

# The Structure of Cytoplasm in Directly Frozen Cultured Cells.

## II. Cytoplasmic Domains Associated with Organelle Movements

P. C. Bridgman, B. Kachar, and T. S. Reese

Laboratory of Neurobiology, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, Maryland 20205. Dr. Bridgman's present address is Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, Missouri 63110.

**Abstract.** The relationship between organelle movement and cytoplasmic structure in cultured fibroblasts or epithelial cells was studied using video-enhanced differential interference contrast microscopy and electron microscopy of directly frozen whole mounts. Two functional cytoplasmic domains are characterized by these techniques. A central domain rich in microtubules is associated with directed as well as Brownian movements of organelles, while a surrounding domain rich in f-actin supports directed but often

intermittent organelle movements more distally along small but distinct individual microtubule tracks. Differences in the organization of the cytoplasm near microtubules may explain why organelle movements are typically continuous in central regions but usually intermittent along the small tracks through the periphery. The central type of cytoplasm has a looser cytoskeletal meshwork than the peripheral cytoplasm which might, therefore, interfere less frequently with organelles moving along microtubules there.

A previous study of the structure of cytoplasm in whole mounts of cultured cells prepared by direct freezing and freeze substitution demonstrated that the basic organization of the cytomatrix prepared by these methods consists of an interconnected meshwork of several size classes of discrete filaments embedded in a granular ground substance (4). This view of cytoplasmic organization differs somewhat from earlier studies where filaments appear integrated with other cytoplasmic proteins in a continuous network called the microtrabecular lattice (5, 18, 19, 33). Furthermore, freeze-substituted whole mounts appear to contain a greater density of filaments and more numerous connections between filaments than are seen after deep etching of extracted cells (14).

The high concentration and spatial organization of filaments in the cytoplasmic meshwork contrasts, however, with the dynamic nature of the cytoplasm observed in living cells (17, 20, 23, 29). It is especially difficult to envision how organelles rapidly move through these dense meshworks, because many organelles are larger than the spaces between filaments. However, it became apparent to us in viewing whole mounts of cultured cells in the electron microscope that their cytoplasm is not homogeneous, but appeared to have two or more different domains. This was not a unique observation because heterogeneity in cytoplasmic or filament organization in conventionally fixed and thin-sectioned or whole mount cultured cells was well known (5, 6, 9, 22). Freeze-substituted whole mounts may, however, offer new insight into this aspect of cytoplasmic organization because of potentially superior preservation of structure (4). Therefore, a correlation between the distribution of the different domains

and the distribution and movement of organelles might clarify how organelles can move through the cytoplasmic meshwork.

The directed movements of organelles in cultured cells have been related to microtubules through mapping microtubule distribution and disrupting movement using microtubule depolymerizing agents (9). The mechanisms of microtubule-associated organelle movement in cultured cells, or its regulation and relationship to cellular processes such as secretion and endocytosis has, however, remained elusive, although the involvement of cytoplasmic structures called "microtrabeculae" in movement has been proposed in certain highly differentiated cell types (7, 8, 27). Results of a recent study (21), however, suggest that incomplete removal of water or solvent during critical point drying may contribute to the formation of a structural network with an appearance like that of the microtrabecular lattice. The introduction of video-enhanced light microscopical imaging of living cells, on the other hand, has permitted several recent advances (2, 11, 15, 28, 29, 32). For instance, membrane-limited organelles move along distinct tracks in very thinly spread areas of cultured cells that stain with fluorescent antibodies to tubulin (11) and some of these tracks appear to represent single microtubules (12). In addition, agents that affect microtubules influence the directed movements of fluorescently labeled endosomes and lysosomes in cultured cells (13), and single microtubules isolated from squid axons support directed movements of axoplasmic organelles (24, 31).

In spite of previous work, there is still little information that explains the various characteristics of organelle movement in cultured cells. Organelle movement in general has

been characterized as saltatory (20), though small organelles move continuously through axons (1). It has been suggested that the saltatory movements of larger organelles in axons could possibly be attributed to interactions with cytoskeletal elements in the path of movement (1). Indeed, axoplasmic organelles of all sizes move continuously and at the same rate along isolated microtubules, which raises the possibility that a single motile mechanism is present which powers both continuous and saltatory motions (24, 31). If this is indeed true, then an explanation might be found for why both continuous and intermittent movements occur in the cytoplasm of other intact cells.

To study the relationships of microtubule-associated organelle movements within the intact cytoplasm in more detail, we took advantage of the recent technical advances in video-enhanced light microscopy (2, 15) and combined it with our improved method of preparing whole mounts (4). The results of correlated observations of cultured cells suggest that their cytoplasm consists of two functional domains. A central perinuclear domain, which is rich in microtubules and exhibits Brownian motion of small particles as well as frequent continuous organelle movements, is surrounded by a peripheral domain that is devoid of Brownian motion. The peripheral domain stains heavily with an actin ligand and rarely supports organelle movements except along cylindrical extensions of the central type cytoplasm or, less frequently, in an intermittent manner along distinct tracks through thin areas that contain individual microtubules. These domains are identified and their cytoplasmic architecture is characterized by electron microscopic examination of whole mounts, thereby defining the structural characteristics of the cytoplasmic domain in these cells through which organelles move.

## Materials and Methods

### Cultures

Dissociated cell cultures were derived from the somites or ectoderm of *Xenopus laevis* embryos and contained fibroblasts and epithelial cells (4). Dissociated cells were plated onto either acid-cleaned No. 0 coverslips (22 × 22 mm) or Formvar-coated gold grids treated with polylysine or polyornithine. Cells were observed 1–5 d after plating.

### Video-Enhanced Light Microscopy

Cells on coverslips were observed in a simple chamber made by inversion of the coverslip onto a drop of fluid on a longer (22 × 60 mm) No. 0 coverslip; the edges were sealed with Valap (mixture of Vaseline, lanolin, and paraffin [11]). Coated electron microscope grids to be viewed in the light microscope were sandwiched between two No. 0 coverslips (22 × 22 mm and 22 × 60 mm) that were tacked together at their corners. A drop of medium was placed on the edge of the smaller coverslip (after tacking) to maintain the volume of the thin fluid layer.

Light microscopy was performed with either a Zeiss ICM 405 or Axiomat inverted microscope equipped with differential interference contrast (DIC)<sup>1</sup> and epifluorescence optics. Two different video image processing systems were used for enhancement of the image; a Hamamatsu C-1000 Chalcon camera connected to Hamamatsu minicomputer image analysis system with a frame memory (2), and a Dage-MTI Series 68 Newvicon camera connected to an Interactive Video Systems image processor with two frame memories. Real-time background subtraction was used to obtain a mottle-free image (2). Recordings were made on 3/4" video cassettes in real time (Sony VO 5600) or time lapse (Sony VO 9000). Still images were photographed directly from the video screen using a Polaroid CU-5 camera.

### Fluorescence Microscopy

To locate actin, cells were lysed, fixed, and labeled in a single step. A solution

containing  $4 \times 10^{-8}$  M rhodamine phalloidin (Molecular Probes Inc., Junction City, OR), 0.05% saponin, and 2% acrolein in a lysis buffer (30 mM Pipes, 25 mM Hepes, 10 mM EGTA, 1.7 mM MgSO<sub>4</sub>, pH 6.9) was applied to cells (previously rinsed once with lysis buffer) for 20 min. Control cells were lysed and fixed in the presence of unlabeled phalloidin ( $8 \times 10^{-8}$  M) (Sigma Chemical Co., St. Louis, MO), rinsed after 20 min, and then incubated with  $4 \times 10^{-8}$  M rhodamine phalloidin.

To visualize microtubules, cells were permeabilized and fixed by sequential dipping into cold (−20°C) acetone and then methanol. They were labeled with a mouse monoclonal antibody to alpha-tubulin (Amersham Corp., Arlington Heights, IL) followed by a fluorescein-conjugated second antibody (E-Y Laboratories, Inc., San Mateo, CA).

### Electron Microscopy

Cultures on grids were frozen by rapid injection of the grid into a rapidly stirred mixture of propane/ethane (3:1) cooled by liquid nitrogen (4). Freeze substitution and critical point drying were as previously described (4) except where noted.

Some cultures were fixed at room temperature before freezing in 0.5% glutaraldehyde and 0.2% tannic acid in 0.05 M Hepes buffer, pH 7.4. After 1 h the cultures were rinsed with buffer; they were then further fixed with 2% glutaraldehyde in the same buffer for 2 h at room temperature. Thin sections of freeze-substituted cells were made as previously described (4). Electron microscopy was performed on a JEOL-200 CX with a high magnification pole piece operating at 200 KV for whole mounts and 120 KV for thin sections.

## Results

Two contiguous regions of cytoplasm were distinguished in thinly spread cells examined by video-enhanced DIC microscopy. The first was a dynamic, organelle-packed central region surrounding the nucleus and the second a more static peripheral region that included the cell margins (Fig. 1). Peripheral regions typically lacked large organelles and contained networks of linear elements.

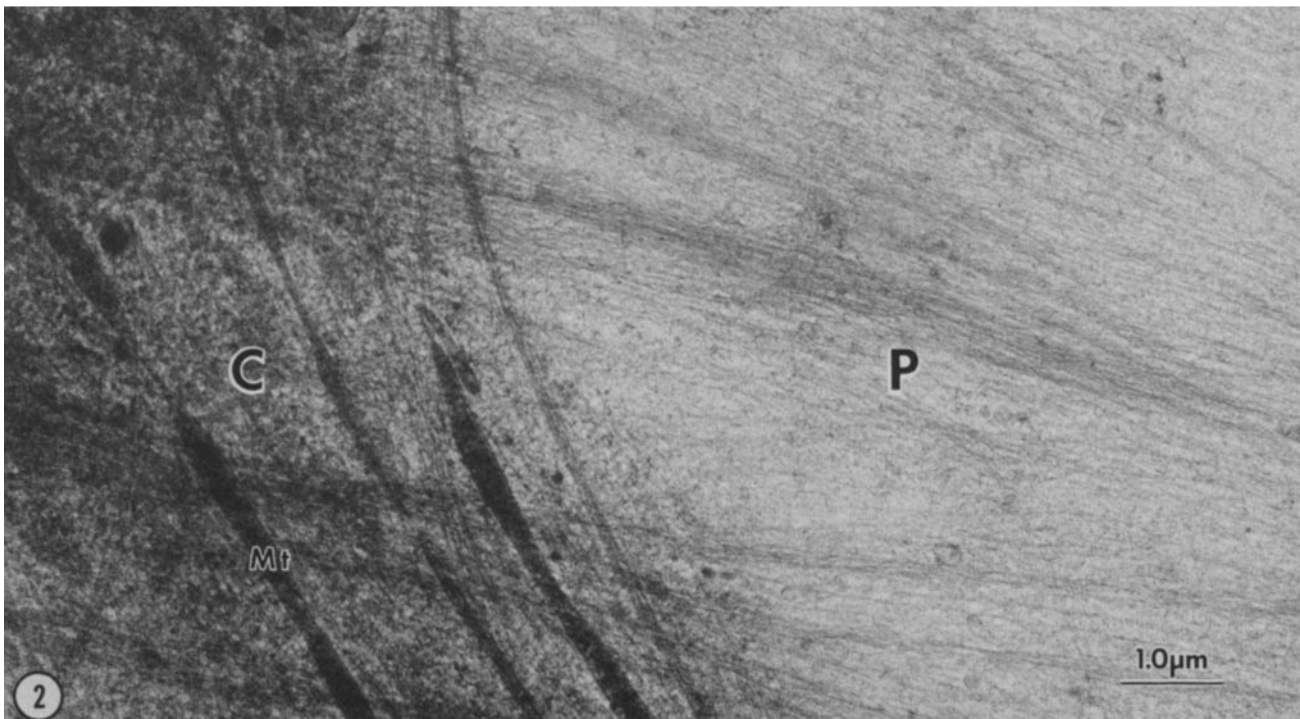
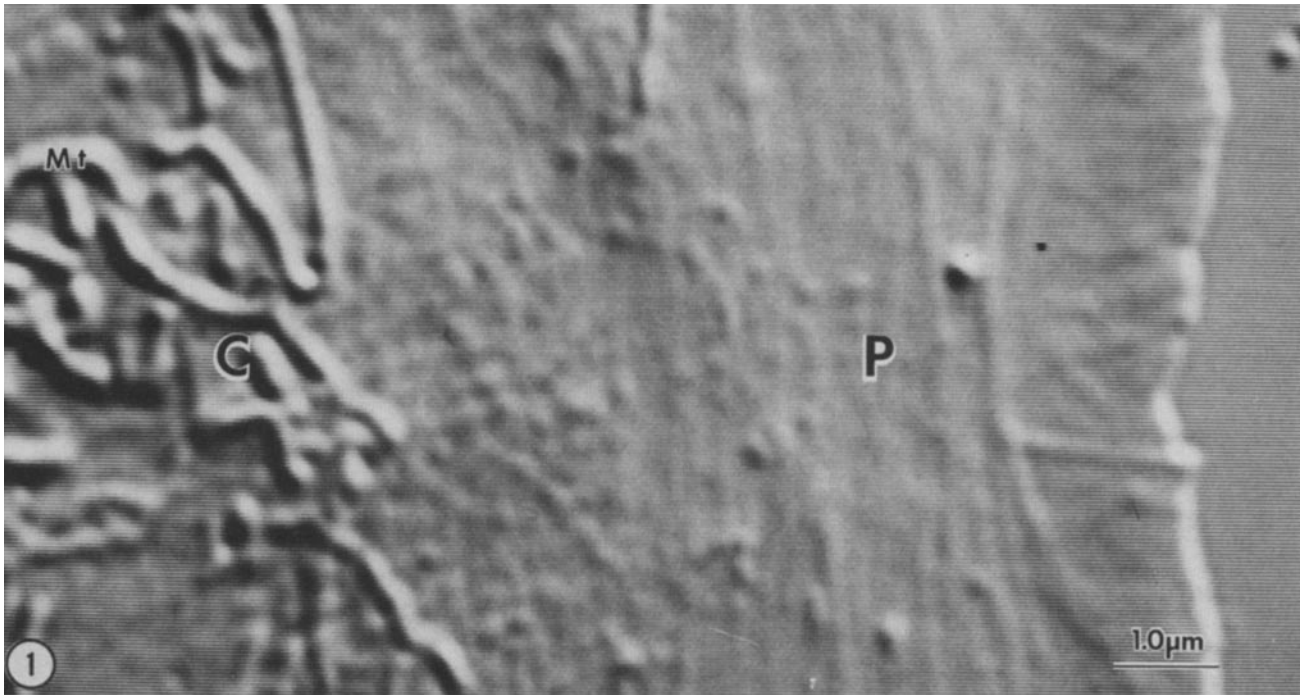
The two cytoplasmic regions identified by video-enhanced DIC microscopy were also distinguishable in direct frozen, freeze-substituted, critical point-dried whole mounts. The relatively thick (typically >1 μm thick) central region contained many mitochondria, Golgi particles, smooth and rough endoplasmic reticulum, and it was also packed with a granular material which included many ribosomes (Fig. 2). Stress fibers ran through central regions but were usually confined to the cytoplasm adjacent to the substratum (Fig. 2). The thin peripheral regions (typically 0.1 μm thick) contained a dense meshwork of filaments, stress fibers, and scattered vesicles (Fig. 2).

Table I. Particle Velocity

| Location             | Particle Size | Average Velocity ±S.D. | Range    | Number of Particles |
|----------------------|---------------|------------------------|----------|---------------------|
|                      | nm            | μm/s                   | μm/s     |                     |
| Central cytoplasm    | ≥500          | 1.40 ± 0.4             | 0.9–2.0  | 22                  |
| Central cytoplasm    | <500          | 2.45 ± 1.1             | 0.9–5.0  | 30                  |
| Peripheral cytoplasm | <500          | 1.21 ± .42             | 0.11–1.8 | 14                  |

Average velocity was determined by measuring the time it took a particle to move between two points on the video screen. Only tracks where movement was linear were chosen for measurement and only particles that moved at least 8 μm were included. Measurements on particles <500 μm in diameter are probably biased towards the larger sizes since the smaller particles (≤200 nm) were sometimes difficult to follow for such lengths. Results were compiled from recordings made on a single day on three different cells from a single batch of cultures.

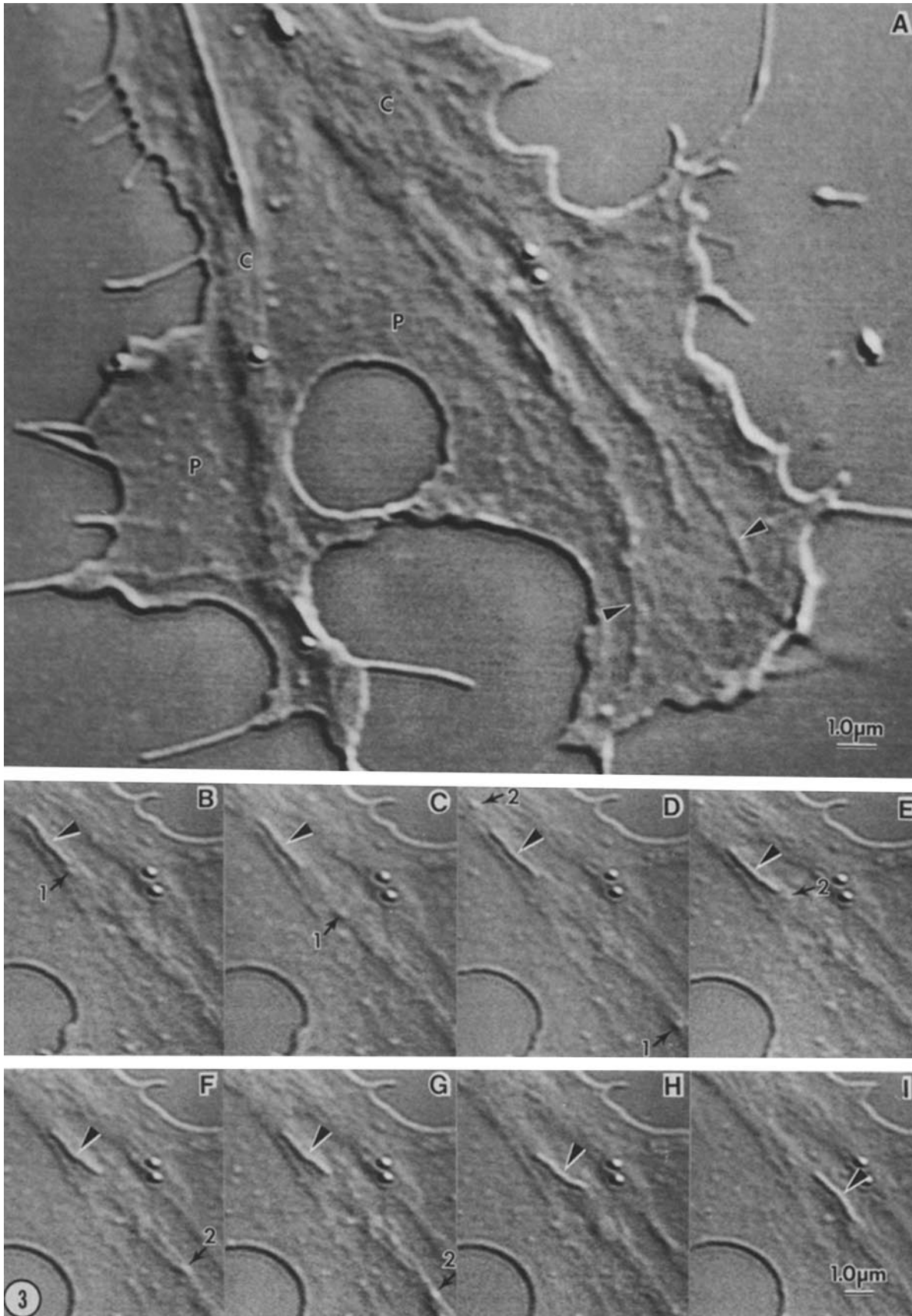
<sup>1</sup> Abbreviation used in this paper: DIC, differential interference contrast.



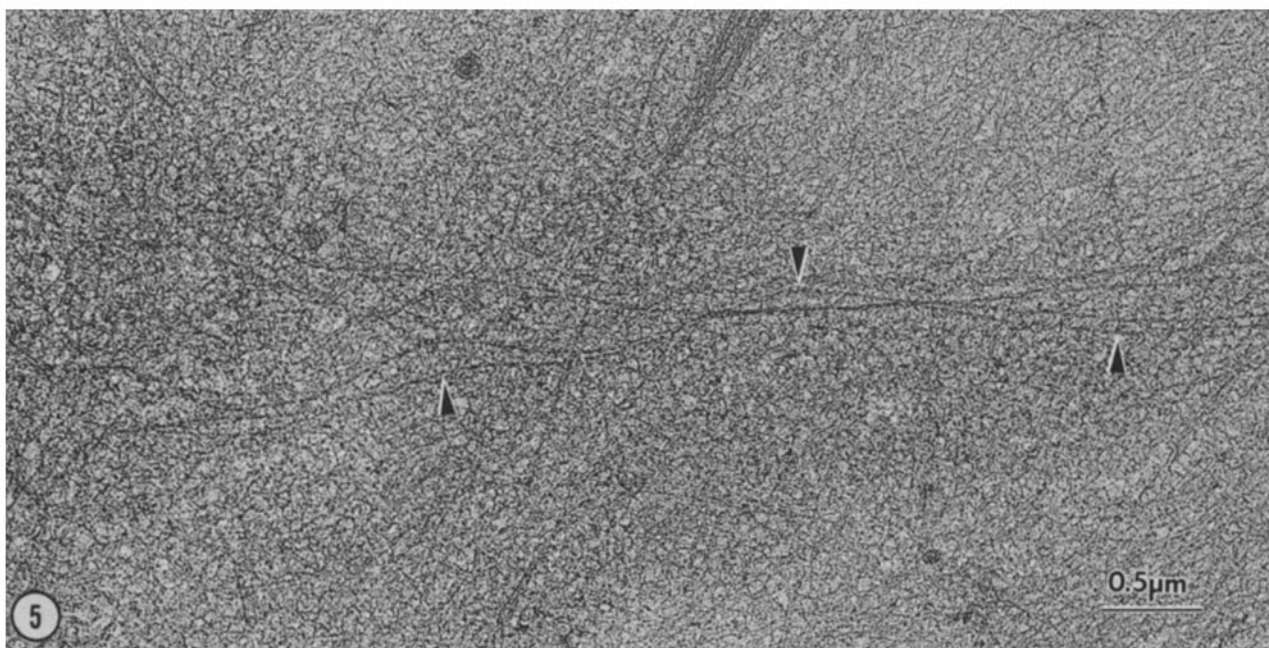
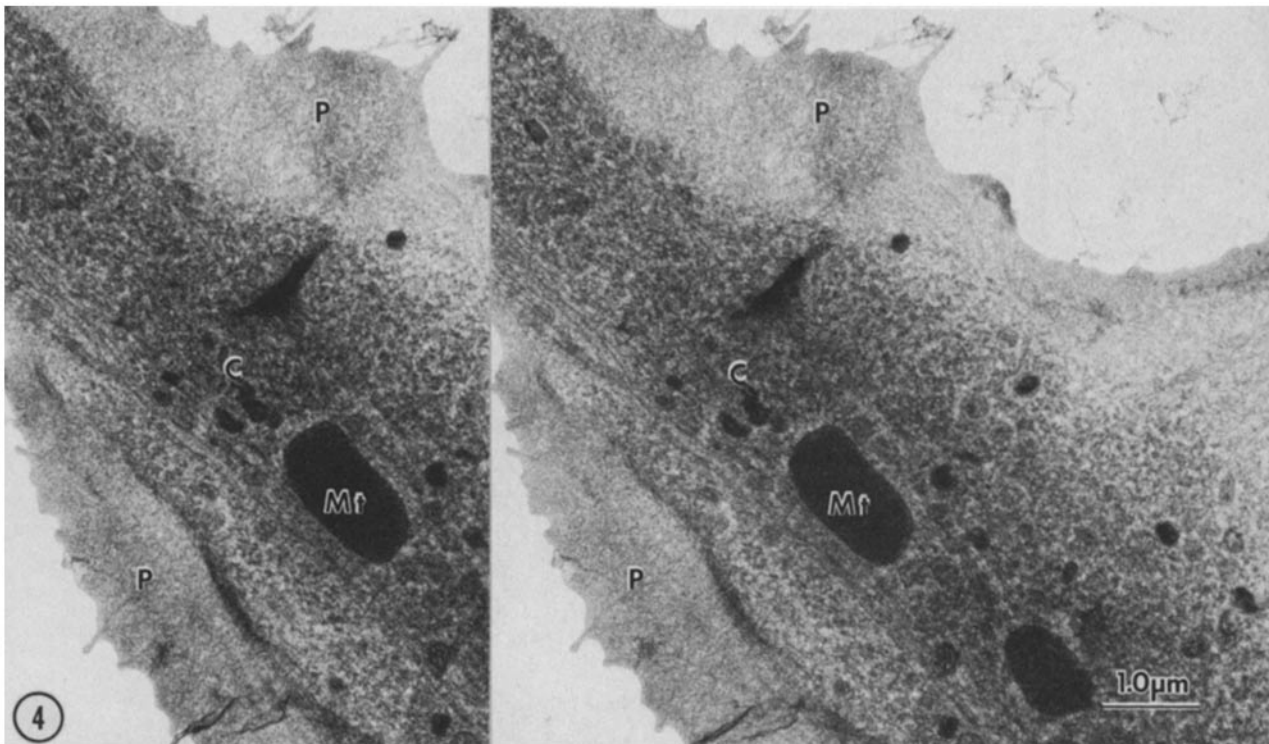
*Figures 1 and 2.* (Fig. 1) A video-enhanced differential contrast micrograph showing the perinuclear, organelle-packed central region (C) and the thinner largely organelle free peripheral region (P). *Mt*, mitochondrion. (Fig. 2) Low magnification view of a directly frozen, freeze-substituted whole mount showing the boundary between the thick central (C) region and thin peripheral (P) region. This is a different cell than that shown in Fig. 1. *Mt*, mitochondria.

Also apparent from video-enhanced DIC was that large cell processes contained a continuous core of the central type of cytoplasm which often narrowed down to individual tracks (Fig. 3). Central regions had three types of cytoplasmic movement that became apparent with high magnification and high gain video enhancement: (a) smooth, linear movements of medium to small (<500 nm in diameter) vesicular organelles

(Fig. 3); (b) short back-and-forth or intermittent movements of mitochondria and large organelles (>500 nm) (Fig. 3); and (c) a barely detectable background movement that appeared to be a combination of directed and Brownian movement of very small particles (<200 nm; it is not possible to show examples of Brownian motion because the very small particles were not detectable in still photographs taken from the video



*Figure 3.* A living cell showing the two different regions of cytoplasm as detected by video-enhanced DIC microscopy. (A) An extended process contains the central (C) type of cytoplasm, appearing as pathways along which organelles move, while the thin peripheral (P) type of cytoplasm lies in surrounding sheets. Extending from the central type of cytoplasm are distinct tracks (arrowheads) along which organelles move intermittently. (B–I) Examples of movements made by medium (1), small (2), and large organelles (mitochondrion indicated by the arrowhead). Time (in seconds): (B) 0:0, (C) 1:4, (D) 10:2, (E) 20:0, (F) 30:0, (G) 30:1, (H) 50:2, (I) 60:2.



Figures 4 and 5. (Fig. 4) A directly frozen, freeze-substituted whole mount showing a cell process that contains a core of the central (C) type cytoplasm bordered by peripheral (P) cytoplasm. *Mt*, mitochondria. (Fig. 5) Directly frozen, freeze-substituted whole mount showing a bundle of filaments (arrowheads) passing through a particularly thin portion of the peripheral region. The diameter and gentle curving of these filaments suggests that they are microtubules. A slightly darker-staining, irregularly shaped halo of granular material surrounds this filament bundle. It was rare in directly frozen cells to find areas sufficiently thin to show such detail.

screen). Organelles in peripheral regions, however, almost always had intermittent movements. Occasionally very small particles (<200 nm in diameter) made continuous movements in peripheral regions along particularly distinct tracks. Table I indicates the average velocity of organelles in the two different regions.

In whole mounts it was also apparent that large cell proc-

esses contained the central type of cytoplasm. In stereo micrographs, it was clear that the core of such processes were thickened, contained organelles, a granular material, and many parallel linear elements (Fig. 4).

Bundles of microtubules or, more rarely, single microtubules occasionally ran through peripheral regions (Fig. 5). Microtubules were identified on the basis of their diameter

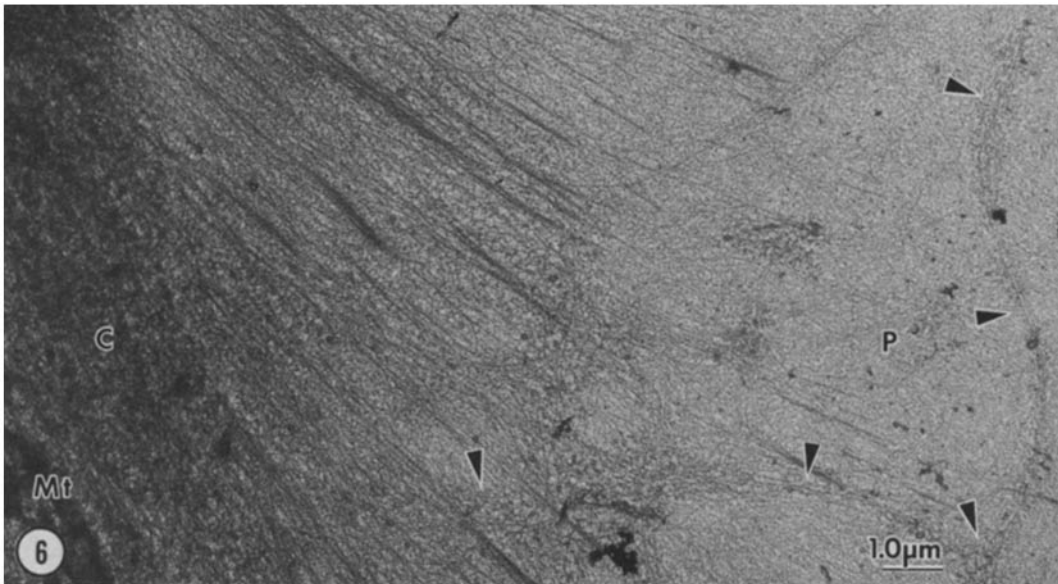


Figure 6. A fixed, freeze-substituted whole mount showing the continuity between central (C) regions and microtubule-associated extensions of central type cytoplasm into the periphery (P). Arrowheads indicate one continuous path from the central (C) region into the periphery (P). Mt, mitochondria.

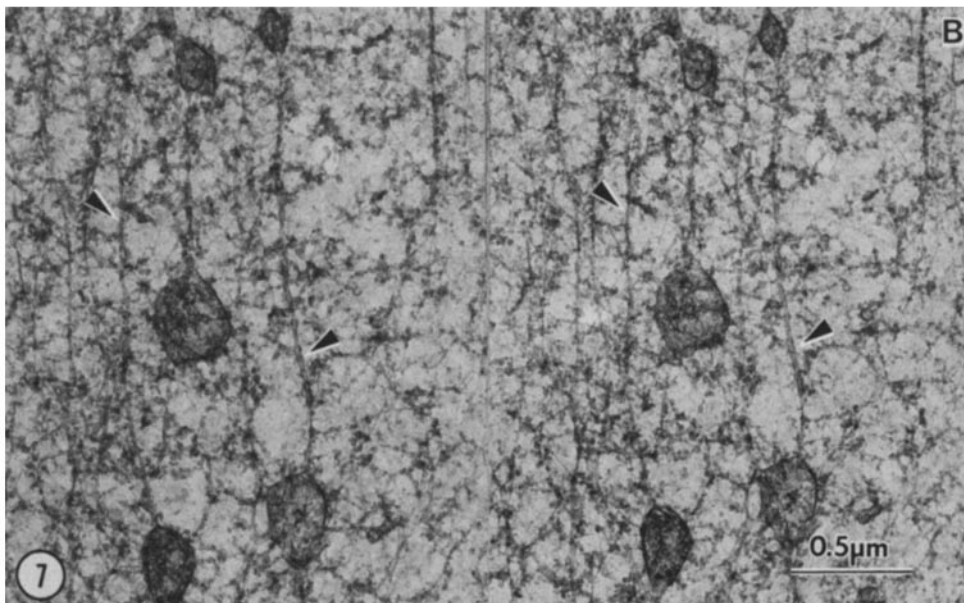
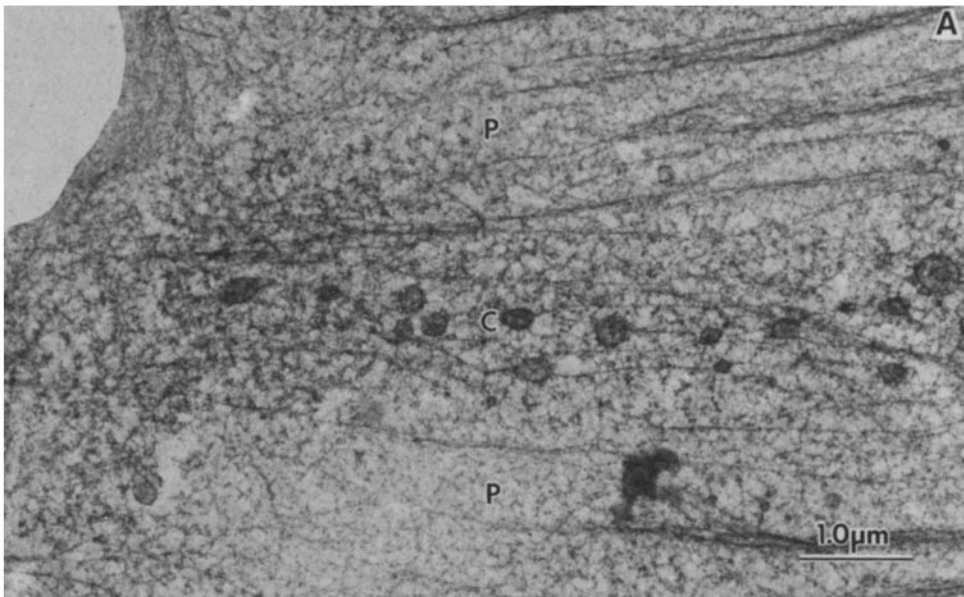
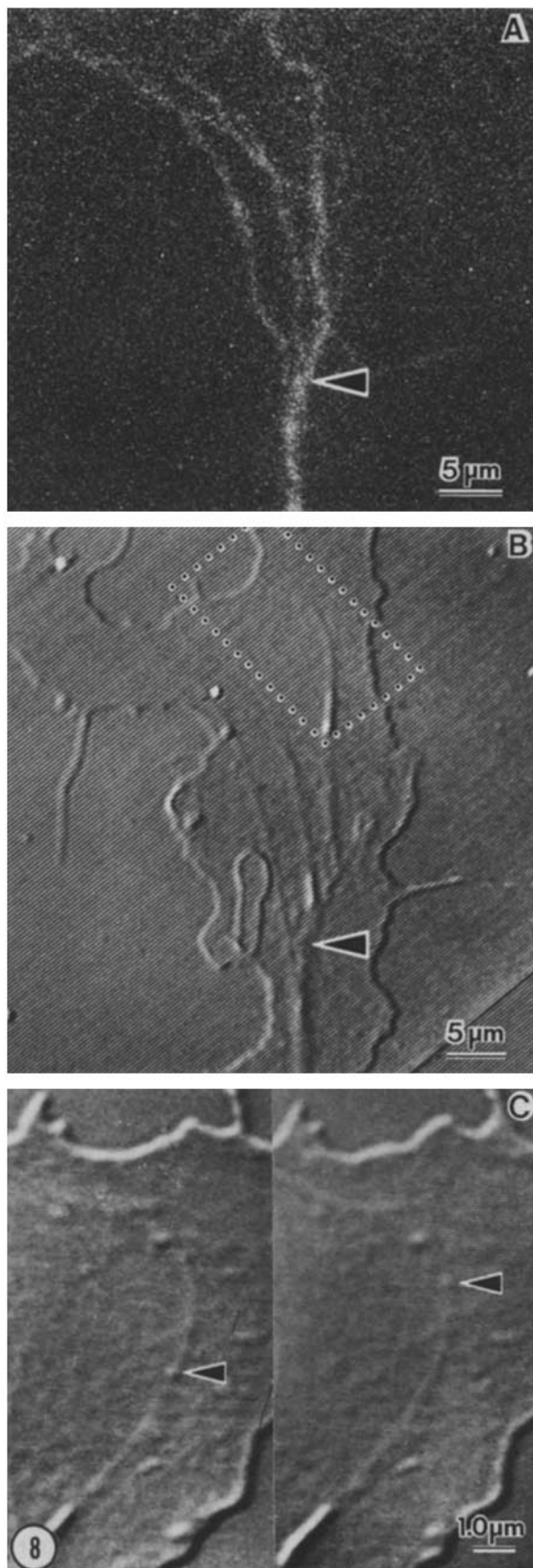


Figure 7. (A) A fixed and freeze-substituted whole mount showing an extension of central (C) cytoplasm into the periphery (P). (B) Stereo view at higher magnification of a portion of the cell shown in A. Microtubules (arrowheads) are closely associated with vesicular organelles, but fixation could have distorted the relationships.



**Figure 8.** The distribution of microtubules fluorescently labeled with anti-tubulin antibodies (*A*) correlated with the paths of organelle movements seen in video-enhanced DIC (*B*). At large arrowheads the bundle of microtubules splays into five separate tracks. (*C*) A

and characteristic appearance as long, gently curving filaments. The cytoplasm sometimes appeared denser along microtubule bundles because of a subtle increase in the amount of dark staining granular material (Fig. 5). The cell was also thicker in areas of increased density as determined by viewing stereo electron micrographs (not shown).

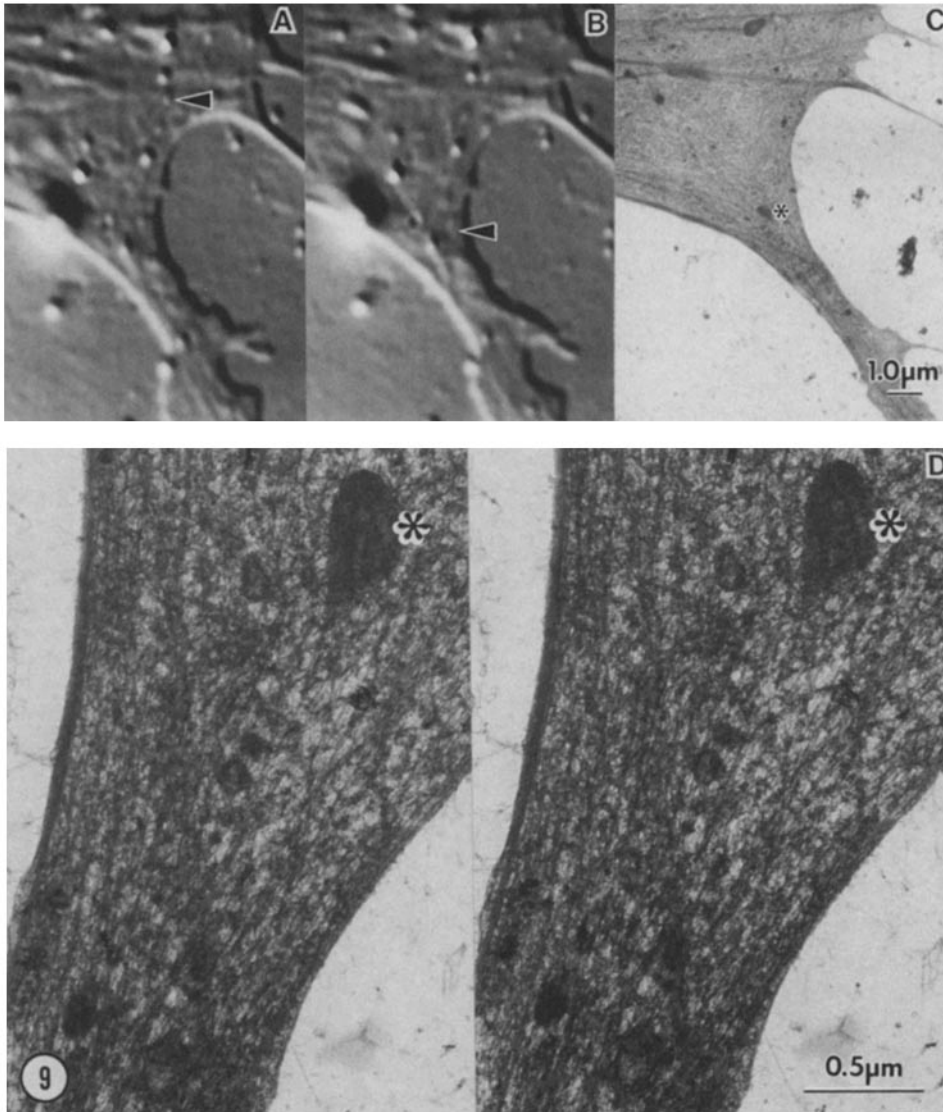
If microtubule bundles in peripheral regions were continuously traced, they were found to originate in central regions (Fig. 6). This suggests that areas of increased density in peripheral regions that follow microtubule bundles, or constitute the core of large cell processes, are extensions of the central type of cytoplasm.

To visualize cytoplasmic filaments more clearly, we fixed some cells with glutaraldehyde and tannic acid in a hypotonic buffer before freezing. Whole mounts prepared in this manner show much less granular material compared to directly frozen whole mounts (4); the reduced background density allows the organization of filaments and microtubules to be seen more clearly. Under these fixation conditions, some distortion of filament organization may occur, although we believe the distortion has been minimized (4). Distinctions between central and peripheral regions based on the concentration of organelles and background density of the cytoplasm were still detectable, although they were less prominent than in directly frozen cells (compare Figs. 4 and 7). However, microtubules and filaments could now be detected in thicker regions of central cytoplasm.

Prominent extensions of the central type of cytoplasm into peripheral regions were apparent in the fixed-frozen cells (Fig. 7). These extensions presumably correspond to the distinct pathways into the periphery along which continuous organelle movements occur because they had the same distribution whether viewed by video or electron microscopy. Stereo micrographs of such regions showed that microtubules were closely associated with vesicular organelles (Fig. 7). By correlating the paths of organelle movement with the fluorescence staining of microtubules labeled with a monoclonal antibody to alpha-tubulin (Fig. 8), we confirmed that the extensions of the central cytoplasm do indeed contain microtubules. In such preparations, it was also apparent that the finer tracks along which organelles move intermittently (Fig. 8*C*) correlate with single fluorescent microtubule elements (Fig. 8*A*).

To determine even more precisely the correspondence between tracks for organelle movement through peripheral regions of a cell and the structure of cytoplasm along these tracks, we made video recordings of organelle movements in cells grown on coated grids and then examined the same cells by electron microscopy of directly frozen or fixed-frozen whole mounts. Twenty-six cells directly frozen from the living state were re-identified in whole mounts in the electron microscope. Directly frozen specimens should allow the precise relationship of organelles to microtubules or other filamentous elements to be seen without the artifacts associated with initial chemical fixation. In each of the directly frozen cells, the path of organelle movement corresponded in stereo electron micrographs with thickened regions of the cytoplasm containing parallel filamentous elements, membraneous or-

sequence from a video recording of the cell shown in *A* and *B* showing that the movement of a small organelle (arrowheads) follows one of the tracks labeled with anti-tubulin antibodies. Area shown in *C* is indicated by the outlined rectangle in *B*. Elapsed time: 0.03 s.



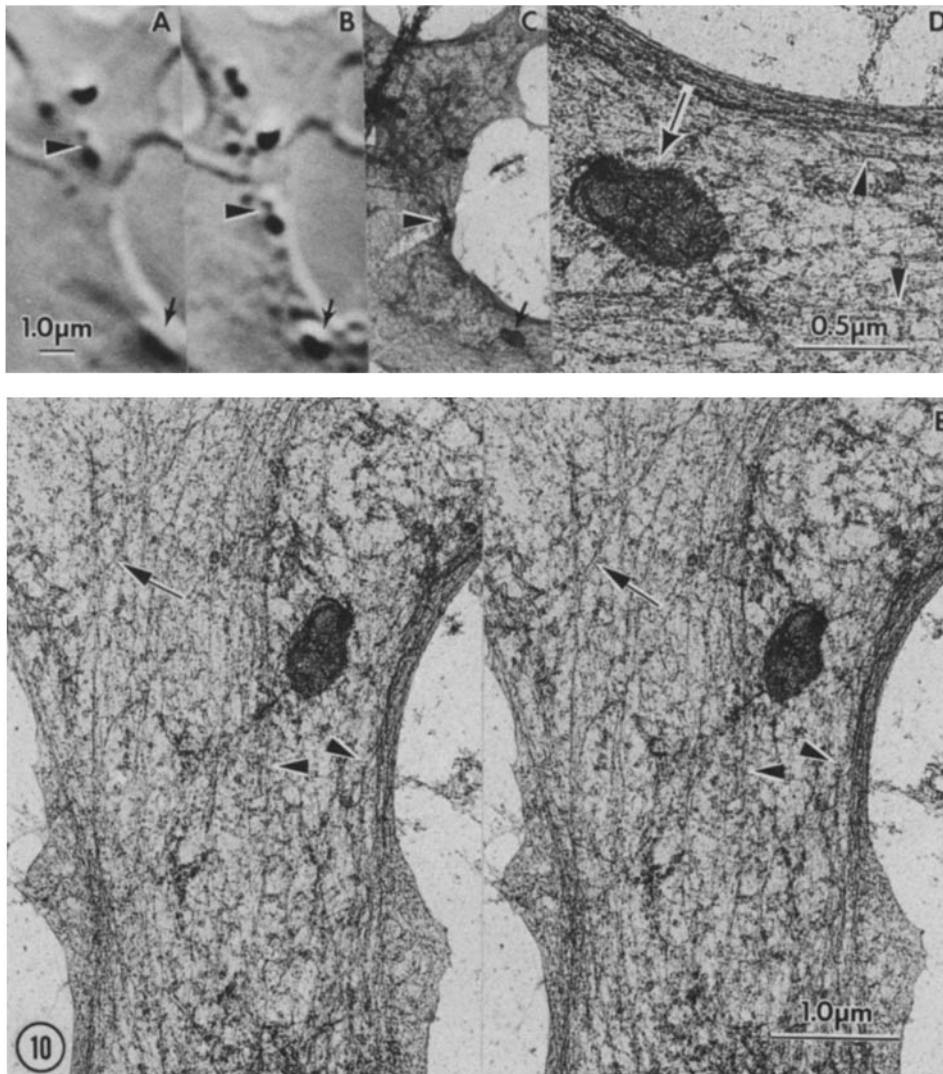
*Figure 9.* A directly frozen, freeze-substituted whole mount and the corresponding video-enhanced DIC image. (A and B) Sequence showing an organelle moving into a cell process. The image is somewhat compromised by the diffraction of light by the grid bars. (C) Corresponding whole mount view of the area seen by video-enhanced DIC. Approximately 90 s elapsed between recording and freezing. Some changes in organelle position and cell outline have occurred. (D) Higher magnification stereo pair of region through which organelles moved. Asterisk indicates organelle used for orientation (this organelle was stationary). Dark staining granular material largely obscures details, although a number of parallel filamentous elements can be seen oriented along the path through which movement occurred.

ganelles, and densely staining granular material (Fig. 9). However, in no preparation was the cytoplasm around the path of organelle movement sufficiently thin to identify clearly the filamentous elements on the basis of their diameter and appearance or to see their relationship with organelles. We, therefore, returned to using glutaraldehyde fixation before freezing because the densely staining granular material did not obscure the associated filamentous elements in fixed-frozen cells.

Seven cells that were fixed and then frozen were re-identified in the electron microscope. In these preparations the paths of frequent, continuous organelle movements also corresponded to thickened areas of the cytoplasm that contained parallel bundles of microtubules and other thinner filaments (Fig. 10). In places, these bundles formed a channel-like path through the dense filamentous meshwork in the peripheral areas of the cytoplasm. The paths of especially infrequent and intermittent organelle movement corresponded to single microtubules or sometimes microtubule pairs that passed through the thinner areas of the peripheral cytoplasmic meshwork without any noticeable increase in the thickness of the cell along their path (Fig. 10).

At this point, it became necessary to determine the overall distributions of various filaments that make up the cytoplasmic meshwork to see whether any were more concentrated in thin peripheral regions than in central regions. The distribution of f-actin, which is thought to be a major component of the peripheral meshwork (22), was compared to the distribution of microtubules by fluorescent-labeling techniques. As judged from staining with rhodamine-phalloidin, peripheral areas contained a meshwork of f-actin while central areas had little or no detectable staining suggestive of such a meshwork (Fig. 11). Central areas did, however, have prominent actin stress fibers that often extended into peripheral areas (Fig. 11). As expected, microtubules were present in greater numbers in central regions than in peripheral regions, as judged by immunofluorescent labeling of alpha-tubulin (Fig. 12). To test further the possibility that the meshwork is largely actin based and especially concentrated in peripheral regions, we lysed some cells with saponin (with phalloidin in the buffer to stabilize f-actin) before freezing them. The concentration of filaments in the meshwork was even greater (Fig. 13) than when cells were lysed without phalloidin (not shown, but see reference 4). The density of the meshwork was again greatest





**Figure 10.** (A) Video-enhanced DIC image of a region of a cell mapped for subsequent examination by electron microscopy. (B) Same area at time of fixation. Time elapsed was 35 s. (C) The same region of the same cell in a fixed and freeze-substituted whole mount. Arrowhead indicates organelle that was seen to move between A and B; unfortunately the cell has partially collapsed during critical point drying at this point. (D) Microtubules (arrowhead) and intermediate filaments in a thickened portion of the same cell. Many continuous particle movements along microtubule pathways seen by video-enhanced DIC occurred in the central region of this cell process. Arrows point to the same mitochondrion in all micrographs. Although this mitochondrion underwent shape changes, it remained stationary. (E) The thickening of the cytoplasm in the central region of the mapped cell is seen more clearly in a stereo view (the mitochondrion is the one indicated by arrows in A-D). Continuous particle movements occurred in the thickened, central region of this cell process while intermittent movements occurred in adjacent thinner areas. A path traveled by organelles moving in an intermittent manner corresponded to the position of a microtubule in a thin region (arrowhead, left). Arrowheads in center and right indicates position of the microtubules indicated in D where continuous organelle movements were observed.

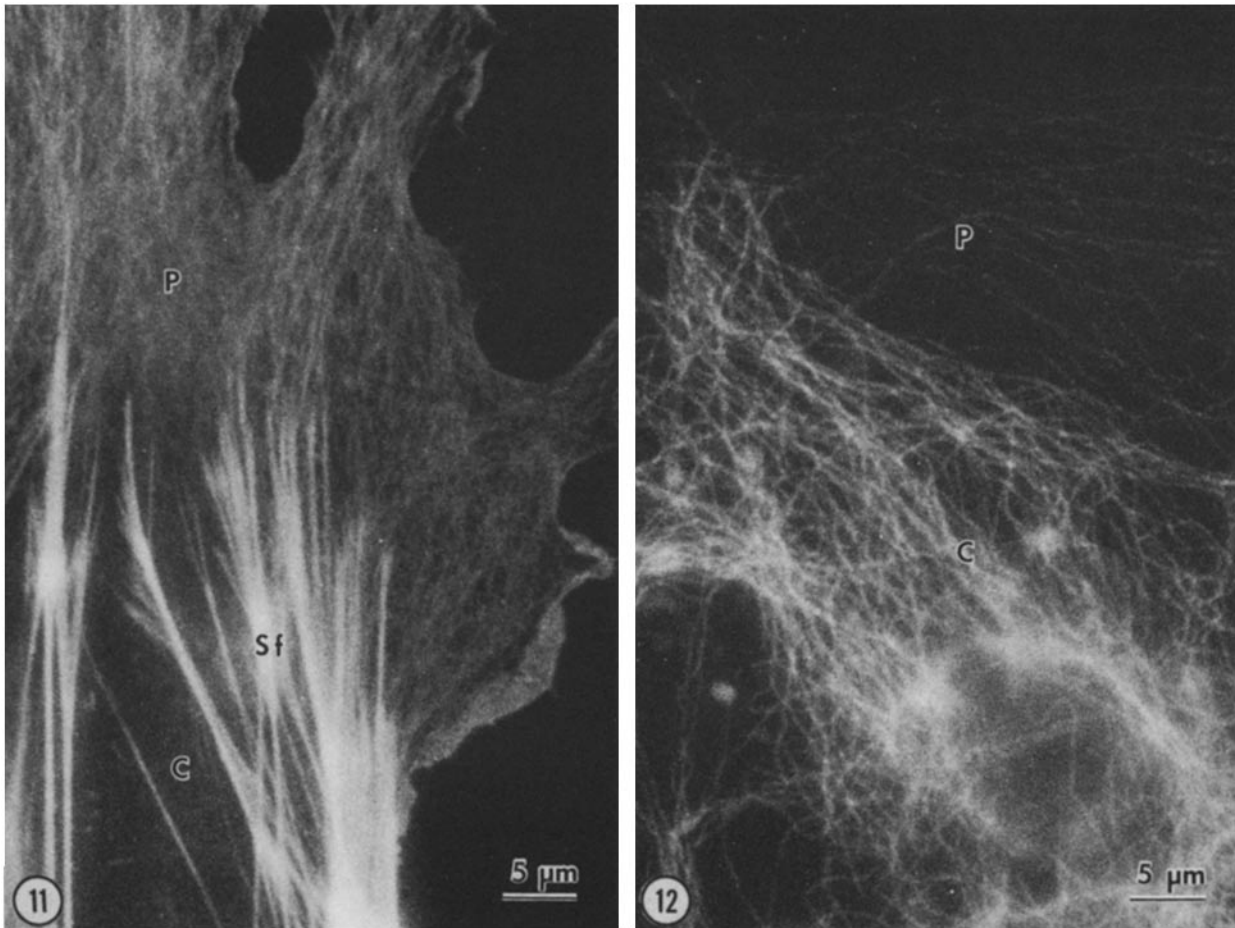
in thin peripheral regions. Microtubules typically passed through the meshwork in thin regions without any local changes in the organization of the actin meshwork but without making any regular contacts with the individual filamentous components (Fig. 13).

Although the paths of organelle movement seen with video microscopy clearly correspond with paths of microtubules seen in whole mounts, it was difficult to see in whole mounts the precise relationship between organelles and microtubules. The images in the fixed-frozen whole mounts were not to be relied on because the initial fixation could distort the relationships between organelles and microtubules. Thin sections were therefore made through some of the directly frozen cultures subsequently prepared by freeze substitution. Numerous examples of close appositions between organelles and microtubules were found in central areas of the cytoplasm (Fig. 14). Typically there was also a deformation of the microtubule at the point of organelle contact (Fig. 14). Similar appositions, while less frequent, could also be found in the peripheral regions.

## Discussion

Cultured fibroblasts or epithelial cells appear to contain two functional domains that can be defined by organelle movements which are typically either continuous or intermittent. These functionally defined domains correlate with structurally distinct regions distinguished in whole mounts by differences in the concentration of specific filamentous elements and cell thickness. Thick central regions are characterized primarily by the presence of many microtubules while thin peripheral regions are characterized by a dense actin meshwork. The paths of directed organelle movement remain associated with microtubules in both central and peripheral regions. The environment of a microtubule differs, however, in the different regions of the cell. It may pass through a loosely woven network of fibers and microtubules in central regions, join parallel arrays of microtubules in extensions of the central cytoplasm, and penetrate a tightly woven, interconnecting meshwork of microfilaments in peripheral regions.

Organelles moving along microtubule tracks might make



Figures 11 and 12. (Fig. 11) Fluorescence micrograph of a cell stained with rhodamine-phalloidin, an actin-specific probe. Fluorescence staining of a meshwork is apparent in thin peripheral (*p*) areas. Thick central (*c*) regions show no staining except along stress fibers (*Sf*). (Fig. 12) The distribution of microtubules in a cell as indicated by fluorescence staining using a monoclonal antibody to  $\alpha$ -tubulin. Central (*C*) areas have many more microtubules than peripheral (*P*) areas. Microtubules sometimes run in bundles away from the perinuclear central (*C*) region.

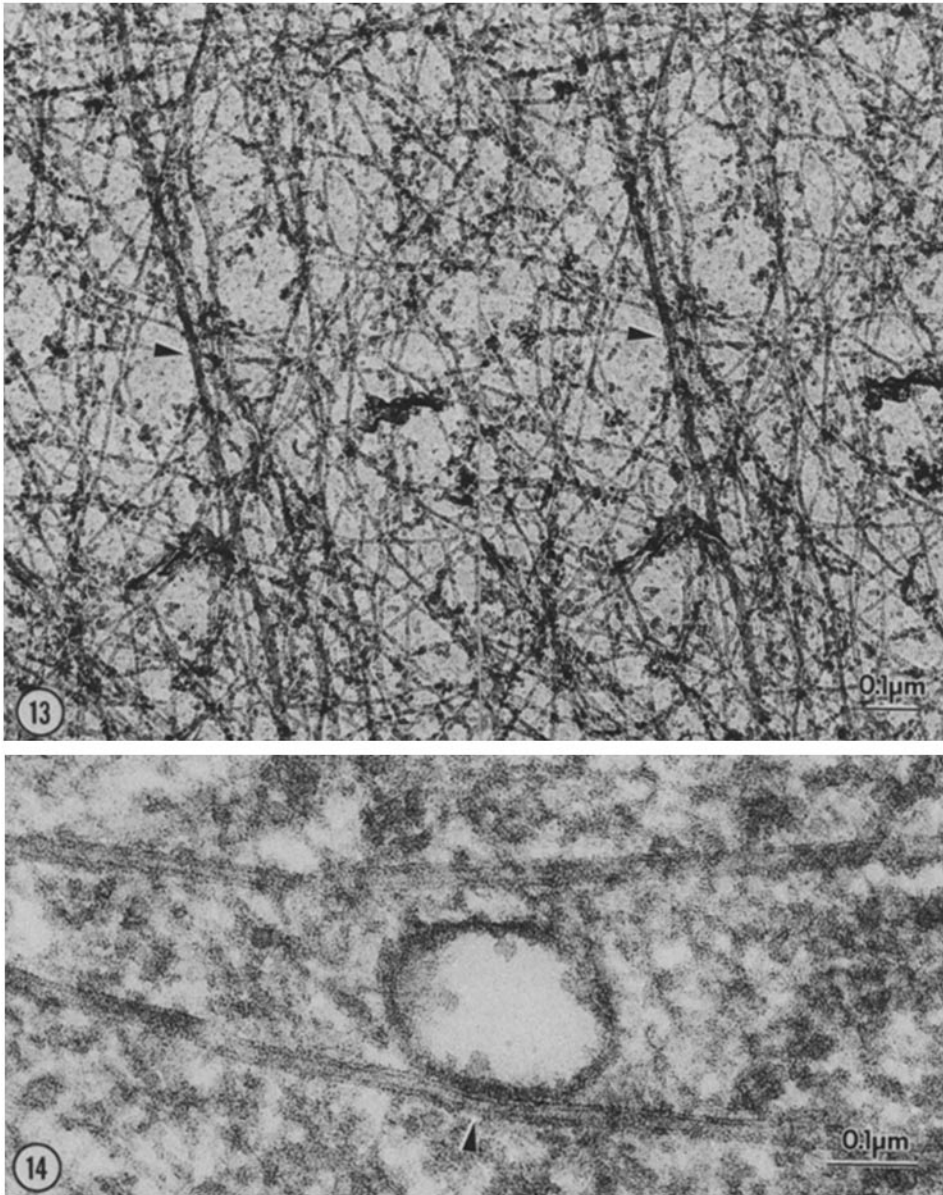
more collisions with crossing filamentous elements in thin peripheral regions than when traveling along the same microtubule tracks in central regions. Differences in the environment of the microtubules could explain why organelle movement is typically continuous in central regions while intermittent or saltatory in peripheral regions. Although we cannot rule out the possibility that intermittent movement arises from intermittent breakages in the link between an organelle and a microtubule, this possibility would not explain the correlation between each type of movement and the region of the cell in which it usually occurs.

Larger organelles, such as mitochondria, often make intermittent movements, even in the thick central regions, but their larger size could make collisions or interactions with other cytoskeletal elements likely even within the loose cytoplasmic meshwork in these regions. A similar explanation may apply to the saltatory movements of larger organelles in squid axoplasm (10, 26). The Brownian motion of the smallest particles in central regions might also reflect structural characteristics of this region of the cytoplasm if the loose network of filaments there leaves large enough fluid spaces between filaments to allow small organelles not attached to cytoskeletal elements to undergo Brownian movements.

Organelles can also move along tracks corresponding to

single microtubules (12) but such tracks are infrequent in the cultured fibroblasts and epithelial cells. No other filamentous structural elements are associated with these microtubule tracks, suggesting that these organelle movements depend only on interactions between the microtubule and an associated organelle. We infer from these close associations, and from the invariant associations between regions supporting organelle movements and their microtubule content in thicker regions of cytoplasm, that microtubules indeed constitute the tracks for movement in all regions of these cells. Images with higher resolution are needed to ascertain the nature of any connections between the organelles and microtubules. These connections appear to involve close apposition of the organelle to the microtubule in another microtubule-based system for moving organelles where the large organelles are deformed to maximize their area of contact with the microtubule (16). The microtubule bends around the contour of the organelle suggesting strong attractive forces between them; similar images are found in cells studied here.

The microtubule-associated movement of organelles in cultured cells is presumably the same as that supporting fast axonal transport (10, 26, 30). Extension of a central type of cytoplasm into the periphery of the cell along a discretely compartmentalized pathway could also explain the cyto-



Figures 13 and 14 (Fig. 13) Stereo pair showing a microtubule (arrowheads) passing through a dense meshwork of filaments in a thin peripheral area of a cell. The cell was extracted with saponin in the presence of phalloidin ( $0.7 \mu\text{g}/\text{ml}$ ) to stabilize microfilaments before freezing. Freeze substitution was in acetone with 10% acrolein followed by 10% glutaraldehyde as fixatives. No staining was performed during freeze substitution; after critical point drying, the cell was rotary shadowed with a thin layer of platinum/carbon to give contrast to the filaments. (Fig. 14) Thin section through a central region of a cell after direct freezing and freeze substitution. A membrane-limited organelle makes close contact (arrowheads) with a microtubule. The microtubule bends slightly at the point of contact.

plasmic organization in the greatly extended processes of nerve cells. Indeed, organelles are associated with the microtubule domains in axons in a manner analogous to that in the cultured cell (25). Organelles in the squid axoplasm also move along filamentous elements which can even be dissociated from the axoplasm (3, 31). These filamentous elements turn out to be single microtubules lacking any associated actin or other filaments (24). It is now becoming increasingly clear that fast axonal transport represents a special case of the more general mechanism for organelle transport present in most cells (1, 23). In particular, the fast axonal transport of membrane-limited organelles seems to be represented in non-nerve cells, such as those studied here, as directed or polarized movement of organelles between central and peripheral regions of the cytoplasm (20).

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