The Mechanism of Cytokinesis: Reconsideration and Reconciliation

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ABSTRACT. The widely held models of cytokinesis contend that signals for cleavage are transmitted by astral microtubules, and that such signals elicit the assembly and contraction of an equatorial band of actin-myosin II filaments. However, experiments during the past decade have painted an increasingly complex picture, including strong evidence for the involvement of chromosomal passenger proteins and interzonal microtubules, and the involvement of not only cortical contraction but also cytoskeletal disintegration. The purpose of this article is to consider alternative models that might better accommodate both old and new observations. It is proposed that chromosomal passenger proteins undergo dynamic associations at centromeres during metaphase and are recruited from the cytoplasm to both astral and interzonal microtubules during anaphase. In addition, cytokinesis may be driven by global inward contractions coupled to a localized collapse of the equatorial cortex.

Key words: actin/myosin/microtubules/mitosis/chromosomes/cleavage

The two major issues in cytokinesis are the signaling, and the ingression, of the cell cortex. In the conventional astral microtubule stimulation-equatorial cortical contraction model, astral microtubules carry the signals to the cortex, which in turn stimulate the assembly and contraction of a band of actin-myosin II filaments along the equator (discussed in Satterwhite and Pollard, 1992). Although it is highly plausible, recent progress at cellular, structural, and molecular levels has provided some conflicting observations that challenge the validity of this model in its simplest form. This article is not intended as a review, but an updated and comprehensive “Discussion” for a number of papers published by this group during the past decade. It will reexamine the implications of several experiments and discuss related experiments performed by other laboratories, in search of alternative models that may better accommodate a number of puzzling observations.

The stimulation of cytokinesis: microtubules and chromosomes

Much of the current thinking of cytokinesis is influenced by the classic studies of Rappaport (1961), who used elegant micromanipulations to create a torus-shaped sand dollar egg with two spindles near each other. Cleavage furrows formed not only along the equator of each spindle, but also between the two spindles where there were abundant overlapping astral microtubules but no chromosome. The results argue convincingly that, at least under some conditions, interactions of astral microtubules with the cortex are sufficient for the stimulation of cytokinesis. They also argue strongly that the proximity to chromosomes is not required. Given the large size of the embryos and the relatively small, centrally located spindle (Fig. 1), this model is not only plausible but may be essential for the successful stimulation of cytokinesis. However, using microperforation to bring the cortex near the spindle, Rappaport has also discovered a stimulatory effect near the metaphase plate region (Rappaport and Rappaport, 1974), which suggests that some components near the chromosomes or the spindle interzone may carry additional stimuli for cytokinesis.

Compared with embryos, most other types of cells contain a larger spindle relative to the size of the cell (Fig. 1). The distance between the two is also much shorter than in embryos. Even though the signals for cytokinesis may be identical, the different geometries likely dictate a different strategy for their transport to the cortex. Studies with cul-
tured epithelial cells have indeed yielded results with important similarities and differences. Microperforation of cultured epithelial cells indicated that cytokinesis requires unobstructed communications between the spindle and the cortex, and that the access to astral microtubules alone is insufficient for the stimulation (Cao and Wang, 1996). The unique organization of interzonal microtubules relative to the cleavage furrow suggests that these microtubules may be involved in transporting the signals. In subsequent experiments with topoisomerase II inhibitors, interzonal microtubules were found to emanate laterally from tangled chromosomes and to stimulate ingestion as they reach the cortex (Wheatley et al., 1998).

However, using fusion techniques to create cultured cells with multiple spindles, Rieder et al. observed, in a small but significant fraction of cells, the formation of ectopic furrows between neighboring spindles similar to what was observed with sand dollar eggs (Rieder et al., 1997). Therefore, in both large embryos and small cultured cells, signals for cytokinesis may be carried by either astral microtubules or interzonal microtubules. The difference between the two systems appears to be not with the basic mechanism, but with the relative contributions of the two subsets of microtubules. All the observations may be explained if the signals for cytokinesis are captured from the cytoplasm onto both sets of microtubules during anaphase, and if the two types of cells have a different relative mass of astral to interzonal microtubules.

Before considering a mechanism for spindle-cortical communication, it is important to address the apparently conflicting results concerning the role of chromosomes in cytokinesis. The torus experiments by Rappaport (1961) argue that, since the site of cleavage is determined primarily by the location of the spindle poles and astral microtubules, chromosomes cannot play a direct role in signaling cytokinesis. This notion was supported by Hiramoto (1971) with echinoderm embryos, and by Zhang and Nicklas (1996) with insect spermatocytes. In both studies microsurgical removal of the nucleus or metaphase chromosomes showed no effect on cytokinesis. However, there are equally strong arguments for the involvement of chromosomes. The most compelling evidence is the identification of a number of “chromosome passenger proteins”, such as the Aurora-B kinase and INCENP, which are required for cytokinesis (Adams et al., 2001). These proteins are associated with chromosomal centromeres until anaphase onset, when they relocate onto interzonal microtubules and concentrate into the equatorial region. Therefore, one might argue that, if chromosomal passenger proteins are required for cytokinesis, so must be the chromosomes.

The dynamics and functions of one of such chromosomal passenger proteins, the Aurora-B kinase, were recently examined in this laboratory with live cells expressing Aurora-B-GFP (Murata-Hori et al., 2002). Approximately 30–60 seconds after anaphase onset, Aurora-B was found to disassociate from centromeres and to localize along interzonal microtubules. The protein then concentrated into short segments along the equator, and condensed laterally during telophase to form the midbody. The relocation from centromeres to interzonal microtubules was inhibited upon the expression of a non-degradable cyclin B, similar to another chromosomal passenger protein TD60 (Wheatley et al., 1997), suggesting that the association of Aurora-B with centromeres is regulated by CDK1. In the simplest model, Aurora-B and associated proteins are sequestered at centromeres during prometaphase and metaphase, until CDK1 degradation at anaphase onset triggers a direct transfer onto interzonal microtubules. They are then transported into the spindle interzone by an associated kinesin-like protein such as MKLP1 (Nislow et al., 1992).

Although this simple model is direct and effective, it appears inconsistent with the involvement of astral microtubules in cytokinesis, which lie far away from centromeres. It is also difficult to explain the results of chromosomal removal as discussed above. Several observations provide important clues for the modification of this model. First, in cells that form an ectopic furrow between two spindles, the chromosomal passenger protein INCENP is localized along not only interzonal microtubule but also microtubule bundles in the ectopic furrow (Savoian et al., 1999). Second, photobleaching of Aurora-B-GFP shows a relatively high rate of recovery, with fluorescence returning to bleached centromeres within seconds (Murata-Hori and Wang, unpublished observations). Third, the motor protein MKLP1 is diffusely distributed throughout the spindle during metaphase, but becomes colocalized with chromosomal passenger proteins during anaphase and telophase (Nislow et al., 1992). These observations indicate that there is a pool of diffusible chromosomal passenger proteins in the cytoplasm, which exchange on and off centromeres during metaphase at a high rate. In addition, motor proteins may become associated with chromosomal passenger proteins only upon the onset of anaphase and the release from cen-
The function of Aurora-B was further studied with NRK cells transfected with a kinase inactive, dominant negative mutant of Aurora-B (Murata-Hori et al., 2002). Although mutant Aurora-B appeared to affect neither the initial assembly of interzonal microtubules nor its relocation onto interzonal microtubules, the protein disappeared from the equatorial region as if falling off the ends of microtubules. The interzonal microtubules remained poorly organized and cytokinesis also failed. Thus one of the primary functions of Aurora-B appears to be the maintenance of a stable association of chromosomal passenger proteins at microtubule ends, in order to promote the organization of interzonal microtubules.

Together, the above observations suggest a model for the signaling of cytokinesis as shown in Figure 2. It proposes that, prior to anaphase onset, high CDK1 activities promote the dynamic binding of chromosomal passenger protein complexes (CPC) with centromeres, and inhibit their association with a plus end directed microtubule motor such as MKLP1. At anaphase onset, CDK1 deactivation and/or proteolysis cause the inhibition of CPC-centromere binding and the activation of CPC-motor association, which then drives CPC along microtubules toward their plus ends in the spindle interzone. The kinase activity of Aurora-B catalyzes the stable binding of CPC at microtubule ends and promotes their continuous elongation, until microtubules from the two half spindles meet and crosslink near the equatorial cortex to form a stable targeting system for directing the cytokinesis signals. The CPC may also interact directly with the cortex to stimulate cytokinesis. Since centromeres play either no role or possibly a negative role in CPC-motor interactions, in a manner similar to the spindle checkpoint control, this model readily explains the dilemma why CPC, but not chromosomes themselves, are required for cytokinesis. In addition, the presence of a free CPC pool explains why both astral and interzonal microtubules can be used for the activation of cytokinesis.

The ingression of the cortex: cortical contraction and cortical disintegration

Under the conventional model, signals delivered to the equatorial region cause localized organization of cortical actin and myosin II filaments along the equator. Contraction of this “purse string” then shrinks its diameter and drives cell cleavage. This model is supported primarily by structural studies, which showed a band of actin and myosin filaments along the equator of at least some large dividing cells (Satterwhite and Pollard, 1992). In addition, mechanical studies have demonstrated that the cortex is stiffer along the equator than elsewhere (Matzke et al., 2001), and is capable of exerting strong forces (Rappaport, 1967).

While this simple scheme can be very effective, a number of observations suggest that activities along the equator may be more complicated than the simple constriction of a purse

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**Fig. 2.** A model for signaling cytokinesis. During prometaphase and metaphase (A), chromosomal passenger protein complexes (small open beads) undergo rapid on-off reactions at chromosomes (large ovals). A significant fraction of the complexes is diffusible in the cytoplasm. These complexes regulate centromere-microtubule interactions but are otherwise unable to interact with microtubules. During anaphase onset (B), the binding of the complexes to centromeres is inhibited, while their interactions with a cytoplasmic motor protein are activated (filled beads). Motor association causes the complexes to bind to both astral and interzonal microtubules (solid thick lines) and migrate toward the central region (solid arrows). Possibly some unique characteristics of kinetochore microtubules (broken thick lines) preclude their interactions with chromosomal passenger proteins. The complexes remain associated at the tip of microtubules and mediate their stabilization and elongation (open arrows). During telophase (C), extending microtubules from opposite half-spindles are crosslinked by the chromosomal passenger proteins, creating a high concentration of microtubule ends for the localization of cleavage signals. The complexes may also interact directly with the cortex.
string. First, it has long been recognized that cytokinesis involves not only contraction but also disassembly of the equatorial cortex (Schroeder, 1972). The disassembly of actin filaments appears to be an essential step, since cleavage is inhibited by the mutation of coflin (Gunsalus et al., 1995), a protein mediating actin depolymerization and fragmentation. Second, the actin “contractile ring” is often undetectable in cultured cells (Cao and Wang, 1990). Analysis of 3D structures of actin filaments relative to cortical ingression further suggests that the equatorial orientation of actin filaments may be a consequence of cortical resistance to cleavage rather than a prerequisite of cytokinesis (Fishkind and Wang, 1993).

There are equally strong indications that cortical activities may not be limited to the equatorial region. First, despite the convincing demonstration of constriction forces (Rappaport, 1967), contractions may occur over a wide region of the cortex, as suggested by some earlier mechanical measurements (Mitchison and Swann, 1955). Second, structural studies revealed that, in regions flanking the equator, actin filaments are organized predominantly along the spindle axis (Fishkind and Wang, 1993), possibly as a result of active longitudinal contractions. In addition, a set of actin filaments is found to associate end-on with the membrane. These are particularly relevant since forces exerted on these filaments would lead directly to the ingestion of the membrane. Third, upon the application of cytochalasins, cortical actin filaments recoil along random directions throughout the cortex (Wang et al., 1994), suggesting that the entire cortex is loaded with forces. Recent genetic analyses have further identified several essential cytoskeletal proteins, such as coronin, that appear to function throughout the cortex during cytokinesis (Robinson and Spudich, 2000). Together, these observations suggest that the understanding of cytokinesis requires a thoughtful consideration of both contraction and relaxation activities over the entire cortex.

The contribution of different cortical regions to cytokinesis has been probed recently by delivering actin disruption drugs to various regions of the cortex (O’Connell et al., 2001). The solution was released from a microneedle while the medium was removed with a neighboring suction pipette, creating a focused distribution of the compounds. Surprisingly, neither cytochalasin D nor latrunculin A caused inhibition of cytokinesis when released at the equator. A significant fraction of cells even showed an apparent stimulation of the ingression activity. In contrast, equatorial release of jasplakinolide, a compound causing stabilization of actin filaments (Spector et al., 1999), was found to inhibit cytokinesis. Furthermore, when cytochalasin D was released near the spindle pole, cytokinesis was inhibited while the equatorial actin organization appeared to become more robust than in control cells. These surprising observations argue strongly against a simple equatorial contraction model, and suggest that the integrity of the entire cortex is critical for cytokinesis. In addition, they suggest that cytokinesis may be facilitated by weakening of the equatorial cortex.

The above observations may be explained by a model that involves global cortical contraction and equatorial cortical collapse (Fig. 3). The first key element in this model is the presence of actin filaments that associate end-on with the membrane and extend over a range of angles toward the interior of the cell. During cytokinesis, interactions of these filaments with myosin II generate not only inward forces but also lateral interactions across the cortex. The latter may in effect create cortical expansion forces, which has been suggested in some studies as a requirement for cytokinesis (reviewed in Rappaport, 1996; He and Dembo, 1997). The second key element is that signals delivered by astral or interzonal microtubules, as discussed above, cause local weakening of the equatorial cortex without significantly inhibiting actin-myosin II interactions. The weakening may involve severing of actin filaments or inhibition of crosslinking factors. The combination of cortical contraction and weakening then leads to local compression and buckling along the equator (Fig. 3). It should be noted that although this model differs substantially from the prevalent contractile ring hypothesis, both global cortical expansion and equatorial cortical relaxation have been considered as possible mechanisms of cytokinesis during the past century (Rappaport, 1996). According to this model, the cortical flow, the increase in the concentration of cytoskeletal materials, and the weakening of the equatorial cortex work together to drive cytokinesis.
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teins in the furrow, the equatorial organization of actin filaments, the apparent increase in cortical “stiffness” (Matzke et al., 2001), may represent various passive consequences of equatorial collapse and compression.

This model not only explains our puzzling results, but also accounts for a number of observations that are difficult to explain with the purse string model. For example, during the cellularization of Drosophila embryos (reviewed in Rappaport, 1996), the “contractile ring” moves toward the center of the embryo without constriction. This is likely due to the formation of circular patterns of weakened cortex combined with the exertion of forces perpendicular to the surface of the embryo. In addition, in myosin II mutants of Dictyostelium, the lack of cortical forces would inhibit cytokinesis in suspension, while the weakening of the equatorial cortex alone would allow the cell to divide by traction forces (Gerish and Weber, 2000).

Prospectus

One unique feature of cytokinesis is the wide range of variations among different cell types and organisms (Rappaport, 1996), which represents both a serious challenge and a powerful guidance since the correct model should be able to explain many if not all of the variations. The two models discussed in this article lead to a number of testable predictions, which should be carefully verified in a range of biological systems. For example, the global cortical contraction-equatorial cortical collapse model predicts that the spatial and temporal regulation of factors that affect cortical integrity, such as cofilin, tropomyosin, and various actin crosslinking factors, is critical for cytokinesis. In contrast, an enhanced contractility along the equatorial cortex may be helpful but dispensable. Future studies that address when and where specific proteins interact, and how these interactions affect the actin and microtubule cytoskeleton, will be particularly informative for sorting out the validity of a plethora of hypotheses that have been proposed during the past century (Rappaport, 1996).

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References


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