

VERTEBRATE NEURAL INDUCTION

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ABSTRACT

During early vertebrate development, the cells of the ectoderm choose between two possible fates: neural and epidermal. The process of neural induction was discovered nearly 70 years ago in vertebrates, and molecular analyses in recent years using *Xenopus laevis* embryos have identified several secreted factors with direct neural-inducing ability. There is considerable evidence that the mechanism of neuralization by these inducing factors is under inhibitory control and involves derepression. This review focuses on factors involved in the specification of neural fate within the frame of the default model of neural induction.

INTRODUCTION

This review focuses on the molecular, cellular, and embryological basis of neural induction in vertebrates. The process of generating a mature neuron can be divided into two distinct phases, which take place during embryonic development: the specification of the neuroectoderm, which occurs at the onset of gastrulation, and the specification of neural precursors, which takes place during neurula stages and after. Here we focus on the recent advances made in our understanding of the earliest phase of neural induction, the specification of the neuroectoderm. We emphasize both the signaling molecules involved in this process and a default model of neuralization.

In all vertebrates, the development of the nervous system is directly linked to the establishment of the dorso-ventral axis. In amphibians, the dorsal axis is defined during the first cell cycle by cytoplasmic movement known as cortical

rotation. This rotation breaks the radial symmetry imposed maternally in the egg and is believed to mix and activate factors in the cytoplasm that will trigger the dorsal determination (Hausen & Riebesell 1991). During subsequent cleavage stages, cells located at the top of the embryo, or animal pole, are specified as ectoderm, and the cells at the bottom, or vegetal pole, are specified as endoderm. When the embryo contains only 16 or 32 cells, the specification of the third germ layer, the mesoderm, begins in the equatorial region, or marginal zone (Kessler & Melton 1994). Because the dorso-ventral polarity is established during the first cell cycle, blastomeres of each germ layer at this stage also have a dorso-ventral identity, though it is not irreversibly fixed. About 9 h after fertilization, gastrulation begins. The cells of the dorsal marginal zone involute at the blastopore lip on the dorsal side. Soon after, the ectodermal cells make a choice between two possible fates: neural on the dorsal side and epidermal on the ventral side of the embryo (Keller 1991). Thus the origin of the nervous system can be traced to ectodermal cells located on the prospective dorsal side of the embryo at gastrula stages.

LESSONS FROM CLASSICAL EXPERIMENTAL EMBRYOLOGY

In 1924, Spemann & Mangold established the concept of neural induction in their studies of amphibian embryos (Spemann & Mangold 1924). They found that transplantation of a dorsal blastopore lip of a salamander early gastrula into the ventral side of another early gastrula embryo causes the formation of a complete second nervous system. The second nervous system develops, not from the transplanted tissue, but from ventral ectoderm, which in an undisturbed embryo forms epidermis (Spemann 1938). Spemann named the dorsal blastopore lip the “organizer” and proposed that in normal development this region of the embryo induces and organizes the nervous system in the neighboring dorsal ectoderm (Spemann & Mangold 1924). In the absence of this influence, as on the ventral side or in explants made before gastrulation, the ectoderm differentiates as epidermis. Thus, development as epidermis was assumed to be a fall-back, or “default,” fate for the gastrula ectoderm, requiring no cell-cell communication, whereas neural specification was contingent upon the receipt of signals from neighboring cells (Slack 1991, Gilbert 1994). Subsequent experiments showing that the organizer can also induce neural structures when recombined *in vitro* with competent ectoderm provided further support for the idea that the dorsal lip is the source of molecules responsible for neural induction (Smith & Slack 1983). Furthermore, the embryological equivalents of the blastopore lip in other vertebrates, for example Hensen’s node in birds, can induce neural tissue *in vivo* and *in vitro*, suggesting that the mechanism of neural induction is conserved among vertebrates (Waddington & Schmidt

1933). Thus the general conclusions from these embryological experiments were (a) that dorsal mesoderm induces neural tissue in the overlying ectoderm, (b) that the dorsal mesoderm emits an instructive positive signal and that in the absence of this signal, the ectoderm will only make epidermis, and (c) since the ectoderm differentiates as epidermis in the absence of these signals, that epidermis was the default state of the ectoderm. Despite the fact that numerous experimental manipulations and grafts were consistent with this model, a considerable effort over several decades failed to identify the chemical substances responsible for neural induction in the embryo.

The conclusion that neural induction requires instructive signal(s) from mesoderm, and thus necessarily follows mesoderm induction, was difficult to reconcile with cell dissociation experiments performed by Grunz & Tacke (1989), Sato & Sargent (1989), and Godsave & Slack (1989). These authors demonstrated that when animal cap cells (Godsave & Slack 1989, Grunz & Tacke 1989) or whole embryos (Sato & Sargent 1989) are dissociated and then either maintained as dissociated cells or reaggregated after a few hours, they can form neural tissue. In these experiments mesodermal tissues are absent. The observed cell-autonomous neuralization was interpreted in different ways. Whereas Grunz & Tacke had no specific model to explain their findings (Grunz & Tacke 1989), Sato & Sargent postulated the existence of unidentified maternal neural determinants that do require mesoderm or mesodermal induction (Sato & Sargent 1989). Godsave & Slack proposed the possibility of an inhibitor of neural induction in the intact presumptive ectoderm (Godsave & Slack 1989). However, some published data were not immediately consistent with the finding that cell dissociation allowed an epidermal to neural transition (Jones & Woodland 1986, Symes et al 1988). Regardless of the interpretation of their results, these three groups (Grunz & Tacke 1989, Sato & Sargent 1989, Godsave & Slack 1989) provided the first hints that neural induction in vertebrates could occur in the absence of instructive or positive signal(s) from dorsal mesoderm, the tissue thought to be responsible for inducing the nervous system in vivo. Looking back, these experiments (Godsave & Slack 1989, Grunz & Tacke 1989, Sato & Sargent 1989) provided the first clues suggesting the existence of a negative or inhibitory signal within the ectoderm that prevents neuralization and drives cells toward an epidermal fate.

MOLECULAR APPROACHES AND THE NOTION OF THE DEFAULT STATE

The Dominant Negative Type II Activin Receptor

Spemann & Mangold's (1924) observation that the organizer could induce neural tissue focused considerable attention on the isolation of neural-inducing

molecules. Despite much effort, the attempt to find neural inducers using biochemical purification has still not been successful. Our own studies on this problem came about indirectly and accidentally as the result of our studies on the roles of various growth factors in mesodermal induction. We were interested in testing whether activins, members of the transforming growth factor (TGF)- β family of growth factors, are required for mesoderm induction. To this end, we generated a mutant form of the type II activin receptor (Δ IXAR1) that inhibited, in a dominant fashion, the function of the endogenous receptor. When mRNA encoding this mutant protein was injected into embryos, the formation of mesoderm was blocked, demonstrating that signaling through this receptor is required for mesoderm induction in vivo (Hemmati-Brivanlou & Melton 1992). Subsequent analyses have shown that this dominant negative receptor blocks signaling by several members of the TGF- β family, namely Vg1 and bone morphogenetic proteins (BMPs), in addition to activin (Schulte-Merker et al 1994, Hemmati-Brivanlou & Thomsen 1995, Kessler & Melton 1995; G Kelly, submitted).

Careful analysis of the expression of several tissue-specific markers following injection of the truncated activin receptor (Δ IXAR1) yielded surprising observations. First, we reported that the neural cell adhesion molecule (NCAM), a general neural marker, was turned on in ectodermal explants following inhibition of activin receptor signaling (Hemmati-Brivanlou & Melton 1992, 1994). These explants express the activins and their receptors (Thomsen et al 1990, Hemmati-Brivanlou et al 1992, Mathews et al 1992) and, as mentioned above, made epidermis when cultured alone. Second, we found that the dominant negative activin receptor could also neuralize cells located at the bottom of the embryo, or vegetal pole, cells normally fated to become endoderm (Hemmati-Brivanlou & Melton 1994). This result suggested that neuralization, by inhibition of the type II receptor signaling, is not only confined to cells of the ectoderm but can be generalized to other germ layers.

Because this neuralizing activity of the truncated activin receptor was direct, involved inhibition of cell signaling, and occurred in blastomeres of different germ layers, we drew a comparison between our observations and those made in dissociated ectodermal explants, and proposed a "default model of neuralization" for vertebrate embryos (Hemmati-Brivanlou & Melton 1994). One attraction of this model is that it reconciles the apparent contradiction between the classical embryological work and the cell dissociation and Δ IXAR1-induced neuralization.

The Default Model of Vertebrate Neuralization

As mentioned above, early work established that gastrula ectoderm of amphibian and other vertebrate embryos gives rise to the neural plate in response

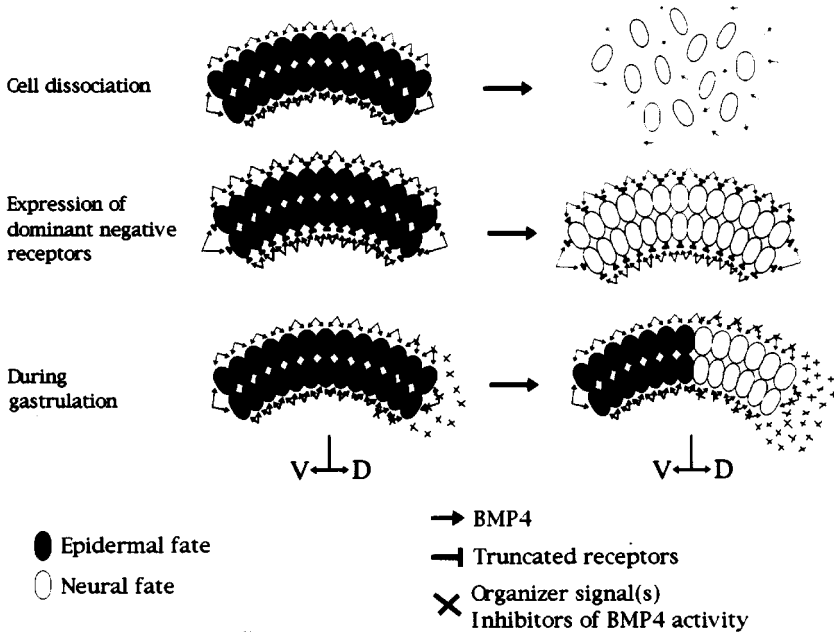


Figure 1 Schematic of the “default model” of vertebrate neuralization. (*Top*) Neuralization by cell dissociation. In the gastrula embryo, BMP4 signaling within the intact ectoderm maintains the epidermal fate (*black cells*) and inhibits the neural fate (*white cells*). Upon dissociation, the soluble inhibitor (BMP4) can no longer signal the cells to maintain their epidermal fate. Thus the default fate of the ectodermal cells, in the complete absence of signaling, is neural. (*Middle*) Neuralization by the dominant negative mutant form of either the type II activin receptor ($\Delta 1XAR1$) or the dominant negative BMP receptor (tBR). Expression of $\Delta 1XAR1$ or tBR in the black epidermal cells inhibits BMP4 signaling; as a result, the epidermal fate can no longer be maintained and the cells become neural by default. (*Bottom*) Establishment of neural fate in the dorsal ectoderm during gastrulation. A secreted inhibitory signal derived from the organizer (dorsal mesoderm) inhibits BMP4 signaling specifically in the dorsal ectoderm and thus unveils the neural fate.

to signals from adjacent dorsal mesoderm (Spemann’s organizer). In the absence of this influence, the ectoderm differentiated as epidermis; such epidermal development was generally assumed to be a default fate that required no cell-cell communication. Based on the experiments described above, however, we proposed that it is the neural and not the epidermal fate that represents the default state of ectodermal cells and that signaling within the ectoderm inhibits neural specification (Hemmati-Brivanlou & Melton 1994). Because cell dissociation and expression of the dominant negative activin receptor in intact ectodermal explants can be interpreted as an interference with

the communication between cells, we suggested that cells of the early gastrula animal cap are predisposed to form neural tissue in the absence of further signals. In this model epidermal specification, and thus the inhibition of neural fate, would result from cell-cell communication within the prospective ectoderm. When this signaling is interrupted, by dispersing the cells or by molecular antagonists, neural tissue forms (Hemmati-Brivanlou et al 1994, Hemmati-Brivanlou & Melton 1994). Neural induction by the organizer *in vivo* would work in the same way, that is, by blocking epidermalizing signals within the animal cap. This view contradicts the commonly held view (Gilbert 1994, Slack 1991) that neural induction requires an instructive or positive signal (Figure 1).

The default model of neural specification makes several predictions. First, the signal from the organizer is an antagonistic secreted signal that inhibits the activity of another inhibitor, the neural inhibitor, specifically in dorsal ectoderm during gastrulation. Second, this factor should be present in the organizer at the onset of gastrulation when neural fate is specified. Third, the hypothesis that epidermal rather than neural specification requires cell-cell communication, in this case local signaling among ectodermal cells, predicts that epidermal fate can be induced in ectodermal cells (Hemmati-Brivanlou & Melton 1994).

ADDITIONAL EVIDENCE IN FAVOR OF THE DEFAULT MODEL OF NEUROGENESIS

As mentioned above, Δ IXAR1 can also block signaling by all TGF- β s tested so far, including mature Vg1 and BMPs (Schulte-Merker et al 1994, Hemmati-Brivanlou & Thomsen 1995, Kessler & Melton 1995). Although the antagonistic range is broad, the mutant receptor apparently directly inhibits only TGF- β signaling and not fibroblast growth factor (FGF) signaling, for example (Hemmati-Brivanlou & Melton 1992). Nevertheless, the observations made with Δ IXAR1 drew our attention to TGF- β ligands that can be inhibited by this mutant receptor as potential neural inhibitors.

Follistatin

The discovery that inhibition of the type II activin receptor signaling led to formation of neural tissue hinted that activin might be the neural inhibitor and epidermal inducer predicted by the default model. This argument was bolstered by the fact that activin can act as a neural inhibitor in murine embryonic carcinoma cells (Hashimoto et al 1990, van den Eijnden-van Raaij et al 1991), where it inhibits the retinoic acid-induced differentiation of P19 cells to neurons and glial cells. In addition, Hashimoto et al (1990) have shown that activin inhibits the differentiation of various neuroblastoma cell lines.

Two activin inhibitors, follistatin and inhibin (Sporn et al 1986), have been identified by endocrinological studies of activin in mammals. We were thus prompted to examine the embryonic distribution and activities of these antagonists to further test the role of activin in early embryos. We were unable to isolate inhibin from embryonic *Xenopus laevis* cDNA libraries, but we were successful in isolating a *X. laevis* follistatin clone (Hemmati-Brivanlou et al 1994). We showed that ectopic expression of follistatin in embryonic ectodermal explants could indeed turn on neural markers. Since both the truncated activin receptor and follistatin could activate expression of neural markers, activin seemed a likely candidate for the factor that mediated epidermal specification. The further discovery that follistatin was expressed in the organizer region in *X. laevis*, from where it could act to block activin signaling in the dorsal ectoderm and thereby permit neural tissue to form, indicated that follistatin was a good candidate for an endogenous neural inducer (Hemmati-Brivanlou et al 1994) and that activin could be the natural neural inhibitor (Hemmati-Brivanlou et al 1994).

Some evidence suggests that follistatin act as a neural inducer in other vertebrates. For example, follistatin was shown to act as a cytokine involved in neural differentiation in P19 cells (Hashimoto et al 1992). In the chick embryo, follistatin is expressed in the Hensen's node (Connolly et al 1995) and subsequently in the region described as the neuralizing area (Connolly et al 1995, Spratt 1952). Hensen's node can induce *X. laevis* ectodermal explants directly (Kintner & Dodd 1991).

Although the experiments with Δ IXAR1 and follistatin point to activin as the neural inhibitor and although follistatin is still considered an activin-specific inhibitor (Nakamura et al 1990), follistatin has not been rigorously checked against all TGF- β ligands, including newly identified members of the family. In fact preliminary evidence shows that follistatin can interfere with the mesoderm-inducing activity of BMP4 (OG Kelly, D Melton & A Hemmati-Brivanlou, unpublished data). Thus, like the truncated activin receptor, follistatin may be a broad antagonist of more than just activin signaling. Nevertheless, follistatin is a secreted factor, a TGF- β antagonist, that can neuralize ectodermal explants and that is expressed at the right time and place in the embryo to be an endogenous neuralizing factor. These observations are all consistent with the default model of neural formation.

*BMP4 and Activin Both Inhibit Neural Fate,
but Only BMP4 Induces Epidermis*

The neural-inducing activity of follistatin, in addition to the activity of the truncated activin receptor, pointed to activin as the endogenous neural inhibitor. Nonetheless, these data provide no direct evidence that activin could specify or

induce epidermis, a third prediction of the default model for neural formation. To address this prediction, a complementation assay was used in which cells of the animal cap were dissociated and incubated in the presence or absence of activin, mature Vg1, and BMP4—which are all TGF- β ligands inhibited by the truncated receptor. Although both activin and mature Vg1 did inhibit neuralization of dissociated ectodermal cells by inducing mesoderm, neither of them induced expression of epidermal markers (Wilson & Hemmati-Brivanlou 1995; PA Wilson & A Hemmati-Brivanlou, unpublished data). Instead, the dissociated cells were shifted from a neural to a mesodermal fate with no specification of epidermal fate. In contrast, BMP4 not only inhibited neuralization but also induced epidermal fate. The two activities of BMP4, neural suppression and epidermal induction, always occur together, leading us to conclude that they represent a single action, as expected from the neural default model of ectodermal specification. Induction of epidermis is inhibited if the dissociated cells express the truncated activin receptor. However, addition of follistatin protein did not prevent BMP4 from inducing epidermis (Wilson & Hemmati-Brivanlou 1995). This result presented a paradox because injection of follistatin mRNA does block the ability of BMP4 to induce ventral mesoderm in animal caps. The endogenous epidermal inducer may not be BMP4, but a related molecule that follistatin antagonizes. Alternatively, since follistatin has only been shown to act when provided early (following mRNA injection in cleavage stages), its effects may reveal an earlier activin-requiring process necessary for later epidermal induction. Recent studies with a variety of BMP4 antagonists favor this latter interpretation (see below).

BMP4 is expressed at the appropriate time and place to be the endogenous neural inhibitor-epidermal inducer. In situ hybridization shows that BMP4 RNA is present in the entire animal cap at the start of gastrulation, as well as in ventral and lateral marginal zone. At later stages, transcripts disappear from the portion of the ectoderm that becomes the neural plate (Hemmati-Brivanlou & Thomsen 1995), suggesting that repression of BMP4 transcription is one of the mechanisms by which BMP4 activity can be inhibited in the prospective neuroectoderm (Fainsod et al 1994, Hemmati-Brivanlou & Thomsen 1995, Schmidt et al 1995). A receptor for BMP4, BMPR, is also expressed in the animal cap (Graff et al 1994, Suzuki et al 1994). Thus the pattern of BMP4 transcription is consistent with a function in the induction of epidermis and the suppression of neural development.

These findings were significant for three main reasons. First, they demonstrated for the first time that epidermis is a fate that can be induced and therefore does not represent the default state of the ectoderm. Second, they demonstrated that a single factor (BMP4) can act as both neural inhibitor and epidermal inducer, the first described in vertebrates. Finally, and perhaps most importantly,

these results provided further evidence that neural specification is under inhibitory control in vertebrates (Wilson & Hemmati-Brivanlou 1995).

Dominant Negative BMP4 Receptors and Ligands

Several groups have reported results supporting the default model of neuralization in *X. laevis* embryos and pointing to BMP4 as an endogenous inhibitor of neural formation. A truncated type I BMP4/2 receptor, tBR, can directly induce neural tissue in intact animal cap explants, as did the truncated activin receptor (Xu et al 1995). However, whereas the truncated activin receptor blocks all TGF- β s tested so far, tBR seems to be more specific in that it cannot inhibit activin or mature Vg1 (Graff et al 1994, Suzuki et al 1994). Dominant negative forms of ligands such as BMP4 and BMP7, but not activin, directly induce neural tissue in ectodermal explants (Hawley et al 1995). The fact that BMP7 dominant negative ligand can also induce neural markers suggests either that other BMPs can fulfill the same neural inhibition activity or that dominant negative BMP ligands have a pleiotropic inhibitory effect on all BMPs. An interesting question is whether or not BMP7 can induce epidermis. Antisense BMP4 RNA, which may block the endogenous activity of BMP4, can also neuralize animal caps (Sasai et al 1995). However, this evidence should be considered carefully, since antisense approaches to inhibiting biological activity in *X. laevis* embryos are controversial. Taken together, these data provide support for both the default model hypothesis of neuralization and the involvement of BMPs in this process. Nonetheless, none of these dominant negative or antisense reagents exist *in vivo*, and as mentioned above, follistatin cannot inhibit the epidermal-inducing ability of BMP4. Thus, there must be a BMP4 inhibitor that is secreted and produced in the organizer.

Chordin

Chordin was originally isolated in a differential screen for genes that are induced by gooseoid and Xnot (Sasai et al 1994). Chordin is a secreted factor localized in the organizer of the early *X. laevis* gastrula. It was later demonstrated that chordin is a vertebrate homologue of the *Drosophila melanogaster* gene *short gastrulation (sog)* (Francois & Bier 1995) and that chordin had direct neural-inducing ability. Sasai et al (1995) suggested that the neuralizing activity of chordin is mediated through the inhibition of BMP4 activity. This hypothesis parallels the genetic evidence demonstrating that *sog* inhibits the activity of the *D. melanogaster* TGF- β protein decapentaplegic (*dpp*) (Francois et al 1994). There is, however, no biochemical evidence to determine whether this inhibition is direct. Nevertheless, neural induction by chordin through an inhibition of the neural inhibitor BMP4 is in agreement with the default model of vertebrate neuralization.

Noggin

Noggin is another secreted factor localized in the Spemann organizer (Smith & Harland 1992). It was originally isolated from a screen aiming to clone genes that can rescue a ventralized phenotype. Smith et al (1993) later demonstrated that noggin, just like follistatin or chordin, has the two activities of the organizer defined by experimental embryologists: the capacity to dorsalize mesoderm and to induce neural tissue directly in embryonic ectoderm. Although both follistatin and chordin induce neural tissue by antagonizing TGF- β s, the mechanism of neural induction by noggin is mostly unknown. Recent evidence suggests that the noggin protein can directly interact with BMP4 protein and interferes with BMP4 receptor binding (R Harland, personal communication). If these results are confirmed, the noggin pathway of neuralization will be directly linked to the BMP4 pathway of ectodermal induction.

Mediators of Neuralization

The default model of vertebrate neural development implies the existence of transcriptional repressors of neural genes in all embryonic cells except the dorsal ectoderm during gastrulation and/or the presence of downstream activators of transcription of neural specific gene in the cells of the neuroectoderm. This model implies that signals will have to be transduced from the membrane to the nucleus in order to relieve the repressed state of neurogenic genes. Although not much is known about the signal transduction pathway involved in neuralization, except that a component of the protein kinase C pathway might be involved (Otte & Moon 1992), candidate transcription factors have been characterized recently in *X. laevis* and higher vertebrates. Dominant negative forms of the *X. laevis* transcription factors, brachyury (Xbra) and lim-1 (Xlim-1), induce neural tissue directly (Rao 1994, Taira et al 1994). The simplest interpretation of these data is that the wild-type Xbra and Xlim proteins (or proteins binding to the same DNA sequence) act as repressors of neuralization in the ectoderm and that their derepression by the dominant negative mutants allows neural specification to occur. Witta et al (1995) reported that the wild-type *Xlpou 2* gene, a noggin inducible gene, is a direct neural inducer and is expressed in the organizer. All these transcription factors, however, might represent neurogenic genes acting later than the original specification events that unfold in the ectoderm during gastrulation.

FGFs AND FRL1

Several recent studies have suggested that FGFs are involved in neural induction and anterior-posterior patterning of the neural tube. bFGF can induce neural tissue and neural crest directly when incubated with gastrula ectoderm

(Kengaku & Okamoto 1993). Lamb & Harland (1995) have also demonstrated that when animal cap cells are dissociated for short periods of time, incubated in the presence of bFGF, and then reaggregated, they will produce neural tissue. Although FGFs and their receptors are expressed in the embryo during ectodermal patterning (Kimelman et al 1988, Musci et al 1990, Tannahill et al 1992, Song & Slack 1994), these results need to be interpreted with care because of evidence against this view: Interfering with the FGF signaling by using a dominant negative FGF receptor, does severely affect the development of posterior structures, including spinal chord, but does not eliminate neural tissue in *X. laevis* embryos, and the brain is relatively unaffected (Amaya et al 1991).

More recently, two additional ligands with no apparent homology to FGFs, called FGF-related ligands 1 and 2 (or FRL-1 and FRL-2), have been characterized in *X. laevis* embryos. When 1 ng of RNA from one of these ligands, FRL-1, was injected into the animal caps, NCAM was induced in the apparent absence of muscle actin, suggesting that FRL-1 might act as a neural inducer (Kinoshita et al 1995). More experiments are required to establish if this neural induction is direct and if it involves an antagonist of BMP4 signaling.

THE DEFAULT MODEL OF NEURALIZATION IN AMNIOTES

Neuralization in the Chick

In chick embryos, the secreted protein hepatocyte growth factor-scatter factor (HGF-SF) has been shown to induce the expression of a neural competence marker (L5) in extra embryonic epiblast cells, which phenotypically differentiate into cells with neuronal morphology expressing neural markers (Stern & Ireland 1993, Streit et al 1995). It is unclear if this induction is direct, and researchers have suggested that HGF-SF is more likely to be involved in the competence to respond to an inducing signal than to be involved in the induction proper. In agreement with an involvement in neural specification, HGF-SF is expressed in the Hensen's node at the primitive streak stage (Streit et al 1995). There is no evidence as of yet that this factor has any antagonist activity vis à vis TGF- β ligands or receptors. Future studies will determine if this factor fits with the default model of neuralization.

Neuralization in Mammals

Most of the knowledge accumulated so far on vertebrate neural induction comes from the study of amphibian embryos. However, several observations made in chick and mouse embryos and in embryonic cell lines are in agreement with the default model conclusions drawn from the study of the amphibian embryo.

The mouse embryonic carcinoma cell line P19 has been used to study neural differentiation. These cells have many characteristics of early mouse epiblast and are capable of differentiating into neurons in response to retinoic acid (RA). Addition of 10^{-7} M RA neuralizes about 15% of the cells in culture (Jones-Villeneuve et al 1982, McBurney et al 1988). Because mesodermal markers such as Xbra are expressed in undifferentiated P19 cells (P Hoodless & A Hemmati-Brivanlou, submitted), it is difficult to establish if this induction is direct. Nevertheless, when these cells are transfected stably with a tagged version of the *X. laevis* dominant negative activin receptor (Δ IXAR1), they differentiate into neurons. Double fluorescence experiments have established that it is the cells that express the mutant receptor that become neural. The number of neurons induced as well as the timing of neuralization is comparable with induction with RA (P Hoodless & A Hemmati-Brivanlou, submitted). In addition, Hoodless & Hemmati-Brivanlou (P Hoodless & A Hemmati-Brivanlou, submitted) have shown that BMP4 can inhibit retinoic acid-induced neuronal differentiation of P19 cells and can induce keratin expression, suggesting that BMP4 can affect epithelial differentiation in these cells. These findings support the idea that neural differentiation in mammalian embryonic cells is also under inhibitory control and requires the inhibition of BMP4. Consistent with this hypothesis, homozygous *BMP4* mutant mice die at gastrulation, at the time when germ layer specification occurs (Winnier et al 1995). This result points to the importance of BMP4 signaling in early mammalian development; however, the relationship between BMP4 knockout and embryonic death needs to be established.

As was the case with the mediators of neuralization in *X. laevis*, specific repressors of neural fate have been reported in the mouse. The default model, if operating in mammals, would also predict that these neural gene repressors should be expressed in all embryonic cells and then selectively inhibited as neuralization proceeds in the ectoderm. One such factor, called either RE1-silencing transcription factor (REST) or neuron-restrictive silencer factor (NRSF), has been recently characterized in mammalian PC12 cells (Chong et al 1995, Schoenherr & Anderson 1995). This transcription factor has been shown to be a negative regulator of the neuronal phenotype; its transcript is detected in neuronal progenitor but not in differentiating neurons, and thus has been proposed to be a master negative regulator of neurogenesis. In agreement with this suggestion, a dominant negative form of REST induces the expression of neural-specific genes in nonneuronal cells (Chong et al 1995). The determination of the timing of action of these factors in the embryo will allow an assignment of the role in the early specification or the late differentiation events.

COMPETENCE OF THE ECTODERM AND THE DEFAULT MODEL

Competence refers to a cell's ability to respond to an inductive signal. In *X. laevis*, there is evidence that the ectoderm is not specified to form neural tissue until the onset of gastrulation (Servetnick & Grainger 1991). The responsiveness of the ectoderm to neuralizing signals emanating from the organizer is lost by the end of gastrulation. With the proposal of a default model for neuralization, the molecular biology of a cell's competence to respond to neuralizing or epidermal inducing signals can be reexamined.

Because the period of competence is determined by the responding cells, factors influencing competence can include receptors, members of the signal transduction pathway, and/or transcription factors. Control of competence at the receptor level is unlikely for several reasons. First, most type I and type II receptors characterized thus far for the TGF- β family interact with more than one ligand (Letsou et al 1995; Yamaji et al 1994, 1995). Thus the control of competence to respond to a given signal by receptor inhibition would have pleiotropic consequences. Second, TGF- β receptors are expressed in the neuroectoderm after the end of the period of competence to neural induction (Graff et al 1994, Hemmati-Brivanlou et al 1992, Suzuki et al 1994; C Chang, P Wilson, L Mathews & A Hemmati-Brivanlou, submitted). Although there is no direct evidence that these receptors are functional, at all stages, their presence suggests that regulation must occur downstream. Sekelsky et al (1995) have characterized some members of the signal transduction pathway downstream from TGF- β molecules. These proteins, related to the *D. melanogaster* "mothers against dpp," or *Mad*, gene have been called DOTs, for downstream of TGF- β . The cytoplasmic proteins also have different functions, and one, DOT1 (or XMad1), appears to relay signals for the BMP set of ligands, whereas another, DOT2, relays signals for Vg1, activin, and nodal. Notably, DOT1 inhibits neuralization by tBR and reestablishes epidermal fate when the two RNAs are coexpressed in the ectoderm (GH Thomsen, personal communication; JM Graff, A Bansal & D Melton, submitted). Further dissection of the molecular pathway of ectodermal specification and the roles of the DOT proteins should broaden our understanding of embryonic competence.

ANALOGIES WITH *D. MELANOGASTER*

Molecular and genetic studies in *D. melanogaster* have characterized at least seven genes involved in the early patterning of the ectoderm (Ferguson & Anderson 1991). Among the seven are the *decapentaplegic* (*dpp*) and *short*

gastrulation (sog) genes. *dpp* is a member of a TGF- β family of growth factors, and *BMP4* is one of its closest vertebrate homologues. The homology between these factors extends to both their embryonic function and localization. Holley et al (1995) have shown that, just like *BMP4*, *dpp* can promote ventral fate in *X. laevis* and that *BMP4* can substitute for *dpp* function in *D. melanogaster*. As mentioned above, chordin is the vertebrate homologue of *sog*, and as it was the case with *BMP4* and *dpp*, these two genes can substitute for each other's activity both in *D. melanogaster* and *X. laevis* (Holley et al 1995). In addition to this apparent conservation of function, and despite the fact that the nervous system in *D. melanogaster* forms in the ventral side and in *X. laevis* in the dorsal side, the localization of this factor seems to be conserved also. In both *D. melanogaster* and *X. laevis*, by the late blastula-early gastrula stages, *sog/chordin* is expressed in the side of the embryos where the CNS forms (Francois et al 1994, Sasai et al 1994) and *dpp/BMP4* is expressed in the opposite site. There is evidence that *sog* antagonizes the activity of *dpp* both in *D. melanogaster* and in a heterologous system such as the frog. Chordin antagonizes the effect of *BMP4* in *X. laevis* and can substitute for *sog* activity in *D. melanogaster* (Holley et al 1995). The biochemical mechanism of this antagonism is unclear. *sog/chd* could antagonize *dpp/BMP4* either by direct contact or indirectly via a parallel pathway. Nevertheless, the fact that these factors play similar roles in the specification of embryonic cells in embryos that are so morphologically different suggests that the strategy of specification of the ectoderm has been conserved from arthropods to chordates. Later during *D. melanogaster* development, after *sog* and *dpp* have defined the neurogenic region within the ectoderm, other genes such as *Notch* and *Delta* establish the neurablast fate. Interestingly, the cellular mechanism underlying this later cell fate determination is also under inhibitory control and involves "lateral inhibition." The other genes involved in the *Drosophila* ectodermal patterning include *dorsal rel*-related, *zen*, *tolloid*, and *twisted gastrulation (tsg)* (Ferguson & Anderson 1991). Examination of the embryological functions of the vertebrate homologue of the *D. melanogaster* factors located in the *sog/dpp* pathway will assess to what extent dorsal ventral patterning of the ectoderm has been conserved.

CONCLUSIONS

The recent molecular dissection of inductive embryonic pathways has not only allowed the characterization of factors involved in cell-cell interaction, but has also provided insightful suggestions about the mechanism of action of these factors. Thus, more than 70 years after the discovery of neural induction in vertebrates, ectodermal induction and patterning can be studied at a resolution that

was not previously possible. Recently, the default model of neural specification has emerged from the study of the amphibian embryo and has provided evidence that epidermis is an inducible fate within the ectoderm (Hemmati-Brivanlou & Melton 1994). As is often the case, this change in view raises as many questions as it answers. At the embryological level, it is important to raise two issues. First, why is the specification of neural fate in a repressed state in all cells of the amphibian blastula? What kind of developmental or evolutionary advantage does that strategy of neural specification represent? Second, how common is this strategy among higher vertebrates? Evidence presented above suggests that at least in the context of murine embryonic carcinoma cell lines, the factors, isolated from frogs, can regulate ectodermal fate decisions (P Hoodless & A Hemmati-Brivanlou, submitted). It remains to be seen if this is the case for the mouse embryo and if it can be extended to include all amniotes. At the cellular level, crucial questions await an answer: How is the number of cells involved regulated, and how are the boundaries between epidermal and neural established? Finally, at the molecular level, characterizing a signal transduction pathway connected to the nuclear response will be challenging from the perspective of the default model. It is also imperative to characterize the molecular nature of embryonic competence, which still remains a mystery. If done comparatively to include both the epidermal induction pathway and the neuralization pathway, these approaches will inevitably shed light on the molecular nature of developmental strategies.

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