Redistribution of Microtubules and Pericentriolar Material during the Development of Polarity in Mouse Blastomeres

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Abstract. The distribution of microtubules and microtubule organizing centers (MTOCs) during the development of cell polarity in eight-cell mouse blastomeres was studied by immunofluorescence and immunoelectron microscopy using monoclonal anti-tubulin antibodies and an anti-pericentriolar material (PCM) serum. In early eight-cell blastomeres microtubules were found mainly around the nucleus and in the cell cortex, whereas PCM foci were observed dispersed in the cytoplasm. During the eight-cell stage, microtubules disappeared from the area adjacent to the zone of intercellular contact and accumulated in the apical part of the cell while their number decreased in the basal domain. The PCM also relocalized to the apical domain of the cell, but this occurred after the redistribution of the microtubules by a mechanism that involved the microtubule network. The possible roles of both MTOCs and microtubules in establishing cell polarity are discussed.

A role for microtubules and their associated microtubule organizing centers (MTOCs)[1] has been demonstrated in many important cellular processes such as cell motility, cell division, control of cell shape, and cytoplasmic organization (for reviews see Solomon, 1981; De Brabander, 1982; McIntosh, 1983). Microtubules play a major part in organizing the distribution of various cytoplasmic organelles such as mitochondria (Heggeness et al., 1978), Golgi apparatus (for a review see Thyberg and Moskalewski, 1985), coated vesicles (Imhof et al., 1983; Pfeffer et al., 1983; Johnson and Maro, 1985), and intermediate filaments (Goldman and Knipe, 1972; Singer et al., 1981; Maro et al., 1983). This role of microtubules in the intracellular localization of organelles suggests that they are important for the maintenance of asymmetry within cells and thus for cell polarity. It may well be that a consistent and defined asymmetry of microtubules is dependent upon the positioning of MTOCs since in many cell types the nucleus and the centrosome define an axis that seems to be oriented nonrandomly. In thyroid or mammary epithelial cells for example, the centrosome is located above the nucleus in the apical part of the cytoplasm (Zeligs and Wollman, 1979; Dylewski and Keenan, 1984). Also, in migrating cells the position of the MTOC with respect to the nucleus is often defined, being anterior in neutrophils (Schliwa et al., 1982) and in *Dictyostelium* (Yumura and Fukui, 1983).

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1. *Abbreviations used in this paper*: hCG, human chorionic gonadotrophin; M2, Medium 2; M16, Medium 16; MTOC, microtubule organizing center; PCM, pericentriolar material; PHEM, buffer containing 10 mM EGTA, 2 mM $MgCl_2$, 60 mM Pipes, 25 mM Hepes, pH 6.9.

While the interaction of the microtubule network with various cytoplasmic components has been studied extensively, little is known about changes of the microtubule network itself during extensive natural cellular reorganizations that occur during cell differentiation. It has been proposed that changes in the morphology of the microtubule network in interphase might be due to changes in the location and/or activity of nucleating sites, stabilizing factors or destabilizing factors, or to interactions with other organelles such as the plasma membrane. However, there is little direct evidence to support this speculation.

The early mouse embryo provides a good system in which to study the role of microtubules and MTOCs during the development of cell polarity. At the eight-cell stage a process called compaction takes place. During this process the previously symmetrical blastomeres become asymmetrical. A pole of apical microvilli develops (Ducibella and Anderson, 1975; Handyside, 1980; Lehtonen and Badley, 1980; Reeve and Ziomek, 1981) and many cytoplasmic organelles, such as cytoplasmic microfilaments (Johnson and Maro, 1984), endosomes (Reeve, 1981; Fleming and Pickering, 1985), and clathrin vesicles (Maro et al., 1985) relocalize to the apical part of the cytoplasm. At the same time, gap junctions develop in the basolateral membranes of adjacent cells (Lo and Gilula, 1979; Goodall and Johnson, 1984). Very little is known about the distribution of microtubules (Ducibella et al., 1977) and their associated acentriolar MTOCs (Szollosi, 1972) during compaction. However, numerous studies have used microtubule inhibitors to investigate the role of microtubules during compaction, and these have provided some useful information (Ducibella and Anderson, 1975; Surani et al., 1980; Ducibella, 1982; Pratt et al., 1982; Sutherland and

Calarco-Gillam, 1983; Maro and Pickering, 1984; Johnson and Maro, 1985; Fleming et al., 1986; Goodall and Maro, 1986).

Our aim is to describe the changes occurring in the distribution of the microtubule network during compaction and to relate them to the known effects of microtubule inhibitors on the same process, to understand further the role of the microtubules during the development of cell polarity.

Materials and Methods

Recovery of Embryos

MF1 (Central Animal Services, Cambridge, UK) or Swiss (Animalerie Spécialisée de Villejuif, CNRS, France) female mice (3–6 wk) were superovulated by injections of 5–7.5 IU of pregnant mare's serum gonadotrophin (Intervet, Cambridge, UK) and human chorionic gonadotrophin (hCG; Intervet) 48 h apart. The females were paired overnight with HC-CFLP (Hacking & Churchill, UK) or Swiss (Animalerie Spécialisée de Villejuif) males and inspected for vaginal plugs the next day. Late four-cell embryos were recovered by flushing late two-cell embryos at 46–50 h post-hCG followed by overnight culture in Medium 16 containing 4 mg/ml BSA (M16 + BSA; Whittingham and Wales, 1969) under oil at 37°C in 5% CO_2 in air. Late eight-cell embryos were recovered by flushing at 65–70 h post-hCG.

Preparation and Handling of Single Cells

Late four-cell and late eight-cell embryos were exposed briefly to acid Tyrode's solution (Nicolson et al., 1975) to remove the zona pellucida, rinsed in Medium 2 containing 4 mg/ml BSA (M2 + BSA; Fulton and Whittingham, 1978), and placed in Ca^{++}-free M2 containing 6 mg/ml BSA for 5–45 min, during which time they were disaggregated to single four- or eight-cell blastomeres (1/4 or 1/8 cells) using a flame-polished micropipette. Isolated cells were cultured in tissue culture dishes (Sterilin, Teddington, UK) in drops of M16 + BSA under oil at 37°C in 5% CO_2 in air. Each hour, the cultures were inspected for evidence of division to 2/8 or 2/16 pairs. All newly formed pairs were removed and designated Oh old. Pairs were then cultured in M16 + BSA as natural 2/8 or 2/16 pairs.

Drugs

A stock solution of 10 mM nocodazole (Aldrich Chemical Co., Ltd., Gillingham, UK) in DMSO was used in these experiments and was stored at 4°C. For treatment of the cells, the drug was diluted in M16 + BSA to a final concentration of 10 μM.

Cell Fixation and Immunocytological Staining

Cells were placed in specially designed chambers as described in Maro et al. (1984) except that the chambers were coated first with a solution of 0.1 mg/ml concanavalin A and after the samples were placed in the chambers, they were centrifuged at 450 *g* for 10 min at 30°C.

After a recovery period of 10 min at 37°C, the cells were then treated in one of two ways: (*a*) For tubulin staining with an anti-α-tubulin monoclonal antibody (Amersham International, Amersham, UK) cells were washed quickly in PHEM buffer (10 mM EGTA, 2 mM $MgCl_2$, 60 mM Pipes, 25 mM Hepes, pH 6.9; derived from Schliwa et al., 1981) containing 0.6 μM taxol (PHEM-taxol), extracted for 5 min in PHEM-taxol buffer containing 0.25% Triton X-100, washed in PHEM-taxol buffer, and fixed for 30 min with 1.8% formaldehyde in PHEM-taxol buffer. All these steps were carried out at 30°C. We checked that the use of 0.6 μM taxol in the extraction buffer did not cause alterations in the microtubule network by comparing the effect of taxol between 0 and 20 μM on eight-cell blastomeres and unfertilized eggs. (*b*) For optimal staining of PCM with the anti-PCM serum (Calarco-Gillam et al., 1983), cells were extracted for 2 min in PHEM buffer containing 0.1% Triton X-100, washed in PHEM buffer, and fixed for 20 min with 1.8% formaldehyde in PHEM buffer. All these steps were carried out at 20°C.

Extraction of the cells with a detergent before fixation reduced the cytoplasmic background due to the thickness of the cells (~30-μm diam) to a level that allowed us to observe clearly the microtubules and the PCM foci. The anti-PCM serum used in our study has been shown to stain the MTOCs in various cell types and in many species including cells without centrioles such as plant cells and mouse oocytes (Calarco-Gillam et al., 1983; Clayton et al., 1985; Maro et al., 1985).

Immunocytological staining was performed as described in Maro et al. (1984) using FITC-labeled anti-human Ig antibodies or rhodamine-labeled anti-mouse or anti-rat Ig antibodies (Miles Laboratories Ltd., Slough, UK) as second layers. For double stain experiments, the second layer (anti-rat) was pre-adsorbed against Sepharose 4B beads (Pharmacia, Sweden) coated with human Igs. To stain chromosomes, fixed cells were incubated in Hoechst dye 33258 (5 μg/ml in PBS) for 30 min.

Regrowth Experiments

For microtubule regrowth experiments 2/8 pairs were cultured for 1 h in 10 μM nocodazole to depolymerize all cytoplasmic microtubules. Cells were maintained in 1 μM nocodazole in M2 containing 4 mg/ml polyvinylpyrrolidone during centrifugation of the chambers. The chambers were then rinsed quickly in M2 and microtubules were allowed to regrow in a large excess of M2 for 1–20-min periods at 30°C. Cells were then extracted and fixed before labeling. The fixation procedure designed for tubulin labeling was used in these experiments, and the cells labeled with both an anti-α-tubulin monoclonal antibody ($YL_{1/2}$; Kilmartin et al., 1982) and the anti-PCM serum.

Photomicroscopy

The coverslips were removed from the chambers, and samples were mounted in "Citifluor" (City University, London) and viewed under a Leitz Ortholux II microscope with filter sets L2 for FITC-labeled reagents. N2 for TRITC-labeled reagents, and A for Hoechst dye. Photographs were taken on Kodak Tri-X film using a Leitz Vario-Orthomat photographic system. The three-dimensional structure of the cell is preserved on the whole mount, but as the size of the eight-cell blastomere is large (30-μm diam), it is impossible to photograph the whole cell in the same focal plane. Therefore, we show optical sections with only one plane through the cell in sharp focus (Fig. 1).

Electron Microscopy

For electron microscopy cells were washed quickly in PHEM buffer, extracted for 5 min in PHEM-taxol buffer containing 0.25% Brij 58 (Sigma Chemical Co., Poole, UK), washed in PHEM-taxol buffer, and fixed for 30 min with 0.2% glutaraldehyde in PHEM-taxol buffer. All these steps were carried out at 30°C. Glutaraldehyde was neutralized with 0.1 M lysine (Sigma Chemical Co.) for 10 min at 20°C. Cells were then labeled with an anti-α-tubulin monoclonal antibody ($YL_{1/2}$; Kilmartin et al., 1982) as a first layer followed by an anti-rat Ig conjugated to 10-nm gold particles (Janssen Pharmaceutica, Beerse, Belgium). Cells were postfixed with 3.5% glutaraldehyde in PBS, embedded in TAAB Embedding Resin, stained with uranyl acetate and lead citrate, sectioned on a Reichert Ultramicrotome, and finally viewed under a Philips 300 electron microscope.

To assess the number of microtubules present in different areas of the cell, 1- and 9-h 2/8 pairs of blastomeres were processed for immunoelectron microscopy. Only sections of cells that passed through the nucleus and showed the area of intercellular contact were used for this purpose. Photographs were taken at a magnification of 4,800 and enlarged 3.5 times during printing (final magnification 16,800). Apical, central, and basal areas of the cells were defined as shown in Fig. 2. The number of microtubules passing through each section was counted irrespective of their length and the angle at which they were cut. This method was used in preference to a measure of total microtubule length as the cells are very large and most microtubules do not run parallel to the plane of the section. The possible occurrence of a few very long microtubules (longitudinally sectioned) in any area in addition to the difficulty of assigning a length to the many transversally cut microtubules would lead to great difficulty in interpreting a measurement of density based on length.

Results

Microtubule Distribution

The redistribution of microtubules during the eight-cell stage was examined by immunofluorescence using a monoclonal anti-tubulin antibody. Because the large size of the embryo (80-μm diam) does not allow easy examination of single

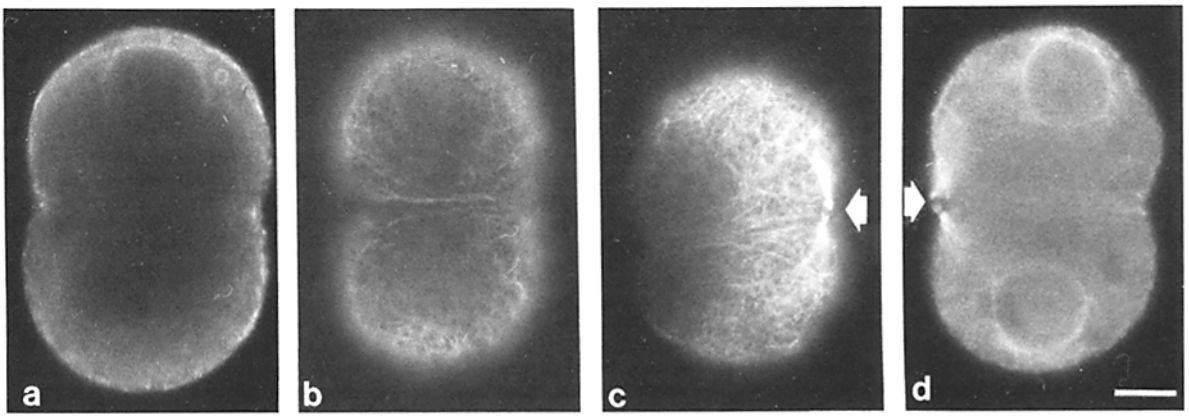


Figure 1. Distribution of microtubules in pairs of 2/8 blastomeres. Cells were stained for tubulin with an anti-tubulin antibody. (*a* and *b*) Pair of 9-h eight-cell blastomeres photographed at two different focal planes: at the level of the nuclei (*a*) and of the cell cortex (*b*). Note the network of cortical microtubules that run parallel to the plane of the optical section (*b*). (*c*) 9-h 2/8 pair. Note network of cortical microtubules. (*d*) 9-h 2/8 pair. Note perinuclear microtubules. Arrows in *c* and *d* point at mid-bodies. Bar, 10 μm.

cells, the experiments were performed on natural pairs of eight-cell blastomeres (2/8) derived in vitro from single four-cell blastomeres (1/4). As the fourth cell cycle of mouse development lasts 10–12 h (Smith and Johnson, 1986), the 2/8 pairs were sampled at 2-h intervals between 1 and 9 h after their formation (time 0 h). In an early pair, microtubules are arranged in a roughly symmetrical manner. There is a cortical network, a layer of perinuclear microtubules, and some cytoplasmic microtubules evenly distributed except for a concentration at the mid-body and a depletion in the areas of cell contact (Fig. 3, *a* and *b*). This pattern is similar to that observed in four-cell blastomeres (data not shown). As the cell cycle progresses, the disappearance of microtubules beneath areas of cell contact extends further into the cell leading to a concentration of microtubules in the apical region (Fig. 3). During this period the cells also flatten upon each other so that the area of contact enlarges (Fig. 3; Lehtonen, 1980), but cell flattening does not necessarily precede the disappearance of microtubules in the basal region (Fig. 3 *b*). After 9 h in culture, 2/8 pairs show a general increase in the concentration of microtubules away from cell contact regions, including an apparent thickening of the cortical microtubule network in the apical part of the cell. The disappearance of many microtubules from the areas of intercellular contact was confirmed by the use of triplets and quartets of blastomeres derived by partial disaggregation from eight-cell embryos or aggregation of two 2/8 pairs of blastomeres. In these clusters microtubules were clearly reduced in the areas of intercellular apposition (Fig. 4). Since a previous study using electron microscopy suggested that microtubules run parallel to the cell contact areas in compacted embryos (Ducibella et al., 1977), we checked our observations at the

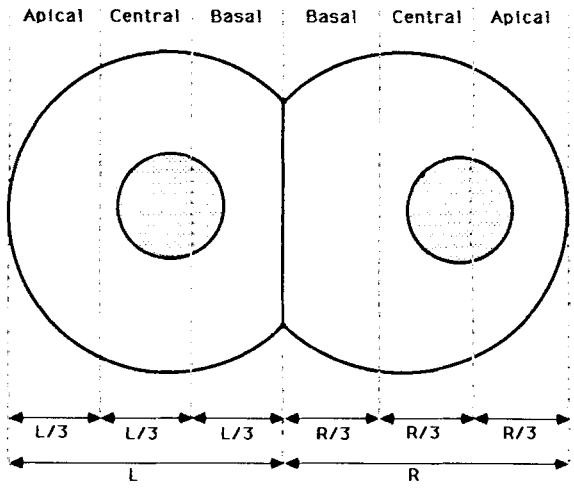


Figure 2. Determination of the three areas (apical, central, and basal) where microtubule counts were performed at the electron microscopic level. Only sections of pairs passing through the nucleus and through the contact area were used.

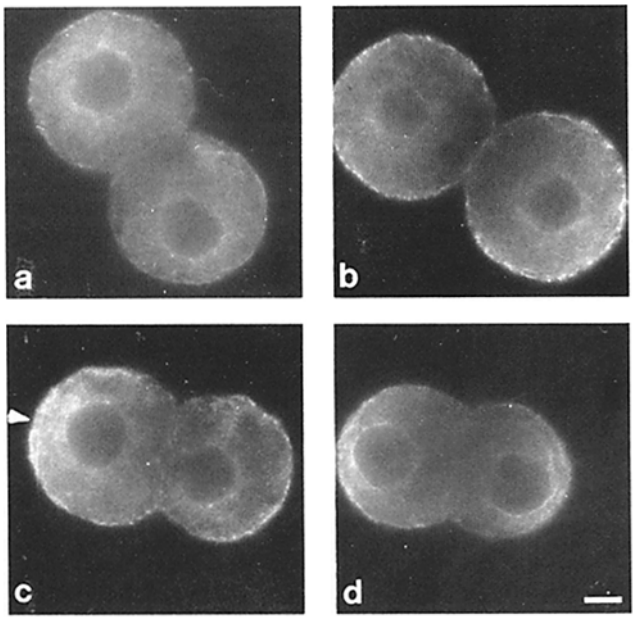


Figure 3. Distribution of microtubules in pairs of 2/8 blastomeres. Cells were stained for tubulin with an anti-tubulin antibody. The sequence shown demonstrates a progressive decrease in the number of basal microtubules and a corresponding concentration of apical microtubules. (*a*) 3-h 2/8 pair. Nonpolar microtubules. (*b*) 3-h 2/8 pair. Note a decreased staining in the basal area of the cells. (*c*) 7-h 2/8 pair. Note polar microtubules in one of the cells (*arrow*). (*d*) 9-h 2/8 pair. Polar microtubules. Bar, 10 μm.

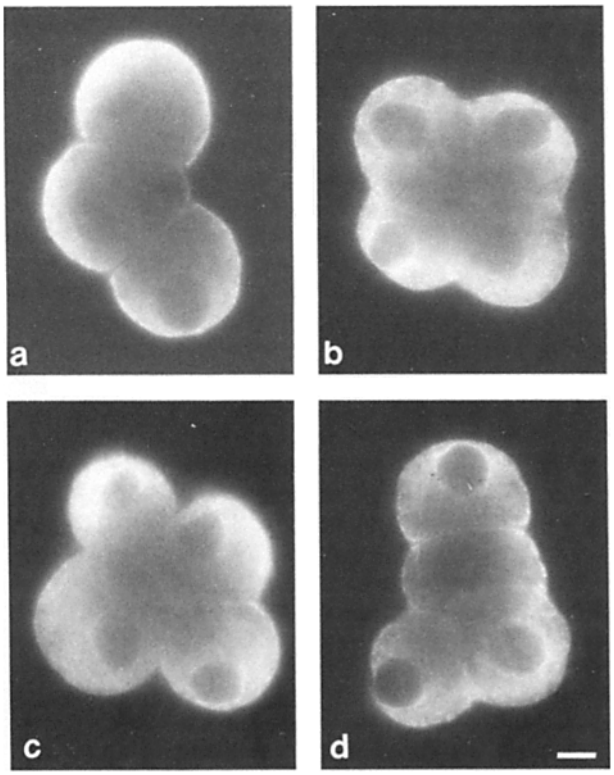


Figure 4. Distribution of microtubules in 9-h triplets and quartets of blastomeres. Cells were stained for tubulin with an anti-tubulin antibody. Note the decreased staining in the areas of intercellular contact. Bar, 10 μm.

electron microscopic level by labeling the microtubules in 1- and 9-h 2/8 pairs with an anti-tubulin antibody followed by a gold-labeled anti-mouse Ig antibody. Most microtubules were seen around the nucleus and in the cell cortex, although they were relatively scarce beneath areas of intercellular apposition (Fig. 5). The decrease in concentration of microtubules in the basal part of the cell and the increase in concentration in the apical part were confirmed when the number of microtubules in different regions of 1- and 9-h blastomeres were compared (Table I). The difference observed between the apical/basal ratios at 1 and 9 h was significant statistically (significance, 0.037; *t* test) even though in the 1-h group two cells out of nine were already polarized, whereas in the 9-h group two cells out of nine were not polarized. These relative ratios of polarized/nonpolarized cells at 1 and 9 h are in good agreement with our immunofluorescence observations (see Fig. 7 *B*).

PCM Distribution

Early cleavage stage mouse blastomeres lack centrioles but have MTOCs consisting of aggregates of pericentriolar material (PCM; Szollosi, 1972; Szollosi et al., 1972; Calarco-Gillam et al., 1983; Maro et al., 1985). A nonimmune sera that recognizes the PCM in a large number of species (Calarco-Gillam et al., 1983) was used to follow the reorganization of the PCM in early blastomeres by immunofluorescence.

At the four-cell stage positive staining was only observed very late in the cell cycle, immediately before the cells were to enter mitosis (cells positive for PCM: 0% at 5 h, 15% at 7 h, 48% at 9 h, and 100% in mitotic cells). The staining consisted of aggregates of PCM localized around the nucleus or at the poles of the mitotic spindle.

At the eight-cell stage, PCM could be detected clearly throughout the cell cycle in many cells with the proportion of cells showing positive staining increasing steadily. In those cells positive for PCM, four different staining patterns were observed: dispersed dots or larger aggregates of PCM (as seen in late four-cells blastomeres), being located either in the apical and/or basal part of the cell. These four different PCM patterns were designated (*a*) nonpolar dispersed (Fig.

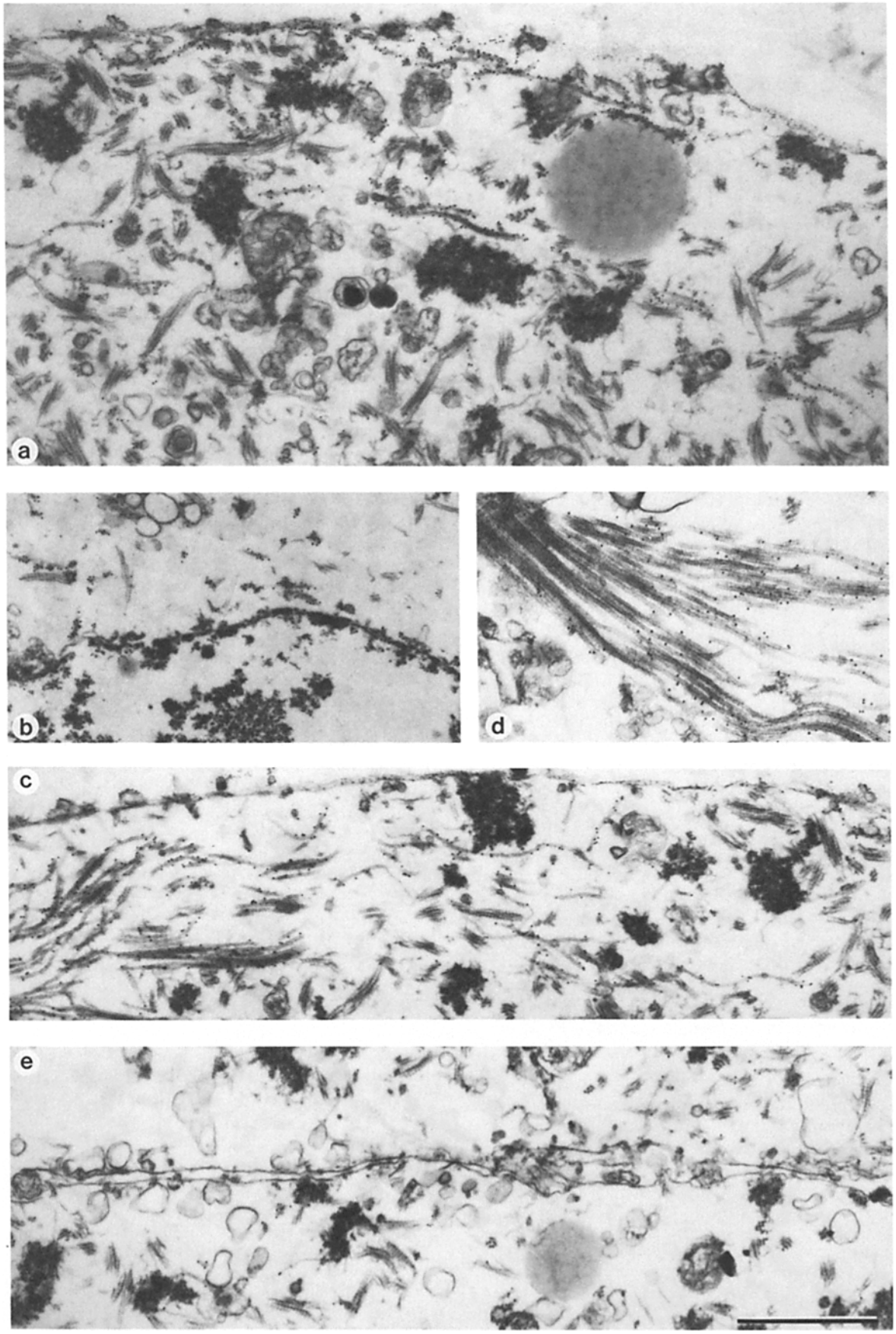
Table I. Localization of Microtubules by Immunoelectron Microscopy in 2/8 Pairs of Blastomeres

Age (in h postdivision)	Area	Cell No.	Surface ± SD	Mean density of microtubules ± SD	Mean relative density of microtubules ± SD	
					Apical/basal	Apical/central
			μm^2	$n/\mu m^2$		
1 h	Total	9	86.1 ± 8.0	5.2 ± 1.3		
	Apical third		20.8 ± 2.0	6.1 ± 2.3		
	Central third		28.0 ± 4.2	4.6 ± 0.7		
	Basal third		28.9 ± 3.9	5.2 ± 2.0		
					1.3 ± 0.6*	1.3 ± 0.4
9 h	Total	9	88.8 ± 11.2	4.4 ± 1.8		
	Apical third		21.6 ± 4.7	7.4 ± 4.0		
	Central third		29.5 ± 3.7	3.8 ± 1.6		
	Basal third		29.9 ± 4.2	3.1 ± 1.8		
					3.5 ± 2.7‡	2.1 ± 1.0

* Two out of nine cells were clearly polarized with apical/basal ratios of 2.2 and 2.4. The apical/basal ratios for the seven other cells were: 0.7, 0.8, 0.8, 0.8, 1.1, 1.3, and 1.4.

‡ Seven out of nine cells were clearly polarized with apical/basal ratios of 1.9, 2.1, 2.1, 2.9, 3.5, 6.8, and 9.6. The apical/basal ratios for the two other cells were 1.1 and 1.4.

Figure 5. Distribution of microtubules in 9-h 2/8 pairs as detected by immunoelectron microscopy. Note microtubules labeled with gold particles in the apical part of the cell (*a*) around the nuclear periphery (*b*), and their relative absence in the areas of intercellular apposition (*e*). In *c*, a bundle of parallel microtubules in the basolateral domain of the cell is shown, which originates from a mid-body (*d*; same cell as *c*, adjacent section). Bar, 1 μm.


a
b
d
c
e

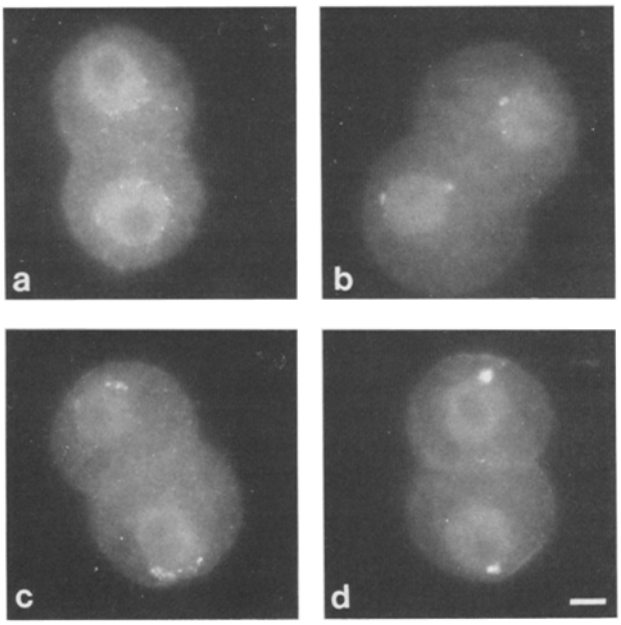


Figure 6. Distribution of PCM in pairs of 2/8 blastomeres. Cells were stained with an anti-PCM serum. (*a*) 3-h 2/8 pair. PCM nonpolar dispersed. (*b*) 7-h 2/8 pair. PCM nonpolar aggregated. (*c*) 9-h 2/8 pair. PCM polar dispersed. (*d*) 9-h 2/8 pair. PCM polar aggregated. Bar, 10 μm.

6 *a*); (*b*) nonpolar aggregated (Fig. 6 *b*); (*c*) polar dispersed (apical dots; Fig. 6 *c*); (*d*) polar aggregated (apical aggregates; Fig. 6 *d*). The kinetics of PCM reorganization is shown on Fig. 7 *A*. The PCM first appears as small dots dispersed in the cytoplasm. These tend to shift towards the apical half of the cell before they finally aggregate. It is clear that the PCM staining appears as the microtubules shift towards the apical part of the cell, while it polarizes after this shift in microtubules has occurred (Fig. 7 *B*). Aggregation only takes place late during the cell cycle (between 5 and 9 h).

At the 16-cell stage, even more cells are positive for PCM, which can be detected in more than 80% of the cells at 1, 3, 5, and 9 h after division. As during the eight-cell stage, PCM tends to be dispersed during the first half of the cell cycle, with aggregation occurring between 5 and 9 h after division.

Microtubule Involvement in the Reorganization of PCM

It has been shown in various systems that experimentally induced relocations of MTOCs are dependent upon microtubules (Malech et al., 1977; Sherline and Mascardo, 1982; Gottlieb et al., 1983; Euteneuer and Schliwa, 1985). Experiments were thus designed to study the role of microtubules on the reorganization of PCM in eight-cell blastomeres. 2/8 pairs were incubated in the presence of nocodazole (10 μM), a drug that blocks tubulin polymerization, leading to a disappearance of cytoplasmic microtubules. Upon addition of the drug at any point during the cell cycle, the process of PCM redistribution (as assessed by the appearance of positive staining, and its polarization and aggregation) was blocked or retarded (Table II). This inhibition was completely reversed upon removal of the drug (Table II). Moreover, by 9 h a polar microtubule network had re-formed, polarity being

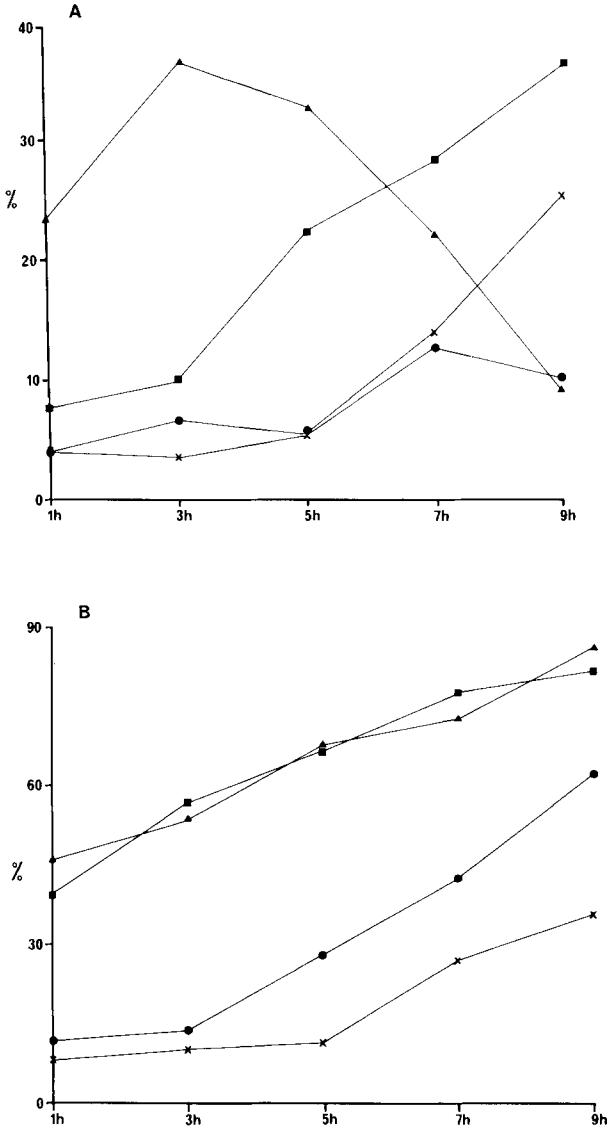


Figure 7. (*A*) Kinetics of PCM distribution in pairs of 2/8 blastomeres. The staining patterns were classified into four groups: nonpolar dispersed (▼); polar dispersed (■); nonpolar aggregates (●); polar aggregates (x). (*B*) Kinetics of PCM and microtubule redistribution in pairs of 2/8 blastomeres. Polar microtubule network (▼); PCM present (■); PCM polarized (●); PCM aggregated (x). The microtubule network was classified as polar if the concentration of cytoplasmic microtubules, away from the cortex and nucleus, was greater in the apical than the basal half of the cell (Fig. 3). The numbers of cells scored for PCM distribution were: 537 at 1 h, 406 at 3 h, 299 at 5 h, 359 at 7 h, and 361 at 9 h. For tubulin, these numbers are: 655 at 1 h, 438 at 3 h, 436 at 5 h, 429 at 7 h, and 454 at 9 h.

88% (n = 32) of the control level after release from the drug at 5 h, and 73% (n = 65) after release at 7 h.

PCM Aggregates Can Act as MTOCs

As the observed relocation of PCM follows the reorganization of the microtubule network in eight-cell blastomeres, it might be suggested that the foci of material detected by the anti-PCM serum do not act as MTOCs. However, examination of the relationship of the PCM foci (as detected by the anti-PCM serum) to microtubules indicates that they are

Table II. Localization by Immunofluorescence of PCM in 2/8 Pairs of Blastomeres after Treatment with Nocodazole

Treatment	Time of analysis	Cell No.	*n*	PCM pattern (%) Present	Polarized	Aggregated
				Mean ± SEM	Mean ± SEM	Mean ± SEM
h	*h*					
Control						
0–1	1	537	7	45.9 ± 6.3	10.6 ± 2.7	7.3 ± 1.8
0–3	3	406	7	56.7 ± 5.8	13.4 ± 2.2	9.6 ± 3.5
0–5	5	299	7	70.3 ± 6.7	23.3 ± 6.6	10.6 ± 1.9
0–7	7	359	7	77.7 ± 4.6	39.1 ± 3.7	25.6 ± 3.4
0–9	9	361	8	79.2 ± 4.9	56.0 ± 6.4	40.9 ± 6.8
Nocodazole (10 μM)						
1–9	9	151	3	43.7 ± 4.4 S*	7.3 ± 3.0 S	0.3 ± 0.3 S
5–9	9	158	3	68.3 ± 6.2 NS‡	26.3 ± 5.2 S	26.3 ± 3.1 NS
7–9	9	168	3	56.7 ± 11.3 NS	42.0 ± 8.3 NS	28.7 ± 3.0 NS
1–5	5	56	3	28.7 ± 8.0	2.0 ± 1.6	8.0 ± 3.3
1–5	9	167	3	85.0 ± 6.6 NS	47.7 ± 7.4 NS	33.0 ± 0.0 NS
1–7	7	22	2	35.0 ± 15.0	4.0 ± 4.0	0.0 ± 0.0
1–7	9	93	3	84.3 ± 10.8 NS	45.3 ± 2.1 NS	30.0 ± 8.5 NS

To assess the statistical significance of the observed differences, the *t* test was used.
Experimental groups were compared to the 9-h control group.
* S, significant ($P < 0.02$).
‡ NS, not significant.

true MTOCs. PCM aggregates are always found at the spindle poles in mitotic blastomeres (Fig. 8, *a* and *b*). In interphase, the abundance of cytoplasmic microtubules prevents direct examination of the relationship of microtubules to PCM, so a series of microtubules regrowth experiments was undertaken. The microtubule network in 9-h 2/8 pairs was completely destroyed by nocodazole treatment (immunofluorescence and electron microscopy revealed only persistent mid-body microtubules). When the drug was washed out microtubules were first seen in the apical cortex and the perinuclear region. By 15–20 min after drug removal, the microtubule network appeared completely restored. It is clear from cells double stained at an intermediate regrowth time (7 min) that microtubules do regrow around PCM aggregates (Fig. 8, *c* and *d*). However, many microtubules also polymerize in the absence of any obvious PCM aggregates. Although PCM aggregates can act as MTOCs, they are not necessarily the preferential site for microtubule regrowth.

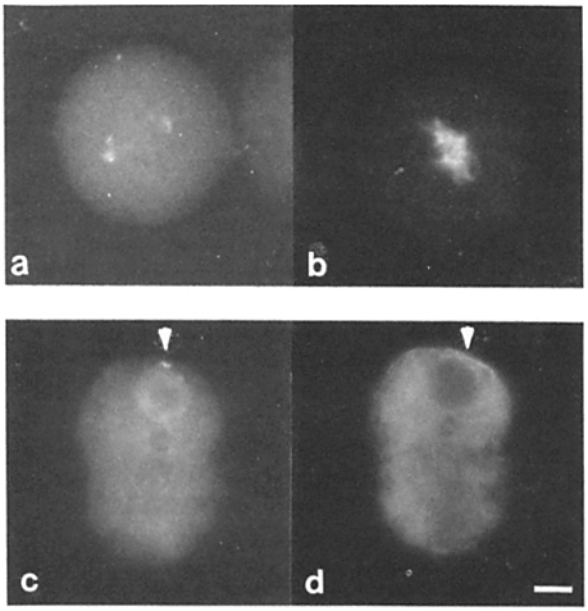


Figure 8. (*a* and *b*) Mitotic cell in a 2/8 pair double stained for PCM (*a*) and for chromatin (*b*). Note PCM at the spindle poles. (*c* and *d*) 9-h 2/8 pair double stained for PCM (*c*) and for tubulin (*d*). Microtubules were first destroyed by treatment with 10 μM nocodazole and then allowed to regrow for 7 min in the absence of the drug. Note microtubules growing around the PCM aggregate (*arrowheads*). Bar, 10 μm.

Discussion

The distribution of microtubules in eight-cell mouse embryos has been studied previously by electron microscopy (Ducibella et al., 1977). In that study, the distribution of microtubules in regions of imminent cell contact and areas of newly formed contacts between blastomeres in partially compacted embryos was considered, and arrays of microtubules located parallel to areas of intercellular apposition were described. Our data from both fluorescence microscopy and electron microscopy do not confirm this distribution. Although we do see some microtubules running parallel to the membrane beneath areas of cell contact, microtubules in all orientations are much more frequently away from such areas, particularly in the apical part of the cell. Possibly the observation of microtubules originating from mid-bodies and running close to contact areas (see Fig. 1, *c* and *d* and 5, *c* and *d*) may have lead to the previous conclusions. The visualization of a network of single microtubules without detergent extraction and use of immunological

markers is very difficult, and it is also difficult to assess the intracellular distribution of microtubules from sections taken through whole embryos where the relationship between the cells in the section is unclear. The use of immunofluorescence on whole mounts and the use of timed pairs of 2/8 blastomeres can reveal a much fuller three-dimensional picture. Microtubules have been visualized in one previous immunofluorescence study (Lehtonen and Badley, 1980), but the changes occurring in the microtubule network during compaction were not described.

We have investigated the redistribution of microtubules and PCM during the development of polarity in mouse eight-cell blastomeres. It is clear from our observations that in early eight-cell blastomeres microtubules are found mainly around the nucleus and in the cell cortex but are relatively deficient from zones of intercellular apposition. PCM, when detected, is observed dispersed in the cytoplasm. During the eight-cell stage, microtubules become further depleted in the area beneath the zone of intercellular contact, and they accumulate in the apical part of the cell while their number decreases in the basal domain. The PCM also relocalizes to the apical domain of the cell, although this occurs after the redistribution of the microtubules and is dependent upon the presence of the microtubule network. The observed accumulation of microtubules in the apical part of the eight-cell stage blastomere could be achieved by a local nucleation of microtubules and/or by local changes in the stability of microtubules. Both the endogenous pattern of microtubules and the pattern of microtubule regrowth after drug-induced depolymerization reveal that PCM aggregates can provide a focus for microtubule nucleation. However, microtubule polymerization also occurs in perinuclear and cortical regions apparently devoid of PCM immunoreactivity. It is possible that PCM exists at these sites but is organized too diffusely to be detected by the anti-serum. It is known that in myotubes, which lack centrioles, perinuclear PCM is indeed the site of microtubule nucleation (Tassin et al., 1985). However, in other situations, microtubule organization that is independent of MTOCs has been observed; for example, in cytoplasts made from confluent L929 cells, most microtubules are not nucleated by the centrosome and are arranged peripherally (Karsenti et al., 1984), and in epithelial cells the microtubules seem to run parallel to each other and to the axis of cell polarity (Gorbsky and Borisy, 1985). Although microtubules appear to exist independently of observable PCM in these situations, regrowth of microtubules in the L929 cytoplasts occurred preferentially around the centrosome after depolymerization (Karsenti et al., 1984). In this regard eight-cell blastomeres differ, since regrowth occurs from PCM foci and also around the nucleus and in the cortex. It will be important to determine how microtubule polymerization is controlled in eight-cell blastomeres. It seems clear, however, that the redistribution of large foci of PCM itself cannot explain the apical concentration of microtubules, since the observed PCM redistribution follows that of microtubules and is dependent upon an intact microtubule network.

A dependence on microtubules of the relocalization of the centrosome has been demonstrated in two other systems. In neutrophils stimulated by a chemoattractant, but not allowed to migrate, the centrosome relocates to a position between the nucleus and the plasma membrane facing the chemoattractant (Malech et al., 1977). Similarly, after an experimentally induced wound made in a confluent monolayer of endothelial cells or fibroblasts, the MTOCs become oriented preferentially towards the edge of the cell facing the wound (Gottlieb et al., 1981; Kupfer et al., 1982). In mouse eight-cell blastomeres the reorganization of the PCM follows that of the microtubules, whereas in neutrophils (Malech et al., 1977) and fibroblasts (Kupfer et al., 1982) microtubules and centrosomes relocate together towards a stimulus. However, in neutrophils and fibroblasts reorientation of the MTOC and of the microtubule network is very rapid, taking place in the first few minutes after experimental stimulation, and it is therefore possible that the centrosome does follow rapidly a primary reorganization of the microtubules (Malech et al., 1977; Kupfer et al., 1982). Indeed the reorientation of the centrosome in neutrophils and fibroblasts does not occur in the absence of microtubules (Malech et al., 1977; Gottlieb et al., 1983), suggesting a leading role for the microtubules, as in eight-cell blastomeres (our data).

Whereas local nucleation provides one route to asymmetry in the microtubule network, an alternative route comes from selective stabilization or destabilization. In blastomeres, a depletion of microtubules occurs in the area of intercellular contact. This effect is also observed at the four-cell stage but becomes marked during the eight-cell stage when the area of apposition between cells also increases. An influence of cell contact on the spatial distribution of microtubules has also been described in some cells in culture. It has been shown that noncentrosomal microtubules appear first in cells at confluence (Karsenti et al., 1984), a change that may be related to a decrease in the critical concentration for tubulin polymerization, allowing the polymerization of free microtubules in confluent cells. Also in other cell types the axis determined by the centrosome and the nucleus is dependent upon an external stimulus (Malech et al., 1977; Gottlieb et al., 1981; Kupfer et al., 1983), but in these cases microtubules seem to be stabilized in the area facing the stimulus (as suggested by Kirschner and Mitchison, 1986) rather than being depleted in a particular domain of the cell. Our results, in contrast, suggest a destabilization of the microtubules in areas of blastomere apposition.

Polarity is one of the major cellular features in which a role for microtubules has been implicated. While it has been supposed generally that microtubules are involved in the maintenance of asymmetries within a cell, they do not seem to be involved in the biogenesis and the maintenance of cell surface polarity, as studied by the budding of enveloped viruses (Salas et al., 1986; for a review see Simons and Fuller, 1985). Moreover, there has been little evidence to substantiate the role of the microtubule network in the setting up of polarity. Recently, it has been suggested that the microtubules may in fact respond to changes taking place at the cell periphery, which direct the development of polarity (Euteneuer and Schliwa, 1985; Kirschner and Mitchison, 1986). Our observations on the changing pattern of the microtubule network taken together with observations from previous studies using drugs that interact with tubulin or microtubules (Ducibella and Anderson, 1975; Surani et al., 1980; Ducibella, 1982; Pratt et al., 1982; Sutherland and Calarco-Gilliam, 1983; Maro and Pickering, 1984; Johnson and Maro, 1985; Fleming et al., 1986; Goodall and Maro, 1986) also suggest that the microtubules respond to changes

Table III. Effect of Microtubule Inhibitors Applied during Interphase on the Various Components of Compaction in the Eight-Cell Mouse Embryo

Treatment	Effect	Cytoplasmic polarity*	Surface polarity‡	Junctional communication§	Intercellular flattening‖
Nocodazole (colcemid)	Microtubule depolymerization	Absent	Broad poles (able to spread)	Normal	Normal but completed more rapidly
Taxol	Microtubule stabilization	Absent	Broad poles	Reduced	Reduced and delayed

* Johnson and Maro, 1985; Fleming et al., 1986.
‡ Ducibella, 1982; Pratt et al., 1983; Maro and Pickering, 1984; plus references under *.
§ Goodall and Maro, 1986.
‖ Ducibella and Anderson, 1975; plus references under ‡ and §.

occurring in the cell cortex. Only data from experiments performed during interphase will be discussed, since microtubule inhibitors block cells in mitosis, and during mitosis the blastomeres round up (Lehtonen, 1980; Maro and Pickering, 1984; Goodall and Maro, 1986), cytoplasmic organelles disperse (Johnson and Maro, 1984; Maro et al., 1985), surface microvilli spread (Johnson and Ziomek, 1981; Dhiman, A., S. J. Pickering, M. H. Johnson, and B. Maro, manuscript in preparation), and gap junctions switch off (Goodall and Maro, 1986). The effects of the microtubule inhibitors on compaction are summarized in Table III. Cytoplasmic polarity is totally dependent upon microtubules since it is inhibited or reversed by nocodazole and taxol (Johnson and Maro, 1985; Fleming et al., 1986), suggesting that the organelles studied are associated closely with the microtubules. Intercellular flattening and junctional communication are reduced by taxol (Maro and Pickering, 1984; Goodall and Maro, 1986), while nocodazole and colcemid do not inhibit either (Ducibella and Anderson, 1975; Ducibella, 1982; Goodall and Maro, 1986) but rather accelerate the completion of flattening (Maro and Pickering, 1984), suggesting a constraining effect of the microtubules on intercellular adhesion and junctional communication. Microtubules are not required for the development of surface polarity but do modify the shape and size of the poles that form (Ducibella, 1982; Maro and Pickering, 1984; Johnson and Maro, 1985). Thus, it seems possible that the relocation of microtubules to the apical part of the cell, concurrent with other changes in the cortex, facilitates the movement of organelles towards the apical part of the cell, the formation of gap junctions in the basolateral domain of the plasma membrane, intercellular flattening, and the loss of basolateral microvilli. A network of apical microtubules may then help to stabilize the microvilli of the surface pole and the organelles of the cytoplasmic pole and also allow the relocalization of the PCM foci, which would itself reinforce the initial asymmetry in the stability of the microtubules by nucleation of new microtubules in the apical domain. It thus seems that, rather than being the driving force during polarization of the eight-cell blastomere, microtubules help to coordinate the various changes taking place during the process of compaction and reinforce an asymmetry set up at the cell periphery.

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