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Expression of the chick Sizzled gene in progenitors of the cardiac outflow tract

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ABSTRACT

The secreted Frizzled receptor related proteins (Sfrp's) belong to a protein family that comprises antagonists and modifiers of Wnt and BMP signalling. Here we report the isolation and expression pattern of the Sfrp gene "Sizzled" in the chick. Sizzled genes, as well as the closely related crescent genes, exist in the genomes of fishes, frogs and chicks, but not of mammals. The chicken Sizzled gene (Szl) is initially expressed in the anterior endoderm of gastrulating and early head fold embryos. An additional, separate expression domain develops at the posterior end of the embryo from the head process stage onwards. Szl transcripts are then detected in precardial mesodermal cells, are transiently transcribed in the straight heart tube, and later prominently in the splanchnic mesoderm surrounding the arterial pole of the developing heart, the anterior heart field. These cells are subsequently recruited to form the cardiac outflow tract. cSzl expression is downregulated when the septation of the outflow tract by neural crest derived cells begins.

1. Results and discussion

Sizzled genes belong to the family of secreted Frizzled related proteins (Sfrp's), which were found in deuterostomes, but not protostomes (Rattner et al., 1997; Tendeng and Houart, 2006). In contrast to initial assumptions, sizzled proteins of frogs and fishes turned out to antagonize BMP rather than Wnt signalling (Martyn and Schulte-Merker, 2003; Yabe et al., 2003; Miller-Bertoglio et al., 1999; Wagner and Mullins, 2002; Collavin and Kirschner, 2003; Lee et al., 2006). The chicken orthologue of Sizzled (Szl) was identified by a whole-mount-in-situ-hybridisation (WMISH) screen of a cDNA library constructed from HH5 prechordal plate/anterior endoderm tissue. The chicken Szl clone coded for a protein of 287 amino acids, which showed 52% amino acid identity with Xenopus Sizzled and 45% amino acid identity with Sizzled from zebrafish. A comparison of the conserved cysteine rich domains showed an amino acid identity of 78% between chick Sizzled and Xenopus Sizzled and 60% identity with zebrafish Sizzled, respectively (Fig. 1A). Together with the Wnt antagonist Crescent, the Sizzled genes form a subgroup of the Sfrp's for which until now no mammalian member has been found (Fig. 1B). Comparison of genomic loci revealed that a Szl gene is located in a synthenic region between MAPK12 and TRABD in chick and Xenopus, strengthening the conclusion that the chicken Szl is a true orthologue of the xenopus Szl gene. In contrast, Szl related sequences are missing in mammalian genomes, although the syntenic region seems to be conserved in humans and mice. The expression pattern of the chicken Szl orthologue was analysed by WMISH analysis on embryos from prestreak stages (Hamburger–Hamilton stage 1, HH1) until HH25 (4 days of incubation).

Before initiation of the primitive streak, Szl transcripts were detected in the extraembryonic hypoblast (Fig. 2A). With the formation of the primitive streak, the Szl expressing cells were detected in the anterior hypoblast (Fig. 2B). In parallel, a second region of Szl expression in the mesoderm and endoderm flanking the posterior primitive streak became evident (Fig. 2C and D). During further development this posterior domain persisted at the caudal end of the primitive streak, and became well defined at the tip of the tail bud and the fringe distal to the future hind limb buds (Fig. 2E, I–K). The anterior endodermal domain became stronger and more focused with the formation of the head process during stages HH4 and HH5 (Fig. 2C and F) and was sustained up to the head fold stage (HH6, Fig. 2G). Additionally, low transcript levels of were detected in two, mesodermal cell populations lateral to the rostral mesoderm (Fig. 2D, arrows). At the four somite stage (HH8), when the bilateral heart fields merge at the midline, Szl was found in the mesoderm and endoderm at the anterior intestinal portal (aip; Fig. 2E and H). After establishment of the straight heart tube, Szl

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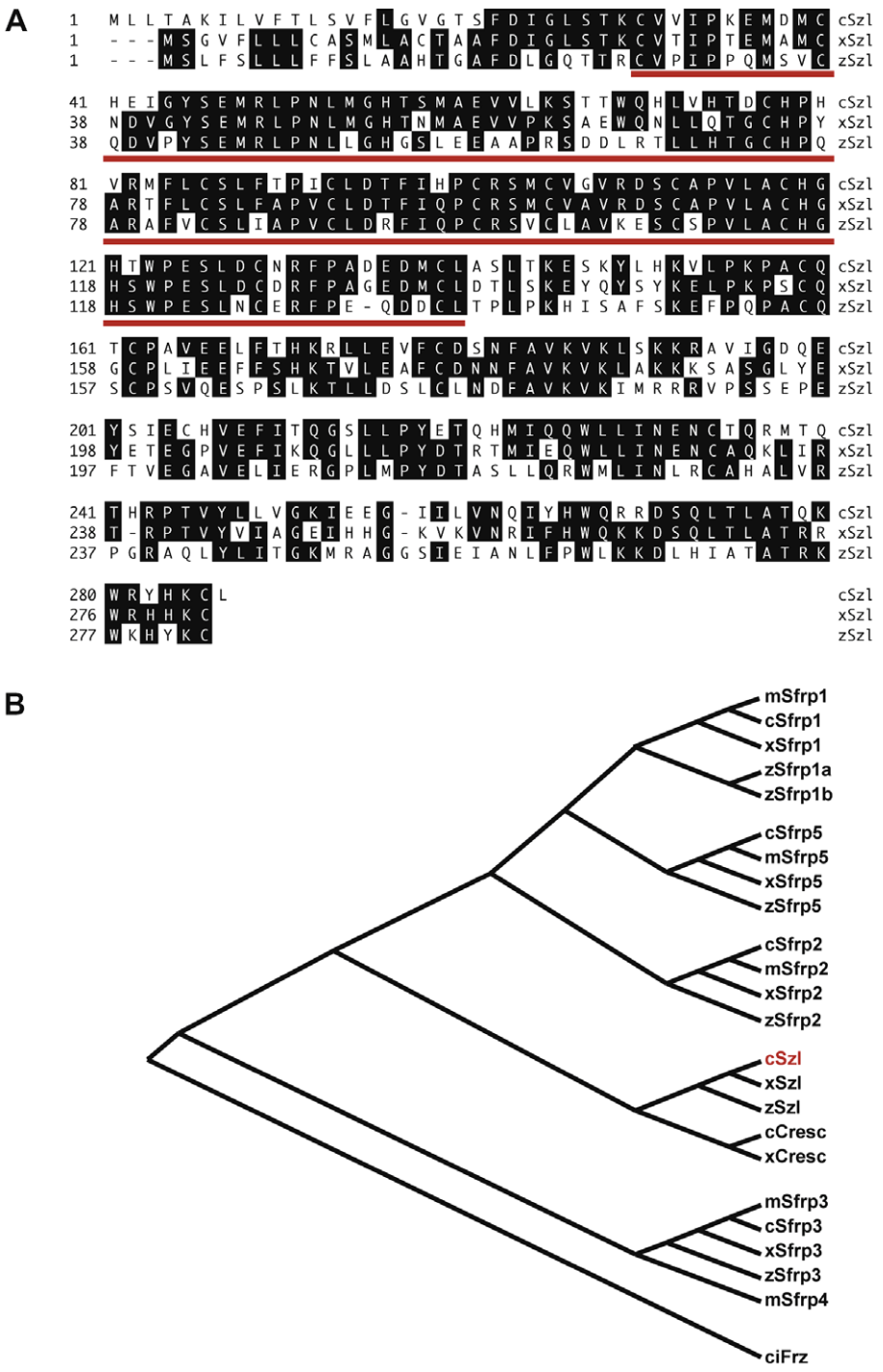

Fig. 1. (A) Comparison of Sizzled proteins from chick (cSzl), Xenopus (xSzl) and zebrafish (zSzl). The cysteine rich, frizzled-like domain is underlined in red. Black boxes indicate identical amino acids. (B) A phylogenetic tree of the known secreted Frizzled related proteins (Sfrp's) of chicken (c), mouse (m), *Xenopus laevis* (x) and zebrafish (z), rooted against frizzled of ciona intestinalis (ciFrz).

transcripts were detected in the cardiogenic area, the splanchnic mesoderm flanking the aip and in the mesoderm at the posterior margin of the subcephalic pocket (Fig. 2I). This expression domain became restricted to the arterial pole of the heart, forming a ring in the splanchnic mesoderm surrounding the emerging outflow tract (Fig. 2J, K and M). Sections of HH14 and HH17 embryos showed Szl transcripts in the base of the pharyngeal endoderm, and mainly in the mesoderm at the transition between splanchnic mesoderm and the outflow tract myocardium (Fig. 2L, N and O). The caudal wall of the outflow tract at HH17 remained free of Szl transcripts (Fig. 2O). At HH21 the area of Szl expression resembled a caudally open horseshoe (Fig. 2P). As such, it corresponded closely to the "anterior heart field" as defined by Mjaatvedt et al. (2001). The recognition of outflow tract progenitor cells, or an additional heart field, outside of the straight heart tube is based on fate mapping studies (Abu-Issa et al., 2004; de la Cruz et al., 1977; Kelly and Buckingham, 2002; Mjaatvedt et al., 2001; Waldo et al., 2001) . These studies identified mesodermal cells migrating towards the arterial pole of the heart tube during cardiac looping (HH9–HH22), collecting around the aortic sac and subsequently forming the myocard of