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The beginning of mammalian development

El comienzo del desarrollo en
los mamíferos

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INTRODUCTION

In a symposium on the Biology of the Oviduct one would expect that any reference to embryos emphasizes the influence of the oviductal milieu on development. As a matter of fact, embryos cultured *in vitro* exhibit reduced cell numbers, diminished viability and, interestingly, their development is arrested at defined stages in diverse species (reviewed by Bavister, 1988). For instance *in vitro* development of early mouse embryos, which are particularly resilient to culture procedures, is blocked at the 2-cell stage but this defect can be overridden by the use of inbred strains or by microinjection of cytoplasm (Biggers, 1971; Muggleton-Harris *et al.*, 1982; Pratt & Muggleton-Harris, 1988). Contrary to expectations, however, this paper does not emphasize the embryo-oviduct interactions; instead, it deals with experiments on mouse embryos *in vitro* which throw some light on the riddle of early development of regulative embryos. (Fig. 1).

EMBRYONIC REGULATION

For classical experimental embryologists the major enigma of early development was embryonic regulation, that is, the process by which normal development is restored after the embryo is subjected to anatomical disturbances. The enigma persists nowadays but the problem of development has been subdivided into more specific questions which are suitable for experimental testing. Morphogenesis and cell differentiation have been uncoupled on the assumption that the former might be an effect of the latter and the process of cell differentiation has been reduced to differential gene expression, which can be analysed in terms of molecular biology. The approach is sound, of course, unless the strategy is mistaken for the problem. Several meanings have been ascribed to morphogenesis and cell differentiation. In this paper, morphogenesis refers to development of spatial heterogeneity in a single cell or group of cells or an embryo, as for instance regionalization, compaction and blastulation. Cell differentiation instead refers to gene expression, involving transcriptional and post-transcriptional processes, as well as to the localization of templates and final products. Since differences are revealed by comparing cells, either with their progenitors or with contemporary cells in diverse positions, cell differentiation may be classified as temporal or spatial.

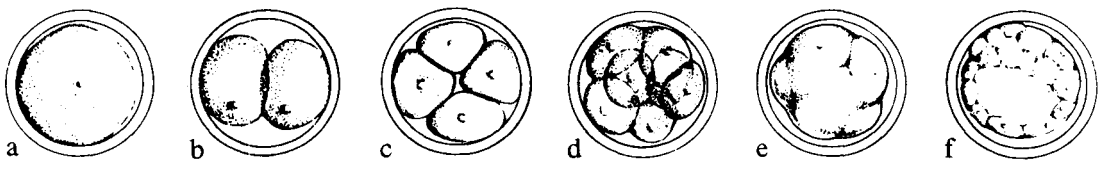

Fig. 1: Preimplantation stages of the mouse. a, 1-cell stage; b, 2-cell stage; c, 4-cell stage; d, 8-cell stage, uncompacted; e, 8-cell stage, compacted; f, early blastocyst.

Embryonic regulation in mammals was not a subject of experimental embryology until reliable methods were available for *in vitro* culture and micromanipulation of early embryos. But as soon as it became possible traditional integrative concepts –such as totipotency, cytoplasmic localizations and embryonic fields were used to explain results on blastomere isolation and embryo aggregations. Dalcq and his coworkers had proposed that the beginning of differentiation in mammals might be caused by a spatial heterogeneity of the fertilized egg which could be detected by certain cytochemical properties (Dalcq, 1957, 1965; Mulnard, 1960). However, the development of normal blastocysts from single blastomeres isolated at the 2-cell stage of the mouse (Tarkowski 1959) and even at the 4- and 8-cell stages (Tarkowski & Wroblewska, 1967) is compatible only with a very labile pre-existing spatial pattern.

Regulation capability of mouse cleaving embryos has also been tested by puncturing one or more blastomeres through the zona pellucida at different stages (Matte *et al.*, 1987) and results reveal that the percentage of blastocysts formed after one half of the embryo is destroyed decreases as development proceeds. (Fig. 2). Earlier experiments with a different method had shown that more living mouse fetuses develop from halved 2-cell embryos than from halved 8-cell embryos (Tsunoda & McLaren, 1983). Embryonic regulation following isolation or destruction of blastomeres prove that cells left unharmed can replace lost parts of the embryo, therefore, subjecting all parts to experimental disturbance is a different test of regulation capability. This has been done by centrifugation of 2-cell mouse embryos, up to the limit of their resistance, which causes a neat stratification of the cytoplasm and elongation of the nuclei (Fig. 3).

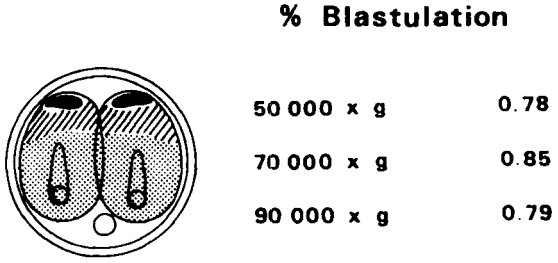

Fig. 3: Cytoplasm stratification of 2-cell embryos submitted to diverse centrifugal forces for 1 h and the development of blastocysts following centrifugation. Blastocyst development is expressed as in Fig. 2. Adapted from Téllez *et al.* (1988).

Results showed that stratification disappears 30 to 40 minutes after centrifugation, except for the lipid droplets at the centripetal pole, and normal-looking blastocysts develop; treatments with cytoskeletal inhibitors delay recovery without preventing it (Téllez *et al.*, 1988). Another procedure that reveals embryonic regulation is the production of chimaeras (reviewed by McLaren, 1976) either by aggregation of embryos (Tarkowski, 1961;

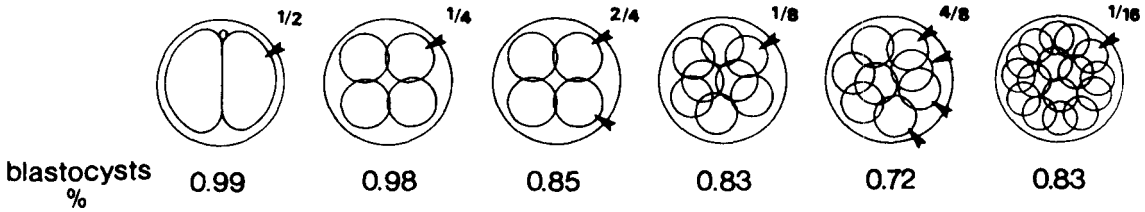

Fig. 2: Effect of puncturing one or more cells in cleavage stages on the development of blastocysts. Superscript indicates the proportion of punctured cells. Blastocyst development is expressed as % of blastocysts developed in operated embryos over % of blastocysts developed in control embryos. Adapted from Matte *et al.* (1987).

Mintz, 1964) or by injection of cells into the blastocoel (Gardner, 1968) (Fig. 4). Since chimaeras also develop from the aggregation of advanced morulae, these experiments suggest that the capability of embryonic regulation is not abated before the blastocyst stage. The aggregation of labelled embryos reveals no cell mingling up to the 8-cell stage (Garner & McLaren, 1974) but the evidence for later stages is less conclusive (Burgoyne & Ducibella, 1977). At these stages cells are becoming molecularly differentiated (Van Blerkom *et al.*, 1976; Handyside & Johnson, 1978; Johnson, 1979) and embryonic regulation might be due to cell sorting and not necessarily to cell reprogramming.

Results on embryonic regulation have been interpreted according to the inside-outside model suggested by diverse authors but most consistently by Tarkowski & Wroblewska (1967). Briefly, it proposes that inner cells differentiate into cell mass and outer cells into trophoblast, because of their location when the blastocoel forms and therefore, if the number of cells at blastulation is reduced a trophoblastic vesicle (devoid of inner cell mass) will form instead of a blastocyst (discussion in Izquierdo, 1977). Many observations support this hypothesis and particularly convincing are experiments with arrangements of either labelled blastomeres or embryos, which show that cells placed outside produce most often trophoblast tissues (Hillman *et al.*, 1972). The model requires a developmental clock that signals blastulation time which does not work on the basis of number of cells nor on number of cell cycles. This has been proven by the development of halved and aggregated embryos (Smith & McLaren, 1977; Fernández & Izquierdo, 1980) by treatments with cytoskeletal inhibitors which arrest cytokinesis (Kimber & Surani, 1981; Izquierdo *et al.*, 1984) and by delaying cleavage with lithium (Izquierdo & Becker, 1982; Becker & Izquierdo, 1982) or accelerating it with progesterone (Roblero & Izquierdo, 1976) (Fig. 5). For a review on time-keeping mechanisms during development see Satoh (1982).

The ability of isolated blastomeres to form a blastocyst does not imply that all of them at a certain stage may originate any

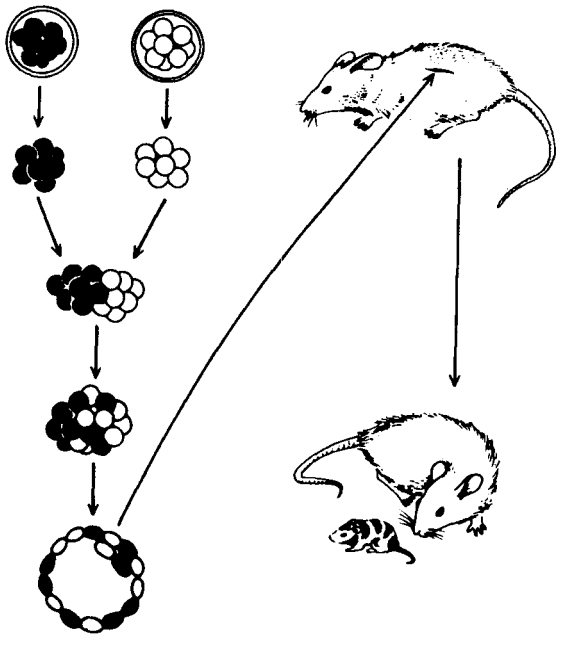

Fig. 4: Construction of aggregation chimaeras.

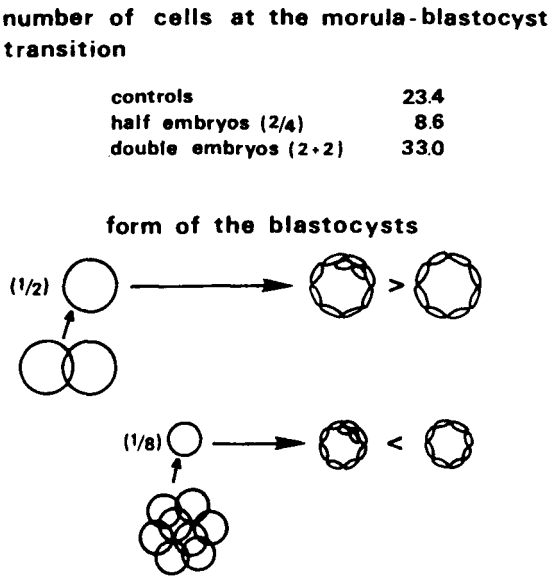

Fig. 5: Above: effect of halving or doubling early embryos on the number of cells of nascent blastocysts. Adapted from Fernández & Izquierdo (1980). *Below:* effect of number of cells at the morula-blastocyst transition on the development of blastocysts or trophoblastic vesicles. Adapted from Fernández & Izquierdo (1980) and from Izquierdo & Becker (1982).

kind of cell. Totipotency has been shown up to the 8-cell mouse embryo by combining labelled blastomeres with carrier cells, grafting these chimaeras in a foster mother and examining advanced embryos for labelled cells in diverse tissues (Kelly, 1975). Maintenance of nuclear totipotency along time is, however, a different matter. The transplantation of nuclei, by microsurgery and fusion (McGrath & Solter, 1983), into enucleated mouse zygotes has shown that nuclei of 2-cell embryos can still support preimplantation development but nuclei of later stages cannot (McGrath & Solter, 1984). The same method applied to the transfer of one or both pronuclei to enucleated eggs has revealed that paternal and maternal genomes are not equivalent and that neither of them, even in diploid condition, can support development (Surani *et al.*, 1984; McGrath & Solter, 1984) thus explaining why mammalian parthenogenones do not develop beyond midgestation (Kaufman *et al.*, 1977).

CELL REGIONALIZATION

Initial mammalian development reveals the regionalization of cytoskeletal and membrane components. Immunofluorescence has shown that cortical myosin concentrates at regions away from cell contact in the 2-cell mouse embryo (Sobel, 1983) whereas spectrin and fodrin are detected at regions of cell apposition (Sobel & Alliegro, 1985; Reima & Lehtonnen, 1985; Schatten *et al.*, 1986); other authors have detected the appearance of spectrin in diverse localizations (Damjanov *et al.*, 1986). During the 2-cell stage, cortical microfilaments and microtubules concentrate opposite to cell contacts (Lehtonen & Badley, 1980; Houliston *et al.*, 1987). Since the cytoskeleton is functionally involved in cell division, different descriptions of it may be related to the cell cycle and not necessarily to morphogenesis; a caution that should also be applied when considering cell membrane components. Actually, some of these components, detected by a variety of antisera, lectins and lipid analogues, localize in the early 2-cell embryo at the pole opposite to the cleavage furrow but their polarization diminishes thereafter during the cell cycle (Handyside *et al.*, 1987). However, cell membrane polarization related to cleavage may be durable and contribute to the regionalization of the embryo; this is likely the case of newly assembled cell membrane that displaces old membrane away from the cleavage furrow causing its localization at the periphery of the morula (Izquierdo, 1977). For a recent review on early embryo topography see Pratt (1989).

We have studied the regionalization of the plasma membrane by means of the cytochemical demonstration of alkaline phosphatase (ALP) or of 5'-nucleotidase (5NUC) activity in early embryos of several mammals. The reaction products are present, from the late 4-cell stage onwards, at the cell surface on areas of cell contact and absent from free surfaces (Izquierdo & Marticorena, 1975; Izquierdo, 1977; Ishiyama & Izquierdo, 1977; Izquierdo *et al.*, 1980; Izquierdo & Ebensperger, 1982; Lois & Izquierdo, 1984). Results suggested that the active enzymes localize on patches of new cell membrane inserted at cleavage furrows; however, 2-cell embryos whose cleavage is arrested by inhibitors of microfilaments or microtubules exhibit a timely appearance of enzyme activity at the cell contact (Izquierdo *et al.*, 1984). We have recently observed that ALP and 5NUC activity also appears on regions of artificial cell contact between embryos that have been aggregated for 2 h, even if they still are at the 2-cell stage. This result was unexpected since these cytochemical tests in single 2-cell mouse embryos do not reveal ALP nor 5 NUC activity and further, because the natural contact between blastomeres of the same embryo showed enzyme activity earlier than the artificial contact between the aggregated embryos (Sepúlveda & Izquierdo, 1990) (Fig. 6). We interpret this as activation rather than synthesis of the enzymes because the appearance of their activity is not prevented by cycloheximide (Sepúlveda & Izquierdo, 1990) and also because ALP has been demonstrated by immunocytochemistry in

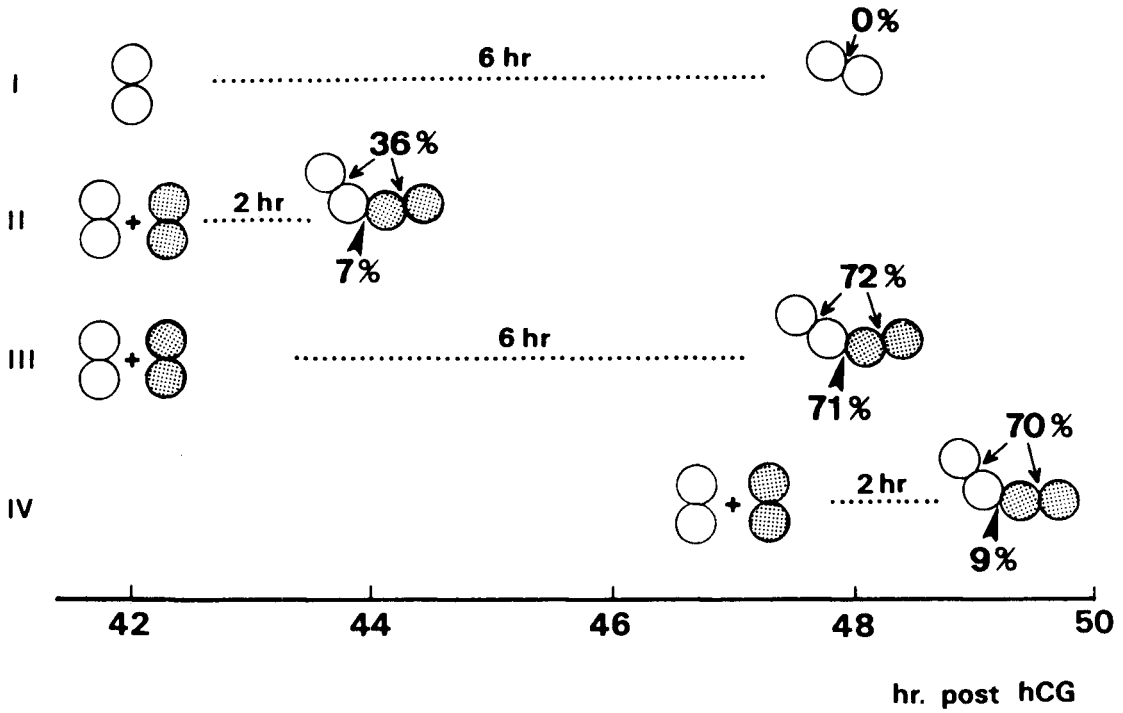

Fig. 6: Effect of aggregating two 2-cell embryos on the regionalization of the cell membrane as recognized by the cytochemical demonstration of ALP or 5NUC activity. I, the natural contact of single 2-cell embryos does not regionalize; II, following aggregation for 2 h, the percentage of regionalized contacts is higher for natural than for artificial contacts; III, following aggregation for 6 h, the percentage of natural and artificial regionalized contacts become similar; IV, aggregates for 2 h of older embryos show that different percentages of regionalization, for natural or artificial contacts, depend on time of aggregation and not on time of development. Adapted from Sepúlveda & Izquiedo (1990).

mouse oocytes and early cleavage stages (Ziomek *et al.*, 1986; Cachicas *et al.*, 1988). Biochemical tests reveal no activity or negligible activity of ALP at the 2-cell stage (Izquierdo & Marticorena, 1975; Kim *et al.*, 1989; Ishikawa, 1990).

New observations from our laboratory (unpublished) suggest that the artificial contact receptor comprises carbohydrates since ALP and 5NUC activity is induced by lectins, either in solution or bound to agarose microspheres. Searching for the signal transmission from the artificial to the natural contact we found that diacylglycerol agonists also induce the enzyme activity at the natural contact and that this effect is suppressed by inhibitors of protein kinase C; therefore, second messengers of the phosphoinositides system are most likely involved (Fig. 7). Work in progress aims at several questions, notably these: do enzyme molecules relocalize on the cell surface as an effect of cell contact? why is it that their activation occurs at cell contacts? how do the second messengers induce activation? During the 8-cell stage, the process of regionalization (or polariza-

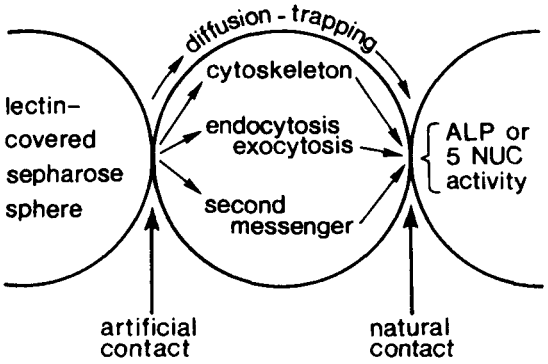

Fig. 7: Aggregation of a microsphere coated with lectin to a 2-cell embryo, representing several possible pathways leading from the artificial to the natural contact. Based on unpublished observations.

tion) is remarkable and has lasting effects on the establishment of embryonic axes. Cells at this stage reveal a clear-cut polarization that comprises the cytoskeleton, diverse organelles and the cell membrane, whose regionalization apparently maintains the asymmetry through cell division (Johnson *et al.*, 1986; Johnson & Maro, 1985; Maro *et al.*, 1985). In rat embryos, the 8-cell stage shows convincingly that cell surface regionalization, detected by ALP or 5NUC activity, precedes the radial polarization of cytoplasmic organelles (Lois & Izquierdo, 1984) (Fig. 8). The role of cell contact on regionalization should be assessed in early embryos so as to define, in the terms of classic experimental embryology, when the capacity of embryonic regulation subsides and determination commences.

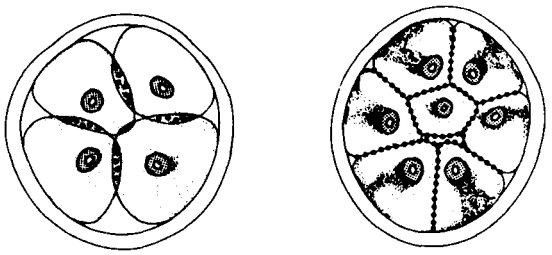

Fig. 8: Diagram of four- and 8-cell rat embryos showing the ALP or 5NUC reaction products on cell contacts. In the 8-cell stage, a column of organelles extends from the nuclei to the free surface of the embryo. Adapted from Lois & Izquierdo (1984) and from earlier reports.

COMPACTION OF THE EMBRYO

An early cleaving embryo has a lobulated contour, due to spherical blastomeres which are only slightly flattened, until compaction at the 8-cell stage. This morphogenetic process has been recognized in many mammalian species since the cinematographic observations by Mulnard (1967) and the formal description by Ducibella & Anderson (1975). Under the light microscope it is characterized by increased cell to cell apposition, effacement of cellular outlines and flattening of blastomeres, while the embryo itself acquires a spherical form (Ducibella, 1977) (Fig. 9). This transformation suggests a process of supracellular integration in which the cytoskeleton plays a leading role (Izquierdo, 1986). Actin microfilaments are involved, as shown by the decompacting effect of cytochalasins, while the involvement of microtubules is less obvious since the effect of inhibitors somehow depends on the cell cycle (Wiley & Eglitis, 1980; Kimber & Surani, 1981; Pratt *et al.*, 1982; Ducibella, 1982; Sutherland & Calarco-Gillam, 1983; Izquierdo *et al.*, 1984; Maro & Pickering, 1984). At the 16-cell stage two distinct cell populations are readily recognized: small inner cells with sparse and uniformely distributed microvilli and larger outer cells with abundant microvilli at their apical pole (Johnson & Ziomek, 1982). These populations, however, are still undetermined and under experimental conditions inner cells may differentiate into trophoblast while outer cells may contribute to the inner cell mass (Rossant & Lis, 1979; Rossant & Vijh, 1980).

We studied by scanning electron microscopy blastomeres disaggregated from 8- and 16-cell morulae which were fixed immediately and found that free surfaces were microvillous while contact surfaces

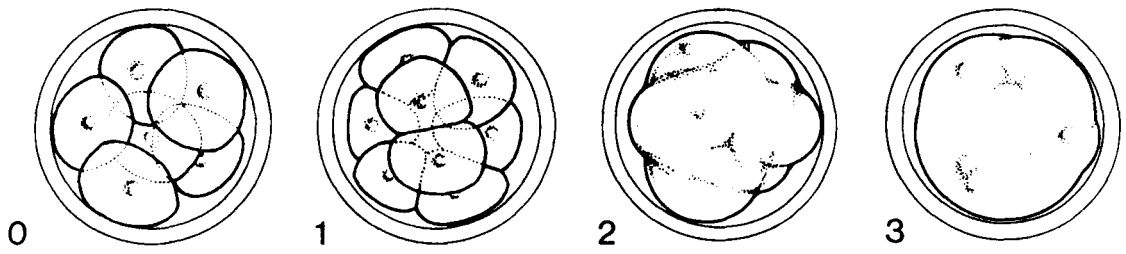

Fig. 9: Substages of compaction in 8-cell mouse embryos. 0, prior to compaction; 1 and 2, intermediate stages; 3, compacted morula. Substages 1 and 2 last for about 1 h each. Based on unpublished observations.

became smooth during compaction (Sepúlveda *et al.*, 1985), thus confirming an earlier report (Reeve & Ziomek, 1981). When blastomeres were fixed at different times after isolation we found that microvilli reappear on the smooth surface completely or partially, depending on whether blastomeres were disaggregated from 8- or 16-cell morulae (Sepúlveda *et al.*, 1985). These results are in conflict with observations based on the staining pattern detected by fluorescent ligands, which showed that blastomeres isolated from 8-cell morulae retain their polarity (Ziomek & Johnson, 1980, 1981). The conjecture that smoothing of adjoining surfaces during compaction is due to cell contact is supported by results of experiments in which embryos were aggregated for 1-3 h and then forced apart: microvilli disappeared completely on the surface which had been in contact when 8-cell embryos were aggregated with 2- to 8-cell embryos, though considerably less when aggregated with later stages (Sepúlveda *et al.*, 1985) (Fig. 10). The asymmetry induced by cell contact on the distribution of microvilli at the 8-cell stage is most likely related to the embryonic regulation of isolated blastomeres or aggregated embryos and to the inside-outside differentiation of undisturbed embryos.

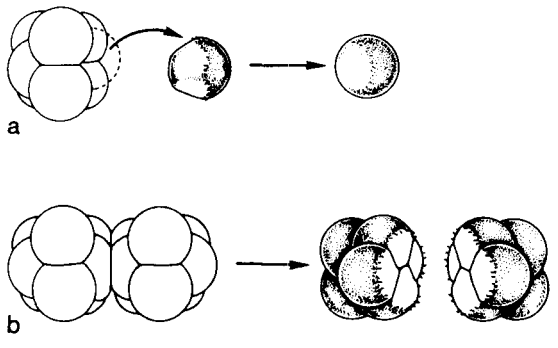

Fig. 10: Drawing of disaggregation of blastomeres and aggregation of embryos in the 8-cell stage as observed by scanning electron microscopy. In a, one dislodged blastomere shows smooth and flat contact surfaces which become spherical and microvillous after a few hours. In b, two embryos aggregated for 2 h and then forced apart show the flattening and smoothing of the surfaces that were in contact; note the long microvilli around the smooth surfaces. Adapted from Sepúlveda *et al.* (1985).

A ring of long microvilli develops during compaction around smooth fields on the cell surface (Reeve & Ziomek, 1981; Sepúlveda *et al.*, 1985) and at the same time, long microvilli appear on embryos which have been cleavage-arrested by means of cytoskeletal inhibitors (Sutherland & Calarco-Gillam, 1983; Izquierdo *et al.*, 1984). We have recently studied, by scanning microscopy, compacting morulae that were detergent-extracted and found that these long microvilli correspond to microfilament-packed processes which extend between the cortical cytoskeleton of adjoining blastomeres (Mayor *et al.*, 1989) (Fig. 11). Cytoskeletal connections in such a position might be physically involved in compaction and therefore we have studied when they form and how their formation might be affected. Connections appear 4 h after the beginning of compaction in synchronized cultures and at 6 h they are already established between all cells in all morulae, which at this stage have 8-12 blastomeres. Since connections develop after the beginning of compaction, treatments that interfere with compaction may suppress their establishment and therefore, we tested the effect of low Ca^{2+} and Mg^{2+}, of EGTA, cytochalasin D, colchicine and α-lactalbumin, which inhibits galactosyltransferase (Bayna *et al.*, 1988). Only cytochalasin D consistently prevents their formation and when already formed partially disrupts them (Mayor *et al.*, 1989).

The prevalent interpretation of compaction states that it is mainly an effect of the Ca-dependent cell surface adhesion molecule (called uvomorulin) which regionalizes at the 8-cell stage, thus explaining why absence of calcium as well as presence of antibodies raised against uvomorulin inhibit compaction (Ducibella & Anderson, 1979; Hyafil *et al.*, 1980; Hyafil *et al.*, 1981). This interpretation does not include explicitly the cytoskeleton as a major responsible of compaction, however, cytochalasins are potent inhibitors of compaction and uvomorulin has a transmembrane peptide that probably links with the cytoskeleton (Kemler & Ozawa, 1989).

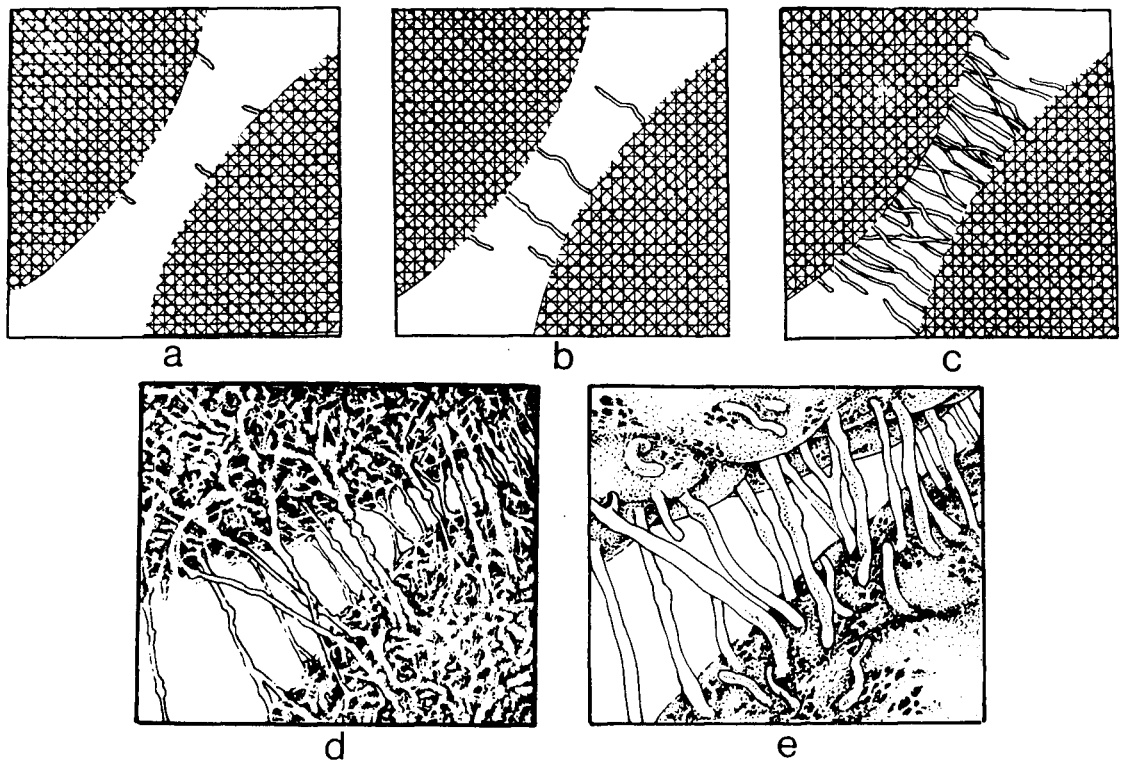

Fig. 11: Artist's drawing of development of cytoskeletal connections between blastomeres in 8-cell embryos: at the beginning of compaction (a), 2 h later (b), 6 h after the beginning (c). Cytoskeletal connections observed by scanning microscopy in detergent-extracted material (d) correspond to long microvilli in non-extracted material (e). Adapted from Mayor *et al.* (1989).

In view of this data and attributing a role in compaction to the cytoskeletal connections described above, we have been studying recompaction kinetics before and after connections appear. Decompaction with cytochalasin followed by transfer to fresh medium reveals a slow recompaction kinetic which is similar for morulae that had been compacted for less than 4 h or more than 6 h. A brief decompacting treatment with EGTA, instead, reveals in the first case a lag phase and a slow recompaction whereas in the second case recompaction starts immediately and proceeds swiftly. Most interestingly, this difference in recompaction kinetics is also observed when calcium channels are blocked by diverse drugs and therefore we think that this ion affects compaction extracellularly by its interaction with uvomorulin but also intracellularly, by inducing a contraction of the cytoskeleton that tenses the connections (unpublished observation) (Fig. 12).

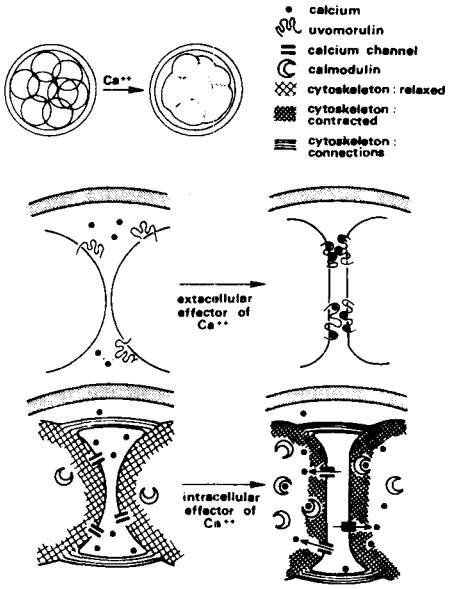

Fig. 12: A model representing extracellular and intracellular roles for calcium in compaction. *Above:* Ca++ interacts extracellularly with the Ca-dependent molecule uvomorulin causing increased cell adhesion. *Below:* Ca++ enters the cell and interacts with calmodulin causing contraction of the cytoskeleton and cytoskeletal connections. Based on unpublished observations.

Fast cell to cell spreading of the compaction process may be due to gap junctions which, become functional precisely at this stage (Lo & Gilula, 1979), by allowing the diffusion of low molecular weight signals. Functional gap junctions have not been demonstrated earlier but this should not be an impediment since blastomeres remain coupled through cell bridges for a long time during the protracted cell cycle of early cleavage stages (Lo & Gilula, 1979).

MORPHOGENESIS AND GENE EXPRESSION

Solving the riddle of embryonic regulation at the beginning of development would demand understanding the transition from maternal to zygotic control of gene expression and certainly, investigating how gene expression is modulated by age and by the position of cells within the developing cellular edifice. In analysing gene expression the usual tool is gel electrophoresis which reveals a sort of disembodied portrait of the phenotype where molecules are defined by weight or isoelectric point while their function is seldom identified (Fig. 13).

The electrophoretic pattern of protein synthesis changes continuously during early mammalian development according to stage (Levinson *et al.*, 1978); for instance, actin increases abruptly from the 8-cell stage

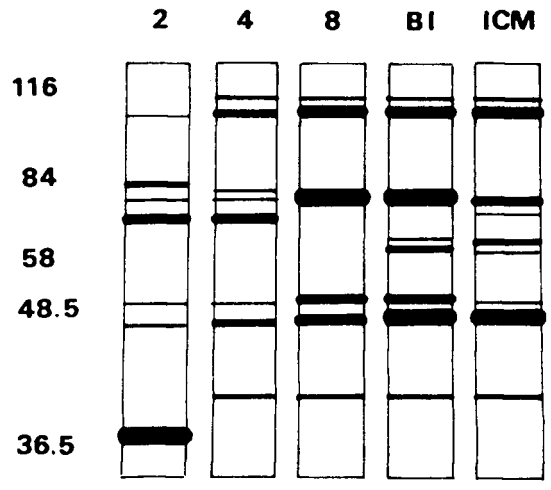

Fig. 13: SDS-PAGE, simplified patterns of protein synthesis in different stages: 2-, 4- and 8-cell embryos, early blastocyst and inner cell mass. First column, molecular weights. Based on unpublished observations.

onwards (Abreu & Brinster, 1978), ribosomal proteins increase 11 fold from 1-to 8-cell stage (Lamarca & Wassarman, 1979) and heat shock proteins appear early at the 2-cell stage (Bensaude *et al.*, 1983); however, at least 50 polypeptides whose synthesis change during preimplantation have not been identified (Howlett *et al.*, 1988).

RNA synthesis is scarce at the 1-cell stage of the mouse (Piko & Clegg, 1982) and neither physical nor chemical enucleation affect molecular changes earlier than the 2-cell stage; therefore, early molecular changes depend on maternal templates and regulation is post-transcriptional (Braude *et al.*, 1979; Van Blerkom, 1981; Schultz *et al.*, 1981; Flach *et al.*, 1982; Howlett & Bolton, 1985; Braude *et al.*, 1988). These results imply that expression of the embryonic genome begins at the same stage; in fact, at the mid 2-cell stage in the mouse, 4- to 8-cell stage in humans and 8-cell stage in the sheep (Braude *et al.*, 1979; Braude *et al.*, 1988; Crosby *et al.*, 1988). Out of the maternal polyadenylated RNA in the mouse oocyte, 40% is degraded in the 2-cell stage and another 30% in the early blastocyst (Bachvarova & De Leon, 1980; Paynton *et al.*, 1988). As to total Poly A, it increases in the mouse 5 fold from the 2-cell stage to the early blastocyst due, generally, to faster synthesis and higher stability (Levey *et al.*, 1978; Kidder & Pedersen, 1982; Piko & Clegg, 1982; Clegg & Piko, 1982, 1983).

A modulating function on gene expression of diverse cellular processes has been explored in early mammalian embryos. For instance, if cleavage of mouse 1-cell eggs is arrested by cytochalasin, they become tetraploid on the next day but the two-dimensional electrophoretic pattern of protein synthesis corresponds to that of control embryos of the same age (Petzoldt *et al.*, 1983) and continuous treatment with the drug does not affect normal protein synthesis in morulae or blastocysts (Pratt *et al.*, 1981). Similarly, the drastic changes in cell number and size of chimaeras formed by aggregation of 2 to 16 embryos do not affect lactate dehydrogenase isozyme expression (Schwarzpaul

& Petzoldt, 1988). Furthermore, modifying the nucleocytoplasmic ratio, through injection or extraction of cytoplasm or bisection or mouse eggs, does not alter the normal pattern of protein synthesis nor the expression of a stage specific antigen (Petzoldt & Muggleton-Harris, 1987). These and earlier observations suggest that gene expression at the beginning of mamalian development is determined by the genetic programme without epigenetic modulations. However, changes in cell form and contacts have been shown to regulate gene expression in a variety of differentiated cell types (review in Ben-Ze'ev, 1986). For instance, the expression of cytoskeletal genes in hepatocytes (Ben-Ze'ev *et al.*, 1988), the transcription of c-myc (Dean *et al.*, 1986), the expression of growth-associated genes in fibroblasts (Dike & Farmer, 1988) or of the interleukin 2 receptor in T cells (Komada *et al.*, 1987) or the synthesis of proteoglycan in chondrocytes (Newman & Watt, 1988).

Recently, we compared the effect on protein synthesis of diverse treatments which interfere with the changes in cell form and contacts which are typical of compaction in 8-cell mouse morulae. The assumption was that different effects should be ascribed to each treatment while common effects may be ascribed to inhibition of compaction. We tested cytochalasin D, EGTA, α-lactalbumin and Con A (which also halts compaction; Reeve, 1982) and found that interference with compaction does not cause common qualitative effects on protein synthesis, as demonstrated by one- or two-dimensional gel electrophoresis. On the contrary, drugs which inhibit transcription (α-amanitin) or DNA replication (aphidicolin) but do not affect compaction, cause profound qualitative changes in protein synthesis (unpublished observations). Our results with drugs that interfere with compaction suggests that the cytoskeleton of mammalian morulae plays no significant role in the control of gene expression, eventhough such a control has been reported for several differentiated cell types (Nielsen *et al.*, 1983; Ornelles *et al.*, 1986; Bag & Pramanik, 1987); and our results with aphidicolin support previous reports on the role of DNA replication on gene expression in early mammalian embryos (Bolton *et al.*, 1984; Smith & Johnson, 1985; Howlett, 1986) (Fig. 14).

A fitting coda to this paper might be this conjecture: embryonic regulation in mammals depends on epigenetic signals that control spatial differentiation, eventhough the analysis of morula-blastocyst morphogenesis has not disclosed as yet signals that modulate gene expression in presumptive trophectoderm or inner cell mass; temporal differentiation instead, seems to be based on a rather fixed genetic programme. To my best knowledge the only significant disturbance of developmental time that has been reported is the sudden acceleration of compaction and blastulation induced in 2-cell embryos by the lectin WGA (Johnson, 1986) in which, according to unpublished observations of ours, second messengers of the phosphoinositides system are involved. Though physiologically interesting, this is likely a case of pseudomorphogenesis, that is, the elicitation of some isolated functions that happen to mimic a developmental stage.

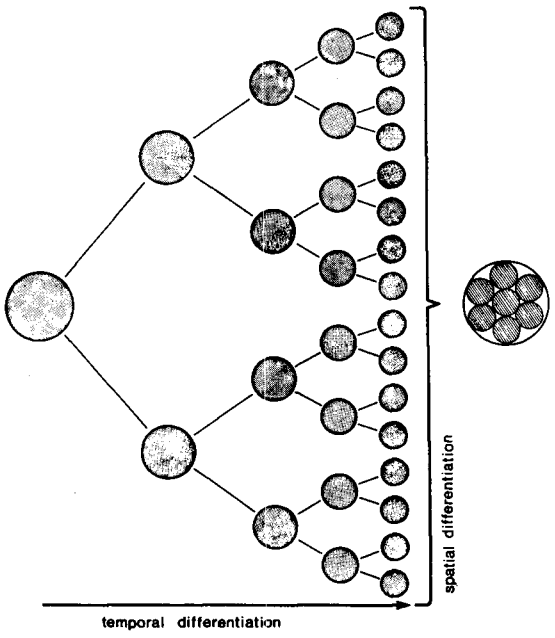

Fig. 14: Geometric diagram of early mammalian development. Temporal differentiation: diameter of cells corresponds to one half the volume of the progenitor cell. Spatial differentiation: tight packing of spherical cells exhibit inner and outer positions in the embryo.

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