Posttranslational Modification of Distinct Microtubule Subpopulations During Cell Polarization and Differentiation in the Mouse Preimplantation Embryo

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Abstract. During the course of preimplantation development, the cells of the mouse embryo undergo both a major subcellular reorganization (at the time of compaction) and, subsequently, a process of differentiation as the phenotypes of trophectoderm and inner cell mass cell types diverge. We have used antibodies specific for tyrosinated (Kilmartin, J. V., B. Wright, and C. Milstein. 1982. *J. Cell Biol.* 93:576–582) and acetylated (Piperno, G., and M. T. Fuller. 1985. *J. Cell Biol.* 101:2085–2094) α-tubulin in immunofluorescence studies and found that subsets of microtubules can be distinguished within and between cells during the course of these events. Whereas all microtubules contained tyrosinated α-tubulin, acetylated α-tubulin was detected only in a subpopulation, located predominantly in the cell cortices. Striking differences developed between the distribution of the two populations during the course of development. Firstly, whereas the microtubule population as a whole tends to redistribute towards the apical domain of cells as they polarize during compaction (Houliston, E., S. J. Pickering, and B. Maro. 1987. *J. Cell Biol.* 104:1299–1308), the microtubules recognized by the antiacetylated α-tubulin antibody became enriched in the basal part of the cell cortex. After asymmetric division of polarized cells to generate two distinct cell types (termed inside and outside cells) we found that, despite the relative abundance of microtubules in outside cells, acetylated microtubules accumulated preferentially in inside cells. Treatment with nocodazole demonstrated that within each cell type acetylated microtubules were the more stable ones; however, the difference in composition of the microtubule network between cell types was not accompanied by a greater stability of the microtubule network in inside cells.

Although the successive differentiative events of embryonic development depend upon the expression of a genetic program, cellular mechanisms exist that modulate that program and can direct the fate of a cell or of its progeny. During the preimplantation development of the mouse, it has been found that a series of such mechanisms are involved in the diversification of the first two cell types, inner cell mass and trophectoderm. The first is a dramatic cellular polarization which takes place during compaction at the eight-cell stage. At this time the blastomeres flatten upon each other and become polarized both at the surface and in the cytoplasm, such that by the end of the eight-cell stage the organization of the blastomeres has been changed from being radially symmetric to polarized, with the axis of polarity being oriented orthogonal to cell contacts. The second cellular mechanism is one of asymmetric cell division which operates on some of the polarized cells when they divide, and occurs as a consequence of their polarized organization. If the orientation of the cleavage furrow falls parallel to the axis of polarity, division results in the formation of two polarized daughter cells; however, if the cleavage is orthogonal to the axis of polarity it produces two different daughter cells: one polar cell which has inherited the apical region of the mother cell and one nonpolar cell derived from the basal part. Thirdly, the unequal adhesive properties of the apical and basal surfaces of the polar cells result in the formation and maintenance of a complete cover of nonpolar cells (inside cells) by polarized ones (outside cells). This process is important for the divergence of cellular phenotypes because without this cover, the nonpolar cells tend to develop a polar phenotype. In the intact embryo, outside cells always give rise to trophectoderm and may give rise to inner cell mass as well, whilst inside cells tend to give rise to the inner cell mass, but may, in certain circumstances contribute to the trophectoderm if for example moved to the outside of the embryo. (For detailed discussion of these events see Johnson, 1985).

It is obviously of great interest to understand the nature of the intercellular interactions and mechanics of the cellular responses that underlie these cellular mechanisms. Microtu-

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bules have become the subject of some interest in this regard since they are known to be involved in many cellular processes such as cell division, control of cell shape, and cytoplasmic organization (see De Brabander, 1982); and indeed they play a part in both the main cellular processes described above that are involved in the diversification of the inner cell mass and trophectoderm. Firstly, they are involved in the setting up of asymmetries within cells during compaction (Maro and Pickering, 1984; Johnson and Maro, 1985; Fleming et al., 1986; Houliston et al., 1987; for review see Maro et al., 1988). Secondly, of course, they form the mitotic spindles of the cleaving embryo, and thus are involved in the asymmetric cell divisions which generate different cell types. The relationship between the organization of the microtubule network in cells and the subsequent divergence of cellular phenotypes has not been described previously.

It seems likely that changes in the organization of the microtubule network, such as those that occur during compaction, could be accompanied by changes in the functional properties of the microtubules in different areas of the cells; and it is possible that such a functional diversity could arise from a structural diversity, perhaps at the level of microtubule components such as tubulin itself or some microtubule-associated protein(s). In this study we have addressed this possibility by examining the distribution of microtubules containing posttranslationally modified forms of α-tubulin, since it has been shown in other cell types that certain modifications such as detyrosination (Gundersen et al., 1984) or acetylation (Piperno and Fuller, 1985) allow the identification of microtubule subsets that, in some cell types, are dynamically different from the rest of the microtubule population (LeDizet and Piperno, 1986; Piperno et al., 1987; Gundersen et al., 1987; Kreis, 1987; Bré et al., 1987; de Pennart et al., 1988). Until now, however, these subsets of microtubules have not been shown to be located in particular areas of the cells or to bear any spatial relation to changes in cell physiology, except in the cases of particularly stable microtubule organelles such as cilia and flagella (Piperno and Fuller, 1985; Sasse et al., 1987). In this study, we demonstrate the existence of a subset of acetylated microtubules in the cells of the preimplantation mouse embryo that develops an asymmetric distribution during compaction. This is transformed into a difference in pattern between daughter cells after a functionally asymmetric division. At both these stages the distribution of acetylated microtubules is spatially distinct from that of the other microtubules in the cells.

Materials and Methods

Recovery of Oocytes and Embryos

Swiss female mice (3-6 wk; Animalerie Spécialisée de Villejuif, Centre National de la Recherche Scientifique [CNRS], France) were superovulated by injections of 5-7.5 IU of pregnant mare's serum gonadotrophin (Intervet, Cambridge, UK) and human chorionic gonadotrophin (Intervet) 48 h apart. They were paired overnight with Swiss males (Animalerie Spécialisée de Villejuif, CNRS, France) and inspected for vaginal plugs the next day. Late four-cell embryos were recovered by flushing late two-cell embryos at 46-50 h after human chorionic gonadotrophin 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 after human chorionic gonadotrophin.

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 their zonae pellucidae, 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 singe four- or eight-cell blastomeres using a flame-polished micropipette. Isolated cells were cultured in polystyrene culture dishes (Falcon, Becton, Dickson, Grenoble, France) 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 0 h 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, Strasbourg, France) in dimethylsulphoxide was used in these experiments and was stored at 4°C. For treatment of the cells, it was diluted in M16+BSA to final concentrations of 0.1-10 μM. A stock solution of 12 mM taxol in DMSO (gift of The National Institutes of Health (NIH); Lot T-4-112, NIH Bethesda, MD) was also stored at 4°C. It was diluted to a final concentration of 2 μM in M16+BSA for treatment of embryos.

Cell Fixation and Immunocytological Staining

Cells were placed in specially designed glass or stainless steel 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 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 have checked in previous studies that the use of 0.6 μM taxol in the extraction buffer does not cause alterations in the microtubule network (Houliston et al., 1987).

Immunocytological staining was performed as described in Maro et al. (1984). The primary antibodies used were YL1/2, specific for tyrosinated α-tubulin (Kilmartin et al., 1982), diluted 1/2,000-1/4,000, and 6-11B-1, specific for acetylated α-tubulin (Piperno and Fuller, 1985), diluted 1/5-1/10. Fluorescein-labeled anti-mouse immunoglobulin antibodies (Kirkegaard & Perry Laboratories, Gaithersburg, MD) or rhodamine-labeled anti-rat immunoglobulin antibodies (Miles Laboratories, Ltd., Slough, UK) were used as second layers. To visualize chromatin, Hoechst dye 33258 (5 μg/ml in PBS) was included with the second antibody.

Photomicroscopy

The coverslips were removed from the chambers and samples were mounted in "Citifluor" (City University, London, UK) and viewed on a Diaplan microscope (E. Leitz, Wetzlar, FRG) with filter sets L2 for FITC-labeled reagents, N2 for TRITC-labeled reagents, and A for Hoechst dye. Photographs were taken on Kodak T-Max film using a Leitz Orthomat photographic system. The three-dimensional structure of the cell is preserved on the whole mount, but as the size of the blastomeres is large (for instance 30 μm in diameter at the eight-cell stage), 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.

Results

Distribution of Tyrosinated and Acetylated Microtubules

In early cleavage-stage (two- and four-cell) embryos the pattern of microtubules containing tyrosinated α-tubulin consisted of a cortical network, a layer of perinuclear microtubules, and a few cytoplasmic microtubules. A depletion in the density of cytoplasmic microtubules close to areas of cell

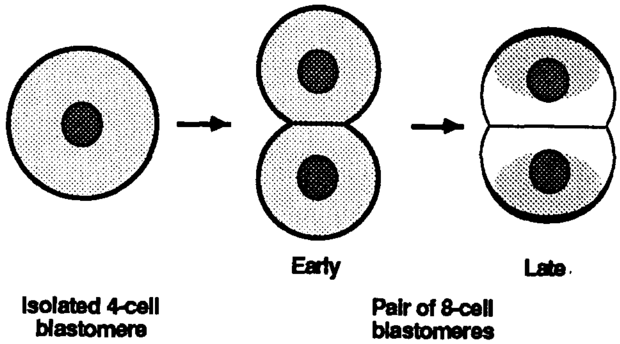

Figure 1. Schematic representation of the experimental procedure used to study compaction at the eight-cell stage. During the eight-cell stage, the two cells in a pair flatten on each other, with both surface and cytoplasmic features becoming polarized along an axis orthogonal to the plane of cell contact.

contact was also seen. The similarity between this pattern and our previous observations (Houliston et al., 1987) using an antibody recognizing all α-tubulin (DM1A; Blose et al., 1984), taken together with the immunoelectron microscopy previously performed with the antityrosinated α-tubulin antibody (Houliston et al., 1987) suggest that essentially all microtubules are visualized with this antibody. In contrast, a distinct subpopulation of microtubules was detected at all stages of development with the antibody recognizing acetylated α-tubulin. These microtubules were found predominantly in the cortices of interphase cells during early cleavage stages (data not shown).

Since the distribution of microtubules in whole mount preparations of mouse preimplantation embryos was difficult to distinguish (these embryos are ~70 μm in diameter and cytoplasmic background staining presents a major difficulty), we used small groups of cells for more detailed examination of events at later stages, in particular pairs of cells derived by division in culture of isolated blastomeres in which the spatial relationships of cells to each other can be determined easily (see Figs. 1 and 3 for experimental protocols). In such pairs it is possible to distinguish clearly individual microtubules by focusing through the samples. Photographs taken in focal planes passing through the cell cortex demonstrate the nature of the staining (Fig. 2 *d*), although focal planes passing through the centers of cells are shown elsewhere in order to enable comparison of cytoplasmic microtubule distributions (e.g., Fig. 2, *a–c* and *e*).

Eight-cell Stage. At the eight-cell stage, during the process of compaction, cells flatten on each other and polarize both in the cytoplasm and at the surface (for review see Johnson and Maro, 1986). Immunofluorescence staining with antibody YL1/2 confirmed that cytoplasmic microtubules

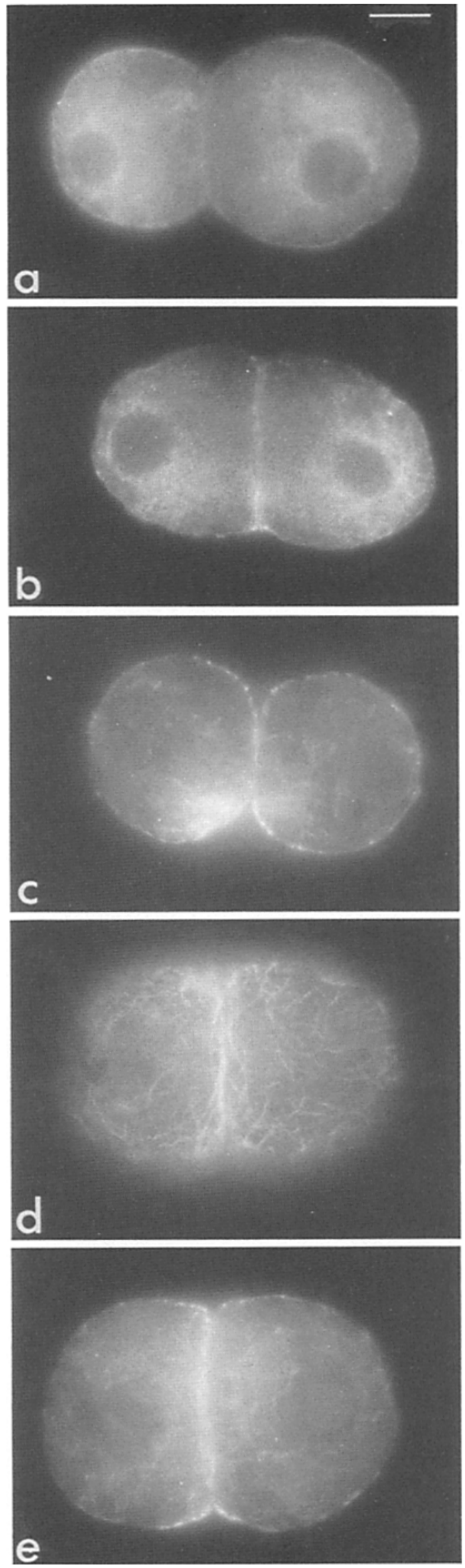

Figure 2. Pairs of eight-cell blastomeres stained with the antityrosinated α-tubulin monoclonal antibody YL1/2 (*a* and *b*) or with the antiacetylated α-tubulin monoclonal antibody 6-11B-1 (*c–e*). (*a* and *c*) 2-h-old pairs; (*b*, *d*, and *e*) 9-h-old pairs. (*d* and *e*) The same pair at different focal planes. Note that in 9-h-old blastomeres cytoplasmic microtubules containing tyrosinated α-tubulin are found in the apical domain of the cell (*b*) while microtubules containing acetylated α-tubulin are found in the basal part of the cell, close to the surface (*d* and *e*). Bar, 10 μm.

Table I.

	Time postdivision	Number of cells	Percentage of cells in which the microtubule network was enriched in apical cytoplasm	depleted in cytoplasm near contact areas*	augmented in cortex near contact areas‡
	h				
Tyrosinated α-tubulin (YL1/2)	2	56	41.1	78.6	0.0
	5	108	72.2	92.6	0.0
	9	154	77.9	79.9	0.0
Acetylated α-tubulin (6-11B-1)	2	62	6.5	0.0	14.5
	5	47	6.4	2.6	52.6
	9	167	6.6	0.0	59.9

* When compared with other areas of the cytoplasm.
‡ When compared with other areas of the cell cortex.

containing tyrosinated α-tubulin redistribute to become relatively concentrated in the apical domain of the cell, leaving the more basal regions of the cytoplasm, especially those away from the cell cortex, relatively depleted in microtubules (Table I and Fig. 2, *a* and *b*; Houliston et al., 1987). In contrast, microtubules recognized by the antiacetylated α-tubulin antibody were found to accumulate progressively in the basal part of the cell, close to the surface. Thus, whereas acetylated microtubules were observed distributed evenly around the cortex in early (2-h-old) eight-cell blastomeres, they were found concentrated near the contact region in older (9-h-old) blastomeres (Table I and Fig. 2, *c–e*).

16-cell Stage. When polarized eight-cell blastomeres divide, two types of pairs of 16-cell blastomeres can be generated: polar/polar pairs or polar/nonpolar pairs (see Fig. 3). Cells in a polar/polar pair will tend to flatten on each other (giving two outside cells) while the polar cell in an polar/nonpolar pair will tend to enclose the nonpolar cell (giving one inside and one outside cell). This reflects the fact that the apical surface of polar cells is less adhesive than the basolateral surface while nonpolar cells are uniformly adhesive.

When such pairs were stained with the antityrosinated α-tubulin antibody, the following pattern of microtubules was observed: cortical networks were predominant in all cells (Fig. 4, *a* and *b*), however cytoplasmic and perinuclear microtubules were much more abundant in outside than inside cells (Fig. 4 *b*). Cortical microtubules were again preferentially acetylated, with this effect being much more marked in the inside cells than the outside ones, giving the impression that there were more acetylated microtubules in inside cells (Fig. 4 *c*). These observations were confirmed when pairs were double stained with the two antibodies (Fig. 5).

Since the pattern of microtubules (both acetylated and tyrosinated) was different between inside and outside cells, we checked that these differences were not due to an artifact linked to the geometry of the cell cluster. To do this, we separated enveloped pairs (polar/nonpolar) from nonenveloped pairs (polar/polar) 8 h after division and cultured both groups in Ca^{++}-free M16+BSA for a further hour in order to inhibit cell adhesion and reverse the enclosure process (Fig. 4, *d–g*). This treatment was successful in reversing enclosure in ~75% of enclosed pairs. When these pairs were stained with the two antibodies the asymmetries observed in control pairs tended to be maintained; in pairs stained with YL1/2, one cell had fewer cytoplasmic and perinuclear tyrosinated microtubules (Fig. 4 *e*) while in pairs stained with 6-11B-1 one cell had more cortical acetylated microtubules (Fig. 4 *g*). Similarly, nonenveloped pairs retained their more symmetrical patterns of staining (Fig. 4, *d* and *f*).

32-cell Stage. A similar pattern of microtubule staining was observed in clusters of four cells derived by the culture of isolated eight-cell blastomeres to the 32-cell stage (Fig. 6, *a–d*). Inside cells were enriched in microtubules containing

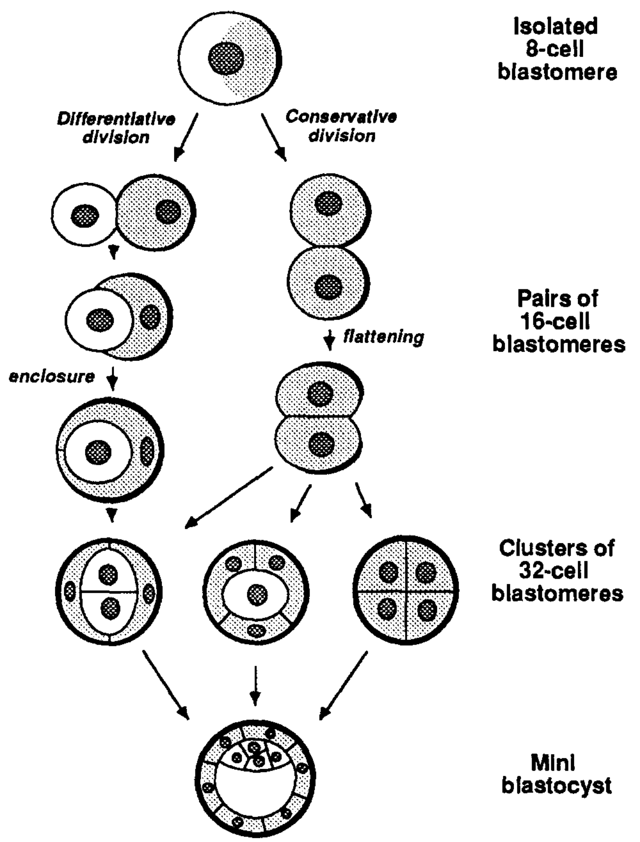

Figure 3. Schematic representation of the experimental procedure used to study cell diversification at the 16- and 32-cell stages. At the 16-cell stage, after a differentiative division, the polar cell encloses the nonpolar cell; while after a conservative division, the cells flatten on each other. Differentiative division can also occur at the 16–32-cell transition. Each interphase lasts ~10–12 h.

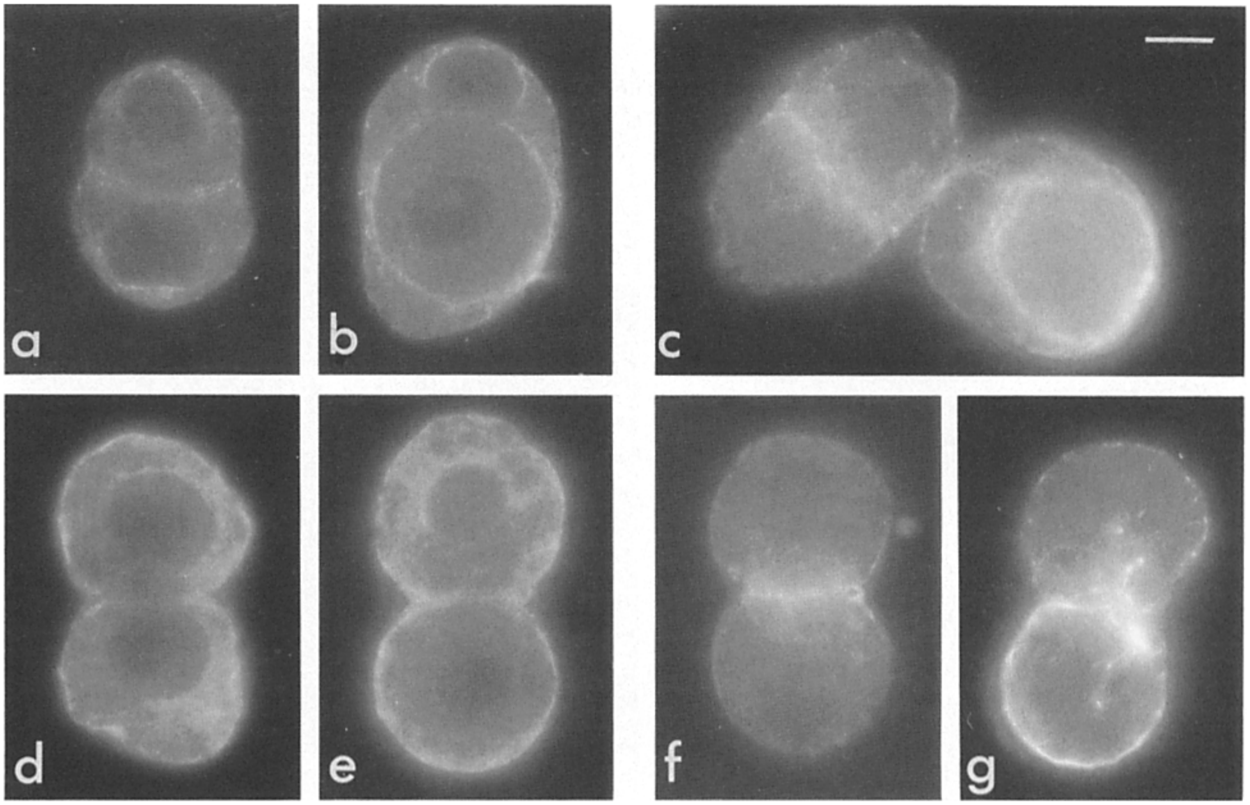

Figure 4. Pairs of 9-h-old 16-cell blastomeres stained with the antityrosinated α-tubulin monoclonal antibody YL1/2 (*a*, *b*, *d*, and *e*) or with the antiacetylated α-tubulin monoclonal antibody 6-11B-1 (*c*, *f*, and *g*). (*a–c*) Control pairs. (*d–g*) Pairs exposed to Ca^{++}-free medium. *d* and *f* are polar/polar pairs while *e* and *g* are enclosed pairs (polar/nonpolar) where the enclosure process has been reversed. Note that cytoplasmic and perinuclear microtubules containing tyrosinylated tubulin are more abundant in outside than inside cells (*b*) and that cortical microtubules are preferentially acetylated (*c*), this effect being more marked in inside cells. Also, note that the asymmetries observed in control pairs are maintained when the enclosure process is reversed (*e* and *g*). Bar, 10 μm.

acetylated α-tubulin whereas outside cells had more cytoplasmic and perinuclear microtubules than inside cells (Fig. 6).

Acetylated Microtubules Are More Stable

We examined the stability of microtubules at various stages of development by use of the drug nocodazole. This drug inhibits microtubule polymerization and thus if used at low doses can be used to assess the stability of microtubules. Various doses of nocodazole ranging from 0.1–10 μM were tested. 1 μM was used routinely. At this dose, many microtubules were still present after 15 min in the drug but only a few after a 60-min treatment.

In 9-h-old pairs of eight-cell blastomeres, exposure to nocodazole for 60 min resulted in the loss of most microtubules detected by the antityrosinated α-tubulin antibody. Those microtubules that remained tended to be located in the basal cell cortex (Fig. 7 *a*). Although the number of acetylated microtubules also decreased, a noticeable proportion remained present (Figs. 7 *b* and 8). Double immunofluorescence experiments revealed that most of the remaining microtubules were acetylated (data not shown).

At the 16-cell stage, it was interesting to note that the greater abundance of acetylated α-tubulin in inside cells was not accompanied by a greater resistance of the microtubules containing it to depolymerization; treatment with 1 μM nocodazole for 60 min resulted in the depolymerization of almost all microtubules in inside cells, while some remained in outside cells (Figs. 7, *c* and *d*, and 9). The microtubules that did remain did, however, contain acetylated α-tubulin. Similar results were found at the 32-cell stage; inside cells had a greater number of acetylated microtubules but these were less stable than the ones present in outside cells (Fig. 6, *e–h*). Thus, it seems that acetylation may correlate with

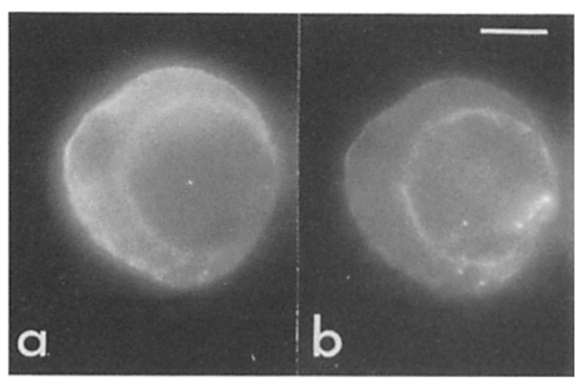

Figure 5. Enclosed pair of 16-cell blastomeres double stained with the antityrosinated α-tubulin monoclonal antibody YL1/2 (*a*) and with the antiacetylated α-tubulin monoclonal antibody 6-11B-1 (*b*). Bar, 10 μm.

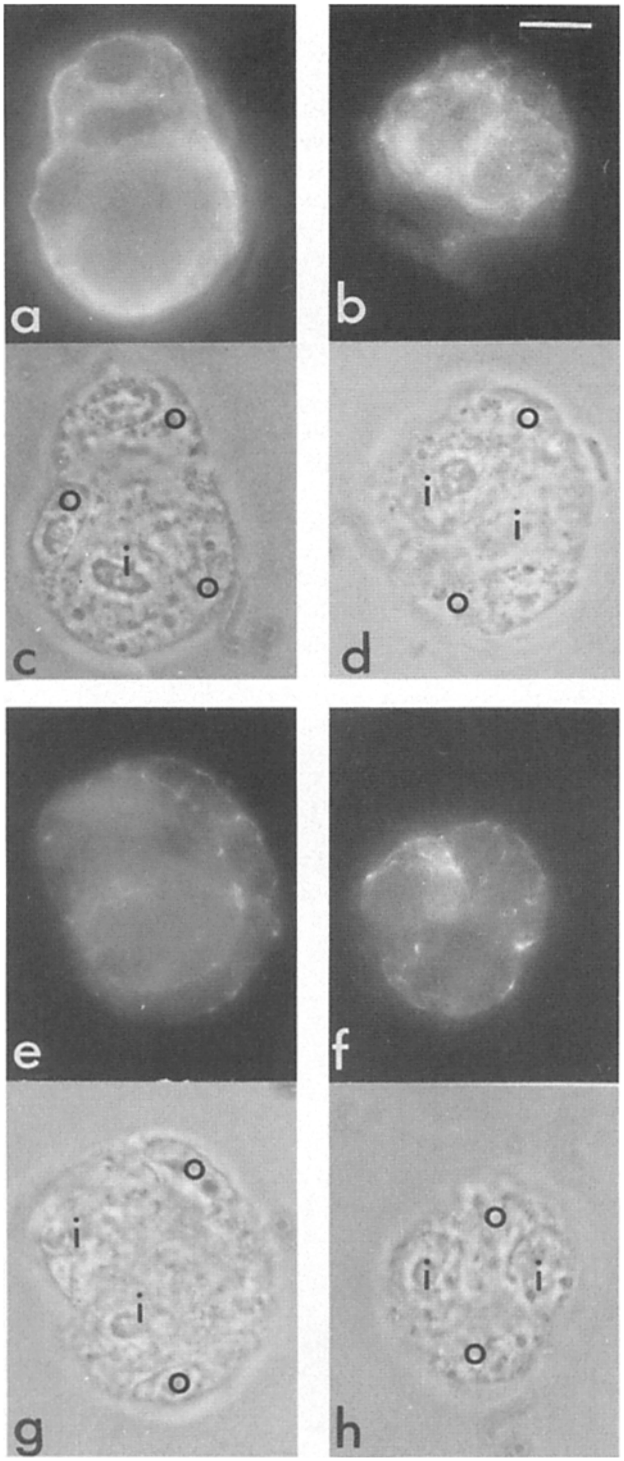

Figure 6. Quartets of 32-cell blastomeres stained with the antityrosinated α-tubulin monoclonal antibody YL1/2 (*a* and *e*) or with the antiacetylated α-tubulin monoclonal antibody 6-11B-1 (*b* and *f*). (*c*, *d*, *g*, and *h*) The corresponding phase-contrast pictures of *a*, *b*, *e*, and *f*, respectively. (*i*) Inside cell; (*o*) outside cell. (*a–d*) Control quartets; (*e–h*) quartets treated with 1 μM nocodazole for 60 min at 37°C. Note that cytoplasmic microtubules containing tyrosinylated tubulin are more abundant in outside than inside cells (*a*) and that inside cells are enriched in cortical microtubules containing acetylated α-tubulin (*b*). Bar, 10 μm.

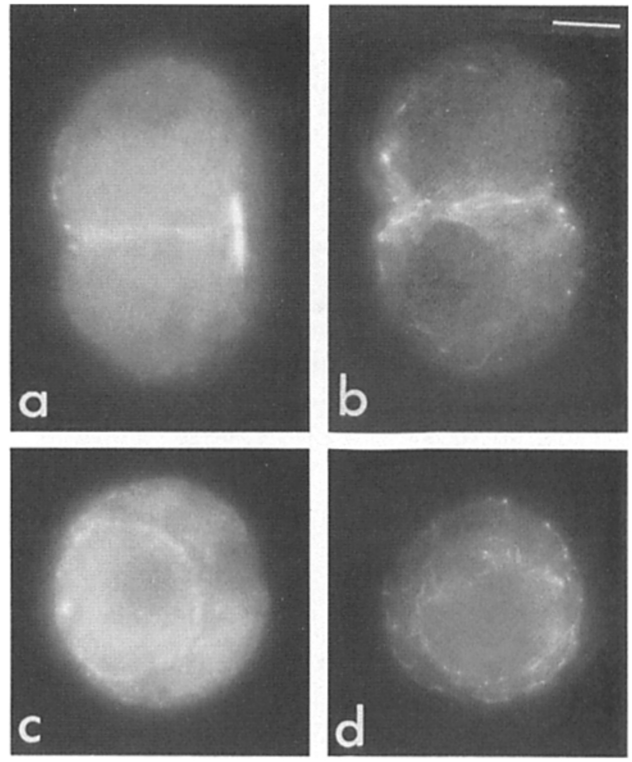

Figure 7. Pairs of 9-h-old eight-cell blastomeres (*a* and *b*) or 16-cell blastomeres (*c* and *d*) treated with nocodazole. *a* and *b* were treated with 1 μM for 15 min at 37°C; *c* and *d* with 1 μM for 60 min at 37°C. Pairs were stained with the antityrosinated α-tubulin monoclonal antibody YL1/2 (*a* and *c*) or with the antiacetylated α-tubulin monoclonal antibody 6-11B-1 (*b* and *d*). Bar, 10 μm.

relative stability of microtubules within a cell but cannot be used as an indicator of relative microtubule stability in different cell types.

Microtubule Acetylation Is Not Restricted to the Basal Part of the Cell

One possible explanation for the relative enrichment of acetylated microtubules in the basal part of eight-cell blastomeres could be that the enzyme responsible for the acetylation, α-tubulin acetyltransferase, is preferentially located or preferentially active in this area. Alternatively, the experiments with nocodazole described above suggest that microtubules are more stable in the basal cortex of the cell, and this may render them more susceptible to modification by the enzyme than less stable microtubules (located apically). To discriminate between these possibilities, we treated eight-cell blastomeres with a low dose of taxol (2 μM) for a short period of time (15 min) in order to stabilize briefly all cellular microtubules. After this treatment, basal and apical microtubules tended to be uniformly acetylated, indeed the anti-acetylated and antityrosinated α-tubulin antibodies gave very similar staining patterns (Fig. 10). This result suggests that basal cortical microtubules become acetylated because they are more stable than apical ones and not because of an asymmetric distribution of acetyltransferase activity.

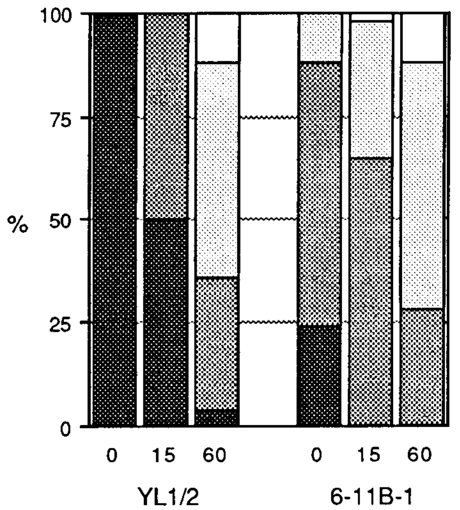

Figure 8. Histogram showing the effect of 1 μM nocodazole on the distribution of cortical microtubules in 9-h-old pairs of eight-cell blastomeres. Cells were observed under the fluorescence microscope and the microtubule distribution scored in the following way: (*white space*) no microtubules; (*light grey bars*) some microtubules; (*medium gray bars*) network; (*dark gray bars*) dense network. The results are expressed as the percentage of cells with a given score in control cells (*0*) and after 15-min (*15*) and 60-min (*60*) treatments with nocodazole. The numbers of cells scored for tyrosinated α-tubulin (*YL1/2*) distribution were 205 controls, 80 after 15-min nocodazole treatment, and 76 after 60-min nocodazole treatment. For acetylated α-tubulin (*6-11B-1*), these numbers are 234 controls, 162 after 15-min nocodazole treatment, and 122 after 60-min nocodazole treatment.

Discussion

In this paper, the redistribution of microtubule subpopulations during the process of cell polarization has been described. One population of cytoplasmic microtubules containing tyrosinated α-tubulin redistributes towards the apex of the cell during the eight-cell stage (Houliston et al., 1987). During the same period, a population of cortical microtubules containing acetylated α-tubulin accumulate near the zone of intercellular contact in the basal part of the cell. This is the first time, to our knowledge, that the progressive segregation of two populations of microtubules into different parts of a cell has been described. The relative concentration of acetylated microtubules in basal regions appears not to be a consequence of a localized enzyme activity, since apical microtubules become acetylated after brief stabilization by taxol. Given that these acetylated microtubules are preferentially resistant to the depolymerizing effect of nocodazole, it appears that they constitute a more stable population of microtubules in the blastomere, as has been noted for acetylated microtubules in other cell types (LeDizet and Piperno, 1986; Piperno et al., 1987) including mouse oocytes (De Pennart et al., 1988). These observations suggest that it is perhaps a difference in the dynamic behavior of the microtubule populations between apical and basal regions that results in the accumulation of acetylated microtubules basally, their increased stability allowing time for the acetyltransferase to modify the tubulin subunits. It is known that polymerized α-tubulin is a better substrate for tubulin acetyltransferase than the dimer (Maruta et al., 1986).

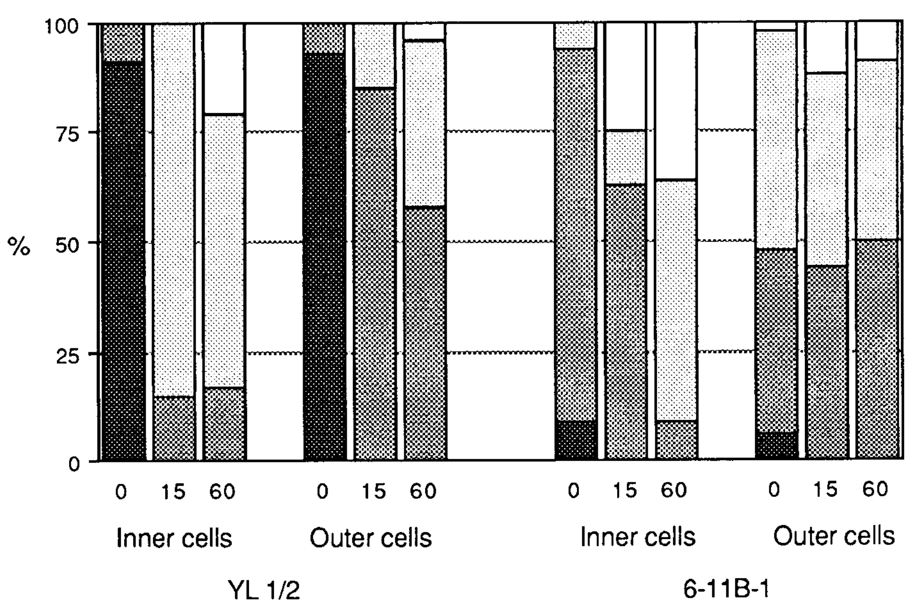

Figure 9. Histogram showing the effect of 1 μM nocodazole on the distribution of cortical microtubules in 9-h-old pairs of 16-cell blastomeres. Only polar/nonpolar pairs are included. Cells were observed under the fluorescence microscope and the microtubule distribution scored in the following way: (*white bars*) no microtubules; (*light gray bars*) some microtubules; (*medium gray bars*) network; (*dark gray bars*) dense network. The results are expressed as the percentage of cells with a given score in control cells (*0*), and after 15-min (*15*) and 60-min (*60*) treatment with nocodazole in inside and outside cells. The numbers of polar/nonpolar pairs of cells scored for tyrosinated α-tubulin distribution were 70 (out of 176 pairs) controls, 13 (out of 38 pairs) after 15-min nocodazole treatment, and 24 (out of 61 pairs) after 60-min nocodazole treatment. For acetylated α-tubulin, these numbers are 66 (out of 149 pairs) controls, 16 (out of 42 pairs) after 15-min nocodazole treatment, and 22 (out of 63 pairs) after 60-min nocodazole treatment.

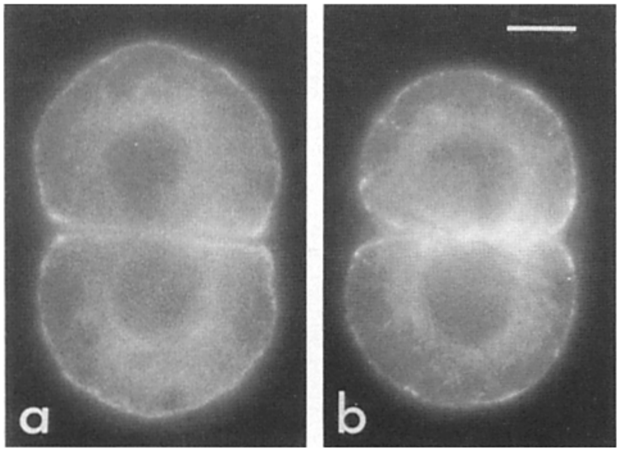

Figure 10. Pairs of 9-h-old eight-cell blastomeres treated with 2 μM taxol for 15 min at 37°C and stained with the antityrosinated α-tubulin monoclonal antibody YL1/2 (*a*) or with the antiacetylated α-tubulin monoclonal antibody 6-11B-1 (*b*). Note that the two antibodies give very similar staining patterns. Bar, 10 μm.

The pattern of microtubules observed in outside and inside cells at the 16-cell stage was strikingly similar to the one observed in the apical and basal regions of polarized eight-cell blastomeres, perhaps reflecting the origin of the two cell types at division. Cortical networks of microtubules containing tyrosinated α-tubulin were again predominant in both cell types, with other cytoplasmic and perinuclear microtubules being much more abundant in outside than inside cells. The only obvious difference from the eight-cell stage was that the depletion of cytoplasmic microtubules near cell contacts seen in eight-cell blastomeres was less dramatic in 16-cell blastomeres. Whether this difference reflects a change in the response of microtubules to some contact-induced signal, a change in that signal, or simply the reduced volume of the cells is as yet unclear. As with the overall microtubule pattern, we found that the pattern of α-tubulin acetylation in 16-cell blastomeres corresponded to that in different parts of the eight-cell blastomere. Cortical microtubules were preferentially acetylated, especially those adjacent to regions of cell contact. However, inside cells, which when enveloped were in contact over their entire surface, seemed to have more acetylated microtubules than did the enveloping outside cells. It is interesting to note that the greater abundance of acetylated α-tubulin in inside cells was not accompanied by a greater resistance of the microtubules containing it to depolymerization. A dose of nocodazole was found which resulted in the depolymerization of almost all microtubules in inside cells, while some remained in outside cells. The microtubules that did remain did, however, contain acetylated α-tubulin. Thus, it seems that acetylation may correlate with relative stability of microtubules within a cell but cannot be used as an indicator of relative microtubule stability when different cell types are compared. We might imagine that high degree of acetylation in inside cells results from a higher proportion of stable (cortically located) microtubules in the cell because of a lower density of rapidly turning over (cytoplasmic) microtubules.

The translation of the asymmetric microtubule organization of a polarized cell into differences between cells could provide a neat mechanism by which differences between cells can be created during cell diversification. It should be noted, however, that particular regional features of the microtubule network cannot themselves be directly passed on from mother to daughter cells because the interphase microtubule network is replaced by a spindle during mitosis. It will be interesting to discover how differences in the microtubule network become established as the cell types of the early embryo diverge in phenotype, and what the cellular consequences (if any) of these differences are.

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Note Added in Proof: After the acceptance of this paper, Schatten et al. (Schatten, G., C. Simerly, D. J. Asai, E. Szöke, P. Cooke, and H. Schatten. 1988. *Dev. Biol.* 130:74–86) published a paper dealing with acetylated α-tubulin during early development of the mouse. In the cleaving embryo, they observed acetylated microtubules only in the extremely stable midbodies, with no cytoplasmic network. They found no relationship between acetylation and microtubule stability, even in oocytes. The differences between their results and ours (this paper covering cleavage stages, and De Pennart et al. [De Pennart, H., E. Houliston, and B. Maro. 1988. *Biol. Cell.* 64:375–378] concerning the egg) are probably due to differences in handling procedures.

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