Identification and cloning of a unique somatostatin receptor in the brain of the spiny dogfish shark, *Squalus acanthias*

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Somatostatin receptors (sst, SSR) are present in numerous tissues, where their activation inhibits a wide variety of exocrine and endocrine secretions. They belong to the rhodopsin superfamily of G-protein coupled receptors, all of which contain seven transmembrane domains. Somatostatin receptors contain no introns. To date, five SSR subtypes have been described, each having specific agonist affinity, mechanisms of action, tissue distribution and function. There is a 39-57% amino acid sequence identity among the subtypes, with a highly conserved sequence in the seventh transmembrane domain[1]. In 1999, Florian Plesch in our laboratory obtained partial sequence of four somatostatin receptors in shark tissues (rectal gland and brain)[2]. We report here the full length cloning and analysis of one of these receptors from shark brain which we have designated as SSR3/5.

Tissue was extracted from adult *S. acanthias* brain, and total RNA was obtained by the standard TRIzol (Gibco BRL, Carlsbad, CA) method. To perform RACE PCR (Clontech BD Biosciences Marathon RACE PCR, Palo Alto, CA), messenger RNA was extracted and double stranded cDNA was produced using AMV reverse transcriptase (Super Script-II, BD Biosciences Clontech, Palo Alto, CA) followed by a Second-Strand Enzyme cocktail containing *E. coli* DNA polymerase I and *E. coli* DNA ligase. Finally, an adaptor was ligated to the double strand cDNA using a T4 DNA ligase. Degenerate primers were designed from our sst partial sequence using Codehop and gene specific primers were designed using DNA Star software.

The following degenerate primers were used on brain cDNA: *5'-GGGATTCTTTATTCCATTTACTATTATTTGTYTNTGYTAYHT-3' and 5'-TGAAAAATCCCATCAC AAAAGTGTANAYNAYRAA-3'*. A gradient PCR was run with initial denaturation at 95° for 2 min followed by 35 cycles of a denaturating step at 95° for 45 seconds, annealing temperature of 61° for 1 min- gradient +/- 10° (Gradient Mastercycler, Eppendorf), extension at 72° for 2 minutes. The receptor was isolated using gene specific primers that were designed based on a 522 bp known segment of the receptor[2]: for sst 3/5, *5'-GCCCCGGATAGCCAAGATG-3'* and *5'-TAAAACGGGAGCCAGCAAATC-3'* followed by *5'-CCCGCTGTTAATAATATGCCTCTG-3'* and *5'-CGTAAACCAGATGACTTGAC TTTGAT-3'*.

These primers yielded 5' 719 and 3' 522 bp fragments that were extracted from a 1% agarose gel (QIAquick Gel Extraction Kit, QIAGEN Sciences, Valencia, CA) and sequenced by the MDIBL sequencing facility and identified as known somatostatin receptors. Start to stop primers-*5'-* CAATGGACATGAGTACAGTTTTGTAGAGA *-3'* and *5'-* TGCAGTAAACGCT CATATTTAGCC -3' were used to amplify both reactions using the same cycle program, and sequence on both strands was confirmed. These were cloned into the Plac lacZ site of the pCR II-TOPO 4.0 kb (TOPO TA Cloning, Invitrogen, Carlsbad, CA) and sequenced again.

RACE PCR gave multiple bands (not shown). Nested PCR reaction using RACE PCR as substrate resulted in a 522 bp 3' band and a 719 bp 5' band. Both overlapped with our known partial sequences. Start to stop primers were designed and a PCR reaction yielded a 1074bp sequence (Figure 1) that had highest homology to both SSR3 and SSR 5 in other species.

Figure 1. Full length clone of the somatostatin 3/5 receptor (1074 bp) using shark brain as cDNA template.

The longest open reading frame predicted a 366 aa protein sequence, with molecular weight of 41117. The translated protein contains 7 TM domains. The highly conserved region in the 7th TM domain is conserved in this receptor as well (YANSCANPI/VLY). (Figure 2).

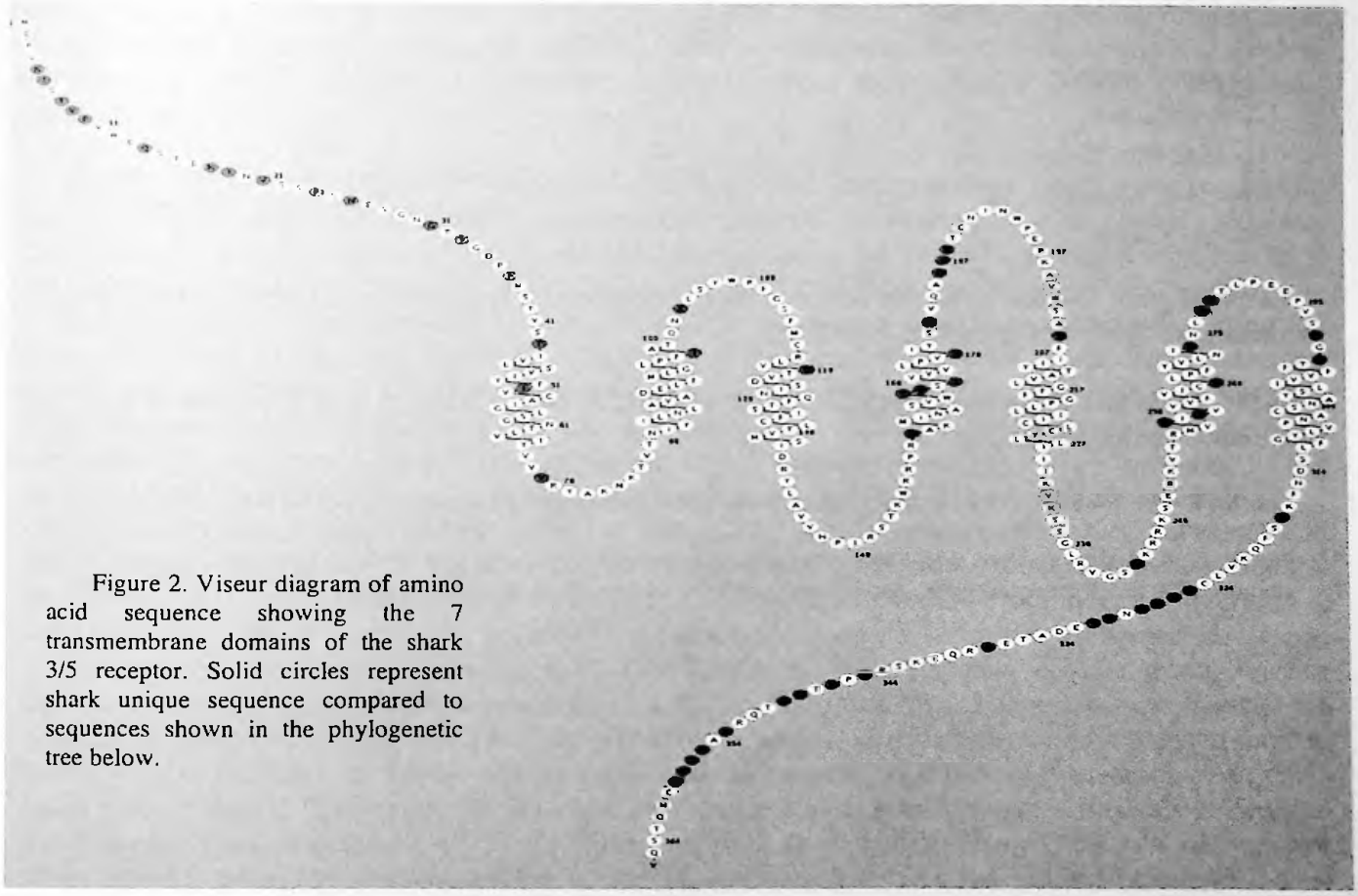

Figure 2. Viseur diagram of amino acid sequence showing the 7 transmembrane domains of the shark 3/5 receptor. Solid circles represent shark unique sequence compared to sequences shown in the phylogenetic tree below.

As expected, the highest homology to mammalian receptors was in the transmembrane domains where only 14 of 140 (10%) amino acids were unique. In constrast, 33% of the amino acids in the N and C termini were unique in shark. This protein had highest homology with SSR from *Takifugu*

rubripes (75% homology, 62% identity). Compared to mammalian SSRs, the highest homology was with SSR3 from human (58%), followed by SSR5 from rat (57%), SSR5 from human (56%, SSR3 from rat (49%) and SSR3 from mouse (47%).

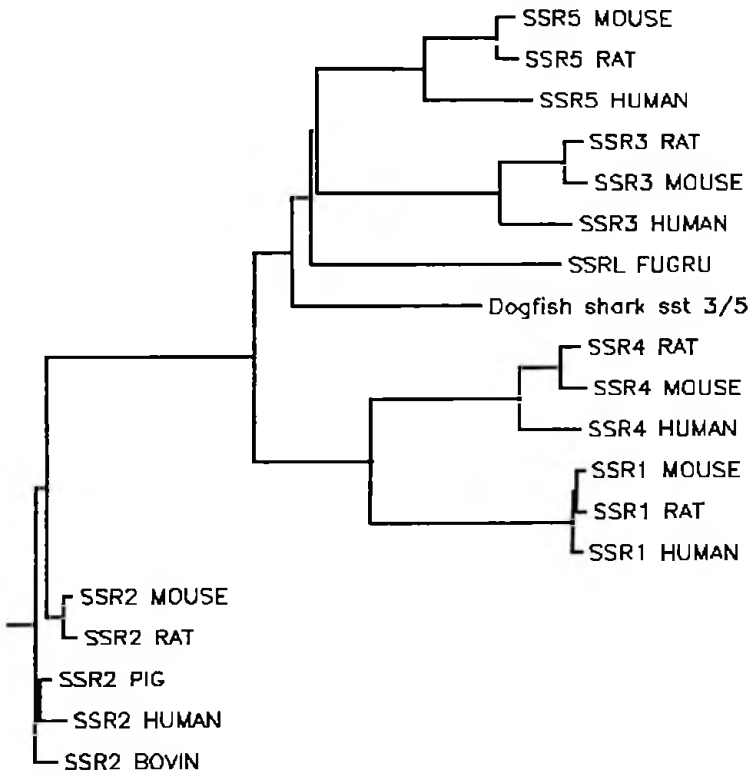

Figure 3: The shark somatostatin receptor roots tree of SSR 3 and 5 subtypes.

A phylogram (Figure 3) shows that this shark somatostatin receptor forms the root of the tree for mammalian SSRs 3 and 5 and the fugu SSR.

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1. **Lin, X., R. Peter**, Somatostatins and their receptors in fish. *Comparative Biochemistry and Physiology Part B* 129: 543-550, 2001.
2. **Plesch, F., C. Smith, S. Aller, J.N. Forrest Jr**. Identification and partial sequence of six somatostatin receptors expressed in the shark rectal gland, shark brain, and skate kidney. *Bull. Mt Desert Isl. Biol. Lab.* 38: 107-109, 1999.