

Polarization of the Golgi apparatus and the microtubule-organizing center in cultured fibroblasts at the edge of an experimental wound

(cell motility/cytoskeleton/membrane growth/double immunofluorescence microscopy)

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ABSTRACT We have used the technique of experimental wounding of confluent monolayers of normal fibroblasts to induce essentially unidirectional and synchronous cell movement at the edge of the wound. The intracellular location of the Golgi apparatus and the microtubule-organizing center was determined by double indirect immunofluorescence microscopy, using antibodies specific for the membranes of the Golgi apparatus and antibodies specific for tubulin, respectively. In cells at the wound edge, the immunolabeled Golgi apparatus and microtubule-organizing center were in close proximity to one another and located predominantly forward of the cell nucleus facing the wound. In the same cultures in cells removed from the wound, the two organelles were also coordinately located; however, they were randomly oriented with respect to the wound edge. This reorientation of the two organelles in cells at the wound edge was evident within minutes after wounding and persisted as cell extension subsequently occurred into the wound. These results suggest that both the Golgi apparatus and the microtubule-organizing center may participate in directing cell movement. The possible mechanisms involved are discussed in the light of previous hypotheses and experimental evidence concerning cell motility.

The migration of tissue cells over biological substrata is an important physiological process involved in such diverse phenomena as embryological development, the formation of metastases by malignantly transformed cells, responses to chemotactic stimuli, and wound healing. The molecular mechanisms involved in the motility of vertebrate cells, particularly in generating the force required for directed cell movement, are not yet understood. Two general types of mechanisms for such force generation have been proposed. (i) The production of new surface membrane at the leading edge of the moving cell and the removal of surface membrane at other sites propels the cell forward, with the cytoplasm flowing passively into the regions of cell extension (1–3). (ii) Cytoplasmic events, particularly those involving cytoskeletal elements polarized in the direction of cell extension, in some manner generate the force required to move the cell forward, membrane turnover being only indirectly related to the motility (4–7). The experimental evidence leading to these views is considered in the *Discussion*.

To study this problem, we have concentrated on the location and orientation of two organelles inside motile fibroblasts in monolayer cultures: the Golgi apparatus and the microtubule organizing center (MTOC). The Golgi apparatus is of interest because it is a critical structure in the intracellular pathways leading to plasma membrane growth and recycling as well as to secretion (8). The MTOC in interphase cells is a region of the cell near the cell nucleus and including the centrioles, out of

which the cytoplasmic microtubules emanate (9–11). The MTOC plays an important role in the internal polarization of the cytoskeleton. It is also known from electron microscopic (12) and much earlier light microscopic (13) studies that the Golgi apparatus is usually found in proximity to the centrioles (and hence the MTOC) in interphase cells. We have detected these organelles in individual cells by double indirect immunofluorescence in the light microscope, by using one antibody preparation that specifically bound to the membranes of the Golgi apparatus (14) and a second antibody directed to tubulin that labeled microtubules and the MTOC (10, 11). Cell motility was induced by the technique of experimental “wounding” (15). If a swath a few millimeters wide is cut in a confluent monolayer of normal cells, during the first several hours, the leading lamellae of the cells at the edge of the swath move into the emptied zone and later the entire cell detaches from its neighbors and migrates into the wound area. This technique allows one to initiate and follow the essentially unidirectional and synchronous movements of many cells at the wound edge in a single experiment. Cells at the wound edge at different times after wounding, and cells well removed from the wound, were examined by double immunolabeling of their Golgi apparatus and MTOC.

Our findings are as follows. (i) In cells remote from the wound, in the unperturbed confluent monolayer, the immunolabeled Golgi apparatus and MTOC in any single cell were invariably localized close to one another near the cell nucleus, as expected (12, 13), and randomly oriented in the plane of the cell layer. (ii) In cells at the wound edge, however, by 5 hr after wounding the Golgi apparatus and the MTOC were located together predominantly forward of the nucleus in the direction of the lamellar extension of the cell. (iii) This coordinate redistribution of the two organelles was evident in many cells at the wound edge within a few minutes after producing the wound, long before any extension of the cell edge was apparent. From these and earlier observations, we infer that both the Golgi apparatus and the MTOC play important roles in directing cell movement. These roles may be concerted: the oriented MTOC and cytoskeleton may serve to direct the traffic of vesicles derived from the cooriented Golgi apparatus to the plasma membrane at the leading edge of the cell, thereby resulting in growth and extension of the leading edge.

MATERIALS AND METHODS

Antibody Reagents. Rabbit antibodies specific for membranes of the Golgi apparatus were prepared as described (14), by subjecting an immunoglobulin fraction of an antiserum made

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Abbreviation: MTOC, microtubule-organizing center.

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against purified rat liver Golgi membranes to careful absorptions with other cell fractions. This absorbed antibody preparation is directed predominantly to a Golgi-specific 135-kilodalton protein component (14). The affinity-purified guinea pig antibodies to chicken brain tubulin (16), affinity-purified and cross-absorbed goat antibodies to rabbit IgG and to guinea pig IgG, and rhodamine and fluorescein conjugates of the goat antibodies were prepared as described (17).

Cells and Cell Manipulations. NRK cells were the gift of Peter K. Vogt. The cells were maintained at 37°C in Coon's modified F-12 medium/10% fetal calf serum supplemented with antibiotics in humid 10% CO₂/90% air. Cells were plated on 18 × 18 mm glass coverslips and allowed to grow to confluency for 4 to 5 days. The wound was made by cutting a swath a few millimeters wide through the cell layer with a rubber "policeman." Except for those experiments carried out within 30 min after wounding, the cell layer was then washed with fresh medium. For the double immunofluorescence labeling experiments, the wounded cell layers were first permeabilized for 20 sec with a microtubule-stabilizing buffer (0.5% Triton X-100/1 mM GTP/1 mM MgSO₄/1 mM EGTA/4% polyethylene glycol 6000/0.1 M PIPES, pH 6.9) and then fixed with 3% paraformaldehyde. This procedure gave better delineation of the MTOC than did fixation followed by permeabilization. For some experiments involving single immunolabeling of the Golgi apparatus, the cells at various times after wounding were first fixed with 3% paraformaldehyde and then permeabilized with Triton X-100 as described (16, 17). The Golgi apparatus was clearly visualized in such cells.

Immunolabeling. Permeabilized and fixed cells were doubly immunolabeled by first applying a mixture of the two primary antibodies (the affinity-purified guinea pig antibodies to tubulin and the absorbed rabbit antibodies to the Golgi membranes) and then applying a mixture of the two secondary antibody reagents. Immunofluorescence was observed in a Zeiss Photoscope III instrument as described (16, 17).

After immunolabeling, cells at the wound edge were selected for observation that, by Nomarski optics, (i) exhibited uninterrupted contact with adjacent cells except at the wound edge, (ii) remained attached to the substratum, and (iii) appeared to be intact. Such cells accounted for 20–50% of all cells at the wound edge. To record the position of the immunolabeled Golgi apparatus and MTOC in each cell, the cell was divided into three 120° sectors centering on the nucleus, one of which was bisected by the perpendicular to the edge of the wound. The Golgi apparatus or MTOC was assigned to that sector in which 50% or more of its fluorescent image was confined. For each time point in Fig. 3, at least 100 cells were examined.

RESULTS

Cells at the edge of a wound were at least 90% viable by the criterion of trypan blue exclusion. Five hours after wounding, most of these cells had extended lamellae into the wound. At that time, 78 ± 5% of the first row of cells at the wound edge exhibited their immunolabeled Golgi apparatus and MTOC within the sector facing the wound edge (Fig. 1A). The two organelles in any one cell were always in close proximity to one another near the cell nucleus. Inside the cells in the still confluent portions of the same cultures, the Golgi apparatus and MTOC were in close proximity to one another but randomly oriented, with ≈33% in each of the three sectors (Fig. 1B). It was of interest to know how soon after wounding this apparent reorientation of the two organelles occurred and whether they retained their codistribution. If cultures were similarly examined within 30 min after wounding, in cells at the wound edge,

the Golgi apparatus and the MTOC were coordinately positioned with a bias in the forward-facing sector. The 5-min experiment is shown in Fig. 2. The time course of this effect was then examined in greater detail with specimens immunolabeled only for the Golgi apparatus (Fig. 3). By the earliest time point we could fix the wounded cultures, 41 ± 5% of the cells at the wound edge already showed the Golgi apparatus in the forward-facing sector, significantly elevated over the random value of 33.3%, and, by 5 min after wounding, this value was 60 ± 5%.

DISCUSSION

The term microtubule organizing center (MTOC) was proposed by Pickett-Heaps (9) to denote the regions inside cells from which microtubules appear to be initiated. In animal cells in interphase, it has been shown by electron microscopy that these regions are near the nucleus and contain a pair of centrioles surrounded by irregular masses of granular material including microtubules and intermediate filaments (see figure 3.2 in ref. 12). In such micrographs, elements of the Golgi apparatus are often found in proximity to the centrioles, confirming the spatial relationships of the Golgi apparatus and the centrioles that was recognized much earlier by light microscopy (13). More recently, it was shown that the MTOC in interphase cells could be recognized by immunofluorescence microscopy using antibodies directed to tubulin (10, 11).

The Golgi apparatus is a critical organelle in secretion (8) and, as has recently been directly demonstrated (18), in the intracellular pathway of integral proteins destined for the plasma membrane. Vesicles containing these integral proteins presumably bud from the *trans* face of the Golgi apparatus and, by unknown mechanisms, arrive at and fuse with the plasma membrane to produce new membrane growth.

To study the roles of the Golgi apparatus and the MTOC in cell motility, we have used the experimental wound technique (15, 19) to synchronize and orient the motile behavior of cultured cells at the edge of a wound and have carried out double immunofluorescent labeling of the Golgi apparatus and the MTOC in the same cells. In any one cell, the immunolabeled Golgi apparatus and MTOC were always found in close proximity to one another. Our principal observation is that, in cells at the edge of the wound, the Golgi apparatus and MTOC were generally facing forward of the nucleus in the direction of cell extension into the wound area (Fig. 1A). By contrast, in cells well removed from the wound within the confluent monolayer, the Golgi apparatus and MTOC remained randomly oriented in the plane of the monolayer (Fig. 1D and E). This reorientation was evident in individual viable cells at the edge of the wound within min after wounding (Figs. 2 and 3), long before any lamellar extension of the cell into the wound was discernible. From the number of cells exhibiting such early internal reorientation, the indications are that, had they not been fixed for observation, these same cells would have subsequently undergone lamellar extension and motility.

We suggest that the repositioning of the Golgi apparatus and the MTOC is a real effect and is important in specifying the subsequent direction of motion of a motile fibroblast. An alternative possibility, that the changes are artifacts of the wounding process occurring in those cells that are exposed at the edge of the wound and that they have nothing to do with cell motility, cannot be entirely ruled out by our results but seems to us unlikely.

A function for the Golgi apparatus in cell motility is consistent with the proposal (1–3) that the force for directed cell movement is derived from growth of new plasma membrane at the leading edge of a cell and the removal of membrane at other sites. In

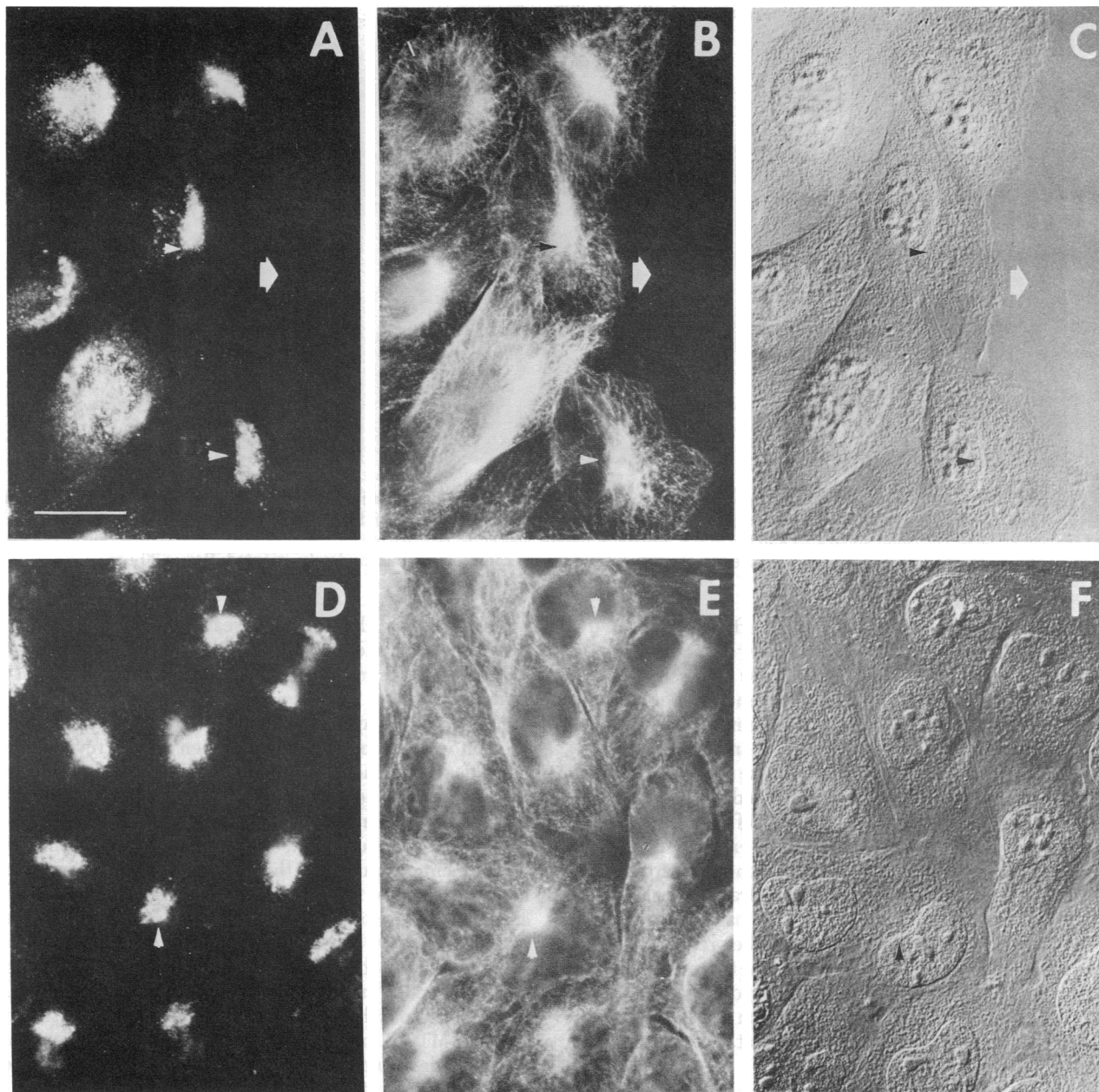


FIG. 1. NRK cells at the edge of an experimental wound (A–C) 5.5 hr after wounding and within the confluent portion of the same monolayer (D–F). The same cells were examined in Nomarski optics (C and F) and by double indirect immunofluorescence with antibodies that labeled the Golgi apparatus (A and D) and the microtubules and MTOC (B and E). \uparrow , Direction perpendicular to the edge of the wound; \triangleright and \triangleleft , same positions in the different fields. Note that, in A–C, the Golgi apparatus and the MTOC in all three cells at the wound edge are coordinately positioned forward of the nucleus in the general direction of the wound whereas, in D–F, the organelles in different cells are coordinately but randomly oriented. Bar = 20 μ m.

the classic experiments of Abercrombie *et al.* (2) on the motility of cultured cells, it was shown that cells moving on a substratum strewn with small particles could pick up these particles at the leading edge of the cells and transport them backward over their dorsal surfaces. Some particles were also transported backward on the ventral cell surface (3). These results when analyzed quantitatively suggested that new membrane mass added at the leading edge produced a backward flow of old membrane mass that carried along the attached particles, while old membrane mass was removed at other sites to keep the overall membrane mass constant. The experiments of Marcus (20) were also consistent with the incorporation of new membrane at the leading edge of a cell. The rapid reorientation of the Golgi apparatus

in the direction of subsequent cell movement could therefore be involved in the production of new membrane at the leading edge. It could also be involved in the directed secretion of extracellular matrix components, such as fibronectin and collagen (21) at the leading edge. These components could help induce the formation of successive transient adhesions of the leading edge to the substratum (22, 23) and thus promote cell movement. An orientation of the Golgi apparatus in embryonic cells *in vivo* has been shown (24, 25) to be coordinated with the directed secretion of components from these cells.

A role for the MTOC in cell motility has already been inferred from two other kinds of experiments.

(i) Observations of the “phagokinetic tracks” left by cells

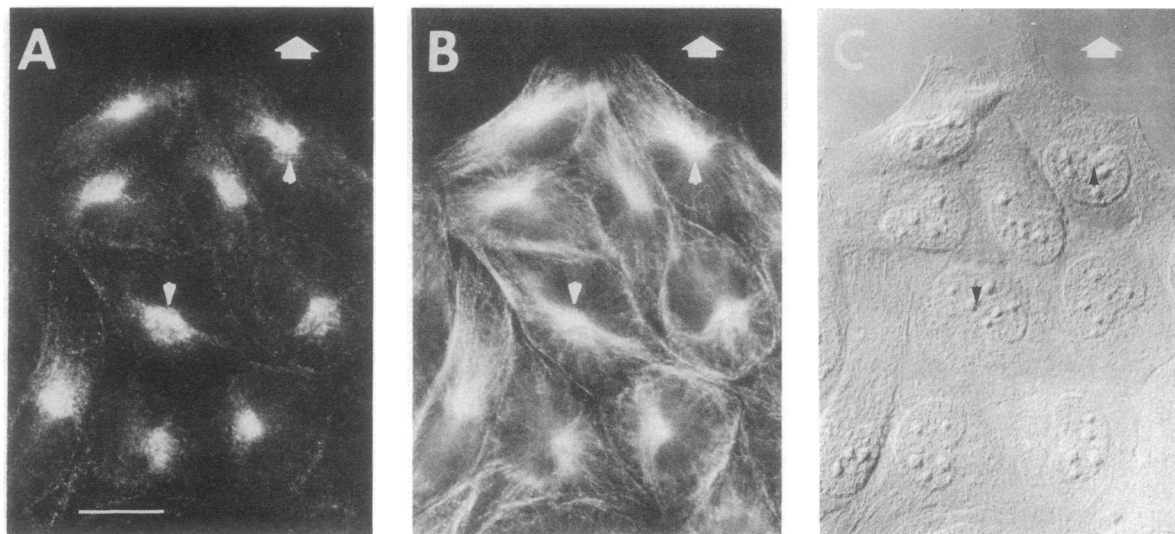


FIG. 2. Part of a sheet of NRK cells near the edge of a wound permeabilized and fixed 5 min after wounding. The same cells are shown in Nomarski optics (C) and immunolabeled for the Golgi apparatus (A) and for the microtubules and the MTOC (B). Symbols are as in Fig. 1. In the cells at the wound edge, the Golgi apparatus and the MTOC are coordinately positioned forward of the nucleus in the general direction of the wound whereas, in the confluent cells behind the wound edge, the two organelles are coordinately but randomly oriented. Bar = 20 μ m.

migrating on a solid substratum covered by a film of evaporated gold particles (7) showed that the two daughter cells arising from a cell division often migrated apart for some distance along a straight line. It was suspected that this pattern of migration was a reflection of the alignment of the centrioles in the two daughter cells that was imposed during cell division. In subsequent experiments, a number of isolated interphase cells whose phagokinetic tracks were recorded were then fixed and serially sectioned for electron microscopic localization of their centrioles. In the relatively few cells observed in these experiments, only a slightly larger incidence of centrioles positioned forward of the nucleus in the direction of the phagokinetic track, as compared with other orientations, was observed. The phagokinetic track, however, measures the overall direction of the previous migration of a cell but does not effectively record the transient cellular extensions and retractions that rapidly occur locally around the cell border. In view of the rapid reorientation of the MTOC in response to a signal for cellular extension that was observed in

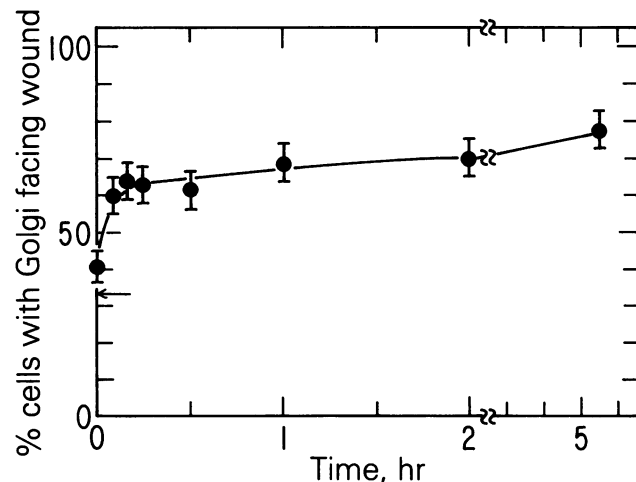


FIG. 3. After single immunolabeling of the Golgi apparatus, the percent of cells at the wound edge having their Golgi apparatus in the forward-facing 120° sector was measured at different times after wounding. Random orientation of the Golgi apparatus with respect to the wound edge corresponds to 33% on the ordinate (\leftarrow).

our experiments, the relatively weak correlation observed between the centriole disposition and the phagokinetic track of a cell in the experiments of Albrecht-Buehler and Bushnell (26) is not surprising.

(ii) By using a layer of neutrophils deposited on a millipore filter, it was possible to apply and then rapidly reverse a chemotactic gradient across the filter (27). The attached cells were fixed and serially sectioned for electron microscopy to localize the nuclei and centrioles with respect to the direction of the gradient. It was found that, within min after establishment of the gradient, in many cells the nuclei had moved rearward and the centrioles had moved forward in the gradient well before any cellular extension was observed. These results are closely similar to those we have obtained with the experimental wound technique in the absence of an applied chemotactic gradient.

What function might be served by reorienting the MTOC in the direction of subsequent cell movement? One possibility is that the MTOC might orient a cytoskeletal force-generating system in the cytoplasm to exert force against the leading edge of the cell. The cytoplasmic microtubules emanating from the MTOC are probably not themselves responsible for force generation, since the microtubule-dissociating agent colchicine disrupts the directed movement of cultured cells but not their amoeboid-like motile activity (4, 5). However, different elements of the cytoskeleton [e.g., intermediate filaments (17, 28, 29)] may interact with microtubules. A reorientation of the MTOC and microtubules may then serve to orient properly whatever cytoskeletal system is responsible for force generation in the cytoplasm so that it acts to move the leading edge forward.

On the other hand, the primary function of the reorientation of the MTOC may be to direct new membrane growth to the leading edge of the cell. Cytoskeletal elements oriented by the MTOC may serve as tracks along which vesicles derived from the Golgi apparatus are directed to the plasma membrane at the leading edge. In this scheme, no other force-generating function need be ascribed to the MTOC and cytoskeleton in cell motility (2). Such a functional relationship between the MTOC and the Golgi apparatus may be the reason why the two organelles are usually (12, 13), although not always (24), found in proximity to one another in interphase cells, whether motile or not.

Finally, the rapid reorientation of the MTOC and Golgi apparatus inside a cell at the edge of a wound raises questions about the molecular mechanisms that produce the reorientation and the nature of the transmembrane signal that activates these mechanisms. In some manner, the exposure of one edge of a cell while the rest of its perimeter remains in contact with neighboring cells in the monolayer, must provide a directional transmembrane signal that activates the mechanisms for reorientation. The effect of this signal may be to cause a transient change in the cytoskeleton in the region of the exposed cell edge that exerts a force on the MTOC and causes the fibrillar network, of which the MTOC is a part (12), to rotate toward the exposed edge. These speculations suggest that transmembrane and cytoskeletal events occurring in cells at the wound edge during the first minutes after wounding should be investigated in detail.

While this work was in progress, Gotlieb *et al.* (30) reported that, in a monolayer culture of endothelial cells from pig thoracic aorta subjected to the experimental wound technique, reorientation of the MTOC in the direction of cell extension was observed in cells at the edge of the wound within 4 hr after wounding.

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