The possible mechanism of cell positioning in mouse morulae: an ultrastructural study

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SUMMARY

Ultrastructure of the mouse morula at the time of formation of the outer cell layer is described.

Analysis of relative positions of sister blastomeres, still connected with cytoplasmic bridge, suggests that cell division may occur both tangentially and perpendicularly in relation to the embryo surface. However, there are observations suggesting that active cell movement may be involved in cell positioning already after mitosis. These observations include: (1) the flexure of cytoplasmic bridge, (2) the presence of bundles of filaments in external blastomeres at the late cytokinesis and (3) a striking difference in loose versus tight packing of blastomeres within the embryo.

INTRODUCTION

The morula is the transitory stage between the 8-cell embryo and the blastocyst. At this stage, a new spatial organization arises, based upon formation of inner and outer cells. There are two possible ways by which formation of the outer cell layer can be explained, i.e. delamination and epiboly. In the delamination process, formation of the outer cell layer depends on the orientation of cleavage planes which take tangential position in respect to the embryo surface. In epiboly, cell movements are mainly responsible for the outer cell layer's constitution. Delamination would ensure more or less fixed positions of cytoplasmic areas within the embryo, whereas epiboly would result in mixing cytoplasmic regions.

Observations and experiments performed on mouse embryos suggest that both the delamination (Johnson & Ziomek, 1981) and the epiboly (Kimber & Surani, 1981) may play a role in the cell positioning. In order to discriminate between two possible processes, ultrastructural analysis of mouse morulae was accomplished. A special attention has been given to a relative position of sister cells still connected with cytoplasmic bridge, and to those cell structures which might indicate an active movement of embryonic cells.

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MATERIAL AND METHODS

CBA-T6T6 female mice were induced to ovulate with 5 i.u. each of PMSG and HCG given 48 h apart and were mated with CBA-T6T6 males. Embryos were flushed from oviducts and uteri 83.5 h post HCG and divided into three classes on the basis of their morphology: (1) flattened morulae, (2) spherical morulae with irregular outlines (semi-compact morulae), (3) spherical morulae with regular outlines (compact morulae). Morulae were fixed for 30 min in 2-5 % w/v glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2), post-fixed for 40 min in 2 % w/v osmium tetroxide in the same buffer, quickly dehydrated in a series of ethanol dilutions, and embedded in Epon. Thin sections were stained with uranyl acetate followed by lead citrate.

Three embryos of each class of morulae were used in a pilot study. Eight flattened morulae and eight semi-compact morulae were then studied on serial or nearly serial sections.

About 300 sections were analysed from each embryo. The section thickness was about 30 nm.

RESULTS

Preliminary observations have shown that the true (= continuous) layer of outer cells, fastened together with tight junctions, occurs in the compact morulae. In flattened and semi-compact morulae, the outer layer is still incomplete, and partially internal cells (see below) are interposed between external cells. These observations have been interpreted as showing that cell positioning had been already terminated in compact morulae, and only flattened and semi-compact embryos have been further studied in detail.

Analysis of serial sections allowed the reconstruction of the spatial organization of cells within the embryos.

Figs. 1–5: sections from the flattened morulae.
Fig. 1. The general appearance of the flattened morula. Note large intercellular spaces. Two external cells (E) have flattened shape. Intercellular bridge in apical position (arrow). ZP, Zona pellucida, PS, perivitelline space. × 2400.
Fig. 2. Intercellular bridge (IB) joining both sister cells of variant 1 is situated externally. Tight junction (arrow) visible underneath the bridge. PS, perivitelline space. × 18000.
Fig. 3. Sister cells of variant 2. External sister cell (E) takes a crescent-like form and displays highly bent intercellular bridge in apical position (arrow). Internal cell (I) display circular outline; its surface is partly covered with a finger-like projection belonging to another cell which is not in the process of cytokinesis (arrowhead). × 3000.
Fig. 4. Portion of the surface of sister cells from variant 2 in the vicinity of the intercellular bridge. E, External sister cell; I, Internal sister cell; Mv, microvilli. Arrows indicate cortical filamentous layer, PS, Perivitelline space. × 36000.
Fig. 5. Portion of the surface of partly internal sister cell of variant 2 (I) in the vicinity of the intercellular bridge is covered with a finger-like projection (FP) of the adjacent cell. PS, Perivitelline space. × 32000.
Cell positioning in mouse morula

[Images of cell positioning in mouse morula]
Morulae of both studied classes are characterized by the presence of three types of cells: external, internal and partially internal cells. The last type comprises the blastomeres, which are exposed to the perivitelline space by a small portion of their surface.

In all embryos examined, some cells are found which pass through the terminal phase of cytokinesis, as one can judge from the presence of two sister blastomeres connected with each other by the intercellular bridge containing the midbody.

The following variants of spatial arrangement of sister blastomeres are distinguished: (1) both sister cells are external; (2) one sister blastomere is external, the second one is internal or partially internal; (3) both sister cells are internal.

**Flattened morulae**

Flattened morulae display a 'loose' appearance because of the presence of intercellular spaces separating the blastomeres. Intercellular spaces are especially extensive at the point where three or four cells meet together (Fig. 1).

The cell shape and the cell surface of sister blastomeres, at the terminal phase of cytokinesis, differ, depending on the position of cells.

If both cells are external (variant 1), they have a flattened appearance. Intercellular bridge is situated superficially and protrudes above the surface of sister cells (Fig. 1, 2). The cell surface in the vicinity of the intercellular bridge is almost smooth, covered with few microvilli. Just underneath, a tight junction between sister cells occurs, closing a small intercellular space (Fig. 2). Sister cells do not differ in respect to their cortical layer structure.

If one of the sister cells is situated externally, and the second one internally or almost internally (variant 2), significant differences are found in respect to their

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Fig. 6. section from the flattened morula; figs. 7–10: sections from the semi-compact morulae.

Fig. 6. Lobe-like projection formed by external cell (E) establishing contact with underlying internal cell (I). The layer of oriented filaments (arrows) is visible in both cells. × 48000.

Fig. 7. General appearance of the semi-compact morula. Close apposition of cells and almost complete disappearance of intercellular spaces. External cells (E, E,) are spherical. Internal cell (I) of variant 2 is placed underneath its sister external cell (E). External cells are covered with numerous microvilli (arrowheads). ZP, Zona pellucida; PS, Perivitelline space. × 2800.

Fig. 8. Details of the cell contact area. Sister external cells (E, E,) make contact with each other by the tight junction (arrow). × 36000.

Fig. 9. Portion of surfaces of cells A and B (see Fig. 11) displaying tight contacts. An arch of filaments is visible in the cytoplasm of cell A (arrow). × 14000.

Fig. 9A. Inset from fig. 9. Intracytoplasmic filaments. × 66000.

Fig. 10. Area of the contact established between cytoplasmic projection of the external cell (E) and the internal 'sister' of variant 2. External cell is not engaged in cytokinesis. Microvillus of the projection (arrow). × 66000.
shape (Fig. 3), surface organization and cortical structure (Fig. 4). An internal cell displays an oval or circular outline. Its free surface, adjacent to the intercellular bridge, is covered with numerous folds and microvilli. The filamentous cortical layer in this area is thick, and filaments are disordered (Fig. 4). Sister external cell is flattened or takes a crescent-like shape, thus embracing a part of the internal cell. Intercellular bridge of arched appearance is found in the apical position (Fig. 3). The region around the bridge, as well as the entire surface facing the perivitelline space, are sparsely covered with microvilli. The thick layer of cortical filaments is limited to that area around the intercellular bridge which faces the perivitelline space (Fig. 4).

In cases of the internal position of both sister cells (variant 3), the surface around the intercellular bridge is covered with few microvilli, and the intercellular bridge is surrounded by intercellular space. No difference has been found in the structure of cortical layer in both cells.

Two types of cytoplasmic projections of external cells can be found in flattened morulae.

1. **Finger-like projections** which on both the external surface (facing perivitelline space) and the internal surface (facing the underlying cell) are covered with microvilli (Fig. 3). The cortical layer of both surfaces shows an irregular network of filaments. This type of projection is found in non-dividing cells. Projections partly cover cell surfaces in the vicinity of the bridge of sister internal cell from variant 2 (Fig. 3, 5).

2. **Lobe-like projections** display a folded external surface. Their internal surface adheres tightly to the underlying cell. The cortex of the external surface is supported with irregular filamentous network. The cortex of the internal surface shows a layer of filaments, which are oriented parallel to each other, as well as to the cell membrane (Fig. 6). This layer is separated from the underlying cytoplasm by a bundle of filaments. The cortical layer of the cell underlying the lobe-like projection is supplied with filaments which are ordered just alike those in the lobe.

Lobe-like projections were observed sporadically and it was not possible to establish whether or not they are formed by cells undergoing cytokinesis.

In flattened morulae the tight junction areas between the external cells which are not engaged in cell division appear to be poorly developed.

**Semi-compact morulae**

These morulae have spherical outlines; blastomeres are in close apposition; there is no intercellular space between adjacent cells (Fig. 7). When compared to the 'loose' cell arrangement in the flattened morulae, semi-compact morulae can be described as 'coherent' embryos.

In all variants of spatial organization of sister cells in advanced cytokinesis, the surface adjacent to intercellular bridge is covered with numerous microvilli (Fig. 8).
In variant 1 sister blastomeres are not flattened, but they are rather of spherical shape.

In variant 2, external cell lies over the internal sister cell (Fig. 7). Surfaces of both sister cells adjacent to the intercellular bridge remain in a close contact. Microvilli penetrating this area are squeezed around the bridge.

In variant 3, the only difference in comparison with the flattened morulae consists in the tight packing of cells within the embryo which results in the disappearance of intercellular space and compression of microvilli and folds.

In three cases, a peculiar organization of three or four pairs of cells connected with cytoplasmic bridges and localized at one pole of the embryo has been found. In two cases the organization of such a spatial system has been reconstructed with certainty (Fig. 11). Two pairs of sister cells belong to variant 1, i.e. all of them are external. The external cell of the pair of variant 2 lies between
them, thus being partially covered with the former cells. The contact between the external cell of variant 2 and any cell of variant 1 is realized in three clearly defined zones (Fig. 11). The zone ‘1’ corresponds to the leading area of external cell where the membranes of both cells are clearly separated, and contact with each other only by numerous microvilli. The zone ‘2’ corresponds to the region of close contact. In both cells a layer of well-organized cortical filaments showing parallel orientation to each other as well as to the cell membranes is present. The layer is bordered by the bundle of filaments displaying the same orientation as cortical filaments. This complex structure is identical with that observed in the lobe-like projections in the flattened morulae (Fig. 6). Within the zone ‘3’, membranes of both apposing cells remain separated by a narrow intermembranous space; the cortex is free of filamentous layer. In the area where cells of variant 1 approach each other along the surface of external cell belonging to variant 2, the latter cell displays the presence of filamentous layer limited to a small area and immersed within the cytoplasm. The layer forms an arch, which is oriented parallel to the free cell surface (Fig. 9, 9A).

Cytoplasmic projections

It is sometimes observed that in the cell of variant 2 undergoing cytokinesis a part of the surface of internal sister cell in the vicinity of the intercellular bridge is covered with protrusion of the adjacent peripheral non-dividing cell. The contact established between the cell being covered and the projection itself is close. Microvilli expanding from the projection are squeezed into the surface of underlying cell like a press-stud device. Cortical cytoplasm of both cells remaining in contact is filled with ordered filaments (Fig. 10).

In the semi-compact morulae the area of tight junction between non-dividing peripheral cells is always more extensive than in the flattened morulae.

DISCUSSION

Mouse embryo, at the morula stage, acquires a new spatial organization which is manifested in the formation of inner and outer cells. New architecture may result as a consequence of oriented cleavage divisions, and/or positioning of sister cells in the advanced phase of cytokinesis.

Cell divisions leading to the formation of external cells are found to occur in both the perpendicular (variant 1) and tangential (variant 2) direction in relation to the embryo surface. There are also ‘intermediate’ cases, when the external cell covers its sister internal cells, and the intercellular bridge is apparently bent (variant 2, cf. Fig. 3). These observations exclude delamination as the only way of cell positioning, and seem to indicate that bridge flexibility may be a part of the mechanism responsible for constitution of external cells.

This is the hypothesis of epiboly, which considers the existence of cell motility as causative for constitution of the external cell layer. Results of this study seem
to indicate that during the advanced stage of cytokinesis such motility of cells may indeed occur, although its range differs, depending on the stage of morula studied.

A prerequisite for the movement of one sister cell in advanced cytokinesis (while the second 'sister' remains immobile, 'inheriting' a part of the intercellular junctions of maternal cell), is some elasticity of intercellular bridge. Such elasticity seems to be confirmed by pictures displaying a pronounced flexure of the bridge joining the cells of variant 2. This is in agreement with conclusions coming from studies on cytokinesis of isolated blastomeres (Graham & Deussen, 1978; Lehtonen, 1980). The presence of ordered filaments and of bundles of filaments, both in external cells during advanced cytokinesis and in cell protrusions establishing the contacts, are very suggestive of an active cell movement. Bundles represent a form of spatial organization of actin filaments related with movement (Goldman, Bushnell, Schloss & Wang, 1974). Bundles or 'cables' of filaments are typical for crawling fibroblasts (Abercrombie, 1980), invasive trophoblast cells (Sobel, Cooke & Pedersen, 1980) as well as many other actively moving cells. The cortical filaments found in mouse blastomeres are built up of actin (Opas & Soltyńska, 1978; Lehtonen & Badley, 1980). The layer of cortical filaments is able to undergo spatial reorganization during cytokinesis (Ducibella, Ukena, Karnovsky & Anderson, 1977; Opas & Soltyńska, 1978). Blastomeres have far less bundles, and of different spatial organization, than do the cells engaged in crawling movement. Moreover, the characteristic contact of the front edge of the bundle with the cytoplasmic side of the membrane has never been observed in blastomeres. This may be due to a specificity of blastomere movement at an advanced stage of cytokinesis, since 'sisters' are held together with the intercellular bridge.

Can the bundles be exclusively considered as structures important for cell propulsion, or do they participate in a cell-to-cell pressing phenomenon? Do filament bundles occurring in the cortex of internal cell underlying the external moving cell participate in movement – or do they appear as a response of the cortex to pressure or movement phenomena? Participation of bundles in a presumed mechanical pressure of cells towards each other is confirmed by a strong apposition of cell membranes of contacting cells. The presence of intracytoplasmic filaments in the cell covered by two cells which are undergoing cytokinesis in the semi-compact morulae can be considered as a counteraction to the external pressure.

In the literature available, only scarce information has been found regarding the movement of blastomeres. Cinematographic analysis of 8-cell embryos demonstrates that blastomeres can spread over each other (Mulnard, 1967) showing membrane activity similar to ruffling membrane activity (Granholm & Brenner, 1976). Experimental aggregation of mouse embryos has shown that cell spreading occurs at the stage of 8–16 blastomeres, leading to cell mixing (Burgoyne & Ducibella, 1977). An important role in the blastomere
movements has been attributed to microvilli (Ducibella & Anderson, 1975; Ducibella et al. 1977; Graham & Lehtonen, 1979). The present results suggest that microvilli are essential in establishing and stabilizing cell contacts, whereas bundles of sub-membrane filaments are among candidates for accomplishing cell movements.

Those morula substages where external cell layer is in statu nascendi appear as flattened and semi-compact embryos. It seems that loose packing of cells and the occurrence of intercellular spaces in flattened morulae, together with underdeveloped tight junctions, favours cell motility. The evidence for extensive cell movements is, of course, indirect and the movement can be deduced from the flattened, crescent-like form of the external cell, which embraces a large portion of the surface of the internal sister cell (cf. Fig. 3). Organization of both the surface and the cortex in such cells favours the idea of the dynamic behaviour of the cell surface. In external cell, microvilli adjacent to the bridge usually disappear as a consequence of changing the cell shape from spherical (early cytokinesis) to flattened (late cytokinesis). At the same time, the internal sister cell retains microvillous surface around the bridge and the thick layer of cortical filaments.

Cytoplasmic projections which are formed by cells not undergoing cytokinesis might be taken as evidence of an active movement of peripheral parts of non-dividing cells.

In the semi-compact morulae, cells adhere strongly to each other and tight junctions are well developed, which results in compression of the cell mass and reduction of the free surface of the embryo. When the surface is reduced, there is also a smaller distance which must be covered by external cells of variants 1 and 2 of cytokinesis, in order to form the outer layer.

In conclusion, it is postulated that establishment of the spatial arrangement, founded upon formation of inner and outer cells at the morula stage, proceeds gradually and is associated with the alteration of cell-to-cell contacts. Cyto-kinetic events as well as dynamic changes of cortical structure, which are found to occur in moving periphery of external cells, may represent sufficient mechanisms for the formation of the peripheral cell layer in the mouse morula.

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REFERENCES


Cell positioning in mouse morula


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