

CLEAVAGE FURROW FORMATION IN A TELOLECITHAL EGG (*LOLIGO PEALII*)

I. Filaments in Early Furrow Formation

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ABSTRACT

Formation of the first cleavage furrow in the telolecithal egg of *Loligo* was studied with the electron microscope. Before the actual furrow forms, a dense filamentous band develops below the plasma membrane from membrane-bounded dense bodies which appear to be Golgi-derived. The egg surface is thrown into a number of longitudinal folds which parallel the furrow and eventually become incorporated into it. These longitudinal folds contain a network of tubules and vesicles. Frequently, multivesiculate bodies are associated with the furrow and possibly give rise to the network of tubules and vesicles. Apparently part of the membrane between the two new blastomeres is derived from the surface of the longitudinal folds. The theory of furrow formation by contraction is discussed in light of the filamentous band.

INTRODUCTION

The problems involved in mitosis and cytokinesis have attracted a great number of investigators, but relatively little work has been done on the fine structure and the mechanism of cleavage furrow formation. The elegant micromanipulation experiments of Rappaport (15-19), Hiramoto (10), Rappaport and Conrad (20), and Rappaport and Ratner (21) have recently shed considerable light on the mechanism of cytokinesis in invertebrate eggs of several species. Essentially, they demonstrated that at the time of mitosis the asters influence the surface of the egg so that it becomes regionally specialized into a future active furrow and a passive polar region. This strongly supports the proposal of Marsland and Landau (13) that cleavage furrow formation involves a contractile ring mechanism which forms the furrow by active constriction. Mercer and Wolpert (14), Weinstein and Herbert (27), Robbins and Gonatas (23), and Buck and Krishan (7) have demonstrated a dense

layer immediately below the furrow in several different types of cells. Arnold (3), Schroeder,¹ and Goodenough, Ito, and Revel² have all demonstrated this dense layer to be composed of filaments or fibrils, and Arnold (4) has experimentally implicated these filaments in contraction during formation of the furrow. This paper is the first of a proposed series which will describe the fine structure of cleavage furrow formation and give evidence for a contractile role of a specialized filamentous region in the surface of the squid egg.

MATERIALS AND METHODS

The eggs used in these studies were obtained by stimulating adult *Loligo pealii* to copulate and lay their

¹ T. E. Schroeder. 1968. Cytokinesis: filaments in the cleavage furrow. *Exp. Cell Res.* 53:272.

² D. Goodenough, S. Ito, and J. P. Revel. 1968. Electron microscopy of early cleavage in *Arbacia punctulata*. *Biol. Bull.* 135:420.

eggs in tanks at the Marine Biological Laboratory at Woods Hole, Mass. (1). By watching the adults it was possible to select eggs in the process of fertilization, just after the egg strings were deposited by the females. After a few minutes these eggs were mechanically removed from their jelly and closely observed until the desired stage of cleavage was reached. In all cases part of the egg string was kept as a control to check on synchrony of division.

The eggs were fixed in a variety of fixatives, but Palade's Veronal-acetate-buffered 1% OsO₄ was found to produce the most consistent and satisfactory results even though there was some leaching out of the background cytoplasm. The embryos were fixed at room temperature for 15–20 min at pH 6.8 and transferred directly into 50% ethanol. It was found that the microtubules of the mitotic apparatus could be preserved at this temperature and pH (2). Glutaraldehyde and glutaraldehyde-formaldehyde combinations were also tried but despite many variations and precautions the plasma membrane was frequently stripped from parts of the blastodisc and shrinkage of the cytoplasm was evident. It was still possible to check the reported results against the results obtained with these other methods of fixation and to confirm the conclusions thereby drawn. The embryos were embedded in hard Epon (12) and carefully oriented and sectioned either parallel or perpendicular to the cleavage furrow. The sections were stained with either lead citrate (22) or double stained with uranyl acetate in methanol (25) followed by lead citrate.

OBSERVATIONS

The egg of *Loligo pealii* is large (1.6 × 1 mm), ovate, and telolecithal, and has bilateral, meroblastic cleavage. Fertilization occurs just at the time of egg laying and approaches 100% in all cases. The blastodisc forms rapidly after fertilization and, if naturally fertilized, the eggs of a given egg string show remarkable synchrony. About 3 hr after fertilization the first cleavage furrow formation takes place. It divides the blastoderm into future right and left halves. About 25 min before cleavage the mitotic apparatus is evident as a birefringent area at the apex of the egg slightly displaced from the polar bodies. Approximately 5 min before the appearance of the cleavage furrows a faintly birefringent band appears at the egg surface exactly in the position of the future furrow (4). There seems to be a correlated slight flattening of the normal curvature of the egg in this region. When the first furrow actually begins to form, it suddenly appears as a line rather than a point and rapidly extends across and down into the cytoplasm. Because the furrow does begin

in the center and spreads toward the edges, the more distal furrow is "younger" than the central furrow; thus a temporal sequence of furrow formation is evident in serial sections of the entire furrow. By fixing embryos in a timed sequence from disappearance of the spindle birefringence to visible appearance of the furrow, it was possible to confirm that this spatial-temporal sequence was the same as a simple-temporal one. However, all but two of the micrographs presented here are from a series of approximately 1200 micrographs made of one embryo fixed about 10 min after the first visible indication of the furrow. These observations were carefully checked against many micrographs taken from other embryos of similar or later ages.

The sequence of formation of the cleavage furrow can be seen in Figs. 1 and 5–9 which represent different levels of the same furrow. The most distal furrow region is differentiated from the surrounding areas by the presence of many blebs of the surface membrane and by a dense, apparently granular layer immediately below this region (Figs. 1 and 2). These surface blebs are actually discontinuous longitudinal folds (Fig. 3) which run the entire length of the forming furrow. In micrographs of areas like those shown in Fig. 1 there is an average of 37 such small folds per cross section and the dense layer averages 18 μ in width. There are many small, irregular vesicles, tubules, and granules in this layer (Fig. 2). In longitudinal sections of the forming furrow the dense layer can be seen to be composed of a filamentous band which underlies the entire furrow region (Fig. 4). These filaments are about 45 Å thick, have a beaded appearance with a periodicity of about 40 Å, and are generally reminiscent of similar filaments found by Cloney (8) in ascidian tadpole tail epithelium, by Goodenough, Ito, and Revel², and by Schroeder¹ in invertebrate egg cleavage furrows. Fig. 1 also shows the uniformly dense chorion and the highly granular intrachorionic material. This granularity is apparently due to fixation and occasionally seems to have a fibrillar nature (Figs. 5 and 6).

Sections through the inbending furrow show the dense filamentous band to be localized only under the area of the longitudinal folds (Fig. 5). It averages 6.75 μ in width and varies between 100 and 150 mμ in thickness. The intrachorionic substance does not extend into the furrow proper but bends downward in that region. There is no evi-

dence of any interconnection between this layer and the plasma membrane of the furrow. The cytoplasm of the blastodisc has small, regular mitochondria but is mainly occupied by a massive network of intraconnecting vesicles. Because it is not possible to satisfactorily relate this network to either smooth or rough endoplasmic reticulum, the noncommittal term "vesiculate reticulum" will be used until further information elucidates its nature. Frequently associated with the furrow itself, and most commonly below the basal region of the furrow, are large multivesiculate bodies (Figs. 6, 7, 10, 13, 14). Occasionally these multivesiculate bodies have regions of electron opacity (Figs. 7, 13, 14).

Figures 6, 7, 8, and 9 show progressively deeper regions of the forming furrow in which the membranes come into apposition. There is no evidence of tight junctions, desmosomes, or other specialized regions of the membrane. Although the region between the nuclei was carefully examined in selected serial sections, there was no evidence of a midbody at this stage or at later stages of first cleavage furrow. Fig. 9 represents the maximum extent of the furrow at 10 min and is from the region between the nuclei. The basal region of the furrow is curved and surrounds an empty area into which the longitudinal folds project (compare Figs. 7-9). In some regions the plasma membranes from both sides of the furrow are in close apposition, but in other places considerable gaps exist

(Figs. 8, 9). In all of the inbending furrows there are longitudinal folds but only in the region of the filamentous band. In 24 counted and measured sections there is an average of 14.4 cross sections (range 12-17) of the longitudinal fold per section, regardless of the degree of inbending.

In most sections there are dense membrane-bounded bodies 0.15-0.3 μ in diameter which are closely associated with the filamentous band of the furrow base (Figs. 8, 10, 11, 14). In Fig. 8, for example, these bodies occur in small groups in the cytoplasm or singly associated with the plasma membrane of the furrow. Fig. 10 shows an example in which the membrane of the dense body closest to the furrow (arrow) has apparently broken down and the internal material is being associated with the filamentous band. This seems to occur slightly below the plasma membrane and the vesicles and tubules mentioned above. Occasionally, in thinner sections, filamentous strands can be seen in these dense bodies (Fig. 11). These filaments measure approximately 45 A in thickness and in general resemble the filaments seen below the forming cleavage furrow. The membrane-bounded dense bodies apparently originate in the Golgi apparatus (Figs. 11, 12) and move as groups to the region of the cleavage furrow and there break down to form the filamentous band. This filamentous band is continuous below the "necks" of the flask-shaped longitudinal folds and occasionally can be seen in

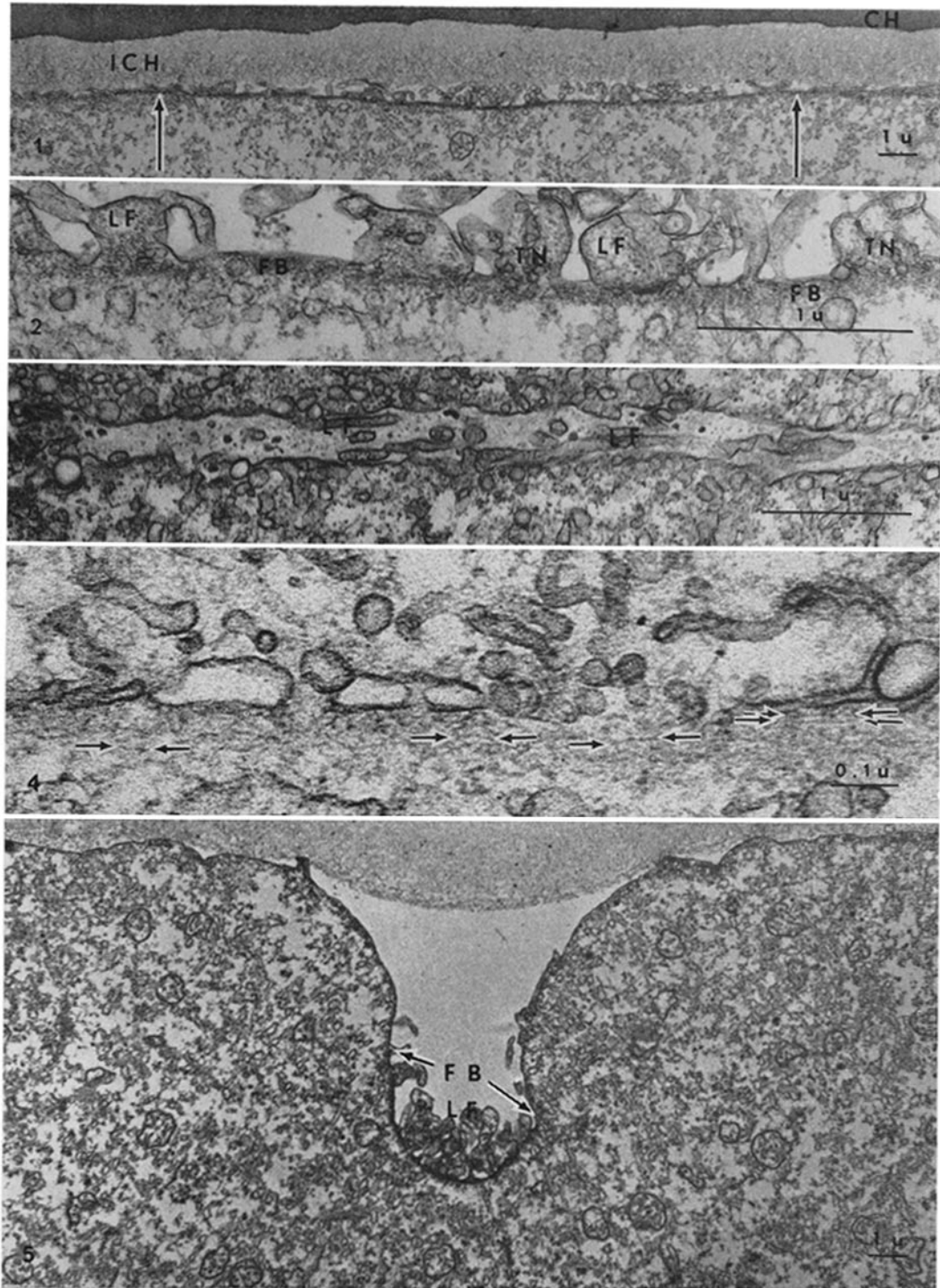
FIGURE 1 Cross section of the future furrow region distal to the incurved furrow. The future furrow region is characterized by the many longitudinal folds and the presence of a dense layer below the plasma membrane. The arrows indicate the lateral margin of the dense layer. The chorion (*CH*) and intrachorionic substance (*ICH*) are also evident. $\times 4,800$.

FIGURE 2 Higher magnification of the area marked in Fig. 1. Note the presence of many vesicles and tubules (*TN*) in the many longitudinal folds (*LF*). The filamentous band (*FB*) below the plasma membrane is quite prominent but is not present in the longitudinal folds. $\times 32,000$.

FIGURE 3 Frontal section of an early furrow showing the discontinuous nature of the longitudinal folds (*LF*) at this stage. $\times 22,000$.

FIGURE 4 Longitudinal section of the very early second furrow showing the presence of many filaments approximately 45 A thick (arrows) in the filamentous band. $\times 100,000$.

FIGURE 5 Inbending furrow proximal to Fig. 1. Many longitudinal folds (*LF*) are evident and the filamentous band (*FB*) is quite prominent (lateral margin at arrows). $\times 6,000$.



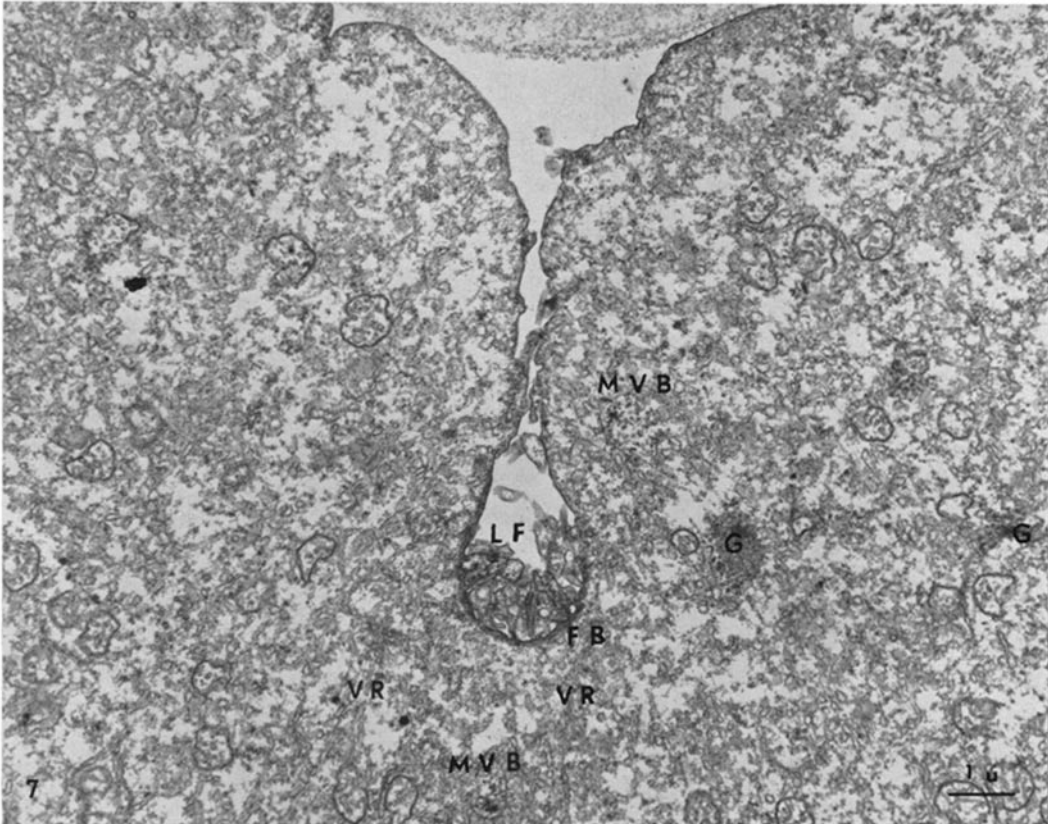
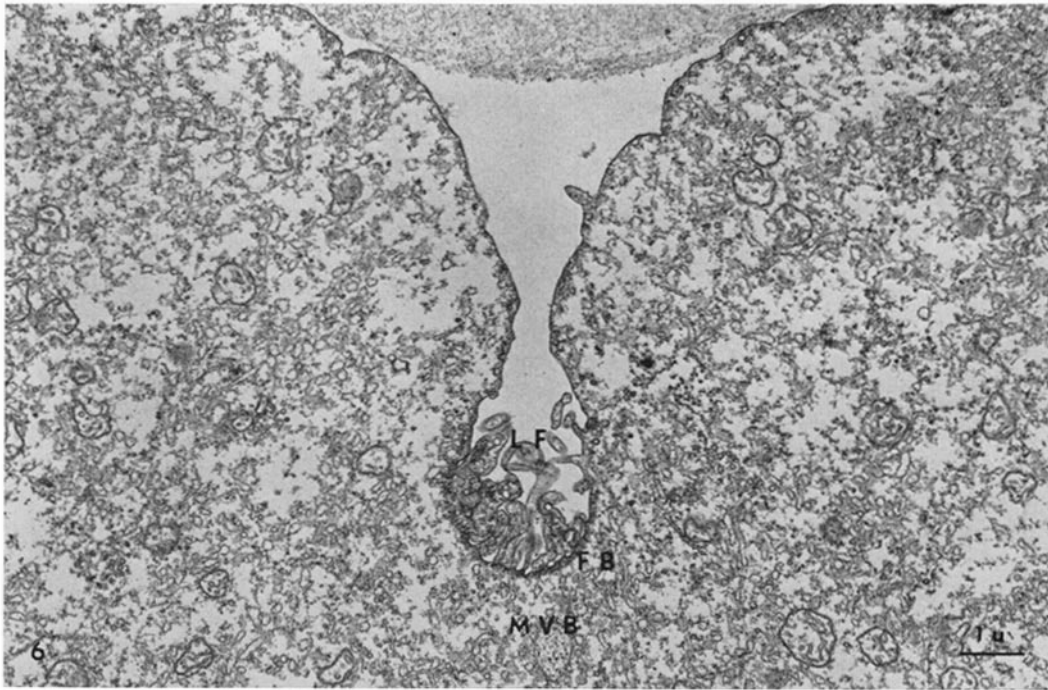


FIGURE 6 Deeper region of the furrow shown in Fig. 5. Note the multivesiculate body (*MVB*) below the furrow. $\times 8,750$.

FIGURE 7 Still deeper region of the furrow of the previous figures. Note the general orientation of the vesiculate reticulum (*VR*) toward the base of the furrow. Golgi regions are evident (*G*). $\times 8,750$.

the basal portion of the longitudinal folds themselves (Fig. 10).

In many instances the multivesiculate bodies associated with the furrow appear to be breaking down and emptying their contents toward the furrow (Fig. 10). Also frequently seen in sections of the longitudinal folds is what appears to be a network of vesicles and tubules. In a few cases the multivesiculate bodies contain tubules similar in size to those in the longitudinal folds (Figs. 13, 14). These vesicles and tubules are characteristic of the longitudinal folds and will be referred to here as the "tubular network." The tubular network occupies much of the folds; the tubules vary between 23 and 27 $m\mu$ in diameter, and the vesicles between 25 and 40 $m\mu$. There are many transitional forms between the vesicles in the folds and the tubules (Figs. 10, 13, 14); however, the tubules occur only between the plasma membrane and either the dense bodies or the filamentous band.

Most of the micrographs show a nonrandom orientation of the long axis of the individual components of the vesiculate reticulum toward the base of the furrow (Figs. 13, 8, 7, 6), but any direct relationship of these vesicles in the furrow is not obvious from these micrographs. The mitochondria seem to be randomly distributed through the cytoplasm but are never closely associated with the furrow. However, the dense bodies and the Golgi complexes found in association with them seem to occur preferentially in the cytoplasm near and directly below the forming furrow (Fig. 15). Although not enough of these highly favorably oriented sections were obtained to make counts which could be dealt with statistically, all the micrographs examined were in essential agreement. There is a concentration of the dense bodies in the cytoplasm below and around the forming furrow and close to the egg surface (Fig. 15).

DISCUSSION

Obviously, two related major problems are involved in the formation of this telolecithal cleavage furrow: the mechanism of formation of the cleavage furrow and the source of the plasma membrane of the furrow. The second problem will be dealt with in another paper and will only be mentioned here as it relates to the discussion of the furrowing mechanism.

In theory, the filamentous band forms the cleavage furrow by contraction while remaining fixed at both ends and thereby cutting through the

curved blastodisc as a cheese is cut by a wire. Evidence for such a contraction on the cleavage furrow was presented by Arnold (4), and by Noden in personal communication, on squid embryos and by Rappaport (19, 20) on several invertebrate eggs and will be presented in detail in a later paper. In the process of cutting through the cytoplasm the width of the filamentous band decreases from about 18 μ in the flattened region beyond the furrow proper to a rather uniform 6.7 μ in the curved region of the furrow. In order for the filamentous band to pull the plasma membrane downward through the cytoplasm it must be linked to the plasma membrane. When the decrease in width occurs the plasma membrane is thrown into a series of longitudinal folds which discontinuously run the length of the furrow. As the width of the filamentous band decreases even further the number of folds seen in cross section goes down because they fuse to form fewer but larger folds. Once the minimal width is attained, the number of folds remains relatively constant during early cleavage, but by the time the furrow is completely formed only two or three folds remain (3). Although this amount of plasma membrane will not account for all of the new surface necessary, obviously it forms part of the new surface. The linkage of the filamentous band to the plasma membrane, together with the resistance of the cytoplasm to the cutting, might also account for the curvature of the base of the furrow. The individual filaments of the filamentous band must be associated or interconnected with each other in some way not obvious in the micrographs. This could be accomplished either by a cross-linkage between individual filaments, by being embedded in a matrix of low electron density, or simply by overlapping in very slightly nonparallel paths. None of these possibilities can be eliminated by the data thus far gathered. The whole band must be anchored at its ends or contraction would merely cause a surface distortion. The nature of these anchor points is as yet unknown.

The dense bodies are quite reminiscent of similar structures seen by Robbins and Gonatas (23) in the division of the HeLa cells. However, those authors presented evidence that HeLa cell membrane-bounded dense bodies are derived from multivesiculate bodies and, from histochemical evidence, they suggested that these dense bodies functioned as lysosomes. They did observe filament-like structures in these membrane-bounded

dense bodies but interpreted them as myelin figures. From serial sections it is obvious that this is not the case in the dense bodies described here. Although the two bodies are similar in appearance and are also similar in that they concentrate near the cleavage furrow, it is not possible to conclude whether they are identical.

Evidence for a similar layer of contractile filaments in resorption of the ascidian tadpole tail has been presented by Cloney (8). In that case the filaments measured 50–70 Å in thickness and became highly oriented when the epithelium contracted to cause tail resorption. Baker and Schroeder (5) have also found 40–60 Å filaments in the apical contact zone of the cells of the invaginating neural plate of *Xenopus* and *Hyla*. Tilney and Marsland³ and Szollosi (26) have found similar filaments in the cleavage furrows of *Arbacia* and the coelenterate *Aequorea*, respectively.

The roles of the multivesiculate bodies and the tubular network remain obscure. It is suggested that the vesicles of the multivesiculate bodies form the tubules which may possibly be continuous with the plasma membrane. Although these tubules have the same dimensions as cytoplasmic microtubules (11, 24), their highly convoluted nature and their apparent origin from or formation of vesicles make them seem quite different from the usual four classes of microtubules (6). However, this evidence is too tenuous to erect a new class of microtubules. The function of the dense material frequently seen in the multivesiculate bodies is also unknown. Possibly it may contribute to the filamentous band but, unlike the material in the dense bodies of Robbins and Gona-

tas (23), it is never seen to be closely membrane-bounded.

Since Johnson and Porter (10) have recently covered the literature of cleavage furrow formation and since a detailed discussion will be included in another paper, a long literature review is not appropriate here. However speculative, it is necessary to relate the data and theory presented here to a general mechanical mechanism of cleavage furrow formation. The data strongly support the many variations of the contractile ring theory (18, 19, 28) and provide a possible structural basis for it. The filamentous band would divide the cytoplasm by shortening while remaining attached to the surface, if the ends of the band were either anchored or if the band ran completely around the cell. In holoblastic cleavage a separate final step would be necessary to divide the blastomeres since the filamentous band could not contract to infinity. Possibly, this could be caused by surface tension, vesicle fusion, or the formation of the midbody. In a telolecithal egg the problem of contraction to infinity is not present since furrowing stops at the yolk. The location of the furrow might be related to the concentration of the membrane-bounded dense bodies which could, in turn, be determined by the mitotic apparatus as appears to be the case in echinoderm eggs. Heavy compression prior to and during early cleavage causes disruption of cytoplasmic organization and results in delayed and disorganized cleavage patterns discussed in reference 4 and by Noden in a personal communication. Undoubtedly other mechanisms of cytokinesis operate in many types of cells (10) but in this case the mechanism seems particularly amenable to experimental attack.

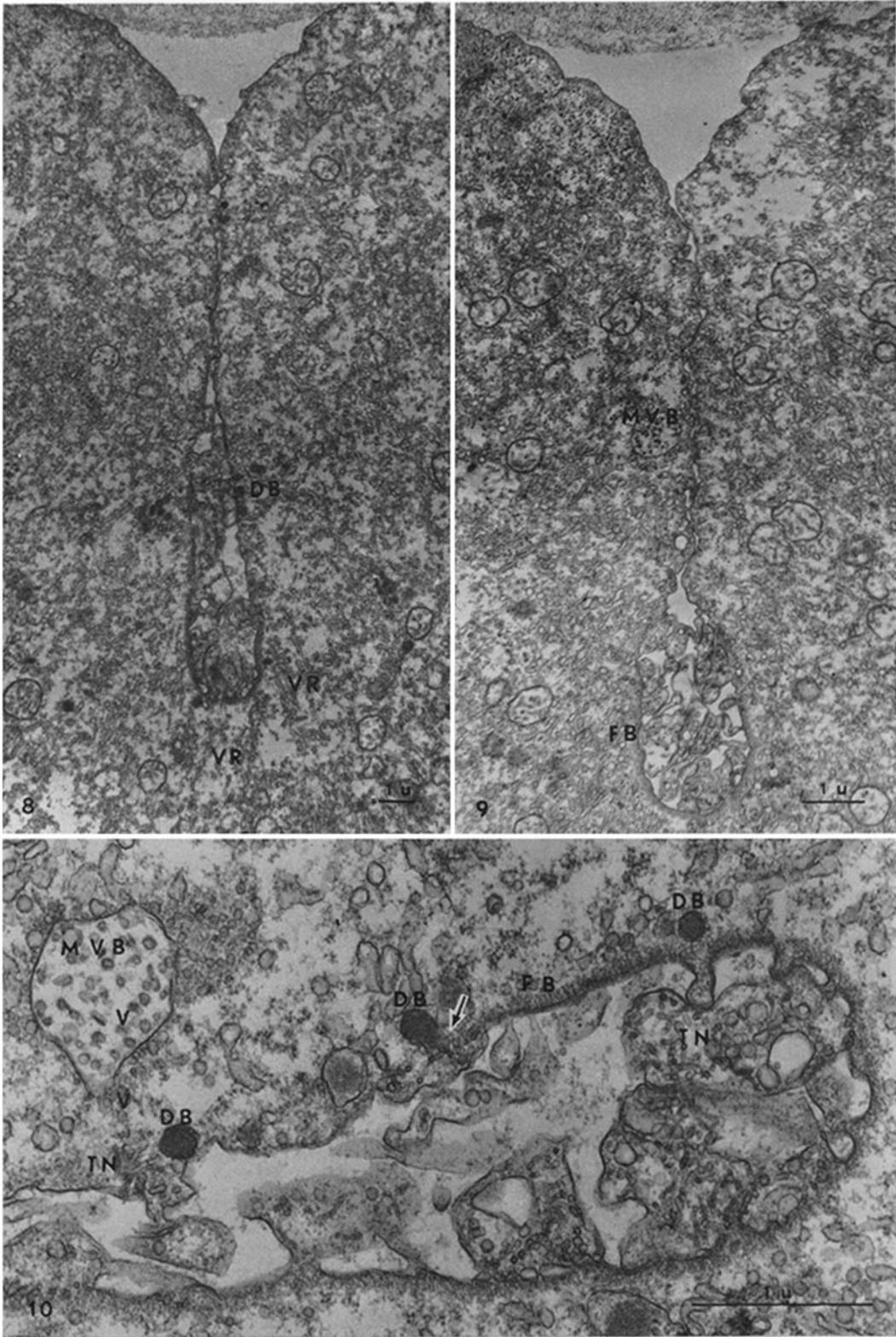
I would like to thank Drs. R. E. Kane and I. R. Gibbons for their continual help and encouragement,

³ L. Tilney and D. Marsland. Data to be published.

FIGURE 8 Membrane apposition in the deeper furrow. Many membrane-bounded dense bodies (*DB*) are present near and around the furrow. $\times 6,000$.

FIGURE 9 Furrow between the nuclei after roughly 10 min of cleavage. Many gaps exist between the membranes. $\times 10,000$.

FIGURE 10 Association of the dense bodies (*DB*) and multivesiculate bodies (*MVB*) with the forming furrow. One of the dense (arrow) bodies appears to be giving rise to the filamentous band (*FB*) while immediately below this there are segments of the tubular network (*TN*). The multivesiculate body apparently is open on the side toward the furrow, and many vesicles (*V*) appear to be coming from it. $\times 29,000$.



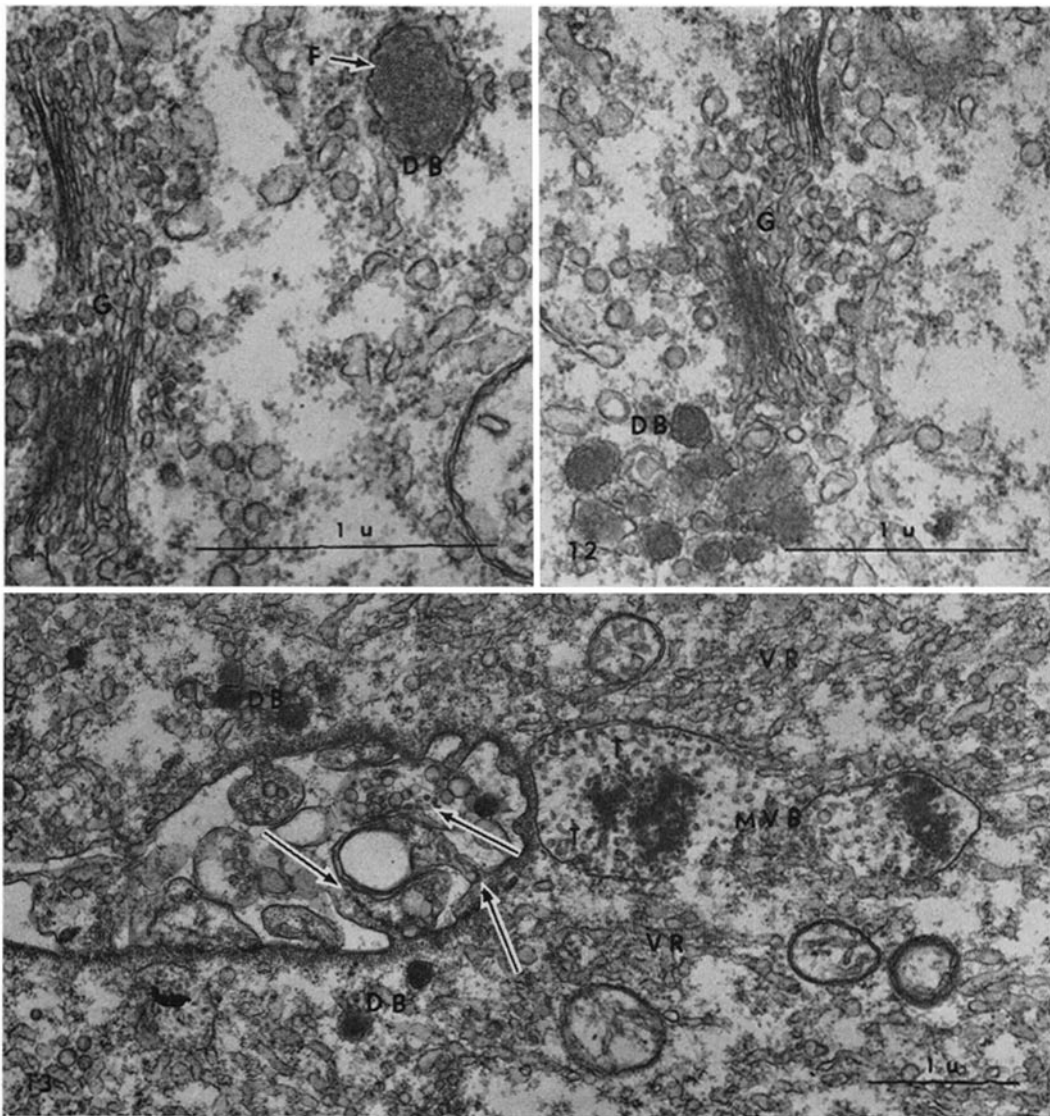


FIGURE 11 Higher magnification of a dense body in the cytoplasm below the furrow. A region of filaments (*F*) is evident at the arrow. This Golgi apparatus had many dense bodies associated with it. $\times 40,000$.

FIGURE 12 Formation of the membrane-bounded dense bodies by the Golgi apparatus. $\times 32,000$.

FIGURE 13 Base of a furrow showing a large multivesiculate body with many vesicles and a few tubules (*T*) and transitional forms of vesicles and tubules (arrows). The multivesiculate body contains masses of dense material which are not membrane-bounded. The vesiculate reticulum (*VR*) is oriented toward the furrow. $\times 19,500$.

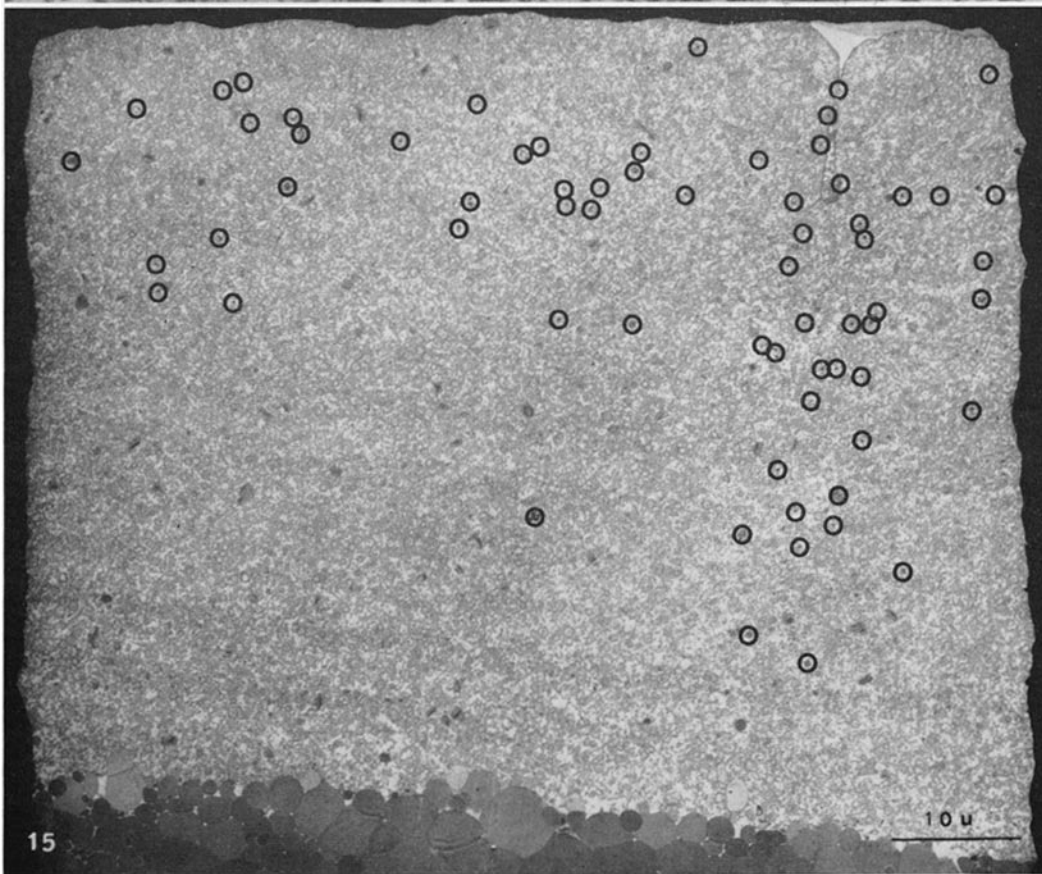
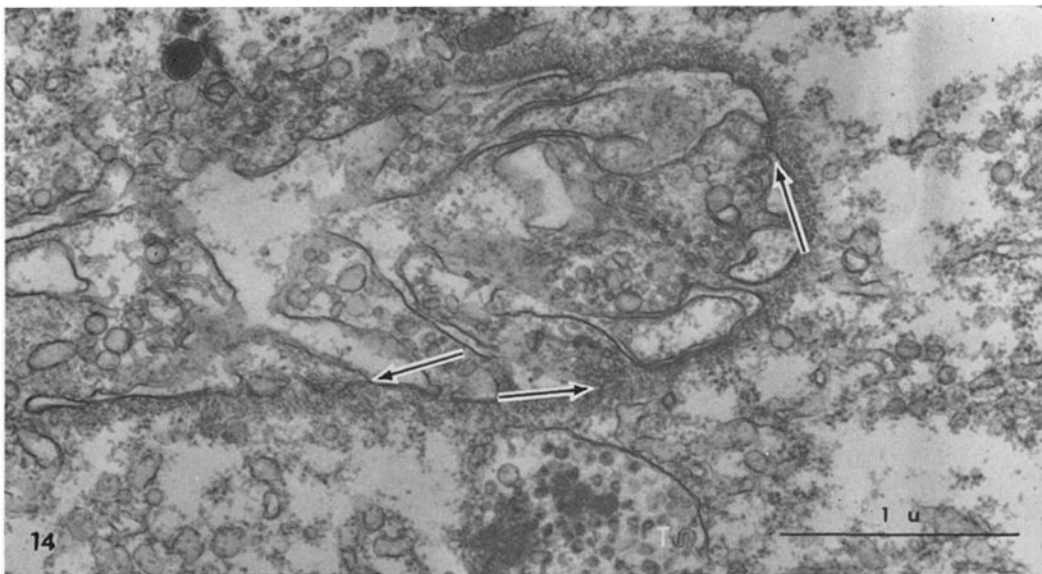


FIGURE 14 Base of a furrow showing possible continuity between the plasma membrane and the tubular network (arrows). Note in particular the small segment of tubule in the multivesiculate body (T). $\times 32,000$.

FIGURE 15 Low-power micrograph of an entire grid space showing the distribution of the dense bodies (circles). $\times 1,700$.

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