

High expression of the HMG box factor Sox-13 in arterial walls during embryonic development

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Received October 3, 1997; Revised and Accepted November 25, 1997

DDBJ/EMBL/GenBank accession no. AJ000740

ABSTRACT

Members of the Sox gene family of transcription factors are defined by the presence of an 80 amino acid homology domain, the High Mobility Group (HMG) box. Here we report the cloning and initial analysis of murine Sox-13. The 984 amino acids Sox-13 protein contains a single HMG box, a leucine zipper motif and a glutamine-rich stretch. These characteristics are shared with another member of the Sox gene family, Sox-6. High level embryonic expression of Sox-13 occurs uniquely in the arterial walls of 13.5 days post coitum (dpc) mice and later. Low level expression was observed in the inner ear of 13.5 dpc mice and in a limited number of cells in the thymus of 16.5 dpc mice, from which Sox-13 was originally cloned. At 18.5 dpc, Sox-13 is expressed in the tracheal epithelium below the vocal cord and in the hair follicles. The Sox-13 protein binds to the consensus HMG box motif, AACAAAG, but does not transactivate transcription through a concatamer of this motif. Sox-13, like other members of the Sox family likely plays an important role in development.

INTRODUCTION

The cloning of the mammalian sex-determining gene, SRY, has led to the identification of a large number of related genes (1–6). These so called Sox (Sry-related HMG box) genes are characterized by the presence of a DNA-binding domain, the HMG box (High Mobility Group). The HMG box is a 80 amino acids motif, which mediates sequence specific DNA binding (7). A key role for Sox genes in development has been identified via the analysis of individuals with naturally occurring mutations as well as via the analysis of mice created by targeted gene-disruption.

Mutations in the sex-determining gene SRY lead to sex reversal in XY individuals and have been extensively documented (1,2). Development of the indifferent gonads into testis depends on the presence of an intact HMG box in the SRY protein. SRY from XY individuals suffering from gonadal dysgenesis exhibits an

impaired DNA binding or bending activity caused by mutations in its HMG domain (8,9).

The human autosomal XY sex reversal locus, SRA1, has been associated with the skeletal malformation syndrome campomelic dysplasia (CMPD1). This locus has been placed at distal 17q (10). Analysis of this region reveals that patients suffering from these linked syndromes display inactivating mutations within or near the SOX-9 gene (11). Expression of Sox-9 in embryonic cartilage and genital ridges of XY embryos strengthen this observation and imply an essential role for Sox-9 in development of cartilage, and sex determination. It has been suggested that Sox-9 functions downstream of Sry (12).

The transcriptional activator Sox-4 is expressed at several sites in the embryo, but in adult mice its expression is restricted to immature B and T lymphocytes (5). Sox-4^{-/-} mice display a severe defect in cardiac outflow tract formation and die due to a circulatory failure at 14 days post coitum (dpc). Detailed analysis of Sox-4^{-/-} hearts revealed a lack of fusion of the endocardial ridges, impaired development of the semilunar valves and common arterial trunk (13). The arrangement of the ventriculo-arterial connection corresponds to transposition of the great arteries (Yin Ya and W.Lamers, submitted). The study of haematopoiesis in lethally irradiated mice reconstituted with Sox-4^{-/-} fetal liver cells revealed a specific block in B cell development at the pro-B cell stage (13). In addition to an impaired B cell development, the Sox-4 mutation also affects the T lineage. Explanted fetal thymic organ cultures (FTOC) of Sox-4^{-/-} thymi yielded 10–50-fold fewer maturing thymocytes than FTOC of littermates (14).

In recent years other Sox genes with possible roles in differentiation and development have been molecularly characterized (15). Sox-1, -2 and -3 are mainly expressed in the developing central nervous system (16). Sox-3 is located on the X chromosome and appears to be the closest relative of Sry, together arguing for an evolutionary relationship (17). In the adult mouse, spermatogenesis is associated with expression of Sox-5 and Sox-6, suggesting overlapping functions of these genes in the testis (3,18,19). Sox-6 may also play a role in the developing nervous system based on its neuronal expression in 9.5–12.5 dpc embryos.

In this study we have searched for Sox genes expressed in 15.5 dpc thymus using degenerate PCR. Besides Sox-4, we identified the

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expression of *Sox-13*, a fragment of which had previously been described by Wright *et al.* (6). We have cloned full length *Sox-13* cDNA. The developmental expression of murine *Sox-13* was studied by *in situ* hybridization from 10.5 dpc onwards. Low level expression was observed in thymus, inner ear, vocal cord and hair follicles. High level expression was restricted to arteries during late embryogenesis. *Sox-13* binds to the consensus HMG box DNA motif, but does not transactivate transcription from multimeric sites.

MATERIALS AND METHODS

Production of cDNA from 15.5 dpc thymocytes

Embryos (15.5 dpc) were collected from timed-pregnant mice to dissect embryonic thymi. Thymi were pressed through nylon to recover only thymocytes. After NP-40 lysis mRNA was isolated using oligo-d(T)beads according to manufacturer's instructions (Dynal). The bound mRNA was directly reverse transcribed using the heat stable reverse transcriptase RtTh (Perkin Elmer) and the oligo-d(T)bead as primer at 72°C for 60 min. After removal of the mRNA a second strand was generated using random 9mers and Klenow at 42°C for 45 min. The two strands were melted, the first strand was extracted using a magnet and stored for re-use at 4°C, the second strand was used for PCR.

Cloning of Sox homologues by guess-mer PCR

PCR was performed using oligonucleotides 5'-gggaattcatga(tc)gc(gatc)tt(tc)at(gatc)gt(gatc)tgg-3' and 5'-gggaagctt(gatc)gg(gatc)cg(ag)ta(ct)tt(ga)ta(ga)t(tc)(gatc)gg-3' in 50 µl reaction volumes containing 2 U SuperTaq according to the manufacturer's instructions (HT Biotechnology Ltd). Cycle conditions were 40 s at 94°C, 40 s at 50°C, 60 s at 72°C with a final extension of 10 min at 72°C after 45 cycles. The PCR reaction was checked by gel electrophoresis, size selected and purified. The amplified material was subsequently blunted and cloned into *EcoRV*-digested pBluescript. After transformation into DH10β, colonies were screened on insert-size and analysed for Sox-related products by sequencing.

Isolation of cDNA clones and RACE

An incomplete murine *Sox-13* cDNA clone was isolated from a 15 dpc whole mouse embryo cDNA library in lambda-gt11, by standard screening using the *Sox-13* PCR fragment as a probe. For

the isolation of the missing 5'-end of the cDNA the RACE technique was applied as described by Frohman *et al.* (20) with minor adaptations. In short, after first strand synthesis, the product was spun over an S-400 column (Pharmacia). After TdT-tailing, the second strand was synthesized using an oligo-d(T)-*XbaI* primer at 42°C annealing temperature, subsequently the temperature was raised to 56°C and nested primers were added. Cycle conditions: 5 min 94°C, 3 min 42°C, 3 min 72°C, 5 min 94°C and 40 cycles (30 s 94°C, 30 s 56°C, 2 min 72°C) with a final extension of 10 min at 72°C. Primers used for the RACE experiment; oligo-d(T)-*XbaI*: 5'-ctcgaacctgtctagatc(t)₁₆-3', Lo-*XbaI*: 5'-ctcgaacctgtctagatc-3', *Sox-13*-primers: 5'-atccatgtcacagctcag-3', 5'-ctgcttacagggaatggcctg-3' and 5'-ctctgctgctgctgccc-3', 5'-tctgctcagagtgccg-3'.

Production of Sox-13 protein

Full length *Sox-13* was cloned into pGLOMYC by ligation of *NcoI* blunted-*KpnI* RACE fragment and a *KpnI-XhoI* cDNA fragment into *EcoRV-XhoI* digested pGLOMYC. pGLOMYC was constructed by inserting a *HindIII-BamHI* fragment containing a MYC-tag and the 5' untranslated region of β-globin into *HindIII-BamHI* digested pCDNA. Subsequently, a *SpeI-XbaI* fragment comprising the 3' untranslated region of β-globin was inserted into this product digested with *XbaI*. *In vitro* transcribed and translated *Sox-13* was made according to the manufacturer's instructions (Promega) using T7 RNA polymerase and pGLOMYC-*Sox-13* (S) or pGLOMYC (L).

Gel retardation assays

Oligonucleotides were labelled by T4 kinase with [γ-³²P]ATP and annealed to their complementary strand. For a typical binding reaction, 1 µl of *in vitro* transcribed and translated protein was pre-incubated with 1 ng of poly-dIdC at room temperature for 15 min. Labelled probe (10 000–20 000 c.p.m.) was added in 13 µl buffer containing 10 mM HEPES, 60 mM KCl, 1 mM EDTA, 1 mM DTT and 10% glycerol and the mixture was incubated for an additional 30 min at room temperature. The samples were electrophoresed through a non-denaturing 4.5% polyacrylamide gel run in 0.25× TBE at room temperature. Oligonucleotides used were: 56, 5'-ACTGAGAACAAGCGCTCT-3' annealed to 3'-TGACTCTTGTTCGCGAGA-5' and 56mut, 5'-ACTGAGGGCAAAGCGCTCT-3' annealed to 3'-TGACTCCCGTTTCGCGAGA-5'.

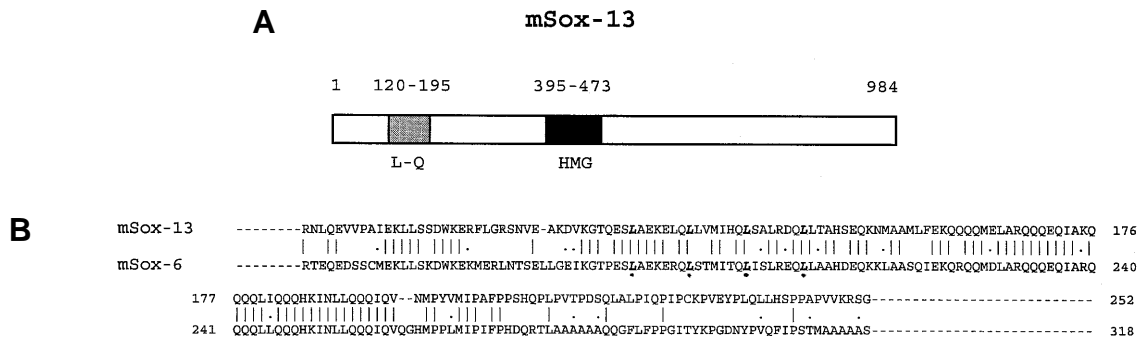


Figure 1. (A) Graphic representation of the murine *Sox-13* protein. Numbers show amino acid positions. The centrally located HMG box is indicated by a black box, the grey box represents the leucine zipper-Q domain. The predicted amino acid sequence was deduced from cDNA and RACE clones, accession no. AJ000740. (B) Amino acid sequence of the leucine zipper-Q region of *Sox-13* (upper sequence) compared to *Sox-6* (lower sequence) (19). Identical residues are shown as '|', similar residues as '.'. The conserved leucine zippers are indicated by marked italics.

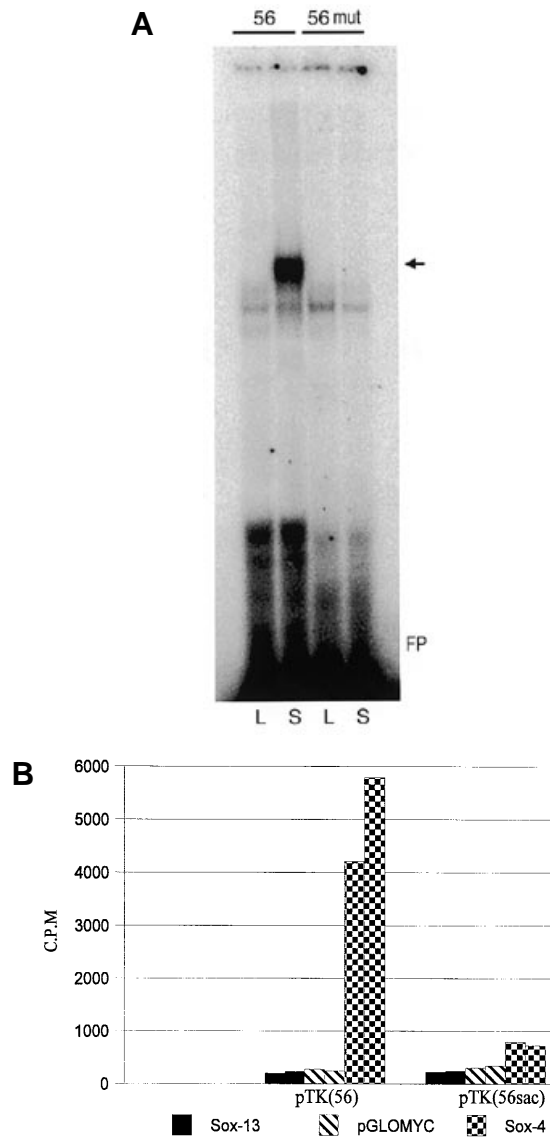


Figure 2. (A) Sequence specific binding of the Sox-13 HMG box to the AACAAAG motif. *In vitro* translated Sox-13 protein (S) or control lysate (L) were incubated with labelled retardation probes 56 (ACTGAGAACAAGCGCTCT) or 56mut (ACTGAGGGCAAAGCGCTCT). A specific shift of labelled oligonucleotide was seen only in the case of Sox-13 protein in combination with the 56 motif (arrow). (B) Transactivation assay of Sox-13. Co-transfection of pGLOMYC-Sox-13 with an AACAAAG multimerized motif in a reporter-CAT construct [pTK(56)] does not lead to an increase in CAT-activity, therefore Sox-13 does not transactivate transcription by itself (nor does pGLOMYC alone). pCDM8-sox-4 shows a 6-fold increase in extracted c.p.m.. No transactivation of transcription was seen with the mutated pTK(56sac) reporter. Transfected cells were the Sox-13 negative B cell line AZU II.

CAT assays

Cells were transfected by electroporation. In short, 2.5×10^6 cells were transiently transfected with 1 μ g of CAT reporter plasmid and 5 μ g of pGLOMYC/pGLOMYC-Sox-13 or 5 μ g of pCDM8-Sox-4 expression plasmid in a volume of 250 μ l. Pulse conditions were 960 μ F and 250 V using a Gene Pulser Apparatus

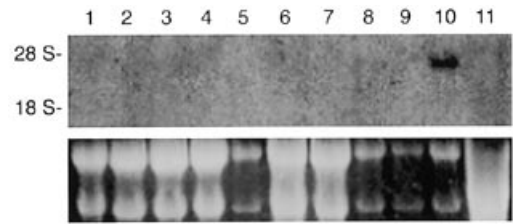


Figure 3. Expression of Sox-13 in murine cell lines. Sox-13 mRNA as measured by Northern blotting on total RNA extracted from various murine cell lines using a 3' orientated probe. Positions of the ribosomal bands are indicated by the 28S and 18S labels. Sox-13 mRNA runs as a 4 kb message. Thymocytes/T cell lines: lane 1, RBL5; lane 2, EL4, pre-B cell lines; lane 3, 1881; lane 4, 30BA; lane 5, 40EI, B cell lines; lane 6, AZU II; lane 7, NS-1, day 14 fetal thymus-derived pro-thymocyte cell line; lane 8, 34.1 early, day 14 fetal thymus-derived pre-thymocyte cell line; lane 9, 34.1 late, day 18 fetal thymus-derived thymocyte cell line; lane 10, 18.2, fibroblast cell line; lane 11, 3T6. Equal amounts of RNA were present in each lane as judged by examination of the ethidium bromide-stained gel (bottom gel).

(Bio-rad). Cells were harvested after 48 h and freeze-thawed in 60 μ l of 100 mM NaCl, 10 mM Tris, pH 7.4, 1 mM EDTA, 50 μ l of lysate were added to 50 μ l CAT cocktail ([¹⁴C]chloramphenicol 1 μ Ci/ml [60 mCi/mmol], 2.5% glycerol, 250 mM Tris, pH 7.5, 3 mM butyryl-CoA) and incubated for 2 h at 37°C. Pristane-xylene extractable c.p.m. representing butyrylated [¹⁴C]chloramphenicol were determined by liquid scintillation counting.

pCDM8-Sox-4 and the two different reporter constructs were identical as described previously by van de Wetering *et al.* (5).

Cell-lines

Cells were grown in RPMI-1640 supplemented with 5% fetal calf serum and antibiotics. All cell-lines are of murine origin and described by Oosterwegel *et al.* (21). The 34.1 early, 34.1 late and 18.2 are all derived from fetal thymus organ culture and transformed with a retroviral myc-raf oncogene.

Northern blot analysis

For the cell-line blots total RNA was isolated according to Chomczynski and Sacchi (22). RNA (15 μ g) was electrophoresed, transferred to nitrocellulose and hybridized with Sox-13 cDNA probes, labelled by random oligo priming, all according to standard procedures (23). For the cell-line blot an EcoRV fragment (1593–3181) was used.

In situ hybridization

Embryos of sequential developmental stages were collected from timed-pregnant mice. Embryos of 10.5, 13.5, 16.5 and 18.5 dpc and day 1 neonates were used for *in situ* hybridization and treated as described by Jonker *et al.* (24). In short, ³⁵S-labelled transcripts were made by *in vitro* transcription of linearized pBluescript (SK) containing a 725 bp, 5' located, EcoRI–NsiI fragment (546–1271) of Sox-13. 20 000 c.p.m./ μ l hybridization solution was added per section. As a control, sections were incubated with a sense Sox-13 probe.

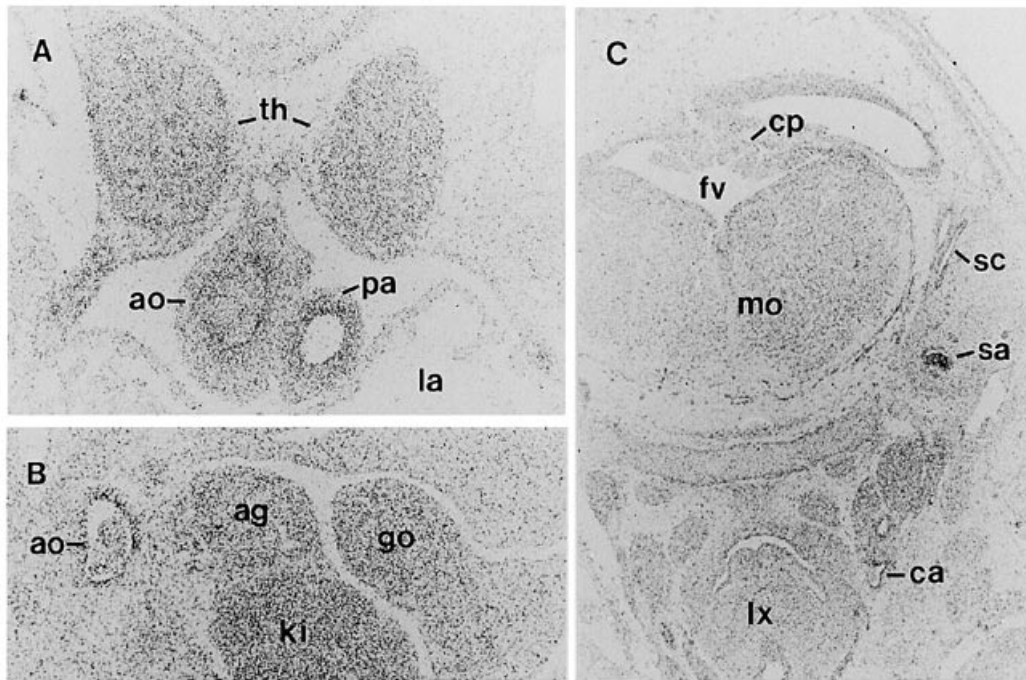


Figure 4. *In situ* hybridization of *Sox-13* on frontal sections of 13.5 dpc murine embryos. Low expression of *Sox-13* was observed in the wall of the great arteries, but not in the wall of the veins. The saccular component of the inner ear also showed reactivity. (A) 13.5 dpc thymus and outflow tract (100 \times). (B) Frontal section at the abdominal aorta level (100 \times). (C) Frontal section through the head (50 \times). ao, aorta; ag, adrenal gland; cp, choroid plexus; ca, carotid artery; fv, fourth ventricle; go, gonad; ki, kidney; la, left atrium; lx, larynx; mo, medulla oblongata; pa, pulmonary artery; sa, saccule; sc, semicircular canal; th, thymus.

RESULTS AND DISCUSSION

Cloning of *Sox-13*

The developmental characteristics of several *Sox* genes prompted us to search for *Sox* homologues with possible roles in thymocyte development, possibly acting in concert with established lymphoid transcription factors (25). To this end, we isolated RNA from 15.5 dpc thymuses. Low stringency PCR with degenerate primers based on homologies between the HMG boxes of *SRY* and *Sox-1-4* (1) was performed. Products of the predicted size were subcloned into pBluescript (SK) and sequenced. Of 40 clones analyzed, 32 encoded the HMG box of *Sox-4* (5), while eight encoded the HMG box of *Sox-13*, originally identified by Wright *et al.* (6). Screening of a 15 dpc whole mouse embryo lambda-gt11 library with the *Sox-13* HMG box yielded a single incomplete *Sox-13* cDNA clone. The 5' sequence was cloned by RACE (Accession no. AJ000740). The sequence predicted an open reading frame of 984 amino acids (Fig. 1A). The ATG at position bp 3, most likely represented the translation initiation codon, based on comparison with partial human SOX-13 cDNAs which have stop codons preceding this conserved ATG (Roose and Clevers, unpublished). A consensus polyadenylation signal (AATAAA) is positioned at bp 3157. The original cDNA clone starts at nucleotide position 546. Of the RACE clones, 70% start at position bp 1, others at bp 27 and bp 324. The encoded protein contained a central HMG box with high homology to *Sox-5* and *Sox-6*, 92 and 91% respectively (6). A putative leucine zipper motif was identified at amino acid position 120–141, immediately followed by a glutamine-rich region from AA 159 to 195 (Fig. 1A). This leucine zipper-Q region was highly homologous to a similar stretch in the *Sox-6/Sox-lz* and SOX-LZ proteins

(Fig. 1B) (19,26). The leucine zipper was clearly amphipatic and, therefore, likely mediates dimerisation of the protein. Indeed, the homologous zipper region in the, *Sox-6* orthologue, rainbow trout protein SOX-LZ mediates homodimerisation, as was shown by Takamatsu *et al.* (26). The conservation of the leucine zipper-Q region indicates a conserved role for this domain in the three proteins, in addition to providing a dimerisation interface.

DNA-binding and transactivating characteristics of *Sox-13*

To establish whether *Sox-13* can bind to the consensus HMG box motif as observed for other *Sox* proteins, we performed a gel retardation analysis (5). The full length *Sox-13* cDNA was cloned in the expression vector pGLOMYC and subsequently transcribed and translated *in vitro*. As a control, pGLOMYC, containing only the MYC-tag flanked by β -globin untranslated regions was transcribed and translated. The two programmed extracts were incubated with radio labelled gel retardation probes containing either the optimal motif (AACAAAG) or a mutated motif (GGCAAAG). As analysed by gel retardation, *Sox-13* (S) bound specifically to the AACAAAG motif (Fig. 2A, lane 2) while no shift was seen using the mutated motif or control extract (L) (lanes 1, 3 and 4). Only one shifted band was observed in the gel retardation of *Sox-13*, suggesting that *Sox-13* binds as a monomer or as a homodimer to the consensus motif in this assay. Homodimerisation of *Sox-13* may be mediated by the leucine zipper-Q region in the same fashion as described for SOX-LZ (26).

Having established the binding capacity of *Sox-13* to the AACAAAG motif, we subsequently studied its transactivating characteristics. *Sox-13* was readily expressed as a nuclear protein

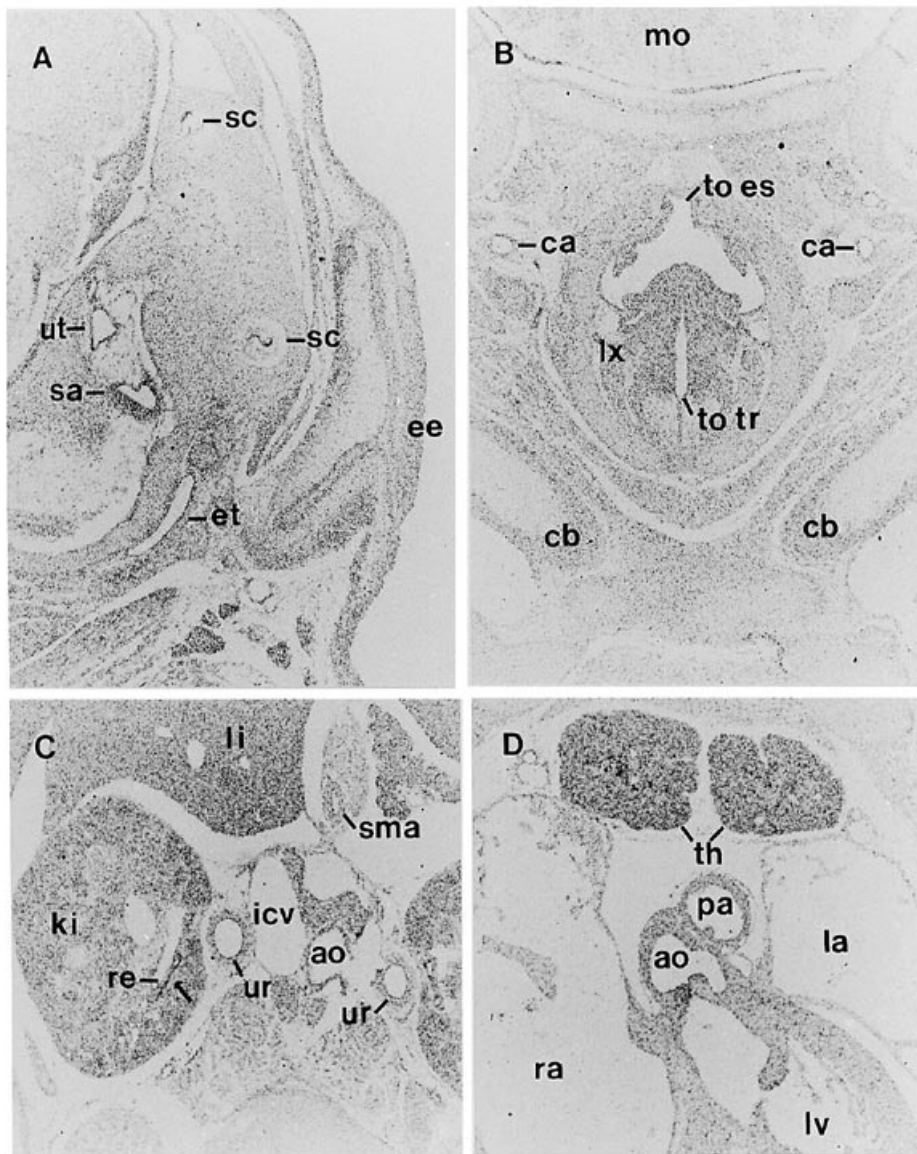


Figure 5. Expression of *Sox-13* at 16.5 dpc. The expression in the blood vessels as well as in the different components of the inner ear resembled that seen at 13.5 dpc. The 16.5 dpc larynx showed no *Sox-13* expression. Strongly positive cells were seen in the thymus. (A) Frontal section through the 16.5 dpc brain (50 \times). (B) 16.5 dpc larynx (50 \times). (C) Section through both kidneys. Note the signal seen in the renal artery indicated by the arrow (50 \times). (D) Frontal section of the heart and thymus at 16.5 dpc (50 \times). Strong expression was seen in a small population of cells in the thymus. cb, collar bone; es, esophagus; et, eustachian tube; ee, external ear; icv, inferior caval vein; lv, left ventricle; li, liver; ra, right atrium; re, renal artery; sma, superior mesenteric artery; tr, trachea; ut, utricule; ur, ureter. Other structures are mentioned in Figure 4.

from the pGLOMYC vector as analysed in transiently transfected COS cells stained with an anti-myc tag antibody (not shown). pGLOMYC-*Sox-13* was co-transfected with pTK(56)7, a reporter-CAT plasmid containing seven copies of the AACAAAG motif inserted upstream of a minimal herpes simplex thymidine kinase (TK) promoter (27). As a control, we used pTK(56sac)7, a TK-CAT vector in which the seven copies of AACAAAG had been replaced by seven CCGCGGT copies (27). CAT transfections were carried out in duplicate and assayed by organic phase separation. *Sox-13* did not transactivate transcription through the AACAAAG concatamer in the murine B cell line, AZU II (Fig. 2B), nor in the murine B cell

line NS1 (not shown). *Sox-4* strongly transactivated transcription from pTK56(CAT) in the same assay as reported previously (5).

Although some HMG box factors, such as *Sox-4* (5), can transactivate transcription from multimerized motifs, it is clearly not a feature of all members of this family. As has been extensively documented for LEF-1, the DNA bending characteristics may endow HMG box factors with potent context dependent transactivation properties (28,29). Such properties can only be studied in the context of natural, complex promoters, that remain to be identified for *Sox-13*. Alternatively, *Sox-13* may need a partner molecule to form a bipartite transcriptional complex, as was shown

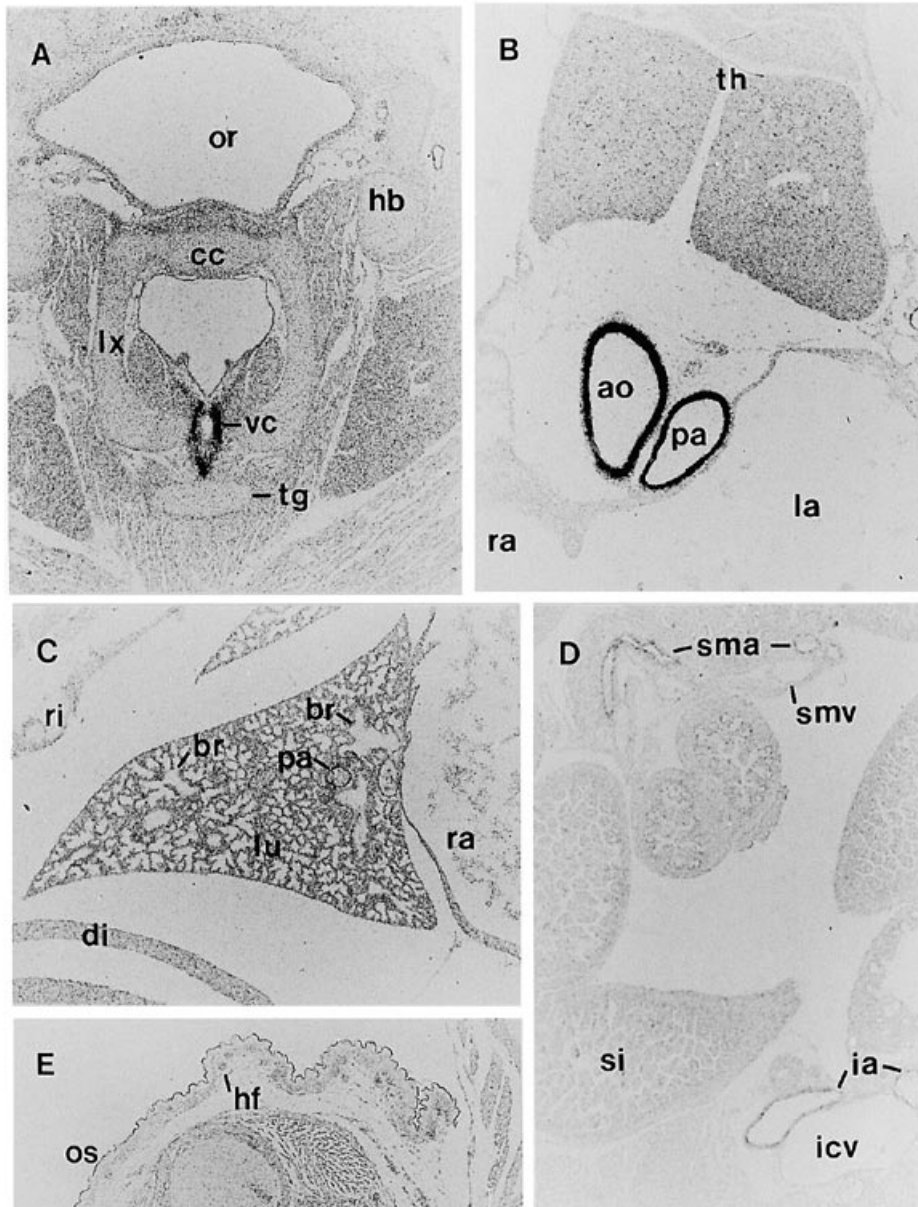


Figure 6. *In situ* hybridization analysis of *Sox-13* expression at 18.5 dpc. High expression was seen in the walls of all arteries, the walls of veins were negative. At this stage the tracheal epithelium below the vocal cord showed expression of *Sox-13*. Thymic expression remained confined to few cells. (A) Expression of *Sox-13* in the 18.5 dpc larynx, notice the low signal in the small arteries (50 \times). (B) Both the aorta and the pulmonary artery showed high expression of *Sox-13* (50 \times). (C) Frontal section of a 18.5 dpc lung. The pulmonary artery was positive, in contrast to the bronchi (50 \times). (D) Frontal section of the abdomen. Striking is the difference between positive arteries and negative veins (50 \times). (E) Outer skin of a 18.5 dpc embryo. Hair follicles in the skin showed low expression of *Sox-13* (50 \times). br, bronchus; cc, cricoid cartilage; di, diaphragm; hb, hyoid bone; hf, hair follicle; ia, iliac artery; lu, lung; or, oropharynx; os, outer skin; ri, rib; smv, superior mesenteric vein; si, small intestine; tg, tracheal ring; vc, vocal cord. Other structures are mentioned in Figures 4 and 5.

for *Sox-2* (30). *Sox-2* is able to form a complex with either the ubiquitous Oct-1 or the embryonic Oct-3, but only in combination with the latter is the ternary complex capable of promoting transcriptional activation (30). As another example, members of the Tcf/LEF family do activate transcription from multimerized motifs when complexed with the Wnt/Wingless effector β -catenin/Armadillo which provides an essential transactivation domain (31).

Lymphoid expression of *Sox-13*

We next analysed expression of *Sox-13* by Northern blot analysis on a selection of well-defined precursor and mature lymphoid cell lines. A single T lineage cell line expressing a *Sox-13* species of 4 kb was identified. This cell line, 18.2, was derived from a day 18 fetal thymus (Fig. 3, lane 10). In addition, Northern blot

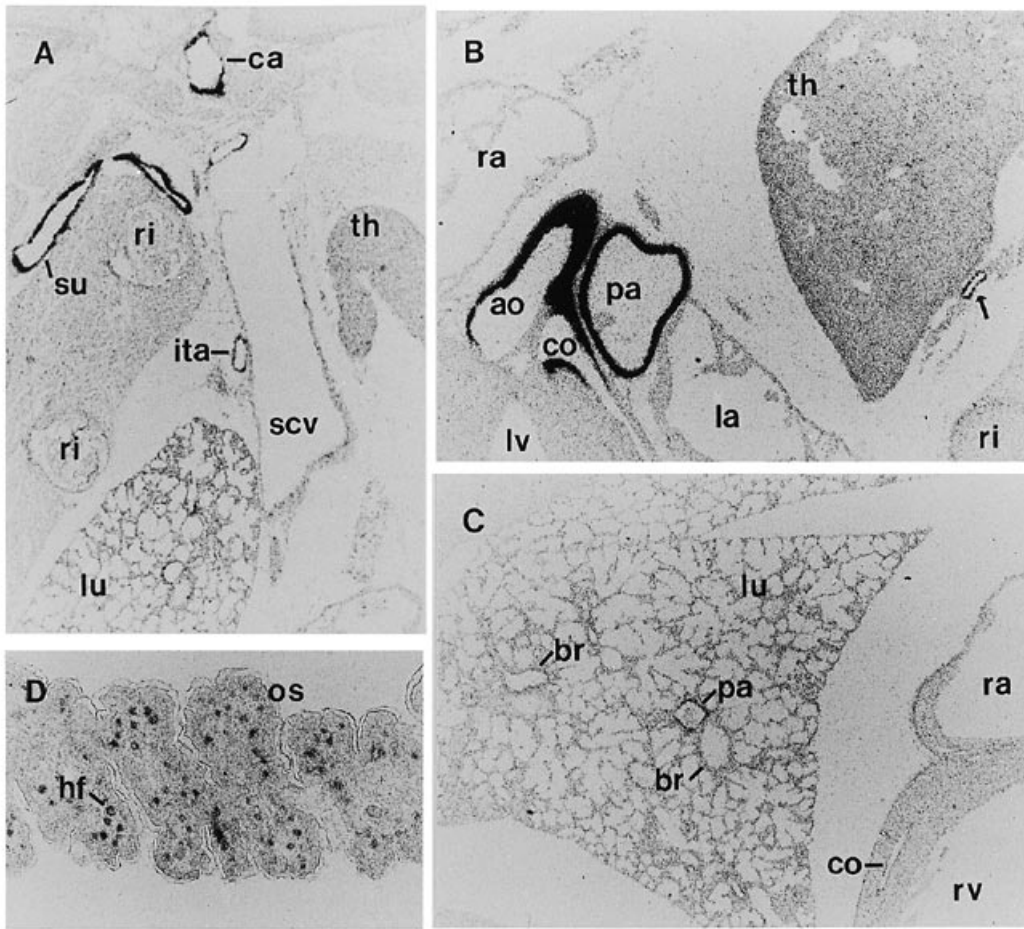


Figure 7. Expression of *Sox-13* in neonates day 1, analyzed by *in situ* hybridization. Expression of *Sox-13* in the wall of arteries further increased in intensity. The hair follicles in the skin also showed a stronger signal. (A) Frontal section of a neonate mouse (50 \times). (B) Outflow tract of the heart and thymus of a neonate. Note the signal in the root of the coronary artery as well as the small artery beside the thymus indicated by the arrow (50 \times). (C) Lung of a neonate mouse day 1 (50 \times). (D) Expression of *Sox-13* in the hair follicles increased in comparison to 18.5 dpc (50 \times). co, coronary artery; ita, internal thoracic artery; rv, right ventricle; scv, superior caval vein; su, subclavian artery. Other structures are mentioned in Figures 4–6.

analysis of sorted thymocyte precursors from adult thymuses revealed low levels of *Sox-13* expression at all stages (not shown).

Expression of *Sox-13* during embryonic development

We investigated *Sox-13* expression during embryonic development by *in situ* hybridization. At 10.5 dpc, no expression of *Sox-13* was observed. *Sox-13* was first detected in the wall of the great arteries at 13.5 dpc (Fig. 4A and B). In contrast, the vein walls did not express *Sox-13*. *Sox-13* signal was also found in the saccular component of the inner ear (Fig. 4C). In 16.5 dpc embryos, the intensity of *Sox-13* expression in the blood vessels resembled that seen in 13.5 dpc (Fig. 5C and D). In the inner ear, a slight but significant signal was seen in the saccular and utricular components as well as in the ampullae of the semicircular canals (Fig. 5A). Finally, a small number of strongly staining cells were observed in the thymus (Fig. 5D). The hybridization signal seen in 16.5 dpc thymus is most likely caused by early thymocytes situated there, given that we amplified the *Sox-13* HMG box originally from 15.5 dpc thymocytes and the fact that it is highly expressed in a day 18 thymocyte cell line. No expression was found in various sections

of the 16.5 dpc larynx (Fig. 5B). Just before birth (18.5 dpc, Fig. 6), staining in the wall of the arteries had strongly increased. Staining appeared confined to the media and intima. In addition to the great vessels (Fig. 6B), *Sox-13* expression included smaller vessels such as the carotid artery, the basilar artery, the pulmonary arteries, the mesenteric artery and the iliac artery (Fig. 6A–D), but remained absent from the wall of the veins. Staining in the thymus remained intense and confined to relatively few cells (Fig. 6B). *Sox-13* expression in the inner ear was no longer above background (not shown). New tissues to acquire *Sox-13* expression were the tracheal epithelium below the vocal cord (Fig. 6A) and, to a much lesser extent, the hair follicles in the skin (Fig. 6E). In neonates of 1 day old, expression of *Sox-13* in the wall of most arteries further increased in intensity (Fig. 7A and B). However, *Sox-13* expression in the coronary arteries was confined to their roots even though small arteries elsewhere strongly expressed *Sox-13* (Fig. 7B, arrow). In the lung, the pulmonary arteries showed *Sox-13* expression, the bronchi or lung epithelium were negative (Fig. 7C). Furthermore, the wall of the veins remained negative for *Sox-13* expression. *Sox-13* expression in the thymus

was comparable to that seen in earlier stages (Fig. 7B), while it increased in intensity in the hair follicles (Fig. 7D).

The specific high level expression suggests that the prime role of *Sox-13* involves the development of arterial walls from generic vessels. Little is known about the formation of arteries from endothelial tubes and smooth muscle cells. Few specific markers discriminating developing venous and arterial vessels at an early stage in embryogenesis have been described to date (32). In aortic smooth muscle cells from fetuses of 8–10 and 20–22 weeks of gestation, expression of α -smooth muscle myosin and smooth muscle-myosin heavy chain is observed (33). Expression of two other markers, calponin and caldesmon, is absent at this stage, but comes up relatively late in embryogenesis, resulting in expression of all four markers in the adult aortic media (33). During the process of vessel-formation, the initial endothelial tubes become invested by mesenchymal cells of unknown lineage (32). Most perivascular tissue arises from mesodermal cells. However, smooth muscle cells of some arteries originate from neural crest (34). In such arteries, i.e. the common carotid vessels, pulmonary trunk, and part of the definitive aortic arch, investment of mesenchymal cells with endothelial cells coincides with the expression of smooth muscle actin and myosin (32). *Sox-13* expression was observed in the media of arteries from both neural crest and mesodermal origin in 13.5 dpc embryos and later stages. Gene knock-out experiments are underway to shed more light on the exact function of *Sox-13* in the developing mouse embryo.

ACKNOWLEDGEMENTS

We thank Marco Schilham for technical assistance, Frits Meijlink for providing us with the cDNA library and Johan van Es and Nick Barker for reviewing the manuscript. We're grateful for the advice and criticism of all the co-worker of the Clevers lab. This project was supported by a PIONIER grant from NWO-GMW.

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