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Anterior-Posterior Polarity in *Caenorhabditis elegans*: Establishment of Asymmetries at the One-Cell Stage and Beyond

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ABSTRACT

The *Caenorhabditis elegans* one-cell embryo is a model system for understanding how cells are polarized during development. Polarity establishment is regulated with the cell cycle and involves sperm donated products and changes in the actomyosin cytoskeleton. Sperm entry results in completion of meiosis II in the embryo and the sperm donated centrosome polarizes the axis by triggering local destabilization of the cortical actomyosin cytosketon. This leads to cortical flows and localization of the cortical PAR proteins to distinct domains. Following the establishment of cortical polarity, the cytoplasm is polarized through protein movement and selective degradation of developmental regulators. The establishment of polarity in *C. elegans* is complete prior to the first cell division and is crucial for cell fate decisions in daughter cells. Further AP polarities in the embryo require cell-cell communication and the Wnt signaling pathway plays a pivitol role in many of the AP cell fate decisions throughout development.

Keywords: actomyosin cytoskeleton, centrosome, PAR proteins, proteolysis, Wnt
Abbreviations: AP, anterior-posterior; **APC**, anaphase-promoting complex; **PAR**, partitioning defective; **SPCC**, sperm pronuclear-centrosome complex

CONTENTS

INTRODUCTION

In the nematode *Caenorhabditis elegans* (*C. elegans*) the anterior-posterior (AP) axis is established prior to the first cell division. Asymmetries are present very early in the single cell, thus making *C. elegans* an excellent model for studying axis formation, cell polarity, and asymmetric cell division. Much of what has been uncovered about polarity in the one-cell *C. elegans* embryo has been shown to also play a role in other cell types in divergent phyla. Important players such as the PAR proteins and the actomyosin cytoskeleton are conserved in their polarity functions in numerous organisms (Baas *et al*. 2004; Betschinger *et al*. 2004). Thus, elucidating the mechanisms underlying AP axis formation in the worm should lead to a better understanding of fundamental principles in polarity establishment.

The AP axis is established shortly after fertilization and is dictated by the position of the sperm pronuclear/centrosome complex (SPCC) (**Fig. 1**). Several events occur concurrently during axis establishment: the segregation of the actomyosin cytoskeleton leading to a contractile anterior and non-contractile posterior, segregation of PAR proteins to anterior and posterior cortical domains, and cytoplasmic flows. These events result in visible signs of polarity such as smoothing of the posterior cortex close to the SPCC, anterior cortical ruffling, and pseudocleavage, a furrow that forms between the anterior and posterior cortical domains. Critical polarity players, the PAR (partitioning-defective) proteins, become asymmetrically localized prior to the first mitosis in response to the SPCC (**Fig. 1**). The PDZ domain proteins PAR-3 and PAR-6 move to the anterior pole with the atypical protein kinase C PKC-3, while the serine-threonine kinase PAR-1 and the ring-finger protein PAR-2 localize at the posterior cortex (**Fig. 1**). Localization of these

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Invited Review

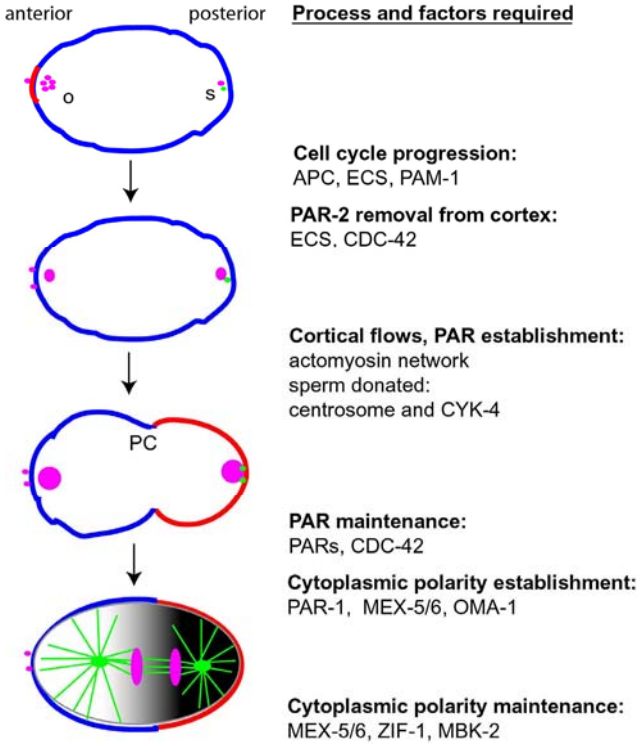

Fig. 1 Overview of polarity establishment. After fertilization, the oocyte chromosomes (o) complete meiosis and two polarbodies are extruded. The sperm chromosomes (s) and associated centrosome sit near the future posterior during this time (chromosomes in purple, centrosome in green). The APC and ECS ubiquitin ligase complexes are necessary for completion of meiosis whereas the PAM-1 aminopeptidase is necessary to exit meiosis. During meiosis, PAR-3 and PAR-6 (blue) are found throughout the cortex and PAR-2 (red) at the anterior cortex near the meiotic spindle. Removal of this PAR-2 patch requires the ECS as well as CDC-42. Around meiotic exit, the centrosome and sperm donated CYK-4 act to destabilize the actomyosin network at the posterior, resulting in cortical flows to the anterior and a visible pseduocleavage furrow (PC) in the embryo. This flow results in PAR-3, PAR-6, and PKC-3 movement to the anterior, followed by PAR-2 and PAR-1 localization to the posterior. These domains are maintained through PAR protein interactions and CDC-42. Following PAR localization, cytoplasmic determinants are localized by PAR-1, MEX-5 and ZIF-1 mediated degradation in the anterior.

proteins is absolutely critical as further asymmetries in the embryo are mediated by the PAR proteins. PARs mediate localization of some cytoplasmic determinants as well as posterior displacement of the first mitotic spindle (Schneider and Bowerman 2003; Cowan and Hyman 2004a), resulting in an asymmetric first cleavage (Etemad-Moghadam *et al*. 1995; Guo and Kemphues 1995; Boyd *et al*. 1996; Watts *et al*. 1996). After the first axis is established, the Wnt signaling pathway plays a role in organizing the embryo and signaling further AP asymmetries. This review will detail the mechanisms governing polarization of the axis, establishment and maintenance of PAR protein localization, and the resulting of cytoplasmic asymmetries in the one-cell *C. elegans* embryo. In addition, the role of the Wnt pathway in later AP polarities of the embryo will be examined.

CELL CYCLE REGULATION AND POLARITY ESTABLISHMENT

Fertilization triggers completion of meiosis and axis polarization

Prior to fertilization, oocytes arrest in diakinesis of meiosis I and appear to be unpolarized, although some asymmetries exist (Kimble and White 1981). During oocyte maturation, which requires signals from the sperm, the oocyte nucleus migrates to the distal end of the cell, the nuclear envelope breaks down, the chromosomes enter metaphase I as the oocyte is ovulated (McCarter *et al*. 1999; Miller *et al*. 2001). Due to the architecture of the gonad, when the oocyte enters the spermatheca, it is immediately fertilized resulting in most cases in the oocyte chromosomes at the future anterior and the sperm chromosomes on the opposing end, which will become posterior. The fertilization event is required for the oocyte chromosomes to continue meiosis (McNally and McNally 2005). Once exiting the spermatheca, the new zygote must complete two meiotic divisions, pinching out two polar bodies. During this time, the sperm donated chromosomes remain tightly compacted and close to the site of sperm entry and the sperm donated centriole does not yet nucleate microtubules (Albertson 1984). After extrusion of the polar bodies, the embryo exits meiosis. During this stage, the 1N chromosomes of sperm and egg decondense, pronuclear envelopes form around the two opposing chromosome masses and the centrosomes begins to nucleate microtubules (**Fig. 1**). Despite apparent asymmetries in the early embryo, polarity is not thought to be established until the onset or completion of meiotic exit.

Regulation of proteolysis in both meiosis and polarity

The timing of polarity establishment appears to be tightly controlled and tied to cell cycle progression. In addition, these events seem to be regulated in part through the proteolytic machinery of the cell. Evidence for these connections has come from the study of numerous mutants in proteins required for protein degradation that show defects in both meiotic completion and polarity establishment (Rappleye *et al*. 2002; Shakes *et al*. 2003; Liu *et al*. 2004; Sonneville and Gönczy 2004; Lyczak *et al*. 2006). In some cases, these defects are separable, suggesting that cell cycle progression and polarity establishment are controlled independently by common proteolytic regulators (Liu *et al*. 2004; Sonneville and Gönczy 2004; Lyczak *et al*. 2006). Defects in meiosis and polarity are seen in mutants for the anaphase promoting complex (APC), a E3 ubiquitin ligase complex (Rappleye *et al*. 2002), however the polarity defects correlate with specific meiotic defects (Rappleye *et al*. 2002; Shakes *et al*. 2003). Null mutants in the APC result in arrest of embryos at the metaphase to anaphase transition of meiosis I, clearly illustrating that this complex is essential for this transition (Golden *et al*. 2000). However, reduction of function and temperature sensitive mutations show a range of phenotypes and temperature shift experiments have revealed that embryos that complete meiosis II normally, show normal polarity, while embryos that bypass meiosis II, fail to polarize the AP axis (Shakes *et al*. 2003). These experiments suggest that the APC is not directly required for AP polarity and that polarity defects are secondary to the meiotic difficulties. However, the polarity defects in these embryos are severe with loss of PAR-2 at the cortex and a symmetric first division. This raises the possibility that the completion of meiosis II and a normal meiotic exit are necessary steps in polarity.

While the role of the APC in polarity is unclear, another E3 ubiquitin ligase complex, the ECS (for the elongin B/C, cullin 2, SOCS box subunits), seems to be necessary for both meiotic progression and polarity establishment (Liu *et al*. 2004; Sonneville and Gönczy 2004). CUL-2, a E3 ligase scaffold, and ZYG-11, a putative adaptor protein, are both necessary for timely progression through the metaphase to anaphase transition of meiosis II and to prevent inappropriate and premature axis polarization during meiosis (Liu *et al*. 2004; Sonneville and Gönczy 2004) (**Fig. 1**). A mutation in either of these genes results in a prolonged metaphase II, meiotic exit problems and reversal of polarity toward the site of the meiotic spindle. In these mutants, PAR-2 localizes to the anterior cortex, instead of the posterior localization found in wild-type, and some embryos go on to divide with reversed polarity (**Fig. 2**).

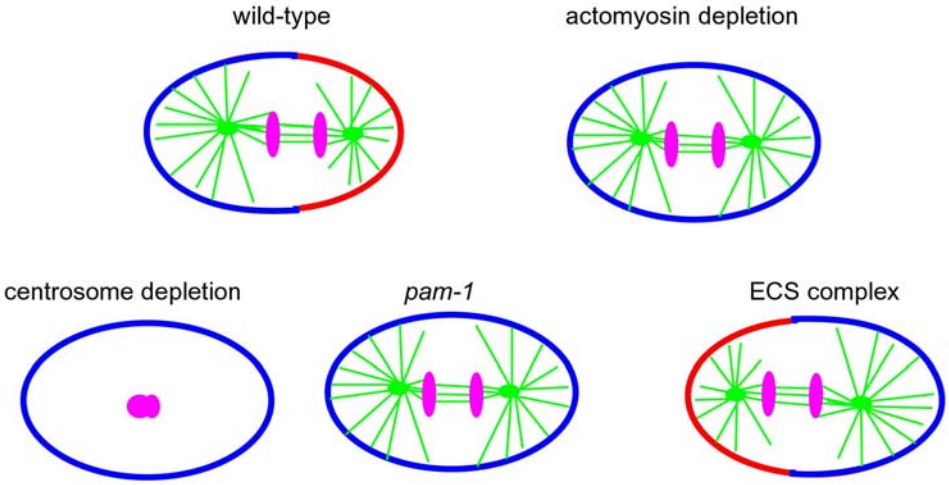

Fig. 2 Polarity establishment requires the actomyosin cytoskeleton, regulation of proteolysis and the centrosome. Anterior is to the left and posterior is to the right. PAR-3/PAR-6/PKC-3 localization is shown in blue, PAR-2 localization in red, chromosomes in purple and centrosomes/microtubules in green. In wild-type embryos, the spindle orients toward the posterior to result in an asymmetric cleavage. These asymmetries require the actomyosin cytoskeleton. Depletion of actin, profilin, or non-muscle myosin prevent PAR-3 restriction to the anterior and PAR-2 fails to reach the cortex. The result is a symmetric spindle orientation. The centrosome must contact the posterior cortex to illicit changes in the actomyosin network and establishment of polarity. Thus ablation of the centrosome or mutations in *spd-2* or *spd*-5 which hamper centrosome maturation, completely block axis polarization. Similarly, the puromycin sensitive aminopeptidase, PAM-1, is necessary for the same asymmetries through regulation of centrosome positioning in the embryo. The ECS ubiquitin ligase complex is necessary for removal of PAR-2 from the anterior cortex. In the absence of *cul-2* or *zyg-11,* some embryos show reversed polarity.

Interestingly, the CUL-2/ZYG-11 complex appears to control the meiotic and polarity processes independently (Liu *et al*. 2004; Sonneville and Gönczy 2004). Potential targets of this complex for cell cycle progress include the B type cyclins CYB-1 and CYB-3 which are upregulated in *cul-2* and *zyg-11* mutants respectively (Liu *et al*. 2004; Sonneville and Gönczy 2004). However, the targets for polarity regulation have yet to be discovered.

In addition to the ECS, the PAM-1 aminopeptidase is also involved in regulating both meiotic progression and polarity (Lyczak *et al*. 2006). *pam-1* mutants delay in meiotic exit and fail to polarize the AP axis. PAR-2 is often absent from the cortex or mislocalized and many embryos divide symmetrically (**Fig. 2**). While the polarity defect differs some from that of *zyg-11* and *cul-2* mutants, they are similarly separable from the meiotic defects. Meiotic exit defects are rescued through inactivation of CYB-3, but polarity defects remain (Lyczak *et al*. 2006). The polarity defects are thought to be due to failure of the SPCC to contact the posterior. This finding has provided further evidence that proteolytic machinery controls both meiotic progression and polarity establishment. A key area of future research will be to discover the targets of these proteins in regulating SPCC positioning and polarity.

ESTABLISHMENT OF CORTICAL POLARITY

The role of the sperm in polarity establishment

The key initiator of axis polarization in *C. elegans* is the sperm. At fertilization, the sperm donates chromosomes as well as the centriole that will become the microtubule organizing center of the cell. While the sperm usually enters at the presumptive posterior, it is not the site of entry, but the site the SPCC rests that will determine the posterior pole (Goldstein and Hird 1996). Evidence suggests that the SPCC must contact the cortex to polarize the axis. In detailed studies, it has been noticed that the first signs of polarity are only manifested in the embryo after the SPCC contacts the presumptive posterior cortex (Cowan and Hyman 2004b). Additionally, embryos that fail to show this contact, such as APC and *pam-1* mutants, lack signs of AP polarity (Rappleye *et al*. 2002; Lyczak *et al*. 2006). Much research has been done to discover the sperm donated product that cues polarity in the embryo. As the sperm donated chromosomes are dispensible for polarity (Sadler and Shakes 2000), the centrosome and associated microtubules have been an area of intense focus.

Probing a role for the centrosome in polarity establishment

Early attention was focused on the microtubules as a polarity cue. Loss-of function mutations in the APC cause embryos to arrest in metaphase of meiosis I (Golden *et al*. 2000). These embryos have a meiotic spindle that remains near the anterior cortex for a long time. In these embryos, a partial axis reversal was observed, including anterior localization of cortical proteins PAR-1 and PAR-2, which are normally localized to the posterior (Wallenfang and Seydoux 2000). This suggested that the meiotic spindle was acting as a mislocalized polarity cue. Reversal of polarity was also observed in *zyg-11* and *cul-2* mutants, beginning during meiotic stages (Liu *et al*. 2004; Sonneville and Gönczy 2004). However, the reversal of polarity observed in *zyg-11* mutants, did not required microtubules, suggesting that there are other mechanisms at play and that microtubules may not be the polarity cue (Sonneville and Gönczy 2004).

A role more specifically for the centrosome has come from numerous studies. Mutations or inactivation of *spd-2* or *spd-5*, critical scaffolding components of the centrosome, result in polarity defects (O'Connell *et al*. 2000; Hamill *et al*. 2002; Cowan and Hyman 2004b). These mutants are severely hampered in centrosome assembly and microtubule nucleation. Due to these defects, early signs of polarity such as cortical flows and pseudocleavage are absent and PAR-2 fails to localize properly to the cortex (O'Connell *et al*. 2000; Hamill *et al*. 2002; Cowan and Hyman 2004b) (**Fig. 2**). However, the polarity defects in these mutants could be interpreted to be caused by the delay in microtubule nucleation by the centrosome or the assembly of centrosome components key to polarity establishment. Evidence for the later has come from studies of centrosome ablation and depletion of microtubules. Axis polarization occurs at the time the centrosome begins to assemble pericentrosomal components such as SPD-5 and ablation of the centrosome completely prevents axis polarization (Cowan and Hyman 2004b). Additionally, the role of the centrosome in axis polarization can be separated from its role in microtubule nucleation, as depletion of tubulin components directly does not

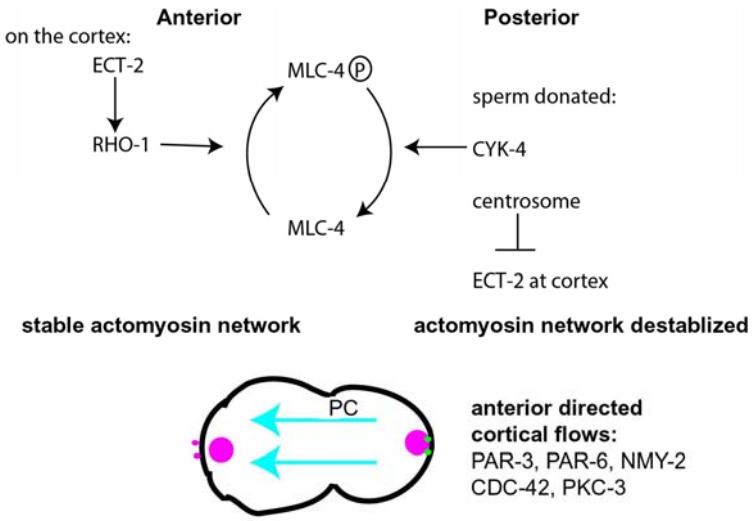

Fig. 3 Regulation of the actomyosin cytoskeleton. At the posterior pole, sperm donated CYK-4 leads to local actomyosin destabilization through dephosphorylation and inactivation of myosin light chain (MLC-4). The centrosome also acts to destabilize the posterior cortex through interactions that move ECT-2 to the anterior where it activates RHO-1, which promotes MLC-4 activation through phosphorylation. The result is a destabilized actomyosin network in the posterior and cortical flows directed anteriorly (light blue arrows) with NMY-2, driving PAR-3, PAR-6, CDC-42, and PKC-3 to the anterior. PC: pseudocleavage.

interfere with axis establishment (Cowan and Hyman 2004b; Sonneville and Gönczy 2004). Although it can not be certain that these experiments result in a complete absence of all microtubules, these studies strongly suggest that that loss of microtubules alone do not cause polarity defects, whereas, loss of the centrosome does. While SPD-2 and SPD-5 appear to critical components of the centrosome required to initiate polarity, further research must be done to identify additional players and to determine how they act to trigger axis polarization.

The role of the actomyosin cytoskeleton in polarity establishment

The actomyosin cytoskeleton is key in initiation of polarity. The nonmuscle myosin II heavy chain, NMY-2, and the small GTPase CDC-42 are found in foci throughout the cortex of the early embryo (Munro *et al*. 2004; Schonegg and Hyman 2006). Integrity of this network is crucial to axis polarization, as depletion of actin, the actin nucleator profilin, *nmy-2,* or the nonmuscle myosin *mlc-4,* using drugs or via RNA interference completely blocks PAR polarity establishment (Guo and Kemphues 1996; Shelton *et al*. 1999; Severson and Bowerman 2003) (**Fig 2**). Similarly, the RHO-1 GTPase, its guanine nucleotide exchange factor ECT-2, and the guanosine triphosphate activating protein CYK-4 are required for the organization of the myosin cytoskeleton and PAR polarity (Jenkins *et al*. 2006; Motegi and Sugimoto 2006; Schonegg and Hyman 2006). Thus, a functional actomyosin network is essential for polarity establishment. In contrast, mutations in PAR proteins do not affect the establishment of contractile polarity (Kirby *et al*. 1990), suggesting that the contractile polarity is upstream of PAR polarity and regulates the distribution of the PAR proteins along the cortex.

How is polarity established?

To establish polarity, the axis of symmetry must be broken. In *C. elegans* the symmetry is broken by a local destabilization of the actomyosin cytoskeleton. The current model suggests that while the SPCC contacts the future posterior cortex, it destabilizes the microfilaments at the local cortex (Munro *et al*. 2004). How the centrosome does this is unknown, but the initial change elicited by the centrosome appears to be exclusion of ECT-2 from the posterior cortex (Motegi and Sugimoto 2006) (**Fig 3**). Exclusion of ECT-2 requires the centrosome but no other factor tested, including MLC-4 and the PAR proteins. ECT-2 in turn is required to enrich RHO-1 at the anterior cortex (Motegi and Sugimoto 2006). The asymmetric distribution of RHO-1 is predicted to decrease myosin dependent contraction in the posterior (Motegi and Sugimoto 2006). Recent studies have suggested that in addition to the centrosome, sperm donated CYK-4 is necessary for this local destabilization of the actomyosin network (Jenkins *et al*. 2006). CYK-4 localizes to the posterior near the sperm pronucleus and acts in opposition to RHO-1 and ECT-2 to promote local actomyosin destabilization. While RHO-1 and ECT-2 are necessary for the organization and contractility of the cortical actomyosin cytoskeleton in the anterior, CYK-4 downregulates the actomyosin cytoskeleton in the posterior (Munro *et al*. 2004; Motegi and Sugimoto 2006; Schonegg and Hyman 2006). This is done in part through regulation of MLC-4 activity. RHO-1 and ECT-2 promote MLC-4 phosphorylation and activity while CYK-4 promotes dephosphorylation of MLC-4 and thus inactivation of the protein in the posterior (Jenkins *et al*. 2006). The result is posterior destabilization of the actomyosin cytoskeleton and asymmetric contractility. It is not known whether depletion of ECT-2 from the posterior, or posterior enrichment of CYK-4 after fertilization is the initial step in polarity establishment as both depend on sperm donated products (**Fig. 3**).

Changes in actomyosin contractility cause dramatic flows at the cortex from the posterior to anterior, while the internal cytoplasm moves in the opposite direction toward the posterior (Hird and White 1993; Goldstein and Hird 1996; Munro *et al*. 2004; Jenkins *et al*. 2006) (**Fig. 2**). The cortical flows can be visualize both through movement of yolk granules, or through fluorescent fusions to NMY-2, PAR-6, or CDC-42, which move dramatically toward the anterior (Munro *et al*. 2004; Motegi and Sugimoto 2006; Schonegg and Hyman 2006). The integrity of the flows depends on NMY-2 and MLC-4 as well as the anterior PAR proteins (Munro *et al*. 2004). This dramatic reorganization of the cortex is the first visible sign of polarity in the embryo. The destabilization of the cytoskeleton in the posterior cortex results in a more relaxed and smooth appearance in this cortical region. However, contractile abilities still remain in the anterior, where the cortex is stabilized by CDC-42 and is seen to actively move during this time (Munro *et al*. 2004; Schonegg and Hyman 2006). The division between these two cortical domains is a temporary furrow in the cell, called pseudocleavage. In most cases, absence of pseudocleavage is a sign that axis polarization is aberrant. While it is clear that sperm donated CYK-4 is necessary for these events, it is still poorly understood how the centrosome also acts to elicit these changes. An interesting future direction will be to understand how both CYK-4 and the centrosome exert their influence only after completion of meiosis to polarize the axis and how the centrosome influences ECT-2 localization.

ASYMMETRIC PROTEIN LOCALIZATION

Establishment of PAR protein domains

During cortical flows, the PAR proteins become localized

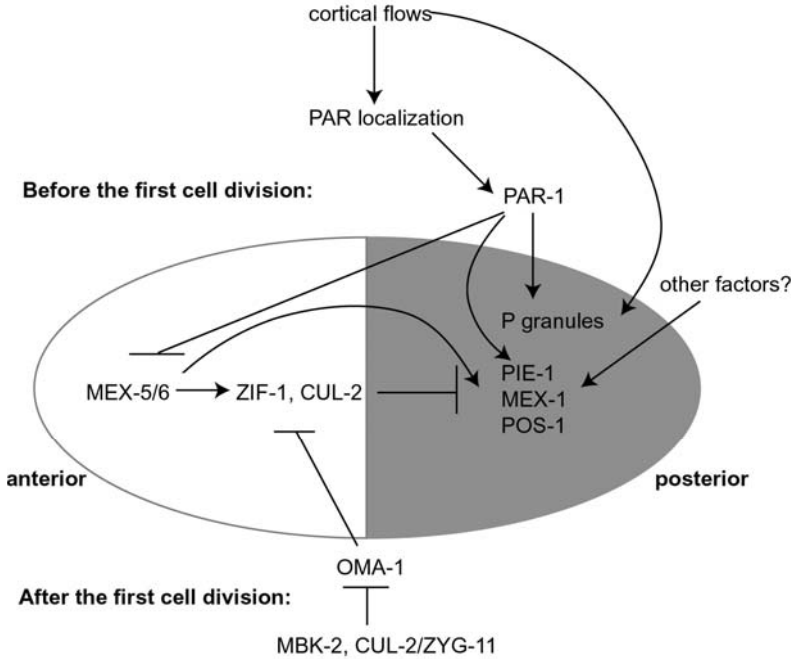

Fig. 4 Localization of cytoplasmic determinants. Prior to the first cell division, cortical flows lead to establishment of PAR localization, including PAR-1 to the posterior cortex. PAR-1 then acts to localize additional determinants through both positive and negative interactions. MEX-5/6 is necessary to localize posterior determinants before the first mitosis through an unknown mechanism. After division and OMA-1 degradation, MEX-5/6 in the anterior activates ZIF-1 mediated proteolysis of remaining posterior determinants.

to restricted domains at the cortex. Localization of the PAR proteins is crucial for later asymmetries in the embryo. Prior to this stage, PAR-3 and PAR-6, and PKC-3 are found throughout the cortex, and during meiosis, PAR-2 is found at the anterior cortex (Cuenca *et al*. 2003) (**Fig. 1**). Widespread localization of PAR-2 at this stage seems to be prevented due to inhibitory phosphorylation by PKC-3 (Hao *et al*. 2006). First, removal of PAR-2 from the anterior cortex near the meiotic spindle must be accomplished. This removal requires the ECS complex components CUL-2 and ZYG-11 as well as CDC-42 (Liu *et al*. 2004; Sonneville and Gönczy 2004; Schonegg and Hyman 2006) (**Fig. 1**). The removal of PAR-2 from the anterior cortex is independent of the asymmetric movements of the cortex or the sperm centrosome signal (Liu *et al*. 2004; Sonneville and Gönczy 2004; Schonegg and Hyman 2006). Establishment of initial PAR asymmetry requires centrosome association with the posterior cortex and cortical flows (Cuenca *et al*. 2003; Munro *et al*. 2004). With the anterior cortical flow, PAR-3, PAR-6, CDC-42, and PKC-3, all move toward the anterior pole. It is still uncertain whether the anterior PARs also require CDC-42 for their initial localization, specifically PAR-6 which is known to interact with CDC-42 directly (Gotta *et al*. 2001). While numerous studies have shown that depletion of CDC-42 does not result in an initial difference in anterior PAR localization (Gotta *et al*. 2001; Kay and Hunter 2001; Aceto *et al*. 2006; Motegi and Sugimoto 2006), one study suggests that more complete loss of CDC-42 function does prevent initial PAR-6 localization (Schonegg and Hyman 2006). A caveat of these experiments is that CDC-42 can not be completely inactivated with RNAi due to the resulting sterility (Schonegg and Hyman 2006). One possibility is that the lack of PAR-6 to the cortex in strong RNAi embryos may be due in part to the remaining PAR-2 at the anterior cortex (Schonegg and Hyman 2006), as a direct interaction between the CDC-42 and PAR-6 does not seem to be required (Aceto *et al*. 2006). PAR-6 mutants unable to interact with CDC-42 can still localize initially to the anterior cortex (Aceto *et al*. 2006). Thus, the role for CDC-42 in PAR establishment is still unclear.

As the PAR-3/PAR-6/PKC-3 complex is swept anteriorly, it frees a portion of the cortex in the posterior. With the movement of PKC-3 to the anterior, the ring-finger domain of PAR-2 becomes active to allow localization to the posterior cortex (Hao *et al*. 2006). With posterior PAR-2 localization, and the serine theronine kinase PAR-1 also accumulates at the posterior pole, near the site of the SPCC (Cuenca *et al*. 2003; Munro *et al*. 2004; Hao *et al*. 2006). Because anterior complex proteins localize in response to the SPCC, they do not require PAR-2 for their initial asymmetry. In contrast, PAR-2 requires asymmetric localization of the anterior proteins to become asymmetrically localized to the posterior (Cuenca *et al*. 2003). Additionally both anterior and posterior cortical PARs require the 14-3-3 protein PAR-5 during the establishment phase to polarize correctly (Cuenca *et al*. 2003). In addition to the PARs, MEX-5/6 acts in a feedback loop during the establishment phase to ensure a fully extended PAR-2 domain (Cuenca *et al*. 2003).

Maintenance of PAR protein domains

Once the initial asymmetry in PAR localization is established, their cortical domains are maintained through mutual exclusion interactions between the PAR proteins (Morton *et al*. 1992; Etemad-Moghadam *et al*. 1995; Boyd *et al*. 1996; Watts *et al*. 1996; Tabuse *et al*. 1998; Cuenca *et al*. 2003). The anterior proteins all rely on each other to localize as well as PKC-3 (Watts *et al*. 1996; Tabuse *et al*. 1998; Hung and Kemphues 1999). PKC-3 levels are maintained by the co-chaperone protein CDC-37 which also contributes to PAR-6 localization by modulating PAR-6 cortical complexes (Beers and Kemphues 2006). In addition, PAR-6 also requires a direct interaction with CDC-42 to maintain its anterior localization (Gotta and Ahringer 2001; Kay and Hunter 2001; Aceto *et al*. 2006; Beers and Kemphues 2006; Schonegg and Hyman 2006). CDC-42 additionally is required to maintain cytoskeletal stability in the anterior (Schonegg and Hyman 2006). PAR-2 is also an important player in the maintenance phase and its function at this time requires both PAR-1 and PAR-5 (Hao *et al*. 2006). One mechanism by which PAR-2 may act is through preventing flows back toward the posterior (Munro *et al*. 2004), thus preventing accumulation of the anterior protein complex back to the posterior (Cuenca *et al*. 2003) (**Fig. 1**).

Establishment of cytoplasmic asymmetries

Following PAR protein localization, cytoplasmic proteins become localized to anterior and posterior domains (**Fig. 4**). Many of these cytoplasmic asymmetries depend in part on cytoplasmic flows and the PAR proteins for their localization. Important determinants that become localized at this time include the P granules, and the CCCH proteins MEX-5 and -6, and PIE-1. MEX-5 and 6 localize to the anterior of the cell while the other factors localize to the posterior (Strome and Wood 1982; Mello *et al*. 1992; Hird *et al*.

1996; Mello *et al*. 1996; Tenenhaus *et al*. 1998; Schubert *et al*. 2000). Internal flux of cytoplasm toward the posterior is necessary to localize ribonucleoprotein particles, called germline P granules to the posterior (Hird *et al*. 1996; Cheeks *et al*. 2004), however this is most likely not the only mechanism. While internal cytoplasm seems to move P granules posteriorly, it is unknown as to why P granules do not also move anteriorly with the cortical flows. Many of the PAR proteins are also required for P granule localization. This may be due to a direct role of the PARs in their localization, as is the case for PAR-1, which stabilizes posteriorly localized granules, or due to the PARs role in regulating cytoplasmic flows (Cheeks *et al*. 2004; Munro *et al*. 2004). In addition to its role in P granule stabilization, PAR-1 is also necessary to restrict localization and activity of MEX-5 and the highly related MEX-6 to the anterior (Schubert *et al*. 2000; Cuenca *et al*. 2003), and PIE-1 to the posterior (Tenenhaus *et al*. 1998). Posterior PIE-1 localization is important for germline development (Mello *et al*. 1992, 1996; Seydoux *et al*. 1996), while MEX-5/6 localization is necessary to ensure asymmetry of additional factors.

Many cytoplasmic factors additionally rely on regulated degradation to be localized appropriately (Hird *et al*. 1996; Reese *et al*. 2000). For instance, in addition to movement posteriorly, P granules are degraded anteriorly (Hird *et al*. 1996). Additional CCCH finger proteins MEX-1, POS-1 and PIE-1 all localize to the posterior at this time (Mello *et al*. 1996; Guedes and Priess 1997; Tabara *et al*. 1999). Localization of MEX-5 and MEX-6 to the anterior is key to localization of these posterior determinants. While it is unclear how this is carried out before the first mitosis, after the first cell division it is controlled through asymmetric protein degradation. If MEX-5 is ectopically expressed it leads to degradation of posterior determinants (Schubert *et al*. 2000). After the first cell division, MEX-5/6 activates ZIF-1 dependent proteolysis of PIE-1, MEX-1, and POS-1, ensuring posterior localization of these factors (DeRenzo *et al*. 2003) ZIF-1 acts with Elongin C and CUL-2 to degrade the proteins through ubiquitin mediated proteolysis in the anterior (DeRenzo *et al*. 2003) (**Fig. 4**). Importantly, MEX-5/6 activates ZIF-1 only after asymmetric distribution of the proteins occurs; early activation of MEX-5 would result in degradation of posterior determinants throughout the one-cell embryo.

How then is this coordinated? Researchers discovered this important piece of the puzzle through study of the oocyte maturation protein OMA-1. OMA-1 and the highly related OMA-2, both CCCH Zinc finger proteins, are necessary for oocyte maturation and are normally found at high levels in the most proximal oocyte, adjacent to the spermatheca (Detwiler *et al*. 2001; Shimada *et al*. 2002). At the onset of the first mitosis, OMA-1 is degraded in the embryo. OMA-1 degradation depends on numerous factors, including phosphorylation by MBK-2, a Drky protein kinase, the known cell cycle regulators CYB-3, and CDK-1, and ECS complex components ZYG-11 and CUL-2 (Pellettieri *et al*. 2003; Quintin *et al*. 2003; Pang *et al*. 2004; Nishi and Lin 2005; Shirayama *et al*. 2006; Stitzel *et al*. 2006). A gain of function mutation in *oma-1*, or depletion of proteins necessary for OMA-1 degradation lead to OMA-1 stabilization and mislocalization of PIE-1, POS-1 and other cell determinants (Lin 2003; Pellettieri *et al*. 2003; Shirayama *et al*. 2006). OMA-1 acts to prevent ZIF-1 mediated proteolysis in the embryo, thus interfering with the degradation of residual posterior determinants in the anterior of the embryo (**Fig 4**). Thus, by tying the degradation of OMA-1 to the start of mitosis, the embryo ensures that ZIF-1 proteolysis will not be active until MEX-5/6 and other determinants have been localized in the cell (Shirayama *et al*. 2006). This degradation then cleans up residual proteins that have not been initially localized in the cell. However, as this proteolysis does not occur until after the first cell division, it is still unclear how posterior determinants are initially localized during the one-cell stage.

ROLE OF THE WNT SIGNALING PATHWAY IN LATER AP ASYMMETRIES

After the initial asymmetries are set up prior to the first cell division, other factors are necessary to provide further AP polarity in the embryo, many of which require cell-cell signaling. One pathway that mediates much of this polarity is the Wnt signaling pathway (reviewed in Korswagen 2007). The Wnt pathway in *C. elegans* is complex and both canonical and noncanonical pathways are present. In addition, there are multiple Wnt ligands and pathway components used at different times and places in the embryo. Despite this complexity, important roles for this pathway in AP cell fate decisions throughout embryogenesis have emerged.

Recent work suggests that the Wnt pathway acts as a global organizer of AP polarity (Bischoff and Schnabel 2006). MOM-2/Wnt signals originate from the posterior cell P_1 and its descendents. This polarizing center originating from the posterior acts to orient axes of cleavage along the anterior posterior axis in descendents of both P_1 and AB. Because of its influence on multiple cell divisions, it is thought at the Wnt signal could be a global organizer of the embryo throughout development (Bischoff and Schnabel 2006).

In addition to its role as a global organizer, Wnt acts on individual cells to regulate levels of the transcription factor POP-1, a TCF homolog. POP-1 is a transcriptional regulator that acts as an activator at low levels, but acts as a transcriptional repressor at high levels (reviewed in Korswagen 2007). Interestingly, all divisions that occur along the anterior-posterior axis during development result in a remarkable asymmetry of POP-1 levels. Anterior daughter show high levels of POP-1 in the nucleus, while posterior daughters show low nuclear levels of this factor (Lin *et al*. 1995; Lin *et al*. 1998). While only a subset of these asymmetries depend on MOM-2/Wnt, they all require the Wnt receptor MOM-5/Frizzled (Park and Priess 2003; Park *et al*. 2004). Specifically a Wnt signal from the posterior, is needed to reduce POP-1 levels in the posterior daughter and thus trigger transcriptional activation of targets. In many of these divisions that have been examined, the POP-1 differences are necessary for the two daughter cells to accept different fates (Lin *et al*. 1995, 1998). Thus, regulation of POP-1 levels appears to be crucial for many AP polarity decisions throughout development.

One of the best studied examples of the regulation of POP-1 levels occurs at the four-cell stage. MOM-2/Wnt signals originating from P_2, polarize the adjacent sister cell EMS (Rocheleau *et al*. 1997; Thorpe *et al*. 1997; Goldstein *et al*. 2006). As a result, the spindle in EMS aligns along the AP axis, the posterior daughter of EMS, E exhibits low levels of POP-1 and is fated to endoderm, and the anterior daughter MS, exhibits high levels of POP-1 and gives rise to mesoderm fates (Goldstein 1995; Rocheleau *et al*. 1997; Thorpe *et al*. 1997; Schlesinger *et al*. 1999; Goldstein *et al*. 2006). This example nicely illustrates the ability of Wnt to signal cell division plane, POP-1 levels and cell fate.

The Wnt pathway works in multiple AP cell divisions, including those in the larva. One example is the epidermal T cell that divides along the AP axis resulting in a high level POP-1 anterior daughter, which becomes a hypodermal cell, and a low level POP-1 posterior daughter, which becomes sensory phasmid. As in embryonic AP divisions, the difference in POP-1 levels is necessary for the fates of the cells and depends upon Wnt signaling components (Herman *et al*. 1995; Sawa *et al*. 1996; Herman 2001). The source of the Wnt signal, in this case the Wnt molecule LIN-44, is a cell just posterior to the T cell, such that the T cell is polarized and the posterior daughter shows low levels of POP-1 (Goldstein *et al*. 2006). Thus, the Wnt pathway acts to polarize numerous AP divisions.

CONCLUDING REMARKS

The initial asymmetries established just following meiotic completion are essential for establishing the body plan of *C. elegans*. The sperm-donated centrosome is pivotal in eliciting changes in the actomyosin cell cortex which initiates polarity. These events quickly lead to localization of cortical and cytoplasmic proteins as well as asymmetric spindle positioning (reviewed in Cowan and Hyman 2004a; Schneider and Bowerman 2003). Once localized in the cell, these determinants are asymmetrically distributed to the two daughter cells after mitosis. This establishes immediate and important differences between the two cells which lead to their different cell fates. Many later AP asymmetries in the embryo are mediated by the Wnt signaling pathway that organizes the embryo, orients cell divisions, and mediates AP cell fate decisions.

While much progress has been made in understanding the mechanisms underlying axis establishment in *C. elegans*, there is still much that remains to be discovered. The relationship between cell cycle regulation and polarity is still unclear. While some common proteolytic machinery regulate both processes, the targets regulated for polarity establishment remain elusive. Similarly how the centrosome acts at a specific time in the cell cycle and how it elicits changes in the actomyosin network at the cortex remains a mystery. A full understanding of all the players necessary for PAR protein localization and maintenance is still necessary and the mechanism governing initial localization of cytoplasmic determinants is still poorly understood. Additionally, the mechanisms governing the Wnt pathway organization of the axis are only beginning to be worked out. Work in the coming years should provide more insights into the mechanisms governing cell polarity in *C. elegans*.

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