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

Claudio D. Stern

Department of Anatomy and Developmental Biology, University College London, Gower Street, London WC1E 6BT, UK
e-mail: c.stern@ucl.ac.uk

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, ingression can occur through a blastopore (as in amphibians), around and through an embryonic shield (as in teleosts), or through a primitive streak (as in amniotes: 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

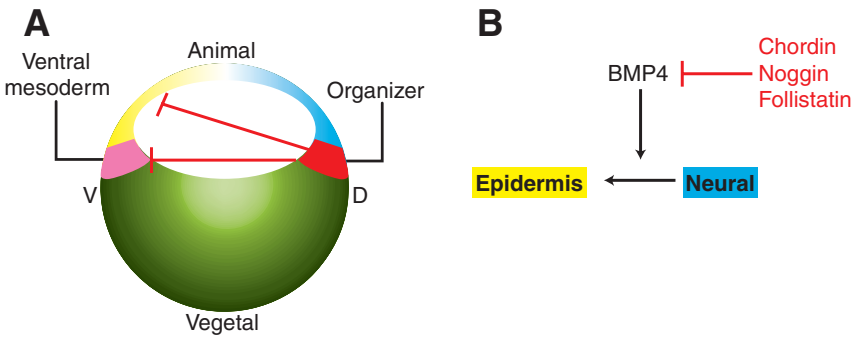


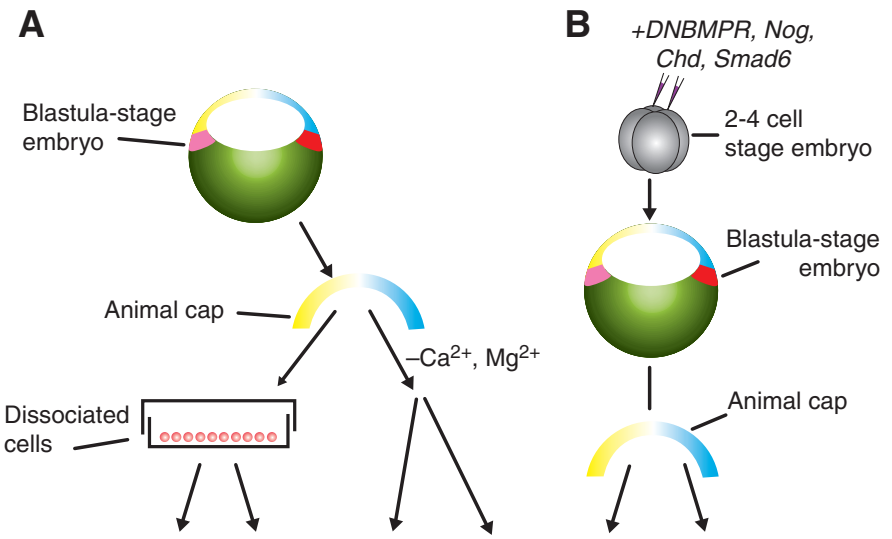
Fig. 1. The 'default model' in *Xenopus*. (A) A rough fate map of a blastula-stage embryo. Prospective territories are organizer in red, ventral mesoderm in pink, neural tissue in blue, epidermis in yellow and yolky endoderm in green. The red lines represent BMP antagonist activity emanating from the organizer. (B) A 'genetic' diagram of the inductive interactions proposed by the model: ectoderm cells have an autonomous tendency to differentiate into neural tissue, but are prevented from doing this and are directed instead to epidermis by BMP4, which is expressed ubiquitously. Near the organizer, BMP antagonists block BMP4 signalling, allowing neighbouring ectoderm cells to develop according to their 'default' neural fate. D, dorsal; V, ventral. Modified, with permission, from Stern (Stern, 2004).

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 neuralize 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, depletion 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.



Tissue markers	–BMP4	+BMP4	No additive	BMP antag.	Control	Injected embryo
Epidermis	–	+	+	↓	+	↓
Neural	+	–	–	+	–	+
Cement gland	+	?	+	+	+	+
Neural crest	+	?	+?	+	?	+
Mesoderm	–	–	–	–	–	–
Organizer	–	–	–	–	–	–

Fig. 2. Experiments in *Xenopus* that support the default model. (A) Cell-dissociation experiments (results shown in the two left-most columns in the table) and intact animal cap assays with or without BMP antagonists (antag.) (results shown in the third and fourth columns). $–Ca^{2+}$, Mg^{2+} indicates that the protein was applied in low Ca^{2+}/Mg^{2+} medium. (B) In the most common type of animal cap experiment, a two- to four-cell stage embryo is injected with RNA encoding a protein to be tested (here, BMP antagonists: *DNBMPR*, dominant-negative BMP receptor; *Nog*, Noggin; *Chd*, Chordin; *Smad6*, the inhibitory effector Smad6). After incubating the embryo to the blastula stage, the animal cap is excised and grown in isolation overnight before assessing its marker gene expression. The table shows the usual results of these experiments: + indicates expression of markers for the 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 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 by a grafted node and in prospective neural plate explants (Streit et al., 2000; Wilson et al., 2000). Consistent with these findings in chick, co-injecting either *chordin* (Sasai et al., 1996; Pera et al., 2003) or *noggin* (Launay et al., 1996) together with a dominant-negative FGFR1 construct into the animal pole of *Xenopus* embryos does not lead to the neuralisation of the animal caps taken from these embryos. This suggests that FGF signalling is required for BMP antagonists to induce neural markers. The same conclusion was reached more recently using a different approach (Linker and Stern, 2004; Delaune et al., 2005). In these studies, the intracellular BMP antagonist Smad6 was injected into the most-ventral blastomere (A4) of *Xenopus* embryos, the progeny of which do not contribute to either the prospective neural plate or to its border. This treatment was found to be insufficient to neuralise the descendants of the injected cell unless FGF4 was co-injected with it.

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; Godsave 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

Table 1. Results of chick misexpression experiments

(A) Experiments on embryos

	Marker genes					
Factor/tissue	*SOX3*	*ERNI*	*OTX2*	*ChCh*	*SOX2*	*Brachyury*
Node	+ (2 hours)	+ (1 hour)	+ (2 hours)	+ (4 hours)	+ (9 hours)	–
Hypoblast	+ (2 hours)	+ (1 hour)	+ (2 hours)	–	–	–
FGF8	+ (2 hours)	+ (1 hour)		+ (4 hours)	–	–
Chordin	–	–	–	–	–	–[†]
Noggin	–	–	–	–	–	–
Smad6	–	–	–	–	–	–
BMP4 electroporation*	No effect (<6 hours)	No effect			Inhibition	
Node (5 hours) + Chordin	+ (Maintenance)	+			–	–
FGF8 (5 hours) + Chordin	+ (Maintenance)	+			–	–
Smad6 + FGF8	+	+			–	–
Smad6 + α-Wnt	–	–	–	–	–	–
Smad6 + FGF8 + α-Wnt + Chordin + Noggin	+	+			–	–
FGF8 + α-Wnt	+	+			–	–

(B) Experiments on explants

	Marker genes									
	SOX3		*OTX2*		*MSX1/2*		*GATA2*		*SOX2*	
Explant	Neur	Epi	Neur	Epi	Neur	Epi	Neur	Epi	Neur	Epi
No factor	+	–	+	–	–	+	–	+	+	–
BMP4	–		–		+		+		–	
DN-BMPR + SU5402	+		+		–		–		+	
Wnt3A (1×)	–		–		+		+		–	
Wnt3A (1×) + FGF2	–		–		+		+/–		–	
Wnt3A (1×) + Noggin	+		+		–		–		+	
SU5402	–		–		+		+		–	
SU5402 + Noggin	–		–		+		+		–	
Wnt3A (3×)	–		–		+		+		–	
Wnt3A (3×) + Noggin	–		–		+		+		–	
Fz8CRD		+		+		–		–		+
Fz8CRD + SU5402		–		–		+		+		–
Fz8CRD + BMP4		–		–		+		+		–
Fz8CRD + SU5402 + Noggin		+		+		–		–		+
Fz8CRD + SU5402 (5 μM)		–		–		+		+		–
Fz8CRD + SU5402 (5 μM) + Noggin		–		–		+		+		–

(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. *ERNI*, *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).

*BMP4 misexpressed by electroporation in the neural plate inhibits *SOX2* but not the early marker *SOX3*.

[†]Chordin induces an ectopic primitive streak when misexpressed inside the embryo but does not induce neural markers in competent extra-embryonic epiblast.

(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 FGF signalling; 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).

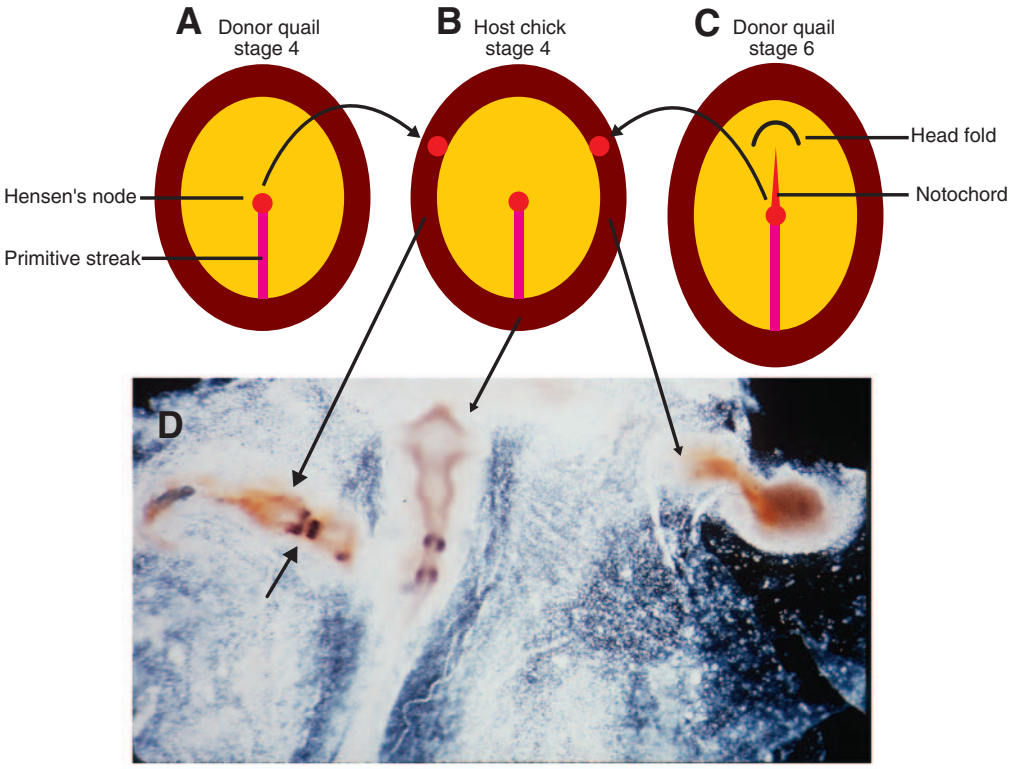


Fig. 3. Chick organizer graft experiments. (A-C) Chick organizer graft experiments showing changes in inducing ability with increasing age of the donor. (A,C) Dorsal views of early quail donor embryos. (B) Dorsal view of host chick embryo, which simultaneously receives a graft of a quail stage 4 node on its left and a quail stage 6 node on its right. Both grafts are placed in the extra-embryonic area opaca (brown), just outside the embryonic area pellucida (yellow). (D) Results of this experiment after in situ hybridization for the hindbrain marker *Krox20* (purple), which is expressed in rhombomeres 3 and 5 (upwards arrow), and after staining with an anti-quail antibody (reddish-brown). The young (stage 4) graft has induced a complete axis including the head (expressing *Krox20*), while the older graft on the right has generated a short axis, mostly derived from the graft itself (reddish-brown indicating quail cells), which lack rostral structures, including the hindbrain (*Krox20*-expressing region). (A-C) Modified, with permission, from Stern (Stern, 2004). (D) Reproduced, with permission, from Storey et al. (Storey et al., 1992).

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

Table 2. Early and late markers of the neural plate

Species	Marker gene expression	
	In early prospective neural plate	In definitive neural plate
Xenopus	*SoxD* (*Sox31*) *Geminin* *Opl* (*Zic1*) *Zic3*	*Sox3* *Sox2* *NCAM*
Chick	*SOX3* *ERNI* *OTX2* (pre-streak only) *NOT1*/*GNOT1* (pre-streak only)	*SOX2*
Mouse	*Sox2* *Otx2* (pre-streak only)	*Sox3*

Markers useful for identifying early (pre-neural, before end of gastrulation) and late (definitive, after gastrulation) neural plate in three model organisms. Early genes expressed later are not repeated in the final column. *Xenopus* SoxD (Sox31) is unique to *Xenopus* and has not been identified in other species (Mizuseki et al., 1998; Schepers et al., 2002), and homologues of ERNI have not yet been found outside of birds (Streit et al., 2000; Acloque et al., 2001; Acloque et al., 2004). In chick, *SOX3* is expressed before gastrulation, whereas *SOX2* appears later (stage 4), when cells commit to a neural plate fate. In mouse, this expression pattern is reversed (Uwanogho et al., 1995; Rex et al., 1997; Uchikawa et al., 1999; Wood and Episkopou, 1999) (see Kuroiwa et al., 2002). *Otx2* (and *NOT1*/*GNOT1* in chick) is an early pre-ne ural' marker in pre-streak chick and mouse embryos, but at primitive streak stages, it is expressed mainly in the node and later in anterior (prospective forebrain) tissues (Bally-Cuif et al., 1995; Knezevic and Mackem, 2001).

that additionally requires the inhibition of the Wnt pathway (Wilson and Edlund, 2001; Wilson et al., 2001) (Fig. 4). In chick, the cells of the prospective neural plate (the medial epiblast) would use predominantly the first pathway, whereas the second pathway might coax prospective epidermis (lateral epiblast) to acquire a neural fate. The evidence for this idea has mainly come from experiments in which explants have been exposed to these factors or to blocking reagents (see Table 1) (Wilson et al., 2000; Wilson et al., 2001). It has also been shown that Wnt antagonism can stimulate neural differentiation in stem cells under certain conditions (Aubert et al., 2002). Conversely, another group has reported that neural induction in *Xenopus* requires activation of the canonical (β-catenin) Wnt pathway, by showing that this pathway represses BMP expression (Baker et al., 1999). These apparently contradictory findings can be reconciled most easily by taking into account differences in timing. At early stages of development, when the embryo is acquiring dorsoventral polarity, Wnt signals are required to specify 'dorsal' character; the study of Baker et al. (Baker et al., 1999) provides an attractive mechanism by which Wnt signalling (a very early dorsal determinant) controls the distribution of BMP activity (which is required for the dorsoventral patterning of the embryo). By the blastula/early gastrula stage, it may be necessary to inhibit Wnt signals for FGF to downregulate BMP expression (Bainter et al., 2001; Wilson and Edlund, 2001). Despite this, a recent study showed that even a combination of FGFs (FGF2, FGF3, FGF4 or FGF8) together with the BMP antagonists Smad6, Chordin and/or Noggin and three different Wnt antagonists, was still unable to induce expression of the neural marker SOX2 directly in chick epiblast in intact embryos (Linker and Stern, 2004). This finding strongly suggests that, in vivo, signals other than FGF, 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

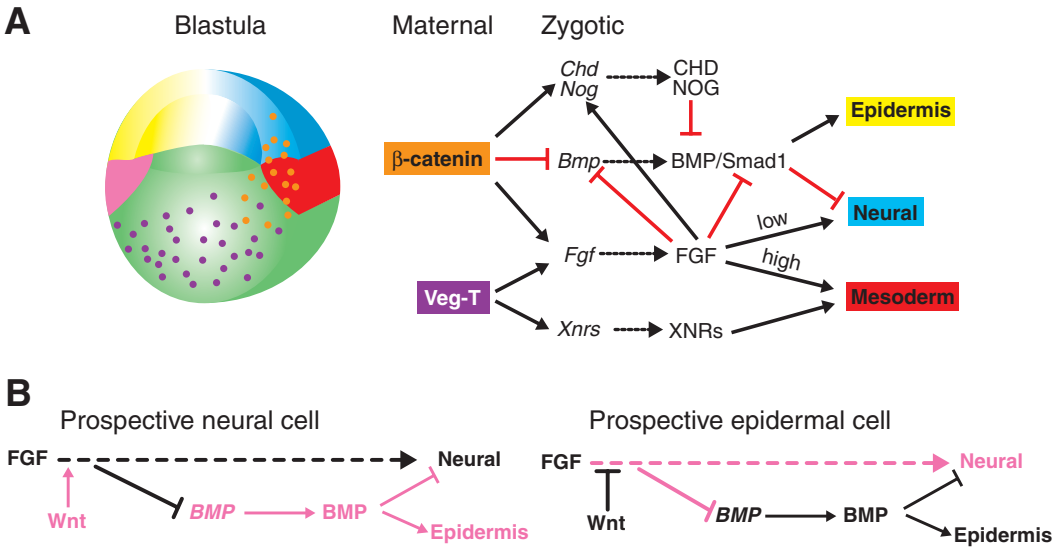


Fig. 4. Two models of neural induction. Models based on studies in (A) *Xenopus* and (B) chick, proposed to reconcile findings on the roles of BMP, FGF and Wnt signalling in neural induction. (A) In this model, at the late blastula/early gastrula stage, FGF signalling cooperates with BMP inhibition to induce a neural (blue) fate by inhibiting Smad1 phosphorylation, repressing *Bmp* transcription, and inducing expression of the BMP antagonists Chordin (*Chd*) and Noggin (*Nog*). At low levels, FGF seems to induce a neural fate directly. High BMP activity induces epidermis (yellow), while high FGF signalling cooperating with Nodal-related factors (XNRs) induces mesoderm (red). At earlier (pre-blastula) stages, *Fgf*, *Xnrs*, *Bmp*, *Chd* and *Nog* distribution are determined by both the nuclear localisation of β-catenin (orange dots) and the vegetal localisation of the T-box transcription factor VegT (purple dots), which pattern the early embryo. Modified, with permission, from Delaune et al. (Delaune et al., 2005). (B) An alternative but similar model (Wilson and Edlund, 2001) from explant experiments in chick. At the blastula stage, medial epiblast cells (prospective neural cell) express FGFs but not Wnts. FGF signalling activates two transduction pathways in epiblast cells: repression of BMP expression and the promotion of neural fate by an independent pathway (broken line from FGF). Lateral epiblast cells (prospective epidermal cell) express both FGFs and Wnts. High Wnt levels block the response of epiblast cells to FGFs, BMPs are expressed, and BMP signals promote epidermal fate and repress neural fate. When Wnt signalling is attenuated, Wnts block the ability of FGFs to repress BMP expression, but the independent pathway (broken line) promoting neural fate is preserved. Under these conditions, BMP antagonists are able to induce neural fate. Modified, with permission, from Wilson and Edlund (Wilson and Edlund, 2001).

lost at the end of the gastrula stage, between stages 12 and 13 (Waddington and Needham, 1936; Gurdon, 1987; Sharpe and Gurdon, 1990; Servetnick and Grainger, 1991). As for the start of the process, it has generally been assumed that it begins at the early gastrula stage, as the organizer is difficult to define before then. However, studies in chick embryos have suggested that the earliest neural induction steps occur before the start of gastrulation, are marked by *ERNI* and *SOX3* expression, and can be mimicked by, and require, FGF signalling (Streit et al., 2000; Wilson et al., 2000). Similar conclusions have now been reached for amphibians (Kuroda et al., 2004; Delaune et al., 2005).

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.

Hensen's node derives from two cell populations, the 'posterior' and 'central' cells, that are present before gastrulation. The posterior cells lie deep to the epiblast in the medial part of a crescent-shaped ridge of middle-layer cells called Koller's sickle, which is situated at the posterior edge of the embryo. These cells express *chordin* and *goosecoid* (Izpisúa-Belmonte et al., 1993; Streit et al., 1998; Streit et al., 2000), and move anteriorly with the extending primitive streak (Hatada and Stern, 1994; Bachvarova et al., 1998; Robb and Tam, 2004). Before this stage, 'central cells' are present in the epiblast and are defined as being node progenitors by fate mapping (Hatada and Stern, 1994; Foley et al., 2000), although they do not uniquely express any known marker at this stage. Central cells move anteriorly as part of the Polonaise movements of the epiblast, long before primitive streak formation begins (Foley et al., 2000). The two cell populations meet and acquire full neural-inducing ability, as well as organizer markers, by the mid-primitive streak stage (stages 3-3^{+}). Before this stage, the posterior cells have low neural-inducing ability and the central cells have none (Izpisúa-Belmonte et al., 1993; Tam and Steiner, 1999; Streit et al., 2000; Robb and Tam, 2004), 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)

(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 *Erni*- 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 Nieukwoop (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

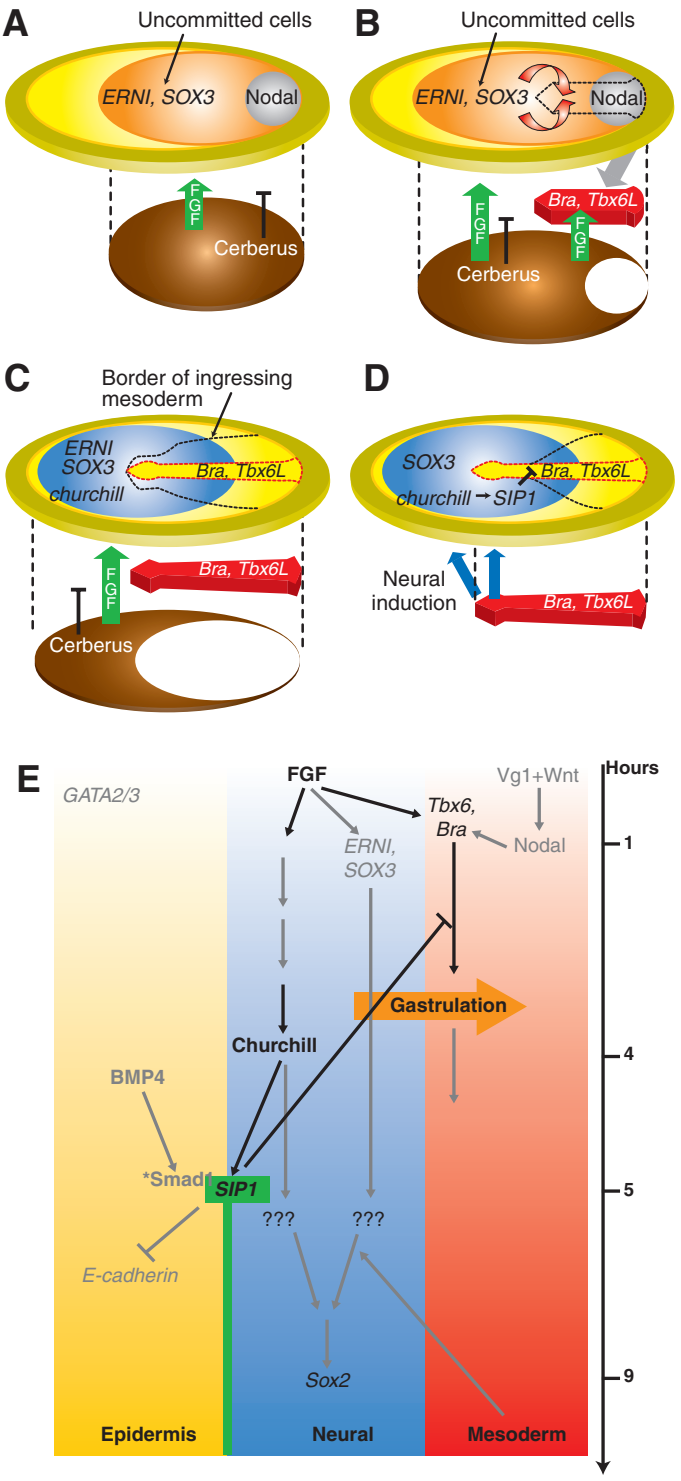


Fig. 5. A model of Churchill functions and regulation during early development in chick. (A-D) The embryologist's view, showing embryos at four stages, viewed obliquely from the dorsal side (posterior towards the right), with their germ layers teased apart (brown represents hypoblast, yellow the epiblast). (A) Stages XI-XII. The hypoblast emits FGF8, which induces the early pre-neural genes *ERNI* and *SOX3* (orange) in the overlying epiblast (yellow). At this stage, cells in this domain are still uncommitted. Nodal is expressed in the posterior epiblast but is inhibited by Cerberus secreted by the hypoblast. (B) Stages XIII-2. The hypoblast is displaced from the posterior part of the embryo by the endoblast (white), which allows Nodal signalling, in synergy with FGF, to induce *Brachyury* (*Bra*) and *Tbx6L* and ingression (red arrows) to form the primitive streak (red, its position is outlined with a broken line in the epiblast layer). (C) Stages 3^{+}-4. Continued FGF signalling now induces *churchill* in a domain of the epiblast (light blue). The border of the epiblast territory destined to ingress to form mesoderm is shown with a broken black line. (D) At the end of stage 4, *churchill* induces *SIP1*, which blocks *Bra*, *Tbx6L* and further ingression of epiblast into the anterior streak. The epiblast remaining outside the streak (blue) is now sensitized to neural-inducing signals emanating from the node (blue arrows). (E) The same model as a genetic cascade. Black represents interactions described by Sheng et al. (Sheng et al., 2003). Grey indicates other interactions from published data. The time axis (right) shows time in hours after a graft of Hensen's node, and the colour gradients indicate progressive commitment to epidermis (yellow), neural (blue) and mesoderm (red). BMP/Smad/Sip1 interactions regulate the epidermis-neural plate border, while ChCh/Sip1/FGF/Bra/Tbx6 regulate the mesoderm-neural decision. The asterisk indicates phosphorylated Smad1. ??? represents as yet unknown components. Modified, with permission, from Sheng et al. (Sheng et al., 2003).

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, ingression 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; Papin 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 *MSX1*). 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.

Box 1. Differences between species or between approaches?

Are there real differences in the mechanisms of neural induction between species, or are these a consequence of the experimental approaches that can be used in each? As cross-species organizer grafts always lead to neural induction, I believe that the key mechanisms will turn out to be conserved. *Xenopus* benefits from the ease with which molecules can be misexpressed or downregulated with a morpholino, which can be injected into a blastomere up to the 32-cell stage. This approach led to the rapid identification of BMP signalling as a key player in neural induction. However, this technique does not allow one to restrict the gain or loss of gene function to a particular cell group at a particular stage, which is a problem when the molecules of interest have multiple sequential roles (as do BMPs, FGFs and Wnts). The introduction of focal electroporation in the chick (Nakamura et al., 2004), coupled with its large size and the ease with which transplants can be performed, has enabled spatiotemporally controlled misexpression and knockdown studies that are now starting to reveal the complexity of the signalling processes that underlie neural induction. The chick also possesses a very large territory that does not contribute cells to the neural plate or to its border, a prerequisite for any assay of induction, which requires an assessment of whether cells can be diverted to a new fate or whether the normal neural territory is expanded or compressed. This contrasts with the *Xenopus* animal cap assay, which was originally designed to test for mesoderm induction and is not a good test for neural induction because it contains cells fated to form part of the neural plate and/or its border (see Streit and Stern, 1999c; Linker and Stern, 2004). However, extra-embryonic chick epiblast assays also need to be interpreted with caution to exclude the possibility that the induction being investigated is artificial. Thus, each system has its advantages and disadvantages, and a thorough understanding of neural induction can only come from knowledge derived from different models.

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 1) 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 responsible for directing 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

Acloque, H., Risson, V., Birot, A. M., Kunita, R., Pain, B. and Samarut, J. (2001). Identification of a new gene family specifically expressed in chicken embryonic stem cells and early embryo. *Mech. Dev.* **103**, 79-91.

Acloque, H., Mey, A., Birot, A. M., Gruffat, H., Pain, B. and Samarut, J. (2004). Transcription factor cCP2 controls gene expression in chicken embryonic stem cells. *Nucleic Acids Res.* **32**, 2259-2271.

Agathon, A., Thisse, C. and Thisse, B. (2003). The molecular nature of the zebrafish tail organizer. *Nature* **424**, 448-452.

Alvarez, I. S., Araújo, M. and Nieto, M. A. (1998). Neural induction in whole chick embryo cultures by FGF. *Dev. Biol.* **199**, 42-54.

Amaya, E., Musci, T. J. and Kirschner, M. W. (1991). Expression of a dominant negative mutant of the FGF receptor disrupts mesoderm formation in Xenopus embryos. *Cell* **66**, 257-270.

Ang, S. L. and Rossant, J. (1994). HNF-3 beta is essential for node and notochord formation in mouse development. *Cell* **78**, 561-574.

Aubert, J., Dunstan, H., Chambers, I. and Smith, A. (2002). Functional gene screening in embryonic stem cells implicates Wnt antagonism in neural differentiation. *Nat. Biotechnol.* **20**, 1240-1245.

Aubin, J., Davy, A. and Soriano, P. (2004). In vivo convergence of BMP and MAPK signaling pathways: impact of differential Smad1 phosphorylation on development and homeostasis. *Genes Dev.* **18**, 1482-1494.

Bachvarova, R. F., Skromne, I. and Stern, C. D. (1998). Induction of primitive streak and Hensen’s node by the posterior marginal zone in the early chick embryo. *Development* **125**, 3521-3534.

Bainter, J. J., Boos, A. and Kroll, K. L. (2001). Neural induction takes a transcriptional twist. *Dev. Dyn.* **222**, 315-327.

Baker, J. C., Beddington, R. S. and Harland, R. M. (1999). Wnt signaling in Xenopus embryos inhibits bmp4 expression and activates neural development. *Genes Dev.* **13**, 3149-3159.

Barth, K. A., Kishimoto, Y., Rohr, K. B., Seydler, C., Schulte-Merker, S. and Wilson, S. W. (1999). BMP activity establishes a gradient of positional information throughout the entire neural plate. *Development* **126**, 4977-4987.

Beddington, R. S. and Robertson, E. J. (1999). Axis development and early asymmetry in mammals. *Cell* **96**, 195-209.

Belo, J. A., Bouwmeester, T., Leyns, L., Kertesz, N., Gallo, M., Follettie, M. and de Robertis, E. M. (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.

Bertocchini, F. and Stern, C. D. (2002). The hypoblast of the chick embryo positions the primitive streak by antagonizing nodal signaling. *Dev. Cell* **3**, 735-744.

Bertrand, V., Hudson, C., Caillol, D., Popovici, C. and Lemaire, P. (2003). Neural tissue in ascidian embryos is induced by FGF9/16/20, acting via a combination of maternal GATA and Ets transcription factors. *Cell* **115**, 615-627.

Biehs, B., Francois, V. and Bier, E. (1996). The Drosophila short gastrulation gene prevents Dpp from autoactivating and suppressing neurogenesis in the neuroectoderm. *Genes Dev.* **10**, 2922-2934.

Blum, M., Gaunt, S. J., Cho, K. W., Steinbeisser, H., Blumberg, B., Bittner, D. and de Robertis, E. M. (1992). Gastrulation in the mouse: the role of the homeobox gene goosecoid. *Cell* **69**, 1097-1106.

Born, J., Janeczek, J., Schwarz, W. and Tiedemann, H. (1989). Activation of masked neural determinants in amphibian eggs and embryos and their release from the inducing tissue. *Cell Differ. Dev.* **27**, 1-7.

Bouwmeester, T., Kim, S.-H., Sasai, Y., Lu, B. and de Robertis, E. M. (1996). Cerberus is a head-inducing secreted factor expressed in the anterior endoderm of Spemann’s Organizer. *Nature* **382**, 595-601.

Brown, J. M. and Storey, K. G. (2000). A region of the vertebrate neural plate in which neighbouring cells can adopt neural or epidermal fates. *Curr. Biol.* **10**, 869-872.

Bylund, M., Andersson, E., Novitch, B. G. and Muhr, J. (2003). Vertebrate neurogenesis is counteracted by Sox1-3 activity. *Nat. Neurosci.* **6**, 1162-1168.

Chapman, S. C., Schubert, F. R., Schoenwolf, G. C. and Lumsden, A. (2003). Anterior identity is established in chick epiblast by hypoblast and anterior definitive endoderm. *Development* **130**, 5091-5101.

Christen, B. and Slack, J. M. (1999). Spatial response to fibroblast growth factor signalling in Xenopus embryos. *Development* **126**, 119-125.

Cox, W. G. and Hemmati-Brivanlou, A. (1995). Caudalization of neural fate by tissue recombination and bFGF. *Development* **121**, 4349-4358.

Curran, K. L. and Grainger, R. M. (2000). Expression of activated MAP kinase in Xenopus laevis embryos: evaluating the roles of FGF and other signaling pathways in early induction and patterning. *Dev. Biol.* **228**, 41-56.

Dale, L. and Wardle, F. C. (1999). A gradient of BMP activity specifies dorsal-ventral fates in early Xenopus embryos. *Semin. Cell Dev. Biol.* **10**, 319-326.

Darnell, D. K., Stark, M. R. and Schoenwolf, G. C. (1999). Timing and cell interactions underlying neural induction in the chick embryo. *Development* **126**, 2505-2514.

Darras, S. and Nishida, H. (2001). The BMP/CHORDIN antagonism controls sensory pigment cell specification and differentiation in the ascidian embryo. *Dev. Biol.* **236**, 271-288.

De Robertis, E. M. and Kuroda, H. (2004). Dorsal-ventral patterning and neural induction in Xenopus embryos. *Annu. Rev. Cell Dev. Biol.* **20**, 285-308.

Delaune, E., Lemaire, P. and Kodjabachian, L. (2005). Neural tissue

development in Xenopus requires early BMP/Smad1-independent FGF signalling, supporting a unified view of neural induction in chordates. *Development* **132**, 299-310.

Dias, M. S. and Schoenwolf, G. C. (1990). Formation of ectopic neurepithelium in chick blastoderms: age-related capacities for induction and self-differentiation following transplantation of quail Hensen's nodes. *Anat. Rec.* **228**, 437-448.

Dionne, M. S., Skarnes, W. C. and Harland, R. M. (2001). Mutation and analysis of Dan, the founding member of the Dan family of transforming growth factor beta antagonists. *Mol. Cell Biol.* **21**, 636-643.

Dufort, D., Schwartz, L., Harpal, K. and Rossant, J. (1998). The transcription factor HNF3 beta is required in visceral endoderm for normal primitive streak morphogenesis. *Development* **125**, 3015-3025.

Eimon, P. M. and Harland, R. M. (2001). Xenopus Dan, a member of the Dan gene family of BMP antagonists, is expressed in derivatives of the cranial and trunk neural crest. *Mech. Dev.* **107**, 187-189.

Fainsod, A., Steinbeisser, H. and de Robertis, E. M. (1994). On the function of BMP-4 in patterning the marginal zone of the Xenopus embryo. *EMBO J.* **13**, 5015-5025.

Fainsod, A., Dibler, K., Yelin, R., Marom, K., Epstein, M., Pillemer, G., Steinbeisser, H. and Blum, M. (1997). The dorsalizing and neural inducing gene follistatin is an antagonist of BMP-4. *Mech. Dev.* **63**, 39-50.

Faure, S., de Santa Barbara, P., Roberts, D. J. and Whitman, M. (2002). Endogenous patterns of BMP signaling during early chick development. *Dev. Biol.* **244**, 44-65.

Foley, A. C., Storey, K. G. and Stern, C. D. (1997). The prechordal region lacks neural inducing ability, but can confer anterior character to more posterior neuroepithelium. *Development* **124**, 2983-2996.

Foley, A. C., Skromne, I. and Stern, C. D. (2000). Reconciling different models of forebrain induction and patterning: a dual role for the hypoblast. *Development* **127**, 3839-3854.

Furthauer, M., Thisse, C. and Thisse, B. (1997). A role for FGF-8 in the dorsoventral patterning of the zebrafish gastrula. *Development* **124**, 4253-4264.

Furthauer, M., Thisse, B. and Thisse, C. (1999). Three different noggin genes antagonize the activity of bone morphogenetic proteins in the zebrafish embryo. *Dev. Biol.* **214**, 181-196.

Furthauer, M., van Celst, J., Thisse, C. and Thisse, B. (2004). Fgf signalling controls the dorsoventral patterning of the zebrafish embryo. *Development* **131**, 2853-2864.

Gallera, J. (1970). Inductions cérébrales et médullaires chez les Oiseaux. *Experientia* **26**, 886-887.

Gallera, J. (1971). Différence de la reactivité à l'inducteur neurogène entre l'ectoblaste de l'aire opaque et celui de l'aire pellucide chez le poulet. *Experientia* **26**, 1953-1954.

Gallera, J. and Ivanov, I. (1964). La compétence neurogène du feuillet externe du blastoderme de poulet en fonction du facteur 'temps'. *J. Embryol. Exp. Morphol.* **12**, 693.

Gallera, J. and Nicolet, G. (1969). Le pouvoir inducteur de l'endoblaste presomptif contenu dans la ligne primitive jeune de l'embryon de poulet. *J. Embryol. Exp. Morphol.* **21**, 105-118.

Glavic, A., Maris Honore, S., Gloria Feijoo, C., Bastidas, F., Allende, M. L. and Mayor, R. (2004). Role of BMP signaling and the homeoprotein Iroquois in the specification of the cranial placodal field. *Dev. Biol.* **272**, 89-103.

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

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

Godsave, S. F. and Durston, A. J. (1997). Neural Induction and patterning in embryos deficient in FGF signaling. *Int. J. Dev. Biol.* **41**, 57-65.

Godsave, S. F. and Slack, J. M. (1989). Clonal analysis of mesoderm induction in Xenopus laevis. *Dev. Biol.* **134**, 486-490.

Grunz, H. and Tacke, L. (1989). Neural differentiation of Xenopus laevis ectoderm takes place after disaggregation and delayed reaggregation without inducer. *Cell Differ. Dev.* **28**, 211-217.

Gurdon, J. B. (1987). Embryonic induction – molecular prospects. *Development* **99**, 285-306.

Hallonet, M., Kaestner, K. H., Martin-Parras, L., Sasaki, H., Betz, U. A. and Ang, S. L. (2002). Maintenance of the specification of the anterior definitive endoderm and forebrain depends on the axial mesendoderm: a study using HNF3beta/Foxa2 conditional mutants. *Dev. Biol.* **243**, 20-33.

Hamburger, V. (1988). *The Heritage of Experimental Embryology: Hans Spemann and the Organizer*. Oxford, UK: Oxford University Press.

Hardcastle, Z., Chalmers, A. D. and Papalopulu, N. (2000). FGF-8 stimulates neuronal differentiation through FGFR-4a and interferes with mesoderm induction in Xenopus embryos. *Curr. Biol.* **10**, 1511-1514.

Hatada, Y. and Stern, C. D. (1994). A fate map of the epiblast of the early chick embryo. *Development* **120**, 2879-2889.

Hatta, K. and Takahashi, Y. (1996). Secondary axis induction by heterospecific organizers in zebrafish. *Dev. Dyn.* **205**, 183-195.

Hawley, S. H., Wunnenberg-Stapleton, K., Hashimoto, C., Laurent, M. N., Watabe, T., Blumberg, B. W. and Cho, K. W. (1995). Disruption of BMP signals in embryonic Xenopus ectoderm leads to direct neural induction. *Genes Dev.* **9**, 2923-2935.

Hemmati-Brivanlou, A. and Melton, D. A. (1992). A truncated activin receptor inhibits mesoderm induction and formation of axial structures in Xenopus embryos. *Nature* **359**, 609-614.

Hemmati-Brivanlou, A. and Melton, D. A. (1994). Inhibition of activin receptor signaling promotes neuralization in Xenopus. *Cell* **77**, 273-281.

Hemmati-Brivanlou, A. and Melton, D. (1997). Vertebrate embryonic cells will become nerve cells unless told otherwise. *Cell* **88**, 13-17.

Hemmati-Brivanlou, A., Kelly, O. G. and Melton, D. A. (1994). Follistatin, an antagonist of activin, is expressed in the Spemann organizer and displays direct neuralizing activity. *Cell* **77**, 283-295.

Henrique, D., Tyler, D., Kintner, C., Heath, J. K., Lewis, J. H., Ish-Horowicz, D. and Storey, K. G. (1997). Cash4, a novel achaete-scute homolog induced by Hensen's node during generation of the posterior nervous system. *Genes Dev.* **11**, 603-615.

Holowacz, T. and Sokol, S. (1999). FGF is required for posterior neural patterning but not for neural induction. *Dev. Biol.* **205**, 296-308.

Hongo, I., Kengaku, M. and Okamoto, H. (1999). FGF signaling and the anterior neural induction in Xenopus. *Dev. Biol.* **216**, 561-581.

Hsu, D. R., Economides, A. N., Wang, X., Eimon, P. M. and Harland, R. M. (1998). The Xenopus dorsalizing factor Gremlin identifies a novel family of secreted proteins that antagonize BMP activities. *Mol. Cell* **1**, 673-683.

Hudson, C. and Lemaire, P. (2001). Induction of anterior neural fates in the ascidian Ciona intestinalis. *Mech. Dev.* **100**, 189-203.

Hudson, C., Darras, S., Caillol, D., Yasuo, H. and Lemaire, P. (2003). A conserved role for the MEK signalling pathway in neural tissue specification and posteriorisation in the invertebrate chordate, the ascidian Ciona intestinalis. *Development* **130**, 147-159.

Inazawa, T., Okamura, Y. and Takahashi, K. (1998). Basic fibroblast growth factor induction of neuronal ion channel expression in ascidian ectodermal blastomeres. *J. Physiol.* **511**, 347-359.

Ishimura, A., Maeda, R., Takeda, M., Kikkawa, M., Daar, I. O. and Maeno, M. (2000). Involvement of BMP-4/msx-1 and FGF pathways in neural induction in the Xenopus embryo. *Dev. Growth Differ.* **42**, 307-316.

Izpisúa-Belmonte, J. C., de Robertis, E. M., Storey, K. G. and Stern, C. D. (1993). The homeobox gene goosecoid and the origin of organizer cells in the early chick blastoderm. *Cell* **74**, 645-659.

Jang, Y. K., Park, J. J., Lee, M. C., Yoon, B. H., Yang, Y. S., Yang, S. E. and Kim, S. U. (2004). Retinoic acid-mediated induction of neurons and glial cells from human umbilical cord-derived hematopoietic stem cells. *J. Neurosci. Res.* **75**, 573-584.

Kawasaki, H., Mizuseki, K. and Sasai, Y. (2002). Selective neural induction from ES cells by stromal cell-derived inducing activity and its potential therapeutic application in Parkinson's disease. *Methods Mol. Biol.* **185**, 217-227.

Kazanskaya, O., Glinka, A. and Niehrs, C. (2000). The role of Xenopus dickkopf1 in prechordal plate specification and neural patterning. *Development* **127**, 4981-4992.

Kengaku, M. and Okamoto, H. (1995). bFGF as a possible morphogen for the anteroposterior axis of the central nervous system in Xenopus. *Development* **121**, 3121-3130.

Khokha, M. K., Hsu, D., Brunet, L. J., Dionne, M. S. and Harland, R. M. (2003). Gremlin is the BMP antagonist required for maintenance of Shh and Fgf signals during limb patterning. *Nat. Genet.* **34**, 303-307.

Khokha, M. K., Yeh, J., Grammer, T. C. and Harland, R. M. (2005). Depletion of three BMP antagonists from Spemann's organizer leads to a catastrophic loss of dorsal structures. *Dev. Cell* **8**, 401-411.

Kida, Y., Maeda, Y., Shiraishi, T., Suzuki, T. and Ogura, T. (2004). Chick Dach1 interacts with the Smad complex and Sin3a to control AER formation and limb development along the proximodistal axis. *Development* **131**, 4179-4187.

Kim, G. J. and Nishida, H. (2001). Role of the FGF and MEK signaling pathway in the ascidian embryo. *Dev. Growth Differ.* **43**, 521-533.
Kintner, C. R. and Dodd, J. (1991). Hensen's node induces neural tissue in Xenopus ectoderm. Implications for the action of the organizer in neural induction. *Development* **113**, 1495-1505.
Knezevic, V. and Mackem, S. (2001). Activation of epiblast gene expression by the hypoblast layer in the prestreak chick embryo. *Genesis* **30**, 264-273.
Knoetgen, H., Teichmann, U. and Kessel, M. (1999a). Head-organizing activities of endodermal tissues in vertebrates. *Cell Mol Biol (Noisy-Le-Grand)* **45**, 481-492.
Knoetgen, H., Viebahn, C. and Kessel, M. (1999b). Head induction in the chick by primitive endoderm of mammalian, but not avian origin. *Development* **126**, 815-825.
Knoetgen, H., Teichmann, U., Wittler, L., Viebahn, C. and Kessel, M. (2000). Anterior neural induction by nodes from rabbits and mice. *Dev. Biol.* **225**, 370-380.
Koshida, S., Shinya, M., Nikaido, M., Ueno, N., Schulte-Merker, S., Kuroiwa, A. and Takeda, H. (2002). Inhibition of BMP activity by the FGF signal promotes posterior neural development in zebrafish. *Dev. Biol.* **244**, 9-20.
Kroll, K. L. and Amaya, E. (1996). Transgenic Xenopus embryos from sperm nuclear transplantations reveal FGF signaling requirements during gastrulation. *Development* **122**, 3173-3183.
Kudoh, T., Concha, M. L., Houart, C., Dawid, I. B. and Wilson, S. W. (2004). Combinatorial Fgf and Bmp signalling patterns the gastrula ectoderm into prospective neural and epidermal domains. *Development* **131**, 3581-3592.
Kuroda, H., Wessely, O. and Robertis, E. M. (2004). Neural induction in Xenopus: requirement for ectodermal and endomesodermal Signals via Chordin, Noggin, beta-Catenin, and Cerberus. *PLoS Biol.* **2**, E92.
Kuroiwa, A., Uchikawa, M., Kamachi, Y., Kondoh, H., Nishida-Umehara, C., Masabanda, J., Griffin, D. K. and Matsuda, Y. (2002). Chromosome assignment of eight SOX family genes in chicken. *Cytogenet. Genome Res.* **98**, 189-193.
LaBonne, C. and Whitman, M. (1997). Localization of MAP kinase activity in early Xenopus embryos: implications for endogenous FGF signaling. *Dev. Biol.* **183**, 9-20.
Lamb, T. M. and Harland, R. M. (1995). Fibroblast growth factor is a direct neural inducer, which combined with noggin generates anterior-posterior neural pattern. *Development* **121**, 3627-3636.
Lamb, T. M., Knecht, A. K., Smith, W. C., Stachel, S. E., Economides, A. N., Stahl, N., Yancopolous, G. D. and Harland, R. M. (1993). Neural induction by the secreted polypeptide noggin. *Science* **262**, 713-718.
Launay, C., Fromentoux, V., Shi, D. L. and Boucaut, J. C. (1996). A truncated FGF receptor blocks neural induction by endogenous Xenopus inducers. *Development* **122**, 869-880.
Leclerc, C., Daguzan, C., Nicolas, M. T., Chabret, C., Duprat, A. M. and Moreau, M. (1997). L-type calcium channel activation controls the in vivo transduction of the neuralizing signal in the amphibian embryos. *Mech. Dev.* **64**, 105-110.
Leclerc, C., Lee, M., Webb, S. E., Moreau, M. and Miller, A. L. (2003). Calcium transients triggered by planar signals induce the expression of ZIC3 gene during neural induction in Xenopus. *Dev. Biol.* **261**, 381-390.
LeSueur, J. A., Fortuno, E. S., 3rd, McKay, R. M. and Graff, J. M. (2002). Smad10 is required for formation of the frog nervous system. *Dev. Cell* **2**, 771-783.
Linker, C. and Stern, C. D. (2004). Neural induction requires BMP inhibition only as a late step, and involves signals other than FGF and Wnt antagonists. *Development* **131**, 5671-5681.
Luther, W. H. (1935). Entwicklungsphysiologische Untersuchungen am Forellenkeim: die Rolle des Organisationszentrums bei der Entstehung der Embryonalanlage. *Biol Zentralbl* **55**, 114-137.
Mangold, O. (1933). Über die Induktionsfähighkeit der verschiedenen Bezirke der Neurula von Urodelen. *Naturwissenshaften* **21**, 761-766.
Marchant, L., Linker, C., Ruiz, P., Guerrero, N. and Mayor, R. (1998). The inductive properties of mesoderm suggest that the neural crest cells are specified by a BMP gradient. *Dev. Biol.* **198**, 319-329.
Mizuseki, K., Kishi, M., Shiota, K., Nakanishi, S. and Sasai, Y. (1998). SoxD: an essential mediator of induction of anterior neural tissues in Xenopus embryos. *Neuron* **21**, 77-85.
Moreau, M., Leclerc, C., Gualandris-Parisot, L. and Duprat, A. M. (1994). Increased internal Ca2+ mediates neural induction in the amphibian embryo. *Proc. Natl. Acad. Sci. USA* **91**, 12639-12643.
Nakamura, H., Katahira, T., Sato, T., Watanabe, Y. and Funahashi, J. (2004). Gain- and loss-of-function in chick embryos by electroporation. *Mech Dev* **121**, 1137-1143.
Nakamura, O. and Toivonen, S. (ed.) (1978). Organizer: a milestone of a half-century from Spemann. Amsterdam: Elsevier/North Holland.
Nguyen, V. H., Schmid, B., Trout, J., Connors, S. A., Ekker, M. and Mullins, M. C. (1998). Ventral and lateral regions of the zebrafish gastrula, including the neural crest progenitors, are established by a bmp2b/swirl pathway of genes. *Dev. Biol.* **199**, 93-110.
Nguyen, V. H., Trout, J., Connors, S. A., Andermann, P., Weinberg, E. and Mullins, M. C. (2000). Dorsal and intermediate neuronal cell types of the spinal cord are established by a BMP signaling pathway. *Development* **127**, 1209-1220.
Niehrs, C. (2004). Regionally specific induction by the Spemann-Mangold organizer. *Nat. Rev. Genet.* **5**, 425-434.
Nieuwkoop, P. D. and Nigtevecht, G. V. (1954). Neural activation and transformation in explants of competent ectoderm under the influence of fragments of anterior notochord in urodeles. *J. Embryol. Exp. Morphol.* **2**, 175-193.
Nieuwkoop, P. D., Botternenbrood, E. C., Kremer, A., Bloesma, F. F. S. N., Hoessels, E. L. M. J., Meyer, G. and Verheyen, F. J. (1952). Activation and organization of the central nervous system in amphibians. *J. Exp. Zool.* **120**, 1-108.
Oppenheimer, J. M. (1936a). Structures developed in amphibians by implantation of living fish organizer. *Proc. Soc. Exp. Biol. Med.* **34**, 461-463.
Oppenheimer, J. M. (1936b). Transplantation experiments on developing teleosts (*Fundulus* and *Perca*). *J. Exp. Zool.* **72**, 409-437.
Otte, A. P. and Moon, R. T. (1992). Protein kinase C isozymes have distinct roles in neural induction and competence in Xenopus. *Cell* **68**, 1021-1029.
Otte, A. P., Koster, C. H., Snoek, G. T. and Durston, A. J. (1988). Protein kinase C mediates neural induction in Xenopus laevis. *Nature* **334**, 618-620.
Otte, A. P., van Run, P., Heideveld, M., van Driel, R. and Durston, A. J. (1989). Neural induction is mediated by cross-talk between the protein kinase C and cyclic AMP pathways. *Cell* **58**, 641-648.
Palma, V., Kukuljan, M. and Mayor, R. (2001). Calcium mediates dorsoventral patterning of mesoderm in Xenopus. *Curr. Biol.* **11**, 1606-1610.
Papin, C., van Grunsven, L. A., Verschueren, K., Huylebroeck, D. and Smith, J. C. (2002). Dynamic regulation of Brachyury expression in the amphibian embryo by XSIP1. *Mech. Dev.* **111**, 37-46.
Pearce, J. J., Penny, G. and Rossant, J. (1999). A mouse cerberus/Dan-related gene family. *Dev. Biol.* **209**, 98-110.
Pera, E. M., Wessely, O., Li, S. Y. and de Robertis, E. M. (2001). Neural and head induction by insulin-like growth factor signals. *Dev. Cell* **1**, 655-665.
Pera, E., Ikeda, A., Eivers, E. and de Robertis, E. M. (2003). Integration of IGF, FGF and anti-BMP signals via Smad1 phosphorylation in neural induction. *Genes Dev.* **17**, 3023-3028.
Perea-Gómez, A., Vella, F. D., Shawlot, W., Oulad-Abdelghani, M., Chazaud, C., Meno, C., Pfister, V., Chen, L., Robertson, E., Hamada, H. et al. (2002). Nodal antagonists in the anterior visceral endoderm prevent the formation of multiple primitive streaks. *Dev. Cell* **3**, 745-756.
Piccolo, S., Sasai, Y., Lu, B. and de Robertis, E. M. (1996). Dorsoventral Patterning in Xenopus: Inhibition of ventral signals by direct binding of chordin to BMP-4. *Cell* **86**, 589-598.
Postigo, A. A. (2003). Opposing functions of ZEB proteins in the regulation of the TGFbeta/BMP signaling pathway. *EMBO J.* **22**, 2443-2452.
Postigo, A. A., Depp, J. L., Taylor, J. J. and Kroll, K. L. (2003). Regulation of Smad signaling through a differential recruitment of coactivators and corepressors by ZEB proteins. *EMBO J.* **22**, 2453-2462.
Pownall, M. E., Welm, B. E., Freeman, K. W., Spencer, D. M., Rosen, J. M. and Isaacs, H. V. (2003). An inducible system for the study of FGF signalling in early amphibian development. *Dev. Biol.* **256**, 90-100.
Rentzsch, F., Bakkers, J., Kramer, C. and Hammerschmidt, M. (2004). Fgf signaling induces posterior neuroectoderm independently of Bmp signaling inhibition. *Dev. Dyn.* **231**, 750-757.
Rex, M., Orme, A., Uwanogho, D., Tointon, K., Wigmore, P. M., Sharpe, P. T. and Scotting, P. J. (1997). Dynamic expression of chicken Sox2 and Sox3 genes in ectoderm induced to form neural tissue. *Dev. Dyn.* **209**, 323-332.
Ribisi, S., Jr, Mariani, F. V., Aamar, E., Lamb, T. M., Frank, D. and Harland, R. M. (2000). Ras-mediated FGF signaling is required for the formation of posterior but not anterior neural tissue in Xenopus laevis. *Dev. Biol.* **227**, 183-196.

Robb, L. and Tam, P. P. (2004). Gastrula organiser and embryonic patterning in the mouse. *Semin. Cell Dev. Biol.* **15**, 543-554.
Rodríguez-Gallardo, L., Climent, V., Garcia-Martinez, V., Schoenwolf, G. C. and Alvarez, I. S. (1997). Targeted over-expression of FGF in chick embryos induces formation of ectopic neural cells. *Int. J. Dev. Biol.* **41**, 715-723.
Rowan, A. M., Stern, C. D. and Storey, K. G. (1999). Axial mesendoderm refines rostrocaudal pattern in the chick nervous system. *Development* **126**, 2921-2934.
Saint-Jeannet, J. P., Huang, S. and Duprat, A. M. (1990). Modulation of neural commitment by changes in target cell contacts in Pleurodeles waltl. *Dev. Biol.* **141**, 93-103.
Sasai, Y., Lu, B., Steinbeisser, H., Geissert, D., Gont, L. K. and de Robertis, E. M. (1994). Xenopus chordin: A novel dorsalizing factor activated by organizer-specific homeobox genes. *Cell* **79**, 779-790.
Sasai, Y., Lu, B., Steinbeisser, H. and de Robertis, E. M. (1995). Regulation of neural induction by the Chd and Bmp-4 antagonistic patterning signals in Xenopus. *Nature* **376**, 333-336.
Sasai, Y., Lu, B., Piccolo, S. and de Robertis, E. M. (1996). Endoderm induction by the organizer-secreted factors chordin and noggin in Xenopus animal caps. *EMBO J.* **15**, 4547-4555.
Sato, S. M. and Sargent, T. D. (1989). Development of neural inducing capacity in dissociated Xenopus embryos. *Dev. Biol.* **134**, 263-266.
Schepers, G. E., Teasdale, R. D. and Koopman, P. (2002). Twenty pairs of sox: extent, homology, and nomenclature of the mouse and human sox transcription factor gene families. *Dev. Cell* **3**, 167-170.
Schulte-Merker, S., Lee, K. J., McMahon, A. P. and Hammerschmidt, M. (1997). The zebrafish organizer requires chordino. *Nature* **387**, 862-863.
Servetnick, M. and Grainger, R. M. (1991). Changes in neural and lens competence in Xenopus ectoderm: evidence for an autonomous developmental timer. *Development* **112**, 177-188.
Sharpe, C. R. and Gurdon, J. B. (1990). The induction of anterior and posterior neural genes in Xenopus laevis. *Development* **109**, 765-774.
Sheng, G., dos Reis, M. and Stern, C. D. (2003). Churchill, a zinc finger transcriptional activator, regulates the transition between gastrulation and neurulation. *Cell* **115**, 603-613.
Smith, W. C. and Harland, R. M. (1992). Expression cloning of noggin, a new dorsalizing factor localized to the Spemann organizer in Xenopus embryos. *Cell* **70**, 829-840.
Smith, W. C., Knecht, A. K., Wu, M. and Harland, R. M. (1993). Secreted noggin protein mimics the Spemann organizer in dorsalizing Xenopus mesoderm. *Nature* **361**, 547-549.
Spemann, H. (1921). Die Erzeugung thierischer Chimären durch heteroplastische Transplantation zwischen Triton cristatus und taeniatus. *Wilh. Roux's Arch. EntwMech. Org.* **48**, 533-570.
Spemann, H. and Mangold, H. (1924). Über Induktion von Embryonalanlagen durch Implantation artfremder Organisatoren. *Roux's Arch. EntwMech. Org.* **100**, 599-638.
Stern, C. D. (2001). Initial patterning of the central nervous system: how many organizers? *Nat. Rev. Neurosci.* **2**, 92-98.
Stern, C. D. (2004). Neural induction. In *Gastrulation: From Cells to Embryo* (ed. C. D. Stern), pp. 419-432. New York: Cold Spring Harbor Press.
Stewart, R., Christie, V. B. and Przyborski, S. A. (2003). Manipulation of human pluripotent embryonal carcinoma stem cells and the development of neural subtypes. *Stem Cells* **21**, 248-256.
Storey, K. G., Crossley, J. M., de Robertis, E. M., Norris, W. E. and Stern, C. D. (1992). Neural induction and regionalisation in the chick embryo. *Development* **114**, 729-741.
Storey, K. G., Selleck, M. A. and Stern, C. D. (1995). Neural induction and regionalisation by different subpopulations of cells in Hensen's node. *Development* **121**, 417-428.
Storey, K. G., Goriely, A., Sargent, C. M., Brown, J. M., Burns, H. D., Abud, H. M. and Heath, J. K. (1998). Early posterior neural tissue is induced by FGF in the chick embryo. *Development* **125**, 473-484.
Streit, A. (2002). Extensive cell movements accompany formation of the otic placode. *Dev. Biol.* **249**, 237-254.
Streit, A. (2004). Early development of the cranial sensory nervous system: from a common field to individual placodes. *Dev. Biol.* **276**, 1-15.
Streit, A. and Stern, C. D. (1999a). Mesoderm patterning and somite formation during node regression: differential effects of chordin and noggin. *Mech. Dev.* **85**, 85-96.
Streit, A. and Stern, C. D. (1999b). Establishment and maintenance of the border of the neural plate in the chick: involvement of FGF and BMP activity. *Mech. Dev.* **82**, 51-66.
Streit, A. and Stern, C. D. (1999c). Neural induction. A bird's eye view. *Trends Genet.* **15**, 20-24.
Streit, A., Sockanathan, S., Perez, L., Rex, M., Scotting, P. J., Sharpe, P. T., Lovell-Badge, R. and Stern, C. D. (1997). Preventing the loss of competence for neural induction: HGF/SF, L5 and Sox-2. *Development* **124**, 1191-1202.
Streit, A., Lee, K. J., Woo, I., Roberts, C., Jessell, T. M. and Stern, C. D. (1998). Chordin regulates primitive streak development and the stability of induced neural cells, but is not sufficient for neural induction in the chick embryo. *Development* **125**, 507-519.
Streit, A., Berliner, A. J., Papanayotou, C., Sirulnik, A. and Stern, C. D. (2000). Initiation of neural induction by FGF signalling before gastrulation. *Nature* **406**, 74-78.
Strong, C. F., Barnett, M. W., Hartman, D., Jones, E. A. and Stott, D. (2000). Xbra3 induces mesoderm and neural tissue in Xenopus laevis. *Dev. Biol.* **222**, 405-419.
Suzuki, A., Chang, C., Yingling, J. M., Wang, X. F. and Hemmati-Brivanlou, A. (1997a). Smad5 induces ventral fates in Xenopus embryo. *Dev. Biol.* **184**, 402-405.
Suzuki, A., Ueno, N. and Hemmati-Brivanlou, A. (1997b). Xenopus msx1 mediates epidermal induction and neural inhibition by BMP4. *Development* **124**, 3037-3044.
Tam, P. P. and Steiner, K. A. (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. and Beddington, R. (1996). Anterior primitive endoderm may be responsible for patterning the anterior neural plate in the mouse embryo. *Curr. Biol.* **6**, 1487-1496.
Tribulo, C., Aybar, M. J., Nguyen, V. H., Mullins, M. C. and Mayor, R. (2003). Regulation of Msx genes by a Bmp gradient is essential for neural crest specification. *Development* **130**, 6441-6452.
Uchikawa, M., Kamachi, Y. and Kondoh, H. (1999). Two distinct subgroups of Group B Sox genes for transcriptional activators and repressors: their expression during embryonic organogenesis of the chicken. *Mech. Dev.* **84**, 103-120.
Uchikawa, M., Ishida, Y., Takemoto, T., Kamachi, Y. and Kondoh, H. (2003). Functional analysis of chicken Sox2 enhancers highlights an array of diverse regulatory elements that are conserved in mammals. *Dev. Cell* **4**, 509-519.
Uchikawa, M., Takemoto, T., Kamachi, Y. and Kondoh, H. (2004). Efficient identification of regulatory sequences in the chicken genome by a powerful combination of embryo electroporation and genome comparison. *Mech. Dev.* **121**, 1145-1158.
Umbhauer, M., Penzo-Mendez, A., Clavilier, L., Boucaut, J. and Riou, J. (2000). Signaling specificities of fibroblast growth factor receptors in early Xenopus embryo. *J. Cell Sci.* **113**, 2865-2875.
Uwanogho, D., Rex, M., Cartwright, E. J., Pearl, G., Healy, C., Scotting, P. J. and Sharpe, P. T. (1995). Embryonic expression of the chicken Sox2, Sox3 and Sox11 genes suggests an interactive role in neuronal development. *Mech. Dev.* **49**, 23-36.
Varlet, I., Collignon, J. and Robertson, E. J. (1997). nodal expression in the primitive endoderm is required for specification of the anterior axis during mouse gastrulation. *Development* **124**, 1033-1044.
Verschueren, K., Remacle, J. E., Collart, C., Kraft, H., Baker, B. S., Tylzanowski, P., Nelles, L., Wuytens, G., Su, M. T., Bodmer, R. et al. (1999). SIP1, a novel zinc finger/homeodomain repressor, interacts with Smad proteins and binds to 5′-CACCT sequences in candidate target genes. *J. Biol. Chem.* **274**, 20489-20498.
Waddington, C. H. (1932). Experiments on the development of chick and duck embryos cultivated in vitro. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **221**, 179-230.
Waddington, C. H. (1933). Induction by the primitive streak and its derivatives in the chick. *J. Exp. Biol.* **10**, 38-48.
Waddington, C. H. (1934). Experiments on embryonic induction. *J. Exp. Biol.* **11**, 211-227.
Waddington, C. H. (1936). Organizers in Mammalian Development. *Nature* **138**, 125.
Waddington, C. H. (1937). Experiments on determination in the rabbit embryo. *Arch. Biol.* **48**, 273-290.
Waddington, C. H. and Needham, J. (1936). Evocation and individuation and competence in amphibian organizer action. *Proc. Kon. Akad. Wetensch. Amsterdam* **39**, 887-891.
Wagner, D. S. and Mullins, M. C. (2002). Modulation of BMP activity in

dorsal-ventral pattern formation by the chordin and ogon antagonists. *Dev. Biol.* **245**, 109-123.

Weinstein, D. C., Ruiz i Altaba, A., Chen, W. S., Hoodless, P., Prezioso, V. R., Jessell, T. M. and Darnell, J. E., Jr (1994). The winged-helix transcription factor HNF-3 beta is required for notochord development in the mouse embryo. *Cell* **78**, 575-588.

Wilson, P. A. and Hemmati-Brivanlou, A. (1995). Induction of epidermis and inhibition of neural fate by Bmp-4. *Nature* **376**, 331-333.

Wilson, P. A., Lagna, G., Suzuki, A. and Hemmati-Brivanlou, A. (1997). Concentration-dependent patterning of the Xenopus ectoderm by BMP4 and its signal transducer Smad1. *Development* **124**, 3177-3184.

Wilson, S. I. and Edlund, T. (2001). Neural induction: toward a unifying mechanism. *Nat. Neurosci. Suppl.* **4**, 1161-1168.

Wilson, S. I., Graziano, E., Harland, R., Jessell, T. M. and Edlund, T. (2000). An early requirement for FGF signalling in the acquisition of neural cell fate in the chick embryo. *Curr. Biol.* **10**, 421-429.

Wilson, S. I., Rydstrom, A., Trimborn, T., Willert, K., Nusse, R., Jessell, T. M. and Edlund, T. (2001). The status of Wnt signalling regulates neural and epidermal fates in the chick embryo. *Nature* **411**, 325-330.

Wilson, S. W. and Houart, C. (2004). Early steps in the development of the forebrain. *Dev Cell* **6**, 167-181.

Withington, S., Beddington, R. and Cooke, J. (2001). Foregut endoderm is required at head process stages for anteriormost neural patterning in chick. *Development* **128**, 309-320.

Wood, H. B. and Episkopou, V. (1999). Comparative expression of the mouse Sox1, Sox2 and Sox3 genes from pre-gastrulation to early somite stages. *Mech. Dev.* **86**, 197-201.

Xu, R. H., Kim, J., Taira, M., Zhan, S., Sredni, D. and Kung, H. F. (1995). A dominant negative bone morphogenetic protein 4 receptor causes neuralization in Xenopus ectoderm. *Biochem. Biophys. Res. Commun.* **212**, 212-219.

Xu, R. H., Kim, J., Taira, M., Sredni, D. and Kung, H. (1997). Studies on the role of fibroblast growth factor signaling in neurogenesis using conjugated/aged animal caps and dorsal ectoderm- grafted embryos. *J. Neurosci.* **17**, 6892-6898.

Yabe, S., Tanegashima, K., Haramoto, Y., Takahashi, S., Fujii, T., Kozuma, S., Taketani, Y. and Asashima, M. (2003a). FRL-1, a member of the EGF-CFC family, is essential for neural differentiation in Xenopus early development. *Development* **130**, 2071-2081.

Yabe, T., Shimizu, T., Muraoka, O., Bae, Y. K., Hirata, T., Nojima, H., Kawakami, A., Hirano, T. and Hibi, M. (2003b). Ogon/Secreted Frizzled functions as a negative feedback regulator of Bmp signaling. *Development* **130**, 2705-2716.

Ying, Q. L., Nichols, J., Chambers, I. and Smith, A. (2003a). BMP induction of Id proteins suppresses differentiation and sustains embryonic stem cell self-renewal in collaboration with STAT3. *Cell* **115**, 281-292.

Ying, Q. L., Stavridis, M., Griffiths, D., Li, M. and Smith, A. (2003b). Conversion of embryonic stem cells into neuroectodermal precursors in adherent monoculture. *Nat. Biotechnol.* **21**, 183-186.

Zhang, H., Wang, J. Z., Sun, H. Y., Zhang, J. N. and Yang, S. Y. (2004). The effects of GM1 and bFGF synergistically inducing adult rat bone marrow stromal cells to form neural progenitor cells and their differentiation. *Chin. J. Traumatol.* **7**, 3-6.

Zimmerman, L. B., de Jesus-Escobar, J. M. and Harland, R. M. (1996). The Spemann organizer signal noggin binds and inactivates bone morphogenetic protein 4. *Cell* **86**, 599-606.