Neural induction: old problem, new findings, yet more questions

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Development 132, 2007-2021
Published by The Company of Biologists 2005
doi:10.1242/dev.01794

Summary

During neural induction, the embryonic neural plate is specified and set aside from other parts of the ectoderm. A popular molecular explanation is the ‘default model’ of neural induction, which proposes that ectodermal cells give rise to neural plate if they receive no signals at all, while BMP activity directs them to become epidermis. However, neural induction now appears to be more complex than once thought, and can no longer be fully explained by the default model alone. This review summarizes neural induction events in different species and highlights some unanswered questions about this important developmental process.

Introduction

During gastrulation, cells ingress from the surface ectoderm into the interior of the embryo to give rise to the mesodermal and endodermal germ layers. In vertebrates, ingress can occur through a blastopore (as in amphibians), around and through an embryonic shield (as in teleosts), or through a primitive streak (as in amnioxes: reptiles, birds, mammals). Dramatically, transplantation of the most-dorsal lip of the amphibian blastopore to the ventral side (prospective belly) of another embryo at the gastrula stage generates a second axis, in which almost all of the central nervous system (CNS) (with the exception of regions of the floor plate) is derived from the host ectoderm rather than from the graft. It was this experiment, performed by Hans Spemann’s student Hilde Mangold (Spemann, 1921; Spemann and Mangold, 1924) between differently pigmented species of newt, that firmly established the concept of neural induction as an instructive interaction between the dorsal lip of the blastopore (the ‘organizer’) and the neighbouring ectoderm. This instructive interaction leads to the induction of the nervous system. Soon thereafter, the equivalent region was discovered in most vertebrate classes: the shield of teleosts (Luther, 1935; Oppenheimer, 1936b) and Hensen’s node (the distal tip of the primitive streak) in birds and mammals (Waddington, 1932; Waddington, 1933; Waddington, 1936; Waddington, 1937). Each of these will induce a neural plate not only within the same species but also when transplants are performed across classes (e.g. Waddington, 1934; Oppenheimer, 1936a; Kintner and Dodd, 1991; Blum et al., 1992; Hatta and Takahashi, 1996), strongly indicating that the mechanisms of neural induction are conserved throughout the vertebrates.

A first molecular explanation: the default model

For more than six decades, many laboratories tried very hard to uncover the molecular nature of the signals emitted from the organizer, always with the expectation that a single molecule might be ‘the neural inducer’. This met with little success, partly because in the newt, where most of the experiments were carried out, many heterologous substances can generate ectopic neural structures (reviewed by Nakamura and Toivonen, 1978; Hamburger, 1988; Stern, 2004). The turning point only came in the mid-1990s, when several groups made a number of observations that at first seemed unconnected. First, it was observed that the dissociation of Xenopus gastrula-stage animal caps into single cells for a short time before reaggregating them led to the formation of neural tissue (Born et al., 1989; Godsave and Slack, 1989; Grunz and Tacke, 1989; Sato and Sargent, 1989; Saint-Jeannet et al., 1990). Then, it was found that misexpression of a dominant-negative ‘activin’ receptor (later discovered to inhibit several TGFβ-related factors) in Xenopus embryos blocked mesoderm formation, but also unexpectedly generated ectopic neural tissue (Hemmati-Brivanlou and Melton, 1992; Hemmati-Brivanlou and Melton, 1994). These findings were later connected by the idea that neural tissue might be induced by the removal of some unknown inhibitory substance (Hemmati-Brivanlou and Melton, 1994). Soon, three genes encoding proteins with neuralizing activity were isolated and found to be expressed in the organizer: Noggin (Smith and Harland, 1992; Lamb et al., 1993; Smith et al., 1993; Furthauer et al., 1999), Follistatin (Hemmati-Brivanlou et al., 1994) and Chordin (Sasai et al., 1994; Sasai et al., 1995). These turned out to be binding partners of bone morphogenetic proteins (BMPs) that antagonize BMP signalling (Piccolo et al., 1996; Zimmerman et al., 1996; Fainsod et al., 1997). That the postulated inhibitory substance was BMP4 was also supported by the finding that BMP4 is an effective inhibitor of neural fate while promoting epidermal differentiation, even in dissociated cells (Hawley et al., 1995; Wilson and Hemmati-Brivanlou, 1995). These findings led to the ‘default model’ of neural induction (Hemmati-Brivanlou and Melton, 1997) (Fig. 1), which proposes that cells within the ectoderm layer of the frog gastrula have an autonomous tendency to differentiate into neural tissue, which is inhibited by BMPs (in particular, BMP4, which acts as an epidermal inducer).

In support of this model (Fig. 2), neuralization does not occur after dissociation of animal caps obtained from embryos that have been previously injected with RNA encoding effectors of BMP4 (Msx1, Smad1 or Smad5) (Suzuki et al., 1997a; Suzuki et al., 1997b; Wilson et al., 1997), consistent...
with the view that neural fates are inhibited by an endogenous BMP activity. Moreover, the expression pattern of Bmp4 in Xenopus conforms to its proposed anti-neural function: in the early gastrula, Bmp4 transcripts are widely expressed in the entire ectoderm and then are lost from the future neural plate when the organizer appears (Fainsod et al., 1994). The transcription of BMP genes is maintained by the activity of BMP protein (Biehs et al., 1996; Schulte-Merker et al., 1997). This accounts for the disappearance of Bmp4 and Bmp7 expression from the vicinity of the organizer, the source of BMP antagonists, at the late gastrula stage (Fainsod et al., 1994; Hawley et al., 1995; Baker et al., 1999). Animal caps cut from embryos injected with dominant-negative BMP receptors (Sasai et al., 1995; Xu et al., 1995), with non-cleavable forms of BMP4 or BMP7 (Hawley et al., 1995), or with antisense Bmp4 RNA (Sasai et al., 1995) adopt a neural, rather than an epidermal, fate. Finally, Chordin and Noggin proteins can neutralize isolated animal caps (although this seems to work best if the animal caps are first exposed briefly to a low Ca\(^{2+}\)/Mg\(^{2+}\) medium – the rationale given for this is that this treatment helps the protein penetrate between the cells) (Lamb et al., 1993; Sasai et al., 1995). Other secreted, BMP-antagonizing molecules have been found that are expressed in the organizer or in its proximity, such as Cerberus (Bouwmeester et al., 1996; Belo et al., 1997), Gremlin, Dan and Drm (Hsu et al., 1998; Pearce et al., 1999; Dionne et al., 2001; Eimon and Harland, 2001; Khokha et al., 2003), and Ogon/Sizzled (Wagner and Mullins, 2002; Yabe et al., 2003b). Furthermore, depletions of three BMP antagonists (Chordin, Noggin and Follistatin) from Xenopus tropicalis embryos using morpholino oligonucleotides causes a dramatic ventralization of the embryo, which includes almost complete loss of the neural plate (Khokha et al., 2005).

Together, these findings provide compelling evidence that BMPs and their modulation by endogenous inhibitors are involved in the specification of neural and non-neural domains in Xenopus. As such, the default model proved very attractive both because of its simplicity and also because it was the first to explain neural induction since the discovery of the organizer by Spemann and Mangold. One might therefore indeed be tempted to consider the whole problem of neural induction as being solved. But many of the most important issues remain, including whether BMP inhibition is really sufficient to specify neural fate. The following sections summarize some of the current controversies and unanswered questions.

Is BMP inhibition sufficient for neural induction?

One might argue that the ideas of ‘default’ and ‘sufficient’ are inappropriate terms to describe any biological process, mainly because both concepts imply that the previous developmental history and present state of the cell are unimportant. Not surprisingly, more recent research on neural induction has started to uncover new players, as well as complex interactions between them. A first challenge to the default model came from observations in amphibian embryos, where it was found that neither Chordin (Sasai et al., 1996) nor Noggin (Launay et al., 1996) could induce neural tissue in embryos in which FGF signalling had been blocked by the injection of a dominant-negative FGF receptor. Further challenges to the default model came from studies in chick embryos (Table 1, Fig. 3). These studies reported several key findings: that the expression patterns of Bmps and their antagonists do not fit the default model, that misexpressing BMP antagonists in competent epiblast does not induce the expression of any neural markers, and that a grafted source of BMP protein does not inhibit neural plate development (except for a slight narrowing of the neural plate) (Streit et al., 1998; Streit and Stern, 1999a; Streit and Stern, 1999b). Moreover, although both Bmp4 mRNA and phospho-Smad1 (an effector of BMP signalling) are downregulated in the forming chick neural plate (Streit and Stern, 1999a; Streit and Stern, 1999b; Faure et al., 2002), this occurs at a relatively late stage of development, when compared with the timing of their downregulation in Xenopus, and only after the neural plate markers SOX3 and SOX2 have begun to be expressed. However, exposure of the chick epiblast to a grafted organizer for 5 hours (11-13 hours are required for neural induction) (Gallera and Ivanov, 1964; Gallera, 1971) transiently induces the early neural plate marker SOX3 (Table 2), the expression of which can be stabilized by Chordin after removal of the grafted organizer (Streit et al., 1998). This finding implies that the ectoderm must be exposed for 5 hours to signals from the organizer before it can respond to BMP antagonists.
A few years ago, a differential screen was conducted to define the events that take place during the first 5 hours following the grafting of an organizer into a chick embryo. This screen identified several genes, including ERNI (early response to neural induction), which is induced after 1 hour (Streit et al., 2000), and Churchill (which is induced after 4-5 hours (Sheng et al., 2003)). Both are expressed in the prospective neural plate, but neither is induced by BMP antagonists, although both genes are induced by fibroblast growth factor (FGF). In fact, inhibiting FGF signalling completely blocks neural induction (early response tissue shown; – indicates no expression; arrows indicate downregulation; ? indicates a fate that has not been tested in published experiments. Modified, with permission, from Stern (Stern, 2004).

A role for FGFs in neural induction

Strikingly in ascidians, which are basal chordates, FGFs, rather than BMP inhibition, are the endogenous factors responsible for generating the nervous system (Inazawa et al., 1998; Darras and Nishida, 2001; Hudson and Lemaire, 2001; Kim and Nishida, 2001; Bertrand et al., 2003; Hudson et al., 2003). In vertebrates, there has been more controversy about the role of FGFs in neural induction. It has been claimed that FGFs can direct ectodermal cells to a neural pathway in amphibians (Kengaku and Okamoto, 1995; Lamb and Harland, 1995; Hongo et al., 1999; Strong et al., 2000), zebrafish (Kudoh et al., 2004) and chick (Rodríguez-Gallardo et al., 1997; Alvarez et al., 1998; Storey et al., 1998) in the absence of other signals. However, in Xenopus, this activity requires special experimental conditions (either partial dissociation of the cells or the isolation of animal caps) (Pera et al., 2003; Linker and Stern, 2004; Delaune et al., 2005), while in chick, the induced neural tissue is of a posterior character; whether or not the induction is direct (without the prior induction of mesoderm and/or endoderm) has not been firmly established. It is now
generally believed that FGFs are probably not direct neural inducers in vertebrates, or at least not by themselves.

There has also been controversy concerning whether or not FGFs are required at all for neural induction in amphibians. This is because the injection of dominant-negative FGFR1 inhibits mesoderm formation and posterior axis development but not neural induction (Amaya et al., 1991; Cox and Hemmati-Brivanlou, 1995; Kroll and Amaya, 1996; Godslove and Durston, 1997; Holowacz and Sokol, 1999; Curran and Grainger, 2000; Ishimura et al., 2000; Ribisi et al., 2000; Pownall et al., 2003). However, it has been proposed that neural induction may involve FGFR4 rather than FGFR1 (Hongo et al., 1999; Hardcastle et al., 2000; Umbhauer et al., 2000), and a more recent study using a general inhibitor of FGFRs has uncovered a clear requirement for FGF signalling in neural induction in *Xenopus* (Delaune et al., 2005). Overall, it is now generally accepted that FGF signalling is required for neural induction both in amphibians (Launay et al., 1996; Sasai et al., 1996; Xu et al., 1997; Hardcastle et al., 2000; Strong et al., 2000; Pera et al., 2003; Linker and Stern, 2004; Delaune et al., 2005) and in chick (Streit et al., 2000; Wilson et al., 2000; Linker and Stern, 2004).

### Relationships between MAPK and BMP signalling in neural induction

To reconcile the default model with findings implicating FGFs in neural induction (Fig. 4), it has been proposed that the activation of MAP kinase (MAPK) by FGF [or by other factors](#1)

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**Table 1. Results of chick misexpression experiments**

(A) Experiments on embryos

<table>
<thead>
<tr>
<th>Factor/tissue</th>
<th>Marker genes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SOX3</td>
</tr>
<tr>
<td>Node</td>
<td>+ (2 hours)</td>
</tr>
<tr>
<td>Hypoblast</td>
<td>+ (2 hours)</td>
</tr>
<tr>
<td>FGF8</td>
<td>+ (2 hours)</td>
</tr>
<tr>
<td>Chordin</td>
<td>–</td>
</tr>
<tr>
<td>Noggin</td>
<td>–</td>
</tr>
<tr>
<td>Smad6</td>
<td>–</td>
</tr>
<tr>
<td>BMP4 electroporation*</td>
<td>No effect (-6 hours)</td>
</tr>
<tr>
<td>Node (5 hours) + Chordin</td>
<td>+ (Maintenance)</td>
</tr>
<tr>
<td>FGF8 (5 hours) + Chordin</td>
<td>+ (Maintenance)</td>
</tr>
<tr>
<td>Smad6 + FGF8</td>
<td>+</td>
</tr>
<tr>
<td>Smad6 + α-Wnt</td>
<td>–</td>
</tr>
<tr>
<td>Smad6 + α-Wnt + Chordin + Noggin</td>
<td>+</td>
</tr>
<tr>
<td>FGF8 + α-Wnt</td>
<td>+</td>
</tr>
</tbody>
</table>

(B) Experiments on explants

<table>
<thead>
<tr>
<th>Explant</th>
<th>Marker genes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SOX3</td>
</tr>
<tr>
<td>Neur</td>
<td>Epi</td>
</tr>
<tr>
<td>No factor</td>
<td>+</td>
</tr>
<tr>
<td>BMP4</td>
<td>–</td>
</tr>
<tr>
<td>DN-BMPR + SU5402</td>
<td>+</td>
</tr>
<tr>
<td>Wnt3A (1×)</td>
<td>–</td>
</tr>
<tr>
<td>Wnt3A (1×) + FGF2</td>
<td>–</td>
</tr>
<tr>
<td>Wnt3A (1×) + Noggin</td>
<td>+</td>
</tr>
<tr>
<td>SU5402</td>
<td>–</td>
</tr>
<tr>
<td>SU5402 + Noggin</td>
<td>–</td>
</tr>
<tr>
<td>Wnt3A (3×)</td>
<td>–</td>
</tr>
<tr>
<td>Wnt3A (3×) + Noggin</td>
<td>–</td>
</tr>
<tr>
<td>Fz8CRD</td>
<td>–</td>
</tr>
<tr>
<td>Fz8CRD + SU5402</td>
<td>–</td>
</tr>
<tr>
<td>Fz8CRD + BMP4</td>
<td>–</td>
</tr>
<tr>
<td>Fz8CRD + SU5402 + Noggin</td>
<td>+</td>
</tr>
<tr>
<td>Fz8CRD + SU5402 (5 μM)</td>
<td>–</td>
</tr>
<tr>
<td>Fz8CRD + SU5402 (5 μM) + Noggin</td>
<td>–</td>
</tr>
</tbody>
</table>

(A) Experiments in whole embryos in which a candidate signalling tissue, or COS cells transfected with a secreted factor, are grafted into a test region of the epiblast; or a plasmid encoding a test protein is electroporated into a test region. Data are taken from Streit et al. and Linker and Stern (Streit and Stern, 1999a; Streit and Stern, 1999b; Streit et al., 1998; Streit et al., 2000; Linker and Stern, 2004). In the node, hypoblast and FGF8 experiments, the length of exposure in hours required to obtain marker induction is shown in brackets. *Brachyury* is a mesoderm marker and SOX2, a ‘definitive’ neural plate marker. *ERN1, OTX2, SOX3 and ChCh* are expressed in the early epiblast, including the prospective neural territory, but do not indicate commitment to a neural fate. No combination of factors mimics SOX2 induction by the node. +, induction; –, no induction; Inhibition, endogenous expression inhibited; Maintenance, treatment maintains otherwise transient expression; α-Wnt, three Wnt antagonists (crescent, NFz8 and Dkk1) together with Cerberus (a multifunctional Wnt, BMP and Nodal antagonist).

*α-Wnt, three Wnt antagonists (crescent, NFz8 and Dkk1) together with Cerberus (a multifunctional Wnt, BMP and Nodal antagonist).

(B) Experiments in which small epiblast explants from the prospective neural territory (Neur) or rostral, prospective epidermal domain (Epi) from pre-streak embryos are isolated and cultured for 48 hours with or without factors. +/– indicates modest induction. Fz8CRD, a truncated Wnt receptor that inhibits Wnt signalling; SU5402, a synthetic inhibitor of FGFRY, DLL-4 signaling; DN-BMPR, dominant-negative BMP receptor. Blank cells indicate that the experiment was not carried out. Data from Wilson et al. (Wilson et al., 2000; Wilson et al., 2001).
that activate this pathway, such as IGF (insulin-like growth factor) and Nodal acting through its EGF-CFC co-factors] can inhibit the downstream targets of BMP (Furthauer et al., 1997; Wilson et al., 2000; Bainter et al., 2001; Pera et al., 2001; Wilson and Edlund, 2001; Wilson et al., 2001; Koshida et al., 2002; LeSueur et al., 2002; Pera et al., 2003; Yabe et al., 2003a) [see De Robertis and Kuroda (De Robertis and Kuroda, 2004) for a comprehensive review]. In particular, it has been shown that FGF signalling can phosphorylate a linker region in the middle of the BMP effector Smad1 (a modification that inhibits Smad1), whereas BMP signalling causes the Smad1 C-terminal domain to be phosphorylated (which activates it) (Pera et al., 2003; De Robertis and Kuroda, 2004). This interesting finding greatly helps to explain some of the apparently contradictory results in *Xenopus*. In agreement with this, it has been reported that merely wounding an amphibian embryo can activate MAPK (LaBonne and Whitman, 1997; Christen and Slack, 1999), which might partly account for the apparent 'sufficiency' of BMP inhibition for neural induction in animal cap assays (Streit and Stern, 1999c). However, three recent studies have suggested that FGF signalling is required for neural induction independently of its ability to downregulate BMP targets (Aubin et al., 2004; Linker and Stern, 2004; Delaune et al., 2005). Thus, although one effect of MAPK signalling is to downregulate BMP signalling, this function alone does not explain completely why FGF is required for neural induction.

In zebrafish, it has been proposed that both BMP inhibition and FGF signalling can act as direct neural inducers, with BMP antagonists functioning to induce the anterior CNS, while FGFs induce posterior neural plate; the combination of both specifies intermediate regions (Furthauer et al., 1997; Furthauer et al., 2004; Kudoh et al., 2004; Rentzsch et al., 2004). However, these experiments were conducted by injecting constructs at very early stages of development, and it cannot be excluded that the induction by either signal is indirect, that cell movement patterns are altered (causing cells from the normal neural plate to be recruited into the ectopic neural plate) or that each initiates a complex cascade of events that culminates in ectopic neural marker expression, but only as a consequence of multiple cooperating signals.

**Does Wnt signalling play a role in neural induction?**

In a second attempt to reconcile the default model with findings in the chick, it has been proposed that two separate pathways are initiated by FGF: one by which FGF induces neural fates independently of BMP inhibition; and another through which FGF represses BMP transcription, a pathway
BMP inhibition and/or Wnt inhibition are required for neural induction, at least in the chick.

**Other players: Ca^{2+} and PKC**

In addition to the signals discussed above, many other proteins have been implicated in neural induction (reviewed by Stern, 2004); however, most of them seem to act directly or indirectly by modulating BMP or MAPK signalling. Apart from these, an intracellular rise in Ca^{2+}-mediated by L-type Ca^{2+} channels has been proposed as a neural-inducing signal (Moreau et al., 1994; Leclerc et al., 1997; Leclerc et al., 2003), although the possibility that it too regulates BMP signalling or acts through the mesoderm has not been excluded (Palma et al., 2001; Leclerc et al., 2003). Finally, the balance between protein kinase C (PKC) and cAMP (Otte et al., 1988; Otte et al., 1989; Otte and Moon, 1992), which does not seem to relate to the pathways of any of the known players, is a possible signal that can trigger neural specification in amphibians. Surprisingly, this has not been followed up and probably deserves more attention.

In conclusion, therefore, findings from recent years have revealed more complexity in the mechanism of neural induction than has been proposed by the default model. Clearly, the initial hope that a single secreted factor might encapsulate all of the inducing and patterning activities of the organizer (dorsalization, neural induction and anteroposterior patterning) has all but vanished. One reason why it has been so difficult to identify the key players in this process is that embryos appear to generate complexity with only a handful of extracellular signals, each of which has multiple roles at different times in development. The use of assays limited to the misexpression of constructs only at early cleavage stages, followed by an analysis of the consequences only at a much later stage of development, will reveal the cumulative effects of the injected molecule for all stages previous to that being studied, including complex or unknown interactions with other pathways. To progress further, we need to know more about the embryological aspects of neural induction.

### When does neural induction occur?

For timed misexpression experiments, it is essential to know when neural induction normally occurs. However, it is not easy to determine which step in a cascade represents the inductive event: is it the initial specification that biases cells to their new fate, but does not irreversibly commit them, or is it the final commitment step? It is therefore relatively easy to determine when neural induction ends, but much more difficult to establish when it begins. In the chick, carefully timed node transplantation experiments have established that the node loses its inducing ability gradually, starting immediately after stage 4 (full primitive streak stage, just before the emergence of the head process; see Fig. 3). Competent regions of host embryos not fated to become neural plate rapidly lose their ability to respond to a node transplant between stages 4 and 4°, strongly suggesting that the induction of a complete CNS by the organizer normally ends between these two stages (Gallera and Ivanov, 1964; Gallera and Nicolet, 1969; Gallera, 1970; Gallera, 1971; Dias and Schoenwolf, 1990; Storey et al., 1992; Storey et al., 1995; Streit et al., 1997; Darnell et al., 1999). Likewise, in amphibians, it is generally believed that the competence of the ectoderm to respond to neural induction is
Development

Gastrulation, are marked by ERNI. This suggests that the earliest neural induction steps occur before the start of gastrulation. However, studies in chick embryos have suggested that the early gastrula stage, as the organizer is difficult to define at this time, it has generally been assumed that it begins at the anterior margin of the blastoderm (Waddington and Needham, 1936; Gurdon, 1987; Sharpe and Tam, 2004). Before this stage, ‘central cells’ are present in the posterior epiblast, as assessed by grafts into a remote site of a host embryo. Recently, a very similar conclusion was reached in *Xenopus*, based on the early expression and inducing activity of Chordin (Kuroda et al., 2004). In this study, the cells equivalent to the posterior cells were called the blastula Chordin- and Noggin-expressing cells (BCNE).

Sources of signals

If neural induction begins before gastrulation, when there is no morphological organizer (Hensen’s node or dorsal lip), what tissues emit the inducing signals? Before chick gastrulation, FGF8 is expressed in the hypoblast (which underlies the expression domains of *SOX3* and *ERNI* in the epiblast). Hypoblast tissue grafted to a remote region of the chick embryo can induce the ectopic expression of these markers (as well as of *OTX2* and *NOT1*) (Foley et al., 2000; Streit et al., 2000; Knezevic and Mackem, 2001) but only transiently. However, there also appears to be some constitutive expression of FGF3 in the epiblast itself (Wilson et al., 2000). The relative contributions of these factors to the normal expression of *SOX3* and *ERNI* have not been elucidated.
(although it should be pointed out that in the chick Noggin is not expressed at all until after the end of gastrulation, stage 4+).

Together, these observations suggest that the earliest signals for neural induction originate in part from the hypoblast (visceral endoderm in the mouse) (Thomas and Beddington, 1996; Belo et al., 1997; Varlet et al., 1997; Beddington and Robertson, 1999), and partly from organizer precursor cells. However, by the end of gastrulation, most regions of the prospective neural plate have never been close to the organizer (Hensen's node, embryonic shield or dorsal lip). This is particularly true for the anterior nervous system (prospective forebrain) in the chick and mouse, and for the posterior nervous system in zebrafish and perhaps frog (Agathon et al., 2003; Kudoh et al., 2004; Wilson and Houart, 2004). In amniotes, node derivatives (prechordal mesendoderm and head process) migrate anteriorly from the node to underlie the midline of the anterior neural plate, but are still far from the lateral regions. Some of these node derivatives have some, but reduced, neural-inducing ability when compared with the earlier-stage node from which they arose (Storey et al., 1995; Foley et al., 1997; Rowan et al., 1999). The definitive endoderm, which is also derived from the node, has been suggested to be an important source of signals for the forebrain (Knoetgen et al., 1999a; Knoetgen et al., 1999b; Withington et al., 2001; Hallonet et al., 2002; Chapman et al., 2003). Given that neither the hypoblast nor node precursor cells can induce the definitive neural marker Sox2, it remains unknown which tissues are responsible for emitting the signals that reinforce or complete the neural induction process and cause Emx- and Sox3-expressing cells to become neural and acquire Sox2 expression.

How many organizers?

The above discussion raises the issue of whether the embryo possesses more than one organizer. Certainly, no part of the embryo other than the gastrula-stage node in amniotes, the dorsal lip in amphibians or the shield in teleosts can induce a complete ectopic nervous system without also inducing mesoderm that includes an organizer. However, Otto Mangold (Mangold, 1933) proposed that separate inducing activities may exist for the head, trunk and tail regions of the axis, which reside in different tissues (or at least within the organizer and its derivatives at different times). The idea of multiple organizers, each inducing one part of the axis, still has some followers (reviewed by Stern, 2001; Niehrs, 2004), and recent findings in zebrafish suggest that the shield and the more ventral marginal region emit different signals responsible for inducing the nervous system in the head and in the trunk/tail (Agathon et al., 2003; Furthauer et al., 2004; Kudoh et al., 2004; Rentzsch et al., 2004). In the mouse, the anterior visceral endoderm (AVE) is required for head development, but not for the formation of the more-posterior CNS, and some have suggested that it might correspond to Mangold’s ‘head organizer’, perhaps through its secretion of BMP and Wnt antagonists (Bouwmeester et al., 1996; Belo et al., 1997; Glinka et al., 1997; Glinka et al., 1998; Knoetgen et al., 1999b; Kazanskaya et al., 2000). However, it has now been shown that despite being required for head development, the AVE does not possess neural-inducing activity unless combined with ‘early gastrula organizer’ (prospective organizer) and the appropriate responding tissue (future forebrain) (Tam and Steiner, 1999; Robb and Tam, 2004). This suggests that the AVE plays only a permissive or indirect role in neural induction. These results are consistent with findings in chick and mouse, which implicate the hypoblast/AVE in the positioning of the primitive streak, in directing its elongation and in the transient induction of early, but not definitive, neural markers (Foley et al., 2000; Streit et al., 2000; Bertocchini and Stern, 2002; Perea-Gómez et al., 2002).

One of the arguments that led to the idea that the mouse AVE might be an independent ‘head organizer’ came from findings that mouse mutants lacking a node and its derivatives (for example, HNF3β mutants) (Ang and Rossant, 1994; Weinstein et al., 1994; Dufort et al., 1998) still have a fairly complete nervous system. It was also suggested that the mammalian node cannot induce a forebrain, while the chick node can (Knoetgen et al., 1999b), although it now appears that the difference was due to experimental design; the node of rabbit and mouse embryos can induce forebrain markers just like the chick node (Foley et al., 2000; Knoetgen et al., 2000). Likewise, ablation and exogastrula experiments in other species have suggested that the Spemann organizer is not required for nervous system development. As discussed above, tissues other than the shield/dorsal lip/node do emit signals that can induce the expression of some neural markers, but no single tissue other than the classical organizer can induce them all. Despite this, it is also clear that there are regions of the nervous system that are never close to the organizer (see above); combinations of signals emanating from different tissues at different times might account for these tissues acquiring a neural fate.

At present, therefore, there is no conclusive evidence that separate organizers exist for different parts of the axis, and published results are at least equally supportive of the alternative ‘activation/transformation’ model of Nieuwkoop (Nieuwkoop et al., 1952; Nieuwkoop and Nigtevecht, 1954). This model proposes that the nervous system that is initially induced is of ‘anterior’ (forebrain) character, and that later signals ‘transform’ parts of it to more caudal fates.

Neural induction: a decision between epidermis and neural plate?

The default model proposes that high BMP activity defines epidermis, while absence of BMP specifies neural plate. It has also been proposed that intermediate concentrations specify the border of the neural plate, including the region fated to give rise to placodes and neural crest (Wilson et al., 1997; Marchant et al., 1998; Nguyen et al., 1998; Barth et al., 1999; Dale and Wardle, 1999; Nguyen et al., 2000; Tribulo et al., 2003; Glavic et al., 2004). However, at the end of gastrulation in the chick BMP4 and BMP7 are most highly expressed at the border of the neural plate, as are the BMP target genes MSX1 and DLX5 (Streit and Stern, 1999b; Streit, 2002). Indeed, the border of the chick neural plate is the only region that is sensitive to the application of BMP protein or BMP antagonists (Chordin and Noggin): BMP causes the inwards displacement of the border (narrowing the neural plate), while antagonists cause the reverse (Streit and Stern, 1999b). Likewise, it has recently been shown that the misexpression of BMP antagonists in Xenopus only enlarges the neural plate when injected into blastomeres, the progeny of which include the border of the neural plate; in a blastomere that does not consistently give rise to neural crest or placodes, BMP antagonists do not induce ectopic neural
markers (Linker and Stern, 2004; Delaune et al., 2005). These findings suggest that BMP activity is crucial in positioning the border between neural plate and epidermis, defining the territory from which neural crest and placodes will arise (reviewed by Streit, 2004).

Indeed, neural induction has always been viewed primarily as a decision between epidermal and neural fates, particularly because in the Spemann and Mangold (Spemann and Mangold, 1924) transplants, the fate of the ventral (belly) epidermis is transformed to a neural fate under the influence of the organizer. A recent study, however, emphasized that defining the neural plate during normal development also requires establishing a boundary between the region of the ectoderm destined to ingress into mesendoderm during gastrulation and the medial edge of the neural plate (Sheng et al., 2003). The epiblast gives rise to all three germ layers of the embryo – at the midline, cells ingress through the primitive streak (in amniotes) or through the blastopore to give rise to mesendoderm. At the end of gastrulation, ingestion stops and the epiblast that remains adjacent to the streak is destined to form the medial (future ventral) part of the neural plate. The zinc-finger transcriptional activator Churchill, isolated in the early response screen described above, starts to be expressed in the future neural plate domain of the chick epiblast at around the end of gastrulation (stage 4). One of its direct targets is the transcription factor SIP1 (verschueren et al., 1999; Papi et al., 2002; Postigo, 2003; Postigo et al., 2003), which is expressed in the same domain (not only in chick but also in Xenopus, fish and mouse). Through Sip1, churchill downregulates the expression of brachyury, which is essential for cell ingression through the primitive streak (Sheng et al., 2003). Sip1 gained its name (Smad-interacting protein 1) because of its ability to bind to phospho-Smad1, a BMP effector. Given that it takes 4-5 hours for FGF to induce Churchill, followed by the activation of Sip1 by Churchill, this might explain why epiblast cells need to receive signals from the organizer (or FGF signals) for 5 hours before they can respond to BMP antagonists (Streit et al., 1998; Sheng et al., 2003) (see above). These results emphasize the critical importance of the precise timing and
spatial distribution of (1) the signals that regulate key genes and (2) the pattern of expression of these genes for gastrulation and neural induction (Fig. 5). As only a handful of secreted signals seem to be important for controlling multiple events during early development (each of them with diverse and sometimes opposing roles), the timing of these signals and the state of the responding cells (that is, their competence) are of crucial importance.

This brings us back to the border of the neural plate. Short exposure of gastrula-stage chick epiblast to either an organizer or to FGF induces a characteristic genetic signature that is reminiscent of the genes expressed at the border of the neural plate during neurulation (e.g. ERNI and MSXI). Likewise, cutting a Xenopus animal cap (which activates MAPK, see above), often induces cement gland markers (Fig. 2; the cement gland normally forms at the anteriormost border of the neural plate of amphibians) (e.g. Smith and Harland, 1992; Lamb et al., 1993). For these reasons, it has been proposed (Streit and Stern, 1999b) that the earliest events in neural induction are equivalent to the induction of a ‘pre-border’ state, within which cells still retain the ability to give rise to neural, epidermal, neural crest or placodal fates.

Transcriptional networks

In chick, the expression of the transcription factor SOX2 begins at the end of gastrulation. It is expressed throughout the neural plate. To date, neither single factors (including FGFs, BMPs, Wnts or their antagonists) nor any combinations of these, whether applied simultaneously or sequentially, has been able to induce the ectopic expression of Sox2 directly in cells that normally do not express this gene (Linker and Stern, 2004). By contrast, early ‘pre-neural’ markers such as SOX3, ERNI and churchill are induced by FGF alone, which is required both for the expression of these early genes and for the later responses, including SOX2 expression (Table 2). Perhaps our best chance of identifying the missing signals will come from analysing the regulatory elements controlling SOX2 expression. An impressive analysis of the chick SOX2 promoter by Hisato Kondoh’s group (Uchikawa et al., 2003; Uchikawa et al., 2004) has revealed two crucial elements that together account for the early expression pattern of SOX2. One of them, N2, drives expression in the prospective anterior neural plate (the largest part of the neural plate at stage 4-5); the other, N1, is responsible for the more posterior expression that elongates caudally as the node regresses and the spinal cord is laid down (Henrique et al., 1997; Storey et al., 1998; Brown and Storey, 2000). Both are conserved between chick and mammals (human, mouse and rat), and each is extremely complex: N1 contains conserved putative binding sites for at least 12 known transcription factors, whereas N2 contains more than 39. Important clues must be embedded in these complex enhancers, the detailed analysis of which will undoubtedly yield interesting answers to some of the unanswered questions.

What is the role of BMP inhibition in neural development?

From the above evidence, there is no question that the modulation of BMP activity (including the control of Smad1 phosphorylation at its linker and C-terminal regions) is crucially important for neural development to occur normally (see Khokha et al., 2005). However, I propose that in order to understand neural induction, we need first to acknowledge the multiple roles of this important signalling pathway and to design experiments that can distinguish between them. BMP signalling apparently needs to be inhibited at least three times during early development to generate a normal neural plate. First, at very early stages of development (at the blastula stage or even earlier), nuclear β-catenin at the dorsal side of the embryo (in amniotes, this may involve Wnt ligands) regulates BMP expression so that BMP transcription is repressed dorsally. This repression establishes the initial dorsoventral polarity of the embryo and contributes to the positioning of the organizer, and perhaps also to establish differential competence of different ectodermal regions to respond to later signals. Chordin is probably the most important BMP antagonist for this step. Second, at the mid-/late-gastrula stage, BMP levels need to be regulated near the border of the neural plate, to fine-tune the position and perhaps width of the neural/non-neural border. Here, both BMP and BMP antagonists have an effect even in amniotes, suggesting that an intermediate concentration of BMP is required. This process is probably coordinated with the dorsoventral patterning of the underlying mesoderm, and is likely to involve Noggin. Third, at the late gastrula/early neurula stage, BMP needs to be kept downregulated within the neural plate proper to allow for the continued expression of Sox2 in this domain. There are no secreted BMP antagonists expressed appropriately for this step,
but it is likely that intracellular factors (such as Sox2 itself and Dach1) (Kida et al., 2004) play an important role in this maintenance step. These three roles of BMP signalling will all be affected in experiments in which factors that ultimately activate or repress the BMP pathway are misexpressed or downregulated by injection of morpholinos (e.g. Khokha et al., 2005) during very early (pre-gastrula) development. As such, their interpretation should depend both on the markers being assessed and on the timing of the analysis. To examine each of these steps independently, both the location and the timing of gene misexpression needs to be controlled. Acknowledging that neural induction consists of several steps, and that BMP and other signalling pathways need to be modulated appropriately in each step, should help to reconcile results from the different experimental systems and approaches (see Box I) used to study neural induction.

Conclusions

We are only now beginning to understand the true complexity of neural induction. The emerging view is of a cascade of sequential events and of cooperation between different signalling pathways, which together allow cells to make not one, but several, decisions. It has become apparent that concentrating on the signalling molecules alone, without considering the intricacies of the embryological processes they control, can lead to models that are too simplistic to account for the complexity that the embryo must generate during development. There has been much recent interest in the possibility of causing cultured stem cells (whether adult or embryonic) to acquire neural fates, with the aim of producing certain neuronal subtypes, such as dopaminergic neurons, that can be used to treat neurodegenerative disease. The conditions required to achieve this are still being debated (Aubert et al., 2002; Kawasaki et al., 2002; Bylund et al., 2003; Stewart et al., 2003; Ying et al., 2003a; Ying et al., 2003b; Jang et al., 2004; Zhang et al., 2004), but it now seems likely that an understanding of normal neural induction, including the dissection of enhancers controlling the expression of the key ‘commitment’ genes like Sox2, will be an invaluable tool towards making real progress in this direction.

I am indebted to Les Dale, Claudia Linker, Roberto Mayor and Andrea Streit for invaluable comments on the manuscript, and to Laurent Kodjabachian and Patrick Lemaire for sharing unpublished information. The research by my group on neural induction is funded by grants from the National Institute of Mental Health (NIMH, USA), the Wellcome Trust and the Medical Research Council.

References


development in Xenopus requires early BMP/Smad-independent FGF signalling, supporting a unified view of neural induction in chordates.


Development


dorsal-ventral pattern formation by the chordin and ogon antagonists. 


