Chromosomal localization of three somatostatin genes in zebrafish. Evidence that the [Pro²]-somatostatin-14 isoform and cortistatin are encoded by orthologous genes

Hervé Tostivint, Lucille Joly, Isabelle Lihrmann, Marc Ekker and Hubert Vaudry

European Institute for Peptide Research (IFRMP 23), Laboratory of Cellular and Molecular Neuroendocrinology, INSERM U-413, UA CNRS, University of Rouen, 76821 Mont-Saint-Aignan, France

1Center for Advanced Research in Environmental Genomics, Department of Biology, University of Ottawa, Ottawa, Ontario, Canada, K1N 6N5

(Requests for offprints should be addressed to H Vaudry; Email: hubert.vaudry@univ-rouen.fr)

Abstract

There is now evidence for the existence of two somatostatin genes in most vertebrate species, and even three somatostatin genes in teleosts. To help clarify the evolutionary relationships between the different somatostatin isoforms currently known, we characterized the somatostatin loci in a teleost species, the zebrafish Danio rerio, and compared them with the corresponding regions in the human and pufferfish genomes. The occurrence of three somatostatin genes, termed SS1, SS2 and SSII, has been previously demonstrated in the zebrafish. Radiation hybrid mapping assigned these three genes to linkage groups 15, 23 and 2, respectively. Conserved synteny of the zebrafish SS2 gene and the human cortistatin gene was revealed by comparative genomic analysis, indicating that mammalian cortistatin is orthologous to the SS2 variant of non-mammalian species. In contrast, using a similar approach, it was not possible to identify the evolutionary relationships between the atypical SSII gene of zebrafish and the other teleost SSII genes.

Journal of Molecular Endocrinology (2004) 33, R1–R8

Introduction

Somatostatin (SS1) is a cyclic tetradecapeptide that was originally identified in ovine hypothalamic extracts from its ability to inhibit growth hormone release (Brazeau et al. 1973). It has been subsequently shown that SS1 is widely distributed in the central nervous system and in various peripheral organs where it acts as both a neurotransmitter/neuromodulator and a hormone (Epelbaum et al. 1994, Barnett 2003). SS1 is synthesized as part of a larger precursor molecule which is proteolytically cleaved to generate the biologically active peptides somatostatin-14 (SS1) and the N-terminally extended form somatostatin-28 (Esch et al. 1980, Pradayrol et al. 1980). The primary structure of SS1 is identical in all vertebrate species investigated so far, from agnathans to mammals (Conlon et al. 1997, Tostivint et al. 2004).

In addition to prepro-SS1 (PSS1), a second somatostatin precursor, encoded by a distinct gene, has been characterized in several representative species, including sturgeon (Trabucchi et al. 2002), goldfish (also named PSSIII; Lin et al. 1999), zebrafish (also named PSS3; Devos et al. 2002), lungfish (Trabucchi et al. 1999), frog (Tostivint et al. 1996), chicken (Trabucchi et al. 2003), mouse (de Lecce et al. 1997a), rat (de Lecce et al. 1996) and human (de Lecce et al. 1997a, Fukusumi et al. 1997). Peptides derived from this second precursor (named SS2 in non-mammalian species and...
The occurrence of a third somatostatin precursor (PSSII) has also been established in teleost fish (Conlon et al. 1997). Most peptides derived from PSSII contain the [Tyr7,Gly10]-somatostatin-14 sequence at their C-terminal extremity. However, in some species such as the catfish (Magazin et al. 1982, Andrews et al. 1984) and zebrafish (Devos et al. 2002), this third precursor generates a peptide that shows only very limited sequence similarity to other teleost SSIIIs (Fig. 1).

Several lines of evidence suggest that PSS2 and PCST are derived from orthologous genes (Tostivint et al. 2004): (1) their processing products, SS2 and CST, both exhibit the Gly→Pro substitution at position 2 (Fig. 1); (2) they are almost exclusively expressed in the brain but not in the pancreas or gut and (3) the mouse, rat and human genomes do not appear to encompass any sequence that would be more related to PSS2 than PCST. It should be noted however that, apart from their C-terminal region, PCST and PSS2 possess very little sequence similarity. In addition, their distributions in the brain are relatively different: while the SS2 gene is expressed in various subdivisions of the brain in all species studied so far (Tostivint et al. 1996, Lin et al. 1999, Trabucchi et al. 1999, 2002, 2003), the CST gene is almost exclusively expressed in the telencephalon (de Lecea et al. 1996, 1997a, 1997b). Although the parentage between the PSS2 gene and the PCST gene is supported by phylogenetic analysis (Trabucchi et al. 1999, 2003, Devos et al. 2002), the orthology of the two genes has not yet been demonstrated. The question of the evolutionary significance of the genes encoding the atypical SSII in catfish and zebrafish is also currently unanswered.

The recent development of comparative genomics now provides new opportunities for identifying orthologous genes in vertebrates (O’Brien et al. 1999, Postlethwait et al. 2000). In the present study we applied a comparative approach utilizing the zebrafish, in which three distinct somatostatin isoforms have been previously identified (Argenton et al. 1999, Devos et al. 2002), to help understanding of the evolutionary history of the somatostatin genes in vertebrates (Barbazuk et al. 2000, Woods et al. 2000). The chromosomal localization of these three genes is compared with those of the somatostatin genes in the human (Lander et al. 2001, Venter et al. 2001) and Fugu (Aparicio et al. 2002) genomes.

Materials and methods

Sequences

The sequences used for this study were obtained from GenBank. Accession numbers were as follows: AF435965 for the zebrafish PSS1 sequence; BI472739 and BI473045 for the zebrafish PSS2 (also named PSS3) sequence; AY8017 for the zebrafish PSSII (also named PSS2) sequence; CAAB01010628 for the Fugu PSSII sequence.
Linkage analysis by radiation hybrid mapping

Radiation hybrids of the LN54 panel (zebrafish DNA in a mouse background; Hukriede et al. 1999, 2001) were used to map the three somatostatin genes, i.e. the SS1, SS2 and SSII genes, to a specific zebrafish linkage group by PCR. DNA (100 ng) from each of the 93 zebrafish × mouse radiation hybrids was amplified using a pair of gene-specific primers which amplify part of the 3'-untranslated region sequence of the three genes (Table 1). The reactions contained 1×PCR buffer, 1·5 mM MgCl2, 0·25 µM each forward and reverse primer, 0·2 mM each dNTP and 1 U Taq DNA polymerase. The PCR templates for the controls were 100 ng DNA from the two parental cell lines. Following an initial denaturation at 94 °C for 4 min, PCR was performed for 32 cycles: 30 s at the appropriate annealing temperature for a given primer set, 30 s at 72 °C, 30 s at 94 °C and a final extension at 72 °C for 7 min. The entire reaction (20 µl) was fractioned by electrophoresis in 1·5% (w/v) agarose. The radiation hybrid panel was scored based on the absence (0) or presence (1) of the expected DNA fragments, or an ambiguous result (2) to generate the radiation hybrid vector.

Phylogenetic analysis

The amino acid sequences of the N-termini of all teleost somatostatin precursors were aligned using CLUSTAL X (Thompson et al. 1994) and optimized manually using the SEAVIEW program (Galtier et al. 1996). Tree construction and bootstrap analysis were carried out with the PHILO_WIN program (Galtier et al. 1996). Distances were calculated according to Saitou & Nei (1987).

Table 1 Sequence of the oligonucleotides used for PCR amplification

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5'→3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>Somato II F1</td>
<td>CTC CAC CTG ACA GCA ACT CTT CTC</td>
</tr>
<tr>
<td>Somato II B1</td>
<td>TCT CAC CTG GTA ACA GCA ACT CTT CAT CCG CAG</td>
</tr>
<tr>
<td>Somato 2 F2</td>
<td>TCA CTA CTC TTA TTA CTG ACC TTT CGC C</td>
</tr>
<tr>
<td>Somato 2 B5</td>
<td>TGC TAT CTC TCC AGC CAG AAC G</td>
</tr>
<tr>
<td>Somato 1 F10</td>
<td>AAC TCG CCA GAT ACA CAC TCG CAG</td>
</tr>
<tr>
<td>Somato 1 B3</td>
<td>GGG AAA CCA GAT ACA CAC TCG CAG</td>
</tr>
</tbody>
</table>

Results and discussion

Chromosomal localization of the three zebrafish somatostatin genes

To localize the three somatostatin genes to zebrafish linkage groups (LGs), radiation hybrid mapping using the LN 54 panel of radiation hybrids (Hukriede et al. 1999, 2001) was performed. The SS1 mapped to LG 15 at a distance of 6·72 cR from the marker z3760. The SS2 gene (also named SS3) was assigned to LG 23 at 18·03 cR from nad1·2. The SSII gene (also named SS2) mapped to LG 2 at 2·02 cR from fa56a06. Primary data and the radiation hybrid vector for linkage analysis is available upon request to the corresponding author.

Evolutionary relationships between the somatostatin 2 and CST genes

It has been previously reported that the human chromosomal region 3q29, where the SS1 gene is located, shares only limited conserved synteny with zebrafish LG 15 (Woods et al. 2000). Accordingly, we did not detect any zebrafish counterparts of human genes or other genetic markers located in 3q29 in the vicinity of the zebrafish SS1 gene locus. In contrast, the zebrafish SS2 gene appears closely linked to at least eight loci, namely PMSCL2, SDR1, HKR3, FLJ20321, PGD, TARDBP, CTNNBIP and FLJ10737, each of which possesses an apparent orthologous locus in the human genome (http://fisher.wustl.edu/fish_lab/cgi-bin/human_int_map.cgi). Interestingly, in human, all of these eight loci are located at 1p36, a region where the CST gene has been mapped previously (de Lecca et al. 1997a). Although the order of these loci...
has not been totally conserved, probably due to intrachromosomal rearrangements (Postlethwait et al. 2000), the chromosomal environments of the SS2 gene and the CST gene appear very similar (Fig. 2). The occurrence of this conserved synteny provides strong evidence that the CST gene is the mammalian counterpart of the SS2 gene known in fish, birds and amphibians, thus corroborating the results previously obtained from phylogenetic analysis (Trabucchi et al. 1999, 2003, Devos et al. 2002). The fact that the cryptic sequences of the SS2 and CST precursors are very divergent, as compared with the cryptic sequences of the SS1 precursors, indicates that the SS2/CST gene underwent a more rapid evolution in the mammalian lineage than in other vertebrates (Trabucchi et al. 2003) resulting in important changes in both its structure and expression pattern (Tostivint et al. 2004). The origin of the SS2/CST gene remains uncertain. The occurrence of an SS2 gene has been demonstrated in all osteichthyes (see Introduction), suggesting that it appeared before the transition from chondrichthyes to osteichthyes. The exact time of the duplication event from which it arose may have coincided with one of the whole-genome duplications that occurred during early vertebrate evolution (Furlong & Holland 2002; Fig. 3). It is interesting to note that lampreys, like all extant osteichthyes, also possess a second somatostatin gene. The lamprey [Ser\(^{12}\)]-somatostatin-14 variants (Andrews et al. 1988, Conlon et al. 1995a, 1995b) may thus represent an SS2 gene product. Alternatively, these two isoforms may be the result of a gene duplication within the lamprey lineage (Escriva et al. 2002).

**Figure 2** Map showing chromosomal position of the SS2 gene in zebrafish and conserved synteny on zebrafish LG 23 (left) and on human chromosome 1p36 (right). The distances are expressed in cR on the zebrafish map and in Mb on the human map. CTNNBIP1, catenin β-interacting protein 1; HKR3, Homo sapiens GLI-Kruppel family member 3; PGD, phosphogluconate dehydrogenase; PMSCL2, polymyositis/scleroderma autoantigen 2; TARDBP, TAR DNA-binding protein; SDR1, short-chain dehydrogenase/reductase 1.

**LG23**

Journal of Molecular Endocrinology (2004) 33, R1–R8

R4 H TOSTIVINT and others · Chromosomal localization of somatostatin genes in zebrafish

Downloaded from Bioscientifica.com at 08/25/2019 12:57:43AM via free access
Evolutionary significance of the atypical zebrafish somatostatin II gene

In contrast to other teleost SSIIIs, which generally contain the [Tyr7, Gly10]-somatostatin-14 sequence (Conlon et al. 1997), zebrafish SSII exhibits a totally atypical structure (Devos et al. 2002; Fig. 1). In order to clarify the evolutionary history of the SSII gene, we compared its chromosomal localization in zebrafish with that of the gene encoding the classical SSII in the pufferfish Fugu rubripes. In silico screening of the pufferfish genome (Aparicio et al. 2002) made it possible to identify one sequence that corresponds to a PSSII-encoding gene (GenBank accession no. CAAB01010628). Although the sequence of this precursor is truncated at its C-terminus and does not contain the SSII sequence, as a result of the lack of the second exon of the corresponding gene, its identity as a PSSII gene is attested by phylogenetic analysis. Indeed, the tree presented in Fig. 4 shows that the Fugu PSSII sequence is more closely related to the [Tyr7, Gly10]-somatostatin-14-containing-PSSIIIs than to the atypical PSSII isoforms from zebrafish (Devos et al. 2002) and catfish (Magazin et al. 1982). The absence of the second exon in the Fugu SSII gene sequence can likely be ascribed to an erroneous annotation. Interestingly, in the pufferfish genome, the SS1 gene and the SSII gene are localized in tandem. This organization suggests that the SSII gene arose by duplication of the SS1 gene, and probably reflects the initial position of the two genes in the common ancestor of the teleost lineage. In contrast, in zebrafish, we found that the SS1 gene and the SSII gene are located on distinct chromosomes. Two hypotheses can be proposed to explain this observation: (1) the zebrafish atypical SSII gene and other teleost SSII genes are not orthologous or (2) the two genes are orthologous, in agreement with a previous report (Su et al. 1988), but, in zebrafish, the SS1 and SSII genes have been physically separated by chromosomal rearrangement. In support of the latter hypothesis, it should be noted that, up to now, no species has been shown to possess both the SSII gene and its atypical variant. However, although the species possessing an atypical SSII gene – i.e. the catfish, the zebrafish and probably the pacu (Ferraz de Lima et al. 1999) – are all members of a single group, the ostariophysi, surprisingly the goldfish, which belongs to the same group, possesses the classical SSII gene (Lin et al. 1999). Teleosts are known to possess more genes than other vertebrates. Recent studies provide evidence that these additional genes may have been produced during a complete fish-specific genome-duplication event (Amores et al. 1998, 2004, Vandepoele et al. 2004). However, the fact that, in Fugu, the SS1 and SSII genes are arranged in tandem indicates that the classical SSII gene has not been generated by tetraploidization. In support of this view, we have recently reported the occurrence in Fugu of a second isoform of the SS1 gene (Tostivint et al. 2004) that is probably a product of this whole-genome duplication event.

In conclusion, the present results show for the first time that SS2 and CST are encoded by

Figure 3 Schematic representation illustrating the phylogenetic relationships among the somatostatin-encoding genes in vertebrates. The possible emergence of the various somatostatin isoforms is shown relative to the established phylogeny of vertebrates. This model is based on the most parsimonious scenario of the history of the somatostatin family assuming that the lamprey [Ser12]-somatostatin-14 variant and the ratfish [Ser5]-somatostatin-14 are both orthologous to SS2. Black line, SS1 gene; dark-grey line, SS2/CST gene; dark-grey arrow, duplication generating the SS2/CST gene; light-grey line, SSII gene; light-grey arrow, duplication generating the SSII gene; broken line, non characterized genes. The atypical SSII gene is not shown.

Evolutionary significance of the atypical zebrafish somatostatin II gene

In contrast to other teleost SSIIIs, which generally contain the [Tyr7, Gly10]-somatostatin-14 sequence (Conlon et al. 1997), zebrafish SSII exhibits a totally atypical structure (Devos et al. 2002; Fig. 1). In order to clarify the evolutionary history of the SSII gene, we compared its chromosomal localization in zebrafish with that of the gene encoding the classical SSII in the pufferfish Fugu rubripes. In silico screening of the pufferfish genome (Aparicio et al. 2002) made it possible to identify one sequence that corresponds to a PSSII-encoding gene (GenBank accession no. CAAB01010628). Although the
orthologous genes. These data demonstrate that the [Pro² Met¹³]-somatostatin-14 variant isolated from the frog brain (Vaudry et al. 1992) was actually the first CST-like peptide to be identified.

**Acknowledgements**

We thank Dr Philippe Vernier (Institut Alfred Fessard, Gif sur Yvette, France) for helpful discussions. This work was supported by grants from INSERM (U-413), the Natural Sciences and Engineering Research Council of Canada and the Conseil Régional de Haute-Normandie.

**Note Added in Proof**

The Genbank accession number of the [Tyr⁷, Gly¹⁰]somatostatin-14 encoding sequence of the fugu PSSII gene is CAAB01010582. At the time this manuscript went to press, the chromosomal localization of this sequence was not yet known.

**References**


