Cell polarity and microtubule organisation during mouse early embryogenesis

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Summary

We have studied the distribution and the role of microtubules in the major developmental events occurring during early development of the mouse. These events are the setting up of asymmetries within blastomeres, the process of asymmetrical cell division and the changes in cellular organisation taking place during epithelial differentiation.

Key words: microtubule, post-translational modifications, acetylation, centriole, polarity, asymmetric division, epithelial differentiation, gp330.

Introduction

Although the successive differentiative events of embryonic development depend upon the expression of a genetic program, cellular mechanisms exist that modulate that program and can direct the fate of a cell or of its progeny. During the preimplantation development of the mouse, it has been found that such mechanisms are involved in the diversification of the first two cell lineages: the inner cell mass and the trophectoderm. In particular, the existence of asymmetries within cells is a crucial factor in the diversification of these two lineages (Fleming and Johnson, 1988; Johnson and Maro, 1986).

After fertilisation and the first cleavage division, the activation of the embryonic genome takes place and it is only at the 8-cell stage that the first major change in the morphology of the embryo takes place: compaction during which cell polarisation occurs. At the 16-cell stage, for the first time, two phenotypically distinct cell populations are found in the embryo: non-polarised inner cells and polarised outer cells. By the 32-cell stage the blastocoele cavity forms and by the 64- to 128-cell stage, a blastocyst with two cell subpopulations has formed: an outer layer of epithelial trophectoderm cells, derived from outer cells of earlier stages, surrounds an inner cluster of cells, the inner cell mass (or ICM), located eccentrically within the blastocoele cavity and derived largely from the inner cells of earlier stages.

During early development of the mouse, the setting up of asymmetries within blastomeres serves a dual purpose. (1) It is involved in the process of cell diversification that takes place during the preimplan-
distributions of many surface features and cytoplasmic components of blastomeres have changed from being radially symmetric to polarised. The axis of polarity forms with respect to contact with other cells, the apical pole of each cell developing in the most distant possible position from adjacent cells. Moreover, there is strong evidence that it is cell contact that orients polarity since the axis of polarity in pairs of cells formed by the reassociation of isolated blastomeres develops with respect to the new contact area rather than the old one (Johnson and Ziomek, 1981b). The mechanisms involved in this process are still unclear, but there is some evidence that transmembrane signalling involving the protein kinase C pathway may be responsible for this process (Bloom, 1989; Winkel et al. 1990). We know more about the changes taking place within the cell, where the cytoskeleton plays a major role. Microfilaments, which are located in both the surface and cytoplasmic poles (Johnson and Maro, 1984), are involved in compaction since cytochalasin D inhibits and reverses intercellular flattening and inhibits the formation of surface and cytoplasmic poles, but does not destroy fully formed poles (Fleming et al. 1986b; Johnson and Maro, 1985; Pratt et al. 1982). The distribution of various actin-associated proteins has been studied at the 8-cell stage and a redistribution of myosin, vinculin and spectrin in the cortex underlying and adjacent to the contact zone between blastomeres has been described (Lehtonen and Reima, 1986; Reima and Lehtonen, 1985; Sobel, 1983; Sobel and Alliegro, 1985).

**Microtubules and compaction**

Microtubules redistribute during compaction. As the blastomeres flatten upon each other, the slight asymmetry in the microtubule distribution resulting from the depletion of cytoplasmic microtubules near cell contacts becomes progressively more marked and the density of microtubules in the apical half of the blastomeres is much greater than in the basal half (Houliston et al. 1987; Fig. 2). This process is facilitated by the flattening of cells that also occurs at this time. In addition, a subpopulation of more stable acetylated microtubules was found mainly in the basal cortex, near the cell contact area (Houliston and Maro, 1989). Apical nucleation of microtubules by microtubule organising centres does not seem to be involved in the redistribution of the dynamic microtubules, since it has been demonstrated that, although discrete microtubule organising centres exist, they are not involved in the dynamic movement of microtubules in the apical half of the blastomeres (Fleming et al. 1986a; Houliston et al. 1987; Johnson and Maro, 1985; Maro and Pickering, 1984). Microtubule depolymerisation induced by nocodazole, an inhibitor of microtubule polymerisation, allows the formation of gap junctions, intercellular flattening and the development of surface polarity while it inhibits the formation of cytoplasmic poles. In contrast, microtubule stabilisation by taxol inhibits intercellular flattening, the formation of gap junctions and cytoplasmic poles but allows the development of surface polarity (although poles of microvilli extend over all of the exposed surface of the cell). These results suggest a constraining effect of the microtubules on most of the components of compaction studied and their absolute requirement for cytoplasmic organisation. It is possible that the reduction in the number of microtubules in the basal half of the cell, concurrent with other changes in the cortex, facilitates the formation of gap junctions in the basolateral domain, intercellular flattening and the loss of basolateral microvilli. The relocation of microtubules to the apical part of the cell facilitates the movement of organelles towards the apical domain. A network of apical microtubules may then help to stabilise the microvilli of the surface pole and the organelles of the cytoplasmic pole. In that case, rather than being the driving force in compaction, microtubules may help to coordinate the various changes taking place during compaction and reinforce asymmetries set up in the cell cortex (Houliston et al. 1987). Microfilaments seem to be more instrumental in the setting up of asymmetries and their redistribution seems to be under the control of
Microtubules and early mouse development

Fig. 2. Four optical sections through a compacted 8-cell embryo stained with an anti-tubulin antibody (gift of J. Kilmartin) and observed under a confocal microscope. Note that there is an increased staining in the apical part of the blastomeres.

the microtubule network (Fleming et al. 1986a; Johnson and Maro, 1985).

Alternative routes for the establishment of surface polarity during compaction of the mouse embryo

If the process of intercellular flattening is prevented by the use of anti-uvomorulin antibodies, polarisation can still occur, but does so in a lower proportion of cells, and without the normal contact-directed orientation (Houliston et al. 1989; Johnson et al. 1986). In these non-flattened cells polarisation is favoured in cells whose nuclei are located close to the cell surface, and the positions of surface poles and of nuclei tend to coincide (Houliston et al. 1989). In these cells, microtubules mediate this association between poles and nuclei and are required for surface polarisation to occur as demonstrated by the use of nocodazole (Houliston et al. 1989). In contrast, cells treated with nocodazole but allowed to flatten polarise at the surface (Houliston et al. 1989; Johnson and Maro, 1985). It must also be noted that the enrichment of acetylated microtubules in the basal part of the cell cortex during compaction is contact dependent (Houliston and Maro, in preparation; see Fig. 3). Thus, surface polarisation of mouse blastomeres can be accomplished by at least two alternative routes: one, related to cell–cell interactions, requires flattening but is independent of microtubules; the other, intrinsic, can occur without flattening but involves a microtubule-mediated interaction between the nucleus and the cell cortex. Both these pathways operate in the undisturbed embryo (Houliston et al. 1989).

Unequal divisions

Until the third cleavage, cell divisions within the embryo are equal, asynchronous and non-oriented. But, after the setting up of asymmetries within cells at the 8-cell stage, the formation of an outer layer of polarised cells takes place at the 16-cell stage because of the existence of asymmetric divisions. During mitosis, gap junctions switch off, blastomeres round up (Goodall and Maro, 1986) and the interphase microtubule network disassembles and is replaced by the mitotic spindle (Fig. 4). Regardless of the loss of cytoplasmic polarity, a polarised organisation of the microvilli at the cell surface can still be detected (Johnson and Ziomek, 1981a). This polarity is retained during division to sixteen cells so that, depending on the orientation of the division plane with respect to the polar axis of the cell, either 2 polar cells or 1 polar cell and 1 nonpolar cell result. In the latter case, the division
is said to be 'differentiative'. The rapid re-establishment of polarity in outside cells (derived from the apical region) but not inside cells (derived from the basal region) also indicates that some aspect of the asymmetric organisation persists during division. There is considerable variation among 8-cell embryos in the number of polarised blastomeres that divide differentiatively to give one polar and one nonpolar cell (Fleming, 1987). Whether or not a blastomere divides differentiatively seems to be determined randomly, since cell interactions by themselves do not influence significantly the overall pattern of division planes within the population (Pickering et al. 1988). Interactions between the cells influence the type of progeny generated at division to the 16-cell stage only via an effect on the size of the surface pole of microvilli. Because the orientation of the axis of division at this stage is randomly determined, the proportion of differentiative division is directly related to the size of the surface pole: the smaller the surface poles, the greater the proportion of differentiative division (Pickering et al. 1988). There is a higher incidence of differentiative divisions among early dividing blastomeres as a consequence of their more extensive intercellular contacts leading to a smaller surface pole. At the 16-cell stage, polarised cells tend to envelope nonpolar cells because of differences in their adhesive properties: in polar cells, the apical surface is less adhesive than the basolateral surface but nonpolar cells are uniformly adhesive and make the maximum possible contact with other cells (Johnson, 1985).

Another interesting feature of the early mouse embryo, in which cell asymmetries play an important role, is the regulation of the ratio between inside and outside cells at the 32-cell stage. Because the axis of division at the 8-cell stage is determined randomly, the ratio between differentiative and conservative cell divisions is variable and the number of inside cells varies greatly at the 16-cell stage (Fig. 5A). This is not true at the 32-cell stage where the number of inside cells is less variable. The compensation mechanism involved in this process is linked to the shape of the outer cells at the 16-cell stage (Fleming, 1987; Johnson and Ziomek, 1983). When there are few inside cells, the outside cells are elongated along the radial axis of the embryo, the spindle will tend to be parallel to this axis and the divisions to be differentiative, thus increasing the number of inside cells. In contrast, when there are many inside cells at the 16-cell stage, the outside cells will be flattened at the surface of the embryo, the spindles will tend to be parallel to the surface and the

![Fig. 3. The enrichment of acetylated microtubules in the basal part of the cell cortex during compaction is contact dependent. Pairs of 8-cell blastomeres were cultured in the presence or in the absence of ECCD-1 (gift of M. Takeichi), an anti-uvomorulin monoclonal antibody that inhibit intercellular flattening (Yoshida-Noro et al. 1984). fixed and stained with an anti-acetylated tubulin antibody (gift of G. Piperno). Bars represent the percentage of cells in which acetylated microtubules are asymmetrically distributed.

![Fig. 4. Two optical sections through a dividing 8-cell embryo stained with an anti-tubulin antibody (gift of J. Kilmartin) and observed under a confocal microscope. Note that cell divisions are asynchronous and that the mitotic spindles are randomly oriented.](image-url)
Fig. 5. (A) Variability in the number of inside cells at the 16-cell stage. (●) Experimental data pooled from Fleming (1987) and from our own unpublished observations (n=64); (○) calculated data assuming that the percentages of differentiative and conservative cell divisions at the 8-cell stage are 65% and 35% respectively (n=500). (B) Compensation mechanism involved in the regulation of the number of inside cells at the 32-cell stage. See text for more details.

Changes in microtubule organisation during cell diversification

From the 8-cell stage to the blastocyst, the outer cells differentiate and acquire the characteristic features of a transporting epithelium. Gap junctional communication between blastomeres is first detectable just prior to compaction, at the 8-cell stage (Goodall and Johnson, 1982; Lo and Gilula, 1979). During later stages, all cells within the embryo communicate through these junctions and it is only after implantation that domains of restricted communication form. The constitution of focal tight junctions takes place in late 8-cell blastomeres, after compaction (Fleming et al. 1989). If cytokeratin genes are first expressed at the four cell stage in a few cells (Chisholm and Houliston, 1987; Duprey et al. 1985), their expression ceases in inner cells and becomes progressively restricted to outer cells during further development (Chisholm and Houliston, 1987; Fig. 6), suggesting that a down regulation of these genes takes place due to regulatory mechanisms controlled by cellular interactions. gp330, a protein associated with coated pits in epithelial cells (Kerjaschki and Farquhar, 1982), is another example of a protein whose expression is restricted to trophectoderm cells in the blastocyst (Buc-Caron et al. 1987). In contrast to cytokeratin, gp330 starts to be expressed at the 16-cell stage and is restricted to outer cells during further development (Gueth-Hallonet, Verroust and Maro, in preparation; Fig. 7). Desmosomes are present...
in 32-cell embryos, a stage when the \( \text{Na}^+/\text{K}^+\)-ATPase becomes located at the basolateral surface (Watson et al. 1990) and when a vectorial transport of ions and water towards the inside of the embryo starts, forming first intracellular vesicles inside some outer cells and later, the blastocoele cavity.

Various changes in microtubule behavior also take

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Fig. 8. Electrophoretogram showing the regulation of \( \beta \)-tubulin synthesis during preimplantation development of the mouse. 240 oocytes or 64 blastocysts were cultured for 3 hours in either control medium (C), medium containing 10 \( \mu \)M taxol (T) or medium containing 10 \( \mu \)M nocodazole (N), then labeled with \( ^{35} \text{S} \)-methionine for 2 hours in presence of the drug. Immunoprecipitations with an anti-\( \beta \)-tubulin antibody were performed using whole cell extracts. Immunoprecipitates were then electrophoresed on a 10\% SDS-PAGE. Note that the synthesis of \( \beta \)-tubulin is decreased after nocodazole treatment in blastocysts but not in oocytes.

Fig. 9. The enrichment of acetylated microtubules in inside cells is independent of cell contact at the 16-cell stage. Pairs of 16-cell blastomeres were cultured in calcium-free medium to inhibit intercellular flattening, fixed and stained with an anti-acetylated tubulin antibody (gift of G. Piperno). Dark grey bars represent the percentage of pairs in which acetylated microtubules are differentially distributed, light grey bars the percentage of pairs resulting from an asymmetric cell division and white bars the percentage of asymmetric pairs in which acetylated microtubules are differentially distributed.
Fig. 10. Centriole appearance in early mouse embryos. Young blastocysts were sorted with respect to the size of their blastocoele cavity: less than 25% of the size of the embryo (A), around 50% (B) and around 75% (C). The embryos were fixed with glutaraldehyde, stained with DAPI, observed under a fluorescence microscope to count the nuclei (D) and then processed for transmission electron microscopy (E). Centrioles were only observed in some outside cells in the third group (arrow), around the 64-cell stage. Bar is 1 μm.
place during this period of development. In order to investigate these changes, experiments were performed, firstly to study the regulation of tubulin synthesis during early development. Auto-regulation of β-tubulin synthesis due to the modulation of the messenger RNA stability by the level of free tubulin monomers has been demonstrated in differentiated cells in vitro (Cleveland et al. 1981; Gay et al. 1989; Gay et al. 1987). When this mechanism is active, the synthesis of β-tubulin is decreased after a treatment with a drug that induces microtubule depolymerisation (nocodazole, colcemid) and thus increases the concentration of free β-tubulin monomers. Regulation does not exist in oocytes (Fig. 8) and early embryos until the third cleavage division. It seems to appear around the 8-cell stage and, in blastocysts a level of auto-regulation similar to the one described in differentiated cells is observed (Aghion and Maro, in preparation; Fig. 8). At the moment, we cannot exclude the possibility that this lack of regulation is due to the low level of protein synthesis existing in oocytes and early embryos (Abreu and Brinster, 1978), rather than to a modification of the regulatory mechanism itself. Secondly, differences in the organisation of the microtubule network between inside and outside cells were observed at the 16- and 32-cell stages: despite the relative abundance of microtubules in outside cells, acetylated microtubules accumulated preferentially in inside cells (Houliston and Maro, 1989). Treatment with nocodazole demonstrated that within each cell type acetylated microtubules were the more stable ones, however, the difference in composition of the microtubule network between cell types was not accompanied by a greater stability of the microtubule network in inside cells (Houliston and Maro, 1989). In contrast to what happens at the 8-cell stage when the enrichment of acetylated microtubules in the basal part of the cell cortex is contact dependent, the difference in composition of the microtubule networks of inside and outside cells becomes an intrinsic property of the cells at the 16-cell stage (Houliston and Maro. in preparation: Fig. 9). Thirdly, the microtubule organising centres present in oocytes and early embryos do not contain any centriole (Szollosi, 1972; Szollosi et al. 1972). A systematic examination of blastomeres between the 8-cell stage and the blastocyst stage at the electron microscope level revealed that centrioles appeared first in outside cells at the 64-cell stage (Antony, Aghion, Gueth-Hallonet and Maro, in preparation; Fig. 10). The late appearance of centrioles in outer cells, at a stage where these cells are fully functional as a transporting epithelium, suggests that centrioles are not involved in the differentiative events leading to the formation of epithelial cells within the embryo.

Conclusion

It must be noted that, although cells within the early mouse embryo differentiate along different lineages according to their position on the inside or the outside of the cellular aggregate, they still retain some plasticity for at least two or three cell cycles such that a change of relative position may still be accommodated by a change of developmental fate: for example, when an inside cell at the 16-cell stage is moved to the surface of the embryo, it will tend to develop a polar phenotype (Ziomek et al. 1982). Asymmetric cell interactions are thus able to modify the fate of the cells by using some regulatory mechanisms working at the cytoplasmic level and are able to control the expression of some specific genes (see above). This stresses the role of epigenetic processes during early mouse development where microtubules play an important role.

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through modulated messenger RNA stability reside within exon 1 of a beta-tubulin messenger RNA. Cell 50, 671-679.


