNAi)	Kamath et al., 2003
C01B7.6	*rpm-1*, *rpm-3*, *sam-1*,	defective synapse formation	Schaefer et al., 2000; Zhen

Cosmid designation	Gene name	Phenotype	References
	sad-3, syd-3	and morphology (mut)	et al., 2000
C01G6.4	-	WT (RNAi)	Kamath et al., 2003; Moore and Boyd, 2004
C02B8.6	-	WT (RNAi)	Maeda et al., 2001
C06A5.8	-	WT (RNAi)	Jones et al., 2002; Maeda et al., 2001
C06A5.9	*rnf-1, tag-54*	WT (RNAi)	Jones et al., 2002
C09E7.5	-	WT (RNAi)	Gonczy et al., 2000
C09E7.8	-	WT (RNAi)	Kamath et al., 2003; Gonczy et al., 2000
C09E7.9	-	-	-
C11H1.3	-	Adl; Mlt; Unc; Dpy; Gro; Pvl (RNAi)	Kamath et al., 2003; Simmer et al., 2003
C12C8.3	*lin-41*	heterochronic defect in which hypodermal cells adopt the adult fate at the L3/L4 molt (mut)	Slack et al., 2000
C15F1.5	-	WT (RNAi)	Kamath et al., 2003; Moore and Boyd, 2004
C16C10.5	-	WT (RNAi)	Kamath et al., 2003; Gonczy et al., 2000
C16C10.7	*rnf-5*	disorganized body wall muscle dense bodies but normal movement (mut)	Broday et al., 2004
C17E4.3	-	Emb (RNAi)	Piano et al., 2000
C17H11.6	-	WT (RNAi)	Kamath et al., 2003
C18B12.4	-	WT (RNAi)	Kamath et al., 2003; Moore and Boyd, 2004
C18H9.7	*rpy-1, rap-1*	WT (RNAi)	Kamath et al., 2003
C26B9.6	-	WT (RNAi)	Kamath et al., 2003
C28G1.5	-	-	-
C28G1.6	-	-	-
C30F2.2	-	WT (RNAi)	Kamath et al., 2003
C32D5.10	-	Lva (RNAi)	Moore and Boyd, 2004
C32D5.11	-	WT (RNAi)	Kamath et al., 2003; Maeda et al., 2001
C32E8.1	-	WT (RNAi)	Jones et al., 2002
C34E10.4	*wrs-2*	Gro (RNAi)	Kamath et al., 2003; Simmer et al., 2003; Gonczy et al., 2000
C34F11.1	-	WT (RNAi)	Kamath et al., 2003; Maeda et al., 2001
C36A4.8	*brc-1*	Him phenotype; elevated levels of germ cell death; germ cell chromosome fragmentation upon irradiation (RNAi)	Boulton et al., 2004

Cosmid designation	Gene name	Phenotype	References
C36B1.9	-	-	-
C39F7.2	-	WT (RNAi)	Kamath et al., 2003
C45G7.4	-	WT (RNAi)	Kamath et al., 2003
C49H3.5	*ntl-4*	Emb; Gro; Slu (RNAi)	Maeda et al., 2001; Simmer et al., 2003
C52E12.1	-	-	-
C53A5.6	-	Sck Ste; Lva Lvl Ste (RNAi)	Kamath et al., 2003; Simmer et al., 2003
C53D5.2	-	WT (RNAi)	Jones et al., 2002
C55A6.1	-	WT (RNAi)	Kamath et al., 2003
C56A3.4	-	Gro (RNAi)	Kamath et al., 2003; Moore and Boyd, 2004
D2089.2	-	Emb; Lvl; Pch; Slu; Gro; Unc (RNAi)	Kamath et al., 2003; Simmer et al., 2003
EEED8.16	-	-	-
F08B12.2	*prx-12*	Clr; L1 stage larval arrest (RNAi)	Kamath et al., 2003; Petriv et al., 2002; Thieringer et al., 2003
F08G12.5	-	WT (RNAi)	Kamath et al., 2003; Moore and Boyd, 2004
F10D7.5	-	Emb; Sck; Ste (RNAi)	Maeda et al., 2001
F10G7.10	-	WT (RNAi)	Kamath et al., 2003
F11A10.3	-	WT (RNAi)	Moore and Boyd, 2004
F16A11.1	-	WT (RNAi)	Jones et al., 2002; Maeda et al., 2001
F19G12.1	-	WT (RNAi)	Kamath et al., 2003
F23B2.10	-	-	-
F26E4.11	-	Emb (RNAi)	Simmer et al., 2003
F26F4.7	*nhl-2*	Ste; Stp (RNAi)	Maeda et al., 2001
F26G5.9	*tam-1*	expression of genes in non-complex transgenic arrays is reduced (mut)	Hsieh et al., 1999
F32A6.3	-	Emb (RNAi)	Piano et al., 2002
F35G12.9	*apc-11*	one-cell stage arrest during meiosis I; mitotic delays in escapers (RNAi)	Gonczy et al., 2000; Davis et al., 2002; Moore and Boyd, 2004
F36F2.3	-	Emb; Led (RNAi)	Jones et al., 2002; Simmer et al., 2003
F40G9.12	-	WT (RNAi)	Kamath et al., 2003; Gonczy et al., 2000; Moore and Boyd, 2004
F40G9.14	-	WT (RNAi)	Kamath et al., 2003; Gonczy et al., 2000
F42C5.4	-	WT (RNAi)	Kamath et al., 2003
F42G2.5	-	WT (RNAi)	Kamath et al., 2003
F43C11.7	-	-	-

Cosmid designation	Gene name	Phenotype	References
F43C11.8	-	-	-
F43G6.8	-	WT (RNAi)	Kamath et al., 2003; Maeda et al., 2001
F44D12.10	-	WT (RNAi)	Kamath et al., 2003
F45G2.6	*trf-1*	WT (RNAi)	Gonczy et al., 2000
F46F2.1	-	WT (RNAi)	Kamath et al., 2003
F47G9.4	-	WT (RNAi)	Kamath et al., 2003; Maeda et al., 2001
F53F8.3	-	WT (RNAi)	Kamath et al., 2003
F53G2.7	-	Emb; Ste (RNAi)	Maeda et al., 2001
F54B11.5	-	WT (RNAi)	Kamath et al., 2003
F54G8.4	*nhl-1*	WT (RNAi)	Kamath et al., 2003; Gonczy et al., 2000
F55A3.1	-	WT (RNAi)	Kamath et al., 2003
F55A11.3	-	none	Moore and Boyd, 2004
F55A11.7	-	WT (RNAi)	Kamath et al., 2003
F56D2.2	-	WT (RNAi)	Kamath et al., 2003; Gonczy et al., 2000
F58B6.3	*par-2*	defective embryonic anterior-posterior polarity (mut; RNAi)	For review: Schneider and Bowerman, 2003
F58E6.1	-	WT (RNAi)	Kamath et al., 2003; Maeda et al., 2001
H05L14.2	-	WT (RNAi)	Jones et al., 2002
H10E21.5	-	WT (RNAi)	Kamath et al., 2003; Gonczy et al., 2000
K01G5.1	-	Emb; Lva (RNAi)	Simmer et al., 2003; Gonczy et al., 2000; Moore and Boyd, 2004
K02B12.8	*zhp-3*	Him (RNAi)	Jones et al., 2002; Piano et al., 2002
K04C2.4	*brd-1*	Him phenotype; elevated levels of germ cell death; germ cell chromosome fragmentation upon irradiation (RNAi)	Boulton et al., 2004
K09F6.7	-	WT (RNAi)	Kamath et al., 2003
K11D12.9	-	-	-
K12B6.8	-	WT (RNAi)	Kamath et al., 2003
M02A10.3	*sli-1*	suppress hypomorphic alleles of let-23; low penetrance head morphology defect (mut)	Jongeward et al., 1995
M88.3	-	WT (RNAi)	Kamath et al., 2003; Gonczy et al., 2000

Cosmid designation	Gene name	Phenotype	References
M110.3	-	WT (RNAi)	Kamath et al., 2003
M142.6	-	Clr; Gro (RNAi)	Kamath et al., 2003; Moore and Boyd, 2004
R02E12.4	-	WT (RNAi)	Kamath et al., 2003
R05D3.4	*rfp-1*	Gro; Lva; Pvl; Rup; Stp; Unc; Egl (RNAi)	Kamath et al., 2003; Piano et al., 2002; Simmer et al., 2003; Crowe and Candido, 2004
R06F6.2	-	Bmd; Lvl; Mlt; Emb; Gro; Sma (RNAi)	Kamath et al., 2003; Simmer et al., 2003;
R10A10.2	*rbx-2*	WT (RNAi)	Jones et al., 2002; Maeda et al., 2001; Piano et al., 2002; Moore and Boyd, 2004
T01C3.3	-	WT (RNAi)	Kamath et al., 2003; Piano et al., 2002; Moore and Boyd, 2004
T01G5.7	-	WT (RNAi)	Kamath et al., 2003
T02C1.1	-	WT (RNAi)	Kamath et al., 2003; Gonczy et al., 2000
T02C1.2	-	-	-
T05A12.4	-	WT (RNAi)	Kamath et al., 2003; Maeda et al., 2001
T08D2.4	-	WT (RNAi)	Kamath et al., 2003; Moore and Boyd, 2004
T13A10.2	-	-	-
T13H2.5	-	-	-
T20F5.6	-	WT (RNAi)	Jones et al., 2002; Piano et al., 2002
T20F5.7	-	WT (RNAi)	Jones et al., 2002
T22B2.1	-	WT (RNAi)	Kamath et al., 2003
T23F6.3	-	WT (RNAi)	Kamath et al., 2003
T24D1.2	-	WT (RNAi)	Jones et al., 2002; Piano et al., 2002; Moore and Boyd, 2004
T24D1.3	-	Emb (RNAi)	Piano et al., 2002
T24D1.5	-	-	-
T26C12.3	-	WT (RNAi)	Kamath et al., 2003; Maeda et al., 2001
W02A11.3	-	WT (RNAi)	Jones et al., 2002; Moore and Boyd, 2004
W04H10.3	*nhl-3*	WT (RNAi)	Kamath et al., 2003
W09G3.6	-	WT (RNAi)	Jones et al., 2002
Y4C6A.3	-	WT (RNAi)	Kamath et al., 2003
Y6D1A.2	-	WT (RNAi)	Maeda et al., 2001
Y7A9C.1	-	WT (RNAi)	Kamath et al., 2003
Y38C1AA.6	-	-	-

Cosmid designation	Gene name	Phenotype	References
Y38F1A.2	-	-	-
Y38H8A.2	-	-	-
Y45F10B.8	-	WT (RNAi)	Kamath et al., 2003
Y45F10B.9	-	WT (RNAi)	Kamath et al., 2003
Y45G12B.2	-	-	-
Y47D3A.22	-	-	-
Y47D3B.11	-	-	-
Y47G6A.14	-	WT (RNAi)	Jones et al., 2002
Y51F10.2	-	-	-
Y52E8A.2	-	-	-
Y53G8AM.4	-	-	-
Y54E10A.11	-	-	-
Y54E10BR.3	-	WT (RNAi)	Jones et al., 2002
Y55F3AM.6	-	WT (RNAi)	Kamath et al., 2003
Y57A10B.1	-	WT (RNAi)	Kamath et al., 2003
Y67D8B.1	-	-	-
Y71F9AL.10	-	-	-
Y73C8C.7	-	WT (RNAi)	Kamath et al., 2003
Y73C8C.8	-	WT (RNAi)	Kamath et al., 2003
Y75B8A.10	-	WT (RNAi)	Kamath et al., 2003; Gonczy et al., 2000
Y105C5B.11	-	-	-
Y105E8A.14	-	WT (RNAi)	Jones et al., 2002
Y119C1B.5	-	-	-
ZC13.1	-	WT (RNAi)	Kamath et al., 2003
ZK287.5	*rbx-1*	one cell stage embryonic arrest (RNAi)	Moore and Boyd, 2004; Sasagawa et al., 2003
ZK637.14	-	WT (RNAi)	Kamath et al., 2003; Gonczy et al., 2000
ZK809.7	*prx-2*	Gro (RNAi)	Kamath et al., 2003; Simmer et al., 2003;
ZK993.2	-	-	-
ZK1240.1	-	Lvl; Sck; Gro; Unc (RNAi)	Kamath et al., 2003; Maeda et al., 2001; Simmer et al., 2003
ZK1240.2	-	WT (RNAi)	Kamath et al., 2003
ZK1240.3	-	WT (RNAi)	Kamath et al., 2003
ZK1240.6	-	WT (RNAi)	Kamath et al., 2003; Moore and Boyd, 2004
ZK1240.8	-	-	-
ZK1240.9	-	-	-
ZK1320.6	*arc-1*, *arl-4*	WT (RNAi)	Maeda et al., 2001; Moore and Boyd, 2004

Cosmid designation	Gene name	Phenotype	References
Only the more severe phenotypes are listed. Phenotypes derived from RNAi or mutant analysis are denoted (brackets). Phenotype abbreviations are defined in WormBase. References for the phenotype listed are given.			

Twenty one of the RING finger proteins in *C. elegans* that do not function as components of known multisubunit E3 complexes have been the focus of genetic studies. (see named genes in Table 4). Most of these have not been studied for possible E3 activity. Five of the potentially monomeric RING finger proteins have been implicated as E3s, and are discussed below.

RNF-5 is the ortholog of the mammalian E3 RNF5 (Didier et al., 2003). RNAi depletion of *rnf-5* causes a disorganization of body wall muscle dense bodies (Broday et al., 2004). RNF-5 binds and negatively regulates the protein level of UNC-95, a LIM-domain protein that is required for the integrity of dense bodies (Didier et al., 2003; Broday et al., 2004). Mutations of the RING finger domain of RNF-5 severely reduce its ability to lower UNC-95 protein levels upon overexpression (Broday et al., 2004). These results suggest that RNF-5 directly targets UNC-95 for ubiquitin-mediated proteolysis.

SLI-1 is the ortholog of mammalian c-Cbl. c-Cbl functions as an E3 to ubiquitinate active receptor tyrosine kinases (RTKs) to induce their endocytosis or degradation (Shtiegman and Yarden, 2003). SLI-1 negatively regulates the LET-23 RTK, which mediates vulva differentiation (Yoon et al., 1995). A *sli-1* mutant that lacks the RING finger domain has a significantly reduced ability to inhibit LET-23 activity, suggesting that SLI-1 functions as an E3 to regulate LET-23 (Yoon et al., 2000).

RFP-1 was identified as a binding partner for the E2 UBC-1 in a yeast two-hybrid screen (Crowe and Candido, 2004). RNAi depletion of *rfp-1* produces an L1-stage larval arrest, while escapers exhibit vulval and egg-laying defects (Crowe and Candido, 2004). Interestingly, RNAi depletion of *ubc-1*, the predicted E2, does not produce any phenotypes, suggesting that RFP-1 can function with additional E2s (Crowe and Candido, 2004).

BRC-1 and BRD-1 are RING finger proteins that are orthologs of the mammalian breast cancer susceptibility gene BRCA1 and the BRCA1-associating protein BRD-1, respectively (Boulton et al., 2004). In mammals, a complex of BRCA1 and BRD1 exhibits E3 activity *in vitro*, and mutations in the RING finger of BRCA1 that abolish E3 activity are associated with breast cancer (Ohta and Fukuda, 2004). In *C. elegans*, BRC-1 also binds BRD-1 (Boulton et al., 2004). RNAi depletion of either gene produces a high incidence of males (Him) phenotype, elevated levels of p53-dependent germ cell death, and chromosome fragmentation after irradiation, suggesting a role in DNA repair (Boulton et al., 2004). Intriguingly, mammalian BRCA1 and BRD1 have been implicated in the ubiquitination of p53 (Dong et al., 2003), suggesting that the p53-dependent germ cell death observed upon BRC-1 or BRD-1 RNAi results from elevated p53 levels. In concordance with this, loss of p53 (*cep-1*) suppressed the *brc-1* and *brd-1* germ cell deaths (Boulton et al., 2004).

RPM-1 encodes a protein with RING finger and guanine nucleotide exchange domains. *rpm-1* mutants have varied defects in neuron branching, and synapse organization and structure, indicating a critical role in presynaptic development (Schaefer et al., 2000; Zhen et al., 2000). RPM-1 controls presynaptic development by negatively regulating DLK-1, which functions as the initial MAP kinase (MAPK) in a MAPK cascade (Nakata et al., 2005). Co-expression of RPM-1 and DLK-1 in mammalian cells promotes the ubiquitination of DLK-1, and DLK-1 is co-immunoprecipitated by the C-terminus of RPM-1, suggesting that RPM-1 directly binds and mediates DLK-1 ubiquitination (Nakata et al., 2005). These results identified the first ubiquitinated substrate of an RPM-1 family member, which also control synapse formation in *Drosophila* and mammals (Chang and Balice-Gordon, 2000; Burgess et al., 2004). RPM-1 has also been shown to physically associate with an SCF E3 complex (Liao et al., 2004), and this will be discussed in the CUL-1 section of the review.

6.4. Multisubunit RING finger complexes: cullin-based E3s

Cullins are a conserved family of E3 components that were first identified in *C. elegans* and budding yeast (Kipreos et al., 1996; Mathias et al., 1996). There are six cullins in *C. elegans*. The crystal structure of a human CUL1-based SCF complex reveals that CUL1 forms a rigid scaffold in which the C-terminus binds the RING-H2 finger protein Rbx1/Roc1, and the N-terminus binds to the adaptor Skp1 (Zheng et al., 2002; Figure 2). Skp1 binds the substrate-recognition subunit (SRS), through an F-box motif of the SRS. The SRS binds substrates and positions them for ubiquitination. The E2 binds the complex through interaction with Rbx1 (Figure 2). Other cullins are also

predicted to function as scaffolds with similar tertiary structure (Wu et al., 2003). All cullin-based E3s components interact with multiple SRSs, each of which form distinct E3 complexes that direct the ubiquitination of distinct sets of substrates (Guardavaccaro and Pagano, 2004). Based on the large number of potential SRS genes in metazoan genomes, cullin-based E3s may comprise the most abundant class of E3 in metazoa.

Each class of cullin-based E3 complexes includes a small RING finger protein, either Rbx1/Roc1 or Rbx2/Roc2 (Ohta et al., 1999). *C. elegans* RBX-1 and RBX-2 share 36% identity. Partial inactivation of the *C. elegans rbx-1* gene produces phenotypes that resemble a mixture of different cullin loss-of-function phenotypes (Sasagawa et al., 2003; Moore and Boyd, 2004; data not shown). Complete inactivation of *rbx-1* causes a one-cell stage arrest that is more severe than any individual cullin loss-of-function phenotype, and may reflect the simultaneous loss of multiple cullin complexes (Sasagawa et al., 2003; Moore and Boyd, 2004). In contrast, inactivation of *rbx-2* by RNAi does not cause any apparent defects (Moore and Boyd, 2004), suggesting a more limited role.

Cullin-based complexes are themselves regulated by the covalent addition of a ubiquitin-like protein, Nedd8, onto the cullin. Nedd8 promotes cullin activity, at least in part, by blocking the binding of the cullin inhibitor CAND1 (Pan et al., 2004). NED-8 is removed from cullins by the COP9 Signalosome complex (CSN; Cope and Deshaies, 2003). In *C. elegans*, inactivation of either the Nedd8 homolog NED-8 or CSN components produce embryonic phenotypes similar to *cul-3* RNAi, suggesting that both neddylation and deneddylation are required for CUL-3 activity (Pintard et al., 2003). In contrast, the early embryonic phenotypes observed in *cul-2* mutants are not seen upon inactivation of NED-8 or CSN, suggesting that NED-8 modification is not essential for CUL-2 function (Pintard et al., 2003). The functional requirement for NED-8 conjugation of other cullins has not been studied.

6.4.1. CUL-1-based complexes

CUL-1-based E3 complexes are known as SCF complexes to reflect the components: Skp1 (the adaptor); CUL1/Cdc53; and an F-box protein (the substrate recognition subunit; Figure 2). Distinct SCF complexes are formed by the combination of core components (Skp1, CUL1, and Rbx1) with different F-box proteins. *C. elegans* has an extremely large number of F-box proteins relative to other metazoa: at least 326 in *C. elegans* compared to 13 in budding yeast and 68 in humans (Kipreos and Pagano, 2000; Jin et al., 2004). This larger number of F-box protein genes is matched by a larger number of Skp1-related genes in *C. elegans* (21 SKR genes) relative to only a single Skp1 gene in budding yeast and humans (Nayak et al., 2002; Yamanaka et al., 2002). Seven of the SKRs were found to interact with CUL-1: SKR-1, -2, -3, -7, -8, -9, and -10 (Nayak et al., 2002; Yamanaka et al., 2002). The observation of multiple SKRs that can bind CUL-1, along with the large number of F-box proteins, suggests that *C. elegans* will contain many distinct SCF complexes. The cellular functions associated with known SCF complexes are discussed below.

SCF^{LIN-23}: LIN-23 is an F-box protein with WD-repeats. *lin-23* mutants have hyperplasia in all somatic lineages caused by a failure of dividing blast cells to cease cell division at the appropriate time (Kipreos et al., 2000). This cell cycle exit defect is also the primary phenotype associated with *cul-1* mutants and with *skr-1* and *skr-2* RNAi, suggesting that the closely related SKR-1 and SKR-2 proteins function as adaptors in the SCF^{LIN-23} complex (Kipreos et al., 1996; Nayak et al., 2002). LIN-23 has a separate function to promote the proper outgrowth of axons (Mehta et al., 2004).

SCF^{SEL-10}: SEL-10 is an F-box protein with WD-repeats that negatively regulates signaling by the Notch family member LIN-12 (Hubbard et al., 1997). SEL-10 was shown to physically bind the LIN-12 receptor (a Notch family member), suggesting that it directly mediates LIN-12 ubiquitination (Hubbard et al., 1997). This pathway was subsequently shown to be conserved in mammals, with the SEL-10 ortholog responsible for the ubiquitin-mediated degradation of Notch (Wu et al., 2001; Oberg et al., 2001). SEL-10 also binds and negatively regulates the presenilin SEL-12 (Wu et al., 1998). Studies with human cells have subsequently shown that the degradation of presenilin by SCF^{SEL-10} is conserved (Li et al., 2002). Finally, SEL-10 binds and facilitates the degradation of FEM-1 and FEM-3, which function in the sex determination pathway to promote male development (Jager et al., 2004).

SCF^{FSN-1}: FSN-1 is an F-box protein with a SPRY domain that is required for regulating neuromuscular junction (synapse) formation in different classes of neurons (Liao et al., 2004). Co-immunoprecipitation (co-IP) analysis indicates that FSN-1 physically associates with CUL-1 and SKR-1, suggesting that it functions as the SRS for an SCF complex (Liao et al., 2004). FSN-1 binds and negatively regulates the level of SCD-1/ALK (Liao et al.,

2004). An *scd-1* mutant suppresses the *fsn-1* synapse defect, suggesting that SCD-1 is the critical substrate of the SCF^{FSN-1} complex for regulating synapse formation (Liao et al., 2004).

FSN-1 has been shown by co-IP analysis to physically associate with the large RING finger protein RPM-1, and it has been proposed that RPM-1 functions as the RING finger component of the SCF^{FSN-1} complex (Liao et al., 2004). However, there is evidence that suggests that RPM-1 does not function analogously to RBX-1 in an SCF^{FSN-1} complex. In particular, *rpm-1* null mutants are not functionally equivalent to *fsn-1* null mutants, and RPM-1 requires the presence of FSN-1 to associate with SCF components, which would not be expected for a component that binds directly to CUL-1 (Liao et al., 2004).

6.4.2. CUL-2-based complexes

CUL-2-based E3 complexes have a structure very similar to that of SCF complexes (Wu et al., 2003; Figure 2). CUL-2 complexes employ the Skp1-related protein elongin C as an adaptor in combination with elongin B, which contains a ubiquitin-like domain (Kim and Kaelin, 2003). Substrate recognition subunits bind elongin C through a BC-box/VHL-box motif (Kamura et al., 2004). The *C. elegans cul-2* gene is the only metazoan *cul-2* ortholog whose functions have been analyzed genetically. *cul-2* mutants have a large number of phenotypes reflecting diverse cellular functions. **1)** CUL-2 is required for the G1-to-S phase transition in germ cells, which is caused, at least in part, by a failure to negatively regulate the levels of the CDK-inhibitor CKI-1 (Feng et al., 1999). **2)** CUL-2 is required for the meiosos II metaphase to anaphase transition and meiosis II exit. The failure/delay of the metaphase II to anaphase II transition is correlated with a failure to degrade the cell cycle regulator cyclin B1, while the delay in meiosis exit is correlated with a failure to degrade cyclin B3 (Liu et al., 2004; Sonneville and Gonczy, 2004). **3)** CUL-2 is required for proper anterior-posterior (A-P) polarity. In *cul-2* mutants, A-P polarity is often reversed due to the perduring meiotic spindle acting as a catalyst for the ectopic placement of the PAR-2 polarity protein onto the anterior cortex (Liu et al., 2004; Sonneville and Gonczy, 2004). Additionally, CUL-2 restricts the localization of PAR-2 in regions distant from microtubule-organizing centers (Liu et al., 2004; Sonneville and Gonczy, 2004). **4)** CUL-2 is required for defects in mitotic chromosome condensation and mitotic progression (Feng et al., 1999). **5)** CUL-2 is required to prevent cytoplasmic extensions/blebbing in the early embryo (Feng et al., 1999). **6)** A CUL-2 complex containing the SRS ZIF-1 is required for the degradation of five CCCH Zn finger polarity proteins (PIE-1, POS-1, MEX-1, MEX-5, and MEX-6) in non-germ cell embryonic lineages (DeRenzo et al., 2003).

In mammals, CUL-2 functions in a complex with the von Hippel-Lindau tumor suppressor protein (VHL) as the SRS to target the degradation of hypoxia inducible factor-1α (HIF-1α; Kim and Kaelin, 2003). In *C. elegans*, VHL-1 also promotes HIF-1 degradation, so it is likely that *C. elegans* VHL-1 functions as a component of a CUL-2 complex (Epstein et al., 2001).

6.4.3. CUL-3-based complexes

CUL-3 has a slightly different structure from that of CUL-1 and CUL-2-based complexes in that a single BTB/POZ-domain protein functions as both the substrate recognition subunit and adaptor, i.e., the BTB protein binds CUL-3 and the substrate (van den Heuvel, 2004; Figure 2). *C. elegans* contains over 100 BTB-domain proteins, indicating the possibility for multiple CUL-3-based complexes (Furukawa et al., 2003; Xu et al., 2003). *C. elegans* was the first organism in which a functional CUL-3-based E3 complex and its substrate were identified. A *C. elegans* CUL-3 complex that contains the BTB protein MEL-26 was shown to degrade the microtubule-severing katanin MEI-1 (Pintard et al., 2003; Furukawa et al., 2003; Xu et al., 2003). MEI-1 is degraded in the one-cell embryo after meiosis (Pintard et al., 2003). Presumably, the microtubule-severing activity of MEI-1 is required during meiosis to restrict the size of the meiotic spindle, but must be destroyed to allow the larger mitotic spindle to form after meiosis. In *cul-3* RNAi animals or *mel-26* mutants, mitotic aster microtubules are disorganized and shorter compared to wild type, and this is associated with defects in spindle positioning and elongation, and cytokinesis (Kurz et al., 2002; Pintard et al., 2003; Dow and Mains, 1998).

6.4.4. CUL-4-based complexes

The structure of CUL-4-based E3 complexes has not been fully worked out. In mammals, the CUL-4 complex includes the DDB1 protein, which appears to be capable of functioning either as an adaptor or as a substrate recognition subunit (Wertz et al., 2004; Hu et al., 2004). In *C. elegans*, CUL-4 has a central role in the regulation of DNA replication by restricting replication to only once per cell cycle (Zhong et al., 2003). RNAi depletion of *cul-4* produces an L2-stage larval arrest in which blast cells undergo unrestrained re-replication and attain elevated DNA contents up to 100 C (Zhong et al., 2003). CUL-4 is required for the degradation of the replication licensing factor

CDT-1 during S phase (Zhong et al., 2003). The degradation of CDT-1 precludes it from reloading the MCM complex onto replication origins, thereby preventing the re-initiation of DNA replication at origins during the same cell cycle (Zhong et al., 2003; Feng and Kipreos, 2003). The degradation of CDT-1 by CUL-4 was subsequently shown to be conserved in *Drosophila* and mammals (Higa et al., 2003).

6.5. Multisubunit RING finger complexes: APC/C

The APC/C is a conserved multisubunit E3 complex that functions during meiosis, mitosis, and G1 phase (Yeong, 2004). *C. elegans* contains nine core APC/C components, as well as two accessory components, FZY-1 (also known as CDC20) and FZR-1 (CDH1; Yeong, 2004). *C. elegans* and budding yeast were the first organisms in which a role for the APC/C in chromosome separation during meiosis I was demonstrated (Salah and Nasmyth, 2000; Yeong, 2004). Mutations of APC/C^{FZY-1} components produce failures of chromosome separation during mitosis and meiosis I of both oocyte and sperm lineages (Furuta et al., 2000; Davis et al., 2002; Shakes et al., 2003; Kitagawa et al., 2002). Inactivation of APC/C^{FZY-1} components causes a one-cell arrest at metaphase of meiosis I that is similar to what is observed upon inactivation of proteasome components, suggesting that APC/C^{FZY-1} mediates this initial requirement for ubiquitin proteolysis in the embryo (Furuta et al., 2000; Davis et al., 2002; Shakes et al., 2003; Kitagawa et al., 2002; Gonczy et al., 2000).

C. elegans APC/C is required for the degradation of IFY-1, the proposed Securin that functions to release Separase, SEP-1, which is a conserved protease that separates sister chromatids at anaphase (Siomos et al., 2001; Kitagawa et al., 2002). However, the degradation of Securin cannot be the sole essential function of APC/C^{FZY-1} in promoting meiosis I, as experimentally-inducing a loss of chromosome cohesion in APC/C mutants (which should bypass the requirement for Securin degradation) does not rescue the meiosis I arrest (Davis et al., 2002). The APC/C-mediated release of active SEP-1 has been suggested to directly affect anterior-posterior polarity in the one-cell stage embryo by ensuring that the paternal pronucleus/centrosome complex remains in tight association with the posterior cortex of the embryo, where it promotes the cortical association of the PAR-2 polarity protein (Rappleye et al., 2002). Hypomorphic alleles of APC/C or RNAi depletion of SEP-1 are associated with a lack of embryonic polarity due to a failure of PAR-2 to localize to the posterior cortex (Rappleye et al., 2002). However, another study has suggested that the polarity defects are secondary consequences of a failure of meiosis and do not imply a direct regulation of polarity by APC/C or SEP-1 (Shakes et al., 2003).

Genetic experiments have implicated APC/C^{FZR-1} in the negative regulation of cyclins during G1 phase (Fay et al., 2003). In mammals, the Rb protein negatively regulates the transcription of cyclins in G1 phase (Peters, 2002). Weak alleles of *fzr-1* have no overt phenotypes by themselves, but produce a synthetic hyperplasia phenotype when combined with homozygous *lin-35* Rb mutant alleles. Additionally, overexpression of cyclins A and E produce more extensive hyperplasia in a *fzr-1* mutant background, suggesting that APC/C^{FZR-1} degrades S phase and mitotic cyclins during G1 phase, as occurs in other metazoa (Fay et al., 2003). More complete inactivation of *fzr-1* by RNAi reveals severe pleiotropic effects on cell proliferation and development (Fay et al., 2003). Biochemical and genetic experiments indicate that APC/C functions with the E2 UBC-2 to promote both meiosis and mitosis (Frazier et al., 2004).

The APC also has a non-cell cycle function to regulate the abundance of GLR-1 glutamate receptors in ventral cord nerve cells (Juo and Kaplan, 2004). The endocytosis of GLR-1 is induced by the covalent attachment of one or a few Ub to GLR-1 (Burbea et al., 2002). APC/C promotes GLR-1 endocytosis; however, APC/C does not directly ubiquitinate GLR-1, and the critical target of APC/C in regulating GLR-1 endocytosis is not yet known (Juo and Kaplan, 2004).

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8. References

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