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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*

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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).

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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.