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Cell fate regulation during preimplantation development: A view of adhesion-linked molecular interactions

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ABSTRACT

In the developmental process of the early mammalian embryo, it is crucial to understand how the identical cells in the early embryo later develop different fates. Along with existing models, many recently discovered molecular, cellular and developmental factors play roles in cell position, cell polarity and transcriptional networks in cell fate regulation during preimplantation. A structuring process known as compaction provides the "start signal" for cells to differentiate and orchestrates the developmental cascade. The proper intercellular junctional complexes assembled between blastomeres act as a conducting mechanism governing cellular diversification. Here, we provide an overview of the diversification process during preimplantation development as it relates to intercellular junctional complexes. We also evaluate transcriptional differences between embryonic lineages according to cell- cell adhesion and the contributions of adhesion to lineage commitment. These series of processes indicate that proper cell fate specification in the early mammalian embryo depends on junctional interactions and communication, which play essential roles during early morphogenesis.
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Introduction

Mammalian development starts with the relatively long voyage of the embryo along the oviduct toward the uterus. The duration of this step varies according to the species, but it has a single purpose: forming a blastocyst to implant in the uterus. However, the majority of human embryos cannot complete this journey and fail to reach the blastocyst stage and/or implant (Ellish et al., 1996). Thus, this dynamic period is the most vulnerable process in mammalian development. During this period, the embryo undergoes a sequence of cellular, molecular and epigenetic changes, leading to the lineage segregation of blastomeres inside the developing blastocyst (Albert and Peters, 2009; Fujimori, 2010). Following these changes, specialization occurs, and three distinct lineages can be distinguished within the blastocyst: the pluripotent epiblast (EPI) and two extraembryonic lineages—the trophoblast (TE) and primitive endoderm (PE).

Expansion and differentiation of embryonic cell lineages occur as a series of events. These events work in perfect sequence during the developmental process. During the initial rounds of cleavage divisions, blastomeres morphologically have the same identity (Duranthon et al., 2008). Morphological differentiation is first observed during compaction, when blastomeres become adhesive and polarized. First, the junctional complexes are gradually formed at exclusively apicolateral and lateral sites; second, polarization is established within the outer cells (Eckert and Fleming, 2008; Johnson et al., 1986; Johnson and Ziomek, 1981). Both compaction and polarization processes in the 8-cell-stage mouse embryo generate cellular asymmetry leading to cellular diversification. As a result of cellular asymmetries, two differentiative cell divisions take place at the fourth (8–16-cell transition) and fifth (16–32-cell transition) cleavages and generate the outer (polarized) and inner (non-polarized) progenitors of the TE and inner cell mass (ICM), which later form the PE and EPI lineages (Can, 2014). Although human embryos go through these stages later in development compared with mouse embryos, as evidenced by embryonic genome activation (EGA) or blastocyst formation, both ultimately form the same lineages, which will contribute to the same layers (Fig. 1). To reveal the cellular origins of blastocyst lineages, it is important to understand the morphological changes controlled by compaction and polarization events that lead to blastocyst formation. Here, we briefly review how the intercellular junctional complexes contribute to this cellular diversification at the 8–16-cell stage onwards in the mouse, which is the most well-studied animal model of early embryo development, and in humans.

Preimplantation development: from fertilization to implantation

The developmental period between fertilization and implantation is defined as preimplantation, and it lasts approximately

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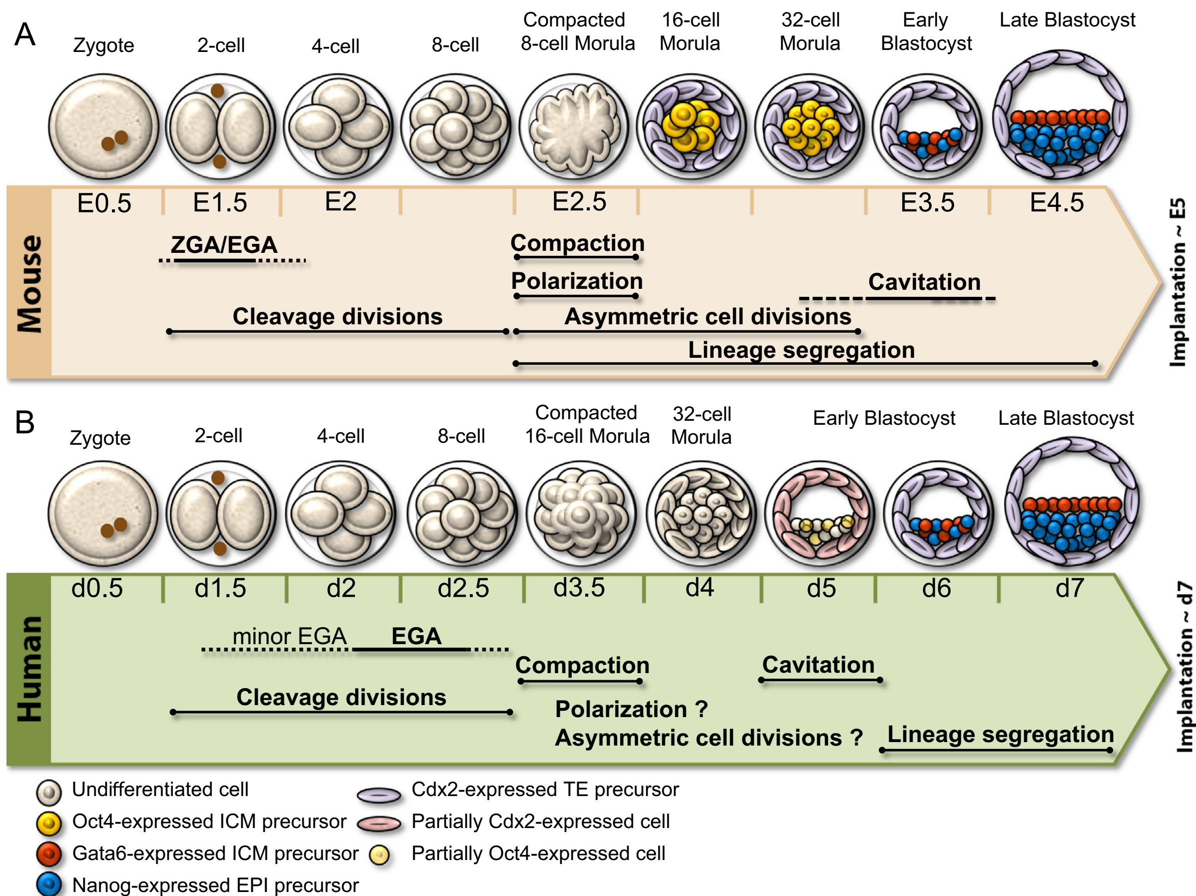

Fig. 1. Timing of preimplantation development events in mouse (A) and human embryos (B). (A) In the mouse, the zygote undergoes three rounds of cleavage to become an 8-cell embryo, and the compaction occurs at E2.5, mediated by E-cadherin activation. Lineage segregation concurrently begins at E2.5 with TE differentiation during compaction. Establishment of the intercellular complexes generates polarized blastomeres and results in asymmetric (differentiative) cell divisions (from E2.5 to E3.5) that produce daughter cells of different sizes that inherit different lineage-specific transcriptional regulators; these cells are considered precursors. After the formation of the cavitation at E3.5, randomly distributed PE and epiblast precursors within the ICM are sorted into their ultimate localization at E4.5, when all three embryonic lineages can be distinguished clearly. (B) Although the human preimplantation period progresses through the same sequence of events, the timing is quite different, with human embryos showing delayed development compared to mouse embryos. In humans, the zygote undergoes four rounds of cleavage, existing as a 16-cell embryo when the compaction occurs at day 3.5 (mediated by E-cadherin activation). Precursor cells first appear during cavitation at day 6, two days later than in mice. Lineage segregation is completed at approximately day 7, when all three embryonic lineages can be distinguished. Retardation of lineage segregation in human embryos occurs due to the prolonged coexpression of Cdx2 and Oct4 in the TE. Polarization and asymmetric cell division events in human preimplantation embryos have not yet been identified.

4.5 days in mice and approximately one week in humans (Fig. 1) (Niakan et al., 2012). Fertilization forms a diploid cell called a zygote, which will give rise to a new organism. The zygote is not an ordinary cell; rather, it has an enormous potential that can reflect all features of cellular components. Once fertilization is accomplished, the zygote undergoes successive cleavage divisions within the following days that occur at intervals of 12–24 h. The first cleavage division takes place approximately 16–20 h after fertilization in mice and 30 h after fertilization in humans. Although there are different opinions about the position of the first cleavage axis (Davies and Gardner, 2002; Plusa et al., 2002), the most commonly accepted model is that the first cleavage axis is associated with the position of the polar bodies; thus, it is assumed that polar bodies guide the first cleavage. One of the major events in preimplantation development is the zygotic or, more appropriately, embryonic genome activation (ZGA or EGA), which begins at the 2-cell stage in mice and approximately the 4- to 8-cell stage in humans; minor human EGA may also occur at the 2-cell stage (Fig. 1) (Taylor et al., 1997). This process constitutes *de novo* nuclear reprogramming and ensures the transcriptional competence of the early embryo. Activation of embryonic transcription is accompanied by the deadenylation of DNA and finally the degradation of maternally provided RNAs. Extensive reviews on EGA can be found elsewhere (Duranthon et al., 2008; Hamatani et al., 2004; Li et al., 2010; Zeng and Schultz, 2005).

Preimplantation development can be divided in two successive phases with respect to the transcriptional profile of the embryo: phase I represents the oocyte-to-embryo transition (until the late 2-cell stage in mouse embryos or the 4-8-cell stage in human embryos), and phase II represents the cellular differentiation from EGA until the blastocyst stage (Zernicka-Goetz et al., 2009). In the mouse embryo, initially, transcriptional profile of cells becomes dissimilar by the orientation and order of second cleavage division (Piotrowska-Nitsche and Zernicka-Goetz, 2005; Plachta et al., 2011; Torres-Padilla et al., 2007). Such differentiated profile allows them to gain a different developmental fate and potency (Tabansky et al., 2013; Torres-Padilla et al., 2007). On the other side, the first structural alteration of embryos within phase II is the compaction process, which occurs at the mid-8-cell stage in mouse (E2.5) and at the 16-cell stage in humans (day 3.5). Compaction is the triggering event of morphogenetic and cellular differentiation. The most important event occurring during compaction is the emergence of two different cell populations: the outer blastomeres residing outside the embryo are selected to form the trophectoderm (TE) layer, whereas blastomeres that are situated inside are selected to form the ICM. Upon completion of

compaction, trophectodermal cells are gathered by newly assembled tight junctional constituents and begin to form a flattened epithelial cell layer structure outside the developing embryo. When a mouse embryo reaches the 32-cell stage (approximately), trophectodermal cells begin pumping the environmental fluids through a transcellular pathway to fill the forming blastocyst cavity. Several genes control the cavitation process, including E-cadherin, catenin, tight junction proteins and the sodium-potassium ATPase transport system, all of which are also responsible for lineage specification in developing blastocytes (Watson and Barcroft, 2001). The active Na/K-ATPase transport mechanism located on the basolateral surface of the trophectoderm cells plays an important role in forming an ion concentration gradient throughout the epithelium, thus ensuring the flow of water to the blastocoel. In addition, the emergence of tight junction complexes provides an impermeable barrier between the trophectodermal cells, contributing to the polarization of Na/K-ATPase distribution and allowing fluid collection and organization of paracellular transport (Watson and Barcroft, 2001). Establishment of intercellular complexes is therefore directly linked with embryonic cell differentiation, which is a consequence of unique cellular events including compaction and polarization, which will be discussed in detail in the following sections.

Timing of compaction: origin of TE/ICM/PE lineages

A common feature of preimplantation embryos of all eutherian mammalian species is the process known as 'compaction', which is the formation of protein complexes that are linked to the cytoskeleton, resulting in 'flattening' of the blastomeres. Until the mid-8-cell stage in mouse or the 16-cell stage in humans, cell boundaries are distinct and clearly visible in each blastomere. During the first three cleavages (the first four cleavages for humans), each blastomere is morphologically identical. The zygote and each blastomere of early embryo (from 2-cell to 4-cell) are assumed to be totipotent (Mitalipov and Wolf, 2009). However, after compaction, cell adhesion dramatically increases, which ultimately results in the polarization and thus differentiation of cells, causing the regression of totipotency. Those different identities give rise to specialized layers: first the TE layer and later the EPI and PE layers become established. Therefore, compaction is considered the most critical event for altering the uniform cell appearance and allowing cellular diversity.

During the compaction period, *de novo* intercellular junctional complexes consisting of apicolateral tight junctions (zonula occludens), intermediate adherens junctions (zonula adherens), lateral membrane gap junctions (nexus) and desmosomal junctions (macula adherens) have been started to form, each possessing distinct structural and molecular properties and performing fundamental roles in cellular communication, adhesion, and differentiation.

Gap junctions (GJs)

A gap junction (GJ) or nexus is a specialized form of intercellular connection. It is a complex of proteins spanning the plasma membrane of neighboring cells and maintaining cellular homeostasis by allowing the passage of low-molecular-weight (<1 kDa) signaling molecules or ions (Makowski et al., 1977). This type of junction is composed of proteins of the connexin (Cx) family; a hexameric assembly of Cx proteins forms a 'connexon', which forms half of the channel (hemichannel) in each cell. Two connexons constitute one GJ connecting across the intercellular space. Connexons are divided into two groups: homomeric connexons, consisting of the same type of connexin, and heteromeric connexons, consisting of multiple connexins. Over 20 different connexin genes have been identified to date, and connexin mRNA expression in the preimplantation embryo varies by developmental stage. For example, in mice, Cx30, Cx31, Cx36, Cx43, Cx45 and Cx57 are expressed beginning in the 2-4-cell stage; Cx30.3, Cx31.1 and Cx40 are expressed beginning in the 8-cell stage (reviewed by (Houghton, 2005). Cx32 and Cx43 are the essential connexins contributing to GJ formation in both mouse and human embryos (Hardy et al., 1996). Translation of connexin mRNAs to proteins occurs shortly after their mRNA synthesis, and the proteins then accumulate in the cytoplasm. After the cell-cell adhesion is fully established between blastomeres, compaction occurs, and Cx43 becomes phosphorylated and accumulates at the plasma membrane to form GJs. Functional GJ formation is first observed during the 8-cell stage in mice (Lo and Gilula, 1979) but not until the early blastocyst stage in humans (Dale et al., 1991). Studies have shown that compaction and GJ formation events in early embryos are independent but temporally correlated processes (Kidder et al., 1987). However, the precise functions of GJs are still controversial throughout preimplantation development because the genetic variation of Cx genes makes GJs difficult to investigate.

Desmosomal junctions (DJs)

A desmosomal junction (DJ) or macula adherens (MA) is one of the specialized cellular junctions classified as 'anchoring junctions', which are highly organized, disk-shaped adhesions arranged randomly at the cell-contact sites of the lateral membrane, where intermediate filaments are anchored. DJ proteins belong to three gene families: cadherins, armadillo proteins, and plakins (Huber, 2003). Desmosomal cadherins are composed of two single-pass transmembrane glycoproteins: desmogleins (Dsg1-4) and desmocollins (Dsc1-3). These glycoproteins mediate Ca^{2+}-dependent cell adhesion. Whereas the extracellular domains of desmosomal cadherins form adhesive links between cells, the cytoplasmic domains of desmosomal cadherins associate with the armadillo proteins, including plakoglobin and plakophilins. The plakin family proteins include desmoplakin, which is linked to the intermediate filament cytoskeleton (Garrod and Chidgey, 2008). The structure, described as a 'desmosomal plaque', has a unique anchoring feature and provides cells with great mechanical integrity, allowing them to resist mechanical stress.

In early embryos, cytokeratins are initially synthesized at the 4-cell stage and assemble into intermediate filaments at the 8-cell stage (Chisholm and Houliston, 1987). Then, desmosomes are formed in the trophectoderm but not in the inner cell mass (Fleming et al., 1991; Hardy et al., 1996). Desmosome formation during preimplantation is related to stabilization of the trophectoderm layer during blastocyte expansion. Accordingly, whereas plakoglobin and desmoplakin are expressed during cleavage and compaction, desmosome assembly occurs only after the activation of desmosomal cadherin expression at the late morula stage (approx. 32-cell) (Bloor et al., 2002; Fleming et al., 1991; Hardy et al., 1996). Dsc and Dsg are the key glycoproteins for the formation of functional DJs between TE cells, and delays in their expression are associated with the transcriptional activation of the embryonic genome (Collins et al., 1995). Desmosome formation in human embryos shows a similar pattern to that observed in the mouse (Bloor et al., 2002; Ghassemifar et al., 2003; Hardy et al., 1996).

Adherens junctions (AJs)

An adherens junction (AJ) or zonula adherens (ZA) is a belt-like protein complex limiting paracellular permeability on the apicolateral region of cell membranes. Its main function is to maintain

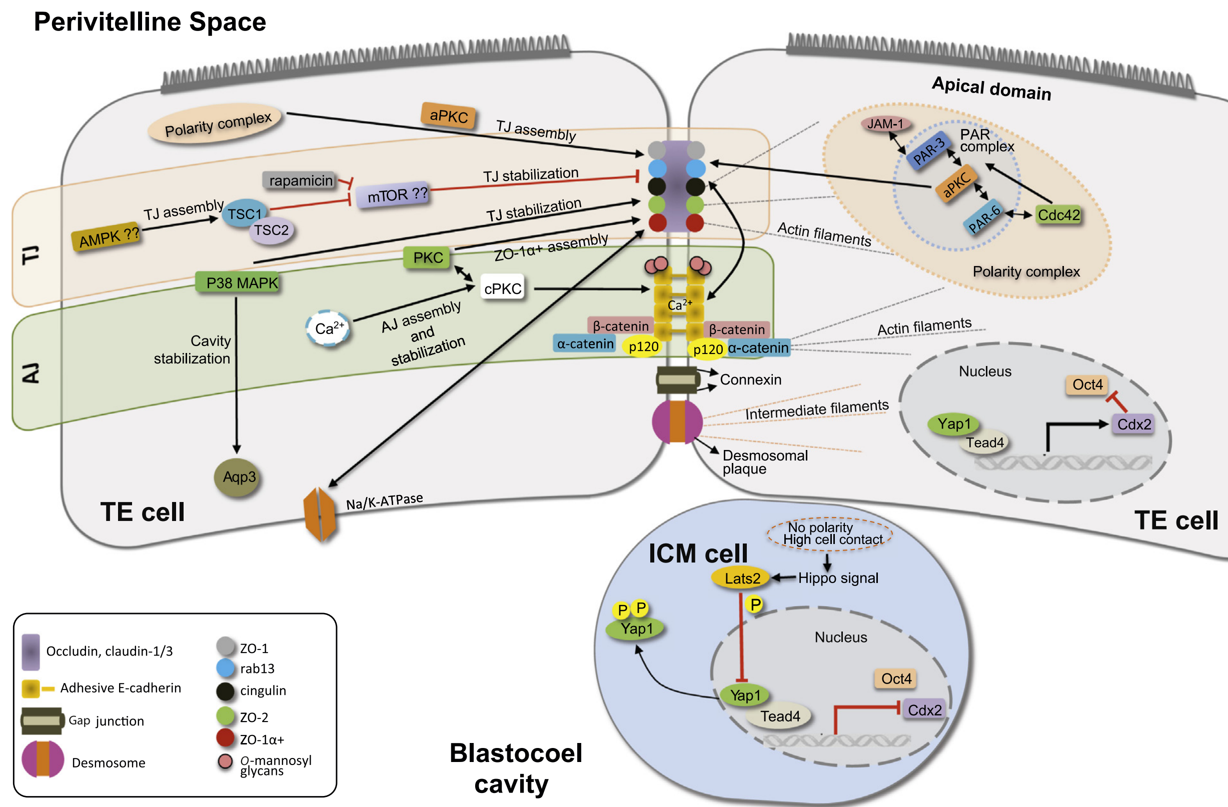

Fig. 2. Schematic overview of the intercellular network that stabilizes and maintains the junctional complexes, cavitation and transcriptional circuits in early embryos. The E-cadherin-catenin adhesion complex (E-cadherin, α/β-catenin, p120 proteins) is activated posttranslationally by cPKC signaling and *O*-mannosyl glycans in the presence of Ca^{2+} ions. The extracellular domain of the E-cadherin complex interacts with E-cadherin molecules on the adjacent cells; its intracellular domain interacts with β-, p120-, and α-catenins, and the latter connect directly to the actin cytoskeleton. Cdc42 binds the polarity complex via PAR-6, which activates aPKC. JAM-1, another binding partner for the polarity complex, binds via PAR-3 and helps localize the complex on the apical membrane, ultimately generating apical polarity in TE cells. TJ construction is a gradual process at both the morphological and molecular levels. TJ formation depends on functional E-cadherin-catenin adhesion but can be inhibited by multiple intracellular signal pathways. Activated aPKC regulates the membrane assembly of TJ constituents. The p38 MAPK pathway specifically contributes to the cavitation by maintaining Aqp3 expression and stabilizing TJ constituents. The crosstalk between AMPK and mTOR signaling may be an important regulator of TJ stabilization, but that mechanism has not yet been elucidated during preimplantation. Correct TJ membrane assembly permits the membrane localization and function of the Na/K-ATPase active transport mechanism in TE cells. Connexin proteins generate a 'connexon' on each cell, and two connexons constitute one GJ connecting across the intercellular space. Desmosomal plaques at the cell contact sites anchor the intermediate filaments and provide mechanical integrity. In the outer cells, nuclear Yap-1 activates Tead4 transcription, resulting in Cdx2 upregulation and promoting differentiation toward the TE fate. Increased cell-cell contact sites and loss of apical polarity in insider cells leads to differentiation toward the ICM fate. The ICM fate is also induced by the activity of the Hippo pathway, which phosphorylates Yap-1 by Lats2 and results in the exclusion of Yap-1 from the nucleus and the inhibition of Tead4 transcription, thereby increasing the level of Oct4.

the E-cadherin/catenin mediated cell-cell adhesion system. The extracellular part of E-cadherin, specifically the Ca^{2+}-binding domain, interacts with other E-cadherin molecules of neighboring cells. Without Ca^{2+} ions, they are nonfunctional and cannot promote cohesion of the epithelium. The cytoplasmic domain of E-cadherin interacts with a group of proteins composed of α-catenins, β-catenins, and p120, of which the former has a direct connection to the actin cytoskeleton (Fig. 2) (Hirano et al., 1987).

Synthesis, accumulation and membrane assembly of E-cadherin, also known as uvomorulin, and catenins occur throughout cleavage both in mice and humans. However, they are nonfunctional as an adhesion system before compaction. In mice, E-cadherin is uniformly distributed over the cell surface until the early 8-cell stage, at which point it becomes restricted to regions of cell-cell contact and becomes functional as part of a stable adhesion system (Vestweber et al., 1987). In humans, E-cadherin is expressed mainly at the cell surface and becomes functional at approximately 3.5–4 days (the 16-32-cell stage) (Campbell et al., 1995; Nikas et al., 1996). Although the mechanism underlying the suppression of stable cell-to-cell adhesion during early embryonic stages remains unclear, there is evidence that E-cadherin proteins activated by posttranslational modifications are involved in cell contact-mediated protein kinase C α (PKCα) signaling during compaction. Studies have shown that stimulation of PKCα can prematurely activate E-cadherin adhesion, particularly in the 4-cell-stage mouse embryo (Winkel et al., 1990). Recently, an important discovery revealed that O-mannosylation managed by O-mannosyl glycans is an essential posttranslational modification activating the E-cadherin complex proteins during preimplantation in mice (Lommel et al., 2013). O-mannosylation-deficient embryos fail to form E-cadherin-mediated cell adhesions and tight junctions and thus cannot reach the blastocyst stage (Lommel et al., 2013). However, proteins responsible for the posttranslational modifications of E-cadherin during the compaction period in mice and especially in humans still require further investigation.

Studies conducted in mice demonstrated that functional E-cadherin expression is the first essential step of the trophectoderm formation. Whereas the morphological appearance and cell divisions of E-cadherin null mutant embryos are unaltered at early cleavage stages, these embryos fail to form a functional trophectoderm at approximately E4.5 and retain the zona pellucida and the earlier compacted morphology (Larue et al., 1994). A similar phenotype is observed in embryos lacking maternal E-cadherin. Adhesion does not occur until the morula stage, when E-cadherin protein expression from the paternal allele is first detected (De Vries et al., 2004). Maternal E-cadherin null embryos undergo compaction only after the paternal protein is expressed, which occurs only after ZGA is completed (De Vries et al., 2004).

Consequently, although E-cadherin is not required for early embryonic stages, the establishment and function of E-cadherin adhesion sites between blastomeres by posttranslational modification of E-cadherin from the newly activated embryonic genome is an irreplaceable step for trophectoderm differentiation. Thus, it is possible to conclude that the posttranslational activation of E-cadherin is the first domino to start the lineage differentiation in the embryo.

Tight junctions (TJs)

A tight junction (TJ) or zonula occludens (ZO) is a multi-protein complex that makes up the belt-like structures between cells, forming a virtually impermeable barrier to fluid exchange between compartments. These junctions are typically located along the upper lateral sides of epithelial cells, and although they are composed of several transmembrane proteins, the major protein types are claudins (Furuse et al., 1998), occludins (Furuse et al., 1993) and junctional adhesion molecules (JAMs) (Bazzoni, 2003; Martin-Padura et al., 1998). TJs are linked with the cytoskeletal elements of adjacent cells by anchoring the strands to the actin cytoskeleton (Fig. 2). AJs and TJs are composed of different proteins, but both provide important adhesive contacts between neighboring epithelial cells and have intercellular links to the actin cytoskeleton (Fig. 2) (See reviews by Gonzalez-Mariscal et al. (2003)).

During preimplantation development, the establishment of TJs between embryonic cells occurs; this process is called TJ maturation (Eckert and Fleming, 2008). By the onset of the E-cadherin-mediated compaction, differentiation of the TE layer begins, and TJ proteins accumulate on the membranes of TE cells as small densities. That is, TJ protein membrane accumulation coincides with TE differentiation and blastocyte biogenesis. Normal TJ accumulation does not occur in E-cadherin null embryos; thus, this membrane accumulation process depends on prior activation of the E-cadherin adhesion system (Ohsugi et al., 1997). Three consecutive endogenous stages have been identified in TJ biogenesis in mouse preimplantation development. First, during compaction, the peripheral membrane scaffold protein ZO-1 accumulates along with rab13 and JAM-1, and these proteins localize at the apical microvillus pole during the 8-cell stage. Next, the peripheral membrane proteins cingulin and ZO-2 accumulate at the apicolateral contact sites during the 16-cell stage. Lastly, ZO-1α+, occludin and claudin-1/3 accumulate during the 32-cell stage (Eckert and Fleming, 2008). During these TJ protein accumulation stages, paracellular permeability decreases. Accumulation of occludin, claudin-1/3 and ZO-1α+ during the final phase is a complementary event in TJ biogenesis; this step is required for the establishment of a seal between TE cells, and without it, the blastocoel cavity fails to form. Ultrastructural analyses of mouse embryos showed that during the first and second accumulation stages (8- and 16-cell stages), blastomeres generate electron-dense plaques resembling adherens junctions along the apicolateral contact sites with clear intercellular space. In contrast, in the final accumulation phase (~32-cell stage), blastomeres display electron-dense regions clearly situated above the adherens junctions, resembling tight junctions without intercellular space. Until the 32-cell stage, TJ components colocalize with the AJ components; after the final accumulation stage, TJs and AJs separate into distinct but closely aligned domains (Fig. 2) (Eckert and Fleming, 2008; Sheth et al., 2000). TJ biogenesis in human preimplantation embryos is regulated during the equivalent period. Human embryos display similar patterns of TJ protein expression and membrane accumulation of key junctional components, including delayed accumulation of ZO-1α+ (Ghassemifar et al., 2003). Although human embryos lag behind mouse embryos in the timing of TJ maturation, the correct accumulation and separation is essential for both mouse and human preimplantation embryos because any deficiencies in these events can result in lethality (Eckert et al., 2007; Ghassemifar et al., 2003).

The development of stable and functional TJs is a distinct form of epithelial differentiation, specifically that of TE formation in the developing blastocyte. Suppressing the accumulation of TJ components results in the inhibition or regression of embryonic morphology, particularly the process of cavitation (Kim et al., 2004; Thomas et al., 2004). Although the essential roles of the claudin and occludin families are directly associated with TJ biogenesis, JAMs, the other transmembrane proteins in TJs, make distinct contributions to lineage specification, and these contributions will be described in subsequent sections. We next consider the intracellular control mechanisms influencing junctional complexes during preimplantation development.

Control of junctional complex biogenesis

Because the formation and function of junctional complexes are directly associated with embryo viability and lineage differentiation, biogenesis and stabilization of junctional complexes within the preimplantation embryo is governed by more than one control mechanism. To date, several studies have been performed to identify these control mechanisms. However, our knowledge is mostly limited to the mouse because of the accessibility of mouse embryos.

Assembly of apical Junctions and polarized membranes

One such intracellular control mechanism involves the protein kinase C (PKC) family, which is divided based on structure and activation requirements. It has been known that 10 isotypes of PKC, including conventional PKCs (cPKCs; α, βI, βII, γ), novel PKCs (nPKCs; δ, ε, η, θ), and atypical PKCs (aPKCs; ι/λ, ζ), are expressed during preimplantation development in mouse (Dehghani and Hahnel, 2005; Pauken and Capco, 2000). Specific suppression of PKC showed that this pathway is involved in regulating the membrane assembly of TJ constituents, particularly by affecting the accumulation of ZO-1 and ZO-1α+ proteins (Eckert et al., 2004). For example, Ca^{2+}-sensitive cPKCs function in the presence of Ca^{2+} ions. As described above, E-cadherin, the AJ constituent required for the commencement of intercellular adhesion at compaction, becomes functional only when the intercellular Ca^{2+} concentration reaches a certain threshold (Aghion et al., 1994; Vinot et al., 2005). Thus, Ca^{2+}, via cPKCs, is an indispensable initiator for the proper and timely establishment of AJ and TJ accumulation/maturation (Fig. 2).

Evidence obtained from epithelial cell lines has suggested that 5′-adenosine monophosphate-activated protein kinase (AMPK) activation acts in TJ membrane assembly (Zhang et al., 2006b). Although the upregulation of AMPK activity coincides with the Ca^{2+}-induced assembly of TJs and ZO-1 on the membrane, inhibition of AMPK disrupts TJ membrane assembly. The mammalian target of rapamycin (mTOR) signaling pathway, as one of the downstream targets of AMPK, is directly inhibited by AMPK via the activated TSC1-TSC2 complex (Xu et al., 2012). A comparison of the rate of Ca^{2+}-induced TJ assembly in the presence or absence of rapamycin shows a direct inhibition of mTOR activity by rapamycin, and this accelerates the assembly of tight junctions in wild-type MDCK cells (Zhang et al., 2006b).

Junction stabilization and maintenance

Further support for the involvement of the mTOR pathway in the regulation of tight junction assembly and stabilization has

recently been shown by Mok et al. (2013) in Sertoli cells. Crosstalk between AMPK and mTOR is important in the regulation of the paracellular TJ seal between cell membranes. However, the existence of crosstalk in mouse or human embryos remains controversial.

The entire p38 MAPK family is expressed throughout the preimplantation development period in mice (Natale et al., 2004). Most recently, a pharmacological approach has been used to demonstrate the specificity of the p38 MAPK pathway and its contribution to the cavitation and blastocyst formation (Bell and Watson, 2013). Suppression of p38 MAPK causes decreases in Aqp3 mRNA levels and AQP3 expression. This pathway has also been shown to participate in regulating TJ permeability between TE cells in mouse blastocysts by affecting TJP1 protein localization in cells with suppressed p38 MAPK activity. These results indicated that p38 MAPK is an intracellular signaling mediator of stabilization and maintenance for the function of proteins involved in establishing TE layer differentiation and polarity as well as the trans-trophectoderm ionic gradient (Fig. 2) (Bell and Watson, 2013).

Acquisition of cell polarity and asymmetric divisions

In some organisms, such as *Caenorhabditis elegans* and *Drosophila melanogaster*, entry of a sperm into an ovum at fertilization induces the asymmetric distribution of determinants for the development of embryonic polarity (Wodarz, 2002). However, in mouse and human embryos, there is no such asymmetric distribution in response to fertilization. Thus, a separate induction event is necessary for generating the polarized phenotype in mouse and human embryos. Compaction appears to be a triggering event in lineage allocation in early embryonic development. Blastomeres become polarized, while flattening between blastomeres proceeds.

As in the other cell line and developmental model organism examples of *de novo* cell polarity, the onset of polarity in the mouse embryo is mediated by proteins of the PAR complex (Johnson and McConnell, 2004; Macara, 2004a). Cytoskeletal proteins are phosphorylated by the PAR (partition defective) protein complex to induce a polarized morphology and permit differentiative (asymmetric) divisions (Ahringer, 2003; Macara, 2004b). This process, defined in epithelial cell lines, briefly links atypical PKC (aPKC) with PAR-3 and then PAR-6, forming the PAR-3–aPKC–PAR-6 complex, which then binds to Cdc42. The binding of active Cdc42 via PAR-6 results in aPKC activation. JAMs anchor the PAR-3–aPKC–PAR-6–Cdc42 complex via PAR-3, which localizes the complex to the apical membrane (Fig. 2) (Ebnet et al., 2001; Ebnet et al., 2004; Suzuki et al., 2002). The identification of JAM-1 (also known as JAM-A, (Bazzoni, 2003)) as a binding partner for PAR-3 strongly indicates the involvement of JAM-1 in polarity formation. Blocking the JAM-1 localization to cell-cell contacts or the formation of a functional PAR-3–aPKC–PAR-6–Cdc42 complex contributes to arrest of cell polarization and apical-basal formation. Expression of JAM-1 protein starts at precompaction (8-cell stage) at the sites of cell-cell contact and rapidly increases in subsequent stages at the apical pole of blastomeres in the mouse embryo (Thomas et al., 2004). Although the first detection of JAM-1 protein in cell-cell contact sites is associated with TJ biogenesis, the increasing density of JAM-1 protein at the newly formed apical pole during compaction gives credence to its role in the acquisition of polarization rather than its junctional function (Thomas et al., 2004). During compaction, PAR proteins perform their functions by localizing to the apical or basolateral surfaces of blastomeres. The homologs of the different members of the PAR/aPKC complex, including PARD6b (mouse homologs of PAR6) and EMK1 (mouse homologs of PAR1), are present in the nucleus and cytoplasm from the 2-cell stage to the early 8-cell stage, but the localization of both proteins becomes asymmetric at the middle of the 8-cell stage, when blastomeres flatten upon each other (Vinot et al., 2005). Concurrently with compaction, PARD6B is localized on the apical membrane, whereas EMK1 is distributed along the basolateral membrane of the newly polarized cells (Vinot et al., 2005). Asymmetric distribution of these molecules also allows asymmetric divisions and the segregation of the first two germline cells in the 8-cell mouse embryo. Suppression of normal aPKC and Par-3 function causes the control of asymmetric cell division and trophectoderm formation to fail (Plusa et al., 2005).

Morphologically, a polarized blastomere possesses three unique features: (1) a non-adhesive apical pole with ezrin-rich microvilli (Dard et al., 2001; Houliston et al., 1989; Nikas et al., 1996), (2) an adhesive basolateral surface with intercellular junctions but no microvilli (Houliston et al., 1989; Nikas et al., 1996; Sheth et al., 2000) and (3) reorganized cytoplasmic organelles and cytoskeleton along the apico-basal axis (Houliston et al., 1989; Johnson and Maro, 1984). In a developing embryo, each blastomere gains these features during compaction. The formation of surface polarity comprises two important processes at compaction; (1) 'flattening', with the increase in the cell–cell contact constituents between the blastomere membranes, and (2) microtubule-mediated interaction between the blastomere nucleus and the apical domain.

Many studies have shown that there is a direct link between compaction and polarization events during preimplantation. Examination of a blastomere pair at the 8-cell stage by scanning electron microscopy revealed that in the absence of cell contact, fewer cells polarized (Houliston et al., 1989). Whereas microvilli were normally localized at the surface of the apical pole at 9 h post-division, ECCD-1 (E-cadherin-blocking antibody) blocks flattening and spreads the formation of microvilli over the entire blastomere surface at 9 h post-division (Houliston et al., 1989). Furthermore, when 8-cell embryos are cultured in Ca^{2+}-free medium to inhibit intercellular flattening, the polarized localization of PARD6b and EMK1 is altered (Vinot et al., 2005). Cell polarity mediated by PARD6b and EMK1 is determined by cell-cell contacts during compaction; therefore, without compaction, polarization is never established.

The formation of intercellular complexes during compaction also results in reorganization of the microtubule network in blastomeres. A population of stable acetylated microtubules is gathered in the basolateral domain, whereas dynamic microtubules located at the sites of cell-cell contact disappear (Houliston et al., 1989; Maro et al., 1990). The mitotic spindle is positioned closer to the posterior pole, which leads to the generation of daughter cells of different sizes after cleavage (Houliston et al., 1989). Polarization breaks the radial symmetry and results in two types of cell division depending on the orientation of the mitotic spindle. Differentiative or asymmetric cytokinesis occurs tangentially to the surface of the embryo and produces one polarized outer-daughter cell, inheriting a large part of the cytoplasm, and one non-polarized inner-daughter cell, inheriting less cytoplasm. Conservative or symmetric cytokinesis arises perpendicular to the surface of the embryo and produces two identical (similar amounts of cytoplasmic content) polarized outer-daughter cells. Thus, generation of inside-daughter cells requires polarized outer cells and a tangentially positioned mitotic spindle. Cells formed after differentiative divisions have different developmental fates; the outer cells contribute to the TE lineage, whereas the inside cells form the inner cell mass (ICM) that later segregates into the EPI and PE lineages (Johnson and Ziomek, 1981). Interestingly, cells formed after conservative divisions mostly share the same fate: they contribute to the TE lineage (Sasaki, 2010). Asymmetric

divisions during the fourth (8-16-cell) and fifth (16-32-cell) cleavages in mouse lead to the lineage segregation by generating polar outer (TE progenitor) and non-polar inner (ICM progenitor) cells (Johnson et al., 1986; Johnson and McConnell, 2004).

Three-dimensional live-cell imaging analysis, which has increased in importance in recent years, has allowed the tracing of the orientation of the cell division axis of blastomeres during embryonic development. Through this analysis, our knowledge about determination of the cell fate in early embryo is constantly being updated. A series of noninvasive lineage-tracing experiments reveals that there is a correlation between blastocyst patterning and specific positioning of blastomeres at 4-cell stage in mouse (Piotrowska-Nitsche and Zernicka-Goetz, 2005). The second cleavage can be orientated in two alternative ways: either meridian (M; parallel to the AV axis) or equatorial (E; perpendicular to the AV axis), which generates four types of embryos: ME, EM, MM and EE (Piotrowska-Nitsche and Zernicka-Goetz, 2005). It has been found that in a significant group of embryos individual 4-cell stage blastomeres differ in the extent of specific epigenetic modifications such as histone H3 arginine 2, 17 and 26 methylation, pluripotency transcription factors such as Oct4, Nanog, Sox2, and subsequent cell fate (Plachta et al., 2011; Tabansky et al., 2013; Torres-Padilla et al., 2007). Taken together, the first transcriptional differences between cells start from the 4-cell stage in mouse, which later contributes to lineage specification. That analysis also reveals the spatial and temporal relationship between symmetric and asymmetric divisions to clarify the generation of inside and outside cells after compaction. According to the noninvasive lineage-tracing studies of unmanipulated embryos, blastomeres undergo waves of asymmetric division, resulting in different proportions of progeny cells (Bischoff et al., 2008; Morris et al., 2010; Zernicka-Goetz et al., 2009). Consequently, after reaching polarization, waves of asymmetric divisions provide guidance to the generation of first TE/ICM lineages that follow the EPI/PE lineages. Lineage size is closely regulated by asymmetric divisions (Zernicka-Goetz et al., 2009).

Transcriptional circuits of lineage commitment

Once an embryo gains compaction and polarization, asymmetric divisions occur in successive waves, and the cells of developing blastocysts possess very striking features, which identify them as the precursors. After this initial segregation, asymmetrically distributed transcriptional regulators in the precursor cells amplify and transform the gene expression circuitries, and precursors become fully committed. For commitment, the proper specific transcriptional networks must be established to reinforce the cell fate decision.

TE/ICM

The trophectoderm (TE), the first differentiated cell lineage in the embryo, forms the outer layer of a blastocyst as an epithelial sheet enclosing the ICM. While the ICM retains pluripotency, the TE is restricted, undergoes epithelialization and plays essential roles in implantation. It interacts with the decidualized maternal uterus and later contributes to the fetal portions of the placenta. Commitment for TE or ICM is controlled by the expression patterns of several transcription factors.

Once TE and ICM precursors are separated, transcription factors including Cdx2, Tead4 and Eomes are upregulated in outer cells (TE precursors) to enable epithelial specification (Nishioka et al., 2008; Strumpf et al., 2005). By contrast, inner cells develop a stable regulatory circuit including the transcription factors Oct4, Sox2 and Nanog to promote pluripotency and resist differentiation (Avilion et al., 2003; Mitsui et al., 2003). Although Cdx2 and Oct3/4 are ubiquitously coexpressed to maintain cells in an undifferentiated state, interactions between these factors promote TE and ICM fates during blastocyst formation. In mice, following the fourth cleavage, downregulation of Oct3/4 and upregulation of Cdx2 in the outer cells trigger the differentiation of cells toward TE (Niwa et al., 2005). By contrast, in inner cells, downregulation of Cdx2 and upregulation of Oct3/4 trigger ICM differentiation (Niwa et al., 2005). Although embryos lacking the Pou5F1 gene, which encodes Oct4, generate ICM that expresses TE markers (Nichols et al., 1998), loss of Cdx2 causes an altered localization of ICM markers in the TE and an inability to sustain TE development (Strumpf et al., 2005). That reciprocal repression between lineage-specific transcription factors is the crucial event for establishing and maintaining the TE/ICM segregation. Similarly, negative regulation of Nanog and Cdx2 expression affects the segregation of the ICM and the TE (Chen et al., 2009). The transcriptional profile for TE/ICM segregation in human embryos is quite similar to that in mice but shows differences in timing (Galan et al., 2010; Niakan and Eggan, 2013). Oct4 restriction to the ICM occurs two days later than in the mouse, at day 6 (approximately at 128-256-cell blastocysts). Interestingly, Cdx2 expression is first detectable in the TE layer following cavitation (day 5), whereas its expression is detectable prior to cavitation in mice and shows prolonged coexpression with Oct4 in TE (Niakan and Eggan, 2013). These results display retardation of lineage segregation in human preimplantation development relative to mouse preimplantation development.

Cdx2 is the earliest known marker for TE specification, and its expression plays a pivotal role in promoting the TE fate in both mouse and human embryos (Nishioka et al., 2009; Strumpf et al., 2005). Cdx2 expression is regulated by Tead4 and its coactivation partners Yap-1 and Wwtr1 (TAZ), defining Tead4 as the first in the hierarchy of transcription factors establishing the TE fate. Differential function of Tead4 rather than its expression regulates specification of the first two cell lineages (Home et al., 2012). Tead4 is ubiquitously expressed in all cells of the preimplantation embryo but becomes active only in outer blastomeres because nuclear Yap-1 are present only in the developing TE. The restriction of Yap-1 causes Cdx2 expression to be restricted to outer cells. Nuclear localization of Yap-1 depends on the Hippo pathway member Lats2. Phosphorylated Yap-1 by Lats2 results in exclusion of Yap-1 from the nucleus and inhibition of Tead4 transcription (Nishioka et al., 2009). Yap$^{-/-}$ mutant embryos exhibit normal TE development (Morin-Kensicki et al., 2006) implying the function of another Yap-related cofactor, Wwtr1 (TAZ), which could compensate the absence of Yap during early development (Nishioka et al., 2009). Results are consistent with the hypothesis that Wwtr1 plays a Yap-like role in the early embryo (Nishioka et al., 2009). Consequently, Yap and Wwtr1 act together with Tead4 to regulate Cdx2 expression in outsider cells during blastocyst formation in mouse. According to the results from delayed Cdx2 upregulation in human embryos (Niakan and Eggan, 2013), it will be important to determine whether human TE specification requires the identified pathways in mouse, such as Hippo signaling and Tead4 activity.

Differential signals leading to changes in cell fate between inside and outside cells are associated with the density of cell-cell contacts. The Hippo pathway controls the localization of Yap/Wwtr1 and therefore Tead activity in response to cell-cell contact in cultured cells (Ota and Sasaki, 2008; Zhao et al., 2007). Hippo signaling is activated by increased cell-cell contacts in insider cells, resulting in the exclusion of Yap1 from nucleus and inhibition of Tead4 transcription (Nishioka et al., 2009). Consequently, cells adopt an ICM fate rather than a TE fate. Similar interactions between cell polarity and the Hippo pathway have been identified during preimplantation development in other contexts (Genevet

and Tapon, 2011; Hirate et al., 2013; Leung and Zernicka-Goetz, 2013).

Implications for the direct links between cell-cell contact and cell fate can be found in isolated ICM studies. Isolated ICMs from early blastocysts are able to regenerate the TE layer (Eckert et al., 2004; Hogan and Tilly, 1978). Loss of cell-cell contacts between outsider cells from isolated ICMs leads to differentiation toward TE, possibly as a result of the inactive Hippo pathway, decreased cell polarity and increased levels of Cdx2. These studies suggest that establishing the intercellular junctional complexes and most likely the acquisition of cell polarity affects the expression of lineage-specific markers, thus affecting cell fate in the developing embryo.

PE/EPI

After embryonic day 3.5 in mice and day 6 in humans, the blastocyst undergoes the second lineage segregation to form the primitive endoderm (PE, future yolk sac), situated as a monolayer on the surface of the ICM, and the epiblast (all future tissues of the organism), situated more deeply in the ICM. Similar to the first segregation, the second segregation and commitment to epiblast and PE lineages is determined by a number of lineage-specific transcription factors that are preferentially expressed in precursor cells. Whereas Gata4, Gata6 and Sox17 lead to differentiation toward the PE fate (Morris et al., 2010; Morrisey et al., 1998; Niakan et al., 2010), Nanog, Oct4/Pou5f1, Sox2 and Sall4 have been shown to be essential for establishing epiblast fate by helping to promote pluripotency and escape differentiation (Avilion et al., 2003; Chambers et al., 2003; Zhang et al., 2006a). Similar to the first segregation (TE/ICM), a reciprocal repression between the lineage-specific transcription factors Nanog and Gata6 is present for the second segregation and is controlled by FGF signaling (Chazaud et al., 2006; Yamanaka et al., 2010). Originally, ICM cells express both the growth factor Fgf4 and its receptor Fgfr2, but at the 64-cell stage, Fgf4 is restricted to the epiblast. However, Fgfr2 is restricted to the PE and activates the PE-specifying genes Gata6, Sox17 and Gata4. Although Fgfr2 expression decreases in Nanog-positive epiblast progenitors, PE progenitor cells upregulate Fgf4 expression. Fgf4 secretion retains the expression of Fgfr2 and reinforces the Gata6 transcription in PE progenitors, thereby repressing Nanog (Yamanaka et al., 2010). The expression profile of transcription factors required for PE/epiblast specification, such as Nanog, Gata4, Gata6 and Sox17, shows a conserved expression pattern between human and mouse embryos (Niakan and Eggan, 2013; Roode et al., 2012). However, unlike that in mouse, formation of PE in human embryos is not dependent upon FGF signaling (Roode et al., 2012). Alternative pathways may be responsible for PE and epiblast specification in human embryos.

To date, two segregation hypotheses have been proposed to explain the mechanism underlying this second cell fate decision in mouse embryos. The first is the "position-based model", which suggests that cell fate is determined by the cells' position within the embryo (Gardner, 1983; Rossant, 1975). Together with the formation of the cavitation at E3.5, a population of cells in the newly formed ICM contacts the blastocoel. Therefore, outsider cells form a polarized epithelium and a basement membrane, and deeper cells (insiders) in the ICM remain undifferentiated and become progenitors for all cells in the organism. The position-based model was conceptually quite similar to ICM/TE segregation and conceivable. However, a more recent model called the "cell-sorting model" offers a different explanation, suggesting that the pre-specified cells at E3.5, which express certain lineage specific markers within the ICM, are sorted into composite layers until E4.5 (Chazaud et al., 2006; Plusa et al., 2008). This model was strongly supported by the gene expression profiles of individual ICM cells showing that PE and epiblast fates are already established by the 64-cell stage (Guo et al., 2010). Apparently, pre-specified cells expressing the PE and epiblast markers, Gata6 and Nanog, respectively, are distributed randomly within the ICM at E3.5 (Plusa et al., 2008). The expression is aligned after cells form distinct layers in their correct positions at E4.5 (Guo et al., 2010; Plusa et al., 2008).

Therefore, the fundamental question remaining is how PE and epiblast progenitors in the ICM are allocated to their correct positions in the E4.5 blastocyst. Although additional studies are needed to answer this question, noninvasive lineage-tracing studies of unmanipulated embryos offer some insights. According to the results of these studies, each wave of asymmetric divisions tends to generate different proportions of PE and epiblast progeny. As the proportion of epiblast progeny during the first (the fourth cleavage), second (the fifth cleavage), and third (the sixth cleavage) waves decreases, the proportion of PE progeny increases (Morris et al., 2010). Furthermore, these progenitor cells undergo positional changes by moving during cavitation. Bidirectional cell movement, which takes place from approximately 7 h after the fifth cleavage until the seventh cleavage begins, has been observed to generate correct cell segregation. Within the ICM, the outsider cells can move to the deep layers, and the insider cells can relocate to the surface (Meilhac et al., 2009; Morris et al., 2010). It has been demonstrated that cells expressing certain transcription markers show differential adhesion to each other, and this mechanism has been identified as controlling cell movement within the ICM (Krupinski et al., 2011; Meilhac et al., 2009; Zernicka-Goetz et al., 2009). Consistent with this result, Gata6-expressing cells have high adhesion, whereas Nanog-expressing cells show low cohesion. That differential adhesion pattern allows the randomly distributed progenitor cells within the ICM to recognize each other, thus generating two clusters of cells within the ICM. Although this mechanism regulates cell sorting, it alone is insufficient to correctly position cells. Correct positioning of these two clusters is also achieved by the forced attraction of the blastocoel or the TE.

Conclusions

In all mammalian species examined, the 8-cell embryo is formed after three rounds of cleavage divisions; totipotency is regressed, and the radial symmetry of the embryo and therefore cell identity is broken. For many years, the generation of embryonic cell identities and the formation of specialized cell lineages have been major questions among researchers. To address this essential question in the field of developmental biology, it is crucial to understand the cell–cell interactions and gene networks that govern cell fate in preimplantation development.

Lineage specification after the 8-cell stage is achieved by compaction, which is the first link of this nested chain of events. Initial establishment of the intercellular junctional complexes provides the cellular polarity that results in the generation of progenies that are physically separated, are associated with different microenvironments and inherit diverse proteins or RNA. If the proper collaboration of compaction-polarization-asymmetric division events are established and completed accurately, transcriptional regulators are distributed asymmetrically in the precursor cells, allowing those transcriptional regulators to ensure full commitment to specific lineages. Although recent studies have explained that cell adhesion, polarity and cell position are linked with proper lineage allocation, many details still require clarification. For example, cell polarization regulates the orientation of cell divisions leading to the establishment of TE or ICM lineage (Plusa et al., 2005), but after this initial segregation,

the effect of cell polarity on lineage-specific markers remains largely unknown.

All three embryonic lineages can be distinguished in the mouse embryo at E4.5 or at day 7 in humans. The final determined cell fate, called commitment, is achieved by the amplification of the unique properties of each progeny cell by specific transcriptional circuits (Chazaud et al., 2006; Nishioka et al., 2008; Niwa et al., 2005; Roode et al., 2012; Strumpf et al., 2005). It is widely accepted that the PE and epiblast progenitor cells are initially generated within the ICM in a position-independent manner and later move to their correct positions (Guo et al., 2010; Morris et al., 2010; Plusa et al., 2008; Zernicka-Goetz et al., 2009). However, the exact mechanism guiding the movements of these progenitor cells remains unknown. Additional studies are required to uncover these mechanisms of PE and epiblast lineage segregation.

Groundbreaking studies using fluorescent tracers and dyes combined with time-lapse imaging enable to show features of individual blastomeres. Recent studies revealed that transcriptional difference arises from second cleavage, 4-cell stage embryo in mouse (Piotrowska-Nitsche and Zernicka-Goetz, 2005; Plachta et al., 2011; Tabansky et al., 2013; Torres-Padilla et al., 2007). Although the embryonic blastomeres appear similar before compaction, their probability of giving rise to either the TE or ICM is not equal because of transcriptional difference. On the other hand, human embryos remain a mystery, as these experiments have not yet been applicable to human embryos for ethical reasons.

Mouse embryos are easily accessible for studies; thus, remarkable progress has been made toward the understanding of lineage segregation events in the mouse. Although studies in the mouse embryo provide key insights into the regulation of mammalian preimplantation development, not all findings can be directly applied to the human embryo due to species-specific differences. Due to the practical difficulties and ethical concerns related to obtaining suitable human eggs and embryos, very little is known about lineage segregation in humans. How human embryos gain a polarized phenotype, when asymmetric cell division occurs and which intracellular signaling mechanisms are responsible for the control of transcriptional circuits remain unclear. Detailed analysis of the molecular mechanisms of lineage segregation during human preimplantation will undoubtedly lead to significant advances in developmental biology and stem cell biology.

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References

Aghion, J., Gueth-Hallonet, C., Antony, C., Gros, D., Maro, B., 1994. Cell adhesion and gap junction formation in the early mouse embryo are induced prematurely by 6-DMAP in the absence of E-cadherin phosphorylation. Journal of Cell Science 107, 1369–1379 (Pt 5).

Ahringer, J., 2003. Control of cell polarity and mitotic spindle positioning in animal cells. Current Opin. Cell Biol. 15, 73–81.

Albert, M., Peters, A.H., 2009. Genetic and epigenetic control of early mouse development. Curr. Opin. Genet. Dev. 19, 113–121.

Avilion, A.A., Nicolis, S.K., Pevny, L.H., Perez, L., Vivian, N., Lovell-Badge, R., 2003. Multipotent cell lineages in early mouse development depend on SOX2 function. Genes Dev. 17, 126–140.

Bazzoni, G., 2003. The JAM family of junctional adhesion molecules. Curr. Opin. Cell Biol. 15, 525–530.

Bell, C.E., Watson, A.J., 2013. p38 MAPK regulates cavitation and tight junction function in the mouse blastocyst. PloS One 8, e59528.

Bischoff, M., Parfitt, D.E., Zernicka-Goetz, M., 2008. Formation of the embryonic-abembryonic axis of the mouse blastocyst: relationships between orientation of early cleavage divisions and pattern of symmetric/asymmetric divisions. Development 135, 953–962.

Bloor, D.J., Metcalfe, A.D., Rutherford, A., Brison, D.R., Kimber, S.J., 2002. Expression of cell adhesion molecules during human preimplantation embryo development. Mol. Hum. Reprod. 8, 237–245.

Campbell, S., Swann, H.R., Seif, M.W., Kimber, S.J., Aplin, J.D., 1995. Cell adhesion molecules on the oocyte and preimplantation human embryo. Hum. Reprod. 10, 1571–1578.

Can, A., 2014. Kök Hücre. Biyolojisi, Türleri ve Klinik Kullanımları. Akademisyen Yayınevi, Ankara.

Chambers, I., Colby, D., Robertson, M., Nichols, J., Lee, S., Tweedie, S., Smith, A., 2003. Functional expression cloning of Nanog, a pluripotency sustaining factor in embryonic stem cells. Cell 113, 643–655.

Chazaud, C., Yamanaka, Y., Pawson, T., Rossant, J., 2006. Early lineage segregation between epiblast and primitive endoderm in mouse blastocysts through the Grb2-MAPK pathway. Dev. Cell 10, 615–624.

Chen, L., Yabuuchi, A., Eminli, S., Takeuchi, A., Lu, C.W., Hochedlinger, K., Daley, G.Q., 2009. Cross-regulation of the Nanog and Cdx2 promoters. Cell Res. 19, 1052–1061.

Chisholm, J.C., Houliston, E., 1987. Cytokeratin filament assembly in the preimplantation mouse embryo. Development 101, 565–582.

Collins, J.E., Lorimer, J.E., Garrod, D.R., Pidsley, S.C., Buxton, R.S., Fleming, T.P., 1995. Regulation of desmocollin transcription in mouse preimplantation embryos. Development 121, 743–753.

Dale, B., Gualtieri, R., Talevi, R., Tosti, E., Santella, L., Elder, K., 1991. Intercellular communication in the early human embryo. Mol. Reprod. Dev. 29, 22–28.

Dard, N., Louvet, S., Santa-Maria, A., Aghion, J., Martin, M., Mangeat, P., Maro, B., 2001. In vivo functional analysis of ezrin during mouse blastocyst formation. Dev. Biol. 233, 161–173.

Davies, T.J., Gardner, R.L., 2002. The plane of first cleavage is not related to the distribution of sperm components in the mouse. Hum. Reprod. 17, 2368–2379.

De Vries, W.N., Evsikov, A.V., Haac, B.E., Fancher, K.S., Holbrook, A.E., Kemler, R., Solter, D., Knowles, B.B., 2004. Maternal beta-catenin and E-cadherin in mouse development. Development 131, 4435–4445.

Dehghani, H., Hahnel, A.C., 2005. Expression profile of protein kinase C isozymes in preimplantation mouse development. Reproduction 130, 441–451.

Duranthon, V., Watson, A.J., Lonergan, P., 2008. Preimplantation embryo programming: transcription, epigenetics, and culture environment. Reproduction 135, 141–150.

Ebnet, K., Suzuki, A., Horikoshi, Y., Hirose, T., Brickwedde, Meyer Zu, Ohno, M.K., Vestweber, D., S., 2001. The cell polarity protein ASIP/PAR-3 directly associates with junctional adhesion molecule (JAM). EMBO J. 20, 3738–3748.

Ebnet, K., Suzuki, A., Ohno, S., Vestweber, D., 2004. Junctional adhesion molecules (JAMs): more molecules with dual functions? J. Cell Sci. 117, 19–29.

Eckert, J.J., Fleming, T.P., 2008. Tight junction biogenesis during early development. Biochim. Biophys. Acta 1778, 717–728.

Eckert, J.J., Houghton, F.D., Hawkhead, J.A., Balen, A.H., Leese, H.J., Picton, H.M., Cameron, I.T., Fleming, T.P., 2007. Human embryos developing in vitro are susceptible to impaired epithelial junction biogenesis correlating with abnormal metabolic activity. Hum. Reprod. 22, 2214–2224.

Eckert, J.J., McCallum, A., Mears, A., Rumsby, M.G., Cameron, I.T., Fleming, T.P., 2004. PKC signalling regulates tight junction membrane assembly in the preimplantation mouse embryo. Reproduction 127, 653–667.

Ellish, N.J., Saboda, K., O'Connor, J., Nasca, P.C., Stanek, E.J., Boyle, C., 1996. A prospective study of early pregnancy loss. Hum. Reprod. 11, 406–412.

Fleming, T.P., Garrod, D.R., Elsmore, A.J., 1991. Desmosome biogenesis in the mouse preimplantation embryo. Development 112, 527–539.

Fujimori, T., 2010. Preimplantation development of mouse: a view from cellular behavior. Dev., Growth Differ. 52, 253–262.

Furuse, M., Fujita, K., Hiiragi, T., Fujimoto, K., Tsukita, S., 1998. Claudin-1 and -2: novel integral membrane proteins localizing at tight junctions with no sequence similarity to occludin. J. Cell Biol. 141, 1539–1550.

Furuse, M., Hirase, T., Itoh, M., Nagafuchi, A., Yonemura, S., Tsukita, S., 1993. Occludin: a novel integral membrane protein localizing at tight junctions. J. Cell Biol. 123, 1777–1788.

Galan, A., Montaner, D., Poo, M.E., Valbuena, D., Ruiz, V., Aguilar, C., Dopazo, J., Simon, C., 2010. Functional genomics of 5- to 8-cell stage human embryos by blastomere single-cell cDNA analysis. PloS one 5, e13615.

Gardner, R.L., 1983. Origin and differentiation of extraembryonic tissues in the mouse. Int. Rev. Exp. Pathol. 24, 63–133.

Garrod, D., Chidgey, M., 2008. Desmosome structure, composition and function. Biochim. Biophys. Acta 1778, 572–587.

Genevet, A., Tapon, N., 2011. The Hippo pathway and apico-basal cell polarity. Biochem. J. 436, 213–224.

Ghassemifar, M.R., Eckert, J.J., Houghton, F.D., Picton, H.M., Leese, H.J., Fleming, T.P., 2003. Gene expression regulating epithelial intercellular junction biogenesis during human blastocyst development in vitro. Mol. Hum. Reprod. 9, 245–252.

Gonzalez-Mariscal, L., Betanzos, A., Nava, P., Jaramillo, B.E., 2003. Tight junction proteins. Prog. Biophys. Mol. biol. 81, 1–44.

Guo, G., Huss, M., Tong, G.Q., Wang, C., Li Sun, L., Clarke, N.D., Robson, P., 2010. Resolution of cell fate decisions revealed by single-cell gene expression analysis from zygote to blastocyst. Dev. Cell 18, 675–685.

Hamatani, T., Carter, M.G., Sharov, A.A., Ko, M.S., 2004. Dynamics of global gene expression changes during mouse preimplantation development. Dev. Cell 6, 117–131.

Hardy, K., Warner, A., Winston, R.M., Becker, D.L., 1996. Expression of intercellular junctions during preimplantation development of the human embryo. Mol. Hum. reprod. 2, 621–632.

Hirano, S., Nose, A., Hatta, K., Kawakami, A., Takeichi, M., 1987. Calcium-dependent cell-cell adhesion molecules (cadherins): subclass specificities and possible involvement of actin bundles. J. Cell Biol. 105, 2501–2510.
Hirate, Y., Hirahara, S., Inoue, K., Suzuki, A., Alarcon, V.B., Akimoto, K., Hirai, T., Hara, T., Adachi, M., Chida, K., Ohno, S., Marikawa, Y., Nakao, K., Shimono, A., Sasaki, H., 2013. Polarity-dependent distribution of angiomotin localizes Hippo signaling in preimplantation embryos. Curr. Biol.: CB 23, 1181–1194.
Hogan, B., Tilly, R., 1978. In vitro development of inner cell masses isolated immunosurgically from mouse blastocysts. I. Inner cell masses from 3.5-day p.c. blastocysts incubated for 24 h before immunosurgery. J. Embryol. Exp. Morphol. 45, 93–105.
Home, P., Saha, B., Ray, S., Dutta, D., Gunewardena, S., Yoo, B., Pal, A., Vivian, J.L., Larson, M., Petroff, M., Gallagher, P.G., Schulz, V.P., White, K.L., Golos, T.G., Behr, B., Paul, S., 2012. Altered subcellular localization of transcription factor TEAD4 regulates first mammalian cell lineage commitment. Proc. Natl. Acad. Sci. USA 109, 7362–7367.
Houghton, F.D., 2005. Role of gap junctions during early embryo development. Reproduction 129, 129–135.
Houliston, E., Pickering, S.J., Maro, B., 1989. Alternative routes for the establishment of surface polarity during compaction of the mouse embryo. Dev. Biol. 134, 342–350.
Huber, O., 2003. Structure and function of desmosomal proteins and their role in development and disease. Cell. Mol. Life Sci.: CMLS 60, 1872–1890.
Johnson, M.H., Maro, B., 1984. The distribution of cytoplasmic actin in mouse 8-cell blastomeres. J. Embryol. Exp. Morphol. 82, 97–117.
Johnson, M.H., Maro, B., Takeichi, M., 1986. The role of cell adhesion in the synchronization and orientation of polarization in 8-cell mouse blastomeres. J. Embryol. Exp. Morphol. 93, 239–255.
Johnson, M.H., McConnell, J.M., 2004. Lineage allocation and cell polarity during mouse embryogenesis. Semin. Cell Dev. Biol. 15, 583–597.
Johnson, M.H., Ziomek, C.A., 1981. Induction of polarity in mouse 8-cell blastomeres: specificity, geometry, and stability. J. Cell Biol. 91, 303–308.
Kidder, G.M., Rains, J., McKeon, J., 1987. Gap junction assembly in the preimplantation mouse conceptus is independent of microtubules, microfilaments, cell flattening, and cytokinesis. Proc. Natl. Acad. Sci. USA 84, 3718–3722.
Kim, J., Gye, M.C., Kim, M.K., 2004. Role of occludin, a tight junction protein, in blastocoel formation, and in the paracellular permeability and differentiation of trophectoderm in preimplantation mouse embryos. Mol. Cells 17, 248–254.
Krupinski, P., Chickarmane, V., Peterson, C., 2011. Simulating the mammalian blastocyst–molecular and mechanical interactions pattern the embryo. PLoS Computat. Biol. 7, e1001128.
Larue, L., Ohsugi, M., Hirchenhain, J., Kemler, R., 1994. E-cadherin null mutant embryos fail to form a trophectoderm epithelium. Proc Natl. Acad. Sci. USA 91, 8263–8267.
Leung, C.Y., Zernicka-Goetz, M., 2013. Angiomotin prevents pluripotent lineage differentiation in mouse embryos via Hippo pathway-dependent and -independent mechanisms. Nat. Commun. 4, 2251.
Li, L., Zheng, P., Dean, J., 2010. Maternal control of early mouse development. Development 137, 859–870.
Lo, C.W., Gilula, N.B., 1979. Gap junctional communication in the preimplantation mouse embryo. Cell 18, 399–409.
Lommel, M., Winterhalter, P.R., Willer, T., Dahlhoff, M., Schneider, M.R., Bartels, M.F., Renner-Muller, I., Ruppert, T., Wolf, E., Strahl, S., 2013. Protein O-mannosylation is crucial for E-cadherin-mediated cell adhesion. Proceedings of the National Academy of Sciences of the United States of America.
Macara, I.G., 2004a. Par proteins: partners in polarization. Curr. Biol.: CB 14, R160–R162.
Macara, I.G., 2004b. Parsing the polarity code. Nat. Rev. Mol. Cell Biol. 5, 220–231.
Makowski, L., Caspar, D.L., Phillips, W.C., Goodenough, D.A., 1977. Gap junction structures. II. Analysis of the x-ray diffraction data. J Cell biol. 74, 629–645.
Maro, B., Kubiak, J., Gueth, C., De Pennart, H., Houliston, E., Weber, M., Antony, C., Aghion, J., 1990. Cytoskeleton organization during oogenesis, fertilization and preimplantation development of the mouse. Int. J. Dev Biol. 34, 127–137.
Martin-Padura, I., Lostaglio, S., Schneemann, M., Williams, L., Romano, M., Fruscella, P., Panzeri, C., Stoppacciaro, A., Ruco, L., Villa, A., Simmons, D., Dejana, E., 1998. Junctional adhesion molecule, a novel member of the immunoglobulin superfamily that distributes at intercellular junctions and modulates monocyte transmigration. J. Cell Biol. 142, 117–127.
Meilhac, S.M., Adams, R.J., Morris, S.A., Danckaert, A., Le Garrec, J.F., Zernicka-Goetz, M., 2009. Active cell movements coupled to positional induction are involved in lineage segregation in the mouse blastocyst. Dev. Biol. 331, 210–221.
Mitalipov, S., Wolf, D., 2009. Totipotency, pluripotency and nuclear reprogramming. Adv. Biochem. Eng./Biotechnol. 114, 185–199.
Mitsui, K., Tokuzawa, Y., Itoh, H., Segawa, K., Murakami, M., Takahashi, K., Maruyama, M., Maeda, M., Yamanaka, S., 2003. The homeoprotein Nanog is required for maintenance of pluripotency in mouse epiblast and ES cells. Cell 113, 631–642.
Mok, K.W., Mruk, D.D., Cheng, C.Y., 2013. Regulation of blood-testis barrier (BTB) dynamics during spermatogenesis via the "Yin" and "Yang" effects of mammalian target of rapamycin complex 1 (mTORC1) and mTORC2. Int. Rev. Cell Mol. Biol. 301, 291–358.
Morin-Kensicki, E.M., Boone, B.N., Howell, M., Stonebraker, J.R., Teed, J., Alb, J.G., Magnuson, T.R., O'Neal, W., Milgram, S.L., 2006. Defects in yolk sac vasculogenesis, chorioallantoic fusion, and embryonic axis elongation in mice with targeted disruption of Yap65. Mol. Cell. Biol. 26, 77–87.
Morris, S.A., Teo, R.T., Li, H., Robson, P., Glover, D.M., Zernicka-Goetz, M., 2010. Origin and formation of the first two distinct cell types of the inner cell mass in the mouse embryo. Proceedings of the National Academy of Sciences of the United States of America 107, 6364–6369.
Morrisey, E.E., Tang, Z., Sigrist, K., Lu, M.M., Jiang, F., Ip, H.S., Parmacek, M.S., 1998. GATA6 regulates HNF4 and is required for differentiation of visceral endoderm in the mouse embryo. Genes Dev. 12, 3579–3590.
Natale, D.R., Paliga, A.J., Beier, F., D'Souza, S.J., Watson, A.J., 2004. p38 MAPK signaling during murine preimplantation development. Dev. Biol. 268, 76–88.
Niakan, K.K., Eggan, K., 2013. Analysis of human embryos from zygote to blastocyst reveals distinct gene expression patterns relative to the mouse. Dev. Biol. 375, 54–64.
Niakan, K.K., Han, J., Pedersen, R.A., Simon, C., Pera, R.A., 2012. Human preimplantation embryo development. Development 139, 829–841.
Niakan, K.K., Ji, H., Maehr, R., Vokes, S.A., Rodolfa, K.T., Sherwood, R.I., Yamaki, M., Dimos, J.T., Chen, A.E., Melton, D.A., McMahon, A.P., Eggan, K., 2010. Sox17 promotes differentiation in mouse embryonic stem cells by directly regulating extraembryonic gene expression and indirectly antagonizing self-renewal. Genes Dev. 24, 312–326.
Nichols, J., Zevnik, B., Anastassiadis, K., Niwa, H., Klewe-Nebenius, D., Chambers, I., Scholer, H., Smith, A., 1998. Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4. Cell 95, 379–391.
Nikas, G., Ao, A., Winston, R.M., Handyside, A.H., 1996. Compaction and surface polarity in the human embryo in vitro. Biol. Reprod. 55, 32–37.
Nishioka, N., Inoue, K., Adachi, K., Kiyonari, H., Ota, M., Ralston, A., Yabuta, N., Hirahara, S., Stephenson, R.O., Ogonuki, N., Makita, R., Kurihara, H., Morin-Kensicki, E.M., Nojima, H., Rossant, J., Nakao, K., Niwa, H., Sasaki, H., 2009. The Hippo signaling pathway components Lats and Yap pattern Tead4 activity to distinguish mouse trophectoderm from inner cell mass. Dev. Cell 16, 398–410.
Nishioka, N., Yamamoto, S., Kiyonari, H., Sato, H., Sawada, A., Ota, M., Nakao, K., Sasaki, H., 2008. Tead4 is required for specification of trophectoderm in preimplantation mouse embryos. Mech. Dev. 125, 270–283.
Niwa, H., Toyooka, Y., Shimosato, D., Strumpf, D., Takahashi, K., Yagi, R., Rossant, J., 2005. Interaction between Oct3/4 and Cdx2 determines trophectoderm differentiation. Cell 123, 917–929.
Ohsugi, M., Larue, L., Schwarz, H., Kemler, R., 1997. Cell-junctional and cytoskeletal organization in mouse blastocysts lacking E-cadherin. Dev. Biol. 185, 261–271.
Ota, M., Sasaki, H., 2008. Mammalian Tead proteins regulate cell proliferation and contact inhibition as transcriptional mediators of Hippo signaling. Development 135, 4059–4069.
Pauken, C.M., Capco, D.G., 2000. The expression and stage-specific localization of protein kinase C isotypes during mouse preimplantation development. Dev. Biol. 223, 411–421.
Piotrowska-Nitsche, K., Zernicka-Goetz, M., 2005. Spatial arrangement of individual 4-cell stage blastomeres and the order in which they are generated correlate with blastocyst pattern in the mouse embryo. Mech. Dev. 122, 487–500.
Plachta, N., Bollenbach, T., Pease, S., Fraser, S.E., Pantazis, P., 2011. Oct4 kinetics predict cell lineage patterning in the early mammalian embryo. Nat. Cell Biol. 13, 117–123.
Plusa, B., Frankenberg, S., Chalmers, A., Hadjantonakis, A.K., Moore, C.A., Papalopulu, N., Papaioannou, V.E., Glover, D.M., Zernicka-Goetz, M., 2005. Down-regulation of Par3 and aPKC function directs cells towards the ICM in the preimplantation mouse embryo. J. Cell Sci. 118, 505–515.
Plusa, B., Piliszek, A., Frankenberg, S., Artus, J., Hadjantonakis, A.K., 2008. Distinct sequential cell behaviours direct primitive endoderm formation in the mouse blastocyst. Development 135, 3081–3091.
Plusa, B., Piotrowska, K., Zernicka-Goetz, M., 2002. Sperm entry position provides a surface marker for the first cleavage plane of the mouse zygote. Genesis 32, 193–198.
Roode, M., Blair, K., Snell, P., Elder, K., Marchant, S., Smith, A., Nichols, J., 2012. Human hypoblast formation is not dependent on FGF signalling. Dev. Biol. 361, 358–363.
Rossant, J., 1975. Investigation of the determinative state of the mouse inner cell mass. II. The fate of isolated inner cell masses transferred to the oviduct. J. Embryol. Exp. Morphol. 33, 991–1001.
Sasaki, H., 2010. Mechanisms of trophectoderm fate specification in preimplantation mouse development. Dev. Growth Differ. 52, 263–273.
Sheth, B., Fontaine, J.J., Ponza, E., McCallum, A., Page, A., Citi, S., Louvard, D., Zahraoui, A., Fleming, T.P., 2000. Differentiation of the epithelial apical junctional complex during mouse preimplantation development: a role for rab13 in the early maturation of the tight junction. Mech. Dev. 97, 93–104.
Strumpf, D., Mao, C.A., Yamanaka, Y., Ralston, A., Chawengsaksophak, K., Beck, F., Rossant, J., 2005. Cdx2 is required for correct cell fate specification and differentiation of trophectoderm in the mouse blastocyst. Development 132, 2093–2102.
Suzuki, A., Ishiyama, C., Hashiba, K., Shimizu, M., Ebnet, K., Ohno, S., 2002. aPKC kinase activity is required for the asymmetric differentiation of the premature junctional complex during epithelial cell polarization. J. Cell Sci. 115, 3565–3573.
Tabansky, I., Lenarcic, A., Draft, R.W., Loulier, K., Keskin, D.B., Rosains, J., Rivera-Feliciano, J., Lichtman, J.W., Livet, J., Stern, J.N., Sanes, J.R., Eggan, K., 2013. Developmental bias in cleavage-stage mouse blastomeres. Curr. Biol.: CB 23, 21–31.
Taylor, D.M., Ray, P.F., Ao, A., Winston, R.M., Handyside, A.H., 1997. Paternal transcripts for glucose-6-phosphate dehydrogenase and adenosine deaminase are first detectable in the human preimplantation embryo at the three- to four-cell stage. Mol. Reprod. Dev. 48, 442–448.

Thomas, F.C., Sheth, B., Eckert, J.J., Bazzoni, G., Dejana, E., Fleming, T.P., 2004. Contribution of JAM-1 to epithelial differentiation and tight-junction biogenesis in the mouse preimplantation embryo. J. Cell Sci. 117, 5599–5608.
Torres-Padilla, M.E., Parfitt, D.E., Kouzarides, T., Zernicka-Goetz, M., 2007. Histone arginine methylation regulates pluripotency in the early mouse embryo. Nature 445, 214–218.
Vestweber, D., Gossler, A., Boller, K., Kemler, R., 1987. Expression and distribution of cell adhesion molecule uvomorulin in mouse preimplantation embryos. Dev. Biol. 124, 451–456.
Vinot, S., Le, T., Ohno, S., Pawson, T., Maro, B., Louvet-Vallee, S., 2005. Asymmetric distribution of PAR proteins in the mouse embryo begins at the 8-cell stage during compaction. Dev. biology 282, 307–319.
Watson, A.J., Barcroft, L.C., 2001. Regulation of blastocyst formation. Frontiers in bioscience: a journal and Virtual Libr. 6, D708–D730.
Winkel, G.K., Ferguson, J.E., Takeichi, M., Nuccitelli, R., 1990. Activation of protein kinase C triggers premature compaction in the four-cell stage mouse embryo. Dev. Biol. 138, 1–15.
Wodarz, A., 2002. Establishing cell polarity in development. Nat. Cell Biol. 4, E39–E44.
Xu, J., Ji, J., Yan, X.H., 2012. Cross-talk between AMPK and mTOR in regulating energy balance. Crit. Rev. Food Sci. Nutr. 52, 373–381.
Yamanaka, Y., Lanner, F., Rossant, J., 2010. FGF signal-dependent segregation of primitive endoderm and epiblast in the mouse blastocyst. Development 137, 715–724.
Zeng, F., Schultz, R.M., 2005. RNA transcript profiling during zygotic gene activation in the preimplantation mouse embryo. Dev. Biol. 283, 40–57.
Zernicka-Goetz, M., Morris, S.A., Bruce, A.W., 2009. Making a firm decision: multifaceted regulation of cell fate in the early mouse embryo. Nat. Rev. Genet. 10, 467–477.
Zhang, J., Tam, W.L., Tong, G.Q., Wu, Q., Chan, H.Y., Soh, B.S., Lou, Y., Yang, J., Ma, Y., Chai, L., Ng, H.H., Lufkin, T., Robson, P., Lim, B., 2006a. Sall4 modulates embryonic stem cell pluripotency and early embryonic development by the transcriptional regulation of Pou5f1. Nat. Cell Biol. 8, 1114–1123.
Zhang, L., Li, J., Young, L.H., Caplan, M.J., 2006b. AMP-activated protein kinase regulates the assembly of epithelial tight junctions. Proc. Natl. Acad. Sci. USA 103, 17272–17277.
Zhao, B., Wei, X., Li, W., Udan, R.S., Yang, Q., Kim, J., Xie, J., Ikenoue, T., Yu, J., Li, L., Zheng, P., Ye, K., Chinnaiyan, A., Halder, G., Lai, Z.C., Guan, K.L., 2007. Inactivation of YAP oncoprotein by the Hippo pathway is involved in cell contact inhibition and tissue growth control. Genes Dev. 21, 2747–2761.