

Cloning and embryonic expression of five distinct *sfrp* genes in the zebrafish *Danio rerio*

Christian Tendeng, Corinne Houart *

MRC Centre for Developmental Neurobiology, New Hunt's House, King's College London, London SE1 9RT, United Kingdom

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Abstract

Recently, a new member of the secreted frizzled-related protein (sFRP) family, named *tlc*, has been identified as expressed by the anterior neural border (ANB) cells in the zebrafish *Danio rerio*. *Tlc* plays an important role in telencephalic induction and patterning. In absence of *Tlc*, formation of the telencephalon is severely delayed, but not abolished. This prompted us to clone the other zebrafish *sfrp* family members and analyse their expression patterns, in search of a family member that may partly functionally overlap with *Tlc*. Except *sizzled*, expression profile of *sfrp* genes in zebrafish has not been reported so far. Here, we describe the cloning of full-length cDNA for *sfrp1a*, *sfrp1b*, *sfrp2*, *sfrp3* and *sfrp5* gene transcripts and we examine their expression at different embryonic stages. Only *sfrp1a* is expressed in the anterior neural plate including the ANB cells where and when *tlc* is expressed. Interestingly, compared to both *tlc* and *sfrp1a*, *wnt* genes are complementary expressed more posteriorly in the neural plate. Later, both *sfrp1a* and *sfrp5* expression profiles are overlapping, in particular at pharyngula stage these genes are expressed in the ventral part of the forebrain, midbrain and hindbrain. *sfrp1b*, *sfrp2* and *sfrp3* are mainly expressed in mesodermal and endodermal embryonic tissues. Expression profiles of these different genes in zebrafish gave interesting clues on the possible function and evolution of sFRPs in zebrafish and other organisms.

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Keywords: Secreted frizzled-related protein; sFRP; *Tlc*; *Sizzled*; Wnt antagonist; Anterior neural plate; Telencephalon; Eyes; Hypothalamus; Midbrain; Tegmentum; Tectum; Hindbrain; Spinal cord; Otic vesicles; Lateral line; Somite; Adaxial cells; Gut; Fins

The first identified secreted frizzled-related protein, Frzb-1 now called sFRP3, has been independently identified from the bovine cartilage extracts and the Spemann's organizer cells from *Xenopus laevis* (Hoang et al., 1996; Leyns et al., 1997; Wang et al., 1997). sFRP3 has been described as involved in antagonizing the Wnt-signalling pathway (Leyns et al., 1997; Wang et al., 1997). Following the identification of sFRP3, many other sFRP family members have been characterized in *X. laevis* as well as in other organisms such as *Mus musculus* (mouse), *Homo sapiens* (human) and *Gallus gallus* (chick), reviewed in Jones and Jomary (2002) and Kawano and Kypta (2003). Although sFRPs have mostly been proposed as negative modulators

of the Wnt/ β -catenin pathway (Jones and Jomary, 2002; Kawano and Kypta, 2003), an agonist effect has also been described for sFRP1, depending upon its concentration (Uren et al., 2000). In addition, sFRP1 has been recently described as involved in axonal guidance by direct binding to a Frizzled receptor (Rodriguez et al., 2005). Except for the latter example and sFRP3, the role of most sFRPs and the molecular mechanisms by which these proteins act are poorly understood.

In the zebrafish *Danio rerio*, two sFRP members have been characterized so far. *sizzled* has previously been described as expressed in the ventral side of the zebrafish embryo (Martyn and Schulte-Merker, 2003; Yabe et al., 2003). Interestingly, zebrafish *Sizzled* did not inhibit Wnt8-dependent transcription and acts as a negative regulator of Bmp signalling (Martyn and Schulte-Merker, 2003; Yabe et al., 2003). On the other hand, a new

* Corresponding author. Tel.: +44 0 207 848 6409; fax: +44 0 207 848 6798.

E-mail address: corinne.houart@kcl.ac.uk (C. Houart).

member of sFRP family, named *tlc*, has been identified as expressed by the anterior neural border (ANB) cells in zebrafish (Houart et al., 2002). Indeed, cells located at the margin of the anterior neural plate play a crucial role in telencephalic induction and patterning (Houart et al., 1998; Shimamura and Rubenstein, 1997). In that process, Tlc has been identified as one of the essential players secreted by the ANB cells (Houart et al., 2002). Tlc is acting as a Wnt/ β -catenin antagonist secreted from the anterior margin of the neural plate. Expression of both Wnt antagonist in the ANB and Wnt agonists in the prospective midbrain may establish an antero-posterior positional information initiating the subdivision of the anterior neural plate into discrete territories (Houart et al., 2002). However, if, in absence of Tlc, formation of the telencephalon is severely delayed, it is not completely abolished (Houart et al., 2002). This prompted us to clone the other zebrafish *sfrp* family members and analyse their expression pattern, in search of a family member that may partly functionally overlap with Tlc. Here, we describe the cloning of full-length cDNA for five *sfrp*

gene transcripts and we examine their expression at different embryonic stages.

1. Results and discussion

1.1. Phylogenic distribution of sFRPs

To date, eight distinct sFRPs have been identified, mostly in vertebrates (i.e., amphibians, birds, fishes and mammals), but also in the urochordate *Ciona intestinalis* (Dehal et al., 2002). One sFRP has been characterized in the sea urchin *Strongylocentrotus purpuratus* (Illies et al., 2002). Finally, no sFRP has been identified so far in protostome organisms, suggesting that sFRP arose after the separation between protostome and deuterostome lineages. In silico analyses of amino-acid sequences suggest existence of four subgroups: sFRP1/sFRP5, sFRP2, sFRP3/sFRP4 and Sizzled/Crescent (Fig. 1). Interestingly, the phylogenetic position of Tlc suggests that this protein, although closest to the sFRP1/5 subgroup, is possibly belonging to a separate subgroup (Fig. 1).

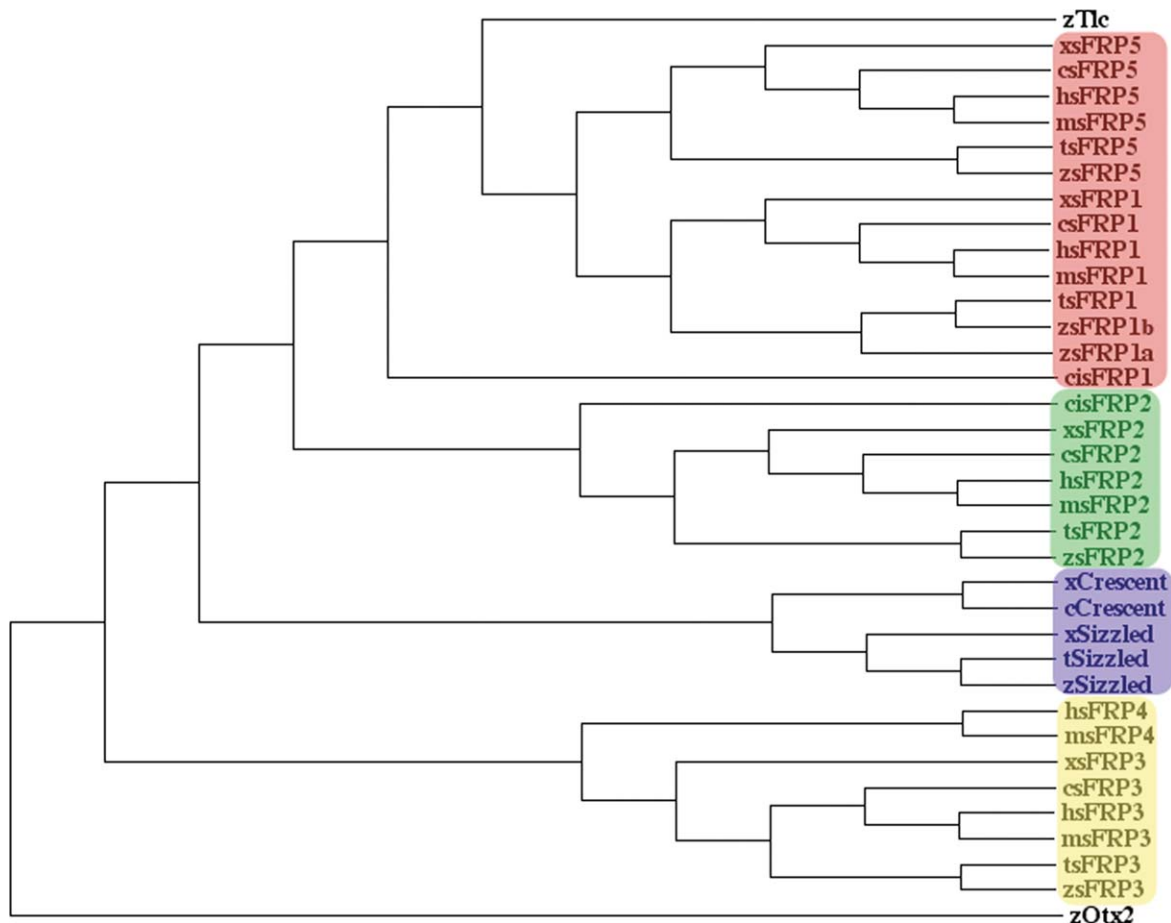


Fig. 1. Phylogenic distribution of sFRPs. From an alignment of sFRPs performed with ClustalW (Thompson et al., 1994), a consensus phylogenetic tree was constructed using the Maximum Parsimony method included in the software Phylowin (Galtier et al., 1996). In terms of amino-acid sequence, sFRP members could be subdivided in four subgroups: sFRP1/5 (red box), sFRP2 (green box), Crescent/Sizzled (blue box) and sFRP3/4 (yellow box). Interestingly, Tlc has not been found clustered in one of these subgroups on the basis of sequence similarity. The zebrafish protein Otx2 is used as an outgroup sequence. Species abbreviations: c, chick (*G. gallus*); ci, *C. intestinalis*; h, human (*H. sapiens*); m, mouse (*M. musculus*); t, pufferfish (*T. nigroviridis*); x, xenopus (*X. laevis*); z, zebrafish (*D. rerio*).

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