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Microvillar extensions and microfilament system in early mouse embryogenesis

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Scanning and transmission electron microscopy were used to study the distribution of microvillar extensions of mouse oocytes and embryos ranging from the 2-cell stage to the 16-cell morula. During early development up to the uncompacted 8-cell stage, numerous microvilli were found on all aspects of the blastomeres, including the areas of intercellular contacts. A distinct redistribution of microvilli took place during compaction: polar concentrations of microvilli were present on the outward-facing surfaces of the blastomeres, and simultaneously, the number of microvilli in the intercellular contacts was significantly reduced. On the other hand, on the outer surface of the compacted embryos, many rather long microvillar processes extended across the cell borders. When compacted embryos were exposed to the microfilament disrupting agent cytochalasin B, they decompacted. In the decompacted embryos, the blastomeres showed distinct concentrations of microvilli together with nonmicrovillous surface areas. We also studied the microfilament system of compacted and decompacted embryos and found changes which seemed to be closely related to the simultaneous alterations in the microvillus distribution. Our results suggest that cell surface specializations such as microvilli together with the cortical microfilament system may be involved in the regulation of early mouse development.

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1. Introduction

During early mammalian embryogenesis, the developmental fate of cleavage-stage blastomeres depends on their relative positions (Tarkowski & Wroblewska 1967, Hillman et al. 1972, Kelly 1977, Graham & Deussen 1978, Graham & Lehtonen 1979). At the morula stage, the more centrally located blastomeres tend to contribute more cells to the inner cell mass (ICM) of the blastocyst than do the peripheral blastomeres, which in turn tend to contribute to the trophectoderm (TE) (Wilson et al. 1972, Graham & Deussen 1978, see also Fig. 1). The ICM and TE cell populations eventually form different parts of the conceptuses: the ICM gives rise to the foetal tissues, amnion, and most of the yolk sac, whereas the TE gives rise to the extra-embryonic ectoderm, the ectoplacental cone, and the trophoblast giant cells and contributes to the parietal yolk sac (Gardner & Papaioannou 1975, Gardner 1983).

The exact mechanisms controlling the preimplantation morphogenesis are not known. However, cytoskeletal and cell surface structures may be involved in the regulation of blastocyst differentiation. This suggestion is largely based on experiments with agents interfering with microfilaments (cytochalasins) (Kimber & Surani 1981, Pratt et al. 1982, Sutherland & Calarco-Gillam 1983, Johnson & Maro 1984), microtubules (colcemid) (Pratt et al. 1982, Sutherland & Calarco-Gillam 1983) or cell surface structures (antibodies to cell surface proteins involved in adhesion of early embryo cells) (Peyrieras et al. 1983, Shirayoshi et al. 1983, Richa et al. 1985), all of which disturb the normal development. The blocking of morphogenesis is especially clear in experiments with 8- to 16-cell embryos. The morphogenetic process of compaction, which normally takes place at this stage, is hindered by the above treatments and

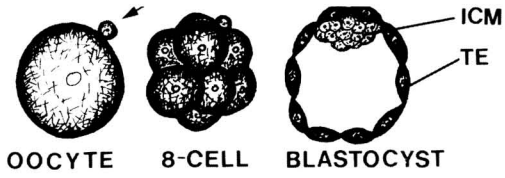


Fig. 1. Mouse preimplantation stages: unfertilized oocyte with a polar body (arrow), 8-cell-stage embryo, blastocyst. The blastocyst consists of two cell populations, the inner cell mass (ICM) and the trophectoderm (TE).

also by lowered levels of calcium in the culture medium (Johnson & Ziomek 1982, Pratt et al. 1982). In compaction, the embryo cells flatten on each other, thereby maximizing their intercellular contacts, and spread over each other (Lewis & Wright 1935, Lehtonen 1980, Kimber et al. 1982). Cell surface specializations, including intercellular junctions and microvillar extensions of embryo cells have been suggested to contribute to the regulation of the morphogenetic movements and the allocation of cells to the two tissues of the blastocyst (Ducibella & Anderson 1975, Ducibella et al. 1975, 1977, Nadijcka & Hillman 1974, Kimber & Surani 1981, Soltynska 1982, Lehtonen et al. 1984).

In the present study we have used scanning (SEM) and transmission (TEM) electron microscopy to study cellular relationships during the preimplantation development of the mouse embryo. We have also examined the distribution of two cytoskeletal proteins, F-actin and vinculin, in compacting embryos. Vinculin is a 130 K cytoskeletal protein. It has been shown to be located close to the cell membrane at the sites where microfilament bundles terminate at the cell membrane and to contribute to the formation of focal cell-substratum contacts and adherent-type intercellular junctions (Geiger 1979, 1983, Burridge & Feramisco 1980, Geiger et al. 1980, 1981). Vinculin has consequently been proposed to be a microfilament-membrane linking protein (Geiger 1983, Geiger et al. 1984, Mangeat & Burridge 1984). Mouse oocytes and embryos ranging from the 2-cell stage to the late blastocyst contain vinculin (Lehtonen & Reima, submitted). Our present results suggest that cell surface specializations such as microvilli together with the microfilament system may participate in the regulation of the preimplantation morphogenesis.

2. Materials and methods

2.1. Oocytes, embryos and culture conditions

Oocytes were obtained from superovulated mice and embryos from natural matings. The granulosa cells were separated from oocytes with hyaluronidase and the zona pellucida was removed from oocytes and embryos with acid Tyrode's solution. The oocytes and embryos were cultivated on bacteriological grade dishes in drops of embryo culture medium under paraffin oil. The methods are described in detail elsewhere (Lehtonen et al. 1984, Reima & Lehtonen 1985). For experiments with cytochalasin B (CB), a stock solution made in DMSO was used. The final concentrations of CB and DMSO in the culture medium were 5 γg/ml and 5 γl/ml, respectively. The embryos were exposed to CB for 20–30 min. Control embryos were cultured in the presence of 5 γl/ml DMSO; this treatment had no visible effect on the embryos as compared with normally cultured embryos.

2.2. Fluorescence microscopy

Oocytes and embryos were fixed and simultaneously permeabilized in a phosphate-buffered solution containing 1.75% paraformaldehyde and 0.05% saponin at 4°C for 20 min. For double fluorescence studies, the specimens were stained with 7-nitrobenz-2-oxa-1,3-diazole-phallacidin (NBD-phallacidin), specifically binding to F-actin, and with rabbit antibodies against chick gizzard vinculin, followed by tetramethyl-rhodamine-isothiocyanate (TRITC)-coupled swine anti-rabbit IgG. The specificity of the anti-vinculin serum was confirmed by immunoblotting experiments (Lehtonen & Reima, submitted). Control oocytes and embryos stained with nonimmune serum gave negative results at all stages. The procedures used for the immunostainings and -blottings are described in detail elsewhere (Reima & Lehtonen 1985).

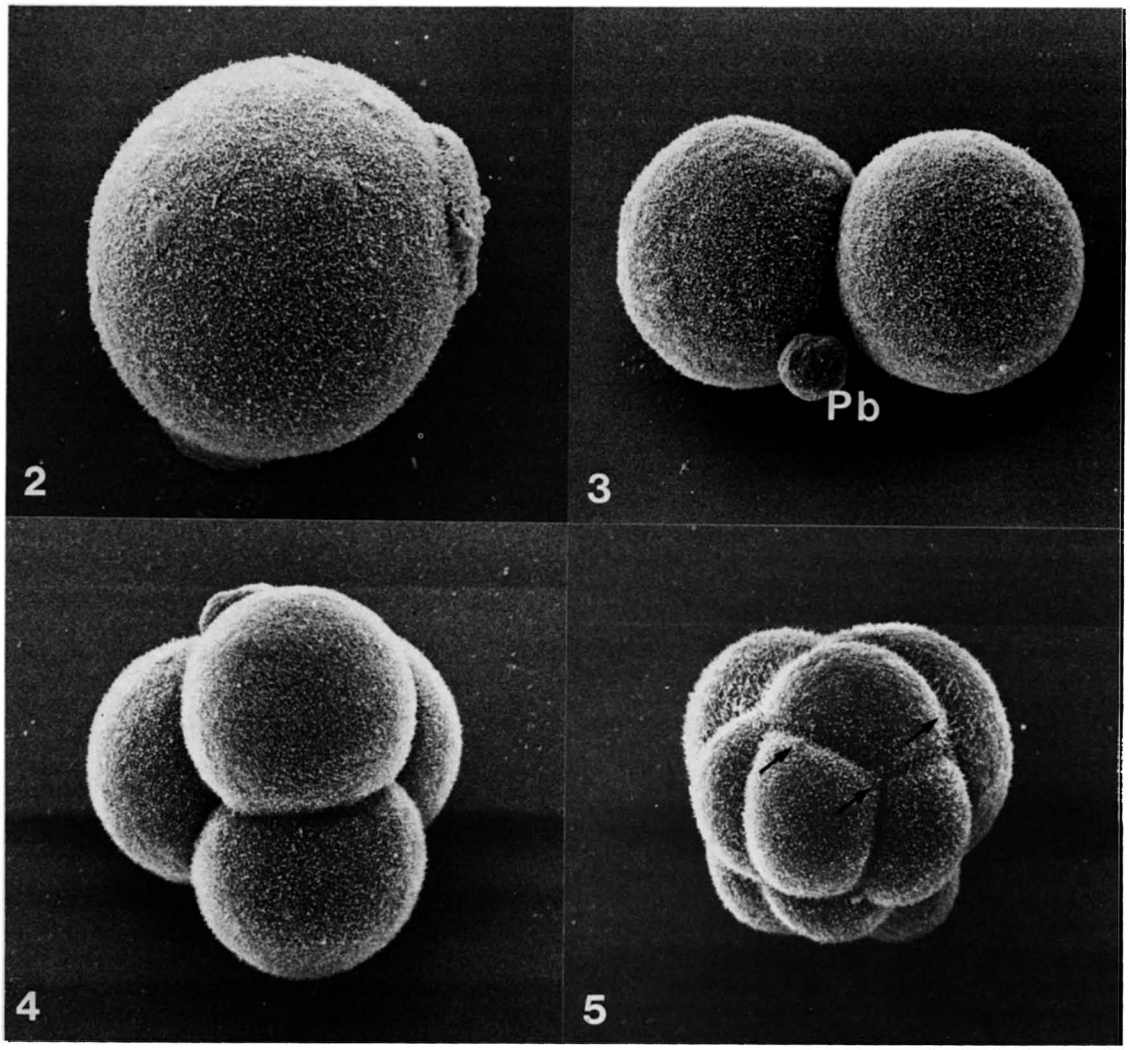
2.3. Electron microscopy

Oocytes and embryos were fixed in phosphate-buffered 2.5% glutaraldehyde and processed for SEM and TEM as described previously (Lehtonen et al. 1984). Briefly, for SEM, the samples were attached to phytohemagglutinin-treated glass coverslips and then fixed, dehydrated, critical-point dried from ethanol, and coated with gold using an ion sputter apparatus. For TEM, the glutaraldehyde fixative was supplemented with 1 mg/ml ruthenium red, and the samples were postfixed in 1% OsO_4, stained *en bloc* with 2% uranyl acetate during dehydration, and embedded in epon. Thin sections were poststained with uranyl and lead.

3. Results

3.1. Cell surface specializations

The outer surface of the oocytes and pre-compaction cleavage-stage embryos was covered with rather evenly distributed micro-



Figs. 2–5. An unfertilized oocyte (Fig. 2) and embryos of the 2-cell stage (Fig. 3), 4-cell stage (Fig. 4), and morula stage (Fig. 5). Up to the 4-cell stage the distribution of the surface microvilli is relatively even (Figs. 2–4). Note the microvilli in the contact lines between morula-stage blastomeres (arrows in Fig. 5). Pb, polar body. Fig. 1, ×1200; Fig. 2, ×1000; Fig. 3, ×1300; Fig. 4, ×1100.

villi (Figs. 2–4). Starting from compaction, the distribution of the surface microvilli was often uneven or polar in many cells (Figs. 5 and 7). Polar concentration of microvilli was also detected in polar bodies (Fig. 6). Many microvillar processes were detected in the vicinity of cell-cell contact lines; these processes were often rather long and extended across the cell borders (Figs. 5 and 7).

The cell contacts inside the embryos were covered with microvilli at the 2- to 4-cell-stages (Fig. 8). In thin sections, the polar body also showed numerous microvilli in the area of contact with the embryo (not shown; indicated by the black arrow in Fig. 6). In 8-cell-stage embryos, the amount of microvillar processes in the intercellular contact areas (Fig. 10) was reduced as compared with the distinct concentration of microvilli at the free outer surface of polarized 8-cell-stage blastomeres (Fig. 9). Specialized membrane contact areas such as adherent-type junctions were also regularly found close to the outward-facing surface of the embryo cells (Fig. 10). The microvilli of

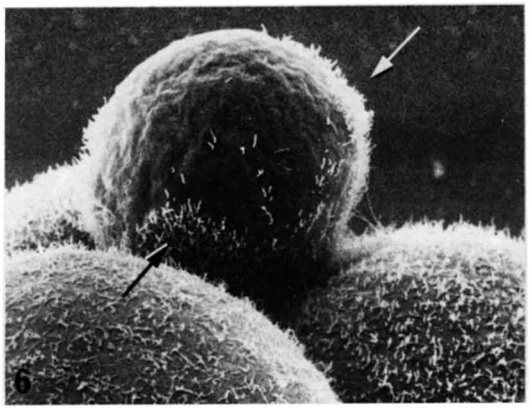


Fig. 6. Four-cell-stage embryo. The polar body (Pb) shows a concentration of microvilli associated with the Pb-blastomere contact (black arrow). Note the concentration of microvilli on the outward-facing surface of the Pb (white arrow). ×3000.

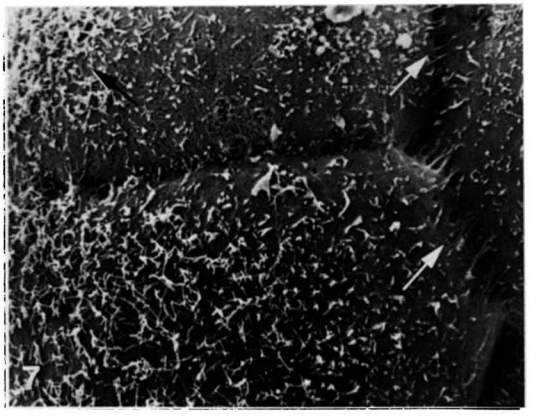
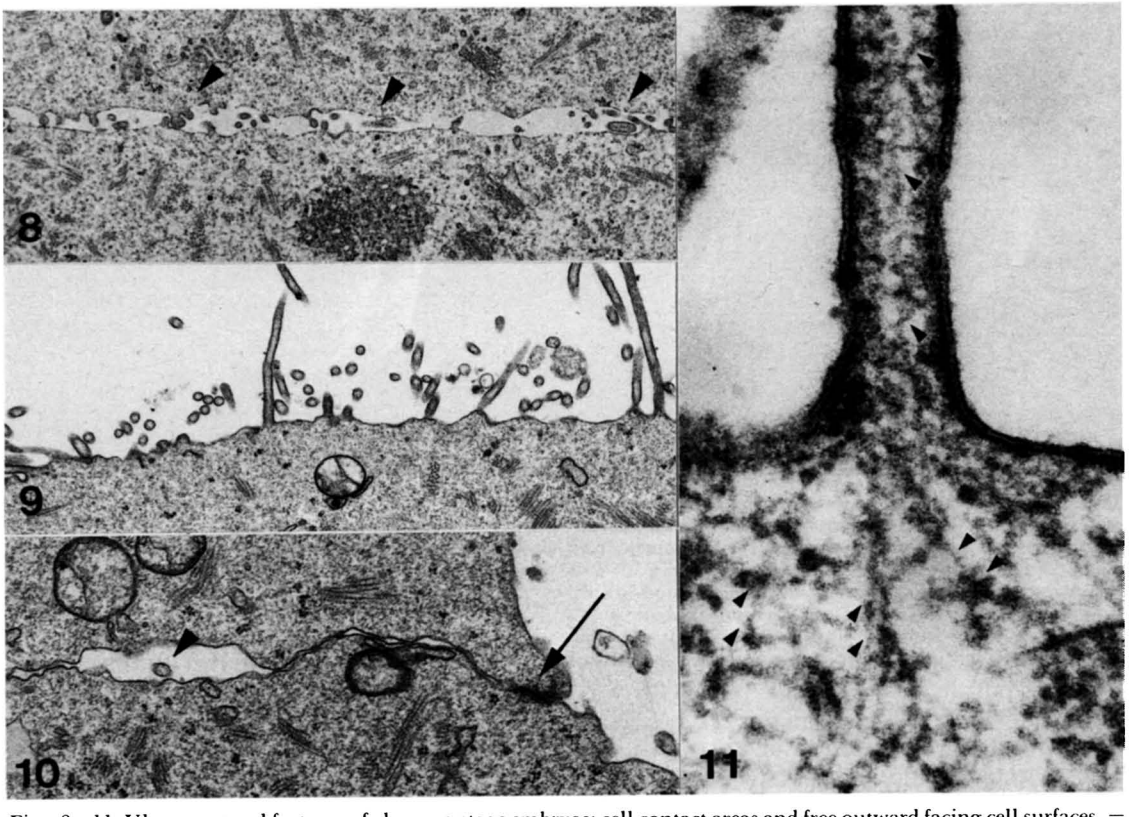


Fig. 7. Compacting 8-cell-stage embryo. Note the concentration of microvilli at the cell contact line (white arrows) and the polar concentration of microvilli on the free outer surface of the blastomeres (black arrow). ×2500.



Figs. 8–11. Ultrastructural features of cleavage-stage embryos: cell contact areas and free outward facing cell surfaces. – Fig. 8, the cell contact area in a 2-cell-stage embryo. Note the numerous microvilli between the cells (arrow heads). – Fig. 9, abundant cell surface microvilli in an 8-cell-stage embryo. – Fig. 10, the contact area between two 8-cell-stage blastomeres. Note the specialized membrane junction close to the outward-facing surface of the embryo cells (arrow). Very few microvilli (arrow head) can be detected in this area of intimate contact between compacting blastomeres. – Fig. 11, a microvillus with microfilaments (arrow heads) terminating in the base of the microvillus. Note the regular arrangement of the microfilaments inside the microvillus. Fig. 8, ×11 000; Fig. 9, ×12 000; Fig. 10, ×17 000; Fig. 11, ×200 000.

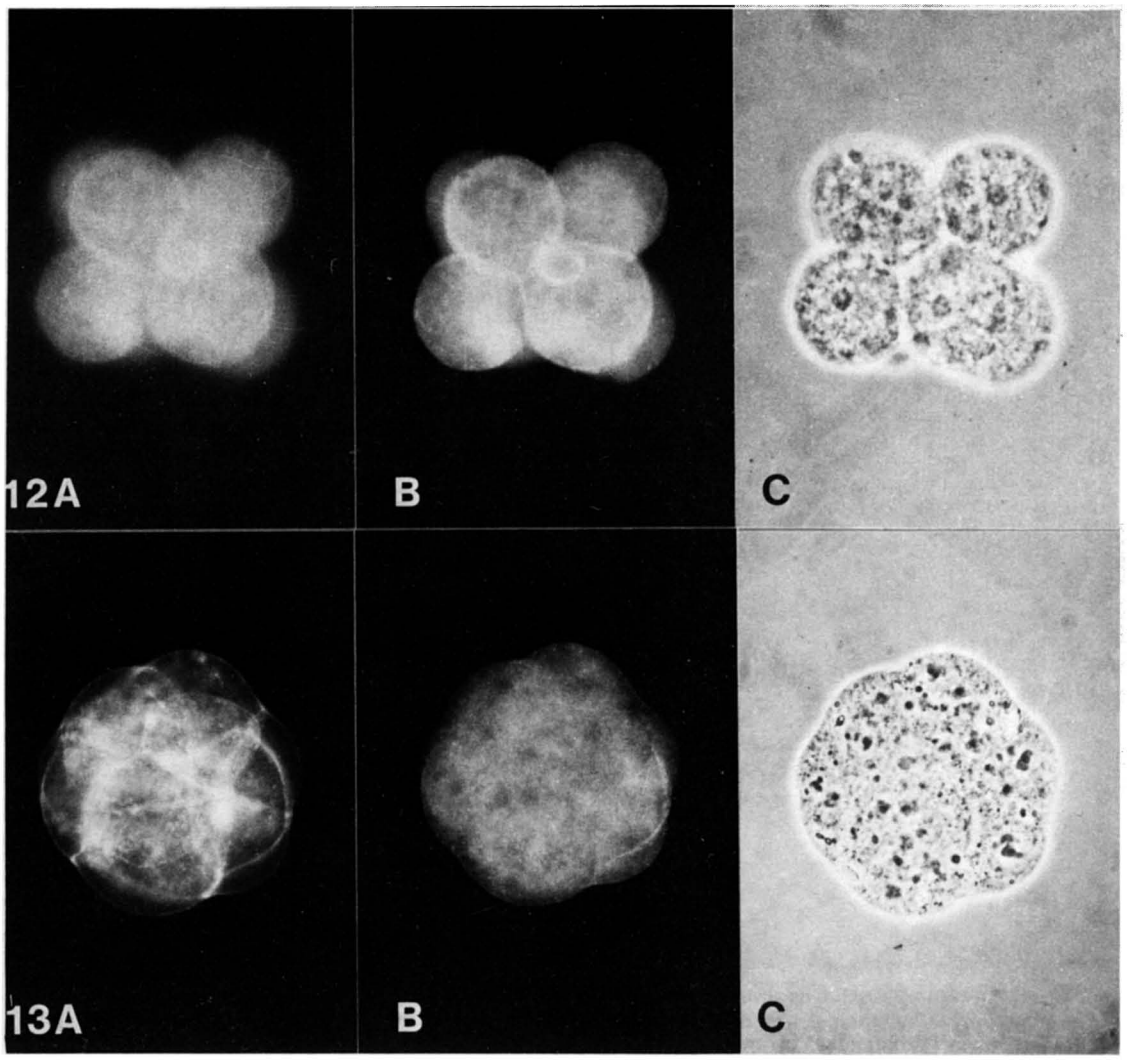


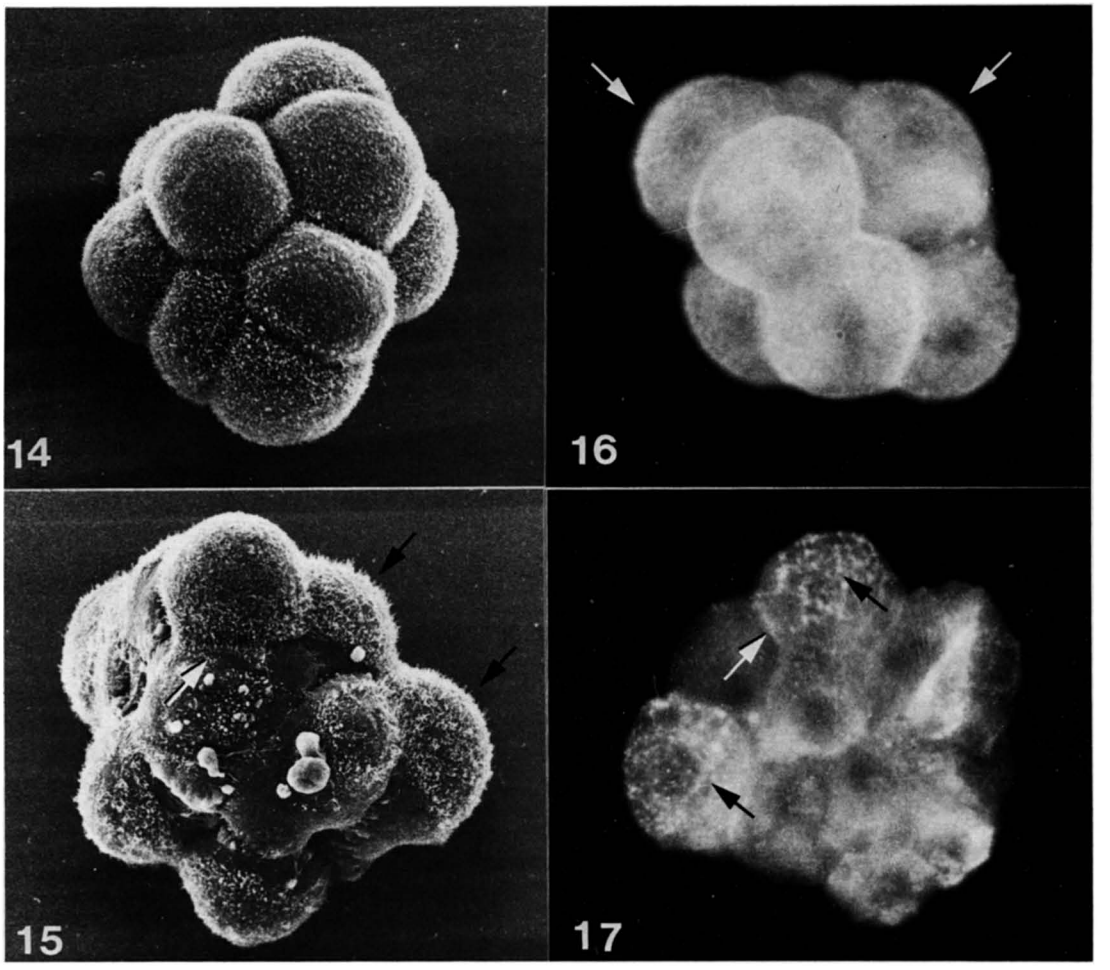
Fig. 12. Double-staining for actin (A) and vinculin (B) in a precompaction 8-cell-stage embryo. Note the similarity of the distribution of actin- and vinculin-specific fluorescence in cell cortex and contacts. ×450.

Fig. 13. Double-staining for actin (A) and vinculin (B) in a compacted morula-stage embryo. Note the differential distribution of the components: only some cell contacts show vinculin-specific fluorescence while a distinct actin-specific staining can be seen in most contacts. ×400.

the embryo cells showed parallel microfilaments terminating in a network of cytoskeletal structures at the bases of the microvilli (Fig. 11).

3.2. Distribution of vinculin and actin in pre- and postcompaction embryos

In precompaction 8-cell embryos, vinculin-specific and actin-specific fluorescence showed concentration to a submembraneous layer in the free outer cell surface and the intercellular contact areas of the blastomeres (Fig. 12). In compacting 8-cell-stage embryos, actin and vinculin often showed a polarized distribution at the free outer surfaces of the blastomeres (Fig. 16). In most of the intercellular contacts in compacted morulae, the concentrations of vinculin-specific fluorescence were indistinct or undetectable as compared with the corresponding actin-specific staining (Fig. 13).



Figs. 14–17. Morula-stage embryos, untreated (Figs. 14 and 16) and after treatment with cytochalasin B (Figs. 15 and 17). – Fig. 14, normal distribution of microvilli in SEM. – Fig. 15, CB treatment results in reorganization of the microvilli on the free outer cell surface (black arrows) and in the contact line (white arrow). Note the non-microvillous surface areas revealed in blastomeres by CB-induced decompaction. – Fig. 16, typical staining pattern of vinculin in a normal 8-cell-stage embryo. Punctate cortical fluorescence includes polar concentrations of the label in some blastomeres (arrows). – Fig. 17, CB treatment results in distinct aggregations of vinculin-specific fluorescence at the free outer surface (black arrows) and at the lines of intercellular contacts (white arrow) of the embryo cells. Figs. 14 and 15, ×1100; Figs. 16 and 17, ×900.

3.3. Effect of cytochalasin B treatment on postcompaction embryos

In SEM, the CB-treated embryos showed drastic changes: the embryos decompacted, and the characteristic microvillus distribution of normal compacted embryos (Fig. 14) changed into one with distinct aggregations of microvilli in the treated embryos (Fig. 15). These concentrations of microvilli were regularly found in two localizations, on the free outer surface and at the intercellular contact areas of the blastomeres. The CB-treated embryos regularly showed large areas of non-microvillous cell surface.

The punctate appearance of cortical vinculin-specific fluorescence (Fig. 16) changed radically during CB treatment. The treated embryos showed submembranous aggregations of vinculin on the free outer surface of the blastomeres and concentrations of specific fluorescence in the contact lines between the blastomeres as well (Fig. 17).

4. Discussion

In early cleavage-stage embryos, the microvilli are rather evenly distributed. They are numerous even between contacted blastomeres (Fig. 8), although there may be patches of smooth membrane in this localization (Calarco & Epstein 1973). Interdigitation of these microvilli may contribute to the adhesion of the early blastomeres. During further development, striking reorganizations occur in the distribution of microvilli. Thus, consistently with earlier results (Calarco & Epstein 1973, Ducibella et al. 1977, Lehtonen et al. 1984), we found polarization of microvilli towards the apical blastomere surface during compaction. Furthermore, unlike the precompaction embryos, inside the compacting embryos the actual contact areas were almost free of microvilli. On the surface of the embryos the compacting blastomeres showed rather long microvillar processes which made contacts across the cell borders on the embryo surface.

Experiments with drugs, or culture conditions affecting the distribution of cell surface extensions have suggested that morula-stage embryos have two distinct populations of microvilli (Johnson & Ziomek 1982, Sutherland & Calarco-Gillam 1983): One group of microvilli is found on the outer surface of the treated embryos, while another population has been reported either to encircle the treated blastomeres laterally (Sutherland & Calarco-Gillam 1983) or actually to cover the inward-facing surface of the outer cells of the morula (Johnson & Ziomek 1982). Our results confirm the presence of ring-like concentrations of microvilli on the surface of CB-treated blastomeres. These concentrations may correspond to the microvilli found in the vicinity of intercellular contact lines on the surface of intact embryos. Many microvilli are also seen in the contact areas remaining after the CB treatment (Fig. 15). In sections of intact embryos, corresponding extensions are found in the peripheries of intercellular contact areas inside the embryo (Lehtonen et al. 1984, Lehtonen & Reima, unpublished).

The surface microvilli as well as wider cell processes may be involved in the spreading of cells on each other in the compacting morula in vivo (Kimber & Surani 1981, Kimber et al. 1982, Soltyńska 1983, Lehtonen et al. 1984). This suggestion is supported by results from aggregation experiments. When 8-cell embryos were aggregated with inner cell masses (ICM) isolated from blastocysts, the ICMs were regularly engulfed by blastomeres. Wide cell extensions were involved in this process (Kimber et al. 1982). Similarly, microvilli and wider cell surface extensions seem to play a role in the aggregation of 8-cell-stage embryos with embryonal carcinoma cells, which results in the formation of chimaeric blastocysts consisting of embryo and embryonal carcinoma cells (Lehtonen et al. 1984).

Cytochalasin B treatment decompacts morula-stage embryos and simultaneously causes an extensive re-organization of their microvillar processes and cytoskeletal components (see also Ducibella & Anderson 1975). We now show that the redistribution of vinculin-specific fluorescence is closely related to the redistribution of microvilli in morulae. Our results suggest that vinculin, as well as actin, localizes partially in microvillar extensions of blastomeres. The presence of these cytoskeletal components in microvilli may be a prerequisite for the formation of close contacts and adherent-type junctions between the cleavage-stage cells. Well-developed adherent-type junctions with associated concentrations of microfilaments are regularly found close to the periphery of each intercellular contact area in late, compacted 8-cell embryos (Lehtonen et al. 1984). The exact relationship of microvilli and adherent junctions to cytoskeletal components such as actin and vinculin in early embryos remains to be shown in immunoelectron microscopy.

The present observations show a correlation between the organization of the cell surface microvilli and the microfilament system of mouse embryos during early development. Interference with the microfilament system results in distinct redistribution of microvilli and, simultaneously, in the inhibition and even reversal of morphogenesis. It thus appears that interactions between cell surface extensions and cytoskeletal structures may be involved in the regulation of intercellular adhesion during early cleavage and in the spreading of cells onto each other during compaction. The cell surface extensions and the microfilament system seem to play an important role in the control of preimplantation development, eventually leading to the segregation of cells to the ICM and the TE of the blastocyst.

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References

Burridge, K. & Feramisco, J. R. 1980: Microinjection and localization of a 130K protein in living fibroblasts: a relationship to actin and fibronectin. — Cell 19:587—595.

Calarco, P. G. & Epstein, C. J. 1973: Cell surface changes during preimplantation development in the mouse. — Devel. Biol. 32:208—213.

Ducibella, T. & Anderson, E. 1975: Cell shape and membrane changes in the eight-cell mouse embryo: prerequisities for morphogenesis of the blastocyst. — Devel. Biol. 47:45—48.

Ducibella, E., Albertini, D. F., Anderson, E. & Biggers, J. D. 1975: The preimplantation mammalian embryo: characterization of intercellular junctions and their appearance during development. — Devel. Biol. 45:231—250.

Ducibella, T., Ukena, T., Karnovsky, M. & Anderson, E. 1977: Changes in cell surface and cortical cytoplasmic organization during early embryogenesis in the preimplantation mouse embryo. — J. Cell. Biol. 74:153—167.

Gardner, R. L. 1983: Origin and differentiation of extraembryonic tissues in the mouse. — Int. Rev. Exp. Pathol. 24:63—133.

Gardner, R. L. & Papaioannou, V. E. 1975: Differentiation in the trophectoderm and inner cell mass. — In: Balls, M. & Wild, A. E. (eds.), The early development of mammals. Cambridge University Press, Cambridge, pp. 107—132.

Geiger, B. 1979: A 130K protein from chicken gizzard: its localization at the termini of microfilament bundles in cultured chicken cells. — Cell 18:193—205.

-"- 1983: Membrane-cytoskeleton interaction. — Biochim. Biophys. Acta 737:305—341.

Geiger, B., Avnur, Z., Rinnerthaler, G., Hinssen, H. & Small, V. J. 1984: Microfilament-organizing centers in areas of cell contact: Cytoskeletal interactions during cell attachment and locomotion. — J. Cell. Biol. 99:83—91.

Geiger, B., Dutton, A. H., Tokuyasu, K. T. & Singer, S. J. 1981: Immunoelectron microscopic studies of membrane-microfilament interactions: the distribution of α-actinin, tropomyosin, and vinculin in intestinal epithelial brush border and chicken gizzard smooth muscle cells. — J. Cell. Biol. 91:614—628.

Geiger, B., Tokuyasu, K. T., Dutton, A. H. & Singer, S. J. 1980: Vinculin, an intracellular protein localized at specialized sites where microfilament bundles terminate at cell membranes. — Proc. Natl. Acad. Sci. USA 77:4127—4131.

Graham, C. F. & Deussen, Z. A. 1978: Features of cell lineage in preimplantation mouse development. — J. Embryol. Exp. Morphol. 48:53—72.

Graham, C. F. & Lehtonen, E. 1979: Formation and consequences of cell patterns in preimplantation mouse development. — J. Embryol. Exp. Morphol. 49:277—294.

Hillman, N., Sherman, M. J. & Graham, C. 1972: The effect of spatial arrangement on cell determination during mouse development. — J. Embryol. Exp. Morphol. 28:263—278.

Johnson, M. H. & Maro, B. 1984: The distribution of cytoplasmic actin in mouse 8-cell blastomeres. — J. Embryol. Exp. Morphol. 82:97—117.

Johnson, M. H. & Ziomek, C. A. 1982: Cell subpopulations in the late morula and early blastocyst of the mouse. — Devel. Biol. 91:431—439.

Kelly, S. J. 1977: Studies on the developmental potential of 4- and 8-cell stage mouse blastomeres. — J. Exp. Zool. 200:365—376.

Kimber, S. J. & Surani, A. H. 1981: Morphogenetic analysis of changing cell associations following release of 2-cell and 4-cell mouse embryos from cleavage arrest. — J. Embryol. Exp. Morphol. 61:331—345.

Kimber, S. J., Surani, M. A. H. & Barton, S. C. 1982: Interactions of blastomeres suggest changes in cell surface adhesiveness during the formation of inner cell mass and trophectoderm in the preimplantation mouse embryo. — J. Embryol. Exp. Morphol. 70:133—152.

Lehtonen, E. 1980: Changes in cell dimensions and intercellular contacts during cleavage-stage cell cycles in mouse embryonic cells. — J. Embryol. Exp. Morphol. 58:231—249.

Lehtonen, E., Wartiovaara, J. & Reima, I. 1984: Cell relationships during aggregation between preimplantation embryos and teratocarcinoma-derived cells. — J. Embryol. Exp. Morphol. 81:17—35.

Lewis, W. H. & Wright, E. S. 1935: On the early development of the mouse egg. — Contr. Embryol. Carnegie Inst. 148:115—143.

Mangeat, P. & Burridge, K. 1984: Actin-membrane interaction in fibroblasts: what proteins are involved in this association? — J. Cell Biol. 99:95—103.

Nadijcka, M. & Hillman, N. 1974: Ultrastructural studies of the mouse blastocyst substages. — J. Embryol. Exp. Morphol. 32:675—695.

Peyriéras, N., Hyafil, F., Louvard, D., Ploegh, H. L. & Jacob, F. 1983: Uvomorulin: A nonintegral membrane protein of early mouse embryo. — Proc. Natl. Acad. Sci. USA 80:6274—6277.

Pratt, H. P. M., Ziomek, C. A., Reeve, W. J. D. & Johnson, M. H. 1982: Compaction of the mouse embryo: an analysis of its components. — J. Embryol. Exp. Morphol. 70:113—132.

Reima, I. & Lehtonen, E. 1985: Localization of nonerythroid spectrin and actin in mouse oocytes and preimplantation embryos. Differentiation 30:68—75.

Richa, J., Damsky, C. H., Buck, C. A., Knowles, B. B. & Solter, S. 1985: Cell surface glycoproteins mediate compaction, trophoblast attachment, and endoderm formation during early mouse development. — Devel. Biol. 108:513—521.

Shirayoshi, Y., Okada, T. S. & Takeichi, M. 1983: The calcium-dependent cell-cell adhesion system regulates inner cell mass formation and cell surface

polarization in early mouse development. — Cell 35:631—638.

Soltyńska, M. S. 1982: The possible mechanism of cell positioning in mouse morulae: an ultrastructural study. — J. Embryol. Exp. Morphol. 68:137—147.

Sutherland, A. E. & Calarco-Gillam, P. G. 1983: Analysis of compaction in the preimplantation mouse embryo. — Devel. Biol. 100:328—338.

Tarkowski, A. K. & Wróblewska, J. 1967: Development of blastomeres of mouse eggs isolated at the 4- and 8-cell stage. — J. Embryol. Exp. Morphol. 18:155—180.

Wilson, I. B., Bolton, E. & Cuttler, R. H. 1972: Preimplantation differentiation in the mouse egg as revealed by microinjection of vital markers. — J. Embryol. Exp. Morphol. 27:467—479.

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