EPITHELIAL POLARITY AND SIGNALING DURING EARLY MAMMALIAN EMBRYOGENESIS

IVAN BEDZHOV
EARLY MOUSE DEVELOPMENT

- ZYGOTE
- MORULA
- BLASTOCYST
- EGG CYLINDER

Stages:
- E0.5
- E1.5
- E2.0
- E3.0
- E4.5
- E5.0
- E5.5
EARLY MOUSE DEVELOPMENT

- **ZYGOTE**
- **MORULA**
- **BLASTOCYST**
- **EGG CYLINDER**

Development stages:
- **E0.5**
- **E1.5**
- **E2.0**
- **E3.0**
- **E4.5**
- **E5.0**
- **E5.5**
EARLY MOUSE DEVELOPMENT

- ZYGOTE
- OVARY
- OVIDUCT
- MORULA
- BLASTOCYST
- EGG CYLINDER
- E0.5
- E1.5
- E2.0
- E3.0
- E4.5
- E5.0
- E5.5
- FETUS
- PLACENTA
EARLY MOUSE DEVELOPMENT
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- ZYGOTE
- MORULA
- BLASTOCYST
- EGG CYLINDER
- OVIDUCT
- OVARY
- EPIBLAST
- TROPHECTODERM
- FETUS
- PLACENTA

Embryonic stem (ES) cells
CELL TYPES BUILDING THE EMBRYO

<table>
<thead>
<tr>
<th>Cell Type</th>
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<tbody>
<tr>
<td>Stem Cells</td>
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<td>Bone Cells</td>
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<td>Blood Cells</td>
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</table>
SHAPE AND FUNCTION

CELL

TISSUE

ORGAN
SHAPE AND FUNCTION

OOCYTE

EPITHELIAL CELL

ASYMMETRIC

MIGRATORY CELL

SYMMETRIC

NEURON
CELL POLARITY

OOCYTE

NON-POLARIZED

EPITHELIAL CELL

POLARIZED

MIGRATORY CELL

NEURON

Dendrites
Nucleus
Axon
Centrosome
Actin
Microtubules
PAR complex
Crumbs complex
Soluble complex
Basal membrane
Apical membrane
CELL POLARITY

- OOCYTE
  - Non-polarized

- EPITHELIAL CELL
  - Apical / Basal (epithelial) polarity

- MIGRATORY CELL
  - Front / Rear polarity

- NEURON
  - Neuronal polarity
    - Dendrites
    - Axon
    - Nucleus
EPITHELIAL POLARITY
EPITHELIAL POLARITY

- APICAL
- BASAL
- LATERAL
- EXTRACELLULAR MATRIX
  (BASAL LAMINA / BASAL MEMBRANE)
- INTEGRINS
- ADHERENS JUNCTIONS
- TIGHT JUNCTIONS
- PAR complex
- Crumbs complex
LOSS OF EPITHELIAL POLARITY

Lindsay J Talbot et al., Int J Biochem Mol Biol 2012
EMT / MET

Epithelial to Mesenchymal Transition

APICAL / BASAL (EPITHELIAL) POLARITY

EMT

FRONT / REAR POLARITY

MIGRATORY CELL

FRONT

REAR
EMT / MET

Epithelial to Mesenchymal Transition (EMT)

Mesenchymal to Epithelial Transition (MET)
EMT IN EMBRYONIC DEVELOPMENT

To the adherens junctions, producing rapid changes in cell shape in conjunction with RhoGEF2, a Rho GTP-exchange factor and cytoskeletal regulator that concentrates at the site of apical constriction (Kolsch et al., 2007). Snail is also required for ventral furrow formation, the cells of which express string, a cdc25 homolog essential for entry into mitosis. Snail-dependent string inhibition generates the mitotic block necessary for gastrulation to occur (Grosshans and Wieschaus, 2000). Simultaneously, Snail represses **E-cadherin** transcription (Oda et al., 1998) and generates the pulses of myosin contraction required for apical constriction while Twist maintains the constricted state between pulses (Martin et al., 2009). In vertebrates, T48 is not conserved, and Twist is not crucial for gastrulation, suggesting that Snail may fulfill all of these functions.

Gastrulation in Vertebrates

In **Xenopus**, the Spemann organizer is induced by the Nieuwkoop center, a group of dorsal blastula cells characterized by the nuclear accumulation of **β-catenin**. Wnt signaling initiates the process, and Goosecoid is induced in the Spemann organizer by its target, Siamois, and by several transforming growth factor β (TGFβ) superfamily members, including Nodal (Gilbert, 2006). In amniotes, activation of Wnt signaling confers competence to the posterior part of the embryo in the formation of the primitive streak (Figure 2C). Subsequently, members of the TGFβ superfamily, including Nodal and Vg1, induce gastrulation. Nodal signaling, together with **fibroblast growth factor** (FGF), controls the specification of the mesendoderm in all vertebrates (Figure 2C). Thus, in preparation for EMT, numerous signaling pathways help establish an organizing center that in turn controls morphogenetic movements and specification (Heisenberg and Solnica-Krezel, 2008).

There are two main Snail genes in vertebrates, **Snail1** and **Snail2** (called **SNAI1** and **SNAI2** in humans). They are induced by TGFβ superfamily members, and FGF signaling is necessary to maintain their expression and for gastrulation to proceed (Barrallo-Gimeno and Nieto, 2005; Ciruna and Rossant, 2001).

Snail-deficient embryos fail to gastrulate, and “mesodermal” cells are unable to downregulate E-cadherin accumulate at the streak (Carver et al., 2001; Nieto et al., 1994). Snail proteins are not essential for mesodermal fate specification as a “mesodermal” population expressing the appropriate markers still forms in Snail mutant mice, although cells fail to migrate because it cannot undergo EMT (Carver et al., 2001). Furthermore, diploblasts (animals derived from only two germ layers) that do not form mesoderm also express snail in the regions of involution or ingression during endoderm formation. Hence, Snail activity is not associated with...
EMT IN EMBRYONIC DEVELOPMENT

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Figure 1. Successive EMT during Embryonic Development

(A) Primary EMT occurs early during embryonic development, even before implantation such as during the formation of the parietal endoderm in mice. The first EMT after implantation is that undergone by the mesendodermal progenitors during gastrulation, whereas the delamination of neural crest cells from the dorsal neural tube is a later event.

(B) Early mesodermal cells are subdivided into axial, paraxial, intermediate, and lateral plate mesodermal cells that will condense into transient epithelial structures: the notochord, the somites, and the somatopleure and splanchnopleure, respectively. These transient structures will undergo secondary EMT, leading to the generation of mesenchymal cells that differentiate into specific cell types. Endodermal tissues, including the pancreas bud and the liver diverticulum, exhibit morphological changes reminiscent of a secondary EMT to induce the dissociation of endocrine cells and hepatoblasts from their respective epithelial primordia.

(C) An example of tertiary EMT arises during the formation of the cushion mesenchyme in the heart from the atrioventricular canal (AV) or the outflow tract (OT). The cushion mesenchyme is the precursor of the cardiac valves.

EMT IN EMBRYONIC DEVELOPMENT

Thiery et al, Cell, 2009
EMT IN EMBRYONIC DEVELOPMENT

**Figure 1.** The sites of origin, migration, and arrival of cranial neural crest cells. (A) Embryonic neural tube showing the mesencephalon, metencephalon, and rhombomeres, with the dorsal face of tube coloured to show the location of neural crest before migration. (B) Sagittal view of embryo, showing paths of migration of cranial crest cells. (C) Sagittal view of adult human, showing the origins of various cranial crest derivatives.
EMT / MET IN CANCER

**EMT inducers as metastasis promoting agents.** In the derived mesenchymal stem cells. Another challenge is to understand whether 

EMT is now thought to play a fundamental role in tumor progression and 

chemoresistance. Oncogenes and Snail induces immunosuppression, immunoresistance, and 

vates the cellular safeguard mechanism of cellular senescence triggered by 

and differentiate into secondary carcinomas. In addition, Twist also inacti-

favors the self-renewal of a small population of cells that can colonize 

the metastatic potential. Both Twist and Snail confer stem cells properties, 

have been recently described that help to understand their implication in 

cell delamination and invasion of adjacent tissues, new facets of the EMT 

EMT inducers. In addition to promoting tumor dissemination by inducing 

combination of these two possibilities. BV/LV, blood vessels/lymphatic vessels. 

that have undergone EMT to acquire stem cell-like properties (red cells), or some 

primary tumor (blue cells), if they are derived from somatic epithelial tumor cells 

lignant migratory cells are cancer stem cells acting as tumor-initiating cells in the 

cancerous environment or tissue. TGFβ correlates with invasion and metastasis (Figure 6A) (Yang et al., 2008). TGFβ 

correlates with invasion and metastasis (Figure 6A) (Yang et al., 2008). TGFβ 

be associated with the concomitant induction of E47, Zeb fac-

Novoa and Nieto, 2009), although the cellular response may 

in many cellular and tumoral contexts (reviewed in López-

al., 2008). TGFβ and VHL loss is associated with renal clear cell carcinoma 

negatively regulates the hypoxia-inducible factor-1 (HIF-1), 

system, the von Hippel-Lindau (VHL) tumor suppressor also 

the conversion of kidney cells to myo-

tors, and, in particular, Twist, a direct target of HIF1-

assembly or development and in normal and transformed cell lines. The signal-

Many signaling pathways trigger EMT in both embryonic develop-

Complexity in EMT Signaling Pathways

Weinberg, 2008), here, we focus on the recent additions to this 

of recent reviews (e.g., Thiery and Sleeman, 2006; Yang and 

many others (Figure 5). As this has been the topic of a number 

the TGFβ signaling pathways include those triggered by different members of 

opment and in normal and transformed cell lines. The signal-

EMT converge at the induction of the E-cadherin repressors, 

impllications for renal 

ürate this pathway with the cooperation of the early growth 

EMT, including cell scattering and reorganization of cortical 

filament remodeling proteins, ful-

MDCK cells. MRTFs also activate the transcription of actin 

nucleus in a Rho-dependent manner to activate Snail2 in 

scription factors (MRTFs), and it is translocated to the 

Egr-1), which binds directly to the SNAIL1 

response factor-1 (Egr-1), which binds directly to the SNAIL1 

SF usually activates MAPK-independent pathways, it can acti-

tyrosine kinases can induce Snail1 and Snail2. Although HGF/

MAPK pathway activated by stimulation of different receptor 

and in particular, of the 

EMT / MET IN CANCER

Thiery et al , Cell, 2009
EMT / MET IN CANCER

**B**  
**Cancer stem cells**  
**Migratory cancer stem cells**

**PRIMARY TUMOR**  
**EMT**  
Single migratory tumor cells

**METASTASIS**  
**EMT**  
**MET**

**Tumor cell**  
**Cancer-associated fibroblasts**  
**Cancer stem cell**  
**Mesenchymal stem cells**  
**Migratory stem cell**  
**Extracellular matrix**

**BV/LV**

**C**

**Amniotes**

- **Nodal**
- **EGF**
- **Wnt**
- **TGF-β/BMP (Nodal/Vg1)**
- **FGF**

**FATE INDUCTION**

**EMT PROGRAM**

- **Eomes**
- **Snail**
- **Mesp**
- **FLRT3**
- **Net1/RhoA**

- **Gain of endodermal markers**
- **Cytoplasmic/ nuclear β-catenin**
- **Loss of epithelial markers**
- **Gain of mesenchymal markers**
- **Cytoskeletal changes**
- **Basement membrane degradation**
- **Gain of mesodermal markers**

- **Endoderm formation**

- **Loss of cell-cell adhesion and polarity**
- **Changes in cell shape, motility, invasion**

**Mesoderm formation**

**Thiery et al., Cell, 2009**
MET IN SOMATIC CELL REPROGRAMING
MET IN SOMATIC CELL REPROGRAMMING

Diagram showing the process of somatic cell reprogramming:
- Blastocyst → Inner cell mass → ESCs culture → Muscle cells, Blood cells, Neurons, Intestinal cells, Liver cells, Pancreatic Islet cells.
MET IN SOMATIC CELL REPROGRAMING

Adult Fibroblast Cell → MET → Reprogram Cells

- KLF4, SOX2, c-Myc, Nanog, Oct-3/4, LIN-28

Reprogram Cells:
- iPS cells
- Cardiomyocytes
- Adipocytes
- Neural Cells
- Motoneurons
- Hematopoietic Progenitor Cells
- Pancreatic β-Cells
- Dopaminergic Neurons
EPITHELIAL POLARITY DETERMINES CELL FATE
EPITHELIAL POLARITY DETERMINES CELL FATE

EGG CYLINDER

BLASTOCYST

MORULA

OVIDUCT

Ovary

ZYGOTE

EPIBlast

TROPHECTODERM

INSIDE

OUTSIDE

FETUS

PLACENTA
ESTABLISHMENT OF INSIDE AND OUTSIDE CELLS DURING COMPACTION

INSIDE CELLS

OUTSIDE CELLS

8-cell stage 16-cell stage 32-cell stage

ICM

TE
ESTABLISHMENT OF INSIDE AND OUTSIDE CELLS DURING COMPACTION

8-cell stage 16-cell stage 32-cell stage

(Bedzhov et al., Plos Genetics 2012)
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INSIDE CELLS

OUTSIDE CELLS

ICM

TE

8-cell stage

16-cell stage

32-cell stage

(Bedzhov et al., Plos Genetics 2012)
MORULA COMPACTION

INSIDE CELLS

OUTSIDE CELLS

8-cell stage 16-cell stage

(Zenker et al., Cell 2018)
MORULA COMPACTION

Actin rings form after cell division

16-cell embryo

cytokinitic furrow
central spindle

Actin rings expand to cell-cell junctions

cell shape change

Myosin II

Actin rings zipper the embryo for blastocyst expansion

blastocyst cavity

Blastocyst

Mature junctions

Myosin II
E-cadherin
α-catenin
ZO1

(Zenker et al., Cell 2018)
EPITHELIAL POLARITY DETERMINES CELL FATE VIA HIPPO / YAP SIGNALLING

Cockburn et al, 2010
EPITHELIAL POLARITY DETERMINES CELL FATE VIA HIPPO / YAP SIGNALLING

Jung-Soon Mo et al, EMBO Reports 2014

Wicklow et al., Plos Genetic 2014
EPITHELIAL POLARITY DETERMINES THE FIRST CELL FATE DECISION