Induction of Polarity in Mouse 8-Cell Blastomeres: Specificity, Geometry, and Stability

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ABSTRACT We studied the cellular mechanisms underlying the induction of polarity in individual blastomeres of the 8-cell mouse embryo. The ability to induce polarity is lacking in the membranes of unfertilized and newly fertilized mouse eggs, then develops during the 2-cell stage, and is present in membranes of cells from 4-, 8-, and 16-cell stages. The axis of polarity takes 3–5 h to become established and thereafter appears to be stable. Multiple cell contacts affect the orientation of the axis of polarity, and no polarity develops in cells which are totally surrounded. Polarized cells show evidence of a limited capacity for slight adjustments in their position relative to other cells. The implications of these results for the mechanisms by which a blastocyst is generated are discussed briefly.

The first overt cellular differentiation of the mouse embryo occurs at the 8-cell stage. Individual cells become functionally and morphologically polarized along a radial axis through the embryo as judged by a variety of criteria (1, 3, 15, 16). We have shown that polarization of an individual ⅛ cell is induced as a result of interaction with another ⅛ cell,[1] and that the point of contact between these cells appears to determine the axis of polarity (19). This contact-mediated polarization of each cell of the late 8-cell embryo imparts to the embryo as a whole a radially organized mosaicism (6), which is preserved during the subsequent division to the 16-cell stage, thereby generating two distinct cell populations. The outer, polar, 1/16 cells incorporate the apices of the polarized 8-cells and are thought to found the trophectodermal lineage; whereas, the inner, apolar, 1/16 cells are derived from the bases of the polarized cells and are thought to found the inner cell mass (ICM) lineage (4, 10). Thus, the cell interactions at the 8-cell stage which induce polarization mark the initiation of the divergent differentiation that culminates in the blastocyst with two distinct, committed, and differentiated tissues: the trophectoderm and ICM (9).

We used, as a marker for polarization, the development of an apical pole of fluorescein isothiocyanate (FITC)–concanavalin A (Con A) binding (3, 19) which has been shown previously to coincide with an apical pole of microvilli (16). We show that there is cell specificity in the interactions leading to polarization, that polarity once generated appears to be stable for the life of the ⅛ cell, and that multiple cell contacts influence the orientation of the axis of polarity and the interactions among polarized cells.

[1] Throughout this paper a single blastomere from the 2-, 4-, 8-, or 16-cell embryo is called ½, ¼, ⅛, or 1/16, respectively. The pair of cells arising from the division of a ½, ¼, or ⅛ blastomere is called a ¾, ⅜, or 3/16 natural couplet, respectively.

MATERIALS AND METHODS

Preparation of Embryos

Embryos were recovered after superovulation of HC-CFLP mice (Hacking and Churchill Ltd., Alconbury, England), aged 3–5 wk (5 IU each of pregnant mare's serum [PMS] and human chorionic gonadotrophin [hCG]) or 5–8 wk (10 IU each of PMS and hCG), followed by mating. All embryonic ages are expressed in hours post-hCG. Embryos were recovered from the oviducts in phosphate-buffered medium 1 plus 0.4% (wt/vol) bovine serum albumin (PBI + BSA) (18). Unfertilized eggs were recovered from nonmated females at 12–15 h post-hCG and exposed briefly to hyaluronidase (1 mg/ml in buffered saline containing 1% (wt/vol) polyvinylpyrollidone [PVP]) to remove cumulus cells. Fertilized eggs were recovered at 22 h post-hCG, 2-cell embryos at 34–41 h post-hCG, and 4-cell embryos at 48–52 h post-hCG. 8-cell embryos were obtained by flushing oviducts at 45 h post-hCG and culturing 2-, 3-, and 4-cell embryos for 14 h in medium 16 plus 0.4% BSA (M16 + BSA) (17) at 37°C in 5% CO_2 in air. 16-cell embryos were recovered at 68 h post-hCG.

Preparation of Single Cells

The zona pellucida was removed by a brief exposure to acid Tyrode's solution (13), and the embryos were placed in prewarmed Ca^{++}-free M16 plus 0.6% (wt/vol) BSA pre-equilibrated with 5% CO_2 in air under oil (3). After 15–20 min, the 2-, 4-, and 8-cell embryos were disaggregated by gentle pipetting with a flame-polished micropipette. Isolated blastomeres were either used immediately as described in Results, or, as in the case of some ¼ and ⅛ blastomeres, were placed in culture in groups of six in drops of M16 + BSA under oil pre-equilibrated at 37°C and 5% CO_2 in air. At hourly intervals, the cultures were examined and any ¼ or ⅛ cells that had divided to ⅜ or 3/16 were harvested as natural ⅜ or 3/16 couplets. They were either used as such, or disaggregated, as described above, into 2 × ⅛ cells or 2 × 1/16 cells.

The midbody, which is the remnant of the material forming the cleavage furrow at the previous division and which persists for several hours post-division,

must be broken during disaggregation of ¾, ⅜, and 7/16 natural couplets. In many cases, the midbody elongates slightly during the early phase of disaggregation, giving a dumbbell appearance to the couplet. The midbody then snaps, usually adjacent to one cell, and the remnant of it is stable over several hours and clearly identifiable on the other cell of the couplet. Its shape characteristically is of a short stalk terminating in a small sphere of cytoplasm (e.g., see Fig. 1 *h*), and is quite distinct in shape and size from any of the surface blebbing often seen around the time of division (10).

Aggregation of Blastomeres

Single blastomeres or natural coupletes were aggregated together in the various combinations and spatial arrays, described in the text, after a brief exposure to a 1/20 dilution of stock phytohemagglutinin (PHA, Gibco Laboratories, Grand Island Biological Co., Grand Island, N.Y.) in Ca^{++}-free M16 + 0.6% BSA. PHA has been shown not to affect polarization (19), and this finding was confirmed in a few pilot experiments in which there was no difference in polarization when PHA-mediated aggregates were compared with naturally aggregated pairs. Since PHA greatly facilitates aggregation, it was therefore used throughout. Aggregates were cultured in M16 + BSA in Sterilin tissue-culture dishes (19).

Fluorescent Surface Labeling of Cells

Throughout these experiments, cells were stained with the FITC-Con A (Miles Laboratories Inc., Miles Research Products, Slough, England) at a concentration of 0.7 mg/ml in PBI + BSA + 0.02% (wt/vol) sodium azide at room temperature for 10–15 min. Cells were washed through three changes of PBI + BSA + sodium azide and mounted under oil in microdrops of this medium on microcytotoxicity slides (Baird & Tatlock Ltd., Romford, England). Under these conditions, it has been shown that FITC-Con A uniformly labels the surfaces of fertilized 1-cell, 2-cell, 4-cell, and early 8-cell blastomeres (3). It also labels unfertilized egg surface membranes weakly in the region overlying the second metaphase spindle, but strongly elsewhere (8); and it labels late ⅛ cells in a polarized pattern, which is observed with or without prior fixation, with mono- or divalent fluorescent ligands and in the presence of azide, cytochalasins, or colcemid (3). It has been demonstrated that the polarized FITC-Con A binding to 8-cells is coincident with a polar concentration of microvilli (16) that increases the membrane density and thus the Con A receptor density at the pole.

Fluorescent Marking of Cells

Individual ⅛ cells were marked for later identification by incubation for 10 min at room temperature in FITC at a final concentration of 0.5 mg/ml in ungassed M16 containing 3 mg/ml PVP. The FITC-blastomeres were washed thoroughly through two large volumes of PBI + BSA and aggregated with nonlabeled blastomeres as described in the text. The FITC-marked cells were later recovered from the aggregates, surface labeled with rhodamine-succinyl Con A (TRITC-succ-Con A; Polysciences, Inc., Warrington, Pa.) at 1 mg/ml in PBI + BSA, and scored for presence or absence of a fluorescent pole of Con A binding. FITC-labeling does not appear to interfere with the polarization of blastomeres in intact preimplantation embryos (in preparation).

Fluorescence Microscopy

A Zeiss epifluoresence microscope, incident source HBO 200 with filter set 487709 + LP425, was used to examine cells for fluorescein labeling. Filter set 487715 was used for observation of TRITC-succ-Con A binding. Photographs were taken on Kodak Tri-X 35mm film, and all exposures were for 2 min. Samples were scored blind by two observers independently, and only agreed scorings were used. Typically, between 98 and 100% of scorings were agreed in each experiment.

RESULTS

Is the Induction of Polarity Cell-specific?

Newly formed ⅛ cells were aggregated with unfertilized and fertilized eggs, ½, ¼, ⅛, or 1/16 blastomeres and cultured for 8 or 9 h. The incidence of polarity developed in the ⅛ blastomeres is recorded in Table I (columns 2–4). A clear trend towards an increased incidence of polarity in the ⅛ cells is observed with more mature carrier cells. During our observations, we noticed that there seemed to be a correlation between the development of polarity in ⅛ cells aggregated with fertilized or unfertilized eggs and the position of the residual midbody on the ⅛ cell. We therefore examined all aggregates for evidence of persistent midbody remnants on the ⅛ cell and, where its presence was detected, scored its position as either being between the ⅛ cell and the carrier cell (Fig. 1 *a* and *b*) or being directed away from the carrier cell (Fig. 1 *c* and *d*). The results of this analysis are presented in Table I (columns 5–7). For combinations of ⅛ plus ⅛ cells, the position of the midbody is irrelevant, as was demonstrated previously (19; see also Fig. 3 *b* and *c*). A similar finding was made for ⅛ plus ¼ combinations (Fig. 1 *g* and *h*). An effect of the midbody position appeared to occur in combinations of ⅛ plus ½ (Fig. 1 *e* and *f*) and a strong effect was observed for combinations of ⅛ plus fertilized or unfertilized eggs (Fig. 1 *a–d*). Polarity was induced in ⅛ blastomeres aggregated to fertilized or unfertilized eggs if the midbody intervened between the ⅛ blastomere and the egg (Fig. 1 *a* and *b*), but rarely if the midbody did not intervene (Fig. 1 *c* and *d*). This result suggested that the ability to induce polarity was lacking from egg membranes but develops during the 2-cell stage to be present in 4- and 8-cell membranes, and that the ⅛ cell midbody membrane not surprisingly has an inducing capacity, but one which, for its expression, requires attachment to another surface; a hint that this might be the case was reported previously (19).

TABLE I

Incidence of Polarity Generated in Newly Formed 1/8 Blastomeres that Were Aggregated with Various Carrier Cells for 8–9 h before Analysis

	Incidence of polarity*			Percent of polarity in relation to orientation of midbody‡		
Carrier cell	P	AP	P	Away	Between	Not visible
			%			
Unfertilized egg	30	70	30	12 (5/40)	91 (21/23)	11 (4/37)
Fertilized egg	15	53	22	14 (3/21)	46 (6/13)	18 (6/34)
½ cell	77	85	48	35 (18/52)	75 (12/16)	50 (47/94)
¼ cell	96	60	62	60 (23/38)	74 (17/23)	60 (56/95)
⅛ cell	83	16	84	86 (32/37)	89 (25/28)	76 (26/34)
1/16 cell§	104	19	85	79 (19/24)	None	86 (85/99)

* P = polar, AP = apolar.

‡ Midbody classified as being between ⅛ and carrier cell, directed away from contact with the carrier cell or not visible.

§ No difference in inducing capacity was observed between polar and apolar 1/16 blastomeres, and the data for each group were therefore pooled.

In no case where unfertilized, fertilized, or ½ cells were used as carriers was polarity induced in the carrier by the ⅛ cell. 2 out of 154 ¼ cells did show pole development opposite the point of contact with the ⅛ cell, but it is probable that these were ¼ cells that had failed to divide and were in fact equivalent in age to mid-⅛ cells. We have shown that suppression or failure of cytokinesis need not affect expression of other features of the developmental programme (9, 14, 20). It therefore seems that, while the capacity to induce polarity develops during the 2-cell stage, the capacity to respond by polarizing does not develop until the time of the 8-cell stage.

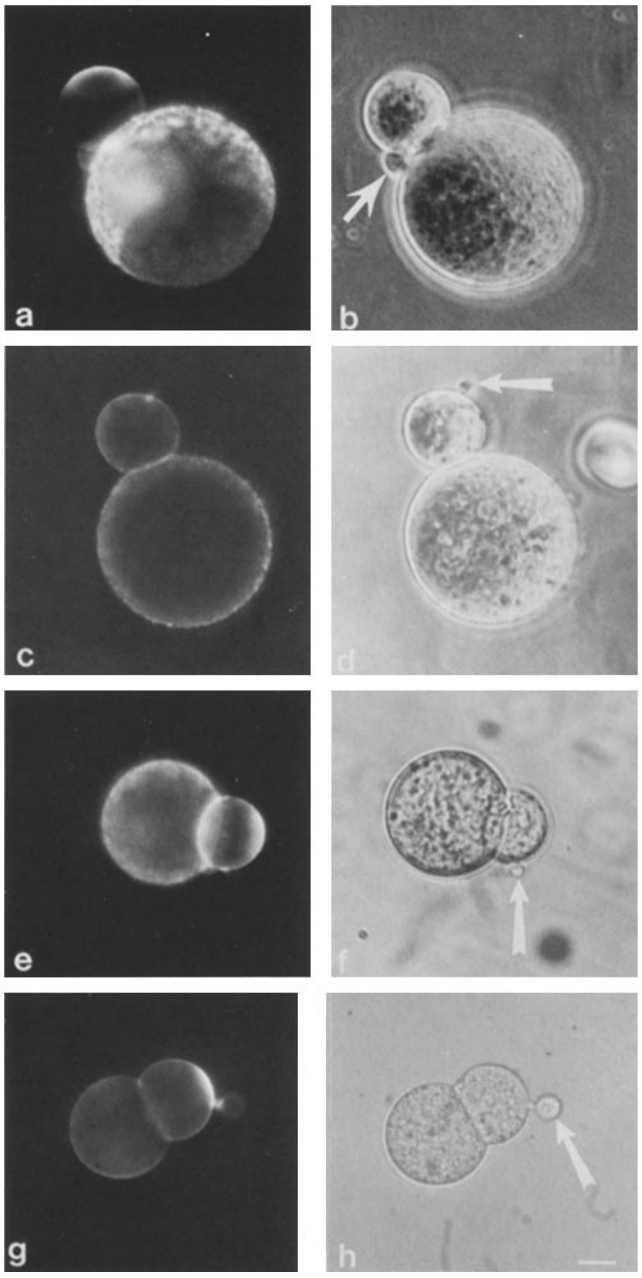

FIGURE 1 Fluorescence (*a*, *c*, *e*, and *g*) and bright-field (*b*, *d*, *f*, and *h*) photomicrographs of examples of ⅛ blastomeres aggregated to unfertilized (*a* and *b*), fertilized (*c* and *d*), ½ blastomere (*e* and *f*) and ¼ blastomere (*g* and *h*), and then cultured for 8–9 h. Arrows point to midbodies. Note in *a* and *b* that the unfertilized egg has an area of its surface which is deficient in FITC-Con A binding and which overlies the second metaphase spindle (8). Also, the midbody of the ⅛ cell is between the ⅛ blastomere and the egg surface, and the pole on the ⅛ cell has formed opposite the point of contact with the midbody. In *c* and *d*, the ⅛ cell is not polarized, and the midbody is directed away from the carrier surface. In *e*, *f*, *g*, and *h*, the ⅛ cell has polarized and the midbody is directed away from the carrier cell. For incidence of polarity in ⅛ blastomeres on different carrier cells, see Table I. Bar, 10 μm. × 580.

The Stability of the Axis of Polarity

We next enquired as to the stability of the polarity in ⅛ cells. Individual, newly formed ⅛ cells were isolated from ⅜ couplets and aggregated together in pairs. Where an obvious midbody was present, the aggregation was arranged such that the midbody was directed away from the point of contact between the cells. Aggregates were cultured for 3, 4, or 5 h, then disaggregated to ⅛ cells and immediately reaggregated together again, either randomly or, if a midbody was obvious, in such a way that the midbody was directed inward towards the new point of contact. The reaggregates were then cultured for a further 6, 5, or 4 h before analysis for the positions of the poles. As controls, aggregated pairs of ⅛ cells were cultured for 3, 4, or 5 h, exposed to Ca^{++}-free medium and micropipetting inadequate to separate them, and then placed back in culture for 6, 5, or 4 h, respectively.

Pairs of cells were then analyzed for FITC–Con A binding. Where the poles of fluoresence were directly opposite the point of intercellular contact, the cells were scored as being "strictly on-axis" (see Fig. 2 *a* and legend). Where the poles of fluorescence were not so arranged, the cells were scored as being "not strictly on-axis" (Fig. 2 *b–c*). If, following the rearrangement of cells at 3, 4, or 5 h, the axis of polarity in individual cells

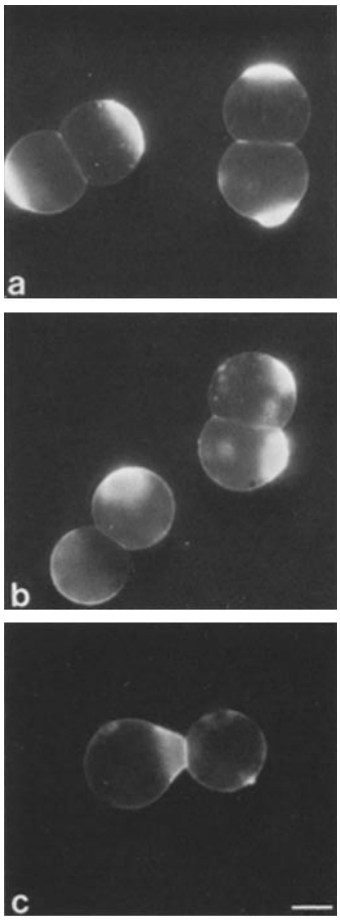

FIGURE 2 Fluorescence photomicrographs of pairs of ⅛ cells aggregated together with PHA within an hour of their formation. After 5 h in culture, the pairs were exposed to Ca^{2+}-free medium and pipetted either insufficiently to separate them (*a*) or sufficiently to separate them into two ⅛ blastomeres (*b* and *c*); each pair was then reaggregated together with a new point of contact. All pairs were cultured for a further 4 h and then stained with FITC-Con A. In *a*, note that one pair is strictly on-axis, whereas the other is almost so, although classified here as not strictly on-axis. In *b*, both pairs consist of cells in which the poles are way off-axis. In *c*, one cell is apolar, the other is stuck to it by its pole and must therefore have been turned through 180° at 5 h. Bar, 10 μm. × 580.

cannot be shifted, then the proportion of cells scored as on-axis should decline. The results, presented in Table II and Fig. 2, show that even after 3 h the incidence of cells off-axis is elevated over controls and that this trend increases with time. This result suggests that the axis of polarity is established in different cells at different times, thereby confirming previous results (19), and that, once established, not only is the axis of polarity stable for the rest of the cell cycle, but also that the two polarized cells do not undergo major shifts in their relative positions.

Orientation of Polarity in Groups of 1/8 Cells

So far we have presented results only for pairs of cells. In the embryo, cells are present in a cluster. To investigate the effect of multiple cell contacts on the organization of polar cells, we aggregated together newly formed 1/8 cells in different spatial arrays of 2, 3, or 4 cells, and cultured the aggregates for 9 h. As demonstrated previously, cells in pairs were not polarized initially (Fig. 3 *a*), but after seven or more hours in culture, both cells developed poles of FITC-Con A binding opposite the point of contact (Fig. 3 *b*). When more than two cells were aggregated, each cell appeared to recognize all cell contacts and developed a pole of FITC-Con A binding at a point geometrically most distant from all of them (Fig. 3 *d*, *e*, and *g*). Where cells were arranged in a linear array, the central cell tended to generate FITC-Con A binding sites at its equator (Fig. 3 *f*).

In 21 experiments, one newly formed 1/8 cell was surrounded by 20–30 other cells, and the aggregates were cultured for 9 h. The 1/8 cell was then recovered by disaggregation of the aggregate and analyzed for presence of a pole of TRITC-succ-Con A binding. Four variations of experimental protocol were used in the 21 experiments: (*a*) a 1/8 cell was labeled with FITC and surrounded by nonlabeled 1/8 cells: (*b*) a nonlabeled 1/8 cell was surrounded by FITC-labeled 1/8 cells; (*c*) a nonlabeled 1/8 cell was surrounded by late 1/2 or 1/4 cells; (*d*) a nonlabeled 1/8 cell was surrounded by 1/16 cells. In the first group, it was possible to examine the aggregate after 9 h and determine whether the labeled cell had in fact become located in an internal position (Fig. 4 *a* and *b*). Of 21 enclosed cells, only three developed a typical pole, and all three of these were from aggregates in which a final surface location was suspected. Of the remainder, 15 showed no polarity whatsoever (Fig. 4 *d*), and three showed a small, diffuse concentration of bound TRITC-succ-Con A at one end of the cell which was quite unlike a typical pole. Over the same incubation period, control, nonsurrounded 1/8 cells with an exposed surface developed poles in 25 cases out of 34. Surrounding a 1/8 cell thus seems to suppress the development of polarity.

TABLE II

Stability of Polarity of 1/8 Cells of Various Ages *

Age of pairs at disaggregation and reaggregation (hours postdivision)	Cells polarized at time of disaggregation	Analysis of orientation of poles at 9–10 h‡			
		Control§		Experimental§	
		On-axis	Off-axis (%)	On-axis	Off-axis (%)
h	%				
3–4	38	15	2 (12)	11	8 (42)
4–5	55	24	2 (8)	13	12 (48)
5–6	57	26	5 (16)	11	19 (63)

* Two 1/8 cells were aggregated together within an hour of their formation, cultured for 3, 4, or 5 h before being placed in Ca^{++}-free medium for 15 min and either pipetted with integrity maintained (control) or pipetted until separation into 2 × 1/8 blastomeres occurred. The 1/8 blastomeres were then reaggregated randomly in pairs and they and the controls were cultured for a further 6, 5, or 4 h before analysis.

‡ Pairs were scored as strictly on-axis only if the poles were opposite the point of contact, and all other staining patterns were scored as off-axis. Note that this is a stricter definition of on-axis than used previously (19).

§ At each time-point, controls differed highly significantly from experimentals by the Fisher exact test ($P = 0.04$, 0.001, and 0.001 at successive time-points). When the three control and three experimental groups were analysed for changing trend with time, the Pearson correlation coefficients were 0 and 1.0, respectively, indicating an increasing incidence of off-axis cells with time in the experimental group.

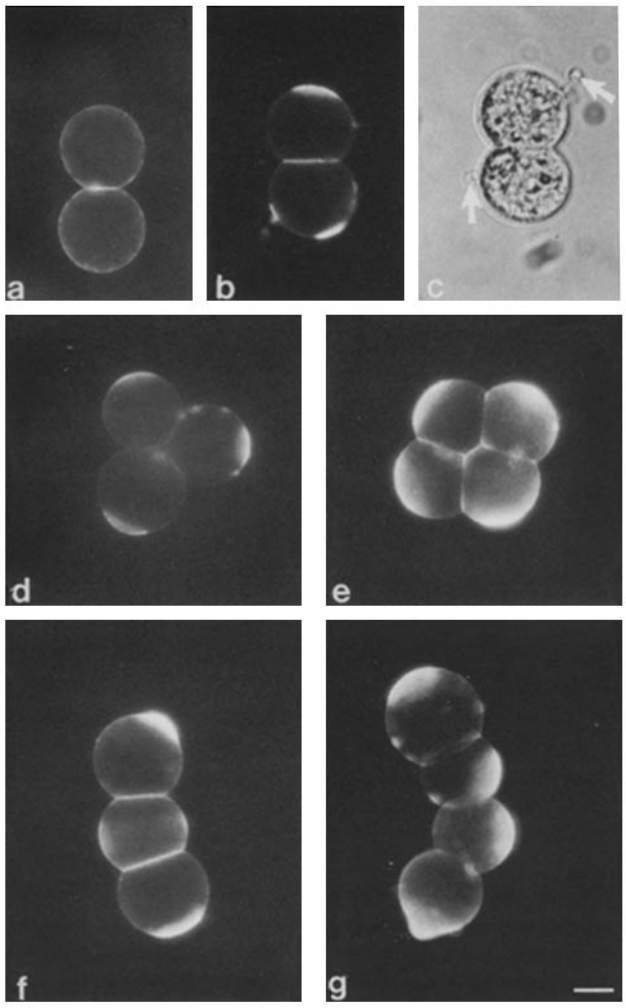

FIGURE 3 Fluorescence photomicrographs (*a*, *b*, and *d–g*) and a bright-field photomicrograph (*c*) of groups of 1/8 blastomeres. (*a*) Pair 2 h after aggregation, no poles present. (*b* and *c*) Pair 7 h after aggregation; note poles opposite points of contact—arrows point to midbodies. (*d*) Triplet. (*e*) Quartet. (*f*) Linear triplet; note equatorial binding of FITC-Con A by center cell. (*g*) Linear quartet. Note throughout how the position of the pole appears to respect all contacts. Bar, 10 μm. × 580.

Is the Effect of Multiple Cell Contacts on Orientation of Polarity Related Exclusively to Inductive Effects?

The cell-specific signal transmitted to a 1/8 cell could both induce polarity and determine its axis. Alternatively, the signal to induce could lead to a polarizing response by the 1/8 cell

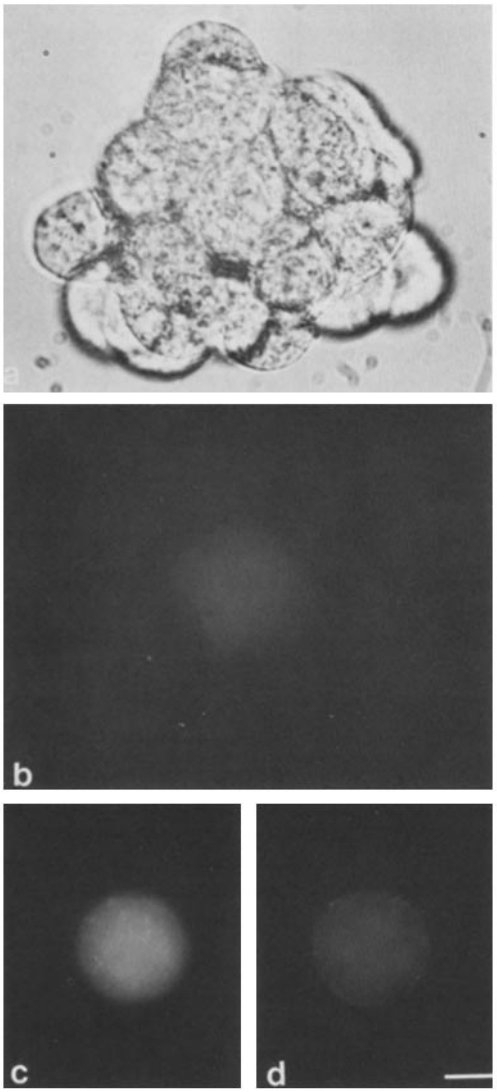

FIGURE 4 A newly formed ⅛ blastomere was labeled with FITC, surrounded by 20–30 other ⅛ blastomeres and cultured for 9 h. The aggregate is shown under bright-field in *a* and fluorescence in *b*, which shows a diffuse image of the fluorescent cell in the center of the aggregate. (*c*) The same cell isolated from aggregate after exposure to Ca^{2+}-free medium, and incubation in TRITC-succ-Con A. (*d*) The surface binding of rhodamine-labeled Con A is uniform and non-polar. Bar, 10 μm. × 840.

which then recognized all cell contacts regardless of whether they possessed specific inducing capacity. To examine this question, we performed two types of experiments. First, we aggregated newly formed natural ⅜ pairs (with an intact mid-body) onto a third carrier blastomere of various types, cultured the aggregates for 9 h, and examined the axes of polarity which developed in the cells of the ⅜ couplet (Fig. 5). The results are summarized in Table III and show that cells which induce are ignored less than cells which do not. However, even noninducing fertilized eggs can have some effects on the orientation of poles in a third of the cells. This result hints that surface-signaling features, in addition to those directly involved in the induction of polarity, are recognized by a cell undergoing polarization.

A second experimental approach supported this conclusion. Natural pairs of ⅜ were aggregated to carrier cells and cultured for 5 h. The ⅜ pair was then separated from the carrier

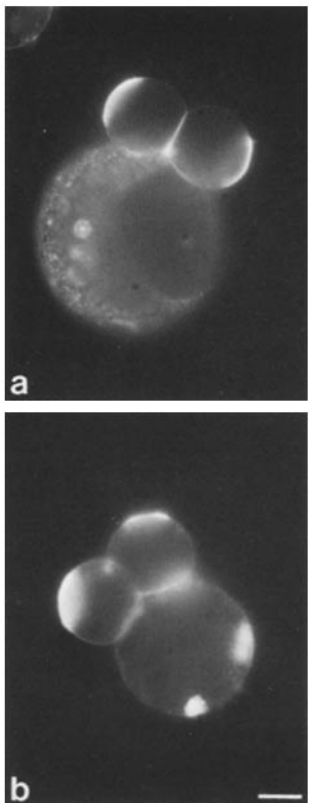

FIGURE 5 Fluorescence photomicrographs of natural ⅜ pairs aggregated onto unfertilized (*a*) and ½ blastomere (*b*), cultured for 9 h and incubated in FITC-Con A. Note that in *a* the polarization appears to ignore the carrier egg, whereas in *b* the carrier ½ blastomere is respected. Bar, 10 μm. × 580.

TABLE III

Influence of Various Carrier Cells on the Orientation of Polarity Developed in the Cells of 2/8 Natural Pairs Aggregated to Them for 8 h

	Number of 1/8 cells that develop poles with orientations which ignore or recognize carriers*	
Carrier cell	Ignore‡	Recognize (%)§
Unfertilized egg	56	11 (16)
Fertilized egg	67	30 (31)
1/2 blastomere	4	46 (92)
1/4 blastomere	10	21 (68)
1/8 blastomere	0	32 (100)

* When unfertilized plus fertilized eggs as a group were compared with 1/2, 1/4, and 1/8 blastomeres as a group, the χ^2 value was 104.92, $P < 0.001$, indicating a highly significant difference.
‡ See Fig. 5 *a* for example.
§ Fig. 5 *b* for example.

and cultured for a further 2–4 h. The orientation of the poles in the couplet was then scored as being on-axis or off-axis. The results are given in Table IV and show that, regardless of inducing capacity, the prior presence of a third cell is evidently not remembered by the cells of the ⅜ pair. The cells within the couplet must adjust their relative position after removal of the carrier cell. Thus, although multiple inducing contacts may influence the initial orientation of polarity, minor shifts in relative cell position in response to increased or decreased

TABLE IV

Stability of Axes of Cell Polarity within Natural Couplets (Aggregated with Carrier for 5 h before Separation)

Carrier cell	Ignore carrier	Respect carrier	Respect
			%
1/8 blastomere	24	4	14
Unfertilized egg	14	1	7
Fertilized egg	30	2	6

numbers of general cell contacts can influence the final orientation of cells within an aggregate.

DISCUSSION

The mechanism by which polarity is induced in ⅛ blastomeres may be important since the suggestion has been made that a major consequence of polarization is the foundation of the two cell lineages of the blastocyst—the ICM and the trophectoderm. Here we have presented evidence that the ability to induce polarization is absent in the membrane of the 1-cell egg and zygote and develops at some point during the 2-cell stage. Because we have evidence that the embryonic genome first becomes transcriptionally active during the 2-cell stage (7), it is tempting to speculate that the development of inducing capacity reflects the activity of embryonic genes and is not a result of the use of maternally inherited information. This notion is currently being tested directly.

The inducing signal appears to act over a period of only a few hours (Fig. 2 *b* and *c;* Table II). Once polarity is induced, turning the two cells in a couplet through 90–180° with respect to each other and sticking them together with PHA does not cause them to shift their axis of polarity correspondingly. Rather, pairs persist in which the poles are greatly off-axis with respect to their new point of contact. This relative stability of the axis of polarity in individual cells concords with previous data on the stability of polarity of single cells in culture and during division to 5/16 cells (10, 19).

However, although the axis of polarity is induced specifically and is stable, two types of experiment suggest that other cell contacts are not ignored during or after the development of polarity. If newly formed natural couplets of ⅜ cells, in which the integrity of the midbody is retained, are aggregated onto carrier cells, then the axis of polarity which develops is influenced strongly by ⅛, ¼, and ½ cells and weakly by unfertilized and fertilized eggs. In the latter case, in which the carrier cells themselves cannot induce polarity, it seems possible that the ⅛ cells induced polarity in each other as a result of contact at their persistent midbody, but that subsequently in some pairs the ⅛ cells swing towards each other and away from the carrier, thus altering the apparent axis in relation to the point of contact. Support for this idea comes from examination of ⅜ natural pairs aggregated with a ⅛ cell for 5 h before disaggregation to ⅜ + ⅛. Despite the fact that in the triplet all cells respect all contacts (Fig. 3 *d*), and that the axis of polarity in individual cells is stable after 5 h (Fig. 2), nontheless the separated pair of ⅜ were found to be polarized on-axis with respect to each other. Presumably, polarized pairs of cells can swing a little about their midbodies. Direct evidence for a limited capacity for this sort of swinging movement has come from time-lapse analysis of cell aggregates (2, 11). Such a mechanism would facilitate the packing of a set of polar cells *in situ* and permit minor spatial adjustment either to a loss of cells by death or to a gain of cells at division.

A complete and continuous set of cell contacts over the whole cell surface, completely suppresses polarization. In artificially large aggregates of several early 8-cell embryos, such as occurs in the production of experimental chimaeras (12), internally placed cells would therefore fail to polarize. If the hypothesis that the trophectodermal lineage is derived from the apical region of polarized ⅛ cells is correct (6, 9), then any totally surrounded, and, thus, nonpolarized cells in such aggregates should not contribute to the trophectoderm. In experiments in which aggregation chimaeras have been made by placing genetically or physically marked cells in either inside or outside positions, totally enclosed cells did indeed contribute predominantly to the ICM (5) in most cases and exclusively in some. Such experiments, taken together with the results reported here, support the notion that polarization might play a role in the foundation of the ICM and trophectodermal lineages (9).

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