

Analysis of the Role of Microfilaments and Microtubules in Acquisition of Bipolarity and Elongation of Fibroblasts in Hydrated Collagen Gels

JAMES J. TOMASEK and ELIZABETH D. HAY

Department of Anatomy, Harvard Medical School, Boston, Massachusetts 02115. Dr. Tomasek's present address is Department of Anatomy, New York Medical College, Valhalla, New York 10595.

ABSTRACT Fibroblasts *in situ* reside within a collagenous stroma and are elongate and bipolar in shape. If isolated and grown on glass, they change from elongate to flat shape, lose filopodia, and acquire ruffles. This shape change can be reversed to resemble that *in situ* by suspending the cells in hydrated collagen gels. In this study of embryonic avian corneal fibroblasts grown in collagen gels, we describe for the first time the steps in the acquisition of the elongate shape and analyze the effect of cytoskeleton-disrupting drugs on filopodial activity, assumption of bipolarity, and cell elongation within extracellular matrix. We have previously shown by immunofluorescence that filopodia contain actin but not myosin and are free of organelles. The cell cortex is rich in actin and the cytosol, in myosin. By using antitubulin, we show in the present study that microtubules are aligned along the long axis of the bipolar cell body. The first step in assumption of the elongate shape is extension of filopodia by the round cells suspended in collagen, and this is not significantly affected by the drugs we used: taxol to stabilize microtubules; nocodazole to disassemble microtubules; and cytochalasin D to disrupt microfilaments. The second step, movement of filopodia to opposite ends of the cell, is disrupted by cytochalasin, but not by taxol or nocodazole. The third step, extension of pseudopodia and acquisition of bipolarity similarly requires intact actin, but not microtubules. If fibroblasts are allowed to become bipolar before drug treatment, moreover, they remain so in the presence of the drugs. To complete the fourth step, extensive elongation of the cell, both intact actin and microtubules are required. Retraction of the already elongated cell occurs on microtubule disruption, but retraction requires an intact actin cytoskeleton. We suggest that the cell interacts with surrounding collagen fibrils via its actin cytoskeleton to become bipolar in shape, and that microtubules interact with the actin cortex to bring about the final elongation of the fibroblast.

The extracellular matrix (ECM)¹ in or upon which cells reside, plays an important role in regulating the cytoskeleton, shape, migration, growth, and differentiation of various types of cells (32). Epithelial cells, whose basal surfaces contact collagen, organize their basal cortical cytoplasm in a predictable way (52) and remain on top of collagen gels, whereas mesenchymal cells tend to burrow into hydrated collagen lattices and, once inside the gel, they assume morphologies that are appropriate

for their particular tissue type (8, 10–13, 20, 25, 42, 46, 48). Mesenchymal cells may interact with collagen indirectly via fibronectin, or directly with collagen and/or fibronectin (30, 32, 35, 42, 47). The ability to suspend such cells within gelling solutions of collagen provides an opportunity to examine cell-matrix interactions *in vitro* that closely resemble the *in vivo* counterpart and to analyze the influence of ECM on cell shape and cytoskeleton. In this paper, we use microfilament- and microtubule-disrupting drugs to study the manner in which components of the fibroblast cytoskeleton respond to collagen.

ECM has been demonstrated to have a dramatic effect

¹ *Abbreviations used in this paper:* CMTC, cytoplasmic microtubular complex; ECM, extracellular matrix; MTOC, microtubule-organizing center.

upon the shape of cultured fibroblasts. Elsdale and Bard (25) first demonstrated that fibroblasts suspended within hydrated collagen lattices adopt a bipolar, elongate morphology comparable to that of fibroblasts in their original connective tissue stroma. By using the embryonic avian corneal fibroblast, a cell that migrates into the collagen-rich primary stroma early in corneal development (33), we have previously described the shape and migration of fibroblasts in hydrated collagen lattices (8) and the effect of such lattices on the organization of the fibroblast cytoskeleton (54). The elongate, bipolar shape of fibroblasts cultured in ECM is quite different from the flattened, polygonal appearance of fibroblasts cultured on a planar substratum. In collagen, the fibroblast migrates by extending a cylindrical cell process, the pseudopodium, that possesses very thin cell processes called filopodia at its tip. On glass, however, the leading cell process, or lamellipodium, is flat and broad and has an upward-swinging leading edge, the ruffled border, which may derive from aborted filopodia (8, 54, 55).

These differences in the morphology of fibroblasts cultured in ECM, as opposed to that of fibroblasts on planar substrata, can be correlated with cytoskeletal differences as revealed by electron microscopy and immunohistochemistry. The large bundles of actin microfilaments in parallel array, termed stress fibers (16), which characterize fibroblasts cultured on planar substrata, are not present in fibroblasts cultured within ECM. Most of the actin is present in the microfilamentous cell cortex, whereas myosin is prevalent in the cytosol, or so-called cytomatrix (37), surrounding the abundant endoplasmic reticulum (11, 54). Both filopodia and ruffled borders are rich in actin and poor in myosin. The highly developed close and focal contacts of cells with planar substrata, which probably reflect strong adhesion to plastic and glass, are not present on fibroblasts grown in three-dimensional ECM lattices (42, 43). Also, as we demonstrate in this paper, microtubules are present as a cytoplasmic microtubular complex (CMTC) oriented along the long axis of the fibroblast in ECM, rather than in a radially dispersed network around the microtubule-organizing center (MTOC).

In this study, we report that embryonic avian corneal fibroblasts cultured within ECM, which are rounded in shape after isolation from the stroma, go through a series of four discrete steps as they assume the bipolar, elongate morphology. To assess the role that microfilament and microtubules play in these cell shape changes, we treated fibroblasts with cytochalasin D, nocodazole, or taxol at different stages during assumption of the final shape. Cytochalasin D is similar to cytochalasin B, but lacks the side effects of the latter; it fragments actin filaments by a mechanism that is not yet completely understood (45). Nocodazole depolymerizes microtubules, whereas taxol aggregates microtubules into stable bundles that may or may not connect to the MTOC (21, 22, 34).

MATERIALS AND METHODS

Cell Culture within Collagen Gels: Embryonic fibroblasts were isolated from developing chick corneas and cultured within hydrated collagen gels as previously described (54). Briefly, corneas from days 9–11 chick embryos (Spafas, Norwich, CT) were treated with 0.1% collagenase (Sigma Chemical Co., St. Louis, MO) and 0.1% trypsin (Sigma Chemical Co.) in Hanks' solution (Gibco Laboratories, Grand Island Biological Co., Grand Island, NY) for 9–12 min at 37°C to facilitate removal of epithelium and endothelium. Isolated corneal stromas were digested with 0.25% collagenase in calcium-magnesium-free Hanks' solution for 30 min at 37°C to release individual corneal fibroblasts

(19). Fibroblasts were resuspended in Ham's F12 medium (Gibco Laboratories) with 10% fetal calf serum (Flow Laboratories, Inc., McLean, VA). Medium was supplemented with 50 µg/ml ascorbic acid and 1% antibiotic antimycotic (penicillin, fungizone, and streptomycin; Gibco Laboratories). Observations were also made on a gerbil fibroma cell line, CCL-146, which we obtained from Dr. L. B. Chen (54), and some fibroblasts were cultured in 1.3% methocellose (methocellose, 4000 CSP, Dow Chemical Co., Midland, MI) in this same medium.

Fibroblasts were cultured in collagen gels prepared by a slight modification of the method of Elsdale and Bard (25) as previously described (54). Type I collagen was acetic acid extracted from rat tail tendons and dialyzed extensively against $\frac{1}{10}$ F12. Collagen gels were prepared by mixing 0.7 ml of collagen (2 mg/ml), 0.1 ml of F12 (0.1 g/ml), 0.1 ml of sodium bicarbonate (12 mg/ml), and 0.1 ml fetal calf serum at 4°C. Corneal fibroblasts were mixed 1:1 with the collagen solution for a final density of 2×10^6 cells/ml and a collagen concentration of 0.7 mg/ml. The collagen solution plus cells (0.25 ml) was immediately placed onto 25-mm circular coverslips in 35-mm petri dishes (Falcon Div., Becton, Dickinson & Co., Sunnyvale, CA) and incubated for 30 min to gel before the addition of 1 ml of media to each dish.

Microscopy of Living Fibroblasts: For observations of living fibroblasts cultured within collagen gels, coverslips with attached gels were transferred to a Dvorak-Stotler chamber (Nicholson Precision Instruments, Inc., Bethesda, MD) (Dvorak and Stotler [24]). Fibroblasts were examined on a Zeiss inverted microscope (Carl Zeiss, Inc., New York) fitted with Nomarski differential interference contrast optics. The stage was heated to 37°C by a Sage air curtain (Sage Instruments, Inc., White Plains, NY). For immunofluorescence, fixed tissues were examined in a Zeiss photoscope fitted for epifluorescence.

Drug Treatments: Fibroblasts were treated with the cytoskeletal-disrupting drugs cytochalasin D (Sigma Chemical Co.), nocodazole (Aldrich Chemical Co., Milwaukee, WI), or taxol (obtained from Dr. John Douras, National Cancer Institute, Bethesda, MD). These drugs were applied at three different times: (a) mixed with the collagen-cell suspension immediately before plating onto coverslips; (b) added to culture medium after the cells were within the collagen gel for 6 h; or (c) added after 18 h in culture. Penetration into the hydrated gel was excellent as judged by the effect of the drugs on the cells.

Drugs were kept as stock solutions of 2 mM cytochalasin D, 1 mg/ml nocodazole, or 10 mM taxol in dimethyl sulfoxide (DMSO) at -20°C and added to the culture medium so that their final concentrations were 2, 4, or 6 µM cytochalasin D, 1 µg/ml nocodazole, or 1 µM taxol. In general, only one drug was administered, but in one set of experiments, fibroblasts cultured for 18 h within collagen gels were first treated with 2 µM cytochalasin D for 30 min, and then with 2 µM cytochalasin D plus 1 mg/ml nocodazole. Control preparations received 0.1% DMSO. After 30 min, 1 h, 3 h, 6 h, or 18 h of drug treatment the cultures were either placed into the Dvorak-Stotler chamber and their morphologies were observed and photographed, or the cultures were fixed for immunofluorescence to examine the cytoskeletal organization of the cells. We also monitored the cells by conventional transmission electron microscopy (54).

Immunostaining: Corneal fibroblasts cultured within collagen gels were fixed and stained with rabbit antibodies to human platelet actin as previously described (54). To examine the distribution of microtubules in these cells, a slightly different procedure was employed. Collagen gels and cells were fixed in 0.1 M PIPES, 0.1% glutaraldehyde, 2% (wt/vol) paraformaldehyde, 2 mM EGTA, 2 mM MgCl₂, pH 6.8, at room temperature for 15 min (15). The gels were then rinsed with PBS (three times, 5 min) and stored in 0.15 M Tris buffer, pH 7.4, at 4°C overnight, to quench any free aldehyde sites. Just before staining, the gels were cut into small pieces and treated with 0.2% Triton X-100 in PBS for 2 min to permeabilize the cells. The pieces were rinsed with PBS (three times, 5 min) and incubated for 1 h in a 1:100 dilution to a purified rabbit antibody to sea urchin egg tubulin prepared by Dr. Keigi Fujiwara (26). After three rinses in PBS, the pieces of collagen gel were incubated again for 1 h with a 1:100 dilution of rhodamine-conjugated goat anti-rabbit IgG (N. L. Cappel Laboratories Inc., Cochranville, PA). After three more washes in PBS, the gels were mounted in 90% glycerol in PBS and examined as whole mounts as described elsewhere (54).

RESULTS

Steps in Acquisition of the Bipolar, Elongate Shape

Hydrated collagen lattices, as we noted above, restore the *in vivo* morphology to cultured embryonic fibroblasts isolated from the chick corneal stroma (8, 54). These fibroblasts, which are spherical in shape after isolation, become bipolar in shape

and extend cylindrical cellular processes into the surrounding matrix. With the use of Nomarski optics we have analyzed, at various time intervals, the process by which cells alter their shape within the collagen gel.

The first event observed was the projection of long, straight filopodia from the entire surface of the freshly suspended, spherical fibroblasts (Fig. 1A). Filopodia were seen on some of the cells 15 min after the beginning of culture in the collagen gel, and within 30 min almost all the cells had extended the thin cell processes. These projections have a diameter of 0.2–0.3 μm and vary in length from 5 to 15 μm . They appear to be relatively rigid structures that probe the surrounding matrix. By electron microscopy and immunofluorescence, it can be seen that filopodia have an actin-rich microfilamentous core and lack cell organelles (see reference 54). The absence of filopodia on the surface of spherical fibroblasts suspended in methocellulose (data not shown) suggests that their projection is a response to the surrounding substratum.

The second event in the assumption of the bipolar shape is the restriction of filopodia to certain regions on the cell surface, usually the two opposite ends of the cell. Examination of fibroblasts cultured in ECM for 2 h reveals that the long, thin filopodia originally projecting from all over the cell surface become shorter and less regular in shape in the process of translocation (Fig. 1, B and C). Filopodial restriction appears to determine the locations at which pseudopodia will subsequently form. When fibroblasts begin to extend pseudopodia, after 3 h in culture, the filopodia are concentrated on the distal ends of the pseudopodial extensions (Fig. 1D).

The third step in the assumption of the bipolar morphology is the initial extension of pseudopodia (Fig. 1E). The pseudopodia participating in this process are usually two in number and the cell becomes bipolar in shape. A few multipolar cells are observed and competing cell processes may project temporarily from the side of an otherwise bipolar cell (*p*, Fig. 1E). Cell organelles, such as mitochondria, tend to be clustered around the nucleus, but electron microscopy reveals the pseudopodia to contain rough endoplasmic reticulum and to possess a microfilamentous cortex (data not shown). 6 h after suspension in the gel, most of the cells are distinctly bipolar, with lengths ranging between 35 and 60 μm (Fig. 1E).

After 18 h in the collagen gel, most of the bipolar cells have completed the fourth step in the process and are now highly elongate in shape, with lengths ranging between 80 and 120 μm (Fig. 1F). The nucleus is located at the center of the cell surrounded by mitochondria, and the pseudopodia at the two poles are now quite attenuated. The rough endoplasmic reticulum extends almost to their tips, where the actin-rich cortex merges with the filopodial cytoskeleton (54). Competing cell processes come and go (*p*, Fig. 1F), but very few truly multipolar cells are observed in these 18-h cultures, suggesting that most of the cells observed earlier will become bipolar. Similar results were obtained with a gerbil fibroma fibroblast cell line cultured in collagen gels, except that most of these are initially multipolar and later withdraw cell processes to assume a bipolar, elongate configuration (data not shown). After the fibroblast elongates, it may begin to migrate through the collagen lattice restricting most of its filopodia to the leading edge (8).

The distribution of tubulin in corneal fibroblasts cultured in collagen gels was examined by indirect immunofluorescence. Staining with antitubulin after 18 h of culture (Fig. 1,

G and H) reveals a highly organized CMTC. Most of the microtubules are oriented along the long axis of the cell and appear to originate from a MTOC located lateral to the nucleus. Electron micrographs suggest that some of the microtubules insert into the microfilament-rich cell cortex (54). The cell cortex, as we noted above, stains intensely with antiactin as visualized by indirect immunofluorescence, but myosin seems distributed mainly in the cytosol (54).

Thus, our observations indicate that fibroblasts go through four sequential steps in order to assume a bipolar, elongate shape: (a) filopodial probing; (b) subsequent filopodial restriction to opposite poles of the cell; (c) extension of pseudopodia from these same cell poles; and (d) final cell elongation. In the experiments to be described next, corneal fibroblasts cultured for different intervals within collagen gels were treated with cytoskeletal disrupting drugs for 30 min to 18 h.

Effect of Drugs Administered at the Beginning of Culture

Freshly isolated fibroblasts were treated with cytochalasin D at the time the cells were placed within collagen lattices. These spherical cells project filopodia from their surfaces at concentrations of 2, 4, and even 6 μM cytochalasin D (Fig. 2, B–D). However, these filopodia differ from the filopodia projected by the DMSO-treated control cells in several respects. The drug-treated filopodia have a larger diameter, $\sim 0.5 \mu\text{m}$ as compared with 0.2–0.3 μm for the control cells (Fig. 2A) and untreated cells (Fig. 1A). Also these projections are often curved rather than straight (Fig. 2B) and may even have a corkscrew appearance (Fig. 2C). Whereas the control filopodia have tapered tips, the drug-treated processes have blunt endings and may terminate in a very small loop (Fig. 2, B and D).

Electron microscopy reveals that the cytochalasin-insensitive filopodia are highly variable in their morphology. They may contain microtubules (Fig. 3A), ribosomes (Fig. 3, A and B), aggregated actin (Fig. 3B), and/or intermediate filaments (Fig. 3C). Cytochalasin D removes the actin cortex (curved arrow, Fig. 3A) and leads to poor preservation of the plasmalemma (Fig. 3). Aggregates of actin (45) are present in the cytoplasm, often at the base of the aberrant filopodia (*agg*, Fig. 3).

Cytochalasin D prevents the restriction of these filopodia to opposite poles of the cell, and the cells do not extend pseudopodia or elongate, even after 18 h in culture. They retain the appearance shown above (Fig. 2, B–D, Fig. 3). The inhibition of pseudopodial extension and fibroblast elongation within collagen lattices by cytochalasin D is not due to a cytotoxic effect of the drug. Cytochalasin D can be washed out of the culture, even after 18 h of treatment, and the cells thereafter assume a bipolar, elongate shape. Moreover, the solvent (DMSO) for cytochalasin D when added alone at the same concentration has no effect on the ability of corneal fibroblasts to become bipolar and elongate within the collagen lattice.

Quite a different result was obtained when freshly isolated fibroblasts in collagen gels were treated with nocodazole or taxol. Spherical fibroblasts treated with either drug project normal filopodia and then extend pseudopodia from opposite poles of the cell (Fig. 2, E and F) in the same fashion as untreated cells in collagen gels (Fig. 1). After 18 h in culture, however, these bipolar cells have still not entered the cell

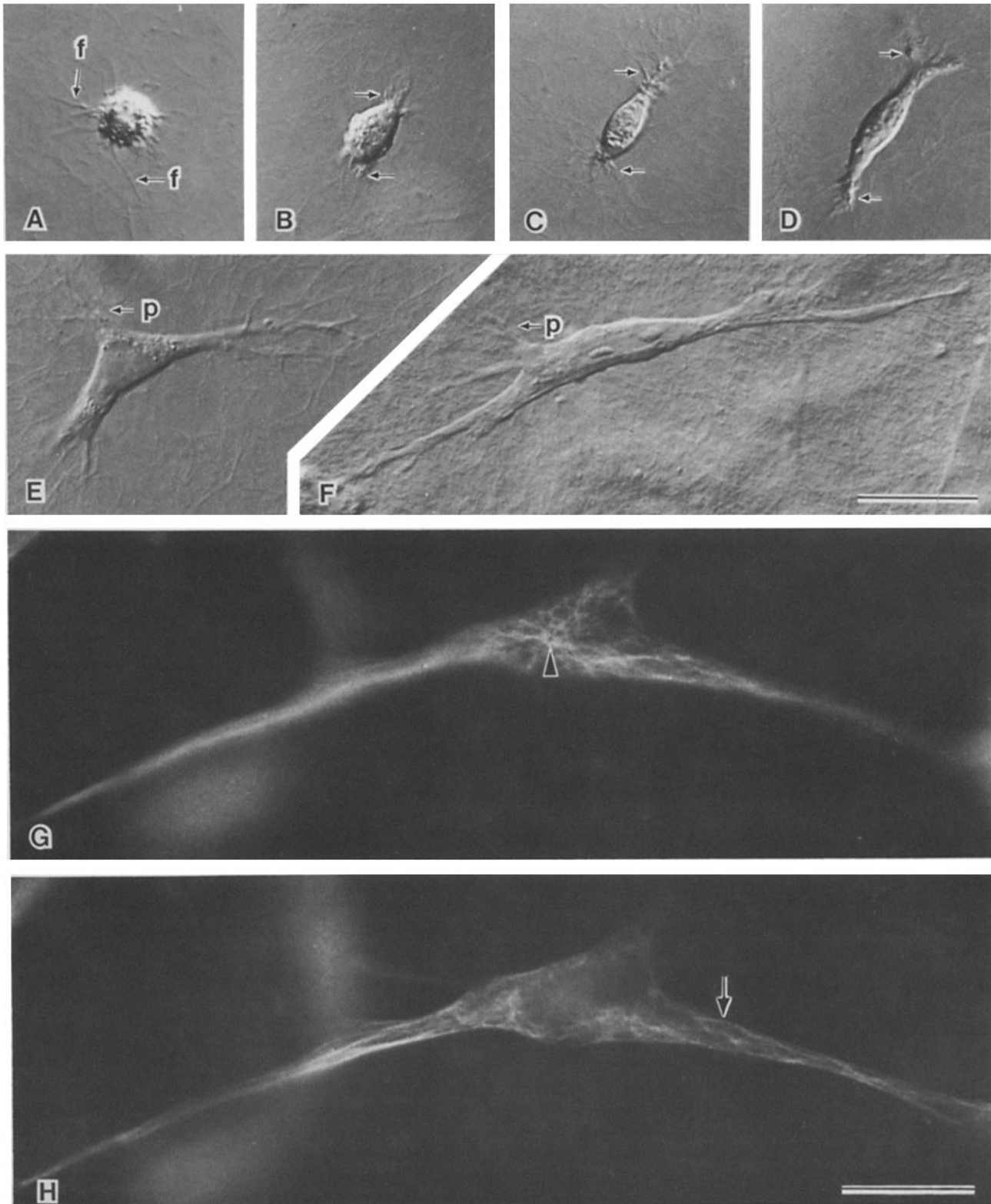


FIGURE 1 Living corneal fibroblasts cultured within collagen gels for various time intervals were examined by Nomarski optics (A–F). After 30 min in a collagen gel, numerous filopodia (*f*) project circumferentially into the surrounding collagen matrix (A). Within 2 h, filopodia (arrows) are restricted to opposite poles of the cell (B and C). Pseudopodia begin to extend from the filopodia-rich poles (arrows) after 3 h in culture (D). After 6 h, pseudopodia extend further into the matrix (E). A small lateral process (*p*) can be seen on this otherwise bipolar cell (E). By 18 h in culture, the bipolar fibroblast has elongated extensively (F). Occasionally, a minor lateral cell process may appear (*p*) and then be withdrawn. On the bottom half of the plate is shown a corneal fibroblast cultured in a collagen gel for 18 h and then stained with antitubulin (G and H). Fluorescence micrographs are of the same cell at two different planes of focus. Microtubules oriented along the long axis of the cell (arrow, H) can be seen emanating from an MTOC located lateral to the nucleus (arrowhead, G). Bars, 20 μm . (A–F) $\times 1,000$; (G and H) $\times 1,200$.

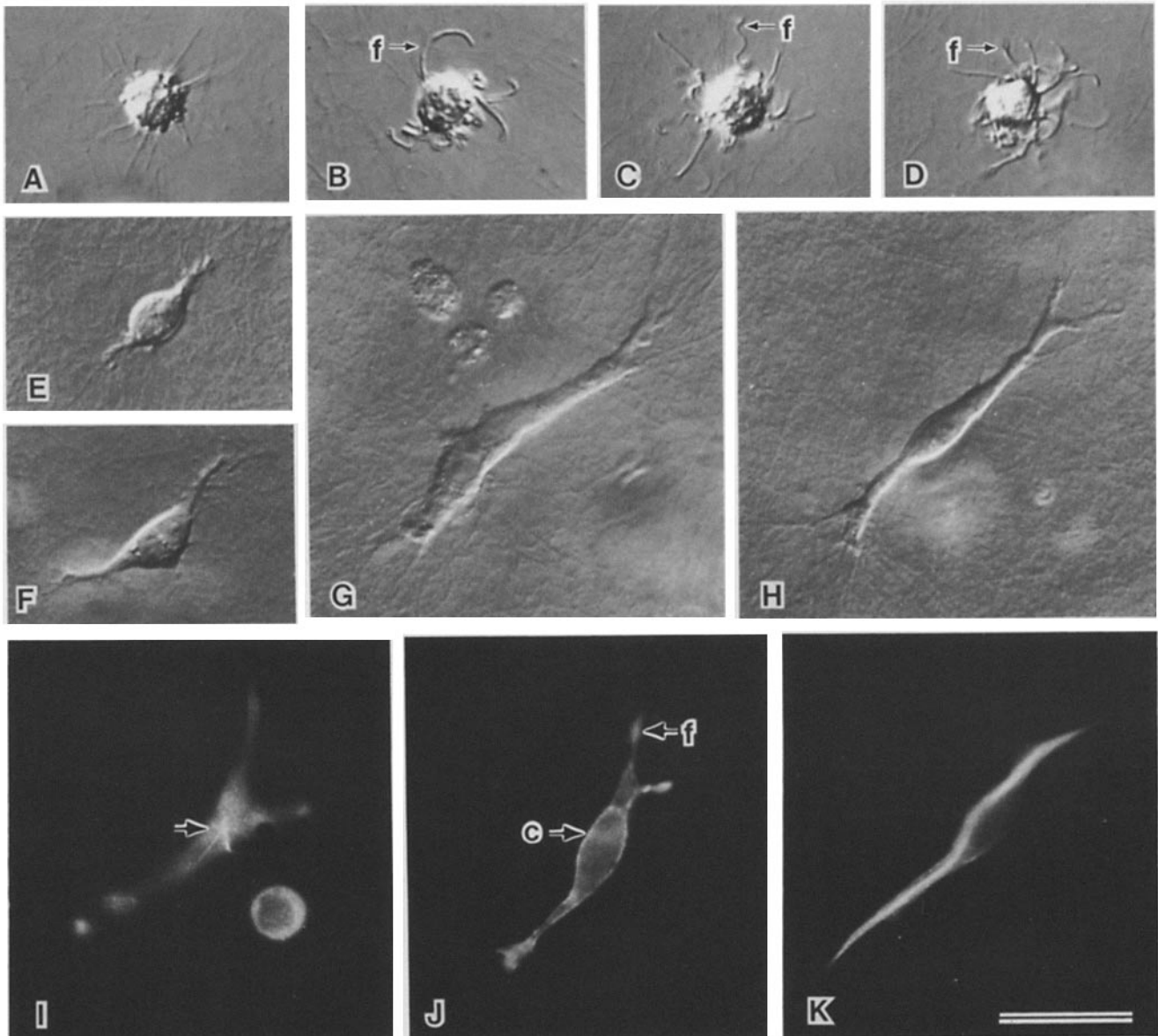
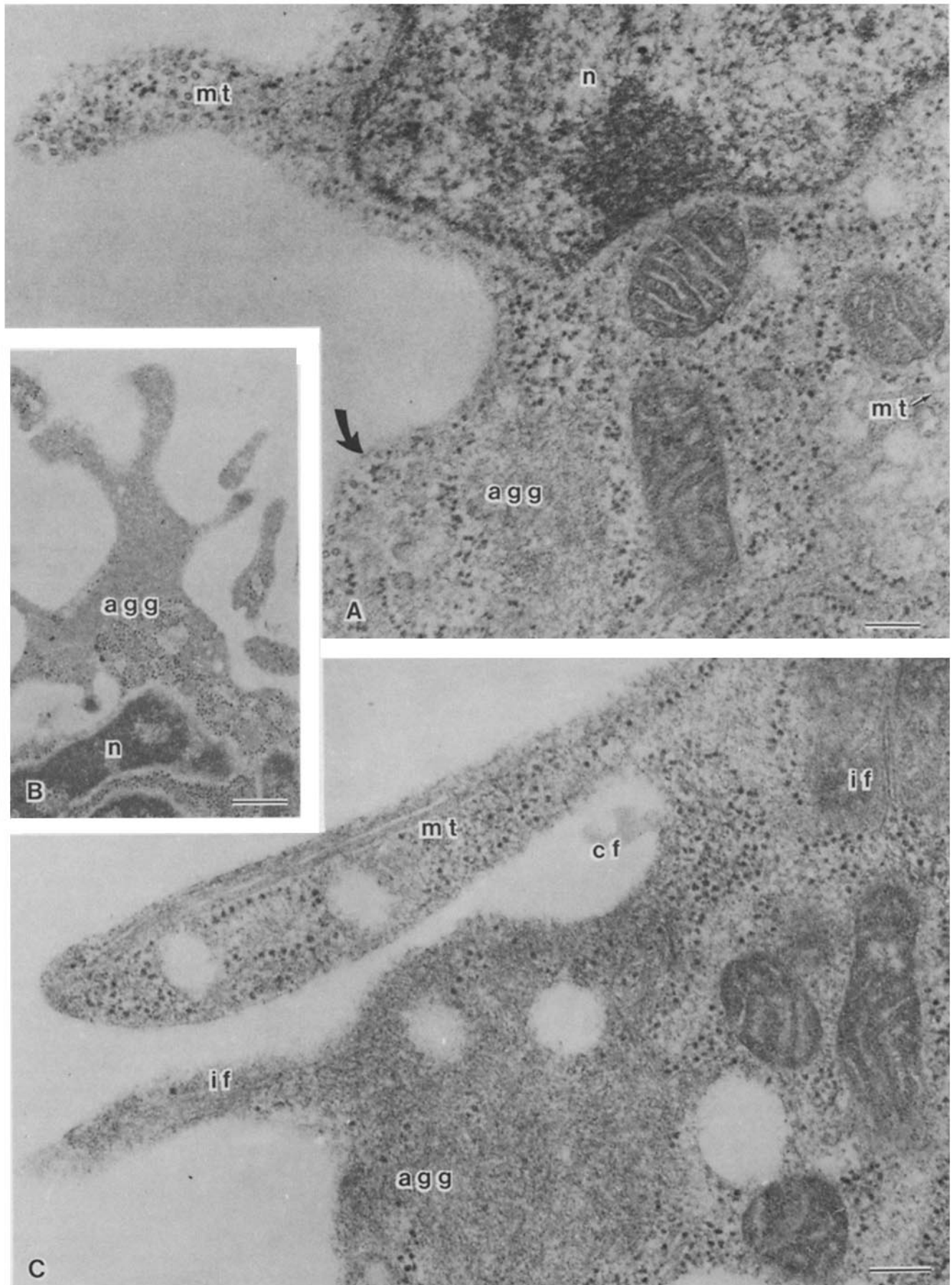


FIGURE 2 Corneal fibroblasts were treated with DMSO (A) or cytochalasin D, at 2 μ M (B), 4 μ M (C), or 6 μ M (D) in DMSO at the beginning of culture and photographed with Nomarski optics 1 h later. Filopodia (*f*) are projected in the presence of cytochalasin D (B–D). They may be curved (B) or have a corkscrew appearance (C), or the tips may loop back at the distal ends (D). The middle part of the plate shows corneal fibroblasts treated with nocodazole (E and G) or taxol (F and H) at the beginning of culture and photographed with Nomarski optics either 3 h (E and F) or 18 h (G and H) later. After 3 h in culture, pseudopodia extend from filopodia-rich opposite poles of the cell, which gives these drug-treated fibroblasts a bipolar appearance (E and F). After 18 h in culture, the drug-treated fibroblasts are more clearly bipolar in shape, but have not elongated (G and H) as extensively as the nontreated fibroblast (Fig. 1H). A corneal fibroblast treated with nocodazole, cultured for 18 h, and then stained with antitubulin is shown in I. Only a few short microtubules (arrow) emanating from the MTOC can be seen in this bipolar cell. The corneal fibroblast shown in J was cultured in nocodazole and then stained with antiactin. Staining is present in both the filopodia (*f*) and cell cortex (*c*), as would be expected in a normal fibroblast (54). The corneal fibroblast in K was cultured in taxol similar to H, and then stained with antitubulin. Individual microtubules are not observed, but instead there appears to be a taxol-stabilized bundle of microtubules running the length of the cell. Bar, 20 μ m. \times 1,000.

FIGURE 3 Corneal fibroblasts were treated with 2 μ m cytochalasin D at the time they were placed within collagen lattices, cultured for 1 h, and then prepared for electron microscopy. (A) Microtubules (*mt*) are present both in the aberrant filopodia and cytosol of treated fibroblasts. The microfilament-rich cortex normally associated with the cell surface of these fibroblasts has been lost (curved arrow). Microfilaments have been replaced by aggregates (*agg*) of fibrillogranular material. (B) In this fibroblast, a large actin aggregation (*agg*) is present at the base of several filopodia. The aggregate extends into the projected filopodia. (C) Intermediate filaments (*if*) are also present in the cytosol in some filopodia of treated fibroblasts. An aggregate of actin (*agg*) can be seen in the cytosol of this cell. A collagen fibril (*cf*) is closely associated with the nearby cell surface. *n*, nucleus. Bars: (A and C) 200 nm; (B) 500 nm. (A) \times 50,000; (B) \times 20,000; (C) \times 55,000.



elongation stage (Fig. 2, *G* and *H*). Their lengths at 18 h range from 40 to 60 μm , similar to those of control cells after 6 h in culture. The effect of these drugs is reversible, inasmuch as cell elongation will occur if the drug is washed out of the culture. In parallel experiments, the same result was obtained when nocodazole was replaced by 1 μM colchicine (data not shown).

The effects of nocodazole and taxol on the CMTC, MTOC, and actin distribution were examined by indirect immunofluorescence. In contrast to untreated fibroblasts (Fig. 1, *G* and *H*), only a few, very short microtubules project from the MTOC in nocodazole-treated fibroblasts after 18 h in culture (Fig. 2*J*). Electron micrographs of these bipolar cells (Fig. 4) reveal that intermediate filaments (Fig. 4*C*) and microfilaments (Fig. 4*B*) are present in the expected locations, but microtubules are absent and the Golgi apparatus is fragmented (Fig. 4, *A* and *C*).

Taxol has the expected effect (see opening paragraphs of this article) of aggregating microtubules into stable bundles, in this case apparently not associated with MTOC (Figs. 2*K* and 5). Neither nocodazole nor taxol appears to affect the distribution of actin in these cells. Both the filopodia and the cortex stain intensely, whereas the cytosol of the cell shows little staining (Fig. 2*J*), the same pattern we have previously reported for untreated fibroblasts (54). By electron microscopy, microfilaments can be detected in the cell cortex (Fig. 5*A*), and prominent microtubules in juxtannuclear zones (Fig. 5, *A* and *B*).

Thus, a normal actin cytoskeleton is sufficient for acquisition of the bipolar shape, but further elongation requires microtubules that are able to function normally. Corneal fibroblasts in collagen gels can project filopodia and extend pseudopodia from opposite poles of the cell in the presence of either nocodazole or taxol, indicating that a normally organized CMTC is not necessary for the early stages of cell shape change in three-dimensional collagen lattices.

Effect of Drugs Administered after 6 h of Culture

The effect of cytochalasin D, nocodazole, and taxol on the elongation of bipolar fibroblasts after 6 h in culture was next evaluated. Bipolar fibroblasts in collagen gels treated with cytochalasin D do not elongate. They remain bipolar, filopodia disappear or become distorted, and pseudopodia appear thinner with bulbous accumulations along their lengths (Fig. 6*A*). Cytochalasin D has a dramatic effect upon actin distribution in these bipolar cells. After only 30 min of treatment, intracellular actin has formed aggregates of fibrillogranular material (as illustrated in electron micrographs in Fig. 3) and antiactin staining is greatly diminished in the cortex (Fig. 6*B*). Microtubules are still present, but are insufficient without the actin cortex for cell elongation.

Nocodazole and taxol also inhibit the elongation of bipolar fibroblasts (Fig. 6, *C* and *D*), as would be expected from the

results above (Fig. 2). Although the cells can no longer elongate, pseudopodia of normal thickness remain extended from opposite poles of these cells, and filopodia are present as usual at their distal ends. These results taken together suggest that both actin microfilaments and an organized CMTC are necessary for elongation.

Effect of Drugs Administered after 18 h of Culture

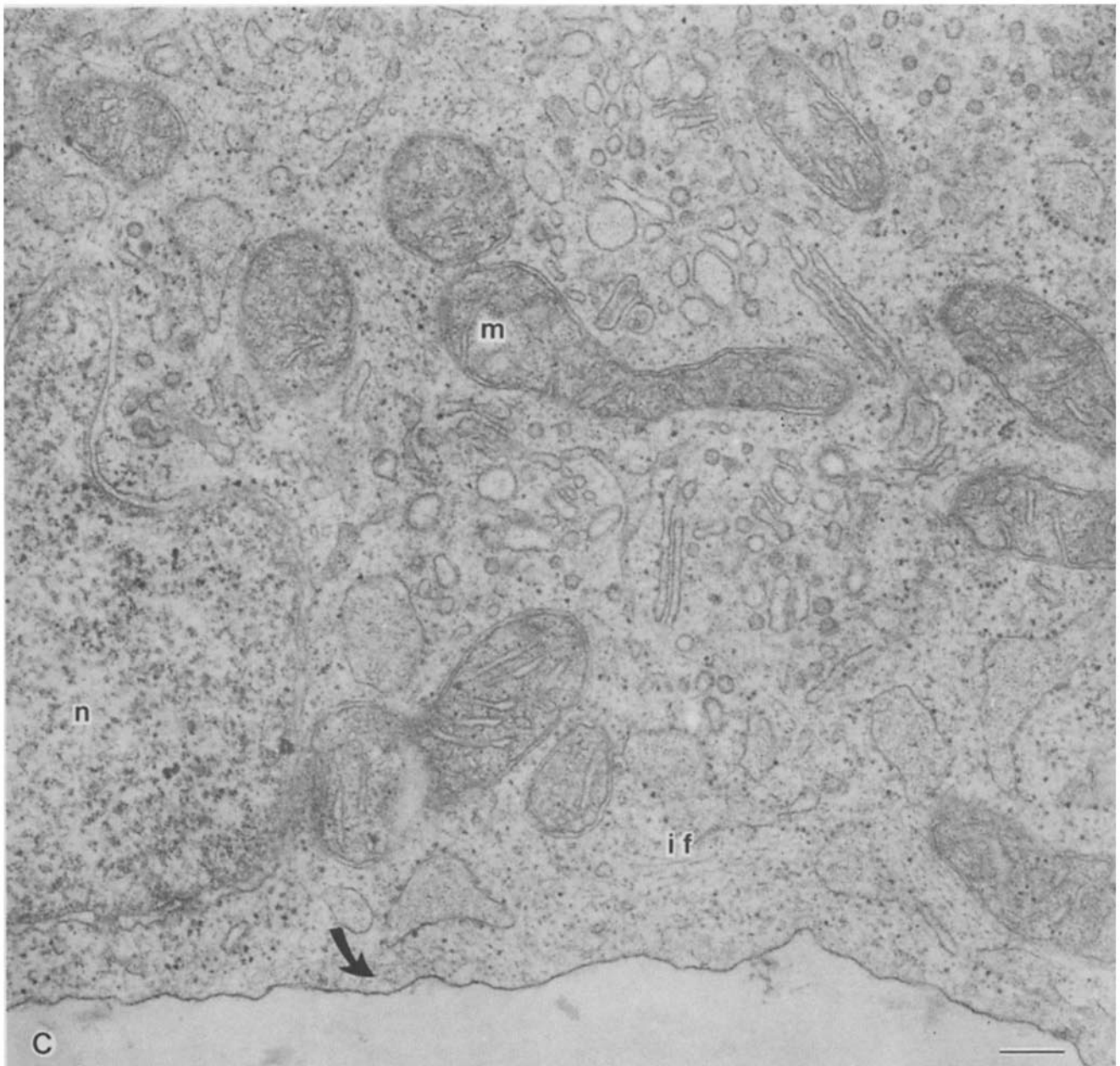
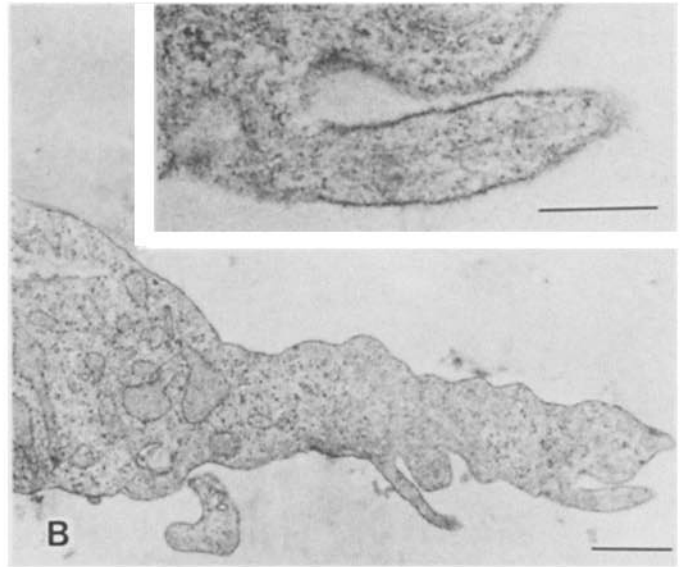
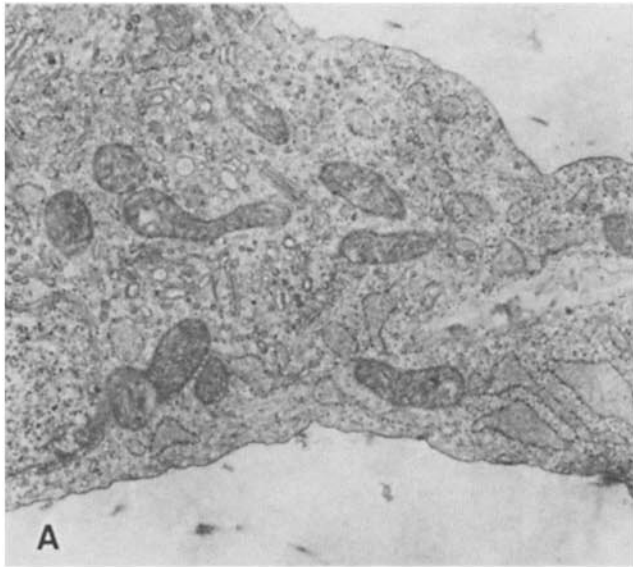
To examine the stability of the highly elongate cell shape (Fig. 1*H*), corneal fibroblasts were treated with cytochalasin D, nocodazole, or taxol after 18 h of culture in collagen gels. When elongate fibroblasts are exposed to cytochalasin D, their pseudopodia become thinner and irregular and filopodia disappear or become abnormal in shape, but the elongate cell configuration is maintained (Fig. 7*A*). Cytochalasin D causes aggregations of actin to appear in these cells (Fig. 7*B*), which are ultrastructurally similar to the aggregates illustrated above (Fig. 3). Cytochalasin D does not cause any dramatic changes in the CMTC, at least in the period (18 h) of observation. Although the cell is thinner, making it difficult to see individual microtubules, they are present in the extended pseudopodia as observed by immunofluorescence (Fig. 7*C*) and electron microscopy (data not shown).

In contrast, when nocodazole or taxol is administered to highly elongated fibroblasts, almost all treated cells shorten within 3 h to $<60 \mu\text{m}$ in length (Fig. 7, *D-G*). These cells, however, continue to be bipolar in shape. As expected, nocodazole rapidly depolymerizes microtubules in these fibroblasts, whereas taxol bundles microtubules, as assayed by indirect immunofluorescence staining with antitubulin (Fig. 7, *E* and *G*) and by electron microscopy. Thus, the highly elongated form of the corneal fibroblast within a collagen lattice is relatively stable to treatment with cytochalasin D, but is not maintained in the presence of nocodazole or taxol, indicating that maintenance of the elongated shape is dependent, in part at least, on a normally organized CMTC.

Finally, we inquired as to whether or not the shortening of the bipolar fibroblast that occurs in the absence of a normally organized CMTC (Fig. 7, *D-G*) requires assembled actin. We found that cytochalasin D does inhibit nocodazole-induced retraction. Elongated fibroblasts treated for 30 min with cytochalasin D were then exposed to nocodazole and filmed for 3 h. Fig. 7*H* illustrates a cell treated in such a manner. The cell has maintained its elongate shape, although the pseudopodia appear as thin threads with bulbous accumulations of cytoplasm (actin aggregates) along their length. These fibroblasts revert back to their typical morphology when placed into control media, demonstrating that the effect of these drugs used together is reversible.

The results are summarized diagrammatically in Fig. 8. The stages in elongation at which drug treatment was initiated are shown at the top, left to right: 0 h; 6 h, by which time filopodial probing, restriction to opposite poles of the cell,

FIGURE 4 Corneal fibroblasts were treated with 1 $\mu\text{g}/\text{ml}$ nocodazole at the time they were placed within collagen lattices, cultured for 18 h, and then prepared for electron microscopy. *A* and *B* are low-power electron micrographs of adjacent areas of the same cell. The rough endoplasmic reticulum is dilated and the Golgi components are fragmented in this treated cell. Filopodia projected from the distal end of pseudopodia contain a normal meshwork of microfilaments (*B* and *inset*). *C* is a higher power view of the Golgi region (*A*) of the same cell. Nocodazole does not disrupt the microfilament-rich cortex (curved arrow) present near the cell surface. Intermediate filaments (*if*) can be seen in the cytosol of the cell. No microtubules are present. *n*, nucleus; *m*, mitochondria. (*A* and *B*) Bar, 1 μm . $\times 15,000$. (*inset*) Bar, $\sim 200 \text{ nm}$. $\times 50,000$. (*C*) Bar, 250 nm . $\times 40,000$.



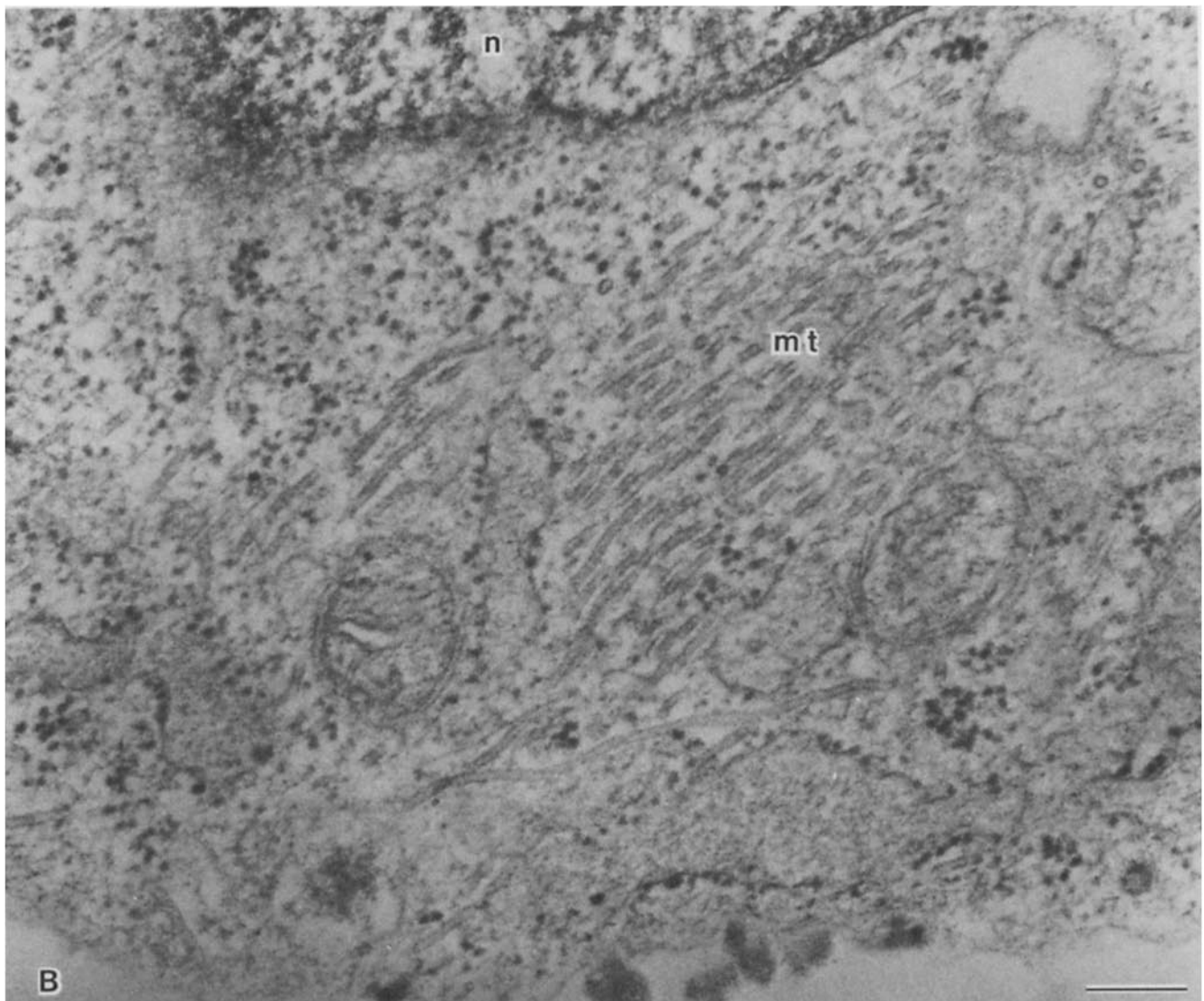
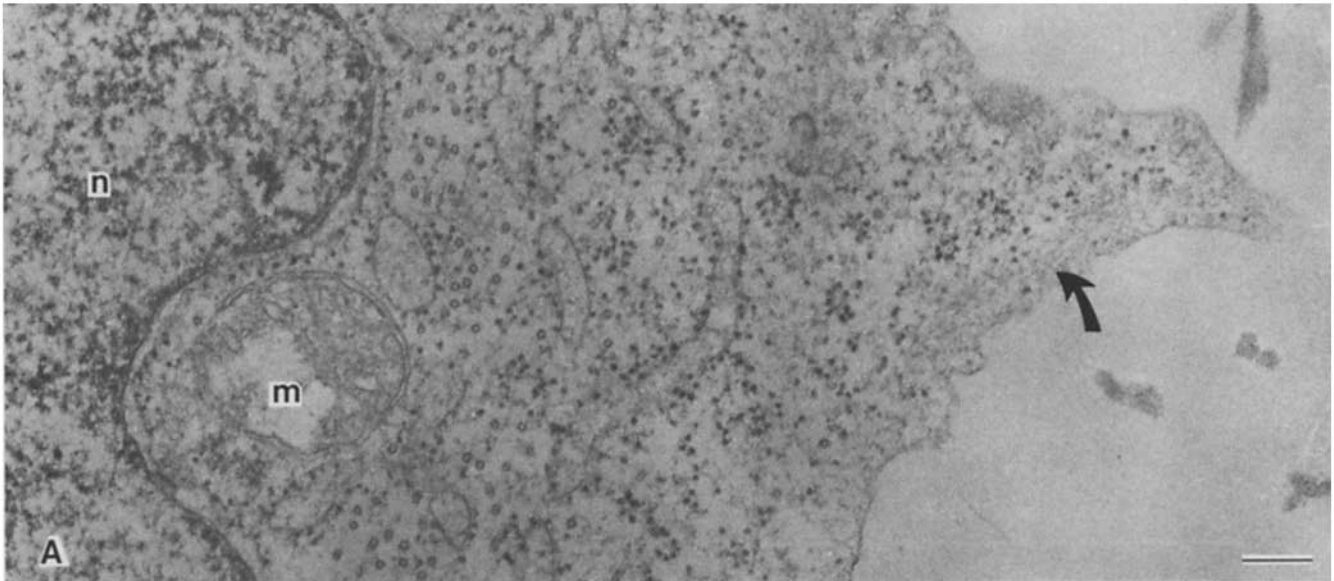


FIGURE 5 Corneal fibroblasts were treated with $1\ \mu\text{m}$ taxol at the time they were placed within collagen lattices, cultured for 18 h, and then prepared for electron microscopy. (A) A large number of microtubules cut in cross section is present near the nucleus (*n*) and throughout the cytosol of this cell. Microfilaments are present in the cell cortex (curved arrow). *m*, mitochondrion. (B) A large bundle of obliquely sectioned microtubules (*mt*) is present near the nucleus (*n*) of this cell. Bars, 200 nm. (A) $\times 45,000$; (B) $\times 75,000$.

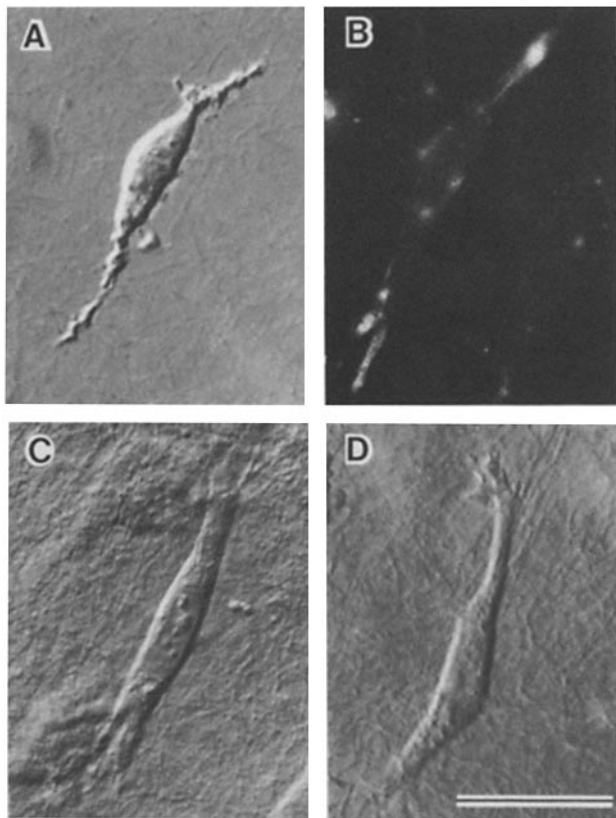


FIGURE 6 Corneal fibroblasts were cultured in collagen gels for 6 h and then treated with cytochalasin D (A and B), nocodazole (C), or taxol (D). The cell shown in A, photographed 12 h later with Nomarski optics, is still bipolar in shape, as at the beginning of treatment, but cell processes have an irregular appearance presumably due to actin clumping. The clumping of actin that occurs after cytochalasin D treatment is shown in B by immunofluorescence, using antiactin to stain a cell fixed 30 min after the beginning of treatment. The fibroblasts that were allowed to become bipolar before nocodazole (C) or taxol (D) treatment are similar in appearance to untreated controls after 6 h in culture. During the subsequent 12 h, they were unable to elongate, but remained bipolar. Bar, 20 μm . $\times 1,000$.

and pseudopodial extension have occurred; and 18 h, after the cell has become highly elongate in the collagen gel. Although aberrant filopodia are extended at time 1 h, disruption of the actin cortex by cytochalasin arrests the cell in the general configuration that it had assumed at the time of treatment (column A, left to right). Microtubules are not required for the fibroblast to become bipolar (column B, left). After the cell has become elongated, treatment with nocodazole or taxol causes it to shorten in the presence of intact actin to the bipolar stage (columns B and C, right). It was surprising, considering the role in cell elongation usually attributed to microtubules alone, to find that both the actin cortex and an intact CMTC are required for cell elongation, and that the actin cytoskeleton without the CMTC is sufficient for filopodial restriction and initial pseudopodial extension leading to the bipolar stage.

DISCUSSION

We have previously demonstrated that the shape and cytoskeleton of corneal fibroblasts respond dramatically to a surrounding extracellular matrix (54). We report here that cor-

neal fibroblasts cultured in collagen gels assume their characteristic bipolar, highly elongate shape by the following sequential steps: (a) freshly isolated spherical cells placed within a polymerizing gel project filopodia from the entire cell surface into the surrounding ECM; (b) filopodia become restricted to opposite poles of the cell; (c) short pseudopodia extend from the regions of restricted filopodia, giving the fibroblast a bipolar shape; (d) the pseudopodia lengthen greatly and the cell becomes highly elongate.

The role of the cytoskeleton in the acquisition of this characteristic morphology was examined with the use of cytoskeletal disrupting drugs. Cytochalasin D does not inhibit the initial projection of filopodia. It does inhibit both filopodial restriction and the extension and elongation of pseudopodia. In contrast, nocodazole and taxol do not affect filopodial restriction or the initial pseudopodial extension, but do inhibit subsequent elongation. Moreover, these microtubule-disrupting drugs cause highly elongated fibroblasts to retract to step c, the short bipolar form. Cells treated with nocodazole following cytochalasin D are unable to retract, suggesting that cell shortening after microtubule disruption requires microfilament function.

Filopodial Projection

Filopodia rapidly project from freshly isolated fibroblasts placed on a planar substratum (6, 37, 40) and ruffled borders form that may represent anomalous filopodia (54). We report here that filopodia project quickly from the surface of corneal fibroblasts cultured in a hydrated collagen lattice, but do not form on fibroblasts suspended in methocellulose. These long, thin processes form around the entire surface of spherical cells cultured in ECM, whereas on a planar substratum the filopodia and ruffled borders tend to arise from the cell perimeter nearest the underlying substratum (3, 41, 62). Presumably, these cell processes are stimulated to form by contact with either a surrounding or underlying substratum and are the means by which the cell explores its substratum (2, 3).

Cytochalasin and microtubule-disrupting drugs do not inhibit projection of filopodia by fibroblasts cultured in collagen gels (Fig. 8) or on planar substrata (2, 3, 6). The filopodia that form on fibroblasts cultured in ECM in the presence of cytochalasin D, however, are not normal. They are thick and floppy, resembling the flaccid, filament-poor microvilli projected after fertilization by sea urchin eggs in the presence of cytochalasin (7, 9). Albrecht-Buehler (3) reported that filopodia forming on 3T3 cells in the presence of cytochalasin contain 100-Å filaments, but we found they contain variable amounts of different cell organelles, including intermediate filaments, actin aggregates, microtubules, and ribosomes.

That filopodial protrusion is not affected by drugs disrupting microtubules is not unexpected since microtubules are not a normal component of these structures (3, 33, 54, 59). Filopodia, however, are composed of a meshwork of actin microfilaments (33, 54, 59) and so it is surprising that their formation is not more sensitive to cytochalasin. If cytochalasin is administered to bipolar cells, however, the already formed filopodia do become irregular in shape and exhibit actin clumping. Filopodia contain no detectable myosin (54), suggesting that the mechanism of force production for filopodial protrusion by cells is not an actin-myosin interaction in the usual sense. The orientation of microfilaments in filopodia is unknown, but their ultrastructure more closely

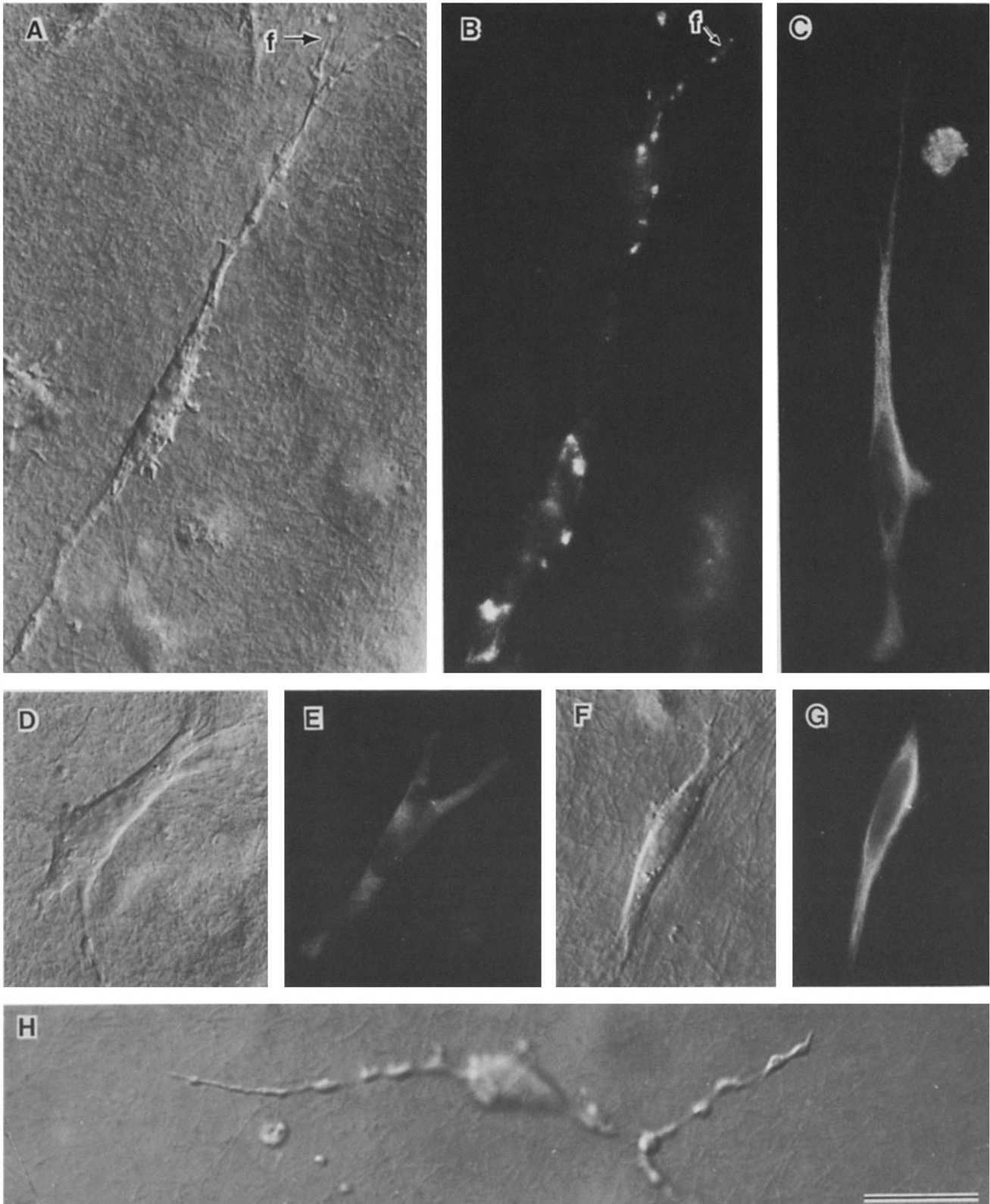


FIGURE 7 Corneal fibroblasts were allowed to elongate in collagen gels for 18 h and then treated for 3 h with cytochalasin D (A–C), nocodazole (D and E), taxol (F and G), or a combination of cytochalasin D and nocodazole (H). A, D, F, and H were photographed with Nomarski optics. For immunofluorescence, cells were stained with antiactin (B), or with antitubulin (C, E, and G). Cytochalasin D treatment clumps intracellular actin (B), resulting in a knotted-appearing cell exterior (A), and irregular filopodia (f). However, the cell remains elongate, presumably due to its complement of microtubules (C). Drugs that depolymerize (E) or clump (C) microtubules cause the cells to shorten to the bipolar configuration (D and F), provided that microfilaments are still present. Pretreatment with cytochalasin D before adding nocodazole to 18-h cultures, however, prevents retraction to the bipolar stage and causes the microtubule-poor cell processes to become very clumped in appearance (H). This cell (H) was photographed 3 h after adding nocodazole to the cytochalasin-treated fibroblast. Bar, 20 μm . $\times 1,000$.

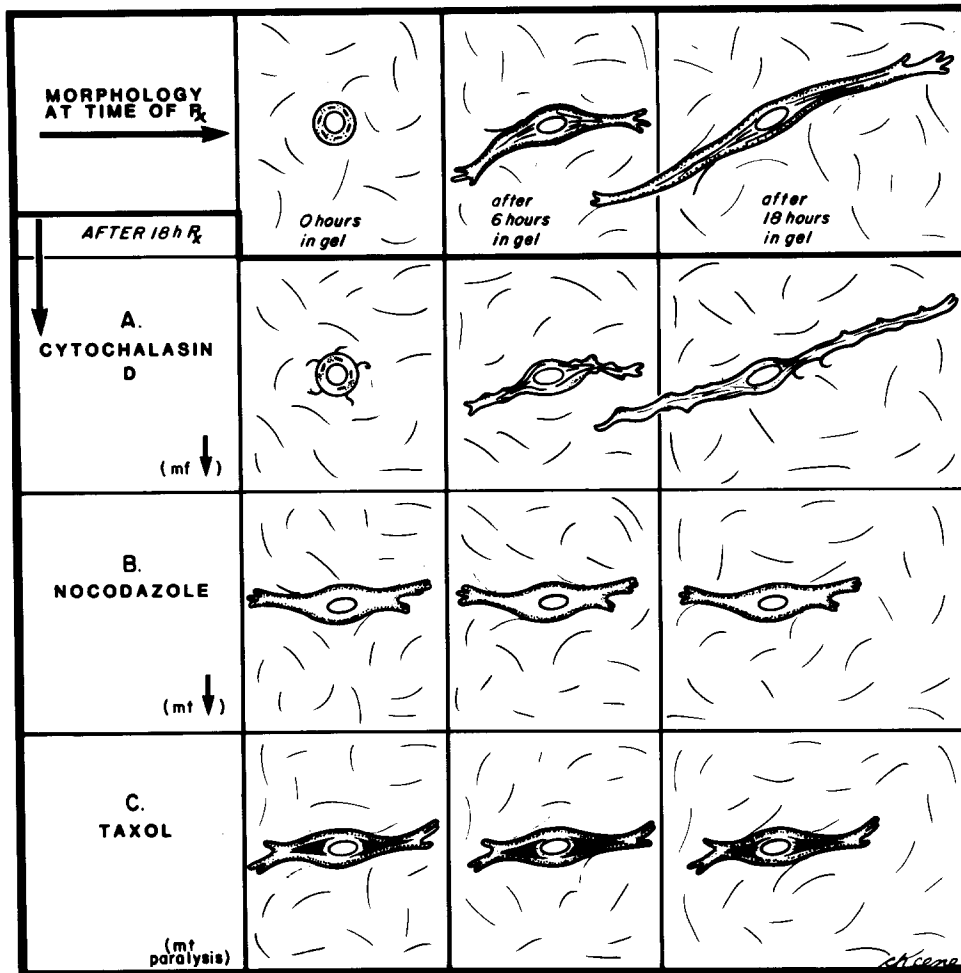


FIGURE 8 Diagram summarizing the effect of cytoskeletal disrupting drugs on acquisition of bipolarity and elongation of fibroblasts cultured in hydrated collagen lattices. See text for further description.

resembles actin meshworks in motile blebs on basal epithelial surfaces (52) than the actin columns that occur in microvilli (53). These observations call attention to our lack of understanding of both the mode of organization of actin in filopodia and the mechanism of force generation in protrusion of these cell processes.

Restriction of Filopodia to Opposite Ends of the Cell

The next event we observed in the assumption of the bipolar shape by the corneal fibroblast is restriction of filopodia to opposite poles of the cell. Cytochalasin D inhibits this process. It is easy to believe that restriction of filopodia involves the microfilamentous actin-rich cortex, for other movements of the cell surface, such as capping, are cytoskeletal dependent (1, 14, 17). "Capping" of filopodia, however, is not dependent on microtubules, as judged by the inability of nocodazole and taxol to prevent it.

The actual mechanism by which actin helps to mediate the restriction of filopodia to the opposite poles of the fibroblast is unclear. It may involve an interaction of the actin-rich filopodia and cell cortex across the plasmalemma with the adjacent ECM. A contact guidance phenomenon has been postulated for the alignment of fibroblasts along extracellular fibrils in ECM containing fibrin, collagen, or fibronectin (25, 35, 58). It is difficult, however, to attribute the elongation of the cells entirely to the orientation of the fibrils, because fibroblasts can by themselves orient ECM fibrils (31). These

cells are bipolar in shape in the original corneal stroma, and it is possible that this anisotropic organization is remembered during the isolation of the cells (4, 49, 50). Certain epithelial cells, however, that are not normally elongate in shape can become bipolar when suspended in collagen gels (28). Resolution of this question is fundamental to our understanding of the assumption of the bipolar state because the site of pseudopodial formation is directly related to the restricted location of the filopodia.

Not all cells follow exactly the same pattern as does the typical corneal fibroblast (Fig. 9). We observed that gerbil fibroma fibroblasts restrict their filopodia to more than two regions of the cell surface, extend pseudopodia in these areas, and later retract one or more of the pseudopodia. Human foreskin fibroblasts show a similar behavior (23). The reason for this difference in the number of pseudopodia from cell to cell is not clear, but the close relation of pseudopodial and lamellipodial extensions to filopodial projections, on the one hand, and to ruffled borders, on the other, seems to be a general phenomenon.

Assumption of Bipolar Shape

After translocation of filopodia to opposite ends of the cell, the corneal fibroblast suspended within ECM forms pseudopodia at the two poles to which the filopodia are now restricted (Fig. 9). Extension of pseudopodia is a highly cytochalasin-sensitive process and undoubtedly requires the presence of intact microfilaments in the pseudopodial cell cortex. Inas-

CELL SHAPE IN COLLAGEN LATTICES

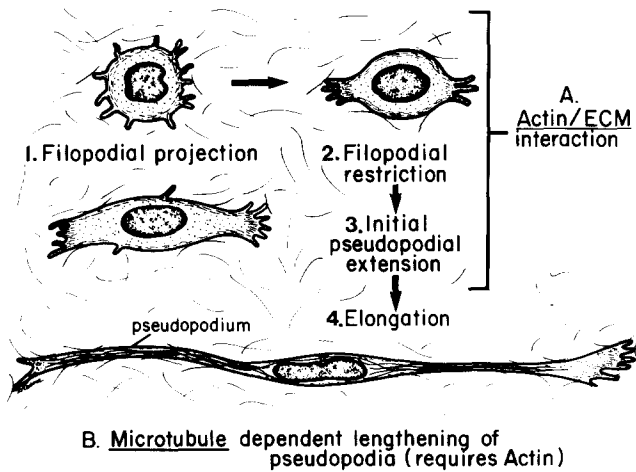


FIGURE 9 Diagram summarizing the steps in acquisition of bipolarity and elongation of fibroblasts cultured in hydrated collagen lattices. The first steps require an intact actin cytoskeleton that is presumably interacting with the ECM via putative intramembrane proteins. The final step of cell elongation requires an assembled actin cortex in contact with collagen and an intact CMTC that seemingly inserts into the cortex.

much as the adjacent cytosol, or so-called cytomatrix (38), is rich in myosin (54), the flowing of cytoplasm into pseudopodia may involve sliding of the microfilament-rich cell cortex along the myosin-rich cytomatrix in a manner similar to that proposed for force production in cells migrating on flat substrata (60, 61), perhaps involving actin binding proteins (18).

The extension of pseudopodia from the poles of the cell into the surrounding collagen matrix is not sensitive to either nocodazole or taxol. Similarly, antimetabolic drugs do not inhibit the extension of lamellipodia on a planar substratum (44, 56). Rather than being involved in the initial extension of cell processes, microtubules seem to play a role in maintaining an asymmetric cell shape in the case of fibroblasts grown on planar substrata. These cells do not form a leading edge in the presence of colchicine (56); membrane ruffling occurs around the periphery of the cell and directed movement of the cell as a whole is inhibited (57). Whether or not the bipolar cell in ECM that has been treated with microtubule-disrupting agents is capable of so-called directional movement, however, is not yet clear.

MTOCs seem to be preferentially located toward the front end of some migrating cells, suggesting their involvement in directional locomotion of cells on planar substrata (5, 27, 36). In fibroblasts grown in collagen lattices, however, we find that the MTOC is generally located lateral to the nucleus. Yet the elongating fibroblast in ECM does show "direction," in the sense that one pseudopodium becomes the leading edge, whereas the other trails and retracts from time to time to permit forward movement (8, 20, 55). Further study of the role of the MTOC and CMTC in fibroblast migration is needed.

Thus, the extension of pseudopodia from opposite poles of the cell to produce the bipolar fibroblast is a cytochalasin-sensitive process requiring intact microfilaments. Both filopodial "capping" and pseudopodial extension seem to involve an interaction between microfilaments, the cell surface, and the surrounding ECM. Neither the initial restriction of filo-

podia nor extension of pseudopodia at the filopodia-rich poles of the cell seems to require microtubules, but we have not ruled out a role for microtubules in establishing the leading end of the fibroblast when it begins to migrate in the collagen lattice.

Fibroblast Elongation

The elongation of pseudopodia by corneal fibroblasts in ECM, in contrast, is dependent upon both the microfilament-rich cortex and the cell's CMTC. Microtubules that originate from the MTOC become oriented along the long axis of the cell during the assumption of the bipolar shape; these microtubules lengthen greatly during the fibroblast elongation phase (Fig. 9, stage 4). We found that depolymerization of microtubules with nocodazole inhibits the ability of the cell to become highly elongate. Taxol also inhibits elongation of the bipolar cell, although it has a very different effect upon the CMTC, inducing the assembly of very stable bundles of microtubules (21, 22, 44) that seem to be free in the cytoplasm (21). Clearly the bundles of microtubules present in taxol-treated cells are unable to function normally in cell elongation. The CMTC also appears to play an important role in maintenance of the elongated shape. Nocodazole and taxol administered to already highly elongated cells induce retraction of pseudopodia to the shorter length characteristic of the original bipolar cell and this retraction is sensitive to cytochalasin D, suggesting that microfilament integrity is required. Similarly, the nocodazole induced retraction of axonal processes is cytochalasin sensitive (51).

It was of some interest to find that elongation of pseudopodia appears to involve more than just the microtubules, being sensitive to both cytochalasin D and to drugs affecting microtubules. Microtubules have been observed by electron microscopy to be closely associated with the microfilament-rich cell cortex in corneal fibroblasts cultured in collagen gels (54). This morphological association of the two cytoskeletal systems and the requirement for both elements during elongation, as judged by drug treatment, suggests that the two interact during fibroblast elongation. Interactions between microtubules and the cell cortex seem to control the location of the contractile ring of microfilaments during the cell cleavage (39). There is reason, moreover, to believe that microtubule-associated proteins could mediate microfilament-microtubule interaction (29, 43). Thus, it is tempting to speculate that interaction between microtubules and the microfilament-rich cortex of mesenchymal cells occurs during and after pseudopodial lengthening. It will be interesting in the future to explore the possible interactions of MTOC, CMTC, and actin during fibroblast elongation and migration within gels of extracellular matrix.

We are indebted to Dr. Keigi Fujiwara and Dr. L. B. Chen (Harvard Medical School) for the tubulin and actin antibodies and the fibroma cells, described in Materials and Methods, to Ms. Veronica Lee for technical assistance, to Ms. Susan Hunt for preparing the manuscript on a Wang word processor, and to Dr. John Douros (National Institutes of Health) for providing taxol.

This research was supported by grant HD-00143 from the United States Public Health Service. Dr. Tomasek was a National Institutes of Health postdoctoral fellow, 5 F32 EY05495, during the tenure of this work.

Received for publication 16 September 1983, and in revised form 27 March 1984.

REFERENCES

1. Albertini, D. F., and E. Anderson. 1977. Microtubule and microfilament rearrangements during capping of concanavalin A receptors on cultured ovarian granulosa cells. *J. Cell Biol.* 73:111-127.
2. Albrecht-Buehler, G. 1976. Filopodia of spreading 3T3 cells. Do they have a substrate-exploring function? *J. Cell Biol.* 69:275-286.
3. Albrecht-Buehler, G. 1976. The function of filopodia in spreading 3T3 mouse fibroblasts. In *Cell Motility*. R. D. Goldman, T. Pollard, and J. Rosenbaum, editors. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 247-264.
4. Albrecht-Buehler, G. 1977. Daughter 3T3 cells. Are they mirror images of each other? *J. Cell Biol.* 72:595-603.
5. Albrecht-Buehler, G., and A. Bushnell. 1979. The orientation of centrioles in migrating 3T3 cells. *Exp. Cell Res.* 120:111-118.
6. Albrecht-Buehler, G., and R. D. Goldman. 1976. Microspike-mediated particle transport towards the cell body during early spreading of 3T3 cells. *Exp. Cell Res.* 97:329-339.
7. Banzhaf, W. C., R. H. Warren, and D. R. McClay. 1980. Cortical reorganization following fertilization of sea urchin eggs sensitive to cytochalasin B. *Dev. Biol.* 80:506-515.
8. Bard, J. B. L., and E. Hay. 1975. The behavior of fibroblasts from the developing avian cornea: morphology and movement in situ and in vitro. *J. Cell Biol.* 67:400-418.
9. Begg, D. A., and H. A. Hyatt. 1982. Changes in actin organization associated with microvillar growth in the sea urchin egg. *J. Cell Biol.* 95:392a. (Abstr.)
10. Bell, E., B. Ivanson, and C. Merrill. 1979. Production of a tissue-like structure by contraction of collagen lattices by human fibroblasts of different proliferative potential in vitro. *Proc. Natl. Acad. Sci. USA.* 76:2268-2272.
11. Bellows, C. G., A. H. Melcher, and J. E. Aubin. 1981. Contraction and organization of collagen gels by cells cultured from periodontal ligament, gingiva, and bone suggest functional differences between cell types. *J. Cell Sci.* 50:299-314.
12. Bellows, C. G., A. H. Melcher, U. Bhargava, and J. E. Aubin. 1982. Fibroblasts contracting three-dimensional collagen gels exhibit ultrastructure consistent with either contraction or protein secretion. *J. Ultrastruct. Res.* 78:178-192.
13. Bermanke, D. H., and R. R. Markwald. 1982. Migratory behavior of cardiac cushion tissue cells in a collagen lattice culture system. *Dev. Biol.* 91:235-245.
14. Bourguignon, L. W., and S. J. Singer. 1977. Transmembrane interactions and the mechanism of capping of surface receptors by their specific ligands. *Proc. Natl. Acad. Sci. USA.* 74:5031-5035.
15. Byers, H. R., K. Fujiwara, and K. Porter. 1980. Visualization of microtubules of cells in situ by indirect immunofluorescence. *Proc. Natl. Acad. Sci. USA.* 77:6657-6661.
16. Byers, H. R., G. E. White, and K. Fujiwara. 1983. Organization and function of stress fibers in vitro and in situ: A review. In *Cell and Muscle Motility*. J. W. Shaw, editor. Vol. 5. Plenum Press, New York. 83-132.
17. Condeelis, J. 1979. Isolation of concanavalin A caps during various stages of formation and their association with actin and myosin. *J. Cell Biol.* 80:751-758.
18. Condeelis, J. 1983. Rheological properties of cytoplasm: significance for the organization of spatial information. In *Modern Cell Biology*, Vol. 2. J. R. McIntosh, editor. Alan R. Liss, Inc., New York. 225-240.
19. Conrad, G. W., G. Hart, and Y. Cehn. 1977. Differences in vitro between fibroblast-like cells from cornea, heart, and skin of embryonic chicks. *J. Cell Sci.* 26:119-137.
20. Davis, E. M. 1980. Translocation of neural crest cells within a hydrated collagen lattice. *J. Embryol. Exp. Morphol.* 55:17-31.
21. DeBrabander, M., G. Geuens, R. Nuydens, R. Willebrords, and J. DeMey. 1981. Taxol induces the assembly of free microtubules in living cells and blocks the organizing capacity of centrosomes and kinetochores. *Proc. Natl. Acad. Sci. USA.* 78:5608-5612.
22. DeBrabander, M., G. Geuens, R. Nuydens, R. Willebrords, and J. DeMey. 1981. Microtubule assembly in living cells after release from nocodazole block: the effects of metabolic inhibitors, taxol and pH. *Cell Biol. Int. Rep.* 5:913-920.
23. Dodd, N. J. F., S. L. Schor, and G. Rushton. 1982. The effects of a collagenous extracellular matrix on fibroblast membrane organization: an ESR spin label study. *Exp. Cell Res.* 141:421-431.
24. Dvorak, J. A., and W. F. Stotler. 1971. A controlled-environmental culture system for high resolution light microscopy. *Exp. Cell Res.* 68:144-148.
25. Elsdale, T. R., and J. B. L. Bard. 1972. Collagen substrata for studies on cell behavior. *J. Cell Biol.* 54:626-637.
26. Fujiwara, K., and T. D. Pollard. 1976. Simultaneous localization of myosin and tubulin in human tissue culture cells by double antibody staining. *J. Cell Biol.* 77:182-195.
27. Gotlieb, A. I., L. McBurnie May, L. Subrahmanyam, and U. I. Kalnins. 1981. Distribution of microtubule-organizing centers in migrating sheets of endothelial cells. *J. Cell Biol.* 91:589-594.
28. Greenburg, G., and E. D. Hay. 1982. Epithelia suspended in collagen gels can lose polarity and express characteristics of migrating mesenchymal cells. *J. Cell Biol.* 95:333-339.
29. Griffith, L. M., and T. D. Pollard. 1978. Evidence for actin filament-microtubule interaction mediated by microtubule-associated proteins. *J. Cell Biol.* 78:958-965.
30. Grinnell, F., and M. H. Bennett. 1981. Fibroblast adhesion on collagen substrata in the presence and absence of fibronectin. *J. Cell Sci.* 48:19-34.
31. Harris, A. K., D. Stopack, and P. Wild. 1981. Fibroblast traction as a mechanism for collagen morphogenesis. *Nature (Lond.)* 290:249-251.
32. Hay, E. D. 1981. *Cell Biology of Extracellular Matrix*. Plenum Press, New York. 417 pp.
33. Hay, E. D., and J. P. Revel. 1969. *Fine Structure of the Developing Cornea*. S. Karger, Basel. 144 pp.
34. Herman, B., M. A. Langevin, and D. F. Albertini. 1983. The effects of taxol on the organization of the cytoskeleton in cultured ovarian granulosa cells. *Eur. J. Cell Biol.* 31:34-45.
35. Hsieh, P., and L. B. Chen. 1983. Behavior of cells seeded in isolated fibronectin matrices. *J. Cell Biol.* 96:1208-1217.
36. Kupfer, A., D. Louvard, and S. J. Singer. 1982. Polarization of the Golgi apparatus and the microtubule-organizing center in cultured fibroblasts at an edge of an experimental wound. *Proc. Natl. Acad. Sci. USA.* 79:2603-2607.
37. Porter, K. R., A. Claude, and E. F. Fullam. 1945. A study of tissue culture cells by electron microscopy. *J. Exp. Med.* 81:233-246.
38. Porter, K. R., M. Beckerle, and M. McNiven. 1983. The cytoplasmic matrix. In *Modern Cell Biology*, Vol. 2. J. R. McIntosh, editor. Alan R. Liss, Inc., New York. 257-302.
39. Rappaport, R. 1975. Establishment and organization of the cleavage mechanism. In *Molecules and Cell Movement*. S. Inoue and R. E. Stephens, editors. Raven Press, New York. 287-303.
40. Revel, J. P., P. Hoch, and D. Ho. 1974. Adhesion of cultured cells to their substratum. *Exp. Cell Res.* 84:207-218.
41. Rovinsky, V. A., and I. L. Slavnyaya. 1974. Spreading of fibroblast-like cells on grooved surfaces: a study by scanning electron microscopy. *Exp. Cell Res.* 84:199-206.
42. Sanders, E. J., and S. Prasad. 1983. The culture of chick embryo mesoderm cells in hydrated collagen gels. *J. Exp. Zool.* 226:81-92.
43. Sattilaro, R. E., W. L. Dentler, and E. L. LeCluyse. 1981. Microtubule-associated proteins and the organization of actin filaments in vitro. *J. Cell Biol.* 90:467-473.
44. Schiff, P. B., and S. B. Horwitz. 1980. Taxol stabilizes microtubules in mouse fibroblast cells. *Proc. Natl. Acad. Sci. USA.* 77:1561-1565.
45. Schliwa, M. 1982. Action of cytochalasin D on cytoskeletal networks. *J. Cell Biol.* 92:79-91.
46. Schor, S. 1980. Cell proliferation and migration on collagen substrate in vitro. *J. Cell Sci.* 41:159-175.
47. Schor, S. L., A. M. Schor, and G. W. Bazill. 1981. The effects of fibronectin on the migration of human foreskin fibroblasts and syrian hamster melanoma cells into three-dimensional gels of native collagen fibres. *J. Cell Sci.* 48:301-314.
48. Schor, S. L., T. D. Allen, and B. Winn. 1983. Lymphocyte migration into three-dimensional collagen matrices: a quantitative study. *J. Cell Biol.* 96:1089-1096.
49. Solomon, F. 1979. Detailed neurite morphologies of sister neuroblastoma cells are related. *Cell* 16:165-169.
50. Solomon, F. 1980. Neuroblastoma cells recapitulate their detailed neurite morphologies after reversible microtubule disassembly. *Cell* 21:333-338.
51. Solomon, F., and M. Magendantz. 1981. Cytochalasin separates microtubule disassembly from loss of asymmetric morphology. *J. Cell Biol.* 89:157-161.
52. Sugrue, S. P., and E. D. Hay. 1981. Response of basal epithelial cell surface and cytoskeleton to solubilized extracellular matrix molecules. *J. Cell Biol.* 91:45-54.
53. Tinley, L. G. 1983. Interactions between actin filaments and membranes give spatial organization to cells. In *Modern Cell Biology*, Vol. 2. J. R. McIntosh, editor. Alan R. Liss, Inc., New York. 163-199.
54. Tomasek, J. J., E. D. Hay, and K. Fujiwara. 1982. Collagen modulates cell shape and cytoskeleton of embryonic corneal and fibroblast fibroblasts: Distribution of actin, α actinin, and myosin. *Develop. Biol.* 92:107-122.
55. Trinkaus, J. P. 1976. On the mechanism of metazoan cell movements. In *The Cell Surface in Animal Embryogenesis and Development* (G. Poste and G. L. Nicolson, eds.) Elsevier North-Holland Publishing Co., Amsterdam. 225-329.
56. Vasiliev, J. M., and I. M. Gelfand. 1976. Effects of colcemid on morphogenetic processes and locomotion of fibroblasts. In *Cell Motility*. R. D. Goldman, T. Pollard, and J. Rosenbaum, editors. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 247-264.
57. Vasiliev, J. M., I. M. Gelfand, L. V. Domnina, O. Y. Ivanova, S. G. Komm, and L. V. Olshevskaja. 1970. Effect of the colcemid on the locomotory behavior of fibroblasts. *J. Embryol. Exp. Morphol.* 24:625-640.
58. Weiss, P., and Garber, B. 1952. Shape and movement of mesenchymal cells as functions of the physical structure of the medium: contributions to a quantitative morphology. *Proc. Natl. Acad. Sci. USA.* 38:264-280.
59. Wessels, N. K., B. S. Spooner, and M. A. Luduena. 1973. Surface movements, microfilaments and cell locomotion. In *Locomotion of Tissue Cells*. R. Porter and D. W. Fitzsimmons, editors. Elsevier North-Holland, Amsterdam. 53-77.
60. Willingham, M. C., S. S. Yamada, P. J. A. Davies, A. V. Rutherford, M. G. Gallo, and I. Pastan. 1981. Intracellular localization of actin in cultured fibroblasts by electron microscopic immunocytochemistry. *J. Histochem. Cytochem.* 29:17-37.
61. Willingham, M. C., S. S. Yamada, P. J. Bechtel, A. V. Rutherford, and I. Pastan. 1981. Ultrastructural immunocytochemical localization of myosin in cultured fibroblastic cells. *J. Histochem. Cytochem.* 29:1289-1301.
62. Witkowski, J. A., and W. D. Brighton. 1971. Stages of spreading of human diploid cells on glass surfaces. *Exp. Cell Res.* 68:372-380.