CHANGES IN CELL SURFACE AND CORTICAL CYTOPLASMIC ORGANIZATION DURING EARLY EMBRYOGENESIS IN THE PREIMPLANTATION MOUSE EMBRYO

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ABSTRACT

Membrane topography and organization of cortical cytoskeletal elements and organelles during early embryogenesis of the mouse have been studied by transmission and scanning electron microscopy with improved cellular preservation. At the four- and early eight-cell stages, blastomeres are round, and scanning electron microscopy shows a uniform distribution of microvilli over the cell surface. At the onset of morphogenesis, a reorganization of the blastomere surface is observed in which microvilli become restricted to an apical region and the basal zone of intercellular contact. As the blastomeres spread on each other during compaction, many microvilli remain in the basal region of imminent cell-cell contact, but few are present where the cells have completed spreading on each other. Microvilli on the surface of these embryos contain linear arrays of microfilaments with lateral cross bridges.

Microtubules and mitochondria become localized beneath the apposed cell membranes during compaction. Arrays of cortical microtubules are aligned parallel to regions of apposed membranes. During cytokinesis, microtubules become redistributed in the region of the mitotic spindle, and fewer microvilli are present on most of the cell surface. The cell surface and cortical changes initiated during compaction are the first manifestations of cell polarity in embryogenesis. These and previous findings are interpreted as evidence that cell surface changes associated with trophoblast development appear as early as the eight-cell stage. Our observations suggest that morphogenesis involves the activation of a developmental program which coordinately controls cortical cytoplasmic and cell surface organization.

During the development of the preimplantation mouse blastocyst, a major period of membrane differentiation takes place at approx. the eight-cell stage. At this time, changes occur in membrane transport systems (4, 16), surface glycoproteins (33), surface antigens (2, 30, 50), and intercellular junctions (12).

At the two- and four-cell stages, cell shape changes are limited to blastomere cleavage, whereas at the eight-cell stage morphogenetic al-

terations in the structure of the embryo are initiated by the process of compaction (12, 23, 31). Adjacent blastomeres in tangential contact are observed to flatten against one another, extending the initial points of cell-cell contact outward toward the surface of the embryo and providing the necessary appositional membrane required for the development of tight and gap junctions (12). This event is considered the initial step in the future segregation of inner and outer blastomeres (31) (presumptive inner cell mass and trophoblast) and is required for the establishment of the zonula occludens at the morula stage (11). The development of a permeability barrier mediated by tight junctions is necessary for transcellular fluid transport in the development of the blastocyst (11, 13, 15) and may help create a micro-environment necessary for the determination of presumptive inner cell mass cells (11, 20, 45).

The establishment of extensive areas of close membrane apposition and new intercellular junctions after a dramatic change in cell shape suggests that the cell surface and cortex may play an active role in this process. By exploring the preimplantation mouse embryo by transmission and scanning electron microscopy, we find that during compaction striking changes occur in the areas of the blastomere surface and cortical cytoplasm where new cell-cell contacts are established. Our results indicate that cell surface changes associated with the development of the trophoblast begin as early as the eight-cell stage.

MATERIALS AND METHODS

Embryo Collection

Female Swiss mice (Charles River Breeding Laboratories, Wilmington, Mass.) were injected with 2-5 immunizing units (IU) of pregnant mare's serum (National Institute of Arthritis, Metabolism, and Digestive Diseases, Rat Pituitary Hormone Distribution Program [NIADD]) and 2-5 IU of human chorionic gonadotropin (HCG) (Sigma Chemical Co., St. Louis, Mo.) 2 days later. The superovulated females were mated with male mice of the same species. Four- to eight-cell embryos and early morulae (approx. 8–16 cells) were collected at 56–62 and 66 h post-HCG, respectively, by flushing the oviduct and uterus with a 30-gauge syringe. Usually, 10–20 embryos were collected per female. In several cases, females were naturally mated (without prior hormonal stimulation), and their embryos were found to be identical to those obtained with hormonal stimulation.

Embryos were cultured in the defined medium of Biggers et al. (5) as previously described (12). All embryos were pooled first in medium under paraffin oil and then incubated at 37°C in a humidified atmosphere of 5% CO_2 in air.

Electron Microscopy

Embryos were either flushed from the fallopian tubes with fixative or fixed after culture in vitro. In the latter case, embryos were transferred in approx. 25 μl of culture medium to a glass dish which was flooded immediately with fixative.

In previous work on the early cleavage stages of the mouse embryo, membranes and microfilaments were not always well preserved. In this connection, we have used two methods for transmission electron microscopy which significantly improve fixation: (*a*) thiocarbohydrazide fixation and (*b*) *en bloc* staining. They may be used independently or together.

THIOCARBOHYDRAZIDE FIXATION: Embryos were fixed in 3% glutaraldehyde, 0.5% paraformaldehyde in 0.05 M phosphate buffer, pH 7.2 at 20°C for 20 min and washed in phosphate buffer. They were washed again three times in 0.1 M cacodylate buffer, pH 7.2 and incubated in 0.5% thiocarbohydrazide (Eastman Kodak no. 7372) in cacodylate buffer at 20°C for 20 min in the dark. (Just before use, the thiocarbohydrazide was solubilized by heating to 50°–60°C for 5 min with stirring and was then cooled.) Thereafter, the embryos were washed in cacodylate buffer and osmicated (1%) in cacodylate buffer at 4°C for 1 hr.

EN BLOC STAINING: Aldehyde fixation and osmication in phosphate buffer was followed by *en bloc* staining in 0.5% uranyl acetate in distilled water for 15 min at 4°C.

Subsequently, embryos were dehydrated in a graded ethanol series, and infiltrated and embedded in Epon-Araldite (1). Grey-to-silver sections were collected on 3% Formvar-coated 150-mesh grids and briefly stained in uranyl acetate (49) followed by lead (41).

For scanning electron microscopy, embryos were fixed in 3.0% glutaraldehyde and 0.5% paraformaldehyde in Hanks' balanced salt solution (Gibco 406, Grand Island Biological Co., Grand Island, N.Y.), pH 7.2, at 20°C for 15 min. After extensive washing, the zona pellucida of fixed embryos was removed by a 10-min incubation in 0.5% pronase (Sigma) dissolved in Hanks' buffered saline at room temperature (27). (All solutions were passed through Millipore filters [Millipore Corp., Bedford, Mass.] before use.) Embryos were thoroughly washed in Hanks', osmicated (1%) in Hanks' at 4°C for 20 min, washed, and micropipetted onto Flotronics silver metal membranes (0.8-μm pores) (Selas Flotronics, Wellesley, Mass.). These were prepared by immersing in a solution of poly-L-lysine (Sigma) in water (1 mg/ml) (25). Immediately before use, the filters were placed on a small Buchner funnel and rinsed with 0.1 M cacodylate buffer with suction. Embryos were adsorbed onto the filters with gentle suction, and the filters were

immersed immediately in buffer. Filters with adsorbed embryos were dehydrated through a graded series of ethanols and into acetone, critically point dried in a Sandri PVT-3 apparatus (Tousimis Research, Inc., Rockville, Md.) out of liquid CO_2, coated with gold and palladium in a Technics sputtering device (Technics, Inc., Alexandria, Va.), and examined in an ETEC Autoscan scanning electron microscope.

The number of embryos observed at each stage of development is: four-eight cell (uncompacted), 15; eight-cell (partially compacted), 38; eight-cell (compacted), 21; postcompaction cleavage, 12; and morulae, 10.

RESULTS

In order to follow the cell surface and cortical changes occurring during morphogenesis, embryos were collected at the following developmental stages: late four-cell to uncompacted (early) eight-cell, compacted (late) eight-cell, and early morula. Although development in a group of embryos is not strictly synchronous and there is some overlap of stages from one group to the next, the following observations apply to most of the embryos at each stage examined. Similar observations were made on embryos fixed in vivo and in vitro. The distribution of microvilli on zona-less embryos visualized by scanning microscopy was also observed in thin section on embryos with intact zonae. All observations on cell surface topography were verified by both scanning and transmission electron microscopy, ruling out artifactual changes induced by pronase treatment of glutaraldehyde-fixed embryos. Organelles typically found in early mouse embryos have been described previously (9): these include vacuolated mitochondria (43, 52), crystalloids, "jigsaw vesicles," and fibrous lamellae which may contain RNA (8).

Normal Development

FOUR- TO EIGHT-CELL STAGES: At 56 h post-HCG, four-cell and early eight-cell embryos contain relatively spherical blastomeres (uncompacted appearance) as described previously (12). Scanning electron microscopy demonstrates that the outer surfaces of the blastomeres of these embryos are characterized by a relatively uniform population of microvilli (Fig. 1). In four-cell embryos, mitochondria are randomly distributed within the cytoplasmic matrix.

Embryos beginning compaction have blastomeres which are more wedge-shaped than spherical (Figs. 2 and 3). The nonappositional surfaces (normally facing the zona pellucida) are regionalized into a relatively nonmicrovillous area bordering the areas of cell-cell contact and a dense "apical" localization of microvilli on the external face of the blastomeres (Figs. 2–5). These apical localizations of microvilli develop asynchronously, appearing on one or two blastomeres at the initiation of compaction (Fig. 2) and later on the remaining cells (Fig. 4). Although there frequently appears to be an increased density of microvilli at the border of the apical localization, closer inspection indicates that this appearance may be due to flattening of microvilli in the central region by the closely apposed zona pellucida (Figs. 3 and 5).

Examination of the area of newly established cell-cell contact demonstrates a separate "basal" localization of microvilli (Figs. 9, 12, and 13). All stages of compacting embryos demonstrate a circumferential band of these basal microvilli in regions where cell-cell contacts are developing. Thin sections of microvilli reveal an internal parallel linear array of microfilaments approx. 60 Å in diameter which appear to terminate in a dense area at the tip of the microvillus (Figs. 10 and 11). The basal ends of the filaments frequently penetrate into the subplasmalemmal cortex (Fig. 10). Lateral filament-filament and filament-membrane cross bridges are observed (Fig. 11).

Cellular polarity is established not only by a rearrangement of surface microvilli but also by a redistribution of cortical cytoplasmic structures observed with transmission electron microscopy. In addition to microfilaments within microvilli, the cell cortex is characterized by a network of nonlinear filamentous material and by microtubules. This network appears to exclude cell organelles and inclusions immediately beneath the plasma membrane (Fig. 10). In the cortex underlying appositional membrane, as well as in the region of new cell-cell contacts, microtubules usually are arrayed in a direction parallel to the cell surface (Figs. 10, 12, and 13). In contrast, the microtubules underlying the apical microvilli facing the zona pellucida are arrayed more orthogonally with respect to the membrane (Fig. 14). Also, fewer long profiles of microtubules are observed, suggesting that these microtubules are not oriented in parallel or that they may be shorter in length than those underlying appositional membrane.

During compaction, mitochondria become localized in a band under the cell cortex where

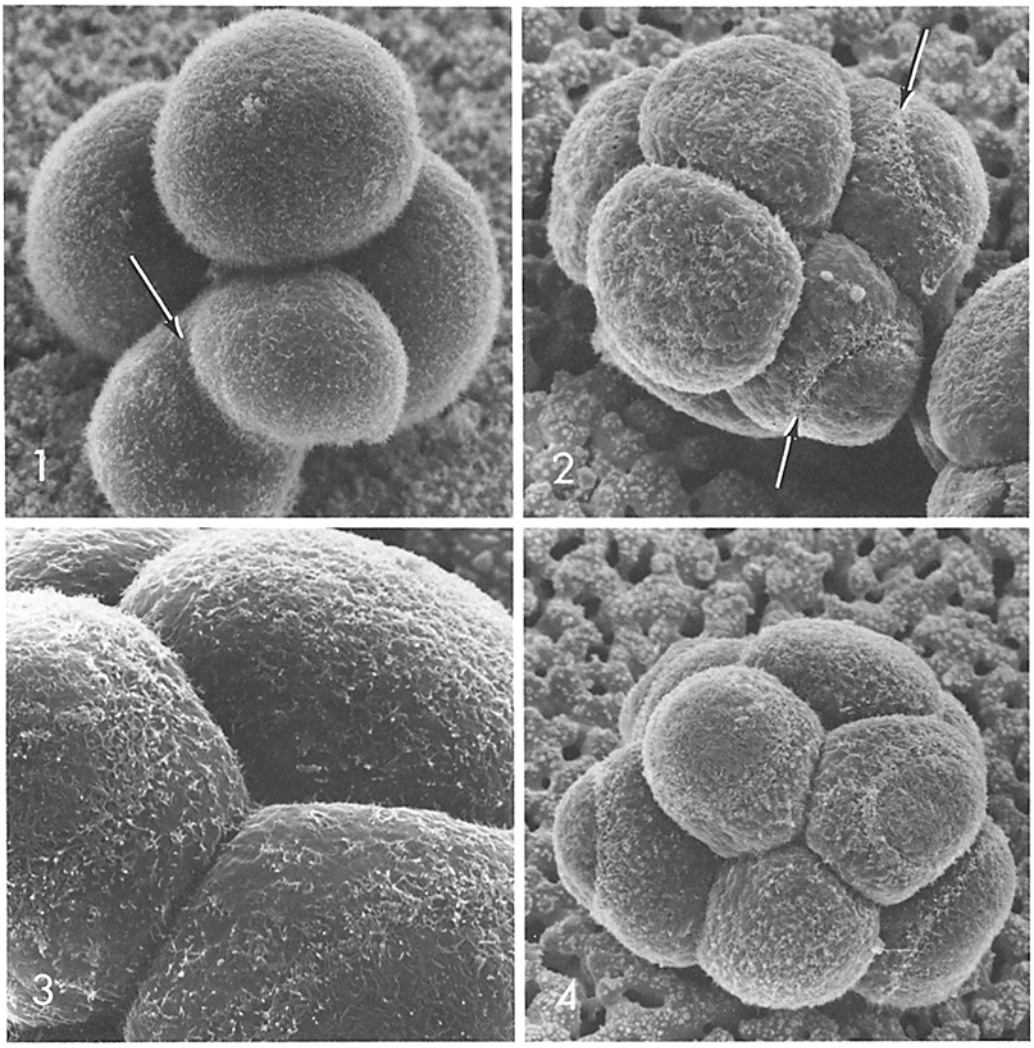


FIGURE 1 Four-cell embryo with one dividing blastomere. The arrow indicates the cleavage furrow. The surfaces of the blastomeres are uniformly covered with microvilli. × 1,500.

FIGURE 2 Eight-cell embryo beginning to compact. The blastomeres are flattening on each other, and two cells show localizations of microvilli (arrows). In the blastomeres of some compacting embryos, there appears to be a groove in the membrane surrounding the apical microvilli (lower arrow). × 1,700.

FIGURE 3 Three blastomeres of a compacting embryo. Microvilli are seen at the apices of two blastomeres (left side and lower right corner). × 3,200.

FIGURE 4 Compacting eight-cell embryo. An apical localization of microvilli is present on four blastomeres. × 1,500.

blastomeres are spreading on each other (Figs. 9 and 13). Relatively few are observed in the remainder of the cytoplasm. These localizations were observed in a total of 13 blastomeres in five different embryos (not serially sectioned). This mitochondrial shift may be of a transient nature since occasionally blastomeres were observed without mitochondrial localizations. Mitochondria are of the vacuolated type (Fig. 13), typical of early stages of mouse embryos (43, 52).

Late eight-cell embryos (62 h post-HCG) which have almost completed compaction demonstrate a

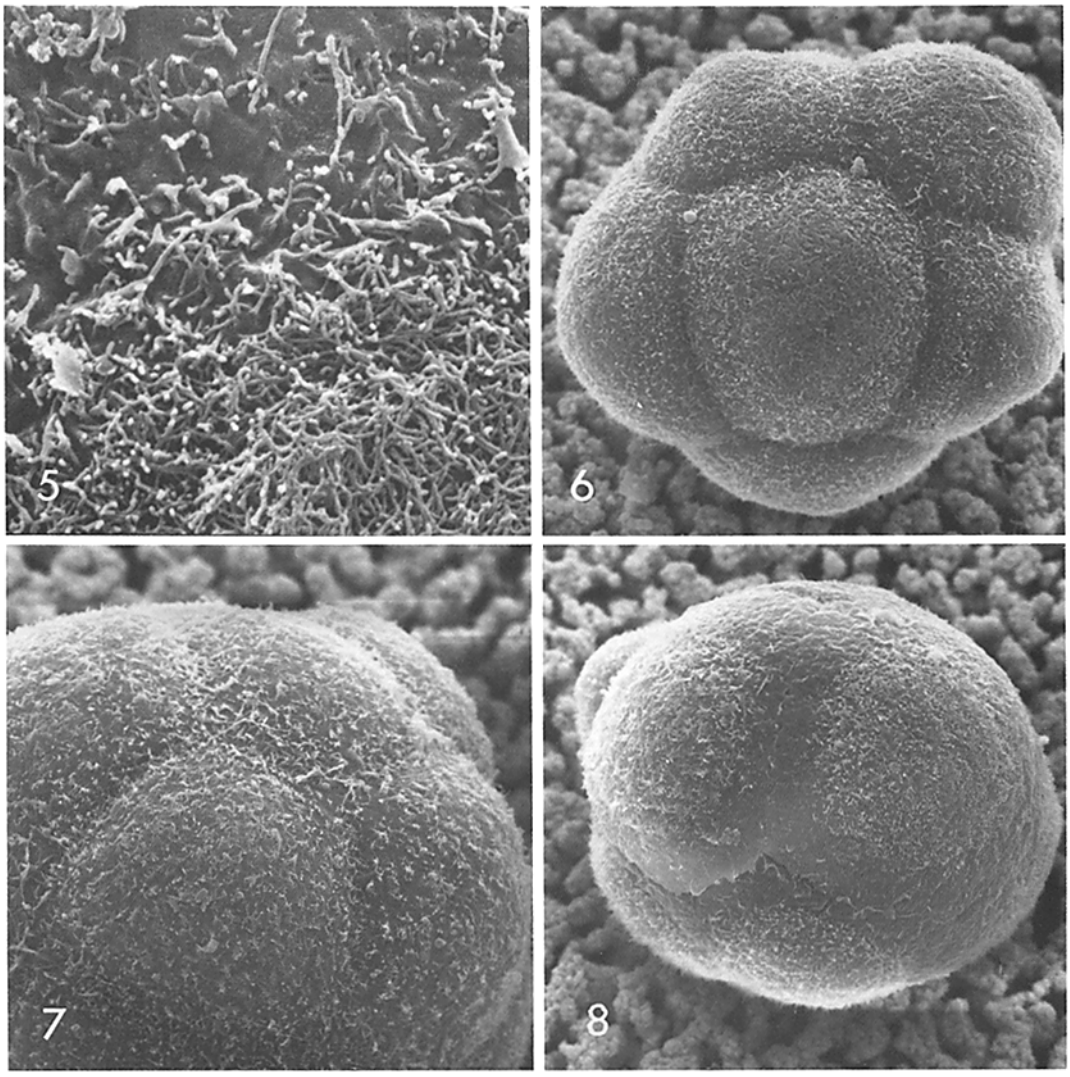


FIGURE 5 High magnification of a compacting blastomere at the border region between the apical localization of microvilli (bottom) and the smooth area (above). × 8,000.

FIGURE 6 Embryo nearing completion of compaction. Only small areas of smooth membrane remain exposed. × 1,600.

FIGURE 7 Compacted embryo in which no smooth surface is visible. × 2,200.

FIGURE 8 Compacted embryo showing smooth areas between the apical localizations of microvilli and regions of cell-cell contact. × 1,500.

narrow smooth region of cell surface between the apical and basal microvilli (Fig. 6). Completely compacted embryos observed by scanning electron microscopy show that this region of smooth membrane varies in size (Figs. 7 and 8) and that basal microvilli are frequently sparse (Fig. 8). Whether or not this variability represents different stages in terms of time before the next series of cleavage divisions is unknown.

POSTCOMPACTION CLEAVAGE (8-16 CELLS): Although the entire surface of dividing cells is relatively smooth with respect to microvilli (Figs.

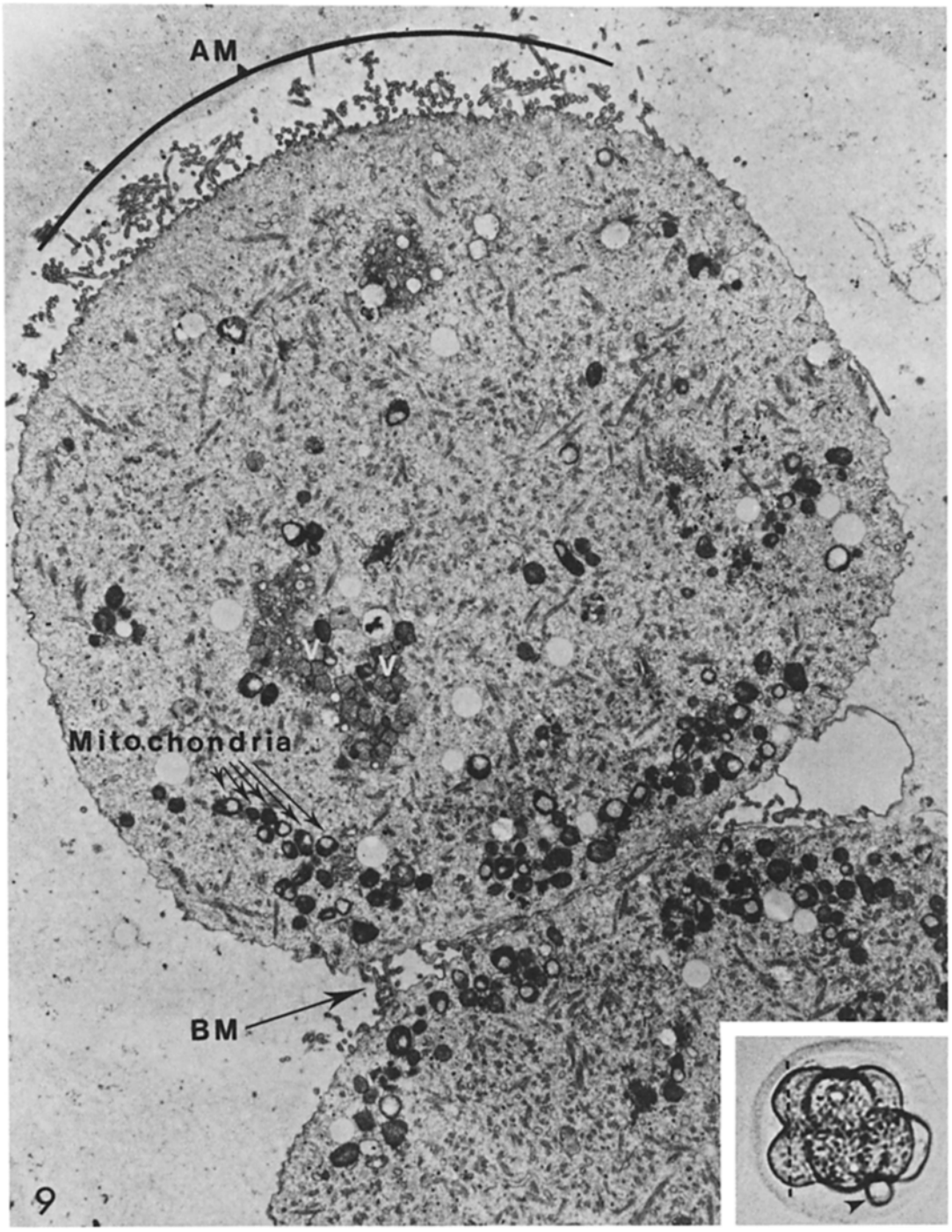


FIGURE 9 Two blastomeres of compacting eight-cell embryo (the dashed line in *inset* indicates the plane of section). Basal microvilli (*BM*) are localized in the regions of newly forming cell-cell contacts and apical microvilli (*AM*) are present immediately beneath the zona pellucida. Mitochondria, both vacuolated and nonvacuolated, are asymmetrically located in the cell cortex where the blastomeres are closely apposed. The cytoplasm also contains lipid droplets and closely packed vesicles (*V*). × 6,900. *Inset*, Light micrograph of a compacting mouse embryo (arrowhead, polar body). × 275.

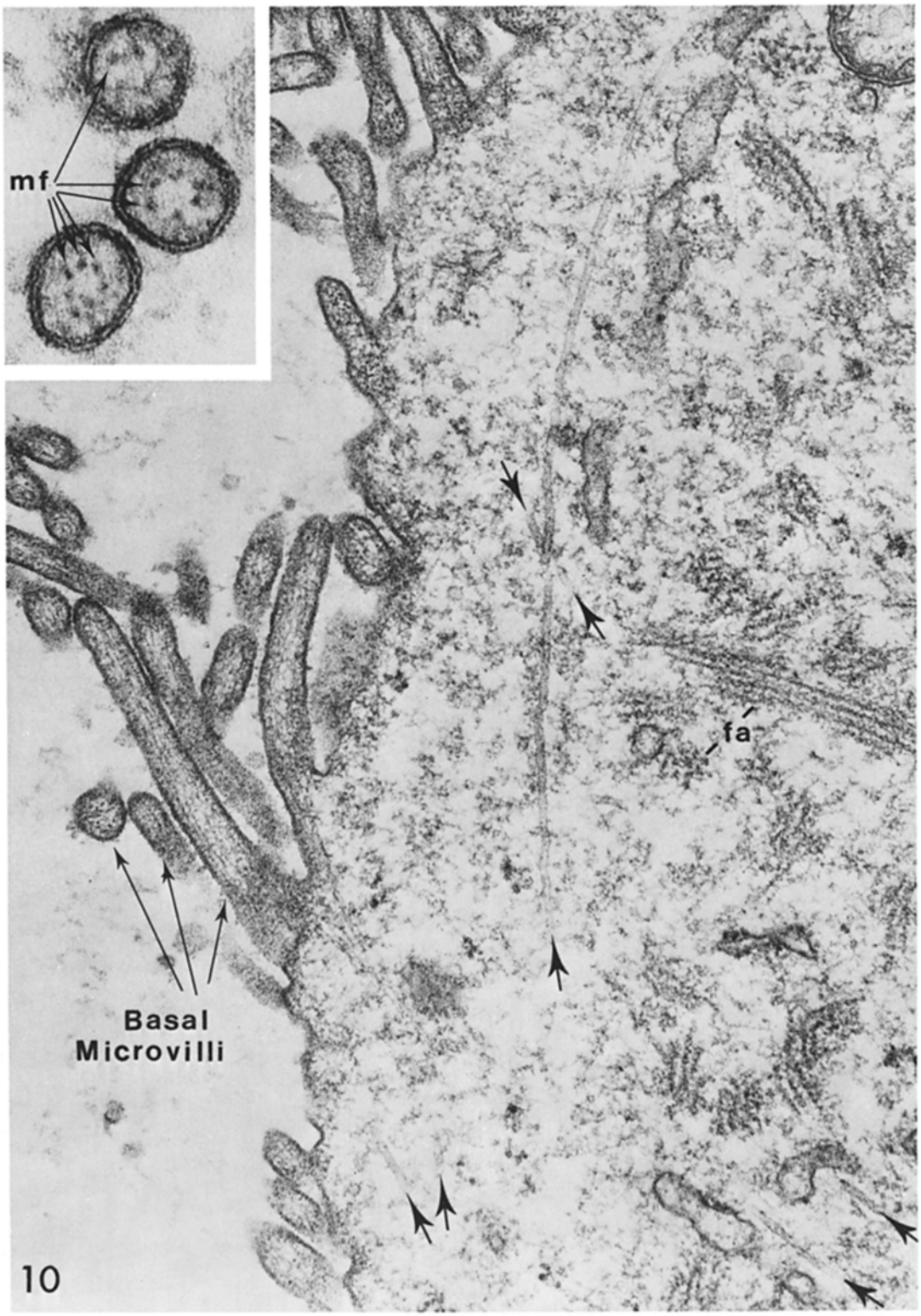


FIGURE 10 Basal microvilli and cortical cytoplasm in the region of imminent cell-cell contact. Except for microtubules (arrows), organelles and other cytoplasmic constituents appear to be excluded from the region immediately underneath the plasma membrane. Microfilaments course from the tips of microvilli into the cortical cytoplasm. Fibrous lamellae (*fa*) are also found in cortical cytoplasm. × 62,000. *Inset*, Cross section of microvilli and their 65-Å filaments. × 226,000.

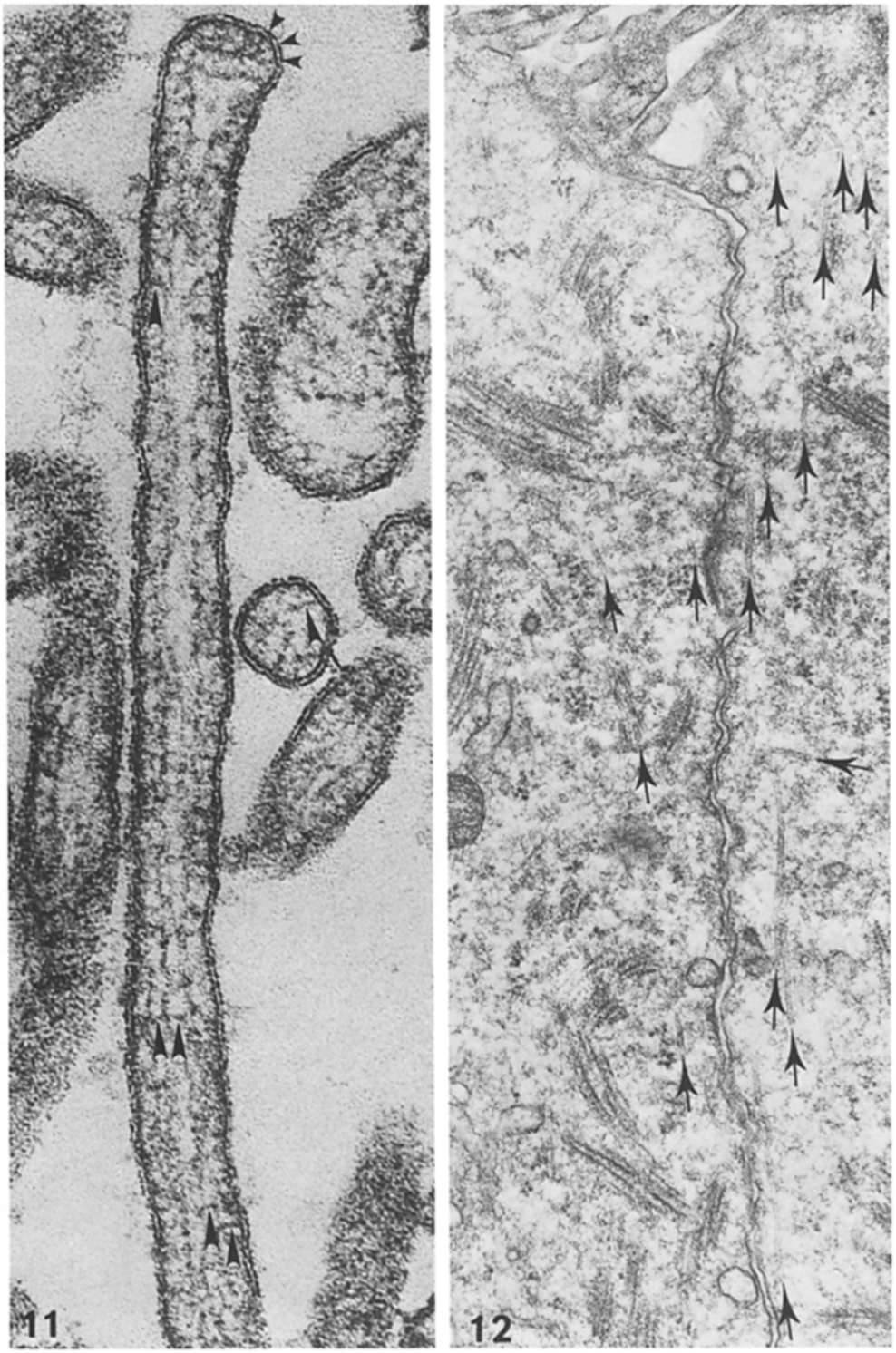


FIGURE 11 Microvilli demonstrating lateral filament-filament and filament-membrane cross-links (arrows). Filaments appear to insert into dense area at the tip of the microvillus (small arrows). × 161,000.

FIGURE 12 Appositional region between two blastomeres with parallel longitudinal profiles of microtubules in a partially compacted embryo. Bordering the intercellular cleft (top) the cell surface is characterized by basal microvilli, whereas below it is smooth where the membranes of adjacent cells are closely apposed. × 43,200.

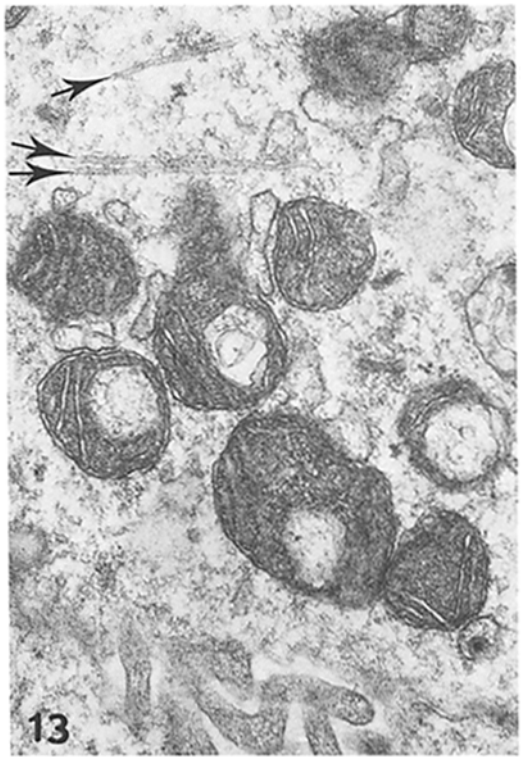


FIGURE 13 Mitochondrial localization in the cell cortex in region of newly formed contacts between blastomeres. Microvilli in lower left corner show location of intercellular furrow. Microtubules (arrows) generally course parallel to the cell surface and in between groups of mitochondria. Many mitochondria appear to be of an intermediate type, possessing both a vacuole and cristae, and frequently are associated with smooth endoplasmic reticulum. × 43,200.

15 and 16), cells which have just initiated cytokinesis display microvilli in the developing cleavage furrow (Figs. 16 and 17). At high magnification (Fig. 17), two regions of the cell cortex can be identified in the cleavage furrow. That part of the cortex which underlies nonmicrovillar membrane is characterized by a well-developed contractile ring of microfilaments approx. 0.5 μm in width (Figs. 17 and 18). In contrast, the contractile ring is not observed under regions of the cortex subjacent to areas of the cell surface where many microvilli remain (Fig. 17). In some instances, microfilaments from microvilli and the contractile ring appear in close association with each other (Fig. 18). During cytokinesis, most of the organelles assume a position below and on either side of the cleavage furrow. Note in Fig. 16 that few mitochondria remain in the areas of cell-cell contact compared to compacting embryos.

EARLY MORULA STAGE: Early morulae of approx. 16 cells appear compacted (Fig. 19). Both scanning and transmission electron microscopy reveal a dense zonular localization of microvilli in the intercellular furrows and a relatively sparse population covering the rest of the cell surface facing the zona pellucida (Figs. 19 and 20).

DISCUSSION

This study has shown that cells of the eight-cell mouse embryo acquire epithelial polarity, apical microvilli and smooth lateral borders, early in compaction and long before the blastocyst stage. The surface changes in the distribution of microvilli are accompanied by a redistribution of mitochondria and cortical cytoskeletal elements. Since

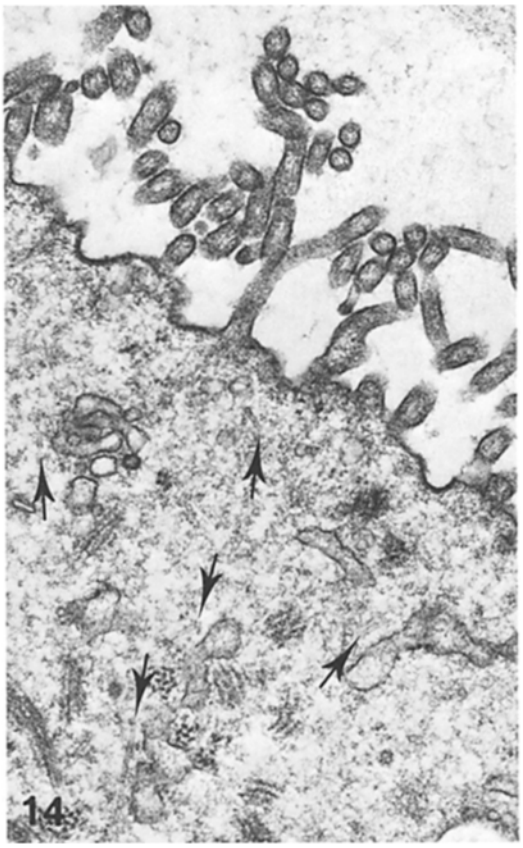
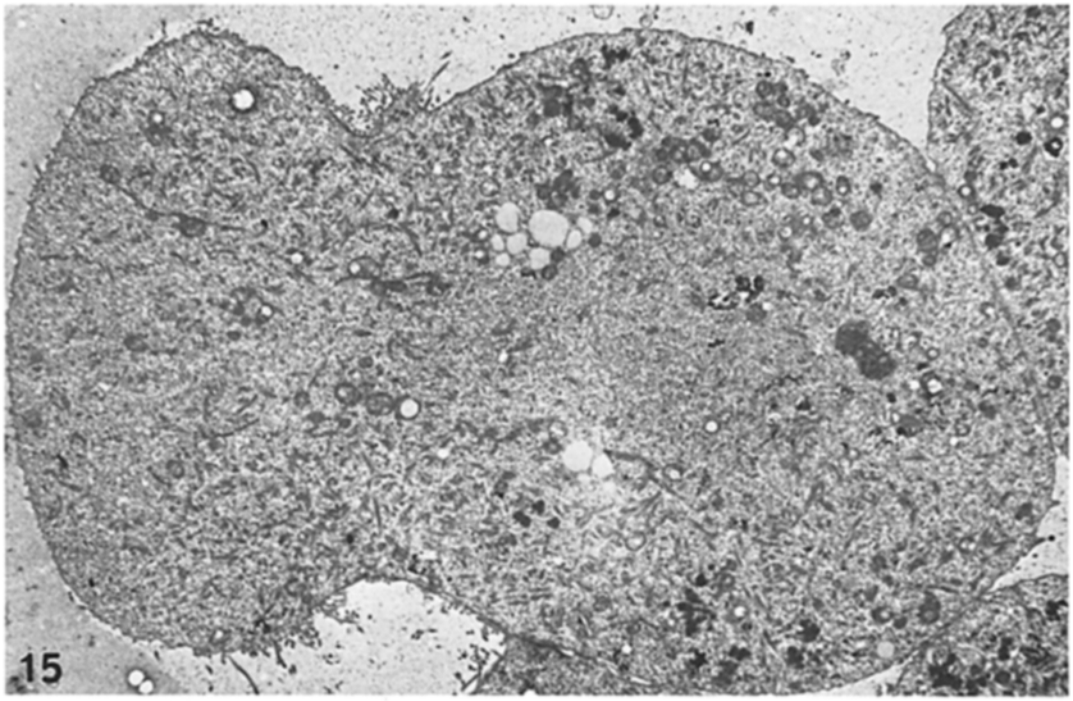


FIGURE 14 Apical microvilli and microtubules (arrows) in the region facing the zona pellucida. Microtubules do not usually lie parallel to the membrane in this region of the cortex. × 33,800.



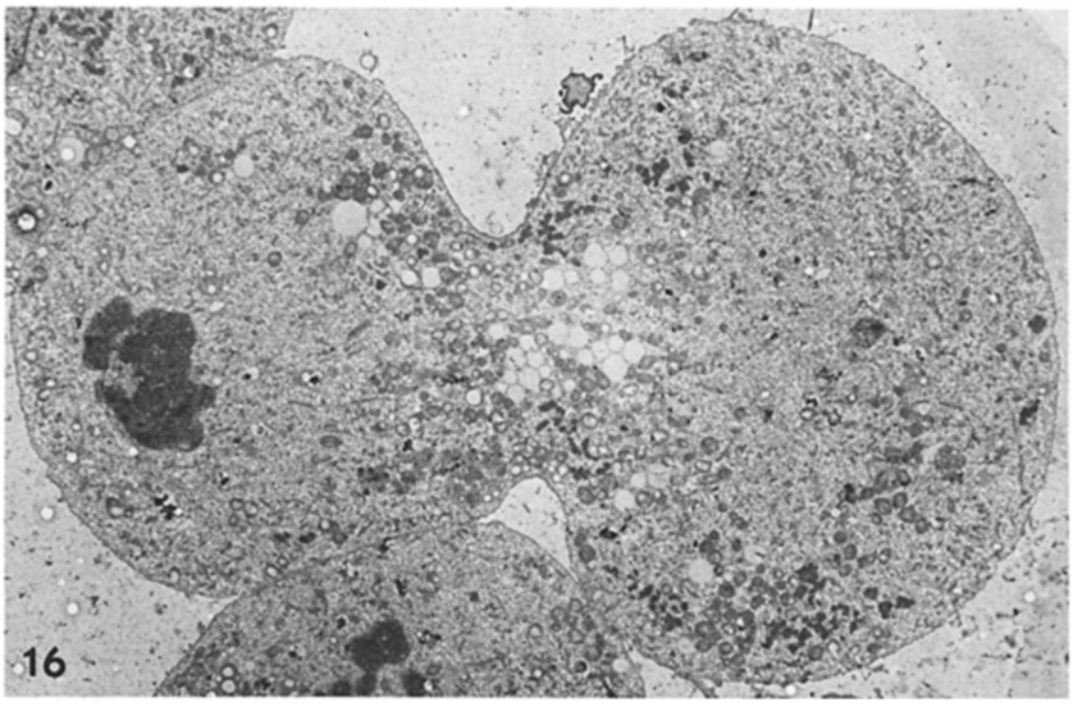


FIGURE 15 Eight-cell stage blastomere in early phase of cytokinesis. Microvilli are frequently observed in the cleavage furrow, but the remainder of the cell surface is relatively smooth. × 4,000.

FIGURE 16 Eight-cell blastomere in later phase of cytokinesis. Cytoplasmic organelles are localized in region near the cleavage furrow. × 3,200.

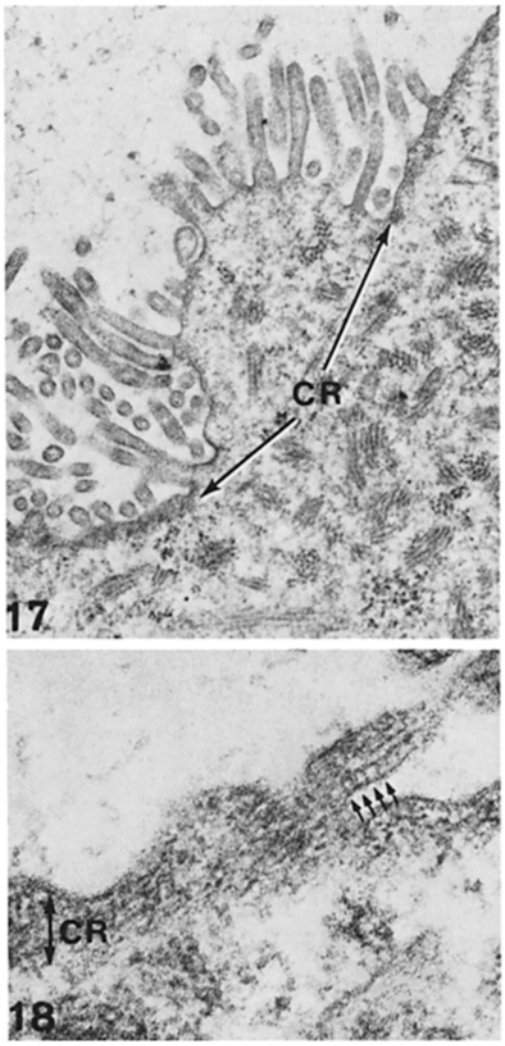


FIGURE 17 A sector of the cleavage furrow from the cell shown in Fig. 15. The contractile ring (*CR*) at this early stage is discontinuous. × 24,700.

FIGURE 18 High magnification of the base of the cleavage furrow of Fig. 16. A microvillus and its microfilaments are shown in close association with the contractile ring (*CR*). Lateral cross-links (small arrows) appear to connect microvillar microfilaments with the plasma membrane. × 110,000.

these alterations of surface and cytoplasmic organization occur during a relatively short period during normal development of the preimplantation mouse embryo, this system offers a unique opportunity to study membrane-cytoplasmic interactions.

Microvilli

The presence of basal microvilli in the region of imminent cell-cell contact, previously observed by Calarco and Epstein (10), suggests that they play a role in approximating the plasma membranes of adjacent blastomeres. This concept is consistent with the involvement of microvilli in cell-cell interactions and adhesion in other systems (19, 22, 24, 47). After compaction, the newly apposed areas of membrane are relatively smooth. This sequence of events suggests that a zone of microvilli moves apically in the intercellular furrow during compaction. For example, microvilli may be formed on the blastomere surface at the points where apposition is occurring and subsequently disappear as adjacent membranes come into contact, or a wave of surface activity may move up the furrow as compaction proceeds. Our observations do not permit a dynamic interpretation of the way in which the cell surface changes proceed.

The motile properties of microvilli (28, 40, 46) would allow them to extend from one cell across to the adjacent blastomere, leading to the development of intercellular adhesions. Retraction of the microvilli would then bring the membranes of adjacent cells into closer apposition. The mechanism of microvillar motility is most likely related to its internal filamentous structure (29). The array of internal cross-linked microfilaments attached at the tip of the microvillus is similar to that observed by other investigators (26, 29). Recent experiments also demonstrate that microfilaments react with an antibody to alpha-actinin (42). Changes in the surface topography of microvilli suggest that there may be a redistribution of cortical alpha-actinin if this unique protein serves as a binding and nucleating site for microvillar microfilament polymerization.

Microtubules in Compacting Embryos

The appearance of numerous microtubules oriented parallel to the apposed membranes of compacting blastomeres indicates that these structures may also be involved in establishing or maintaining the compacted state of the embryo. Microtubules are often observed underlying regions of plasma membrane of cells that are attached to an artificial substratum (18), and they appear in this location when cells are induced to flatten onto the substratum by addition of analogues of cyclic AMP (6, 36, 51). Microtubules that underlie regions of apposed blastomere membrane may thus

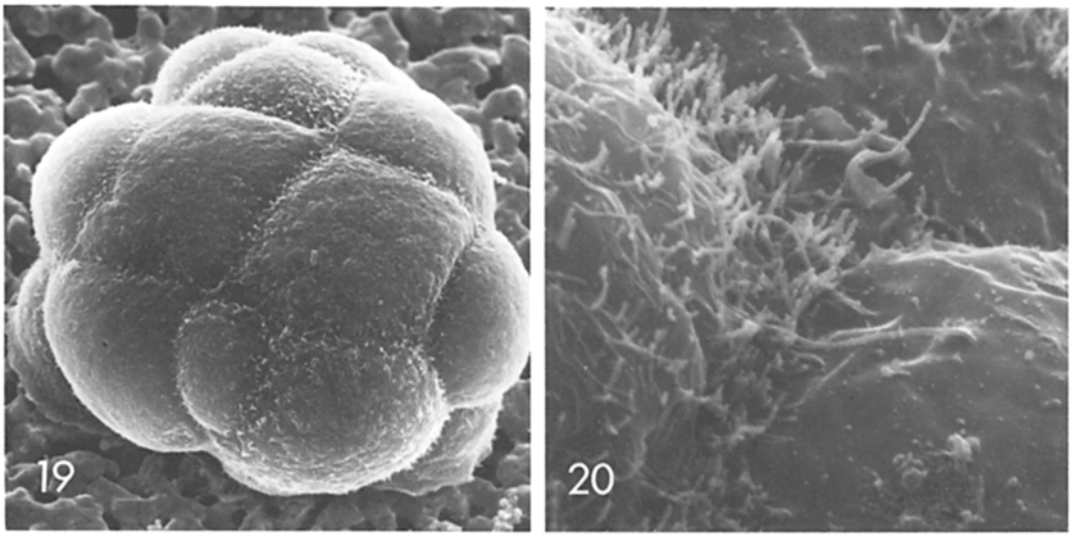


FIGURE 19 Morula. Most of the outer cell surface appears smooth since microvilli have been flattened. × 1,600.

FIGURE 20 Higher magnification of a morula. Microvilli are concentrated in the intercellular furrow, and some cells are more microvillous than others which may be due to cell cycle differences (see Discussion). × 7,900.

function in membrane stabilization. Moreover, it has been proposed that the spatial organization of microtubules may be important in determining their effects on cell shape (6, 51) and cytoplasmic organelles (17, 34). A possible illustration of this effect in the mouse embryo occurs in the cortical cytoplasm beneath the apical tuft of microvilli, where the tubules do not acquire the parallel alignment observed under the smooth appositional membrane. Microtubules may also be involved in determining the location of mitochondria in these cells (see below). Although compaction is not inhibited at the light microscope level when embryos are treated with low doses of colchicine (12), the effect of the drug on microtubules was not monitored.

Distribution of Mitochondria in Compacting Embryos

Before compaction, a relatively random distribution of mitochondria is observed in the blastomere cytoplasm. In contrast, during compaction mitochondria become localized in the cortex associated with newly established cell-cell contacts. During mitoses subsequent to compaction, mitochondria are observed in the region of the cleavage furrow. These dramatic shifts suggest that the location of mitochondria may be related to the organization of microtubules in the cytoplasm. Both microfilaments and microtubules have been proposed to mediate the movements and distribution of cytoplasmic organelles (for a review, see reference 38), including mitochondria (17, 37). Thus, in the mouse embryo, microtubules could serve as an intracellular boundary to maintain the mitochondria in their cortical location and provide a "guidance system" for the association of mitochondria with advancing regions of cell-cell contact.

These shifts of mitochondria may reflect major organizational changes in the cytoplasmic matrix as well as the changing metabolic needs of the embryo. Metabolic studies are consistent with the hypothesis that the rate of ATP metabolism is higher at the late four-cell and early eight-cell stages than at the two-cell stage (4). Moreover, mitochondria begin to undergo transformation from the unorthodox vacuolated type with few cristae to the orthodox, nonvacuolated type with many cristae (43). By analogy with the localization of mitochondria near energy-requiring transport systems of epithelial cells (3), we suggest that the localization of mitochondria in regions of new membrane apposition may function to provide locally high concentrations of ATP. During mitosis,

their localization near the cleavage furrow may be necessary to insure an equitable distribution to the daughter cells since few new mitochondria appear to arise as evidenced by a lack of extensive mitochondrial DNA synthesis during cleavage (32).

Cytokinesis

Since cells of compacted mouse embryos round up before division and later resume their flattened appearance, the cell surface and cortex might be expected to undergo reversible changes in their organization. In fact, the cell surface changes associated with cytokinesis of blastomeres of the mouse embryo are remarkably similar to those observed by Knutton et al. (21) on mouse mastocytoma cells. During most of interphase the microvilli are found on the blastomere surface, whereas cells undergoing cytokinesis have a surface with fewer microvilli except for the region of the cleavage furrow.

The close association between microvilli and the microfilaments comprising the contractile ring suggests that there may be a recycling of microfilaments or actin between the two structures (44). For example, the early cleavage furrow is regionalized into extensive smooth areas of cell surface with underlying contractile ring filaments and occasional microvillous patches without contractile ring filaments. Since microvillous regions bulge from the surface of the cleavage furrow and disappear later in cytokinesis, they may be incorporated into the furrow overlying the contractile ring. The dynamics of microvillous unfolding, formation of the contractile ring, and later reappearance of microvilli imply coordinate control over cell surface topography and cortical filament polymerization.

Control of Cell Polarity

Elucidation of the earliest events in the establishment of cell polarity is of central importance to the understanding of epithelial cell development, in this case the developing trophoblast. By scanning and transmission electron microscopy, we have shown that cells of the compacting mouse embryo acquire polarity by virtue of a reorganization of their surface microvilli and associated cytoplasmic constituents, microtubules, and mitochondria. These cell surface and cortical changes are the first manifestations of polarity in embryogenesis. At the morula stage, the outer cells of the embryo become increasingly polarized with the appearance of zonular tight junctions (11), an underlying filament bed (11), and decreased adhesiveness of the outer cell surface (7). These events culminate in the differentiation of mature transporting epithelial trophoblast cells at the blastocyst stage (4, 11, 14, 15).

Although control over cell polarity and differentiation can be exerted extracellularly (by ligands in reference to capping and by embryonic induction), several lines of evidence suggest that the development of polarity in early mouse embryo cells is controlled to some extent intracellularly: (*a*) blastomeres of a single uncompacted eight-cell embryo initiate cell surface changes and cytoplasmic reorganization at different times, which is probably related to the fact that cell division is asynchronous with each blastomere on its own, slightly different developmental time table. In addition, the development of the apical localization of microvilli with the surrounding smooth nonapositional membrane may indicate that cells are able to regulate the topography of areas of membrane independent of direct involvement of neighboring cells. (*b*) In a separate study on development of the mouse embryo in medium with low Ca^{+2}, it was shown that when morphogenesis and the normal cell-cell relationships are prevented, isolated cells still develop the ability to transport fluid (13). These findings indicate that some aspects of development are programmed at the level of individual cells.

However, cell-cell contact may be required for some of the observed developmental changes in the surface topography of mouse embryo cells as in cultured cells (35, 39). The maintenance of the basal zone of microvilli and smooth closely apposed surfaces of adjacent blastomeres may depend upon cell-cell contact. Changes in cell surface topography which are not dependent on cell-cell contact could be studied by following the development of isolated blastomeres from dissociated uncompacted eight-cell embryos.

The development of cell polarity may also be expressed by the appearance of new membrane proteins or the redistribution of existing ones. It is noteworthy that the $+t^{12}$-regulated antigen, a surface component thought to be involved in cell-cell recognition (48), becomes prominent at the eight-cell stage during mouse embryo development (2). The finding that cleavage-stage embryos of the same developmental age (in time) are not a homogeneous population with respect to lysis by antiserum (2) suggests that embryos in different stages

of compaction or of the cell cycle have different affinities for the antiserum.

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