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Degrade to create: developmental requirements for ubiquitin-mediated proteolysis during early *C. elegans* embryogenesis

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The ubiquitin protein conjugation system tags proteins with the small polypeptide ubiquitin. Most poly-ubiquitinated proteins are recognized and degraded by the proteasome, a large multi-subunit protease. Ubiquitin-dependent protein degradation is used as a regulatory tool for many essential processes, the best studied of which is eukaryotic cell cycle progression. More recently, genetic studies in *C. elegans* have identified multiple roles for the ubiquitin system in early development, where ubiquitin-dependent protein degradation governs such diverse events as passage through meiosis, cytoskeletal regulation and cell fate determination.

Introduction

Ubiquitin-mediated proteolysis, discovered over 25 years ago (Ciechanover et al., 1980; Hershko et al., 1980), covalently attaches ubiquitin polypeptide chains to proteins, targeting them for degradation by the 26S proteasome (see Box 1). The proteasome recycles ubiquitin-tagged proteins by hydrolyzing them into their constituent amino acids, allowing for their subsequent reincarnation into newly translated protein (Voges et al., 1999). Originally viewed as simply mediating the disposal of mis-folded or otherwise spent proteins, we now know that ubiquitin-mediated proteolysis is much more than a cellular composting program. Like phosphorylation, it is an ubiquitous form of post-translational regulation.

Regulatory roles for ubiquitin first emerged when cell cycle transitions were found to depend extensively on the rapid, ubiquitin-mediated degradation of cyclins, conserved proteins that bind to, activate and provide target specificity for regulatory protein kinases (Glotzer et al., 1991; Hershko et al., 1991). Most of these insights came from biochemical studies of frog oocyte extracts and from genetic studies in budding and fission yeast (Morgan, 1997; Murray, 2004). Here, we summarize recent advances from genetic studies in the soil nematode *Caenorhabditis elegans* that have identified additional roles. As discussed in this review, *C. elegans* exploits ubiquitin-mediated proteolysis to regulate several essential processes during early embryogenesis, including cell cycle progression, cytoskeletal function and cell fate determination.

Ubiquitination and cell cycle progression

The mitotic cell cycle involves a sequence of transitions that include entry into DNA replication and the subsequent duplication of chromosomes (S phase), followed by entry into mitosis and the subsequent segregation of duplicated chromosomes to each of two daughter cells (M phase). Progression through the cell cycle requires the activity of cell division kinases, which must bind to cyclin proteins to be active (Morgan, 1997; Murray, 2004). Cyclins were so named because they accumulate to peak levels at specific times during the cell cycle, thereby activating cell division kinases at appropriate moments. A regulatory role for ubiquitin in the cell cycle was discovered when it was shown that cyclins are targeted for rapid degradation during the cell cycle by poly-ubiquitination (Glotzer et al., 1991; Hershko et al., 1991).

Additional roles for ubiquitination emerged from studies of the metaphase-to-anaphase transition during mitosis (Peters, 2002). Early in mitosis, the duplicated chromosomes (sister chromatids) condense and are held together by a multi-protein cohesin complex. As the bipolar mitotic spindle forms, sister chromatid pairs become captured by microtubules such that each spindle pole is attached to only one sister. Oppositely directed forces applied to the chromatid pairs cause them to align midway between the poles at the metaphase plate. Subsequently, during anaphase, sister chromatids are pulled apart and segregated to opposite poles (Gadde and Heald, 2004).

The transition to anaphase during mitosis requires the proteolytic cleavage of one cohesin subunit, called Scc1 in budding yeast, by a cysteine protease called separase. This protease is tightly regulated, as premature cleavage would lead to early anaphase onset and to chromosome-segregation defects. To prevent premature cleavage, separase is kept inactive by a protein called securin. Only after the poly-ubiquitination of securin by an ubiquitin E3 ligase called the anaphase promoting complex/cyclosome (APC/C; see Fig. 1 and Box 1) is separase released to cleave Scc1 (Nasmyth, 2001). APC/C E3 ligase activity is controlled by spindle assembly checkpoint proteins (Nasmyth, 2005; Cleveland et al., 2003; Amon, 1999). These regulators have the remarkable ability to sense whether all sister chromatid pairs are properly captured by microtubules, with tension indicating bipolar attachment and alignment at the metaphase plate. As long as there are unattached chromatids, the spindle checkpoint prevents activation of the APC/C.

The APC/C is a widely conserved, multi-subunit RING-type E3 ubiquitin ligase (see Box 1 and Fig. 1), with well-documented roles in cell cycle progression (Peters, 2002). In budding yeast, the APC/C consists of at least 12 subunits (see Table 1 and Fig. 1). Although the functions of many subunits remain unclear, two activators called Cdc20/Fizzy and Cdh1/Fizzy-related are likely substrate-specific adaptors that recruit target proteins to the APC/C for poly-ubiquitination (Kraft et al., 2005; Pfleger et al., 2001) (see Box 1). Progression through meiosis in budding yeast also requires the APC/C, when securin ubiquitination frees separase to cleave the meiosis-specific cohesin Rec8, triggering meiotic anaphase (Salah and Nasmyth, 2000; Nasmyth, 2001). Curiously, the APC/C does not appear to be required for *Xenopus* meiotic divisions (Peter et al., 2001; Taieb et al., 2001).

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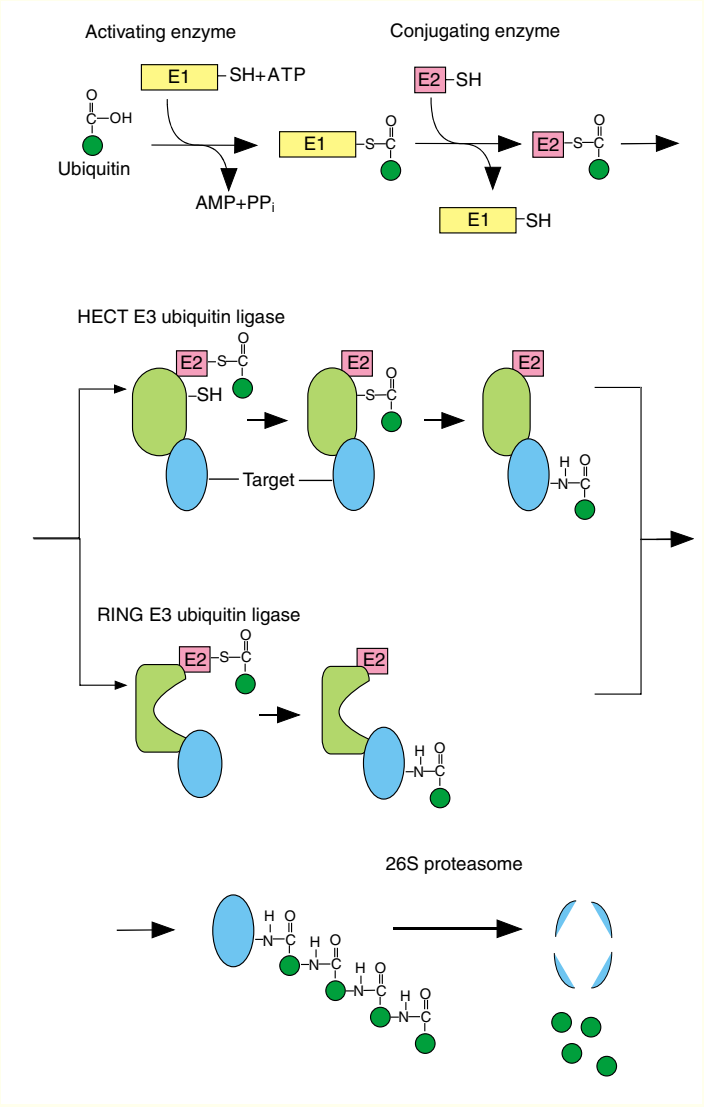
Box 1. The ubiquitin proteasome system

Ubiquitination entails the covalent modification of target proteins with a chain of at least four ubiquitin (Ub) proteins (see figure), each only 76 amino acids in size, that target a protein for recognition and degradation by the 26S proteasome (Chau et al., 1989; Thrower et al., 2000). Ubiquitin conjugation requires three enzymatic steps (see figure). First, an E1-activating enzyme hydrolyzes ATP to produce a high-energy E1~Ub thiol ester between the C terminus of Ub and an E1 cysteine (Cys) residue. Second, the activated Ub is passed from the E1 to a Cys residue of an E2-conjugating enzyme. Third, the Ub-charged E2 is recruited by an E3 ligase, which also binds the substrate that will be poly-ubiquitinated by the E2-conjugating enzyme. E3 ligases regulate proteolysis by selectively binding only a subset of substrates (Pickart, 2001). The two main types are called HECT and RING E3s (after domains found within key components). RING E3 ligases bring the substrate and the Ub-charged E2-conjugating enzyme into close proximity, promoting Ub transfer directly from the E2 to the target. HECT E3s form a thiol ester intermediate with Ub before transferring it to the target (Pickart, 2001). The first Ub tag forms an isopeptide bond through its C-terminal glycine residue with an internal lysine (Lys), or rarely with the N terminus, of the target protein. Linkage of another Ub onto Lys48 of the previous Ub extends the chain. The proteasome recognizes the poly-ubiquitin chain and, in an ATP-dependent manner, unfolds the tagged substrate and translocates it into the proteasome, where hydrolysis ensues (Pickart, 2000; Voges et al., 1999). De-ubiquitinating enzymes remove Ub chains from the substrate prior to translocation, preventing Ub degradation (Guterman and Glickman, 2004). Specificity in Ub target selection ensures that proteins are degraded at the right time and place. Some substrates need modification, e.g. by phosphorylation, to be recognized by an E3 ligase (Ang and Harper, 2004). The Ub system itself also provides substantial intrinsic variability and thus specificity. Although there is only one Ub E1-activating enzyme in any one organism, E2 conjugating enzyme numbers vary from 11 in *S. cerevisiae* to 20 in *C. elegans,* and many more in mammals. E3 ligase enzymes are far more numerous. Thus, many possible E2 and E3 combinations can influence substrate specificity, and some E3 ligases are themselves multi-subunit complexes with interchangeable substrate recognition modules, allowing for even more diversity (Willems et al., 2004) (see Fig. 1).



The APC/C and cell cycle progression in *C. elegans*

As in other organisms, the APC/C is required for progression through meiosis and mitosis in *C. elegans*. Meiosis begins during oogenesis, in the U-shaped hermaphrodite ovary, a syncytial tube of peripherally located nuclei (Albertson et al., 1997; Hubbard and Greenstein, 2000) (see Fig. 2). After fertilization, meiosis is completed, extruding chromosomes into two small polar bodies to generate a haploid female pronucleus (Fig. 2). Several groups have identified *C. elegans* APC/C mutants that arrest in metaphase of meiosis I, or exhibit germline mitosis and other meiosis defects (Table 1). Genome-wide RNAi screens also have implicated the APC/C and ubiquitin-mediated proteolysis in meiotic progression (Kamath et al., 2003; Sonnichsen et al., 2005; Gunsalus et al., 2005).

Most of the mutant APC/C alleles identified in *C. elegans* are temperature sensitive, and at semi-permissive temperatures these mutations can cause abnormalities during meiosis I or II, suggesting that the APC/C functions in both meiotic divisions (Shakes et al., 2003). The defects during meiosis II include a failure to separate sister chromatids and an elongated spindle. However, no metaphase arrests have been observed, leaving it unresolved whether the metaphase-to-anaphase transition in meiosis II requires the APC/C. Indeed, there is evidence that anaphase onset during meiosis II is controlled by another ubiquitin E3 ligase (Liu et al., 2004; Sonneville and Gonczy, 2004) (see below). Finally, no defects in mitotic cell cycle progression in early embryonic cells have been documented in APC/C mutants shifted to fully restrictive temperatures after the completion of meiosis (Golden et al., 2000). Thus, it remains unknown whether the APC/C is required for anaphase onset during mitosis in early embryonic cells. Furthermore, substrates targeted for degradation by the APC/C in *C. elegans* have not yet been identified.

Cullin-based E3 ligases

Progression through the cell cycle is also regulated by other multi-subunit E3 ligases that use proteins called cullins as scaffolds (see Fig. 1). Most animal genomes encode five cullin family members (cullin 1 to cullin 5), with one member of each subfamily present in *C. elegans* (Pintard et al., 2004). Cullin 1-based SCF (after the

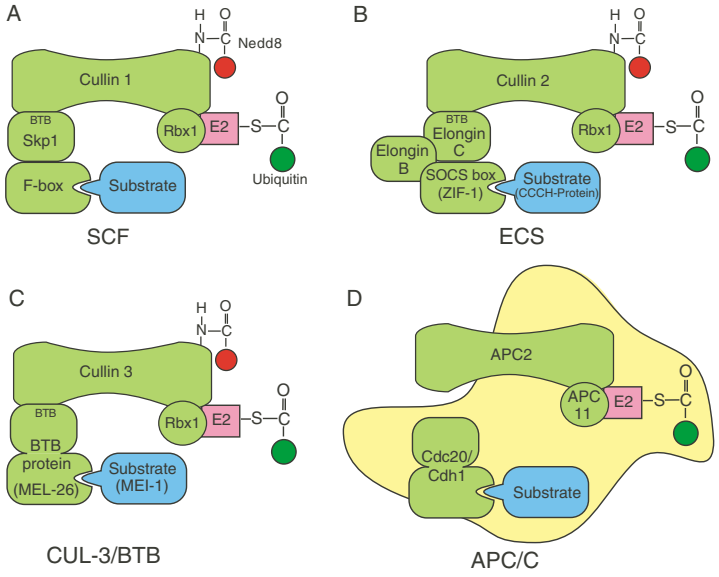


Fig. 1. Schematic representations of different cullin RING ubiquitin ligases and the APC/C. All cullin complexes are Nedd8 modified (see Box 2) and recruit E2-bound ubiquitin through their association with the RING-finger protein Rbx1. (**A**) The SCF complex is the best-characterized cullin ligase. Cullin 1 proteins bind at their N terminus to the BTB-fold protein Skp1. Skp1, in return, interacts with different F-box proteins, which specifically recognize substrates (blue). (**B**) Cullin 2-based ECS complexes recruit their substrate through their interaction with elongin B and elongin C and a variable SOCS-box protein, such as ZIF-1 (hence the name ECS complex). (**C**) Cullin 3-based complexes are unique in using only one protein to bind the cullin and the substrate. (**D**) The APC/C has many more subunits than the cullin ligases, many of which have not been assigned a function and are shown as a single yellow mass. Although not Nedd8 modified, APC2 has sequence similarity to cullin proteins and interacts with the APC11 RING-finger protein. Different WD40 repeat proteins, e.g. Cdc20 or Cdh1, mediate substrate recognition by the APC/C. APC/C, anaphase promoting complex/cyclosome; BTB, Bric-a-Brack, Tramtrack and Brahma; Cdc20, cell division cycle 20; Cdh1, Cdc20 homolog 1; ECS, elongin B/C, cullin 2, SOCS box; MEI-1, meiosis defective 1; MEL-26, maternal effect lethal; Rbx1, ring box protein 1; SCF, Skp1, cullin 1 and F-box subunits; Skp1, suppressor of kinetochore protein 1; ZIF-1, zinc-finger interacting factor 1.

Skp1, cullin 1 and F-box subunits) and cullin 2-based ECS ligases (after the elongin B/C, cullin 2 and SOCS box subunits) constitute two distinct E3 subclasses (see Fig. 1). In SCF complexes, the cullin 1 scaffold binds through its N terminus to a substrate-recognition module consisting of Skp1 (suppressor of kinetochore protein 1) and an F-box protein. The small ring-finger protein Rbx1 (ring box protein 1) binds to the C terminus of cullin 1 and recruits an E2 ubiquitin-conjugating enzyme, positioning the E2 ubiquitin moiety near the substrate (Willems et al., 2004; Deshaies, 1999) (see Box 1 and Fig. 1A). One well-characterized SCF E3 ligase is required for the G1 to S transition that initiates DNA replication prior to mitosis in budding yeast (Willems et al., 2004; Deshaies, 1999).

Cullin 2-based ECS E3 ligases also recruit Rbx1 proteins, ubiquitin E2-conjugating enzymes and substrate-recognition adaptor modules. The adaptors include Elongin B and Elongin C proteins, which bind to the cullin 2 N terminus, and substrate-binding proteins called SOCS boxes (Kile et al., 2002; Kim and Kaelin, 2003) (Fig. 1B). A well-characterized SCF E3 ligase is the human von Hippel-Lindau (VHL) tumor suppressor complex, which degrades the hypoxia-inducible factor HIF1 under normoxic conditions. Mutations in VHL complex components have been implicated in kidney cancer (Kim and Kaelin, 2003).

C. elegans cullin 2 and meiosis II

In *C. elegans,* cullin 2 (CUL-2) E3 ligases, in addition to the APC/C, are required for cell cycle progression. For example, worms lacking CUL-2 fail to enter S phase in the germline (Feng et al., 1999). Depletion of the cell division kinase CKI-1 rescues this cell cycle arrest, suggesting that CKI-1 may be a target of a CUL-2-based E3 ligase (Feng et al., 1999). Loss of CUL-2 function also impairs meiosis in the early embryo, delaying both the onset of meiosis II anaphase and exit from meiosis II (Liu et al., 2004; Sonneville and Gönczy, 2004). Similar meiosis defects are observed after depletion of RBX-1 or of ELB-1/ELC-1 (Elongin B1/Elongin C1) (Liu et al., 2004; Sasagawa et al., 2005; Sasagawa et al., 2003). Furthermore, ELB-1 and ELC-1 form a stable complex, and ELC-1 binds CUL-2, indicating that an ECS E3 ligase is required for progression through meiosis II (Liu et al., 2004; Sasagawa et al., 2005). The meiosis II defects in mutants lacking CUL-2 are not a result of a failure to degrade the meiosis-specific cohesin REC-8 (Liu et al., 2004), and thus the targets of CUL-2 during meiosis II remain unknown. Meiosis I progresses normally in mutants lacking CUL-2 function (Liu et al., 2004; Sonneville and Gönczy, 2004), while the APC/C appears to promote the transition to anaphase only during meiosis I (see Table 1 and above). Presumably the two meiotic divisions require different sets of E3 targets, accounting for the use of two distinct ligases. Nevertheless, CUL-2 may act redundantly with the APC/C in meiosis I, as CUL-2 loss of function enhances the meiosis I defects in temperature-sensitive APC/C mutants (Sonneville and Gönczy, 2004).

The Armadillo repeat protein ZYG-11 is potentially a substrate-specific adaptor for CUL-2, as *zyg-11(–)* mutants exhibit the same meiosis II defects that result from CUL-2 inactivation (Liu et al., 2004; Sonneville and Gönczy, 2004). However, germline proliferation requires only CUL-2 and not ZYG-11 (Liu et al., 2004). Thus, ZYG-11 may recruit only a subset of the CUL-2 targets. Like some adaptors in other cullin-based E3 ligases, ZYG-11 is itself degraded in a CUL-2-dependent manner (Sonneville and Gönczy, 2004). However, ZYG-11 does not contain a SOCS box, a hallmark of CUL-2 substrate adaptors (Kile et al., 2002), and thus far there is no evidence for a physical interaction between ZYG-11 and a potential substrate, or between ZYG-11 and both ELC-1 and CUL-2. Hence, ZYG-11 may activate CUL-2 through an as yet unidentified mechanism, rather than acting as a substrate-specific adaptor.

Although the targets of CUL-2 and ZYG-11 remain unknown, the two B-type cyclins B1 and B3 have been shown to accumulate during meiosis in the absence of CUL-2 (Liu et al., 2004; Sonneville and Gönczy, 2004). This result may explain the observed M-phase delay in *cul-2(–)* mutants, as the destruction of B-type cyclins is known to be required for mitotic exit (Glotzer et al., 1991; Peters, 1999). The inactivation of either cyclin B1 or cyclin B3 in *cul-2(–)* and *zyg-11(–)* mutants alleviates the meiotic exit delay, but not the delay in anaphase onset (Liu et al., 2004; Sonneville and Gonczy, 2004). Thus, cyclins B1 and B3 may be substrates for a putative $CUL2^{ZYG\text{-}11}$ ligase. Alternatively, the upregulation of cyclins B1 and B3 could be an indirect result of a failure to degrade other CUL-2 targets.

Table 1. APC components

	S. cerevisiae	*C. elegans*	Structural motifs	*C. elegans* references
APC subunits	Apc1	MAT-2	RPN1/2 homolgy (proteasome subunits)	Davis et al., 2002; Shakes et al., 2003; Golden et al., 2000; Rappleye et al., 2002
	Apc2	APC-2	Cullin homology	Davis et al., 2002
	Cdc27	MAT-1	Tetratrico peptide repeats	Davis et al., 2002; Shakes et al., 2003; Golden et al., 2000; Rappleye et al., 2002
	Apc4	EMB-30	WD40 repeats	Cassada et al., 1981; Furuta et al., 2000; Davis et al., 2002; Shakes et al., 2003; Rappleye et al., 2002
	Apc5	APC-5	Tetratrico peptide repeats	Davis et al., 2002
	Cdc16	EMB-27	Tetratrico peptide repeats	Cassada et al., 1981; Golden et al., 2000; Davis et al., 2002; Shakes et al., 2003; Rappleye et al., 2002
	Cdc23	MAT-3	Tetratrico peptide repeats	Davis et al., 2002; Shakes et al., 2003; Golden et al., 2000; Rappleye et al., 2002
	Apc9	Not known	Not known	
	Apc10	APC-10	APC10/DOC domain	Davis et al., 2002
	Apc11	APC-11	RING-H2 domain	Davis et al., 2002
	Cdc26	Not known	Not known	
	Apc13	Not known	Not known	
APC activators	Cdc20	FZY-1	WD40 repeats	Kitagawa et al., 2002; Rappleye et al., 2002
	Cdh1	FZR-1	WD40 repeats	Fay et al., 2002
	Ama1	Not known	WD40 repeats	

See text for details and Peters, 2002 for a review.

In summary, multiple E3 ligases regulate cell cycle progression in the early *C. elegans* embryo, while the substrates that are targeted for degradation remain unidentified. Finding the substrates would greatly improve our understanding of these regulatory events.

Ubiquitination and cytoskeletal dynamics

The transition from oogenesis to embryogenesis is a key step in animal development. In *C. elegans,* an oocyte remains diploid until fertilization by a haploid sperm. Subsequently, oocyte meiosis is completed to generate a haploid maternal complement of chromosomes and thus a diploid zygote. As in other animals, the female meiotic spindles are small and acentrosomal, although the much larger mitotic spindles that assemble shortly after meiosis are organized by centrosomes (Figs 2 and 3). Thus, the transition from oogenesis to embryogenesis includes the proper regulation of microtubule dynamics and spindle assembly during meiosis and then mitosis. In *C. elegans,* a key player in this transition is the microtubule severing complex called katanin (McNally and Vale, 1993; Srayko et al., 2000). Katanin is required for meiotic spindle assembly, presumably by promoting the presence of short microtubules, but must be downregulated after meiosis to permit the assembly of much larger mitotic spindles (Mains et al., 1990; Clark-Maguire and Mains, 1994a; Clark-Maguire and Mains, 1994b; Srayko et al., 2000; Kurz et al., 2002; Pintard et al., 2003a; Yang et al., 2003).

C. elegans CUL-3 and katanin downregulation

Katanin downregulation requires a cullin 3-based E3 ubiquitin ligase. Depletion of *C. elegans* CUL-3 prevents katanin degradation after meiosis, causing defects during mitosis in microtubule stability, spindle positioning and chromosome segregation (Kurz et al., 2002; Pintard et al., 2003a; Pintard et al., 2003b). Although cullin 1 and cullin 2 scaffolds were known to be required for assembling SCF and ECS ligases (see Fig. 1), little was known about the other cullin sub-families. Thus, the discovery that CUL-3 is required for katanin downregulation suggested that a novel class of ubiquitin E3 ligases targets katanin for proteolysis.

Analysis of a *C. elegans* protein called MEL-26 has substantially advanced our understanding of the CUL-3 E3 ligase that targets katanin (Pintard et al., 2003b). Previous work had shown that MEL-26 is required for katanin downregulation and that it contains a BTB (Bric-a-Brack, Tramtrack and Brahma) domain, which is also found in several transcription factors but was of unknown function (Dow and Mains, 1998). Subsequent crystallographic studies revealed that the adaptor proteins Skp1 and Elongin C bind to cullin 1 and cullin 2, respectively, through diverged BTB folds (Stebbins et al., 1999; Schulman et al., 2000; Stogios et al., 2005; Zheng et al., 2002b). These data suggested that MEL-26 functions as a substrate-specific adaptor. Consistent with this model, MEL-26 binds to the N terminus of CUL-3 through its BTB fold (Furukawa et al., 2003) (Pintard et al., 2003b; Xu et al., 2003), and to one katanin subunit, called MEI-1, through a C-terminal MATH (meprin and TRAF homology) domain (Pintard et al., 2003b; Xu et al., 2003) (see Fig. 1C). MEI-1 contains a high-scoring PEST motif, a sequence present in many short-lived proteins. A dominant mutation in this PEST motif results in a loss of binding to MEL-26 and post-meiotically stable MEI-1/katanin activity (Pintard et al., 2003b; Xu et al., 2003). Moreover, MEL-26, like some substrate-specific adaptors for SCF E3 ligases, is itself targeted for degradation by CUL-3 (Luke-Glaser et al., 2005; Pintard et al., 2003b). Finally, CUL-3 and MEL-26 can mediate the poly-ubiquitination of MEI-1 in vitro (Furukawa et al., 2003), confirming that MEL-26 is a substrate-specific adaptor for an ubiquitin E3 ligase.

The direct interaction of MEL-26 with both CUL-3 and MEI-1 distinguishes it from SCF and ECS adaptors, in which one component binds to the cullin, while a second binds to the first component and to the substrate (Fig. 1). Intriguingly, the *C. elegans* genome encodes 47 proteins with both a BTB domain and another protein-protein

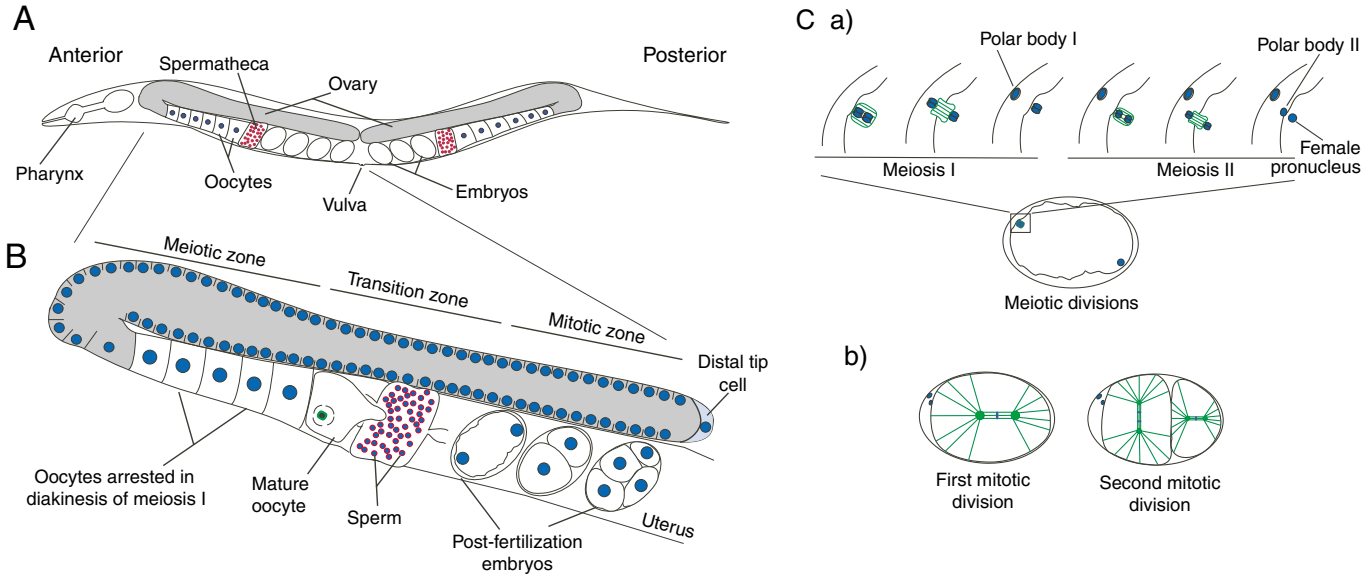


Fig. 2. Oogenesis and early embryogenesis in *C. elegans*. (**A**) A schematic of an adult *C. elegans* hermaphrodite to show the bilaterally symmetrical U-shaped gonad arms (grey). Anterior is towards the left, dorsal is upwards. The major anatomical features are indicated. (**B**) One arm of the U-shaped *C. elegans* gonad. After dividing mitotically in the distal arm of the gonad, germ cell nuclei (in blue), partially enclosed by membrane cubicles, migrate proximally, enter meiosis and cellularize into oocytes. Mature oocytes enter the spermatheca, are fertilized and exit as zygotes into the uterus. The fertilized zygotes form an eggshell, finish meiosis and undergo the first mitotic divisions in the uterus. (**C**) (a) Meiotic divisions I and II, and (b) the first two mitotic divisions of a *C. elegans* embryo. DNA, blue; meiotic and mitotic spindles, green. (a) After fertilization, the *C. elegans* embryo executes the first and second meiotic division in the anterior of the zygote, resulting in the extrusion of two polar bodies and the formation of a haploid female pronucleus (top). The female meiotic spindles are small and lack centrosomes. After meiosis, the male and female pronuclei fuse, and the zygote enters mitosis. (b) The mitotic spindle is organized by two centrosomes (green circles) at the spindle poles. The first division is asymmetric, producing larger anterior and smaller posterior daughter cells that differ in cell cycle timing and mitotic spindle orientation during the next round of mitosis.

interaction domain, while the human genome encodes 103 such proteins (Xu et al., 2003). Many of the *C. elegans* MEL-26-like proteins specifically bind to CUL-3 and not to other cullins (Furukawa et al., 2003; Xu et al., 2003), suggesting that CUL-3 may form multiple ligases with different MEL-26-like BTB proteins. Thus, the CUL-3^{MEL-26} E3 ligase appears to be the founding member of a third class of cullin-based RING-type E3 ligases. One MEL-26-like human protein is mutated in an axonal neuropathy (Bomont et al., 2000).

MEL-26 and cortical actomyosin

In addition to regulating microtubule stability, MEL-26 also influences the contractile forces generated by the cortical actomyosin cytoskeleton during cell division (Luke-Glaser et al., 2005) (see Fig. 4). Depletion of CUL-3 results in excessive cortical contractility both during the migration of the egg and sperm pronuclei, and during cytokinesis (Kurz et al., 2002) (Fig. 4D). Normally, pronuclear migration is accompanied by modest membrane invaginations in the anterior half of the zygote, after which a single cleavage furrow ingresses during cytokinesis. In *cul-3(–)* mutant embryos, extra furrows form during both pronuclear migration and cytokinesis. However, excess cortical contractility is not observed in *mel-26(–)* mutants, or in the dominant *mei-1/katanin* mutant that resists degradation (Luke-Glaser et al., 2005). Thus, the extra furrowing is not due simply to the persistence of katanin and microtubule instability during mitosis. Instead, the excess actomyosin contractility in *cul-3(–)* embryos requires MEL-26, as the cortex appears normal in *cul-3(–); mel-26(–)* double mutants. Indeed, MEL-26 localizes to ingressing cytokinetic furrows in wild-type embryos, and cytokinetic furrows ingress more slowly and sometimes fail in *mel-26(–)* mutant embryos. Finally, MEL-26 is present at substantially higher levels in *cul-3(–)* mutants (Luke-Glaser et al., 2005). Thus, MEL-26 is an activator of cortical actomyosin contractility, and MEL-26 degradation modulates this activity.

MEL-26 promotes actomyosin forces through a direct interaction with POD-1 (Luke-Glaser et al., 2005), a cortically localized, coronin-like, microfilament-binding protein required for cytokinesis (Rappleye et al., 1999). The MATH domain of MEL-26 binds POD-1, and this interaction is required for the cortical localization of MEL-26 (Luke-Glaser et al., 2005). A point mutation in MEL-26 that prevents POD-1 binding also blocks MEL-26 cortical localization but does not promote excess cortical contractility upon inactivation of CUL-3, even though POD-1 remains at the cortex (Luke-Glaser et al., 2005). Thus, the excessive furrowing observed in *cul-3(–)* mutants is mediated by MEL-26 through its interaction with POD-1. Importantly, POD-1 is not a target of the CUL-3^{MEL-26} ligase, as POD-1 levels are not elevated in *cul-3(–)* mutants (Luke-Glaser et al., 2005). Instead, the failure to lower MEL-26 levels in *cul-3(–)* mutants promotes excess furrowing, although the mechanism by which MEL-26 stimulates POD-1/coronin remains unknown. These findings highlight the indirect defects that can occur when E3 ligases are inactivated, owing to some E3 components having additional functions unrelated to substrate ubiquitination.

Cytoskeleton defects and the Nedd8 ubiquitin-like protein conjugation pathway

The discovery that katanin is degraded by the proteasome after meiosis followed from studies of a mutant called *rfl-1* (for membrane ruffling) (Kurz et al., 2002). In *rfl-1(–)* mutant embryos,

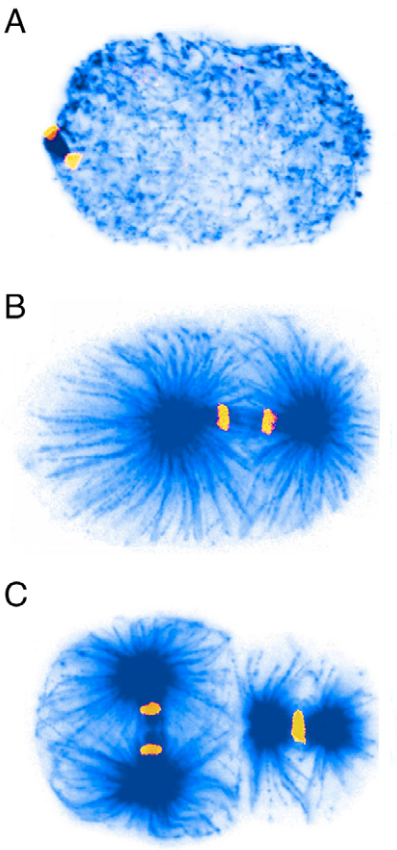
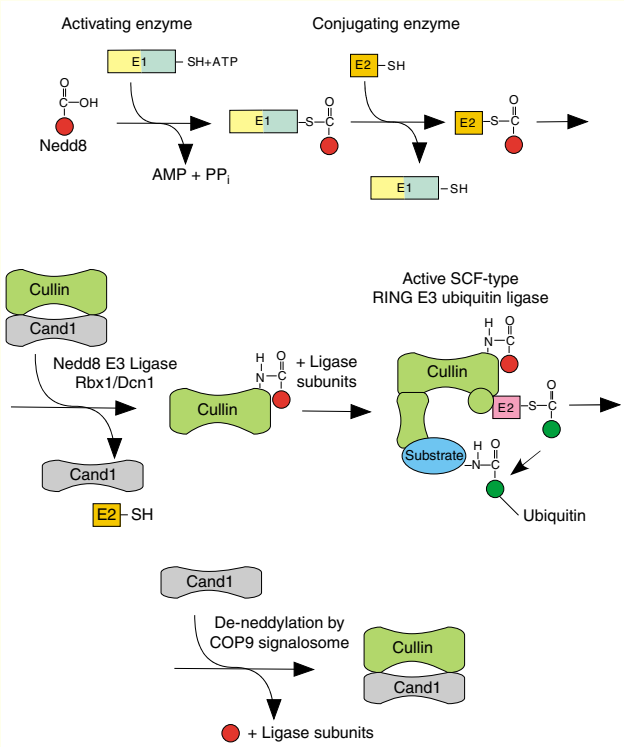


Fig. 3. Meiosis and mitosis in early *C. elegans* embryos. (A-C) *C. elegans* embryos stained for tubulin (blue) and DNA (yellow). (**A**) Shortly after fertilization, a small meiotic spindle assembles at the anterior cortex. At this stage, most tubulin is not recruited to the spindle, but is scattered throughout the cytoplasm. (**B**) During the first mitosis, a large and asymmetric spindle forms. The first mitosis produces daughter cells of unequal size. (**C**) The anterior somatic precursor AB is shown undergoing anaphase of mitosis, while the posterior germline precursor P1 is still in metaphase.

Box 2. The Nedd8 conjugation pathway



Nedd8 and ubiquitin are about 57% identical in amino acid sequence, and Nedd8 is transferred to target proteins by E1 and E2 enzymes related to the E1 and E2s that mediate ubiquitin conjugation (Liakopoulos et al., 1998) (see figure). Unlike ubiquitin, which modifies many proteins, the only well-characterized targets of Nedd8 modification (neddylation) are cullins; all cullin subtypes, except for the cullin-like APC2 protein, have been shown to be neddylated (Hori et al., 1999) (see also Fig. 1). Neddylation is believed to promote E3 assembly by removing from cullin proteins an assembly inhibitor called Cand1 (see figure and main text for more details).

as in *cul-3(–)* mutants, mitotic microtubules are unstable because of the persistence of MEI-1/katanin after meiosis, and extra MEL-26-dependent membrane ingressions appear during pronuclear migration and cytokinesis (Kurz et al., 2002; Luke-Glaser et al., 2005). Positional cloning revealed that *rfl-1* is the *C. elegans* ortholog of *UBA3*, a *S. cerevisiae* gene that encodes one subunit of the heterodimeric E1-activating enzyme for an ubiquitin-like protein called Rub1p in yeast and NEDD8 in humans (Kurz et al., 2002) (see Box 2). In contrast to ubiquitin, NEDD8 neither forms chains on, nor triggers the proteasomal degradation of, its cullin targets. Rather, cullin modification by a single NEDD8 moiety is thought to promote E3 assembly by removing from the cullin an assembly inhibitor called Cand1/TIP120A (Goldenberg et al., 2004; Liu et al., 2002; Oshikawa et al., 2003; Zheng et al., 2002a) (see Box 2). Cullin neddylation may also assist Rbx1 in recruiting ubiquitin E2-conjugating enzymes to the E3 ligase (Kawakami et al., 2001).

Loss of RFL-1, or of any other Nedd8 conjugation pathway component in *C. elegans*, results in a loss of CUL-3 function (Kurz et al., 2002) (see Table 2). By contrast, the defects observed after inactivation of CUL-2 do not overlap with those caused by inactivation of the Nedd8 conjugation pathway (Liu et al., 2004; Sonneville and Gönczy, 2004; Kurz et al., 2002) (see Table 2). Thus, CUL-2 probably does not require neddylation. Similarly, the budding yeast CUL-1 homolog Cdc53p is neddylated but does not require Nedd8 to function in essential processes (Liakopoulos et al., 1998; Lammer et al., 1998). It seems clear that cullin subfamilies differ substantially in their requirements for neddylation, and therefore perhaps differ in their potential for regulation by neddylation.

A screen for additional factors required for katanin degradation in *C. elegans* recently identified a new and widely conserved Nedd8-conjugation pathway component called DCN-1 (for defective in cullin neddylation) (Kurz et al., 2005). RNAi depletion of DCN-1 in early embryos reduces CUL-3 neddylation, and katanin persists into mitosis to cause the same cytoskeletal defects observed in *rfl-1(–)* and *cul-3(–)* mutant embryos. The *S. cerevisiae* ortholog Dcn1p is also required for cullin neddylation (Kurz et al., 2005). The requirement for DCN-1/Dcn1p in neddylation appears to be direct, as Dcn1p binds to the C terminus of the cullin Cdc53p, near the neddylation site, and DCN-1 also directly binds Nedd8. Moreover, *S. cerevisiae* Dcn1p can increase the rate of neddylation in vitro

Table 2. E3 ligases acting during *C. elegans* embryogenesis

E3 ligase	Subunits	Regulators	Substrates	Processes
APC/C*	see Table 1	FZY-1 (fizzy)	IFY-1 (interactor of fizzy)?	Meiosis I and II, embryonic cortical polarity?
CUL-2^{ZYG-11}*	CUL-2,ELC-1, ELB-1, RBX-1, ZYG-11	Not known	CYB-1, CYB-3 (cyclin B), ZYG-11	Meiosis I and II, embryonic cortical polarity?
CUL-2^{ZIF-1}*	CUL-2, ELC-1, ELB-1, RBX-1, ZIF-1	MBK-2, MEX-5/6	PIE-1, POS-1, MEX-1, MEX-5, MEX-6, ZIF-1	Embryonic cytoplasmic polarity
CUL-3^{MEL-26}	CUL-3, MEL-26, RBX-1	MBK-2, Nedd8 pathway (NED-8, RFL-1, ULA-1, UBC-12, DCN-1), COP9 signalosome (CSN-1 to CSN-6)	MEI-1, MEL26	Meiosis-to-mitosis transition; regulation of cortical contractility
Not known	Not known	MBK-2, GSK-3	OMA-1/2	Oocyte to embryo transition; cell polarity

*The substrates, subunits and activators of these ligases are inferred from sequence homology, mutant phenotype, genetics or binding studies, and have not been confirmed biochemically. See main text for references.

(Kurz et al., 2005). Thus, DCN-1/Dcn1p has been proposed to function as an E3 ligase for Nedd8 (see Box 2), possibly by cooperating with the RING protein Rbx1, which also promotes cullin neddylation (Morimoto et al., 2003).

Deneddylation and cullin function

Cullin de-neddylation, mediated by a protein complex called the COP9 signalosome (Lyapina et al., 2001; Schwechheimer et al., 2001), is also required for CUL-3^{MEL-26} E3 ligase function (Pintard et al., 2003a) (see Box 2). The signalosome consists of eight subunits (CSN1 through CSN8) in other organisms, but only six subunits (CSN1 through CSN6) have been identified in *C. elegans* (Pintard et al., 2003a). The inactivation of any one *C. elegans* subunit by RNAi increases the steady-state levels of Nedd8-modified CUL-3, and prevents the degradation of MEI-1/katanin after meiosis (Pintard et al., 2003a). Thus, both hypo- and hyper-neddylation of CUL-3 result in a loss of ligase function.

Interestingly, the lethality caused by a partial reduction of CUL-3 neddylation can be rescued by simultaneously reducing COP9 signalosome function (Pintard et al., 2003a). Thus, at least for the CUL-3^{MEL-26} E3 ligase, a balance of neddylation and de-neddylation is required for the optimal function of this ligase. One interpretation of this finding is that cycles of neddylation and deneddylation may promote E3 function. For example, neddylated CUL-3 might recruit an ubiquitin-charged E2-conjugating enzyme to the E3 ligase, with de-neddylation subsequently promoting expulsion of the spent E2. Alternatively, a Nedd8-modified E3 ligase might undergo multiple rounds of substrate ubiquitination, with de-neddylation promoting the exchange of a poly-ubiquitinated substrate for a new substrate molecule.

A recent study supports a more static model for neddylation and deneddylation (Wee et al., 2005). In this model, Nedd8 modification of the cullin is required for ligase assembly and activation. Once a substrate has been ubiquitinated, cullin de-neddylation shuts down the ligase to prevent ubiquitination and degradation of the substrate-specific adaptor. Consistent with this model, some BTB substrate adaptors are rapidly ubiquitinated and degraded in *S. pombe* de-neddylation defective mutants, preventing degradation of the corresponding substrates (Wee et al., 2005). However, not all adaptors exhibit this property (Wee et al., 2005), and whether MEL-26 is degraded in signalosome-defective mutants is not known. In vitro studies of CUL-3 ligase assembly and function will be required to distinguish between these cycling and static models.

The discovery and analysis of the CUL-3^{MEL-26} E3 ligase in *C. elegans* has provided mechanistic insight into the regulation of microtubule stability and actomyosin contractility during the transition from oogenesis to early embryogenesis. These studies also have provided substantial insight into conserved mechanisms that regulate ubiquitin-mediated proteolysis, highlighting the utility of *C. elegans* for studies of this fundamentally important regulatory system.

Ubiquitination and cell fate determination

The rapid development of the early *C. elegans* embryos is characterized by a sequence of five asymmetric cell divisions that are largely responsible for establishing the nematode body plan (Sulston et al., 1983) (reviewed by Lyczak et al., 2002). These asymmetric cell divisions require proper positioning of mitotic spindles, which depends in part on the destruction of katanin after meiosis (see above). These early divisions also require the proper regulation of cell polarity, with developmental determinants asymmetrically inherited by daughter cells. Recent studies have implicated ubiquitin-dependent proteolysis both in the initial establishment of cell polarity along the AP axis in the one-cell *C. elegans* zygote, and in subsequent events that further influence the asymmetric inheritance of cell fate determinants by early embryonic cells.

The APC/C and axis specification

In addition to its requirements for progression through meiosis (see above), loss of APC/C function in *C. elegans* can also result in a failure to establish the anteroposterior (AP) body axis (Wallenfang and Seydoux, 2000; Rappleye et al., 2002) (see Fig. 4). In wild-type embryos, the AP axis is specified by the position of the sperm-donated pronucleus and its associated centrosomes, which become closely apposed to the plasma membrane and specify the posterior pole (Albertson, 1984; Goldstein and Hird, 1996). Axis specification does not require the sperm pronucleus (Sadler and Shakes, 2000), but fails in centrosome maturation-defective mutants (Hamill et al., 2002; O'Connell et al., 2000; Wallenfang and Seydoux, 2000), and after laser ablation of the centrosome in wild-type embryos (Cowan and Hyman, 2004) (see Fig. 4A). The duration of the close apposition of the sperm pronucleus with the cell cortex is substantially reduced in APC/C or separase mutants, leading to suggestions that the APC/C is directly required for this close apposition and thus for the specification of the posterior pole (Rappleye et al., 2002).

The arrest at metaphase of meiosis I in *C. elegans* APC/C mutants frequently results not just in a loss of polarity but in a partial reversal of the AP axis, and this reversal depends on the assembly of a meiotic spindle (Wallenfang and Seydoux, 2000). Thus, it appears that whereas the sperm-donated centrosomes normally specify the posterior pole, in APC/C mutants the meiotic spindle, which usually forms at the pole opposite the site of sperm entry (see Fig. 2), can ectopically (but only partially) specify a posterior pole. Indeed, even in wild-type embryos, posterior-specific cortical proteins sometimes transiently accumulate near the meiotic spindle, at what will ultimately become the anterior pole (Boyd et al., 1996; Cuenca et al., 2003). This temporary reversal is often stabilized in APC/C mutants, presumably owing to the persistence of the meiotic spindle and the failure of the sperm pronucleus-associated asters to mature (Wallenfang and Seydoux, 2000). Apparently, the transient nature of the meiotic spindle, and the subsequent maturation of the sperm-donated centrosomes, results in the normal specification of a posterior pole in wild-type embryos (reviewed by Schneider and Bowerman, 2003; Cowan and Hyman, 2004).

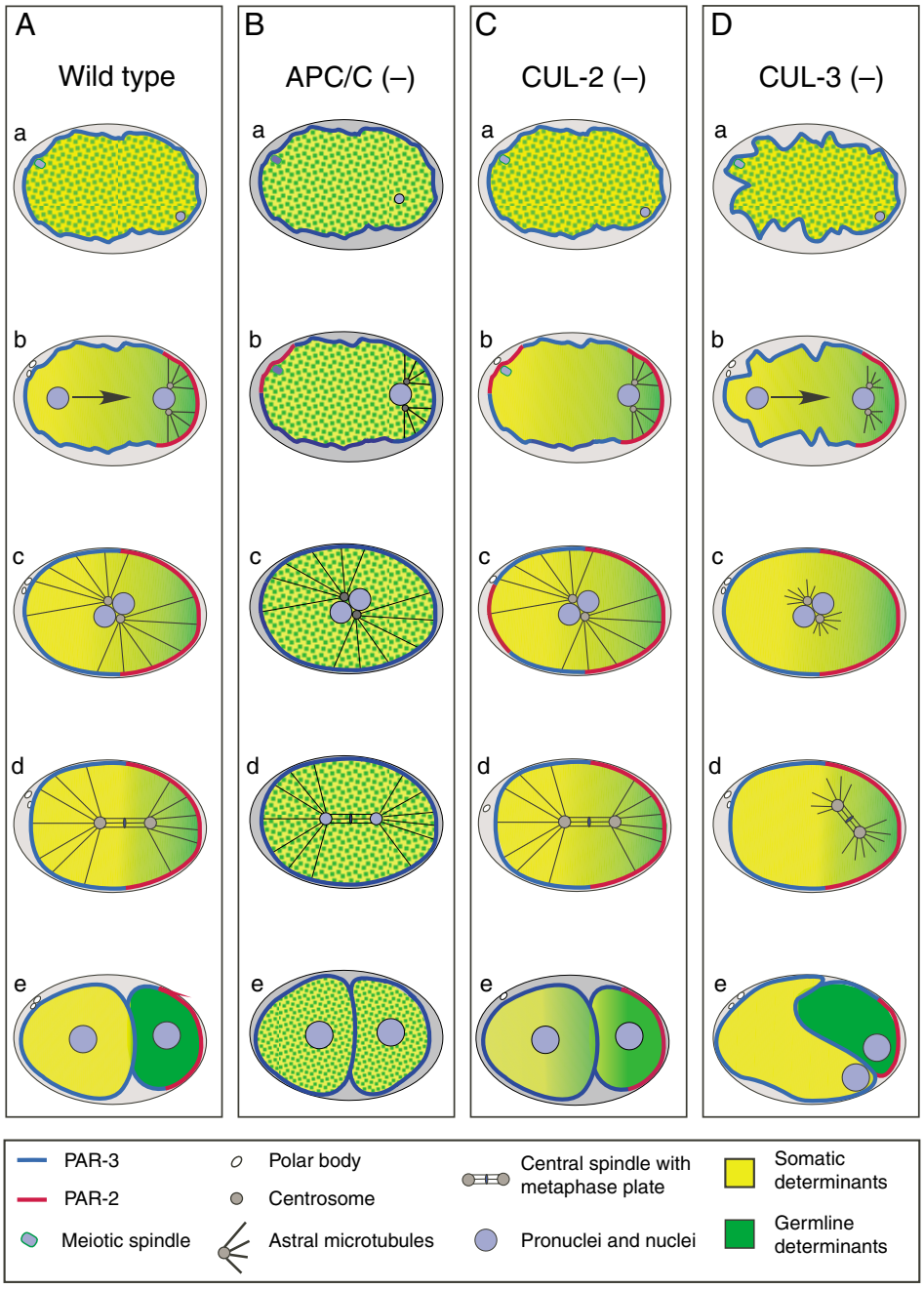


Fig. 4. A schematic of *C. elegans* embryos undergoing meiosis, axis specification and the first mitotic division in wild-type and different E3 ubiquitin ligase mutants. (**A**) During meiosis in wild-type embryos, cytoplasmic cell fate determinants are uniformly distributed in the cytoplasm and the PAR-3 protein (blue) occupies the entire cortex (a). After meiosis, the centrosomes that accompany the male pronucleus trigger the establishment of anteroposterior (AP) polarity: starting at the site of sperm aster-cortex contact, the posterior pole is specified and PAR-2 (red) spreads, replacing PAR-3 at the posterior cortex. (b,c) During this process, cytoplasmic cell fate determinants are also actively partitioned to the anterior (yellow; e.g. MEX-5 and MEX-6) and posterior (green; e.g. MEX-1, PIE-1, POS-1 and germline P granules) cytoplasm. (d) The first mitotic spindle aligns along the AP axis and cytokinesis cleaves the cell asymmetrically. (e) The respective cell fate determinants are confined to the anterior or posterior cell, and residual 'mis-localized' determinants are degraded by the ubiquitin proteasome system late at the two-cell stage. (**B**) Partial loss-of-function APC/C mutants do not arrest in meiosis, but go on to divide mitotically. (a,b) In these embryos, the meiotic spindle often persists longer than in wild type, the sperm pronucleus does not properly associate with the cortex and an aberrant cortical PAR-2 crescent often forms. (c-e) Subsequently, polarity is lost and the embryo divides symmetrically. (**C**) (a-c) Loss of function of CUL-2 results in a delayed exit from meiosis II and aberrant establishment of the cortical PAR-2 domain. (c,d) In some embryos, this polarity reversal is corrected and the first division is executed properly. (d,e) Although cortical polarity is normal, the cytoplasm is not polarized properly, owing to impaired degradation of cell fate determinants. (**D**) No polarity defects are observed in embryos in which CUL-3 has been inactivated. (a,b) Rather, extensive cortical contractions are apparent during pronuclear migration, owing to ectopic activation of the acto-myosin cytoskeleton by MEL-26. (c,d) The failure to degrade MEI-1/katanin results in the severing of mitotic microtubules and spindle orientation defects. (e) During and after cytokinesis, MEL-26-dependent ectopic furrows appear again.

The early requirements for the APC/C during meiosis make it difficult to determine whether the subsequent polarity defects observed in APC/C mutants are purely an indirect consequence of the meiotic delays, or are due to direct requirements for this E3 ligase. In one study, the duration of the meiotic delay did not correlate with defects in polarity, as most of the mutant embryos with polarity defects had only modest delays (Rappleye et al., 2002). However, a more recent study has found that APC/C mutant embryos with polarity defects always fail to extrude one polar body, owing to a bypass of meiosis II (Shakes et al., 2003). Mutants in which meiosis occurred more normally produced two polar bodies and never exhibited severe polarity defects. Thus, the nature of the meiotic progression defect appears to indirectly cause the loss of polarity, possibly owing to a failure to signal centrosome maturation properly after meiosis II (Shakes et al., 2003).

CUL-2 and axis specification

Reducing CUL-2 function also results in a failure to properly establish the AP body axis (Liu et al., 2004; Sonneville and Gönczy, 2004) (see Fig. 4C). In wild-type embryos, establishment of the AP axis by the sperm-donated centrosomes (see above) results in: (1) the cortical actomyosin cytoskeleton becoming enriched anteriorly; (2) the cortical polarity regulators PAR-3, PAR-6 and PKC-3 becoming restricted to the anterior pole; and (3) PAR-1 and PAR-2 occupying the posterior cortex (reviewed by Schneider and Bowerman, 2003). The polarized distribution of these cortical regulators then establishes subsequent asymmetries in the distributions of cytoplasmic cell-fate determinants. These include multiple proteins with CCCH-type zinc-finger domains: the nearly identical proteins MEX-5 and MEX-6 are restricted to the anterior, while the germline determinant PIE-1 is restricted to the posterior cytoplasm (reviewed by Lyczak et al., 2002) (see also Fig. 4). The polarized distribution of these determinants is required for the daughters of the first mitotic division to produce distinct patterns of cell fate as embryogenesis proceeds.

As in APC/C mutants, the AP axis is reversed in some *cul-2(–)* and *zyg-11(–)* mutant embryos, such that cortical PAR-2 becomes localized to the pole opposite the sperm pronucleus (Liu et al., 2004; Sonneville and Gönczy, 2004) (see Fig. 4C). Sometimes this reversal is maintained throughout the first mitotic cell cycle. In other mutant embryos, a wild-type PAR-2 distribution is established after the initial reversal, while in still other mutant embryos cortical PAR-2 localization and polarity are lost entirely (Sonneville and Gönczy, 2004).

The persistence of the meiotic spindle in *cul-2(–) and zyg-11(–)* mutants may be indirectly responsible for the AP axis defects, as in APC/C mutants (Wallenfang and Seydoux, 2000) (see above). However, the polarity requirements for CUL-2 and ZYG-11 may be more direct, as PAR-2 still localizes abnormally in *cul-2(–)* and *zyg-11(–)* mutant embryos, even after depleting α- or β-tubulin to disrupt meiotic spindle assembly (Liu et al., 2004; Sonneville and Gönczy, 2004). Furthermore, RNAi-mediated depletion of the cyclin CYB-3, like CUL-2 or ZYG-11 depletion, also delays meiotic spindle disassembly but does not cause polarity reversals (Sonneville and Gönczy, 2004). Conversely, entirely bypassing meiosis and hence the meiotic delays in *zyg-11(–)* mutant embryos, by depletion of the *C. elegans* Cdc2 homolog NCC-1, does not prevent axis reversal (Sonneville and Gönczy, 2004). Finally, depletion of CUL-2 or ZYG-11 in APC/C mutants results in an arrest during metaphase of meiosis I, but the polarity defects resemble those in *cul-2(–)* and *zyg-11(–)* mutants (Sonneville and Gönczy, 2004). These results suggest that a CUL-2 E3 ligase may be directly required for the proper establishment of the AP body axis. Nevertheless, it remains possible that the meiotic defects in *cul-2(–)* or *zyg-11(–)* mutants delay centrosome maturation, and thereby favor reversed polarity. An examination of centrosome maturation in *cul-2* (–) and *zyg-11(–)* mutants is needed to rule out an indirect effect. Ultimately, the identification of ubiquitin substrate(s) will be required to conclude that CUL-2 and ZYG-11 function directly in the establishment of AP polarity.

CUL-2 and cytoplasmic polarity

In addition to possibly influencing the initiation of cortical polarity, CUL-2 is required later in development to polarize the cytoplasm after the asymmetric division of the one-cell zygote (DeRenzo et al., 2003) (see Fig. 4). The posterior daughter of this first division inherits higher levels of PIE-1 and two additional cytoplasmic CCCH-type zinc finger proteins called POS-1 and MEX-1 (Guedes and Priess, 1997; Mello et al., 1996; Tabara et al., 1999). The anterior daughter inherits higher levels of MEX-5 and MEX-6 (Schubert et al., 2000). Subsequently, all five proteins are degraded in the anterior daughter and its descendants. Residual PIE-1, POS-1 and MEX-1 are degraded more rapidly, while MEX-5 and MEX-6 are degraded more slowly (DeRenzo et al., 2003; Reese et al., 2000). In each of these proteins, one of their two CCCH-type zinc fingers is required and sufficient for degradation (DeRenzo et al., 2003). Furthermore, the SOCS box protein ZIF-1 (zinc-finger interacting factor 1) binds specifically to the zinc-finger in each protein, and is required for their degradation (DeRenzo et al., 2003). ZIF-1 also interacts with ELC-1, suggesting that somatic degradation of CCCH-finger proteins requires the action of a CUL-$2^{ZIF\text{-}1}$ ligase, with ZIF-1 being the substrate-specific adaptor (DeRenzo et al., 2003). Consistent with this conclusion, partial RNAi depletions of CUL-2, RBX-1 and the ubiquitin-conjugating enzyme UBC-5 also results in a failure to degrade PIE-1, POS-1, MEX-1, MEX-5 and MEX-6 in the anterior embryo (DeRenzo et al., 2003). Although CUL-2, ZYG-11 and ZIF-1 are required to degrade the CCCH-finger proteins well after the first cell division, they are not required for the polarized distributions of these proteins that are observed in the cytoplasm just prior to this asymmetric division (DeRenzo et al., 2003). Thus, at least two mechanisms appear to generate a polarized cytoplasmic distribution of these embryonic determinants. One unknown mechanism appears to move proteins posteriorly prior to division, while ubiquitin-mediated proteolysis degrades any posterior determinants that remain in the anterior daughter.

Curiously, this putative CUL-$2^{ZIF\text{-}1}$ E3 ligase requires MEX-5 and MEX-6 to be functional. In mutant embryos lacking both of these redundant proteins, the ZIF-1-dependent degradation of POS-1, MEX-1 and PIE-1 is inhibited (DeRenzo et al., 2003). Thus, it appears that MEX-5 and MEX-6 are activators of the CUL-$2^{ZIF\text{-}1}$ ligase, as well as being its substrates. Regulation of the ligase by these two proteins may explain why CCCH-finger proteins are stabilized in the posterior daughter, where MEX-5 and MEX-6 levels may be too low to activate the CUL-$2^{ZIF\text{-}1}$ ligase.

Temporal and spatial regulation of protein degradation

It is clear that multiple ubiquitin E3 ligases operate during early embryogenesis in *C. elegans*. It is less clear how the ubiquitin machinery is activated at the right time and place. The recent identification of the DYRK (dual-specificity YAK1-related kinase) kinase called MBK-2 provides important insights into the regulation of these early proteolytic events. MBK-2 is required both for the CUL-$3^{MEL\text{-}26}$ E3 ligase to degrade katanin after meiosis, and for the

CUL-2^{ZIF-1} ligase to degrade PIE-1 after the first asymmetric division of the zygote (Pang et al., 2004; Pellettieri et al., 2003; Quintin et al., 2003). The cell polarity defects occur independently of katanin regulation, as *mbk-2(–); mei-1(–)* double mutants do not exhibit the microtubule stability defects observed in *mbk-2(–)* single mutants, but still fail to degrade PIE-1 properly (Pang et al., 2004; Quintin et al., 2003). Earlier steps in axis formation appear normal in *mbk-2(–)* mutant embryos: the cortical PAR proteins polarize normally, and MEX-5 and MEX-6 still localize to the anterior (Pang et al., 2004; Pellettieri et al., 2003; Quintin et al., 2003).

Substrate phosphorylation often is a pre-requisite for recognition by an E3 ligase (Ang and Harper, 2004). Consistent with MBK-2 playing such a role, MEI-1/katanin contains an MBK-2 phosphorylation site within its PEST motif and MBK-2 phosphorylates MEI-1 in vitro (Stitzel et al., 2006). Furthermore, a mutant MEI-1 protein with an alanine substitution at the MBK-2 phosphorylation site is no longer degraded after meiosis, and a phospho-specific MEI-1 antibody detects high levels of MBK-2-dependent phosphorylation only after the completion of meiosis (Stitzel et al., 2006). Thus, MEI-1 phosphorylation by MBK-2 may provide the temporal trigger for degrading katanin after meiosis, although it remains to be determined if phosphorylation promotes MEI-1 recognition by the CUL-3^{MEL-26} E3 ligase.

Another target of MBK-2 is the oocyte maturation factor OMA-1. Remarkably, OMA-1 also is a CCCH-type zinc-finger protein that contains an MBK-2 phosphorylation site in a PEST motif, and MBK-2 phosphorylates OMA-1 in vitro and in vivo (Nishi and Lin, 2005; Stitzel et al., 2006). Normally, OMA-1 functions redundantly with the almost identical protein OMA-2 during oocyte maturation, and both are degraded during the first mitotic division in an ubiquitin-dependent manner by an unknown ligase (Detwiler et al., 2001; Shimada et al., 2002; Shirayama et al., 2006). Like MEI-1, OMA-1 degradation also requires phosphorylation by MBK-2 (Nishi and Lin, 2005; Shirayama et al., 2006; Stitzel et al., 2006). A gain-of-function OMA-1 mutant that is resistant to degradation persists after meiosis and interferes with the degradation of PIE-1 and other cell fate determinants (Lin, 2003). Thus, the failure to degrade developmental determinants in *mbk-2(–)* mutant embryos may be a secondary consequence of OMA-1 stabilization (Lin, 2003; Shirayama et al., 2006). Nevertheless, MBK-2-dependent phosphorylation is required for at least two independent degradation events: MEI-1 after meiosis and OMA-1 during the first mitotic division.

Recent studies indicate that the glycogen synthase kinase GSK-3 also is required for OMA-1 degradation (Nishi and Lin, 2005; Shirayama et al., 2006). GSK-3 phosphorylates OMA-1 at a residue different from MBK-2, OMA-1 phosphorylation by MBK-2 is required for GSK-3 to phosphorylate OMA-1, and both phosphorylation events are required for OMA-1 degradation (Nishi and Lin, 2005). Thus, MBK-2 may act as a priming kinase for GSK-3. Intriguingly, GSK-3 appears to be specific for OMA-1 degradation, as no defects reminiscent of MEI-1 stabilization are seen in embryos lacking GSK-3 (Nishi and Lin, 2005; Schlesinger et al., 1999). Two additional kinases also are required, directly or indirectly, for OMA-1 degradation (Shirayama et al., 2006). It will be interesting to learn whether MEI-1 also requires additional kinase(s) to be efficiently degraded. Although phosphorylation clearly regulates proteasomal degradation in the early *C. elegans* embryo, it remains to be determined how MBK-2 and GSK-3 act together to achieve the timely degradation of their respective substrates. Intriguingly, progression through meioisis, but not fertilization, is required for MBK-2 activation, suggesting that meiotic cell cycle regulators play a key role in mediating the oocyte-to-embryo transition (Stitzel et al., 2006).

Conclusion

Recent studies of early embryogenesis in *C. elegans* have converged to a remarkable degree on the discovery of ubiquitin E3 ligases that mediate several different cell cycle and developmental transitions. Moreover, it seems likely that still more ubiquitin ligases with essential roles remain to be identified. For example, embryonic lethality ensues when RNAi is used to deplete a HECT domain ubiquitin ligase (Huang et al., 2000), and the cortical polarity regulator PAR-2 is itself a RING finger protein and thus potentially an ubiquitin ligase. Understanding how these regulatory complexes function should substantially advance our understanding of *C. elegans* development, and contribute to a general understanding of E3 ligase function and regulation. A key goal for future studies will be the identification of substrates that are ubiquitinated and degraded, as most remain unknown or unproven. Finally, it will be interesting to see if ubiquitin-mediated proteolysis emerges as an important regulator of the cytoskeleton, cell polarity and cell fate determination during the development of other organisms.

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