Gastrulation

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https://reactome.org
Introduction

Reactome is open-source, open access, manually curated and peer-reviewed pathway database. Pathway annotations are authored by expert biologists, in collaboration with Reactome editorial staff and cross-referenced to many bioinformatics databases. A system of evidence tracking ensures that all assertions are backed up by the primary literature. Reactome is used by clinicians, geneticists, genomics researchers, and molecular biologists to interpret the results of high-throughput experimental studies, by bioinformaticians seeking to develop novel algorithms for mining knowledge from genomic studies, and by systems biologists building predictive models of normal and disease variant pathways.

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Literature references


Reactome database release: 82

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Gastrulation is the reorganization of the blastula to form the multilayered gastrula. During gastrulation, a portion of cells from the exterior of the epithelial epiblast layer migrate to the interior, where they form mesoderm and endoderm which, together with the outer layer of ectoderm (arising from epiblast cells that have not migrated), comprise the three cell layers that are characteristic of triploblastic metazoans (reviewed in Technau and Scholz 2003). In the human peri-implantation embryo, epiblast cells ingress through the primitive streak located in the posterior region of the embryonic disc to form the mesoderm and endoderm of the embryo proper (reviewed in Bardot and Hadjantonakis 2020, Ghimire et al. 2021, Zhai et al. 2021, Rossant and Tam 2022). In the mouse, a mammalian model organism, the embryo is cup-shaped instead of being disc-shaped (reviewed in Kojima et al. 2014). However, the morphogenetic process of germ layer formation is broadly conserved in both species.

The primitive streak forms at the posterior region of the epiblast where there is high signaling activity of NODAL, BMP, FGF and WNT pathways, which drive the allocation of cells to the mesoderm and endoderm lineages. In the primitive streak, the ingressing cells undergo epithelial-to-mesenchymal transition (reviewed in Amack 2021). Cells allocated to the mesoderm acquire a mesenchymal phenotype. Endodermal cells are reputed to revert back to an epithelial architecture through a mesenchymal-to-epithelial transition as they are integrated into the pre-existing layer of hypoblast. A recent study in mouse, however, revealed that ingressing cells that are destined for the endoderm undergo an incomplete or partial-EMT and retain some epithelial features prior to re-acquiring epithelialization (Scheibner et al. 2021).

During gastrulation in mice, extraembryonic mesoderm is formed first and is followed by mesoderm that populates the anterior structures, the head, face and heart of the embryo and next the mesoderm to the trunk. Endoderm is also formed in an anterior to posterior sequence, with endoderm emerging early in gastrulation populating the foregut, followed by the mid- and hind-gut. Along the anterior-posterior axis of the primitive streak, endoderm and axial mesoderm emerge from the anterior region, whereas mesoderm emerging from the mid- to posterior regions is allocated in a medial-lateral order to paraxial, intermediate and lateral plate mesoderm. Cells remaining in the overlying epiblast contribute to the ecto-
derm. Cells of the ectoderm are allocated to the neural ectoderm and to the surface ectoderm and the neural border cells that give rise to the neural crest cells. Patterning of the neuroectoderm is facilitated by the inductive interaction with prechordal plate and the notochord derived from the axial mesoderm.

Before ingression, cells of the primitive streak express genes such as TBXT (T, BRACHYURY) and EOMES that are characteristic of nascent mesoderm and endoderm. It is not known if there are common progenitors that give rise to all types of mesodermal derivatives, such as lateral plate mesoderm and paraxial mesoderm. The knowledge to this date indicates that the different types of mesodermal derivatives are allocated in accordance to the timing and locality of emergence from the primitive streak. The existence of bipotential mesendoderm progenitors in the gastrulating embryo is unresolved but unlikely (Probst et al. 2021), though a bipotential cell population may be derived from mouse embryonic stem cells in vitro.

**Literature references**


**Editions**

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Due to ethical considerations, most research on mammalian gastrulation has been performed on mouse embryos. Therefore most of the reactions described in this section are the results of research in mouse embryos. Significant research has also been performed on non-human primates such as cynomolgus monkeys (Macaca fascicularis) (Nakamura et al. 2016, Sasaki et al. 2016). More recently, human gastrula-like cell assemblages ("gastruloids") generated from pluripotent stem cells have been developed (Moris et al. 2020) and are now being compared with mouse embryos (reviewed in Rossant and Tam 2021, Ghimire et al. 2021).

At the beginning of gastrulation in the mouse, the primitive streak forms in a region of BMP, WNT, FGF, and NODAL signaling. In the mouse embryo, NODAL is expressed throughout the epiblast before anterior-posterior axis induction and is required for pluripotency (reviewed in Robertson 2014). NODAL signaling is restricted to the posterior side of the embryo by the secretion of NODAL and WNT antagonists (CER1, LEFTY1) from the anterior visceral endoderm (AVE) (reviewed in Stower and Srinivas 2014). In human embryonic stem cells (hESCs) NODAL is also crucial for maintenance of pluripotency (James et al. 2005, Vallier et al. 2004). In mouse embryos, NODAL and WNT3 are required for formation of the primitive streak (Conlon et al. 1994, Brennan et al. 2001, Liu et al. 1999) and NODAL expression subsequently becomes restricted to the node at the anterior end of the primitive streak (Zhou et al. 1993). Pro-NODAL secreted by the epiblast in response to BMP4 signalling from the extraembryonic ectoderm is converted to mature NODAL by furin (PCSK3) secreted from the extraembryonic ectoderm. NODAL maintains BMP4 expression in the extraembryonic ectoderm which then activates WNT3 in the posterior epiblast. WNT signaling, in turn, amplifies NODAL expression (Brennan et al. 2001). The order of events in this signaling cascade may be different in human embryos due to differences in early embryo architecture.
NODAL, BMP, and WNT show similar effects on human 2D gastruloids (Martyn et al. 2019). Mesoderm and definitive endoderm progenitors appear to be already separate and distinct in the primitive streak, therefore bipotential mesendoderm progenitors may be transitory if they exist (Probst et al. 2021). This is an area of ongoing research.

Mesoderm is formed by an epithelial-mesenchymal transition that produces an ingress of cells through the primitive streak. Endoderm does not show a complete epithelial-mesenchymal transition and instead forms by cell plasticity (a partial epithelial-mesenchymal transition in which both E-cadherin and N-Cadherin are expressed) (inferred from mouse embryos in Scheibner et al. 2021). However, in mouse embryos endoderm progenitors still ingress through the anterior region of the primitive streak, migrate with mesoderm cells, and eventually integrate into the visceral endoderm layer to give rise to the definitive endoderm (Viotti et al. 2014).

Specific types of mesoderm are formed sequentially according to the time and position of ingress of cells through the primitive streak. This patterning is caused by gradients of NODAL, WNT, and BMP signaling that activate transcriptional programs in the mesoderm progenitors.

T-box transcription factor T (TBXT, T, Brachyury) and Eomesodermin (EOMES) are two of the first transcription factors expressed in mesoderm and endoderm progenitors in the primitive streak (reviewed in Probst and Arnold 2016). The two factors combined are required for formation of all mesoderm and endoderm (Arnold et al. 2008, Tosic et al. 2019).

TBXT is activated by WNT signaling (via beta-catenin acting with LEF1 or TCF1) and BMP4 and is expressed in mesodermal and axial mesodermal progenitors and in the primitive streak during gastrulation, later becoming localized to the notochord and tailbud. TBXT is an early marker of mesodermal differentiation and is often used in studies of embryonic stem cells. In hESCs TBXT is expressed in both mesodermal and endodermal progenitors, it regulates different sets of target genes depending on the signaling environment (Faial et al. 2015).

Expression of EOMES is activated by NODAL via SMAD2 and SMAD3 and is observed in the posterior epiblast prior to formation of the primitive streak and in mesoderm and endoderm progenitors during the first day of gastrulation. EOMES in combination with SMAD2,3 is crucial for the activation of definitive endoderm genes (Teo et al. 2011). TBXT and EOMES generally activate expression of mesoderm genes and repress expression of genes associated with pluripotency such as SOX2 and NANOG.

Some transcription factors are particularly important for regulating gastrulation and are also used as markers for particular stages and morphological features. For example, Goosecoid (GSC) expression marks the onset of gastrulation, is first observed in the primitive streak, and becomes localized to the anterior end of the primitive streak and then the axial mesoderm (Blum et al. 1992). SMAD2 and SMAD3 activated by NODAL are recruited to the GSC promoter by FOXH1, which is already located at the promoter. MIXL1 also binds the GSC promoter and activates expression. In mice, GSC is a regulator of head development.

MIXL1 is required for formation of both mesoderm and definitive endoderm (Hart et al. 2002) and is expressed early throughout the primitive streak and in nascent mesoderm cells exiting the streak. Expression of MIXL1 is mediated downstream by NODAL through SMAD2 and SMAD3 binding to the promoter of MIXL1. EOMES also plays a direct role in activating MIXL1 and GSC expression in hESCs (Teo et al. 2011) and in mouse embryos (Tosic et al. 2019).

Developing mesoderm becomes specified by expression of transcription factors such as MESP1, a marker of cardiac progenitors. (See the Reactome pathway "Cardiogenesis").
Literature references


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During the epithelial-mesenchymal transition (EMT) during gastrulation, epithelial cells in the primitive streak transition to dissociated mesenchymal cells, allowing them to leave the epithelial epiblast (reviewed in Francou and Anderson 2020). EMT is induced by FGF, WNT, NODAL, and BMP signaling pathways that are present on the posterior side of the embryo (reviewed in Morgani and Hadjantonakis 2021). The FGF pathway in particular has been implicated in the regulation of EMT during gastrulation (inferred from mouse embryos in Ciruna and Rossant 2001). In later stage cancer cells the TGFbeta signaling pathway is a major inducer of EMT that leads to metastasis (reviewed in Hao et al. 2019). During gastrulation BMP4 and NODAL of the TGFbeta pathways are also probably involved in EMT (Martyn et al. 2018).

This epithelial-mesenchymal transition (EMT) is responsible for formation of mesoderm. An incomplete EMT appears to be responsible for formation of endoderm (inferred from mouse embryos in Viotti et al. 2014, Scheibner et al. 2021). Prospective definitive endoderm cells leave the epiblast layer together with mesoderm cells and eventually integrate and displace the extraembryonic visceral endoderm layer (inferred from mouse embryos in Viotti et al. 2014).

SNAIL (SNAI1), a transcription factor activated in the primitive streak (inferred from the mouse homolog in Carver et al. 2001), participates in crucial events in the EMT that creates mesoderm: the downregulation of cell adhesion proteins E-cadherin (Cadherin-1, CDH1), Occludin (OLCN), and Claudins that results in loss of contact between cells. Instead, cells switch to expression of N-cadherin and mesenchymal gene programs.

Both EOMES and TBXT activate expression of SNAI1 at the primitive streak but not in definitive endoderm progenitors. SNAI1 represses CDH1 expression (reviewed in Bardot et al. 2020), OCLN expression (inferred from mouse homologs in Ikenouchi et al. 2003), and expression of Claudins (inferred from mouse homologs in Ikenouchi et al. 2003). Downregulation of CDH1 also occurs posttranslationally through an incompletely characterized mechanism involving NIK, p38 MAPK, and EBP41L5 (inferred from mouse homologs in Lee et al. 2007, Hirano et al. 2008). SNAI1 but not SNAI2 is required for proper EMT during gastrulation. Other factors required for EMT during gastrulation include p120-catenin,
which regulates WNT signaling and EMT (inferred from mouse homologs in Hernandez-Martinez et al. 2019); Crumbs2, which promotes cell ingression (inferred from mouse homologs in Ramkumar et al. 2016); RhoA and microtubules, which control cell basement interactions (inferred from mouse homologs in Nakaya et al. 2008); and p38 and p38 interacting protein, which are critical for downregulating E-Cadherin (Zohn et al. 2006).

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