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Neural induction and factors that stabilize a neural fate

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Abstract

The neural ectoderm of vertebrates forms when the BMP signaling pathway is suppressed. Herein we review the molecules that directly antagonize extracellular BMP and the signaling pathways that further contribute to reduce BMP activity in the neural ectoderm. Downstream of neural induction, a large number of "neural fate stabilizing" (NFS) transcription factors are expressed in the presumptive neural ectoderm, developing neural tube, and ultimately in neural stem cells. Herein we review what is known about their activities during normal development to maintain a neural fate and regulate neural differentiation. Further elucidation of how the NFS genes interact to regulate neural specification and differentiation should ultimately prove useful for regulating the expansion and differentiation of neural stem and progenitor cells.

Keywords

BMP; FGF; Wnt; neural stem cells; transcriptional network; neural fate stabilization

Introduction

Nearly 100 years ago, Hilde Mangold and Hans Spemann (1924) excised small bits of tissue from the point at which cells first involute in the amphibian gastrula and grafted these into the ventral belly region of host embryos. Surprisingly, a secondary body axis formed at the transplant site, and it included a neural tube and axial mesoderm (notochord, somites). By using explants and hosts from differently pigmented species so that the donor cells could be identified, they demonstrated that the transplanted piece, now known as Spemann's "organizer", did not give rise to neural ectoderm, but instead formed dorsal mesoderm. They reasoned that the transplanted cells signaled adjacent host cells to become the neural tissue of the secondary axis; thus, the phenomenon of neural induction was discovered. Equivalent organizing regions with neural inducing capacity are found in zebrafish (shield), chick (Henson's node), and mouse (node) embryos (Beddington, 1994; Shih and Fraser, 1996; Smith and Schoenwolf, 1998; Boettger et al., 2001). Hence, neural induction is one of the primary steps in vertebrate neural development, and understanding how it is accomplished is of fundamental importance.

Since this landmark observation, numerous studies focused on identifying the inducing molecule(s) that are necessary to establish neural fate in vertebrate embryos. In addition, a large number of transcription factors whose expression is initiated by neural inductive signaling was identified. How these factors cooperatively stabilize the neural fate of embryonic cells is only beginning to be appreciated. While understanding the acquisition of

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neural fate during embryogenesis is exciting in its own right, it also appears that these same signaling factors and transcriptional regulators may be expressed in endogenous niches of neural stem cells in the adult brain. Therefore, the developmental program that specifies a neural fate in the embryo may prove to be critically important for coaxing neural stem cells to repopulate damaged tissue. This review will summarize the current state of knowledge of the process of neural induction, the genes involved in neural fate stabilization, and speculate on how this information may play a role in regulating the differentiation of neural stem cells.

Neural Induction

The organizer forms in the dorsal mesoderm in response to secreted factors from a signaling center located in the adjacent presumptive dorsal endoderm, which in amphibians is called the Nieuwkoop Center (Nieuwkoop, 1967; Gerhart et al., 1989; Agius et al., 2000; Vonica and Gumbiner, 2007). In *Xenopus*, the Nieuwkoop Center forms where the expression domains of VegT, Vg1, Nodal-related (Nr) proteins, and β-Catenin overlap (Fig. 1). The Spemann-Mangold experiment predicted that a neural inducing substance emanated from the organizer. While several studies sought to identify the neural inducer(s), more recent work led to the hypothesis that ectoderm forms neural tissue not by an activating signal, but by default (Sato and Sargent, 1989; Wilson and Hemmati-Brivanlou, 1995; Hawley et al., 1995; Weinstein and Hemmati-Brivanlou, 1997). Pieces of naïve embryonic ectoderm dissected from the animal pole of *Xenopus* blastulae (called animal cap [AC] ectodermal explants) form non-neural epidermis when cultured without the addition of growth factors (Fig. 2). However, if the AC ectodermal explant is first dissociated into single cells, the cells become neural, indicating that: (1) short range cell-to-cell signaling is required for the formation of epidermis, and (2) in the absence of this signaling, neural tissue forms by default (Sato and Sargent, 1989; Wilson and Hemmati-Brivanlou, 1995). In support of this hypothesis, experiments showed that the inhibition of transforming growth factor-β (TGF-β) signaling through the use of a dominant-negative Activin receptor caused AC ectodermal explants to express neural marker genes (Hemmati-Brivanlou et al., 1992; Hemmati-Brivanlou and Melton, 1994; Hemmati-Brivanlou and Thomsen, 1995; Hawley et al., 1995). However, the dominant-negative Activin receptor interferes with signaling of all of the members of the TGF-β family, including TGF-β, Activin, Nr, and bone morphogenetic proteins (BMPs). Further work demonstrated that BMPs are the important ligands for neural *versus* epidermal fate induction.

BMP signaling

Several studies pointed to the BMP signaling pathway as key for neural induction, but as a negative regulator, not a positive inducer. First, using expression screening techniques to identify novel genes that induce a neural fate in AC ectodermal explants, secreted antagonists of BMPs were discovered. These proteins are secreted from the organizer (Fig. 1), and include Noggin (Zimmerman et al., 1996), Chordin (Piccolo et al., 1996), Follistatin (Fainsod et al., 1997), Cerberus (Bouwmeester et al., 1996), and XNr3 (Smith et al., 1995). They bind to BMP in the extracellular space and inhibit activation of its receptor (Fig. 3); when this occurs in the naïve embryonic ectoderm, neural tissue forms instead of epidermis (Meinhardt, 2001;Vonica and Gumbiner, 2007). Second, when expression of one of these secreted proteins in the organizer is blocked, BMP signaling is maintained on the dorsal side of the embryo and the neural plate does not form (Wessely et al., 2004). Third, when dominant-negative BMP4 protein, dominant-negative BMP receptors, or any of the BMP antagonists are expressed in AC ectodermal explants, anterior neural genes are directly induced. Similarly, when these proteins are expressed on the ventral side of embryos, a secondary axis containing neural tissue forms (Hawley et al., 1995).

Mutational analysis and knock-down experiments in zebrafish, mouse, and frog embryos also demonstrate that BMP inhibition is required to form neural tissue. In zebrafish, double mutants for the BMP antagonists, *chordino* (a Chordin homolog) and *ogon* (a secreted Frizzled homolog), have a ventralized phenotype (Miller-Bertoglio et al., 1999; Yabe et al., 2003). *Xenopus tropicalis* embryos depleted of Chordin, Noggin, and Follistatin protein have nearly a complete loss of the central nervous system (CNS) (Khokha et al., 2005). Mouse mutants lacking the BMP antagonists, Chordin (Bachiller et al., 2000), Noggin (McMahon et al., 1998), or Cerberus (Belo et al., 2000), express only posterior neural genes, and double mutants for Noggin and Chordin fail to develop anterior brain structures (Bachiller et al., 2000). These studies indicate that across vertebrates there is a conserved and necessary role for BMP inhibition in the induction of anterior neural tissue.

There are more than 30 known BMP proteins (Balemans and Van Hul, 2002), but it is BMP4 that is mainly involved in epidermal induction and neural inhibition (Sasai et al., 1995; Wilson and Hemmati-Brivanlou, 1995; Xu et al., 1995). Its expression is restricted to the non-neural ectoderm and ventral mesoderm, and over-expression ventralizes embryos (Fainsod et al., 1994; Hemmati-Brivanlou and Thomsen, 1995). A number of BMP target genes have been identified and these include *Msx1* (Foerst-Potts and Sadler, 1997; Tucker et al., 1998; Feledy et al., 1999; Ishimura et al., 2000; Takeda et al., 2000; Yamamoto et al., 2000; Yamamoto et al., 2001; Tribulo et al., 2003), *Msx2* (Foerst-Potts and Sadler, 1997), *Gata2* (Walmsley et al., 1994; Friedle and Knochel, 2002), *Vent1* (Gawantka et al., 1995; Onichtchouk et al., 1998; Friedle et al., 1998; Rastegar et al., 1999), and *Vent2* (Ladher et al., 1996; Onichtchouk et al., 1996; Onichtchouk et al., 1998; Rastegar et al., 1999; Trindade et al., 1999; Friedle and Knochel, 2002). *Msx2* is not likely to be involved in inhibiting the onset of expression of early neural genes because it is not expressed until the mid-gastrula stage, which is after neural induction occurs. In contrast, *Msx1*, *Gata2*, *Vent1*, and *Vent2* are expressed throughout the embryonic ectoderm of the animal pole of blastula embryos, and as gastrulation proceeds their expression domains are restricted to the ventral tissues in a pattern similar to that of *BMP4* (Hemmati-Brivanlou and Thomsen, 1995).

Gain-of-function studies indicate that Msx1, Vent1, and Vent2 are involved in restricting the expression of neural genes to the dorsal ectoderm. Injection of *Msx1* mRNA inhibited neural tissue formation induced by Noggin (Ishimura et al., 2000), and over-expression of a dominant-activator form of Msx1 prevented ventralization by BMP. However, whereas knock-down of Msx1 function with anti-sense morpholino oligonucleotides (MOs) demonstrated that it is required for the ventralizing activity of BMP4, it is not required for epidermal development, in hibition of neural induction, or axis formation (Suzuki et al., 1997; Yamamoto et al., 2000). Knock-down experiments have not been reported for the Vent proteins, but over-expression of Vent1 (Gawantka et al., 1995) or Vent2 (Ladher et al., 1996; Onichtchouk et al., 1996; Friedle and Knochel, 2002) induces epidermis and inhibits the formation of both dorsal mesoderm and neural tissue. Additionally, both Vent proteins are required to restrict expression of reporter constructs of *Geminin* (Taylor et al., 2006) and *Sox3* (Rogers et al., 2008), two early neural ectoderm transcription factors (discussed below), to the dorsal ectoderm.

Even though inhibition of BMP signaling is sufficient to induce neural tissue in AC ectodermal explants, it is not sufficient to induce it in the ventral, non-neural ectoderm of the embryo (Wilson and Hemmati-Brivanlou, 1995; Hawley et al., 1995; Rogers et al., 2008). For example, inhibition of BMP signaling in ventral ectoderm via over-expression of Smad6, an inhibitor of the BMP effector Smad1, repressed epidermis formation but failed to induce ectopic expression of neural markers (Delaune et al., 2005; Chang and Harland, 2007). It is possible that either a second downstream pathway needs to be silenced. For example, BMP inhibition is sufficient to induce neural tissue in ventral ectoderm if signaling

through both Smad1 and Smad2 are inhibited (Chang and Harland 2007). There also is evidence that additional instructive or permissive signals are required. Several experiments in frog and chick demonstrate that neural tissue is induced in non-neural ectoderm when BMP signaling is inhibited in the presence of fibroblast growth factor (FGF) (Delaune, Lemaire et al. 2005; McMahon and Moon, 1989; Christian et al., 1991a; Rodriguez-Gallardo et al., 1997; Alvarez et al., 1998; Wilson et al., 2001).

FGF signaling

In frog, there are four known FGF receptors (FGFR1-4α) implicated in neural development due to their expression in the ectoderm prior to neural induction and the subsequent restriction of their expression to neural tissue. Because *FGFR2* and *FGFR4α* are up-regulated at the onset of neural induction, they may play a specific role in the process (Hongo et al., 1999). To determine if FGF signaling is required for neural induction, signaling was inhibited by incubation in SU5402, a chemical inhibitor of FGF signaling (Mohammadi et al., 1997), or by over-expression of a dominant-negative FGFR1 receptor (XFD) (Amaya et al., 1991) or a dominant-negative FGFR4α receptor (Δ4α) (Hongo et al., 1999). XFD inhibits FGF3 and FGF4 signaling through FGFR1, FGFR2, and FGFR3, whereas Δ4α blocks FGF8 signaling through FGFR4α (Ueno et al., 1992; Hongo et al., 1999; Bainter et al., 2001). XFD and SU5402 inhibited the induction of neural tissue in neuralized AC ectodermal explants as indicated by a loss of the pan-neural markers, *Ncam* and *Sox2* (Launay et al., 1996; Ribisi et al., 2000; Delaune et al., 2005). Over-expression of Δ4α led to a loss of anterior neural tissue (marked by *Nrp1*, *Bf1*, and *En2*) in whole embryos and in neuralized AC ectodermal explants (Hongo et al., 1999). Furthermore, although dissociated AC ectodermal explants are thought to be neuralized by the reduction in levels of BMP protein due to cell dispersal, recent studies showed that BMP signaling still occurs in these cultures (Hurtado and De Robertis, 2007), and that MAP kinase signaling may drive the conversion of cells to neurons (De Robertis and Kuroda, 2004; Kuroda et al., 2005). While these studies suggest that FGF signaling is required for the formation of neural tissue in dissociated AC ectodermal explants, disruption of FGF signaling via the MAP kinase pathway by inhibiting Ras did not affect the induction of anterior neural genes, but inhibited the posteriorization of neural tissue in AC ectodermal explants (Ribisi et al., 2000). This is consistent with several previous studies indicating that FGF is required for the anterior-posterior patterning of the neural plate (reviewed in Gould and Grainger, 1997; Gamse and Sive, 2000). Moreover, treatment of embryos with SU5402 decreased some dorsal mesodermal markers, including *Noggin*; thus, the decrease in neural tissue may be accomplished indirectly by reducing the amount of dorsal mesoderm available to induce it (Delaune et al., 2005). Taken together, these experiments demonstrate that signaling through FGFR4α may be required for the induction of anterior neural tissue, and that FGFR1, FGFR2, and MAP kinase signaling are required for posteriorizing neural tissue.

Other studies demonstrate a role for FGF in neural induction; however, they do not demonstrate that the function of FGF is independent of its ability to inhibit *BMP* transcription or the BMP signaling pathway (Baker et al., 1999; Hongo et al., 1999; Ishimura et al., 2000; Wilson et al., 2000; Wilson et al., 2001; Pera et al., 2003; Sheng et al., 2003; Rentzsch et al., 2004; Wittler and Kessel, 2004; Linker and Stern, 2004; Delaune et al., 2005). In chick and zebrafish, FGF signaling inhibits *BMP* gene expression (Furthauer et al., 1997; Wilson et al., 2000), and in frog and zebrafish, FGF signaling inhibits BMP signaling through the phosphorylation and inactivation of its effector protein, Smad1 (Schier, 2001; Pera et al., 2003). In this manner, FGF may cooperate with or be redundant with the activity of extracellular BMP antagonists. Taken in total, these data support a role for FGF in patterning the anterior-posterior axis of neural tissues and in reinforcing the antagonism of the BMP pathway, but its requirement for neural induction independent of

BMP inhibition remains in question (Lamb and Harland, 1995; Wilson et al., 2000; Takemoto et al., 2006; Rogers et al., 2008; Weisinger et al., 2008).

Wnt signaling

Maternal Wnt signaling and the inhibition of zygotic Wnt are important for the formation of neural tissue. The establishment of the dorsal-ventral axis and formation of the Nieuwkoop Center are dependent on the dorsal accumulation of the Wnt effector, β-Catenin (Larabell et al., 1997). However, it is unclear if there is a specific role for Wnt signaling in neural induction. Supporting a requirement for Wnt signaling in axis formation, over-expression of either Wnt1 or Wnt3a, which are expressed in neural tissue, induced a secondary axis (Steinbeisser et al., 1993). However, their ability to induce neural tissue in naïve ectoderm has not been tested. The strongest evidence for a role for Wnt signaling in neural induction were experiments demonstrating the induction of *Ncam* in AC ectodermal explants in the absence of mesoderm formation in response to mRNA injections of either *Wnt8* or an activated form of *β-Catenin*. Both inhibited *BMP4* expression, leading to the proposal that Wnt signaling sensitizes AC ectodermal explants to extracellular BMP inhibition (Baker et al., 1999). However, it is worth noting that *Wnt8* normally is expressed in the ventral ectoderm, not the dorsal, presumptive neural ectoderm (Christian et al., 1991b), and in whole embryos it induces dorsal mesoderm (Otte and Moon, 1992), indicating an indirect effect. Also, loss of Wnt signaling by over-expression of a dominant-negative transcriptional effector (TCF3) or a dominant-negative receptor (Frizzled8) did not interfere with the induction of neural markers by Noggin (Rogers et al., 2008). Thus, there is little evidence for a direct role of Wnt signaling in neural induction.

In fact, studies in both frog and chick support a requirement for the inhibition of Wnt signaling for neural induction. In frog, over-expression of a dominant-active form of β-Catenin inhibited the expression of *Sox2* and *Sox3* in whole embryos and inhibited the induction of *Sox3* by Noggin in AC ectodermal explants (Heeg-Truesdell and Labonne, 2006). Similarly, over-expression of Wnt3a in chick embryos inhibited both the neuralization of ectoderm by BMP inhibition and FGF signaling (Wilson et al., 2001). However, like FGF, Wnt signaling does play an important role in the anterior-posterior patterning of the neural plate (Gould and Grainger, 1997; Gamse and Sive, 2000).

Neural Fate Stabilization

As the presumptive neural ectoderm is established via neural inductive signaling, a large number of transcription factors is co-expressed in broad overlapping domains (Fig. 4; Sasai, 1998;Moody and Je, 2002). The mRNAs of several of these genes (*Geminin, Sox3, Sox11, SoxD*) are found throughout the dorsal ectoderm at the onset of gastrulation. The transcripts of others (*FoxD5, Sox2, Zic1, Zic2, Zic3*) are concentrated in a broad band near the blastoporal lip. The transcripts of a third group (*Xiro1, Xiro2, Xiro3*) are detected in two dorso-lateral bands near the blastoporal lip. Although a few are expressed maternally, most are expressed around the onset of gastrulation and continue to be expressed in the neural tube (Fig. 4). Experiments in *Xenopus* demonstrated that while most of these transcription factors do not induce ectopic neural tissue on their own, when they are over-expressed dorsally by mRNA injection, they all expand the neural plate. Previous studies propose that some of these genes modulate the competence of neural ectoderm to respond to signaling molecules (*Sox2, Sox3, Zic1, Zic3*), some maintain an immature neural state (*Geminin, FoxD5, Zic2*), whereas others promote the expression of neural determination/differentiation bHLH genes (*SoxD, Sox11, Xiro1, Xiro2, Xiro3*). How these transcription factors relate to each other to stabilize a neural fate and regulate the progression from the initially-induced neural ectoderm to neural progenitor cells has yet to be revealed.

Sox genes

Sox genes are named for their similarity to the testis determination factor Sry, and a conserved high-mobility group (HMG) (Sry-related HMG-box) domain that confers their DNA binding ability (Gubbay et al., 1990; Sinclair et al., 1990; Wegner, 1999). They are transcription factors that bind to the minor groove of DNA, inducing a sharp bend and regulating gene transcription (Guth and Wegner, 2008). Of the ten groups of Sox proteins, only SoxD (a *Xenopus* member of the SoxG group; Wegner, 1999), SoxB (Sox1, 2, 3, 14, 21), SoxC (Sox4 and 11), and SoxE (Sox8, 9, 10) groups are involved in neural induction and development (Guth and Wegner, 2008).

Sox2 and *Sox3* are well documented early pan-neural markers (Uwanogho et al., 1995; Collignon et al., 1996; Penzel et al., 1997; Rex et al., 1997; Mizuseki et al., 1998a; Uchikawa et al., 1999; Graham et al., 2003; Linker and Stern, 2004; Takemoto et al., 2006; Rogers et al., 2008), and are part of the SoxB1 (*Sox1, Sox2, Sox3*) subfamily of transcriptional activators (Penzel et al., 1997; Uchikawa et al., 1999). They are both expressed in neural progenitor cells throughout CNS development and are required for neural progenitor maintenance (Graham et al., 2003; Ellis et al., 2004; Pevny and Placzek, 2005; Rogers et al., 2009). Their expression patterns are similar but not identical (Fig. 4). *Sox3* is expressed maternally and is pan-ectodermal until the mid-gastrula stage when expression is restricted to the dorsal ectoderm (Penzel et al., 1997). In contrast, *Sox2* expression begins at the onset of neural induction only in the neural ectoderm (Nitta et al., 2006). Other differences include: *Sox3* is repressed in the floor plate of the neural tube prior to *Sox2*, expressed much more strongly in the otic placodes, and expressed in the developing lens, while *Sox2* is expressed in the retina (Penzel et al., 1997; Elkins and Henry, 2006; Nitta et al., 2006; Rogers et al., 2008). These differences in their spatio-temporal expression patterns indicate that they are regulated differently, and promoter studies and bioinformatic analysis indicate that this is true (Uchikawa et al., 2003; Rogers et al., 2008). The *Sox2* regulatory region has a neural induction module that is conserved across species, and in chick this module responds to both FGF and Wnt signaling for activation in the posterior neural plate (Takemoto et al., 2006). This module is present upstream of *Xenopus tropicalis Sox2*, but is not in the flanking sequence of *Sox3*. Thus far, reporter studies in transgenic frog embryos reveal that *Xenopus Sox3* expression is induced by the inhibition of BMP signaling and is restricted to the neural plate by the BMP targets, Vent1 and Vent2 (Rogers et al., 2008).

Rescue experiments indicate that the SoxB1 proteins are redundant in tissues in which they are co-expressed. In mouse, loss of *Sox2* in the CNS is compensated for by the up-regulation of *Sox3* expression. Neurogenesis is decreased in these mice, but neural stem cells retain multipotency (Miyagi et al., 2008). In chick, over-expression of *Sox1* rescues the loss of *Sox2* (Graham et al., 2003). Although the SoxB1 proteins can compensate for each other, each gene is also expressed in unique tissues during development and loss in that tissue can result in severe phenotypes. Mice mutant for either *Sox2* or *Sox3* have relatively normal CNS formation (Malas et al., 2003; Taranova et al., 2006). However, loss of *Sox2* results in diminished neurogenesis in the mouse retina (Ferri et al., 2004) and anophthalmia in humans (Fantes et al., 2003). *Sox3*-null mice have craniofacial abnormalities and defective pituitary development (Rizzoti et al., 2004; Rizzoti and Lovell-Badge, 2007), and *Sox1*-null mice suffer from epilepsy due to a complete loss of neurons in the ventral striatum (Malas et al., 2003; Ekonomou et al., 2005).

In early neural development, vertebrate SoxB1 proteins function as transcriptional activators (Bylund et al., 2003; Graham et al., 2003; Uchikawa et al., 1999). It has been proposed that they counteract neuronal differentiation induced by the SoxB2 subfamily of repressor proteins (Sox14 and Sox21) (Bylund et al., 2003). In this way, the two subgroups of SoxB

proteins together may maintain a balance between proliferation and differentiation. In concordance, over-expression of both *Sox2* and *Sox3* in frog and zebrafish leads to a loss of neurogenesis in the cranial placodes (Dee et al., 2008; Schlosser et al., 2008) and expansion of neural progenitors in the frog and chick neural tube at the expense of epidermal development and neuronal differentiation (Kishi et al., 2000; Graham et al., 2003; Rogers et al., 2009).

The SoxC gene, *Sox11*, is expressed in the developing nervous system of chick (Uwanogho et al., 1995), mouse (Hargrave et al., 1997), zebrafish (de Martino et al., 2000), and frog (Hyodo-Miura et al., 2002; Brugmann et al., 2004). *Sox11* is induced in response to BMP inhibition by Chordin (Hyodo-Miura et al., 2002) and by ectopic expression of the FoxD5 transcription factor (Yan et al., 2009). In *Xenopus* animal cap assays, *Sox11* expression induces pan-neural (*Ncam*) and anterior neural (*Otx2*, *En2*) markers through an interaction with the MAP kinase, NLK (Hyodo-Miura et al., 2002). Sox11 is thought to be functionally redundant with the other SoxC proteins, Sox4 and Sox12, due to their overlapping expression patterns, ability to bind to the same DNA gene sequence, and weak single gene knockout phenotypes in mouse (Hoser et al., 2008; Dy et al., 2008). In chick, while *Sox11* is expressed in the neural epithelium, it also is up-regulated in maturing neurons (Uwanogho et al., 1995), and functions in the differentiation of neural progenitors into neurons (Bergsland et al., 2006).

SoxD, a member of the SoxG group, is unique to amphibians (Mizuseki et al., 1998b, Guth and Wegner, 2008,). It is first detected at late blastula stages throughout the embryonic ectoderm, and like *Sox3*, it is then restricted to the dorsal ectoderm during gastrulation. *SoxD* is induced in response to the inhibition of BMP signaling, and like *Sox2*, can be induced in AC ectodermal explants by *Zic1*. Little is known about the requirement for FGF or Wnt signaling for the expression of *SoxD*, although the MEK5/ERK5 pathway has been implicated in its neuralizing activity (Nishimoto et al., 2005). Over-expression of *SoxD* in whole embryos expanded neural progenitors marked by *Nrp1*, induced the neural determination bHLH gene *Ngnr1*, and inhibited epidermal gene expression. Inhibition of *SoxD* by a dominant-negative construct suppressed neural tissues, a phenotype that was not rescued by *Sox2*, indicating that *SoxD* acts downstream (Mizuseki et al., 1998b).

Geminin

Geminin is a novel coiled-coil protein whose mRNA is expressed in a similar pattern as *Sox3* (Fig. 4); it is maternally expressed and ubiquitous in the animal pole ectoderm until gastrulation when it is first enriched dorsally, and then restricted to the neural ectoderm (Kroll et al., 1998). After gastrulation, *Geminin* expression is pan-neural with an anterior bias and encompasses a region wider than that of either *Sox2* or *Sox3* (Kroll et al., 1998;Kroll, 2007). *Geminin* expression can be induced by inhibiting BMP signaling, and it is maintained in the proliferative regions of the developing nervous system (Kroll, 2007). Geminin maintains a neural progenitor population by inhibiting re-initiation of DNA replication, thereby maintaining chromosomal integrity and preventing cell cycle exit (Seo and Kroll, 2006). The latter is controlled by an antagonistic interaction with the catalytic subunits of the SWI/SNF complex, Brg1 and Brahma (McGarry and Kirschner, 1998;Seo et al., 2005). Over-expression of *Geminin* inhibits epidermal development and neuronal differentiation, and expands the neural progenitor population (Kroll et al., 1998;Papanayotou et al., 2008). It has been suggested that *Geminin* maintains cell cycling during neural plate stages and represses various aspects of differentiation, allowing for the expansion of neural ectoderm (Luo and Kessel, 2004;Pitulescu et al., 2005;Seo and Kroll, 2006).

Experiments using a human *Geminin* regulatory region driving GFP expression indicated that as for *Sox3*, Vent1 and Vent2 restrict *Geminin* to the dorsal side of the embryo (Kroll et

al., 1998). Dorsal expression of this *Geminin* reporter construct required positive regulation by the Wnt signaling effector, TCF3 (Taylor et al., 2006). Additionally, recent experiments demonstrated that *Geminin* expression is activated directly by two other early neural proteins, Sox3 (Rogers et al., 2009) and FoxD5 (Yan et al., 2009).

FoxD5

FoxD5 is a member of the *forkhead/winged helix* family of transcription factors (Solter et al., 1999; Fetka et al., 2000; Sullivan et al., 2001). Although there are homologous genes in zebrafish (*fkd8*; Odenthal and Nusslein-Volhard, 1998), mouse (*FoxD4/Fkh2*; Kaestner et al., 1995), and human (*FOXD5* [2q13]; Katoh and Katoh, 2004), no functional information is currently available for any of these. In *Xenopus*, *FoxD5* maternal transcripts are localized to the animal pole, similar to those of *Sox3* and *Geminin*. However, at gastrulation, *FoxD5* zygotic transcripts are more restricted than those of *Sox3* and *Geminin* in the presumptive neural ectoderm (Fig. 4). Unlike the other early neural transcription factor genes, *FoxD5* expression is not maintained throughout neural development, but is extinguished as the neural folds elevate and fuse, except at the midbrain/hindbrain junction and the tail bud. Also, unlike the other neural genes, *FoxD5* is only weakly induced by Noggin, not induced by Chordin, but is induced strongly by the maternally regulated Siamois pathway via Cerberus (Sullivan et al., 2001).

It is proposed that FoxD5, like Geminin and the SoxB1 proteins, functions to maintain a proliferating neural progenitor population. Dorsal injections of *FoxD5* mRNA expanded expression of other early neural genes (*Sox2, Sox3, Otx*2), and repressed neural patterning (*En2, Krox20*) and neural differentiation (*Ngnr1, NeuroD, N-tub*) genes (Sullivan et al., 2001). While expression of *FoxD5* induced several neural genes in AC ectodermal explants (Sullivan et al., 2001), it only induces a few of these in the ventral ectoderm of embryos (Yan et al., 2009), suggesting that it acts downstream of neural induction. Microinjections of *FoxD5* dominant-activator and dominant-repressor constructs revealed that FoxD5 functions as a transcriptional repressor to expand the neural plate and deletion analysis revealed that it is dependent on the C-terminal domain of the protein. These results indicate that *FoxD5* contributes to maintaining an undifferentiated neural ectoderm during the early steps of neural plate formation.

Zic genes

Three *Zic* genes (*Zic1, Zic2, Zic3*), members of the zinc finger family of transcription factors, are expressed in the early neural ectoderm (Fig. 4). All three are expressed in the presumptive neural ectoderm during early gastrulation, and later become restricted to the lateral margins of the neural plate in mouse, fish, and frog (reviewed in Moody and Je, 2002). In *Xenopus*, *Zic1* and *Zic3* are transcribed 30-60 minutes after the *Chordin* gene, and can be induced in AC ectodermal explants by BMP antagonism (Nakata et al., 1997;Kuo et al., 1998;Mizuseki et al., 1998a;Nakata et al., 1998), indicating that they are likely transcribed in immediate response to neural induction. Expression of *Zic1* allows ectoderm to be more sensitive to neural induction by Noggin (Kuo et al., 1998), confirming its role in neural fate stabilization. Over-expression of *Zic1* and *Zic3* expands the neural plate and neural crest, and concomitantly represses epidermal fate. In AC ectodermal explants, Zic1 activates other early neural markers (*Nrp1, SoxD*) in the absence of mesoderm induction. Zic1 and Zic3 both expand the expression of neural determination/differentiation bHLH genes (Mizuseki et al., 1998a), suggesting that they promote the transition to neural differentiation.

Zic2 is expressed in a similar pattern to *Zic1* and *Zic3* (Brewster et al., 1998; Nakata et al., 1998), and it similarly expands the neural plate and neural crest, and reduces epidermal

markers (Brewster et al., 1998). In contrast, *Zic2* over-expression represses neural determination/differentiation bHLH genes, and can counteract the formation of ectopic neurons produced by *Ngnr1* mRNA injection. These studies suggest that during neural plate formation Zic1 and Zic3 promote the onset of neural differentiation, whereas Zic2 maintains cells in a more immature progenitor state.

Iroquois genes

Genes within the *Drosophila Iroquois* complex (*Iro/Irx*) encode homeodomain proteins that are required for the activation of proneural bHLH genes (Gomez-Skarmeta et al., 1996; Gomez-Skarmeta and Modollel, 2002). Several vertebrate homologues have been cloned, three of which in *Xenopus* (*Xiro1, Xiro2, Xiro3*) are expressed in restricted bands in the neural ectoderm (Fig. 4) just prior to the earliest expressed neural determination bHLH genes (*Ngnr1* and *NeuroD*). Later in development, *Xiro1-3* are expressed only in the dorso-lateral neural tube and neural crest. All three *Xiro* genes are induced only weakly by Noggin, but their expression is greatly enhanced by the addition of posteriorizing factors, such as Wnt and FGF. These data indicate that they are expressed downstream of the afore-described early neural genes, which do not require posteriorizing factors for their induction. In *Xenopus*, over-expression of each of the three *Xiro* genes causes the neural plate to expand, and promotes the onset of neural differentiation (Bellefroid et al., 1998; Gomez-Skarmeta et al., 1998), but suppresses terminal differentiation into neurons (de la Calle-Mustienes et al., 2002). Thus, these genes are predicted to function in a position intermediate between the above-described early neural genes and the neural determination/differentiation bHLH genes (Fig. 5).

Do early neural transcription factors interact to regulate neural fate stabilization?

Because of their overlapping expression patterns and their common over-expression phenotype of expanding the neural plate, it seems likely that together these early neural genes coordinately stabilize the newly induced neural fate, regulate neural plate formation, and control the onset of neural differentiation. However, our understanding of how this is accomplished is woefully incomplete. Several studies indicate that one aspect of stabilizing neural fate is by modifying the signaling pathways involved in neural induction. For example: (1) in the presence of Zic1 a lower concentration of Noggin is required to induce neural ectoderm (Kuo et al., 1998); (2) Sox11 induces neural marker genes by antagonizing Wnt signaling (Hyodo-Miura et al., 2002); and (3) Geminin, Sox3, and Xiro1 each antagonize some aspect of the BMP4 signaling pathway (Kroll et al., 1998; Glavic et al., 2001; Gomez-Skarmeta et al., 2001; Rogers et al., 2008), and FoxD5 reduces the nuclear localization of the BMP signaling effectors, phosphorylated SMAD1/5/8, reduces the ventral epidermal expression of BMP4 target genes, and up-regulates the expression of Szl, a secreted BMP antagonist (Yan et al., 2009). Thus, one aspect of neural fate stabilization appears to be the regulation of the strength of signaling pathways, in particular that of BMP4, in the neural ectoderm. However, we know virtually nothing about how these several early neural genes, henceforth called neural fate stabilizing (NFS) genes, may cooperatively cause these effects.

The over-expression studies reviewed above indicate that the NFS genes can be placed into two functional groups: those that promote an immature, undifferentiated neural state (*Geminin, Sox2, Sox3, FoxD5, Zic2*), and those that promote the onset of neural differentiation (*SoxD, Sox11, Zic1, Zic3, Xiro1, Xiro2, Xiro3*). Are the genes within each group simply redundant, or do they have distinct roles in the transition from neural induction to neural differentiation? Answers to these questions are only just beginning to be experimentally addressed.

Recently, as a first step in putting the NFS genes into transcriptional order, we found that when endogenous FoxD5 levels in the neural ectoderm were reduced by targeted injection of anti-sense MOs, the expression of each of the 11 other NFS genes was down-regulated (Yan et al., 2009). This suggests that FoxD5 is necessary for either the induction or the maintenance of their expression. As expected from previous studies (Sullivan et al., 2001), over-expression of FoxD5 expanded the neural plate and thus the domains of each of the 11 other NFS genes; this was a cell non-autonomous effect because the neural plate was expanded equally well in regions devoid of the FoxD5-expressing cells, which were marked by a lineage tracer. However, when only the lineage-tagged cells expressing elevated levels of FoxD5 were examined, several responses were observed: (1) some genes that promote an immature neural fate (*Geminin, Zic2*) were up-regulated; (2) *Sox11*, which previously had been characterized as promoting neural differentiation (Bergsland et al., 2006), also was up-regulated; (3) some genes that promote an immature neural fate but are required for neural differentiation (*Sox2, Sox3*) were transiently and weakly repressed; and (4) genes that promote the onset of neural differentiation (*Zic1, Zic3, SoxD, Xiro1, Xiro2, Xiro3*) were strongly repressed. Thus, FoxD5 appears to maintain an immature neural state by differentially regulating genes that either repress or promote neural differentiation. Further experiments demonstrated that FoxD5 directly regulates *Geminin, Sox11*, and *Zic2* transcription, and that these three genes together phenocopy FoxD5 over-expression and rescue FoxD5 MO-mediated knock-down phenotypes.

These results indicate that the NFS genes coordinately stabilize neural fate and regulate the initiation of neural differentiation, and data from several laboratories predict a NFS gene regulatory network (Fig. 5). FoxD5 acts upstream of the other NFS genes, primarily by regulating *Geminin, Sox11*, and *Zic2*. This transcriptional triad cooperatively carries out most of the effects of FoxD5 on the other NFS genes. They also regulate each other's expression, indicating a network rather than a linear path of transcriptional regulation. There also are mutual interactions between Geminin and SoxB1 genes. Chick Geminin interacts in a complex at the N2 enhancer to promote *Sox2* transcription (Papanayotou et al., 2008), and frog Sox2 and Sox3 directly regulate *Geminin* expression (Rogers et al., 2009). As mentioned above, Zic1 up-regulates both *Sox2* and *SoxD* expression. In addition, those genes that promote the onset of neural differentiation (*Zic1, Zic3, SoxD, Xiro1, Xiro2, Xiro3*) feedback to repress *FoxD5* expression, perhaps to allow the neural ectodermal cells to initiate differentiation (Yan et al., 2009). These studies are just the beginning of defining the regulatory network that promotes the transition from neural induction to differentiation. Much more work is needed to fully understand the gene interactions that comprise this network, and to determine what other factors are involved.

How Might NFS Genes Regulate Neural Stem Cells?

Stem cell therapy is expected to have widespread benefits to patients suffering from neurodegenerative diseases and neuronal loss due to trauma, stroke and congenital disease. Although the mature CNS harbors niches of self-renewing neural stem cells (Doetsch et al., 1999; Alvarez-Buylla et al., 2001), these make a significant contribution to only a few areas of brain (Cameron and McKay, 2001; Pencea et al., 2001). Therefore, many laboratories world-wide are researching how to manipulate different sources of stem cells to produce specific types of neurons for therapeutic treatments. It is most exciting that many of the NFS genes have already been implicated in controlling the differentiation of neural stem and progenitor cells. *Sox2* and *Sox3* are necessary for neural differentiation (Kishi et al., 2000; Wegner and Stolt, 2005; Wang et al., 2006). Each maintains neural stem/progenitor cells in a proliferative state upstream of neuronal terminal differentiation genes (Li et al., 1998; Zappone et al., 2000; Bylund et al., 2003; Graham et al., 2003; Ellis et al., 2004; Chung et al., 2006; Wang et al., 2006). *Sox2* is required for adult neural stem cell maintenance and its

loss causes cells to leave the ventricular zone and exit the cell cycle (Graham et al., 2003; Ellis et al., 2004; Episkopou, 2005). In contrast, *Sox1* and *Sox3* appear to regulate neural progenitor cell states (Kan et al., 2004; Barraud et al., 2005; Chung et al., 2006; Wang et al., 2006). *Sox11* is reported to be up-regulated as neural stem cells transition to neural progenitor cells, and later it maintains pan-neural genes in neuronal progenitors downstream of bHLH differentiation factors (Uwanogho et al., 1995; Wegner and Stolt, 2005; Bergsland et al., 2006). Thus, together these *Sox* genes may function downstream of *FoxD5*, *Geminin*, and *Zic2* to promote the initial steps from neural stem to neural progenitor cell. FoxD5 transiently represses *Sox2*, *Sox3*, and *Sox11* (Yan et al., 2009), suggesting that it may act to delay the stem-to-progenitor transition. *Zic* genes, which promote the expression of neural determination/differentiation bHLH factors, are expressed in proliferative neural progenitor cells and are required for their expansion (Aruga et al., 2002; Ebert et al., 2003; Inoue et al., 2007). *Iro/Irx* genes also are involved in the initiation of expression of the neural determination/differentiation bHLH factors. Later, they play important roles in patterning the CNS (Rodriguez-Sequel et al., 2009; Stedman et al., 2009), and appear to regulate the number of cranial placode progenitors (Feijoo et al., 2009).

Conclusions

Much has been learned about the endogenous signaling pathways and transcriptional factors that induce the neural ectoderm and stabilize its neural fate. However, new experimental tactics will be needed to sort out how the various signaling pathways intersect to produce a neural fate, and likewise how the several NFS transcription factors regulate one another. Ultimately, producing neurally-committed stem and progenitor cells in sufficient quantities and at the appropriate state of differentiation for clinical applications requires that we understand the molecular mechanisms that regulate the normal process of *in vivo* neural development in greater detail.

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References

Agius E, Oelgeschlager M, Wessely O, Kemp C, De Robertis EM. Endodermal nodal-related signals and mesoderm induction in *Xenopus*. Development. 2000; 127:1173–1183. [PubMed: 10683171]

Alvarez IS, Araujo M, Nieto MA. Neural induction in whole chick embryo cultures by FGF. Dev Biol. 1998; 199:42–54. [PubMed: 9676191]

Alvarez-Buylla AJ, Garcia-Verdugo JM, Tramontin AD. A unified hypothesis on the lineage of neural stem cells. Nature Rev. 2001; 2:287–293.

Amaya E, Musci TJ, Kirschner MW. Expression of a dominant negative mutant of the FGF receptor disrupts mesoderm formation in *Xenopus* embryos. Cell. 1991; 66:257–270. [PubMed: 1649700]

Aruga J, Tohmonda T, Homma S, Mikoshiba K. Zic1 promotes expansion of dorsal neural progenitors in spinal cord by inhibiting neuronal differentiation. Dev Biol. 2002; 244:329–341. [PubMed: 11944941]

Bachiller D, Klingensmith J, Kemp C, Belo JA, Anderson RM, May SR, McMahon JA, McMahon AP, Harland RM, Rossant J, De Robertis EM. The organizer factors Chordin and Noggin are required for mouse forebrain development. Nature. 2000; 403:658–661. [PubMed: 10688202]

Bainter JJ, Boos A, Kroll KL. Neural induction takes a transcriptional twist. Dev Dyn. 2001; 222:315–327. [PubMed: 11747068]

Baker JC, Beddington RS, Harland RM. Wnt signaling in *Xenopus* embryos inhibits bmp4 expression and activates neural development. Genes Dev. 1999; 13:3149–3159. [PubMed: 10601040]

Balemans W, Van Hul W. Extracellular regulation of BMP signaling in vertebrates: a cocktail of modulators. Dev Biol. 2002; 250:231–250. [PubMed: 12376100]

Barraud P, Thompson L, Kirik D, Bjorklund A, Parmar M. Isolation and characterization of neural precursor cells from the Sox1-GFP reporter mouse. Eur Neurosci. 2005; 22:1555–1569.

Beddington RSP. Induction of a secondary neural axis by the mouse node. Development. 1994; 120:613–620. [PubMed: 8162859]

Bellefroid EJ, Kobbe A, Gruss P, Pieler T, Gurdon JB, Papalopulu N. *Xiro3* encodes a *Xenopus* homolog of the *Drosophila Iroquois* genes and functions in neural specification. EMBO J. 1998; 17:191–203. [PubMed: 9427753]

Belo JA, Bachiller D, Agius E, Kemp C, Borges AC, Marques S, Piccolo S, De Robertis EM. Cerberus-like is a secreted BMP and nodal antagonist not essential for mouse development. Genesis. 2000; 26:265–270. [PubMed: 10748465]

Bergsland M, Werme M, Malewicz M, Perlmann T, Muhr J. The establishment of neuronal properties is controlled by Sox4 and Sox11. Genes Dev. 2006; 20:3475–3486. [PubMed: 17182872]

Boettger T, Knoetgen H, Witte L, Kessel M. The avian organizer. Int J Dev Biol. 2001; 45:281–287. [PubMed: 11291858]

Bouwmeester T, Kim S, Sasai Y, Lu B, De Robertis EM. Cerberus is a head-inducing secreted factor expressed in the anterior endoderm of Spemann's organizer. Nature. 1996; 382:595–601. [PubMed: 8757128]

Brewster R, Lee J, Ruiz i Altaba A. Gli/Zic factors pattern the neural plate by defining domains of cell differentiation. Nature. 1998; 393:579–583. [PubMed: 9634234]

Brugmann SA, Pandur PD, Kenyon KL, Pignoni F, Moody SA. Six1 promotes a placodal fate within the lateral neurogenic ectoderm by functioning as both a transcriptional activator and repressor. Development. 2004; 131:5871–5881. [PubMed: 15525662]

Bylund M, Andersson E, Novitch BG, Muhr J. Vertebrate neurogenesis is counteracted by Sox1-3 activity. Nat Neurosci. 2003; 6:1162–1168. [PubMed: 14517545]

Cameron HA, McKay RD. Adult neurogenesis produces a large pool of new granule cells in the dentate gyrus. J Comp Neurol. 2001; 435:406–417. [PubMed: 11406822]

Chang C, Harland RM. Neural induction requires continued suppression of both Smad1 and Smad2 signals during gastrulation. Development. 2007; 134:3861–3872. [PubMed: 17933792]

Christian JL, Gavin BJ, McMahon AP, Moon RT. Isolation of cDNAs partially encoding four *Xenopus* Wnt-1/int-1-related proteins and characterization of their transient expression during embryonic development. Dev Biol. 1991a; 143:230–234. [PubMed: 1991549]

Christian JL, McMahon JA, McMahon AP, Moon RT. Xwnt-8, a Xenopus Wnt-1/int-1-related gene responsive to mesoderm-inducing growth factors, may play a role in ventral mesodermal patterning during embryogenesis. Development. 1991b; 111:1045–1055. [PubMed: 1879349]

Chung S, Shin BS, Hedlund E, Pruszak J, Ferree A, Kang UJ, Isacson O, Kim KS. Genetic selection of sox1 GFP-expressing neural precursors removes residual tumorigenic pluripotent stem cells and attenuates tumor formation after transplantation. J Neurochem. 2006; 97:1467–1480. [PubMed: 16696855]

Collignon J, Sockanathan S, Hacker A, Cohen-Tannoudji M, Norris D, Rastan S, Stevanovic M, Goodfellow PN, Lovell-Badge R. A comparison of the properties of Sox-3 with Sry and two related genes, Sox-1 and Sox-2. Development. 1996; 122:509–520. [PubMed: 8625802]

de la Calle-Mustienes E, Glavic A, Modolell J, Gomez-Skarmeta JL. Xiro homeoproteins coordinate cell cycle exit and primary neuron formation by upregulating neuronal-fate repressors and downregulating the cell-cycle inhibitor XGadd45-γ. Mech Dev. 2002; 119:69–80. [PubMed: 12385755]

de Martino S, Yan YL, Jowett T, Postlethwait JH, Varga ZM, Ashworth A, Austin CA. Expression of sox11 gene duplicates in zebrafish suggests the reciprocal loss of ancestral gene expression patterns in development. Dev Dyn. 2000; 217:279–292. [PubMed: 10741422]

De Robertis EM, Kuroda H. Dorsal-ventral patterning and neural induction in *Xenopus* embryos. Annu Rev Cell Dev Biol. 2004; 20:285–308. [PubMed: 15473842]

Dee CT, Hirst CS, Shih YH, Tripathi VB, Patient RK, Scotting PJ. Sox3 regulates both neural fate and differentiation in the zebrafish ectoderm. Dev Biol. 2008; 320:289–301. [PubMed: 18572157]

Delaune E, Lemaire P, Kodjabachian L. Neural induction in *Xenopus* requires early FGF signalling in addition to BMP inhibition. Development. 2005; 132:299–310. [PubMed: 15590738]

Doetsche F, Caille I, Lim DA, Garcia-Verdugo JM, Alvarez-Buylla A. Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. Cell. 1999; 97:703–716. [PubMed: 10380923]

Dy P, Penzo-Mendez A, Wang H, Pedraza CE, Macklin WB, Lefebvre V. The three SoxC proteins--Sox4, Sox11 and Sox12--exhibit overlapping expression patterns and molecular properties. Nucleic Acids Res. 2008; 36:3101–3117. [PubMed: 18403418]

Ebert PJ, Timmer JR, Nakada Y, Helms AW, Parab PB, Liu Y, Hunsaker TL, Johnson JE. Zic1 represses Math1 expression via interactions with the Math1 enhancer and modulation of Math1 auto-regulation. Development. 2003; 130:1949–1959. [PubMed: 12642498]

Ekonomou A, Kazanis I, Malas S, Wood H, Alifragis P, Denaxa M, Karagogeos D, Constanti A, Lovell-Badge R, Episkopou V. Neuronal migration and ventral subtype identity in the telencephalon depend on SOX1. PLoS Biol. 2005; 3:e186. [PubMed: 15882093]

Elkins MB, Henry JJ. Isolation and characterization of a novel gene, xMADML, involved in *Xenopus* laevis eye development. Dev Dyn. 2006; 235:1845–1857. [PubMed: 16607642]

Ellis P, Fagan BM, Magness ST, Hutton S, Taranova O, Hayashi S, McMahon A, Rao M, Pevny L. SOX2, a persistent marker for multipotential neural stem cells derived from embryonic stem cells, the embryo or the adult. Dev Neurosci. 2004; 26:148–165. [PubMed: 15711057]

Episkopou V. SOX2 function in adult neural stem cells. Trends Neurosci. 2005; 28:219–221. [PubMed: 15866195]

Fainsod A, Deissler K, Yelin R, Marom K, Epstein M, Pillemer G, Steinbeisser H, Blum M. The dorsalizing and neural inducing gene follistatin is an antagonist of BMP-4. Mech Dev. 1997; 63:39–50. [PubMed: 9178255]

Fainsod A, Steinbeisser H, De Robertis EM. On the function of BMP-4 in patterning the marginal zone of the *Xenopus* embryo. EMBO J. 1994; 13:5015–5025. [PubMed: 7957067]

Fantes J, Ragge NK, Lynch SA, McGill NI, Collin JR, Howard-Peebles PN, Hayward C, Vivian AJ, Williamson K, van Heyningen V, FitzPatrick DR. Mutations in SOX2 cause anophthalmia. Nat Genet. 2003; 33:461–463. [PubMed: 12612584]

Feijoo CG, Saldias MP, De la Paz JF, Gomez-Skarmeta JL, Allende ML. Formation of posterior cranial placode derivatives requires the Iroquois transcription factor *irx4a*. Mol Cell Neurosci. 2009; 40:328–337. [PubMed: 19111617]

Feledy JA, Beanan MJ, Sandoval JJ, Goodrich JS, Lim JH, Matsuo-Takasaki M, Sato SM, Sargent TD. Inhibitory patterning of the anterior neural plate in *Xenopus* by homeodomain factors Dlx3 and Msx1. Dev Biol. 1999; 212:455–464. [PubMed: 10433834]

Ferri AL, Cavallaro M, Braida D, Di Cristofano A, Canta A, Vezzani A, Ottolenghi S, Pandolfi PP, Sala M, DeBiasi S, Nicolis SK. Sox2 deficiency causes neurodegeneration and impaired neurogenesis in the adult mouse brain. Development. 2004; 131:3805–3819. [PubMed: 15240551]

Fetka I, Doederlein G, Bouwmeester T. Neuroectodermal specification and regionalization of the Spemann organizer in *Xenopus*. Mech Dev. 2000; 93:49–58. [PubMed: 10781939]

Foerst-Potts L, Sadler TW. Disruption of Msx-1 and Msx-2 reveals roles for these genes in craniofacial, eye, and axial development. Dev Dyn. 1997; 209:70–84. [PubMed: 9142497]

Friedle H, Knochel W. Cooperative interaction of Xvent-2 and GATA-2 in the activation of the ventral homeobox gene Xvent-1B. J Biol Chem. 2002; 277:23872–23881. [PubMed: 11964398]

Friedle H, Rastegar S, Paul H, Kaufmann E, Knochel W. Xvent-1 mediates BMP-4-induced suppression of the dorsal-lip-specific early response gene XFD-1′ in Xenopus embryos. EMBO J. 1998; 17:2298–2307. [PubMed: 9545242]

Furthauer M, Thisse C, Thisse B. A role for FGF-8 in the dorsoventral patterning of the zebrafish gastrula. Development. 1997; 124:4253–4264. [PubMed: 9334274]

Gamse J, Sive H. Vertebrate anteroposterior patterning: the *Xenopus* neurectoderm as a paradigm. Bioessays. 2000; 22:976–986. [PubMed: 11056474]

Gawantka V, Delius H, Hirschfeld K, Blumenstock C, Niehrs C. Antagonizing the Spemann organizer: role of the homeobox gene Xvent-1. EMBO J. 1995; 14:6268–6279. [PubMed: 8557046]

Gerhart J, Danilchik M, Doniach T, Roberts S, Rowning B, Stewart R. Cortical rotation of the *Xenopus* egg: consequences for the anteroposterior pattern of embryonic dorsal development. Development. 1989; 107(Suppl):37–51. [PubMed: 2699856]

Glavic A, Gomez-Skarmeta JL, Mayor R. Xiro-1 controls mesoderm patterning by repressing bmp-4 expression in the Spemann organizer. Dev Dyn. 2001; 222:368–376. [PubMed: 11747072]

Gomez-Skarmeta J, de La Calle-Mustienes E, Modolell J. The Wnt-activated Xiro1 gene encodes a repressor that is essential for neural development and downregulates Bmp4. Development. 2001; 128:551–560. [PubMed: 11171338]

Gomez-Skarmeta JL, Diez del Corral R, de la Calle-Mustienes E, Ferre-Marco D, Modolell J. Araucan and caupolican, two members of the novel iroquois complex, encode homeoproteins that control proneural and vein-forming genes. Cell. 1996; 85:95–105. [PubMed: 8620542]

Gómez-Skarmeta JL, Glavic A, de la Calle-Mustienes E, Modolell J, Mayor R. Xiro, a *Xenopus* homolog of the *Drosophila Iroquois* complex genes, controls development at the neural plate. EMBO J. 1998; 17:181–190. [PubMed: 9427752]

Gómez-Skarmeta JL, Modollel J. *Iroquois* genes:genomic organization and function in vertebrate neural development. Curr Opin Genet Dev. 2002; 12:403–408. [PubMed: 12100884]

Gould SE, Grainger RM. Neural induction and antero-posterior patterning in the amphibian embryo: past, present and future. Cell Mol Life Sci. 1997; 53:319–338. [PubMed: 9137624]

Graham V, Khudyakov J, Ellis P, Pevny L. SOX2 functions to maintain neural progenitor identity. Neuron. 2003; 39:749–765. [PubMed: 12948443]

Gubbay J, Collignon J, Koopman P, Capel B, Economou A, Munsterberg A, Vivian N, Goodfellow P, Lovell-Badge R. A gene mapping to the sex-determining region of the mouse Y chromosome is a member of a novel family of embryonically expressed genes. Nature. 1990; 346:245–250. [PubMed: 2374589]

Guth SI, Wegner M. Having it both ways: Sox protein function between conservation and innovation. Cell Mol Life Sci. 2008; 65:3000–3018. [PubMed: 18516494]

Hargrave M, Wright E, Kun J, Emery J, Cooper L, Koopman P. Expression of the Sox11 gene in mouse embryos suggests roles in neuronal maturation and epithelio-mesenchymal induction. Dev Dyn. 1997; 210:79–86. [PubMed: 9337129]

Hawley SH, Wunnenberg-Stapleton K, Hashimoto C, Laurent MN, Watabe T, Blumberg BW, Cho KW. Disruption of BMP signals in embryonic *Xenopus* ectoderm leads to direct neural induction. Genes Dev. 1995; 9:2923–2935. [PubMed: 7498789]

Heeg-Truesdell E, Labonne C. Neural induction in *Xenopus* requires inhibition of Wnt-beta-catenin signaling. Dev Biol. 2006; 298:71–86. [PubMed: 16879817]

Hemmati-Brivanlou A, Melton DA. Inhibition of activin receptor signaling promotes neuralization in *Xenopus*. Cell. 1994; 77:273–281. [PubMed: 8168134]

Hemmati-Brivanlou A, Thomsen GH. Ventral mesodermal patterning in *Xenopus* embryos: expression patterns and activities of BMP-2 and BMP-4. Dev Genet. 1995; 17:78–89. [PubMed: 7554498]

Hemmati-Brivanlou A, Wright DA, Melton DA. Embryonic expression and functional analysis of a *Xenopus* activin receptor. Dev Dyn. 1992; 194:1–11. [PubMed: 1384808]

Hongo I, Kengaku M, Okamoto H. FGF signaling and the anterior neural induction in *Xenopus*. Dev Biol. 1999; 216:561–581. [PubMed: 10642793]

Hoser M, Potzner MR, Koch JM, Bosl MR, Wegner M, Sock E. Sox12 deletion in the mouse reveals nonreciprocal redundancy with the related Sox4 and Sox11 transcription factors. Mol Cell Biol. 2008; 28:4675–4687. [PubMed: 18505825]

Hurtado C, De Robertis EM. Neural induction in the absence of organizer in salamanders is mediated by MAPK. Dev Biol. 2007; 307:282–289. [PubMed: 17540356]

Hyodo-Miura J, Urushiyama S, Nagai S, Nishita M, Ueno N, Shibuya H. Involvement of NLK and Sox11 in neural induction in *Xenopus* development. Genes Cells. 2002; 7:487–496. [PubMed: 12047350]

Inoue T, Ota M, Ogawa M, Mikoshiba M, Aruga J. Zic1 and Zic3 regulate medial forebrain development through expansion of neuronal progenitors. J Neurosci. 2007; 27:5461–5473. [PubMed: 17507568]

Ishimura A, Maeda R, Takeda M, Kikkawa M, Daar IO, Maeno M. Involvement of BMP-4/msx-1 and FGF pathways in neural induction in the *Xenopus* embryo. Dev Growth Differ. 2000; 42:307–316. [PubMed: 10969730]

Kaestner KH, Monaghan AP, Kern H, Ang SL, Weitz S, Lichter P, Schutz G. The mouse fkh-2 gene. Implications for notochord, foregut, and midbrain regionalization. J Biol Chem. 1995; 270:30029–30035. [PubMed: 8530406]

Kan L, Israsena N, Zhang Z, Hu M, Zhao LR, Jalali A, Sahni V, Kessler JA. Sox1 acts through multiple independent pathways to promote neurogenesis. Dev Biol. 2004; 269:580–594. [PubMed: 15110721]

Katoh M, Katoh M. Human FOX gene family. Int J Oncol. 2004; 25:1495–1500. [PubMed: 15492844]

Khokha MK, Yeh J, Grammer TC, Harland RM. Depletion of three BMP antagonists from Spemann's organizer leads to a catastrophic loss of dorsal structures. Dev Cell. 2005; 8:401–411. [PubMed: 15737935]

Kishi M, Mizuseki K, Sasai N, Yamazaki H, Shiota K, Nakanishi S, Sasai Y. Requirement of Sox2-mediated signaling for differentiation of early *Xenopus* neuroectoderm. Development. 2000; 127:791–800. [PubMed: 10648237]

Kroll KL. Geminin in embryonic development: coordinating transcription and the cell cycle during differentiation. Front Biosci. 2007; 12:1395–1409. [PubMed: 17127390]

Kroll KL, Salic AN, Evans LM, Kirschner MW. Geminin, a neuralizing molecule that demarcates the future neural plate at the onset of gastrulation. Development. 1998; 125:3247–3258. [PubMed: 9671596]

Kuo JS, Patel M, Gamse J, Merzdorf C, Liu X, Apekin V, Sive H. Opl: a zinc finger protein that regulates neural determination and patterning in *Xenopus*. Development. 1998; 125:2867–2882. [PubMed: 9655809]

Kuroda H, Fuentealba L, Ikeda A, Reversade B, De Robertis EM. Default neural induction: neuralization of dissociated *Xenopus* cells is mediated by Ras/MAPK activation. Genes Dev. 2005; 19:1022–1027. [PubMed: 15879552]

Ladher R, Mohun TJ, Smith JC, Snape AM. Xom: a *Xenopus* homeobox gene that mediates the early effects of BMP-4. Development. 1996; 122:2385–2394. [PubMed: 8756284]

Lamb TM, Harland RM. Fibroblast growth factor is a direct neural inducer, which combined with noggin generates anterior-posterior neural pattern. Development. 1995; 121:3627–3636. [PubMed: 8582276]

Larabell CA, Torres M, Rowning BA, Yost C, Miller JR, Wu M, Kimelman D, Moon RT. Establishment of the dorso-ventral axis in *Xenopus* embryos is presaged by early asymmetries in beta-catenin that are modulated by the Wnt signaling pathway. J Cell Biol. 1997; 136:1123–1136. [PubMed: 9060476]

Launay C, Fromentoux V, Shi DL, Boucaut JC. A truncated FGF receptor blocks neural induction by endogenous *Xenopus* inducers. Development. 1996; 122:869–880. [PubMed: 8631265]

Li M, Pevny L, Lovell-Badge R, Smith A. Generation of purified neural precursors from embryonic stem cells by lineage selection. Curr Biol. 1998; 8:971–974. [PubMed: 9742400]

Linker C, Stern CD. Neural induction requires BMP inhibition only as a late step, and involves signals other than FGF and Wnt antagonists. Development. 2004; 131:5671–5681. [PubMed: 15509767]

Luo L, Yang X, Takihara Y, Knoetgen H, Kessel M. The cell-cycle regulator geminin inhibits Hox function through direct and polycomb-mediated interactions. Nature. 2004; 427:749–753. [PubMed: 14973489]

Malas S, Postlethwaite M, Ekonomou A, Whalley B, Nishiguchi S, Wood H, Meldrum B, Constanti A, Episkopou V. Sox1-deficient mice suffer from epilepsy associated with abnormal ventral forebrain development and olfactory cortex hyperexcitability. Neuroscience. 2003; 119:421–432. [PubMed: 12770556]

McGarry TJ, Kirschner MW. Geminin, an inhibitor of DNA replication, is degraded during mitosis. Cell. 1998; 93:1043–1053. [PubMed: 9635433]

McMahon AP, Moon RT. Ectopic expression of the proto-oncogene int-1 in *Xenopus* embryos leads to duplication of the embryonic axis. Cell. 1989; 58:1075–1084. [PubMed: 2673541]

McMahon JA, Takada S, Zimmerman LB, Fan CM, Harland RM, McMahon AP. Noggin-mediated antagonism of BMP signaling is required for growth and patterning of the neural tube and somite. Genes Dev. 1998; 12:1438–1452. [PubMed: 9585504]

Meinhardt H. Organizer and axes formation as a self-organizing process. Int J Dev Biol. 2001; 45:177–188. [PubMed: 11291845]

Miller-Bertoglio V, Carmany-Rampey A, Furthauer M, Gonzalez EM, Thisse C, Thisse B, Halpern ME, Solnica-Krezel L. Maternal and zygotic activity of the zebrafish ogon locus antagonizes BMP signaling. Dev Biol. 1999; 214:72–86. [PubMed: 10491258]

Miyagi S, Masui S, Niwa H, Saito T, Shimazaki T, Okano H, Nishimoto M, Muramatsu M, Iwama A, Okuda A. Consequence of the loss of Sox2 in the developing brain of the mouse. FEBS Lett. 2008; 582:2811–2815. [PubMed: 18638478]

Mizuseki K, Kishi M, Matsui M, Nakanishi S, Sasai Y. *Xenopus* Zic-related-1 and Sox-2, two factors induced by chordin, have distinct activities in the initiation of neural induction. Development. 1998a; 125:579–587. [PubMed: 9435279]

Mizuseki K, Kishi M, Shiota K, Nakanishi S, Sasai Y. SoxD: an essential mediator of induction of anterior neural tissues in *Xenopus* embryos. Neuron. 1998b; 21:77–85. [PubMed: 9697853]

Mohammadi M, McMahon G, Sun L, Tang C, Hirth P, Yeh BK, Hubbard SR, Schlessinger J. Structures of the tyrosine kinase domain of fibroblast growth factor receptor in complex with inhibitors. Science. 1997; 276:955–960. [PubMed: 9139660]

Moody SA, Je HS. Neural induction, neural fate stabilization, and neural stem cells. ScientificWorldJournal. 2002; 2:1147–1166. [PubMed: 12805974]

Nakata K, Nagai T, Aruga J, Mikoshiba K. *Xenopus* Zic3, a primary regulator both in neural and neural crest development. Proc Natl Acad Sci U S A. 1997; 94:11980–11985. [PubMed: 9342348]

Nakata K, Nagai T, Aruga J, Mikoshiba K. *Xenopus* Zic family and its role in neural and neural crest development. Mech Dev. 1998; 75:43–51. [PubMed: 9739105]

Nieuwkoop PD. The “organization centre”. 3. Segregation and pattern formation in morphogenetic fields. Acta Biotheor. 1967; 17:178–194. [PubMed: 4967351]

Nishimoto S, Kusakabe M, Nishida E. Requirement of the MEK5-ERK5 pathway for neural differentiation in *Xenopus* embryonic development. EMBO Rep. 2005; 6:1064–1069. [PubMed: 16179948]

Nitta KR, Takahashi S, Haramoto Y, Fukuda M, Onuma Y, Asashima M. Expression of Sox1 during *Xenopus* early embryogenesis. Biochem Biophys Res Commun. 2006; 351:287–293. [PubMed: 17056008]

Odenthal J, Nusslein-Volhard C. Fork head domain genes in zebrafish. Dev Genes Evol. 1998; 208:245–258. [PubMed: 9683740]

Onichtchouk D, Gawantka V, Dosch R, Delius H, Hirschfeld K, Blumenstock C, Niehrs C. The Xvent-2 homeobox gene is part of the BMP-4 signalling pathway controlling dorsoventral patterning of *Xenopus* mesoderm. Development. 1996; 122:3045–3053. [PubMed: 8898218]

Onichtchouk D, Glinka A, Niehrs C. Requirement for Xvent-1 and Xvent-2 gene function in dorsoventral patterning of *Xenopus* mesoderm. Development. 1998; 125:1447–1456. [PubMed: 9502725]

Otte AP, Moon RT. Ectopic induction of dorsal mesoderm by overexpression of Xwnt-8 elevates the neural competence of *Xenopus* ectoderm. Dev Biol. 1992; 152:184–187. [PubMed: 1385790]

Papanayotou C, Mey A, Birot AM, Saka Y, Boast S, Smith JC, Samarut J, Stern CD. A mechanism regulating the onset of Sox2 expression in the embryonic neural plate. PLoS Biol. 2008; 6:e2. [PubMed: 18184035]

Pencea V, Bingaman KD, Freedman LJ, Luskin MB. Neurogenesis in the subventricular zone and rostral migratory stream of the neonatal and adult primate forebrain. Exp Neurol. 2001; 172:1–16. [PubMed: 11681836]

Penzel R, Oschwald R, Chen Y, Tacke L, Grunz H. Characterization and early embryonic expression of a neural specific transcription factor xSOX3 in *Xenopus laevis*. Int J Dev Biol. 1997; 41:667–677. [PubMed: 9415486]

Pera EM, Ikeda A, Eivers E, De Robertis EM. Integration of IGF, FGF, and anti-BMP signals via Smad1 phosphorylation in neural induction. Genes Dev. 2003; 17:3023–3028. [PubMed: 14701872]

Pevny L, Placzek M. SOX genes and neural progenitor identity. Curr Opin Neurobiol. 2005; 15:7–13. [PubMed: 15721738]

Piccolo S, Sasai Y, Lu B, De Robertis EM. Dorsoventral patterning in *Xenopus*: inhibition of ventral signals by direct binding of chordin to BMP-4. Cell. 1996; 86:589–598. [PubMed: 8752213]

Pitulescu M, Kessel M, Luo L. The regulation of embryonic patterning and DNA replication by geminin. Cell Mol Life Sci. 2005; 62:1425–1433. [PubMed: 15870955]

Rastegar S, Friedle H, Frommer G, Knochel W. Transcriptional regulation of Xvent homeobox genes. Mech Dev. 1999; 81:139–49. [PubMed: 10330491]

Rentzsch F, Bakkers J, Kramer C, Hammerschmidt M. Fgf signaling induces posterior neuroectoderm independently of Bmp signaling inhibition. Dev Dyn. 2004; 231:750–757. [PubMed: 15532058]

Rex M, Orme A, Uwanogho D, Tointon K, Wigmore PM, Sharpe PT, Scotting PJ. Dynamic expression of chicken Sox2 and Sox3 genes in ectoderm induced to form neural tissue. Dev Dyn. 1997; 209:323–332. [PubMed: 9215646]

Ribisi S Jr, Mariani FV, Aamar E, Lamb TM, Frank D, Harland RM. Ras-mediated FGF signaling is required for the formation of posterior but not anterior neural tissue in *Xenopus laevis*. Dev Biol. 2000; 227:183–196. [PubMed: 11076686]

Rizzoti K, Brunelli S, Carmignac D, Thomas PQ, Robinson IC, Lovell-Badge R. SOX3 is required during the formation of the hypothalamo-pituitary axis. Nat Genet. 2004; 36:247–255. [PubMed: 14981518]

Rizzoti K, Lovell-Badge R. SOX3 activity during pharyngeal segmentation is required for craniofacial morphogenesis. Development. 2007; 134:3437–3448. [PubMed: 17728342]

Rodriguez-Gallardo L, Climent V, Garcia-Martinez V, Schoenwolf GC, Alvarez IS. Targeted over-expression of FGF in chick embryos induces formation of ectopic neural cells. Int J Dev Biol. 1997; 41:715–723. [PubMed: 9415491]

Rodriguez-Seguel E, Alarcon P, Gomez-Skarmeta. The *Xenopus Irx* genes are essential for neural patterning and define the border between prethalamus and thalamus through mutual antagonism with the anterior repressors *Fezf* and *Arx*. Dev Biol. 2009; 329:258–268. [PubMed: 19268445]

Rogers CD, Archer TC, Cunningham DD, Grammer TC, Casey EM. Sox3 expression is maintained by FGF signaling and restricted to the neural plate by Vent proteins in the *Xenopus* embryo. Dev Biol. 2008; 313:307–319. [PubMed: 18031719]

Rogers CD, Harafuji N, Archer T, Cunningham DD, Casey ES. *Xenopus* Sox3 activates *sox2* and *geminin* and indirectly represses *Xvent2* expression to induce neural progenitor formation at the expense of non-neural ectodermal derivatives. Mech Dev. 2009; 126:42–55. [PubMed: 18992330]

Sasai Y. Identifying the missing links: genes that connect neural induction and primary neurogenesis in vertebrate embryos. Neuron. 1998; 21:455–458. [PubMed: 9768831]

Sasai Y, Lu B, Steinbeisser H, De Robertis EM. Regulation of neural induction by the Chd and Bmp-4 antagonistic patterning signals in *Xenopus*. Nature. 1995; 376:333–336. [PubMed: 7630399]

Sato SM, Sargent TD. Development of neural inducing capacity in dissociated *Xenopus* embryos. Dev Biol. 1989; 134:263–266. [PubMed: 2731653]

Schier AF. Axis formation and patterning in zebrafish. Curr Opin Genet Dev. 2001; 11:393–404. [PubMed: 11448625]

Schlosser G, Awtry T, Brugmann SA, Jensen ED, Neilson K, Ruan G, Stammler A, Voelker D, Yan B, Zhang C, Klymkowsky MW, Moody SA. Eya1 and Six1 promote neurogenesis in the cranial placodes in a SoxB1-dependent fashion. Dev Biol. 2008; 320:199–214. [PubMed: 18571637]

Seo S, Herr A, Lim JW, Richardson GA, Richardson H, Kroll KL. Geminin regulates neuronal differentiation by antagonizing Brg1 activity. Genes Dev. 2005; 19:1723–1734. [PubMed: 16024661]

Seo S, Kroll KL. Geminin's double life: chromatin connections that regulate transcription at the transition from proliferation to differentiation. Cell Cycle. 2006; 5:374–379. [PubMed: 16479171]

Sheng G, dos Reis M, Stern CD. Churchill, a zinc finger transcriptional activator, regulates the transition between gastrulation and neurulation. Cell. 2003; 115:603–613. [PubMed: 14651851]

Shih J, Fraser SE. Characterizing the zebrafish organizer: microsurgical analysis at the early-shield stage. Development. 1996; 122:1313–1322. [PubMed: 8620858]

Sinclair AH, Berta P, Palmer MS, Hawkins JR, Griffiths BL, Smith MJ, Foster JW, Frischauf AM, Lovell-Badge R, Goodfellow PN. A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. Nature. 1990; 346:240–244. [PubMed: 1695712]

Smith WC, McKendry R, Ribisi S Jr, Harland RM. A nodal-related gene defines a physical and functional domain within the Spemann organizer. Cell. 1995; 82:37–46. [PubMed: 7606783]

Smith JL, Schoenwolf GC. Getting organized: new insights into the organizer of higher vertebrates. Curr Top Dev Biol. 1998; 40:79–110. [PubMed: 9673849]

Solter M, Koster M, Hollemann T, Brey A, Pieler T, Knochel W. Characterization of a subfamily of related winged helix genes, XFD-12/12′/12" (XFLIP), during *Xenopus* embryogenesis. Mech Dev. 1999; 89:161–165. [PubMed: 10559492]

Spemann, H.; Mangold, H. Induction of embryonic primordia by implantation of organizers from different species. In: Willier, BH.; Oppenheimer, JM., editors. Foundations of Experimental Embryology. Hafner, NY: 1924. p. 144-184.

Stedman A, Lecaudey V, Havis E, Anselme I, Wassef M, Gilardi-Hebenstreit P, Schneiderr-Maunoury S. A functional interaction between Irx and Meis patterns the anterior hindbrain and activates *krox20* expression in rhombomere 3. Dev Biol. 2009; 327:566–577. [PubMed: 19152797]

Steinbeisser H, De Robertis EM, Ku M, Kessler DS, Melton DA. *Xenopus* axis formation: induction of goosecoid by injected Xwnt-8 and activin mRNAs. Development. 1993; 118:499–507. [PubMed: 7900991]

Sullivan SA, Akers L, Moody SA. *foxD5a*, a *Xenopus* winged helix gene, maintains an immature neural ectoderm via transcriptional repression that is dependent on the C-terminal domain. Dev Biol. 2001; 232:439–457. [PubMed: 11401404]

Suzuki A, Ueno N, Hemmati-Brivanlou A. *Xenopus* msx1 mediates epidermal induction and neural inhibition by BMP4. Development. 1997; 124:3037–3044. [PubMed: 9272945]

Takeda M, Saito Y, Sekine R, Onitsuka I, Maeda R, Maeno M. *Xenopus* msx-1 regulates dorso-ventral axis formation by suppressing the expression of organizer genes. Comp Biochem Physiol B Biochem Mol Biol. 2000; 126:157–168. [PubMed: 10874163]

Takemoto T, Uchikawa M, Kamachi Y, Kondoh H. Convergence of Wnt and FGF signals in the genesis of posterior neural plate through activation of the Sox2 enhancer N-1. Development. 2006; 133:297–306. [PubMed: 16354715]

Taranova OV, Magness ST, Fagan BM, Wu Y, Surzenko N, Hutton SR, Pevny LH. SOX2 is a dose-dependent regulator of retinal neural progenitor competence. Genes Dev. 2006; 20:1187–1202. [PubMed: 16651659]

Taylor JJ, Wang T, Kroll KL. Tcf- and Vent-binding sites regulate neural-specific *geminin* expression in the gastrula embryo. Dev Biol. 2006; 289:494–506. [PubMed: 16337935]

Tribulo C, Aybar MJ, Nguyen VH, Mullins MC, Mayor R. Regulation of *Msx* genes by a Bmp gradient is essential for neural crest specification. Development. 2003; 130:6441–6452. [PubMed: 14627721]

Trindade M, Tada M, Smith JC. DNA-binding specificity and embryological function of Xom (Xvent-2). Dev Biol. 1999; 216:442–456. [PubMed: 10642784]

Tucker AS, Al Khamis A, Sharpe PT. Interactions between Bmp-4 and Msx-1 act to restrict gene expression to odontogenic mesenchyme. Dev Dyn. 1998; 212:533–539. [PubMed: 9707326]

Uchikawa M, Ishida Y, Takemoto T, Kamachi Y, Kondoh H. Functional analysis of chicken Sox2 enhancers highlights an array of diverse regulatory elements that are conserved in mammals. Dev Cell. 2003; 4:509–519. [PubMed: 12689590]

Uchikawa M, Kamachi Y, Kondoh H. Two distinct subgroups of Group B Sox genes for transcriptional activators and repressors: their expression during embryonic organogenesis of the chicken. Mech Dev. 1999; 84:103–120. [PubMed: 10473124]

Ueno H, Gunn M, Dell K, Tseng A Jr, Williams L. A truncated form of fibroblast growth factor receptor 1 inhibits signal transduction by multiple types of fibroblast growth factor receptor. J Biol Chem. 1992; 267:1470–1476. [PubMed: 1309784]

Uwanogho D, Rex M, Cartwright EJ, Pearl G, Healy C, Scotting PJ, Sharpe PT. Embryonic expression of the chicken Sox2, Sox3 and Sox11 genes suggests an interactive role in neuronal development. Mech Dev. 1995; 49:23–36. [PubMed: 7748786]

Vonica A, Gumbiner BM. The *Xenopus* Nieuwkoop center and Spemann-Mangold organizer share molecular components and a requirement for maternal Wnt activity. Dev Biol. 2007; 312:90–102. [PubMed: 17964564]

Walmsley ME, Guille MJ, Bertwistle D, Smith JC, Pizzey JA, Patient RK. Negative control of *Xenopus* GATA-2 by activin and noggin with eventual expression in precursors of the ventral blood islands. Development. 1994; 120:2519–2529. [PubMed: 7956828]

Wang TW, Stromberg GP, Whitney JT, Brower NW, Klymkowsky MW, Parent JM. Sox3 expression identifies neural progenitors in persistent neonatal and adult mouse forebrain germinative zones. J Comp Neurol. 2006; 497:88–100. [PubMed: 16680766]

Wegner M. From head to toes: the multiple facets of Sox proteins. Nucleic Acids Res. 1999; 27:1409–1420. [PubMed: 10037800]

Wegner M, Stolt CC. From stem cells to neurons and glia: a Soxist's view of neural development. Trends Neurosci. 2005; 28:583–588. [PubMed: 16139372]

Weinstein DC, Hemmati-Brivanlou A. Neural induction in *Xenopus laevis*: evidence for the default model. Curr Opin Neurobiol. 1997; 7:7–12. [PubMed: 9039789]

Weisinger K, Wilkinson DG, Sela-Donenfeld D. Inhibition of BMPs by follistatin is required for FGF3 expression and segmental patterning of the hindbrain. Dev Biol. 2008; 324:213–225. [PubMed: 18823972]

Wessely O, Kim JI, Geissert D, Tran U, De Robertis EM. Analysis of Spemann organizer formation in *Xenopus* embryos by cDNA macroarrays. Dev Biol. 2004; 269:552–566. [PubMed: 15110719]

Wilson PA, Hemmati-Brivanlou A. Induction of epidermis and inhibition of neural fate by Bmp-4. Nature. 1995; 376:331–333. [PubMed: 7630398]

Wilson SI, Graziano E, Harland R, Jessell TM, Edlund T. An early requirement for FGF signalling in the acquisition of neural cell fate in the chick embryo. Curr Biol. 2000; 10:421–429. [PubMed: 10801412]

Wilson SI, Rydstrom A, Trimborn T, Willert K, Nusse R, Jessell TM, Edlund T. The status of Wnt signalling regulates neural and epidermal fates in the chick embryo. Nature. 2001; 411:325–330. [PubMed: 11357137]

Wittler L, Kessel M. The acquisition of neural fate in the chick. Mech Dev. 2004; 121:1031–1042. [PubMed: 15296969]

Xu RH, Kim J, Taira M, Zhan S, Sredni D, Kung HF. A dominant negative bone morphogenetic protein 4 receptor causes neuralization in *Xenopus* ectoderm. Biochem Biophys Res Commun. 1995; 212:212–219. [PubMed: 7612010]

Yabe T, Shimizu T, Muraoka O, Bae YK, Hirata T, Nojima H, Kawakami A, Hirano T, Hibi M. Ogon/Secreted Frizzled functions as a negative feedback regulator of Bmp signaling. Development. 2003; 130:2705–2716. [PubMed: 12736214]

Yamamoto TS, Takagi C, Hyodo AC, Ueno N. Suppression of head formation by Xmsx-1 through the inhibition of intracellular nodal signaling. Development. 2001; 128:2769–2779. [PubMed: 11526082]

Yamamoto TS, Takagi C, Ueno N. Requirement of Xmsx-1 in the BMP-triggered ventralization of *Xenopus* embryos. Mech Dev. 2000; 91:131–141. [PubMed: 10704838]

Yan B, Neilson KM, Moody SA. *foxD5* plays a critical upstream role in regulating neural ectodermal fate and the onset of neural differentiation. Dev Biol. 2009; 329:80–95. [PubMed: 19250931]

Zappone MV, Galli R, Catena R, Meani N, De Biasi S, Mattei E, Tiveron C, Vescovi AL, Lovell-Badge R, Ottolenghi S, Nicolis SK. Sox2 regulatory sequences direct expression of a (beta)-geo transgene to telencephalic neural stem cells and precursors of the mouse embryo, revealing regionalization of gene expression in CNS stem cells. Development. 2000; 127:2367–2382. [PubMed: 10804179]

Zimmerman LB, De Jesus-Escobar JM, Harland RM. The Spemann organizer signal noggin binds and inactivates bone morphogenetic protein 4. Cell. 1996; 86:599–606. [PubMed: 8752214]

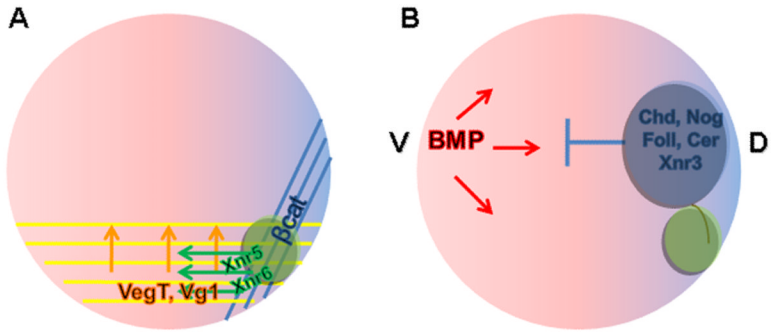

Figure 1.

The dorsal-ventral axis is specified by the Nieuwkoop center and the Spemann organizer in *Xenopus*. (A) Prior to gastrulation the dorsal axis is specified by the Nieuwkoop center (green circle), formed as a result of overlapping expression of VegT, Vg1, Nodals (Xnr), and β-Catenin (βcat). (B) The Nieuwkoop center secretes dorsalizing molecules that induce the formation of the Spemann organizer (blue oval) in the dorsal mesoderm. The organizer expresses Chordin (Chd), Noggin (Nog), Follistatin (Foll), Cerberus (Cer), and in *Xenopus*, Nodal related-3 (Xnr3). These molecules actively antagonize BMP, which is secreted from the ventral side (red arrows). V is ventral; D is dorsal. Line intersecting the Nieuwkoop center represents the dorsal blastopore lip.

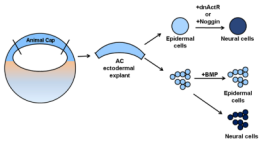

Figure 2.

The default model of neural induction in *Xenopus*. An ectodermal explant dissected from the animal cap of a blastula stage embryo forms epidermis (light blue). If BMP signaling is inhibited in those cells by expressing a dominant-negative Activin receptor (dnActR) or the extracellular antagonist Noggin, then neural tissue forms (dark blue). Dissociation of the explant into single cells without the addition of exogenous factors also leads to the formation of neural tissues. However, if exogenous BMP is added to the dispersed cells, they adopt an epidermal fate (light blue).

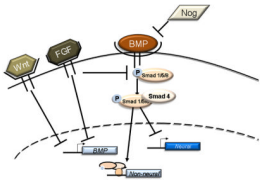

Figure 3.

Pathways involved in neural induction. BMP2, 4, or 7 dimers bind to the serine-threonine receptor complex, leading to phosphorylation of the type I receptor by the type II receptor, which in turn phosphorylates (P) an appropriate Receptor regulated (R)-Smad (Smad1, 5, or 8). This phosphorylation enables the R-Smad to form a complex with the Common-Smad, Smad4, and this complex translocates to the nucleus to activate (non-neural) or repress (neural) target genes. The binding of secreted factors from the organizer, such as Noggin (Nog), to extracellular BMP prevents activation of its receptor complex. In addition, FGF and Wnt signaling lead to the inhibition of *bmp4* transcription. FGF signaling also can prevent the phosphorylation of Smads1/5/8.

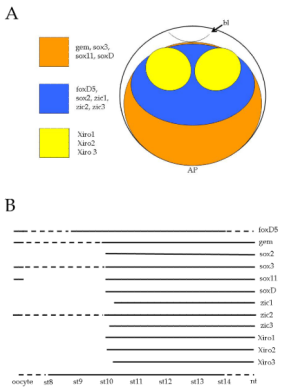

Figure 4.

Twelve neural genes have overlapping expression patterns during the establishment of the *Xenopus* neural plate. (A) At the onset of gastrulation (st10), some NE genes are expressed throughout the dorsal ectoderm (orange: *geminin* [*gem*], *sox3*, *sox11*, *soxD*), some are expressed only in a broad dorsal band adjacent to the blastopore lip (bl) (blue: *foxD5*, *sox2*, *zic1-3*), and the *Xiro* genes (yellow) are expressed weakly in two posterior-lateral bands. Dorsal view with animal pole (AP) to the bottom. (B) The temporal expression patterns of these genes also overlap extensively. Four genes (*foxD5*, *gem*, *sox3*, *zic2*) are expressed maternally (oocyte) with detectable mRNAs through blastula stages; *sox11* is expressed in oocytes but is not detected at blastula stages. The zygotic expression of most of these genes begins at late blastula (st9) to early gastrula (st10). The expression of nearly all is maintained through neural tube (nt) stages; in contrast, *foxD5* is lost as the neural plate is established (st14).

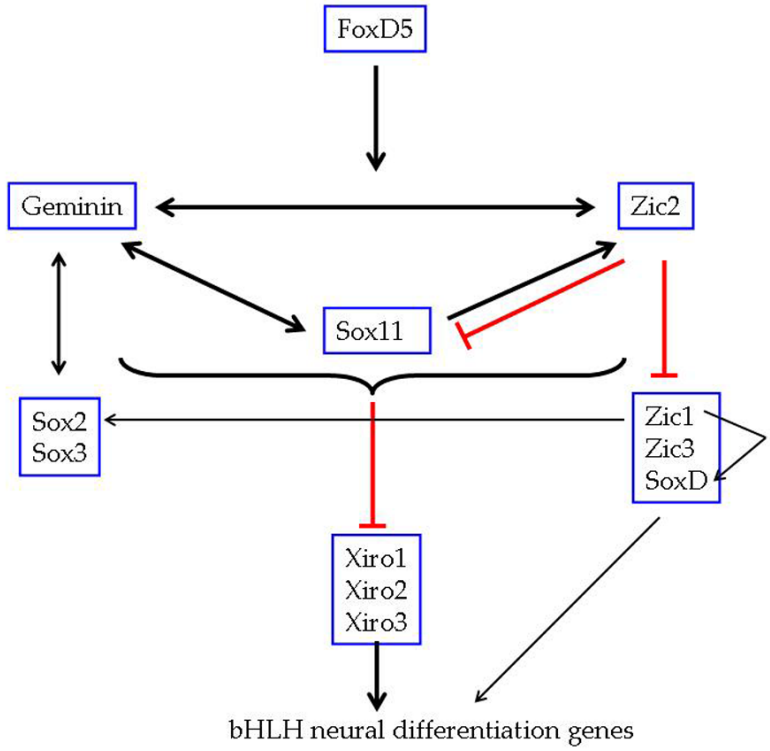

Figure 5.

A summary of the data discussed in the text predicts a gene regulatory network that results in the stabilization of neural fate. After these 12 NFS genes are induced, FoxD5 up-regulates *Geminin*, *Sox11*, and *Zic2* expression. These three genes regulate each other and together (bracket) they differentially affect the expression of the other 8 NFS genes. Geminin and Sox2/Sox3 directly regulate each other, and increased Zic1 up-regulates *Sox2* and *SoxD*. FoxD5, Geminin, Sox11, and Zic2 maintain neural ectodermal cells in an immature, stem-like state, whereas Zic1, Zic3, SoxD, and Xiro1-3 promote the onset of neural differentiation.