Epithelial differentiation and intercellular junction formation in the mouse early embryo

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Summary

Trophectoderm differentiation during blastocyst formation provides a model for investigating how an epithelium develops in vivo. This paper briefly reviews our current understanding of the stages of differentiation and possible control mechanisms. The maturation of structural intercellular junctions is considered in more detail. Tight junction formation, essential for blastocoele cavitation and vectorial transport activity, begins at compaction (8-cell stage) and appears complete before fluid accumulation begins a day later (approx 32-cell stage). During this period, initial focal junction sites gradually extend laterally to become zonular and acquire the peripheral tight junction proteins ZO-1 and cingulin. Our studies indicate that junction components assemble in a temporal sequence with ZO-1 assembly preceding that of cingulin, suggesting that the junction forms progressively and in the 'membrane to cytoplasm' direction. The protein expression characteristics of ZO-1 and cingulin support this model. In contrast to ZO-1, cingulin expression is also detectable during oogenesis where the protein is localised in the cytocortex and in adjacent cumulus cells. However, maternal cingulin is metabolically unstable and does not appear to contribute to later tight junction formation in trophectoderm. Cell-cell interactions are important regulators of the level of synthesis and state of assembly of tight junction proteins, and also control the tissue-specificity of expression. In contrast to the progressive nature of tight junction formation, nascent desmosomes (formed from cavitation) appear mature in terms of their substructure and composition. The rapidity of desmosome assembly appears to be controlled by the time of expression of their transmembrane glycoprotein constitutents; this occurs later than the expression of more cytoplasmic desmosome components and intermediate filaments which would therefore be available for assembly to occur to completion.

Key words: mouse preimplantation embryos, cell polarity, trophectoderm, inner cell mass, tight junctions, ZO-1, cingulin, desmosomes.

Introduction

Mammalian development begins with three consecutive and binary cell diversification events that are controlled by cell-cell interactions and give rise to the embryonic and extraembryonic lineages of the conceptus (Fig. 1). The first diversification takes place during cleavage where one group of cells differentiate into the outer trophectoderm epithelium of the blastocyst while the other group of cells differentiate into the inner cell mass (ICM). Secondly, as trophectoderm generates the blastocoele by vectorial fluid transport, this epithelium segregates into polar (proliferative) and mural (non-proliferative) subpopulations. Thirdly, the ICM segregates into primary endoderm (epithelium; at blastocoele interface) and primary ectoderm (ICM core), the latter forming the foetal lineages after implantation. These diversification processes generate firstly a radial polarity in the embryo and subsequently the embryonic-abembryonic axis (discussed in Johnson et al., 1986a).

In this paper, we are concerned with the first segregation step, which underpins all subsequent morphogenesis. There has been a number of recent reviews on the mechanisms and characteristics of trophectoderm differentiation and tissue diversification in the mouse embryo, some of which focus on specific issues (Johnson and Maro, 1986; Johnson et al., 1986a; Wiley, 1987; Fleming and Johnson, 1988; Maro et al., 1988; Pratt, 1989; Kimber, 1990; Wiley et al., 1990; Fleming, 1992). Here, we shall briefly review some of the main features and proposed models for early development before considering in more detail our more recent work on the lineage-specific maturation of the trophectoderm intercellular junctional complex. Junction maturation is clearly essential for nascent epithelial cells to integrate as a functional tissue and, we argue, is therefore crucial for the sequence of differentiative decisions outlined above.

Epithelial biogenesis: a brief overview

The earliest stage at which trophectoderm differentiation is

detectable is compaction in the 8-cell embryo. Each blastomere becomes adhesive and polarises along an apicobasal axis (apical on outer embryo surface). Polarisation embraces an extensive reorganisation of cytocortical and cytoplasmic domains of the cell. For example, in the cytoplasm, the distribution of actin filaments, microtubules and their organising centres, clathrin and endosomal organelles are all affected, generally by accumulating in the apical area. In the cortex, polarity results in the formation of an apical pole of microvilli, a basolateral distribution of uvomorulin (E-cadherin) responsible for cell-cell adhesion, and basolateral gap and nascent tight junctions. Various actin-associated cytocortical proteins also polarise and there is limited evidence that certain membrane proteins become distributed asymmetrically (reviewed in Johnson and Maro, 1986; Johnson et al., 1986a; Fleming and Johnson, 1988; Wiley et al., 1990; Fleming, 1992).

Biosynthetic inhibitors have been used to investigate the temporal regulation of compaction. Collectively, these studies indicate that compaction takes place at the 8-cell stage largely in response to post-translational changes in components synthesised as early as the 2-cell stage (about 24 hours earlier), rather than by contemporary transcriptional or translational activity (Kidder and McLachlin, 1985; Levy et al., 1986). Cell contact signalling at the time of compaction appears to regulate the orientation and spatial features of polarisation in blastomeres that are already in a permissive state for this cue (Ziomek and Johnson, 1980; Johnson and Ziomek, 1981a). The cell contact signal is most likely to be mediated by uvomorulin homotypic binding, although, in the absence of this signal, polarisation can still take place but later than normal, presumably as a result of a non-specific cue (Peyrieras et al., 1983; Johnson et al., 1986b; Blaschuk et al., 1990). Evidence from different experimental approaches indicate that, upon receipt of the uvomorulin inductive signal, aspects of cytocortical polarisation are initiated as a primary response and lead secondarily to polarisation within the cytoplasm (reviewed in

Johnson and Maro, 1986; Johnson et al., 1986a; Fleming and Johnson, 1988; Fleming, 1992).

Two mechanisms have been proposed for the establishment of a stable primary axis of polarity within the cytocortex resulting from uvomorulin adhesion. One involves the generation of a transcellular ion current driven by membrane channels and pumps (influx apically, efflux basolaterally) that would cause charged molecules in the membrane and cytoplasm, and ultimately organelles, to be mobilised, leading to a polarised state. Circumstantial evidence in support of this model has been reported (Nuccitelli and Wiley, 1985; Wiley and Obasaju, 1988, 1989; Wiley et al., 1990, 1991). A second mechanism proposed to explain axis determination in polarising blastomeres involves the phosphatidylinositol (PI) second messenger system. There is evidence that protein kinase C activation is required both for initiating uvomorulin adhesivity at compaction and for establishing cytocortical polarity in blastomeres (Bloom, 1989; Winkel et al., 1990). Moreover, specific protein phosphorylation events coincide with compaction (Bloom and McConnell, 1990; Bloom, 1991). It is possible that both a transcellular ion current and protein kinase C activation may integrate to achieve polarisation (discussed in Fleming, 1992).

In succeeding 16- and 32-cell cycles, daughter cells inheriting the apical domain of polarised 8-cell blastomeres (see Fig. 1) continue to differentiate into a functional epithelium, achieved by the late 32-cell stage when cavitation occurs. This progression embraces various cellular features, including maturation of cell-cell adhesion systems and junction formation (see later), basement membrane formation, cytoplasmic reorganisation, and the establishment of membrane polarity. These topics have been reviewed in detail elsewhere (Johnson et al., 1986a; Fleming and Johnson, 1988; Fenderson et al., 1990; Kimber, 1990; Wiley et al., 1990; Fleming, 1992). In consequence, the nascent trophectoderm at the late 32-cell stage acquires the capacity for vectorial fluid and selective molecular transport. Blas-
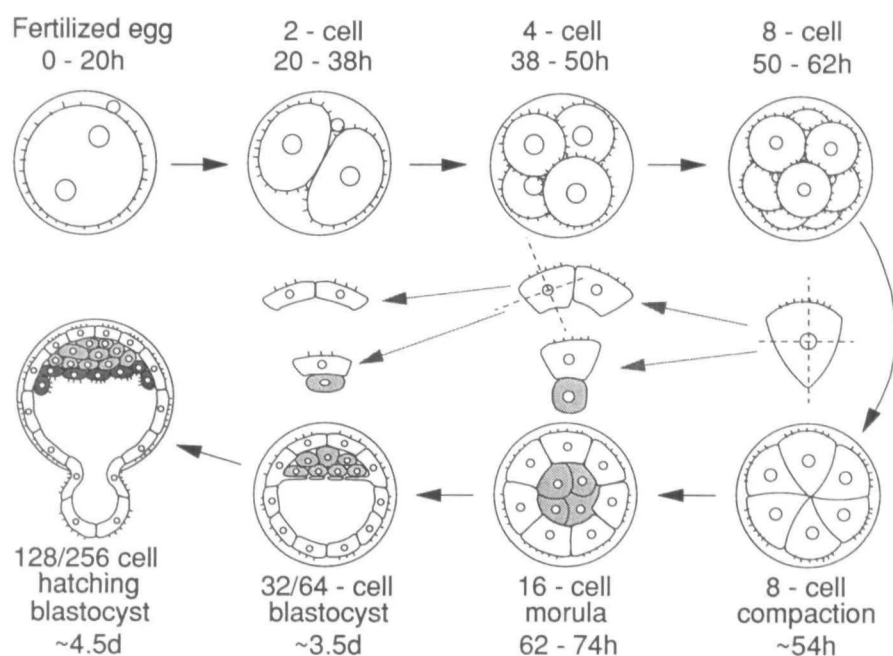

Fig. 1. A schematic representation of mouse preimplantation development. Embryo stage and time elapsed since fertilization are indicated. In the centre, the conservative and differentiative division planes (dotted lines) of polar 8- and 16-cell blastomeres are shown, which lead to distinct trophectoderm and ICM cell lineages. Shaded cells belong to the ICM lineage, which, in the hatching blastocyst, differentiates primary endoderm at the blastocoele surface (darker shading). Reprinted from Fleming (1992) with permission of publishers, Chapman and Hall.

tocoele formation is largely a result of Na⁺ influx at the apical membrane via several transporters, including the Na⁺/H⁺ exchanger and Na⁺-channel (Manejwala et al., 1989), and active Na⁺ efflux basally, mediated by functional Na⁺,K⁺-ATPase (reviewed in Wiley, 1987), thereby generating passive water flow in the apicobasal direction. Regulation of this important early function of the epithelium is indeed complex. Regulatory processes include biosynthetic control of Na⁺,K⁺-ATPase expression (Gardiner et al., 1990; Kidder and Watson, 1990; Watson et al., 1990b) and subsequent basal polarisation (Watson et al., 1990a), the formation of a permeability seal to prevent blastocoele leakage (Magnuson et al., 1978; Van Winkle and Campione, 1991), and a physiological control mediated by cAMP (Manejwala et al., 1986; Manejwala and Schultz, 1989).

Intercellular junction formation

The junctional complex of epithelial cells is a fundamental characteristic of this phenotype, essential for polarised cellular function and tissue formation (see reviews by Staehelin, 1974; Garrod and Collins, 1992). The complex consists of an apicolateral tight junction (zonula occludens), an intermediate zonula adherens, and lateral membrane gap and desmosomal junctions, each possessing distinct structural and molecular properties. In the mouse embryo, the formation of these specialised junctions is temporally regulated and, with the exception of gap junctions, tissue-specific. Gap junctions form from the 8-cell stage (Lo and Gilula, 1979; Goodall and Johnson, 1984), following expression of different junctional components either from maternal genes or during early cleavage (McLachlin et al., 1983; McLachlin and Kidder, 1986; Barron et al., 1989; Nishi et al., 1991). Inhibition of gap junctional communication at the time of compaction by different means fails to prevent cell polarisation (Goodall, 1986; Levy et al., 1986) but does inhibit the adhesion and integration of such cells into the morula and blastocyst (Lee et al., 1987; Buehr et al., 1987; Bevilacqua et al., 1989). The zonula adherens is not a prominent junction type in the embryo and its maturation has received little attention. However, the adherens junction components, vinculin and α-actinin, begin to localise apicolaterally from the compaction stage (Lehtonen and Reima, 1986; Lehtonen et al., 1988; Reima, 1990). Tight junction and desmosome formation have been studied in greater detail and are discussed below.

Tight junctions

The tight junction is a belt-like structure around the cell apex where the membranes of adjacent epithelial cells are closely apposed, and possibly partially fused. The freeze-fracture image of the tight junction, composed of an anastomosing network of intramembraneous cylindrical fibrils (P-face) and complementary grooves (E-face) has been well documented (eg, Staehelin, 1974). These junctions restrict uncontrolled paracellular transport between mucosal and serosal compartments by occlusion of the intercellular space, thereby contributing in large part to the transepithelial electrical resistance. They are also thought to act as a

barrier to the mixing of apical and basolateral integral membrane proteins and exoplasmic leaflet lipids, thereby helping to preserve membrane polarity. For recent reviews of tight junction structure and function, see Stevenson et al. (1988) and Cereijido (1991).

Tight junctions are multimolecular complexes that are as yet poorly understood in terms of their composition (reviewed in Anderson and Stevenson, 1991). The integral membrane component providing the freeze-fracture image is undefined. However, a total of four peripheral membrane proteins have been proposed as tight junction components, two of which have been characterised in some detail. ZO-1, originally identified in mouse liver membrane fractions, was the first protein reported as a ubiquitous and specific component of the tight junction in a variety of epithelia (Stevenson et al., 1986). ZO-1 is a high relative molecular mass (215-225×10³) protein that, from extraction and immunogold studies, is avidly associated with the cytoplasmic face of the junction, positioned very close to the membrane domain (Anderson et al., 1988; Stevenson et al., 1989). The biophysical properties of ZO-1 suggest that it is an asymmetric monomer and is phosphorylated on serine residues (Anderson et al., 1988). Cingulin (140×10³ M_r) is a second ubiquitous component of tight junctions from various sources, originally identified in avian intestinal epithelium (Citi et al., 1988, 1989). A purified 108×10³ M_r polypeptide region of cingulin occurs as a heat-stable elongated homodimer organised in a coiled-coil configuration similar to the myosin rod. Cingulin, like ZO-1, is a phosphoprotein, and the assembly of both molecules at the tight junction may be regulated by kinase activity (Denisenko and Citi, 1991; Nigam et al., 1991). Cingulin is localised more cytoplasmically than ZO-1 at the tight junction of various epithelia following double labelling immunogold analysis (Stevenson et al., 1989). Other likely tight junction-associated proteins are a 192×10³ M_r polypeptide in rodent liver recognised by monoclonal antibody BG9.1 (Chapman and Eddy, 1989) and 160×10³ M_r protein that co-immunoprecipitates with ZO-1 from MDCK cells (Gumbiner et al., 1991). In addition, actin filaments have been visualised in ultrastructural preparations at the cytoplasmic face of tight junctions (Madara, 1987).

Temporal expression and assembly of tight junction proteins

The development of the tight junction in mouse embryos has been examined structurally and immunologically. Freeze-fracture and thin-section studies have shown that at compaction in the 8-cell embryo, focal apicolateral contact sites are formed exhibiting an intramembraneous particle organisation and associated cytoplasmic density typical of tight junctions. During the morula stage, outer blastomeres gradually acquire a belt-like tight junction organisation as focal sites extend laterally, becoming facial and then zonular in appearance before cavitation begins (Ducibella and Anderson, 1975; Ducibella et al., 1975; Magnuson et al., 1977; Pratt, 1985). Immunolocalisation of ZO-1 follows a similar pattern to these structural events. ZO-1 sites first appear at compaction as a series of punctate foci distributed along the apicolateral contact region; in morulae, these sites become a series of discontinuous lines before appear-

ing zonular (Fleming et al., 1989). In the blastocyst, tro-phectoderm cells are bordered by a prominant belt-like ZO-1 distribution (Fig. 2). Immunoblotting and cellular experiments involving biosynthetic inhibitors suggest that ZO-1 is first synthesised from the late 4-cell/early 8-cell stage, although it appears that the gene is transcribed earlier in the third cell cycle (Fleming et al., 1989). See Fig. 3 for a summary of tight junction protein expression patterns during early development.

The expression and membrane assembly of cingulin in the early embryo shows a pattern both complex and quite distinct from that of ZO-1 (Javed et al., 1992; Fleming et al., 1992). The complexity of cingulin expression is due mainly to the fact that the protein is synthesised not only by the embryonic genome but also by the maternal genome. One consequence of this is that cingulin localisation is not restricted to developing tight junctions, a factor that must be borne in mind when considering biosynthetic control of junction assembly. Maternally expressed cingulin is detectable as a 140×10^3 M_r protein in unfertilised eggs following either immunoblotting or immunoprecipitation. Synthesis of maternal cingulin is unaffected by fertilisation but runs down after first cleavage, corresponding with the time of global maternal transcript degradation (Schultz, 1986), and the initiation of embryonic gene activity (Flach et al., 1982). Maternal cingulin is not associated with tight junctions but is localised in the egg cytocortex as a uniform submembraneous layer in the microvillous domain of the egg, being depleted or absent in the smooth membrane domain overlying the metaphase II spindle (Fig. 3). We envisage that maternal cingulin may function during oogenesis as a cytocortical component in the interaction between cumulus cell processes and the oocyte surface where uvomorulin (Vestweber et al., 1987), and gap and desmosome junctions (Anderson and Albertini, 1976) have been identified previously. Indeed, cingulin is detectable at both the cumulus and oocyte sides of this contact site in ovarian pre-ovulatory follicles (Fleming et al., 1992).

Cingulin synthesis from the embryonic genome occurs at very low levels during early cleavage (approx. tenfold less than in unfertilised eggs) but is enhanced significantly at compaction in the 8-cell embryo (Javed et al., 1992). This enhancement therefore occurs later than the period identified for ZO-1 synthesis (see above). The time at which cingulin is detectable at the developing tight junction is also later than that of ZO-1, in most embryos (or synchronised cell clusters) being during the 16-cell stage (Fleming et al., 1992). This delay suggests that junction maturation at the molecular level is a sequential process, at least partly controlled by the varied time of synthesis of different junction components.

Although such a model is attractive, we must also consider whether maternal cingulin contributes to tight junction formation in the early embryo. This possibility is validated by the facts that proteins in the egg or embryo tend to have a long half-life (eg, Merz et al., 1981; Barron et al., 1989) and that cytocortical cingulin (characteristic of the egg) is also detectable during cleavage up to the morula stage (Fleming et al., 1992). Significantly, cytocortical cingulin is associated preferentially with the outer, more microvillous, membranes of the embryo (e.g., apical poles

of 8- and 16-cell blastomeres, see Fig. 3), which, in undisturbed embryos, would be relatively older membrane inherited from the egg (see Pratt, 1989). However, two factors argue against maternal cingulin contributing to junction development in the embryo. First, pulse-chase metabolic labelling and immunoprecipitation data suggest that newly synthesised maternal cingulin is turned over quite rapidly ($t_{\frac{1}{2}}\sim4$ hours) compared with that of embryonic cingulin at the blastocyst stage ($t_{\frac{1}{2}}\sim10$ hours), and is therefore unlikely to persist during the 24-36 hour period between the decline of maternal expression (2-cell) and the time of cingulin assembly at junctions. Second, cytocortical cingulin during early cleavage does not appear to be a stable component of the membrane but rather characterises those sites where microvillous growth takes place. Thus, if 4-cell or early 8-cell embryos are disaggregated and then reaggregated such that original cell orientations are randomised, by the time of compaction (6-12 hours later), cytocortical cingulin is localised not at its original site but at the new outer surface where microvillous poles develop. Moreover, prior to compaction, the low level of embryonic cingulin turns over with similar rapid kinetics ($t_{\frac{1}{2}}\sim4$ hours) to maternal cingulin.

Collectively, these and other experiments indicate that the cytocortex represents a labile site of assembly that is available for cingulin expressed either from maternal or embryonic genomes. However, it is conceivable that this site may still act as a pool for tight junctional assembly during the morula period. This is unlikely since tight junctional assembly of cingulin is sensitive to cycloheximide treatment (Fleming et al., 1992), indicating incorporation exclusively of newly synthesised protein. The function of cytocortical cingulin during cleavage is therefore elusive but may be explained by the retention of binding sites associated with microvillous membrane that were required for cumulus cell interactions earlier in development. Cytocortical cingulin persists up until the late morula/early blastocyst stage when endocytic activity at the apical membrane results in the internalisation and gradual disappearance (and presumably degradation) of this site (Fig. 3; Fleming et al., 1992).

Regulation of tight junction protein membrane assembly

Although biosynthetic events appear to influence the sequence of molecular assembly at the tight junction, cell-cell contacts clearly provide spatial control of assembly. Thus, if uvomorulin-mediated cell adhesion at compaction is inhibited by specific antibody incubation or extracellular calcium depletion, ZO-1 assembly at the membrane is delayed and, significantly, is randomly distributed rather than apicolateral with respect to cell contact position (Fleming et al., 1989). A permissive role for uvomorulin adhesion in ZO-1 assembly has also been identified in MDCK cells (Siliciano and Goodenough, 1988) and Caco-2 cells (Anderson et al., 1989), and is likely to reflect the need for close and stable cell contact for tight junction formation to occur (Gumbiner et al., 1988). Our recent finding that cingulin synthesis is enhanced significantly at compaction (Javed et al., 1992), and that ZO-1 protein level in Caco-2 cells increases following provision of cell adhesion (Anderson et al., 1989), both suggest that cell adhesion may promote the expression of tight junctional components as well
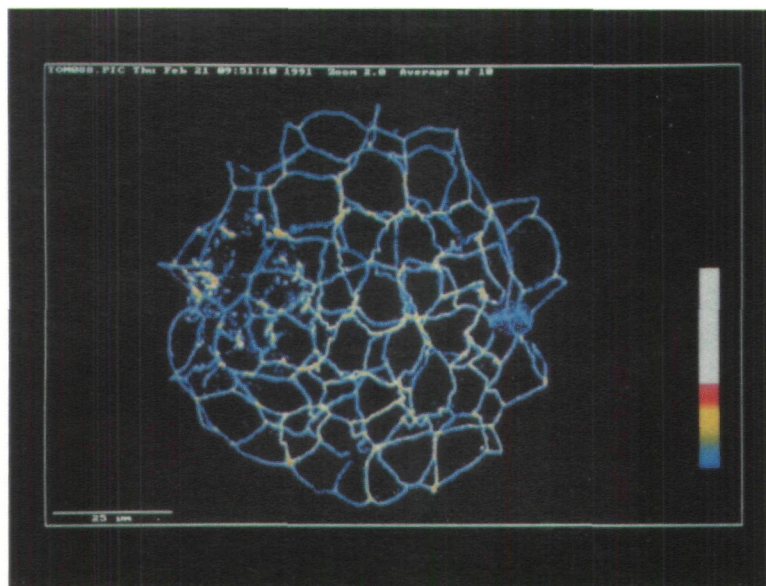
Fig. 2. ZO-1 immunolocalisation in a complete blastocyst viewed by confocal microscopy. Intensity of ZO-1 immunofluorescence is indicated by the colour code (white, highest; blue, lowest). Note the zonular network of ZO-1 that is associated with the trophectoderm (from S. Spong and T. Fleming, in preparation).
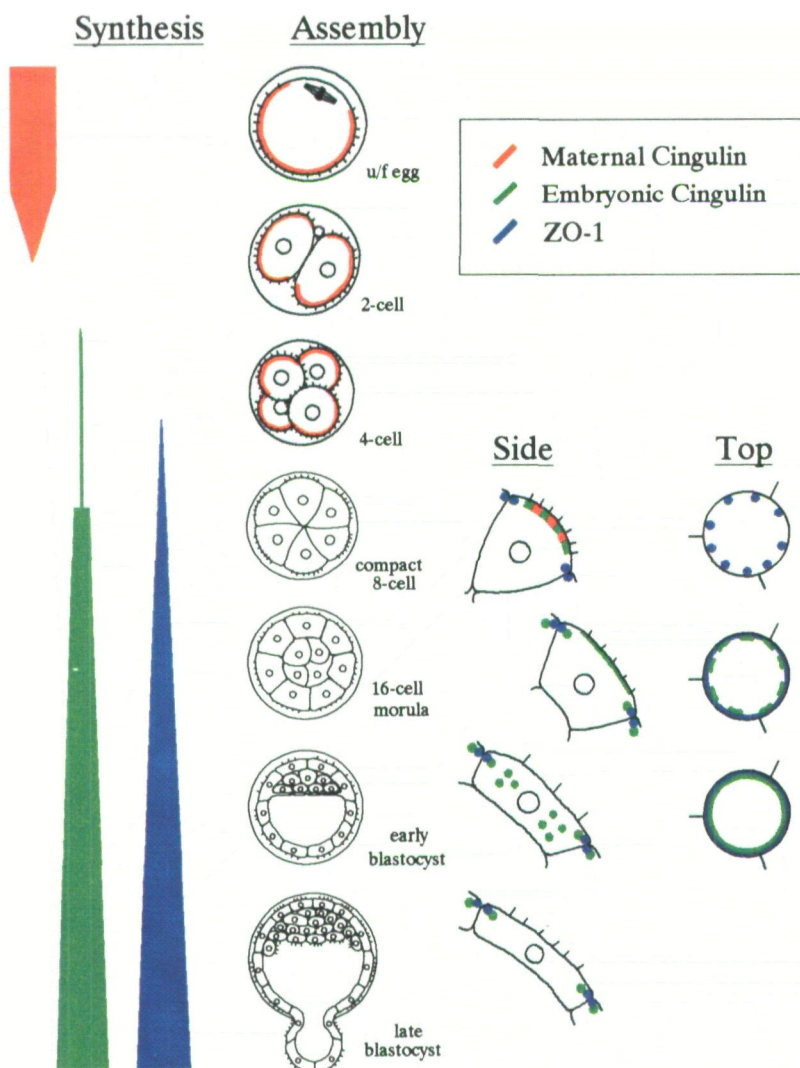

Fig. 3. Summary model of tight junction protein expression in the embryo. Relative levels of synthesis at each stage are shown on the left, measured directly (immunoprecipitation) for maternal and embryonic cingulin, but only estimated for ZO-1 from immunoblotting and localisation studies involving cycloheximide treatments. Assembly sites are shown on the right, with side and top views of the trophectoderm lineage at later stages enlarged. Cytocortical sites have been excluded from top views for clarity. The heterogeneous pattern in the apical cytocortex at compaction is intended to represent the run down of maternal cingulin and its replacement by embryonic cingulin. Cytoplasmic foci of embryonic cingulin in blastocysts represent endocytosed cytocortical sites. See text for details.

as their membrane assembly. In addition to cell adhesion, we have identified three other requirements for membrane assembly of ZO-1 in the embryo (Fleming et al., 1989; Fleming and Hay, 1991). First, assembly is dependent upon intact microfilaments but not microtubules. Second, the adjacent cell must be equally competent to form tight junctions (demonstrated in heterogeneous 4-cell + 8-cell couplets), suggesting that apicolateral assembly is dependent upon molecular interactions that traverse the intercellular boundary. Third, a contact-free membrane surface, but not necessarily apical in terms of molecular character, must be preserved to maintain ZO-1 membrane assembly. This last requirement appears to contribute to tissue-specific control of ZO-1 expression in the embryo and is discussed below.

In addition to the regulation of tight junction formation per se, it is also important to understand how this process integrates with other features of epithelial maturation occurring in the trophectoderm lineage. Since experimental evidence has suggested that cell polarisation at compaction is established initially in the cytocortex and leads secondarily to polarisation within the cytoplasm (see earlier), it is therefore of interest to determine whether the specification of the tight junctional domain in the cytocortex may be a primary event in cell polarisation. The close temporal correlation between the onset of tight junction formation and microvillous polarisation at compaction (Ducibella and Anderson, 1975; Fleming et al., 1989) is consistent with an important role for the tight junction in global cell polarisation. However, it has been possible in two experimental situations to test this proposition. First, in the 4-cell + 8-cell heterogeneous couplets referred to above, tight junction formation is perturbed (assayed by ZO-1 assembly) yet the establishment of uvomorulin adhesion and microvillous polarisation in the 8-cell blastomere is not affected. Second, cycloheximide treatment of synchronised 8-cell couplets can inhibit expression and assembly of ZO-1, again without disturbing the onset of adhesion and microvillous polarisation (Fleming et al., 1989). Thus, tight junction formation appears not to be an essential causal event in the generation of a polarised phenotype in the embryo, a conclusion consistent with experimental evidence derived from other epithelial systems (Vega-Salas et al., 1987; McNeill et al., 1990).

Another regulative feature of tight junction development in the embryo is that during blastocyst formation these junctions are tissue-specific, being confined to the trophectoderm lineage. In our analysis of cingulin synthesis during early development, we have indicated that net synthesis rates are also tissue-specific, being up to fifteen-fold higher in the trophectoderm than in the ICM of expanding blastocysts (Javed et al., 1992). To investigate the cellular mechanisms underlying tissue-specificity of tight junction formation and related synthetic activity, we have monitored ZO-1 immunolocalisation during and after cell divisions leading to the distinct lineages of the blastocyst (Fleming and Hay, 1991). These lineages are established by differentiative divisions of polarised 8- and 16-cell blastomeres such that daughter cells inherit either the apical (polar; trophectoderm lineage) or the basal (nonpolar; ICM lineage) region of their parent cell (Johnson and Ziomek, 1981b; Pedersen et al., 1986; Fleming, 1987; see Fig. 1). During

differentiative divisions, ZO-1 is inherited by both polar and nonpolar daughter cells and localises to the periphery of the contact site. Once nonpolar cells are fully enclosed by polar cells (as occurs in the embryo interior), their ZO-1 sites are destabilised, first by dispersing into punctate foci distributed randomly on the cell membrane and then disappearing altogether. Thus, loss of cell contact asymmetry initiates ZO-1 down-regulation, a process that is fully reversible by re-exposing nonpolar cells to conditions of asymmetric contacts (Fleming and Hay, 1991). By culturing ICMs isolated from early blastocysts in the presence of biosynthetic inhibitors, it has been possible to investigate the level at which ZO-1 down-regulation is controlled. Data from these experiments indicate that the ICM lineage retains transcripts for ZO-1 that can be utilised to re-express the protein rapidly upon restoration of cell contact asymmetry. Down-regulation at the translational rather than the transcriptional level may be advantageous for the developing ICM in terms of processing efficiency if such transcripts become available for primary endoderm differentiation at the blastocoele interface (Fleming and Hay, 1991).

Desmosomes

The disc-shaped desmosome junction is characterised by membrane-associated cytoplasmic plaques positioned in register in apposed cells and to which intermediate filaments attach, and an intercellular adhesive domain possessing a dense midline. These junctions are thought to contribute to epithelial tissue formation and integration. Desmosomes are composed of numerous interacting components that can be broadly classified into desmosomal proteins (dp) and transmembrane glycoproteins (dg). The principal constituents have been characterised and include dp1 and dp2 (250 and 215×10^3 M_r, desmoplakins), dp3 (83×10^3 M_r, plakoglobin), dg1 ($175-150\times10^3$ M_r, desmoglein I) and dg2 and dg3 (115 and 107×10^3 M_r, desmocollins) (reviewed in Garrod et al., 1990; Schwarz et al., 1990). Dp and dg constituents occupy distinct morphological domains of the desmosome complex. In bovine epidermis, dg2 and dg3 are located mainly in the intercellular space, dg1 spans the intercellular space and the plaque, dp3 is in the plaque, and dp1 and dp2 are at the cytoplasmic side of the plaque where intermediate filaments attach (Miller et al., 1987). Although desmosome assembly has been studied in considerable detail in cultured epithelial cells by manipulating extracellular calcium levels (reviewed in Garrod et al., 1990; Garrod and Collins, 1992), little is known of how assembly might be regulated in vivo, as part of a morphogenesis programme.

In the mouse embryo, desmosomes first appear in the trophectoderm once cavitation is initiated (32- to 64-cell stage) (Ducibella et al., 1975; Magnuson et al., 1977; Fleming et al., 1991). Ultrastructurally, nascent desmosomes appear mature, containing an intercellular midline organisation, cytoplasmic plaques and associated cytokeratin filaments. The latter are first formed earlier during cleavage, predominantly in the 16-cell embryo (Chisholm and Houliston, 1987). The mechanisms controlling desmosome biogenesis have been studied by monitoring the expression of the principal desmosomal proteins and glycoproteins using specific antibodies (Fleming et al., 1991). Dp1+2, dg1, and

dg2+3 are all first detectable by immunofluorescent labelling at the 32-cell stage, specifically in the trophectoderm after cavitation has begun, at punctate sites along apposed lateral membranes. Dp3 is also first detectable at the 32-cell stage, but initially is not tissue-specific, is linear in appearance at membrane contact sites, and is evident before cavitation. This distinction is transitory and may reflect the wider distribution of dp3 at non-desmosomal locations.

These results imply that nascent desmosomes in the embryo contain all the principal molecular components identified for these junctions, consistent with their mature appearance ultrastructurally. However, no indication is evident for the timing of desmosome assembly at this stage of development. Immunoprecipitation of desmosome constituents from staged embryos has shown that, in contrast to membrane assembly events, their time of initial synthesis is quite variable. Thus, dp3 synthesis is detectable at least from the 8-cell stage, dp1+2 from the 16-cell stage, and dg1 and dg2+3 from cavitation onwards (Fleming et al., 1991). This temporal sequence implicates a key role for desmosomal glycoprotein expression (either transcription or translation) in junction assembly. The intriguing correlation between cavitation and desmosome formation awaits further investigation. It will be of interest to establish the causal sequence if any in this relationship.

Conclusions

The mouse embryo continues to be an exciting model in which to study mammalian cell differentiation. Trophectoderm biogenesis takes place rather slowly with long cell cycles and can do so in the face of considerable experimental abuse, an ideal combination for the mechanistic approach. It is also a 'real' tissue and, despite the limitation of material for biochemical studies, offers some opportunities not available in parallel epithelial cell culture systems. For example, the focus in this review has been on cell junctions, and it is clear that consideration of maturational events along a time axis can be instructive for identifying how assembly might be controlled. At the molecular level, it appears that the tight junction is formed sequentially, with the assembly of membrane and membrane-associated constituents (intramembraneous particles, ZO-1) preceding that of more cytoplasmic constituents (cingulin). Moreover, the assembly of ZO-1 at compaction coincides with an upsurge in the synthesis of cingulin. Are these events causally linked? In contrast, the desmosome appears to form at the membrane in a single step, and may be regulated by the sequential expression of intrinsic components, with the last to be synthesised in this case being the membrane constituents (dgs) which trigger assembly. Our future goals will include the inhibition of specific proteins from participating in assembly events and monitoring the consequences for junction formation and early morphogenesis.

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