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Gene expression pattern

The novel transcription factor gene *Sp5* exhibits a dynamic and highly restricted expression pattern during mouse embryogenesis

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Abstract

We describe the sequence and expression pattern of *Sp5*, a novel member of the vertebrate *Sp1* transcription factor gene family which consists of at least five members. This gene family is characterized by a highly conserved domain which is formed by three Zn fingers, which bind to the GC box or the GT/CACC box in the promoter of many genes. These boxes are important cis-acting elements required for the expression of the respective genes. In vitro experiments indicate that the Sp1 transcription factors act by influencing the methylation state of the DNA, or by direct interactions with other promoter specific transcription factors. Despite intensive research, the results from in vivo experiments, including targeted gene inactivation, have been difficult to explain. This may be due to possible redundancies and interferences with other transcription factors of this gene family. Here, we report the isolation of the mouse *Sp5* gene, a novel *Sp1* homolog. Its sequence indicates that *Sp5* is a possible link between *Sp1* and the closely related *BTEB/KLF* gene family. We provide detailed information of its highly dynamic expression pattern during mouse embryogenesis in the developing brain, the spinal cord, the trigeminal ganglia, the somites and additional sites outside the nervous system starting from embryonic day 7.25 (E7.25) up to E10.5.

Keywords: *Sp5*; *Sp1*; *BTEB*; *EKLF*; *LKLF*; Mouse; Embryogenesis; Tail bud; Branchial arches; Pharyngeal pouch; Pharyngeal cleft; Central nervous system; Pretectum; Tuberal hypothalamus; Mammilary body; Mesencephalon; Red nucleus; Rubral nucleus; Midbrain–hindbrain boundary; Spinal cord; Trigeminal ganglia; Somites; Otic vesicle; Limbs

1. Results

A PCR based approach was used to clone Sp1 gene family homologs. Degenerate PCR primers were designed on the basis of the conserved Zn finger domain. The pair cgccaggtgatcrctickcatraaic (5′) and cacatctgccayatycciggit-gygarmg (3′) amplified on genomic mouse DNA a characteristic Sp1-type Zn finger box of 231 nucleotides (annealing at 47°C; 40 cycles). This fragment was used to screen an E8.5 mouse cDNA library (kindly provided by Brigid Hogan). We obtained a 1255 bp fragment of a cDNA sequence including the poly(A). From the NCBI entry *AF279479*, 636 bp were added 5′ to our sequence (length of the entry, 1825). Our sequence is identical to *AF279479*, except for an additional 53 bp at the 3′end (TTCCAGGGTCTCCTCGGGAAGACCCCCACAGAA-AAAAAAAAAAAAAAAAAAAA).

As shown in Fig. 1A,B, the *Sp5* gene belongs to the *Sp1* transcription factor family, which is characterized by typical Zn finger and buttonhead regions (El-Baradi and Pieler, 1991; Wimmer et al., 1993, 1996). Outside of these domains, the Sp5 protein shows little similarity to other Sp1 homologs, which contain S/T-rich and Q-rich stretches. Instead, in the latter region, Sp5 is similar to the evolutionary homologous but distinct BTEB/KLF group, characterized by a high content of proline residues (about 20%; Fig. 1C; Philipsen and Suske, 1999). Members of the BTEB/KLF group have a distinct Zn finger domain and lack the buttonhead domain. Therefore, the *Sp5* gene has probably been generated during evolution by a domain swapping between a *Sp1* and a *BTEB/KLF* homolog.

The in situ hybridization (ISH) revealed a highly dynamic and specific expression pattern, which appears to be subdivided into two phases (Figs. 2 and 3): an early activity which is connected to the process of gastrulation, and a second wave of expression during organogenesis.

1.1. Early expression of Sp5

At E7.25 (Figs. 2A,B and 3A), *Sp5* is expressed in the entire region of the primitive streak. In the cells of the embryo

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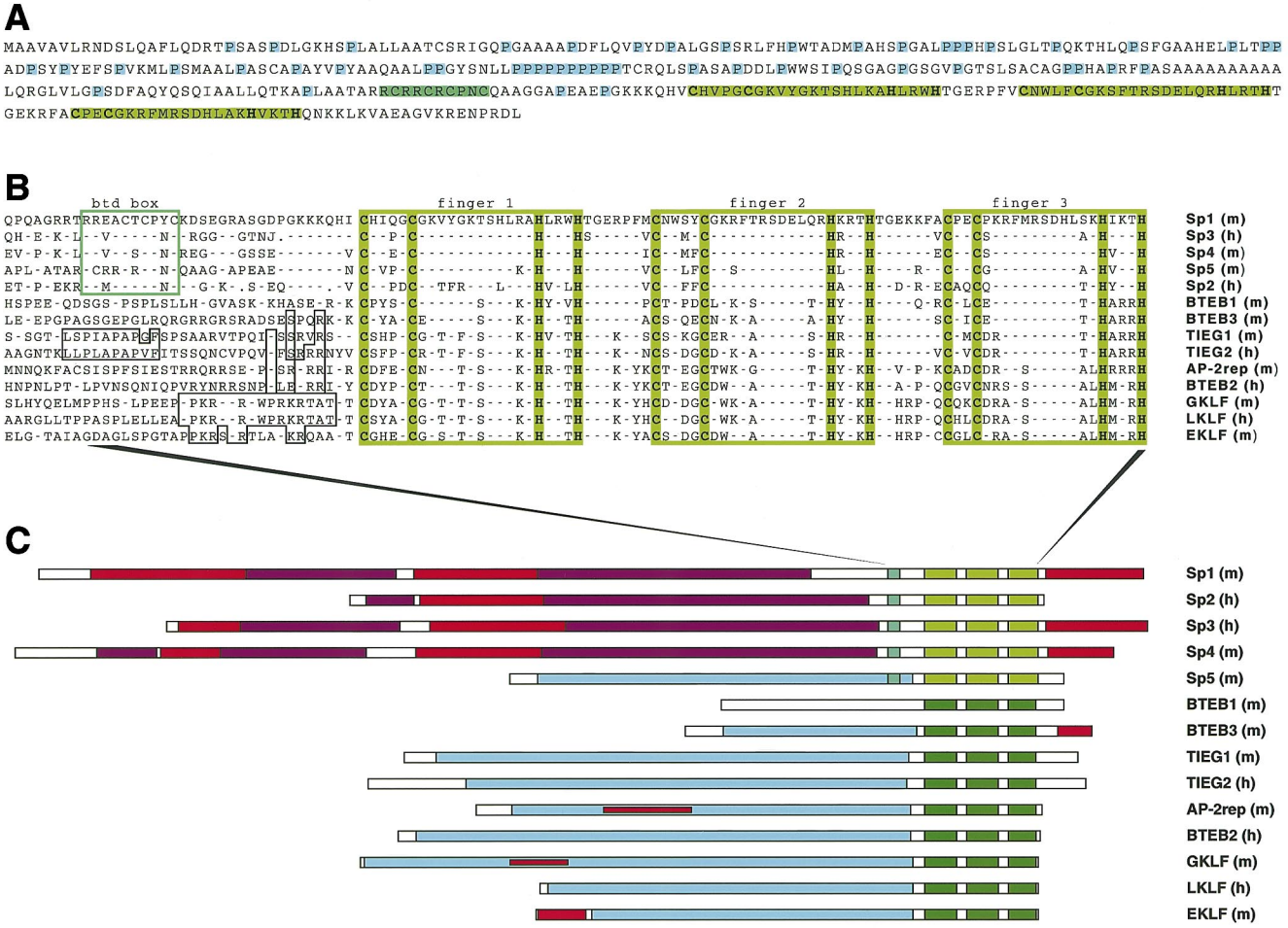

Fig. 1. (A) Deduced amino acid sequence of the mouse Sp5 protein. The three Zn finger domains are highlighted in light green, the btd domain (RCRRCRCPNC) is in a darker green, and the proline residues are highlighted in light blue. (B) Comparison of the Zn finger and btd protein domains of different Sp1-like and BTEB/KLF-like proteins. All sequences are according to the protein translations given at NCBI for the mentioned genes (m, mouse; h, human). Note that the proteins Sp1–Sp5 all contain the conserved btd box about 18 amino acids in front of the Zn finger domains, while the more distantly related genes belonging to the BTEB/KLF family lack this domain. In contrast, they contain conserved sequences (black boxes) that are not found in the Sp1-like proteins. (C) Schematic drawing representing the deduced proteins of the Sp1 and the BTEB/KLF family. The scheme is drawn to scale. The region of the btd and Zn finger domains is symbolized by green boxes and represents the deduced sequences in (B). The purple boxes represent glutamine-rich domains, the red boxes serine/threonine-rich stretches, and the blue boxes represent proline-rich domains. Note that Sp5 is homologous to Sp1 regarding the btd and Zn finger domain, while the rest of the protein is homologous to the BTEB/KLF family like BTEB2, BTEB3, TIEG1, TIEG2, EKLF or LKLF.

proper (primitive ectoderm and ingressing mesoderm), *Sp5* mRNA is transcribed, but not in the underlying visceral endoderm (arrows in Fig. 3A). The lateral constriction of the expression is unclear, since the lateral most mesoderm and ectoderm are also negative (data not shown). Later on, the signal remains confined to the primitive streak at the end of the embryo (Fig. 2E–J). At E8.25, the gene is expressed in the posterior end of the neural plate and the underlying mesoderm (Fig. 3C). The visceral endoderm is still negative (data not shown). With the formation of the gut, the definitive endoderm also becomes positive (arrow in Fig. 3C), as well as the notochord (double arrow in Fig. 3C). At E10.25 and later stages, the primary gastrulation has finished and the secondary gastrulation starts in the posterior tip of the embryo, the tail bud. Its mesenchymal tissue strongly expresses *Sp5* (Figs. 2J and 3D,E), while the surrounding ectoderm is negative (arrow in Fig. 3E). Interestingly, after the switch in the gastrulation and notochord formation, the notochord still expresses *Sp5* (arrow in Fig. 3D), but exclusively in a single cell (double arrow in Fig. 3F) surrounded by *Sp5*-negative notochordal cells (arrows in Fig. 3F).

1.2. Sp5 expression in the developing brain

At E8.0, the expression during organogenesis starts in the CNS as a faint ISH signal in the mesencephalon and diencephalon (arrows in Figs. 2E,F and 4A). At E8.5, the mRNA level becomes very strong in the dorso-lateral regions of the same domains (Fig. 4B,I). In the diencephalon, the *Sp5*-mRNA is restricted to the region of the prospective pretectum and epithalamus (Fig. 4B). The telencephalon shows no staining up to E10.5.

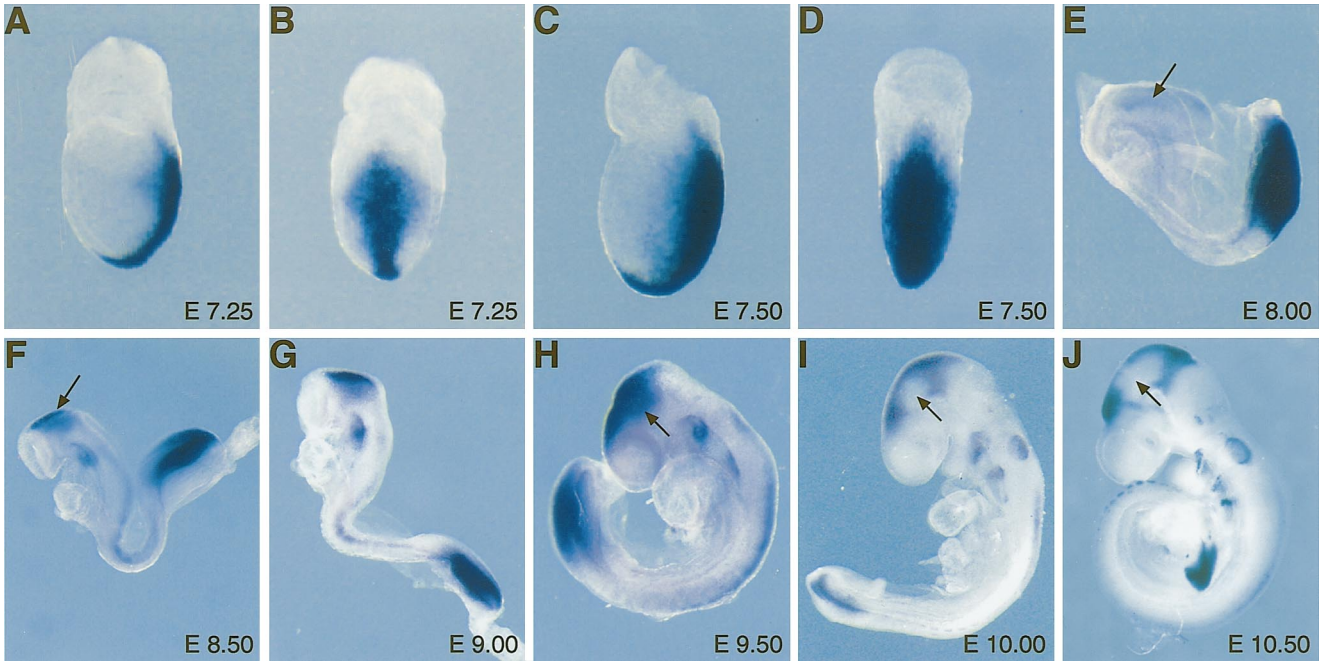

Fig. 2. *Sp5* shows two phases of expression during gastrulation and organogenesis. Whole-mount in situ hybridizations on mouse embryos from E7.25 to E10.5. The hybridizations were done with a PmlI fragment containing the 3′ part of the cDNA up to nt 676 as previously described. In (A–D) the anterior is to the left, and the posterior is to the right. The arrows in (E,F) point to the starting CNS expression, the arrows in (H–J) point to the fading expression in the lateral mesencephalon.

Until stage E9.5, both the dorsal and lateral regions of the mesencephalon are *Sp5*-positive (arrow in Fig. 2H), but the lateral expression gradually disappears between E10.0 and E10.5 (arrows in Figs. 2I,J and 4C). However, the midbrain–hindbrain boundary (MHB) shows a strong hybridization signal throughout its entire dorso-ventral axis, except for the roofplate (arrow in Fig. 4D) and the floorplate (arrow in Fig. 4F). In addition, in the midbrain, *Sp5* starts to be expressed from E10.0 onwards in the tegmentum (tm in Fig. 4F–H), while the mesencephalic floorplate is *Sp5*-negative (arrow in Fig. 4F).

In contrast to the mesencephalon, the pretectum also expresses *Sp5* in lateral and ventral regions, at least until E10.5. Here, the transcripts are detectable as a stripe at the base of the pretectum and hypothalamus, within the anlage of the mammilary bodies (mb in Fig. 4F–H,J). Noteworthy, in the entire floorplate of the brain, only the hypothalamus is Sp5-positive (Fig. 4J).

1.3. Sp5 expression in the developing spinal cord

In the spinal cord, *Sp5* expression is first detectable around E8.0. The hybridization signal has a peak within the upper thoraxic region and fades towards the rhomben-

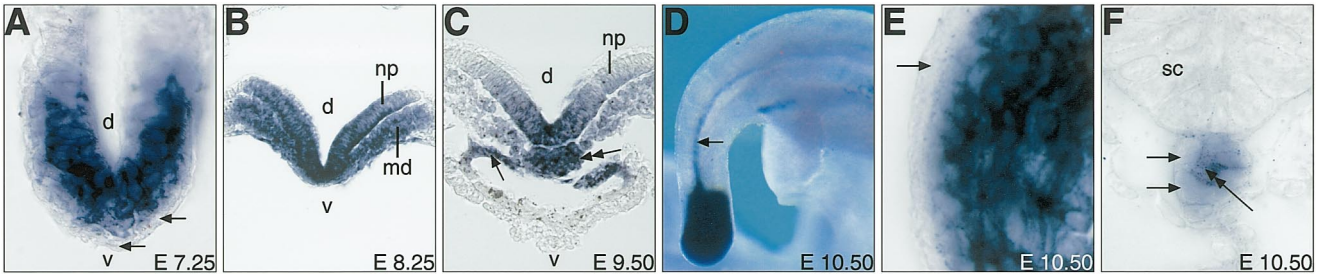

Fig. 3. *Sp5* expression during: (A–C), primary; and (D–F), secondary gastrulation. (A) Cross-section through the embryo in (2A). The arrows are pointing to individual visceral endoderm cells. (B) Cross-section through the posterior end of an E8.25 embryo. (C) Cross-section through the posterior end of the embryo in (2H). The arrow points to the definitive endoderm, the double arrow to the notochord. (D) Tail bud of a whole-mount in situ hybridized embryo; the notochord expression is marked with an arrow. (E) Cross section through the tail in (D). The arrow points to the ectoderm. (F) Cross-section through the notochord at the level of the arrow in (D). The double arrow points to the single *Sp5*-positive cell inside the notochord, the arrows to individual surrounding *Sp5*-negative cells. (A–C,E,F) Fifteen micrometer paraffin sections through whole-mount in situ hybridized embryos. d, dorsal; md, mesoderm; np, neural plate; v, ventral.

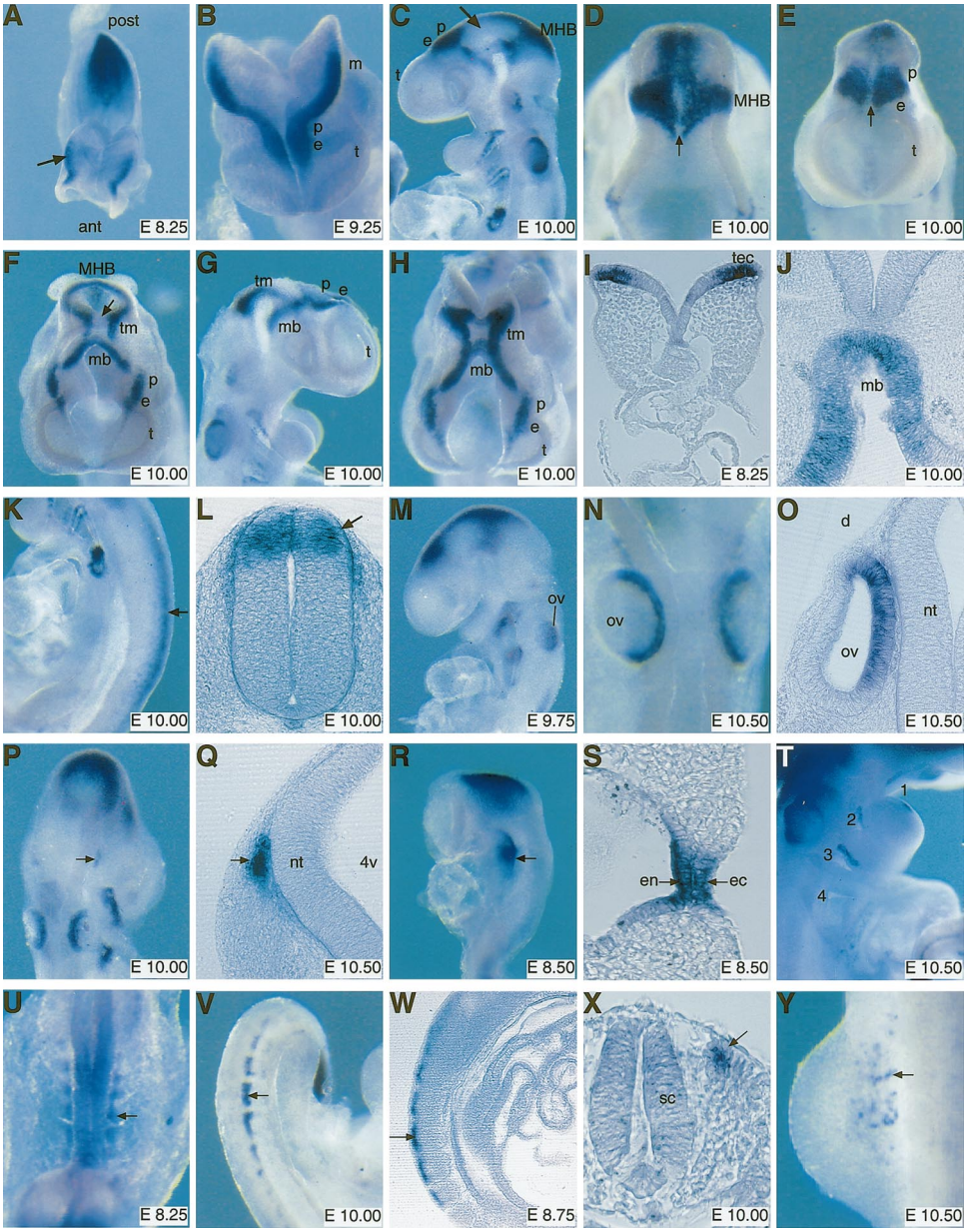

Fig. 4. *Sp5* expression during organogenesis. *Sp5* expression in: (A–J) the brain; (K,L), the spinal cord; (M–O), the otic placode/vesicle; (P,Q), the trigeminal ganglia; (R–T), the branchial pouches and clefts; (U–X), the somites; and (Y), the limbs (Y) at various stages of embryonic development. (A,B) Top view of the developing brain. The arrow in (A) indicates the expression in the neural folds of the mesencephalon/diencephalon. (C) Lateral view of the head. The arrow points to the lateral mesencephalon which is at this stage *Sp5*-negative. (D) The arrow indicates the *Sp5*-negative roofplate at the MHB. (E) The arrow points to the *Sp5*-negative roofplate of the anlage of the epithalamus and pretectum. (F) Top view of the developing brain after removal of the dorsal telencephalon. The arrow points to the *Sp5*-negative floorplate of the MHB. (G,H) Lateral and top view of the head after removal of the dorsal telencephalon, mesencephalon and anterior rhombencephalon, respectively. (I) Frontal section within the mesencephalon through the head of the embryo shown in (A). (J) Section showing specimen in the plane of the tuberal hypothalamus and the anlage of the mammilary bodies. (K) Dorso-lateral view of the spinal cord of a stained embryo. (L) Cross section through the embryo shown in (K) at the level of the rostral spinal cord. The arrow points to the expression in the dorsal most alar plate of the spinal cord. (M) Lateral view of the embryo showing the otic vesicle as an additional site of expression. (N) Dorso-lateral view of the otic vesicle. (O) Frontal section through the otic vesicle in (N). (P) Dorso-lateral view of the embryo head. The arrow points to the beginning of expression in the trigeminal ganglia. (Q) Cross section through the trigeminal ganglion of an E10.50 embryo. (R) Lateral view of the embryo head. The arrow points to the first branchial cleft. (S) Frontal section through the branchial cleft and pouch in (R). (T) Lateral view showing *Sp5* expression into the branchial clefts 1, 2 and 3. (U) Dorsal view of E8.25; and (V), dorso-lateral view of E10.00. The arrows point to the *Sp5* expressing somites. (W) Sagittal section through the embryo at the level of the heart. The arrow points to the *Sp5* expression in the somites. (X) Cross-section through the embryo at the level of the arrow in (V). The arrow points to the epaxial dermomyotome lip. (Y) dorsal view of the forelimb. The arrow points to *Sp5* expressing cells within the mesenchymal tissue of the limb bud. (A–H,K,M,N,P,R,T–W,Y) Whole-mount ISH; (I,S,X), paraffin sections (18 μm); and (J,L,O,Q,W), vibratome sections (35 μm). ant, anterior; d, dorsal; e, anlage of the epithalamus; ec, ectoderm; en, endoderm; m, mesencephalon; mb, anlage of the mammilary body; nt, neural tube; ov, otic vesicle; p, pretectum; post, posterior; sc, spinal cord; t, telencephalon; tec, prospective tectum of the mesencephalon; tm, tegmentum of the mesencephalon; 1, 2, 3, 4, branchial arches; 4v, fourth ventricle. ISH was performed according to Wilkinson (1992). The anatomical analysis was done according to Puelles and Rubenstein (1993).

cephalon and the tail bud. Along the dorso-ventral axis, the expression is confined to the dorsal most part of the alar plate (Fig. 4K,L).

1.4. Additional sites of Sp5 expression

At around E10.0, the trigeminal ganglia are strongly positive (arrow in Fig. 4P,Q). Furthermore, the neurogenic otic placodes/vesicles express *Sp5*. The initially (E9.00) faint ISH signal becomes very strong in the otic vesicles at E10.0, and is confined to its medio-dorsal part (Fig. 4M–O).

In addition, the branchial (pharyngeal) arch system, the somites and the limbs show a dynamic *Sp5* expression. While the first three branchial pouches (endoderm) and clefts (ectoderm) express the gene continuously between E8.5 and 10.5 (Fig. 4R–T), the fourth pouch and cleft are *Sp5*-negative (Fig. 4T). In the somites, the mRNA is only briefly detectable. About 2 h after the generation of an individual somite, *Sp5* is expressed in its dorsal tip which will develop into the epaxial dermomyotome lip. *Sp5*-mRNA levels reach a peak after about 15 h (arrows in Fig. 4U–X) and gradually fade away during the maturation of the individual somite.

In the limb buds, the *Sp5* gene is first expressed dorsally in scattered cells within the mesenchyme at E10.50 (arrow in Fig. 4Y). Later on, *Sp5*-positive cells are also found in the ventral part (data not shown).

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