JCB Mini-Review

Cleavage furrow positioning

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To complete the cell cycle, the cleavage furrow draws the plasma membrane toward the cell center, pinching the cytoplasm into two lobes that are subsequently separated into two cells. The position of the cleavage furrow is induced by the mitotic spindle during early anaphase. Although the mechanism of cleavage furrow positioning is not understood at a molecular level, recent results suggest that it might be mediated by local relief from the inhibitory effects of microtubules.

Faithful propagation of the genome requires more than high fidelity DNA replication and equal segregation of the replicated chromosomes on the mitotic spindle. The chromosomes must also be stably partitioned into the two daughter cells by cytokinesis. In animal cells, the mechanical force for cell division is generated by myosin II as it translocates actin filaments within the contractile ring. The contractile ring assembles in the cell cortex, an actin-rich layer juxtaposed to the cell membrane, in a position that is determined by the mitotic spindle during anaphase. The mechanism by which the spindle positions the division plane remains an important open question in cell biology.

Supramolecular control of furrow formation

Microdissection, genetic, and inhibitor experiments have been used to define the parts of the spindle that are required for cleavage furrow induction. Chromosomes have been shown to be dispensable for cytokinesis (Rappaport, 1996; Zhang and Nicklas, 1996; Bucciarelli et al., 2003; Dekens et al., 2003). Likewise, centrosomes can be ablated or genetically disrupted without preventing cytokinesis (Khodjakov and Rieder, 2001; Megraw et al., 2001). Though chromosomes and centrosomes are dispensable, they may influence the process when they are present (Piel et al., 2001). In addition to chromosomes and centrosomes, the spindle contains a large array of microtubules. Microtubule depolymerization during metaphase or very early anaphase prevents cleavage furrow formation, indicating that microtubules are essential (Hamaguchi, 1975). However, furrow formation can occur if the mitotic spindle is depolymerized later in anaphase, but before ingression has begun (Hamaguchi, 1975). Thus, mitotic spindle microtubules are required to induce furrow formation, but they are not, per se, required for ingression.

Further insight into the mechanism of cleavage furrow induction has come from experiments in which cells, usually embryos, are physically manipulated and their potential to cleave assessed. These perturbations include alteration of the position of the spindle with respect to the cell cortex, cell shape deformation, and removal of parts of the spindle. For example, the classic "torus experiment" in which two spindles in a common cytoplasm induce an additional furrow indicates that opposing asters are sufficient to induce a furrow (Rappaport, 1961). Additionally, repositioning of the spindle during anaphase results in multiple cleavage furrows whose positions are dictated by the spindle (Rappaport, 1985). Results of numerous experiments of this type have led to the astral stimulation model (Fig. 1 A; Rappaport, 1996). This concept assumes that astral microtubules provide a cleavage stimulus, which, for example, could be a factor that is transported along astral microtubules. This model proposes that because the equatorial cortex is influenced by astral microtubules from two poles, the strength of this stimulus would be highest at the cell equator. With some assumptions concerning the nature of the signal, its mode of delivery, and the distribution of microtubules, computer modeling indicates that a cleavage stimulus could reach a maximum at the equatorial region (Devore et al., 1989; Harris and Gewalt, 1989).

A second hypothesis, termed astral relaxation, asserts that astral rays (i.e., microtubules) cause a reduction of cortical contractility in a density-dependent manner. According to this model, the density of astral rays is higher near the poles than at the equator, assuming spherically symmetric asters in spherical cells. This would cause the polar regions to be less contractile than the equator, and this difference in contractility would induce equatorial furrowing (Fig. 1 B; Wolpert, 1960). Quantitative modeling confirmed that this model could, in principle, allow furrow formation, but indicated that a positive feedback loop during contractility would be required to allow complete ingression (White and Borisy, 1983; Yoshigaki, 1999).

These two models come to opposite conclusions regarding the role of astral microtubules because they differ in their underlying assumptions about the distribution of microtubules, their lengths, and the way in which they interact with the cell cortex. In addition, it is now apparent that activities exist that bundle microtubules from opposing asters and generate a structure that is called the central spindle (also known as the

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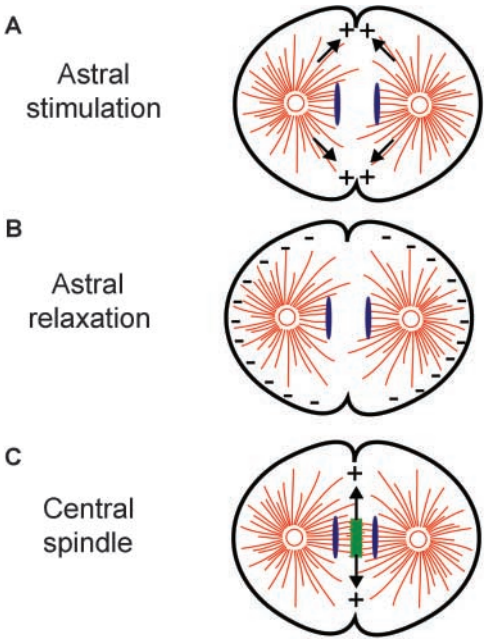

Figure 1. **Three schematic models for cleavage furrow positioning.** See text for details.

spindle midzone). The evolutionarily conserved centralspindlin complex containing a kinesin-like protein Mklp1 and a Rho family GAP, HsCYK-4/MgcRacGAP (Mishima et al., 2002), is one such factor. Centralspindlin is directly involved in central spindle assembly because it localizes to the central spindle and has microtubule-bundling activity (Mishima et al., 2002). Another important factor in central spindle assembly is the microtubule-binding protein PRC1 (Mollinari et al., 2002). Because there is evidence that antiparallel microtubule bundles can regulate furrowing (see below), some of the micromanipulation experiments that have led to the astral stimulation and relaxation models may need to be reinterpreted.

Indeed, observations in *Drosophila* provide compelling evidence that astral microtubules may not be critical for furrow formation and that the central spindle is necessary and sufficient to induce furrow formation (Fig. 1 C; Giansanti et al., 2001). In particular, cells deficient in the kinesin-like protein Pavarotti (the orthologue of Hs MKLP1/Ce ZEN-4) fail to form a central spindle, have rather normal appearing astral microtubules, and do not form cleavage furrows (Adams et al., 1998; Somma et al., 2002). Conversely, asterless mutants, which lack most astral microtubules but retain a central spindle, are still capable of forming cleavage furrows (Bonaccorsi et al., 1998). These data fit neither the astral stimulation nor the astral relaxation model, and suggest that the central spindle is responsible for furrow induction.

Additional evidence supports the notion that the central spindle is involved in furrow formation. In cultured rat cells, if a small perforation is created adjacent to the central spindle, furrow formation occurs on the side of the perforation adjacent to the central spindle and not at the cortical site where furrow formation would have occurred in an unmanipulated cell (Cao and Wang, 1996). Furthermore, grasshopper spermatocytes have been manipulated to simultaneously remove centrosomes and chromosomes, and the remaining microtubules self-organize into bundles that resemble the central spindle and appear to induce furrow formation (Alsop and Zhang, 2003). These results, combined with the fact that many key regulators of mitotic events localize to the central spindle, have lead to the proposal that central spindle microtubules (or more generally, antiparallel microtubule bundles) are a principle regulator of furrow formation.

However, there is also compelling evidence that the central spindle is dispensable for cleavage furrow formation. In *Caenorhabditis elegans* embryos, disruption of the central spindle does not prevent cleavage furrow ingression. Under these conditions cleavage furrows form and constrict, but they fail to complete cytokinesis (Powers et al., 1998; Raich et al., 1998; Jantsch-Plunger et al., 2000).

The dramatically different requirement for the central spindle in furrow formation in *Drosophila* and *C. elegans* could result from differences in cell size in these systems. Alternatively, the critical determinant for furrow formation may not be evolutionarily conserved. Indeed, some variation has been reported in the localization of critical factors that regulate cytokinesis. For example, in *Drosophila*, in addition to the central spindle localized pool of Pavarotti, there is also a cortically localized pool that is not detected in other organisms (Sellitto and Kuriyama, 1988; Adams et al., 1998; Powers et al., 1998; Raich et al., 1998; Minestrini et al., 2003). Conversely, in mammalian cells, ECT2 (a GEF for RhoA) is readily detected in association with both the cell cortex and the spindle, but its orthologue in *Drosophila* is primarily associated with the cell cortex (Prokopenko et al., 1999; Tatsumoto et al., 1999). However, recent results suggest that neither cell size nor lack of conservation underlies the variable degree to which the central spindle controls furrow formation, and indicate that this process is controlled by two parallel pathways. In *C. elegans* embryos, the central spindle is not generally essential for furrow formation. However, if the extent of spindle elongation during anaphase is reduced by one of several genetic perturbations, the central spindle becomes essential (Dechant and Glotzer, 2003). In addition, although furrow formation can occur in the absence of the central spindle, initiation of cytokinesis is slightly delayed under these circumstances. Thus, perhaps different cell types use both astral microtubules and the central spindle for furrow formation, albeit to varying degrees. Indeed, there is evidence for plasticity in the induction of cleavage furrows in mammalian cells. Microsurgical experiments indicate that the central spindle has furrow-inducing activity, yet cells depleted for key central spindle components, such as MKLP1 or PRC1, still form furrows (Cao and Wang, 1996; Matuliene and Kuriyama, 2002; Mollinari et al., 2002).

Molecular control of furrow formation

Given that both the central spindle and astral microtubules can contribute to induction of cleavage furrows, at least under some circumstances, proteins that localize to these structures are potential clues to the mechanism of furrow induction. The central spindle in particular contains numerous factors implicated in cytokinesis. In principle, these factors could regulate furrow formation in two ways: they could be positive inducers of furrow formation, or they could inhibit a negative regulator of furrow formation.

Delivery of an activator of furrow formation

There are several factors that concentrate on the central spindle that have been suggested to be inducers of cleavage furrow formation. One candidate is the ABI complex consisting of Aurora B, INCENP, and Survivin/BIR-1 (Adams et al., 2000; Kaitna et al., 2000; Kang et al., 2001; Bolton et al., 2002; Cheeseman et al., 2002; Honda et al., 2003; Romano et al., 2003). In nematodes this complex contains a fourth protein, CSC-1 (Romano et al., 2003). In mammalian cells, INCENP first localizes to chromosomes during prometaphase, then it concentrates on centromeres during metaphase, and then, upon anaphase onset, it localizes to both the central spindle and, interestingly, the overlying cell cortex (Cooke et al., 1987). Both astral microtubules and the central spindle contribute to cortical localization of Aurora B (Murata-Hori and Wang, 2002), presumably due to interactions with INCENP and Survivin, whose sole function appears to be to activate and localize Aurora B. Interestingly, Aurora B localizes to the central spindle in cells that lack chromosomes (Bucciarelli et al., 2003), indicating that these subcellular targeting events are independent. The cortical localization of the ABI complex precedes the early stages of cytokinesis (Eckley et al., 1997). Although this localization of the ABI complex suggests that it may direct cleavage furrow formation, cells deficient in Aurora B (due to mutation, RNAi-mediated depletion, or chemical inhibition) are competent to form cleavage furrows (Schumacher et al., 1998; Fraser et al., 1999; Kaitna et al., 2000; Hauf et al., 2003). Therefore, Aurora B is not essential for cleavage furrow formation. However, functional redundancy may obscure a role for Aurora B in furrow formation.

A second potential activator of cleavage furrow formation that could link the central spindle to cleavage furrow formation is the RhoGEF, Pebble. Pebble was recently shown to associate with *Drosophila* centralspindlin (Somers and Saint, 2003). Pebble (*Hs* ECT2/*Ce* LET-21) is essential for furrow formation, presumably because it is the critical activator of RhoA in cytokinesis (Prokopenko et al., 1999; Tatsumoto et al., 1999). Two-hybrid analysis indicates that the NH_2 terminus of Pebble binds to the NH_2-terminal region of the fly orthologue of CYK-4, RacGAP50C. Concentration of centralspindlin in the spindle midzone could thereby recruit Pebble and induce the local activation of RhoA, followed by actin polymerization and cleavage furrow formation. If this were the case, then cells defective in central spindle formation would also be expected to be defective in furrow formation. Although coupling of these two processes is observed in *Drosophila*, this is not the case in *C. elegans* embryos or in mammalian cells. Moreover, overexpression of the NH_2-terminal domain of the Pebble orthologue, ECT2, causes a late defect in cytokinesis (Tatsumoto et al., 1999), not the early defect expected if the association of Pebble with centralspindlin was essential for spatial regulation of Pebble function. Thus, although Pebble is critical for furrow formation, its association with the central spindle does not appear to be critical in all species. The association of Pebble with centralspindlin might promote the continued ingression of the cleavage furrow by maintaining RhoA in an active state. It will certainly be interesting to understand the interplay between the RhoGAP and the RhoGEF in this unusual protein complex.

Local inhibition of a negative regulator of furrow formation

An alternative way to regulate furrow formation is through local inhibition of a negative regulator. Experiments in mammalian cells and *C. elegans* embryos suggest that during cytokinesis, microtubules inhibit the contractility of the cell cortex. When microtubules are induced to be unusually short by prolonged activation of the katanin microtubule-severing complex, additional furrows are observed at cell poles (Fig. 2 A; Kurz et al., 2002). Similarly, mammalian cells forced to exit mitosis in the absence of microtubules undergo vigorous unorganized contractions (Canman et al., 2000). These data indicate that microtubules inhibit furrow formation. Given that there is compelling evidence that microtubules control furrow formation, it is conceivable that modulation of their distribution or properties could control furrow formation. In principle, bundling of microtubules to form the central spindle could quantitatively and/or qualitatively regulate the inhibitory effects of microtubules. Thus, a second mechanism by which the central spindle could promote furrow formation is by inhibiting this negative regulation.

Spindle elongation and central spindle assembly act together to create a local minimum of microtubule density at a position in the cell equator equidistant from the two spindle poles (Dechant and Glotzer, 2003). Importantly, these two processes also act in parallel to promote furrow formation (Fig. 2 B). These observations are consistent with a model in which the position of the cleavage furrow in the equatorial region is determined by a site where the inhibitory effect of microtubules reaches a local minimum (Fig. 2 D).

Central spindle assembly may not only affect the spatial organization of microtubules—it could also alter the capacity of microtubules to inhibit contractility by changing the properties of the microtubules. For example, inhibition of cortical contractility could rely on the dynamics of microtubules or microtubule-associated proteins. Binding of factors to the ends of the microtubules could alter their properties or dynamics. Indeed, early work in mammalian cells indicated that midzone microtubules are more stable than elsewhere in the cell (Saxton and McIntosh, 1987), and more recent observations confirm and extend these findings. In particular, before furrow ingression, a subset of microtubules in the vicinity of the presumptive furrow are significantly more stable than microtubules near the cell poles (Fig. 2 C; Canman et al., 2003). It is not yet known if the stabilization of microtubules in the equatorial region is mediated by the centralspindlin complex, but this seems likely because it is present there, and overexpression of the kinesin subunit of centralspindlin (MKLP1/Pavarotti) induces hyperstabilization of microtubules (Minestrini et al., 2002). Thus, binding of centralspindlin to microtubules could induce central spindle assembly and simultaneously prevent the microtubule-dependent inhibition of furrow formation.

The mechanism by which microtubules inhibit cortical contractility and control cytokinesis is not known. The RhoA exchange factor, *Dm* Pebble (*Hs* ECT2, *Ce* LET-21) is one of the most upstream molecules in this pathway and is a candidate for regulation by microtubules. However, little is known about how this critical exchange factor is regulated, except that its activity requires phosphorylation (Tatsumoto et al., 1999). Additionally, although RhoA and some of its

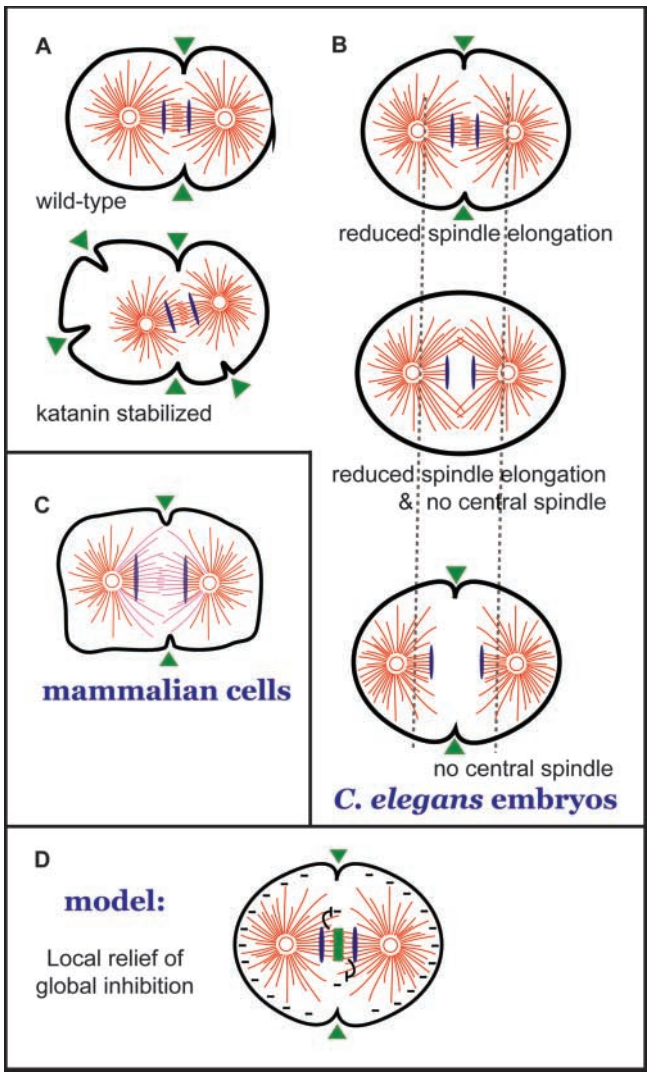

Figure 2. **Experimental evidence that furrow positioning may result from local relief from microtubule-dependent inhibition of furrow formation.** (A) Stabilization of the microtubule-severing complex katanin leads to microtubule shortening and ectopic furrowing. (B) Either central spindle assembly or spindle elongation are sufficient to induce furrow formation, but when both pathways are inhibited, no furrow formation occurs. (C) In mammalian cells, a subset of the microtubules in the vicinity of the furrow are less dynamic (purple) than elsewhere in the cell. (D) Model for furrow positioning.

effectors concentrate at the cleavage furrow, there is no direct evidence for local differences in RhoA activity early in cytokinesis. Recently, a FRET-based approach to observe active RhoA during cytokinesis was reported. These probes did not reveal detectable amounts of active RhoA during early cytokinesis, but active RhoA did appear late in cytokinesis (Yoshizaki et al., 2003). Because RhoA activity is required for the initial stages of furrow formation, it is possible that low levels of active RhoA drive furrow formation, and these levels of RhoA were below the detection limit of these probes. Second generation probes specific for active forms of RhoA and other molecules essential for cytokinesis may provide further insight into this important question.

However, given that there are several precedents for local activation of GTPase signaling complexes, a reasonable working model is that a local increase in RhoA signaling induces furrow formation. If so, furrow positioning could simply be explained if astral microtubules inhibit RhoA activation, thereby inhibiting furrow formation at ectopic sites, and central spindle assembly and spindle elongation conspire to provide local relief from these inhibitory effects and allow RhoA activation at the equatorial region. Interestingly, microtubule depolymerization in interphase cells causes activation of RhoA-GTP (Ren et al., 1999), implying that microtubules can, directly or indirectly, inhibit RhoA. Moreover, a particular RhoGEF, GEF-H1, is inhibited by microtubule-mediated sequestration (Krendel et al., 2002), illustrating one such mechanism. Importantly, RhoA may not be the only factor whose activity is spatially restricted. Further analysis of the biochemical events that occur during furrow initiation is absolutely essential, with particular attention paid to how these events might be regulated by microtubules.

Concluding remarks

At this juncture, furrow positioning does not appear to be solely due to induction by astral microtubules or the central spindle, but rather, both components contribute. Cleavage furrow induction through local relief from the inhibitory effects of microtubules is an appealing model because it explains how two pathways, spindle elongation and central spindle assembly, could control furrow formation through a common molecular mechanism. In addition, it accounts for the fact that the central spindle has a positive (though nonessential) role in furrow formation. It also has predictive value in that local depolymerization of microtubules should induce cleavage furrows.

Much remains to be learned about the regulation of furrow positioning by microtubules. In particular, it is tempting to speculate that the signals discussed here are important for patterning of the cortex in response to a local inhomogeneity in the distribution of microtubules. Subsequent reactions may be required to refine this positional information and to amplify the signal that directs assembly of the contractile ring. Alternatively, contractile ring assembly might be a cooperative process that is self-refining and amplifying. Many mysteries remain concerning this critical step in the cell division cycle.

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