



HAL
open science

Morphogenesis of the mammalian blastocyst

Nicolas Dard, Manuel Breuer, Bernard Maro, Sophie Louvet-Vallée

► **To cite this version:**

Nicolas Dard, Manuel Breuer, Bernard Maro, Sophie Louvet-Vallée. Morphogenesis of the mammalian blastocyst. *Molecular and Cellular Endocrinology*, 2008, 282 (1-2), pp.70. 10.1016/j.mce.2007.11.004 . hal-00531959

HAL Id: hal-00531959

<https://hal.science/hal-00531959>

Submitted on 4 Nov 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

Title: Morphogenesis of the mammalian blastocyst

Authors: Nicolas Dard, Manuel Breuer, Bernard Maro, Sophie Louvet-Vallée

PII: S0303-7207(07)00420-0
DOI: doi:10.1016/j.mce.2007.11.004
Reference: MCE 6747

To appear in: *Molecular and Cellular Endocrinology*



Please cite this article as: Dard, N., Breuer, M., Maro, B., Louvet-Vallée, S., Morphogenesis of the mammalian blastocyst, *Molecular and Cellular Endocrinology* (2007), doi:10.1016/j.mce.2007.11.004

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Morphogenesis of the mammalian blastocyst

Nicolas Dard¹, Manuel Breuer¹, Bernard Maro^{1,2} and Sophie Louvet-Vallée¹

¹*Laboratoire de Biologie Cellulaire du Développement, UMR 7622, CNRS - Université Pierre et Marie Curie, 9 Quai St Bernard, 75252 Paris cedex 05, France*

^{1,2}*Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv, 69978, Israel*

Correspondence should be addressed to S.L-V.: Sophie.louvet@snv.jussieu.fr

Abstract

The first four days of mouse pre-implantation development is characterized by a period of segmentation including morphogenetic events that are required for the divergence of embryonic and extra-embryonic lineages. These extra-embryonic tissues are essential for the implantation into the maternal uterus and for the development of the foetus. In this review, we first discuss data showing unambiguously that none essential axis of development is set up before the late blastocyst stage, and explain why the pre-patterning described during the early phases (segmentation) of development in other vertebrates cannot apply to mammalian pre-implantation period. Then, we described important cellular and molecular events that are required for the morphogenesis of the blastocyst.

1) An overview of mammalian pre-implantation development

During most part of mammalian development, the embryo is attached to the maternal uterine epithelium. Thus, a special feature of mammalian development is that the zygote generates two groups of cells with distinct fates: embryonic and extraembryonic tissues (see Figure). Therefore, the first period, called pre-implantation development, is devoted to the formation of extra-embryonic tissues that will be required for the implantation of the embryo into the uterus. It has been suggested to use the term of conceptus rather than embryo from fertilization until implantation, which takes place 4.5 days later (Johnson and Selwood, 1996).

Fertilization marks the beginning of pre-implantation development that corresponds to the cleavage of the zygote into smaller and smaller blastomeres. In mammals, the segmentation is total, asynchronous with long cell cycles (see below). Due to the small amount of maternal stores, the zygotic genome of the mouse is activated at the end of the 1-cell stage by a minor burst followed by a major burst at the end of the 2-cell stage (Aoki et al., 1997; Hamatani et al., 2004). The maternal mRNAs are degraded at the 2-cell stage (Bachvarova and Moy, 1985; Paynton et al., 1988; Schultz, 1993).

Epigenetic and morphogenetic events lead to the formation of two different cell types at the 16-cell stage (E 3.0): apolar inner blastomeres and polar outer blastomeres. The outer blastomeres will give rise mainly to the trophectoderm (TE) while the inner blastomeres will form the inner cell mass (ICM) of the blastocyst (E 3.5). At E 4.5, a third tissue differentiates on the surface of the ICM facing the blastocœle cavity: the primitive endoderm (PE). The remaining ICM cells forming the epiblast (EPI) contributes exclusively to the formation of the embryo proper. The extra-embryonic tissues derive from the TE and PE.

2) Setting up asymmetries in the conceptus

Morphological asymmetries are visible in the female gamete and at the blastocyst stage (see Figure). In the mature oocyte, which is blocked in metaphase of the second meiotic division (MII oocyte), the meiotic spindle is located off-center. Upon fertilization, this leads to the extrusion of a small polar body, which has been proposed to mark the animal pole. At the blastocyst stage, the conceptus displays a clear polarity axis known as the embryonic-abembryonic (Em-Ab) axis: the ICM is located to one pole called the embryonic pole while the trophectoderm facing the cavity defines the abembryonic pole. This Em-Ab axis corresponds to the dorso-ventral (D-V) axis of the post-implantation embryo (review in (Beddington and Robertson, 1999)).

Over the past decade, several groups have tried to correlate the animal-vegetal axis of the zygote to the Em-Ab axis of the blastocyst but these studies lead to a controversy. The reasons of the discrepancy are presented in two recent reviews (Hiragi et al., 2006; Zernicka-Goetz, 2006) and will not be exposed here. It is rather important to understand whether these putative axes have any influence on later development, and several essential simple experimental manipulations of the conceptus performed recently and more than 30 years ago, indicate unambiguously that none essential axis of development is set up before the blastocyst stage :i) removal of the animal or vegetal pole of the zygote has no deleterious effect on development (Zernicka-Goetz, 1998), (ii) a single 2- or 4-cell blastomere can give rise to a normal and fertile mouse (Kelly, 1975; Kelly, 1977; Tarkowski and Wroblewska, 1967) (iii) finally, chimerae resulting from the fusion of two normal 4- or 8-cell conceptus lead to the formation of a blastocyst with a single axis of development (Garner and McLaren, 1974). These experiments indicate that no essential developmental cue is heterogeneously localized in the zygote, and all blastomeres are equivalent until the 8-cell stage. This flexibility is maintained until the early blastocyst stage since aggregation of two blastocysts leads to the formation of normal adult chimerae when the orientation of the blastocysts enables the two ICM to integrate into a single ICM (Tarkowski et al., 2005). All these data show that mouse early development is very regulative and cannot be compared to the development of either vertebrates (*Xenopus*) and non vertebrates (*Drosophila*) embryos. Indeed, one fundamental difference is that the

product of fertilization is not equivalent in these species since in *Xenopus* and *Drosophila* all the cells derived from the zygote will form the embryo. According to this point, P. O' Farrell *et al.* (O'Farrell *et al.*, 2004) have suggested that the “*use of fertilization as a reference point misaligns the earliest stage and masks parallels that are evident when development is aligned at conserved stages*”. They have proposed that “*during evolution of mammals, fertilization was advanced to an earlier stage such that events that occurred late in the cell lineage of the oocyte in the progenitors of mammals were displaced and modified to become earliest events of post-fertilization development in mammals*”. Which arguments lead to this proposition?

First, the comparison of early cell cycles characteristics indicates that the *Xenopus* blastula corresponds to the mouse gastrula. Indeed, in *Xenopus* and *Drosophila* embryos, segmentation is characterized by rapid cell cycles (30 min and 8.3 to 23 min respectively) with no gap phases and a lack or modification of most checkpoint controls. In the mouse conceptus, the first cell cycle is very long, 18 hrs, and the duration of the next four cycles is 12 hrs. These cycles possess gap phases and a G1/S checkpoint control. Interestingly, during development of mammals, cells with the characteristic features of embryonic cell cycle are present during gastrulation in the egg cylinder (E 6.5): embryonic ectoderm cells have a short cell cycle (2.2 hrs) with short gap phases (0-30 min) (Mac Auley *et al.*, 1993; Snow and Bennett, 1978) and lack a G1/S checkpoint (Heyer *et al.*, 2000).

Second, the comparison of the expression pattern of conserved signaling molecules involved in body plan formation leads also to put the mouse gastrula into the same category as the *Xenopus* blastula. In the mouse embryo, the morphological distinction of the antero-posterior axis (A/P) is marked by the formation of the primitive streak at the posterior end of the epiblast at E 6.5. However, molecular asymmetries in the visceral endoderm identify a proximal-distal axis (P/D) at E 5.5. The migration of the distal visceral endoderm toward the future anterior side at E 6.0 converts the P/D to an A/P axis (Ang and Constam, 2004). We will consider two signaling pathways important for axis determination: Wnt and TGF β . In *Xenopus*, dorso-ventral polarity is established after fertilization, by a canonical Wnt signal that leads to the accumulation of maternal β -catenin on the prospective dorsal side of the embryo (Heasman *et al.*, 2000). The *Wnt11* mRNA crucial for the activation of Wnt pathway, is already localized at the vegetal cortex of the oocyte (Tao *et al.*, 2005). In the mouse embryo, the Wnt pathway is responsible for the A/P axis determination. At E 5.5, *Wnt3* mRNA is first detectable in the posterior visceral endoderm before the movements of anterior visceral endoderm (AVE) (Rivera-Perez and Magnuson, 2005). Moreover, at E 6.5, β -catenin is enriched on the posterior side of the epiblast. Some recent data revealed an asymmetric nuclear localization of β -catenin in restricted cells of the trophectoderm facing the primitive endoderm during a transient phase around E 4.5. Interestingly, during the same period, the homeobox gene *Hex* (a marker of AVE) is strongly expressed in the PE on

the opposite side to β -catenin (Chazaud and Rossant, 2006). This means that Wnt signaling and asymmetries leading to later axis development may begin as early as E 4.5 and might be driven by extra-embryonic tissues. The same parallel could be done with members of the TGF β family: *Xenopus* Vg1, required for the formation of the dorso-ventral axis is localized at the vegetal cortex of the oocyte and is then enriched in the dorsal vegetal quadrant of the embryo (Birsoy et al., 2006). While in mouse, *Nodal* expression is initiated in the proximal epiblast at E5.5 (Brennan et al., 2001).

These examples illustrate the analogy of the *Xenopus* blastula with the pre-gastrula mouse embryo rather than the blastocyst. The first days of mouse development are thus devoted to the divergence of embryonic (epiblast) and extra-embryonic lineages (TE and PE). Once this segregation has occurred, the conserved signalling pathways can regionalize the embryo (epiblast). It is also evident that the A/P axis of the mouse is established via interactions between the extra-embryonic tissues and the epiblast (review in (Ang and Constam, 2004). This is reminiscent of the establishment of the A/P axis of *Drosophila* oocyte by the surrounding follicle cells (review in (Huynh and St Johnston, 2004).

3) Establishment of the embryonic and extra-embryonic lineages

3.1) Segregation between the TE and ICM

Compaction, the first morphogenetic event of mammalian development occurs during the 8-cell stage. For the first time all the blastomeres change their shape and their cellular organization. Each blastomere polarizes its cytoplasm and its cortex along an apico-basal axis, leading to intercellular flattening through the development of an adhesive baso-lateral cortical domain. These morphological events (asymmetrical contacts and polarity) are essential processes that contribute to the generation of trophectoderm (TE) and inner cell mass (ICM) through differentiative divisions (see Figure).

3.1.1) Setting up asymmetries in the blastomeres

Blastomeres flattening

The blastomere flattening is due to the formation of adherent junctions between adjacent blastomeres. It occurs only when Ca^{2+} ions are present in the culture medium (Ducibella, 1977) since the major molecule involved is the transmembrane cell adhesion molecule E-cadherin. The first evidence for the involvement

of this protein comes from experimental approaches since functionally interfering anti E-cadherin antibodies inhibits the compaction of the conceptus (Hyafil et al., 1980) (Vestweber and Kemler, 1984). E-cadherin is expressed on the cell surface of oocytes and early blastomeres, and during compaction homophilic interactions are established in membrane domains involved in cell-cell contacts (Vestweber et al., 1987). To determine the precise role of E-cadherin, knock out (KO) mice have been generated (Larue et al., 1994; Riethmacher et al., 1995). E-cadherin null conceptus compact normally but fail to form an intact trophectodermal epithelium. This late phenotype is due to the maternal store of protein that is sufficient for compaction to occur at the 8-cell stage. Indeed, in conceptus lacking maternal E-cadherin, flattening fails at the 8-cell stage but occurs at the 16-cell stage when the paternal protein is expressed (De Vries et al., 2004). The cytoplasmic region of E-cadherin binds to β - or γ - catenin, which binds to α -catenin and links the complex to the actin cytoskeleton (Perez-Moreno et al., 2003). KO mice for α -catenin display a phenotype similar to that of E-cadherin KO mice (Torres et al., 1997).

In epithelial cell, some other transmembrane proteins are involved in adherent junctions formation. Although they are expressed in mouse conceptus, their roles in blastomeres flattening remain elusive. It is the case of Nectin-2 that is undetectable before compaction and is recruited at the cell-cell contact during compaction (Thomas et al., 2004). Nectin-2 forms a complex with the cytoplasmic protein afadin (Tachibana et al., 2000), but the role of this complex in blastomeres flattening has not been investigated. The transmembrane protein, vezatin, is localized all around the blastomeres cortex before compaction and accumulates in adherent junctions thereafter, like E-cadherin (Hyenne et al., 2005). Specific inhibition of vezatin translation by morpholino-oligonucleotide induces an arrest of development and a decrease in the amount of E-cadherin at the membrane. However, the precise role of vezatin during compaction has not been clarified by this approach due to the arrest of cell cycle and needs further investigations. Epithin, a type II membrane serine protease, colocalizes with E-cadherin in mouse conceptus (Khang et al., 2005). Inhibition of its expression results in absence of blastomere flattening at the 8-cell stage and suggests that epithin may cooperate with E-cadherin. However, the mechanism of cooperation remains to be elucidated.

Regulation of flattening

Although all the known components of adherent junctions are localized all around the blastomeres by the 2-cell stage, cell flattening occurs only at the 8-cell stage. Post-translational modifications are likely to maintain the E-cadherin complex inactive and to inhibit premature flattening. Indeed, inhibition of translation does not inhibit flattening but rather induces a premature compaction (Levy et al., 1986). Furthermore, cell flattening is associated with important phosphorylation events and premature compaction can also be achieved at the 2-cell and 4-cell stages by activators of protein kinase C (Ohsugi

et al., 1993; Winkel et al., 1990). β -catenin, which is phosphorylated on ser/thr residues at the time of compaction, is likely to be a target of this signaling cascade. (Goval et al., 2000; Pauken and Capco, 1999). The cytoskeleton protein ezrin is another good candidate (Louvét-Vallée et al., 2001). We have shown that phosphorylation of ezrin on T567 regulates its localization at the time of compaction and that maintenance of ezrin at the basolateral cortex at the 8-cell stage, interferes with the activity of E-cadherin and inhibits the formation of normal cell-cell contacts (Dard et al., 2004).

The Rho-family GTPases, required for cadherin-mediated cell-cell adhesion in culture cell lines (for review see (Noritake et al., 2005)) are also involved in compaction. Inhibition of their function by the C3 botulinum toxin disturbs intercellular flattening, and expression of a constitutively activated form of Cdc42 induces a premature flattening (Clayton et al., 1999). Moreover, Rac1 shifts from the cell cortex of blastomeres during the first stages of development to the cytoplasm at the 8-cell stage (Natale and Watson, 2002). The best-studied effector through which Rac1 and Cdc42 may regulate E-cadherin activity is IQGAP1. It is expressed in early mouse conceptus and its localization is similar to Rac1 (Natale and Watson, 2002), suggesting that the E-cadherin complex in the embryo may be regulated like in culture cell lines. Until compaction IQGAP1 may negatively regulate E-cadherin-mediated cell-adhesion through its interaction with β -catenin, thereby causing α -catenin to dissociate from the cadherin complex. Then activated Rac1 and Cdc42 may positively regulate flattening by inhibiting the interaction of IQGAP1 with β -catenin. Flattening is also impaired by the inhibition of the myosin light-chain kinase (Kabir et al., 1996), and myosin 2, a key effector of Rho-kinase signaling, regulates cell-cell adhesion by controlling the ability of cells to concentrate cadherin molecules at contact cells (Shewan et al., 2005).

Blastomeres polarization

The reorganization of cytoplasmic components is the first sign of blastomere polarization. It is characterized by the accumulation of endosomes and clathrin-coated vesicles under the apical membrane while the nucleus localizes more basally (Fleming and Pickering, 1985; Maro et al., 1985). The microtubule network is also modified: stable acetylated microtubules become enriched at the basal pole while a population of more dynamic microtubules is found apically (Houliston and Maro, 1989). Two parallel pathways mediate the polarization of blastomeres at the 8-cell stage: one requires flattening and can occur in absence of microtubules; the other is independent of flattening but involves a microtubule-mediated interaction between the nucleus and the cell surface (Houliston et al., 1989).

Up-to-date, the molecular mechanism involved in the reorganization of microtubules network remains unknown. However, blastomere polarization is associated with a change in the distribution of a serine-threonine kinase belonging to the MARK family (Microtubules Affinity Regulating Kinases) called EMK1 in the mouse. Before polarization, EMK1 is located only in nuclei and after compaction it is

targeted to the basolateral domain (Vinot et al., 2005). EMK1 is a homologue of the PAR1 protein (Partitioning defective 1) involved in the regulation and/or maintenance of cell polarity in *Drosophila* and *C. elegans*. Its role in the regulation of the microtubule network has been clearly established in *Drosophila* oocytes (Cox et al., 2001) and in follicular epithelial cells (Doerflinger et al., 2003). It will be interesting to investigate whether EMK1 is also involved in the reorganization of microtubules in the mouse conceptus.

Compaction is also associated with a reorganization of the cell surface, characterized by the formation of a dense pole of microvilli at the apical membrane. The removal of microvilli at the basolateral membrane, is crucial to allow flattening (Dard et al., 2004). Ezrin, a cytoskeleton protein linking actin to the plasma membrane in diverse types of membrane protrusions, follows exactly the distribution of microvilli: before compaction it is distributed all around the cell cortex and during polarization, it is removed from cell-cell contacts and relocates exclusively at the apical pole of blastomeres (Louvet et al., 1996).

As described in other model systems (Ohno, 2001), cortical polarization in mouse conceptus may be regulated at the molecular level by the PAR proteins. Indeed PAR proteins are expressed in mouse conceptus and some of them become asymmetrically distributed at the onset of polarization (Vinot et al., 2005). During the 8-cell stage, PARD6b (one homologue of the PAR6 protein) becomes associated with the apical cortex while EMK1 accumulates at the basolateral cortex (Vinot et al., 2005). PAR6 cooperates with atypical protein kinase C (aPKC) to establish cell polarity and protein aPKC ζ is also found at the apical cortex of the 8-cell compacted conceptus (Thomas et al., 2004).

3.1.2) Asymmetric division and cell diversification

Although all blastomeres are identical until the 8-cell stage, two populations appear at the 16-cell stage. At this stage, blastomeres differ in their position, phenotype and fate: the inner non-polarized blastomeres will give rise to the inner cell mass of the blastocyst, while the outer polarized blastomeres will form the trophectoderm. This cell diversification results from the polarization of blastomeres at compaction and from asymmetric divisions occurring in some 8-cell blastomeres. In fact, although the cytoplasmic and cytoskeletal polarities are lost during mitosis, the pole of microvilli remains stable (Johnson and Maro, 1985) and experimental evidences suggest that the cyto-cortex associated with this microvillus pole acts as a stable memory (Wiley and Obajasu, 1988). The inheritance of this pole by a blastomere permits the re-establishment of polarity and the engagement of the cell into the epithelial differentiation program. At the 16-cell stage, polarized cells tend to engulf non-polar cells because of their different adhesive properties: in polar cells, the apical surface is less adhesive than the basolateral surface while non-polar cells are uniformly adhesive and establish a maximum of contacts with other cells (Johnson and Maro, 1985).

The number of polarized blastomeres that divide asymmetrically (giving rise to one polar and one apolar cell) (Fleming, 1987) is very variable during the 8- to 16-cell stage transition, showing that spindle orientation is not controlled. Therefore in the 16-cell stage conceptus, the outer/inner cell ratio lies in the range 8/8-16:0, with being most of the time between 10/6 and 12/4 (Johnson and McConnell, 2004). By contrast, this ratio is much less variable at the 32-cell stage. Indeed, spindle orientation can be biased during the 16- to 32-cell stage transition, when the outer/inner cell ratio reaches extreme values (near 8/8 or 16/0). Indeed, the number of inner and outer cells influences the cell shape at the 16-cell stage, outer cells having a large apical pole when the ratio is low, and reversely, being more elongated along the apico-basal axis with a small apical pole when the ratio is higher. Then, when outer cells display a small pole and a large embedded basolateral membrane (low number of inner cells) the mitotic spindle tends to orient along the apico-basal axis and cells tend to divide asymmetrically, thereby increasing the number of inner cells. Reversely, when outer cells are flattened at the surface of the conceptus displaying a large apical domain (high number of inner cells), the mitotic spindle tends to orientate perpendicular to the apico-basal axis, and outer cells tend to divide symmetrically, thus maintaining the inner/outer cell ratio (since dividing inner cells always give rise to two inner daughter cells).

Up to date, only two proteins, ezrin and PARD6b, remain associated with the apical pole of the dividing blastomeres (Louvet et al., 1996; Vinot et al., 2005) and only EMK1 stays at the basolateral membrane when blastomeres enter mitosis. It is attractive to speculate that in the mouse conceptus PAR proteins contribute to the early lineage diversification and act as cell fate determinants like in other model systems. This idea is reinforced by the behavior of these proteins before cell polarization since PARD6b and EMK1 are on the spindle during mitosis allowing an equal segregation of these proteins in the two daughter cells. We have also observed a correlation between the shape of the spindle and the dynamic localization of PARD6a, another homologue of the PAR6 protein (Louvet-Vallée et al., 2005): until the third cleavage division the spindle elongates throughout the cell and reached the cortex at metaphase while PARD6a accumulates at the spindle poles and at the cortex facing the spindle poles. During the fourth cleavage divisions when the orientation of the spindle is dependent upon cell shape, the spindle never reaches the cortex and no accumulation of PARD6a at the cortex is observed.

3.1.3) Establishment and maintenance of the epithelial fate

The position of blastomeres within the conceptus at the 16-cell stage determines their fate: outer blastomeres acquire progressively epithelial features to eventually form a functional epithelium while inner blastomeres remain undifferentiated. The formation of a functional epithelium depends on a set of specialized cell junctions, especially tight junctions (TJ) and desmosomes. The formation of (TJ) in outer blastomeres is temporally and spatially regulated (Sheth et al., 2000): first, at the end of the 8-cell stage,

ZO-1 α^- and Rab13 assemble at the most apical part of the adherent junctions; then ZO-2 and cingulin are addressed to this domain at the 16-cell stage; finally, the recruitment of ZO-1 α^+ and occludin at the 32-cell stage leads to the formation of mature TJ as attested by the apparition of the cavity. The biogenesis of TJ requires the activities of aPKC: aPKC δ and ζ . are required for the localization of ZO-2 but only aPKC ζ is involved in the localization of ZO-1 α^+ (Eckert et al., 2005). The desmosomes are observed by electron microscopy at the 16-cell stage onward (Fleming et al., 1991). At the molecular level, the plakoglobin is present at the 16-cell stage while desmoplakin, desmoglein and desmocollin are detected only when the cavity forms (Fleming et al., 1991). Moreover, the epithelial phenotype is repressed in inner blastomeres: it has been shown that inside cells can differentiate as epithelial cells as soon as they are exposed to asymmetrical cell contacts, without synthesis of new mRNAs, demonstrating that they contain a store of untranslated mRNAs coding for all the proteins required for epithelial differentiation (Johnson, 1979) ;(Eckert et al., 2004; Louvet-Vallée et al., 2001; Spindle, 1978)

If the determination of the ICM and TE fate depends on the position of the blastomeres, the maintenance of these fates require mainly the expression of two transcription factors *Cdx2* and *Oct4*. *Cdx2* (*Caudal related homeobox 2*) is specifically expressed in TE blastomeres (Yamanaka et al., 2006). Conceptus lacking *Cdx2* zygotic expression form a blastocyst but the integrity of the epithelium is not maintained and they are unable to implant (Strumpf et al., 2005). *Oct4* (called also *Pou5f1*) expression is restricted to the ICM after the initiation of the formation of the blastocyst. Embryos homozygous mutant for this gene develop to the blastocyst stage but their isolated ICM cells express TE markers (Nichols et al., 1998). Although these transcription factors are TE (*Cdx2*) and ICM (*Oct4*) specific, they are expressed in both inner and outer cells at the morula stage in a stochastic way and become progressively restricted to their specific lineage only in the blastocyst. A negative feedback loop on each other expression associated to a positive feedback loop on themselves has been described. In addition asymmetric cell contacts favor *Cdx2* expression (Yamanaka et al., 2006). Finally, blastocyst morphogenesis can take place in their absence but they are required to stabilize the structure and the two lineages, ICM and TE, once they are formed.

3.2) Segregation between epiblast and primitive endoderm

The formation of the primitive endoderm (PE) is the second event differentiation occurring during mammalian pre-implantation development. The PE is an epithelial layer formed on the surface of the ICM facing the blastocœl at E 4.0. Until recently, the model suggested that the position of cells within ICM drives their fate, i.e. ICM cells facing the blastocœl differentiated into epithelial primitive endoderm cells. However, recent genetic data has shown that *Nanog* (homeodomain) and *Gata* family transcriptional factors are involved in specifying epiblast versus PE fate. In ES cells and ICM, loss of *Nanog* leads to an absence of epiblast specification, all cells following a PE fate (Mitsui et al., 2003).

Overexpression of *Gata6* is sufficient to shift ES cells to the PE lineages (Fujikura et al., 2002) and *in vivo* *Gata6* mutant initiate PE lineage formation but fail to form functional visceral endoderm in peri-implantation embryos (Koutsourakis et al., 1999). At E 4.5, expression of *Gata6* is restricted to PE and *Nanog* to EPI. At E 3.5, *Gata6* and *Nanog* proteins are present in subsets of ICM cells. The domains of *Gata6* and *Nanog* mRNA expression are mainly exclusive suggesting that ICM is a mosaic of *Nanog* and *Gata6* expressing cells (Chazaud et al., 2006). In *Grb2* mutants (an adaptator molecule that plays an important role in signal transduction downstream of several different tyrosin kinases), all ICM cells become *Nanog* positive and no PE form. Consequently, the current model for the segregation of epiblast versus PE is the following. A *Grb2* dependant signals induce *Gata* and repress *Nanog* in PE progenitors (although the mechanism of selective activation of this pathways in PE is unknown). The absence of *Grb2*-dependant signals allows *Nanog* expression in epiblast progenitors. In PE progenitors, *Gata* induces target genes such as lamin and *Dab2*, which modulate cellular adhesive function to initiate the sorting of the two lineages by cell movements (*Gata6* expressing cells moving to the edge of the ICM facing the blastocoele). By E 4.5, the basal lamina forms and separates the 2 lineages (Chazaud et al., 2006). Alternatively, the two factors may be expressed stochastically in all ICM cells, the expression of *Gata6* being stabilized in cells facing the blastocoele and *Nanog* in cells surrounded by other cells.

4) Conclusion

Changes in cellular organization, cell shape and intercellular contacts all play a major role during preimplantation development: cell polarization allows the existence of asymmetric division, the divergence of the first two lineages and the formation of a structure with an outer layer of polarized cells and an inner core of non-polarized cells; cell shape influences the type of divisions, asymmetric or symmetric, and thus regulates the number of cells within the different lineages; the asymmetry of intercellular contacts allows the synthesis of epithelial proteins from stored mRNAs and the differentiation of epithelial cells leading to an epithelial seal and the formation of the blastocoele. The stochastic expression of transcription factors (*Cdx2*, *Oct4*) required for the stabilization of these cell lineages (TE, ICM) is also finally restricted by the asymmetry of intercellular contacts (inside versus outside position). Whether similar mechanisms are at work for the formation of the PE remains to be established. Dynamic studies concerning the expression of *Gata* and *Nanog* should help to solve this problem.

References

- Ang, S. L., and Constam, D. B. (2004). A gene network establishing polarity in the early mouse embryo. *Semin Cell Dev Biol* **15**, 555-61.
- Aoki, F., Worrad, D. M., and Schultz, R. M. (1997). Regulation of transcriptional activity during the first and second cell cycles in the preimplantation mouse embryo. *Dev Biol* **181**, 296-307.
- Bachvarova, R., and Moy, K. (1985). Autoradiographic studies on the distribution of labeled maternal RNA in early mouse embryos. *J Exp Zool* **233**, 397-403.
- Beddington, R. S., and Robertson, E. J. (1999). Axis development and early asymmetry in mammals. *Cell* **96**, 195-209.
- Birsoy, B., Kofron, M., Schaible, K., Wylie, C., and Heasman, J. (2006). Vg 1 is an essential signaling molecule in *Xenopus* development. *Development* **133**, 15-20.
- Brennan, J., Lu, C. C., Norris, D. P., Rodriguez, T. A., Beddington, R. S., and Robertson, E. J. (2001). Nodal signalling in the epiblast patterns the early mouse embryo. *Nature* **411**, 965-9.
- Chazaud, C., and Rossant, J. (2006). Disruption of early proximodistal patterning and AVE formation in *Apc* mutants. *Development* **133**, 3379-87.
- Chazaud, C., Yamanaka, Y., Pawson, T., and Rossant, J. (2006). Early lineage segregation between epiblast and primitive endoderm in mouse blastocysts through the Grb2-MAPK pathway. *Dev Cell* **10**, 615-24.
- Clayton, L., Hall, A., and Johnson, M. H. (1999). A role for Rho-like GTPases in the polarisation of mouse eight-cell blastomeres. *Dev Biol* **205**, 322-31.
- Cox, D. N., Lu, B., Sun, T. Q., Williams, L. T., and Jan, Y. N. (2001). *Drosophila* par-1 is required for oocyte differentiation and microtubule organization. *Curr Biol* **11**, 75-87.
- Dard, N., Louvet-Vallee, S., Santa-Maria, A., and Maro, B. (2004). Ezrin phosphorylation on Thr567 plays a crucial role during compaction in the mouse early embryo. *Developmental Biology* **271**, 87-97.
- De Vries, W. N., Evsikov, A. V., Haac, B. E., Fancher, K. S., Holbrook, A. E., Kemler, R., Solter, D., and Knowles, B. B. (2004). Maternal beta-catenin and E-cadherin in mouse development. *Development* **131**, 4435-45.
- Doerflinger, H., Benton, R., Shulman, J. M., and St Johnston, D. (2003). The role of PAR-1 in regulating the polarised microtubule cytoskeleton in the *Drosophila* follicular epithelium. *Development* **130**, 3965-75.
- Ducibella, T. (1977). Surface changes of the developing trophoblast cell. *Development in Mammals* **1**, 5-30.
- Eckert, J. J., McCallum, A., Mears, A., Rumsby, M. G., Cameron, I. T., and Fleming, T. P. (2004). Specific PKC isoforms regulate blastocoel formation during mouse preimplantation development. *Dev Biol* **274**, 384-401.
- Eckert, J. J., McCallum, A., Mears, A., Rumsby, M. G., Cameron, I. T., and Fleming, T. P. (2005). Relative contribution of cell contact pattern, specific PKC isoforms and gap junctional communication in tight junction assembly in the mouse early embryo. *Dev Biol* **288**, 234-47.
- Fleming, T. P. (1987). A quantitative analysis of cell allocation to trophectoderm and inner cell mass in the mouse blastocyst. *Developmental Biology* **119**, 520-531.
- Fleming, T. P., Garrod, D. R., and Elsmore, A. J. (1991). Desmosome biogenesis in the mouse preimplantation embryo. *Development* **112**, 527-539.
- Fleming, T. P., and Pickering, S. J. (1985). Maturation and polarization of the endocytotic system in outside blastomeres during mouse preimplantation development. *Journal of Embryology and experimental Morphology* **89**, 175-208.
- Fujikura, J., Yamato, E., Yonemura, S., Hosoda, K., Masui, S., Nakao, K., Miyazaki Ji, J., and Niwa, H. (2002). Differentiation of embryonic stem cells is induced by GATA factors. *Genes Dev* **16**, 784-9.

- Garner, W., and McLaren, A. (1974). Cell distribution in chimaeric mouse embryos before implantation. *J Embryol Exp Morphol* **32**, 495-503.
- Goval, J. J., Van Cauwenberge, A., and Alexandre, H. (2000). Respective roles of protein tyrosine kinases and protein kinases C in the upregulation of beta-catenin distribution, and compaction in mouse preimplantation embryos: a pharmacological approach. *Biol Cell* **92**, 513-26.
- Hamatani, T., Carter, M. G., Sharov, A. A., and Ko, M. S. (2004). Dynamics of global gene expression changes during mouse preimplantation development. *Dev Cell* **6**, 117-31.
- Heasman, J., Kofron, M., and Wylie, C. (2000). Beta-catenin signaling activity dissected in the early *Xenopus* embryo: a novel antisense approach. *Dev Biol* **222**, 124-34.
- Heyer, B. S., MacAuley, A., Behrendtsen, O., and Werb, Z. (2000). Hypersensitivity to DNA damage leads to increased apoptosis during early mouse development. *Genes Dev* **14**, 2072-84.
- Hiiragi, T., Alarcon, V. B., Fujimori, T., Louvet-Vallee, S., Maleszewski, M., Marikawa, Y., Maro, B., and Solter, D. (2006). Where do we stand now? Mouse early embryo patterning meeting in Freiburg, Germany (2005). *Int J Dev Biol* **50**, 581-6; discussion 586-7.
- Houliston, E., and Maro, B. (1989). Posttranslational modification of distinct microtubule subpopulations during cell polarization and differentiation in the mouse preimplantation embryo. *Journal of Cell Biology* **108**, 543-551.
- Houliston, E., Pickering, S. J., and Maro, B. (1989). Alternative routes for the establishment of surface polarity during compaction of the mouse embryo. *Developmental Biology* **134**, 342-350.
- Huynh, J. R., and St Johnston, D. (2004). The origin of asymmetry: early polarisation of the *Drosophila* germline cyst and oocyte. *Curr Biol* **14**, R438-49.
- Hyafil, F., Morello, D., Babinet, C., and Jacob, F. (1980). A cell surface glycoprotein involved in the compaction of embryonal carcinoma cells and cleavage stage embryos. *Cell* **21**, 927-934.
- Hyenne, V., Louvet-Vallee, S., El-Amraoui, A., Petit, C., Maro, B., and Simmler, M. C. (2005). Vezatin, a protein associated to adherens junctions, is required for mouse blastocyst morphogenesis. *Dev Biol* **287**, 180-91.
- Johnson, M. H. (1979). Molecular differentiation of inside cells and inner cell masses isolated from preimplantation mouse embryos. *Journal of Embryology and experimental Morphology* **53**, 335-344.
- Johnson, M. H., and Maro, B. (1985). A dissection of the mechanisms generating and stabilising polarity in mouse 8 and 16-cell blastomeres: the role of cytoskeletal elements. *Journal of Embryology and experimental Morphology* **90**, 311-334.
- Johnson, M. H., and McConnell, J. M. L. (2004). Lineage allocation and cell polarity during mouse embryogenesis. *Seminars in Cell and Developmental Biology* **15**, 583-97.
- Johnson, M. H., and Selwood, L. (1996). Nomenclature of early development in mammals. *Reprod Fertil Dev* **8**, 759-64.
- Kabir, N., Yamamura, H., Takagishi, Y., Inouye, M., Oda, S., and Hidaka, H. (1996). Regulation of preimplantation development of mouse embryos: effects of inhibition of myosin light-chain kinase, a Ca²⁺/calmodulin-dependent enzyme. *J Exp Zool* **274**, 101-10.
- Kelly, S. J. (1975). Studies of the potency of early cleavage blastomeres of the mouse blastomeres. In "The early development of mammals" (M. Balls and A. Wild, Eds.), pp. 97-105. Cambridge University Press, Cambridge.
- Kelly, S. J. (1977). Studies of the developmental potential of 4- and 8-cell stage mouse blastomeres. *J Exp Zool* **200**, 365-76.
- Khang, I., Sonn, S., Park, J. H., Rhee, K., Park, D., and Kim, K. (2005). Expression of epithin in mouse preimplantation development: its functional role in compaction. *Dev Biol* **281**, 134-44.
- Koutsourakis, M., Langeveld, A., Patient, R., Beddington, R., and Grosveld, F. (1999). The transcription factor GATA6 is essential for early extraembryonic development. *Development* **126**, 723-32.
- Larue, L., Ohsugi, M., Hirchenhain, J., and Kemler, R. (1994). E-cadherin null mutant embryos fail to form a trophoblast epithelium. *Proceedings of the National Academy of Sciences of the United States of America* **91**, 8263-8267.

- Levy, J. B., Johnson, M. H., Goodall, H., and Maro, B. (1986). Control of the timing of compaction: a major developmental transition in mouse early embryogenesis. *Journal of Embryology and experimental Morphology* **95**, 213-237.
- Louvet, S., Aghion, J., Santa-Maria, A., Mangeat, P., and Maro, B. (1996). Ezrin becomes restricted to outer cells following asymmetric division in the preimplantation mouse embryo. *Developmental Biology* **177**, 568-579.
- Louvet-Vallée, S., Dard, N., Santa-Maria, A., Aghion, J., and Maro, B. (2001). A major posttranslational modification of ezrin takes place during epithelial differentiation in the early mouse embryo. *Developmental Biology*, 190-200.
- Louvet-Vallée, S., Vinot, S., and Maro, B. (2005). Mitotic spindles and cleavage planes are oriented randomly in the 2-cell mouse embryo. *Current Biology* **in press**.
- Mac Auley, A., Werb, Z., and Mirkes, P. E. (1993). Characterization of the unusually rapid cell cycles during rat gastrulation. *Development* **117**, 873-83.
- Maro, B., Johnson, M. H., Pickering, S. J., and Louvard, D. (1985). Changes in the distribution of membranous organelles during mouse early embryogenesis. *Journal of Embryology and experimental Morphology* **90**, 287-309.
- Mitsui, K., Tokuzawa, Y., Itoh, H., Segawa, K., Murakami, M., Takahashi, K., Maruyama, M., Maeda, M., and Yamanaka, S. (2003). The homeoprotein Nanog is required for maintenance of pluripotency in mouse epiblast and ES cells. *Cell* **113**, 631-42.
- Natale, D. R., and Watson, A. J. (2002). Rac-1 and IQGAP are potential regulators of E-cadherin-catenin interactions during murine preimplantation development. *Gene Expr Patterns* **2**, 17-22.
- Nichols, J., Zevnik, B., Anastasiadis, K., Niwa, H., Klewe-Nebenius, D., Chambers, I., Scholer, H., and Smith, A. (1998). Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4. *Cell* **95**, 379-91.
- Noritake, J., Watanabe, T., Sato, K., Wang, S., and Kaibuchi, K. (2005). IQGAP1: a key regulator of adhesion and migration. *J Cell Sci* **118**, 2085-92.
- O'Farrell, P. H., Stumpff, J., and Su, T. T. (2004). Embryonic cleavage cycles: how is a mouse like a fly? *Curr Biol* **14**, R35-45.
- Ohno, S. (2001). Intercellular junctions and cellular polarity: the PAR-aPKC complex, a conserved core cassette playing fundamental roles in cell polarity. *Curr Opin Cell Biol* **13**, 641-8.
- Ohsugi, M., Ohsawa, T., and Semba, R. (1993). Similar responses to pharmacological agents of 1,2-OAG-induced compaction-like adhesion of two-cell mouse embryo to physiological compaction. *J Exp Zool* **265**, 604-8.
- Pauken, C. M., and Capco, D. G. (1999). Regulation of cell adhesion during embryonic compaction of mammalian embryos: roles for PKC and beta-catenin. *Mol Reprod Dev* **54**, 135-44.
- Paynton, D. V., Rompel, R., and Bachvarova, R. (1988). Changes in state of adenylation and time course of degradation of maternal mRNA during oocyte maturation and early embryonic development in the mouse. *Developmental Biology* **129**, 304-314.
- Perez-Moreno, M., Jamora, C., and Fuchs, E. (2003). Sticky business: orchestrating cellular signals at adherens junctions. *Cell* **112**, 535-48.
- Riethmacher, D., Brinkmann, V., and Birchmeier, C. (1995). A targeted mutation in the mouse E-cadherin gene results in defective preimplantation development. *Proceedings of the National Academy of Sciences of the United States of America* **92**, 855-859.
- Rivera-Perez, J. A., and Magnuson, T. (2005). Primitive streak formation in mice is preceded by localized activation of Brachyury and Wnt3. *Dev Biol* **288**, 363-71.
- Schultz, R. M. (1993). Regulation of zygotic gene activation in the mouse. *Bioessays* **15**, 531-8.
- Sheth, B., Fontaine, J. J., Ponza, E., McCallum, A., Page, A., Citi, S., Louvard, D., Zahraoui, A., and 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.

- Shewan, A. M., Maddugoda, M., Kraemer, A., Stehbins, S. J., Verma, S., Kovacs, E. M., and Yap, A. S. (2005). Myosin 2 is a key Rho kinase target necessary for the local concentration of E-cadherin at cell-cell contacts. *Mol Biol Cell* **16**, 4531-42.
- Snow, M. H., and Bennett, D. (1978). Gastrulation in the mouse: assessment of cell populations in the epiblast of tw18/tw18 embryos. *J Embryol Exp Morphol* **47**, 39-52.
- Spindle, A. I. (1978). Trophoblast regeneration by inner cell masses isolated from cultured mouse embryos. *Journal of Experimental Zoology* **203**, 483-489.
- Strumpf, D., Mao, C. A., Yamanaka, Y., Ralston, A., Chawengsaksophak, K., Beck, F., and Rossant, J. (2005). Cdx2 is required for correct cell fate specification and differentiation of trophectoderm in the mouse blastocyst. *Development* **132**, 2093-102.
- Tachibana, K., Nakanishi, H., Mandai, K., Ozaki, K., Ikeda, W., Yamamoto, Y., Nagafuchi, A., Tsukita, S., and Takai, Y. (2000). Two cell adhesion molecules, nectin and cadherin, interact through their cytoplasmic domain-associated proteins. *J Cell Biol* **150**, 1161-76.
- Tao, Q., Yokota, C., Puck, H., Kofron, M., Birsoy, B., Yan, D., Asashima, M., Wylie, C. C., Lin, X., and Heasman, J. (2005). Maternal wnt11 activates the canonical wnt signaling pathway required for axis formation in *Xenopus* embryos. *Cell* **120**, 857-71.
- Tarkowski, A. K., Jagiello, K., Czolowska, R., and Ozdzenski, W. (2005). Mouse chimaeras developed from electrofused blastocysts: new evidence for developmental plasticity of the inner cell mass. *Int J Dev Biol* **49**, 909-14.
- Tarkowski, A. K., and Wroblewska, J. (1967). Development of blastomeres of mouse eggs isolated at the 4- and 8-cell stage. *Journal of Embryology and experimental Morphology* **18**, 155-180.
- Thomas, F. C., Sheth, B., Eckert, J. J., Bazzoni, G., Dejana, E., and Fleming, T. P. (2004). Contribution of JAM-1 to epithelial differentiation and tight-junction biogenesis in the mouse preimplantation embryo. *J Cell Sci* **117**, 5599-608.
- Torres, M., Stoykova, A., Huber, O., Chowdhury, K., Bonaldo, P., Mansouri, A., Butz, S., Kemler, R., and Gruss, P. (1997). An alpha-E-catenin gene trap mutation defines its function in preimplantation development. *Proc Natl Acad Sci U S A* **94**, 901-6.
- Vestweber, D., Gossler, A., Boller, K., and Kemler, R. (1987). Expression and distribution of cell adhesion molecule uvomorulin in mouse preimplantation embryos. *Developmental Biology* **124**, 451-456.
- Vestweber, D., and Kemler, R. (1984). Rabbit antiserum against purified surface glycoprotein decompacts mouse preimplantation embryos and reacts with specific adult tissues. *Experimental Cell Research* **152**, 169-178.
- Vinot, S., Le, T., Ohno, S., Pawson, T., Maro, B., and Louvet-Vallee, S. (2005). Asymmetric distribution of PAR proteins in the mouse embryo begins at the 8-cell stage during compaction. *Dev Biol* **282**, 307-19.
- Wiley, L. M., and Obajasu, M. F. (1988). Induction of cytoplasmic polarity in heterokaryons of mouse 4-cell/16-cell stage blastomeres heterokaryons. *Developmental Biology* **133**, 375-384.
- Winkel, G. K., Ferguson, J. E., Takeichi, M., and Nucitelli, M. (1990). Activation of protein kinase C triggers premature compaction in the four-cell stage mouse embryo. *Developmental Biology* **138**, 1-15.
- Yamanaka, Y., Ralston, A., Stephenson, R. O., and Rossant, J. (2006). Cell and molecular regulation of the mouse blastocyst. *Dev Dyn* **235**, 2301-14.
- Zernicka-Goetz, M. (1998). Fertile offspring derived from mammalian eggs lacking either animal or vegetal poles. *Development* **125**, 4803-8.
- Zernicka-Goetz, M. (2006). The first cell-fate decisions in the mouse embryo: destiny is a matter of both chance and choice. *Curr Opin Genet Dev* **16**, 406-12.

Figure legend

Schematic representation of the mouse early development. The vertical line represents the approximate timing in days of development starting from fertilization (E 0). Upon fertilization of the mature MII oocyte, the second polar body is extruded, defining the animal pole of the animal – vegetal axis (A/V). Segmentation of the zygote occurs during the travel of the conceptus in the mother oviduct. Development depends on maternal stores until the zygotic genome activation phase at the 2-cell stage. At the 8-cell stage, compaction takes place. This morphogenetic event is characterized by the flattening and the polarization of blastomeres. Cellular and molecular modifications occurring during compaction are depicted in the insert. During the following mitosis, asymmetric divisions take place, resulting in the divergence of the inner cell mass (ICM) and of the trophectoderm (TE). The formation of the segmentation cavity, called the blastocœl, coincides with the arrival of the blastocyst into the uterus. At E 3.5, the eccentric location of the ICM defines the Embryonic (Em) – Abembryonic (Ab-em) axis: the ICM and the polar TE represent the embryonic pole while the mural TE corresponds to the abembryonic pole. Before implantation, the blastocyst hatches from the zona pellucida allowing the mural trophectodermal cells to invade the uterine epithelium. In parallel, a third tissue differentiates from the ICM to form the primitive endoderm (PE), while the remaining ICM cells form the epiblast (EPI). After implantation, at E 5.5, molecular asymmetries in the visceral endoderm (VE) result in the formation of a proximal-distal axis (P/D) that will become the anterior – posterior axis during gastrulation.

