Int. J. Dev. Biol. 45: 321-326 (2001)

Roles of Sox factors in neural determination: conserved signaling in evolution?

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ABSTRACT Neural differentiation in amphibian embryos is initiated by the neural inducers emanating from the Spemann-Mangold organizer. The fate of uncommitted ectoderm is determined by graded BMP activity along the dorsal-ventral axis. Several transcriptional regulators acting in early neural differentiation have been identified, including Sox, Zic, Pou, HLH and Fox factors. In this paper, I review recent molecular studies on neural determination, focusing mainly on Sox factors. I also discuss the possible conservation of regulatory factors in neural differentiation, comparing Xenopus and Drosophila counterparts.

KEY WORDS: *Neural inducers, Sox2, SoxD, SoxN, evolution.*

Introduction

During the last decade, much progress has been made in elucidating the molecular basis of neural induction (Hemmati-Brivanlou and Melton, 1997). Neural inducers such as Noggin, Follistatin and Chordin (Chd) have been isolated (Lamb et al, 1993; Hemmati-Brivanlou et al, 1994; Sasai et al, 1995). These organizer-specific secreted factors induce neural differentiation while BMP4 promotes epidermogenesis when acting on uncommitted animal cap ectoderm (Wilson and Hemmati-Brivanlou, 1995). Interestingly, the neural inducers act by antagonizing BMP signaling by binding to BMP protein in the extracellular space (Piccolo et al, 1996; Zimmerman et al, 1996; Fainsod et al, 1997). This idea is supported by the fact that neural differentiation is caused by manipulations that block BMP signaling, such as overexpression of dominant-negative BMP receptor, dominant-negative BMP ligand and antisense BMP RNA (Sasai et al, 1995; Hawley et al, 1996). Cerberus, which binds to BMP, Nodal and Wnts, also induces neural differentiation in the animal cap ectoderm (Boumeester et al, 1996).

Several types of transcription factors mediating early neuralizing signals have been identified. They can be categorized into two classes. The first class consists of primary neuron-specific transcriptional regulators (Lee, 1997), such as Xngnr-1 (neurogenin-related), an HLH factor expressed in the primary neuron primodia (Ma et al, 1996). Xngnr-1 is capable of inducing neuronal differentiation in the animal cap ectoderm and in the embryo. Another example is MyT1, a zinc finger protein activated by Xngnr-1 signaling in the primary neuron (Bellefroid et al, 1996). One function of MyT1 in the primary neuron is that MyT1 makes the cell resistant to lateral inhibition from neighboring cells.

The other class consists of the factors widely expressed in the gastrula neuroectoderm. These include Sox family genes (Wegner, 1999), Zic-related genes (Nagai et al, 1997; Mizuseki et al, 1998a; Kuo et al, 1998), Pou (Witta et al, 1995) and Fox family genes (Bourguignon and Papalopulu, 1998; Mariani and Harland, 1998; Gomez-Skarmeta et al, 1999). These factors play roles in the formation and specification of the neuroectoderm (Sasai, 1998). In this review, I focus on the activities and roles of Sox family genes in early neural differentiation.

Sox family members as transcriptional regulators

Sox genes encode a group of factors structurally related to the sex-determination factor Sry (Pevny and Lovell-Badge, 1997; Wegner, 1999). They carry a single HMG (high mobility group) domain that binds to the DNA in a sequence-specific manner (Kamachi et al, 2000). The HMG motives are also found in other groups of genes, such as HMG1/2 and TCF/LEF family genes. The HMG domains of Sox factors are 79-amino acids long, and recognize a 6-7 base pair sequence (typically ATTGTT or CTTTGTT) located in the minor groove of the target DNA. When the HMG domain binds to the DNA, it causes a bending of the DNA molecule, suggesting that it functions as an architectural element in the assembly of nucleoprotein structures (Prior and Walter, 1996).

Abbreviations used in this paper: CNS, central nervous system; HMG, high mobility groups; BMP, bone morphogenetic protein; Chd, Chordin.

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0214-6282/2001/$25.00

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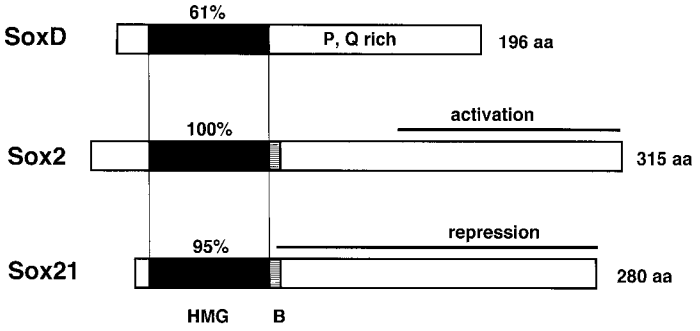

Fig. 1. Structural comparison of Sox2, Sox21 and SoxD. *The percentages of identical amino acid residues in the HMG domain (filled box) are shown. Following the HMG domain, Sox2 and Sox21 have a conserved sequence among Group B Sox factors (B, striped box). The carboxyl parts of these Sox factors are not conserved. SoxD has a high proline and glutamin content in the carboxyl half. Sox2 and Sox 21 have a activation and a repression domain, respectively.*

More than 20 Sox factors have been identified in vertebrates, and they are classified into seven groups, Groups A-G, according to mutual similarities in their HMG domains (Wegner, 1999). The HMG domains of Sox factors share more than 50% amino acid identity across the groups, and approximately 90% identity within an individual group (except for Group G). Outside of the HMG domain, however, little conservation is observed in the primary structure across the groups (Fig. 1).

Because of the conservation in the DNA-binding domains, Sox factors generally show a similar binding selectivity on the target sequence when examined in vitro (Kamachi et al, 2000). However, different Sox factors do have clear preferences for target genes in the cell. For instance, Sox9 (Group E) cannot mimic Sox2 (Group B) in the transactivation of the ∂-crystalline gene (Kamachi et al, 1999). Therefore, it is likely that the regions outside of the HMG domains are crucial to the selection of the target genes.

One promising explanation for the target selectivity is that many Sox factors require particular partner factors for transactivation (or transrepression). The target site of Sox2 in the regulatory region of the ∂-crystalline gene is located just adjacent to the binding site of ∂EF3, which is essential for transactivation

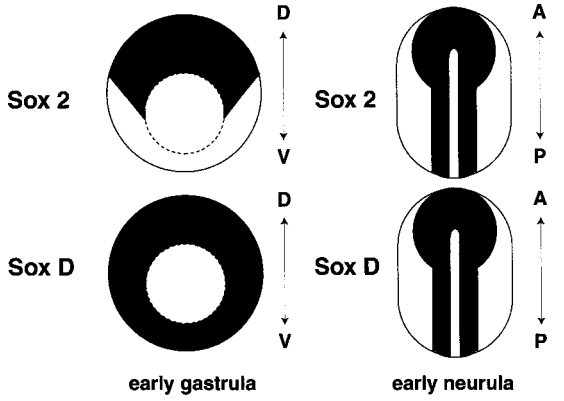

Fig. 2. Expression of *Sox2* and *SoxD* in early ectoderm. *At the early gastrula stage (left), Sox2 (top) is expressed in the dorsal ectoderm while SoxD mRNA (bottom) is distributed throughout the ectoderm. At the neurula stage (right), both genes are expressed in a neural-specific manner.*

by Sox2 (Kamachi *et al.,* 1995). Sox2 seems to utilize distinct partners in a cell type-specific manner. In ES (EC) cells, Sox2 interacts with Oct3 protein on binding to the enhancers of Fgf4 (Yuan et al, 1995) and UTF1 genes (Nishimoto et al, 1999). It has been shown that the Sox2-Oct3 complex forms even in the absence of the target DNA (Ambrosetti et al, 1997). These facts suggest that the Sox-partner interaction accounts for the target-specific and the cell type-specific functions of Sox factors, at least in part.

Sox family factors in neural development

Sox genes are expressed in a large variety of embryonic and adult tissues, and involved in many aspects of tissue development (Wegner, 1999). In early development, at least three distinct classes of Sox genes are expressed in the forming neural tissues (Fig. 1). The first class consists of Sox1, 2 and 3 (Group B, subgroup B1)(Uwanogho et al, 195; Kamachi et al, 1995; Collignon et al, 1996). They are closely related in structure throughout the protein length, and are expected to have largely overlapping functions. Cotransfection studies have shown that these subgroup B1 factors are transcriptional activators and that the activation domains are located in the C-terminal half (Kamachi et al, 1999)(Fig. 1, middle). The subgroup B1 genes are expressed broadly in the frog neuroectoderm from the late blastula stage onward (Penzel et al, 1997 ; Mizuseki et al, 1998a). Later, they are also expressed in the forming lens, neural crest and lateral line cells.

The second class of neural Sox genes is subgroup B2, to which Sox 14 and Sox 21 belong (Rex et al, 1997; Uchikawa et al, 1999; Rimini et al, 1999). Sox 14 and 21 carry an HMG domain and a short sequence that is conserved among Group B (Fig. 1, bottom; striped box). However, the rest of the protein regions show little homology to other Sox proteins, including subgroup B1. Sox 21 are widely expressed in the developing CNS while Sox 14 expression is limited to small regions in the brain. The unique feature of the subgroup B2 Sox factors is that they are transcriptional repressors (Uchikawa et al, 1999). Since expression patterns of Sox2 and Sox21 largely overlap in the neural tube, it is possible that transcription of the target genes in the cell is regulated by a fine balance of the activator (Sox2) and the repressor (Sox21).

The third class of Sox genes expressed in early neuroectoderm is SoxD (Group G)(Mizuseki et al, 1998b), which is only distantly related to Sox2 (Fig. 1, top and middle). SoxD expression is first detected in the entire ectoderm at late blastula stages. By the mid-gastrula stage, SoxD expression is restricted to the dorsal ectoderm (neuroectoderm) and is neural-specific during the neurula stage. It remains to be known whether SoxD is a transcriptional activator or repressor as no direct target genes have been identified for SoxD.

In Xenopus, both Sox2 and SoxD are expressed specifically in the neuroectoderm of late gastrulae. The expression of both genes is regulated by Chd and BMP4 in a positive and negative manner, respectively (Mizuseki et al, 1998a, b). However, Sox2 and SoxD show different expression patterns at the early gastrula stage when the Spemann-Mangold organizer starts to work (Fig. 2). Sox2 expression at this stage is restricted to dorsal ectoderm while SoxD expression is pan-ectodermal. This indicates the existence of two distinct signaling modes for the neuroectoderm determination; one is already active at early gastrulation and the other is engaged at mid-gastrulation.

SoxD as an essential regulator of anterior neural formation

SoxD has a strong neuralizing activity on uncommitted ectoderm (Mizuseki et al, 1998b). When overexpressed in the animal cap explant, SoxD induces neural and neuronal markers as well as neural crest markers. Misexpression of SoxD in the ectoderm of developing embryos results in the ectopic formation of a neural mass under the skin.

A dominant-negative mutant of SoxD (dnSoxD) is generated by deleting the DNA-binding domain. This mutant is likely to function by competing for endogenous partner factors in the nucleus. Overexpression of dn SoxD in the animal cap suppresses neural differentiation caused by the blockade of BMP signals. This shows that SoxD acts in the essential signaling pathway leading to neural differentiation of the ectoderm. In the developing embryo, the inhibition of SoxD function results in agenesis of forebrain structures including the neural retina. However, the CNS tissues posterior to the midbrain seem intact, indicating that SoxD function is not essential in the caudal area (Fig. 3, middle).

Thus, SoxD is an essential regulator of forebrain development in Xenopus (Mizuseki et al, 1998b).

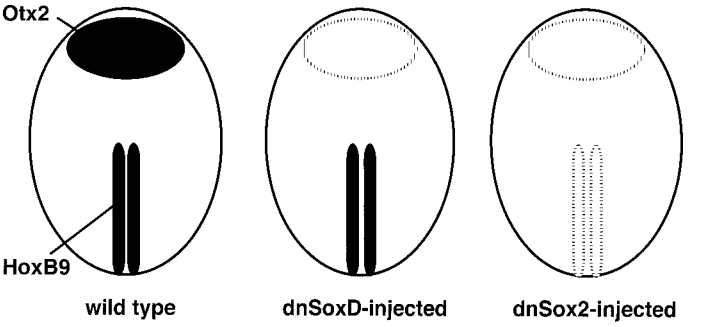

Fig. 3. Effects of dominant-negative *Sox2* and *SoxD* on anterior and posterior neural marker genes. *Effects of dominant-negative Sox mRNAs on Otx2 (forebrain) and HoxB9 (spinocaudal) markers are shown (left, wild type embryo). Overexpression of dnSoxD (middle) inhibits only Otx2 while that of dnSox2 (right) suppresses both anterior and posterior markers.*

Sox2 is required for differentiation of the neuroectoderm

Overexpression of Sox2, unlike that of SoxD, does not induce neural differentiation of animal cap ectoderm. Instead, Sox2 modifies the competence of the gastrula animal cap so that the ectoderm can respond to the FGF neuralizing signal (Mizuseki et al, 1998a). When FGF is added to Sox2-injected animal caps, neural tissues with anterior and posterior characters are induced. This is in contrast to the induction by SoxD, which promotes only anterior neural differentiation in the explant.

On the other hand, it has been shown in mammalian cell culture studies that overexpression of Sox1 (a subgroup B1 member) promotes neural differentiation in aggregated P19 embryonic carcinoma cells (Pevny et al, 1998). These results suggest that the subgroup B1 genes play positive roles in neural differentiation, although the mode of action is not clear from these data.

The in vivo role of Sox2 has been investigated in dominant-negative studies on Xenopus embryos (Kishi et al, 2000). Dominant-negative Sox2 (dnSox2) constructs can be generated either by deleting the HMG domain or by fusing the HMG domain to the repressor domain of Engrailed. When Sox2 function is inhibited, the animal cap explant cannot differentiate into neural tissues in response to the BMP blockade. Overexpression of dnSox2 in the ectoderm of the developing embryo strongly inhibits neural development in general. dnSox2 suppresses all the neural markers tested in the neurula embryo (Fig. 3, right), including neural, neuronal and neural crest markers. However, at the early gastrula stage, early neural markers such as Zic and Sox2 genes are normally expressed in the embryo injected with dnSox2 mRNA. These genes become suppressed when the injected embryo reaches the late gastrula stage. This indicates that Sox2 signaling is required for the maintenance rather than for the initial induction of neural differentiation.

This idea is supported by the fact that the ectoderm tissues injected with dnSox2 fail to adopt either neural or epidermal fates. The temporal requirement of Sox2 has been examined with inducible dominant-negative Sox2 fused with a glucocorticoid receptor domain. A similar extent of inhibition of NCAM expression is seen when Sox2 signaling is shut off from early and late gastrula stages onwards. These data suggest that *Sox2*-class factors are essential for early neuroectoderm cells to consolidate their neural identity during secondary steps of neural differentiation (Fig. 4)(Kishi et al, 2000).

Roles of Sox genes in neural differentiation of vertebrates

Differential roles of Sox2 and SoxD are summarized in Fig. 5. When the ectodermal cells receive the dorsal positional information (provided by BMP/neural inducers), the dorsal cells start to express transcription factors that have neuralizing activities, such as SoxD and Zic-r1. Sox2 is needed for the neuroectodermal cells to further differentiate and is required for the formation of all the neural tissues. In contrast, SoxD is essential only for anterior neural development (Fig. 3), although SoxD is expressed through-

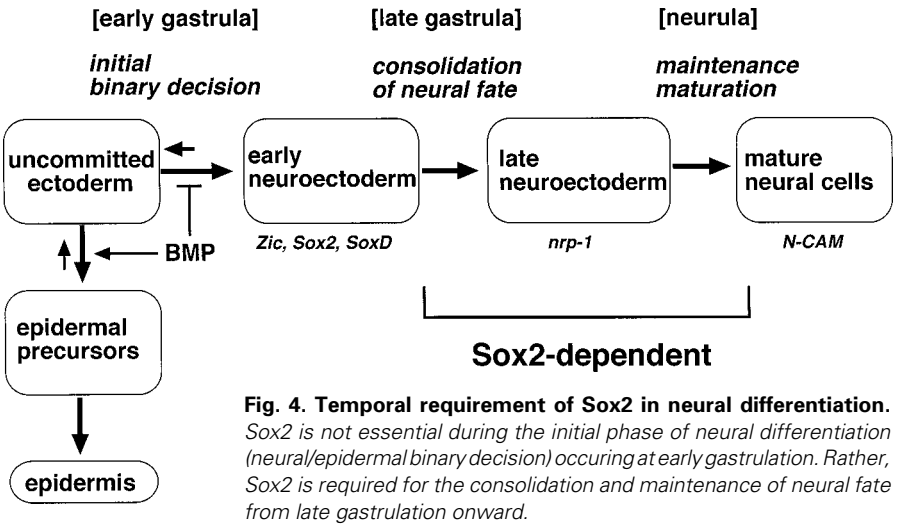

Fig. 4. Temporal requirement of Sox2 in neural differentiation. *Sox2 is not essential during the initial phase of neural differentiation (neural/epidermal binary decision) occuring at early gastrulation. Rather, Sox2 is required for the consolidation and maintenance of neural fate from late gastrulation onward.*

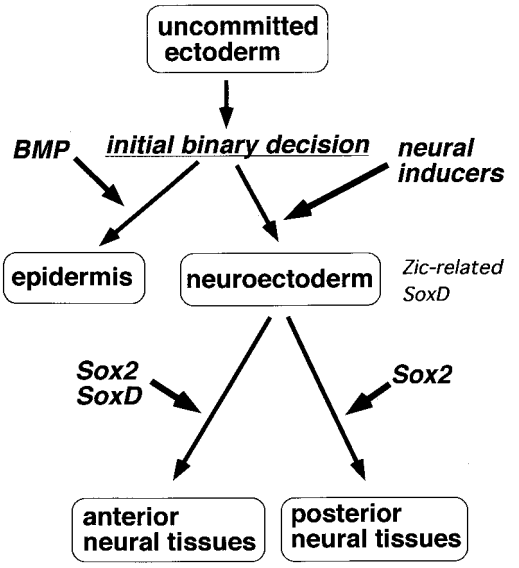

Fig. 5. Summary of roles of Sox2 and SoxD. *Uncommitted ectoderm makes the binary decision (neural or epidermal) on the balance of the BMP and the neural inducer signals. SoxD and Zic factors, which have neuralizing activities, are induced in the presumptive neuroectoderm of early gastrulation. Sox2 are required at secondary steps of neural differentiation in both anterior and posterior tissues while SoxD is essential only in the anterior region.*

out the forming CNS (Fig. 2). In this regard, it is important to know which genes compensate for SoxD function in the posterior neural development.

Evolutional consideration of neuralzing pathways

Over the last few years, important roles of Sox genes in invertebrate development have been suggested. Several Sox genes have been isolated in Drosophila (see below) and C. elegans (Hanna-Rose and Han, 1999) by conventional methods, and more members are found in the genome project databases.

In Drosophila, at least two Sox genes are expressed widely in the forming nervous tissues. *Fish-hook/Dichaete* (*fish*) encodes a Sox protein with significant homology to Group B Sox factors (Nambu and Nambu, 1996; Russell et al, 1996). The HMG domains of FISH and Sox2 are 88% identical in amino acid residues. These two factors share 42% overall sequence identity. Expression of *fish* starts at the central domain of the syncitial blastoderm embryo. In stage 5 cellular blastoderm embryos, *fish* is expressed in seven ectodermal stripes and in the procephalic region (Fig. 6, left top). Thus, the initial phase of *fish* expression shows a "segmentation gene" pattern (gap and pair-rule type). As the gastrulation proceeds, the pair-rule-type *fish* distribution is replaced by strong neuroectoderm expression (Fig. 6, right top). In stage 9 germ-band-extended embryos, prominent expression is found in the ventral and cephalic neuroectoderm. The loss-of-function phenotypes of fish include severe malfomation in the CNS in addition to segmentation defects.

Another Sox gene expressed widely in the fly neuroectoderm is *Sox Neuro* (*SoxN*)(Crémazy et al, 2000). *SoxN* also encodes an HMG protein belonging to Group B. The HMG domain of SOXN shows 92% amino acid identity to that of vertebrate Sox2. The role of *SoxN* in fly development remains to be investigated, but the regulation of this gene is intriguing in the terms of evolutional conservation. In the neuroectoderm of late syncitial blastoderm embryos (Fig. 6, left bottom), the distribution of *SoxN* is largely overlapping with that of *sog*, the fly counterpart of vertebrate *Chd* (Holley et al, 1995; De Robertis and Sasai, 1996). Furthermore, *SoxN* is downregulated in *sog* mutants and upregulated in *dpp* mutants. Ectopic expression of *sog* and *dpp* results in induction and repression of *SoxN*, respectively. This demonstrates that *SoxN* is regulated by the antagonistic signals of *sog* and *dpp* in the fly neuroectoderm, just like Xenopus *Sox2* and *SoxD*, which are regulated by Chordin/BMP4 signals (Fig. 7).

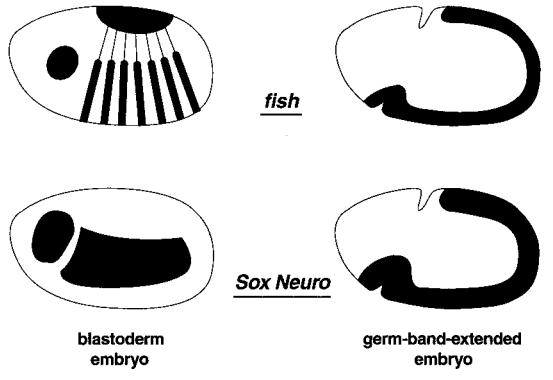

Fig. 6. Expression of Drosophila Sox genes in the neuroectoderm. *The RNA distribution of two fly Sox genes is illustrated in a simplified fashion at the blastoderm stage (stage 4-5; left) and at late gastrulation (stage 9-10; right). At the earlier stage, the fish genes (top) is expressed in the seven stripes and in the procephalic region while Sox Neuro (bottom) is expressed specifically in the cephalic and ventrolateral neuroectodermal regions. Both genes are expressed in the ventral and cephalic CNS at the end of gastrulation. The Sox Neuro expression domain extends more laterally than the fish expression domain.*

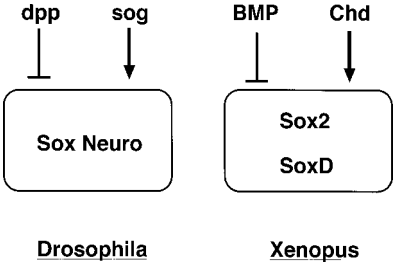

Fig. 7. Conserved regulation of neural-specific Sox genes by the BMP-antagonist signals. *In fly (left), Sox Neuro is controlled negatively and positively by dpp and sog, respectievly. In Xenopus (right), Sox2 and SoxD are regulated in a similar manner by the vertebrate counterparts, BMP and Chd.*

The Sox genes acting downstream of the common signals (Chd/sog) may represent the conserved neuralizing pathways across species, although further study is needed to prove it.

Conclusion

I reviewed recent studies on the roles of Sox genes in early neural development. An important challenge for future studies,

both in vertebrates and fly, is to find the bona fide target genes and, possibly, partner factors for Sox genes in early neural differentiation. Also, it is intriguing to isolate neural-specific Sox genes in much lower animals, such as in planaria and even in hydra. This may clarify the extent of mechanistic conservation of neural development and may provide important data on the origin of the CNS.

Acknowledgments

I am grateful to Dr Eddy De Robertis for encouragement while writing this article. I thank Miss Takako Yuasa for her secretarial assistance in the manuscript preparation. Parts of my work shown here were supported by grants from the Ministry of Education, the Organization of Pharmaceutical Safety and Research and HFSPO.

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