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Gene expression pattern

Cloning and expression of *CSAL2*, a new member of the spalt gene family in chick

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Abstract

In this study we describe cloning and expression of *CSAL2*, a second member of the spalt gene family in chick. All spalt proteins are characterized by the presence of multiple zinc-finger motifs, which are highly conserved. Mutations in *HSAL1*, a human *spalt* gene result in Townes–Brocks syndrome (TBS). We show here that *CSAL2* is expressed in many of the tissues affected in TBS, including neural tissue, limb buds, mesonephros and cloaca.

Keywords: *spalt*; *SAL*; Townes–Brocks syndrome; Limb; Kidney; Central nervous system; Cloaca; Zinc finger

1. Results

1.1. CSAL2 is a member of the Spalt family of zinc finger proteins

We used degenerate RT-PCR to isolate a novel chick *spalt*, *CSAL2*. Sequence alignment of CSAL2 with other Spalt proteins revealed that it is closely related to Xenopus XSAL1 (76.6%), mouse MSAL (70%, Fig. 1A) and human HSAL3 (67%).

Phylogenetic comparison of all full-length vertebrate Spalt proteins currently isolated (Hollemann et al., 1996; Kohlhase et al., 1996, 1999, 2000; Ott et al., 1996; Koster et al., 1997; Onuma et al., 1999; Farrell and Münsterberg, 2000) suggests that the Spalt family can be divided into at least four subgroups (Fig. 1B). All Spalt proteins contain highly conserved zinc finger motifs and a glutamine-rich region close to the amino terminal end.

1.2. Expression of CSAL2 in the central nervous system

We examined embryos from Hamburger–Hamilton stage HH1–HH 35 (HH; Hamburger and Hamilton, 1951). We found that*CSAL2* is first expressed in the neural plate at HH7 (Fig. 2A). Subsequently, expression extends more posteriorly and anteriorly during neural fold and neural tube formation (Fig. 2B–F). From HH11 *CSAL2* is expressed throughout the neural tube and developing brain (Fig. 2D). Sections demonstrate that *CSAL2* expression is restricted to neural tissue (Fig. 2G) where it becomes confined to the ventricular zone (Figs. 2H and 3B,D,E). Expression cannot be detected in ectoderm and mesoderm (Fig. 2G). At HH15 staining becomes apparent at the mid-hindbrain and mid-forebrain boundaries. This staining becomes more prominent by stage HH17 (Fig. 3A) and sections demonstrate that *CSAL2* is expressed in the ventricular zone of these regions (Fig. 3B). *CSAL2* is also expressed at the base of the optic stalk (Fig. 3C), in symmetric regions in the mantle layer of the hindbrain and in the ventricular zone of the hindbrain (Fig. 3D). In the neural tube *CSAL2* is expressed in the ventricular zone (Fig. 3E). The neural pattern (from HH17) appears to be similar to that found for mouse *MSAL* (Ott et al., 1996). We did not observe expression of *CSAL2* in the otic vesicle or developing ear in the stages examined (up to HH35).

1.3. Expression of CSAL2 during limb development

Expression can first be detected in wing buds at HH18, in the posterior mesenchyme (Fig. 4A). There is no expression in leg buds at this stage. Wing bud expression at HH18 is transient; it begins to be downregulated at HH19 and cannot be detected by HH21 (Fig. 4B). There is no expression in limb buds from HH21 to late stage HH23.

Subsequently, *CSAL2* begins to be expressed in posterior regions of both wing and leg buds, with strong expression

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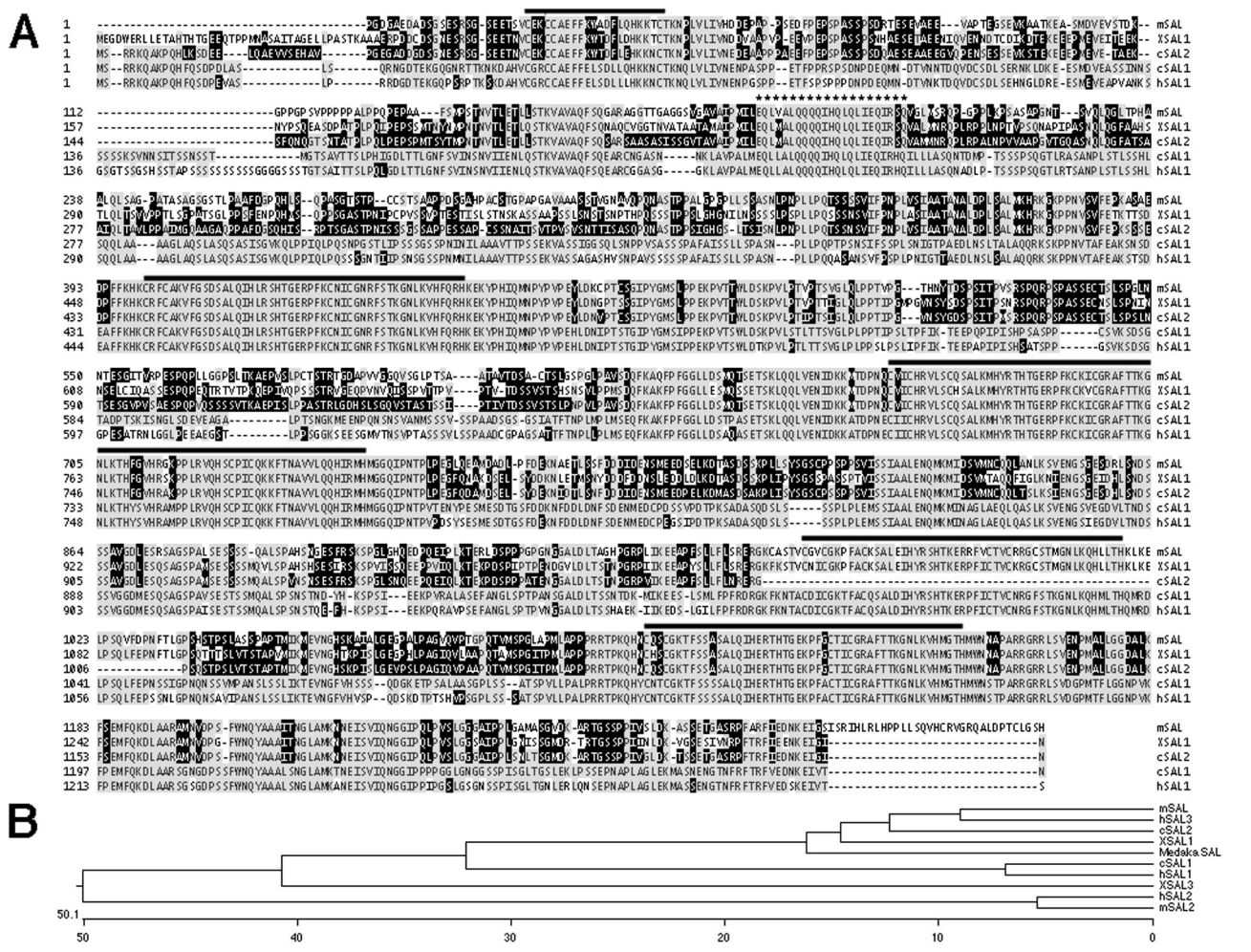

Fig. 1. Sequence alignment of Spalt family members. (A) The predicted amino acid sequence of CSAL2 shows closest homology to Xenopus XSAL1 and mouse MSAL. Amino acids identical to CSAL2 are shown in black. Amino acids identical to CSAL1 are shown in grey. CSAL1 is closest to HSAL1, mutations in which cause Townes–Brocks syndrome (Kohlhase et al., 1998). Black bars indicate the highly conserved zinc finger motifs. Note that the CSAL2 cDNA isolated encodes a protein in which the 3rd double zinc-finger motif is deleted. This form of the protein has also been described in Xenopus XSAL1 (Hollemann et al., 1996). A highly conserved glutamine rich region is indicated by asterisks (*). (B) Phylogenetic comparison of all vertebrate spalt genes currently available in the data base. For simplification the SAL acronym was used. We kept the numbers that were originally given to the gene. These reflect the chronology in which the genes were cloned in different species and not necessarily their relationship to each other. We propose that in future the human genes dictate the numbering of their respective orthologues in other species. (Thus, CSAL2 should be renamed CSAL3 and XSAL1 should be renamed XSAL3, etc.)

evident by HH24 (Fig. 4C,E). By HH26, expression in the leg bud becomes confined to a 'C' shaped domain in the posterior mesenchyme (Fig. 4F), while expression in the wing bud is more diffuse (Fig. 4D). *CSAL2* is expressed predominantly in the mesenchyme of both dorsal and ventral regions of the limb (Fig. 4G). Expression begins to be downregulated at HH27 and is no longer detectable from HH28 onwards. Embryos up until HH35 were examined. In contrast, *CSAL1* is expressed continuously throughout distal limb mesenchyme and the apical ridge from HH17 to HH25 (Capdevila et al., 1999; Farrell and Münsterberg, 2000).

1.4. Expression of CSAL2 in the tail bud, the mesonephros and the cloaca

CSAL2 is expressed in the tailbud from HH14 until HH 27 (Fig. 5A). It is expressed in the mesonephros (Fig. 5A) from late HH23 until HH27. Transverse sections show that *CSAL2* expression in the mesonephros is located in epithelial cells of the nephric ducts (Fig. 5B). *CSAL2* is expressed in the cloaca from stage 23/24 until HH26). In contrast, *CSAL1* is not expressed in the mesonephros or the cloaca (Farrell and Münsterberg, unpublished observations).

2. Materials and methods

RT-PCR was performed on 3.5 day chick embryo cDNA using primers derived from conserved regions of *spalt* genes. The 1 kb product was cut with HindIII to eliminate *CSAL1* clones. Sequencing confirmed that most of the remaining clones represented a novel chick *spalt* gene.

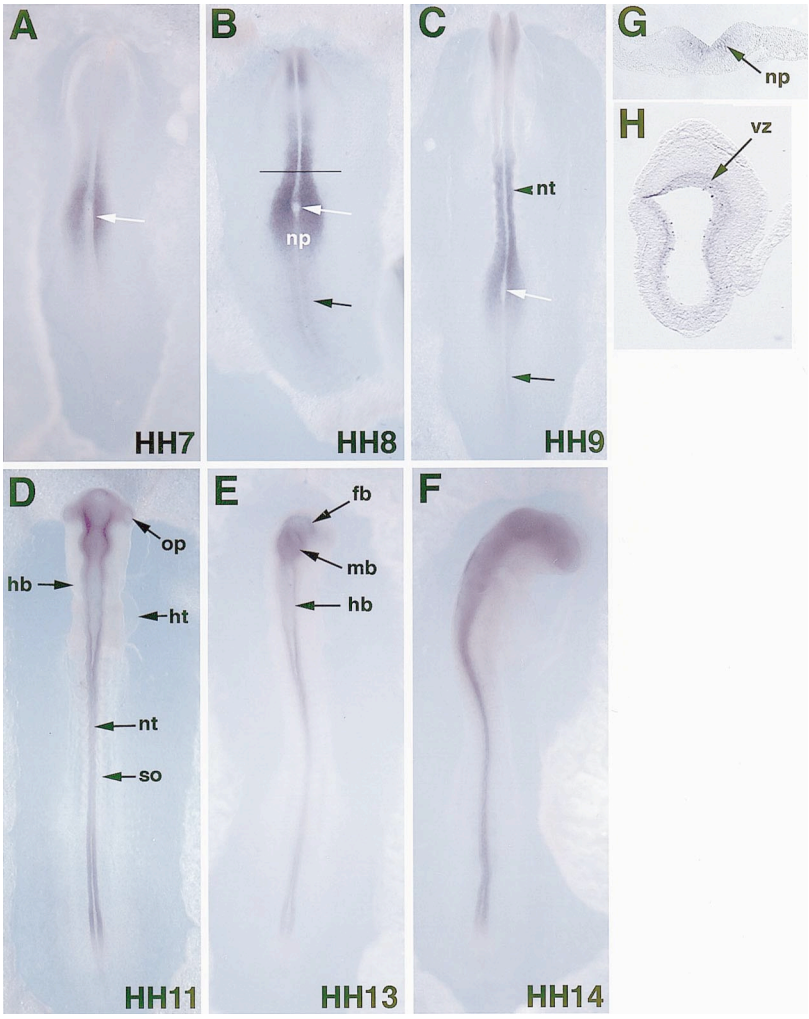

Fig. 2. *CSAL2* expression in the nervous system. (A) Whole-mount in situ hybridization of a HH7 embryo, showing expression in the neural plate. (B) HH8, expression expands in the neural plate (np) and (C) the forming neural tube at HH9. (D–F) *CSAL2* is expressed throughout the neural tube and brain from HH11. (G) Transverse section through the neural plate of a HH8 embryo. Level of section indicated by a horizontal line in (B). (H) Transverse section through the forebrain of a HH10 embryo. White arrows, Hensen's node; black arrows, primitive streak; fb, forebrain; hb, hindbrain; ht, heart; mb, midbrain; np, neural plate; nt, neural tube; op, optic vesicle; so, somite; vz, ventricular zone.

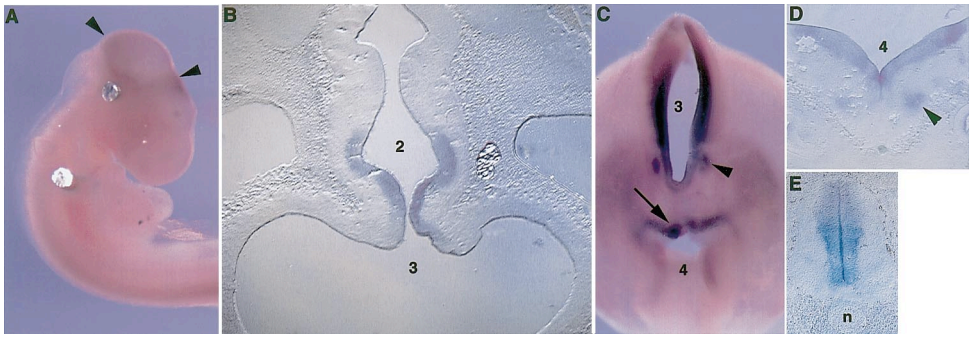

Fig. 3. Expression of *CSAL2* in the brain. (A) Whole-mount in situ hybridization of a HH17 embryo, showing expression in the spinal cord and at the mid-hindbrain and mid-forebrain boundaries (arrowheads). (B) Frontal section through the brain of an HH26 embryo, showing expression in the ventricular zone between the 2nd and 3rd ventricle (the mid-forebrain boundary). (C) Transverse razor section along the floor of the 3rd ventricle, showing expression at the base of the optic stalk (arrow head) and at the isthmus (arrow). (D) Transverse section through the hindbrain of a HH26 embryo, showing expression in the ventricular zone (arrow) and in two domains symmetrically around the midline (arrow head). (E) Transverse section (HH26) demonstrates that expression in the neural tube is located in the ventricular zone; n, notochord.

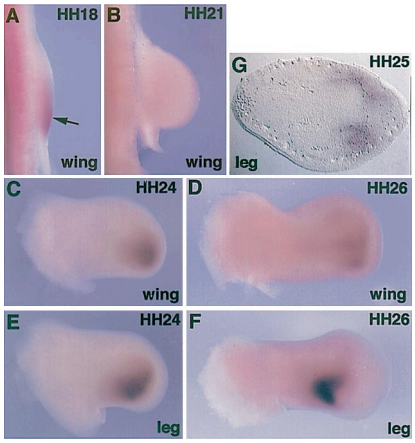

Fig. 4. Expression of *CSAL2* during limb development. (A) Whole-mount in situ hybridization of a HH18 embryo, showing expression in the posterior mesenchyme of the emerging wing bud (arrow). (B) A wing bud at HH21 shows no expression of *CSAL2*. (C) Expression of *CSAL2* in the wingbud at HH24 (D) and HH26. (E) Expression of *CSAL2* in the legbud of HH24 (F) and HH26. (G) Transverse section of HH26 legbud; only the posterior half is shown. Expression of *CSAL2* is predominantly in the mesenchyme.

This fragment was used to screen a chick cDNA library as described in Church and Gilbert (1984). The complete sequence of *CSAL2* has been deposited with the Genbank database under accession number AF304358.

Whole-mount in situ hybridization was performed as described by Henrique et al. (1997) with the following modifications: for stages older than HH24, the concentration of Proteinase K was doubled and wash time was increased. Sectioning, microscopy and photography were as previously described (Farrell and Münsterberg, 2000), except embryos were sectioned at 30 μm. For stages HH1–HH5 a probe detecting chick Delta (kindly provided by Kate Storey) was used as a positive control, for all other stages chick Shh (Sonic hedgehog) was used.

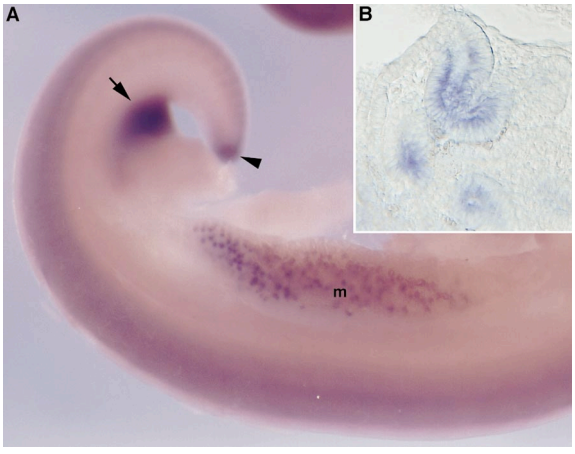

Fig. 5. Expression of *CSAL2* in the mesonephros, tailbud and cloaca. (A) Whole-mount in situ hybridization of a HH26 embryo, lateral view. The limb buds have been removed to reveal *CSAL2* expression in the cloaca (arrow), tail bud (arrowhead), mesonephros (m) and neural tube. (B) Transverse section through the mesonephros of a HH26 embryo, showing expression in epithelial cells of the nephric ducts.

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