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Ectodermal Patterning in Vertebrate Embryos

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Recent molecular insights on how the ectodermal layer is patterned in vertebrates are reviewed. Studies on the induction of the central nervous system (CNS) by Spemann's Organizer led to the isolation of noggin and chordin. These secretory proteins function by binding to, and inhibiting, ventral BMPs, in particular BMP-4. Neural induction can be considered as the dorsalization of ectoderm, in which low levels of BMP-signaling result in CNS formation. At high levels of BMP signaling the ectoderm adopts a ventral fate and skin is formed. In *Xenopus* the forming neural plate already has extensive dorsal-ventral (D-V) patterning, and neural induction and D-V patterning may share common molecular mechanisms. At later stages sonic hedgehog (shh) plays a principal role in D-V patterning, particularly in the neural tube of the amniote embryo. A great many transcription factor markers are available and mouse knockouts provide evidence of their involvement in the regional specification of the neural tube. Recent evidence indicating that differentiation of posterior CNS is promoted by FGF, Wnt-3a, and retinoic acid is reviewed from the point of view of the classical experiments of Nieuwkoop that defined an activation and a transformation step during neural induction.

INTRODUCTION

Each somatic cell of the vertebrate body is derived from one of the three germ layers, ectoderm, mesoderm, and endoderm, which are established during gastrulation. The ectoderm, which forms the outer layer, gives rise to the epidermis, the central nervous system (CNS), the peripheral nervous system (PNS), the placodes (nasal, lens, otic, and lateral line), and various glandular tissues. These different tissues are produced and patterned from ectodermal precursor cells as a result of inductive interactions during early embryogenesis. Inductive signals that act on the ectodermal region can originate in neighboring mesodermal, endodermal, and/or ectodermal cells. In Amphibia the dorsal blastopore region, or Spemann's organizer, is known to possess strong inducing activities on the ectoderm. The organizer, a relatively small dorsal region of the embryo, when grafted to the ventral side of another embryo, can induce a secondary axis containing CNS, PNS, placodes, and cement gland. The induced tissues have a well-organized arrangement along the dorsal-ventral (D-V) and anterior-posterior (A-P) axes, showing that the organizer graft can trigger a cascade leading to induction and patterning of the entire ectoderm (Spemann and Mangold, 1924). Traditionally the induction of CNS by the organizer is called ''primary induction,'' whereas the term ''secondary induction'' is reserved for later inductive phenomena evoked by tissues resulting from primary induction, such as induction of lens by the optic cup or auditory vesicles by the hindbrain (Hamburger, 1988).

Embryonic tissues that have inductive activities similar to Spemann's organizer are presumably present in gastrulae of all vertebrate species. In chick and mice, the primitive node (Hensen's node) is considered as the organizer. During early gastrulation the organizer tissue is located at the anterior end of the primitive streak (reviewed by De Robertis *et al.*, 1994). This region can induce neural structures when grafted ectopically not only in an embryo of the same species (Waddington, 1933; Storey *et al.*, 1992; Beddington, 1994) but also in *Xenopus* ectoderm (Kintner and Dodd, 1991; Blum *et al.*, 1992). In fish, the embryonic shield, which is located on the dorsal side, is functionally homologous to the organizer (Oppenheimer, 1936; Shih and Fraser, 1996).

In this review, we discuss recent progress in vertebrate ectodermal patterning, focusing on primary and secondary induction initiated by the organizer. Although we place more emphasis on data from *Xenopus* studies, we attempt

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to integrate data from mammalian, chick, and zebrafish studies which provide complementary information.

D-V PATTERNING I: NEURAL INDUCERS AND ANTINEUROGENIC FACTORS

The biochemical isolation of the molecules that mediate primary induction has been the Holy Grail for amphibian embryologists for decades (Hamburger, 1988). One of the biggest obstacles was the size of the organizer, which is too small to isolate material in amounts useful for biochemical studies. Another difficulty was that the animal cap ectoderm of the newt, which was the preferred material during early days, is very sensitive to chemical and physical change, puzzling researchers with nonspecific initiation (autoneuralization) of neural differentiation (Hamburger, 1988). Recent molecular biological studies on neural induction have used mostly animal cap explants of *Xenopus* which have less of a tendency to undergo autoneuralization than those of the newt.

So far three secreted factors have been identified as bona fide neural inducers which are expressed at the right time and in the right place to function in *Xenopus* primary induction. Noggin (Smith and Harland, 1992; Lamb *et al.*, 1993), chordin (Sasai *et al.*, 1994, 1995), and follistatin (Hemmati-Brivanlou *et al.*, 1994) can induce neural tissues from animal cap cells when injected as mRNA and are expressed in the dorsal lip of frog gastrulae and in the axial mesoderm of neurulae, tissues known to possess strong neuralizing activity. The neural tissue induced by these organizer factors expresses anterior neural markers (Lamb *et al.*, 1993; Hemmati-Brivanlou *et al.*, 1994; Sasai *et al.*, 1995) such as Xanf-1 (anterior neural plate and pituitary gland) and Otx-2 (forebrain), but does not express spinal cord markers such as Hoxb-9 (XlHbox6). In the terminology of classical embryology, these three organizer factors are archencephalic (forebrain-type) neural inducers (Hamburger, 1988).

Noggin and chordin were initially identified as dorsalizing factors (Smith and Harland, 1992; Sasai *et al.*, 1994) that induced dorsal mesoderm (muscle and notochord) from precursor tissue of ventral mesoderm (blood, mesothelium, and mesenchyme). Both factors have dose-dependent activity. Interestingly, follistatin (which has been traditionally considered only an activin antagonist) also has dorsalizing activity when injected as mRNA (Sasai *et al.*, 1995). These data suggest that a neural inducer and a mesoderm dorsalizing factor represent two sides of the same coin, contrary to the reasonable expectation that these two distinct activities would result from independent signals.

A similar correlation of effects on mesoderm and ectoderm has been found in the case of BMP-4, a TGF-β family molecule which is a strong ventralizing factor of mesoderm (Dale *et al.*, 1992; Jones *et al.*, 1992; Fainsod *et al.*, 1994), and has also been shown to have antineurogenic activity. BMP-4 can suppress neural induction by noggin, chordin, and follistatin in *Xenopus* animal caps at the gastrula stage (Sasai *et al.*, 1995). BMP-4 can also inhibit neuralization of dissociated animal caps, promoting the formation of epidermis (Wilson and Hemmati-Brivanlou, 1995). When endogenous BMP-4 signaling is blocked by using a dominant-negative BMP receptor, antisense BMP-4 RNA (but not by BMP-2 antisense) or a dominant-negative form of BMP-4 ligand (and of its heterodimer partner BMP-7), animal caps undergo neural differentiation in the absence of organizer-derived neural inducers (Sasai *et al.*, 1995; Xu *et al.*, 1995; Suzuki *et al.*, 1995; Hawley *et al.*, 1995). BMP-4 is expressed widely in frog gastrulae, except for the organizer and dorsal animal cap regions (Fainsod *et al.*, 1995; Schmidt *et al.*, 1995a) where the neural plate forms. Thus, BMP-4 is a bona fide antineurogenic factor that is expressed at the right time and in the right place during ectodermal patterning.

The molecular data described above suggest that an antagonistic signaling system involving organizer secreted factors and BMP-4 regulates neural differentiation in *Xenopus*. This model is supported by studies on neurogenic ectoderm formation in *Drosophila*. The *Drosophila* homologue of BMP-4 is the product of *decapentaplegic* (*dpp*), which is a gene expressed in the dorsal side of the embryo at the cellular blastoderm stage (St. Johnson and Gelbart, 1987). *dpp* plays a central role in the establishment of D-V polarity in the fly. The loss-of-function phenotype of *dpp* mutation involves expansion of the neurogenic ectoderm at the expense of dorsal tissues such as the amnioserosa (Wharton *et al.*, 1993). Ectopic expression of *dpp* mRNA leads to expansion of dorsal tissues and reduction of the neurogenic ectoderm (Ferguson and Anderson, 1992a; Wharton *et al.*, 1993). Thus, dpp acts as a suppressor of neurogenesis in the fruit fly.

Recently a *Drosophila* homologue of *chordin* was identified as the product of the gene *short-gastrulation* (*sog*) (François *et al.*, 1994; François and Bier, 1995; Holley *et al.*, 1995), which is required for proper D-V development in the fly (Zusman *et al.*, 1988). *sog* is expressed on the ventral side of the fly embryo (François *et al.*, 1994) and gene dosage studies have shown that *sog* antagonizes the function of the *dpp* morphogen in D-V patterning (Ferguson and Anderson, 1992b). In null mutants of *sog*, dorsal epidermis expands at the cost of partial loss of the neurogenic ectoderm (Zusman *et al.*, 1988; Ferguson and Anderson, 1992b; François *et al.*, 1994). Microinjection of *sog* mRNA leads to ectopic formation of CNS tissue in *Drosophila* embryos (Holley *et al.*, 1995). Furthermore, *dpp* and *sog* have been shown to be the functional homologues of BMP-4 and chordin, respectively. Human BMP-4 (and the closely related molecule BMP-2) can rescue the *dpp* phenotype in fly (Padgett *et al.*, 1993) and *dpp* has potent ventralizing activity in *Xenopus* (Holley *et al.*, 1995). *sog* has strong mesoderm dorsalizing and neural inducing activities in *Xenopus* (Holley *et al.*, 1995; Sasai *et al.*, 1995; Schmidt *et al.*, 1995b) and *chordin* partially mimics the ventralizing activity of *sog* in the fly embryo (Holley *et al.*, 1995).

These results lead to two important conclusions. First, both in insects and vertebrates a conserved system of an-

tagonistic secreted factors regulates initiation of neural differentiation: *chordin*/*sog* promotes the formation of the CNS while *BMP-4*/*dpp* suppresses it. Second, the data provide support for the hypothesis of Geoffroy Saint-Hilaire, who proposed from comparative anatomy studies that the D-V axes of the vertebrate and arthropod body plans were inverted (Geoffroy Saint-Hilaire, 1822; Arendt and Nübler-Jung, 1994; De Robertis and Sasai, 1996). *Chordin* is expressed on the dorsal side of the frog embryo while *sog* is expressed on the ventral side of the fly. BMP-4 is expressed strongly on the ventral side of *Xenopus* gastrula and neurula while *dpp* expression is limited to the dorsal side of *Drosophila*. Thus, a pair of antagonistic upstream regulatory genes for CNS formation and dorsoventral patterning are expressed in an inverted manner between vertebrates and arthropods, suggesting that the dorsal side of one is homologous to the ventral side of the other (Hogan, 1995; Jones and Smith, 1995; Ferguson, 1996). This idea is further supported by the expression patterns of vertebrate netrin, an axon guidance molecule, and its fly homologue. Vertebrate netrin-1 is expressed specifically in the midline cells of the CNS (floor plate) while its *Drosophila* homologue is expressed in the midline of the ventral CNS (C. Goodman, personal communication). In conclusion, the regions of ectoderm that will give rise to CNS in vertebrates and in arthropods are specified by a system of diffusible signals involving *sog*/*chd* and *dpp*/*BMP-4* that has been conserved in evolution (De Robertis and Sasai, 1996).

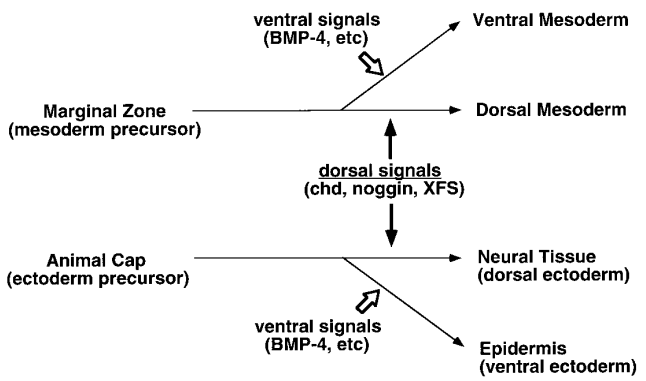

FIG. 1. Dorsal and ventral signals change the fate of tissues by providing varying dorsal–ventral positional information (Sasai *et al.*, 1995). (A) The dorsal signals from the frog organizer, chordin, noggin, and follistatin (XFS) act on both marginal zone cells (mesodermal precursors) and animal cap cells (ectodermal precursors) and induce dorsal-type tissues: dorsal mesoderm (notochord and muscle) and neural tissues, respectively. Ventral signals such as BMP-4 also change the fate of both mesoderm and ectoderm, generating ventral mesoderm (blood, mesothelium, and mesenchyme) and epidermis. Thus, the same set of antagonizing regulatory signals, the organizer factors vs BMP-4, can pattern both germ layers.

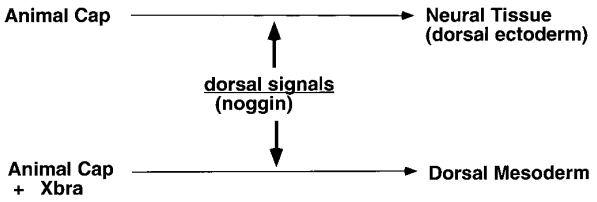

FIG. 2. Experiment by Cunliffe and Smith (1994) illustrating how a differential response to the same signal can be generated. Animal caps treated with noggin became neural. When animal caps were injected with *Xbra*, a mesoderm-specific transcription factor, the explants became dorsal mesoderm in response to the same noggin signal.

D-V PATTERNING II: NEURAL INDUCTION AS DORSALIZATION OF ECTODERM

As discussed in the previous section the same set of signals, the organizer factors (chordin, noggin, follistatin), and BMP-4 can pattern both ectoderm and mesoderm. In this view, neural induction may be considered to be the dorsalization of ectoderm in the same sense as formation of notochord and muscle is considered dorsalization of mesoderm. A model has been proposed (see Fig. 1) in which the organizer factors impart dorsal positional information to tissues while the ventralizing factor BMP-4 provides ventral positional values (Sasai *et al.*, 1995; De Robertis and Sasai, 1996). When a high dorsal value is specified, ectodermal precursor tissues undergo neural differentiation and mesodermal precursor tissues form dorsal mesoderm structures such as notochord and muscle. At high ventral values ventral ectoderm (epidermis) and ventral mesodermal tissues (blood, mesenchyme, and mesothelium) are formed.

Several questions are raised by such a model. First, if the signaling molecules utilized for dorsal differentiation of both ectoderm and mesoderm are the same, then the differences must reside in the responding tissues. What is the molecular mechanism underlying the predisposition to become either dorsal ectoderm or mesoderm? One hint on how this differential response may come about was provided by an experiment by Cunliffe and Smith (1992), shown in Fig. 2, in which injection of *noggin* mRNA induced neural tissues, whereas injection of *noggin* together with *Xbra* mRNA led to the formation of dorsal mesoderm in animal cap explants. Xbra is a transcription factor expressed in the mesoderm but not in the animal cap. Although Brachyury is essential only for posterior mesodermal differentiation in mice and zebrafish, it appears likely that a small number of transcription factors activated by mesodermal inducers, including Xbra, could provide mesodermal specification in the embryo. In this context, it is worth noting that a mutated form of Xbra, when overexpressed in *Xenopus* animal caps, can promote neural differentiation (Rao, 1994).

A second question concerns how a spectrum of dorsoventral positional values forms during gastrulation. Do the organizer factors produce concentration gradients from the dorsal to the ventral side? Do the chordin and noggin proteins diffuse to different degrees? Is there a concentration gradient of BMP-4 in the reverse orientation? These questions are of importance with respect to the morphogen theory, and will be addressed once suitable antibodies become available. *In situ* hybridization studies show that BMP-4 mRNA is distributed quite uniformly in the animal cap and marginal zone except for the organizer region from which it is absent (Fainsod *et al.*, 1994) and a similar observation has been made for *BMP-7*, which is expressed in a related, but not identical, domain (Hawley *et al.*, 1995). It is therefore likely that a gradient of BMP activity is formed by diffusion of organizer factors that antagonize ventralizing signals rather than by graded differences in gene activity.

A third question concerns the mode of action of the organizer factors. As blockade of endogenous BMP-4 signaling by dominant-negative BMP receptors and BMP-4 antisense RNA results in neural differentiation of animal cap cells (Sasai *et al.*, 1995), one possibility is that organizer factors work by blocking BMP-4 signaling. Possible levels at which this might occur from a mechanistic point of view are: (1) blocking of processing or secretion of mature BMP-4 protein. (2) Direct binding to BMP-4 in the extracellular space. (3) Binding to and blocking of the BMP receptor. (4) Through a parallel receptor system (initiating an intracellular signal that antagonizes the BMP signaling downstream of the BMP-4 receptor). At present, there are no data available in favor of membrane receptors for the organizer factors. Instead, it has become apparent that the organizer factors Chordin and Noggin function by direct binding to BMP-4 in the extracellular space.

Cross-linking and immunoprecipitation experiments have shown that the chordin and BMP-4 proteins can physically interact with high affinity (Piccolo *et al.*, 1996). This affinity ($K_d = 3 \times 10^{-10}$ *M*) is directly comparable to those of BMP-4 and dpp for their cognate receptors (9 and 2.5×10^{-10} *M*, respectively; Graff *et al.*, 1994; Penton *et al.*, 1994). The addition of chordin protein inhibits radiolabeled BMP-4 protein from binding to its receptors on $10T\frac{1}{2}$ cells (Piccolo *et al.*, 1996), indicating that chordin traps BMP-4 and prevents receptor binding. Similar data have been obtained for noggin and BMP-4 (Zimmerman *et al.*, 1996), showing that both chordin and noggin interact with BMP-4 in a similar way *in vitro*. The affinity of the BMP-4–noggin interaction is 15 times higher than that of BMP-4–receptor or BMP-4–chordin binding (Zimmerman *et al.*, 1996). Both noggin and chordin dorsalize ventral mesodermal explants at 1 n*M*, but only chordin can neuralize animal caps at this low concentration (Lamb *et al.*, 1993; Piccolo *et al.*, 1996). Thus, although both molecules act by binding BMPs, differences that are not detected by the biochemical binding assays exist in their mode of action *in vivo*. In addition, E. L. Ferguson and his collaborators have shown that *Xenopus noggin*

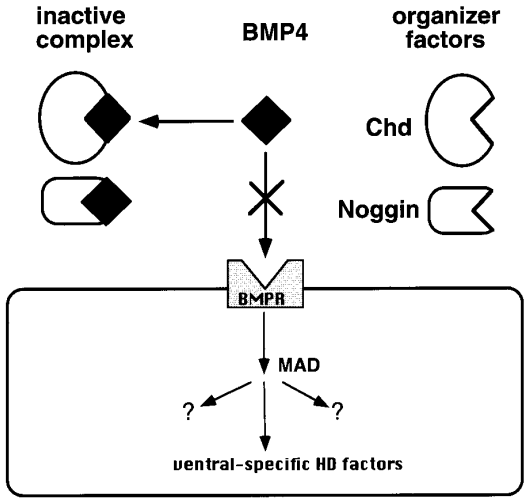

FIG. 3. The organizer factors inactivate BMP-4 by binding it in the extracellular space. Both chordin and noggin bind to BMP-4 and inhibit BMP-4 protein from binding to its own receptor. Downstream of the BMP receptor, the vertebrate homologue of *Drosophila mother against dpp* (MAD) seems to play a fundamental role in the signal transmission to the nucleus. Among the target genes in the BMPR signaling pathways are ventral-specific homeodomain (HD) proteins. Both MAD and ventral HD factors can mimic the ventralizing activity of BMP-4 by microinjection.

mRNA injected into eggs ventralizes *Drosophila* embryos by preventing dpp from activating its receptor (Holley *et al.*, 1996). Furthermore, these authors showed that the double mutant of *dpp* and *sog* is indistinguishable from the *dpp* mutant in early phenotype, demonstrating that *dpp* is epistatic to *sog*. In other words, in the absence of dpp the presence or absence of sog does not cause any difference in phenotype, suggesting that sog functions through dpp. Taken together, these data (Zimmerman *et al.*, 1996; Piccolo *et al.*, 1996; Holley *et al.*, 1996) suggest that the main function of the organizer factors chordin and noggin is to inactivate ventral BMP signals in the extracellular space, as depicted in Fig. 3.

Follistatin might also act through direct binding to ventralizing BMPs. Although follistatin was discovered because it binds to another TGF-β molecule, activin, recent results suggest that activin must not be the only binding molecule of follistatin *in vivo*. Both follistatin and activin can induce a similar partial secondary axis when ectopically expressed in the *Xenopus* embryo (Sasai *et al.*, 1995; Thomsen *et al.*, 1990); this fact is hard to reconcile with follistatin being a specific activin antagonist. By using cultured cells, Miyazono and his collaborators showed that follistatin can antagonize another BMP molecule, BMP-7, albeit at a 10-fold higher concentration than that required against activin (Yamashita *et al.*, 1995). Furthermore, a dominant-negative activin receptor, which can induce neural differentiation in *Xenopus* animal caps (Hemmati-Brivanlou and Melton,

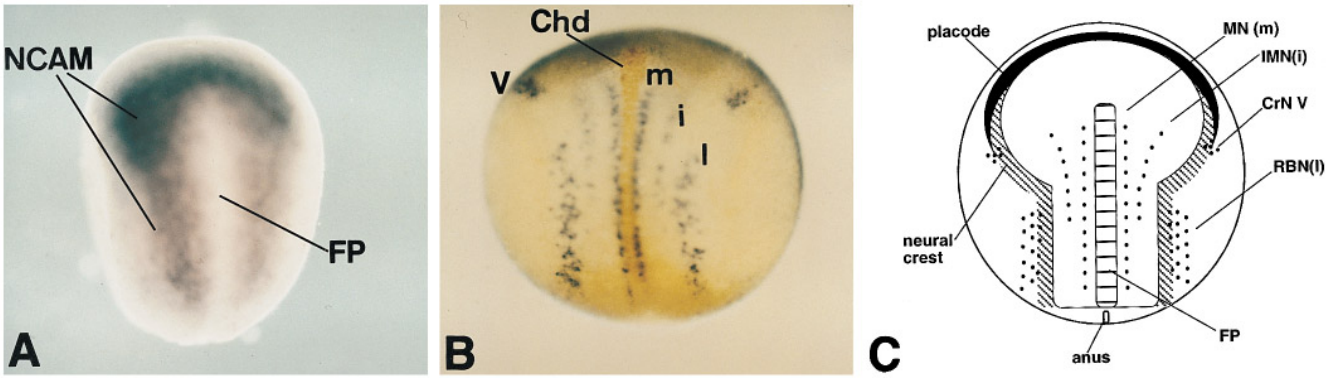

FIG. 4. Expression of gene markers during early patterning. (A) Whole-mount *in situ* hybridization of N-CAM in the early *Xenopus* neurula. The N-CAM staining demarcates the early neural plate. Note that the presumptive floor plate is devoid of N-CAM transcript, indicating that the floor plate has a distinct pattern of differentiation that can be traced back to this early stage. (B) Double labeling *in situ* hybridization of *chordin* (brown) and the neuronal marker β-tubulin (blue) at the early neurula. *Chordin* expression is detected in axial mesoderm (notochord) and the initial D-V arrangement of the primary neurons has already been established by the neural plate stage. m, medial neurons (motoneurons); i, intermediate neurons (interneurons); l, lateral neurons (Rohon-Beard neurons). These neurons are involved in the escape reflex of the tailbud tadpole. V, trigeminal ganglion. (C) Schematic map of the early D-V arrangement of the ectoderm at the neural plate stage in *Xenopus*. In the neural plate (from medial to lateral), the presumptive floor plate (FP), the motoneuron (MN), and intermediate neurons (IMN) are found. The trunk neural plate is flanked by the presumptive neural crest (hatched area) while in the head the placode-forming region (black area) borders the neural plate. In the posterior, sensory Rohon-Beard neurons (RBN) form in the ectoderm just outside of the neural plate. Photographs kindly provided by Bin Lu.

1994), blocks not only signals of activin but also of those of BMP-4 (Wilson and Hemmati-Brivanlou, 1995). These data, together with data from mouse knockouts (Matzuk *et al.*, 1995a and b), call into question the role of endogenous activin as an antineurogenic factor (Kelly and Melton, 1995) and suggest that follistatin may function by binding to other TGFβ molecules such as ventralizing BMPs.

Finally, can the same principles be applied to neural induction of amniotes? Detailed studies on follistatin expression in mice and chick have been reported (Albano *et al.*, 1994; Connolly *et al.*, 1995). Unlike its expression pattern in *Xenopus*, mouse follistatin has not been detected in axial mesoderm or node (which are derived from the organizer), but is expressed in the paraxial mesoderm. In chick, follistatin expression is similar to that in mice except that transient expression is found in the early node (Connolly *et al.*, 1995). Gene disruption of mouse follistatin does not show defects in early neural development (Matzuk *et al.*, 1995c). So far similar loss-of-function data for noggin and chordin in amniotes have not been reported; they will be important because all the data available at present derives from gain-of-function studies. The BMP-4 gene was disrupted in mice (Winnier *et al.*, 1995), and gastrulation and formation of posterior body and ventral mesoderm (such as blood islands) is strongly affected. However, specific defects in the CNS have not been reported. In chick, HGF/SF (hepatocyte growth factor or scatter factor) is expressed in Hensen's node and was shown to induce neural differentiation in extraembryonic epiblast (Streit *et al.*, 1995). As in this system one must add high concentration of serum to the culture medium, it is difficult to determine whether HGF/SF is a direct neural inducer or acts by potentiating other neural inducing activities present in the medium (Streit *et al.*, 1995; Bronner-Fraser, 1995). The *Xenopus* homologue of HGF/SF has been cloned; its transcripts are not detected until late gastrula stages, when the neuroectoderm is already formed, and at neurula stages it is expressed on the ventral (not dorsal) side (Nakamura *et al.*, 1995). In conclusion, at present we do not have enough data to address the mechanisms of amniote neural induction, although the *sog*/*chd* and *dpp*/*BMP-4* conservation between *Drosophila* and *Xenopus* suggests that common mechanisms may eventually be found in most animals.

D-V PATTERNING III: D-V PATTERNING OF THE NEURAL TUBE

The secondary neural tube induced by the grafted dorsal lip has a clear D-V polarity, demonstrating that the organizer not only induces neural tissues but also patterns them. By the neural plate stage, a very accurate pattern of dorsoventral differences has been established in *Xenopus* ectoderm. The D-V arrangement of frog ectoderm at the open neural plate stage is illustrated in Fig. 4. The dorsal midline of the ectoderm (from the posterior up to the midbrain primordium) is a specialized tissue that gives rise to floor plate. Thus, the floor plate is the most dorsal ectoderm, even though it becomes topologically the ventral midline of the

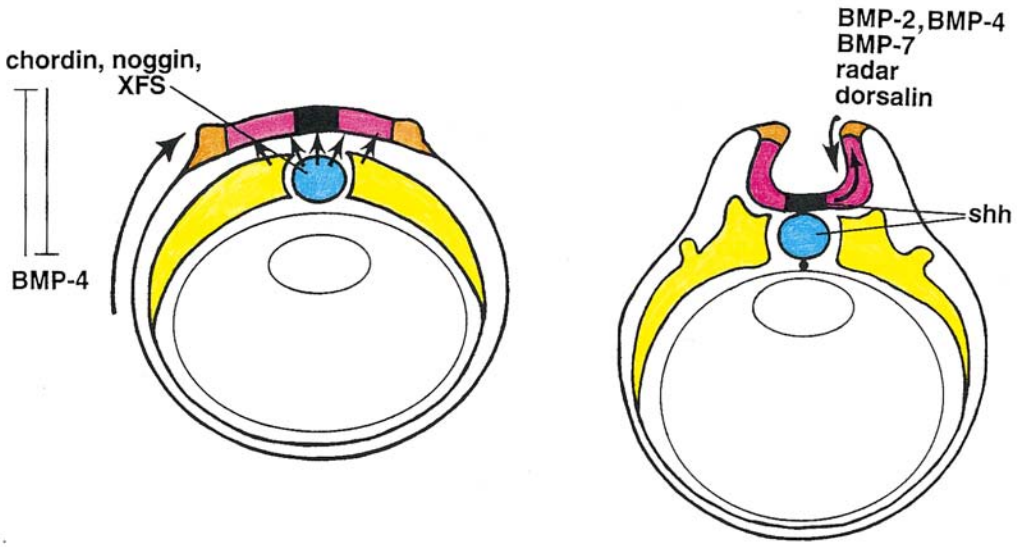

FIG. 5. Diagram of how the initial neural induction and D-V patterning of the neural tube might share common mechanisms. At the early neurula, BMP-4 in the ventral ectoderm and mesoderm is antagonized by chordin, noggin, and follistatin (XFS), which are secreted by dorsal chordamesoderm derived from Spemann's organizer (notochord, blue, and somite, yellow). These signals could pattern the ectoderm forming floor plate (thick black layer), neural plate (pink), and neural crest (orange) at different concentrations. At high BMP-4 concentrations BMP-4 leads to skin development (ventral ectoderm). At the late neurula stage (left), the notochord and floor plate produce the shh signal, which is opposed by a number of BMP-related molecules expressed in the dorsal neural tube and nearby ectoderm.

CNS after the neural tube closes. The floor plate primordium is devoid of N-CAM expression, which is a pan neural marker staining neurons and glia (Fig. 4A), and starts expressing HNF3-β-like genes and sonic hedgehog (shh) by neurulation (Dirksen and Jamrich, 1992; Ruiz i Altaba and Jessel, 1992; Ekker *et al.*, 1995). A very useful marker is a neuron-specific β-tubulin (Richter *et al.*, 1988) that marks the first neurons that differentiate in the neural plate and has been characterized in detail by Chitnis *et al.* (1995). Three rows of neurons are formed at the neural plate stage: a row of motoneurons is formed next to the floor plate, interneurons appear in the intermediate region, and large Rohon–Beard neurons are born in the neural crest and flanking ectoderm of the spinal cord region (see Figs. 4B and 4C). In the anterior, sensory neurons of the trigeminal (V) ganglion are formed (Fig. 4B). These very convenient markers of D-V patterning are expressed so early in *Xenopus* development in order to generate the escape reflex circuit of the tailbud tadpole. The Rohon–Beard neurons are sensory cells present in larvae of fishes and amphibians; after the aquatic phase they are functionally replaced by dorsal root ganglia in Amphibia.

The border of the neural plate forms the neural fold, which gives rise to neural crest cells and the dorsal roof of the spinal cord (Fig. 5A). Finally, the ectoderm ventral to the neural fold becomes epidermis. At these early stages, the D-V patterning of the epidermis does not exhibit specific landmarks, except that the region just anterior to the head neural fold forms placodes. Interestingly, expression studies indicate that the arrangement of the floor plate and primary neurons is established as early as late gastrula in amphibians (Chitnis *et al.*, 1995), when the neural or epidermal fates of the ectoderm are also determined (Spemann, 1918).

Studies from experimental biology as well as from genetics have shown a central role of the notochord in the establishment of the D-V polarity of the vertebrate neural tube. In Amphibia, a piece of young notochord has strong neural-inducing activity in animal cap assays (for review, Kintner, 1992). The notochord is a major derivative of Spemann's organizer, and the amount of notochord tissue is very sensitive to dorsalizing and ventralizing agents such as LiCl and UV treatments, which increase and decrease, respectively, the amount of organizer tissue (Kao and Elinson, 1988). A mild ventralizing treatment, e.g., by brief UV irradiation can eliminate the notochord but not the neural tube (Youn and Malacinski, 1981). In a notochord-less embryo the neural tube does not have a floor plate and the D-V arrangement is disrupted (Holftreter and Hamburger, 1955). In chick, ectopic grafts of notochordal tissues lateral to the neural tube induces ectopic formation of a floor plate and motoneurons (Yamada *et al.*, 1991, 1993). Removal of part of the notochord aborts or delays formation of the floor plate (van Straaten and Hekking, 1991; Yamada *et al.*, 1991; Artinger and Bronner-Fraser, 1993; Catala *et al.*, 1996).

An excellent candidate for the patterning molecule emanating from the notochord is the secreted protein sonic hedgehog (shh) (Riddle *et al.*, 1993; Echelard *et al.*, 1993; Krauss *et al.*, 1993; Roelink *et al.*, 1994), a vertebrate homo-

logue of the *Drosophila* segment polarity gene hedgehog. Throughout the vertebrates, shh is expressed in the notochord and also in the floor plate (Fig. 5), which has also been shown, like the notochord, to possess D-V patterning activity on the neural tube. Shh-overproducing COS cells (Roelink *et al.*, 1994; Tanabe *et al.*, 1995) and the amino terminal 19 kDa of the autocleavage product of shh (Lee *et al.*, 1994; Roelink *et al.*, 1995; Martí *et al.*, 1995) mimic the activity of notochord and floor plate, inducing floor plate and motoneuron from dorsal and lateral neural tube explants cultured in collagen gels. *Drosophila* hedgehog is a segment-polarity gene that plays an essential role in the establishment of anterior–posterior polarity of fruit fly parasegments (Nüsslein-Volhard and Wieschaus, 1980). In vertebrates, shh plays roles in the establishment of D-V polarity of the neural tube (discussed above) and somites (Fan *et al.*, 1995), of A-P polarity in limb buds (Riddle *et al.*, 1993), and of left-right polarity in the internal organs (Levin *et al.*, 1995). Thus, hedgehog molecules function in the establishment of polarity in many tissues.

Next we will address the mechanism by which shh regulates the determination of CNS D-V polarity *in vivo*. Shh seems to lie downstream of the transcription factor HNF-3β, which is also expressed in the notochord and the floor plate. In mice, HNF-3β is required for the formation of the notochord and the floor plate and for *shh* expression in these tissues (Ang and Rossant, 1994; Weinstein *et al.*, 1994). Misexpression of HNF-3β in the dorsal neural tube results in the ectopic expression of floor plate markers in mouse and *Xenopus* (Sasaki and Hogan, 1994; Ruiz i Altaba *et al.*, 1993). HNF-3β induces *shh* in the neural tube and, interestingly, *shh* can in turn induce expression of HNF-3β (Echelard *et al.*, 1993; Roelink *et al.*, 1994). From studies on the temporal and spatial expression of shh and HNF-β, a possible scenario emerging for *shh* gene regulation is: (1) dorsal mesoderm inducers (Nieuwkoop center factors) turn on expression of HNF-3β in the organizer and expression continues while the organizer involutes as chordal mesoderm, (2) at a certain point, HNF-3β switches on expression of *shh* in the notochordal tissue, (3) *shh* emanating from the notochord induces HNF-3β in the overlying part of neural tube and, (4) HNF-3β in the floor plate would in turn induce *shh* in the floor plate. In the downstream pathway of *shh*, repression by Protein kinase A (PKA) signals seems to play a crucial role (Hammerschmidt *et al.*, 1996) as is the case for *Drosophila* hedgehog (reviewed by Perrimon, 1995).

An important question concerns the *in vivo* role for *shh*. In frogs, *shh* expression is first detected at low levels during gastrula stages (Ekker *et al.*, 1995) and levels increase during neurula stages, at which strong signals are detected in floor plate as well as in the notochord. *Shh* per se cannot induce neural tissues from presumptive ectoderm cells, but can change the D-V pattern of preexisting neural tissue (Ekker *et al.*, 1995). It is still to be clarified whether *in vivo shh* is involved in the initial D-V patterning of the CNS or in the maintenance of the pattern once it is established. The latter role for *shh* could be particularly important because signals that antagonize the activity of *shh* have been recently shown to emanate from the dorsal neural tube and the epidermis overlying it. In chick, the epidermal ectoderm can induce dorsal CNS markers (such as *Wnt-1*) from lateral neural tube explants (Dickinson *et al.*, 1995; Selleck and Bronner-Fraser, 1995), and several BMP factors expressed in the dorsal neural tube and/or the overlying epidermis can mimic this activity. These are BMP-4, BMP-7 (Liem *et al.*, 1995), and dorsalin-1 (Basler *et al.*, 1993). In mice, BMP-2 is expressed in a similar region. In zebrafish, another BMP-related molecule, radar, is expressed in the dorsal midline of the embryonic CNS (Rissi *et al.*, 1995). The possible interactions among these factors are illustrated in Fig. 5B.

Which factors initiate early D-V patterning in the *Xenopus* neural plate? As mentioned above, the onset of *shh* expression appears to be too late for such a role in *Xenopus*. On the other hand, the *Xenopus* organizer factors *chordin* and *noggin* are expressed in the chordal mesoderm from late blastula to neurula stages (Smith and Harland, 1992; Sasai *et al.*, 1994, 1995). There are several lines of evidence suggesting that these organizer factors could pattern the CNS. When an animal cap has been treated with noggin, both dorsal and ventral CNS markers are induced in different parts of the explant, suggesting that the neural tissue induced in the explant is somewhat patterned (Knecht *et al.*, 1995). When an animal cap is treated with chordin and bFGF (Sasai *et al.*, 1996), it expresses the floor plate marker F-spondin (chordin alone cannot induce this marker in the caps probably because the induced tissue is that of the forebrain type, which does not have a floor plate). More importantly, *BMP-4* and its related molecules, which are antagonistic signals to chordin and noggin, seem to play a role in the D-V patterning of the CNS in the chick (Liem *et al.*, 1995).

Since molecules of the BMP family have opposite activities to both the organizer factors and *shh* in neural induction and CNS patterning, respectively, an attractive possibility is that neural induction (i.e., dorsalization of the ectoderm) and D-V patterning of the CNS are, at least in part, the consequence of the same signaling mechanisms. In this view, the D-V patterning of the CNS would be under the control of a unifying D-V positional information system that patterns the ectoderm and also the mesoderm. To investigate this hypothesis, it will be important to determine whether chordin and noggin, or their combination, can induce markers for the floor plate, motoneurons, interneurons, neural crest, and epidermis in a dose-dependent manner, and whether *BMP-4* can reverse this in a dose-dependent way. An important difference between chordin/noggin and shh is that shh cannot induce neural tissues from animal cap cells. This is probably not due to a simple lack of shh receptors in the explant as shh can induce cement glands in animal caps (Ekker *et al.*, 1995). It would be intriguing to test whether or not the PKA pathway acting downstream of shh is responsible for this lack of neuralization.

D-V PATTERNING OF THE ECTODERM IV: A PLETHORA OF TRANSCRIPTION FACTORS

The last aspect of D-V ectoderm patterning that we would like to discuss is recent progress on the signal transduction and intracellular events that occur during neural induction and D-V patterning. There are at least two kinds of transcription factors expressed in the early vertebrate neural plate: the pou-domain factor Xlpou2 (a frog homologue of mouse Brn-4) and Sox factors (Sry-related HMG factors). In *Xenopus,* Xlpou2 can be induced in animal caps by *noggin,* and the effect of microinjection of Xlpou2 mRNA is to cause neural differentiation in animal caps (Witta *et al.,* 1995). The chromatin proteins Sox-1, -2, and -3 are closely related to one another in structure, contain an HMG box (Grosschedl *et al.,* 1994), and are among the earliest pan-neural markers so far available. Neural crest precursors express the zinc-finger gene *slug* from very early stages (Nieto *et al.,* 1994). *slug* belongs to the same family as the transcription factor *snail* of fly and vertebrates (Boulay and Dennefield, 1987; Sargent and Bennet, 1990) and *scratch.* In *Drosophila, scratch,* a pan-neural marker, is required for neurogenesis (Roark *et al.,* 1995). In chick, differentiation of the neural crest is impaired when accumulation of *slug* is inhibited by antisense oligonucleotides against slug mRNA (Nieto *et al.,* 1994). Thus, the Pou, Sox, and *slug* factors discussed above are good candidates for effector genes acting closely downstream of the neural inducing signaling pathways.

In *Drosophila,* several basic Helix-Loop-Helix (bHLH) transcription factors function as proneural genes (Campos-Ortega, 1993). Vertebrate homologues have been identified for *AS-C* (*Mash-1, Xash-1, Xash-3*) (Johnson *et al.,* 1990; Ferreiro *et al.,* 1994; Turner and Weintraub, 1994), *atonal* (*NeuroD, Math-1, -3,* and *Nex-1*) (Lee *et al.,* 1995; Akazawa *et al.,* 1995; Bartholoma and Nave, 1994) and *daughterless* (E12) (Murre *et al.,* 1989). Vertebrate homologues for negative regulators of the *Drosophila* proneural or neurogenic genes are also available (*Id* family for *emc, HES* family for *E(spl)*) (Benezra *et al.,* 1990; Sasai *et al.,* 1992). Many of them display intriguing expression patterns in the developing CNS of vertebrates, suggesting that they may be involved in the regulation of vertebrate neural development (Simpson, 1995; Kageyama *et al.,* 1995).

Interesting examples are provided by the *NeuroD* and *Mash-1* bHLH factors. *Xenopus NeuroD* is expressed in developing sensory neurons and cranial ganglia (Lee *et al.,* 1995). Mouse *Mash-1* is expressed in the sympathetic and enteric ganglia, olfactory sensory cells, and parts of the CNS during early neurogenesis (Lo *et al.,* 1991). Injection of *NeuroD* mRNA will initiate neural differentiation in animal caps; however, expression of *NeuroD in vivo* starts relatively late and is not detectable in the neuroectoderm at the stage when neural induction takes place (Lee *et al.,* 1995). To date we have no pan-neural bHLH factors expressed in the entire neural plate, although this does not preclude that one might be found in the near future. The observations to date may imply that the main *in vivo* roles for bHLH factors during neurogenesis are regional specification and temporal regulation of neuronal differentiation. In accordance with this possibility, when the *Mash-1* gene is disrupted in mice, sympathetic and enteric ganglion precursors are produced but fail to differentiate properly (Guillemot *et al.,* 1993; Sommer *et al.,* 1995). Vertebrate bHLH family members are presumably regulated by vertebrate homologues of *Drosophila* proneural or neurogenic genes, such as those of the Notch/Delta/Serrate/Jagged signaling pathway (Coffman *et al.,* 1990, 1993; Lindsell *et al.,* 1995; Chitnis *et al.,* 1995; Myat *et al.,* 1996).

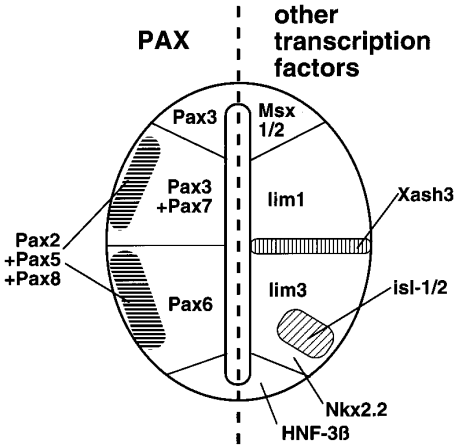

FIG. 6. Transcription factors involved in the D-V specification of the CNS in amniotes. On the left half of the scheme, the differential expression of seven Pax genes is indicated (modified after Gruss and Walther, 1992). Other classes of transcription factors are shown on the right half, including Msx-1/2 (roof plate and neural crests), lim-1 (alar plate), lim-3 (basal plate), isl-1/2 (motoneurons), Xash3 (sulcus limitans), Nkx 2.2 (region between the floor plate and motoneurons, area of expression varies slightly among species), and HNF-3β (floor plate). Many of these transcription factors have been shown to play essential roles in the development of the regions that express them (see text).

D-V specification of the neural tube also involves several additional classes of transcription factors: (1) the winged-Helix class (such as HNF-3β, Dirksen and Jamrich, 1992; Ruiz i Altaba and Jessell, 1992), (2) the Pax family (e.g., Pax-3, for reviews, see Gruss and Walther, 1992; Chalepakis *et al.,* 1994), (3) the Lim family (such as lim-1 and islet-1, Tsuchida *et al.,* 1994; Dawid *et al.,* 1995), (4) the Msx family (Davidson and Hill, 1991), and (5) the Nkx class (e.g., Nkx 2.2, for review see Price, 1993). This plethora of transcription factors serve as very useful markers for the D-V axis of the neural tube, as depicted in Fig. 6. Loss-of-function studies in mice have demonstrated that these transcription factors have important roles for the development of specific regions of the CNS. For example, Pax-3, which is expressed

in the dorsal part of the CNS, corresponds to the locus responsible for the *Splotch* mutation in mice (Epstein *et al.*, 1991) and of Waardenburg syndrome in human (Tassabehji *et al.*, 1992). The Splotch mutation impairs the development of the dorsal side of the neural tube, causing spina bifida, meningocele, and various neural crest cell-associated deficiencies (Epstein *et al.*, 1991). Targeted disruption of the *islet-1* gene, which is expressed in the motoneurons, has shown that *islet-1* is required for the generation of motoneurons as well as of interneurons that depend on secondary signals from motoneurons for their formation (Pfaff *et al.*, 1996). In future an important challenge will be to elucidate the mechanisms that bridge the early patterning action of the organizer factors such as chordin and noggin and the regional specifications dependent on transcription factors such as those of the *Pax* and *Lim* families.

A-P PATTERNING I: FORMATION OF POSTERIOR CNS

The organizer can pattern the neural tube not only in the D-V direction but also along the A-P axis. A common feature of the *Xenopus* neural inducers chordin, noggin, and follistatin is that they induce exclusively anterior neural tissues (forebrain type) but not posterior ones (hindbrain and spinal cord type). Until recently, little was known about the molecular mechanisms underlying posterior CNS formation except for the fact that Hox genes act in the specification of the hindbrain and spinal cord (for review, McGinnis and Krumlauf, 1992; Keynes and Krumlauf, 1994).

The mechanisms that have been proposed for the formation of posterior neural tissue can be classified into two categories (Fig. 7). The first model postulates the presence of distinct anterior (archencephalic) neural inducers and posterior (deuterencephalic) neural inducers (Fig. 7A). In this model, anterior CNS tissues are induced by the archencephalic inducers and posterior ones by the deuterencephalic/spinocaudal inducers. The ratio of the two kinds of factors would define the A-P specification of the CNS tissues (Tiedemann, 1959; Saxén and Toivonen, 1961). This kind of model may be designated as the two inducer model.

The second model is the two step model, shown in Fig. 7B, in which neural development is initiated by neural inducers (first step: ''activation'' or ''induction'') and then a later signal provides posterior specification to the induced neural tissues (second step: ''transformation''). There is much experimental support for the two-step model (reviewed by Saxén, 1989), with the strongest evidence coming from the famous neural fold experiments of Pieter Nieuwkoop (1952a and b). By implanting folds of competent ectoderm at different anteroposterior levels of the neural plate of *Triturus* and *Amblystoma*, Nieuwkoop found that in all cases anteriormost neural structures (such as nasal pits, eyes, pineal gland, and forebrain) were present in the induced grafts. However, the nature of the tissue that formed at the base of the fold was dependent of the anteroposterior level of the graft. Thus, a graft placed in the anterior would have forebrain at its base, one placed in the hindbrain would have forebrain distally and hindbrain at its base, and those grafts placed at the level of the spinal cord would differentiate forebrain distally, hindbrain in the middle, and spinal cord at the base. The interpretation of these experiments is that all neural tissues are submitted first to an activation or neural induction step by which archencephalic structures are induced. After this, the posterior values are imparted upon this tissue by a second signal, the transformation step, so that hindbrain and spinal cord are generated. Because the grafts of ectodermal folds were placed at the neural plate stage, long after the prechordal endomesoderm had involuted, a graft placed at the level of the spinal cord should never come in contact with an anterior inducer. This indicates that before becoming transformed into spinal cord, all neural tissues are activated (induced) to form archencephalic structures. This work represents a masterpiece of experimental embryology and reading the original papers is highly recommended (Nieuwkoop, 1952a and b).

There are three kinds of candidate factors that may be involved in the development of posterior CNS. Retinoic acid (RA) is the best known candidate molecule. RA can transform prospective anterior CNS into posterior CNS (Sharpe, 1991; Ruiz i Altaba and Jessell, 1991). In *Xenopus*, RA concentration in the posterior quadrant of the late gastrula and early neurula is 10 times higher than in the anterior quadrant (Chen *et al.*, 1994). Since RA per se is unable to induce neural tissues in animal cap explants, RA is a candidate molecule for a posterior transformation signal in Nieuwkoop's model. However, our knowledge about spatial and temporal distribution of RA is fragmentary and the *in vivo* roles for RA remain unclear at this time.

Recently two kinds of secreted protein factors, FGFs and Wnts, have been suggested as candidate molecules for the posterior transformation signal (for review, see Doniach, 1995). bFGF protein can transform a frog anterior neural plate explant into posterior CNS *in vitro* (Cox and Hemmati-Brivanlou, 1995). When animal caps are treated with bFGF and one of the archencephalic inducers (noggin, follistatin, or chordin), posterior neural tissues (e.g., hindbrain) are induced in addition to forebrain tissues (Lamb and Harland, 1995; Cox and Hemmati-Brivanlou, 1995; Sasai *et al.*, 1996). Block of FGF signaling *in vivo* by a dominant-negative FGF receptor results in posterior truncation of the *Xenopus* embryo (Amaya *et al.*, 1991). Although FGF signaling seems to be essential for posterior (trunk-tail) development, it is not yet clear which FGF molecule is responsible. At present, eFGF seems most promising because it is strongly expressed in the posterior mesoderm of the *Xenopus* neurula, including the prospective tailbud region (Isaacs *et al.*, 1992). *Wnt-3a* is another good candidate for a posterior transformation signal. Coinjection of *Wnt-3a* and *noggin* mRNAs induces posterior neural markers in animal caps while *Wnt-3a* alone cannot induce neural tissue (McGrew

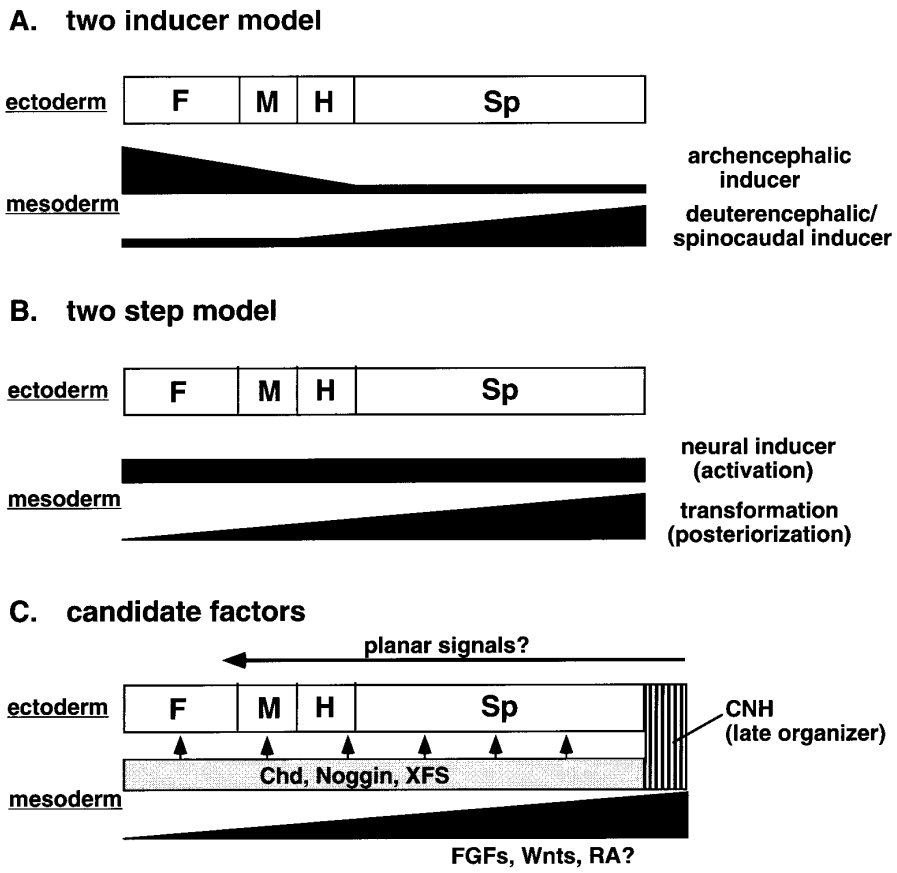

FIG. 7. Schematic models for the formation of posterior CNS. (A) A two inducer model. The archencephalic and deuterencephalic inducers promote the formation of anterior CNS and posterior CNS, respectively. The concentration gradient and/or combination of the two kinds of inducers determine the fine pattern. In the context of our discussion, the two inducer model stands for the existence of posterior neural inducers that can directly initiate posterior-type neural differentiation from presumptive ectodermal tissues. (B) The two step model. First, the neural inducers initiate neural differentiation of the ectoderm. The neural inducers, when acting alone, promote formation of archencephalic neural tissues. In a second transformation step, posteriorizing factors act on the induced neural tissue and give various posterior values depending on concentration timing. (C) A possible model for the involvement of known inducers and modulators. The dorsal mesoderm releases chordin, noggin, and follistatin (XFS), which can act as archencephalic neural inducers. The posterior mesoderm expresses FGFs (e.g., eFGF), Wnts (e.g., Wnt3a), and contains RA.

et al., 1995). Mouse gene targeting has shown that *Wnt-3a* is essential for posterior development (Takada *et al.*, 1994). Both *chordin* and *noggin* are expressed in chordamesoderm from the anterior to the posterior during neural plate formation (Smith and Harland, 1992; Sasai *et al.*, 1994) and are therefore reasonable candidates for inducers working at the activation step of Nieuwkoop's model. RA, FGFs, and *Wnt-3a* seem to satisfy the criteria for the transformation step. In conclusion, the activities of the factors discussed above support the view of Nieuwkoop's "two step model" at the molecular level (Fig. 7C).

The two inducer model, however, cannot be entirely ruled out at this time, for two groups reported that bFGF can induce posterior neural tissues in *Xenopus* animal caps explants under certain conditions (Kengaku and Okamoto, 1993 and 1995; Lamb and Harland, 1995). bFGF does not change the fate of untreated gastrula animal caps explants (at earlier stages blastula caps respond to bFGF by forming mesoderm, Slack *et al.*, 1987), but it has recently been noted that gastrula animal caps can undergo neural differentiation in response to bFGF when animal cap cells are pretreated either by brief disaggregation followed by reaggregation (Kengaku and Okamoto, 1995) or by incubation in very low Ca^{2+}, Mg^{2+} medium (Lamb and Harland, 1995). In these pretreated animal caps, high concentrations of bFGF induce posterior neural markers while lower concentrations tend to activate more anterior ones. It is worth noting that animal cap explants pretreated as above are not necessarily naive, as pointed out by Lamb and Harland (1995). The caps pretreated with transient disaggregation or in low divalent cation medium spontaneously express cement gland markers (but not neural markers), showing that these sensitized

cells have a different state of differentiation from that of untreated gastrula caps which are resistant to bFGF. In *Xenopus*, cement gland formation often accompanies neural induction although the mechanism underlying cement gland formation is still to be clarified (Sive and Bradley, 1996). One possible model is that cement gland induction and neural induction share the first step of differentiation cascade but require distinct signals for later steps (Sive and Bradley, 1996). Treatment of animal caps by transient disaggregation or with low Ca^{2+}, Mg^{2+} medium may mimic the signals that promote the first differentiation step, probably by attenuating BMP signaling (Lamb and Harland, 1995; Wilson and Hemmati-Brivanlou, 1995). In such conditions low FGF may cooperate with the activation step. At higher concentrations FGFs may mimic the transformation signal. A role for endogenous FGFs in the initial step of neural induction is supported by the observation that blocking FGF signaling by a dominant-negative FGF receptor in the animal cap prevents neural induction initiated by the organizer factors noggin and chordin in *Xenopus* animal caps (Launay *et al.*, 1996; Sasai *et al.*, 1996).

A-P PATTERNING II: VERTICAL VS PLANAR INDUCTION

It is believed that the organizer induces and patterns the neural plate in two different ways: by vertical signals emanating from the underlying chordamesoderm and by planar signals spreading through the plane of the neural plate (Ruiz i Altaba, 1992; Doniach, 1993). One of the unanswered questions in neural induction and patterning is to which extent vertical and planar signals function *in vivo*. Most of the molecular data discussed above on frog neural induction favor the idea of the vertical signals (Figs. 5 and 7). Chordin and noggin are expressed in the underlying chordamesoderm and encode soluble factors with strong neuralizing activities. In addition to chordin and noggin, the posterior chordamesoderm expresses eFGF (called FGF-4 in mammals), which could posteriorize the neural tissues induced by the organizer factors. Moreover, it has been shown that anterior axial mesoderm induces preferentially anterior neural structures while the posterior notochord induces spinocaudal tissue both in Einsteck experiments and animal cap sandwiches (Mangold, 1933; Hemmati-Brivanlou *et al.*, 1990). Similar observations have been reported in mice using ectoderm explants (Ang *et al.*, 1994).

The role of planar signals in amphibian neural induction is derived mostly from experiments with exogastrulae and Keller explants. In Keller explants the dorsal marginal zone is prevented from invaginating and the ectoderm proximal to the mesoderm expresses posterior neural markers while the distal ectoderm shows archencephalic characters and a cement gland (Doniach, 1993). In the exogastrula experiment invagination of the mesoderm is impaired by placing the embryo in high salt. While in salamanders exogastrulation blocks neural induction (Holtfreter, 1933), in *Xenopus* this is not always the case. In an important recent study Nieuwkoop and Koster (1995) have argued that in *Xenopus* planar induction can account for the transforming signal, but not for the initial neural induction. It has been long known that in *Xenopus* the prechordal endomesoderm has undergone extensive migration by stage $10\frac{1}{2}$ (when the external dorsal lip becomes visible) and underlies the supposedly naive ectoderm (Nieuwkoop and Florschütz, 1950; see also Bouwmeester *et al.*, 1996). When care was taken to prevent vertical induction by prechordal endomesoderm in *Xenopus* (for example by making exogastrulae at stage 9 before mesoderm involution), no neural differentiation was observed (Nieuwkoop and Koster, 1995).

Last, we would like to discuss another experiment that may shed light on the vertical vs planar issue. In *Rana pipens*, it is possible to disturb the normal involuting movement of mesoderm by using an integrin recognition peptide (Saint-Jeannet and Dawid, 1994). When the RGD oligo peptide is injected into the blastocoele of this frog, the migration of axial mesoderm does not occur in the direction from vegetal to animal as normal. Rather, it splits into two streams that involute horizontally along the equator, resulting in the formation of two ectopic notochords in the lateral region. In this case, two neural plates form along the two lateral notochords but not in the dorsal ectoderm where the planar signals would have spreaded (Saint-Jeannet and Dawid, 1994). This result suggests that the planar signals are not sufficient to direct the formation of the neural plate in the right place, at least in *Rana*. However, it is still conceivable that the planar signals alone could initiate neural differentiation but not maintain it *in vivo*. The vertical vs planar neural induction issue remains unresolved at this point in time.

CONCLUSIONS AND PROSPECTS

In this article ectodermal patterning of early vertebrate embryos has been reviewed in light of the ability of Spemann's organizer to impart D-V and A-P polarity. Due to space limitations, we did not touch on topics such as cement gland and placode induction, for which good reviews are available (Grainger *et al.*, 1992; Sive and Bradley, 1996). Several interesting molecular players in neural patterning have emerged and more probably will follow. The BMP signaling pathway may regulate both neural induction (the activation step on Nieuwkoop) and D-V patterning of the neural tube, raising the possibility that these two processes are related mechanistically. The signals emanating from the organizer and its derivatives, chordin, noggin, and follistatin, counteract BMP signals. The balance between organizer vs ventral BMP signals provides the ectodermal germ layer with its D-V positional information. Studies on the A-P patterning signals from the mesoderm have just begun, but data on the posteriorizing (or transformation signal of

Nieuwkoop) factors FGF, Wnt-3a, and RA hold great promise. Prepatterning of the animal cap ectoderm (Sharpe *et al.*, 1987) is an important issue and in future it will be worth investigating how much of the predisposition can be attributed to differential distribution of known factors such as BMP-4. On the other hand, very little is known about the regional specification of the skin ectoderm during early embryogenesis; new region-specific early markers, such as those available in the neural plate itself, will be necessary to address this question. In this review, we discussed ectoderm patterning signals emanating from the mesoderm or from the ectoderm itself. The other germ layer, the endoderm, is also a classical source of inductive signaling (reviewed in Jacobson, 1966), whose molecular character remains to be clarified. In this context, *Xenopus* cerberus (Bouwmeester *et al.*, 1996), a new neuralizing factor secreted by the anterior endomesoderm of Spemann's Organizer, is an attractive molecule for future studies.

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Note added in proof. After this review was completed we learned that Professor Pieter Nieuwkoop passed away in September 1996. We dedicate this review to his memory.

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