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Developmental Expression Pattern

Expression of *csal1* in pre limb-bud chick embryos

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ABSTRACT The spalt family of transcriptional repressors has been implicated in limb, heart, ear and kidney development and truncating mutations in a human gene, *SALL1,* result in the autosomal dominant disorder Townes-Brocks syndrome. Here we show the expression pattern of the chick orthologue of the *SALL1* gene, *csal1,* during early development. We found *csal1* expression in the heart and in the pharynx, a source of inductive signals during heart development. Expression was also seen in involuting mesoderm and later in presegmented paraxial mesoderm. We also describe expression in the ectoderm and neural plate of the early embryo and subsequent expression in the neural tube.

KEY WORDS: *chick, csal1, Townes-Brocks syndrome, spalt, heart*

The spalt zinc finger proteins, some of which have been shown to act as transcriptional repressors, have been implicated in various patterning events during development. A number of orthologues have been identified in *Drosophila* (Barrio *et al*., 1996), human (Kohlhase *et al.*, 1996, Kohlhase *et al.*, 1999a, Kohlhase *et al.*, 2002), mouse (Ott *et al.*, 1996, Buck *et al.*, 2000, Kohlhase *et al.*, 2000, Kohlhase *et al.*, 2002), *Xenopus* (Hollemann *et al*., 1996, Onuma *et al.*, 1999, Onai *et al.*, 2004), zebrafish (Camp *et al*., 2003), chick (Capdevila *et al.*, 1999, Farrell and Munsterberg, 2000, Farrell *et al.*, 2001) and *Medaka* (Koster *et al*., 1997). In *Drosophila* the related genes *sal* and *salr* are involved in the determination of the embryonic termini, wing patterning and tracheal branching (Kuhnlein *et al*., 1994, Kuhnlein and Schuh, 1996, de Celis and Barrio, 2000, Barrio and de Celis, 2004). In human the autosomal dominant condition Townes-Brocks syndrome (TBS) is caused by mutations in *SALL1* and patients present with defects in kidney, ear, anogenital, heart and limb development (Kohlhase *et al*., 1998). Most of the phenotypic features in TBS patients are variable, including heart defects which have been reported in some familial and spontaneous cases of TBS (Surka *et al*., 2001). The mutations resulting in TBS are premature stop codons predicted to lead to the production of a truncated SALL1 protein (Kohlhase *et al*., 1999b). Recent work has strongly suggested that such a truncated protein can act as a dominant negative allele interfering with the function of full length SALL1 and possibly other spalt proteins (Netzer *et al.*, 2001, McLeskey Kiefer *et al*., 2002, Sweetman *et al.*, 2003). This view has been confirmed by the production of mice expressing a truncated Sall1 protein. Malformations in mice expressing this truncated protein are similar to those seen in TBS patients (McLeskey Kiefer *et al.*, 2003).

In the chick three members of the spalt family have been described so far, *csal1*, *csal3* and *csal4*. We have previously characterized the expression of *csal1* at later developmental stages where transcripts were detected in the CNS, tail bud and developing limb buds. Furthermore, we demonstrated that csal1 expression is regulated by members of the FGF and Wnt families of proteins during limb development (Farrell and Munsterberg, 2000). *csal3* is expressed in the nervous system, developing kidney, cloaca and limb bud (Farrell *et al*., 2001) and *csal4* is expressed in the neural tube, migrating neural crest and branchial arches (Barembaum and Bronner-Fraser, 2004).

In order to address the potential role of *csal1* in early development we have undertaken a detailed expression analysis in chick embryos from pre-streak to Hamburger-Hamilton (HH, Hamburger and Hamilton, 1951) stage 16. Transcripts were found in ectoderm, involuting mesoderm and presegmented mesoderm. We also observed *csal1* expression in the heart, the neural plate and the pharynx.

csal1 *expression during gastrulation and neurulation*

Transcripts of *csal1* were first detected at HH stage 3 embryos with expression restricted to Hensen's node and the primitive streak (Fig. 1A). At HH stage 5 expression was seen in the ectoderm of the head fold, the head process and in the involuting mesoderm in the primitive groove (Fig. 1B - E). At HH stage 6 *csal1* was expressed in the head fold, neural and non-neural

Abbreviations used in this paper: TBS, Townes-Brocks syndrome.

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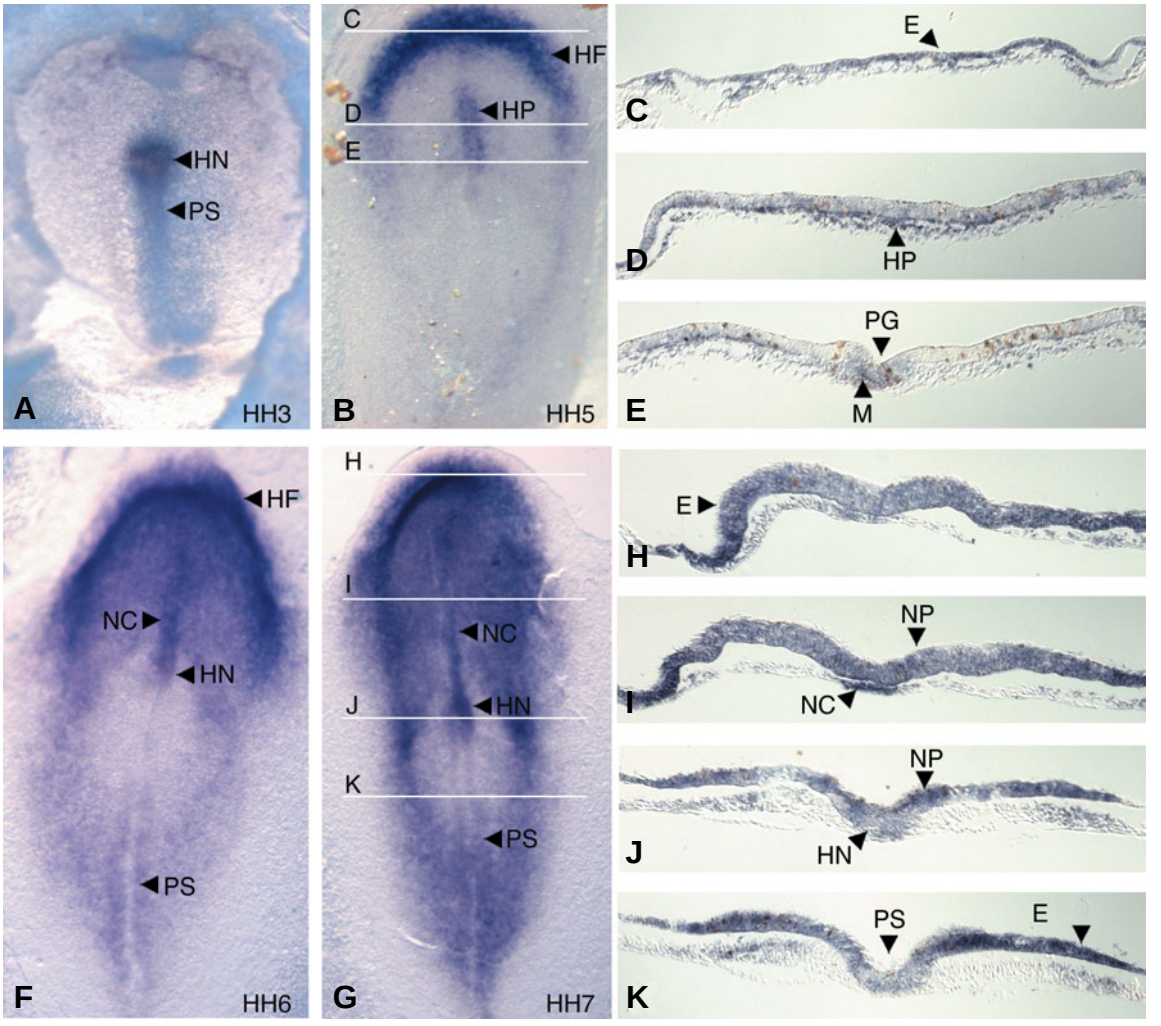

Fig. 1. *In situ* hybridisation of *csal1* in early gastrula embryos. (A) *Wholemount staining of HH st 3 embryo.* **(B)** *Wholemount staining of HH st 5 embryo.* **(C,D,E)** *tranverse sections of HH st 5 embryo, levels indicated in* **(B)**. **(F)** *Wholemount staining of HH st 6 embryo.* **(G)** *Wholemount staining of HH st 7 embryo.* **(H,I,J,K)** *transverse sections of HH st 7 embryo, levels indicated in* **(G)**. *E, ectoderm; HF, head fold; HN, Hensen's node; HP, head process; M, mesoderm; NC, notochord; NP, neural plate; PG, primitive groove; PS, primitive streak.*

ectoderm, the notochord, Hensen's node and posterior primitive streak (Fig. 1F). By HH stage 7 expression was still present in the notochord and node while the expression in the ectoderm has expanded throughout the embryo with the exception of the ectoderm posterior to the node (Fig. 1 G - K).

Expression during somitogenesis stages and heart morphogenesis

At HH stage 10 in anterior regions *csal1* was expressed in the head and stomatodaeum (Fig. 2A, B). At the level of the heart tube expression was seen in the ectoderm, paraxial mesoderm and dorsal mesocardium (Fig. 2A, C). Posterior to the heart tube *csal1* was expressed in lateral endoderm (Fig. 2A, D). Just posterior to the level of neural tube fusion *csal1* was observed in the dorsal neural folds and notochord (Fig. 2A, E) while in more posterior regions expression was seen throughout the neural folds and the presegmented mesoderm (Fig. 2A, F). At HH stage 10 csal1 transcripts were still observed in the node, primitive streak and involuting mesoderm (Fig. 2A, G). Strong expression was observed in the sinus venosus at HH stage 12. At the same axial level expression was seen in the overlying ectoderm, somatic mesoderm and the keel of the pharynx (Fig. 3A, B). At HH stage 14 expression was still present in the sinus venosus and maintained in the pharynx (Fig. 3C, D). By HH stage 16 *csal1* was only expressed in the presegmented mesoderm and the underlying endoderm. The region of the presomitic mesoderm which will give rise to the next formed somite does not express *csal1* (Fig. 3E, F). In other vertebrate species where the expression of orthologues of *csal1* has been reported expression has been observed in both the developing kidney and the otic vesicle (Buck *et al.*, 2000, Buck *et al.*, 2001, Ott *et al.*, 2001). However in the chicken embryo *csal1* is not expressed in either of these tissues. Given the other similarities in expression in the limb bud, ectoderm, heart and neural plate this may imply some divergence in function between species. A paralogue of *csal1*, *csal3*, is expressed in the mesonephros and CNS and may functionally substitute for *csal1* in these areas

(Farrell *et al.*, 2001). Diversification of *csal1* orthologues has also been reported in zebrafish where gene duplication has produced two similar *sal1* alleles (Camp *et al.*, 2003). However, it is clear that many of the tissues affected in TBS patients are those where *csal1* is expressed and studies in chick embryos would complement those in other model systems to help understand the function of these important genes in normal development and disease. For example a potential role of the *spalt* genes in heart development has not yet been elucidated. Heart defects have been reported in some TBS patients (Kohlhase *et al.*, 1999b, Kohlhase *et al.*, 2003) and expression of *spalt* genes has been detected in the hearts of both mouse (Ott *et al.*, 1996, Buck *et al.*, 2001, Ott *et al.*, 2001) and zebrafish embryos (Camp *et al.*, 2003). The expression within the developing heart in the chick suggests a possible role for *csal1* in cardiac morphogenesis. In addition expression of *csal1* in the pharynx raises the possibility that expression of *csal1* may be required for the mutual signalling events that occur between this tissue, cardiac neural crest and myocardium and this could be tested in a vertebrate model system.

Materials and Methods

Whole mount *in situ* hybridisations, sections and photography were performed as described (Schmidt *et al.*, 2000). Probes used were as described in (Farrell and Munsterberg, 2000).

The EMBL/GenBank accession number of *csal1* is: AF_288697.

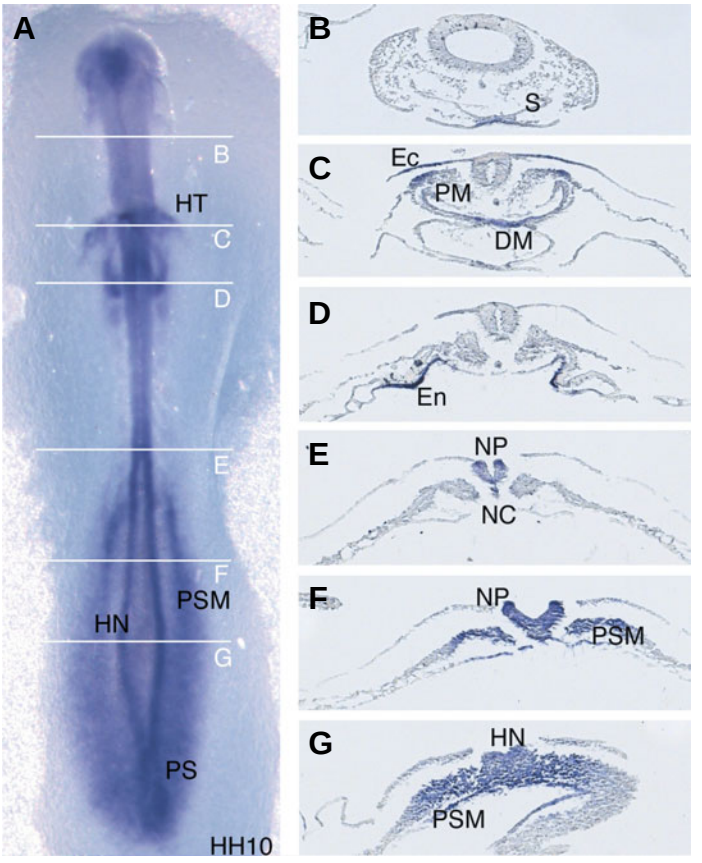

Fig. 2. ***In situ*** **hybridisation of** ***csal1*** **in HH st 10 embryo. (A)** *Wholemount ventral view of HH st 10 embryo,* **(B,C,D,E,F,G)** *transverse sections of HH st 10 embryo, levels indicated in* **(A)**. *DM, dorsal mesocardium; Ec, ectoderm; En, endoderm; HN, Hensen's node; HT, heart tube; NC, notochord; PM, paraxial mesoderm; PS, primitive streak; PSM, presegmented mesoderm; S, stomatodaeum.*

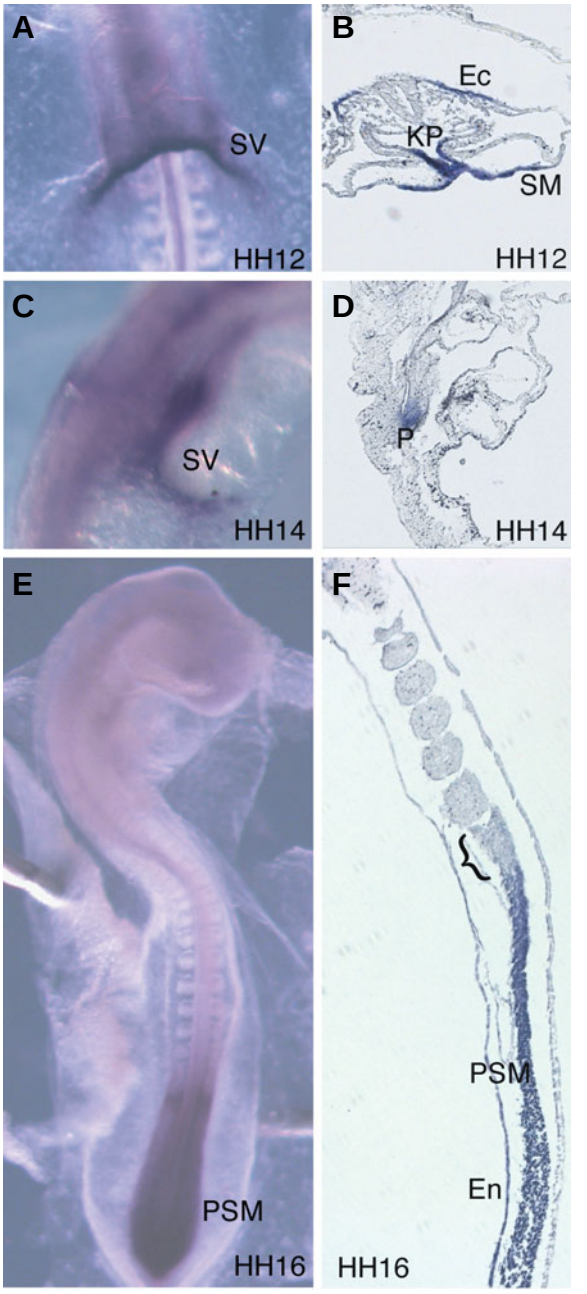

Fig. 3. ***In situ*** **hybridisation of** ***csal1*** **in HH st 12 to 16 embryos. (A)** *Wholemount ventral view of HH st 12 heart tube.* **(B)** *Transverse section of HH st 12 embryo at heart level.* **(C)** *Lateral view of HH st 14 embryo at heart level.* **(D)** *Sagittal section of HH st 14 embryo at heart level.* **(E)** *Wholemount staining of HH at 16 embryo.* **(F)** *Sagittal section of HH st 16 embryo at the boundary of the somites / presegmented mesoderm. Ec, ectoderm; En, endoderm; KP, keel of the pharynx; P, pharynx; PSM, presegmented mesoderm; SM, somatic mesoderm; SV, sinus venosus. The "{" symbol indicates region of next formed somite.*

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