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Gene expression pattern

Expression patterns of zebrafish *sox11A*, *sox11B* and *sox21*

Rebecca Rimini[a,1], Monica Beltrame[b], Francesco Argenton[c], Dimitri Szymczak[c], Franco Cotelli[d], Marco E. Bianchi[a,b,*]

[a]*DIBIT, San Raffaele Scientific Institute, via Olgettina 58, 20132 Milan, Italy*
[b]*Dipartimento di Genetica e di Biologia dei Microrganismi, Università di Milano, via Celoria 26, 20133 Milan, Italy*
[c]*Dipartimento di Biologia, Università di Padova, via U. Bassi 58/B, 35131 Padua, Italy*
[d]*Dipartimento di Biologia, Università di Milano, via Celoria 26, 20133 Milan, Italy*

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Abstract

We have cloned three *sox* genes in zebrafish (*Danio rerio*), one related to human and chicken *SOX21*, and two related to mammalian and chicken *Sox-11*. Zebrafish *sox21*, *sox11A and sox11B* transcripts are accumulated in the egg, are present in all cells until gastrulation and become restricted later to the developing central nervous system (CNS); expression in adults is undetectable. *sox21* is expressed in the forebrain, midbrain and hindbrain, but maximally at the midbrain–hindbrain junction; *sox11A,B* have a widespread and dynamic expression in the CNS, but in contrast to *sox21* are absent at the midbrain–hindbrain boundary.

Keywords: Brain; *noi*; Midbrain; Hindbrain; HMG-box; Maternal genes; Transcription factors

1. Results and discussion

Sox transcription factors are related to the mammalian testis determining gene *Sry* (Gubbay et al., 1990) through the possession of a DNA binding motif of about 70 amino acids known as the high mobility group (HMG) box. HMG boxes bind to prestructured or kinked DNA molecules irrespective of their sequence, and bend the DNA to a sharp angle when binding to specific sequences (reviewed by Bianchi and Beltrame, 1998). There are more than a dozen different *Sox* genes in mammals, and many of them are tightly regulated during the development of the central nervous system (Pevny and Lovell-Badge, 1997).

We obtained several zebrafish *sox* cDNAs by screening a cDNA library with Sry-related HMG boxes. Two cDNAs coded for closely related proteins of 354 and 368 amino acids, both of which are very similar to human and chicken Sox-11 proteins (Jay et al., 1995; Uwanogho et al., 1995). The similarity to Sox-11 is maximal in the HMG box, but also includes the first four amino acids, a segment consisting mainly of glutamic and aspartic acids, and a C-terminal region of 54 amino acids (Fig. 1). We therefore called the corresponding genes *sox11A* and *sox11B* (collectively, *sox11A/B*). The third cDNA codes for a protein of 240 amino acids, which at the time of its submission to the database had no similarity outside the HMG box to any other protein; recently, however, the sequence of SOX21, a human protein with 54% identity to our zebrafish protein, and the homologous sequence from chicken have appeared in the databank (accession numbers AF1070441, Malas et al., and AB026623, Uchikawa et al., respectively). Zebrafish *sox11A*, *sox11B* and *sox21* are single-exon genes, and map on linkage groups 17, 20 and 6, respectively (P. Haffter, pers. commun.).

sox11A/B and *sox21* transcripts are already present at 2- and 4-cell stages (Fig. 2C,D) and are evenly distributed to all blastomeres. Their presence prior to the midblastula transition suggests that they are maternally inherited. In fact, their transcripts are present in oocytes (Fig. 2A,B): hybridization is stronger at primary growth stage and at the cortical alveolus stage, while it is weaker at the vitellogenesis stage, due to the accumulation of storage proteins (Selman et al., 1993).

At blastula stage (Fig. 2E) and during gastrulation (Fig. 2F), *sox11A/B* and *sox21* are ubiquitously expressed throughout the blastoderm. At 60% epiboly there is stronger hybridization in the embryonic shield (ES; Fig. 2F). Transcripts are uniformly distributed to the two germ layers, but

* Corresponding author. Dipartimento di Genetica e di Biologia dei Microrganismi, Università di Milano, via Celoria 26, 20133 Milano, Italy. Tel.: +39-02-2643-4780; fax: +39-02-266-4551.

E-mail address: marco.bianchi@unimi.it (M.E. Bianchi)

[1] Present address: Glaxo-Wellcome Ricerche, via Fleming 4, 37100 Verona, Italy.

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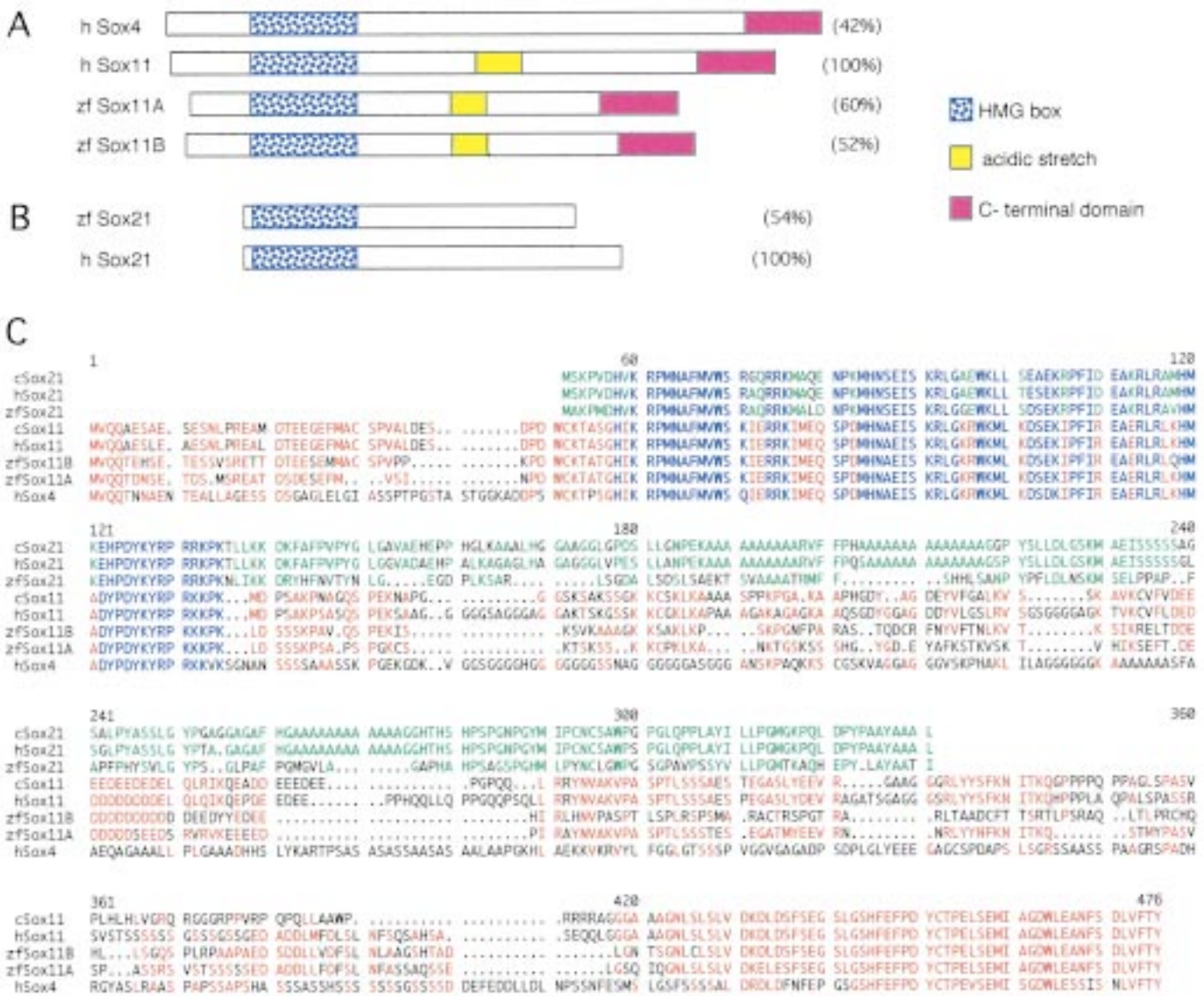

Fig. 1. Comparison of Sox proteins (h, human; zf, zebrafish; c, chicken). Nucleotide sequences of *sox11A*, *sox11B* and *sox21* have accession numbers U85090, U85091 and U85092 respectively. (A) Diagrammatic representation of proteins of the Sox-4/Sox-11 subfamily. The identity level to human Sox-11 is indicated between brackets. (B) Diagrammatic representation of Sox21 proteins. The identity level to human Sox-21 is indicated between brackets. (C) Comparison of the protein sequences. Blue color indicates residues that are conserved in all listed proteins, and identifies the HMG boxes. Green color indicates residues conserved in at least two Sox-21 proteins. Red color indicates residues conserved in at least 3 members of the Sox-4/Sox-11 subfamily. Dots indicate gaps in the alignment.

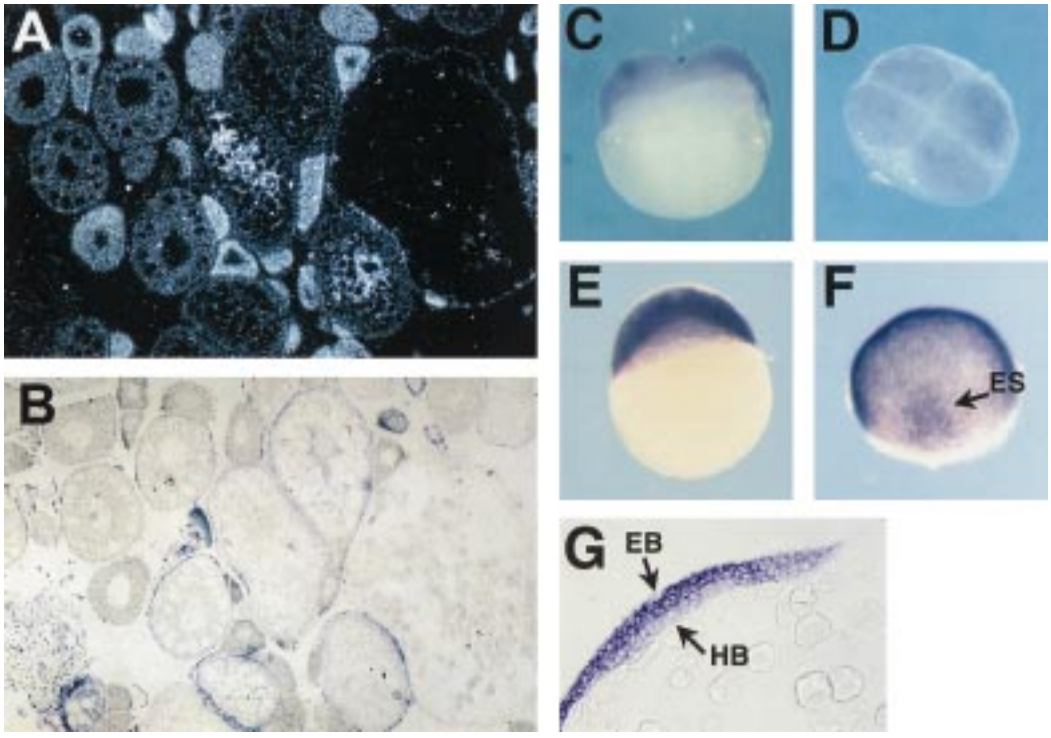

Fig. 2. Whole-mount in situ hybridization of *sox11A*, *sox11B* and *sox21* during early embryogenesis. Transcripts are ubiquitously distributed during early stages. (A) Section through zebrafish ovary, in dark field. (B) Bright field; same section as in (A). (C) Two-cell stage, (D) 4-cell stage, (E) blastula, (F) 60% epiboly. Notice the strong staining in the embryonic shield (ES). (G) Sagittal section through the ES at 70% epiboly. Hybridization is stronger in the epiblast (EB) than in the hypoblast (HB). No staining is detected in the yolk cell adjacent layer. Embryos and sections shown were analyzed with the *sox11B* probe; *sox11A* and *sox21* probes gave identical results.

already at 70% epiboly a significantly stronger signal is detected in the epiblast with respect to the underlying hypoblast (Fig. 2G). Expression becomes definitively confined to the epiblast as gastrulation proceeds (see below). No staining is detected in the yolk cell adjacent layer (Fig. 2G). The expression pattern of the three genes starts to diverge at 90% epiboly.

At 9 h.p.f., *sox11A/B* transcripts are absent from the ventral part of the embryo, the evacuation zone, and are expressed in the dorsal part. *sox11A* is expressed in a cruciform pattern (Fig. 3A), whereas *sox11B* is expressed in the middle embryonic axis (Fig. 3C, arrowhead). At 10 h.p.f., *sox11A* is expressed in three bands in the anterior portion of the embryo (Fig. 3B). Morphologically, the rostral curved band (arrows) corresponds to the limit of the anterior neural plate. The *noi* gene, that codes for the pax2.1 protein, is expressed between the middle (black arrowhead) and posterior (white arrowhead) bands, while *krox-20* is expressed just behind the posterior band (data not shown). *sox11B* (Fig. 3D) is expressed in three longitudinal stripes within the prospective trunk and cephalic regions. The midline longitudinal stripe is located in the neuroectoderm overlying the axial mesoderm and the two lateral stripes are in the ectoderm at the border of the neural plate (Fig. 3I). Two transverse stripes of expression are also present (Fig. 3D, black and white arrowheads), which by double staining coincide with the middle and posterior bands of expression of *sox11A* (not shown). In contrast, *sox21* is expressed at 10 h.p.f. only in two small areas flanking the midline (Fig. 3E), that at 11 h.p.f. merge into a solid transverse band (Fig. 3F), rostral to expression domains of *krox-20* (Fig. 3H) and colocalizing with *noi* transcripts (Fig. 3G).

During somitogenesis the expression patterns of *sox11A/B* are restricted exclusively to the developing CNS. At the 16-somite stage (Fig. 4F,G) and at the 30-somite stage (24 h.p.f., Fig. 4H,I), *sox11B* is expressed in the forebrain, in the midbrain and in the hindbrain, but far from uniformly. In particular, the expression domains in the midbrain and hindbrain are separated by a gap region at the mese-metencephalic border, where *noi* is expressed (Fig. 4J,K, red signal). *sox11A* shows a very similar expression pattern, save that the forebrain signal is fainter (data not shown). Neither *sox11A* or *sox11B* are expressed in the floor plate (not shown). At 36 h.p.f., *sox11A/B* expression patterns are similar to the patterns at 24 h.p.f., but signals are fainter and more widespread; at 48 h.p.f. and later, no expression was detected (not shown).

sox21 is expressed in 16-somite embryos (Fig. 4A–C) in the midbrain (Fig. 4B) and, to a lower extent, in the hindbrain and in the basal anterior diencephalon (Fig. 4A,C). In 30-somite embryos (24 h.p.f., Fig. 4D,E) expression of *sox21* does not vary significantly, except that staining in the olfactory placodes becomes clearly visible. Maximal expression of *sox21* is always in the posterior midbrain

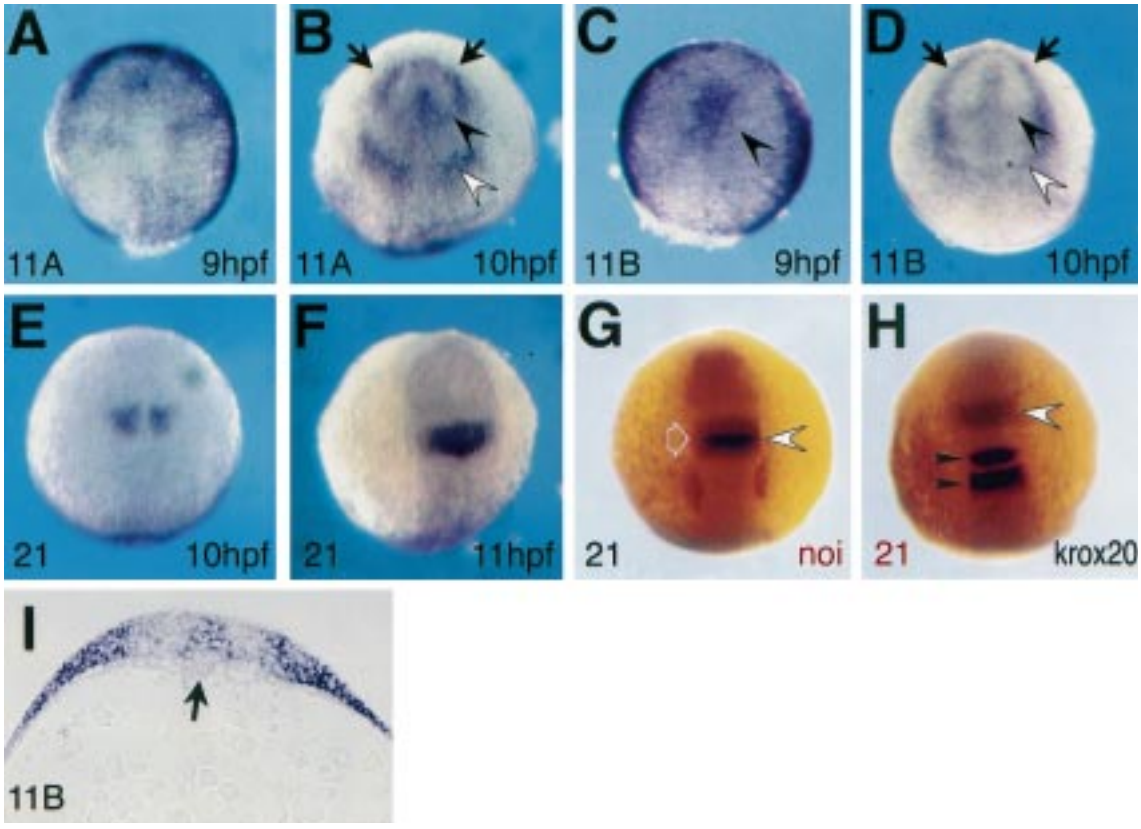

Fig. 3. Expression of *sox11A*, *sox11B* and *sox21* after gastrulation. (A–H) Zebrafish embryos (dorsal views, anterior is top, posterior is bottom) at the indicated post-fertilization times (9 h.p.f. is equivalent 90% epiboly, 10 h.p.f. to tail bud stage), hybridized with the probes indicated in each panel (11A is *sox11A*, 11B is *sox11B* and 21 is *sox21*). (A) Faint signal for *sox11A* is widespread, stronger signal forms a cruciform pattern. (B) *sox11A* expression is detected in the outline of the neural keel (arrows) and in the prospective mid- and hindbrain (black and white arrowhead, respectively). (C) Faint signal for *sox11B* is widespread, stronger signal is present in the middle embryonic axis (arrowhead). (D) *sox11B* transcripts are present in the neural plate in one midline and two lateral stripes (arrows) which fuse anteriorly forming an arch. Two transverse stripes of expression are localized in the prospective mid- and hindbrain (black and white arrowheads, respectively). (E,F) *sox21* expression is first detected in two small areas, that soon fuse into a transverse band. (G) Double labeling of 11 h.p.f. embryos: *sox21* expression (dark color, white arrowhead) is located within the area of expression of *noi* (red color, large outlined arrow) in the prospective posterior midbrain. (H) Double labeling of 11 h.p.f. embryos with *sox21* (red color, white arrowhead) and *krox-20* (dark color, black arrowheads). (I) Section of the embryo shown in (D). The longitudinal midline stripe of expression of *sox11B* corresponds to the neuroectoderm overlying the axial mesoderm (arrow) and the two lateral longitudinal stripes correspond to the ectoderm at the border of the neural plate.

(Fig. 4D), where *noi* is also expressed, but *sox11A/B* are not. *sox21* expression fades at 36 h.p.f., and is undetectable later on.

In conclusion, *sox11A/B* genes code for related proteins and share a complex expression pattern consistent with their similarity to chicken and human *SOX-11*. *sox21* defines a new *sox* subfamily, is expressed like *sox11A/B* until the end gastrulation, but later on is notably present at the midbrain–hindbrain junction, where *sox11A/B* are absent.

2. Methods

2.1. Isolation of cDNAs

A randomly primed cDNA library, prepared in λZAPII from 18–40 h.p.f. zebrafish embryos was screened at low stringency with PCR products amplified from zebrafish genomic DNA. PCR was performed using oligonucleotides SOXboxdir, ATGAAYGCNTTYATNGTNTGG, and SOXboxrev, GGNYKRTAYTTRTARTYNGG, and 35 cycles of 30 s at 94°C, 60 s at 50°C, and 60 s at 72°C. Several independent clones were isolated from 2×10^6 plaques, and recombinant pBluescript DNAs were excised as recommended by the manufacturer (Stratagene). Plasmids were sequenced in full.

2.2. In situ hybridization

Sense and antisense RNA probes were produced using the DIG RNA labeling kit SP6/T7 (Boehringer Mannheim) from selected *sox11A/B* and *sox21* subclones in pBluescript. The *noi* and *krox-20* probes were previously described (Krauss et al., 1991; Oxtoby and Jowett, 1993).

In situ hybridization on zebrafish ovary sections was carried out as described (Gulisano et al., 1996). Whole-mount in situ hybridization was performed as described (Oxtoby and Jowett, 1993). RNA probes for two-color

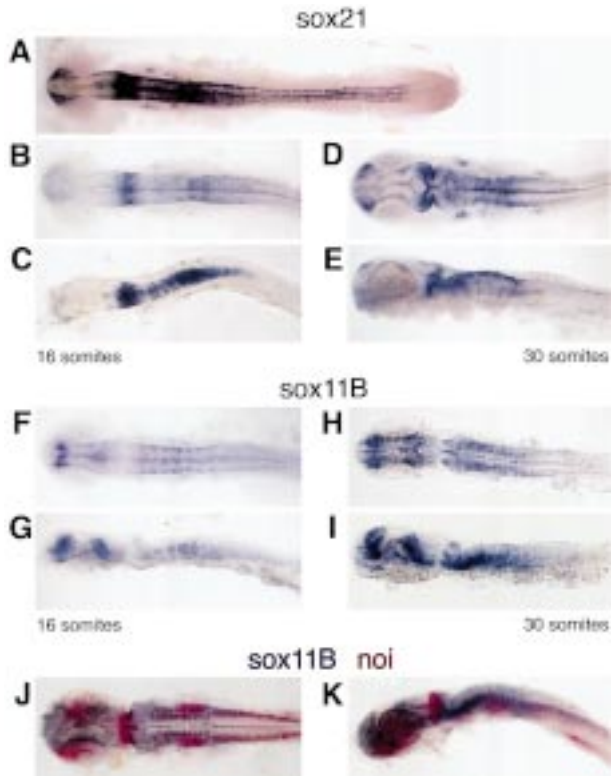

Fig. 4. Expression of *sox11A*, *sox11B* and *sox21* during somitogenesis. Embryos were dissected away from the yolk and flattened. (A–E) Expression of *sox21* at 16 somites (A–C) and 30 somites (D,E). (A,B) Dorsal views at two different staining levels, to highlight the stronger expression in the mid-hindbrain junction region (B) but also weaker expression in the most anterior basal diencephalon, in the hindbrain and along the axis in three longitudinal stripes (A). (C) Lateral view. (D,E) Dorsal and lateral views of 30 somite-stage embryos (24 h.p.f.), respectively. Overall expression does not vary significantly from the previous stage: the posterior midbrain is the major site of expression. Additional signals are now detectable in the olfactory placodes, and in the regions of the trigeminal and lateral line ganglia. (F–K) Expression of *sox11B* (expression of *sox11A* is similar, save that the forebrain signal is fainter). Dorsal and lateral views at 16 somites (F,G) and 30 somites (H,I) are shown. A double staining with *noi* (red color) shows an expression gap of *sox11B* (blue color) in the mid-hindbrain junction region, where *noi* is expressed (J,K dorsal and lateral views, respectively, at 30 somites).

hybridizations (Hauptmann and Gerster, 1994) were labeled with either digoxigenin or fluorescein.

Specimens were analyzed using a Zeiss SV11 microscope and photographed with Kodak Ektachrome 64T film. Images were scanned on a Nikon Coolscan scanner and processed using Adobe Photoshop software.

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