**RESEARCH ARTICLE**

# Evolutionary history of the somatostatin and somatostatin receptors

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## Abstract

Somatostatin and its receptors have a critical role in mammalian growth through their control pattern of secretion of growth hormone, but the evolutionary history of somatostatin and somatostatin receptors are ill defined. We used comparative whole genome analysis of *Danio rerio, Carassius auratus, Xenopus tropicalis, Gallus gallus, Monodelphis domestica, Homo sapiens, Sus scrofa, Bos taurus, Mus musculus, Rattus norvegicus, Canis lupus familiaris, Ovis aries, Equus caballus, Pan troglodytes* and *Macaca mulatto* to identify somatostatin and somatostatin receptors in each species. To date, we have identified a minimum of two genes of somatostatin and five somatostatin receptor genes in mammalian species with variable forms. We established a clear evolutionary history of the somatostatin system and traced the origin of the somatostatin system to 395 million years ago (MYA), identifying critical steps in their evolution.

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## Introduction

Somatostatin (SST) also abbreviated as SRIF (somatotropin release inhibiting factor) or SS (somatostatin), is a polypeptide that was first isolated by Brazeau *et al*. 1973 from mammalian hypothalamus. It was identified as an inhibitor of growth hormone (GH) secretion. In mammals, SRIF is known to be a multifunctional peptide that is widely distributed throughout the central nervous system and peripheral tissues (Reisine 1995; Reisine and Bell 1995). Its functions include the modulation of neurotransmission, cell secretion, cell proliferation (Patel 1999) and inhibition of various hormone secretions (Florio *et al*. 1994). In mammals, somatostatin 1 (commonly recognized as somatostatin), exists in two predominant biologically-active forms: SRIF-14 and its NH2-terminal extension of 14 amino acids called SRIF-28. Both SRIF-14 and SRIF-28 are encoded by a common gene and processed from a single precursor (Patel and Srikant 1998). SRIF-14 has been more extensively studied and identified, with the same amino acid sequence, in representative species of all classes of vertebrate (Conlon *et al*. 1997).

The actions of SST are mediated by a family of G protein-coupled receptors (GPCRs) named somatostatin receptors (SSTRs) with six subtypes, encoded by separate genes except SSTR2A (Patel 1999). Based on structural features and pharmacological properties, and keeping in view with their sequence homology, the receptor subtypes can be classified into two subgroups, SRIF1 and SRIF2 (Olias *et al*. 2004). Further, SRIF1 receptor group comprises of SSTR2, SSTR3 and SSTR5 (SRIF1A, SRIF1B and SRIF1C), while SSTR1 and SSTR4 (SRIF2A and SRIF2B) belong to the SRIF2 group. Five SSTRs are variably expressed in brain e.g. in the rat brain, mRNA for SSTR1 is the most abundant followed by SSTR2, SSTR5, SSTR3, and SSTR4 (Kong *et al*. 1994; Patel 1999). The expression level is also different in different parts of the brain. A high density of neurons positive for SSTR1 and SSTR2, and moderate density of SSTR5-expressing neurons occur in the deeper layers of the cerebral cortex (Hervieu and Emson 1998), but SSTR3 localizes mainly in the cerebellum (Patel 1999). All five SSTRs are also expressed in the pituitary, islets, gut and aorta (Kumar *et al*. 1999).

Somatostatin is a phylogenetically ancient and multigene family of peptides in vertebrates, including cortistatin that

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has two forms viz. CST-17 and CST-29, and interestingly binds with high affinity to all somatostatin receptor subtypes (Baranowska *et al*. 2006).

To date, the somatostatin system has not been completely characterized in any mammalian species, although there is fragmentary information from several mammalian and non-mammalian species, including goldfish, suggesting that the somatostatin system arose early in vertebrate evolution (Lin *et al*. 1999). The availability of draft genomes for many mammalian and non-mammalian vertebrate species now allows us to systematically identify somatostatin and somatostatin receptor genes across the species and infer their evolutionary origin. Here, we used the draft genomes of *D. rerio, C. auratus, X. tropicalis, G. gallus, M. domestica, H. sapiens, S. scrofa, B. taurus, M. musculus, R. norvegicus, C. l. familiaris, O. aries, E. caballus, P. troglodytes* and *M. mulatto* to systematically identify all somatostatin and somatostatin receptors in each species and infer the evolutionary origins and history of somatostatin and somatostatin receptors in mammals.

## Materials and methods

### *Identification of putative SST and SSTRs within the draft genomes of various species*

All SST and SSTRs for *G. gallus, H. sapiens, S. scrofa, B. taurus, M. musculus, C. l. familiaris, O. aries, E. caballus, P. troglodytes M. mulatto* and SST for *D. rerio, X. tropicalis* and *M. domestica* were identified from National Center for Biotechnology Information (www.ncbi.nlm.nih.gov), while somatostatin receptors for *D. rerio*, *X. tropicalis* and *M. domestica* were from UCSC Genome Bioinformatics (http://genome.ucsc.edu) . All known SST and SSTRs were compared with predicted and known protein sequences from the databases by basic local alignment search tool (expected score: 0.0001). Only hits that, when compared back to the Entrez Protein Clusters database, National Center for Biotechnology Information, returned known SST or SSTRs as their top scoring hit were retained. In addition to examining automated gene predictions, we also examined genomic sequence direction by comparing known SSTR and SSTRs to draft genomes by TBLASTN (expected score: 0.1), and putative peptide fragments were identified. Putative peptide fragments that returned a known SST or SSTR when compared with the Entrez Protein Clusters database were retained. Sequences were then compared with the National Center for Biotechnology Information dbEST database (www.ncbi.nlm.nih.gov/dbEST/index.html) and any matches were used to refine predictions.

Only putative receptors that placed phylogenetically within the somatostatin receptor subgroup of GPCRs were considered putative SSTRs for this study. All putative SST and SSTRs and their genomic locations are listed in tables 1 and 2.

### *Pair-wise homology of putative SST and SSTRs*

All SST and SSTRs were compared with each other including both known and putative forms from the examined species (tables 1 and 2) by Needlemen–Wunsch pairwise alignment using the *needle* program from the EMBOSS package with default parameters and the per cent identity of maximum scoring pair listed (Rice *et al*. 2000). To avoid penalizing incomplete sequences, terminal 3′ and 5′ gaps were not scored.

**Table 1.** The identified somatostations.

| Somatostatin (SST1) | | | | | | |
|---|---|---|---|---|---|---|
| Name | Scientific name | Length | High ID (to) | Low ID (to) | Chromosome | GS |
| Drs | *Danio rerio* | 324 | 48.1 mms | 41.2 caus2 | 15 | Unknown |
| Hss | *Homo sapiens* | 351 | 99.7 pts | 45.6 drs | 3 | 188869547-188870773 |
| Pts | *Pan troglodytes* | 351 | 99.7 hss | 45.8 drs | 3 | 193254703 193255899 |
| Mus | *Macaca ulatto* | 351 | 98.6 pts | 46.4 drs | 2 | 180010228 180011435 |
| Bts | *Bos Taurus* | 351 | 98.6 oas | 46.3 drs | 1 | 72569474 72570664 |
| Oas | *Ovis aries* | 322 | 98.6 bts | 46.3 drs | | Unknown |
| Sss | *Sus scrofa* | 351 | 97.4 ecs | 47.8 drs | | Unknown |
| Ecs | *Equus caballus* | 351 | 97.4 sss | 46.6 drs | 19 | 21598681 21599899 |
| Cfs | *Canis l. familiaris* | 351 | 96.9 ecs | 44.0 drs | 34 | 23071618 23072819 |
| Rns | *Rattus norvegicus* | 351 | 97.7 mms | 47.6 drs | 11 | 79125091 |

**Table 1** (*contd.*)

| Somatostatin (SST1) | | | | | | |
|---|---|---|---|---|---|---|
| Name | Scientific name | Length | High ID (to) | Low ID (to) | Chromosome | GS |
| | | | | | | 79126062 |
| Mms | *Mus musculus* | 351 | 97.7 rns | 48.1 drs | 16 | 23889815<br>23890830 |
| Mds | *Monodelphis domestica* | 351 | 84.9 cfs | 44.9 drs | | Unknown |
| Ggs | *Gallus gallus* | 351 | 83.8 sss and cfs | 44.9 drs | 9 | 15910976<br>15911190 |
| Xls | *Xenopus tropicalis* | 348 | 76.7ggs | 42.6 drs | 81 | 2228109<br>2229325 |
| Cau1 | *Carassius auratus* | 345 | 69.3 ggs | 45.7 drs | | Unknown |
| Cau 2 | *Carassius auratus* | 366 | 52.7 mms and hss | 40.7 drs | | Unknown |
| Cortistatin (SST2/CST) | | | | | | |
| Name | Scientific name | Length | High ID (to) | Low ID (to) | Chromosome | GS |
| Drcs | *Danio rerio* | 324 | 44.8 rncs | 35.2 eccs | 23 | Unknown |
| Hscs | *Homo sapiens* | 468 | 60.4 btcs | 26.7 cfcs | 1 | 10432718<br>10434239 |
| Ptcs | *Pan troglodytes* | 474 | 90.8 mucs | 29.4 cfcs | 1 | 10612665<br>10613138 |
| Mucs | *Macaca ulatto* | 489 | 90.8 ptcs | 28.9 cfcs | 1 | 13537550<br>13538038 |
| Btcs | *Bos Taurus* | 363 | 66.6 rncs | 32.9 cfcs | 16 | 41932255<br>41933690 |
| Eccs | *Equus caballus* | 276 | 45.2 mmcs | 32.8 ptcs | 2 | 39006976<br>39008557 |
| Cfcs | *Canis l. familiaris* | 255 | 44.7 drcs | 26.7 hscs | 1 | 21179319<br>21179862 |
| Rncs | *Rattus norvegicus* | 339 | 85.1 mmcs | 29.6 cfcs | 5 | 166199057<br>166200399 |
| Mmcs | *Mus musculus* | 330 | 85.1 rncs | 32.8 mucs | 4 | 148499373<br>148500755 |
| Ggcs | *Gallus gallus* | 306 | 51.1 xlcs | 33.7 cfcs | 21 | 4100420<br>4103982 |
| Xlcs | *Xenopus tropicalis* | 348 | 52.5 rncs | 33.2 eccs | 81 | 2228109<br>2229325 |

***Phylogenetic analysis of SST and SSTRs***

All known and putative SST and SSTRs were aligned using ClustalX (CIBBR, Dublin, Ireland) and phylogenetic analysis was performed using both protein parsimony and nearest neighbour methods with a bootstrap score of 100 using the PHYLIP software package (Phylogeny Inference Package, version 3.5c; J. Felsenstein, University of Washington, Seattle, USA) and Mega 4. All known and putative sequences used were: *S. scrofa* (pig) SST (NM_001009583), SSTR1 (AY138806), SSTR2 (NM_001011694), SSTR3 (AY156054), SSTR4 (AY156053), SSTR5a (AY156052), SSTR5b (DQ234796) and SSTR5c (DQ234798); *B. taurus* (cow) SST (NM_173960), SSTR1 (XM_001250796), SSTR2 (NM_174467), SSTR3 (XM_596779), SSTR4 (GS44949459-44950270) and SSTR5 (XM_587471); *R. norvegicus* (rat) SST (NM_012659), SSTR1(X62314 X61630), SSTR2a (EDM06514.1), SSTR2b (EDM06515), SSTR3 (NM_133522 XM_346802), SSTR4 (NM_013036) and SSTR5 (L04535 S53287); *M. musculus* (mouse) SST (NM_009215), SSTR1(M81831), STR2a (CAA48766), SSTR2b (NM_009217 XM_990740), SSTR3 (NM_009218), SSTR4 (NM_009219) and SSTR5 (U82697); *C. l. familiaris* (dog) SST (NM_001003307), SSTR1 (AY702069), SSTR2 (AY702068), SSTR3 (AY643737), SSTR4a (GS 19578333-19582486), SSTR4b (GS 19578957-19579787), SSTR4c (GS 10000450-10001292) and SSTR5 (XM_547202); *H. sapiens* SST (NM_001048), SSTR1 (M81829), SSTR2a (AAF42809), SSTR2a

**Table 2.** Identified somatostatin receptors.

| Name | Scientific name | Chromosome /genomic scaffold | Length | High ID (to) |
|---|---|---|---|---|
| xlr1 | *Xenopus tropicalis* | GS248 | 870 | 90.3 xl2r4 |
| Xl1r2 | *Xenopus tropicalis* | GS23 | 902 | 96.5 xlr5 |
| Xl2r2 | *Xenopus tropicalis* | GS176 | 807 | 56.0 ggr2 |
| Xlr3 | *Xenopus tropicalis* | GS23 | 516 | 55.7 xl1r4 |
| Xl1r4 | *Xenopus tropicalis* | GS23 | 927 | 98.7 xlr5 |
| Xl2r4 | *Xenopus tropicalis* | GS248 | 786 | 90.3 xlr1 |
| xlr5 | *Xenopus tropicalis* | GS23 | 939 | 98.7 xl1r4 |
| Dr1r1 | *Danio rerio* | GC17 | 1116 | 80.9 dr1r4 |
| Dr2r1 | *Danio rerio* | GC17 | 1101 | 92.2 cau1r1 and cau2r1 |
| Drr2 | *Danio rerio* | GC12 | 1140 | 85.6 caur2 |
| Drr3 | *Danio rerio* | GC12 | 1442 | 76.1 cau1r3 |
| Dr1r4 | *Danio rerio* | GC7 | 978 | 93.5 dr2r4 |
| Dr2r4 | *Danio rerio* | GC2 | 918 | 93.5 dr1r4 |
| Dr3r4 | *Danio rerio* | GC17 | 921 | 83.7 dr2r1 |
| Drr5 | *Danio rerio* | GC14 | 1212 | 81.1 cau3r5 |
| Cau1r1 | *Carassius auratus* | Unknown | 1104 | 95.8 cau2r1 |
| Cau2r1 | *Carassius auratus* | Unknown | 1104 | 95.8 cau1r1 |
| Caur2 | *Carassius auratus* | Unknown | 1143 | 85.6 drr2 |
| Cau1r3 | *Carassius auratus* | Unknown | 1643 | 76.1 drr3 |
| Cau2r3 | *Carassius auratus* | Unknown | 1359 | 61.5 drr3 |
| Cau1r5 | *Carassius auratus* | Unknown | 1173 | 88.3 cau2r5 |
| Cau2r5 | *Carassius auratus* | Unknown | 1175 | 88.3 cau1r5 |
| Cau3r5 | *Carassius auratus* | Unknown | 1134 | 81.1 drr5 |
| Ggr1 | *Gallus gallus* | GC5 | 1179 | 80.5 ssr1 |
| Ggr2 | *Gallus gallus* | GC18 | 1116 | 82.4 ssr2 |
| Ggr3 | *Gallus gallus* | GC1 | 1209 | 69.5 mdr3 |
| Ggr4 | *Gallus gallus* | GC3 | 1191 | 73.1 mur4 |
| Ggr5 | *Gallus gallus* | GC14 | 1080 | 75.4 mdr5 |
| Mdr1 | *Monodelphis domestica* | GC1 | 1596 | 65.1 ecr1 & ecr4 |
| Mdr2 | *Monodelphis domestica* | GC2 | 1599 | 58.1 cf3r4 |
| Mdr3 | *Monodelphis domestica* | GC8 | 1245 | 74.7 mu1r3 |
| Mdr4 | *Monodelphis domestica* | GC1 | 1223 | 74.7 ptr4 |
| Mdr5 | *Monodelphis domestica* | GC6 | 1071 | 75.4 ggr5 |
| Ssr1 | *Sus scrofa* | Unknown | 1173 | 95.6 btr1 |
| Ssr2 | *Sus scrofa* | GC7 | 1110 | 93.4 cf2r4 |
| Ssr3 | *Sus scrofa* | Unknown | 1266 | 88.3 Cfr3 |
| Ssr4 | *Sus scrofa* | Unknown | 760 | 70.2 btr4 |
| Ss1r5 | *Sus scrofa* | Unknown | 1116 | 89.0 btr5 |
| Ss2r5 | *Sus scrofa* | Unknown | 906 | 79.7 ss1r5 |
| Ss3r5 | *Sus scrofa* | Unknown | 591 | 65.2 ss2r5 |
| Rnr1 | *Rattus norvegicus* | GC6 | 1176 | 90.0 mmr1, mu1r1, mu2r1, btr1, |
| Rn1r2 | *Rattus norvegicus* | GC28 | 1041 | 93.6 mm1r2 |
| Rn2r2 | *Rattus norvegicus* | GC11 | 1110 | 93.3 rn1r2 |
| Rnr3 | *Rattus norvegicus* | GC28 | 1287 | 93.8 mmr3 |
| Rnr4 | *Rattus norvegicus* | GC22 | 1155 | 94.9 mmr4 |
| Rnr5 | *Rattus norvegicus* | GC28 | 1092 | 91.7 mmr5 |
| Mmr1 | *Mus musculus* | GC12 | 1263 | 90.0 rnr1 |
| Mm1r2 | *Mus musculus* | GC11 | 1041 | 98.8 mm2r2 |
| Mm2r2 | *Mus musculus* | GC11 | 1041 | 98.8 mm1r2 |
| Mmr3 | *Mus musculus* | GC2 | 1287 | 93.8 rnr3 |
| Mmr4 | *Mus musculus* | GC2 | 1158 | 94.9 rnr4 |
| Mmr5 | *Mus musculus* | GC17 | 1105 | 91.7 rnr5 |
| Cfr1 | *Canis l. familiaris* | GC8 | 1176 | 95.2 btr1 |
| Cfr2 | *Canis l. familiaris* | GC9 | 1110 | 99.5 cf2r4 |

**Table 2** (*contd.*)

| Name | Scientific name | Chromosome /genomic scaffold | Length | High ID (to) |
|---|---|---|---|---|
| Cfr3 | *Canis l. familiaris* | GC10 | 1278 | 88.5 Ssr3, hsr3 |
| Cf1r4 | *Canis l. familiaris* | GC8 | 1068 | 90.8 cfr1 |
| Cf2r4 | *Canis l. familiaris* | GC8 | 1110 | 99.5 cfr2 |
| Cfr5 | *Canis l. familiaris* | GC6 | 1299 | 78.5 ecr5 |
| Hsr1 | *Homo sapiens* | GC14 | 1176 | 99.5 ptr1 |
| Hs1r2 | *Homo sapiens* | GC17 | 1110 | 99.6 pt1r2 |
| Hs2r2 | *Homo sapiens* | GC17 | 1110 | 99.6 pt2r2 |
| Hsr3 | *Homo sapiens* | GC22 | 1257 | 99.2 ptr3 |
| Hsr4 | *Homo sapiens* | GC20 | 1167 | 99.0 hs2r4 |
| Hs2r4 | *Homo sapiens* | GC20 | 1170 | 99.0 hsr4 |
| Hs3r4 | *Homo sapiens* | GC20 | 1155 | 98.7 hs2r4 |
| Hsr5 | *Homo sapiens* | GC16 | 1095 | 98.5 ptr5 |
| Ptr1 | *Pan troglodytes* | GC14 | 1176 | 99.5 hsr1 |
| Pt1r2 | *Pan troglodytes* | GC17 | 1110 | 99.6 hs1r2 |
| Pt2r2 | *Pan troglodytes* | GC17 | 1110 | 99.6 hs2r2 |
| Ptr3 | *Pan troglodytes* | GC22 | 1257 | 99.2 hsr3 |
| Ptr4 | *Pan troglodytes* | GC20 | 1167 | 98.6 hs2r4 |
| Ptr5 | *Pan troglodytes* | GC16 | 1095 | 98.9 hsr5 |
| Mur1 | *Macaca ulatto* | GC7 | 1176 | 98.4 ptr1 |
| Mu1r2 | *Macaca ulatto* | GC16 | 1010 | 91.0 mu2r2 |
| Mu2r2 | *Macaca ulatto* | GC16 | 1110 | 98.0 hs1r2, hsr2 |
| Mu1r3 | *Macaca ulatto* | GC10 | 1257 | 95.8 hsr3 |
| Mu2r3 | *Macaca ulatto* | GC10 | 1815 | 69.3 mu1r3 |
| Mur4 | *Macaca ulatto* | GC10 | 1167 | 95.2 ptr4 |
| Mur5 | *Macaca ulatto* | GC20 | 1184 | 88.6 hsr5, ptr5 |
| Btr1 | *Bos taurus* | GS2931 | 1173 | 95.6 ssr1 |
| Btr2 | *Bos taurus* | GC19 | 1107 | 92.5 ssr2 |
| Btr3 | *Bos taurus* | GC5 | 1482 | 79.3 ecr3 |
| Btr4 | *Bos taurus* | GC19 | 813 | 72.9 cf1r4 |
| Btr5 | *Bos taurus* | GC25 | 1107 | 94.8 oar5 |
| Oar1 | *Ovis aries* | Unknown | 1400 | 69.7 cf1r4 |
| Oar2 | *Ovis aries* | Unknown | 212 | 28.2 xlr3 |
| Oar5 | *Ovis aries* | Unknown | 1104 | 94.8 btr5 |
| Ecr1 | *Equus caballus* | GC1 | 1449 | 75.3 hsr1 |
| Ecr2 | *Equus caballus* | GC11 | 1051 | 89.4 cf2r4 |
| Ecr3 | *Equus caballus* | GC28 | 1476 | 79.3 btr3 |
| Ecr4 | *Equus caballus* | GC1 | 1449 | 70.0 cf1r4 |
| Ecr5 | *Equus caballus* | Unknown | 1098 | 88.9 btr5 |

(EAW89112), SSTR3 (NM_001051), SSTR4a (EAX10168), SSTR4b (EAX10169), SSTR4c (EAX10170) and SSTR5 (NM_001053); *Ovis aries* (sheep) SST (NM_001009196), SSTR1 (AJ314853), SSTR2 (AF335550) and SSTR5 (NM_001009265); *E. caballus* SST (XM_001499693), SSTR1 (XM_001492597), SSTR2 (XM_001498230), SSTR3 (XM_001499436), SSTR5 (LOC100067398); *P. troglodytes* SST (XM_526419), SSTR1(XM_522831), SSTR2a (XM_001167728), SSTR2b (XM_511653), SSTR3a (XM_001160416), SSTR3b (XM_001160468), SSTR4 (XM_525282) and SSTR5 (XM_510725); *M. mulatta* SST (XM_001103516), SSTR1a (XM_001091300), SSTR1b (XM_001091429), SSTR2a (XM_001085452), SSTR2b (XM_001085574), SSTR3a (XM_001086007), SSTR3b (XM_001086124), SSTR4 (XM_001095303) and SSTR5 (XR_010595); *M. domestica* (gray short-tailed opossum) SST (XM_001362104), SSTR1 (XM_001379121), SSTR2 (XM_001378502), SSTR3 XM_001376353.1, SSTR4 (XM_001382059.1) and SSTR5 (XM_001373774.1); *D. rerio* SST (AJ238017), SSTR1a (XM_680740.2), SSTR1b (XM_691574.2), SSTR2 (XM_689793.2), SSTR3 (XM_690273.2), SSTR4a GS12224236-12224763), SSTR4b (GS46643961-46644788), SSTR4c (8218146-8218979), SSTR5 (XM_683665.1); *C. auratus* SST1 (U40754), SST2 (AF025686), SSTR1a (AF097726), SSTR1b (AF097727), SSTR2 (AF139597), SSTR3a (AF311307), SSTR3b (AF252879), SSTR5b (AF272949) and SSTR5c (AF472593); *X. tropicalis* SST (BC135242), SSTR1 (512538-513423), SSTR2a (448935-449867), SSTR2b (1792971-1793883), SSTR3 (449358-449883), SSTR4a

(448953-449892), SSTR4b (512541-513339) and SSTR5 (448912-449906) and *G. gallus* SST (NM_205336), SSTR1 (NM_001113167.1), SSTR2 (AY954511), SSTR3 (DQ003337), SSTR4 (DQ069274) and SSTR5 (AY954512). Cortistatin included human (NM_001302), mouse (NM_007745 XM_905725), rat (NM_012835), cow (XM_001250130), chimpanzee (GS 10612614-10613214), rhesus (GS 13537499-13538099), horse (GS 39006976-39008557), dog (GS 21179319-21179802), chicken (DQ279789), frog (AY319522) and zebrafish (NM_131727). The protein parsimony and nearest neighbour methods both yielded consistent trees. Genes were considered orthologous to a human SST or SSTR if they placed phylogenetically with SST or SSTR protein (bootstrap score of 70 or higher) and shared higher homology to an individual SST or SSTR (by amino acid identity) than any other known human protein or if they displayed characteristics unique to a particular human gene (figures 2 and 3).

## Results

To reconstruct the evolutionary history of the SST system and to provide a catalog of the SST system for important mammalian species, we sought to systematically identify all SST and SSTRs within the draft genomes of *D. rerio, C. auratus, X. tropicalis, G. gallus, M. domestica, H. sapiens, S. scrofa, B. taurus, M. musculus, R. norvegicus, C. l. familiaris, O. aries, E. caballus, P. troglodytes* and

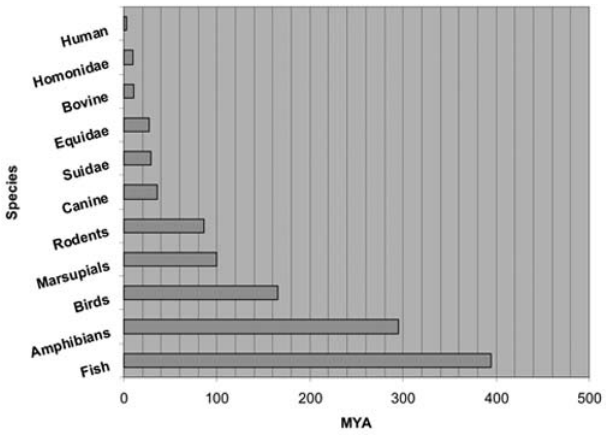


**Figure 1.** Phylogenetic history of somatostatin gene in different mammalian and non-mammalian vertebrates.

*M. mulatto*. We performed *de novo* SST and SSTRs gene prediction where necessary, otherwise, public databases and expressed sequence tag information was used to get SSTR and its receptors sequences. Orthology to human SST and SSTRs was determined by phylogenetic and discriminating feature analysis as described in materials and methods.

***SST and SSTRs in fishes, amphibians and birds***

In the current study, genes were named as follows: species abbreviation derived from its scientific name, s for somatostatin, cs for cortistatin and r for receptor, whereas digit before alphabets indicates isoform of somatostatin or its receptor, while digit after alphabets indicates type of receptor. For example, pt2r3 should be read as *Pan troglodytes* form two of receptor three. We identified and annotated all known and putative SSTs and SSTRs in the draft genome of the zebrafish (*D. rerio*), goldfish (*C. auratus*), frog (*X. tropicalis*), chicken (*G. gallus*), opossum (*M. domestica*), human (*H. sapiens*), pig (*S. scrofa*), cow (*B. Taurus*), mouse (*M. musculus*), rat (*R. norvegicus*), dog (*C. l. familiaris*), sheep (*O. aries*), horse (*E. caballus*), chimpanzee (*P. troglodytes*) and monkey (*M. mulatto*) (tables 1 and 2).

Unexpectedly, zebrafish SST1 molecule was more like mouse (48.1%) while somatostatin 2/cortistatin (SST2) was more identical to rat (44.8%). As for as SSTRs were concerned; in zebrafish SSTR1 and 4 had two and three forms, respectively, while the rest had only one form. SSTRs 2, 4 and 5 were highly identical to their counterparts in goldfish. Out of the two forms of SSTR1, dr1r1 (first) was highly identical to dr1r4 (receptor four isoform one) in zebrafish while second was identical to its counterpart in goldfish. Among three forms of SSTR4, first and second were more identical to each other (93.5%) but, third form (dr3r4) was identical to isoform two of receptor one (83.7) as shown in table 2.

Two forms of SST identified in goldfish were quite different from each other. SST1 isoform one was more identical to chicken while SST1 isoform two was more identical to human and mouse (table 1). We could not identify SSTR4 in goldfish while SSTRs 1, 3 and 5 had two, two and three isoforms, respectively, and SSTR2 had only one. SSTR2 was highly similar to its counterpart in zebrafish (85.6%) and same was true for two isoforms of receptor three. SSTR1 and SSTR5 both had two isoforms which were highly identical with each other (95.8% and 88.3%, respectively). SSTR5 isoform three was highly identical to its counterpart in zebrafish (SSTR5).

Frog SST1 was closer to that of birds (76.7%), but SST2/CST was more identical to mammals (52.5% with rat), as shown in table 1 and figure 4. In frog, there were two isoforms each of SSTRs 2 and 4 while SSTRs 1, 3 and 5 had only one form. SSTR1 was highly similar to receptor four isoform two (90.3%). SSTR2 isoform one was highly identical to receptor five (96.5%) and isoform two to chicken (56.0%). SSTR3 was more identical to isoform one of receptor four (55.7%) (table 2; figure 4). Although we identified five forms of somatostatin receptors, we could not clearly establish its orthology to birds or mammals (tables 1 and 2; figures 4 and 5). We identified SST and SSTRs genes in the draft genome of the chicken (*G. gallus*) (tables 1 and 2). Pair-wise alignment showed that SST1 was more identical to mammals (83.8% to dog and swine) while SST2/CST to its counterpart in frog (table 1).

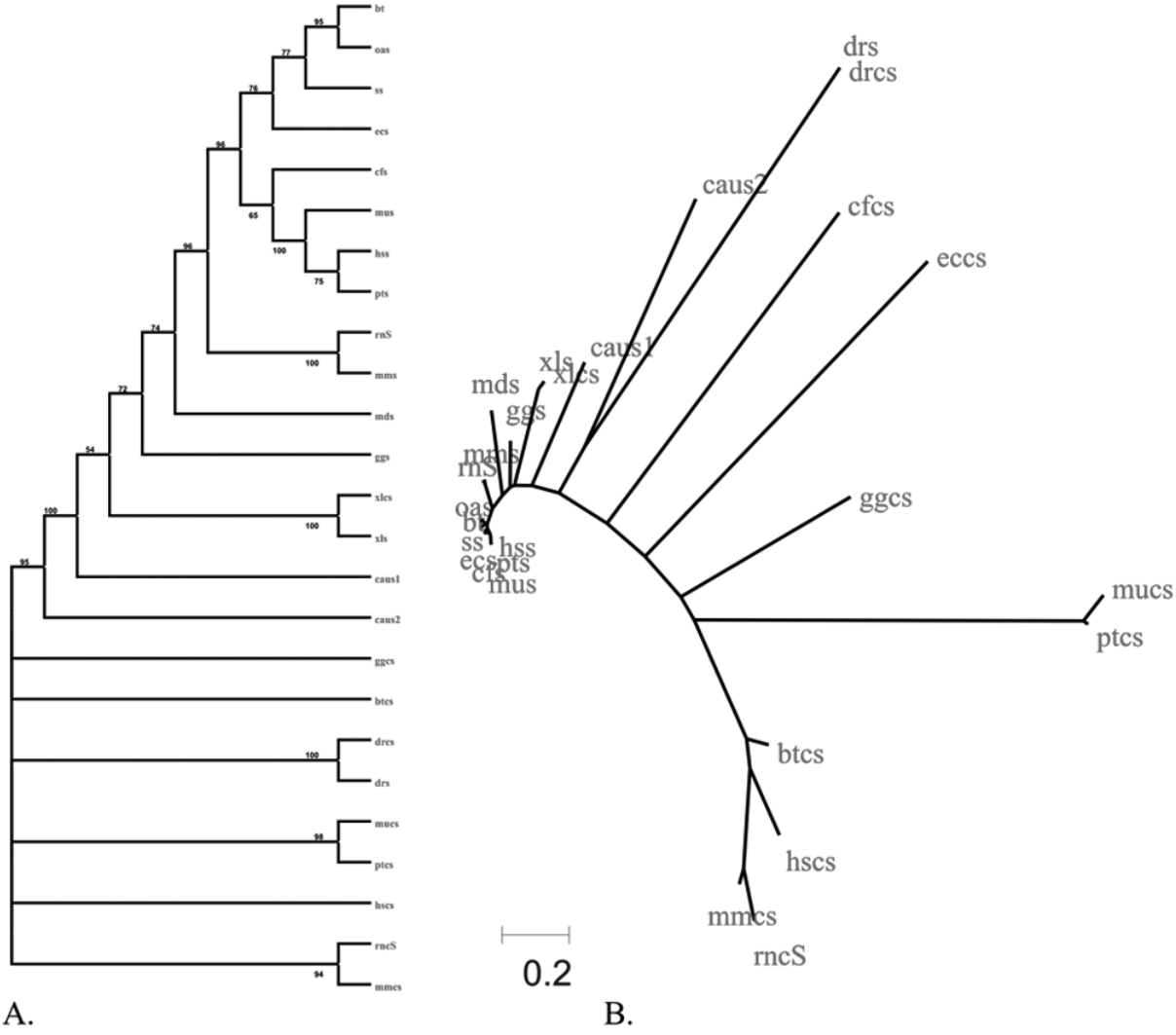


**Figure 2.** Phylogenetic tree for somatostatin genes. (A) Rooted tree with bootstrap value of 100. (B) Unrooted tree (abbreviation given should be read as first two letters showing the scientific name of animal e.g. 'hs' stands for *Homo sapiens*, 's' for somatostatin/SST1 and 'cs' for cortistatin/SST2.

Based on homology and phylogenetic placement, there were five receptors of somatostatin which were highly identical to mammals, SSTR1 and SSTR2 were highly identical to their counterparts in swine; SSTR3 and 5 were identical to their counterparts in opossum while SSTR4 to its counterpart in monkey (table 2).

***Somatostatin and somatostatin receptors in mammals***

Primate is the most recently evolved class of mammals present on the globe whose evolutionary history is 63 MYA. The infraorder Simiiformes contains the two parvorders including new world monkeys in one, and the old world monkeys, humans and the other apes in the other. This division took place about 40 MYA (Goodman *et al*. 1990). We identified and annotated all putative SST and SSTR genes in the draft genome of the human, chimpanzee and monkey (tables 1 and 2).

Human SST1 was 99.7% identical to its counterpart in chimpanzee while monkey somatostatin 1 was 98.6% identical to chimpanzee. So, as for as identity is concerned; the equation would be like this; monkey → chimpanzee → human. But we could not find such relationship in case of SST2/CST (table 1; figure 5). As for as SSTRs were concerned; each human, chimpanzee and monkey has five forms (SSTR1–5). SSTRs 2 and 4 have further two and three isoforms, respectively in human. In chimpanzee, SSTR2 had two isoforms and monkey, SSTR2 and 3 had two isoforms. Human SSTR2 isoforms one and two were more identical to their counterparts in chimpanzee (99.6%). Human SSTRs 1, 3 and 5 were highly identical to their counterparts in chimpanzee. Out of three forms of SSTR4, first and second were more identical to each other (99.0%) while third was highly identical to second

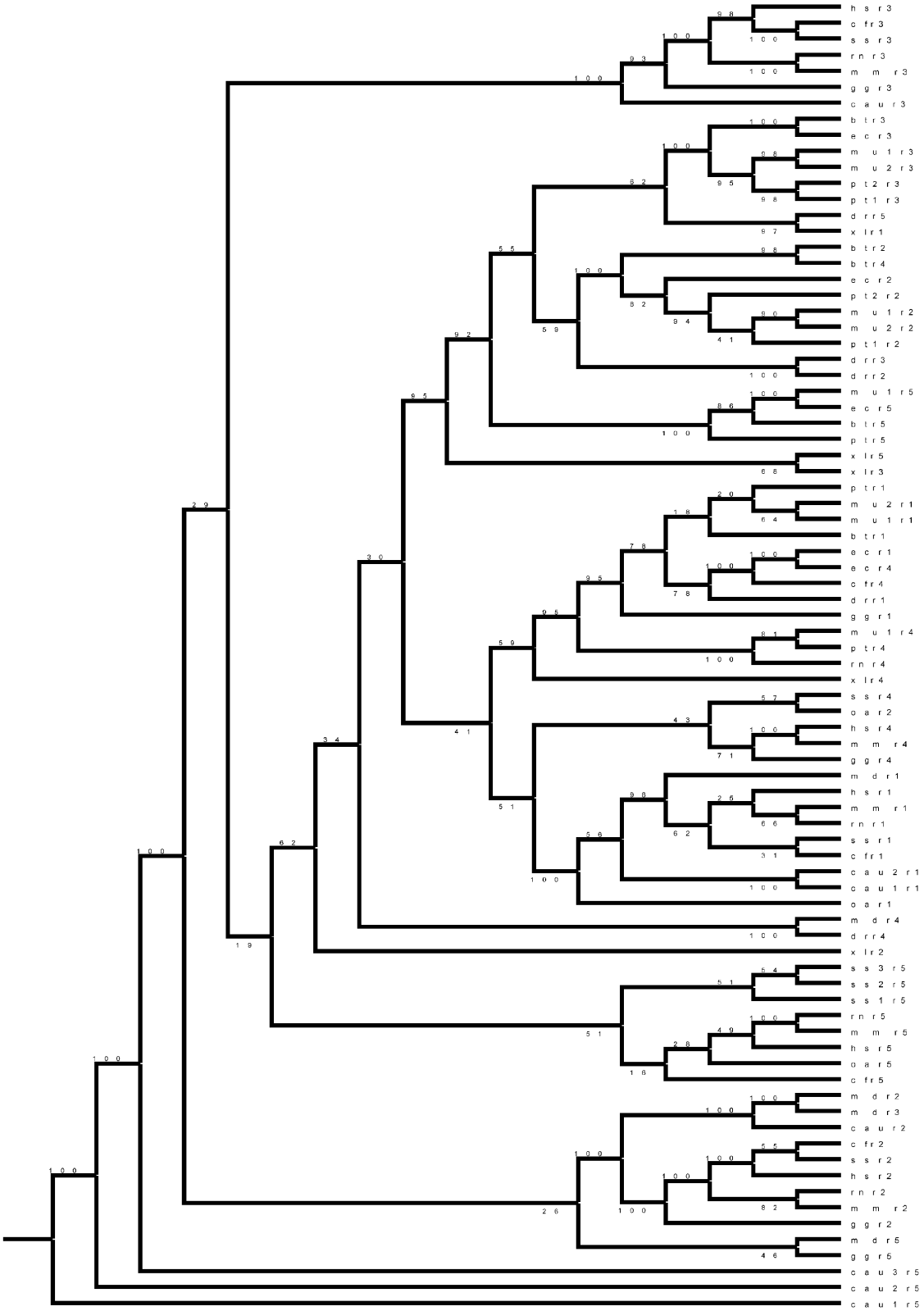


**Figure 3.** Phylogeny of putative somatostatin receptors. Bootstrap values (of 100) are shown (abbreviation given should be read as first two letters showing the scientific name of animal e.g. 'hs' stand for *Homo sapiens*, digit before 'r' which stands for receptor indicates the iso-form of receptor while digit after 'r' indicates receptor type).

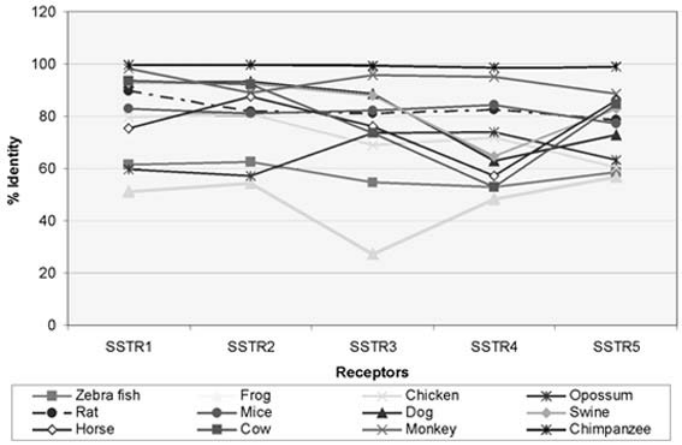


**Figure 4.** Closest homolog to human somatostatin receptors.

one (98.7%) (table 2). In chimpanzee, SSTR4 was highly identical to somatostatin receptor four isoform two in human (98.6%) as shown in table 2. In monkey, SSTR1, 4 and 5 were highly identical to their counterparts in chimpanzee while SSTR2 isoform two and SSTR3 isoform one were highly identical to their counterpart in human as indicated in table 2. SSTR2 isoform one and SSTR3 isoform two were more identical to SSTR2 isoform two (91.0%) and SSTR3 isoform one (69.3%), respectively.

In opossum, a primitive mammal, SSTR5 was identical to its counterpart in chicken while SSTRs 1, 2 3 and 4 were more identical to their counterparts in horse, dog, monkey and chimpanzee, respectively (table 2; figure 4).

Rat and mice (rodents) SST1, SST2/CST and SSTRs 1, 3, 4 and 5 were similar between the two species. Rat SSTR2 isoform one was identical to its counterpart in mouse (93.6%), but isoform two was identical to its isoform one (93.3%). Two isoforms of SSTR2 in mouse were 98.8% identical to each other (tables 1 and 2).

Among the SSTRs of family Bovidae (sheep and cow), only SSTR5 was similar between two species while all others were more identical to other mammals (table 2). Cow SST1 was more identical to its counterpart in sheep (98.6%) but, SST2/CST to rat (66.6%). SST1 was highly identical (97.4%) between Equidae (horse) and Suidae (pig). We could not identify SST2/CST in Suidae, and SST2/CST of Equidae was identical to mice. SSTRs differred in identity to other mammals in these species (table 2). Similarly in dog, SST1 was identical to that of horse but SSTRs were more diverse as far as their identity was concerned.

### *Phylogeny of somatostatin*

History of somatostatin in vertebrates is 395 MYA as shown in figure 1 with the most recent being in humans (3 MYA). Figure 2 shows the phylogenetic tree of somatostatin. In the rooted tree (figure 2,A), the bootstrap values are given and somatostatin 1 shows clade formation which is not in case of somatostatin 2. This is further explained in figure 2,B. In which, SST1 are grouped among different species while SST2 is more dispersed.

In figure 3, rooted tree of somatostatin receptors is shown, and figure 4 shows the relationship of human somatostatin receptors with other mammalian and non-mammalian species. Over all, chimpanze receptors are the most similar to that of human (figure 4).

### *Comparative genomic analysis of invertebrates failed to identify SST or STRs and SSTRs*

In order to trace back the origin of somatostatin to the members of chordate phylum, we blasted sea squirt (*C. intestinalis*) genome on http://genome.ucsc.edu, but failed to identify any of somatostatin or its receptors.

## Discussion

### *Evolutionary history of SST and SSTRs*

In our study, we found two members of somatostatin gene family: somatostatin and cortistatin. Somatostatin has been extensively studied among the vertebrate species, particularly in mammals while relatively few studies have focussed on cortistatin. In mammals, SS has two biologically active forms viz. SS14 and its NH2-terminal extension of 14 amino acids to give rise to SS28. Both SS14 and SS28 are encoded by a common gene and processed in a tissue-specific manner from a single precursor, preprosomatostatin I (PSSI) (Lin *et al*. 1998). SS14 is the most extensively studied (with the same amino acid sequence in all vertebrate species) form of somatostatin (Conlon *et al*. 1997). Cortistatin also has two forms icluding CST-17 and CST-29 that have striking commonality to bind to all somatostatinreceptor subtypes with high affinity (Baranowska *et al*. 2006).

Based on our current knowledge of somatostatin and its different forms in all vertebrate classes, we can confidently conclude that there are at least two somatostatin genes, encoding two distinct somatostatin isoforms, namely SS1 and

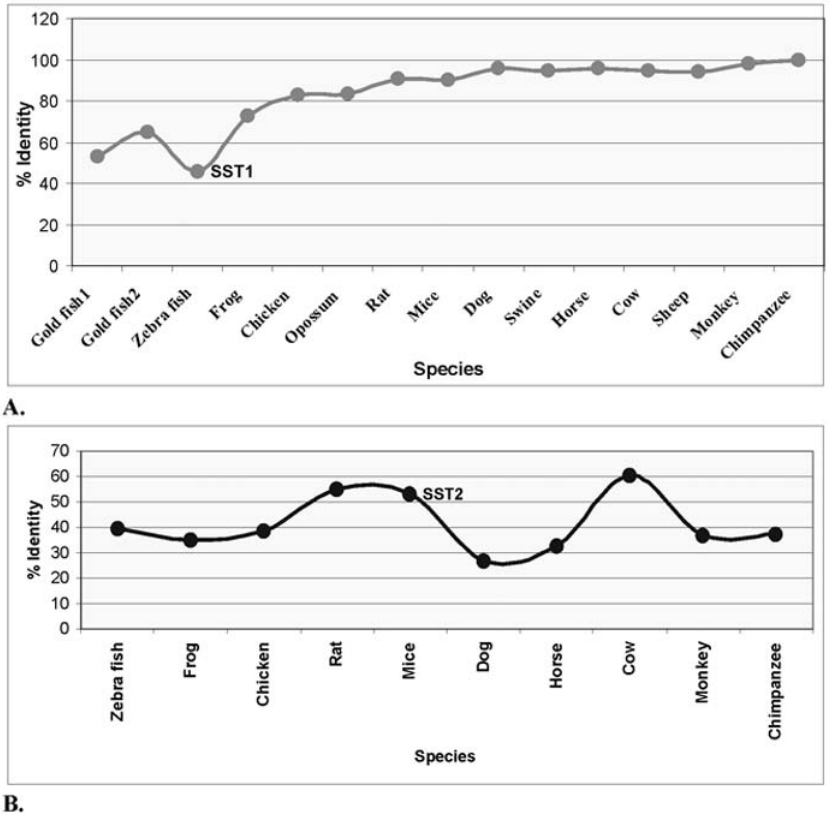


**Figure 5.** Closest homolog to human somatostatin A, somatostatin I B and somatostatin II/cortistatin.

somatostatin 2 (SS2), also termed cortistatin (CST), in mammals. Although, it is generally accepted that the SS2/CST gene arose through duplication of the SS1 gene some 450 MYA, Lin *et al*. (1999) found a third gene encoding another somatostatin isoform named somatostatin II (SSII) in teleost fish.

Sea squirts possess typical characteristics of vertebrates during some stage of their life i.e. vertebrate dorsal notochord in the larval stage and the rudiments of multiple organ systems. Consequently, they have been commonly used to examine evolutionary trends. The last common ancestor of sea squirts and mammals were around 650 MYA and represents an important stage in the evolution of vertebrates (Kunwar and Lehmann 2003). Sea squirt is the only extant invertebrate belonging to the Chordate phylum to have its genome completed today (Davidson *et al*. 2002), but we failed to identify somatostatin or any of its receptors in this member of Chordate group.

In fish, class Osteichthyes (ray-finned fish) are the most abundant vertebrate class and shared a last common ancestor with mammals some 450 MYA (Kumar and Hedges 1998). Zebrafish is a member of class Osteichthyes and has rapidly become an important model species for different scientific studies (Trede *et al*. 2004). The evolutionary history of somatostatin was very old as indicated by model animal zebrafish. In zebrafish somatostatin evolved 395 MYA (figure 1). This class of fish gives us the oldest history of somatostatin as well as maximum members of somatostatin gene family. In teleosts, in addition to PSSI, there is also a second somatostatin precursor named PSSII, a molecule that is thought to be processed to a large form of somatostatin with [Tyr7, Gly10]-SS14 sequence at its C-terminus. The first cDNA sequence of PSSII was identified in anglerfish (Hobart *et al*. 1980), and then in rainbow trout and goldfish, strengthening the notion that somatostatin arose from a multigene family (Moore *et al*. 1995, 1999; Kittilson *et al*. 1999). PSSIII, a third type of preprosomatostatin was first reported in frog (Vaudry *et al*. 1992; Tostivint *et al*. 1996) and then [Pro2]-SS14 was reported in sturgeon followed by African lungfish (Nishii *et al*. 1995; Trabucchi *et al*. 1999, 2002). These three different somatostatin genes (PSS) have been characterized in goldfish brain. Two of them encode for PSSI and PSSII, respectively, whereas the third cDNA encodes for a precursor abbreviated PSSIII with a SS14 variant [Pro2]-SS14 at its C-terminus (Lin *et al*. 1999). There are more than two genes of somatostatin in more than one members of teleost fish viz. goldfish and orange-spotted grouper (Xing *et al*. 2005), while there are four distinct genes in zebrafish (Tostivint *et al*. 2008). It is established that PSS2 and PCST (preprocortistatin) are derived from orthologous genes

on the basis of processing products of SS2 and CST exhibiting the Glyà→Pro substitution at position two, based on their exclusive expression in the brain and similarity of sequence (Tostivint *et al*. 2004).

Class Amphibia shares the last common ancestor with mammals living 360 MYA (Kumar and Hedges 1998). In vertebrate evolution, amphibians possess special importance because this class established the evolutionary shift to a terrestrial environment and marked the emergence of tetrapods. The evolutionary history of somatostatin is about 300 MYA in amphibians (figure 1). Tostivint *et al*. (1996) characterized two isoforms of somatostatin (SS1 and SS2) from frog brain for first time in 1996. These two forms of somatostatin induced a modest but significant reduction in cAMP formation in dispersed distal lobe cells of brain but, did not affect spontaneous growth hormone GH release (Jeandel *et al*. 1998); instead, the inhibition of GH by somatostatin was mediated through inhibition of synthetic human growth hormone releasing factor (hGRF). The hGRF could induce a significant increase in cAMP accumulation and GH release in frog brain. A possible reason for this mechanism is that somatostatin could affect release of pituitary hormones other than GH, as GH cells are exclusively located in the dorsal area of the frog adenohypophysis but, somatostatin receptors are present throughout the pars distalis.

A specific pattern for evolution of somatostatin was exhibited in current study. In each class of vertebrates, somatostatin evolution began about 50 MY after the evolution and emergence of respective vertebrate group which indicates that somatostatin might have been associated with an adaptive radiation for each individual group of vertebrates (figure 1). SST2/CST was evolved after SST1 and was more dispersed on a radiating phylogenetic tree as compared to SST1 which was grouped together almost in all species (figures 2 and 5). This indicates that SST2/CST might not have been descended from ancesters in various species, but rather evolved later on during the radiation of species. Alternatively, if it evolved from SST1 earlier in the evolutionary history, then initially it might have been a pseudogene which later evolved to a functional one.

Birds and mammals diverged from reptiles at least 310 MYA (Kumar and Hedges 1998). The evolutionary history of somatostatin in birds is 165 MYA. It is theorized and accepted by most of the evolutionists that the opossum was the most primitive ancestor of the mammals before the continental breakup of a southern landmass of Gondwanaland, after which the opossum developed into the various marsupials which exist today in Australia. The last common ancestor of mammals with class Metatheria existed on the globe about 125 MYA (Ji *et al*. 2002) while the phylogenetic history of somatostatin in mammals is 100 MYA (figure 1).

Somatostatin receptors are members of G protein coupled receptors family (GPCRs), which is very diverse and important to physiology, and also pursued as drug targets. There are about 1000 to 2000 members of this group in vertebrates, and the family comprises >1% of the human genome. Even invertebrates cannot live without them or may even require them more as compared to vertebrates and mammals because more than 1000 GPCRs make up to 5% of the nematode genome (Bargmann 1998), five times more than that of human genome. According to Fredriksson *et al*. (2003), the second family of the GRAFS classification system is the rhodopsin family. This is the biggest family so far and is further subdivided into four main groups including $\alpha$, $\beta$, $\gamma$ and $\delta$. The $\gamma$-group of rhodopsin receptors has three main types. Type I has somatostatins, opioids and galanin; type II has melanin-concentrating hormones (MCH), and finally type III has chymokines.

Gene duplication events occurred among GPCRs through creation of paralogons (Fredriksson *et al*. 2003). By definition, paralogons are the regions on certain sets of chromosomes that contain sets of homologous genes. One such group has been identified by Lundin (1993) that is located between human chromosomes four and five, and contains GPCRs as well as channel proteins, hormones, and enzymes. Human chromosome three contains chymokine receptors, angiotensin (AT1 receptor), and purinergic receptors (P2Y12, P2Y1) as a tandem array and can be paired with the X chromosome, which contains genes grouped as P2Y4, CXCR3, P2Y9, P2Y10, and then the AT2 receptor (Fredriksson *et al*. 2003). One likely explanation for this 'paralogous region' is whole chromosomal duplication. DeVries *et al*. (2005) studied such *en bloc* duplication in chymokine and chymokine receptors.

Here, in case of somatostatin and somatostatin receptors which are the members of GPCRs, as compared to paralogons as in case of other members of GPCRs discussed above, there is convincing evidence that tandem duplication (the copying of a chromosomal segment containing a locus to yield two adjacent copies) is a common source of gene duplication and likely played an important role in the formation of somatostatin and somatostatin receptor repertoires. Human chromosome 17 has two forms of receptor two, chromosome two has three forms of receptor four, and same chromosome has not only more than one isoform of a receptor but also has two or more different receptors, for example monkey chromosome 10 has two isoforms of receptor three and receptor four as well (table 2). In fact, examining individual genomes in different vertebrate classes demonstrates that tandem duplication is an ongoing process, with each species exhibiting somatostatin and somatostatin receptors apparently duplicated in a lineage-specific fashion. For example, two of the putative somatostatins of zebrafish occur in a single cluster on chromosome one and form their own phylogenetic group (table 1). Lineage-specific duplication is also an important factor in humans; multiple receptors from the chromosome two loci and corresponding ones from chromosome 17 are not present in chicken, frog, or fish genomes. Although we cannot discount the possible role of gene deletion in all non-mammalian species, the comparative genomic

analysis of multiple species suggests that a number of these genes are specific to the mammalian lineage.

In conclusion, if there is chance of *en bloc* gene duplication, then this is likely to have occurred through the creation of gene clusters (paralogons) as a result of *en bloc* duplication or chromosomal duplication, eventually leading to smaller clusters through gene duplication and chromosomal translocations. The more scattered the genes of family are, the further back in history the gene duplication took place. This seems to have occurred for somatostatin and other members of GPCRs, which do have many smaller clusters on many different chromosomes and often are located around the chromosomal ends (Fredriksson *et al*. 2003). However, some well known gene clusters such as the $\beta$-globin, Hox, and ParaHox gene clusters have remained intact for million of years (Brooke *et al*. 1998; Holland 1999). The phylogenetic analysis of patterns of gene duplications seen on the chromosomes suggest that somatostatin and somatostatin receptors like other members of GPCRs are derived from an ancestral source.

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