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# Unusual Cleavage Furrows in Vertebrate Tissue Culture Cells: Insights into the Mechanisms of Cytokinesis

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In cultures of the epithelial cell lines, PtK2 and LLC-PK, some cells assume unusually large flattened morphologies and, during cell division, produce unusual cleavage furrows. We have microinjected some of these large cells with fluorescent actin and myosin probes to determine how the cell's shape and the position of its mitotic spindle affect the deposition of actin and myosin in the forming cleavage furrow. In cells with two spindles, contractile proteins were recruited not only to the cortex bordering the former metaphase plates but also to the cortex midway between each pair of adjacent nondaughter poles or centrosomes. The furrowing between adjacent poles seen in these cultured epithelial cells conformed to the furrows seen when echinoderm eggs were manipulated into a torus shape so that the poles of two mitotic spindles were adjacent to one another [Rappaport, 1961]. The recruitment of contractile proteins and the formation of furrows between adjacent centrosomes was a function of the distances between them. When adjacent centrosomes were positioned too close together neither contractile protein recruitment nor furrow formation occurred. If a normal-sized spindle was present in a very large cell, fibers of contractile protein assembled in the cortex above the former metaphase plate but they did not extend to the cell periphery, resulting in an inhibition of cytokinesis. In all injected cells, the recruitment of actin and myosin to the cell surfaces could first be detected at mid-anaphase before there was any visible sign of furrowing. Our results suggest that vertebrate cells share common mechanisms for the establishment of the cleavage furrow with echinoderm cells. The results are consistent with a model in which (1) the positions of the centrosomes and their linearly connected microtubules determine the position for the assembly of the cleavage furrow, and (2) the signal arrives at the surface within a few minutes after the initiation of anaphase. We speculate that an interaction of the ends of microtubules from adjacent centrosomes with the cell surface promotes a clustering of integral membrane protein(s) that interact with and target contractile proteins to a position midway between centrosomes where furrowing occurs. Cell Motil. Cytoskeleton 39:95–106, 1998. 

**Key words: cytokinesis; mitotic spindle; actin; myosin, mitotic poles, mitosis; cell division; daughter centrosomes; cousin centrosomes**

## INTRODUCTION

The mitotic spindle and the cleavage furrow are intimately linked, with furrow placement dictated by the position of the metaphase plate [reviewed in Rappaport 1971, 1986, 1996]. As a consequence when the mitotic spindle is in the center of the cell, a symmetrical cleavage

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furrow is formed around the periphery of the cell, the so-called purse string arrangement [reviewed in Schroeder, 1975]. When the spindle is asymmetrically positioned in a cell, the furrow is displaced from the cell equator producing daughter cells of unequal size. In some large eggs, when a small spindle lies parallel to the membrane at one side of the cell the resultant cleavage furrow forms at that side over the former metaphase plate, and as it contracts, the furrow propagates around the equator of the cell [Rappaport, 1996]. In tissue culture cells when the spindle is positioned asymmetrically, actin and myosin assemble over the former metaphase plate in an asymmetric pattern and contraction is asymmetric, beginning at the side of the cell closest to the spindle [Sanger et al., 1989b, 1994].

The correlation of cleavage furrow position with metaphase plate position becomes more complicated when multiple spindles are present in one cell. In a series of seminal experiments, Rappaport demonstrated that when an echinoderm zygote was manipulated so that the cell formed a doughnut or torus containing two mitotic spindles, cleavage furrows formed not only around the center of each spindle, but furrows could also form between the centrosomes of the adjacent spindles [summarized in Rappaport 1971, 1986]. Other experiments showed that if a centrally positioned spindle was made smaller, it did not induce the formation of a cleavage furrow unless it was moved closer to the cell surface [Rappaport, 1996]. Although there have been suggestions that the cleavage furrow stimulus emanates from the chromosomes and not from the centrosomes [Earnshaw and Cooke, 1991], it is difficult to interpret the Rappaport torus experiment in those terms unless there are several ways in which the cleavage furrow can be induced and assembled in different types of cells.

This paper describes the effect of cell shape, spindle position, and multiple mitotic poles on the recruitment of actin and myosin in the formation of cleavage furrows in the tissue culture cell lines, PtK2 and LLC-PK. In many experiments, our results on these vertebrate-derived tissue culture cells are almost identical to those obtained for the experimentally manipulated echinoderm blastomere cells. We have injected all of these PtK2 and LLC-PK cells with actin or myosin fluorescent probes so that we could determine not only if a furrow formed, but when and where the contractile cytoskeletal proteins first appeared in the furrow region after the initiation of anaphase. We find that cleavage furrows form not only between separating daughter chromosomes but also between centrosomes of adjacent spindles as in the torus experiment of Rappaport [1961]. Our injection results demonstrate that contractile proteins appear in the future cleavage furrows before any sign of surface contractions. These experiments support the hypothesis that the mechanism for the establishment of cleavage furrows is the same for all types of cells and that the centrosomes and their linearly connected microtubules may play an important role for the assembly of the cleavage furrow. This work was presented in a preliminary form at the American Society for Cell Biology meeting [Sanger et al., 1995].

## MATERIALS AND METHODS

### Cells and Injected Fluorescent Cytoskeletal Probes

PtK2 and LLC-PK cells were obtained from the American Tissue Culture Collection (Rockville, MD) and cultured on coverslips under conditions previously described [Sanger et al., 1989a; Zurek et al., 1990]. Rhodamine-labeled phalloidin was obtained from Molecular Probes (Eugene, OR), concentrated and used as we have recently reported [Sanger et al., 1994]. Myosin light chains were purified from chicken skeletal muscles [Mittal et al., 1987]. We have demonstrated that the fluorescently labeled myosin light chains (myosin light chains 1, 2, and 3) can be used as a mixture or used separately to localize the positions of the native myosin molecules in living muscle and nonmuscle cells [Mittal et al., 1987; Sanger et al., 1989, 1994]. These cytoskeletal probes were microinjected during either interphase (myosin light chains) or in prophase (fluorescently labeled phalloidin) using a specially designed pressure injection device coupled to a Leitz micromanipulator [Pochapin et al., 1983; Sanger et al., 1985].

### Imaging of the Microinjected Cells

The coverslips were mounted on glass slides using a stiff and nonmelting silicone lubricant (high vacuum grease; Dow Corning, Midland, MI) to form a closed chamber. The microscopic stage on the microscope was heated to a stable temperature of 37°C before images were recorded. This procedure prevented shifting of the slides and images during the recording procedures. The images were obtained using a 63× Zeiss Phase Contrast Planapochromat lens (NA 1.3) mounted on an Olympus Vanox microscope. A Dage-MTI-SIT camera (Dage-MTI, Michigan, IN) was mounted on the top of the microscope. The images were recorded using an Image-I processing system (Universal Imaging, West Chester,

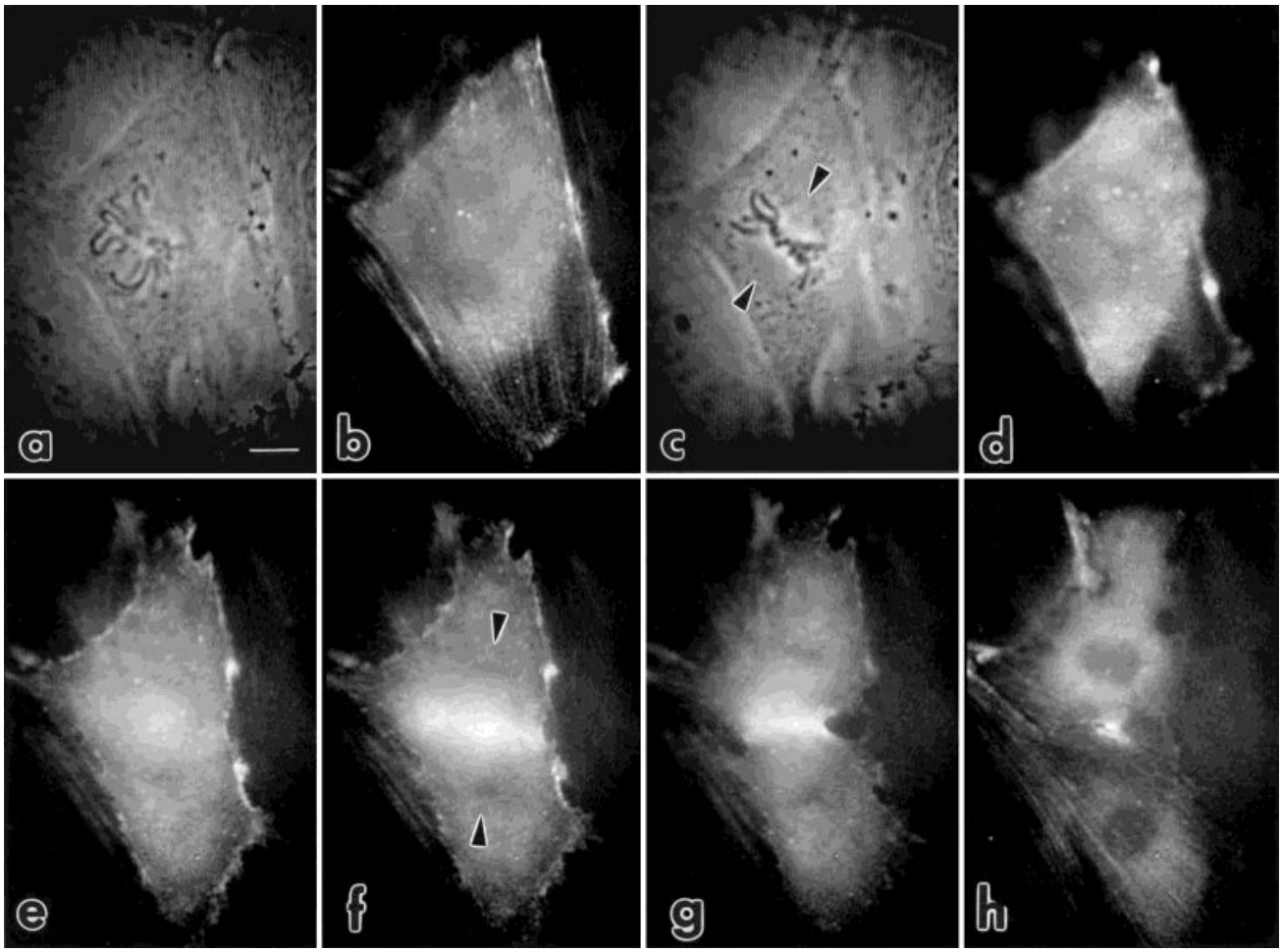


Fig. 1. Time points of a mitotic PtK2 cell previously microinjected with fluorescent myosin II light chains. **a:** Phase-contrast image of the cell in prophase. **b:** Fluorescent myosin light chains in the same prophase cell. Striated stress fibers are visible in the bottom half of the cell. **c:** Same cell at metaphase. The arrows indicate the position of the centrosomes and point along the long axis of the spindle. Compare the axis of the spindle at this time point to its final position during anaphase (*arrowheads*, **f**). **d:** Fluorescent image of the same metaphase cell. Note that the stress fibers have disassembled by this time point. **e,f:** In anaphase, myosin accumulates in the area between the separating chromosomes, which appear as dark bodies. **f:** *Arrowheads*, positions of the centrosomes and point along the spindle axis. Note how the spindle has rotated from its original position in the cell at metaphase (c). **g:** During cytokinesis, the band of myosin narrows, and. the polar regions of the cell begin to spread. **h:** Double gasket of myosin in the midbody connecting the two daughter cells at the end of cytokinesis. Note the reformation of the stress fibers in the lower cell. Times: a,b: 0; c,d: 10 min, 50 sec; e: 21 min, 32 sec; f: 22 min, 54 sec; g: 27 min, 22 sec; h: 54 min, 12 sec. Scale = 10 μm.

PA). Images were averaged over 15–30 frames. Some images were filtered to enhanced the fibrous structure of the fibers in the cells. Images were recorded from the monitor as previously described [Waterman-Storer et al., 1993].

## RESULTS

### Cytokinesis in Cells With Bipolar Spindles

The first reorganization of the actin cytoskeleton in mitosis occurs in prophase when those stress fibers underneath the condensing chromosomes disassemble (Fig. 1a,b). The remaining peripheral stress fibers usually disassemble by metaphase resulting in a diffuse distribution of actin and myosin in the cell (Fig. 1c,d).The first indication of recruitment of myosin (Fig. 1e) and actin in the future furrow region occurs in mid-anaphase (Fig. 1e,f). The plane of the furrow is determined by the final position of the metaphase plate, which in this cell shifted counterclockwise between the time (Fig. 1c) and anaphase onset. During contraction of the furrow the myosin band becomes narrower (Fig. 1e–g), with the amount of

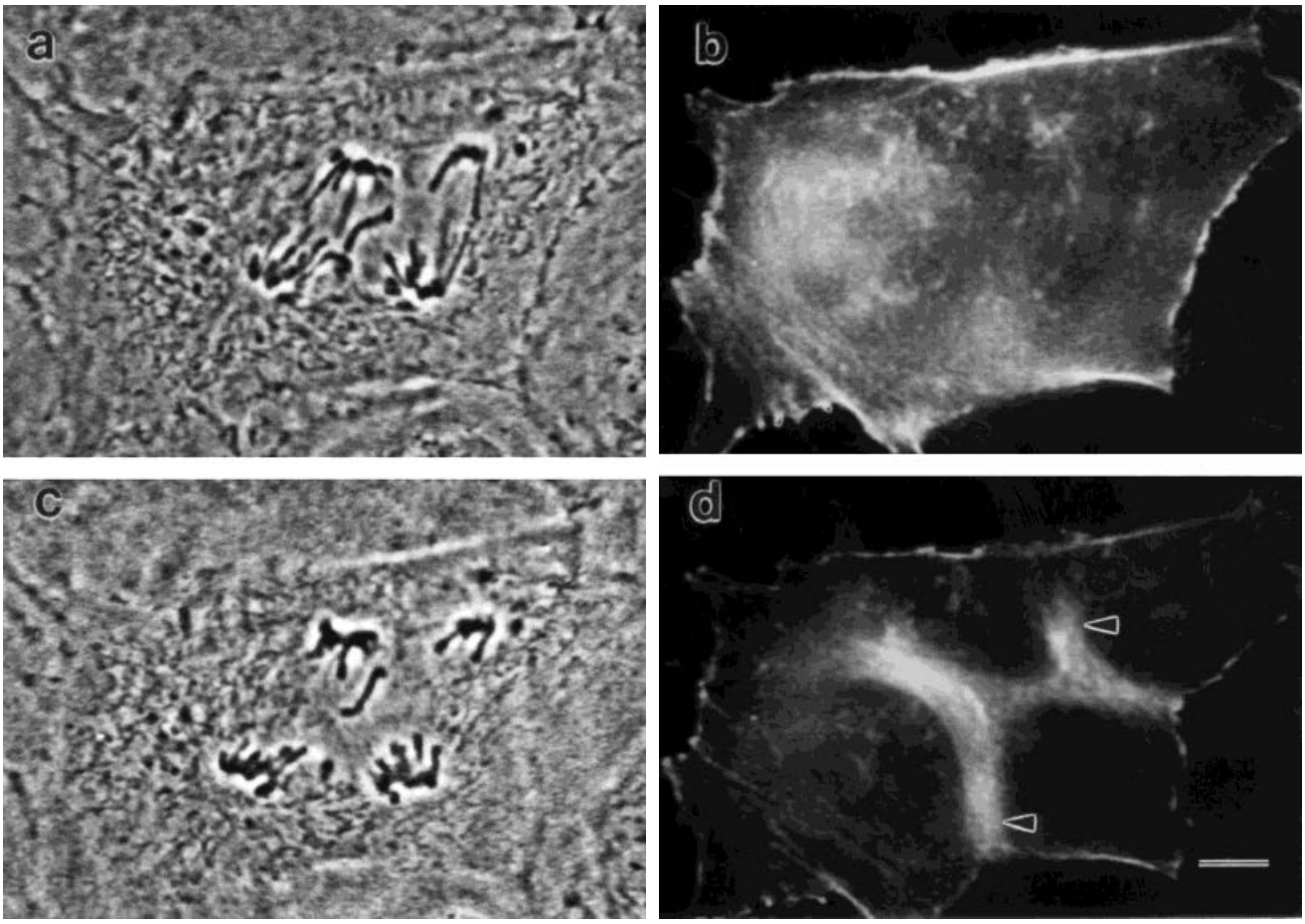


Fig. 2. Living PtK2 cell previously injected with rhodamine-labeled phalloidin that contains two mitotic spindles. The positions of the chromosomes can be detected in the phase-contrast images **(a,c)** and that of the phalloidin labeled F-actin in the fluorescence images **(b,d)**. The width of this injected cell was 73 μm **(a,b)**. The center of the right-most spindle was 15.5 μm from the right side of the cell, while the center of the left spindle was 40 μm from the left edge of the cell. The actin was recruited to the positions of the two former metaphase plates and in the areas *(arrowheads)* between the two pairs of adjacent poles **(c,d)**. Because the two spindles are positioned asymmetrically in the cell, the cleavage furrow that extended across the spindle equators did not reach the most distant side of the cell. Fibers, some of which are banded, can be seen in the furrows (d). The images in (a,b) are separated from (c,d) by 7 min, 25 sec. Scale = 10 μm.

myosin in the cleavage ring decreasing as contraction proceeds At the end of cytokinesis, two small gaskets of myosin (Fig. 1h) and actin are associated with the midbody connecting the two daughter cells. Stress fiber reformation in the spreading daughter cells is sometimes asynchronous (Fig. 1h).

### Cytokinesis in Cells With Multipolar Spindles

When a binucleate cell, injected during prophase with fluorescent phalloidin, reached early anaphase, two spindles were present adjacent to one another, and displaced closer to one side of the cell (Fig. 2a) with F-actin diffusely distributed in the cytoplasm (Fig. 2b). During cytokinesis two sets of actin fibers formed at the equator of the two spindles and merged in a cleavage furrow that reached from the proximal edge of the cell part way across the middle of the cell (Fig. 2c,d). Actin fibers were also recruited, in two bands, to the areas midway between the adjacent poles of the two spindles (Fig. 2d, arrowheads) with the band between the upper pair of spindle poles extending all the way to the cell periphery where the membrane was pulled inward. The lower band extended only part way towards the edge of the cell and furrowing was not evident at that margin. Although furrowing activity was apparent between each

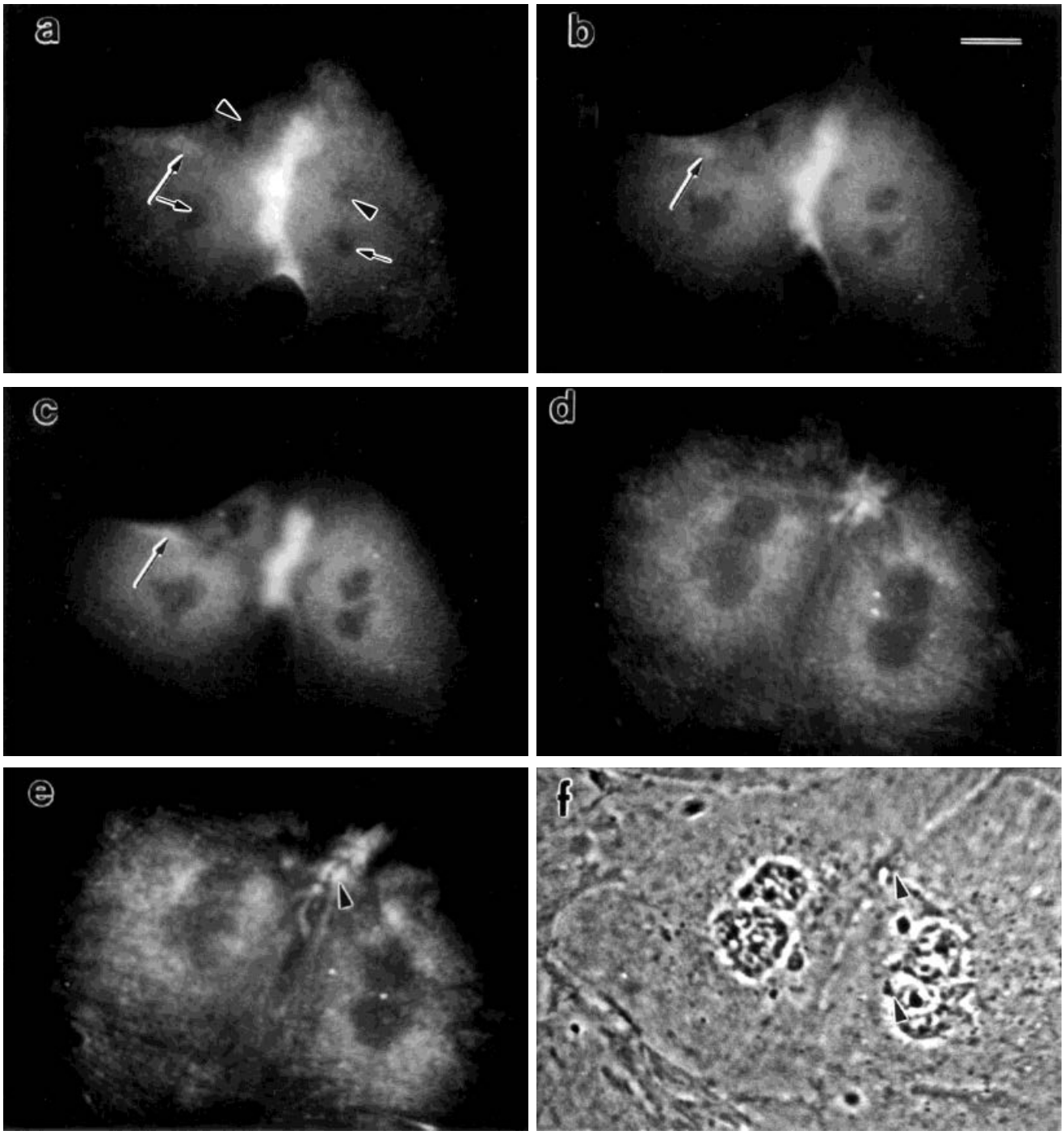


Fig. 3. A binucleate PtK2 cell was microinjected with myosin light chains. The cleavage furrow that formed across the metaphase plates extended from the bottom edge of the cell almost to the opposite side of the cell. Positions of the separated chromosomes: the upper spindle *(arrowheads)*; lower spindle *(small arrows)*. The two sets of telophase chromosomes on the right were separated by only 8 μm, and a cleavage furrow did not form between them. On the other side of the main furrow, a small furrow (*large arrows*, **a–c**) formed between the two sets of telophase chromosomes that were 18 μm apart. This furrow contracted but then regressed and disappeared. The metaphase plate furrow constricted the cell to form a narrow isthmus connecting the two daughter cells, each containing two nuclei **(d–f)**. The remnant of the cleavage furrow can still be detected in the isthmus region (**e,f**, *arrowheads*), as the stress fibers reform in the two binucleate cells (e). Times: a: 0; b: 3 min, 3 sec; c: 4 min, 46 sec; d: 56 min, 14 sec; e,f: 1 hr, 11 min, 26 sec. Scale = 10 μm.

set of daughter chromosomes and between the adjacent poles of the two spindles, cytokinesis was not completed to form four mononucleated cells, rather a tetranucleate cell resulted.

In another multipolar cell, fluorescent myosin light chains assembled in a prominent band across the midzone of two adjacent spindles (Fig. 3). No fluorescence or furrow formed between one of the pairs of adjacent poles that were separated by 8 μm, whereas a small amount of myosin label and a correspondingly small furrow formed between the second pair that were separated by 18 μm (Fig. 3). This small furrow did not cleave completely and eventually relaxed with the concomitant loss of fluorescent probe. The prominent furrow cleaved asymmetrically progressing from one side of the cell toward the other with the midbody forming at one side.

### Influence of Cell Shape on Cytokinesis

When a cell was very large with a centrally positioned spindle of normal size, as in the LLC-PK cell in Figure 4, contractile proteins were recruited to the surfaces above and below the former metaphase plate but did not reach from one edge of the cell to the other (Fig. 4a–c). The furrowing that took place was from the top and bottom of the cell, rather than from the sides inward. The furrow eventually relaxed and the cell re-entered interphase with the reformation of the stress fibers (Fig. 4d–i). During furrowing the band of contractile protein present in the early stages of cytokinesis became organized into individual fibers (Fig. 4e–h). Several of the furrow fibers remained in place as the cell re-entered interphase and were indistinguishable from the interphase stress fibers (Fig. 4g,h). A greater number of stress fibers formed in the lower half of the cell (Fig. 4 h,i) reflecting the higher concentration of cortical actin localized there in metaphase and anaphase (Fig. 4b,c).

In one unusual PtK2 cell with a bipolar spindle, injected fluorescent myosin light chains extended in a band beyond the midzone of the spindle into an arm of cytoplasm that projected from the main body of the cell at a site bordering the cleavage furrow (Fig. 5a). The myosin probes extended from the cleavage furrow about 10 μm, along the cortex of the shelf-like extension (Fig. 5b). The thickness of the band of myosin that was recruited initially to the cortex of the extension was about one-half that of the myosin band in the centrally positioned furrow Fig. 5b). There was only partial contraction of the furrow (Fig. 5 b–d) with fibers forming in the cytoplasm and persisting in the cortex of the extension (Fig. 5e,f).

## DISCUSSION

The classic ''torus'' experiments of Rappaport [1961] on echinoderm blastomeres defined the relationship between the poles or centrosomes of mitotic spindles and the cell surface that lead to the formation of cleavage furrows. In those experiments, a glass bead was pressed into a fertilized egg resulting in the formation of a torus-shaped cell with a mitotic spindle positioned between the bead and one side of the cell. The cleavage furrow formed midway between the spindle poles, extending from the edge of the cell to the bead. After cytokinesis, the resultant cell had two nuclei in a horseshoe-shaped cytoplasm surrounding the glass bead. In the second embryonic division, two mitotic spindles formed in opposite arms of the horseshoe, but in addition to the two expected cleavage furrows, a third cleavage furrow formed between the adjacent poles of the two spindles, provided that the two poles were close together in the common cytoplasm. In some cases, the furrows that formed between the adjacent poles did not cleave completely. Rappaport concluded that centrosomes and not chromosomes [Swann and Mitchison, 1958] were the important elements in the establishment of the furrow regions.

When mitotic cells are microinjected with fluorescent probes for actin and myosin, concentrations of these essential proteins are detected in the cleavage furrow [Mittal et al., 1987; Sanger et al., 1989b, 1994]. The concentration of the proteins is first detected by mid-anaphase and, in cells with a single mitotic spindle, it is always in a plane midway between the poles of the spindle, regardless of the position of the spindle in the cell [Mittal et al., 1987; Sanger et al., 1989], as expected from

Fig. 4. LLC-PK cell, previously injected with rhodamine phalloidin, with a normal-sized spindle in a very large cell. **a,b:** Phase-contrast and fluorescent images of metaphase cell. The cell was 61 μm wide at the metaphase plate with the center of the spindle 25.5 μm from the right edge of the cell and 35.5 μm from the left edge. **c:** The actin band that formed initially in anaphase in the furrow was about 21 μm long. During the prolonged contraction in this cell, the fluorescent band gradually became fibrous in substructure **(d–f)** with attachment plaque-like structures at the ends of the band (*arrowheads*, **f**). Although the length of the fluorescent band increased by about 6 μm in length, it never reached the two lateral boundaries of the cell (c–f). Some of the cell's cleavage furrow fibers persist in their position as the binucleate cell entered interphase and reformed its stress fibers **(f–i)**. Times given from the anaphase image in (c): c: 0; d: 8 min, 36 sec; e: 19 min, 13 sec; f: 45 min, 32 sec; g: 2 hr, 3 min, 59 sec; h: 5 hr, 11 min, 9 sec; i: 6 hr, 35 min, 36 sec. Scale = 10 μm.

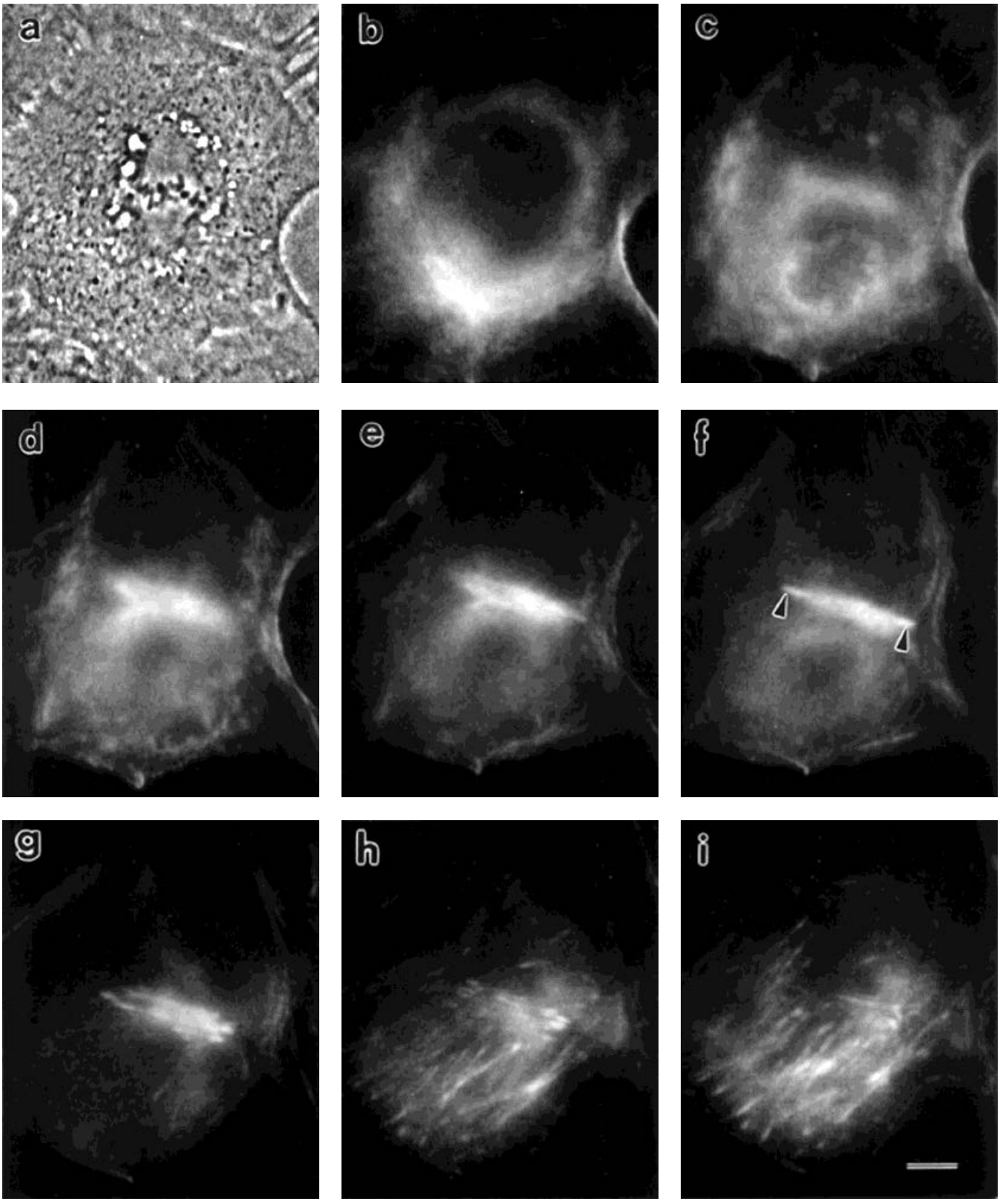


Figure 4.

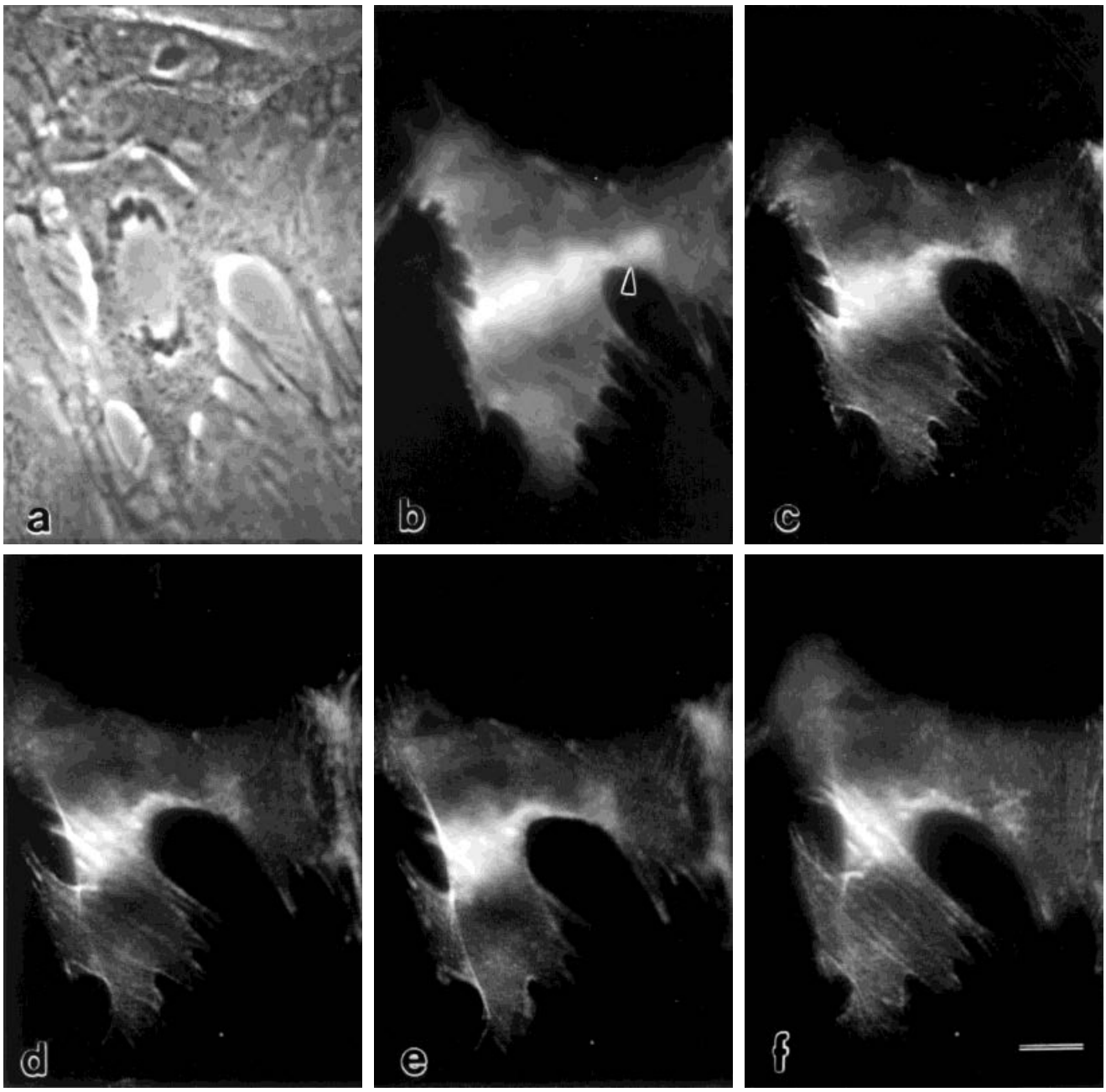


Fig. 5. This unusually shaped PtK2 cell exhibits the normal recruitment of the myosin light chain to the former metaphase plate **(a)**. Myosin is also recruited to a ledge of cytoplasm extending *(arrow)* from the dividing cell. This unusual cell shape presumably resulted from the neighboring interphase cells holding the mitotic surfaces in place (a,b). The positions of the chromosomes can be seen in the phase-contrast image (a) and in the subsequent fluorescent images as a negative image **(b–f)**. b: The thickness of the band of fluorescent myosin probe (3–4 μm) recruited to the ledge extension is about one-half that of the myosin recruited to the region over the former metaphase plate (6–9 μm). Cytokinesis is greatly prolonged with the result that cleavage is not completed and the stress fibers reform in the binucleate cell (b–f). Times: a,b: 0; c: 10 min; d: 14 min, 30 sec; e: 30 min, 51 sec; f: 44 min, 55 sec. Scale = 10 μm.

the extensive literature of cytokinesis [Rappaport, 1996]. The metaphase mitotic spindle often changes its orientation before anaphase onset [Sanger et al., 1989; Rappaport, 1996] so that the targeting of the contractile furrow protein is determined not by the initial position of the metaphase plate but by its position at anaphase onset (Fig. 1c–f). This indicates that there is some interaction between a component of the spindle and the cell surface occurring at anaphase onset that initiates the assembly of cytoskeletal proteins into the cleavage furrow. This

interaction is distance dependent with respect to the spindle and the cell surface [Rapaport, 1996]. In very large cells with asymmetrically displaced spindles, the cytoskeletal proteins assemble in the cortex nearest the spindle equator and fail to extend laterally to the margins of the cell (Figs. 2, 4). Future work will have to determine whether this relationship is linked to the interaction of spindle microtubules with the cell cortex.

In the present study, PtK2 cells with two spindles showed a positioning of cleavage furrow protein between adjacent spindle poles similar to that seen in the echinoderm torus cells, supporting the suggestion of Rappaport [1996] that the centrosomes play an important role in the formation of the cleavage furrow. We also found that if adjacent poles of the sister spindles in these tissue culture cells were very close together (separated by less than 8 μm), no furrow protein assembled between those adjacent poles (Fig. 3). However there was no clear correlation between centrosome separation and furrow formation when the centrosomes were greater than 8 μm apart. In the cell shown in Figure 3, only a small amount of contractile protein assembled between the pair of adjacent poles that were 18 μm apart. Yet in the cell illustrated in Figure 2, the two pairs of adjacent spindle poles were separated by 15 and 20 μm and protein assembly, as well as contraction, occurred between them. To discover whether microtubules emanating from the centrosomes might interact with the cortex where the cleavage furrow proteins assemble, it will be important in future studies to determine the distribution of the microtubules that extend from adjacent spindle poles.

Recent evidence showing that some proteins located on metaphase chromosomes can be detected in the furrow region starting at mid-to late anaphase [Andreassen et al., 1991; Cooke et al., 1987; Earnshaw and Cooke, 1991], has revived the idea that chromosomes determine the position of the cleavage furrow [reviewed by Swann and Mitchison, 1958]. Experiments in which furrow formation was inhibited when perforations were introduced between the mitotic spindle and the cortex suggest that a signal from the spindle midzone is required for furrowing to occur [Cao and Wang, 1996]. However, earlier experiments with similar perforations adjacent to the spindle of mitotic echinoderm cells were interpreted as illustrating the importance of the ''astral rays'' (i.e., microtubules) radiating from the spindle poles [Dan, 1943]. Additional evidence that furrowing depends on the spindle midzone comes from observations of Wheatley and Wang [1996] that in multipolar cells, only when midzone microtubules were present between poles of multipolar spindles did cleavage occur. They hypothesized that the signal for cleavage furrow formation requires interaction between midzone microtubules and the cell cortex.

Several lines of evidence would seem to rule out the chromosomes as the source of a signal for furrowing. In syncitial *Drosophila*, embryos when the centrosomes and associated microtubules were dissociated from the replicating chromosomes by injection of aphidicolin, an inhibitor of DNA polymerase, the centrosomal structures moved to the cell surface, where they induced actin caps to form between the centrosomes [Glover et al., 1989]. At the posterior poles of the treated embryos, furrowing occurred in the absence of any chromosomes to form polar ''cells.'' The torus experiments of Rappaport [1961] and the furrows in double spindle cells reported in this paper also demonstrate that furrows can form in areas where chromosomes are absent. A recent report of Rieder et al. [1997] also shows examples of cleavage furrows forming between two independent spindles in PtK1 cells; and in insect spermatocyte spindles lacking chromosomes, furrowing occurred normally [Zhang and Nicklas, 1996]. Thus, it seems unlikely that proteins released from the chromosomes determine where furrows form. Moreover, in the case of oddly shaped cells (e.g., Fig. 5), it is difficult to image how putative chromosome signaling molecules could influence or direct the assembly of contractile proteins along a surface of the cell far removed from the former metaphase plate. As it is unlikely that microtubules from the lower pole can reach this surface, we assume that any microtubules impinging on this region must come from the upper centrosome on the right side of the dividing cell. It is also possible that the actin-myosin band propagated from the cleavage furrow along the membrane of the cell extension.

## Model Proposed to Explain Targeting of Cytoskeletal Proteins in Cells Undergoing Cytokinesis

A model that could account for recruitment of furrow proteins between centrosomes is outlined in Figure 6. If microtubules from pairs of centrosomes intersect at the cortex midway between spindle poles, a concentration of plus ends of the two sets of microtubules could interact directly or indirectly with the membrane and promote a clustering of protein(s) that allows targeting of actin filaments. There is evidence that tubulin binds to the transmembrane glycoprotein, CD2, a member of the immunoglobulin superfamily [Offringa and Bierer, 1993]. A concentration of microtubules impinging on the cell membrane could promote localized foci of a molecule like CD2, which, in turn, could provide a link to actin-binding proteins that would recruit actin filaments.

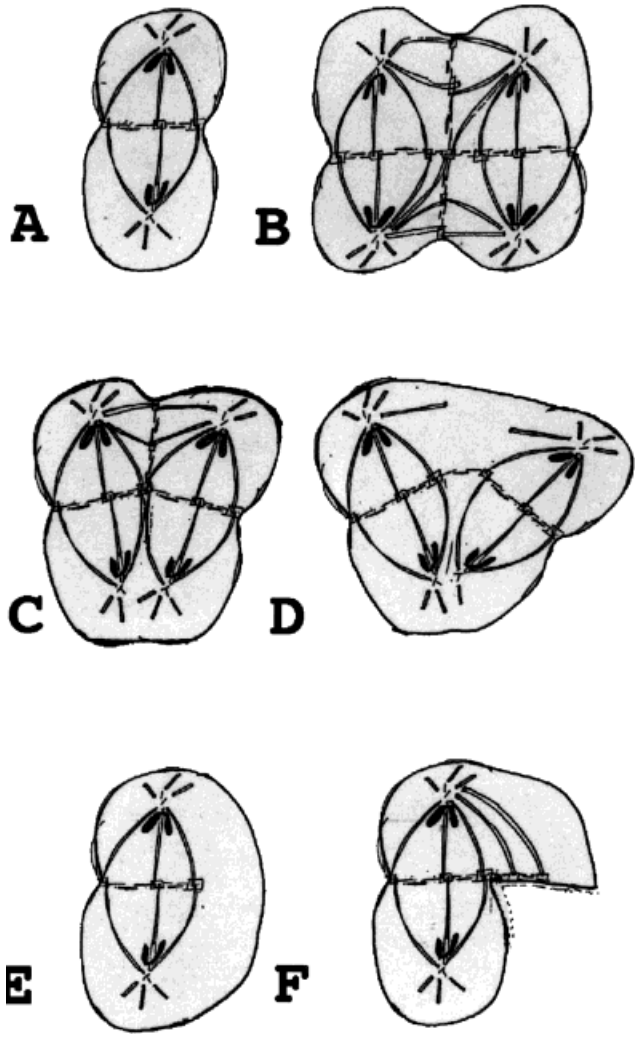


Fig. 6. Model based on the idea that interdigitating centrosomal microtubules interact with the cell surface to stabililze clusters of transmembrane proteins that bind actin filaments, which in turn interact with myosin filaments to form contractile units. Diagrammatic representations of cells show the postulated relationship between microtubules *(thin lines)* and contractile proteins *(thick dotted lines)*. **A:** In cells in which the mitotic spindle is symmetrically positioned, the microtubule ends are predicted to target a circular array of contractile protein, i.e., a pursestring. **B:** In cells containing two spindles, contractile protein would assemble at the membranes above and below the spindle midzones as well as at the midway between adjacent centrosomes. **C,D:** In cells containing two spindles, no furrows will form between centrosomes that are too close so that the two pairs of centrosomes act as one centrosome, or too far apart for the centrosomal microtubules to interact together to stabilize this region for actin interaction with the transmembrane proteins. **E:** In cells where the spindle is asymmetrically placed, the microtubules would not interact with the distal cell surface resulting in the assembly of an incomplete cleavage furrow. **F:** In a cell in which microtubules from only one centrosome can interact with part of the cortex, contractile protein can be targeted to that region.

Some integrins are concentrated in the furrows of certain cell types [Rogalski and Singer, 1985; Yonemura et al., 1993], and although they are not known to interact with microtubules, they do interact with alpha-actinin and talin [Pavalko et al., 1991; Simon et al., 1991], two proteins that are in the cleavage furrows of PtK2 cells [Fujiwara et al., 1978; Sanger et al., 1987, 1994, 1997]. A linking of foci of actin filaments by antipolar myosin filaments would result in the formation of a sarcomeric structure [Schroeder, 1975; Sanger and Sanger, 1980; Mabuchi, 1986; Sanger et al., 1994] whose contractile activity would lead to local furrowing activity (Fig. 6). The evidence that repositioning an anaphase spindle along the length of an elongated cell will induce furrowing at each new position of the spindle midzone [Rappaport, 1971], suggests that a transitory interaction of signal molecules at the cortex is sufficient for recruitment of furrow proteins. The signal molecules themselves might not remain in the furrow once it was initiated.

The deposition of myosin in the unusually shaped cell in Figure 5 suggests that even one set of uniformly polarized microtubules may be sufficient for recruiting proteins to a cleavage furrow. The geometry of the large outpocketing of cytoplasm on one side of the cell isolates it from the centrosome microtubules of the lower pole of the mitotic spindle. The amount of contractile protein in the cortex of the cell extension bordering the mitotic spindle is significantly lower than that in the cortex above the center of the mitotic spindle. The proposal that microtubule interactions with the cell cortex initiate furrow formation, implies that this interaction is absent between centromeres that are very close together as in the cell in Figure 3, where there is no furrowing or contractile protein concentration between one of the two pairs of centrosomes. The microtubules radiating from the adjacent centrosomes may not contact the cortex midway between the centrosomes as they presumably do in the plane of the metaphase plate and between centrosomes of adjacent spindle poles that are not so close together. A knowledge of the disposition of the microtubules at the time of actin and myosin recruitment is necessary to determine if microtubles that extend from the centrosomes are positioned to influence the targeting of cleavage furrow proteins.

The connection of actin filaments to the cell surface may be the same in both the cleavage furrow and stress fibers [Sanger et al., 1987, 1994, 1997; Sato et al., 1991]. In one injected cell (Fig. 4), some of the cleavage fibers remained in place after the cell failed to complete cytokinesis, and when the binuceate cell entered interphase additional stress fibers formed which were indistin-

guishable from the fibers in the furrow region. If actin, via α-actinin or talin, does interact with the transmembrane proteins (e.g., integrins) localized in these stabilized regions, a linking of these actin islands by antipolar myosin filaments would result in the formation of a sarcomeric structure whose contractile activity would lead to local furrowing activity. The linear alignment of the sarcomeric units around the middle of the mitotic cell would lead to the assembly of a circular contractile furrow.

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