

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)¹ 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).

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

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

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; Sellitto 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 $\mu\text{g/ml}$ 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 warm DME containing 15% DMSO (Sigma Chemical Co.; Lewis and Albrecht-Buehler, 1987) and then allowed >24 h to recover in growth medium, F12K (Sigma Chemical Co.) supplemented with 10% FCS (JRH Biosciences, Lenexa, KS), before experimentation.

Cells were treated, at the stages indicated, with 2.5 μM nocodazole or 10 μM cytochalasin B (both from Sigma Chemical Co.) diluted directly into warm medium from DMSO stocks held at -20°C .

Preparation and Microinjection of Rhodamine-Tubulin

Twice-cycled bovine brain tubulin, a gift from Dr. C. Wilkerson (Worcester Foundation for Biomedical Research, Shrewsbury, MA), was prepared by the method of Williams and Lee (1982) and stored as droplets at -80°C . Tubulin was labeled with tetramethyl rhodamine succinimidyl ester (Molecular Probes, Inc., Eugene, OR) as described by Sammak and Borisy (1988). Rhodamine-tubulin was drop-frozen and stored in liquid nitrogen at a concentration of 8–12 mg/ml in microinjection buffer (50 mM potassium glutamate, 0.5 M MgCl_2 , pH 6.5–6.7) and then diluted to 4–6 mg/ml immediately before use. The molar ratio of conjugated rhodamine to tubulin dimer was 1.5, based on rhodamine absorption at 550 nm, an extinction coefficient of 50,000, and protein concentration assay (Lowry et al., 1951). Microinjection was performed by continuous flow using custom drawn glass needles (Kopf Vertical Pipette Puller 720; Kopf Instruments, Tujunga, CA) fitted to a custom-designed pressure control system mounted on a micromanipulator (Leica, Inc., Deerfield, IL) as described by Wang (1992).

Immunofluorescence and Phalloidin Staining

All fixatives were prepared in warm cytoskeleton buffer (137 mM NaCl, 5 mM KCl, 1.1 mM Na_2HPO_4 , 0.4 mM KH_2PO_4 , 2 mM MgCl_2 , 2 mM EGTA, 5 mM Pipes, 5.5 mM glucose, pH 6.1; Small, 1981; Small et al., 1982). Cells were rinsed with warm PBS before fixation. For optimal preservation of microtubules and F-actin, cells were immersed for 1 min in 0.5% glutaraldehyde (Polysciences Inc., Warrington, PA) with 0.15% Triton X-100, rinsed thoroughly in cytoskeleton buffer, and postfixed for 10 min in 1% glutaraldehyde (Small, 1981; Small et al., 1982). Cells were again washed in cytoskeleton buffer and then incubated for 5 min in 0.5% 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, or with 4% formaldehyde for 10 min and then extracted in -20°C acetone for 5 min. Fixed 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. Balczon, 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 galate [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-phalloidin (Sigma Chemical Co.) or FITC-phalloidin (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 \times , NA 0.75, plan-achroplan phase contrast lens, a 63 \times , NA 1.30, Neofluar phase lens, or a 100 \times , NA 1.30 Neofluar lens. Fluorescence was detected using epifluorescent optics and cooled CCD camera (TE/CCD-576EM; Princeton Instruments, Trenton, NJ). Images were processed by subtraction of camera dark noise and filed 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 $\sim 0.7\%$ ($n = 1,000$). 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 phalloidin (Fishkind and Wang, 1993).

Cleavage Pattern Varies with the Configuration of Chromosomes

Upon entry into prophase, multinucleated cells typically had four or more centrosomes with associated microtubules. These centrosomes 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 chromosomes. 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 tripolar 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

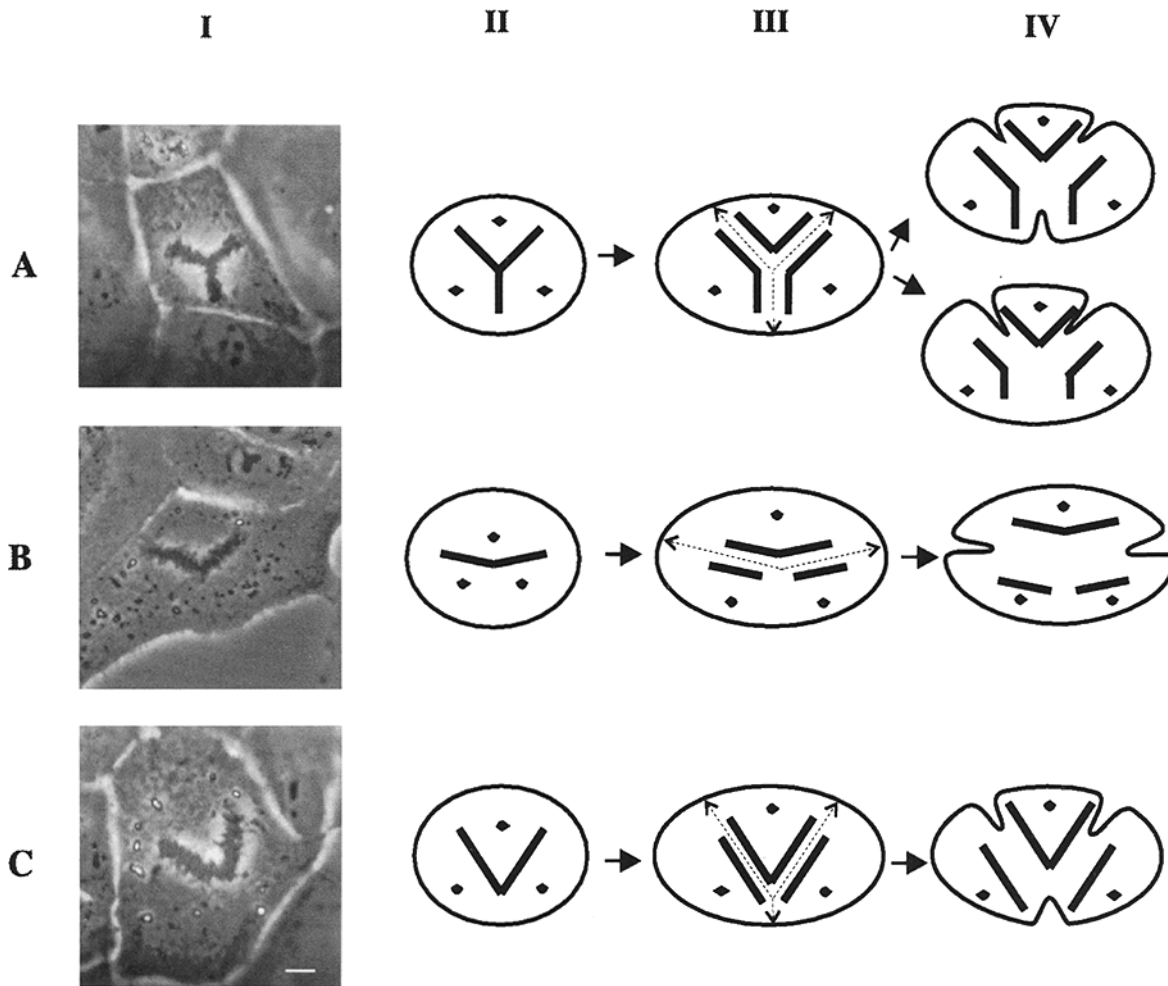


Figure 1. Chromosomes are arranged into a “Y” (A), shallow “V” (B), or sharp “V” (C) shape at metaphase, shown as phase images (I) and schematically in (II). Progression through anaphase (III) to cleavage (IV) is illustrated for each. Chromosomes are represented by thick black lines, spindle poles as 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 .

between the chromosomes: cells with a sharp V (range $\sim 70^\circ$ to 125°) typically divided into three ($n = 9/11$), while those with a shallow V (range $\sim 125^\circ$ to 180°) always divided into two ($n = 24/24$). Cleavage pattern in relation to chromosome organization is summarized in Fig. 1 (II–IV).

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 chromo-

somes (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.

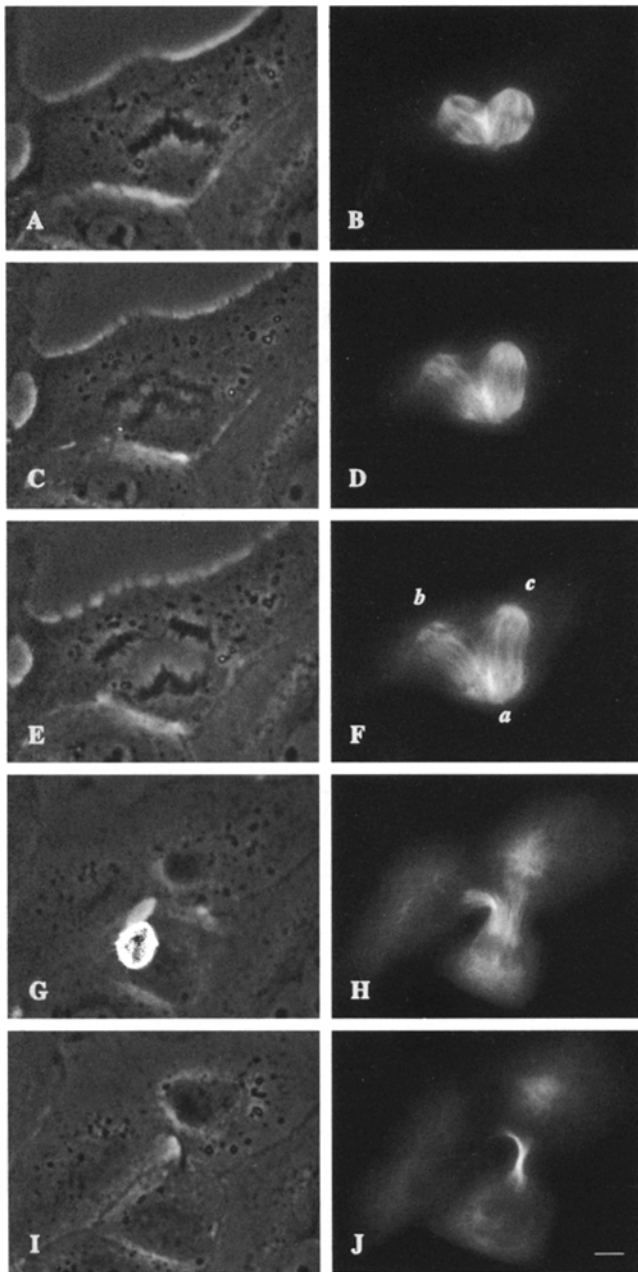


Figure 2. Cleavage and microtubule dynamics in a tripolar NRK cell with a shallow V-shaped metaphase plate (A). The cell was injected during prometaphase (A) with rhodamine-tubulin (B) and monitored as it progressed through anaphase onset (C and D), anaphase (E and F) to telophase (G and H). The cell was then fixed and stained with FITC-phalloidin (I) to reveal F-actin accumulation as a marker for cleavage activity. Multiple spindle poles were apparent during metaphase (B, arrowheads). By anaphase onset these had converged into three functional spindle poles (D, arrows) with prominent kinetochore microtubule bundles emanating towards the chromosomes. The chromosomes split into three groups (C, E, and G) and arrays of interzonal microtubules developed in the midzones (F and H). Among the three functional spindle poles (H, a, b, and c), two were equivalent (a and b, and a and c), containing a normal configuration of intervening chromosomes and typical midzone microtubule bundles. The third pair (b and c) had chromosomes only on one side, with a small number of microtubule bundles located between the two sets of chromosomes (H, arrow). F-actin localization (I) reflected closely the organization of midzone microtubules. Bar, 10 μ m.

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 symmetrical 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,

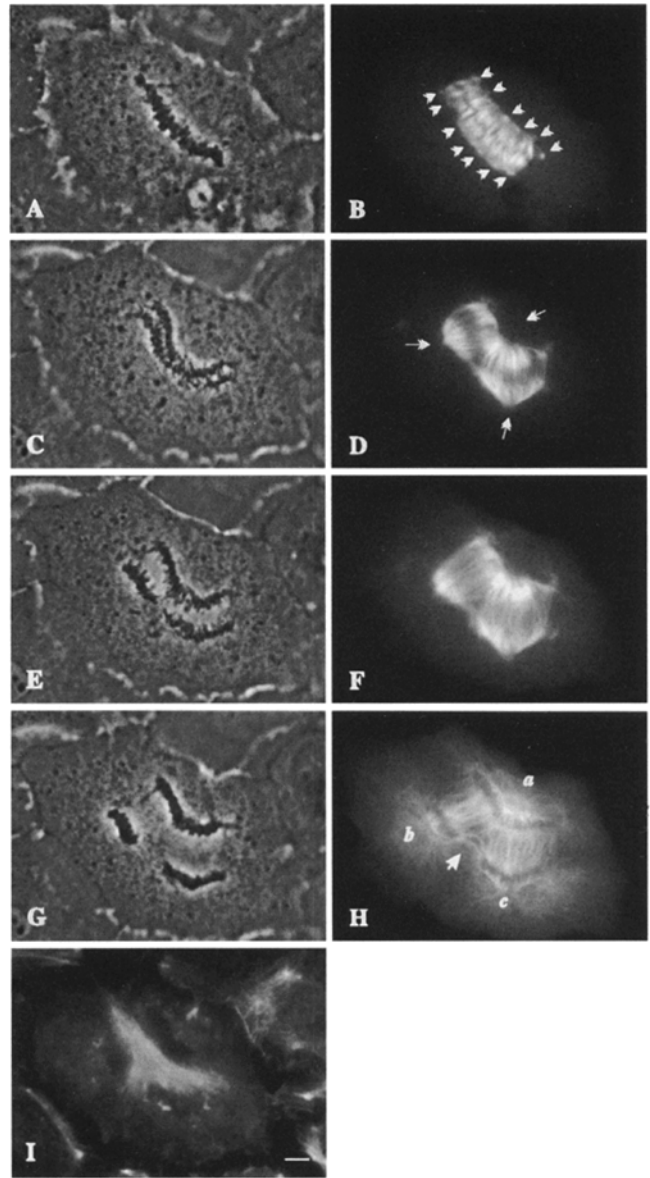


Figure 3. Cleavage activity and microtubule dynamics in a tripolar NRK cell with a shallow V-shaped metaphase plate. The cell was injected during prometaphase (A) with rhodamine-tubulin (B) and monitored as it progressed through anaphase onset (C and D), anaphase (E and F) to telophase (G and H). The cell was then fixed and stained with FITC-phalloidin (I) to reveal F-actin accumulation as a marker for cleavage activity. Multiple spindle poles were apparent during metaphase (B, arrowheads). By anaphase onset these had converged into three functional spindle poles (D, arrows) with prominent kinetochore microtubule bundles emanating towards the chromosomes. The chromosomes split into three groups (C, E, and G) and arrays of interzonal microtubules developed in the midzones (F and H). Among the three functional spindle poles (H, a, b, and c), two were equivalent (a and b, and a and c), containing a normal configuration of intervening chromosomes and typical midzone microtubule bundles. The third pair (b and c) had chromosomes only on one side, with a small number of microtubule bundles located between the two sets of chromosomes (H, arrow). F-actin localization (I) reflected closely the organization of midzone microtubules. Bar, 10 μ m.

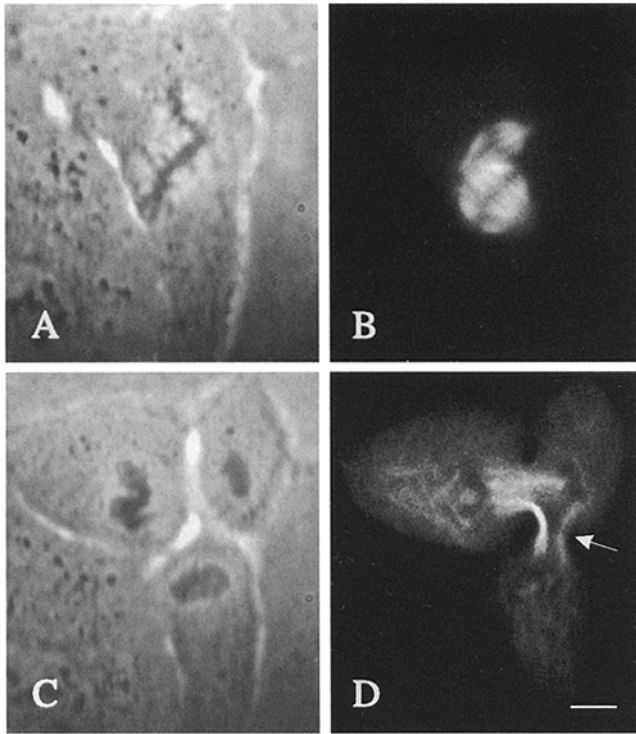


Figure 4. Cleavage of a tripolar NRK cell with a sharp V-shaped metaphase plate. The cell was injected at prometaphase with rhodamine-tubulin. Despite the absence of shared metaphase chromosomes between one pair of poles (compare *A* and *B*), three cleavage planes developed (*C*). Cleavage was accompanied 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 three-fold 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 for-

mation 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 (Andreassen et al., 1991). CHO1 staining varied among furrows, with no detectable concentration found in $\sim 10\%$ 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

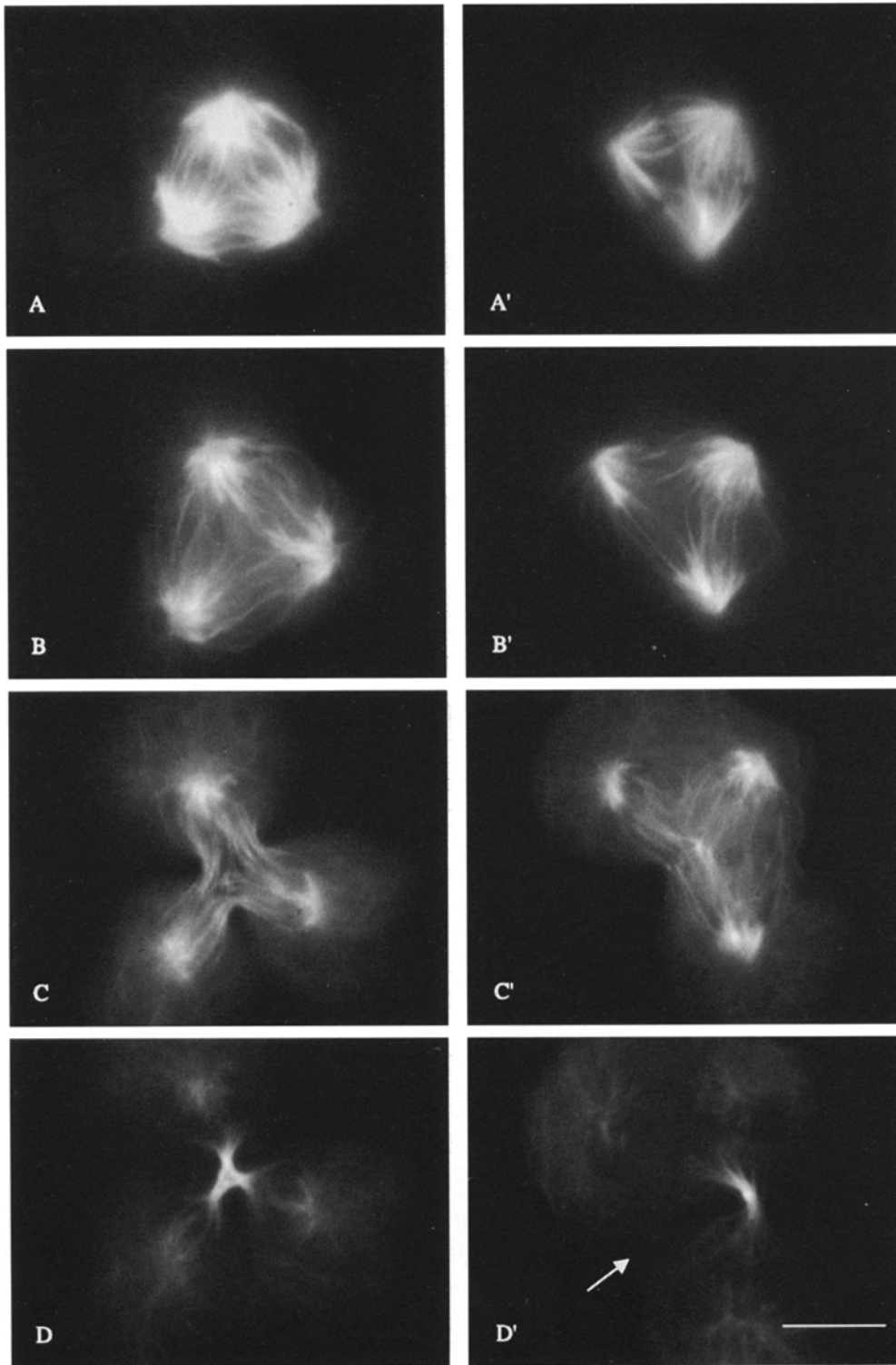


Figure 5. Midzone microtubule dynamics in relation to cleavage. Spontaneously formed tripolar cells were injected at prometaphase with rhodamine-tubulin and observed as they completed cell division (judged by midbody formation). In the first series (A–D), the cell exhibited threefold symmetry in the organization of its microtubules and spindle poles as it progressed from metaphase (A), through anaphase (B), to telophase (C). Ultimately, this cell divided into three equal portions (D). By contrast, in the second series (A'–D') the threefold symmetry apparent at metaphase (A') was lost as microtubule bundles between the top two spindle poles relocated to other regions during anaphase and telophase (B' and C'). No furrowing was observed in this region, and the cell cleaved into two unequal parts (D', arrow indicates the direction of furrowing). Bar, 10 μ m.

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; Mastronarde 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

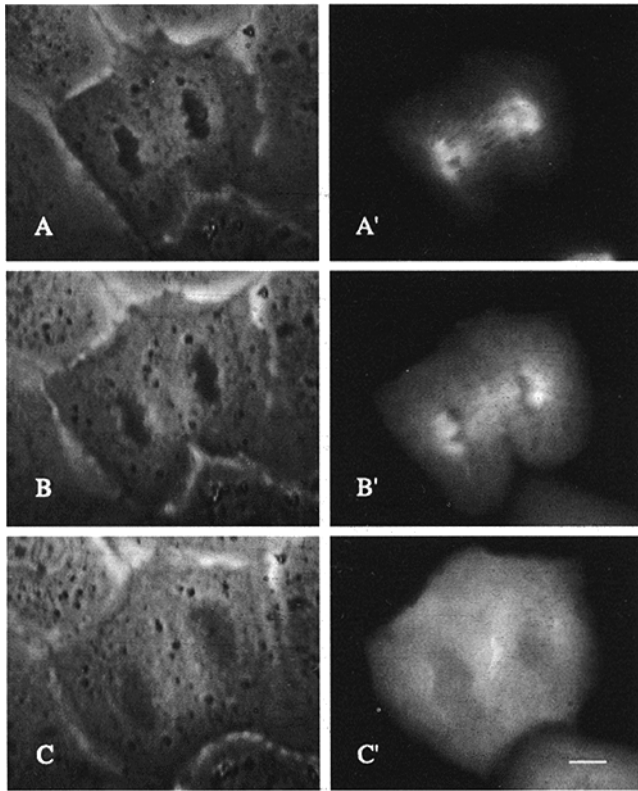


Figure 6. Effects of nocodazole on midzone bundles and cleavage after the initiation of furrowing. A cleaving bipolar NRK cell (A) was treated with 2.5 μ M nocodazole (B) and viewed after 20 min incubation (C). All microtubules were removed from the vicinity of cell cortex (C') and the cleavage furrow regressed (C). Bar, 10 μ m.

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 micro-

tubule 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 ingressions. 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 Bernat, 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

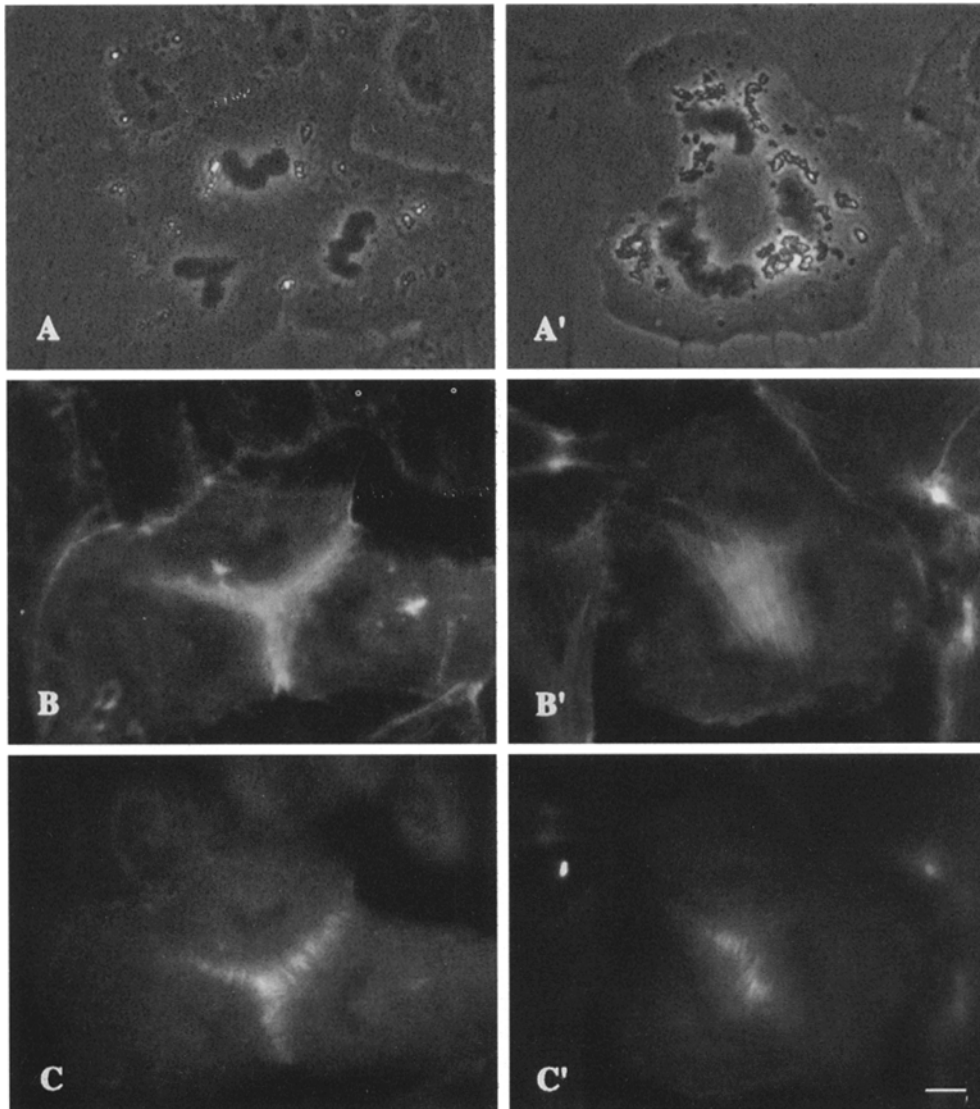


Figure 7. TD60 localization in tripolar NRK cells. Cleavage activity was assessed by F-actin accumulation (*B* and *B'*). One cell (*A–C*) was undertaking a three-way cleavage at time of fixation; F-actin (*B*) and TD60 (*C*) were found in each furrow. A second cell (*A'–C'*), which had a shallow V-shaped 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 substitutes. 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.

This study was prompted by discussion with members from the labs of Drs. David Burgess, Bill Earnshaw, Tony Hyman, Dan Kiehart, Issei Mabuchi, Fumio Matsumara, Tom Pollard, and John White during a Human Frontier Meeting with Ray Rappaport as invited discussant. We thank all attendees for their stimulating thoughts and ideas. We are also grateful to Dr. Kip Sluder for his comments on the manuscript.

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