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Review article

Mouse gastrulation: the formation of a mammalian body plan

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Abstract

The process of gastrulation is a pivotal step in the formation of the vertebrate body plan. The primary function of gastrulation is the correct placement of precursor tissues for subsequent morphogenesis. There is now mounting evidence that the body plan is established through inductive interactions between germ layer tissues and by the global patterning activity emanating from embryonic organizers. An increasing number of mouse mutants have been described that have gastrulation defects, providing important insights into the molecular mechanisms that regulate this complex process. In this review, we explore the mouse embryo before and during gastrulation, highlighting its similarities with other vertebrate embryos and its unique characteristics. © 1997 Elsevier Science Ireland Ltd.

Keywords: Mouse; Gastrulation; Mammalian body plan

1. The anatomy of mouse gastrulation

The purpose of gastrulation is to generate a body plan that serves as a blueprint for the subsequent morphogenesis of the embryo. In the mouse, gastrulation is a constantly changing, three-dimensional puzzle that has unique features when compared to non-mammalian and even other mammalian embryos. After implantation in the uterus, the mouse embryo initially develops as a cylindrical structure that is quite different from most mammalian embryos.

During the immediate post-implantation period (5–6 days p.c.), the mouse embryo changes dramatically in size and shape. The embryonic tissue volume increases by about 40-fold, largely due to the cell proliferation of the tissue that gives rise to the extraembryonic ectoderm (Snow, 1976). Because of the physical block imposed by the attachment of the blastocyst to the uterine wall and the space constraints imposed by the implantation site, the inner cell mass (ICM) and its associated trophectoderm grow into the space available in the blastocoel cavity (Fig. 1) (Copp, 1979).

Epithelialization of the ICM into a layer of epiblast cells occurs concomitantly with the formation of a central cavity called the proamniotic cavity (Fig. 1). The embryo thus acquires the shape of a cup made up of two cell layers, the inner epiblast and the outer visceral endoderm. Proamniotic cavity formation is believed to occur by cell death. This process appears to be regulated by the adjacent visceral endoderm as suggested by in vitro studies using embryonal carcinoma (EC) and embryonic stem (ES) cell lines that have been differentiated into embryoid bodies (Coucouvanis and Martin, 1995). Cell death, however, does not appear to have a significant morphogenetic role during gastrulation because the incidence of cell death in the epiblast is low (Poelmann, 1980) and the apoptotic cells that are present are randomly distributed in the germ layers (Sanders et al., 1997).

The mouse embryo at this stage, called the egg cylinder, has well delineated extraembryonic and embryonic regions that further define a polarized proximal-distal axis (Fig. 1). At this stage, the prospective dorsal-ventral (D-V) axis of the embryo becomes apparent with the proamniotic cavity surface of the epiblast corresponding to the dorsal side of the embryo and the outer surface of the visceral endoderm corresponding to the ventral side of the embryo (Fig. 1).

Gastrulation utilizes a set of morphogenetic movements

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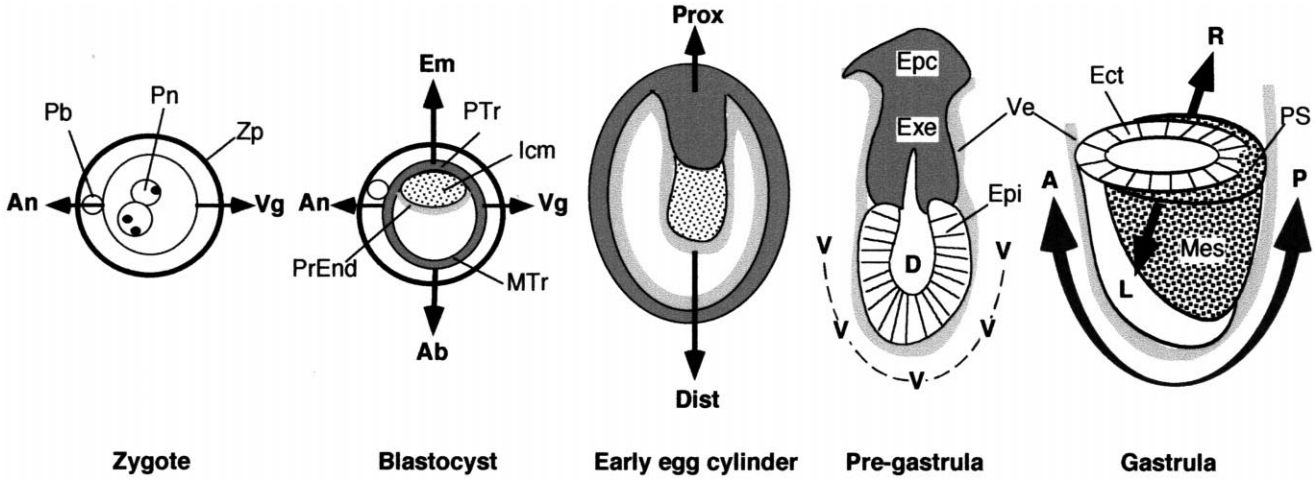


Fig. 1. Orientation of the embryonic axes; the animal-vegetal (An-Vg) axis of the zygote and the blastocyst, the embryonic-abembryonic (Em-Ab) axis of the blastocyst, the proximal (Prox)-distal (Dist) axis of the egg cylinder and the prospective dorso-ventral (D-V), anterior-posterior (A-P) and left-right (L-R) axes of the pre-gastrula and gastrula. Ect, ectoderm; Epc, ectoplacental cone; Epi, epiblast; Exe, extraembryonic ectoderm; Icm, inner cell mass; Mes, Mesoderm; MTr, mural trophectoderm; Pb, polar body; Pn, pronucleus; PrEnd, primitive endoderm; PS, primitive streak; PTr, polar trophectoderm; Ve, visceral endoderm; Zp, zona pellucida.

coupled with cell proliferation and differentiation to convert an embryo with two germ layers into one with three, namely the ectoderm, mesoderm and endoderm. The mouse, like the chick, gastrulates by the recruitment of embryonic ectoderm (epiblast) cells to a transient embryonic structure called the primitive streak. With the formation of the primitive streak, the anterior-posterior (A-P) axis becomes morphologically obvious with the streak located on the posterior side of the embryo. With the definition of the A-P axis, the left-right (L-R) axis becomes apparent, perhaps by default (Fig. 1). At the primitive streak, the epiblast cells undergo an epithelial to mesenchymal transition, then ingress in-between the epiblast and endoderm to become incorporated into either the mesoderm or the definitive endoderm germ layers (Bellairs, 1986; Hashimoto and Nakatsuji, 1989; reviewed by Tam et al., 1993). The mesoderm is formed as a new tissue sheet expanding from both sides of the primitive streak and is often referred to as the mesodermal ‘wings’ (Fig. 1).

At the conclusion of gastrulation, cell populations that have been allocated to different germ layers but are required for the formation of specific organs or body parts are physically brought together. Such juxtapositions of tissues, either transiently or permanently, facilitate inductive interactions that are critical for lineage specification and tissue patterning. The complex movements of the germ layer tissues in the cup-shaped mouse gastrula are difficult to reconcile spatially with the more familiar gastrulation movements of amphibian and avian embryos. Another unique feature of the mouse embryo is that it initially develops ‘inside-out’, with the internally-located ectoderm surrounded by the mesoderm and endoderm. After gastrulation, this necessitates an inversion of the germ layers to bring the ectoderm to the outside of the embryo and the endoderm to the inside with the mesoderm in the middle.

In this review, we will examine the formation of the body plan before and during gastrulation and the cellular and molecular aspects of germ layer morphogenesis in the mouse embryo.

2. The overture to gastrulation

2.1. The axes of the pre-implantation embryo

To generate the primitive streak on one side of the mouse embryo, the embryo must have established asymmetries prior to gastrulation. In other mammals such as marsupials, axis formation clearly occurs prior to implantation and thus appears to be intrinsic to the embryo (Selwood, 1992; Tyndale-Biscoe and Renfree, 1987). However, the idea that the specification of the mouse embryonic axes occurs prior to implantation is controversial (Gardner et al., 1992).

The formation of the blastocoel cavity and the localization of the ICM at one side of an enveloping single layered epithelium called the trophectoderm of the blastocyst is perhaps the first overt manifestation of morphological asymmetry (Fig. 1). This results in a more intimate association of the ICM with a subset of trophectoderm cells (polar trophectoderm) than with the rest of the trophectoderm (the mural trophectoderm). The asymmetric localization of the ICM/polar trophectoderm relative to the mural trophectoderm reveals the orientation of the embryonic-abembryonic (Em-Ab) axis (Fig. 1). Subsequently, at 4.0 days p.c., a second epithelial layer called the primitive endoderm differentiates on the blastocoelic surface of the ICM further emphasizing this asymmetry. The primitive endoderm will differentiate further into visceral endoderm that remains associated with the epiblast and parietal endoderm that moves out onto the blastocoelic surface of the mural trophectoderm. The visceral and parietal endoderm contribute

to extraembryonic tissues and are distinct from the definitive endoderm of the embryo proper (Gardner and Beddington, 1988).

Another asymmetry that is distinct from the Em-Ab axis has been recently identified in the mouse blastocyst (Gardner, 1997). During oogenesis the formation of the polar body marks an animal-vegetal (An-Vg) axis. In preimplantation embryos of the PO mouse strain, the second polar body often remains tethered to a specific blastomere. In the blastocyst, the polar body remains attached to the trophectoderm adjacent to the margin of the ICM (Gardner, 1997). The position of the polar body defines a previously unrecognized axis of bilateral symmetry in the early mouse embryo that is orthogonal to the Em-Ab axis (Fig. 1). These intriguing findings indicate that the initiation of the embryonic axes of the mouse occurs during pre-implantation development. Whether or not the An-Vg and Em-Ab axes are aligned with any body axes of the postimplantation mouse embryo remains to be determined. Embryonic tissue mass can be altered experimentally by addition to or removal of cells from the pre-implantation embryos. If an axis-determination system is indeed operational during pre-implantation development, it is likely to act in a regulative manner so that any departures from the normal pattern can be reconciled. How these experimental embryos might assimilate the superfluous signals to achieve normal axis development remains enigmatic.

2.2. The axes of the pre-gastrula

Other morphological asymmetries are detected upon blastocyst implantation and in the egg cylinder stage mouse embryo. First, the mouse blastocyst displays an asymmetrical inner cell mass (Fig. 2A) and it often attaches to the uterine epithelium with its lateral mural trophectoderm (Smith, 1980). Second, the ectoplacental cone develops asymmetrically relative to the long axis of the egg cylinder (Fig. 2B). Third, the peri-implantation embryo does not have a symmetrical cylindrical shape and different curvatures are found on opposing sides of the embryo (Fig. 2C). The tilted orientation of the ectoplacental cone and these asymmetrical curvatures are indicative of the plane of the A-P axis but not its polarity (Smith, 1985; Gardner et al., 1992). The existence of morphological asymmetry in the mouse pre- and peri-implantation embryo suggests that the initial determination of the embryonic axes occurs prior to gastrulation. However, the A-P axis of the gastrulating embryos is often aligned orthogonally to the longitudinal axis of the uterine horn. This suggests that either there is a yet unknown uterine influence on the establishment of the A-P axis or that the embryos align their axes to the uterus during the pre-gastrulation period.

A growing number of genes have been identified that are expressed in patterns that indicate the A-P axis in pre-gastrula stage mouse embryos. An endoderm-associated antigen (VE-1) is first detected in the visceral endoderm on one side of the 5.0-day embryo and is later localized to the anterior visceral endoderm, providing one of the earliest markers of A-P polarity (Rosenquist and Martin, 1995). Posteriorly-restricted expression patterns in pre-gastrula mouse embryos include those of *Evx1*, *Fgf8*, *goosecoid* (*Gsc*) and *nodal*. *Evx1* and *Fgf8* are expressed in the posterior epiblast and the extraembryonic ectoderm shortly before the formation of the primitive streak (Dush and Martin, 1992; Crossley and Martin, 1995). *Gsc* transcripts are detected prior to gastrulation in the proximal-posterior epiblast, the prospective site of primitive streak formation (Faust et al., 1995). Nodal activity has been detected, using a *lacZ* reporter, in the presumptive posterior region of the epiblast prior to the formation of the primitive streak (Varlet et al., 1997). The loss of *Evx1* or *nodal* results in early embryonic lethalities prior to gastrulation (Table 1) (Conlon et al., 1991; Spyropoulos and Capecchi, 1994). Furthermore, studies by Conlon et al. (1994) suggest that nodal has a primary role in the induction and/or maintenance of the primitive streak. *Gsc* has been shown to be dispensable for gastrulation in the mouse (Rivera-Pérez et al., 1995; Yamada et al., 1995). *Fgf8* mutant mice have gastrulation defects but the precise nature of these abnormalities are still under investigation (G. Martin, pers. commun.). Although the precise roles of the genes discussed above in axis determination are yet to be determined, the observations that discrete A-P restricted expression domains in visceral endoderm and the epiblast exist in the pre-gastrula mouse embryo demonstrate that a molecular A-P asymmetry exists before the initiation of gastrulation.

2.3. Cell proliferation and the initiation and progression of gastrulation

The attainment of a threshold number of epiblast cells and the maintenance of active epiblast proliferation are critical for the initiation and progression of gastrulation. Mouse embryos that lack normal numbers of cells due to the disruption of cell proliferation or to cell losses delay gastrulation until the appropriate number of epiblast cells has been attained (Snow and Tam, 1979; Tam, 1988; Power and Tam, 1993). Mutant embryos that fail to sustain epiblast cell proliferation typically do not initiate gastrulation or arrest shortly after the formation of a rudimentary primitive streak (Table 1).

The attainment of a threshold number of epiblast cells, however, is not sufficient to initiate gastrulation. Double-sized mouse embryos generated by the aggregation of two morulae attain the critical cell number and form a proper pre-gastrula embryo earlier than normal embryos but do not initiate gastrulation at this stage. The double-sized embryos reduce cell proliferation and initiate gastrulation at the same time as their normal-sized littermates, suggesting that there is a chronological control of gastrulation (Buehr and McLaren, 1974; Lewis and Rossant, 1982; Rands, 1986). Thus, the onset of gastrulation is sensitive to, but not entirely

dependent upon, cell number or tissue volume of the epiblast (Henery et al., 1992). It will be interesting to determine if the schedule of morphogenetic events in under-sized and over-sized embryos is tightly coupled to the expression of genes that are activated prior to and/or during gastrulation.

Cell proliferation is almost certainly the major morphogenetic force that leads to the expansion of the germ layers. Indeed, the mitotic activity of the epiblast has been calculated to be sufficient to account for the entire increase in embryonic tissue mass during gastrulation (Snow, 1976, 1977; Poelmann, 1980; Poelmann, 1981a; MacAuley et al., 1993). The expansion of the mesodermal layer, however, may be driven by other form-shaping forces such as the propulsion generated by the incoming prospective mesoderm at the primitive streak and the tendency of the cells to disperse away from the primitive streak.

3. Regionalization of cell fate in the gastrula

The developmental fates of cells in the gastrulating mouse embryo have been extensively studied by the analysis of the cellular progeny of single marked epiblast cells or those derived from an orthotopically transplanted cell population (Beddington and Lawson, 1990). The results from these experiments reveal that the germ layers can be partitioned into domains of progenitor cells that give rise to the major tissue types. Fate maps are constructed for the germ layers of the gastrulating mouse embryo by collating the geographical distribution of the progenitors of foetal and

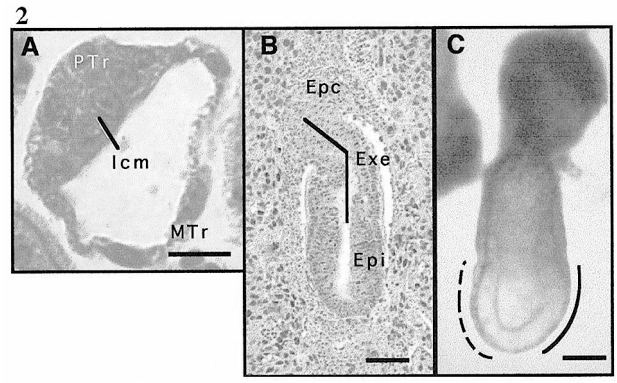


Fig. 2. Histology of mouse embryos showing (A) the asymmetrical shape of the inner cell mass (Icm) of the blastocyst, (B) the lopsided attachment of the ectoplacental cone (Epc) to the decidual tissues and (C) the unequal curvatures (solid versus dashed line) of the egg cylinder at 6.5 days p.c. shortly before the appearance of the primitive streak. Epi, epiblast; Exe, extraembryonic ectoderm; Mtr, mural trophectoderm; PTr, polar trophectoderm. Bar (A) 20 μm, (B,C) 50 μm.

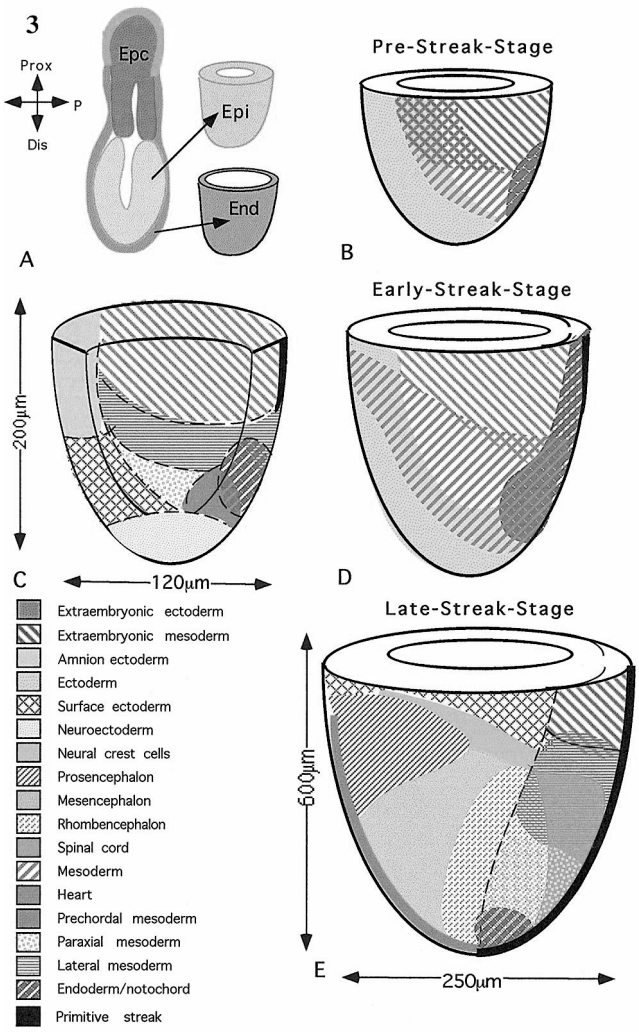


Fig. 3. Fate maps of the epiblast/ectoderm of mouse embryos at the pre-primitive-streak (pre-streak, 6.25 days p.c.), early-streak (6.5 days p.c.) and late-streak (7.5 days p.c.) stages (data from Beddington, 1981; Beddington, 1982; Tam and Beddington, 1987; Tam and Beddington, 1992; Tam, 1989; Lawson et al., 1991; Lawson and Pedersen, 1992a; Parameswaran and Tam, 1995; Quinlan et al., 1995; Quinlan and Tam, 1996; Tam and Quinlan, 1996). (A) The epiblast (Epi) and the primitive endoderm (End) of the egg cylinder can be separated into two cup-shaped epithelial layers. A-P, anterior posterior axis; Prox-Dist, proximal-distal axis; Epc, ectoplacental cone. (B) At the pre-streak stage, the epiblast can be broadly divided into domains of progenitors for tissues of the three classical germ layers (ectoderm, mesoderm and endoderm). The domains of different progenitors overlap, indicating that clonal descendants of cells localized in the overlapping zone contribute to multiple tissue lineages. (C,D) The regionalized pattern of cell fate in the early-streak epiblast resembles that of the pre-streak stage. (C) shows the right half of the epiblast and depicts a finer resolution of the fate map into domains of progenitors of specific ectodermal and mesodermal tissues. The delineation of these domains is based upon the predominant fate of cells in specific locations in the epiblast and has not taken the overlapping domains of tissue progenitors into account (shown in (D)). (E) The ectoderm of the late-streak stage embryo contains the progenitors of the neuroectoderm, the surface ectoderm and the neural crest cells. The precursor tissues for major segments of the neural tube can be mapped in their respective cranio-caudal order along the A-P axis. Mesodermal progenitors are still present in the ectoderm, mainly in the posterior region adjacent to the primitive streak (the dark bar). Not shown in these fate maps are the precursors of the primordial germ cells, which are localized amongst the progenitors of the extraembryonic mesoderm in the proximal epiblast of the early-streak embryo (Lawson and Hage, 1994; Tam and Zhou, 1996) and in the posterior segment of the primitive streak of the late-streak embryo (Ozdzenski, 1967; Snow, 1981; Copp et al., 1986; Ginsburg et al., 1990).

Table 1

Gene activity essential for gastrulation and formation of the body plan revealed by the impact of mutation

Gene/mutation	Gene structure or product	Expression during gastrulation	Mutant phenotype
Pre-gastrulation embryonic development: growth of the epiblast			
Brca1	Nuclear phosphorylation protein with amino-terminal ring finger motif	Unknown	Disorganized egg cylinder and no mesoderm formation (Liu et al., 1996; Ludwig et al., 1997)
Evx1	Homeobox-containing gene of the pair-rule *even-skipped* class	Posterior epiblast and proximal primitive streak (Bastian and Gruss, 1990; Dush and Martin, 1992)	Poor growth of the epiblast, may be due to defective primitive endoderm (Spyropoulos and Capecchi, 1994)
Fgf4	Secreted growth factor	Distal region of the primitive streak (PS) (Niswander and Martin, 1992; Bueno et al., 1996)	Inner cell mass fails to differentiate (Feldman et al., 1994)
fug1	Homologous to *RNA1* gene of the yeast	Ubiquitous in all tissues at early-PS stage	Arrested development of the egg cylinder (DeGregori et al., 1994)
rad51	Homologue of *E. coli* RecA that involves with DNA recombination repair	Unknown	Epiblast fails to proliferate and excessive apoptosis (Lim and Hasty, 1996)
Hnf4	Transcription factor of the hepatocyte nuclear factor family	Visceral endoderm of extraembryonic and proximal regions over the epiblast (Duncan et al., 1994; Taraviras et al., 1994)	Abnormal endoderm differentiation, degenerated epiblast and deficient extraembryonic mesoderm (Chen et al., 1994)
Requirement for initiation of gastrulation			
Bmpr	Type I serine/threonine kinase TGF-β family receptor	Ubiquitous in germ layers	Arrested at early gastrulation and no mesoderm formation (Mishina et al., 1995)
nodal	Member of the TGF-β family	Primitive streak and asymmetrical expression in perinodal endoderm (Zhou et al., 1993; Collignon et al., 1996; Lowe et al., 1996)	Hyperplasia but reduced cell size and extensive cell death, failure to form primitive streak and abnormal expression of mesoderm-specific genes in the epiblast (Iannaccone et al., 1992; Conlon et al., 1994)
msd	Gene within the albino (*c*)-deletion complex on Chr 7	Unknown	No primitive streak formation and absence of mesoderm (Holdener et al., 1994)
Hdh	Cytoplasmic protein (350 kDa), gene contains an N-terminal portion of polyglutamine repeat	Epiblast (early-PS) and then restricted mesoderm (late-PS) (Zeitlin et al., 1995)	Apoptosis of epiblast, primitive streak forms but fails to extend (Duyao et al., 1995; Zeitlin et al., 1995)
Global organization of the body plan at early gastrulation			
TRAFamn	Signal transducing protein associated with tumour necrosis factor receptors	Ubiquitous in all tissue of mid-PS stage embryo	Arrested in gastrulation, lacks amnion and no patterning of ectoderm and mesodermal derivatives (Wang et al., 1996)
Bmp4	Bone morphogenetic protein of the TGF-β family	Proximal region of the primitive streak	Variable penetrance, failure to initiate primitive streak formation to truncated or disorganized posterior structures (Winnier et al., 1995)
eed	Homologue of *Drosophila extra sex comb* of the *polycomb* group	Ubiquitous in germ layers	Reduced anterior primitive streak function and ectopic expression of node- and mesoderm-specific gene. Hypomorph shows posterior transformation of the skeleton (Faust et al., 1995; Schumacher et al., 1996)
Hβ58	Unknown function, identified by transgenic insertion on Chr 10, conserved among mammals and birds	Ubiquitous, weak expression in epiblast, strong in visceral endoderm (Lee et al., 1992)	Arrested epiblast growth, poor ectoderm and mesoderm differentiation, developmental retardation at organogenesis and overall reduction in embryonic size (Radice et al., 1991)
Hnf3β	Forkhead-domain protein	Proximal posterior epiblast (early-PS), anterior midline tissues (late-PS) and node notochord, floor plate and gut (head-fold stage) (Sasaki and Hogan, 1993; Ang and Rossant, 1994)	Absence of node and notochord, lack of dorso-ventral pattern in neural tube, discernible anterior-posterior pattern in the rudimentary neural tube (Ang and Rossant, 1994; Sasaki and Hogan, 1994), defective synthesis of growth factor and secreted protein of primitive endoderm (Farrington et al., 1997)

Table 1 (*continued*)

Gene/mutation	Gene structure or product	Expression during gastrulation	Mutant phenotype
inv	Identified by transgene insertion on Chr 4	Unknown	Complete reversal of left-right polarity of heart and abdominal viscera (Yokoyama et al., 1993), altered expression of TGF-β factors *lefty* (Meno et al., 1996) and *nodal* (Lowe et al., 1996)
Formation and migration of mesoderm			
t^{w18}	Recessive mutation of the *t*-complex (Crossley and Little, 1991)	Unknown	Mesoderm accumulates at the primitive streak (Snow and Bennett, 1978)
T	Transcription factor	Primitive streak and node	Retarded migration of the mesoderm through the primitive streak, defective formation of posterior mesoderm and notochord (Yanagisawa et al., 1981; Hashimoto et al., 1987; Wilson et al., 1995)
T^{wis}	Possibly a gain-of-function mutation of the *T* gene	Normal *T* expression in primitive streak and early node, diminishes by head-fold stage (Herrmann, 1991)	Poor differentiation of notochordal precursor (Conlon et al., 1995)
Brca2	Protein with 3418 amino acid, putative co-factor in Rad51-dependent repair process	No expression at 6.5 days, ubiquitous expression in all germ layers at 7.5 days	Mutant has initiated mesoderm formation but fails to sustain morphogenesis and displays extensive apoptosis (Sharan et al., 1997; Ludwig et al., 1997; Suzaki et al., 1997)
Fgfr1	Tyrosine kinase linked FGF receptor	Ectoderm and mesoderm of the anterior-distal part and the primitive streak of late-PS embryo (Yamaguchi et al., 1992)	Failure to form paraxial mesoderm but expansion of axial mesoderm (Deng et al., 1994; Yamaguchi et al., 1994)
Wnt3a	Secreted putative signalling molecule of the WNT family	Primitive streak including tissue immediately anterior of the node (late-PS)	Affects dorsal mesoderm but not lateral and caudal mesoderm of the trunk, lacks caudal somites, disorganized notochord and tailbud formation (Takada et al., 1994; Greco et al., 1996)
Patterning of anterior structures			
Lim1	Homeodomain protein with LIM domains	Proximal posterior epiblast (early-PS stage), mesoderm and primitive streak (mid-PS stage) and prechordal mesoderm and primitive streak (late-PS) (Barnes et al., 1994; Shawlot and Behringer, 1995)	Malformed egg cylinder, absence of a morphological node and lack of fore- and midbrain (Shawlot and Behringer, 1995)
Otx2	Homeobox gene related to *Drosophila orthodenticle*	Uniformly in epiblast (early-PS stage) and restricted to anterior ectoderm (late-PS stage), may be in anterior mesendoderm (Ang et al., 1996)	Malformed egg cylinder, lacks head structures anterior to the middle-hindbrain level (Acampora et al., 1995; Matsuo et al., 1995; Ang et al., 1996)

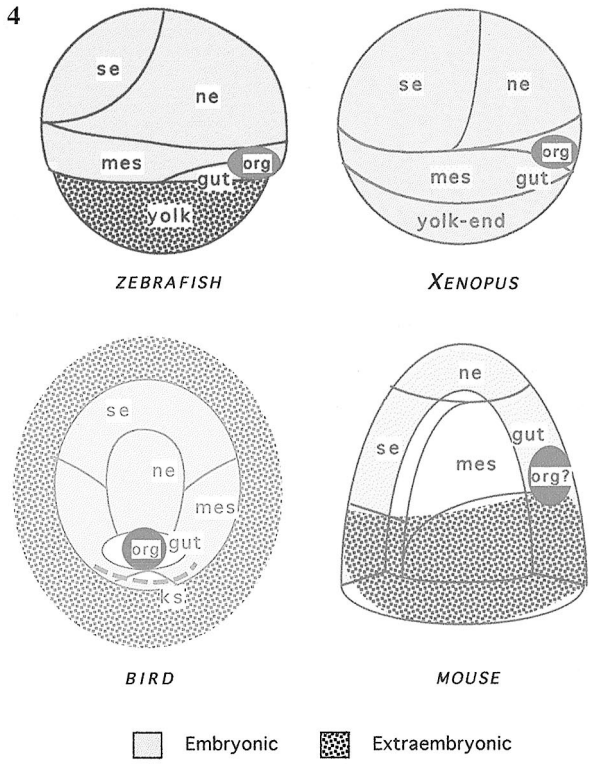
extraembryonic tissues. Fig. 3 shows a series of fate maps for the epiblast/ectoderm at the pre- to late-primitive streak stages of development.

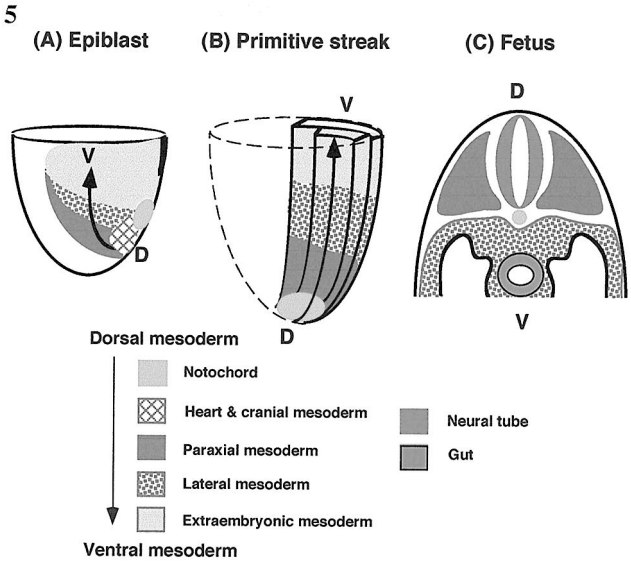
3.1. Delineation of the prospective embryonic and extraembryonic domains

In addition to the mouse, the body plan has been studied extensively in the gastrulae of the zebrafish, *Xenopus* and the chick. The fish and frog gastrulae are comprised of two major tissue compartments, i.e. the animal and the vegetal hemispheres (Fig. 4). The animal hemisphere of the zebrafish embryo contains all of the progenitors of the embryonic tissues including the endoderm, while the endoderm of the frog embryo arises from the vegetal cell mass. Unique to the avian and the mouse gastrulae is the formation of the extraembryonic tissues. A morphologically defined 'embryonic' region can be discerned in the mouse (the epiblast) and the chick (area pellucida). However, the mouse epiblast and the chick area pellucida contain not only the progenitors of the embryonic tissues but also those that form tissues of the extraembryonic membranes. The extraembryonic domains thus include the area opaca and part of the posterior area pellucida in the chick and the extraembryonic ectoderm, visceral endoderm and the proximal region of the epiblast in the mouse (Figs. 3C,D, and 4).

Comparisons between the body plans of these four vertebrate embryos suggest that the domains occupied by the progenitors of the major germ layer tissues in the blastoderm of the bird or in the epiblast of the mouse may be equivalent to the blastoderm in the animal hemisphere of the fish and frog gastrulae (Fig. 4). In the chick, the central and anterior regions of the area pellucida, which contain the prospective neuroectoderm and surface ectoderm, would be

equivalent to the frog embryo animal cap. In the mouse, the distal and anterior epiblast of the gastrula may be regarded as the animal cap equivalent. In the fish and frog gastrulae, the mesoderm progenitors are localized to the germ ring or the lateral marginal zone, respectively. The equivalent region in the chick gastrula would be the posterior and lateral part of the area pellucida. In the mouse gastrula, the posterior-lateral epiblast and the primitive streak would be the equivalent region. Therefore, in the mouse, like the fish, amphibian and avian gastrulae, the prospective mesoderm is found between the progenitors of the ectoderm and the vegetal/extraembryonic tissues.





3.2. Identification of the mouse gastrula organizer

Classical embryological experiments have identified the existence of a specific population of cells in the gastrulae of the zebrafish (the dorsal embryonic shield), the amphibian (dorsal blastopore lip), the bird (Hensen's node) and the mouse (the node) that act to organize the body plan (reviewed by Lemaire and Kodjabachian, 1996). A unique property of the organizer is its ability to induce the formation of an ectopic axis or to alter the pattern of the pre-existing neural and mesodermal tissues into a new embryonic axis following its transplantation to heterotopic sites. The node of the late-primitive-streak stage mouse gastrula has been shown to be able to induce the formation of a second axis that contains host-derived neural and somitic tissues (Beddington, 1994). However, the duplicated axis did not contain anterior structures, suggesting that either the host tissues have limited competence to respond to the inductive activity of the node or that the node at this stage only has partial organizer activity and is equivalent to the late-gastrula organizer of the frog and the bird (Dais and Schoenwolf, 1990; Lane and Keller, 1997). This implies that an organizer, capable of inducing more anterior structures, may exist earlier in mouse gastrulation prior to the morphological appearance of the node.

Fate mapping studies reveal that cells from the organizer can differentiate into axial mesoderm, such as the prechordal mesoderm and the notochord (or chordamesoderm). Other derivatives include the neuroectoderm (ventral tissues in the neural tube of the zebrafish and the floor plate in the chick and mouse), the gut endoderm (pharyngeal endoderm in the zebrafish and *Xenopus* and fore- to midgut endoderm in the chick and the mouse) and the somites (in zebrafish, *Xenopus* and chick) (Smith and Slack, 1983; Selleck and Stern, 1991, 1992; Beddington, 1994; Sulik et al., 1994; Shih and Fraser, 1995). The variable contribution of the organizer to non-notochordal tissues may be due to spe-

Fig. 4. A comparison of the fate maps of the early gastrulae of four vertebrate embryos showing similar regionalisation of the tissue precursors. The fish and frog embryos are shown in left-lateral views and the bird blastoderm is viewed dorsally. For the mouse embryo, the left half of the epiblast is presented in an inverted position with the proximal side down. References: zebrafish (Warga and Kimmel, 1990; Woo and Fraser, 1995; Woo et al., 1995; Melby et al., 1996), *Xenopus* (Keller, 1975, 1976), chick (Hatada and Stern, 1994), mouse (see Fig. 3 legend). ks, Koller's sickle; mes, mesoderm; ne, neuroectoderm; org, organizer; se, surface ectoderm; yolk-end, yolk-containing endoderm.

Fig. 5. (A) Regionalized cell fate of the epiblast of early-streak stage embryo suggest that a dorso-ventral (D-V) pattern of the mesodermal progenitors may be established in the epiblast before germ layer formation. (B) This early D-V relationship of the progenitors in the epiblast is maintained during the ingression of cells through the primitive streak of the late-streak stage embryo (Tam and Beddington, 1987; Smith et al., 1994). The heart mesoderm which has already ingressed is no longer present in the primitive streak at this stage. (C) The distribution of the different types of mesodermal tissues in the fetus is shown in a cross-section of the trunk region.

cies-specific differences in the constituents of the cell population in the organizer. The apparent differences in cell fates might also arise because the various vertebrate organizers are at different developmental stages when their lineage potencies are tested. It has been shown that the composition of the organizer of the *Xenopus* embryo changes during development (Lane and Keller, 1997). Currently, it appears that the organizer, if it is a single entity (see below), changes its cellular composition during gastrulation.

In non-mammalian embryos, cells that display organizer activity are found at the site of germ layer formation at the beginning of gastrulation (Fig. 4). In the mouse, the node which functions as an embryonic organizer is present only when gastrulation is well underway. If there is indeed a conserved mechanism of pattern formation in vertebrate gastrulae, then an organizer would be expected to be present earlier in mouse gastrulation. Several organizer-specific genes (*Sek2*, *Gsc*, *Hnf3β Follistatin* and *Lim1*; Fig. 7B,D,I) are expressed in the posterior epiblast at the junction of the domains for embryonic mesoderm and extraembryonic mesoderm at the initiation of gastrulation (Blum et al., 1992; Sasaki and Hogan, 1993; Ruiz and Robertson, 1994; Shawlot and Behringer, 1995). Fate mapping studies show that cells from this region of the epiblast contribute to typical node derivatives and post-nodal primitive streak cells, including the definitive endoderm, the notochord, the floor plate and the head process mesoderm (Fig. 3B,D) (Lawson et al., 1991). This group of cells can induce the formation of a second axis upon ectopic transplantation to the late-primitive-streak stage embryo, though still without any anterior (forebrain) characteristics (Tam et al., 1997b). Thus, the cell fate, gene activity and patterning activity of this population of cells suggest that it may be an embryonic organizer of the early gastrula. The lack of anterior structures in the induced axis suggests that a separate head organizer may be present outside of this population (see below).

3.3. Mesoderm patterning in the primitive streak

Fate mapping studies of the primitive streak in the mouse embryo have revealed that cells in different regions of the streak display different mesodermal fates (Tam and Beddington, 1987; Lawson et al., 1991; Smith et al., 1994; Wilson and Beddington, 1996). In the primitive streak of the late gastrula, the axial mesoderm (Axm) arises from the nodal region of the primitive streak, the paraxial mesoderm from the perinodal (anterior) segment of the streak, the lateral mesoderm from the mid-segment and the extraembryonic mesoderm (Exm) from the posterior segment (Fig. 5). Thus, the prospective dorsal and progressively more ventral mesoderm pattern aligns with the A-P axis of the primitive streak.

It is not known if there is a signalling mechanism that specifies the mesodermal lineage characteristics of cells ingressing through the different segments of the primitive streak (Sasaki and Hogan, 1993; Tam and Trainor, 1994). However, the phenotypes of mice with mutations affecting fibroblast growth factor (FGF), transforming growth factor-beta (TGF-β) and Wnt signalling suggest that these pathways may play a role in this process. Mice with mutations in the FGF receptor 1 (*Fgfr1*) gene are deficient in the formation of paraxial mesoderm (Table 1) (Deng et al., 1994; Yamaguchi et al., 1994). These observations could be interpreted as a lack of dorsalization of the mesoderm. In contrast, the loss of bone morphogenetic protein-4 (BMP-4) activity leads to a deficiency of ventral mesoderm derivatives in some embryos that develop to the organogenesis stage (Table 1) (Winnier et al., 1995). Interestingly, BMP-4 transcripts are detected in the posterior primitive streak of the gastrula (Winnier et al., 1995). Mutation of the *Wnt3a* gene results in a deficiency of trunk paraxial mesoderm and although epiblast cells can migrate through the primitive streak they do not move laterally and differentiate into ectopic neural tissue (Takada et al., 1994; Yoshikawa et al., 1997). These mutant phenotypes are consistent with an inappropriate allocation of cells to mesodermal lineages during cellular ingression in the primitive streak (Table 1).

4. Developmental potency of the germ layers

4.1. Changes during the differentiation of the epiblast to definitive ectoderm

Orthotopic transplantation of epiblast cells into the mouse gastrula show that cells from neighbouring regions of the epiblast often contribute to similar foetal tissues and differ only by their relative contributions to the various tissue types. Epiblast cell populations that are located further apart, however, tend to display more divergent cell fates and have less overlap in the types of tissues that they can produce (Fig. 3). Because between 5 and 20 cells are usually transplanted and analyzed in these types of experiments, the outcome represents the collective fate of the cell population. Therefore, it is not possible to determine if every cell in this population is pluripotent or if there is a mixed population of progenitors with heterogeneous cell fates. A more rigorous test of cell potency has been performed by the clonal analysis of epiblast cells. Individual cells in the epiblast have been found to contribute clonal descendants to more than one tissue type, demonstrating that these epiblast cells at the time of marking have not been restricted to a single lineage outcome and are therefore multipotent (Lawson et al., 1991). Thus, it appears that epiblast cells at the early-streak stage are not restricted in their potency. Interestingly, at this stage there are even epiblast cells that can give rise to both germline and somatic tissues (Lawson and Hage, 1994).

Another test of cell potency is to determine if cells can assume a different fate when they are confronted with a novel tissue environment, such as by transplanting them to heterotopic sites in the embryo. Results from these heterotopic transplantation experiments have shown that epi-

blast cells are capable of displaying the fate that is characteristic of the cells in the new location (Parameswaran and Tam, 1995; Tam and Zhou, 1996). Although cell plasticity has only been tested for cells localized at the anterior versus posterior and proximal versus distal regions of the epiblast, it is conceivable that cells throughout the epiblast may have equivalent potency. The finding that distal epiblast cells that normally are destined to form neuroectoderm can differentiate into cells with germline characteristics further suggests that epiblast cells are not only flexible in differentiation but are likely to be pluripotent.

Ectodermal cells of the late-primitive-streak stage embryo are able to differentiate into mesodermal tissues when they are tested by heterotopic transplantation (Beddington, 1982). However, the anterior (presumptive neural) ectodermal cells tend to differentiate mostly into neural tissues (Beddington, 1982). The clonal distribution of late-primitive-streak ectodermal cells marked by a retrovirally-introduced *lacZ* reporter gene has been examined. The clonal descendants were found, in contrast to the single cell clones derived from the early-primitive-streak epiblast (Lawson et al., 1991), to contribute to only one germ layer tissue type (Carey et al., 1995). The more limited fates of late-primitive-streak ectodermal cells suggest that there may be a restriction in their potency.

A further indication of the change in tissue potency is revealed by the inability of the anterior ectoderm to undergo haematopoiesis autonomously in vitro (Kanatsu and Nishikawa, 1996). It is not known if such lineage bias is caused by a restriction of potency or the selection against cells that display inappropriate differentiation in culture or at ectopic sites. The anterior ectoderm, however, can be induced to form blood cells by exposure to activin or BMP-4 (Johansson and Wiles, 1995; Kanatsu and Nishikawa, 1996), suggesting that for some tissues the apparent loss of potency may be due to the withdrawal of inductive factors from specific cell populations during gastrulation.

4.2. Potency and transit through the primitive streak

A restriction of potency may occur after ingression of epiblast cells through the primitive streak. Mesodermal cells when transplanted back to the epiblast are capable of re-ingressing through the primitive streak. Interestingly, although the transplanted mesoderm has acquired most of the potency that is characteristic of the epiblast, they are unable to colonize the lateral mesoderm (Tam et al., 1997a). This suggests that cells that have ingressed through the primitive streak are unable to regain a full range of tissue potency (Beddington et al., 1992). In contrast, epiblast cells that have been transplanted directly into the mesoderm display full differentiation potential and can contribute to more tissue lineages than the native mesodermal cells (Tam et al., 1997a). Therefore, cells that have not experienced ingression through the primitive streak can retain their original potency even though they are now amongst a post-ingression cell population in a different germ layer. The weight of evidence so far favours the maintenance of pluripotency of epiblast cells before ingression but there may be a restriction in potency associated with their transit through the primitive streak.

We are therefore confronted with an apparent paradox. At early gastrulation, individual cells are flexible in their potency but their location in the germ layer may influence their allocation to certain tissue lineages. It is possible that the apparent regionalization of cell fate is not a reflection of the state of cell fate determination but is an indication of how cells are distributed to different parts of the body during germ layer formation. The fate map therefore describes the allocation and not the determination of cell fate. The specification of tissue lineages may take place during the gastrulation movements of cells, or in specific cases, only after the cells have reached their final destinations in the embryo.

5. Morphogenetic movements during mouse gastrulation

The formation of the germ layers is accomplished by extensive morphogenetic movements of cell populations. Cell movement in the gastrulating mouse embryo has been analyzed primarily by marking or transplanting cells in embryos followed by mapping of the positions of the labelled cellular progeny at different times after culture in vitro. Alternatively, the pattern of cell movement for specific lineages can be inferred from the location of the lineage precursors in fate maps of consecutive developmental stages. There is, however, concern about the consistency and comparability of results obtained from different embryos for the collation of a consensus pattern of cell movement. Although the direct visualization of cell movement in the mouse embryo has been achieved in vitro, it can only be performed during a small window of development (Nakatsuji et al., 1986). The availability of stable and easily visualized vital cell markers (such as green fluorescent protein, GFP) should allow for the direct tracking of cell movement in live mouse embryos (Zernicka-Goetz et al., 1997; Zhuo et al., 1997).

5.1. The order of epiblast recruitment and movement through the primitive streak

The primitive streak forms as a site of cellular ingression in the posterior epiblast at the junction with the extraembryonic ectoderm (Hashimoto and Nakatsuji, 1989). As gastrulation progresses, the primitive streak lengthens, ultimately extending to the distal tip of the egg cylinder. It has been suggested that the extension of the primitive streak is caused by the intercalation of newly recruited cells between the proximal and the distal ends of the primitive streak. In older embryos, cells recruited to the primitive streak intercalate between those derived from the proximal

epiblast and those recruited earlier, now in the distal region of the primitive streak, resulting in the distal (or anterior, with regard to the body axis) extension of the primitive streak (Lawson et al., 1991).

Epiblast cells are recruited for ingression when the primitive streak has extended into their vicinity. The primitive streak at its inception is localized in the epiblast domain that contains the precursors of the extraembryonic mesoderm. This suggests that this will be the first tissue type that will emerge from the primitive streak of the early-primitive-streak embryo. Consistent with this idea is the finding that the mesoderm formed during the first 12 h of gastrulation is primarily composed of extraembryonic mesoderm (Parameswaran and Tam, 1995). By the mid-primitive-streak stage, the primitive streak has extended to encroach upon the precursors of the embryonic mesoderm and definitive endoderm in the epiblast. At this stage, precursor cells of the heart and the cranial mesenchyme are found in the distal region of the mesodermal layer near the anterior extremity of the primitive streak (Fig. 5) (Frohman et al., 1990; Parameswaran and Tam, 1995). For the endoderm, epiblast-derived cells that are destined for definitive endoderm of the foregut first appear in the endodermal layer at this stage (Lawson et al., 1986; Lawson and Pedersen, 1987).

There is also a sequential recruitment to the primitive streak of epiblast cells located at different distances from the primitive streak. Cells nearer to the primitive streak, such as those in the posterior and lateral epiblast, are recruited to the streak ahead of those that are located further away in the anterior epiblast (Lawson et al., 1991; Lawson and Pedersen, 1992a, 1992b). The epiblast cells are recruited first to cranial mesoderm and foregut endoderm and then to caudal mesoderm and hindgut endoderm (Tam and Beddington, 1987, 1992). The progenitors of the mesodermal tissues are positioned in a posterior to anterior order in the lateral epiblast of the early gastrula that is in opposite polarity to the A-P body axis of the embryo. Therefore, a reversal of A-P polarity of the mesodermal precursors occurs as they move through the streak to form the mesoderm.

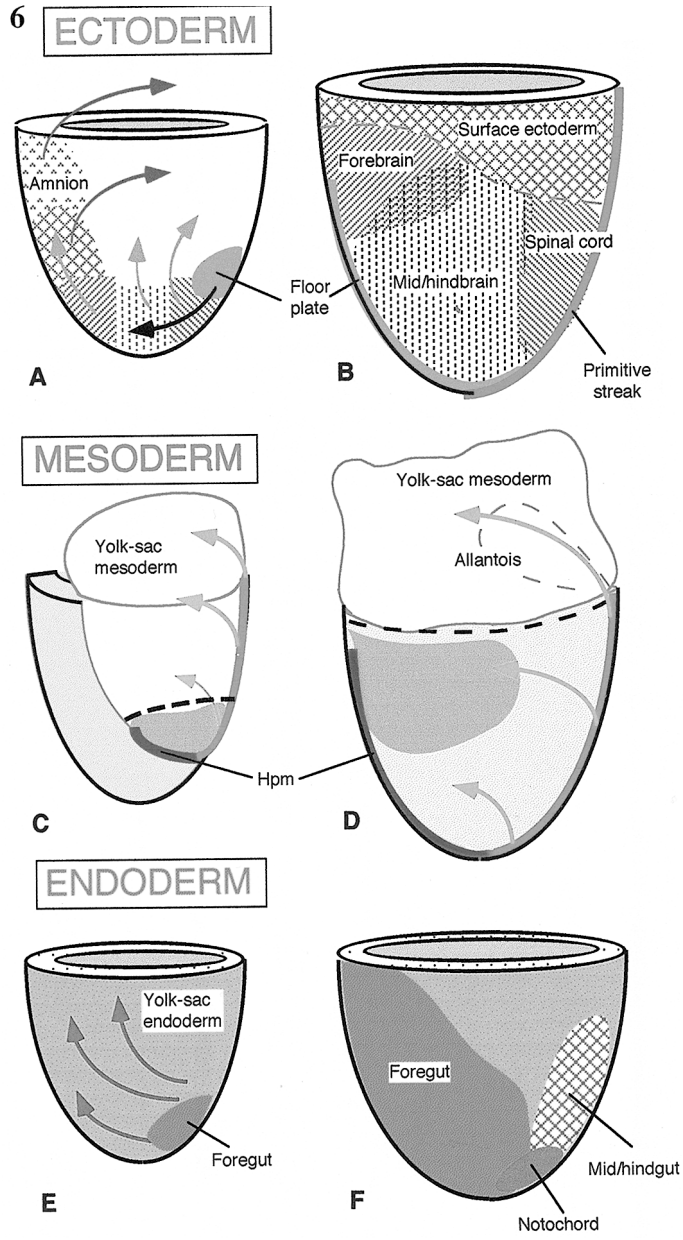
The migration of prospective mesodermal cells through the primitive streak appears to be regulated by the activity of growth factors. Blocking FGF activity by a mutation in one of the FGF receptors (FGFR1) results in the retention of the ingressed mesodermal cells at the primitive streak (Deng et al., 1994; Yamaguchi et al., 1994; Ciruna et al., 1997). This may be caused by inhibitory effects on cell migration that may be similar to the blockage of dorsolateral mesoderm migration in *Drosophila Heartless* (DFR1/DFGF-R2) mutants (Gisselbrecht et al., 1996). Mesoderm migration abnormalities are also associated with the *Brachyury* mutation in mice (Wilson et al., 1995), which may be mediated through alterations in FGF signalling.

If the emergence of the mesoderm from the primitive streak represents the order of mesoderm induction during gastrulation, then this implies that the extraembryonic and ventral mesoderm are the first to be induced followed later by the dorsal mesoderm. Unfortunately, there is little known about the process of mesoderm induction in the mouse. Growth factors, such as those of the TGF-β and FGF families, are effective at inducing mesoderm in *Xenopus* gain-of-function assays (Cunliffe and Smith, 1994; Slack, 1994; Smith, 1995). A number of these growth factors are expressed in the mouse gastrula (FGF-3, Wilkinson et al., 1988; FGF-4, Feldman et al., 1994; Bueno et al., 1996; FGF-5, Haub and Goldfarb, 1991; Hèbert et al., 1991; FGF-8, Crossley and Martin, 1995; Bueno et al., 1996; BMP-4, Winnier et al., 1995). However, these factors, with the exception of BMP-4 (Table 1) and possibly FGF-8, do not appear to have an essential role in mesoderm formation in mouse embryos. Mouse embryos that lack activin or the type II activin receptor display no disruption in gastrulation or mesoderm formation, indicating that zygotic expression of activins is not essential for mesoderm induction (Schrewe et al., 1994; Vassalli et al., 1994; Matzuk et al., 1995a, 1995b). However, there may be a maternal source of activins that is important for mesoderm induction in the mouse (Albano et al., 1994). Mutations that affect the formation of mesoderm in the mouse gastrula have been identified (Table 1) but the precise cause of the mutant phenotype is not fully understood. Thus, the factors that are essential for mesoderm induction in the mouse embryo remain essentially unknown.

5.2. Epiblast cells are displaced proximally and posteriorly towards the primitive streak

The descendants of single cells originally located in the proximal and the lateral regions of the epiblast are distributed in trails leading towards the primitive streak (Lawson et al., 1991). This pattern of epiblast cell displacement is consistent with the recruitment of prospective mesodermal cells to the primitive streak. However, clonal descendants of single cells seldom remain together and labelled progeny originating from different sectors are often intermingled (Lawson et al., 1991). This suggests that there may be frequent changes in the relationships between epiblast cells as they are displaced towards the primitive streak.

During gastrulation, the prospective neuroectodermal cells in the distal epiblast are displaced proximally and expand into the area previously occupied by the prospective mesodermal cells (Fig. 6A,B) (Quinlan et al., 1995). Expansion of the neuroectodermal precursors is not random. Cells from different sections of the distal epiblast along the A-P axis are allocated to specific segments of the neural tube, suggesting that there may already be an early A-P pattern set up in the progenitor population. The prospective surface ectoderm moves from anterior to proximal sites in the epiblast during gastrulation. The displacement of the prospective ectoderm is therefore directed proximally and posteriorly and the whole epiblast expands as a coherent sheet of tissue.



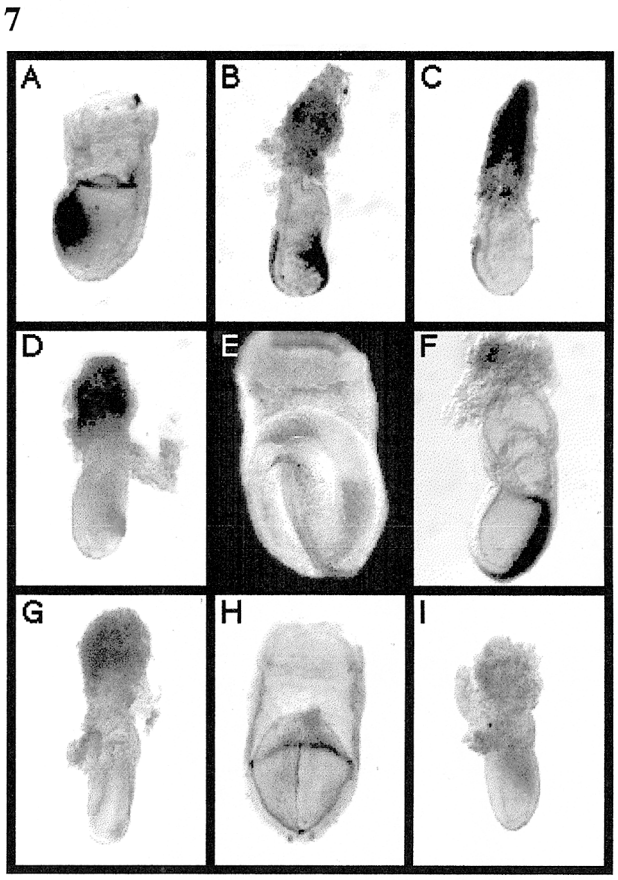


Fig. 6. Morphogenetic movement of germ layer tissues during gastrulation of the mouse embryo (data from Lawson et al., 1986; Lawson et al., 1991; Nakatsuji et al., 1986; Lawson and Pedersen, 1987; Tam and Beddington, 1992; Parameswaran and Tam, 1995; Quinlan et al., 1995; Thomas and Beddington, 1996; Tam et al., 1997a). (A) Ectoderm. The progenitors of the surface ectoderm and the amnion move proximally and posteriorly to the proximal region of the ectoderm (green arrows). The epiblast in the distal region expands proximally and posteriorly (red arrows). Cells from different sectors of the distal epiblast move to occupy different segments of the neural tube in the ectoderm of the late-streak embryo (B). The progenitors of the floor plate are initially localized posterior to those of the spinal cord. The descendants of this population are distributed anteriorly along the midline of the neural primordium (black arrow). (C) Mesoderm. Cells emerging from the primitive streak move away from the streak over the epiblast and cross over to the extraembryonic ectoderm. The shaded region in the mesoderm (C,D) represents the heart and cranial mesoderm which are displaced proximally and anteriorly (purple curved arrows) during gastrulation. The dashed lines indicate the position of the frontier of the embryonic mesoderm at the (C) mid-streak and (D) late-streak stage. Some cells that are positioned near the edge of the mesoderm are later found in the midline and may become part of the head process mesoderm (hpm). (E) Endoderm. At the early- to mid-streak stages, the definitive endoderm is recruited from the epiblast and ingressed through the anterior region of the primitive streak. The new endodermal population is integrated into the pre-existing visceral endoderm by cellular intercalation. The visceral endoderm is progressively displaced in an anterior-proximally direction (purple arrows) to the yolk sac by the incoming definitive endoderm. (F) Cells destined for different segments of the gut are recruited sequentially and occupy different regions of the endodermal layer at the late-streak stage.

Fig. 7. Molecular heterogeneity of the germ layer tissues in the early mouse embryo as revealed by regionalized gene expression. (A) *Otx2*, neural plate stage; (B) *Lim1*, late-streak stage; (C) *Cerr1*, early-streak stage; (D) *gsc*, mid-streak stage; (E) *Hnf3β*, head-fold stage; (F) *Brachyury*, neural plate stage; (G) *chordin*, mid- to late-streak stage; (H) *nodal*, neural plate stage; (I) follistatin, mid-streak stage. All embryos are lateral views with anterior to the left and posterior to the right, except for *nodal* in which the neural plate faces the viewer. Embryos are staged according to Downs and Davies (1993).

5.3. Midline ectoderm is displaced anteriorly

The floor plate of the neural tube is a derivative of midline ectoderm that is derived from the distal epiblast and also from the posterior epiblast of the early gastrula (Lawson et al., 1991). Cells derived from the posterior epiblast are found to contribute to the floor plate of the brain (Lawson et al., 1991; Tam et al., 1997b). This colonization of the anterior floor plate by cells originating from the posterior epiblast necessitates an anterior movement of prospective floor plate cells in the opposite direction of the general posterior movement of the prospective neuroectoderm (Fig. 6A,B).

During gastrulation, the A-P axis of the mouse embryo grows by about 3.5-fold in length. This is achieved by the proliferation of epiblast cells and the elongation of the primitive streak (Lawson et al., 1991). In the chick, *Gsc*- and *Hnf3β*-expressing cells (presumptive node cells) first found near the posterior margin of the blastoderm are translocated anteriorly as the primitive streak elongates (Izpisúa-Belmonte et al., 1993; Ruiz i Altaba et al., 1995). Similarly, anterior extension of the primitive streak might also account for the relocation of the precursors of the midline ectoderm from the proximal to the distal position in the cylindrical mouse gastrula. Further extension of midline tissues anterior to the node of the late-primitive-streak embryo requires a different morphogenetic mechanism such as convergent extension of the midline mesoderm found in the frog embryo (Wilson and Keller, 1991; Keller et al., 1992; Shih and Keller, 1994; Domingo and Keller, 1995). It is currently not known if convergent-extension of midline cells occurs in the mouse gastrula. However, the distribution of labelled cells in the notochord after the node region has been marked appears to be consistent with the involvement of these morphogenetic movements (Beddington, 1994).

5.4. Maintenance of tissue relationships during gastrulation

A concerted movement during gastrulation of the mesoderm and the definitive endoderm germ layers has been discovered by fate map comparisons and the tracking of cell movements. The analysis of the direction of tissue expansion reveals that the mesoderm and the definitive endoderm move in concert in a direction opposite to that of epiblast expansion.

In the mesoderm, the precursors of the heart and cranial mesoderm move from the distal to the anterior and proximal region of the germ layer and displace the precursors of the extraembryonic mesoderm to the yolk sac (Fig. 6C,D) (Parameswaran and Tam, 1995). In the endoderm, the cells recently recruited from the epiblast are incorporated into the endoderm in the posterior distal region of the gastrula. This new population of definitive endoderm then expands in an anterior-proximal direction and displaces most of the pre-existing visceral endoderm to the yolk sac (Fig. 6E,F) (Lawson et al., 1986; Lawson and Pedersen, 1987; Tam and Beddington, 1992). The morphogenetic force required to displace the visceral endoderm towards the extraembryonic region may be derived from the propulsion generated by the newly recruited endodermal cells inserting into the visceral endoderm. Although yet unproven, it is also possible that the expansion of the proximally-located yolk sac cavity may provide an additional traction force that draws the visceral endoderm and extraembryonic mesoderm away from the embryonic region of the gastrula. This model for germ layer morphogenesis predicts that a functional deficiency of the endoderm (Farrington et al., 1997) and the failure to sustain the expansion of the extraembryonic cavity will disrupt the morphogenesis of the germ layers and lead to abnormal embryonic shape during gastrulation. Consistent with this idea are the findings that mutations in several genes such as *Hnf3β*, *Lim1* and *Otx2* result in abnormal morphogenesis of the mouse gastrulae characterized by a constriction between the embryonic and extraembryonic regions of the gastrula (Ang and Rossant, 1994; Shawlot and Behringer, 1995; Ang et al., 1996).

Although the co-migration of heart mesoderm and foregut endoderm has not been demonstrated directly in the mouse, the temporal and spatial coincidence of these two tissues as revealed by fate mapping studies suggests that they maintain a constant relationship that is established while they are in the epiblast through every stage of gastrulation movement (Keller and Tibbetts, 1989; Garcia-Martinez and Schoenwolf, 1992; Schoenwolf et al., 1992). The maintenance of a constant tissue relationship suggests that tissue movement is highly organized during gastrulation and that the correct juxtaposition of progenitor tissues may facilitate the inductive interactions that are critical for morphogenesis (Jacobson and Sater, 1988; Sugi and Lough, 1994; Nascone and Mercola, 1995).

The mechanisms that regulate the assortment of ingressing cells to the mesoderm and endoderm are not known. However, there are indications that selective cell adhesivity (Takeichi, 1988) and integrin-mediated interactions with extracellular matrix (Burdsal et al., 1993; Hatta and Takahashi, 1996) may be involved in this process. The epithelial to mesenchymal transformation of cells at the primitive streak is accompanied by the novel expression of a combination of cadherins and integrins (Takeichi, 1988; Burdsal et al., 1993). Although the activity of these cell adhesion molecules may influence mesoderm migration it does not appear to be essential for the progression of gastrulation. For example, N-cadherin is expressed during mouse gastrulation in the mesoderm. However, N-cadherin mutant embryos do not have gastrulation defects (Radice et al., 1997). The deletion of integrin $\beta 1$ leads to a pre-gastrulation embryonic death (Fässler and Meyer, 1995; Stephens et al., 1995). Although the loss of fibronectin does not affect the specification of mesodermal cells it impairs the morphogenesis of the somites and the notochord (Georges-Labouesse et al., 1996). Furthermore, a null mutation of the focal adhesion kinase (FAK) that mediates cell adhesion impedes

mesoderm movement but not gastrulation (Furuta et al., 1995). Thus, genetic evidence for an adhesion molecule essential for germ layer formation has yet to be described.

6. Inductive interactions during gastrulation

6.1. The induction of the primitive streak

In *Xenopus*, the formation of the organizer is postulated to result from inductive interactions between the dorsal vegetal cells (the Nieuwkoop centre) and the dorsal marginal cells in the animal hemisphere (Vodicka and Gerhart, 1995). It has been suggested that this is mediated between the Wnt signalling pathways and the activity of TGF-β molecules such as activin, BMP and Vg1 (Watabe et al., 1995; Cui et al., 1996). In the chick embryo, the *Vg1* homologue (*cVg1*) is expressed in the cells of the posterior marginal zone and in the adjacent Koller's sickle (Selerio et al., 1996). Ectopic expression of *cVg1* results in the formation of an ectopic primitive streak, suggesting that cells in the posterior marginal zone may act as a source of signals that induce the formation of the primitive streak (Stern, 1990; Eyal-Giladi et al., 1994). The *cVg1* expression domain encompasses the *Gsc*-expressing cell populations in and anterior to Koller's sickle (De Robertis et al., 1994). Lineage tracing experiments show that these *Gsc*-expressing cells are the precursors of the cells that later colonize Hensen's node of the stage 3–4+ gastrula (Izpisúa-Belmonte et al., 1993). If the formation of the ectopic primitive streak is a response that is similar to the induction of the organizer, then the equivalent of the chick Nieuwkoop centre would therefore be localized to the posterior marginal zone of the blastoderm that is immediately posterior to the early organizer.

Little is known about the molecular mechanisms that induce the formation of the primitive streak in the mouse. If an analogy can be drawn between the chick and the mouse, both of which utilize the primitive streak as the conduit for gastrulation, then fate map comparisons between these two species would indicate that the tissue (the mouse Nieuwkoop centre) that would induce the formation of the mouse primitive streak should be localized to the most proximal region of the posterior side of the epiblast and may also include the adjacent extraembryonic ectoderm (Fig. 4). As described earlier, these embryonic regions will give rise to extraembryonic tissues. If the above hypothesis is true, then these tissues would be expected to express the mouse homologues of genes that induce the organizer in other species (Lemaire et al., 1995). If these molecules have essential functions in a mouse Nieuwkoop centre equivalent, then a loss-of-function situation would be predicted to lead to a lack of primitive streak formation. A possible outcome of the constitutive over-expression of these molecules would be the development of supernumerary body axes.

In pre- and early-primitive-streak stage embryos, cells of the proximal posterior epiblast express *Brachyury*, *Evx1*, *Fgf8* and *nodal* (Herrmann, 1991; Dush and Martin, 1992; Crossley and Martin, 1995; Thomas and Beddington, 1996; Varlet et al., 1997). *Brachyury* mutants form a primitive streak. *Evx1* has been found to be essential for development beyond the egg cylinder stage (Spyropoulos and Capecchi, 1994). As mentioned above *Fgf8* mutants have gastrulation defects (G. Martin, pers. commun.). Interestingly, *nodal* mutants do not form a primitive streak, a predicted phenotype for the loss of a mouse Nieuwkoop centre (Conlon et al., 1991, 1994). However, *nodal* mutants express *Gsc*, an organizer-specific marker (Conlon et al., 1994). Obviously, there are still many studies needed to determine if a Nieuwkoop centre equivalent truly exists in the mouse.

6.2. The inductive activity of the mesendoderm

Recent studies have implicated the combined mesoderm and endoderm (the mesendoderm) as an important source of signals for the organization of the mammalian body plan. In the early epiblast, cells that are allocated to the neuroectoderm remain pluripotent during gastrulation (Lawson et al., 1991; Tam and Zhou, 1996). Regionalization of the neuroectoderm has been shown to be the result of inductive interactions with the underlying mesendoderm. Tissue recombination experiments in the mouse have shown that a positive signal generated by the anterior mesendoderm can induce or maintain the expression of anterior neural markers, *Otx2* and *engrailed*, in the ectoderm (Ang and Rossant, 1993; Ang et al., 1994). In contrast, posterior mesendoderm fails to induce *engrailed* expression in ectoderm and suppresses *Otx2*. The patterning of the neural primordium as revealed by the progressive restriction of *Otx2* to the prospective cranial neuroectoderm during gastrulation therefore appears to be regulated by inductive and inhibitory signals produced by the mesendoderm that are later associated with the cranial neural plate. *Otx2* mutations in the mouse result in the loss of the forebrain and midbrain regions (Acampora et al., 1995; Matsuo et al., 1995; Ang et al., 1996). Expression of *Otx2* is observed not only in the ectoderm but also in the mesoderm and endoderm in the midline underneath the prospective neural plate (Fig. 7A, Table 1). Whether *Otx2* intrinsically defines the neural characteristics of the ectoderm and/or the inductive ability of the mesendoderm remains to be clarified.

The induction of neural markers in the epiblast by the combined mesendoderm raises the possibility that inductive signals may emanate from either the mesoderm or the endoderm. A functional deficiency of the endoderm caused by mutations in *Hnf3β*, *Lim1*, *nodal* and *Otx2* (Table 1) may be the probable cause of the abnormal morphology of these mutant embryos during gastrulation (Ang and Rossant, 1994; Shawlot and Behringer, 1995; Ang et al., 1996; Varlet et al., 1997). The homeobox gene *Hesx1*/*Rpx* is transiently expressed in a patch of anterior visceral endoderm in the early mouse gastrula (Hermesz et al., 1996; Thomas and Beddington, 1996). *Hesx1*/*Rpx* expression subsequently is found in the anterior ectoderm overlying the visceral endo-

derm expression domain suggestive of a homeogenetic induction. Surgical removal of the *Hesx1*/*Rpx* expressing visceral endoderm leads to abnormal anterior neural development (Thomas and Beddington, 1996), suggesting a role for anterior visceral endoderm in anterior neural patterning. Consistent with this idea are the findings that *Hesx1*/*Rpx* mutant mice have anterior neural abnormalities (K. Mahon, pers. commun.). However, it is not yet clear if these defects are caused by the mutant endoderm, the mutant prospective prosencephalic ectoderm, or both tissues. A role for anterior visceral endoderm in anterior neural development has also received support from studies of mouse chimeras generated with *nodal* mutant cells that have abnormal forebrain and midbrain development (Varlet et al., 1997). Further evidence to support the idea of the mesendoderm as a source of inductive signals comes from the discovery of an inductive activity in the anterior endomesoderm of the *Xenopus* embryo encoded by the *Cerberus* gene (Bouwmeester et al., 1996; Sasai et al., 1996). A mouse *Cerberus*-related cDNA (*Cerr1*) has recently been shown to be expressed in the anterior visceral endoderm and the prospective definitive endoderm of the gastrula (Fig. 7C) (W. Shawlot, pers. commun.). Whether this mouse gene is the functional homologue of the *Xenopus Cerberus* gene is not known.

6.3. Formation of the head process

At late gastrulation, a condensed strip of tissue is found underlying the midline of the prospective forebrain and midbrain ectoderm and joins posteriorly with the node at the anterior tip of the primitive streak. Cells in this midline tissue, known as the head process, are closely apposed to one another and acquire a polygonal shape (Poelmann, 1981b; Tam et al., 1993). The endodermal cells that are immediately subjacent to the head process are organized into rosettes and are morphologically distinct from the endoderm located more laterally (Tam and Meier, 1982). During neurulation, the head process cells flatten and form the midline mesoderm underlying the neural groove of the cephalic neural tube (Meier and Tam, 1982). The anterior end of the midline mesoderm merges with the foregut endoderm and forms a localized patch of mesoderm called the prechordal plate (mouse, Meier and Tam, 1982; chick and quail, Seifert et al., 1992, 1993). In the avian embryo, the prechordal plate contributes to cells of the muscles (Couly et al., 1992). Although a distinction between the head process and the prechordal mesoderm has been made in the avian embryo (Seifert et al., 1992, 1993), it is not clear if these two structures can be distinguished anatomically in the mouse (Poelmann, 1981b). Nothing is known about the fate of the head process in the mouse but it is thought to become part of the notochord of the head region (Seifert et al., 1993). The anterior midline mesoderm expresses genes that are also found in the notochord such as *Brachyury* (Fig. 7F) (Herrmann, 1991), *cordon-bleu* (Gasca et al., 1995), *Gsc* (Filosa et al., 1997), *Hnf3β* (Fig. 7E) (Sasaki and Hogan, 1993; Ang and Rossant, 1994; Ruiz i Altaba et al., 1995), *Notch2* (Williams et al., 1995) and *shh* (Echelard et al., 1993). At this moment, in the mouse, it may be more appropriate to refer to the midline mesoderm anterior to the node at the late gastrula stage as the head process mesoderm.

It is not entirely clear how the head process is formed during gastrulation. In the chick, the continuity of the head process with the node suggests that head process cells might be produced by the anterior movement of node-derived cells (Psychoyos and Stern, 1996a). The formation of the head process by an extension of the mesodermal tissue is clearly divergent from the formation of the rest of the midline mesoderm of the body axis. During post-gastrulation development in the avian embryo, the primitive streak recedes to the posterior region of the embryo as the axis elongates (Bellairs, 1986). The node as an integral part of the anterior end of the primitive streak regresses along the cranio-caudal axis and as it does so, it leaves a trail of cells which forms the notochord (Catala et al., 1996) that extends posteriorly from the junction with the head process mesoderm. Two other possibilities for head process formation may be considered. First, the head process may be formed by cells derived from the two opposing mesodermal wings as they merge at the midline from the two sides of the embryo (Tam et al., 1993, 1997b). However, some genes that are expressed later in the head process (*Brachyury*, *Gsc*, *Lim1* and *Otx2*) are expressed in the tissues at the anterior midline of the gastrulating embryo before the mesodermal wings have met at the midline (e.g. *Lim1*; Fig. 7B). This suggests that tissues other than the head process are expressing these genes or certain components of the head process are derived from sources other than the node and the primitive streak. This raises the second possibility that the head process may be derived locally from the anterior endoderm. During the formation of the notochord, the prospective notochordal cells are first organized into an epithelial structure immediately anterior to the node (the notochordal plate). The notochordal plate is contiguous with the endoderm and the notochord is formed as the cells of the notochordal plate move to a mesodermal position and are re-organized into a cord (Jurand, 1974; Poelmann, 1981b; Sulik et al., 1994). It is possible that the head process is formed by a similar mechanism from the anterior endoderm and that the early expression of head process genes is in the endodermal precursor of the head process. This concept is compatible with the results of cell lineage studies of the endoderm that show that not all visceral endoderm is replaced by the primitive streak-derived endoderm and that some descendants of the visceral endoderm may remain in the embryonic foregut (Lawson et al., 1986; Tam and Beddington, 1992).

6.4. The head and trunk organizers

Several lines of evidence suggest that the refinement of the basic body plan involves separate organizing activities

for the head and the trunk. The *Xenopus* organizer is comprised of cell populations that are fated to become chordamesoderm of different parts of the body (Lane and Keller, 1997). Cells in the organizer can also be segregated into domains characterized by the expression of a set of genes such as *Gsc*, *noggin*, *Siamois*, *Xlim-1*, *Xbra* and *Xnr3* (Taira et al., 1992; Lemaire et al., 1995; Vodicka and Gerhart, 1995). The organizer also expresses these genes in a stage- and region-specific manner and a combinatorial genetic activity may be responsible for the developmental changes in the patterning and inducing activity of the organizer (Gilbert and Saxen, 1993; Vodicka and Gerhart, 1995). These data suggest that there are separable head- and trunk-inducing activities in the organizer.

In the mouse embryo, some genes that are expressed in the putative early organizer such as *eck*/*Sek2* (Ruiz and Robertson, 1994), *Gsc* (Blum et al., 1992; De Robertis et al., 1992) and *Lim1* (Barnes et al., 1994; Shawlot and Behringer, 1995) are later shifted to the anterior midline tissues and are no longer expressed in the node by late gastrulation (Fig. 7B,D). A parallel therefore can be drawn between the developmental change in the organizer of the mouse and *Xenopus* regarding the transition from a 'late blastula' to a 'gastrula' organizer as head organizing activity diminishes (Vodicka and Gerhart, 1995). This suggests that two discrete populations of cells arise in the organizer whose derivatives may have distinct organizer activity for the head and trunk. Transplantation of the node of late-primitive-streak stage mouse embryo to the lateral region of a synchronous host embryo can induce a secondary neural axis, demonstrating that the mouse node has organizer activity (Beddington, 1994; Tam et al., 1997b). Interestingly, the node at this stage was not able to induce the most anterior neural tissues, indicating that at this stage the node may function primarily as a trunk organizer. However, these observations could also be due to the reduced competence of the late-primitive-streak embryo to respond to head organizer activity. It might be informative to test the late-primitive streak stage node's organizing activity in the epiblast of early-primitive-streak stage embryos or perhaps in chick embryos.

The identification of genes expressed in embryonic organizers and their subsequent mutation by gene targeting using mouse ES cells has been of great benefit to the characterization of the genetic pathways that regulate the formation of the body plan in the mouse (reviewed by St.-Jacques and McMahon, 1996). Recently, genetic evidence for distinct head and trunk organizers in the mouse embryo has been accumulating. Mutation of the *Lim1* gene demonstrates that its activity is critical for the formation of anterior head structures (Shawlot and Behringer, 1995). In these mutants all head structures anterior to rhombomere 3 of the hindbrain are missing yet the rest of the body axis is normal. These results suggest that *Lim1* defines a genetic pathway for the head organizer. In addition, mice lacking *Otx2* have a mutant phenotype that is strikingly similar to the *Lim1* mutant phenotype, in that they lack structures anterior to rhombomere 3 (Acampora et al., 1995; Matsuo et al., 1995; Ang et al., 1996). The similar phenotypes of the *Lim1* and *Otx2* mutants suggest that these genes may be in the same genetic pathway for head organizer function. The recent observations of *Hesx1*/*Rpx*, *nodal* and *Cerberus*/*Cerr1* discussed above suggest that, in the near future, these genes will be ordered in a genetic pathway defining the head organizer.

One gene that has provided some surprises is *Gsc* (Fig. 7D). *Gsc* was originally identified in *Xenopus* in a screen for novel homeobox genes (Blumberg et al., 1991). *Gsc* homologues have been identified in many vertebrates species as well as in *Drosophila* (Blum et al., 1992; Izpisúa-Belmonte et al., 1993; Stachel et al., 1993; Schulte-Merker et al., 1994; Goriely et al., 1996; Hahn and Jäckle, 1996). In vertebrate gastrulae, *Gsc* expression marks tissues with organizer activity. In the mouse gastrula, *Gsc* is expressed in the anterior primitive streak, suggesting a role in anterior development (Blum et al., 1992). In gain-of-function assays performed with *Xenopus* embryos, *Gsc* mRNA can induce the formation of a secondary body axis, mimicking the activity of the organizer (Cho et al., 1991). Surprisingly, mice lacking *Gsc* have no apparent defects in gastrulation or axis formation (Rivera-Pérez et al., 1995; Yamada et al., 1995). It is possible that other genes may compensate for the absence of *Gsc*.

Gsc and *Hnf3β* have recently been shown to be co-expressed in all three germ layers in the anterior region of head-fold to early somite stage mouse embryos (Filosa et al., 1997). *Gsc* −/−; *Hnf3β* +/− mouse embryos have anterior defects, including abnormalities in the forebrain, optic vesicles, neural tube, branchial arches and heart, that are not observed in either *Gsc* −/− or *Hnf3β* +/− mice, suggesting that *Hnf3β* can compensate for a lack of *Gsc* in head-fold and early somite stage embryos (Filosa et al., 1997). Recently, a second *GSC*-related gene (*GSX*) has been isolated in the chick (Lemaire et al., 1997). Initially, *GSC* and *GSX* are co-expressed in the primitive streak but during gastrulation *GSX* expression segregates to the neural plate and posterior primitive streak while *GSC* expression is found in the node and prechordal plate. Intriguingly, a *Gsc*-related (*Gscl*) gene has also been reported in the mouse that maps to chromosome 16 in a region that is syntenic with human chromosome 22q11.2 that contains the minimal critical region that is deleted in patients with the DiGeorge and velocardiofacial syndromes (Galili et al., 1997; Gottlieb et al., 1997). *Gscl* transcripts are detected in 6.5 days p.c. mouse embryos by RT-PCR, indicating that *Gscl* may interact with *Gsc* during gastrulation (Wakamiya et al., 1997).

Targeted mutagenesis in mouse ES cells (*Hnf3β*, *sonic hedgehog* (*Shh*) and *Wnt3a*), one classical mouse mutant (*Brachyury*) and one zebrafish mutant (*floating head* (*flh*)) have begun to define the genetic pathway of the trunk organizer. Mutations for the *Brachyury* and *Wnt3a* genes do not affect the formation of anterior structures but have severe

posterior deficiencies (Takada et al., 1994; Herrmann, 1996). In addition, *Brachyury* mutants are unable to differentiate a node or notochord. The expression of *Tbx6*, a *Brachyury*-related gene, overlaps with *Brachyury* expression in the primitive streak (Chapman et al., 1996). It will be interesting to determine the role of *Tbx6* in trunk development and its potential interaction with *Brachyury*. In zebrafish, *flh* is the homologue of the homeobox gene *Xnot* (Gont et al., 1993; von Dassow et al., 1993; Talbot et al., 1995). *Flh* mutants specifically lack a notochord but in contrast to *Brachyury* mutants, *flh* mutants have posterior structures such as the tail. In addition, the floor plate forms and motor neurons differentiate in the neural tube which correlates with transient *Shh* expression. These results suggest that *flh* is essential for notochord differentiation. Chick homologues of *Xnot*/*flh* have been identified (Knezevic et al., 1995; Stein and Kessel, 1995) but the identification of a mouse homologue has not been reported. *Hnf3β* mutants lack a node and notochord and have head and trunk abnormalities, including a loss of D-V patterning of the neural tube and somite patterning defects (Ang and Rossant, 1994; Weinstein et al., 1994). Despite the absence of the node, A-P polarity is still present in the neural tube of the *Hnf3β* mutants. *Shh* mutants have many interesting defects, including cyclopia and the absence of a floor plate and vertebrae (Chiang et al., 1996). Interestingly, *Shh* mutants initially express both *Brachyury* and *Hnf3β* in notochordal tissue which is later reduced and ultimately undetectable, suggesting that *Brachyury* and *Hnf3β* are upstream of *Shh* in notochord development. Thus, *Shh* appears to be primarily required for the maintenance of the notochord. As discussed above, *Wnt3a* mutants clearly have a deficiency in the formation of trunk structures (Takada et al., 1994). Another gene, originally identified in *Xenopus*, that may be involved in head and trunk development is *chordin*, which encodes a secreted factor (Sasai et al., 1994). In *Xenopus*, *chordin* is expressed in the dorsal lip, prechordal plate, notochord and tail. Gain-of-function assays demonstrate that chordin has potent dorsalizing activity (Sasai et al., 1994). In the mouse, *chordin* can be detected by whole mount in situ hybridization at the mid- to late-primitive-streak stage in the anterior primitive streak, a pattern that is reminiscent of *Gsc* (Fig. 7G).

It appears that the phenotypes of the 'trunk' mutants discussed above can be sorted into two general classes, i.e. one that has posterior truncations (*Brachyury* and *Wnt3a*) and one with notochordal defects with D-V polarity alterations in the neural tube, somites and gut (*Hnf3β*, *Shh* and *flh*). What these two classes of mutants are telling us about the trunk organizer remains to be determined.

6.5. Ablation of the mouse gastrula organizer

The surgical removal of the organizer has produced intriguing results. Zebrafish embryos (at 50% epiboly) apparently develop normal body axes after the embryonic shield has been removed (Shih and Fraser, 1996). In the chick, ablation of up to 40% of the anterior portion of the early gastrula (stage 3 + to 4 −) has no impact on axis development. The remaining epiblast cells surrounding the region of surgical deletion differentiate to replenish the tissues of the organizer by an inductive interaction involving the epiblast lateral to the original node and cells along the anterior region of the primitive streak (Yuan et al., 1995a; Yuan et al., 1995b; Psychoyos and Stern, 1996b). This reconstitution of organizer cells is accompanied by the up-regulation of specific genes such as *Gsc*, *Hnf3β* and *Shh*. It appears that the organizer induces the differentiation and patterning of embryonic tissues and at the same time inhibits the expression of organizing activity in the surrounding epiblast. Interestingly, a small proportion of *Lim1* mutant embryos develop axial duplications (Shawlot and Behringer, 1995). Because the node has been shown to be abnormal in these mutants, these axial duplications may be due to the loss of anti-organizing activity emanating from the node region, leading to an expansion and subsequent splitting of the organizer field. A similar situation may occur in mice homozygous for *Fused* that can develop duplicate posterior axes (Theiler and Gluecksohn-Waelsch, 1956; Perry et al., 1995). The discovery of anti-dorsalizing morphogenetic factor (ADMF), a member of the TGF-β family, that counteracts the dorsalizing activity of the organizer in *Xenopus* (Moos et al., 1995) and the observation that Hensen's node in the chick can suppress the cardiogenic inductive activity of the endoderm on mesodermal cells (Schultheiss et al., 1995) suggest that the organizer can exert inhibitory effects on cell differentiation (Yuan et al., 1995a, 1995b).

Removal of the mouse gastrula organizer does not appear to affect axial development. However, ablations of the organizer and the adjacent epiblast tissues result in the splitting of the embryonic midline but have no effect on the formation of axial and paraxial structures (Snow, 1981; G. Schoenwolf and P. Tam, unpublished data). Whether the reconstitution of axial structures after organizer ablation involves the de novo differentiation of the epiblast into organizer cells is not known. It would be interesting to determine if organizer-specific genes are up-regulated following the ablation of the mouse organizer. Furthermore, the ability to regenerate the organizer after surgical ablation could be tested in mutant embryos that lack organizer-specific genes such as *Gsc*, *Hnf3β* and *Lim1*. It would also be interesting to test the regenerated organizer regions of wild-type and mutant embryos for patterning activity by heterotopic transplantation. This organizer ablation and regeneration model may provide novel insights into the genetic activity that may be involved in the formation and/or maintenance of the organizer.

6.6. Summary

The study of mouse embryogenesis is driven by a fundamental curiosity about the development of mammalian spe-

cies, notably our own. Information generated from the study of the embryos of model organisms such as the mouse has provided important insights into the mechanisms that lead to the pathogenesis of human diseases, some of which can be related to defects in morphogenesis and organogenesis (Bedell et al., 1997a, 1997b).

In this review, we have emphasized the importance of gastrulation in the formation of a body plan. As stated earlier, the mouse gastrula is unique in its morphology among mammalian species. The mouse embryo develops into the egg cylinder prior to gastrulation with the epiblast on the inside and the visceral endoderm on the outside. To overcome this topographical variation in germ layer organization, the mouse embryo that has completed gastrulation subsequently undergoes a turning process with a concomitant inversion of the germ layers to generate the typical C-shaped foetus with ectoderm on the outside, mesoderm in the middle and endoderm on the inside (Kaufman, 1990). The egg cylinder morphology is also found in the rat, guinea pig and Chinese hamster (Long and Burlingame, 1938; ten Donkelaar et al., 1979; Ilgren, 1981). In contrast, most mammalian embryos, including humans, develop as planar structures (like the avian embryo) with the epiblast on top (the prospective dorsal side of the foetus) and the endoderm below (Waterman, 1943; Phillips, 1976; Tamarin, 1983; O'Rahilly and Müller, 1987; Tarara et al., 1987; Selwood, 1992). Despite this significant morphological difference between the mouse and most mammals and birds, the finer details of the molecular and cellular aspects of germ layer formation are likely to be similar because the embryos of all of these species utilize the primitive streak as the conduit for germ layer formation during gastrulation. However, one must be cognizant of the unique aspect of mouse development as general conclusions are drawn from experimental data. Other mammals less commonly employed for embryological studies, such as the marsupial mouse, laboratory opossum, tree shrew and rabbit, that gastrulate more like human embryos, should probably be utilized more actively (Daniel and Olson, 1966; Selwood, 1992; Yasui, 1992; Hrabe de Angelis and Kirchner, 1993; Mate et al., 1994).

Despite the caveat mentioned above, the advantages of the mouse system greatly outweigh these species-specific differences in gastrulation. The most significant advantage of the mouse system in the study of gastrulation is the availability of classical spontaneous mutants and the ability to generate targeted or randomly induced mutations. The generation and description of a mutant gastrulation phenotype in the mouse, though very important, should not be considered the final word on the analysis of the mutant. These mutants are important genetic resources for subsequent embryological studies using various chimera methodologies (Guillemot et al., 1994; Chen and Behringer, 1995; Wilson et al., 1995; Duncan et al., 1997; Varlet et al., 1997), embryo manipulation (Beddington and Lawson, 1990; Darnell and Schoenwolf, 1997; Quinlan et al., 1997; Trainor et al., 1997), germ layer recombination experiments (Ang and Rossant, 1993; Ang et al., 1994) and controlled gain-of-function approaches (Arkell and Beddington, 1997). In addition, these mutants provide extremely useful resources for differential screens to isolate downstream genes. Finally, because most of the targeted mutations are typically loss-of-function alleles, their mutant phenotypes only provide useful information regarding their essential roles in the establishment of the mammalian body plan. A further analysis of the possible changes in the expression of other genes that are likely to be involved in the cascade of activity that regulates gastrulation will certainly be rewarding. Another way to exploit the potential of these genetic resources will be to examine compound mutants to test for genetic interactions. Whether these genes are in a single pathway or parallel pathways may be difficult to ascertain. However, the cumulative information generated from these mouse mutants may for the first time allow us to begin to define the endogenous genetic pathways that regulate vertebrate gastrulation.

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