Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 351 (2006) 287–293
BBRC
www.elsevier.com/locate/ybbrc

Expression of *Sox1* during *Xenopus* early embryogenesis

Kazuhiro R. Nitta [a], Shuji Takahashi [b], Yoshikazu Haramoto [c], Masakazu Fukuda [a], Yasuko Onuma [d], Makoto Asashima [a,b,c,d,e,*]

[a] *Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Tokyo, Japan*
[b] *Center for Structuring Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, Tokyo, Japan*
[c] *Department of Life Sciences (Biology), Graduate School of Arts and Sciences, The University of Tokyo, Tokyo, Japan*
[d] *Network for Life Science Education, The University of Tokyo, Tokyo, Japan*
[e] *International Cooperative Research Project (ICORP) Japan Science and Technology Agency (JST), Tokyo, Japan*

Received 2 October 2006
Available online 16 October 2006

Abstract

Sox B1 group genes, *Sox1*, *Sox2*, and *Sox3* (*Sox1–3*), are involved in neurogenesis in various species. Here, we identified the *Xenopus* homolog of *Sox1*, and investigated its expression patterns and neural inducing activity. *Sox1* was initially expressed in the anterior neural plate of *Xenopus* embryos, with expression restricted to the brain and optic vesicle by the tailbud stage. Expression subsequently decreased in the eye region by the tadpole stage. *Sox1* expression in animal cap explants was induced by inhibition of BMP signaling in the same manner as *Sox2*, *Sox3*, and *SoxD*. In addition, overexpression of *Sox1* induced neural markers in ventral ectoderm and in animal caps. These results implicate *Xenopus Sox1* in neurogenesis, especially brain and eye development.

Keywords: *Xenopus laevis*; *Sox1*; *Sox2*; *Sox3*; *Sox* B1; *SoxD*; Neural induction

Sox genes encode Sry-related transcription factors containing an HMG DNA-binding domain [1], and are grouped into several subfamilies based on sequence similarity [2]. *Sox* B1 group genes, *Sox1*, *Sox2*, and *Sox3* (*Sox1–3*), are expressed in neural tissues and are implicated in neurogenesis in many species.

In mouse, *Sox2* is first expressed in the inner cell mass, the epiblast, the extra embryonic ectoderm, and the chorion, and subsequently in neural precursors, the ependyma, the neuron, and the thalamus [3,4]. *Sox2* ablation causes early embryonic lethality [4]. Deletion of a neural cell-specific enhancer in the *Sox2* regulatory sequence affects proliferation of neural precursor cells and generation of neurons in the adult mouse neurogenic region, suggesting that *Sox2* plays an important role in mouse neural development [5]. In chick, *Sox2* expression is first detected in the presumptive neuroectoderm [6]. In *Xenopus*, *Sox2* is initially expressed on the dorsal side of gastrula embryos, and the expression remains restricted to the central nervous system (CNS) through early development. Overexpression of *Xenopus Sox2* induces neural differentiation markers in animal caps treated with basic fibroblast growth factor. In addition, neural differentiation markers are reduced by overexpression of a dominant-negative mutant of *Sox2*, suggesting that *Sox2* is required for neural differentiation in *Xenopus* development [7].

Mouse *Sox3* is expressed early in the epiblast, the primitive streak, the neuroectoderm, and neural precursors. *Sox3* is also expressed in the placode epithelium and the gut endoderm [3,8]. *Sox3*-knockout mice show defects in oocyte development, testis differentiation, and gametogenesis [9]. Chick *Sox3* is expressed in the epiblast, ventricular zone of the spinal cord, and the brain [6,10], while *Xenopus Sox3* is expressed in the unfertilized eggs and becomes

* Corresponding author. Fax: +81 3 5454 4330.
E-mail address: asashi@bio.c.u-tokyo.ac.jp (M. Asashima).

0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2006.10.040

restricted to the neural region through embryogenesis, similar to *Xenopus Sox2* [11,12].

SoxD, the Sox group I gene, is also involved in neurogenesis in *Xenopus* [13,14]. *SoxD* is expressed in the neuroectoderm and neural tissue, and overexpression of *SoxD* induces neural marker genes in animal caps [14]. A dominant-negative mutant of *SoxD* and antisense morpholino oligonucleotides against *SoxD* inhibit neural development, suggesting that *SoxD* is essential for neurogenesis in *Xenopus* [14,15].

Mouse *Sox1* is initially expressed in the neural plate ectoderm at the late headfold stage of development. Overexpression of *Sox1* induces the expression of neuronal markers in cultured cells [16,17], and *Sox1* is important for neuronal maturation, and for maintaining neural precursors in the ventricular zone [18]. Chick *Sox1* is expressed during neural fold closure, and is detected in the brain and spinal cord during embryogenesis [10]. *Xenopus Sox1*, however, has not been reported. Here, we identify *Xenopus Sox1* expression in the CNS and the optic vesicle of embryos, and show that *Xenopus Sox1* is induced by inhibition of BMP signaling and has neural inducing activity.

Materials and methods

Embryos. *Xenopus laevis* embryos were obtained by artificial fertilization and were cultured in 10% Steinberg's solution (SS) at 20 °C. The embryos were staged according to Nieuwkoop and Faber [19].

Isolation of Sox1. *Xenopus laevis Sox1* was isolated by PCR using primers, forward 5′-ATGTACAGCATGATGATGG-3′ and reverse 5′-TCAGATGTGTGTCAGTGGC-3′. These primers were designed from the Contig 034028 sequence in the National Institute for Basic Biology in Japan (NIBB) XDB database and the amplified region corresponded to the putative open reading frame of *Sox1*. Amplified cDNA was cloned into pGEM®-T Easy vector (Promega)(pGEM-*Sox1*). Using pGEM-*Sox1* as a probe, we isolated a clone from a *X. laevis* stage-33 cDNA library. *In vivo* excision of the clone was performed according to the manufacturer's instruction (pBluescript(SK-)-*Sox1*). Sequences of both pGEM-*Sox1* and pBluescript(SK-)-*Sox1* were identical in the overlapping regions.

Multiple sequence alignment and a phylogenetic tree were computed using ClustalW analysis (UPGMA) in MacVector version 7 (Accelrys).

Semi-quantitative RT-PCR analysis. Total RNA was extracted from *Xenopus* embryos using Isogen (Nippon Gene). First-strand cDNA was synthesized from 1 μg total RNA using SuperScript™ II RT (Invitrogen Corp.). One-twentieth of the cDNA was used for RT-PCR. Elongation factor 1α (EF-1α) and ornithine decarboxylase (ODC) were used as internal controls. Reverse transcriptase negative (RT-) reactions showed absence of genomic DNA contamination. Primer sequences, sizes of PCR products, and cycling numbers are described in Table 1.

Whole-mount in situ hybridization. *Xenopus Sox2* was amplified by PCR using primers, forward 5′-TCTGCCAGCCTTTGCTCC-3′ and reverse 5′-CACATGTGCGACAGAGGC-3′, and cloned into pGEM®-T Easy vector (Promega). *Sox3* was amplified by PCR using primers, forward 5′-AAAGAATTCATGTATAGCATGTTGGACAC-3′ and reverse 5′-AAACTCGAGTTATATGTGAGTGAGCGGTAC-3′, and the amplified fragment was digested by *Eco*RI and *Xho*I for cloning into pBluescript(KS+)™ vector (Stratagene). Whole-mount in situ hybridization analysis was performed according to Harland [20] using albino embryos (Fig. 3) or pigmented type embryos (Fig. 4). Antisense RNA probes were synthesized using the following plasmids: pGEM-*Sox1*, pGEM-*Sox2*, pBluescript(KS+)-*Sox3*, and pBluescript(SK-)-*SoxD*. Signals were detected using BM purple (Roche). Wild-type embryos were bleached using 10% hydrogen peroxide in methanol.

Microinjection and animal cap dissection. To construct pCS2-*Sox1*, the insert of pGEM-*Sox1* was isolated with *Eco*RI and subcloned into the *Eco*RI site of pCS2+ vector. mRNAs were synthesized using SP6 mMESSAGE mMACHINE (Ambion) with linearized pCS2-*Sox1*, pCS2-*chordin* [21], and pCS2-*NLS-lacZ* [22].

Microinjection was performed in 100% SS containing 5% Ficoll. Dissected animal caps at stage 9 were cultured in 100% SS containing 0.1% BSA, and were analyzed by RT-PCR.

LacZ staining and whole-mount immunohistochemistry. Wild-type embryos were coinjected with *Sox1* and *NLS-lacZ* mRNA, and were prestained with Red-Gal (Research Organics). These embryos were bleached using 10% hydrogen peroxide in methanol. Antibodies used were anti-neural tissue monoclonal antibody, NEU-1 [23] and a goat anti-mouse IgG + IgM-alkaline phosphatase conjugate as secondary antibody (AMI0705, Biosource International).

Results and discussion

We found an EST contig (034028) encoding a *Sox1*-related sequence with highly conserved N- and C-terminal sequences in a *X. laevis* EST project database provided by the NIBB XDB database. We isolated *X. laevis Sox1*, which encodes a putative 393-amino acid protein containing a Sox1-type HMG-box (GenBank Accession No. AB219572; Fig. 1). The deduced amino acid sequence is highly conserved in Sox1 proteins of other species (71%

Table 1
RT-PCR primer sequences

Name	Sequence	Length	Cycles	Correspondence	Origin
Sox1	F: 5′-TCCAGCCAACAGCAGCAC-3′ R: 5′-CTGTCCTGCTTCAGAGAG-3′	372	32	3′-UTR	New
Sox2	F: 5′-GAGGATGGACACTTATGCCCAC-3′ R: 5′-GGACATGCTGTAGGTAGGCGA-3′	214	28	3′-Proximal coding region	New
Sox3	F: 5′-AGCGCAGGTATGACATGAGCG-3′ R: 5′-TATCTCGCAGGTCTCCCAGGC-3′	233	27	3′-Proximal coding region	Penzel et al. [12]
SoxD	F: 5′-TCAGCAACAGGTCCAGTACC-3′ R: 5′-TCTAACAAGATCCGACGCC-3′	315	27 or 23	3′-UTR	Yabe et al. [35]
EF-1α	F: 5′-TTGCCACACTGCTCACATTGCTTGC-3′ R: 5′-ATCCTGCTGCCTTCTTTTCCACTGC-3′	297	21	3′-Proximal coding region	Krieg et al. [36]
ODC	F: 5′-GTCAATGATGGAGTGTATGGATC-3′ R: 5′-TCCAATCCGCTCTCCTGAGCAC-3′	385	27	3′-Proximal coding region	XMMR

XMMR, *Xenopus* molecular marker resource (http://www.cbrmed.ucalgary.ca/prize/html/WWW/Welcome.html). The cycling numbers of *SoxD* are given in Fig. 2 or Fig. 5, respectively.

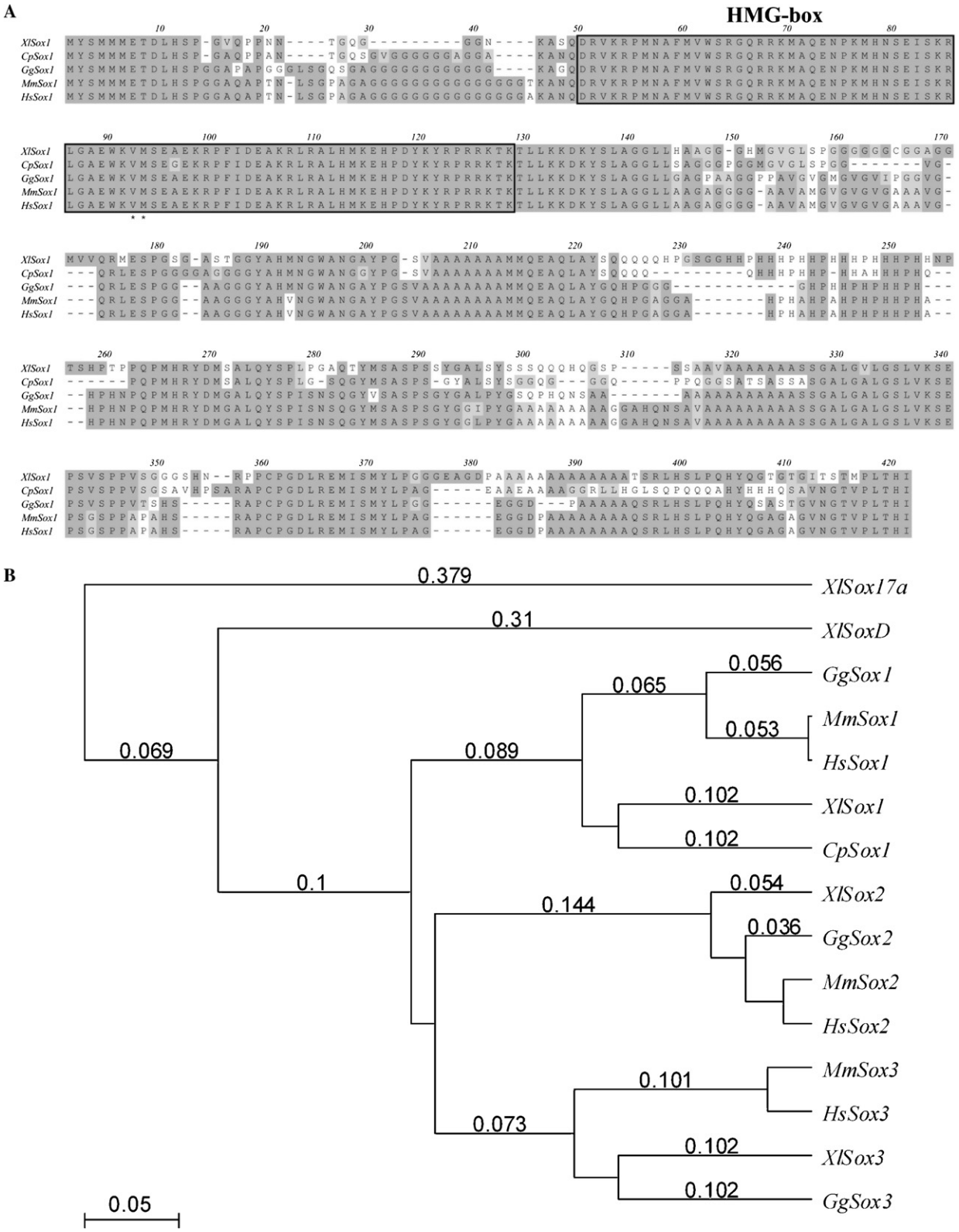

Fig. 1. (A) Alignment of Sox1 amino acid sequences in various species. Identical amino acids are shaded in gray, and conserved amino acid substitutions are shaded in light gray. The conserved Sox1-type HMG-box domain is boxed. *Xenopus* Sox1 shares 71% sequence identity with newt Sox1, 69% with chick, 68% with mouse, and 69% with human. (B) Rooted phylogeny of Sox1–3, SoxD, and Sox17α amino acid sequences. *X. laevis* Sox17α was used as the outlier. Xl, *X. laevis*; Cp, *Cynops pyrrhogaster*; Gg, *Gallus gallus*; Mm, *Mus musculus*; Hs, *Homo sapiens*. Sequence sources (GenBank Accession Nos.) are as follows: *X. laevis Sox2* (AAB62821) [24], *Sox3* (CAA68828) [12], *SoxD* (AB013896) [14], *Sox17α* (CAA04957) [25]; *Cynops pyrrhogaster Sox1* (AB154820) [26]; *G. gallus Sox1* (BAA25092) [27], *Sox2* (P48430) [27,28], *Sox3* (P48433) [28]; *Mus musculus Sox1* (AB108672) [29], *Sox2* (P48432) [30], *Sox3* (P53784) [8,29,31]; *Homo sapiens Sox1* (O00570) [32], *Sox2* (CAA83435) [33], *Sox3* (P41225) [34].

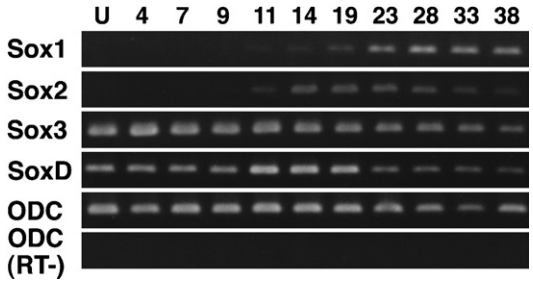

Fig. 2. Temporal expressions of *Sox1–3* and *SoxD* during *Xenopus* development. RT-PCR analysis was performed at various developmental stages. *Sox1* and *Sox2* transcripts appeared at stage 11 and were detected until stage 38. *Sox3* and *SoxD* transcripts were detected in unfertilized eggs and in embryos up to stage 38. Numbers indicate developmental stages. U, unfertilized egg.

amino acid identity to newt, 69% to chick, 68% to mouse, and 69% to human; Fig. 1A).

We performed RT-PCR to examine the temporal expression pattern of *Sox1* during *Xenopus* embryogenesis. *Sox1* expression appeared first at the early gastrula stage and was maintained throughout development (Fig. 2). In contrast, expression of *Sox2* and *Sox3* transcripts was detected from stage 11 to stage 38 and from unfertilized eggs to stage 38, respectively, consistent with previous studies [7,11,12]. We detected *SoxD* transcripts in unfertilized eggs, although it has been reported to be a zygotic gene [14].

Next, we performed whole-mount in situ hybridization to determine the spatial expression pattern of *Sox1*

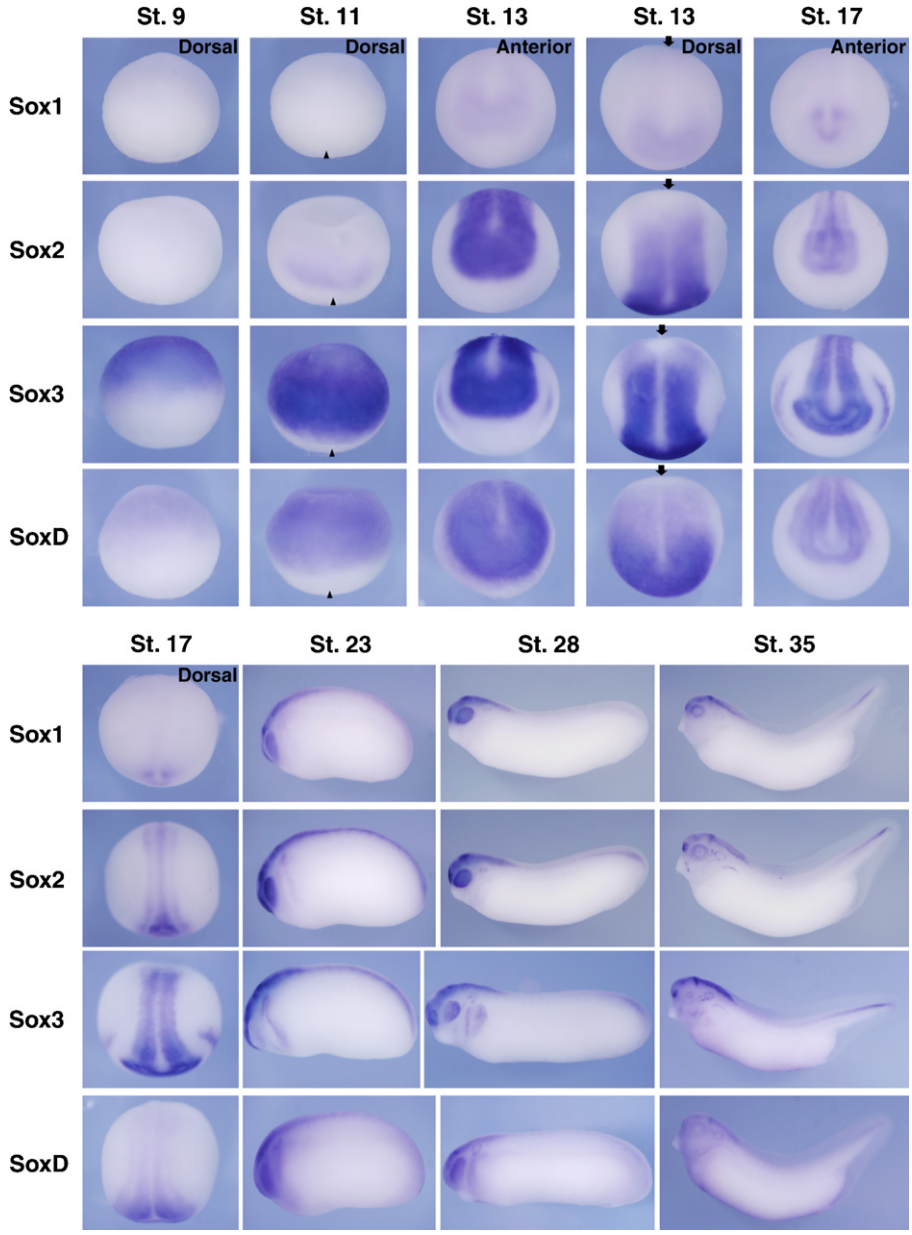

Fig. 3. Spatial expression patterns of *Sox1–3* and *SoxD* revealed by whole-mount in situ hybridization. In dorsal views of stage 13 and 17, the anterior is at the bottom of the panel. Arrowheads and arrows indicate the dorsal lip and the yolk plug, respectively. *Sox1* expression was not detected at stage 11. At stage 35, *Sox1* expression was detected throughout the brain and the tail.

(Fig. 3). *Sox1* mRNA was not detected during gastrula stages, although gene expression was detected at stage 11 by RT-PCR (Fig. 2). It is possible that the expression level is insufficient for detection with whole-mount in situ hybridization at this early stage. *Sox1* expression appeared in the anterior neural plate at stage 13 (Fig. 3), and was restricted to the prospective brain and eye by stages 17 and 23 (Fig. 3). *Sox1* was expressed through the forebrain to hindbrain and in the optic vesicle at stage 28 (Fig. 3), and by stage 35, *Sox1* transcripts were detected only in the brain and the tail (Fig. 3).

Xenopus Sox1 was expressed in the CNS, similarly to *Sox2*, *Sox3*, and *SoxD*. To examine the expression of *Sox1* in detail, we sectioned fixed embryos for in situ hybridization analysis (Fig. 4). At stage 28, *Sox1*, *Sox2*, and *SoxD* were expressed in the brain and the optic vesicle (Fig. 4C, D and F), while *Sox3* was expressed in the brain and the epidermis of the optic vesicle (Fig. 4E, arrowhead). At stage 35, *Sox1* mRNA was strongly expressed in the brain (Fig. 4G), but only weakly in the eye. Transcripts of *Sox2* and *SoxD* were detected in the brain, the neural retina, and the lens (Fig. 4H and J), while *Sox3* was expressed in the brain and the lens, but not in the neural retina (Fig. 4I, arrowhead). These observations are consistent with a previous report showing different expression levels of each of the *Sox* genes in the neural retina and the lens in chick embryos [10]. In addition, expression of *SoxD* was reduced after the neurula stage (Figs. 2–4F,J), suggesting that *SoxD* mainly functions in early neurogenesis.

Previous studies showed that early neural genes including *Sox2* and *SoxD* are induced following inhibition of BMP signaling by neural-inducing genes such as *chordin* [7,14,21]. Semi-quantitative RT-PCR analysis in this study showed that overexpression of *chordin* in animal caps induced *Sox1* expression at stages 13, 18, and 25, as well as the expression of *Sox2*, *Sox3*, and *SoxD* (Fig. 5). Expression of *Sox1* and *Sox2* in uninjected animal caps may not depend on inhibition of BMP signaling, while the expression of *Sox3* and *SoxD* in uninjected animal caps may be derived from maternal transcripts.

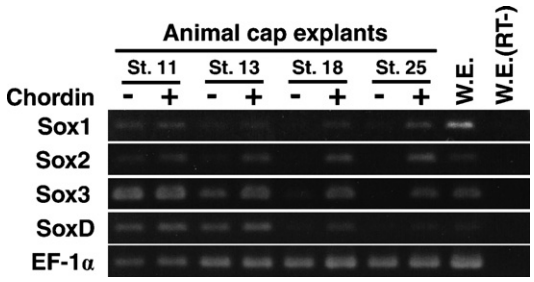

Fig. 5. *Sox1–3* and *SoxD* are induced by inhibition of BMP signaling in animal caps. Animal caps were dissected at stage 9 from embryos uninjected (−) or injected with 50 pg of *chordin* mRNA (+), and were cultured until the sibling embryos reached stages 11, 13, 18, and 25. W.E., whole embryos.

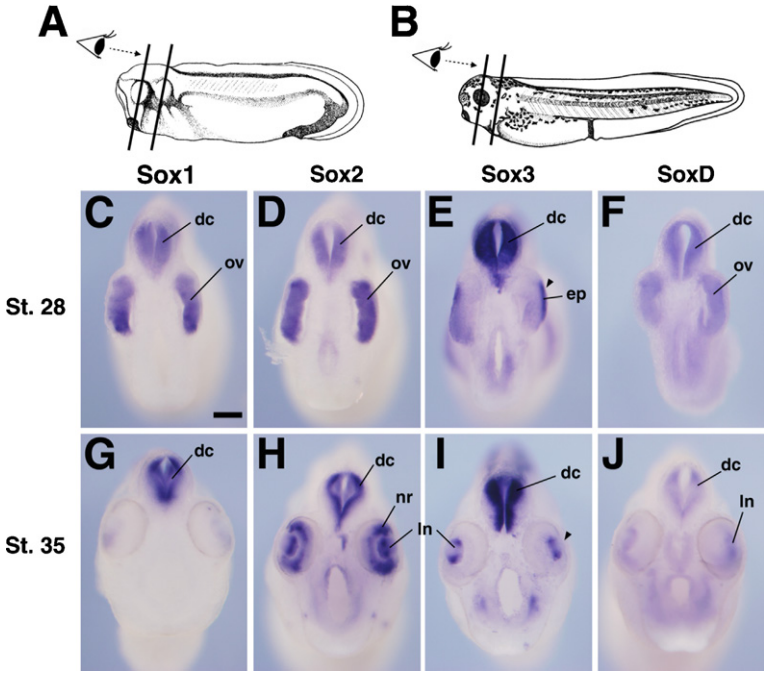

Fig. 4. Expression patterns of *Sox1-3* and *SoxD* in sectioned embryos. Fixed embryos were dissected at the line indicated in (A) (stage 28) and in (B) (stage 35). *Sox1–3* and *SoxD* were expressed in the diencephalon at stage 28 (C–F). *Sox1*, *Sox2*, and *SoxD* were also expressed in the optic vesicle (C,D,F). *Sox3* was expressed strongly in the epidermis of the optic vesicle (E, arrowhead). At stage 35, *Sox1* was expressed in the diencephalon and was weakly expressed in the eye (G). *Sox2* and *SoxD* were expressed in the diencephalon, the neural retina, and the lens (H,J). *Sox3* was expressed in the diencephalon and the lens, but not in the neural retina (I, arrowhead). A bar in (C) indicates 200 μm. dc, diencephalon; ep, epidermis; nr, neural retina; ov, optic vesicle; ln, lens.

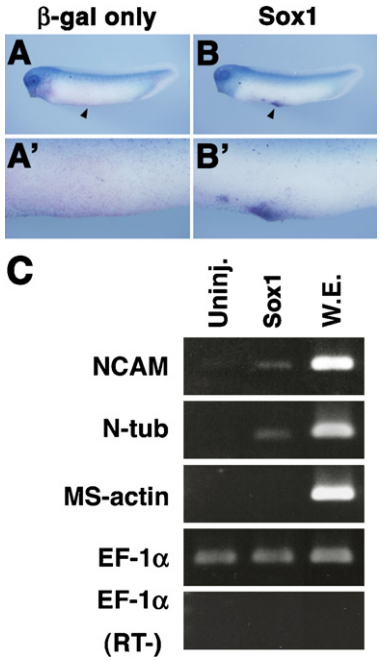

Fig. 6. *Sox1* induces neural tissue and neural gene expression. (A,B) Embryos were coinjected with 100 pg of *Sox1* mRNA and β-gal mRNA into both A4 blastomeres of 32-cell stage embryos. Overexpression of *Sox1* induced neural tissue recognized by NEU-1. (A′,B′) High magnification views of (A) and (B). Arrowheads indicate overexpressed regions. (C) Overexpression of *Sox1* mRNA induces neural genes, *NCAM*, and *N*-tublin (*N*-tub), without inducing a mesodermal gene, *MS-actin* in animal caps. Animal caps were dissected from embryos injected with 100 pg of *Sox1* mRNA, and cultured until sibling embryos reached stage 32. W.E., whole embryos.

Next, we investigated whether *Sox1* induces neural tissue and neural gene expression in *Xenopus* development. *Sox1* mRNA was coinjected with a lineage tracer, β-gal mRNA, into A4 blastomeres of 32-cell embryos. Immunohistochemistry using NEU-1 antibody indicated that *Sox1* induced ectopic neural tissue in the ventral epidermal region derived from the A4 blastomeres (Fig. 6B and B′; 100%, $n = 49$). RT-PCR analysis also showed that overexpression of *Sox1* induced a pan-neural marker gene, *N-CAM* and a neuron-specific gene, *N*-tubulin in animal caps without mesoderm induction (Fig. 6C). These results suggest that *Sox1* in *Xenopus* participates in neural induction, similar to *Sox1* in other species.

Acknowledgments

We thank Dr. Y. Sasai for his generous gift of plasmid. This work was supported by Grants-in-Aid for Science Research from the Ministry of Education, Science, Sports, Culture and Technology of Japan.

References

[1] L.H. Pevny, R. Lovell-Badge, Sox genes find their feet, Curr. Opin. Genet. Dev. 7 (1997) 338–344.
[2] M. Wegner, From head to toes: the multiple facets of Sox proteins, Nucleic Acids Res. 27 (1999) 1409–1420.
[3] H.B. Wood, V. Episkopou, Comparative expression of the mouse Sox1, Sox2 and Sox3 genes from pre-gastrulation to early somite stages, Mech. Dev. 86 (1999) 197–201.
[4] A.A. Avilion, S.K. Nicolis, L.H. Pevny, L. Perez, N. Vivian, R. Lovell-Badge, Multipotent cell lineages in early mouse development depend on SOX2 function, Genes. Dev. 17 (2003) 126–140.
[5] A.L. Ferri, M. Cavallaro, D. Braida, A. Di Cristofano, A. Canta, A. Vezzani, S. Ottolenghi, P.P. Pandolfi, M. Sala, S. DeBiasi, S.K. Nicolis, Sox2 deficiency causes neurodegeneration and impaired neurogenesis in the adult mouse brain, Development 131 (2004) 3805–3819.
[6] M. Rex, A. Orme, D. Uwanogho, K. Tointon, P.M. Wigmore, P.T. Sharpe, P.J. Scotting, Dynamic expression of chicken Sox2 and Sox3 genes in ectoderm induced to form neural tissue, Dev. Dyn. 209 (1997) 323–332.
[7] K. Mizuseki, M. Kishi, M. Matsui, S. Nakanishi, Y. Sasai, Xenopus Zic-related-1 and Sox-2, two factors induced by chordin, have distinct activities in the initiation of neural induction, Development 125 (1998) 579–587.
[8] J. Collignon, S. Sockanathan, A. Hacker, M. Cohen-Tannoudji, D. Norris, S. Rastan, M. Stevanovic, P.N. Goodfellow, R. Lovell-Badge, A comparison of the properties of Sox-3 with Sry and two related genes, Sox-1 and Sox-2, Development 122 (1996) 509–520.
[9] J. Weiss, J.J. Meeks, L. Hurley, G. Raverot, A. Frassetto, J.L. Jameson, Sox3 is required for gonadal function, but not sex determination, in males and females, Mol. Cell. Biol. 23 (2003) 8084–8091.
[10] M. Uchikawa, Y. Kamachi, H. Kondoh, Two distinct subgroups of Group B Sox genes for transcriptional activators and repressors: their expression during embryonic organogenesis of the chicken, Mech. Dev. 84 (1999) 103–120.
[11] S. Koyano, M. Ito, N. Takamatsu, S. Takiguchi, T. Shiba, The *Xenopus* Sox3 gene expressed in oocytes of early stages, Gene 188 (1997) 101–107.
[12] R. Penzel, R. Oschwald, Y. Chen, L. Tacke, H. Grunz, Characterization and early embryonic expression of a neural specific transcription factor xSOX3 in *Xenopus laevis*, Int. J. Dev. Biol. 41 (1997) 667–677.
[13] J. Bowles, G. Schepers, P. Koopman, Phylogeny of the SOX family of developmental transcription factors based on sequence and structural indicators, Dev. Biol. 227 (2000) 239–255.
[14] K. Mizuseki, M. Kishi, K. Shiota, S. Nakanishi, Y. Sasai, SoxD: an essential mediator of induction of anterior neural tissues in *Xenopus* embryos, Neuron 21 (1998) 77–85.
[15] K.R. Nitta, K. Tanegashima, S. Takahashi, M. Asashima, XSIP1 is essential for early neural gene expression and neural differentiation by suppression of BMP signaling, Dev. Biol. 275 (2004) 258–267.
[16] L.H. Pevny, S. Sockanathan, M. Placzek, R. Lovell-Badge, A role for SOX1 in neural determination, Development 125 (1998) 1967–1978.
[17] L. Kan, N. Israsena, Z. Zhang, M. Hu, L.R. Zhao, A. Jalali, V. Sahni, J.A. Kessler, Sox1 acts through multiple independent pathways to promote neurogenesis, Dev. Biol. 269 (2004) 580–594.
[18] A. Ekonomou, I. Kazanis, S. Malas, H. Wood, P. Alifragis, M. Denaxa, D. Karagogeos, A. Constanti, R. Lovell-Badge, V. Episkopou, Neuronal migration and ventral subtype identity in the telencephalon depend on SOX1, PLoS Biol. 3 (2005) e186.
[19] P.D. Nieuwkoop, J. Faber, Normal Table of *Xenopus laevis* (Daudin), ed., North-Holland, Amsterdam, 1956.
[20] R.M. Harland, In situ hybridization: an improved whole-mount method for *Xenopus* embryos, Methods Cell Biol. 36 (1991) 685–695.
[21] Y. Sasai, B. Lu, H. Steinbeisser, D. Geissert, L.K. Gont, E.M. De Robertis, *Xenopus chordin*: a novel dorsalizing factor activated by organizer-specific homeobox genes, Cell 79 (1994) 779–790.

[22] S. Takahashi, C. Yokota, K. Takano, K. Tanegashima, Y. Onuma, J. Goto, M. Asashima, Two novel nodal-related genes initiate early inductive events in *Xenopus* Nieuwkoop center, Development 127 (2000) 5319–5329.
[23] K. Itoh, H.Y. Kubota, Expression of neural antigens in normal *Xenopus* embryos and induced explants, Dev. Growth Differ. 31 (1989) 563–571.
[24] E.M. De Robertis, S. Kim, L. Leyns, S. Piccolo, D. Bachiller, E. Agius, J.A. Belo, A. Yamamoto, A. Hainski-Brousseau, B. Brizuela, O. Wessely, B. Lu, T. Bouwmeester, Patterning by genes expressed in Spemann's organizer, Cold Spring Harb Symp. Quant. Biol. 62 (1997) 169–175.
[25] C. Hudson, D. Clements, R.V. Friday, D. Stott, H.R. Woodland, Xsox17alpha and -beta mediate endoderm formation in *Xenopus*, Cell 91 (1997) 397–405.
[26] T. Hayashi, N. Mizuno, Y. Ueda, M. Okamoto, H. Kondoh, FGF2 triggers iris-derived lens regeneration in newt eye, Mech. Dev. 121 (2004) 519–526.
[27] Y. Kamachi, M. Uchikawa, J. Collignon, R. Lovell-Badge, H. Kondoh, Involvement of Sox1, 2 and 3 in the early and subsequent molecular events of lens induction, Development 125 (1998) 2521–2532.
[28] D. Uwanogho, M. Rex, E.J. Cartwright, G. Pearl, C. Healy, P.J. Scotting, P.T. Sharpe, Embryonic expression of the chicken Sox2, Sox3 and Sox11 genes suggests an interactive role in neuronal development, Mech. Dev. 49 (1995) 23–36.
[29] S. Tanaka, Y. Kamachi, A. Tanouchi, H. Hamada, N. Jing, H. Kondoh, Interplay of SOX and POU factors in regulation of the Nestin gene in neural primordial cells, Mol. Cell. Biol. 24 (2004) 8834–8846.
[30] H. Yuan, N. Corbi, C. Basilico, L. Dailey, Developmental-specific activity of the FGF-4 enhancer requires the synergistic action of Sox2 and Oct-3, Genes. Dev. 9 (1995) 2635–2645.
[31] J. Gubbay, J. Collignon, P. Koopman, B. Capel, A. Economou, A. Munsterberg, N. Vivian, P. Goodfellow, R. Lovell-Badge, 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 346 (1990) 245–250.
[32] S. Malas, S.M. Duthie, F. Mohri, R. Lovell-Badge, V. Episkopou, Cloning and mapping of the human SOX1: a highly conserved gene expressed in the developing brain, Mamm Genome 8 (1997) 866–868.
[33] M. Stevanovic, O. Zuffardi, J. Collignon, R. Lovell-Badge, P. Goodfellow, The cDNA sequence and chromosomal location of the human SOX2 gene, Mamm Genome 5 (1994) 640–642.
[34] M. Stevanovic, R. Lovell-Badge, J. Collignon, P.N. Goodfellow, SOX3 is an X-linked gene related to SRY, Hum. Mol. Genet. 2 (1993) 2013–2018.
[35] S. Yabe, K. Tanegashima, Y. Haramoto, S. Takahashi, T. Fujii, S. Kozuma, Y. Taketani, M. Asashima, FRL-1, a member of the EGF-CFC family, is essential for neural differentiation in Xenopus early development, Development 130 (2003) 2071–2081.
[36] P.A. Krieg, S.M. Varnum, W.M. Wormington, D.A. Melton, The mRNA encoding elongation factor 1-alpha (EF-1 alpha) is a major transcript at the midblastula transition in Xenopus, Dev. Biol. 133 (1989) 93–100.