Single Particle Tracking of Surface Receptor Movement during Cell Division

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Abstract. We have used fluorescent latex beads to label membrane receptors on cultured NRK cells. Movement of individual beads during cell division was recorded with digital imaging techniques. Surface-bound beads showed no organized movement during metaphase but started to migrate toward the equator ~1 min after anaphase onset, when chromosomes moved out of the equatorial region to create the interzone. The movement was most active in the central region of the cell near separating chromosomes, while beads located near the poles of the cell underwent primarily random motion. Most beads showed a surge in speed upon the passage of chromosomes, suggesting a possible link between chromosome separation and cortical reorganization. Furthermore, treatment of anaphase cells with cytochalasin D induced a rapid, simultaneous collapse of beads and cortical actin filaments into aggregates, indicating that the movement of beads was closely related to the reorganization of the actin cortex. In contrast to normal directional movement, cytochalasin-induced movement occurred in random directions and caused some beads in the equatorial region to move toward poles. Our results indicate that cytokinesis involves contractile activities, not only along the equator, but over a wide area of the actin-containing cortex. In addition, organized cortical activities appear to be temporally activated at anaphase onset, and spatially modulated by the spindle interzone or separating chromosomes.

DESPITE many years of investigation, the mechanism of cytokinesis in animal cells remains poorly understood. It is generally recognized that the contractility of the cell cortex plays an important role (for reviews see Mabuchi, 1986; Rappaport, 1986; 1991; Salmon, 1989; Satterwhite and Pollard, 1992; Conrad and Schroeder, 1990; Fukui, 1993). Moreover, numerous ultrastructural, microinjection, and molecular biological studies have provided strong evidence for the involvement of actin and myosin II in the generation of cortical forces (Perry et al., 1971; Fujiwara and Pollard, 1976; Mabuchi and Okuno, 1977; Knecht and Loomis, 1987; DeLozanne and Spudich, 1987). Since both actin and myosin II become transiently organized in the equatorial region during cytokinesis, they may effect cell cleavage by generating a strong contractile force along the equatorial plane.

What remains unclear is how the structure of actin and myosin becomes reorganized before and during cell division. Recent studies indicate that preexisting actin filaments can incorporate into the equatorial cortex of dividing cells (Cao and Wang, 1990*a*; Sanger et al., 1994), and that such incorporation involves the transport of cortical actin filaments toward the equator (Bray and White, 1988; Cao and Wang, 1990*b*). Consistent with this process, a set of endogenous actin filaments have been found to align along the long axis of the cell in the region flanking the equator (Fukui and Inoue, 1991; Fishkind and Wang, 1993). These observations indicate that, to reach a better understanding of cytokinesis, it is important to address not only contractility along the cleavage furrow, but also a more global restructuring of the cell cortex.

Important clues about the mechanism and regulation of the cortical reorganization during cell division may be provided by a detailed analysis of its spatial and temporal characteristics. For example, when does the transport start? How does the transport relate to chromosome separation? Does it occur over the entire cortex, or only over a limited region? Since cytokinesis is known to be tightly coupled, both spatially and temporally, to the separation of chromosomes (Rappaport, 1986, 1991), it is possible that cortical movement is also linked to the onset of anaphase. Through the regulation of the timing or pattern of cortical reorganization, the mitotic spindle may then effectively dictate the onset and site of cell cleavage.

To analyze motile activities of the cortex, we have chosen to follow the movement of surface receptors in dividing cultured cells. It has long been recognized that in interphase cells, the movement of many surface receptors is coupled to the motility of the underlying actin-containing cortex (for a recent review see Sheetz, 1993). In dividing cells, surface

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Concanavalin A (Con A)[1]-binding proteins have been shown to concentrate in the equatorial region (Koppel et al., 1982; McCaig and Robinson, 1982), with a spatial and temporal pattern similar to that manifested by actin and myosin. Furthermore, as will be shown in this study, surface receptors and cortical actin in dividing cells colocalize into aggregates after the treatment of cytochalasin D, indicating that they do move together as a complex structure.

Because of the ease that surface receptors can be labeled and tracked with fluorescent particles or latex beads, it is possible to determine precisely the characteristics of their movement (Gross and Webb, 1988). In the present study, we have analyzed the velocity of individual beads as a function of time and location. We show that receptors start to move toward the equator ~ 1 min after the onset of anaphase. The characteristics of the movement suggest that cortical reorganization may be regulated spatially and temporally by components associated with the spindle interzone or separating chromosomes.

Materials and Methods

Cell Culture

All experiments were performed with a well-spread subclone of normal rat kidney (NRK) cells (Fishkind and Wang, 1993). Many cells in this subclone maintain their association with neighbors during cytokinesis, without showing appreciable lateral constriction. Cells were cultured on glass coverslips at 37°C in 5% CO_2 for 48–72 h, using the F12K medium (JRH Biosciences, Lenexa, KS) supplemented with 10% fetal bovine serum (JRH Biosciences), 1 mM L-glutamine, 50 μg/ml streptomycin, and 50 μg/ml penicillin.

Processing of Latex Beads

Yellow-green fluorescent, carboxylate-modified beads (0.1 μm diameter, 2% suspension in water; Molecular Probes, Inc., Eugene, OR) were used either without modification or after covalent conjugation with Con A (Sigma Immunochemicals, St. Louis, MO), fibronectin (Sigma), or BSA (Sigma). Control beads, without surface carboxylate groups, were also obtained from Molecular Probes. Before the conjugation of proteins, beads were washed twice in 20 mM KH_2PO_4 by centrifugation in an airfuge (Beckman Instruments, Inc.) and sonication. They were then resuspended in 20 mM KH_2PO_4 and mixed 1:1 with 20 mg/ml 1-ethyl-3-(3-dimethylaminopropy) carbodiimide (Sigma) dissolved in the same buffer. The suspension was shaken at room temperature for 3–4 h. After three washes and resuspension in 200 mM borate buffer, pH 8.5, beads were mixed with the protein to be coupled and incubated for 6 h on ice. They were then washed and resuspended in 10 mg/ml BSA in either 2 mM Tris-acetate or PBS. Beads without protein conjugation were similarly washed and resuspended in BSA solutions before use.

Labeling of Living Cells and Treatment with Cytochalasin

Cells were maintained in an incubator built on the microscope stage (McKenna and Wang, 1989). To label surface receptors with fluorescent Con A, cells were washed several times with PBS/BSA and incubated with Texas red–conjugated Con A (Molecular Probes) at a concentration of 0.1 mg/ml in PBS/BSA for 3 min. Fluorescence images were recorded either with live cells or with cells fixed in 4% formaldehyde in PBS after 5 min of incubation.

To label surface receptors with beads, a well-spread metaphase cell was located and a suspension of beads, diluted fivefold in culture medium, was applied to the dish. After incubation for 2 min, the dish was washed with several changes of culture medium. Beads were then imaged during subsequent stages of cell division at an interval of 10–60 s. To simplify the interpretation of bead movement, cells that divided without extensive lateral constriction during the period of observation were chosen for analysis. In most experiments, cells were coilluminated for simultaneous epifluorescence and phase-contrast optics.

To study the effects of cytochalasin D, cells were labeled with beads as above and incubated until shortly after anaphase onset, when directional bead movement became detectable. The medium was then replaced with a solution of cytochalasin D (Sigma) in F12K medium at a concentration of 2 or 5 μM.

Fixation and Staining of Cells

A glutaraldehyde-based fixative was used to maintain the beads on the cell surface. Cells were rinsed twice with 37°C cytoskeleton buffer (Small et al., 1981), and were rapidly immersed into 0.5% glutaraldehyde, 0.3% Triton X-100 in cytoskeleton buffer for 1 min, and into 1% glutaraldehyde in cytoskeleton buffer for an additional 15 min. The dish was then treated with 0.1% $NaBH_4$ for 5 min to reduce autofluorescence and was washed with PBS/BSA. Cells were stained with 200 nM rhodamine phalloidin (Molecular Probes) in PBS for 1 h.

Microscopy

Microscope images were acquired with an IM35 or Axiovert 10 inverted microscope (Carl Zeiss, Inc., Thornwood, NY), a 100× NA 1.3 Neofluar oil immersion phase objective, and a cooled CCD camera (Princeton Instruments Inc., Trenton, NJ) linked to a PC-based custom image processing system (Gateway 2000, N. Sioux City, SD, and Alacron, Nashua, NH) for data storage, display, and mathematical operations. Fluorescence of yellow-green beads and rhodamine phalloidin was detected with Zeiss No. 17 and 15 filter sets, respectively. The filter set for Texas red was obtained from Omega Optical (Brattleboro, VT). Optical sectioning microscopy and three-dimensional imaging were performed as described in Fishkind and Wang (1993).

Bead movement was visualized by displaying sequences of images as movie loops. The movement was quantified by defining the position of beads as a function of time in a Cartesian coordinate system, using a computer mouse and custom-developed software. Velocity vectors and paths were constructed using CorelDraw, Microsoft Excel, and Adobe Photoshop, based on bead coordinates at successive time points. It should be noted that the actual path is likely to be more complicated than that shown in Fig. 3 because of the limitation in temporal and spatial resolution. The speed of directional movement was calculated from the distance of net translocation within a time interval of 1–2 min, using beads located near the long axis of the cell to minimize complications resulting from shape change.

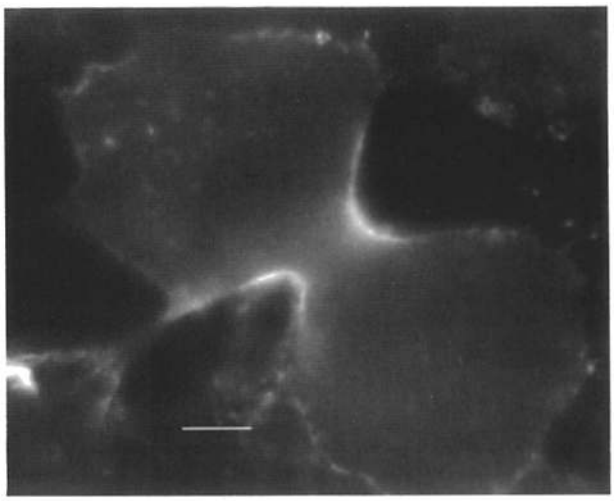

Figure 1. The concentration of Con A receptors during cytokinesis. A dish was labeled with Texas red Con A for 3 min and incubated for an additional 5 min. The dish was then fixed with formaldehyde and the image of a telophase cell was recorded. The fluorescence is concentrated in the equatorial region. Bar, 5 μm.

1. *Abbreviations used in this paper*: Con A, concanavalin A; NRK, normal rat kidney.

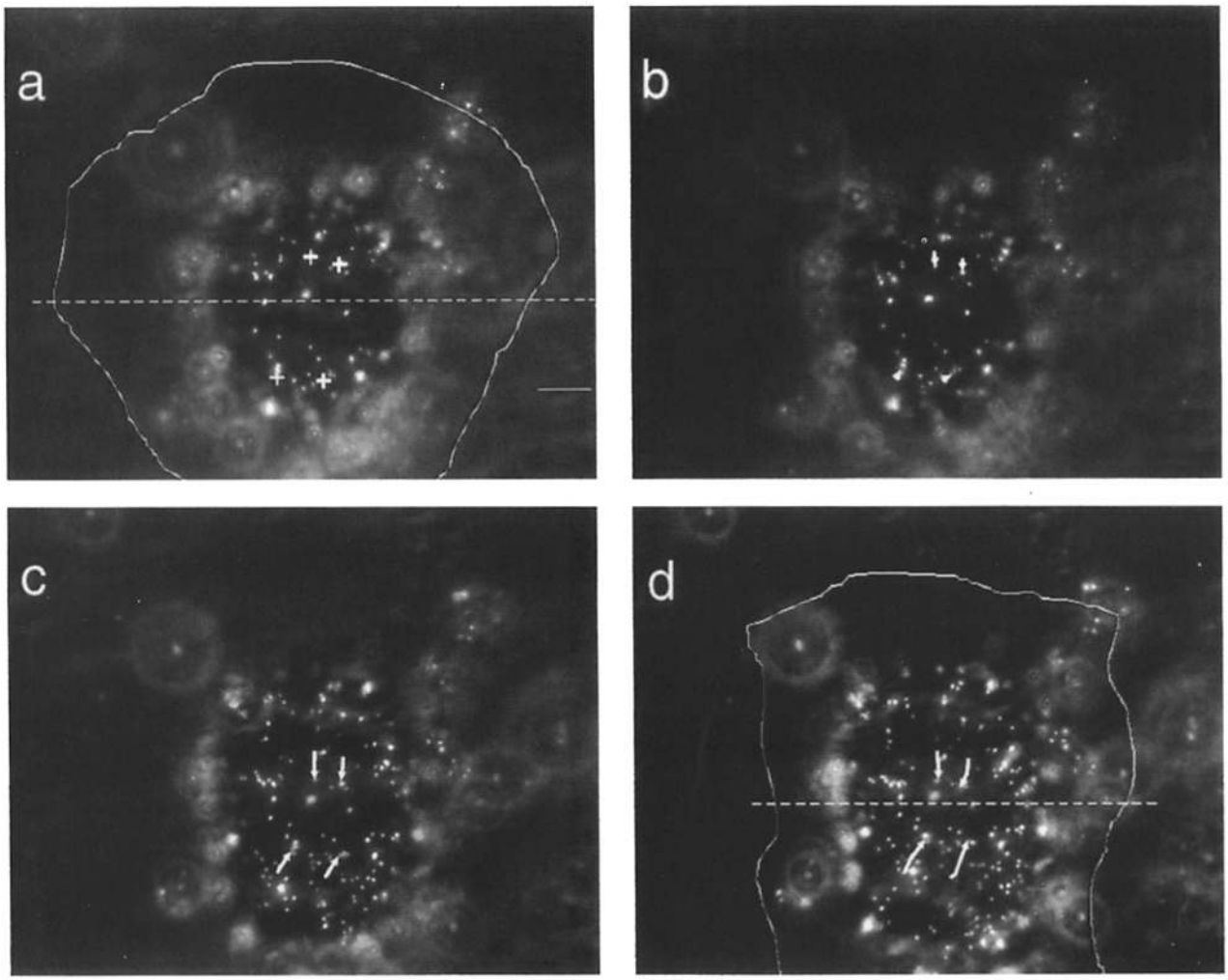

Figure 2. Directional movement of surface receptors during anaphase and telophase. Fibronectin-coated beads were used to label cell surface receptors. The central region of a cell was imaged from anaphase onset through cytokinesis using simultaneous phase and fluorescent optics. Images shown were recorded at 0, 2.6, 5.2, and 7.8 min after anaphase onset. Initial positions of several beads are marked by crosses (*a*). Subsequent movements are indicated as arrows (*b–d*) drawn from the position at time 0 to the position at each time point. The approximate boundary of the cell is marked by solid lines, and the equator is marked by dotted lines (*a* and *d*). Bar, 5 μm.

Results

The concentration of Con A receptors into the equatorial region of dividing cells has been shown previously in sea urchin eggs and a macrophage-like cell line (McCaig and Robinson, 1982; Koppel et al., 1982). Fig. 1 shows that a similar process occurs when a dividing NRK cell was labeled with Texas red Con A.

To determine how this redistribution of surface receptors takes place, we labeled the surface of living NRK cells with 0.1-μm fluorescent beads and observed their movement by tracking individual beads with fluorescence imaging. To maximize the number of beads that fall within the same plane of focus and to alleviate complications in interpretation caused by changes in cell shape, we used a well-spread subclone of NRK cells and focused on cells that maintained the adhesion to their neighbors throughout cell division. These cells showed only limited lateral constriction during cytokinesis and divided primarily from the dorsal surface toward the ventral surface (Fishkind and Wang, 1993). However, similar results were obtained with more typical dividing cells showing lateral constrictions. To facilitate the monitoring of bead movement, cell shape and the progress of chromosome separation at the same time, we recorded most cells under simultaneous illumination for phase and epifluorescence optics.

We first used carboxylate-modified fluorescent beads covalently conjugated with Con A. Similar results were obtained subsequently with fibronectin-conjugated beads in serum-free medium, and with unconjugated carboxylate-modified beads. On the other hand, plain beads without surface carboxylate groups, fibronectin beads in the presence of serum, and beads conjugated with BSA bound poorly to the cell surface. These observations suggest that the results described below reflect the behavior of multiple classes of surface receptors.

Characteristics of Bead Movement

As shown in Figs. 2 and 3 *a*, surface-bound beads moved to-

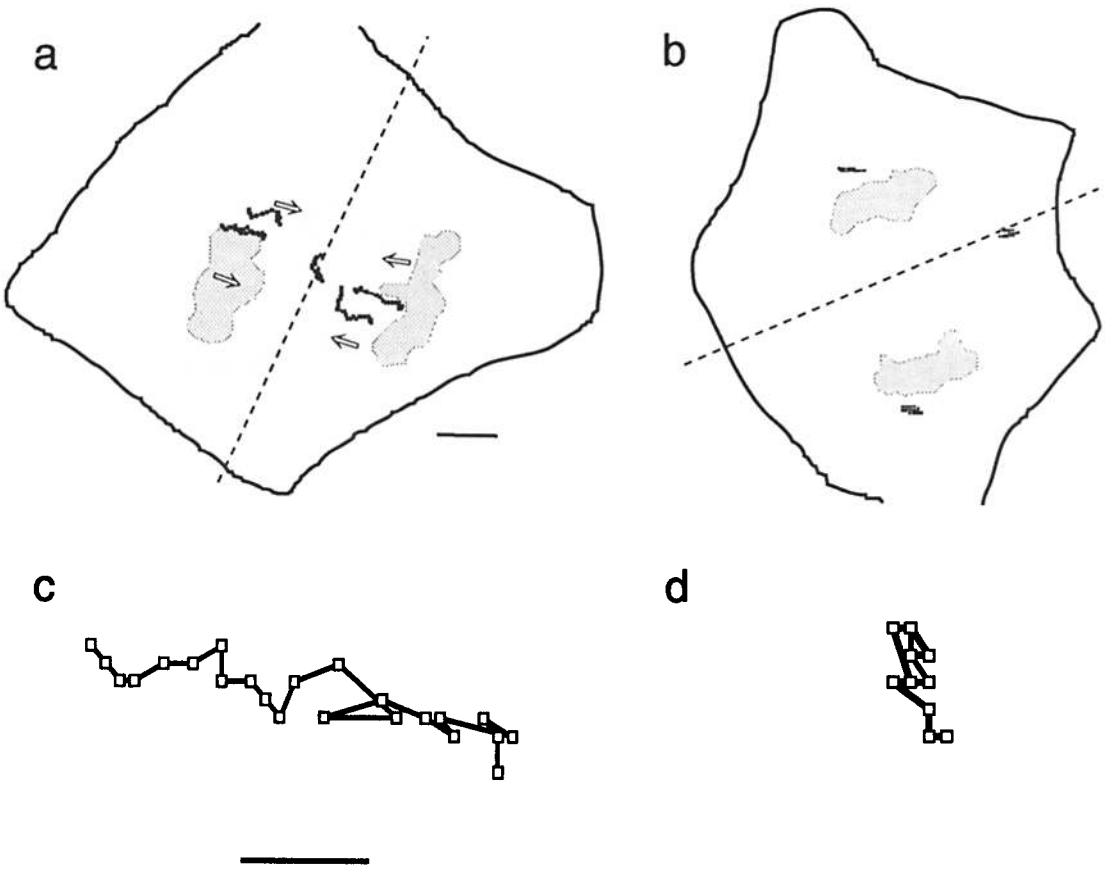

Figure 3. Tracks of bead movement in different regions of the cell. Cells were labeled with Con A–conjugated beads during metaphase, and the position of several beads in two separate cells was plotted after anaphase onset, in regions above (*a*) or further away from (*b*) the mitotic spindle. The time interval between successive measurements was 10–15 s. The direction of movement is indicated by arrows. The final location of chromosomes is shown as shaded areas (*a* and *b*). Beads in the central region, except those already in the equatorial zone, moved toward the equator, whereas most beads in the peripheral region showed primarily random movement. The bottom panels show more detailed paths of beads in the central (*c*) or peripheral (*d*) region. Bar, 5 μm (*a* and *b*) and 1 μm (*c* and *d*).

ward the equator during anaphase and telophase. The movement of individual beads contained a small degree of random movement superimposed upon the directional component toward the equator (Fig. 3 *c*). The most active movement was observed between the surface above separating chromosomes and the equator. Although there were variabilities in direction and speed, essentially all the beads in this general area moved toward the equator (Figs. 3 *a* and 4, *b* and *c*). The average speed obtained from 429 beads, 0.68 ± 0.34 (SD) μm/min, would allow beads located within ~7 μm of the equator to reach the equatorial region during a 10-min period of anaphase and telophase.

Beads located near the equator showed initially only random movement (Fig. 3 *a*). During later stages of anaphase and telophase, there appeared to be a converging pattern of bead movement toward the center of the cell (Fig. 4 *c*). Although it is difficult to rule out entirely the contribution of cell shape change, this converging movement has been observed in cells showing little or no lateral constriction. Toward the poles of the cell, most beads moved randomly during anaphase (Fig. 3, *b* and *d*).

Relationship between Bead Movement and Chromosome Separation

Individual beads on metaphase cells showed no persistent directionality in their movements (Fig. 5 *a*, *open squares*). Based on the mean square displacement, we estimated the diffusion coefficient to be in the order of 10^{-12} cm^2/s. As shown in Fig. 4 *a*, there was also no definable pattern of bead movement over the surface, although some beads within a small area or strip may show the same direction of movement. The first detectable directional movement occurred ~1 min after anaphase onset (Figs. 4 *b* and 5 *a*), when chromosomes moved out of the equatorial region. In cells where anaphase onset was arrested by reducing the culture temperature, the directional movement of beads was also inhibited until chromosome separation was recovered by raising the temperature (data not shown).

The influence of chromosomes was further indicated by the speed of bead movement in relation to the distribution of chromosomes. About 75% of the beads that encountered the passage of chromosomes, which moved with an average

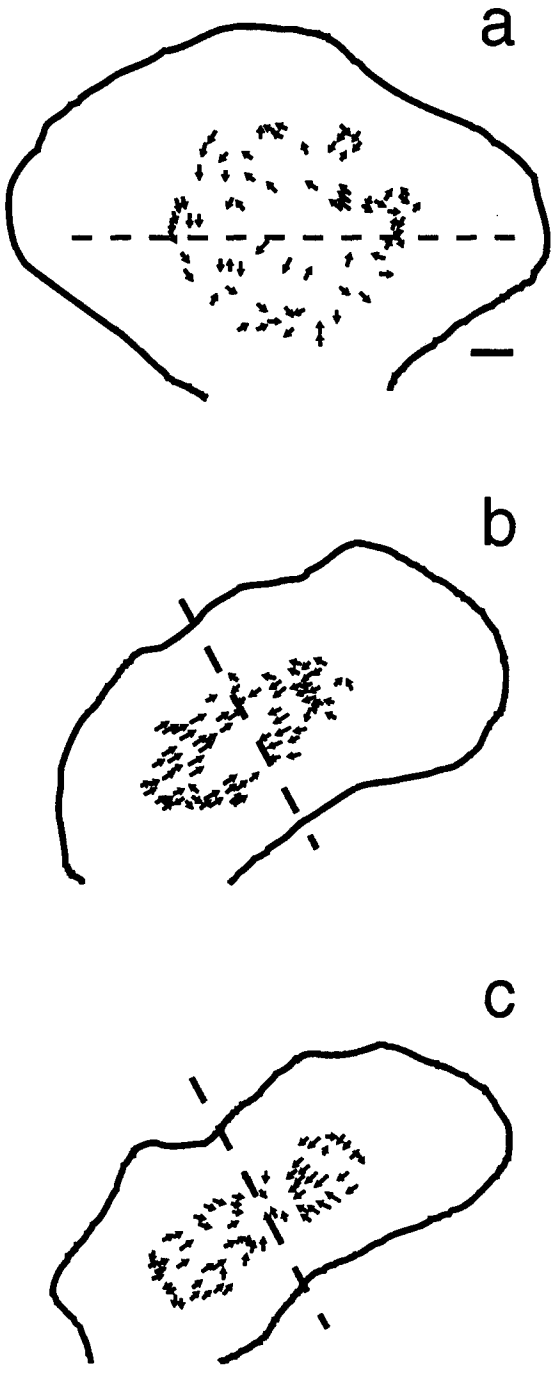

Figure 4. Vector diagram of bead movement during different stages of cell division. Surface receptors were labeled with carboxylate-modified beads (*a*) or Con A–conjugated beads (*b* and *c*). Velocity vectors of the movement are shown as arrows during metaphase (*a*), and 1.5 min (*b*) and 5.4 min (*c*) after anaphase onset. The size of the arrow reflects the rate of movement. The equator is indicated by dotted lines. the movement of beads showed no clear pattern in the metaphase cell (*a*), but became strongly directional toward the equator during early (*b*) and late anaphase (*c*). A converging pattern was evident in the late anaphase cell (*c*), even though the boundary showed only limited constriction. Bar, 5 μm.

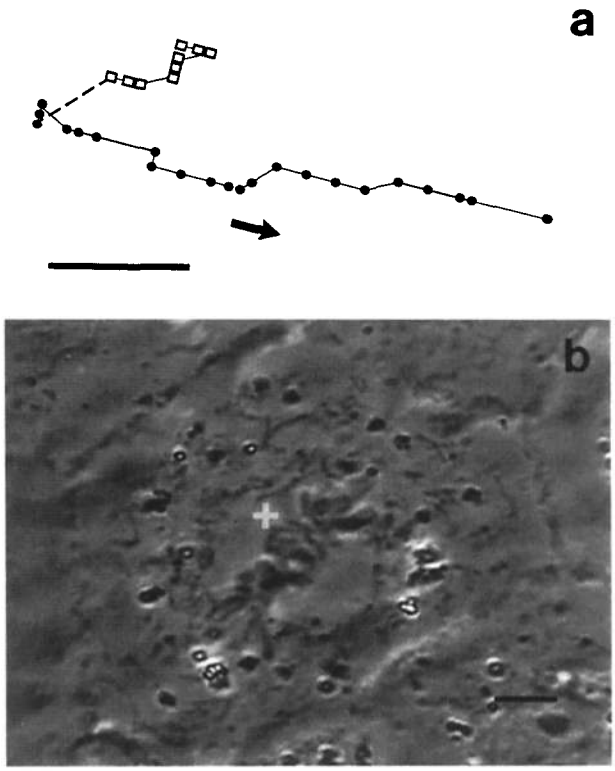

Figure 5. Movement track of a bead during metaphase to anaphase transition. (*a*) The position of the bead was plotted every 10–20 s before and after anaphase onset, except for the dotted segment where the interval is 3 min. During metaphase (*squares*), there was no persistent directionality. The movement then became directed toward the equator during anaphase (*round dots*). (*b*) The phase image of the cell during metaphase, with the starting position of the bead marked with a cross. Bars, 1 μm for *a*; 5 μm for *b*.

speed of 1.5 μm/min, showed a transient surge in speed by as much as fourfold (Figs. 6 and 7). As shown in Fig. 7, the increase in speed can occur either during or shortly after the passage of chromosomes. The acceleration also resulted in a reduction in the number of beads on the surface above chromosomes (Fig. 6).

It is possible that the observed change in two-dimensional speed may be caused by a rise of the dorsal surface near chromosomes, which might give an illusion of acceleration when beads move across regions of different vertical slopes. However, such slopes have to be very sharp, 60° to give a twofold increase in projected speed, while three-dimensional reconstruction of beads during this stage showed no such rise or fall of the dorsal surface near chromosomes (not shown).

Effects of Cytochalasin on Bead Movement

To determine the involvement of actin in bead movement, we labeled metaphase cells with fluorescent beads and applied 2–5 μM of cytochalasin D shortly after anaphase onset, when directional bead movement first became detectable. As shown in Fig. 8, cytochalasin D induced a rapid redistribution of beads into aggregates. This process occurred over the entire dorsal surface in random directions, and it caused many beads to move out of the equatorial region into aggregates closer to the poles (Fig. 8, *arrows*).

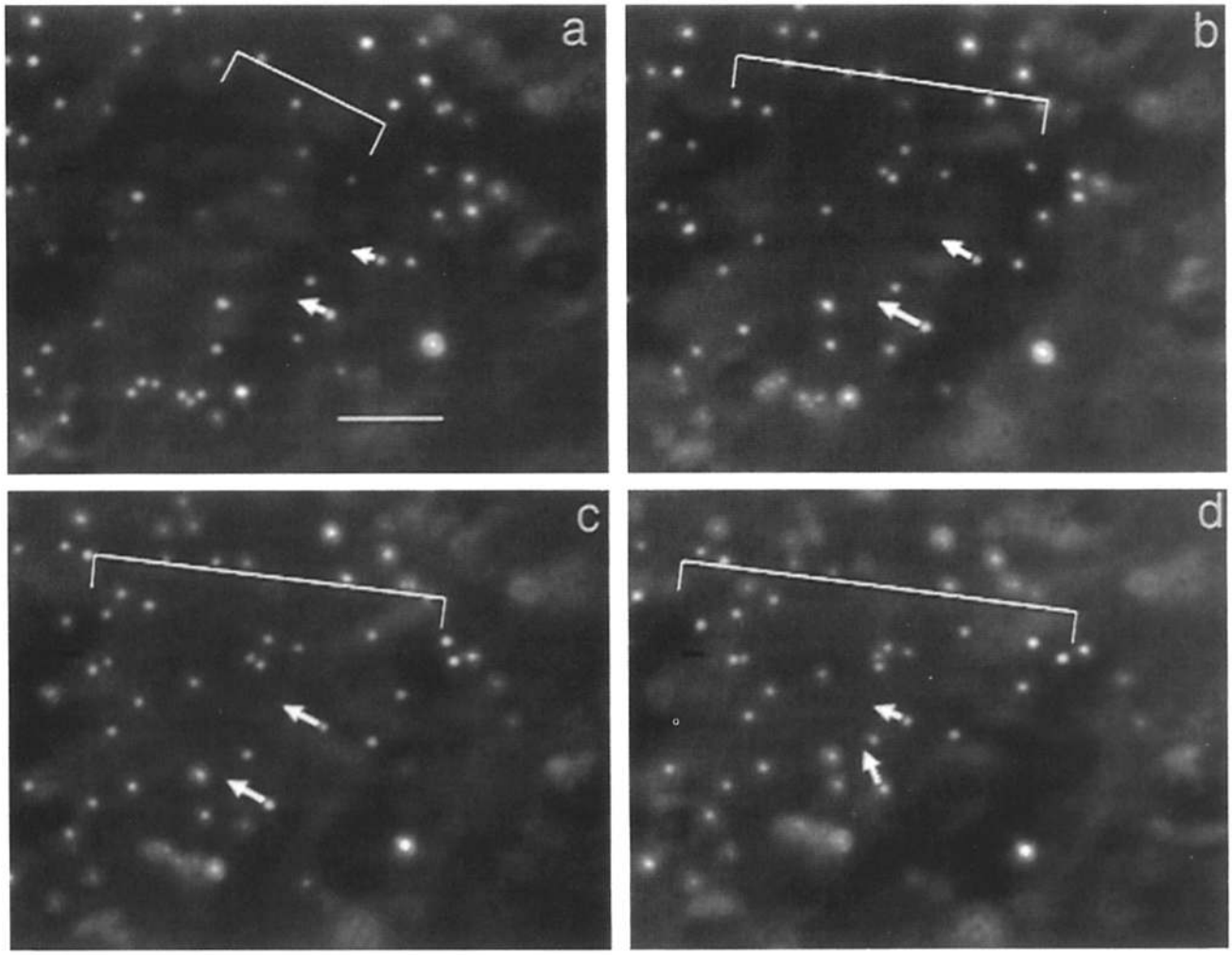

Figure 6. Acceleration of bead movement upon the passage of chromosomes. The experiment was performed as in previous figures. Images shown were recorded at 1.6 (*a*), 3.6 (*b*), 5.5 (*c*), and 6.8 min (*d*) after anaphase onset. The velocity vectors of several beads before and after the passage of chromosomes are marked with arrows. The length of the arrow reflects the rate of movement. A surge of speed occurred as the chromosomes passed beneath the beads. As a result, regions directly above the chromosomes were depleted of beads. The bracket in each frame indicates the approximate position of the separating chromosomes. Bar, 5 μm.

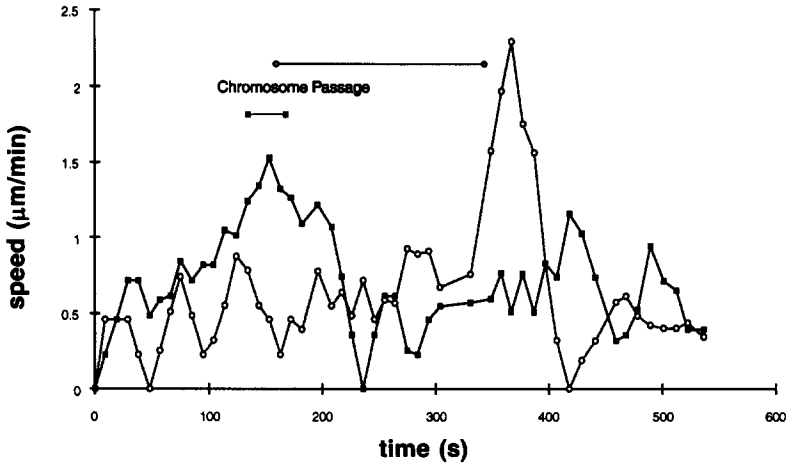

Figure 7. The rate of bead movement in relation to chromosome separation. The speed for two beads is shown as a function of time after anaphase onset. The time of chromosome passage is indicated above the graphs. The speed reached a peak either during (*squares*) or shortly after (*circles*) the passage of chromosomes.

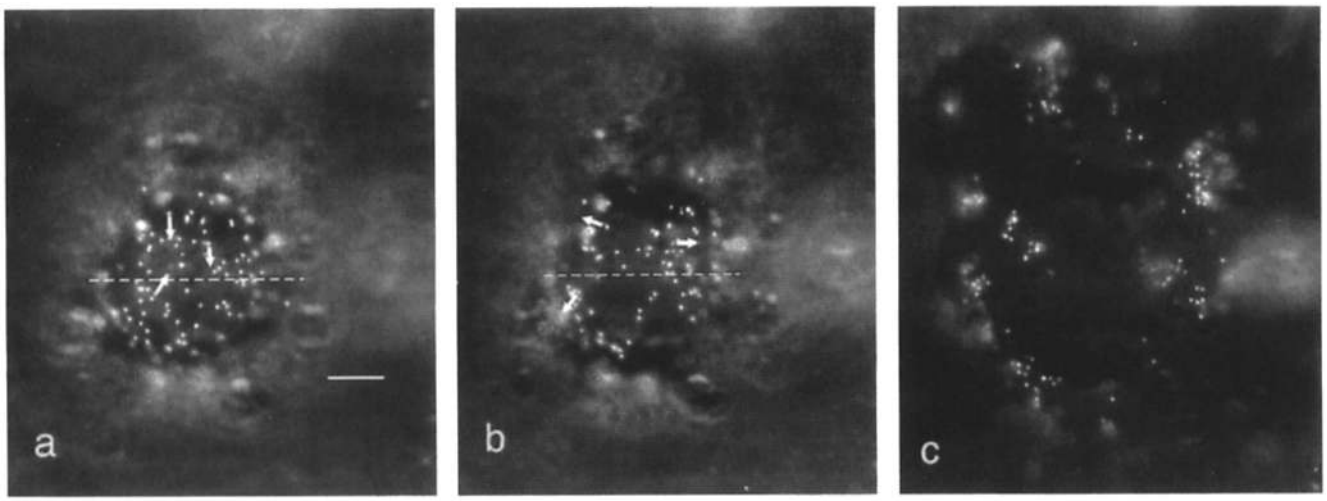

Figure 8. Disruption of bead movement by cytochalasin D. Cells were labeled during metaphase with carboxylate-modified beads, and treated with 5 μM cytochalasin D 2.6 min after the onset of anaphase. Images shown were recorded at 3.0 (*a*), 6.0 (*b*), and 9.1 min (*c*) after the application of cytochalasin D. Arrows indicate the direction of movement for three beads and dotted lines indicate the equator. The drug caused the beads to aggregate into a number of clusters located randomly over the cell surface. Some beads were observed to move out of the equatorial region. Bar, 5 μm.

The distribution of actin filaments in cytochalasin-treated cells was examined in two- and three-dimensional images after fixation and staining with fluorescent phalloidin. In contrast to control cells, where actin filaments were located throughout the cell (Fishkind and Wang, 1993), in cytochalasin-treated cells, the filaments were concentrated in aggregates on the dorsal and ventral surfaces (Fig. 9 *a*). Aggregates of beads were invariably colocalized with large aggregates of actin filaments on the dorsal cortex (Fig. 9 *b*).

Discussion

More than 40 years ago, Dan and Ono (1954) observed the movement of kaolin particles toward the equator of dividing sea urchin eggs. They interpreted the results as indicating surface expansion at the poles and shrinkage at the equator. However, based on the current understanding, it is likely that they have made the first observation of membrane receptor movement in dividing cells. More recently, Koppel et al. (1982) analyzed the photobleaching recovery kinetics of fluorescent succinyl Con A molecules on the surface of dividing J774 cells, and concluded that Con A receptors must undergo directional movement toward the equator during late anaphase. Byers and Armstrong (1986) labeled surface proteins of dividing *Xenopus* embryos by radioiodination, and found the proteins to concentrate into the leading edge of the cleavage furrow. In these earlier studies, it was already recognized that membrane/cortical reorganization can play an universal and important role in animal cell division.

However, to fully understand the significance of this event in cell division, it is important to determine its temporal and spatial relationship to other processes, such as mitosis and cytokinesis. In the present study, we have used fluorescent latex beads as a powerful tool for visualizing and tracking the receptor movement in living cells. In combination with the use of a well-adhered cell line, this method has allowed us to map receptor movement during different stages of cell division, and in different regions of the cell relative to chromosomes and the mitotic spindle.

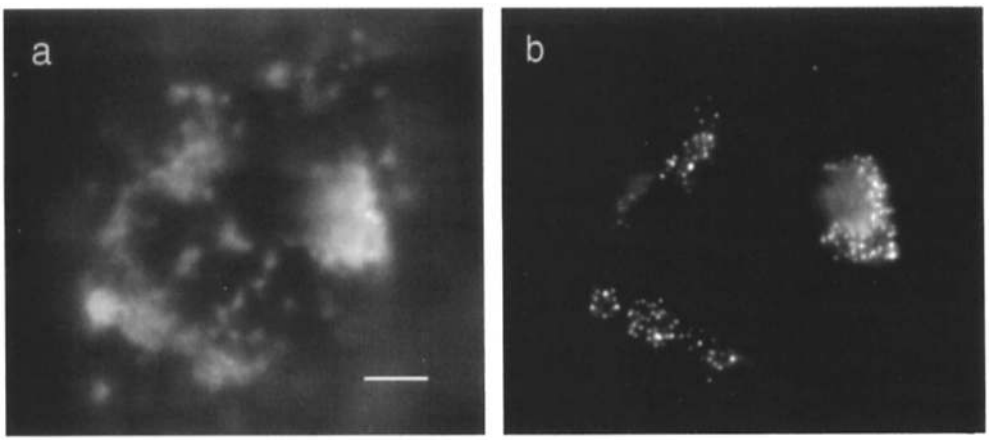

Figure 9. Colocalization of aggregates of beads with aggregates of cortical actin. A dividing cell was labeled with fluorescent beads and treated with cytochalasin D as in the previous figure. The cell was then fixed and stained with rhodamine phalloidin. Actin filaments became concentrated into discrete cortical aggregates (*a*). (*b*) The corresponding distribution of beads. All aggregates of beads colocalize with aggregates of actin filaments. Bar, 5 μm.

Several observations suggest that the movement of beads reflects, not only receptor dynamics, but also the movement of the underlying actin cortex. First, receptor movement occurs during the same stage of cell division as the reorganization of cortical actin and myosin II, as reported in a number of studies (Fujiwara and Pollard, 1976; Koppel et al., 1982; McCaig and Robinson, 1982). Second, the behavior of beads is similar to that of microinjected fragments of actin filaments, which show directional movements at a similar rate after the onset of anaphase (Cao and Wang, 1990*b*). Third, as shown in Fig. 8, the treatment of dividing cells with cytochalasin D causes a rapid redistribution and colocalization of beads and cortical actin into aggregates, suggesting that receptors associate directly or indirectly with actin filaments. These observations indicate that the present approach can be used as a means for studying the reorganization of cortical actin in living cells during division.

Spatial and Temporal Characteristics: Relationship to Mitosis

The close relationship between the mitotic spindle and cytokinesis has long been recognized (for reviews see Rappaport 1986, 1991; Salmon 1989). In addition, previous studies have indicated that early anaphase represents the most critical point for the determination of the site of cell cleavage (reviewed by Rappaport, 1986). Our observations suggest that the mitotic spindle, particularly the spindle interzone, may play an important role in regulating cortical reorganization. For example, the directional movement of beads starts as soon as chromosomes move out of the equatorial region, and the movement occurs primarily in the region above the interzone. It is possible that the timing and location of cell cleavage is dictated by this pattern of cortical movement. In a recent study, Hird and White (1993) reported that the movement of cortical granules in early *Caenorhabditis elegans* embryos is also strongly influenced by the position of spindles. They observed a continuous change in the direction of cortical flow in concert with the movement of attenuated spindles in nocodazole-treated cells.

Of special interest is the acceleration of beads upon the passage of chromosomes, which suggests that signals for directional movement may originate near separating chromosomes. Although the nature of such signals is still unclear, microtubules have often been regarded as a primary candidate for mediating the interactions between the spindle and the cortex (Devore et al., 1989; Harris and Gewalt, 1989). However, based on the pattern of cortical movement, it is unlikely that the cortex simply follows the direction of any microtubule toward its plus end. Furthermore, so far, no tight correlation between microtubules and actin filaments has been detected in ultrastructure or three-dimensional immunofluorescence images (Asnes and Schroeder, 1979; Fishkind, D., and Y.-l. Wang, unpublished observations). It remains as a possibility that cortical interaction may be mediated by a subset of microtubules in cultured cells, particularly the interpolar microtubules that extend between chromosomes and the equator (Mastronarde et al., 1993). The interaction may involve a number of microtubule-associated proteins found near the plus ends of these microtubules (reviewed by Margolis and Andreassen, 1993), and/or signals that are targeted to the cortex along microtubules (Devore et al., 1989; Harris and Gewalt, 1989).

Role of Receptor and Cortical Movement

It is commonly held that cytokinesis is a result of strong cortical contraction along the equatorial zone (Rappaport, 1986). However, from the present results and from the behavior of cortical organelles, it is clear that forces and movements are not limited to the equatorial region alone (Dan, 1954; Rappaport, 1991; Hird and White, 1993). A general cortical contractility is further indicated by the global collapse of receptors and cortical actin into aggregates after cytochalasin treatment, which most likely induces disruptions in local cortical structures and in the balance of cortical forces (Miranda et al., 1974). The random direction of movement, including the movement away from the equatorial region, indicates that forces are present on the cortex along multiple directions. Together, these results suggest that cytokinesis involves a fine balance and coordination of forces across the cortex considerably more complex than a localized equatorial contractile mechanism.

What might be the functional role of cortical movement in cell division? First, the transport may simply reflect the network nature of the cortex and a gradient of cortical contractile forces during cell division, with the strongest contraction and/or rigidity located in the cleavage furrow (White and Borisy, 1983). However, this explanation does not readily account for some of the characteristics, such as the acceleration of beads upon the encounter of chromosomes. Alternatively, it is possible that cytokinesis may involve separate processes of cortical transport of actin and myosin into the equatorial region, followed by active actin-myosin interactions. The transport, possibly regulated by separating chromosomes, may thus represent a crucial step in the assembly of a contractile apparatus. It is also possible that the movement of the cortex plays an active role in the separation of daughter cells or the disassembly of equatorial cortical cytoskeleton (Schroeder, 1972), by exerting counter forces on the equatorial cortex toward the poles.

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