

CORTICAL CYTOPLASMIC FILAMENTS OF CLEAVING EGGS: A STRUCTURAL ELEMENT CORRESPONDING TO THE CONTRACTILE RING

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ABSTRACT

A sheath consisting of filaments 50–70 Å in diameter has been demonstrated in association with the expanded, leading margins of the cleavage furrow in unilaterally and symmetrically cleaving eggs of a jellyfish and a polychaete worm, respectively. The observations suggest that the filament system might provide a structural basis for the existence of the contractile gel that, according to a hypothesis by Marsland and Landau, accomplishes cleavage. The filamentous sheath is present only in the furrow region and is arranged in an arcuate manner in unilaterally cleaving eggs and circumferentially in symmetrical cleavage. The filaments appear to be of finite length, and a number of them must overlap to span the length of the furrow. Contraction may be accomplished if the filaments slide relative to each other. However, contraction per se was experimentally not demonstrated in the studied systems. The disappearance of microvilli and the merocrine type secretion of mucoid droplets at the interdigitating or “spinning” membrane region of unilateral cleavage suggest that the unfolding of a pleated membrane and the insertion of intracytoplasmic membranes might contribute, at least in part, to the necessary extra cell membrane.

INTRODUCTION

A prevailing hypothesis (24) “assumes that the furrowing potency in animal cells depends on the structural state (and hence contractile capacity) of the gelled cortical cytoplasm in the furrow region.” Although alternate hypotheses have been proposed by other authors (11, 21, 32, 41, 49), this “contractile ring hypothesis” still accounts most satisfactorily for most experimental data and theoretical considerations concerning furrowing. More recently a number of other experiments have emphasized the significance of the egg cortex in furrowing (33–35), while others have demonstrated that disruption or replacement of the endoplasm had no deleterious effect on it (8, 15).

One of the more serious objections to the hypothesis formulated by Marsland and Landau

has been that initial fine structure studies failed to locate any structural component that would have corresponded to the contractile ring, other than a particle-free, homogeneous zone (12, 28) or an amorphous cortical cytoplasmic layer (1, 37). By contrast, contractile processes and movement in living cells have usually been attributed to proteinaceous cytoplasmic filaments, which presumably contract when exposed to appropriate chemical environment (39). In various morphogenetic movements and in the sudden cell shape changes of *Ascidian* metamorphosis (3, 4, 9, 46), oriented cytoplasmic filaments have been implicated by fine structural analyses. The retraction of the pigment mass in the chromatophore organ of squids also is apparently accomplished by fila-

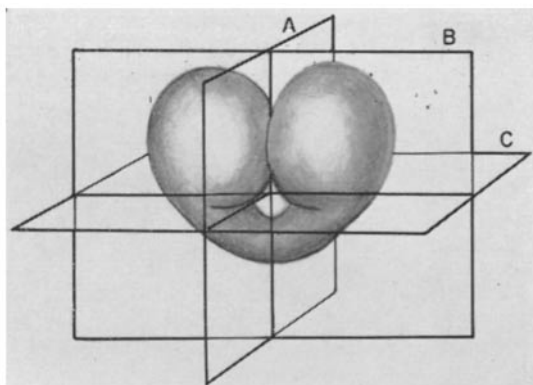


FIGURE 1 A schematic drawing of the unilaterally cleaving *Aequorea* egg. Plane *A* is passing exactly through the plane of the furrow and is designated median furrow plane. Plane *C* is designated as the "frontal plane" when sections are cut perpendicular to the animal-vegetal pole axis. Plane *B* or the longitudinal plane represents sections cut parallel to the long axis of the spindle apparatus.

ments composing the cytoelastic sacculus (10). Oriented intracytoplasmic filaments are reportedly responsible for maintenance of over-all cell form and dynamic behavior (7, 29, 47, 48). The locomotor pseudopods, spikelike "acanthopodia" and surface microprojections of *Acanthamoeba castellanii* contain fine fibrillar material (5) as do the pseudopods of *Diffugia corona* (51).

This report expands upon preliminary communications (42, 43) on the existence of an oriented filament system related specifically to the advancing cleavage furrow in symmetrically cleaving eggs of *Armandia brevis* and unilaterally dividing eggs of *Aequorea aequorea*. Some comments also pertain to symmetrical cleavage of rat eggs. The observations suggest that a cleavage furrow filament system corresponds, at least in part, to the cortical contractile ring of Marsland and Landau (24).

MATERIALS AND METHODS

Naturally spawned eggs of the polychaete worm *Armandia brevis* (Polychaeta) and hydromedusa *Aequorea aequorea* (Cnidaria) were washed briefly in filtered sea water and inseminated with a dilute suspension of motile spermatozoa. Development of the eggs was followed by phase-contrast microscopy. Batches of eggs were fixed at the slightest indication of first cleavage and in various phases during cleavage. The fixative employed was a 3% glutaraldehyde solu-

tion in 0.125 M cacodylate buffer at pH 7.2 or 6.4. In the case of *Aequorea* eggs, 0.25% formaldehyde was also included in the fixation mixture. Osmolarity of the solution was adjusted to 960 milliosmols by the addition of appropriate amounts of sodium chloride. Fixed eggs were next rinsed briefly in sea water or in an isotonic solution of buffer and sodium chloride. Samples of the various cleavage stages were pooled and incubated for the localization of ATPase (30). Cleaving rat eggs were fixed at various times after mating in a mixture of 1% glutaraldehyde and 0.5% acrolein in 0.1 M cacodylate buffer. Postosmication followed in a 1% osmium tetroxide solution in isotonic cacodylate buffer : sodium chloride mixture with or without ruthenium red (23). Rapid dehydration of all the eggs in an ethyl alcohol series was followed by three changes of propylene oxide and infiltration and embedding in Epon 812 (24). The specimens were flat embedded and oriented on metal chucks for sectioning in various planes (see Figs. 1 and 2).

The section passing exactly through the plane of the furrow is designated as being in the *median furrow plane* (Plane *A*). The term *frontal plane* (Plane *C*) is used when the sections are cut at right angles to the median furrow plane and in a sequence that first grazes the vegetal pole and then progresses to the animal pole. *Longitudinal plane* (Plane *B*) refers to sections cut parallel to the long axis of the eggs and spindle apparatus and at right angles to both the above planes (i.e., in the plane of the paper in Fig. 1).

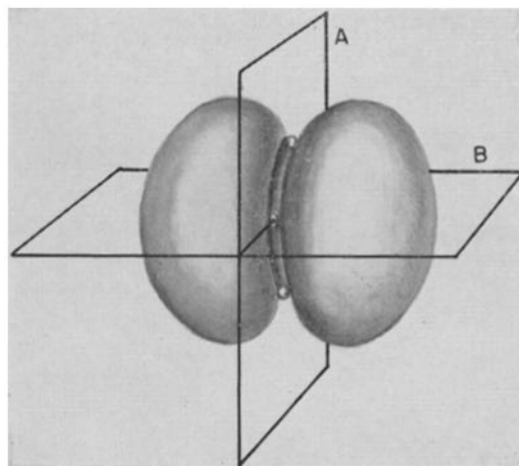


FIGURE 2 A schematic drawing of symmetrical cleavage in *Armandia* eggs. The median furrow plane and longitudinal plane are indicated as Planes *A* and *B*, respectively.

RESULTS

Eggs of *Aequorea* and *Arandia* initiate their first cleavage approximately 2 hr after insemination. The former are spawned in a pronucleate stage, while in the latter the extrusion of polar bodies is initiated only following sperm penetration (14). Because of these differences, the pronucleate eggs were used as the initial stage in these studies. There is no distinct cortical plasm in pronucleate eggs of either species, although the cytoplasm adjacent to the cell membrane usually lacks mitochondria and large yolk granules. The cnidarian egg surface is covered by microvilli with a regular distribution prior to cleavage. A bundle of thin, 50–70 Å diameter filaments projects radially into the egg cytoplasm from each microvillus (Fig. 3). At the apex a thin layer of dense material is found which is penetrated by the filaments in a fashion similar to that reported for intestinal absorptive cells (25). The length of the filaments must be from one to several microns long but cannot be determined accurately from the sectioned material. A very prominent filamentous “fuzz” layer is associated with the external leaflet of the egg cell membrane (Fig. 3), particularly when ruthenium red is utilized (Fig. 14) (17). In polychaete eggs, microvilli were irregular in distribution; no filaments were associated with them.

Asymmetrical Cleavage (Aequorea)

Phase-contrast microscopy readily reveals the beginning cleavage furrow at the animal pole of *Aequorea* eggs; shallow surface folds are observed there having an orientation roughly perpendicular to the initial furrow (Fig. 4). Such folds become much more prominent with continued furrowing (Fig. 5). Electron micrographs of randomly oriented thin sections of the furrow region disclose the following two obvious changes: (a) microvilli are lacking from the furrow, and the plasma membrane has become relatively straight; (b) associated with the inner dense layer of the cell membrane, an electron-opaque component appears, which at higher magnifications discloses a filamentous nature. When sections are cut in the median furrow plane the points discussed above are particularly clearly demonstrated and the electron-opaque layer can be resolved distinctly into filaments oriented parallel to the cell membrane and each measuring approximately 50–70 Å in di-

ameter. The filaments are limited exclusively to the advancing region of the furrow; no indication for a filament layer with similar orientation can be found at the vegetal pole or at any other part along the cell membrane of the cleaving eggs. Occasionally a shortened microvillus is found in the furrow region, and a cluster of the filaments enters it (Fig. 6).

Sections cut parallel to the median furrow plane are particularly suitable for study of the orientation and distribution of the filaments and their possible anchoring. Along the entire length of the cell membrane in the deepest portion of the furrow, filaments 50–70 Å in diameter form a layer 600–800 Å. The number of filaments (i.e., the thickness of the filamentous layer) seems to vary slightly in adjacent areas. All the 50–70 Å filaments are of uniform thickness, and no substructure or periodicity can be detected. No other cytoplasmic elements were found between the filaments.

On critical examination of the *Aequorea* egg surface in the above sections, the following three morphologically different regions are recognizable: (a) the microvillous portion over the greatest extent of the egg surface (this area basically resembles the whole egg surface prior to cleavage); (b) the “smooth” membrane of the furrow, nearly lacking microvilli; (c) a transitional region between the preceding two. The transitional region possesses large membrane folds, each of which may carry several typical microvilli and their filaments (Figs. 7 and 8). The filamentous layer is reduced in thickness in the transitional region, while in the microvillous region it is lacking. Here small numbers of filaments bend sharply and enter individual microvilli (Fig. 8, *insert*).

The plasma membrane within the furrow, lacking microvilli is slightly undulating and is nearly smooth. Where the membranes of the future blastomeres come into close proximity, they interdigitate in a complex manner (Fig. 9). A number of straight or slightly curved filament bundles are seen in the cytoplasm adjacent to the lateral walls of the furrow, near the interdigitating membrane portion. They usually project from the occasional, small microvilli of the area (Figs. 6 and 10) toward the advancing margin of the furrow.

Groups of microtubules are always found in proximity to and frequently abutting directly on the filamentous layer in the furrow (Fig. 6). They are seen in cross-sectional profiles in sections cut

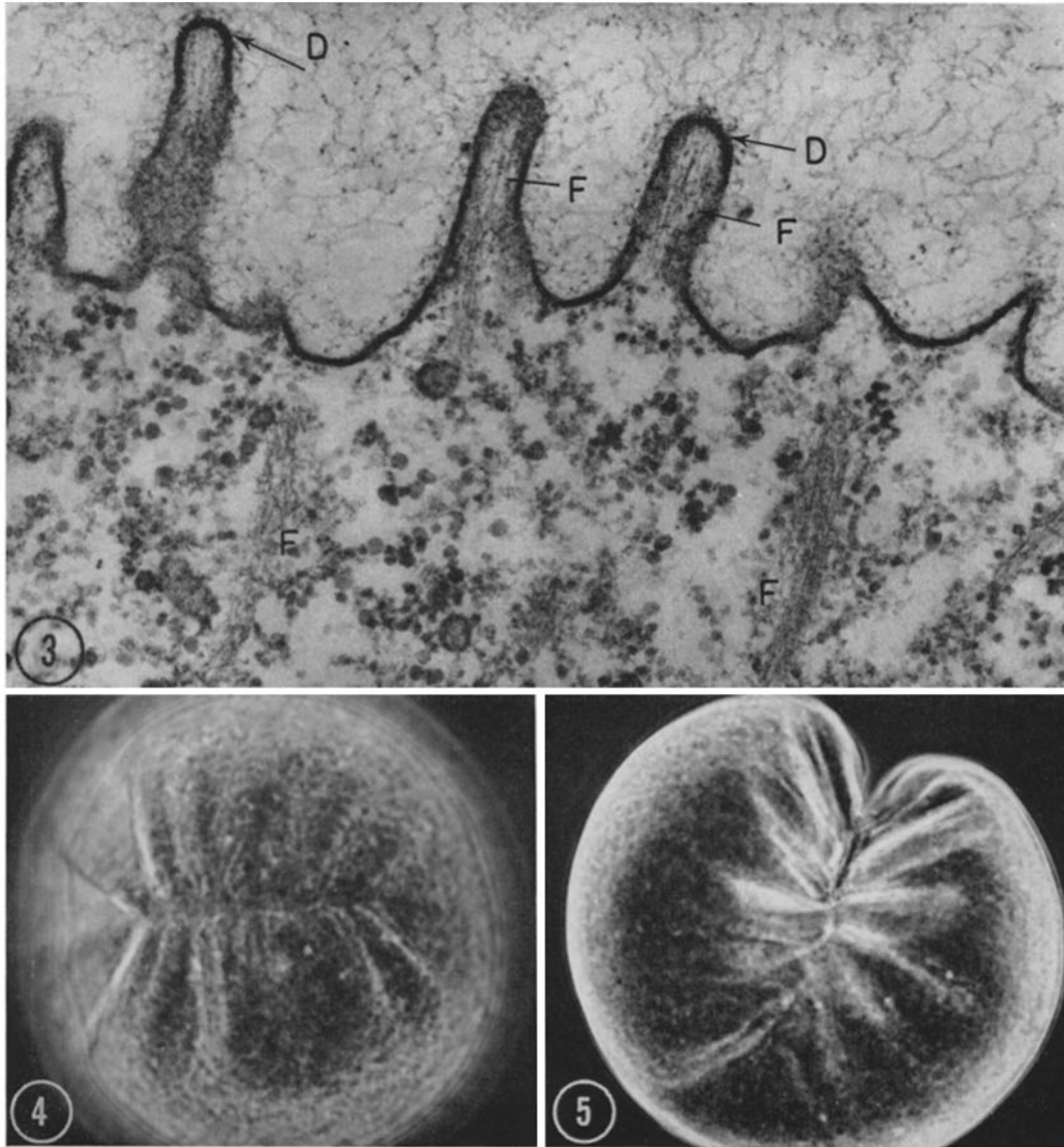


FIGURE 3 Regularly spaced microvilli with a filamentous core (F) cover the surface of *Aequorea* eggs prior to initiation of cleavage. A thin electron-opaque layer is at the apices of the microvilli (D). $\times 63,000$.

FIGURES 4 and 5 An early stage and a more advanced cleavage stage of *Aequorea* eggs observed in phase contrast. Large surface folds project perpendicular or in a slight angle toward the furrow. $\times 70$.

along the median furrow plane. The tubules are embedded in an electron-opaque matrix, and their number per group varies. They are elements of the continuous fibers of the spindle apparatus and represent portions of the forming mid-body (17).

Frontal sections were also taken from *Aequorea* eggs. However, the furrow is curved and, hence, when its most peripheral portions are sectioned, the filament layer is cut quite obliquely. In sections which include the furrow center, a larger segment of the furrow is contained in the same

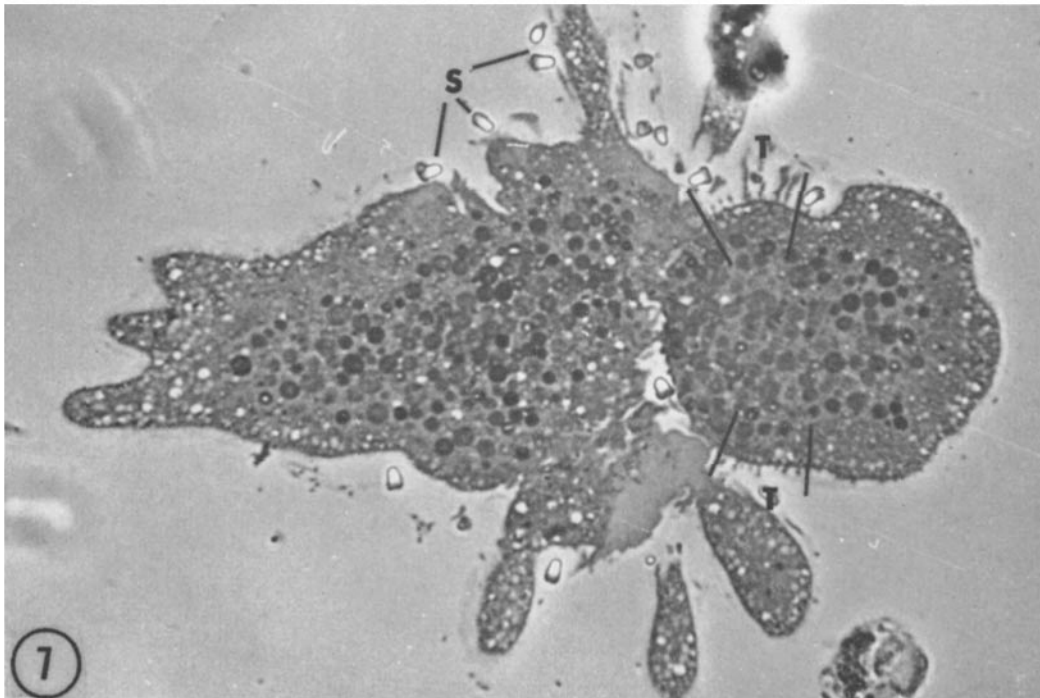
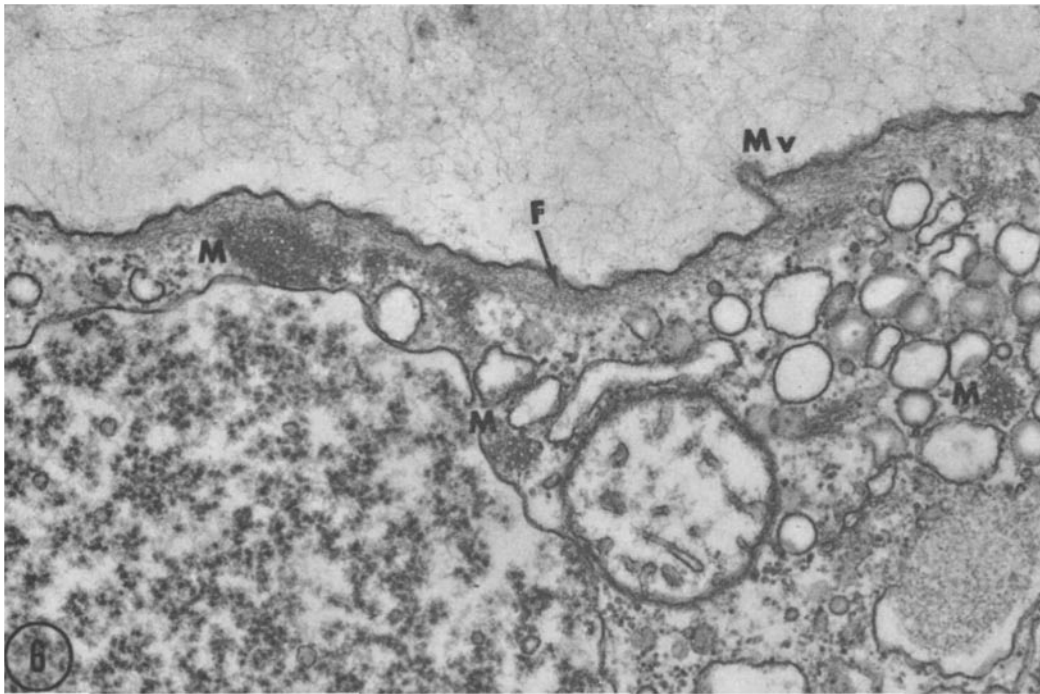


FIGURE 6 In the median furrow plane of an *Aequorea* egg the cell membrane is undulating and lacks most microvilli. Filaments 50–70 Å in diameter (*F*) are oriented parallel to the cell membrane. The filaments seem to course into the remaining, sole microvillus (*Mv*). Small clusters of microtubules are sectioned transversely, which represent elements of the mid-body (*M*). $\times 34,000$.

FIGURE 7 A phase-contrast micrograph of a $0.5\ \mu$ thick section, stained with Richardson's dye, close to the median furrow plane of an *Aequorea* egg depicts the cytoplasmic stalk between the forming blastomeres and a portion of one of the blastomeres. A large number of extranumerary spermatozoa (*S*) are visible in this plane of section. The transitional region of the egg surface is demarcated (*T*). $\times 950$.

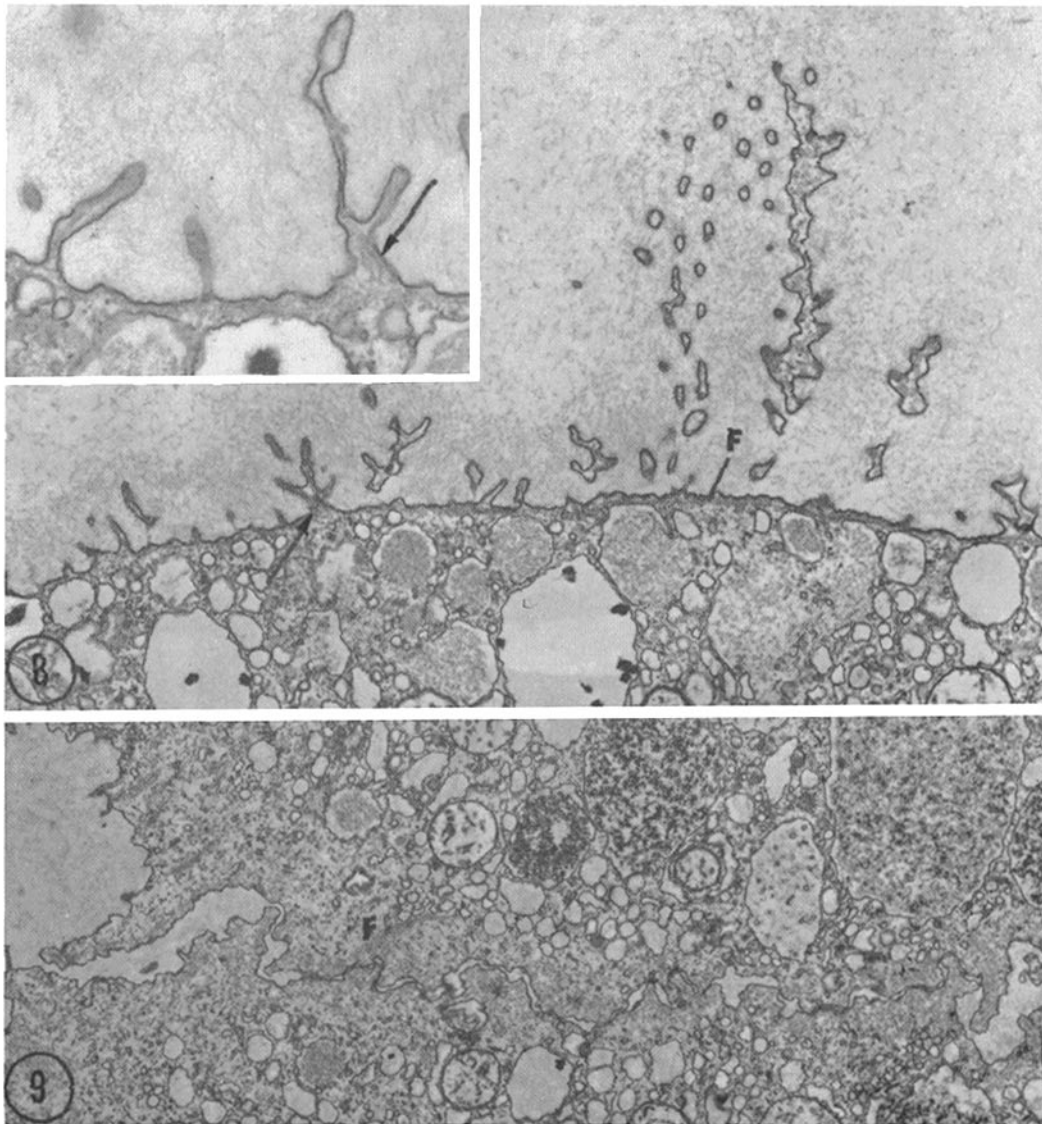


FIGURE 8 An electron micrograph of the transitional region of *Aequorea* egg surface demonstrates the microvillous portion to the left, irregular small folds more centrally, and large membrane folds to the right. The longitudinally oriented components of the filament system (*F*) become less pronounced toward the left. Few filaments seem to bend and course into the microvilli (arrow). $\times 12,000$; *insert*. $\times 38,000$.

FIGURE 9 The cell membranes of the forming blastomeres interdigitate in an intricate manner in a frontal section (*Aequorea*). Occasionally a bundle of microfilaments (*F*) courses close to the cell membrane. $\times 10,500$.

plane, and in the cytoplasm, adjacent and parallel to the cell membrane, a band of 50–70 Å filaments are observed. The width of the band is oriented in a plane perpendicular to the direction of the

advancing furrow. Such orientation would be expected from observations made on serial sections parallel to the median furrow plane or from longitudinal sections (Fig. 12). The floor of the

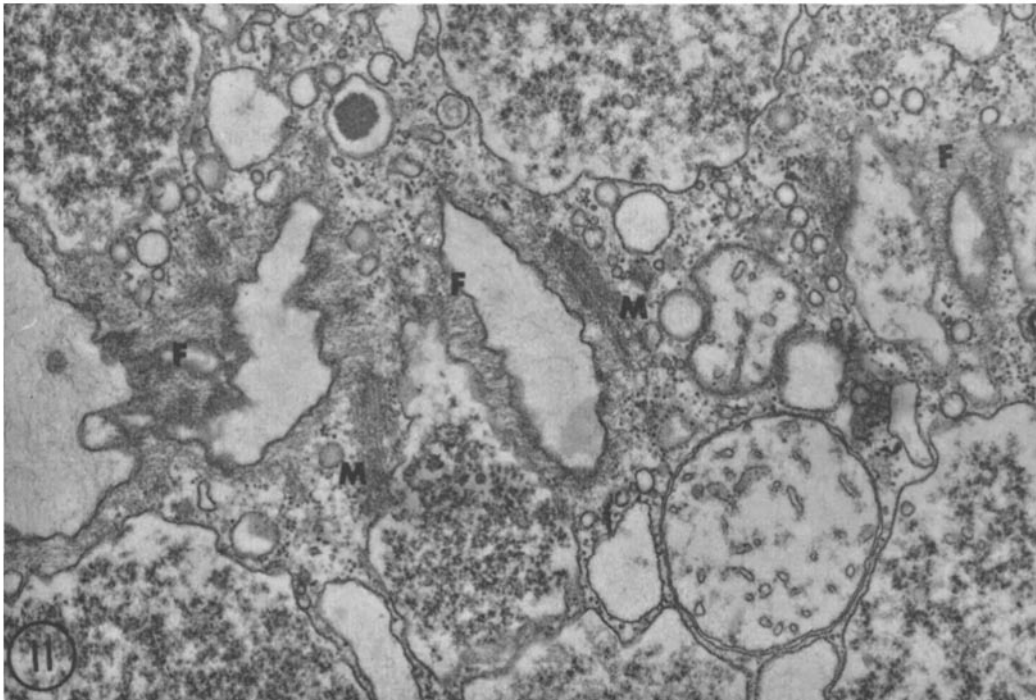
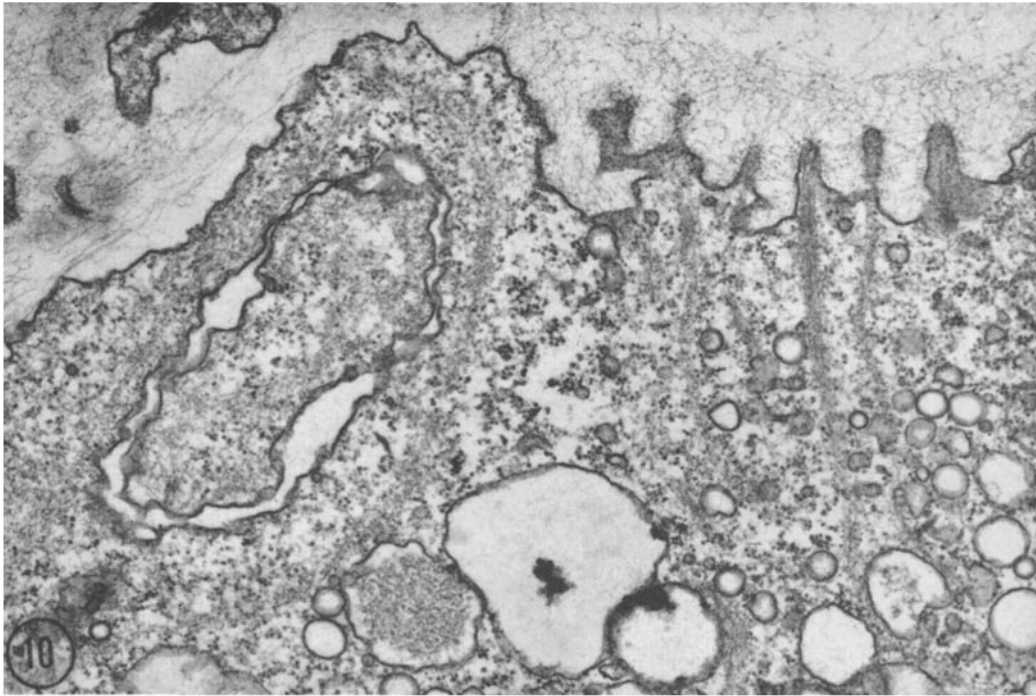


FIGURE 10 On the lateral surface of the furrow wall occasionally few microvilli remain. Bundles of microfilaments regularly course into them. The filaments project toward the leading margin of the furrow. $\times 25,500$.

FIGURE 11 A frontal section approximating the leading margin of the furrow in an *Aequorea* egg demonstrates the longitudinal orientation of filaments (*F*) within the filamentous sheath parallel to the margin. Perpendicular to the filaments are seen small groups of microtubules, components of the forming mid-body (*M*). $\times 29,000$.

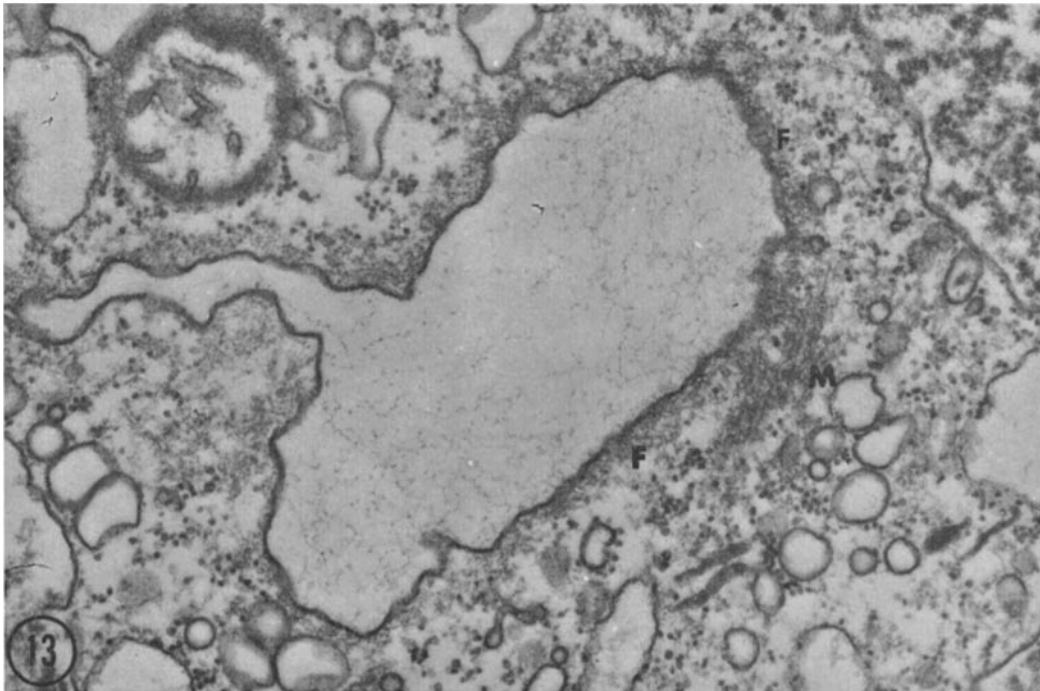
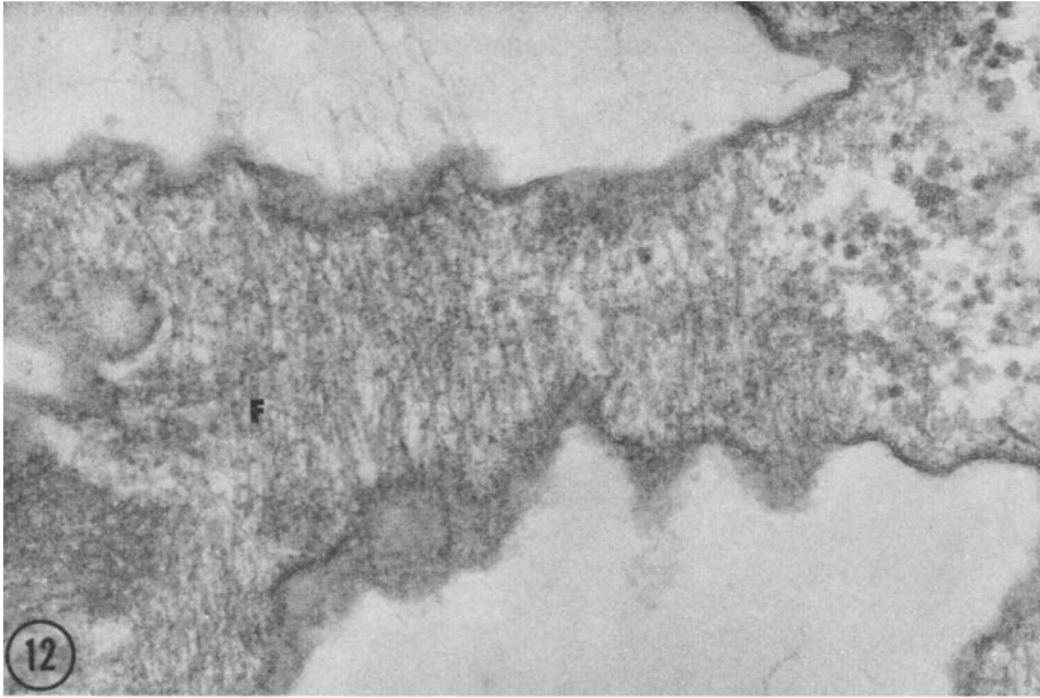


FIGURE 12 The thin filaments (*F*) are clearly recognized in frontal sections subjacent to the egg cell membrane. $\times 109,000$.

FIGURE 13 The cell membranes of the forming blastomeres are in regions close together in an advanced furrow. Longitudinal sections demonstrate that the leading margin is enlarged while cross to obliquely cut profiles of the components of the filament system (*F*) outline the enlarged margin. Microtubule components of forming mid-body, *M*. $\times 44,000$.

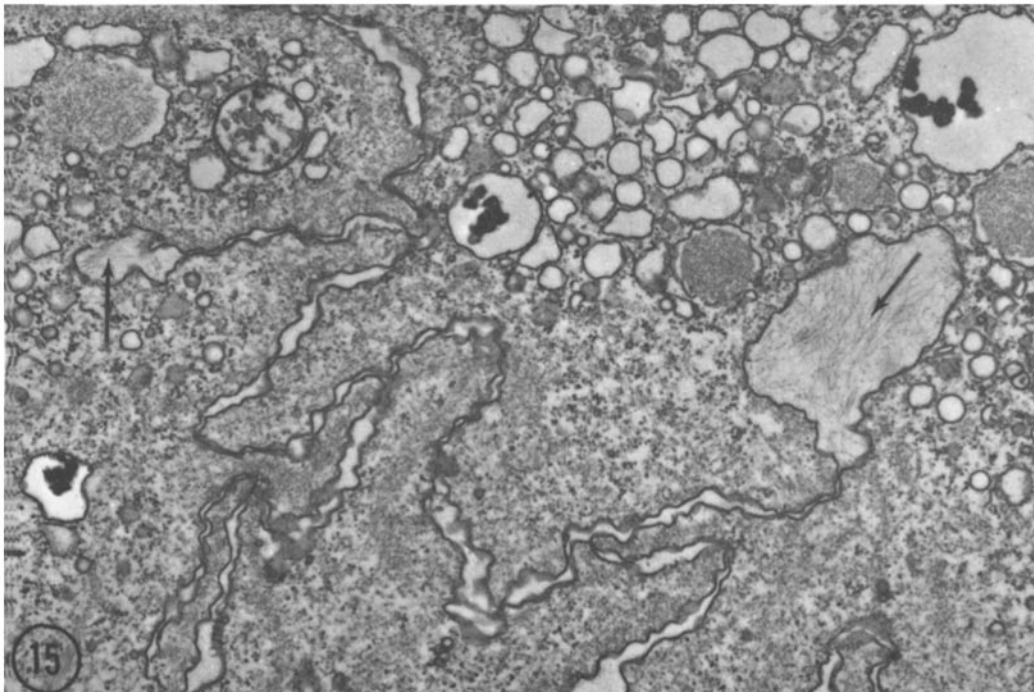
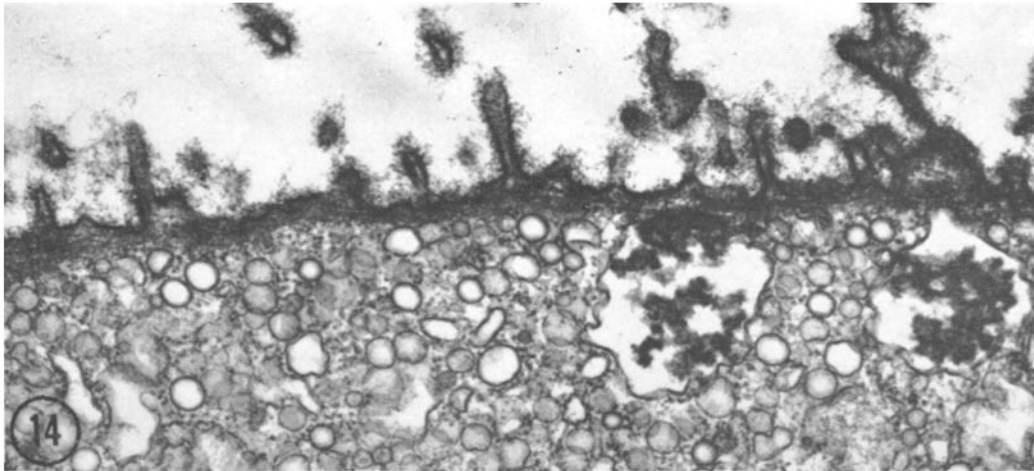


FIGURE 14 Ruthenium red stains a thin cytoplasmic matrix surrounding the filamentous complex in the transitional region of *Aequorea* eggs. The extracellular "fuzz" stains also more intensely with this dye. $\times 27,500$.

FIGURE 15 In the interdigitating region of the forming *Aequorea* blastomeres the membranes invaginate at various points. Near the base of these invaginations filamentous material is found in the extracellular space (arrows). $\times 17,000$.

furrow is slightly undulating, and therefore, a section may pass several times in and out of the extracellular space (Fig. 11). At each point, just entering the cytoplasm, the oriented, parallel

filaments of the band can be seen to advantage (Fig. 11). Often the cell membrane of the furrow is cut tangentially, and the filaments are displayed as they overlie it. Stereo electron micrographic

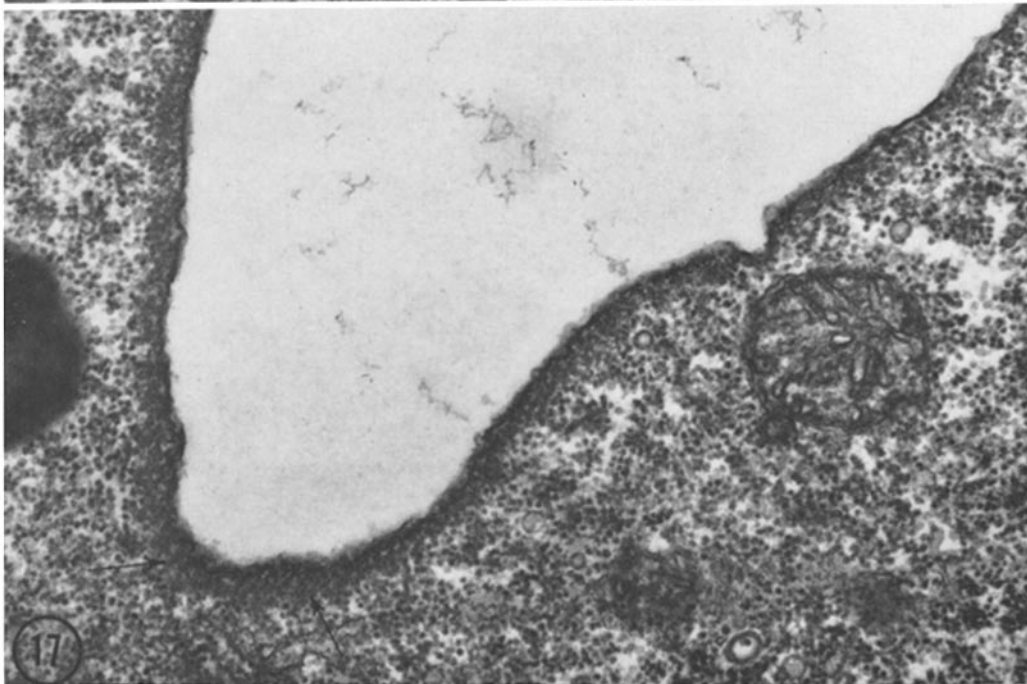
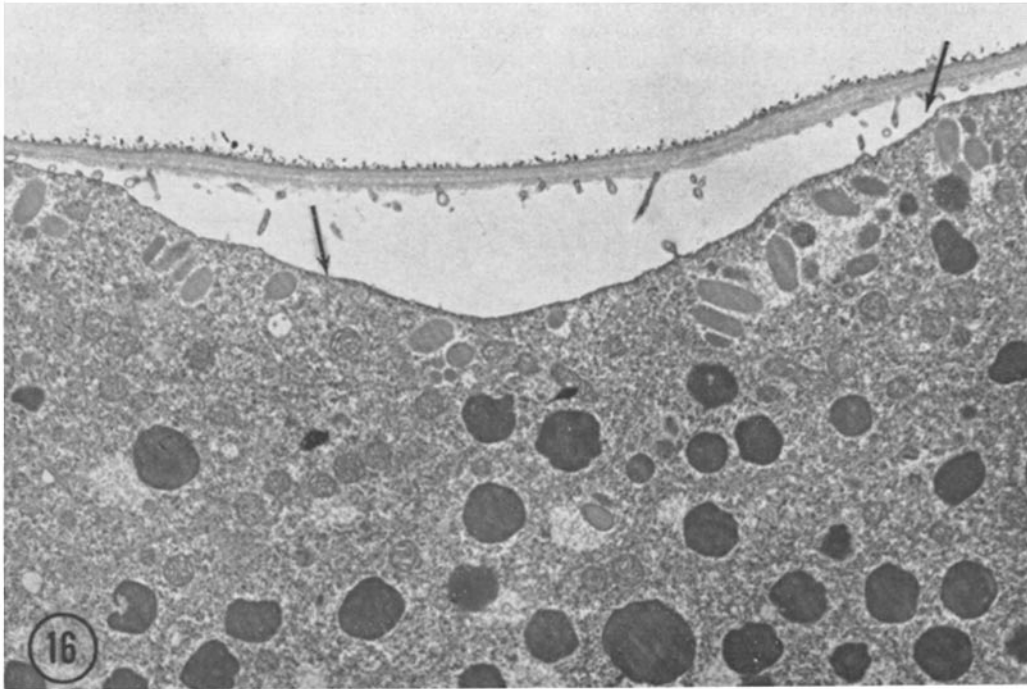


FIGURE 16 Longitudinal section of an *Armandia* egg in early cleavage stage. A broad band of electron-opaque layer is seen in the equatorial region (arrows). $\times 8,500$.

FIGURE 17 In more advanced furrow of *Armandia* egg cut longitudinally, the electron-opaque material can be seen along the infolding membrane portion. This layer is composed of obliquely cut thin filaments (arrows). $\times 38,000$.

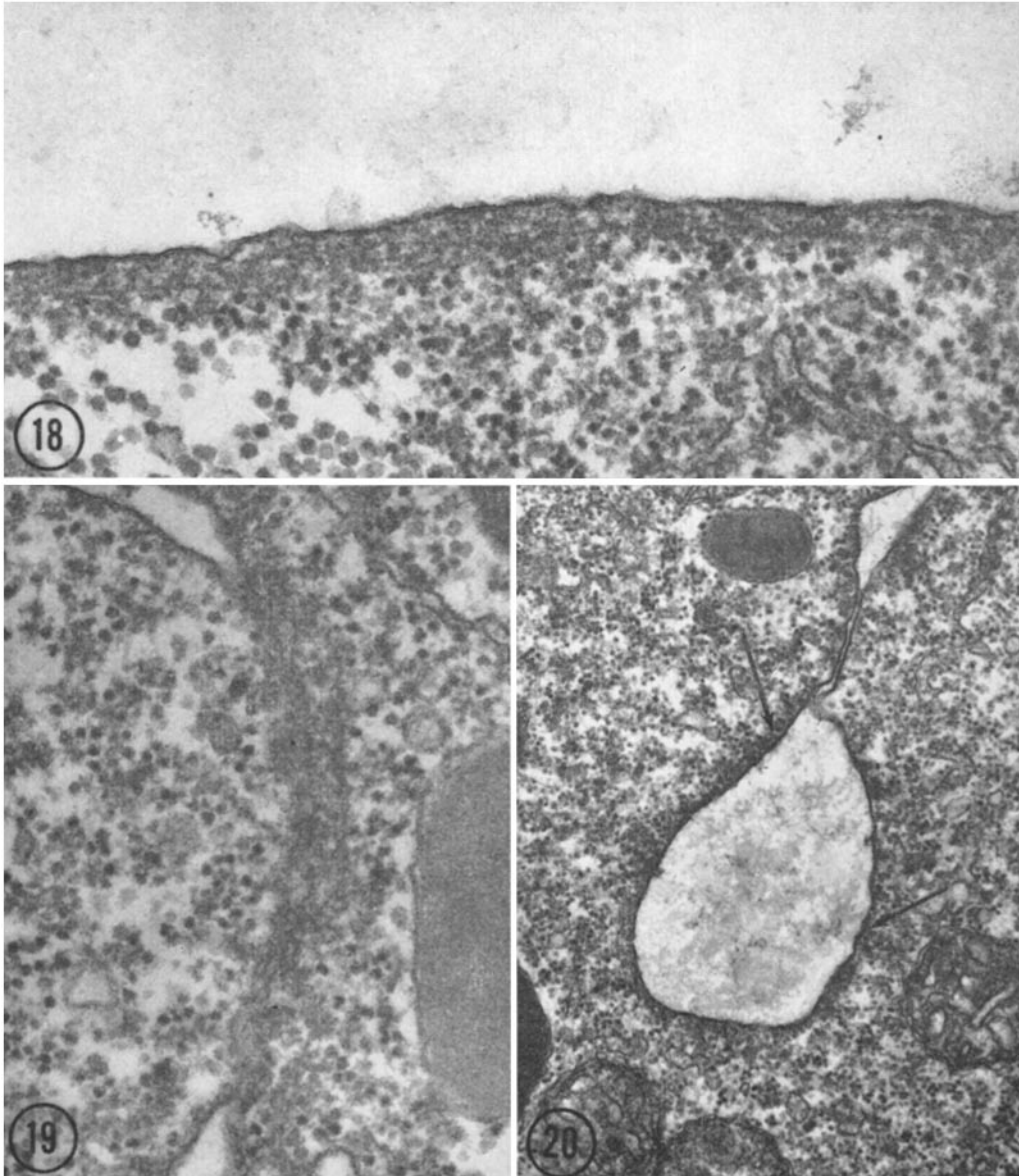


FIGURE 18 Longitudinal filament profiles are seen in sections cut in the median furrow plane of *Armandia* eggs. $\times 78,000$.

FIGURE 19 The base of a cytoplasmic bleb in the furrow appears to be spanned by filaments, but no filaments are found inside the bleb. $\times 74,000$.

FIGURE 20 The leading margin of the furrow is broad. The electron-opaque margin is associated only with this expanded area (arrows). $\times 34,000$.

analyses of such images gave no indication of an attachment mechanism between the filaments and cell membrane, other than a sparse amount of electron-opaque matrix among the filaments and

between them and the cell membrane. This matrix has a slightly greater electron opacity in eggs stained with ruthenium red, indicating that it could be an adhesive acid mucopolysaccharide

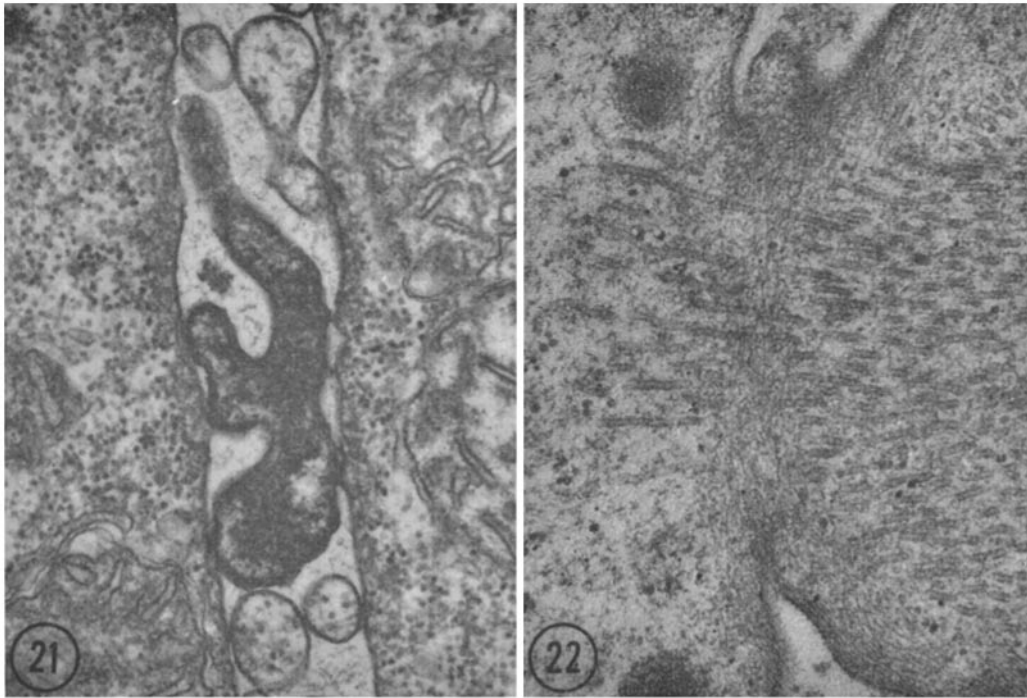


FIGURE 21 The mid-body becomes entrapped in a membrane-bounded vesicle between the two blastomeres upon completion of cytokinesis in *Armandia* eggs. $\times 50,000$.

FIGURE 22 Thin filaments form a band in the furrow plane between two forming blastomeres in a rat egg. $\times 34,000$.

(Fig. 14). Notably, the filament complex gave a negative reaction for ATPase.

Microtubules that are embedded in an electron-opaque matrix and which constitute the mid-body are also cut longitudinally in these sections, but they are oriented perpendicularly to the long axis of the filaments (Fig. 11, 13).

Longitudinal sections of the unilaterally cleaving *Aequorea* eggs demonstrate clearly that the filaments form a continuous sheath enwrapping the expanded, leading margin of the furrow. The filaments appear as 50 Å punctate components when sectioned transversely or as short filaments when cut slightly obliquely (Fig. 12).

In the interdigitation region of the furrow, where the surfaces of the future blastomeres come into contact, invaginating cavities can be seen (Fig. 15). Within the extracellular spaces a filamentous material is found, particularly near the base of the invaginations. Similar material occupies other regions of the furrow even where the membranes are quite closely apposed. In the cyto-

plasm near such invaginating cavities, membrane-bounded granules 100–300 m μ with tightly packed filamentous contents are found. The granule contents are seemingly secreted by exocytosis into the extracellular space. In the furrow region extranumerary spermatozoa are preferentially entrapped within the extracellular filamentous material, the only region of the egg surface where such secretory activity occurs (Fig. 7).

Symmetrical Cleavage (Armandia and Rat)

Eggs of the polychaete worm *Armandia brevis* divide symmetrically (14), becoming dumbbell-shaped during the process. In longitudinal sections a thin electron-opaque layer can be seen occupying a broad band around the equatorial region and closely associated with the egg cell membrane. It is thinner toward the spindle poles and somewhat thicker at the equator (Fig. 16), but is symmetrically distributed in cross-section at every point. As the furrow proceeds, this layer becomes

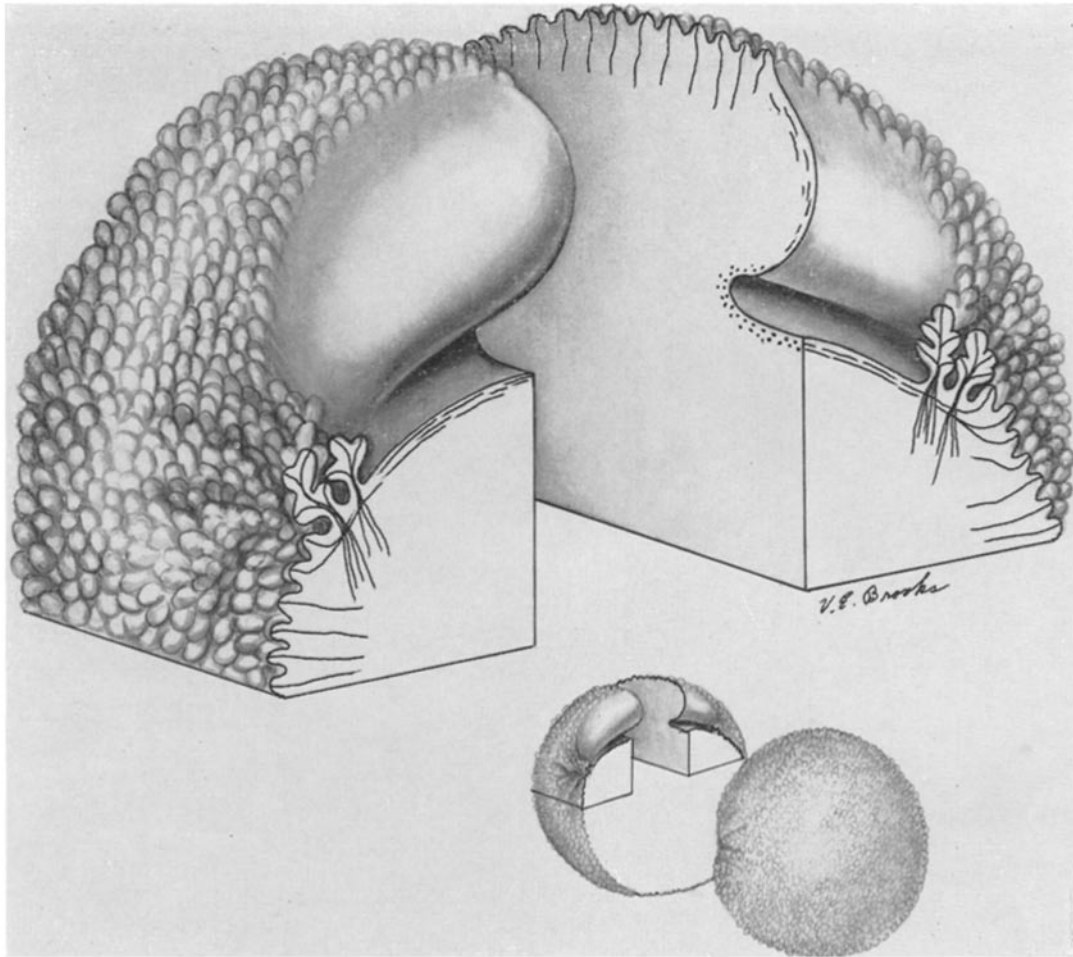


FIGURE 23 A schematic representation of the arrangement of the filament system in the unilaterally cleaving *Acquorea* egg.

limited to the infolding portion of the membrane. Short, obliquely cut filaments are detectable within the electron-opaque layer at higher magnifications (Fig. 17, arrows). In sections cut in the median furrow plane, the band is oriented circumferentially along the cell membrane, with the long axis of the filaments oriented parallel to the median furrow plane (Fig. 18). Measurement of the filaments cannot be made accurately because they are embedded in an electron-opaque matrix, but some appear to be about 50–70 Å in diameter. Thicker filaments appearing to be 80–100 Å in diameter have also been observed occasionally, but it cannot yet be stated unequivocally that two filament populations are present in the furrow of

Armandia eggs. The larger dimension might represent some material deposited onto the surface of smaller filaments during the preparatory procedures or may be due to overlap of adjacent filaments in the thickness of the section.

Cytoplasmic blebs were frequently found along the furrow. Filaments appear to span the bases of these blebs but do not accompany the cell membrane into the blebs themselves (Fig. 19).

When the furrow is more complete, its leading edge is broad rather than sharp or angular (Fig. 20). The dense substance enclosing the filaments (which are cut in cross-section and would be visible at best as small dots) is distinctly associated only with this expanded area of the furrow mem-

brane. Cell membranes of the forming blastomeres become closely associated and parallel more peripherally, and small condensations of electron-opaque material occupy positions along them. These areas comprise junctional complexes similar to focal intermediate junctions (19).

During the last stages of furrowing, the mid-body becomes compressed by a final circle of cell membrane formed by the advancing furrow. Upon completion of cytokinesis the mid-body apparently becomes entrapped in a membrane-bounded cytoplasmic remnant isolated between the blastomeres (Fig. 21). A similar observation has been made at the first cleavage of *Limnaea* eggs (W. Berendsen, 1968 unpublished observations) and in mitotic erythroblasts of rat liver (18).

In *Armandia* the second cleavage originates nearly synchronously in both daughter blastomeres. Initially this cleavage appears to be unilateral, advancing from the periphery toward the first cleavage plane. But after a brief delay, furrowing also starts from the more central surface of each blastomere. Likewise the equatorial cortical filament system and its dense matrix is first seen peripherally and later centrally.

Cleavage divisions in rat eggs are also symmetrical. In grazing sections of the furrow membrane, thin filaments measuring 50–70 Å in diameter are found (Fig. 22). The filaments seem to be oriented along the furrow in a circumferential manner identical to that seen in *Armandia* eggs.

DISCUSSION

These observations on symmetrical and unilateral cleavages indicate that, in each instance when there was any morphological indication for furrowing, 50–70 Å filaments were detected in close proximity to the egg cell membrane with their long axes oriented in the plane of the furrow. The oriented filaments were present only in that region of the egg surface where furrowing had already started. From the evidence presented, it appears that the leading, expanded margin of the furrow is enwrapped by a sheath or ribbon composed of these 50–70 Å filaments. The position of the filament system corresponds well to the postulated location of a cortical contractile gel by Marsland and Landau (24), while the individual filaments demonstrated in this study may represent the important structural components of that gel.

The contractile ring hypothesis has been greatly

strengthened by experimental work demonstrating that a furrowing egg reverted to a spheroidal shape when cuts were made through the base of the furrow (33). By the use of connective tissue stains a stratification was demonstrated in the sea urchin egg cortex (20) that may correspond to a dense layer observed, in early electron microscope studies (26), to be parallel with the surface of the egg and to have a distribution similar to that of the postulated contractile gel. Studies with the electron microscope have more recently indicated the existence of thin filaments immediately beneath the cell membrane at the cleaving furrow of squid (2), *Limnaea* (W. Berendsen, 1968 unpublished observations), sea urchin (13, 45), and a jellyfish (40). In the only published report with accompanying electron micrographs (40), the filaments were interpreted to “constitute the fine structural manifestation of the ‘contractile gel.’” Very likely, the dense layer associated with the furrow in electron micrographs of mitotic cells represents the same structural component (37, 40) even though the individual filaments were not resolved.

The origin of filaments is difficult to determine in the symmetrically cleaving *Armandia* and rat eggs. Prior to cleavage no filaments can be observed in any part of the egg cortex. By contrast, nearly the entire surface of *Aequorea* eggs is covered with microvilli, each with a bundle of filaments projecting for several microns into the cytoplasm. At the apex of these microvilli a thin layer of electron-opaque material is found which could act as an anchoring device for the filaments, as had been suggested for microvilli of intestinal absorptive cells (25).

Individual filaments that originally projected radially as bundles into the egg cytoplasm from the microvilli may well appear to be redistributed and may interact to establish the filamentous sheath of the median furrow plane. The thickness of the sheath in different parts of the furrow varies slightly, which may reflect regional random interaction of filament bundles from microvilli. However, the overall thickness of the sheath does not vary significantly between early, mid, and late cleavage stages. This suggests that as cleavage progresses some filaments may dissociate from the sheath. The filament bundles found in the granule-free cytoplasm close to the furrow membrane of the forming blastomeres, where the membranes interdigitate and lack microvilli, may represent

those filaments that are disassociating from the sheath, having completed their role earlier in furrowing activity. The filaments still seem to be attached to the cell membrane even though no dense matrix, typical of attachment sites, was seen in those regions. The filaments found in the microvilli of *Aequorea* eggs may be short, terminating in the egg cytoplasm a few microns from the cell membrane. From studies of thin sections it cannot be excluded, however, that these filaments may be long, looping from microvillus to cytoplasm and back again a number of times. The assumption can be made that the filaments are of finite lengths and that they overlap to constitute the necessary length in the furrow.

The filaments are distributed circumferentially along the entire perimeter of the furrow in symmetrically cleaving eggs while they are arranged in an arcuate manner in the unilateral (also referred to as "heart-shaped" (11)) cleavage, and this is expected from theoretical considerations. Fig. 23 represents diagrammatically the arrangement of the oriented filament system at the leading margin of the furrow and demonstrates the relationship of the filaments to the egg *in toto* and its microvilli.

The consistent association of the oriented filaments organized into a sheath surrounding and parallel to the advancing margin of the furrow suggests that, in both symmetrical and unilateral egg types, furrowing occurs via an interaction of filaments and the cell membrane. The interaction apparently manifests itself as a contraction, as originally proposed by Marsland and Landau (24), but the mechanism of contraction cannot be determined from present studies. There are no obvious supercoiled or thickened segments of the filaments and, therefore, some kind of a sliding model would seem preferred even though no lateral bridges were observed on the filaments. From available electron micrographs a decision cannot be made whether continuous filaments extend along the entire furrow in the unilaterally cleaving eggs or whether individual filaments form continuous rings in the symmetrically cleaving egg.

Experimentally, no proof exists for contraction *per se* in the furrow of the eggs observed in these studies. A contraction was demonstrated in other eggs (2, 36), and the force exerted by the contraction of the cortical gel was measured on a calibrated needle introduced into sea urchin eggs

(34). ATPase activity could not be demonstrated in association with the filamentous sheath in these experiments on the light and electron microscope level of cytochemistry. This finding is in accord with those of Sakai (38) who extracted from fertilized eggs a protein in which contraction can be induced by oxidation of —SH bonds but not by ATP.

Contraction can be inferred indirectly from observations on *Aequorea* eggs. The surface folds observed at the animal pole by phase-contrast microscopy imitate a partially pulled purse-string effect in precisely that portion of the egg where the oriented filaments are found adjacent to the furrow membrane. The folds can be thought of as the expression of contractile forces of the filamentous sheath exerted onto the advancing margin of the furrow. Disappearance of microvilli in the furrow region probably occurs in response to forces translated from the filament system to the cell membrane. This interpretation is supported by recent experiments (O. Brunser, and J. H. Luft; submitted for publication 1969) in which microvilli disappeared completely or partially from the luminal surface of absorptive cells lining an inflated segment of intestine. The filamentous core material was still recognizable in the apical cytoplasm of the cells, but the filaments were reoriented more or less parallel to the cell surface. In some cells, on which the stress apparently was less, few shortened, irregularly oriented microvilli remained with otherwise little structural alterations. The undulating membrane of the cleavage furrow and the occasional shortened microvilli with approaching or inserting filament bundles are quite similar to these partially or fully stretched regions of intestinal epithelium.

If any force is to be translated onto the cell membrane, it is obligatory that the filaments adhere in some manner to the inner surface of the plasma membrane. The dense matrix embedding the filaments could represent such an adhesive material. In *Armandia* eggs the existence of an electron-opaque matrix embedding the filaments is more prominent than in *Aequorea* eggs, but after ruthenium red treatment the matrix in the latter also appears densely staining. Because the matrix stains with ruthenium red (23), it could be an acid mucopolysaccharide that acts as an excellent glue under certain conditions. The attachment would not have to be along the entire length of the filaments. Cytoplasmic blebs

and large folds may be the result of the contraction of the system of filaments which are alternately anchored firmly and less firmly as they course along the cell membrane. The orifices of the blebs in *Armandia* eggs are indeed spanned by filaments while no filaments are found inside the blebs. The large membrane folds of the transitional portion of the furrow in *Aequorea* possess microvilli. Their base is also spanned by several filaments. Such membrane folds could arise if filaments from more peripheral microvilli became involved first in the filamentous sheath associated with the furrow, and if those filaments contracted, the plasma membrane would buckle in the intervening region where the filaments have not interacted with the forming filament sheath. The membrane fold would vary in extent depending on the number of microvilli whose filaments failed to participate in the sheath.

In *Aequorea* eggs the filaments of the filamentous sheath associated with the cleavage furrow seemed anchored in the microvilli at the periphery of the furrow. They bend sharply as they enter the cortical cytoplasm and probably remain attached via some adhesive material (possibly the same material found at the apexes of microvilli) to the cell membrane even after the disappearance of microvilli. The number of filaments in the sheath is reduced peripherally in the furrow as expected, if they terminated in microvilli scattered over a large surface area.

Filament systems of similar dimensions have been implicated in a variety of contractile processes, cell dynamics, and in maintenance or change in cell shapes (3-5, 7, 9, 10, 29, 46-48). Cleavage could be considered as one form of cell shape change. If cleavage of eggs and cytokinesis as a general phenomenon take place by similar mechanisms, an equatorial filament system should be found at least during division in every cell capable of division. Examination of a variety of published reports supports this assumption even though in some the emphasis and interpretation differed or were not considered. In sea urchin eggs (26), dividing HeLa cells (37), grasshopper spermatocytes (6, 44), liver parenchymal cells (12), embryonic chick cells (1), and rat thymocytes (28), either densities in association with the cell membrane of the equatorial region or a rim of granule-free, homogeneous cortical material were described. In dividing somatic cells, filaments were demonstrated by Dales (1965, pub-

lished in Ham's *Histology*, J. B. Lippincott, Philadelphia and Montreal, 5th edition), that were purported to accomplish cytoplasmic division and to be of contractile nature.

The postulate has generally been accepted that during cleavage the volume of forming daughter cells stays constant but that there is a concomitant 26-28% surface increase (31, 50). It cannot be decided, from the experimental evidence presented thus far, whether this surface increase might arise by stretching of the existing membrane, by insertion of new material, or by unfolding of a pleated membrane. No dimensional change of the cell membrane is obvious during cleavage, which would be expected if the surface increase were to be accomplished by stretching. The disappearance of microvilli in the region of the furrow is most compatible with the suggestion of the unfolding of a pleated membrane, the forces acting upon the cell membrane bringing about the incorporation of the membranes of microvilli into the total egg surface. The tortuous or interdigitating regions of the membrane in the furrow, referred to as the "spinning" membranes by Dan (11), are possibly an expression of the insertion of new membrane into the furrow. The presence of a flocculent to faintly filamentous material in the extracellular spaces of the same region indicates that a merocrine-type secretion or exocytosis of mucoids may accompany furrowing. In fact, extranumerary spermatozoa are commonly found, apparently entrapped, exclusively within the extracellular mucoids in this area. Exocytosis is, of course, one mechanism by which membrane may be added. The secretion of mucus during cleavage has also been observed in sea urchin eggs by Motomura (27) by means of histochemical techniques.

Addendum

Two papers were published recently reporting that thin filaments appear in the cytoplasmic cortex during naturally-occurring or artificially-induced cleavage. The major findings in these papers are in agreement with the findings of the current report:

ARNOLD, J. M. 1969. Cleavage furrowing formation in a telolecithal egg (*Loligo pealii*). I. Filaments in early furrow formation. *J. Cell Biol.* 41:894.

TILNEY, L. G., and D. MARS LAND. 1969. A fine structural analysis of cleavage induction and furrowing in the eggs of *Arbacia punctulata*. *J. Cell Biol.* 42:170.

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