Molecular characterization of three forms of putative membrane-bound progestin receptors and their tissue-distribution in channel catfish, *Ictalurus punctatus*

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Abstract

Membrane-bound progestin receptors (mPR) were recently cloned and characterized as a new class of steroid receptors that transduce cell-signals through alteration of MAP kinase- and cAMP-dependent pathways. To further develop our understanding of this new class of steroid receptors, we characterized the cDNAs and genes of the α , β and γ forms of the channel catfish mPRs (lpmPR). The predicted α and β proteins have 49% sequence identity, whereas they only have 30% and 27% identities, respectively, with the γ form. Furthermore, lpmPR α and lpmPR β genes have similar structures featuring intronless coding regions, while lpmPR γ gene is composed of 8 exons and 7 introns. The 5'-flanking region of each lpmPR gene differs, but each contains putative transcriptional regulatory elements of factors known to influence reproductive physiology and endocrine disruption, for example, responsive elements for cAMP and steroids and the recognition sites for steroidogenic factor-1 and for the aryl hydrocarbon receptor. The lpmPR α gene was detected in all the tissues tested with relatively greater expression in brain, pituitary, muscle and testis. The expression of lpmPR β was much lower than that of lpmPR α and the transcript was predominantly observed in brain, pituitary, ovary and testis. In contrast, the lpmPR γ transcript was mainly detected in gill, ventral aorta, intestine, and trunk kidney. In conclusion, all the structural features of the lpmPRs strongly suggest that the closely related α and β forms are distantly related to the γ form. Additionally, regulatory features of the 5'-flanking regions and the differences in tissue-specific expression of each lpmPR gene suggest that they are involved in different endocrine functions in catfish.

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Introduction

Steroid hormones play important roles in many physiological processes, including reproduction. The classic mechanism of steroid action involves alteration of gene transcription, which typically is a relative slowly process, by diffusing into the cell and binding to ligand-activated transcription factors belonging to the nuclear steroid receptor superfamily (Beato 1989, Tsai & O'Malley 1994). Steroids have also been shown to exert rapid effects in many target tissues that are initiated at the cell surface by binding to steroid membrane receptors and frequently occur via nongenomic mechanisms (Falkenstein *et al.* 2000, Losel & Wehling 2003, Simoncini & Genazzani 2003). However, the identities of the steroid membrane receptors mediating most of these nonclassical actions remain unknown, and there is evidence for the existence of both unique receptors and nuclear receptor-like forms on the plasma membranes of steroid target cells (Bagowski *et al.* 2001, Losel & Wehling 2003, Xu *et al.* 2003).

Meiotic maturation of fish and amphibian oocytes is one of the most well-characterized nongenomic steroid

actions mediated by membrane-bound steroid receptors at both the biochemical and cytological levels (Yamashita *et al.* 2000). The precise mechanism for the activation and/or stabilization of the maturation-promoting factor by the maturation-inducing steroid (MIS) appears to differ among species. For example, the MIS-receptor (MIS-R), which in fish is a membrane-bound progestin receptor (mPR), appears to be coupled to an inhibitory G-protein (G_i) in three fish species: rainbow trout, Atlantic croaker and spotted seatrout, (Yoshikuni & Nagahama 1994, Thomas *et al.* 2002, Zhu *et al.* 2003a), whereas in zebrafish and an amphibian, *Xenopus*, it is likely that progestins activate a stimulatory G-protein (Gallo *et al.* 1995, Kalinowski *et al.* 2004). However, many other processes initiated through activation of the membrane-bound MIS-R, such as a decrease in intracellular cAMP levels, have been shown to be conserved (Yamashita *et al.* 2000).

Until recently, despite intensive research in several laboratories, efforts to characterize MIS-Rs in vertebrates at the molecular level have been unsuccessful (Maller 1998). For the first time in any species, a novel

mPR cDNA was recently isolated and characterized in a teleost fish, the spotted seatrout, and several lines of evidence suggest it is the MIS-R in this species (Zhu *et al.* 2003a). Subsequently, three forms of mPR (α , β and γ) have been identified in several other species of vertebrates, from fish to mammals (Zhu *et al.* 2003b). The finding that antisense oligonucleotides for both mPR α and β blocked MIS induction of oocyte maturation in zebrafish provided an initial indication that both mPRs may serve as the MIS-Rs in this species (Zhu *et al.* 2003a, Thomas *et al.* 2004). However, there are still many unanswered questions concerning the precise physiological roles of the three members of this new class of steroid receptors in fish and other vertebrates.

In this paper, we report the characterization of the channel catfish, *Ictalurus punctatus*, cDNAs encoding three forms of mPR (mPR α , β and γ), their genomic organization, the structures of their 5'-flanking regions, and their tissue distributions. Our findings clearly suggest that the catfish mPRs are related to the seatrout mPR and that the highly related α and β forms are involved in catfish reproductive physiology, but their roles as MIS-Rs remain to be demonstrated.

Materials and methods

Animals

All fish were maintained and tissue samples were taken in accordance with protocols approved by the University's Institutional Animal Care and Use Committee

Oligonucleotides and DNA sequencing

Oligonucleotide PCR primer synthesis and nucleotide sequence analysis were provided by the BioAnalytical Services Laboratory at the Center of Marine Biotechnology. Nucleotide sequences were determined with the PRISM Cycle sequencing kit using an ABI Model 377 DNA Sequencer (PE Applied Biosciences, Foster City, CA, USA).

Cloning of cDNA encoding putative channel catfish mPRs (IpmPRs)

Total RNA was extracted from ovary, testis and posterior kidney of channel catfish using Trizol Reagent (Life Technologies Inc, Gaithersburg, MD, USA), from which poly(A)+-RNA was purified with Straight A's mRNA isolation system (Novagen, Madison, WI, USA). The poly(A)+-RNA was reverse-transcribed into cDNA using PowerScript (BD Bioscience, Palo Alto, CA, USA) after priming with a clamped oligo(dT) primer. Three

sets of degenerate primers were designed to separately amplify each type of mPR by PCR (Table 1). The channel catfish mPR (IpmPR) α and β cDNA fragments were amplified from ovary and testis cDNA with mPRS1 and A1, and mPRS2 and A2, respectively, while the isolation of an IpmPR γ cDNA fragment was carried out using kidney cDNA with mPRS3 and A3 primers. The resultant PCR products were inserted into a TA cloning vector (pDrive; Qiagen, Valencia, CA, USA) and sequenced. Subsequently, 5'- and 3'-RACE of testis cDNA amplified the sequences of the 5' and 3' ends of each cDNA encoding IpmPR α and β using the SMART RACE cDNA Amplification kit (BD Bioscience) according to the manufacturer's instructions. Posterior kidney cDNA was used for the RACE PCR cloning of the cDNA for the γ form. Gene-specific primers, mPRA4 and S4, were used for 5'- and 3'-RACE to obtain full length cDNA of the α form. Due to low transcript abundance, nested RACE PCRs were required for the isolation of the β form cDNA. The first 5'- and 3'-RACE PCRs were conducted with mPRA5 and S5 and were followed by a second set of RACE PCRs using mPRA6 and S6, respectively. RACE PCRs for the γ form were carried out using mPRA7 and S7. Finally, to isolate amplicons, including the entire open reading frames for each type of IpmPR, PCRs were conducted with the following three sets of primers: mPRS8 and A8, mPRS9 and A9, and mPRS10 and A10 for IpmPR α , β and γ , respectively, using a proof reading PfuTurbo DNA polymerase (Stratagene, La Jolla, CA, USA). The resultant amplicons were cloned and sequenced as described above.

Phylogenetic analysis of the mPRs characterized to date was carried out by the neighbor-joining method using the Clustal W and njplot programs (Saitou & Nei 1987). Potential transmembrane domains, N-glycosylation sites and phosphorylation sites in mPRs were predicted by analyses posted on the web sites, www.ch.embnet.org/software/TMPRED_form.html (Hofmann & Stoffel 1993), www.cbs.dtu.dk/services/NetNGlyc/ and www.cbs.dtu.dk/services/NetPhos/ (Blom *et al.* 1999), respectively.

Characterization of genomic organization and the 5'-flanking regions of the mPRs

Genomic DNA was extracted from catfish brain using a Blood & Cell Culture DNA Starter Kit (Qiagen). A catfish GenomeWalker library was constructed with a Universal GenomeWalker Kit (BD Bioscience) according to the manufacturer's protocol. 'Genome walking' PCRs using the GenomeWalker library was carried out using Advantage-GC genomic polymerase and the gene-specific primers (mPRA11-A16) listed in Table 1 in order to characterize the 5'-flanking region of the three types of IpmPR genes. Typical PCRs using genomic

Table 1 Oligonucleotides and fluorescent probes used for PCR

Name	Nucleotide sequence	Use and its target DNA
mPRS1	5'-GTVTACCARTWYGGNAGYGC-3'	lpmPR α cDNA fragment
mPRA1	5'-TGGAAGANYTGRTGNCCYTG-3'	lpmPR α cDNA fragment
mPRS2	5'-CTGTTCRNGARCTNTAYAT-3'	lpmPR β cDNA fragment
mPRA2	5'-GGCACNGGRCANGARAARAA-3'	lpmPR β cDNA fragment
mPRS3	5'-ACYCTSAAYATDTGGACNCA-3'	lpmPR γ cDNA fragment
mPRA3	5'-CACACRTGRAANAGYGRTG-3'	lpmPR γ cDNA fragment
mPRS4	5'-ACAGTTCACAGCAGGGTGCACCGACCC-3'	3'-RACE for lpmPR α cDNA
mPRA4	5'-GCCCAGGGAACCAGCACTCTGGATGAG-3'	5'-RACE for lpmPR α cDNA
mPRS5	5'-CGGCTCTGACCTACCTCACCTGTAGCAC-3'	3'-RACE for lpmPR β cDNA
mPRA5	5'-GATCTTCCTGCGCAGCGGGTATGGACG-3'	5'-RACE for lpmPR β cDNA
mPRS6	5'-GTGCCCCTAGCCTGGCGTACATGTTGG-3'	3'-RACE for lpmPR β cDNA
mPRA6	5'-CAGAAGCGTAGTACAGCACTGATACCC-3'	5'-RACE for lpmPR β cDNA
mPRS7	5'-GGCACCTGGACGTTTCGACTTAGTAGGCC-3'	3'-RACE for lpmPR γ cDNA
mPRA7	5'-CAGGTGCCAGCCTCTCGGGCAGATGTG-3'	5'-RACE for lpmPR γ cDNA
mPRS8	5'-CACCTTCATCATGGCGACCGTGGTGATGG-3'	ORF of lpmPR α cDNA
mPRA8	5'-CGCCTTCCCAAGTCTCTCACTCCTGC-3'	ORF of lpmPR α cDNA
mPRS9	5'-CGCTGGACATAACCCAGAAAGGAGCTGATC-3'	ORF of lpmPR β cDNA
mPRA9	5'-CCCTCCAGAACTGGAGGTAAACTCCC-3'	ORF of lpmPR β cDNA
mPRS10	5'-GCAGTCTCACCAGCAACCTGGAACCTG-3'	ORF of lpmPR γ cDNA
mPRA10	5'-AAGCTCTAGTTGGTGCCCTTAGTTC-3'	ORF of lpmPR γ cDNA
mPRA11	5'-GACCTGAAGCATATACCTGAAAGGCC-3'	1st Genome walking for lpmPR α 5'-flanking region
mPRA12	5'-TGTTCCAGCGGGTGAGGCTCCTGTCA-3'	2nd Genome walking for lpmPR α 5'-flanking region
mPRA13	5'-AGGTCAACTGTCCATGTAGTGCACCTG-3'	1st Genome walking for lpmPR β 5'-flanking region
mPRA14	5'-GTAAGTGTCTGGTTCACAAGATGAAGCC-3'	2nd Genome walking for lpmPR β 5'-flanking region
mPRA15	5'-GTACATTGAAGACGGGAGGCAGCTTGA-3'	1st Genome walking for lpmPR γ 5'-flanking region
mPRA16	5'-CAGGTTCCAGGTTGCTGGTGAGACTGCC-3'	2nd Genome walking for lpmPR γ 5'-flanking region
mPRS11	5'-AGAGCGTATAAAAGGTCTGTCATAA-3'	TaqMan of lpmPR α
mPRA17	5'-CACGGTCGCCATGATGAA-3'	TaqMan of lpmPR α
mPRP1	FAM-5'-CTGATTGGCTCTTGAAAAGATCCCAGAAAGTTC-3'-TAMRA	Probe of lpmPR α for TaqMan
mPRS12	5'-GAGGCCGAATCCCTCCAT-3'	TaqMan of lpmPR β form
mPRA18	5'-CAGACGGCCTAAAACTCCACTT-3'	TaqMan of lpmPR β form
mPRP2	FAM-5'-CTGGACATAACCCAGAAAGGAGCTGATCACT-3'-TAMRA	Probe of lpmPR β for TaqMan
mPRS13	5'-GGCAGTATTCAACGGGAGGCAGTTATC-3'	TaqMan of lpmPR γ
mPRA19	5'-GTGAAGGCTAAACCCGCTAGCA-3'	TaqMan of lpmPR γ
mPRP3	FAM-5'-TGGCCTGTTACTCCAGATTTGCCGAA-3'-TAMRA	Probe of lpmPR γ for TaqMan
mPRS14	5'-TCTTCACTGCCTGGCTGAGATA-3'	TaqMan of DpmPR α form
mPRA20	5'-GAACAGCGCCCAATCTG-3'	TaqMan of DpmPR α form
mPRP4	FAM-5'-TCCTCACCATGGCGACGGTTGTG-3'-TAMRA	Probe of DpmPR α for TaqMan
mPRS15	5'-GAAGAGCTACTTTCTGCATAGAGTTTCA-3'	TaqMan of DpmPR β
mPRA21	5'-AGACGAGGCAACCAGTTCTGA-3'	TaqMan of DpmPR β
mPRP5	FAM-5'-TGCAGCAGCTGGGTCAAGTACCACAC-3'-TAMRA	Probe of DpmPR β for TaqMan

DNA or 'genome walking' PCRs were also conducted using proof-reading DNA polymerases (PfuTurbo DNA polymerase (Stratagene), Advantage-GC genomic polymerase or Advantage 2-GC polymerase (BD Biosciences)) and appropriate primers based on the cDNA sequences for each mPR under the thermal cycling conditions recommended by the manufacturer. Intron/exon boundary sites were determined by comparing the genomic sequence with the respective cDNA sequence.

Putative transcriptional factor binding sites located on the 5'-flanking region of each lpmPR gene were identified with the aid of analyses posted on the web site, <http://motif.genome.ad.jp/> with an 80% cut off score.

Additionally, consensus core sequences for transcriptional factor binding sites potentially related to reproductive physiology or endocrine disruption, i.e., cAMP responsive element (CRE: TKACGTMA), estrogen responsive element (ERE: AGGTCANNNTGACCT), glucocorticoid/progestin/androgen responsive element (GRE/PRE/ARE: AGAACANNNTGTTCT), steroidogenic factor-1 binding site (SF-1: TCAAGGTYA) and the responsive element for aryl hydrocarbon receptor and its nuclear transfer (AhR/Arnt: TYGGTG), were manually searched using software, DNASIS-Mac v3.5. All potential elements are more than 80% identical to their corresponding core sequences.

Gene-specific expression of catfish and zebrafish mPRs

Total RNA (1 µg) prepared from various tissues (i.e., parts of the brain (telencephalon, cerebellum, diencephalon, hypothalamus and hindbrain), pituitary, gill, ventral aorta, intestine, muscle, heart, spleen, liver, posterior kidney, head kidney and ovary) from a female adult catfish and from testis from a male adult catfish was primed with a clamped oligo(dT) primer and reverse-transcribed using M-MLV reverse transcriptase (Life Technologies Inc). Real-time, quantitative RT-PCR, (rtqRT-PCR; PE Applied BioSystems) was employed to measure transcript abundance of each *IpmPR* gene. The primers and probes used for each gene are listed in Table 1. The primer/probe sets for *IpmPR* α and *IpmPR* β were mPRS11, A17 and mPRP1, and mPRS12, A18 and mPRP2, respectively. The set of primer/probes for the γ form was mPRS13, A19 and mPRP3. Transcript abundance of each mPR was normalized to the abundance of catfish β -actin (GenBank accession number AY555575; Kazeto *et al.* 2003). The same type of analysis was conducted in zebrafish to examine the tissue-specific distribution of the zebrafish mPR (*DrmPR*) α and β genes. The primer/probe sets to measure transcript abundance of *DrmPR* α and *DrmPR* β by TaqMan were mPRS14, A20 and mPRP4, and mPRS15, A21 and mPRP5, respectively (Table 1). The detailed procedure to design and synthesize the primer/probe sets, and to validate similar rtqRT-PCR assays has been described elsewhere (Trant *et al.* 2001).

Identification of putative mPRs and their genomic organization in human and *Fugu*

BLAST searches of human and *Fugu* genome databases were conducted with each form of catfish mPRs using the analyses posted on the web site of The Wellcome Trust Sanger Institute (www.sanger.ac.uk) in order to identify cognate cDNAs and their genomic organization, and to compare them with the corresponding *IpmPR*s characterized in this report.

Results

Isolation of the cDNAs encoding *IpmPR*s

PCR-based isolation of cDNAs encoding putative *IpmPR* α (GenBank accession number AY533540), β (GenBank accession number AY533541) and γ (GenBank accession number AY533542) were completed with the aid of 5'- and 3'-RACE PCRs. The length of the nucleotide sequences of *IpmPR* α , β and γ are 2303, 3487 and 1186 bp, respectively. Coding regions of α , β and γ forms consist of 1062, 1068 and 1017 bp encoding 354, 356 and 339 amino acid residues, respectively. The degree of

amino acid sequence identity between the α form and the β and γ forms is 49% and 30%, respectively, while the β and γ forms have 27% sequence identity

Identification of potential cognate cDNAs of mPRs from human and *Fugu* genome databases

A BLAST search of the human and *Fugu* genome databases was carried out using the deduced amino acid sequences of catfish mPRs to identify the homologous genes. Six putative mPRs, including α (Ensembl ID. SINFRUP00000149401) and γ (ID. SINFRUP00000127469) forms reported previously (Zhu *et al.* 2003b) were retrieved from the *Fugu* database. *Fugu* mPR β (ID. SINFRUP00000160848) was also newly identified. Three other cognate forms named *Fugu* mPR-like protein (FmPRLP 1–3) appear to be novel forms. FmPRLP 1 (SINFRUP00000145530) and FmPRLP 2 (SINFRUP00000145528) are structurally closely related and share 78% identity. The predicted amino acid sequences of FmPRLP 1 and 2 show approximately 50%, 40% and 30% identities to *Fugu* mPR α , β and γ , respectively. The degree of identity between FmPRLP 3 (SINFRUP00000156015) and FmPRLP 1 and 2 is 44% and 38%. FmPRLP 3 shares 39%, 46% and 25% homology with *Fugu* mPR α , β and γ , respectively. Three forms of mPR α (ENSP00000329071), β (ENSP00000302785) and γ (ENSP00000260380) were identified by this search in the human genome as reported previously (Zhu *et al.* 2003b).

Structural and phylogenetic analysis of *IpmPR*s

Phylogenetic analysis of the mPR-related proteins clearly shows that the catfish mPRs are clustered together with their respective mPR types amongst the fish representatives and that the γ forms appear to be highly diversified from the other two groups (Fig. 1). FmPRLP 1 and 2 appear to be phylogenetically derived from mPR α , although they are not tightly clustered with fish mPR α s. In contrast, FmPRLP 3 is clearly placed together with fish mPR β , especially the *Fugu* form.

The amino acid sequences of each *IpmPR* and their related forms from the other species are aligned in Figures 2–4. The deduced amino acid sequence of *IpmPR* α showed high homology with other fish forms (higher than 70%) and 49–54% sequence identity with mammalian forms and FmPRLP 1 and 2. *IpmPR* β has 72% sequence identity to zebrafish mPR β and 48–55% identity to other mPR β -related forms, including FmPRLP 3. *Fugu* mPR γ shares the closest identity to *IpmPR* γ (71%). Transmembrane and lipophilicity analyses identified seven potential transmembrane domains in all mPR α - and β -related forms except for FmPRLP 1 and zebrafish mPR β that appeared to lack the sixth and second transmembrane domains,

Figure 1 Phylogenetic analysis of mPR-related proteins. The analysis was performed by the neighbor-joining method using full-length protein sequences. The numbers beside the branches indicate bootstrap values from 100 replicates.

respectively. IpmPR γ possesses seven potential transmembrane domains like mPR α and β , but other γ forms only have five or six potential transmembrane domains by this analysis.

One potential N-linked glycosylation site (N $\times$ S/T) located in the N-terminal extra-cellular region was identified in the α and γ forms, but not in the β forms. Several phosphorylation sites that are conserved among the fish forms and among all forms of each type of mPR were identified, however all sites are located in extra-cellular regions or transmembrane domains. Two highly conserved regions were identified in all forms of mPR α , β and γ (Figs 2–4). One region, composed of sixteen amino acid residues that span the N-terminal region and first transmembrane domain and includes a potential N-linked glycosylation site, is highly conserved and the identity between this region of IpmPR α and those of all other mPR forms is over 70%. The second conserved region (fifteen amino acids) is located on the putative boundary of the third cytoplasmic loop and sixth transmembrane domain in the α and β forms, while this region corresponds to the fourth extracellular region of IpmPR γ . This conserved region in IpmPR γ is over 60% identical to the other forms of mPR.

Comparison of IpmPR α , β and γ gene structure to human and *Fugu* mPRs

The genomic organizations of each IpmPR, as determined by PCR-based strategy, are illustrated in Figure 5. Intron/exon junctions of each IpmPR gene were identified by comparison with the corresponding IpmPR cDNA sequence. Interestingly, both the IpmPR α and β genes do not possess any introns on their coding regions. The IpmPR α gene is over 4331 bp with a large single intron (only 2061 bp has been sequenced)

interrupting the 5'-UTR. The untranslated exon I is 577 bp in length, whereas exon II contains 33 nucleotides of the 5'-UTR as well as the entire coding region (1062 bp) and 3'-UTR (598 bp). The genomic organization of the IpmPR β gene is comparable to that of the IpmPR α gene, except for an additional intron in the 5'-UTR. The entire sequence of this gene has been determined to be 5118 bp in length. First and second exons of the IpmPR β gene encode 582 bp and 125 bp of the 5'-UTR, respectively. Exon III consists of the remaining 9 bp of the 5'-UTR, the whole coding region (1068 bp) and the 3'-UTR (1681 bp). Intron I and II are 756 bp and 897 bp, respectively. In contrast, the structure of IpmPR γ gene spanning approximately 15.7 kbp is quite different from the other IpmPR forms and includes 8 exons (with a non-coding exon I) and 7 introns. The sizes of introns are 263, 1727, 7585, 243, 1883, 1397 and 1431 bp, respectively. All the nucleotide sequences of the intron/exon boundary sites are consistent with the classical GT/AG rule (Breathnach & Chambon 1981) involved in the recognition for intron splice site.

The structures of the mPR α and β genes of human and *Fugu* share the basic characteristics of their respective IpmPR genes, i.e., these genes do not contain introns in their open reading frames. Furthermore, human and *Fugu* mPR γ genes possess 6 introns in the coding region like the IpmPR γ gene and the position for all intron/exon junctions are identical to those in the IpmPR γ gene. Additionally, there are no introns in the coding regions of FmPRLP 1 and 2 genes that are structurally cognate to mPR α at the phylogenetic level. In contrast, the FmPRLP 3 gene is structurally different from all three IpmPRs (i.e., 4 intron/exon junctions that are not identical to those in mPR γ genes) even though the predicted protein is highly similar to mPR β .

Catfish mPR α	MATVVMVQIG RLFINVQQLR QIPQLLESAP PTLPTCVSIS DVPRVFRESH ILTGYPDPDH	60
Zebrafish mPR α	-----E-----A-----RF-----K-----MV-----W-----I-----Q	60
Seatrout mPR α	-----E-----A-----KV-----W-----R-----Q-----Q	60
Fugu mPR α	-----E-----T-----KV-----W-----R-----Q-----Q	60
PFMSR 1	--S--LERT-- --SL--IQ EF-RM-TQ-A -SM-G--RDT E--HY--Q-F VF-----LHQ	60
PFMSR 2	--S--LERT-- --SL--IQ EF-RM-TQ-A -SM-G--RDT E--HY--Q-F VF-----LHQ	60
Mouse mPR α	--MA-AQKEN H-LSSLWHVG -K-P...QPE -VF...-DRA Q--PL-WKPY -YA-----LHQ	55
Human mPR α	--M...AQKLS H-LPSLR-VI -E---LQPE -VF...-DRA E--PL-WKPY -YA-----LHQ	56
I		
Catfish mPR α	NWRYFYLSLF QRHNETINVM THLLASLVIL VKFQEVSETV DFLRDSHAQP LFLIGLLTAFT	120
Zebrafish mPR α	-----T-----SV-----I-----L-----P-----M--L--A---	120
Seatrout mPR α	S-----T-----L-----AFI-----W--I-----P-----V--A---	120
Fugu mPR α	S-----T-----L-----S-----AFI-----W--M-----P-----V--A---	120
PFMSR 1	H-----I-----I-----LFIF- F-LLQL-AE- -V--P-SW- -LVL-VSSL-	120
PFMSR 2	H-----I-----I-----LFIF- F-LLQL-AE- -V--P-SW- -LVL-VSSL-	120
Mouse mPR α	--CF--RT-----AV-----A-AL- LRLIGLAAS -RE-P-L- -FIV-AS-	115
Human mPR α	T--F--RT-----Q--AV-----A--L- LRLALFV--- -WG-P--L- -IV-AS-	116
II		
III		
Catfish mPR α	YLTCSFLAHL LSAKSELSNY TFYFLDYGV AVYQYGSALV HFYVIEETW HAYIRGFPLP	180
Zebrafish mPR α	--G--A-----H-----A-----V--E- --QV-T-----	180
Seatrout mPR α	--SF-A-----Y-----A-----Y--A--KE- -TKVQ-L---	180
Fugu mPR α	--SF-A-----Y-----A-----A--Y--A--KE- -NRVQRL---	180
PFMSR 1	-TA--VA-----AG--CH- -Q--F--V- -SVD-DL- -RQVQ-V-M-	180
PFMSR 2	-TA--VA-----AG--CH- -Q--F--V- -SVD-DL- -RQVQ-I---	180
Mouse mPR α	--SF-AV--- -Q--FWH- S-F-----F-----A-----A--PS- -DKVQAI-	175
Human mPR α	--SF-A-----Q--FWH- S-F-----F-----A-----A--PA- -QVQAV---	176
IV		
V		
Catfish mPR α	TAAFMALWSC AGCCYGYKAS RRLPRFGHKF CQMVPSSALAY CLDISPVVHR IYTCYSSQQG	240
Zebrafish mPR α	AS--L-----T-----PK--K-V--L F-V-----G-----L--R--E--EHW	240
Seatrout mPR α	A--L--T--F-----PE--KVAN-L F-V-----S--S--E--	238
Fugu mPR α	-----L--T--F-----R ADMSKLAQ-L L-V-----V-R--E--	238
PFMSR 1	L--LLCV--- L-S-C-N-CT HSQ-SWVRNV A-VT-----VW-S--FL- LWSWT-AR-	238
PFMSR 2	V-MVFSC--- L-----K-CN HDQ-TLL--A G-LI--V--F LW-----IAK- LMFPAD--	237
Mouse mPR α	-----L-----S--N--SQ K...GLLGRI F-EA-----V-----L--IV...PLPA	231
Human mPR α	M--L-----I-S--N--IQ K...GLLGRT -E-----A-----FV...DPT	232
VI		
Catfish mPR α	CTDPAVTFHF YQILFFLVSA FFFTYPHPEC WHPGRCDFIG QGHQIFHLFL VLCTLMQIEA	300
Zebrafish mPR α	-A-Q--VY-C -V-----I--Y--S-----R -V-----V-----V-----	300
Seatrout mPR α	-S--V-AY-- -HVV--IG- Y--CC--S L--K-----L--V-V -V--T-V--	298
Fugu mPR α	-S--V-AY-- -HVV--IG- Y--CC--S L-----F--A-V -V-----	298
PFMSR 1	.E--MLY-I G-VV--S-G ---C-L--R C-----L- -S--L--VLI SC--S--H-	297
PFMSR 2	.Q--AIY-L S-VVLS-STT ---V-ML-S C--W--M- -S--L--VCI SCE--S--HS	296
Mouse mPR α	EE---LLY-K C-VV--LA- A--STVM--S ---S-HIF--V--VL-----A-L--	291
Human mPR α	TD---LLY-K C-VV--LA- A--STFM--R ---S-HVF--L--I-----A-L--	292
VII		
Catfish mPR α	VRMDYTERR LYERLHGDLA HD.AAALFVF TTCCSALTAF YVRKQVKAYL EEEKQE	354
Zebrafish mPR α	--L-----R-----H-----.-V--I- -A-----R--T-----	354 82%
Seatrout mPR α	L-T-F-----.-V--I- -A-----QR-R-S-H--G-	352 76%
Fugu mPR α	L-T-F-----.-V--I- -A-----G--C--QR-R-S-Q--K-	352 71%
PFMSR 1	TYL-FVG--D --GP--SYE AALFLG-Y--LLACT-VMA FMLRRIQQT- -A-NKHN	354 51%
PFMSR 2	AHL-FMG--K --S--QSRE AALF-R-Y-A NSVLCV-VVA FMLRK-RQM- DQ-SKSK	353 49%
Human mPR α	-TL--QA--G I--P--ARWP RN.FSG--LL -VAS-S--L LLSQL-RRK- HQ-TK	345 51%
Mouse mPR α	-AL--EA--I --P--THWP -N.FSG--LL -VGS-I--L LLSQL-QRK- DQ-TK	346 54%

Figure 2 Alignment of the deduced amino acid sequence of IpmPR α with the mPR α related proteins of other animal species. Dashes indicate residues that are identical to IpmPR α . Dots indicate gaps introduced to facilitate alignment. Hatched boxes indicate highly conserved regions in all mPR-related proteins. The arrow shows the potential N-linked glycosylation site. I-VII indicate the putative transmembrane domains. *, the putative phosphorylation sites.

Structure of 5'-flanking regions of the IpmPR genes

Sequence analysis of the GeneWalker amplicons generated 3045 bp (GenBank Accession number AY587768), 3701 bp (GenBank accession number AY587769) and 3382 bp (GenBank accession number AY587770) of unique sequences representing the 5'-flanking regions of IpmPR α , β and γ genes, respectively. Figure 6 is a schematic diagram showing potential transcription regulatory elements located within the 3.0 kbp upstream of the end of the 5'-UTR for each IpmPR gene. The full lengths of the 5'-UTRs were determined by sequence analysis of 5'-RACE amplicons. A TATA box was identified within 90 bp of the end of the 5'-UTR of each IpmPR. The 5'-flanking region of the IpmPR α gene contained the consensus sequences of two CREs, two binding sites for SF-1, five former-half sequences of GRE/PRE/AREs and two

latter-half sequences of EREs. The regulatory 5'-flanking region of the IpmPR β gene showed the consensus sequences of three binding sites for SF-1, four half-GRE/PRE/AREs, three half-EREs, a binding site for AhR/Arnt and a binding site for early growth response-4 (Egr-4). Four half palindromic sequences for GRE/PRE/AREs, a binding site for SF-1, a binding site for AhR/Arnt, and an AhR/Arnt binding site overlapped with a recognition site for Egr-2 and Egr-3 were located on the 5'-flanking region of IpmPR γ gene.

Tissue distribution of transcripts of mPRs in catfish and zebrafish

Differential tissue-distribution of the transcripts for mPRs in catfish (Fig. 7A) and for zebrafish (Fig. 7B) was determined by sensitive real-time, quantitative RT-PCR

Catfish mPRβ	MSSGVLGRSL	TLTLNIKQVV	GLPHLSPSLH	.SLPSLQATV	EVSNNVPSLFR	*EPYIQSGYRP	59
Zebrafish mPRβ	---SLQ-LG	Q---NM-P	R---RR---	HA-E---	---	---	59
Fugu mPRβ	--TPI-Q---	---S-H-N	R-HRI-DYMP	F-I--PSP--	G-Q-T--Q	---LT---	60
PFMSR 3			PSV-D RL---	PP---	GDTE--P---	K-F-V---	
Xenopus mPRβ	-TTAI-ECI-	--SISFQ-LR	R--RF...	E GGTTKMPL--	TD-D-R---	---QT---	57
Mouse mPRβ	-TTAI-E---	--SMSGQ-LR	R--KI...	E EG--KMPC--	PETD-Q---	---HA---	57
Human mPRβ	-TTAI-E---	--SVSQ-LR	R--KI...	E DG--KMPC--	PETD-Q---	---RT---	57
Catfish mPRβ	VGQPHWCYAL	SLFORHNELL	NVWSHLI	GVP	AVLLRFWLFA	..ATRSLTLD AS.SLPLFLY	116
Zebrafish mPRβ	--H-E-RS-FC	---C---	---T---AI-	---Q-SF--	..GAWG---N	LA.---	116
Fugu mPRβ	T-D-S--LF	---C---	---T---AA-	IL-IHW-AKV	..EALGF--N	-A.---Y-F	117
PFMSR 3	---R-R--V-	---V---TI	---AAV	C-AI--MV--	VLQGGG-SV-	V.---V-H	
Xenopus mPRβ	TD-D-KY-F	--KK--SV	---T---VAL	---VVA-V	..EAG--S-N	VV.-F-Y--	114
Mouse mPRβ	T-HE-RY-FF	--K--VV	---T---AAL	---A-V	..EAGA-QWA	SPHT---L-F	115
Human mPRβ	T-HE-RY-FF	--K--VV	---T---AAL	---A-A	..EAEA-PWA	STHS---L-F	115
Catfish mPRβ	TLSTALTYLTC	STLAHLQSH	SELSHYSLFF	LDYVGVAVYQ	YGSALAHYFY	CAEEEWKQSL	176
Zebrafish mPRβ	V--S---SF	--VA---	---A---	V---	---CSMG---	--S-P--RH--	176
Fugu mPRβ	LV-S---MFF	--VV---H-	C-H---IV-	---	---CS-G---	SS-AA-RE-I	177
PFMSR 3	H--TV---S	--AA---PR	--PARR--VL	V-R---I-	---C-V-LCL	SVDTA-T--M	
Xenopus mPRβ	V--S---S	--I---K	--A-TFY-	I---ST-	---C---Y-	TSN-A-YD.K	173
Mouse mPRβ	V--SI---	--L---K	---TFY-	V---S---	---F---	SSDQA-YE.L	174
Human mPRβ	I--SI---	--L---K	---TFY-	V---S---	---F---	SSDQA-YD.R	174
Catfish mPRβ	VGALFLPGAA	LLGWLSCASC	CFAKLHYRRP	YPLRRKIFQI	VPTSLAYMLD	ISPVALRLAR	236
Zebrafish mPRβ	--V-----	M-A-----	--YS-FR---	--FH---C--	I-----L--	---H--LT	236
Fugu mPRβ	---	---	--LG-	--IS-FR-Q-	--WQ---C-L	I-----L--	237
PFMSR 3	L-QVY--A--	--A-C-GTI-	PC-Q-CFQHR	.R-CHMVC-V	LLVGV-HVWV	-V---VY--T	
Xenopus mPRβ	ACVYF---	E---YV-	--Y--YR-	--VM--L-V	--AG--I-	---VH-IVT	233
Mouse mPRβ	FWLF---	FC-----G-	--Y--YR-	--VM--C-V	--AG--FV--	---H-V-L	234
Human mPRβ	FWLF---	FC-----G-	--Y--YR-	--VM--C-V	--AG--FI--	---H-V-L	234
Catfish mPRβ	RPC...HEPV	LVLHALQVAF	FLMAAFFFSC	PVPERFPGK	CDIVGHGHQI	FHIFLVMCTM	293
Zebrafish mPRβ	KSW...D---	--P--M---	--L--L---	--R---	---	---	293
Fugu mPRβ	VSNW...Q-S	--PE---I-C	--LS---	--I---	--Q---F-Q---	---VL-SL-I	295
PFMSR 3	CWWS...NSA	AP--I---VL	--LS-L--G	---C---	--H---Q---	---VL-AL--L	
Xenopus mPRβ	CHMEDYTDKA	VM--S--MI-	--IIG-Y---	---KY---	--SI---	---V--GL--L	293
Mouse mPRβ	CHLAG-Q-QA	ANY-T--IL-	--VS-Y---	---KY---	--S---	---A--SV--L	294
Human mPRβ	CHLAG-Q-QA	ANY-T--IL-	--VS-Y---	---KY---	--S---	---A--SI--L	294
Catfish mPRβ	QOMEAVFKDF	LVQRSILVVH	HGEWSLWLAV	GSFFVLVLS	ILTAARMRQR	VQRQLQERLKSE	356
Zebrafish mPRβ	--L--M-R---	--HQQS--DA	--HFIL--S	---	--VL--GA	---R-KD	352
Fugu mPRβ	F--L--L-Q-Y	ARR-DTM-E	F--GR--W-C	VGLPL-F-GC	LI--LLTMKH	-KNHRTTTKKDK	357
PFMSR 3	V-Q--L-R--	--WR-PGL-RQ	Y--ER-L-VC	T-VPC-A--C	AAT	---	48%
Xenopus mPRβ	S-L--L-I-Y	QTRQEVFSAR	YSSNYITLMCC	A---L-I---	TF--VVA-R-	IKEK-ARKE-	353
Mouse mPRβ	S-L--TL-L-Y	QGRHEIFLQR	--PL-VYS-C	L---AA--	AA--SLL-HK	-KDR-I-RKDS	354
Human mPRβ	S-L--I-L-Y	QGRQEIFLQR	--PL-VHM-C	L---F-AA--	AA--LL-HK	-KAR-T-KSD	354

Figure 3 Alignment of the deduced amino acid sequence of lpmPRβ with the mPRβ related proteins of other animal species. The key for the symbols is shown in Figure 2 legend.

Catfish mPRγ	MLGLVKLPPV	FNHVQVPKDF	QEDCIISGYR	HPRSSATDCL	LSLFQLTNET	LNWTHFLPT	60
Fugu mPRγ			V- H-G----	-----A---	W-----	-----	
Mouse mPRγ	--S...--RL	-RID--QV-	H-QG-LF---	--Q---A-I	---M---	-----L-F	59
Human mPRγ	--S...--RL	-SID-I-QV-	H-QG-LF---	--Q---A-I	---M---	-----L-F	59
Catfish mPRγ	WYFLYKLLTV	IFIQDVWTD	YTWPLLVFL	SCCMYPLASS	CAHTFSTMT	RARHICYFFD	120
Fugu mPRγ	---W--V-	VL--TA-Q-S	F-----M---	T--I-----	-----S--A	-----F---	
Mouse mPRγ	-F-VWRFM-A	LYVT-IQN-S	-S--M--YMC	TS-V-----	-----S--K	N-----L-	119
Human mPRγ	-F-AWRFV-A	LYMT-IKN-S	-S--M--YMC	TS-V-----	-----S--K	N-----L-	119
Catfish mPRγ	YGALSVSYSLG	SAIAYSAYIV	PDAWVNSLFH	VYVVPVAVFN	TALSTTMACY	SRFAEDKSLR	180
Fugu mPRγ	-----I---	-----F-	-K--GV--	RW-LAL--L-	-VVC-SLS--	---P-YQ-PK	
Mouse mPRγ	---VNLF---	-----TF	---L-C-T-	EC--AL--L-	-I---GLS--	---L-LQKP-	179
Human mPRγ	---VNLF---	-----TF	---LMCTT--	D---A---L-	-I---GLS--	---L-VQKP-	179
Catfish mPRγ	LAKVLRVLAF	TYPYLFDPNP	LFYRIFLCVG	EGCSANKVNS	LHVYHILLAF	LTGFLFATHL	240
Fugu mPRγ	FS-----V-	A-----	---V---E-	D--TD-DT-I	--YN--A---	-----	
Mouse mPRγ	-C-L-----	A---TW-SL-	I---L--FP-	-S.-R-EAML	Y-QK-MGMTL	-AS-FYSA--	238
Human mPRγ	-C--I-----	A---TW-SL-	I---L--FP-	-S.AQ-EAT-	Y-QK-MIMTL	-AS--YSA--	238
Catfish mPRγ	PERLAGRGRFD	LVGHSHQLFH	VCGIATHFQ	MQAIEMDMTL	RRPWLHDHGP	*DITFRGTLGA	300
Fugu mPRγ	-----S--	YI-----	--A-LG---	-K--K--AT	-----LE-SR	PL-SVN-VWP	
Mouse mPRγ	-----YI-	-----	--V-L---L-	-E--LL-K-	--E--LATS	PFS-POIAA-	298
Human mPRγ	-----YI-	-----	--V-L---M-	-E--LL-K-	--E--LATS	PFS-SQIA--	298
Catfish mPRγ	TMLSMVISLG	IICLFSYPLF	QSPFWGKATR	EGSGAAKKN	339	71%	
Fugu mPRγ	ALFCVLVNF	--V--L-					
Mouse mPRγ	ML-CIIF--S	N-IY--AA-Y	RI-EPELHEK	-T	330	56%	
Human mPRγ	IL-CIIF--S	N-IY--AA-Y	RI-KPELHKK	-T	330	55%	

Figure 4 Alignment of the deduced amino acid sequence of lpmPRγ with the mPRγ related proteins of other animal species. The key for the symbols is shown in Figure 2 legend.

Figure 5 Schematic structures of the genes encoding IpmPR α , β and γ . Open boxes indicate untranslated regions of exons. Closed boxes indicate translated regions of exons. Bars represent introns.

assays. Expression of the IpmPR α gene was detected in all tissues analyzed. However relatively high expression was evident in all parts of the brain, pituitary, muscle and the testis. The IpmPR β transcript was generally lower in abundance than IpmPR α and was observed primarily in parts of the brain (especially the hypothalamus and hindbrain), the pituitary, the ovary and the testis. Even though the IpmPR β transcript was detected in other parts of the brain, gill, ventral aorta (including the surrounding thyroid follicles), and head kidney, abundance was very low. On the other hand, the IpmPR γ transcript was mainly detected in the gill, ventral aorta, intestine, and trunk kidney with lower levels of expression in the cerebellum, hypothalamus and testis. The tissue expression of the mPR α gene in zebrafish (DrmPR α) and catfish differed. DrmPR α mRNA was detected primarily in zebrafish gonadal tissues and was less abundant than the transcript of DrmPR β . DrmPR β was predominantly detected in the

same tissues that the mPR β transcript was found in catfish (i.e., brain, pituitary and gonadal tissues).

Discussion

In the present study, the cDNAs encoding three catfish putative mPRs, IpmPR α , β and γ , were isolated, and their secondary structures phylogenetic relationship to other mPR forms were determined. IpmPR α and β showed relatively high identity at the amino acid sequence level, approximately 50%, however, the γ form shared less than 30% identity with the other IpmPRs. Furthermore, phylogenetic analysis clearly placed each form of IpmPR together with their respective mPR subtype groups and showed greater divergence between the clusters of mPR γ s compared with those of the α and β forms. These findings strongly suggest that mPR α and mPR β are closely related, whereas it is likely that

Figure 6 Diagram of structures and potential transcription regulatory elements located in the 5'-flanking regions of the catfish mPR genes. AhR/Arnt, responsive element for aryl hydrocarbon receptor and its nuclear translocation factor; CRE, cAMP responsive element; Egr, binding site for early growth response genes; SF-1, steroidogenic factor-1 binding site; ERE, estrogen responsive element; GRE/PRE/ARE, glucocorticoid/progesterin/androgen responsive element.

Figure 7 Tissue-specific expression of mPRs in (A) catfish and (B) zebrafish. The transcript abundance of each mPR was determined by real-time quantitative RT-PCR and normalized to the abundance of β -actin. Data are expressed as percentage of the transcript abundance of each mPR gene in the tissue representing the highest expression level.

mPR γ evolutionally diversified from other groups of mPRs.

Interestingly, six mPR-related sequences including mPR α , β and γ , and three uncharacterized forms (FmPRLP 1–3) were retrieved from the *Fugu* genome database. Structure analysis of these novel forms demonstrated that two of them, FmPRLP 1 and 2, are closely related to mPR α , while the predicted amino acid residue of FmPRLP 3 shares high identity with the β form. These conclusions are supported by the phylogenetic analysis showing their relationships to other mPRs. These findings suggest that FmPRLPs are potential members of mPR family, although further characterization of them is required, including the demonstration that their mRNAs are expressed in fish tissues and are functional genes. The mPRLPs are possibly species-specific or teleost-specific forms of mPR-related genes, since the human genome appears to contain only three forms of mPRs from this phylogenetic

analysis. Further information on the number of mPR-related proteins in other animal species would provide valuable insight into the evolution of this new steroid receptor family.

The deduced amino acid sequences of each IpmPR showed high homology to other animal forms; in two regions in particular. The amino acid residues 69–84 and 273–287 in IpmPR α are quite highly conserved among all mPR forms, including FmPRLPs. The former conserved region contains a portion of the N-terminal extra-cellular region and a potential N-linked glycosylation site identified in mPR α and γ . These data may imply that these conserved regions are involved in fundamental functions of mPR, for example, the ligand-binding, coupling with G-proteins.

Interestingly, IpmPR α and β show comparable genomic organizations without any introns in their coding regions. In contrast, the structure of the mPR γ gene is quite different in that it includes six intron

junctions within the coding region. This difference in the gene structure between *IpmPR* γ and those of the α and β forms appears to coincide with the secondary structural and phylogenetic divergence of the γ from the α and β forms. Furthermore, these features of genomic structure are completely conserved in the corresponding human and *Fugu* mPR genes. This finding strongly suggests that the mPR genes are phylogenetically conserved throughout the vertebrates based on a high degree of conservation of their genomic organization as well as their coding sequences.

The putative transcriptional regulatory elements located on the unique 5'-flanking regions of each *IpmPR* gene were also identified. 5'-flanking regions of each *IpmPR* show a TATA box within 90 bp of the end of the 5'-UTR, which suggests that these 5'-termini are close to the transcription start sites for each *IpmPR* gene. The 5'-flanking region of each *IpmPR* gene differentially exhibits the consensus regulatory elements for transcription factors related to reproductive physiology (CRE, SF-1 binding site; Morohashi *et al.* 1992), steroid hormone regulation, and cellular growth and differentiation (Egr; Tourtellotte *et al.* 1999 and AhR/Arnt; Poland & Knutson 1982). Therefore, these transcription factors may be partly responsible for the direct transcriptional modulation of *IpmPR* genes. In fact, we recently have shown that the catfish mPR α gene is down-regulated by a progestin, 17 α , 20 β -dihydroxy-4-pregnene-3-one (the MIS in this species) while estradiol up-regulates its expression (Kazeto *et al.* 2005). Furthermore, it was reported that the protein abundance of mPR α was enhanced in seatrout ovary by hCG (Zhu *et al.* 2003a) whose effects are generally mediated by cAMP (Ascoli *et al.* 2002), although recent studies have shown that the catfish and zebrafish mPRs are not regulated by gonadotropins (Kazeto *et al.* 2005). The transcriptional regulatory elements identified in this study adds significantly to the paucity of information that is currently available concerning the gene regulation of mPRs and it provides valuable insights into the potential mechanisms of mPR gene regulation.

The different tissue distributions of the three *IpmPR* genes was expected based on the significant structural differences in their 5'-flanking regions. The transcript for *IpmPR* α was detected in all tissues examined whereas the β form, although poorly expressed, was in greatest abundance in the brain, pituitary and gonads. The γ form was primarily expressed in the posterior kidney, gill and intestine. The more sensitive RT-PCR procedure used in the present study demonstrates that the distribution of *IpmPR* α transcript is ubiquitous in catfish whereas Northern blot analysis only showed significant expression of the mPR α gene in the brain, pituitary and gonads in seatrout (Zhu *et al.* 2003a), and in various reproductive tissues and the kidney in humans (Zhu *et al.* 2003b). Similarly, the RT-PCR procedure reveals that

the mPR β gene is also expressed in the gonads in catfish, whereas only neural expression of the gene was detected in human tissues by Northern blot analysis (Zhu *et al.* 2003b). However, these two methods showed similar tissue expression of mPR γ genes in catfish and humans, with expression primarily in kidney and intestinal tissue (Zhu *et al.* 2003b). Interestingly, catfish gill also expresses *IpmPR* γ , which suggests that *IpmPR* γ may be involved in ion regulation.

In order to determine if the apparent differences seen in tissue-specific expression of mPR α and β in catfish are specific to this species, the tissue distribution of mPR α and β expression was examined in a second teleost species, the zebrafish. The tissue-specific gene expression of mPR α differed in zebrafish, where the mPR β transcript was expressed almost exclusively in gonadal tissues. The apparent differences in tissue expression of the mPR α gene among the different fish species investigated to date may indicate possible species-specific differences in the extra-gonadal functions of the mPR α in fish. In contrast, the tissue-specific distribution of the mPR β transcript in zebrafish is essentially the same as in the catfish, that is in brain, pituitary and gonadal tissues, and potentially could reflect some conserved functions of mPR β among these teleost species.

In conclusion, the cDNAs and the genes encoding three putative mPRs, the α , β and γ forms, were isolated from catfish and structural analyses strongly suggest that the highly related α and β forms of the catfish mPR are distantly related to the γ form. Furthermore, structural analysis of the 5'-flanking regions of the mPR genes and the differences in the tissue-specific distributions of their transcripts suggest that expression of these genes is differentially modulated by multiple regulators in catfish. Currently, only a few studies have been published on this new class of steroid receptors. The molecular characteristics of the mPRs described in this study provide valuable insights into the possible functions and physiology of these novel steroid receptors.

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