The Cortical Microfilament System of Lymphoblasts Displays a Periodic Oscillatory Activity in the Absence of Microtubules: Implications for Cell Polarity

Michel Bornens, Michel Paintrand,* and Claude Celati

Centre de Genetique Moléculaire, Centre National de la Recherche Scientifique, 91190 Gif-sur-Yvette, France; and *Centre de Biologie Cellulaire, Centre National de la Recherche Scientifique, 94200 Ivry sur Seine, France

Abstract. For an understanding of the role of microtubules in the definition of cell polarity, we have studied the cell surface motility of human lymphoblasts (KE37 cell line) using video microscopy, time-lapse photography, and immunofluorescent localization of F-actin and myosin.

Polarized cell surface motility occurs in association with a constriction ring which forms on the centrosome side of the cell: the cytoplasm flows from the ring zone towards membrane veils which keep protruding in the same general direction. This association is ensured by microtubules: in their absence the ring is conspicuous and moves periodically back and forth across the cell, while a protrusion of membrane occurs alternately at each end of the cell when the ring is at the other. This oscillatory activity is correlated with a striking redistribution of myosin towards a cortical localization and appears to be due to the alternate flow of cortical myosin associated with the ring and to the periodic assembly of actin coupled with membrane protrusion. The ring cycle involves the progressive recruitment of myosin from a polar accumulation, or cap, its transportation across the cell and its accumulation in a new cap at the other end of the cell, suggesting an assembly–disassembly process. Inhibition of actin assembly induces, on the other hand, a dramatic microtubule-dependent cell elongation with definite polarity, likely to involve the interaction of microtubules with the cell cortex.

We conclude that the polarized cell surface motility in KE37 cells is based on the periodic oscillatory activity of the actin system: a myosin-powered equatorial contraction and an actin-based membrane protrusion are concerted at the cell level and occur at opposite ends of the cell in absence of microtubules. This defines a polarity which reverses periodically as the ring moves across the cell. Microtubules impose a stable cell polarity by suppressing the ring movement. A permanent association of the myosin-powered contraction and the membrane protrusion is established which results in the unidirectional activity of the actin system.

Microtubules exert their effect by controlling the recruitment of cytoplasmic myosin into the cortex, probably through their direct interaction with the cortical microfilament system.

A basic property of animal cells is their polarity; i.e., the expression of a morphological and functional asymmetry relative to a polar axis defining a front and a rear edge. This holds even for unattached cells such as lymphocytes where uropod and protopod were defined long ago (Bessis and De Boisfleury, 1976) and in which surface receptor redistribution occurs towards the area facing cytoplasmic organelles such as the Golgi apparatus or the centrosome (Oliver and Berlin, 1982). From early observations of moving lymphoid cells (Lewis, 1931) to more recent works (Senda et al., 1975; Keller and Cottier, 1981; Haston and Shields, 1984; Shields and Haston, 1985), the involvement of a constriction ring in lymphoid cell movement has been emphasized. Moreover, several authors have stressed that the constriction was particularly pronounced when microtubules (Mts)[1] were disrupted by drugs (Oliver and Berlin, 1982; Keller et al., 1984). This effect was interpreted as a permanent "polarizing effect" of Mt-disrupting drugs.

However, the respective contributions of Mts and microfilaments (mfs) to the movement of such cells, or to the ligand-induced capping of their surface receptors, are not yet clarified (for a review see Lackie, 1986). We have decided to undertake a study of the cell surface motility of the human lymphoblasts KE37 as these cells show active surface movements even when cultured on plastic; i.e., when they do not migrate. Using video-recording and time-lapse photography, we have carried out a comparative study of the surface motil-

1. *Abbreviations used in this paper*: CD, cytochalasin D; mf, microfilament; Mt, microtubule; NBD-phallicidin, 7-nitrobenz-2-oxa-1,3-diazole-phallicidin; NZ, Nocodazole; TX, taxol.

ity of normal and Nocodazole (NZ)-treated cells, in an attempt to reveal the role of Mts. This study, together with the immunofluorescent localization of F-actin and myosin, showed that surface movements, as well as actin and myosin distribution, were quite modified by NZ treatment. Normal cells displayed a polarized cell surface activity associated with a constriction ring, whereas cells deprived of Mts demonstrated an oscillatory surface motility which involved a periodic building up and propagation of a pronounced myosin-associated ring from one end of the cell to the other, and a periodic actin assembly coupled with membrane protrusion. Both actin-dependent activities appeared to be concerted at the cell level. As they occurred at the two opposite ends of the cell, they defined a polarity, the direction of polarity reversing periodically with respect to external references. The results indicate that Mts exert a profound effect on the characteristics of the acto-myosin system resulting in the definition of a stable cell polarity. We also observed that inhibition of actin assembly induced a dramatic Mt-dependent cell lengthening with definite polarity. All together, the results clearly point to the critical role of Mts in the control of cell surface polarity. The mechanism of this effect involves the control of the distribution of cellular myosin.

Materials and Methods

Cells

The KE37 cell line of T-lymphoblastic origin was cultivated in RPMI 1640 containing 7% fetal calf serum (Boehringer GmbH, Mannheim, FRG), using Falcon Tissue culture dishes (Becton, Dickinson and Co., Oxnard, CA). Cells were maintained in closed flasks after equilibration in 5% CO_2 and observed on an inverted Diaphot microscope (Nikkon Corp, Tokyo, Japan) thermostated at 37°C.

Chemicals

NZ was purchased from Janssen Pharmaceutica (Beerse, Belgium). Stock solution was 10^{-2} M in DMSO. Taxol (TX) was a generous gift from Dr. D. Guenard (Institut des Substances Naturelles, Gif-sur-Yvette, France). Stock solution was made 10^{-2} M in DMSO. Cytochalasin D (CD) was purchased from Sigma Chemical Co. (St. Louis, MO) and stock solution was made 5 mg/ml in DMSO.

Immunofluorescence

Cells were sedimented at 400 *g* for 10 min on round coverslips previously treated for 30 min with poly-lysine. The temperature was maintained at 37°C during sedimentation. Cells were then fixed in methanol at −20°C for 6 min and further incubated for 30 min with one or a mixture of two antibodies: antimyosin heavy chain monoclonal antibody was obtained in the laboratory (Klotz et al., 1986); monoclonal antitubulin and antiactin antibodies were from Amersham SA (Les Ulis, France); and the anticentrosome antibody was from a rabbit serum previously characterized in the laboratory (Gosti et al., 1986, 1987). The second antibodies were either rhodamine-labeled goat anti-mouse antibody or a mixture of the latter antibody with fluorescein-labeled goat anti-rabbit antibody.

Alternatively, cells were fixed in acetone at −20°C for 6 min and further incubated with a 10 U/ml solution of 7-nitrobenz-2-oxa-1,3-diazole-phallicidin (NBD-phallicidin) (Molecular Probes, Inc., Junction City, OR) in PBS for 20 min.

Electron Microscopy

Transmission. Cells were fixed in their culture medium by dilution (1/1) with 5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, for 30 min at room temperature. They were then postfixed in 2% OsO_4, dehydrated in ethanol, and embedded in Epon. Sections parallel or perpendicular to the coverslip on which cells were sedimented (10 min, 1,500 *g*) after glutaraldehyde fixation, were contrasted with lead citrate and uranyl acetate and observed with a Philips Electronic Instruments, Inc. (model 201; Mahwah, NJ) electron microscope.

Scanning. Cells were processed as above up to ethanol dehydration. Then acetone was progressively substituted for ethanol. Samples were dried by the critical point method after substitution of acetone by liquid carbon dioxide, at 72.9 atm and 31.1°C in a Balzers S.p.A. (Milan, Italy) CPD 010 apparatus. Specimens were coated with a 200-Å-thick layer of gold in an Edwards High Vacuum, Inc. (Grand Island, NY) S150-PA sputter coater. They were observed with a Philips Electronic Instruments, Inc. 505 scanning electron microscope.

Results

KE37 Lymphoid Cells Show Polarized Cell Surface Motility Associated With a Constriction Ring

The most frequent shape of KE37 cells (Fig. 1 *a*) was characterized by a large spherical domain where the nucleus is located and a constriction ring (Fig. 1 *a*, *long arrows*) at the base of an area where cell protrusion and membrane activity were evident. The latter was often made of a single slightly curved filopodium (Fig. 1 *a*, *short arrows*). The ring was not very pronounced, its size being ~4/5 of the cell diameter. Several other cell shapes could be seen which might be similar to the previous one observed from different orientations. In a plastic dish where cells were unable to migrate, the membrane protrusion often occurred in a slowly rotating manner around the cell body which acted as an anchor (rows *I* and *II*, Fig. 1 *a*). A new filopodium could protrude before the preceding one had disappeared. Differential-interference contrast (DIC) microscopy revealed that each filopodium was sustaining a unique and large veil (Fig. 2 *A*). In all cases, the leading cytoplasm was free of organelles and seemed to flow into the membrane veil. The constriction ring did not propagate much and often vanished in an unpredictable manner.

Double staining with centrosome and tubulin antibodies showed that the motile surface side of cells coincided with the centrosome side (Fig. 2, *B* and *C*).

Mt Disruption Leads to Periodic Movement of the Constriction Ring Across the Cell

Cells observed after complete disassembly of Mts with NZ-treatment for 15 min (Fig. 1 *B*) were slightly more elongated than the untreated cells and displayed a pronounced ring-shaped constriction (Figs. 1 *b* and 3). In each case, the ring appeared to move smoothly from one end of the cell to the other, maintaining the same diameter (~1/5 of the cell diameter). A remarkable feature of this movement was that the ring went to one end of the cell and moved back to the other end. A complete back and forth movement of the constriction ring is shown for two individual cells in Fig. 1 *b* (rows *III* and *IV*; 15-s interval between each view). A partial sequence is shown for two cells in Fig. 3 *A* (cells *a* and *b*; 7-s interval between each view) and the successive outlines of one of them (Fig. 3 *A*, cell *a*) is recorded with respect to external references in Fig. 3 *B*. The periodic movement of the constriction ring apparently involved the disappearance of the ring at the end of the cell and the persistence of a small "knob" protruding from the cell surface during a few seconds (Figs. 1 *b* and 3, *black arrowheads*). A new ring apparently formed from this knob and started to propagate in the oppo-

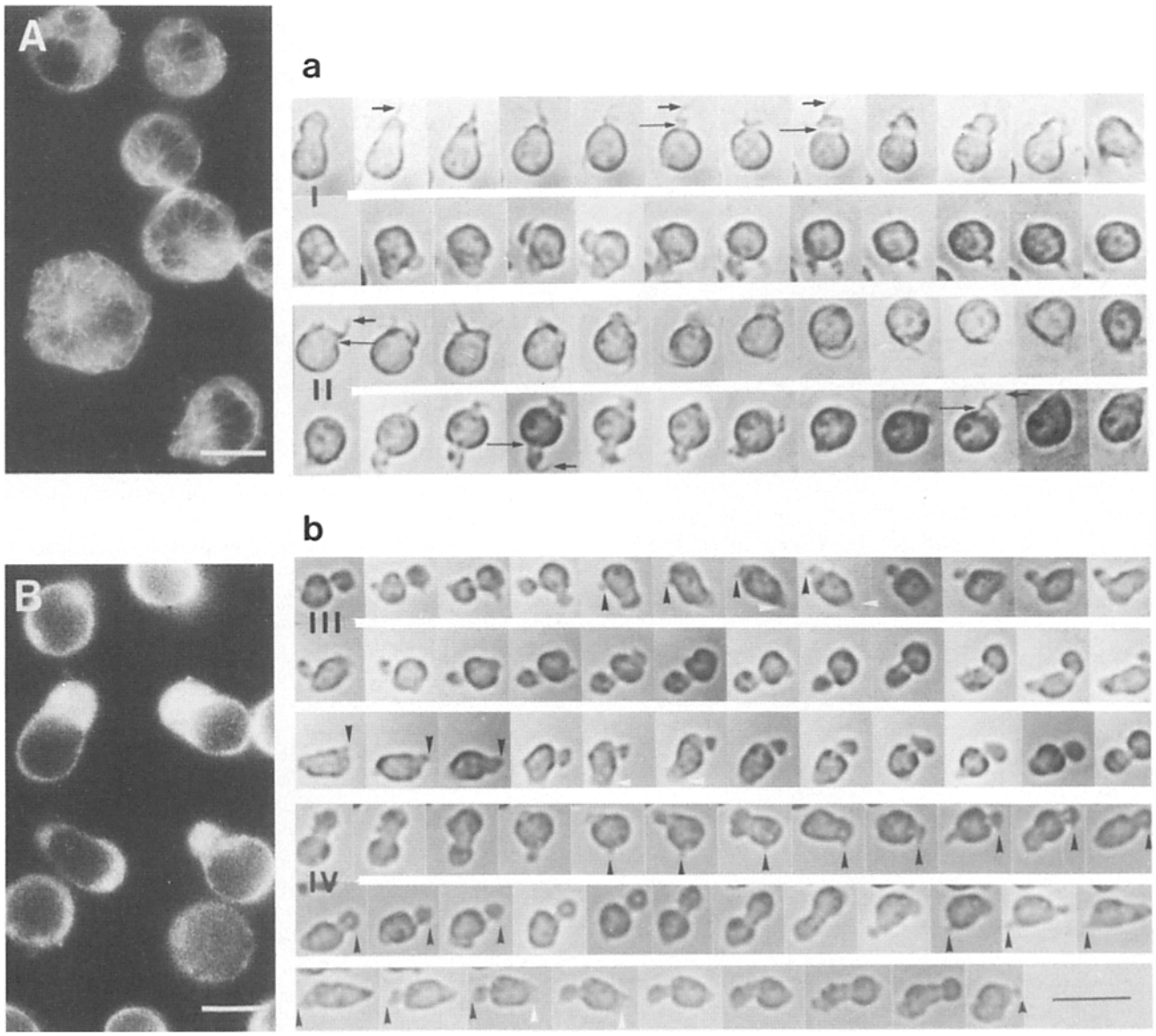


Figure 1. Cell surface motility of KE37 lymphoblasts in the presence (*a*) and in the absence (*b*) of Mts. (*A* and *B*) Immunofluorescent staining of tubulin before (*A*) or after (*B*) NZ treatment for 15 min. Note that Mts are totally disassembled by the treatment. Note also that cell shapes are significantly modified by the treatment: a constriction ring can be observed even after sedimentation of the cells on a coverslip. (*a*) Examples of cell surface movements in two individual untreated cells (*I* and *II*; from left to right and from top to bottom). The most frequent shape is characterized by a large spherical domain where the nucleus is located and a constriction ring (*long arrows*) at the base of an area where cell protrusion (*short arrows*) and membrane activity are evident. Several other cell shapes could be seen which might be similar to the previous one observed from different orientations. In a plastic dish where cells are unable to migrate, the membrane protrusion often occurs in a slowly rotating manner around the cell body which acts as an anchor. A new filopodium sustaining a veil could protrude before the preceding one has disappeared. (*b*) Examples of cell surface movements in two individual NZ-treated cells (*III* and *IV*; from left to right and from top to bottom). A back and forth movement of a pronounced constriction ring is obvious in each case. Upon reaching a cell end, the ring disappears, leaving a small protruding knob (*black arrowheads*) from which the new ring seems to start. A membrane activity is visible at one end of the cell (*white arrowheads*), when the ring is at the other end. Cells were cultured at 37°C and micrographs were taken every 15 s. Bars, 10 μm.

site direction; i.e., in the only possible direction. The movement of the cytoplasm and nucleus through the ring sometimes appeared to be hampered. Extreme cases could be observed. One is illustrated in Fig. 3 *A* by cell *b* (see Fig. 3 legend). From one cell to the other, it took from 2.5 to 4.5 min for the ring to propagate from one end to the other. A uniform propagation at a constant speed of ~0.1 μm/s along the cell could be estimated in favorable cases (Fig. 3, cell *a*).

The other striking feature of the movement was that, whenever the constriction ring was approaching one end of the cell, the other end displayed a membrane activity (formation of veils or protrusions; Figs. 1 *b* and 3, *white arrowheads*). This lasted until the new ring had covered about the same distance on its way back. When the ring was closer to the middle part of the cell, membrane activity was apparently low or absent at either side of the cell. This is also the

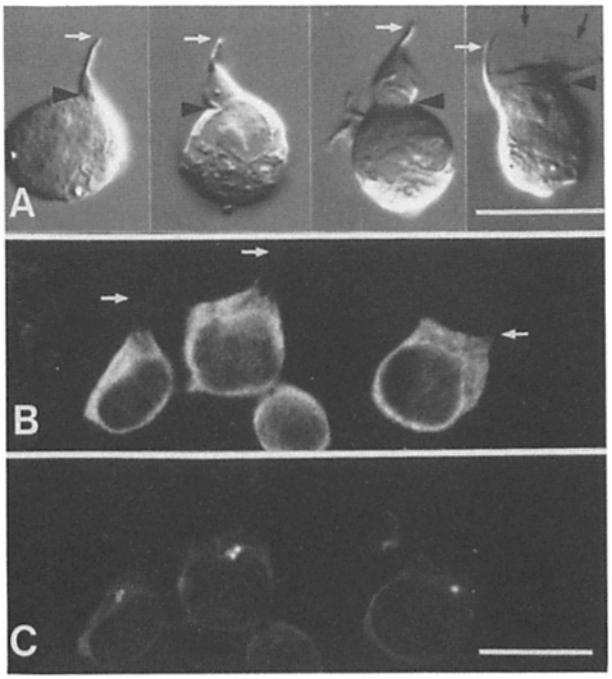


Figure 2. Untreated KE37 cells observed by DIC microscopy (*A*) and by double immunofluorescence for tubulin (*B*) and centrosome (*C*). (*A*) Typical polarized surface activity: a filopodium (*white arrow*) sustaining a unique veil (*black arrows*) protrudes and some organelle-free cytoplasm flows from the constriction ring zone (*black arrowheads*) into the membrane veil. (*B* and *C*) Membrane activity of cells (*B, white arrows*) occurs on the centrosome side (*C*). Bars, 10 μm.

conclusion reached when NZ-treated cells were observed by scanning electron microscopy (Fig. 4 *B*). However, the total cell length did not change much during the ring movement (Fig. 3 *B*).

Since the constriction ring in untreated cells was not pronounced and did not move significantly across the cell, we conclude that Mts reduce the equatorial constriction and its longitudinal propagation. As the constriction ring is likely to be an actin-dependent process, it was of interest to study the effect of mf disorganization on cell shape and cell surface motility.

Mf Disorganization Inhibits Cell Surface Motility and Leads to Cell Elongation

When KE37 cells were treated with 0.8 μg/ml or higher CD for 15–20 min at 37°C, a dramatic change in cell shape, shown on Fig. 5, was observed for 80–100% of cells depending on the culture: cells displayed a stereotypic elongated shape characterized by a thick extension of fairly constant diameter on one side of the nucleus, which most often ended with a spherical bleb, and by a shorter tapering extension on the other side of the nucleus. These elongated cells displayed, therefore, a definite polarity. The size of the CD-treated cells was fairly constant and represented a cell lengthening of two to three times the original size.

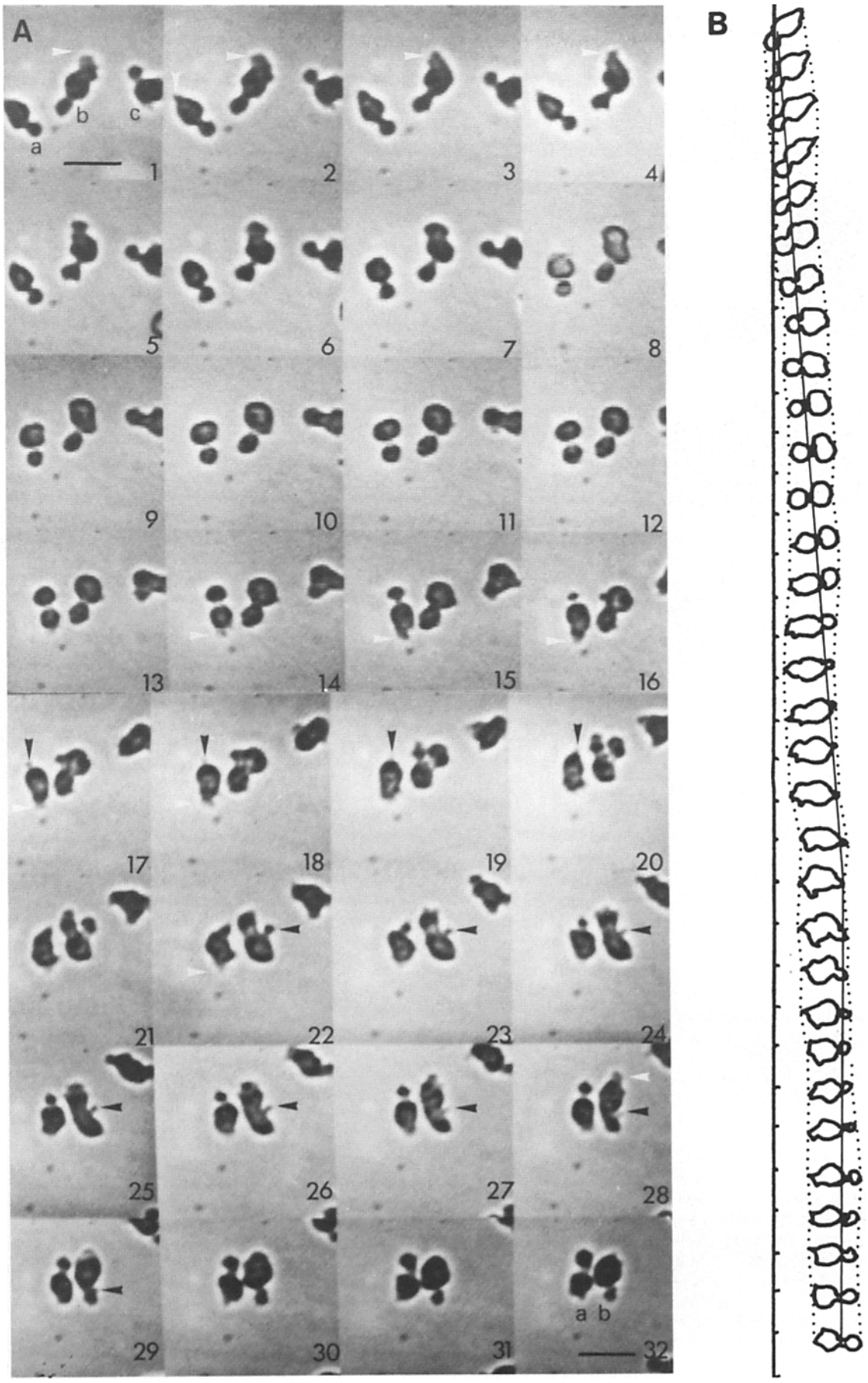
Video recording of the CD effect (not shown) revealed that the first feature was the arrest of surface movements and the disappearance of membrane protrusion. After several minutes, one could observe the slow outgrowth of exceedingly thin extensions which almost reached their maximum length before thickening. The swollen segments on the large cellular extension could move tipward and erratic whipping movements of the whole extension were sometimes observed. The CD effect was complete after 20–30 min and was rapidly reversible after several hours of treatment, indicating the absence of toxic effects.

From this result, we conclude that the round cell shape of untreated cells is mainly imposed by the mf system. In the absence of organized mfs, Mts could elongate and be responsible for the elongated shape of the cells. This is also suggested by the organization of the centrosome–Mt network in CD-treated cells: double staining for centrosome and tubulin revealed that Mts were present in the extensions and were originating from the centrosome which had an axial localization, at the base of one of the two cellular extensions (Fig. 6, *A* and *B*). The role of Mts might be ascertained with tubulin drugs.

CD-induced Cell Elongation Is Mt Dependent

NZ totally inhibited the CD effect when added before CD or at time zero. It suppressed the CD effect once established. Cells treated with both drugs showed arborization and blebbing and apparently lost fractions of membrane-bound cytoplasm (Fig. 5). In the presence of TX, CD action lead to elongated cells which were obviously more rigid than when treated by CD alone, with a significantly shorter length (Fig. 5). Double staining for centrosome and tubulin demonstrated numerous Mts in both cellular extensions (Fig. 6, *C* and *D*). Additional Mts induced by TX were all apparently associated with the centrosome, which as a rule was localized at the base of one of the extensions. Protracted pretreatment of cells with TX, which induced a characteristic lemon-like shape (not shown), did not inhibit subsequent CD action. The final shape of cells treated with both drugs was identical whether TX was added before or after CD, and was obtained with similar ki-

Figure 3. Cell surface motility of NZ-treated cells. (*A*) Movements of three cells recorded every 7 s (from left to right and from top to bottom). Note the protruding knob where a ring has disappeared (*black arrowheads*) and the membrane activity at the cell end opposed to the ring position (*white arrowheads*). Cell *b* showed a peculiar behavior during ~20 s (frames *19–21*): the ring has split in two parts during its progression. When the cell had retrieved a unique convex volume (frames *22* and *23*), the knob had an unusual position: in the middle of the long edge of the cell (frame *23* and *24*). It then slid along the cell edge while decreasing in size (frame *25–28*) and finally vanished before it reached the end of the cell where a ring was clearly formed. Such a case strongly supported the view that the knob was instrumental in forming a new ring. (*B*) The successive outlines of cell *a* recorded with respect to external references (the two black dots visible in each frame in *A*) demonstrate that the cell length does not change much and that the back and forth movement of the constriction ring has a uniform speed (~0.1 μm/s in this case). Automatic video-recording of 32 time-lapse frames were carried out using a Cytix (Digital Design, France) and contrast enhancement was achieved to reinforce cell outlines. Bars, 10 μm.


A
B
a
b
c
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
a b
32

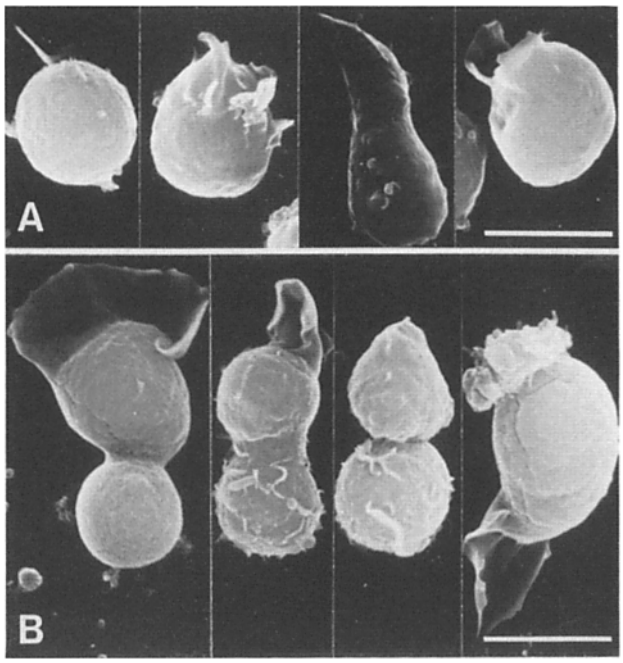


Figure 4. Scanning electron microscopy of untreated (*A*) and NZ-treated (*B*) cells. In the absence of Mts, cells have a simple shape with a unique and deep constriction ring in the middle of the cell or a unique veil present at one end when the ring is at the other. We have tentatively reconstructed a plausible sequence from the observations on living cells. Bars, 5 μm.

netics. By contrast, cells treated with CD until completion (30 min) in the absence of Mts (with NZ added), were unable to establish an elongated shape when Mts were allowed to reassemble (by removal of NZ) precluding the naive scheme mentioned above which would see cell elongation as due to Mt elongation in the absence of a constraining mf system.

From these results we conclude that cell extensions are sustained by Mts and that the process of cell elongation requires interaction of mfs and Mts. The latter conclusion lead us to look at the ultrastructural localization of Mts in the extensions. Cells treated with CD with or without TX were sectioned according to three orientations after being sedimented on a coverslip (Fig. 7). Mts, which were apparently all anchored at the centrosome, directly reached a peripheral localization where they lay parallel to the cell surface. The pictures suggested an interaction with a cortical meshwork,

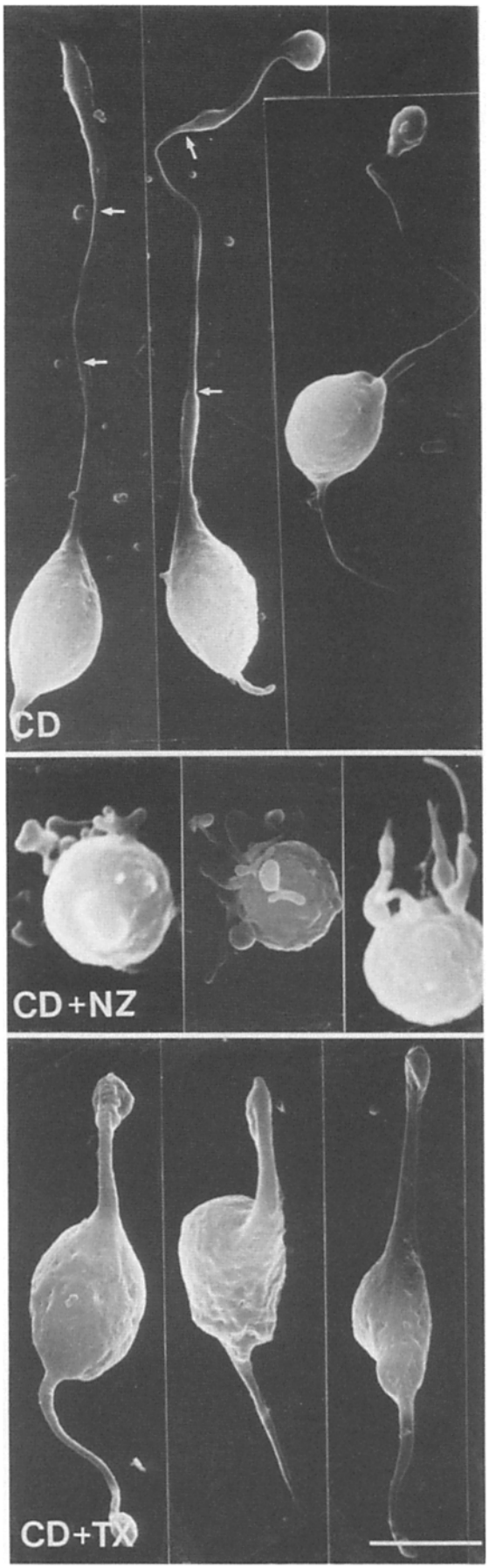


Figure 5. KE37 cells shapes induced by cytoskeleton drugs, as observed by scanning electron microscopy. Cells treated for >30 min at 37°C with CD alone, or in association with NZ or TX displayed a shape characteristic of each treatment: a very elongated shape, with diametrically opposed extensions of different sizes, for CD treatment (*CD*); a complicated shape with many blebs of various sizes, for treatment with both CD and NZ (*CD + NZ*); a rigid and stereotyped elongated shape, with the nucleus off-side of the axis and half way between the two ends, for treatment with CD and TX (*CD + TX*). Note that the main extensions of CD-treated cells often displayed variations in the diameter (*arrows*). Untreated and NZ-treated cells are shown in Fig. 4. Bars, 5 μm.

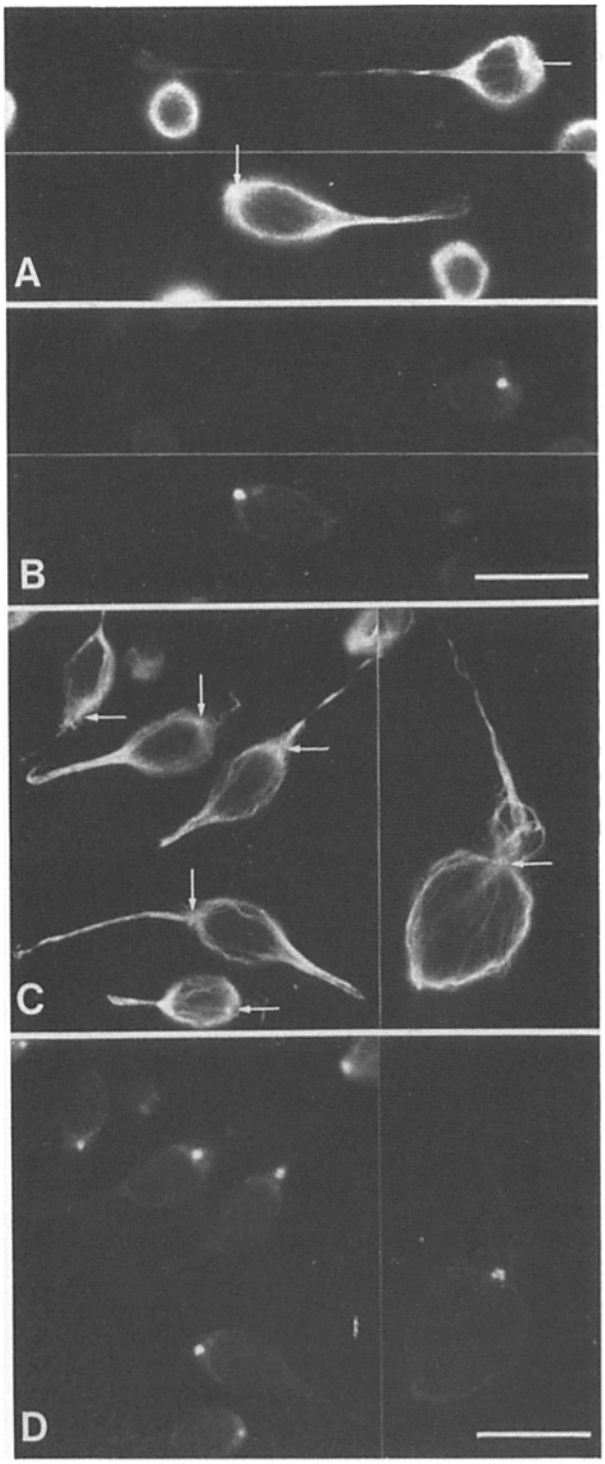


Figure 6. Double immunofluorescence for tubulin (*A* and *C*) and for centrosome (*B* and *D*) on cells treated with CD alone (*A* and *B*) and with both CD and TX (*C* and *D*). Note that in both cases, Mts are present in the extensions and originate from the centrosome which has an axial position (*arrows*, *A* and *C*). The apparently larger cell on the right in *C* and *D* was near the edge of the coverslip and thus pulled apart in all directions by capillary forces: one can see that Mts run close to the cell surface. Bars, 10 μm.

particularly when tangential sections were observed (lower right corner in Fig. 7 *A*). Transverse sections (Fig. 7, *B* and *E*) demonstrated that Mts were very close to the plasma membrane, except in the narrow terminal parts of the extensions of cells treated with both CD and TX, where Mts were probably too numerous to all interact with the cortex (Fig. 7 *F*). The distal ends of Mts were not easily identified. Mts seemed to end at the base of the terminal bleb in the principal extension (Fig. 7 *D*).

From these data, we conclude that Mts in the cellular extensions are closely associated with the cortex with which they probably interact. This is further suggested by the effect of additional Mts, induced by TX, on the shape of CD-treated cells: as a rule, sections perpendicular to the long axis showed almost perfect circular profiles anywhere along the axis (Fig. 7, compare *B* and *E*). This is probably the result of increasing Mt interactions with the cell cortex.

Effect of NZ and CD on Actin and Myosin Organization in KE37 Cells

Using a monoclonal antibody against myosin heavy chain, we observed an even and slightly granular staining of the cytoplasm in KE37 cells (Fig. 8, *A* and *B*). The nuclear area was left unstained. No particular accumulation of myosin could be detected at the plasma membrane or elsewhere. NZ-treated cells displayed a striking redistribution of myosin, either as a ring or as a bright spot protruding out of the cell surface (Fig. 8, *C* and *D*). In each case, myosin appeared associated with the plasma membrane. Whenever myosin was accumulated in a knob, the corresponding phase-contrast image showed that it was a tight cap associated with the plasma membrane (Fig. 8, *C* and *D*, and *insets*). Double immunofluorescence for myosin and for centrosome showed that the centrosome had no specific localization with respect to the constriction ring (Fig. 9). CD-treated cells most often displayed large accumulations of myosin on short segments along the cell extensions or at their base (Fig. 8 *E* and *F*).

Actin, as assessed by the use of a monoclonal antibody, displayed an even distribution in KE37 cells (not shown). Filopodia were the only distinctly decorated features. NZ or CD treatment did not induce massive cellular redistribution of cellular actin. The state of organization of polymerized actin could be assessed by NBD-phallicidin. In KE37 cells, F-actin was preferentially accumulated at one pole of the cell, from the ring to the active zones of the plasma membrane (Fig. 10, *A* and *B*). In NZ-treated cells, F-actin was observed either at one end of the cell, associated with the constriction ring area, or at both ends, associated with the constriction ring at one end and underlying the plasma membrane at the other (Fig. 10, *C* and *D*). In such cells therefore, F-actin was accumulated in two separate areas. In CD-treated cells, the acetone fixation required for NBD-phallicidin was quite deleterious. However, a thin cortical staining along the cellular extensions and at their base could be observed (Fig. 10, *E* and *F*), suggesting that cortical mfs were still present after CD action.

From these results, we conclude that NZ induces a dramatic redistribution of myosin towards a cortical localization. Cortical myosin can be organized as a ring or accumulated in a protruding cap. Both situations can also be observed on the same cell. NZ also induces a redistribution

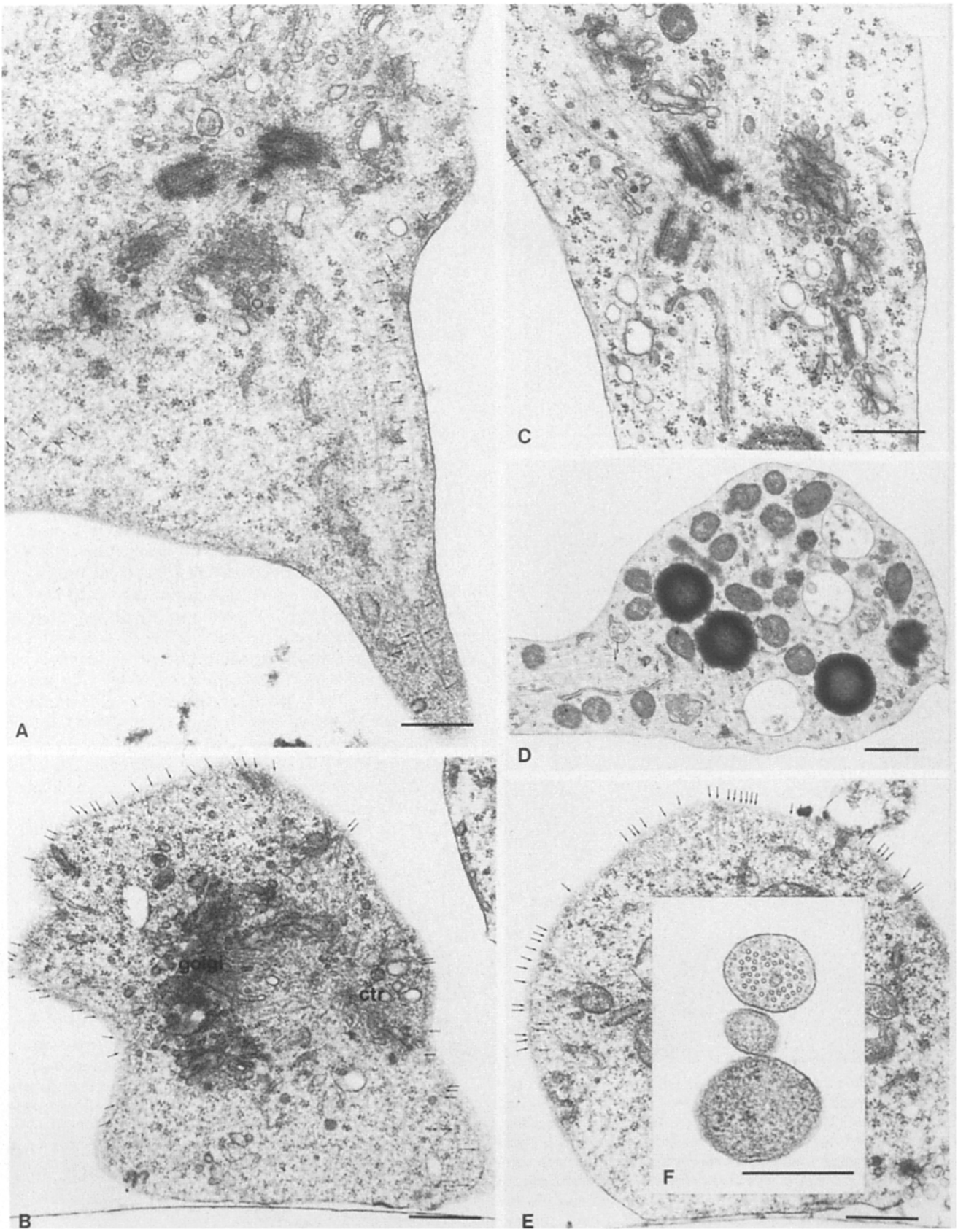


Figure 7. Transmission electron microscopy of KE37 cells treated with CD (*A* and *B*) or with CD and TX (*C–E*). Sections parallel (*A*, *C*, and *D*) and perpendicular (*B* and *E*) to the coverslip on which cells were sedimented, were realized. (*A*) Note that Mts emanating from the centrosome reach the plasma membrane where they run parallel to the cortex (*small arrows*). The pictures suggest an interaction with the cortex, particularly when tangential sections are observed (lower right corner in *A*). Transverse sections (*B* and *E*) also demonstrate that Mts (*arrows*) are very close to the plasma membrane. (*C*) Note that additional Mts induced by TX treatment are also anchored at the centrosome (*ctr*) and that transverse sections of TX-treated cells have a circular profile (compare *E* to *B*). (*D*) A longitudinal section of a main extension shows that Mts seem to end at the base of the terminal bleb (*small arrows*) which apparently contains mitochondria, vesicles of various kinds, and lipid droplets. (*F*) Transverse sections of narrow segments of extensions showing either a bundle of Mts surrounded by a cortical meshwork, or a zone deprived of Mts, perhaps at the end of the smallest extension. Bars, 0.5 μm.

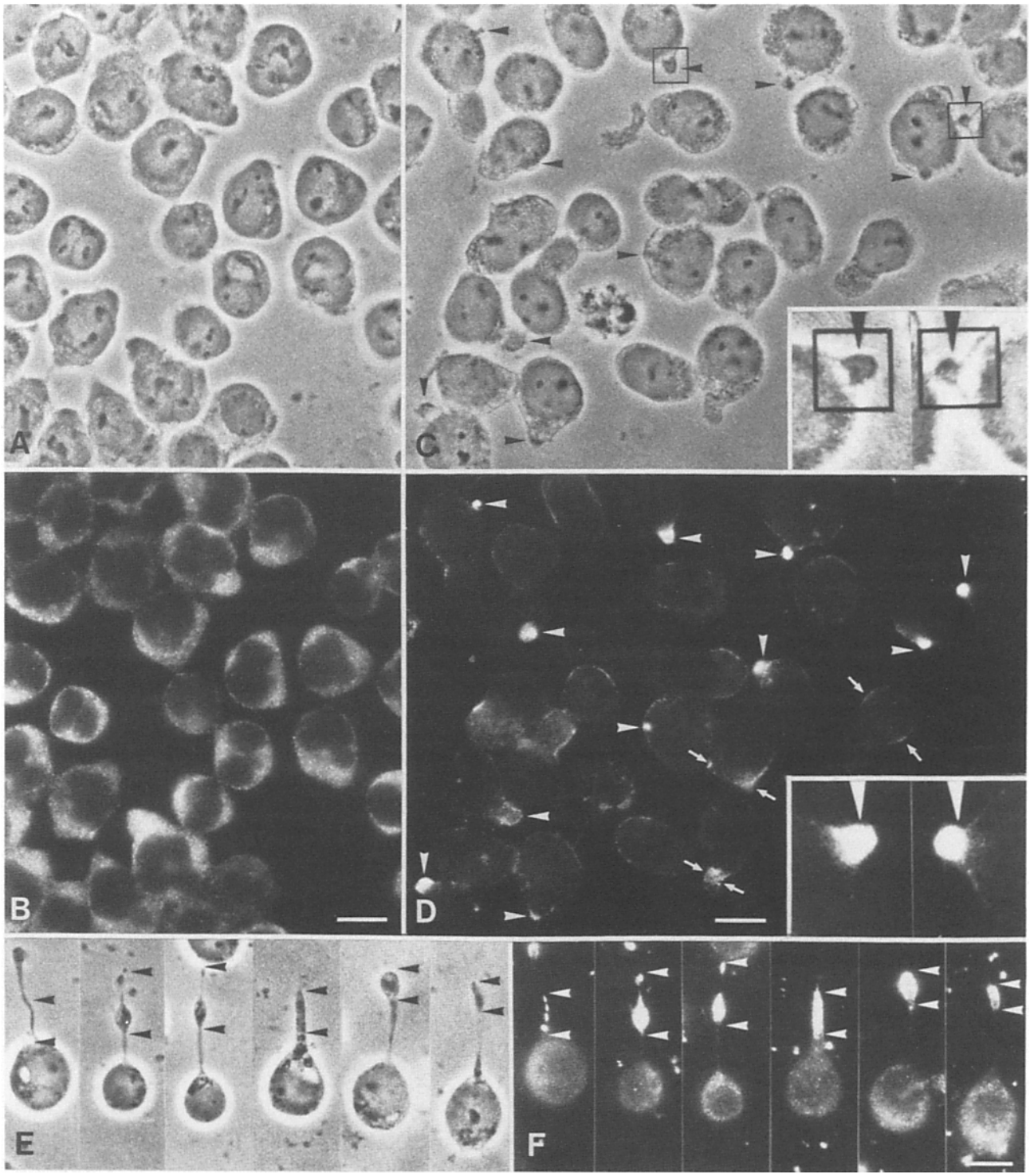


Figure 8. Myosin distribution in untreated (*A* and *B*), NZ-treated (*C* and *D*), and CD-treated (*E* and *F*) cells, as demonstrated with a monoclonal antimyosin heavy chain antibody. Untreated cells display an even and slightly granular staining of the cytoplasm (*B*) with only the nuclear area left unstained (see phase contrast in *A*). This distribution precludes the observation of cortical myosin. By contrast, NZ-treated cells showed a clear accumulation of myosin at the plasma membrane (*D*, with corresponding phase contrast in *C*) either as a ring (*white arrows*, *D*) or a tight cap (*arrowheads*, *C* and *D*). Note that nuclear outlines are no longer visible due to the absence of cytoplasmic myosin. The caps outlined with a square in *C* have been blown up in the inserts in the lower right corners of *C* and *D*. Note that they are visible by phase contrast together with the cell cortex. In CD-treated cells, most of the cellular myosin was observed on segments (*arrowheads*, *E* and *F*), sometimes interrupted, of the cellular extensions, but also at their base (not shown). Bars, 5 μm.

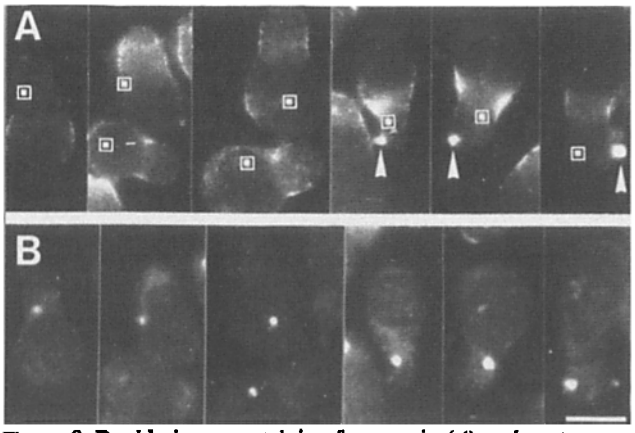


Figure 9. Double immunostaining for myosin (*A*) and centrosome (*B*) on NZ-treated cells. Myosin accumulates in a membrane-associated ring and/or a cap (*arrowheads*). The centrosome, marked with a white square in *A*, has no specific location with respect to the constriction ring. Bar, 5 μm.

of the cortical F-actin which can become associated with two separate domains of the plasma membrane, a situation which is not observed in untreated cells. CD also induces a major redistribution of myosin which accumulates at the base or along cellular extensions. The pictures also suggest a cortical localization within these extensions although, due to their narrowness and the large amount of myosin, this conclusion is not certain. Finally, cortical mfs apparently escape CD action as judged by NBD-phallicidin staining.

Discussion

Cell surface movements of KE37 cells show a basic polarized pattern: a constriction ring makes a limit between the cell body and a domain where membrane protrusion takes place. Internally, the active side corresponds to the centrosome side: the ring zone moves little and separates the organelle-containing cytoplasm from an organelle-free cytoplasm. The latter flows from the ring zone into the membrane veils which are sustained by filopodia and which keep protruding in the same general direction. The nucleus does not move through the ring and seems to be rocked by the movements of the active side. This could take place through Mts anchored at the centrosome which faces the active side and which is itself associated with the nucleus (Bornens, 1977; Maro and Bornens, 1980; Fais et al., 1984). Accordingly, in the absence of Mts the cell is more elongated, the nucleus passing back and forth through the ring, and the centrosome has no particular location with respect to the ring.

The association between the constriction ring and the membrane protrusion is apparently the important functional feature for cell surface motility and cell polarity in KE37 cells. It could be necessary for example to allow the coupling between solation and contraction of the actin system (Hellewell and Taylor, 1979). Our data indicate that this association is ensured by Mts.

In the absence of Mts, the ring and the membrane protrusion, when present on the same cell, are always separated from each other, at opposite ends of the cell. The ring is conspicuous and the membrane protrusion appears as a unique

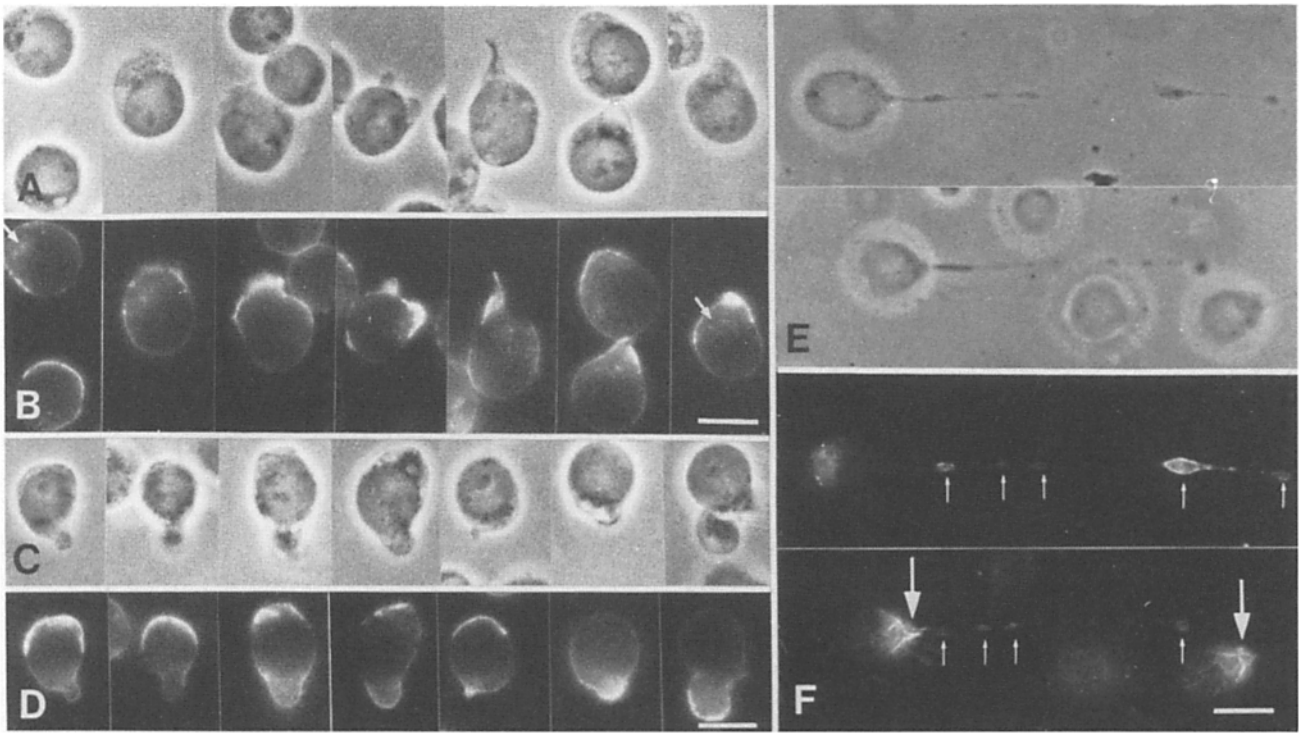


Figure 10. F-actin distribution in untreated (*A* and *B*), NZ-treated (*C* and *D*) and CD-treated (*E* and *F*) cells, using NDB-phallicidin labeling. F-actin was mainly localized at one pole of the untreated cells. The staining either corresponds to evident membrane activity on the corresponding phase-contrast microscopy, or appears as a large continuous cap on spherical cells. Filopodia were stained (*small arrows, B*). In NZ-treated cells (*D*), F-actin was observed either at one end of the cells, associated with the constriction ring area, or at both ends, associated with the constriction ring at one end and underlying the plasma membrane at the other, where activity was suggested by phase-contrast images (*C*). In CD-treated cells, NDB-phallicidin (*F*) demonstrated that actin filaments underlying the plasma membrane were still present (*small arrows*) and often accumulated at the base of the extensions (*large arrows*). Bars, 5 μm.

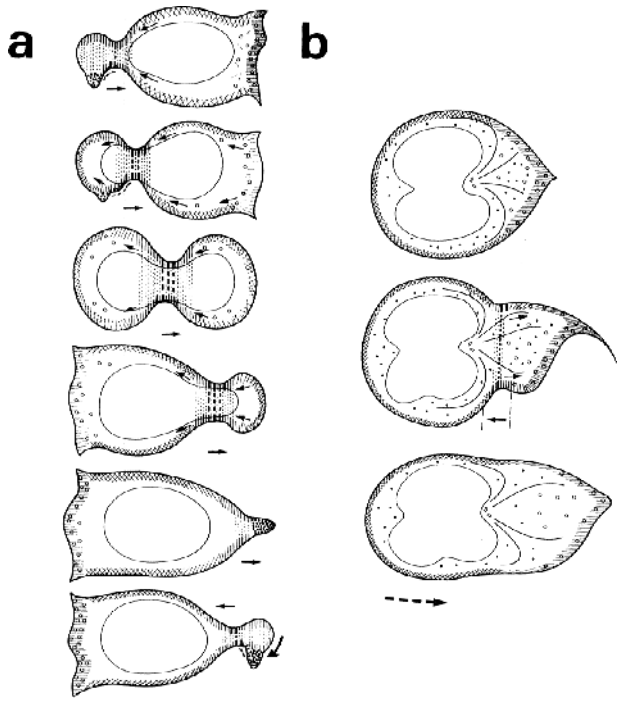


Figure 11. Schematic drawing of an hypothetical model for the cell surface movements of KE37 cells. Longitudinal sections, encompassing the nucleus, have been represented. The cortical mfs are represented as short and thin straight lines. The degree of parallelism between mfs is supposed to indicate a higher level of assembly. Black dots represent myosin heavy chain molecules, or filaments. When assembled in acto-myosin fibrils, myosin is depicted as short and fat straight lines. Open circles represent actin-binding proteins involved in actin assembly that takes place where membrane protrusion occurs. (*a*) Mts are disrupted. Myosin has an exclusive cortical localization and flows continuously back and forth along the cell. (top to bottom) The contractile ring moves from left to right (arrow outside the cell). Myosin is recruited into the ring (small dotted arrow along the cell), from a cap left over by the preceding ring movement, up to a full state of assembly. During ring movement, upstream mfs are recruited into the ring and others are released downstream (arrows inside the cell). When the ring has reached the right end of the cell and disappeared, a membrane-associated cap of myosin is left over. It will be used to assemble a new ring which will move from right to left. Usually assembly of a new ring leads to a displacement of the myosin cap to the side (curved arrow at bottom), suggesting an osmotic phenomenon. When the ring is not yet fully assembled, or disassembled, cortical mfs can participate in membrane protrusion at the other end of the cell, through appropriate network building mediated by actin-binding proteins other than myosin. (*b*) Untreated cells. Mts are present (represented as long lines emanating from the juxtanuclear centrosome which is depicted as two perpendicular rectangles). Most of the myosin is not associated with the plasma membrane. A contractile ring can assemble anywhere from the pool. The level of assembly is always low in such a way that there are always mfs available for interaction with other actin-binding proteins to build up filopodia and veils. The ring is the site of a motive force production, leading to forward protrusion. (top to bottom) Unidirectional streaming of solate cytoplasm is produced (arrows within the cell), while the ring moves in the other direction a short distance (arrow between dotted lines outside the cell). Protrusion of the leading edge occurs by assembly of actin meshwork. When the ring disassembles, forward movement of the cell body could occur (dotted arrow outside the cell) if transient interaction with the cell environment is established. A new cycle could start elsewhere before complete relaxation of the preceding one: several filopodia and veils would be superimposed, as often observed.

veil. A complete cycle of formation and disappearance of both the ring and the membrane veil is apparently concerted in time and space at the cell level: it involves a periodic movement of the ring across the cell, back and forth, and the alternate protrusion of a membrane veil at one end of the cell when the ring is at the other. F-actin is associated with both the ring and the membrane veil, whereas myosin is associated with the constriction ring only. A striking redistribution of myosin towards a cortical localization is the landmark of the absence of Mts. Within the limits of the method, the whole complement of cellular myosin seems associated with the plasma membrane either as a ring or as a small protruding cap. This pattern corresponds nicely to the surface movements observed on living cells: when the ring disappears at one end of the cell, a protruding knob is left over which slowly disappears as a new ring is reforming. The ring assembly–disassembly cycle seems therefore to involve the progressive recruitment of myosin from the cap to the ring, its transportation across the cell and its reaccumulation in a new cap at the other end of the cell. To our knowledge, this alternate flow of cortical myosin across the cell has never been described and the existence of a myosin ring is documented in interphasic cells for the first time. It would be of interest to know what regulates the cortical oscillator. Durham (1974) has argued that Ca^{++} flux could create contraction waves along the cell surface. Preliminary experiments, however, using agonists and antagonists of Ca^{++} channels, have failed to reveal any effect on the features of the ring cycle. The alternate capping of myosin observed during the ring cycle could bear some relevance to the capping phenomenon of membrane receptors in lymphoid cells (Hewitt, 1979; Oliver and Berlin, 1982).

The demonstration that the amount of myosin associated with the cell cortex depends upon the presence of Mts is also new. Untreated cells apparently have most of the myosin in a cytoplasmic localization, precluding the observation of the cortex-associated myosin. This could have important implications for the understanding of cell motility. The presence of Mts in KE37 cells reduces the equatorial contraction and suppresses its longitudinal propagation. This suggests that Mts are able to tone down the acto-myosin system. A constraining effect of Mts on motility has often been noted in various cell systems (Malech et al., 1977; Solomon and Magendantz, 1981; Rich and Hoffstein, 1981; Malawista and De Boisfleury, 1982; Forman et al., 1986). Our results indicate that Mts do not act mechanically but rather by maintaining most of the myosin in a cytoplasmic localization; i.e., by controlling the recruitment of myosin filaments from the pool. This effect might involve a direct interaction between Mts and the cortex. How it is brought about in biochemical terms awaits further study. Evidence for a direct binding between tubulin and myosin has been reported in the past (Shimo-Oka et al., 1980). Also, Toyama et al. (1982) were able to generate pseudosarcomeres in which myosin filaments and Mts interdigitate in a specific pattern. The effect of Mts on myosin distribution could bear some relevance to cytokinesis which is achieved by a contractile ring (see Oliver and Berlin, 1982). In mitotic cells there are no Mts interacting with the cell periphery: according to the observations reported in this work, this would lead to the recruitment of myosin into the cell cortex.

Another role of Mts is apparently to guide the flow of cytoplasm from the ring zone towards the protruding veils.

In the absence of Mts, it is the ring which moves and we did not observe any evidence for cytoplasmic flow into the membrane veils. We have sketched the surface movements of KE37 in the presence and in the absence of Mts on Fig. 11 and proposed a plausible mechanism in both cases. All together, the data suggest a critical role for Mts in the establishment of cell surface polarity through an elaborate damping of the cortical acto-myosin activity.

The round shape of KE37 cells requires Mts. It also requires the mf system: when the latter is disorganized, the cells elongate considerably. This suggests that the shape of lymphoid cells is obtained through the interaction of Mts with the mf system. The curvature of Mts from the centrosome to the periphery in normal conditions would be imposed by the actin system and particularly by its cortical domain. Cell elongation provoked by the disorganization of the actin system depends upon the presence of Mts. It cannot however be explained as the direct result of the elongation of Mts in the absence of constraints for the following reasons: (*a*) the stereotypic shape of CD-treated cells implies the existence of a specific equatorial constraint on the spatial distribution of the Mt network resulting in two diametrically opposed bundles; (*b*) the two extensions are different, defining a polarity for the elongated cell; and (*c*) the elongation process cannot take place upon reassembly of Mts if CD treatment has been carried out in the absence of Mts.

A mechanism for cell elongation would be possible if CD had a differential effect on the two basic features of cell surface motility, the membrane protrusion and the constriction ring. The first one would be inhibited as it requires actin assembly. The second one, which only requires acto-myosin contraction, would not be inhibited. The latter possibility is supported by the presence of cortical F-actin in the extensions (cortical mfs anchored at the cell surface via their barbed ends may escape drug action). It is known that among cytochalasins, CD, which binds actin with the highest affinity (Sheterline, 1983), provokes effects at concentrations lower than those capable of disrupting acto-myosin contraction (Schliwa, 1982; McLean-Fletcher and Pollard, 1980; Hartwig et al., 1985). The cortical mfs could participate in myosin-powered contraction. They could even slowly interact with most of the cellular myosin as they are the only mfs present within the cell. This is suggested by the segmental accumulation of myosin observed in the extensions. A similar situation has been substantiated by Miranda et al. (1974*a*,*b*).

The contraction is equatorial only when Mts are present. In their absence, the cortical contraction provokes arborization and blebbing. Cell elongation involves a movement of the cytoplasm into the extensions and the swollen tip contained a significant amount of cytoplasm and numerous cellular organelles. The interaction of Mts with the constriction ring is apparently necessary for this to occur and we have obtained evidence for a direct interaction of Mts with the cell cortex in the extensions. One may reasonably suppose that the polarity of elongated cells corresponds to the native cell polarity. Although the slow movement of the whole cytoplasm into the extensions is different from the directed cytoplasmic flow observed in normal conditions which involves the disassembly and reassembly of actin filaments, the direction of movement in both situations is imposed by Mts.

Mts are highly dynamic structures (Soltys and Borisy, 1985; Schulze and Kirschner, 1986). Within the dynamic instability model for tubulin assembly, Kirschner and Mitchison (1986) have proposed that cell polarity would be obtained by selective stabilization of the Mt network in one preferential direction upon a local extracellular signal. Our data indicate that in lymphocytes, Mts impose a permanent endogenous cell polarity by interfering with the dynamics of the cortical mf system. The latter involves an oscillatory movement of a constriction ring across the cell, the direction of which reverses periodically. By suppressing the ring movement through their interaction with the cell cortex, Mts force the acto-myosin system to adopt a unidirectional activity.

We wish to thank Dr. G. Keryer, Dr. S. Brown, and one anonymous referee for careful reading of the manuscript, and Dr. D. Pantaloni for allowing us to use his facilities for observations of living cells and for his help in the use of the Cytix. We thank also I. Gaspard, C. Pouget, and D. Meur for the art work, and J. Brizard for editing the manuscript.

This work has been supported by Centre National de la Recherche Scientifique and by a grant (87.C.0555) from Ministere de la Recherche et de l'Enseignement Supérieur.

Received for publication 5 December 1988 and in revised form 26 April 1989.

References

Bessis, M., and A. De Boisfleury. 1976. A catalogue of white blood cell movements (normal and pathologic). *Blood Cells (Berlin).* 2:365–410.

Bornens, M. 1977. Is the centriole bound to the nuclear membrane. *Nature (Lond.).* 270:80–82.

Durham, A. C. H. 1974. A unified theory of the control of actin and myosin in non-muscle movements. *Cell.* 2:123–136.

Fais, D., E. S. Nadeshdina, and Y. S. Chentsov. 1984. Evidence for the nucleus–centriole association in living cells obtained by ultracentrifugation. *Eur. J. Cell Biol.* 33:190–196.

Forman, D. S., W. G. Shain, and D. A. Fuchs. 1986. Slow pulsatile movements of Schwann cells in vitro. A time-lapse cinematotographic study. *Cell Motil. Cytoskeleton.* 6:595–603.

Gosti-Testu, F., M. C. Marty, J. Berges, R. Maunoury, and M. Bornens. 1986. Identification of centrosomal proteins in a human lymphoblastic cell line. *EMBO (Eur. Mol. Biol. Organ.) J.* 5:2545–2550.

Gosti, F., M. C. Marty, J. C. Courvalin, R. Maunoury, and M. Bornens. 1987. Centrosomal proteins and lactate dehydrogenase possess a common epitope in human cell lines. *Proc. Natl. Acad. Sci. USA.* 84:1000–1004.

Hartwig, J. H., R. Niederman, and S. E. Lind. 1985. Cortical actin structures and their relationship to mammalian cell movements. *Subcell. Biochem.* 11:1–49.

Haston, W. S., and J. M. Shields. 1984. Contraction waves in lymphocytes locomotion. *J. Cell Sci.* 68:227–241.

Hellewell, S. B., and D. L. Taylor. 1979. The contractile basis of ameboid movement. VI. The solation-contraction coupling hypothesis. *J. Cell Biol.* 83:633–648.

Hewitt, J. A. 1979. Surf-riding model for cell capping. *J. Theor. Biol.* 80:115–127.

Keller, H. U., and H. Cottier. 1981. Crawling-like movements and polarisation in non-adherent leucocytes. *Cell Biol. Int. Rep.* 5:3–7.

Keller, H. U., A. Naef, and A. Zimmermann. 1984. Effects of colchicine, vinblastine and nocodazole on polarity, motility, chemotaxis and cAMP levels of human polymorphonuclear leukocytes. *Exp. Cell Res.* 153:173–185.

Kirschner, M. W., and T. Mitchison. 1986. Beyond self-assembly: from microtubules to morphogenesis. *Cell.* 45:329–342.

Klotz, C., N. Bordes, M. C. Laine, D. Sandoz, and M. Bornens. 1986. Myosin at the apical pole of ciliated epithelial cells as revealed by a monoclonal antibody. *J. Cell Biol.* 103:613–619.

Lackie, J. M. 1986. Cell Movement and Cell Behaviour. Allen and Unwin, Inc. Winchester, MA. 316 pp.

Lewis, W. H. 1931. Locomotion of lymphocytes. *Bull. Johns Hopkins Hosp.* 49:29–36.

Malawista, S. E., and A. De Boisfleury. 1982. The cytokinetoplast: purified, stable, and functional motile machinery from human blood polymorphonuclear leukocytes. *J. Cell Biol.* 95:960–973.

Malech, H. L., R. K. Root, and J. I. Gallin. 1977. Structural analysis of human neutrophyl migration. Centriole, microtubule, and microfilament orientation and function during chemotaxis. *J. Cell Biol.* 75:666–693.

Maro, B., and M. Bornens. 1980. The centriole–nucleus association: effect of cytochalasin B and nocodazole. *Biol. Cell.* 39:287–290.

McLean-Fletcher, S., and T. Pollard. 1980. Mechanisms of action of cytochalasin B on actin. *Cell.* 20:329–341.

Miranda, A. F., G. C. Godman, A. D. Dietch, and S. W. Tanenbaum. 1974*a*. Action of cytochalasin D on cells of established lines. I. Early events. *J. Cell Biol.* 61:481–500.

Miranda, A. F., G. C. Godman, and S. W. Tanenbaum. 1974*b*. Action of cytochalasin D on cells of established lines. II. Cortex and microfilaments. *J. Cell Biol.* 62:406–423.

Oliver, J. M., and R. D. Berlin. 1982. Mechanisms that regulate the functional architecture of cell surfaces. *Int. Rev. Cytol.* 74:55–95.

Rich, A. M., and S. T. Hoffstein. 1981. Inverse correlation between neutrophil microtubule numbers and enhanced random migration. *J. Cell Sci.* 48:181–191.

Schliwa, M. 1982. Action of cytochalasin D on cytoskeletal networks. *J. Cell Biol.* 92:79–91.

Schulze, E., and M. W. Kirschner. 1986. Microtubule dynamics in interphase cells. *J. Cell Biol.* 102:1020–1031.

Senda, H., H. Tamura, N. Shibata, J. Yoshitake, K. Kondo, and K. Tanaka. 1975. The mechanism of the movement of leukocytes. *Exp. Cell Res.* 91:393–407.

Sheterline, P. 1983. Mechanisms of Cell Motility: Molecular Aspects of Contractility. Chap. 8. Academic Press, New York. 167–171.

Shields, J. H., and W. S. Haston. 1985. Behavior of neutrophil leucocytes in uniform concentration of chemotactic factors: contraction waves, cell polarity and persistence. *J. Cell Sci.* 74:75–93.

Shimo-Oka, T., M. Hayashi, and Y. Watanabe. 1980. Tubulin–myosin interaction. Some properties of binding between tubulin and myosin. *Biochemistry.* 19:4921–4926.

Solomon, F., and M. Magendantz. 1981. Cytochalasin separates microtubule disassembly from loss of asymmetric morphology. *J. Cell Biol.* 89:157–161.

Soltys, B. J., and G. G. Borisy. 1985. Polymerization of tubulin in vivo: direct evidence for assembly onto microtubules ends and from centrosomes. *J. Cell Biol.* 100:1682–1689.

Toyama, Y., S. Forry-Schaudies, B. Hoffman, and H. Holtzer. 1982. Effects of taxol and colcemid on myofibrillogenesis. *Proc. Natl. Acad. Sci. USA.* 76:6556–6560.