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Spindle Positioning by Cortical Pulling Forces

Commentary

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Summary

Proper spatial control of the cell division plane is essential to any developing organism. In most cell types, the relative size of the two daughter cells is determined by the position of the mitotic spindle within the geometry of the mother cell. We review the underlying mechanisms responsible for positioning of the mitotic spindle, both in cases where the spindle is placed in the center of the cell and in cases where the spindle is placed away from the center of the cell. We discuss the idea that cortical pulling forces are sufficient to provide a general mechanism for spindle positioning within symmetrically and asymmetrically dividing cells.

How is the position of the cleavage plane specified? In eukaryotic cells, the cleavage furrow bisects the middle of the mitotic spindle (Albertson, 1984; Rappaport, 1971; Strome, 1993) with exceptions from this general rule (Kaltschmidt et al., 2000). Consequently, during equal cell division, the spindle needs to be positioned in the center of the cell. For an unequal cell division, the spindle has to be positioned away from the center.

As was first realized by Sachs (Wilson, 1925), a mitotic spindle will tend to place itself in the center of a cell: it must therefore respond to the geometry of the cell because this defines the center. Within an asymmetrically dividing cell, mechanisms must exist that place the spindle eccentrically. It seems likely that asymmetric spindle positioning would be accomplished by modulation of the principles that symmetrically position spindles during most cell divisions. Spindles are thought to position themselves by astral microtubules nucleated from both of their poles (Sharp et al., 2000; Strome, 1993). The microtubule cytoskeleton can generate force both by virtue of the intrinsic properties of the polymer and by acting as tracks for microtubule-based motors (see Desai and Mitchison [1997] and Howard and Hyman [2003] for a more extensive discussion on this issue). For a spindle to position itself, these force-generating mechanisms must be coupled to the geometry of the cell cortex. Consequently, a direct interaction between astral microtubules and the cell cortex is a likely mechanism for spindle positioning: microtubules contact the cell cortex at various points, and forces are created at these points of interaction (Pearson and Bloom, 2004). How could such force generating mechanisms be coupled to the cell cortex to place the spindle in the center of the cell? We can distinguish between two general possibilities: pushing forces generated at the cortex and pulling forces also generated at the cortex (see Figure 1).

Pushing at the Cortex

Microtubule polymerization can provide a pushing force at an unspecific site of interaction between astral microtubules and the cell cortex. Tubulin dimers are added on to the plus end of a microtubule, while the microtubule plus end itself is touching the cell cortex (Figure 1A). This mechanism can result in a stable force equilibrium at the center of the cell (Dogterom and Yurke, 1998) (Figure 3A).

The centering capability of such a mechanism was demonstrated in elegant in vitro experiments (Holy et al., 1997): microtubule asters that were placed within a microfabricated chamber utilized polymerization-based pushing forces to move toward the center of the chamber. However, the actual force that can be exerted decreases with the length of the microtubule because of buckling (Dogterom and Yurke, 1997), and there is little evidence outside yeast (Tolic-Norrelykke et al., 2004; Tran et al., 2001) that such a mode of MTOC or spindle positioning based only on pushing forces generally applies (Dogterom et al., 2005).

Pulling at the Cortex

In most cell types examined, specific sites at the cortex are able to capture astral microtubules. The microtubule is subsequently reeled inward, thus a pulling force is exerted upon the respective spindle pole (Figure 1B). There are many examples of positioning the spindle via pulling forces ranging from budding yeast (Pearson and Bloom, 2004) and *C. elegans* embryos (Gönczy, 2002) to vertebrates (Dujardin and Vallee, 2002). Direct evidence for pulling forces in spindle positioning comes from laser-cutting experiments. Here, the mitotic spindle was severed with a laser, and an outward movement of the two spindle poles demonstrates the existence of pulling forces (Aist and Berns, 1981; Aist et al., 1993; Grill et al., 2001).

The detailed molecular mechanisms of force generation generally remain obscure. Pulling forces could rely on microtubule depolymerization. When the microtubule contacts the cortical site, depolymerization of the microtubule generates a pulling force, providing that the microtubule does not detach from the cortex. Kinetochore-microtubule interactions provide ideas as to how a depolymerizing microtubule can stay attached to a cortical site (Biggins and Walczak, 2003; Howard and Hyman, 2003; Mitchison and Salmon, 2001; Westermann et al., 2005). There is molecular evidence for the existence of such a force-generating microtubule-end-on interaction with the cortex in budding yeast, involving the action of Bim1, Kar9, and the microtubule destabilizer Kip3 (Pearson and Bloom, 2004; Schuyler

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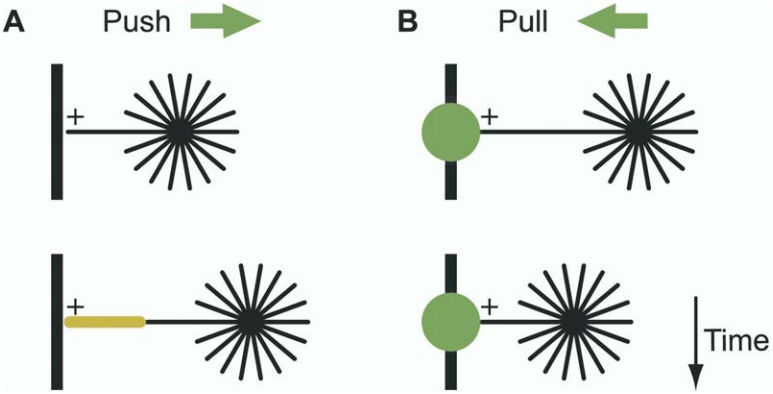

Figure 1. Force Generation at the Cortex
(A) Forces resulting from microtubule polymerization result in a pushing force exerted on the spindle pole at an unspecific cortical site. Yellow, newly inserted tubulin subunits.
(B) A cortical force generator (green) exerts a pulling force on the spindle pole at a specific cortical site.

and Pellman, 2001). Pulling forces can rely on cortical-anchored microtubule-based minus-end-directed motors. Upon capture, the motor walks into the minus-end direction of the microtubule and thus pulls the respective spindle pole toward the cortex. Dynein, anchored to the cortex via dynactin, is the most prominent example for such a motor (Dujardin and Vallee, 2002; Sharp et al., 2000). There are further possibilities. For example, a microtubule can be captured at a specific site, with the site itself then moving along the cortex, as proposed in budding yeast via the action of Myo2 (Beach et al., 2000; Hwang et al., 2003; Yin et al., 2000). In this case, the mechanical work is performed by an actin- rather than a microtubule-based motor. For the remainder of our discussion, we do not consider the detailed physical and molecular mechanisms of force generation. We presume that pulling forces are exerted upon the respective spindle pole after astral microtubule capture at specific cortical sites (Figure 1B). We discuss the idea that such pulling forces are sufficient to provide a general mechanism for spindle positioning during equal and unequal cell division.

Spatial Control of Cortical Force Generation: Equal Cell Division

How are cortical pulling forces distributed to position a spindle in the center of a cell? It would seem that if these sites were evenly distributed, a force balance at the center of the cell is guaranteed. However, there is a complication with this idea: the spindle must actually be capable of both finding and staying at the center of a cell. This means that any displacement away from the center must result in a force pointing back toward the center, a situation referred to as a "stable equilibrium." Furthermore, the geometric center of the cell must be the only position with a stable force balance throughout the whole cell.

As an example, if every microtubule radiating out from a MTOC is capable of attaching to a cortical site where a pulling force is generated, there is no reason to move to the center of a cell because the forces are balanced even if the centrosome is at an eccentric position (Figure 2A). In this case, cell geometry is not capable of defining a single point of force balance. This example raises the question as to how, in situations of cortical pulling forces present, a cell cortex can define and allow a spindle or MTOC to find a single point of stable force equilibrium at all.

Stable Equilibrium by Length-Dependent Forces

Hamaguchi and coworkers provided the first example of a mechanism involving pulling forces but circumventing the problem denoted above (Hamaguchi and Hiramoto, 1986). Here, length-dependent forces define a mechanism in which the center of the cell is stably defined. If force generators can attach along the whole length of a microtubule, the number of attached and pulling complexes increases with the length of the microtubule. This causes the aster to experience a net force in direction of longest microtubules and thus toward the center of the cell, as illustrated in Figure 2B.

This mechanism has been proposed to exist as shown by colcemid-UV experiments in sand dollar eggs (Hamaguchi and Hiramoto, 1986). Colcemid is a microtubule depolymerization agent that can be locally inactivated by irradiation with UV light. Cells were globally treated with colcemid, preventing the sperm aster from growing. Inactivation of colcemid by UV light in a circular region containing the sperm aster resulted in the outgrowth of microtubules from the sperm aster concomitant with the centering of the initially displaced aster within that circular region, thus elegantly providing the evidence for a length-dependent mechanism of force generation.

Although length-dependent forces have remained an appealing model, there are a number of problems with this idea. First, force generators need to be evenly anchored in the cytoplasm and not on the cell cortex. Only in flat cells, astral microtubules can align along the cortex and thus experience cortical length-dependent forces (O'Connell and Wang, 2000). In spherical or ovoid-shaped cells that extend equally in all three dimensions, astral microtubules need to bend if they align with the cortex, thus transmitting only a fraction of the cortical force to the centrosome. A length-dependent mechanism of cortical force generation is unlikely in these cases.

Stable Equilibrium by a Limited Number of Cortical Force Generation Sites

We propose another solution to the problem denoted above in which the number of force generator sites on

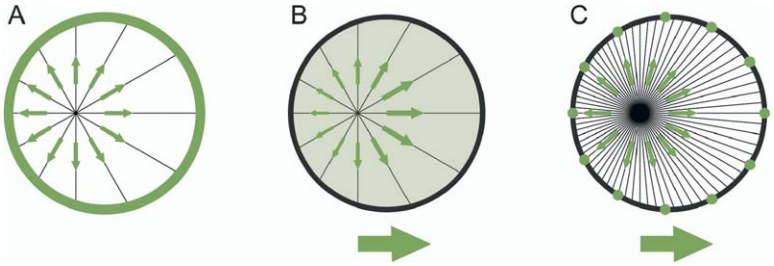

Figure 2. Conveying Cortical Geometry to Spindle Position in Situations of Cortical Pulling

(A) Cortical pulling paradox. If every astral microtubule that reaches the cell cortex is captured and pulled upon, the MTOC will always experience a force balance independent of MTOC position and should, thus, not move.

(B) Length-dependent forces provide a solution in the case of flat cells. If microtubules align along the cortex, they will experience a force that is proportional to their length. The MTOC will thus move in the direction of longest microtubules toward the center of the cell.

(C) A limited number of cortical force generation sites provides a general solution for cells extending equally in all three dimensions: if cortical sites are saturated by high densities of astral microtubules at the cortex, any displacement of the MTOC will lead to a net force pointing back toward the center of the cell. In the situation depicted, the MTOC is displaced to the left. The net force acting, however, points to the right because there are more cortical sites to the right than to the left of the MTOC.

the cortex is limiting. If these sites are sparsely and evenly distributed along the cortex, and there are always more microtubules reaching out to the cortex than there are possible capture sites, cortical geometry alone defines a single point of force balance. To understand this idea, consider Figure 2C (and compare to Figure 2A). If the number of cortical sites rather than the number of microtubules reaching out to the cortex limits the number of connections where forces are generated, there is always a force pointing to the center of the cell, independent of where the spindle pole is positioned. In Figure 2C, the MTOC is displaced from the geometric center of the cell. However, there are more cortical force generation sites to the right of the MTOC than there are to the left of the MTOC. As all of these cortical sites are saturated and pulling, the net force points back to the center of the cell. The mechanism is not as static as implied in the picture; astral microtubules stochastically search for cortical attachment sites by the use of dynamic instability with the requirement that there are always more microtubules radiating out to the cortex than there are microtubules attached to a cortical force generator.

Experiments from *C. elegans* embryos suggest that indeed the total number of cortical force generators is significantly lower than the number of microtubules reaching out to the cell cortex (Grill et al., 2003). Another hint that such a mechanism exists comes from *Dictystelium*, where only a few of the microtubules reaching out to the cortex are attached to cortical dynein, as indicated by a kink in the microtubules that are attached and pulled upon (see Figure 5 in Koonce et al. [1999]). However, it will be hard to distinguish such models of spindle positioning by conventional cytology. Rather, biophysical approaches will be essential to determine the precise number and distribution of active force generators in a cell.

Spatial Control of Cortical Force Generation: Unequal Cell Division

When a cell divides unequally, it is likely that the basic principles determining spindle position still operate. Therefore, any mechanism for asymmetric spindle positioning must be either layered on top of the basic mechanisms determining symmetric spindle position or act via a modulation of these. How does a cell position the spindle eccentrically? One obvious option is to change the distribution of cortical force generators. This shifts the point of force balance toward the region of increased cortical force generator density (Figure 3B), thus moving the spindle or MTOC off center. Evidence for eccentric positioning via a change in the distribution of cortical force generation sites comes from the *C. elegans* embryo. By fragmenting centrosomes with a UV laser and following the movement of centrosomal fragments toward the cell cortex (Grill et al., 2003), it was possible to first show that force generators are positioned all over the cell cortex. Second, an analysis of the fluctuations in the speeds of centrosomal fragments suggested that the same force generator operates throughout the embryo, with about 50% more of these force generators acting in the posterior compared to the anterior cell cortex. This discrepancy in cortical force generator number explains the eccentric position of mitotic spindle at the end of anaphase in the 1-cell stage *C. elegans* embryo. The actual displacement occurs before these experiments were conducted but is likely to reflect an increase in the extent of asymmetry in the distribution of cortical force generators from metaphase to anaphase.

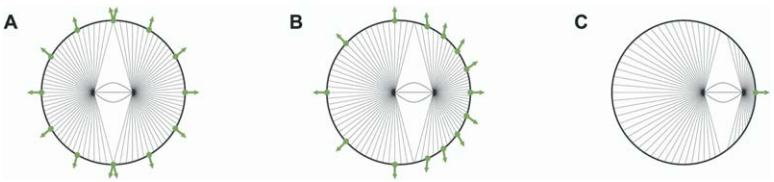

Figure 3. Spindle Positioning via Cortical Pulling and Limiting the Number of Force Generation Sites

(A) Symmetric spindle positioning via an isotropic distribution of cortical force generation sites.

(B) Eccentric spindle position via an anisotropic distribution of cortical force generation sites.

(C) Very eccentric spindle position via pulling from a single confined cortical site.

The clearest evidence for an asymmetric distribution of force generator sites comes from the extreme cases of very eccentric spindle positioning in a meiotic division. The most elegant experiments are those performed in *Chaetopterus* oocytes undergoing meiotic cell division (Lutz et al., 1988). In this study, the spindle was physically detached from the site with a microneedle. Positioning the spindle close to the cortex in other regions of the oocyte resulted in a movement away from the cortex. Only if either spindle pole were placed within close proximity of the cortical site, the spindle would migrate toward it at increasing rates. Spindle reorientation in *C. elegans* is another example of such a "confined pull" (Figure 3C), where a cortical site containing dynein and dynactin located at the boundary of the P1 and the AB cells is responsible for the proper rotation of the centrosomal-nuclear complex onto the axis of division in the P1 cell (Gönczy et al., 1999; Hyman, 1989; Skop and White, 1998).

There are further possibilities besides changing the distribution of force generators to achieve a point of force balance that is off center. The distribution of their activity can be changed, for example, by modulating the average time that a microtubule stays attached. Indeed, a measurement of mean cortical residency times of astral microtubules showed that there is a difference between the times that astral microtubules spent at the posterior compared to the anterior cortex in *C. elegans* (Labbe et al., 2003). Finally, a cell may rely on a combination of different strategies to achieve the task. Also, more elaborate mechanisms of spindle positioning could exist that act on top of the simple geometric concepts we have presented here. Examples are the consideration of the dependence of force on the angle between the astral microtubule and the cortex (Tsou et al., 2003) and the consideration of the dynamicity of astral microtubules and the whole spindle structure. Microtubules will reform a single, centered microtubule aster in cell fragments that are cut off with a microneedle (Rodionov and Borisy, 1997), and a theoretical analysis shows that microtubule turnover is essential to this process (Cytrynbaum et al., 2004). Further mechanical perturbation experiments and detailed theoretical models will be required to understand how exactly the task of generating a single point of force balance in a central or an eccentric position within a cell is achieved.

It is clear that cell polarity must provide the spatial control in all the cases where the spindle is positioned off center (Horvitz and Herskowitz, 1992). In *C. elegans* and *Drosophila*, cell polarity is communicated to the cytoskeleton via hererotrimeric *G*-protein-mediated signaling (Betschinger and Knoblich, 2004; Gönczy, 2002). Although the detailed mechanism of regulation down to the level of force generation sites remains to be elucidated, these events take place in the right location: at the cell cortex (Cowan and Hyman, 2004). The regulatory machinery should thus be able to utilize the fundamental mechanisms we have presented here to control daughter cell size.

Conclusions

For a spindle to position itself within the cell, it is necessary to convey the geometry of the cell cortex to spindle position. We have discussed several solutions to this problem. However, we suggest that cortical pulling forces provide a general mechanism for positioning spindles and MTOCs during interphase and both in equal and unequal cell division. During central spindle or MTOC positioning in equal cell division or during interphase, a limited number of cortical force generator sites can allow the spindle or MTOC to move to the center of the cell. During eccentric spindle positioning in unequal cell division, an asymmetric distribution of active force generators shifts the balance to an off-center location. A limited number of cortical sites is still required to convey a single point of force balance, and the extent of asymmetry in the distribution will determine the extent of spindle displacement.

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