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Zoological Research

Temporal and Spatial Expression Patterns of *Sox*1 Gene in *Xenopus laevis* Embryo

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Abstract: We describe the temporal and spatial expression pattern of *Sox*1 gene during *Xenopus laevis* early development and compare the expression patterns of *Sox*1-3 in the developing eye and brain. Alignment of *Sox*1-3 amino acid sequences shows a high conservation within the HMG-box DNA binding domains. RT-PCR analysis indicates that *Sox*1 is expressed throughout development from the unfertilized egg to at least the tadpole stage, although at different expression levels. The transcripts of *XSox*1 are detected in the animal pole at cleavage and blastrula stages and mainly in the central nervous system (CNS) and the developing eye at neurula stages. The study of the developmental expression of *XSox*1 will aid in the elucidation of the function of *SoxB*1 subgroup genes in vertebrate neurogenesis.

Key words: *Sox*1; *Sox*2; *Sox*3; *Xenopus laevis*; Expression pattern

*Sox*1 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 *Sox*1 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 *Sox*1—3 基因在发育的脑和眼中的表达图式。序列比对分析显示 *Sox*1—3 蛋白在其 HMG 框结构域具有高度的保守性。通过 RT-PCR 方法分析了 *Sox*1 基因在爪蟾早期不同发育时段的表达情况, 结果显示 *Sox*1 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, *Sox*1 基因主要在动物极表达; 从神经板期开始, *Sox*1 基因主要在中枢神经系统和眼原基中表达。在蝌蚪期, *Sox*1 与 *Sox*2、*Sox*3 在脑部和眼睛的表达区域有所不同。对于爪蟾 *Sox*1 基因时空表达图式的研究将有助于阐明 *SoxB*1 基因家族在脊椎动物神经系统发生过程中的作用。

关键词: *Sox*1 基因; *Sox*2 基因; *Sox*3 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 *SoxB*1 subfamily proteins (*Sox*1, *Sox*2 and *Sox*3) 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 *SoxB*1 genes has been evolutionarily conserved in the neural primordium during early embryonic development (for review see Pevny &

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Placzek, 2005). 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 *Sox*2 is expressed in the pyramidal cells of the cerebral cortex, some striatal neurons and many thalamic neurons (Ferri et al, 2004) and *Sox*3 is preferentially expressed in the ventral hypothalamus (Rizzoti et al, 2004). The *SoxB*1 factors are also differentially expressed in the developing eye and are crucial for eye development (Kamachi et al, 1998). *In vitro*, the *SoxB*1 factors also show some differences in their activity. For example, overexpression of *Sox*1, but not *Sox*2 or *Sox*3, in neural progenitor cells is sufficient to promote neural differentiation (Pevny et al, 1998; Kan et al, 2004). In *Xenopus* embryos, *Sox*2 and *Sox*3 are similarly expressed in the newly induced neural plate and their expression is regulated by neural inducing signals (Mizuseki et al, 1998; Penzel et al, 1997; Koyano et al, 1997). *XSox*2 plays important roles in establishing neural fate and injection of dominant interfering forms of *Sox*2 into *Xenopus* embryos inhibits neural differentiation (Kishi et al, 2000). In addition, *XSox*3 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 Sox*1 and studied in detail its temporal and spatial expression pattern during early development. *XSox*1 is highly expressed maternally and then in the developing central nervous system, overlapping with that of *Sox*2 and *Sox*3. In the brain and eye, the three *SoxB*1 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′-TAAATACCGGCCGAGGCGAAAAAC-3′ and reverse 5′-GCGGGTGGTGATGCGGGTGAT-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′-GGGGCAATAAAGCCAGTCAG-3′ and reverse: 5′-TTCCATGCGTTGTACCACCA-3′; XSox3, forward: 5′-ACA ACCCTATGATGACCTCTG-3′; reverse: 5′-AGTCTGATAGTTGCCAGCAGG-3′. The primers for *XSox2* and *H4* were used as reported (Matsuo-Takasaki 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 Nieuwkoop & Faber (1967). The *Sox*1 probe was a 1.9 kb fragment including the 5′ untranslated region and the coding region. The 3′ untranslated regions of *XSox*2 and *XSox*3 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 *Sox*1 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 *Sox*1-3 during *Xenopus* embryogenesis (Fig. 2). The *XSox*1 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 *XSox*1 can be detected at stage18, stage20 and stage30. In contrast, *Sox*2 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). *Sox*3 is detected strongly throughout the stages tested (Fig. 2, see also Penzel 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 ectoderm in the late blastula embryos (Fig. 3 E). At gastrula stage (St. 10.5), it seemed to be expressed weakly in the anterior ectoderm distant from the blasto-

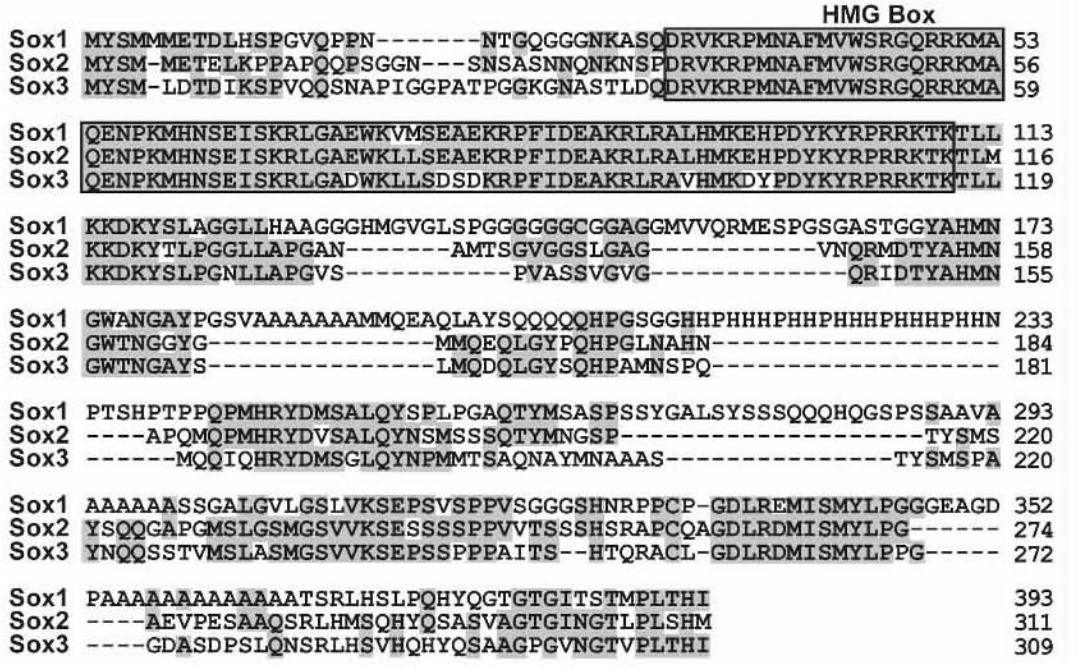

Fig. 1 Alignment of *Xenopus laevis Sox*1, *Sox*2 and *Sox*3 amino acid sequences.
The DNA-binding HMG domains are boxed. Identical amino acids are highlighted by gray background.

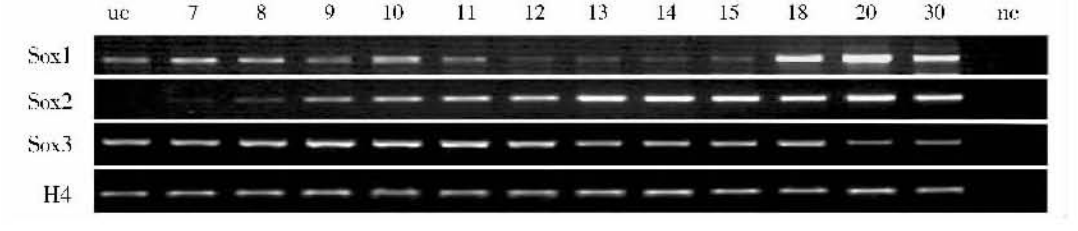

Fig. 2 Temporal expression of *Sox*1-3 gene during *Xenopus* early development.
Temporal expression of *Sox*1-3 gene was analyzed by RT-PCR. Numbers indicate the developmental stages. H4 was used as an internal control. The product from a RT reaction without reverse transcriptase using stage 30 embryo total RNA was used as templates for the negative controls (nc); ue, unfertilized egg.

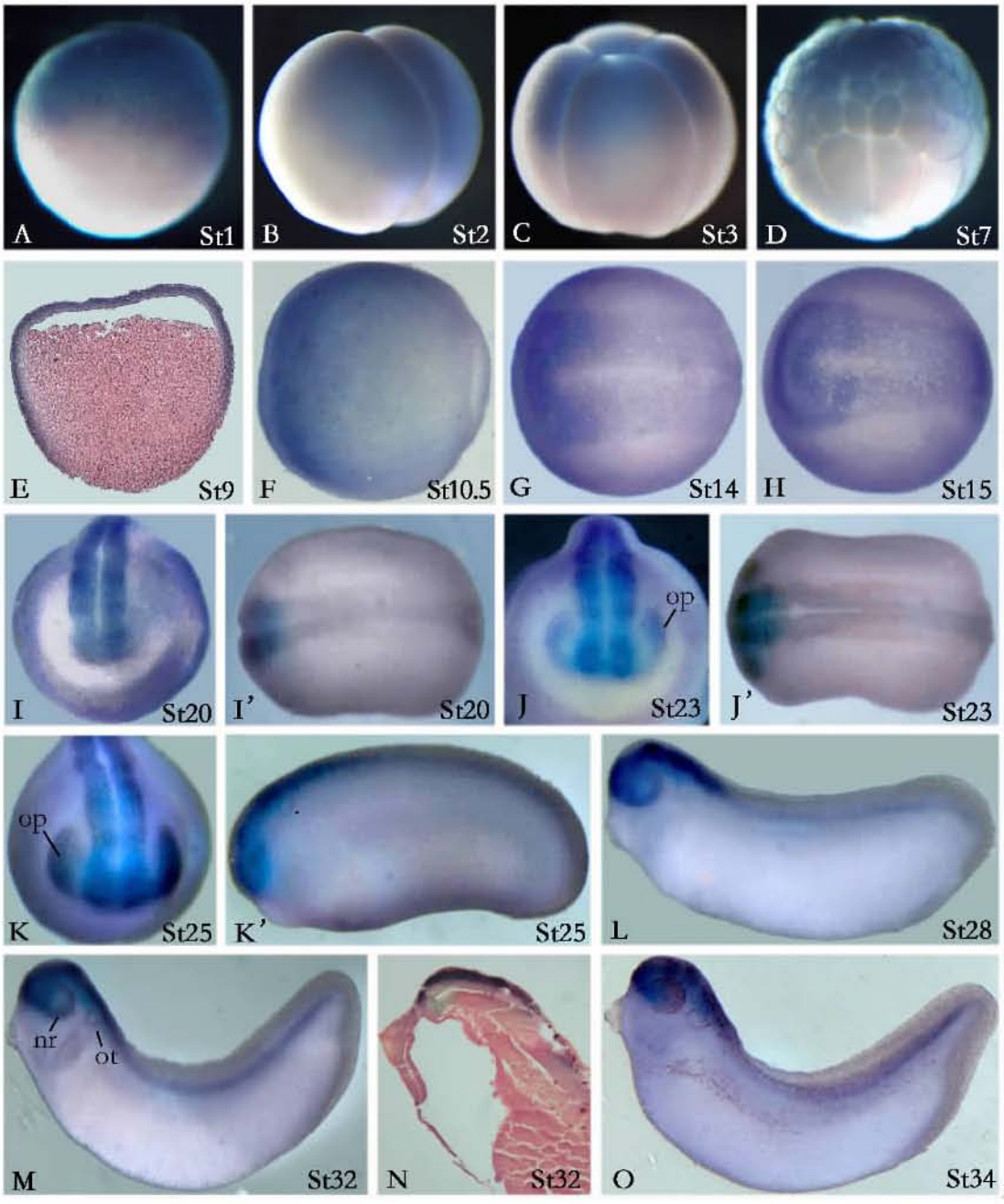

Fig. 3 Embryonic expression of *Xenopus laevis* Sox1 gene.

Whole-mount in situ hybridization results at indicated development stages. A-D, K', L, M, O, lateral views; F-H, I', J', dorsal views, anterior to the left; I, J, K, anterior views, dorsal to the top. (E) Section of a fine-cell blastula stage embryo (St9), animal pole to the top. (N) Longitudinal section of the head region of an embryo at stage 32. nr, neural retina. op, optic vesicle. ot, otic vesicle.

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, *Sox*1 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 *Sox*1 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 *XSox*1-3 in the brain and eye

At stage23, both Sox1 and Sox2 were expressed in

the primitive brain and the optic vesicle but the expression of *Sox*2 at the anterior neural tube was stronger and broader than *Sox*1 (Fig. 4 A, B). *Sox*3 was only expressed in the neural tube but not the primitive eye domain at this stage. In addition, *Sox*3 is also expressed in a crescent-shaped domain surrounding the anterior neural plate (Fig. 4 C). At tail-bud stages, *Sox*1, 2 and 3 showed slightly different expression patterns in the brain. *Sox*1 seemed to be strongly expressed in the dorsal part of the brain while *Sox*2 was strong in several patches along the anterior-posterior axis in the telencephalon, midbrain-hindbrain boundary and hindbrain. *Sox*3 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. *Sox*2 and *Sox*3 but not Sox1 are expressed in the branchial arches (Fig. 4 D, E, F). In the eye, *Sox*1 is expressed in the neural retina but not the lens (Fig. 4 D, G) while *Sox*2 can be detected both in the neural retina and the lens (Fig. 4 E, H). *Sox*3 expression was not detected in the neural retina and weakly in the lens at stage 30 (Fig. 4 F, I).

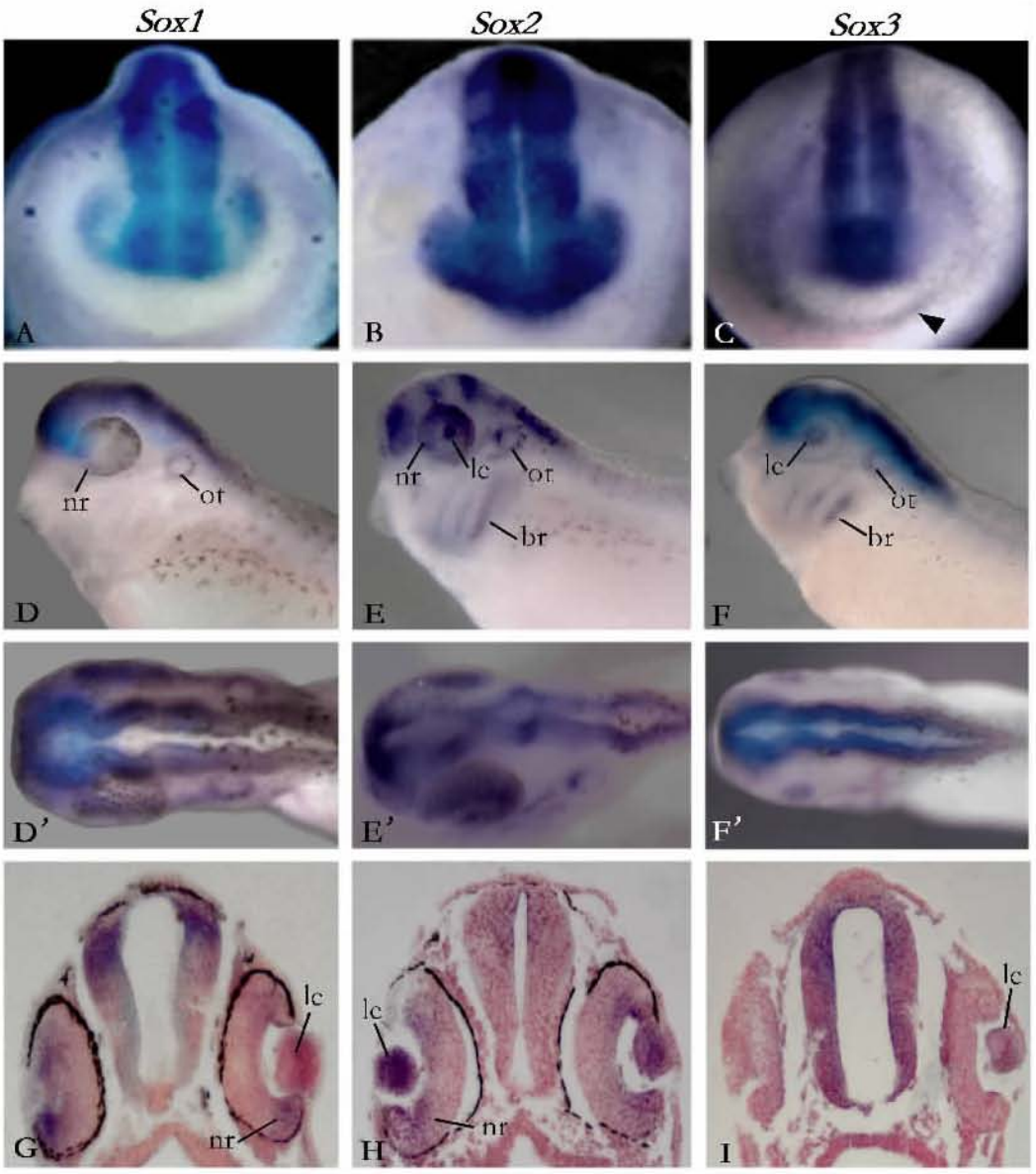

Fig. 4 Comparison of the expression of *XSox*1-3 in the brain and eye.

(A-C) Expression of *XSox*1 (A), *XSox*2 (B), and *XSox*3 (C) at stage23, anterior views. Arrowhead in C mark the crescent-shaped expression domain of *XSox*3 surrounding the anterior neural plate. (D-F, D′-F′) Expression of *XSox*1 (D, D′), *XSox*2 (E, E′) and *Sox*3 (E, E′) at stage33, D-F, lateral views; D′-F′, dorsal views; (G-I) Transversal sections through the eye region showing the expression of *XSox*1 (G, Stage33), *XSox*2 (H, Stage33) and *XSox*3 (I, Stage30) in the brain and eye. br, branchial aches. le, lens. nr, neural retina. ot, otic vesicle.

3 Discussion

We show here strong maternal expression of *XSox*1 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. *XSox*3 is also strongly maternally expressed and has been shown to be important in germ layer formation (Zhang et al, 2004). The maternal expression of *XSox*1 might suggest a similar role in the early patterning of the ectoderm in *Xenopus*. The widely overlapping expression of the *SoxB*1 genes in the central nervous system might suggest redundant roles of the three genes in neural patterning and differentiation.

The expression of the SoxB1 genes in the eye shows some differences in different species. In the chick, *Sox*1, *Sox*2 and *Sox*3 are all expressed in the neural retina and lens (Uchikawa et al, 1999; Kamachi et al, 1998; Rex et al, 1997). In the mouse, however, *Sox*2 and *Sox*1 but not *Sox*3 have been detected in the embryonic lens (Collignon et al, 1996; Kamachi et al, 1998). These results might suggest different roles of the three *SoxB*1 genes in vertebrate eye development.

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