Neural induction

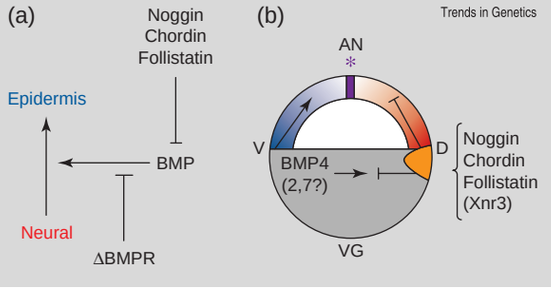
a bird's eye view

Since the discovery of the phenomenon of neural induction by Spemann and Mangold in 1924, considerable effort has been invested in identifying the signals produced by the organizer that are responsible for diverting the fate of cells from epidermal to neural. Substantial progress has been made only recently by the finding in amphibians that BMP4 is a neural inhibitor and epidermal inducer, and that endogenous antagonists of BMPs are secreted by the organizer. However, recent results in the chick point to the existence of other, upstream events required before BMP inhibition stabilizes neural fates. Here we take a critical view of the evidence for and against the view that BMP inhibition is a sufficient trigger for neural induction in different vertebrates.

In vertebrates, the neural plate is initially established during gastrulation, when the ectoderm becomes subdivided into neural and non-neural domains. The border region between the two will later give rise to the cement gland (in amphibians), neural crest cells and the sensory placodes of the head. Spemann and Mangold established that the neural plate can be induced in the ectoderm by signals from the dorsal lip of the amphibian blastopore, a region they called the 'organizer'. When transplanted to an ectopic position, the organizer can change the fate of the adjacent ectoderm from epidermis to neural. Subsequently, Hensen's node (the tip of the primitive streak; see Ref. 1) and the embryonic shield[2] were established as its functional homologues in amniotes and teleosts, respectively. The organizer not only induces neural tissue, but also emits signals that pattern the mesoderm and can confer axial character to lateral mesoderm ('dorsalization'; see Ref. 3). The cells of the organizer eventually differentiate into notochord, gut endoderm and prechordal mesoderm, and several of these descendants themselves retain neural-inducing activity[1].

Soon after the discovery of neural induction, interspecies grafting experiments[2,4] indicated that at least some of the signals are conserved between different vertebrate classes, a finding that has since been confirmed with more modern techniques (e.g. Ref. 5). However, it is only recently that some of the molecular players have started to be identified. The most widely accepted view (the 'default model') is derived from experiments in *Xenopus*. In this review, we take a critical viewpoint on the evidence for this model, and argue that the phenomenon of neural induction will turn out to involve cooperation between different classes of signals during normal development.

FIGURE 1. The default model



In *Xenopus*, ectoderm cells have a natural tendency to differentiate into neural tissue, but are inhibited from doing so under the influence of BMP signalling. The endogenous BMP antagonists chordin, noggin, follistatin and Xnr3 are expressed in the organizer and the first three of these bind to BMP directly. Inhibition of BMP signalling by injection of a dominant-negative BMP receptor (ΔBMPR) also causes neural differentiation. In addition to epidermal (anti-neural) induction, the BMPs also play a role in ventralization of the mesoderm. (a) The proposed pathway. (b) Spatial relationships of these signals at late blastula/early gastrula stages. AN, animal; VG, vegetal; D, dorsal; V, ventral; *, position of the future cement gland (anterior border of neural plate). The organizer region is shown in orange, prospective neural tissue in red, epidermis in blue and the border region between the latter two in purple.

The 'default model' and the evidence supporting it

This model (Fig. 1) proposes that cells within the ectoderm layer of the frog gastrula have an autonomous tendency to differentiate into neural tissue, and that this tendency is inhibited by bone morphogenetic proteins (BMPs) – in particular, BMP4 (for reviews see Refs 6, 7). To date, most neural-inducing treatments that affect signalling by secreted factors appear to act through inhibition of BMP signalling.

The idea that release from inhibitory signals is sufficient to cause neural development in the ectoderm ('autoneuralization') was originally suggested by the observation that, in amphibians, dissociation of gastrula-stage animal caps leads to the formation of neural tissue (see Refs 3, 6, 7). This can be suppressed by BMP4 (Ref. 8), or if the animal cap is obtained from embryos previously injected with RNA encoding one of the effectors of BMP4 (*msx1* or *smad1*; Refs 9, 10), consistent with the view that the neural pathway is naturally inhibited by an endogenous BMP-like activity. 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 clear from the future neural plate at the time when the organizer appears[11].

The default model gains further support from experiments in which the BMP4 signalling pathway is inhibited (Fig. 2). Animal caps cut from embryos injected with RNA

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encoding dominant-negative receptors that bind BMPs (Refs 12, 13), or non-cleavable forms of BMP4 or 7 (Ref. 14), or antisense *BMP4* RNA (Ref. 15), adopt a neural, instead of epidermal, fate.

The existence of several endogenous molecules that antagonize BMP signalling also suggests that BMP inhibition plays a role *in vivo*. Genes encoding the secreted factors noggin, chordin, follistatin and Xnr3 are all expressed in the *Xenopus* organizer at the gastrula stage, when neural induction is thought to occur. In *Xenopus*, ectopic expression of any of them by RNA or DNA injection results in the development of neural tissue from blastula-stage animal caps that have been allowed to age beyond the gastrula stage[15–18]. Chordin, noggin and follistatin all counteract BMP signalling by direct binding to BMP2, 4 and 7, preventing them from interacting with their receptor(s)[19–21]. In addition, transcription of *BMP* RNA is maintained by the activity of BMP protein[22], which explains the disappearance of *BMP4* and *7* expression from the vicinity of the organizer (which secretes BMP inhibitors) at the gastrula stage[11,14].

In addition to its role in neural induction, the organizer can also pattern the mesoderm at the gastrula stage ('dorsalization'). This activity can also be attributed to BMP inhibition. BMPs can modify dorsal mesoderm to give ventral cell types[11,23,24], while their inhibitors can generate notochord and muscle from ventral mesoderm[25,26]. BMP inhibitors can also regulate the dorsoventral polarity of the whole embryo before gastrulation. For example, UV-irradiated embryos lack dorsoventral polarity and fail to gastrulate, but can be rescued fully by injection of RNA encoding any of the BMP inhibitors: the blastopore (dorsal) will form close to the site of injection[26,27].

Together, these findings provide compelling evidence that BMPs and their modulation by endogenous inhibitors are involved in all the activities of the organizer, including the establishment of neural and non-neural domains of the *Xenopus* gastrula. However, closer examination of some of the experimental data reveals that this interpretation might be too simplistic.

Additional complexity in neural induction

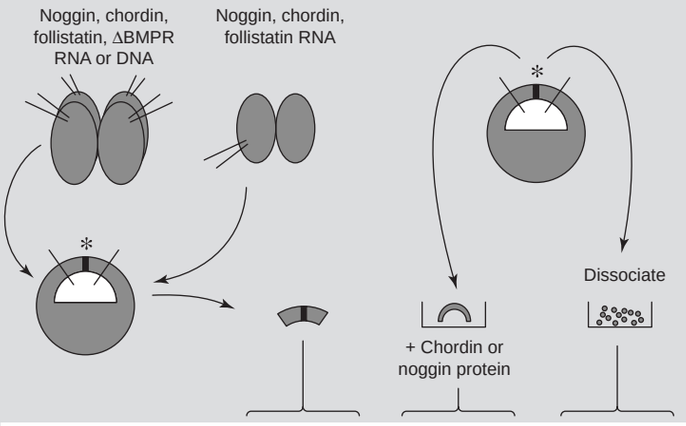
One phenomenon that the default model currently fails to explain is 'homoiogenetic induction' (the induction of neural plate by previously induced neural plate[1]). The neural plate itself does not express any of the BMP antagonists identified to date and there is, therefore, no obvious molecular basis for its inducing activity.

Other results reveal unexpected differences in the properties of the various BMP antagonists. For example, chordin and noggin differ in their affinity for BMP4 protein: chordin has a K_D of 300 pM, while noggin binds the same ligand with a K_D of 20 pM (Refs 19, 20). When assayed for dorsalization of the mesoderm, 1 nM of either chordin or noggin will suffice. However, for neural induction, noggin is 10–20 times less efficient than chordin, the opposite of what might be expected from their affinity for BMP4 (Refs 3, 16). This indicates that the situation *in vivo* is probably more complex than predicted by the default model in its simplest form.

Although neither noggin nor chordin are mesoderm inducers, both can modify the state of cells that have previously received mesoderm inducing signals. There is some evidence that weak mesoderm inducing signals have reached the animal cap at the gastrula stage and that noggin can enhance this to produce dorsal mesoderm[16]. This is a particular concern because of the greater potency of noggin in dorsalization than in neuralization[3,16] (see above). Of course, most authors have been careful to test for expression of a panel of mesodermal markers in their excised animal caps. However, the markers rarely include genes expressed in the endoderm. It would also be interesting to examine the expression patterns of dorsal mesoderm/endoderm markers in the remaining part of the embryo after excision of the animal cap, to see if an expanded or ectopic organizer territory was present adjacent to the excised region.

BMP inhibitors, such as noggin and chordin, are not the only molecules that have been reported to have direct neuralizing activity. There is evidence that FGF can act as a direct neural inducer of animal caps cut from gastrula-stage embryos[28] and similar findings have been made in chick embryos[29,30]. In addition, two experiments showed that the neuralizing activity of chordin and noggin in animal caps requires an intact FGF signalling pathway. In

FIGURE 2. Assays in the frog

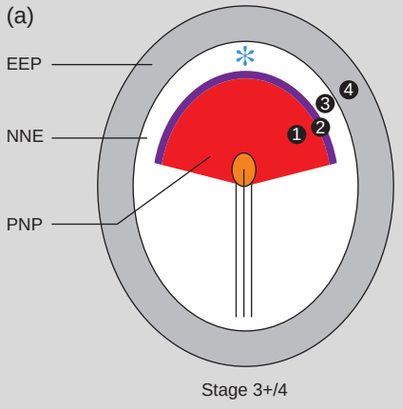


Tissue	Markers	C	E	C	E	–BMP4	+BMP4
Epidermis	Keratin	+	–	+	–	–	+
Neural	N-CAM	–	+	–	+	+	–
	N-tubulin	–	+	–	+	+	–
	NF-M	–	+	–	+	+	–
	Otx-2	–	+	–	+	+	–
	En2	–	–	–	–	–	–
	Krox-20	–	–	–	–	–	–
	Hox b9	–	–	–	–	–	–
Cement gland	XAG, XCG	+	+	+	+	+	+
Organizer, mesoderm							
	goosecoid	–	–	–	–	–	–
	brachyury	–	–	–	–	–	(+)
	actin	–	–	–	–		

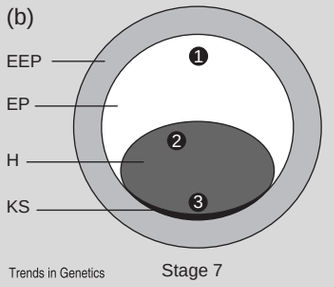
Trends in Genetics

The assays begin in one of three ways (top row of diagrams). RNA or DNA encoding noggin, chordin, follistatin or BMPR is injected either into the animal pole of all blastomeres at the four-cell stage (left) or into the ventral vegetal side of one blastomere at the two-cell stage (middle). These embryos are allowed to grow to the late blastula or early gastrula stage, the animal cap excised and matured *in vitro* before assaying for various markers by reverse transcriptase polymerase chain reaction (RT-PCR). Alternatively, animal caps are excised from late blastula or early gastrula embryos. The caps are either treated with chordin or noggin protein, or dissociated, treated with BMP4 and reaggregated. Following culture, they are assayed as above. The table shows typical results obtained for each of these experiments. C, control (uninjected embryos or untreated caps); E, experimental. The + in brackets under brachyury refers to the finding that high concentrations of BMP4 act as a mesoderm inducer[8]. Note that cement gland markers are expressed after all treatments as well as in controls.

FIGURE 3. Assays in the chick



Site	Factor	*SOX2/3*
❶	– BMP4	+ +
❷	– BMP4 CHD	+/– – ++
❸	– CHD/NOG	– –
❹	– CHD/NOG	– –



Site	Factor	*BRA*	*NOT1*	*SOX2/3*
❶	– CHD	– +	– +	– +
❷	– BMP			+ +
❸	– BMP	+ –	+ –	+ ND

(a) Mis-expression of chordin, noggin and BMP4 in the primitive streak stage, analyzed in terms of the expression of the neural markers *SOX2* and *SOX3*. (b) Results of mis-expression at pre-primitive streak stages, analyzed with neural (*SOX2*, *SOX3*) as well as mesodermal markers (*BRA*, brachyury for primitive streak mesoderm; *NOT1* for organizer; orange). EEP, extraembryonic epiblast; EP, embryonic epiblast; H, hypoblast; KS, Koller's sickle; NNE, non-neural ectoderm; PNP, prospective neural plate (red). The prospective neural crest/placode territory is shown in purple.

the first experiment, animal caps cut from embryos expressing a dominant-negative FGF receptor were found to be insensitive to neural induction by noggin[31]. The second experiment showed that in the absence of FGF signalling, chordin or noggin generate predominantly endoderm, while the same proteins induce both endoderm and neural tissue when FGF signalling is intact[32]. However, it is puzzling that when FGF signalling is inhibited in whole embryos neural tissue nevertheless develops[33]. Taken together, these experiments suggest that some form of FGF signalling is involved in neural induction together with BMP inhibitors. Both noggin and chordin promote the differentiation of endoderm, raising the additional possibility that the endoderm mediates some aspect of neural induction. In line with this, the anterior endoderm in *Xenopus* also expresses *cerberus*, another secreted factor with neural-inducing activity[34].

It is interesting that mere cutting of an animal cap, or brief treatment with low-calcium and -magnesium medium, activates the FGF signalling pathway[35] and, at least transiently, induces expression of the cement gland marker *XAG1* (Ref. 28) and the early neural marker *Sox2* (Y. Sasai, pers. commun.). These observations must be taken into account when considering that both noggin and chordin protein are only effective in neuralization if the animal cap has been sensitized by incubation in low-calcium and -magnesium medium[15,16]. Although the rationale of this pretreatment is that it promotes access of the protein to all cells in the animal cap, it is also possible that the partial loss of cell contact activates other intracellular responses (as shown for FGF signalling), which are then enhanced by the BMP inhibitors, an argument that is equally valid for experiments where dissociation is used alone as a means of promoting neural differentiation.

The interpretation of some animal cap experiments is complicated by the existence of a 'prepattern' in the animal cap at gastrula stages. By the late blastula stage in *Xenopus*, the dorsal ectoderm is biased in favour of a neural fate[36,37], and the boundary between neural and non-neural ectoderm appears to be positioned before gastrulation[38]. Competing planar signals within the ectoderm, emanating from the dorsal and ventral vegetal cells respectively, might be responsible for this early subdivision[36,38]. Dorsal and ventral portions of the animal cap of the early gastrula differ in their sensitivity to neural-inducing stimuli[39–41]. These findings could indicate that the dorsal ectoderm has received some neural-inducing signals before gastrulation, and that it merely requires additional permissive, or stabilizing signals (such as BMP inhibition) to complete its differentiation in a neural direction.

Multiple cooperating signals from the organizer

Recent results in the chick embryo support the idea that BMP inhibition is not sufficient for neural induction but, rather, acts downstream of, or in conjunction with, other signals from the organizer. Mis-expression of either chordin (Ref. 42) or noggin (A. Streit and C.D. Stern, unpublished) in the extraembryonic or embryonic non-neural ectoderm (Fig. 3) does not generate an ectopic neural plate or expression of any neural markers. However, when ectopically expressed before primitive streak formation, chordin can generate the formation of a second primitive streak expressing organizer markers[42]. Moreover, unlike in *Xenopus*, cell dissociation of the chick epiblast does not lead to neural differentiation, but promotes muscle development[43].

The expression patterns of BMPs and their inhibitors in chick and mouse embryos are also at odds with their proposed roles in *Xenopus*. Neither *BMP2*, *4* nor *7* is expressed in the future neural plate at the early primitive streak stage, when neural induction is thought to begin[42,44,45]. *Follistatin* is not expressed in the mouse node[46], and only weakly in the chick[47]. Chick *noggin* (Ref. 48 and A. Streit and C.D. Stern, unpublished) only starts to be expressed in the node just as its inducing ability begins to diminish[49], and although *chordin* is present in the chick node before this stage[42], its expression persists and even increases long after the node has virtually lost its neural-inducing ability. These patterns of expression do not explain the changes in neural-inducing activity of the organizer and suggest that these inhibitors might be required for other developmental processes.

Unfortunately, loss of function mouse mutants for BMPs, their receptors and inhibitors have not helped us to elucidate the roles of BMPs in neural induction. Noggin mutants develop a fairly normal neural plate and show patterning defects only at later stages[50]. Null mutants for follistatin[51], BMP7 (Ref. 52) or BMP2 (Ref. 53) have no early neural phenotype. A proportion of mutants lacking BMP4 die before gastrulation, but a few survive to early limb bud stages; these do not appear to have an enlarged nervous system or absence of epidermis[44]. Mutations in the BMP receptor (Bmpr-Ia; Ref. 54) lead to very early death and are therefore uninformative.

A further experiment in the chick strengthens the proposal that BMP inhibition by chordin is only effective in neural induction on cells that have previously received other organizer-derived signals. Chordin mis-expression is insufficient to induce expression of neural markers in the extraembryonic area opaca. However, it is able to stabilize the expression of *Sox3* if cells are exposed to a graft of Hensen's node for five hours (too brief for induction of a neural plate), the node is then removed and replaced by chordin-secreting cells[42]. Together, these results suggest that BMP inhibition acts downstream or in conjunction with other neural-inducing signals from the organizer.

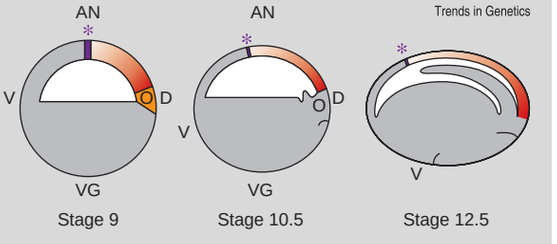
A role for BMP signalling at the edge of the neural plate

In the chick embryo, the border between neural and non-neural ectoderm seems to be the only region that can respond to excess BMP4 or to its inhibition (Fig. 3). The prospective neural plate delimited by this border is not inhibited by BMP4 or 7, and the non-neural ectoderm outside the border is not induced by chordin or noggin. By contrast, mis-expression of chordin at the border leads to a widening of the neural plate, while mis-expression of BMP4 in the same region leads to a narrowing of the neural plate[42,55].

In *Xenopus*, the anterior boundary of the neural plate (prospective cement gland) has been located to the animal pole of the ectoderm at stages 7–9 (Ref. 38). However, other fate-mapping studies[56] done at the early gastrula stage place it more dorsally and there is, therefore, a pressing need for new, detailed fate maps of the gastrula stage so that this important issue can be resolved. During convergence and extension movements (which begin at stages 9–10), this border shifts anteriorly[57] (towards the original ventral side of the embryo; see Fig. 4). It is likely, therefore, that animal caps, which are usually excised at stages 9–10 for studies on neural induction, contain this border. Furthermore, isolated animal caps, even when obtained from UV-ventralized embryos, express the cement gland marker *XAG1* in the absence of BMP inhibition[10].

It is interesting in this context to return to the differences in the pattern of expression of *BMP*s in amniotes and anamniotes. In frog and zebrafish, *BMP2* and *4* are expressed ubiquitously in the ectoderm before gastrulation, and then cleared from the prospective neural plate. In chick and mouse, there is little or no expression in the central epiblast before gastrulation. By early neurulation, the expression of *BMP*s in both groups becomes strongly localized to the boundaries of the neural plate. Thus, although the expression patterns differ at the early stages, they converge just after the appearance of the neural plate, suggesting that a role for BMP at this border is important and has been strongly conserved in evolution.

FIGURE 4. Movements of the neural plate anterior boundary



Movements of the neural plate (red) and the position of the cement gland (*) at the border between neural and non-neural territories (purple) according to Zhang and Jacobson[38] from the late blastula (stage 9) to late gastrula/early neurula (stage 12.5). AN, animal; VG, vegetal; D, dorsal; V, ventral; O, organizer (orange).

Conclusions

Having outlined some apparently contradictory results, we are left with the question of whether amniotes and anamniotes have evolved different mechanisms for generating their nervous systems, or whether the differences are intrinsic to the assays employed and their interpretation. The finding that organizer grafts can induce neural tissue across different vertebrate classes appears to argue in favour of the second possibility.

An open question is: when does neural induction begin? Without answering it, we will be unable to ascertain how many signals and cell states are required before BMP inhibition can effectively neuralize. This is particularly important in assays that include some cells whose normal fate is to give rise to the neural plate or to its border and which might, therefore, have received other signals before the start of the assay.

However, species differences might also contribute to the different results. For example, it is conceivable that in the frog, a larger domain (perhaps even the entire animal cap) behaves as the border of the neural plate before gastrulation and that, as in the chick, this region is particularly sensitive to BMP inhibition.

We believe that neural induction will turn out to consist of a hierarchy of events, where each step in the cascade stabilizes the previous ones. The identification of these steps (including signals and responses) provides some interesting challenges for the future.

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mRNA degradation

a tale of poly(A) and multiprotein machines

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The *Escherichia coli* RNA degradosome is a multiprotein complex containing an endoribonuclease, polynucleotide phosphorylase and a DEAD-box RNA helicase. A related complex has been described in the spinach chloroplast. The exosome and the mtEXO complex have recently been described in yeast and it is likely that related complexes also exist in animal cells. This research suggests the widespread existence of sophisticated machines for the efficient degradation of messenger RNA. The DEAD-box helicase in the degradosome can unwind regions of RNA structure that interfere with 3′–5′ degradation. The polyadenylation of RNA 3′ ends is also known to promote degradation by creating a 'toehold' for the degradation machinery. Much remains to be learned about the regulation of mRNA stability. The complexity of the degradation process, both in the eubacteria and in the eukaryotes, suggests that many steps are possible points of control.

All RNAs can be classified by their stability in the cell. The best known stable RNAs are the transfer and ribosomal RNAs. Messenger RNAs (mRNAs) are unstable with half-lives in *Escherichia coli* ranging from 30 seconds to 20 minutes. In eukaryotic cells, mRNA turnover is slower, but the half-lives are usually shorter than the generation time. Until recently, the degradation of mRNA was not generally considered in discussions of the regulation of gene expression. RNA degradation was mainly viewed as a nuisance in the laboratory, causing problems when trying to do a northern blot or construct a cDNA library. With the growing appreciation of the complexity of gene regulation, interest in the mechanism of the degradation of mRNA has rapidly increased during the past decade. The instability of