Involvement of oocyte-coded message in cell differentiation control of early human embryos

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Summary

Considerable evidence indicates that the first phenotypical diversification of embryonic cells during mammalian preimplantation development is achieved in two successive steps: (i) generation of cell asymmetry and (ii) unequal cell division. This paper shows that ultrastructural signs of blastomere surface regionalization in human preimplantation embryos are evident as early as the 2-cell stage when modifications of the plasma membrane (loss of microvilli and endocytotic activity, formation of cell junctions) are induced in places of blastomere contact. The capacity of the plasma membrane to undergo these cell-contact-dependent changes precedes any detectable activity of the embryonic genome. The area of the modified plasma membrane shows a continuous increase during the first three cleavage stages. The progression of these membrane modifications is the same in embryos that have properly enhanced their transcriptional activity at the 8-cell stage and in those that have not. In spite of the failure of this early-cleavage-progressed-cleavage transition of gene activity, the formation of zonula adherens and gap junctions goes on apparently normally in the respective embryos and morphologically distinct inner cell mass and trophectoderm cell lineages are subsequently segregated in 16-cell morulae. However, tight junctions do not develop under these conditions. The occurrence of the progressed-cleavage pattern of gene activity in the majority of embryonic cells is a necessary prerequisite for the appearance of the blastocyst cavity. Thus, oocyte-coded message is apparently involved in the control of relatively late stages of human preimplantation development including the differentiation of the first two embryonic tissues, but the embryonic genome is required for the full achievement of this early differentiative event.

Key words: cell differentiation control, embryonic genome activation, blastomere surface regionalization, cell lineage segregation, preimplantation development, human.

Introduction

Fertilization of the mammalian oocyte triggers a series of mitotic divisions which, at the beginning, give rise to phenotypically identical progeny, followed by a period during which cell diversity is created by means of unequal blastomere divisions (Davidson, 1986; Johnson, 1986; Johnson et al. 1986). This mechanism of cell diversification obviously requires the previous development of a clear asymmetry of cell organization. In the mouse embryo, which represents the most studied subject in this regard, structural asymmetries involving the distribution of microvilli (Ducibella et al. 1977; Handyside, 1980; Reeve & Ziomek, 1981) and cell junctions (Ducibella & Anderson, 1975; Magnuson et al. 1977; McLachlin et al. 1983; Goodall & Johnson, 1984), polarization of the endocytotic system (Reeve, 1981; Fleming & Pickering, 1985), the position of nuclei (Reeve & Kelly, 1983) and distribution of various cytoplasmic membranous organelles (Maro et al. 1985; Batten et al. 1987) appear for the first time at the 8-cell stage of the preimplantation development. These morphological changes are preceded by regionalization of the plasma membrane at the molecular level, involving polarized distribution of membrane-associated enzymes (Izquierdo et al. 1980; Izquierdo & Ebensperger, 1982), membrane lipids (Pratt, 1985) and antibody- and lectin-binding sites (Handyside et al. 1987), which can be traced back up to the 2-cell stage.

As it is known that the early animal embryonic development is controlled by proteins/enzymes coded for by two different genomic sources: the products of oocyte genome transmitted to the embryo via the oocyte cytoplasm and the products of the embryonic genome proper (reviewed in Tesafik, 1988), a question arises about the genetic origin of factors that are responsible for these very early differentiative events. This question can hardly be answered by studies carried...
out on mouse embryos, since the activation of the mouse embryonic genome, largely occurring at the 2-cell stage (e.g. Flach et al. 1982), precedes the appearance of the first signs of blastomere polarization in this species. While the oocyte-derived message in the form of maternal mRNA may be rapidly eliminated after the onset of embryonic genome transcription (Bachvarova & De Leon, 1980; Pikó & Clegg, 1982), longevity of some oocyte gene products, such as glucose phosphate isomerase (West & Green, 1983), in the cytoplasm of embryonic cells is remarkable, which makes the possibility of oocyte-derived influences on some relatively late embryonic processes quite real.

It is evident that the study of the impact of oocyte-coded factors on the early embryonic development would be more feasible in those mammalian species in which the activation of the embryonic genome occurs relatively late in development, so as to avoid misinterpretations relating to the superposition of oocyte- and embryo-coded factors. As demonstrated recently, the human preimplantation embryo represents such a kind of material. The first limited transcription can be detected in human embryos at the 4-cell stage, followed by a major outburst of transcriptional activity at the 8-cell stage (Tesafík et al. 1986a,b; Tesafík, 1987). The first signs of human embryonic genome expression can also be seen during the transition between the 4- and 8-cell stages (Tesafík, 1987; Tesafík et al. 1988; Braude et al. 1988). Moreover, the existence of two possible patterns of gene activity in blastomeres of human 8- to 16-cell embryos (Tesafík et al. 1986a), each reflected by distinct qualitative (Tesafík, 1987) and quantitative (Tesafík et al. 1988) ultrastructural cytological characteristics, enables a direct study of correlations between the activity of embryonic genome and the progression of differentiation.

Here we use a morphometric ultrastructural analysis of human preimplantation embryonic cells to examine the relationship between the development of cell-contact-dependent surface regionality and the activity of the embryonic genome. It is shown that the generation of cell surface heterogeneity precedes any overt embryonic gene expression and that the persistence of the limited gene activity characterizing the early-cleavage transcriptional pattern is compatible with the differentiation of the first two embryonic cell lineages. We conclude that cytoplasmic factors inherited from the oocyte contribute a message utilized in the control of these early differentiative events in human preimplantation embryos.

The decision concerning embryo allocation to the experimental group was made on day 3 after in vitro insemination (day 1 was the day of insemination) when the embryos were at the 2- or 4-cell stages; some of the selected embryos were then cultured for an additional 1 to 3 days to achieve more advanced developmental stages, from 8-cell to blastocyst. When the culture was finished, embryos were fixed and processed for electron microscopy as described (Tesafík et al. 1987). Individual embryonic cells were examined in thin sections for the presence of the qualitative (Tesafík, 1987) and quantitative (Tesafík et al. 1988) ultrastructural markers of embryonic gene activity. Details of morphometric measurements of fractional volumes of cell organelles were reported earlier (Tesafík et al. 1988). For determination of the fractional surface of different plasma membrane domains thin sections were photographed and printed at the final magnification of ×10000. The prints were plotted against a lattice consisting of test lines whose intersections served as test points in a coherent stereological test system (Weibel, 1969). Fractional surfaces ($S_V$) of different types of the plasma membrane (fractional surface is the surface of a membrane constituting unit cell volume) were calculated using the formula

$$S_V = 2I_L$$

where $I_L$ represents intersection density of membrane profiles on unit test line length (Weibel, 1969). Practically, $I_L$ values were determined as fractions obtained by dividing the number of intersections of test lines with the plasma membrane of a given type by the number of test points of the coherent stereological system falling on intracellular areas of measured blastomere sections. The plasma membrane surfaces were calculated from the fractional surfaces using the values of fixed embryo volume determined as described earlier (Tesafík et al. 1986a).

Beginning with the 4-cell stage, when the embryonic genome transcription is first detectable (Tesafík et al. 1986a,b), all embryonic cells were classified into two categories, based on the character of their gene activity, determined using the qualitative and quantitative ultrastructural markers. These two types, denoted as the early-cleavage and progressed-cleavage patterns, were characterized in a previous study (Tesafík et al. 1988). Brie fy, the early-cleavage-type blastomeres show a low level of extranucleolar RNA synthesis and the absence of nucleolar transcriptional activity, whereas the progressed-cleavage-type blastomeres, first appearing in the fourth cell cycle after fertilization, are characterized by intense transcription of both nucleolar and extranucleolar genes. Morphologically, the transition from the early-cleavage to the progressed-cleavage pattern is signalled by the development of nucleolar structure, disappearance of vesicles from the perinuclear space, increase in the number of tubules of the endoplasmic reticulum and lysosomes and reduction of the Golgi apparatus and vesicular endoplasmic reticulum profiles. Fractional surfaces of different types of the plasma membrane were expressed separately for the two types of blastomeres. Significance levels were ascertained by t-test.

Materials and methods

Embryos used in this study were 55 supernumerary concepti from an in vitro fertilization programme. Criteria for embryo selection for experimental purposes were those reported earlier (Tesafík et al. 1986a,b). All of the embryos had developed at normal rates and showed normal morphology when examined under a dissecting microscope. Delayed embryos and those with irregularly sized blastomeres or abundant cell fragments were excluded from this analysis.

Results

Definition of basic plasma membrane patterns

From the topographical viewpoint, the cell surface of blastomeres of cleaving embryos shows two well-defined regions: the free surface — denoted as apical, and that making contact with adjacent embryonic cells denoted as basolateral. From the morphological view-
Cell differentiation in early embryos

Fig. 1. Electron micrographs showing the morphology of the two basic patterns of the plasma membrane in human 2-cell (C) and 4-cell (A, B) embryos. The microvillar type shows the presence of numerous microvilli (mv) and characteristic pits (arrows) giving rise to endocytotic vesicles; it can be observed at both the apical (A) and basolateral (B) blastomere surfaces. The junctional type (C) lacks microvilli and signs of endocytosis, develops focal submembrane densities (arrowheads) and is restricted to the basolateral blastomere surface. (A) \( \times 25,000 \); (B and C) \( \times 60,000 \).

Point, two distinct patterns of the blastomere surface can be distinguished again, based on the ultrastructural properties of the corresponding plasma membrane (Fig. 1). The **microvillar type** of the plasma membrane is characterized by the presence of microvilli and signs of active endocytotic membrane transport. This type of membrane can be observed at both the apical (Fig. 1A) and basolateral (Fig. 1B) surfaces. On the other hand, the **junctional type** of the plasma membrane is free of microvilli, lacks the signs of endocytotic activity and shows a tendency to develop focal submembrane densities and cell junctions. Under normal circumstances it appears at the basolateral blastomere surfaces only (Fig. 1C).

**Plasma membrane modifications and cell differentiation during preimplantation development**

Fig. 1C shows that the junctional type of the plasma membrane can be recognized as blastomere basolateral surfaces as early as the 2-cell stage of human preimplantation development. The microvillar and junctional plasma membrane domains display a proportional growth at the basolateral surfaces between the 2- and 8-cell stages, followed by an isolated marked increase in the junctional type at the 16-cell stage, when the segregation of the inner cell mass and trophectoderm cell lineages begins (Fig. 2). The area of the microvillar plasma membrane at the apical blastomere surfaces remains the same throughout the first four cleavage stages.

Beginning with the 8-cell stage, the differentiation of the cell surface was evaluated separately for the blastomers having undergone the early-cleavage-progressed-cleavage transition of gene activity and for those in which this process had not occurred. A clear difference in the microvillar/junctional membrane ratio was found between outer and inner cells of 16-cell morulae (Table 1). However, the extents of the microvillar and junctional types of the plasma membrane in blastomers of 8-cell embryos and in both the outer and inner cells of 16-cell morulae did not depend on the proper stage-specific enhancement of embryonic gene activity (Table 1), as was previously shown to be the case for the process of compaction of human morulae (Tesafik *et al.* 1986a). These findings indicate that morphologically distinct (as to their plasma membrane at least) populations of inner and outer cells are...
Table 1. Quantitative representation of different types of plasma membrane (PM) in relation to actual pattern of gene activity

<table>
<thead>
<tr>
<th>Pattern of gene activity</th>
<th>Fractional surface of microvillar and junctional PM at different developmental stages ($\times 10^{-2}$) ($\mu m^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8-cell</td>
</tr>
<tr>
<td></td>
<td>Microvillar</td>
</tr>
<tr>
<td>Early-cleavage</td>
<td>10-1 ± 1-2</td>
</tr>
<tr>
<td>Progressed-cleavage</td>
<td>9-7 ± 0-9</td>
</tr>
<tr>
<td>$P$</td>
<td>&gt;0-05</td>
</tr>
</tbody>
</table>

Values are means ± standard deviations. Each type of blastomere was represented by at least 25 cells.

Table 2. Relationship between formation of specialized cell junctions, cavitation and actual pattern of gene activity

<table>
<thead>
<tr>
<th>Pattern of gene activity</th>
<th>Occurrence of gap junctions (GJ), tight junctions (TJ) and development of blastocyst cavity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8-cell</td>
</tr>
<tr>
<td>Early-cleavage</td>
<td>+</td>
</tr>
<tr>
<td>Progressed-cleavage</td>
<td>+</td>
</tr>
</tbody>
</table>

Fig. 3. Electron micrograph showing the contact of two 8-cell blastomeres both of which have failed to assume the progressed-cleavage pattern of gene activity. Note a structurally well-developed gap junction (arrows). X65 000.

segregated in human 16-cell embryos, regardless of the previous acquisition of the progressed-cleavage transcriptional pattern.

The same approach was used for the examination of the relationship between the pattern of blastomere gene activity and the ability to form specialized cell junctions and to support the development of the blastocyst cavity (Table 2). The formation of zonula-adherens-type junctions and gap junctions was observed in 8-cell and older embryos, irrespective of the actual pattern of gene activity (Fig. 3). By contrast, the presence of the progressed-cleavage pattern in both neighbouring cells was necessary for the development of tight junctions in the nascent trophectoderm layer and this pattern of gene activity was always detected in cells facing the blastocyst cavity. When the progressed-cleavage pattern had not developed in the majority of embryonic cells, a normal blastocyst cavity was never formed (Table 2). It is worth mentioning that, on the other hand, cavitation was observed in all five embryos that succeeded in developing the progressed-cleavage pattern in >50% of cells and that were cultured for the appropriate time (≥5 days after in vitro insemination).

Discussion

The appearance of distinct plasma membrane domains in blastomeres of preimplantation embryos represents the first step towards the development of cell asymmetry, a necessary prerequisite for future establishment and diversification of cell lineages. The development of uneven distribution of microvilli and cell junctions on the blastomere surface are two aspects of this process commonly denoted as blastomere surface polarization. Originally, the term 'polarization' was used for gener-
ation of cell polarity in situ (e.g. Ducibella et al. 1977).

Later this term was usually reserved for the designation of changes that were not dependent upon continuing cell contact and were maintained when embryos were disaggregated to single cells (e.g. Handyside, 1980; Reeve & Ziomek, 1981). In order to avoid misinterpretation, we therefore use the term plasma membrane 'regionalization' for the changes described in this study as no attempt was made to examine the stability of these changes after embryo disaggregation. Nevertheless, as judged by analogy with the murine system, the asymmetries in the distribution of microvilli, endosomes and cell junctions described here are most probably stable ones which may have been preceded by less stable asymmetries at the molecular level, similar to those described in mice (Izquierdo et al. 1980; Izquierdo & Ebensperger, 1982; Pratt, 1985; Handyside et al. 1987).

In mouse embryos, morphological polarization of the blastomere surface is first evident at the 8-cell stage. Thus microvilli (Ducibella et al. 1977; Handyside, 1980; Johnson & Ziomek, 1981; Reeve & Ziomek, 1981) and endosomes (Reeve, 1981; Fleming & Pickering, 1985) become localized apically, whereas cell junctions develop at apposed cell surfaces (Ducibella & Anderson, 1975; Magnuson et al. 1977; McLachlin et al. 1983; Goodall & Johnson, 1984). At the same time, blastomeres gain the capacity to respond to contact between blastomeres by changes in cytocortical organization (Johnson et al. 1988). Fluorescent ligand (Handyside, 1980; Reeve & Ziomek, 1981) and electron microscopic studies (Calcaro & Epstein, 1973; Ducibella et al. 1977; Van Blerkom & Motta, 1979; Reeve & Ziomek, 1981) of intact mouse embryos did not reveal any asymmetries in the distribution of microvilli prior to the 8-cell stage. Also the distribution of the endocytotic system in the cortical cytoplasm of blastomeres of 2- and 4-cell mouse embryos is random (Fleming & Pickering, 1985). Surprisingly, in human embryos, morphological regionalization of the plasma membrane, corresponding to that occurring at the 8-cell stage of the mouse development, is evident as early as the 2-cell stage. Together with the delayed activation of human embryonic genome transcription (Tesafik et al. 1986a,b; Tesafik, 1987) and expression (Tesafik, 1987; Tesafik et al. 1988; Braude et al. 1988), as compared with the mouse, this finding is proof of a role of oocyte-coded message in the control of this process. Information concerning the stage at which morphological heterogeneity of the plasma membrane first appears in embryos of other mammalian species is actually lacking; neither is it known, except for human and mouse embryos, how these changes temporally relate to the embryonic gene activation.

It is believed that the development of cell surface polarity is induced by interactions occurring in places of blastomere contact (Ziomek & Johnson, 1980). We have shown here that the process of cell-contact-mediated transformation of the plasma membrane from the microvillar to the junctional type and the segregation of the first two embryonic cell lineages are propagated at virtually constant rates in embryos showing different patterns of gene activity. This finding represents a further argument supporting the view that the message necessary for the adequate reaction of preimplantation embryonic cells to positional stimuli is apparently coded for, at least in part, by the maternal genome and transmitted to the early embryo from the oocyte. It is not clear whether this information is stored in the form of maternal RNA or other cytoplasmic components. Informational signals stored in the egg cytocortex, whose persistence during cleavage of mouse embryos up until the blastocyst stage has been suggested (Pratt, 1985), are also possible candidates for this regulatory role.

On the other hand, the embryonic transcripts newly appearing at the 8-cell stage of human development seem to be implicated in the activation of vectorial transport mechanism and/or tight junctional assembly necessary for establishing a permeability seal capable of sequestering the nascent blastocyst fluid within the embryo. These two conditions are known to be required for blastocyst formation (Magnuson et al. 1978; Wiley, 1984; Fleming & Pickering, 1985). In this study, we observed no blastocysts among embryos in which most blastomeres failed to undergo the early-cleavage-progressed-cleavage transition of gene activity by 5 days in culture. It is possible that the failure of proper gene activation at the 8-cell stage of human embryogenesis, which represents a rather frequent anomaly in cultured human embryos (Tesafik et al. 1986a, Tesafrk, 1988), may be the main cause of the generally poor development of human blastocysts in vitro.

In conclusion, the present analysis performed on human embryos, in which the activation of the embryonic genome is delayed as compared with mouse embryos, has shown the persistence of an oocyte-derived regulatory mechanism involved in the control of early cellular differentiative events up to relatively late stages of preimplantation development. Whether this delayed maternal effect is a specific feature of human embryos or whether it is shared by embryos of other mammalian species remains to be elucidated.

References


organization during early embryogenesis in the preimplantation mouse embryo. Dev. Biol. 74, 252-261.


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