

The Organizational Fate of Intermediate Filament Networks in Two Epithelial Cell Types during Mitosis

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ABSTRACT Intermediate filaments (IF) appear to be attached to the nuclear envelope in various mammalian cell types. The nucleus of mouse keratinocytes is enveloped by a cage-like network of keratin-containing bundles of IF (IFB). This network appears to be continuous with the cytoplasmic IFB system that extends to the cell surface. Electron microscopy reveals that the IFB appear to terminate at the level of the nuclear envelope, frequently in association with nuclear pore complexes (Jones, J. C. R., A. E. Goldman, P. Steinert, S. Yuspa, and R. D. Goldman, 1982, *Cell Motility*, 2:197–213). Based on these observations of nuclear-IF associations, it is of interest to determine the fate and organizational states of IF during mitosis, a period in the cell cycle when the nuclear envelope disassembles. Immunofluorescence microscopy using a monoclonal keratin antibody and electron microscopy of thin and thick sections of mitotic mouse keratinocytes revealed that the IFB system remained intact as the cells entered mitosis and surrounded the developing mitotic spindle. IFB were close to chromosomes and often associated with chromosome arms. In contrast, in HeLa, a human epithelial cell, keratin-containing IFB appear to disassemble as cells enter mitosis (Franke, W. W., E. Schmid, C. Grund, and B. Geiger, 1982, *Cell*, 30:103–113). The keratin IFB in mitotic HeLa cells appeared to form amorphous nonfilamentous bodies as determined by electron microscopy. However, in HeLa, another IF system composed primarily of a 55,000-mol-wt protein (frequently termed vimentin) appears to remain morphologically intact throughout mitosis in close association with the mitotic apparatus (Celis, J. E., P. M. Larsen, S. J. Fey, and A. Celis, 1983, *J. Cell Biol.*, 97:1429–34). We propose that the mitotic apparatus in both mouse epidermal cells and in HeLa cells is supported and centered within the cell by IFB networks.

Intermediate filament (IF)¹ organization during mitosis varies in different cell types. For example, in primary vascular endothelial cells, IF are arranged in interphase as a perinuclear ring that is divided between daughter cells without apparent disassembly into subunits (1). In contrast, it has been reported that in the continuous cell lines, e.g., HeLa, Chinese hamster ovary, baby hamster kidney (BHK)-21, and cultured rat kangaroo cells (PtK₂), a cage or a perispindle aggregation of IF containing a major IF structural protein, vimentin, appears to form during cell division and surrounds the mitotic appa-

ratus (2–5). In addition, keratin-containing IF bundles (IFB) are found throughout mitosis in PtK₂ cells, but Aubin et al. (5) suggest that these IFB are not associated with the mitotic apparatus. In this latter case they appear to be restricted to the substrate-attached side of the cell (5). Franke et al. (6) have shown that in PtK₂ cells, there is a rearrangement of keratin IF during mitosis involving masking and unmasking of immunologic determinants. Recently, it has been reported that during mitosis in certain epithelial cell types (HeLa and Madin-Darby bovine kidney), keratin- and vimentin-containing IF disappear and are converted into electron-dense, relatively amorphous granules (7, 8). Moreover, Lane et al. (9) have reported that the disruption of keratin IF during mitosis is a phenomenon restricted to cells rich in keratin filaments such as keratinocytes. In summary, several investigators claim

¹ *Abbreviations used in this paper:* BHK, baby hamster kidney; MES, 2-(*N*-morpholino)ethane sulfonic acid; IF, intermediate filaments; IFB, intermediate filament bundles; PtK₂, cultured rat kangaroo cells.

that in some cells during mitosis, a cage of IF forms around the mitotic spindle, whereas other investigators claim that IF break down or disassemble as cells enter mitosis. In addition, different laboratories obtain different results with the same cell type. For example, Franke et al. (7) report that in mitotic HeLa cells vimentin-containing IF unravel during mitosis to form spheroidal aggregates, whereas in the same cell type Celis et al. (3) have shown that vimentin IF remain morphologically intact during mitosis. Thus, the literature is very confusing with regard to IF reorganization during mitosis.

We have demonstrated recently that there is an elaborate cage of keratin-containing IFB closely surrounding the interphase nucleus of cultured mouse keratinocytes (10). These IFB appear frequently to associate closely with the nuclear envelope at the site of nuclear pore complexes (10). Based on the close relationship between IFB and the interphase nucleus in mouse keratinocytes and in every other epithelial cell type that we have examined (J. C. R. Jones and R. D. Goldman, unpublished observations), we have proposed that the IFB system, which courses from the nuclear surface to the plasma membrane, functions in nuclear positioning and centration within the cytoplasm and perhaps even as a cell surface-nucleus signal transducer (10, 21). Since the mitotic apparatus usually forms at the cell center and the nuclear envelope breaks down and disappears during mitosis, a knowledge of the exact organizational fate of IF and their relation to the mitotic apparatus during and immediately after mitosis is a prerequisite to determining the functions of these major cytoskeletal components. We, therefore, carried out light and electron microscopic observations on the organization and fate of IF and their constituent proteins through the cell division cycle in two epithelial cell types, mouse keratinocytes and HeLa cells.

MATERIALS AND METHODS

Cell Cultures

Mouse keratinocytes were prepared and maintained in culture as previously reported (10, 11). HeLa cells were grown as described in a previous publication (12). The high mitotic indexes of these two cell types enabled us to look at many mitotic cells without using any chemical treatments (e.g., colchicine arrest) to synchronize cell populations.

IF Network Isolation

Native IF networks were isolated from mouse keratinocytes by a method previously described (13). The final IF-enriched pellet was washed three times in phosphate-buffered saline (6 mM Na⁺, K⁺ phosphate, 171 mM NaCl, 3 mM KCl, [pH 7.4]) with 0.1 mM phenylmethylsulfonyl fluoride and stored at -80°C as a pellet for future use.

Whole Cell Protein Preparation

One 100-mm petri dish of confluent mouse keratinocytes was washed briefly with phosphate-buffered saline, and then 1 ml sample buffer (8 M urea in 0.19 M Tris-HCl [pH 6.8], 1% SDS, 1% β -mercaptoethanol) was added to the cells. The cells were scraped off the dish in the sample buffer. This solution was stirred at room temperature for 30 min and was subsequently stored at -20°C for later use.

Antibodies

MONOCLONAL ANTIBODY PRODUCTION: Two 150-g Sprague-Dawley rats were injected with 1 mg protein derived from mouse keratinocyte IF networks in PBS emulsified with an equal volume of Freund's complete adjuvant. The rats were boosted with 0.5 mg protein in PBS emulsified with an equal volume of Freund's incomplete adjuvant 21 d after initial injection. After an additional 10 d, the rats were injected with 0.5 mg of the same protein

preparation in PBS and 2 d later, the rats were killed and their spleens removed. Spleen cells were fused with the mouse myeloma line, Sp2, according to the procedure of Galfre and Milstein (14). Cells were plated out in 24-well culture dishes (Costar, Data Packaging, Cambridge, MA). Medium from wells containing cell colonies was tested by immunofluorescence on mouse epidermal cells and by Western blotting using the Towbin procedure (15) (see below). Hybridoma cells in wells that contained antibodies as detected by these above two procedures were then cloned twice by limiting cell dilution in 96-well Costar plates and medium from resulting colonies was tested by both immunofluorescence and Western blotting (see below).

RABBIT ANTISERA: A rabbit antiserum directed against the 60,000-mol-wt K2 mouse keratin subunit (10), and a rabbit serum directed against the BHK-21 55,000-mol-wt IF subunit (vimentin) (12) were used in these studies.

Western Blotting Procedure

SDS-PAGE using 3-12% gradient acrylamide slab gels with 4.5% acrylamide stacking gels was performed on mouse keratinocyte IF networks and keratinocyte whole cell protein solubilized in sample buffer (see above). After this one-dimensional separation, the resulting proteins were transferred to nitrocellulose (15). Immunoblotting was carried out according to Zackroff et al. (16) using the monoclonal and the rabbit antikeratin antibody preparations.

Indirect Immunofluorescence

Cells growing on glass coverslips were fixed for 2 min in -20°C methanol and air dried. Monoclonal antibody medium was added to cover the cells. The coverslips were incubated in a moist chamber for 1 h at 37°C, then washed 10 times in beakers containing distilled water and incubated for an additional 30 min at 37°C with fluorescein-conjugated goat anti-rat IgG (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD). Coverslips were washed thoroughly in several changes of distilled water and mounted on glass slides in gelvatol (Monsanto Co., St. Louis, MO). For double indirect immunofluorescence, cells were fixed as described above. Rabbit antitubulin antiserum (prepared according to the method of Brinkley et al. [17]) was mixed with monoclonal antibody medium to a final dilution of 1:20, overlaid on the cells, and incubated for 1 h at 37°C. Coverslips were washed in distilled water and then overlaid with a mixture of fluorescein-conjugated goat anti-rat and rhodamine-conjugated goat anti-rabbit IgG (Kirkegaard & Perry Laboratories, Inc.) for 30 min at 37°C, washed extensively in water and mounted in gelvatol.

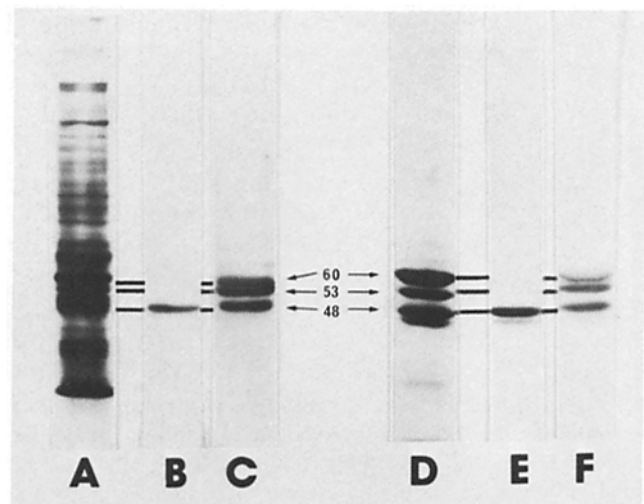


FIGURE 1 Specificity determination of the rat monoclonal anti-keratin by the immunoblotting technique. Polypeptides of whole mouse keratinocyte extracts (lanes A-C), and polypeptides of mouse keratinocyte IF network (lanes D-F) were subjected to SDS PAGE and electrically transferred to nitrocellulose. Lanes A and D show amido black stains of the transferred polypeptides. The rat monoclonal antikeratin reacts with a 48,000-mol-wt polypeptide in both the whole cell extracts and the IF network preparations (lanes B and E). The rabbit anti-mouse keratin reacts with this same polypeptide in addition to two other keratin polypeptides with molecular weights of 60,000 and 53,000 (lanes C and F).

Light Microscopy

A Zeiss Photomicroscope III equipped with epifluorescence and phase-contrast optics was used for observation of fixed and stained cells. A Zeiss III RS epi-illumination system was equipped with narrow band filter sets for selective observation of fluorescein and rhodamine. Observations of cells labeled with fluorescein were made with a xenon 75W DC lamp, and those labeled with rhodamine with a 100W DC mercury arc source. Fluorescence micrographs were taken on Kodak Plus-X film. All films were exposed for development in Diafine (Acufine, Inc., Chicago, IL) two-stage developer.

Electron Microscopy

Cells in petri dishes were fixed and processed for electron microscopy according to Starger et al. (18). Thin sections (50 nm) and thick sections (between 0.25 and 0.5 μm) were prepared using an LKB IV Ultratome (LKB Instruments, Gaithersburg, MD). Sections were stained in 3% aqueous uranyl acetate, followed by Reynolds lead citrate. Thin sections were viewed in a JEOL 100S electron microscope at an accelerating voltage of 60 kV. Thick sections were viewed in a JEOL 200CX electron microscope at an accelerating voltage of 200 kV.

Immunogold localization was carried out according to the procedure of Yang, Lieska, Goldman, and Goldman (*J. Cell Biol.*, in press). HeLa cells grown in 35-mm dishes were washed twice in PBS and then fixed in 0.1% glutaraldehyde, 0.2% Triton X-100 in 2, (*N*-morpholino)ethane sulfonic acid (MES) buffer (0.1 M MES [pH 6.6], 0.5 mM MgSO_4 , 2 mM EGTA) for 30 s. After three washes over a 20-min period in MES buffer, cells were further permeabilized in this buffer plus 0.2% Triton X-100. Cells were then treated with 0.5 mg/ml NaBH_4 in MES buffer for 20 min to reduce the free aldehyde groups of glutaraldehyde that can nonspecifically bind colloidal gold. After an additional three washes over a 30-min period in MES buffer, cells were incubated for 60 min at 37°C in primary antibody (i.e., either the rabbit antikeratin serum or the rabbit anti-55,000-mol-wt IF subunit serum). Cells were washed extensively in MES buffer and then incubated for an additional 60 min at 37°C in 5 nm colloidal gold-conjugated goat anti-rabbit IgG (Janssen Pharmaceutical, Beerse, Belgium). After washing in MES buffer, cells were fixed and processed for electron microscopy according to the procedure of Starger et al (18).

RESULTS

Intermediate Filament Networks in Interphase and Mitotic Mouse Keratinocytes and HeLa Cells

IMMUNOFLUORESCENCE OBSERVATIONS: The monoclonal antibody reacted by the immunoblotting technique with a single 48,000-mol-wt polypeptide found in total protein isolated from mouse keratinocytes and also in enriched form in the IF network preparations from mouse epidermal cells (Fig. 1). The keratinlike nature of this polypeptide has been confirmed by its reaction with a rabbit antibody preparation directed against the 60,000-mol-wt (K2) keratin subunit extracted from mouse skin tissue (Fig. 1) (also see references 10, 19). The monoclonal antibody did not react by immunoblotting with similar IF preparations isolated from BHK-21 fibroblasts, nor did it stain the cytoplasmic arrays of IF within these cells as determined by immunofluorescence (results not shown).

By indirect immunofluorescence, the monoclonal antibody stained a complex network of keratin-containing tonofibrils in interphase keratinocytes (Fig. 2). The pattern is typical of epithelial cells stained with keratin antibodies (20) and, furthermore, the pattern is indistinguishable from that observed using the rabbit K2 antiserum (see Fig. 1a in reference 10). By observing epidermal cells at various focal levels, one can see that the centrally situated nucleus was enveloped by a cage of keratin-containing fluorescent tonofibrils (Figs. 2, a-c). This cage of tonofibrils was continuous with similar fibrils that span the cytoplasm and associate with the cell surface.

The juxtannuclear cage of fluorescent tonofibrils remained in place as the nuclear envelope broke down during the

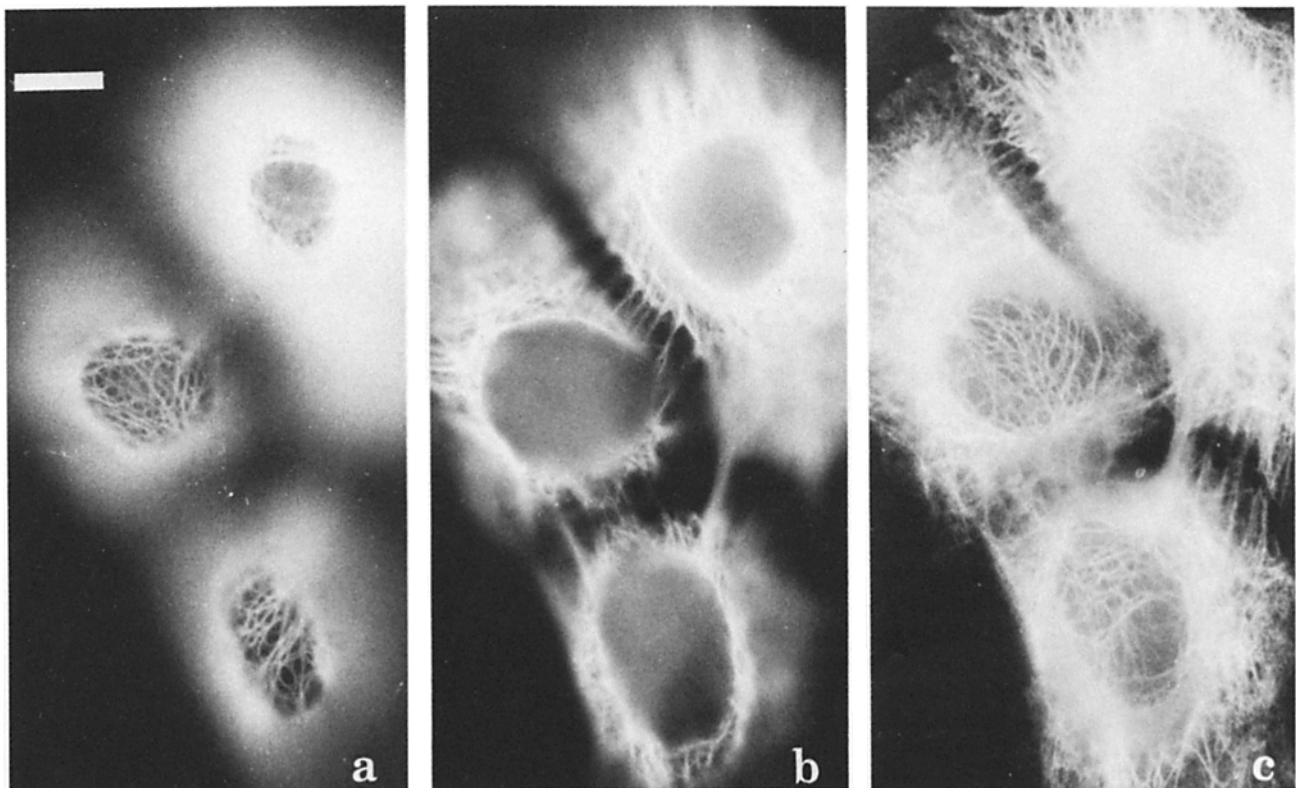


FIGURE 2 Interphase mouse keratinocytes prepared for indirect immunofluorescence using the monoclonal antikeratin. A through-focal series shows that a cage-like network of fluorescent fibers surrounds the centrally situated nucleus of these cells. Bar, 10 μm . $\times 1,350$.

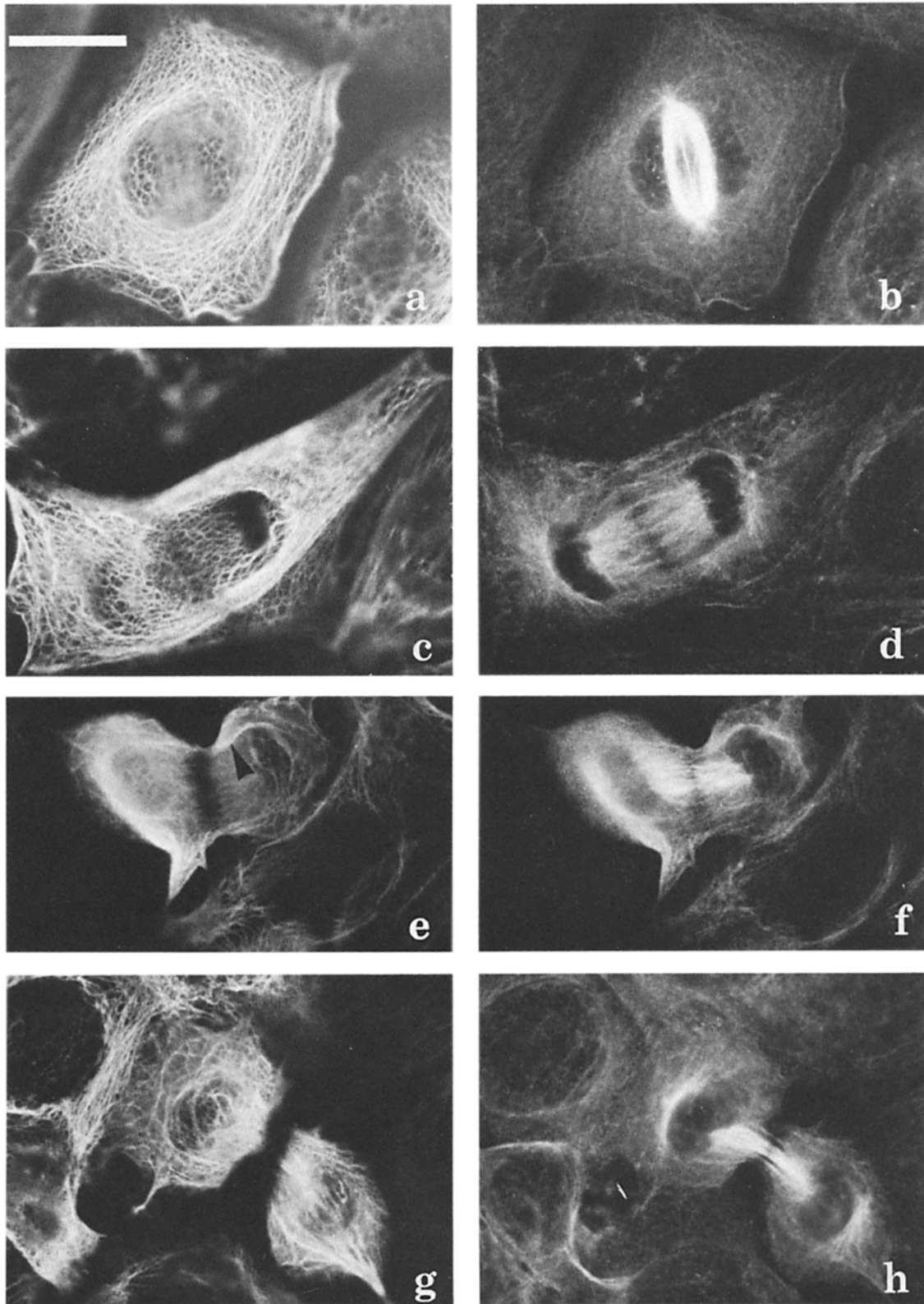


FIGURE 3 Mitotic mouse keratinocytes prepared for double indirect immunofluorescence using the rat monoclonal antikeratin (a, c, e, and g) and an antiserum directed against tubulin (b, d, f, and h). A cage of fluorescent fibers surrounding the spindle is present throughout mitosis. At telophase, the cage appears to be constricted in the region of midbody formation (arrow in e). By very late telophase, two complete cages surround the two new daughter nuclei (g). Bar, 10 μm . $\times 1,900$.

formation of the mitotic spindle as determined by indirect immunofluorescence using either the monoclonal keratin (Fig. 3a) or the mouse skin keratin antiserum (results not shown). An extensive network of fluorescent tonofibrils re-

mained closely associated with the spindle throughout mitosis. This was visualized most dramatically when double-indirect immunofluorescence was employed using the monoclonal keratin and rabbit antitubulin preparations (Fig. 3). In early

telophase, as the nuclear envelopes of daughter cells reformed, the tonofibrils appeared to be constricted in the region of the midbody (Fig. 3*e*). By late telophase, no fluorescent tonofibrils were seen in the midbody region and the two daughter cells contained apparently noncontinuous systems of keratin-containing tonofibrils (Figs. 3*g*).

Keratin-containing IFB did not undergo a dramatic reorganization during mitosis in mouse keratinocytes; this result, different from that reported by Lane et al. (9), led us to look at the fate of keratin- and vimentin-containing IF during mitosis in HeLa cells to determine whether our results would again conflict with those already reported in the literature (e.g., that keratin-containing IFB break down during mitosis). However, our immunofluorescence observations using the mouse skin keratin subunit antiserum and the vimentin antiserum on the organization of IF during mitosis in HeLa cells confirmed those results reported by others (3, 7, 8). For example, as HeLa cells entered mitosis, the majority of keratin-containing IF appeared to be altered to form discrete aggregates and at late telophase they appeared to reform from these aggregates (results not shown). This was determined by indirect immunofluorescence using the mouseskin keratin subunit antiserum, the same antiserum that stains a cage-like system of tonofibrils surrounding the mitotic apparatus in mouse keratinocytes. The other IF system in HeLa cells that

reacts with vimentin antiserum did not undergo any obvious reorganization during mitosis (data not shown).

ELECTRON MICROSCOPICAL OBSERVATIONS: To determine the detailed morphology of the tonofibrils surrounding the interphase nucleus and the mitotic apparatus of keratinocytes, we carried out an ultrastructural analysis of thin-sectioned preparations. The structures that most readily account for the tonofibrils seen by indirect immunofluorescence were the IFB located throughout the cytoplasm and concentrated in the perinuclear and perispindle areas (Figs. 4–7).

The IFB comprising the nuclear cage frequently appeared to be closely associated with the nuclear envelope at the site of nuclear pore complexes (Fig. 4*b*, see reference 10). Thin sections of a variety of stages of mitosis revealed that IFB were always present in the region peripheral to the spindle at each stage of mitosis (Fig. 5, *a* and *b*) and that they frequently lie close to arms of chromosomes that extend from the mitotic apparatus into the surrounding cytoplasm (Fig. 6, *a* and *b*). To more readily examine the extent of the juxtannuclear IFB network throughout mitosis in mouse keratinocytes, we prepared thicker sections (up to $\sim 0.5 \mu\text{m}$) and viewed them at an accelerating voltage of 200 kV. In general, these showed a similar, but a more comprehensive view of the IFB system (Fig. 7). At anaphase, IFB that are normally wavy frequently appear to straighten in the region between the separating poles

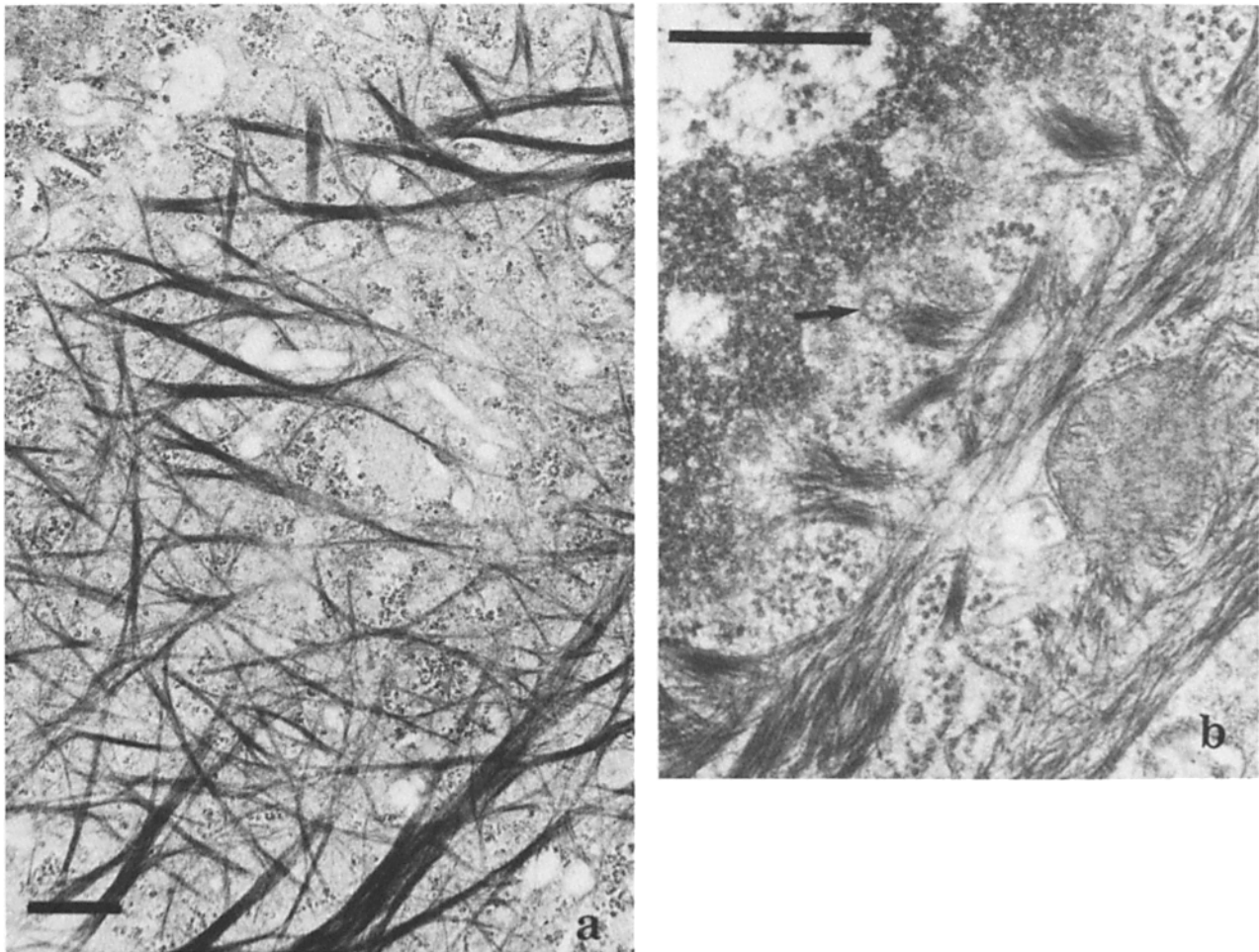


FIGURE 4 Electron micrographs of thin sections of interphase mouse keratinocytes. (a) The extensive tonofilament (IF) network in this cell type. (b) IFB are closely associated with the nuclear envelope. One bundle is immediately adjacent to a nuclear pore complex (arrow). (a) Bar, $1 \mu\text{m}$. $\times 10,600$. (b) Bar, $0.5 \mu\text{m}$. $\times 54,000$.

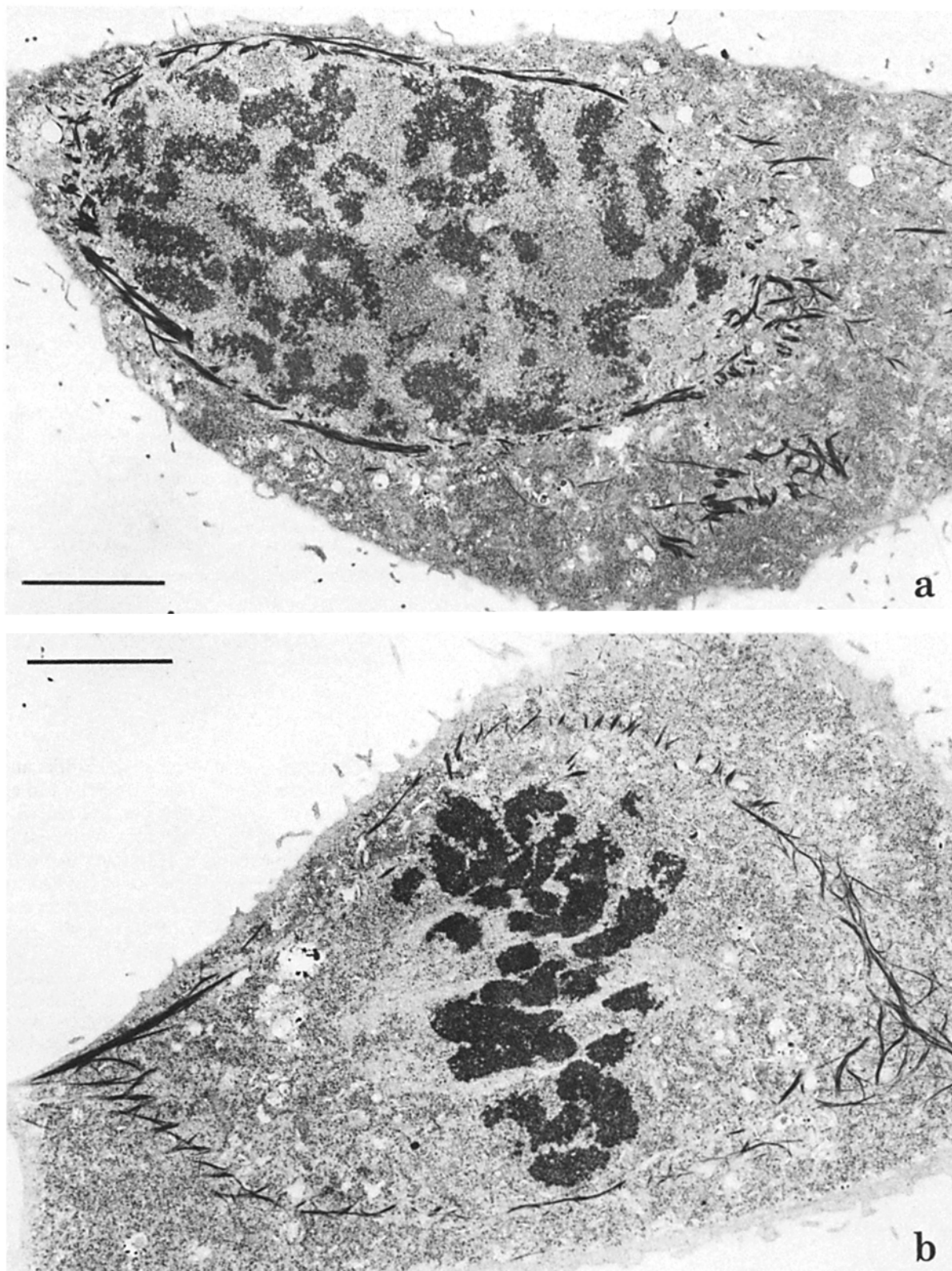


FIGURE 5 Electron micrographs of thin sections of two mitotic mouse keratinocytes. (a) Tonofilaments (IFB) surround the condensing chromatin at prophase. (b) IFB surround the mitotic spindle in metaphase. Bars, 5 μ m. (a) \times 4,800; (b) \times 5,000.

of the mitotic apparatus (Fig. 7d). This latter observation has been confirmed *in vivo* by phase-contrast microscopy (data not shown).

In contrast to mouse keratinocytes, IFB in mitotic HeLa appear to disassemble and form amorphous nonfilamentous

bodies in the cytoplasm (Fig. 8). To show that these bodies are keratin-enriched and that the fibroblastlike IF remain intact throughout mitosis as reported by Celis et al. (3), we processed HeLa cells for IF antibody localization in electron microscope preparations (see Material and Methods). Using

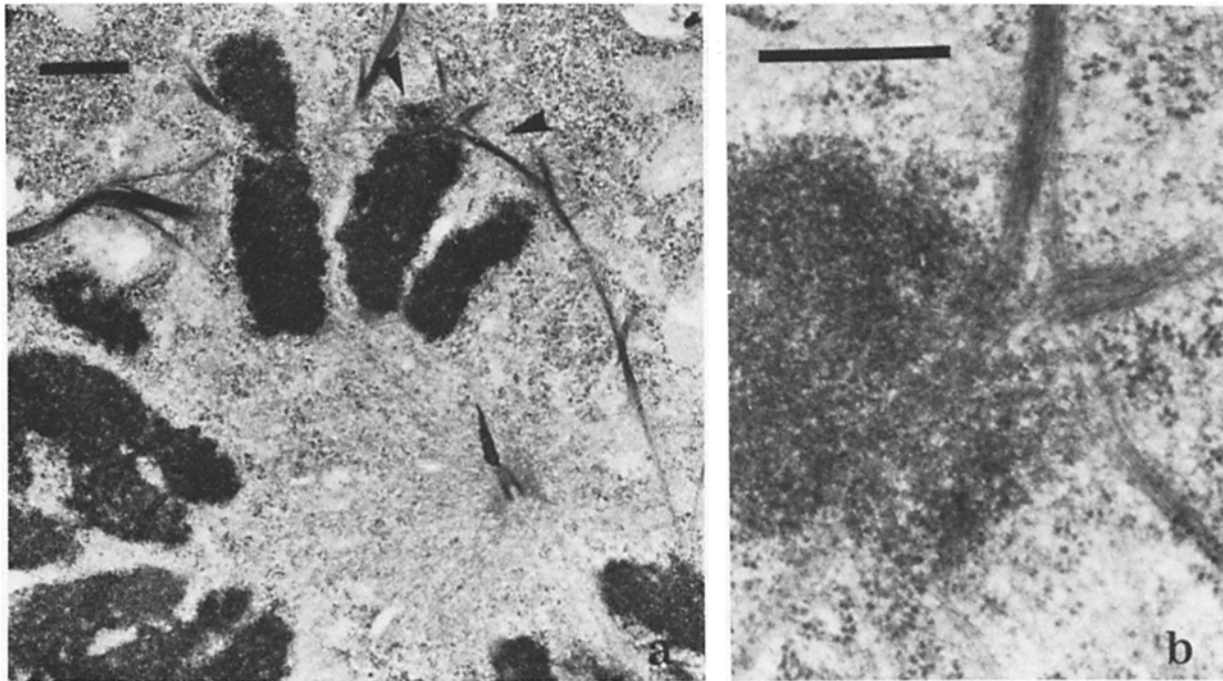


FIGURE 6 Electron micrographs of a thin section of a mitotic mouse keratinocytes show the close association of IFB and the arms of chromosomes (a). The area between the arrowheads in a has been enlarged in b. In b, IFB appear close to the chromosome arm. Bars, 0.5 μ m. (a) \times 12,500; (b) \times 50,000.

the BHK 55,000-mol-wt IF subunit antiserum, gold particles are seen closely associated with loosely aggregated polymerized IF just outside the spindle, but are not seen in or near the amorphous bodies (Fig. 8*b*). Using the keratin antiserum, 5-nm gold particles are localized at the periphery of the amorphous bodies in mitotic HeLa (Fig. 8*c*).

DISCUSSION

Mouse keratinocytes possess complex networks of keratin-containing IFB coursing from the cell periphery to the nucleus. These IFB frequently appear to terminate in close association with the nuclear surface, especially in regions adjacent to nuclear pore complexes (Fig. 4*b*) (10). These observations have led us to propose that the IFB system plays important roles in the attainment and maintenance of cell shape, in the positioning of the nucleus towards the center of epidermal cells (10), and perhaps with conduction of various types of signals from the cell surface to the nucleus (21). In this study, we extended these observations to include the fate of this complex IFB network during mitosis. The results demonstrate that IFB remain closely associated with the mitotic spindle throughout mitosis in epidermal cells. This finding would appear to contradict Lane et al. (9), who have reported that IFB unravel during mitosis in human keratinocytes.

In HeLa, unlike the situation in mouse keratinocytes, the keratin enriched system of IFB undergoes a dramatic reorganization during mitosis, since IFB disassemble and form non-filamentous bodies (7, 8). However, in contrast, a second IF network present in these cells containing the IF protein more characteristic of fibroblasts (i.e., vimentin) (12) appears to remain morphologically intact throughout interphase and mitosis (3). We confirmed these observations by gold-labeled antibody localization that revealed that polymerized vimen-

tin-containing IF are arranged in a perinuclear position during interphase, and that as HeLa cells enter mitosis this type of IF remains closely associated with the mitotic spindle (see Fig. 8).

Thus, we have shown that there are similarities and differences in the organizational fate of IF in these two epithelial cell types. Both cell types retain polymerized IF systems throughout mitosis. However, although both cells in interphase possess keratin-containing IF systems, in one cell type, HeLa, this IF system is disassembled during mitosis (7, 8), whereas in the other cell type, mouse keratinocytes, this IF system is retained throughout mitosis. Therefore, we suggest that no generalized statements concerning IF organization throughout the cell cycle can be made from a study of any one cell type. Furthermore, on the basis of preliminary results with other cell systems (e.g., BHK-21 [26]), we are convinced that the organizational fate of IF through the cell cycle differs from cell to cell and this has led to numerous confusing general statements in the literature.

While no specific functions for IF in mitosis have been determined, it is intriguing to speculate that retention of IF networks throughout the cell cycle in the cell types that we have studied is an essential requirement for normal mitotic events. In the case of mouse keratinocytes, since they possess only one IF system (22), this requirement would be met of necessity by the keratin IFB network. However, in HeLa cells that possess both a keratin system and a second IF (i.e., vimentin-enriched) system, the latter is retained (3). In this connection we speculate that HeLa with its two networks is programmed to retain the least complex network. In support of this, this laboratory has recently demonstrated that the keratin system of HeLa cells appears to contain up to nine IF structural polypeptides (12). Thus it may be that a polymerized IF system consisting of just one major structural protein (i.e., vimentin) may be more easily divided between daughter

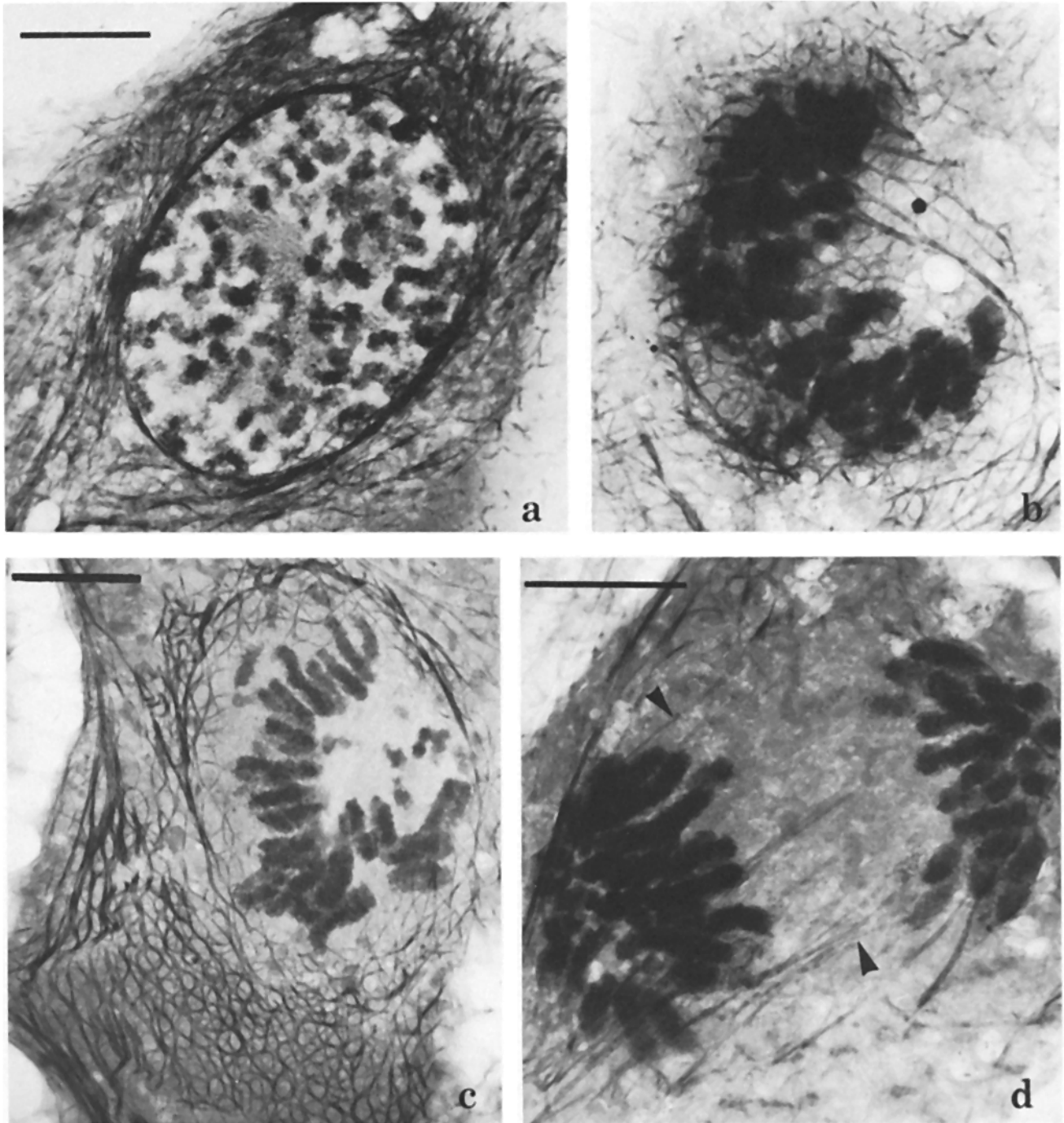


FIGURE 7 Electron micrographs of thicker sections ($\leq 0.5 \mu\text{m}$ thick) of mitotic mouse keratinocytes. (a) IFB remain intact as the chromatin condenses and the nuclear envelope breaks down during prophase. (b) The network of IFB appears to surround the chromosomes. (c) The extensive network of IFB remains in mitotic cells. (d) At anaphase, IFB aligned parallel to the long axis of the spindle appear "stretched out" between the spindle poles (arrowheads). (a and b) Bar, $5 \mu\text{m}$; $\times 4,000$. (c) Bar, $10 \mu\text{m}$; $\times 2,100$. (d) Bar, $5 \mu\text{m}$; $\times 4,900$.

cells than the more complex keratin-enriched IF system. It follows that to facilitate the distribution of this latter network in daughter cells, the keratin-containing IF system in mitotic HeLa cells is disassembled and packaged into discrete amorphous bodies (7, 8), that can be readily divided into daughter cells as is the case with other organelles (e.g., mitochondria).

Since both epithelial cells that we have studied retain a polymerized IF system throughout mitosis, both cell types are faced with the problem of the distribution of these apparently stable IF networks to daughter cells. Therefore, a mechanism

must exist that permits locally controlled breakdown of IF, perhaps within the midbody structure, before the final separation of daughter cells. It is possible that Ca^{2+} -activated proteases are involved in this phenomenon (1), as proteases that specifically degrade IF have been reported in both tissues and in cultured cells (23, 24).

The arms of chromosomes appear to be closely associated with IFB throughout each stage of the mitotic process in mouse keratinocytes; and in HeLa cells, IF are frequently seen closely associated with the mitotic spindle. Although, we

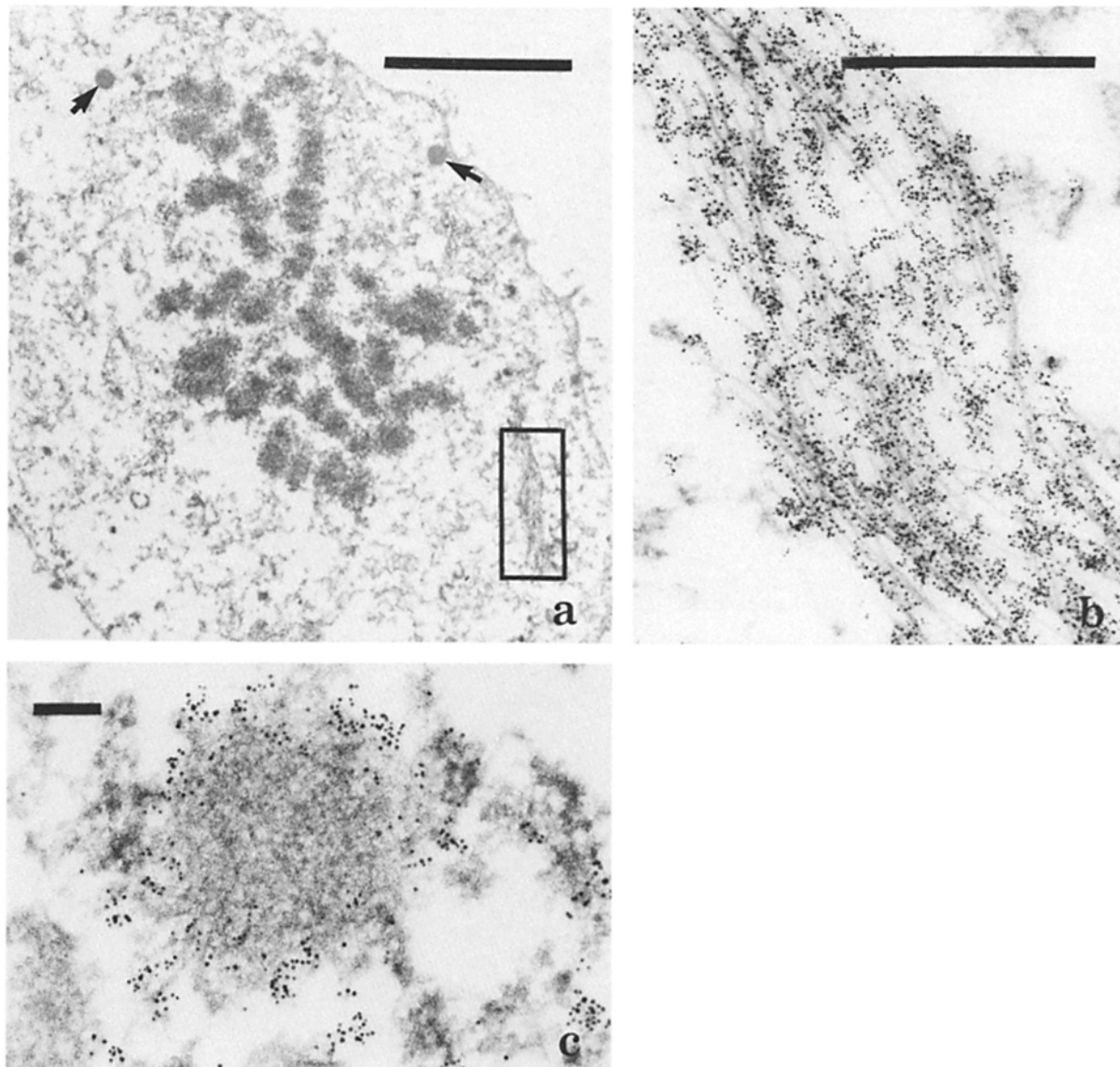


FIGURE 8 Immunogold localization of keratin and the fibroblast form of IF in mitotic HeLa cells. (a) A thin section of a mitotic cell processed for indirect immunogold localization using the BHK 55,000-mol-wt IF antiserum. Note the presence of amorphous bodies (arrows) and also IF bundles (e.g., within the rectangle). (b) An enlargement of the area within the rectangle reveals that gold particles are specifically localized to the IF within the bundle. (c) The amorphous body is from a mitotic HeLa cell processed for immunogold localization using the keratin antiserum. Gold particles are located primarily around the edge of the body. (a) Bar, 5 μm ; \times 5,300. (b) Bar, 0.5 μm ; \times 72,000. (c) Bar, 0.1 μm ; \times 100,000.

have no evidence that IF are directly involved in chromosome movement, we believe that their presence must be taken into account when proposing mechanisms for chromosome movement and pole-to-pole separation in mitosis. Since IFB are found so close to chromosome arms, they may act passively, perhaps as a brake, to impede the movement of chromosomes. Alternatively, if there is a direct attachment of IFB to the arms of chromosomes as some of our micrographs indicate (e.g., Fig. 6*b*), and if IFB are capable of "stretching," as indicated by the straightening of some IFB during anaphase (see Fig. 7*d*), then it is possible that IFB may play a more active role in chromosomal movements. In any case, their proximity to the mitotic apparatus must be taken into account when determining mechanisms of chromosome movement and mitotic spindle functions.

The possibility that there is a biochemical association of IF with the nuclear envelope in interphase cells and also with

the spindle and chromosomes in mitotic cells is currently under investigation. We have already reported that there is a group of 60,000–70,000-mol-wt proteins that co-isolate with IF and that these appear to be localized at the periphery of the nucleus (25). These polypeptides may be involved in linking IF to the nuclear surface and consequently may also be involved in the chromosome-IF interactions reported in this study. We are now attempting to isolate IF and chromosomes from mitotic cells to examine this possibility.

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