# Effects of Mechanical Tension on Protrusive Activity and Microfilament and Intermediate Filament Organization in an Epidermal Epithelium Moving in Culture

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*Abstract.* Mechanical tension influences tissue morphogenesis and the synthetic, mitotic, and motile behavior of cells. To determine the effects of tension on epithelial motility and cytoskeletal organization, small, motile clusters of epidermal cells were artificially extended with a micromanipulated needle. Protrusive activity perpendicular to the axis of tension was dramatically suppressed. To determine the ultrastructural basis for this phenomenon, cells whose exact locomotive behavior was recorded cinemicrographically were examined by transmission electron microscopy. In untensed, forward-moving lamellar protrusions, microfilaments appear disorganized and anisotropically oriented. But in cytoplasm held under tension by micromanipulation or by the locomotive activity of other cells within the epithelium, microfilaments are aligned parallel to the tension. In nonspreading regions of the epithelial margin, microfilaments lie in tight bundles parallel to apparent lines of tension. Thus, it appears that tension causes alignment of microfilaments. In contrast, intermediate filaments are excluded from motile protrusions, being confined to the thicker, more central part of the cell. They roughly follow the contours of the cell, but are not aligned relative to tension even when microfilaments in the same cell are. This suggests that the organization of intermediate filaments is relatively resistant to physical distortion and the intermediate filaments may act as passive structural support within the cell. The alignment of microfilaments under tension suggests a mechanism by which tension suppresses protrusive activity: microfilaments aligned by forces exerted through filament–surface or filament–filament interconnections cannot reorient against such force and so cannot easily extend protrusions in directions not parallel to tension.

By definition, the essential feature of multicellular organisms is the long-term contacts that exist between cells. These contacts provide channels of chemical and electrical communication and serve as physical connections through which mechanical stresses are transmitted from cell to cell. Since a number of observations suggest that cells can respond to purely physical signals, the possibility exists that such mechanical linkages also function in intercellular communication. For example, the development of a callous in the skin is a hyperproliferative response to pressure. Direct responses to purely physical signals have also been observed at the cellular level. Fibroblasts subjected to oscillatory tension have a higher rate of mitosis than unstressed cells (14), chondrocytes exhibit an increase in proteoglycan synthesis in response to mechanical stress (15), and cultured myotubes are stimulated to synthesize myosin by stretching (47). The observation of Folkman and Moscona (19) that highly flattened cells divide more frequently than rounded cells might also be an effect of tension, since highly flattened cells are subject to the mechanical pull exerted by their spreading margins. Spreading is itself affected by tension as demonstrated by Takeuchi (46), who showed that explants of corneal epithelium display an increased degree of spreading when stretched.

The last observation suggests that physical tension transmitted through cell–cell attachments can affect motile behavior. This may play an important role in the extensive cellular translocations of embryogenesis, during which dramatic reorganization of tissues creates many distinct tensile fields (4). Odell et al. (37) have proposed that stretching of tissues during embryogenesis could trigger cell contractions that, in turn, would produce the foldings and pocketings of embryonic epithelia. Their computer models of a number of morphogenetic movements illustrate how these shape changes could be brought about in a simple, direct manner purely by the cell-to-cell propagation of tensile signals. Harris et al. (24) have shown that mesenchymal cells can create large tensile fields by virtue of the tractional forces they exert on the extracellular matrix and that, in tissue culture, these forces can create highly polarized structures (45). These forces may be responsible for such morphogenetic movements as the wrapping of anatomical structures with collagen (e.g., organ capsules, tunica medea, periostea, etc.) and also the alignment of polarized

tissues such as ligaments, tendons, and muscles (38). Regarding the latter, Beloussov (3) has shown that organ primordia transplanted into different tensile fields frequently undergo axial reorientation so as to align relative to the new direction of tension. Thus, tension is probably of general importance in the morphogenesis of polarized structures.

The underlying causes of these phenomena are unknown. Indeed, very little is known about how physical forces exerted on and by a cell effect cell movement. Inasmuch as the ultimate basis of cell movement is the generation of force and its transmission to the environment, detailed analysis of this relationship is needed, if only as part of the general effort to understand the mechanism of tissue cell movement. As a model system in which to study the effects of physical interconnections on cell behavior, I have adopted the epithelial clusters observed in cultures of fish epidermis (30). These cells display both a very high level of locomotive activity and stable, intercellular adhesions: like the epidermal sheets cultured from *Xenopus* tadpoles (5, 40), they move extremely rapidly, extending from their margins broad lamellar protrusion which serve as the sheet's sole adhesions to the substratum (30). And like all epidermal keratinocytes, they are repleat with tonofilaments and desmosomes (unpublished observations) presumably providing adhesion between cells. In a previous investigation, it was proposed that the highly directional movement of these epidermal clusters might be due to the peculiar tensions that arise when cells that are attached to one another have the opportunity to move in opposing directions (30). In the present study, this possibility is probed directly by using a micromanipulated tungsten needle to apply tension in a controlled manner. It is shown that mechanical stress can indeed have profound effects on the polarity of a cell's locomotive activity. Examining manipulated and unmanipulated cells in the electron microscope after cinemicrography of the living cells before and during fixation, so that the exact movement of the regions under examination is known, demonstrates that tension within the cell is also correlated with changes in the organization of filamentous elements of the cytoskeleton. These observations suggest specific roles for microfilaments and intermediate filaments in a cell's response to physical stress.

# Materials and Methods

## Cells

Cells for these studies were obtained from the skin of the poecelid fish *Xiphophorus maculatus* (platyfish). The fish were anaesthetized in a solution of ethylaminobenzoate (1:3,000 in filtered well water), and single scales were plucked from the fish with forceps. Up to 16 scales could be removed at a time without visible impairment of the health of the fish. The scales were immediately transferred to Dulbecco's modified Eagle's medium, pH 7.4, containing 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml Fungizone, rinsed in three changes of this medium to reduce contamination, and then placed in the inverted lid of a Permanox plastic culture dish (Lux Scientific, Palo Alto, CA) and surrounded by small drops of culture medium (Leibovitz's L-15 medium, pH 7.4, supplemented with 10% horse serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml Fungizone). The bottom of the dish was then placed inside the lid, pinning the explant to the lid and flattening the drops of medium so that they covered the explant. The cultures were maintained in a water-saturated atmosphere at room temperature (22°C). After a 3–6-h culture, the bottom of the dish could be very gently lifted off the explant without disturbing the epidermis that had spread onto the lid. The medium was changed daily thereafter, and the cells were examined either through an inverted microscope or a standard microscope equipped with water immersion objectives. If cells spread onto the bottom of the dish, rather than on the lid, they could be viewed in the microscope after turning the dish over and cutting off its sides with scissors or a razor blade. The epidermis that spreads from the explant gradually breaks up into smaller, translocating groups of cells, or clusters, by a process already described (30). It is these epithelial clusters whose movements and morphology were examined in this study.

## Micromanipulation

Micromanipulations were performed on cells in culture using a fine tungsten needle (tip diameter = ~5 μm) mounted on a Leitz micromanipulator, which has a "joy stick" mechanism converting coarse hand movements directly into fine displacements of the tip of the needle at a ratio of 800:1. The needle was mounted in the micromanipulator nearly parallel to the chamber holding the cells, i.e., so that the needle made an angle of <5° with the substratum on which the cells were moving. Using the micromanipulator's horizontal coarse adjustments, the needle was positioned directly above the cell to be pulled. Then the side of the needle was pressed against the upper surface of the cell by lowering the needle with the vertical fine adjustment until the cell or cells directly beneath the needle began to be squashed as indicated by the first distortion of the nucleus and displacement of intracellular organelles. Further pressure can cause the cell beneath the needle to rupture, whereupon the cell's locomotive activity ceases immediately and completely. Rupture also results in the cell tearing when pulled instead of effectively transmitting force to adjacent cells. However, with practice, excessive pressure is easily avoided and the cell(s) beneath the needle can then be forcibly moved about by moving the needle sideways, i.e., so that the side of the needle rather than its tip pushes against the cell. This stage of the manipulation was performed with the "joy stick." It is generally easiest to push a cell by placing the needle alongside the nucleus and pushing against the thick, central part of the cell. Because these clusters adhere to the substratum almost exclusively at the underside of the lamellar protrusions at the periphery of the cluster, cells away from the cluster's margin can easily be pushed about with the microneedle, and, through the attachments of these cells to the rest of the epithelium, it is possible to pull on other cells in the sheet without direct intrusion of the needle. Cell–cell and cell–substratum adhesions are generally quite strong in this system, and it was possible to perform the desired manipulation well over 50% of the time without tearing the cells or pulling them off the substratum.

## Cinemicrography

Time-lapse films were made with either an Arriflex 16-mm motion picture camera and intervalometer (Arriflex Corporation of America, New York) or a Bolex H16 movie camera driven by a Sage Series 500 Cinemicrographic Apparatus (Arenberg Ultrasonic Inc., Jamaica Plains, MA). When filming cells during micromanipulation or prior to preparation for electron microscopy, the interval between successive frames was 0.5 s or less. The film (Kodak TP2415 negative or Kodak Plus-X reversal, type 7276) was processed commercially and projected for analysis with a Vanguard Motion Analyzer (Vanguard Instrument Co., Melville, NY).

## Electron Microscopy

For transmission electron microscopy, the location of the cells to be examined was marked by scratching a circle around the cells in the plastic dish. Cells were fixed by drawing off as much medium as possible without uncovering the cells, then flushing the dish with 0.1 M phosphate buffer, pH 7.3, containing 3% gluteraldehyde, 0.2% tannic acid, and 0.1 M sucrose. The movement of the cells was always recorded cinemicrographically as they were being fixed so that their exact locomotive behavior was known. After 1–2 h in fixative at room temperature, the cells were rinsed with three changes of the fixation buffer, postfixed for 10 min on ice in 0.5% osmium tetroxide in 0.1 M phosphate buffer, pH 6.0 (36), and rinsed 4–5 times with distilled water. The cells were then stained en bloc with 1% aqueous uranyl acetate for 30–60 min at room temperature, rinsed three times with distilled water, and embedded in a thin layer of Epon 812 or Polybed 812 plastic. The entire operation was carried out in the Permanox dishes. When the hardened plastic with the embedded cells was peeled off the dish, the circle etched on the dish appeared in relief in the embedding plastic, permitting easy location of the original cells. The cells could also be discerned through phase optics to facilitate orientation and precise location for block trimming. When cells were to be sectioned in a plane parallel to the substratum, the plastic film was fastened to a larger Epon block with epoxy to make it easier to handle during trimming and sectioning. When cells were to be sectioned in a plane perpendicular to the substratum, the plastic film containing the cells was re-embedded in an additional portion of the original embedding medium so that, when the new block was trimmed and sectioned, the embedded cells did not lie on the very edge of the section,

where it is difficult to cut through the specimen cleanly and without distortion.

Serial thin sections were cut on a Sorval MT-2 ultramicrotome and picked up on formvar-coated slot grids. All sections were stained for 20 min in 2% uranyl actetate in 50% ethanol and 1.5 min in Reynold's lead citrate, and examined in a Phillips 300 transmission electron microscope at 60–80 kV.

## Results

### Motile Behavior under Tension

In primary cultures of fish epidermis, small clusters of cells can break off from the epithelium and translocate as separate, miniature motile epithelia (30). Mechanical tension was applied to specific cells within clusters by pulling on other cells within the epithelium with a microneedle (see Materials and Methods). Direct observation of over 100 such manipulations revealed the following characteristic behavior of cells under tension. When a cluster is pulled in a direction perpendicular to its free edge, i.e., so as to pull the cell backward with respect to the direction in which its lamellar margin could spread, it elongates in the direction of pull (Fig. 1). As the cell elongates, the cell body narrows and spreading from the more lateral edges of its lamellar protrusions is suppressed (Fig. 1, *2:00*). If tension on the cell is sufficiently great, these lateral edges will retract while the middle part of the protrusion remains spread and sometimes even continues to move forward (Fig. 1, *5:00*). If tension is maintained on the cell, protrusive activity becomes confined to the middle part of the leading margin in a direction parallel to the pull on the cell. There is little or no spreading from the cell's lateral margin even as the cell separates from its neighbors. If the cell is pulled backward, retraction fibers are formed and the cell moves slowly away from its old adhesion points, all the while remaining flat against the substratum. Jerking sharply on the cell or pulling smoothly but very rapidly causes sudden detachment of the cell from the substratum.

It was also found that spreading is suppressed when tension is exerted in a direction parallel to the cell's free edge, i.e., sideways relative to the direction in which spreading could occur (Fig. 2). When a microneedle is pressed against a cell on the margin of a cluster and pulled tangentially to the margin, the immediately adjacent cell is slightly elongated. This is accompanied by an immediate cessation of protrusive activity along the entire margin of the tensed cell. Increasing the tension, i.e., pulling the needle so as to further elongate the cell, eventually induces retraction of the cell's lamellar protrusion. Retraction of the protrusion permits more elongation of the cell, and the cell can be drawn out further by pulling with the microneedle as the protrusion retracts. If tension is maintained in this fashion, respreading of the cell

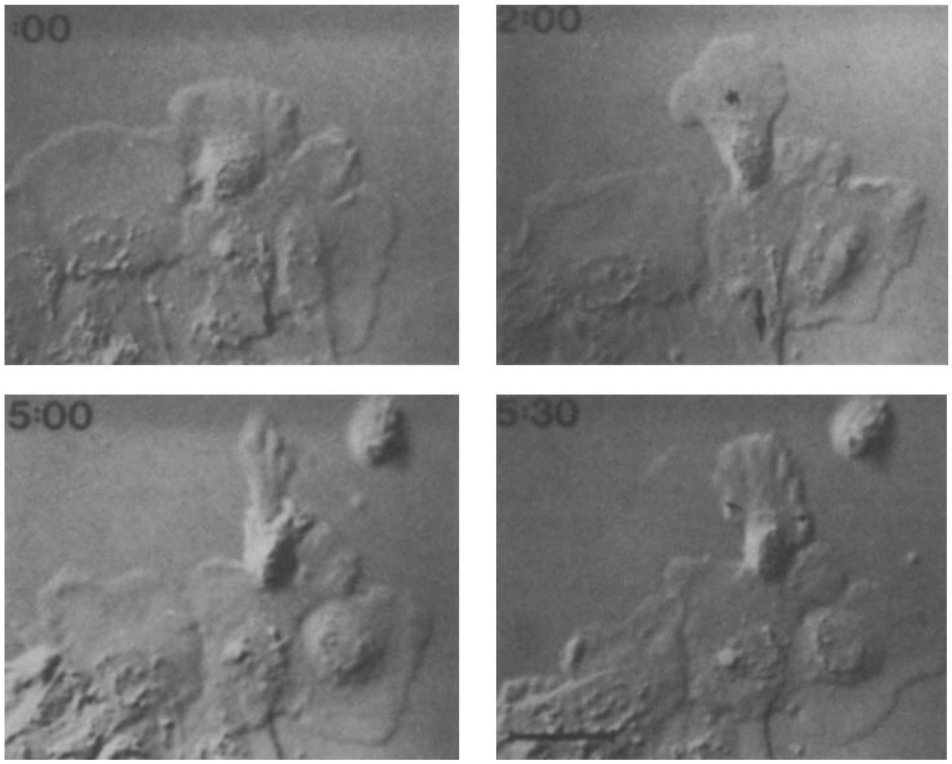



*Figure 1.* Cellular response to tension exerted perpendicular to the spreading edge. These micrographs are taken from a time-lapse movie using Nomarski-DIC optics of a cluster moving on a glass coverslip. Time is in min:sec. At :00, the edge of a cluster is shown spreading towards the top of the micrograph. After 1 min, a microneedle was placed against the sheet directly below the point of the arrow (just at the edge of the field of the micrograph) and then pulled in the direction of the arrow. At 2:00, the cell marked by the asterisk was still under tension from the pull of the needle and had been stretched by approximately one third. Note that the cells on either side of the marked cell are not directly linked with the cell being pulled and so are not being subjected to significant tension. The leading edge of the marked cell continued to advance towards the top of the micrograph, while spreading from its lateral margins decreased. At 5:00, the microneedle was removed, releasing the cell from tension. By this time, the cell had elongated by half again its original length and had noticeably narrowed. Its leading edge had moved still further towards the top of the picture, but lateral spreading is completely eliminated, i.e., no lamellar protrusions extended from the cell's long axis. At 5:30, 30 s after release of tension, large lamellae had begun to spread from the cell's lateral edges (arrows). Bar, 20 $\mu$m.

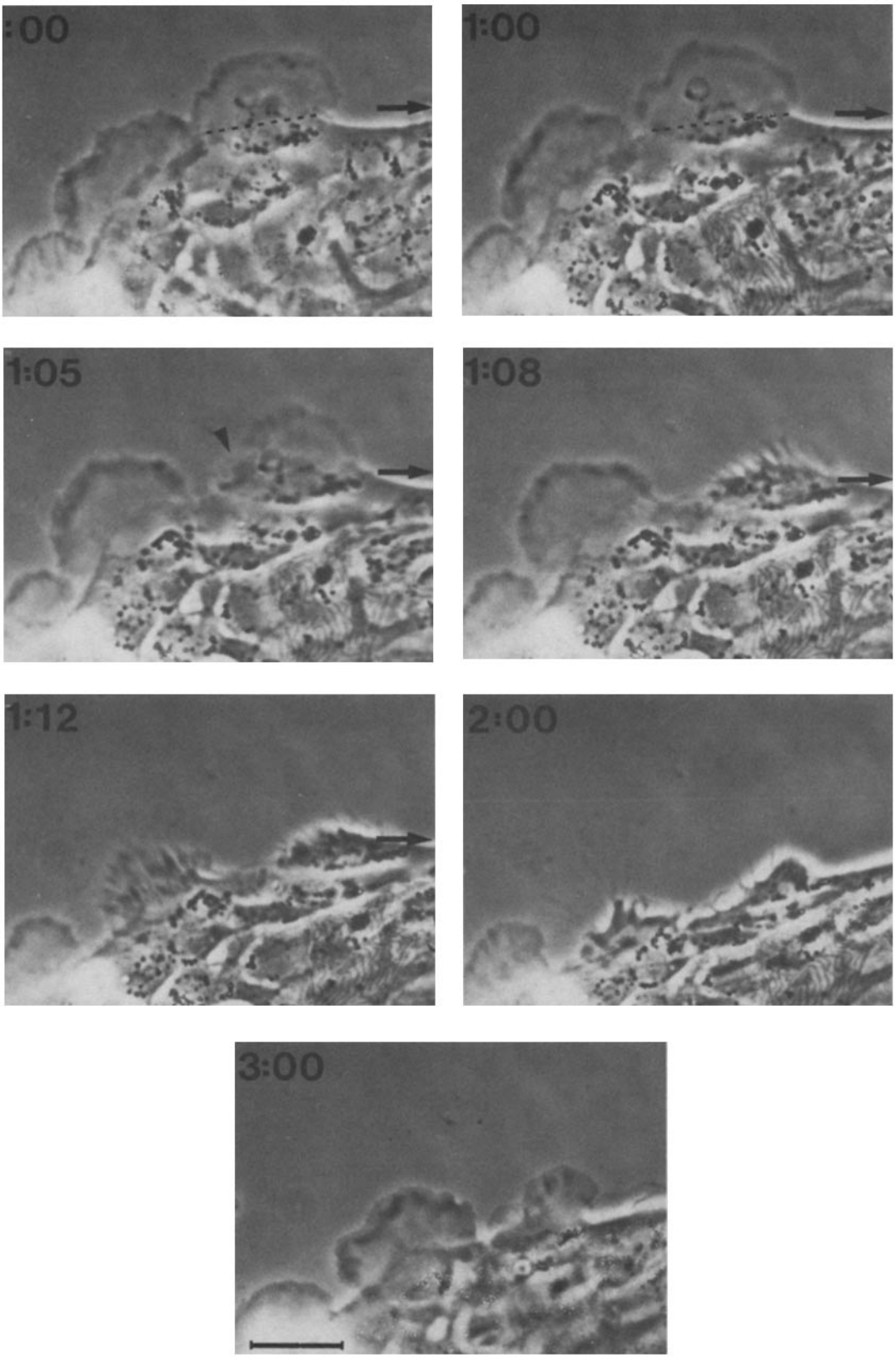



*Figure 2.* Cellular response to tension exerted parallel to the spreading edge. These phase-contrast micrographs are taken from a time-lapse movie of clusters moving on a glass coverslip. The time is indicated in min:sec. At :00, the cells were moving towards the top of the picture. A dotted line is drawn across the cell body along the axis in which the cell was about to be stretched. At :01 (not shown), a microneedle was placed against the cluster sheet immediately to the right of the arrow (i.e., coincident with the edge of the micrograph) and pulled in the direction of the arrow. The margin of the marked cell stopped advancing. At 1:00, the cell was still under tension from the pull of the needle. Compare the length of the cell body with the length at :00. The needle was then pulled slightly further and, at 1:05, the cell's well-spread lamella began to retract (arrowhead). Rapid retraction of the the entire lamella ensued (1:08). With the loss of this lamella, tension was exerted directly on the next cell, and retraction of its lamella rapidly followed (1:12). At 2:00, the microneedle was removed, releasing the sheet from tension. Well-spread lamellar protrusions are absent from the "stretched" edge of the sheet at this time. By 3:00, large lamellae had respread from the margins of the manipulated cells. Bar, 20 $\mu$m.

is prevented. If the cell is only pulled just far enough to initiate retraction, its lamellar protrusion will usually respread, albeit with a longer and slightly narrower shape. Attempts to cause the gradual retraction of a cell's lamellar protrusion, such as was often observed when cells were pulled perpendicular to their leading edges, were unsuccessful. However, it is considerably more difficult to pull on a cell parallel to its free edge because tension must be transmitted through marginal cells, which are generally well-spread and relatively difficult to displace with the microneedle. There is also a smaller region of cell–cell contact between adjacent marginal cells than between a marginal cell and its submarginal neighbors, and this contact must transmit the tensile force without separating. Thus, the failure to observe graded changes in motility when exerting tension parallel to a cell's free edge may be due to the crudeness of the micromanipulation procedure.

### *Cytoskeletal Organization*

The cytoplasm of these epidermal cells consists of two distinct domains: the thicker, central part of the cell, or cell body, which is filled with a dense matrix of intermediate filaments, and the cell's lamellar protrusions, which contain a fine network of microfilaments. There are no specialized junctions on the cell surface covering the latter, which, in any case, are mostly confined to the free margins of the cluster. There are, however, numerous, well-developed desmosomes linking the intermediate filament–laden cell body of each cell to the cell bodies of its neighbors.

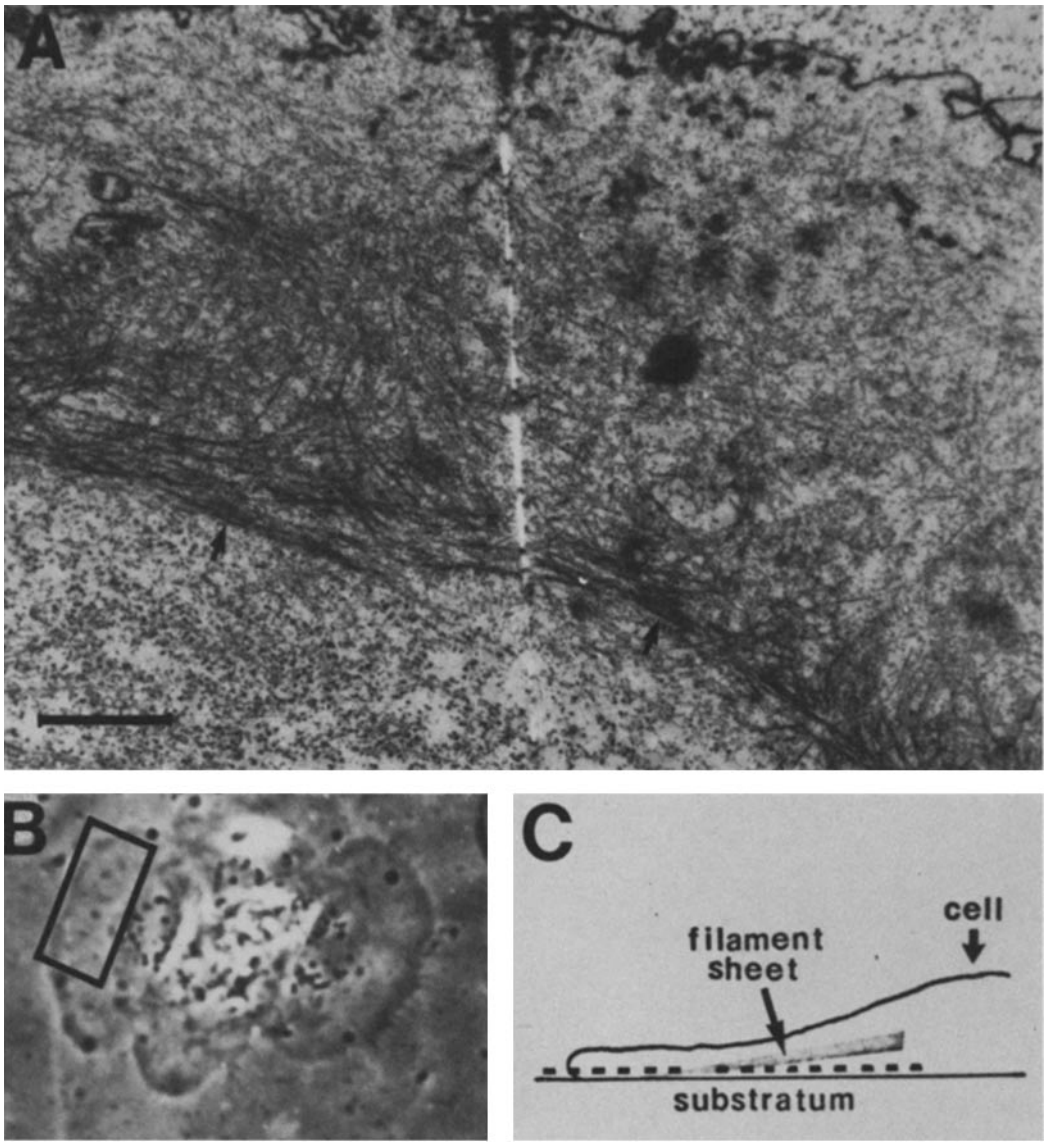



*Figure 3.* Microfilament orientation in the distal portion of an advancing lamella. (*A*) Electron micrograph of a thin section taken from the region indicated in the phase-contrast micrograph in *B* in a plane parallel to the substratum at the height indicated by the dotted line on the schematic cross section in *C*. At the moment of fixation, the margin of the cell was moving towards the top of the micrograph (in *B* the cluster is moving from right to left). In the middle part of the lamella, the plane of section passes through the dense planar array of microfilaments (arrows), which slants through the lamella. The microfilaments in this dense array tend to be oriented parallel to the cell's leading edge. More distally, the microfilaments are sparser and splayed apart to such a degree that no preferential orientation is evident. Bar, 2 μm.

In search of a structural basis for the phenomena described in the previous section, the organization of the filamentous elements of the cytoplasm was examined in cells that were subject to an external tensile force. First, the highly motile, lamellar protrusions of cells on the margins of unmanipulated clusters were examined. A cluster moves most rapidly when its leading margin faces the least amount of resistance in the form of spreading by the cells on the opposite margin (30). Presumably this is also the condition in which there is the least amount of tension on the spreading protrusions of the leading margin. When rapidly moving clusters (i.e., those moving at speeds > 1.5 $\mu$m/min) were fixed and examined in thin sections, it was found that the microfilaments in the lamellar protrusions along the cluster's leading margin appear highly disorganized with a slight tendency to lie parallel to the protrusion's advancing edge (Fig. 3). This morphology was observed in 21 different cells in 7 different clusters. It was also observed in rapidly translocating, individual epidermal cells and at the margin of epithelial spreading from large epidermal explants (not shown).

The cytoskeleton of cells under more pronounced tension was examined in three different situations: (*a*) in cells on the lateral and trailing edges of moving clusters, where the lamellar protrusions are held back or dragged backwards over the substratum by the collective pull of the cells on the leading margin of the cluster; (*b*) in cells subjected to an artificial tension by pulling on a cluster with a microneedle, and (*c*) in cells pulled taut between two cells or groups of cells moving in opposing directions.

(*a*) In thin sections taken through the immotile (11 cases) or retracting (16 cases) lamellar protrusions of moving clusters, microfilaments were found to be aligned perpendicular to the cell's free edge, i.e., parallel to the presumed direction of tension (Fig. 4). The aligned microfilaments tend to lie in a plane slanting through the lamellar protrusion from the dorsal surface of the cell in the proximal part of the protrusion to the ventral, cell–substratum contacts at the protrusion's distal edge. Aligned filaments extend to within 0.5 $\mu$m of the protrusion's most distal edge, but microfilaments in the more distal cytoplasm and in cytoplasm above and below the plane of aligned filaments are less organized and often appear as an anisotropic meshwork.

(*b*) An identical alignment of microfilaments parallel to tension was observed when tension was applied artificially.

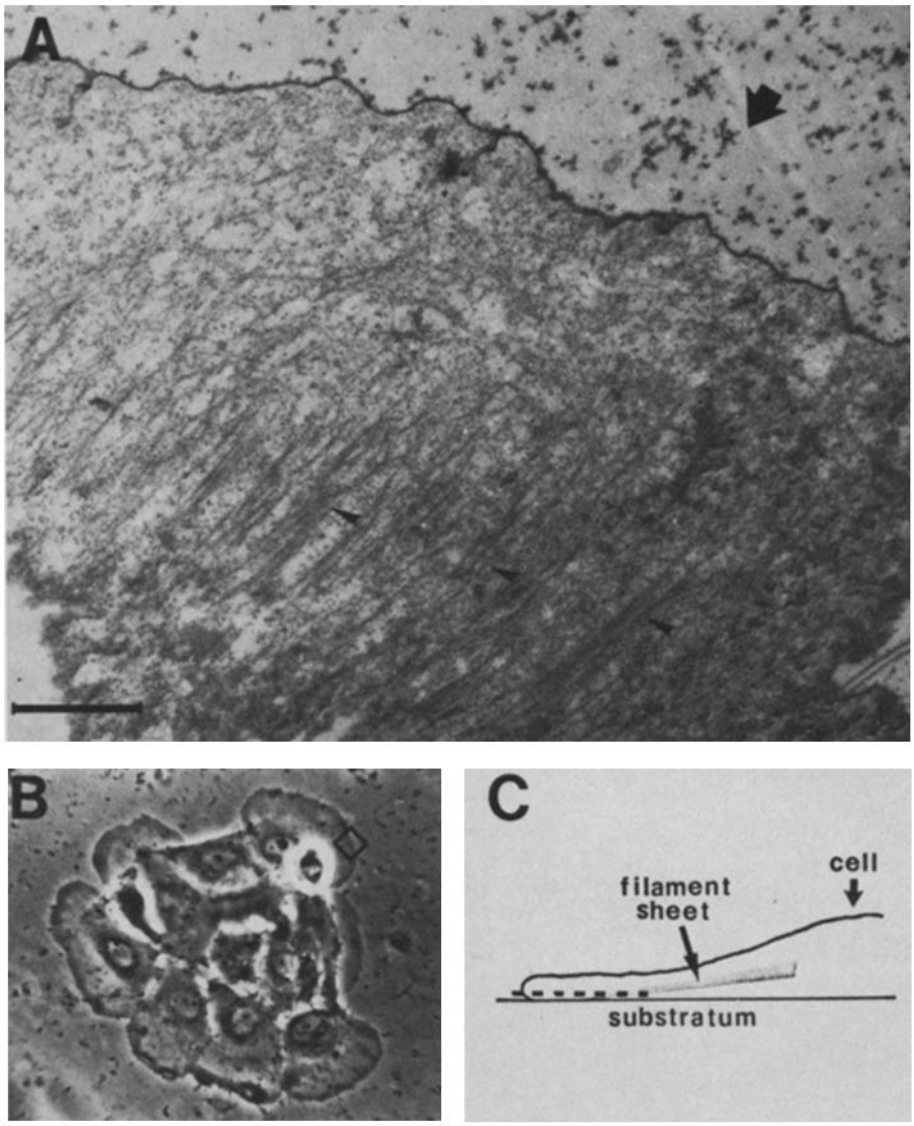
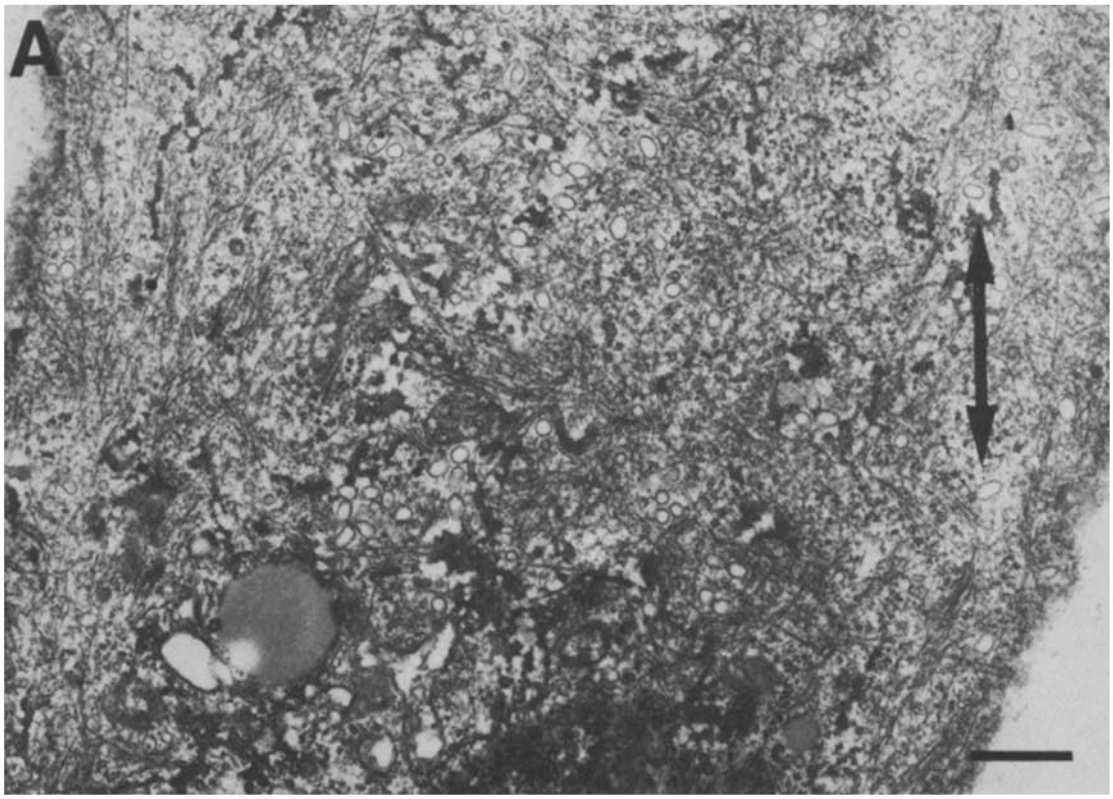



*Figure 4.* Microfilament orientation in the distal portion of a retracting lamella. (*A*) Electron micrograph of a thin section taken from the trailing edge of a moving cluster (from the region indicated on the phase-contrast micrograph in *B*) in a plane parallel to the substratum (at the height indicated by the dotted line on the schematic cross section in *C*). Movement at the time of fixation was in the direction of the arrow (in *B* the cluster is moving toward the lower left). A great many microfilaments (arrowheads) are aligned strictly perpendicular to the cell's edge. Note, however, that microfilaments in the most distal lamellar cytoplasm (which is above the dense sheet of microfilaments) form a loose meshwork with no preferential orientation in evidence. Bar, 0.5 $\mu$m.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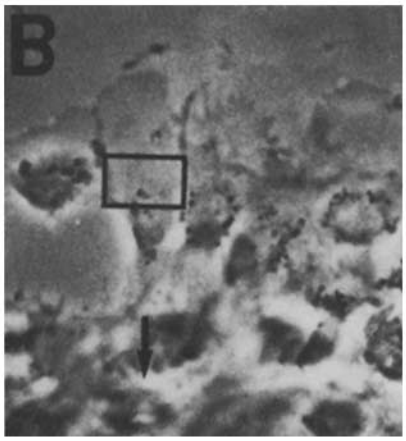
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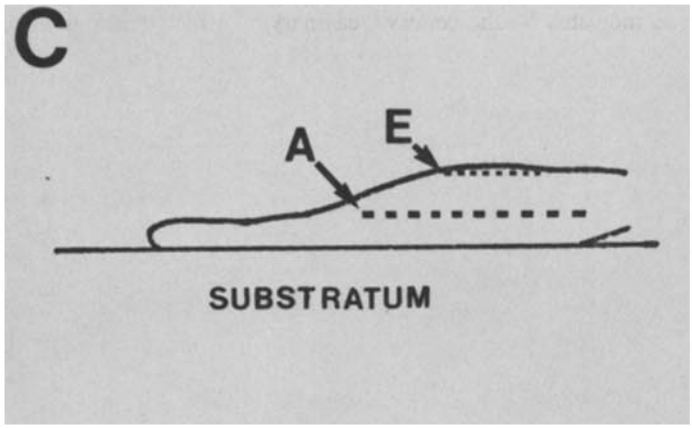
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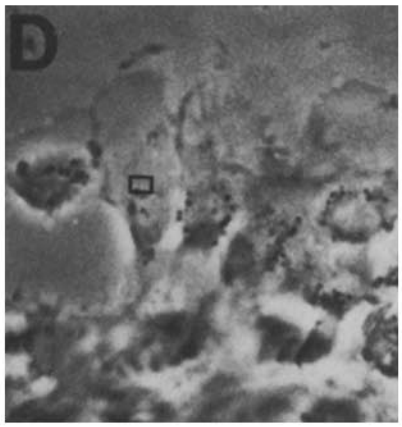
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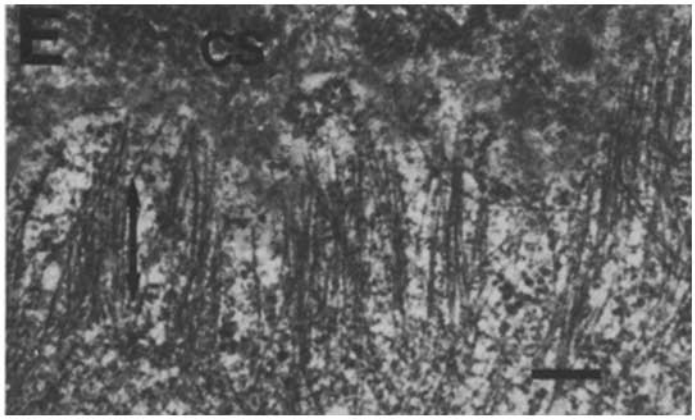
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The spreading of cells at the leading margin of a cluster could be stopped by using a microneedle to pin submarginal cells to the substratum. Thin sections through cells fixed in this tethered position revealed a morphology indistinguishable from that described above for cells on the rear and lateral margins of moving clusters. However, it was necessary to hold the cluster for 2–3 min before motion at the leading edge was brought to a complete halt. Under these circumstances, filament alignment could be a gradual reorganization brought about by an intermediate regulator of motility. To be more certain that tension caused filament realignment directly, a similar manipulation was performed in which the microneedle was placed against a moving cluster and then pulled backwards against the direction of movement (Fig. 5*e*). This stopped forward motion of the leading edge virtually instantaneously (within a single frame in time-lapse records, i.e., <0.5 s). Cells manipulated in this fashion were immediately flushed with fixative so that the entire procedure from application of the needle to fixation of the cells took no more than 5 s. In eight separate trials, thin sections revealed that microfilaments in the lamellar protrusions and in the microfilamentous cytoplasm immediately subjacent to the plasma membrane that overlies the cell body were aligned parallel to the tensile force in every case.

(*c*) Microfilaments were also found to be aligned when tension occurs parallel to the cell's free edge, i.e., perpendicular to the direction in which cell spreading occurs. This is readily apparent in unmanipulated cells. A cell on the cluster's margin that is located between two cells or groups of cells that are spreading in opposing directions appears to be under considerable tension, as indicated by the concave, catenary contours of the cell's free margin and the sharp retraction of the cell when tension is released by breaking cell–cell or cell–substratum adhesions (30). Such alamellar marginal cells display no protrusive activity, despite their position on the free edge of the epithelium. The organization of the cytoskeleton in these alamellar regions is shown in Fig. 6. Microfilaments are aligned in tight bundles just beneath the cell surface and run parallel to the cell's free edge and to the axis of tension.

This morphology was always found when taut, catenary contours of alamellar marginal cells were examined (17 cases). Alamellar margins created artificially by pulling on a cell in a direction parallel to its free edge (as in Fig. 2) were also found to contain bundles of parallel microfilaments subjacent to the plasma membrane and oriented in the direction of tension (3 cases).

The organization of 8–10-nm intermediate filaments, in contrast, is very different. The intermediate filaments are slightly thicker than microfilaments, which are 6–7 nm in these preparations, and are also distinguished by the uniformity of their fixation and staining—there is less variation in the intensity of staining and the thickness of the filament along the length of intermediate filaments than along microfilaments. Using these morphological criteria, intermediate filaments were found in great abundance in the thicker, central part of the cell, or cell body, but were never detected in lamellar protrusions. The demarcation between the two domains is generally quite clear. Thus, filaments were usually not measured individually to distinguish intermediate filaments from microfilaments. Rather, it was assumed that the narrower, unevenly staining filaments, which were found in the lamellar cytoplasm and the peripheral regions of the cell, were all microfilaments and the thicker, more evenly staining filaments located exclusively in the cell body were all intermediate filaments. Periodic measurements of filaments in high magnification electron micrographs of thin sections invariably proved these relatively subjective criteria to be in agreement with the identification made on the basis of filament diameter. In thin sections taken through cells representing all of the various locations within clusters—the leading edge, the trailing edge, the lateral margins, and the central regions—intermediate filaments were found to lie with many different orientations and to follow tortuously curving paths which tended to lie roughly parallel to the contours of the cell (Fig. 7). No specific orientation of intermediate filaments relative to the direction of cell movement or to the location of protrusive activity was evident.

Intermediate filaments were found to display little or no alignment in response to tension. In taut, alamellar regions of the epithelial margins the orientation of intermediate filaments is extremely irregular even among filaments lying immediately adjacent to strictly aligned, tightly bundled microfilaments (Fig. 6). Likewise, when tension is applied to cells by pulling on an epithelium with a microneedle, intermediate filaments retain their loose, irregular orientation, while nearby microfilaments are aligned parallel to the tensile force (Fig. 5).

*Figure 5.* Filament orientation in a micromanipulated cell. (*A*) Electron micrograph of a thin section taken from the area in the box in the phase-contrast micrograph in *B*. The height of the plane of section is indicated by the broken line (*A*) in the schematic in *C*. Intermediate filaments near the cell's lateral edges are loosely oriented parallel to the cell surfaces, which lie parallel to the axis of tension (double-headed arrow). But the majority of intermediate filaments are not aligned parallel to tension, particularly in the more central parts of the cell. Bar, 1.0 μm. (*B*) Phase-contrast micrograph taken from a time-lapse movie of the micromanipulation and fixation showing the location of the electron micrograph in *A*. It was taken immediately after fixation and is printed in the same orientation as *A*. The arrow marks where the needle was placed against the sheet and points in the direction in which the needle was pulled. The needle was oriented parallel to the margin of the cluster and pressed against the surface of the four cells immediately submarginal to those on the cluster's edge shown here. The cells were fixed immediately, while still under tension. The shape and size of the cell under examination prior to manipulation were approximately the same as that of the adjacent cell to the left, which was not stretched because of the tear in the epithelium along the cell's submarginal edge. (*C*) Schematic diagram showing the plane of section of the micrographs in *A* and *E*. (*D*) Phase-contrast micrograph showing the exact location of the electron micrograph shown in *E*. (*E*) Electron micrograph of the region indicated in *C* and *D*. Microfilaments lie within a thin layer immediately subjacent to the plasma membrane—at the top of the micrograph the plane of section is seen to enter the cell surface (*CS*) at an extremely shallow angle. This section lies directly above that shown in *A*, and shows that, in contrast to the intermediate filaments, the microfilaments lie strictly parallel to the axis of tension (double-headed arrow). Bar, 0.2 μm.

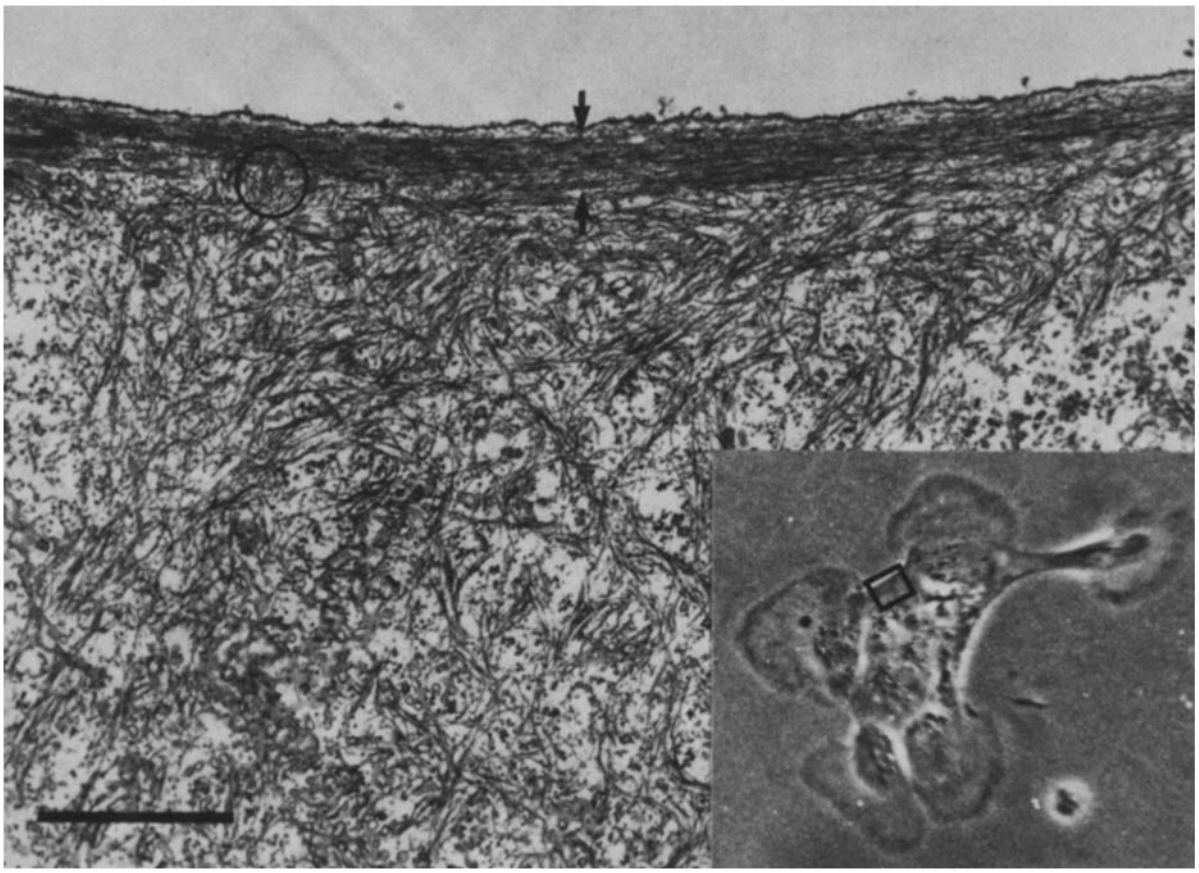

*Figure 6.* Filament orientation in a cell under tension. Electron micrograph of a thin section taken in a plane parallel to the substratum from the region indicated on the phase-contrast micrograph (*inset*). The concave, catenary contours of this cell suggests that it is under tension—presumably held taut by the outward movement of the cells on either side. A dense bundle of microfilaments (arrows) runs along the cell's free edge, with the individual microfilaments aligned parallel to the edge. Many intermediate filaments immediately adjacent to the microfilament bundles run roughly parallel to the cell surface, as always, but they are not highly oriented, and some even lie at right angles to the bundle (circle). Notice that the intermediate filaments in the lower left of the micrograph, which are closer to the leftmost edge of the cell than to the cell's free edge, tend to run parallel to that nearest surface—almost perpendicular to the predominant orientation of the filaments near the free edge. Bar, 1 $\mu$m.

## *Discussion*

The observations that stretching an epithelial cell causes it to withdraw its lateral protrusions, and that this suppression of spreading activity is reversed upon release of tension, indicate that simple mechanical forces can regulate motile behavior. That this is indeed a mechanical effect is strongly implied both by the precise temporal correlation between the application of a simple mechanical stimulus and changes in motility and by the consistent spatial relationship between the direction of tension and the direction in which motility is suppressed. That protrusive activity perpendicular to tension is dramatically suppressed when a cell is stretched, whereas spreading parallel to tension is not (Fig. 8), cannot be explained on a purely chemical basis.

An absence of locomotive activity on taut surfaces can be observed in many cells (for example, nerve axons, the elongate tails of chick heart fibroblasts, the rear margins of *Fundulus* "fan cells" [23], and the bodies of cultured myoblasts). Moreover, at least in the case of fibroblasts and nerve axons, the release of tension is followed by the onset of extensive locomotive activity (6, 11). In the nerve, Bray has shown that extension of the growth cone can be "steered" by applying tension to the axon: extension is greatest in a direction parallel to the axis of tension and reduced in the perpendicular directions (6). Thus, the behavior observed in these rather specialized epidermal cells may well be common among cells that extend locomotive protrusions.

### *Tension and the Microfilamentous Cytoplasm*

Inside the cell, tension produces obvious physical alterations in the microfilamentous cytoskeleton. In cytoplasm under tension, whether pulled taut by other cells or by a microneedle, microfilaments are invariably aligned parallel to tension. Furthermore, this alignment can be induced within seconds in rapidly moving protrusions where microfilaments are normally not aligned. There is considerable precedent for the observation that microfilaments organize under tension. It has long been proposed that stress fibers—the dense microfilament bundles that frequently develop in cultured cells—are the result of intracellular tension (8, 9, 25, 34). Fleischer and Wohlfarth-Botterman (18) have shown that microfilament bundles are generated in isolated strands of cytoplasm contracting under isometric conditions, i.e., under sufficient tension to maintain the length of the strand against the force of contraction, but that filament bundles are absent during

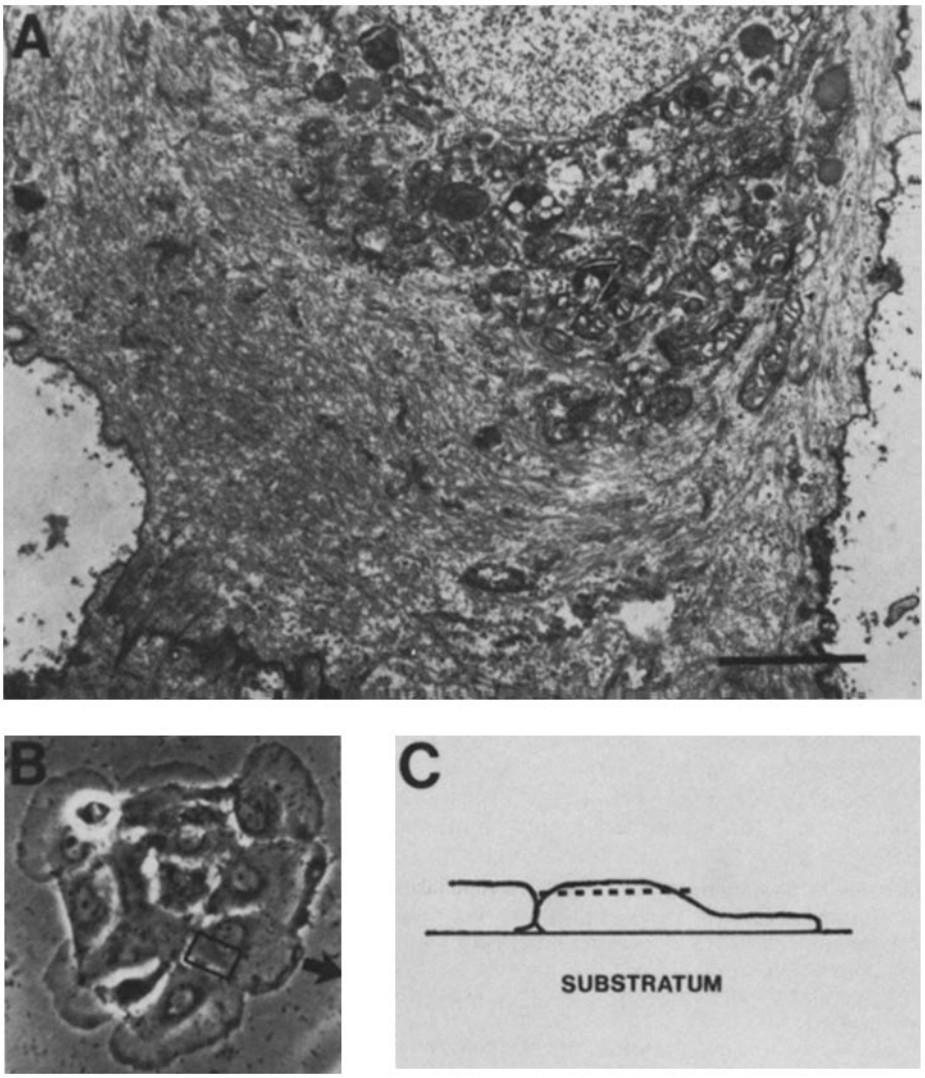


*Figure 7.* Intermediate filament orientation. The electron micrograph in *A* was taken from the region indicated in the phase-contrast micrograph in *B*. It was taken from a thin section cut in a plane parallel to the substratum at the height indicated by the dotted line in the schematic diagram in *C*. Note that the plane of section cuts only the upper part of the cell body and so passes above the cell's lamellar protrusions. At the moment of fixation, the cluster was translocating in the direction of the arrow in *B*, so the direction of movement of the cell in *A* is from left to right. The cell body is filled with intermediate filaments, which have no specific orientation relative to the direction of movement. Also, there are no dramatic differences between the leading, trailing, and lateral edges of the cell in terms of the organization of filaments. In general, the intermediate filaments appear to follow roughly the contours of the cell. Bar, 2 $\mu$m.

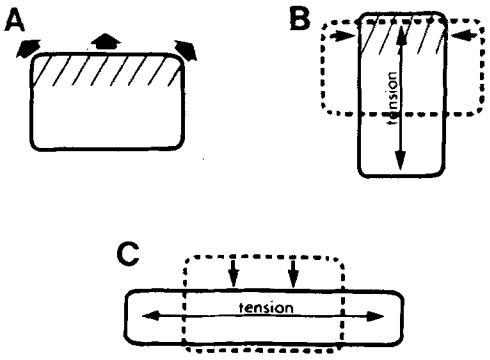


*Figure 8.* Schematic representation of the antagonism between tension and protrusive activity. Under normal circumstances (*A*), a cell extends a broad lamellar protrusion (hatched region) from its free margin, and the cell spreads or moves in the direction of the arrows. Tension applied to the cell (double-headed arrows in *B* and *C*), suppresses lateral protrusive activity (single-headed arrows in *B* and *C*), but does not affect spreading parallel to tension (hatched region in *B*). In other words, tension has a much greater effect on protrusive activity that is perpendicular to the axis of tension (*C*) than on that which is parallel to tension (*B*).

isotonic contractions, i.e., when the cytoplasmic strand is allowed to shorten under a constant, and necessarily smaller, tensile load. Chen (11) found strictly aligned microfilament bundles in the taut "tails" that are frequently present on the trailing edge of fibroblasts moving on glass or plastic. Upon distraction of the tail from the substratum, the microfilament bundles disappear and are replaced by a meshwork. In addition, fibroblasts placed in collagen gels develop stress fibers when they are contracting the collagen matrix, but the stress fibers disappear when the collagen fibers tear and tension is released (16).

How microfilaments become aligned throughout the tensed cytoplasm is unclear. When tension is applied to the cell it is presumably transmitted to the microfilaments via associations between microfilaments and the cell surface at cell–cell and cell–substratum attachments (1, 12, 21, 25), where tension is ultimately transmitted to and from the cell's environment. Microfilaments may also have physical associations with the intermediate filament network (10, 29, 41) that would permit tension transmitted from cell to cell via the intermediate filament–desmosome network (see below and reference 31) to reach the microfilament network. Once tension is trans-

mitted to the microfilaments, the microfilaments must also interact with one another in order for tension to be transmitted deeper into the microfilamentous cytoplasm. Thus, the alignment of microfilaments upon the external application of tension supports the hypothesis that at least a portion of a cell's microfilaments exist as a meshwork or lattice, as depicted in electron micrographs from a great many laboratories (1, 2, 8, 39, 41, 42, 43, 48). Of course, microfilaments need not be actually cross-linked to one another; they could also interact transiently via force-generating molecules such as myosin or, if individual filaments are long enough, simply by pushing against one another laterally as do individual straws in a bundle.

If indeed the microfilamentous cytoplasm may be regarded as a network of interacting filaments, there exists a straightforward mechanism for how the effects of tension on spreading are mediated. Consider any filamentous network, such as the strands of an onion bag. If one pulls on the ends of the bag, the fabric stretches in the direction of pull and narrows perpendicular to the pull as the individual strands of the onion bag fabric become aligned parallel to tension. The greater the tension on the bag, the more difficult it is to spread the fabric out. The microfilamentous cytoplasm may behave in a similar fashion: spreading may be absent from such regions as the aligned microfilaments on the edge of an alamellar cell because tensile forces exerted on the ends of the microfilament network keep it in a tightly bundled state. Similarly, tension exerted on spreading cytoplasm could suppress, and ultimately cause the retraction of, lateral protrusions by realigning the microfilament network into a narrower, more parallel arrangement. At the same time, spreading along the axis of tension could still continue, as has been observed in the growth cones of nerve axons under tension (6, 7) and also in some instances in the course of this study. Spreading might even be stimulated, as has been reported by Takeuchi (46) and Bray (6).

### *Tension and Intermediate Filaments*

A much different relationship is observed between mechanical tension and a cell's intermediate filaments. Intermediate filaments tend to roughly parallel the contours of the nearest cell surface (Fig. 6 and 7), and even in cells under great mechanical tension, this shape-related distribution of intermediate filaments is relatively undisturbed. In both the taut, alamellar edges of unmanipulated cluster cells and in cells stretched with a microneedle, intermediate filaments retain their loose organization and tendency to follow roughly the contours of the cell, even as microfilaments in the same cell are dramatically aligned parallel to tension. It is possible that tensile force is simply not transmitted to the intermediate filaments and is transmitted from cell to cell only through the thin layer of microfilamentous cytoplasm surrounding the intermediate filament–filled cell body. However, this seems unlikely for two reasons. First, these cells are held together by numerous desmosomes which are, in turn, directly associated with intermediate filaments that are interwoven with the bulk of the cell's intermediate filaments (unpublished observations). Second, the cell body and nucleus are sharply displaced when a cell is being micromanipulated through its connections to neighboring cells. This indicates that some force is being very efficiently transmitted to the central regions of the cell. The tendency of the lamellar protrusions to be drawn out and narrowed during such manipulations suggests that the plasma membrane and microfilamentous cytoplasm are too malleable to account for all the force transmitted to the center of the cell, particularly since the microfilamentous cytoplasm overlying the cell body is much thinner than in the lamellar protrusions.

If indeed the intracellular matrix of intermediate filaments is less easily distorted by external physical forces than is the microfilamentous cytoplasm, one function of the intermediate filaments could be to give the cell its tensile strength and rigidity of form. This would be consistent with the particularly great abundance of tonofilaments in epidermis in general, where strength and resiliency are such essential properties. In other cells, including other epithelial cells, smooth muscle cells, and fibroblasts, at least some structural stability of the intermediate filament matrix is suggested by the fact that the intermediate filaments left behind after detergent extraction faithfully retain the shape of the cell and the location of the nucleus (13, 17, 22, 26, 33, 44). However, it remains to be seen if the intermediate filament network in these other cell types display mechanical properties similar to those observed in the present study. It is also important to be aware that the observations reported in this study reveal nothing about how intermediate filaments become arranged in a particular spatial distribution, only that the arrangement resists deformation by external stress. Other studies have shown that cell shape is not disturbed when intermediate filaments are disrupted by the injection of antibodies against intermediate filament or intermediate filament-associated proteins in fibroblasts (20, 27, 32, 35) and epithelial cells (28, 32). Thus, intermediate filaments may be acting simply as bulk structural material—"intracellular clay"—the actual sculpting of which is dependent on other factors such as contractile proteins, the cell's adhesive relationships, or intracellular transport mechanisms.

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