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Biogenesis of structural intercellular junctions during cleavage in the mouse embryo

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SUMMARY

The preimplantation embryo differentiates the trophectoderm epithelium which, from the 32-cell stage, generates the blastocoel of the blastocyst and, after implantation, gives rise to most extraembryonic lineages of the conceptus. Trophectoderm differentiation begins at compaction (8-cell stage) when cell-cell adhesion, mediated by uvomorulin, and epithelial cell polarisation first occur. Here, we review our work on the biogenesis of tight junctions and desmosomes during epithelial differentiation. Tight junction construction begins at compaction and appears to be a gradual process, both at morphological and molecular levels. This maturation pattern may be due in part to sequential expression of tight junction constituents from the embryonic genome. Tight junction formation is dependent upon uvomorulin adhesion but can be inhibited by different means without apparently disturbing cell adhesion or polarisation. Cell interactions appear to regulate tight junction tissue specificity, in part by controlling the level of synthesis of constituents. Desmosome formation begins at the 32-cell stage, particularly as the embryo initiates blastocoel accumulation, and, in contrast with tight junction formation, does not appear to be a gradual process. Thus, nascent desmosomes appear mature in terms of their molecular composition. Desmosomal proteins are synthesised well in advance of desmosome formation but the synthesis of the principal glycoprotein components begins at the blastocyst stage and may regulate the timing of junction assembly. Implications of these differing patterns of biogenesis for the embryo are discussed.

Key words: epithelium, cell adhesion, cell polarity, differentiation, mouse embryo, trophectoderm, ICM, blastocyst, uvomorulin, E-cadherin, intercellular junctions, tight junction, zonula occludens, ZO-1, cingulin, desmosome

INTRODUCTION

In our laboratory, we study the process of epithelial differentiation and polarization in the cleaving mouse embryo. The epithelium in question, the trophectoderm, forms the wall of the blastocyst at about 3.5 days post-fertilization and encloses the ICM (inner cell mass; progenitor of foetus) and blastocoelic cavity. The first developmental function of the nascent trophectoderm is to generate the blastocoel by vectorial transport (Wiley, 1987; Manejwala et al., 1989; Wiley et al., 1990) and to regulate metabolic exchange with the ICM. After further expansion of the blastocoel, the trophectoderm contributes to embryo hatching from the zona pellucida by enzymic secretion (Perona and Wassarman, 1986; Sawada et al., 1992). It then engages, at 4.5-5.5 days post-fertilization, in specific adhesive and invasive cellular interactions with maternal tissue to accomplish implantation (Holmes and Lindenberg, 1988; Lindenberg et al., 1990). After implantation, trophectoderm (trophoblast) cell lineages contribute exclusively to extra-embryonic tissues, notably giant cells, extraembryonic ectoderm, ectoplacental cone and chorionic ectoderm (Gardner and Beddington, 1988). Our interest in trophectoderm lies in understanding its derivation from non-epithelial and non-polar blastomeres and how the epithelial phenotype is propagated selectively in this lineage of the blastocyst (Fleming and Johnson, 1988; Fleming, 1992).

There are three main constraints in studying the trophectoderm as a model for epithelial differentiation: preimplantation embryos are expensive to generate, there is only limited material to work with and, because of inherent developmental asynchrony, staging of embryos must be tackled carefully. However, we believe that there are some real advantages. First, the trophectoderm is a native tissue with important consequences for morphogenesis and development, and hence is of clear medical interest. Second, its formation is rather slow (about 24 hours), providing time for analysis of mechanisms. Third, and perhaps most important, the epithelium differentiates de novo after normal cell cycling and 'housekeeping' functions have been reestablished in the embryo following release from oocyte meiotic arrest (Pratt, 1989; Fleming, 1992). Thus, it is possible to study gene and protein expression events required for epithelial differentiation, in addition to changes in cell

organisation. Recently, we have studied maturation of cell-cell adhesion during trophectoderm differentiation, in particular the formation of structural intercellular junctions. Here, we provide a brief review of this work.

ADHESION AND POLARISATION

Cell-cell adhesion between blastomeres begins at the 8-cell stage when the embryo compacts and each cell polarises along its apicobasal axis, generating a proto-epithelial phenotype. Adhesion is achieved by uvomorulin (120×10^3 M_r, E-cadherin) intercellular binding (Hyafil et al., 1980; Peyrieras et al., 1983). Uvomorulin is synthesised and is present in the membrane in a non-polar and non-adhesive state from early cleavage; at compaction, it becomes adhesive and progressively basolateral in distribution (i.e. at cell-cell contact sites) (Vestweber et al., 1987). The mechanism causing uvomorulin adhesion at compaction is not known but coincides with the onset of uvomorulin phosphorylation (Sefton et al., 1992). Moreover, treatment of pre-compact embryos with phorbol ester to activate protein kinase C causes premature compaction (Winkel et al., 1990), further suggesting a role for phosphorylation in initiating adhesion.

As adhesion commences, the proto-epithelial organisation generated in 8-cell blastomeres includes changes in the distribution of cytoskeletal elements (actin filaments, microtubules), cytoplasmic organelles (mostly endocytic vesicles) and components of the membrane and underlying cortex (e.g. microvilli, actin-binding proteins) (reviewed by Fleming and Johnson, 1988; Fleming, 1992; Gueth-Hallonet and Maro, 1992). Experimental evidence suggests that certain, as yet undefined, aspects of polarisation in the membrane and cortex (cytocortex) are of fundamental importance in guiding the reorganisation of other cellular structures (Johnson and Maro, 1985, 1986; Fleming et al., 1986; Johnson et al., 1986a; Wiley and Obasaju, 1988). This primary cytocortical state appears also to act as a stable 'memory' of the polar axis in subsequent cell cycles, since during mitosis aspects of polarity are transiently run down before being reestablished in the next interphase (Johnson et al., 1988). What is the relationship between cell adhesion and polarisation at compaction? If the onset of adhesion is prevented (by, for example, incubation with uvomorulin antibody), polarisation is delayed, disoriented with respect to cell contact patterns, but is not inhibited (Johnson et al., 1986b). This suggests that adhesion may act normally to induce and orientate polarisation but the molecular pathway involved is not known. In the absence of adhesion, however, other non-specific factors may induce polarisation, reflecting the 'programmed' state of blastomeres to begin differentiation. 'Programming' for polarisation at compaction requires RNA, DNA and protein synthesis in the 2-cell embryo, 24 hours earlier, but apparently not subsequently (Kidder and McLachlin, 1985; Smith and Johnson, 1985; Levy et al., 1986).

TIGHT JUNCTION CONSTRUCTION

The first epithelial-type junctions to form in the embryo are gap and tight junctions during the 8-cell stage, although an analysis of adherens junction formation has not yet been conducted systematically (Reima, 1990). The process of tight junction (zonula occludens) formation will be considered here; gap junction formation and function during preimplantation development have been discussed elsewhere recently (Barron et al., 1989; Bevilacqua et al., 1989; Nishi et al., 1991; Fleming, 1992; Fleming et al., 1992).

Ultrastructural analysis of embryos indicates that tight junctions begin to form at compaction; however, this process is not completed until the late morula stage (16- to 32-cell stage), up to 24 hours later (Ducibella and Anderson, 1975; Ducibella et al., 1975; Magnuson et al., 1977; Pratt, 1985). Once completed, vectorial transport and blastocoel accumulation can begin, dependent upon the segregation of Na^+,K^+-ATPase to basolateral trophectoderm membranes (Watson and Kidder, 1988). During the tight junction construction period, the apicolateral contact region between outside blastomeres displays sites of apparent membrane fusion and lanthanum exclusion, corresponding with a freeze-fracture morphology of anastomosing strands and grooves in complementary faces. These sites first appear as a discontinuous series along the apicolateral border and gradually extend laterally to become continuous (zonular) during the morula stage.

We have examined the molecular maturation of the tight junction in embryos using antibodies to peripheral membrane (cytoplasmic face) tight junction proteins. For a review of tight junction molecular composition, see Anderson and Stevenson (1991). Since embryos develop asynchronously, to improve accuracy in the relative timing of assembly of different components, we have used natural 8-cell couplets (2/8 cells), synchronised from their time of division from single 4-cell blastomeres (1/4 cells). These couplets engage in adhesion and polarisation at compaction in a similar way to blastomeres in intact embryos. The assembly of ZO-1 (225×10^3 M_r; Stevenson et al., 1986; Anderson et al., 1988) at the junction begins 1-2 hours after compaction initiates and precedes the assembly of cingulin (140×10^3 M_r; Citi et al., 1988, 1989) which, on average, assembles about 10 hours later, usually during the 16-cell stage (Fleming et al., 1989, 1993). For both proteins, assembly is at first punctate before gradually becoming zonular. Thus, in double-labelled 2/8 couplets recently compacted, ZO-1 but not cingulin is detectable at the tight junction site (Fig. 1A,B). Our results imply, therefore, that junction formation is progressive, not only in morphological terms but also at the molecular level. Double immunogold analysis of ZO-1 and cingulin localisation at the tight junction in chick and rat epithelia indicate that ZO-1 is positioned closer to the membrane than cingulin (Stevenson et al., 1989). Assuming these proteins occupy similar relative positions in the mouse embryo junction, our results are also consistent with molecular maturation proceeding in the membrane-to-cytoplasm direction (Fleming et al., 1992).

What factors might influence the sequential nature of tight junction protein assembly during cleavage? To investigate this, we have studied the ontogeny of expression of

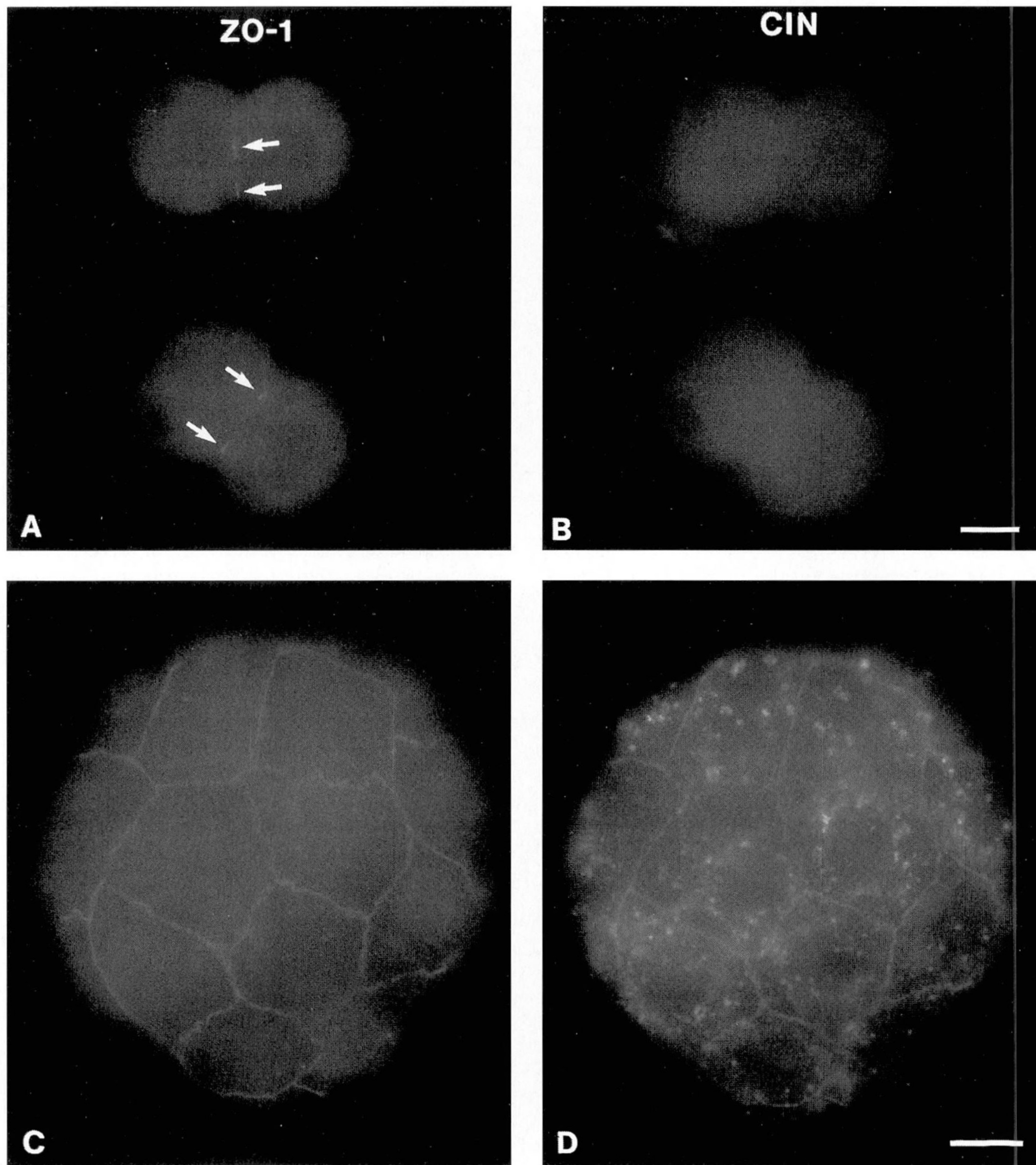

Fig. 1. Double immunofluorescence labelling of whole-mount embryo samples for the tight junction proteins ZO-1 (A,C) and cingulin (B,D). (A,B) Synchronised 2/8 couplets 8 hours post-division from 1/4 cells and 2 hours after blastomere adhesion initiated (compaction). The onset of ZO-1 assembly is detectable (arrows, A) as a weak discontinuous reaction at the periphery (apicolateral region) of the adhesive contact site between blastomeres. At this early stage in tight junction biogenesis, cingulin is not detectable (B). (C,D) Early expanding blastocyst (32- to 64-cell stage) viewed tangentially. Trophectoderm cells are bordered by a continuous (zonular) distribution of ZO-1 (C) and cingulin (D) that appear colocalised. Perinuclear cytoplasmic foci of cingulin, but not ZO-1, are evident, derived from endocytic turnover of cingulin sites in the apical cytocortex. Bar, 20 µm.

junction components at the protein level. Immunoblot analysis indicates detectable expression of ZO-1 from the late 4-cell stage, some 5-6 hours before ZO-1 incorporation into junctions is evident cytochemically (Fleming et al., 1989). The biosynthesis of cingulin is more complex than that of ZO-1 because this protein is expressed from both the maternal and embryonic genomes (Javed et al., 1993). Thus, immunoblot and immunoprecipitation data show synthesis of cingulin, but not ZO-1, during oogenesis. Maternal cingulin has a short metabolic half-life, ceases synthesis during the 2-cell stage when maternal transcripts degrade globally, and localises in the oocyte cortex where it may participate in the oocyte-cumulus cell interaction (Javed et al., 1993; Fleming et al., 1993). Biochemical and embryo manipulation experiments indicate that this maternal pool does not contribute to tight junction formation as trophectoderm differentiates. However, the cortical binding site for maternal cingulin persists during cleavage (on embryo outer surface) long after the maternal expression programme has ceased, and presumably embryonic cingulin

replaces maternal cingulin at this binding site. Cortical cingulin is finally degraded by endocytic turnover of the embryo outer surface in late morulae and early blastocysts (Fleming et al., 1993), resulting in cingulin cytoplasmic foci (Fig. 1C,D). Synthesis of cingulin from the embryonic genome is detectable at trace levels during early cleavage (about ten-fold less than in the egg) but a significant increase occurs at compaction, and continues to increase during later cleavage (Javed et al., 1993). The significant enhancement in cingulin synthesis from compaction at the 8-cell stage precedes by approx 5-10 hours the detection of cingulin assembly at the tight junction. This surge in cingulin synthesis is clearly later than the onset of detectable ZO-1 protein expression in the late 4-cell embryo. These differing expression patterns for ZO-1 and embryonic cingulin during cleavage are consistent with, and may control, the sequential membrane assembly pattern at the tight junction. Future work will investigate whether tight junction protein expression is regulated by sequential transcription and/or translation.

What is the relationship between the onset of tight junction formation at compaction and the concurrent differentiation of blastomeres into a proto-epithelial phenotype? Different experimental situations employing synchronised 2/8 cell couplets cultured during the fourth cell cycle have been used to investigate this question (Fig. 2A; Fleming et al., 1989). If uvomorulin adhesion is inhibited (anti-uvomorulin antibody; calcium removal), ZO-1 membrane assembly is both delayed and distributed randomly (i.e. not at apicolateral contact site), while microvillus polarisation is similarly delayed and oriented randomly, as mentioned earlier (Fig. 2A). Thus, adhesion is necessary both for normal tight junction assembly and for regulating the timing and orientation of cell polarity. If 2/8 couplets are treated with cycloheximide from the time of their division from 1/4 cells, adhesion and microvillus polarisation occur as normal at compaction but ZO-1 membrane assembly is inhibited. Also, if newly formed 1/8 blastomeres are contacted with newly formed 1/4 blastomeres, these heterogeneous couplets will subsequently adhere together with the 1/8 cell polarising apparently normally, but ZO-1 assembly in the 1/8 cell is delayed and randomly distributed (Fig. 2A). These two latter treatments divorce tight junction formation from epithelial polarisation and intercellular adhesion in the embryo (Fleming et al., 1989). Junction formation, but not polarisation or adhesion, requires (i) proximate

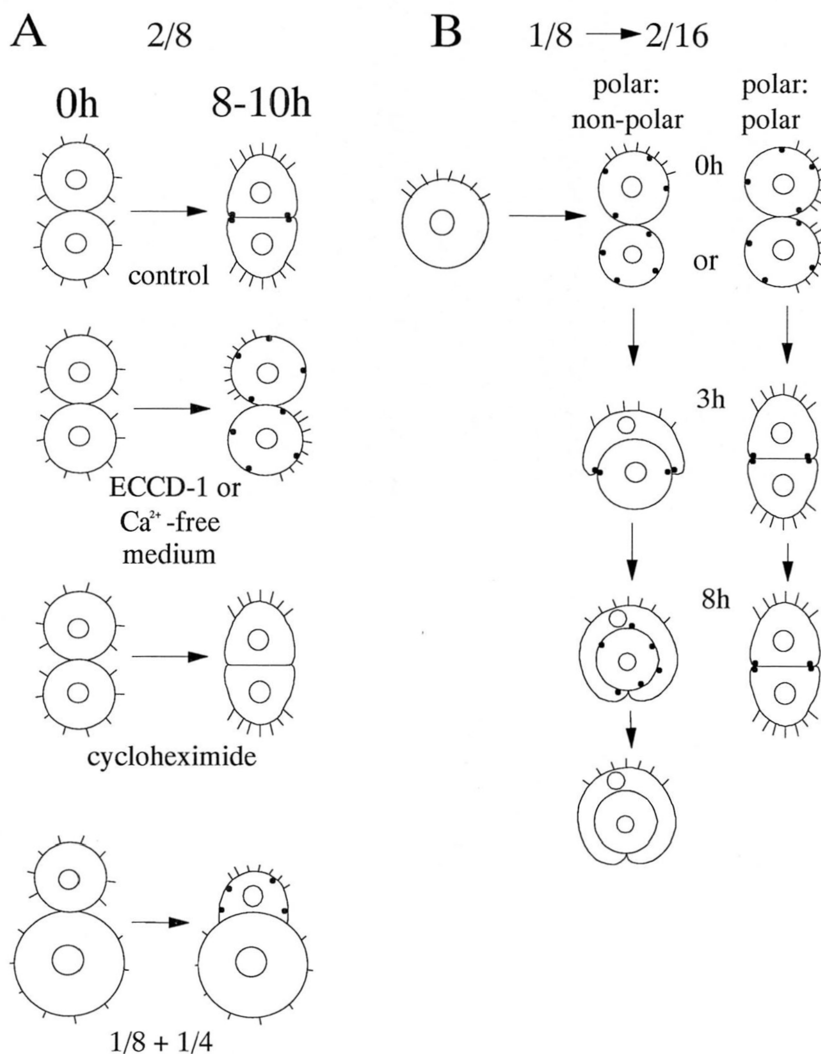

Fig. 2. (A) Schematic representations of ZO-1 distribution (black dots) in 2/8 and heterogeneous couplets in different experimental situations. Control couplets, by 8-10 hours post-division, have adhered together, polarised (here shown by apical microvilli), and assembled ZO-1 at periphery of contact site. Inhibition of uvomorulin function by ECCD-1 uvomorulin antibody or calcium-free medium treatment prevents adhesion and randomises both the orientation of polarity and the membrane distribution of ZO-1. Cycloheximide treatment does not perturb adhesion or polarity but inhibits ZO-1 assembly. 1/8 cells combined with 1/4 cells become adhesive and polarise but ZO-1 membrane assembly is randomly distributed. After Fleming et al. (1989). (B) ZO-1 distribution in 2/16 couplets at different times post-division from 1/8 cell. Differentiative or conservative divisions yield polar:non-polar or polar:polar couplets, depending on whether the apical pole is inherited by one or both cells. Polar:polar couplets assemble ZO-1 at the periphery of their contact site and this pattern is maintained throughout the cell cycle. Polar:non-polar couplets display a similar ZO-1 distribution until the non-polar cell is enveloped, at which time membrane-associated ZO-1 is run down. Equivalent cell interactions regulate maintenance or loss of ZO-1 membrane assembly in trophectoderm and ICM lineages respectively of intact embryos (after Fleming and Hay, 1991).

translation and (ii) adjacent blastomeres to be equally competent to assemble ZO-1 at the correct membrane site. The capacity to inhibit or perturb normal tight junction formation at compaction without apparently affecting epithelial polarisation or cell-cell adhesion argues against the tight junction having a fundamental role in the establishment of a proto-epithelial phenotype in the embryo, data consistent with other systems (e.g. McNeill et al., 1990).

Why does the tight junction develop only in the trophectoderm lineage of the blastocyst? To investigate this question, we have compared cingulin synthesis in trophectoderm and ICM tissues and found that, as blastocyst expansion progresses, the level of cingulin synthesis declines in the ICM and increases in trophectoderm such that the difference between these two tissues reaches fifteen-fold (Javed et al., 1993). Differential translation of tight junction proteins may therefore provide a biosynthetic mechanism to explain tight junction tissue specificity. How might blastomeres perceive their tissue type and modulate their expression pattern accordingly? Trophectoderm and ICM lineages derive from differentiative divisions of certain polarised 8- and 16-cell blastomeres (Johnson and Ziomek, 1981; Pedersen et al., 1986; Fleming, 1987). Daughter cells (at 16- or 32-cell stage) inheriting the apical microvillus domain (plus the cytocortical 'memory' of epithelial polarity, see earlier) remain on the outside of the embryo and continue to differentiate into trophectoderm. Daughter cells not inheriting this domain occupy an internal position in the embryo and differentiate into non-polar ICM cells (Fig. 2B). Polarised 8- and 16-cell blastomeres may also divide conservatively such that both daughters inherit part of the apical domain and continue to differentiate into trophectoderm (Fig. 2B). In newly formed 16- and 32-cell couplets following either differentiative or conservative cleavage, ZO-1 protein is present in both cells and soon associates with the apicolateral region of cell-cell contact between them (Fig. 2B; Fleming and Hay, 1991). Thus, tight junction tissue specificity cannot be explained by differential inheritance of junctional proteins and their membrane binding site (cf. the apical microvillus domain). Following differentiative cleavage, non-polar cells gradually become enveloped by polar cells until all contact-free membrane is lost. Once this has occurred, membrane-associated ZO-1 in non-polar cells is rapidly run down whilst at polar:polar contacts, apicolateral ZO-1 assembly is maintained (Fig. 2B). If contact-free membrane is regained by non-polar cells (e.g. by isolating the ICM), ZO-1 membrane assembly is re-established within a few hours. This assembly process does not require transcription but does require protein synthesis (Fleming and Hay, 1991). These results suggest the following model for tight junction tissue specificity which is currently being tested in our laboratory. Cell position, and hence tissue type, is intepreted by cell-cell contact patterns which regulate tight junction assembly competence. In the absence of membrane assembly (ICM lineage), expression is down-regulated at the protein level but appropriate transcripts are retained. These putative transcripts would allow for rapid formation of a new tight junction network once contact asymmetry is provided. This mechanism, if substantiated, is perhaps required for rapid delamination of primary endoderm by the ICM at the time of implantation, or for replacing damaged trophectoderm cells (Fleming and Hay, 1991).

DESMOSOME FORMATION

Desmosome junctions, characterised by their disc-shaped, membrane-associated plaques and inserted cytokeratin filaments, have been reviewed recently at cellular (Garrod and Collins, 1992) and molecular (Buxton and Magee, 1992; Legan et al., 1992) levels. Desmosomes form relatively late in the process of trophectoderm differentiation, at the 32-cell stage once blastocoel formation has begun (Ducibella et al., 1975; Magnuson et al., 1977; Jackson et al., 1980; Fleming et al., 1991). In contrast to the tight junction, the first desmosomes do not appear to form gradually, but rather display a mature morphology and molecular composition. Thus ultrastructurally, nascent desmosomes, although rather small, contain membrane-associated cytoplasmic plaques with inserted cytokeratin filaments and an intercellular adhesive domain possessing a dense midline (Fleming et al., 1991). These early desmosomes show immunoreactivity for the major desmosomal proteins, desmoplakin (dp1+2) and plakoglobin (dp3), and glycoproteins, desmoglein (dg1) and desmocollins (dg2+3). In time-course assays, all of these components first assemble at basolateral membranes from the 32-cell stage and, with the exception of plakoglobin, only after blastocoel fluid accumulation has initiated. Plakoglobin may assemble slightly earlier in the same cell cycle, before fluid accumulation begins. The close temporal relationship between the assembly of desmosomes and the start of blastocoel formation suggests that desmosomes may have an important role in blastocyst expansion, perhaps to stabilise the trophectoderm layer as the cavity enlarges.

What mechanisms may control the timing of desmosome formation in the embryo? We have monitored the pattern of synthesis of desmosomal constituents and found that plakoglobin and desmoplakin are both synthesised significantly in advance of their time of membrane assembly at the 32-cell stage. However, synthesis of desmoglein and desmocollins is not detectable before the 32-cell stage, suggesting that their synthesis may be regulatory for desmosome formation at cavitation (Fleming et al., 1991). Currently, we are investigating the time at which transcription of desmocollins begins using the reverse transcriptase-PCR technique. Our preliminary data indicate that desmocollin transcripts are first expressed from the embryonic genome in the 16-cell morula (J. Collins and T. Fleming, unpublished), an event that may therefore regulate desmocollins synthesis and the timing of desmosome assembly. Finally, the close link between desmosome formation in trophectoderm and the start of blastocoel accumulation suggests that other non-biosynthetic processes may 'fine-tune' assembly timing. Cavitation, like compaction, is an integrated differentiation event. Tight junction formation is completed, apical and basolateral membrane domains become segregated, Na^+,K^+-ATPase localises on basal membranes and vectorial transport initiates (e.g. DiZio and Tasca, 1977; Kaye et al., 1982; Watson and Kidder, 1988; Watson et al., 1990). Under these circumstances, it is plausible that newly

synthesised desmosomal glycoproteins, restricted to basolateral membranes by the 'fence' activity of the tight junction, would rapidly accumulate to a threshold level appropriate for desmosome assembly.

CONCLUSIONS

Programmes of expression of junctional constituents in the embryo, in combination with cell-cell interactions, appear to control the timing, characteristics and tissue specificity of assembly of structural intercellular junctions. Biogenesis of the tight junction network is gradual (approximately 24 hours), both in morphological and molecular terms. In contrast, the first desmosomes are constructed, with apparent full molecular complexity, over a relatively short time period. The slower maturation of the tight junction may reflect in part its larger, zonular configuration, requiring a sequential pattern of expression of constituents to achieve efficient assembly. In contrast, the formation of smaller, disc-shaped desmosomes may be controlled by a different mechanism, the later expression of specific constituents (desmosomal glycoproteins) that act as limiting factors for complete assembly. Both of these junction types, however, appear to function for the first time when the embryo accumulates blastocoelic fluid. Their varied patterns of biogenesis may therefore explain why they begin to form at different times during trophectoderm differentiation and perhaps why compaction must occur a day in advance of cavitation.

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REFERENCES

Anderson, J. M. and Stevenson, B. R. (1991). The molecular structure of the tight junction. In *Tight Junctions* (ed. M. Cereijido), pp. 77-90. Florida: CRC Press.

Anderson, J. M., Stevenson, B. R., Jesaitis, L. A., Goodenough, D. A. and Mooseker, M. S. (1988). Characterization of ZO-1, a protein component of the tight junction from mouse liver and Madin-Darby canine kidney cells. *J. Cell Biol.* **106**, 1141-1149.

Barron, D. J., Valdimarsson, G., Paul, D. L. and Kidder, G. M. (1989). Connexin32, a gap junction protein, is a persistent oogenetic product through preimplantation development of the mouse. *Dev. Genet.* **10**, 318-323.

Bevilacqua, A., Loch-Caruso, R. and Erickson, R. P. (1989). Abnormal development and dye coupling produced by antisense RNA to gap junction protein in mouse preimplantation embryos. *Proc. Nat. Acad. Sci. USA* **86**, 5444-5448.

Buxton, R. S. and Magee, A. I. (1992). Structure and interactions of desmosomal and other cadherins. *Semin. Cell Biol.* **3**, 157-167.

Citi, S., Sabanay, H., Jakes, R., Geiger, B. and Kendrick-Jones, J. (1988). Cingulin, a new peripheral component of tight junctions. *Nature* **333**, 272-276.

Citi, S., Sabanay, H., Kendrick-Jones, J. and Geiger, B. (1989). Cingulin: characterization and localization. *J. Cell Sci.* **93**, 107-122.

DiZio, S. M. and Tasca, R. J. (1977). Sodium-dependent amino acid transport in preimplantation embryos. III. Na^+,K^+-ATPase linked mechanism in blastocysts. *Dev. Biol.* **59**, 198-205.

Ducibella, T., Albertini, D. F., Anderson, E. and Biggers, J. D. (1975). The preimplantation mammalian embryo: characterization of intercellular junctions and their appearance during development. *Dev. Biol.* **45**, 231-250.

Ducibella, T. and Anderson, E. (1975). Cell shape and membrane changes in the 8-cell mouse embryo: prerequisites for morphogenesis of the blastocyst. *Dev. Biol.* **47**, 45-58.

Fleming, T. P. (1987). A quantitative analysis of cell allocation to trophectoderm and inner cell mass in the mouse blastocyst. *Dev. Biol.* **119**, 520-531.

Fleming, T. P. (1992). Trophectoderm biogenesis in the preimplantation mouse embryo. In *Epithelial Organization and Development* (ed. T. P. Fleming), pp. 111-136. London: Chapman and Hall.

Fleming, T. P., Cannon, P. M. and Pickering, S. J. (1986). The cytoskeleton, endocytosis and cell polarity in the mouse preimplantation embryo. *Dev. Biol.* **113**, 406-419.

Fleming, T. P., Garrod, D. R. and Elsmore, A. J. (1991). Desmosome biogenesis in the mouse preimplantation embryo. *Development* **112**, 527-539.

Fleming, T. P. and Hay, M. J. (1991). Tissue-specific control of expression of the tight junction polypeptide ZO-1 in the mouse early embryo. *Development* **113**, 295-304.

Fleming, T. P., Hay, M. J., Javed, Q. and Citi, S. (1993). Localization of tight junction protein cingulin is temporally and spatially regulated during early mouse development. *Development* **117**, 1135-1144.

Fleming, T. P., Javed, Q. and Hay, M. (1992). Epithelial differentiation and intercellular junction formation in the mouse early embryo. *Development. Suppl.*, 105-113.

Fleming, T. P. and Johnson, M. H. (1988). From egg to epithelium. *Annu. Rev. Cell Biol.* **4**, 459-485.

Fleming, T. P., McConnell, J., Johnson, M. H. and Stevenson, B. R. (1989). Development of tight junctions de novo in the mouse early embryo: control of assembly of the tight junction-specific protein, ZO-1. *J. Cell Biol.* **108**, 1407-1418.

Gardner, R. L. and Beddington, R. S. P. (1988). Multi-lineage 'stem' cells in the mammalian embryo. *J. Cell Sci. Suppl. 10*, 11-27.

Garrod, D. R. and Collins, J. E. (1992). Intercellular junctions and cell adhesion in epithelial cells. In *Epithelial Organization and Development* (ed. T. P. Fleming), pp. 1-52. London: Chapman and Hall.

Gueth-Hallonet, C. and Maro, B. (1992). Cell polarity and cell diversification during early mouse embryogenesis. *Trends Genet.* **8**, 274-279.

Holmes, P. V. and Lindenberg, S. (1988). Behaviour of mouse and human trophoblast cells during adhesion to and penetration of the endometrial epithelium. In *Eukaryotic Cell Recognition: Concepts and Model Systems* (ed. G. P. Chapman, C. C. Ainsworth and C. J. Chatham), pp. 225-237. Cambridge: Cambridge University Press.

Hyafil, F., Morello, D., Babinet, C. and Jacob, F. (1980). A cell surface glycoprotein involved in the compaction of embryonal carcinoma cells and cleavage stage embryos. *Cell* **21**, 927-934.

Jackson, B. W., Grund, C., Schmid, E., Burki, K. and Franke, W. W. (1980). Formation of cytoskeletal elements during mouse embryogenesis. *Differentiation* **17**, 161-179.

Javed, Q., Fleming, T. P., Hay, M. J. and Citi, S. (1993). Tight junction protein cingulin is expressed by maternal and embryonic genomes during early mouse development. *Development* **117**, 1145-1151.

Johnson, M. H., Chisholm, J. C., Fleming, T. P. and Houliston, E. (1986a). A role for cytoplasmic determinants in the development of the mouse early embryo? *J. Embryol. Exp. Morph.* **97** *Suppl.*, 97-121.

Johnson, M. H. and 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. and 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 and R. A. Pedersen), pp. 35-66. New York: Cambridge University Press.

Johnson, M. H., Maro, B. and Takeichi, M. (1986b). The role of cell adhesion in the synchronisation and orientation of polarisation in 8-cell mouse blastomeres. *J. Embryol. Exp. Morph.* **93**, 239-255.

Johnson, M. H., Pickering, S. J., Dhiman, A., Radcliffe, G. S. and Maro, B. (1988). Cytocortical organisation during natural and prolonged mitosis in mouse 8-cell blastomeres. *Development* **102**, 143-158.

Johnson, M. H. and Ziomek, C. A. (1981). The foundation of two distinct cell lineages within the mouse morula. *Cell* **24**, 71-80.

Kaye, P. L., Schultz, G. A., Johnson, M. H., Pratt, H. P. and Church, R. B. (1982). Amino acid transport and exchange in preimplantation mouse embryos. *J. Reprod. Fertil.* **65**, 367-380.

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

Legan, P. K., Collins, J. E. and Garrod, D. R. (1992). The molecular biology of desmosomes and hemidesmosomes: 'what's in a name?' *BioEssays* **14**, 385-393.

Levy, J. B., Johnson, M. H., Goodall, H. and Maro, B. (1986). Control of the timing of compaction: a major developmental transition in mouse early development. *J. Embryol. Exp. Morph.* **95**, 213-237.

Lindenberg, S., Kimber, S. and Hamberger, L. (1990). Embryo-endometrium interaction. In *From Ovulation to Implantation* (ed. J. H. L. Evers and M. H. Heineman), pp. 285-295. Elsevier Science Publishers B.V. (Biomedical Division).

Magnuson, T., Demsey, A. and Stackpole, C. W. (1977). Characterization of intercellular junctions in the preimplantation mouse embryo by freeze-fracture and thin-section electron microscopy. *Dev. Biol.* **61**, 252-261.

Manejwala, F. M., Cragoe, E. J. and Schultz, R. M. (1989). Blastocoel expansion in the preimplantation mouse embryo: role of extracellular sodium and chloride and possible apical routes of their entry. *Dev. Biol.* **133**, 210-220.

McNeill, H., Ozawa, M., Kemler, R. and Nelson, W. J. (1990). Novel function of the cell adhesion molecule uvomorulin as an inducer of cell surface polarity. *Cell* **62**, 309-316.

Nishi, M., Kumar, N. M. and Gilula, N. B. (1991). Developmental regulation of gap junction gene expression during mouse embryonic development. *Dev. Biol.* **146**, 117-130.

Pedersen, R. A., Wu, K. and Balakier, H. (1986). Origin of the inner cell mass in mouse embryos: cell lineage analysis by microinjection. *Dev. Biol.* **117**, 581-595.

Perona, R. M. and Wassarman, P. M. (1986). Mouse blastocysts hatch *in vitro* by using a trypsin-like proteinase associated with cells of mural trophectoderm. *Dev. Biol.* **114**, 42-52.

Peyrieras, N., Hyafil, F., Louvard, D., Ploegh, H. L. and Jacob, F. (1983). Uvomorulin: a non-integral membrane protein of early mouse embryo. *Proc. Nat. Acad. Sci. USA* **80**, 6274-6277.

Pratt, H. P. M. (1985). Membrane organization in the preimplantation mouse embryo. *J. Embryol. Exp. Morph.* **90**, 101-121.

Pratt, H. P. M. (1989). Marking time and making space: chronology and topography in the early mouse embryo. *Int. Rev. Cytol.* **117**, 99-130.

Reima, I. (1990). Maintenance of compaction and adherent-type junctions in mouse morula-stage embryos. *Cell Differen. Devel.* **29**, 143-153.

Sawada, H., Hoshi, M., Someno, T., Suzuki, R. and Yamazaki, K. (1992). Inhibition of mouse blastocyst hatching by subsite-specific trypsin inhibitors, peptidyl argininals. *Dev. Growth Differ.* **34**, 357-362.

Sefton, M., Johnson, M. H. and Clayton, L. (1992). Synthesis and phosphorylation of uvomorulin during mouse early development. *Development* **115**, 313-318.

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

Stevenson, B. R., Heintzelman, M. B., Anderson, J. M., Citi, S. and Mooseker, M. S. (1989). ZO-1 and cingulin: tight junction proteins with distinct identities and localizations. *Amer. J. Physiol.* **257**, C621-C628.

Stevenson, B. R., Siliciano, J. D., Mooseker, M. S. and Goodenough, D. A. (1986). Identification of ZO-1: a high molecular weight polypeptide associated with the tight junction (zonula occludens) in a variety of epithelia. *J. Cell Biol.* **103**, 755-766.

Vestweber, D., Gossler, A., Boller, K. and Kemler, R. (1987). Expression and distribution of cell adhesion molecule uvomorulin in mouse preimplantation embryos. *Dev. Biol.* **124**, 451-456.

Watson, A. J., Damsky, C. H. and Kidder, G. M. (1990). Differentiation of an epithelium: factors affecting the polarized distribution of Na^+,K^+-ATPase in mouse trophectoderm. *Dev. Biol.* **141**, 104-114.

Watson, A. J. and Kidder, C. M. (1988). Immunofluorescence assessment of the timing of appearance and cellular distribution of Na/K-ATPase during mouse embryogenesis. *Dev. Biol.* **126**, 80-90.

Wiley, L. M. (1987). Trophectoderm: the first epithelium to develop in the mammalian embryo. *Scanning Microsc.* **2**, 417-426.

Wiley, L. M., Kidder, G. M. and Watson, A. J. (1990). Cell polarity and development of the first epithelium. *BioEssays* **12**, 67-73.

Wiley, L. M. and Obasaju, M. F. (1988). Induction of cytoplasmic polarity in heterokaryons of mouse 4-cell-stage blastomeres fused with 8-cell- and 16-cell-stage blastomeres. *Dev. Biol.* **130**, 276-284.

Winkel, G. K., Ferguson, J. E., Takeichi, M. and Nuccitelli, R. (1990). Activation of protein kinase C triggers premature compaction in the four-cell stage mouse embryo. *Dev. Biol.* **138**, 1-15.