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Developmental Biology 308 (2007) 247–256

DEVELOPMENTAL BIOLOGY

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Review

Proposal of a model of mammalian neural induction

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Received for publication 21 February 2007; revised 20 May 2007; accepted 24 May 2007
Available online 2 June 2007

Abstract

How does the vertebrate embryo make a nervous system? This complex question has been at the center of developmental biology for many years. The earliest step in this process – the induction of neural tissue – is intimately linked to patterning of the entire early embryo, and the molecular and embryological of basis these processes are beginning to emerge. Here, we analyze classic and cutting-edge findings on neural induction in the mouse. We find that data from genetics, tissue explants, tissue grafting, and molecular marker expression support a coherent framework for mammalian neural induction. In this model, the gastrula organizer of the mouse embryo inhibits BMP signaling to allow neural tissue to form as a default fate—in the absence of instructive signals. The first neural tissue induced is anterior and subsequent neural tissue is posteriorized to form the midbrain, hindbrain, and spinal cord. The anterior visceral endoderm protects the pre-specified anterior neural fate from similar posteriorization, allowing formation of forebrain. This model is very similar to the default model of neural induction in the frog, thus bridging the evolutionary gap between amphibians and mammals.

Keywords: Embryo; Neural induction; Default; Organizer; Anterior visceral endoderm

Introduction

In the past century, a powerful combination of embryological, genetic, and molecular approaches has illuminated many of the complicated processes that guide the fertilized egg through development to produce the mature organism. However, we still have not resolved the critical question of how vertebrate animals first form the neural tissue that will become their brains and intricate nervous system—one of the hallmark features of these 'higher' organisms. Many experiments have been done in the classic organisms of embryology: the fly, the frog, fish, chicken, and mouse and from these data, many different models have been proposed, including the unlikely concept that perhaps evolution has found independent strategies for neural induction in different phyla. We present here a re-analysis of older data from several classic studies, together with some very new findings. We suggest that neural induction in mice proceeds through a neural default model similar to that previously formulated to describe frog neural induction, thus bridging the evolutionary gap from amphibians to mammals.

Our model for the formation of neural tissue in the mouse states that: (1) The early mouse embryo exists in a pre-anterior neural state and that this cell fate must be blocked to allow the formation of other tissues. This occurs in the posterior side of the gastrulating mouse embryo to allow formation of mesoderm and endoderm through activation of BMP, Nodal, Wnt, and FGF signals. (2) The actual 'induction' of neural tissue during early gastrulation begins when the early/mid-gastrula organizer inhibits these posterior signals (a double negative) and thus protects a local region of the epiblast, allowing it to remain as prospective anterior neural tissue. (3) The specified anterior neural cells move from the distal epiblast to the anterior epiblast, to be juxtaposed with the anterior visceral endoderm that expresses inhibitors of posteriorizing factors to protect the pre-specified anterior neural tissue from acquiring posterior character. (4) More posterior types of neural tissue are subsequently induced by sequential derivatives of the gastrula organizer. (5) The ultimate derivatives of the gastrula organizer and node form the anterior mesendoderm that stabilizes and maintains the overlying neural tissue.

Our model is based in many ways on models of neural induction in the frog embryo. The first and second statements of our model draw directly from the organizer and neural default

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0012-1606/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.ydbio.2007.05.036

models. Historically, the first experimental embryology on neural induction was done by Hans Spemann and his group. In his seminal work with Hilde Mangold, published in 1924, they proposed the organizer model. Spemann and Mangold found that the dorsal blastopore lip of the gastrulating *Triturus* (newt) embryo "exerts an organizing effect on its environment in such a way that, following its transplantation to an indifferent region of another embryo, it there causes the formation of a secondary embryo" (Spemann and Mangold, 1924). This 'secondary embryo' consisted of graft-derived notochord but other tissues, such as neural tube, were derived from the host. The donor graft was derived from dorsal mesoderm and is now referred to as the organizer in frog embryos.

After decades of work to discover the molecular mechanism of the organizer's ability to induce neural tissue, the neural default model was articulated by Brivanlou and colleagues (reviewed in Munoz-Sanjuan and Brivanlou, 2002). This model proposes that the organizer secretes BMP inhibitors that induce and 'organize' neural tissue in the neighboring ectoderm, revealing the 'default,' or automatic fate.

The neural default model was based on two linked initial observations, both of which were conducted in the animal cap region of the frog embryo. This region is fated to give rise to ectoderm (both epidermis and neural tissue). When explanted and cultured alone, the animal cap forms epidermis. However, if the cells of the animal cap are dispersed, thereby inhibiting cell–cell communication, these cells become neural tissue—this is the first important observation that led to the neural default model (Grunz and Tacke, 1989). The second observation was that overexpression of a dominant negative TGF-β type II receptor in the animal cap gives rise to neural tissue (Hemmati-Brivanlou and Melton, 1994). Together, these two findings suggested that a TGF-β related signal was normally signaling to cells of the animal cap to inhibit differentiation to neural tissue. It was found that this factor is BMP signaling, as very low doses of exogenous BMPs can convert dispersed animal cap cells back into epidermis (Wilson and Hemmati-Brivanlou, 1995).

Subsequent work showed that BMP/TGF-β inhibitors can induce neural tissue in ectodermal explants and whole embryos and that loss of BMP signal transduction or depletion of multiple BMP ligands can convert the entire ectoderm into neural tissue (Hemmati-Brivanlou and Melton, 1994; Henry et al., 1996; Lamb et al., 1993; Reversade et al., 2005). These data suggest that the default state for frog ectodermal cells is neural and that BMP signaling is required to prevent neural fate acquisition in non-neural regions of the ectoderm.

The fourth statement of our model is derived from Nieuwkoop's 'activation-transformation model,' which proposes that signals from the organizer induce anterior neural tissue (activation) that is subsequently posteriorized to elaborate the anterior–posterior axis of brain and spinal cord tissue (Nieuwkoop, 1954). Nieuwkoop formulated this model in opposition to an earlier theory of Spemann and Otto Mangold that explains anterior and posterior neural induction as separable events regulated by distinct inducing centers (Mangold, 1933; Spemann, 1931). These two centers exist consecutively in both space and time such that the early blastopore lip ("the head organizer") gives rise to the anterior axial mesoderm and can induce both anterior and posterior neural structures, while the late blastopore lip ("trunk organizer") gives rise to more posterior axial mesoderm and induces only posterior neural structures. Instead, Nieuwkoop believed that anterior neural tissues are "activated" (induced) first and may be subsequently transformed (posteriorized) into more caudal structures and that the primary step of any neural induction is the formation of forebrain. He also postulated that the organizer may be the source for both the activation and subsequent transformation signals (Nieuwkoop, 1954).

The early mouse embryo is 'pre-anterior neural'

Several recent findings in the mouse embryo evoke the neural default model. These data show that the default state for all cells in the early mouse epiblast is to become neural and that other cell types are formed through active signaling that inhibits neural formation.

Once the mouse embryo implants into the uterus of the mother, it is composed of an extra-embryonic region, an embryonic epiblast, and extra-embryonic layers surrounding the epiblast. At this point, the epiblast is essentially totipotent, in that it will form all of the cell types of the final organism, beginning during gastrulation with the establishment of separate mesoderm, endoderm, and ectoderm tissues, in addition to germ cells. However, it can no longer form extra-embryonic tissues.

The first hint of a pre-neural state throughout the early mouse embryo may be the broad expression of Sox2 and of Otx2, markers of inner cell mass/epiblast, then neural tissue and anterior neural tissue, respectively. In pre-gastrula mouse embryos, Sox2 is expressed throughout the entire epiblast and becomes restricted to the anterior ectoderm by mid to late streak stages (Avilion et al., 2003). Otx2 is expressed throughout the epiblast of pre-streak embryos, then shifts to cover the anterior/distal half of the embryo by mid-streak stages, when the posterior-proximal limit of Otx2 expression is marked by the anteriorly advancing edge of the primitive streak (Fig. 1) (Ang et al., 1994; Kinder et al., 2001). During pre-gastrulation stages, Sox2 is a general inner cell mass marker, but beginning at early streak stages, it is possible that Sox2 and Otx2 reflect the first indication of a broad neural transcriptional program.

To generate non-neural tissues, signals from the proximal extra-embryonic and proximal-posterior epiblast promote the formation of mesoderm and endoderm. Signals that induce non-neural tissues are BMPs, Nodal, and Wnts (Tam, 2004). During early gastrulation, BMP signaling is active in the proximal epiblast and then later shifts to the proximal-posterior epiblast, in a pattern complementary to that of Otx2 (Fig. 1) (de Sousa Lopes et al., 2003; Hayashi et al., 2002; Yang and Klingensmith, 2006). In contrast, Nodal signaling is active in cells throughout the early embryo and extra-embryonic visceral endoderm (de Sousa Lopes et al., 2003). On a descriptive level, these data are consistent with the understanding that neural induction occurs by the inhibition of neural-blocking posterior signals, such as BMPs.

These marker expression data are correlative, but two important recent functional reports support this model. It was found that loss of the BMP receptor Alk3 (A. di Gregorio and T.

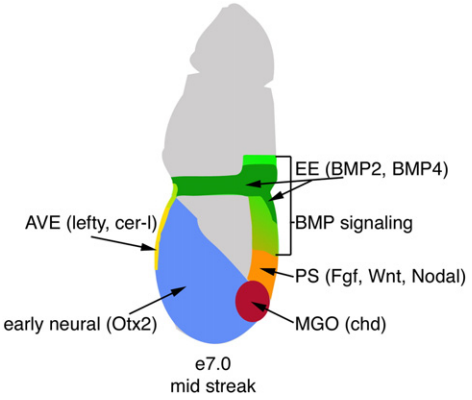

Fig. 1. Signaling centers and molecules implicated in neural induction. A 7.0 mid-streak embryo is shown in gray. In the proximal extra-embryonic region (EE), BMP4 and BMP2 are expressed (dark green). These factors activate signaling through phosphorylation of Smad1 (light green) in the primitive streak and some extra-embryonic tissues. At the anterior end of the embryo, the anterior visceral endoderm (AVE, yellow) expresses Lefty and Cerberus (cer-l). In the embryonic region, the anterior and distal epiblast expresses early neural markers such as Otx2 (blue). At the posterior end of the embryo, the mesendoderm forms from the primitive streak (PS, orange), which expresses Fgfs, Wnts, and Nodal. At the anterior end of the primitive streak, the mid-gastrula organizer (maroon) expresses Chordin (chd), a BMP inhibitor.

Rodriguez, personal communication) or of Nodal (Camus et al., 2006) each results in a dramatic and precocious conversion of almost the entire epiblast into anterior neural tissue that expresses Otx2 and Sox1 as well as markers of anterior forebrain such as Six3, Dlx5, and Hesx1.

Thus, it seems that, normally, Nodal *and* BMP signaling are required during pre-gastrulation stages to prevent a default acquisition of neural fate and to maintain a pluripotent epiblast that also can form epidermis, mesoderm, and endoderm. Interestingly, both Nodal and BMP signaling have a role in maintaining the pluripotent state of embryonic stem cells and the inner cell mass from which they are derived (James et al., 2005; Vallier et al., 2005; Ying et al., 2003).

A direct challenge of the default state of embryonic stem cells has recently shown that, with no external signals, mouse embryonic stem cells rapidly and efficiently differentiate into a primitive neural cell type (Smukler et al., 2006). This experiment was performed by culturing cells at very low density (to avoid cell–cell communication) and in buffered saline to avoid any growth factors or other signaling factors present in cell culture media. Similar experiments in minimal media demonstrated that, at low density culture, treatment with the BMP inhibitor noggin also converts mouse embryonic stem cells into neural tissue (Tropepe et al., 2001). This finding confirms both the default neural state of mouse embryonic stem cells and the role of BMP inhibition in directing neural cell fate.

It is important to note that the molecular basis for this extreme neural default state is conserved from frogs to mammals and it is primarily an inhibition of BMP pathway signaling. The loss of Nodal, another TGF-β family member that signals through a distinct pathway, also allows default formation of neural tissue, potentially expanding the molecular basis of TGF-β family members in neural inhibition. This would be similar to the observation that, in frog embryos, BMP inhibition is sufficient to induce neural tissue within the ectoderm, but combined BMP and Activin/Nodal inhibition is required to induce neural tissue within the endoderm (Henry et al., 1996).

However, Nodal is required for the maintenance of BMP4 expression (Ben-Haim et al., 2006; Brennan et al., 2001) and it is possible that this indirect loss of BMP signaling allows neural default differentiation to occur. In addition, the Nodal mutant embryos do not contain a primitive streak, but do express markers of the organizer, such as Brachyury, FoxA2 and Gsc and these are located more broadly than in wild-type embryos (Brennan et al., 2001; Camus et al., 2006; Conlon et al., 1994). Therefore, it is possible that neural induction in the absence of Nodal signaling proceeds through the same organizer-based molecular mechanism as in normal neural induction.

While BMP inhibition is the major molecular determinant of neural induction, other pathways cooperate to establish competence, regulate inducing signals, and maintain the induced state. In addition to a possible pre-gastrulation role for Nodal signaling, FGF signaling is another important pathway for establishing proper neural induction (reviewed in Stern, 2005). In the mouse embryo system, two key experiments (the loss of BMP receptor (A. di Gregorio and T. Rodriguez, personal communication) and the embryonic stem cell experiments (Smukler et al., 2006)) found that pharmacological inhibition of FGF signaling had no effect on acquisition of neural fates. However, these findings are somewhat limited and it is possible that FGF signaling plays a role in mammalian neural induction.

Two likely mechanisms for FGF's potential role in neural induction reveal the complex interactions between signaling pathways in the regulation of embryonic development. First, FGF signaling before gastrulation may be an important competence factor for neural induction and FGF signaling later could act as a maintenance factor for neural tissue; this would explain the many studies that reveal a requirement for FGF signaling in neural induction in frog and chick embryos (Delaune et al., 2005; Launay et al., 1996; Sheng et al., 2003; Streit et al., 1998, 2000).

Second, FGF pathway activation may impact neural induction through direct and indirect regulation of the BMP pathway. It has been shown that FGF signaling through MAPK itself can inhibit signaling through the BMP signal transducer Smad1 (Kuroda et al., 2005; Pera et al., 2003). Therefore, it is possible that, in the normal embryo, FGF signaling could play a similar role, enhancing intracellular inhibition of BMP signaling to cooperate with extracellular inhibitors such as noggin and chordin. In addition, FGF may regulate expression of BMP ligands themselves, providing another level of coordinated regulation (Delaune et al., 2005).

A descriptive analysis of neural induction in the mouse embryo

While the molecular mechanism of BMP inhibition is conserved in the mouse as the primary mode of neural induction, the embryological basis for neural tissue formation is still not clearly explained. In understanding this process, it is helpful to

consider classical embryological concepts such as specification and determination. Specification means that cells have received the appropriate inducing signals to become a given tissue but are not yet committed to this fate. Determination indicates a fully committed state that cannot be inhibited by other signals.

The timing of specification and determination can be revealed by three types of experiments: explants, grafting, and analyzing molecular markers of cell fate. Before beginning a summary of the data from explants, it is important to note that these experiments are not performed in neutral media and usually include serum with unknown signaling factors, unlike the default culture experiments performed in saline solutions for frog embryo explants or mouse embryonic stem cells. However, growth and survival of mammalian tissue in non-serum containing media are poor, necessitating this experimental compromise.

Explants of fragments of the early mouse embryo show that epiblast from early streak embryos can express anterior neural markers if cultured alone, although only a fraction of explants do this and only with the marker Otx2 (Ang and Rossant, 1993; Ang et al., 1994). This induction of Otx2 cannot distinguish between Otx2 as a marker of epiblast and as a marker of anterior neural tissue. In contrast, mid and late streak explants will consistently express Engrailed (an unambiguous marker of anterior neural tissue) and Otx2 upon culture (Ang and Rossant, 1993; Ang et al., 1994), while explants from slightly later stages also express markers of forebrain such as Six3 (Yang and Klingensmith, 2006). This anterior neural character of anterior epiblast explants can be inhibited by ectopic BMP signaling until early head fold stages, at which point it is resistant to outside signaling and fully determined to be anterior neural tissue (Yang and Klingensmith, 2006).

Grafting experiments reveal a similar time of determination in that grafts of early streak distal epiblast can contribute to many cell types, depending on the site of transplantation (Tam and Zhou, 1996), but anterior ectoderm from late streak embryos is somewhat restricted to form neural tissue, despite local signaling (Beddington, 1982).

An analysis of molecular markers of early neural tissue shows that Otx2 is not expressed in the pre-streak embryo but its expression is induced throughout the epiblast at very early streak stages. By mid to late streak stages, Otx2 expression has shifted to the anterior-distal third of the embryo (Ang et al., 1994), as discussed above. Throughout gastrulation, the pattern of Otx2 is complementary to that of phosphorylated Smad1, an indicator of BMP signaling, that is known to inhibit neural induction (Fig. 1).

The anterior-distal epiblast locations for the earliest markers of neural tissue are consistent with labeling and grafting experiments that show the prospective neural tissue resides in the distal epiblast. The distal cells of the pre-streak and early streak embryo move anteriorly during gastrulation to give rise to the anterior neural tissue (Quinlan et al., 1995; Tam, 2004).

In summary, and as depicted in Fig. 2, it seems that neural tissue is first specified in the distal epiblast by mid-streak stages and is determined during late streak and bud stages. The specifically anterior character of neural tissue is determined by head fold stages.

The mouse early/mid-gastrula organizer specifies prospective forebrain in the distal epiblast

Most previous models of neural induction in the mouse embryo have focused on the role of the node or the anterior

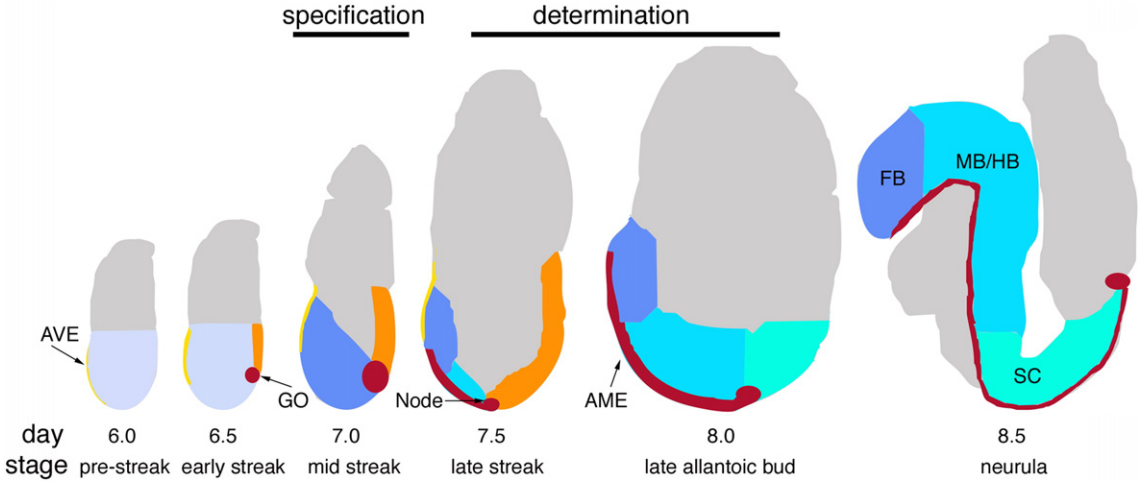

Fig. 2. Neural induction in the mouse embryo from embryonic day 6.0 to 8.5. The epiblast of the early mouse embryo (day 6.0–6.5) exists in a pre-neural state (light blue). At pre-streak stages, the only evidence of embryonic polarity is the anterior visceral endoderm (AVE, yellow) overlying the anterior epiblast. As gastrulation initiates, the early primitive streak (orange) forms in the posterior embryo, with the early gastrula organizer (GO, maroon) anterior to the streak. By mid-streak stages, the AVE has migrated proximally, the primitive streak has elongated distally, and early neural markers are expressed (blue) in the distal/anterior half of the embryo. These markers represent the specification of neural tissue. As gastrulation proceeds, anterior neural precursors (blue) migrate anteriorly, to become juxtaposed with the AVE. The node is located at the distal end of the embryo by late streak stages and anterior mesendoderm (AME, maroon) extends anteriorly from the node during late streak and allantoic bud stages. Determination of anterior neural tissue occurs during late streak stages and is complete by the late allantoic bud stage. By e8.5, neural induction is mostly completed and the neural plate begins to form a tube, through neurulation. The neural tissue is comprised of forebrain (FB, blue), midbrain (MB, light blue), hindbrain (HB, light blue), and spinal cord (SC, turquoise). The anterior mesendoderm underlies the neural tissue and is required for its maintenance. This image is not drawn to scale.

visceral endoderm because loss of either tissue results in anterior neural truncations. In fact, many models of neural induction suggest that there could be multiple tissues that induce neural tissue in the embryo—one that could induce anterior neural tissue (hypothesized to be the anterior visceral endoderm) and one that could induce posterior neural tissue (hypothesized to be the node). This explanation is similar to a model suggested in the 1930s by Hans Spemann and Otto Mangold (reviewed in Stern, 2005).

However, the literature shows that prospective forebrain is specified at early to mid-streak stages—well before the formation of the definitive node, and independently of the anterior visceral endoderm. We have re-examined published literature of neural induction in the mouse, considering which tissues are present at the relevant time, place, and with the appropriate molecular components to induce the neural tissue. We conclude that anterior neural tissue is induced by the early/mid-gastrula organizer and that posterior neural tissue is induced subsequently by its derivative, the node, as is the case for frog embryos (Table 1).

The anterior visceral endoderm

The anterior visceral endoderm has been described as a possible 'head' organizer and is thought to induce anterior neural tissue, possibly in collaboration with the gastrula organizer or node. The anterior visceral endoderm certainly exists at the right time to induce neural tissue, being present beginning shortly after implantation, and it is located over the proximal anterior epiblast during neural induction stages, not far removed from the prospective forebrain region (Thomas et al., 1998). Molecularly, the anterior visceral endoderm expresses Lefty, a Nodal inhibitor, and Cerberus, an inhibitor of both Wnt and BMP signaling that could account for its partial ability to cooperate in the induction of anterior neural tissue (Belo et al., 1997; Meno et al., 1997).

The evidence for this function of the anterior visceral endoderm comes principally from loss of function analysis, which shows that several anterior visceral endoderm factors are required for formation of anterior neural tissue. Removal of the anterior visceral endoderm or genetic loss of Otx2, Lim1, FoxA2 and other factors from the anterior visceral endoderm results in dramatic anterior neural truncations (Martinez-Barbera and Beddington, 2001; Thomas and Beddington, 1996).

Of note, despite loss of the anterior visceral endoderm in the Nodal mutant, anterior neural induction occurs throughout the epiblast (Camus et al., 2006). This demonstrates that anterior visceral endoderm is not always required for anterior neural formation, but more likely plays an indirect or secondary role.

The mouse anterior visceral endoderm is not sufficient to induce neural tissue in total epiblast explants (Kimura et al., 2000) or in anterior explants of early streak embryos, although it can act on anterior explants of mid or late streak embryos to induce forebrain markers (Yang and Klingensmith, 2006). This last observation does not demonstrate the ability of the anterior visceral endoderm to induce neural tissue because at mid to late streak stages, the forebrain precursors have already been specified and have migrated to the anterior epiblast. Similarly, anterior visceral endoderm is not sufficient to induce any neural tissue upon heterochronic, heterotropic grafting (Tam and Steiner, 1999), although it can cooperate with the early gastrula organizer and the anterior epiblast to induce a full secondary axis upon such grafting. However, the anterior visceral endoderm of rabbits can induce forebrain when transplanted into chick epiblast (Knoetgen et al., 1999; Tam and Steiner, 1999).

These partial and cooperative sufficiency findings may reflect the fact that the anterior visceral endoderm expresses Cerberus, a combined BMP and Wnt inhibitor. However, it seems that strong BMP inhibition is required for neural induction, such as that produced by multiple BMP inhibitors and that Cerberus alone, and located in the anterior visceral endoderm, is not sufficient in the context of the mouse embryo. In addition, it may be possible that some aspects of neural induction in the chick are distinct and that the chick embryo is more conducive to neural induction due to unknown factors.

Furthermore, mouse mutants (Wnt3 and β-catenin) with normal anterior visceral endoderm induction, but that lack the gastrula organizer and node, do not form neural tissue. These observations strengthen the argument that the anterior visceral endoderm is not sufficient by itself for neural inducing activity (Huelsken et al., 2000; Liu et al., 1999). In addition, loss of the node factors chordin and noggin results in forebrain truncations despite normal initial patterning of the anterior visceral endoderm (Bachiller et al., 2000). As discussed below, we think that the basis of the anterior neural truncations observed upon loss of the anterior visceral endoderm reflects a requirement for this tissue in protecting the anterior character of the future brain from posteriorizing influences.

The node

Much interest regarding neural induction in mammals has focused on the node because it is thought to be the cellular and

Table 1
Summary of critical characteristics of tissues implicated in mouse neural induction

	Necessary	Sufficient	Correct time/place	Signaling factors
AVE	NO (Camus et al., 2006)	NO (Kimura et al., 2000; Kinder et al., 2001)	YES (Thomas et al., 1998)	Lefty, Cerberus (Belo et al., 1997; Meno et al., 1997)
Node	NO (Klingensmith et al., 1999)	YES (Beddington, 1994; Klingensmith et al., 1999)	NO (Kinder et al., 2001)	Chordin, Noggin (Bachiller et al., 2003)
GO	YES (Liu et al., 1999)	YES (Klingensmith et al., 1999)	YES (Kinder et al., 2001)	Chordin (Kinder et al., 2001)

molecular equivalent of the *Xenopus* organizer that causes neural induction in the frog. The node is located at the distal tip of the embryo (Beddington and Robertson, 1999), placing it in the appropriate location to induce anterior neural tissue, then later posterior neural tissue, but the true node of the mouse embryo does not form until late streak stages, after neural induction has commenced and at a time when forebrain precursors are already located proximally. The node possesses a full complement of the signaling molecules involved in neural induction, including chordin and noggin (Bachiller et al., 2000; Zhu et al., 1999).

The mouse node is sufficient for neural induction as nodes from late streak (but not head-fold stage) can induce expression of anterior neural markers, such as Engrailed, in early streak ectoderm explants (Klingensmith et al., 1999). In addition, the mouse node can induce a full secondary neural axis when transplanted into the chick embryo and a partial secondary neural axis when transplanted into the mouse embryo (Beddington, 1994; Knoetgen et al., 2000).

The node factors noggin and chordin are even sufficient as purified proteins to induce anterior neural tissue (as indicated by Sox2, Six3, and Hesx1) in explants of mid-streak 'naive' ectoderm, thus providing a molecular explanation for the sufficiency of the node in neural induction (Yang and Klingensmith, 2006). However, these 'naive' ectoderm explants may have already received the inducing signals that specify neural tissue around the mid-streak stage, so these results cannot prove the sufficiency of noggin/chordin-mediated direct neural induction.

Absence of the node, as occurs in the FoxA2 mouse mutant, allows limited neural induction in a fraction of embryos (Klingensmith et al., 1999). Importantly, this mutation causes a loss of the definitive node and of the molecules that it normally expresses (for instance noggin and chordin). Loss of the node from similar stages of gastrulation in Fgf8 and Cripto mutants allows robust anterior neural induction (Ding et al., 1998; Sun et al., 1999). Therefore, the node is not required for neural induction.

The gastrula organizer

While the node itself may not play a role in inducing the first neural tissue, its predecessor, the early/mid-gastrula organizer, fulfills this function. This tissue is located at the anterior edge of the primitive streak (Kinder et al., 2001), closely juxtaposed to the distal epiblast at mid-streak stages (Fig. 2). Furthermore, the early/mid-gastrula organizer expresses chordin, a key BMP inhibitor (Kinder et al., 2001).

The primary role of the early gastrula organizer in anterior neural induction is supported by the observations that the only mouse mutations that result in complete failure to induce neural tissue are those that prevent formation of the gastrula organizer. For instance, loss of the mesoderm inducer Wnt3 (Liu et al., 1999), or its signal transducer β-catenin (Huelsken et al., 2000), completely abrogates primitive streak formation, including the gastrula organizer, and no neural markers are expressed in these embryos. In contrast, the anterior visceral endoderm is induced normally. While these data could support a model in which Wnt signaling is required directly for neural induction, this is unlikely as activated Wnt pathways inhibit anterior neural tissue in the mouse, through Wnt8C overexpression (Popperl et al., 1997) or loss of the Wnt inhibitor Dkk1 (Mukhopadhyay et al., 2001). Furthermore, treatment of mouse embryonic stem cells with the inhibitor Dkk1 cooperates in inducing neural cell fates (Watanabe et al., 2005). Therefore, it is more likely that these data demonstrate a requirement for Wnt signaling in inducing the organizer, which in turn is required for all neural induction in the mouse embryo.

The ability to induce neural tissue can be specifically assigned to the early/mid-gastrula organizer (as opposed to the node) because preservation of the organizer allows neural formation in several mouse mutants that prevent formation of the definitive node.

For instance, in the FoxA2 mouse mutant, neural induction proceeds, albeit in a compromised manner and only in a fraction of embryos. In these mutants, the definitive node is missing but a brief phase of early chordin expression in the mid-gastrula organizer is preserved (Klingensmith et al., 1999). These embryos also have distal localization of anterior visceral endoderm markers that may protect the limited amount of neural tissue formed from posteriorization (Klingensmith et al., 1999). Similarly, loss of Fgf8 or Cripto preserves some proximal expression of organizer markers, while interrupting formation of the node, and in these mutants, anterior neural tissue is formed robustly (Ding et al., 1998; Sun et al., 1999).

Mutation of Nodal disrupts formation of the anterior visceral endoderm, the primitive streak, and node. However, the organizer markers Brachyury, FoxA2, and Gsc indicate possible broad formation of the organizer (Camus et al., 2006; Conlon et al., 1994) and most of the epiblast is converted into anterior neural tissue. It would be very interesting to see if chordin expression in the organizer is preserved in the Nodal mutant and to determine whether these markers represent a functional organizer.

The early/mid-gastrula organizer is sufficient to induce anterior neural tissue and probably fulfills this function in the mouse mutants just described (FoxA2, Fgf8, Cripto, Nodal). In a more direct sufficiency test, the organizer can induce Engrailed in explants of early streak embryos (Klingensmith et al., 1999). It has previously been suggested that the early gastrula organizer is not sufficient for anterior neural induction because heterochronic, heterotropic grafts of the early gastrula organizer induce a secondary axis that does not contain forebrain (Tam and Steiner, 1999). However, the most likely explanation for this observation is that the early gastrula organizer does induce anterior neural tissue but that it is posteriorized by local influences in the host embryo as the gastrula organizer can induce anterior neural markers in explants of early epiblast (Klingensmith et al., 1999). Another possibility is that the grafting procedure only includes a partial organizer, one that is insufficient for full organizer activity.

In summary, our model suggests that the first, anterior neural tissue is induced during early to mid-streak stages by the gastrula organizer through its secretion of a BMP inhibitor that protects the distal epiblast from BMP posteriorizing signals and allows the formation of default anterior neural tissue.

The anterior visceral endoderm protects pre-specified anterior neural tissue from posteriorization

Default anterior neural tissue specified in the distal epiblast then moves anteriorly to lay under the anterior visceral endoderm in the proximal epiblast (Quinlan et al., 1995). This is a critical placement because the anterior visceral endoderm is required to inhibit signals such as Wnts, Nodals, and BMPs that are all expressed in the posterior embryo and can posteriorize neural tissue. To this end, the anterior visceral endoderm expresses the Wnt and BMP inhibitor Cerberus and the Nodal inhibitor Lefty (Belo et al., 1997; Meno et al., 1997). Removal of the anterior visceral endoderm through surgical (Thomas and Beddington, 1996) or genetic means results in loss of anterior neural tissues including the forebrain, midbrain, and hindbrain (Martinez-Barbera and Beddington, 2001). This is consistent with a general role for the anterior visceral endoderm in restricting posterior cell fates, as mesoderm markers that are typically only expressed in the posterior embryo are found throughout the anterior and posterior proximal embryo in anterior visceral endoderm mutants (Kimura et al., 2000).

While the anterior visceral endoderm cannot induce neural tissue in explants, it can suppress markers of posterior tissue (Kimura et al., 2000). In the Cripto and Fgf8 mutants, the anterior visceral endoderm is located distally and the underlying ectoderm that would normally form posterior neural tissue such as spinal cord instead is converted into anterior neural tissue, most likely through organizer-induced anterior neural formation followed by anterior visceral endoderm mediated protection of this tissue from posteriorizing influences (Ding et al., 1998; Sun et al., 1999).

The node induces caudal neural tissue

The anterior neural tissue is formed first and moves away from the gastrula organizer. The molecularly identifiable gastrula organizer then progressively becomes the node—a morphologically distinct structure that retains the neural inducing capability of its predecessor. The late streak/early bud node still possesses the ability to induce anterior neural tissue, as determined by its activity upon co-culture with early streak epiblast explants (Klingensmith et al., 1999). This is in accordance with Nieuwkoop's activation-transformation model, in which the first step in neural induction is the formation of anterior neural tissue, which we now know is the default fate (Nieuwkoop, 1954). However, neural tissue induced by the definitive node is located distally in the late gastrula embryo where it is influenced by posteriorizing signals emanating from the primitive streak, so this tissue is 'transformed' into caudal neural tissue.

As the node normally induces neural tissue that is posteriorized by local influences, it can also induce an ectopic posterior neural axis upon transplantation to the posterior region of a host (Beddington, 1994; Tam and Steiner, 1999). However, transplantation of the node to the anterior region of the chick embryo induces a secondary neural axis with both anterior and posterior neural tissue (Knoetgen et al., 2000). This may be a species-specific difference, but it is also possible that this is a site-specific difference, in that the anterior regions of embryos are somewhat shielded from the posteriorizing influences of the primitive streak, and thus neural tissue induced here may include anterior structures.

The node is required for the induction of caudal neural tissue. As discussed above, in mutants that preserve early gastrula organizer function, but in which the node does not form (FoxA2, Fgf8, Cripto), no posterior neural tissue develops. This observation can support our model in which early anterior neural tissue is induced by the gastrula organizer and migrates anteriorly, while the gastrula organizer then forms the node which continues the same function but in proximity to posteriorizing factors. If the definitive node tissue is not present, continuous induction of neural tissue will fail after formation of the early anterior tissue.

However, it is unclear what molecular factors in the node account for its ability to induce neural tissue that is posteriorized because removal of the classic BMP inhibitors chordin and noggin abrogates anterior neural formation but preserves posterior neural development (Bachiller et al., 2000). As more posterior fates are produced sequentially, it is possible that another BMP inhibitor is expressed in the late definitive node and can rescue chordin and noggin function only during these later stages. GDF-3, a BMP-inhibitory TGF-β ligand, could play this role as it is expressed beginning at late e7.5 in the node, suggesting that it could account for later and more posterior rescue of BMP inhibition-mediated neural induction (Levine and Brivanlou, 2006).

Alternatively, the data from the FoxA2, Fgf8, and Cripto mutants could be explained by the multiple organizer model in which the gastrula organizer induces head neural tissue through one strategy and the definitive node induces caudal neural tissue separately. We do not favor this model because in the absence of posteriorizing factors, for instance in the context of an early epiblast explant on in the anterior chick embryo, the late streak node has full ability to induce anterior neural tissue (Klingensmith et al., 1999; Knoetgen et al., 2000).

The anterior mesendoderm derivatives of the node maintain forebrain character

Following the specification and determination of anterior neural tissue during gastrulation, this tissue must be maintained by the underlying anterior mesendoderm, the final derivatives of the node. This maintenance likely occurs through multiple pathways, including continued BMP inhibition. The ultimate outcome of this regulation is the induction and specification of the anterior neural ridge, a signaling center that expresses Fgf8, Shh, and Foxg1.

The role of the anterior mesendoderm was first appreciated following the observation that co-culture of anterior mesendoderm explants from head-fold stage embryos with naive ectoderm induces anterior neural tissue (Ang and Rossant, 1993). Anterior mesendoderm from mid to late streak embryos also possesses this ability, but induces anterior neural tissue with lower efficiency (Ang and Rossant, 1993). In contrast, co-culture of posterior mesendoderm with early ectoderm suppresses endogenous Otx2

expression (Ang et al., 1994). While anterior mesendoderm can induce anterior neural tissue, it appears to be more important for maintenance of this fate. In explants of 'late-bud' stage embryos, the forebrain has already been specified but markers of forebrain disappear after prolonged culture or upon treatment with exogenous BMPs unless these explants are co-cultured with anterior mesendoderm (Yang and Klingensmith, 2006).

Several mouse mutants reveal the necessity of this ongoing maintenance as defects in mesendoderm generally result in anterior neural truncations and post-gastrulation loss of forebrain markers that were normally induced during gastrulation. This phenotype is typified by the Gdf1−/−;Nodal+/− double mutant that does not properly induce mesendoderm and as a result displays forebrain defects (Andersson et al., 2006). In the Hex1 mutant, anterior mesendoderm is induced but does not migrate anteriorly. The subsequent lack of anterior mesendoderm under the forebrain results in failure to maintain this tissue (Martinez Barbera et al., 2000). The signaling factors chordin and noggin are also required in the mesendoderm to continuously inhibit BMP signaling for the maintenance of forebrain. In Chordin−/−;Noggin+/− embryos, lack of these factors in the anterior mesendoderm results in failure to maintain forebrain tissue (Anderson et al., 2002). In support of a requirement for BMP inhibition in forebrain maintenance, it has been shown that treatment of anterior neural tissue with exogenous BMPs results in decreased expression of markers of the anterior neural ridge (Anderson et al., 2002).

Conclusion

Our hypothesis opposes the model that, in the mouse embryo, "there appear to be two signaling centers, one in the node and one in the anterior visceral endoderm. The latter center is critical for generating the forebrain, while the former is critical in inducing axial structures caudally from the midbrain" (Gilbert, 2003). Many previous reviews have considered both the multiple organizer model and a model based on the cooperative induction of neural tissue by the anterior visceral endoderm and node/organizer together (Stern et al., 2006; Tam, 2004).

Instead, we find that the cumulative data on neural induction in the mouse embryo point to a model in which the gastrula organizer antagonizes BMP signaling to allow induction of anterior neural tissue through a default mechanism and that this tissue is subsequently maintained by the anterior visceral endoderm, then anterior mesendoderm. The derivative of the gastrula organizer, the node, induces posterior neural tissue through the same BMP-inhibitory mechanism. This model is supported by data from genetics, explants, grafting, and molecular marker expression. Our model is reminiscent of neural induction in 'lower' vertebrates, suggesting an evolutionarily conserved strategy for this important step in development.

Nearly one hundred years of developmental biology research has finally discovered the fundamental mechanisms driving the induction of neural tissue, a principal question of development since the original frog organizer experiments of Spemann and Mangold. However, the findings and understanding presented in our review represent only the most elemental first step in the formation of the amazingly complex nervous system of vertebrates. Once neural induction is complete, this tissue must further differentiate, undergo complicated morphogenesis processes, and form the vast network of axons and dendrites that make up the final nervous system. It is these later processes that form the uniquely capable human brain. But concerning the most basic mechanisms of neural induction, there is nothing new under the sun.

Acknowledgments

We gratefully acknowledge the thoughtful and critical suggestions on this review by Dr. Richard Harland, Dr. Francesca Spagnoli and Dr. Richard Levine. We also sincerely appreciate the generosity of Dr. Tristan Rodriguez who allowed us to include his unpublished observations, including work done by Aida di Gregorio.

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