Temporal and Spatial Expression Patterns of Sox1 Gene in *Xenopus laevis* Embryo

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Abstract: We describe the temporal and spatial expression pattern of Sox1 gene during *Xenopus laevis* early development and compare the expression patterns of Sox1–3 in the developing eye and brain. Alignment of Sox1–3 amino acid sequences shows a high conservation within the HMG-box DNA binding domains. RT-PCR analysis indicates that Sox1 is expressed throughout development from the unfertilized egg to at least the tadpole stage, although at different expression levels. The transcripts of XSox1 are detected in the animal pole at cleavage and blastula stages and mainly in the central nervous system (CNS) and the developing eye at neurula stages. The study of the developmental expression of XSox1 will aid in the elucidation of the function of SoxB1 subgroup genes in vertebrate neurogenesis.

Key words: Sox1; Sox2; Sox3; *Xenopus laevis*; Expression pattern

 Sox1 基因在爪蟾早期发育中的时空表达图式

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摘要：克隆了非洲爪蟾的 Sox1 基因并研究了它在非洲爪蟾早期发育过程中的时空表达图式，比较了 Sox1–3 基因在发育的脑和眼中的表达图式。序列比对分析显示 Sox1–3 蛋白在其 HMG 域结构域具有高度的保守性。通过 RT-PCR 方法分析了 Sox1 基因在爪蟾早期不同发育时段的表达情况，结果显示 Sox1 基因从未受精卵到尾芽期均有表达，但表达强度有所差异。原位杂交结果显示，在早期卵裂阶段和囊胚期，Sox1 基因主要在动物极表达；从神经板期开始，Sox1 基因主要在中枢神经系统和眼原基中表达。在蝌蚪期，Sox1 与 Sox2、Sox3 在脑部和眼睛的表达区域有所不同。对于爪蟾 Sox1 基因时空表达图式的研究将有助于阐明 SoxB1 基因家族在脊椎动物神经系统发生过程中的作用。

关键词：Sox1 基因; Sox2 基因; Sox3 基因; 非洲爪蟾; 表达图式

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The Sox family transcription factors are characterized by their DNA-binding high-mobility group (HMG) domains and play crucial roles in neural development (for reviews see Wegner & Stolt, 2005; Pevny & Placzek, 2005). Among them, the SoxB1 subfamily proteins (Sox1, Sox2 and Sox3) share more than 90% identity within their HMG domains and significant homology outside. All three factors are co-expressed in proliferating neural progenitors of the embryonic and adult central nervous system (CNS) and play important roles in neural cell fate determination and differentiation.

The expression of the SoxB1 genes has been evolutionarily conserved in the neural primordium during early embryonic development (for review see Pevny &
In the mouse, Sox2 and Sox3 are expressed in the epiblast and extraembryonic ectoderm of the egg cylinder and then restricted to the neuroepithelium at the onset of gastrulation. Sox1 expression then appears in the neural plate ectoderm at the headfold stage. After neural induction, their expression is confined to the proliferating neural precursors along the entire antero-posterior axis of the developing embryo and subsequently in adult neural stem cells (Wood & Episkopou, 1999). Compelling evidence suggests that the SoxB1 factors function in neural precursors to maintain neural progenitor identity by counteracting neurogenesis (Bylund et al., 2003). Because of their biochemical similarities and largely overlapping expression pattern, the SoxB1 proteins are believed to play redundant roles in neural cell fate determination.

In addition to their roles in maintaining neural precursor identity, the SoxB1 factors also have late subtype-specific functions in postmitotic neurons. The expression of SoxB1 proteins overlaps much less in the mature brain than during embryonic CNS development, suggesting different roles of the individual factors. In the mouse brain, Sox1 expression is particularly strong in the GABAergic neurons of the ventral striatum (Ekonomou et al., 2005), while Sox2 is expressed in the pyramidal cells of the cerebral cortex, some striatal neurons and many thalamic neurons (Ferri et al., 2004) and Sox3 is preferentially expressed in the ventral hypothalamus (Rizzoti et al., 2004). The SoxB1 factors are also differentially expressed in the developing eye and are crucial for eye development (Kamachi et al., 1998). In vitro, the SoxB1 factors also show some differences in their activity. For example, overexpression of Sox1, but not Sox2 or Sox3, in neural progenitor cells is sufficient to promote neural differentiation (Peuny et al., 1998; Kan et al., 2004). In Xenopus embryos, Sox2 and Sox3 are similarly expressed in the newly induced neural plate and their expression is regulated by neural inducing signals (Mizuseki et al., 1998; Pennel et al., 1997; Koyano et al., 1997). XSox2 plays important roles in establishing neural fate and injection of dominant interfering forms of Sox2 into Xenopus embryos inhibits neural differentiation (Kishi et al., 2000). In addition, XSox3 is also strongly maternally expressed and play an important role in germ layer formation (Zhang et al., 2004).

In this study, we have cloned the Xenopus Sox1 and studied in detail its temporal and spatial expression pattern during early development. XSox1 is highly expressed maternally and then in the developing central nervous system, overlapping with that of Sox2 and Sox3. In the brain and eye, the three SoxB1 factors show overlapping but different expression domains. Our results are similar as recently reported (Nitta et al., 2006) but with some differences.

1 Materials and Methods

1.1 Isolation of Xenopus Sox1 gene

Xenopus laevis Sox1 was isolated from a St. 30 X. laevis head cDNA library (gift from Dr. C. Niehrs) by PCR screening using the following primers designed according to a XSox1 EST clone (GenBank accession number: CA986222): forward 5’-TAAATACCGGCGGGCGAAAAAC-3’ and reverse 5’-GGGTTGAGTGGCTGGTGCTGAT-3’. The insert of the clone was full-length sequenced and the sequence was submitted to the GenBank under accession number EF672727.

1.2 Reverse transcription-PCR assay

Reverse transcription was carried out using the RevertAid H minus first strand cDNA Synthesis kit (Fermentas) and PCR assays were carried out in the linear phase of amplification. H4 was used as an internal control (Glinka et al., 1997). Primers used for RT-PCR were: XSox1, forward: 5’-CGGCGATATGCTGGGACGTCG-3’ and reverse: 5’-TTCCATGCGGTTACGACCA-3’; XSox3, forward: 5’-ACA ACCCTATGATGACCTG-3’; reverse: 5’-AGTCTGATTGGCAGGCGGAA-3’. The primers for XSox2 and H4 were used as reported (Matsutoh et al., 2005; Glinka et al., 1997).

1.3 Embryos. in situ hybridization and sections

In vitro fertilization, embryo culture and whole-mount in situ hybridization of Xenopus embryos were carried out as described by Gawantka et al. (1995). Developmental stages were determined according to Nieuwoop & Faber (1967). The Sox1 probe was a 1.9 kb fragment including the 5′ untranslated region and the coding region. The 3′ untranslated regions of XSox2 and XSox3 were used for probe preparation for in situ hybridizations. Stained embryos were embedded in paraffin, sectioned at 30 μm and the sections were counter-stained with eosin.

2 Results

2.1 Alignment of Sox1–3 amino acid sequences

An XSox1 clone was isolated from a St. 30 Xenopus laevis head cDNA library by PCR screening. The
XSox1 cDNA contains an open reading frame of 393 amino-acid residues, showing 71% homology with newt, 69% with chick, 68% with mouse and 69% with human Sox1 genes (see also Nitta et al., 2006). Alignment of the Xenopus Sox1, 2 and 3 proteins shows a high conservation of the HMG box (Fig. 1) and that Sox1 is less related to Sox2 and Sox3.

2.2 Temporal expression of Sox1 during Xenopus development

Reverse transcription PCR (RT-PCR) analysis was performed to examine the temporal expression pattern of Sox1–3 during Xenopus embryogenesis (Fig. 2). The XSox1 transcripts are clearly detected in unfertilized eggs and blastula stages. Its expression remained at a relatively high level till late gastrula stage (St. 11) but became weaker at early neurula stages (St. 12 to 15, Fig. 2). Strong expression of XSox1 can be detected at stage 18, stage 20 and stage 30. In contrast, Sox2 is not detected maternally and its zygotic expression started at late blastula stage and remained relatively constant at later developmental stages (Fig. 2, see also Mizuseki et al., 1998). Sox3 is detected strongly throughout the stages tested (Fig. 2, see also Pennel et al., 1997; Koyano et al., 1997).

2.3 Spatial expression of Sox1 during Xenopus laevis early development

The maternal transcripts of Sox1 could be clearly detected in the animal hemisphere at early cleavage stages (Fig. 3 A, B, C, D) and in the presumptive endoderm in the late blastula embryos (Fig. 3 E). At gastrula stage (St. 10.5), it seemed to be expressed weakly in the anterior endoderm distant from the blasto-
pore (Fig. 3 F). At neural plate stages (St. 14, 15), weak expression of Sox1 could be detected broadly in the forming neural plate but was absent in the midline (Fig. 3 G, H). At neural fold stage (St. 20), Sox1 showed relatively strong expression in the anterior region of the neural tube (Fig. 3 I, I'). At stages 23 and 25, its expression became stronger in the presumptive brain area and appeared in the forming eye-anlagen (Fig. 3 J, J', K, K'). At tail-bud stages, Sox1 was strongly expressed in the brain, eye and weakly in the spinal cord (Fig. 3 L, M, N, O). At tadpole stages, the expression of Sox1 was much stronger in the dorsal roof of the brain vesicles than in the ventral part (Fig. 3 N).

2.4 **Comparison of the expression of X Sox1–3 in the brain and eye**

At stage 23, both Sox1 and Sox2 were expressed in
the primitive brain and the optic vesicle but the expression of Sox2 at the anterior neural tube was stronger and broader than Sox1 (Fig. 4 A, B). Sox3 was only expressed in the neural tube but not the primitive eye domain at this stage. In addition, Sox3 is also expressed in a crescent-shaped domain surrounding the anterior neural plate (Fig. 4 C). At tail-bud stages, Sox1, 2 and 3 showed slightly different expression patterns in the brain. Sox1 seemed to be strongly expressed in the dorsal part of the brain while Sox2 was strong in several patches along the anterior-posterior axis in the telencephalon, midbrain-hindbrain boundary and hindbrain. Sox3 is more or less continuously expressed in the brain region (Fig. 4 D, E, F, D', E', F'; Fig. 3 N). The three genes are all expressed in the otic vesicle at this stage. Sox2 and Sox3 but not Sox1 are expressed in the branchial arches (Fig. 4 D, E, F). In the eye, Sox1 is expressed in the neural retina but not the lens (Fig. 4 D, G) while Sox2 can be detected both in the neural retina and the lens (Fig. 4 E, H). Sox3 expression was not detected in the neural retina and weakly in the lens at stage 30 (Fig. 4 F, I).

Fig. 4 Comparison of the expression of XSox1–3 in the brain and eye.

(A-C) Expression of XSox1 (A), XSox2 (B), and XSox3 (C) at stage 23, anterior views. Arrowhead in C mark the crescent-shaped expression domain of XSox3 surrounding the anterior neural plate. (D-F, D'-F') Expression of XSox1 (D, D'), XSox2 (E, E') and XSox3 (E, E') at stage 33. D-F, lateral views; D'-F', dorsal views; (G-I) Transversal sections through the eye region showing the expression of XSox1 (G, Stage 33), XSox2 (H, Stage 33) and XSox3 (I, Stage 30) in the brain and eye. br., branchial arches; le., lens; nr., neural retina; ot., optic vesicle.
3 Discussion

We show here strong maternal expression of XSox1 by RT-PCR and in situ hybridization analysis, which was not shown by Nitta et al. (2006). This could be due to different primers and probes used in the experiments. XSox3 is also strongly maternally expressed and has been shown to be important in germ layer formation (Zhang et al., 2004). The maternal expression of XSox1 might suggest a similar role in the early patterning of the ectoderm in Xenopus. The widely overlapping expression of the SoxB1 genes in the central nervous system might suggest redundant roles of the three genes in neural patterning and differentiation.

References:


Wood HB, Episkopou V. 1999. Comparative expression of the mouse Sox1, Sox2 and Sox3 genes from pre-gastrulation to early somite stages [J]. Mech Dev., 86 (1–2); 197–201.