Determination of the Cleavage Plane in Early *C. elegans* Embryos

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Abstract

Cells split in two at the final step of each division cycle. This division normally bisects through the middle of the cell and generates two equal daughters. However, developmental signals can change the plane of cell cleavage to facilitate asymmetric segregation of fate determinants and control the position and relative sizes of daughter cells. The anaphase spindle instructs the site of cell cleavage in animal cells, hence its position is critical in the regulation of symmetric vs asymmetric cell division. Studies in a variety of models identified evolutionarily conserved mechanisms that control spindle positioning. However, how the spindle determines the cleavage site is poorly understood. Recent results in *Caenorhabditis elegans* indicate dual functions for a Gα pathway in positioning the spindle and cleavage furrow. We review asymmetric division of the *C. elegans* zygote, with a focus on microtubule-cortex interactions that position the spindle and cleavage plane.

INTRODUCTION TO THE TOPIC AND MODEL

Asymmetric divisions: divisions that give rise to different daughters. Here, we only consider intrinsically asymmetric divisions, in which different fates are determined by the division process

Cell division is one of the most fascinating and fundamental processes in the life of an organism. The actual division process takes place in two coordinated steps during the final phase of the cell cycle, named M phase (**Figure 1*a***). During nuclear division or mitosis, the duplicated genetic material is separated into two identical sets. Next, the cytoplasm is cleaved during cytokinesis, thereby forming two separate daughter cells. With only rare exceptions, cells cleave in coordination with nuclear division and in between the segregated chromosomes. This coupling between mitosis and cytokinesis supports the maintenance of an intact genome through many rounds of cell division and helps the equal distribution of cellular components to the progeny cells. However, not all cell divisions are meant to form identical daughters: Certain asymmetric divisions segregate cellular components such as fate determinants specifically to one of the daughter cells, which often also differs in size from the other daughter. Such "intrinsically asymmetric divisions" promote cell diversity and are one of the mechanisms by which stem and progenitor cells combine self-renewal with production of differentiating daughters (10, 33). The position of the cleavage plane forms a critical aspect of such asymmetric divisions (**Figure 1*b*,*c***).

The protein apparatus that segregates the chromosomes, the mitotic spindle, also determines the plane of cell cleavage. Because the cleavage plane bisects the spindle, chromosomes end up in different daughter cells. In some cells, such as plant and fungal cells, the division plane is established prior to mitosis and the mitotic spindle is oriented with respect to this predetermined site (reviewed in Reference 7). In contrast, in animal cells the position of the spindle determines where the cell will cleave. Hence, the position or symmetry of the mitotic spindle is regulated in order

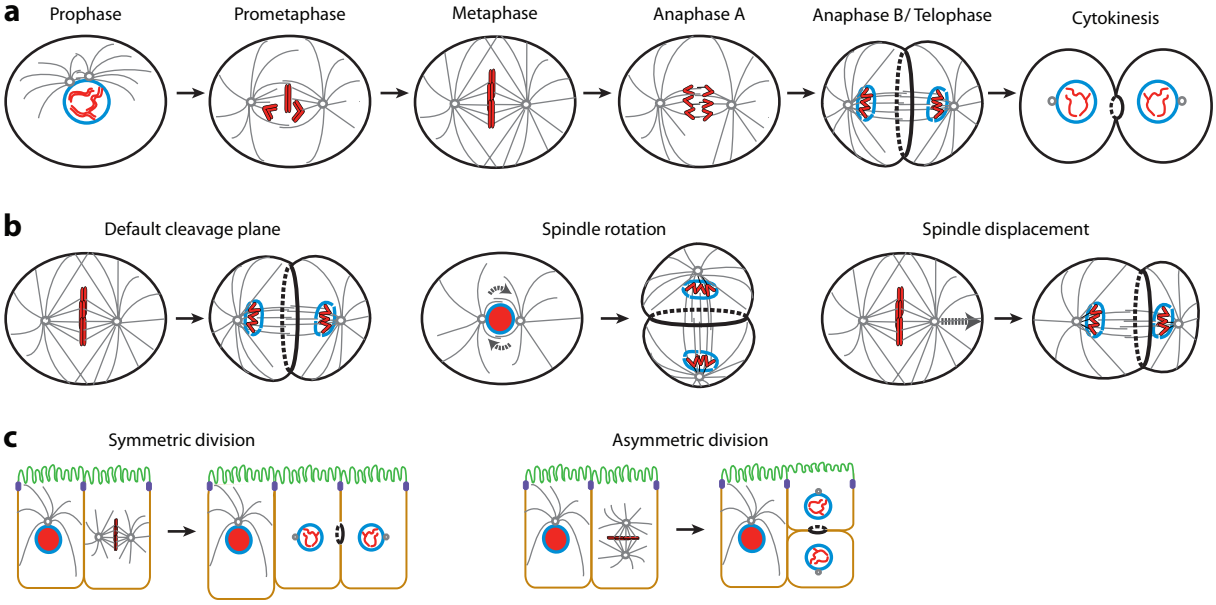

Figure 1

(*a*) Schematic representation of the sequential stages of mitosis. Chromosomal DNA (*red*), microtubules (*gray lines*), centrosomes (*gray circles*), and the nuclear envelopes (*blue*). (*b*) Illustration of how changes in the position of the spindle alter the plane of cell cleavage and thereby the size and placement of daughter cells. (*c*) Polarized cells can switch from symmetric to asymmetric divisions by altering their cleavage plane. The apical domain (*green*), basal domain (*tan*), and cell junctions (*blue*) are shown.

to control the cell cleavage plane (**Figure 1*b***). Rotation of the spindle changes the position of daughter cells and distribution of determinants, whereas displacing the spindle off-center leads to daughter cells of unequal sizes. As the position of the spindle dictates the plane of cleavage, it is of fundamental importance to understand what molecular mechanisms control the spindle position, and how the spindle controls the place of cell cleavage.

Since the classical experiments by Rappaport with sand dollar eggs (85), much has been learned about the interconnection between spindle positioning and cell cleavage determination. Genetic studies in the fruit fly *Drosophila melanogaster* and nematode *Caenorhabditis elegans* revealed several molecular pathways that control the cleavage plane during animal development (reviewed in References 10, 33). As an example, during stem cell divisions in the *Drosophila* male germline, the mother centrosome maintains a fixed position so that the spindle forms away from the stem cell niche (116, 117). Consequently, the subsequent division produces another stem cell in contact with the niche and one daughter that initiates a differentiation program. Stem cell-like divisions of the larval neuroblasts in *Drosophila* use a fixed centrosome as well, and this centrosome acts also as the major microtubule-nucleation center (86). Hence, this spindle is asymmetric itself, and places the cleavage plane off center to promote production of another large neuroblast as well as a smaller neural precursor.

In *C. elegans*, the positions of the spindle and cell cleavage plane are tightly regulated and guide a reproducible pattern of asymmetric and symmetric divisions throughout development. Decades of extensive studies have revealed evolutionarily conserved mechanisms that control the position of the spindle and cleavage plane. Several recent results start to shed light on the possible connection between the spindle apparatus and placement of the acto-myosin contractile ring in cytokinesis. In this review, we summarize the current understanding of asymmetric division of the one-cell *C. elegans* embryo, focusing on the connections between the acto-myosin network at the cortex and microtubules of the spindle in determining the cell cleavage plane.

Cell Cleavage in *C. elegans*

In a relatively short time, *C. elegans* has arguably become the leading model in studies of cleavage plane determination. Reasons for this status include the reproducible cell lineage, which incorporates asymmetric cell division and cytokinesis from the very first division onward. Thus, cleavage can be studied when the cells are still large (length/width of the zygote ~50/30 μm) and spindles and chromosomes easily observable. Equally important is the genetic tractability. Embryonic functions of genes with general roles in cytokinesis have been discovered though relatively rare mutations (including temperature-sensitive and maternal-effect lethal mutants) (56, 72, 78) as well as large-scale and genome-wide RNA interference (RNAi) screens (35, 81, 99; **http://nematoda.bio.nyu.edu**). Several more specialized techniques can be added to such experiments, including the introduction of drugs (e.g., 46, 104), culturing of blastomeres that have been removed from eggs (e.g., 30, 50), and severing of the spindle with a UV laser, as introduced by Hyman (40, 49). Green fluorescent protein (GFP)-tagged proteins can be expressed in the germline and early embryos through the introduction of low-copy transgenes (83), and followed by time-lapse fluorescence microscopy. The combination of these approaches allows for detailed analysis of the molecular basis of cleavage-plane regulation in *C. elegans*.

The framework for all cell division studies is the *C. elegans* cell lineage (105). This lineage starts with fertilization, followed by two consecutive highly asymmetric meiotic divisions. These divisions produce two small polar bodies, as the compact meiotic spindle segregates the chromosomes in close proximity of the cortex (**Figures 2**, **3** top). Subsequently, the maternal and paternal pronuclei meet in the posterior and migrate toward the center

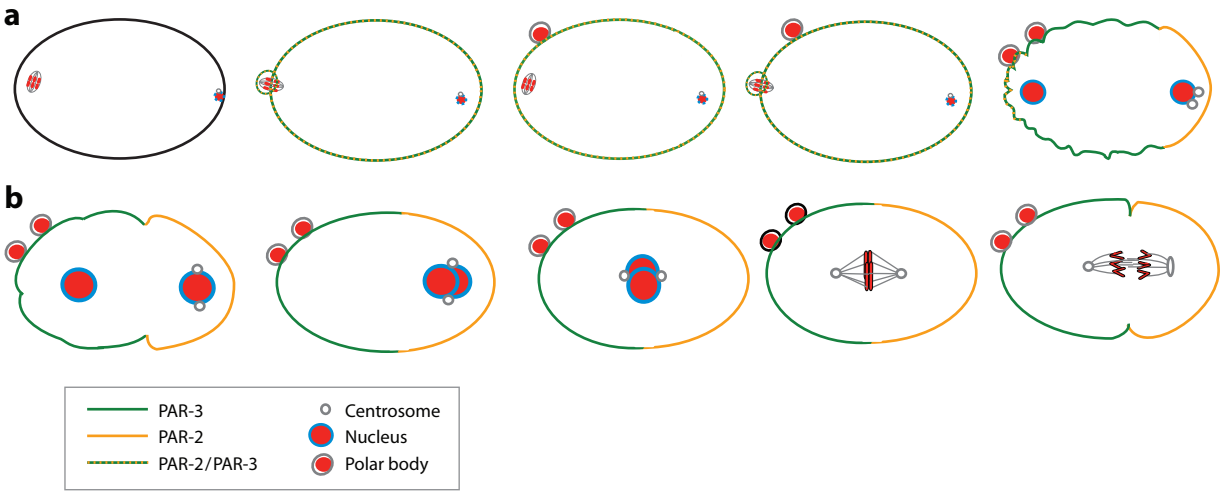

Figure 2

The establishment of polarity in the early *C. elegans* embryo. For details, see text.

(centration), while they rotate and the mitotic spindle assembles along the anterior-posterior axis of the zygote (P_0). Following degradation of the nuclear envelopes, the spindle aligns the chromosomes at the metaphase plate in the middle of the zygote. The spindle is slightly displaced toward the posterior in metaphase and anaphase. During this relocation, the posterior pole shows extensive lateral oscillations, called "rocking," while the anterior pole remains relatively fixed. The off-center placement of the spindle results in an unequal first division that gives rise to a larger anterior blastomere (AB) and smaller posterior daughter (P_1) (**Figure 3**). During prophase of the next division, the spindle in the P_1 blastomere rotates 90°, resulting in a subsequent transverse division. P_1 and subsequent precursors of the germline (P_2–P_4) continue cell-autonomously controlled asymmetric divisions. In contrast, asymmetric division of EMS, the precursor of endoderm (intestine) and mesoderm, requires signaling from its neighbor P_2 at the four-cell stage (**Figure 3**) (31). The AB daughters ABa and ABp divide left right under a slight angle, which creates reproducible left-right asymmetry (9). Asymmetric and symmetric divisions continue throughout embryonic and larval development.

Supplemental Material

Cleavage furrow/contactile ring: contains actin and myosin filaments and other proteins. Forms during anaphase and ingresses during cytokinesis

In summary, the position of the cleavage plane is highly regulated in *C. elegans* and critical in establishing the body axes in the early embryo as well as in generating the proper cell lineages throughout development.

Below, we review some of the events that lead to asymmetric mitotic division of the zygote. This involves establishment of anterior-posterior polarity, which guides the positioning of the mitotic spindle. Subsequently, the position of this spindle dictates the plane of cell cleavage. We focus on the recently discovered overlap in regulators of spindle positioning and molecules that determine cleavage furrow positioning [for a summary of these regulators, see **Supplemental Table 1** and references therein. Follow the **Supplemental Material link** from the Annual Reviews home page at **http://www.annualreviews.org**]. We propose a model that may explain the data currently available.

POSITIONING THE MITOTIC SPINDLE

Studies of the *C. elegans* zygote helped define a paradigm for asymmetric cell division. This involves several coordinated steps: establishment

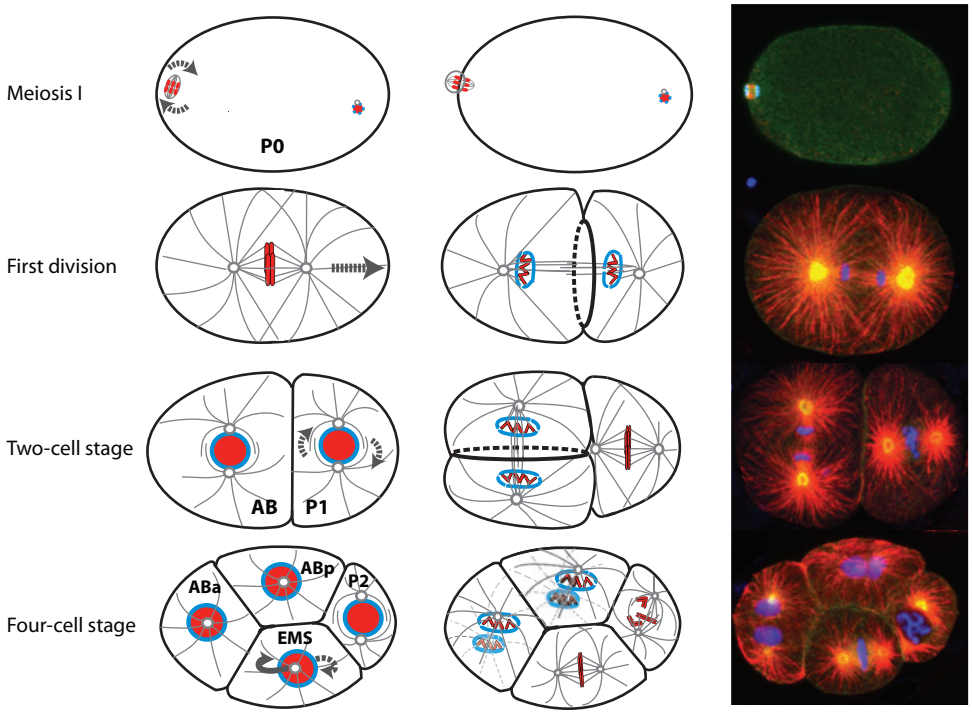

Figure 3

Spindle positioning events in the early *C. elegans* embryo. The first spindle positioning events occur during female meiosis I, when the meiotic spindle translocates to the cortex and then rotates, so that a small polar body can be pinched off. During the first mitotic division, the spindle is displaced toward the posterior, and cleavage results in a larger AB and a smaller P_1 blastomere. At the two-cell stage, the P_1 cell rotates its centrosomes 90°, so that P_1 divides anterior-posteriorly, creating P_2 and EMS. At the four-cell stage, signaling from the P_2 blastomere induces rotation of the nucleus-spindle complex in EMS, so that EMS divides along the anterior-posterior axis.

of cell polarity, asymmetric localization of determinants, and determination of the proper cell cleavage plane (reviewed in References 10, 33). The establishment of cell polarity precedes and directs the localization of determinants and the spindle. It involves reorganization of the cell cortex in association with localization of anterior- and posterior-specific protein complexes. Experiments with microfilament and microtubule inhibitors provided the first insights in the critical roles for the actin cytoskeleton during asymmetric division. Strome & Wood showed that migration of the maternal and paternal pronuclei and chromosome segregation require microtubules (104). By contrast, cortical contractions that include pseudocleavage (see below), asymmetric localization of germline-specific P granules (particles of RNA and associated proteins), and the asymmetric position of the cleavage plane all depend on the actin cytoskeleton. Importantly, a brief pulse of the actin filament drug cytochalasin D during the period of cortical contractions results in a symmetrical cleavage plane (46). In a landmark study, Kemphues isolated a series of *par* (partitioning defective) maternal-effect lethal mutants in which the cleavage plane was similarly displaced (56). Characterization of these *par* genes has revealed a conserved protein complex with critical functions in the polarization of the cytoskeleton in animal cells. Establishment of cell polarity has been discussed in several excellent reviews (e.g., 22, 90). Here, we provide a short summary to emphasize how the

Cell cortex: layer underneath the plasma membrane that contains actin, myosin, and other proteins

establishment of cell polarity relates to spindle positioning and how it is driven by several of the same regulators of acto-myosin contraction as cytokinesis.

Polarizing the Fertilized Egg

The *C. elegans* oocyte is not polarized; the anterior-posterior (AP) axis is formed after fertilization, with the place of sperm entry defining posterior (32) (**Figure 2**). However, the first signs of polarization do not appear until approximately 30 min. after fertilization. The oocyte nucleus completes meiosis I and II during this time period, and the cell cortex forms "ruffles", local contractions followed by relaxation (19). Upon completion of meiosis, the contractions of the cortex and localizations of PAR proteins become asymmetric. Starting close to the sperm centrosomes, contractions disappear from the posterior cortex and a smooth domain expands toward the anterior (**Figure 2**). The anterior cortex continues to ruffle and a deep ingression (pseudocleavage) forms at the boundary of these domains. An equilibrium is reached when the rough anterior and smooth posterior cortex each occupy about half of the surface of the zygote.

Coincident with the establishment of these cortical domains, anterior and posterior PAR protein complexes form two opposing domains (23). The conserved anterior PAR complex contains the PDZ-domain proteins PAR-3 and PAR-6 in association with atypical protein kinase C (PKC-3). PAR-6 also contains a CRIB-like domain that mediates functional and physical interaction with CDC-42 (1, 36, 54, 93). The anterior PAR proteins are evenly distributed over the cortex at the onset of the first mitotic cell cycle, but then retract with the contractile cortex toward the anterior (23). The cleared part of the cortex recruits the posterior PAR proteins PAR-2, a ring finger protein, and PAR-1, a Ser/Thr kinase of the MARK family (90). Importantly, PAR proteins of the anterior and posterior complex antagonize each other's localization, which results in the establishment of stable cortical domains. This interdependence is clearly visible when the localization of GFP-tagged PAR proteins is studied in various *par* mutants (23). The anterior PAR domain extends toward the posterior when *par-2* is inactive. Vice versa, mutations in the anterior *par* genes allow PAR-1 and PAR-2 to localize uniformly around the cortex and the embryo is "posteriorized."

Confirming earlier observations (47, 58, 103), live-imaging by Munro showed that cortical nonmuscle myosin (NMY-2) in conjunction with filamentous actin powers the cortical ruffling and localization of the anterior PAR complex (75). F-actin and NMY-2 form part of a meshwork with dense foci that covers the fertilized egg. Upon completion of meiosis II, the meshwork contracts toward the future anterior pole while moving PAR-3, PAR-6, and PKC-3 along. PAR-2 localizes to the posterior cortex and prevents reappearance of myosin and return of the anterior PAR complex to the posterior. Inactivation of anterior *par* genes severely reduced anterior movement of myosin foci, which demonstrates that the anterior complex promotes its own transport. How the anterior PAR complex associates with the acto-myosin meshwork is poorly understood. One factor is the small GTPase CDC-42, which binds PAR-6 and contributes to its localization (1). In summary, acto-myosin contractions in conjunction with the anterior PAR proteins polarize the zygote, while the posterior PAR proteins stabilize the asymmetry.

The driving forces in acto-myosin reorganization during polarity establishment are closely related to those used in cytokinesis (see below). The key regulator is the Rho subfamily of small GTPases (52, 73, 93). Formation of NMY-2 contractile foci can be prevented by inactivation of *rho-1* RhoA or *ect-2/let-21*, which encodes a putative Rho guanine nucleotide exchange factor (GEF). Thus, RHO-1 and its activator ECT-2 GEF are needed to form a contractile NMY-2/actin meshwork. In contrast, disassembly of the meshwork requires the Rho GTPase activating protein (GAP) CYK-4 (52). *ect-2*, *rho-1*, and *cyk-4* are all required for anterior movement of the meshwork and PAR

localization, showing that the Rho GTPase cycle drives this process. RHO-1 promotes phosphorylation of the myosin light chain MLC-4 (52), likely through activation of a Rho-binding kinase (presumably LET-502). This in turn promotes acto-myosin contractility.

What breaks the symmetry of the acto-myosin meshwork and initiates its anterior contraction? Not fertilization per se (91), but the mature sperm-derived centrosome is a critical factor (20, 21, 44, 55, 75, 79). NMY-2 retraction from the posterior cortex and initiation of the posterior domain correspond in time and position to alignment of the duplicated centrosomes with the cortex. In addition, genes required for centrosome maturation, such as *spd-2*, *spd-5*, *cdk-2*, and *cye-1* Cyclin E, are essential for polarity establishment (21, 44, 79). Hence, the lag time between fertilization and cortical polarity may be determined by maturation of the centrosomes. It is still a subject of debate whether microtubules emanating from these centrosomes are the polarity determining factor (113, 117a), or if polarization can take place in the absence of microtubules (20, 98a).

Repositioning of the centrosomes near the cortex coincides with a local removal of ECT-2 RhoGEF (73). Moreover, the sperm contributes not only the centrosome but also a pool of CYK-4 GAP (52). Paternally provided CYK-4 in conjunction with centrosome maturation and ECT-2 removal likely reduce RHO-1 activity in the posterior. As a result, the acto-myosin contractile activity is reduced locally, which leads to retraction of the acto-myosin meshwork toward the anterior. Approximately 8 min after this process initiates, cortical domains with distinct PAR complexes are firmly established and the anterior-poster (AP) body axis is secured.

Generating the Pulling Forces that Position the Spindle

Once polarity is established, cell fate determinants need to become unequally segregated and the spindle positioned according to the AP axis. Spindle positioning in the one-cell embryo involves two distinct phases (reviewed in References 19, 22). During prophase, the paternal and maternal pronuclei move in close association to the center of the cell while undergoing a 90° rotation (**Figure 2**). This centration/rotation results in alignment of the centrosomes and spindle along the AP axis. The second phase occurs coincident with chromosome segregation during metaphase and anaphase, and positions the spindle toward the posterior of the cell. Below, we focus predominantly on the second process, as this is most directly linked to cleavage-plane determination. However, centration and posterior displacement are controlled by polarity and driven by a similar cast of players (e.g., 39, 80, 115). A major difference is that the microtubule-dependent pulling forces are higher in the anterior during centration/rotation, whereas the posterior forces are higher during metaphase-anaphase movements (63).

Ablation of spindle structures in combination with gene inactivation has provided important insights into the contribution of PAR proteins in cleavage-plane determination. Grill and coworkers used a UV laser beam to sever the central spindle during anaphase of the first mitosis (40). After ablation, both spindle poles with attached chromosomes moved faster and further toward the cortex than normal, indicating that the spindle midzone opposes rather than promotes spindle-pole movement and chromosome segregation. As in normal embryos, the posterior spindle pole traveled a greater distance and moved faster toward the cortex than the anterior spindle pole. Thus, the net forces that pull along astral microtubules are higher at the posterior compared to the anterior cortex, resulting in posterior displacement of the spindle (**Figure 4**). When these experiments were repeated in *par-2* mutants, both spindle poles traveled the same distance and with the same speed as the anterior pole in the wild type. In *par-3* mutants, the opposite was seen: Both spindle poles behaved as the wild-type posterior pole (40). Thus the "anteriorization" or "posteriorization" of the

Midzone: structure based on bundled antiparallel microtubules, formed in between separating chromosomes in anaphase

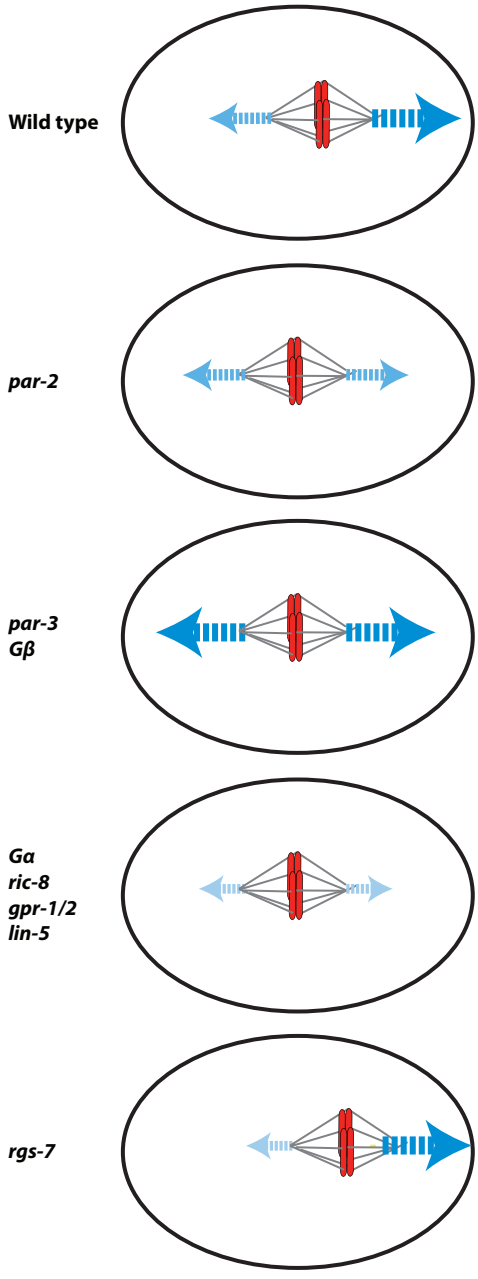

Figure 4

The distribution of spindle pulling forces in *C. elegans* mutants. High forces are depicted by a large dark blue arrow, whereas low forces are depicted by a small light blue arrow. Anterior is to the left, posterior to the right.

different *par* mutants is reflected in the cortical forces that act on astral microtubules.

An important question that arises from these findings is how PAR polarity controls the cortical forces that act on astral microtubules. A number of genes have been identified that are likely intermediates between polarity and the spindle. Mutation and/or RNAi of such genes cause defects in cleavage-plane positioning while polarity is established and chromosomes become segregated in the first division. One of the genes involved, *let-99*, was defined by a maternal-effect lethal mutation with cell cleavage-plane defects (89). Another one, *lin-5*, was found in a screen for cell lineage-abnormal mutants (5). Yet others were identified in studies of Gα signaling in neurotransmitter release (70) and in large-scale RNAi screens (35). The characterizations of these genes in parallel with studies in *Drosophila* revealed the control of spindle positioning by a noncanonical heterotrimeric G-protein pathway (10).

In the absence of *let-99* function the spindle shows several positioning abnormalities (89). Upon meeting of the maternal and paternal pronuclei, the nuclear-centrosome complex starts to swing hyperactively and fails to move to the center. In contrast, anaphase oscillations of the spindle are reduced or absent in *let-99* embryos, and pole separation is limited (108). Because of the initial posterior displacement of the spindle, part of the *let-99* embryos show exaggerated asymmetry of the first division. The LET-99 protein contains an N-terminal DEP (Disheveled, EGL-10, and Plekstrin) domain, which has been implicated in membrane localization and G-protein signaling (15). Starting at the onset of pronuclear migration, LET-99 is asymmetrically enriched in a cortical band in the central-posterior region of the embryo (108). This band pattern depends on anterior PAR-3/PAR-6/PKC-3 and posterior PAR-1 functions, which both oppose cortical LET-99 localization (108, 115). Removal of the eggshell creates a spherical P_0 cell in which the spindle aligns according to the intrinsic A/P polarity in wild-type, but not in *let-99*, embryos (50, 108). Hence, the LET-99 band is

required for the spindle rotation during centration. These and many other observations indicate that LET-99 likely acts downstream of the PAR proteins in spindle orientation.

lin-5 was originally identified because of its general role in postembryonic cell division and, more specifically, chromosome segregation (5). However, the first defects in early *lin-5*(*RNAi*) and *lin-5*(*ev571*ts) embryos are abnormal movements of the spindle and incorrect positioning of the meiotic and mitotic cleavage plane (64). Inactivation of specific Gα subunits causes similar defects. Miller & Rand observed that *goa-1* $G\alpha_o$ and *ric-8* (Resistance to Inhibitors of Cholinesterase) loss of function causes embryonic lethality and abnormal spindle positioning (70). These effects are partial, but Gotta & Ahringer observed redundancy between GOA-1 $G\alpha_o$ and another Gα subunit, GPA-16. RNAi of *goa-1* and *gpa-16* (together referred to as Gα) causes fully penetrant spindle positioning defects (37). Equivalent defects result from inactivation of two closely related genes, *gpr-1*/F22B7.13 and *gpr-2*/C38C10.4 (together:*gpr-1/2*), as first observed in a chromosome III-wide RNAi screen by Gonczy et al. (35). *gpr-1* and *gpr-2* encode GoLoco domain proteins that associate with Gα subunits and with the coiled-coil protein LIN-5 (102). This Gα/GPR/LIN-5 pathway is evolutionarily conserved and related to *Drosophila* Gα/Pins/Mud and human Gα/LGN-AGS3/NuMA (26).

The exact functions of *lin-5*, *gpr-1/2*, *goa-1/gpa-16*, and *ric-8* are only partly understood. Upon inactivation of these genes, the mitotic spindle in the zygote fails to migrate to the posterior during anaphase, pole separation is reduced, and the posterior pole does not flatten or oscillate. These defects all suggested reduced cortical pulling forces on astral microtubules, which was indeed observed after severing the spindle midzone or centrosomes with a UV laser (**Figure 4**) (2, 17, 76). Gα, GPR-1/2, and LIN-5 colocalize at the cell cortex, and Gα is needed for the cortical localization of the other proteins. LIN-5 is also present at meiotic and mitotic spindle poles and, specifically at metaphase, diffusely around microtubules between the poles (64). The localizations of GPR-1/2 coincide with and depend on LIN-5, while localization of LIN-5 to the cortex—but not spindle poles—requires GPR-1/2. Thus, Gα, GPR-1/2, and LIN-5 show dependence and colocalization specifically at the cortex, indicating that this location reflects their contribution in the pulling forces that act at the spindle poles. In support of this view, loss of LIN-5 from the spindle poles does not affect these pulling forces (M. van der Voet & S. van den Heuvel, submitted).

A variety of functional and physical interactions connect Gα, GPR-1/2, LIN-5, and RIC-8 in pulling force generation. Importantly, the Gα subunits are thought to contribute in this process independently of a heterotrimeric G protein–coupled receptor and trans-membrane signaling. Gα subunits act as molecular switches that change between active GTP-bound and inactive GDP-bound states. GPR-1 and -2 contain GoLoco motifs, which bind GDP-bound $G\alpha_{i/o}$ and act as GDP-dissociation inhibitor (GDI) (57). $G\alpha \cdot GDP$ normally forms an inactive heterotrimeric complex with Gβγ. *gpb-1* Gβ and *gpc-2* Gγ also affect spindle movements (37, 122). The spindle oscillates hyperactively in Gβγ RNAi embryos; however, this is suppressed by $G\alpha_{i/o}$ inhibition (2, 109). These results indicate that the Gβγ phenotype results from excessive Gα activity, and that Gβγ inhibits spindle force generation (**Figure 4**). In contrast, *gpr-1/2* and $G\alpha_{i/o}$ RNAi cause identical loss-of-function phenotypes (17, 38, 102). Thus, GPR-1/2 bind $G\alpha_{i/o} \cdot GDP$ and yet promote $G\alpha_{i/o}$ function.

Paradoxically, *ric-8* also acts similar to *gpr-1/2* and Gα, yet encodes a conserved guanine nucleotide exchange factor (GEF), which triggers release of GDP from the Gα subunit (2, 69, 107). The similar loss-of-function phenotypes of $G\alpha_{i/o}$, *gpr-1/2* GDI, and RIC-8 GEF may indicate that $G\alpha_{i/o}$ needs to switch between GDP- and GTP-bound conformations. RGS-7 acts as a GTPase activating Protein (GAP) for GOA-1 and its inactivation shows partial overlap with Gα loss of function (45). In

Asters: radial arrays of microtubules nucleated from microtubule-organizing centers (centrosomes in animal cells)

fact, *rgs*-7 mutant embryos show hyperactive, rather than reduced, movements of the posterior pole during anaphase. However, spindle-severing experiments revealed that this results from reduced net forces acting on the anterior pole (45) (**Figure 4**). While a G-protein cycle appears to be involved in force generation, the contributions of RIC-8 and RGS-7 remain unclear. It has been suggested that RIC-8 GEF activity frees Gα of Gβγ to allow interaction with GPR-1/2. However, *C. elegans* RIC-8 shows in vitro GEF activity toward GOA-1 but not toward GPA-16 (2, 3). In contrast, RIC-8 determines cortical localization of GPA-16 Gα similar to the function of Ric-8 in *Drosophila* (3, 66). Rat RIC-8 does not use heterotrimeric Gα · GDP-Gβγ as a substrate, but triggers dissociation of a $G\alpha_i$ · GDP/LGN/NuMa complex (106, 107). Thus, *ric-8* has been implicated in Gα localization, Gα · GDP/GPR complex formation, and Gα · GDP/LGN dissociation. Further experiments will be needed to reveal the *ric-8* substrate.

What are the downstream targets of cortical $G\alpha_{i/o}$, GPR-1/2, and LIN-5? Their role in generating cortical pulling forces could reflect control of microtubule dynamics, motor protein activity, and/or cortical anchoring of microtubules. Based on similarity with mammalian Gα/LGN/NuMA, a function in connecting microtubule ends to the cell cortex is likely (26). In mammals, NuMA binding promotes unfolding of LGN and allows interaction with cortical $G\alpha_i$ · GDP. NuMA interacts with microtubules and also with a dynein motor complex (68). Recent studies have also connected a dynein motor complex to the Gα/GPR/LIN-5 pathway. One study detected genetic and physical interactions between the dynein light chain DYRB-1 and LIN-5/GPR (18). Another study demonstrated interaction between the dynein motor–associated LIS-1 and LIN-5 (76). Importantly, *lin-5*, *gpr-1/2*, and Gα promote cortical localization of LIS-1 and DHC-1 dynein heavy chain, while dynein activity promotes pulling forces. In addition, cortical pulling forces also require microtubule turnover (76). Live imaging demonstrated rapid shrinking of microtubules upon cortical contact (59). Therefore, a model has been proposed that unites microtubule dynamics, microtubule-cortex anchoring, and motor protein activity (59, 76). In this model, interaction between Gα · GDP, GPR-1/2 and LIN-5 promotes the localization of a cortical DHC-1/LIS-1 complex. The dynein motor then attaches to microtubule ends, while microtubule depolymerization together with minus end–directed movement generates the pulling forces on astral microtubules (59, 76).

Creating Asymmetry in Cortical Pulling Forces

The net forces that pull at the anterior vs posterior asters are different, which leads to displacement of the spindle toward the posterior and asymmetric division of P_0. As the Gα/GPR/LIN-5 pathway is the major force generator, some form of asymmetry in this pathway should be expected. One reported asymmetry is the localization of GPR-1/2, which becomes slightly enriched at the posterior cortex in late mitosis (17, 38, 109). This may indicate a greater number of force generators at the posterior cortex and supports the conclusion from experiments in which the asters where fragmented with a UV-laser (41). In addition LET-99 is asymmetrically localized in a central-posterior band (108). LET-99 counteracts Gα function and GPR-1/2 localization, hence its enrichment in a central-posterior domain could lead to increased net forces in the posterior direction (39, 109).

The localizations of GPR-1/2 and LET-99 depend on the anterior and posterior PAR complexes (17, 38, 108). The PAR proteins themselves could directly affect spindle force generation. PAR-1 appears to be a particularly attractive candidate, as it is a kinase of the MARK family, which regulates microtubule dynamics by phosphorylating MAPs (25, 42). However, *par-2*;*par-3* and *par-3*;*par-1* double mutants are "posteriorized," and resemble *par-3* single mutants at the one-cell stage (16). Thus, pulling forces appear high at both poles at the one-cell stage, and both

spindles rotate in the absence of anterior and posterior PAR complexes in two-cell embryos. These results do not support the hypothesis that the posterior PAR proteins promote spindle forces directly. Rather, it has been suggested that the PAR-3/PAR-6/PKC-3 complex represses the default "high" spindle forces, while PAR-2 normally restricts the localization of this complex to the anterior (90).

As another level of asymmetry, Labbe et al. observed *par*-dependent higher microtubule turnover at the posterior cortex (62). A recent study did not observe a difference in the overall number of microtubules at the anterior vs posterior cortex (59). This latter study used measured values in combination with parameter variation to generate a computer simulation of spindle positioning with remarkable resemblance to live observations. One of the critical asymmetries considered in the modeling was higher rigidity of the anterior cortex. Differences in cortical rigidity, for instance, as a consequence of the anterior accumulation of actin, could have major effects on net force generators (59).

Positioning the Spindle in Subsequent Divisions

At least partly overlapping mechanisms control division of the zygote and subsequent asymmetric divisions. Although these divisions are beyond the scope of this review, we mention a few relevant points. All precursor cells that give rise to the germline (P_0–P_4) go through autonomously controlled asymmetric division (**Figure 3**). Similar to P_0, P_1–P_4 undergo cortical reorganization, asymmetrically localize PAR proteins, and restrict LET-99 to a cortical band (28, 48, 75, 108). Prior to asymmetric division of P_1, the centrosomes and associated nucleus rotate by 90° to orient the spindle along the AP axis. This rotation is also dependent on the Gα/GPR/LIN-5 pathway as well as on dynein (17, 64, 76, 97, 102).

Cell-cell signaling contributes to cleavage-plane positioning as early as the four-cell stage. The P_2 blastomere induces rotation of the nucleus-spindle in its anterior sister EMS. This causes EMS to divide along the AP axis, rather than left-right, which is critical in the generation of unequal daughters (**Figure 3**). Partly redundant Wnt/Frizzled and Src signaling pathways control the EMS cleavage plane and endoderm daughter cell fate (8). LIN-5, GPR-1/2, and dynactin become enriched at the P_2/EMS cell contacts (102, 121), while the amount of LET-99 is reduced (109). These asymmetric localizations depend on signaling and mutual interactions and determine the EMS cleavage plane.

Another important spindle orientation event takes place at the four-cell stage. The AB daughters ABa and ABp undergo synchronous left-right divisions, while their spindles rotate counter-clockwise by 20° just before cytokinesis (9). This process results in reproducible left-right asymmetry, starting at the six-cell stage throughout development. The *spn-1* mutation randomizes the ABa and ABp spindle positions. This mutation was found to be a *gpa-16* allele, again emphasizing Gα-dependent spindle control (9).

Meiosis

Of particular interest are the highly asymmetric meiotic divisions of the fertilized oocyte (**Figure 2**). Both spindle formation and localization are considerably different between meiosis and mitosis, yet the general regulators of cytokinesis are shared (see below). During meiosis, the spindle and associated chromosomes position in close proximity to the cortex. This supports the formation of two small polar bodies and a large diploid zygote (6). As centrosomes are absent, the microtubule arrays of the meiotic spindle form initially around the chromosomes and subsequently become organized into poles. This results in a compact "barrel-shaped" spindle, which lacks astral microtubule arrays. How this spindle induces a cytokinetic furrow is poorly understood (see below); however, its exact position with respect to the cortex is critical in this process.

Meiotic spindle positioning involves two independent steps (118, 119). First, *unc-116* kinesin-1 translocates the acentrosomal bipolar spindle sideways to the cortex. Subsequently, the spindle shortens and then rotates by 90° in an anaphase promoting complex (APC)-dependent fashion. This allows segregation of a set of chromosomes into a polar body during anaphase and subsequent cytokinesis. The two steps are repeated during meiosis II. *lin-5* acts in meiotic spindle rotation apparently independently of *goa-1;gpa-16* and *gpr-1/2* (64, 102). Recently, we identified a functional complex for LIN-5 together with ASPM-1, related to *Drosophila* Asp and human ASPM (abnormal spindle-like, microcephaly-associated) and CMD-1 Calmodulin. This complex localizes to meiotic and mitotic spindle poles, and controls meiotic spindle rotation in conjunction with dynein. In contrast, cortically localized Gα/GPR/LIN-5 is critical for mitotic spindle positioning (M. van der Voet, S. van den Heuvel et al., submitted).

POSITIONING THE CLEAVAGE FURROW

Several of the above-mentioned regulators of spindle positioning appear to be closely associated with positioning of the cleavage furrow. Although it is not fully understood how the spindle determines the cleavage plane, recent publications provide important insights and help clarify some long-standing issues. This includes the question: Which part of the spindle determines the cleavage plane, astral microtubules or the spindle midzone? Experiments that have pointed in different directions include the groundbreaking studies by Rappaport with sand dollar eggs (85). Upon manipulation, cytokinesis occurred between the asters of two distinct spindles, thus not overlapping with the location of the spindle midzone. However, experiments in other systems pointed to a critical contribution of the midzone. For instance, *Drosophila asterless* mutants complete cytokinesis despite a virtual absence of astral microtubules (11). More recent studies have indicated regulation by the asters as well as midzone (e.g., 24). A recent landmark study in *C. elegans*, discussed below, clearly demonstrated that the asters and midzone both contribute to furrow formation and act redundantly in the one-cell embryo (13).

A related long-standing question has not been fully resolved: What signals are provided by the astral and midzone microtubules to determine the cleavage site? Localized assembly of an acto-myosin contractile ring is central in this process. However, it is still unclear whether microtubules promote or inhibit formation and contraction of this ring. The models that are often compared and contrasted are the equatorial stimulation and polar relaxation models (reviewed in 27). The first model suggests that astral microtubules deliver a furrow-stimulating signal to the cortex, specifically at the cortical site of future cleavage. The polar relaxation model implies that the astral microtubules send a negative signal to the cell cortex, which does not reach the equator. Support for both positive and inhibitory microtubule functions has been obtained in studies of the one-cell *C. elegans* embryo. Astral microtubules provide negative regulation (74, 114), possibly after an initial furrow-stimulating function (74). In addition, the midzone contributes both positive and inhibitory cleavage signals (13). Recent data indicate that critical regulators of spindle positioning, the GoLoco proteins GPR-1/2, Gα subunits GOA-1/GPA-16 and DEP domain protein LET-99, all promote cytokinetic furrow formation as part of the astral signal.

Genes Essential for Cytokinesis

Cytokinesis involves several sequential steps: site determination and assembly of an acto-myosin contractile ring, furrow ingression, and completion, which involves membrane growth through vesicle fusion during abscission. We focus on contributions of the spindle in the first steps of cytokinesis and refer to excellent recent reviews for a more general overview (7, 27, 29). Inactivation of the general cytokinesis machinery by mutation or RNAi causes a

clear cytokinesis-defective (Cyk) phenotype in the early embryo: Anaphase spindles form and segregate the chromosomes, nuclei reform during telophase, yet cleavage fails and the nuclei rejoin in the center of the cytoplasm and start the next cell cycle. The first cytokinesis defects appear as early as meiosis and prevent expulsion of polar bodies. Hence, rather than a single haploid maternal pronucleus, two or three maternal pronuclei form in the anterior, which migrate to the posterior to meet the paternal pronucleus. The additional chromosomes usually align at the metaphase spindle and two nuclei are formed during the first division. During the subsequent mitotic divisions, the nuclei remain separate, leading to the accumulation of many nuclei within a common cytoplasm and early embryonic lethality.

Several classes of genes essential for cytokinesis in *C. elegans* can be distinguished (see **Supplemental Table 1**). Inactivation of genes of the first class prevents furrowing. This class includes *nmy-2* nonmuscle myosin, *mlc-4* myosin regulatory light chain, *rho-1* RhoA, *let-21/ect-2* RhoGEF, *act-4* actin, *let-502* Rho Kinase, *cyk-1* formin, and *pfn-1* profilin (13, 35, 81, 82, 94, 96, 99). These genes have evolutionarily conserved functions in assembly and contraction of the contractile ring (29). Local activation of the small GTPAse RHO-1 RhoA is the driving force in this process, as in polarity establishment (described above). RHO-1 accumulates at the equatorial cortex before furrowing can be detected (74). ECT-2 GEF and CYK-4 GAP likely control the RHO-1 GTPase cycle and thereby activation of downstream effectors. Activated RhoA · GTP is known to activate the actin-polymerizing protein formin in other systems. This relieves formin auto-inhibition and promotes assembly of unbranched actin filaments. Association with profilin increases the rate of formin-mediated actin filament elongation (94). Another downstream effector of RhoA · GTP is the Rho binding Kinase (ROCK), encoded by *let-502* in *C. elegans* (82). ROCK activates the nonmuscle myosin motor through phosphorylation of the myosin regulatory light chain rMLC and inhibition of myosin light chain phosphatase, MLC-4 and MEL-11, respectively, in *C. elegans* (82). Phosphorylation of rMLC promotes assembly of myosin motors into filaments that translocate actin filaments and promote constriction of the contractile ring. Thus, data from *C. elegans* studies agree with and have contributed to the view that RhoA localization and activation at the equatorial cortex promotes formation of circumferential actin-filaments and myosin motor activity.

The second class of cytokinesis genes is not required for furrow initiation but rather for completion. These genes encode components of the "centralspindlin" and "chromosomal passenger" complexes. The centralspindlin complex consists of the kinesin ZEN-4 MKLP1 of the kinesin-6 family in association with the CYK-4 RhoGAP (51, 84). This complex promotes the bundling of overlapping microtubules in the midzone during anaphase. The localization and activation of ZEN-4 is mediated by the chromosomal passenger complex (CPC), which localizes at the central spindle at the onset of anaphase (53, 95). The conserved CPC consists of the Aurora B homologue AIR-2, the INCENP-like protein ICP-1, the survivin homologue BIR-1, and the Borealin-related protein CSC-1. ICP-1, BIR-1, and CSC-1 are interdependent for their localization to the midzone and all three are required for the localization of AIR-2 to the central spindle (53, 95, 100). AIR-2 in turn phosphorylates and activates ZEN-4 during cytokinesis (43). Phosphorylation by CDK-1 prevents premature ZEN-4 activation, which subsequently is countered by the proline-directed phosphatase CDC-14 (71). The function of *cdc-14* is normally not rate-limiting, as it is required for completion of cytokinesis in a sensitized *zen-4::GFP* background, but not in wild-type embryos (92).

Supplemental Material

Two additional conserved proteins, SPD-1 PRC1 and KLP-7 KIF4/MCAK, promote microtubule bundling in the midzone (40, 111, 112). Loss of *spd-1* and *klp-7* function does not prevent completion of cytokinesis, in contrast to the centralspindlin and CPC phenotypes. Residual midzone microtubules in *spd-1* and *klp-7* mutants may suffice in providing a

midzone signal. However, cleavage also completes when the midzone is destroyed with a UV laser beam (12). Therefore, the cytokinesis defects after centralspindlin inactivation likely indicate functions in addition to midzone microtubule bundling. ZEN-4 also localizes at the furrow in *C. elegans* and other animals and part of its activity may reside at this location (see below) (77, 111, 112).

Asters vs Midzone Signals

Clearly, the genes that are needed for midzone formation are not essential for cleavage-plane specification and initial furrowing. Does this mean that a signal from the asters is the critical determining factor? A study by Dechant & Glotzer indicated redundancy between midzone and aster-related regulation of furrow formation (24). Subsequently, Bringmann & Hyman provided further insights and conclusive support for such dual functions (13). The latter study used a UV laser beam to sever the anaphase spindle asymmetrically between one aster and its associated chromatin. The other aster remained associated with the chromosomes and spindle midzone. Because the cortical pulling forces moved the asters further apart, the midzone was no longer positioned in the middle of the two asters. This procedure resulted in the initiation of two furrows. A first furrow formed midway between the two asters, ingressed but did not complete cleavage. A second furrow was induced later and formed at the position of the midzone. This second furrow intersected with the first furrow and completed cytokinesis. These results provide strong support for furrow-inducing activity of the midzone. Moreover, when asymmetric spindle severing was combined with ablation of the isolated aster, the first furrow formed further away from the remaining aster. Thus, the spindle asters and midzone provide two consecutive signals to specify the cleavage plane.

The combination of asymmetric spindle severing and selected gene inactivations led to several important observations (13). For instance, genes with known functions in midzone formation formed two different classes. Upon inactivation of *air-2*, *zen-4*, or *cyk-4* only weak ingression of the first furrow was observed, and no midzone furrow formed. Apparently, these genes affect not only midzone-induced furrowing but also contraction of the aster-induced furrow. ZEN-4::GFP has been observed at the ingressing furrow in *C. elegans*, and the same is true for the centralspindlin complex in other species (77, 111, 112). This observation is important for the interpretation of *zen-4* double mutant combinations (see below) (24, 112, 114). Following inactivation of *spd-1* PRC1 and *klp-7* MCAK, which act specifically in microtubule bundling, only the aster-induced furrow was formed. This furrow ingressed rapidly and completed abscission independently of the DNA, generating one cell with two nuclei and one without a nucleus. Thus, the midzone signal also delays aster-induced furrowing and allows correction of the cleavage plane to maintain genome integrity (13).

The Nature of the Midzone Signal?

The experiments described above clearly established that both the midzone and asters provide cleavage furrow-inducing signals. However, the question remains: What is the nature of these signals? The microtubule signals likely contribute or control the critical regulators of the acto-myosin contractile ring, in particular, the ECT-2/LET-19 RhoGEF and CYK-4 RhoGAP. Additional possible targets include the kinases AIR-2 aurora B, PLK-1 Polo-like kinase, and CDK-1 cyclin-dependent kinase, as well as antagonizing phosphatases CDC-14 and MEL-11. The ZEN-4/CYK-4 centralspindlin complex is a good candidate for providing a furrow-inducing signal. ZEN-4/CYK-4 recruits the RhoA GEF ECT-2 in other systems (98, 120), and both CYK-4 and ECT-2 are essential for accumulation of RHO-1 RhoA at the furrow (74). Because of the considerable distance between the midzone and cortex in the one-cell egg, the plus end–directed ZEN-4 motor activity may be needed to transport centralspindlin to the cortex. This transport

could involve microtubules nucleated from the midzone, as has been described in other systems (27). As an alternative mechanism for a furrow-inducing midzone function, it has been proposed that the bundling of microtubules into a midzone by ZEN-4/CYK-4 helps create a local microtubule minimum at the equator (24).

Apart from a presumed positive cue, the midzone also inhibits furrow initiation and ingression (13, 112). Inhibition of *mel-11* myosin light chain phosphatase causes a similar increase in aster-induced cleavage as *spd-1* and *klp-7* RNAi (13). This may indicate that the midzone slows down progression of cleavage through inhibition of myosin motor activity. Thus, the combination of a positive positional cue and a negative progression signal may be used as a correction mechanism for a mispositioned aster-induced furrow.

Repression or Induction of Furrow Formation by Astral Microtubules?

The microtubule asters provide the first cleavage signal in the one-cell embryo (13). Soon after anaphase onset, RHO-1 RhoA and filamentous actin (F actin) accumulate at the equatorial cortex (74). Foci of NMY-2 and phosphorylated myosin regulatory light chain accumulate at approximately the same time (114). Depletion of *tba-2* tubulin by RNAi prevents these accumulations, which confirms that astral microtubules promote assembly of the contractile ring (74). Still in dispute is whether the cue from the astral microtubules is strictly inhibitory, so that the furrow forms at the site where this inhibition is locally relieved. As an alternative possibility, microtubule plus ends at the cortex may initially promote assembly of a contractile ring as suggested by equatorial stimulation.

A critical question is whether contractile ring assembly coincides with a minimum or maximum of microtubule ends at the cortex. Despite the high quality of quantitative analyses in different studies, the conclusions vary greatly. Dechant & Glotzer found that initial furrowing is associated with a relative local minimum of microtubule density at the cortex (24). This local minimum depends on the separation of the spindle poles during anaphase B. Double inactivation of *par-2;zen-4* or *Ga;zen-4* prevents midzone formation and reduces aster separation. These doubly-depleted embryos fail to form a local microtubule minimum and do not show furrow ingression. These results fit with a model in which the local microtubule minimum at the equator created by aster separation acts as the cue to astral furrow initiation.

The measurements in this study were based on the timing of initial furrow ingression, which succeeds actin-ring assembly. Motegi and coworkers observed that RHO-1 and F-actin accumulate at the equatorial cortex before ingression could be detected (74). Their measurements of GFP-tubulin and GFP-EB1 indicated that RHO-1 accumulation takes place at the region of highest microtubule density. Actual furrowing followed a decrease in GFP-EB1. In mutants that fail to induce furrowing, such as the *par-2;zen-4* double mutants, RHO-1 still localized temporarily at the equator. These results lead to the proposal of a two-step model: RHO-1 initially accumulates at the equatorial cortex coincident with a local maximum in microtubule density. Next, the separation of the spindle poles creates a local minimum of microtubule density, which activates furrowing.

A third study observed neither a peak nor a low in microtubule density coincident with contractile ring assembly (112). This study also contested the correlation between spindle aster separation and lack of furrowing of double mutants without spindle midzone. The absolute timing and readout for furrow initiation may explain part of the differences between these studies. However, it appears unlikely that the density of microtubule plus ends at the cortex is the single determining factor, and other contributors need to be considered. For instance, *par-2* mutations and *zen-4* or *cyk-4* RNAi certainly have defects in addition to reduced aster segregation and midzone formation, respectively. It has also been unclear why *air-2;par-3* double mutants fail to undergo furrowing, despite extensive aster segregation (24, 122). Recently, mutations in *par-3* have been observed to

reduce PLK-1 Polo kinase levels (14, 87). AIR-2 and PLK-1 each promote cytokinesis, which may explain the *air-2;par-3* synthetic cytokinesis defect of double mutants.

Mutations and drugs that reduce microtubule length or abundance have long been known to cause ectopic furrowing (e.g., 4, 34, 61, 67, 104). Several such mutants affect the switch from meiotic to mitotic spindle formation. *mei-1* and *mei-2* act to maintain short microtubules during meiosis. The MEI-1 and MEI-2 proteins form a heterodimer with similarity to the p60 and p80 subunits of katanin, a microtubule-severing complex (101). The MEI-1/MEI-2 complex remains present during mitosis in *mei-1(gf)* mutants, and also in mutants defective in the MEI-1 degradation pathway, which includes *rfl-1* and *cul-3* cullin (110). Consequently, the mitotic spindle microtubules are abnormally short, coinciding with hyperactive ruffling of the cortex. These cortical contractions depend on cytokinesis components and have been considered as ectopic furrowing in response to reduced inhibition by astral microtubules.

Again, the explanation for this furrowing phenotype may be more complex. MEL-26 is the substrate-specificity factor for the CUL-3 ubiquitin ligase that targets MEI-1, but it is also a substrate for degradation itself (110). In addition, MEL-26 has been observed to promote furrowing in association with the actin-binding protein POD-1 Coronin (65). Thus, *rfl-1* and *cul-3* mutations could promote cortical ruffling as a result of increased levels of MEL-26 (65). In a recent study, *mel-26* RNAi embryos were further characterized (114). At the one-cell stage, the abnormal mitotic spindle becomes abnormally positioned in a transverse orientation in the posterior. Inactivation of *zyg-9* XMAP215 or partial loss of function of *tbb-2* β-tubulin creates very similar phenotypes. The microtubule free anterior cortex in such embryos accumulates the myosin reporter NMY-2::GFP and induces a second cleavage furrow. These data support that astral microtubules negatively regulate cleavage furrow formation and inhibit recruitment of myosin foci.

The Nature of the Astral Signal?

What is the astral microtubule signal and how is it communicated to the cortex? A recent screen focused specifically on identifying the astral signal (12). Gene inactivation by RNAi was used to identify genes with functions essential for cytokinesis in a midzone defective *spd-1* mutant, and not in the wild type. RNAi of *gpr-1/2* and *goa-1/gpa-16* Gα showed this phenotype, consistent with previous results (24, 112). Two additional genes were found in the screen, *cls-2* CLASP, and, most surprisingly, *let-99*. PAR-3 and PAR-1 restrict the localization of the LET-99 DEP domain protein to a cortical band (described above). However, independent of PAR-3, a LET-99 band forms during anaphase to overlay the cleavage plane (12, 108). GPR-1/2, Gα, and LET-99 all act in spindle positioning. CLS-2 CLASP is a plus end microtubule binding protein, which could help microtubules reach the cortex or more directly to contact cortical Gα/GPR/LIN-5. Thus, the joint activity of Gα, GPR-1/2, LET-99, and CLS-2 CLASP may provide a furrow-inducing cue.

A surprise, however, is the equivalent phenotypes of *let-99*, *gpr-1/2*, and *goa-1/gpa-16* inactivation in furrow determination. Previous data indicated that LET-99 and Gα/GPR/LIN-5 oppose each other's localizations (80, 109). In fact, this relationship was also observed during cytokinesis (12). While the LET-99 anaphase band correlates with the site of furrow formation, GPR-1/2 maxima flank this region. LET-99 localization was lost upon Gα RNAi and, vice versa, GPR-1/2 accumulated at the furrow position when LET-99 was inactivated. Thus, Gα/GPR and LET-99 are mutually inhibitory, and yet these components show similar contributions to furrow formation. Gα/GPR/LIN-5 likely mediate temporary microtubule-cortex interactions, which should then be reduced by LET-99 localization in the region of furrow formation. In combination with the results described above, these data appear to fit with a modified version of the polar inhibitory model. In the modified model, astral microtubules connecting to the cortex through Gα/GPR/LIN-5 inhibit furrow formation. Hence, the furrow

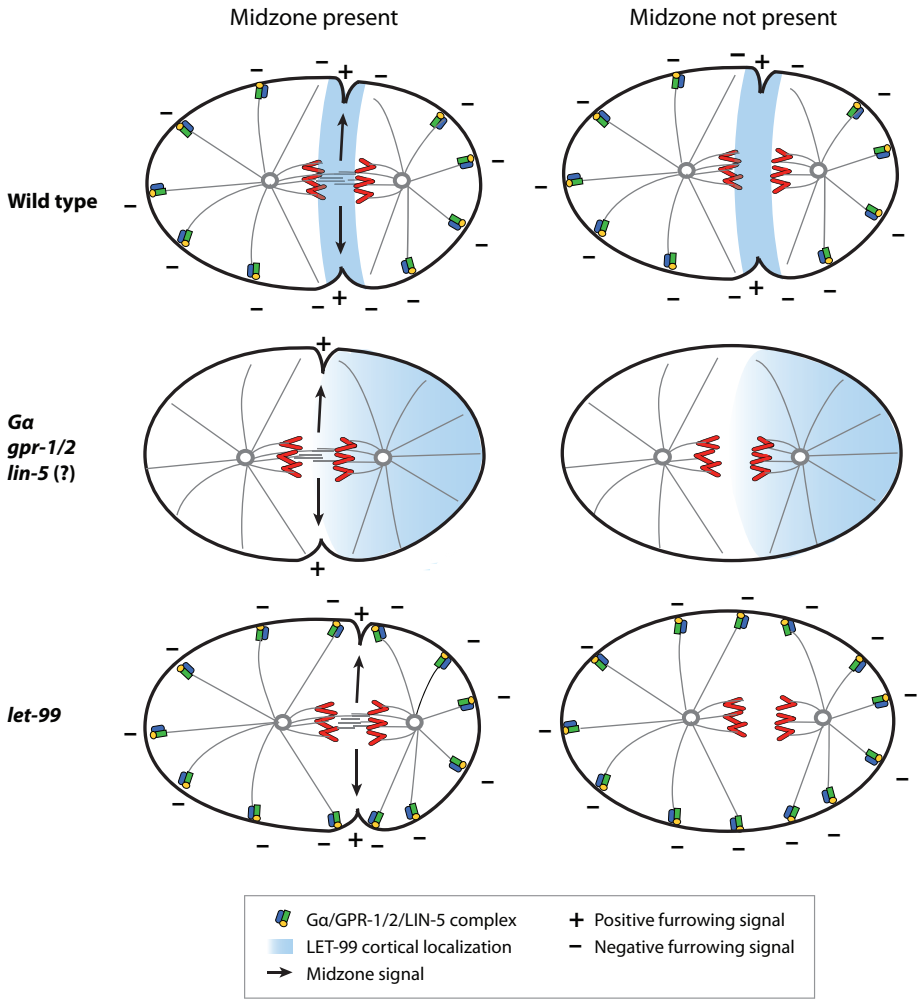

Figure 5

Model for the induction of furrowing in *C. elegans* and the relative contributions of an astral signal and a midzone signal. The light blue band represents LET-99 localization at the cortex. The + indicates the place of furrow induction, which is promoted by an antagonism between LET-99 and a Gα/GPR/LIN-5 complex, as well as a signal from the midzone. LET-99 may act positively in furrow induction by creating a local minimum of astral microtubules that inhibit furrowing through contacts with Gα/GPR/LIN-5 at the cortex (-).

forms at the site where this inhibition is locally relieved, in part through LET-99 localization (**Figure 5**). Alternatively, LET-99 could have a furrow-inducing activity and be restricted to the equatorial cortex through inhibition by Gα/GPR/LIN-5 in contact with astral microtubules.

Although several components of the astral furrow-inducing signal have been identified, how they connect to localized RHO-1 activation is unclear. Given the nature of the currently identified components, physical pulling at the cortex may contribute to this process. Microtubule-cortical connections may affect NMY-2 movement through Gα (39, 114) or sequester factors such as ECT-2 RhoGEF away from the cortex, as shown in other systems (60, 88). Alternatively,

microtubules that interact with Gα/GPR/LIN-5 may trigger relocalization of LET-99 to the equatorial cortex, while LET-99 activates the RHO-1 pathway. *C. elegans* Gα/GPR/LIN-5, *Drosophila* Gα/Pins/Mud, and mammalian Gα/LGN/NuMA act similarly in spindle positioning. It will be interesting to see whether this functional similarity extends to cleavage-plane determination. More research is still needed to further define the pathways that provide microtubule cues to the cortex.

SUMMARY POINTS

1. Reorganization of the cortical acto-myosin network in association with PAR proteins polarizes the one-cell egg.
2. Cell polarity creates asymmetry in the forces that act from the cortex on astral microtubules.
3. These forces are mediated by a Gα/GPR/LIN-5/dynein pathway and antagonized by the DEP domain protein LET-99.
4. While cortical pulling forces determine the position of the spindle, the spindle apparatus dictates the position of the acto-myosin contractile ring at the cortex.
5. Sequential and functionally redundant signals from the asters and midzone control cleavage furrow positioning.
6. The furrow-inducing cue from the astral microtubules is mediated by LET-99, Gα, and GPR-1/2. Hence, the same regulators may act in two directions, first from the cortex to control the spindle and then from the spindle to instruct the cortex.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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