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Discovery of a Novel Human G Protein-Coupled Receptor Gene (*GPR25*) Located on Chromosome 1

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We amplified human genomic DNA by the polymerase chain reaction (PCR) using oligonucleotides based on the primary sequence of the genes encoding the somatostatin receptors (SSTR) and the somatostatin-like receptor gene *SLC-1*. One resultant DNA fragment was used to screen a genomic DNA library resulting in the isolation of a gene, *GPR25*, encoding an additional member of the G protein-coupled receptor family (GPCR). *GPR25* is intronless throughout its open reading frame (ORF) and encodes a protein of 360 amino acids. The receptor encoded by *GPR25* shares highest identity to the receptor encoded by *GPR15*, angiotensin II type 1A receptor, and somatostatin receptor 5. Northern analysis found no transcripts expressed in liver or any of the 12 brain regions analyzed. Fluorescence *in situ* hybridization analysis localized *GPR25* to chromosome 1q32.1. © 1997 Academic Press

Members of the superfamily of GPCRs are essential participants in certain biological signaling pathways, transducing extracellular stimuli to diverse intracellular responses. There have now been identified close to 200 unique human genes each encoding a distinct GPCR. We have continually sought to discover novel members of this family, and in particular opioid and other peptide-binding receptors, as their characterization and knowledge of their distribution will contribute significantly to our understanding of G protein-linked systems and may assist in novel endogenous ligand discovery. Recently, we cloned *SLC-1*, a gene encoding a receptor bearing sequence remarkably homologous to the 5 SSTRs (1). We are conducting experiments to determine whether the receptor encoded by *SLC-1* has a somatostatin-like pharmacology, although preliminary studies thus far have failed to show specific binding to somatostatin ligands. We have now performed a search for subtypes related to *SLC-1* and the somatostatin receptor genes using PCR with degenerate oligonucleotides based on regions of conserved sequence. Our methods have previously been successful in identifying many GPCR genes, including 19 other genes encoding receptors for which the endogenous ligand has yet to be identified, as follows: *APJ* (2), *GPR1*, *GPR2*, *GPR3* (3), *GPR4*, *GPR5*, *GPR6* (4), *GPR7*, *GPR8* (5), *GPR9*, *GPR10*, *GPR14* (6), *GPR15* (7), *GPR19* (8), *GPR20*, *GPR21*, *GPR22*, *GPR23* (9) and *SLC-1* (a.k.a. *GPR24*)(1). In this report, we describe the discovery and characterization of another gene in this series, *GPR25*, encoding for an additional member of the receptor family.

MATERIALS AND METHODS

Amplification of genomic DNA. Human genomic DNA was amplified by PCR using degenerate oligonucleotides designed based on the sequences encoding transmembranes (TM) regions TM3 (P1: 5′-CTGACCGYCATGRSCATTGACSGCTAC; Y = C or T, R=A or G, S = C or G) and TM7 (P2: 5′-GGGGTTGRSGCAGCTGTTGGCRTA) of somatostatin receptors and the receptor encoded by the somatostatin-related gene, *SLC-1*, recently discovered in our laboratory. The PCR conditions were as follows: denaturation at 95°C for 1 min, annealing at either 55°C, 45 °C, or 38 °C for 1 min and extension at 72°C for 2.5 min for 30 cycles, followed by a 7 min extension at 72°C. The resultant PCR products were phenol/chloroform extracted, precipitated with ethanol, phosphorylated with T4 polynucleotide kinase, and blunt-ended with Klenow enzyme. Subsequently, they were electrophoresed on a 0.5% low-melting point agarose and a fragment of the expected size was subcloned into the *Eco*RV site of pBluescript SK(−) (Stratagene, La Jolla, CA). Colonies were selected, plasmid DNA was purified, and the inserts sequenced.

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TABLE 1

Classification of Products Obtained from a Search for Genes Encoding for Novel GPCR

Receptors	Annealing temperature 55°C	45°C	38°C
Clone 37	5	4	0
GPR24 (SLC-1)	13	36	1
SSTR2	5	0	0
SSTR3	1	6	6
SSTR4	0	0	1
SSTR5	4	15	5
GPR7	13	22	5
GPR14	1	0	1
Bradykinin B2	1	0	0
Olfactory I7	0	0	1
Non-GPCR encoding fragments	4	5	10
Total	47	88	30

Note. Human genomic DNA was amplified by PCR using primers P1 and P2 at three different annealing temperatures.

Library screening. DNA fragments radiolabelled with [^{32}P]dCTP (ICN) by nick translation (Amersham) were used as a probe to screen a λ EMBL3 SP6/T7 human genomic library (Clontech, Palo Alto, CA). Positive phage clones were plaque purified, DNA was prepared, restriction enzyme digested, electrophoresed on an agarose gel, transferred to nylon membrane, and hybridized with the same probe used to screen the library, as described by Marchese *et al*, 1994 (3).

Northern blot analysis. mRNA from several human tissues were extracted as described previously (3). Briefly, total RNA was extracted by the method of Chomczynski and Sacchi (10), and poly(A)$^+$ RNA was isolated using olig-dT cellulose spin columns (Pharmacia). RNA was denatured and size fractionated on a 1% formamide agarose gel, transferred onto nylon membrane and immobilized by UV irradiation. The blots were hybridized with a [^{32}P]-labeled DNA fragment, washed with 2X SSPC and 0.1% SDS at 50°C for 20 min and with 0.1X SSPE and 0.1% SDS at 50°C for 2hr and exposed to X-ray film at −70°C in the presence of an intensifying screen.

Fluorescence in situ hybridization (FISH). FISH of metaphase spread chromosomes derived from human lymphocytes together with DAPI banding patterns was used to map *GPR25* to its chromosome, as described (11, 12).

RESULTS AND DISCUSSION

Our recent discovery of a somatostatin-like gene *SLC-1* (1) prompted us to perform a search for related receptor genes. Using degenerate oligonucleotides based on TM3 and TM7 of the receptors encoded by *SLC-1* and the somatostatin receptor genes, human genomic DNA was amplified by PCR at various annealing temperatures to search for GPCR-encoding fragments. The DNA fragments were sequenced and analyzed for homology with known GPCRs (see Table 1 for summary). The overall population of clones yielded suggested that the oligonucleotides were not optimal, since not all the SSTR genes were amplified. One fragment, clone 37, partially encoded a novel member of the GPCR family and was used to screen a human genomic library to obtain the full-length ORF. Six positive phage clones were plaque purified, restriction enzyme digested and analyzed by Southern blot. A 4 kb *Pst*I fragment detected in two of the phage clones was subsequently isolated and found to contain the gene. This genomic clone, named *GPR25*, with overlapping sequence with the

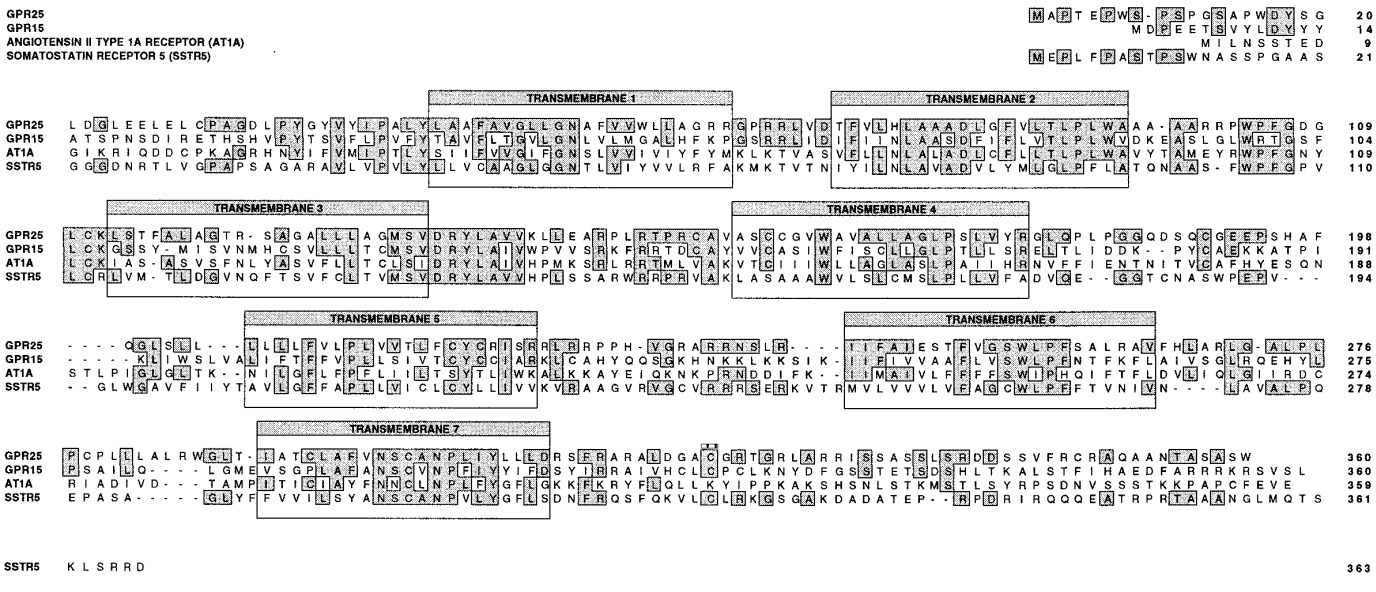

FIG. 1. Amino acid comparison of the receptor protein encoded by *GPR25* with receptors exhibiting greatest primary sequence identity. Amino acids identical to the protein encoded by *GPR25* are boxed and shaded. The predicted seven transmembrane domains are indicated. Gaps (−) have been introduced to maximize the alignments among the sequences. The putative site for palmitoylation is indicated (ii/home2/milesgrsym/8point/gs147,l(2,0)).

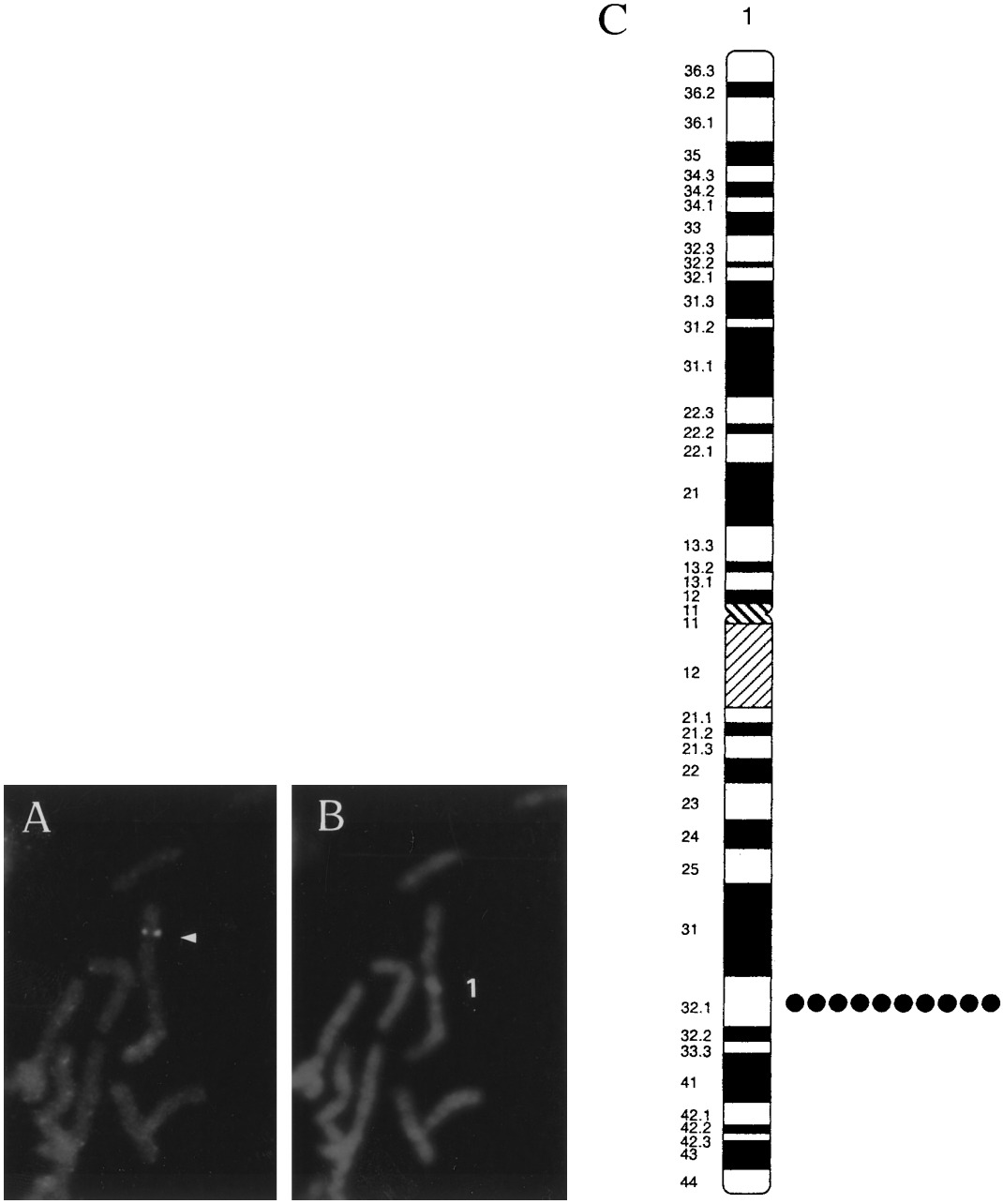

FIG. 2. FISH analysis of *GPR25*. (**A**) Results of metaphase spread chromosomes probed with a phage clone encoding *GPR25*. The arrowhead points to the FISH signals on a pair of chromosomes. (**B**) DAPI banding patterns were used to identify the chromosome and localization. (**C**) Summary of the FISH data. Each dot represents the location of a fluorescent signal on the chromosome.

PCR product, contained an intronless ORF of 1080 bp, encoding a protein of 360 amino acids. The initiation methionine conforms to the Kozak consensus sequence (13) and is preceded by an in-frame stop codon. Hydropathy analysis indicated seven putative TM regions, a characteristic feature of GPCRs. The protein encoded by *GPR25* contains no N-linked glycosylation consensus sites in the amino terminus or in any of the external loops. A cysteine residue Cys322 was found in the carboxy tail in an analogous position to other GPCR that has been demonstrated to be palmitoylated (14).

BLAST analysis (15) showed the receptor encoded by *GPR25* was related to the receptor encoded by *GPR15* (41% TM regions, 30% overall), followed by angiotensin II type 1 receptor (16) (38% TM regions, 27% overall) and somatostatin receptor 5 (17) (34% TM regions, 33% overall) (Fig. 1). The identity to the other somatostatin receptors and the *SLC-1*-encoded receptor is lower.

Northern blot analysis of poly(A)$^+$ mRNA extracted

from human tissues revealed no expression of *GPR25* in liver, or in the 12 brain regions examined: basal forebrain, frontal cortex, thalamus, hypothalamus, amygdala, caudate, putamen, hippocampus, midbrain, medulla, cerebellum and pituitary (data not shown). Many of these tissues had been analyzed using *GPR15* as a probe and also found not to express this related receptor(7).

FISH of human metaphase spread chromosomes was used to identify the specific chromosomal localization of *GPR25*. The phage clone was biotinylated and used as a probe for FISH mapping. Of 100 mitotic figures checked, 97 displayed signals on one pair of chromosomes (Fig. 2A). Since the DAPI banding was used to identify the specific chromosome, the assignment between signal from probe and the long arm of chromosome 1 was obtained. The detailed position was further determined based on the summary from 10 photographs (Fig. 2B). There were no additional loci picked by FISH detection under the conditions used. Therefore, *GPR25* is located at human chromosome 1, region q32.1 (Fig. 2C), in close proximity to other GPCR genes, adenosine A1 receptor (18) at q32.1, and muscarinic 3 cholinergic receptor (19) at q41-q44.

In summary, we have discovered an additional member of the GPCR gene family. The significant amino acid identity between the receptor encoded by *GPR15* suggests that the receptor encoded by *GPR25* may bind a similar endogenous ligand. These two related receptors likely represent the first of a subfamily of receptors predicted to bind an endogenous peptide ligand.

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