

Control of asymmetric cell division in early *C. elegans* embryogenesis: teaming-up translational repression and protein degradation

Sue-Yun Hwang^{1,2} & Lesilee S. Rose^{3,*}

¹Department of Animal Biotechnology, Graduate School of Informatics and Biotechnology, ²Institute of Genetic Engineering, Hankyong National University, Ansong 456-749, Korea, ³Department of Molecular and Cellular Biology, University of California, Davis, CA 95616, USA

Asymmetric cell division is a fundamental mechanism for the generation of body axes and cell diversity during early embryogenesis in many organisms. During intrinsically asymmetric divisions, an axis of polarity is established within the cell and the division plane is oriented to ensure the differential segregation of developmental determinants to the daughter cells. Studies in the nematode *Caenorhabditis elegans* have contributed greatly to our understanding of the regulatory mechanisms underlying cell polarity and asymmetric division. However, much remains to be elucidated about the molecular machinery controlling the spatiotemporal distribution of key components. In this review we discuss recent findings that reveal intricate interactions between translational control and targeted proteolysis. These two mechanisms of regulation serve to carefully modulate protein levels and reinforce asymmetries, or to eliminate proteins from certain cells. [BMB reports 2010; 43(2): 69-78]

INTRODUCTION

The early stage of metazoan development is a crucial period during which body axes are determined and the blueprints of cell fates are established. In most species these early events are carried out with little or no *de novo* transcription, relying instead on regulated translation of stored maternal mRNAs and modulation of protein activity (1-4). Such spatiotemporal regulation of maternal factors generates polarized distributions of cell fate determinants in the zygote and early blastomeres, and in many organisms asymmetric cleavage promotes unequal segregation of these determinants into daughter cells. A leading model for studies of asymmetric division is the nematode

Caenorhabditis elegans (5-10). In this organism, early blastomeres attain distinct fates through the differential inheritance of cell fate determinants during a series of asymmetric cleavages.

During asymmetric division, the first step is to break the symmetry of cellular components in the mother cell. Symmetry breaking is initiated at fertilization in *C. elegans* (5-8, 10). An unidentified signal from the sperm with its associated centrosome and microtubule aster triggers a cortical flow of acto-myosin away from the site of the sperm entry, which results in the formation of anterior and posterior cortical domains containing distinct PAR polarity proteins (Fig. 1A). By the end of this "establishment phase", the two PDZ-domain proteins, PAR-3 and PAR-6, localize to the anterior, where they form a complex with an atypical protein kinase C (aPKC-3). On the other hand, a RING-finger protein PAR-2 and a serine-threonine kinase PAR-1 are placed at the posterior cortex. The anterior cortical movements are controlled by a Rho family GTPase and its regulators, and PAR-3 itself is needed for a robust establishment period through feedback on acto-myosin movement. PAR-5, a 14-3-3 protein family member, is also required for establishment of PAR polarity, in particular to prevent overlapping of the anterior PARs with PAR-2. Once established, the anterior and posterior domains maintain the partition through mutual exclusion. A serine/threonine kinase PAR-4, although not asymmetric itself, participates in the maintenance of PAR domains as well as the establishment of downstream asymmetries.

The polarization of PAR proteins instigates a panoply of downstream events (1, 8). First, PAR polarization results in the differential localization of a number of molecules that influence cell fate, including MEX-5, MEX-6, MEX-3 and GLP-1 to anterior cells and PIE-1, POS-1, and PAL-1 to posterior cells (Fig. 1B). Some of these asymmetries are visible in the one-cell, others are manifest slightly later, as further asymmetric divisions reinforce their unequal distribution among progeny cells. Second, the PAR proteins regulate the position of the mitotic spindle through asymmetric localization of cortical components of a G protein signaling pathway (6-8). Consequently the cleavage plane is formed perpendicular to the polarity axis

*Corresponding author. Tel: 530-754-9884; Fax: 530-752-3085; E-mail: lsrose@ucdavis.edu

Received 16 February 2010

Keywords: Asymmetric division, *Caenorhabditis elegans*, PAR proteins, Translational control, Ubiquitin proteolysis

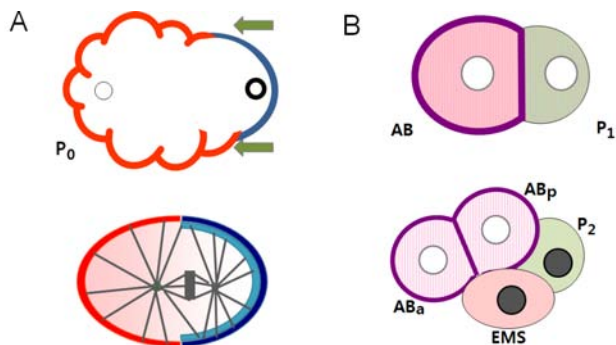


Fig. 1. PAR polarity and cell fate determination. (A) Establishment of PAR polarity and spindle displacement. In the newly fertilized zygote (P_0), the position of the sperm (bold circle at right) defines the posterior pole of the cell. A sperm associated signal triggers a directed flow of actomyosin cortical contraction towards the future anterior (arrows), accumulating PAR-3, PAR-6 and PKC-3 proteins (red) at the anterior half of the cortex. The absence of these PARs at the posterior allows cortical association of PAR-2 (dark blue) and PAR-1 (light blue). Establishment of PAR polarity domains results in the asymmetric distribution of MEX-5 and MEX-6 (pink) in the cytoplasm. PAR domains also regulate alignment of the spindle along the A/P axis and its displacement towards the posterior. (B) Distribution of Cell Fate Determinants. The first asymmetric cleavage produces the larger anterior AB cell and the smaller posterior P_1 cell. The amount of MEX-5/6 is higher in the AB cell. MEX-3 is present in both cells (pink hatch marks), while POS-1 and PIE-1 (light green) are more abundant in the P_1 cell. The anterior determinant GLP-1 (purple) is localized to the periphery of the AB cell and its descendants. By the 4-cell stage, MEX-3 is restricted to the anterior cells and the posterior determinant PAL-1 (grey) appears in the nuclei of the two posterior cells. MEX-5 is actually dynamic, with higher levels in the EMS daughter, but decreasing levels in the anterior by the end of the 4-cell stage.

and off center, dividing the P_0 zygote into the larger anterior AB cell and the smaller posterior P_1 cell.

While the morphological aspects of early asymmetric divisions have been extensively studied, the molecular nature of their regulation has only begun to be understood. Several recent reviews describe the regulation of PAR localization by Rho family GTPases, and other aspects of asymmetric division such as the role of the conserved G protein signaling pathway in cleavage plane specification in *C. elegans* (5-8, 10). In this review, we focus on recent findings in the regulation of early asymmetric division that reveal an intriguing interplay of translational repression and targeted protein degradation within a given pathway. In the absence of *de novo* transcription, these modes of regulation provide a means to fine tune the levels of key modulators in rapidly dividing early blastomeres.

Regulation of PAR protein levels by the worm homologs of Nanos, Pumilio and Brat

The anterior PAR complex proteins exclude PAR-2 from the cortex. Functional studies showed that this exclusion depends

on its phosphorylation by PKC-3 (11). By analogy to other systems, phosphorylation of PAR-2 could generate a binding site for the 14-3-3 protein PAR-5, which would cause PAR-2 to dissociate from the cortex. Similarly, exclusion of the anterior PARs from the posterior PAR-1/PAR-2 domain during the maintenance phase could involve phosphorylation of anterior PARs by PAR-1 and binding by PAR-5. Such mutual exclusion via phosphorylation has been demonstrated for PAR proteins in other systems (8-10) but these ideas have not yet been tested in the early *C. elegans* embryo. In addition, the role of PAR-2 in the maintenance of PAR polarity at the molecular level remains to be elucidated. PAR-2's identity as a putative E3 ligase raises the possibility that it could cause degradation of the anterior PARs in a localized fashion, but it could also act indirectly by affecting modulators of PAR proteins.

Recently, novel insight into the regulation of PAR polarity via protein degradation and translation has come from a genome-wide search for suppressors of *par-2* mutations (12). In *par-2* mutants, posterior expansion of PAR-3/PAR-6/PKC-3 results in polarity defects and embryonic lethality. Yet the disrupted equilibrium can be restored if function of the anterior complex is compromised. For example, RNAi knock-down of *par-3*, *par-6* or *pkc-3* can all suppress the lethal phenotype of *par-2* mutant. Interestingly, the list of recently identified *par-2* suppressors includes the homologs of Drosophila nanos (*nos-3*), pumilio (*FBF-1/2*) and brat (*Cebrat*) (12, 13). The Nanos-Pumilio-Brat complex controls the development of posterior structures in Drosophila embryos, by inhibiting the translation of *hunchback* mRNA in the posterior (14). However, the effect of their worm homologs in early *C. elegans* embryos is not directly on repressing translation of posterior determinants, but rather on modulating PAR protein levels through an intersection of translational repression and ubiquitin-mediated degradation.

NOS-3 regulates PAR-6 protein level through the CBC^{FEM-1} ubiquitin ligase

In theory, suppressor mutations of *par-2* can reverse the lethality in two ways; by reducing the level of PAR-3/PAR-6/PKC-3 in the anterior, or by increasing the residual activity of PAR-2 in the posterior. NOS-3 appears to regulate polarization mainly by modulating the levels of PAR-6 protein (15). The levels of PAR-6 protein, but not mRNA, were decreased in *nos-3* mutant worms to 60% of the wild type levels. Moreover, restoring PAR-6 protein levels by the expression of a transgene in the *nos-3*; *par-2* double mutant reversed the suppression of the *par-2* phenotype. What then would be the contribution of NOS-3 in this process? NOS-3, together with a Pumilio homolog FBF-1, has been proposed to bind the *fem-3* 3'-UTR and repress its translation in the germ line (16). FEM-1, FEM-2 and FEM-3 are subunits of the CBC^{FEM-1} complex, a CUL-2 based ubiquitin ligase (17). The ubiquitin ligase, also called E3 complex, marks the target protein for degradation by attaching a poly-ubiquitin tag. In the CBC^{FEM-1} complex, CUL-2 serves as the main scaffold protein, while FEM-1 is the target-binding

adaptor subunit. Interestingly, suppression of *par-2* lethality in *nos-3*; *par-2* double mutants required not only the presence of CUL-2 and FEM-1, but also FEM-2 and FEM-3 (15). Moreover, a physical interaction between ^{FEM-1} and PAR-6 has been demonstrated, suggesting that PAR-6 is subjected to degradation by the CBC ^{FEM-1} E3 ligase. A model emerging from these data is that the NOS-3/FBF-1 complex may prevent the assembly of CBC ^{FEM-1} E3 ligase by inhibiting the translation of the FEM-3 subunit, and as a result increase PAR-6 protein levels (Fig. 2A).

Because FEM-1 has only been shown to be present during oogenesis thus far (17), it is possible that regulation of PAR-6 protein levels mainly occurs in oocytes. However, other CUL-2 based E3 complexes that utilize ZIF-1 and ZYG-11 as substrate-recruiting adaptors participate in polarity establishment by degrading proteins in the zygote and 2-cell embryo (18, 19). Thus, it would be worthwhile to determine if CBC ^{FEM-1} can persist beyond fertilization, and whether its own localization takes an asymmetric pattern. In any case, the consecutive actions of the NOS-3/FBF-1 and CBC ^{FEM-1} complexes in PAR polarity establishment demonstrate a novel regulatory circuit where the levels of a key protein are modulated by targeted degradation, which in turn is under the control of translational repression.

C. elegans Brat homologs may regulate PAR-2 polarity independently of NOS-3

C. elegans has 5 homologs of Brat, the third component of the Nanos-Pumilio-Brat complex in *Drosophila*. Mutations in 4 out of the 5 *Brat* homologs (*cebrats*; *ncl-1*, *nhl-1*, *nhl-2* and *nhl-3*) can individually suppress the lethality of *par-2* mutants to varying degrees (13). Interestingly, none of these mutations affected the level of PAR-6 protein as the *nos-3* mutant did (15). However, disruption of the cortical PAR-2 domain in a *par-2*

strain was partially restored in double mutants with *nos-3*, *ncl-1*, *nhl-2* *pnhl-3*. Given the interplay between the anterior PARs and PAR-2 in restricting each other's domain, these results raise the possibility that CeBRATs act via regulation of PAR-2 levels. Significantly, double mutant analysis suggested that at least NHL-2 functions in a separate pathway from NOS-3 and FEM-3 (13). It is possible that CeBRATs are responsible for the modulation of PAR-2 protein levels independently of NOS-3, while the main job of NOS-3 is to control PAR-6 levels. Functional segregation of Brat from Nanos and Pumilio has also been noticed in *Drosophila*. For example, Nanos and Pumilio inhibit the translation of Cyclin B mRNA without the participation of Brat; and Brat alone is known to play a role in asymmetric division of the neuroblast without Nanos/Pumilio (20).

What could be the nature of modulation exerted by CeBRATs on the polarity of *C. elegans* zygote? With the exception of NCL-1, which functions as a cell growth inhibitor and has been suggested as the ortholog of *Drosophila* Brat (21), the other three (NHL-1, NHL-2, NHL-3) all contain a RING finger domain found in most E3 ligases. Among these, the ubiquitinating activity of NHL-1 has been verified *in vitro* (22). Also, an ubiquitin-conjugating enzyme UEV-1 bound to NHL-1 and PAR-2 in a yeast 2-hybrid assay. These data suggest the possibility that PAR-2 protein levels are regulated directly by NHL-1 mediated ubiquitin proteolysis. The presence of RING finger domains in NHL-2 AND NHL-3 suggest that they may affect PAR-2 protein levels through a similar mechanism (Fig. 2B). Interestingly, expression of NCL-1 and NHL-2 was decreased in *par-2* mutants, and *ncl-1* mutants exhibited reduced levels of NHL-2 (13). Such complex feedback among PAR-2 and CeBRATs may be required for prompt and accurate adjustment of PAR-2 levels within the cell.

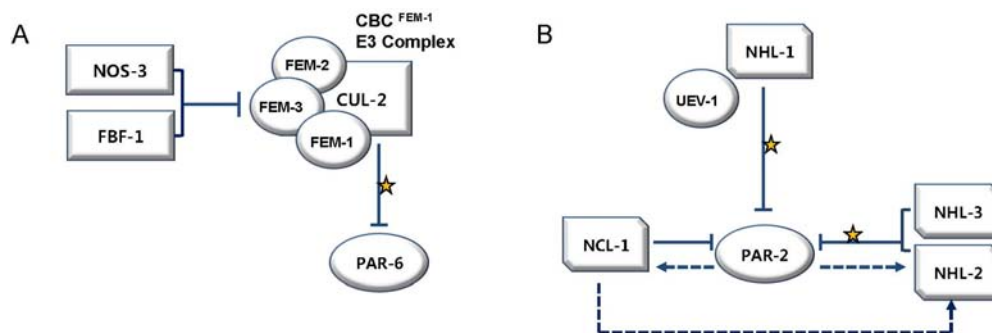


Fig. 2. Proposed Regulation of Anterior and Posterior PAR Proteins by Ubiquitin Proteolysis. (A) Regulation of PAR-6 protein levels by NOS-3 and FBF-1. A Nanos homolog NOS-3, together with the Pumilio homolog FBF-1, inhibit translation of the FEM-3 subunit of the CBC ^{FEM-1} ubiquitin ligase complex. The FEM-1 subunit of the complex recognizes PAR-6 for ubiquitin-mediated proteolysis. (B) Regulation of PAR-2 by CeBRATs. 4 out of the 5 CeBRATs affect PAR-2 protein levels. Three RING finger proteins (NHL-1, NHL-2, NHL-3) may target PAR-2 for ubiquitin-mediated proteolysis. PAR-2 itself affects the level of NCL-1 and NHL-2 through unknown mechanisms, and NHL-2 may also be positively regulated by NCL-1, presumably to ensure prompt adjustment of PAR-2 protein levels. (arrows: activation, blocked lines: suppression, yellow stars: ubiquitination, dashed lines: potential activation/suppression).

Translating the PAR polarity cue into AP-axis determination

One major outcome of PAR protein segregation in the zygote is the interpretation of this polarity into the asymmetric distribution of downstream cell fate determinants (Fig. 1) (1, 8). These asymmetries are mediated in large part through the action of MEX-5 and MEX-6, two very similar CCCH Zn-finger proteins that are enriched in the anterior cytoplasm of the one-cell in response to the PAR proteins. MEX-5 and MEX-6 are highly redundant and will be referred to hereafter as MEX-5/6 where their localizations or functions appear to be the same. Although the precise molecular function of MEX-5/6 remain to be determined, recent work implicates these proteins in a network of protein degradation and translational regulation that leads to asymmetric localization of the cell-fate determinants GLP-1 and PAL-1 to specific blastomeres by the 4-cell stage (1, 4, 23, 24).

Mutual regulation between MEX-5/6 proteins and their targets sharpens the asymmetric distribution of cell fate determinants

The best studied role of MEX-5/6 is in triggering the asymmetric protein degradation of the posterior acting factors PIE-1 and POS-1. Like MEX-5/6, PIE-1 and POS-1 are tandem CCCH Zn finger proteins of the TTP family, but they are required for different aspects of posterior development. In particular PIE-1 inhibits the transcription of somatic genes in the germ lineage cells and has other germ-line roles, while POS-1 is a key translational regulator of the anterior determinant, GLP-1 (1, 25).

The levels of PIE-1 and POS-1 become enriched in the posterior during the first cell cycle, and this gradient is further sharpened during subsequent divisions. Degradation by CUL-2^{ZIF-1} is responsible, at least in part, for differential accumulation of these proteins. MEX-5/6 are required for the activation of the CUL-2^{ZIF-1} E3 ligase. Because MEX-5/6 are themselves enriched in the anterior, this results in the degradation of any remaining PIE-1 and POS-1 in the progeny of anterior blastomeres. Degradation requires the first CCCH finger of each of these proteins, which mediates binding to the ZIF-1 substrate adaptor for CUL-2 (18, 26). How MEX-5/6 activate degradation of the posterior CCCH proteins remains to be elucidated, but the Zn-finger domains of MEX-5 were shown to bind mRNA non-specifically (27). Thus, an intriguing model to test is that MEX-5/6 activates the translation of ZIF-1 or other components of the CUL-2^{ZIF-1} E3 ligase asymmetrically in the anterior.

Interestingly, MEX-5 is itself subjected to both degradation and translational control. The initial anterior enrichment of MEX-5/6 established by the PAR proteins may be re-enforced by translational control by POS-1. Although loss of *pos-1* in a wild-type background does not affect MEX-5/6 asymmetry, reduction of *pos-1* gene function rescued the phenotype of *mex-5* single mutants (28). Furthermore, POS-1 bound the 3'-UTR of *mex-6* (28). Whether binding of POS-1 inhibits the

translation of these mRNAs was not tested directly, but POS-1 has been shown to act as a translational repressor (25). By the 4-cell stage, the levels of MEX-5/6 begin to decrease in the anterior cells. This change is mediated by the second Zn-finger of MEX-5 and the CUL-2^{ZIF-1} E3 ligase (18). The fact that MEX-5/6 are both activators and targets of the CUL-2^{ZIF-1} is reminiscent of the mutual regulation between MEX-5/6 and POS-1. Perhaps such a complex circuit of regulation serves to precisely tune the activities of these proteins during rapid cleavage divisions.

Localization of PAL-1 and GLP-1 by a circuit of translational regulation

The selective degradation of posterior CCCH proteins via MEX-5/6 leads to further asymmetries in several cell fate regulators, the best studied of which are GLP-1 and PAL-1 (1, 3, 4). GLP-1 is one of the two members of the *C. elegans* LIN-12/Notch family, and it is expressed on plasma membrane of the AB cells beginning at the 2-cell stage (29). *glp-1* mRNA is found in all cells, thus the anterior confinement of GLP-1 protein must involve selective translation or degradation. In this case, GLP-1 accumulation is governed by several RNA binding proteins that function as translational regulators. POS-1 and GLD-1 suppress GLP-1 translation in the posterior blastomeres by binding to distinct 3'-UTR elements (25, 30). At the same time, SPN-4, an RNP-type RNA binding factor that also binds the *glp-1* 3'-UTR, is required for the activation of GLP-1 translation in the anterior (25). SPN-4 is present throughout the embryo at the two-cell stage when GLP-1 translation is first seen. Because POS-1 is being degraded in the anterior via the MEX-5/6 pathway at this point, the ratio of SPN-4 to POS-1 is higher and thus activation prevails (25).

In contrast, the homeobox protein PAL-1 is localized to the posterior nuclei of the 4-cell embryo where it activates transcription of genes involved in specifying muscle cell fates (31, 32). The posterior localization of PAL-1 in early *C. elegans* embryo resembles its *Drosophila* homolog Caudal in many ways (33). While *pal-1* mRNA is evenly distributed in the oocyte and early embryos, PAL-1 protein is not synthesized until the 4-cell stage and then, only in posterior blastomeres (34, 35). As with Caudal, posterior expression of PAL-1 is placed under a multistep translational control. First, translation of *pal-1* is repressed by a STAR/Maxi-KH domain protein GLD-1 in the germ line (36). After fertilization, MEX-5/6 and MEX-3, a KH domain RNA binding protein, are all required for restriction of PAL-1 to the posterior (37).

Since MEX-3, MEX-5 and MEX-6 all contain RNA-binding motifs, a plausible hypothesis would be that they bind directly to *pal-1* mRNA and repress translation. Indeed the translation of *pal-1* mRNA was dictated by its 3'-UTR in a *lacZ* reporter assay (34), and recent work identified MEX-3 binding sites in the *pal-1* 3'-UTR, as well as in the 3'-UTRs of other developmentally regulated genes (38, 39). However, MEX-5/6 act through MEX-3, at least in part, because MEX-5/6 are required for the enrichment of MEX-3 to the anterior (Fig. 3). In addition,

tion, PAL-1 protein was ectopically present in all blastomeres of *mex-5*; *mex-6* double mutants whether MEX-3 was present or not, suggesting that MEX-5/6 facilitate MEX-3 activity. Further, MEX-6 bound to MEX-3. Together these results suggest that MEX-5/6 could either function as an upstream activator of MEX-3 or as a co-repressor on the *pal-1* 3'UTR, or both (37). How MEX-5/6 localize MEX-3 has been unclear, but recent studies show that POS-1 can bind to the 3'UTR of *mex-3* mRNA, consistent with translation repression in the posterior (40). Thus a simple model to test is that MEX-5/6 affects MEX-3 accumulation through restriction of POS-1 to the posterior. At the same time, reporter assays revealed that POS-1 binding to the *mex-3* 3'UTR was not sufficient for repression (40). Therefore, as seen for GLP-1 regulation above, it is likely that there is a complex interplay among RNA binding proteins to generate different translational outputs.

Positioning of the mitotic spindle relative to the polarity axis

Once PAR polarity is established in the one-cell embryo, it instructs the orientation and asymmetric movement of the mitotic spindle along the AP axis to ensure proper segregation of cell fate determinants into daughter cells. The mechanisms by which the PAR proteins regulate spindle positioning are an active area of study (6, 7). PAR polarization results in asymmetric activation of a heterotrimeric G protein signaling pathway, which creates unequal pulling on the astral microtubules of the mitotic spindle. Asymmetries in the pulling forces serve first to orient the spindle along the polarity axis, then displace it towards the posterior end of the embryo (Fig. 1). In addition to cortical pulling, proper movement of mitotic spindles also

requires regulation of microtubule length and dynamics so that the microtubules can interact with the cortex. A well studied case is the targeted proteolysis of the microtubule severing protein MEL-1/MEI-2 (katanin) after fertilization (23). Interestingly, recent studies suggest that the levels of MEL-1 and other microtubule associated proteins are also controlled at the translational level.

Timely degradation of MEL-1 by a network of protein degradation pathways

One of the critical prerequisites for the asymmetric cleavage of the *C. elegans* one-cell embryo is the switch from a small meiotic spindle to a larger mitotic spindle with astral microtubules that contact the cortex. Meiotic spindle microtubules are maintained short by the action of the MEL-1/MEI-2 katanin complex, while the prolonged presence of MEL-1/MEI-2 after fertilization causes shorter mitotic spindles that are mispositioned with respect to the polarity axis (41-44). Since meiotic and mitotic spindles are assembled in the same cytoplasm, it is a challenge to achieve rapid inactivation of the MEL-1/MEI-2 complex after fertilization without attenuating its activity during the second division of meiosis. *C. elegans* solves this problem by subjecting the MEL-1 subunit to multiple levels of regulation, including ubiquitin proteolysis (23, 42, 45-51).

In the zygote, MEL-26 binds and targets MEL-1 for degradation by a CUL-3 based E3 ligase (Fig. 4A) (50). The levels of MEL-26 protein are kept low in the oocyte by the action of an unknown CUL-2 based E3 ligase, although it is not known if this interaction is direct (45). Equally unclear is the mechanism of keeping CUL-3^{MEL-26} turned off during meiosis, but one candidate for the switch is RFL-1 which activates E3 ligases by neddylation. RFL-1 is required for CUL-3^{MEL-26} to degrade

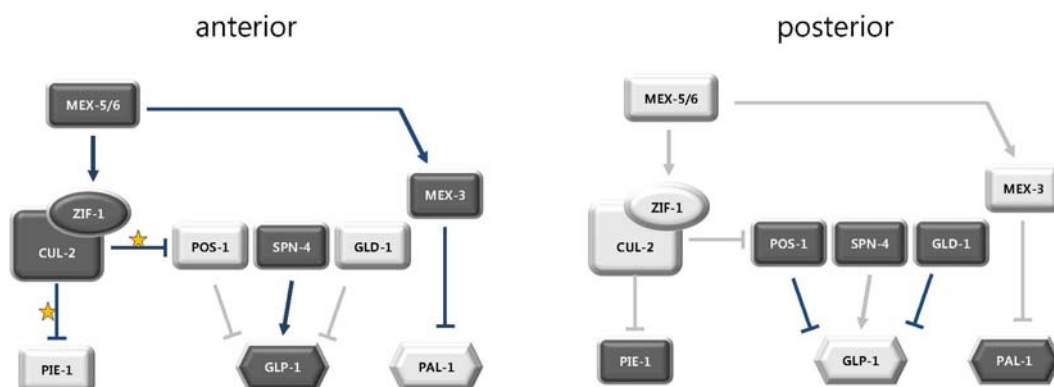


Fig. 3. Regulation of anterior vs. posterior fates by CCCH proteins. Posterior PAR proteins are responsible for the localization of MEX-5/6, resulting in higher concentrations of these CCCH proteins in the anterior where they activate CUL-2^{ZIF-1}. The major targets of ubiquitination by CUL-2^{ZIF-1} in the anterior are two other CCCH proteins PIE-1 and POS-1. MEX-5/6 also activate a KH domain protein MEX-3, which inhibits translation of the posterior determinant PAL-1 in the anterior. The RNA binding protein SPN-4 activates GLP-1 translation in the anterior, while the GLD-1 RNA binding protein represses GLP-1 in the posterior. (arrows: activation, blocked lines: suppression, yellow stars: ubiquitination, dark shapes and lines: high amount of proteins and robust regulation, faint shapes and lines: low amount or absence of proteins and regulation).

MEI-1 (49), and it may also activate the CUL-2 based E3 ligase in the oocyte since abnormal accumulation of MEL-26 was observed in both *rfl-1* and *cul-2* mutants (45). Perhaps RFL-1 alternates its activation target from CUL-2 in the oocyte to CUL-3 in the zygote, postponing degradation of MEI-1 until after fertilization.

Another pathway of inactivation targets MEI-1 exclusively in the zygote. Substrates of E3 ligases are often phosphorylated before ubiquitination, and the MBK-2 kinase was shown to be required for timely degradation of MEI-1 (47, 48, 51, 52). Initially phosphorylation of MEI-1 by MBK-2 was believed to prime MEI-1 for degradation by CUL-3^{MEL-26}, but it appears that MBK-2 and MEL-26 act in parallel pathways (46). While the nature of MBK-2 mediated degradation remains to be identified, it nonetheless serves as an elegant case of a molecular timer (Fig. 4A). During meiotic arrest before fertilization, MBK-2 is sequestered at the cortex by EGG-3 and is thus unable to phosphorylate MEI-1 in the cytoplasm (53). When meiosis is resumed upon fertilization, the meiotic APC degrades EGG-3 and MBK-2 is released into the cytoplasm. Thus the inactivation of microtubule severing complex during first mitosis appears to be insured by dual regulatory pathways, one through the degradation of MEI-1 by CUL-3^{MEL-26} and the other by an MBK-2 associated degradation pathway. In each case, the activity of the degradation machinery itself is regulated by targeted protein degradation to confer temporal control.

Negative regulation of MEI-1 translation by a novel 4E-BP, SPN-2

In addition to the elaborate panoply of protein degradation, the activity of MEI-1 was recently proposed to be controlled by translational repression after fertilization. Because *mei-1* mRNA persists in embryos after meiosis (44), a means to prevent its translation would be required in addition to degradation of existing protein. A regulator suitable for this function was identified from the recent characterization of a spindle orientation defective mutant, *spn-2* (54). The *spn-2* gene encodes a novel eIF4E binding protein (4E-BP); 4E-BPs in other systems disrupt the translation initiation complex by competing with the eIF4G initiation factor (1, 2). The abnormal nuclear and spindle positioning phenotypes observed in early *spn-2* embryos were caused by ectopic expression of MEI-1 (54). SPN-2 bound in vitro to the RNA binding protein OMA-1, which interacted with the 3'-UTR of the *mei-1* in an RNA pull-down assay. Further, RNAi knock-down of *oma-1*, together with functionally redundant *oma-2*, similarly caused ectopic MEI-1 in early cleavage divisions (54). In *Drosophila*, translational repression by a 4F-BP (Cup) is mediated by gene-specific adaptors, for example Bruno for *oskar* mRNA (55) and Smaug for *nanos* mRNA (56). In the case of *C. elegans* SPN-2, OMA-1 may serve as a gene-specific adaptor for *mei-1*.

Both SPN-2 and OMA-1 are present in oocytes and meiotic embryos (57), and thus it is necessary to suppress their activity on *mei-1* mRNA during these stages. Because 4E-BPs are often activated upon phosphorylation, a specific kinase may trigger

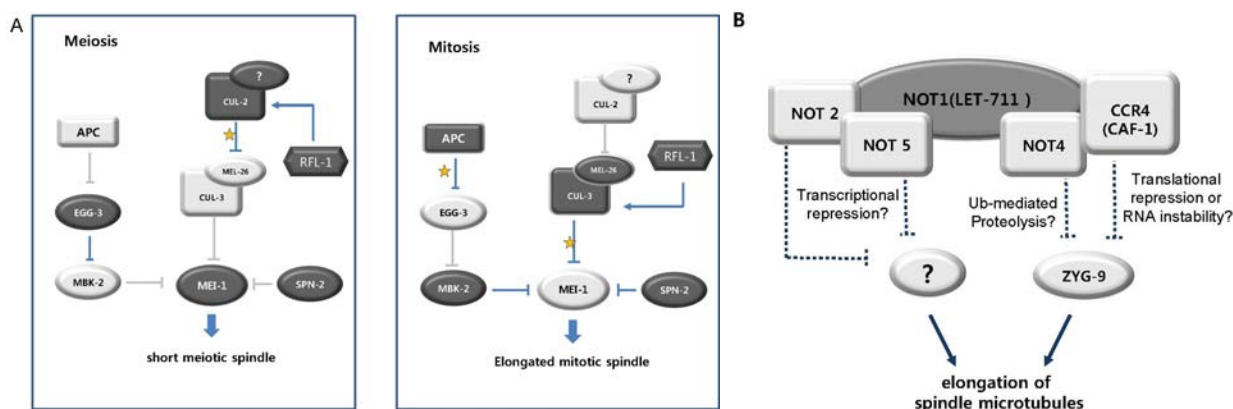


Fig. 4. Regulation of microtubule length during asymmetric division. (A) Differential regulation of MEI-1 during meiosis and mitosis. The microtubule severing activity of MEI-1 is required for a short meiotic spindle but is removed before the first mitotic cleavage. RFL-1 may serve as a switch in this transition by activating an unknown E3 ligase in the oocyte which degrades CUL-3^{MEL-26}, thus preventing ubiquitination of MEI-1. During mitosis, RFL-1 activates CUL-3^{MEL-26}, and robust degradation of MEI-1 ensues. Removal of MEI-1 is also achieved via translational repression by SPN-2 as well as MBK phosphorylation. Interaction with MBK-2 is prevented in the oocyte because this kinase is sequestered at the cortex by EGG-3, which is degraded once APC is activated after fertilization. (arrows: activation, blocked lines: suppression, yellow stars: ubiquitination, dark shapes and lines: high amount of proteins and robust regulation, faint shapes and lines: low amount or absence of proteins and regulation). (B) Opposing effects of LET-711 and ZYG-9 LET-711 negatively regulates the localization of ZYG-9 at centrosomes. As a scaffold protein of the CCR4-NOT complex, LET-711 may affect ZYG-9 and thus microtubule organization through any of several mechanisms as indicated. (dashed lines: potential suppression).

translational repression by SPN-2 after fertilization. Alternatively, the interaction between SPN-2 and OMA-1, or the binding of OMA-1 to *mei-1* 3'-UTR, may be blocked until after meiosis. Interestingly OMA-1/OMA-2 were shown to repress the translation of the *nanos* homolog *nos-2* in the oocyte (39). Since SPN-2 is also required for the translational repression of *nos-2* in the oocyte (Li and Rose, unpublished data), a likely hypothesis is that SPN-2/OMA-1 is active in the oocyte but somehow is prevented from binding to the 3'-UTR of *mei-1* mRNA. SPN-2 may also have functions independent of OMA-1, because OMA-1 is phosphorylated by MBK-2 and begins to be degraded after the one cell stage (52, 57, 58), while the level of SPN-2 protein remains high until 8-cell stage (54). Taken together, timely elimination of the microtubule severing protein MEI-1 in the mitotic embryo is achieved through a combined effort of ubiquitin-mediated degradation pathways and translational repression by SPN-2 (Fig. 4A).

LET-711, the *C. elegans* ortholog of NOT-1, may promote the degradation of ZYG-9

Spindle positioning is thought to be defective in *mei-1* over-expression mutants due to shorter astral microtubules that fail to interact with the cortex. A similar spindle mispositioning defect is seen with loss of function mutations in *zyg-9*, which encodes the *C. elegans* homolog of the microtubule polymerization protein, XMAP215 (6). On the other hand, abnormally long microtubules appear to be equally detrimental because *let-711* mutant embryos also have misoriented spindles, but astral microtubules were found to be longer and more resistant to depolymerization by cold temperatures than in wild type (59). The level of ZYG-9 protein was increased at centrosomes in *let-711* embryos. In addition, the spindle orientation phenotype was partially rescued in *let-711; zyg-9* double mutants, suggesting that LET-711 and ZYG-9 have opposing functions. Surprisingly though, LET-711 is the *C. elegans* homolog of the NOT1, the core component of the CCR4/NOT complex, which is involved in a wide range of transcriptional and post-transcriptional control (60). In yeast and humans, the NOT4 component of the complex has been demonstrated to function as an E3 ligase, while the CCR4 subcomplex functions in deadenylation of mRNA. Other roles in transcription and phosphorylation have also been implicated for this versatile complex (60-63). It is not known which of these activities is important for the role of *let-711*, although a clear possibility is that degradation modulates ZYG-9 levels (Fig. 4B). In contrast to MEI-1, ZYG-9 is needed for meiosis and mitosis. Further, during early mitotic cell divisions, the size of cells and thus astral microtubules get smaller over time, which would necessitate the modulation of ZYG-9 levels in proportion. In this regard, partial degradation would be required in order to fine tune protein levels, rather than eliminating ZYG-9 altogether.

An equally possible scenario is that the CCR4 subunit of the LET-711/NOT1 complex could serve to degrade the *zyg-9* mRNA or repress its translation. In support of this role, *let-711*

(*RNAi*) causes derepression of *nos-2* translation in the oocyte (64). However, the localization of proteins to microtubules and centrosomes has many interdependencies, and thus it is also possible that the effects of LET-711 on ZYG-9 are through an unidentified microtubule-associated protein. It will be important in the future to test for regulation of *zyg-9* at the mRNA level and to determine if it binds the CCR4 subunit of the NOT complex or any of the known translational repressors.

Summary and perspectives

During early *C. elegans* embryogenesis, translational regulation and targeted proteolysis play important roles in cell fate determination among rapidly dividing blastomeres. The intersection of these two modes of post-transcriptional modulation is observed in the asymmetric localization of PAR proteins and downstream determinants, and in the positioning of the spindle along the polarity axis. However, many of the molecular details remain to be elucidated. An important area for future study will be determining how the timing of these various pathways are controlled, as mentioned already for the effect of the degradation pathway on PAR-6, and the effect of SPN-2 on *mei-1* mRNA. In particular, the timing of degradation of the CCCH proteins is an important mystery to be solved. PIE-1 and POS-1 are present in maturing oocytes as are their activators of degradation, MEX-5/6. However, degradation of the posterior determinants is not apparent until late in the first cell cycle. Clearly, there must be activation of the pathway in response to fertilization or cell cycle advance. While this could be accomplished through modulation of protein activity, it will not be surprising if translational control is involved. Indeed, it has been proposed that OMA-1/2 could regulate the translation of *zif-1* mRNA (58). The nature of this regulation could be repression, as observed for the effect of OMA-1/2 on *nos-2* mRNA. In this scenario, phosphorylation of OMA-1/2 by MBK-2 could act as the switch that inactivates and/or removes OMA-1/2, allowing ZIF-1 translation and thus degradation of PIE-1 and POS-1. With the broad range of techniques available for use in this system, *C. elegans* will continue to pave the way for probing the interplay of translational and degradation pathways that act during early asymmetric divisions and will provide a model to understand corresponding processes in higher organisms.

Acknowledgements

We regret that we were unable to cite all the primary references for the work described here due to space considerations. We refer the reader to the excellent reviews cited for more detail. We thank Monica Gotta and Alan Rose for critical reading of the manuscript. This work was supported by an NIH ARRA award (R01GM068744) to L. S. R. and a sabbatical research award from Hankyong National University to S-Y. H.

REFERENCES

1. Farley, B. and Ryder, S. (2008) Regulation of maternal mRNAs in early development. *Crit. Rev. Biochem. Mol. Biol.* **43**, 135-162.
2. Vardy, L. and Orr-Weaver, T. (2007) Regulating translation of maternal messages: multiple repression mechanisms. *Trends Cell Biol.* **17**, 547-554.
3. Evans, T. C. and Hunter, C. P. (2005) Translational control of maternal RNAs (November 10, 2005), WormBook, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.34.1, <http://www.wormbook.org>
4. Kuersten, S. and Goodwin, E. (2003) The power of the 3'-UTR: translational control and development. *Nat. Rev. Genet.* **4**, 626-637.
5. Cowan, C. and Hyman, A. (2007) Acto-myosin reorganization and PAR polarity in *C. elegans*. *Development* **134**, 1035-1043.
6. Galli, M. and van den Heuvel, S. (2008) Determination of the cleavage plane in early *C. elegans* embryos. *Annu. Rev. Genet.* **42**, 389-411.
7. Gonczy, P. (2008) Mechanisms of asymmetric cell division: flies and worms pave the way. *Nat. Rev. Mol. Cell Biol.* **9**, 355-366.
8. Goldstein, B. and Macara, I. (2007) The PAR proteins: fundamental players in animal cell polarization. *Dev. Cell* **13**, 609-622.
9. Suzuki, A. and Ohno, S. (2006) The PAR-aPKC system; lessons in polarity. *J. Cell. Sci.* **119**, 979-998.
10. Nance, J. (2005) PAR proteins and the establishment of cell polarity during *C. elegans* development. *BioEssays* **27**, 126-135.
11. Hao, Y., Boyd, L. and Seydoux, G. (2006) Stabilization of cell polarity by the *C. elegans* RING protein PAR-2. *Dev. Cell* **10**, 199-208.
12. Labbe, J.-C., Pacquelet, A., Marty, T. and Gotta, M. (2006) A genome-wide screen for suppressors of *par-2* uncovers potential regulators of PAR-protein dependent cell polarity in *Caenorhabditis elegans*. *Genetics*. **174**, 285-295.
13. Hyenne, V., Desrosiers, M. and Labbe, J.-C. (2008) *C. elegans* Brat homologs regulate PAR protein-dependent polarity and asymmetric cell division. *Dev. Biol.* **321**, 368-378.
14. Sonoda, J. and Wharton, R. (2001) *Drosophila* Brain Tumor is a translational repressor. *Genes. Dev.* **15**, 762-773.
15. Pacquelet, A., Zanin, E., Ashiono, C. and Gotta, M. (2008) PAR-6 levels are regulated by NOS-3 in a CUL-2 dependent manner in *Caenorhabditis elegans*. *Dev. Biol.* **319**, 267-272.
16. Kraemer, B., Crittenden, S., Gallegos, M., Moulder, G., Barstead, R., Kimble, J. and Wickens, M. (1999) NANOS-3 and FBF proteins physically interact to control the sperm-oocyte switch in *Caenorhabditis elegans*. *Cur. Biol.* **9**, 1009-1018.
17. Starostina, N., Lim, J.-M., Schvarzstein, M., Wells, L., Spence, A. and Kipreos, E. (2007) A CUL-2 ubiquitin ligase containing three FEM proteins degrades TRA-1 to regulate *C. elegans* sex determination. *Dev. Cell* **13**, 127-139.
18. DeRenzo, C., Reese, K. and Seydoux, G. (2003) Exclusion of germ plasm proteins from somatic lineage by cullin-dependent degradation. *Nature* **424**, 685-689.
19. Liu, J., Vasudevan, S. and Kipreos, E. (2004) CUL-2 and ZYG-11 promote meiotic anaphase II and the proper placement of the anterior-posterior axis in *C. elegans*. *Development* **131**, 3515-3525.
20. Lee, C.-Y., Wilkinson, B., Siegrist, S., Wharton, R. and Doe, C. (2006) Brat is a Miranda cargo protein that promotes neuronal differentiation and neuroblast self renewal. *Dev. Cell* **10**, 441-449.
21. Frank, D. and Roth, M. (1998) *ncl-1* is required for the regulation of cell size and ribosomal RNA synthesis in *Caenorhabditis elegans*. *J. Cell. Sci.* **110**, 1321-1329.
22. Gudgeon, M., Chandrasekaran, A., Frazier, T. and Boyd, L. (2004) Interactions within the ubiquitin pathway of *Caenorhabditis elegans*. *Biochem. Biophys. Res. Comm.* **325**, 479-486.
23. Bowerman, B. and Kurz, T. (2006) Degrade to create: developmental requirements for ubiquitin mediated proteolysis during early *C. elegans* embryogenesis. *Development* **133**, 773-784.
24. Schubert, C., Lim, R., de Vries, D., Plastert, R. and Priess, J. (2000) MEX-5 and MEX-6 function to establish somatogermine asymmetry in early *C. elegans* embryos. *Mol. Cell* **5**, 671-682.
25. Ogura, K.-I., Kishimoto, N., Mitani, S., Gengyo-Ando, K. and Kohara, Y. (2003) Translational control of maternal *glp-1* mRNA by POS-1 and its interacting protein SPN-4 in *Caenorhabditis elegans*. *Development* **130**, 2495-2503.
26. Reese, K., Dunn, M., Waddle, J. and Seydoux, G. (2000) Asymmetric segregation of PIE-1 in *C. elegans* is mediated by two complementary mechanisms that act through separate PIE-1 protein domains. *Mol. Cell*. **6**, 445-455.
27. Pagano, J., Farley, B., McCoig, L. and Ryder, S. (2007) Molecular basis of RNA recognition by the embryonic polarity determinant MEX-5. *J. Biol. Chem.* **282**, 8883-8894.
28. Tenlen, J., Schisa, J., Diede, S. and Page, B. (2006) Reduced dosage of *pos-1* suppresses Mex mutants and reveals complex interaction among CCCCH zinc-finger proteins during *Caenorhabditis elegans* embryogenesis. *Genetics* **174**, 1933-1945.
29. Mello, C., Draper, B. and Priess, J. (1994) The maternal genes *apx-1* and *glp-1* and establishment of dorsal-ventral polarity in the early *C. elegans* embryo. *Cell* **77**, 95-106.
30. Marin, V. and Evans, T. (2003) Translational repression of a *C. elegans* Notch mRNA by the STAR/KH domain protein GLD-1. *Development* **130**, 2623-2632.
31. Lei, H., Liu, J., Fukushige, T., Fire, A. and Kraus, M. (2009) Caudal-like PAL-1 directly activates the bodywall muscle module regulator *hlh-1* in *C. elegans* to initiate the embryonic muscle gene regulatory network. *Development* **136**, 1241-1249.
32. Edgar, L., Carr, S., Wang, H. and Wood, W. (2001) Zygotic expression of the caudal homolog *pal-1* is required for posterior patterning in *Caenorhabditis elegans* embryogenesis. *Dev. Biol.* **229**, 71-88.
33. Mlodzik, M., Gibson, G. and Gehring, W. (1990) Effects of ectopic expression of caudal during *Drosophila* development. *Development* **109**, 271-277.

34. Hunter, C. and Kenyon, C. (1996) Spatial and temporal controls target *pal-1* blastomere specification activity to a single blastomere lineage in *C. elegans* embryo. *Cell* **87**, 217-226.
35. Bowerman, B., Ingran, M. and Hunter, C. (1997) The maternal *par* genes and the segregation of cell fate specification activities in early *Caenorhabditis elegans* embryos. *Development* **124**, 3815-3826.
36. Mootz, D., Ho, D. and Hunter, C. (2004) The STAR/Maxi-KH domain protein GLD-1 mediates a developmental switch in the translational control of *C. elegans* PAL-1. *Development* **131**, 3263-3272.
37. Huang, N., Mootz, D., Albertha, J., Walhout, J., Vidal, M. and Hunter, C. (2002) MEX-3 interacting proteins link cell polarity to asymmetric gene expression in *Caenorhabditis elegans*. *Development* **129**, 747-759.
38. Pagano, J., Farley, B., Essien, K. and Ryder, S. (2009) RNA recognition by the embryonic cell fate determinant and germline totipotency factor MEX-3. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 20252-20257.
39. Jadhav, S., Rana, M. and Subramaniam, K. (2008) Multiple maternal proteins coordinate to restrict the translation of *C. elegans* *nanos-2* to primordial germ cells. *Development* **135**, 1803-1812.
40. Farley, B., Pagano, J. and Ryder, S. (2008) RNA target specificity of the embryonic cell fate determinant POS-1. *RNA* **14**, 2685-2697.
41. McNally, K., Audhya, A., Oegema, K. and McNally, F. (2006) Katanin controls mitotic and meiotic spindle length. *J. Cell. Biol.* **175**, 881-891.
42. Kurz, T., Pintard, L., Willis, J., Hamill, D., Gonczy, P., Peter, M. and Bowerman, B. (2002) Cytoskeletal regulation by the Nedd8 ubiquitin-like protein modification pathway. *Science* **295**, 1294-1298.
43. Srayko, M., Buster, D., Bazirgan, O., McNally, F. and Mains, P. (2000) MEI-1/MEI-2 katanin-like microtubule severing activity is required for *Caenorhabditis elegans* meiosis. *Genes. Dev.* **14**, 1072-1084.
44. Clark-Maguire, S. and Mains, P. (1994) Localization of the *mei-1* gene product of *Caenorhabditis elegans*, a meiotic-specific spindle component. *J. Cell. Biol.* **126**, 199-209.
45. Johnson, J-L., Lu, C., Raharjo, E., McNally, K., McNally, F. and Mains, P. (2009) Levels of the ubiquitin ligase substrate adaptor MEL-26 are inversely correlated with MEI-1/katanin microtubule-severing activity during both meiosis and mitosis. *Dev. Biol.* **330**, 349-357.
46. Lu, C. and Mains, P. (2007) The *C. elegans* anaphase promoting complex and MBK-2/DYRK kinase act redundantly with CUL-3/MEL-26 ubiquitin ligase to degrade MEI-1 microtubule-severing activity after meiosis. *Dev. Biol.* **302**, 438-447.
47. Pang, K., Ishidate, T., Nakamura, K., Shirayama, M., Trzepacz, C., Schubert, C., Priess, J. and Mello, C. (2004) The minibrain kinase homolog, *mbk-2*, is required for spindle positioning and asymmetric cell division in early *C. elegans* embryos. *Dev. Biol.* **265**, 127-139.
48. Pellettieri, J., Reinke, V., Kim, S. K. and Seydoux, G. (2003). Coordinated activation of maternal protein degradation during the egg-to-embryo transition in *C. elegans*. *Dev. Cell.* **5**, 451-462.
49. Pintard, L., Kurz, T., Glaser, S., Willis, J., Peter, M. and Bowerman, B. (2003) Neddylation and deneddylation of CUL-3 is required to target MEI-1/katanin for degradation at the meiosis-to-mitosis transition in *C. elegans*. *Curr. Biol.* **13**, 911-921.
50. Pintard, L., Willis, J., Willems, A., Johnson, J., Srayko, M., Kurz, T., Glaser, S., Mains, P., Tyers, M. and Bowerman, B. (2003). The BTB protein MEL-26 is a substrate-specific adaptor of the CUL-3 ubiquitin-ligase. *Nature* **425**, 311-316.
51. Quintin, S., Mains, P., Zinke, A. and Hyman, A. (2003). The *mbk-2* kinase is required for inactivation of MEI-1/katanin in the one-cell *Caenorhabditis elegans* embryo. *EMBO Rep.* **4**, 1175-1181.
52. Stitzel, M., Pellettieri, J. and Seydoux, G. (2006) The *C. elegans* DYRK Kinase MBK-2 marks oocyte proteins for degradation in response to meiotic maturation. *Curr. Biol.* **16**, 56-62.
53. Stitzel, M., Cheng, K. and Seydoux, G. (2007) Regulation of MBK-2/DYRK kinase by dynamic cortical anchoring during the oocyte-to-zygote transition. *Curr. Biol.* **17**, 1545-1554.
54. Li, W., DeBella, L., Guven-Ozkan, T., Lin, R. and Rose, L. (2009) An eIF4E-binding protein regulates katanin protein levels in *C. elegans* embryos. *J. Cell. Biol.* **187**, 33-42.
55. Nakamura, A., Sato, K. and Hanyu-Nakamura, K. (2004) *Drosophila* Cup is an eIF4E binding protein that associates with Bruno and regulates *oskar* mRNA translation in oogenesis. *Dev. Cell.* **6**, 69-78.
56. Nelson, M., Leidal, A. and Smibert, C. (2004) *Drosophila* Cup is an eIF4E-binding protein that functions in Smaug-mediated translational repression. *EMBO J.* **23**, 150-159.
57. Nishi, Y. and Lin, R. (2005) DYRK2 and GSK-3 phosphorylate and promote the timely degradation of OMA-1, a key regulator of the oocyte-to-embryo transition in *C. elegans*. *Dev. Biol.* **288**, 139-149.
58. Shirayama, M., Soto, M., Ishidate, T., Kim, S., Nakamura, K., Bei, Y., van den Heuvel, S. and Mello, C. (2006) The conserved kinases CDK-1, GSK-3, KIN-19, and MBK-2 promote OMA-1 destruction to regulate the oocyte-to-embryo transition in *C. elegans*. *Curr. Biol.* **16**, 47-55.
59. DeBella, L., Hayashi, A. and Rose, L. (2006) LET-711, the *Caenorhabditis elegans* NOT1 ortholog, is required for spindle positioning and regulation of microtubule length in embryos. *Mol. Biol. Cell.* **17**, 4911-4924.
60. Collart, M. and Timmers, H. (2004) The eukaryotic Ccr4-Not complex: a regulatory platform integrating mRNA metabolism with cellular signaling pathways. *Prog. Nucleic. Acid. Res. Mol. Biol.* **77**, 289-322.
61. Cui, Y., Ramnarain, D., Chiang, Y., Ding, L., McMahon, J. and Denis, C. (2008) Genome wide expression analysis of CCR4-NOT complex indicates that it consists of three modules with the NOT module controlling SAGA-responsive genes. *Mol. Genet. Genomics.* **279**, 323-337.
62. Panasenko, O., Landrieux, E., Feuermann, M., Finka, A., Paquet, N. and Collart, M. (2006) The yeast Ccr4-Not complex controls ubiquitination of the nascent-associated polypeptide (NAC-EGD) complex. *J. Biol. Chem.* **281**, 31389-31398.

63. Albert, T., Hanzawa, H., Legtenberg, Y., de Ruwe, M., van den Heuvel, F., Collart, M., Boelens, R. and Timmers, H. (2002) Identification of a ubiquitin-protein ligase subunit within the CCR4-NOT transcription repressor complex. *EMBO J.* **21**, 355-364.
 64. Gallo, C., Munro, E., Rasoloson, D., Merritt, C. and Seydoux, G. (2008) Processing bodies and germ granules are distinct RNA granules that interact in *C. elegans* embryo. *Dev. Biol.* **323**, 76-87.
-