Midzone Microtubule Bundles Are Continuously Required for Cytokinesis in Cultured Epithelial Cells

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Abstract. The current model of cytokinesis proposes that spindle poles and associated microtubules determine the cleavage plane, and, once the signal has been delivered to the cortex, the entire mitotic apparatus can be removed without affecting cell division. While supported by compelling data from Echinoderm embryos, recent observations suggest that the model may not be universally applicable. In this study, we have examined the relationship(s) among microtubules, chromosomes, and cleavage activity in living normal rat kidney (NRK) cells with multipolar mitotic figures. We found that cleavage activity correlated with the distribution of midzone microtubule bundles and Telophase Disc 60 protein (TD60) rather than the position of spindle poles. In addition, reduction of midzone microtubules near the cortex, by either nocodazole treatment or spontaneous reorganization in tripolar cells, caused inhibition or regression of furrowing. These results demonstrate that continuous interaction between midzone microtubule bundles and the cortex is required for successful cleavage in tissue culture cells.

Cytokinesis, the final stage in cell division, is a tightly regulated process (for reviews see Mabuchi, 1986; Salmon, 1989; Satterwhite and Pollard, 1992; Fishkind and Wang, 1995). It normally occurs only after chromosome separation, along a plane perpendicular to the spindle axis and equidistant between the segregated chromosomes. Past observations have provided strong evidence that the cleavage plane is dictated by the position of the mitotic apparatus (MA) or, more specifically, by the spindle poles (Rappaport, 1961; Rappaport and Ebstein, 1965; Rappaport, 1985; for reviews see Rappaport, 1991; Strome, 1993). The most elegant demonstration involved manipulation of a sand dollar egg into a horseshoe shape with one spindle located in each arm (Rappaport, 1961). In addition to cleavage at the spindle midplanes, division occurred between the asters without intervening chromosomes. Such convincing studies from Echinoderm eggs have led to a general view that the spindle poles represent the key cleavage plane determinant in all animal cells (for reviews see Mabuchi, 1986; Rappaport, 1991). Furthermore, as the spindle poles are distant from the cortex, the anaphase astral microtubules are proposed to transmit the cleavage signal to the cortex (Schroeder, 1981; White and Borisy, 1983; Rappaport and Rappaport, 1988; Harris and Gewalt, 1989; Devore et al., 1989; Rappaport, 1991).

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Abbreviations used in this paper: MA, mitotic apparatus; NRK, normal rat kidney; PEG, polyethylene glycol; TD60, Telophase Disc 60 protein.

A second widely held view is that there is a narrow time window in which the cleavage signal is transmitted to the cortex (Hamaguchi, 1975; Rappaport and Rappaport, 1993), and once the signal is received, the entire MA can be discarded without any consequence to the ensuing division (Swann and Mitchison, 1953; Hiramoto, 1956). In these early works, however, it was difficult to ascertain whether all microtubules had been removed. Moreover, there are indications that the structural organization and regulation of cytokinesis vary substantially between cell types: while the MA of marine invertebrate eggs has extensive asters and a small central spindle, mammalian cells in monolayer culture have a comparatively large spindle, large chromosomes, and small asters. In cultured cells, the interzonal microtubules that develop between the separating chromosomes during anaphase are the microtubules closest to the cortex, and various observations have implicated them in stimulating cleavage (Kawamura, 1977; Williams et al., 1995; Cao and Wang, 1996). Even in Echinoderm eggs, artificially bringing the cortex near the central spindle can stimulate cleavage (Rappaport and Rappaport, 1974), implying that the ability to induce cleavage is not limited to astral microtubules.

Surprisingly, despite the consensus that microtubules play an important role in stimulating cytokinesis, few observations have been made to characterize directly the relationship between microtubule organization and cleavage (Asnes and Schroeder, 1979; Wadsworth and Sioboda, 1983; Hamaguchi et al., 1985; Conrad et al., 1992; Oka et al., 1994; Fishkind et al., 1996). Most informative would be a detailed knowledge of microtubule organization in various alternative forms of cytokinesis, e.g., in cells with multiple
spindle poles (Ghosh and Paweletz, 1984). Here we report that the location of the cleavage plane correlates more directly with the position of midzone microtubules than the spindle poles or polar microtubules. Moreover, we show that the persistence of midzone microtubule organization is essential for successful completion of cleavage in these cells.

Materials and Methods

Culture and Treatment of Normal Rat Kidney (NRK) Cells

Two subclones (NRK2 and 13) of NRK-52E epithelial cells (American Type Culture Collection, Rockville, MD), selected for their spread morphology during division, were cultured on glass chamber dishes as described previously (McKenna and Wang, 1989). Multipolar spindles were generated by colcemid treatment (Sigma Chemical Co., St. Louis, MO; Ghosh and Paweletz, 1984; Keryer et al., 1984; Solitto and Kuriyama, 1988) or polyethylene glycol (PEG)-induced fusion (PEG 1500; BDH Lab Supplies, Lutterworth, UK; Davidson and Gerald, 1976). For colcemid treatment, low density cultures were grown (24-48 h) and then incubated in medium containing 0.04 μg/ml colcemid. After 24 h, cells were washed thoroughly with fresh medium and allowed 48 h to recover. For PEG-induced fusion, cells were grown until large colonies formed (48 h), the medium was then removed, and cells were bathed in 1 ml warm PEG 1500 (50% wt/vol, in DME; 37°C) for 60 s. Cells were then rinsed gently but thoroughly with cytoskeleton buffer (137 mM NaCl, 5 mM KCl, 1.1 mM Na2HPO4, 0.4 mM KH2PO4, 2 mM MgCl2, 2 mM EGTA, 5 mM Pipes, 5.3 mM glucose, pH 6.1; Small; Small et al., 1982). Cells were rinsed with warm PBS before fixation. For optimal preservation of microtubules and F-actin, cells were immersed in 1 ml of 0.5% glutaraldehyde (Polysciences Inc., Warrington, PA) with 0.15% sodium borohydride. For best preservation of CHO1 and Telophase Disc 60 protein (TD60) antigens, cells were fixed with 2% formaldehyde (EM Sciences, Gibbstown, NJ) plus 0.5% Triton X-100 for 10 min. Then cells were rinsed with cytoskeleton buffer and then blocked for >20 min with 1% BSA in PBS (Boehringer Mannheim Corp., Indianapolis, IN). Antibodies were diluted in 1% BSA/PBS and clarified in a microcentrifuge before use. Primary antibodies: human JH autoimmune serum against TD60 (kindly provided by Dr. D. Palmer, University of Washington, Seattle, WA; 1/50 dilution); monoclonal anti-β tubulin (Amersham Corp., Arlington Heights, IL; 1/10 dilution); and monoclonal antimitotic kinesin-like protein (CHO1; kindly provided by Dr. R. Kuriyama, University of Minnesota, Minneapolis, MN; 1/500 dilution). All antibody incubations were performed for 1 h at 37°C or overnight at 4°C. Secondary antibodies: FITC-anti-human IgG (a kind gift from R. Balezon, University of South Alabama, Mobile, AL; for TD60, 1/20 dilution); FITC-sheep anti-mouse IgG (Sigma Chemical Co.; for tubulin, 1/50 dilution); and rhodamine B-goat anti-mouse IgG and IgM (TAGO Inc., Burlingame, CA; for CHO1; 1/50 dilution). Finally, cells were rinsed and mounted in PBS or antibleaching medium (100 mg/ml DABCO [Sigma Chemical Co.], 1 mg/ml n-propyl galactol [Sigma Chemical Co.], 80% glycerol, 100 mM Tris-HCl [pH 8]).

Phalloidin staining was performed by incubation for 1 h at room temperature with 200 nM TRITC-phallolidin (Sigma Chemical Co.) or FITC-phallolidin (Molecular Probes) diluted in PBS.

Microscopy and Data Collection

Cells were viewed using an inverted microscope (Axiovert 10; Carl Zeiss, Inc., Thornwood, NY) with a 40×, NA 0.75, plan-achroplan phase contrast lens, a 63×, NA 1.30, Neofluar phase lens, or a 100×, NA 1.30 Neofluar lens. Fluorescence was detected using epifluorescent optics and cooled CCD camera (TE/CCD-596EM, Princeton Instruments, Trenton, NJ). Images were processed by subtraction of camera dark noise and field using custom designed computer software. Hard copies were prepared on a Kodak Color Ease PS Printer (Rochester, NY).

Results

The incidence of spontaneous binucleation in NRK cells is ~0.7% (n = 1,000). Multinucleation increased dramatically after transient treatment with colcemid (23%; n = 1,000) or PEG-induced fusion (3%; n = 2,000). While most experiments were performed on colcemid-treated cells, consistent results were obtained with spontaneous or PEG-fused cells.

Chromosome movement and cleavage pattern were monitored by time-lapse phase imaging and, after microinjection of rhodamine-tubulin, microtubule and spindle pole dynamics were monitored with epifluorescence. As cytokinesis in adherent cells does not always occur by lateral ingression of the cell margins, cleavage activity was also assessed by F-actin accumulation after fixation and staining with fluorescent phallolidin (Fishkind and Wang, 1993).

Cleavage Pattern Varies with the Configuration of Chromosomes

Upon entry into prophase, multinucleated cells typically had four or more centroosomes with associated microtubules. These centroosomes tended to migrate and merge during prometaphase (see Fig. 3 B and D, arrowheads). By metaphase, the majority of multipolar spindles consisted of three poles connected by microtubules to a common set of centroosomes. We focused our attention on spindles with pole-pole and pole to cell boundary distances comparable to those seen in cleaving bipolar cells.

The metaphase plates of triporal cells were typically "Y"-(Fig. 1 A, I) or "V"-shaped (Fig. 1, B and C, I). Among cells with a Y-shaped metaphase plate (n = 37), 50% underwent a symmetrical mitosis and divided into three parts while the other 50% cleaved into two (described below). Cells with a V-shaped metaphase plate also divided into two or three daughters. The pattern of cleavage appeared to depend on the sharpness of the angle
Chromosomes are arranged into a "Y" (A), shallow "V" (B), or sharp "V" (C) shape at metaphase, shown as phase images (I) and schematically in (II). Progression through anaphase (III) to cleavage (IV) is illustrated for each. Chromosomes are represented by thick black lines, spindle poles as black dots, and cleavage as invaginations of the cell outline. Dashed lines (III) indicate the predicted cleavage sites if determined by the position of the metaphase plate. In 50% of cells with a Y configuration (A), one furrow fails due to the rearrangement of midzone microtubules (IV, lower figure; see also Fig. 5). Cells with a shallow V configuration (B) always cleave into two (B, IV), whereas cells with a sharp V divide into three (C, IV). Bar, 10 μm.

Cleavage Plane Is Defined by the Position of the Midzone Microtubules

Cells with shallow V-shaped metaphase plates appeared to divide in a pattern contradicting that predicted by Echinoderm experiments (Rappaport, 1961) and thus were examined in detail with microinjected rhodamine-tubulin. In this configuration, two pairs of poles had intervening chromosomes while the third had chromosomes only on one side (Figs. 2 and 3). Arrays of midzone microtubules started to form in the expanding midzones during anaphase (Figs. 2, D and F, and 3 F) and subsequently developed into discrete bundles (Figs. 2, H and J, and 3 H). By contrast, between the two poles without intervening chromosomes (Figs. 2 F and 3 H, poles b and c), microtubule bundles were either absent (Fig. 2 F) or present only to a limited extent (Fig. 3 H, arrow). In such cells, cleavage activity was only apparent along the two planes defined by the midzone microtubules (n = 24/24), as judged by ingression of the lateral margins (Figs. 2, G–J) or by F-actin organization (Fig. 3 I). In some cases, a short band of cleavage activity was found between the poles without intervening chromosomes (Fig. 3 I). This region, however, always contained a small band of bundled microtubules interconnecting the neighboring chromosomes (Fig. 3 H, arrow).

In addition to the midzone microtubules, numerous polar microtubules extended throughout the cell during late anaphase (Fig. 3 H). The distribution of their plus ends, however, showed no defined relationship to cleavage, unless incorporated into a midzone region as seen in cells with a sharp V (Fig. 4 D, arrow). These results suggest that determination of the cleavage plane is governed by the location of the metaphase plate and midzone microtubules.
Figure 2. Cleavage and microtubule dynamics in a tripolar NRK cell with a shallow V-shaped metaphase plate (A). The cell was injected at prometaphase with rhodamine-tubulin (B). As anaphase progressed (C–F), interzonal microtubules developed between two pairs of poles (F; a and b, and a and c) but not between the third pair (F; b and c). Cleavage, visible by ingression (G and I), occurred only where midzone microtubule bundles were present (H and J). Bar, 10 μm.

Continued Presence of Midzone Microtubule Bundles Is Required for Successful Cleavage

To assess the role of microtubules after cleavage stimulation, we microinjected rhodamine-tubulin into symmetric tripolar cells with Y-shaped metaphase plates (Fig. 5). Microtubule organization was then compared between cells that divided into three equal parts and those that divided into two parts of differing size. In the former (Fig. 5, A–J),...
Cleavage of a tripolar NRK cell with a sharp V-shaped metaphase plate. The cell was injected at prometaphase with rhodamine-tubulin. Despite the absence of shared metaphase chromosomes between one pair of poles (compare A and B), three cleavage planes developed (C). Cleavage was accompanied by the formation of midzone microtubule bundles between each pair of poles including the pair with no intervening chromosomes (D, arrow). Bar, 10 μm.

A-D), both the distribution of chromosomes and the organization of the midzone microtubules maintained a threefold symmetry throughout mitosis and cytokinesis (n = 19/37). By contrast, in the latter (Fig. 5, A'-D'), the distribution of midzone microtubules became progressively asymmetric as bundles from one branch of the tripolar spindle merged into the other two branches (n = 18/37). Cleavage occurred only where midzone microtubule bundles were present (Fig. 5, D', arrow).

We then asked whether cytokinesis could continue in normal bipolar cells after microtubule disruption. Compared to interphase and polar microtubules, midzone microtubule bundles are more resilient to depolymerizing agents; this resilience increases as cells progress through division. To monitor the distribution of residual microtubules in relation to cleavage, cells were injected with rhodamine-tubulin before treatment with nocodazole. When all microtubule bundles were removed from the cortical vicinity (n = 14/19), cleavage was inhibited. Moreover, ingressing furrows regressed (n = 5) when all microtubule bundles were removed from near the cortex (Fig. 6). On the other hand, if some microtubules remained in close proximity to the cortex after treatment (n = 5/19), cleavage completed with the active margin located adjacent to the residual microtubules.

To determine whether midzone microtubule bundle formation was a cause or effect of furrowing, we applied cytochalasin B to cells in late metaphase and anaphase (n = 10). Despite the inhibition of cleavage, microtubule bundles formed normally, although they never compacted into a midbody (see also Schroeder, 1970; Martineau et al., 1995).

**Relationship between Cleavage and the Distribution of Spindle Midzone Proteins**

A number of microtubule- and chromosome-associated proteins have been implicated in stimulating cytokinesis based on their relocation to the midzone during telophase (for reviews see Rattner, 1992; Earnshaw and Mackay, 1994). We examined the distribution of two such proteins: mitotic kinesin-like protein identified by CHO1 antibody (Sellitto and Kuriyama, 1988), and TD60 recognized by a human autoimmune serum (Andreasen et al., 1991). CHO1 staining varied among furrows, with no detectable concentration found in ~10% of the actively cleaving regions (n = 13/115). By contrast, TD60 distribution paralleled that of F-actin and midzone microtubules (n = 115/115) in all multipolar cells (Fig. 7). In cells with a shallow V configuration, neither TD60 nor F-actin was found between pairs of poles with no intervening chromosomes (Fig. 7, A'-C'). However, both were abundantly present in the region occupied by midzone microtubule bundles, suggesting that TD60 is likely involved in the stimulation of cleavage.

**Discussion**

The classic view of cytokinesis is derived primarily from experiments with *Echinoderm* eggs. Although these large cells are amenable to manipulations, their size also obstructs precise observation of cytoskeletal architecture (Asnes and Schroeder, 1979; Wadsworth and Sloboda, 1983; Hamaguchi et al., 1985; Oka et al., 1994). In addition, they are morphologically distinct from somatic cells and may not be as prototypic as biologists first anticipated. In this study, we induced multipolarity in well-spread mammalian epithelial (NRK) cells. The favorable optical quality of these cells, together with the abnormal mitotic configurations, allowed us to probe the relationships among cleavage, microtubules, spindle poles, and chromosomes.

**The Roles of Spindle Poles and Microtubules in Cleavage Stimulation**

While the currently favored model of cytokinesis emphasizes the importance of the spindle poles in stimulating cleavage (Rappaport, 1991), it has also been speculated that microtubules probably play a more direct role (Devore et al., 1989; Harris and Gewalt, 1989).

In this study, we first asked whether a pair of poles with associated microtubules is sufficient to stimulate cleavage as originally proposed for *Echinoderm* eggs. We found that the cleavage plane is not determined solely by the organization of the spindle poles, as tripolar cells with a shallow V-shaped metaphase plate always divide into two (Fig. 1 B, IV), whereas most cells with a sharp V divide into three. Furthermore, by monitoring microtubule dynamics in living cells, we found a strong correlation between...
cleavage and midzone microtubules: cleavage only occurred near regions populated by midzone microtubule bundles, which are comprised primarily of overlapping interzonal microtubules (Saxton and McIntosh, 1987; Mas-tronarde et al., 1993). These observations suggest that, in cultured mammalian cells, midzone microtubules play a more direct role in stimulating cytokinesis than other parts of the MA.

Midzone Microtubules Are Continuously Required for Cytokinesis in NRK Cells

It is widely held that after cortical stimulation, the entire MA, including chromosomes and associated microtubules, can be removed without any consequence to the developing furrow. This was shown first by colchicine treatment (Swann and Mitchison, 1953) and later by aspirating the
MA of a sea urchin egg during anaphase (Hiramoto, 1956); in each case, cytokinesis completed in the absence of the MA. It is possible, however, that in either experiment, some microtubules remained associated with the cortex and provided continuous stimulation. Indeed, after initiation of furrowing in sand dollar eggs, the cleavage plane can be relocated by moving the MA (Rappaport, 1985). Our experiments with nocodazole indicate that, although cytokinesis can occur after disassembly of a large portion of microtubules during anaphase, the process continues only where midzone microtubule bundles remain in sufficient number or in close proximity to the cortex. Even after initiation of cytokinesis, furrowing can be reversed if all midzone microtubule bundles near the cortex are eliminated (Fig. 6). These observations may explain the variable response of dividing PtK1 cells to nocodazole treatment (Mullins and Snyder, 1981).

Further evidence comes from cells with Y-shaped metaphase plates. Cleavage fails and the cell divides into two unequal parts if microtubules from one midzone become incorporated into the other midzones (Fig. 5). Thus, contrary to the current opinion, cleavage progression and completion appear to require continuous interaction of the cortex with a subset of microtubules. This finding is independently supported by two recent studies. First, in snail eggs, regression of polar lobes correlated with microtubule loss and persistent furrowing correlated with taxol stabilization of microtubules (Conrad et al., 1992). Second, during recovery of HeLa cells from dihydrocytochalasin B treatment, the ability of cells to cleave appeared to be determined by the retention of a "postmitotic spindle," comprised of interzonal microtubules and the TD60 protein (Martineau et al., 1995; discussed below).

**The Roles of Chromosomes and Spindle Midzone Proteins in Cytokinesis**

Chromosomes are generally thought to play no active part in mitosis or cytokinesis (Zhang and Nicklas, 1996). However, in cultured somatic cells, numerous observations point to the region occupied by the chromosomes as the potential source of the cytokinetic signal (see Margolis and Andreassen, 1993). Consistent with this view, perforation of NRK cells between the chromosomal region and cortex at metaphase inhibited division on the operated side and caused microtubules to assume an interphase-like network (Cao and Wang, 1996). However, similar perforations made during anaphase were not inhibitory, indicating that the influence of the chromosomes is restricted to late metaphase/early anaphase (Cao and Wang, 1996). In the present experiments, we found the pattern of cleavage to be predicted by the location of the metaphase plate in tripolar cells (Fig. 1, III, arrowheads). Particularly informative was the pattern of cleavage in cells with V-shaped metaphase plates. Presumably, in cells with a small angle between the chromosome branches (Fig. 4), a signal from the chromosomal region impinges on the cortex at three points, generating three ingestions. When the angle is large (Figs. 2 and 3), signals from the two branches are expected to merge at the center, resulting in only two furrows. Together these observations indicate that while microtubule bundles may interact more directly and continuously with the cortex, signals for bundle formation originate transiently from the vicinity of the metaphase plate. This view appears to conflict with the recent finding that cleavage in grasshopper spermatocytes is uncompromised following removal of all the chromosomes during early mitosis (Zhang and Nicklas, 1996). However, the equatorial region of these manipulated cells contained prominent microtubule bundles as did control cells, and it is possible that the manipulation exposed microtubule plus ends, thereby permitting cytokinesis.

Recently, a number of proteins have been found to relocate from various parts of the spindle to the midzone during telophase, including inner centromere proteins (INCENPs; Cooke et al., 1987), TD60 (Andreassen et al., 1991), and members of the kinesin family. The behavior of these proteins suggest that they may contribute to the signaling of cytokinesis (for reviews see Earnshaw and Bornat, 1991; Rattner, 1992; Earnshaw and Mackay, 1994). In the present study, we found TD60 distribution to correlate tightly with cleavage activity (Fig. 7); however, the kinesin-like CHO1 antigen was absent from some cleavage sites. This was surprising as it has been suggested that kinesins, including CHO1 (Sellitto and Kuriyama, 1988; Nislow et al., 1992) and centromeric polypeptide-E (Yen et al., 1991) are capable of organizing microtubules and delivering molecules to their plus ends. It is possible that
Figure 7. TD60 localization in tripolar NRK cells. Cleavage activity was assessed by F-actin accumulation (B and B'). One cell (A–C) was undertaking a three-way cleavage at time of fixation; F-actin (B) and TD60 (C) were found in each furrow. A second cell (A’–C’), which had a shallow V-shaped metaphase plate, was watched as it progressed from metaphase to telophase. It was then fixed and stained to reveal F-actin (B’) and TD60 (C’) distributions. F-actin and TD60 localizations were always tightly linked. Bar, 10 μm.

CHO1-negative furrows were in an early stage of regression; alternatively there may be redundancy within the system such that when one motor is absent another substitute. Moreover, both midzone microtubules and cytokinesis were found to be disrupted when a kinesin-related protein, KLP3A, was mutated in Drosophila (Williams et al., 1995). With a clear view of the relationship between microtubules and cytokinesis, the key future task would be to identify and characterize signaling components that migrate from the spindle to the equatorial cortex.

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References

Andreassen, P.R., D.K. Palmer, M.H. Wener, and R.L. Margolis. 1991. Telophase Disc: a new mammalian mitotic organelle that bisects telophase cells with a possible function in cytokinesis. J. Cell Sci. 99:523-534.

Asnes, C.F., and T.E. Schroeder. 1979. Cell cleavage ultrastructural evidence against equatorial stimulation by aster microtubules. Exp. Cell Res. 122:327-338.

Cao, L.-G., and Y.-L. Wang. 1996. Signals from the spindle midzone are required for the stimulation of cytokinesis in cultured epithelial cells. Mol. Biol. Cell. 7:225–232.

Conrad, A.H., A.Q. Paulsen, and G.W. Conrad. 1992. The role of microtubules in contractile ring function. J. Exp. Zool. 262:154-165.

Cooke, C.A, M.S. Heck, and W.C. Earnshaw. 1987. The inner centromere protein (INCENP) antigens: movement from inner centromere to midbody during mitosis. J. Cell Biol. 105:2053–2067.

Davidson, R.L., and P.S. Gerald. 1976. Improved techniques for the induction of mammalian cell hybridization by polyethylene glycol. Somatic Cell Genet. 2:165–176.

Devore, J.J., G.W. Conrad, and R. Rappaport. 1989. A model for astral stimulation of cytokinesis in animal cells. J. Cell Biol. 109:2225–2232.

Earnshaw, W.C., and R.L. Bernstein. 1991. Chromosomal passengers: towards an integrated view of mitosis. Chromosoma (Berl.). 100:139–146.

Earnshaw, W.C., and A.M. Mackay. 1994. Role of nonhistone proteins in the chromosomal events of mitosis. FASEB J. 8:947–956.

Fishkind, D.J., and Y.-L. Wang. 1993. Orientation and three-dimensional organization of actin filaments in dividing cultured cells. J Cell Biol. 123:837–848.

Fishkind, D.J., and Y.-L. Wang. 1995. New horizons for cytokinesis. Curr. Opin. Cell Biol. 7:23–31.

Fishkind, D.J., J.D. Silverman, and Y.-L. Wang. 1996. Function of spindle mi-
crotubules in directing cortical movements and actin filament organization in dividing cultured cells. J. Cell Sci. 109:2041-2051.

Ghosh, S., and N. Pawletz. 1984. Synchronous DNA synthesis and mitosis in multinucleate cells with one chromosome in each nucleus. Chromosoma (Berl.). 89:197-200.

Hamaguchi, Y. 1975. Microinjection of colchicine into sea urchin eggs. Dev. Growth Differ. 17:111-117.

Hamaguchi, Y., M. Toriyama, H. Sakai, and Y. Hiramoto. 1985. Distribution of fluorescently labeled tubulin injected into sand dollar eggs from fertilization through cleavage. J. Cell Biol. 100:1262-1272.

Harris, A.K., and S.L. Gewalt. 1989. Simulation testing of mechanisms for inducing the formation of the contractile ring in cytokinesis. J. Cell Biol. 109:2215-2223.

Hiramoto, Y. 1956. Cell division without mitotic apparatus in sea urchin eggs. Exp. Cell Res. 11:630-636.

Kawamura, K. 1977. Microdissection studies on the dividing neuroblast of the grasshopper, with special reference to the mechanism of unequal cytokinesis. Exp. Cell Res. 106:127-137.

Keryer, G., H. Ris, and G.G. Borisy. 1984. Centriole distribution during tripolar mitosis in Chinese hamster ovary cells. J. Cell Biol. 98:2222-2229.

Lewis, L., and G. Albrecht-Buehler. 1987. Distribution of multiple centrospheres determines migration of BHK syncitia. Cell Mot. Cytoskeleton. 7:282-290.

Lowry, O.H., N.J. Rosebrough, A.L. Farr, and R.J. Randall. 1951. Protein measurements with Folin phenol reagent. J. Biol. Chem. 193:265-275.

Mabuchi, I. 1986. Biochemical aspects of cytokinesis. Int. Rev. Cytol. 101:175-213.

Margolis, R.L., and P.R. Andreassen. 1993. The telophase disc: its possible role in mammalian cell cleavage. Bioessays. 15:201-207.

Martin, S.N., P.R. Andreassen, and R.L. Margolis. 1995. Delay of HeLa cell cleavage into interphase using dihydrocytochalasin B: retention of a postmitotic spindle and telophase disc correlates with synchronous cleavage recovery. J. Cell Biol. 131:191-205.

Mastronarde, D.N., K.L. McDonald, R. Ding, and J.R. McIntosh. 1993. Inter-polar spindle microtubules in PTK cells. J. Cell Biol. 123:1475-1489.

McKenna, N.M., and Y.-L. Wang. 1989. Culturing cells on the microscope stage. Meth. Cell Biol. 29:195-205.

Mullins, J.M., and J.A. Snyder. 1981. Anaphase progression and furrow establishment in nocodazole-arrested PtK cells. Chromosoma (Berl.). 83:493-505.

Nislow, C., V.A. Lombillo, R. Kuriyama, and J.R. McIntosh. 1992. A plus-end-directed motor enzyme that moves antiparallel microtubules in vitro localizes to the interzone of mitotic spindles. Nature (Lond.). 359:543-547.

Oka, M.T., T. Araki, and Y. Hamaguchi. 1994. Different reactivity with monoclonal anti-tubulin antibodies between native and fixed mitotic microtubules in sea urchin eggs. Cell Motil. Cytoskeleton. 29:241-249.

Rappaport, R. 1961. Experiments concerning the cleavage stimulus in sand dollar eggs. J. Exp. Zool. 148:81-89.

Rappaport, R. 1985. Repeated furrow formation from a single mitotic apparatus in cylindrical sand dollar eggs. J. Exp. Zool. 234:167-171.

Rappaport, R. 1991. Cytokinesis. In Oogenesis, Spermatogenesis and Reproduction, Comparative Physiology. Vol. 10. H.H. Kinne, editor. Karger, Basel, Switzerland. 1-36.

Rappaport, R., and R.P. Ebstein. 1965. Duration of stimulus and latent periods preceding furrow formation in sand dollar eggs. J. Exp. Zool. 158:373-382.

Rappaport, R., and B.N. Rappaport. 1974. Establishment of cleavage furrows by the mitotic spindle. J. Exp. Zool. 189:189-196.

Rappaport, R., and B.N. Rappaport. 1988. Reversing cytoplasmic flow in nucleated constricted sand dollar eggs. J. Exp. Zool. 247:92-98.

Rappaport, R. and B.N. Rappaport. 1993. Duration of division-related events in cleaving sand dollar eggs. Dev. Biol. 158:265-273.

Rattner, J.B. 1992. Mapping the mammalian intracenular bridge. Cell Motil. Cytoskeleton. 23:231-235.

Salmon, E.D. 1989. Cytokinesis in animal cells. Curr. Opin. Cell Biol. 1:541-547.

Sammak, P.J., and G.G. Borisy. 1988. Detection of single fluorescent microtubules and methods for determining their dynamics in living cells. Cell Motil. Cytoskeleton. 10:237-245.

Satterwhite, L.L., and T.D. Pollard. 1992. Cytokinesis. Curr. Opin. Cell Biol. 4:43-52.

Saxton, W.M., and J.R. McIntosh. 1987. Interzone microtubule behavior in late anaphase and telophase spindles. J. Cell Biol. 105:875-886.

Schroeder, T.E. 1970. The contractile ring. J. Fine structure of dividing mammalian (HeLa) cells and the effects of cytochalasin B. Z. Zellforsch. Mikrosk. Anat. 100:431-449.

Schroeder, T.E. 1981. The origin of cleavage forces in dividing eggs. Exp. Cell Res. 134:231-240.

Selitto, C., and K. Kuriyama. 1988. Distribution of a matrix component of the midbody during the cell cycle in Chinese hamster ovary cells. J. Cell Biol. 106:431-439.

Small, J.V. 1981. Organization of actin in the leading edge of cultured cells: influence of osmium tetroxide and dehydration on the ultrastructure of actin meshworks. J. Cell Biol. 91:695-705.

Small, J.V., G. Rinnerthaler, and H. Hissken. 1982. Organization of actin meshworks in cultured cells: the leading edge. Cold Spring Harbor Symp. Quant. Biol. 46:599-611.

Strome, S. 1993. Determination of cleavage planes. Cell. 72:3-6.

Swann, M.M., and J.M. Mitchison. 1953. Cleavage of sea-urchin eggs in colchicine. J. Exp. Zool. 129:209-216.

Wadsworth, P., and R.D. Sloboda. 1983. Microinjection of fluorescent tubulin into dividing sea urchin cells. J. Cell Biol. 97:1249-1254.

Wang, Y.-L. 1992. Fluorescence microscopic analysis of cytoskeletal organization and dynamics. In The Cytoskeleton: A Practical Approach. K.L. Carraway and C.A.C. Carraway, editors. IRL Press at Oxford University Press, Oxford, UK. 1-22.

White, J.G., and G.G. Borisy. 1983. On the mechanisms of cytokinesis in animal cells. J. Theor. Biol. 101:289-316.

Williams, R.C., and J.C. Lee. 1982. Preparation of tubulin from brain. Methods Enzymol. 85:376-381.

Williams, B.C., M.F. Riedy, E.V. Williams, M. Gatti, and M.L. Goldberg. 1995. The Drosophila kinesin-like protein KLP3A is a midbody component required for central spindle assembly and initiation of cytokinesis. J. Cell Biol. 129:709-723.

Yen, T.J., D.A. Compton, D. Wise, R.P. Zinkowski, B.R. Brinkley, W.C. Earnshaw, and D. Cleveland. 1991. CENP-E, a novel human centromere-associated protein required for progression from metaphase to anaphase. EMBO (Eur. Mol. Biol. Organ.) J. 10:1245-1254.

Zhang, D., and B. Nicklas. 1996. "Anaphase" and cytokinesis in the absence of chromosomes. Nature (Lond.). 382:466-468.