Germ layer formation at gastrulation

May, B., Probst, S., Salehin, N., Tam, PPL., Zissel, L.

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This is just an excerpt of a full-length report for this pathway. To access the complete report, please download it at the Reactome Textbook.

18/11/2022
**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

This document contains 1 pathway and 11 reactions (see Table of Contents)

https://reactome.org
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 MESPI, a marker of cardiac progenitors. (See the Reactome pathway "Cardiogenesis").
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**LEF1 or TCF7 (TCF1), CTNNB1, and TBPL2 (TRF3) bind the TBXT (T, Brachyury) gene**

**Location:** Germ layer formation at gastrulation

**Stable identifier:** R-HSA-9754191

**Type:** binding

**Compartments:** nucleoplasm

**Inferred from:** Lef1 or Tcf7 (tcf1) and Ctnnb1 bind the Tbxt (T, Brachyury) gene (Mus musculus)

In the mouse embryo Tbxt (T, Brachyury) expression in the primitive streak is induced by WNT3 and WNT3A. In vitro studies of human embryonic stem cells (ESCs) show CTNNB1 binding to upstream regions of the TBXT (T, Brachyury) gene after WNT activation (Funa et al. 2015). Expression of the mouse homolog of human TBXT is accompanied by LEF1 binding the TBXT promoter and binding of CTNNB1 (beta-catenin) (inferred from mouse homologs). LEF1 and CTNNB1 are downstream effectors of WNT signaling. TCF7 (TCF1) can also perform the same function as LEF1 (inferred from mouse homologs). During mesendodermal differentiation of human ESCs, TBPL2 (TRF3 binds the TBXT promoter (Liang et al. 2020).

**Followed by:** Expression of TBXT (T, Brachyury) in the primitive streak

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Expression of TBXT (T, Brachyury) in the primitive streak

Location: Germ layer formation at gastrulation

Stable identifier: R-HSA-452331

Type: omitted

Compartments: nucleoplasm

Inferred from: Expression of T (Tbxt) in the primitive streak (Mus musculus)

The TBXT (T, Brachyury) gene is transcribed to yield mRNA and the mRNA is translated to yield protein. During early gastrulation, TBXT is expressed in cells in the primitive streak and in mesodermal cells near the primitive streak (inferred from the mouse homolog, inferred from the non-human primate Macaca fascicularis in Nakamura et al. 2016, Sasaki et al. 2016). After node formation, TBXT is expressed in the node and notochord and at later stages in the tailbud region. Expression of TBXT is required for initiating expression of posterior mesoderm gene programs (inferred from mouse homologs). Pluripotency factors are involved in the regulation of factors regulating gastrulation and mesoderm and definitive endoderm cell fate specification. In particular, OCT4 has been suggested to be involved in mesoderm induction and regulation of TBXT (Babaie et al. 2007, Funa et al. 2015, and inferred from mouse homologs). During gastrulation, WNT signaling activates expression of TBXT via LEF1 or TCF7 and beta catenin (CTNNB1) (inferred from mouse homologs). BMP4 also activates TBXT (Lu et al. 2008, Purpura et al. 2008, Zhang et al. 2008). TBPL2 (TRF3) binds the TATA-box during transcription in mesendoderm (Liang et al. 2020).

Preceded by: LEF1 or TCF7 (TCF1), CTNNB1, and TBPL2 (TRF3) bind the TBXT (T, Brachyury) gene

Followed by: EOMES and TBXT bind the NANOG gene, EOMES and TBXT bind the POU5F1 (OCT4) gene, EOMES and TBXT bind the SOX2 gene

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p-2S-SMAD2,3:SMAD4, CTNNB1, and TBPL2 bind FOXH1:SMAD3:EOMES gene

Location: Germ layer formation at gastrulation

Stable identifier: R-HSA-9754480

Type: binding

Compartments: nucleoplasm

Inferred from: p-2S-Smad2,3:Smad4 bind Foxh1:Smad3:Eomes gene (Mus musculus)

NODAL-SMAD2,3 signaling is crucial for induction of EOMES expression, as EOMES is absent in NODAL-deficient mouse embryos. NODAL signaling causes phosphorylation of SMAD2 which transits to the nucleus with SMAD4 and binds and activates expression of the EOMES gene. Beta-catenin (CTNNB1) activated by Wnt signaling binds the EOMES gene and interacts directly with SMAD2,3 to yield full activation of EOMES (Funa et al. 2015). The pluripotency factors OCT4 and NANOG have also been implicated in activation of EOMES expression (Teo et al. 2011, Funa et al. 2015). In mesendoderm cells derived from human embryonic stem cells, the basal transcription factor TBPL2 (TRF3) binds the EOMES gene (Liang et al. 2020). In mouse embryonic stem cells, FOXH1 and SMAD3 are pre-bound to the EOMES gene (inferred from mouse homologs in mesendoderm progenitors generated from embryonic stem cells in vitro).

Followed by: Expression of EOMES in the primitive streak

Literature references


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Expression of EOMES in the primitive streak

Location: Germ layer formation at gastrulation

Stable identifier: R-HSA-452353

Type: omitted

Compartments: nucleoplasm

Inferred from: Expression of Eomes in the primitive streak (Mus musculus)

The Eomesodermin (EOMES) gene is transcribed to yield mRNA and the mRNA is translated to yield protein. EOMES is required for formation of mesoderm. In embryonic stem cells, pluripotency factors POU5F1 (OCT4), SOX2, and NANOG bind the promoter of the EOMES gene (Boyer et al. 2005). OCT4 and SOX2 repress EOMES transcription (Babaie et al. 2007, Teo et al. 2011) while NANOG positively regulates EOMES expression (Teo et al. 2011). During gastrulation EOMES is expressed in the posterior epiblast, in the primitive streak, and in mesoderm and definitive endoderm progenitors (inferred from the mouse homolog, inferred from the non-human primate Macaca fascicularis in Nakamura et al. 2016, Sasaki et al. 2016). EOMES-expressing cells are present in anterior mesoderm derivatives and are excluded from posterior mesoderm derivatives (inferred from the mouse homolog). Expression of EOMES is seen in human embryonic stem cells (Ginis et al. 2004 Tsankov et al. 2015).

Preceded by: p-2S-SMAD2,3:SMAD4, CTNNB1, and TBPL2 bind FOXH1:SMAD3:EOMES gene

Followed by: EOMES and TBXT bind the NANOG gene, EOMES and TBXT bind the POU5F1 (OCT4) gene, EOMES and TBXT bind the SOX2 gene

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EOMES and TBXT bind the NANOG gene

**Location:** Germ layer formation at gastrulation

**Stable identifier:** R-HSA-9756671

**Type:** binding

**Compartments:** nucleoplasm

**Inferred from:** Eomes and Tbxt bind the Nanog gene (Mus musculus)

EOMES and TBXT bind and repress the expression of NANOG (inferred from mouse homologs).

**Preceded by:** Expression of TBXT (T, Brachyury) in the primitive streak, Expression of EOMES in the primitive streak

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EOMES and TBXT bind the POU5F1 (OCT4) gene

**Location:** Germ layer formation at gastrulation

**Stable identifier:** R-HSA-9756670

**Type:** binding

**Compartments:** nucleoplasm

**Inferred from:** Eomes and Tbxt bind the Pou5f1 (Oct4) gene (Mus musculus)

EOMES and TBXT bind and repress the expression of POU5F1 (OCT4) (inferred from mouse homologs).

**Preceded by:** Expression of TBXT (T, Brachyury) in the primitive streak, Expression of EOMES in the primitive streak

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EOMES and TBXT bind the SOX2 gene

Location: Germ layer formation at gastrulation

Stable identifier: R-HSA-9756661

Type: binding

Compartments: nucleoplasm

Inferred from: Eomes and Tbxt bind the Sox2 gene (Mus musculus)

EOMES and TBXT bind and repress the expression of SOX2 (inferred from mouse homologs).

Preceded by: Expression of TBXT (T, Brachyury) in the primitive streak, Expression of EOMES in the primitive streak

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p-2S-SMAD2,3:SMAD4, p-2S-SMAD2,3:TRIM33, CTNNB1, EOMES, and TBPL2 bind FOXH1:SMAD3:GSC gene

**Location:** Germ layer formation at gastrulation

**Stable identifier:** R-HSA-9756948

**Type:** binding

**Compartments:** nucleoplasm

**Inferred from:** p-2S-Smad2,3:Smad4 binds Foxh1:Smad3:Gsc gene (Mus musculus)

In mesendoderm progenitors differentiated from embryonic stem cells in vitro, FOXH1 and SMAD3 are pre-bound to the GSC gene (inferred from mouse homologs). The same situation is inferred to occur in cells of the primitive streak. NODAL signaling causes phosphorylation of SMAD2 and SMAD3, which transit to the nucleus with SMAD4. SMAD2 interacts with SMAD3 at the promoter of the GSC gene (Martín-Malpartida et al. 2017, Funa et al. 2015, and inferred from mouse homologs). After activation by NODAL, phosphorylated SMAD2 and SMAD3 are also complexed with TRIM33 in the nucleus and the p-2S-SMAD2,3:TRIM33 complex binds methylated histone H3 lysine-9 and acetylated histone H3 lysine-18 at the promoter of GSC, which may open chromatin at the promoter (inferred from mouse homologs). Beta-catenin (CTNNB1) activated by Wnt signaling also binds the GSC gene and interacts directly with SMADs to yield full activation of GSC (Funa et al. 2015). EOMES also binds regulatory regions of the GSC gene and is crucial for its activation (Teo et al. 2011, and inferred from mouse homologs). The basal transcription factor TBPL2 (TRF3) also binds the promoter of the GSC gene (Liang et al. 2020).

**Followed by:** Expression of Goosecoid (GSC) in the primitive streak

**Literature references**


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The Goosecoid (GSC) gene is transcribed to yield mRNA and the mRNA is translated to yield GSC protein. Expression of GSC is first observed in the region of the epiblast where the primitive streak forms (inferred from the mouse homolog). Expression then becomes restricted to the anterior primitive streak and the anterior midline mesoderm. Phosphorylated SMAD2 and SMAD3 in complexes with SMAD4 and TRIM33 activate transcription of GSC (inferred from mouse homologs). Beta-catenin (CTNNB1) also binds the GSC promoter and interacts with SMAD2 and SMAD3 to enhance transcription of GSC (Funa et al. 2015). GSC can bind its own promoter and downregulate expression (Danilov et al. 1998). POU5F1 (OCT4), SOX2, and NANOG bind the promoter of the GSC gene (Boyer et al. 2005), OCT4 and SOX2 repress transcription of GSC, and NANOG activates transcription of GSC in human embryonic stem cells (Babaie et al. 2007, Teo et al. 2011).

**Preceded by:** p-2S-SMAD2,3:SMAD4, p-2S-SMAD2,3:TRIM33, CTNNB1, EOMES, and TBPL2 bind FOXH1:SMAD3:GSC gene

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p-2S-SMAD2,3:TRIM33, p-2S-SMAD2,3:SMAD4, CTNNB1, EOMES, and TBPL2 bind FOXH1:MIXL1 gene

**Location:** Germ layer formation at gastrulation

**Stable identifier:** R-HSA-9756724

**Type:** binding

**Compartments:** nucleoplasm

**Inferred from:** p-2S-Smad2,3:Trim33 and p-2S-Smad2,3LSmad4 bind Foxh1:Mixl1 gene (Mus musculus)

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In differentiating human embryonic stem cells (ESCs), beta-catenin (CTNNB1):LEF1, SMAD2,3, and OCT4 bind to an upstream enhancer region (Estarás 2015, Funa 2015) and both SMAD2 and FOXH1 bind to the immediate promoter region of MIXL1 to mediate MIXL1 expression driven by WNT and NODAL signaling. CTNNB1 from WNT signaling binds the promoter and interacts directly with SMAD proteins (Funa et al. 2015). Knockdown of CTNNB1, SMAD2, or OCT4 results in reduced MIXL1 expression (Funa et al. 2015). EOMES binds the enhancer and promoter regions of the MIXL1 gene and is important for transcriptional activation (Teo et al. 2011, and inferred from mouse embryos). The basal transcription factor TBPL2 (TRF3) also binds the promoter of the MIXL1 gene (Liang et al. 2020). NODAL causes phosphorylation of SMAD2 and SMAD3 which then complex with SMAD4 and transit to the nucleus. Phosphorylated SMAD2 and SMAD3 are also present in complexes with TRIM33 in the nucleus (inferred from mouse homologs). SMAD2,3:TRIM33 complexes bind methylated histone H3 lysine-9 and acetylated histone H3 lysine-18 to open chromatin at the MIXL1 promoter (inferred from mouse homologs). SMAD2,3:SMAD4 also binds the MIXL1 promoter (inferred from mouse homologs).

**Followed by:** Expression of MIXL1 in the primitive streak

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Expression of MIXL1 in the primitive streak

Location: Germ layer formation at gastrulation

Stable identifier: R-HSA-9758501

Type: omitted

Compartments: nucleoplasm

Inferred from: Expression of Mixl1 in the primitive streak (Mus musculus)

During gastrulation, MIXL1 is expressed throughout the primitive streak and the nascent mesoderm, but then becomes localized to the anterior and posterior ends of the streak (inferred from the mouse homolog in mouse embryos, inferred from the non-human primate Macaca fascicularis in Nakamura et al. 2016, Sasaki et al. 2016). Transcription of MIXL1 is activated by NODAL via SMAD2,3, EOMES, and by WNT via beta-catenin (CTNNB1) (Teo et al. 2011, Funa et al. 2015, and inferred from mouse homologs).

Preceded by: p-2S-SMAD2,3:TRIM33, p-2S-SMAD2,3:SMAD4, CTNNB1, EOMES, and TBPL2 bind FOXH1:MIXL1 gene

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