Cytokinesis series

Site selection for the cleavage furrow at cytokinesis

David R. Burgess¹,² and Fred Chang²,³

¹Boston College, Department of Biology, Higgins Hall, 140 Commonwealth Ave, Chestnut Hill, MA 02167-3811, USA
²Cytokinesis Group, Marine Biological Laboratory, Woods Hole, MA 02543, USA
³Columbia University College of Physicians and Surgeons, Department of Microbiology, 701 W. 168th St., New York, NY 10032, USA

The question of how the site for division of the cytoplasm is determined at the end of mitosis has been studied for over a century, and it remains an active, controversial and fascinating problem in cell biology. This problem draws on the use of several model cell types, with the goal of understanding and identifying how the cell cycle regulates signals between the mitotic apparatus and the cell cortex. Studies in different cell types and using a vast array of techniques reveal different answers: these might reflect differences in experimental approaches, multiple and redundant mechanisms and, importantly, diversity in biology. In this article (which is part of the Cytokinesis series), we present a summary and critique of the major models for the roles of the mitotic apparatus microtubules in stimulating furrow formation at cytokinesis.

Ray Rappaport, a pioneer of the cytokinesis field, has remarked that no new theory of cytokinesis can be proposed because they have all been proposed in the last hundred years [1]. Indeed, in the long and colorful history of cytokinesis, theories have been proposed and knocked down, only to reappear in new clothes. This trend continues. Despite the advent of new experimental technologies and model genetic systems, little is known about this interesting and complex process. In this review, we discuss first the major models to describe how microtubules (MTs) of the mitotic apparatus (MA) specify the division plane in animal cells. Second, we discuss recent developments in fission yeast that are beginning to reveal the molecular signals in this model organism. It is worth noting that the later events of cytokinesis, including new membrane addition, the mechanics of furrowing, late furrow progression, myosin II regulation, disassembly of the contractile ring and abscission, are discussed in other reviews in the Trends in Cell Biology series on cytokinesis [2].

Role of the mitotic apparatus in cell-division placement in animal cells

The specification of the cell-division site by the mitotic apparatus provides a way to spatially and temporally coordinate cell division with chromosomal segregation. The furrow forms at the equatorial site between the centrosomes, in a plane that bisects the former metaphase plate. Early studies using either MT inhibitors or cold to depolymerize MTs established that the positioning of the cleavage furrow requires MTs [3]. The MTs in the MA send ‘signals’ to the cortex that regulate the assembly and contractility of the contractile ring. However, most aspects of this signaling process either remain unknown or are controversial. Not only are the molecular aspects of this process unknown, but the parts of the MA that ‘emit’ these signals and whether these signals either activate or inhibit contractility of the cell cortex are under debate.

We suggest a nomenclature for MTs in the anaphase MA so that they can be discussed more readily (Figure 1). Our nomenclature reflects the different geometrical relationships with the future cleavage furrow rather than functional attributes such as dynamics, stability and associated proteins. Kinetochore MTs are the bundled MTs from the centrosomes that attach to the chromosomes at the kinetochores. The spindle midzone is an extensive network of overlapping bundles of non-kinetochore MTs in the equatorial region of the spindle. Astral MTs are defined loosely as MTs outside these categories. In many animal cells, astral MTs grow rapidly at the onset of anaphase from the centrosomes to the cortex, one consequence of the requirement of anaphase onset for cytokinesis. [4] The astral MTs that grow towards the equatorial region are termed ‘equatorial astral MTs’, whereas those that grow towards the pole are termed ‘polar astral MTs’. The geometrical relationships of the MT to the cell cortex vary between the major model cell types. In large eggs, the spindle midzone is in the interior of the spherical cell, whereas in smaller cells, it may be closer to the cortex. It is not clear whether the molecular details of spindle midzone MTs are different to those of equatorial and polar astral MTs. The different nomenclature used by investigators for these sets of MTs can be a source of confusion, and so the definitions must be carefully considered.

Here, we present current models (Figure 2) for how the MA specifies the placement of the division site and discuss some key experiments that either favor or disfavor each. Each model emphasizes a different part of the MA; the equatorial astral MTs, the polar astral MTs, the spindle midzone and the kinetochores.
The equatorial stimulation model

This model proposes that equatorial astral MTs impart a positive signal to the cortex for formation of the cleavage furrow [1]. The strongest support for this model is Ray Rappaport's classic series of micromanipulation experiments using echinoderm eggs [1]. Rappaport and his predecessors termed the MA MTs as astral 'rays' or 'fibers' because they did not have the techniques to image MTs directly, but observed the MA mostly by brightfield and phase-contrast light microscopy.

Many elegant experiments show that the MA imparts an activating signal for furrow formation, not a negative one (as proposed in the polar relaxation model below). For example, asymmetrical placement of the MA leads to cleavage in the vicinity of the MA. This often occurs naturally, for example in Caenorhabditis elegans and amphibian zygotes, micromere formation in sea urchin embryos, and in polar body and polar lobe formation in oocytes [5–9]. In addition, moving the MA to one part of the cell by either displacing the MA with a needle close to one side of an egg [10] or by centrifuging the cell [11–13] also leads to initiation of the cleavage furrow at the midpoint of the MA at the new site. Rappaport formed echinoderm eggs into cylindrical shapes in which it was unlikely that astral 'rays' could contact the polar cortex and showed that they divided always at the plane between the spindle poles [14]. By further manipulation of cylindrical cells, he moved a single MA to multiple sites, and demonstrated that it could induce up to 13 furrows, always at the site occupied by the former plane of the metaphase plate [15,16]. To test whether signals were emitted towards either the equator or the poles, oil droplets and holes in the cell were introduced into cells to block the putative signal. Blocks between the asters and equatorial region inhibit cleavage on the normal surface, whereas those between the asters and the poles have little effect [17,18]. Many experiments of this type that support equatorial stimulation were used by Rappaport [1] to rule out competing models, such as polar relaxation (see below).

Rappaport’s experiments indicate some aspect of the asters induces the furrow in eggs. In Rappaport’s famous torus experiment, he examined cytokinesis in torus-shaped cells from sand dollars that have two MAs [19]. He discovered that furrows form between the asters of each MA in the normal equatorial position as well as between the asters of different MAs, termed a ‘Rappaport furrow’ by others in the field. These furrows form at sites that lack chromosomes and spindle midzone MTs, which demonstrates that chromosomes (including kinetochores)
and midzone MTs are not required for furrowing. Hiramoto used micro-aspiration to remove parts of the MA, such as the chromosomes, spindle midzone and asters, and showed that asters alone are necessary and sufficient for induction of cleavage [20]. Similar Rappaport furrows are also induced in multipolar mammalian cells and between cytasters with no associated chromosomes [21–23]. Interestingly, analysis of the microtubule bundles associated with Rappaport furrows in vertebrate cells reveals densities in the electron microscope and association of some chromosomal passenger proteins (see below) [23]. There are many examples in different species of pairs of asters that lack chromosomal material and have the ability to induce a furrow [24–27].

One challenge of the equatorial stimulation model is how to account for placement of the site at the geometric center between a pair of asters. Two asters might increase the local concentration of inductive signals that is sufficient for furrow formation. Increased MT densities at the cortex might be responsible for this effect (although such attribution is not part of the original model). Additionally, MTs from pairs of asters might form bundles of anti-parallel MTs at the equatorial cell surface, which have the potential to provide the inductive signals (see spindle midzone model). One significant weakness of hypothesizing higher densities of MTs at the equatorial cortex is that direct observations of preserved cells by transmission electron microscopy and immunofluorescence microscopy shows fewer, not more, MTs in this zone [28,29]. Furrows can also be made to form in locations other than at the equatorial point between two centrosomes. Rappaport produced ice cream cone-shaped eggs that formed furrows in a plane perpendicular to the spindle axis on the small side of the cone [30]. Single asters, which do not appear to have overlapping MTs, also induce furrows [31,32]. Using sand dollar eggs shaped in the form of a cylinder, Rappaport disassociated the two asters by micromanipulation [31]. Furrows formed at both the region where the nucleus remained with one aster (monopolar spindle) and also at the region occupied by the single aster. In a recent paper, Canman et al. [33] have confirmed and extended this result by generating monopolar asters using the kinesin inhibitor monasterol in vertebrate cells. By inhibiting the spindle checkpoint, they found that these monopolar asters induced furrows distal to the chromosomes.

One interpretation of the astral stimulation model is that these MTs might transport to the cortex factors that induce contractile ring assembly in a spatially distinct manner. Recent studies demonstrate that plus-ends of MTs are slightly more stable at the future cleavage site (MT dwell time of 2–3 min rather than 1 min), which indicates that factors on the MT plus-ends interact with the cortex during this process [33], although dynamic microtubules do not appear to be necessary to induce furrowing [34]. It is also possible that MTs interact with the cortex through lateral interactions (see later). However, the function and molecular basis of MT-cortical interaction has not been tested. Although the role of proteins that interact with the plus-ends of MTs in this process is yet to be examined, they are likely candidates for communicating with the cell cortex [35,36].

The polar relaxation model

The polar relaxation model states that astral MTs relay signals to the polar cortex that locally inhibit cortical tension [37,38]. This relieves cortical tension locally only in the poles in a cell that was under overall global tension [39]. This model is supported by mathematical modeling and direct measurements of MT distribution, which indicate that equatorial astral MTs in eggs are less dense at the cortex of the future division site than polar astral MTs in the polar regions [28,29,38,40]. However, one caveat to the MT-density measurements are that they were obtained in fixed specimens, which might not reflect the true dynamic interactions of MTs with the cell cortex. Nevertheless, assuming that astral MTs are equivalent and preserved adequately for immunofluorescence, there might be more signals from astral MTs at the poles, not at the equatorial regions as proposed by the equatorial stimulation model.

Consistent with the polar relaxation model, MTs have been associated with a ‘relaxing’ effect on the cortex. During the early cleavages in C. elegans, extensive ruffling (contractile events?) occur in cortical regions in which MTs are less dense [41]. In addition, in a mutant with shortened astral MTs (caused by overactivity of katanin, a MT-severing protein), the whole cortex seems to be overly contractile and ‘furrows’ form all over the cortex [41].

Rappaport spent much of his career testing the polar relaxation model in echinoderm eggs. For example, by physically altering the geometry of the cell, he found that cells still divided between the astral poles even after the polar cortical regions were moved far from the MA [14]. In another experiment, constricting the equatorial cortex inward at metaphase to make the density of astral fibers at the poles either less than or equal to that in the equatorial region also resulted in normal cleavage [42]. If it were possible, a similar experiment in C. elegans would be informative. In total, Rappaport’s experiments make a strong argument that polar relaxation by itself is not sufficient to explain the effects of the MA on furrow specification, at least in echinoderm eggs.

Although there is some dispute over whether MTs induce or inhibit contractility, it is likely that regulation of contractility during cytokinesis is considerably more complex than any single change at the pole or equator. In cells in tissue culture, local application of the actin inhibitor cytochalasin at the pole inhibits cleavage, whereas application at the equator does not [43]. The opposite results were found by Rappaport in echinoderm eggs; cytochalasin inhibits cleavage only at the equator, not the poles [44]. Experiments and modeling are beginning to indicate that both global and local changes in cell-surface tension might occur in several steps and might be different in different cell types.

The spindle midzone model

This model states that the spindle midzone, which comprises bundles of anti-parallel, non-kinetochore MTs, induces the cleavage furrow [45,46]. Early studies using micromanipulated grasshopper neuroblasts showed that bending the MA causes the furrows to form initially near the spindle midzones, not at the asters [47]. Disruption of
the spindle midzone by either micromanipulation or genetics cause cytokinesis defects of varying degrees in different cell types [28,45,48,49]. In insect cells, the close interaction between the spindle midzone and contractile ring is shown by their co-dependent relationship: the spindle midzone is required for ring assembly and ring-assembly factors affect the organization of MTs at the spindle midzone. These studies also call into question the requirement for the astral microtubule array because asterless mutants undergo furrowing [49].

Bundles of overlapping, anti-parallel MTs themselves might be sufficient for furrow induction. Using microsurgery to selectively remove components of the MA, Alsop and Zhang have tested the ability of asters, spindle midzomes and MTs to induce a cleavage furrow [50,51]. This shows that a MT bundle, disassociated from the rest of the MA, can induce a cleavage furrow. Although the spindle midzone contains the most impressive array of overlapping MTs in the MA, it is possible that in Rappaport’s experiments described above overlapping MTs from two (either related or unrelated) asters form the equivalent of spindle midzones at the cortex, and might be the genesis of Rappaport furrows. This perspective provides a way to view the spindle midzone model as a modification of the equatorial stimulation model, which might reconcile some results. However, this overlapping MT model seems inconsistent with observations that monopolar asters, which apparently lack overlapping MTs, still position the inner centromere protein INCENP and induce cleavage [31,33].

The spindle midzone might contribute to cytokinesis by localizing many proteins that regulate cytokinesis. Spindle midzone factors include MLP kinesin, which contributes to MT bundling, a Rho GTPase-activating protein (GAP) and a Rho GDP–GTP exchange factor (GEF) [49,52–57]. Numerous chromosomal passenger proteins including INCENP, aurora kinase and survivin locate to this region and affect aspects of cytokinesis [58,59]. In general, however, these factors are not absolutely required for the initial contraction of the contractile ring because depleted cells usually begin to cleave, but they appear to be important for furrow stability and completion.

The kinetochore signaling model
For the proponents of the equatorial stimulation model, one challenge is to address the fact that there is often no local increase in MTs at the future division site on the cortex. One solution is that not all astral MTs are equivalent. Despite evidence in several systems that chromosomes are not required for cleavage, recent molecular and imaging studies indicate a kinetochore signaling model. Canman et al. observed that stabilized MTs at the future division site either ‘pass through’ or emanate from kinetochores, and suggested that factors might travel directly from the kinetochore to the cortex via these MTs [33]. These factors might stabilize the MTs but might also induce assembly of the contractile ring at the cortex. The polarity of these MTs is unknown in this experiment. In Rappaport’s experiment with single, detached asters, the aster with chromosomes (monopole) induced furrows more strongly than its sister aster with no chromosomes [31], which supports a kinetochore signaling model. The geometrical relationships of kinetochore-associated MTs and the future division site makes sense in normal cell division and provides an attractive way of specifying a subset of equatorial astral MTs. However, the data relating kinetochores to cytokinesis are correlative, and many experiments and observations discussed previously, which show that furrows form in the absence of chromosomes, argue against such a model.

However, consistent with this model, is the fact that kinetochores and the contractile ring share many proteins in common. A set of ‘chromosome passenger’ proteins localizes to kinetochores, the spindle midzone and then the contractile ring (see above). Several regulate dynamics of the MT plus-end, including dynein/dynactin, EB1 and CLIP170 [35,36]. These might function to stabilize the MT plus-end at the kinetochore and the cortex. To date, however, no chromosome passenger protein has been shown definitively to affect furrow specification. The recent report of the formin mDia3 at the kinetochore and the contractile ring makes it an attractive candidate for a ring-assembly factor that emanates from the kinetochores [60]. Formins have been implicated in actin assembly at contractile rings and in stabilization of MTs in different cell types [61–63]. Although depletion of mDia3 causes chromosomal segregation defects [60], its possible role in cytokinesis remains to be tested. In fission yeast, molecular mechanisms that describe how MT plus-end factors and formins regulate the spatial distribution of actin assembling are being revealed [64]. The characterization of MT plus-end factors in animal cell cytokinesis is an important area of future research.

Division-site determination by the nucleus in fission yeast
Other eukaryotic cells might use different cellular structures as spatial cues for placement of the division sites. Fission yeast, *Schizosaccharomyces pombe*, and some plant cells might use the nucleus rather than the MA [65]. In haploid budding yeast, bud scars on the cell surface that are deposited from previous cell cycles might specify the bud site and the future division site in G1 of the current cell cycle [66].

*S. pombe* is an established model for the molecular genetic analysis of cytokinesis [66], which has helped to identify a set of conserved cytokinesis factors that point the way towards understanding molecular mechanisms of division-site placement. In contrast to animal cells, fission yeast cells appear to use the nucleus for positioning the division plane and do not use MTs (Figure 3) [65]. Although cells with disrupted MTs do have problems in the medial placement of the division plane [67], the role of MTs in this process might be indirect because they have a primary function in the placement of the nucleus. Mutants that are defective in spindle assembly have little effect on contractile ring assembly or its placement [68]. Unlike animal cells, fission yeast has a closed mitosis in which spindle components do not contact the future division site. Furthermore, the contractile ring is established before astral MTs appear. Cell centrifugation methods to physically manipulate the position of the nucleus have been
mid1p appears to be attached to the cortex near the nucleus via a structure that is, as yet, not described. The structures that connect the nucleus to the cortex might be analogous to the equatorial astral MTs in animal cells (in the equatorial stimulation model). There is limited similarity between mid1p and the contractile ring protein anillin, which share features such as a C-terminal PH domain, interaction with myosin and nuclear localization [71]. Although anillin has properties expected of a ring ‘organizer,’ there is little evidence that it has an analogous function in signaling from the MA.

A second possible way for the nucleus to influence ring assembly is through the spindle pole bodies (centrosome equivalents) that are embedded in the nuclear envelope. During the early stages of actin ring assembly an ‘actin aster’ forms either near to or at the spindle pole body, which appears to be a source of actin filaments for the ring [72]. Several ring factors, including the formin cdc12p, the IQGAP rng2p and myosin (myo2p and rlc1p) can move on MTs and might associate transiently with the spindle pole body [73–75]. Although some of these factors still associate with the nucleus (and possibly the spindle pole body) in the absence of mid1p [68,69,76], the functional significance of these spindle pole body and microtubule interactions have not been demonstrated. In addition, the spindle pole body has a well characterized role in localizing many components of the septation initiation network (SIN) pathway, which primarily regulates ring contraction and septation, rather than initial assembly of the ring [77]. Thus, the spindle pole body, like the centrosome in animal cells, contributes to several aspects of cytokinesis.

**Concluding remarks**

It is clear that, even after over a hundred years of research, there is no unifying model for the mechanism of furrow placement at cytokinesis. In fact, each model can be ‘disproved’ in some context or another. How can these models be reconciled? One easy route is to plead cell type differences. It is true that large marine eggs have different geometric consideration than small cells from humans, insects and yeast. For example, different cell types possess different densities and geometries of MA MTs. Furthermore, the geometry of flat, adherent cells places different constraints on cell division than those of spherical cells in suspension.

One attractive way of reconciling some of the models for induction of cytokinesis is to suggest that multiple, overlapping mechanisms operate in a single cell. The cytokinesis signaling machinery must be robust because it is essential for cell reproduction. The relative contribution of functionally redundant mechanisms might be cell type-dependent. Indeed, overlapping mechanisms have been demonstrated by analyzing synthetic genetic effects in *C. elegans* [28]. Although single disruptions of either the spindle midzone or astral–cortical interactions do not lead to strong defects in cytokinesis, disruption of both produces a strong cytokinesis defect. Thus, both equatorial stimulation (and spindle midzone) and polar relaxation mechanisms could operate in the same cell.

These overlapping mechanisms might provide a new way of interpreting previous experiments. Under this
assumption, a structure such as a spindle midzone (which is, perhaps, analogous at the molecular level to interacting equatorial asters) might be sufficient to induce cleavage but might not be required in a loss-of-function experiment. Because cytokinesis is essential to cell reproduction, it is possible that nature has resulted in significant functional redundancy to allow maximal robustness and the flexibility to accommodate different cell types and environmental situations. The ultimate test of the diverse models will come with the characterization of the key signals that specify the cleavage site. As Rappaport said in a keynote address at a recent meeting on cytokinesis, ‘When I began working on cytokinesis, I thought I was tinkering with a beautifully made Swiss watch, but what I was really working on was an old Maine fishing-boat engine: over-built, inefficient, never-failed and repaired by simple measures’ [78].

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