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ASYMMETRIC CELL DIVISION IN *C. ELEGANS*: Cortical Polarity and Spindle Positioning

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■ **Abstract** The one-cell *Caenorhabditis elegans* embryo divides asymmetrically into a larger and smaller blastomere, each with a different fate. How does such asymmetry arise? The sperm-supplied centrosome establishes an axis of polarity in the embryo that is transduced into the establishment of anterior and posterior cortical domains. These cortical domains define the polarity of the embryo, acting upstream of the PAR proteins. The PAR proteins, in turn, determine the subsequent segregation of fate determinants and the plane of cell division. We address how cortical asymmetry could be established, relying on data from *C. elegans* and other polarized cells, as well as from applicable models. We discuss how cortical polarity influences spindle position to accomplish an asymmetric division, presenting the current models of spindle orientation and anaphase spindle displacement. We focus on asymmetric cell division as a function of the actin and microtubule cytoskeletons, emphasizing the cell biology of polarity.

CONTENTS

INTRODUCTION

Three steps are required for a successful asymmetric cell division. First, a polarity cue determines the position of the cell-axis. Second, this polarity cue triggers the formation of cortical domains, which define the polarity of the cell. Finally, polarity is communicated to the downstream cytoskeleton in order to polarize the cytoplasm.

Twenty years of work have elucidated, in outline, the process by which the one-cell *Caenorhabditis elegans* embryo undergoes an asymmetric division. Shortly after fertilization, a cue issuing from the sperm centrosome-pronucleus complex induces the formation of a polarity axis (Goldstein & Hird 1996). This axis is revealed by the formation of two cortical domains consisting of PAR proteins: an anterior domain defined by the presence of a complex of PAR-3, PAR-6, and atypical protein kinase C (aPKC) (Cuenca et al. 2003, Etemad-Moghadam et al. 1995, Hung & Kemphues 1999, Tabuse et al. 1998); and a posterior domain defined by PAR-1 and PAR-2 (Boyd et al. 1996, Cuenca et al. 2003, Guo & Kemphues 1995) (Figure 1). The formation of these cortical domains determines the segregation of determinants along the anterior-posterior axis and the orientation and translocation of the mitotic spindle. The events and the molecules that are associated with the PAR proteins have been discussed in a number of excellent and recent reviews (Doe 2001, Doe & Bowerman 2001, Gomes & Bowerman 2002, Gotta & Ahringer 2001a, Lyczak et al. 2002, Macara 2004, Pellettieri & Seydoux 2002, Schneider & Bowerman 2003). Here we discuss what is known about the underlying cell-biological mechanism involved in establishing asymmetry in the one-cell embryo, focusing on issues of cortical polarization.

ESTABLISHING CORTICAL POLARITY

Introduction

The *C. elegans* oocyte is arrested in meiotic prophase, at which point the anterior and posterior PAR proteins are distributed uniformly around the cell periphery (Boyd et al. 1996, Cuenca et al. 2003, Etemad-Moghadam et al. 1995, Hung & Kemphues 1999). Fertilization triggers the completion of meiosis in the egg, and

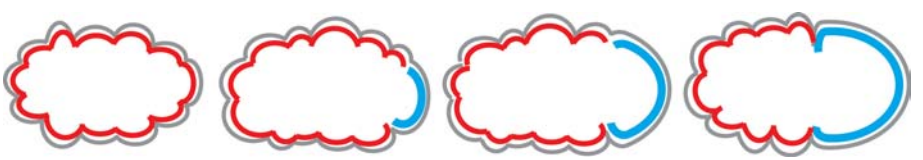

Figure 1 Establishment of PAR protein polarity. PAR-3, PAR-6, and aPKC localization is shown in red; PAR-1 and PAR-2 localization is shown in blue. Anterior is to the left.

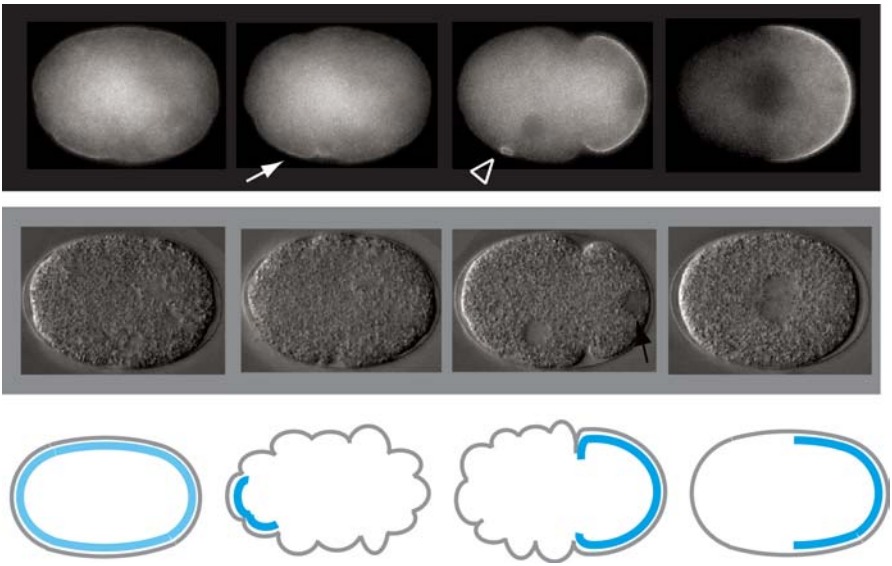

Figure 2 PAR-2 localization cycle. After fertilization, PAR-2 localizes weakly to the entire cell periphery. When the meiotic spindle lies adjacent to the cortex, PAR-2 becomes concentrated at the cortex overlying the spindle (*white arrow*). PAR-2 remains on the cortex of the polar body as it is extruded from the egg (*white outline arrow head*). PAR-2 in the embryo is restricted to the posterior cortex, spreading from the site of polarity initiation near the centrosome-male pronucleus complex (*black arrow*). Anterior is to the left.

PAR-2 localizes to the region overlying the meiotic spindle (Figure 2). Following the completion of meiosis, an anterior-posterior polarity axis is established that will be used for the development of the embryo. The establishment of anterior-posterior polarity depends on the formation of two cortical domains with different properties. The PAR proteins represent one type of cortical polarization: PAR-3, PAR-6, and aPKC (PKC-3) form a complex at the anterior cortex (Cuenca et al. 2003, Etemad-Moghadam et al. 1995, Hung & Kemphues 1999, Tabuse et al. 1998); PAR-1 and PAR-2 localize to the posterior cortex (Boyd et al. 1996, Cuenca et al. 2003, Guo & Kemphues 1995) (Figures 1 and 2).

Loss of *par-3*, *par-6*, or *pkc-3* leads to the uniform distribution of cortical PAR-1 and PAR-2 (Cuenca et al. 2003, Etemad-Moghadam et al. 1995, Hung & Kemphues 1999, Tabuse et al. 1998, Watts et al. 1996), suggesting that the anterior PAR domain may antagonize the posterior PAR proteins. Although we know little about how the PAR proteins antagonize each other in *C. elegans*, work in *Drosophila* embryos and neuroblasts and in mammalian tissue culture cells has suggested phosphorylation-based mechanisms (Benton et al. 2002, Benton & St. Johnston 2003, Betschinger et al. 2003, Hurd et al. 2003, Plant et al. 2003, Yamanaka et al. 2003). For instance, aPKC-dependent phosphorylation of the

polarity determinant Lethal (2) giant larvae (Lgl) results in its exclusion from the PAR-3/PAR-6/aPKC domain, thereby restricting Lgl activity to the opposite cortex (Betschinger et al. 2003). Similar mechanisms could operate in *C. elegans* embryos. In the *Drosophila* follicular epithelium, PAR-1-dependent phosphorylation of PAR-3 results in 14–3-3 (PAR-5) binding to PAR-3, and thus inhibition of PAR-3/PAR-6/aPKC activity (Benton & St. Johnston 2003), suggesting reciprocal antagonism of the PAR domains. In *C. elegans*, loss of PAR-1 activity does not affect the segregation of cortical PAR domains (Cuenca et al. 2003, Guo & Kemphues 1995). One possibility is that a redundant kinase or distinct mechanism is involved. However, this type of mechanism may be more important in *C. elegans* for stabilizing the domains once formed rather than triggering their formation in the first place (Cuenca et al. 2003). In summary, PAR proteins have emerged as key players in cortical asymmetry, and their mutually antagonizing properties may allow for the maintenance of distinct cortical domains. Understanding how the asymmetric PAR domains are initially established remains an outstanding question.

Contractile Polarity

A second type of cortical domain in the one-cell *C. elegans* embryo is represented by the difference in contractile activity of the anterior and posterior cortex, which we term contractile polarity (Figure 3). Coincident with the completion of both meiotic divisions, the embryo cortex begins to ruffle, a motion visible as numerous temporary invaginations of the cortex. Ruffles last from five seconds to several minutes. Some ruffles are shallow dips in the surface of the cortex but others are deeper ingressions that occasionally intersect, resulting in a single deep ruffle. When the polarizing signal is received, cortical smoothing begins in the area around the sperm centrosome, located adjacent to the cortex, and spreads toward the opposite end of the embryo. Smoothing continues until this posterior domain occupies half of the embryo cortex, but the anterior cortex continues to ruffle (Figure 3). During expansion of the posterior cortex, the leading edge of the smooth domain forms a deeper and more stable ingression than the other ruffles in the cortex. This pseudocleavage furrow reaches its maximal ingression as the smooth domain reaches its maximum extension. Ruffles arising both in the anterior cortex and immediately posterior to the pseudocleavage furrow appear to feed into the furrow as it advances (Figure 4).

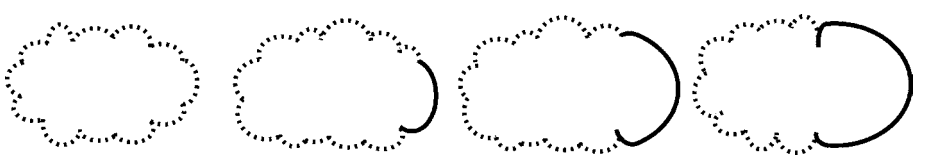

Figure 3 Establishment of contractile polarity. The ruffling cortex is indicated by dotted lines; the smooth cortex is indicated by the solid black line. Anterior is to the left.

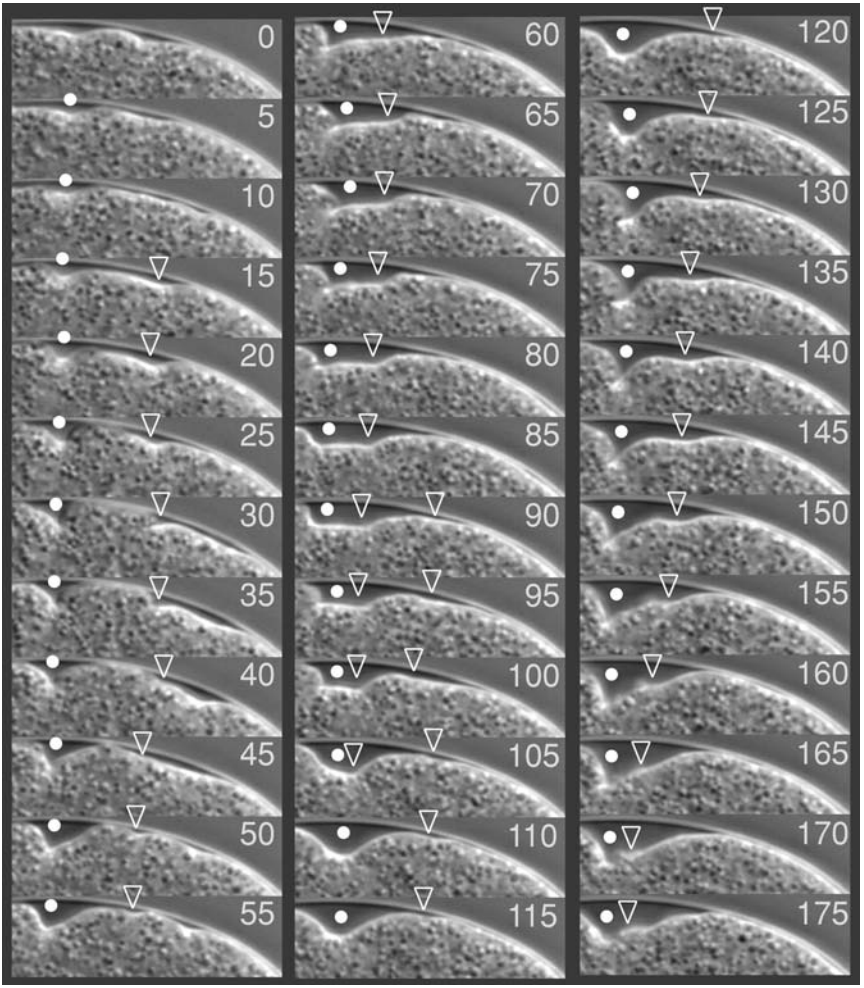

Figure 4 Ruffles feed into the advancing pseudocleavage furrow. Time-lapse differential interference contrast (DIC) images from ~3 min during posterior domain extension. Moving ruffles are indicated by white outline arrow heads; the forming pseudocleavage furrow is indicated by a white dot. This furrow is located approximately one fourth of the embryo length from the posterior pole. Anterior is to the left.

Ruffling is apparently driven by contraction of the acto-myosin cortex. Inhibition of actin filament polymerization with cytochalasin abolishes all cortical activity (Hill & Strome 1988, 1990; Strome & Wood 1983). The formin CYK-1 and profilin (PFN-1), both of which are proposed to promote actin nucleation/assembly in the embryo, are required for ruffling (Severson et al. 2002, Swan et al. 1998), as is nonmuscle myosin (NMY-2) and its regulatory light chain (MLC-4) (Guo &

Kemphues 1996, Shelton et al. 1999). ARP-2/3 depletion does not affect ruffling in the one-cell embryo, suggesting that the ruffling process is a formin-dependent rather than an ARP-2/3 dependent nucleation-based process (Severson et al. 2002) and therefore independent of the small GTPase Cdc42 (Welch 1999). In conclusion, ruffles appear to be independent events, probably arising from the self-organization of actin and myosin into contractile units, which then undergo futile cycles of contraction and relaxation.

Contractile polarity corresponds precisely with the domains defined by the PAR proteins (Cuenca et al. 2003) (Figure 5), suggesting that the two processes may be linked. This coordination continues into cytokinesis when the edge of the PAR-2 domain corresponds to the position of the cytokinesis furrow and PAR-2 associates with the ingressing cleavage furrow. Importantly, however, current

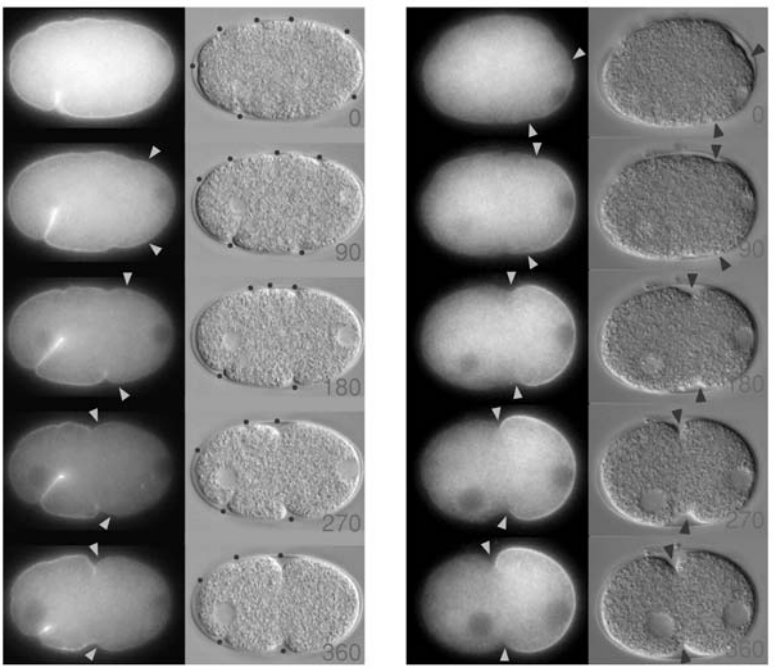

Figure 5 Establishment of GFP::PAR-6 (*left panel*) and GFP::PAR-2 (*right panel*) asymmetry corresponds to establishment of contractile polarity domains. Time-lapse green fluorescent protein (GFP) and DIC images (90-s intervals) document a period of 6 min during which cortical polarity is established. Light gray arrow heads indicate the boundaries of the PAR-6 (anterior) and PAR-2 (posterior) domains. Black dots (*left panel*) indicate ruffle ingressions. Black arrows (*right panel*) indicate the boundary of the smooth posterior domain. Elapsed times (s) are indicated. Anterior is to the right.

evidence suggests that the PAR proteins do not dictate cortical activity. In *par-2*, *par-3*, and *par-6* mutants, normal ruffling asymmetry can be observed (Kirby et al. 1990) despite the mislocalization of the remaining PAR proteins. Furthermore, in the absence of PAR-2, smoothing of the posterior cortex is sufficient to displace the anterior PAR proteins (Cuenca et al. 2003). Although these findings are not unequivocal owing to the difficulty in establishing the molecular null phenotype of Par mutants, they suggest that contractile polarity and PAR protein asymmetry are parallel responses to the polarity cue. More difficult to establish is whether PAR polarity is downstream of contractile polarity. The ruffling process itself is not necessary for cortical polarization. Weak mutants in contractile processes can abolish pseudocleavage and reduce ruffling without affecting the formation of the PAR domains (Rose et al. 1995) (C.R. Cowan & A.A. Hyman, unpublished observations) (Figure 6). However, stronger mutants abolish both ruffling and PAR protein asymmetry (Severson et al. 2002, Severson & Bowerman 2003) (Figure 6).

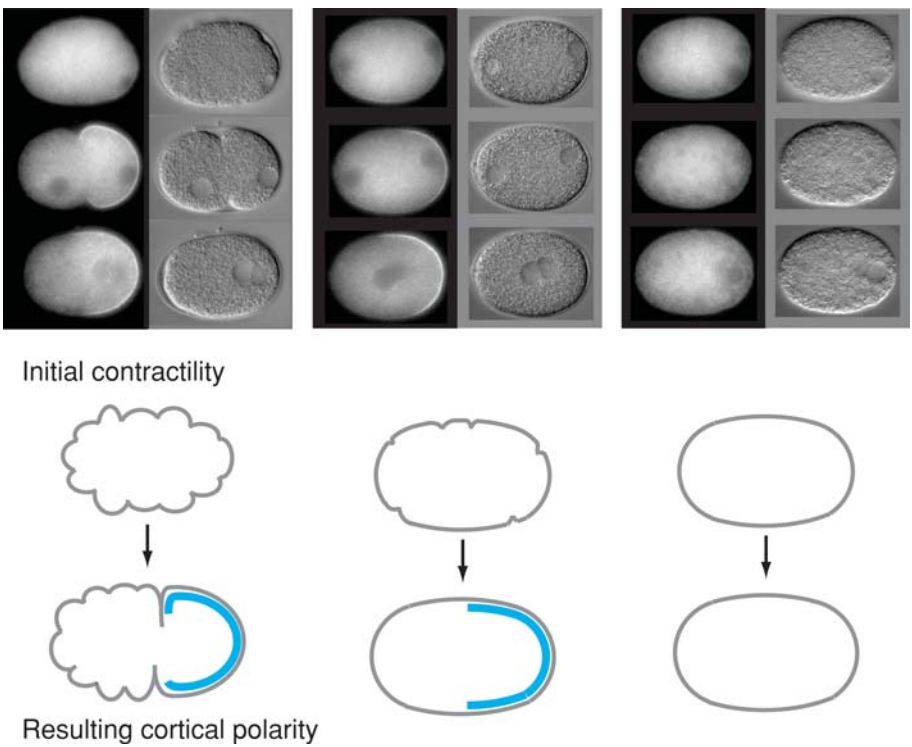

Figure 6 Contractility thresholds for posterior domain formation. PAR-2 polarity can be established independently of ruffling, but severe disruption of the cortex eliminates PAR-2 polarity. GFP::PAR-2 and DIC images of polarity establishment in control, *nop-1(it142)*, and cytochalasin-treated embryos. Diagrams summarizing the correlation between contractility and the resulting cortical polarity; PAR-2 localization is shown in blue. Anterior is to the left.

To us it seems likely that ruffling is a manifestation of, rather than a requirement for, the basic underlying cell biology necessary for cortical polarization. The ruffles appear to be the result of the local concentration of contractile activity surpassing a threshold, thus resulting in contraction. A reduction in contractile activity prevents the contraction cycles (ruffles) but maintains cortical polarity. This reduction in contractility also prevents cytoplasmic flow (see below). Eliminating the contractile activity prevents cortical polarization altogether. In *C. elegans*, the underlying cell biological mechanism of cortical polarization may be the establishment of contractile polarity, distinct, however, from contraction. Alternatively, some other, as yet unidentified, manifestation of cortical polarity may be responsible. The PAR proteins can be viewed as signaling modules that respond to an established cortical polarity and transduce signals to the various downstream effectors necessary for asymmetric cell division.

Cortical Flow

The establishment of contractile polarity coincides with the cortical flow of yolk granules moving along the cortex from the posterior pole to the pseudocleavage furrow and then returning through the center of the cell (Hird & White 1993) (Figure 7). In *C. elegans* embryos, P-granule segregation (Cheeks et al. 2004, Strome & Wood 1983), pronuclear migration (O'Connell et al. 2000), and mitochondria redistribution (Badrinath & White 2003) may occur at least in part through forces generated by cytoplasmic flow, although it has been hard to obtain unequivocal evidence for such a mechanism. Similarly, one-cell ascidian eggs exhibit waves of contraction emanating from one pole, coincident with a large cytoplasmic rearrangement that segregates fate determinants and organelles (Roegiers et al. 1999). Therefore it seems likely that the cortical flow reorganizes the cytoplasm, thereby establishing the required distribution of organelles and fate determinants in the developing embryo.

What are the forces that generate cortical flow? Cortical flow accompanies the migration of many types of animal cells, including macrophages, amoebae, and fibroblasts (Bray & White 1988). In general, these types of migrating cells create a tension gradient within the cortex by establishing a highly contractile region at the rear toward which cortical flow is directed (Bray & White 1988).

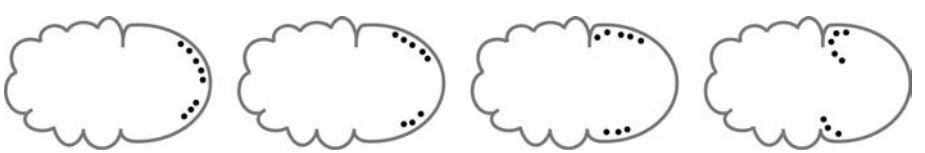

Figure 7 Polarized cortical flow during posterior domain establishment. Yolk granules and filamentous actin foci flow along the cortex from the posterior pole to the pseudocleavage furrow. There is a corresponding posterior-directed flow of central cytoplasm. Yolk granules are indicated by black dots; the cortex is indicated by a gray outline. Anterior is to the left.

In vitro, a fully cross-linked gel of actin filaments and myosin motors can be induced to undergo localized solation either through reduction of actin filament length or through removal of myosin cross-links (Janson & Taylor 1993). The resulting contraction of the gel results in collapse of the actin–myosin meshwork and generates cytoplasmic flow (Janson et al. 1991, Janson & Taylor 1993). Thus flows can be generated in a simple system consisting of a tension gradient. By analogy to migrating cells, contractile polarization in *C. elegans* embryos may create the tension gradient required to produce cortical and cytoplasmic flows and thus polarization of the cytoplasm. The establishment of contractile polarity could serve two functions in the embryo: formation of distinct cortical domains to specify asymmetric PAR protein localization, as discussed above, and generation of a tension gradient to drive cortical flow and thus cytoplasmic polarization and axis alignment.

An indication as to the possible function of cortical flow comes from the observation that the orientation of the cortical polarity axis can be realigned during the cortical flow period. The initial polarity cue in the embryo can occur anywhere on the egg cortex, but the anterior-posterior axis subsequently aligns with the long axis of the egg (Goldstein & Hird 1996). In this process of posteriorization (Rappleye et al. 2002), the entire posterior domain, including the smooth cortex, centrosomes, and pronucleus, migrates into the pole of the egg (Cuenca et al. 2003, Goldstein & Hird 1996, Rappleye et al. 2002) during which the centrosome-male pronucleus maintains its position in the center of the posterior cortical domain (Cuenca et al. 2003) (Figure 8). Mutants with weak or absent cortical flow often fail to undergo posteriorization. Similar posterior movement can be seen after partial depolymerization of microtubules: These nocodazole-treated metaphase spindles lie adjacent

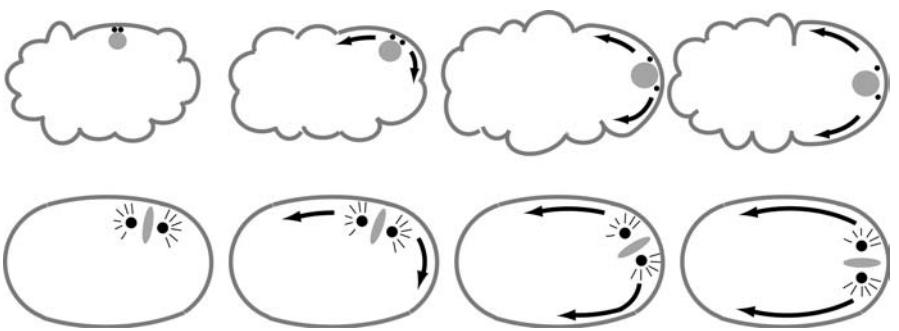

Figure 8 Posteriorization and cortical flow. Posteriorization aligns the polarity axis with the long axis of the cell coincident with the expansion of the posterior domain and cortical flow. Mitotic spindles that form adjacent to the cortex (following microtubule depolymerization) also undergo a migration similar to posteriorization, which is accompanied by cortical flow. Centrosomes are indicated by black dots, microtubules are thin black lines, the male pronucleus and metaphase chromosomes are gray circles and ovals, respectively, and the cortex is indicated by a gray outline. The direction of cortical flow is indicated by the black arrows.

the posterior cortex but not necessarily at the pole of the egg. Coincident with a period of cortical flow, the spindles migrate along the cortex to sit in the extreme posterior pole (Hird & White 1993) (Figure 8).

The Centrosome and Axis Specification

How is the cortical polarity axis of the embryo determined? A combination of experiments suggest that the sperm-supplied centrosome establishes both the contractile polarity and the PAR-polarity of the embryo: (*a*) The *C. elegans* sperm contributes a pronucleus, centrosome, and cytoplasm to the egg, but the sperm pronucleus is dispensable for polarity establishment (Sadler & Shakes 2000). (*b*) The embryo posterior, including the smooth domain and cortical PAR-2, correlates with the position of sperm entry (Goldstein & Hird 1996). Posterior polarity appears to spread symmetrically from the site of polarity initiation (Cuenca et al. 2003). (*c*) The centrosome-male pronucleus complex is found adjacent to the cortex during the initiation and expansion of the posterior domain (Cuenca et al. 2003). Prior to polarity establishment, the position of the centrosome within the egg is variable, sometimes up to 10 μm from the nearest point on the cortex. The centrosome is repositioned to lie near the cortex coincident with polarity onset (C.R. Cowan & A.A. Hyman, manuscript in preparation). (*d*) Mutants in *spd-2* and *spd-5*, which disrupt centrosome function, exhibit polarity defects (Hamill et al. 2002, O'Connell et al. 2000). (*e*) Laser-ablation of the centrosome prior to polarity establishment prevents the establishment of polarity (C.R. Cowan & A.A. Hyman, manuscript in preparation). Laser ablation has allowed a more thorough analysis of the type of signal that the centrosome provides: Ablation of the centrosome more than two minutes after the centrosome is on the cortex does not affect the subsequent expansion of the cortical domains (C.R. Cowan & A.A. Hyman, manuscript in preparation) (Figure 9). Therefore, the centrosome appears to use a "kiss and run" mechanism to initiate cortical polarity, presumably delivering a signal that changes the activity of the cortex and induces cortical polarization (see below).

It is still unclear whether the centrosome takes up a random position on the cortex or whether a predetermined site on the egg cortex, such as a membrane domain created by sperm entry, attracts the centrosome. Axis specification in several organisms, including mouse (Piotrowska & Zernicka-Goetz 2001) and *Xenopus* (Gerhart et al. 1981, 1984; Scharf & Gerhart 1980; Vincent et al. 1986), is determined by the position of sperm entry. In *C. elegans* there is a spatial correlation between the sperm entry site and the posterior domain (Goldstein & Hird 1996), but this proximity could arise either because the centrosome does not move far from the site of sperm entry or because the sperm entry site recruits the centrosome.

Centrosomes and Microtubules in Polarization

The most obvious possibility for the role of the centrosome in polarity is a requirement for microtubules. Consistent with this idea, microtubules are implicated in

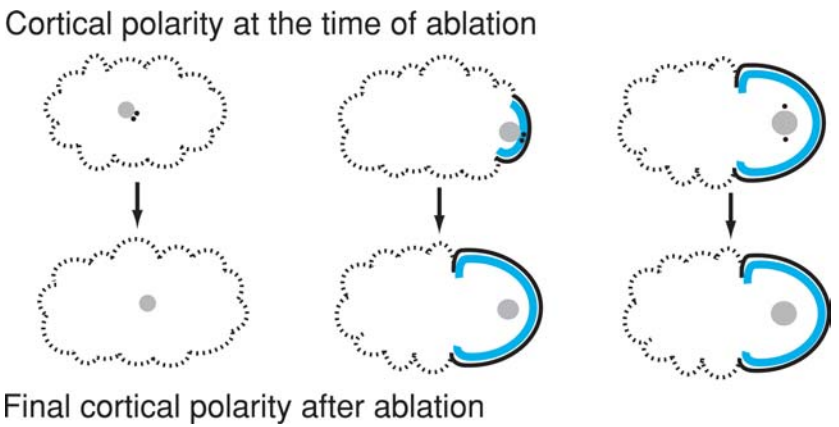

Figure 9 Time-resolved centrosome ablation and cortical polarity establishment. Ablation of the centrosome prior to cortical polarity initiation abolishes embryo polarity; later ablation does not affect polarization. The ruffling cortex is indicated by dotted lines, the smooth cortex is indicated by the solid black line, and PAR-2 localization is shown in blue. Centrosomes are indicated by black dots, and the male pronucleus is indicated by a gray circle. Anterior is to the left.

establishing a PAR-2 domain during meiosis (Wallenfang & Seydoux 2000). However, during the subsequent formation of the anterior-posterior axis, depletion or depolymerization of the detectable microtubules in the embryo does not affect cortical polarity establishment (Strome & Wood 1983; C.R. Cowan & A.A. Hyman, manuscript in preparation). Thus embryo polarization appears to be controlled directly by the centrosome rather than through microtubules.

Centrosomes appear to control the direction of cortical flow (Figure 10), presumably by specifying a region of high cortical tension, as discussed above. The centrosomes could modify tension either positively, by creating greater contractility, or negatively, by suppressing contractility, and these possibilities are discussed

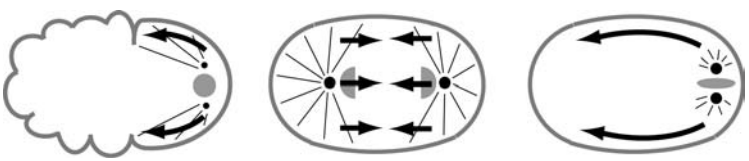

Figure 10 The direction of cortical flow is determined by the centrosomes. The cortical flow (*black arrows*) accompanying pseudocleavage, cytokinesis, and cortical metaphase spindle positioning is directed away from the centrosomes (*black dots*) and associated microtubules (*thin black lines*). The male pronucleus or chromosomes are shown as gray circles/ovals.

further in the following section. Cortical flows are directed away from the centrosomal asters during pseudocleavage, and the unidirectionality of the flows may reflect the asymmetric positioning of the centrosomes along the anterior-posterior axis (Hird & White 1993, White 1990, White & Borisy 1983). During cytokinesis, when the centrosomes are located on either side of the center of the egg, cortical flow is directed away from the anterior and posterior asters and converges in the middle (Hird & White 1993). However, if the metaphase spindle forms adjacent to the cortex, as occurs after nocodazole treatment, cortical flow is unidirectional, moving away from the asymmetrically placed centrosomes that form the poles of the spindle (Hird & White 1993). Cortical flow in *Xenopus* eggs also relies on an asymmetrically placed microtubule-organizing center for directionality (Benink et al. 2000).

Cortical flow, in contrast to polarity establishment, is influenced by microtubules. Microtubule depolymerization reduces the rate of cortical flow, and there is a corresponding decrease in the extent of ruffling and pseudocleavage (Hird & White 1993). As discussed above, the amount of contractility required to generate cortical polarity appears to be less than the contractility threshold required for pseudocleavage, and the contractility requirements for pseudocleavage and cytoplasmic flow appear to be similar (Hird & White 1993). Thus whereas microtubule-independent mechanisms (most likely acto-myosin contractility) can generate tension in the cortex for the establishment of contractile polarity, microtubules may be required to generate an increased cortical tension to drive cortical flow and pseudocleavage. The cortical microtubules, rather than the astral microtubules, may be responsible for increasing cortical contractility, given that ruffling is relatively unchanged in mutants that lack astral microtubules but have cortical microtubules [for example, *spd-2* and *spd-5* (Hamill et al. 2002, O'Connell et al. 2000)]. These mutants, however, do not establish polarity, and thus it is unclear whether cortical microtubule contractility alone is responsible for pseudocleavage and cortical flow. Alternatively, astral microtubules may contribute to generating the localized region of cortical tension involved in these later events, perhaps similar to the formation of the cytokinesis furrow in *C. elegans*, which requires nonuniform astral microtubule interactions with the cortex (Dechant & Glotzer 2003).

Cortical Relaxation and Polarity Establishment

How does the centrosome induce cortical polarity? A popular model proposes that the smoothing of the posterior cortex, pseudocleavage, and cortical flow are triggered by a sudden change in cortical tension at the posterior pole (Hird & White 1993, White 1990, White & Borisy 1983). Before polarization, the early embryo cortex would be under tension generated by a uniform meshwork of acto-myosin contractile units. Polarization would be initiated by releasing the tension in a limited region of the posterior cortex. The contractile units of the posterior cortex and the subcortical cytoplasm would flow away from the initiating site until tension in the cortex had been relieved, thus creating a relaxed (smooth) domain

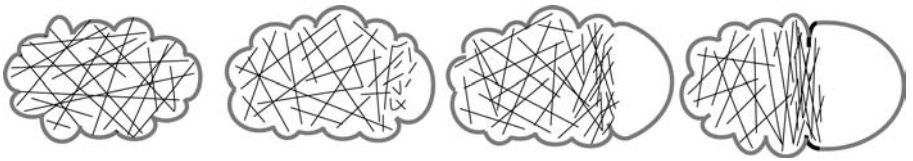

Figure 11 The cortical relaxation model. Uniform contractility, caused by a acto-myosin meshwork (*black lines*), is released in a local area causing the remaining meshwork to collapse. The contractile units accumulate and realign in the contractile-noncontractile boundary, creating an area of high tension capable of ingression.

in the process (Figure 11). An analogy is a mesh stocking stretched over a ball (Bray & White 1988): release of the tension would be equivalent to cutting several strings of the stocking in a localized area. The tension in the stocking would pull the remaining mesh away from the relaxed region until tension was relieved. The width of the contractile region should depend on the tenseness of the network before the relaxation event; high tension would result in a narrow margin, but low tension would not promote much contraction. An extension of this idea is that the size of the smooth domain would be determined by the tension in the cortex before the relaxation trigger.

By extending the cortical relaxation model to include a requirement for the centrosome, it seems that a possible role of the centrosome is to down-regulate the contractility of the acto-myosin meshwork at the posterior of the embryo by either inhibiting myosin activity or severing actin filaments. This would release the tension in the acto-myosin network, which would then contract toward the anterior. The size of the smooth domain would be determined by the contractility of the acto-myosin network itself.

The contractility of an acto-myosin meshwork is thought to be controlled by the level of myosin activity. High myosin activity results in more contractility, and greater contractility creates an increase in cortical tension (Pasternak et al. 1989). The regulation of myosin activity is therefore an important parameter in modifying cortical tension. In general, the phosphorylation state of the regulatory light chain of myosin is responsible for the regulation of myosin activity (Adelstein & Conti 1975, Craig et al. 1983). Myosin is active when the light chain is phosphorylated and inactive when dephosphorylated (Umemoto et al. 1989). Phosphorylation of myosin light chain is regulated positively by Rho-associated kinase (ROCK) (Amano et al. 1996, Kureishi et al. 1997) or myosin light chain kinase (Frearson & Perry 1975, Pires & Perry 1977) and negatively by myosin light chain phosphatase (Morgan et al. 1976). ROCK leads to myosin activity both directly and indirectly; it phosphorylates myosin light chain, leading to myosin activation, and it phosphorylates myosin light chain phosphatase, inhibiting phosphatase activity and thus preventing myosin deactivation (Kawano et al. 1999, Kimura et al. 1996). The cortical relaxation model predicts that contractility drives cortical domain establishment, and consistent with this idea, *C. elegans* embryos depleted of

either myosin (NMY-2) or its regulatory light chain (MLC-4) fail to establish polarity. *nmy-2* and *mlc-4* mutant embryos establish a small patch of cortical PAR-2 over the centrosomes, but the PAR-2 domain fails to expand (Cuenca et al. 2003, Guo & Kemphues 1996, Shelton et al. 1999). In *C. elegans*, regulators of myosin activity, ROCK (LET-502) and myosin light chain phosphatase (MEL-11), are required for cytokinesis in the early embryo (Piekny & Mains 2002), suggesting they may also be important for contractility. Similarly, both *C. elegans* RhoA (RHO-1) (Jantsch-Plunger et al. 2000) and the Rho activator CYK-4 (Gonczy et al. 2000; Jantsch-Plunger et al. 2000; Piano et al. 2000, 2002) affect cortical contractility. It is not known, however, whether these modulators of contractility affect cortical polarity in the embryo.

A Scaffold Model for Cortical Domain Formation

An alternative model to explain formation of the smooth posterior cortex is the assembly of a cortical scaffold under the posterior cortex (Figure 12). The scaffold could consist of a noncontractile actin structure or a spectrin-like polymer network that would impose rigidity on the cortex and thereby suppress ruffling. Assembly of the scaffold would be initiated by a signal from the centrosome but would be capable of self-organization. For instance, neutrophils and keratocytes appear to self-organize contractile and noncontractile cortices following a stochastic symmetry-breaking event (Verkhovsky et al. 1999, Xu et al. 2003). This self-organization, mediated by Rho and Cdc42, depends on positive feedback loops within the contractile and noncontractile cortices in conjunction with mutual exclusion of the two domains (Xu et al. 2003). In the embryo, the size of the smooth domain could be regulated in several ways: (*a*) by limiting the amount of scaffold structure available; (*b*) by an opposing activity from the anterior cortex; or (*c*) by noncortical spatial information, for example, from microtubules. The formation of a pseudocleavage furrow could be explained by a number of possible mechanisms. For instance, the apposition of the contractile cortex and the noncontractile scaffold could create a tension gradient, causing the accumulation of more contractile units and furrow ingression (Oegema & Mitchison 1997), as proposed by the cortical relaxation model (Hird & White 1993, White 1990, White & Borisy 1983). Alternatively, posterior scaffold assembly might push a contractile ring along the anterior-posterior axis. Finally, astral microtubules might pull the

Figure 12 A scafflod model of cortical polarization. A rigid scaffold (*black x*) is assembled, suppressing ruffling, and creating a noncontractile domain.

cortex into the cell, creating furrows, but the posterior scaffold could inhibit these pulling interactions. In all these scenarios, tension would be greatest in the pseudocleavage furrow, and such a local area of high contractility could still account for cortical flows. However, to date, there is no molecular evidence for such a scaffold, and distinguishing between different models for contractile polarity will require a further dissection of the molecular basis of cortical polarity, in general.

TRANSMITTING CORTICAL POLARITY TO THE SPINDLE

To ensure the correct segregation of cell fate determinants, the spindle must align along the axis of cell polarity. This process is often accompanied by an unequal division, in which one daughter differs in size from the other. In *C. elegans* the process of spindle positioning starts after pronuclear meeting as the pronuclei migrate toward the center of the embryo (Albertson 1984, Hyman & White 1987). During this process, the centrosome-pronuclear complex executes a 90° rotation to align the centrosomes along the anterior-posterior axis in the center of the cell (Albertson 1984, Hyman & White 1987). Around anaphase, the spindle moves toward the posterior of the embryo (Albertson 1984), generating an asymmetrically positioned spindle, which defines an unequal cleavage.

Spindle positioning in *C. elegans* can be defined as two processes: (*a*) alignment of the spindle along the anterior-posterior axis and (*b*) asymmetric displacement of the spindle toward the posterior. Both processes are linked to cortical polarity. In *par-2* mutants, the spindle sets up on the short axis of the embryo (Cheng et al. 1995), 90° to the anterior-posterior axis (see below). In all Par mutants, asymmetric anaphase movement of the spindle fails (Cheng et al. 1995, Kemphues et al. 1988), therefore, the cortical polarity determined by the PAR proteins must communicate in some way with the mitotic spindle to position it. The astral microtubules extending from the centrosomes to the cortex are required to transmit cortical information to the spindle. Mutations that result in short astral microtubules fail dramatically in spindle positioning (Albertson 1984, Bellanger & Gonczy 2003, Hyman & White 1987, Le Bot et al. 2003, Matthews et al. 1998, Srayko et al. 2003). The most likely mechanistic basis for both rotation and spindle displacement is the interaction of astral microtubules with pulling forces generated at the cortex, acting through microtubule-based motors, coupling the dynamics of microtubules to movement, or through a combination of the two (Hyman & Karsenti 1996, Pearson & Bloom 2004). In order for the cortex to pull a microtubule, a force generator physically attached to the cortex must move toward the minus-end of the microtubules. The force generator must be processive or of high affinity, or numerous pulling units must act in a concerted manner in order to generate force before the microtubule depolymerizes away from the cortex. Additionally, cortical integrity must be sufficiently high to result in the displacement of the microtubule aster toward the cortex rather than movement of the cortex down the microtubule.

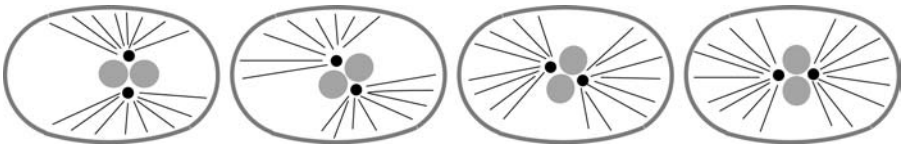

Figure 13 Centrosome rotation. The centrosomes (*black dots*) and pronuclei (*gray circles*) rotate 90° so that the centrosomal axis is aligned with the anterior-posterior axis of the embryo. Astral microtubules (*black lines*) contact the cortex, driving rotation.

Centrosome Rotation

The process of rotation (Hyman & White 1987) aligns the centrosomes with the anterior-posterior axis of the embryo (Figure 13). As the cell enters prometaphase and the nuclear envelopes break down, the spindle will form between the two centrosomes, where they incorporate into the spindle poles. Thus the position of the centrosomes defines the axis of the spindle. Work from various systems has generated several theories to explain how spindle orientation is determined with respect to a cellular axis.

GEOMETRY Spindles exhibit a tendency to align along the long axis of the cell, which suggests that cell shape can control spindle orientation. Given that the geometric and polarity axes of the *C. elegans* embryo are aligned, spindle orientation could be accomplished by cell shape. Shape-dependent spindle orientation requires that several requirements be met: force generators at the cortex must be evenly distributed and exert equal force, the number of force generators must be limiting relative to the number of microtubules, and the force exerted on the asters is dependent on microtubule length. Despite evidence for geometry-determined spindle alignment in rat epithelial cells (O'Connell & Wang 2000) and *Xenopus* eggs (Bjerknes 1986, Denegre et al. 1998), the one-cell *C. elegans* embryo does not use its geometry to align the spindle. Instead, the perception of geometry may be prevented (Tsou et al. 2003b). Rounded one-cell embryos, which lack a long axis, align the spindle according to the axis of cortical polarity (Hyman & White 1987, Tsou et al. 2002). *par-2* embryos, which have normal geometry, are largely unable to align the spindle with the long axis of the cell (Cheng et al. 1995).

THE CENTRIOLIC PRINCIPLE The "centriolic principle" (Costello 1961) proposes that spindle orientation is a direct consequence of centrosome separation around the nuclear envelope, assuming the duplicated centrosomes migrate symmetrically with respect to their common starting point. If this rule governs spindle orientation, each spindle is rotated 90° (in any direction) relative to the previous spindle axis, as seen in spiral cleavage patterns. In the one-cell *C. elegans* embryo, centrosome separation around the male pronucleus usually results in placement

of the centrosomes perpendicular to the polarity axis of the cell (Hyman & White 1987). Thus the centriolic principle does not account for spindle alignment in the one-cell embryo although it may apply in later divisions.

LOCALIZED PULLING Spindle alignment can occur through directed rotation toward a predetermined region of the cell cortex. Cell polarity cues could establish a specialized cortical region that either attracts or repels microtubules emanating from the spindle poles. Re-alignment of the spindle by such a site requires that there is an imbalance in the forces acting on the two poles of the spindle. The force imbalance results in one pole being selectively pulled toward (or away from) the cortical region. Two different models of cortical inhomogeneity have been proposed in *C. elegans* to explain alignment of the spindle with the anterior-posterior axis: (*a*) Cortical pulling sites: These are localized regions of the cortex that actively pull in centrosomes, analagous to reeling in a fish with a fishing rod; (*b*) down-regulation of pulling forces: Here the pulling forces in regions of the cortex are suppressed. Both models rely on a base-line pulling activity at the cortex. The difference between these two ideas is that in the first case, a cluster of force generators is imposed on top of the generalized pulling force. In the second case, the generalized pulling force is down-regulated in different areas.

Cortical Pulling Sites

Cortical pulling sites are discrete locations where pulling forces on the microtubules are strongest. A cortical site could be enriched with minus-end-directed microtubule motors or plus-end-binding proteins, providing a concerted pulling force. In order to position a spindle, a cortical site must ensure that it interacts with only one spindle pole. The site may be located slightly asymmetric relative to the anterior-posterior axis (up or down on the axis) to spatially favor interaction with one spindle pole over the other. Alternatively, the cortical site could be sufficiently small to limit the number of effective contacts, necessarily creating an imbalance in the number of pulling units exerting force on either of the two spindle poles (Hyman 1989). Ultimately, only one centrosome would undergo active rotation, whereas the other centrosome would be held in place by counter-forces (Figure 14).

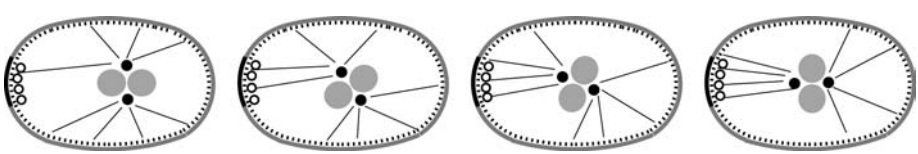

Figure 14 Cortical pulling site. A small region of the cortex (*black cortical patch*) is enriched with pulling units (*white dots*), although the entire cortex is capable of exerting pulling forces (*dotted line*). One centrosome will rotate toward the cortical site; the other centrosome is held in place, acting as a pivot.

A cortical site has been proposed to drive spindle alignment in *C. elegans* at the two-cell stage (Hyman 1989, Keating & White 1998). During rotation, the anterior centrosome migrates in an arc, whereas the posterior centrosome remains in a fixed position, acting like a pivot (Hyman & White 1987), a behavior consistent with a cortical site mechanism. Ablation of the area between one centrosome and the cortical site, presumably severing microtubules that connect the centrosome and the cortical site, led to rotation of the opposite centrosome toward the site (Hyman 1989). Had pushing forces been acting from the opposite cortex, ablation would have had no effect. A similar asymmetry of rotation force appears to operate in the first cleavage embryo (Figure 15). Following pronuclear meeting, the centrosomes and associated pronuclei undergo a simultaneous migration to the center of the egg and rotation to align the centrosomes with the long axis of the embryo (Albertson 1984, Hyman & White 1987). There are no convincing data about the molecular nature of the cortical site, but the minus-end-directed microtubule motor dynein is enriched in the proposed cortical site in both one-cell embryos and the posterior (P1) cell at the two-cell stage (Skop & White 1998, Waddle et al. 1994), suggesting a molecular means of generating the observed pulling force. Cortical sites are the most prevalent mechanism for spindle pole attachment to the cortex in other organisms. In budding yeast, the spindle aligns along the mother-bud axis through pulling interactions at the bud cortex (Lee et al. 2000, Yeh et al. 2000); microtubules are actively guided to pulling sites located at the bud tip. Embryos from annelids [*Tubifex* (Shimizu et al. 1998), *Chaetopterus* (Lutz et al. 1988)], brown algae

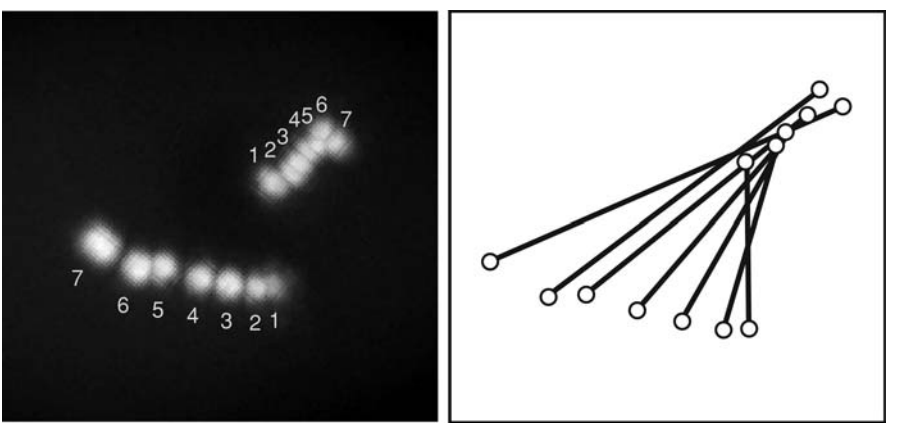

Figure 15 Pivot point behavior is observed during rotation in the one-cell embryo. Time-lapse images of GFP::γ-tubulin-labeled centrosomes undergoing rotation. The individual images are superimposed and the temporal sequence is indicated by the numbers (each corresponding to a 15-s interval). The upper centrosome remains in a relatively fixed position, whereas the lower centrosome migrates in a broad arc toward the anterior pole of the embryo. The corresponding diagram indicates the paired centrosomes (*white dots*) as they rotate.

[*Pelvetia* (Bisgrove & Kropf 2001)], marine shrimp [*Sicyonia* (Wang et al. 1997)], and ascidians [*Halocynthia* (Hibino et al. 1998)] as well as *Drosophila* germline stem cells (Yamashita et al. 2003) all use a cortical site for spindle alignment.

Localized Down-Regulation of Pulling Force

The recent identification of LET-99, a cortical protein required for spindle alignment along the anterior-posterior axis, suggests a novel mechanism of spindle orientation. LET-99 localizes to a wide ring around the embryo middle, slightly displaced toward the posterior pole (Tsou et al. 2002). The position of the LET-99 band is determined by the PAR proteins (Tsou et al. 2002). If LET-99 down-regulated the pulling forces (Tsou et al. 2002, 2003a), this would ensure that pulling forces would be favored either toward the anterior half or toward the posterior pole. The LET-99 band model (Tsou et al. 2002) relies on a stochastic shift of one centrosome toward one pole and the corresponding shift of the opposite centrosome toward the opposite pole. This imbalance would be propagated until alignment along the anterior-posterior axis created a stable orientation when microtubules from both centrosomes would be subject to maximum pull from their respective poles (Figure 16). An important distinction from the cortical site model (above) is that both spindle poles would be pulled and thus both poles would undergo rotation (Tsou et al. 2002), in contrast to the observed pivoting of the centrosome-pronuclear complex. More work on the molecular function of LET-99 will be required to further investigate these ideas.

Anaphase Spindle Displacement

By metaphase, the spindle is aligned on the anterior-posterior axis and centered in the cell. As the cell enters anaphase, the spindle moves toward the embryo posterior (Figure 17). First, the posterior spindle pole begins to move toward the embryo posterior; second, the posterior spindle pole starts to oscillate; third, the posterior spindle pole flattens (Albertson 1984, Keating & White 1998) (Figure 18).

Microtubule-based force generation during anaphase in the *C. elegans* embryo involves cortically anchored pulling forces acting on astral microtubules. Direct evidence for these pulling forces came from experiments in which the midzone of the early anaphase spindle was physically destroyed with a laser beam (Grill

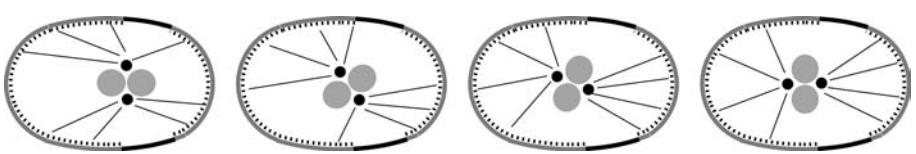

Figure 16 Down-regulation of forces. The LET-99 band (*black cortical patches*) suppresses pulling forces on the cortex. A basal pulling force (*dotted line*) is active outside of the LET-99 region, generating torque on both centrosomes and thus driving rotation.

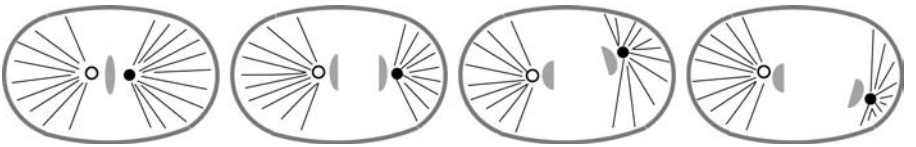

Figure 17 Anaphase spindle displacement. The posterior spindle pole (*black dot*) moves toward the embryo posterior, displacing the spindle middle and thus leading to an asymmetric division. Oscillations accompany some of this posterior movement. The anterior pole (*white dot*), in contrast, remains relatively fixed. Pulling forces at the cortex act through astral microtubules (*black lines*) from both spindle poles. Anterior is to the left.

et al. 2001). This experiment demonstrated that there is an asymmetry in the forces exerted on the anterior and posterior spindle poles; the greater displacement of the posterior spindle pole is due to a greater net force pulling on the posterior aster (Grill et al. 2001). This force difference is under the control of cortical polarity: Mutants in the PAR proteins equalize the forces acting on the poles (Grill et al. 2001). Recent experiments suggest that each pulling unit on the cortex works at its maximum capacity in both the anterior and posterior of the cell and that the force each pulling unit exerts is equal (Grill et al. 2003). The stronger force on the posterior centrosome must therefore come at least in part from an increase in the number of force generators on the posterior cortex (Grill et al. 2003).

Microtubule dynamics may contribute to the posterior displacement of the spindle. The time that an individual microtubule remains near the cortex is lower in the posterior than the anterior of the embryo (Labbe et al. 2003), suggesting that microtubules are more dynamic at the posterior cortex. This difference is controlled by PAR protein polarity (Labbe et al. 2003). Additionally, if astral microtubules are induced to shorten during metaphase/anaphase, either by microtubule

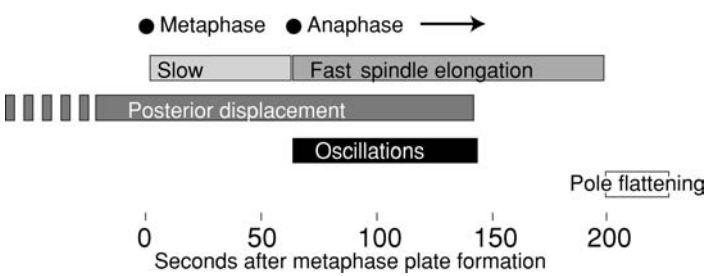

Figure 18 Temporal sequence of spindle displacement (Oegema et al. 2001; C.R. Cowan & A.A. Hyman, unpublished observations). The time frames over which spindle elongation (anaphase B), posterior movement of the spindle, and posterior spindle pole oscillations and flattening are shown relative to the onset of metaphase and anaphase. Posterior displacement occurs throughout metaphase and anaphase, although oscillations of large magnitudes are restricted to anaphase. The initiation of posterior displacement relative to metaphase is variable, indicated by the broken bar.

depolymerizing drugs or the *zyg-8* mutation, the spindle moves from the cell center to the posterior pole (Gonczy et al. 2001, Hyman & White 1987), which suggests that the posterior cortex can use the shrinking microtubules to generate pull, similar to the kinetochore-generated pulling forces exerted on spindle microtubules following nocodazole treatment (Cassimeris et al. 1990, Cassimeris & Salmon 1991). Therefore, the preferential posterior displacement of the spindle probably results from a combination of a greater number of force generators at the posterior cortex (Grill et al. 2003) together with a greater frequency of shrinking microtubules in the posterior (Labbe et al. 2003).

The force generator(s) at the cortex has not been unambiguously identified, but pulling forces are under the control of a receptor-independent G protein pathway. Mutants that reduce the activity of Gα-dependent signaling reduce the forces acting on the spindle (Gotta & Ahringer 2001b, Grill et al. 2003), and at least one of the regulators of G protein signaling, GPR-1/2, is asymmetrically localized when force on the posterior spindle pole is at a maximum (Colombo et al. 2003, Gotta et al. 2003). Therefore, in some way still to be determined, the cortical polarity of the embryo acts through the PAR proteins (Colombo et al. 2003, Gotta et al. 2003, Srinivasan et al. 2003) to up-regulate G protein signaling in the posterior, increasing the total force (Colombo et al. 2003) and thereby generating asymmetric spindle position.

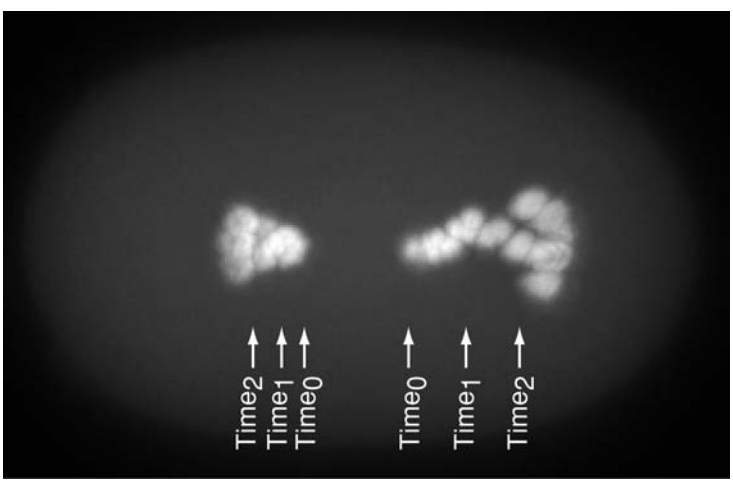

Figure 19 Displacement and oscillations of the posterior spindle pole. Time-lapse images of GFP::γ-tubulin-labelled spindle poles during anaphase. The individual images (each representing a 15-s interval) are superimposed. The anterior and posterior spindle pole positions at three equivalent time points are indicated, showing the greater movement of the posterior spindle pole relative to the anterior in a given time frame. Oscillations are visible as centrosome movement up and down after $time_2$. Anterior is to the left.

One of the interesting aspects of asymmetric spindle movement is that the spindle oscillates as it moves. This is observed not only in the asymmetric divisions of the *C. elegans* embryo but also in asymmetrically dividing surf clam zygotes (Dan & Inoué 1987) and *Drosophila* neuroblasts (Kaltschmidt et al. 2000, Savoian & Rieder 2002). Although earlier models suggested that oscillation itself could drive asymmetric spindle movement (Grill et al. 2001), much of the posterior displacement occurs before oscillations start (Figure 18, Figure 19). It seems more likely that oscillation occurs at a threshold of force generation. At low force, the spindle can displace, although not to its full extent. As the force increases, the spindle displaces further until a threshold of force generation causes cooperative detachment of microtubules from the cortex, which results in oscillations.

The idea that a threshold in pulling force would result in oscillations is similar to the idea that thresholds in contractile activity drive ruffling and suggests that *C. elegans* embryos use robust mechanisms to establish and transduce asymmetry. More contractile activity and more force than necessary are provided. Therefore, these activities go over a threshold level, producing phenomena (ruffling and spindle oscillations) that are not necessary for the processes (contractile polarity and spindle displacement) but are important for understanding the underlying mechanisms that drive them.

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