

Making a commitment: cell lineage allocation and axis patterning in the early mouse embryo

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Abstract | Genetic studies have identified the key signalling pathways and developmentally regulated transcription factors that govern cell lineage allocation and axis patterning in the early mammalian embryo. Recent advances have uncovered details of the molecular circuits that tightly control cell growth and differentiation in the mammalian embryo from the blastocyst stage, through the establishment of initial anterior–posterior polarity, to gastrulation, when the germ cells are set aside and the three primary germ layers are specified. Relevant studies in lower vertebrates indicate the conservation and divergence of regulatory mechanisms for cell lineage allocation and axis patterning.

Gastrulation

The embryonic process during which the three germ layers of the embryo are specified.

Blastocyst

The spherical embryo at the time of implantation. The blastocyst consists of the primary tissue types: trophoblast, epiblast and the primitive endoderm.

Blastomere

The cell type of the early embryo that is generated by cleavage of the zygote.

Early post-fertilization embryos of different vertebrate species show considerable variation in form, size and timing of development. These can be understood as the result of evolutionary adaptation to environmental requirements for rapid extrauterine (such as frog and fish), intrauterine (mouse) or *in ovo* (chicken) development. Despite initial gross architectural differences of early embryos, the basic signalling pathways that control cell lineage allocation and axis patterning, such as the Wnt, transforming growth factor- β (TGF β) and fibroblast growth factor (FGF) pathways, are conserved. Variations in embryonic geometry and/or tissue morphogenesis can account for differences in susceptibility to experimental disturbances of these pathways that lead to phenotypes.

Vertebrate development has been extensively studied in frogs and chicks because these embryos are readily accessible, easy to visualize and amenable to direct manipulation in tissue transplantation or extirpation experiments. Although the mouse embryo is less accessible because it develops in the maternal uterine environment, it offers the advantage that gene-expression patterns can be manipulated readily using transgenic and embryonic stem (ES) cell technology. More recently, ES cell differentiation protocols were developed that allow for detailed molecular studies of signalling processes that guide cell fate allocation during development¹.

Mammalian development is characterized by slow progression prior to implantation in the uterine wall. For example, frog embryos complete approximately

12 rounds of cell division within 10 hours of fertilization (depending on the temperature) to generate around 8,000–10,000 cells before gastrulation. By contrast, the onset of gastrulation in mouse embryos occurs at embryonic day 6 (E6) after approximately one-third of the 19–20 day gestation period. This delay in development reflects the small size of the egg, and the length of time that is required to generate extraembryonic tissues, which mediate implantation and subsequently support and protect the embryo. Importantly, extraembryonic cell tissues also have an important instructive role in patterning the emerging body axis and specification of the germ line.

This Review summarizes our understanding of the signalling pathways and transcriptional networks that control reciprocal interactions between the embryonic and extraembryonic lineages that establish the basic body plan of the early mouse embryo. Understanding the molecular mechanisms of early patterning and cell fate allocation will be of key importance in efforts towards inducing the directed differentiation of cells in research and medicine.

The cell lineages of the blastocyst

The mouse embryo develops from fertilization to implantation into the spherical structure of the blastocyst, which contains three distinct tissue types. These develop through successive steps of transcription factor-regulated cell fate specification. After three rounds of cell division, the eight blastomeres that are present in the mouse zygote show no overt morphological differences.

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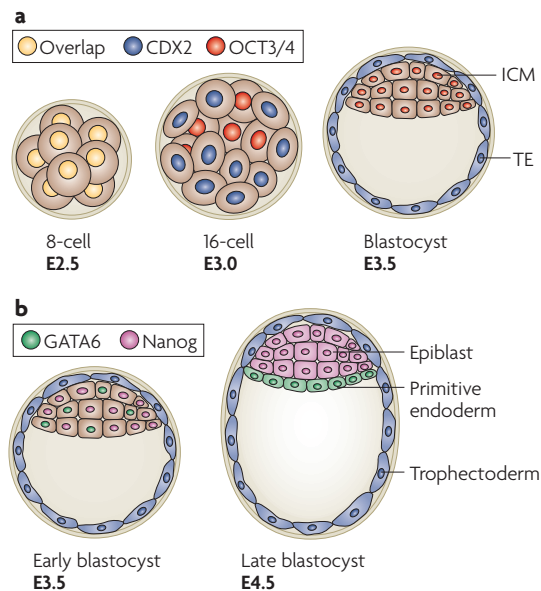


Figure 1 | Lineage segregation in the blastocyst.

The primary tissue types of the mouse embryo — trophoblast, epiblast and primitive endoderm — are established before implantation at around embryonic day 4.5 (E4.5). **a** | At E2.5, the eight blastomeres initially show overlapping expression of two transcription factors, caudal-type homeobox protein 2 (CDX2) and octamer-binding transcription factor 3/4 (OCT3/4; also known as POU5F1), both of which are instructive for the first binary cell fate decision to form trophoblast (TE) or inner cell mass (ICM), respectively. The next round of cell divisions generates larger outer and smaller inner cells. Reciprocal negative regulation leads to the exclusive expression of CDX2 in outer blastomeres and OCT3/4 in inner blastomeres, thereby specifying the cells as TE and ICM, respectively. **b** | The primitive endoderm and the epiblast lineages segregate from the ICM at the blastocyst stage. At E3.5, the ICM shows mosaic and random ‘salt and pepper’ expression of the transcription factors nanog and GATA-binding factor 6 (GATA6). GATA6-positive cells are subsequently sorted to the distal surface of the ICM, where they give rise to the primitive endoderm. Nanog-positive cells exclusively give rise to the pluripotent epiblast, the founder tissue of the embryo proper.

Inner cell mass

Pluripotent tissue inside the blastocyst that gives rise to the embryo proper and yolk sac tissue.

Trophectoderm

An extraembryonic, outside tissue layer of the early embryo that connects the embryo to the uterus and forms the placenta.

Pluripotency

The ability of a stem cell to give rise to many different cell types.

Primitive endoderm

Extraembryonic tissue that initially covers the epiblast surface and later gives rise to the yolk sac tissue.

Epiblast

The founding tissue of the embryo proper that gives rise to all fetal tissues.

Asymmetrical cell divisions at the morula stage along a basolateral cleavage plane generate two visibly distinct subpopulations²: the smaller inner cells that will comprise the so-called inner cell mass (ICM) and larger polarized outer cells that will become allocated to the trophoblast (TE) lineage.

Two transcription factors, octamer-binding transcription factor 3/4 (OCT3/4; also known as POU5F1) and caudal-type homeobox protein 2 (CDX2), mediate this binary cell fate decision³. OCT3/4 and CDX2 are initially co-expressed throughout all cells of the compacted morula³ and subsequently establish a mutually exclusive expression pattern (FIG. 1a). CDX2 expression is slightly enhanced in the outer cells by a mechanism that might depend on asymmetrical cell divisions of the morula^{4,5}. Subsequently, levels of CDX2 increase through a positive autoregulatory feedback mechanism that leads

directly to the termination of OCT3/4 expression in the same cells. Conversely, OCT3/4 that is expressed in the inner cells represses *Cdx2* transcription³. CDX2 expression is essential for the expansion of the TE lineage, whereas OCT3/4 maintains pluripotency in the ICM³. A second transcription factor, TEA-domain family member 4 (TEAD4), is also required for specification of the TE lineage. Embryos of *Tead4*-mutants fail to initiate CDX2 expression, and all cells adopt an ICM fate⁶.

Coincident with segregation of the TE and the ICM, the embryo cavitates to form the blastocyst. Over the next few hours, the outermost layer of cells that overlies the ICM forms the primitive endoderm (PE). Gene expression and lineage-tracing experiments have shown that the early ICM already contains distinct subpopulations of cells. These selectively express the transcription factors *nanog* (a homeobox transcription factor) or GATA-binding factor 6 (GATA6) in a position-independent, random and mosaic ‘salt and pepper’ pattern⁷, and are committed to become either epiblast or PE, respectively (FIG. 1b). Live-cell imaging experiments suggest that these lineage-restricted expression patterns are established by the 64-cell stage⁸. *Nanog*^{9,10}, together with *Sal-like 4* (*SALL4*)¹¹, maintains pluripotency in epiblast progenitors. GATA6 drives endoderm differentiation, and forced expression of GATA6 in cultured ES cells is sufficient to promote differentiation to the PE lineage¹². GATA6 expression depends on growth-factor-receptor-bound protein 2 (*GRB2*), a mediator of receptor Tyrosine kinase–Ras–mitogen-activated protein kinase (MAPK) signalling. Embryos that lack *GRB2* (REFS 7, 13), *FGF4* or *FGF receptor 2* (*FGFR2*) — the upstream components of the signalling pathway — fail to express GATA6 and cannot form PE^{14–16}. It is unknown whether mutually exclusive expression of GATA6 and *nanog* is regulated by a reciprocal feedback mechanism, as is the case for CDX2 and OCT3/4.

The cell-sorting process that controls segregation of the endoderm to the free surface of the ICM remains poorly understood, but differences in cell movements and adhesion properties between ICM and PE cells, in combination with selective apoptosis, are thought to be involved⁸. Live embryo imaging using lineage-specific markers suggests that the bias of cells in the ICM towards epiblast or PE fates, which are initiated by *nanog* and GATA6, respectively, requires additional reinforcement by position-dependent mechanisms to manifest cell lineage choices. When cells that are committed to the PE do not reach their appropriate destination at the surface of the ICM, they are forced to undergo apoptosis⁸. Low-density lipoprotein receptor-related protein 2 (*LRP2*; also known as megalin and GP330), LRP-associated protein 1 (*LRPAP1*; a LRP2 chaperone) and disabled homologue 2 (*DAB2*; a LRP adaptor protein) are selectively activated in the early PE and also have essential roles in this tissue. However, their mode of action needs further clarification¹⁷. Gene targeting experiments have also identified numerous molecules (such as hepatocyte nuclear factor 4A (HNF4A), laminin C1, $\beta 1$ integrin and maspin) that are required in the PE at later stages⁷.

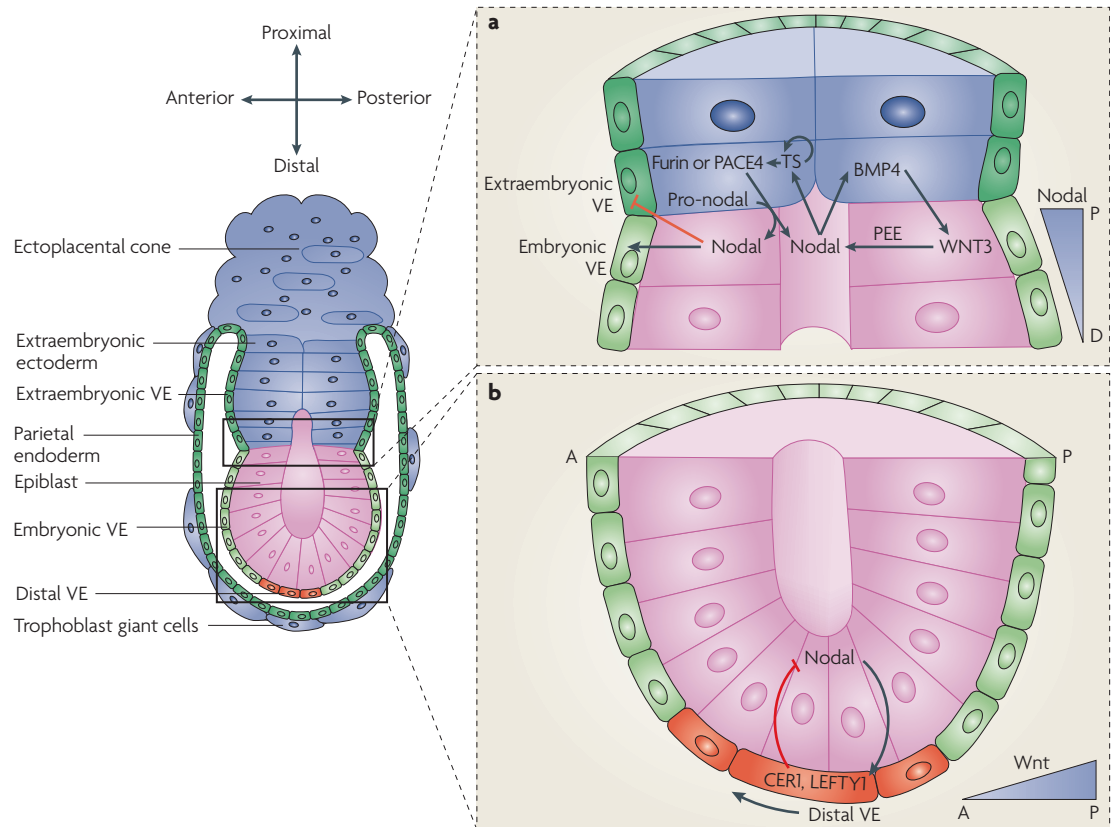


Figure 2 | The proximo–distal axis of the pre-gastrulation embryo is established through reciprocal tissue interactions. **a** | In the embryonic day 5.5 (E5.5) embryo, a gradient of nodal signalling levels preconfigures the proximal–distal axis. Two independent feedback loops enhance the strength of nodal signalling at the proximal epiblast. Nodal becomes activated through prodomain cleavage by the secreted proprotein-convertases furin (also known as PCSK3) and SPC4 (also known as PCSK6 and PACE4) at the interface of the extraembryonic ectoderm (including trophoblast stem (TS) cells) and epiblast. Nodal produced by the epiblast also upregulates the levels of bone morphogenetic protein 4 (BMP4) in the extraembryonic ectoderm, which in turn signals back to the epiblast to enhance WNT3 expression. The nodal proximal epiblast enhancer (PEE) is a direct target of the canonical Wnt- β -catenin pathway. The visceral endoderm (VE) acquires a distinctive regional pattern that is dependent on local signals from the underlying extraembryonic ectoderm or the epiblast. **b** | A few cells at the distal tip of the pre-gastrula embryo become specified as distal VE cells (red) and initiate the expression of the extracellular nodal and Wnt-signalling inhibitors cerberus-like-1 (CER1) and left–right determination factor 1 (LEFTY1), which attenuate nodal signalling in the adjacent epiblast to contribute to the formation of a proximal–distal gradient of nodal signalling.

The late blastocyst stage embryo contains three distinct lineage-restricted subpopulations (FIG. 1). The TE mediates implantation and then expands to form progenitors of the placenta — namely, the extraembryonic ectoderm (ExE) and the ectoplacental cone. The PE diversifies and gives rise to the parietal endoderm, which migrates from the surface of the ICM and directly contacts the maternal tissue, and the visceral endoderm (VE), which remains in contact with the embryo and expands along the surface of the ExE and epiblast, giving rise to the endoderm of the visceral yolk sac. Finally, the early epiblast retains pluripotency and gives rise to both the somatic tissues and the germ cell lineage of the embryo proper.

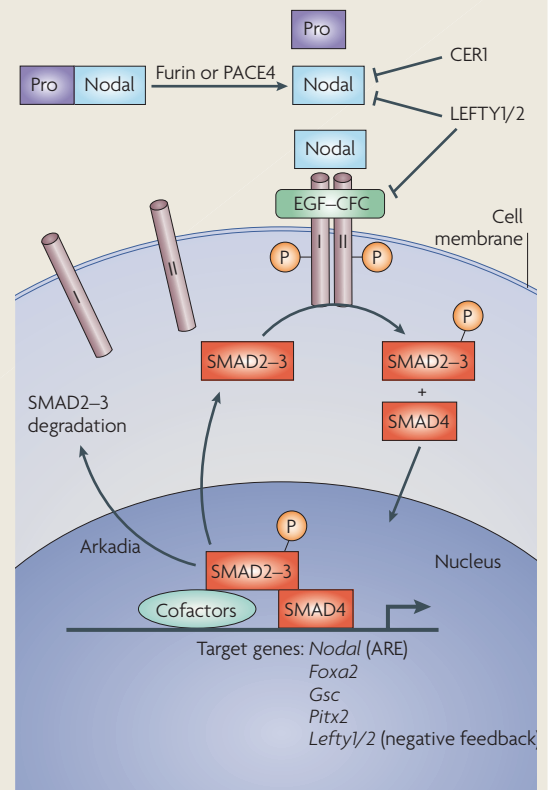
Early molecular asymmetries

Establishing the proximal–distal axis. Shortly after implantation, a cavity forms in the centre of the epiblast and the conceptus elongates along the proximal–distal (P–D) axis to form the ‘egg cylinder’ stage embryo

(FIG. 2). The ExE forms a discrete cup-shaped layer of epithelial cells at the proximal aspect of the embryo, directly juxtaposed to the distally positioned epiblast cells. The VE forms a continuous cell monolayer that overlies both the ExE and the epiblast. Reciprocal signalling between these three cell populations by secreted growth factors of the TGF β family, including *nodal* (BOX 1) and bone morphogenetic protein (BMP), and the Wnt (BOX 2) and FGF families, leads to regionalized gene-expression patterns in the epiblast and the ExE and VE tissues. The first signs of tissue regionalization are seen as differences in marker gene expression along the P–D axis of the embryo. Soon afterwards, the radial symmetry is broken and marker genes indicate anterior and posterior tissue identities. Setting up the embryonic axes can be regarded as the starting point of embryonic pattern formation and is required for all successive steps of embryogenesis, including cell lineage allocation and tissue differentiation.

Box 1 | Fine-tuning the nodal-SMAD signalling pathway

Nodal precursors are cleaved to generate the carboxy-terminal ligand by the subtilisin-like proprotein convertases furin (also known as PCSK3) and SPC4 (also known as PCSK6 and PACE4). The nodal proprotein functions as a partial agonist by binding to its receptors in a co-receptor (epidermal growth factor (EGF)-CFC)-independent manner. The extracellular inhibitors cerberus-like 1 (CER1) and left-right determination factor 1 (LEFTY1) or LEFTY2 directly bind to nodal ligand or receptor complexes, respectively. Mature nodal, together with its co-receptor cripto (the founding member of the EGF-CFC family), activates the type I-II receptor complexes, which phosphorylate the downstream effectors SMAD2 and SMAD3. Activated receptor SMADs associate with the co-SMAD, SMAD4, and are translocated to the nucleus to regulate target gene expression. Additional transcription factors, including the winged helix factors FOXH1 and FOXA2, or homeodomain proteins, such as MIX/BIX family members, function cooperatively to target downstream genes. Nodal upregulates its own expression through a SMAD-FOXH1-dependent autoregulatory enhancer, but also activates a negative-feedback circuit by inducing the expression of its antagonists *Lefty1* and *Lefty2* to attenuate nodal signalling. Activated phosphorylated SMAD2 and SMAD3 are subject to ubiquitylation by the E3 ubiquitin ligase arkadia, marking them for degradation by the proteasome. Efficient SMAD2 and SMAD3 turnover is required for enhancing the maximal signalling levels of these proteins^{144,145}. ARE, the intronic nodal enhancer; *Gsc*, goosecooid; *Pitx2*, pituitary homeobox 2.



Starting at E5, nodal signals induce P-D patterning in the epiblast. Nodal that is initially expressed throughout the epiblast¹⁸ activates its intracellular effector *SMAD2* in the overlying VE¹⁹. Phosphorylated SMAD2 complexes are the key component of the transcriptional network that is required for formation of the distal VE (DVE), a specialized signalling centre (FIG. 2). Nodal-SMAD2 signals induce expression of the transcription factors forkhead box A2 (*FOXA2*) and LIM homeobox protein 1 (*LHX1*), which, together with SMAD2, govern the production of extracellular antagonists of Wnt and nodal signalling²⁰, including Dickkopf homologue 1 (*DKK1*), cerberus-like protein 1 (CER1) and left-right determination factor 1 (LEFTY1). The DVE inhibits nodal and Wnt signalling in the overlying epiblast, it restricts target gene activation to the most proximal region and it maintains the anterior character of the distal epiblast cells. Functional loss of SMAD2 leads to failure to induce nodal antagonists²¹, and as a consequence unabated nodal signalling throughout the epiblast causes ectopic activation of proximal genes, including *Wnt3*, *Brachyury* (also known as T protein) and *Fgf8* (REFS 21,22).

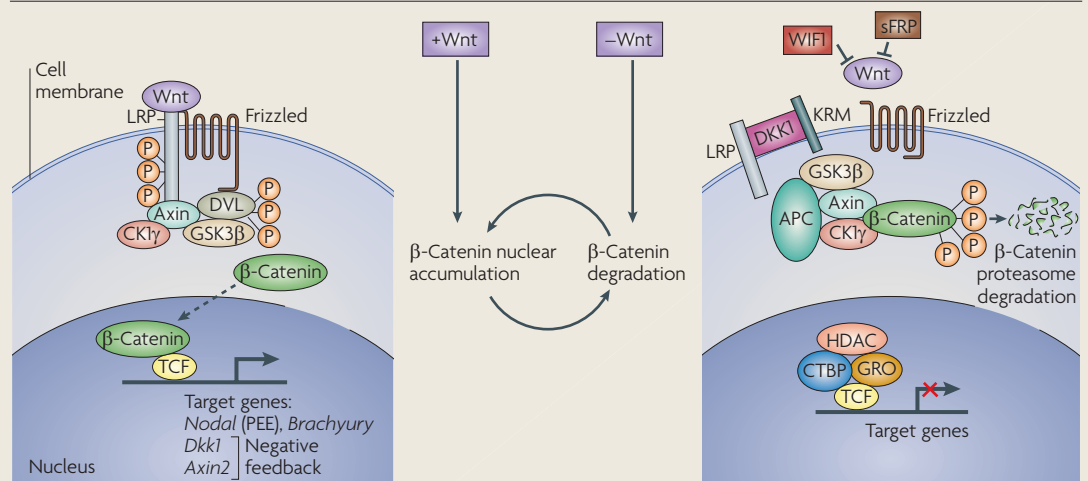
Canonical Wnt-β-catenin signalling is also required for maintenance of the P-D axis. Loss of adenomatous polyposis coli (APC), a negative regulator of Wnt-β-catenin leads to constitutive activation of the pathway and failure to form the DVE²³. By contrast, the DVE is induced in β-catenin mutants, as judged by expression of LHX1 and CER1. However, other DVE markers, such as HEX, fail to be activated²⁴. The nodal-SMAD2 and Wnt-β-catenin

pathways thus activate discrete target genes in the DVE that generate P-D polarity in the epiblast.

Besides its role in establishing the DVE, nodal signalling also regulates interactions with the proximal ExE¹⁹. In collaboration with FGF4, which is secreted by the epiblast, nodal maintains the pool of trophoblast progenitors²⁵. In turn, signals from the ExE tissue, including BMPs, are required to pattern the proximal epiblast and the adjacent VE. Physical removal of the extraembryonic region of the E5.5 embryo results in expansion of DVE markers throughout the VE and the loss of proximal epiblast marker gene expression²⁶. Similarly, loss of the ETS (erythroblast transformation specific) transcription factors ELF5 or ETS2 results in failure to maintain the ExE, and mutants exhibit epiblast and VE patterning defects^{27,28}. Unidentified inhibitory signals from the ExE prevent activation of DVE markers in the proximal VE, thereby restricting DVE induction to the distal tip^{26,29} (FIG. 2). Interactions of the VE with the ExE are also required for the localized expression of *GATA4*, *HNF4A* and α-fetoprotein (AFP), which are normally confined to the proximal VE²⁹.

Conversion to anterior-posterior polarity. The rapid and directed migration of the DVE to the prospective anterior side of the embryo at E6.0 establishes the anterior-posterior (A-P) axis of the embryo³⁰⁻³². The gross movement of the DVE to form the anterior VE (AVE), breaks radial symmetry by repositioning the source of nodal and Wnt antagonists.

Box 2 | The canonical Wnt-β-catenin pathway



When Wnt ligands bind to frizzled receptors (see the figure, left panel), the low-density-lipoprotein receptor-related protein (LRP) co-receptors are phosphorylated by casein kinase 1 γ (CK1 γ) and glycogen synthase kinase 3 β (GSK3 β). Dishevelled (DVL) and axin proteins are recruited via interactions with frizzled and LRPs, thereby preventing the formation of a β -catenin phosphorylating ‘destruction complex’. β -Catenin is found in the cytoplasm and translocates to the nucleus. Nuclear β -catenin interacts with the T-cell-specific factor/lymphoid enhancer-binding factor (TCF/LEF) transcription factors to regulate target gene transcription. In the absence of Wnt ligands, the presence of secreted inhibitors (Wnt inhibitory factor 1 (WIF1) and soluble frizzled-related proteins (sFRPs)) or the inhibition of LRP co-receptors (by secreted Dickkopf protein (DKK1)), the active β -catenin destruction complex, which contains the core components axin, adenomatous polyposis coli (APC) and GSK3 β , recruits and phosphorylates β -catenin (see the figure, right panel). Phosphorylated β -catenin is rapidly ubiquitinated by β -transducin repeat-containing protein (β TRCP) and degraded by proteasomes. The nucleus becomes depleted of β -catenin and TCF-mediated complexes silence Wnt target genes via transcriptional repressors, such as groucho (GRO) and carboxy-terminal binding protein (CTBP), that recruit histone deacetylases (HDACs)⁴⁶. Krm, kremen; PEE, proximal epiblast enhancer.

Nodal signalling has an important role in driving DVE migration, and reducing the level of *Nodal* transcription prevents DVE migration^{18,33}. The DVE also remains distal in embryos that lack the nodal co-receptor *cripto* (also known as teratocarcinoma-derived growth factor precursor (TDGF1)), which is required for maximal pathway activity in the epiblast³⁴. Orthodenticle homologue 2 (OTX2), a downstream target of SMAD2-induced FOXA2 complexes that are formed in the VE, is also essential for axis rotation^{35–37}. One model proposes that enhanced cell proliferation in response to the bias in nodal signalling on the prospective posterior side passively ‘pushes’ DVE cells towards the anterior³⁸. However, real-time imaging using a HEX–green fluorescent protein (GFP) reporter allele has revealed localized filopodia projections on the surface of HEX–GFP-positive actively migrating DVE cells³⁹. Loss of NAP1 (also known as NCKAP1), a regulatory component of the WAVE–WASP1 (Wiskott–Aldrich syndrome protein family member 1) complex that is required for the reorganization of the actin cytoskeleton and the formation of filopodia, results in DVE migration defects⁴⁰. Thus, rapid DVE cell movement is also likely to be mediated by active mechanisms of migration. These might be directed by Wnt signalling as a repulsive cue at the posterior pole and by the Wnt inhibitor DKK1 acting as an attractive signal on the anterior⁴¹. Despite the evidence for molecular asymmetries in the expression of several components of the Wnt and nodal signalling cascades,

the identity of the earliest determinants of axis polarity are currently under debate⁴².

Relocation of the DVE to the anterior side of the embryo establishes an A–P gradient of nodal and Wnt signals in the epiblast. The antagonists secreted by the AVE block signalling and impart neurectodermal character, whereas signals on the prospective posterior side of the embryo instruct cells to acquire mesodermal and endodermal fates.

Forming the embryonic germ layers

At around E6.0, the embryo is prepatterned by regional differences in gene expression. The molecular pattern is followed by gross changes in morphology as the three embryonic germ layers are generated during gastrulation. Ectoderm, mesoderm and definitive endoderm (DE) constitute the progenitor cells from which all fetal tissues will develop. What are the mechanisms that lead to gastrulation and how are the germ layers specified?

Initiation of primitive streak formation. Although molecular evidence for A–P axis formation becomes evident at around E6.0, the mouse embryo remains morphologically radially symmetrical until the onset of gastrulation. Extensive cell mixing is known to occur in the epiblast before gastrulation⁴³. By contrast, cells in the extraembryonic tissues are rather static and form coherent clonal patches⁴⁴. At around E6.0, epiblast cells begin to converge towards the posterior proximal pole of the

Ectoderm

The founding germ layer of neural tissues, neural crest and skin.

Mesoderm

The middle sheet of mesenchymal cells that forms blood and vasculature, muscle, bone, cartilage and connective tissues. Mesoderm contributes to many cell types of internal organs.

Definitive endoderm

The outside tissue layer that gives rise to the gut tube and associated organs, such as the lungs, liver, pancreas and the intestinal tract.

embryo to form the primitive streak⁴⁵. Chimaera experiments reveal that nodal-deficient cells preferentially contribute to the anterior compartment of the embryo⁴⁶. Similarly, the ability to contribute to posterior tissues is compromised in cells that lack the nodal receptor ALK4 (also known as ACVR1B)⁴⁷. In zebrafish, cells that lack both of the nodal homologues cyclops and squint tend to disperse rather than cohere⁴⁸. Nodal activities thus seem to govern cell-sorting behaviours in the proximal epiblast that direct cells towards the site of primitive streak formation.

Nascent mesoderm is formed when epiblast cells that are ingressing at the primitive streak undergo an epithelial–mesenchymal transition (EMT) and subsequently emerge to form a new cell layer between the epiblast and the overlying VE. BMP signals from the ExE, as well as nodal–SMAD2 and SMAD3 and canonical Wnt signals in the epiblast, are required for mesoderm induction. Embryos that lack the BMP receptor BMPRI⁴⁹, nodal⁵⁰ or its intracellular effectors SMAD2 and SMAD3 (REF. 51) fail to form mesoderm and become blocked before gastrulation. WNT3 (REF. 52) and β -catenin mutants²⁴, as well as mutants that lack both of the Wnt co-receptors LRP5 and LRP6 (REF. 53), similarly fail to form mesoderm. Conversely, loss of axin 2, a negative regulator of the Wnt pathway⁵⁴, or misexpression of chicken WNT8C⁵⁵ leads to ectopic streak induction. Loss of both nodal antagonists CER1 and LEFTY1 similarly results in the formation of multiple streaks or enlarged primitive streak regions²².

The high levels of nodal–SMAD2 and SMAD3 signals that are focused in the proximal posterior epiblast closest to the ExE initiate primitive streak formation. Several regulatory mechanisms that establish this signalling gradient have been described (FIG. 2). First, uncleaved nodal precursor that is secreted by the epiblast activates the expression of BMP4 and the proprotein convertases SPC4 (also known as PCSK6 and PACE4) and furin (also known as PCSK3) in the ExE⁵⁶. Nodal maturation by furin and SPC4 rapidly upregulates local nodal expression through the SMAD–FOXH1 autoregulatory enhancer, which is present in the first intron of the *Nodal* gene^{57,58}. Second, nodal-dependent upregulation of BMP4 in the ExE directly activates WNT3 in posterior epiblast cells⁵⁶ (FIG. 2b). Wnt signalling in turn maintains high levels of nodal expression selectively in the posterior cells through a second TCF/LEF (T-cell-specific factor/lymphoid enhancer-binding factor)-dependent 5' *Nodal* enhancer^{18,56}. In the absence of WNT3, *Nodal* transcription is induced but not maintained. The WNT3-mutant embryos⁵² have more severe disturbances in comparison with embryos that lack the 5' *Nodal* enhancer⁵⁹, which suggests that additional WNT3 targets are required to promote mesoderm formation. Consistent with this idea, in *Xenopus laevis*, WNT3a stabilizes BMP–SMAD1 signals⁶⁰ to promote crosstalk between the nodal, BMP and Wnt pathways during mesoderm specification.

Cell lineage allocation in the primitive streak. At around E6.25, the primitive streak is initially induced at the proximal posterior pole of the epiblast and over

the next 36 hours, it elongates and extends to the distal tip of the embryo (FIG. 3). Distinct mesodermal cell lineages become allocated according to the time and site of ingress through the streak⁶¹. The earliest, most posterior mesoderm subpopulations, which are patterned in response to BMP4 signalling from the ExE⁶², give rise to the extraembryonic tissues, including the mesodermal layer of the chorion and the visceral yolk sac mesoderm and blood islands. Genetic studies show that BMP4 and its downstream effector SMAD1 are required for the formation of the allantois^{62,63}. Lateral plate, paraxial and cardiac mesoderm emerge slightly later from the intermediate and anterior levels of the streak. Finally, epiblast cells that migrate through the extreme anterior tip of the primitive streak (termed the APS progenitors) give rise to midline axial mesoderm tissues that comprise the prechordal plate (PCP), the notochord and the node, as well as the DE cell lineage. Starting at E6.5, nascent DE cells move onto the outer surface of the embryo by intercalation into the overlying VE. A recent study has questioned the long-held view that DE cells entirely displace the AVE and most of the VE to the extraembryonic regions⁶⁴. Instead, real-time imaging studies suggest that VE cells become dispersed but persist until later stages in the gut tube tissues. Future studies are needed to clarify if there is a functional significance of the endurance of VE cells in the gut tissues and how long they persist.

Derivatives of the APS, including the anterior DE, midline mesoderm and the node, all strongly express antagonists of the nodal and Wnt pathways as well as the BMP antagonists chordin and noggin, which function collectively to maintain the overlying neuroectoderm (NE). The thin strip of notochord cells that precisely underlies the ventral midline of the neural plate is also an important source of sonic hedgehog signals, which function as morphogens and govern dorsal–ventral patterning in the emerging central nervous system⁶⁵.

Morphogenesis of the notochord has been analysed by live-cell imaging⁶⁶. The anterior notochord is formed by aggregation of dispersed cells along the midline, whereas the notochordal plate of the node gives rise to the trunk notochord. Cells on the posterior margin of the node are progenitors of the posterior notochord. Real-time imaging studies to chart cell movements and behaviours, in combination with molecular lineage mapping, are required to provide insight into how these diverse anterior streak derivatives are precisely specified.

Spatial separation of the mesodermal and endodermal progenitors along the animal–vegetal axis is readily visualized in the *X. laevis* embryo. However, in the mouse anterior streak, mesodermal derivatives — namely the head mesenchyme, cardiac mesoderm and anterior paraxial mesoderm — and the emerging DE progenitors are all present in close proximity as they ingress through the anterior streak. The mechanisms that guide segregation of these distinct cell lineages are still poorly understood. Genetic studies have identified several transcription factors that function cooperatively to pattern APS derivatives and orchestrate DE

Primitive streak

The site on the posterior side of the embryo where epiblast cells ingress to form the mesoderm and definitive endoderm.

Epithelial–mesenchymal transition

A process during which cells change their shape from an epithelium to mesenchyme by loss of epithelial cell adhesion properties and epithelial cell polarity.

Morphogen

A signalling molecule that generates dose-dependent morphogenetic responses during development.

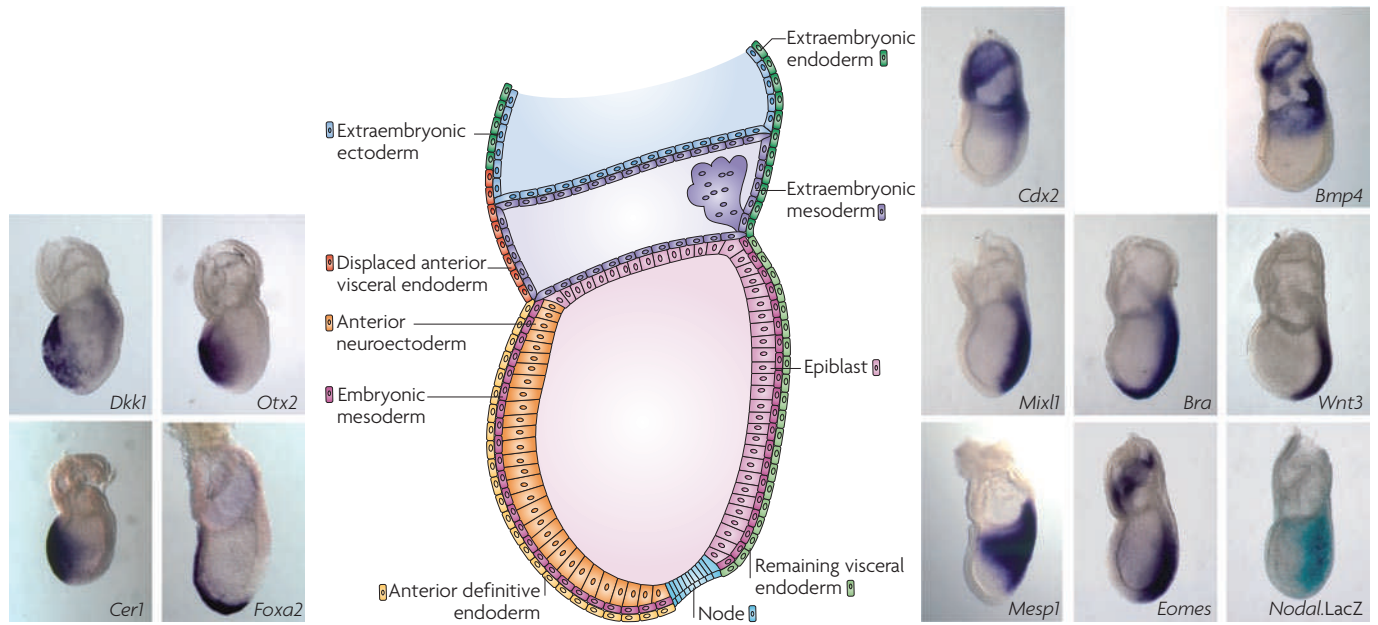


Figure 3 | Formation of different cell types at gastrulation. At embryonic day 6.5–7.5 (E6.5–E7.5), anterior–posterior polarity is demarcated by the anterior visceral endoderm (AVE) on the anterior side (red), and the onset of gastrulation at the opposite posterior proximal pole of the embryo. Cells of the epiblast (pink) converge towards the posterior of the embryo and ingress at the primitive streak to form the nascent embryonic mesoderm (violet) and extraembryonic mesoderm (purple). As the primitive streak expands to the distal tip of the embryo, cells that are present in the anterior primitive streak give rise to the definitive endoderm (yellow), which emerges on the surface of the embryo and gradually replaces cells of the visceral endoderm (light green). Cells that remain in the epiblast cell layer by the end of gastrulation constitute the neuroectoderm (orange). A specialized population of cells at the anterior tip of the primitive streak form the node (light blue), which is an important signalling centre for embryonic patterning and development of left–right handedness. RNA *in situ* hybridization has revealed the localized expression of signalling molecules and transcriptional regulators at gastrulation. At E7.5, expression of bone morphogenetic protein 4 (*Bmp4*), *Wnt3* and *Nodal* (with LacZ staining) is restricted to the posterior side, whereas the inhibitors cerberus-like 1 (*Cer1*) and Dickkopf homologue 1 (*Dkk1*) are selectively expressed on the anterior side of the gastrulating embryo. Graded signals differentially activate transcriptional regulators that confer positional information and initiate cell fate commitment. Caudal-type homeobox protein 2 (*Cdx2*) is a posterior mesoderm marker, MIX1 homeobox-like 1 (*Mixl1*) is expressed in the intermediate primitive streak and mesoderm posterior 1 (*Mesp1*) marks cardiac progenitors. The T-box transcription factor brachyury (*Bra*; also known as T protein) is broadly expressed throughout the entire streak, the node and the notochord. The related T-box gene eomesodermin (*Eomes*) is restricted to the anterior streak region and the chorion. Forkhead box A2 (*Foxa2*) expression is confined to derivatives of the anterior mesendoderm, the prechordal plate and the anterior midline endoderm, and underlies the anterior neuroectoderm marked by orthodenticle homologue 2 (*Otx2*) expression. The specific roles of these transcription factors in discrete subsets of streak derivatives have been defined by loss-of-function studies and cell-fate-mapping experiments.

specification in the early mouse embryo. DE formation is disrupted by the loss of SMAD2 (REFS 59,67) or SMAD4 (REF. 68), the forkhead transcription factors FOXH1 (REFS 69,70) or FOXA2 (REF. 71), or the T-box gene eomesodermin (*EOMES*)⁷². These factors function as components of the nodal pathway (SMAD2 and SMAD4), or are considered to be primary targets of SMAD2 and SMAD4 signalling (FOXH1, FOXA2 and *EOMES*).

Graded nodal signals have been shown to govern lineage allocation in the APS. Genetic manipulations that reduce nodal or SMAD2–SMAD3 levels result in the progressive loss of APS derivatives. Highest levels of activated SMAD2–SMAD3–SMAD4 complexes are necessary to specify DE and PCP, whereas the formation of the node requires intermediate levels^{59,68}. Allocation of the paraxial and the lateral plate mesoderm require

lower thresholds of nodal signals that are not dependent on SMAD4 (REFS 51, 68). Similarly, during gastrulation in *X. laevis*, dose-dependent nodal–activin signals generate concentration-dependent domains of brachyury and gooseoid (*Gsc*) expression, which mark all mesoderm, or specifically mesendoderm, respectively⁷³. Low levels of nodal–activin are sufficient to induce brachyury expression through its high-affinity promoter-binding sites. Brachyury activates *Xvent2* (also known as *Xom*, a *X. laevis* homeobox gene that mediates the early effects of BMP4), which mediates the repression of *Gsc* expression. However, increased levels of nodal–activin downregulate *Xvent2* and simultaneously activate *Gsc*, which in turn represses brachyury. These feedback regulatory circuits lead to mutually exclusive and spatially restricted expression domains in a nodal–activin concentration-dependent manner⁷⁴.

The transcriptional networks that govern the allocation of the DE lineage are broadly conserved across vertebrates, but the fine details can differ. In *X. laevis*, the maternally expressed T-box gene *vegt* activates nodal signals that are required for mesoderm and endoderm formation. A VegT homologue is not present in the mammalian genome, but another T-box gene product, EOMES, acts downstream of nodal to regulate endoderm formation⁷². Additional factors, including members of the SRY box (SOX), GATA and MIX/BIX-type homeobox gene families, are known to have crucial roles in DE formation and are broadly conserved⁷⁵. Constitutive expression of SOX17 drives the differentiation of human ES cells predominantly to DE⁷⁶, whereas mouse SOX17 is not required for early DE specification but rather functions later to promote endoderm survival⁷⁷. In *X. laevis*, Sox17 is a direct target of the Smad2 and VegT transcriptional complex⁷⁸, but transcriptional hierarchies in mouse remain ill-defined.

In vitro differentiation protocols, in combination with ES cell lines with reporter cassettes that have been knocked into specific loci and loss-of-function mutant cell lines, have been used to study lineage segregation and to reveal developmental potential¹. Cell culture systems should facilitate the isolation of intermediate cell types that only transiently exist in the embryo, and might prove to be a useful tool for studying the signalling pathways that regulate cell fate decisions. High doses of activin mimic nodal signalling and promote DE differentiation^{79,80}. Unexpectedly, *Brachyury*, which was previously viewed to be a bona fide mesoderm marker, was used to identify DE progenitors in ES cell differentiation protocols⁷⁹. Furthermore, SOX17, which has been used as a DE marker, is associated (in a cell non-autonomous manner) with development of the cardiomyocyte lineage *in vitro*⁸¹. This is consistent with a close lineage relationship between cardiac mesoderm and DE progenitors in the embryo. Wnt signalling might influence this cell fate decision, as the conditional loss of β -catenin in mesoderm cells disrupts lineage allocation to DE and instead favours the development of cardiac mesoderm⁸².

EMT and cell migration at the primitive streak. The EMT that allows nascent mesoderm to delaminate and migrate away from the primitive streak involves the loosening of epithelial adherens junctions, loss of association with the basement membrane and rearrangement of the cytoskeletal architecture (reviewed in REF. 83). Downregulation of E-cadherin expression is required for disruption of the adherens junctions. Both E-cadherin transcripts and protein are rapidly lost as cells enter the primitive streak (FIG. 4). Transcription is downregulated by FGF signals through FGFR1 that induce the expression of the zinc-finger transcriptional repressor snail, which binds directly to E-box sequences in the E-cadherin promoter^{84,85}. FGF8, FGFR1 and snail loss-of-function mutations disrupt EMT to varying degrees. FGF8-deficient epiblast cells ingress at the streak, but failure to migrate blocks the formation of mesoderm and DE cell lineages⁸⁶. Loss of FGFR1 also disrupts gastrulation, resulting in the expansion of the primitive streak^{87,88}. Snail-mutant embryos

develop nascent mesoderm but these cells retain an epithelial morphology and fail to efficiently downregulate E-cadherin expression⁸⁹.

MAPK signalling also has an important role in regulating EMT during gastrulation. Embryos that lack the p38-interacting protein (p38IP; also known as FAM48A), which is thought to control p38 MAPK activity, also exhibit impaired degradation of E-cadherin protein⁹⁰. p38IP functions at the post-translational level as FGF-dependent activation of snail remains intact⁹⁰. The resulting phenotype is less severe and mesoderm forms with reduced efficiency, causing delayed axis elongation and defective morphogenesis. MAPK kinase kinase 4 (MAP4K4) acts upstream of p38 (REF. 91), and functional loss similarly causes a paucity of mesoderm derivatives.

EOMES is crucial in EMT and mesodermal cell migration^{72,92}. Genetic evidence suggests that it is a nodal target⁷². In embryos that lack EOMES, epiblast cells are correctly induced (as judged by expression of nascent mesoderm markers, including brachyury and WNT3) but accumulate at the posterior side of the epiblast and form a thickened primitive streak, thereby failing to delaminate. E-cadherin transcripts and protein are not effectively downregulated but expression of FGF8 and its targets snail and TBX6 are unaffected⁷². It remains unclear whether EOMES functions independently or acts in concert with snail to repress E-cadherin expression. The basic helix-loop-helix transcription factors mesoderm posterior 1 (MESP1) and MESP2 might have additional roles in controlling EMT at the streak^{93,94}, but their ability to regulate E-cadherin expression has not been examined. MESP1 also regulates specification of cardiac progenitors, which shows an interesting connection between cell fate specification and the regulation of morphogenesis linked by the same transcription factor^{95,96}.

Both the mesodermal and DE cell lineages are programmed to execute a complex set of migratory behaviours. Guidance cues have been extensively examined in zebrafish and frog embryos. Interactions between mesoderm that expresses stromal-derived factor 1 (SDF1), and the endoderm that expresses the corresponding cytokine receptor C-X-C-chemokine receptor 4 (CXCR4), are required for endoderm migration in fish^{97,98}. By contrast, loss of SDF1 or CXCR4 has no noticeable effect on mouse development. Similarly, disruption of the Wnt-mediated non-canonical planar cell polarity pathway in zebrafish and frog interferes with convergence and extension movements that are required for elongation of the embryo⁹⁹. The planar cell polarity pathway in chick regulates early movement of primitive ectoderm cells before ingress through the streak¹⁰⁰. By contrast, mouse planar cell polarity genes were proven to be non-crucial for tissue morphogenesis during gastrulation¹⁰¹. TGF β signals directly regulate cell adhesion properties and govern tight junction assembly via phosphorylation of partitioning-defective 6 (PAR6) and occludin^{102,103} and govern EMT in numerous developmental contexts, such as neural crest delamination by BMP signals and cardiac valve formation by TGF β ⁸³. TGF β signalling also controls the early breakdown of the basement membrane

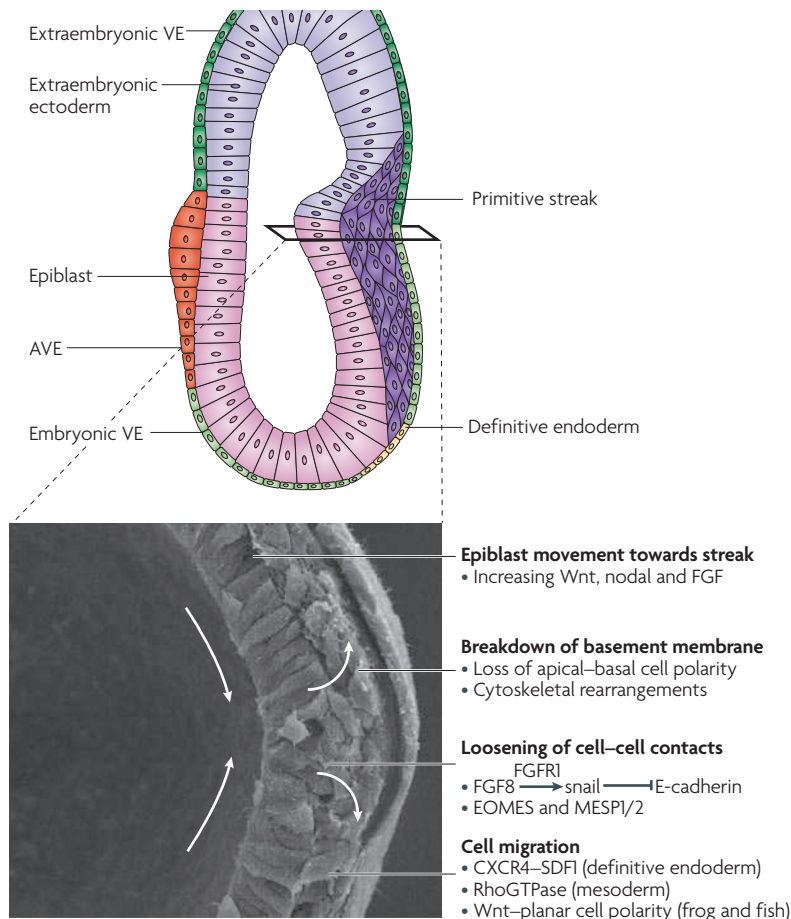


Figure 4 | Epithelial-mesenchymal transition in the primitive streak. Formation of nascent mesoderm during gastrulation is a result of an epithelial-mesenchymal transition (EMT) and tissue migration. Epithelial cells of the epiblast sheet converge towards the primitive streak, where increasing concentrations of signalling molecules, such as WNT3, fibroblast growth factor 8 (FGF8) and nodal, influence cell behaviour. Cells in the primitive streak detach from the basement membrane, lose their characteristic apical-basal cell polarity and undergo rapid and drastic cytoskeletal rearrangements that enable them to delaminate and migrate. A signalling cascade that involves FGF8 and the zinc-finger transcription factor snail causes the downregulation of the epithelial cell-adhesion molecule E-cadherin from adherens junctions, allowing mesodermal cells to migrate away from the streak. Additional activities of the transcription factors eomesodermin (EOMES), mesoderm posterior 1 (MESP1) and MESP2 are required for CDH1 downregulation and EMT, respectively. Nascent mesoderm cells migrate laterally and anteriorly between the epiblast and overlying visceral endoderm (VE). In lower vertebrates, chemoattractant-receptor interactions, such as stromal-derived factor 1 (SDF1)-C-X-C-chemokine receptor 4 (CXCR4), cytoskeletal rearrangements regulated by RhoGTPases or convergence-extension movements that are governed by the Wnt or planar cell polarity pathway orchestrate these complex cell movements. AVE, anterior VE.

in tumour cells⁸³. The fibronectin Leu-rich repeat transmembrane protein Flrt3 and the small GTPase Rnd1 were recently identified as nodal targets in *X. laevis*. Coexpression of Flrt3 and Rnd1 in the involuting cells of the marginal zone promotes localized E-cadherin downregulation, which causes cell internalization and migration along the inner surface of the blastocoel cavity¹⁰⁴. In mouse, FLRT3 is predominantly localized to the VE and DE¹⁰⁵. FLRT3 is non-essential for EMT during gastrulation, but is required for DE migration and the closure of the ventral midline¹⁰⁵.

NE — the default state of epiblast differentiation?

Epiblast cells that fail to migrate through the streak give rise to the NE and eventually the central nervous system⁶¹. Considerable evidence suggests that NE represents the default state of epiblast differentiation. Loss of either the BMPRI1A receptor¹⁰⁶ or nodal¹⁰⁷ results in precocious neuronal differentiation and premature loss of pluripotency within the epiblast. The combined activities of the antagonists CER1 and LEFTY1 are required to maintain NE precursors on the anterior side of the epiblast. Loss of either CER1 or LEFTY1 fails to disrupt NE specification, but double-mutant embryos show expansion of the mesoderm at the expense of the NE²². Sustained expression of antagonists in the anterior DE tissue and the midline mesendoderm during gastrulation maintains the overlying neurectoderm. Embryos that lack APS progenitors develop characteristic anterior central nervous system truncations^{51,59}. Similar phenotypes are seen in mutant embryos that specifically lack the Wnt inhibitor DKK1 (REFS 108,109), or both of the known BMP inhibitors chordin and noggin¹¹⁰. Recent experiments suggest that additional retinoic acid signals activated by SMAD-FOXH1 complexes in the anterior DE are equally required to maintain and pattern anterior NE¹¹¹.

Arkadia, a RING-domain E3 ubiquitin ligase, is essential for transducing maximal nodal signals that are required for APS progenitor specification in the mouse¹¹² and mesoendoderm specification in the frog¹¹³. Arkadia ubiquitylates phosphorylated SMAD2-SMAD3 complexes, and serves to couple activation of transcription with subsequent SMAD2-SMAD3 degradation through the proteasome¹¹⁴. Similarly, the BMP receptor SMADs 1, 5 and 8 are also downregulated by ubiquitylation¹¹⁵, and in *X. laevis* the Smad4 ubiquitin ligase ectoderm regulates the cell fate switch between ectoderm and mesoderm¹¹⁶.

Segregation of the germ cell lineage

In lower organisms, segregation of the somatic cell lineages versus the germ cell lineages is controlled by the partitioning of maternal determinants that globally repress transcription¹¹⁷. By contrast, in the mammalian embryo, early epiblast cells are all competent to adopt either a somatic or a germ cell fate. Primordial germ cells (PGCs) are specified in response to extrinsic signalling cues coincident with axis patterning at gastrulation stages¹¹⁸.

Prospective germ cells are selected from their somatic neighbours by dose-dependent BMP signals that originate from the ExE¹¹⁹. Loss of BMP4 prevents the formation of PGCs¹¹⁹. Similarly, loss of BMP8B and BMP2 expression in the ExE and VE lineages, respectively, also quantitatively affects PGC formation¹¹⁸. BMP ligands that signal through cell-surface receptors in proximal epiblast cells activate the SMAD1 and SMAD5 effectors. Both SMAD1 and SMAD5 homozygous null embryos fail to form germ cells^{63,120}. PGC specification is also compromised in SMAD1 and SMAD5 double heterozygotes¹²¹, which suggests that these transcriptional regulators act cooperatively. Conditional inactivation

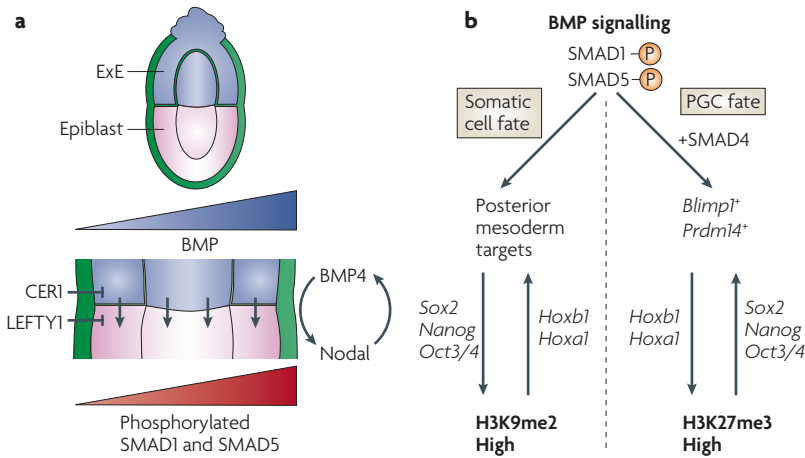


Figure 5 | Dose-dependent BMP-SMAD signals are required for germ cell lineage specification. **a** | Graded bone morphogenetic protein (BMP) signals from the extraembryonic ectoderm (ExE, blue) generate an anterior-posterior gradient of activated SMAD1 and SMAD5 regulatory complexes in the adjacent epiblast (pink). Highest BMP signalling levels are reached at the posterior pole through a feed-forward loop, which involves nodal, WNT3 and BMP4 signals. Transforming growth factor- β (TGF β)-BMP signalling is counteracted at the anterior pole by the expression of the inhibitors cerberus-like 1 (CER1) and left-right determination factor 1 (LEFTY1) from the anterior visceral endoderm (AVE). **b** | BMP signalling, in conjunction with nodal pathway activities, promotes somatic cell fates and leads to the formation of posterior mesoderm. Somatic cells downregulate pluripotency markers, including SRY-box-containing 2 (SOX2), nanog and OCT3/4 (octamer-binding transcription factor 3/4; also known as POU5F1) that are expressed in the early epiblast, and activate expression of mesodermal markers, including *Hox* genes. Phosphorylated SMAD1 and SMAD5 complexes, in association with SMAD4, activate expression of the transcription factors BLIMP1 (also known as PRDM1) and PRDM14 in a subset of cells to specify primordial germ cell (PGC) fate. BLIMP1 and PRDM14 repress the expression of somatic genes, including *Hoxa1* and *Hoxb1*, and reactivate pluripotency genes. Combined BLIMP1 and PRDM14 activities also initiate global reprogramming of PGCs to erase H3K9 histone marks and increase levels of H3K27me3 during PGC maturation.

of the co-Smad, SMAD4, in the epiblast blocks PGC specification but fails to perturb early mesoderm formation or patterning⁶⁸, which suggests that there are selective requirements for SMAD4 in BMP signal transduction. Collectively, these genetic studies show that dose-dependent BMP-SMAD signals cause a few epiblast cells to adopt a germ cell fate (FIG. 5).

PGCs characteristically express high levels of endogenous alkaline phosphatase activity. This subpopulation of cells first becomes apparent at E7.5 as a discrete cluster of 30–40 cells at the base of the incipient allantois. The localized induction and positioning of the PGCs depends on the reciprocal signalling cues that occur between the epiblast and extraembryonic tissues. These cues also function to establish initial A-P polarity. The high levels of BMP signals on the prospective posterior side of the embryo result from the expression of antagonists by the AVE (CER1 and LEFTY1), in combination with upregulated BMP4 expression that is localized to the posterior ExE by the nodal-WNT3-BMP4 feed-forward loop (FIG. 2; see above). These A-P signalling gradients act cooperatively, and maximal levels of phosphorylated SMAD complexes selectively activate germ-cell-specific target genes in the posterior proximal epiblast. PGCs have been induced in epiblast

tissue that is co-cultured with ExE and proximal VE tissues. Similarly, the removal of the VE inhibits PGC formation^{122,123}. In SMAD2-deficient embryos that lack A-P polarity, PGCs form in random patches owing to uniformly high levels of BMP signalling from the overlying ExE⁶⁷.

As gastrulation proceeds, the PGCs become localized to the hindgut endoderm to facilitate cell homing to the genital ridges. A family of interferon-induced transmembrane proteins (IFITMs) have been implicated in both PGC clustering and localization to the gut endoderm¹²⁴. IFITM3 (also known as fragilis), is activated in the posterior pre-gastrulation epiblast during PGC specification, and with time becomes confined to PGCs. IFITM1, which is initially co-expressed with IFITM3 in the PGCs, becomes localized to posterior mesoderm. Overexpression and siRNA-knockdown experiments suggest that IFITM1 probably repels germ cells from the mesoderm, whereas IFITM3 promotes their entry into the hindgut¹²⁴. However, the IFITM proteins are not required for germ cell development as the animals that carry a deletion of the *Ifitm* gene cluster are fully fertile¹²⁵. Other mechanisms presumably guide PGC localization in the absence of *Ifitm* expression. Similarly, *stella* (also known as DPPA3) is selectively expressed in germ cells¹²⁶, but it is non-essential for germ cell development. Instead, *stella* is required maternally for oocyte development¹²⁷.

BLIMP1 and PRDM14 are required for programming PGCs. An important hallmark of the PGC lineage is its ability to undergo orderly and extensive epigenetic reprogramming^{118,128}. As the pool of progenitors expands and migrates, PGCs show a loss of DNA methylation and H3K9me2 histone modifications and concomitantly acquire high levels of H3K27me3 histone marks, which is indicative of the pluripotent state. Expression of somatic genes, such as *Hoxa1* and *Hoxb1*, is repressed, whereas pluripotency transcription factors including, OCT3/4, nanog and SOX2, are re-activated.

Single-cell transcriptional profiling experiments have provided considerable insight into the early germ cell programme^{126,129,130}. Two members of the PRDM family of zinc finger transcriptional repressors **BLIMP1** (also known as PRDM1) and PRDM14 are required for PGC specification^{131–133}. Their onset of expression in the epiblast requires BMP-SMAD signalling and is strictly localized to the emerging PGCs^{131,133}. Fate-mapping experiments show that BLIMP1-positive cells are lineage-restricted to become PGCs¹³¹. Loss of the *Blimp1* or *Prdm14* gene acutely impairs initial PGC specification, and the few PGCs that form are rapidly lost. PRDM14 is exclusively required for germ cell specification, as *Prdm14*-null mice of both sexes are viable but sterile. By contrast, *Blimp1* was cloned as a master regulator of B-cell development and has multiple cell-type-specific essential roles in the embryo and adult. *Blimp1*-null embryos die at mid-gestation owing to placental insufficiency¹³². Conditional inactivation strategies have revealed the essential BLIMP1 functions in the patterning of the forelimb buds, in the pharynx, heart and sensory vibrissae of the developing embryo¹³⁴ and in the sebaceous gland¹³⁵ and skin¹³⁶ of adults.

Are the same transcriptional targets controlled by both these key regulators? BLIMP1 is required for the extinction of *Hox* gene expression¹³¹, whereas loss of PRDM14 results in both the failure to reactivate pluripotency genes and in defects in genome-wide epigenetic reprogramming, which is associated with increased expression of GLP1 (also known as euchromatic histone methyltransferase 1 (EHMT1)) and shifted ratios of H3K9me2 and H3K27me3 (REF. 133). BLIMP1 DNA-binding specificity has been extensively studied¹³⁷. In B cells, BLIMP1 directly blocks transcription at the promoters of target genes such as *Myc*, which is required for cell-cycle progression and globally redirects patterns of gene expression by silencing transcription factors, such as paired box 5 (PAX5), class II major histocompatibility complex transactivator (CIITA) and interferon regulatory factor 4 (IRF4), that are all required to maintain B-cell identity¹³⁸. In PGCs, BLIMP1 forms complexes with the Arg methyltransferase PRMT5 to selectively regulate epigenetic reprogramming¹³⁹. By contrast, PRDM14 partnerships and binding site specificities have not been reported. It will be important to further describe the shared and divergent roles of these closely related transcription factors in germ cell allocation and in the silencing of the default somatic programme.

Conclusions and future perspectives

Studies of vertebrate development have uncovered complex tissue interactions that coordinate early embryonic patterning and cell fate allocation. In mice, the extra-embryonic tissues are instrumental in setting up the basic body plan and for the initiation of developmental hallmarks, such as axis determination and primitive streak formation. Gene targeting in mice has provided an invaluable strategy to identify and study the main signalling molecules, their pathways and the transcriptional regulators that mediate tissue crosstalk.

However, it is poorly understood how the plethora of signals in the developing embryo is conclusively interpreted on the subcellular level. The molecular analysis of embryonic signalling is often grossly limited by size and heterogeneity of embryonic tissues that do not allow for biochemical analysis. Considerable progress has been made over the past few years in the generation of cell lines that represent embryonic progenitor cells^{140–143}, and in the development of specific ES-cell differentiation protocols that to some degree mimic embryonic cell differentiation¹. The availability of genetically modified ES cells that carry targeted deletions, reporter cassettes or transgenic overexpression constructs will grant useful tools to analyse the regulatory networks that guide development. It will also be particularly interesting to see the degree to which epigenetic control mechanisms, such as histone modifications, DNA methylation and non-coding RNAs, contribute to developmental gene regulation.

The process of embryonic morphogenesis includes rapid changes in cell behaviour through changes in cell shapes, migration, proliferation and apoptosis. Classic studies were mostly unable to reflect these dynamics. Recent advances in imaging techniques now offer possibilities to visualize development on a single-cell resolution in living embryos. Using gene-specific promoters that drive the expression of fluorescent markers, or recombinases to permanently label cell progeny, precise correlations of marker gene expression and cell fate can be made. Therefore, these techniques will be instrumental in defining the characteristics of progenitor cells during development — for example, the various progenitor cells in the primitive streak region. Defining the signals and intracellular mediators that are responsible for the propagation and differentiation of progenitor cell populations will be of key importance in the efforts to promote directed differentiation of cells for scientific or therapeutic purposes.

- Murry, C. E. & Keller, G. Differentiation of embryonic stem cells to clinically relevant populations: lessons from embryonic development. *Cell* **132**, 661–680 (2008).
- Sutherland, A. E., Speed, T. P. & Calarco, P. G. Inner cell allocation in the mouse morula: the role of oriented division during fourth cleavage. *Dev. Biol.* **137**, 13–25 (1990).
- Niwa, H. *et al.* Interaction between Oct3/4 and Cdx2 determines trophoctoderm differentiation. *Cell* **123**, 917–929 (2005).
Describes reciprocal interactions between OCT3/4 and CDX2 that might underlie the first embryonic cell lineage decision between ICM and TE.
- Dietrich, J. E. & Hiiragi, T. Stochastic patterning in the mouse pre-implantation embryo. *Development* **134**, 4219–4231 (2007).
- Ralston, A. & Rossant, J. Cdx2 acts downstream of cell polarization to cell-autonomously promote trophoctoderm fate in the early mouse embryo. *Dev. Biol.* **313**, 614–629 (2008).
- Yagi, R. *et al.* Transcription factor TEAD4 specifies the trophoctoderm lineage at the beginning of mammalian development. *Development* **134**, 3827–3836 (2007).
- Chazaud, C., Yamanaka, Y., Pawson, T. & Rossant, J. Early lineage segregation between epiblast and primitive endoderm in mouse blastocysts through the Grb2–MAPK pathway. *Dev. Cell* **10**, 615–624 (2006).
Shows that epiblast and PE lineages are already separated at the early blastocyst stage in a position-independent manner. It is suggested that nanog-positive cells give rise to the epiblast and that the PE derives from GATA6-positive cells in a GRB2–Ras–MAPK-dependent manner.
- Plusa, B., Piliśzek, A., Frankenberg, S., Artus, J. & Hadjantonakis, A. K. Distinct sequential cell behaviours direct primitive endoderm formation in the mouse blastocyst. *Development* **135**, 3081–3091 (2008).
- Chambers, I. *et al.* Functional expression cloning of Nanog, a pluripotency sustaining factor in embryonic stem cells. *Cell* **113**, 643–655 (2003).
- Mitsui, K. *et al.* The homeoprotein Nanog is required for maintenance of pluripotency in mouse epiblast and ES cells. *Cell* **113**, 631–642 (2003).
- Zhang, J. *et al.* Sall4 modulates embryonic stem cell pluripotency and early embryonic development by the transcriptional regulation of Pou5f1. *Nature Cell Biol.* **8**, 1114–1123 (2006).
- Fujikura, J. *et al.* Differentiation of embryonic stem cells is induced by GATA factors. *Genes Dev.* **16**, 784–789 (2002).
- Cheng, A. M. *et al.* Mammalian Grb2 regulates multiple steps in embryonic development and malignant transformation. *Cell* **95**, 793–803 (1998).
- Arman, E., Haffner-Krausz, R., Chen, Y., Heath, J. K. & Lonai, P. Targeted disruption of fibroblast growth factor (FGF) receptor 2 suggests a role for FGF signaling in pregastrulation mammalian development. *Proc. Natl Acad. Sci. USA* **95**, 5082–5087 (1998).
- Feldman, B., Poueymirou, W., Papaioannou, V. E., DeChiara, T. M. & Goldfarb, M. Requirement of FGF-4 for postimplantation mouse development. *Science* **267**, 246–249 (1995).
- Wilder, P. J. *et al.* Inactivation of the FGF-4 gene in embryonic stem cells alters the growth and/or the survival of their early differentiated progeny. *Dev. Biol.* **192**, 614–629 (1997).
- Gerbe, F., Cox, B., Rossant, J. & Chazaud, C. Dynamic expression of Lrp2 pathway members reveals progressive epithelial differentiation of primitive endoderm in mouse blastocyst. *Dev. Biol.* **313**, 594–602 (2008).
- Norris, D. P. & Robertson, E. J. Asymmetric and node-specific nodal expression patterns are controlled by two distinct *cis*-acting regulatory elements. *Genes Dev.* **13**, 1575–1588 (1999).
- Brennan, J. *et al.* Nodal signalling in the epiblast patterns the early mouse embryo. *Nature* **411**, 965–969 (2001).
Indicates the central role of nodal signalling in patterning the early mouse embryo through reciprocal tissue interactions.
- Perea-Gomez, A., Shawlot, W., Sasaki, H., Behringer, R. R. & Ang, S. HNF3 β and Lim1 interact in the visceral endoderm to regulate primitive streak formation and anterior–posterior polarity in the mouse embryo. *Development* **126**, 4499–4511 (1999).
- Waldrup, W. R., Bikoff, E. K., Hoodless, P. A., Wrana, J. L. & Robertson, E. J. Smad2 signaling in extraembryonic tissues determines anterior–posterior polarity of the early mouse embryo. *Cell* **92**, 797–808 (1998).
- Perea-Gomez, A. *et al.* Nodal antagonists in the anterior visceral endoderm prevent the formation of multiple primitive streaks. *Dev. Cell* **3**, 745–756 (2002).

23. Chazaud, C. & Rossant, J. Disruption of early proximodistal patterning and AVE formation in *Apc* mutants. *Development* **133**, 3379–3387 (2006).
24. Huelsenken, J. *et al.* Requirement for β -catenin in anterior–posterior axis formation in mice. *J. Cell Biol.* **148**, 567–578 (2000).
25. Guzman-Ayala, M., Ben-Haim, N., Beck, S. & Constam, D. B. Nodal protein processing and fibroblast growth factor 4 synergize to maintain a trophoblast stem cell microenvironment. *Proc. Natl Acad. Sci. USA* **101**, 15656–15660 (2004).
26. Rodriguez, T. A., Srinivas, S., Clements, M. P., Smith, J. C. & Beddington, R. S. Induction and migration of the anterior visceral endoderm is regulated by the extra-embryonic ectoderm. *Development* **132**, 2513–2520 (2005).
27. Donnison, M. *et al.* Loss of the extraembryonic ectoderm in *Elf5* mutants leads to defects in embryonic patterning. *Development* **132**, 2299–2308 (2005).
28. Georgiades, P. & Rossant, J. *Ets2* is necessary in trophoblast for normal embryonic anteroposterior axis development. *Development* **133**, 1059–1068 (2006).
29. Mesnard, D., Guzman-Ayala, M. & Constam, D. B. Nodal specifies embryonic visceral endoderm and sustains pluripotent cells in the epiblast before overt axial patterning. *Development* **133**, 2497–2505 (2006).
30. Beddington, R. S. & Robertson, E. J. Axis development and early asymmetry in mammals. *Cell* **96**, 195–209 (1999).
31. Lu, C. C., Brennan, J. & Robertson, E. J. From fertilization to gastrulation: axis formation in the mouse embryo. *Curr. Opin. Genet. Dev.* **11**, 384–392 (2001).
32. Thomas, P. & Beddington, R. Anterior primitive endoderm may be responsible for patterning the anterior neural plate in the mouse embryo. *Curr. Biol.* **6**, 1487–1496 (1996).
Describes, for the first time, the functional significance of the AVE as an important signalling centre that defines anterior identity in the mouse.
33. Lowe, L. A., Yamada, S. & Kuehn, M. R. Genetic dissection of nodal function in patterning the mouse embryo. *Development* **128**, 1831–1845 (2001).
34. Ding, J. *et al.* *Cripto* is required for correct orientation of the anterior–posterior axis in the mouse embryo. *Nature* **395**, 702–707 (1998).
35. Ang, S. L. *et al.* A targeted mouse *Otx2* mutation leads to severe defects in gastrulation and formation of axial mesoderm and to deletion of rostral brain. *Development* **122**, 243–252 (1996).
36. Kimura-Yoshida, C. *et al.* Crucial roles of *Foxa2* in mouse anterior–posterior axis polarization via regulation of anterior visceral endoderm-specific genes. *Proc. Natl Acad. Sci. USA* **104**, 5919–5924 (2007).
37. Perea-Gomez, A. *et al.* *Otx2* is required for visceral endoderm movement and for the restriction of posterior signals in the epiblast of the mouse embryo. *Development* **128**, 753–765 (2001).
38. Yamamoto, M. *et al.* Nodal antagonists regulate formation of the anteroposterior axis of the mouse embryo. *Nature* **428**, 387–392 (2004).
39. Srinivas, S., Rodriguez, T., Clements, M., Smith, J. C. & Beddington, R. S. Active cell migration drives the unilateral movements of the anterior visceral endoderm. *Development* **131**, 1157–1164 (2004).
40. Rakeman, A. S. & Anderson, K. V. Axis specification and morphogenesis in the mouse embryo require *Nap1*, a regulator of WAVE-mediated actin branching. *Development* **133**, 3075–3083 (2006).
41. Kimura-Yoshida, C. *et al.* Canonical Wnt signaling and its antagonist regulate anterior–posterior axis polarization by guiding cell migration in mouse visceral endoderm. *Dev. Cell* **9**, 639–650 (2005).
42. Takaoka, K., Yamamoto, M. & Hamada, H. Origin of body axes in the mouse embryo. *Curr. Opin. Genet. Dev.* **17**, 344–350 (2007).
43. Lawson, K. A. & Pedersen, R. A. Clonal analysis of cell fate during gastrulation and early neuroulation in the mouse. *Ciba Found. Symp.* **165**, 3–21 (1992).
44. Gardner, R. L. & Cockroft, D. L. Complete dissipation of coherent clonal growth occurs before gastrulation in mouse epiblast. *Development* **125**, 2397–2402 (1998).
45. Lawson, K. A., Meneses, J. J. & Pedersen, R. A. Clonal analysis of epiblast fate during germ layer formation in the mouse embryo. *Development* **113**, 891–911 (1991).
46. Lu, C. C. & Robertson, E. J. Multiple roles for Nodal in the epiblast of the mouse embryo in the establishment of anterior–posterior patterning. *Dev. Biol.* **273**, 149–159 (2004).
47. Gu, Z. *et al.* The type I activin receptor ActRIB is required for egg cylinder organization and gastrulation in the mouse. *Genes Dev.* **12**, 844–857 (1998).
48. Warg, R. M. & Kane, D. A. One-eyed pinhead regulates cell motility independent of Squint/Cyclops signaling. *Dev. Biol.* **261**, 391–411 (2003).
49. Mishina, Y., Suzuki, A., Ueno, N. & Behringer, R. R. *Bmpr* encodes a type I bone morphogenetic protein receptor that is essential for gastrulation during mouse embryogenesis. *Genes Dev.* **9**, 3027–3037 (1995).
50. Conlon, F. L. *et al.* A primary requirement for nodal in the formation and maintenance of the primitive streak in the mouse. *Development* **120**, 1919–1928 (1994).
51. Dunn, N. R., Vincent, S. D., Oxburgh, L., Robertson, E. J. & Bikoff, E. K. Combinatorial activities of *Smad2* and *Smad3* regulate mesoderm formation and patterning in the mouse embryo. *Development* **131**, 1717–1728 (2004).
52. Liu, P. *et al.* Requirement for *Wnt3* in vertebrate axis formation. *Nature Genet.* **22**, 361–365 (1999).
53. Kelly, O. G., Pinson, K. I. & Skarnes, W. C. The Wnt co-receptors *Lrp5* and *Lrp6* are essential for gastrulation in mice. *Development* **131**, 2803–2815 (2004).
54. Zeng, L. *et al.* The mouse *fused* locus encodes *Axin*, an inhibitor of the Wnt signaling pathway that regulates embryonic axis formation. *Cell* **90**, 181–192 (1997).
Establishes axin as an important regulator of Wnt signalling. It shows, for the first time, that elevated levels of Wnt signalling induce formation of the primitive streak in mice.
55. Popperl, H. *et al.* Misexpression of *Cwnt8C* in the mouse induces an ectopic embryonic axis and causes a truncation of the anterior neuroectoderm. *Development* **124**, 2997–3005 (1997).
56. Ben-Haim, N. *et al.* The nodal precursor acting via activin receptors induces mesoderm by maintaining a source of its convertases and BMP4. *Dev. Cell* **11**, 313–323 (2006).
Defines two independent feed-forward loops that ensure the highest nodal signalling level on the posterior pole of the embryo at the site of primitive streak formation.
57. Norris, D. P., Brennan, J., Bikoff, E. K. & Robertson, E. J. The *Foxh1*-dependent autoregulatory enhancer controls the level of Nodal signals in the mouse embryo. *Development* **129**, 3455–3468 (2002).
58. Saijoh, Y. *et al.* Left–right asymmetric expression of *lefty2* and *nodal* is induced by a signaling pathway that includes the transcription factor *FAST2*. *Mol. Cell* **5**, 35–47 (2000).
59. Vincent, S. D., Dunn, N. R., Hayashi, S., Norris, D. P. & Robertson, E. J. Cell fate decisions within the mouse organizer are governed by graded Nodal signals. *Genes Dev.* **17**, 1646–1662 (2003).
Shows that cell lineage allocation in the APS is governed by graded levels of nodal–SMAD2 signalling. The conditional deletion of SMAD2 in the epiblast, or lowering nodal expression in the primitive streak by targeted deletion of the primitive streak enhancer element, results in the selective loss of the anterior DE and PCP mesoderm but not other APS derivatives.
60. Fuentealba, L. C. *et al.* Integrating patterning signals: *Wnt/GSK3* regulates the duration of the BMP/*Smad1* signal. *Cell* **131**, 980–993 (2007).
61. Lawson, K. A. Fate mapping the mouse embryo. *Int. J. Dev. Biol.* **43**, 773–775 (1999).
62. Winnier, G., Blessing, M., Labosky, P. A. & Hogan, B. L. Bone morphogenetic protein-4 is required for mesoderm formation and patterning in the mouse. *Genes Dev.* **9**, 2105–2116 (1995).
63. Tremblay, K. D., Dunn, N. R. & Robertson, E. J. Mouse embryos lacking *Smad1* signals display defects in extra-embryonic tissues and germ cell formation. *Development* **128**, 3609–3621 (2001).
64. Kwon, G. S., Viotti, M. & Hadjantonakis, A. K. The endoderm of the mouse embryo arises by dynamic widespread intercalation of embryonic and extraembryonic lineages. *Dev. Cell* **15**, 509–520 (2008).
Using elegant cell fate analysis and fluorescent markers, this study shows the persistence of VE cells in the layer of DE in the post-gastrulation embryo.
65. Echelard, Y. *et al.* Sonic hedgehog, a member of a family of putative signaling molecules, is implicated in the regulation of CNS polarity. *Cell* **75**, 1417–1430 (1993).
66. Yamanaka, Y., Tamplin, O. J., Beckers, A., Gossler, A. & Rossant, J. Live imaging and genetic analysis of mouse notochord formation reveals regional morphogenetic mechanisms. *Dev. Cell* **13**, 884–896 (2007).
67. Tremblay, K. D., Hoodless, P. A., Bikoff, E. K. & Robertson, E. J. Formation of the definitive endoderm in mouse is a *Smad2*-dependent process. *Development* **127**, 3079–3090 (2000).
68. Chu, G. C., Dunn, N. R., Anderson, D. C., Oxburgh, L. & Robertson, E. J. Differential requirements for *Smad4* in TGF β -dependent patterning of the early mouse embryo. *Development* **131**, 3501–3512 (2004).
69. Hoodless, P. A. *et al.* *FoxH1 (Fast)* functions to specify the anterior primitive streak in the mouse. *Genes Dev.* **15**, 1257–1271 (2001).
70. Yamamoto, M. *et al.* The transcription factor *FoxH1 (FAST)* mediates Nodal signaling during anterior–posterior patterning and node formation in the mouse. *Genes Dev.* **15**, 1242–1256 (2001).
71. Dufort, D., Schwartz, L., Harpal, K. & Rossant, J. The transcription factor *HNF3 β* is required in visceral endoderm for normal primitive streak morphogenesis. *Development* **125**, 3015–3025 (1998).
72. Arnold, S. J., Hofmann, U. K., Bikoff, E. K. & Robertson, E. J. Pivotal roles for eomesodermin during axis formation, epithelium-to-mesenchyme transition and endoderm specification in the mouse. *Development* **135**, 501–511 (2008).
73. Saka, Y. & Smith, J. C. A mechanism for the sharp transition of morphogen gradient interpretation in *Xenopus*. *BMC Dev. Biol.* **7**, 47 (2007).
74. Schmierer, B. & Hill, C. S. TGF β –SMAD signal transduction: molecular specificity and functional flexibility. *Nature Rev. Mol. Cell Biol.* **8**, 970–982 (2007).
75. Grapin-Botton, A. & Constam, D. Evolution of the mechanisms and molecular control of endoderm formation. *Mech. Dev.* **124**, 253–278 (2007).
76. Seguin, C. A., Draper, J. S., Nagy, A. & Rossant, J. Establishment of endoderm progenitors by SOX transcription factor expression in human embryonic stem cells. *Cell Stem Cell* **3**, 182–195 (2008).
77. Kanai-Azuma, M. *et al.* Depletion of definitive gut endoderm in *Sox17*-null mutant mice. *Development* **129**, 2367–2379 (2002).
78. Howard, L., Rex, M., Clements, D. & Woodland, H. R. Regulation of the *Xenopus Xsox17 α* , promoter by co-operating *VegT* and *Sox17* sites. *Dev. Biol.* **310**, 402–415 (2007).
79. Kubo, A. *et al.* Development of definitive endoderm from embryonic stem cells in culture. *Development* **131**, 1651–1662 (2004).
80. Tada, S. *et al.* Characterization of mesoderm: a diverging point of the definitive endoderm and mesoderm in embryonic stem cell differentiation culture. *Development* **132**, 4363–4374 (2005).
81. Liu, Y. *et al.* *Sox17* is essential for the specification of cardiac mesoderm in embryonic stem cells. *Proc. Natl Acad. Sci. USA* **104**, 3859–3864 (2007).
82. Lickert, H. *et al.* Formation of multiple hearts in mice following deletion of β -catenin in the embryonic endoderm. *Dev. Cell* **3**, 171–181 (2002).
83. Yang, J. & Weinberg, R. A. Epithelial–mesenchymal transition: at the crossroads of development and tumor metastasis. *Dev. Cell* **14**, 818–829 (2008).
84. Batlle, E. *et al.* The transcription factor snail is a repressor of E-cadherin gene expression in epithelial tumour cells. *Nature Cell Biol.* **2**, 84–89 (2000).
85. Cano, A. *et al.* The transcription factor snail controls epithelial–mesenchymal transitions by repressing E-cadherin expression. *Nature Cell Biol.* **2**, 76–83 (2000).
86. Sun, X., Meyers, E. N., Lewandoski, M. & Martin, G. R. Targeted disruption of *Fgf8* causes failure of cell migration in the gastrulating mouse embryo. *Genes Dev.* **13**, 1834–1846 (1999).
87. Ciruna, B. & Rossant, J. FGF signaling regulates mesoderm cell fate specification and morphogenetic movement at the primitive streak. *Dev. Cell* **1**, 37–49 (2001).
Establishes that the transcriptional repressor snail functions downstream of FGF signalling to repress E-cadherin expression at the primitive streak. Loss of E-cadherin is a prerequisite for EMT and migration of nascent mesoderm from the primitive streak.

88. Yamaguchi, T. P., Harpal, K., Henkemeyer, M. & Rossant, J. *Jgfr-1* is required for embryonic growth and mesodermal patterning during mouse gastrulation. *Genes Dev.* **8**, 3032–3044 (1994).
89. Carver, E. A., Jiang, R., Lan, Y., Oram, K. F. & Gridley, T. The mouse *snai* gene encodes a key regulator of the epithelial–mesenchymal transition. *Mol. Cell. Biol.* **21**, 8184–8188 (2001).
90. Zohn, I. E. *et al.* p38 and a p38-interacting protein are critical for downregulation of E-cadherin during mouse gastrulation. *Cell* **125**, 957–969 (2006).
91. Xue, Y. *et al.* Mesodermal patterning defect in mice lacking the Ste20 NCK interacting kinase (NIK). *Development* **128**, 1559–1572 (2001).
92. Russ, A. P. *et al.* Eomesodermin is required for mouse trophoblast development and mesoderm formation. *Nature* **404**, 95–99 (2000).
93. Kitajima, S., Takagi, A., Inoue, T. & Saga, Y. *MesP1* and *MesP2* are essential for the development of cardiac mesoderm. *Development* **127**, 3215–3226 (2000).
94. Saga, Y. *et al.* *MesP1* is expressed in the heart precursor cells and required for the formation of a single heart tube. *Development* **126**, 3437–3447 (1999).
95. Bondue, A. *et al.* *MesP1* acts as a master regulator of multipotent cardiovascular progenitor specification. *Cell Stem Cell* **3**, 69–84 (2008).
96. Lindsley, R. C. *et al.* *MesP1* coordinately regulates cardiovascular fate restriction and epithelial–mesenchymal transition in differentiating ESCs. *Cell Stem Cell* **3**, 55–68 (2008).
97. Fukui, A., Goto, T., Kitamoto, J., Homma, M. & Asashima, M. *SDF-1 α* regulates mesodermal cell migration during frog gastrulation. *Biochem. Biophys. Res. Commun.* **354**, 472–477 (2007).
98. Mizoguchi, T., Verkade, H., Heath, J. K., Kuroiwa, A. & Kikuchi, Y. *Sdf1/Cxcr4* signaling controls the dorsal migration of endodermal cells during zebrafish gastrulation. *Development* **135**, 2521–2529 (2008).
99. Wallingford, J. B., Fraser, S. E. & Harland, R. M. Convergent extension: the molecular control of polarized cell movement during embryonic development. *Dev. Cell* **2**, 695–706 (2002).
100. Voiculescu, O., Bertocchini, F., Wolpert, L., Keller, R. E. & Stern, C. D. The amniote primitive streak is defined by epithelial cell intercalation before gastrulation. *Nature* **449**, 1049–1052 (2007).
101. Klein, R. S. & Rubin, J. B. Immune and nervous system CXCL12 and CXCR4: parallel roles in patterning and plasticity. *Trends Immunol.* **25**, 306–314 (2004).
102. Barrios-Rodiles, M. *et al.* High-throughput mapping of a dynamic signaling network in mammalian cells. *Science* **307**, 1621–1625 (2005).
103. Ozdamar, B. *et al.* Regulation of the polarity protein Par6 by TGF β receptors controls epithelial cell plasticity. *Science* **307**, 1603–1609 (2005).
104. Ogata, S. *et al.* TGF- β signaling-mediated morphogenesis: modulation of cell adhesion via cadherin endocytosis. *Genes Dev.* **21**, 1817–1831 (2007).
105. Maretto, S. *et al.* Ventral closure, headfold fusion and definitive endoderm migration defects in mouse embryos lacking the fibronectin leucine-rich transmembrane protein FLRT3. *Dev. Biol.* **318**, 184–193 (2008).
106. Di-Gregorio, A. *et al.* BMP signalling inhibits premature neural differentiation in the mouse embryo. *Development* **134**, 3359–3369 (2007).
Shows that BMP signalling in the epiblast is required to maintain epiblast pluripotency and to repress precocious NE differentiation.
107. Camus, A., Perea-Gomez, A., Moreau, A. & Collignon, J. Absence of Nodal signaling promotes precocious neural differentiation in the mouse embryo. *Dev. Biol.* **295**, 743–755 (2006).
108. Lewis, S. L. *et al.* *Dkk1* and *Wnt3* interact to control head morphogenesis in the mouse. *Development* **135**, 1791–1801 (2008).
109. Mukhopadhyay, M. *et al.* *Dickkopf1* is required for embryonic head induction and limb morphogenesis in the mouse. *Dev. Cell* **1**, 423–434 (2001).
110. Bachiller, D. *et al.* The organizer factors Chordin and Noggin are required for mouse forebrain development. *Nature* **405**, 658–661 (2000).
111. Silvestri, C. *et al.* Genome-wide identification of Smad/Foxh1 targets reveals a role for Foxh1 in retinoic acid regulation and forebrain development. *Dev. Cell* **14**, 411–423 (2008).
112. Episkopou, V. *et al.* Induction of the mammalian node requires Arkadia function in the extraembryonic lineages. *Nature* **410**, 825–830 (2001).
113. Niederlander, C., Walsh, J. J., Episkopou, V. & Jones, C. M. Arkadia enhances nodal-related signalling to induce mesendoderm. *Nature* **410**, 830–834 (2001).
114. Mavrikis, K. J. *et al.* Arkadia enhances Nodal/TGF- β signaling by coupling phospho-Smad2/3 activity and turnover. *PLoS Biol.* **5**, e67 (2007).
115. Zhu, H., Kavsak, P., Abdollah, S., Wrana, J. L. & Thomsen, G. H. A SMAD ubiquitin ligase targets the BMP pathway and affects embryonic pattern formation. *Nature* **400**, 687–693 (1999).
116. Dupont, S. *et al.* Germ-layer specification and control of cell growth by Ectoderm, a Smad4 ubiquitin ligase. *Cell* **121**, 87–99 (2005).
117. Seydoux, G. & Braun, R. E. Pathway to totipotency: lessons from germ cells. *Cell* **127**, 891–904 (2006).
118. Hayashi, K., de Sousa Lopes, S. M. & Surani, M. A. Germ cell specification in mice. *Science* **316**, 394–396 (2007).
119. Lawson, K. A. *et al.* *Bmp4* is required for the generation of primordial germ cells in the mouse embryo. *Genes Dev.* **13**, 424–436 (1999).
Shows for the first time that BMP signals from extraembryonic tissues are required for the generation of PGCs in a dose-dependent manner.
120. Chang, H. & Matzuk, M. M. Smad5 is required for mouse primordial germ cell development. *Mech. Dev.* **104**, 61–67 (2001).
121. Arnold, S. J., Maretto, S., Islam, A., Bikoff, E. K. & Robertson, E. J. Dose-dependent *Smad1*, *Smad5* and *Smad8* signaling in the early mouse embryo. *Dev. Biol.* **296**, 104–118 (2006).
122. de Sousa Lopes, S. M. *et al.* BMP signaling mediated by ALK2 in the visceral endoderm is necessary for the generation of primordial germ cells in the mouse embryo. *Genes Dev.* **18**, 1838–1849 (2004).
123. de Sousa Lopes, S. M., Hayashi, K. & Surani, M. A. Proximal visceral endoderm and extraembryonic ectoderm regulate the formation of primordial germ cell precursors. *BMC Dev. Biol.* **7**, 140 (2007).
124. Tanaka, S. S., Yamaguchi, Y. L., Tsoi, B., Lickert, H. & Tam, P. P. IFITM/Mil/fraxilis family proteins IFITM1 and IFITM3 play distinct roles in mouse primordial germ cell homing and repulsion. *Dev. Cell* **9**, 745–756 (2005).
125. Lange, U. C. *et al.* Normal germ line establishment in mice carrying a deletion of the *Ifitm/Fragilis* gene family cluster. *Mol. Cell. Biol.* **28**, 4688–4696 (2008).
126. Saitou, M., Barton, S. C. & Surani, M. A. A molecular programme for the specification of germ cell fate in mice. *Nature* **418**, 293–300 (2002).
127. Bortvin, A., Goodheart, M., Liao, M. & Page, D. C. *Dppa3 / Pgc7 / stella* is a maternal factor and is not required for germ cell specification in mice. *BMC Dev. Biol.* **4**, 2 (2004).
128. Seki, Y. *et al.* Extensive and orderly reprogramming of genome-wide chromatin modifications associated with specification and early development of germ cells in mice. *Dev. Biol.* **278**, 440–458 (2005).
129. Kurimoto, K. *et al.* Complex genome-wide transcription dynamics orchestrated by Blimp1 for the specification of the germ cell lineage in mice. *Genes Dev.* **22**, 1617–1635 (2008).
130. Yabuta, Y., Kurimoto, K., Ohinata, Y., Seki, Y. & Saitou, M. Gene expression dynamics during germline specification in mice identified by quantitative single-cell gene expression profiling. *Biol. Reprod.* **75**, 705–716 (2006).
131. Ohinata, Y. *et al.* Blimp1 is a critical determinant of the germ cell lineage in mice. *Nature* **436**, 207–213 (2005).
132. Vincent, S. D. *et al.* The zinc finger transcriptional repressor Blimp1/Prdm1 is dispensable for early axis formation but is required for specification of primordial germ cells in the mouse. *Development* **132**, 1315–1325 (2005).
References 131 and 132 show that BLIMP1 (also known as PRDM1) is a marker for lineage restricted PGCs. Both papers show that targeted deletions of BLIMP1 lead to the complete loss of PGCs.
133. Yamaji, M. *et al.* Critical function of *Prdm14* for the establishment of the germ cell lineage in mice. *Nature Genet.* **40**, 1016–1022 (2008).
134. Robertson, E. J. *et al.* Blimp1 regulates development of the posterior forelimb, caudal pharyngeal arches, heart and sensory vibrissae in mice. *Development* **134**, 4335–4345 (2007).
135. Horsley, V. *et al.* Blimp1 defines a progenitor population that governs cellular input to the sebaceous gland. *Cell* **126**, 597–609 (2006).
136. Magnusdottir, E. *et al.* Epidermal terminal differentiation depends on B lymphocyte-induced maturation protein-1. *Proc. Natl Acad. Sci. USA* **104**, 14988–14993 (2007).
137. Shaffer, A. L. *et al.* Blimp-1 orchestrates plasma cell differentiation by extinguishing the mature B cell gene expression program. *Immunity* **17**, 51–62 (2002).
138. Martins, G. & Calame, K. Regulation and functions of Blimp-1 in T and B lymphocytes. *Annu. Rev. Immunol.* **26**, 133–169 (2008).
139. Ancelin, K. *et al.* Blimp1 associates with Prmt5 and directs histone arginine methylation in mouse germ cells. *Nature Cell Biol.* **8**, 623–630 (2006).
140. Brons, I. G. *et al.* Derivation of pluripotent epiblast stem cells from mammalian embryos. *Nature* **448**, 191–195 (2007).
141. Tanaka, S., Kunath, T., Hadjantonakis, A. K., Nagy, A. & Rossant, J. Promotion of trophoblast stem cell proliferation by FGF4. *Science* **282**, 2072–2075 (1998).
142. Tesar, P. J. *et al.* New cell lines from mouse epiblast share defining features with human embryonic stem cells. *Nature* **448**, 196–199 (2007).
143. Yamanaka, Y., Ralston, A., Stephenson, R. O. & Rossant, J. Cell and molecular regulation of the mouse blastocyst. *Dev. Dyn.* **235**, 2301–2314 (2006).
144. Massague, J. TGF β in cancer. *Cell* **134**, 215–230 (2008).
145. Shen, M. M. Nodal signaling: developmental roles and regulation. *Development* **134**, 1023–1034 (2007).
146. Clevers, H. Wnt/ β -catenin signaling in development and disease. *Cell* **127**, 469–480 (2006).

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DATABASES

Entrez Gene: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene>
 Brachyury | Fgf8 | vegf | Wnt3 |
 UniProtKB: <http://www.uniprot.org>
 BLIMP1 | BMP4 | CDX2 | crypto | DAB2 | DKK1 | EOMES |
 EGF4 | EGR2 | FOXA2 | furin | GATA4 | GATA6 | GRB2 | LH2 |
 LRPAP1 | LRP2 | nanog | nodal | OCT3/4 | SALL4 | SMAD2 |
 SMAD3 | SMAD4 | TEAD4

FURTHER INFORMATION

Elizabeth J. Robertson's homepage: <http://www.path.ox.ac.uk/dirci/molbiology/robertson>

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