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Duplicate *sfrp1* genes in zebrafish: *sfrp1a* is dynamically expressed in the developing central nervous system, gut and lateral line

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Abstract

The secreted frizzled-related proteins (Sfrp) are a family of soluble proteins with diverse biological functions having the capacity to bind Wnt ligands, to modulate Wnt signalling, and to signal directly via the Wnt receptor, Frizzled. In an enhancer trap screen for embryonic expression in zebrafish we identified an *sfrp1* gene. Previous studies suggest an important role for *sfrp1* in eye development, however, no data have been reported using the zebrafish model. In this paper, we describe duplicate *sfrp1* genes in zebrafish and present a detailed analysis of the expression profile of both genes. Whole mount in situ hybridisation analyses of *sfrp1a* during embryonic and larval development revealed a dynamic expression profile, including: the central nervous system, where *sfrp1a* was regionally expressed throughout the brain and developing eye; the posterior gut, from the time of endodermal cell condensation; the lateral line, where *sfrp1a* was expressed in the migrating primordia and interneuromast cells that give rise to the sensory organs. Other sites included the blastoderm, segmenting mesoderm, olfactory placode, developing ear, pronephros and fin-bud. We have also analysed *sfrp1b* expression during embryonic development. Surprisingly this gene exhibited a divergent expression profile being limited to the yolk syncytium under the elongating tail-bud, which later covered the distal yolk extension, and transiently in the tail-bud mesenchyme. Overall, our studies provide a basis for future analyses of these developmentally important factors using the zebrafish model.

Keywords: Blastoderm; CNS; *Danio rerio*; Ear; Endoderm; Enhancer trap; Eye; Fin-bud; Frizzled; FrzA; Gastrula; Gut; Hindbrain; Interneuromast; Lateral line; Midbrain; Neuroectoderm; Neuromast; Paraxial mesoderm; Presomitic; Primordium; Pronephric duct; Pronephros; Secreted frizzled-related protein; Sfrp; Somite; Tegmentum; Telencephalon; Wnt; Yolk extension; Yolk syncytial layer; Zebrafish

1. Results and discussion

The secreted frizzled-related proteins (Sfrp) are a family of soluble proteins found in vertebrates that possess a cystein-rich domain (CRD), with homology to the Wnt receptor Frizzled, and a netrin (NTR) domain (Rattner et al., 1997; Banyai and Patthy, 1999). Sfrps bind Wnt ligands and consequently can modulate the multifaceted Wnt signalling network which is involved in axis specification, gastrulation, embryonic patterning, organogenesis and neural connectivity (reviewed in Jones and Jomary, 2002; Ciani and Salinas, 2005). More recently, Sfrps have been shown to signal independent of Wnt ligands through the Frizzled receptor (Rodriguez et al., 2005). Based on amino acid sequence similarity, Sfrp1, 2 and 5 form a subgroup of Sfrps (Yamaguchi, 2001). *sfrp1* has been identified in several mammalian species, chick, *Xenopus*, medaka and zebrafish (reviewed in Jones and Jomary, 2002; Esteve

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et al., 2004) and has an important role in eye development including the formation of the eye field and retinal ganglion cell differentiation and axon growth (Esteve et al., 2000, 2003; Rodriguez et al., 2005). Additional roles for *sfrp1* are suggested by its expression profile that has been described in mouse (Leimeister et al., 1998), chick (Esteve et al., 2000; Terry et al., 2000) and *Xenopus* (Xu et al., 1998), however, no data have been reported concerning *sfrp1* expression in zebrafish.

1.1. Enhancer trapping and identification of duplicate sfrp1 genes in zebrafish

From a bank of enhancer trap founders, generated by the technique of Ellingsen et al. (2005), the line CLGY713 was isolated. Fluorescence imaging of live embryos revealed YFP expression in the optic vesicle beginning at the 15 somite stage (ss) (Fig. 1A). At earlier stages YFP expression was detected throughout the embryo (data not

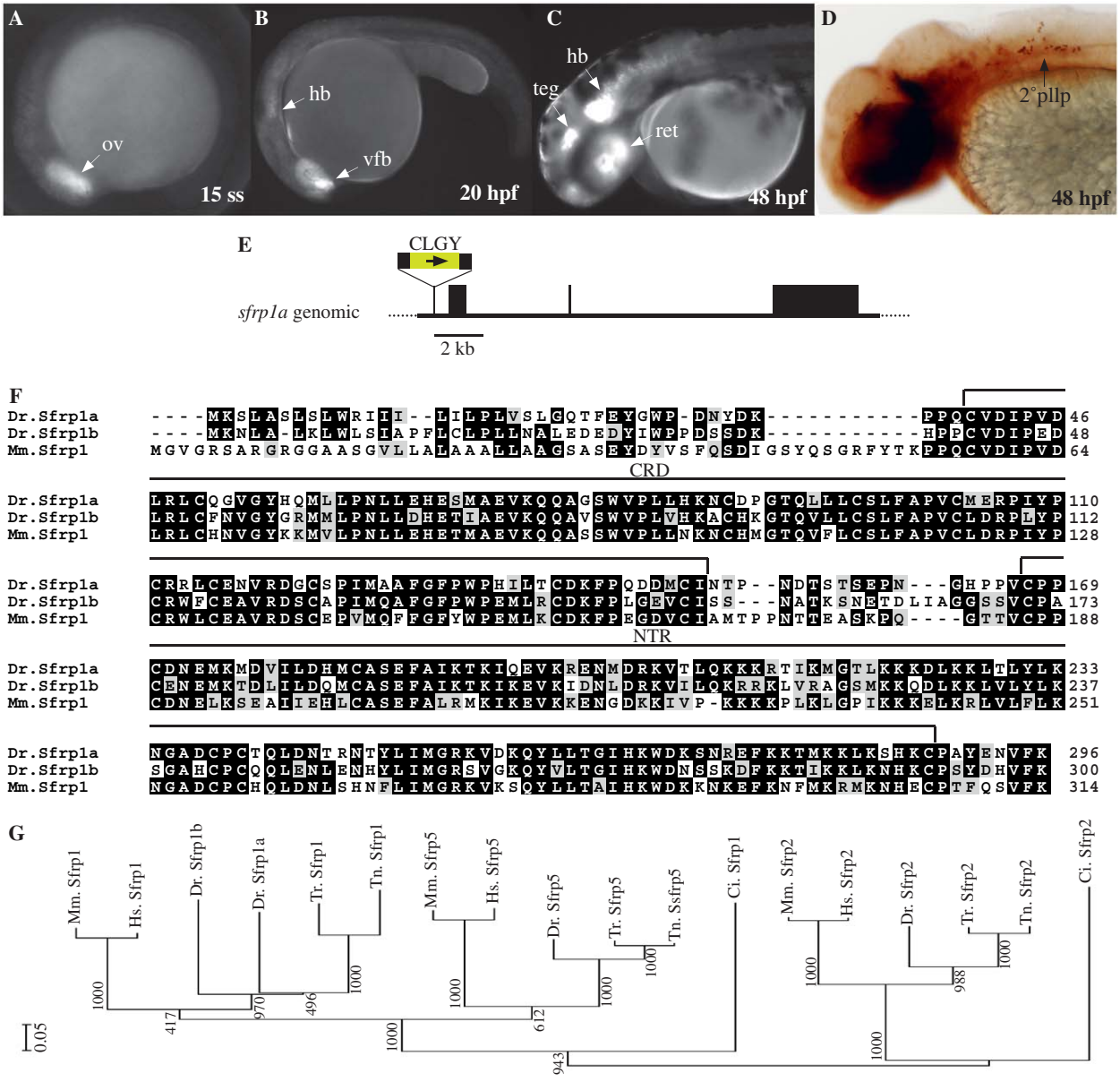

Fig. 1. Analysis of the enhancer trap line CLGY713 and identification of duplicate *sfrp1* genes. Whole mount fluorescent imaging of live embryos (A–C) or YFP-immunostaining (D) of line CLGY713 was performed at the indicated stages. Organisation of the *sfrp1a* genomic locus (formerly *sfrp1*) and CLGY virus integration site (E). Amino acid sequence alignment of Sfrp1 proteins from zebrafish and mouse (F). The single letter code for amino acids is used. Amino acid numbering is indicated on the right. Identical and similar residues are indicated in black and grey, respectively. The conserved N-terminal cystein-rich domain (CRD) and C-terminal netrin (NTR) homology are shown. Phylogenetic tree of the full-length Sfrp1, Sfrp2 and Sfrp5 proteins (G). The degree of relatedness is indicated by the length of the vertical lines. Numbers indicate bootstrap support for the nodes. Ci, *Ciona intestinalis*; Dr, *Danio rerio*; hb, hindbrain; Hs, *Homo sapiens*; hpf, hours post-fertilisation; Mm, *Mus musculus*; ov, optic vesicle; ret, retina; ss, somite stage; teg, tegmentum; Tn, *Tetraodon nigroviridis*; Tr, *Takifugu rubripes*; vfb, ventral forebrain; 2°pllp, secondary posterior lateral line primordium.