

Ten-nanometer filaments and mitosis: Maintenance of structural continuity in dividing endothelial cells

(cytokinesis/cytochalasin B/immunofluorescence/topology/blood vessels)

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ABSTRACT By indirect immunofluorescence the behavior of the 10-nm filaments was studied at various stages of mitosis in guinea pig vascular endothelial cells. Interphase cells contain a ring of 10-nm filaments that encircles the nucleus and is maintained in a plane parallel to the substrate. During prophase and metaphase the cells round up and the 10-nm filament ring becomes wavy though still a closed structure. As anaphase progresses the ring then elongates into a rectangle that contains the spindle apparatus and chromosomes. In late telophase, cytokinesis cleaves the 10-nm filaments into crescents at the site of the contractile ring. These crescents then close into rings in the daughter cells. If cytokinesis is inhibited with 5 μ g of cytochalasin B per ml, then cleavage of the 10-nm filaments is blocked and the daughter nuclei remain surrounded by the parent ring. At no point during mitosis does the array of 10-nm filaments undergo major disassembly. These results indicate that, in contrast to the other major cytoplasmic structures, ventral microfilament bundles and cytoplasmic microtubules, which disassemble and reassemble during mitosis, 10-nm filaments remain intact throughout this process. The possibility is discussed that these filaments may function in transport of organelles and structural proteins, and provide the daughter cells with topological information about placement and assembly of these elements within the microtrabecular lattice.

Although 10-nm filaments are a major cytoplasmic structure of many eukaryotic cells (1-8), few studies (3, 4, 8) have dealt with their fate during mitosis. Initial observations on 10-nm filaments in endothelial cells provided evidence that the filaments were present through several stages of mitosis (8). Recently, the work of Hynes and Destree (9) and Gordon *et al.* (10) has demonstrated that the 10-nm filaments remained conspicuous during mitosis in nonendothelial cells. These findings appear to be in contrast to the behavior of other structural components of the cytoplasm which undergo major reorganization and recycling during mitosis (11-19). For example, in interphase cells the cytoplasmic microtubules form an extensive network that undergoes disassembly in prophase, then reappears in the mitotic spindle (11). At the conclusion of mitosis the daughter cells rearrange their microtubules back into a cytoplasmic network. In an analogous manner the stress fibers or ventral microfilament bundles of interphase cells undergo recycling during mitosis (12-19). The microfilament bundles disassemble during prophase and redistribute their constituent proteins to participate in membrane movements associated with cleavage furrow formation of cytokinesis and separation of daughter cells.

To understand the behavior of 10-nm filaments during mitosis, I carried out studies on cultured guinea pig vascular en-

dothelial cells. The advantage of using these cells is that they arrange a majority of their 10-nm filaments into a single ring or torus that encircles each nucleus (8, 20). Hence, these rings can easily be visualized by polarization microscopy or indirect immunofluorescence. When these rings were followed through various stages of mitosis, it was found that the 10-nm filaments, unlike cytoplasmic microtubules and ventral microfilament bundles, remain intact during mitosis. In late telophase, cytokinesis cleaves the parent 10-nm filament ring into symmetrical crescents that enter the daughter cells.

MATERIALS AND METHODS

Primary cultures of vascular endothelial cells were obtained from the thoracic aortas of 1-month-old guinea pigs and grown on glass coverslips as described (8, 20, 21). At 10-15 days of culture during logarithmic growth (21), approximately 70 cells were selected at various stages of mitosis for study. Cells on coverslips were washed with phosphate-buffered saline, pH 7.4, at 20°C and fixed with 3.5% formalin in phosphate-buffered saline for 30 min at 20°C. Coverslips were then washed 10 times with the buffered saline, once with deionized water, extracted with absolute acetone at -20°C for 10 min, and rinsed in buffered saline. Rabbit antibodies against 10-nm filaments (10) were obtained as the generous gift of William E. Gordon III. They were diluted 1:40 with buffered saline and overlaid on the coverslip. The coverslips were incubated in a humidified atmosphere at 37°C for 30 min, then washed in 10 changes of buffered saline, and stained with fluorescein-labeled goat anti-rabbit IgG at a 1:20 dilution. After 10 washings with buffered saline, coverslips were mounted in Gelvatol (Monsanto brand of polyvinyl alcohol; Monsanto, St. Louis, MO). Cells were photographed on a Zeiss photomicroscope III with a Zeiss $\times 63$ oil phase 3 lens on an epifluorescence nose piece or a Zeiss $\times 40$ POL lens. All cells were examined by optical sectioning (through focus) to assess the continuity of the 10-nm filament ring. Phase micrographs were recorded on Kodak high contrast copy film (5069), E.I. 16-20, and developed in Kodak D-19 for 5 min at 20°C; fluorescent micrographs on Kodak Tri-X film (5063), E.I. 400, were developed in Kodak Microdol-X for 9 min at 20°C; and polarization micrographs on H & W control VTE film (St. Johnsbury, VT), E.I. 50, were developed in Acufine for 4 min at 20°C. All of the light was diverted to the film plane for exposure. Fluorescence exposure times were 5-10 sec.

Cytochalasin B (Aldrich) was dissolved in dimethyl sulfoxide at 1 mg/ml and added to the culture media at a final concentration of 5 μ g/ml.

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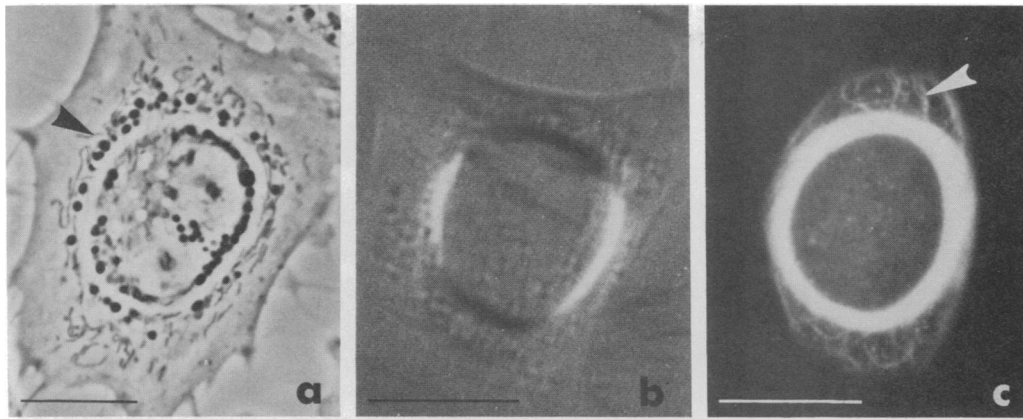


FIG. 1. Interphase endothelial cells. Phase (a) and polarization (b) micrographs of living cells that contain the phase lucent-birefringent ring of 10-nm filaments. In close association with the ring are found lipid vacuoles, lysosomes, and mitochondria (a, arrowhead). (c) When these cells are exposed to anti-10-nm-filament antibody the ring is stained as well as smaller numbers of attending 10-nm filaments (arrowhead). Bars are 10 μm .

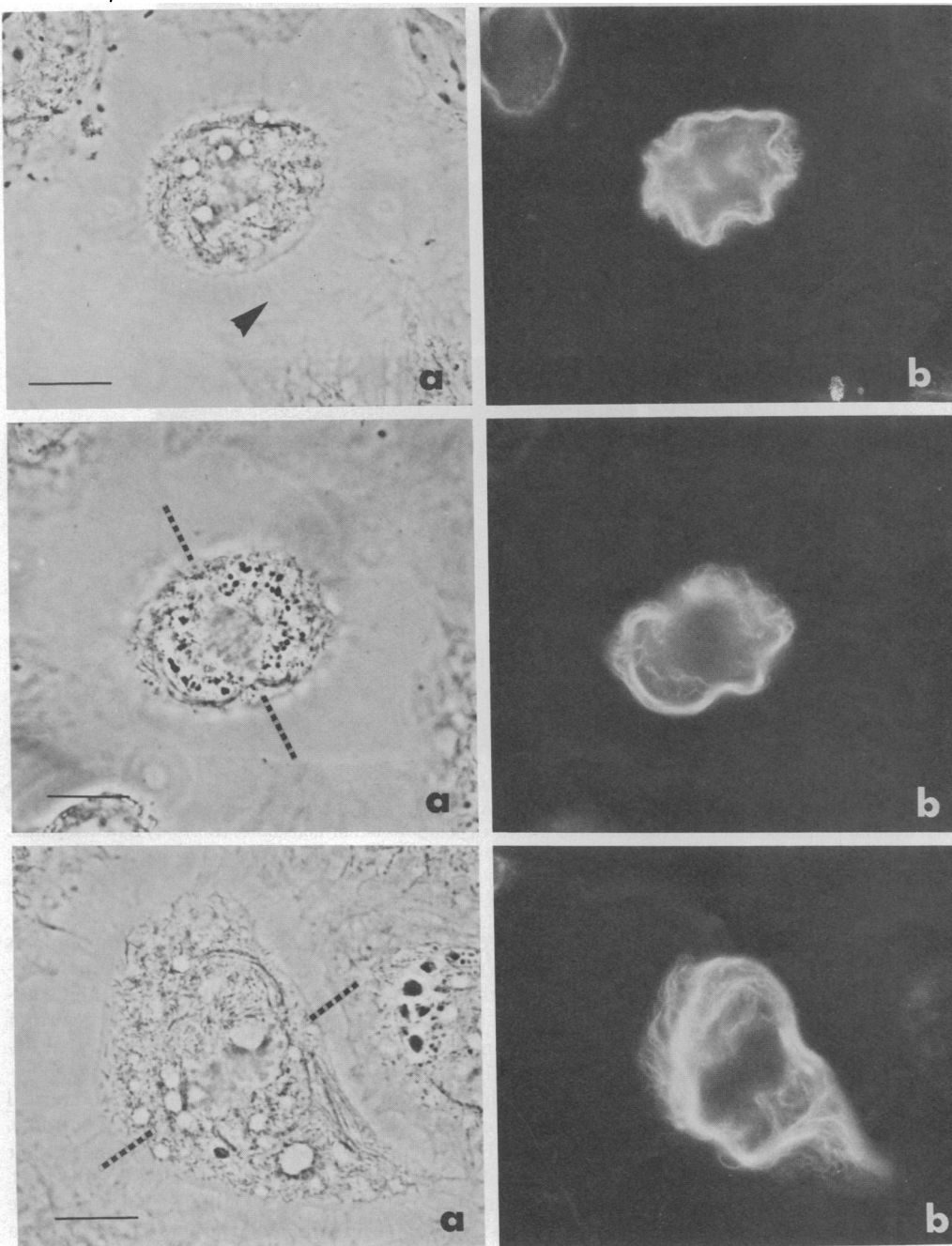


FIG. 2. Phase (a) and immunofluorescence (b) micrographs of the same endothelial cell. Bars are 10 μm . (Top) Prophase. The cell has rounded up producing retraction fibers (arrowhead), and the ring assumes a continuous wavy configuration. (Middle) Early metaphase. The chromosomes are aligning at the metaphase plate (dotted line) seen in the phase micrograph. Immunofluorescence reveals the ring to be continuous and closed, although a small section of it falls out of the plane of focus. (Bottom) Metaphase. Chromosomes at the metaphase plate are indicated by the dotted line in the phase micrograph. Immunofluorescence reveals the ring to be continuous and wavy. Individual filaments fray off the ring.

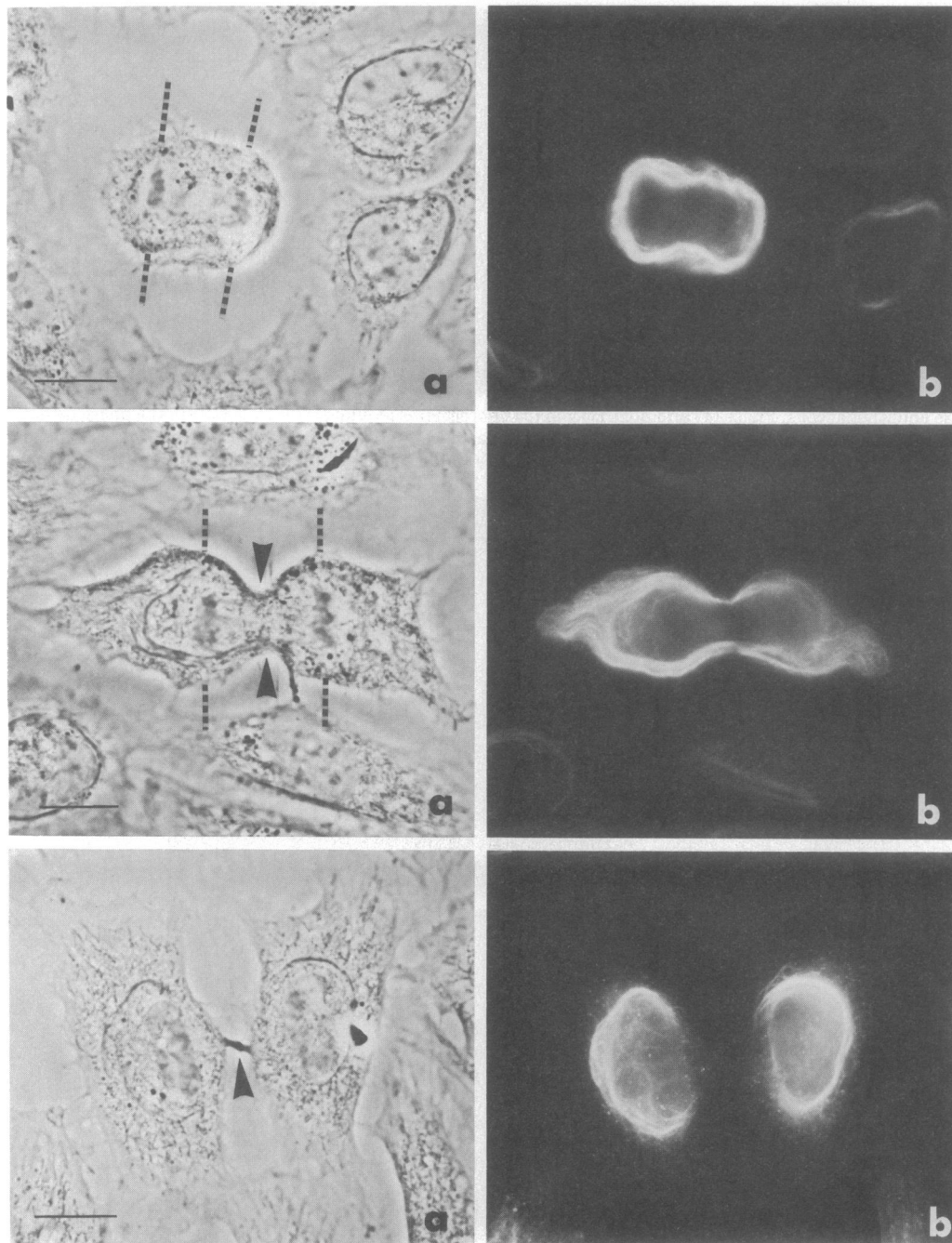


FIG. 3. Phase (a) and immunofluorescent (b) micrographs of the same endothelial cell. Bars are 10 μm . (Top) Anaphase. The dotted lines indicate the position of the chromosomes. (Middle) Telophase. The contractile ring, indicated by arrowheads in the phase micrograph, begins to pinch the 10-nm filaments observed by immunofluorescence. Dotted line in a indicates positions of chromosomes. (Bottom) Late telophase. Cytokinesis has now completely cleaved the ring into two symmetrical crescents that enter the daughter cells. (a) Phase micrograph of daughter cells and midbody (arrowhead). (b) Immunofluorescent micrograph of 10-nm filament crescents.

RESULTS

During interphase endothelial cells contain an intact perinuclear ring of 10-nm filaments (Fig. 1) (8, 20). Associated with the ring are lipid vacuoles, lysosomes, and mitochondria (Fig. 1a and unpublished results), as well as smaller numbers of attending 10-nm filaments (Fig. 1c) as reported (8). At the onset of prophase the cells rounded up and the ring became wavy (Fig. 2 top). In metaphase the ring remained a closed structure that surrounded the chromosomes at the metaphase plate (Fig. 2 middle and bottom). Occasionally, individual filaments appeared to fray from the parent ring (Fig. 2 bottom) during cell rounding. In anaphase the ring elongated symmetrically into

a closed "rectangular-ellipse" that included the chromosomes and mitotic spindle (Fig. 3 top). The bulk of the filaments remained at the cell's circumference outside of the spindle apparatus. In telophase, the now almost rectangular array of 10-nm filaments was pinched by the cleavage furrow of cytokinesis (Fig. 3 middle). Finally, as a result of completed cytokinesis, the filament array was cleaved into symmetrical crescents contained within the daughter cells (Fig. 3 bottom). Subsequently, the newly formed daughter cells arranged their complement of 10-nm filaments back into rings (Fig. 4 top). As determined by optical sectioning, throughout mitosis the array of 10-nm filaments remained continuous, weaving in a

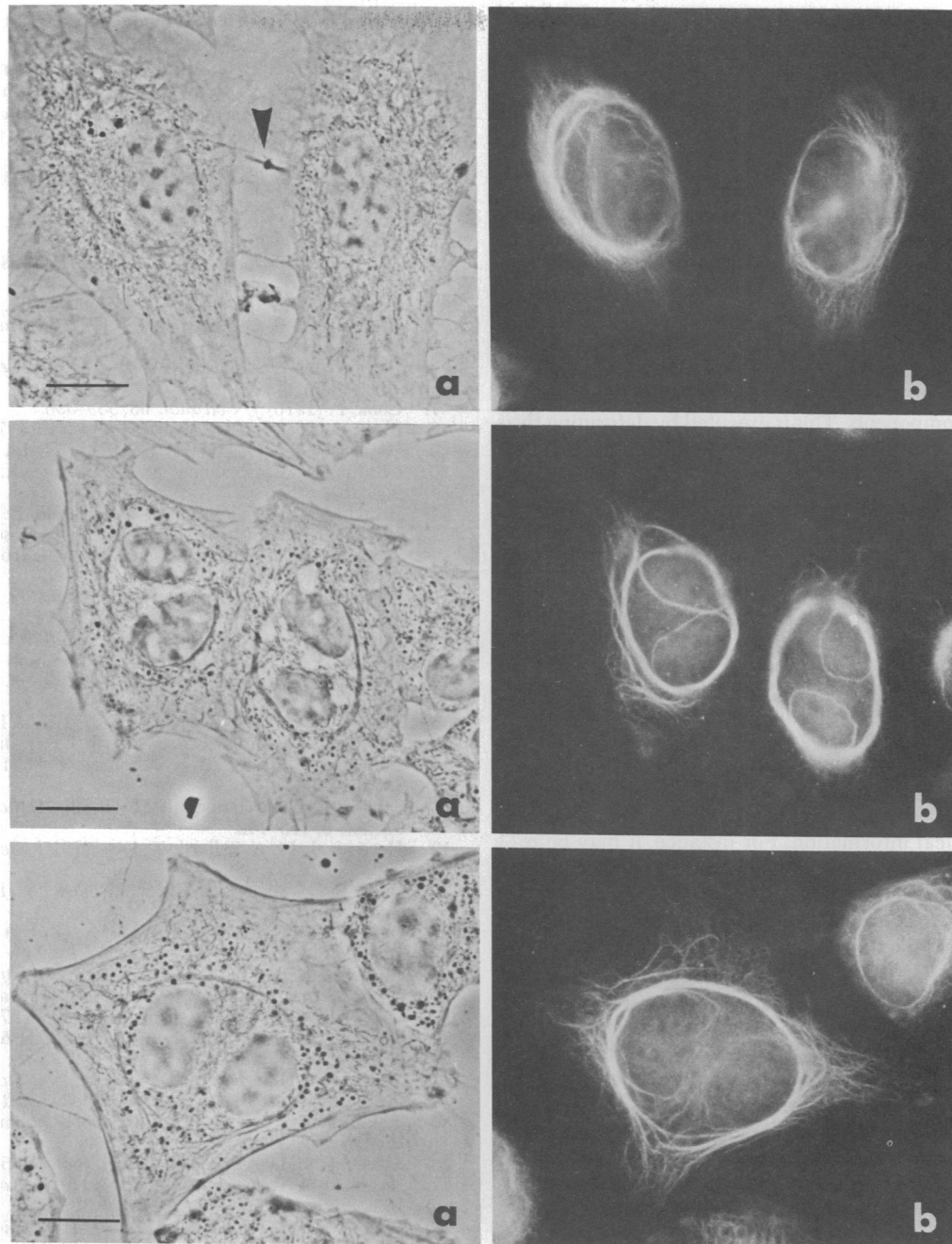


FIG. 4. (Top) Two daughter cells that have recently completed mitosis. Phase micrograph (a) shows that between these cells rests the spent midbody (arrowhead). Immunofluorescence micrograph (b) shows these cells arranging their complement of 10-nm filaments back into rings. Bar is 10 μm .

(Middle) Two endothelial cells in log phase of growth incubated in the presence of cytochalasin B at 5 $\mu\text{g}/\text{ml}$ to block cytokinesis. (a) The phase micrograph shows that both cells are binucleated and have failed to undergo cytokinesis. (b) The immunofluorescence micrograph of these cells reveals that the nuclei are contained within the parent ring and that the ring failed to undergo cleavage. In both of these cells, after nuclear envelope formation, a small bundle of 10-nm filaments has surrounded the nuclei. Bar is 10 μm .

(Bottom) Another example of a binucleated cell that failed to undergo cytokinesis in the presence of cytochalasin B at 5 $\mu\text{g}/\text{ml}$. The parent ring is not cleaved and includes both nuclei. Phase (a) and immunofluorescence (b) micrographs. Bar is 10 μm .

plane roughly parallel to the substrate. The array did not undergo major disassembly. Also throughout this process mitochondria, lipid vacuoles, and lysosomes were observed in close contact with the 10-nm filament bundles (8).

It appeared from these observations that the main cause for dividing the parent ring between daughter cells is the cleavage of the 10-nm filaments by cytokinesis. This notion is supported by the following experiment: Logarithmically growing cells were incubated in the presence of cytochalasin B at 5 $\mu\text{g}/\text{ml}$

for 10–20 hr to block cytokinesis (22). The resulting binucleated cells (Fig. 4 middle and bottom) were then fixed and stained with anti-10-nm-filament antibody. In these cells both daughter nuclei were contained within the intact parent ring. Several binucleated cells had small satellite rings leaving the parent ring and surrounding daughter nuclei (Fig. 4 middle). The formation of these satellites occurred during reformation of the nuclear envelope. As a control to this experiment, interphase cells were observed in the presence of cytochalasin B. The majority

of these control cells exhibited modest retraction of their cell borders; however, the perinuclear rings remained undisturbed. From this experiment it was concluded that blockage of cytokinesis resulted in failure of the parent ring to be cleaved into daughter crescents.

DISCUSSION

Vascular endothelial cells, in contrast to many other eukaryotic cells in culture, arrange the majority of their 10-nm filaments into a perinuclear ring (8, 20). This arrangement greatly facilitates cytological observation of these filaments during various cellular events such as mitosis.

From the observations reported here endothelial cells clearly reveal that throughout mitosis the bulk of their 10-nm filaments are divided between daughter cells without prior disassembly into molecular components. This is executed by deforming the parent ring such that the 10-nm filament array migrates symmetrically ahead of the chromosomes. Cytokinesis then cleaves the filaments into symmetrical crescents that enter the daughter cells. To date, the network of 10-nm filaments is the only known filamentous cytoplasmic structure that stretches out into the cytoplasm of interphase cells (8–10, 23, 24) and retains continuity of its fibers during mitosis. Consequently, one could compare them with a flexible scaffold to which other cellular components may relate their postmitotic positions when the cytoplasm of the daughter cells reorganizes. Despite deformation of this scaffold of 10-nm filaments during mitosis it can provide information about the relative position of the fibrous structures and organelles within the microtrabecular lattice (25) of newly structuring daughter cells. Therefore, the continuity of the 10 nm filament scaffold may provide topological rather than morphological information. For instance, it may be responsible for the symmetrical redistribution between daughter cells of structural proteins, such as actin (26, 27), or organelles, such as mitochondria and nuclear envelope components. It remains to be seen whether it is generally true for eukaryotic cells that the 10-nm filaments remain in an assembled state during mitosis. However, consistent with the results reported in this paper, preservation of the assembled state has been suggested for tonofilaments in various mitotic epithelial cells (28) and in BHK-21 cells (4), although in the latter case the 10-nm filaments formed a cap around the time of mitosis.

Division of the 10-nm filament ring into two daughter crescents by cytokinesis, rather than karyokinesis, appears to be analogous to the cleavage of myofibrils in dividing cardiac muscle described by Kelly and Chacko (29). Central to this mechanism of cleavage might be a local calcium release which would (i) activate the actomyosin system of the cleavage furrow (12, 16) and (ii) activate a calcium-activated neutral protease known to break down 10-nm filaments (30) and Z-lines (31–33).

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