Cloning of partial putative gonadotropin hormone receptor sequence from fish

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A search for the presence of mariner-like elements in the *Labeo rohita* genome by polymerase chain reaction led to the amplification of a partial DNA sequence coding for a putative transmembrane domain of gonadotropin hormone receptor. The amplified DNA sequence shows a high degree of homology to the available turkey and human luteinizing and follicle stimulating hormone receptor coding sequences. This is the first report on cloning such sequences of piscine origin.

1. Introduction

Transposable elements are ubiquitous components of eukaryotic genomes (Labrador and Corces 1997). Among them, mariner like elements (MLEs) belonging to the mariner/ Tc1 superfamily of transposable elements, are perhaps the most successful genetic parasites in evolutionary history. Mariner was originally described in *Drosophila mauritiana* (Jacobson *et al* 1986) and subsequently the presence of MLEs was reported in several invertebrates and mammals (Hartl *et al* 1997). However, its occurrence in fish has not so far been recorded. Typically, the MLE is about 1·3 kb in size with 30 bp of inverted terminal repeats and it moves through DNA intermediates by a cut and paste mechanism (Lampe *et al* 1996). Indian major carps are cultivable freshwater fishes; their growth hormone (GH) cDNAs have been cloned (Venugopal *et al* 1998) and attempts to construct transformation vectors containing the cDNA sequence are being made. Considering the possible use of the mariner element in constructing a germline transformation vector, the genome of rohu (*Labeo rohita*) was scanned for the presence of mariner like elements by PCR amplification using MLE specific primers. Interestingly, as detailed below, these primers have also amplified a partial DNA sequence coding for a putative gonadotropin hormone receptor.

Receptors of luteinizing hormone (LH), follicle-stimulating hormone (FSH) and thyroid stimulating hormone (TSH) constitute the subfamily of glycoprotein hormone receptors within the superfamily of G protein coupled receptors (GPCR). These receptors have been studied in higher vertebrates (Dufau 1998). Whether fish pituitary gonadotropin (GTH), mediating the similar functions of FSH and LH, is of a single type or two types is still controversial (Bhattacharya 1992). High affinity GTH receptors have been identified in the oocyte plasma membrane of fishes such as the Indian murrel (Jamaluddin and Bhattacharya 1986), and binding of gonadotropic hormone to the ovarian plasma membrane of the Indian major carp *Catla catla* (Manna and Bhattacharya 1993) has been demonstrated at different stages of the reproductive cycle. The presence of activin subunits has been demonstrated in various tissues of goldfish and recently genes coding for activin and its receptor have been cloned (Ge *et al* 1997). However, this is the first report on cloning of a gonadotropin hormone receptor (GTHR) coding sequence from a fish.

2. Materials and methods

Based on the consensus residues of the MLEs identified in *Bombyx mori* (Mathavan *et al* 1996), primers BmSMmar124F (TGG GTG CCG CAC GAG TT–17mer) and BmSMmar265R (GCC TAG CTC TGC GGC TTT C–19 mer) were designed. These primers were expected to amplify a 450 bp region representing the internal sequences of MLEs.

Keywords. Glycoprotein hormone receptor; gonadotropin receptor; *Labeo rohita*; luteinizing hormone receptor; mariner transposon; PCR cloning

2.1 *PCR amplification of rohu genome with BmSM MLE primers*

Genomic DNA was extracted from the caudal fin clippings of rohu (Sambrook *et al* 1989). Following the protocol of Mathavan *et al* (1996), 50 ng of DNA was amplified in a PCR thermal cycler (Perkin Elmer 2400) in 50 μl of PCR mix and the amplified product was resolved on a 2% agarose gel and stained with ethidium bromide.

2.2 *Purification and cloning of PCR products*

Using a Qiagen PCR purification column, total PCR products were purified. The purified PCR products were blunted using Pfu DNA polymerase (Costa and Weiner 1994) and ligated to a blunt-ended plasmid vector (pMos blue, Amersham). Ligation was carried out at 12°C overnight and competent *Escherichia coli* JM 109 cells were transformed with the ligation mix following standard protocols. Recombinant clones were selected by blue-white selection on ampicillin plates containing IPTG and X-gal. Plasmid DNA isolated from the recombinant clones was digested with *Eco*RI and *Pst*I to release the insert and analysed on an agarose gel.

2.3 *DNA sequencing and analysis of the nucleotide sequence data*

The recombinant plasmid was sequenced by the dideoxy termination method (Sanger *et al* 1977) in an automated DNA sequencer (ABI prism, Perkin Elmer) using universal and custom-made primers. Nucleotide sequences were analysed using the GCG software version 9·1 (Wisconsin Package 9·1, Wisconsin University, Madison, USA) in a Unix-based server. Dendrograms were constructed using the Pileup, Distances and Growtree modules of the GCG software.

3. Results and discussion

Amplification of the rohu genome with mariner specific primers resulted in the amplification of DNA fragments ranging from 100 bp–450 bp in size (figure 1A). The PCR products were purified and cloned in a plasmid vector. On

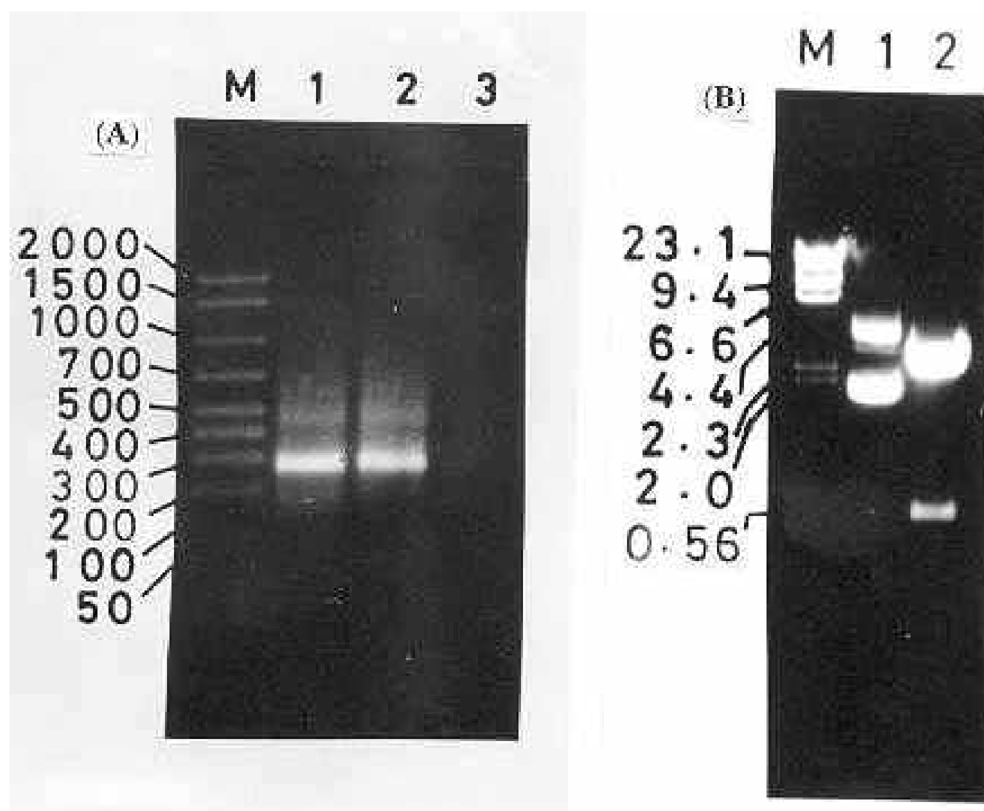

Figure 1. PCR amplification of rohu genome with BmSM mariner primers and cloning of the PCR products. (**A**) PCR amplification of rohu genomic DNA. Lane M, Molecular weight marker; lanes 1 and 2, PCR products from rohu genome; lane 3, negative control. (**B**) Electrophorogram of recombinant clone. Lane M, l *Hin*dIII marker; lane 1, uncut recombinant plasmid; lane 2, recombinant plasmid digested with *Eco*RI and *Pst*I.

Table 1. Comparison (percentage of similarity) of partial amino acid sequence of the rohu putative gonadotropin hormone receptor with available LH/FSH/TSH receptor protein sequences.

Species	LH (%)	FSH (%)	TSH (%)
Homo sapiens	100·0	87·1	71·0
Rattus rattus	87·1	71·0	71·0
Melegaris gallopova	90·0	–	–
Mus musculus	87·1	–	74·2
Bos taurus	87·1	74·2	74·2
Mustela vison	87·1	–	–
Sus scrofa	83·9	71·0	–
Ovis aries	86·7	–	74·2
Equus caballus	–	74·2	–
Equus asinus	–	71·0	–
Macaca fascicularis	–	83·9	–
Gallus gallus	–	74·2	–
Canis familiaris	–	–	74·2
Drosophila melanogaster	–	61·3	–

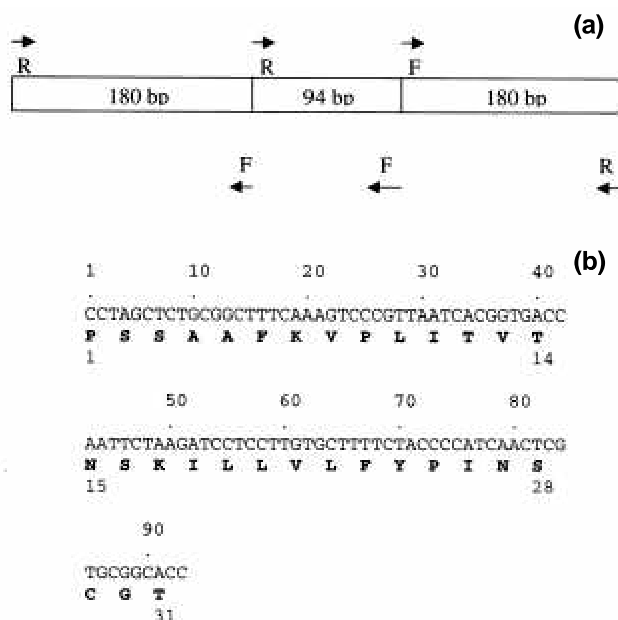

Figure 2. Structure of the cloned insert. (**a**) Diagrammatic representation of the cloned DNA fragment; arrangement of the PCR products are illustrated with primers. Letters 'F' and 'R' indicate the BmSM 124F and BmSM 265R primers; arrows indicate the primer orientation. (**b**) Nucleotide sequence of rohu partial gonadotropin hormone receptor sequence. The nucleotide sequence represents the middle 94 bp fragment in the total insert (Genbank accession no: AF 118849).

digestion with *Eco*RI and *Pst*I, the recombinant clones released inserts of 100 bp–450 bp. One such clone (figure 1B) containing a 450 bp insert (size of a typical mariner amplicon, Mathavan *et al* 1996) was sequenced and analysed. Surprisingly, the sequence shows less than 40% identity, with MLEs, which is not a significant score of homology. On the other hand, it shows more than 60% identity with gonadotropin receptor coding sequences of different animals (60–100% similarity with peptide sequences, table 1). The insert consisted of 3 DNA fragments, a fact that was later confirmed also by PCR analysis of the plasmid with the same primers used for amplification. The full-length insert was a trimer (180 bp–94 bp–180 bp); the identical 180 bp products were flanking a 94 bp fragment (figure 2A). The nucleotide sequence of the 180 bp PCR product shows (data not shown) 57·9% identity with a cosmid library clone of *Caenorhabditis elegans* (EMBL accession no: Z37979) which remains unidentified and yet to be characterized.

The deduced peptide of the 94 bp sequence (figure 2B) showed different percentage of similarity to peptides of different sequences which are available in the Genbank. However, it was chosen to make a comparison with those, whose similarity is more than 60% (table 1); for instance, the similarity values are 100% for LH receptor of human (93% identity), 90% similarity with LH receptor of turkey and 87% similarity with FSH of human (table 1). To clarify the nature of the cloned fish DNA sequence, it was compared with all available LH, FSH and TSH receptor sequences and a dendrogram (figure 3) was constructed with all available LH, FSH and TSH receptor sequences. The fish sequence matched well with FSH/LH receptor coding sequences, substantiating that it could indeed be a putative gonadotropin I or II receptor coding sequence (the LH and FSH analogues in fish, Bhattacharya 1999). To the best of our knowledge this is the first report on cloning a gonadotropin receptor sequence of fish.

Similarly, the deduced amino acid sequence was compared with LH (figure 4A) and FSH (figure 4B) receptor coding protein sequences. The peptide showed 100% similarity with the signal transducing and G protein-coupling module of human and other mammalian FSH/LH/TSH receptor sequences. The deduced peptide seq-

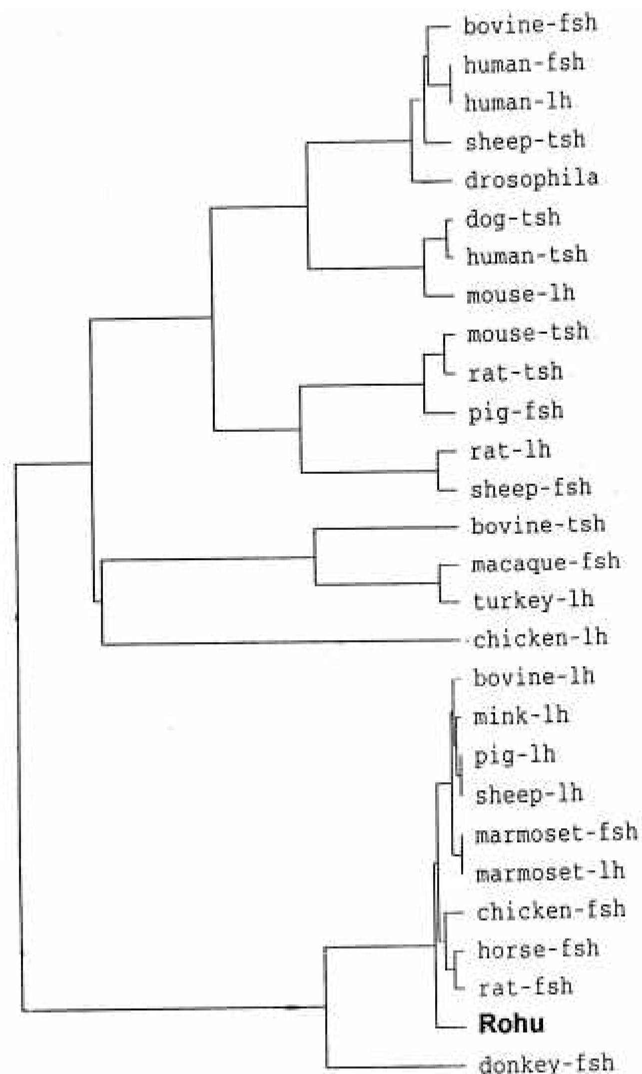

Figure 3. Dendrogram of partial fish putative gonadotropin hormone receptor coding sequence with other available LH, FSH and TSH receptor coding sequences.

(a)

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BOVINE      ISAAFKVPLITVTNSKVLLVLFYPVNSCANP
HUMAN       ISAAFKVPLITVTNSKVLLVLFYPINSCANP
MARMOSET    ISAAFKMPLITVTNSKVLLVLFYPINSCANP
MINK        ISAAFKVPLITVTNSKVLLVLFYPVNSCANP
MOUSE       ISAAFKVPLITVTNSKVLLVLFYPVNSCANP
RAT         ISAAFKVPLITVTNSKILLVLFYPVNSCANP
ROHU        SSAAFKVPLITVTNSKILLVLFYPINSCGT-
TURKEY      ISAAFKVPLITVTNSKILLVLFYPVNSCANP
             *****.*********.*******.***  .
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(b)

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BOVINE      AISASLKVPLITVSKSKILLVLFYPINSCAN
CHICKEN     AISASLRVPLITVSKSKILLVLFYPINSCAN
DONKEY      GISASLKVALITVSKSKILLVLFYPINSCAN
HORSE       AISASLKVPLITVSKSKILLVLFYPINSCAN
HUMAN       AISAAFKVPLITVTNSKVLLVLFYPINSCAN
MACAQUE     AISASLKVPLITVSKAKILLVLFYPINSCAN
MARMOSET    AISAAFKMPLITVTNSKVLLVLFYPINSCAN
PIG         AISASLKVPLITVSKLKILLVLFYPINSCAN
RAT         AISASLKVPLITVSKAKILLVLFYPINSCAN
ROHU        PSSAAFKVPLITVTNSKILLVLFYPINSCGT
SHEEP       AISASLKVPLITVSKSKILLVLFYPINSCAN
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Figure 4. Multiple sequence alignment of putative partial fish gonadotropin receptor sequence with other available (**a**) LH and (**b**) FSH receptor protein sequences.

uence typically matched with the VI and VII transmembrane domains of human LH receptor except for a valine, which was replaced by isolucine in fish. The present study shows that the structure of fish GTHR could be identical to the LH and FSH receptors of other vertebrates. The VI and VII domains are crucial and inactivation of the LH receptor by mutations in these domains leads to histophysiological impairment of testis, like Leydig cell hypoplasia/aplasia in humans (Dufau 1998). The transposons including MLEs occur in T cell receptors (Morgan 1995) and major histocompatibility complex (MHC) molecules of human (Andersson *et al* 1998). Dufau's group (Hu *et al* 1994) has observed an unusual insertion of the LINE R transposon in-between the two putative polyadenylation signals of LH receptor. Mariner transposon specific primers have amplified the gonadotropin receptor sequence, and this finding adds to the earlier observations of Andersson *et al* (1998), Hu *et al* (1994) and Morgan (1995), which remain to be fully explored. Since sex regulation is an important aspect in aquaculture research (Pandian *et al* 1999), cloning and characterization of gonadotropin hormones and their receptors merit a detailed study.

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