Mechanisms of daughter cell-size control during cell division

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Abstract

Daughter cell size is tightly regulated during cell division. In animal cells, the position of the anaphase spindle specifies the cell cleavage site to dictate the relative size of the daughter cells. Although spindle orientation is regulated by dynein-dependent cortical pulling forces exerted on astral microtubules in many cell types, it was unclear how these forces are precisely regulated to center or displace the spindle. Recently, intrinsic signals derived from chromosomes or spindle poles have been demonstrated to regulate dynein-dependent pulling forces in symmetrically dividing cells. Unexpectedly, myosin-dependent contractile forces have also been shown to control spindle position by altering the cellular boundaries during anaphase. In this review, I discuss how dynein- and myosin-dependent forces are coordinately regulated to control daughter cell size.

123 words

Daughter cell size is defined by anaphase spindle position

Cell division is the fundamental process by which two daughter cells are produced from a single progenitor. In animal cells, the site of cell cleavage to “cut” the cell in two is specified during anaphase [1-3] by signals derived from the spindle midzone, which is formed between the separating chromosomes (Fig. 1a, [4]). This mechanism coordinates chromosome segregation with cytokinesis and ensures the equal distribution of the duplicated chromosomes into daughter cells to maintain the genomic information. In contrast, other factors such as polarized cell fate determinants and extrinsic niche signals can be symmetrically or asymmetrically distributed between daughters by controlling spindle orientation (Fig. 1a, see left and center, and; Fig. 1b, reviewed in [5]). Because the distribution of these polarized factors is critical to determine the fate of the daughter cells, and thus the cell division type (symmetric or asymmetric cell division) [6], the mechanisms of spindle orientation have been extensively studied in several organisms during development [7-13] and in vitro cell culture [14]. These studies have established a key concept that polarity signals assemble force-generating machineries at specific sites at the cell cortex to properly orient the spindle along the polarity axis [5].

Similar to the way in which the distribution of polarity factors can be controlled by modulating spindle orientation, the physical size of the daughter cells is varied by controlling spindle position (Fig. 1a, center and right). However, compared to the detailed understanding that exists for the basis of spindle orientation, the mechanisms and roles of spindle positioning and daughter cell-size control were poorly understood. In most vertebrate cells, the spindle is positioned in the center of the cell resulting in equal-sized cell division (Fig. 1b I and III). In contrast, the spindle is displaced to generate unequal-sized daughters in several different cell types, such as the *C. elegans* early embryo [8], *Drosophila* neuroblasts [7], sea urchin blastomeres [2], and mouse oocytes [15-17]. These unequally dividing daughters display different cell fates due to both differences in cell size, as well as asymmetric distribution of cell-fate determinants as a result of controlled spindle orientation (Fig. 1b IV). However, a difference in cell size is not always required for asymmetric cell fate during asymmetric cell division (Fig. 1b III), and the contribution of the relative daughter

cell size to defining cell fate was unclear.

Nonetheless, daughter cell size appears to be tightly regulated during both symmetric and asymmetric cell division. Indeed, the difference in cell volume or mass between two daughter cells in symmetrically dividing L1210 lymphoblasts is less than 10% [18, 19]. Daughter cell size affects the relative amount of cytoplasmic factors and organelles, such as mitochondria, which are critical for cell function and survival [20]. Furthermore, daughter cell size may have critical roles in early interphase events, such as nucleus formation, and may affect the relative volume of the nucleus and cytoplasm, which is maintained as a constant ratio during cell cycle [21]. Thus, it is plausible that daughter cell-size control plays a critical role in cell physiology. Recent studies indicate that altering daughter cell size results in differential behavior of daughter cells in both symmetrically and asymmetrically dividing cells [22-24].

To understand the mechanisms of spindle positioning, it is critical to define the forces acting on the spindle to control its position. Accumulating evidence indicates that the minus-end directed microtubule-based motor cytoplasmic dynein [25, 26] (hereafter called dynein), generates cortical pulling forces that act on astral microtubules to control spindle orientation and position [8, 27, 28]. However, the mechanisms that precisely regulate cortical dynein localization and activity to control spindle position is poorly understood, especially in symmetrically dividing cells. Here, I review the recent advances in understanding how dynein-dependent cortical pulling forces are regulated during metaphase and anaphase to center or displace the spindle, thus generating equal or unequal-sized daughters, respectively.

Direct contacts between astral microtubules and the cell cortex act to generate a physical connection and generate force to move the spindle in most cell types. However, in extremely large cells, such as *Xenopus* zygotes [29], and in cells with anastral spindles such as mouse oocytes [15-17] or plant cells [12], astral microtubules do not reach the cell cortex. In these cells, spindle position must be regulated by different mechanisms such as length-dependent astral microtubule pulling in the cytoplasm [29-32] or actin-based forces both in the cytoplasm [16, 33] and at the cell cortex [34]. As these mechanisms have been reviewed extensively [15, 17, 35, 36], I will instead focus on the mechanisms of

spindle positioning by cortical pulling forces during metaphase and anaphase.

Cortical dynein recruitment in unequally dividing cells

Over the past few decades, the mechanisms of spindle displacement and the resulting unequal-sized cell division have been extensively studied in the asymmetrically dividing one-cell *C. elegans* embryo (reviewed in detail in [8, 28]). In these cells, the maternal and paternal pronuclei and their associated centrosomes are initially positioned in the center of the cell during interphase by microtubule-length dependent pulling mechanisms [31]. Subsequently, the spindle forms and is displaced toward the posterior cell cortex during anaphase (Fig. 2a). Dynein is a key player in the generation of asymmetric pulling forces that act on astral microtubules [8, 28]. In *C. elegans* embryos, a conserved cortical dynein receptor (GPR-1/2) asymmetrically accumulates at the posterior cell cortex, downstream of polarity signals. GPR-1/2 forms a ternary complex with membrane-bound $G\alpha$ proteins (GOA-1 and GPA-16) and LIN-5, which are likely to recruit or activate dynein at the posterior cell cortex [37, 38]. Although the asymmetric enrichment of dynein is not detectable in these one-cell embryos, over-expression of GPR-1 causes the asymmetric cortical enrichment of dynein at the posterior cell cortex [39]. During the second cell division in these embryos, there is a clear cortical enrichment of dynein that is dependent on cortical LIN-5, GPR-1/2, and $G\alpha$ that is observed even without overexpression of GPR-1 [38]. Thus, current models suggest that cortical dynein is asymmetrically recruited and/or activated at the cell cortex via a LIN-5-GPR-1/2- $G\alpha$ ternary complex to displace the spindle in unequally dividing *C. elegans* embryos (Fig. 2a), although an additional $G\alpha$ -independent mechanism for cortical force generation is also proposed [40].

Cortical dynein recruitment in symmetrically dividing HeLa cells

The regulation of cortical dynein localization and activity observed in *C. elegans* provides a generalizable paradigm for motor-driven spindle movement. The ternary complex containing LIN-5, GPR-1/2, and $G\alpha$ (GOA-1, GPA-16) that is present in *C. elegans* is conserved in *Drosophila* (Mud/Pins/ $G\alpha$) and vertebrates (NuMA/LGN/ $G\alpha$) [5]. Dynein is also required for spindle orientation in budding

yeast, but in this case Num1 functions as a cortical dynein receptor [41]. Among the subunits of the ternary complex, NuMA has been implicated as a direct binding partner for dynein or its associated proteins, as the N-terminal region of NuMA is sufficient to recruit the dynein-dynactin complex to the cell cortex in symmetrically dividing human HeLa cells [39].

Whereas the ternary NuMA-LGN-G α i complex represents the lone pathway for cortical dynein recruitment in metaphase HeLa cells [42], NuMA and the dynein-dynactin complex can be targeted to the cell cortex independently of LGN and G α i during anaphase [22, 43-45] (Fig. 2b). This anaphase-specific recruitment of dynein to the cell cortex is negatively regulated in metaphase by CDK-dependent phosphorylation of the C-terminal region of NuMA at T2055 [22, 46, 47], and is activated by dephosphorylation via PPP2CA during anaphase [47]. Two different molecules have been proposed to act as cortical receptors for dephosphorylated NuMA in the anaphase-specific pathway (Fig. 2b). One candidate involves the conserved family of 4.1 proteins [22]. Vertebrates contain 4 isoforms of 4.1, referred to as 4.1R/EPB41, 4.1N/EPB41L1, 4.1G/EPB41L2, and 4.1B/EPB41L3. These 4.1 proteins act as hubs to organize membrane proteins through several domains including FERM and spectrin/actin-binding domains, and a unique C-terminal domain (CTD) [48]. The conserved C-terminal domain of 4.1 (4.1-CTD) interacts directly with the C-terminal region of NuMA [49], and is necessary and sufficient for the cortical targeting of NuMA and dynein during anaphase [22]. Although the precise mode of interaction between the 4.1-CTD and NuMA during anaphase is still unclear, phosphorylated NuMA shows reduced interactions with the 4.1-CTD [22]. A second candidate for recruiting NuMA to the cell cortex during anaphase is phosphoinositides (PIPs), which interact with the C-terminal region of NuMA [43, 45]. At present, two separate regions of NuMA have been proposed as direct binding sites for PIPs. One of these contains the CDK-phosphorylation site (T2055 residue) [43], suggesting that CDK-dependent phosphorylation may prevent NuMA from interacting with phosphoinositides directly by introducing a negative charge. How the other potential PIP-binding region on NuMA is regulated by T2055 phosphorylation remains unclear.

If cortical dynein is widely used as a force generator to control spindle

position or orientation, additional cortical dynein recruitment pathways may exist during asymmetric cell division to respond to different polarity signals. In fact, the LGN-binding partner, Inscuteable, is expressed in asymmetrically dividing mouse epidermal progenitor cells [50] and functions to recruit LGN to the cell cortex by linking LGN with the Par polarity complex [11]. Similarly, Mud/NuMA interacts with Dishevelled, which links Mud/NuMA to the transmembrane receptor Frizzled in *Drosophila* sensory organ precursors [51]. These additional pathways that recruit cortical dynein receptors likely provide distinct and alternative ways of generating cortical pulling forces during asymmetric cell division [5].

Cortical dynein regulation and spindle centering in metaphase

During cell division, most animal cells have a round shape, which ensures proper space for chromosome alignment and spindle assembly [52], and facilitates spindle positioning [53]. Since the spindle is not precisely positioned during prometaphase, dynein-dependent cortical pulling forces must act to properly control spindle position during metaphase and anaphase. Recent studies have demonstrated that chromosome- and spindle pole-derived signals regulate cortical dynein recruitment during metaphase in symmetrically dividing HeLa cells [42]. Chromosome-derived Ran-GTP gradients negatively regulate the interaction between G α i and LGN-NuMA in the vicinity of the metaphase chromosomes (Fig. 3). This helps to restrict cortical dynein to the polar cell cortex, downstream of NuMA-LGN, and maintain the spindle orientation axis [42]. Although the precise molecular mechanisms by which Ran-GTP dissociates NuMA-LGN from G α i remain unclear, NuMA is an excellent target for Ran-GTP regulation, as NuMA is an established downstream target of the Ran gradient that directs spindle assembly [54, 55].

To align the spindle in the center of the cell, cortical pulling force must be properly regulated to rectify spindle mis-positioning. Importantly, Polo-like kinase 1 (Plk1) signals derived from the spindle pole negatively regulate the interaction between the NuMA-LGN complex and dynein-dynactin in a distance-dependent manner [42] (Fig. 3). This mechanism autonomously generates cortical dynein asymmetry when the spindle is displaced from the cell center, resulting in the

generation of asymmetric cortical pulling forces to correct spindle mis-positioning [42] (Fig. 3). Since NuMA and LGN occasionally display asymmetric localization in symmetrically dividing cells ([56, 57], T.K. unpublished observation), the Ran-GTP system may also negatively regulate NuMA-LGN localization at the polar cell cortex to facilitate spindle centering, especially when the spindle is highly displaced from the cell center.

Together, these Ran- and Plk1-dependent negative feedback systems control dynein-dependent cortical pulling forces to achieve spindle centering during metaphase in symmetrically dividing HeLa cells. Such distance-dependent regulation by intracellular gradients are also used in other biological process, such as the correction of kinetochore-microtubule attachment defects by Aurora-B kinase [58, 59], cell size homeostasis in fission yeast by the Pom1 kinase [60-62], and division site specification in *Caulobacter crescentus* by Mip-Z (reviewed in [63, 64]). Intracellular gradients are useful for transmitting positional information to downstream targets and may be widely used for spatial control. While dynein-dependent cortical pulling forces appear to play a dominant role in spindle movement within HeLa cells, other mechanisms such as length-dependent astral microtubule pulling in the cytoplasm [29, 31], astral microtubule buckling [65], spindle length control [66], actin-dependent mechanisms [67], and control of cell size or geometry [53] may also cooperate to center the spindle.

Spindle centering by membrane elongation during anaphase

During early anaphase, cell shape remains constant and the distribution of cortical dynein is regulated similarly to metaphase, with dynein-dependent asymmetric pulling forces moving the spindle to rectify spindle mis-positioning [22] (Fig. 4a-1). In contrast to the presence of checkpoints that monitor spindle assembly and chromosome-segregation [68], checkpoint systems that inhibit anaphase onset until the spindle is properly positioned appear to be absent, at least in HeLa (human), LLC-PK (pig), and MDCK (dog) cells [22, 57, 69]. Instead, vertebrate cells have developed back-up systems to rectify spindle mis-positioning during anaphase by coordinately controlling spindle movement and cortical membrane elongation [22]. During the later stages of anaphase, cell

shape changes dynamically, providing an alternative mechanism to rectify spindle mis-positioning by asymmetrically expanding the polar cell cortex and alter the cellular boundaries relative to the spindle (Fig. 4a-2). This asymmetric cortical expansion occurs in response to spindle positioning defects at the end of early anaphase: only the polar membrane that is closest to the spindle elongates, with the other side remaining stationary (Fig. 4a-2). Since cortical expansion is a rapid process (roughly estimated 1.4 $\mu\text{m}/\text{min}$ in HeLa cells [22]) as compared to dynein-dependent spindle movement (0.05 - 0.40 $\mu\text{m}/\text{min}$ in HeLa cells [42]), this may be an ideal way to rectify spindle mis-positioning within a limited time-period during late anaphase. However, rapid cortical expansion results in a flow of cytosol that moves the spindle toward the growing cell cortex [70]. Thus, cortical dynein must act at the stationary cell cortex to anchor the spindle and prevent spindle displacement [22] (Fig. 4a-2, 4b). In the absence of cortical dynein during anaphase, the spindle becomes displaced due to the cytosolic flow caused by asymmetric membrane elongation. This spindle movement results in unequally sized daughter cells following cell division [22]. Thus, the coordinated action of cortical dynein and asymmetric membrane elongation is critical to center the spindle during late anaphase (Fig. 4b).

Asymmetric membrane elongation during anaphase represents a distinct paradigm for controlling spindle position. As local secretion of new membrane from internal vesicles is not observed at the growing cell cortex [22], cortical remodeling of the pre-existing cell cortex plays a major role in asymmetric cortical expansion. During anaphase, cortical rigidity is largely regulated by actomyosin-based contractile forces acting on the plasma membrane [4]. While actomyosin accumulates at the equatorial region downstream of midzone signals to form a contractile ring for cytokinesis, a substantial amount of actin and myosin remain at the polar cell cortex during anaphase [70]. When the polar actomyosin levels are equal on both sides, the polar membrane expands symmetrically as the cleavage furrow ingresses (Fig. 4c, symmetric). In contrast, when polar actomyosin is asymmetric, unbalanced forces in the polar cell cortex can trigger cell shape instabilities or asymmetric cortical expansion [71] (Fig. 3c, asymmetric). In this case, the myosin-rich cortex contracts, whereas the opposing polar cell cortex with reduced myosin asymmetrically expands to alter

the cellular boundaries [22, 24, 72] (Fig. 3c, asymmetric). Similarly, cortical remodeling by membrane-actin linkers, such as moesin, at the polar cell cortex regulates polar relaxation during anaphase [73, 74]. Thus, the asymmetry of the cortical factors that regulate contraction or relaxation of the polar cell cortex can drive asymmetric cortical membrane elongation during anaphase.

These components of the actin cytoskeleton and cell cortex provide the molecular players to achieve the physical expansion of the plasma membrane to control spindle position. However, the question remains as to what signals generate the polar asymmetry of these cortical factors in response to spindle mis-positioning during anaphase. A possible candidate is the chromosome-derived Ran-GTP gradient, as it inhibits the localization of the cortical scaffold protein, anillin [75], and its downstream target myosin II at the growing cell cortex [22] (Fig. 4b). Although the precise mechanisms are still unclear, anillin is a good candidate for a target of Ran-GTP as it contains a nuclear localization sequence (NLS) and is regulated by Ran-GTP during pseudocleavage formation in *Drosophila* syncytial embryos [76]. Given the complexity of the cortical structure and its regulation, additional intrinsic signals derived from chromosomes, spindle pole, or astral microtubules are also likely to regulate the localization or activity of anillin and other cortical proteins for cortical expansion [4].

In summary, cortical contractile forces at the polar cell cortex are spatially regulated by intrinsic signals in response to the anaphase spindle position during equal-sized cell division. These mechanisms control whether the polar membrane elongates symmetrically or asymmetrically, which contributes to rectifying spindle mis-positioning by altering the cellular boundaries during equal-sized cell division.

Daughter cell-size control by dynein- and myosin-based asymmetric forces

Symmetrically dividing cells have developed intrinsic error-correction systems that autonomously generate dynein- or myosin-based asymmetric forces to rectify spindle mis-positioning to generate equal-sized daughters (Fig. 5a). As these systems and their regulators such as Ran, Plk1, and anillin have multiple

essential roles during cell division [75, 77-79], these components are likely to exist in unequally dividing cells. Thus, another question that arises is how do cells eliminate or modulate these spindle-centering systems to divide unequally?

In unequally dividing *C. elegans* one-cell embryos, the chromosome-derived Ran gradient may not reach the cell cortex due to the relatively long distance between the chromosomes and the polar cell cortex (more than 10 μm). Although the size of the Ran-GTP gradient is likely to differ between cell types [80], the working distance is approximately 2 and 10 μm in HeLa cells and mouse oocytes, respectively [42, 81]. Interestingly, Polo-like kinase 1 (PLK-1) is distributed asymmetrically in the anterior cytoplasm downstream of Par polarity signals [82, 83], which may neutralize opposing pulling forces for spindle centering. In addition, due to the nature of the eggshell in these embryos, which provides a fixed external barrier, drastic asymmetric membrane elongation does not occur at the posterior cell cortex, even if the spindle comes close to the posterior cell cortex. These mechanisms would coordinately promote spindle displacement, although some buffering mechanisms appears to exist so as to not displace the spindle all the way to one side of the cell and generate daughter cells that are too small [28].

As the *C. elegans* one-cell embryo is a relatively large cell, different mechanisms may exist in smaller cells for spindle displacement. Recent studies in *C. elegans* neuroblasts, which are much smaller than the one-cell embryo, revealed that cortical myosin asymmetry is generated during anaphase and drives unequal-sized cell division by asymmetrically elongating the polar cell cortex [24] (Fig. 5b). Similar asymmetric cortical expansion has also been reported in *Drosophila* neuroblasts [72]. Contrary to symmetrically dividing cells, this cortical myosin asymmetry is likely to be generated independently of the chromosome/spindle-derived signals, but does depend on polarity signals [84].

Given that the *Drosophila* dynein receptor, Pins (the LGN ortholog) localizes asymmetrically to the growing apical cell cortex downstream of Par proteins [85-87], it is unclear how the spindle can counteract cytosol flow, which is likely to be generated from the basal to apical cell cortex during asymmetric membrane elongation. As *Drosophila* neuroblasts generate much larger apical centrosomes, and thus longer astral microtubules at the apical side [23, 88, 89],

this spindle asymmetry may function to counteract the cytosol flow and displace the spindle toward the basal side during asymmetric cortical expansion (Fig. 5b). DSas4-mutant flies, which lack astral microtubules, display partial defects in daughter cell-size asymmetry in neuroblasts [90], indicating that asymmetrically longer astral microtubules are likely to contribute to displacing the spindle. Apical complex genes and G β / γ signals also contribute to control daughter cell-size asymmetry in *Drosophila* neuroblasts [23, 88, 89, 91], although the precise mechanisms are still unclear.

Polarity signals must have a key role in generating asymmetric forces, which dominantly function to displace the spindle and/or inhibit spindle-centering mechanisms in unequally dividing cells. In cases where the polarity signals generate cortical pulling forces, but do not inhibit spindle-centering systems, the signals may generate “torque” to orient the spindle, but not displace the spindle away from the cell center, resulting in properly oriented and equally sized daughter cells (Fig. 1b-III).

Concluding remarks

Compared with the current understanding of spindle orientation, the mechanisms of spindle positioning remain poorly understood. However, recent studies have revealed key players and a basic framework for spindle position control, which defines daughter cell size during cell division. The cortical force-generating machinery that acts on astral microtubules is the primary factor required to control spindle orientation. In contrast, cortical forces derived from both dynein-dependent cortical pulling forces acting on astral microtubules and myosin-dependent contractile forces acting at the polar cell cortex act in coordination to control spindle position by moving the spindle or altering cellular boundaries, respectively (Fig. 5).

Intrinsic signals derived from the chromosomes or the spindle autonomously create cortical dynein and myosin asymmetries within the cell in response to spindle mis-positioning. These signals control spindle movement or membrane elongation to center the spindle during anaphase and ensure that cells divide equally (Fig. 5a). In contrast, to divide unequally, polarity signals create cortical asymmetry of these motor proteins within the cell and displace the

spindle by asymmetrically moving the spindle or altering cellular boundaries (Fig. 5b). Defining the precise molecular mechanisms of asymmetric force generation downstream of intrinsic signals and polarity signals, respectively, is critical to understand daughter cell-size control. In particular, how polarity signals disrupt or modulate intrinsic spindle centering systems are important future questions, as Ran, Plk1, and their downstream targets appear to be differently regulated between equally dividing HeLa cells [42] and unequally dividing *C. elegans* embryos [82, 83] or mouse oocytes [81]. In addition to polarity signals, daughter cell size is affected by cell geometry [2, 35, 53] and cell adhesion [18], which are variable between cell types and during development. Understanding the mechanisms by which these factors affect the spindle positioning systems described here is an important open question.

As both kinetochores and the cell cortex function as attachment sites for a microtubule plus-ends, the strategies, techniques and concepts from the kinetochore field [92] are useful to understand mechanisms that specify, assemble, and regulate force-generating machineries at the cell cortex. For example, a proteomics-based approach would be useful to identify binding partners of key cortical proteins. In addition, in vitro reconstitution of astral microtubule-cell cortex interaction would provide useful information to understand the detailed mechanics of force generation and spindle positioning. Centrosome positioning was recently reconstituted in vitro using purified centrosomes and yeast dynein combined within a micro-chamber [93]. This study revealed that dynein induces microtubule catastrophe and generates force by interacting with depolymerizing microtubules, analogous to the Ndc80/Ska1 complex at kinetochores [94]. As dynein is an approximately 1.4 MDa multi-protein complex, it has long been difficult to reconstitute dynein holo-complex in vitro. However, the human dynein holo-complex was recently reconstituted in vitro [95], which revealed that the ternary complex formation of dynein, dynactin and adaptor proteins such as Bicaudal D is required for the activation of human dynein motility [96, 97]. In addition, other dynein-binding partners such as Lis1 and NudE regulate the force producing state and/or the prolonged microtubule attachment of dynein [98, 99]. Investigating the mechanisms by which these dynein-binding partners regulate microtubule

capture, astral microtubule dynamics, and force generation in vitro would provide important mechanistic insights to understand spindle positioning.

Although the relationship between spindle orientation and cell-fate determination has been established, the functional link between daughter cell size and daughter cell fate remains largely unknown in animal cells. To understand this, it is critical to manipulate daughter cell size without affecting other polarized cell-fate determinants. Recently, unequal-sized daughters have been generated in cells, which normally divide symmetrically (Fig. 1b-II), using genetics [22], artificially controlling cell geometry [2, 53], or by directly manipulating spindle position [100]. Analyzing the behavior of resulting smaller- and larger-sized daughters of different cell types could provide additional information to understand the role of daughter cell-size control. Defining the mechanisms and roles of daughter cell-size control in both symmetrically and asymmetrically dividing cells will continue to be an exciting area of future research.

3995 words

Figure legends

Figure 1. Daughter cell-size control by spindle positioning (a) Spindle orientation controls the distribution of polarized factors (compare Left with Center), whereas spindle position defines daughter cell size (compare Center to Right). Left: during symmetric cell division, the spindle is centrally positioned and all cellular components are equally distributed between daughter cells. Center: during asymmetric cell division in most vertebrate cells, the spindle is oriented parallel to the polarity axis to asymmetrically distribute polarized factors, whereas the spindle is positioned in the center of the cell to divide equally in size. Right: in a subset of asymmetrically dividing cells, the spindle is displaced and both, daughter cell size and the distribution of polarized factors become asymmetric. The blue line and green circle indicate chromosomes and centrosomes, respectively. (b) Cell division can be classified into 4 groups in terms of daughter cell size and distribution of polarized factors.

Fig. 2 Cortical dynein recruitment pathways (a) A model showing recruitment of cortical dynein by the conserved ternary complex, LIN-5, GPR-1/2, and G α proteins (GOA-1 and GPA-16), in unequally dividing *C. elegans* embryos. (b) Diagram showing two parallel pathways that target dynein and dynactin at the cell cortex during metaphase and anaphase in symmetrically dividing HeLa cells. NuMA-LGN-G α i recruits dynein during metaphase and anaphase, whereas 4.1- and/or PIPs- NuMA pathways targets dynein specifically during anaphase [22, 43-45].

Fig. 3 Spindle centering systems in metaphase A model showing spindle centering systems in metaphase. Chromosome-derived Ran-GTP gradient (blue) negatively regulates the interaction between G α i and LGN-NuMA and excludes LGN-NuMA from the cell cortex near chromosomes. This system confines LGN, NuMA, and dynein at the polar cell cortex and contributes to maintain the axis for spindle orientation. In contrast, spindle-pole derived Plk1 signals (red) negatively control the interaction between LGN-NuMA and dynein in a distance-dependent manner. This system autonomously regulates cortical

dynein localization in response to spindle mis-positioning and generates asymmetric pulling force to correct spindle mis-positioning.

Fig. 4 Spindle centering systems in anaphase (a) Diagrams summarizing anaphase spindle centering processes. When the spindle is centrally positioned by the end of metaphase, the central spindle position is maintained during anaphase and the polar membrane elongates symmetrically. 1) Spindle mis-positioning is rectified by moving the spindle during early anaphase. Once the spindle is centrally positioned, this is maintained until cytokinesis. 2) When the spindle is mis-positioned at the end of early anaphase, cells autonomously generate cortical dynein and myosin asymmetry in response to the spindle position. Myosin asymmetry causes asymmetrical membrane elongation, whereas dynein asymmetry produces cortical pulling forces to move or anchor the spindle at the stationary cell cortex. 3) When the spindle is fully elongated during anaphase to reach the polar cell cortex, the spindle is naturally placed in the center of the cell. (b) Diagram showing regulation of asymmetrical membrane elongation during anaphase. Ran-GTP functions to initiate the local reduction of polar anillin and myosin at the growing polar cell cortex near the chromosomes. Anillin and myosin actively accumulate at the equatorial cell cortex by midzone-derived signals, which negatively regulate cortical NuMA localization at the equatorial cell cortex during anaphase. (c) When the polar actomyosin level (magenta) is identical on both sides, the polar cell cortex expands symmetrically as the equatorial cell cortex (gray) ingresses during anaphase. In contrast, polar region asymmetrically extends when polar actomyosin level is asymmetrical. Asymmetric cortical contraction causes cytosolic flow (light gray arrows) toward the growing cell cortex (light green) and asymmetrically expands the cellular boundary.

Fig. 5 Mechanisms of equal- and unequal-sized cell division (a) During equal-sized cell division, cortical dynein and polar myosin asymmetry are generated in response to spindle mis-positioning downstream of intrinsic signals. Dynein and myosin asymmetry generates asymmetrical pulling forces that move the spindle and asymmetrical contractile forces that alter cellular boundary,

respectively. These asymmetrical forces are balanced once the spindle is positioned in the center of the cell. (b) During unequally sized cell division, cortical dynein and polar myosin asymmetry are generated by polarity signals. Similarly, in equally sized cell division dynein and myosin asymmetry generates asymmetrical pulling forces and asymmetric contractile forces, respectively, but displace the spindle. In addition, these asymmetric forces are not balanced during anaphase, resulting in unequally sized cell division.

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Fig.1

Fig.2

Symmetric cortical pulling force

(a) Unequally dividing *C.elegans* embryos

(b) Equally dividing HeLa cells

Fig.3

Fig.4

(a)

(b)

(c)

Fig.5

(a) Equal-sized cell division

(b) Unequal-sized cell division