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Gene expression pattern

Cloning and expression of *xSix3*, the *Xenopus* homologue of murine *Six3*

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Abstract

The vertebrate *Six* family of transcription factor genes are homologues of the fruitfly gene *sine oculis* (*so*). Human, murine, avian and fish (medaka, zebrafish) homologues have recently been cloned. We report the cloning and developmental pattern of expression of xSix3, the *Xenopus laevis* homologue of Six3. In addition, we have compared all the known sequences of vertebrate Six3 genes. xSix3 is very homologous to Six3 in other vertebrates in terms of amino acid sequence. The reported developmental pattern of expression of Six3 in chick and mouse includes not only the developing eyes and the ventral diencephalic tissue between them, but also a large, sagittally-oriented telencephalic region. The distribution of xSix3, however, is virtually restricted to the eyes and ventral diencephalon, showing only a very small territory of expression in the telencephalon. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

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Transcription factor Six3 has been shown to be fundamental in the genetic network that controls eye development. Six3 has been recently cloned in a number of species (Oliver et al., 1995; Bovolenta et al., 1998; Loosli et al., 1998; Seo et al., 1998a,b; Granadino et al., 1999; Leppert et al., 1999). A partial sequence of *Xenopus* Six3 has been recently published (Chow et al., 1999).

1. Molecular analysis (Fig. 1)

We have identified (see Section 3) a novel cDNA containing a full length coding region whose predicted amino acid sequence contained Six domain and homeodomain, which made it a member of the Six/so family. The predicted amino acid sequence showed high similarity to that reported for Six3 in other vertebrates. The Six domain of *Xenopus* Six3 shows 97% identity to that of human, mouse and chicken, 80% to that of medaka fish, 96% to that of zebrafish and 77% to that of fruitfly. The homeodomain of xSix3 is completely identical to that of human, mouse, chicken and zebrafish, and shows 94% identity to that of medaka fish and fruitfly. xSix3 encodes a protein with a predicted sequence of 291 amino acids in which the homeo- and Six-domain are basically identical to those of the human, murine, chicken and zebrafish. Overall, the amino acid sequence of xSix3 presented 89% identity with that of chicken, 87% to that of mouse and human, 86% to that of zebrafish, 66% to that of medaka fish and 79% to that of fruitfly. These results indicate that the isolated cDNA is a *Xenopus* homologue of Six3. The GenBank accession number of xSix3 is AF183571. The partial sequence recently published (Chow et al., 1999) corresponds to the last 71 amino acids of the C-terminal side of xSix3. The correspondence between this partial sequence and the one we present here is complete except for two differences: residues 271 and 276 correspond, respectively, to glutamic acid (E) and alanine (A) in our clone, to aspartic acid (D) and glycine (G) in Chow et al. (1999).

2. Expression pattern (Fig. 2)

We have used wholemount in situ hybridization to reveal the developmental distribution of xSix3. The results can be summarized as follows: xSix3 is expressed during gastrulation in a single round patch of cells (st14, Fig. 2A) located in the anterior end of the prospective neural plate (sensorial layer of the neuroectoderm; Fig. 2D–F). By the early differentiation stages of the nervous system (st20, Fig. 2B,G–I) the territory of expression is still continuous, although by

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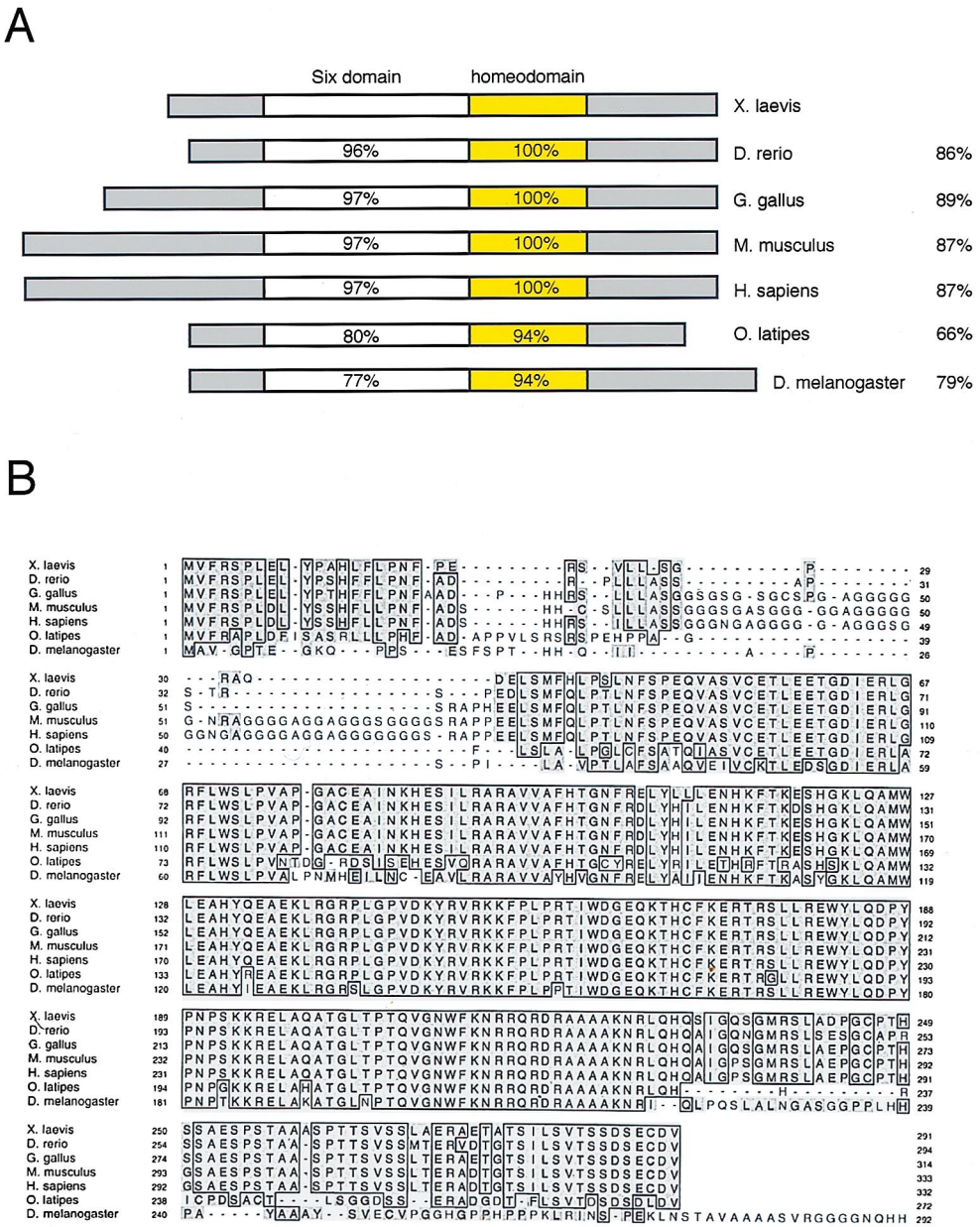

Fig. 1. (A) Diagrammatic representation of the percentage of amino acid homologies between the different species Six3 genes compared with *Xenopus* Six3. The homeodomain is shown in yellow (60 aa), while the conserved upstream Six domain is represented by an open box (110 aa). (B) Comparison between the predicted amino acid sequences of all the Six3 homologues identified to date. Identity of amino acid residues is indicated by a shaded box. Out of the box, shading indicates amino acid homology. Dashed lines represent gaps included for better alignment.

this stage it is cross-shaped, with a sagittal domain starting in the middle part of the ventral diencephalon and insinuating itself rostrally in the telencephalon; the transversal domain includes both eye primordia and the strip of ventral diencephalon between them. During the stages of mantle differentiation of the nervous system (st32, Fig. 2C,J–L), the pattern remains in essence the same; however, small isolated groups of (presumably) differentiating cells in the diencephalon and telencephalon show also xSix3 expression. As is clear from these results, the distribution of xSix3 is almost completely restricted to the eyes and the strip of ventral diencephalon between them (presumably corresponding to that portion of the hypothalamic primordium that will form part of the pituitary gland). This is in contrast with the distribution of Six3 in other vertebrates, where a sagittally oriented region of the telencephalon shows intense developmental expression of Six3 (Bovolenta et al., 1998). In the chick and the mouse, Six3 is early expressed in large areas of the telencephalon, and later in discrete nuclei of the forebrain in principle unrelated to vision (Oliver et al., 1995; Bovolenta et al., 1998). The expression pattern of xSix3 is more similar to, although

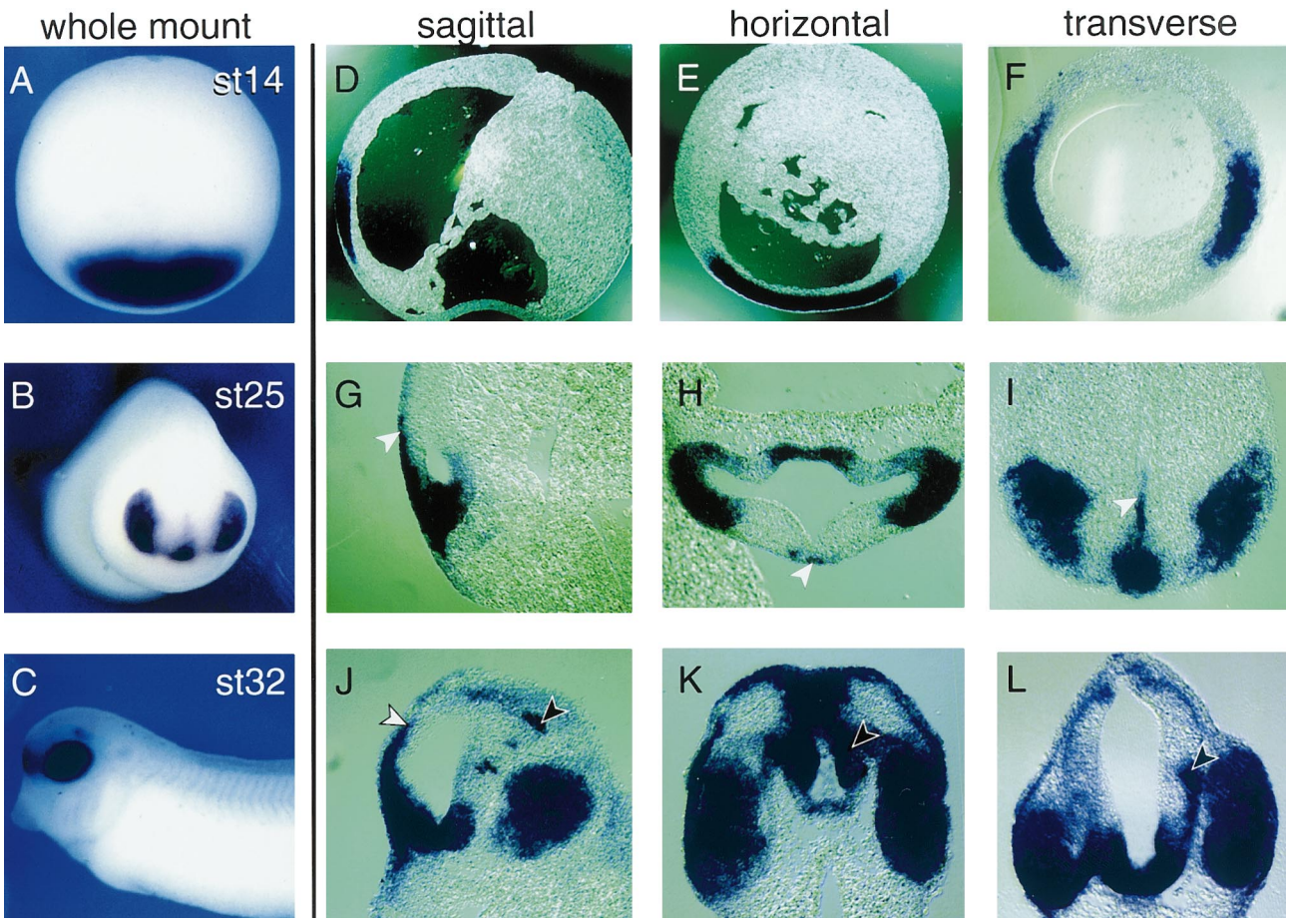

Fig. 2. (A–C) Wholemount in situ hybridization of xSix3 during development, showing the progression of the expression pattern from round patch of cells to a cross-shaped domain in the ventral-rostral forebrain. (D–L) Vibratome sections through the embryos shown in A–C. (D–F) By st14, xSix3 mRNA can be detected in a continuous domain in the sensorial layer of the neuroectoderm. (G–I) At st25, a sagittally-oriented prolongation that extends into the telencephalon (white arrowheads. (J–L) During the early cell differentiation stages of the nervous system, isolated group of cells in the forebrain (black arrowhead in J), and parts of the diencephalic mantle layer (black arrowhead in L) express xSix3. The white arrowhead in J shows the telencephalic prolongation.

not the same as, that of Six3 in fishes. Specifically, the distribution of Six3 in the forebrain of medaka fish (*Oryzias latipes*) shows a large territory of expression in the diencephalic neuroepithelium, beyond the hypothalamic anlage and corresponding to the thalamic primordium (Loosli et al., 1998). The distribution of the zebrafish homologue of Six3 is the most similar to that of xSix3 (being also simpler), because it is restricted to one continuous domain of neuroepithelium comprising the eyecups and the small area of the floor of the third ventricle that connects both eye primordia (Seo et al., 1998a).

3. Methods

A 516 bp fragment of xSix3 cDNA was PCR-amplified from an embryonic *Xenopus* cDNA library using degenerated primers (5′ GAG CAR GTG WGC GTI TGC GAR GTK CTG SAG 3′ and 5′ GTC KCK YTG YCK CCG GTT CTT RAA CAA GTT 3′). This fragment was used as a probe to isolate 12 additional clones from the same library. One of them contained the full length cDNA. For the in situ (Harland, 1991), as well as for the cloning (Sambrook et al., 1989), we have used current protocols.

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