J. Embryol. exp. Morph. 90, 311–334 (1985)
Printed in Great Britain © The Company of Biologists Limited 1985

A dissection of the mechanisms generating and stabilizing polarity in mouse 8- and 16-cell blastomeres: the role of cytoskeletal elements

MARTIN H. JOHNSON AND BERNARD MARO*
Department of Anatomy, Downing Street, Cambridge CB2 3DY, U.K.

SUMMARY

Pairs of 8-cell or 16-cell blastomeres were cultured for up to 9 h after their formation from isolated 1/4 or 1/8 blastomeres respectively. Blastomeres were examined for the incidence and orientation of their surface polarity, as assessed by binding of FITC-Con A and by distribution of microvilli, and of their cytoplasmic polarity, as assessed by distribution of cytoplasmic actin, clathrin and a 100kD antigen associated with the lysosomal/acid vesicle fraction of membranous organelles. The effect on polarity of incubating the pairs of cells in taxol, nocodazole, cytochalasin D or in a combination of nocodazole plus cytochalasin D for different parts of the incubation period was examined.

Neither the development nor the stability of the surface polarity in 8-cell blastomeres was blocked by any treatment and only the use of CCD in combination with nocodazole affected the incidence of surface polarity appreciably. However, with some treatments, the form and position of the surface poles were modified. In the presence of microtubule inhibitors surface poles extended over a larger area of the cell surface, while exposure to CCD led to poles that were not opposite to the contact point between cells. In contrast to surface polarity, the development of cytoplasmic polarity was suppressed by both microtubule- and microfilament-inhibiting drugs, which also reversed it rapidly.

In polar 16-cell blastomeres surface polarity was influenced in a similar manner to that of 8-cell blastomeres, only the combined use of cytochalasin D and nocodazole having any major effect. Polarization of clathrin in polar 16-cell blastomeres was inhibited almost completely by all drug treatments applied including cytochalasin D. The focal concentration of lysosomal antigen that occurs during the 16-cell stage was reduced only in the continuous presence of nocodazole plus cytochalasin D, but once established was not reversed appreciably by any drug. However, the localization of the lysosomal antigen to the basal region of polarized cells did not seem to occur in the presence of any drug. The dissociation of surface and cytoplasmic polarity revealed in these experiments leads us to conclude that either (1) surface polarity is a prerequisite for the organization of cytoplasmic polarity, and mediates the latter via the cytoskeleton, or (2) surface and cytoplasmic polarity develop by parallel but separate mechanisms.

INTRODUCTION

The 8-cell stage of mouse embryogenesis is characterized by several marked morphological changes including the flattening of cells upon each other to

* C.N.R.S., Paris, France.

Key words: microtubules, microfilaments, mouse, morula, compaction, polarization, taxol, nocodazole, cytochalasin D.

maximize cell contact (Lehtonen, 1980), the establishment of intercellular junctions (Ducibella, Albertini, Anderson & Biggers, 1975; Magnuson, Demsey & Stackpole, 1977; Lo & Gilula, 1979; Goodall & Johnson, 1984), and the reorganization of surface and cytoplasmic components at the 8-cell stage to generate polarized cells from cells that were previously radially symmetrical (Handyside, 1980; Johnson & Maro, 1984; Maro, Johnson, Pickering & Louvard, 1985; Fleming & Pickering, 1985). At division to the 16-cell stage two phenotypically and functionally distinguishable cell subpopulations are generated (Johnson & Ziomek, 1981*a*), and these various differences are further elaborated during the 16-cell stage thereby yielding cells with increasingly divergent phenotypes and developmental potentials (Fleming, Warren, Chisholm & Johnson, 1984; Fleming & Pickering, 1985). There is now reasonable evidence to suggest that of these events, the processes of intercellular flattening and of cell polarization are events central to the generation of cell diversity (Johnson, 1985*a*,*b*), and thus an understanding of their regulation is important for an understanding of development.

Since both intercellular flattening and polarization involve major reorganizations of cell constituents, as well as the interaction of one cell with another, it is reasonable to suspect that cytoskeletal elements will be involved in the regulation of these processes. Indeed, there is already compelling evidence to suggest that microfilaments not only redistribute during the 8-cell stage at the time of cell flattening and polarization (Lehtonen & Badley, 1980; Johnson & Maro, 1984), but also that they are involved actively in the process of cell flattening itself (Ducibella, Ukena, Karnovsky & Anderson, 1977; Surani, Barton & Burling, 1980; Pratt, Chakraborty & Surani, 1981; Johnson & Maro 1984). A role for microfilaments in cell polarization is less clearly established and more difficult to prove, since microfilamentous actin constitutes one of the important components by which polarization can be recognized (Johnson & Maro, 1984). However, the available evidence suggests that in the absence of turnover of microfilamentous actin, polarity may nonetheless develop, but in a more disorganized and disorientated way than in control embryos (Johnson & Maro, 1984).

Intermediate filament proteins are detected at these early stages of development (Lehtonen *et al.* 1983; Oshima *et al.* 1983), but do not seem to be assembled into intermediate filaments to any appreciable extent until the very late 8- or early 16-cell stage (Lehtonen *et al.* 1983; J. C. Chisholm and B. Maro, unpublished data).

Microtubules are present throughout early development and changes in the disposition and organization of microtubules occur during the 8-cell and 16-cell stages (Ducibella *et al.* 1977). Use of drugs that impair turnover and/or stability of microtubules has produced major disturbances of development (Surani *et al.* 1980; Pratt *et al.* 1981; Sutherland & Calarco-Gillam, 1983). However, these studies involved the culture of embryos over a period of mitosis, at which the cells blocked. When application of the drugs, and analysis of the consequences of their action, was limited to the period of interphase, remarkably little effect on the processes of flattening and polarization was observed (Ducibella & Anderson,

1975; Ducibella, 1982; Maro & Pickering, 1984). In general, it was concluded that the absence of microtubules was compatible with cell flattening and polarization, whilst in the presence of the uncontrolled assembly of microtubules, cell flattening and polarization were delayed and reduced in incidence (Maro & Pickering, 1984). Thus, microtubules appeared to exercise a constraining role, impairing the initiation of these processes but not being required for their completion.

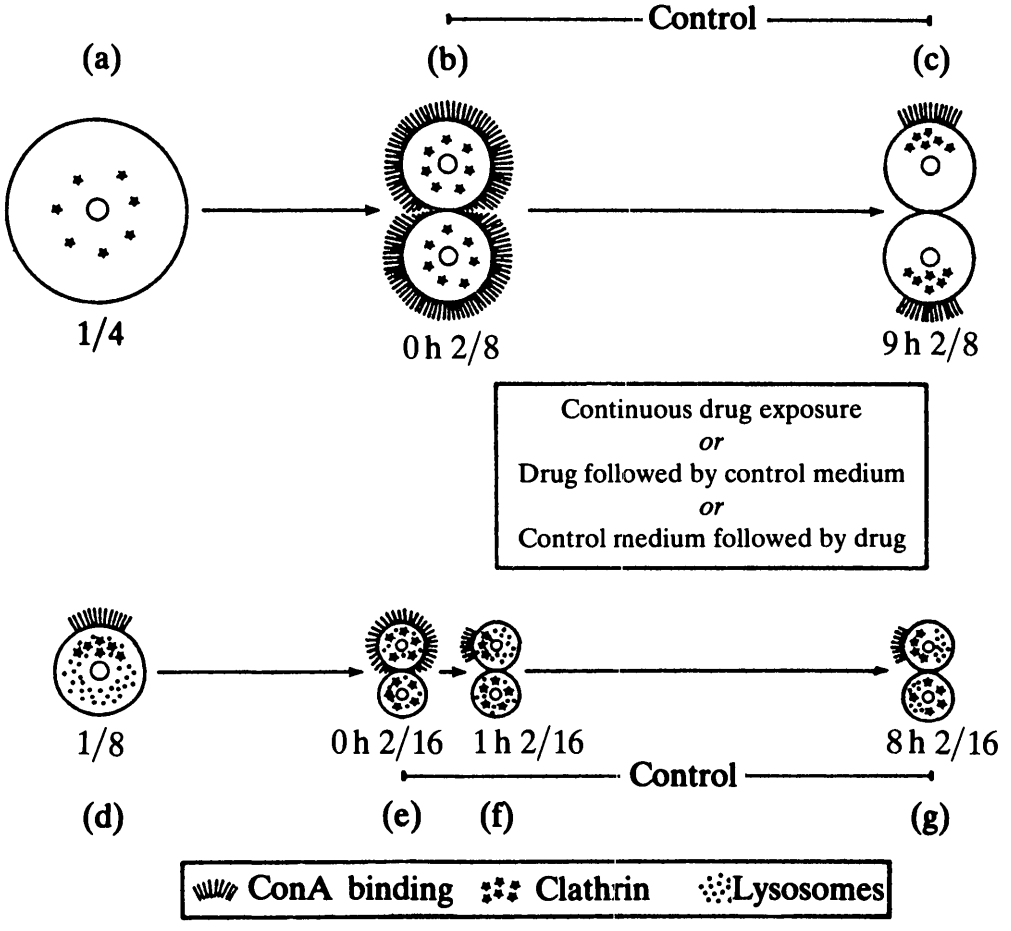


Fig. 1. Schematic outline of protocols used, and a summary of conclusions from previously published work. Quarter blastomeres disaggregated from 4-cell embryos divide to yield 2/8 pairs that are non-polar (a to b). 9 h later the blastomeres are polarized cytoplasmically and at their surface (c). Isolated polarized 1/8 cells taken from late 8-cell embryos (d) divide to yield a 2/16 couplet both cells of which lack cytoplasmic polarity; if the orientation of the division plane is perpendicular to the axis of polarity as shown in d to e, then the polar surface features are restricted to one cell. Within an hour or so both cytoplasmic and surface poles become tightly focused in that cell, the other cell remaining apolar (f). As the 16-cell stage progresses, lysosomal antigen concentrates in a single focus in both cell types but becomes increasingly located opposite to the surface and clathrin poles in the polar cells (g). In the experiments reported here, drug treatments at the 8- and 16-cell stages were applied over the period and in the sequence shown in the large box.

These studies on the effects of microtubule-inhibiting drugs on early embryos were limited in two ways. First, only the 8-cell stage was studied. Second, they were performed on whole embryos, which makes difficulties for experimental protocol and analysis. For example, there is considerable heterogeneity in division time amongst the blastomeres *within* a single embryo (Lehtonen, 1980; Smith & Johnson, 1985). Thus, some 8-cell blastomeres in an embryo may be 3–4 h old by the time the last 4-cell divides to two 8-cells. If drugs are added only in interphase then clearly some blastomeres will have a longer exposure than others. Moreover, the visualization of polarization at light and scanning electron microscope levels is sometimes difficult on mounted whole embryos, in which much of the blastomere is hidden or obscured, by being deep within the cell cluster.

In this paper we report on the action of the two microtubule-inhibiting drugs, nocodazole and taxol, and the microfilament-inhibiting drug, cytochalasin D, on events occurring during interphase of the 8-cell and 16-cell stages. We used pairs of cells, of known age after their formation at the preceding division, and exposed them to drugs for controlled periods of time. We analysed the pairs of cells for evidence of polarization of both surface and cytoplasmic features. A general schematic summary of our approach is illustrated in Fig. 1. Our results indicate a dissociation between surface and cytoplasmic polarity and lead us to propose a model for polarization.

MATERIALS AND METHODS

1. Recovery of embryos

MF1 female mice (3–5 weeks; Olac) were superovulated by injections of 5 i.u. of pregnant mare's serum gonadotrophin (PMSG; Intervet) and human chorionic gonadotrophin (hCG; Intervet) 48 h apart. The females were paired overnight with HC-CFLP males (Hacking & Churchill) and inspected for vaginal plugs the next day. Late 4-cell embryos were recovered by flushing late 2-cell embryos at 46–50 h post-hCG followed by overnight culture in Medium 16 containing 4 mg BSA ml^{-1} (M16+BSA; Whittingham & Wales, 1969) under oil at 37 °C in 5 % CO_2 in air. Late 8-cell embryos were recovered at 65–70 h post-hCG.

2. Preparation and handling of single cells

Late 4-cell and late 8-cell embryos were exposed briefly to acid Tyrode's solution (Nicolson, Yanagimachi & Yanagimachi, 1975) to remove the zona pellucida, rinsed in Medium 2+BSA (Fulton & Whittingham, 1978), and placed in Ca^{2+}-free M2+6 mg BSA ml^{-1} for 5–45 min, during which time they were disaggregated to single 4- or 8-cell blastomeres (1/4 or 1/8 cells) using a flame-polished micropipette. Isolated cells were cultured in Sterilin tissue culture dishes 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. Couplets were then cultured in M16+BSA as natural 2/8 pairs.

3. Immunocytochemistry

Surface polarity was assessed by incubation of cells or embryos in either 50 $\mu g\,ml^{-1}$ tetramethylrhodamine-labelled Succinyl Concanavalin A (TMRTC-S-ConA; Polysciences) or 700 $\mu g\,ml^{-1}$ fluorescein isothiocyanate-labelled Concanavalin A (FITC-Con A; Polysciences) for 5 min at room temperature, followed by two to three washes in M2+BSA. Labelled cells were then placed in specially designed chambers exactly as described in Maro, Johnson, Pickering & Flach (1984) for fixation with 3·7 % formaldehyde followed by extraction with 0·25 % Triton X-

100. After washing, cells were labelled either with affinity-purified rabbit anti-actin antibodies (Gounon & Karsenti, 1981) or rabbit anti-clathrin antibodies or rabbit anti-lysosome antibodies to a 100kD antigen associated with the lysosomal (Louvard *et al.* 1983) and acid vesicle membranous compartment (Reggio *et al.* 1984) or mouse monoclonal anti-tubulin antibodies (Amersham) followed by rhodamine or fluorescein-labelled anti-rabbit or anti-mouse immunoglobulin antibodies. The detailed characteristics of the procedures and of control staining patterns are reported in Maro *et al.* (1984, 1985) and Johnson & Maro (1984).

Samples were mounted in 'Citifluor' (City University, London) and viewed on a Leitz Ortholux Photomicroscope with filter set L2 for FITC-labelled reagents and N2 for TMRTC-labelled reagents. Photography was on Tri-X film with exposures of 3–45 sec. The mounting medium reduces fading of fluorescein labels.

4. Scanning electron microscopy (SEM)

Pairs of 2/8 cells were cultured for 9 h under various conditions and were prepared for SEM analysis exactly as described in Johnson & Ziomek (1982). Samples were viewed in a JSM-35CF Jeol microscope under 20 kilovolts and scored for the presence or absence of microvillous poles. Numbers of cells examined were as follows: (A) controls – 18 (70 % polarized); (B) last hour in nocodazole – 40 (40 % polarized); (C) last hour in taxol – 34 (44 % polarized); (D) last hour in nocodazole+CCD – 26 (16 % polarized); (E) 9 h in nocodazole – 34 (53 % polarized); (F) 9 h in taxol – 20 (36 % polarized); (G) 9 h in CCD – 26 (61 % polarized); (H) 9 h in nocodazole plus CCD – 16 (12 % polarized).

5. Reagents

The following drugs were used: taxol at 1·2, 12 and 24 μM (Lot T-4-112, National Institutes of Health, Bethesda, Stock solution stored at −70°C), nocodazole at 10 μM (Aldrich), cytochalasin D (Sigma, used at 0·5 $\mu g\,ml^{-1}$ after storage at −20°C in DMSO at a concentration of $1\,mg\,ml^{-1}$).

RESULTS

1. *Effect of taxol and nocodazole on microtubules*

We first confirmed that taxol (12 and 24 μM) and nocodazole (10 μM) were effective when used on pairs of cells. Cells were cultured for 1, 2, 4 or 9 h in the drugs, and then analysed immunocytochemically for their distribution of tubulin. Control cells showed a delicate and mainly cortical microtubular mesh, part of which appeared to fan out from the midbody remaining from the previous mitotic division (Fig. 2a,b). Within one hour of exposure to the drugs, all 8-cell blastomeres analysed had either diffuse staining indicating that microtubules had depolymerized (nocodazole; Fig. 2d) or microtubules condensed into large, irregular bundles (taxol; Fig. 2c,e). In addition, we noticed that when taxol-treated blastomeres were examined with antibodies to clathrin, the induced proliferation of microtubules around the midbody remnant of the previous cleavage spindle was associated with heavy clathrin staining (Fig. 2g). An association between clathrin and the microtubules of the mitotic spindle has been reported previously (Louvard *et al.* 1983; Maro *et al.* 1985).

2. *Effects of drugs on stability of polarity in polarized 8-cell blastomeres*

Pairs of 8-cell blastomeres were cultured together for 6, 7 or 8 h at which time some were sampled for analysis of polarity, whilst others were transferred to and

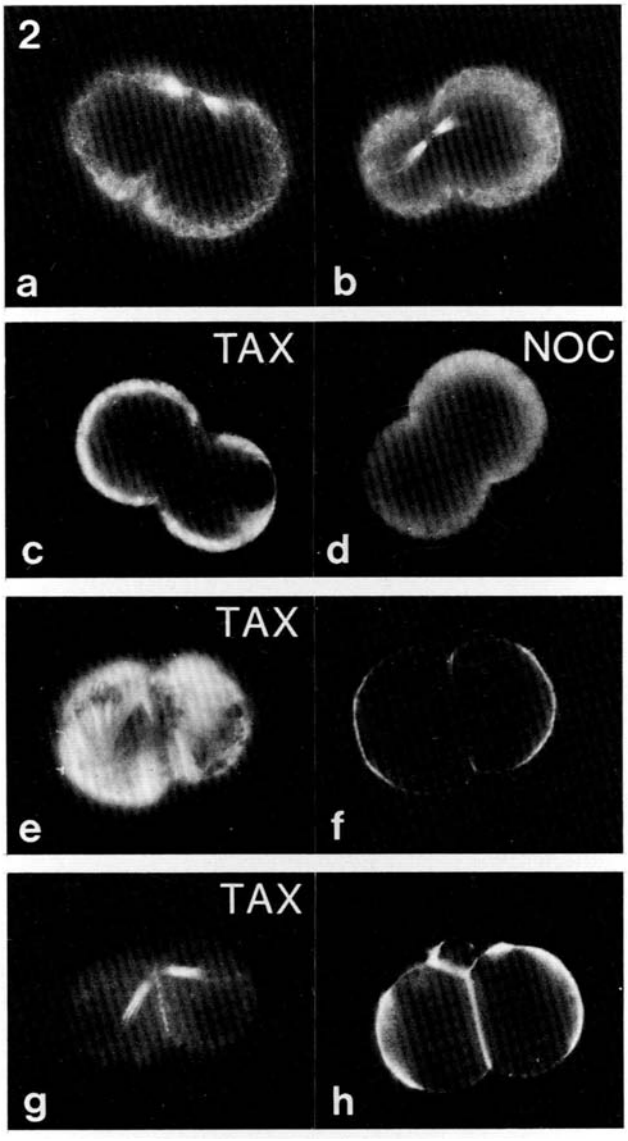


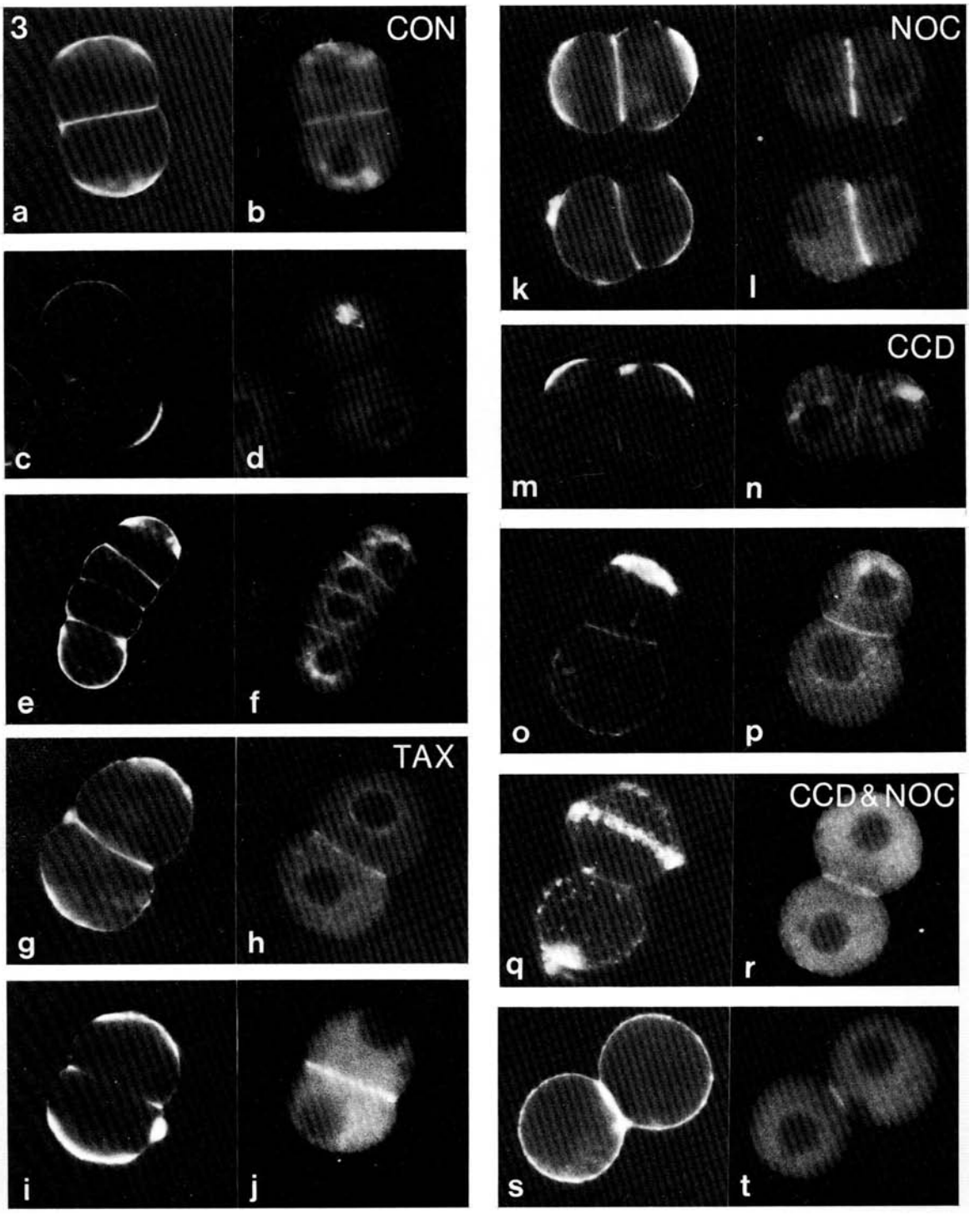
Fig. 2. Pairs of 8-cell blastomeres cultured for 9 h *in vitro* and examined immunocytochemically for distribution of tubulin (a,b,c,d,e), surface Con A binding sites (f,h) or clathrin (g). In controls (a,b), a fine cortical mesh of microtubular tubulin is observed together with the remnant of the previous mitotic spindle (midbody) still linking the two cells. Treatment with nocodazole for 1 or more hours (d) results in loss of microtubular structure yielding a diffuse staining pattern, whilst treatment with taxol for 1 or more hours leads to condensation and bundling of microtubules viewed in optical section (c) or *en face* (e). Note that surface Con A binding remains polar after taxol treatment (f,h) but clathrin is dispersed throughout the cells localizing only to the remnant of the mitotic spindle shown here *en face* (g). (Mag. ×410).

Table 1. *Effect of drugs on the stability of polarity in 2/8 pairs of blastomeres*

Treatment (1)	Age at analysis (h) (2)	No. of cells scored (3)	% of cells: Polar surface and cytoplasm (4)	% of cells: Polar surface only (5)	% of cells: Polar cytoplasm only (6)	% of cells: Non-Polar (7)	% of cells: With surface poles (4+5) (8)	% of cells: With cytoplasmic poles (4+6) (9)
(1) Control	6	40	60	0	35	5	60	95
(2) Control	7	44	61	5	24	12	66	85
(3) Control	8	51	64	0	26	10	64	90
(4) Control	9	144	75	4	2	19	79	77
(5) Taxol (24 μM) 6–9 h*	9	39	3	64	0	33	67	3
(6) Taxol (24 μM) 7–9 h*	9	41	17	54	0	29	71	17
(7) Taxol (24 μM) 8–9 h*	9	48	6	60	7	27	66	13
(8) Taxol (12 μM) 7–9 h*	9	46	13	54	0	33	67	13
(9) Taxol (12 μM) 8–9 h*	9	66	24	48	2	26	72	26
(10) Taxol (1·2 μM) 8–9 h*	9	37	16	54	3	27	70	19
(11) Nocodazole (10 μM) 8–9 h*	9	124	3	64	3	40	67	6
(12) Cytochalasin D(0·5 μg μl^{-1})† 8–9 h*	9	107	36	33	5	26	69	41
(13) Cytochalasin D+ nocodazole† 8–9 h*	9	78	4	35	0	61	39	4

* Period of exposure to drug.

† These samples analysed for cytoplasmic polarity with anti-clathrin antibodies; with anti-actin antibodies no internal localization of microfilaments is evident – see Johnson & Maro (1984).


3
CON
a
b
NOC
k
l
c
d
CCD
m
n
e
f
o
p
TAX
g
h
CCD&NOC
q
r
i
j
s
t

incubated in drugs for a further 3, 2 or 1 h respectively. A control group of blastomeres was cultured for 9 h in the absence of drugs. The results are summarized in Table 1.

Amongst control pairs, cytoplasmic polarity (as assessed by either clathrin or actin distribution) was detectable at earlier times and in more cells than was surface polarity (Table 1, lines 1–4, Fig. 3a,b). This relatively early development of cytoplasmic polarity in relation to surface polarity has been reported previously (Johnson & Maro, 1984; Maro *et al.* 1985; Fleming & Pickering, 1985). The slight fall in the total proportion of cells showing cytoplasmic polarity at 9 h (Table 1, line 4, column 9) reflects incipient division in a small subpopulation of cells, as both actin and clathrin redistribute from polar regions during mitosis whereas the surface pole remains evident (Fig. 3c,d; Johnson & Ziomek, 1981*a*; Johnson & Maro, 1984; Maro *et al.* 1985). Surface poles were also examined by scanning electron microscopy (Fig. 4a,b; 70% of control cells polarized). Polarized cells were characterized by a pole of apical microvilli, that appeared to be longer than the microvilli on apolar cells. The region with long microvilli was sharply demarcated from the remainder of the surface which either lacked microvilli or had short microvilli. These observations suggest that achievement of a polar surface phenotype involves shortening of basolateral microvilli and lengthening of apical microvilli.

Addition of taxol during the terminal stages of polarization (i.e. at 6, 7 or 8 h) produced a consistent and clear set of results regardless of dose. First, the incidence of surface polarity was reduced only marginally compared with that in control blastomeres (Fig. 3i; Table 1, column 8, compare line 4 with lines 5–10). Indeed, in most cases a slight increase over the incidence of surface polarity scored at the time of drug addition was evident (e.g. Table 1, column 8, compare lines

Fig. 3. Pairs of 8-cell blastomeres cultured for 9 h *in vitro* and examined immunocytochemically for distribution of surface Con A binding sites (a,c,e,g,i,k,m,o,q,s) and clathrin (b,d,f,h,j,l,n,p,r,t). Control cells (CON) show a high incidence of (a) surface and (b) cytoplasmic polarity in which the poles coincide and are opposite to the point of intercellular contact. As cells approach division (c,d) they pull apart, the polar clathrin first disperses (lower cell in d) and then concentrates on the spinde (upper cell in d) but surface poles are evident throughout (c) although tending to expand in area (upper cell). After division (e,f) polar cells derived from the apices of the polar 1/8 cells have a polar distribution of clathrin whereas the apolar cells do not; the expanded surface pole of Con A binding receptors (lower cell) contracts down to a more clearly defined pole (upper cell) overlying the polar clathrin within 1–2 h of division. Cells treated with 24 μM-taxol for entire 9 h culture (g,h) or for the last 1 h of a 9 h culture (i,j) show broad surface poles and diffuse non-polar clathrin distributions, as do cells treated in nocodazole in the same way (k and i show four cells treated for 9 h). Cytochalasin D does not reverse polarity appreciably when applied for the last hour (upper cell in o,p). However, cytoplasmic (but not surface) polarity is lost after 9 h in culture in the drug, but is restored rapidly if the drug is removed for the last hour of incubation (n). Note that in the presence of CCD, poles are often not opposite the point of intercellular contact (m,n). In the presence of both CCD and nocodazole (q–t), either continuously present or present for the last hour only, surface polarity is usually lost (s) or severely disturbed (q in which an equatorial ring of stain persists in the upper cell) and cytoplasmic polarity is lost (r,t). (Mag. ×410).

1 and 5, lines 2, 6, and 8, and lines 3, 7, and 9). Second, taxol produced a marked reduction in the incidence of cytoplasmic polarity whether scored by clathrin or actin distribution (Fig. 3j; Table 1, column 9, compare line 4 with lines 5–10). There is a suggestion that longer periods of drug action or higher doses produce a more marked effect. Thus, the stability of cytoplasmic polarity is more sensitive to taxol than is the stability of surface polarity.

Nocodazole produces very similar results (Table 1, columns 8 and 9, compare line 4 with line 11), having little effect on surface polarity, but a major effect on cytoplasmic polarity (Fig. 4d,e).

The effects of cytochalasin D, alone or in conjunction with nocodazole, are also recorded in Table 1 (lines 12 and 13). As reported previously (Johnson & Maro, 1984), cytochalasin alone does not destroy existing surface poles; indeed these become more discretely bounded and tightly organized (Fig. 3o). However, the drug does reduce the incidence of cells in which clathrin is polarized. When both cytochalasin D and nocodazole are present, there is a marked reduction of both surface and cytoplasmic poles (Fig. 3s,t; Table 1, line 13). When examined by SEM, polar cells characteristically have patchy long microvilli at their apices and in some of these cells short dense microvilli are also located in the basal part of the cell. When drug-treated cells were examined by SEM, the incidence of surface poles was slightly lower than that recorded when Con A was used to score surface polarity. The combined use of CCD and nocodazole led to an almost complete dispersion of surface poles. In this case, some cells showed an equatorial band of microvilli (Fig. 4g) and most showed a rather reticular pattern (Fig. 4h).

3. *Effects of drugs on development of polarity in 8-cell blastomeres*

Next we examined the effect of placing newly formed pairs of 2/8 blastomeres in drugs immediately after their formation and then culturing them for 9 h before analysing the incidence of cytoplasmic and surface polarity. In some cases, the drug was washed out at between 4 and 8 h and the final 5 to 1 h were spent in control medium.

The presence of taxol throughout the 9 h had no clear effect on the incidence of surface polarity, although surface poles occupied a greater proportion of the cell surface than in controls, and tended to have shorter microvilli and not to show a clear boundary (Figs 2f, 3g, 4c; Table 2 compare line 1 with lines 2–4, column 7). Cytoplasmic polarity did not develop at the highest dose of taxol, and was reduced greatly at lower doses (Fig. 3h Table 2, lines 1–4, column 8). The effect of taxol on microtubules is not rapidly reversible, and, not surprisingly, withdrawal of taxol at 7 h or 8 h resulted in only a slight increase in the incidence of cytoplasmic polarity by 9 h (data not shown).

The continuous presence of nocodazole was also compatible with the development of surface polarity (Fig. 3k; Table 2, compare line 1 with line 5) although the incidence was reduced slightly (53 % polarized) and again poles were larger,

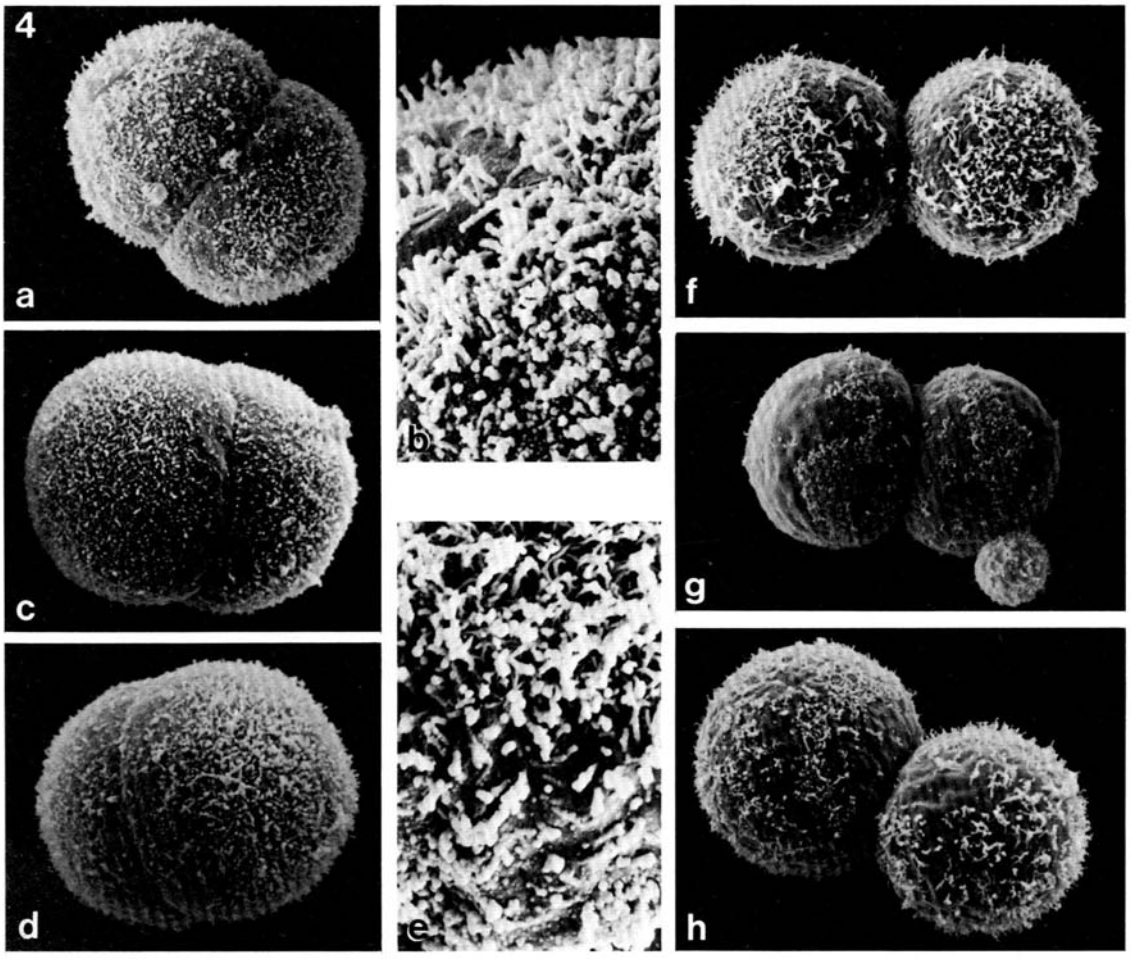


Fig. 4. Scanning electron micrographs of 2/8 pairs of blastomeres 9 h after their formation. (a) Control in which polarization is not quite completed: note apical long microvilli, area relatively depleted of microvilli adjacent to zone of intercellular contact and an intervening zone in which there are short microvilli – the transition from long to short microvilli is sharp as shown in (b). (c) Blastomeres exposed to 24 μM-taxol for 9 h: note that clearing of microvilli from contact zone has occurred but that the apical microvilli are neither as long nor as restricted in distribution as in the control; an intermediate zone of shorter microvilli is evident between the apical microvilli and the non-microvillous area. (d) Blastomeres exposed to 10 μM-nocodazole for 1 h prior to analysis: note similarity to control including sharp transition from long to short microvilli shown in (e). (f) Blastomeres exposed to both CCD and nocodazole for 9 h; in this example clear poles of long microvilli have formed. (g,h) Blastomeres exposed to CCD + nocodazole for 1 h prior to analysis: note band of microvilli around the equator of cells in (g) and reticular, not obviously polarized distribution of microvilli in (h). (Mag. a,c,d,f–g: ×980; Mag. b & e: ×3400).

Table 2. *Effect of drugs on the development of polarity in 2/8 pairs of blastomeres. (Note: all analyses performed at 9 h after formation of 2/8)*

Treatment (1)	No. of cells scored (2)	% of cells: Polar surface and cytoplasm (3)	% of cells: Polar surface only (4)	% of cells: Polar cytoplasm only (5)	% of cells: Non-polar (6)	% of cells: With surface poles (3+4) (7)	% of cells: With cytoplasmic poles (3+5) (8)	% of polar cell with poles 'off axis' to contact point (9)
(1) Control 0–9 h	159	73	4	6	17	77	79	4
(2) 1·2 μM taxol 0–9 h	44	16	57	7	20	73	23	17
(3) 12 μM taxol 0–9 h	87	9	54	1	36	73	10	5
(4) 24 μM taxol 0–9 h	50	0	88	0	12	88	0	0
(5) 10 μM nocodazole 0–9 h	113	12	43	1	44	55	13	4
(6) 10 μM nocodazole 0–8 h	76	9	49	4	38	58	13	0
(7) 10 μM nocodazole 0–7 h	72	21	31	10	38	52	31	4
(8) 10 μM nocodazole 0–6 h	32	25	47	3	25	72	28	0
(9) 10 μM nocodazole 0–5 h	35	40	34	9	17	74	49	3
(10) 10 μM nocodazole 0–4 h	40	40	32	8	20	72	48	0
(11) 10 μM nocodazole 7–8 h	30	17	57	0	26	74	17	3
(12) 0·5 μG ml^{-1} CCD 0–9 h*	52	0	40	0	60	40	0	52
(13) 0·5 μM ml^{-1} CCD 0–8 h*	36	33	28	6	33	61	39	75
(14) 0·5 μg ml^{-1} CCD 0–7 h*	40	25	15	18	42	40	43	57
(15) CCD + nocodazole 0–9 h*	60	0	35	0	65	35	0	38
(16) CCD + nocodazole 0–8 h*	30	0	17	6	77	17	6	30
(17) CCD + nocodazole 0–7 h*	28	7	32	0	61	39	7	26

* Cytoplasmic polarity assessed by clathrin distribution only since actin is dispersed in a nonfilamentous form by CCD – see Johnson & Maro (1984).

more diffuse and with shorter microvilli. Cytoplasmic polarity was markedly depressed. Although the inhibitory action of nocodazole on microtubules is reversed within minutes of the removal of the drug, removal of nocodazole for the last 1–5 h of the 9 h incubation period (Table 2, lines 6 to 10) did not lead to a correspondingly rapid restoration of cytoplasmic polarity. The slight depression in the incidence of surface polarity is not evident when nocodazole is removed at 6 h or earlier, and although the incidence of cytoplasmic polarity is higher with earlier removal of the drug, it does not reach control levels. Moreover, exposure of cells to a pulse of nocodazole between 7 and 8 h (i.e. after polarity is established) does not result in any more rapid reformation of cytoplasmic polarity over the subsequent recovery hour than occurs in cells exposed to nocodazole over the whole 0 h to 8 h period (Table 2, compare lines 6 and 11).

As reported previously (Johnson & Maro, 1984), cytochalasin D does not suppress the development of surface poles (Table 2, line 12) although the morphology of the poles is frequently atypical and the poles often do not develop opposite to the contact point between the cells. The proportion of such poles scored as 'off-axis' with respect to the contact point is indicated in column 9 of Table 2. One problem with the assessment of surface poles in cells treated with CCD is that the assay system for surface poles detects the distribution of microvilli, which themselves contain microfilaments. Although these microvillous microfilaments are relatively resistant to CCD (Pratt *et al.* 1981; Johnson & Maro, 1984), nonetheless some are affected and thus polar morphology is disturbed. Most commonly, a single focus of long, dispersed microvilli is seen in the apical or lateral region, whereas short dense microvilli are located basally. Often a ring of microvilli bounding the limits of the pole is evident at both light and electron microscope level (e.g. like the examples shown in Figs 3q, 4g). Since polar microvilli reform rapidly upon removal of CCD, it is easier to score both the incidence and orientation of surface poles in pairs cultured for 7 or 8 h in CCD and for the final 2 or 1 h in medium 16 (Fig. 3m,n; Table 2 lines 13 & 14). Under these conditions it is possible to confirm that many surface poles are formed in the presence of CCD and that these poles are indeed often off-axis with respect to the point of contact between cells. As CCD also destroys cytoplasmic filamentous actin (Johnson & Maro, 1984), cytoplasmic polarity was assessed only by examining the redistribution of clathrin and was also found to be suppressed totally by CCD (Table 2, line 12, column 8) but was restored rapidly (within 1 h) of removal of CCD (Table 2, lines 13 & 14, column 8; Fig. 3n). The restored cytoplasmic focus of clathrin always located beneath the surface pole (Fig. 3m,n).

The combined use of both CCD and nocodazole produced a major inhibition of the development of both surface and cytoplasmic polarity (Table 2, line 15), although in some cells poles did form (e.g. Fig. 4f. Where polarity was evident, it was often marked by the presence of a ring of long microvilli bounding the pole (Figs 3q, 4g). Removal of the drugs for the terminal period of culture did not reduce the extent of this inhibition (Table 2, lines 16 and 17).

4. *The involvement of cell contact and intercellular continuity in the resistance of surface polarity to drug action*

All the foregoing results were obtained using pairs of 8-cell blastomeres derived by division of a single 4-cell blastomere and remaining in cytoplasmic continuity via a residuum of the cleavage furrow – the midbody (Goodall & Johnson, 1984), containing a remnant of the equatorial spindle that includes tubulin (Fig. 2a,b). It seemed possible that the resistance of the development of surface polarity to drug action might be due to some very early or lingering influence of the midbody remnant on the cytocortex. In order to investigate this possibility, we took newly formed pairs of 8-cell blastomeres, disaggregated them to single cells, and reaggregated them in a new contact orientation so that the midbodies were directed outwards. We then cultured the pairs for 9 h under various conditions and scored the incidence and orientation of their surface poles. Of 44 cells analysed in reaggregated control pairs 73 % were polarized (3 % off-axis), of 57 cells cultured as reaggregates in nocodazole (10 μm) 65 % were polarized (8 % off-axis), and of 44 cells cultured as reaggregates in taxol (24 μm) 41 % were polarized (none off-axis). In all groups surface poles were observed that were coincident with the old midbody remnant and opposite to the new point of contact. Thus, the development of surface polarity in the presence of drugs is not dependent on some 'memory' of orientation based on the previous division.

We were also interested to see whether the ability to establish polarity in the presence of microtubule-disrupting drugs was contingent upon the intercellular flattening that occurs at the same time or whether polarity would nonetheless develop in the absence of flattening. We therefore took advantage of the inhibitory effect on flattening of the monoclonal antibody ECCD-1, directed against the homotypic, calcium-dependent cell surface glycoprotein, cadherin (as described in Maro *et al.* 1985). This antibody does not prevent polarization (Johnson, 1985*a*; Maro *et al.* 1985). Newly formed 2/8 pairs were incubated under various conditions for 9 h and then examined for the incidence of surface polarity. Of 38 cells exposed only to nocodazole, all were flattened and 60 % were polarized; of 44 cells exposed throughout to both nocodazole and ECCD-1, none were flattened and 60 % were polarized; of 46 cells exposed to nocodazole for 9 h and to ECCD-1 for the terminal hour, none were flattened and 75 % were polarized. Thus, surface polarization in the absence of microtubules is not dependent on intercellular flattening.

5. *Effects of drugs on the stability of polarity at the 16-cell stage*

Analysis of polarity at the 16-cell stage of development is complicated by three features summarized and referred to in Fig. 1. First, cell heterogeneity within the embryo becomes evident for the first time at this stage, both outer polar and inner apolar cells being present. This cell heterogeneity arises initially from the division of the polarized 8-cell blastomere; during division, the polarized distribution of clathrin is lost as the antigen redistributes to both cells (see Fig. 3d and Maro *et al.*

1985) whereas the surface polarity is retained although the area occupied by the surface pole expands over the whole apical region of the dividing 1/8 cell (upper cell Fig. 3c) to yield a 1/16 cell which has all or most of its surface brightly stained with FITC Con A and covered with microvilli (lower cells in Fig. 3e and Fig. 5a). Within 1–2 h of division, this area of Con A binding concentrates into a more discretely defined surface pole (Figs 3e, 5c) located over the reformed polar aggregate of the clathrin (Fig. 3e,f). The basolateral region of the polarized 1/8 cell yields an apolar 1/16 cell with weakly staining uniform FITC-Con A binding properties and a homogeneous or non-polar distribution of cytoplasmic clathrin (Fig. 3e,f). Second, one feature of the cell heterogeneity in the 16-cell embryo is that between 5 and 8 h into the 16-cell state, polar cells envelop apolar cells in a process that anticipates trophectodermal envelopment of cells in the inner cell mass. Envelopment makes scoring of cytoplasmic and surface polarity more difficult, but it can be prevented by incubation of the cells in the monoclonal antibody directed against cadherin (see above and Maro *et al.* 1985). Use of this reagent leads to retention of rounded cell outlines, and ready identification of cells as polar or apolar by both surface and cytoplasmic criteria. Third, at the 16-cell stage cytoplasmic re-arrangement of the lysosomal elements of both polar and apolar blastomeres occurs, the lysosomes moving from a dispersed array initially (Fig. 5b) to a concentrated focus, which by 8 h in most polar cells is located basally on the opposite side of the cell from both the pole of surface Con A binding and the apically located clathrin (Fig. 5d, Maro *et al.* 1985; Fleming & Pickering, 1985). Thus, cytoplasmic scoring for both lysosomal and clathrin organization may be undertaken in polar 1/16 cells.

Newly formed 2/16 pairs were recovered and cultured for 8 h as described in Materials and Methods. For the final 1 h taxol, nocodazole, cytochalasin D or a combination of CCD + nocodazole were added. The polar cells in the couplets were then analysed for the extent to which the surface pole of Con A binding, the subjacent focus of clathrin and the opposite focus of lysosomal antigen were affected by the treatments. The results are summarized in Table 3, lines 1–6. The restriction of bright surface staining to discrete poles that normally occurs in the polar 1/16 cells within 2 h of division (compare lower and upper cells in Fig. 3e and lower cells in Fig. 5a,c; Table 3, compare lines 1 & 2, columns 3 & 4) is not reversed by any drug treatment applied (Figs 5e,g,i,k and 6a,c,e,g; Table 3, lines 3–6). In contrast, *all* drug treatments redistributed clathrin from a concentrated polar focus to a diffuse pattern (Fig. 6b,d,f,h; Table 3, columns 5 & 6). The *de novo* concentration of lysosomal antigen into a single focus that occurs in polar cells during the 16-cell stage was only marginally reversed by the various drug treatments (Fig. 5f,h,j,l; Table 3, column 7). However, the localization of the lysosomal antigen was influenced appreciably, the predominantly basal position observed in controls being replaced by a more randomly located position (compare Fig. 5d with Fig. 5f,h,j,l; Table 3, column 8).

Apolar 1/16 cells exposed to the various drugs for the terminal hour of incubation also showed a completely homogeneous clathrin distribution, and did

not differ from the controls in this regard (Fig. 6b,d,f,h; Table 4, column 3, lines 1–6). The focal concentration of lysosomal antigen that occurs in many apolar cells over an 8 h period was, if anything, enhanced by a terminal hour's exposure to nocodazole or CCD, and only marginally reduced by combined use of nocodazole and CCD (Fig. 5d,f,h,j,l; Table 4, column 4, lines 1–6).

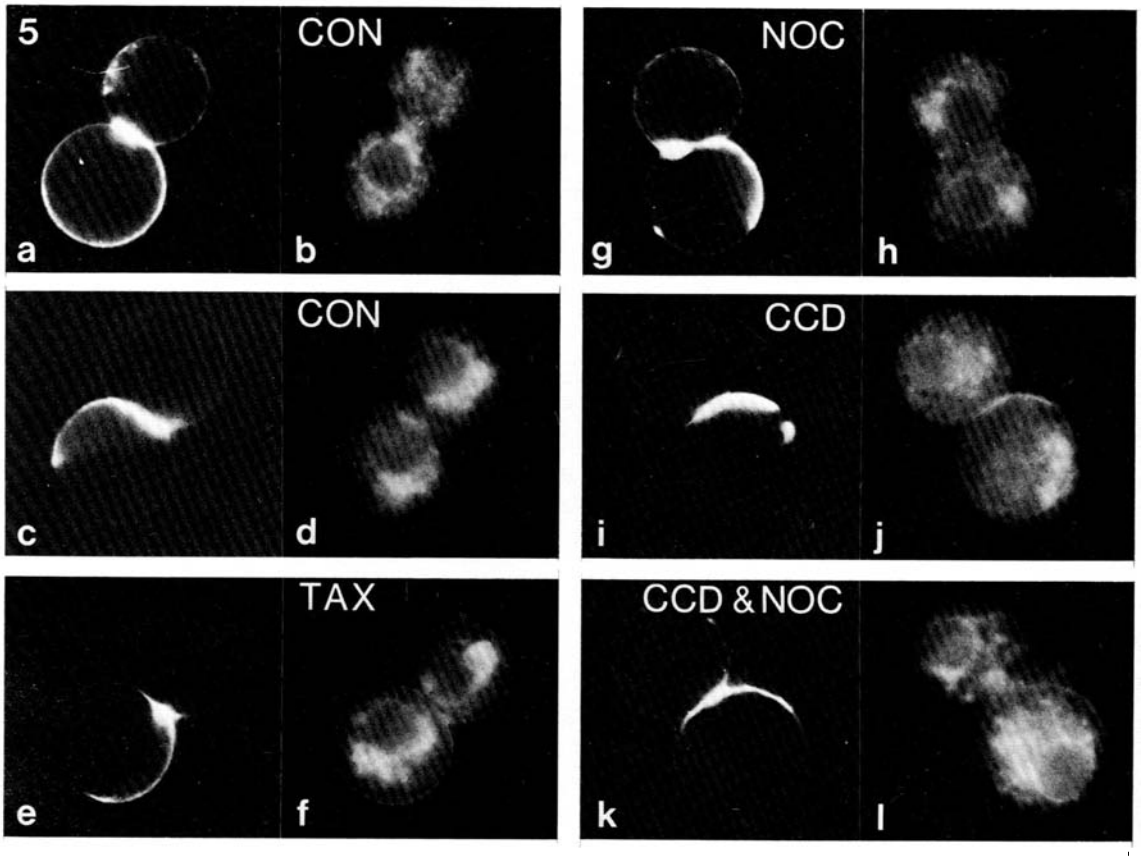


Fig. 5. Pairs of 16-cell blastomeres derived from division of an isolated 8-cell blastomere and examined immediately (a,b) or 8 h later (c to l) for distribution of surface Con A binding sites (a,c,e,g,i,k) or lysosomal antigen (b,d,f,h,j,l). (a,b) Newly formed 2/16 pair with one larger bright and one smaller dim cell, in both cells the lysosomal antigen is dispersed. (c,d) 8 h later a bright polar and a dim apolar cell are evident and in each lysosomal antigen has become concentrated into a major single focus, that in the polar cell being on the opposite side of the cell to the surface pole.(e,f) A similar pair, treated for the terminal hour with 24 μM-taxol, and differing from the control primarily in the location of the slightly more diffuse lysosomal antigen focus beneath the surface pole. (g,h) A 2/16 pair incubated for 8 h in nocodazole – polar foci of lysosomal antigen have formed, but the focus in the polar cell is located adjacent to the surface pole not opposite it. (i,j) A 2/16 pair treated for the terminal hour with cytochalasin D – lysosomal foci are present but more diffuse cytoplasmic staining is also evident; in the polar cell, the lysosomal focus is not opposite to the surface pole. (k,l) A 2/16 pair exposed to both CCD and nocodazole for the terminal hour – note dispersed lysosomal antigen. (Mag. ×600).

Table 3. *Effect of various drugs on the stability and on the generation of surface and cytoplasmic polarity in polar 16-cell blastomeres. All analyses undertaken 8 h after entry into 16-cell stage*

Incubation condition (1)	No. of polar cells analysed (2)	% with dispersed surface poles (3)	% with discrete surface poles (4)	% with localized clathrin (5)	% of these in which clathrin is apical (6)	% with localized lysosomes (7)	% of these in which lysosomes are basal (8)
(1) Control 0 h	173	92	8	3	100	0	—
(2) Control 8 h	36	0	100	87	91	84	56
(3) Taxol 7–8 h	72	6	94	0	—	65	22
(4) Nocodazole 7–8 h	67	4	96	31	100	57	10
(5) CCD 7–8 h	68	8	92	5	100	75	36
(6) CCD + nocodazole 7–8 h	60	7	93	0	—	51	21
(7) Taxol 0–8 h	38	13	87	0	—	65	31
(8) Nocodazole 0–8 h	42	10	90	0	—	54	21
(9) CCD 0–8 h	35	8	92	0	—	53	22
(10) CCD + nocodazole 0–8 h	29	31	69	0	—	15	0

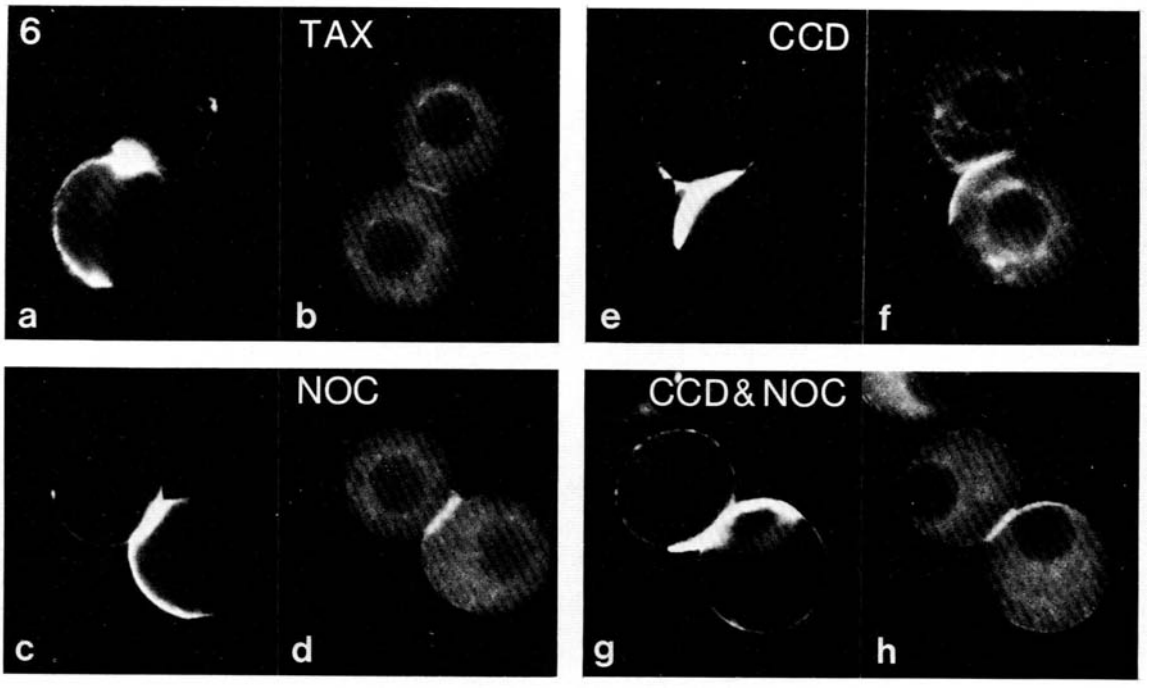


Fig. 6. Pairs of 16-cell blastomeres derived from division of an isolated 8-cell blastomere, cultured for 8 h and examined for distribution of surface Con A binding sites (a,c,e,g) or clathrin (b,d,f,h). In each case the cells were incubated for the terminal hour in (a,b) taxol, (c,d) nocodazole (e,f) cytochalasin D and (g,h) both CCD and nocodazole. All cells show dispersed cytoplasmic clathrin. (Mag. ×600).

Table 4. *Effect of various drugs on the stability and on the generation of cytoplasmic organization in apolar 16-cell blastomeres. All analyses undertaken 8 h after entry into 16-cell stage*

Incubation condition (1)	No. of apolar cells analysed (2)	% with localized clathrin (3)	% with localized lysosomes (4)
(1) Control 0 h	103	0	0
(2) Control 8 h	63	9	42
(3) Taxol 7–8 h	45	0	43
(4) Nocodazole 7–8 h	47	0	60
(5) CCD 7–8 h	45	0	63
(6) CCD + nocodazole 7–8 h	48	0	28
(7) Taxol 0–8 h	22	0	53
(8) Nocodazole 0–8 h	24	0	53
(9) CCD 0–8 h	15	16	55
(10) CCD + nocodazole 0–8 h	13	0	27

6. *Effects of drugs on the development of polarity at the 16-cell stage*

Newly formed pairs of 16-cell blastomeres were placed immediately into monoclonal antibody to cadherin plus one of the drugs under test and cultured for 8 h before analysis for the incidence and orientation of surface and cytoplasmic polarity. The results are summarized in Table 3 for polar blastomeres and in Table 4 for apolar blastomeres.

The restriction of bright surface labelling to discrete poles that normally occurs over the first 1–2 h after division (e.g. compare lines 1 and 2 in Table 3, columns 3 & 4) was not hindered by taxol, CCD or nocodazole alone, but was reduced by a combination of CCD and nocodazole (Table 3, line 10, columns 3 & 4). In contrast, the clathrin redistribution in polar cells that follows division (compare lines 1 & 2, columns 5 & 6 Table 3) does not occur in the presence of any of the drugs (Table 3, lines 7–10). The focal concentration of lysosomal antigen is only slightly inhibited by nocodazole, taxol and CCD, but is greatly depressed by combined application of CCD and nocodazole (Table 3, lines 7–10, column 7). However, although lysosomal antigen does concentrate into a focal clump in the presence of CCD, nocodazole or taxol acting alone, it is not located basally as often (Table 3, column 8). Similarly, the incidence of focal accumulation of lysosomal antigen in apolar cells is reduced by CCD and nocodazole acting together, but not by any drug acting alone (Table 4, lines 2, 7–10).

DISCUSSION

The most remarkable finding reported in this paper is the relative independence from the effects of any of the individual drugs tested of both the development and the stability of surface polarity in interphase 8- and 16-cell blastomeres. Only nocodazole and CCD acting together reduced surface polarity appreciably and even then not completely. Moreover, given that the scoring of surface polarity relies on the relative resistance of microvillous, filamentous actin to CCD, even the latter reduction may represent a scoring difficulty rather than a real effect on surface reorganization. Whilst the drugs did disturb the *form* of the surface pole that developed, both taxol and nocodazole leading to larger, less sharply defined poles, and CCD (with or without nocodazole) leading to patchy, sharply defined poles often with a marked boundary ring of long microvilli, a pole nonetheless was evident. These results are consistent with previous results in which CCD was used on whole 8-cell embryos or pairs of 8-cells (Johnson & Maro, 1984), but do appear to differ from our previous results in which taxol and nocodazole were applied to whole 8-cell embryos (Maro & Pickering, 1984). In the latter paper, taxol (unlike nocodazole) was reported to prevent the development of surface polarity in the majority of blastomeres. However, it is clear from the use here of pairs of blastomeres that taxol merely prevents a tightly organized pole from forming in most cells. In the intact whole-mount embryo, in which only the exposed apical surfaces of cells can be visualized clearly, the heterogeneity of surface organization between basal and apical regions will be observed less readily, especially when

larger, more diffuse poles develop. When intact, taxol-treated embryos are examined by transmission electron microscopy, it is found that indeed the blastomeres are polarized at their surface but that the pole is broader than in controls (S. J. Pickering and B. Maro, unpublished).

The failure of microtubule-inhibitory drugs to prevent surface polarization does not appear to be due to the presence of some residual polar organization relating to the previous cleavage plane, since disaggregated, rotated and reaggregated blastomere pairs also developed surface poles in the drugs. Moreover the poles developed in the drugs quite independently of the cell flattening component of compaction, since blocking the latter did not block the former. We can also be confident that the resistance of surface polarity to the action of the cytoskeletal inhibiting drugs is not a consequence of the failure of drug action, since (i) we have demonstrated a direct, rapid and reversible effect of each drug on its target cytoskeletal element in mouse blastomeres, (ii) the surface poles that develop are modified in their phenotype by the actions of the various drugs, and (iii) the drugs produce clear, and quite distinct, effects on the cytoplasmic components of the polarized cells. Thus, polarization of intracellular clathrin is blocked and reversed by the inhibiting drugs at both the 8- and 16-cell stages. Moreover, polarity of clathrin organization is not restored on removal of nocodazole, despite rapid restoration of microtubules, implying a major disturbance of intracellular organization by the drug. The accumulation of lysosomal antigen into a single focal cluster that occurs in both polar and apolar cells during the 16-cell stage is not appreciably inhibited by any regime except continuous exposure to both CCD and nocodazole. However, the focal accumulations that do occur tend to be unlike those in control cells, being located away from the basal region of polarized 1/16 cells. Very similar results to these have been reported by Fleming, Cannon & Pickering (1985), who have shown that the generation and stabilization of a polarized distribution of endocytic organelles at the 8-cell and 16-cell stages is dependent upon cytoskeletal integrity, whilst surface polarity is not.

The difference in effect that the cytoskeletal-inhibitory drugs have on surface and cytoplasmic polarity is striking. Detectable cytoplasmic polarity develops several hours in advance of overt surface polarity during the 8-cell stage, and it was therefore suggested that this temporal sequence might possibly also be a causal sequence (Johnson & Maro, 1984; Maro *et al.* 1985). The results reported here suggest that this interpretation cannot be correct. It already seems likely from our published work that, once polarity is established in the late 8-cell blastomere, the locus of a 'memory' of polarity resides in the cytocortex. Thus, at mitotic division to two 16-cell blastomeres the surface polarity is evident throughout (Johnson & Ziomek, 1981) whereas cytoplasmic polarity is lost (Johnson & Maro, 1984; Maro *et al.* 1985; Fleming & Pickering, 1985). Moreover, after division is completed polarity of cytoplasmic clathrin is restored *only* in the polar progeny and the cluster of clathrin underlies the surface pole (Maro *et al.* 1985). The surface pole appears to serve as a focus for the redistribution of clathrin from a dispersed to a focal organization. The results reported here (and in Fleming *et al.* 1985) suggest that, at

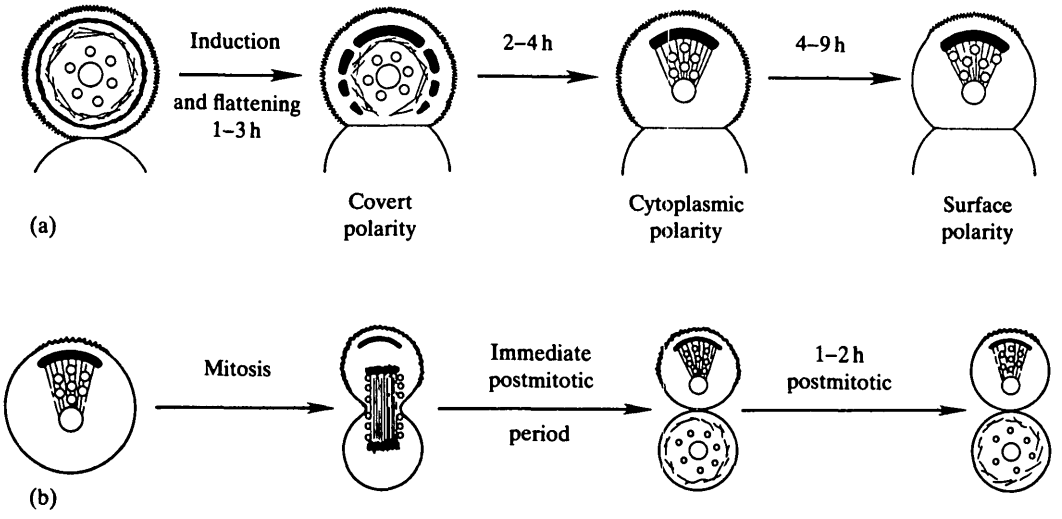


Fig. 7. (a) Schematic representation of hypothesized sequence of polarization events at the 8-cell stage and (b) the proposed changes that occur at division to the 16-cell stage. Newly formed 8-cells have a uniform distribution of microvilli (corrugated surface), microtubules (thin lines), clathrin (open circles) and of a putative cytocortical organizer (solid line). Maintained contact with another cell leads to a redistribution of the putative cytocortical organizer towards the future apical region of the cell opposite to the point of intercellular contact, thereby giving the cell a covert polarity. This redistribution may be regulated by spread of a signal from the region of intercellular contact either in the cytocortical plane or via low molecular weight cytoplasmic messengers (see text). The changing organizer should not necessarily be equated with movement of a physical entity but represents a functional change in the subcortical properties. Once established, the covert polar organizer serves as a focus for cytoplasmic and surface reorganization during both the 8- and 16-cell stages.

the 16-cell stage, cytoskeletal elements are essential (i) for focusing this post-mitotic redistribution of clathrin to the cortical region subjacent to the surface pole, (ii) for the later basally polarized positioning of lysosomal clusters and (iii) for the maintenance of both of these localizations. The results also suggest a similar cytoskeletal requirement for the positioning of clathrin apically at the 8-cell stage. As this cytoskeletal-dependent polar relocation of clathrin in 8-cell blastomeres occurs in advance of detectable surface polarization, we are drawn to the conclusion that the earliest phases of polarization may go undetected by us. We suggest that perhaps there is an early but covert change in the apical cytocortex that serves as (i) a memory for the axis of polarity, (ii) an organizing focus for *overt* surface polarity, via a process that is not prevented by cytoskeletal inhibitors and that might perhaps involve a localized stabilization of existing apical microvillous microfilaments and (iii) an organizing focus for cytoplasmic polarity via a process that *is* sensitive to cytoskeletal inhibitors and that might perhaps involve a localized stabilization of microtubules. A diagrammatic representation of this hypothesis is given in Fig. 7.

Such a putative covert reorganization within the cytocortex would have to develop during the first 3 h of the 8-cell stage and be located opposite to the

point(s) of contact with other blastomeres (Ziomek & Johnson, 1980; Johnson & Ziomek, 1981*b*). The putative change could either be a localized *positive* change apically that permitted stabilization *de novo*, or a localized loss from the basolateral regions of a hitherto uniform stabilizing property. Whichever mechanism applied, our results here show that it would have to operate independently of the cytoskeletal organization within the cell and thus of an organized cytoplasmic matrix. It seems to us that two types of signal might operate to effect the required change (Johnson, 1985*a*). The inductive signal could be transmitted from the point of intercellular contact basally to the apex via ionic currents generated by changes in permeability or in the activity of ionic pumps (Nuccitelli & Wiley, 1985). Alternatively, information might pass in the plane of the cytocortex from the basolateral region of intercellular contact towards the apex. Given the time scale and the cell size involved, it is unlikely that the physical movement of macromolecular proteins within the plane of the membrane could transmit the information, although membrane lipids, submembranous macromolecules or even the spread of a catalytic change such as an action potential or a wave of macromolecular modification could act in the required way (Pratt, 1978; Wolf, 1983). It is possible to test these alternatives and to discover how they might generate a memory focus in the apical cytocortex. Such a putative cytocortical 'memory' is reminiscent of those suggested for other embryological systems (Gerhart *et al.* 1984), and might share features with them. We are now exploring these possibilities.

We wish to acknowledge the help and advice of our research colleagues especially Tom Fleming and Hester Pratt, the gift of monoclonal antibody ECCD-1 from Dr M. Takeichi, the gift of taxol from N.I.H., the technical assistance in the work, and its preparation for publication, of Gin Flach, Sue Pickering, Martin George, Ian Edgar, Sheena Glenister and Francis Woodman. The work was supported by a grant from the Medical Research Council to Martin H. Johnson and Peter R. Braude. Bernard Maro is an EMBO Fellow.

REFERENCES

DUCIBELLA, T. (1982). Depolymerization of microtubules prior to compaction. *Expl Cell Res.* **138**, 31–38.

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

DUCIBELLA, T., ALBERTINI, D. F., ANDERSON, E. & BIGGERS, J. (1975). The preimplantation mammalian embryo: characterization of intracellular junctions and their appearance during development. *Devl Biol.* **45**, 231–250.

DUCIBELLA, T., UKENA, T., KARNOVSKY, M. & ANDERSON, E. (1977). Changes in cell surface and cortical cytoplasmic organization during early embryogenesis in the preimplantation mouse embryo. *J. Cell Biol.* **74**, 153–167.

FLEMING, T. P. & PICKERING, S. J. (1985). Maturation and polarization of the endocytotic system in outside blastomeres during mouse preimplantation development. *J. Embryol. exp. Morph.* **89**, 175–208.

FLEMING, T. P., CANNON, P. & PICKERING, S. J. (1985). 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. *Devl Biol* (in press).

FLEMING, T. P., WARREN, P. D., CHISHOLM, J. C. & JOHNSON, M. H. (1984). Trophectodermal processes regulate the expression of totipotency within the inner cell mass of the mouse expanding blastocyst. *J. Embryol. exp. Morph.* **84**, 63–90.

FULTON, B. P. & WHITTINGHAM, D. G. (1978). Activation of mammalian oocytes by intracellular injection of calcium. *Nature* **273**, 149–151.

GERHART, J. C., VINCENT, J.-P., SCHARF, S. R., BLACK, S. D., GIMLICH, R. L. & DANILCHIK, M. (1984). Localization and induction in early development of *Xenopus*. *Phil. Trans. R. Soc. Lond.* B. **307**, 319–330.

GOODALL, H. & JOHNSON, M. H. (1984). The nature of intercellular coupling within the preimplantation mouse embryo. *J. Embryol. exp. Morph.* **79**, 53–76.

GOUNON, P. & KARSENTI, E. (1981). Involvement of contractile proteins in the changes in consistency of oocyte nucleoplasm of the newt *Pleurodeles waltlii*. *J. Cell Biol.* **88**, 410–421.

HANDYSIDE, A. H. (1980). Distribution of antibody- and lectin-binding sites on dissociated blastomeres from mouse morulae: evidence for polarization at compaction. *J. Embryol. exp. Morph.* **60**, 90–116.

JOHNSON, M. H. (1985*a*). Three types of cell interaction regulate the generation of cell diversity in the mouse blastocyst. In *The Cell in Contact: Adhesions and Junctions as Morphogenetic Determinants* (ed. G. Edelman & J.-P. Thiery), Neurosciences Institute Publication Series, pp. 27–48. New York: John Wiley.

JOHNSON, M. H. (1985*b*). Manipulation of early mammalian development: what does it tell us about cell lineages? In *Manipulation of Mammalian Development* (ed. R. B. L. Gwatkin). New York: Plenum. (In press).

JOHNSON, M. H. & MARO, B. (1984). The distribution of cytoplasmic actin in mouse 8-cell blastomeres. *J. Embryol. exp. Morph.* **82**, 97–117.

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

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

JOHNSON, M. H. & ZIOMEK, C. A. (1982). Cell subpopulations in the late morula and early blastocyst of the mouse. *Devl Biol.* **91**, 431–439.

LEHTONEN, E. (1980). Changes in cell dimensions and intracellular contacts during cleavage-stage cell cycles in mouse embryonic cells. *J. Embryol. exp. Morph.* **58**, 231–249.

LEHTONEN, E. & BADLEY, R. A. (1980). Localization of cytoskeletal proteins in preimplantation mouse embryo. *J. Embryol. exp. Morph.* **55**, 211–225.

LEHTONEN, E., LEHTO, U. P., VARTIO, T., BADLEY, R. A. & VIRTANEN, I. (1983). Expression of cytokeratin polypeptides in mouse oocytes and preimplantation embryos. *Devl Biol.* **100**, 158–165.

LO, C. W. & GILULA, N. B. (1979). Gap junctional communication in the preimplantation mouse embryo. *Cell* **18**, 399–409.

LOUVARD, D., MORRIS, C., WARREN, G., STANLEY, K., WINKLER, F. & REGGIO, H. (1983). A monoclonal antibody to the heavy chain of clathrin. *EMBO J.* **2**, 1655–1664.

MAGNUSON, T., DEMSEY, A. & STACKPOLE, C. W. (1977). Characterization of intercellular junctions in the preimplantation mouse embryo by freeze-fracture and thin-section electron microscopy. *Devl Biol.* **61**, 252–261.

MARO, B. & PICKERING, S. (1984). Microtubules influence compaction in preimplantation mouse embryos. *J. Embryol. exp. Morph.* **84**, 217–232.

MARO, B., JOHNSON, M. H., PICKERING, S. & FLACH, G. (1984). Changes in actin distribution during fertilization of the mouse egg. *J. Embryol. exp. Morph.* **81**, 211–237.

MARO, B., JOHNSON, M. H., PICKERING, S. J. & LOUVARD, D. (1985). Changes in the distribution of membranous organelles during mouse early development. *J. Embryol. exp. Morph.* **90**, 287–309.

NICOLSON, G. L., YANAGIMACHI, R. & YANAGIMACHI, H. (1975). Ultrastructural localization of lectin binding sites on the zonae pellucidae and plasma membranes of mammalian eggs. *J. Cell Biol.* **66**, 263–274.

NUCCITELLI, R. & WILEY, L. (1985). Polarity of isolated blastomeres from mouse morulae: detection of transcellular ion currents. *Devl Biol.* (In press).

OSHIMA, R. G., HOWE, W. E., KLIER, F. G., ADAMSON, E. D. & SHEVINSKY, L. H. (1983). Intermediate filament protein synthesis in preimplantation murine embryos. *Devl Biol.* **99**, 447–455.

PRATT, H. P. M. (1978). Lipids and transitions in embryos. In *Development in Mammals*, **3** (ed. M. H. Johnson), pp. 83–130. Amsterdam: Elsevier.

PRATT, H. P. M., CHAKRABORTY, J. & SURANI, M. A. H. (1981). The effect of cytochalasin D on the differentiation of the mouse blastocyst. *Cell* **26**, 279–292.

REGGIO, H., BAINTON, D., HARRIS, E., CAUDRIER, E. & LOUVARD, D. (1984). Antibodies against lysosomal membranes reveal a 100,000-mol-wt protein that cross-reacts with purified H^+, K^+ ATPase from gastric mucosa. *J. Cell Biol.* **99**, 1511–1526.

SMITH, R. K. W. & JOHNSON, M. H. (1985). Analysis of the third and fourth cell cycles of early mouse development. *J. Reprod. Fert.* (in press).

SURANI, M. A. H., BARTON, S. C. & BURLING, A. (1980). Differentiation of 2-cell and 8-cell mouse embryos arrested by cytoskeletal inhibitors. *Expl Cell Res.* **125**, 275–286.

SUTHERLAND, A. E. & CALARCO-GILLAM, P. G. (1983). Analysis of compaction in the preimplantation mouse embryo. *Devl Biol.* **100**, 328–338.

WHITTINGHAM, D. G. & WALES, R. G. (1969). Storage of two-cell mouse embryos *in vitro*. *Austr. J. biol. Sci.* **22**, 1065–1068.

WOLF, D. E. (1983). The plasma membrane in early embryogenesis. In *Development in Mammals, 5*. (ed. M. H. Johnson), pp 187–208. Amsterdam: Elsevier.

ZIOMEK, C. A. & JOHNSON, M. H. (1980). Cell surface interaction induces polarization of mouse 8-cell blastomeres at compaction. *Cell* **21**, 935–942.

(Accepted 24 June 1985)