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A role for cytoplasmic determinants in the development of the mouse early embryo?

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INTRODUCTION

A role for cytoplasmic determinants in the early development of several organisms has been suggested by results from a variety of studies. Ideally, a demonstration of the existence of determinants requires their transfer between regions of an embryo, but this has been achieved only rarely (see chapters by Nusslein-Volhard and Jäckle, this volume). In many cases, the existence of cytoplasmic determinants is inferred indirectly, for example where procedures that disturb the asymmetric arrangement of the cytoplasm also disrupt the normal allocation of cell types, or where a correspondence exists between the prospective fate of a cell and the cytoplasmic endowment it has received (see chapters by Gurdon, Schierenberg, Strome and Kalthoff, this volume). In these latter examples, a heterogeneity within the cell may indeed exist and influence further development, but its nature may preclude direct testing by cytoplasmic transfer, or a regulatory capacity may exist whereby the localization and/or expression of putative determinants can be modified (Jeffery & Raff, 1983; Johnson & Pratt, 1983). In this paper, we define three minimal operational criteria that must be satisfied for the demonstration of a role for cytoplasmic inheritance in early development. First, a consistent asymmetry in the organization of individual cells of the early embryo should be identifiable. Second, at least some of the properties characterizing the distinct regions of each asymmetric cell should be distributed differentially at cell division. Third, the properties that characterize the asymmetric organization of the parent cell and its daughters should lead causally and predictably to differences in the fates of these cells and their descendants. We examine the evidence as to whether these criteria can be met for the earliest events of mouse development. We conclude that by these criteria cytoplasmic determinants are indeed present, but we question the value of the notion of cytoplasmic determinants for an understanding of developmental mechanisms.

Key words: determinant, mouse, polarity, cytocortex, cell lineage.

AXES OF DEVELOPMENT

The acquisition of developmental axes occurs relatively late in mammalian development when compared with most, if not all, other types of embryo. The earliest identified axis is radial and develops at the 8-cell stage about 48 h into development, the internal or core region of the embryo becoming structurally and developmentally distinguishable from the outer peripheral region (Fig. 1; Tarkowski & Wroblewska, 1967; Wilson, Bolton & Cuttler, 1972; Hillman, Sherman & Graham, 1972; Ducibella & Anderson, 1975). This radial axis marks the positions of future potential allocations to the embryonic and extraembryonic lineages. A secondary axis, also relating to embryonic: extraembryonic divergence, develops around 36 h (or three cell cycles) later when the embryonic: abembryonic axis forms (Fig. 1; Gardner, Papaioannou & Barton, 1973; Gardner & Papaioannou, 1975; Smith & McLaren, 1977; Wiley & Eglitis, 1981). It is not until around 3 days further into development that definitive anteroposterior and dorsoventral embryonic axes become evident. We know little of the mechanism by which the embryonic: abembryonic axis is laid down (Surani & Barton, 1984) and

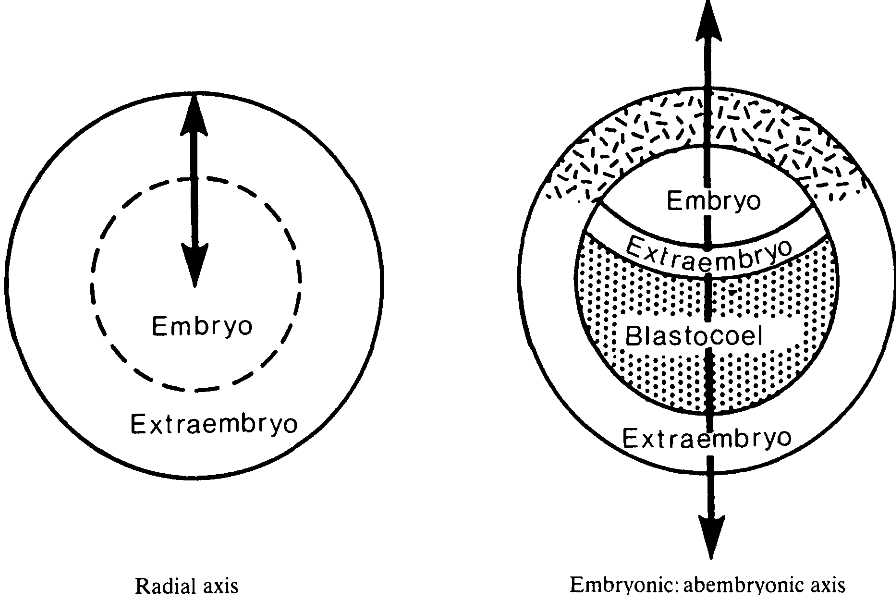


Fig. 1. Schematic view of the first two axes established during mouse development. A radial axis develops first and involves the generation of differences between the centre and the periphery of the embryo, central regions developing into the inner cell mass (ICM) and peripheral regions yielding extraembryonic trophectoderm. With the appearance of the blastocoel (stippled) and development of the ICM and trophectoderm, an axial embryonic: abembryonic axis is established; the ICM lies at the embryonic pole and differentiates primary (yolk sac) endoderm abembryonically (labelled extraembryo) and primary ectoderm towards the embryonic pole (labelled embryo); the 'polar' trophectoderm overlying the embryonic ectoderm of the ICM proliferates (hatched), whilst the 'mural' abembryonic trophectoderm undergoes endoreduplication to yield giant cells.

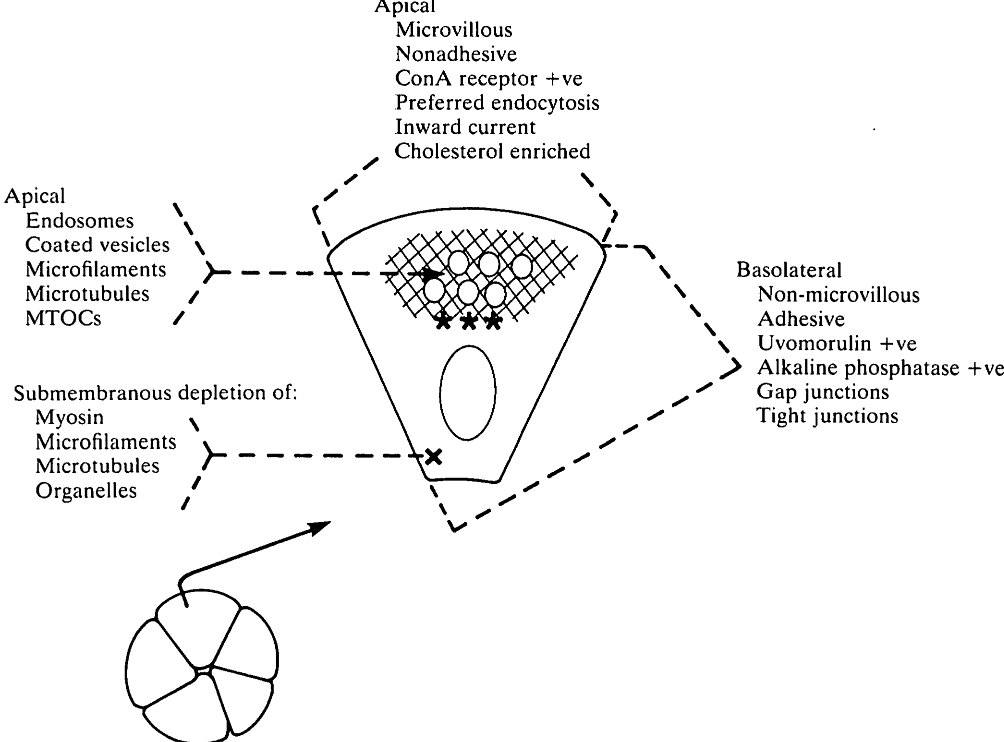


Fig. 2. Schematic view of a compact 8-cell embryo (lower left) from which one cell has been removed and illustrated in more detail to reveal the polarized distribution of many of its components.

less about how the definitive embryonic axes are established (Smith, 1985). This chapter therefore concentrates on the establishment of the earliest, radial axis.

DOES THE RADIAL AXIS OF DEVELOPMENT INVOLVE THE ASYMMETRIC ORGANIZATION OF INDIVIDUAL CELLS?

It is possible to identify many differences in organization between the central and peripheral regions of the embryo in advance of the earliest establishment of internal and external cell populations at the 16-cell stage. These differences include structural and functional regionalization of individual cells (Fig. 2) with regard to their cytoplasmic organelles (Reeve, 1981*a*; Reeve & Kelly, 1983; Lois & Izquierdo, 1984; Fleming & Pickering, 1985; Maro, Johnson, Pickering & Louvard, 1985), the major cytoskeletal elements of the cytoplasmic matrix (Ducibella, Ukena, Karnovsky & Anderson, 1977; Sobel, 1983, 1984; Johnson & Maro, 1984; Sobel & Alliegro, 1985; Houliston, Pickering & Maro, in preparation; Fig. 3) and the cytocortex defined here as embracing the cell membrane plus its associated material including membrane-associated cytoskeletal proteins (Calarco & Brown, 1969; Ducibella & Anderson, 1975; Ducibella, Albertini, Anderson & Biggers, 1975; Izquierdo & Marticorena, 1975; Magnuson, Demsey & Stackpole,

1977; Handyside, 1980; Izquierdo, Lopez & Marticorena, 1980; Reeve & Ziomek, 1981; Izquierdo & Ebensperger, 1982; Kimber & Surani, 1982; Kimber, Surani & Barton, 1982; McLachlin, Caveney & Kidder, 1983; Goodall & Johnson, 1984; Fleming & Pickering, 1985; Nuccitelli & Wiley, 1985; Pratt, 1985; Johnson, Maro & Takeichi, 1986). Some of these differences appear first at the 4-cell stage, but most develop and become stabilized at the 8-cell stage in the mouse. The result is an embryo in which each cell is polarized with apical (outward facing) and basolateral (inward facing) domains. Moreover, when the late 8-cell embryo is disaggregated into its constituent cells, each retains most elements of its polar phenotype (Figs 2, 3). Thus, the first criterion for a role for cytoplasmic inheritance appears to be met.

CAN DIVISION OF A POLARIZED 8-CELL BLASTOMERE GENERATE CELL DIVERSITY?

Most of the features identified as being polarized in individual 8-cell blastomeres are dispersed at entry into mitosis, congregate in and around the mitotic spindle or its poles, and become distributed to both daughter cells. Such features include cytoplasmic organelles (Reeve, 1981*b*; Fleming & Pickering, 1985; Maro *et al.* 1985), cytoplasmic actin (Johnson & Maro, 1984) and both cytoplasmic tubulin and microtubule-organizing centres (MTOCs; Houliston *et al.*, in preparation). It is not possible to say that the distribution to each daughter cell is exactly equivalent, indeed this is unlikely to be the case especially where daughter cells differ in size (Ziomek & Johnson, 1981). However, it is clear that the asymmetric distribution of the main cytoskeletal elements of the cytoplasmic matrix, as well as the organelles associated with them, depends upon the continuing interphase state for its stability. Such a finding is not unexpected for components essential to a cell's survival.

In contrast, at least some features of cytocortical polarity are relatively stable. Throughout mitosis, which lasts some 45–60 min from prophase to cytokinesis, it is possible to discern an inhomogeneity within the cytocortex (Fig. 4; Johnson & Ziomek, 1981*a*). As a result, if the cleavage plane is oriented approximately perpendicular to this axis of polarity, one of the two cells that result inherits an apical domain and remains polar, the other lacks such a domain and is apolar. Alternatively, if the cleavage plane runs approximately parallel to the axis of polarity, two polar cells result, and the poles on each cell are contiguous across the residue of the cleavage furrow connecting the two daughter cells (Johnson & Ziomek, 1981*a*; Reeve, 1981*b*). Immediately after the completion of cytokinesis, the cytoskeletal matrix and cytoplasmic organelles in those 16-cell blastomeres that have inherited an apical cytocortical domain also resume a polarized distribution about an axis of polarity coincident with that of the cytocortex (Fleming & Pickering, 1985; Maro *et al.* 1985). The relative stability during mitosis of the cytocortical organization compared to that of the cytoplasmic matrix has also been inferred from observations on the behaviour of exogenously introduced markers

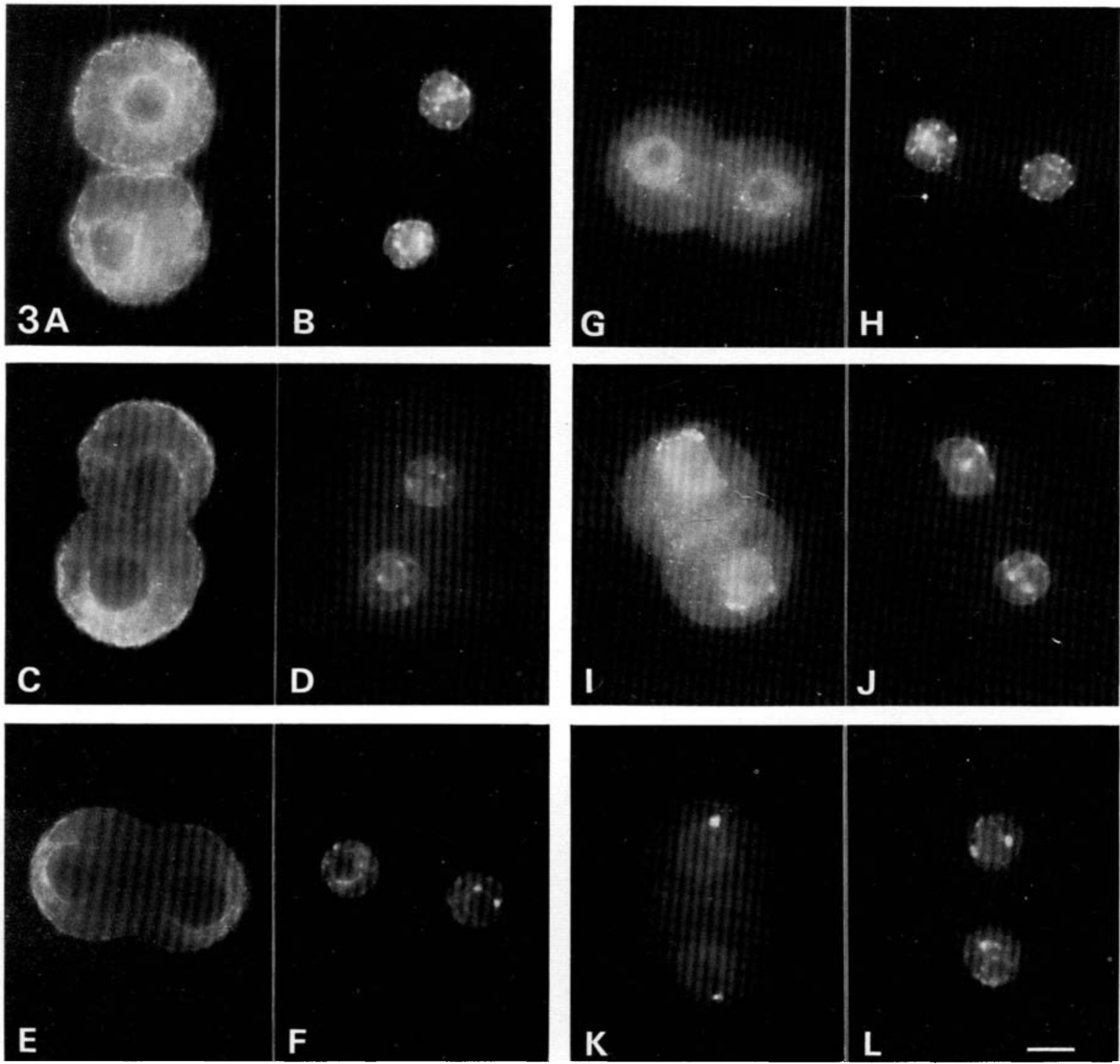


Fig. 3. Polarization of microtubules and MTOCs at the 8-cell stage (from Houliston, Pickering & Maro, in preparation). Immunofluorescent staining of 8-cell couplets with antibody to tubulin (A,C,E) or to pericentriolar material (G,I,K; gift of M. Kirschner; Callarco-Gillam *et al.* 1983). Accompanying panels (B,D,F,H,J,L) show Hoechst nuclear staining. Cells were fixed at 3 h (G,H), 5 h (A,B), 7 h (C,D) and 9 h (E,F,I–L) after their formation by division from isolated 4-cell-stage blastomeres. In all 8-cell-stage blastomeres, microtubules are concentrated around the nucleus and cell periphery but are absent at cell contact sites. During the 8-cell stage their distribution shifts from predominantly uniform (A) to polarized (E), with cytoplasmic microtubules concentrated apically (C and E). MTOC material, which cannot be detected in the previous interphase, appears gradually during the 8-cell stage. Initially it is usually seen as small widely dispersed dots (G). These tend to polarize apically (I) and then concentrate near the nucleus (K) prior to mitosis, when MTOC aggregates are found at the spindle poles. Bar, 10 μm.

(Wilson *et al.* 1972; Graham & Deussen, 1978; Karasiewicz & Modliński, 1985). These results suggest that the cytocortex is characterized by two properties. First, it manifests a polar organization with a long half life in relation to the period of mitosis. Second, some aspect of this cytocortical polarity appears to remain spatially discrete in that it is susceptible to division by the cleavage furrow and serves as a defined organizing focus for the internal axis of cell polarity.

We must ask whether this relatively stable polar cytocortical domain coincides exactly and exclusively with the most prominent observable polarized feature of the cytocortex, namely the pole of microvilli, or whether there is some underlying basis for the various visible manifestations of polarity both cytocortical and cytoplasmic. Three lines of evidence make it unlikely that the observable pole of microvilli *per se* constitutes an exclusive basis for stable polarity. First, during mitosis the clearly demarcated apical pole of microvilli that characterizes the interphase state becomes less-sharply defined. The region of the surface occupied

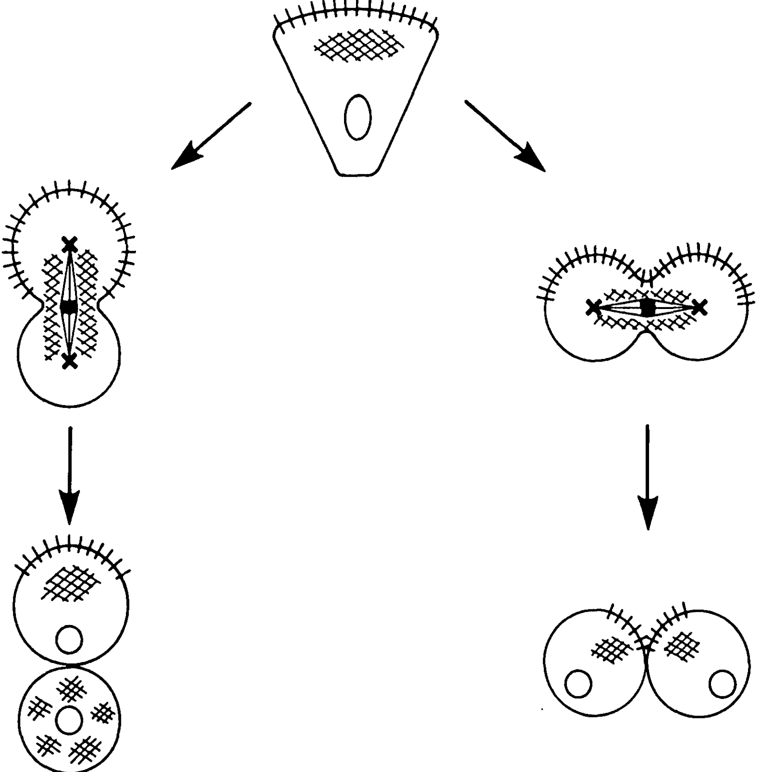


Fig. 4. Schematic view of two possible consequences of the division of a polarized 8-cell (or 16-cell) blastomere. If the plane of division is as shown on the left, the division is differentiative and two cells with different phenotypes result. In contrast, a conservatively oriented division plane, as illustrated on the right, yields two cells both of which are polar, the poles being contiguous across the remnant of the cleavage furrow. Hatching represents microfilaments, endosomes and clathrin; surface spikes represent microvilli.

by microvilli expands, occupying progressively more of the cell surface as division proceeds (Fig. 4). During a normal mitosis of 45–60 min, microvillous pole expansion is relatively limited. However, if mitosis is arrested at metaphase for up to 6 h by placing prophase cells into medium containing the microtubule-depolymerizing agent nocodazole, the dispersal of microvilli extends over the whole surface in many cells, although a gradation remains evident in most cells giving them a continuing inhomogeneity (Fig. 5). If the drug is removed, mitosis resumes immediately and couplets of cells form with identical phenotypes to those derived from nonarrested controls i.e. polar–apolar pairs or polar–polar pairs with polar continuity at the remnant of the cleavage furrow, and in each case the pole that forms is clearly demarcated from the nonmicrovillous areas of the cell (Fig. 5, Table 1; Dhiman, Pickering, Maro & Johnson, in preparation). Thus, the

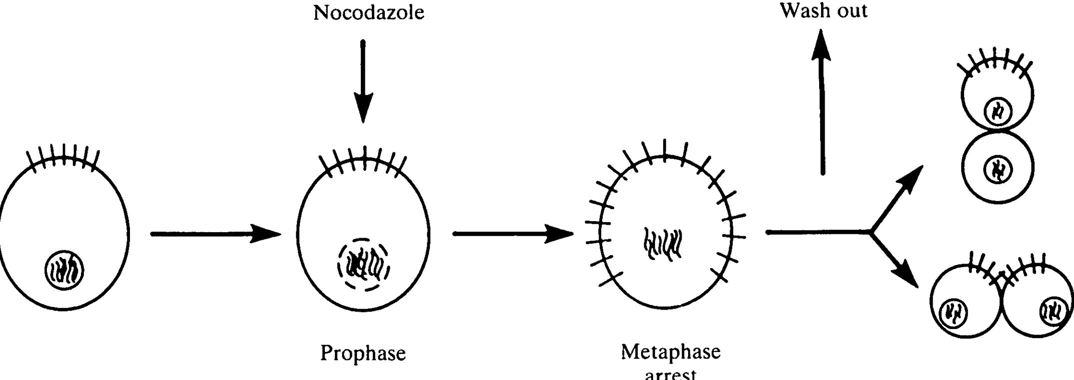


Fig. 5. Schematic illustration of the strategy employed to examine the effect of prolonged mitotic arrest on the stability of the polar axis. Nocodazole was added to isolated polarized late 8-cell blastomeres as they entered mitotic prophase (assessed by loss of nuclear membrane). Cells were held arrested for up to 6 h before either being analysed for evidence of a continuing surface inhomogeneity or removal from the drug and analysis of the 2/16 couplets that resulted. The results are summarized in Table 1 taken from Dhiman *et al.*, in preparation.

Table 1. *Summary of data showing effect on surface polarity of prolonged exposure of polar 1/8 blastomeres to nocodazole*

Hours in nocodazole	% of cells showing polarity	% of 2/16 couplets formed after release from nocodazole with		
		No polar cells	One polar cell	Two polar cells
0	91	0	68	32
2	100	0	64	36
4	93	0	60	40
6	81	7	59	34

Data abstracted from Dhiman, Pickering, Maro & Johnson, in preparation.

observed 'diffusion' of surface polarity did not prevent the restoration of a sharply defined and appropriately oriented polar phenotype subsequently.

A second line of evidence suggesting that the axis of cell polarity is separable from overt microvillous polarity derives from the analysis of interphase polar cells in which the overt polar region of microvilli has been disturbed experimentally in various ways but the underlying polar axis to the cell nonetheless persists (Nuccitelli & Wiley, 1985).

A third line of evidence leading to the same conclusion comes from a study of the generation of polarity *de novo* at the 8-cell stage. When 8-cell blastomeres are cultured together, polarity in each always develops on an axis that is normal to the geometric centre of the various asymmetric points of contact that each cell makes (Ziomek & Johnson, 1980; Johnson & Ziomek, 1981*b*). It has been shown that these asymmetric cell contact patterns do indeed determine the orientation of the axis of polarity that develops. By use of pairs of 8-cell blastomeres of known age after their derivation from a dividing 4-cell blastomere, it has been shown that at any time over the first few hours of the 8-cell stage, changing the contact pattern between the cells changes the axis of polarity that develops, such that it is normal to the new point of contact. However, at later time points, such a regulative response does not occur, and changing the contact patterns results in axes of polarity that are related to the old rather than the new contact points (Fig. 6; Johnson & Ziomek, 1981*b*). The axis of polarity induced within each cell appears to become stabilized as progress through the fourth cell cycle occurs. A similar increase in axial stability with development can be inferred from the results of comparable studies on blastomeres *in situ* (Sepulveda, Doggenweiler & Izquierdo, 1985). The establishment of this stable axis of polarity occurs at about the time that the earliest evidence of polarity in the cytoplasmic matrix can be detected, but develops in advance of the earliest detection of microvillous polarity (Johnson & Maro, 1984; Maro *et al.* 1985; Fleming & Pickering, 1985; Fleming, Pickering, Qasim & Maro, 1986*b*). However, the temporal sequence in which the overt features of polarity develop naturally does not seem to reflect a causal sequence, since the disruption of microtubules and/or microfilaments, and thereby of the organized cytoplasmic matrix, does not prevent the generation of microvillous polarity (Ducibella, 1982; Maro & Pickering, 1984; Johnson & Maro, 1985; Fleming, Cannon & Pickering, 1986*a*).

Taken together with the evidence from mitotic cells, these results suggest that an early event in the process of polarization is the induction and stabilization of an axis of polarity that provides the framework about which the various visible elements of cellular asymmetry are organized (Fig. 7). As the development and persistence of this polar axis appears not to require an organized cytoplasmic matrix, it may reside in whole or in part within the cytocortex. Such a stable polar cytocortical domain would satisfy the second criterion for the existence of a role for cytoplasmic inheritance during early development in the mouse. Our current understanding of the nature of this domain, and how it might be achieved, is discussed in the last section.

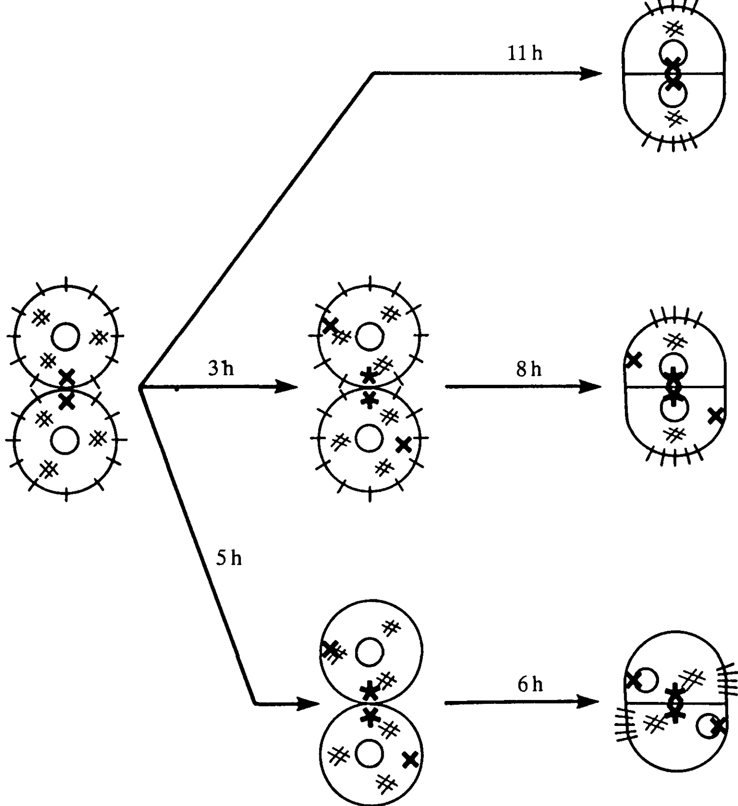


Fig. 6. Summary of experiment in which the contact point between two 8-cell blastomeres was changed at various timepoints during the fourth cell cycle and the orientation of the subsequently developed axis of polarity scored. Note that when contact relations are changed early in the cell cycle (3 h), the polarity develops opposite to the *new* point of contact (star not cross), but that this does not occur later, the *previous* contact relations being 'remembered' (cross not star).

DOES THE GENERATION OF POLAR AND APOLAR 16-CELL BLASTOMERES REPRESENT THE FOUNDATION OF TWO CELL LINEAGES?

The newly formed 16-cell embryo is composed of two cell types: a population of outer, polar cells each derived from the apical region of a polarized 8-cell blastomere and a population of inner, apolar cells each derived from the basal region of a polarized 8-cell blastomere. The ratio of polar to apolar cells will depend on the relative frequency of cleavage plane orientations (see later). Amongst the properties that distinguish the apolar and polar cells, perhaps the most important in the context of lineage establishment is their differing patterns of adhesivity. Apolar cells appear uniformly adhesive, whereas polar cells are preferentially adhesive over their basolateral surfaces (Burgoyne & Ducibella, 1977; Ziomek & Johnson, 1981; Kimber *et al.* 1982; Johnson & Ziomek, 1983). This distinction ensures the maintenance of the relative internal and external positions within the embryo at which each cell type arrives following cleavage (Ziomek &

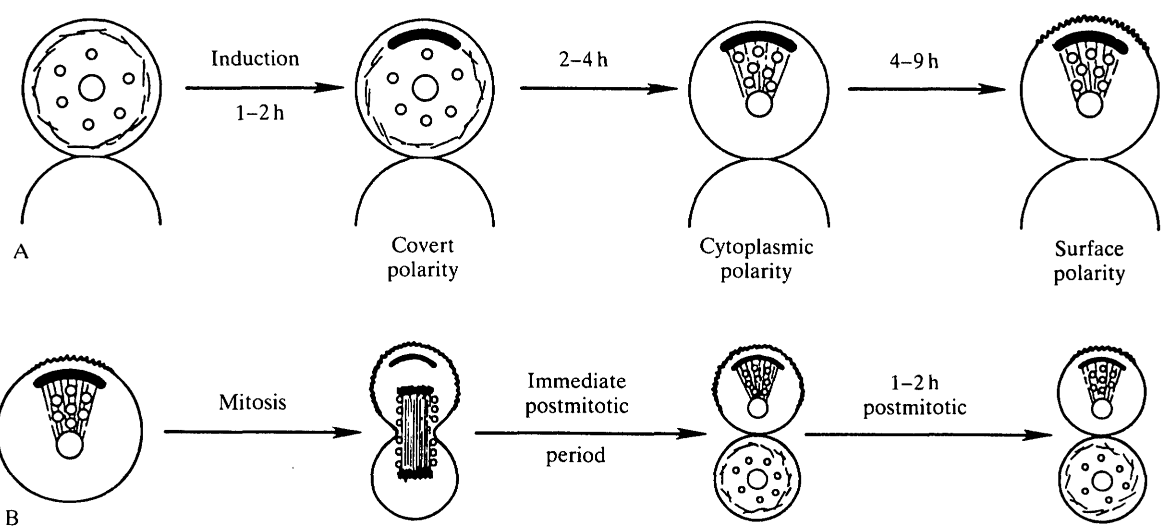


Fig. 7. Schematic representation of stable apical cytocortical domain (represented by a solid black zone), overt surface polarity (surface microvillous corrugations) and cytoplasmic matrix polarity (represented by clathrin and microfilament distribution) in (A) nonpolar, polarizing and polarized blastomeres, and (B) during and after mitosis. Note that the representation of the apical cytocortical domain as a discrete area should not necessarily be equated with an identifiable physical entity, but represents a functional change in the apical cytocortex (taken from Maro & Johnson, 1985).

Johnson, 1982; Randle, 1982; Surani & Handyside, 1983). It also ensures that the polar zone of microvilli is directed outwards at the surface of the embryo, thus orienting each cell radially.

In the definitive blastocyst, which forms at the late 32-cell stage and achieves its mature phenotype by the late 64-cell stage, two cell subpopulations exist that resemble in many ways the subpopulations at the earlier 16-cell stage (Handyside, 1981; Johnson & Ziomek, 1982; Randle, 1982; Edirisinghe, Wales & Pike, 1984; Fleming & Pickering, 1985; Fleming & Goodall, 1986). Thus, the outer trophectodermal cells form a polar epithelium whilst the inner cell mass (ICM) cells are apolar and are totally enveloped within trophectodermal processes (Fleming, Warren, Chisholm & Johnson, 1984). However, comparability of phenotype does not prove continuity of lineage. For this to be established, the fate of the progeny of the apolar and polar cells at the 16-cell stage must be followed. This can be achieved by marking cells in each subpopulation at the 16-cell stage and examining the distribution of the marked cells at later stages. Of direct relevance to the subject of this paper is the role of the stable cytocortical domain. Do all cells possessing this domain contribute all their progeny to the trophectoderm? Conversely, do those cells lacking it ever contribute cells to the trophectoderm?

A number of lineage studies has been undertaken in which a single 16-cell polar blastomere has been marked, the cell incorporated into an unmarked intact embryo or aggregate of unmarked cells, and the distribution of the marked cells examined one to two cell cycles later. These studies have revealed that a polar

16-cell blastomere almost always contributes progeny to the trophectoderm, can under some circumstances contribute cells to the ICM as well, but contributes cells exclusively to the ICM so rarely as to be explicable in terms of misidentification of, or damage to, the marked cell or its progeny (Table 2 – which also includes references). This result indicates that the stable cytocortical domain may place a restriction on developmental fate, in that it evidently debars exclusive contribution to the ICM, but the result does not tell us whether the domain is developmentally determinative in the strictest sense of the word, namely that possession of the zone necessarily results in trophectoderm formation. This question has been addressed by examining the lineage behaviour of a single marked polar 16-cell blastomere cultured either alone or in combination with a second polar or apolar cell. It is clear that when the polar cell is associated with an apolar cell it divides conservatively and generates two polar trophectodermal daughter cells, whereas a polar cell alone or aggregated with a second polar cell can divide differentiatively to yield one polar and one apolar cell (Fig. 8; Johnson & Ziomek, 1983). These results indicate that (i) cell interaction influences cleavage planes and thus lineage, and (ii) transmission of all or part of the apical cytocortical domain, or some closely associated property, does appear to be determinative for a trophectodermal fate.

An equivalent series of studies has been undertaken for apolar 16-cell blastomeres. As a result, two conclusions may be drawn. First, apolar cells by virtue of their adhesive properties tend to locate internally and generate only ICM progeny (Randle, 1982; Ziomek & Johnson, 1982). Second, if an apolar cell is forced to occupy an external position, by, for example, its aggregation with a population of other apolar cells, then it responds to the asymmetric array of contacts in just the same way as early 8-cell blastomeres do, and polarizes. Once polarized, it may then contribute progeny to the trophectoderm (Fig. 9; Ziomek & Johnson, 1981; Ziomek, Johnson & Handyside, 1982; Johnson & Ziomek, 1983; Johnson, 1986). This series of experiments leads to the conclusion that the possession of an apical

Table 2. *Fate of individual marked polar 1/16 blastomeres*

Study	No. of cells examined	Contribution to: Trophectoderm only	ICM only	Trophectoderm + ICM
Ziomek & Johnson (1982)	87	73	0	14
Randle (1982)	11	11	0	0
Johnson & Ziomek (1983)	243	188	0	55
Bałakier & Pedersen (1982)	70	37	2	31
Johnson (1986)	35	25	1	9
Fleming (1986)*	526	379	0	147
% Contribution		73·4	0·3	26·3

*Data derived by inference from study in which all polar 16-cell blastomeres in a single embryo were labelled *in situ*.

cytocortical domain guarantees a contribution to the trophectoderm through its effect on cell interaction, whilst in its continuing absence an ICM fate is assumed.

THE RELATIONSHIP BETWEEN DETERMINACY AND PLASTICITY

The lineage analyses described in the previous section indicate that the possession of an apical cytocortical domain is indeed associated with the determination of cell fate, and is so because of the polar phenotype imposed on the cells that possess it. The analyses also show that the existence of this determinant of trophectodermal development is compatible with the developmental plasticity observed in early mouse development. Indeed, it is now clear that the plasticity within the system revealed by experiment is used regulatively in the intact embryo. Thus, when the number of differentiative divisions amongst the 8-cell blastomeres within a single embryo is low, so that the allocation of apolar cells to the inside of the 16-cell embryo is correspondingly low, then at the next division the frequency of differentiative divisions by polar 16-cell blastomeres is relatively high, yielding a compensatory second allocation of cells to the ICM. The converse also applies (Fig. 10; Fleming, 1986). Moreover, in the intact 16-cell morula, the differential adhesive properties of polar and apolar cells are sufficient to maintain their relative outer and inner positions so that apolar cells do not in fact express their

	Percentage polar cell divisions	
	Conservative	Differentiative
	52	48
	86	14
	100	0

Fig. 8. Summary of results from observations on the distribution of the orientation of division planes relative to the axis of polarity in polar 16-cell blastomeres under various conditions. Top, polar cell cultured alone; middle, two polar cells cultured together; lower, polar cell cultured with apolar cell. Note that the orientation appears to be influenced by cell interaction (data from Johnson & Ziomek, 1983).

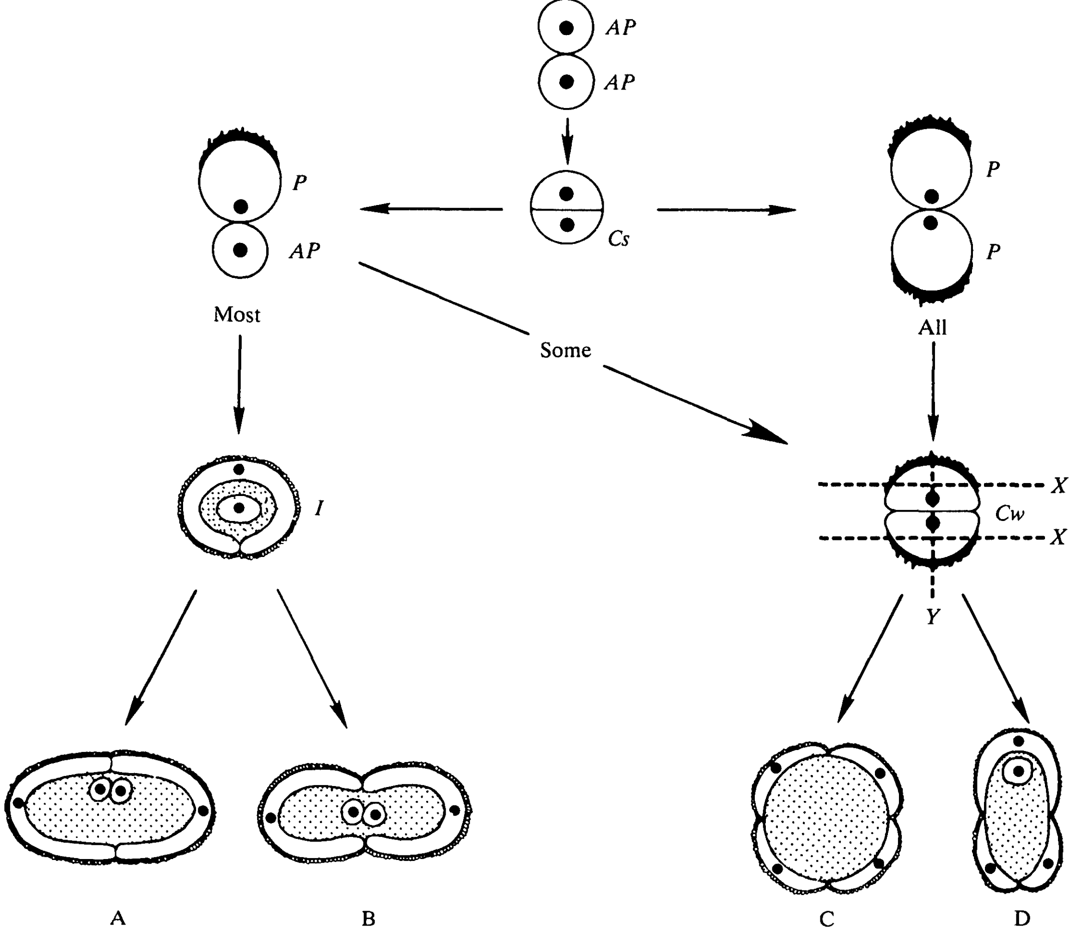


Fig. 9. Summary of possible fates of polar (*P*) and apolar (*AP*) 16-cell blastomeres under different conditions, to illustrate the mechanisms underlying cell plasticity in the morula. In most polar: apolar pairs, the polar cell envelopes the apolar cell (*I*) and each divides to yield progeny that resemble their parent cell. When both cells in a pair are polar, cells flatten on each other (*Cw*) and interact, increasing the incidence of differentiative divisions of one of them, thereby yielding an apolar 1/32 cell. If both cells are apolar, rapid and complete intercellular flattening occurs (*Cs*), inducing polarization in one or both and thence polar cell behaviour. Thus, a polar cell *must* generate at least one polar descendant, whereas an apolar cell *can* generate descendants that are all apolar, all polar or a mixture of polar and apolar (from Johnson & Ziomek, 1983).

potential to polarize and therefore they contribute their progeny exclusively to the ICM (Fleming, 1986).

Taken together, the studies summarized here have (i) established the lineage relationships of the ICM and trophectoderm, (ii) quantified the extent to which, and defined the conditions under which, plasticity is expressed within this pattern of lineage relationships, and (iii) defined the cell dynamics underlying both the determinative and plastic aspects of development. We now ask whether the

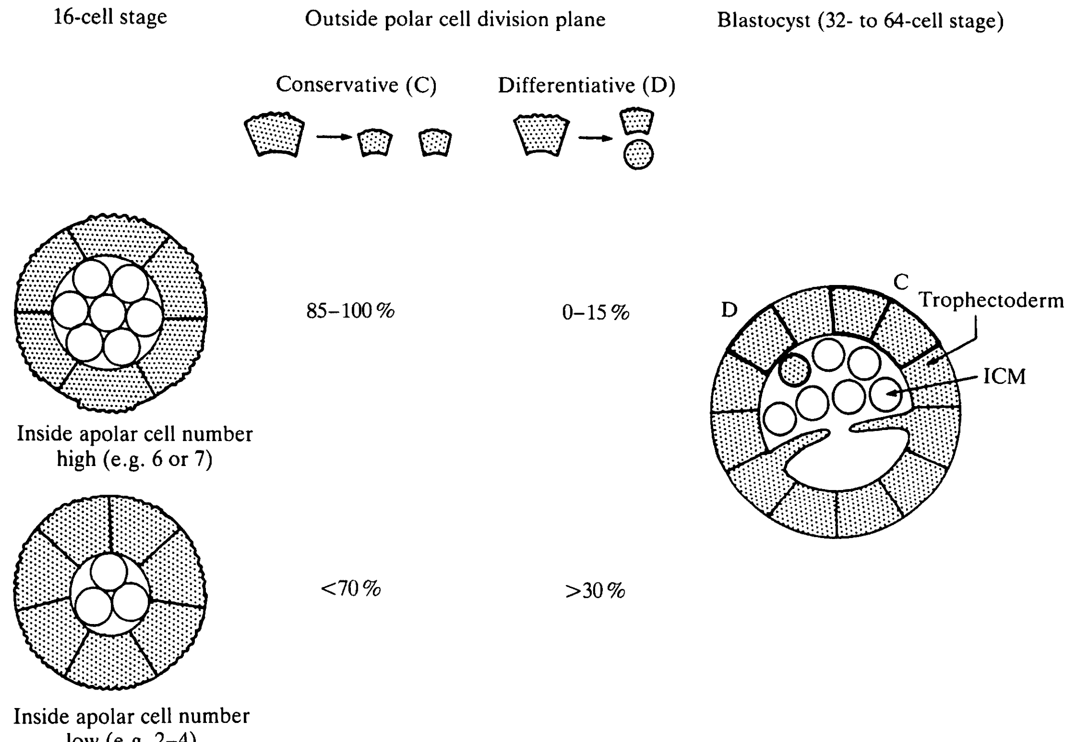


Fig. 10. Schematic representation of cell lineage relationships that occur *in situ* between the 16-cell and early blastocyst stages. All the outside cells at the 16-cell stage were labelled with a fluorescent latex lineage marker (shaded cells; Fleming & George, 1986). In 16-cell embryos with a relatively high number of inside apolar cells, all or most of the polar cells divide conservatively to yield only or mainly trophectodermal descendants (upper embryo). However, when the inside cell number is low (lower embryo), a greater proportion of polar cells divide differentiatively thereby regulating the size of the ICM. Inside apolar cells at the 16-cell stage contribute cells exclusively to the ICM regardless of the relative size of polar and apolar cell populations at the 16-cell stage (Fleming, 1986).

presence or absence of the apical cytocortical domain leads not just to cells with different properties but also to cells with different and stabilized patterns of genetic activity.

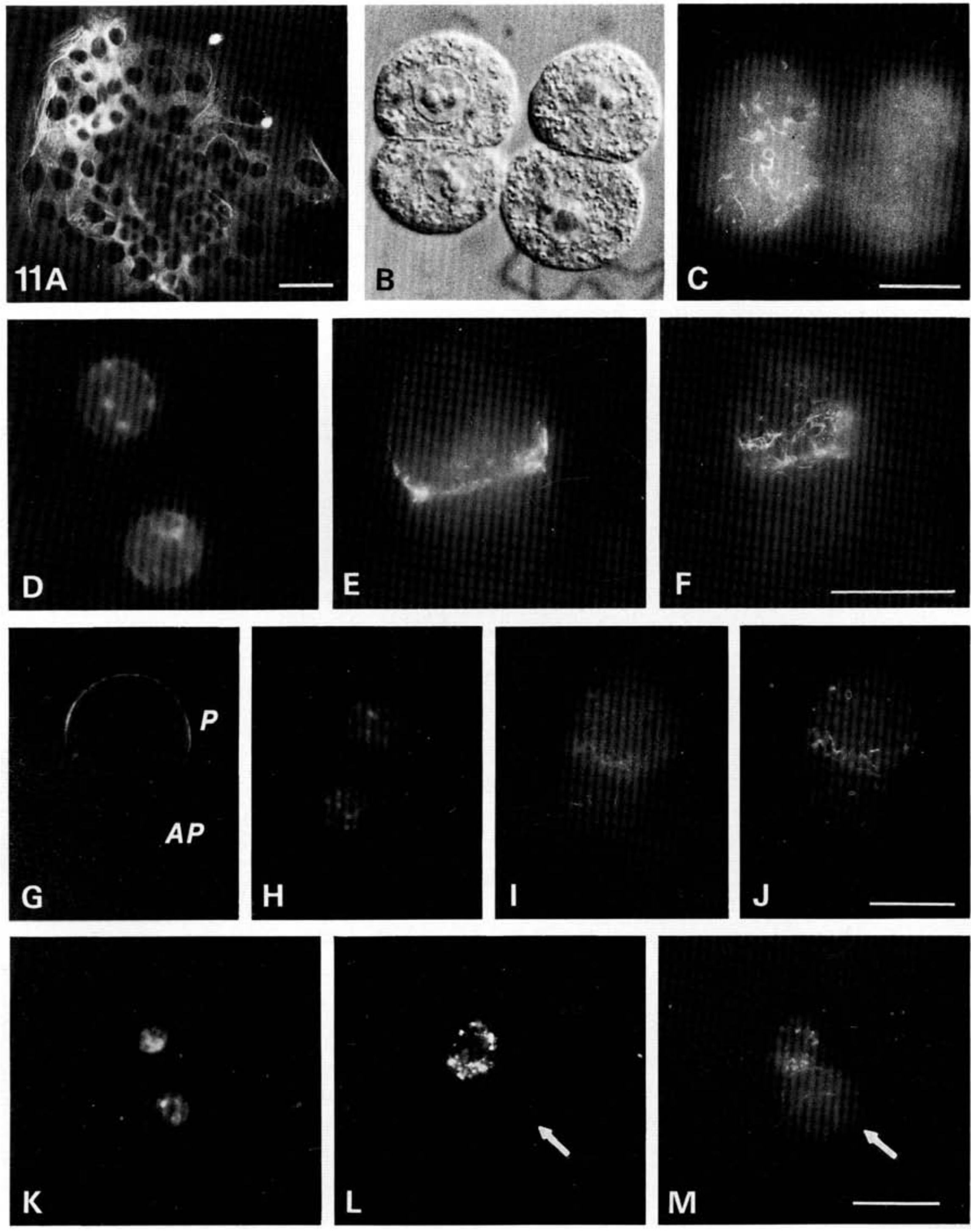
DO THE CELLS IN THE TWO CELL LINEAGES FORMED AT THE 16-CELL STAGE EXPRESS DIFFERENT GENES?

It has been known for a number of years that the cells of the ICM and the trophectoderm synthesize different proteins (Van Blerkom, Barton & Johnson, 1976; Dewey, Filler & Mintz, 1978; Howe & Solter, 1979). These differences in biosynthetic pattern appear to be associated with differences in the prevalence of coding transcripts. Thus, when ICM cells from newly expanding blastocysts are removed from within their enclosed trophectodermal environment and are exposed to *in vitro* culture either in isolation or after aggregation in groups, they

undergo a transformation to yield miniature blastocysts that include trophectoderm cells (Johnson, Handyside & Braude, 1977; Handyside, 1978; Hogan & Tilley, 1978; Spindle, 1978; Surani, Torchiana & Barton, 1978). The earliest events in this transformation involve flattening and polarization of the most superficial cells of the exposed ICM (Fleming *et al.* 1984). Over the ensuing 18 to 24 h period in culture, the polarized cells yield a definitive trophectodermal vesicle that may contain an ICM-like cluster. At the time of their isolation, the ICMs do not synthesize the marker proteins characteristic of trophectoderm. However, the synthesis of these proteins increases with time in culture, corresponding to the phenotypic transformation (Johnson, 1979). If transcription in the newly isolated ICM cells is blocked by addition of α-amanitin, both the morphological transformation to trophectoderm and the synthesis of the mature pattern of trophectodermal proteins are blocked. Some early trophectodermal proteins are, however, synthesized (Johnson, 1979). These results suggest that (i) at least some of the proteins characteristic of trophectoderm are synthesized on templates that are unavailable in ICM cells, and (ii) blocking the synthesis of mRNA transcripts is associated with a failure of the ICM cells to transform to a trophectodermal phenotype. Thus, the segregation of the two cell lineages may indeed have become associated with a difference in prevalence of transcripts. Such a difference in transcript availability could arise *via* differential inheritance, differential synthesis or a combination of both.

Among the various trophectodermal marker proteins, the most prominent of those that are sensitive to the action of α-amanitin have been identified as cytokeratins (Brulet, Babinet, Kemler & Jacob, 1980). Correspondingly, assembled cytokeratin intermediate filaments have been described in trophectodermal cells but are reportedly absent in ICM cells of the early blastocyst (Brulet *et al.* 1980; Jackson *et al.* 1980; Lehtonen *et al.* 1983). Both synthesis and presence of the cytokeratins can be detected at earlier morula stages, although the distribution of cell types responsible for their synthesis has not been ascertained (Handyside & Johnson, 1978; Lehtonen *et al.* 1983; Oshima *et al.* 1983). The cytokeratins therefore seem to offer a potentially useful molecular marker for studying differences in genetic expression.

Interestingly, careful analysis of the distribution of assembled cytokeratin filaments during cleavage has revealed their presence in a few blastomeres as early as the 8-cell stage (Fig. 11; Chisholm, in preparation). Their presence at this stage is preceded by the detection of the 55×10^3 M_r cytokeratin protein in immunoblots at the 4-cell stage (Houliston & Maro, in preparation). In the early 16-cell embryo almost as many inside apolar cells have assembled filaments as have outside polar cells, although in general the filaments are less extensive in the apolar cells. When individual pairs of 16 cells derived by division of a single 8-cell blastomere are analysed, most are found to have either two filament-positive or two filament-negative cells, rather than one negative and one positive cell (Fig. 11). As the embryos progress through the 16- and 32-cell stages, the proportion of outer polar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cells with a filament network rises towards 100 % whilst that of inner apolar cells declines.

These results indicate that the capacity to assemble filaments and to elaborate filament networks is not acquired in all cells of the embryo at the same time. Moreover, different cell phenotypes and/or positions appear not to be correlated with the initiation of differences in filament assembly, but rather with a subsequent modulation up or down of filament protein synthesis and/or assembly as cells diverge further. Differences in genetic expression may derive from, rather than generate, the differences in phenotype and position of cells in the two subpopulations. A somewhat analogous 'product driven' regulation of cytoskeletal protein synthesis and assembly has been reported for other types of cell (Ben-Ze'ev, 1984). Although these analyses on the early mouse embryo are restricted to one identified marker protein, and are far from being complete, they are consistent with the possession of an apical cytocortical domain leading ultimately to the setting up of genetic differences between cell lineages *via* its effect on cell surface properties.

WHAT IS KNOWN ABOUT THE STABLE APICAL CYTOCORTICAL DOMAIN?

The evidence relating to the induction and organization of polarity has been reviewed in detail recently (Johnson & Maro, 1986). Here we will present a summary of the conclusions together with a model based upon them. We need to consider (i) the temporal component of polarization – why does it occur at the 8-cell stage? and (ii) the spatial component of polarization – how is the axis of polarity in each blastomere oriented radially?

Polarization, and the associated events of intercellular flattening and gap and tight junction formation, occur characteristically at the 8-cell stage and together constitute the process of compaction. The proteins required for this major transition are present in the embryo considerably in advance of compaction itself (McLachlin *et al.* 1983; Kidder & McLachlin, 1985; L. Johnson, 1986; Levy, Johnson, Goodall & Maro, 1986). For example, it is possible to elicit cytocortical

Fig. 11. Cytokeratin filaments in mouse blastomeres revealed by immunocytochemistry using the monoclonal antibody TROMA-1 (Brulet *et al.* 1980). (A,C,E,F,I, J,M) TROMA-1 staining pattern, (D,H,K) Hoechst nuclear staining, (G) Rhodamine Concanavalin A labelling pattern, (L) Rhodamine Peanut agglutinin labelling pattern.

(A) Trophoblastic outgrowth; note the extensive networks of cytokeratin filaments in the trophoblast cells.

(B,C) 2/8 pairs 10 h after the division of their isolated parent 4-cell blastomere; one pair has filaments in both cells, the other in neither.

(D–F) 2/16 pair 8 h after division of the isolated parent 8-cell blastomere; filaments are present in both cells and are prominent in the contact region between cells.

(G–I) 2/16 pair 4 h after division; both polar and apolar cells contain filaments, although reduced in the apolar cell.

(K–M) Pair of one outside cell and one inside cell isolated from a disaggregated 32-cell embryo; the outside cells were labelled selectively with fluorescent peanut agglutinin prior to disaggregation; both cells show evidence of cytokeratin filaments (from Chisholm, in preparation).

Bar (A) 50 μm, (F,J,M) 20 μm.

polarization and intercellular flattening prematurely at the early 4-cell stage by inhibiting protein synthesis (Levy *et al.* 1986). The results of this type of experiment tell us two things about the process of polarization. First, it must presumably be organized at a post-translational level as a result of a change in the stability, conformation and/or state of chemical modification of pre-existing proteins. Such a level of response may be important in facilitating the efficient integration of spatial signals in the organization of polarization. Second, since the proteins involved are present in adequate quantities some 12 or more hours in advance of their normal activation, the process of polarization (as well as the other events of compaction) must be prevented from occurring prematurely, and activation will therefore involve release from such a restraint. The temporal component to polarization appears to be permissive.

This permissive temporal component may involve a change in state intrinsic to the cells, since the capacity to polarize seems to develop in the absence of other cells (see below). Moreover, this intrinsic change of state does not appear to depend upon any time-measuring process involving recognition of the number of rounds of cytokinesis or karyokinesis, or to changing nuclear: cytoplasmic ratio, since it is insensitive to proximate manipulations of these cellular functions (Surani, Barton & Burling, 1980; Pratt, Chakraborty & Surani, 1981; Smith & Johnson, 1985; contrast with early *Xenopus* or *Drosophila* development, Newport & Kirschner, 1982; Edgar, Kiehle & Schubiger, 1986). How intrinsic time is measured is not clear.

A basis for the spatial component to polarization is more clearly established. The axis of polarity in a blastomere is evidently determined by the pattern of cell contacts perceived by the cell. As was reported earlier, changing the contact pattern of a cell during the early labile induction period leads to a change in the axis of polarity that develops. Moreover, in the absence of effective cell contact, blastomeres nonetheless polarize but do so more protractedly and with a random axis (Ziomek & Johnson, 1980; Johnson *et al.* 1986). In the intact embryo, the intrinsic and permissive temporal signal must coincide with an extrinsic spatial signal which is instructive and which determines the axis of polarity. In the absence of such a positional signal, the cell appears to generate polarity at random.

What is the nature of the spatial signal? Contact between cells in the early mouse embryo is mediated largely by the Ca^{2+}-sensitive cell adhesion system involving the glycoprotein uvomorulin (also called cadherin, L-CAM, gp120 and cell-CAM 80/120; Hyafil, Morello, Babinet & Jacob, 1980; Damsky, Richa, Solter, Knudsen & Buck, 1983; Gallin, Edelman & Cunningham, 1983; Shirayoshi *et al.* 1984; Vestweber & Kemler, 1984). The neutralization of this system leads to the loss of positional recognition and to delayed and randomly oriented polarization (Shirayoshi, Okada & Takeichi, 1984; Johnson *et al.* 1986). Uvomorulin would therefore appear to be implicated in the induction of polarity, whether or not other cell surface macromolecules are also required. Such a central role for cell adhesion in the earliest events of positional recognition is appropriate, since, as was seen earlier, one important consequence of polarization is the

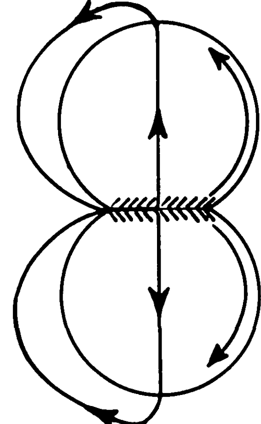

Fig. 12. Scheme to illustrate how a basally located contact signal might generate an apical cytocortical domain. It is proposed that the response to a focal contact changes at the 8-cell stage from a local reaction to a more global reaction that might be propagated around the cytocortex or mediated by an axial gradient through the cell.

establishment of a mosaic pattern of surface adhesivity that can be distributed differentially to the daughter cells at cytokinesis. The difference in adhesivity of the two cell subpopulations so generated then reinforces and stabilizes the lineage differences. Cell adhesion is therefore involved in the establishment, confirmation and regulation of the two cell lineages (Kimber & Surani, 1982).

How is the receipt of an uvomorulin-mediated signal at one end of the cell converted to a stable cytocortical domain at the opposite end of the cell? We have suggested that since this process can be achieved in the absence of any obvious organized cytoplasmic matrix (Johnson & Maro, 1985; Fleming *et al.* 1986*a*), the transduction of information across the cell must rely either on the passage of a signal circumferentially around the cytocortex by, for example, a spreading enzymic activity, or on the setting up within the cell of axial gradients of small molecules such as could be provided by ion currents (Fig. 12; Johnson & Maro, 1985, 1986; Nuccitelli & Wiley, 1985). These mechanisms are not of course mutually exclusive and could be mutually reinforcing. We can gain some clues as to the mechanisms involved by a close examination of the local consequences of intercellular adhesion during earlier cleavage stages.

Uvomorulin is present and active in mediating adhesion on 1-, 2- and 4-cell blastomeres as well as on 8-cell blastomeres (Hyafil *et al.* 1980; Shirayoshi *et al.* 1984; Johnson *et al.* 1986). At these earlier developmental stages, sites of intercellular contact are characterized by a number of features including the reorganization of intramembranous lipid (Pratt, 1985), presence of certain enzymic activities (Lois & Izquierdo, 1984), reduction or loss of microvilli (Calarco & Brown, 1969; Dvorak *et al.* 1985), subcortical depolymerization of myosin, actin and tubulin (Sobel, 1983; Johnson & Maro, 1984; Maro & Pickering, 1984; Houliston *et al.*, in preparation), and absence subcortically of several types of cell organelle (Maro *et al.* 1985). All of these features could arise as a result of

localized subcortical changes in the concentration of relatively nonspecific molecules such as ions or cAMP. These various responses are also observed in the compact 8-cell embryo. However, whereas at earlier stages all the responses are focal and limited to the region of contact, at the 8-cell stage many extend beyond the immediate area of contact which itself becomes more extensive anyway. We have proposed that the remarkable global change of cell organization that occurs at compaction could be achieved quite simply by allowing a hitherto local response to cell contact to become propagated beyond the immediate contact point (Johnson & Maro, 1986). For example, tubulin polymerization is evidently not stabilized in regions of contact compared with elsewhere in the cell. If the failure to stabilize microtubules was extended progressively beyond the immediate point of intercellular contact, perhaps by a propagated change in the organization of the cytocortex, then progressive depolymerization would lead to a rise in the concentration of free monomer. Eventually, the contact-driven depolymerization basally would become balanced by a concentration-driven polymerization apically. With this local apical stabilization of microtubules would come recruitment of MTOCs which could further promote apical polymerization (Maro, Johnson, Webb & Flach, 1986; Houliston *et al.*, in preparation). Cell asymmetry would thereby be achieved. A similar contact-driven propagation of actin depolymerization basally could lead to its polymerization apically, associated with apical cytocortical localization of actin-associated stabilizing and organizing proteins (Fleming *et al.* 1986*a*). In consequence of these cytoskeletal reorganizations, cell organelle and microvillous distribution would also change, the latter promoting intercellular flattening. This model has the advantage that it invokes a relatively small change in the global state of the cell as a trigger for the magnification and propagation of a hitherto local contact-induced response. A major transition in cell organization could be achieved independently of any requirement for protein synthesis or gene expression. Which features of the local response are critical for the successful achievement of the transition?

It is useful to divide the local responses to contact into those that remain restricted to the region of contact at the 8-cell stage and those that extend beyond it. Amongst the former are the activities of surface enzymes and the modified lipid organization, whilst the latter include the redistribution of microvilli and of subcortical organelles and cytoskeletal elements. It is tempting to speculate that a local contact stimulation of, for example, membrane-linked kinase, phosphatase or calcium channel activities constitutes part of a primary response, and that it is the extent to which the secondary consequences of such an activity are allowed to spread beyond the immediate contact zone that determines whether the cell flips to a polarized state. The chemical basis of these earliest contact-dependent responses will hopefully lead us to an understanding of the chemical basis of the stabilized apical polarizing domain that results. Moreover, since organisms as diverse as ascidians, seaweeds and amphibia also seem to generate, stabilize and use defined cytocortical domains to regulate early development (Gerhart *et al.* 1984; Brawley & Robinson, 1985; Jeffery, 1985), the underlying principles may be

universal. It seems to us that we will achieve a more coherent understanding of development by identifying cellular mechanisms, rather than by searching for elusive and possibly illusory determinants. The question posed by this chapter may therefore be false.

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REFERENCES

BALAKIER, H. & PEDERSEN, R. A. (1982). Allocation of cells to inner cell mass and trophectoderm lineages in preimplantation mouse embryos. *Devl Biol.* **90**, 352–362.

BEN-ZE'EV, A. (1984). Cell–cell interaction and cell-shape-related control of intermediate filament protein synthesis. In *Molecular Biology of the Cytoskeleton* (ed. G. G. Borisy, D. W. Cleveland & D. B. Murphy), pp. 435–444. Cold Spring Harbor Laboratory.

BRAWLEY, S. H. & ROBINSON, K. R. (1985). Cytochalasin treatment disrupts the endogenous currents associated with cell polarization in fucoid zygotes: studies of the role of F-actin in embryogenesis. *J. Cell Biol.* **100**, 1173–1184.

BRULET PH., BABINET, CH., KEMLER, R. & JACOB, F. (1980). Monoclonal antibodies against trophectodermal-specific markers during mouse blastocyst formation. *Proc. natn. Acad. Sci. U.S.A.* **77**, 4113–4117.

BURGOYNE, P. S. & DUCIBELLA, T. (1977). Changes in the properties of the developing trophoblast of preimplantation mouse embryos as revealed by aggregation studies. *J. Embryol. exp. Morph.* **40**, 143–157.

CALARCO, P. G. & BROWN, E. H. (1969). An ultrastructural and cytological study of preimplantation development of the mouse. *J. exp. Zool.* **171**, 253–284.

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

DAMSKY, C. H., RICHA, C., SOLTER, D., KNUDSEN, K. & BUCK, C. A. (1983). Identification and purification of a cell surface glycoprotein mediating intercellular adhesion in embryonic and adult tissue. *Cell* **34**, 455–456.

DEWEY, M. J., FILLER, R. & MINTZ, B. (1978). Protein patterns of developmentally totipotent mouse teratocarcinoma cells and normal mouse embryos. *Devl Biol.* **65**, 171–182.

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 organisation during early embryogenesis in the preimplantation mouse embryo. *J. Cell Biol.* **74**, 153–167.

DVORAK, M., CECH, S., STASTNA, J., TESARIK, J. & TRAVNIK, P. (1985). *The Differentiation of Preimplantation Mouse Embryos.* Brno: J. E. Purkyne University Press.

EDGAR, B. A., KIEHLE, C. P. & SCHUBIGER, G. (1986). Cell cycle control by the nucleo-cytoplasmic ratio in early *Drosophila* development. *Cell* **44**, 365–372.

EDIRISINGHE, W. R., WALES, R. G. & PIKE, I. L. (1984). Studies of the distribution of glycogen between the inner cell mass and trophoblast cells of mouse embryos. *J. Reprod. Fert.* **71**, 533–538.

FLEMING, T. P. (1986). Regulation of cell allocation to trophectoderm and inner cell mass in the mouse blastocyst. *Devl Biol.* (submitted).

FLEMING, T. P., CANNON, P. & PICKERING, S. J. (1986*a*). The cytoskeleton, endocytosis and cell polarity in the mouse preimplantation embryo. *Devl Biol.* **113**, 406–419.

FLEMING, T. P. & GEORGE, M. (1986). A new non-invasive lineage marker. *Wilhelm Roux' Arch. devl Biol.* (in press).

FLEMING, T. P. & GOODALL, H. (1986). Endocytic traffic in trophectoderm and polarized blastomeres of the mouse preimplantation embryo. *Anat. Rec.* (in press).

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., PICKERING, S. J., QASIM, F. & MARO, B. (1986*b*). The generation of cell surface polarity in mouse 8-cell blastomeres: the role of cortical microfilaments analysed using cytochalasin D. *J. Embryol. exp. Morph.* **95**, 169–191.

FLEMING, T. P., WARREN, P., 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.

GALLIN, W. J., EDELMAN, G. M. & CUNNINGHAM, B. A. (1983). Characterization of L-CAM, a major cell adhesion molecule from embryonic liver cells. *Proc. natn. Acad. Sci. U.S.A.* **80**, 1038–1042.

GARDNER, R. L. & PAPAIOANNOU, V. E. (1975). Differentiation in the trophectoderm and inner cell mass. In *The Early Development of Mammals* (ed. M. Balls & A. E. Wild), pp. 107–132. Cambridge University Press.

GARDNER, R. L., PAPAIOANNOU, V. E. & BARTON, S. C. (1973). Origin of the ectoplacental cone and secondary giant cells in mouse blastocysts reconstituted from isolated trophoblast and inner cell mass. *J. Embryol. exp. Morph.* **30**, 561–572.

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

GRAHAM, C. F. & DEUSSEN, Z. A. (1978). Features of cell lineage in preimplantation mouse development. *J. Embryol. exp. Morph.* **48**, 53–72.

HANDYSIDE, A. H. (1978). Time of commitment of inside cells isolated from preimplantation mouse embryos. *J. Embryol. exp. Morph.* **45**, 37–53.

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**, 99–116.

HANDYSIDE, A. H. (1981). Immunofluorescence techniques for determining the numbers of inner and outer blastomeres in mouse morulae. *J. Reprod. Immunol.* **2**, 339–350.

HANDYSIDE, A. H. & JOHNSON, M. H. (1978). Temporal and spatial patterns of the synthesis of tissue-specific polypeptides in the preimplantation mouse embryo. *J. Embryol. exp. Morph.* **44**, 191–199.

HILLMAN, B., SHERMAN, M. I. & GRAHAM, C. F. (1972). The effect of spatial rearrangement on cell determination during mouse development. *J. Embryol. exp. Morph.* **28**, 263–278.

HOGAN, B. & TILLY, R. (1978). *In vitro* development of inner cell masses isolated immunosurgically from mouse blastocysts. II. Inner cell masses from 3·5 to 4·0 days p.c. blastocysts. *J. Embryol. exp. Morph.* **45**, 107–121.

HOWE, C. C. & SOLTER, D. (1979). Cytoplasmic and nuclear protein synthesis in preimplantation mouse embryos. *J. Embryol. exp. Morph.* **52**, 209–225.

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

IZQUIERDO, L. & EBENSPERGER, C. (1980). Cell membrane regions in early mouse embryos as demonstrated by 5′-nucleotidase activity. *J. Embryol. exp. Morph.* **69**, 115–126.

IZQUIERDO, L. & MARTICORENA, P. (1975). Alkaline phosphatase in preimplantation mouse embryos. *Expl Cell Res.* **92**, 399–402.

Izquierdo, L., Lopez, T. & Marticorena, P. (1980). Cell membrane regions in preimplantation mouse embryos. *J. Embryol. exp. Morph.* **59**, 89–102.

Jackson, B. W., Grund, C., Schmid, E., Burki, K., Franke, W. W. & Illmensee, K. (1980). Formation of cytoskeletal elements during mouse embryogenesis. Intermediate filaments of the cytokeratin type and desmosomes in preimplantation embryos. *Differentiation* **17**, 161–179.

Jeffery, W. R. (1985). The spatial distribution of maternal mRNA is determined by a cortical cytoskeletal domain in *Chaetopterus* eggs. *Devl Biol.* **110**, 217–229.

Jeffery, W. R. & Raff, R. A. (1983). *Time Space and Pattern in Embryonic Development.* New York: Alan R. Liss, Inc.

Johnson, L. V. (1986). Wheat germ agglutinin induces compaction- and cavitation-like events in two-cell mouse embryos. *Devl Biol.* **113**, 1–9.

Johnson, M. H. (1979). Molecular differentiations of inside cells and inner cell masses isolated from the preimplantation mouse embryo. *J. Embryol. exp. Morph.* **53**, 335–344.

Johnson, M. H. (1986). Manipulation of early mammalian development: what does it tell us about cell lineages? In *Developmental Biology: a Comprehensive Synthesis* (ed. L. Browder). New York: Plenum Press (in press).

Johnson, M. H., Handyside, A. H. & Braude, P. R. (1977). Control mechanisms in early mammalian development. In *Development in Mammals*, vol. 2 (ed. M. H. Johnson), pp. 67–97. Amsterdam: Elsevier/North Holland Biomedical 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. & Maro, B. (1985). A dissection of the mechanisms generating and stabilising polarity in mouse 8- and 16-cell blastomeres: the role of cytoskeletal elements. *J. Embryol. exp. Morph.* **90**, 311–334.

Johnson, M. H. & Maro, B. (1986). Time and space in the mouse early embryo: a cell biological approach to cell diversification. In *Experimental Approaches to Mammalian Embryonic Development* (ed. J. Rossant & R. Pedersen). Cambridge University Press (in press).

Johnson, M. H., Maro, B. & Takeichi, M. (1986). The role of the calcium-dependent cell–cell adhesion system in the generation of cell diversity in the mouse blastocyst. *J. Embryol. exp. Morph.* **93**, 239–255.

Johnson, M. H. & Pratt, H. P. M. (1983). Cytoplasmic localisations and cell interactions in the formation of the mouse blastocyst. In *Time, Space and Pattern in Embryonic Development* (ed. W. J. Jeffery & R. A. Raff), pp. 287–312. New York: Alan R. Liss, Inc.

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 and 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.

Johnson, M. H. & Ziomek, C. A. (1983). Cell interactions influence the fate of blastomeres undergoing the transition from the 16- to the 32-cell stage. *Devl Biol.* **95**, 211–218.

Karasiewicz, J. & Modliński, J. A. (1985). Dislocation of gold particles in the cytoplasm of mouse zygotes during cleavage. *Wilhelm Roux' Arch. devl Biol.* **194**, 495–497.

Kidder, G. M. & McLachlin, J. R. (1985). Timing of transcription and protein synthesis underlying morphogenesis in preimplantation mouse embryos. *Devl Biol.* **112**, 265–275.

Kimber, S. J. & Surani, M. A. H. (1982). Spreading of blastomeres from eight-cell mouse embryos on lectin-coated beads. *J. Cell Sci.* **56**, 191–206.

Kimber, S. J., Surani, M. A. H. & Barton, S. C. (1982). Interactions of blastomeres suggest changes in cell surface adhesiveness during the formation of inner cell mass and trophectoderm in the preimplantation mouse embryo. *J. Embryol. exp. Morph.* **70**, 133–152.

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.

Levy, J. B., Johnson, M. H., Goodall, H. & Maro, B. (1986). Temporal regulation of compaction: a major developmental transition in mouse early development. *J. Embryol. exp. Morph.* **95**, 213–237.

Lois, P. & Izquierdo, L. (1984). Cell membrane regionalisation and cytoplasm polarisation in the rat early embryo. *Wilhelm Roux' Arch. devl Biol.* **193**, 205–210.

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., 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.

Maro, B., Johnson, M. H., Webb, M. & Flach, G. (1986). Mechanism of polar body formation in the mouse oocyte: an interaction between the chromosomes, the cytoskeleton and the plasma membrane. *J. Embryol. exp. Morph.* **92**, 11–32.

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

McLachlin, J. R., Caveney, S. & Kidder, G. M. (1983). Control of gap junction formation in early mouse embryos. *Devl Biol.* **98**, 155–164.

Newport, J. & Kirschner, M. (1982). A major developmental transition in early *Xenopus* embryos. II. Control of the onset of transcription. *Cell* **30**, 687–696.

Nuccitelli, R. & Wiley, L. (1985). Polarity of isolated blastomeres from mouse morulae: detection of transcellular ion currents. *Devl Biol.* **109**, 452–463.

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. (1985). Membrane organisation in the preimplantation mouse embryo. *J. Embryol. exp. Morph.* **90**, 101–121.

Pratt, H. P. M., Chakraborty, J. & Surani, M. A. H. (1981). Molecular and morphological differentiation of the mouse blastocyst after manipulations of compaction with Cytochalasin D. *Cell* **26**, 279–292.

Randle, B. (1982). Cosegregation of monoclonal reactivity and cell behaviour in the mouse preimplantation embryo. *J. Embryol. exp. Morph.* **70**, 261–278.

Reeve, W. J. D. (1981*a*). Cytoplasmic polarity develops at compaction in rat and mouse embryos. *J. Embryol. exp. Morph.* **62**, 351–367.

Reeve, W. J. D. (1981*b*). The distribution of ingested horseradish peroxidase in the 16-cell mouse embryo. *J. Embryol. exp. Morph.* **66**, 191–207.

Reeve, W. J. D. & Kelly, F. (1983). Nuclear position in cells of the mouse early embryo. *J. Embryol. exp. Morph.* **75**, 117–139.

Reeve, W. J. D. & Ziomek, C. A. (1981). Distribution of microvilli on dissociated blastomeres from mouse embryos: evidence for surface polarisation at compaction. *J. Embryol. exp. Morph.* **62**, 339–350.

Sepulveda, M. S., Doggenwieler, C. & Izquierdo, L. (1985). Scanning microscopy of disaggregated preimplantation mouse embryos. *Wilhelm Roux' Arch. devl Biol.* **194**, 445–452.

Shirayoshi, Y., Okada, T. S. & Takeichi, M. (1983). The calcium-dependent cell–cell adhesion system regulates inner cell mass formation and cell surface polarisation in early mouse development. *Cell* **35**, 631–638.

Smith, L. J. (1985). Embryonic axis orientation in the mouse and its correlation with blastocyst relationships to the uterus. II. Relationships from 4·25 to 9·5 days. *J. Embryol. exp. Morph.* **89**, 15–35.

Smith, R. K. W. & Johnson, M. H. (1985). DNA replication and compaction in the cleaving embryo of the mouse. *J. Embryol. exp. Morph.* **89**, 133–148.

Smith, R. & McLaren, A. (1977). Factors affecting the time of formation of the mouse blastocoel. *J. Embryol. exp. Morph.* **41**, 79–92.

Sobel, J. S. (1983). Cell–cell contact modulation of myosin organization in the early mouse embryo. *Devl Biol.* **100**, 207–213.

Sobel, J. S. (1984). Myosin rings and spreading in mouse blastomeres. *J. Cell Biol.* **99**, 1145–1150.

Sobel, J. S. & Alliegro, M. A. (1985). Changes in the distribution of a spectrin-like protein during development of the preimplantation mouse embryo. *J. Cell Biol.* **100**, 333–336.

Spindle, A. I. (1978). Trophoblast regeneration by inner cell masses isolated from cultured mouse embryos. *J. exp. Zool.* **203**, 483–489.

Surani, M. A. H. & Barton, S. C. (1984). Spatial distribution of blastomeres is dependent on cell division order and interactions in mouse morulae. *Devl Biol.* **102**, 335–343.

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.

Surani, M. A. H. & Handyside, A. H. (1983). Reassortment of cells according to position in mouse morulae. *J. exp. Zool.* **225**, 505–511.

Surani, M. A. H., Torchiana, D. & Barton, S. C. (1978). Isolation and development of the inner cell mass after exposure of mouse embryos to calcium ionophore A23187. *J. Embryol. exp. Morph.* **45**, 237–247.

Tarkowski, A. J. & Wroblewska, J. (1967). Development of blastomeres of mouse eggs isolated at the 4- and 8-cell stage. *J. Embryol. exp. Morph.* **18**, 155–180.

Van Blerkom, J., Barton, S. C. & Johnson, M. H. (1976). Molecular differentiation in the preimplantation mouse embryo. *Nature, Lond.* **259**, 319–321.

Vestweber, D. & Kemler, R. (1984). Rabbit antiserum against purified surface glycoprotein decompacts mouse preimplantation embryos and reacts with specific adult tissues. *Expl Cell Res.* **152**, 169–178.

Wiley, L. M. & Eglitis, M. A. (1981). Cell surface and cytoskeletal elements: cavitation in the mouse preimplantation embryo. *Devl Biol.* **86**, 493–501.

Wilson, I. B., Bolton, E. & Cuttler, R. H. (1972). Preimplantation differentiation in the mouse egg as revealed by microinjection of vital markers. (1972). *J. Embryol. exp. Morph.* **27**, 467–479.

Ziomek, C. A. & Johnson, M. H. (1980). Cell surface interactions induce polarization of mouse 8-cell blastomeres at compaction. *Cell* **21**, 935–942.

Ziomek, C. A. & Johnson, M. H. (1981). Properties of polar and apolar cells from the 16-cell mouse morula. *Wilhelm Roux' Arch. devl Biol.* **190**, 287–296.

Ziomek, C. A. & Johnson, M. H. (1982). The roles of phenotype and position in guiding the fate of 16-cell mouse blastomeres. *Devl Biol.* **91**, 440–447.

Ziomek, C. A., Johnson, M. H. & Handyside, A. H. (1982). The developmental potential of mouse 16-cell blastomeres. *J. exp. Zool.* **221**, 345–355.