Presence of Actin During Chromosomal Movement

(mitotic apparatus/chromosomal spindle fibers/fluorescent heavy meromyosin/nucleoli)

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ABSTRACT   Actin has been shown to be present in the nucleoli, kinetochore and centriolar regions, and in the mitotic spindle of rat kangaroo cells which have been stained with fluorescently labeled heavy meromyosin. The actin in the spindle is confined to the fibers that connect the chromosomes with the centriolar region. Actin was not present in astral fibers, in the continuous spindle fibers that connect the poles, or in non-kinetochore regions of the chromosomes. The specific localization of actin in chromosomal spindle fibers suggests an actin–myosin interaction as the force-producing mechanism for chromosomal movement.

Chromosome movement during cell division of eukaryotic cells has been shown to be caused by spindle fibers [reviewed by Nicklas (1)]. Three kinds of fibers can be identified in the spindle with light microscopy: (1) chromosomal fibers which run from kinetochore to pole; (2) continuous fibers which run from pole to pole; and (3) astral fibers which are characteristic of animal cells and radiate from the poles toward the cell periphery (2, 3). An important component of these spindle fibers is the microtubule. Microtubules are invariably seen in electron micrographs of the spindles of glutaraldehyde-osmium fixed cells (4, 5). Furthermore, they are attached to chromosomes at the kinetochores, from which points the chromosomes seem to be pulled poleward. Agents such as colchicine, low temperature, and high pressure which interfere with microtubular integrity also prevent chromosome movement (6–9). These observations have led to the generally accepted hypothesis that microtubules are responsible for the movement of chromosomes during cell division (10). The way in which the microtubules produce the motile force is, however, still a subject for speculation (10, 11, 1).

Actin and myosin, which were once thought to be localized exclusively in muscle cells, have now been identified in a variety of non-muscle cells (12, 13). Actin, in particular, has been found to be associated with motile regions of many cells (14, 15). As a result of these findings, it has been proposed that many types of non-muscle motility (e.g. ameboid movement, cytokinesis, cytoplasmic streaming) are due to actin–myosin interactions (12–14). The following report identifies actin in discrete bundles connecting individual chromosomes to the poles in dividing rat kangaroo cells. These actin bundles shorten as division proceeds and the chromosomes approach the poles. At no time during mitosis was actin observed in astral fibers or in continuous spindle fibers. This specific localization of actin in chromosomal spindle fibers makes it necessary to consider an actin–myosin interaction as the force-producing mechanism for chromosome movement.

MATERIALS AND METHODS

Rat kangaroo (*Potorous tridactylis*) cells, strain Pt-K2, a generous gift of Dr. Yasushi Ohnuki (Pasadena Foundation for Medical Research), were grown on glass coverslips in the following medium: 100 parts by volume Eagle's minimal medium, 10 parts fetal calf serum, 1 part antibiotics (5000 units of penicillin, 5000 μg of streptomycin/ml), 1 part Fungizone. Culture dishes containing the glass coverslips were rinsed with Eagle's minimal medium to remove any remnants of the fetal calf serum and placed in 50% (v/v) glycerol/standard salt/antibiotic solution (4°) (14, 15). The cells were glycerinated at 4° overnight and then rinsed with cold standard salt solution to remove all glycerol. After the wash solution was drained off, fluorescent heavy meromyosin (1 mg/ml) in 25% glycerol/standard salt/antibiotic solution (4°) was added to the culture dish (14). Cells were labeled from 10 to 16 hr, then washed extensively (3–4 hr) with several changes of cold standard salt solution. The cells were then placed in 50% glycerol and stored in the refrigerator until examined in the fluorescent microscope.

RESULTS

Interphase. During mitosis, rat kangaroo cells do not completely round up as do most dividing cells, but remain flattened on the culture dish (Fig. 1). For this reason the position of the chromosomes and thus the stages of mitosis can be easily identified. The cytoplasm of interphase rat kangaroo cells contained a network of long fluorescent fibers after the cells had been stained with fluorescent heavy meromyosin (Fig. 2). These bundles were not evident in the phase microscope. The nucleolus, observable with phase microscopy in interphase cells (Fig. 1), also stained with fluorescent heavy meromyosin. Stain could be removed with 0.01 M ATP solution (14), which breaks heavy meromyosin-actin linkages, but could not be removed by extensive washings with standard salt solution. If glycerinated cells were treated with unlabeled heavy meromyosin before addition of the fluorescent heavy meromyosin, the amount of fluorescence was greatly reduced in both fibers and nucleoli.

Prophase. When prophase begins most of the long, fluorescent, cytoplasmic fibers present during interphase disappear and a diffuse fluorescence appears in the cytoplasm (Fig. 3). A few actin fibers could be observed in the cell, often around the nucleus. The chromosomes that condense during prophase do not bind fluorescent heavy meromyosin. Furthermore, the fluorescent nucleous disappears and two bright dots of fluorescence can be observed adjacent to the nuclear area (Fig. 3).

Metaphase. Fluorescent fibers joining chromosomes to polar regions are the most striking feature of metaphase cells stained with fluorescent heavy meromyosin (Figs. 4 and 10). Five to nine fluorescent chromosomal fibers can be seen converging toward each pole. The fibers are very slender, 0.2 μm
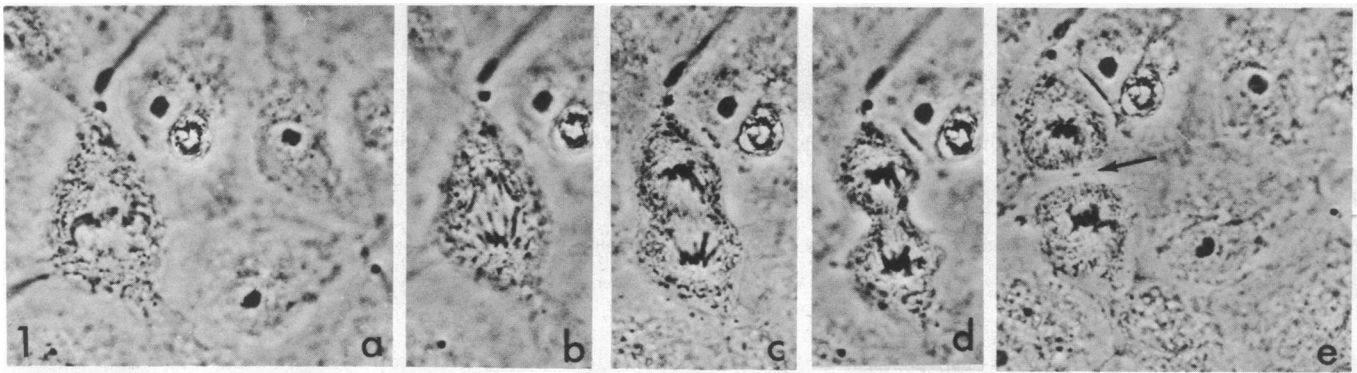
FIG. 1. Phase microscopy of living rat kangaroo cells. Nondividing cells have one or more large nucleoli. A cell is followed through mitosis. (a) Cell at metaphase. (b) Cell at end of anaphase. The chromosomes have reached the pole. (c) Cell in early telophase. The cleavage furrow has been initiated. (d) Cell in mid-telophase: cleavage furrow has contracted halfway. (e) Cell at end of telophase. The cleavage furrow has contracted completely and a mid-body (arrow) is present. 650×.

in diameter (the limit of resolution of our microscope) and as long as 7 μm (Fig. 10). Because of their small diameter and their location in different focal planes, it is difficult to determine the exact number of fibers. In the vicinity of the pole, adjacent fibers become contiguous, resulting in a decreased number of fibers near the pole but an increased amount of fluorescence per fiber. The chromosomes are unstained except for a small dot of fluorescence in the kinetochore region where the fibers meet the chromosomes. At the center or centriolar region of each pole a small round spot of fluorescence is also observed. No stain was observed in the astral fibers or in continuous fibers connecting the two poles.

Anaphase. As the chromosomes move closer to the poles during anaphase the length of the fluorescent fibers shortens correspondingly (Figs. 5–8). The bright fluorescent dots at the poles and kinetochore regions continue to be visible. No fluorescent fibers were ever detected in the interzonal region between sister chromosomes (Figs. 6 and 7). A diffuse fluorescence does appear during late anaphase in the interzonal region (Fig. 8) where a cleavage furrow will form (Fig. 1c).

Telophase. Fluorescent fibers and centriolar staining disappear during telophase and fluorescent staining becomes concentrated in the cleavage furrow (Fig. 9). There is no staining of the unravelling chromosomes. About 30 min after the completion of cleavage the long fluorescent fibers and a fluorescent nucleolus are again present as the cell reenters interphase.

DISCUSSION

Heavy meromyosin binding that is reversible with ATP is considered to be diagnostic of actin (12, 15). Heavy meromyosin will not bind myosin, intermediate-sized (100 Å) filaments, collagen, microtubules, or microtubular protofilaments (12, 14–16). We have previously demonstrated that heavy meromyosin can be coupled with fluorescein isothiocyanate in a manner which protects the actin-binding site and leaves unaltered the binding specificity of the heavy meromyosin (14). This fluorescently labeled heavy meromyosin can then be used for light microscopic identification of actin in glycerinated cells. In the dividing rat kangaroo cells, fluorescent heavy meromyosin staining does not occur in the presence of ATP or when cells have been pretreated

with unlabeled heavy meromyosin. Furthermore, the stained component is stable in cold glycerol for several days and in cold standard salt solution after several hours of washing. We conclude, therefore, that our results indicate that actin is present in nucleoli and chromosomal spindle fibers of rat kangaroo cells. The interphase and cleavage stage staining patterns obtained with these cloned rat kangaroo cells are comparable to the patterns we have observed in chick fibroblasts (14). In both types of cells, long fluorescent fibers are present throughout the cytoplasm during interphase; these fibers are absent during mitosis; during cleavage, fluorescent staining is concentrated primarily in the furrow region (14).

In 1965, Aronson (17) showed that isolated sea urchin mitotic apparatuses bound fluorescent heavy meromyosin most strongly in the chromosomes, with uniform fluorescence present throughout the rest of the apparatus. This nonlocalized binding was attributed by Aronson to alteration of the actin-binding sites of the heavy meromyosin during the labeling process. Unbound fluorescent dye, in addition, was not removed from the fluorescent heavy meromyosin solution and may have contributed to the nonspecific staining. The procedure we have developed (14, 15) allows the actin-binding site to be protected during labeling and removes the unbound fluorescent dye. The rat kangaroo spindles, moreover, have been stained *in situ*, making contamination of the spindle by cytoplasmic actin less likely than in isolated spindle preparations. It is of interest that the chromosomes in the rat kangaroo cells do not bind fluorescent heavy meromyosin except in the kinetochore region. Moreover the fluorescent heavy meromyosin staining was localized in only one type of spindle fiber: the chromosomal spindle fibers.

In only two cases have any filaments similar in diameter to actin been identified in electron micrographs of routinely fixed spindles (18, 19). In glycerinated cells, heavy meromyosin-binding filaments have been reported in spindles of crane fly spermatocytes (20), locust spermatogonia (21), and HeLa cells (22). Based on these observations and on the growing evidence that actin and myosin are involved in many types of cytoplasmic motility, Forer has postulated without, as he notes, definitive evidence that an actin–myosin interaction is also responsible for chromosomal movement (13). In the ultrastructural results cited above it was not clear what relationship the actin filaments had to the spindle fibers. It was not possible to conclude, in fact, that the filaments
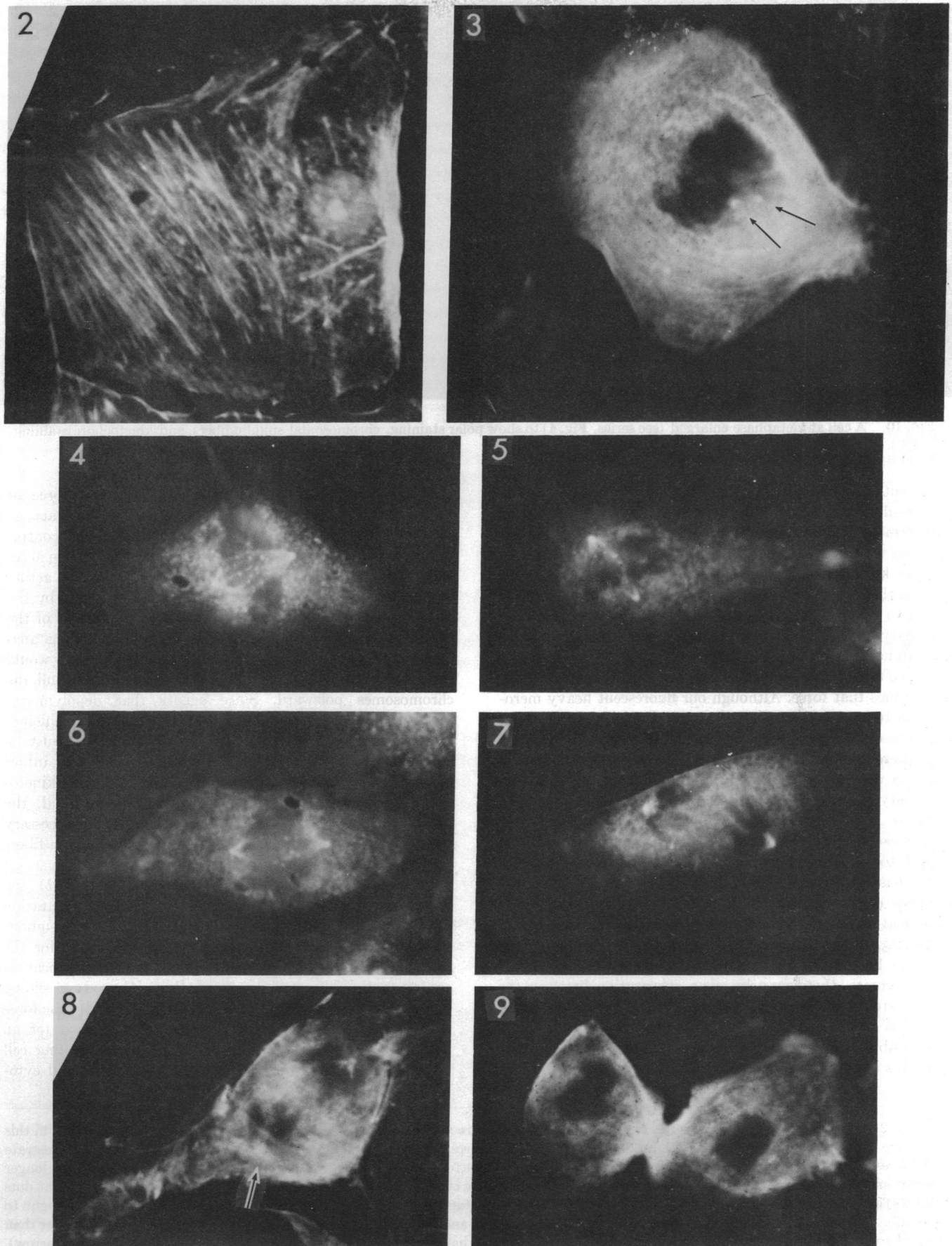
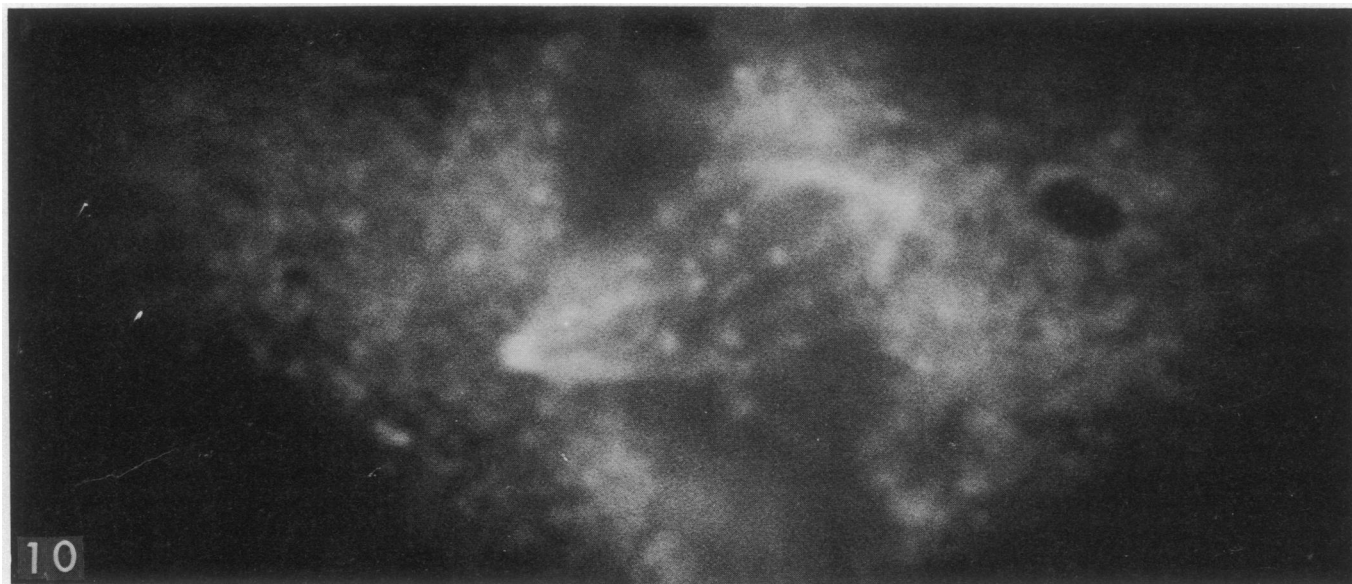FIGS. 2–9.   (*Legend appears at bottom of the next page.*)

FIG. 10.   A cell at metaphase enlarged (see series, Fig. 4) to show polar staining, chromosomal spindle fibers, and kinetochore staining. (4000×).

represented anything more than general cytoplasmic actin. Our results demonstrate that there is actin in the spindle, and furthermore that it is localized in the chromosomal spindle fibers as well as in the centriolar and kinetochore regions.

It is known from micromanipulation and microbeaming studies that the chromosomal spindle fibers pull the chromosomes to the poles (reviewed in refs. 1, 6, and 23). The force necessary to pull one chromosome has been estimated to be 10^{-8} dynes (0.1 pN) (1, 24). Lowy *et al.* (25) have calculated that one thick filament in conjunction with actin can generate 1000 times that force. Although our fluorescent heavy meromyosin technique does not yet permit quantitation of actin, it can be estimated by reference to labeled sarcomeres (14, 15) that there is sufficient actin present in a chromosomal spindle fiber to complex with myosin and produce more than the necessary force needed for chromosome movement. If spindle actin is in fact part of the contractile unit necessary for chromosome movement, we predict that myosin will also be found to be present in chromosomal spindle fibers, since actin has never been observed to cause contraction in the absence of myosin. The localization of myosin in the chromosomal spindle fibers would be compelling support for an actin–myosin role in chromosomal movement.

Since both actin and microtubules are connected to the chromosomes, there may be some cooperation between the two systems to produce a contractile force for shortening. There is no evidence, however, that actin can complex with microtubular protein, and thus it is unlikely that these two proteins directly interact. Forer (13) has suggested that the

spindle microtubules may control the speed and force of actin–myosin contraction. The microtubules could act as rigid rods running from chromosome to pole and thus control the degree of shortening that could be produced by the actin and myosin. At metaphase, the contraction of the actin-myosin chromosomal fibers would be exactly opposed by the chromosomal microtubules, resulting in no movement of the chromosomes (i.e., an isometric contraction). During anaphase, depolymerization of chromosomal microtubules would allow the actin spindle fibers to shorten and thus pull the chromosomes poleward. Since agents that depolymerize microtubules but do not affect actin filaments (e.g., colchicine) also cause chromosomal movement to cease, there must be an additional dependence of actin spindle fibers on intact microtubules. This may occur, for example, in actin–kinetochore or actin–pole attachments. On the other hand, the microtubules of astral or continuous fibers may be necessary for the anchoring of the poles against which the actin fibers presumably contract.

Chromosomal separation has two components: (*1*) the movement of chromosomes to the poles, and (*2*) the separation of the poles due to spindle elongation (1–3). Continuous spindle fibers, which are thought to be responsible for the second of these two movements (1, 23), do not appear to contain actin. Microtubular elongation either via a sliding mechanism (11) or polymerization (10) could supply the force necessary for polar separation, obviating the need for an actin–myosin system. In the spindle, then, as in other cell systems, microtubules could serve as lengthening and cyto-

FIGS. 2–9 (*on preceding page*).   A series of glycerinated rat kangaroo cells stained with fluorescent heavy meromyosin. All cells in this series are shown at the same magnification (1200×).   FIG. 2.   Interphase cell located on the edge of a colony, selected to demonstrate the fluorescent cytoplasmic fibers and fluorescent nucleolus in the same focal plane.   FIG. 3.   A cell in prophase. The nucleolus is no longer present and the condensing chromosomes do not stain. There are a few cytoplasmic fibers in the cell. Note particularly the two bright dots (arrows) close to the condensing chromosomes.   FIG. 4.   A cell in metaphase.   FIG. 5.   Early anaphase. The chromosomes have begun to separate.   FIG. 6.   Early anaphase.   FIG. 7.   Late anaphase. These anaphase spindle fibers connecting the chromosomes are shorter than metaphase spindle fibers.   FIG. 8.   Late anaphase: the chromosomes have separated. One of the two poles is out of the focal plane (arrow). FIG. 9.   A cell in mid-telophase. The stain is concentrated in the cleavage furrow.

skeletal agents (26–28), while actin filaments along with myosin could produce the contractile force for the shortening processes.

Biochemical analyses have demonstrated that actin is present in nuclei and nucleoli in a number of different cells (29–31). This report represents the cytological observation of actin in nucleoli. We have also stained HeLa cells and found the nucleoli to contain actin, a finding confirmed by the biochemical evidence of Le Stourgeon et al. (31). The role of this nucleolar actin is puzzling. Lazarides and Lundberg (32) have recently presented evidence that actin is an inhibitor of DNase I isolated from pancreatic acinar cells. They suggested that actin may control the nucleolytic activity of this enzyme during the cell cycle. DNase I may be localized in the nucleolus and complexed there with actin but there is no evidence that the enzyme occurs other than in lysosomal vesicles. In this location the enzyme would not normally be exposed to actin.

Nucleoli disappear at the beginning of prophase and reappear at the end of telophase (1–3, 33). During this interval, kinetochores, chromosomal spindle fibers, cleavage furrow, and centriolar regions stained positively for actin. We can only speculate that the nucleoli may contribute actin to one or more of these sites. As long ago as 1892, Strasburger suggested that nucleoli contributed some unknown substances from which the spindle fibers were formed (33). Further work will be needed to determine the fate of nucleolar actin during mitosis.

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