Isolation and Partial Characterization of a Cage of Filaments That Surrounds the Mammalian Mitotic Spindle

GARY W. ZIEVE, STEVEN R. HEIDEMANN, and J. RICHARD McINTOSH
Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado 80309, and Department of Physiology, Michigan State University, East Lansing, Michigan 48824. Dr. Zieve's present address is the Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139.

ABSTRACT Mitotic cells have been detergent extracted under conditions that support microtubule assembly. When HeLa cells are lysed in the presence of brain tubulin, mitotic-arrested cells nucleate large asters and true metaphase cells yield spindles that remain enclosed within a roughly spherical cage of filamentous material. Detergent-extracted mitotic Chinese hamster ovary (CHO) cells show a similar, insoluble cage but the mitotic apparatus is only occasionally stabilized. In later stages of mitosis, HeLa cages are observed in elongated and furrowed configurations. In the terminal stages of cell division, two daughter filamentous networks are connected by the intercellular bridge. When observed in the electron microscope the cages include fibers 7–11 nm in diameter. The polypeptide composition of cages isolated from mitotic HeLa cells is complex, but the major polypeptides are a group with mol wt ranging from 43,000–60,000 daltons and a high molecular weight polypeptide. CHO cells contain a subset of these proteins which includes a major 58,000-dalton and a high molecular weight polypeptide. Two different antisera directed against the vimentin-containing intermediate filaments bind to polypeptides in the electrophoretic profiles of isolated HeLa and CHO cages and stain the cages, as visualized by indirect immunofluorescence. These results suggest that the HeLa and CHO cages include intermediate filaments of the vimentin type. The polypeptide composition of HeLa cages suggests that they also contain tonofilaments. The cages apparently form as the cells enter mitosis. We propose that these filamentous cages maintain the structural continuity of the cytoplasm while the cell is in mitosis.

Mitosis in mammalian cells is accompanied by a dramatic reorganization of cytoplasmic and nuclear components. The cytoskeleton of interphase is largely dissolved during prophase, and the distribution of cytoplasmic membranes is grossly perturbed with the breakdown of the nuclear envelope (12, 16, 42, 43, 23). Even overall shape is altered at mitosis, given the tendency of most cells to alter their physical attachment to neighboring surfaces and round up (28, 57). Within the nucleus the nucleolus dissociates and the chromatin condenses into chromosomes. The actions of the mitotic spindle on the organization of the chromosomes and the subsequent segregation of the chromatids are perhaps the best-studied rearrangements in the mitotic cell. Cytoplasmic reorganization, on the other hand, is a less well characterized process. It is clear that the interphase organization breaks down during mitosis, and it is equally clear that the organization is reestablished after telophase with the postmitotic reentry into interphase. Several stimulating papers have recently shown that there is often a similarity between the two daughters of a cell division, suggesting that some factors are inherited that help to set up cytoplasmic organization in the next generation (1, 51). What these components might be and how the cells might remember their interphase organization are at present questions requiring further investigation.

A previously successful approach to the study of factors important in cell architecture has been that of lysing and extracting cells with conditions that preserve the fibrous elements of the cytoplasm (10, 36, 25, 20). There are several methods of lysis applicable to mitotic cells which preserve some or all of the mitotic spindle or the cleavage furrow (37, 39, 41, 48, 58), but little attention has yet been paid to other components of the mitotic cell.

THE JOURNAL OF CELL BIOLOGY · VOLUME 87 OCTOBER 1980 160-169

In this paper and the accompanying one (26), we present the result of a search for new lysis conditions that would extract most of the protein of a mitotic mammalian cell, yet stabilize the microtubule components of the mitotic apparatus (MA). We have found a buffer that extracts >80% of the cell's protein, yet leaves the stabilized spindle enclosed within a fibrous cage of detergent-insoluble proteins. This cage appears to be deformable, as we have isolated it in a sequence of shapes that reflects the geometry of the dividing cell. By microscopic, biochemical, and serological criteria we demonstrate that the cages include 10-nm filaments of the vimentin type (18, 30) in both HeLa and Chinese hamster ovary (CHO) cells. These isolated structures are most likely related to the cages of intermediate filaments that have recently been observed to form around the developing mitotic spindle, using indirect immunofluorescence (6, 18, 30). The cages represent fibrous elements of the cytoplasm that persist throughout mitosis, and we suggest that such a structure might serve as a framework for the reestablishment of interphase organization after cell division.

MATERIALS AND METHODS

Cell Culture

HeLa cells were grown as monolayer cultures in Dulbecco's modified Eagle's medium (Grand Island Biological Co. [GIBCO], Grand Island, New York) supplemented with 7% calf serum (GIBCO). Monolayers of CHO cells were maintained in Ham's F12 (GIBCO) supplemented with 10% fetal calf serum (GIBCO). In some preparations mitotic cells were detached from the monolayer by gently shaking. In others, cells were partially synchronized with a single thymidine block and release (9), followed by accumulation in 0.04 μ/ml Nocodazole and shaking-off as previously described (62). Populations of late stage mitotic cells were obtained by releasing Nocodazole-accumulated cells into drug-free medium for 50 min in the case of HeLa cells and 12 min for CHO cells (62).

Cell Fractionation

All steps of the fractionation procedure were carried out at 37°C. Mitotic cells were collected by centrifugation at 200 *g* for 3 min, resuspended in a hypotonic buffer (1 mM PIPES, pH 6.9, 2 mM EGTA, 1 mM $MgCl_2$, 5% dimethyl sulfoxide [DMSO], 95% D_2O) for 2 min, pelleted by centrifugation at 200 *g* for 2 min, and lysed in microtubule (MT) assembly buffer (0.5 M PIPES, pH 6.9, 1 mM $MgCl_2$, 1 mM EGTA, 5% DMSO, 1% Triton-X 165, 0.5% sodium deoxycholate, 0.2% SDS, 1 mM GTP, 2 mM phenylmethylsulfonyl fluoride [PMSF], 1% Aprotinin [Sigma Chemical Co., St. Louis, Mo.]) (26) containing 2 mg/ml of porcine brain microtuble protein (MTP). The MTP was prepared by the method of Weingarten et al. (59). After the fourth cycle of polymerization, the protein was resuspended at a concentration of 4 mg/ml in 0.5 M PIPES, 1 mM EDTA, depolymerized at 0°C, and centrifuged for 4 h at 225,000 *g* at 4°C to produce MTP which showed a lag in spontaneous assembly (2).

Lysed cells were digested with 20 μg/ml of DNase I (Worthington Biochemical Corp., Freehold, N. J.) to reduce viscosity. Some preparations were further extracted in 1 M KCl, 10 mM Tris, pH 7.5, 1 mM EGTA for 1 h, collected at 1000 *g* for 4 min, and then resuspended in 1 M KI, 100 mM Tris, pH 8.7, 1 mM EGTA, 100 mM β-mercaptoethanol for an additional hour before use (34).

Microscopy

For light microscopy, preparations were sealed under coverslips and observed with a Zeiss Photomicroscope II using phase, differential interference contrast (DIC) and polarization optics. All photographs were taken on Kodak SO 115 film (ASA 100) developed with Diafine.

Whole mounts of the lysed cell preparations were prepared for electron microscopy by fixation for 10 min in 2% glutaraldehyde with 2 mM $MgCl_2$ in 0.1 M PIPES buffer, pH 6.9. Immediately after fixation, lysed cells were centrifuged at 100 *g* for 3 min onto electron microscopy grids that had been mounted on coverslips and covered with Formvar, overlaid with carbon and 0.1% polylysine. After 10 min the grids were rinsed in 0.1 M Na cacodylate, pH 7.4, 10% sucrose, and then stained with 0.2% tannic acid (Mallinckrodt Inc., St. Louis, Mo.) in the same buffer for 2 min (47). The grids were then rinsed extensively in 0.1 M Na cacodylate, pH 7.4, 10% sucrose, stained with 1% osmium in the same buffer for 2 min, and then rinsed in distilled water, dehydrated with a concentration series of acetone or 2-methoxyethanol, and dried by passing through the critical point of CO_2 (3). The specimens were then carbon coated on both sides and examined in a JEM 1000. Thin sections of intact cells were prepared as previously described (40).

SDS Polyacrylamide Electrophoresis

Material to be analyzed by SDS polyacrylamide electrophoresis was dialyzed against 0.1% SDS, precipitated with 10 vol of acetone, and then collected by centrifugation at 20,000 *g* for 10 min. Samples were resuspended in sample buffer and electrophoresed according to the procedure of Laemmli (33). Molecular weight determinations were calculated relative to known polypeptide standard by assuming that mobility is proportional to the log of the molecular weight.

Immunological Analysis

Antigens were identified in SDS polyacrylamide gels by the procedure of Burridge (11). After electrophoresis and fixation without staining, gels were equilibrated with buffer at neutral pH and overlaid with a 1:15 dilution of antiserum for 16–24 h followed by 6 d of rinsing. Gel lanes were then overlaid with Staph A protein (Pharmacia Inc., Piscataway, N. J.) labeled with ^{125}I (29) for 16–24 h and further rinsed for 5 d. The gel slices were then stained with Coomassie Blue, dried, and analyzed by autoradiography.

Indirect immunofluorescence of intact cells was performed as previously described (12). Lysed cells were fixed for immunofluorescence in 0.1 M HEPES, pH 6.9, 3 mM $MgSO_4$, 1.2% formaldehyde, and 0.1% glutaraldehyde for 5 min. Fixed preparations were then collected by centrifugation at 500 *g* for 4 min and rinsed in phosphate-buffered saline (PBS), followed by 30% goat serum. These cells were resuspended in immune serum diluted 1:20 for 25 min at 25°C, rinsed in phosphate buffer saline without calcium (PBS), incubated in fluorescein-conjugated goat anti-rabbit IgG (Miles Laboratories Inc., Elkhart, Ind.) for 20 min at 37°C, and then rinsed extensively. Preparations were settled onto polylysine-coated coverslips and mounted in Elvano (DuPont Instruments, Wilmington, Del.). Micrographs were taken with Kodak Plus X film developed in Diafine using Zeiss epifluorescence optics.

RESULTS

Lysis of Mitotic HeLa and CHO Cells

Treatment of mitotic cells (Fig. 1 *a*) with the hypotonic buffer described above preserves spindle birefringence (BR) while the cells swell (Fig. 1 *b*). The subsequent lysis with a detergent-containing MTP solution solubilizes >80% of the cell protein as judged by colorimetric protein estimation (7) and disperses the chromosomes, leaving MT-containing ghosts (Fig. 1 *c–e*). HeLa cells accumulated with Nocodazole and lysed without release of the block produce large, calcium-sensitive asters (26), while later stages yield highly birefringent, spindle-shaped structures. The size of the asters and the BR of the spindle is affected by the concentration of polymerizable MTP in the lysis mixture. Regardless of this concentration, however, the isolated mitotic centers and associated MTs are enclosed within a thin refractile cage. The cages of lysed, metaphase HeLa cells average 18 μm in diameter and are similar in size to the intact cell before swelling. The MA of CHO cells is more difficult to preserve than that of HeLa cells: identical extraction conditions produce a similar refractile cage, but only occasionally will the cage contain a stabilized MA.

The refractile cage is by far the most stable structure in these extracted mitotic cells. Cells lysed in a wide variety of buffers promoting MT assembly yield labile spindles which readily dissolve, but the cage surrounding the MA invariably persists. Indeed, even under conditions in which we have biased the equilibrium towards a stabilization of the MT component by the addition of MTP at several milligrams per milliliter, no buffers have been found that will solubilize the cage but leave the MA intact.

Lysis of interphase cells using these procedures solubilizes

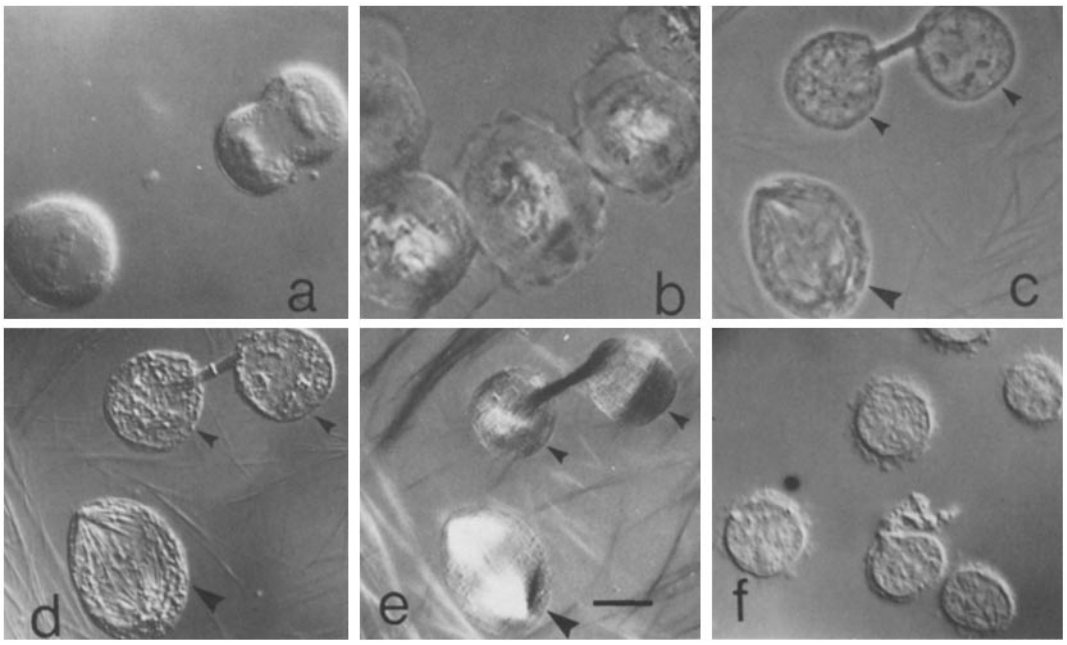

FIGURE 1 Fig. 1 *a* shows isolated mitotic HeLa cells detached from monolayer cultures by gentle shaking (DIC optics). Fig. 1 *b* shows mitotic HeLa cells swollen in the hypotonic buffer described in Materials and Methods (polarization optics). Fig. 1 (*c*–*e*) shows lysed anaphase and early G_1 HeLa cells lysed in the presence of MT protein as described in Materials and Methods, using phase (*c*), DIC (*d*), and polarization (*e*) optics. Cage structures are indicated with arrowheads. Extensive background polymerization of the MT protein has occurred in this preparation. Fig. 1 *f* shows interphase HeLa cells lysed as described in Materials and Methods (DIC optics). Bar, 10 μm. × 750.

>80% of the cellular protein (7). A nuclear structure remains surrounded by limited amounts of fibrillar cytoplasmic material (Fig. 1*f*), but no cage analogous to that of mitotic cells is seen. Similar preparations have been reported by several other laboratories (11, 49, 36, 21, 20). The interphase cells in our lysates are not active in nucleating organized birefringent arrays of MTs when lysed with MTP. Similar variation of MT-organizing ability with time in the cell cycle has been reported previously (60, 38, 50, 56).

Electron Microscopy of Detergent-extracted Mitotic Cells

Fig. 2*a* is a pair of stereo high-voltage electron micrographs (HVEMs) of a prometaphase HeLa cell lysed in assembly buffer containing MTP. We often observe multiple asters in the drug-blocked cells which are always enclosed within a thin fibrous cage. The large asters observed are the result of the elongation of endogenous MTs by the MTP in the lysis solution (26). The fibrous remnant clearly reflects the geometry of the unlysed cell. The asters in the drug-blocked prometaphase cell and the stabilized spindles in the metaphase cells are found within roughly spherical cages (Fig. 2*a* and *b*). Anaphase spindles lie in elongated cages (Fig. 3*a*) while telophase structures show a distinct furrow (Figs. 2*c* and 3*b*). The cages from detergent-extracted telophase cells are often collapsed, which may reflect the geometry of the cages at this stage or a tendency of these structures to deform during preparation. In the terminal phase of cell division, the two sister cell ghosts are connected by an intercellular bridge with a distinct midbody (Fig. 3*c*). At this stage, the cage has divided into two, one-half almost surrounding each daughter nucleus. The cages thus appear to be elastic and can be deformed in concert with the kinetic activities of the mitotic spindle and cleavage furrow. Scanning micrographs of lysed mitotic cells confirm the three-dimensional characteristics of these fibrous cages (data not shown). Interphase cells lysed and prepared for microscopy by similar methods exhibit a distinct nucleus with attached fibrous protein which is often found as a cap on the nucleus (Fig. 3*d*) or as a maze of fibers surrounding the nucleus.

Examination of the cages at higher magnification reveals a heterogeneous population of fibers (Fig. 4). Many fields contain arrays of fibers showing an approximately uniform diameter of 8–11 nm characteristic of intermediate filaments (31, 24, 46). Other fibers, however, appear beaded with diameters ranging from 10 to 30 nm. This nonuniform appearance is not presently understood, but it may result from variable amounts of cytoplasm adhering to the fibers during lysis and fixation. In addition to the MTs extending from the mitotic centers, short segments of MTs are observed extending from other regions of the lysed structures in disorganized arrays. These MTs are most likely the result of nonspecific nucleation of the MTP used in the lysis solutions by the material left in the extracted cells.

Electron micrographs of lysed mitotic CHO cells reveal a similar filamentous component in the detergent-extracted mitotic ghosts (Fig. 5*a*). The cages are composed primarily of beaded fibers although large numbers of disorganized MTs are present. The CHO cages appear to have a lower density of fibers than those prepared from HeLa cells and do not maintain the distinct geometry of the mitotic cell after lysis. Nonetheless, scanning electron micrographs confirm the roughly spherical shape of the mitotic ghosts (Fig. 5*b*).

We have studied the structure of the isolated cages with thin sections and electron microscopy, but the residue of material left after detergent extraction is so sparse that the images are not useful in comparison with the HVEMs presented in Figs. 2 and 3. Thin sections of unlysed and unswollen cells are,

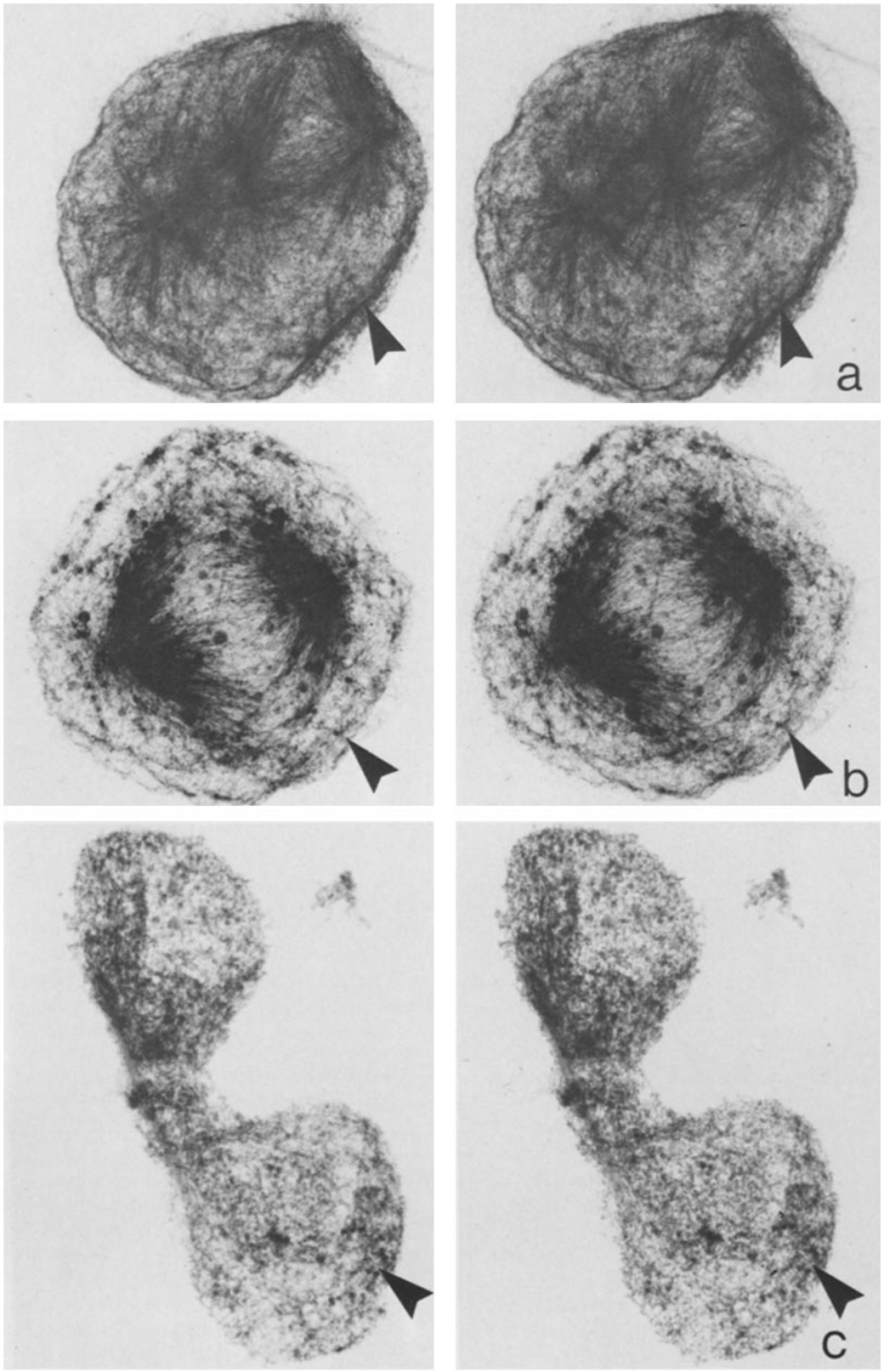

FIGURE 2 Three pairs of stereo micrographs of lysed mitotic HeLa cells. Mitotic cells were lysed in the presence of MTP and processed for high-voltage electron microscopy as described in Materials and Methods. All pairs were tilted by 3° about a vertical axis between the taking of the two pictures. Fig. 2*a* is a pair of micrographs of lysed drug-blocked cells. Fig. 2*b* is of a lysed metaphase cell. Fig. 2*c* is of a lysed telophase cell. The midbody appears to have formed in this cell before the cleavage furrow completed cytokinesis. The cage structures are indicated with arrowheads. × 3,000.

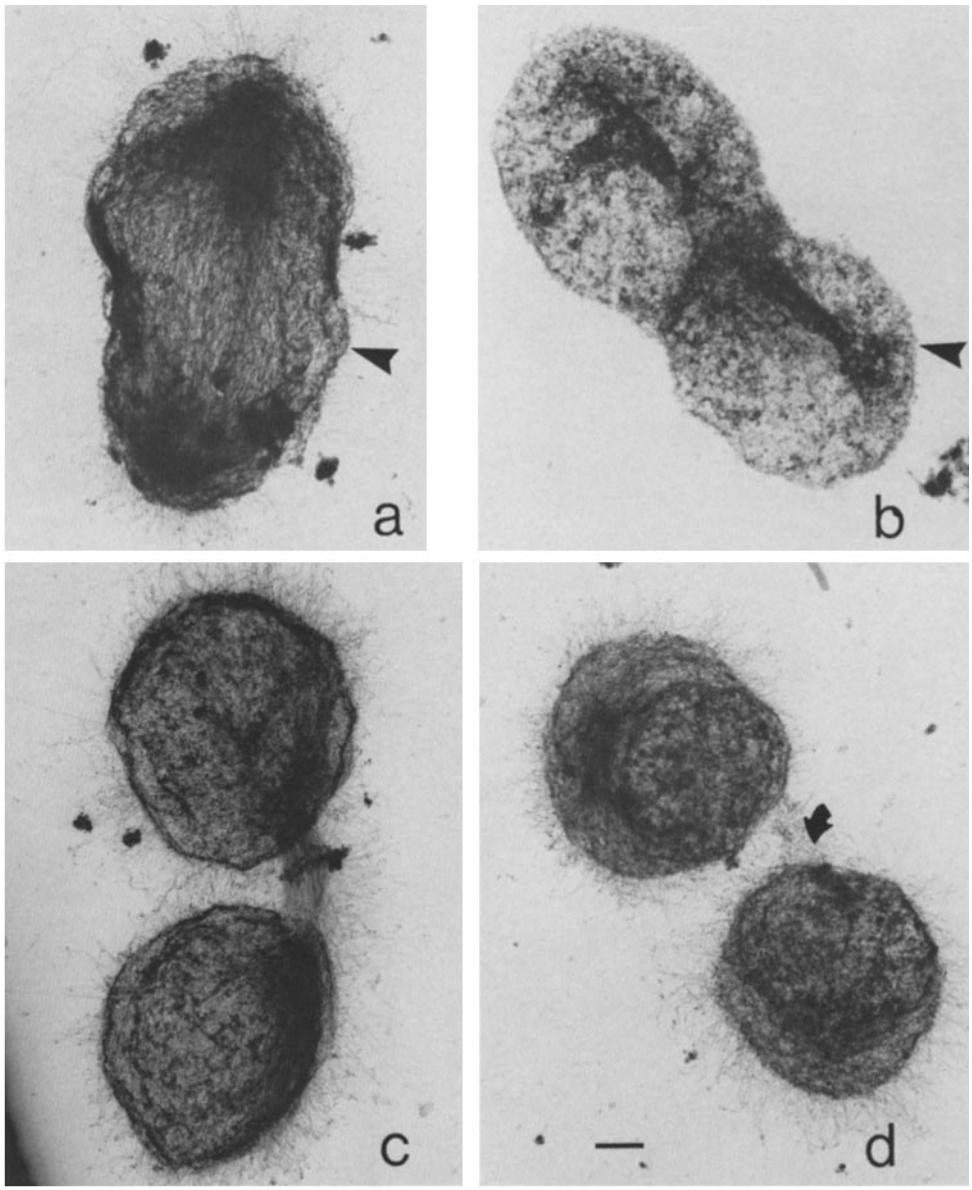

FIGURE 3 HVEMs of lysed mitotic and interphase HeLa cells. Cage structures are indicated with arrowheads. Fig. 3 *a* shows an anaphase cell with the hint of a forming cleavage furrow. Fig. 3 *b* shows a telophase cell with a distinct cleavage furrow. In this preparation the organized MTs in the ghost were lost. Fig. 3 *c* shows a pair of early G_1 cells connected by an intercellular bridge with a distinct midbody ring. Fig. 3 *d* shows a pair of later G_1 daughter cells with distinct nuclei and a cap of filamentous material. The remnant of the midbody is attached to one daughter cell (arrow). The high concentration of MTP in this preparation nucleated what we interpret as a large number of nonspecific MTs. The daughter cells appear to have some symmetry about the midbody. Bar, 2 μm. × 3,000.

however, informative about the physiological distribution of fibrous elements that appear to constitute the cage. We have focused on CHO cells for this work because HeLa cells contain a variety of intermediate filaments that cannot be distinguished in the electron microscope. The cytoplasm of CHO cells in metaphase contains large bundles of 10-nm filaments situated about midway between the periphery of the spindle and the cell cortex (Fig. 5 *c* and *d*). These bundles loosely encircle the spindle and, as such, are likely candidates for components of the cage seen in the lysed cells. The enlarged diameter of the cage relative to the position of the fiber bundles in unlysed cells may be the result of the swelling process that precedes lysis.

Several of the populations of mitotic cells prepared for light and electron microscopy were obtained by accumulating mitotic populations with the use of the readily reversible antimicrotubule drug Nocodazole (27, 62). Antimicrotubule drugs have been shown to produce alterations in cell architecture which, in addition to the depolymerization of MTs, include a rearrangement of intermediate filaments and a disorganization of cellular organelles (14, 24, 31). However, mitotic cells lysed without exposure to Nocodazole display insoluble filamentous cages that appear identical to those collected with the use of low concentrations of Nocodazole (compare Figs. 2 *a*, 3 *b* and Fig. 3 *a*, *c*). It is therefore unlikely that structures observed here are the result of the synchronization procedures employing 0.04 μg/ml of Nocodazole.

Polypeptide Composition of the Fibrous Cage

The electrophoretic profiles of the detergent-extracted mitotic and interphase HeLa cells are similar and reveal several

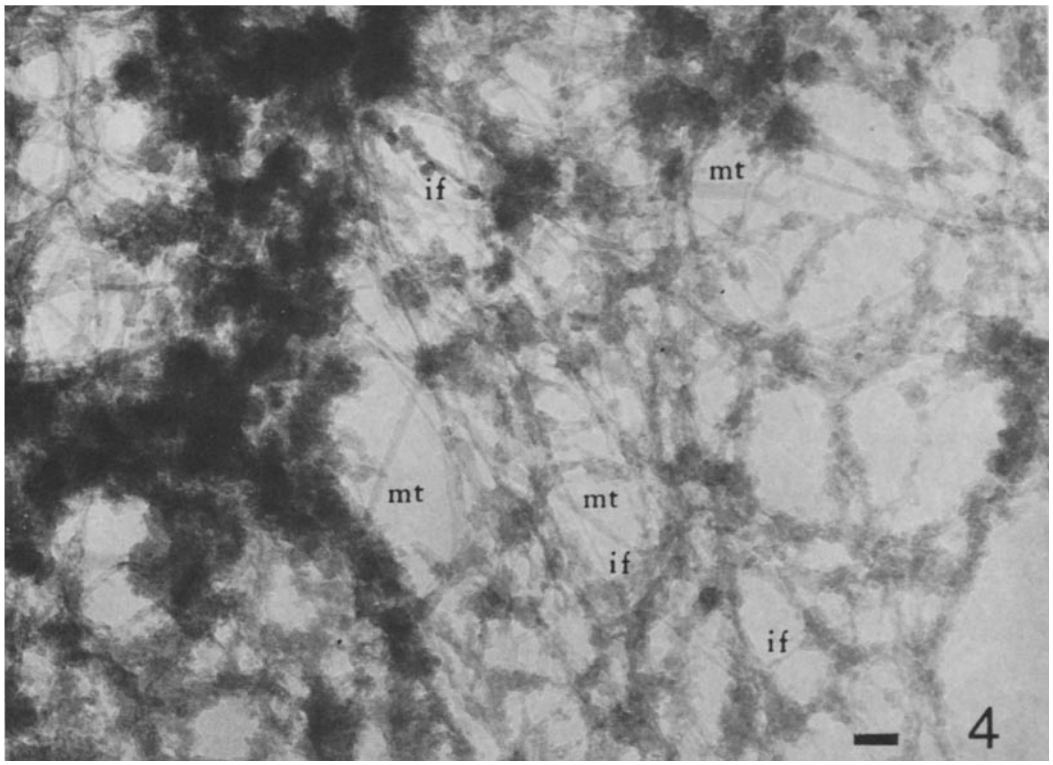

FIGURE 4 A field near the edge of a cage from a detergent-extracted mitotic HeLa cell. Several microtubules (*mt*) and a large number of fibers 8–11 nm in diameter (*if*) are present. Also, globular material appears to be associated with many of the fibers in our preparations. Bar, 0.1 μm. × 60,000.

major polypeptides which range in mol wt from 43,000 to 60,000 daltons (Fig. 6). There is also a major high molecular weight polypeptide of ~260,000 daltons. The profiles of lysed CHO cells are less complex than those of HeLa cells and contain major polypeptides of 58,000 and ~260,000 mol wt. Our morphological studies reveal that the MA is missing from the cell ghosts that have been through two cycles of detergent extraction in the absence of exogenous tubulin (data not shown). The extraction of the spindle is reflected in the absence of major tubulin polypeptides in the isolated ghosts. Comparison of the major polypeptides present in the detergent-extracted cages and those of whole-cell extracts suggests that the proteins of the cage are among the most abundant proteins of the cell in both HeLa and CHO cells.

Immunological Characterization of the Fibrous Cage from Mitotic Cells

In an effort to identify some protein components of the fibrous cage, two different antisera directed against 10-nm filaments from cultured cells were obtained from other investigators. Antisera prepared against intermediate filament proteins from hamster cells were the generous gifts of Robert Goldman (53) and of Richard Hynes (30). Both sera were used to stain electrophoretic profiles of the detergent-extracted mitotic cages according to the procedure of Burridge (11) (Fig. 7).

The serum from Hynes' laboratory binds to the major 58,000-dalton polypeptide from mitotic HeLa and CHO cells. This antiserum was raised against a 58,000-dalton polypeptide from hamster fibroblasts that has been implicated as the subunit of the intermediate filaments by several laboratories and has been named "vimentin" (18). By the same assay, the antiserum of Starger et al. (53), which was raised using intact intermediate filaments as an antigen, recognizes the high 260,000-dalton polypeptide present in both the HeLa and CHO preparations.

Isolated cages prepared from drug-blocked mitotic HeLa and CHO cells stain with both sera (Fig. 8). The fluorescent structures appear as spherical cages with occasional deformations of the surface. No individual filaments can be distinguished in our preparations, probably because of the high density of the fibers in these structures.

DISCUSSION

Stabilized mitotic centers or spindles, as isolated here from HeLa cells, are surrounded by a fibrous cage that is missing or greatly reduced in similarly treated interphase cells. Apparently, the cage forms as the cell enters mitosis. The cage changes shape as the cell proceeds through mitosis to accommodate the elongating spindle, and during cytokinesis it is partitioned into the two daughter structures. Lysed mitotic CHO cells reveal similar fibrous cages, but only occasionally do they contain a stabilized spindle. When visualized in the electron microscope, the cages isolated from HeLa and CHO cells consist of heterogeneous fibrous material that includes fibers 10 nm in diameter.

Analysis of the fibrous cages by SDS gel electrophoresis shows that they are chemically complex, but compared with cell sap they are greatly enriched for polypeptides with mobilities characteristic of intermediate filaments. The rather heterogeneous morphology of the cage fraction is consistent with the biochemical complexity that they display. Our serological studies of the cages from HeLa and CHO cells do, nonetheless, confirm that they contain intermediate (10-nm) filaments of the vimentin type. In HeLa cells, which are of epithelial origin, the cages are particularly well developed and may also contain intermediate filaments of the keratin type.

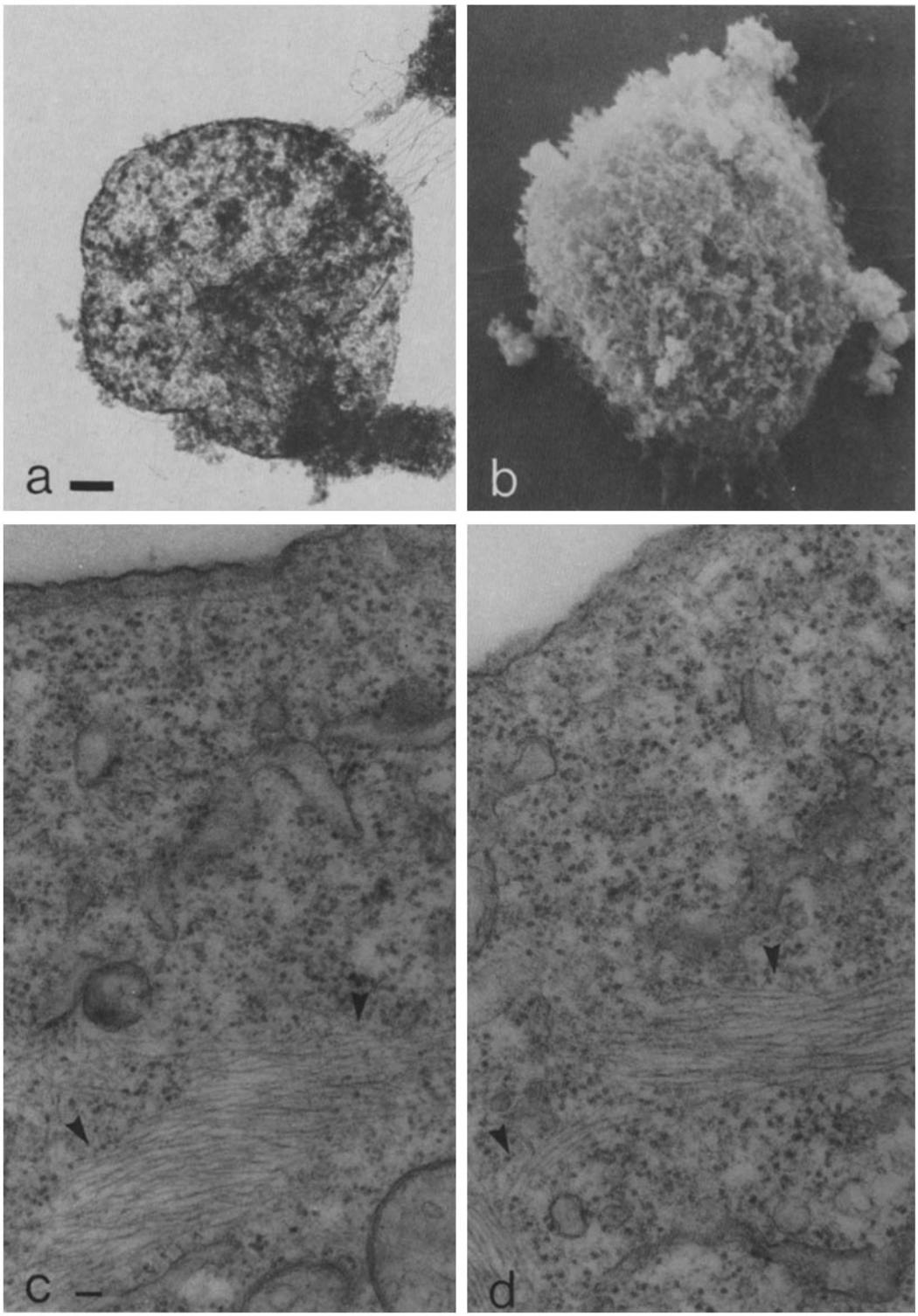

FIGURE 5 Mitotic CHO cells. Fig. 5 *a* shows a HVEM and Fig. 5 *b* shows a scanning electron micrograph of detergent-extracted CHO cells. These cells only occasionally contain a stabilized mitotic spindle. Bar, 1 μm. × 6,000. Fig. 5 *c* and *d* show conventional thin-section electron micrographs of intact mitotic CHO cells showing bundles of 10-nm filaments in the cytoplasm (arrowheads). Bar, 0.1 μm. × 40,000.

Intermediate filaments, defined as unbranched cytoplasmic fibers with a diameter of ~10 nm, have been described in a variety of cells. Biochemical and immunological characterizations of these fibers in several laboratories have led to the conclusion that they occur in at least five distinct classes. Muscle cells, neurons and glial cells all contain filaments that appear 10 nm in diameter (5, 8, 13, 31, 35, 44, 45, 61). The fibers of these cells are immunologically distinct, even comparing cells from the same organism. Cells of epidermal origin contain insoluble filaments 6–8 nm in diameter called tonofilaments (17, 19, 21, 32, 54). These fibers pervade the cytoplasm and are found in several differentiated states. The constituent polypeptides of these fibers have been identified as keratins, and they include a family of at least six polypeptides with mol

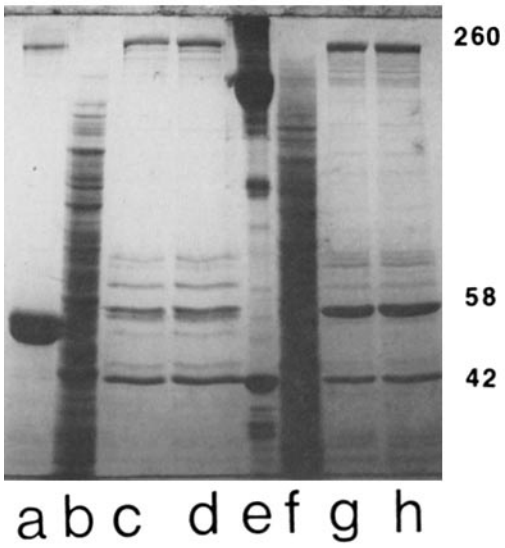

FIGURE 6 Polypeptide composition of whole HeLa and CHO cells compared to detergent-extracted interphase and detergent-extracted mitotic cells. Populations of mitotic and interphase cells were prepared and extracted twice with detergents as described in Materials and Methods. Insoluble mitotic cages and interphase nuclei with associated fibers were analyzed on SDS polyacrylamide gels and compared to whole cells and known markers: (*a*) 10 μg porcine brain tubulin, (*b*) 1×10^5 whole Hela cells, (*c*) 2×10^6 detergent-extracted interphase HeLa cells, (*d*) 1.5×10^6 detergent-extracted mitotic HeLa cells, (*e*) 25 μg rabbit skeletal muscle, (*f*) 5×10^5 whole CHO cells interphase, (*g*) 5×10^6 detergent-extracted interphase CHO cells, (*h*) 4×10^6 detergent-extracted mitotic CHO cells.

wt from 40,000 to 60,000. Antibody produced against soluble keratin (prekeratin) will decorate filamentous arrays in cultured cells of epithelial origin, including HeLa cells, but this probe does not stain the intermediate filaments of fibroblasts (17, 19, 55). A fifth distinct system of intermediate filaments forms networks in the cytoplasm of a number of interphase cells (6, 18, 25, 30, 52). These networks appear to originate from a juxtanuclear position and collapse around the nucleus to form perinuclear whorls when interphase microtubules are depolymerized by antimicrotubule reagents (15, 24). A 58,000-dalton polypeptide has been identified as a subunit of these filaments by several laboratories (18, 25, 30) and has been named vimentin by Franke et al. (18). Vimentin fibers are insoluble in the presence of nonionic detergents at both low and high ionic strength (4, 30, 52, 49). They have been identified as a major component of cytoskeletal preparations from a wide variety of cell types (4, 30, 19).

The immunofluorescent staining of isolated mitotic cages from HeLa and CHO cells and the radioimmune recognition of appropriate polypeptides in the electrophoretic profiles of the isolated cages by antisera directed against the vimentin fibers demonstrates that this class of intermediate filaments is present in our detergent-extracted preparations from both epithelial cells and fibroblasts. The localization of intermediate filaments of this type in the isolated mitotic cages even after extraction of the spindle is not surprising, considering the large number of reports that intermediate filaments remain insoluble after detergent extraction. The metaphase organization of the filaments in a cage that surrounds the MA suggests that the interphase arrays collapse around the mitotic spindle as the cell enters into mitosis.

Previous immunofluorescence studies have described vimentin filaments forming a cage around the MA (18, 30): such filaments are probably a major component of the structure isolated in this investigation. A similar immunofluorescence study has described the intermediate filaments of the vimentin type forming a ring around the MA in endothelial cells (6). The planar geometry may result from the flat shape of these endothelial cells which differs from the rounded shape of the mitotic HeLa and CHO cells we have studied. Blose (6) has demonstrated that the ring of intermediate filaments is not cleaved if the cleavage furrow is inhibited with cytochalasin. Similarly, the cage of intermediate filament in HeLa cells remains spherical when cells are arrested at the onset of mitosis, suggesting that the intermediate filaments are passively deformed by the kinetic activities of the mitotic spindle and cleavage furrow. Thin-section electron micrographs of intact mitotic CHO cells in which the intermediate filaments are readily identifiable demonstrate that the filaments appear in the cytoplasm around the perimeter of the cell, however, they do not appear to interact with the cell membrane.

HeLa cells have recently been identified as containing intermediate filaments immunologically related to the keratin-containing tonofilaments in addition to the vimentin filaments (20). Similar filaments were shown to be lacking in fibroblasts such as the CHO cells. These fibers were demonstrated to be insoluble in detergent and also to collect around the MA to form a "basket" during cell division. It is highly likely that our isolated HeLa cell mitotic cages include prekeratin-containing intermediate filaments as well as vimentin-containing fila-

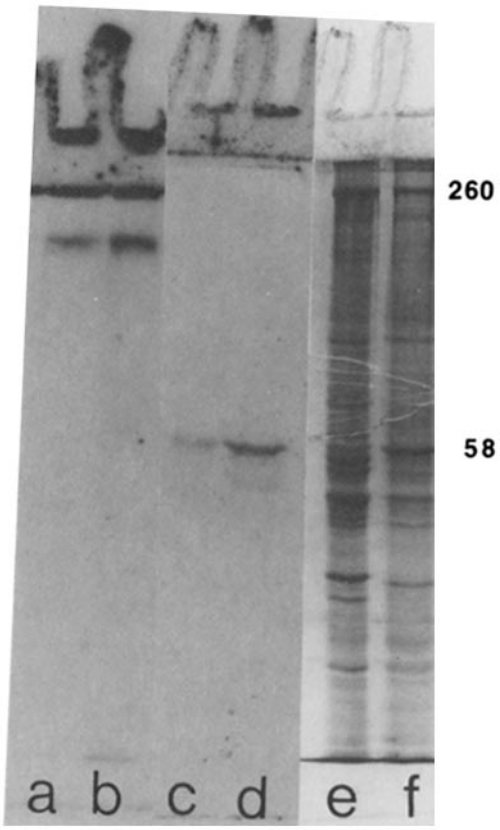

FIGURE 7 Staining of electrophoretic profiles of mitotic cages isolated from HeLa (*a* and *c*) and CHO (*b* and *d*) cells using antisera directed against the vimentin type of intermediate filaments. Fig. 7 *a* and *b* show staining by the serum of Starger et al. (53); Fig. 7 *c* and *d* show staining by the serum of Hynes and Destree (30); Fig. 7 *e* and *f* show the profile of gels *c* and *d* stained with Coomassie blue.

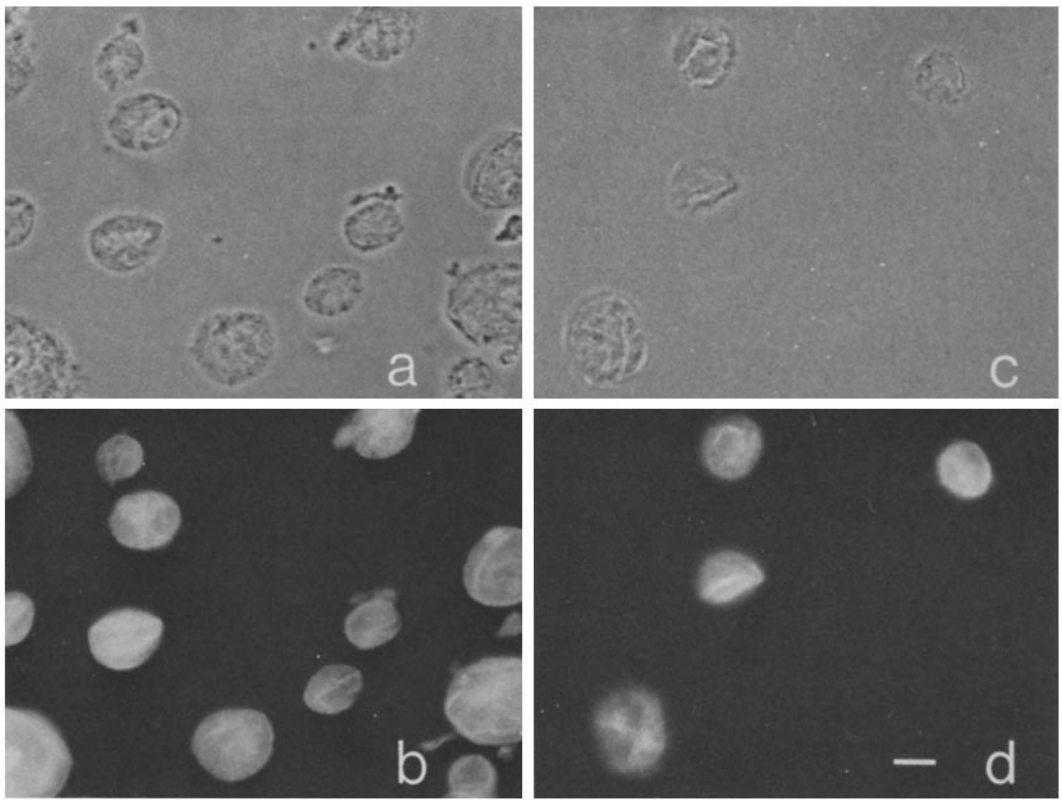

FIGURE 8 Indirect immunofluorescent staining of detergent-extracted mitotic cells with antibodies directed against the vimentin type intermediate filaments. Phase (*a*) and fluorescence micrograph (*b*) of detergent-extracted mitotic HeLa cells using antiserum of Starger et al. (53). Phase (*c*) and fluorescence micrograph (*d*) of detergent-extracted mitotic CHO cells using antiserum of Hynes and Destree (30). Bar, 10 μm. × 625.

ments. These tonofilaments may account for the polypeptides of ~40,000–55,000 daltons present in the lysed HeLa cells and not present in the lysed CHO cells (11). This second class of intermediate filaments may also contribute to the greater stability of the lysed HeLa cells compared to the lysed CHO cells.

Several laboratories have proposed that the distribution of vimentin filaments is closely tied to that of the MTs, a suggestion based both on the similarity of their spatial distribution in many cells and on the rearrangement of intermediate filaments that occurs when MTs are depolymerized by antimicrotubule drugs (19, 24, 30). The rearrangement of the intermediate filaments into a cage surrounding the MA may be an analogous result of the physiological depolymerization of the cytoplasmic MTs as the mitotic spindle assembles. It is noteworthy, however, that intermediate filaments are conspicuously absent from the spindle itself, except for the telophase interzone (40) where they are presumably co-mingled with the spindle tubules by the action of the cleavage furrow. It seems unlikely, therefore, that mitotic MTs interact in any direct way with intermediate filaments.

No specific function for the intermediate filaments in cultured cells has yet been identified, although many investigators have suggested that they play a structural role in the cytoplasm. The filaments may be associated with the well-known exclusion of cytoplasmic organelles from the area of the mitotic spindle (22), or they may form a cage because there is no place else for them to go. More speculative but intriguing is the possibility that the retention of these filaments and whatever additional material is associated with them in the absence of cytoplasmic MTs or microfilament bundles allows them to serve a skeletal role in mitotic cells. When the microfilament bundles, the cytoplasmic MTs, and the nuclear envelope break down in prophase, much of the interphase organization of a cell is lost. Nonetheless, cells reestablish their interphase morphology shortly after division. Perhaps the mitotic cage serves as a framework that helps redefine the cytoplasmic geometry after cytokinesis.

This investigation was supported by grant VCM 154C from the American Cancer Society and grant PCM-77-14796 from the National Science Foundation. G. W. Zieve was supported by a postdoctoral fellowship from the Helen Hay Whitney Foundation.

Received for publication 30 November 1979, and in revised form 16 June 1980.

REFERENCES

1. Albrecht-Buehler, G. 1977. Daughter 3T3 cells: are they mirror images of each other? *J. Cell Biol.* 72:595–603.
2. Allen, C., and G. G. Borisy. 1974. Structural polarity and directional growth of microtubules of *Chlamydomonas* flagella. *J. Mol. Biol.* 90:381–402.
3. Anderson, T. 1951. Techniques for the preservation of 3-D structures in preparing specimens for electron microscopy. *Trans. N. Y. Acad. Sci. Trans. II.* 13:130–134.
4. Ben-Ze'ev, A., S. R. Farmer, and S. Penman. 1979. Mechanisms of regulating tubulin synthesis in cultured mammalian cells. *Cell.* 17:319–325.
5. Bennett, G. S., S. A. Fellini, J. M. Croop, J. J. Otto, J. Bryan, and H. Holtzer. 1978. Differences among 100-A filament subunits from different cell types. *Proc. Natl. Acad. Sci. U. S. A.* 75:4364–4368.
6. Blose, S. H. 1979. Ten-nanometer filaments and mitosis: Maintenance of structural continuity in dividing endothelial cells. *Proc. Natl. Acad. Sci. U. S. A.* 76:3372–3376.
7. Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72:248–254.
8. Brecher, S. 1975. The occurrence and possible role of 80–100 A filaments in PtK1 cells. *Exp. Cell Res.* 96:303–310.
9. Bootsma, D., L. Budke, and O. Vos. 1964. Studies on synchronous division of tissue

culture cells initiated by excess thymidine. *Exp. Cell Res.* 33:310–309.
10. Brown, S., W. Levinson, and J. A. Spudich. 1976. Cytoskeletal elements of chick embryo fibroblasts revealed by detergent extraction. *J. Supramol. Struct.* 5:119–130.
11. Burridge, K. 1978. Direct identification of specific glycoproteins and antigens in sodium dodecyl sulfate gels. *Methods Enzymol.* 50:54–64.
12. Cande, W. Z., E. Lazarides, and J. R. McIntosh. 1977. A comparison of the distribution of actin and tubulin in the mammalian mitotic spindle as seen by indirect immunofluorescence. *J. Cell Biol.* 72:552–567.
13. Cooke, P. 1976. A filamentous cytoskeleton in vertebrate smooth muscle fibers. *J. Cell Biol* 68:539–556.
14. Croop, J., and H. Holtzer. 1975. Response of myogenic and fibrogenic cells to cytochalasir B and to colcemid. *J. Cell Biol.* 65:271–285.
15. DeBrabander, M., F. Aerts, R. Van de Veire, and M. Borgers. 1975. Evidence against interconversion of microtubules and filaments. *Nature (Lond.).* 253:119–120.
16. Franke, W. W. 1974. Structure, biochemistry and functions of the nuclear envelope. *Int. Rev. Cytol.* (Suppl. 4) 72–236.
17. Franke, W. W., K. Weber, M. Osborn, E. Schmid, C. Freudenstein. 1978. Antibody to prekeratin: decoration of tonofilament-like arrays in various cells of epithelial character. *Exp. Cell Res.* 116:429–445.
18. Franke, W. W., E. Schmid, M. Osborn, and K. Weber. 1978. Different intermediate-sized filaments distinguished by immunofluorescence microscopy. *Proc. Natl. Acad. Sci. U. S. A.* 75:5034–5038.
19. Franke, W. W., E. Schmid, M. Osborn, and K. Weber. 1978. Intermediate-sized filaments in rat kangaroo PtK_2 cells. *Cytobiologie.* 17:392–411.
20. Franke, W. W., E. Schmid, K. Weber, and M. Osborn. 1979. HeLa cells contain intermediate-sized filaments of the prekeratin type. *Exp. Cell Res.* 118:95–109.
21. Fuchs, E., and H. Green. 1978. The expression of keratin genes in epidermis and cultured epidermal cells. *Cell.* 15:887–897.
22. Fuge, H. 1977. Ultrastructure of the mitotic spindle. *Int. Rev. Cytol.* (Suppl. 6) 1–58.
23. Gerace, L., A. Blum, and G. Blobel. 1978. Immunocytochemical localization of the major polypeptides of the nuclear pore complex-lamina fraction. *J. Cell Biol.* 79:546–566.
24. Goldman, R. D., and D. H. Knipe. 1973. Functions of cytoplasmic fibers in non-muscle cell motility. *Cold Spring Harbor Symp. Quant. Biol.* 37:523–533.
25. Gordon, W. E., A. Bushnell, and K. Burridge. 1978. Characterization of the intermediate (10 nm) filaments of cultured cells using an autoimmune rabbit antiserum. *Cell.* 13:249–261.
26. Heidemann, S. R., G. W. Zieve, and J. R. McIntosh. 1980. Evidence for microtubule subunit addition to the distal end of mitotic structures in vitro. *J. Cell Biol.* 87:152–159.
27. Hoebeke, J., G. Van Nyen, and M. DeBrabander. 1976. Interaction of Oncodazole, a new anti-tumoral drug, with rat brain tubulin. *Biochem. Biophys. Res. Comm.* 69:319–324.
28. Hughes, A. F., and M. M. Swann. 1948. Anaphase movements in the living cell. *J. Exp. Biol.* 25:45–70.
29. Hunter, W. M., and F. C. Greenwood. 1962. Preparation of ^{131}I-labeled human growth hormone of high specific activity. *Nature (Lond.).* 194:495–496.
30. Hynes, R. O., and A. T. Destree. 1978. 10 nm filaments in normal and transformed cells. *Cell.* 13:151–163.
31. Ishikawa, H., R. Bischoff, and H. Holtzer. 1968. Mitosis and intermediate-sized filaments in developing skeletal muscle. *J. Cell Biol.* 38:538–555.
32. Kelly, D. E. 1966. Fine structure of desmosomes, hemidesmosomes and an adepidermal globular layer in developing newt epidermis. *J. Cell Biol.* 28:51–72.
33. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.).* 227:680–685.
34. Lazarides, E., and B. D. Hubbard. 1976. Immunological characterization of the subunit of the 100 A° filament from muscle cell. *Proc. Natl. Acad. Sci. U. S. A.* 73:4344–4348.
35. Lazarides, E., and D. R. Balzer. 1978. Specificity of desmin to avian and mammalian muscle cells. *Cell.* 14:429–438.
36. Lenk, R., L. Ranson, Y. Kaufman, and S. Penman. 1977. A cytoskeletal structure with associated polyribosomes from HeLa cells. *Cell.* 10:67–78.
37. Mazia, D., and K. Dan. 1952. The isolation and biochemical characterization of the mitotic apparatus of dividing cells. *Proc. Natl. Acad. Sci. U. S. A.* 38:826–838.
38. McGill, M., and B. R. Brinkley. 1975. Human chromosomes and centrioles as nucleating sites for *in vitro* assembly of microtubules from bovine brain tubulin. *J. Cell Biol.* 67:189–199.
39. McIntosh, J. R., W. Z. Cande, and J. A. Snyder. 1975. Structure and Physiology of the Mammalian Mitotic Spindle. *In* Molecules and Cell Movement. S. Inoué and R. E. Stephens, editors. Raven Press, New York. 31–76.
40. McIntosh, J. R., and S. C. Landis. 1971. The distribution of spindle microtubules during mitosis in cultured human cells. *J. Cell Biol.* 49:468–497.
41. McIntosh, J. R. 1977. Mitosis *in vitro*: Isolates and models of the mitotic apparatus. *In* Mitosis—Facts and Questions. M. Little, N. Paweletz, C. Petzelt, H. Ponstingl, D. Schroeter, and H.-P. Zimmerman, editors. Springer-Verlag, New York. 167–195.
42. Pollard, T. D., and R. R. Weihing. 1974. Cytoplasmic actin and myosin and cell movement. *CRC Crit. Rev. Biochem.* 2:1–65.
43. Porter, K. R., and R. D. Machado. 1960. Studies on the endoplasmic reticulum. *J. Biophys. Biochem. Cytol.* 7:167–180.
44. Schachner, M., E. T. Hedley-Whyte, D. W. Hsu, G. Schoonmaker, and A. Binani. 1977. Ultrastructural localization of glial fibrillary acidic protein in mouse cerebellum by immunoperoxidase labeling. *J. Cell Biol.* 75:67–73.
45. Schlaepfer, W. W. 1977. Immunological and ultrastructural studies of neurofilaments isolated from rat peripheral nerves. *J. Cell Biol.* 74:226–240.
46. Shelanski, M. L., S. Albert, G. H. DeVries, and W. T. Norton. 1971. Isolation of filaments from brain. *Science (Wash. D. C.).* 174:1242–1245.
47. Simionescu, N., and M. Simionescu. 1976. Galloylglucoses of low molecular weight as mordant in electron microscopy. *J. Cell Biol.* 70:608–621.
48. Sisken, J. E., E. Walkers, G. M. Donnelly, and T. Kakefuda. 1964. The isolation of the mitotic apparatus from mammalian cells in culture. *J. Cell Biol.* 32:212–216.
49. Small, J. V., and A. Sobieszek. 1977. Studies on the function and composition of the 10 nm filaments of vertebrate smooth muscle. *J. Cell Sci.* 23:243–268.
50. Snyder, J. A., and J. R. McIntosh. 1975. Initiation and growth of microtubules from mitotic centers in lysed mammalian cells. *J. Cell Biol.* 67:744–760.
51. Solomon, F. 1979. Detailed neurite morphologies of sister neuroblastoma cells are related. *Cell.* 16:165–169.
52. Starger, J. M., and R. D. Goldman. 1977. Isolation and preliminary characterization of 10 nm filaments from baby hamster kidney (BHK-21) cells. *Proc. Natl. Acad. Sci. U. S. A.* 74:2422–2426.
53. Starger, J. M., W. E. Brown, A. E. Goldman, and R. D. Goldman. 1978. Biochemical and immunological analysis of rapidly purified 10 nm filaments from baby hamster kidney (BHK-21) cells. *J. Cell Biol.* 78:93–109.
54. Steinert, P. M., and W. W. Idler. 1975. The polypeptide composition of bovine epidermal α-keratin. *Biochem. J.* 151:603–614.
55. Sun, T., and H. Green. 1978. Immunofluorescent staining of keratin fibers in cultured cells. *Cell.* 14:469–476.
56. Telzer, B. R., and J. L. Rosenbaum. 1979. Cell cycle-dependent, *in vitro* assembly of microtubules onto the pericentriolar material of HeLa cells. *J. Cell Biol.* 81:484–488.
57. Terasima, T., and L. J. Tolmach. 1963. Growth and nucleic acid synthesis in synchronously dividing populations of HeLa cells. *Exp. Cell Res.* 30:344–362.
58. Turner, J. L., and J. R. McIntosh. 1977. Isolation of the mitotic apparatus *Methods Cell Biol.* 16:373–379.
59. Weingarten, M. D., M. M. Suter, D. R. Littman, and M. W. Kirschner. 1974. Properties of the depolymerization products of microtubules from mammalian brain. *Biochemistry* 13:5529–5537.
60. Weisenberg, R. C., and A. C. Rosenfeld. 1975. *In vitro* polymerization of microtubules into asters and spindles in homogenates of surf clam eggs. *J. Cell Biol.* 64:146–158.
61. Yen, S., D. Dahl, M. Schachner, and M. L. Shelanski. 1976. Biochemistry of the filaments of brain. *Proc. Natl. Acad. Sci. U. S. A.* 73:529–533.
62. Zieve, G., D. Turnbull, M. Mullins, and J. R. McIntosh. 1980. Production of large numbers of mitotic mammalian cells by use of the reversible microtubule inhibitor Nocodazole. *Exp. Cell Res.* 126:397–405.