Neural induction: toward a unifying mechanism

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Published online: 29 October 2001, DOI: 10.1038/nn747

Neural induction constitutes the initial step in the generation of the vertebrate nervous system. In attempting to understand the principles that underlie this process, two key issues need to be resolved. When is neural induction initiated, and what is the cellular source and molecular nature of the neural inducing signal(s)? Currently, these aspects of neural induction seem to be very different in amphibian and amniote embryos. Here we highlight the similarities and the differences, and we propose a possible unifying mechanism.

Neural tissue is derived from the embryonic ectoderm, which is also the source of the epidermis (skin). The progression from naive ectodermal cells to postmitotic neurons involves several distinct steps (**Table 1**) and requires the integration of a number of signaling pathways. Here we use the term neural induction to denote the step whereby embryonic ectodermal cells are exposed to signals that will instruct the cells to become neural stem or precursor cells unless exposed to signals that divert them to alternative fates. Thus, neural induction is defined as the step when ectodermal cells become 'specified' as neural stem or precursor cells (**Table 1**). Later in development, these specified cells will no longer respond to signals that induce alternative fates, and have thus 'committed' to a neural fate (**Table 1**). Ultimately, these cells will differentiate into neurons (**Table 1**).

The first insight into the mechanism of neural induction came from transplantation studies done in amphibian embryos in the mid 1920s. These studies identified a morphologically distinct group of mesodermal cells called the 'organizer,' formed during gastrulation in vertebrate embryos as a source of neural inducing signals[1,2]. Over the past decade, a considerable effort has been invested in identifying organizer-secreted molecular signals that could induce neural character in ectodermal cells. These studies, performed mainly in the frog *Xenopus laevis*, led to the idea that ubiquitously expressed bone morphogenetic protein (BMP) signals (**Table 2**) normally prevent embryonic ectoderm from executing its natural 'default' tendency to differentiate into neural tissue and instead instruct cells to form epidermis. During gastrulation, as the organizer forms, it emits diffusible inhibitors of BMPs that permit surrounding cells to execute their natural default tendency to generate neural tissue[3]. Collectively, these studies propose that neural induction is initiated during gastrulation when the organizer is formed, and depends on BMP antagonists secreted by cells in the organizer. This model is referred to as the default model of neural induction.

In contrast, more recent studies in amniote embryos (a term used to describe animals that form an amnion, such as humans, rodents and birds, but not amphibians or fish) provide evidence that the organizer is not required for neural induction, and that neural induction occurs much earlier in development, at the blastula stage before the organizer region has formed. In addition, these studies also provide evidence that BMP antagonists are neither sufficient nor required for neural induction[4–11] and that the selection of neural and epidermal fate depends on a cascade of signaling events that is regulated by Wnt signals (**Table 2**)[11].

Thus, key aspects of neural induction seem to differ between amphibians and amniotes. These observations raise a number of questions. Why is the specification of neural cells initiated at different developmental stages in amphibian and amniote embryos? Why are the cellular sources and the molecular nature of neural inducing signals different in amphibian and amniote embryos? Pivotally, have anamniote and amniote embryos developed different mechanisms of neural induction, or do these results reflect differences in experimental approaches or conditions? Here we first briefly summarize the key aspects of the model derived from studies in *Xenopus* as has been discussed extensively in several reviews[3,12–15], and then discuss recent findings derived from studies in amniote embryos in more detail. We highlight the similarities and the differences between these models of neural induction and we suggest some possible explanations for the apparent discrepancies.

Neural induction in amphibians

In amphibians, neural tissue forms on the dorsal side of the embryo (dorsal ectoderm), whereas the epidermis forms on the ventral side of the embryo (ventral ectoderm). Early studies of neural induction were conducted in amphibian embryos. About 75 years ago, it was discovered that when the dorsal lip of the blastopore of gastrula stage newt embryos (later called the organizer) was transplanted to the region that normally forms the epidermis (prospective epidermis) of another gastrula stage embryo, organizer cells followed their normal path of differentiation and generated primarily axial mesoderm[1,2]. Strikingly, however, recipient ectoderm cells surrounding the site of transplantation cells were recruited to form an entire embryo with a fully developed secondary nervous system. The equivalent structure (node, embryonic shield) in other species including amniote embryos is formed on the dorsal side adjacent to the neural territory and was later found to have similar inductive properties[16–23]. Collectively, these findings led to the idea that the organizer region is a local source of inductive signals that impose neural fate on the surrounding dorsal ectoderm at gastrula stages.

Table 1. Major steps in neural differentiation.

Competence:	Cells have the ability to become neural precursors if they are exposed to the right combination of signals.
Specification:	Cells have received the signals to become neural precursors cells but will still respond to signals that repress a neural character.
Commitment:	Cells have received the signals to become neural precursors cells and will progress to become neurons even in the presence of signals that repress a neural character.
Differentiation:	Neural precursors cells exit the cell cycle to become post-mitotic neurons.

Differentiation from pluripotient stem cell to postmitotic neuron can be dissected into at least four major steps.

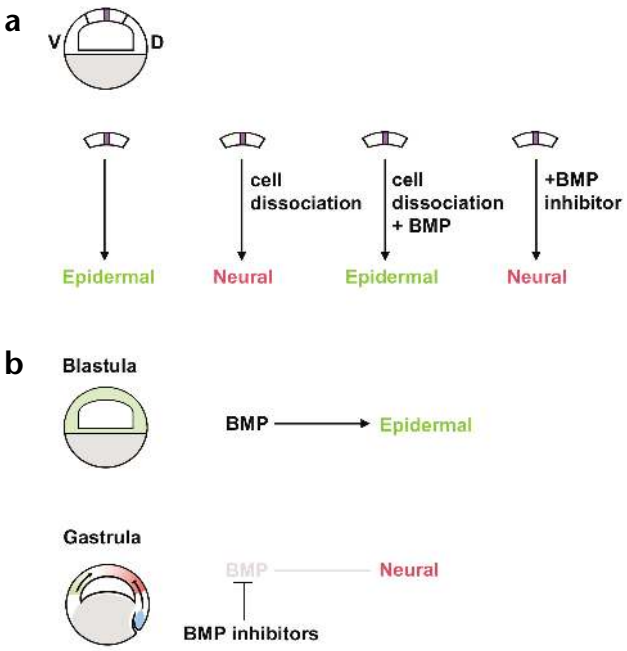
In *Xenopus*, the mechanism by which the organizer region induces neural fate in amphibians was studied using both whole embryos and by *in vitro* culture of explants[3,12]. Primarily, the explants used for such studies encompass a large piece of ectoderm (called an animal cap) excised from the embryonic part (animal hemisphere) of late blastula or early gastrula embryos (**Fig. 1a**)[24–28]. Animal caps from blastula stage embryos generate cells of epidermal character when cultured *in vitro* (**Fig. 1a**). However, dissociation of these explants into single cells generates cells of neural character[29], which raised the possibility that signaling between early ectodermal cells, possibly mediated by a secreted protein, normally suppresses neural differentiation (**Fig. 1a**).

For more than half a century, the neuralizing signals emanating from cells in the organizer region remained elusive. The first clue came from experiments aimed at studying the mechanism of mesoderm formation. Overexpression of a dominant negative form of the activin receptor in *Xenopus* blastula stage ectoderm was found to promote neural differentiation[25]. Activin is a member of a large class of secreted proteins called transforming growth factor beta (TGFβ). Interestingly, a dominant negative form of a receptor that binds BMP[30], another member of the TGFβ family, was soon found to have similar neuralizing properties[31,32]. These results fueled the idea that normally in the *Xenopus* embryo, BMP signaling prevents cells from adopting their default tendency to become neural. Consistent with this idea, it was shown that normally in the *Xenopus* embryo, *Bmp* mRNA is initially expressed ubiquitously, but as gastrulation proceeds, *Bmp* mRNA is cleared from the neural territory (the neural plate) as the organizer begins to form[28,33]. In addition, *Bmp* mRNA expression is maintained in epidermal and repressed in neural cells. Blockade of BMP signals represses *Bmp* mRNA expression, indicating that *Bmp* mRNA is autoregulated in both a positive and a negative feedback loop[33,34]. Finally, the neuralization of dissociated animal cap cells is suppressed if the cells are exposed to BMP4 protein[28] or if the animal cap has been obtained from embryos ectopically expressing an effector of BMP signaling[30] such as Smad1 (ref. 35) or Msx1 (ref. 36). These results triggered the idea that inhibitors of BMP signaling secreted from the organizer act as neural inducing signals in *Xenopus*. Consistent with this idea, BMP inhibitors including Follistatin, Noggin and Chordin are expressed in the organizer region of *Xenopus* embryos and can induce neural markers in blastula stage animal cap explants (**Fig. 1a**)[24,26,27]. Collectively, these results imply that the absence of BMP signaling is sufficient to induce neural differentiation and that naive ectoderm has a natural 'default' tendency to differentiate into neural tissue unless it is instructed by BMP to become epidermis (**Fig. 1b**). Thus, the model of neural induction in amphibians, in its simplest form, suggests that ectodermal cells acquire neural fate during gastrulation in response to BMP antagonists secreted by the cells in the organizer region (**Fig. 1b**).

Are inhibitory signals derived from the organizer the entire basis for neural induction? A number of observations dispute this idea. First, evidence suggests that at least two additional signaling pathways are involved in selecting neural and epidermal fate in *Xenopus*. FGFs are a large class of secreted diffusible glycoproteins that bind to four classes of extracellular receptors to mediate their effects (called FGFR 1–4)[37]. These transmembrane receptors consist of an extracellular FGF ligand binding domain, a transmembrane domain and an intracellular signaling domain[37]. Different FGFs show high affinity to different FGF receptors[37]. It has been suggested that intact FGF signaling is required for neural induction[38,39]. The evidence for this comes largely from overexpression of dominant negative forms of FGF receptors, which contain the FGF-binding domain but lack the intracellular domain, and therefore allow fewer FGF ligands to interact with the natural receptor. Under these conditions, the generation of neural tissue may be blocked. Moreover, expression of these dominant negative FGF receptors in animal cap cells blocks the ability of Noggin or Chordin to induce neural cells[39,40]. Finally, FGF alone may also induce a neural character in animal cap cells[41,42]. However, several studies from *Xenopus* contradict the proposed requirement of FGF signaling in neural induction[43–45]. One possible explanation for the contradictory results may be FGF receptor specificity. In a study that compared the 'neural inhibiting' properties of dominant negative FGF receptors 1 and 4, the dominant negative form of FGFR4 was considerably more effective at inhibiting a neural fate[38]. Thus, although there is evidence to suggest that FGF is involved in neural induction in *Xenopus*, this issue remains contentious, and the precise role of FGF signaling in this process in amphibians and the interaction with BMP signaling remain to be determined.

Wnt signaling has also been implicated in the selection of neural or epidermal fate in *Xenopus*. The acquisition of neural fate is a direct consequence of the establishment of the dorsoventral axis of the embryo[46]. This axis is defined during the first cell cycle by cytoplasmic rearrangements that result in the activation of the Wnt signaling pathway on the dorsal side of the cell[47,48]. Wnts are a large class of secreted glycoproteins, which can be divided into two functionally distinct groups[49]. Introduction of mRNA encoding *Wnts* or their effectors into the animal hemisphere of one-cell embryos generates an 'over-dorsalized' embryo with ectopic neural tissue[46]. Furthermore, animal cap explants excised from such embryos undergo neural differentiation, indicating that early Wnt signaling is important in dorsoventral patterning of the *Xenopus* embryo and consequently in the generation of neural cells[46].

Conversely, there is evidence to suggest that later in development, Wnt signaling may suppress the generation of neural cells. If Wnts are overexpressed in *Xenopus* embryos at blastula stages, the resulting embryos are 'over-ventralized' and the generation of neural tissue is inhibited[46,50]. Furthermore, expression of several different inhibitors of Wnt signaling induces neural markers in animal cap cells[51–53]. It is difficult to assess whether excess Wnt signaling at the one-cell stage directly or indirectly affects the generation of neural cells at a much later stage. Nevertheless, although in *Xenopus* the role of Wnt signaling in neural induction is unclear and the relationship between Wnt and FGF signaling remains to be determined, results suggest that Wnt



signaling is involved in the selection of neural and epidermal fate[39,41,45,46,54,55].

The idea that BMP antagonists are sufficient as neural inducing signals is based on the assertion that blastula ectoderm of *Xenopus* embryos used to isolate animal cap explants is a homogeneous ventral tissue that is specified to generate epidermis. However, a number of observations contradict this idea. At mid-blastula stages, the ectoderm appears to exhibit both ventral (the region from where the epidermis forms) and dorsal (the region from where neural territory forms) character, as it is specified to express markers that are later either selectively expressed in epidermal or neural ectoderm[56]. The early neural markers *Sox3*, *SoxD* and *Geminin*, which result in overt neural differentiation if ectopically expressed, are already expressed in the ectoderm before gastrulation in late-blastula embryos, and *Sox3* expression becomes restricted to dorsal ectoderm before the onset of gastrulation[57–59]. In addition, there is evidence to suggest that by late-blastula stages in *Xenopus*, the dorsal ectoderm (the region from where neural territory forms) may be predisposed to neural differentiation[56]. Consistent with this idea, the border between the future neural and epidermal cells seems also to be established before onset of gastrulation[60]. Moreover, by using an antibody that recognizes the activated (phosphorylated) form of the BMP effectors SMAD1, 5 and 8, which are indicators of ongoing BMP signaling (**Table 2**), it was shown that BMP signaling starts to be restricted ventrally by late blastula, before the organizer has formed[61]. Finally, a recent study demonstrated that neural induction can occur in the absence of mesoderm[55]. Collectively, these studies indicate that blastula stages animal caps contain both prospective neural and epidermal cells. One possible explanation for the fact that experiments using animal caps explants do not detect the predisposition to neural differentiation may be as a result of BMPs derived from prospective epidermal cells in the explant suppressing the fate of prospective neural cells in the same explant. Thus, embryonic ectoderm cells may be exposed to signals that specify neural fate before the generation of the organizer. In this view, BMP antagonists would not induce neural character but rather prevent the suppression of a previously specified neural fate.

Fig. 1. Neural induction in *Xenopus*. (**a**) Position of explants isolated for the animal cap assay. White, animal part of the embryo; gray, vegetal part of the embryo; purple, position of the prospective cement gland (the border between neural and non-neural territories)[60]. D, dorsal; V, ventral; line bisecting circle, dorsal–ventral axis. Animal caps matured *in vitro* differentiate into epidermal tissue. If the animal caps are excised, dissociated for a suitable length of time, reassociated and allowed to mature *in vitro*, they differentiate into neural cells[29]. However, under these conditions, if the dissociated cells are exposed to BMP4 protein, they differentiate into epidermal tissue[28]. If animal caps are allowed to mature in the presence of BMP inhibitors, or if BMP inhibitors are ectopically expressed in animal cap cells, then the explants differentiate into neural tissue[3]. (**b**) The default model of neural induction. At blastula stages, the ectoderm secretes BMP signals (green), which promote epidermal and suppress neural fate. During gastrulation, the organizer (blue) forms and cells in the organizer express BMP inhibitors such as Noggin, Chordin, Follistatin and Xnr3. As a result, BMP signals are blocked, which allows the ectoderm to execute its natural default tendency to differentiate into neural tissue (red).

The organizer is not required for neural induction

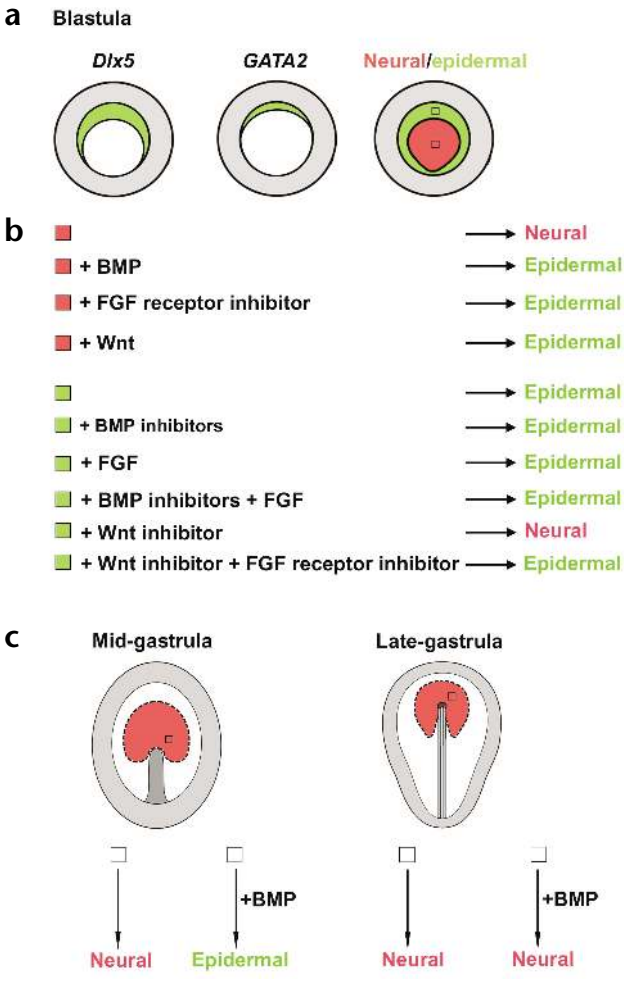
Like the amphibian organizer, the chick and mouse node/organizer can induce ectopic neural cells[19,20]. However, although such transplantation studies demonstrate that the node is sufficient to induce ectopic neural cells, they do not address whether the organizer region is required during the normal process of neural induction. The requirement for the node/organizer in neural induction has been addressed in mouse, by analyzing mutants that fail to generate a node/organizer or its derivatives. In the absence of a functional transcription factor HNF3β or the Arkadia protein, which acts upstream of HNF3β, mouse embryos fail to generate the node and node derivatives[4,5,7]. However, these embryos develop a neural plate with an initial rostrocaudal pattern, providing genetic evidence that the generation of neural cells in mouse embryos does not require a functional node or node derivatives[4,5,7]. Consistent with these results, a neural plate is formed when the gastrula organizer is surgically removed in chick, frog, zebrafish and mouse embryos[21,62–64]. Collectively, these findings indicate that the necessary neural inducing signals derive from tissues other or in addition to the node/organizer.

Neural induction is initiated before gastrulation

The lack of requirement of the node for the generation of neural cells in the mouse leaves open the possibility that the specification of neural cells is initiated before the formation of the node. The stage at which ectodermal cells become specified as neural cells has been addressed in chick[9,10]. At blastula stages, before the onset of gastrulation and formation of the node, chick embryos are flat and disc-shaped. However, already at blastula stages, the embryo is patterned along the mediolateral axis as revealed by the expression of the transcription factors *Dlx5* mRNA and *GATA2* mRNA in lateral but not in medial primitive ectodermal/epiblast cells (**Fig. 2a**)[65,66]. During gastrulation, *Dlx5* mRNA and *GATA2* mRNA are expressed in epidermal ectoderm and excluded from the neural plate[65,66]. Lateral epiblast cells isolated from blastula embryos generate epidermal cells, and medial epiblast cells generate neural cells when grown as explants *in vitro*[9], and using this experimental protocol, a specification map of the blastula chick epiblast has been established (**Fig. 2a**)[9]. This map shows that the medial part of the embryo constitutes a neurogenic region, whereas cells in the lateral region are specified as cells of epidermal character (**Fig. 2a**). The map closely resembles the early mediolateral patterning of the epiblast as depicted by the patterns of expression of *Dlx5* mRNA[65] and

GATA2 mRNA[66]. These findings are also consistent with the genetic evidence in mouse, which shows that the node/organizer is not required for neural induction. Thus, collectively these results provide evidence that the specification of neural cells in amniote embryos is independent of signals provided by the node and is initiated before the onset of gastrulation.

Previously, neural induction in chick was considered to occur during gastrulation, which is initiated when the primitive streak forms in the midline of the embryo. As discussed above, more recent results provide evidence that ectodermal cells become specified as either neural or epidermal cells before the onset of gastrulation. This implies that at this stage, the character of these cells is still flexible and can be changed if exposed to appropriate signals; during gastrulation, some of the medial cells may migrate to the primitive streak and be re-instructed (re-specified) to become either mesodermal or endodermal progenitor cells. Consistent with this idea, ectopic neural tissue is generated in mouse and zebrafish mutants that fail to induce mesoderm[67,68]. Chick embryos at defined stages of development are readily available, making it feasible to isolate prospective neural cells at different stages of development to monitor their response to signals that block neural differentiation and induce alternative fates. Using this approach, it has been shown that at the end of gastrulation, neural precursor cells no longer respond to signals that induce alternative fates, and have thus committed to neural differentiation (**Table 1**, **Fig. 1c**)[9,69,70].



BMP antagonists are not required for neural induction

The ability of BMP signals to block neural and promote epidermal fate in early embryonic cells is conserved among anamniote and amniote embryos[9,28]. In contrast, the sufficiency and requirement of BMP inhibitors to induce neural character have not so far gained support from studies in chick and mouse[4–9]. In mouse, genetic studies provide evidence that a neural plate is formed in embryos lacking functional Follistatin, Noggin and Chordin, or both Noggin and Chordin[6,71,72]. However, these studies do not exclude functional redundancy or yet undiscovered BMP inhibitors. Moreover, mouse embryonic stem (ES) cells acquire neural character when cultivated on stromal feeder cells, but BMP antagonists cannot substitute for the feeder cells. However, under these conditions, the acquisition of neural character can be prevented by BMP signals[73]. Together, these findings indicate that in amniote embryos, although BMP can promote an epidermal cell fate at the expense of a neural cell fate, BMP inhibition is not sufficient to induce a neural fate.

In chick, the node can induce neural tissue when transplanted to recipient embryos[16–19]. However, the temporal patterns of expression of *noggin*, *chordin* and *follistatin* mRNA do not coincide with the ability of the node to induce ectopic neural cells[8,19,74]. In addition, BMP inhibitors are unable to induce neural character in epidermal or extra-embryonic ectoderm of both or blastula and gastrula stage chick embryos[8,9,19,74,75]. Thus, in amniotes, BMP antagonists are neither sufficient nor required to induce neural cells and may instead maintain or stabilize neural character, indicating that signals distinct from BMP antagonists are required for induction of neural fate.

In chick, two studies have indicated that cells in the border region between the prospective neural and epidermal ectoderm are the only cells that acquire neural character in response to inhibition of BMP signaling[70,76]. Exposure of these cells to BMP inhibitors leads to a widening of the neural plate. In contrast, BMP inhibitors do not induce neural character in the prospective epidermal ectoderm or in non-embryonic ectoderm[8,11]. These results indicate that the cells at the border region are simultaneously exposed to signals that promote neural and epidermal character fate and that under these conditions BMP antagonists are sufficient to promote neural fate. This situation

Fig. 2. Neural induction in chick. (**a**) Patterns of expression of the epidermal markers *Dlx5* and *GATA2* in blastula-stage chick embryos (adapted from refs. 65 and 66). Specification map of the blastula stage chick embryos; the neurogenic (red) region is located in the medial part and the epidermal (green) region in the lateral part of the embryo[9]. The medial neurogenic region and the region expressing *Dlx5* are complementary. The positions of medial (M) and lateral (L) explants used (b) are indicated. (**b**) If medial explants are matured *in vitro*, they differentiate into neural tissue unless exposed to either BMP protein, FGF signaling antagonist or Wnt protein[9,11]. If lateral explants are matured *in vitro*, they differentiate into epidermal tissue even in the presence of BMP inhibitors, FGF or a combination of both[9,11]. However, if lateral explants are matured in the presence of a Wnt inhibitor, they differentiate instead into neural tissue. Under these conditions, ongoing FGF signaling is required. (**c**) Mid- and late-gastrula stage chick embryo; the prospective neural plate is shown in red and the primitive streak in the midline of the embryo in gray. Hensen's node (blue) is indicated at the top of the primitive streak of the late-gastrula embryo. Medial explants from both mid- and late-gastrula chick embryos differentiate into neural tissue when matured *in vitro*[9]. However, if these explants are exposed to BMP4 protein, medial explant from mid-gastrula embryos generates epidermal tissue, whereas medial explants from the late-gastrula embryos still generate neural tissue, indicating that prospective neural cells have committed to neural differentiation by the late-gastrula stage[9].

Table 2. Major signaling pathways involved in neural induction.

Signaling pathway	Intracellular effectors
BMP	SMAD[30]
	Msx[30]
FGF	MAPK[37]
	RAS[37]
Wnts	β-catenin[49]

may be mirrored by observations in zebrafish where genetic evidence has demonstrated that BMP signaling is involved in regulating the size of the neural plate. Mutations in the ortholog of the *chordin* gene (*chordinio*)[77] or in *bozozok*[78], which is required for the expression of *chordino* mRNA, generate mutant embryos with a neural plate reduced in size. Consistent with this, ectopic expression of *Bmp4* mRNA phenocopies the *chordino* mutant phenotype. Conversely, mutations in the *Bmp2b* gene (*swirl*)[79], *Bmp7* (*snailhouse*)[80] and *Smad5* (*somitabun*)[81], a mediator of BMP2b (swirl) activity, are associated with a neural plate that is expanded to different degrees, at the expense of neural crest and epidermal ectoderm. Collectively, these results are consistent with the idea that BMP antagonists are involved in maintaining neural fate.

A dual role for FGF signaling in neural induction

As in *Xenopus*, *Bmp* mRNA is expressed ubiquitously at low levels in the epiblast of chick blastula embryos[8,9]. During gastrulation, the expression of *Bmp* mRNA is extinguished in medial prospective neural cells but maintained in lateral prospective epidermal cells[8]. Thus, the exclusion of *Bmp* mRNA expression from the prospective neural plate seems to be a common theme among vertebrate embryos and may represent a common initial step in the specification of neural precursor cells. *Fgf3* mRNA is expressed in medial epiblast cells of blastula chick embryos; when the FGF signaling in medial epiblast cells is inhibited by FGF receptor antagonists, *Bmp* mRNA expression is maintained, neural fate is blocked, and cells acquire epidermal fate (**Fig. 2b**)[9]. Attenuation of FGF signaling also blocks the generation of cells of neural character in pregastrula stage chick whole embryo cultures[10]. Together, these studies indicate that in chick, intact FGF signaling is required for neural induction to proceed. BMP antagonists can restore neural fate in medial epiblast cells exposed to low but not to high concentrations of FGF receptor antagonists[11]. These findings indicate that FGF signaling promotes neural differentiation through the activation of two transduction pathways. The first is the repression of *Bmp* mRNA expression, which requires a high level of FGF signaling[9]. Therefore, blocking FGF signaling at a low level (resulting in lower FGF signaling) can be reversed by BMP inhibitors[9]. The second pathway is independent of repression of *Bmp* mRNA expression and requires a lower level of FGF signaling[9] (**Fig. 3**). Thus, these results provide evidence that FGF signals expressed by the prospective neural cells in chick are required for neural induction by repressing *Bmp* mRNA expression cells and by promoting neural fate (**Fig. 3**).

Wnt signaling regulates neural or epidermal fate

Fgf3 mRNA is also expressed in lateral epiblast cells of blastula-stage chick embryos, which maintain *Bmp* expression and acquire epidermal fate. In these cells, exogenous FGF signals are unable to induce neural character[9]. Collectively, these findings may indicate that lateral epiblast cells are exposed to signals that prevent them from responding to FGF signals[9]; recent results provide evidence that exposure to Wnt signals causes this effect[11].

Wnt3A mRNA and *Wnt8C* mRNA are expressed in lateral but not in medial epiblast cells of blastula chick embryos, and Wnts block neural and induce epidermal character in medial epiblast cells both *in vitro* and *in vivo* in chick embryos[11]. In the presence of Wnt signals, medial epiblast cells maintain *Bmp* mRNA expression; an identical situation is observed when these cells are exposed to FGF receptor antagonists[11]. Wnts also mimic the concentration-dependent action of inhibitors of FGF signaling. At low concentrations of Wnts, BMP antagonists restore neural fate, whereas at high concentrations of Wnts, medial epiblast cells maintain epidermal character in the presence of BMP antagonists. Moreover, Wnts block the ability of added FGF to promote neural fate in medial epiblast cells[11]. Collectively, these results suggest Wnts induce epidermal fate and repress neural fate by attenuating the response of epiblast cells to FGF signaling (**Fig. 3**). It remains to be determined how the how the initial medial–lateral distribution of Wnt activity is established.

Consistent with this idea, a truncated soluble fragment of the mouse Wnt receptor Frizzled 8 (mFrz8CRD-IgG), which antagonizes Wnt signaling[82], induces neural character and blocks epidermal character in prospective epidermal cells both *in vitro* and in intact chick embryos (**Fig. 2b**)[11]. Under these conditions, FGF signaling is required to repress *Bmp* mRNA expression and to induce neural fate, as BMP signals restore epidermal fate (**Fig. 2b**, **Fig. 3**). Thus, blockade of Wnt signaling in lateral epiblast cells initiates a program of neural differentiation that resembles the pathway normally followed by medial epiblast cells. Presumably, lateral epiblast cells are exposed to Wnts at concentrations sufficient to block both FGF signaling pathways, which provides an explanation for why BMP antagonists alone or in combination with FGF are insufficient to induce neural character in lateral epiblast cells. In support of this idea, a reduction in the level of Wnt signaling in later epiblast cells achieved by addition of a concentration of mFrz8CRD-IgG threefold lower than the threshold for neural induction permits both Noggin and FGF to induce neural fate[11].

Thus, these results provide evidence that the status of Wnt signaling regulates the selection of neural or epidermal fate in the chick blastula, proposing a model for how interactions between Wnt, FGF and BMP signals expressed in the embryo before the onset of gastrulation generate cells of neural and epidermal fate. The lack of exposure of medial epiblast cells to Wnts permits FGF signaling both to repress *Bmp* mRNA expression and to activate an independent pathway necessary for progression to neural fate (**Fig. 3**). High-level Wnt signaling in lateral epiblast cells inhibits both FGF transduction pathways and permits *Bmp* mRNA expression and BMP signaling to direct cells to an epidermal fate[11]. The challenge now is to resolve how Wnt activity becomes spatially restricted along the mediolateral axis of the epiblast in the absence of underlying mesoderm and endoderm and how Wnt activity blocks the ability of ectodermal cells to respond to FGF signals (**Fig. 3**).

Cells in the organizer or in organizer-derivatives express Wnt antagonists[52,83,84] in addition to FGFs[85] and BMP antagonists[24,26,27,86], which may provide an explanation for why these tissues but neither BMP antagonists nor FGF signals alone or in combination are sufficient to induce neural character in chick epidermal or extra-embryonic ectoderm[8–11].

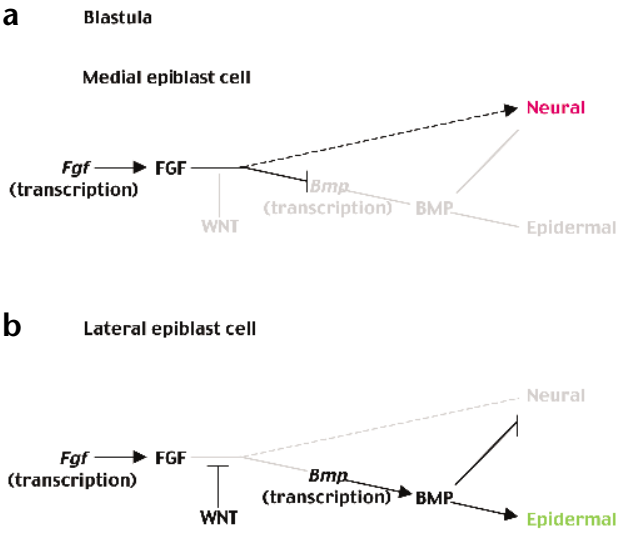


Fig. 3. Proposed signaling pathway for neural induction in the chick embryo. At the blastula stage, medial epiblast cells express FGFs but not Wnts. FGF signaling activates two distinct transduction pathways in epiblast cells: first, the repression of *Bmp* expression (solid line from FGF), and second, the promotion of a neural fate by a pathway independent of the repression of *Bmp* expression (dashed line from FGF)[11]. (**b**) Lateral epiblast cells express FGFs and Wnts. High levels of Wnt signals block the response of epiblast cells to FGFs. Thus, *Bmp*s are expressed, and BMP signals promote epidermal fate and repress neural fate. When Wnt signaling is attenuated, Wnts block the ability of FGFs to repress *Bmp* expression, but the independent pathway involved in promotion of neural fate is preserved. Under these conditions, BMP antagonists are able to induce neural fate[11].

Neural cells exhibit an initial rostral forebrain character

Based on the assumption that neural cells are induced at gastrula stages, several models have been proposed that involve separate head- and trunk-inducing signals derived from the organizer or organizer derivatives[87,88]. However, the finding that neural cells become specified before the onset of gastrulation and the formation of the organizer allows a reassessment of the mechanism of rostrocaudal patterning of neural plate cells.

In chick, medial epiblast cells from blastula embryos generate neural cells that express Sox2, Sox3, Otx2 and Pax6, a combination of neural markers characteristic of the forebrain. However, these cells do not express En1/2, Krox20 or *Hoxb8*, markers characteristic of cells in the midbrain, hindbrain and spinal cord[9] (S.W. and T.E., unpublished observations). These findings support the hypothesis that neural progenitor cells initially possess a rostral 'forebrain-like' character and that cells of caudal character (such as midbrain, hindbrain and spinal cord) are generated by reprogramming of rostral cells[89]. More recent molecular studies have supported this idea[69,90,91]. Neural cells induced by the blockade of Wnt signals in the lateral epiblast express the same combination of neural markers[11]. Thus, in chick, neural cells are initially specified as cells characteristic of the rostral forebrain. However, these prospective neural cells acquire midbrain, hindbrain and spinal cord character at gastrula stages in response to caudalizing signals derived in part from the paraxial mesoderm[92] before the node-derived axial mesoderm of the notochord starts to be generated. Consistent with this observation, the notochord lacks caudalizing activity[92–94]. Previously isolated 'head inducers' expressed by cells in the organizer or in organizer derivatives act as Wnt inhibitors[87]. However, whereas Wnt inhibitors can induce neural cells of forebrain character when misexpressed in early embryos, putative head inducers expressed in the organizer or in organizer-derivatives are likely to maintain rather than induce forebrain character.

The extra-embryonic anterior visceral endoderm (AVE) in mouse has been implicated in the induction of anterior neural fate[95]. Recent results, however, indicate that signals derived from the AVE, rather than inducing forebrain cells, protect prospective forebrain cells from caudalizing signals[88,96]. Thus, specification of neural cells in mouse may also be initiated before the primitive streak is formed and before the AVE is positioned adjacent to the prospective forebrain.

A conserved mechanism of neural induction?

This overview brings us back to the pivotal question raised in the beginning. Are the apparent differences in the time and in the mechanism of specification of neural cells in anamniote and amniote embryos genuine? At least two key aspects of the mechanism of specification of neural and epidermal cells are conserved in amphibian and amniote embryos. First, BMP signals block neural and promote epidermal fate. Second, *Bmp* mRNA expression is excluded from prospective neural cells and present in prospective epidermal cells. However, the time at which cells receive signals that initiate the exclusion of *Bmp* expression from prospective neural cells and the molecular nature of these signals seem to be different in amphibian and chick embryos.

At the blastula stage, the chick embryo is relatively large and already patterned along the mediolateral axis, which makes it feasible to identify and separately isolate medial and lateral explants that contain prospective neural and epidermal cells[9]. In addition, prospective neural and epidermal cells can be isolated from embryos at different stages of development and their response to extracellular signals can be monitored. Using blastula and gastrula stage explants, the results provide evidence that the specification of neural cells is initiated at the blastula stage and prospective neural cells are committed to neural differentiation by the late gastrula stage. These studies also show that in the absence of expression of Wnt signals, FGF signals expressed by medial prospective neural cells both repress *Bmp* mRNA expression and activate an independent pathway necessary for the progression to a neural fate (**Fig. 3**). Wnt signals prevent lateral epiblast cells from responding to FGF signaling, which in turn allows *Bmp* mRNA expression and causes cells to exhibit epidermal character. Thus, in the presence of a high level of Wnt signaling, neither BMP antagonists nor FGF signals alone or in combination are able to induce neural fate in prospective epidermal cells[11]. However, when Wnt signals are partially blocked in prospective epidermal cells, both BMP antagonists and FGF signals can induce neural character in these cells[11].

In *Xenopus*, the prevailing model in its simplest form suggests that BMP antagonists derived from cells in the organizer region inhibit a positive autoregularory loop of *Bmp* expression in prospective neural cells, and that the blockade of BMP signals is sufficient for cells to acquire neural fate (**Fig. 1b**). FGF and Wnt signaling are also implicated in the selection of neural and epidermal fate in *Xenopus*, although the precise roles remain to be determined[38,39,41,44,46,52,53]. However, as discussed above, recent evidence suggests that *Xenopus* blastula stage ectoderm is composed of cells that are specified as either neural or epidermal cells[56]. Moreover, neural induction in *Xenopus* can occur in the absence of mesoderm[55] and both Wnt and FGF signaling seem to be ongoing in the blastula ectoderm[39,51–53]. These

observations raise the possibility that in blastula-stage animal cap explants, low-level Wnt signaling attenuates FGF signaling, which allows *Bmp* mRNA expression, causing cells to acquire epidermal character. Under these conditions, both BMP antagonists and FGF signals would promote neural fate, which may mirror the condition when Wnt signaling is partially inhibited in chick prospective epidermal cells. Thus, neural induction in anamniotes and amniotes may be more similar than would seem at a first glance.

In *Drosophila*, neural cells are derived from a neurogenic region that is generated during the initial dorsoventral patterning of the embryonic ectoderm. Like in vertebrate embryos, the generation of neurogenic domain in *Drosophila* depends on the exclusion of Dpp, the fly homolog of BMPs, from prospective neural cells. Dpp is expressed in dorsal ectoderm, which differentiates into epidermis. The generation of the lateral neurogenic region in the fly embryo is initiated before the onset of gastrulation, depends on maternal effect genes, and requires signals derived from non-embryonic cells[97,98]. The mediolateral axis of the blastula stage chick epiblast defines the future dorsoventral axis of the ectoderm. Thus, the specification of the chick epiblast into a medial neurogenic and a lateral epidermal region before the onset of gastrulation has certain parallels with the generation of neurogenic and epidermal ectoderm in *Drosophila*. In contrast, the prevailing model of neural induction in amphibians suggests that the specification of neural cells is initiated at a much later stage in development in response to signals derived from the organizer. It seems unlikely, however, that amphibians have developed an independent mechanism of specification of neural cells that requires gastrulation and the formation of the organizer. Consistent with this idea, recent evidence suggests that in *Xenopus*, the dorsal ectoderm has already been exposed to signals that specify neural fate at the blastula stage[56] and that neural induction can occur in the absence of mesoderm[55]. Thus, the developmental stage at which exclusion of *Bmp* mRNA expression and specification of neural cells is initiated may be conserved between fly, amphibian and amniote embryos.

Acknowledgements

T.E. is supported by the Swedish Medical Research Council and by the Foundation for Strategic Research. We thank members of the Edlund lab for helpful discussions and we are grateful to T.M. Jessell for comments, suggestions and ideas.

Received 5 July; Accepted 27 August 2001

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