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REVIEW

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Anterior endoderm and head induction in early vertebrate embryos

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Abstract Early work on the formation of the vertebrate body axis indicated the existence of separate head- and trunk-inducing regions in Spemann's organizer of the amphibian gastrula. In mammals some head-organizing activity may be located in anterior visceral (extraembryonic) endoderm (AVE). By analogy, the equivalent structure in the *Xenopus laevis* gastrula, the anterior endoderm, has been proposed to be the amphibian head organizer. Here we review recent data that challenge this notion and indicate that the involvement of AVE in head induction seems to be an exclusively mammalian characteristic. In *X. laevis* and chick, it is the prechordal endomesoderm that is the dominant source of head-inducing signals during early gastrulation. Furthermore, head induction in mammals needs a combination of signals from anterior primitive endoderm, prechordal plate, and anterior ectoderm. Thus, despite the homology of vertebrate anterior primitive endoderm, a role in head induction seems not to be conserved.

Key words Anterior endoderm · *cerberus* · Prechordal plate · Head induction · Gastrulation · *Hex* · Organizer

Introduction

The vertebrate body can be divided along the anteroposterior axis into head, trunk, and tail regions, and this organization is the result of patterning events that happen during gastrulation. In amphibians, a region in the dorsal equator of the gastrula, known as Spemann's organizer, is responsible for patterning the three germ layers: ectoderm, mesoderm, and endoderm. The transplantation of the upper dorsal blastopore lip, which contains the Spemann organizer, to the ventral side of a host embryo can induce a complete secondary body axis. In the frog *Xenopus laevis*, the organizer region can be divided into three parts along the future anteroposterior axis: the anterior endoderm, which gives rise to the liver; the prechordal endomesoderm (or prechordal plate), which includes prechordal mesoderm and pharyngeal endoderm, and the chordamesoderm, which gives rise to the notochord (Fig. 1A). During gastrulation, these axial tissues migrate toward the anterior of the embryo and provide crucial neural-inducing signals that pattern the overlying ectoderm along the anteroposterior axis (Fig. 1A; Harland and Gerhart 1997). Indeed, Spemann (1931) realized that different parts of the organizer differ in their ability to induce head or trunk and/or tail structures. The transplantation of organizers of early gastrulae induces secondary axes containing a head, while the transplantation of organizers from older gastrulae induces only secondary trunks and/or tails. These and other findings discussed below indicate the existence of a rather continuous organizer field, which separates into head and trunk organizers during gastrulation. The prechordal endomesoderm has traditionally been considered to be the head organizer of the amphibian gastrula, while the trunk organizer would be located in the more posterior chordamesoderm (Niehrs 1999). Recently, however, the anterior endoderm of the *X. laevis* organizer has also been implicated in head induction following the finding that it expresses *cerberus*, a gene coding for a secreted factor with head-inducing properties (Bouwmeester et al. 1996).

Similarly, an anterior endodermal tissue of the mouse gastrula is also involved in the induction of the anterior central nervous system (CNS). The early mouse gastrula has a cylindrical shape and consists of an outer and an inner epithelial layer. All embryonic structures are derived from the inner layer (epiblast), while the outer layer, the visceral (primitive) endoderm, does not contribute tissues to the embryo proper (Fig. 1B; Beddington and Robertson 1999). Gastrulation begins at the posterior side with the formation of the primitive streak and the node, a structure equivalent to the frog organizer. Axial endomesoderm derived from the node (gut endoderm,

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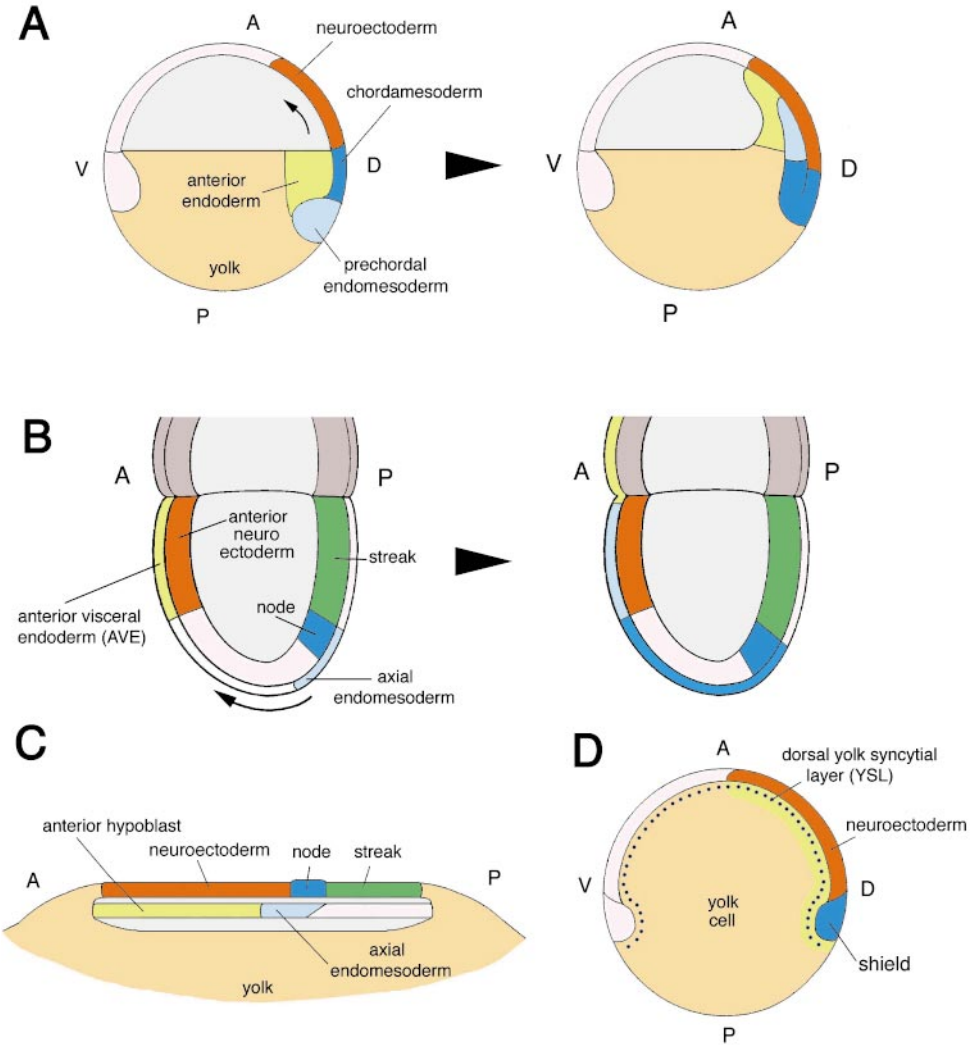

Fig. 1A–D Comparative schemes of vertebrate gastrulae. **A** *Left:* Lateral view of a *Xenopus laevis* gastrula. The organizer comprises the chordamesoderm, prospective prechordal plate and anterior endoderm (prospective liver). *Right:* As gastrulation proceeds, the anterior endoderm and prechordal endomesoderm migrate toward the anterior (*arrow*) and contact the neural plate. **B** *Left:* Embyonic cylinder of a mouse embryo at the beginning of gastrulation. In the anterior side, the anterior visceral endoderm (*AVE*) contacts the future anterior neural plate. Definitive axial endomesoderm (anterior definitive endoderm, prechordal endomesoderm, chordamesoderm) originates in the node (organizer) located at the posterior side of the cylinder. *Right:* As gastrulation proceeds, definitive axial endomesoderm migrates toward the anterior (*arrow*), displacing the visceral endoderm and coming into contact with the anterior neuroectoderm. **C** Lateral view of a chick embryo at the beginning of gastrulation. The anterior portion of the extraembryonic endoderm (hypoblast) contacts the neuroectoderm. Axial endomesoderm derived from the chick organizer (Hensen's node) migrates toward the anterior and displaces the hypoblast. **D** Lateral view of an early zebrafish gastrula, sagital cut. The zebrafish organizer, termed shield, is located in the dorsal side. The equivalent to the anterior endoderm in *X. laevis* and AVE in the mouse is the dorsal yolk syncytial layer (*YSL*), an extraembryonic tissue that contacts the neuroectoderm. The dorsal YSL expresses *Hex* (Ho et al. 1999). (*A* Anterior, *P* posterior, *V* ventral, *D* dorsal)

prechordal endomesoderm, chordamesoderm) migrates toward the anterior and displaces the visceral endoderm (Fig. 1B). The transplantation of the mouse node to an ectopic location can induce secondary axes, but these lack the anterior CNS (Beddington 1994). Recent evidence indicates that the anterior visceral endoderm (AVE), which contacts the future anterior CNS during early gastrulation, is responsible for inducing fore- and midbrain. This has led to the proposal that head and trunk organizers are separated in the mouse embryo, the trunk organizer residing in the node and the head organizer in the AVE (Thomas and Beddington 1996; Bouwmeester and Leyns 1997; Beddington and Robertson 1998).

Since the anterior endoderm of the *X. laevis* organizer and the mouse AVE express homologous genes (Beddington and Robertson 1998) and both regions are implicated in head induction, it has been proposed that the anterior endoderm of *X. laevis* is the topological and functional equivalent of the AVE in the mouse (Bouwmeester and Leyns 1997; Beddington and Robertson 1998). According to this view, anterior endoderm is necessary for head induction in frogs and mice, and perhaps in other vertebrates

as well. This review focuses on recent findings concerning the role of the anterior endoderm in embryonic head induction and genes involved in this process in vertebrates. Other reviews dealing with the role of anterior endoderm in vertebrate head induction (Bouwmeester and Leyns 1997; Beddington and Robertson 1998; Bielinska et al. 1999; Knoetgen et al. 1999a; Niehrs 1999; Viebahn 1999) as well as general vertebrate axis formation (Harland and Gerhart 1997; Tam and Behringer 1997; Arendt and Nübler-Jung 1999; Beddington and Robertson 1999) are available. The head consists of a complex ensemble of tissues derived from all germ layers, but in amniotes the expression „head induction" often refers to the induction of anterior CNS, while in *X. laevis* the induction of a complete head is typically scored. Here we use both definitions.

Conservation of anterior primitive endoderm in vertebrates

The topological equivalence between the *X. laevis* anterior endoderm and the mouse AVE was proposed based on similarities in marker gene expression (Bouwmeester and Leyns 1997; Beddington and Robertson 1998). Recent data on marker gene expression, heart induction, and tissue movements in frogs, mice, and other model vertebrates reinforce this idea.

Marker gene expression

Many marker genes expressed in the mouse AVE have homologues that are similarly expressed in the *X. laevis* anterior endoderm, such as the homeobox gene *Hex* (Newman et al. 1997; Thomas et al. 1998), the zinc finger gene *Xblimp1* (de Souza et al. 1999), the secreted factors cerberus (Bouwmeester et al. 1996; Belo et al. 1997; Biben et al. 1998) and dickkopf1 (*dkk1*; Glinka et al. 1998; Pearce et al. 1999), and others. A problem with the proposal of homology between the AVE and the *X. laevis* anterior endoderm is that the AVE is part of an extraembryonic layer, while the *X. laevis* anterior endoderm is an embryonic tissue that gives rise to liver (Bouwmeester et al. 1996). In the mouse, genes expressed in the AVE are usually also expressed in anterior definitive (embryonic) tissues derived from the node. Thus, *Hex* and *cerberus-like* (*Cer-l*) are expressed both in the AVE and in node-derived definitive endomesoderm, which is fated for liver, foregut, and, in the case of *Cer-l*, prechordal endomesoderm (Belo et al. 1997; Biben et al. 1998; Shawlot et al. 1998; Thomas et al. 1998). In contrast, *Xenopus Hex* (*XHex*) and *cerberus* are not expressed in two distinct domains, but only in anterior endoderm fated to become liver and pharynx (Bouwmeester et al. 1996; Newman et al. 1997; Schneider and Mercola 1999; Zorn et al. 1999).

It could be argued that mammals are unique in having extraembryonic and embryonic anterior endoderm expressing similar genes, but recent data on the expression of *Hex* and *cerberus* homologues in chick and zebrafish suggest that this is a general vertebrate feature. The chick pregastrula is composed of two layers: the epiblast, from which all embryonic structures originate, and the hypoblast. The latter is extraembryonic and can be regarded as homologous to the mouse visceral endoderm (Fig. 1C). During gastrulation, definitive endomesodermal cells originating from the streak and from Hensen's node (the chick organizer) displace the hypoblast in a manner similar to that observed in mammals (Fig. 1C). Chick *Hex* (Yatskievych et al. 1999), as well as a chick *cerberus* homologue (*Caronte*; Rodriguez-Esteban 1999) are first expressed in the hypoblast. At later stages, chick *Hex* is expressed in definitive endoderm of the liver and pharynx, among other structures (Yatskievych et al. 1999). In zebrafish, the embryo develops on top of a big yolk cell, and at the blastula stage cells from the margin of the embryonic blastoderm fuse with and release their nuclei into the yolk cell, forming the yolk syncytial layer (YSL; Fig. 1D). This extraembryonic layer has been suggested, on topological grounds, to be the teleost equivalent of the mammalian visceral endoderm and the avian hypoblast (Bouwmeester and Leyns 1997; Beddington and Robertson 1998; Viebahn 1999). In the early gastrula, zebrafish *Hex* is expressed in the dorsal YSL, and later it is expressed in embryonic structures such as the liver (Ho et al. 1999). Thus, *Hex* expression in the early embryos of teleosts, birds, and mammals is always found in topologically equivalent extraembryonic tissues and later in definitive anterior endoderm (liver, pharynx). In the amphibian gastrula, without a well-defined extraembryonic endoderm, both components of *Hex* and *cerberus* expression probably coincide in the gastrula anterior endoderm.

Heart induction

It is well established that anterior endoderm plays an important role in embryonic heart induction (reviewed by Fishman and Chien, 1997). In the *X. laevis* gastrula, prospective heart mesoderm flanks the organizer. Both the anterior endoderm and the organizer mesoderm are necessary for the induction of heart development during gastrulation (Nascone and Mercola 1995; Schneider and Mercola 1999). The hypoblast of the chick can also induce heart development in the epiblast of the early gastrula in a synergistic manner with the organizer (Yatskievych et al. 1997). Finally, experiments with explants show that, in the mouse also, signals from the AVE are necessary for heart induction during midgastrulation (Arai et al. 1997). Thus, the anterior endoderm of frogs and the extraembryonic endoderm of chick and mice have at least one function in common: to promote heart development at early stages of development in cooperation with the organizer.

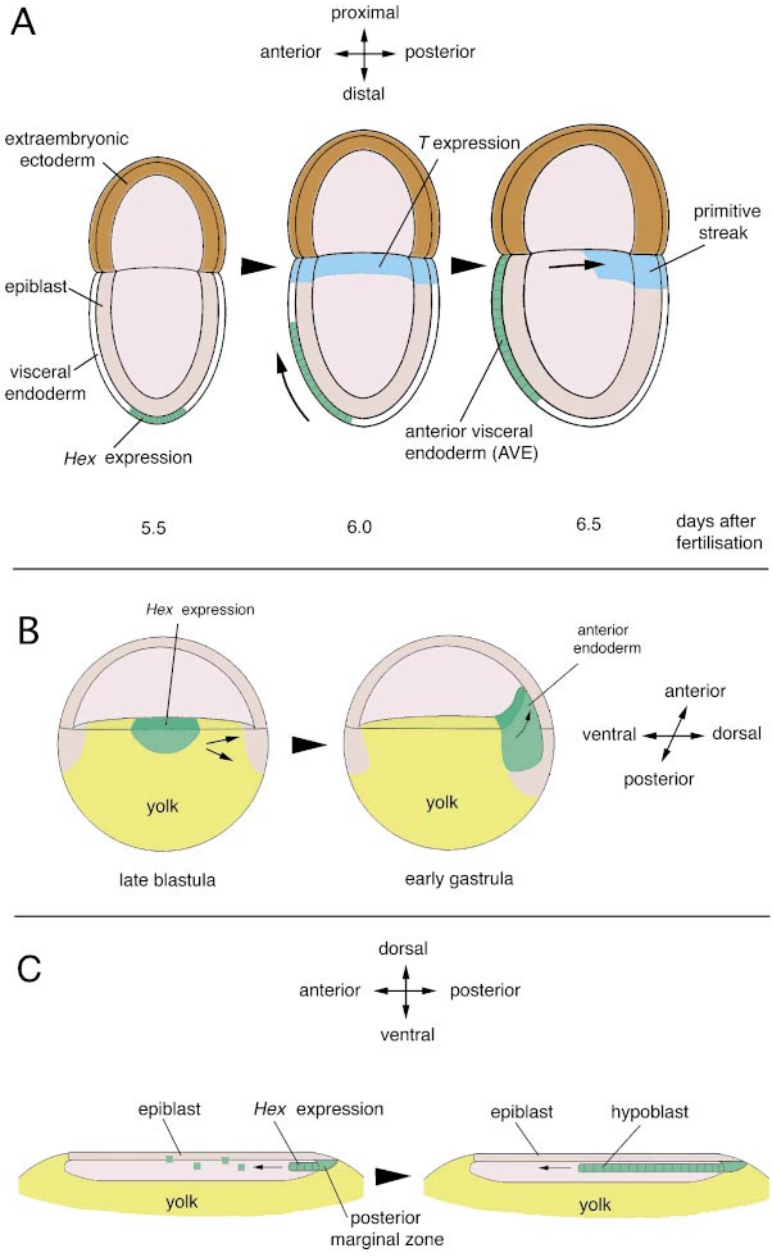

Fig. 2A–C Pre-gastrulation movements of the primitive endoderm in mouse, frog, and chick. *Hex*-expressing cells are colored *dark green* in all panels. **A** In the pregastrula mouse embryo, *Hex* expression begins at the distal portion of the visceral endoderm. *Hex*-expressing cells then move toward the anterior of the embryo (*arrow*), forming the anterior visceral endoderm (*AVE*). At approximately the same time, the expression of *Brachyury* (*T, blue*) at the extraembryonic/embryonic boundary moves to the posterior side of the embryo, where gastrulation begins. **B** In the late *Xenopus laevis* blastula embryo, *XHex* is expressed in yolky endodermal cells of the blastocoele roof. At early gastrulation, extensive endodermal movements (vegetal rotation) position *Hex*-expressing cells in the dorsal side, in the anterior endodermal layer of the organizer. **C** In the chick embryo, the hypoblast is formed by groups of cells that delaminate from the epiblast (*green squares*) and from a sheet of cells that migrate from the posterior marginal zone toward the anterior. Note that *Hex*-expressing anterior primitive endoderm moves toward the anterior of the embryos in all vertebrates shown

Pregastrulation movements

A feature that might also be conserved in vertebrates is pregastrulation movements that position the anterior endoderm. Such movements were initially discovered by studying the AVE marker *Hex*, which starts being expressed in the mouse pregastrula at 5.5 days postcoitum (Thomas et al. 1998). *Hex* is initially expressed in visceral endoderm at the distal part of the egg cylinder and the expression domain moves toward one side of the embryo. In lineage-tracing experiments, Thomas et al. (1998) found that the distal, *Hex*-positive cells migrate to the prospective anterior side of the embryo prior to the onset of gastrulation (Fig. 2A). Approximately at the same time, the expression of the mesodermal marker *Brachyury* (*T*) moves to the presumptive posterior part of the embryo, where the primitive streak will form (Fig. 2A; Thomas et al. 1998). These movements may not take place in mice mutant for *Cripto*, a gene encoding a member of the Cripto/FRL-1/Cryptic family of epidermal growth factor-related proteins (EGF-CFC) class of membrane-bound proteins. In *Cripto*$^{-/-}$ embryos, gastrulation does not occur and AVE marker genes remain expressed in the distal part of the egg cylinder, while primitive streak genes stay expressed in the proximal part (Ding et al. 1998).

Two recent papers indicate that pregastrulation movements are also responsible for positioning the anterior endoderm in *X. laevis*. Winklbauer and Schürfeld (1999) show that the yolky endoderm of the late blastula undergoes an extensive rotational movement (termed „vegetal rotation“) that transports endodermal cells from the mid-

dle of the blastocoele floor toward the early organizer. Jones et al. (1999) draw similar conclusions studying the expression pattern of *XHex*. *XHex* is expressed at late blastula stage in endodermal cells located at the roof of the endodermal cell mass, and *XHex*-expressing cells move toward the anterior portion of the organizer before gastrulation begins (Fig. 2B).

Recently, Arendt and Nübler-Jung (1999) have suggested that the initial movements of the *X. laevis* anterior endoderm („endodermal wedge“) are homologous to movements of hypoblast cells observed in the chick. During pregastrulation stages, the chick hypoblast is formed by two processes: the delamination of small groups of epiblast cells, and the migration of a cohesive cell sheet from the posterior margin to the anterior side of the embryo (Fig. 2C). Based on various morphological criteria, the migration of the hypoblast sheet toward the anterior is considered by Arendt and Nübler-Jung to be equivalent to the active migration of *X. laevis* anterior endodermal cells toward the anterior pole. Considering that chick *Hex* is expressed in the hypoblast as it forms (Yatskievych et al. 1999), an anteriorly directed movement of *Hex*-expressing cells is likely to be observed during the formation of the chick hypoblast (Fig. 2C). Thus, pregastrulation movements transporting primitive endoderm toward the anterior side of the embryo may be conserved in amphibians, birds, and mammals, strengthening the hypothesis that the mouse AVE, the chick hypoblast, and the *X. laevis* anterior endoderm are homologous structures.

The head organizer and anterior endoderm

Molecular mechanism of head induction

The molecular basis of head induction is best understood in *X. laevis*. Many of the molecules secreted by the *X. laevis* organizer act by inhibiting signaling molecules of the BMP, Wnt, and Nodal-related families (Harland and Gerhart 1997). The idea of separate head and trunk organizers has gained support with the finding of secreted organizer factors that induce only heads or trunks (Niehrs 1999). Secreted proteins that inhibit BMP signaling, such as Chordin (Piccolo et al. 1996), Noggin (Zimmerman et al. 1996), and Follistatin (Iemura et al. 1998), can induce ectopic trunks, while molecules that inhibit Wnt signaling, such as Frzb (Leyns et al. 1997; Wang et al. 1997) and Dkk1 (Glinka et al. 1998), can cooperate with BMP inhibitors in inducing secondary trunks containing heads. This observation led to the proposal that, while trunk induction depends on the effective inhibition of BMP signaling, head induction requires the inhibition of both BMP and Wnt signals (Glinka et al. 1997).

The first secreted head-inducing factor discovered was *Cerberus* (Bouwmeester et al. 1996). Cerberus mRNA injections induce heads containing a cement gland (an anterior ectodermal derivative) and forebrain with one eye, along with anterior structures such as heart and liver. No trunk structures such as notochord or somites are induced. Cerberus is a multifunctional protein that binds and inhibits Nodal-related, BMP, and Wnt molecules (Piccolo et al. 1999), suggesting that it also induces heads by inhibiting BMP and Wnt simultaneously and that Nodal inhibition may play a role in this process.

Anterior endoderm may not be involved in head induction in *X. laevis*

Cerberus is expressed in anterior endoderm of the organizer, and hence it has been proposed that this tissue is responsible for head induction in *X. laevis* (Bouwmeester and Leyns 1997). This idea was subsequently supported by the finding that other genes coding for candidate head-inducers, such as *frzb* and *dkk1*, are also initially expressed in this region (Glinka et al. 1997, 1998; Leyns et al. 1997; Wang et al. 1997; F. S. J. de Souza and C. Niehrs, unpublished observations).

Recent evidence for a role of the endoderm and *cerberus* in head formation comes from studies with the panendodermal homeobox gene *Mix.1* (Rosa 1989). *Mix.1* is able to induce *cerberus* in a cooperative manner with anterior endodermal factors such as the homeobox gene *siamois* (Lemaire et al. 1998) and the zinc finger gene *Xblimp1* (de Souza et al. 1999). Injection of RNA coding for a dominant-negative *Mix.1* causes the downregulation of *cerberus* and leads to deficiencies in gut as well as head development, with embryos displaying cyclopic or eyeless phenotypes (Lemaire et al. 1998; Latinkic and Smith 1999). In addition, overexpression of dominant-negative versions of transcription factors expressed in the anterior endoderm such as the homeobox gene *goosecoid* (Ferreiro et al. 1998; Latinkic and Smith 1999) and *Xblimp1* (de Souza et al. 1999) also cause the development of headless embryos. Furthermore, *Xblimp1* can cooperate with the BMP inhibitor *chordin* to induce ectopic heads (de Souza et al. 1999). It is not clear, however, whether these genes are required for head formation specifically in the anterior endoderm, since all of them are expressed in the prechordal endomesoderm, a region implicated in head induction.

There are arguments against an essential role for *X. laevis* anterior endoderm in head induction. The anterior endoderm acts as a poor head inducer in amphibians when transplanted to an ectopic location, for example to the blastocoele of a host embryo (Einsteck experiment; Mangold 1933; Bouwmeester et al. 1996). Stronger evidence suggesting that the anterior endoderm is not involved in head induction is presented in a recent paper by Schneider and Mercola (1999). The authors extirpated the anterior endoderm from dorsal explants and whole embryos at the early gastrula stage. Heart induction was severely affected, but operated specimens developed normal heads. Head formation was only affected when both the anterior endoderm and the prechordal plate were removed. Thus, if the anterior endoderm plays any role in

head induction, it is before or at the beginning of gastrulation. The experiments rule out an essential role for the anterior endoderm in providing vertical signals to the neuroectoderm that confer anterior characteristics, but it could still help pattern the neuroectoderm through planar signals before gastrulation starts (Doniach et al. 1992; Ruiz i Altaba 1992). Alternatively, one could speculate that the anterior endoderm is required to induce head-organizer properties in the adjacent prechordal endomesoderm before gastrulation begins. Evidence in *X. laevis* is still lacking for these hypotheses.

The results of Schneider and Mercola (1999) indicate an essential role for the prechordal plate in head induction. This is in agreement with previous observations that prechordal endomesoderm explanted from early urodele neurulae or early *X. laevis* gastrulae is a potent head inducer when transplanted to the blastocoele of host embryos (Spemann 1931; Mangold 1933; Zoltewicz and Gerhart 1997). Prechordal endomesoderm is also able to induce forebrain in exogastrulae and Keller sandwiches neuralized by planar signals (Dixon and Kintner 1989; Ruiz i Altaba 1992). Although it does not express *cerberus* (Bouwmeester et al. 1996), the prechordal endomesoderm expresses candidate head-inducing genes such as *frzb* and *dkk1*. Indeed, *dkk1* is necessary for head induction in *X. laevis*, although it is not clear whether it is required in anterior endoderm, prechordal endomesoderm, or both (Glinka et al. 1998). It is possible that prechordal endomesoderm and anterior endoderm act cooperatively in the induction of heads, as they may do in the induction of cement glands (Bradley et al. 1996). Such synergism is unlikely to take place during gastrulation, however, since Schneider and Mercola (1999) could not observe any change in the frequency of head induction when explants contained the prechordal endomesoderm alone or prechordal endomesoderm and anterior endoderm. At present, the available evidence points to a more prominent role for the prechordal endomesoderm in head induction in *X. laevis*, compared with the anterior endoderm. Along with giving anterior character to the neuroectoderm, the prechordal endomesoderm is also important to induce the midline of the anterior neural tube (Chiang et al. 1996; Dale et al. 1997; Li et al. 1997; Blader and Strähle 1998).

Does *cerberus* have a role in head induction?

The results of Schneider and Mercola (1999) also suggest that *cerberus* does not participate in head induction during gastrulation, since *cerberus* expression was mostly absent in embryos in which the endoderm was extirpated. Nevertheless, it is possible that *cerberus* expression recovered in time to rescue forebrain induction in their experiments, since this was not tested. In addition, as the authors consider, *cerberus* might act in head induction before gastrulation begins, perhaps in the ectoderm by diffusing through the plane of the animal ectoderm. Indeed, *cerberus* expression is already detectable in anterior endoderm at the late blastula stage (Zorn et al. 1999). Piccolo et al. (1999) suggest that *cerberus* prevents the trunk organizer from forming in anterior regions of the organizer. It is possible that *cerberus* helps to create the head organizer in presumptive prechordal endomesoderm by inhibiting Nodal signaling in this region before gastrulation (Piccolo et al. 1999), although Cerberus overexpression is not sufficient to induce prechordal endomesodermal markers in the ventral side of embryos (Schneider and Mercola 1999).

Studies on *XHex* suggest that endogenous levels of *cerberus* expression are not sufficient to induce ectopic heads in the ventral side of embryos. Jones et al. (1999) found that ventral endoderm expressing *XHex* is able to induce cement glands in naive animal ectoderm, as is anterior endoderm (Bradley et al. 1996), showing that *XHex* can confer some anterior characteristics to ventral endoderm. However, ventral expression of *XHex* cannot induce heads, even though it induces ectopic *cerberus* transcription at levels comparable with endogenous expression (Jones et al. 1999; Zorn et al. 1999). Again, this observation might suggest that, although it represses Wnt and BMP signaling and is sufficient to induce ectopic heads upon mRNA overexpression, *cerberus* needs to synergize with other organizer factors (*frzb*, *dkk1*, *chordin*, *noggin*) to promote head induction in physiological conditions. Thus, the role of *cerberus* in *X. laevis* head formation is still unclear.

In the mouse, an essential role in head induction can be ruled out for a homologue of *cerberus*, *Cerberus-like* (*Cer-l*). *Cer-l* is expressed in the AVE and definitive axial endomesoderm, two tissues involved in head induction (Belo et al. 1997; Biben et al. 1998; Shawlot et al. 1998). Recently it has been shown that mice carrying a deletion in chromosome IV encompassing the *Cer-l* locus develop without any aberrant head phenotype (Simpson et al. 1999). There is a family of *cerberus*-related genes in mammals but, among the known members of the family, only *Cer-l* is expressed in the right place at the right time to be involved in head induction (Pearce et al. 1999). It is possible that *Cer-l* is not the true homologue of *Xenopus cerberus*, since the identity between both genes is low (26%) and *Cer-l* is not able to induce ectopic heads in *X. laevis* embryos (Belo et al. 1997). Alternatively, the presence of other head-inducing genes in the AVE (*mdkk1*; Pearce et al. 1999) and axial endomesoderm (*frzb* and *mdkk1*; Leyns et al. 1997; Glinka et al. 1998) might rescue anterior development in embryos lacking *Cer-l*. A role for *Cer-l* in head formation might become apparent in double mutants with other candidate head-inducing genes.

Mouse head induction requires the AVE and the prechordal endomesoderm

Despite the lack of phenotype in *Cer-l* mutant embryos, evidence of a role for the AVE in mouse head induction is strong (Beddington and Robertson 1998, 1999). The

AVE lies adjacent to the future anterior CNS and its ablation during early gastrulation results in lack of forebrain marker gene expression (Thomas and Beddington 1996). In addition, homologues of genes implicated in head induction in *X. laevis* are expressed in the AVE, such as the previously mentioned *Cer-l* and *mdkk1*. A recent paper by Knoetgen et al. (1999b) shows that the mammalian AVE is also able to induce anterior CNS in cross-species grafts. Taking advantage of the fact that the rabbit AVE can be easily recognized morphologically before gastrulation begins (Viebahn 1999), the authors transplanted the pregastrula rabbit AVE to the epiblast of chick embryos and observed induction of the forebrain homeobox gene *Ganf* (the homologue of mouse *Hesx1* and frog *Xanf*; Zaraisky et al. 1995; Thomas and Beddington 1996). Additional evidence comes from work on *Cripto* (Ding et al. 1998). In *Cripto*$^{-/-}$ mutants, anterior neural markers such as *Otx2*, *BF1*, and *Engrailed1* are expressed in the distal epiblast adjacent to the misplaced AVE, suggesting that the AVE is sufficient to promote anterior CNS development (Ding et al. 1998).

The most convincing evidence linking the mammalian visceral endoderm with head induction comes from experiments with chimeric mouse embryos, in which the embryo proper is composed of wild-type cells but the visceral endoderm is mutant for genes expressed in the AVE, such as the homeobox gene *Otx2* (Rhinn et al. 1998) or the transforming growth factor-β (TGFβ) factor *nodal* (Varlet et al. 1997). Such chimeras display anterior CNS truncations even though all of their embryonic structures are wild-type, indicating that expression of both *Otx2* and *nodal* is required in the visceral endoderm for the induction of the anterior CNS (Varlet et al. 1997; Rhinn et al. 1998). Mouse embryos mutant for the winged helix transcription factor *HNF3β* also lack anterior CNS in some cases (Ang and Rossant 1994; Weinstein et al. 1994), and wild-type visceral endoderm has been shown to rescue this effect (Dufort et al. 1998). Another gene implicated in the mouse head organizer is the LIM domain transcription factor *Lim1* (Shawlot and Behringer 1995). *Lim1* is expressed in both the AVE and in node-derived axial endomesoderm and, as in the case of *Otx2*, *Lim1*$^{-/-}$ embryos lack forebrain and midbrain (Ang et al. 1996; Shawlot and Behringer 1995). In addition, double mutants of *HNF3β* and *Lim1* lack AVE marker gene expression (Perea-Goméz et al. 1999). Recently, the requirement for *Lim1* in different germ layers was investigated by Shawlot et al. (1999). The authors produced chimeric embryos in which *Lim1* was lacking either in the visceral endoderm or in the epiblast. Interestingly, they found that both types of chimeras have severe truncations of the anterior CNS, indicating that neither the AVE nor the axial endomesoderm are sufficient to confer full anterior identity to the CNS. *Lim1* expression in axial endomesoderm is normal in chimeras with *Lim1*$^{-/-}$ visceral endoderm, indicating that *Lim1* is required in the AVE to induce anterior neural tissue directly. In addition, Shawlot et al. observed that *Lim1*$^{-/-}$ anterior endomesoderm, in contrast to wild-type (Ang and Rossant 1994), is unable to maintain *Otx2* expression in ectoderm, indicating that *Lim1* is required for signaling by node-derived structures. The authors suggest that anterior CNS induction happens in a two-step manner, with the AVE inducing a labile anterior identity in the adjacent neuroectoderm, and the axial endomesoderm reinforcing and maintaining this induction (Fig. 3; Thomas and Beddington 1996; Shawlot et al. 1999). These results are consistent with the recent observation that mice mutant for *Wnt3* lack definitive endomesoderm and do not develop anterior neural structures, although AVE marker gene expression is normal (Liu et al. 1999).

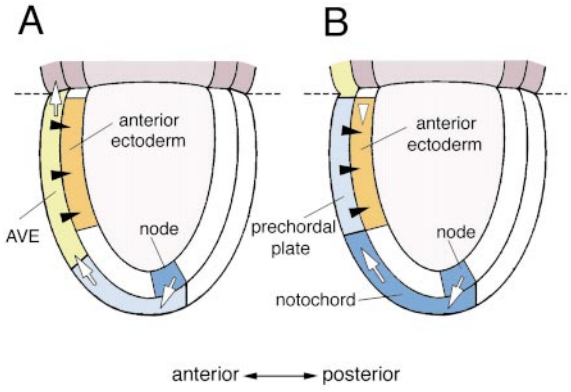

Fig. 3A,B Multistep model for the induction of anterior central nervous system (CNS) in the mouse embryo. Lateral view. The extraembryonic-embryonic boundary is indicated by *dashed lines*. **A** Before and during early gastrulation, the anterior visceral endoderm (*AVE*) sends anteriorizing signals (*black arrowheads*) to the anterior ectoderm (prospective brain). The AVE is displaced by node-derived axial endomesoderm, which migrates to the anterior of the embryo (*white arrows*). **B** Starting at midgastrulation, prechordal endomesoderm comes into contact with the anterior ectoderm and sends signals to the anterior ectoderm (*black arrowheads*) that reinforce the previous anteriorizing signals from the AVE. The anterior ectoderm itself also provides anteriorizing signals (*white arrowhead*) to the CNS

The work by Ding et al. (1998) on *Cripto* mutants seems to contradict the conclusion that axial endomesoderm is necessary for proper anterior CNS induction. *Cripto* mutants do not gastrulate and, although no axial endomesoderm reaches the distal part of the embryo, the AVE seems to be able to induce anterior neural markers (Ding et al. 1998). However, it is possible that the secreted molecules responsible for forebrain induction by node-derived structures are still produced in *Cripto*$^{-/-}$ mutants and can diffuse from the proximal to the distal part of the epiblast. Alternatively, as Shawlot et al. (1999) propose, the AVE signal might lose its labile character in the absence of *Cripto*, causing stable anterior CNS induction by the AVE in *Cripto*$^{-/-}$ embryos.

Independent evidence for the simultaneous requirement for the AVE and the axial endomesoderm derived from the node in anterior CNS induction comes from a recent study by Tam and Steiner (1999). The authors explanted three regions from mouse embryos at the early streak stage: the AVE; the anterior epiblast adjacent to the AVE (EPI, fated to become anterior CNS); and the

early gastrula organizer in the posterior epiblast of the embryo (EGO). The fragments were then grafted alone or in combination into late mouse gastrulae, and ectopic neural induction was examined by morphology and marker gene expression. Tam and Steiner (1999) found that the EGO could induce neural markers of posterior character, but the combination AVE + EPI + EGO was needed to induce an anterior neural marker (*Otx2*). Both the isolated explants or the combinations AVE + EPI, AVE + EGO and EPI + EGO had poor capacity to induce anterior neural markers, suggesting that all three tissues synergize to promote anterior neural induction. In contrast to the results of Knoetgen et al. (1999b), obtained by grafting rabbit AVE in avian epiblast, Tam and Steiner (1999) found that murine AVE alone is unable to induce ectopic neural tissue. This could reflect a difference in competence of avian and mammalian ectoderm to AVE signals. Alternatively, the difference could be stage-related, since the rabbit AVE explants were obtained from prestreak embryos (Knoetgen et al. 1999b) and the mouse AVE explants were from early streak embryos (Tam and Steiner 1999).

It is particularly interesting that Tam and Steiner (1999) reported that the anterior epiblast adjacent to the AVE is also required to induce anterior neural markers in grafting experiments. An early role for the anterior ectoderm in head induction had already been shown by Houart et al. (1998) in the zebrafish. These authors observed that a particular group of cells (row-1), located at the border between prospective neural plate and epidermis in the zebrafish mid-gastrula, is essential for forebrain induction during gastrulation. A somewhat similar forebrain-inducing center, although acting later, is located in the anterior neural fold of early somite stage mice embryos (Shimamura and Rubenstein 1997). The results of Tam and Steiner (1999) suggest that mammalian gastrulae have a forebrain-inducing center located in anterior ectoderm. It is likely that similar inducing centers will be found in the ectoderm of frogs and birds.

Which secreted factors could be responsible for the induction of anterior CNS by AVE and axial endomesoderm? According to the two-inhibitor model derived from studies in *X. laevis* (Glinka et al. 1997), candidates should include BMP inhibitors such as *chordin* and *noggin* and Wnt-inhibitors such as mouse *frzb*, *dkk1*, and *cerberus* homologues (excluding *Cerbl*, which is not necessary for head formation; Simpson et al. 1999). Among these, *dkk1* is at present the best candidate, considering that it is necessary for anterior CNS formation in *X. laevis* (Glinka et al. 1998). Mouse *dkk1* is expressed in both AVE and prechordal plate (Glinka et al. 1998; Pearce et al. 1999) and hence has the potential of being required for head induction in one or both of these regions.

In summary, the results by Shawlot et al. (1999) and Tam and Steiner (1999) suggest that signals from the AVE are part of a multistep process of anterior CNS induction in the mouse (Fig. 3). A first step in induction is mediated by signals emitted by the AVE, and the induction is subsequently reinforced by signals from node-derived axial endomesoderm as it reaches the anterior of the embryo. In addition, signals from the anterior epiblast are also required to confer full anterior identity to the CNS, possibly during gastrula stages (as shown for the zebrafish; Houart et al. 1998) and/or early somite stages (Shimamura and Rubenstein 1997).

Anterior endoderm and head induction in other vertebrates

The evidence linking anterior endoderm and head induction came from studies in *X. laevis* and mouse (Bouwmeester and Leyns 1997; Beddington and Robertson 1998), but at present there are few indications that anterior primitive endoderm has an influence on head induction in chick and zebrafish. Table 1 summarizes the role played by candidate head-inducing molecules and tissues in different vertebrates.

Birds

In the chick, the anterior hypoblast is equivalent to the mouse AVE (Fig. 1E; Arendt and Nübler-Jung 1999; Viebahn 1999). Ablation experiments, however, show that the hypoblast is not required for anterior neural in-

Table 1 Head-organizer activity of tissues and genes in vertebrates (*M* mammals, *C* chick, *X Xenopus laevis*, *Z* zebrafish)

	Required for anterior neural induction	Induces anterior neural tissue or markers
Anterior endoderm	M+[a] C–[b] X–[c] Z?	M+[b]* C–[b] X–[d] Z?
Prechordal endomesoderm	M+[e,g] C+[b] X+[c] Z–[f]	M–[g] C+[b,h*,i*] X+[j] Z?
Cerberus	M–[k]* X?	M+[l] X+[d]
Dickkopf1 (*dkk1*)	X+[m] Z?	X+[m]* Z+[n]

[a] Thomas and Beddington 1996; Varlet et al. 1997; Rhinn et al. 1998; Shawlot et al. 1999
[b] Knoetgen et al. 1999b, *rabbit AVE in avian epiblast
[c] Schneider and Mercola 1999
[d] Bouwmeester et al. 1996; Bradley et al. 1996, *cement gland; Jones et al. 1999
[e] Shawlot et al. 1999
[f] Schier et al. 1997; Gritsman et al. 1999
[g] Tam and Steiner 1999
[h] Foley et al. 1997, *area pellucida
[i] Pera and Kessel 1997, *area opaca
[j] Zoltewicz and Gerhart 1997
[k] Simpson et al. 1999, **Cerberus-like*
[l] Belo et al. 1997
[m] Glinka et al. 1998, *together with BMP inhibitors
[n] Hashimoto et al. 2000

duction (Knoetgen et al. 1999b; Pera et al. 1999). Furthermore, the anterior hypoblast and the anterior definitive endoderm lack neural-inducing capacity (Knoetgen et al. 1999b), although, interestingly, rabbit AVE can induce anterior neural markers in the avian embryo, suggesting that forebrain induction by primitive endoderm might be a specific mammalian characteristic (Knoetgen et al. 1999a, 1999b). As in the case of *X. laevis*, the prechordal endomesoderm is important for anterior CNS induction in the chick. The removal of the young head process (from which the prechordal endomesoderm originates) inhibits anterior CNS formation (Knoetgen et al. 1999b), and the prechordal endomesoderm is able to induce ectopic neural tissue of anterior character in embryonic ectoderm (Foley et al. 1997; Pera and Kessel 1997; Knoetgen et al. 1999b). In addition, the prechordal endomesoderm is able to bestow anterior characteristics on neural tissue induced by Hensen's node in extraembryonic ectoderm (Foley et al. 1997). In summary, the prechordal plate in chick seems to be responsible for conferring anterior character to the neural plate, and it is unlikely that the anterior hypoblast plays a role in this process.

Zebrafish

In zebrafish, it is not clear where the head organizer is located. A region that could be the equivalent to the *X. laevis* anterior endoderm and the mouse AVE is the dorsal YSL (Bouwmeester and Leyns 1996; Beddington and Robertson 1998; Viebahn 1999), as indicated by the expression of zebrafish *Hex* (Ho et al. 1999). Interestingly, *Hex* overexpression in zebrafish embryos downregulates *Wnt8* and *Bmp2* expression, suggesting that the YSL may participate in inhibiting Wnt and BMP signaling in the dorsoanterior side of the embryo, which is necessary to promote head development in *X. laevis* (Glinka et al. 1997; Ho et al. 1999). There is, however, no evidence to date that the YSL is involved specifically in head induction. In fish mutants lacking both maternal and zygotic One-eyed-pinhead (Oep), a protein of the EGF-CFC family such as Cripto (Gritsman et al. 1999), endomesoderm fails to develop but anterior CNS is induced (Schier et al. 1997; Gritsman et al. 1999), suggesting that the prechordal endomesoderm may be dispensable for anterior neural induction in the zebrafish. However, relevant head-inducing molecules may be expressed in these mutants, as is the case for *chordin* (Gritsman et al. 1999). Apart from a possible role for the prechordal endomesoderm, it is known that planar signals emitted by the organizer at late blastula stages (Grinblat et al. 1998) and by an inducing center in the ectoderm (row-1; Houart et al. 1998) are necessary for forebrain induction in the zebrafish.

Conclusions

Mouse AVE, chick anterior hypoblast, *X. laevis* anterior endoderm, and zebrafish dorsal YSL can be regarded as equivalent structures based on similar topology, marker gene expression, and pregastrulation movements. These anterior endodermal tissues also have conserved functions, considering that they play a role in inducing the heart in all vertebrates analyzed. As for a role in head induction in the mouse, new evidence reinforces the idea that signals from the AVE are required. In contrast, the available evidence does not support a role for anterior endoderm in head induction in other vertebrates. In *X. laevis* and chick, the head organizer resides predominantly in the prechordal endomesoderm, indicating that the extraembryonic head organizer may be a characteristic restricted to mammalian embryos. The prechordal endomesoderm seems to have a more conserved role in vertebrate head induction, since node-derived axial endomesoderm is also essential for head induction in the mouse. In mouse and zebrafish, anterior ectoderm is required for head induction, and future experiments should address to what extent this is true in other vertebrates.

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References

Ang SL, Rossant J (1994) HNF-3 beta is essential for node and notochord formation in mouse development. Cell 78:561–74

Ang SL, Jin O, Rhinn M, Daigle N, Stevenson L, Rossant J (1996) A targeted mouse Otx2 mutation leads to severe defects in gastrulation and formation of axial mesoderm and to deletion of rostral brain. Development 122:243–52

Arai A, Yamamoto K, Toyama J (1997) Murine cardiac progenitor cells require visceral embryonic endoderm and primitive streak for terminal differentiation. Dev Dyn 210:344–53

Arendt D, Nübler-Jung K (1999) Rearranging gastrulation in the name of yolk: evolution of gastrulation in yolk-rich amniote eggs. Mech Dev 81:3–22

Beddington RS (1994) Induction of a second neural axis by the mouse node. Development 120:613–20

Beddington RS, Robertson EJ (1998) Anterior patterning in mouse. Trends Genet 14:277–84

Beddington RSP, Robertson EJ (1999) Axis development and early asymmetry in mammals. Cell 96:195–209

Belo JA, Bouwmeester T, Leyns L, Kertesz N, Gallo M, Follettie M, De RE (1997) Cerberus-like is a secreted factor with neuralizing activity expressed in the anterior primitive endoderm of the mouse gastrula. Mech Dev 68:45–57

Biben C, Stanley E, Fabri L, Kotecha S, Rhinn M, Drinkwater C, Lah M, Wang CC, Nash A, Hilton D, Ang SL, Mohun T, Harvey RP (1998) Murine cerberus homologue mCer-l: a candidate anterior patterning molecule. Dev Biol 194:135–51

Bielinska M, Narita N, Wilson D (1999) Distinct roles for visceral endoderm during embryonic mouse development. Int J Dev Biol 43:83–205

Blader P, Strähle U (1998) Casting an eye over cyclopia. Nature 395:112–113

Bouwmeester T, Leyns L (1997) Vertebrate head induction by anterior primitive endoderm. Bioessays 19:855–863

Bouwmeester T, Kim S-H, Sasai Y, Lu B, De Robertis EM (1996) Cerberus is a head-inducing secreted factor expressed in the anterior endoderm of Spemann's organizer. Nature 382:95–601

Bradley L, Wainstock D, Sive H (1996) Positive and negative signals modulate formation of the *Xenopus* cement gland. Development 122:2739–2750

Chiang C, Litingtung Y, Lee E, Young KE, Corden JL, Westphal H Beachy PA (1996) Cyclopia and defective axial patterning in mice lacking Sonic hedgehog gene function. Nature 383: 407–13

Dale K, Vesque C, Lints TJ, Sampath TK, Furley A, Dodd J, Placzek M (1997) Cooperation of BMP7 and SHH in the induction of forebrain ventral midline cells by prechordal plate. Cell 90:257–269

Ding J, Yang L, Yam Y, Chen A, Desai N, Winshaw-Bori A, Shen MM (1998) Cripto is required for correct orientation of the anterior-posterior axis in the mouse. Nature 395:702–707

Dixon JE, Kintner CR (1989) Cellular contacts required for neural induction in *Xenopus* embryos: evidence for two signals. Development 106:749–57

Doniach T, Phillips CR, Gerhart JC (1992) Planar induction of anteroposterior pattern in the developing central nervous system of *Xenopus laevis*. Science 257:542–5

Dufort D, Schwartz L, Harpal K, Rossant J (1998) The transcription factor HNF3beta is required in visceral endoderm for normal primitive streak morphogenesis. Development 125:3015–25

Ferreiro B, Artinger M, Cho KWY, Niehrs C (1998) Antimorphic goosecoids. Development 125:1347–1359

Fishman M, Chien K (1997) Fashioning the vertebrate heart: earliest embryonic decisions. Development 124:2099–2117

Foley AC, Storey KG, Stern CD (1997) The prechordal region lacks neural-inducing ability, but can confer anterior character to more posterior neuroepithelium. Development 124:2983–96

Glinka A, Wu W, Onichtchouk D, Blumenstock C, Niehrs C (1997) Head induction by simultaneous repression of Bmp and wnt signaling in *Xenopus*. Nature 389:517–519

Glinka A, Wu W, Delius H, Monaghan PA, Blumenstock C Niehrs C (1998) Dickkopf-1 is a member of a new family of secreted proteins and functions in head induction. Nature 391:357–362

Grinblat Y, Gamse J, Patel M, Sive H (1998) Determination of the zebrafish forebrain: induction and patterning. Development 125:4403–4416

Gritsman K, Zhang J, Cheng S, Heckscher E, Talbot WS, Schier AF (1999) The EGF-CFC protein one-eyed pinhead is essential for nodal signaling. Cell 97:121–32

Harland RM, Gerhart J (1997) Formation and function of Spemann's organizer. Annu Rev Dev Biol 13:611–667

Hashimoto H, Itoh M, Yamanaka Y, Yamashita S, Shimizu T, Solnica-Krezel L, Hibi M, Hirano T (2000) Zebrafish dkk1 functions in forebrain specification and axial mesendoderm formation. Dev Biol 217:138–152

Ho C-Y, Houart C, Wilson SW, Stainier DYR (1999) A role for the extraembryonic yolk syncytial layer in patterning the zebrafish embryo suggested by properties of the hex gene. Curr Biol 9:1131–1134

Houart C, Westerfield M, Wilson SW (1998) A small population of anterior cells patterns the forebrain during zebrafish gastrulation. Nature 391:788–92

Iemura S-I, Yamamoto T, Takagi C, Uchiyama H, Natsume T, Shimasaki S, Sugino H, Ueno N (1998) Direct binding of follistatin to a complex of bone morphogenetic protein and its receptor inhibits ventral and epidermal cell fates in early *Xenopus* embryos. Proc Natl Acad Sci USA 95:9337–9342

Jones CM, Broadbent J, Thomas PQ, Smith JC, Beddington RSP (1999) An anterior signaling center in *Xenopus* revealed by the homeobox gene XHex. Curr Biol 9:946–954

Knoetgen H, Teichman U, Kessel M (1999a) Head-organizing activities of endodermal tissues in vertebrates. Cell Mol Biol (Noisy-Le-Grand) 45:481–492

Knoetgen H, Viebahn C, Kessel M (1999b) Head induction in the chick by primitive endoderm of mammalian, but not avian origin. Development 126:815–825

Latinkic BV, Smith JC (1999) Goosecoid and Mix.1 repress brachyury expression and are required for head formation in *Xenopus*. Development 126:1769–79

Lemaire P, Darras S, Caillol D, Kodjabachian L (1998) A role for the vegetally expressed *Xenopus* gene Mix1 in endoderm formation and in the restriction of mesoderm to the marginal zone. Development 125:2371–2380

Leyns L, Bouwmeester T, Kim S-H, Piccolo S, De Robertis EM (1997) Frzb-1 is a secreted antagonist of wnt-signals expressed in the Spemann organizer. Cell 88:747–756

Li H, Tierney C, Wen L, Wu JY, Rao Y (1997) A single morphogenetic field gives rise to two retina primordia under the influence of the prechordal plate. Development 124:603–615

Liu P, Wakamiya M, Shea MJ, Albrecht U, Behringer RR, Bradley A (1999) Requirement for Wnt3 in vertebrate axis formation. Nat Genet 22:361–5

Mangold O (1933) Über die Induktionsfähigkeit der verschiedenen Bezirke der Neurula von Urodelen. Naturwissenschaften 21:761–766

Nascone N Mercola M (1995) An inductive role for the endoderm in *Xenopus* cardiogenesis. Development 121:515–23

Newman CS, Chia F, Krieg PA (1997) The XHex homeobox gene is expressed during development of the vascular endothelium: overexpression leads to an increase in vascular endothelial cell number. Mech Dev 66:83–93

Niehrs C (1999) Head in the Wnt- the molecular nature of Spemann's head organizer. Trends Genet:15:314–319

Pearce JJH, Penny G, Rossant J (1999) A mouse cerberus/dan-related gene family. Dev Biol 209:98–100

Pera EM, Kessel M (1997) Patterning of the chick forebrain anlage by the prechordal plate. Development 124:4153–62

Pera E, Stein S, Kessel M (1999) Ectodermal patterning in the avian embryo: epidermis versus neural plate. Development 126:63–73

Perea-Gómez A, Shawlot W, Sasaki H, Behringer RR, Ang S-L (1999) HNF3b and Lim1 interact in the visceral endoderm to regulate primitive streak formation and anterior-posterior polarity in the mouse embryo. Development 126:4499–4511

Piccolo S, Sasai Y, Lu B, De Robertis EM (1996) Dorsoventral patterning in *Xenopus*: inhibition of ventral signals by direct binding of chordin to BMP-4. Cell 86:589–598

Piccolo, S Agius, E Leyns, L Bhattacharyya, S Grunz, H Bouwmeester, T De Robertis, EM (1999) The head inducer Cerberus is a multifunctional antagonist of Nodal, BMP and Wnt signals. Nature 397:707–710

Rhinn M, Dierich A, Shawlot W, Behringer RR, Le Meur M, Ang SL (1998) Sequential roles for Otx2 in visceral endoderm and neuroectoderm for forebrain and midbrain induction and specification. Development 125:845–856

Rodriguez-Esteban C, Capdevila J, Economides AN, Pascual J, Ortiz A, Izpisua BJ (1999) The novel Cer-like protein Caronte mediates the establishment of embryonic left-right asymmetry. Nature 401:243–51

Rosa FM (1989) Mix.1, a homeobox mRNA inducible by mesoderm inducers, is expressed mostly in the presumptive endodermal cells of *Xenopus* embryos. Cell 57:965–74

Ruiz i Altaba A (1992) Planar and vertical signals in the induction and patterning of the *Xenopus* nervous system. Development 116:67–80

Schier AF, Neuhauss SC, Helde KA, Talbot WS, Driever W (1997) The one-eyed pinhead gene functions in mesoderm and endoderm formation in zebrafish and interacts with no tail. Development 124:327–42

Schneider VA, Mercola M (1999) Spatially distinct head and heart inducers within the *Xenopus* organizer region. Curr Biol 9: 800–809

Shawlot W, Behringer RR (1995) Requirement for Lim1 in head-organizer function. Nature 374:25–30

Shawlot W, Deng JM, Behringer RR (1998) Expression of the mouse cerberus-related gene, Cerr1, suggests a role in anterior neural induction and somitogenesis. Proc Natl Acad Sci USA 95:6198–203

Shawlot W, Wakamiya M, Kwan KM, Kania A, Jessell TM, Behringer RR (1999) Lim1 is required in both primitive streak-derived tissues and visceral endoderm for head formation in the mouse. Development 126:4925–4932

Shimamura K, Rubenstein JL (1997) Inductive interactions direct early regionalization of the mouse forebrain. Development 124:709–18

Simpson EH, Johnson DK, Hunsicker P, Suffolk R, Jordan SA, Jackson IJ (1999) The mouse Cer-l (cerberus related or homologue) gene is not required for anterior pattern formation. Dev Biol 213:202–206

Souza FSJ de, Gawantka V, Perea Gómez A, Delius H, Ang SL, Niehrs C (1999) The zinc finger gene Xblimp1 controls anterior endomesodermal cell fate in Spemann's organizer. EMBO J 18:6062–6072

Spemann H (1931) Über den Anteil von Implantat und Wirtskeim an der Orientierung und Beschaffenheit der induzierten Embryonalanlage. W Roux Arch Entwicklungsmech 123:390–516

Tam P, Behringer R (1997) Mouse gastrulation: the formation of a mammalian body plan. Mech Dev 68:183–205

Tam PPL, Steiner KA (1999) Anterior patterning by synergistic activity of the early gastrula organizer and the anterior germ layer tissues of the mouse embryo. Development 126:5171–5179

Thomas P, Beddington R (1996) Anterior primitive endoderm may be responsible for patterning the anterior neural plate in the mouse embryo. Curr Biol 6:1487–96

Thomas PQ, Brown A, Beddington RS (1998) *Hex*: a homeobox gene revealing peri-implantation asymmetry in the mouse embryo and an early transient marker of endothelial cell precursors. Development 125:85–94

Varlet, I Collignon, J Robertson, EJ (1997) nodal expression in the primitive endoderm is required for specification of the anterior axis during mouse gastrulation. Development 124:1033–44

Viebahn C (1999) The anterior margin of the mammalian gastrula: comparative and phylogenetic aspects of its role in axis formation and head induction. Curr Top Dev Biol 46:63–103

Wang S, Krinks M, Lin K, Luyten FP, Moos M (1997) Frzb, a secreted protein expressed in the Spemann organizer, binds and inhibits Wnt-8. Cell 88:757–766

Weinstein DC, Ruiz i AA, Chen WS, Hoodless P, Prezioso VR, Jessell TM, Darnell JJ (1994) The winged-helix transcription factor HNF-3 beta is required for notochord development in the mouse embryo. Cell 78:75–88

Winklbauer R, Schürfeld M (1999) Vegetal rotation, a new gastrulation movement involved in the internalization of the mesoderm and endoderm in *Xenopus*. Development 126:3703–13

Yatskievych TA, Ladd AN, Antin PB (1997) Induction of cardiac myogenesis in avian pregastrula epiblast: the role of the hypoblast and activin. Development 124:561–70

Yatskievych TA, Pascoe S, Antin PB (1999) Expression of the homeobox gene *Hex* during early stages of chick embryo development. Mech Dev 80:107–9

Zaraisky AG, Ecochard V, Kazanskaya OV, Lukyanov SA, Fesenko IV, Duprat AM (1995) The homeobox-containing gene *XANF-1* may control development of the Spemann organizer. Development 121:3839–47

Zimmerman LB, De Jesús-Escobar J-E, Harland RM (1996) The Spemann organizer signal noggin binds and inactivates bone morphogenetic protein-4. Cell 86:599–606

Zoltewicz JS, Gerhart JC (1997) The Spemann organizer of *Xenopus* is patterned along its anteroposterior axis at the earliest gastrula stage. Dev Biol 192:82–491

Zorn AR, Butler K, Gurdon JB (1999) Anterior endomesoderm specification in *Xenopus* by Wnt/beta-catenin and TGF-beta signaling pathways. Dev Biol 209:282–97