Mitotic Architecture of the Cell: The Filament Networks of the Nucleus and Cytoplasm

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ABSTRACT The skeletal framework of cells at the various stages of mitosis are prepared by extraction with nonionic detergent and examined by stereoscopic whole mount electron microscopy. The insoluble filament network remaining after the detergent-extraction and the depolymerization of microtubules is shown. The nonchromatin filament network of the nucleus, or nuclear matrix, becomes visible as the chromatin condenses at prophase. Filaments are associated with the chromosomes throughout mitosis. Parts of the chromosomes are associated with or are near the nuclear lamina at early stages. The nuclear lamina disappears at metaphase while chromosomes remain associated with filaments now continuous with the cytoplasmic network. Microtubules appear to be unnecessary for maintaining the chromosome position in these preparations since comparison of cells with and without microtubules shows no gross change in chromosome arrangement. The cellular filament network at metaphase and anaphase appears continuous from the plasma lamina to the chromosomes. The filament networks visualized here may be responsible for the prometaphase chromosome movement and participate in the formation of the midbody.

The interior architecture of cells as well as their external morphology and motility appears determined by complex structural networks of the cytoplasm and nucleus. The cytoplasmic network is clearly seen in detergent-extracted whole mounts. The filaments of the nuclear matrix, however, are best seen in whole mounts from which chromatin has been removed. These networks are complex, both in structure and composition, and contain hundreds of proteins in addition to the comparatively well known actin and intermediate filament proteins (8). It is possible that developmental alterations in cell morphology as well as gene regulation are mediated by changes in the organization of these structural networks. Of the several kinds of events that alter interior and exterior cell morphology, the most notable are the structural changes accompanying mitosis. In this report we will examine the behavior of the structural networks of the nucleus and cytoplasm in mitotic cells and how they relate to the formation and ordering of chromosomes.

Essentially every organelle within a cell is affected at mitosis (17). The major events visible, at the light microscope level, concern the formation of chromosomes and their subsequent movement (5, 13, 17, 18, 32, 36, 44, 51). In the very broadest of terms, three phases of mitosis may be defined. First, at prophase, chromatin is organized into chromosomes which are initially located near the nuclear envelope. At metaphase, the chromosomes migrate to the cell center at which time the nuclear envelope disappears. Finally, the spindle apparatus attaches to the kinetochore of the chromosomes, effecting their separation to the cell poles at anaphase, after which cells return to interphase morphology. Of these major organizational processes, only the last, the formation and function of the spindle, has been studied in great depth (for examples, see references 5, 18, 28, 44–46, 62). In this report we examine those aspects of chromosome formation and movement which are not mediated by the spindle but rather appear to be affected by the filament networks of the nucleus and cytoplasm.

The study of the filaments of mitotic cells uses detergent-extracted, cell whole mounts. In this procedure, cells growing on electron microscope grids are extracted with nonionic-detergent under buffer conditions which have been found to maximize structure preservation (10, 21, 29, 38, 39). The resulting skeletal structures are then viewed as a whole mount through the entire cell thickness by conventional transmission electron microscopy (6, 7, 20, 21, 30, 41, 48, 49, 53, 57–59, 60). There is no embedding plastic present and images of great clarity and high contrast are obtained even in the absence of heavy metal stains (24, 37). The network, composed of filaments and other detergent-resistant materials, can be viewed as a three-dimensional object by stereo microscopy. The extracted whole mount technique is applicable to relatively thin cells, which precludes examination of most cell types at mitosis since they round up excessively. This problem was solved in

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the present study in part by using cells of the rat kangaroo epithelial line (PTK) in an epithelial cell sheet which remain relatively flat during mitosis. Also, electron microscopes with objective lenses of unusually large depth of field were employed.

The views afforded by whole mount microscopy of detergent-prepared skeletons shown here can not be obtained by conventional embedded thin-section techniques. The embedded section affords clear images of membranes and nucleic acid-containing structures which stain well with heavy metals. However, three-dimensional filament networks are poorly imaged in sections, in part because the section is thin and filaments appear as mere specks, but also because the limited staining of filament proteins often results in their being masked by the electron scattering of the embedding plastic itself (24, 37). Nevertheless, early work with such thin sections, sometimes with the aid of unconventional staining procedures, has clearly indicated the presence of a protein-containing matrix in the nucleus of interphase cells (3). Also, conventional thin-section techniques have shown the presence of "fibrillogranular" material (4, 28, 35) associated with chromosomes of higher eukaryotes, and in a lower eukaryote a protein filament matrix has been identified in association with chromosomes (2). The present report extends these early observations and shows the intimate relation between the cytoplasmic filaments, the nuclear matrix filaments, and the chromosomes of mitotic cells.

MATERIALS AND METHODS

PTK_2 cells were seeded onto gold grids which had been parlodium-coated and carbon-stabilized as described previously (8). The cells were partially synchronized by the double thymidine block method. After removal of the second block, cells proceeded into mitosis, whereupon the grids were removed from growth medium and placed in cytoskeleton buffer for 5 min at 4°C. Cytoskeleton buffer contains 100 mM NaCl, 3 mM $MgCl_2$, 300 mM sucrose, 10 mM Pipes (pH 6.8), 0.5% Triton X-100, 1.2 mM phenylmethylsulfonyl fluoride (PMSF). When skeletons were prepared with microtubules removed, 5 mM $CaCl_2$ was added to the cytoskeleton buffer. Cells were fixed in cytoskeleton buffer made 2% with glutaraldehyde for 30 min at 4°C, washed, and postfixed in 1% OsO_4 in 0.1 M sodium cacodylate for 5 min at 4°C (osmium is necessary to stabilize the skeleton in the electron beam). When skeletons containing microtubules were prepared the 5 mM $CaCl_2$ was removed from the cytosekelton buffer, 0.2 μg/ml Taxol was added to stabilize microtubules, and the extraction was carried out at 23°C. This microtubule-stabilizing cytoskeleton buffer was made 2% with glutaraldehyde for fixation which was carried out for the first 10 min at 23°C and then lowered to 4°C for an additional 20 min; this was followed by postfixation with 1% OsO_4 for 5 min at 4°C. The grids were then processed as described below. The skeletons, still attached to grids, were dehydrated through a series of increasing ethanol concentrations and dried through the CO_2 critical point. Next, some grids containing extracted cells were coated with a thin layer of carbon which further stabilizes the structure against movement in the electron beam. Whole mounts were examined in the following electron microscopes: JEOL JEM 100B, ISI LEM 2000, and a AEI EM7-II HVEM (1.2 million volts).

RESULTS

Whole mount microscopy of detergent-extracted cells is suitable for relatively flat cells. At mitosis, most cells become round, and hence, too thick and dense for whole mount microscopy. One partial solution to this difficulty has been to use cells which remain relatively flat during mitosis. The rat kangaroo kidney epithelial cell lines, PTK_1 and PTK_2, have been used previously in studies of mitosis, for this reason (for example, see references 23, 44, 45). Apparently, when these cells are in epithelial sheets, sufficient connections to neighboring nonmitotic cells persist through mitosis so as to maintain cells in a relatively flat morphology. Even so, cell thickness exceeds the depth of field of conventional short focal length transmission electron microscopes and renders microscopy difficult or impossible. The micrographs shown below were made possible by the significantly greater depth of field afforded by long focal length electron microscopes such as the 100 kV, ISI LEM 2000, and the 1.2 MEV, AEI EM7-II.

The PTK_2 epithelial cells, used in this study, are grown in subconfluent colonies on Parlodium-covered, carbon-stabilized gold grids. Cells were synchronized by a double thymidine block, and a mitotic index of ~20% was achieved without the use of mitotic inhibitors. This double thymidine block procedure was specifically chosen to enrich the mitotic index while avoiding inhibitors which might alter the cell's filament network. Furthermore, all stages of mitosis are obtained which is not the case when metaphase-blocking agents are used.

When the mitotic index of the cells on grids reaches a maximum, they are removed from the culture medium, washed, and then extracted with cytoskeleton buffer containing Triton X-100 (8). Since we desired to prepare the cell filament network both with and without the spindle microtubules, our standard cytoskeleton buffer was modified. When the spindle microtubules were to be retained, the microtubule-stabilizing drug Taxol (47, 55) was added to the buffer and the extraction performed at room temperature. When microtubules were to be removed, our standard extraction procedure, which uses low temperature to depolymerize microtubules, was employed to remove most microtubules. In addition, to be certain that mitotic spindle microtubules were completely depolymerized, we supplemented the cytoskeleton buffer with 5 mM Ca^{++}. The combination of millimolar amounts of calcium ions and extraction at 4°C is reported to completely depolymerize microtubules (19, 31). Neither the extraction with detergent nor the consequent depolymerization of the spindle has any perceptible effect on the gross morphology of chromosome arrangements, when viewed at the light or electron microscope level (see below). This is especially notable when chromosomes are arranged at the metaphase plate or near the spindle poles in anaphase where their characteristic arrangements appear relatively unperturbed by the extraction procedure. Clearly, nonspindle elements of cell structure serve to maintain chromosome position during extraction, and these elements become apparent when the detergent-extracted whole mounts are viewed in the electron microscope.

Several different images of detergent-extracted cell whole mounts have appeared in the literature. Although all cells show a complex three-dimensional anastomosing filament network in the cytoplasmic space, the precise morphology is very dependent on extraction conditions and cell type. The procedure developed in this laboratory uses an ionic strength and pH close to physiological. The resulting skeleton morphology appears richer in filaments, and biochemical analysis indicates greatest polyribosome retention (10, 38, 39). Recent biochemical findings suggest strongly that the "skeleton" (cytoplasmic and nuclear) and "soluble" proteins are biochemically distinct with little cross contamination (8, 38, 39). An example of the comparatively dense network obtained under our conditions in well-spread 3T3 fibroblasts is shown in Fig. 1 *a*. As mentioned above, the cytoplasmic skeletal structure is also very much a function of cell type (39). The PTK_2 cells used in this study are unusual in having the densest concentration of detergent-resistant cytoplasmic filaments as well as detergent-resistant nonfilamentous structures encountered so far. The example in Fig. 1 *b*, at the same magnification as Fig. 1 *a*, shows the closely spaced, anastomosing filaments with polyribosomes attached as well as the lamella structures whose nature is unknown. Because of this density of cytoplasmic structures in PTK_2 cells, many of the low magnification pictures that follow are unable

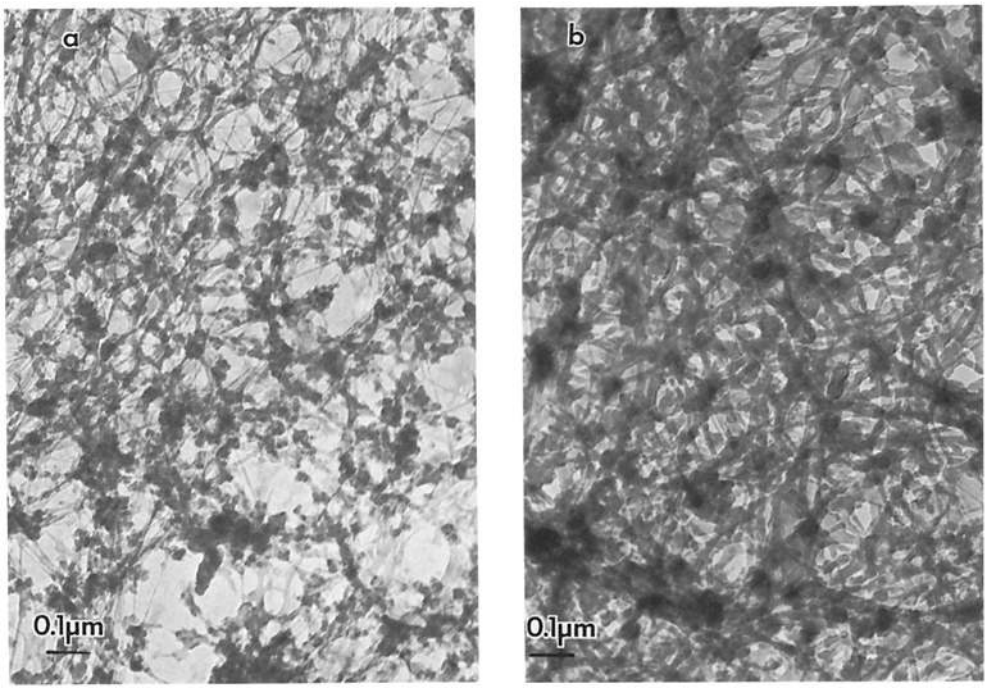

FIGURE 1 Comparison of the cytoskeleton of detergent-extracted 3T3 fibroblasts and PTK_2 epithelial cell whole mounts. Cells grown on gold grids were detergent-extracted as described in Material and Methods. Extraction was performed in the cold in cytoskeleton buffer containing 5 mM $CaCl_2$ to depolymerize microtubules. Extracted cells were fixed, dehydrated and then dried through the CO_2 critical point. *a* is a 3T3 cell, *b* is a PTK_2 cell. Both micrographs are at the same final magnification. Note the greater number of filaments per unit area in the PTK_2 cells. These micrographs were obtained on the JOEL JEM100B electron microscope. × 53,000.

to resolve the individual filaments which can be seen in the higher magnification micrographs.

The extensive reorganization of the cellular structural network at mitosis is best appreciated by first examining the interphase cytoplasmic network and nucleus obtained by extraction of PTK_2 cells with nonionic detergent in the cytoskeleton buffer. Such a whole mount with the typically opaque nucleus is shown in Fig. 2. The PTK_2 cells used in this study have relatively flat, thin nuclei whose chromatin arrangement allows some penetration by the electron beam in less dense areas of the nucleus. The entire interior nuclear structure of the interphase nucleus has been seen previously only by the removal of chromatin with nuclease and high salt. However, mitotic cells provide a natural means of reorganizing the chromatin. We shall see here that the chromatin condensation at early prophase permits views of some of the interior nuclear structure without resorting to harsh extraction techniques.

The earliest detectable nuclear alteration in preparation for mitosis is the condensation of chromatin at early prophase. As the chromatin reorganizes, spaces devoid of chromatin appear within the nucleus and nuclear matrix filaments can be glimpsed within these spaces (Figs. 3 and 4). In these micrographs the cell's microtubules have been removed by calcium ions and low temperature during the detergent-extraction.

Initially, the overall shape of the nucleus, delimited by the nuclear lamina, remains unchanged. Chromatin, together with associated chromatin fibers, begins condensing in a few regions where chromatin-containing "cores" are formed. From these "cores" radiate thick "cords" of chromatin which in some regions appear to consist of underlying filaments composed of supercoiled nucleoprotein or protein filaments or both. Spaces within the nucleus, now devoid of chromatin, can be seen to contain filaments ranging in size from 3 to 30 nm (Figs. 3, 4, and 5). The 3-nm filaments are the clearest candidates for nuclear matrix components, since they have been reported in chromatin-depleted matrix preparations (8, 9) and are smaller than the expected size of chromatin fibers.

The condensation of chromatin continues so that by late-prophase recognizable chromosomes are formed. These are

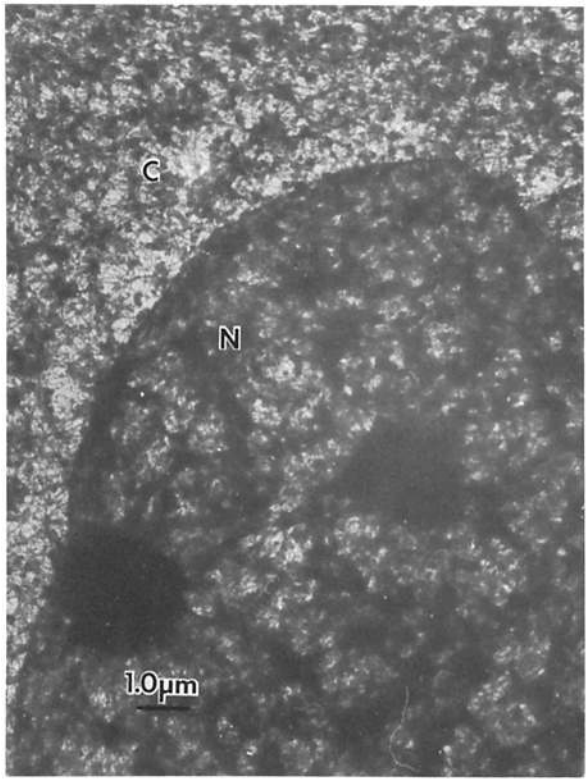

FIGURE 2 Interphase nucleus of a detergent-extracted PTK_2 whole mount. Cells were prepared as described in the legend to Fig. 1. The interphase PTK_2 nucleus has a relatively flat nucleus and allows some penetration of the electron beam. *N*, Nucleus. *C*, Cytoskeleton. This micrograph was obtained using the ISI LEM 2000. × 6,600.

seen in the stereo micrograph of Fig. 5. The nuclear lamina is still present and the stereo view shows the chromosomes arranged at the nuclear periphery with a few chromosome arms extending into the center of the nucleus which is otherwise relatively free of chromatin. The chromosomes are proximally surrounded by granular or fibrogranular material. Since this

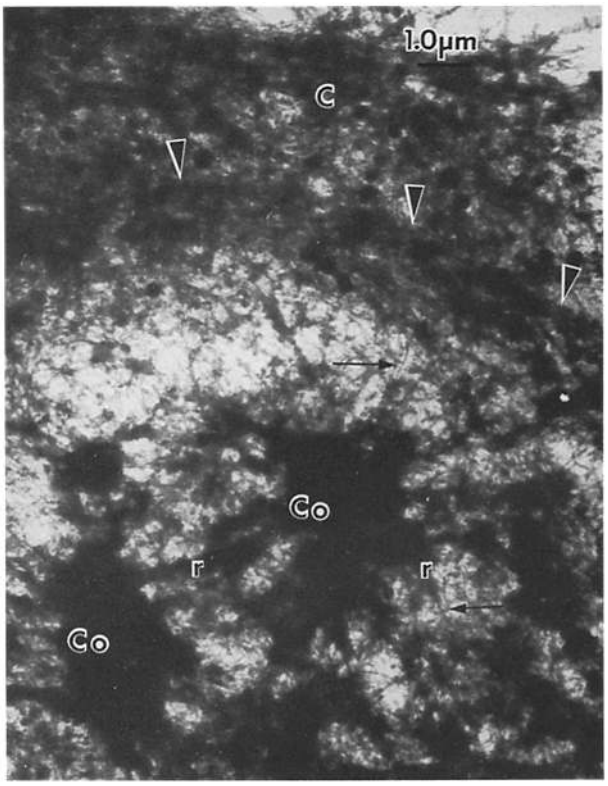

FIGURE 3 Early prophase nucleus of a detergent-extracted PTK_2 whole mount. Cells were prepared as described in the legend to Fig. 1. This micrograph is exposed to illustrate details of the filament network in the nucleus which causes the cytoskeleton to be overexposed. Arrowheads indicate the position of the nuclear lamina. *Co*, Condensing core of chromatin. *r*, Cords of chromatin in the form of radiating arms. The arrow indicates filaments underlying the chromatin in the radiating arms. This micrograph was prepared using the ISI LEM 2000. × 6,600.

material is not observed after prophase, it probably represents the last of the condensing chromatin. In the large spaces between the chromosomes, filaments devoid of granular material can be observed that are possible candidates for nuclear matrix filaments. The stereoscopic view in this micrograph also shows these nuclear matrix filaments joining with the chromosomes themselves. The nuclear lamina, which forms the nuclear matrix boundary, is visible in whole mount as the nuclear periphery curves into the plane of focus. Nearer the cell's center, the sheetlike structure of the nuclear lamina rises out of the plane of focus and ceases to form a distinct image.

Another view of the prophase nucleus is shown in Fig. 6. Here again, the microtubules have been deliberately removed by the presence of calcium ions and low temperature during detergent-extraction. Condensed chromosomes appear to be undergoing the initial positioning for the eventual formation of the metaphase plate. In this micrograph, one of the chromosome arms extends into a bleb of the nuclear lamina. Chromosome edges are fuzzy at this magnification which appears to be due to chromatin strands that extend for short distances from the chromosome arm itself. Once chromosomes are positioned, the spindle microtubules are assumed to exert the tension which partitions the chromosomes to opposite cell poles. However, although the spindle structure is necessary for the polar movement of the chromosomes, these microtubules are not necessary for maintaining chromosome position in the skeleton structure. Once the detergent extract is made, the chromosome position remains unchanged in the presence or absence of the spindle microtubules. Fig. 7 shows the anaphase position of chromosomes in a skeleton whole mount. In this case, the microtubules have been stabilized by adding Taxol to the cytoskeleton buffer and extracting at room temperature. Fig. 8 is a later anaphase whole mount of cells extracted with calcium and at low temperature. In both cases, the chromosomes remain in their anaphase positions, presumably supported by the network of filaments that surround and contact

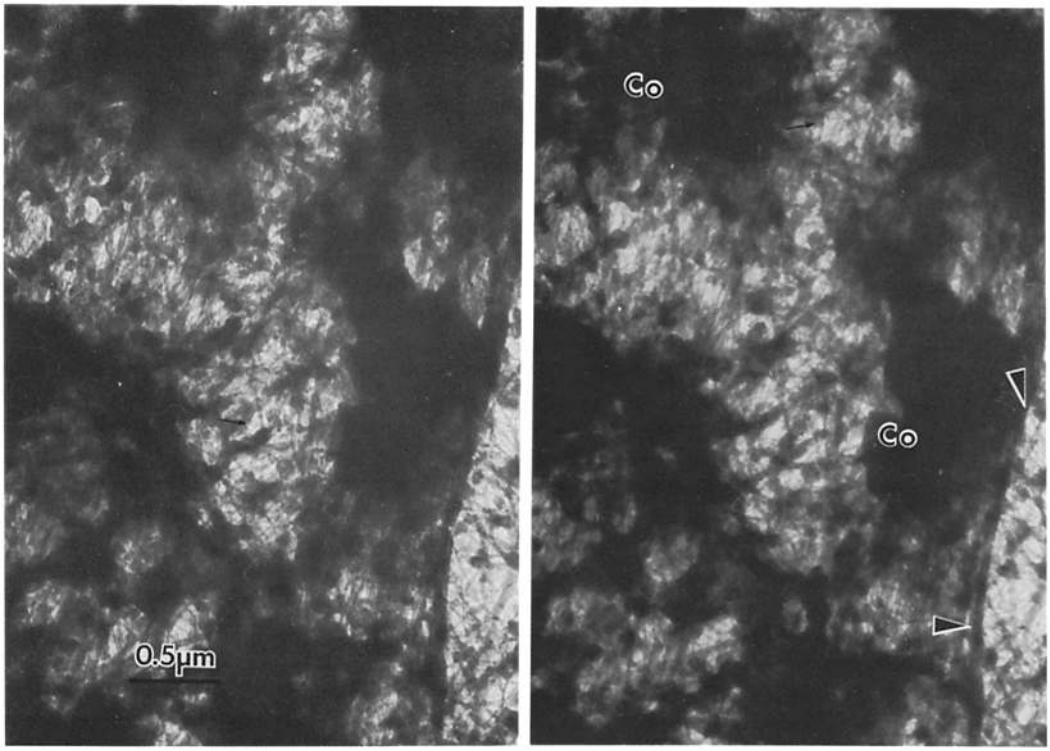

FIGURE 4 Stereo-pair whole mount of detergent-extracted early prophase nucleus. PTK_2 cells were prepared as described in the legend to figure 1. *Co*, Chromatin cores. Arrowhead indicates the nuclear lamina. Arrows indicate thin filaments which are possible nuclear matrix filaments. This micrograph was obtained using the ISI LEM 2000. × 21,600.

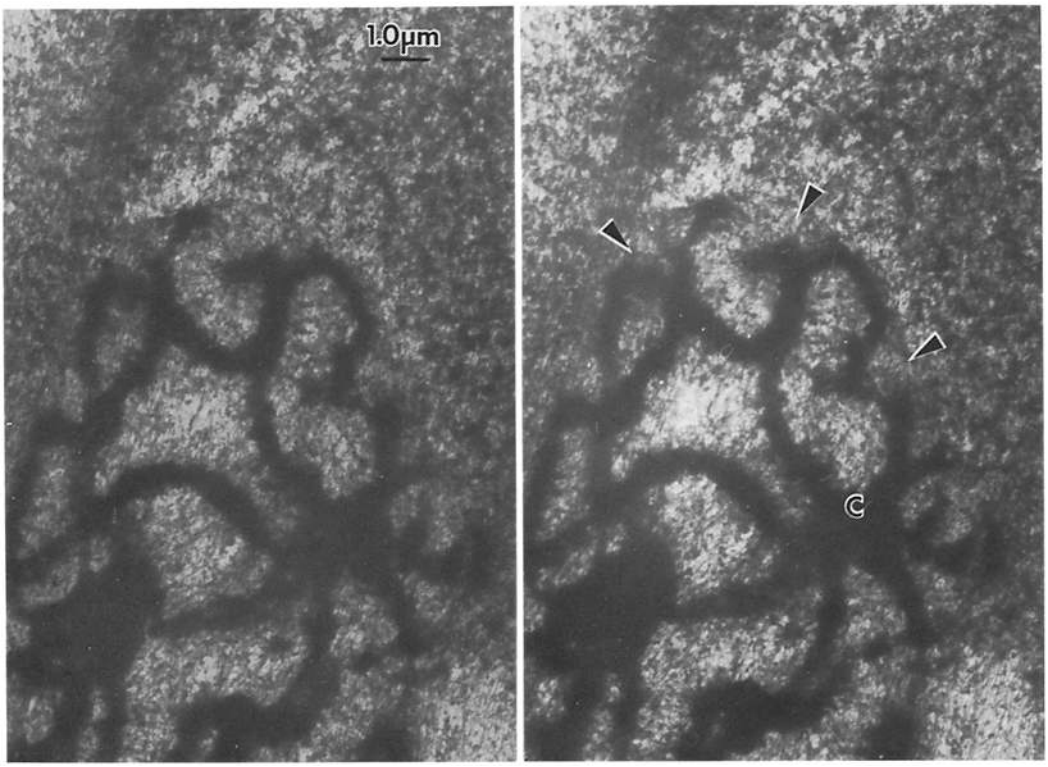

FIGURE 5 Stereo-pair whole mount of detergent-extracted prophase nucleus. The cells were prepared as described in the legend to Fig. 1. Arrowheads indicate the nuclear lamina still present at this stage. Note that the chromosomes, *C*, appear associated with the nuclear lamina in many regions. Nuclear filaments are associated with the chromosomes in many regions. This micrograph was obtained with an ISI LEM 2000. × 6,600.

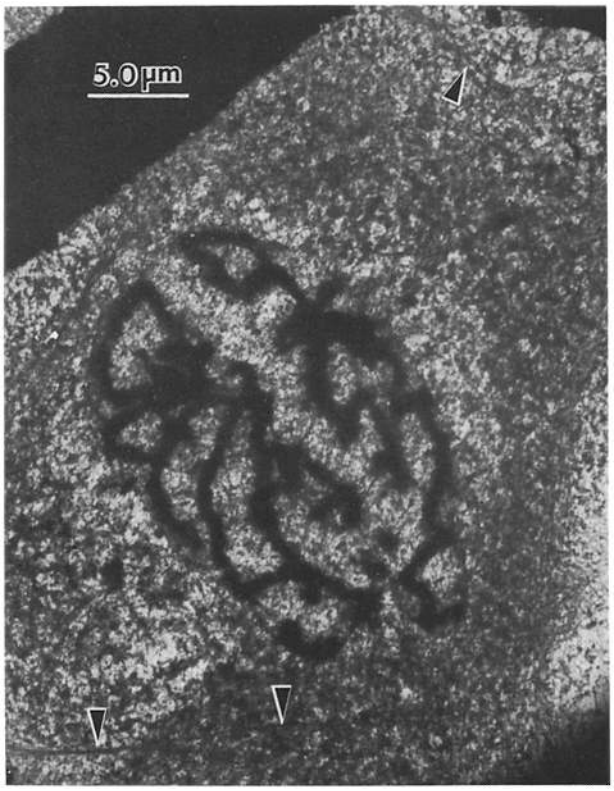

FIGURE 6 Prophase nucleus of a detergent-extracted PTK_2 cell. The cells were prepared as described in the legend to Fig. 1. The nuclear lamina is still present at this stage. The arrowheads mark the edges of the cell. This micrograph was obtained with an ISI LEM 2000. × 2,700.

the chromosome structure. Unfortunately, it is difficult to obtain adequate views of anaphase cells because cell rounding is greatest at this stage. Thus, while Fig. 8 illustrates the chromosome position it is difficult to view the filaments which can be seen clearly at higher magnification in Fig. 12. Fig. 9 is a stereo pair view of a portion of the micrograph shown in Fig. 7. Microtubules and filaments are seen throughout the cell and contacting the chromosomes. The nuclear lamina has dispersed at the end of prophase and is not visible in these anaphase preparations.

Because of the unusual filament density in PTK_2 cells, architectural changes in the cytoplasmic filament network during mitosis are, in general, difficult to observe. A notable exception is the formation of the midbody, midway between the two spindle poles and in the plane that will separate the two daughter cells during cytokinesis. Fig. 10 shows the midbody as a part of the detergent-resistant, microtubule-depleted cytoplasmic filament network. The nuclear lamina has not yet reformed around the daughter nuclei although filaments are observed extending from the nuclear region into the midbody. Normally, the midbody structure is rich in microtubules (14), but in the presence of millimolar amounts of calcium ions and low temperatures these should have been removed and the other structural elements of the midbody revealed. The open region on the lower side of the nuclei is an artifact due to shrinkage that sometimes occurs during dehydration. Near the completion of cytokinesis the nuclear lamina is again visible (Fig. 11). The remnant connection between the two daughter cells is shown in stereo view of Fig. 11 which affords a three-dimensional view of this terminal structural feature of the mitotic process.

After the disappearance of the nuclear lamina the chromosomes are surrounded by the cell's filament network (Fig. 12). The complexity of this network is best appreciated in the stereo view afforded by Fig. 13. The chromosomes appear supported in space by the filament network and the short chromatin strands projecting from the chromosome are clearly seen.

DISCUSSION

There are many marked metabolic and morphological changes in cells at mitosis. The synthesis of nuclear RNA (12, 15, 40,

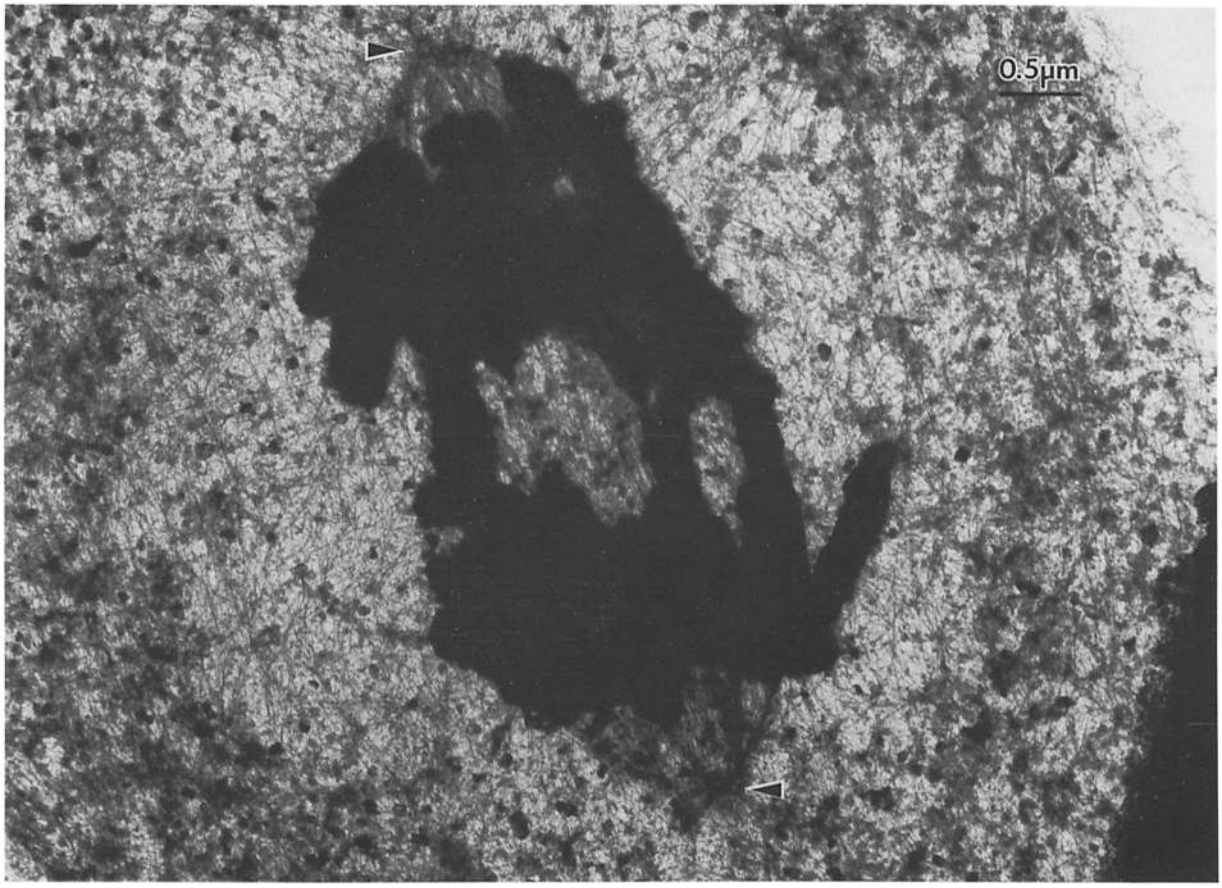

FIGURE 7 Early anaphase of a detergent-extracted PTK_2 whole mount. Cells were extracted in buffer containing Taxol to preserve microtubules (see Materials and Methods). The nuclear lamina is not present at this stage. The chromosomes are positioned between the two spindle poles (arrowheads). See Fig. 9 for a stereo view of this micrograph. This micrograph was obtained with a JEOL JEM 100B electron microscope. × 20,500.

52) is strongly suppressed while protein synthesis drops to 30% of its interphase level (16, 46, 50). Morphological changes include cell rounding, the condensation of chromatin into chromosomes, the disappearance of the nuclear envelope, and the formation of spindle poles. Previous ultrastructural studies of the mitotic process have usually employed conventional embedded thin sections prepared from intact cells. This report shows the first observations of filaments in mitotic cells by whole mount electron microscopy of detergent-extracted cells. This procedure uses no embedding plastic, thus the filament networks of the cytoplasm and nucleus are revealed with great clarity (24, 37). The procedure affords views of the entire cell structure including the three-dimensional arrangements of chromosomes and associated filaments; after the nuclear lamina disappears at metaphase, the filament network appears continuous from the plasma lamina to the chromosomes.

The use of the detergent-extracted cell whole mount technique has heretofor been precluded by the geometry of mitotic cells. The rounding that normally accompanies mitosis usually renders cells too thick and dense for observation. In the present report, this difficulty has been circumvented to a large degree by the use of electron microscopes with long focal length lenses and consequently greater depth of field and by using PTK_2 cells which, when part of a cell sheet, remain relatively flat during division.

The nuclear matrix of the interphase cell can be seen only after the removal of chromatin which requires relatively harsh procedures. In these experiments, the nuclear matrix becomes visible as the chromatin condenses at early prophase. Thus, the nuclear matrix filaments can be observed in cells which have only been extracted with Triton X-100 under conditions which provide good preservation of structure. Previous studies with thin sections have shown fibrous or fibrillogranular structures associated with chromosomes and persistent nucleoli (4, 28, 35) during mitosis. It is possible that much of this material is similar to the detergent-resistant filaments seen here.

The extraction procedures used to remove soluble protein and lipid in these experiments differ from those previously used by this laboratory in one important regard: the addition of calcium to those samples in which the spindle microtubules were depolymerized. The amount of Ca^{++} used completely depolymerizes microtubules (19, 31). In general, although not always, the presence of calcium leads to a small but measurable dissociation of polyribosomes from the cytoskeleton or the appearance some of skeleton specific protein in the soluble phase (E. G. Fey and S. Penman, manuscript in preparation). Some possible perturbation of structure by calcium is accepted in the present experiments so as to remove the spindle completely. This was considered necessary for two reasons. The spindle microtubules obscure many of the nonspindle structural connections and their removal permits maximum clarity in observing nonspindle structures. In addition, the removal of the spindle clearly shows the role of nonspindle proteins in maintaining chromosome organization in the extracted mitotic

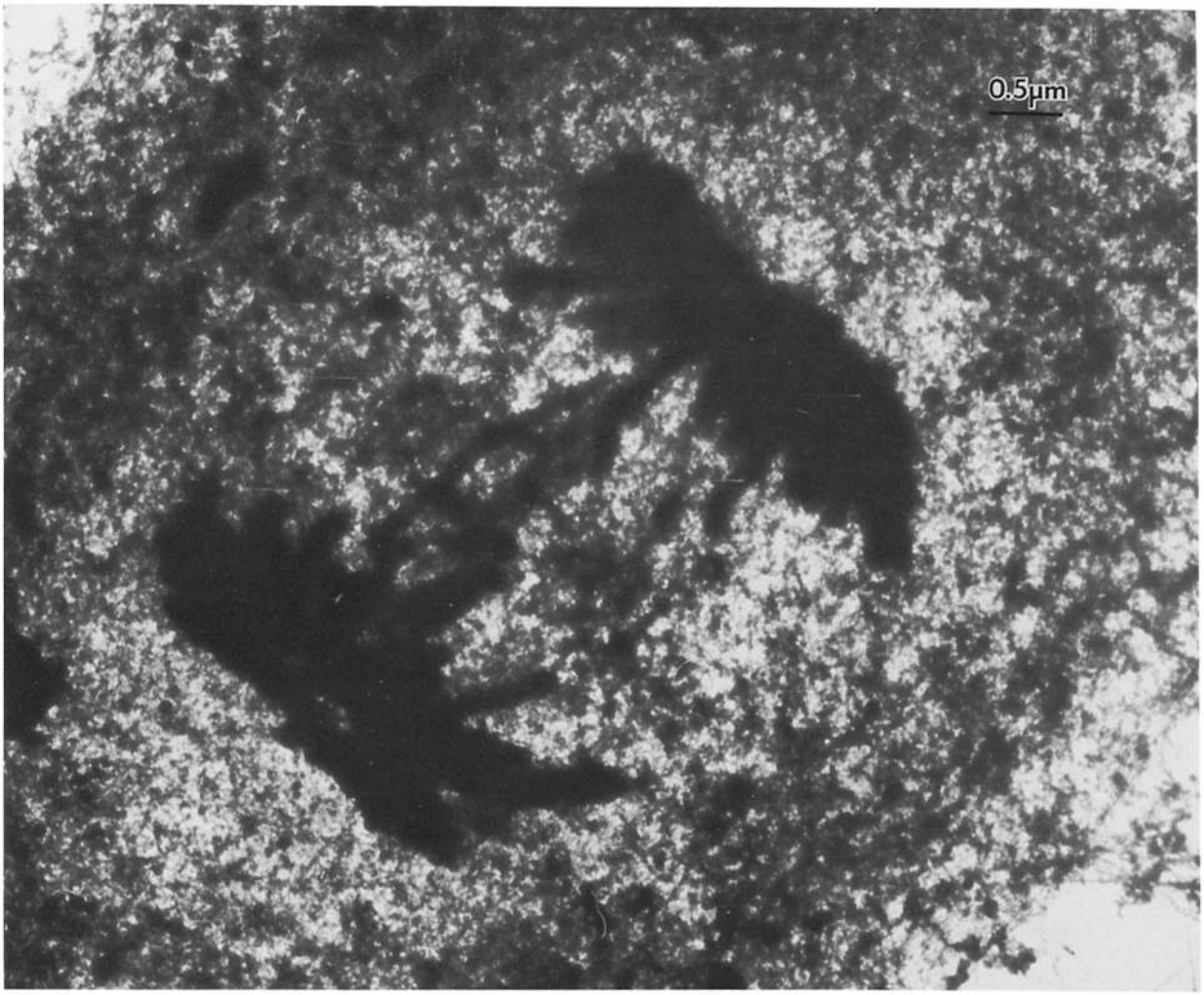

FIGURE 8 Late anaphase of a detergent-extracted PTK_2 whole mount. Cells were extracted as described in figure 1 under the conditions which depolymerize microtubules. The spindle poles and accompanying microtubules are gone, but the chromosomes are in a typical anaphase configuration. This micrograph was obtained with a ISI LEM 2000 electron microscope. × 20,000.

cell. There is an apparent lack of observable filaments in Figs. 7 and 8 due to low magnification and the limitation of the photographic emulsion to reproduce filaments in such a high-contrast specimen. In other cells, and at higher magnification of these cells, the cytoskeletal filaments appear normal (see Figs. 4, 10, 11, 12).

In interphase cells, the nuclear matrix is defined as the filament structure confined to the region within the nuclear lamina. It has many properties that distinguish it from the cytoplasmic filament network. It has a regionally distinct topography and, most importantly, a protein composition very different from that of the cytoskeleton (8). It appears to be much more resistant to dissociation by high ionic strength which accounts for the relative ease of its isolation and separation from chromatin. We see here that at the end of prophase the nuclear lamina disappears and the cytoplasmic and nuclear networks appear to form a single topographically continuous structure. The presumptive nuclear space is identifiable as an area with a relatively low density of filaments which link the chromosomes to the cellular filament network. The remaining nuclear filaments may have rearranged to form the scaffolding fibers of the chromosomes described by Laemmli (1, 27, 32, 36) and others (61). A possible role of the nuclear matrix in chromatin condensation can be suggested. Similarities exist between the organization of DNA in the interphase cell and that in the mitotic cell. In both cases the DNA is attached to a protein network or scaffold and the DNA is reported to be capable of looping out from this protein network both in interphase nuclei and in chromosomes (11, 27, 32, 33, 54). This suggests that one of the events possibly involved in chromatin condensation may be the rearrangement of the DNA binding sites of the interphase nuclear matrix into the scaffold of the chromosome.

The full potential of the whole mount techniques emerges when cell structures are viewed by stereoscopic microscopy. The stereomicrographs shown here afford a perception of the three-dimensional organization of the cell architecture which is not possible with thin sections. More importantly, the stereoscopic microscopy shows the arrangment of the chromosomes in space. There are brief periods during mitosis, i.e., prophase and telophase, when condensed chromosomes and the nuclear lamina coexist. At these times, the chromosomes are located near or at the nuclear lamina, while the central region of the nucleus becomes rather empty. The association of chromosomes with the lamina, or nuclear envelope, is not unexpected and has been reported by several investigators (42, 44, 51). The nuclear lamina disappears at the end of prophase as was observed by Gerace and Blobel (22) using Chinese hamster

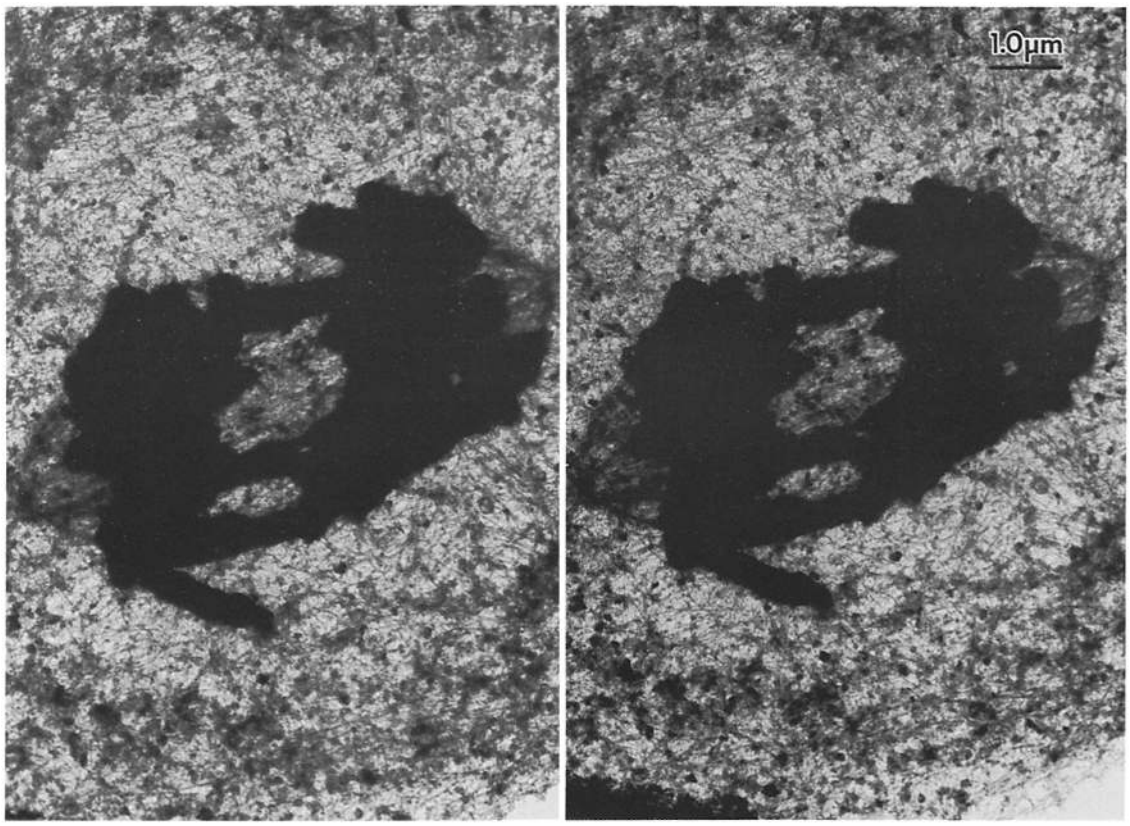

FIGURE 9 Stereo-pair micrographs of detergent-extracted PTK_2 whole mount in early anaphase. This is a stereo pair of the micrograph shown in Fig. 6. Cells were prepared as described in figure 7. × 9,200.

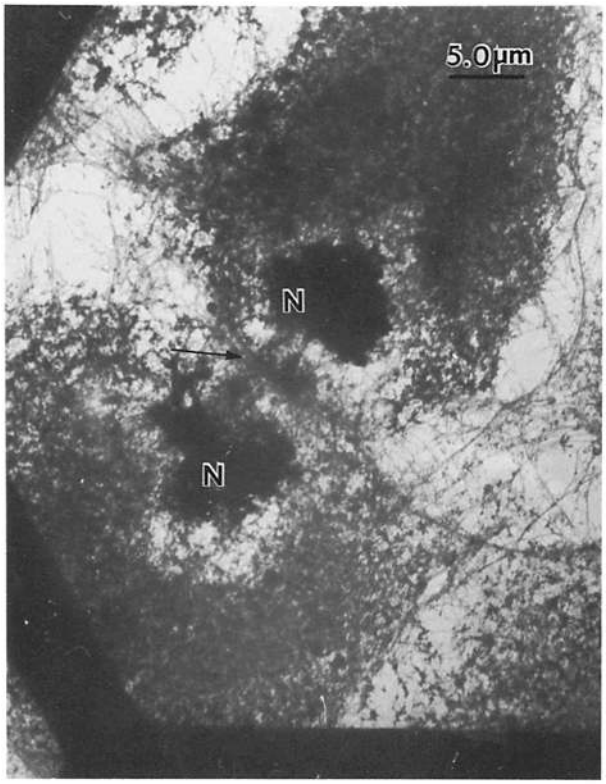

FIGURE 10 Telophase of a detergent-extracted PTK_2 whole mount. Cells were prepared as described in Fig. 1. The space under both daughter cell nuclei is a shrinkage artifact which occurred during critical point drying. This micrograph illustrates that the midbody (arrow) is part of the microtubule-depleted cytoskeleton. *N*, Daughter cell nuclei. The nuclear lamina does not appear to have reformed. Note that filaments extend from the nuclear region to the midbody. This micrograph was obtained with an ISI LEM 2000 electron microscope. × 1,900.

ovary cells and antibodies directed against three rat liver nuclear lamina proteins. They also observed reassembly of the nuclear lamina at telophase as is observed here.

In the lower eukaryote, *Physarum polycephalum*, Bekers et al. (2) have identified nuclear matrix filaments attached to the DNA at all stages of mitosis. In our study we see many filaments or fibrils which attach to the chromosomes, but we cannot be certain whether they are chromatin fibrils or nuclear matrix filaments.

The spindle apparatus can be removed from the detergent-prepared cell structure with no consequent apparent change in cellular filament architecture. In this sense, the spindle appears to be an ancillary organelle whose probable exertion of force in effecting chromosome separation is superimposed on the filament arrangements connected to the chromosomes. Presumably, these filaments must change their connections during chromosome migration, disassembling and reforming as the chromosomes move toward the spindle poles. Such dynamic behavior is in accord with the dynamic properties assumed for the cytoskeleton. However, translocating an object as large as a chromosome through a network that must break and remake many connections could very well result in a resistance to movement. Skeleton behavior could thus give rise to a pseudoviscosity which would account for the shape of chromosome during their polar movement when, by optical microscopy,

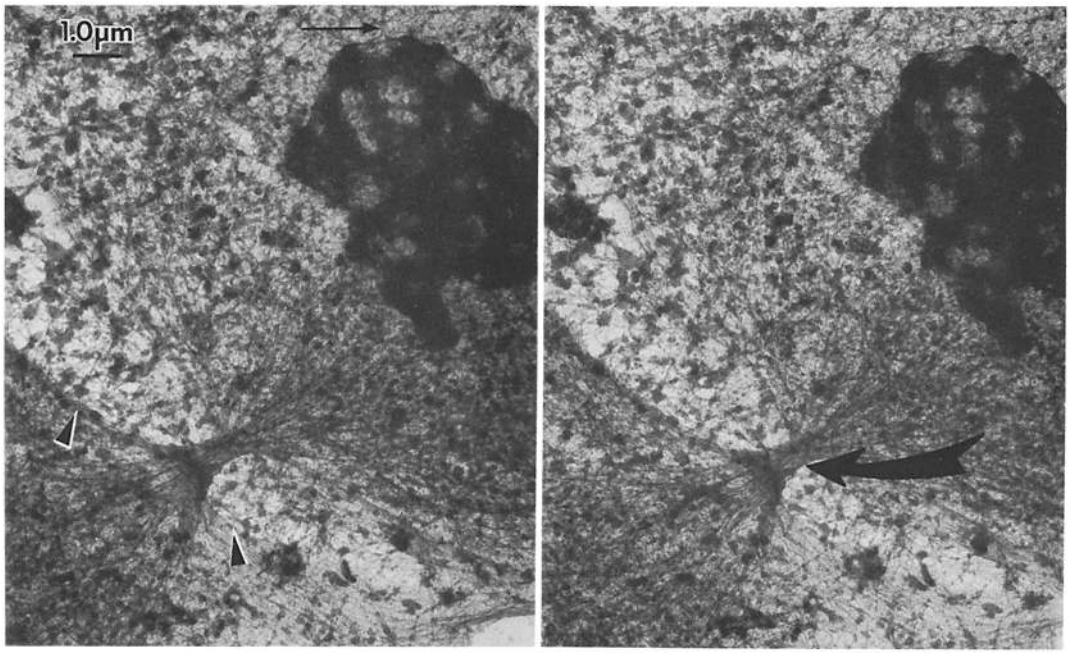

FIGURE 11 Stereo-pair micrographs of late cytokinesis in detergent-extracted PTK_2 whole mounts. Cells were prepared as described in Fig. 1. One daughter cell lies in the upper right corner of the micrograph, the other daughter cell lies in the lower left-hand corner of the micrograph. Its nucleus is not in the field of view. The bold arrow points to the remnant of the midbody. The daughter cell in the upper right corner has moved slightly beneath the daughter cell in the lower left corner. Arrowheads indicate the forward edge plasma lamina of the daughter cell in the lower left-hand corner. The nuclear lamina has reformed at this stage and the thin arrow points to a filament in the cytoplasmic region which appears to become part of the nuclear lamina. This could be a means of the nuclear lamina protein returning to the nuclear region. This micrograph was obtained on a AEI EM 7-II HVEM. × 6,500.

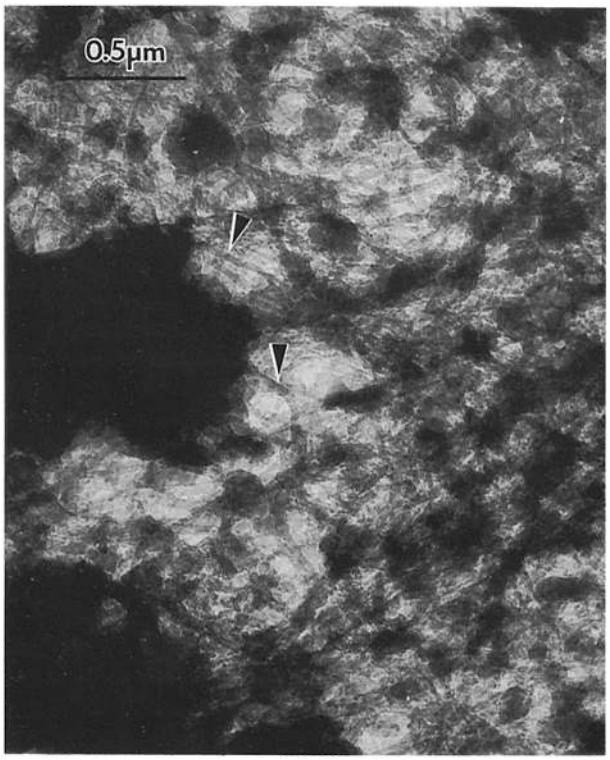

FIGURE 12 Electron micrograph of detergent-extracted cell whole mount of a chromosome arm during anaphase. Cells were prepared as described in Fig. 1. Note the extensive filament network around the chromosome. Arrow points to some filaments which appear to contact the chromosomes. This micrograph was obtained with a AEI-EM 7-II HVEM. × 33,000.

they appear to be dragged through a viscous medium. The elaborate filament architecture of the cell, through which the spindle fibers penetrate, is probably the source of the actin and other structural proteins reported to be associated with the isolated spindle apparatus (62).

The micrographs here indicate the complex networks surrounding and probably interacting with chromosomes as they form and move during mitosis. It is very likely that these structures are responsible for chromosome movements prior to the formation of the mitotic spindle and possibly play a role after the formation of the spindle. These networks also may underlie other important features of cell reorganization during mitosis, such as the reformation of the nuclear lamina at telophase, the location of the poles for the spindle apparatus, and the formation of midbody for cytokinesis. The micrographs of the dividing cell indicate that the midbody region is rich in filaments structures that can be seen when the microtubules are depolymerized as they are here. The many complex events of mitosis suggest an elaborate underlying structure, some aspects of which are shown in the stereoscopic whole mount microscopy reported here.

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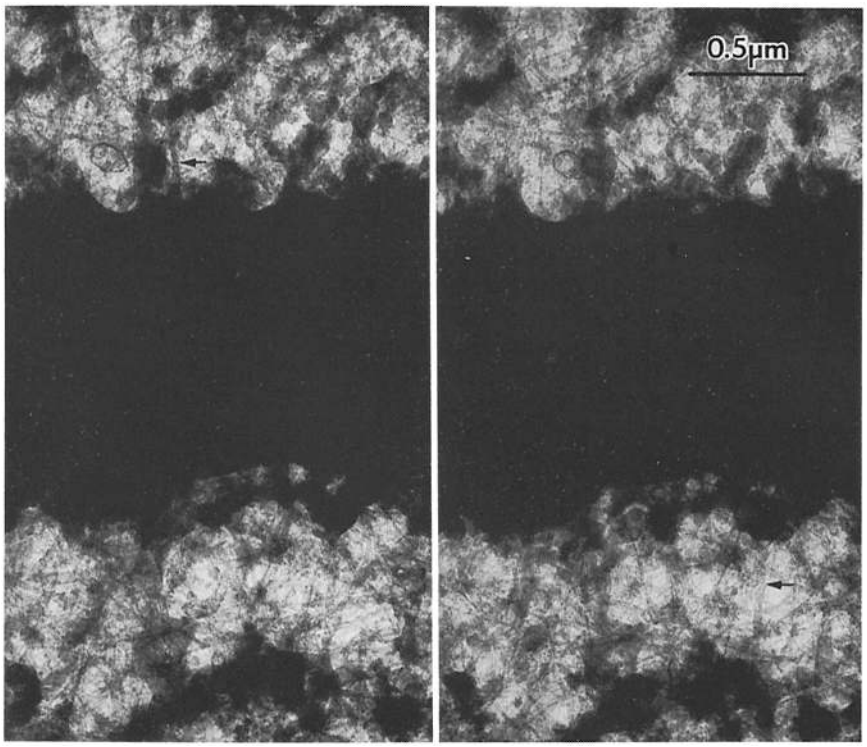

FIGURE 13 Stereo-pair whole mount of detergent-extracted PTK_2 cell chromosome arm during anaphase. Cells were prepared as described in Fig. 1. Note the extensive network of filaments surrounding the chromosomes. Arrows point to some filaments which fuse with the chromosome arm. This micrograph was obtained using the AEI-EM7-II HVEM. × 30,000.

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