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Short Sequence-Paper

Molecular cloning of a fish gene encoding a novel seven-transmembrane receptor related distantly to catecholamine, histamine, and serotonin receptors ☆

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Abstract

A genomic DNA fragment encoding a G protein-coupled seven-transmembrane receptor was isolated from Medaka fish, *Oryzias latipes*. The encoded protein is similar in sequence to other receptors including catecholamine, histamine and serotonin receptors. However, the similarity is much lower than those among members of these receptor subfamilies, thus suggesting this seven-transmembrane receptor to be an orphan receptor whose ligand has not yet been identified. Genomic Southern blot analysis suggested that the fish genome contains additional receptor genes related to the isolated gene, indicating that this novel receptor, possibly with its related receptors, might constitute a novel subfamily of the seven-transmembrane receptor superfamily.

Keywords: G protein-coupled receptor; Seven-transmembrane domain receptor; Transmembrane domain; Gene cloning; (*Oryzias latipes*)

G protein-coupled receptors with seven transmembrane domains (TMD) are known to be activated by various extracellular stimuli including light, hormones and neurotransmitters [1–3]. These receptors are divided into a number of subfamilies in terms of primary structures, molecular natures of ligands and intracellular signalling pathways [1–3]. Among them, several subfamilies respond to extracellular ligands synthesized from amino acids, exemplified by catecholamines and serotonin. Although molecular, cellular and pharmacological mechanisms involving such ligands and receptors have been extensively studied in mammals [1–3], their universality has not always been elucidated in other animals including other vertebrate genera.

In order to identify and characterize genes encoding G protein-coupled seven-transmembrane receptors in fishes, we synthesized several oligonucleotide probes which were based on well-conserved amino acid sequences corresponding to TMDs 3, 6 and 7 of adrenergic receptor subfamilies [2,4–6]. Next, we screened a genomic DNA library of Medaka fish, *Oryzias latipes*, using each of the probes [7]. By using a probe corresponding to TMD 6 (5′-GCNA(A/G)(A/G)AA(A/G)AANGGNA(A/G)CC-A(A/G)CA-3′, antisense), we have obtained three clones encoding 7 TMD receptors until now. Two of them appeared to encode homologues of mammalian adrenergic and dopamine receptors (data not shown), and the other clone, termed λAR31, encoded a 7 TMD receptor whose sequence is novel as described below.

As shown in Fig. 1, the nucleotide sequence of a DNA fragment of λAR31, positive to the TMD 6 probe, contains a single open reading frame of 428 amino acid residues which is not interrupted by any introns as in the case of many 7 TMD receptor genes [3]. Computer search using a protein data base (Swiss Prot., rel. 28) yielded a number of known proteins showing significant similarity, which belonged to various subfamilies of 7 TMD receptors. Relatively higher similarity to the newly identified gene product (p47MNR; 47kDa Medaka Novel Receptor), was observed for catecholamine, histamine and serotonin receptors [8–10]. As shown in Fig. 2, the similarity was

☆ The nucleotide sequence reported in this paper has been submitted to the EMBL/GenBank/DDBJ Data Libraries under the accession number D43633.

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GAATTCACTTATGTTTTTGCTTTCAAGTTGATCCTAAATATTTGCATTAAAGTTGTGAAGGGGTTAAAGATCCCAGAAAATCGGATGCAT    90
GGAACTCGTAATATGAACTTACTTTAAGATAGTTGAAGTATTATTTTTGGGTTAGGAAGTCTTATTGTGTTTTTAGGAAAAGCCAAAGCG   180
ATGAAAGTGAGGTTGGATAATTTCTCCATTACTTTTACCAGTGGCAGTTCAATTACTGGTAGTAGTTTACACAAATGCCTGAACATAATC   270
AATCTATTTTGCTTTCTTTTCTGAGCACAGGAAATCCACATTCGCAAACATCTGAGATGCAGATGGAGGAAAGGCTGGAGGAATGATGGC   360
                                                 *                               M  M  A      3
TGACAAGACTAGCCCCATGATAACCTCTGATCATTCAATTTCTAATTTTTCCACTGGGTTATTTGGGCCCCATCCCACAGTCCCCCCTGA   450
 D  K  T  S  P  M  I  T  S  D  H  S  I  S [N  F  S] T  G  L  F  G  P  H  P  T  V  P  P  D     33
TGTGGGCGTCGTCACAAGCTCCCAGTCTCAAATGAAAGACTtGTTTGGGTTGTTCTGCATGGTAACGCTGAACCTCATAGCATTGTTGGC   540
 V  G  V  V  T  S  S  Q  S  Q  M  K  D  L  F  G  L  F  C  M  V  T  L  N  L  I  A  L  L  A     63
CAACACTGGCGTGATGGTGGCTATTGCTCGTGCCCCTCACCTCAAAAAGTTTGCATTTGTGTGTCACCTCTGTGCAGTGGATGTGCTGTG   630
 N  T  G  V  M  V  A  I  A  R  A  P  H  L  K  K  F  A  F  V  C  H  L  C  A  V  D  V  L  C     93
TGCCATCCTCCTCATGCCTTTGGGAATCATATCCAGCTCCCCCTTCTTTGGCACTGTAGTCTTCACCATTCTGGAATGTCAAGTTTACAT   720
 A  I  L  L  M  P  L  G  I  I  S  S  S  P  F  F  G  T  V  V  F  T  I  L  E  C  Q  V  Y  I    123
CTTCCTCAATGTTTTCCTCATTTGGCTGTCCATTCTCACCATCACAGCCATCAGTGTGGAACGtTATTTCTATATTGTACACCCCATGAG   810
 F  L  N  V  F  L  I  W  L  S  I  L  T  I  T  A  I  S  V  E  R  Y  F  Y  I  V  H  P  M  R    153
ATATGAGGTCAAAATGACCATAAACCTTGTTATTGGTGTCATGCTCTTGATCTGGTTCAAGTCCCTTCTCTTGGCTTTGGTTACGCTTTT   900
 Y  E  V  K  M  T  I  N  L  V  I  G  V  M  L  L  I  W  F  K  S  L  L  L  A  L  V  T  L  F    183
TGGTTGGCCACCTTACGGTCATCAGAGCTCCATTGCAGCCTCTCATTGTTCTCTGCATGCCAGTCACAGTCGGTTGAGAGGAGTCTTTGC   990
 G  W  P  P  Y  G  H  Q  S  S  I  A  A  S  H  C  S  L  H  A  S  H  S  R  L  R  G  V  F  A    213
CGTTCTGTTTTGTGTGATCTGCTTCCTGGCTCCTGTGGTGGTTATTTTTTTCTGTTTACTCCGCTGTGTACAAAGTAGCCCGCTCTGCAGC  1080
 V  L  F  C  V  I  C  F  L  A  P  V  V  V  I  F  S  V  Y  S  A  V  Y  K  V  A  R  S  A  A    243
TTTGCAGCAAGTTCCCGCTGTGCCAACATGGGCAGATGCAAGTCCTGCCAAAGATCGGTCAGACTCCATCAACAGCCAGACCACGATCAT  1170
 L  Q  Q  V  P  A  V  P  T  W  A  D  A  S  P  A  K  D  R  S  D  S  I  N  S  Q  T  T  I  I    273
CACCACCCGCACTTTACCCCAAAGACTATCCCCAGAGAGGGCCTTCAGTGGAGGCAAAGCTGCTCTCACCTTGGCATTCATTGTTGGCCA  1260
 T  T  R  T  L  P  Q  R  L  S  P  E  R  A  F  S  G  G  K  A  A  L  T  L  A  F  I  V  G  Q    303
GTTCTTAGTTTGCTGGCTGCCTTTTTTCATTTTCCACCTTCAAATGTCCCTCACGGGGTCCATGAAGAGCCCTGGAGACTTGGAGGAGGC  1350
 F  L  V  C  W  L  P  F  F  I  F  H  L  Q  M  S  L  T  G  S  M  K  S  P  G  D  L  E  E  A    333
AGTCAACTGGCTTGCCTACTCCTCATTTGCAGTCAACCCATCCTTCTATGGGCTGTTGAACAGACAAATCAGAGATGAACTGGTCAAGTT  1440
 V  N  W  L  A  Y  S  S  F  A  V  N  P  S  F  Y  G  L  L  N  R  Q  I  R  D  E  L  V  K  F    363
TCGACGTTGCTGCGTGACCCAACCTGTGGAGATCGGGCCATCCAGCCTAGAGGGCTCGTTTCAGGAAAACTTTCTTCAGTTTATTCAAAG  1530
 R  R  C  C  V  T  Q  P  V  E  I  G  P  S  S  L  E  G  S  F  Q  E  N  F  L  Q  F  I  Q  R    393
AACCAGCAGCTCATCAGAAACTCATCCAAGTTTTGCCAACTCCAATCCCAGAAACATGGAGAACCAAGCTCACAAGATTCCTGGGCAAAT  1620
 T  S  S  S  S  E  T  H  P  S  F  A  N  S  N  P  R  N  M  E  N  Q  A  H  K  I  P  G  Q  I    423
ACCAGAGGAGCAGGCTTAGACTTTGAACTGTGCAAATCATGGAATTCAGAGCTAATTTGTGGACCTTTCCTTTTCTGGATTTGTTGATCA  1710
 P  E  E  Q  A  *                                                                            428
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Fig. 1. Nucleotide and deduced amino acid sequences of a DNA fragment of λAR31 positive to the TMD 6 probe. The nucleotide and amino acid residues are numbered from the 5′-terminus of the DNA fragment and the putative initiation methionine, respectively. The probe-positive sequence is underlined, and a potential N-glycosylation sequence is boxed. Relevant in-frame termination codons are indicated by asterisks.

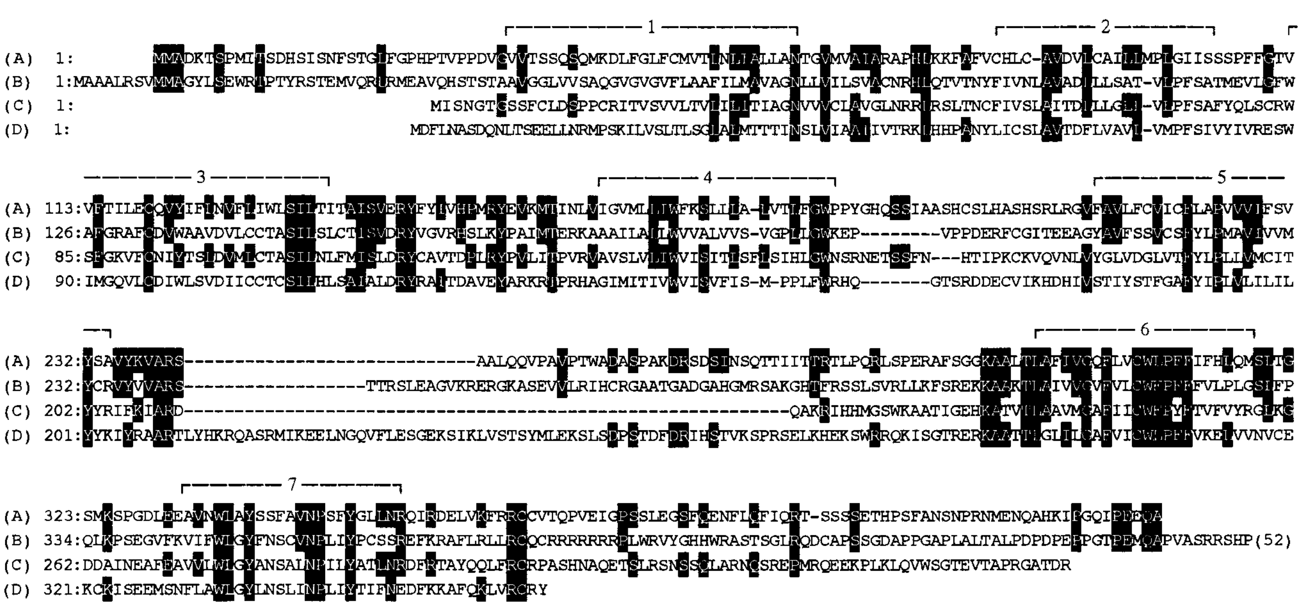

Fig. 2. Comparison of the amino acid sequences of Medaka p47MNR (A), human α1A adrenergic receptor (B) [8], dog histamine H2 receptor (C) [9], and mouse 5-hydroxytryptamine 1F receptor (serotonin receptor) (D) [10]. Residues identical to p47MNR are reversed. Seven transmembrane domains (TMD, 1–7) deduced by the hydrophobicity plot are shown above the sequences. Residue numbers are indicated at the right. C-terminal residues of human α1A adrenergic receptor are omitted and indicated by a parenthesis including the residue number omitted.

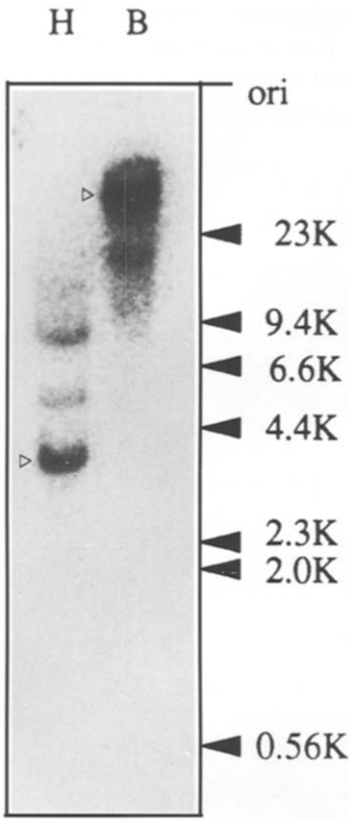

Fig. 3. Genomic Southern blot analysis of p47MNR gene. 10 μg each of Medaka genomic DNA was digested with *Hin*dIII (H) or *Bam*HI (B), electrophoresed in a 1% agarose gel, and blotted onto a nylon membrane [7]. The membrane was hybridized at 50° C with ^{32}P-labeled DNA probe for p47MNR (residues 2–1666, Fig. 1) and washed at 50° C in 2×SSC containing 0.1% SDS [7]. The bands corresponding to the isolated p47MNR gene are marked by open triangles at the left of the two lanes. Positions of length markers are shown at the right.

highest around TMD6 corresponding to the probe used for screening. However, other regions are largely different, and only marginal similarities were detected around the other TMDs between p47MNR and other receptors. In addition, two intracellular domains between TMD 1 and 2 and between TMD 3 and 4 contain also showed some sequence similarity. On the other hand, the other two intracellular domains and four extracellular domains were significantly diverged. On the whole, the similarity between p47MNR and these 7 TMD receptors was below 25%, which was much lower than those among vertebrate sequences belonging to the same subfamily of 7 TMD receptors (above 50%) [2,4–6,8–11]. That is, p47MNR is difficult to categorize as any of known 7 TMD receptor subfamilies. Consequently, this Medaka receptor seems to be novel in terms of the primary sequence, and there is a possibility that this is an orphan receptor whose ligand is unknown.

In order to examine if there are other related genes in the Medaka genome, genomic Southern blot analysis was carried out using the isolated gene as a probe at a less stringent condition [7]; hybridization temperature was 50° C and the final washing was carried out in 2 × SSC containing 0.1% SDS. As shown in Fig. 3, in addition to the positive bands corresponding to the isolated gene of p47MNR, a few weaker signals with different lengths were detected. The result indicates that other related genes would exist in the Medaka genome, suggesting that p45MNR and its related receptors might constitute a novel subfamily of 7 TMD receptors which is distantly related to other receptors such as adrenergic and serotonin receptors [1–3].

On the other hand, Northern analysis using total body mRNA of adult fishes did not yield a significant signal, suggesting that the mRNA is very low abundant.

Further physiological and biochemical studies are needed to know the physiological roles and ligand of this receptor. In addition, it may be also useful to identify and characterize homologues of p47MNR in other genera including mammal.

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