SCIREA Journal of Biology

http://www.scirea.org/journal/Biology

November 6, 2023

Volume 8, Issue 6, December 2023

https://doi.org/10.54647/biology180331

Genesis of Epithelial Polarity in Early Mammalian Embryos

Xiang-Xi Mike Xu, Elizabeth R. Smith

Department of Radiation Oncology; Department of Obstetrics, Gynecology and Reproductive Sciences, University of Miami Miller School of Medicine, Miami, FL 33136, United States

ABSTRACT

Epithelial cells comprise the surface layers that cover tissues and organs, and by definition, exhibit an asymmetric surface domain and hence apical-basal polarity. The development of early mammalian embryos, from a fertilized oocyte to a blastocyst and implanted embryo, provides an excellent system to observe the formation and morphogenesis of several epithelia. From our studies of early mouse embryonic development and morphogenesis, we propose that there are at the least three distinct types of mechanisms for polarization of epithelial cells: the classical tight junction and Par complexes; cell autonomous polarity established by endocytosis; and the subtle polarization caused by the formation of an apical actin cap of adjacent cells. Here, we describe the understanding of the genesis of four embryonic epithelial structures, the trophectoderm, inner cell mass, primitive endoderm, and epiblast, and the genes that are critical for their epithelial polarity and associated morphogenesis.

Key Words: blastocysts, early embryos, morphogenesis, cell sorting, embryonic stem cells, epithelial polarity.

Introduction

Early mammalian embryos, especially mouse blastocysts, provide a relatively simple, excellent model to study developmental lineage differentiation and morphogenesis (Rossant, 2004; Lu et al., 2001; Stephenson et al., 2012; Zhang and Hiiragi, 2018). Cell proliferation within a fertilized oocyte, and subsequent spontaneous assembly of cells in blastocysts and pre-implanting embryos lead to the development of four simple epithelial structures: the trophectoderm, the inner cell mass, and subsequent formation of the primitive endoderm and epiblast (**Figure 1**). Particularly, the embryonic system allows us to observe the emergence of the polarized epithelial structures from the aggregates of precursor cells (Eckert and Fleming, 2008; Zhang and Hiiragi, 2018). Such analyses of the formation of cell polarity may bring the most profound understanding of the simplest and essential features in cell organization into epithelial structure.

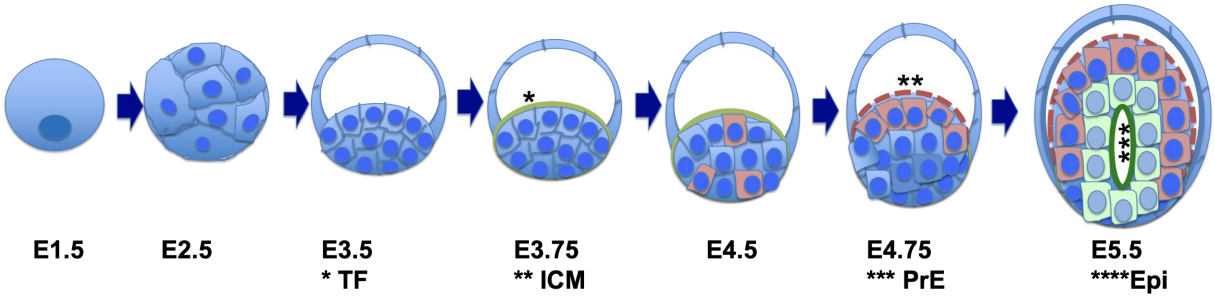

Figure 1. Illustration of the progressive morphogenesis and structure formation in early mouse embryos. One cell embryo (fertilized oocyte) divides to a multiple cell aggregate. At around 38 cell stage, the dividing embryo undergoes compaction to form blastocoel cavity and inner cell mass (ICM), a stage known as the blastocysts. A polarized trophectoderm layer is formed (*). The surface of ICM polarizes, signified by a layer of apical actin (**). Subsequently, primitive endoderm differentiation occurs in cells randomly distributed in ICM. The primitive endoderm cells sort to the surface, forming a polarized epithelium (***). A lumen is then developed, enveloped by a polarized ectoderm epithelium (****).

Here, we discuss the studies supporting our proposal for three distinct types of mechanisms for generating apical-basal polarity. Particularly, we describe the development of subtle polarization by the formation of an apical actin cap on the surface of the inner cell mass, the cell autonomous polarity established by Dab2-dependent directional endocytosis in the primitive endoderm, and the epithelial polarity that occurs due to the classical tight junction and Par complexes of the trophectoderm and epiblast. Our discussion is limited as several excellent reviews on early embryogenesis and morphogenesis will provide more comprehensive reviews of the topics (Leung et al., 2016; Lim and Plachta, 2021; Martin et al.,

2021; Zhang and Hiiragi, 2018; Zhu et al., 2020). Several significant and informative recent studies also provide additional advance beyond the scope of the current concise review (Kim et al., 2021; Lim et al., 2020; Ryan et al., 2019; Shahbazi et al., 2016, 2017; Zenker et al., 2018; Zhu et al., 2017).

Trophectoderm epithelium

The trophectoderm is the first epithelium established in the early mammalian embryos/blastocysts. Extensive studies have mapped the development of the trophectoderm lineage and the generation of a polarized epithelium (Stephenson et al., 2010). Prior to the blastocyst stage, cells of the outer layer of the early mouse embryos (blastomeres) differentiate into trophectoderm, which exhibits apical-basal polarity and envelops the apolar cells of the inner cell mass (Stephenson et al., 2010). After cavitation occurs to form blastocysts, the trophectoderm develops into a single layer of polarized epithelium (Stephenson et al., 2010) (**Figure 2**), where F-actin, PKCzeta, and P-ERM are found at the apical domain, and Na^+/K^+-ATPase, Jam-1, and FGFR2 segregate to the basal bilateral domain (Salas-Vidal and Lomelí, 2004; Stephenson et al., 2010; Anani et al., 2014). E-cadherin is critical for the formation of tight junction in the generation of polarity and organization of the epithelium; however, trophectoderm apical-basal polarity and surface positioning of the epithelial cells are independent of differentiation into the trophectoderm and inner cell mass lineages (Stephenson et al., 2010).

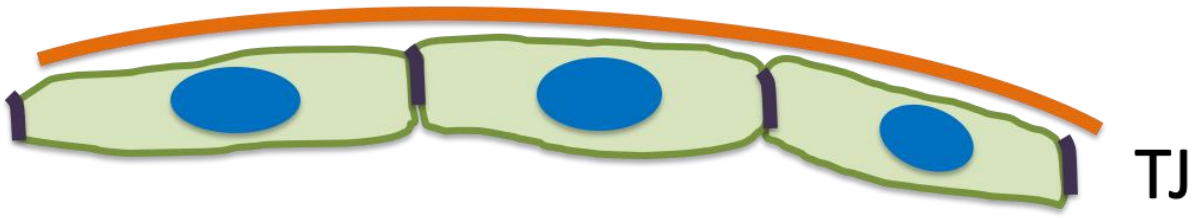

Figure 2. Formation of trophectoderm. An E-cadherin dependent process of cell compaction leads to the progression of the embryo to blastocyst stage. A polarized trophectoderm consisting of single cell layer jointed with tight junction is formed (*).

Prior to the formation of trophectoderm layer, the blastomere shows an outer/inner configuration of cells within the embryonic cell aggregate, in which an actin cap forms on the surface of the outer cell layer (Salas-Vidal and Lomelí, 2004; Stephenson et al., 2010; Anani et al., 2014). Thus, the polarization of the trophectoderm may be initiated by asymmetric distribution of apical actin cap (discussed further in a later section). However, the polarity of the mature trophectoderm epithelium appears to fit the criteria of the classical tight junction containing PKCzeta and ZO-1/ZO-2, components of the polarity complex. Several atypical PKC (aPKC) isoforms appear to participate in the formation of trophectoderm polarity, indicating possible redundancy of the PKC isoforms in early embryogenesis (Carracedo et al., 2014). Disturbing the activity of the aPKC/PAR6 complex using siRNA to down-regulate aPKClambda expression results in an absence of tight junctions. Although polarity of the trophectoderm is not abolished at the earlier (8-cell) stage, it is severely defective at the 16-cell stage (Dard et al., 2009). Thus, likely the actin cap formation with adherent junction accounts for the polarity of the earlier stage, though tight junction is critical for the maintenance of polarity of the more mature trophectoderm. The polarized surface cells generally take on a trophectoderm cell fate, and the nonpolar cells eventually internalize to become cells of the inner cell mass. Thus, polarity rather than cell position is important in cell fate commitment (Anani et al., 2014). Although E-cadherin and the components of the classical polarity complex are not essential for trophectoderm lineage differentiation (Stephenson et al., 2010), the apical domain of the surface cells that contains the actin cap serves as a cue that is required and sufficient to initiate the differentiation (Korotkevich et al., 2017). Therefore, the initial polarization marked by the asymmetric distribution of actin at the apical domain, due to inner/outer configuration, may be the initial cue in the initiation of trophectoderm differentiation. However, polarization by tight junction plays a critical role in maintaining surface positioning and epithelial organization of the trophectoderm.

Surface polarity of the cell of inner cell mass

Prior to differentiation, the cells of the inner cell mass (ICM) are pluripotent and resemble an aggregation of embryonic stem (ES) cells. Studies of ES cell aggregates in culture revealed that the outer layers of the ICM develop a subtle polarity, in which an actin cap involving several adjacent cells can be found upon maturation of the ES cell aggregates (Yano et al., 2017) (**Figure 3**). The presence and function of this polarity in the blastocysts are not understood.

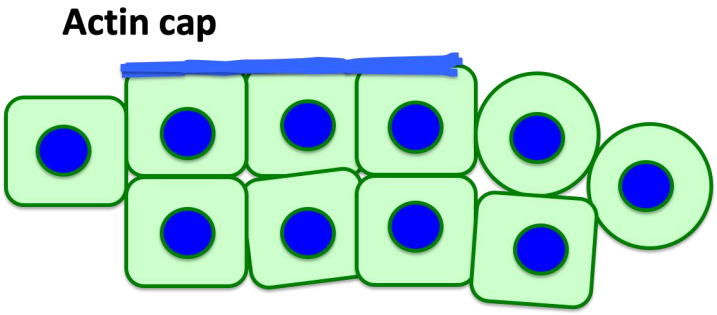

Figure 3. Development of polarity on the surface of inner cell mass. Within the blastocyst covered by a layer of trophectoderm cells, an aggregate of . A subtle polarity is formed on the surface by adjacent cells of the inner cell mass, signified by apical actin cap spanning multiple adjacent cells (**). At this stage, no tight junction is established.

The significance of this subtle epithelial polarity was shown in studies of cell sorting between highly adhesive wild type and less adhesive E-cadherin null ES cells. The highly adhesive ES cells were able to sort and position on surface and envelop the E-cadherin deficient cells (Tse et al., 2021). The sorting pattern was opposite that of predicted by the differential adhesive affinity hypothesis (Steinberg, 2007). The force for the retention of the highly adhesive cells on surface can be attributed to the ability of the cells to develop a subtle apical polarity signified by an actin cap (Lim and Plachta, 2021; Yano et al., 2017). This type of epithelial polarity differs apparently from the classic epithelial polarity that is formed by tight junctions and involves Par6, PKC-alpha, and ZO-1/2. The subtle surface polarity may be transient and the surface layer will then be disrupted and replaced by primitive endoderm that subsequently develops.

The apical actin cap is also seen on the surface of blastomeres (Salas-Vidal and Lomelí, 2004; Stephenson et al., 2010; Anani et al., 2014), and may be the initiation signal that triggers the segregation of the outer trophectoderm from the cells of the inner cell mass lineages (Korotkevich et al., 2017).

Cell sorting and the formation of primitive endoderm epithelium

Immediately prior to implantation at around E4.5, a primitive endoderm epithelial structure develops that covers the epiblast (**Figure 1**). The initial differentiation of the primitive endoderm lineage occurs randomly within the ICM (Rossant, 2004). Differentiation depends on the transcription factor GATA6 (Cai et al., 2008; Bessonnard et al., 2014), and the Fgf/Ras/MAPK signaling pathway (Chazaud et al., 2006; Kang et al., 2013; Krawchuk et al.,

2013; Kuijk et al., 2012; Lanner and Rossant, 2010). Erk1/2 phosphorylation of GATA6 enables the activation of the GATA6 self-promoter in a positive feed forward mechanism (Meng et al., 2018). Subsequently, the differentiated cells sort to the surface to form the primitive endoderm layer (Chazaud and Yamanaka, 2016; Rula et al., 2007). The progressive development of apical polarity has been well documented (Gerbe et al., 2008).

The embryonic phenotype of Dab2-deficient mice provides substantial clues to the mechanism for the cell sorting and formation of the primitive endoderm (Yang et al., 2002; 2007; Moore et al., 2013). Primitive endoderm differentiation occurs in the absence of Dab2. However, the differentiated cells fail to sort and form a surface layer; rather the primitive endoderm cells instead intermingle with the epiblast cells. Dab2 is an endocytosis adaptor, and links endocytic cargos to myosin VI, a motor that travels along actin bundles (Dance et al., 2004; Morris et al., 2002). Dab2 mediates unique directional trafficking of endocytic cargos that gives rise to apical polarity, and the Dab2-dependent polarity enables the sorting and positioning of the primitive endoderm layer (Yang et al. 2007; Moore et al., 2013). Thus, the initial polarity of the primitive endoderm cells is cell autonomous, and cell-cell adhesion of multiple primitive endoderm cells is not required initially (**Figure 4**). This property would allow the differentiated cells to reach surface gradually and independently, and form a continuous outer layer subsequently. The cell-cell adhesion independent polarity of the primitive may also allow the subsequent differentiation and migration of the cells to the parietal endoderm to cover the blastocoel.

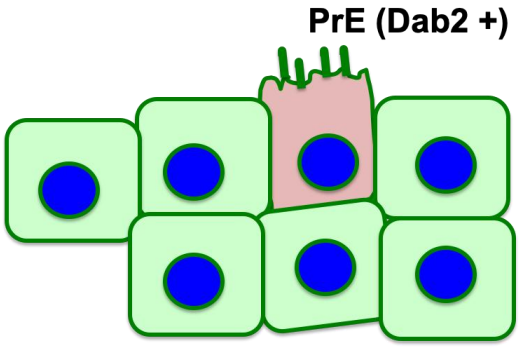

Figure 4. Formation of Apical polarity of primitive endoderm. At around E4.5, primitive endoderm differentiation occurs, and Dab2 is a marker of primitive endoderm cells. The differentiated cells randomly distributed either superficially or internally. Subsequently, the cells sort to surface by a Dab2-dependent mechanism to form a primitive endoderm epithelial layer. The primitive endoderm epithelial cells are thought to position on surface by a cell autonomous mechanism for intrinsic ability to establish polarity once reaching cell surface. The polarization of the primitive endoderm cells is thought to rely on endocytosis by a Dab2-depedent directional endocytic trafficking of cargos along cytoskeletal network.

As this type of apical polarity is considered to depend on Dab2-mediated directional trafficking of endocytic cargos on actin filaments, it would be reasonable to assume that the described polarity depends on the actin cytoskeleton. It has not been tested if the primitive endoderm sorting to surface relies on the actin cytoskeleton.

At a later stage, the primitive endoderm matures into the visceral endoderm, and differentiation involves the classical tight junction dependent apical basal polarity. It is reported that ZO-1 and ZO-2 are required for extraembryonic endoderm in embryoid body models (Phua et al., 2014). Although aPKC and PKCZ gene knockouts present a much later embryonic lethality phenotype (Sengupta et al., 2011; Saiz et al., 2013), suggesting the classical tight junction dependent polarity mechanism is not essential for the formation of primitive endoderm epithelium and neither is required for the formation of a polarized epithelial layer. Likely, these genes participate in the proper function and fine regulation of the primitive endoderm epithelium, but their functions may be redundant and their absence does not stop the formation of the basic epithelial polarity.

Integrin beta1 is essential for embryonic development at the primitive endoderm stages (Fässler and Meyer,1995; Stephens et al., 1995). Further analyses of integrin beta1 null embryos and embryoid bodies indicated that a polarized primitive endoderm layer forms initially; however, the differentiated endoderm cell layer rounds up and detaches because the cells fail to bind the basement membrane (Moore et al., 2014). Although integrin beta1 apparently is required for organization of the primitive endoderm, the cell adhesion molecule is not essential for polarity of the primitive endoderm cells (Moore et al., 2014).

Thus, so far only Dab2 has been clearly shown to be required for primitive endoderm polarization and organization (Yang et al. 2007; Moore et al., 2013). Another, similar-functioning endocytosis adaptor, Arh, is also expressed in the early embryos, but is not essential for primitive endoderm development (Maurer and Cooper, 2005; Tao et al., 2016). The Dab2 protein is found in many epithelia including the kidney, lactating mammary gland, ovary, and other tissues (Tao et al., 2017); however, Dab2 does not appear to be essential for the formation of other epithelium in the embryos, since deletion of Dab2 in the embryo proper but not in extraembryonic tissues using Sox2-Cre can bypass embryonic lethality (Moore et al., 2013).

Embryonic cavitation and the formation of the epiblast epithelium

In mouse embryos at around the E5.5 stage, the proamniotic cavity forms in the center of the epiblast, and the epiblast cells organize into a layer of polarized epithelium (**Figure 1**). Proamniotic cavitation has traditionally been thought to occur by apoptotic cell death (Coucouvanis and Martin, 1995; 1999). However, recent observations indicate that the cavity forms by the expansion of an adhesion rosette, rather than cell death (Bedzhov and Zernicka-Goetz, 2014; Christodoulou et al., 2018). Expansion of the rosette creates a luminal surface and thus establishes polarity of the epithelial cells. One idea is that the polarity is created by ligation of integrins to the basement membrane on the basal side (Bedzhov and Zernicka-Goetz, 2014).

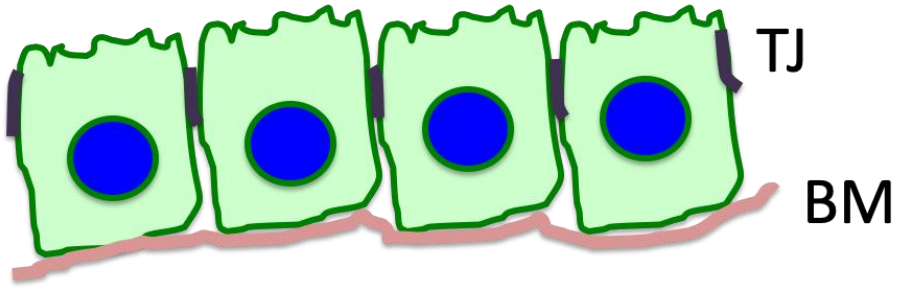

Figure 5. Epithelial polarity of embryonic ectoderm and the formation of proamniotic lumen. At around E5.5 stage, E-cadherin mediated cell–cell adhesion intensifies in the epiblast and stimulates increased Pten focal expression that initiates the formation of a dominant rosette (multiple ZO-1 focal stainings and Pten positive). The epithelial tight junction and apical surface are matured at the rosette, which expands into the proamniotic lumen composed of polarized epithelial cells. Excessive cells located within the lumen may be removed by apoptosis. When Pten is absent, cell adherent junctions form at random cell–cell contact sites, but no concentrated localization of tight junctions are initiated to establish a dominant rosette. Thus, Pten is required for proamniotic cavitation of mouse embryos before gastrulation, by the mechanism of epiblast cell polarization and rosette formation rather than apoptosis. Pten activity initiates apical polarity and subsequent expansion of the rosette into the proamniotic lumen.

The analysis of Pten deficient embryos provides additional clues to the mechanism of cavitation and the origin of epiblast epithelial polarity (Meng et al., 2017) (**Figure 5**). Despite the presence of a well-developed visceral endoderm, a proamniotic lumen fails to form in Pten knockout embryos, suggesting an essential role of Pten in the creation of the lumen (Meng et al., 2017). Pten expression was found to concentrate at the site of a rosette in the pre-cavitation embryos and embryoid bodies, suggesting the localization of Pten there may be an initiating signal of polarity (Meng et al., 2017). PKCa and ZO-1 are also present at the site of

the lumen opening, indicating the formation of tight junction is required for the initiation of epiblast lumen polarity (Christodoulou et al., 2018). The developing luminal epithelium exhibits strong ZO-1 marked tight junctions (Meng et al., 2017), which indicates that the epiblast/ectoderm epithelial polarity is established via a classical tight junction mechanism. Additionally, cortical actin also presents along with tight junction markers as the rosette expands to form a lumen (Meng et al., 2017). The initiation of polarity from the rosette structure may be accounted for by strong lateral E-cadherin bonding of the adjacent cells. Consistently, it is reported that E-cadherin mediates the induction Pten expression (Lau et al., 2011). Besides Pten, we are not aware of rigorous studies of embryos of any additional gene deletions that exhibit a similar phenotype—a failure of cavitation and luminal formation of the epiblast.

Consistently, the development of ectoderm fails in knockout mouse embryos lacking aPKC or PKC zeta (Saiz et al., 2013; Sengupta et al., 2011), key components of the tight junction polarity complex. In embryoid body models, the ectoderm does not organize when both ZO-1 and ZO-2 are absent (Phua et al., 2014). Thus, establishment of ectoderm epithelium requires the classical tight junction polarity complex.

Genes involved in embryonic epithelial polarity

The embryonic phenotypes of gene knockout mice may serve as an indication for the involvement of a gene in the genesis of epithelial polarity (**Table 1**). Only a few genes present an early lethality in knockout mice, and only a subset of these genes may act in morphogenesis rather than cell survival, proliferation, and differentiation. Presumably, genes affect morphogenesis and the assembly of cells, and act by affecting a few defined physical mechanisms, by affecting such as cell-cell adhesion, cell-matrix adhesion, and cell polarity.

TABLE 1. Gene knockout affecting early mouse embryonic morphogenesis

Gene(s)	Manipulation	Phenotype(s)	References
E-cadherin	Gene Knockout	E3.0: failed cell compaction to form blastocyst cavity, failed trophectoderm formation	Larue et al., 1994; Ohsugi et al., 1997; Stephenson et al., 2010
Intigrin beta1	Gene Knockout	E4.5-E5.5: primitive endoderm segregation from epiblast	Moore et al., 2019
Dab2	Gene Knockout	E5.0: extraembryonic endoderm disorganization	Yang et al., 2002; 2007; Moore et al.,

			2013
Pten	Gene Knockout	E5.5 to E6.5: Fail to undergo proamniotic cavitation	Meng et al., 2018
ZO-1 and ZO-2	Embryoid bodies of knockout ES cells	ZO-1 and ZO-2 are required for extra-embryonic endoderm integrity, primitive ectoderm survival and normal cavitation in embryoid bodies.	Phua et al., 2014
ZO-2	Gene knockout	E6.5, Fail to undergo proamniotic cavitation	Xu et al., 2008
PI3KC3	Gene knockout	Failed cavitation at E6.5-7.5	Zhou et al., 2011
Rho-Associated Kinase	Inhibitors, RNAi	Abnormal morphogenesis of ICM	Laeno et al., 2013
Par3	Gene knockout	Mid-gestation (E10.5-11.5)	Hirose et al., 2006
Par6	Gene knockout	Early (no detail)	
CDC42	Gene knockout	Failed cavitation at E6.5-7.5	Chen et al, 2000
aPKC	Inhibitors, RNAi	Deficient PrE maturation and organization	Saiz et al., 2013
aPKCλ: Protein kinase C-zeta (PKCZ)	Gene knockout	Very early embryonic lethality (no details)	Sengupta et al., 2011
PARD6B	siRNA	trophectoderm formation in preimplantation	Alarcon et al., 2010
Prkci	Gene knockout, embryoidbodies	cavitation	Mah et al., 2016

Notes: The knockout of the following genes has later (later than E7.5 stage) embryonic phenotypes, including N-cadherin (Radice et al., 1997), Arh (Tao et al., 2016), FAK (Furuta et al., 1995), collagens (Pöschl et al., 2004), laminins (Li et al., 2003), Rac1 (Sugihara et al., 1998), aPKCζ (Leitges et al., 2001), Crumbs3 (Whiteman et al., 2014), PKCι (Forteza et al., 2016).

The reasons for lack of an early embryonic phenotype may be redundant gene functions or the roles are not critical in morphogenesis.

Here, we do not include knockout of genes that do not primarily impact morphogenesis, but rather affect lineage differentiation and cell growth and survivals (such as GATA6, Grb2, Oct3/4, Nanog, etc.).

The trophectoderm is the first epithelial structure that forms in mammalian embryos, and several of polarity genes are known to affect trophectoderm development, such as PARD6B (Alarcon et al., 2010). At the end, trophectoderm polarity appears to be the classic type that requires formation of tight junctions (Salas-Vidal and Lomelí, 2004; Stephenson et al., 2010; Anani et al., 2014). The trophectoderm epithelium progressively recruits proteins to the tight junction complexes, correlating with an increased loss in permeability (Wiley et al., 1990).

Polarity complex proteins such as ZO-1 and ZO-2, PI3KC3, Rho-Associated Kinase, Par3; Par6, CDC42, and aPKCλ, affect the epiblast (Phua et al., 2014; Zhou et al., 2011; Laeno et

al., 2013; Hirose et al., 2006; Chen et al, 2000; Saiz et al., 2013; Sengupta et al., 2011) (**Table 1**), suggesting that tight junction polarity is critical for the development of the ectoderm layer.

Pten is involved in the formation of epiblast epithelial structure; however, Pten deletion does not affect the trophectoderm layer (Meng et al., 2018). It is possible that Pten plays a critical role in the initiation of polarization in the interior of cell aggregates by providing the first cue, but in trophectoderm differentiation the initiation cue is given by the inner/outer cells configuration.

Dab2 is required for polarization and formation of the primitive endoderm. Surprisingly, however, deletion of Dab2 within the inner cell mass using Sox2-cre does not at all affect the development of embryo proper (Moore et al., 2013). Thus, it appears that Dab2-mediated endocytosis to achieve cell polarization is only required for the development and organization of primitive endoderm. The mechanism may be used again during development, but those are non-essential, likely because of redundant functions from other endocytic adaptor proteins or another redundant mechanism to achieve cell polarization.

Summary: Variations in types and mechanisms of epithelial polarity

Epithelial polarity is commonly recognized as a structural feature signified by the presence of tight junctions (Johnson and McConnell, 2004; Chen and Zhang, 2013; Nance, 2014; Campanale et al., 2017). Indeed, this classic type of polarity is probably present in the majority of epithelial structures, particularly in mature adult tissues (Joberty et al., 2000). Our investigation of polarity in embryonic epithelia recognizes additional distinct types, one of which depends upon Dab2 and the other formed by an apical actin cap. Studies in embryonic tissues have also allowed us to better understand the mechanisms responsible for initiation and formation of polarized epithelial structures.

Thus, we propose that epithelial polarity at the least can be classified into three different types, discussed below.

Epithelial polarity signified by the Par complex, which includes Par6, Par3, ZO-1/2, and aPKC (Joberty et al., 2000; Chen and Zhang, 2013), requires the adhesion of multiple cells to form a layer. The epiblast/embryonic ectoderm epithelium is an example of this classic type of polarity in early mammalian embryos. A second type requires directional endocytic trafficking to establish an asymmetric distribution of cellular components, and is dependent

upon Dab2 (or additional endocytic adaptors). This polarity can be cell autonomous, and may entail asymmetrically arranged actin or microtubule cytoskeletons. We also observe a type of subtle polarity presented by an actin cap spanning the apical surfaces of multiple adjacent cells (Tse et al., 2021). This third type of epithelial polarity often appears to first emerge from E-cadherin-mediated cell-cell adhesion on a tissue surface, but the polarized organization is then replaced by tight junction protein complex (Eckert and Fleming, 2008).

Furthermore, actual polarity in epithelial tissues may be a combination of two, or perhaps all three, of the discussed mechanisms. The uncoupling of these mechanisms in the presentation of epithelial polarity has been observed. For example, apical polarity is not eliminated when tight junctions are eliminated (Vega-Salas et al., 1987). Also, polarity is still retained with actin and microtubule cytoskeletons are disrupted (Salas et al., 1986). These studies indicate various mechanisms and multiple types of epithelial polarity are involved in achieving the asymmetric structure.

Epithelial polarity may be established initially with only minimal and essential features. The early epithelium may be highly dynamic and changeable to allow rapid expansion and development. However, many of the epithelial structures may undergo maturation with increasingly complex features and higher stability, to reach a homeostatic state. Thus, in more established epithelial structure, two or more mechanisms (an apical actin network, directional endocytic transport, and/or formation of tight junctions) may cooperate to facilitate epithelial polarity that is robust and exhibits redundant processes.

Likely, recognition of these mechanisms will allow us to approach understanding the principles in cell assembly and technical ability for application in regenerative medicine.

REFERENCES

[1] Alarcon VB. Cell polarity regulator PARD6B is essential for trophectoderm formation in the preimplantation mouse embryo. Biol Reprod. 2010 Sep;83(3):347-58.

[2] Anani S, Bhat S, Honma-Yamanaka N, Krawchuk D, Yamanaka Y. Initiation of Hippo signaling is linked to polarity rather than to cell position in the pre-implantation mouse embryo. Development. 2014 Jul;141(14):2813-24.

[3] Bedzhov I, Zernicka-Goetz M. Self-organizing properties of mouse pluripotent cells initiate morphogenesis upon implantation. Cell 2014; 156:1032-1044.

[4] Bedzhov I, Zernicka-Goetz M. Cell death and morphogenesis during early mouse

development: are they interconnected? Bioessays. 2015 Apr;37(4):372-8.

[5] Bessonnard S, De Mot L, Gonze D, Barriol M, Dennis C, Goldbeter A, Dupont G, Chazaud C. Gata6, Nanog and Erk signaling control cell fate in the inner cell mass through a tristable regulatory network. Development 2014; 141:3637-3648.

[6] Campanale JP, Sun TY, Montell DJ. Development and dynamics of cell polarity at a glance. J Cell Sci. 2017 Apr 1;130(7):1201-1207.

[7] Cai KQ, Capo-Chichi CD, Rula ME, Yang DH, Xu XX. Dynamic GATA6 expression in primitive endoderm formation and maturation in early mouse embryogenesis. Dev Dyn 2008; 237:2820-2829.

[8] Capo-Chichi CD, Rula ME, Smedberg JL, Vanderveer L, Parmacek MS, Morrisey EE, Godwin AK, Xu XX. Perception of differentiation cues by GATA factors in primitive endoderm lineage determination of mouse embryonic stem cells. Dev Biol 2005; 286:574-586.

[9] Capo-Chichi CD, Smedberg JL, Rula M, Nicolas E, Yeung AT, Adamo RF, Frolov A, Godwin AK, Xu XX. Alteration of Differentiation Potentials by Modulating GATA Transcription Factors in Murine Embryonic Stem Cells. Stem Cells Int. 2010; 2010:602068.

[10] Carracedo S, Sacher F, Brandes G, Braun U, Leitges M. Redundant role of protein kinase C delta and epsilon during mouse embryonic development. PLoS One. 2014 Aug 1;9(8):e103686.

[11] Chazaud C, Yamanaka Y. Lineage specification in the mouse preimplantation embryo. Development 2016; 143:1063-1074.

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

[13] Chen F, Ma L, Parrini MC, Mao X, Lopez M, Wu C, Marks PW, Davidson L, Kwiatkowski DJ, Kirchhausen T, Orkin SH, Rosen FS, Mayer BJ, Kirschner MW, Alt FW. Cdc42 is required for PIP(2)-induced actin polymerization and early development but not for cell viability. Curr Biol. 2000 Jun 29;10(13):758-65.

[14] Chen J, Zhang M. The Par3/Par6/aPKC complex and epithelial cell polarity. Exp Cell Res. 2013 Jun 10;319(10):1357-64.

[15] Cheng AM, Saxton TM, Sakai R, Kulkarni S, Mbamalu G, Vogel W, Tortorice CG, Cardiff RD, Cross JC, Muller WJ, Pawson T. Mammalian Grb2 regulates multiple steps in embryonic development and malignant transformation. Cell 1998; 95:793-803.

[16] Christodoulou N, Kyprianou C, Weberling A, Wang R, Cui G, Peng G, Jing N, Zernicka-Goetz M. Sequential formation and resolution of multiple rosettes drive embryo remodelling after implantation. Nat Cell Biol. 2018 Nov;20(11):1278-1289.

[17] Coucouvanis E, Martin GR. 1995. Signals for death and survival: a two-step mechanism for cavitation in the vertebrate embryo. Cell 83:279-287.

[18] Coucouvanis E, Martin GR. BMP signaling plays a role in visceral endoderm differentiation and cavitation in the early mouse embryo. Development 1999; 126:535-546.

[19] Dance AL, Miller M, Seragaki S, Aryal P, White B, Aschenbrenner L, Hasson T. Regulation of myosin-VI targeting to endocytic compartments. Traffic. 2004 Oct;5(10):798-813.

[20] Dard N, Le T, Maro B, Louvet-Vallée S. Inactivation of aPKClambda reveals a context dependent allocation of cell lineages in preimplantation mouse embryos. PLoS One. 2009 Sep 21;4(9):e7117.

[21] Eckert JJ, Fleming TP. Tight junction biogenesis during early development. Biochimica et Biophysica Acta (BBA) – Biomembranes. 2008; 778:717-728.

[22] Fässler R, Meyer M. 1995. Consequences of lack of beta 1 integrin gene expression in mice. Genes Dev. 9:1896–1908.

[23] Feldman B, Poueymirou W, Papaioannou VE, DeChiara TM, Goldfarb M. Requirement of FGF-4 for postimplantation mouse development. Science 1995; 267:246-249.

[24] Forteza R, Wald FA, Mashukova A, Kozhekbaeva Z, Salas PJ. Par-complex aPKC and Par3 cross-talk with innate immunity NF-κB pathway in epithelial cells. Biol Open. 2013 Oct 8;2(11):1264-9.

[25] Forteza R, Figueroa Y, Mashukova A, Dulam V, Salas PJ. Conditional knockout of polarity complex (atypical) PKCι reveals an anti-inflammatory function mediated by NF-κB. Mol Biol Cell. 2016 Jul 15;27(14):2186-97.

[26] Frankenberg S, Gerbe F, Bessonnard S, Belville C, Pouchin P, Bardot O, Chazaud C. Primitive endoderm differentiates via a three-step mechanism involving Nanog and RTK signaling. Dev Cell 2011; 21:1005-1013.

[27] Frankenberg SR, de Barros FR, Rossant J, Renfree MB. The mammalian blastocyst. Wiley Interdiscip Rev Dev Biol. 2016 Mar-Apr;5(2):210-32.

[28] Fujikura J, Yamato E, Yonemura S, Hosoda K, Masui S, Nakao K, Miyazaki J, Niwa H. Differentiation of embryonic stem cells is induced by GATA factors. Gene Dev 2002; 16:784-789.

[29] Furuta Y, Ilić D, Kanazawa S, Takeda N, Yamamoto T, Aizawa S. Mesodermal defect in late phase of gastrulation by a targeted mutation of focal adhesion kinase, FAK. Oncogene. 1995 Nov 16;11(10):1989-95.

[30] Gardner RL. Cell allocation and lineage in the early mouse embryo. Ciba Found Symp 1989; 144:172-181; discussion 181-186, 208-211.

[31] Gardner RL. Origin and differentiation of extraembryonic tissues in the mouse. Int Rev Exp Pathol 1983; 24:63-133.

[32] Gardner RL. Investigation of cell lineage and differentiation in the extraembryonic endoderm of the mouse embryo. J Embryol Exp Morphol 1982; 68:175-198.

[33] Gerbe F, Cox B, Rossant J, Chazaud C. Dynamic expression of Lrp2 pathway members reveals progressive epithelial differentiation of primitive endoderm in mouse blastocyst. Dev Biol. 2008 Jan 15;313(2):594-602.

[34] Goldin SN, Papaioannou VE. Paracrine action of FGF4 during periimplantation development maintains trophectoderm and primitive endoderm. Genesis 2003; 36:40-47.

[35] Greber B, Wu G, Bernemann C, Joo JY, Han DW, Ko K, Tapia N, Sabour D, Sterneckert J, Tesar P, Schöler HR. Conserved and divergent roles of FGF signaling in mouse epiblast stem cells and human embryonic stem cells. Cell Stem Cell. 2010 Mar 5;6(3):215-26.

[36] Hayashi S, Lewis P, Pevny L, McMahon AP. Efficient gene modulation in mouse epiblast using a Sox2Cre transgenic mouse strain. Mech Dev 2002; 119:S97-S101.

[37] Hermitte S, Chazaud C. Primitive endoderm differentiation: from specification to epithelium formation. Phil Tran Royal Soc of London Series B 2014; 369:1657.

[38] Hirose T, Karasawa M, Sugitani Y, Fujisawa M, Akimoto K, Ohno S, Noda T. PAR3 is essential for cyst-mediated epicardial development by establishing apical cortical domains. Development. 2006 Apr;133(7):1389-98.

[39] Joberty, G., Petersen, C., Gao, L., Macara, I. G. The cell-polarity protein Par6 links Par3 and atypical protein kinase C to Cdc42. Nature Cell Biol. 2: 531-539, 2000.

[40] Johnson MH, McConnell JM. Lineage allocation and cell polarity during mouse embryogenesis. Semin Cell Dev Biol. 2004 Oct;15(5):583-97.

[41] Jones C, Hammer RE, Li WP, Cohen JC, Hobbs HH, Herz J. Normal sorting but defective endocytosis of the low density lipoprotein receptor in mice with autosomal recessive hypercholesterolemia. J Biol Chem. 2003 Aug 1;278(31):29024-30.

[42] Kang M, Piliszek A, Artus J, Hadjantonakis AK. FGF4 is required for lineage restriction and salt-and-pepper distribution of primitive endoderm factors but not their initial

expression in the mouse. Development 2013; 140:267-279.

[43] Kang M, Garg V, Hadjantonakis AK. Lineage Establishment and Progression within the Inner Cell Mass of the Mouse Blastocyst Requires FGFR1 and FGFR2. Dev Cell. 2017 Jun 5;41(5):496-510.e5.

[44] Keramari M, Razavi J, Ingman KA, Patsch C, Edenhofer F, Ward CM, Kimber SJ: Sox2 is essential for formation of trophectoderm in the preimplantation embryo. PLoS One 2010; 5:e13952.

[45] Kim YS, Fan R, Kremer L, Kuempel-Rink N, Mildner K, Zeuschner D, Hekking L, Stehling M, Bedzhov I. Deciphering epiblast lumenogenesis reveals proamniotic cavity control of embryo growth and patterning. Sci Adv. 2021 Mar 10;7(11):eabe1640.

[46] Krawchuk D, Honma-Yamanaka N, Anani S, Yamanaka Y. FGF4 is a limiting factor controlling the proportions of primitive endoderm and epiblast in the ICM of the mouse blastocyst. Dev Biol 2013; 384:65-71.

[47] Korotkevich E, Niwayama R, Courtois A, Friese S, Berger N, Buchholz F, Hiiragi T. The Apical Domain Is Required and Sufficient for the First Lineage Segregation in the Mouse Embryo. Dev Cell. 2017 Feb 6;40(3):235-247.e7.

[48] Kuijk EW, van Tol LT, Van de Velde H, Wubbolts R, Welling M, Geijsen N, Roelen BA. The roles of FGF and MAP kinase signaling in the segregation of the epiblast and hypoblast cell lineages in bovine and human embryos. Development 2012; 139:871-882.

[49] Lanner F, Rossant J. The role of FGF/Erk signaling in pluripotent cells. Development 2010; 137:3351-3360.

[50] Larue, L., Ohsugi, M., Hirchenhain, J. and Kemler, R. (1994). E-cadherin null mutant embryos fail to form a trophectoderm epithelium. Proc. Natl. Acad. Sci. USA 91, 8263-8267.

[51] Lau MT, Klausen C, Leung PC. E-cadherin inhibits tumor cell growth by suppressing PI3K/Akt signaling via β-catenin-Egr1-mediated PTEN expression. Oncogene. 2011 Jun 16;30(24):2753-66.

[52] Leung CY, Zhu M, Zernicka-Goetz M. Polarity in Cell-Fate Acquisition in the Early Mouse Embryo. Curr Top Dev Biol. 2016;120:203-34.

[53] Leitges M, et al. (2001) Targeted disruption of the zetaPKC gene results in the impairment of the NF-kappaB pathway. Mol Cell 8:771-780.

[54] Li L, Sun L, Gao F, Jiang J, Yang Y, Li C, Gu J, Wei Z, Yang A, Lu R, Ma Y, Tang F, Kwon SW, Zhao Y, Li J, Jin Y. Stk40 links the pluripotency factor Oct4 to the Erk/MAPK pathway and controls extraembryonic endoderm differentiation. Proc Natl

Acad Sci USA. 2010; 107:1402-7.

[55] Li S, Edgar D, Fässler R, Wadsworth W, Yurchenco PD. The role of laminin in embryonic cell polarization and tissue organization. Dev Cell. 2003 May;4(5):613-24.

[56] Lim HYG, Alvarez YD, Gasnier M, Wang Y, Tetlak P, Bissiere S, Wang H, Biro M, Plachta N. Keratins are asymmetrically inherited fate determinants in the mammalian embryo. Nature. 2020 Sep;585(7825):404-409.

[57] Lim HYG, Plachta N. Cytoskeletal control of early mammalian development. Nat Rev Mol Cell Biol. 2021 Apr 29. doi: 10.1038/s41580-021-00363-9.

[58] Lu CC, Brennan J, Robertson EJ. From fertilization to gastrulation: axis formation in the mouse embryo. Curr Opin Genet Dev 2001; 11:384-392.

[59] Mah IK, Soloff R, Izuhara AK, Lakeland DL, Wang C, Mariani FV. Prkci is required for a non-autonomous signal that coordinates cell polarity during cavitation. Dev Biol. 2016 Aug 1;416(1):82-97.

[60] Martin E, Girardello R, Dittmar G, Ludwig A. New insights into the organization and regulation of the apical polarity network in mammalian epithelial cells. FEBS J. 2021 Jan 14.

[61] Maurer ME, Cooper JA. Endocytosis of megalin by visceral endoderm cells requires the Dab2 adaptor protein. J Cell Sci. 2005 Nov 15;118(Pt 22):5345-55.

[62] Meng Y, Cai KQ, Moore R, Tao W, Tse JD, Smith ER, Xu XX. Pten facilitates epiblast epithelial polarization and proamniotic lumen formation in early mouse embryos. Dev Dyn. 2017;246(7):517-530.

[63] Meng Y, Moore R, Tao W, Smith ER, Tse JD, Caslini C, Xu XX. GATA6 phosphorylation by Erk1/2 propels exit from pluripotency and commitment to primitive endoderm. Dev Biol. 2018;436(1):55-65.

[64] Mihajlović AI, Bruce AW. The first cell-fate decision of mouse preimplantation embryo development: integrating cell position and polarity. Open Biol. 2017 Nov;7(11). pii: 170210.

[65] Molotkov A, Mazot P, Brewer JR, Cinalli RM, Soriano P. Distinct Requirements for FGFR1 and FGFR2 in Primitive Endoderm Development and Exit from Pluripotency. Dev Cell. 2017 Jun 5;41(5):511-526.e4.

[66] Moore R, Cai KQ, Escudero DO, Xu XX. Cell adhesive affinity does not dictate primitive endoderm segregation and positioning during murine embryoid body formation. Genesis 2009; 47:579-589.

[67] Moore R, Cai KQ, Tao W, Smith ER, Xu XX. Differential requirement for Dab2 in the

development of embryonic and extra-embryonic tissues. BMC Dev Biol 2013; 13:39.

[68] Moore R, Tao W, Smith ER, Xu XX. The primitive endoderm segregates from the epiblast in beta1 integrin-deficient early mouse embryos. Mol Cell Biol 2014; 34:560-572.

[69] Moore R, Tao W, Meng Y, Smith ER, Xu XX. Cell adhesion and sorting in embryoid bodies derived from N- or E-cadherin deficient murine embryonic stem cells. Biol Open. 2014 Feb 15;3(2):121-8.

[70] Morris SM, Arden SD, Roberts RC, Kendrick-Jones J, Cooper JA, Luzio JP, Buss F. Myosin VI binds to and localises with Dab2, potentially linking receptor-mediated endocytosis and the actin cytoskeleton. Traffic. 2002 May;3(5):331-41.

[71] Morris SA, Teo RT, Li H, Robson P, Glover DM, Zernicka-Goetz M. Origin and formation of the first two distinct cell types of the inner cell mass in the mouse embryo. Proc Natl Acad Sci USA 2010; 107:6364-6369.

[72] Morrisey EE, Ip HS, Lu MM, Parmacek MS. GATA-6: a zinc finger transcription factor that is expressed in multiple cell lineages derived from lateral mesoderm. Dev Biol 1996; 177:309-322.

[73] Morrisey EE, Tang Z, Sigrist K, Lu MM, Jiang F, Ip HS, Parmacek MS. GATA6 regulates HNF4 and is required for differentiation of visceral endoderm in the mouse embryo. Genes Dev 1998; 12:3579-3590.

[74] Morrisey EE, Musco S, Chen MY, Lu MM, Leiden JM, Parmacek MS. The gene encoding the mitogen-responsive phosphoprotein Dab2 is differentially regulated by GATA-6 and GATA-4 in the visceral endoderm. J Biol Chem 2000; 275:19949-19954.

[75] Nakai-Futatsugi Y, Niwa H. Epiblast and primitive endoderm differentiation: fragile specification ensures stable commitment. Cell Stem Cell 2015; 16:346-347.

[76] Nance J. Getting to know your neighbor: cell polarization in early embryos. J Cell Biol. 2014 Sep 29;206(7):823-32.

[77] Niakan KK, Schrode N, Cho LT, Hadjantonakis AK. Derivation of extraembryonic endoderm stem (XEN) cells from mouse embryos and embryonic stem cells. Nature protocols 2013; 8:1028-1041.

[78] Nichols J, Zevnik B, Anastassiadis K, Niwa H, Klewe-Nebenius D, Chambers I, Scholer H, Smith A. Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4. Cell 1998; 95:379-391.

[79] Nichols J, Silva J, Roode M, Smith A. Suppression of Erk signalling promotes ground state pluripotency in the mouse embryo. Development 2009; 136:3215-3222.

[80] Ohnishi, Y. et al. Cell-to-cell expression variability followed by signal reinforcement

progressively segregates early mouse lineages. Nature cell biology 16, 27-37, (2014).

[81] Ohsugi M, Larue L, Schwarz H, Kemler R. Cell-junctional and cytoskeletal organization in mouse blastocysts lacking E-cadherin. Dev Biol. 1997 May 15;185(2):261-71.

[82] Phua DC, Xu J, Ali SM, Boey A, Gounko NV, Hunziker W. ZO-1 and ZO-2 are required for extra-embryonic endoderm integrity, primitive ectoderm survival and normal cavitation in embryoid bodies derived from mouse embryonic stem cells. PLoS One. 2014 Jun 6;9(6):e99532.

[83] Plusa B, Piliszek A, Frankenberg S, Artus J, Hadjantonakis AK. Distinct sequential cell behaviours direct primitive endoderm formation in the mouse blastocyst. Development 2008; 135:3081-3091.

[84] Pöschl E, Schlötzer-Schrehardt U, Brachvogel B, Saito K, Ninomiya Y, Mayer U. Collagen IV is essential for basement membrane stability but dispensable for initiation of its assembly during early development. Development. 2004 Apr;131(7):1619-28.

[85] Radice GL, Rayburn H, Matsunami H, Knudsen KA, Takeichi M, Hynes RO. Developmental defects in mouse embryos lacking N-cadherin. Dev Biol. 1997 Jan 1;181(1):64-78.

[86] Rappolee DA, Basilico C, Patel Y, Werb Z. Expression and function of FGF-4 in peri-implantation development in mouse embryos. Development 1994; 120:2259-2269.

[87] Rossant J, Chazaud C, Yamanaka Y. Lineage allocation and asymmetries in the early mouse embryo. Phil Tran Royal Soc of London Series B Biological Sciences 2003; 358:1341-1348; discussion 1349.

[88] Rossant J. Lineage development and polar asymmetries in the peri-implantation mouse blastocyst. Semin Cell Dev Biol 2004; 15:573-581.

[89] Rossant J. Mouse and human blastocyst-derived stem cells: vive les differences. Development. 2015 Jan 1;142(1):9-12.

[90] Rossant J, Tam PP. Emerging asymmetry and embryonic patterning in early mouse development. Dev Cell 2004; 7:155–164.

[91] Rossant J, Tam PP. New Insights into Early Human Development: Lessons for Stem Cell Derivation and Differentiation. Cell Stem Cell 2017; 20:18-28.

[92] Rula ME, Cai KQ, Moore R, Yang DH, Staub CM, Capo-Chichi CD, Jablonski SA, Howe PH, Smith ER, Xu XX. Cell autonomous sorting and surface positioning in the formation of primitive endoderm in embryoid bodies. Genesis 2007; 45:327-338.

[93] Saiz N, Grabarek JB, Sabherwal N, Papalopulu N, Plusa B. Atypical protein kinase C couples cell sorting with primitive endoderm maturation in the mouse blastocyst.

Development. 2013 Nov;140(21):4311-22.

[94] Salas PJ, Misek DE, Vega-Salas DE, Gundersen D, Cereijido M, Rodriguez-Boulan E. Microtubules and actin filaments are not critically involved in the biogenesis of epithelial cell surface polarity. J Cell Biol. 1986 May;102(5):1853-67.

[95] Salas PJ, Rodriguez ML, Viciana AL, Vega-Salas DE, Hauri HP. The apical submembrane cytoskeleton participates in the organization of the apical pole in epithelial cells. J Cell Biol. 1997 Apr 21;137(2):359-75.

[96] Salas PJ, Vega-Salas DE, Hochman J, Rodriguez-Boulan E, Edidin M. Selective anchoring in the specific plasma membrane domain: a role in epithelial cell polarity. J Cell Biol. 1988 Dec;107(6 Pt 1):2363-76.

[97] Schrode N, Xenopoulos P, Piliszek A, Frankenberg S, Plusa B, Hadjantonakis AK. Anatomy of a blastocyst: cell behaviors driving cell fate choice and morphogenesis in the early mouse embryo. Genesis 2013; 51:219-233.

[98] Schröter C, Rué P, Mackenzie JP, Martinez-Arias A. FGF/MAPK signaling sets the switching threshold of a bistable circuit controlling cell fate decisions in embryonic stem cells. Development 2015; 142:4205-4216.

[99] Sengupta A, Duran A, Ishikawa E, Florian MC, Dunn SK, Ficker AM, Leitges M, Geiger H, Diaz-Meco M, Moscat J, Cancelas JA. Atypical protein kinase C (aPKCzeta and aPKClambda) is dispensable for mammalian hematopoietic stem cell activity and blood formation. Proc Natl Acad Sci U S A. 2011 Jun 14;108(24):9957-62. Erratum in: Proc Natl Acad Sci U S A. 2011 Jul 19;108(29):12185.

[100] Shahbazi MN, Jedrusik A, Vuoristo S, Recher G, Hupalowska A, Bolton V, Fogarty NNM, Campbell A, Devito L, Ilic D, Khalaf Y, Niakan KK, Fishel S, Zernicka-Goetz M. Self-organization of the human embryo in the absence of maternal tissues. Nat Cell Biol. 2016 Jun;18(6):700-708.

[101] Shahbazi MN, Scialdone A, Skorupska N, Weberling A, Recher G, Zhu M, Jedrusik A, Devito LG, Noli L, Macaulay IC, Buecker C, Khalaf Y, Ilic D, Voet T, Marioni JC, Zernicka-Goetz M. Pluripotent state transitions coordinate morphogenesis in mouse and human embryos. Nature. 2017 Dec 14;552(7684):239-243.

[102] Ryan AQ, Chan CJ, Graner F, Hiiragi T. Lumen Expansion Facilitates Epiblast-Primitive Endoderm Fate Specification during Mouse Blastocyst Formation. Dev Cell. 2019 Dec 16;51(6):684-697.e4.

[103] Sheth B, Nowak RL, Anderson R, Kwong WY, Papenbrock T, Fleming TP. Tight junction protein ZO-2 expression and relative function of ZO-1 and ZO-2 during mouse

blastocyst formation. Exp Cell Res. 2008 Nov 1;314(18):3356-68.

[104] Smith ER, Capo-chichi CD, He J, Smedberg JL, Yang DH, Prowse AH, Godwin AK, Hamilton TC, Xu XX. Disabled-2 mediates c-Fos suppression and the cell growth regulatory activity of retinoic acid in embryonic carcinoma cells. J Biol Chem 2001; 276:47303-47310.

[105] Smith ER, Smedberg JL, Rula ME, Xu XX. Regulation of Ras-MAPK pathway mitogenic activity by restricting nuclear entry of activated MAPK in endoderm differentiation of embryonic carcinoma and stem cells. J Cell Biol. 2004 Mar 1;164(5):689-99.

[106] Smith ER, Meng Y, Moore R, Tse JD, Xu AG, Xu XX. Nuclear envelope structural proteins facilitate nuclear shape changes accompanying embryonic differentiation and fidelity of gene expression. BMC Cell Biol. 2017; 18(1):8.

[107] Steinberg MS. Differential adhesion in morphogenesis: a modern view. Curr Opin Genet Dev 2007; 17:281-286.

[108] STEINBERG MS. Reconstruction of tissues by dissociated cells. Some morphogenetic tissue movements and the sorting out of embryonic cells may have a common explanation. Science. 1963 Aug 2;141(3579):401-8.

[109] Steinberg MS, Gilbert SF. Townes and Holtfreter (1955): directed movements and selective adhesion of embryonic amphibian cells. J Exp Zool A Comp Exp Biol 2004; 301:701-706.

[110] Stephenson RO, Yamanaka Y, Rossant J. 2010. Disorganized epithelial polarity and excess trophectoderm cell fate in preimplantation embryos lacking E-cadherin. Development 137, 3383–3391.

[111] Stephenson RO, Rossant J, Tam PP. Intercellular interactions, position, and polarity in establishing blastocyst cell lineages and embryonic axes. Cold Spring Harb Perspect Biol. 2012 Nov 1;4(11).

[112] Stephens LE, Sutherland AE, Klimanskaya IV, Andrieux A, Meneses J, Pedersen RA, Damsky CH. 1995. Deletion of beta 1 integrins in mice results in inner cell mass failure and peri-implantation lethality. Genes Dev. 9:1883–1895.

[113] Sugihara K, Nakatsuji N, Nakamura K, Nakao K, Hashimoto R, Otani H, Sakagami H, Kondo H, Nozawa S, Aiba A, Katsuki M. Rac1 is required for the formation of three germ layers during gastrulation. Oncogene. 1998 Dec 31;17(26):3427-33.

[114] Tam PP, Loebel DA. Gene function in mouse embryogenesis: get set for gastrulation. Nat Rev Genet 2007; 8:368-381.

[115] Tao W, Moore R, Smith ER, Xu XX. Endocytosis and Physiology: Insights from Disabled-2 Deficient Mice. Front Cell Dev Biol. 2016 Nov 25;4:129.

[116] Tao W, Moore R, Meng Y, Smith ER, Xu XX. Endocytic adaptors Arh and Dab2 control homeostasis of circulatory cholesterol. J Lipid Res. 2016 May;57(5):809-17.

[117] Tse JD, Moore R, Meng Y, Tao W, Smith ER, Xu XX. Dynamic conversion of cell sorting patterns in aggregates of embryonic stem cells with differential adhesive affinity. BMC Dev Biol. 2021 Jan 6;21(1):2.

[118] Vallier L, Touboul T, Chng Z, Brimpari M, Hannan N, Millan E, Smithers LE, Trotter M, Rugg-Gunn P, Weber A, Pedersen RA. Early cell fate decisions of human embryonic stem cells and mouse epiblast stem cells are controlled by the same signalling pathways. PLoS One. 2009 Jun 30;4(6):e6082.

[119] Vega-Salas DE, Salas PJ, Gundersen D, Rodriguez-Boulan E. Formation of the apical pole of epithelial (Madin-Darby canine kidney) cells: polarity of an apical protein is independent of tight junctions while segregation of a basolateral marker requires cell-cell interactions. J Cell Biol. 1987 Apr;104(4):905-16.

[120] Wang Y, Smedberg JL, Cai KQ, Capo-Chichi DC, Xu XX: Ectopic expression of GATA6 bypasses requirement for Grb2 in primitive endoderm formation. Dev Dyn 2011; 240:566-576.

[121] Whiteman EL, Fan S, Harder JL, Walton KD, Liu CJ, Soofi A, Fogg VC, Hershenson MB, Dressler GR, Deutsch GH, Gumucio DL, Margolis B. Crumbs3 is essential for proper epithelial development and viability. Mol Cell Biol. 2014 Jan;34(1):43-56.

[122] Xie W, Schultz MD, Lister R, Hou Z, Rajagopal N, Ray P, Whitaker JW, Tian S, Hawkins RD, Leung D, Yang H, Wang T, Lee AY, Swanson SA, Zhang J, Zhu Y, Kim A, Nery JR, Urich MA, Kuan S, Yen CA, Klugman S, Yu P, Suknuntha K, Propson NE, Chen H, Edsall LE, Wagner U, Li Y, Ye Z, Kulkarni A, Xuan Z, Chung WY, Chi NC, Antosiewicz-Bourget JE, Slukvin I, Stewart R, Zhang MQ, Wang W, Thomson JA, Ecker JR, Ren B. Epigenomic analysis of multilineage differentiation of human embryonic stem cells. Cell. 2013 May 23;153(5):1134-48.

[123] Xu J, Kausalya PJ, Phua DC, Ali SM, Hossain Z, Hunziker W. Early embryonic lethality of mice lacking ZO-2, but Not ZO-3, reveals critical and nonredundant roles for individual zonula occludens proteins in mammalian development. Mol Cell Biol. 2008 Mar;28(5):1669-78.

[124] Xu XX, Yang W, Jackowski S, Rock CO. Cloning of a novel phosphoprotein regulated by colony-stimulating factor 1 shares a domain with the Drosophila disabled gene product.

J Biol Chem 1995; 270:14184-14191.

[125] Yamanaka Y, Lanner F, Rossant J. FGF signal-dependent segregation of primitive endoderm and epiblast in the mouse blastocyst. Development 2010; 137:715-724.

[126] Yano T, Kanoh H, Tamura A, Tsukita S. Apical cytoskeletons and junctional complexes as a combined system in epithelial cell sheets. Ann N Y Acad Sci. 2017;1405(1):32-43.

[127] Yang DH, Smith ER, Roland IH, Sheng Z, He J, Martin WD, Hamilton TC, Lambeth JD, Xu XX. Disabled-2 is essential for endodermal cell positioning and structure formation during early extraembryonic development. Dev Biol 2002; 251:27-44.

[128] Yang DH, Cai KQ, Roland IH, Smith ER, Xu XX. Disabled-2 is an epithelial surface positioning gene. J Biol Chem 2007; 282:13114-13122.

[129] Yang DH, Smith ER, Cai KQ, Xu XX. C-Fos elimination compensates for disabled-2 requirement in mouse extraembryonic endoderm development. Dev Dyn 2009; 238:514-523.

[130] Yang DH, Cai KQ, Roland IH, Smith ER, Xu XX. Disabled-2 is an epithelial surface positioning gene. J Biol Chem. 2007 Apr 27;282(17):13114-22.

[131] Yang JQ, Leitges M, Duran A, Diaz-Meco MT, Moscat J. Loss of PKC lambda/iota impairs Th2 establishment and allergic airway inflammation in vivo. Proc Natl Acad Sci U S A. 2009 Jan 27;106(4):1099-104.

[132] Yano T, Kanoh H, Tamura A, Tsukita S. Apical cytoskeletons and junctional complexes as a combined system in epithelial cell sheets. Ann N Y Acad Sci. 2017 Oct;1405(1):32-43.

[133] Zenker J, White MD, Gasnier M, Alvarez YD, Lim HYG, Bissiere S, Biro M, Plachta N. Expanding Actin Rings Zipper the Mouse Embryo for Blastocyst Formation. Cell. 2018 Apr 19;173(3):776-791.e17.

[134] Zhang HT, Hiiragi T. Symmetry Breaking in the Mammalian Embryo. Annu Rev Cell Dev Biol. 2018 Oct 6;34:405-426.

[135] Zhu M, Leung CY, Shahbazi MN, Zernicka-Goetz M. Actomyosin polarisation through PLC-PKC triggers symmetry breaking of the mouse embryo. Nat Commun. 2017 Oct 13;8(1):921.

[136] Zhu M, Cornwall-Scoones J, Wang P, Handford CE, Na J, Thomson M, Zernicka-Goetz M. Developmental clock and mechanism of de novo polarization of the mouse embryo. Science. 2020 Dec 11;370(6522):eabd2703.