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The Inner Compass of Spindle Positioning and Orientation

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Polarized cortical cues are known to guide spindle movements to dictate division axis and cleavage site during asymmetric cell division. In a recent issue of *Nature Cell Biology*, Kiyomitsu and Cheeseman (2012) report two novel spindle-intrinsic signals that regulate spindle orientation and position in symmetrically dividing human cells.

Cells can divide symmetrically or asymmetrically, and both modes of division are found in life forms from bacteria to animals (Barák and Wilkinson, 2007; Morin and Bellaïche, 2011). Symmetric cell divisions are important for clonal expansion of cells, whereas asymmetric cell divisions generate cell diversity. In animals, both types of division are required to determine the architectures of tissues and organs. Central to the decisions for cell division axis and site are the spindle orientation and positioning. In asymmetric cell division, polarized cortical cues, which come from various upstream signals, including cell shape, cell-cell contact, cell-extracellular matrix adhesion, and cell cortex-associated polarity proteins, are translated into mechanical forces that drive polarized movements of mitotic spindles (Castanon and González-Gaitán, 2011) (Figure 1A). Information learned from asymmetric cell divisions has led to the general belief that the source of instructive signals for spindle positioning and orientation comes from the cell cortex and that spindle positioning in symmetrically dividing cells may not be under active regulation. A new study by Kiyomitsu and Cheeseman (2012) has fundamentally changed these views.

Kiyomitsu and Cheeseman (2012) uncover the fact that signals intrinsic to the spindle-chromosome complex play essential roles in defining spindle position and orientation in symmetrically dividing human cells (Figure 1B). Through the action of the spindle-pole-localized polo-like kinase 1 (Plk1), the spindle pole proximity negatively regulates the dynamic localization of cortical dynein-dynactin (microtubule-based motor that drives spindle movement). This, in turn, places spindles at the cell center prior to cell division. The authors further demonstrate that the chromosome-derived RanGTP gradient restricts the localization of LGN-NuMA (upstream targeting factors of cortical dynein-dynactin) from cortical regions near the spindle midzone and thereby confines the orientation axis of spindles. The two signals that emanate from the spindle poles and chromosomes act cooperatively to regulate spindle position and orientation in symmetrically dividing cells (Figure 1B).

Kiyomitsu and Cheeseman first notice that dynein-dynactin, but not its cortical targeting factors LGN, NuMA, and Gαi1, localizes asymmetrically to the cell cortex during metaphase. Time-lapse microscopy reveals that dynein is enriched at the cortical region that is distal to the spindle but delocalizes as soon as the spindle is pulled toward it. The spindle consequently oscillates between the two cell poles until the balance of forces finally settles the spindle at the cell center. Furthermore, disrupting cortical dynein localization or spindle-pole-derived astral microtubules impedes spindle oscillations, indicating that the dynamic distribution of cortical dynein-dynactin aligns spindles in the middle of cells through the pulling forces exerted on astral microtubules.

The authors hypothesize that a short-range inhibitory signal is sent forth from the spindle poles to remove dynein-dynactin from the nearby cell cortex. Indeed, inhibiting Plk1, a kinase concentrated at spindle poles, renders cortical dynein-dynactin insensitive to spindle pole proximity. Consistently, artificial targeting of Plk1 to the plasma membrane hinders cortical dynein-dynactin localization, as well as spindle oscillation. No effect is seen on LGN localization under the same condition, suggesting that Plk1 acts to disassociate dynein-dynactin from its targeting factor LGN-NuMA. The authors further demonstrate that dynein-dynactin forms a complex with LGN-NuMA in cell lysates, but the complex is disrupted upon Plk1 addition. Plk1 thus serves as the inhibitory signal through which the spindle pole proximity directly regulates the distribution of cortical dynein-dynactin and thereby adjusts asymmetric pulling forces for spindle centration.

Conceptually, cortical forces that oscillate and center the spindle should come from any direction in symmetrically dividing cells. It is therefore unclear how spindles stably align to the axis at which they are formed during the oscillation. Kiyomitsu and Cheeseman notice that whereas LGN is symmetrically distributed at the cell cortex near spindle poles, it is excluded (together with dynein-dynactin) from the lateral cortex around the spindle midzone (Figure 1B). This raises an idea that the pole-enriched LGN may serve to confine spindle orientation, particularly when the spindles are actively oscillating. Further analyses reveal that LGN is excluded from cortical regions close to chromosomes. Disrupting spindle structure, and hence chromosome organization, changes the localization pattern of LGN accordingly. For example, when chromosomes are kept away from the cell cortex, LGN becomes localized evenly throughout the cell cortex. In instances in which chromosome mass is shifted toward the cell cortex, LGN localization is disrupted locally. This suggests

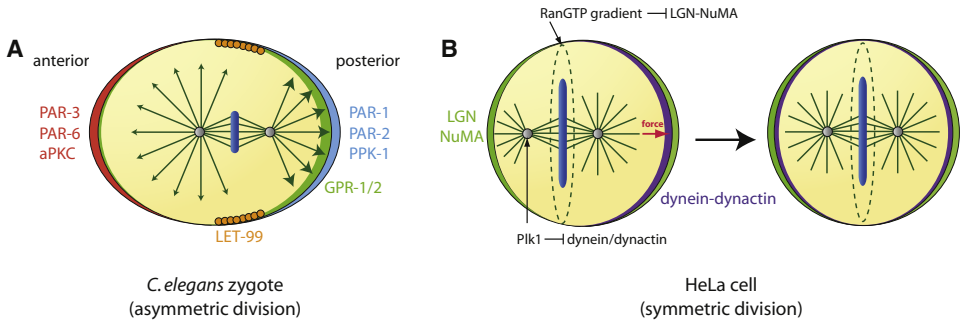

Figure 1. Spindle Orientation and Position Govern Cell Division Axis and Site

(A) An example of asymmetric cell division in one-cell *C. elegans* embryos. The division site and axis are dictated by polarized localization of a group of cortical proteins including Par, aPKC, and LET-99 (Siller and Doe, 2009). Their combined activity results in an enrichment of GPR-1/2 (LGN ortholog) at the posterior cortex. The enriched GPR-1/2 activates the cortical dynein-dynactin complex and generates a net force that displaces the spindle toward the posterior end of the zygote (Siller and Doe, 2009).

(B) In HeLa cells, Plk1 at spindle poles inhibits localization of the dynein-dynactin complex. A RanGTP gradient from chromosomes inhibits localization of LGN-NuMA. The two signals work together to align the spindle at the right position (center) and angle.

the presence of a chromosome-derived, inhibitory signal that locally regulates LGN distribution. Strikingly, disrupting the RanGTP gradient, a concentration gradient of the GTP-bound Ran GTPase centering on chromosomes, induces homogeneous localization of LGN to the cell cortex, even at regions in which chromosomes are in close proximity. Furthermore, disruption of LGN or RanGTP activity randomizes spindle orientation in cells in which spindles are normally aligned to a defined axis on patterned substrates (Théry et al., 2005). Therefore, restricted localization of LGN by RanGTP gradient functions to define or maintain spindle orientation. It will be interesting to check whether spindles continue to rotate or rock in cells in which the RanGTP pathway is disrupted but the pole-localized Plk1 remains active.

RanGTP is involved in a range of cellular processes, including macromolecular transport between cytoplasm and nucleus, assembly of nuclear envelope at mitotic exit, and spindle assembly in mitosis (Quimby and Dasso, 2003). Kiyomitsu and Cheeseman now add spindle orientation to the list of essential roles of RanGTP gradient at chromosomes. Whether this new function of RanGTP is conserved in other systems involving oriented cell division remains to be seen. Interestingly, a negative-feedback loop preventing excess accumulation of cortical LGN has been reported in asymmetrically dividing P2 cells of *C. elegans* embryos (Werts et al., 2011). In this case, signals derived from astral microtubules appear to be responsible for removal of cortical LGN when spindles are pulled close to the cortex. It will be interesting to see whether Plk1 or even RanGTP gradient plays a role there in P2 cells, because both signals associate and move together with spindles. Further analyses are required to carefully resolve the contribution of the two signals.

The work of Kiyomitsu and Cheeseman (2012) introduces an important view on the role of the spindle-chromosome complex in defining cell division axis and position. Instead of being a passive structure that is moved or rotated by external cues, the complex itself is actively controlling its own orientation and position, at least in symmetrically dividing cells. It is not clear whether these spindle-intrinsic signals also function in asymmetric cell division in which cortical cues appear to be dominant. Understanding how the signals from the spindle-chromosome complex act in conjunction with extrinsic signals from the cell's surroundings will undoubtedly yield important insights into the mechanism behind cell fate and tissue architecture determination.

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