

Epithelium-Mesenchyme Transitions Are Crucial Morphogenetic Events Occurring during Early Development

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Abstract

Developmental biology constitutes a unique field to study cell dynamics within an organism. Transitions from epithelial to mesenchymal architectures represent major morphogenetic events during development. In this chapter, trophectoderm and mesoderm formation in the mouse is analyzed in detail to exemplify general features of epithelial to mesenchyme transition at the level of the organism, tissues, cells and molecules. As a conclusion, importance of regulation of these processes in embryos and adults is stressed, dysregulations leading to cancer formation and progression.

Introduction

The term “morphogenesis” originates from the combination of two Greek words, “Morphê” (= form, shape) and “Génésis” (= principle, origin). Consequently, it means “origin of forms”. Because of this etymological definition, the word has numerous domains of application in Biology. Life sciences, by definition, deal with a dynamic environment, and “morphogenesis” designates the formation and organization, and also the deformation, movement and disappearance of biological objects. The biological objects at the sub-cellular level for example are the different organelles. At the cellular level, morphogenesis is at least involved in two major cellular phenomena, cell division and the general shape of the cell. For tissues, morphogenesis refers to the correct arrangement of the cells forming the tissue. Finally, for whole organisms, morphogenesis designates all the morphological transformations of the organism. In embryology in particular, morphogenesis designates the harmonious transformations leading from the zygote to an adult organism. Developmental biology is consequently a field involving the integration of a series of morphogenetic events that depend on molecular events and that can be analyzed at the sub cellular, cellular and tissue levels.

The study of morphogenesis has been fundamental to the classification of animal species and hence to considerations of evolution. Indeed, phylogenetic divisions are based on the appearance of relative morphogenetic characteristics. In particular, the appearance of intercellular adhesion and the subsequent apparition of different types of supracellular architectures were essential. The first division is based on the appearance of multicellular structures (= colonial protists) from unicellular organisms (= protozoans) as shown in Figure 1. Seventeen known types of colonial protists present development phases during which they form multicellular assemblies. An example of this type of protist is *Dictyostelium discoideum* that forms a multicellular assembly, a pseudo-plasmodium, in absence of nutrients. The molecular mechanism of

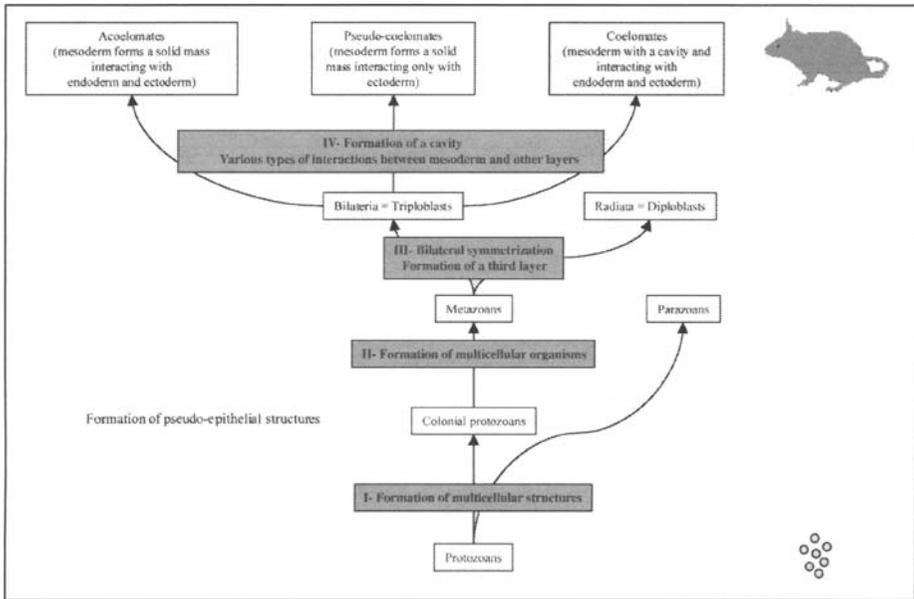


Figure 1. Simplified phylogenetic tree. This simplified phylogenetic tree present the major evolutionary steps from Protozoans to Mammals. The first step consists in the apparition of multicellular structures followed by multicellular organisms. The following steps correspond to the simultaneous apparition of bilateral symmetry and of the first mesenchymal layer, mesoderm. The interaction of mesoderm with the two other layers defines the further subclassification.

intercellular adhesion involves the sequential expression of transmembrane glycoproteins. The initial adhesion is mediated by the expression at the membrane of one glycoprotein.¹ This adhesion is stabilized by the expression of a second membrane glycoprotein.² In a third phase, a third membrane glycoprotein is expressed, and mediates the cohesion of particular cells during the migration of the pseudo-plasmodium. Loomis proposed the hypothesis that this third intercellular adhesion system allowed the separation of two cell types: the future spore cells and the future stem cells. Thus, very early during evolution, intercellular adhesion, morphogenesis and cell differentiation are closely related.

The major second step that occurred during evolution was the formation of multicellular organisms, the Metazoans. A supracellular and organized architecture, the epithelium, had appeared (see Chapter 5, Sarras). It is only from after this step was made that the term “embryo” can be used to describe the first developmental stages of an animal. By definition, an embryo is a multicellular assembly in which distinct cell types become individualized (cell differentiation).

The third accepted criterion appearing during evolution, used for classification, is the appearance of bilateral symmetry. This is associated with the appearance of a third basic layer, the mesoderm, initially a loose tissue. This evolutionary step corresponds to the acquisition of a particular supracellular organization in the embryo, and the appearance of the mesenchyme. Thus, there was a transition from the epithelial architecture to the mesenchymal architecture, called the Epithelial-Mesenchymal Transition (or EMT). The presence of the mesoderm and bilateral symmetry distinguishes triblastics from diblastics. Triblastics can be classified into two groups (coelomates and pseudo-coelomates) according to the presence of a cavity within the embryo and specific interactions of the mesoderm layer with the two other basic epithelial layers (ectoderm and endoderm). The coelomates are defined by the presence of a cavity called the coelom limited by epithelial cells. These epithelial cells are mesoderm cell derivatives,

corresponding to a Mesenchymal-Epithelial Transition or MET. Coelom development is induced by ectoderm and endoderm cells. The pseudo-coelomates have no such cavity in the mesoderm, which forms a solid mass of cells in contact with ectoderm and endoderm.

Thus, the phylogenetic classification of animals is based on morphogenetic events that are closely linked to the apparition and interconversion of two types of cell architectures: epithelium, a tight aggregation of polarized cells, and the mesenchyme, a loose association of poorly polarized cells. Here, we apply a strict dichotomy between epithelium and mesenchyme. Although this is as arbitrary as any binary representation of complex events, it is legitimized by the general outline of evolution, presented above, and is widely accepted. We will first define epithelium and mesenchyme, and then describe MET and EMT as occurs during early mammalian development. We will consider trophoblast formation and gastrulation at all levels from the organism to molecular, using the mouse as a reference animal model. Finally, regulatory processes for EMT in embryos will be compared to those in adults.

Supracellular Architecture: The Dichotomy between Epithelium and Mesenchyme

Epithelial Organization: A Cohesive Assembly of Cells

The word “epithelium” has for etymology “épi” (on) and “thélé” (nipple). Consequently, “epithelium” designated, at the origin, the aggregation of cell recovering the surface of the nipple. The epithelial organization was invented in the animal reign with the diblastics and allowed the production of tissues and organs. Epithelial organization has two fundamental features: (i) separating two distinct biological compartments, the “interior” and the “exterior” and (ii) conferring to an assembly of cells a transportation function vector. The vectorial character results from the structural and functional polarity of the epithelial cells (Fig. 2).

Epithelial characteristics at the cellular level can be defined by the five following criteria in various *in vivo* or *in vitro* study systems: (i) the cohesive interaction between cells allowing the formation of continuous cell layers, (ii) the existence of three types of membrane domains: apical, lateral and basal, (iii) the existence of tight junctions between apical and lateral domains, (iv) the polarized distribution of the different organelles and components of the cytoskeleton and, (v) the quasi immobility of the group of epithelial cells relative to the local environment.

These structural features allow three important types of function: (a) the formation of vast surfaces for exchange (for example, microvilli) and also of cavities by the overall folding of epithelial layers (for example, intestinal tube or nervous tube), (b) the formation of biological compartments of different ionic compositions (low ionic strength and serous), due to selective permeability of the cells, (c) the absorption, the transcytosis and the vectorial secretion of macromolecules.

The ontogeny, the maintenance and the dynamics of epithelial structure require appropriate functioning of the cells individually and the epithelial tissue as a whole. Thus, the functioning of the epithelial cells, and the implementation of their genetic programs, has to be coherent and coordinated both in time and space.

The adhesive systems of the epithelial cells can be classified into cell-cell adhesion involving mainly the lateral sides of the cells, and cell-matrix adhesion involving mainly the basal surfaces of the cells. Cell-cell adhesion confers the cohesiveness of the epithelium, and is a characteristic of epithelia, whereas cell-matrix adhesion is also found for mesenchymal cells. There are several epithelial cell-cell adhesion systems including gap junction, adherens junctions, desmosomes, and tight junctions. Different families of proteins are involved in these different systems.

Mesenchymal Organization

The term “mesenchyme” originates from the Greek words *Mésos* (environment), *In* (in) and *Chymos* (juice). A supracellular mesenchymal architecture can be defined by contrast to a

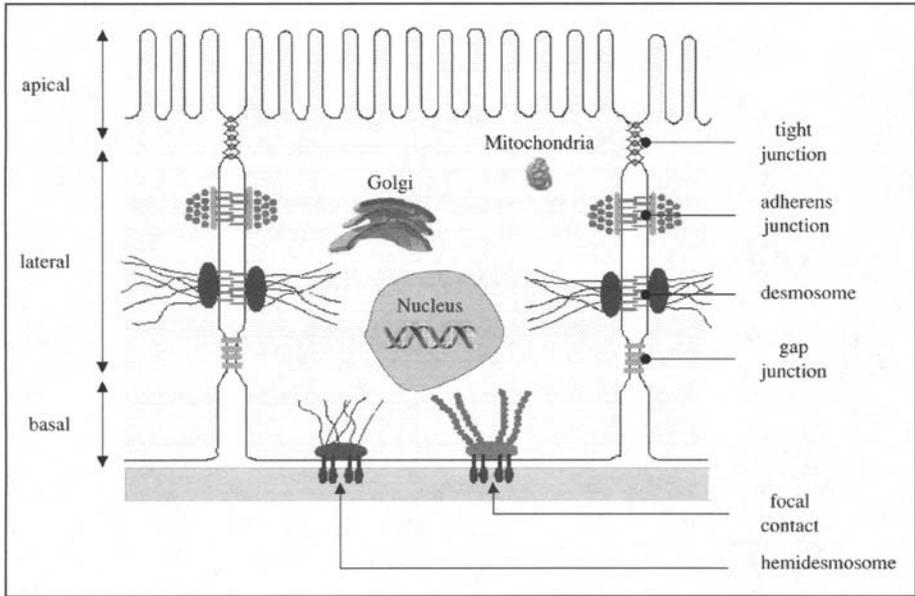


Figure 2. This scheme presents the main characteristics of an epithelial cell: the intercellular adhesion mediated by different types of junctions (tight, adherens, desmosomal, gap), the definition of distinct membrane regions (apical, lateral, basal) and the polarised distribution of organelles. It should be noted that cell-matrix adhesion complexes are located exclusively on the basal region and involves different types of junctions (focal contacts and hemidesmosomes). In addition, an epithelial assembly is characterised by its immobility and its exchanges of molecules between compartments of different composition.

supracellular epithelial organization as: (i) loose or no interaction between the cells, such that a continuous cell layer is not formed. (ii) The absence of clear apical and lateral membranes. (iii) The nonpolarized distribution of the different organelles and components of the cytoskeleton. (iv) The motility and even invasiveness of the cells.

During development and cancer progression, mesenchymes may be temporary intermediates in the formation of an epithelial structure from another epithelial structure. However, the mesenchymal architecture can be a lasting organization. Mesenchymal functions include support and nutrient supply.

In summary, the epithelial organization is mainly dependent on the tightness of the cell-cell junctions. Cell-cell adhesion is dependent on transmembrane glycoproteins, including the epithelial marker E-cadherin. The mesenchymal status can be considered to be the only, and default, alternative of the epithelial status.

During the genesis of epithelium from individualized cells, four phases can be distinguished (i) an intercellular aggregation step, (ii) a polarization cell step, (iii) a cell differentiation step, and (iv) an integration of the differentiated cells into a functional organization. Thus, a parallel can be drawn between embryonic development and evolution. This general scheme applies to embryonic development as well as to physiological and pathological processes in adult; the gain or loss of the epithelial organization associated with the loss or gain of the mesenchymal status.

Embryonic Morphogenesis: A Harmonious Series of Transitions from One Cellular Architectural Type to Another

Here, we will describe the interconversions between epithelium and mesenchyme during early mouse embryonic development, until gastrulation. In mammals, the first MET occurs

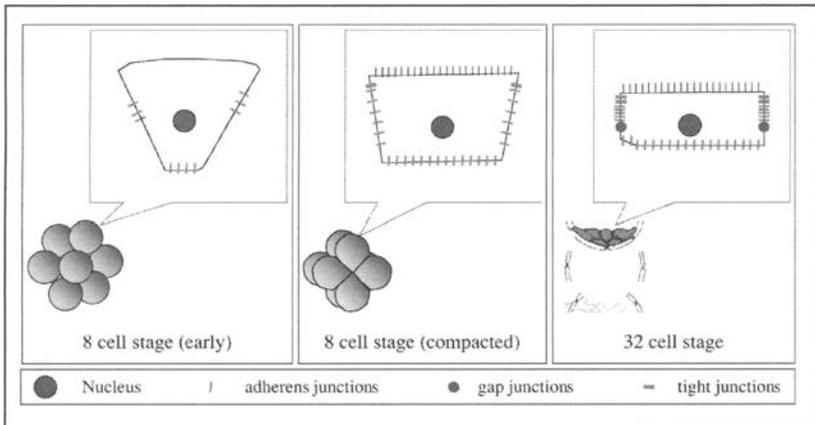


Figure 3. At the early 8 cell stage, blastomeres are characterised by a intercellular adhesion mediated by E-cadherin. At the compacted 8 cell stage, in addition to the formation of additional adherens junctions, some tight junctions appear on the apical side. At the 32 cell stage, gap junctions appear in addition to the existing adherens and tight junctions.

during the preimplantation period with the formation of the trophectoderm and the first EMT during the gastrulation period with the formation of the mesoderm.

The First MET in Embryonic Epithelium Leads to the Formation of the Trophectoderm

The first stages of Metazoans embryonic development involve a series of rapid cell divisions, called the segmentation phase. The cells at this stage are called blastomeres. The morphogenetic characteristics of these first stages are limited, although the cell fates are already determined after the first cell divisions in organisms such as the *Tunicates*, *Caenorhabditis elegans* or sea urchin (see Chapter 6, Wessel). For Mammals, the determination seems to occur at a later stage during development. However, the first real morphogenetic event is the formation of the trophectoderm.

After segmentation, the embryo looks like a small blackberry, *mora* in Latin; thus, the 8-cell stage is termed the “morula”. The shape and morphology of these 8 cells, the blastomeres are highly similar. The morula then becomes a compacted morula with 16 cells; the blastomeres at the periphery flatten progressively, become polarized and all types of junctions appear. These cells become the first embryonic epithelium, the trophectoderm. The cells inside the compacted morula become the inner cell mass (ICM), and are totipotent. The trophectoderm is an active epithelium and gives rise to the blastocoel.^{3,4} There are three axes of symmetry: antero-posterior, dorso-ventral and left-right axis. The dorso-ventral axis is defined at the time of the blastocyst. The future dorsal part of the embryo, contained in the ICM, is located at the contact of the blastocoel. A series of cellular and molecular modifications during early development lead to the formation of the trophectoderm and the ICM.

Modulation of Cell-Cell Adhesion

E-cadherin (or Cdh1) is a cell adhesion molecule important during compaction and the formation of the trophectoderm. E-cadherin is present in the zygote as a maternal protein. At the end of the two-cell stage, zygotic E-cadherin production starts. In noncompacted morulas (Fig. 3B), the blastomeres are linked. The maternal and zygotic E-cadherin is progressively redistributed and concentrated on the future baso-lateral side of the blastomeres at the periphery of the future compacted morula.^{5,6}

By the 16-cell stage (Fig. 3C), the amount of E-cadherin on the baso-lateral side of the blastomere is greater, and cell-cell adhesion tighter. Noncompacted morulas incubated with anti-E-cadherin antibodies are not able to compact or to form a blastocyst.^{5,7-9} E-cadherin knockout mice, *Cdh1*^{-/-}, have been produced. These embryos cannot form a functional epithelium and die at the peri-implantation stage. Interestingly, the compaction of *Cdh1*^{-/-} embryos is not perturbed, indicating that the maternal E-cadherin is sufficient. The zygotic E-cadherin is however required to allow the embryo to form a blastocyst.¹⁰

Cell Polarization

Scanning electron microscopy analysis of compacted and uncompact embryos shows that compaction is associated with morphological modifications, such as formation of microvilli scattered in a polarized manner.¹¹ It appears that the cells polarize after cell-cell contact. When compaction is artificially reproduced by pairing two noncompact embryo blastomeres, the membranes have two distinct fates. The free membranes form microvilli and the cell-cell interacting membranes stay smooth.⁶ The main proteins involved in adhesion at 2-cell stage are the proteins of the cadherin-catenin complex. At the noncompact, 8-cell stage (Fig. 3B), the membranes of the blastomeres are associated by functional gap junctions, allowing the exchange of ions and small molecules between the cells of the forming epithelial layer. These gap junctions are formed by connexin octamers. In parallel, E-cadherin and ZO-1 are recruited at the cell-cell contact site leading to adherens and tight junctions on the baso-lateral sides of the future trophoctoderm epithelial cells. Maternal E-cadherin is redistributed at the cell surface and zygotic E-cadherin and ZO-1 are addressed to the future adherens and tight junctions. On the future apical membrane, endosomes accumulate. Reorganization of cortical and cytoplasmic actin and of microtubules is observed at microvilli.

Cingulin is recruited at the tight junctions at the 16-cell stage (Fig. 3C), before the junctions are functional. Functional adhesive junctions are scattered along the baso-lateral sides, reinforcing regional cell-cell adhesion. Large amounts of protein migrate in the plasma membrane; the apical proteins, such as the Na⁺/glucose carrier, and the baso-lateral proteins, such as EGFR (epidermal growth factor receptor) relocate.^{12,13} The polarized distribution of the organelles is continues within the cells; the mitochondria and lipid vesicles concentrate on the baso-lateral side, and the lysosomes, the Golgi apparatus and the nucleus at the central basal part of the cells.

Function Acquisition by the Active Epithelium

The tight junctions established in the earliest stages become functional at the 32-cell stage (Fig. 3D). Numerous desmosomes form on the baso-lateral sides by desmogleins and desmoplakins which are synthesized during the morula stage.¹⁴ The size of the blastocoele, and therefore the blastocyst, increases continuously due to the activity of the trophoctoderm cells. The resulting pressure on the zona pellucida is responsible, at least in part, for hatching. The hatched embryo then interacts with and implants into the uterus.

The formation of the first embryonic epithelium is associated with the development of both complex cell-cell adhesion machinery and also cellular and the sub-cellular polarization. This first epithelial supracellular architecture subsequently leads to the first mesenchymal supracellular architecture: the mesoderm.

During post-implantation, the trophoctoderm regionalizes into distinct epithelia with various morphologies and different rates of proliferation.¹⁵ The parietal trophoctoderm is the part of the trophoctoderm that is not in contact with the ICM. This cell population stops dividing, although the DNA continues to replicate. The chromosomes of these cells become polytenic, and the cells themselves become giant cells.¹⁶ The visceral trophoctoderm is in contact with the ICM. This population of cells contributes to the formation of the extraembryonic ectoderm during gastrulation.

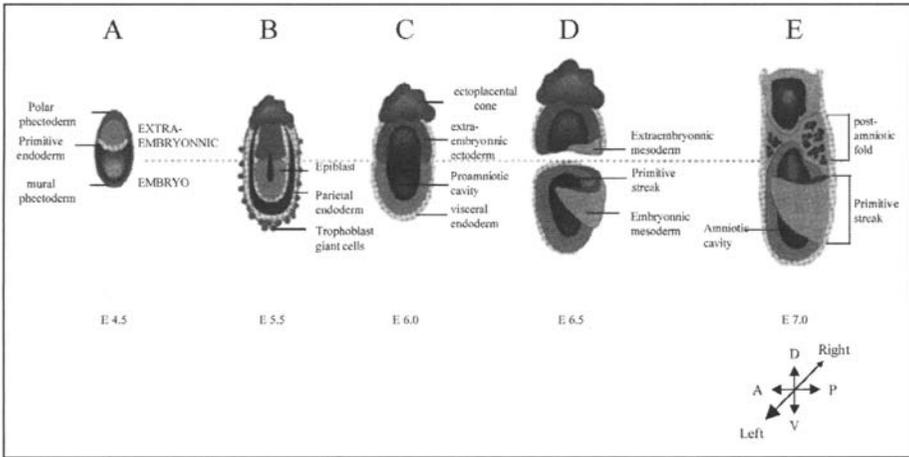


Figure 4. Like in all coelomates, gastrulation in the mouse is characterised by cellular movements occurring between embryonic days E4.5 and E7.5 which enable the reorganisation of the embryonic cells and the formation of the definitive plan of the animal. A to E: the major steps of this reorganisation between E4.5 and E7.0 are shown on schematic sections along the dorso-ventral axis (D-V). In particular, the migration of mesoderm progenitor cells (in red) from the posterior to the anterior side of the embryo between the ectoderm (in blue) and endoderm (in yellow) is presented on sections D and E. Reprinted with permission from Hogan B, Beddington R, Costantini F et al. *Manipulating the mouse embryo: a laboratory manual*, 2nd ed. 1994:58. ©1994 Cold Spring Harbor Laboratory Press.

Formation of the First Embryonic Mesenchyme: The Mesoderm Layer

Following the formation of trophectoderm, at E4.0, a second epithelial layer is formed at the interface between the cells of the ICM and of the blastocoele. This second epithelium is called the primitive endoderm and contributes to the formation of the visceral endoderm (which remains associated with the epiblast) and of the parietal endoderm, which covers the surface of the blastocoele and the parietal trophectoderm. The parietal endoderm and the visceral endoderm do not contribute to the embryonic endoderm.¹⁷ The blastocyst implants into the uterine wall between E4.5 and E5.5 (Fig. 4). Spatial constraints and the attachment of the blastocyst to the uterine wall cause the cells of the ICM and of the trophectoderm to grow towards the interior of the blastocoele cavity.¹⁸ The cells of the ICM then form a third epithelium: the epiblast. This is accompanied by the formation of a central cavity called the amniotic pro-cavity. This cavity is not the result of the formation of epithelium, but rather of localized cell death.¹⁹ At this stage the embryo, called the “egg cylinder”, has the shape of a cup made of two layers: the epiblast, surrounded by the visceral endoderm. The embryonic and extraembryonic regions are clearly distinct, and the dorso-ventral and proximo-distal axes are apparent.

Mouse gastrulation displays highly coordinated epithelium to mesenchyme conversions, cell migration, cell proliferation and differentiation. The general organization of the embryo emerges, with the formation of a new layer, the mesoderm, between the ectoderm and the endoderm. During gastrulation cell populations which were previously separated come together to form tissues and organs, and cells are transiently or permanently changed to allow permissive or instructive induction. The formation of the mesoderm layer involves a transition from the epithelial organization of the epiblast (tight and polarized) to the mesenchymal organization of the mesoderm (loose and apolar).

The mesoderm cells are recruited from the cells of the epiblast, into a structure called the primitive streak. The location of the primitive streak defining the posterior pole and this is the

first assignment of an antero-posterior axis to the embryo. The cells recruited into the primitive streak undergo an epithelium-mesenchyme transition, then ingress between the primitive ectoderm and the endoderm form the mesoderm and of part of the endoderm.²⁰ The newly formed mesoderm cells form a new tissue, which spreads between the endoderm and the ectoderm, symmetrically from the primitive streak like "wings".

Triggering of Gastrulation

Gastrulation does not start before the epiblast contains a certain number of cells.²¹⁻²³ The triggering of gastrulation also depends upon a chronological checkpoint.²⁴⁻²⁶ Cell proliferation is the main driving force leading to the development of the primary layers.²⁷⁻³⁰ Additional morphogenetic forces are involved in the formation of the mesoderm layer, for example ingression of epiblast cells into the primitive streak and their subsequent tendency to move away from it.

Formation of the Primitive Streak

The formation of the primitive streak occurs in the posterior and ventral epiblast, at the junction with the extraembryonic ectoderm.³¹ The streak progresses to the extremity of the cylinder. Initiation of the formation of the primitive streak is poorly understood. The streak may lengthen due to division, recruitment and intercalation of cells of the epiblast between the extremities of the developing primitive streak. Thus, the cells at the distal end of the streak are of posterior origin, having been the first recruited when the streak formed (Lawson et al, 1991).

Epithelium to Mesenchyme Transition

There is little available data about EMT in the mouse. Nevertheless, findings for rabbit and rat appear to apply to the mouse. An epithelium-mesenchyme transition occurs in epiblast cells before they penetrate the primitive streak. In the mouse, the epiblast is a pseudo-stratified epithelium constituted of high and columnar cells.^{28,32,33} The epiblast presents various characteristics of epithelia, including polarization, specialization of the apical surface with short and separated microvilli,^{34,35} junctions (principally adhesive junctions, but also tight junctions, communicating junctions and desmosomes) in the apical portion of the lateral faces and a basal lamina.^{14,33}

Loss of Polarization

When EMT begins, organelles in certain cell populations of the epiblast are relocated to the apical face^{34,36} This step is called "cytoplasmic hyperpolarization". Concomitantly, an apical constriction appears, with an enlargement distorting and destroying the basal lamina at the basal pole (Fig. 5). As in amphibians, these cells are named "bottle cells".³⁶ After EMT, cells which separated from the epiblast show mesenchymal features: irregular outlines and apolar distribution of organelles. The cells that penetrate the primitive streak show a characteristic distribution of the Na⁺-K⁺ ATPase pump. This enzyme is basal in epiblast cells outside of the primitive streak, but is apical in cells in the primitive streak, and is present throughout the cytoplasm of mesoderm cells after they have gone through the primitive streak.³⁷

Modification of the Cytoskeleton

During EMT the intermediary filaments of the cytokeratin cytoskeleton of the epiblast cells are replaced by vimentin.³⁸ If the gene coding for vimentin or cytokeratin 8, an epithelial cytokeratin, is knocked out, the mice do not present any abnormality during gastrulation.^{39,40} In cultures of primitive streak stage mouse embryos *in vitro*, immunoreactivity for desmoplakins and for E-cadherin is lost by epiblast cells going through the primitive streak.⁴¹ Desmoplakins are found in mesoderm cells during migration, suggesting that desmosomal junctions contribute to the cohesion of the migrating mesoderm cell layer.⁴²

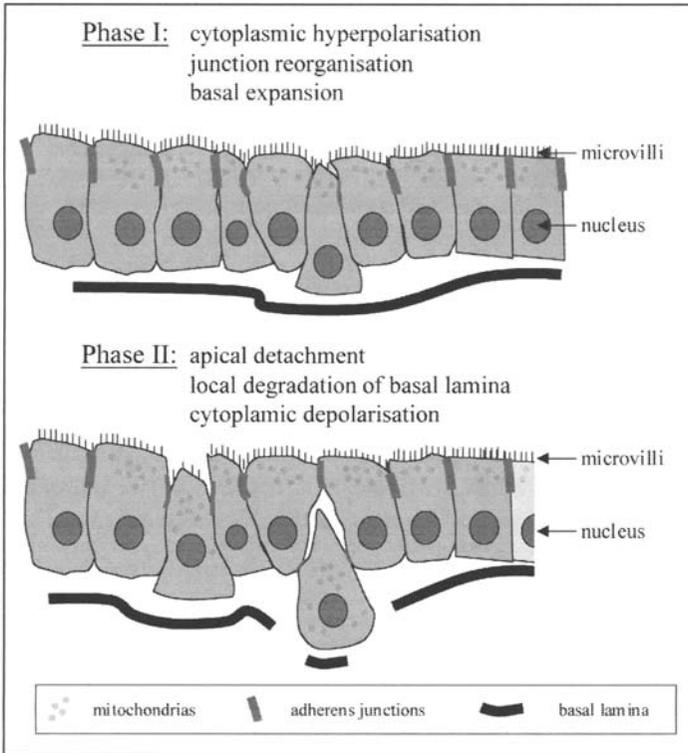


Figure 5. This model for epiblast to mesoderm transition in Mammals was proposed by Viebahn in 1995 from observations using transmission electron microscopy.³⁶ In phase I, organelles are relocated to the apical side of the cells and a basal enlargement appears. In phase II, cytoplasmic polarisation is gradually lost and apical detachment of the cells undergoing epiblast to mesoderm transition occurs. This second phase comprises also a local degradation of the extracellular matrix.

Modification of Cell-Cell Adhesion

The reorganization of adhesive junctions during the formation of “bottle cells” has been studied by electron microscopy.³⁶ The resulting model explains the delamination of mesoderm cells from the epithelium and their ingress into the primitive streak.

The molecular mechanisms of this EMT *in vivo* are poorly understood because functional studies in the embryo *in utero* are particularly difficult. In the mouse, the transformation of the epiblast epithelium into mesoderm mesenchyme involves the loss of E-cadherin immunoreactivity.⁴³ In the chicken, N-cadherin is first expressed by cells penetrating the primitive streak.⁴⁴ In the mouse, it is not clear whether there is similar replacement of E-cadherin by another cadherin. For example, cadherin-11 is expressed by mesoderm cells only after they start to migrate.^{45,46} β -catenin is a protein linking the conventional cadherins to the actin cytoskeleton. Its knockout results in embryonic death at E7.0, associated with the absence of mesoderm formation.⁴⁷ γ -catenin appears to be unable to compensate for β -catenin deficiency at this developmental step. However, in γ -catenin gene knockout mice, functional compensation by β -catenin is possible in certain tissues at E10.5.^{48,49}

Epiblast explants have been cultured *in vitro*.⁵⁰ Under basal conditions, they form epithelial clusters of cells, show characteristic adhesive and migratory behavior and express the characteristic markers of the epiblast. Antibodies blocking E-cadherin activity at the surface of explants of the posterior epiblast destroy the epithelial organization and induce a mesenchymal

organization. This involves radical changes in the cell-cell and cell-extracellular matrix adhesive properties, the acquisition of far more developed migratory properties and the corresponding changes in the expression of molecular markers for mesoderm and epiblast cells. This EMT is not conventional insofar as it leads to the formation of a "mesenchymal layer", in which mesenchyme cells are not individualized. This mesenchymal layer state is preserved during migration after ingression into the primitive streak. This state represents a feature of mesoderm formation in Vertebrates, not found in Invertebrates.

Modification of the Rate of Cell Proliferation

BrdU staining and a stathmokinetic analysis demonstrated that the mean length of the cell cycle in the rat primitive streak is 3 hours, whereas that of an epiblast cell before penetrating into the primitive streak is 7.5 hours.³⁰ The difference is due to shortened G1 and G2 phases.

Modification of the Extracellular Matrix

Initially, there is a thin layer of extracellular matrix in the form of a basal lamina between the epiblast and the primitive endoderm. It is constituted of hyaluronic acid, fibronectin, laminin, type IV collagen and thrombospondin.⁵¹⁻⁵⁵ The basal lamina is discontinuous, even absent, under bottle cells which go on to form the mesoderm, whereas it is intact at the more posterior part of the epiblast (Fig. 5).^{33,35,42}

Aggregation experiments with mutant ES cells with wild-type blastocyst cells demonstrated that the knock-out of the genes coding for the components of the extracellular matrix or for their receptors at the cell membrane such as fibronectin and $\alpha 5$ or $\beta 1$ integrins does not perturb the behavior of the mutant cells during gastrulation.⁵⁶⁻⁵⁸

Modification of Gene Expression

Molecules of the signaling pathways for growth factors such as TGF β (transforming growth factor β) or FGF (fibroblast growth factor) are involved in mesoderm formation in mouse.⁵⁹ T (or brachyury) gene expression is induced and E-cadherin gene expression decreases during mesoderm formation. Knockout mutant mice for T, *msd*, *eed*, *snail* show defects in mesoderm formation and, consequently, defective conversion of the epithelial epiblast into mesenchymal mesoderm see also Chapter 11, Berx and Van Roy.⁶⁰⁻⁶² The brachyury mutant is particularly interesting because T expression appears to require FGF-2 activity for mesoderm formation, as in amphibians.⁶³ In mouse, anterior epiblast explants treated with 10 to 50 ng/ml of FGF-2 behaved in the same way as anterior epiblast explants treated with anti-E-cadherin-blocking antibodies.⁶⁴ Functional compensation by other growth factors of the FGF family or of families resulting in a functionally identical signaling could explain why the *fgf2* knockout does not impair mesoderm formation.^{65,66} Mouse mutants for the major gastrulation FGF-2 receptor, *fgfr1*, show defects in mesoderm formation, due to defective mesoderm cell migration after ingression. Conversely, defects in epithelium to mesenchyme transition could not be demonstrated directly.⁶⁷

Model for the EMT from Epiblast to Mesoderm

The generally accepted model distinguishes two phases (Fig. 5). First, cytoplasmic hyperpolarization and junction remodeling (apical constrictions and basal enlargement) occur. Secondly, there is apical detachment and local destruction of the basal lamina. Two types of molecule directly controlling the EMT leading to mesoderm cell formation have been identified: a growth factor (FGF-2) and a cell-cell adhesion molecule (E-cadherin).

Ingression into the Primitive Streak

Once the primitive streak has been established, cells in contact with it ingress. The first cells to go through the primitive streak are those that will form the extraembryonic mesoderm.⁶⁸ As the streak elongates towards the anterior pole of the epiblast, the cells forming the embryonic mesoderm and endoderm migrate through it; the further from the primitive streak and closer to the anterior pole of the epiblast they are, the later they start.⁶⁸⁻⁷¹

Cell fate determination at gastrulation has been extensively studied in invertebrates such as sea urchin (see Chapter 6, Wessel) or drosophila (see Chapter 7, Ganguly), and vertebrates including amphibians, fish and birds. In mammals and in particular in the mouse, *in vivo* studies are complicated because embryonic development is internal. Cell fate maps have been established in the mouse by the technique of orthotopic transplantation of stained epiblast cells. Like “organizers” in other Vertebrates, the “node” at the posterior end of the primitive streak announces the organization properties of the embryo. Heterotopic transplantation of a node removed from a primitive streak stage gastrula into a gastrula at the same stage induces the formation of a second axis containing neural and somitic structures originating from the host, but induced by the transplant.⁷² In contrast to amphibians, fish and birds, the induced axis in the mouse does not contain any anterior structures. This suggests that there is a structure in the mouse, in addition to the node, able to induce and to organize the anterior structures.⁷³ The mouse node structure only appears during gastrulation, whereas the equivalent structures in other Vertebrates are present before gastrulation.

Regionalization of mesoderm populations induced during the course of the gastrulation in the mouse has been correlated with sites of ingression into the primitive streak.⁷⁴⁻⁷⁷ The dorso-ventral axis of the embryo is the antero-posterior axis of the primitive streak: the axial mesoderm originates from cells crossing the streak primitive in the vicinity of the node region, the paraxial mesoderm originates from cells crossing the primitive streak in the perinodal region, i.e., anterior region of the streak, the lateral mesoderm originates from cells crossing the primitive streak in its middle part, and the extraembryonic mesoderm originates from cells crossing the streak in its distal, and therefore posterior end.

Numerous transcription factors, membrane receptors and growth factors act as mesoderm inducers and regionalizers in lower Vertebrates. However, the molecular mechanisms of the steps between induction of these various mesoderm lineages to their ingression into the streak are mostly unknown.^{78,79} *In vivo* gene knockouts and *in vitro* assays indicate that growth factors control epiblast cell migration through the primitive streak and the consequent cell determination. Growth factors and receptors of the FGF, TGF β and Wnt families have been implicated in certain cell population allocation defects at the time of ingression. FGF-2 elicits the conversion of epiblast explants into cells displaying cellular and molecular features similar to those of mesoderm cells *in vivo*.⁶⁴ Knocking-out the gene encoding the main FGF receptor at this stage, *fgfr1*, results in cells having completed ingression being retained in the neighborhood of the primitive streak, leading to a defect in paraxial mesoderm formation. This has been interpreted as a mesoderm dorsaling defect.^{67,80,81} Cell migration abnormalities after ingression have also been described in embryos carrying T mutations; T is a transcription factor the expression of which in other vertebrates is specific to developing mesoderm cells and is modulated by FGFs.^{82,83}

Likewise, homozygote mutants for *wnt3a* show a paraxial mesoderm formation defect in the trunk: epiblast cells can cross the primitive streak, but cannot migrate laterally or differentiate into neural tissue.^{84,85} Conversely, homozygote mutants for *bmp4* (bone morphogenetic protein 4) show a defect in the formation of ventral mesoderm.⁸⁶

Mesoderm induction and regionalization involve restriction of differentiation potential, corresponding to phases of cell differentiation. Epiblast cells are multipotent.^{68,75,87,88} Epiblast cells of any location grafted heterotopically differentiate with the cells at the site of the graft. Epiblast cell multipotency is such as they can form either somatic tissues, or germ line cells. These are the cells that are used to establish embryonic stem cell lines (ES cells).

Epiblast cells corresponding to the embryonic cells at the late primitive streak stage can differentiate into any mesoderm lineage.⁸⁹ Nevertheless, cells from the most anterior region of the primitive streak contribute only to neural tissues.⁹⁰ Therefore, the transformation of the epiblast into primitive ectoderm during gastrulation is associated with a restriction of the development potential of the cells. Anterior primitive ectoderm cells cannot differentiate into hematopoietic lineage cells *in vitro*.⁹¹ Conversely, ES cells treated with activin or BMP-4,

regain the potential to differentiate *in vitro* into hematopoietic lineage cells.^{91,92} the loss of differentiation potential can also be interpreted as an indicator of the availability of an instructive or permissive inducer in the environment of a cell population.

The cell differentiation potential is restricted when epiblast cells ingress into the primitive streak. Mesoderm cells grafted back into the epiblast recover some of the potential of epiblast cells, including both that to ingress, again, into the primitive streak and that to form mesoderm populations. Nevertheless, they cannot colonize lateral mesoderm.⁹³ Epiblast cells grafted into the mesoderm contribute to all mesoderm populations and also to lineages to which mesoderm cells at the grafting site cannot contribute.⁹³ This supports Beddington's suggestion that ingression of epiblast cells into the primitive streak results in a loss of differentiation potential.⁹⁴ Furthermore, the differentiation of epiblast cells into a given type of tissue is not dependent on the initial location of these cells, but on the route they follow and the molecular and cellular environments they meet during gastrulation.

Migration Associated with Gastrulation

Epiblast cells migrate as a cluster towards the primitive streak, during gastrulation and after ingression. Clonal analysis shows that cells derived from the same parental cells are very rarely close to one another during this migration: the migration of the primitive ectoderm cells is not clonal.⁷⁵ At gastrulation, epiblast cells that will contribute to the future neurectoderm migrate from the distal and anterior part of the epiblast towards the proximal and posterior part of the embryo. These cells migrate as a cluster of cells and they strictly follow a very precise route.⁹⁵ Once ingressed into the primitive streak, the cells contributing to the embryonic mesoderm and endoderm, and those contributing to the extraembryonic mesoderm migrate together in the opposite direction to the presumptive ectoderm cells, i.e., from the postero-distal to the antero-proximal part of the embryo.

After ingression, the migration of the precursors of the cranial and cardiac mesoderm pushes back the precursors of extraembryonic mesoderm towards the extraembryonic region. These are among the first ingressed cells to cross the primitive streak and are localized at the antero-proximal pole during the early phases of gastrulation. Under the pressure of cardiac and cranial mesoderm precursors, this population of cells is pushed back to the extraembryonic region.⁶⁸ The same type of migratory movements by propulsion have been observed for precursor cells of the embryonic endoderm, which move preexisting visceral endoderm cells towards the antero-proximal pole, in the yolk sac.^{70,71,96} The physical basis of this propulsion has not been clearly elucidated. It is currently believed, in the case of the propulsion of the visceral endoderm towards the yolk sac, that the intercalation of newly ingressed endoderm cells into the posterior and distal region of the embryo pushes the preexisting visceral endoderm in the antero-proximal direction; in addition the expansion of the yolk sac in this same direction creates a traction on the visceral endoderm. All mouse mutants presenting a phenotypic defect in the formation of the endoderm, extraembryonic cavity or constrictions at the interface of the embryonic and extra-embryonic regions also present defects in the formation of the primitive layers.⁹⁷⁻¹⁰⁰

Little is known about the molecular mechanisms involved in these cell migrations. Cell-cell adhesion molecules and matrix-cell adhesion molecules may well be involved in this process.^{44,50,101} During the EMT associated with ingression in the primitive streak, the range of cell adhesion molecules expressed at the surface of the cells is modified, and in particular E-cadherin disappears.⁹ After ingression, cohesion between the mesodermal cells is reestablished via N-cadherin and cadherin-11.^{45,46} However, N-cadherin and cadherin-11 knockouts do not present any obvious phenotype at gastrulation.^{102,103} E-cadherin knockout embryos, in contrast die at the time of perimplantation, and this early lethality prevents analysis of the effect during gastrulation.¹⁰ Nevertheless, *Cdh1*^{-/-} ES cells strongly express T, a mesodermal marker.¹⁰⁴ Finally, in the absence of FAK, a protein kinase involved in matrix-cell adhesion, mesodermal cells present clear migratory defects.¹⁰⁵

Table 1. EMT examples during embryonic development

Example	Stage (Mouse)	Transition	Specific Related Factors
Gastrulation	E6.5	Epiblast -> Mesoderm	TGF β /BMP, FGF, Wnt
Prevalvular mesenchyme (Heart formation)	E8	Endothelium -> Atrial and ventricular septum	TGF β , Slug
Neural crest cells	E8	Neural plate -> Numerous derivatives	Notch/delta, shh, BMP Slug
Somitogenesis and sclerotome differentiation	E9	Somite walls -> Sclerotome	Notch/delta, shh, slug?
Palate formation	E13.5	Oral epithelium -> Mesenchymal and epithelial cells + apoptosis	TGF β
Mullerian tract regression	E15	Mullerian tract -> Mesenchymal cells combined with apoptosis	TGF β , anti-Mullerian hormone, β catenin

In conclusion, the formation of the first embryonic mesenchyme is associated with the conversion of some epithelial cells of the primitive ectoderm. One of the main features of this conversion is the loss of the (mostly E-cadherin-dependent) cell-cell adhesion. The neo mesenchymal cells then lose subcellular polarization and their gene expression is modified. The cells present novel properties including higher rates of proliferation and motility. This EMT results in the genesis of novel cell types, and tissues and a new general organization in the embryo. Some of the cells derived from the epiblast do not undergo EMT, and will form the ectoderm. This developmental step is therefore associated with a restriction of multipotentiality.

EMT Later during Embryonic Development and Adult Stages

Several epithelium-to-mesenchyme conversions occur later during embryonic development (Table 1). Examples are the formation of neural crest cells from the neural tube on embryonic day 8 (= E8) (see Chapter 3, Newgreen and McKeown), of the atrial and ventricular septum from the endothelium during the formation of the heart on E8 (see Chapter 4, Runyan et al), of the sclerotome from the somite on E9, of mesenchymal cells of the palate from oral epithelium at E13.5 (see Chapter 1, Hay), and of mesenchymal cells during the regression of the Mullerian tract on E15. The main molecular events associated with these transitions are similar but the regulation is different. These EMT will not be described here.

Regulation of EMT

Soluble factors were thought to be important in EMT for many years, but they are not the only factors involved. Indeed, morphogen gradients are not the sole molecular mechanism of EMT induction. Transmembrane proteins such Notch/Delta or cadherin/catenin are essential in the induction and control of EMT (see Chapter 11, Bex and Van Roy).

During embryonic development, EMT are regulated by the combination of (i) the execution of a genetic program within cells that are undergoing EMT, and (ii) the signals emanating from the environment, such as growth factors, and physical constraints.

Regulation of gastrulation-related EMT arises from the migration of mesenchymal cells to environments in which EMT triggering factors are not produced. In addition, execution of genetic programs is responsible for producing pro-epithelial molecules. EMT is a normal process in the maintenance and repair of tissues (for example maintenance of the intestinal epithelium, and wound healing heightened on Chapter 8, Arnoux et al) in the adult. The regulation of adult EMT is similar to that for gastrulation-related EMT. Some abnormal processes in the

adult, such as primary tumor or metastasis formation, are also associated with EMT. These EMT are not regulated because: (i) EMT triggering signals are produced continuously after mutations in their genes; (ii) cells undergoing EMT have become insensitive to their environment and therefore, the process never stops.

Conclusion

In conclusion, it appears that the embryonic development involves a series of conversions of epithelial architecture to mesenchymal architecture and of mesenchymal architecture to epithelial architecture. These successive MET \leftrightarrow EMT interconversions can affect the same original cell. Consequently, the acquisition of the epithelial or mesenchymal status by a cell is not final. In other words, there is a single mechanism which is defined as MET or EMT according to the direction in which it works.

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