FILAMENTS IN THE DIVISION FURROW OF MOUSE MAMMARY CELLS

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INTRODUCTION

Of the various theories that have been advanced to account for the mechanism of furrow formation and cytokinesis in animal cells (9, 21), the notion of active constriction in the equatorial cortex has recently received support from physical measurements such as those reported by Wolpert (22) and Rappaport (13). A probable structural manifestation of the "contractile ring" has been found through ultrastructural studies of cleaving invertebrate eggs, in which equatorially oriented filaments in the cleavage furrow suggest a region specialized for contraction (3, 7, 15, 17, 18). These bundles of presumably contractile filaments have not been described in dividing cells of vertebrates or in dividing tissue cells of any organism. The present study reports the presence of a well organized filamentous component in the division furrows of mouse mammary epithelial cells.

MATERIALS AND METHODS

Virgin C57 female mice 4–5 wk of age were anesthetized with pentobarbitol and the mammary glands were surgically exposed. Mammary end buds were removed in 1–2 mm pieces and fixed for 2 hr in glutaraldehyde buffered with 0.066 M cacodylate at pH 7.2.

The tonicity of the glutaraldehyde was measured with a vapor pressure osmometer and adjusted to 315 milliosmols by the addition of $CaCl_2$. The tissues were rinsed in buffer, postfixed for 2 hr in cold osmium tetroxide buffered with collidine at pH 7.4 (4), dehydrated in a graded series of cold ethanol to propylene oxide, embedded in Maraglas (16), and sectioned on a Porter-Blum MT2 microtome (Ivan Sorvall, Inc., Norwalk, Conn.). Thick sections (1 μ) were examined with phase contrast. Thin sections were stained in lead citrate for 1–2 min (19), with aqueous uranyl acetate for 1 min, and examined with an RCA EMU-3H electron microscope.

OBSERVATIONS

Active growth of the mouse mammary gland begins in the second to third week after birth (12). In a typical mammary gland from a 4 wk C57 female, the elongating ducts terminate in enlarged end buds, which represent the region of apical growth (Fig. 1). The end bud is characterized by rapid and extensive epithelial cell division and is therefore a favorable structure in which to study cytokinesis in a normal mammalian tissue.

Progressive thick sections of end buds were examined until telophase cells were located whose centriolar axis was parallel to the plane of the section. The tissue was then thin-sectioned serially until sections tangential to the division furrow were observed. In this manner the division furrows were examined in transverse section (those sections passing close to the centriolar axis) and in longitudinal section (those sections tangential to the division furrow).

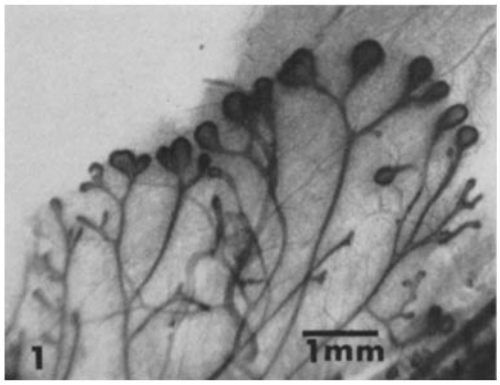

FIGURE 1 Light micrograph of a portion of mammary gland from a 4 wk old C57 female mouse. The ducts terminate in end buds which grow invasively through the fat pad. Whole-mount preparation, hematoxylin stain. × 10.

With one exception, the mitotic cells examined showed equatorial constriction proceeding equally from all sides; the exceptional cell contained a large autophagic vesicle which apparently blocked furrow constriction in the adjacent cortex. At low magnification, the cortical cytoplasm at the base of the division furrow appears more dense than the surrounding cytoplasm (Figs. 2 *a*, 4). Higher magnification reveals that the increased density is due to a punctate array (Fig. 2 *b*). When the base of the furrow is examined in tangential sections, it is apparent that this array is composed of aligned filaments 50–70 A in diameter (Fig. 3). In early telophase (Fig. 2 *b*), the furrow filaments are spaced at 50–100 A, in a layer 0.2 μ thick and extending 0.3–0.4 μ from the deepest point of the constriction along each side of the furrow. This layer of filaments is closely associated with the cell membrane. As equatorial constriction proceeds, the furrow filaments are compacted into a 0.15–0.2 μ bundle (*insert*, Fig. 4). There is virtually no spacing between filaments in the compacted bundle, and intimate contact with the cell membrane is maintained.

A second array of filaments, also 50–70 A in diameter, and oriented at right angles to the filaments in the "contractile ring," is present just within the furrow bundle (Fig. 4). These filaments are often continuous with desmosomes and are typical of the differentiated cells situated close to the end bud lumen. Unspecialized cells close to the basal lamina lack such filaments (Fig. 2 *a*) and do not contain desmosomes (C. Daniel and D. Scott. Data to be published).

DISCUSSION

The contractile ring hypothesis proposed by Marsland and Landau (8) has recently received experimental and morphological support. Active constriction in the cleavage furrow was suggested by the studies of Wolpert (22), who measured the resistance to membrane deformation at the polar and equatorial regions of cleaving sea urchin eggs and found increased membrane stiffness in the furrows relative to the poles. Arnold (2), working with partially flattened or

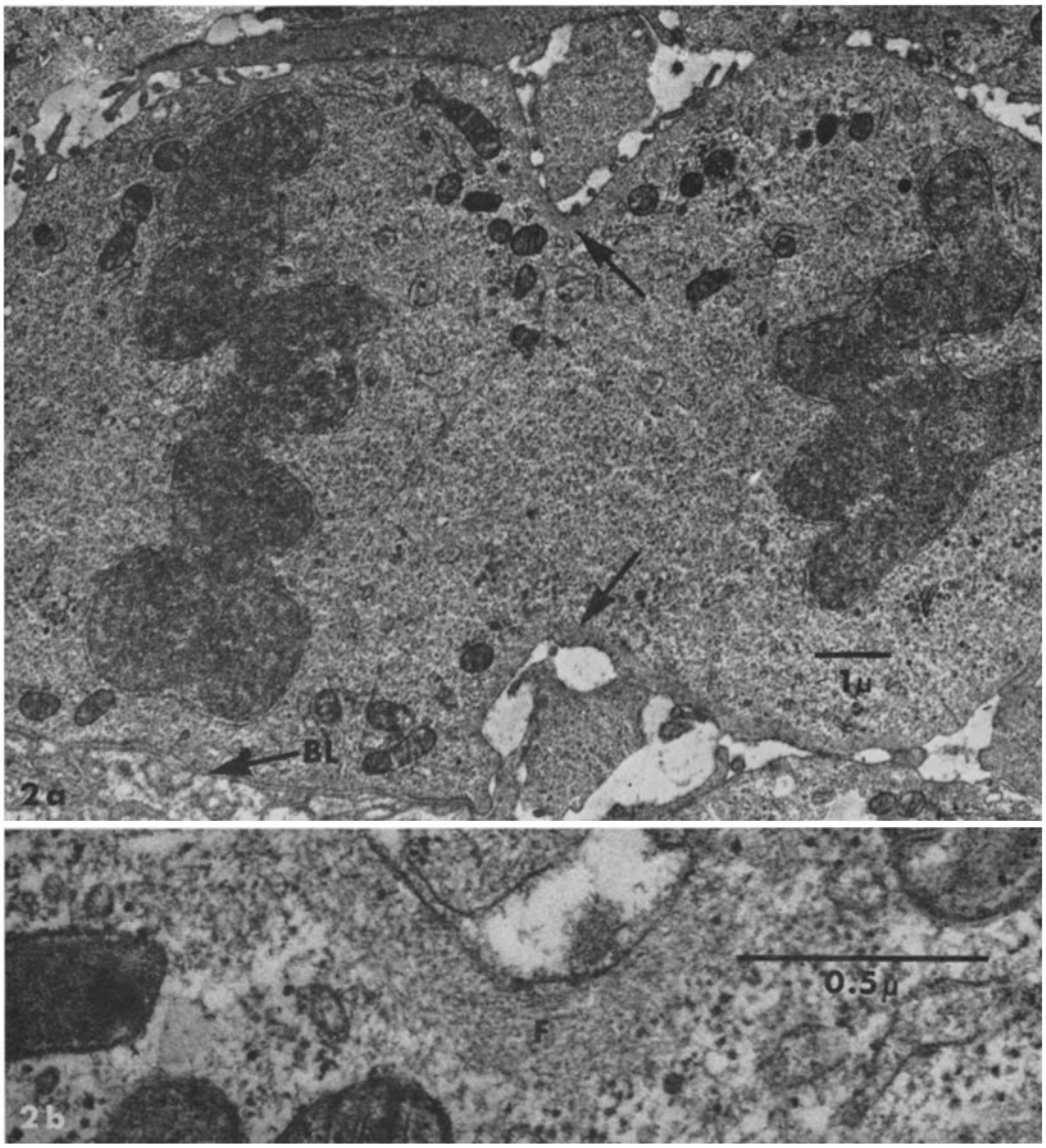

FIGURE 2 *a* Section through the centriolar axis of an early telophase cell, showing the dense cortical cytoplasm at the base of the division furrows (arrows). One portion of the cell adjoins the basal lamina (*BL*). × 9717.

FIGURE 2 *b* Higher magnification of the upper furrow shown in Fig. 2 *a*. The dense cortical area is a cross-sectional array of filaments (*F*). × 66,675.

microbeam-irradiated squid blastodiscs, obtained further evidence that furrow formation is associated with active constriction. Rappaport (13) directly measured the tension exerted by the cleavage furrow in various echinoderm eggs, in which ultrastructural studies have previously demonstrated an electron-opaque layer closely associated with the cell membrane in the cleavage furrow (10). Assuming that this layer was composed of proteins with contractile characteristics

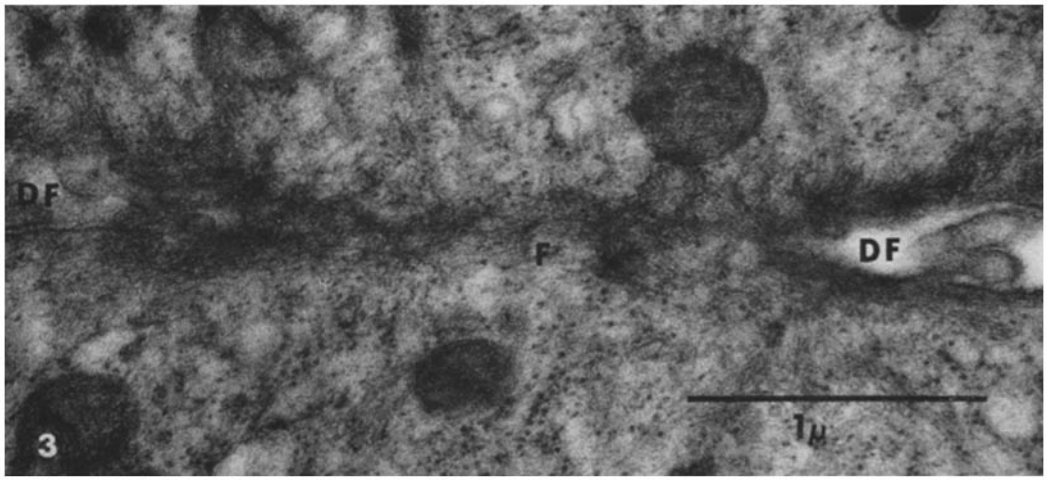

FIGURE 3 Section tangential to the division furrow (*DF*) of a telophase cell, showing the band of furrow filaments (*F*) in longitudinal section. × 40,188.

similar to those of actomyosin threads, Rappaport calculated that the tension that could be generated was sufficient to account for the force actually used in division (13).

Fine structural studies of cleavage furrows in dividing eggs of related Echinoderms have demonstrated the presence of a band of aligned filaments (7, 18), and similar filaments have been described in other cleaving invertebrate eggs (3, 15, 17). Because of the location and orientation of these filaments in the cleavage furrow, and because they resemble cytoplasmic filaments related to other types of cell movement such as larval tail contraction during tunicate metamorphosis (6) and motility in slime molds (20), it is reasonable to postulate that these filaments are functionally significant to the mechanics of cytokinesis in these invertebrates.

Although Robbins and Gonatas reported an increased cortical density in the equatorial region of dividing HeLa cells (14), and a punctate array of suggestive appearance was incidentally shown in the division furrow of a dividing chick mesenchyme cell (1), furrow filaments have apparently been specifically described only in early cleavage stages of invertebrate eggs. The filamentous component of the division furrow in mouse mammary epithelial cells described in the present report indicates that these filaments occur in tissue cells as well. Although we present no experimental evidence regarding the role of these filaments in mammary cells, active constriction of the division furrow is strongly suggested by certain observations. In Fig. 4, the desmosomes, whose filaments underlie the furrow filaments, are apparently under tension as constriction proceeds, and they appear to pull adherent cytoplasmic projections of adjoining cells into the deepening division furrow. The filamentous band in mammary cells is smaller than the bands described in invertebrate eggs. This is probably explained by the large amount of yolk and other cytoplasmic structures found in eggs which resist furrow constriction and would require more force, and presumably more filaments, to successfully cleave.

A different mechanism of division furrow formation, similar to that found in plants, has been described in several mammalian cell types (5, 11). Cytokinesis involves the formation of an equatorial vesicular plate and subsequent fusion of the vesicles to form the division furrows. In these cases, active constriction of the equatorial cortex does not appear to be involved in cytokinesis.

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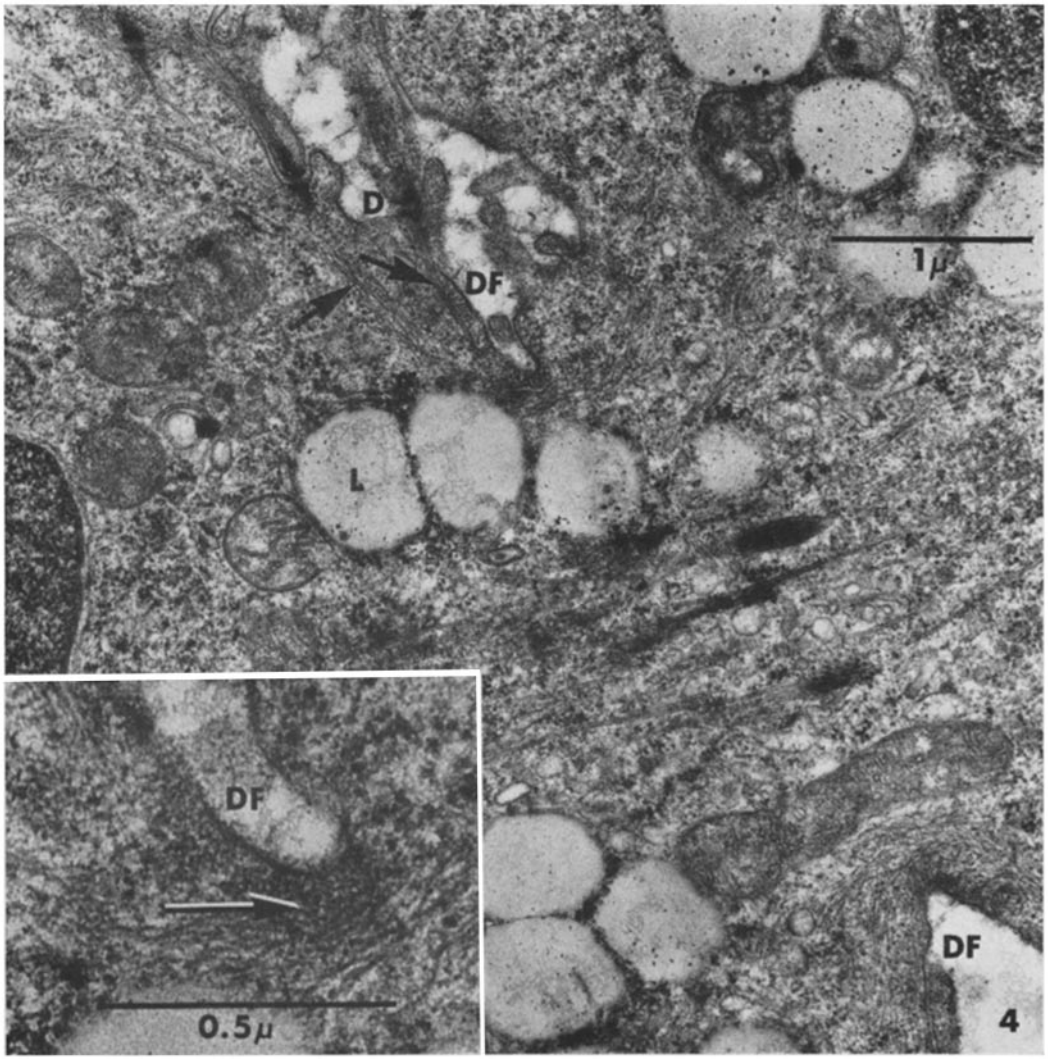

FIGURE 4 Late telophase cell with deep division furrows (*DF*) and lipid vacuoles (*L*). The filaments (arrows) from the desmosomes (*D*) extend beneath the base of the furrows. The *insert* shows details of the division furrow (*DF*) with the closely associated filamentous bundle (arrow). × 26,933; *insert*, × 79,375.

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