Cytoskeleton organization during oogenesis, fertilization and preimplantation development of the mouse

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ABSTRACT The organization and role of the cytoskeletal networks (mainly microtubules and microfilaments) during oogenesis, fertilization and preimplantation development of the mouse are described given the importance of cell-cell interactions and of the subcellular organization in events leading to the formation of the first two lineages of the mouse embryo.

KEY WORDS: *mouse, oocyte, embryo, blastocyst, fertilization, oogenesis, compaction, microtubules, microtubule organizing centers, microfilaments*

Introduction

During the embryonic development of the mouse, as for all mammals, priority seems to be given not to rapid growth of the embryo by frequent divisions (as in the case of *Xenopus laevis*) but rather to the elaboration of extra-embryonic structures. The job of these is to protect the embryo and allow its development *in utero*. The fertilized egg gives rise to the embryo and to these extra-embryonic structures. We describe here the events that take place during preimplantation development, from fertilization to the blastocyst stage, a period during which the embryonic and extraembryonic lineages diverge (Fig. 1).

By the time ovulation takes place, the mouse egg has reached, and becomes arrested at metaphase of the second meiotic division. Fertilization triggers the completion of meiosis and thus the entry into the first mitotic cell cycle that starts with a long G1 phase. It is not before the two-cell stage that the maternal and paternal genomes are enclosed into a single nucleus and that the activation of the embryonic genome takes place (Flach *et al.*, 1982; Piko and Clegg, 1982). The first three cleavage divisions are equal, asynchronous and non-oriented. The first two cell cycles each last about 20 h *in vivo* while the subsequent cycles each last for about 12 h. At the 8-cell stage, the first major change in the morphology of the embryo takes place: the embryo compacts. At the 16-cell stage, for the first time two phenotypically distinct cell populations are found in the embryo: non-polarized inner cells and polarized outer cells. By the 32-cell stage the blastocoelic cavity forms. By the 64- to 128-cell stage a blastocyst with two cell subpopulations has formed: an outer layer of epithelial trophectoderm cells, derived largely from outer cells of earlier stages, surrounds an inner cluster of cells, the inner cell mass (or ICM) located eccentrically within the blastocoelic cavity, and derived largely from the inner cells of earlier stages. In the blastocyst, the trophectoderm will give rise to the extra embryonic tissues and allow the implantation of the embryo in the uterine mucous membrane. The inner cell mass will give rise to the embryo proper.

The key stages in the early development of the mouse appear to be:

(i) The activation of the egg at fertilization leading to the formation and activation of the embryonic genome at the two-cell stage when the male and female chromosomes are enclosed in a single nuclear membrane. The terminal differentiation of the oocyte after resumption of meiosis overlaps with some of the preceding events and will thus be included in our discussion.

(ii) The setting up of asymmetries within the embryo leading to the formation of the first two lineages, between the 8-cell stage and the blastocyst stage (for review see Johnson and Maro, 1986). This corresponds:

- First, to the setting up of asymmetries within cells, a process called polarization, which first takes place during the 8-cell stage at compaction. Cytoskeletal elements and organelles, such as clathrin vesicles and endosomes, accumulate first in an apical focus, while the cell nucleus tends to migrate basally and gap junctions form. Polarity becomes detectable at the cell surface a few hours after the first signs of intracellular

Abbreviations used in this paper: GV, germinal vesicle; ICM, inner-cell mass; MI, first meiotic metaphase; MII, second meiotic metaphase; MIII, third meiotic metaphase; MTOC, microtubule organizing center; PCM, pericentriolar material; MPF, maturation promoting factor.

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polarization. It consists of a "pole" of microvilli located over the apical (outward-facing) part of the cell.

- Secondly, to the existence of asymmetrical (or differentiative) cell divisions giving rise to the two cell types present in the 16-cell stage embryo, polarized outer cells and non-polarized inner cells. This is due to the fact that some elements of polarity are maintained throughout division and that the cleavage plane may be roughly orthogonal to the axis of polarity.

We would like to emphasize two points:

(a) The normal course of early developmental events guiding the unicellular egg on its way to the multicellular embryo requires the coordination of all the mechanisms involved in the spatial and temporal control of cell multiplication and cell differentiation. During this period, events controlled by the cell cycle program overlap with those controlled by the developmental program.

(b) The differentiation of cells within the early mouse embryo depends on their position on the inside or the outside of the cellular aggregate (Tarkowski and Wróblewska, 1967; for review see Johnson, 1985). It must be noted that even if they differentiate along different lineages according to their position, they still retain some plasticity for at least two or three cell cycles, so that a change in relative position may still be accommodated by a change in developmental fate. Asymmetric cell interactions are thus able to modify the fate of the cells by using certain regulatory mechanisms working at the cytoplasmic level. This stresses the role of epigenetic processes during early mouse development.

We will discuss these two points in relation to the organization and the role of the cytoskeleton during oogenesis and early development because it is now clear that cytoskeletal elements (mainly microtubules, but also microfilaments) play a major role in processes such as cell division and cell organization.

Oogenesis and fertilization

In the mouse zygote, the cell cycle consists of the usual succession of four phases: G1, S (DNA synthesis), G2 and M (mitosis) as in somatic cells. Besides these events, mechanisms specific for that stage of development take place, such as the formation of two distinct pronuclei (male and female), their centripetal migration and the formation of the first common metaphase plate on a common spindle, leading to the first cleavage division. These postfertilization-specific features proceed exclusively during the first zygotic cell cycle. The egg must be well equipped to receive the sperm at fertilization and it is mostly its cellular machinery that drives early development, the role of the sperm being to trigger all these events correctly. The egg acquires this machinery during oogenesis.

The entry into the first zygotic interphase is preceded by the meiotic maturation of the oocyte. The latter commences when a fully grown oocyte arrested in meiotic prophase and containing all the necessary equipment for future development undergoes germinal vesicle breakdown, that is, the breakdown of its nuclear membrane and the resumption of meiosis. The cell cycle of oocytes undergoing meiosis is profoundly modified. The first meiotic metaphase (M I) is followed by an unequal division resulting in extrusion of the small, first polar body and the entry into a new metaphase (second meiotic metaphase or M II) without formation of any interphase nucleus. At this stage, the cell cycle is once again arrested, but this time in M phase. The M II arrest is released during oocyte activation, a process normally triggered by a penetrating spermatozoon, but also experimentally by various parthenogenetic treatments. The oocyte undergoes another unequal cleavage leading to the extrusion of the second polar body followed by transition to a normal interphase, in contrast to the first meiotic division. Meiosis is completed and the first mitotic cell cycle follows. Cytoskeletal elements are actively involved in these events, since changes in the spatial organization within the cell are concerned. We will discuss the reorganization of the cytoskeleton during maturation and following fertilization and the way in which this is controlled both in events regulated by cell cycle as well as in those that seem to be developmentally regulated.

Cytoskeleton organization in oocytes and fertilized eggs

Among the three types of cytoskeletal elements, two are clearly present in mouse oocytes: microtubules and microfilaments (Abreu and Brinster, 1978; Wassarman and Fujiwara, 1978), the presence of intermediate filaments being more controversial. In both cases, their organization differs from that observed in somatic cells.

Microfilaments and the formation of the polar body

In the metaphase II egg, microfilaments are found mainly in the cortex but with a greater concentration in the area overlying the meiotic spindle that is located near, and parallel to, the cell surface (Maro *et al.*, 1984; Longo and Chen, 1985). This area of the cell surface is also devoid of microvilli (Eager *et al.*, 1976; Longo and Chen, 1985; Maro *et al.*, 1986), has relatively few binding sites for Concanavalin A, a lectin that binds to some surface molecules (Johnson *et al.*, 1975; Maro *et al.*, 1984), and is free of cortical granules (Ducibella *et al.*, 1988). It seems that this organization of the oocyte influences the process of fertilization. The spermatozoa do not normally fertilize the egg in this microvilli-poor and microfilament-rich area located over the meiotic spindle (Johnson *et al.*, 1975; Nicosia *et al.*, 1977). This functional differentiation of the oocyte surface during fertilization ensures that the spermatozoon will not be extruded from the oocyte cytoplasm together with the second polar body, which could easily happen if it was incorporated in the vicinity of the oocyte chromosomes. After fertilization, the meiotic cleavage furrow forms in this actin-rich domain of the cortex at the equator of the spindle. Two actin-rich shoulders are thereby created on each side of the furrow, and one of these subsequently shrinks while the other expands causing a rotation of the spindle and leading to the formation of the second polar body (Maro *et al.*, 1984). A similar process takes place during extrusion of the first polar body (Longo and Chen, 1985). In addition, following fertilization, an area free of microvilli and rich in microfilaments develops near the decondensing sperm nucleus forming the incorporation cone (Stefanini *et al.*, 1969; Shalgi *et al.*, 1978; Maro *et al.*, 1984). These two actin-rich, microvillous-free domains disappear when the pronuclei form and migrate towards the egg center and, at that time, numerous microfilaments can be observed around the pronuclei (Maro *et al.*, 1984). Experiments using Cytochalasin D, a microfilament inhibitor, suggest that microfilaments are not required for sperm entry at fertilization, but are necessary for spindle rotation, polar body formation and the migration of the pronuclei towards the center of the egg (Maro *et al.*, 1984).

A close association exists between the egg cortex and both the meiotic chromosomes and, after fertilization, the newly introduced male chromatin. In both cases it seems that the chromosomes

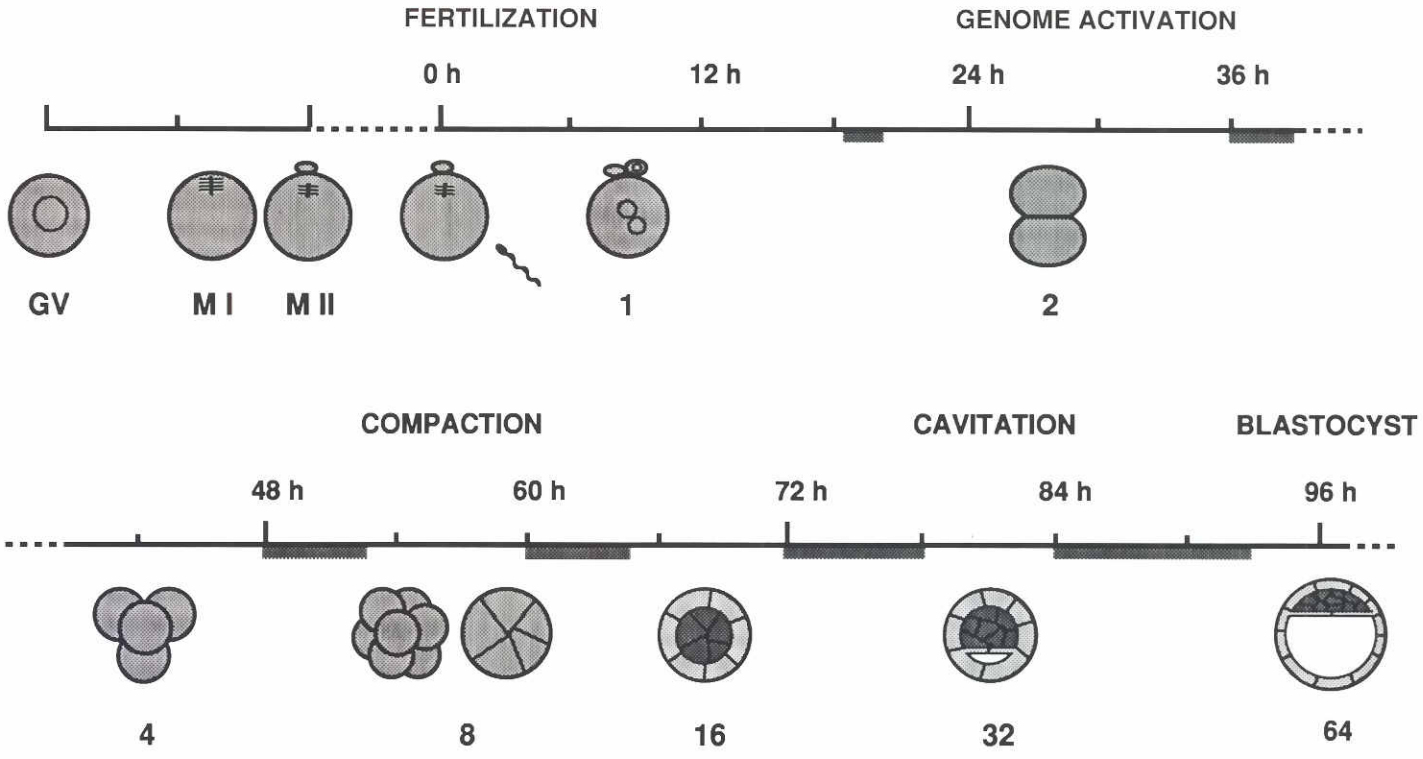

Fig. 1. Schematic view of early mouse development *showing the relationship between major developmental transitions and the cell cycle.*

induce the formation of a cortical focus of stable polymerised actin. The influence of metaphase II chromosomes on their immediate environment was investigated following their nocodazole-induced dispersal (Maro *et al.*, 1986). Each maternal chromosome cluster, as well as the non-dispersed sperm-derived chromosomes, induces a focal accumulation of cortical microfilaments and a loss of surface microvilli in the overlying membrane. If, however, nocodazole is removed later, multiple spindles form. Polar bodies are extruded in many of these cortical domains associated with the chromosomes (if a spindle is present) suggesting that the meiotic cleavage furrow is limited to these areas, thereby yielding unequal cleavage (Maro *et al.*, 1986). The hypothesis that the actin-rich cortical area overlying the meiotic spindle forms a domain to which the meiotic cleavage furrow is restricted is strengthened by experiments in which the cytoskeletal organization of the egg was studied during ageing (Webb *et al.*, 1986), where it was shown that the normal formation of a polar body is dependent upon the existence of a microfilament-rich domain overlying the spindle. The effect of chromatin on the cell cortex also explains the existence of the incorporation cone that develops at the sperm entry site. The sperm nuclear envelope breaks down because of the meiotic environment in the egg during the first 30 min after fertilization (Sato and Blandau, 1979). The presence of the non-enveloped male chromatin then alters the overlying cortical domain.

Microtubules

In most animal cells the microtubule organizing center (MTOC) is composed of a pair of centrioles surrounded by an electron dense material, the pericentriolar material (PCM), and it is within this material that the MTOC activity is located (Gould and Borisy, 1977). The mouse M II oocytes as well as M I and most probably those arrested in the meiotic prophase, do not have centrioles (Szöllösi *et al.*, 1972). The poles of the meiotic spindle are composed of bands of electron dense PCM (Fig. 2; Szöllösi *et al.*, 1972). This material reacts with monoclonal antibodies raised against mitotic phosphoproteins like MPM-2 (Vandre *et al.*, 1984; Maro *et al.*, 1988; Hiraoka *et al.*, 1989), isolated centrosomes (de Pennart, Bornens and Maro, unpublished data) or antibodies produced by some scleroderma patients like those present in the 5051 serum (Calarco-Gillam *et al.*, 1983; Maro *et al.*, 1985a). Using the latter, it was shown that, in addition to the polar bands of PCM, numerous cytoplasmic PCM foci can be observed in the M II oocyte cortex (Maro *et al.*, 1985a; Schatten *et al.*, 1986). However, whereas the PCM foci are dispersed within the egg, microtubules are seen only within the spindle (Fig. 4D; Wassarman and Fujiwara, 1978; Maro *et al.*, 1985a). Although the cytoplasmic PCM foci in the M II oocyte are inactive as MTOCs, they can be shown to have a nucleating capacity by the addition of taxol (a drug that decreases the critical concentration for tubulin polymerization; Schiff *et al.*, 1979), which induces multiple asters to form around each PCM focus (Maro *et al.*, 1985a).

Recently, we have followed the fate of the microtubules and of the PCM foci using MPM-2 during oocyte maturation (Fig. 3; Kubiak *et al.*, 1989). The PCMs present around the germinal vesicle (GV) of prophase oocytes nucleate numerous microtubules (Fig. 4A), which penetrate cytoplasmic invaginations in the wavy nuclear envelope (D. Szöllösi and M. Szöllösi, personal communication). Upon GV breakdown, some of them invade the nuclear area with

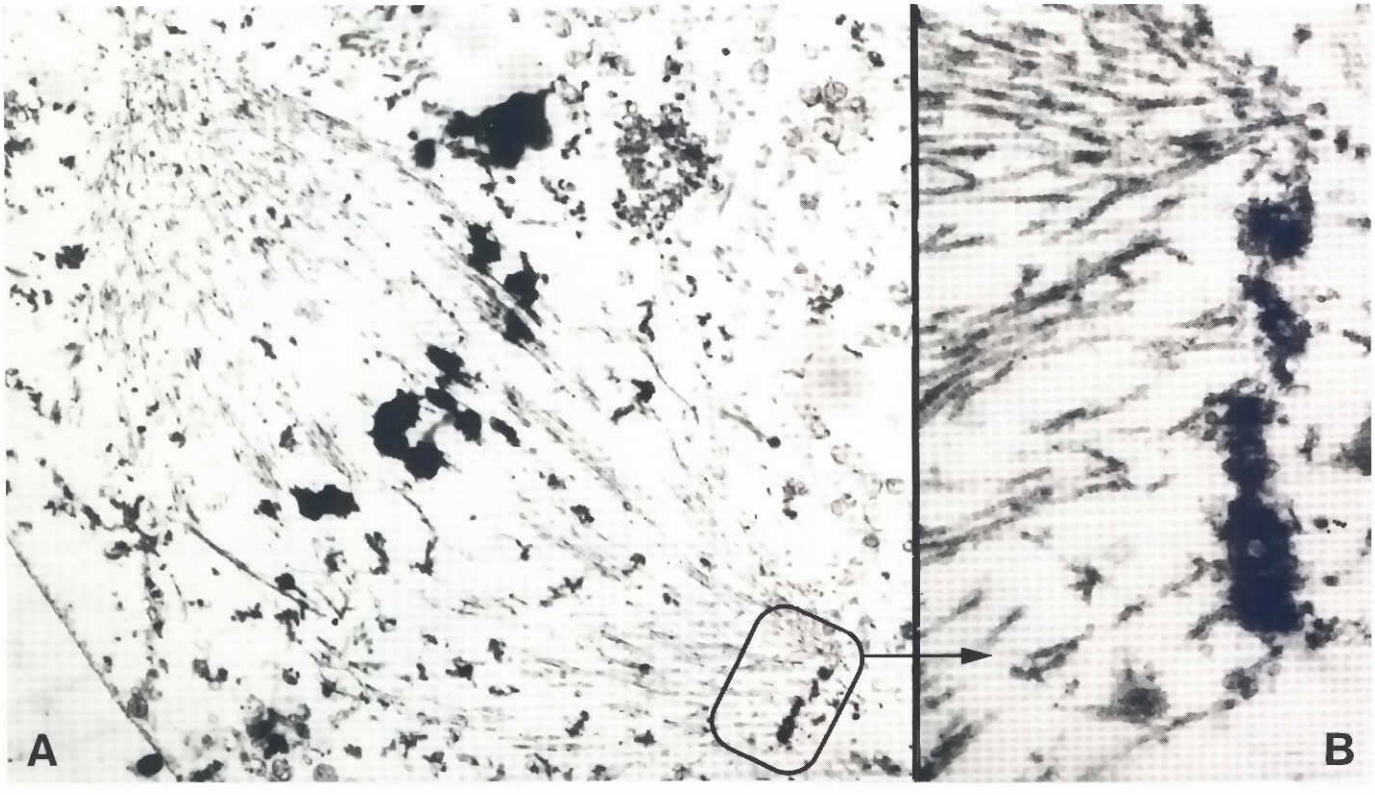

Fig. 2. (A) *Electron micrograph showing a second meiotic spindle.* **(B)** *Enlargement of the spindle pole showing the electron dense band of PCM. The oocyte was first extracted and then fixed as described in Houliston* et al. *(1987).*

many newly assembled microtubules forming around the condensing chromosomes (Fig. 4B). Most microtubules seem to form freely in the direct vicinity of the chromosomes without any specific relationships to the cytoplasmic MTOCs. However, at that point, not all PCM foci are located in the central part of the oocyte near the chromosomes; some are dispersed into the cytoplasm. Then they undergo a microtubule-dependent migration towards the centrally located chromosomes. When GV breakdown takes place in the presence of nocodazole, an inhibitor of microtubule assembly, two (less frequently one or three) relatively large PCM foci are found peripherally near the cell cortex. This suggests that in the absence of microtubules, they do not localize around the chromosomes but merge to form these larger structures which, usually, are never observed during the normal process. During the normal course of maturation, numerous small PCM foci gather around the chromosome mass and then migrate towards two opposite sides forming the poles of the broad M I spindle (Fig. 4C). Further changes include merging of the PCM foci into two or three larger structures at each spindle pole and dispersion of some of them into the cytoplasm of the oocyte. These observations show that the PCM foci are dynamic structures which change their shape, size and activity during the formation of the meiotic spindle.

After fertilization, and during completion of meiosis, G1 phase and early S phase, the cytoplasmic MTOCs stay in a peripheral position near the cell cortex. Numerous asters of microtubules appear and then enlarge to form a dense cytoplasmic network (Fig. 4E) that remains until the end of interphase (Schatten *et al.*, 1985). At that time, microtubules are involved in the migration of the pronuclei towards the center of the egg (Maro *et al.*, 1986). Then, at the end of S phase they migrate centrally towards the pronuclei (Maro *et al.*, 1985a). When the two pronuclear membranes break down, at prometaphase of first mitosis, numerous MTOCs are found around the two sets of chromosomes, and many half spindles originate from these MTOCs and invade the pronuclear area (Zamboni *et al.*, 1972). All these MTOCs then align to form the poles of the barrel-shaped mitotic spindle (Calarco-Gillam *et al.*, 1983; Maro *et al.*, 1985a).

Microtubules are modified in a cell cycle-dependent manner

It seems that usually the critical concentration for tubulin polymerization in the egg is high, so that microtubules can only polymerize in the region of the chromosomes. In contrast, following fertilization and passage of the egg from metaphase II of meiosis to interphase, a drop in the critical concentration for tubulin polymerisation seems to occur and multiple cortical asters form spontaneously around the PCM foci (Maro *et al.*, 1985a; Schatten *et al.*, 1985).

During M phase the cytoplasmic PCMs remain inactive and do not nucleate microtubules (Maro *et al.*, 1985a; Kubiak *et al.*, 1989). The only active PCMs at this stage are those located at the spindle poles. This reflects the effect of the chromosomes, which locally decrease the critical concentration for tubulin polymerization (Maro *et al.*, 1986). This was demonstrated by use of the microtubule inhibitors: nocodazole (which promotes depolymerization of micro

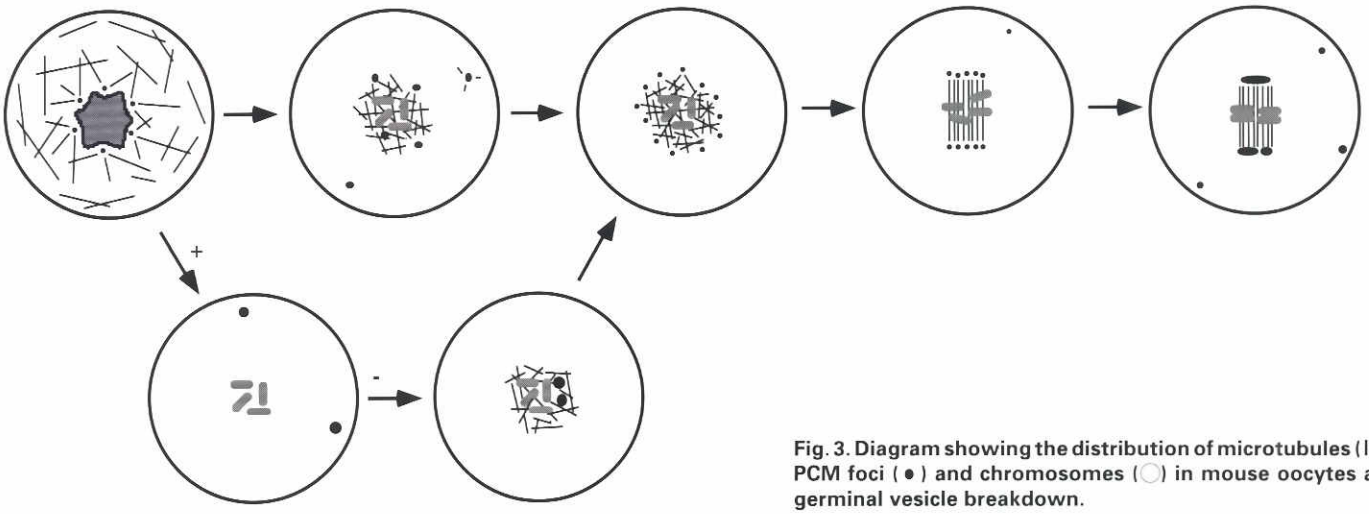

Fig. 3. Diagram showing the distribution of microtubules (|), PCM foci (•) and chromosomes (○) in mouse oocytes at germinal vesicle breakdown.

tubules) and taxol (which promotes microtubule assembly). In the presence of nocodazole, the oocyte becomes devoid of microtubules, and, in addition, the chromosomes disperse and do not remain associated with the PCM foci. However, upon removal of the drug and after a very short incubation (a few minutes) in the presence of taxol, new microtubules form around the PCMs, mainly around the chromosomes (without associated PCM foci). The nucleating activity of free chromosomes is observed also immediately after GV breakdown, when the nuclear area is filled with numerous microtubules without any involvement of the PCM foci.

The opposite properties of the MTOCs are manifested upon transition to interphase. Two examples of the interphase state are provided either by the prophase-arrested oocytes or by the activated, pronuclear eggs. In contrast to M phase oocytes, their cytoplasm is filled with an array of numerous microtubules. This network of microtubules in prophase oocytes is drastically disassembled upon GV breakdown (Alexandre *et al.*, 1989; Kubiak *et al.*, 1989) indicating their sensitivity to the M phase cytoplasmic environment, while the opposite is observed after the activation of M II oocyte (Maro *et al.*, 1985a; Schatten *et al.*, 1986). In the latter, extrusion of the polar body is followed by the transition to interphase and formation of a dense microtubular network clearly nucleated by the cytoplasmic PCM foci. In other words during interphase, the cytoplasmic conditions facilitate microtubule assembly on PCM foci (like taxol), while the M phase conditions have the reverse effect and inhibit microtubule assembly (similarly to the action of nocodazole).

These properties are also illustrated in experiments in which M II oocytes were fused with interphase blastomeres (Kubiak, 1988). Fusion of these two different cells results in premature chromatin condensation (PCC) of the blastomere nucleus if the oocyte is not activated and remains in M phase (Tarkowski and Balakier, 1980). This is due to the presence of chromosome condensation activity of the maturation promoting factor (MPF; Johnson and Rao, 1971; Masui and Markert, 1971). The process of PCC is accompanied by characteristic changes in the microtubular cytoskeleton. During the first few minutes after fusion the interphase network of the blastomere disassembles. This is rapidly followed by microtubule assembly around the prematurely condensed blastomere chromosomes when the nuclear envelope breaks down (Kubiak, 1988). However, if activation takes place around the time of fusion, the microtubule network of the blastomere disassembles since the M phase environment still exists in the oocyte, but later, the hybrid cell reforms its interphase network and the blastomere nucleus remains intact, while the oocyte forms a pronucleus. In this case, the unscheduled changes in the organization of the microtubule network are caused by modifications in the cytoplasmic environment due to the cell fusion.

The relationship between the cell cycle stage and microtubule behavior can also be demonstrated during abortive activation of M II oocytes (Kubiak, 1989). Early M II oocytes when activated can extrude a second polar body, but are unable to undergo the transition to interphase. They enter a subsequent M phase arrest, the M III arrest, third meiotic metaphase. The M III oocytes behave like M II oocytes, the main difference residing in the number of chromosomes: 20 single chromatids instead of 20 pairs of sister chromatids. An M III spindle is present without any other microtubules despite the presence of PCM foci dispersed in the cortical region (Kubiak and Maro, unpublished data). During abortive activation leading to M III formation, the oocyte extrudes a second polar body which also enters M phase. The midbody formed between the second polar body and the M III oocyte disassembles during the first hour after polar body extrusion. In normally activated eggs, the midbody persists for many hours, and most probably until entry into the subsequent M phase (Kidder *et al.*, 1988). This behavior provides some evidence that even the very stable midbody microtubules modify their properties in response to changes in the cell cycle stage.

The changes in the behavior of microtubules during the cell cycle also affect the way tubulin is post-translationally modified. The M II spindle is composed of acetylated and tyrosinated microtubules without any detectable detyrosinated microtubules (de Pennart *et al.*, 1988). This contrasts with activated eggs where all three subclasses of microtubules are detected. These post-translational modifications reflect the stability of the microtubules. Acetylation was observed mostly in spindle kinetochore bundles, midbody and some interphase microtubules, while detyrosination was only observed in the interphase midbody microtubules. The differences

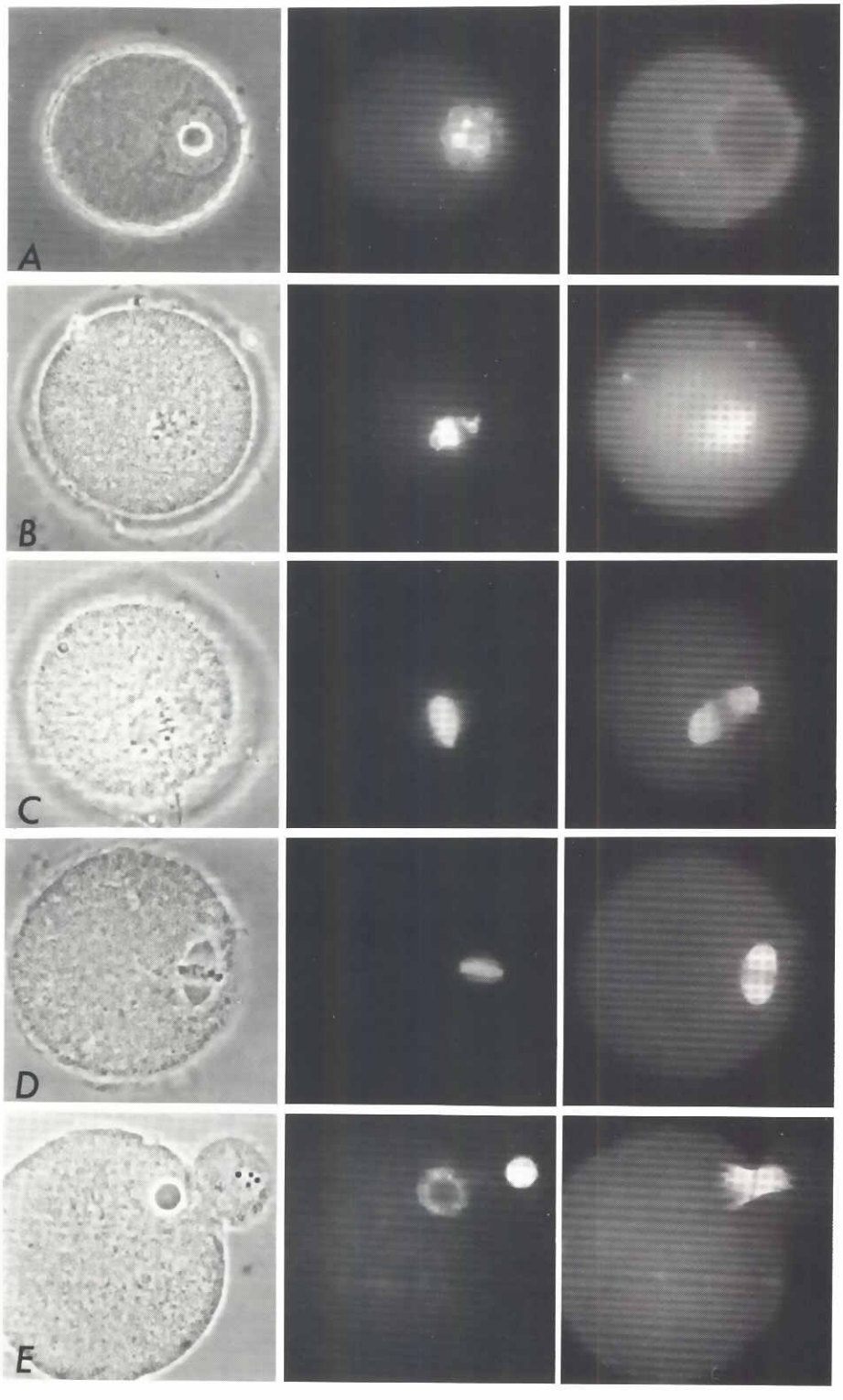

Fig. 4. Microtubule distribution during mouse oocyte maturation and activation. *Left: phase contrast; center: chromatin staining (DAPI); right: tubulin staining.* **(A)** *Meiotic prophase;* **(B)** *germinal vesicle breakdown;* **(C)** *metaphase I;* **(D)** *metaphase II;* **(E)** *parthenogenetically activated egg. Oocytes were fixed and stained as described in de Pennart* et al. *(1988).*

in the stability of M phase and interphase microtubules were tested in M II oocytes (de Pennart *et al.*, 1988) and eight-cell blastomeres (Houliston and Maro, 1989) respectively by means of nocodazole treatment. These experiments showed that the midbody microtubules were the most stable subpopulation of microtubules (half-life >> 1 h) while interphase microtubules (half-life = 30-60 min) were more stable than the microtubules found in the kinetochore fibers of the spindle (half-life = 5-10 min) which were themselves more stable than the pole-to-pole microtubules of the spindle (half-life = 1 min). There is a good correlation between the stability of these

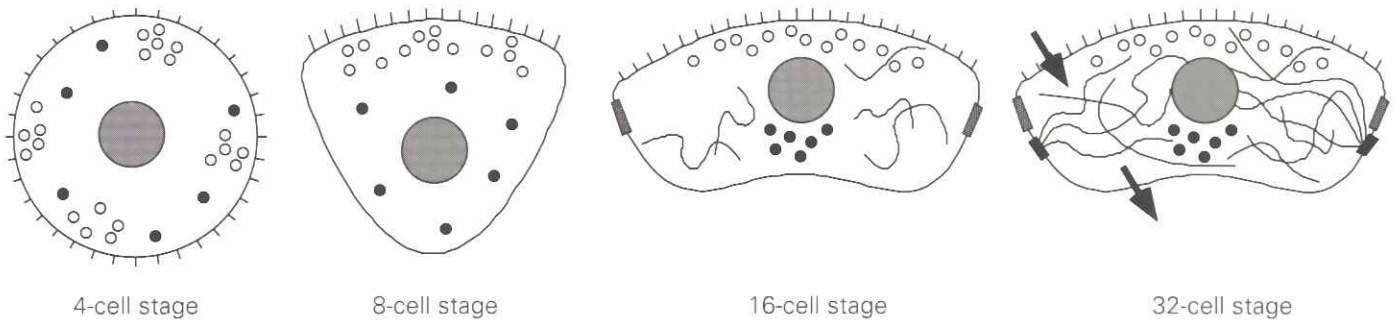

Fig. 5. Diagram showing the acquisition of the epithelial phenotype in the outer layer of cells during preimplantation development of the mouse. *Endosomes, clathrin (○); lysosomes, golgi (●); tight junctions (▬); desmosomes (▬); keratin filaments (∽); vectorial transport (→).*

subclasses of microtubules and their post-translational modifications: detyrosinated microtubules being more stable than acetylated microtubules.

Compaction and cell diversification

Compaction

The first steps involved in the diversification between the first two cell lineages of the mouse embryo occur during compaction at the 8–cell stage. At this time, the blastomeres flatten upon each other, poles of microvilli form on the apical surface and major cytoplasmic reorganization takes place in each cell. First, gap junctions assemble in the basal part of the cell (Lo and Gilula, 1979; Goodall and Johnson, 1982) and an apical pole of endosomes (Reeve, 1981), clathrin vesicles (Maro *et al.*, 1985b), microfilaments (Johnson and Maro, 1984) and microtubules (Houliston *et al.*, 1987) form. Second, an apical pole of microvilli (Ziomek and Johnson, 1980) develops on the apical part of the blastomere while cells flatten on each other (the calcium-dependent cell adhesion molecule involved in this process is uvomorulin; Hyafil *et al.*, 1980; Kemler *et al.*, 1977). By the end of the 8-cell stage, the distributions of many surface features and cytoplasmic components of blastomeres have changed from being radially symmetric to polarized. 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).

Microfilaments

Microfilaments are present in both the surface and cytoplasmic poles (Johnson and Maro, 1984). In addition, microfilaments are involved in compaction since cytochalasin D inhibits and reverses intercellular flattening (Pratt *et al.*, 1982; Johnson and Maro, 1984). Cytochalasin D inhibits the formation of surface poles (although it is difficult to interpret this since they are part of the microvilli themselves; Fleming *et al.*, 1986), but does not destroy fully formed microvillous poles (Johnson and Maro, 1984). Moreover, although it prevents the development of cytoplasmic polarity, it does not destroy it once formed (Johnson and Maro, 1985; Fleming *et al.*, 1986). Thus, it is possible that stabilization of microfilaments in the microvilli and in the cytoplasm takes place after the setting up of asymmetries. This latter process seems to be microtubule-dependent (for review see Johnson and Maro, 1986). The distribution of various actin-associated proteins has been studied at the 8-cell stage and a redistribution of actin, myosin, vinculin and spectrin in the cortex underlying and adjacent to the contact zone between blastomeres has been described (Sobel, 1983; Johnson and Maro, 1984; Lehtonen and Reima, 1986; Reima and Lehtonen, 1985; Sobel *et al.*, 1988).

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). It thus seems that microtubules redistribute during the cell polarization at compaction to concentrate in those regions of the blastomeres that are distant from cell contact zones. This process is facilitated by the cell flattening which also occurs at this time. Functional microfilaments are necessary for the redistribution of microtubules during compaction to occur normally, although this requirement can be accounted for by the involvement of microfilaments in the cell shape change (E. Houliston, S. J. Pickering and B. Maro, unpublished data). Nucleation of microtubules by PCM foci does not seem to be involved in the microtubule redistribution, since it has been demonstrated that although PCM aggregates form in the apical part of the blastomeres during the 8-cell stage and can promote microtubule nucleation, the process of PCM redistribution follows that of microtubules and is microtubule-dependent (Houliston *et al.*, 1987). In contrast, acetylated microtubules do not show the same apical concentration as the total microtubule population in compacted blastomeres. They are found mainly in the basal part of the cell, near the cortex (Houliston and Maro, 1989). When microtubules were depolymerised with nocodazole, these acetylated microtubules were found to be more stable, thus supporting the idea that a subpopulation of microtubules that is less dynamic exists near the cell contact areas. The relative concentration of acetylated microtubules in basal regions is not a consequence of a localized acetylase activity, since apical microtubules become acetylated when sta-

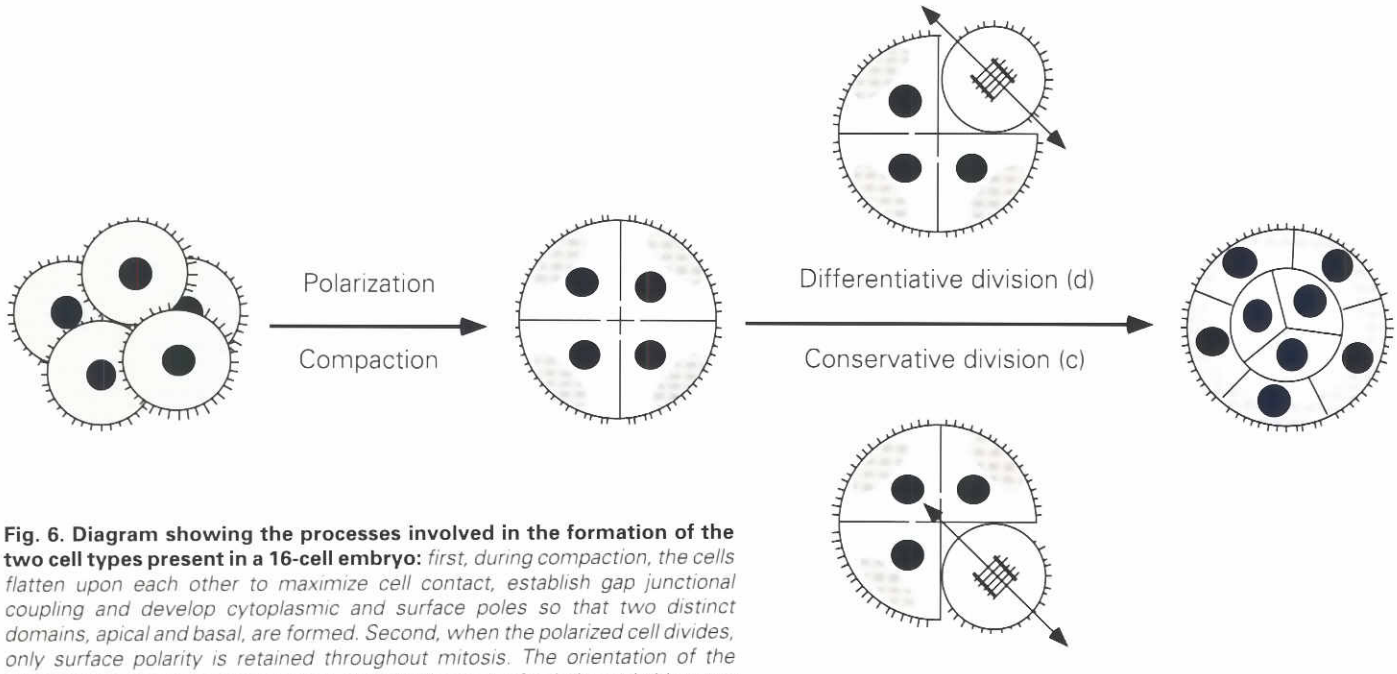

Fig. 6. Diagram showing the processes involved in the formation of the two cell types present in a 16-cell embryo: *first, during compaction, the cells flatten upon each other to maximize cell contact, establish gap junctional coupling and develop cytoplasmic and surface poles so that two distinct domains, apical and basal, are formed. Second, when the polarized cell divides, only surface polarity is retained throughout mitosis. The orientation of the division plane varies according to the longitudinal axis of polarity and either two polar cells, or one polar cell and one nonpolar cell can result. Cytoplasmic polarity is restored only in those cells that have inherited a noticeable part of the surface pole. These cell divisions are called differentiative when the two daughter cells have different phenotypes (for example the two cells labeled d), and conservative when they both inherit the polarized phenotype (for example the two cells labeled c). Third, polar and nonpolar cells differ in their adhesive properties. Apolar cells are uniformly adhesive whereas the polar cells have an adhesive basolateral surface and a nonadhesive microvillous pole. Both cell types attempt to maximize contact between adhesive surfaces and the result is that the polar cells tend to enclose the nonpolar cells.*

bilized by taxol (Houliston and Maro, 1989).

When microtubule inhibitors were used during compaction, the following observations were made. Microtubule depolymerisation by nocodazole allows the formation of gap junctions, intercellular flattening and the development of surface polarity and inhibits the formation of cytoplasmic poles (Ducibella and Anderson, 1975; Ducibella, 1982; Maro and Pickering, 1984; Johnson and Maro, 1985; Fleming *et al.*, 1986; Goodall and Maro, 1986). In contrast, microtubule stabilization 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; Maro and Pickering, 1984; Johnson and Maro, 1985; Goodall and Maro, 1986). These results suggest a constraining effect of the microtubules on most of the components of compaction studied and their absolute requirement for cytoplasmic organization.

The possible involvement of the microtubule network during compaction can be summarized in the following way. The reduction in the number of microtubules in the basal part 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 stabilize the microvilli of the surface pole and the organelles of the cytoplasmic pole. The secondary relocalization of the PCM foci to the apical part of the cell would reinforce the initial asymmetry in the distribution of the microtubules by nucleation of new microtubules in the apical domain. 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.

Alternative routes for surface polarization during compaction

During compaction, 8-cell blastomeres flatten upon each other and polarize along an axis perpendicular to cell contacts. If the process of flattening is prevented, polarization can still occur, but does so in a lower proportion of cells, and without the normal contact-directed orientation (Johnson *et al.*, 1986). In non-flattened cells polarization is favored in cells whose nuclei are located close to the cell surface, and the positions of surface poles and of nuclei tend to coincide. In these cells, microtubules mediate this association between poles and nuclei and are required for surface polarization to occur (Houliston *et al.*, 1989). In contrast, cells treated with nocodazole but allowed to flatten polarize at the surface. In conclusion, surface polarization of mouse blastomeres can be accomplished by at least two alternative routes: one requires flattening but is independent of microtubules; the other 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). It must also be noted that the enrichment of acetylated microtubules in the basal part of the cell cortex during compaction is contact dependent (E. Houliston and B. Maro, unpublished data).

Cell diversification

From the 8-cell stage to the blastocyst, the outer cells differen-

tiate and acquire the characteristic features of an epithelium (Fig. 5; for review see Fleming and Johnson, 1988). After the setting up of asymmeties within cells at the 8-cell stage, the formation of an outer layer of polarized cells takes place at the 16-cell stage because of the existence of asymmetric divisions.

Asymmetric divisions

During mitosis the interphase microtubule network disassembles and is replaced by the mitotic spindle. Regardless of the loss of cytoplasmic polarity, a polarized organization of the cell surface can still be detected (Johnson *et al.*, 1988). 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 two polar cells or one polar and one apolar cell result (Fig. 6; for review see Johnson and Maro, 1986). In the latter case, the division is said to be "differentiative". In 8-cell embryos there is considerable variation in the number of polarized blastomeres that divide differentiatively to give one polar and one apolar cell. 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 a certain aspect of the asymmetric organization persists during division (Johnson and Ziomek, 1981a). Whether or not a blastomere divides differentiatively does not seem to be determined randomly, since early dividing blastomeres tend to do so more frequently (Kelly *et al.*, 1978; Spindle, 1982). This higher incidence of differentiative divisions among the early dividing cells is a consequence of their more extensive intercellular contacts (Kelly *et al.*, 1978; Garbutt *et al.*, 1987). Cell interactions by themselves do not seem to significantly influence the overall pattern of division planes within the population. In contrast, interactions between the cells influence the type of progeny generated at division to the 16-cell stage via an effect on the size of the surface pole of microvilli (Pickering *et al.*, 1988).

Microtubules and cell diversification

At the 16-cell stage, polarized cells tend to envelope apolar cells because of differences in their adhesive properties: in polar cells, the apical surface is less adhesive than the basolateral surface while apolar cells are uniformly adhesive and make the maximum possible contact with other cells. Following asymmetric division of polarized cells, microtubules were found to be more abundant in outside cells while acetylated microtubules accumulated preferentially in inside cells (Houliston and Maro, 1989). These patterns of microtubules observed in outside and inside cells were remarkably similar to those present in the apical and basal domains of polarized 8-cell blastomeres respectively. Cortical microtubules were again preferentially acetylated. It is interesting to note that although there are more acetylated microtubules in inside cells than in outside cells at the 16- and 32-cell stages, this is 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 become an intrinsic property of the cells at the 16-cell stage (E. Houliston and B. Maro, unpublished data). The relationship between the differences in the organization of the microtubule network in inner and outer cells and the differences in cell fate is still unknown. It will be of great interest to elucidate this point.

Conclusion and perspectives

A blastocyst, with its two cell types, is a simple structure, but despite the number of studies dealing with its formation, many questions concerning both compaction and the subsequent cell diversification remain unanswered. Compaction plays a major role in the initiation of cell diversification through intercellular flattening and cell polarization. The way these events are regulated is still poorly understood. It seems that a change, which may correspond to release from a constraint (Levy *et al.*, 1986) and involve dephosphorylations (Aghion and Maro, 1989; Bloom, 1989), initiate these two processes, leading to the observed local destabilization of the cytoskeleton, with loss of microvilli, microfilaments and microtubules in the area of cell contact. If the nature of the molecules involved in the adhesion process is now better understood (Kemler *et al.*, 1977; Hyafil *et al.*, 1980; Hyafil *et al.*, 1981; Mansouri *et al.*, 1988; Ozawa *et al.*, 1989), the regulatory molecules involved in the triggering of compaction and their targets associated with the cytoskeleton have not yet been characterized. A similar observation can be made concerning fertilization, although progress has been made towards this characterization (Maro *et al.*, 1988).

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References

ABREU, S. L. and BRINSTER, R. L. (1978). Synthesis of tubulin and actin during the preimplantation development of the mouse. *Exp. Cell Res. 114:* 134-141.

AGHION, J. and MARO, B. (1989). 6 dimethylaminopurine-induced flattening in mouse preimplantation embryos. *Cell Differentiation 27S:* S141.

ALEXANDRE, H., VAN CAUWENBERGE, A. and MULNARD, J. (1989). Involvement of microtubules and microfilaments in the control of the nuclear movement during maturation of mouse oocyte. *Dev. Biol. 136:* 311-320.

BLOOM, T. L. (1989). The effects of phorbol ester on mouse blastomeres: a role for protein kinase C in compaction? *Development 106*: 159-171.

CALARCO-GILLAM, P. D., SIEBERT, M. C., HUBBLE, R., MITCHISON, T. and KIRSCHNER, M. (1983). Centrosome development in early mouse embryos as defined by an autoantibody against pericentriolar material. *Cell 35:* 621-629.

DE PENNART, H., HOULISTON, E. and MARO, B. (1988). Post-translational modifications of tubulin and the dynamics of microtubules in mouse oocytes and zygotes. *Biol. Cell 64:* 375-378.

DUCIBELLA, T. (1982). Depolymerization of microtubules prior to compaction. *Exp. Cell Res. 138:* 31-38.

DUCIBELLA, T. and ANDERSON, E. (1975). Cell shape and membrane changes in the eight cell mouse embryo: prerequesite for morphogenesis of the blastocyst. *Dev. Biol. 47:* 45-58.

DUCIBELLA, T., ANDERSON, E., ALBERTINI, D. F., AALBERG, J. and RANGARAJAN, S. (1988). Quantitative studies of changes in cortical granule number and distribution in the mouse oocyte during meiotic maturation. *Dev. Biol. 130:* 184-197.

EAGER, D., JOHNSON, M. H. and THURLEY, K. W. (1976). Ultrastructural studies on the surface membrane of the mouse egg. *J. Cell Sci. 22:* 345-353.

FLACH, G., JOHNSON, M. H., BRAUDE, P. R., TAYLOR, R. S. and BOLTON, V. N. (1982). The transition from maternal to embryonic control in the 2-cell mouse embryo. *EMBO J. 1:* 681-686.

FLEMING, T. P., CANNON, P. and PICKERING, S. J. (1986). The role of the cytoskeleton in generating and stabilising an asymmetric distribution of endocytotic organelles during the process of cell polarization in the mouse preimplantation embryo. *Dev.*

Biol. 113: 406-419.

FLEMING, T. P. and JOHNSON, M. H. (988). From egg to epithelium. *Ann. Rev. Cell Biol. 4:* 459-485.

FLEMING, T. P., PICKERING, S. J., QASIM, F. and MARO, B. (1986). The generation of cell surface polarity in mouse 8-cell blastomeres: the role of cortical microfilaments analyzed using cytochalasin D. *J. Embryol. Exp. Morphol. 95:* 169-191.

GARBUTT, C. L., JOHNSON, M. H. and GEORGE, M. A. (1987). When and how does division order influence cell allocation to the inner cell mass of the mouse blastocyst? *Development 100:* 325-332.

GOODALL, H. and JOHNSON, M. H. (1982). Use of carboxyfluorescein diacetate to study formation of permeable channels between mouse blastomeres. *Nature (Lond.) 295:* 524-526.

GOODALL, H. and MARO, B. (1986). Major loss of junctional communication during mitosis in early mouse embryos. *J. Cell Biol. 102:* 568-575.

GOULD, R. R. and BORISY, G. G. (1977). The pericentriolar material in Chinese ovary cells nucleates microtubule formation. *J. Cell Biol. 73:* 601-615.

HIRAOKA, L., GOLDEN, W. and MAGNUSON, T. (1989). Spindle pole organization during early mouse development. *Dev. Biol. 133:* 24-36.

HOULISTON, E., PICKERING, S. J. and MARO, B. (1987). Redistribution of microtubules and peri-centriolar material during compaction in mouse blastomeres. *J. Cell Biol. 104:* 1299-1308.

HOULISTON, E., PICKERING, S. J. and MARO, B. (1989). Alternative routes for the establishment of surface polarity during compaction of the mouse embryo. *Dev. Biol. 134:* 342-350.

HOULISTON, H. and MARO, B. (1989). Posttranslational modification of distinct microtubule subpopulations during cell polarization and differentiation in the mouse preimplantation embryo. *J. Cell Biol. 108:* 543-551.

HYAFIL, F., BABINET, C. and JACOB, F. (1981). Cell-cell interactions in early embryogenesis: a molecular approach to the role of calcium. *Cell 26:* 447-454.

HYAFIL, F., MORELLO, D., BABINET, C. and JACOB, F. (1980). A cell surface glycoprotein involved in the compaction of embryonal carcinoma cells and cleavage stage embryos. *Cell 21:* 927-934.

JOHNSON, M. H. (1985). Manipulation of early mammalian development: what does it tell us about cell lineages? In *Developmental Biology: a Comprehensive Synthesis* (Ed. L. Browder). Plenum Press.

JOHNSON, M. H., EAGER, D., MUGGLETON-HARRIS, A. L. and GRAVES, H. M. (1975). Mosaicism in the organisation of concanavalin A receptors on surface membrane of mouse eggs. *Nature (Lond.) 257:* 321-322.

JOHNSON, M. H. and MARO, B. (1984). The distribution of cytoplasmic actin in mouse 8-cell blastomeres. *J. Embryol. Exp. Morphol. 82:* 97-117.

JOHNSON, M. H. and MARO, B. (1985). A dissection of the mechanisms generating and stabilising polarity in mouse 8 and 16-cell blastomeres: the role of cytoskeletal elements. *J. Embryol. Exp. Morphol. 90:* 311-334.

JOHNSON, M. H. and MARO, B. (1986). Time and space in the early mouse embryo: a cell biological approach to cell diversification. In *Experimental Approaches to Mammalian Embryonic Development* (Eds. J. Rossant and R. Pedersen). Cambridge University Press, Cambridge. 35-65.

JOHNSON, M. H., MARO, B. and TAKEICHI, M. (1986). The role of cell adhesion in the synchronisation and orientation of polarization in 8-cell mouse blastomeres. *J. Embryol. Exp. Morphol. 93:* 239-255.

JOHNSON, M. H., PICKERING, S. J., DHIMAN, A., RADCLIFFE, G. S. and MARO, B. (1988). Cytocortical organisation during natural and prolonged mitosis of mouse 8-cell blastomeres. *Development 102:* 143-158.

JOHNSON, M. H. and ZIOMEK, C. A. (1981a). The foundation of two distinct cell lineages within the mouse morula. *Cell 24:* 71-80.

JOHNSON, M. H. and ZIOMEK, C. A. (1981b). Induction of polarity in mouse 8-cell blastomeres: specificity, geometry and stability. *J. Cell Biol. 91:* 303-308.

JOHNSON, R. T. and RAO, P. N. (1971). Nucleocytoplasmic interactions in the achievement of nuclear synchrony in DNA synthesis and mitosis in multinucleate cells. *Biol. Rev. 46:* 97-155.

KELLY, S. J., MULNARD, J. G. and GRAHAM, C. F. (1978). Cell division and cell allocation in early mouse development. *J. Embryol. Exp. Morphol. 48:* 37-51.

KEMLER, R., BABINET, C., EISEN, H. and JACOB, F. (1977). Surface antigen in early differentiation. *Proc. Natl. Acad. Sci. USA 74:* 4449-4452.

KIDDER, G. M., BARRON, D. J. and OLMSTED, J. B. (1988). Contribution of midbody channels to embryogenesis in the mouse: analysis by immunofluorescence. *Roux Arch. Dev. Biol. 197:* 110-114.

KUBIAK., J.Z. (1988). Investigations on oocyte activation and pronuclear growth during early mouse development. PhD Thesis, University of Warsaw.

KUBIAK, J.Z., DE PENNART, H. and MARO, B. (1989). Formation of the first meiotic spindle (MI) in mouse oocytes. *Cell Differ. 27S:* S137.

KUBIAK, J. Z. (1989). Mouse oocytes gradually develop the capacity for activation during the metaphase II arrest. *Dev. Biol. 136:* 537-545.

LEHTONEN, E. and REIMA, I. (1986). Changes in the distribution of vinculin during preimplantation mouse development. *Differentiation 32:* 125-134.

LEVY, J. B., JOHNSON, M. H., GOODALL, H. and MARO, B. (1986). Control of the timing of compaction: a major developmental transition in mouse early embryogenesis. *J. Embryol. Exp. Morphol. 95:* 213-237.

LO, C. W. and GILULA, N. B. (1979). Gap junctional communication in the preimplantation mouse embryo. *Cell 18:* 411-422.

LONGO, F. J. and CHEN, D. Y. (1985). Development of cortical polarity in mouse eggs: involvement of the meiotic apparatus. *Dev. Biol. 107:* 382-394.

MANSOURI, A., SPURR, N., GOODFELLOW, P. N. and KEMLER, R. (1988). Characterization and chromosomal localization of the gene encoding the human cell adhesion molecule uvomorulin. *Differentiation 38:* 67-71.

MARO, B., HOULISTON, E. and PAINTRAND, M. (1988). Purification of meiotic spindles and cytoplasmic asters from mouse oocytes. *Dev. Biol. 129:* 275-282.

MARO, B., HOWLETT, S. K. and WEBB, M. (1985a). Non-spindle microtubule organizing centers in metaphase II-arrested mouse oocytes. *J. Cell Biol. 101:* 1665-1672.

MARO, B., JOHNSON, M. H., PICKERING, S. J. and FLACH, G. (1984). Changes in actin distribution during fertilization of the mouse egg. *J. Embryol. Exp. Morphol. 81:* 211-237.

MARO, B., JOHNSON, M. H., PICKERING, S. J. and LOUVARD, D. (1985b). Changes in the distribution of membranous organelles during mouse early embryogenesis. *J. Embryol. Exp. Morphol. 90:* 287-309.

MARO, B., JOHNSON, M. H., WEBB, M. and FLACH, G. (1986). Mechanism of polar body formation in the mouse oocyte: an interaction between the chromosomes, the cytoskeleton and the plasma membrane. *J. Embryol. Exp. Morphol. 92:* 11-32.

MARO, B. and PICKERING, S.J. (1984). Microtubules influence compaction in preimplantation mouse embryos. *J. Embryol. Exp. Morphol. 84:* 217-232.

MASUI, Y. and MARKERT, C. L. (1971). Cytoplasmic control of nuclear behavior during meiotic maturation of frog oocytes. *J. Exp. Zool. 117:* 129-146.

NICOSIA, S. V., WOLF, D. P. and INOUE, M. (1977). Cortical granule distribution and cell surface characteristics in mouse eggs. *Dev. Biol. 57:* 56-74.

OZAWA, M., BARIBAULT, H. and KEMLER, R. (1989). The cytoplasmic domain of cell adhesion molecule uvomorulin associates with three independent proteins structurally related in different species. *EMBO J. 8:* 1711-1717.

PICKERING, S. J., MARO, B., JOHNSON, M. H. and SKEPPER, J. (1988). The influence of cell contact on the division of mouse 8-cell blastomeres. *Development 103:* 353-363.

PIKO, L. and CLEGG, K. B. (1982). Quantitative changes in total RNA, total poly A and ribosomes in early mouse embryos. *Dev. Biol. 89:* 362-378.

PRATT, H. P. M., ZIOMEK, C. A., REEVE, W. J. D. and JOHNSON, M. H. (1982). Compaction of the mouse embryo: an analysis of its components. *J. Embryol. Exp. Morphol. 70:* 113-132.

REEVE, W. J. D. (1981). The distribution of ingested horseradish peroxidase in the 16-cell mouse embryo. *J. Embryol. Exp. Morphol. 66:* 191-207.

REIMA, I. and LEHTONEN, E. (1985). Localization of nonerythroid spectrin and actin in mouse oocytes and preimplantation embryos. *Differentiation 30:* 68-75.

SATO, K. and BLANDAU, R. J. (1979). Second meiotic division and polar body formation in mouse eggs fertilized *in vitro*. *Gamete Res. 2:* 283-293.

SCHATTEN, G., SIMERLY, C. and SCHATTEN, H. (1985). Microtubule configuration during fertilization, mitosis and early development in the mouse. *Proc. Natl. Acad. Sci. USA 82:* 4152-4156.

SCHATTEN, H., SCHATTEN, G., MAZIA, D., BALCZON, R. and SIMERLY, C. (1986). Behavior of centrosomes during fertilization and cell division in mouse oocytes and in sea urchin eggs. *Proc. Natl. Acad. Sci. USA 83:* 105-109.

SCHIFF, P. B., FANT, J. and HORWITZ, S. B. (1979). Promotion of microtubule assembly *in vitro* by taxol. *Nature (Lond.) 277:* 665-667.

SHALGI, R., PHILLIPS, D. M. and KRAICER, P. F. (1978). Observations on the incorporation cone in the rat. *Gamete Res. 1:* 27-37.

SOBEL, J. S. (1983). Cell-cell contact modulation of myosin organization in the early mouse embryo. *Dev. Biol. 100:* 207-213.

SOBEL, J. S., GOLDSTEIN, E. G., VENUTI, J. M. and WELSH, M. J. (1988). Spectrin and calmodulin in spreading mouse blastomeres. *Dev. Biol. 126:* 47-56.

SPINDLE, A. I. (1982). Cell allocation in preimplantation mouse chimeras. *J. Exp. Zool. 235:* 361-367.

STEFANINI, M., OURA, C. and ZAMBONI, L. (1969). Ultrastructure of fertilization in the mouse: penetration of sperm into the ovum. *J. Submicrosc. Cytol. 1:* 1-23.

SZÖLLÖSI, D., CALARCO, P. and DONAHUE, R. P. (1972). Absence of centrioles in the first and second meiotic spindles of mouse oocytes. *J. Cell Sci. 11:* 521-541.

TARKOWSKI, A. K. and BALAKIER, H. (1980). Nucleocytoplasmic interactions in cell hybrids between mouse oocytes, blastomeres and somatic cells. *J. Embryol. Exp. Morphol. 55:* 319-330.

TARKOWSKI, A. K. and WRÓBLEWSKA, J. (1967). Development of blastomeres of mouse eggs isolated at the 4- and 8-cell stage. *J. Embryol. Exp. Morphol. 18:* 155-180.

VANDRE, D. D., DAVIS, F. M., RAO, P. N. and BORISY, G. G. (1984). Phosphoproteins are components of mitotic microtubule organizing centers. *Proc. Natl. Acad. Sci. USA 81:* 4439-4443.

WASSARMAN, P. M. and FUJIWARA, K. (1978). Immunofluorescent anti-tubulin staining of spindles during meiotic maturation of mouse oocytes *in vitro*. *J. Cell Sci. 29:* 171-188.

WEBB, M., HOWLETT, S. K. and MARO, B. (1986). Parthogenesis and cytoskeleton organisation in ageing mouse oocyte. *J. Embryol. Exp. Morphol. 95:* 131-145.

ZAMBONI, L., CHAKRABORTY, J. and MOORE-SMITH, D. (1972). First cleavage division of the mouse zygote: an ultrastructural study. *Biol. Reprod. 7:* 170-193.

ZIOMEK, C. A. and JOHNSON, M. H. 1980. Cell surface interactions induce polarization of mouse 8-cell blastomeres at compaction. *Cell 21:* 935-942.