MICROFILAMENTS IN SPERMATOCYTES OF *NEPHROTOMA SUTURALIS*

JAMES R. LaFOUNTAIN, JR. From the Department of Biology, State University of New York, Buffalo, New York 14214

INTRODUCTION

Accumulated evidence from a variety of types of animal cells points to the involvement of a "contractile ring" in constricting cells to form cleavage furrows during cytokinesis (7, 12). Ultrastructural studies of dividing cells showing microfilaments in close proximity to the plasma membrane in regions where furrows are present suggest that microfilaments may be the structural manifestation of the contractile ring (1, 14, 17, 18, 19). This hypothesis has been supported by experiments which show that the disruption of the normal course of cytokinesis by the drug cytochalasin B, is associated with the loss of microfilaments (15, 16).

Our studies on selected spermatocytes from the crane fly, *Nephrotoma suturalis*, have revealed microfilaments in cleavage furrows during cytokinesis of the first meiotic division. The purpose of this report is to document these findings and to discuss their significance in light of recent reports of actin-like filaments in *Nephrotoma* spermatocytes after glycerination and treatment with heavy meromyosin (3, 4).

MATERIALS AND METHODS

Spermatocytes from larvae of *N. suturalis* cultured in the laboratory were prepared as monolayers on agar films and flat-embedded according to methods that have been described elsewhere (6). Ultrathin sections of selected cells were cut with a Porter-Blum ultramicrotome II (Ivan Sorvall Inc., Newtown, Conn.) using glass knives and were collected on carbon-coated Formvar films suspended over the apertures of slotted grids. Sections were stained with uranyl acetate (20) and lead citrate (13) and observed with a Hitachi HU 11C microscope.

Photographs of living cells cultured in vitro (5) and of fixed cells in perfusion chambers (6) were made with a Zeiss universal microscope equipped with Nomarski differential interference optics.

RESULTS AND DISCUSSION

Cytokinesis in primary spermatocytes of *N. suturalis* begins at the completion of anaphase I as the autosomal half bivalents approach the poles. Initially, the cell is constricted in the plane of the spindle equator, and as the cleavage furrow develops through the hyaline cytoplasm adjacent to the plasma membrane, the mitochondrial sheath is forced toward the longitudinal axis of the cell (previously the pole-to-pole axis of the spindle). Cytokinesis is completed when the newly formed secondary spermatocytes separate.

Ultrastructural details of the events of cytokinesis have been studied in spermatocytes prepared as monolayers on agar films, permitting precise identification and ultramicrotomy of individual cells. Cells prepared according to our methods appear morphologically similar to living cells as is evidenced by comparing the living cell in Fig. 1 *a* with the fixed cell in Fig. 1 *b*.

Equatorial sections through telophase cells in the regions of the cleavage furrow reveal microfilaments (50–70 Å thick) in the cytoplasm underlying the plasma membrane (Fig. 4). Filaments are of indefinite length (depending on the plane of section), appear in close association with the plasma membrane (but no definite connections between the membrane and filaments have been seen), and are located within 40–50 nm of the plasma membrane in an amorphous, weakly staining matrix.

Meridional sections made parallel to the plasma membrane at early stages of cytokinesis show that filaments are oriented parallel to the equatorial plane of the cell (Fig. 2). Individual filaments often appear to aggregate with one another, and in some cases, filaments have a particulate appearance (resembling beads on a string) with particles having a diameter of about 60 Å. In later stages, the cortical region appears denser, and individual filaments become difficult to resolve (Fig. 3). It appears that filaments and aggregates thereof form an annulus at the periphery of the cell coinciding with the cleavage furrow.

Examination of cortical regions near the poles at telophase as well as in cells at metaphase and anaphase has not revealed cortical microfilaments.

Previous studies showing microfilaments in the cortical regions of several different types of cells during cytokinesis lead to the conclusion that these structures may be a feature common to all cells that undergo cytokinesis by formation of a cleavage furrow. Moreover, filaments may generate forces resulting in constriction and development of the furrow. Besides observations of microfilaments in intact cells, studies on glycerinated cells treated with heavy meromyosin have shown decorated filaments (similar to actomyosin complexes from extracts of striated muscle) in cortical regions of dividing cells (10). The properties of these filaments are similar to those of actin, but as has been cautioned by others, these properties do not characterize them as *actin*. On this basis, they have been called "actin-like" filaments. Accumulating evidence suggests that actin-like filaments may be involved in contractile processes in several types of nonmuscle cells (2, 9, 11). An actomyosin-like system may also be involved in bringing about cytokinesis (8).

Actin-like filaments have been located in cortical regions of *Nephrotoma* spermatocytes after glycerination and treatment with heavy meromyosin, but they were not found in untreated cells (4). This apparent absence of microfilaments in intact cells was thought to be due to the lability of the filamentous structures, which disorganized upon fixation in intact cells without previous glycerination and/or stabilization with heavy meromyosin.
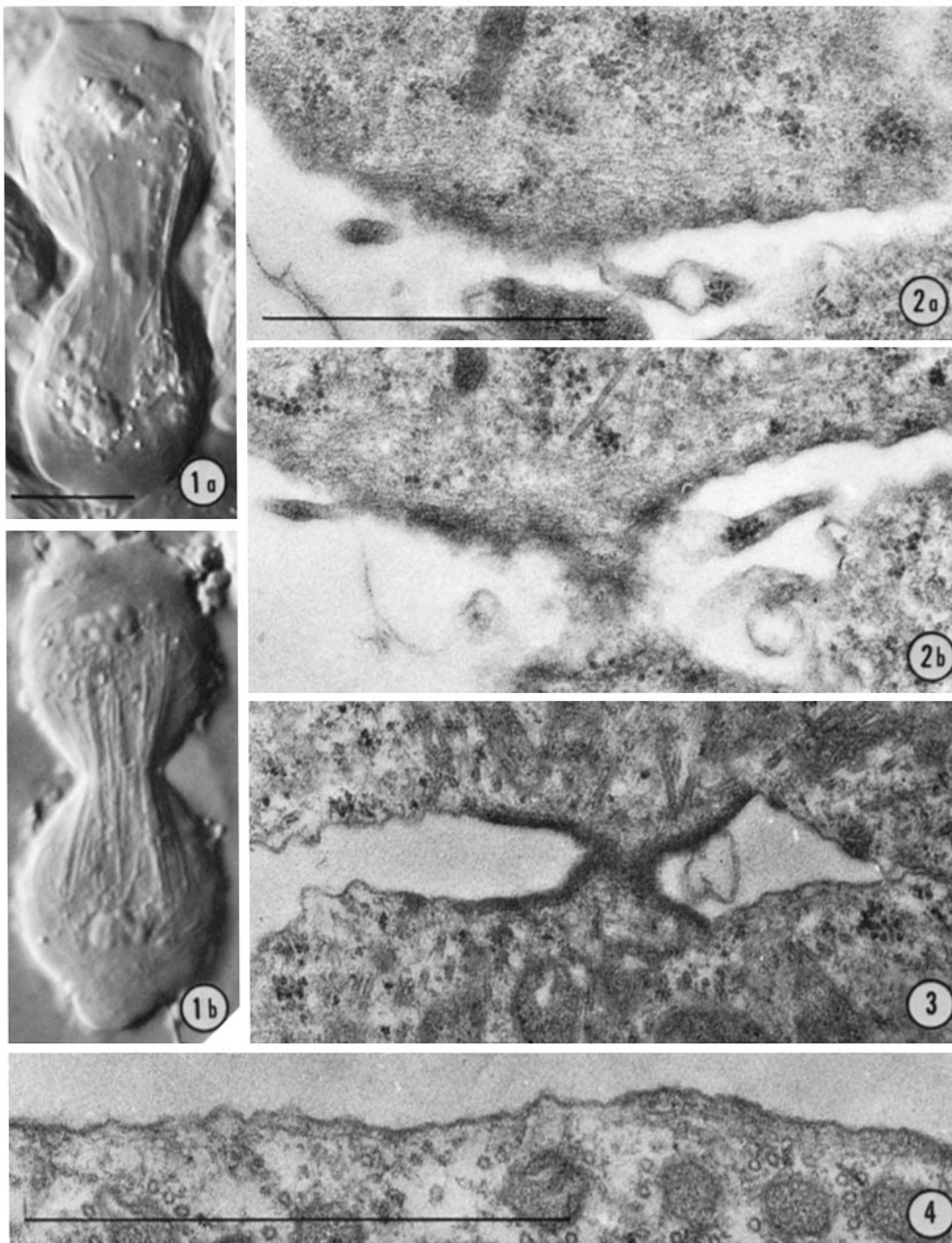
FIGURE 1   Photomicrographs of spermatocytes of *N. suturalis* at telophase I with Nomarski differential interference optics (100/1.25 N.A. oil immersion planachromatic objective). Fig. 1 *a* illustrates a living cell cultured in vitro; Fig. 1 *b* is a glutaraldehyde-fixed cell photographed in a perfusion chamber after fixation. $\times 1,800$; scale indicates 10 μm.

FIGURE 2   Electron micrographs of two adjacent meridional sections made parallel to the plasma membrane of a *Nephrotoma* spermatocyte in the region of the cleavage furrow at telophase. Microfilaments are located in a weakly staining matrix. $\times 50,700$; scale indicates 1 μm.

FIGURE 3   Electron micrograph of a meridional section through the cleavage furrow at a later stage of cytokinesis than Fig. 2. Filaments appear condensed, making identification of individual filaments difficult. Same magnification as Fig. 2.

FIGURE 4   Electron micrograph of an equatorial section through the cleavage furrow at an early stage of cytokinesis similar to that represented in Fig. 1. Microfilaments are located in close proximity to the plasma membrane; mitochondria and microtubules are seen in transverse section. $\times 80,400$; scale indicates 1 μm.
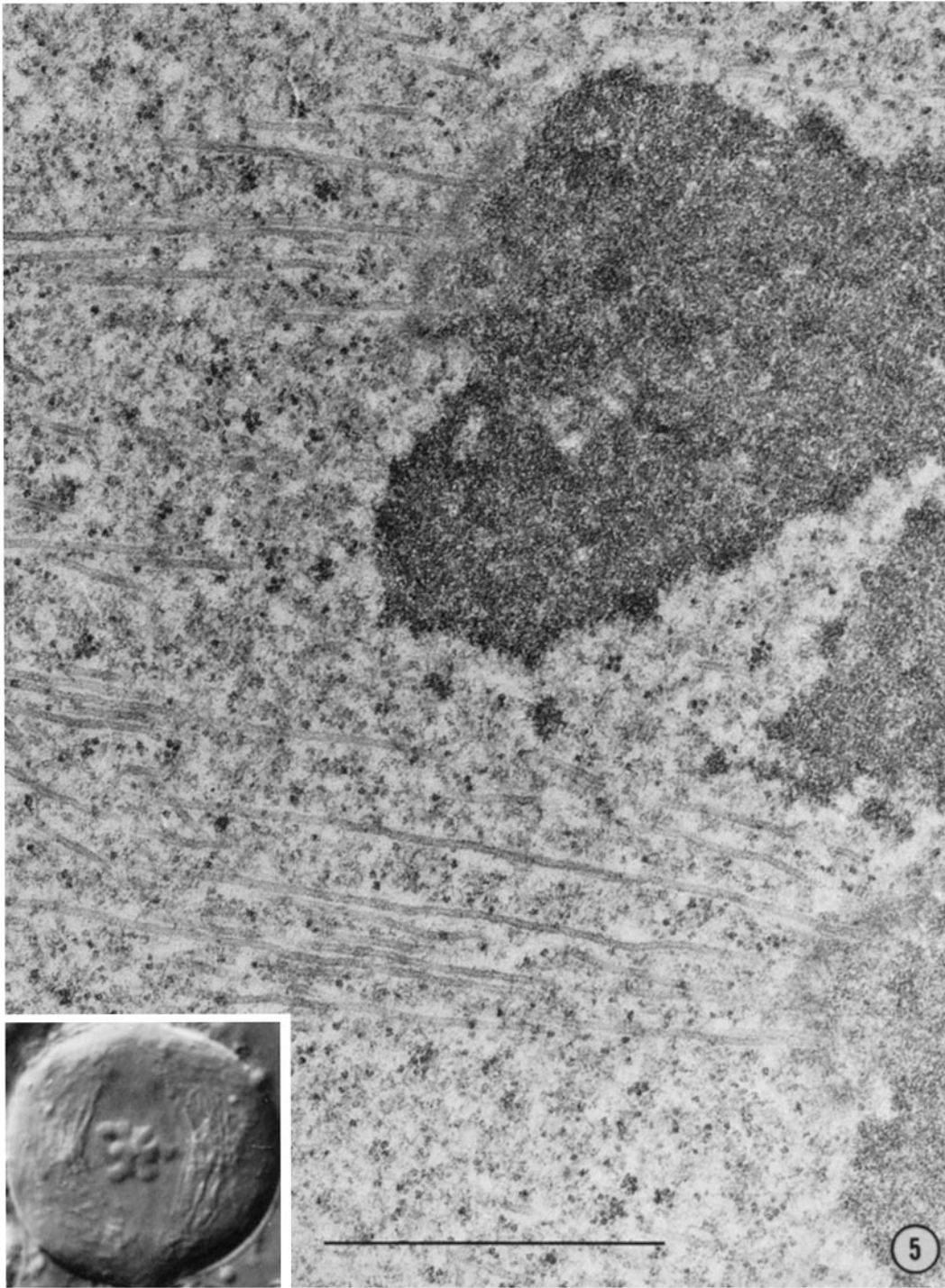
FIGURE 5   Electron micrograph of a thin section of a *Nephrotoma* spermatocyte at metaphase of the first meiotic division (same cell as *insert*) showing portions of bivalent and univalent chromosomes with kinetochores, microtubules, and granular matrix of the spindle. ×50,700; scale indicates 1 μm. For a more detailed description of fine structure of *Nephrotoma* spindles, see reference 6.

Micrographs presented here show microfilaments preserved in cells without glycerination or heavy meromyosin treatment. Although it is not possible to determine whether the filaments seen here represent the entire population of microfilaments that occur in vivo, present results indicate that cortical filaments in *Nephrotoma* spermatocytes are not as labile as was suggested earlier.

These findings take on added significance when considered in light of previous observations of decorated actin-like filaments in spindles of glycerinated spermatocytes and the suggestion that actin-like filaments are not only a component of spindles in vivo, but also may play a role in bringing about movements of chromosomes (3).

We have not been able to recognize microfilaments in spindles comparable to those observed in glycerinated cells in our preparations of untreated cells (Fig. 5, also see reference 6). Our inability to detect microfilaments in *Nephrotoma* spindles has prompted us to question whether spindle microfilaments actually exist in vivo.

Others have claimed that microfilaments can be visualized in *Nephrotoma* spermatocytes only after glycerination and treatment with heavy meromyosin (3, 4). The results presented here show otherwise. Since cortical filaments have been preserved by our techniques, one would expect that at least some microfilaments in spindles would have been preserved and detected, provided they are present in the spindle. This apparent absence of microfilaments in spindles does not rule out the possibility that actin-like molecules in the globular form are present in the spindle (as has been suggested by others [3]), nor does it rule out the possibility that cortical filaments have been preserved in our preparations and filaments in the spindle have not. Experimental evidence regarding the hypothesis that filaments in different regions of the cell may react differently to chemical fixation might shed some light on this problem. Until then, present results cast doubt on the hypothesis that actin-like microfilaments are a component of *Nephrotoma* spindles in vivo.

REFERENCES

1. BLUEMINK, J. G. 1970. The first cleavage of the amphibian egg. An electron microscope study of the onset of cytokinesis in the egg of *Amblystoma mexicanum. J. Ultrastruct. Res.* 32:142.
2. COMLY, L. T. 1973. Microfilaments in *Chaos carolinensis.* Membrane association, distribution, and heavy meromyosin binding in the glycerinated cell. *J. Cell Biol.* 58:230.
3. FORER, A., and O. BEHNKE. 1972. An actin-like component in spermatocytes of a crane fly (*Nephrotoma suturalis,* Loew). I. The spindle. *Chromosoma.* 39:145.
4. FORER, A., and O. BEHNKE. 1972. An actin-like component in spermatocytes of a crane fly (*Nephrotoma suturalis,* Loew). II. The cell cortex. *Chromosoma.* 39:175.
5. LaFOUNTAIN, J. R., JR. 1972. Spindle shape changes as an indicator of force production in crane-fly spermatocytes. *J. Cell Sci.* 10:79.
6. LaFOUNTAIN, J. R., JR. 1974. Birefringence and fine structure of spindles in spermatocytes of *Nephrotoma suturalis* at metaphase of the first meiotic division. *J. Ultrastruct. Res.* In press.
7. MARSLAND, D., and J. V. LANDAU. 1954. The mechanisms of cytokinesis: Temperature-pressure studies on the cortical gel system in various marine eggs. *J. Exp. Zool.* 125:507.
8. MIKI-NOUMURA, T., and H. KONDO. 1970. Polymerization of actin from sea urchin eggs. *Exp. Cell Res.* 61:31.
9. NACHMIAS, V. T., H. E. HUXLEY, and D. KESSLER. 1970. Electron microscope observations on actomyosin and actin preparations from *Physarum polycephalum,* and on their interaction with heavy meromyosin subfragment I from muscle myosin. *J. Mol. Biol.* 50:83.
10. PERRY, M. M., H. A. JOHN, and N. S. T. THOMAS. 1971. Actin-like filaments in the cleavage furrow of newt egg. *Exp. Cell Res.* 65:249.
11. POLLARD, T. D., E. SHELTON, R. R. WEIHING, and E. D. KORN. 1970. Ultrastructural characterization of F-actin isolated from *Acanthamoeba castellanii* and identification of cytoplasmic filaments as F-actin by reaction with rabbit heavy meromyosin. *J. Mol. Biol.* 50:91.
12. RAPPAPORT, R. 1971. Cytokinesis in animal cells. *Int. Rev. Cytol.* 31:169.
13. REYNOLDS, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* 17:208.
14. SCHROEDER, T. E. 1968. Cytokinesis: Filaments in the cleavage furrow. *Exp. Cell Res.* 53:272.
15. SCHROEDER, T. E. 1969. The role of "contrac-

tile ring" filaments in dividing *Arbacia* egg. *Biol. Bull. (Woods Hole).* **137:**413.

16. SCHROEDER, T. E. 1970. The contractile ring. I. Fine structure of dividing mammalian (HeLa) cells and the effects of cytochalasin B. *Z. Zellforsch. Mikrosk. Anat.* **109:**431.

17. SELMAN, G. G., and M. M. PERRY. 1970. Ultrastructural changes in the surface layers of the newt's egg in relation to the mechanism of its cleavage. *J. Cell Sci.* **6:**207.

18. SZOLLOSI, D. 1970. Cortical cytoplasmic filaments of cleaving eggs. A structural element corresponding to the contractile ring. *J. Cell Biol.* **44:**192.

19. TUCKER, J. B. 1971. Microtubules and a contractile ring of microfilaments associated with a cleavage furrow. *J. Cell Sci.* **8:**557.

20. WATSON, M. L. 1958. Staining of tissue sections for electron microscopy with heavy metals. *J. Biophys. Biochem. Cytol.* **4:**475.