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Gene expression pattern

Differential expression of two somatostatin genes during zebrafish embryonic development

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Abstract

We have identified the cDNAs of two new zebrafish preprosomatostatins, *PPSS1* and *PPSS3*, in addition to the previously cloned *PPSS2* (Argenton et al., 1999). *PPSS1* is the orthologue of mammalian *PPSSs*, with a conserved C-terminal SS-14 sequence, *PPSS2* is a divergent SS precursor and *PPSS3* is a cortistatin-like prohormone. Using whole-mount in situ hybridisation, we have analysed the expression of *PPSS1* and *PPSS2* in zebrafish embryos up to 5 days post fertilisation. *PPSS1* was expressed in the developing pancreas and central nervous system (CNS), whereas *PPSS2* expression was exclusively pancreatic. In the CNS, *PPSS1* was detected in several areas, in particular in the vagal motor nucleus and in cells that pioneer the tract of the postoptic commissure. *PPSS1* was also expressed transiently in the telencephalon and spinal motor neurons. In all areas but the telencephalon *PPSS1* was coexpressed with *islet-1*.

Keywords: Somatostatin; Pancreas; Central nervous system; Zebrafish; Development; In situ hybridisation; Masterblind; *islet-1*

1. Results and discussion

Somatostatin (SS), synthesised as preprosomatostatin (PPSS), undergoes a tissue-specific proteolytic cleavage generating two bioactive peptides (SS-14 and SS-28), corresponding to its 14- and 28-aminoacid C-termini, respectively. SS is produced throughout the central and peripheral nervous systems, where it affects cognitive, locomotor, sensory and autonomic functions, and in some peripheral organs, where it inhibits cell proliferation and secretion of a wide range of endocrine and exocrine cells (Patel, 1999; Zeyda et al., 2001). SS is also involved in developmental processes as evidenced by the early onset of *PPSS* transcription and transient expression in mouse embryos (Bendotti et al., 1990) and its capacity to affect neuronal migration in the developing rodent nervous system (Yacubova and Komuro, 2002).

We previously cloned a zebrafish cDNA encoding a SS precursor, hereafter called *PPSS2* (Argenton et al., 1999). Herein, we report the sequences of two additional zebrafish SS-like precursors (Fig. 1A), designated *PPSS1* and *PPSS3*. Several vertebrate genes encoding SS-like precursors have been identified and grouped in four clades (Fig. 1B; Lin et al., 2000). *PPSS1* belongs to the first group, comprising the genes encoding the precursor of a strictly conserved SS-14, while *PPSS2* is grouped with catfish *PPSS2* (Fig. 1B). *PPSS3* (Fig. 1A), belongs to the third group together with the preprocortistatin genes. This mammalian peptide precursor gives rise to cleavage product comparable to SS-14 and SS-28 and seems to play a role in neuronal depression and sleep modulation (Spier and de Lecea, 2000).

Here we have analysed the expression of *PPSS1* and *PPSS2* in zebrafish embryos at various stages up to 5 days post fertilisation (dpf) by whole-mount in situ hybridisation.

At all investigated stages, *PPSS2* expression was exclusively pancreatic, while *PPSS1* was expressed both in the pancreas and central nervous system (CNS). In the pancreatic primordium, the first *PPSS2*-expressing cells appeared at the 16-somite stage (17 hours post fertilisation (hpf)), whereas *PPSS1* expression was first detected in few cells at 24 hpf (Fig. 2A–D). From 24 to 77 hpf, *PPSS1*- and *PPSS2*-expressing cell number increased and they gathered in a single islet (Fig. 2). *PPSS2* was expressed in a larger

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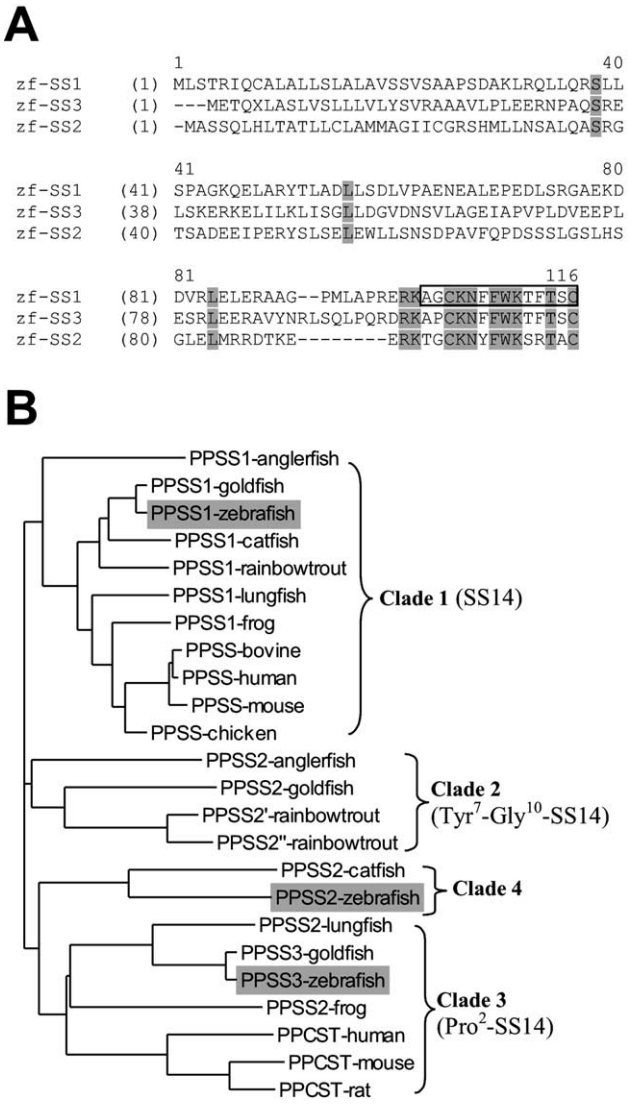

Fig. 1. (A) Comparison of zebrafish *PPSS1*, *PPSS2* and *PPSS3*. Strictly conserved aminoacids are boxed with gray background. SS-14 mature peptide is boxed. The *PPSS1* cDNA is 711 bp long and contains an open reading frame of 114 aminoacids with the conserved SS-14 in its C-terminus. The *PPSS2* corresponds to the previously reported PPSS (Argenton et al., 1999). (B) Full-length aminoacid sequences from all vertebrate SS-related peptide precursors available to date were used to calculate a phylogenetic tree with ClustalX (Page, 1996). PPSS, preprosomatostatin; PPCST, preprocortistatin. Zebrafish PPSSs are boxed. SS-like precursors are grouped according to Lin's description (Lin et al., 2000). The first group is composed of precursors of a strictly conserved SS-14. The three other groups can be characterised by common substitutions found in the deduced mature SS-14. The Tyr^7–Gly^{10}–SS-14 variants constitute group 2. The third group is composed of frog, goldfish and lungfish genes encoding Pro^2-variants of SS-14 but also of the mammalian cortistatin genes. In our phylogenetic analysis, the previously described zebrafish SS cDNA is grouped with the catfish *PPSS2* gene (clade 4). These two genes potentially encode divergent SS-14 with the common substitutions Tyr6, Ser10, Arg11 and Ala13.

number of cells than *PPSS1* (Fig. 2F, H) and double staining showed that most *PPSS1*-expressing cells also expressed *PPSS2* (data not shown). In the CNS, *PPSS1* expression was first detected at the 23-somite stage (19 hpf) in two symmetrical clusters of cells in the rostro-ventral diencephalon (Fig. 3A) and in isolated cells distributed along the ventral spinal cord. At 24 hpf, these expression domains were maintained (Fig. 3B, E, H, I) and, in addition, *PPSS1*-expression was also observed in two bilateral cell clusters in the dorsal telencephalon (Fig. 3E) and in one or two cells on both sides of the caudalmost hindbrain. The telencephalic *PPSS1* labelling, that was reduced at 28 hpf and no longer observed at 30 hpf (Fig. 3F, G), was the only site in which *PPSS1* did not colocalise with *islet-1* expression. At 30 hpf, all *PPSS1*-expressing cells also expressed *islet-1* (Fig. 3C, G, J–L), allowing their precise identification since *islet-1*-expressing cells in zebrafish CNS are well characterised (Korzh et al., 1993; Inoue et al., 1994; Higashijima et al., 2000). In the diencephalon, the *PPSS1* signal was included in the *islet-1* expression domain of the nucleus of the tract of the postoptic commissure (nTPOC) (Fig. 3C, F, G). These results prompted us to check *PPSS1* expression in *masterblind/axin1* embryos, in which the telencephalon and part of the diencephalon are lacking, and the *islet-1*-expressing cells of the nTPOC are absent (Heisenberg et al., 1996, 2001; van de Water et al., 2001). At 24 hpf, *masterblind/axin1* mutants lacked both the telencephalic and diencephalic *PPSS1* staining (Fig. 3D), indicating that the *PPSS1* gene is indeed expressed in a *islet-1*-expressing subpopulation of cells of the nTPOC, responsible for pioneering the postoptic commissure (Heisenberg et al., 1996). In the caudalmost hindbrain, starting from 24 hpf, *PPSS1* was expressed in a subset of neurons of the vagal motor nucleus (nX) (Fig. 3L–O). Expression of the *PPSS1* orthologue in nuclei of the vagus motor nerve has been reported in adult lungfish (Trabucchi et al., 1999). In the spinal cord, *PPSS1* expression was detected, from the 23-somite stage, in several *islet-1* expressing primary motor neurons located bilaterally just above the floor plate (Fig. 3H, I, K). Our results support the hypothesis of a role (direct or not) of this LIM homeobox protein on the SS gene expression (Leonard et al., 1992; Vallejo et al., 1992). From 55 hpf to 5 dpf, the expression of the *PPSS1* gene was maintained in the pancreas (Fig. 2F), diencephalon and motor neurons of the nX (Fig. 3M–O), but not in the spinal cord. From 55 hpf, *PPSS1* expression was also detected in the mesencephalon and cerebellum (Fig. 3M–O), and an additional signal in the hypothalamus was observed at 5 dpf (Fig. 3O).

The dynamic, specific and transient pattern of PPSS1 expression in the embryonic zebrafish CNS is in accordance with the situation observed in other vertebrates where SS peptide is detected transiently in various regions of the developing CNS including forebrain, diencephalic area and spinal cord (Senba et al., 1982; Shiosaka et al., 1982).

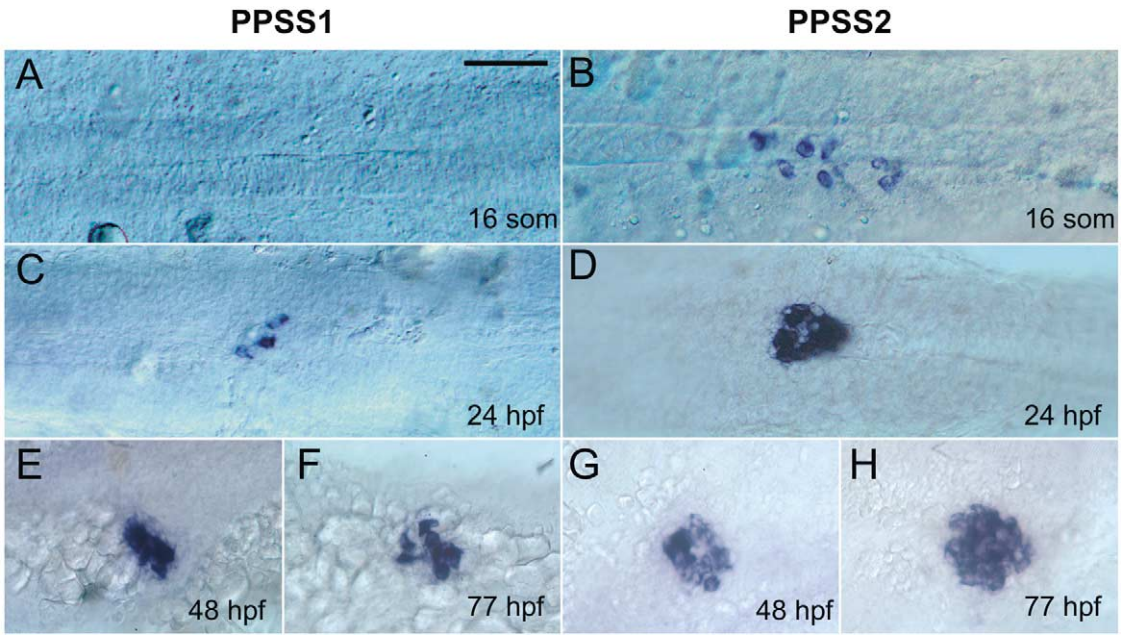

Fig. 2. Expression of *PPSS1* and *PPSS2* in the pancreas of wildtype zebrafish embryos. Ventral views of whole-mount in situ hybridisation with the complete *PPSS1* (A,C,E,F) or *PPSS2* (B,D,G,H) antisense mRNA probes. Embryos were at the 16-somite stage (17 hpf) (A,B), 24 hpf (C,D), 48 hpf (E,G) or 77 hpf (F,H). Scale bar represents 50 μm.

2. Materials and methods

The zf-*PPSS1* cDNA was obtained from the RZPD (http:www.rzpd.de) as EST in the zebrafish databank, sequenced on both strand on an ABI 310 Genetic Analyser and submitted to Genbank (Accession number: AF435965). The zf-*PPSS3* nucleotide sequence (EST fr91b10) was obtained by using the BLAST program with zf-*PPSS1* sequence on the NCBI EST databases (http://www.ncbi.nlm.nih.gov/blast/). Then, the zf-*PPSS3* amino acid sequence was deduced from the fr91b10 (Accession number: BI472739 and BI473045) complete sequence.

2.1. Whole-mount in situ hybridisation

Antisense RNA probes were prepared by transcribing linearised cDNA clones either with SP6 or T7 polymerase and digoxigenin or fluorescein labelling mix (Roche). Single and double whole-mount RNA in situ hybridisations and detection were carried out as previously described (Hauptmann and Gerster, 1994), with a 65°C overnight hybridisation step. Embryos were mounted in 87% glycerol in phosphate buffered saline (PBS) and photographed with Nomarski optics using a Leica digital camera. Digital images were processed using Adobe Photoshop software.

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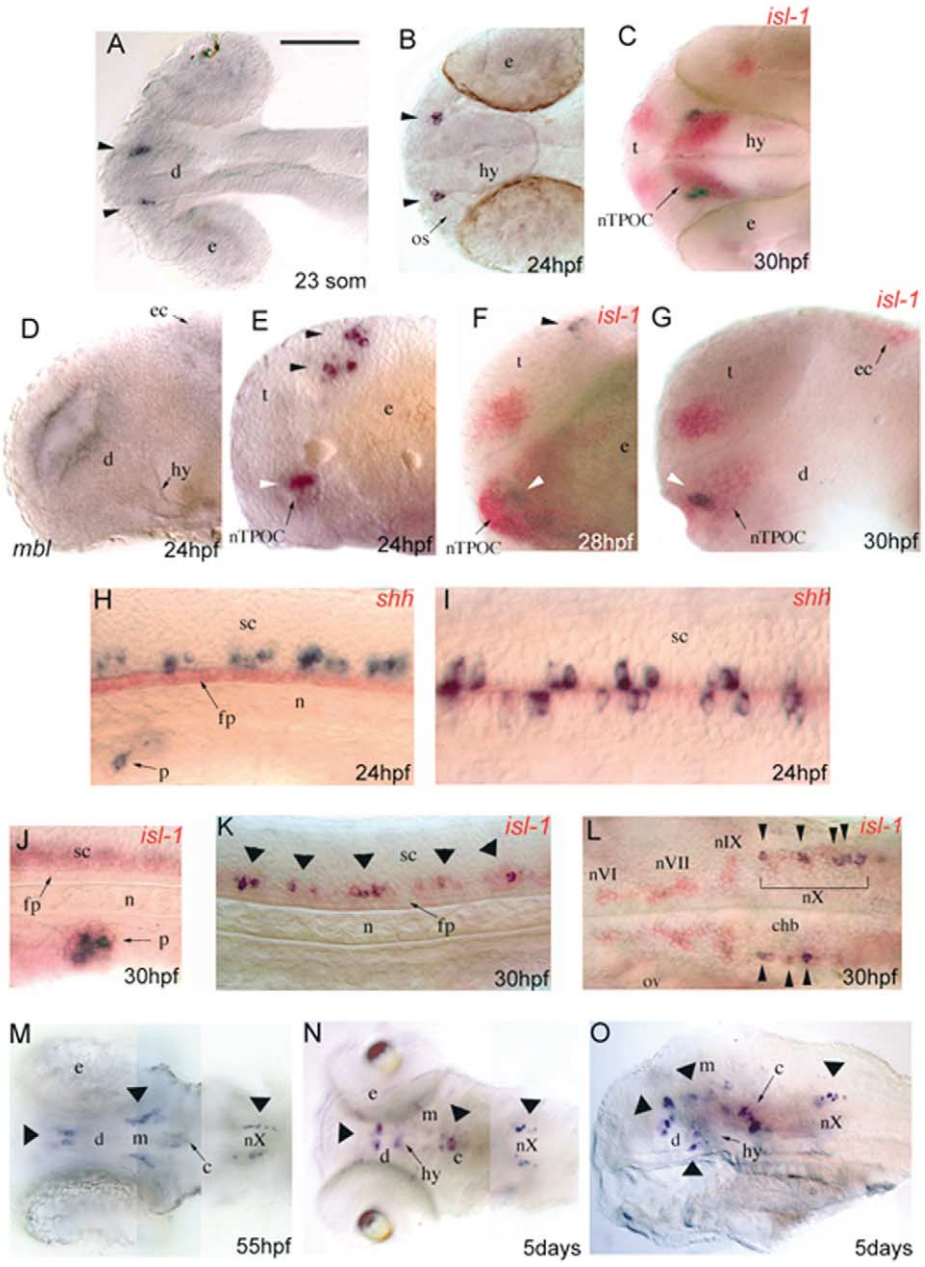

Fig. 3. Whole-mount in situ staining of zebrafish embryos, visualizing the expression pattern of *PPSS1* (blue reaction product), *islet-1* (*isl-1*) and *sonic hedgehog* (*shh*) (red reaction product) mRNAs. The yolk was removed and embryos were mounted between glass coverslips. (A,B) Dorsal views of *PPSS1* expression (arrowheads) in the head at 23-somite stage (19 hpf) and 24 hpf, respectively. (E) Lateral view, at 24 hpf, reveals *PPSS1* expression in the ventral diencephalon (white arrowhead) and dorsal telencephalon (black arrowheads). (D) Lateral view of a 24-hpf *masterblind/axin1* (*mbl*) mutant showing the lack of *PPSS1* staining in the head. (C) Dorsal view of a double staining with *islet-1* at 30 hpf shows that the *PPSS1* signal is found in a subset of *islet-1*-expressing cells in the nTPOC (nucleus of the tract of the postoptic commissure). Lateral views at 28 hpf (F) and 30 hpf (G) show that the telencephalic *PPSS1* labelling does not colocalise with *islet-1* at 28 hpf and is absent at 30 hpf. Lateral (H) and dorsal (I) views of 24-hpf embryos double stained with the floor plate marker *sonic hedgehog* show that *PPSS1* expression is distributed bilaterally along the ventral spinal cord. (J–L) Double staining with *islet-1* at 30 hpf: (J) lateral view, in the pancreatic primordium *PPSS1*-expressing cells are a subpopulation of *islet-1*-positive cells; (K) lateral view, along the ventral spinal cord *PPSS1* expression colocalises with *islet-1*-positive primary motor neurons (arrowheads); (L) dorsal view, in the hindbrain *PPSS1* signal overlaps with *islet-1*-positive neurons of the vagal motor nucleus (nX), but not with those of the abducens (nVI), facial (nVII) and glossopharyngeal (nIX) motor nuclei. (M) Dorsal view at 55 hpf, *PPSS1* expression (arrowheads) is observed in the diencephalon, mesencephalon, cerebellum and vagal motor nucleus. (N) Dorsal and (O) lateral (eyes removed) views at 5 days, *PPSS1* mRNA (arrowheads) is expressed in the same regions of the CNS as observed at 55 hpf and, in addition, also in the hypothalamus. c, cerebellum; chb, caudalmost hindbrain; d, diencephalon; e, eye; ec, epiphysial cluster; fp, floor plate; hy, hypothalamus; m, mesencephalon; n, notochord; nTPOC, nucleus of post optic commisure; os, optic stalk; ov, otic vesicle; p, pancreas; sc, spinal cord; t, telencephalon. In all pictures anterior is to the left; in lateral views dorsal is to the top. Scale bar: A–G and J–L 100 μm, H and I 50 μm, M–O 150 μm.

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