Mar. Biotechnol. 3, 590–599, 2001
DOI: 10.1007/s1012601-0067-5

MARINE BIOTECHNOLOGY

© 2001 Springer-Verlag New York Inc.

Channel Catfish Follicle-Stimulating Hormone and Luteinizing Hormone: Complementary DNA Cloning and Expression During Ovulation

Zhanjiang Liu,* Soonhag Kim, and Attila Karsi

Fish Molecular Genetics and Biotechnology Laboratory, Department of Fisheries and Allied Aquacultures, and Program of Cell and Molecular Biosciences, Auburn University, Auburn, AL 36849, U.S.A.

Abstract: Gonadotropins are important regulators of reproduction. To develop molecular resources for production of recombinant gonadotropins, we have cloned and sequenced complementary DNA encoding the channel catfish (*Ictalurus punctatus*) follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which encode 132 and 140 amino acid proteins, respectively. The deduced amino acid sequences of the catfish FSH and LH are highly conserved with those cloned from other teleosts. Both the FSH and the LH were highly induced during ovulation after injection of carp pituitary extract. Taken together with our previous findings of enhanced expression of growth hormone and other pituitary hormones, this research suggests that a hormonal cocktail may be needed for more efficient manipulation of catfish reproduction. The availability of the catfish FSH and LH cDNAs provides the opportunity for production of immunologically or biologically active recombinant gonadotropins for the study of catfish reproductive physiology and the manipulation of artificial spawning for aquaculture.

Key words: spawning, induction, gene, expression, pituitary, reproduction.

INTRODUCTION

A family of related glycoprotein hormones are produced in mammals: follicle-stimulating hormone (FSH), luteinizing hormone (LH), thyroid-stimulating hormone (TSH), and the chorionic gonadotropin (CG). These glycoproteins all share the same α subunit in a given species in a functional heterodimer composed of one α subunit and one β subunit. The different β subunit confers distinct functions to each of the 4 glycoproteins (Pierce and Parsons, 1981).

Received March 12, 2001; accepted June 27, 2001
Corresponding author: fax 334-844-9208; e-mail zliu@acesag.auburn.edu.

In teleosts, 2 types of gonadotropin were first identified in chum salmon (*Oncorhynchus keta*) (Kawauchi et al., 1986), then referred to as gonadotropin I and II (GTH-I and GTH-II), and now generally accepted as homologues of mammalian FSH and LH, respectively. In the last decade, FSH and LH complementary DNAs have been cloned from various teleost species (Sekine et al., 1989; Lin et al., 1992; Kato et al., 1993; Hassin et al., 1995; Elizur et al., 1996; Nagae et al., 1996; Rosenfeld et al., 1997; Yoshiura et al., 1997). Although FSH and LH are similar in structure, they are synthesized in 2 different cell types (Nozaki et al., 1990a, b; Naito et al., 1991, 1993) and at different stages of the reproductive cycle (Naito et al., 1991). FSH is expressed in

the periphery of the glandular cords of the proximal pars distalis (PPD), in close association with somatotrophs; LH is expressed in the central parts of the glandular cords of the PPD. Temporally, FSH is expressed first in ontogeny and involved in early reproductive events such as steroidogenesis, vitellogenesis, and spermatogenesis, whereas LH is expressed in later stages of reproductive cycle and regulates spermiation and ovulation (Naito et al., 1991).

Mechanisms controlling ovulation and spawning have not been well studied in channel catfish (*Ictalurus punctatus*) despite its significance for aquaculture (Dunham, 1993). Channel catfish spawn once a year during late spring and early summer when water temperature is 24° to 27°C. Under natural conditions, only a small percentage of channel catfish spawn each year. The majority carry their oocytes through the summer but fail to spawn. The major environmental regulator of catfish spawning is temperature (Liu et al., 1997), while photoperiod appears to be more important for other teleosts. This spawning behavior should result from the complex interaction along the brain-pituitary-gonad axis, but the hormone profile during oogenesis is not known (reviewed by Patino 1995, 1997).

For aquaculture, carp pituitary extract (CPE) has been used most successfully for induction of spawning in catfish (Dunham, 1993; Liu et al., 1997; Lambert et al., 1999), but a low hatching rate has been routinely observed for embryos obtained from females treated with artificial spawning induction reagents. This problem is most prominent for production of channel catfish × blue catfish F_1 hybrids. Although the F_1 hybrid catfish derived from the female channel catfish × male blue catfish perform better in essentially all important traits such as growth rate, feed conversion efficiency, disease resistance, fillet yield, and harvestability, mass production of the hybrid fingerlings has not been feasible for the catfish industry. The major problem appeared to be the inconsistent results with induction of using CPE, which often gave very low hatching rates. Two factors have been proposed as the causes of the failure in CPE-induced spawning: first, great variation in the quality of CPE from the commercial sources because of the age, season of harvest, sex, and physiological condition of carps may greatly affect the composition of CPE; second, the heterologous nature of induction hormones may not be optimal for catfish. One could argue that homogeneous hormones should work better for their own systems. In addition, if recombinant FSH and LH can be produced, then the amount of these hormones can be precisely controlled. Toward this end, we report here isolation and sequence analysis of the cDNAs encoding the channel catfish FSH and LH and their expression during spawning induced by CPE. Along with the previous cloning of α subunit, this work should provide molecular resources for production of recombinant GTHs for application in catfish.

Materials and Methods

Animals and Treatments

Channel catfish were obtained from the Fish Genetics Facility of Auburn University. Gravid fish were selected for experiments and randomized in 7 groups of 5 fish each and placed in indoor tanks. The water temperature was kept at 27° to 28°C. Tissues from fish in each treatment group were collected at different times for isolation of RNA. At the beginning of the experiment, tissues from the first group of fish were harvested as 0-hour controls. CPE (Stollers, Spirit Lake, Iowa) was then injected into the fish of the remaining 6 groups at a priming dose of 2 mg/kg of body weight and at a resolving dose of 8 mg/kg of body weight after 12 hours when appropriate (these doses are routinely used for induced spawning in catfish hatcheries). Pituitaries were collected at 4, 8, and 12 hours (before second injection), 16 hours (4 hours after the second injection), 20 hours, and 36 hours after the initial injection. All fish were treated with tricaine methane sulfonate (MS-222) at 300 ppm before harvesting the pituitaries to euthanize the fish.

RNA Isolation

Pituitary glands from the 5 noninjected fish were pooled, and 30 pituitaries after injection with carp pituitary extracts were pooled prior to RNA extraction. The sample of 30 pituitaries included 5 pituitaries each at 4, 8, 12, 16, 20, and 36 hours after the initial injection. The pituitary glands were harvested from freshly killed fish, frozen in liquid nitrogen, then transferred for storage in a −80°C freezer. At the time of RNA extraction, pituitaries were frozen in liquid nitrogen again, pulverized with a mortar and pestle, and then homogenized (model 985-370, Biospec Products, Inc., Wis.) in RNA extraction buffer following the guanidinium thiocyanate method (Chomczynski and Sacchi, 1987). Poly(A)$^+$ RNA was purified by using the Oligotex Spin Column Kit according to the manufacturer's instructions (Qiagen Inc., Chatsworth, Calif.).

Complementary DNA Library Construction and Mass In Vivo Excision

The Unizap λ cDNA library was constructed from the poly(A)$^+$ RNA isolated from channel catfish pituitaries according to the manufacturer's instructions (Stratagene, La Jolla, Calif.) and has been previously described (Liu et al., 1997). The λ Unizap cDNA library was converted into a plasmid library in order to simplify DNA preparation and other procedures, by using the mass in vivo excision procedure (Stratagene) according to the manufacturer's instructions, and as previously described (Karsi et al., 1998).

Amplification and Molecular Cloning

The fixed orientation of cDNAs in the plasmid vector allows use of vector-anchored polymerase chain reaction (PCR) using one primer specific to the gene of interest and one primer with vector sequence (Liu et al., 1997). The upstream portion of a cDNA can be amplified by using the reverse sequencing primer in the cloning vector and a gene-specific primer that is complementary to the RNA sequences. Similarly, the downstream portion of a cDNA can be amplified using the universal primer and a gene-specific primer that has the same sequences as the RNA. To obtain probes, this approach was used to amplify the downstream portions of cDNAs for both FSH and LH. The gene-specific primers used to amplify the FSH and LH downstream sequences were 5′-CCAATCTCCATCACCGTGG-3′ and 5′-CCAAGGAACCTGTGTACAAGAGC-3′, respectively. The sequence designs of these primers were based on conserved regions of alignments of cloned counterparts from other teleosts. After successful amplification of a portion of the cDNAs, the aplicon was cloned in a pCR2.1 (Invitrogen, Carlsbad, Calif.) vector, and only one strand was sequenced to determine the identity of the amplicon by BLAST searches. The verified fragments were then used as probes to screen the cDNA library in order to obtain the complete cDNA clone (Sambrook et al., 1989).

PCR reactions (50 μl) were carried out in 50-mM KCl, 10-mM Tris (pH 9.0 at 25°C), 0.1% Triton X-100, 0.25-mM each of deoxynucleotide triphosphates (dNTPs), 1.5-mM $MgCl_2$, 20-μM each of the upper and lower PCR primers, approximately 10 ng of plasmid library DNA, and 2.5 U of *Taq* DNA polymerase. The temperature profiles used to amplify the cDNAs were 94°C for 30 seconds, 45°C for 1 minute, and 72°C for 2 minutes, for 35 cycles.

DNA Sequencing and Sequence Analysis

All cDNA clones were sequenced by the dideoxy chain-termination method (Sanger et al., 1977) using the cycle sequencing kit (Applied Biosystems, Inc., Foster City, Calif.). Minipreparation plasmid DNA (1 μl, about 200–500 ng) was used for each reaction. The profiles for cycling were 94°C for 1 minute, 72°C for 1 minute, 55°C for 1 minute, for 30 cycles. An initial 2 minutes of extra denaturation at 94°C was always used. After initial sequencing with either the universal or the reverse sequencing primers, the primer-walking method was used to generate complete sequences. Sequencing primers were ordered from Gibco (Bethesda, Md.). DNA sequences were compiled by using microcomputer software packages, DNASIS (Hitachi, version 2.0), or DNASTAR (DNA Star Inc., Madison, Wis.) Protein sequences were aligned by using the MEGALIGN program of the DNASTAR package with CLUSTAL method and residue weight table set at PAM250 (Hein, 1990). Sequences used for alignments other than reported here were extracted from the nonredundant GenBank+EMBL+DDBJ+PDB databases from the National Center for Biotechnology Information using BLAST searches.

Northern Hybridization Analysis

Total RNA isolated from pituitary was electrophoresed on a 1% agarose gel with formaldehyde (Sambrook et al., 1989), then transferred to a piece of Zetabind nylon membrane (Wattman, Wis.) by capillary transfer with 10× SSC overnight. The complete FSH and LH cDNA fragments were gel purified and used as probes. The probes were made using the random primer method (Sambrook et al., 1989) with a labeling kit from Boehringer Mannheim (Indianapolis, Ind.) Membranes were dried at 80°C under vacuum for 2 hours and prehybridized in 50% formamide, 5× SSPE, 0.1% sodium dodecylsulfate (SDS), 5× Denhardt's, and 100 μg/ml sonicated and denatured calf thymus DNA overnight. Hybridizations were carried out at 42°C overnight in the same solution with probes added (more than 10^9 cpm/μg DNA). The Zetabind membranes were washed first in 2× SSC for 10 minutes at room temperature, followed by 3 washes in 0.2 × SSC with SDS at 0.2% at 50°C for 15 minutes each. The membranes were then wrapped by Saran wrap and exposed to Kodak BioMax MS film for 30 minutes to overnight depending on signal intensities. Hybridization signals were quantified using the GS-525 Molecular Imaging System (Molecular Dynamics, Sunnyvale, Calif.). The

magnitude of enhanced gene expression after CPE induction was calibrated by measuring also the levels of β-actin messenger RNA (Liu et al., 1990). In other words, the RNA loading differences were corrected by using a factor that brought β actin to the same level before and after CPE induction.

Results and Discussion

Cloning and Sequencing of the FSH cDNA

Part of the FSH subunit cDNA was initially isolated by PCR amplification using vector-anchored PCR (Liu et al., 1997). The PCR reaction produced a product with the expected size of 670 bp. The PCR product was cloned and partially sequenced to verify its identity by BLAST searches of the GenBank databases. A complete clone was then obtained by screening the pituitary cDNA library.

The nucleotide sequences and the deduced amino acid sequences for the FSH cDNA are shown in Figure 1. The sequences were submitted to GenBank with accession number AF112191. The cDNA consisted of a 399-bp coding region (including the termination codon), an 18-bp 5′ untranslated region (UTR), and a 360-bp 3′ UTR. Three polyadenylation signal sequences AATAAA exist in the 3′ UTR, but all are located about 160 bp upstream from the polyadenylation sequences. This is somewhat longer than the usual distance of approximately 30 bp observed for most genes. The 3 potential poly(A)$^+$ signals are part of a simple sequence repeat located at the nontranslated region with an imperfect sequence of (CA)n(AAAT)n(CCA)n (Figure 1). At 25 bp upstream of poly(A)$^+$ site there are sequences ATTAAA, similar to the consensus polyadenylation signal sequences. It is likely that this sequence is actually used as the poly(A)$^+$ signal. The coding region encodes a putative peptide of 132 amino acids, 17 amino acids of which are a signal peptide, as determined by the alignment of amino acid sequences to other teleosts (Lin et al., 1992; Hassin et al., 1995; Power et al., 1997; Rosenfeld et al., 1997).

Until recently it was widely believed that catfish had only one GTH, although both FSH and LH were found in several teleosts (Hassin et al., 1995; Elizur et al., 1996; Garcia-Hernandez et al., 1997; Yoshiura et al., 1997). The only indication that 2 GTHs are present in catfish was the recent cloning of the FSH and LH receptors (Kumar et al., 2001a, b). This research provided the first concrete evidence for the presence of both FSH and LH in catfish. This finding indicates that the molecular architecture in regulation of catfish gonadal development is similar to that of other teleost fishes.

```
gaattcggca cgagggtgAT GATGCGTGGA GTCACCATGG TGTTGCTGTT GCCGATGCTG 60
                    M   M  R  G   V  T  M   V  L  L  L   P  M  L  14

GTGTGGGCGG GGTCAGAATG CAAGGCACGC TGTTGTCTTA CCAACATCTC CATCACCGTG 120
 V  W  A   G  S  E  C   K  A  R   C  C  L   T  N  I  S   I  T  V  34

GAGAGCGACG AGTGTGGCAG CTGCATCACT GTCAACACCA CGGCCTGCAC TGGCCTCTGC 180
 E  S  D   E  C  G  S   C  I  T   V  N  T   T  A  C  T   G  L  C  54

CGAACCCAGG AGAGAGCATA CCGCAGCCCA GTGGCACCGT ACTTCCAGAA CACCTGTAAT 240
 R  T  Q   E  R  A  Y   R  S  P   V  A  P   Y  F  Q  N   T  C  N  74

TTCAGGGACT GGACGTATGA GACCATCCAG CTGCCGGGCT GTCCTCTGGG GGTTGATTCA 300
 F  R  D   W  T  Y  E   T  I  Q   L  P  G   C  P  L  G   V  D  S  94

TCCTTCACCT ACCCCGTGGC TCTGAGCTGT GAGTGCAGCC AGTGTAACAC AGAGATCACG 360
 S  F  T   Y  P  V  A   L  S  C   E  C  S   Q  C  N  T   E  I  T 114

GACTGTGGCG CTTTCAGCAT GCAGCCGAGT AGCTGCCACA CACACGCCTA CTACTGAacc 420
 D  C  G   A  F  S  M   Q  P  S   S  C  H   T  H  A  Y   Y  *     133

aagtcgcatc agctcagcca acagcccaaa gccctcgcag caatctggat cactttcagc 480

atctttaaga tcattttagg aatattttgt accaagaagt ttcatccttc cttcacgtta 540

attatcctat ctgaccgtga at|ca|ct|ca|ca|ca|ca|aatttc ac|aaat|atg|    589

aaat|aaat|aat|aaat|aaat|aaaaaact|cca|cca|cca|cca|cagtttgatacctt   639

atacta caaatgagat acaggtctgt tctgtttgtc tgtgtgaccc tgaatatcatgaa  699

gggcatg atgtgctgtg ttcagcatca cctgagcatg taaacactgc ttcattaaaaca  759

gctcacat gcagcatt (Poly A)                                        777
```

Figure 1. The nucleotide and deduced amino acid sequences of cDNA encoding channel catfish FSH (GenBank accession number AF112191). Nucleotide sequences are numbered on the top, far right, and the amino acid sequence on the bottom, far right. The coding region is in uppercase letters and the noncoding region in lowercase letters. The first amino acid in the mature peptide is underlined. The stop codon is indicated by a bold asterisk. Three AATAAA sequences and an ATTAAA potential poly(A)$^+$ sequence are underlined. The simple sequence repeats are placed between vertical bars (|). A long arrow indicates the position of the PCR primer.

Cloning and Sequencing of the LH cDNA

With an approach similar as to that used to isolate the FSH, LH cDNA fragments were obtained by vector-anchored PCR. PCR amplification of the downstream portion of the LH yielded a fragment with the expected size of 460 bp, as determined by agarose gel electrophoresis. This fragment was cloned, sequenced, and determined to be the LH. The fragment was then used as a probe to obtain a complete cDNA clone by screening the pituitary cDNA library. The nucleotide sequences and the deduced amino acid sequences of LH are shown in Figure 2. The sequences have been submitted to GenBank with accession number AF112192. The cDNA sequences contained a coding se-

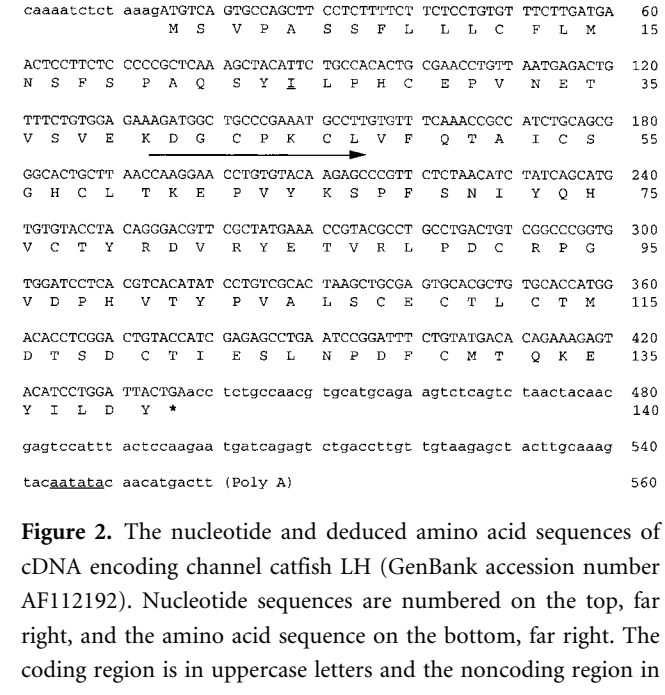

Figure 2. The nucleotide and deduced amino acid sequences of cDNA encoding channel catfish LH (GenBank accession number AF112192). Nucleotide sequences are numbered on the top, far right, and the amino acid sequence on the bottom, far right. The coding region is in uppercase letters and the noncoding region in lowercase letters. The first amino acid in the mature peptide is underlined. The stop codon is indicated by a bold asterisk. The potential poly(A)$^+$ sequences are underlined. The position of the PCR primer is indicated by a long arrow.

quence of 423 bp, a 5′ UTR of 14 bp, and a 3′ UTR of 123 bp. The LH cDNA encodes a polypeptide of 140 amino acids, with 24 amino acids of signal sequence. The mature peptide of LH in channel catfish, therefore, is predicted to be 116 amino acids. A putative poly(A)$^+$ signal sequence (AATATA) was found at 17 bp upstream from the poly(A)$^+$ site.

Structural and Evolutionary Conservation

The deduced amino acid sequences of the channel catfish FSH were compared with those from several teleosts and mammalian species (Figure 3). The carboxyl terminus is more evolutionarily conserved than the amino terminus. The 12 cysteines and the N-linked glycosylation sites are completely conserved in all FSH, indicating functional importance of these residues. The lengths of the teleost FSH vary from 113 amino acids to 137 amino acids. The channel catfish FSH is 132 amino acids long.

The carboxyl terminus of LH is also more evolutionarily conserved than the amino terminus. Similar to FSH, all 12 cysteine residues and the N-linked glycosylation site are completely conserved (Figure 4). These features indicate

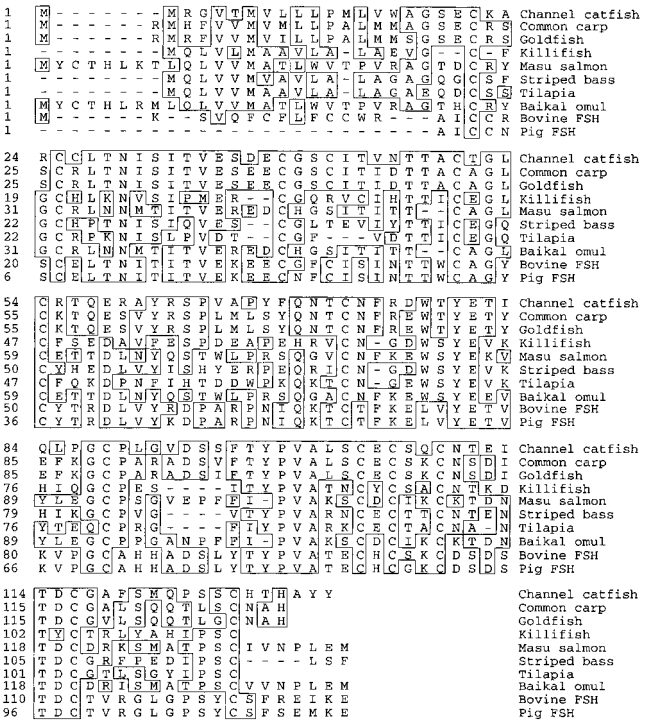

Figure 3. Alignment of the amino acid sequences of FSH. The sequences used for alignment were obtained from the published studies or from the nonredundant Genbank+EMBL+DDBJ+PDB databases. The following reference or accession numbers were used in this alignment: common carp, AB003583; goldfish, Yoshiura et al., 1997; killifish, M87014; masu salmon, Kato et al., 1993; striped bass, L35071, Hassin et al., 1995; tilapia, Rosenfeld et al., 1997; Baikal omul, L23432; bovine FSH, M13383, Esch et al., 1986; pig FSH, M35676, Kato, 1988.

not only the functional importance of these residues, but also the related origin of these molecules in evolution.

Phylogenetic analysis indicated that the channel catfish FSH (Figure 5, A) and LH (Figure 5, B) are highly related to those from African catfish and Cyprinidae, consistent with the phylogenetic relation of these species.

Comparisons of amino acid sequences (Figures 3 and 4) and phylogenetic analysis (Figure 5, A and B) clearly indicated that the channel catfish FSH is more similar to mammalian FSH than to LH. Similarly, the channel catfish LH is more similar to mammalian LH than to FSH. These results provided structural evidence for their identities as FSH and LH. It appeared that the teleost LH exhibited a greater level of divergence (Querat, 1994) than FSH (about 40% vs about 33%). However, this observation awaits confirmation since fewer teleost FSHs are available for comparison than LHs. Although the channel catfish LH clustered with African catfish, Cyprinidae, and Salmonidae

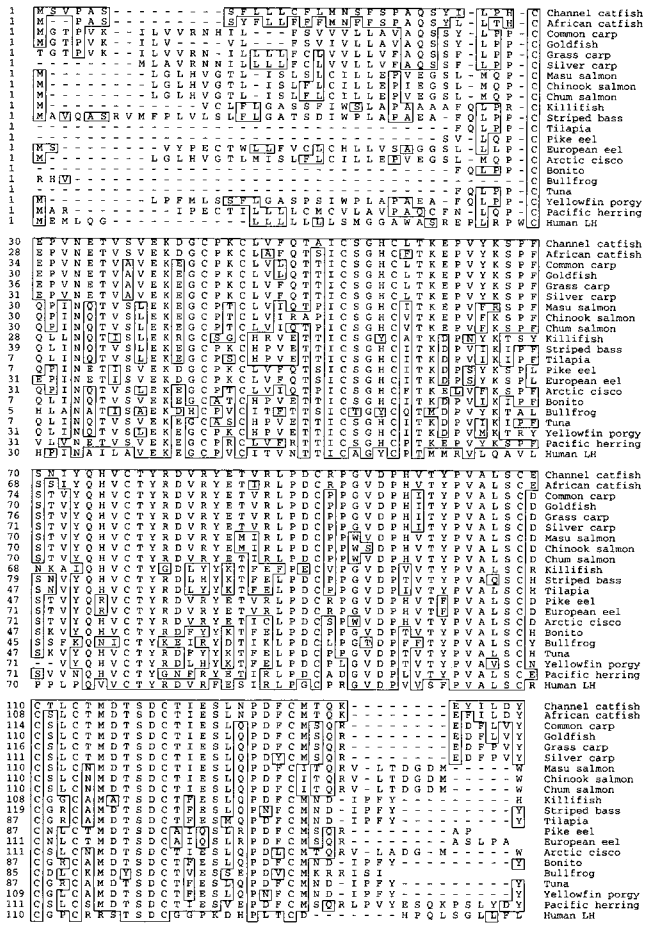

Figure 4. Alignment of the amino acid sequences of LH. The following references or accession numbers were used in this alignment: African catfish, X97761, Rebers et al., 1997; striped bass, L35096; tilapia, Rosenfeld et al., 1997; Chinook salmon, Xiong and Hew, 1993; Chum salmon, M27154, Sekine et al., 1989; European eel, X61039, Querat et al., 1990; killifish, M87015; carp, X59889, Chang et al., 1992; Masu salmon, S69276; yellowfin porgy, L11722; Pacific herring, Power et al., 1997; grass carp, X61051; human LH, X00264, Talmadge et al., 1984; goldfish, D88024; bullfrog, 126481; tuna, 585227, Okada et al. 1994; bonito, 298263, Koide et al., 1993; arctic cisco, 1346202.

(with about 20% divergence), several separate clusters appeared to be prominent: the Pacific herring by itself stands as a group (about 32% divergence), and striped bass, porgy, bonito, tuna, and killifish appeared to be a distinct cluster in the phylogenetic tree (Figure 5, B).

Similarity of the Catfish FSH and LH Subunits

A BLAST search using the channel catfish FSH as a query revealed significant similarities between channel catfish FSH

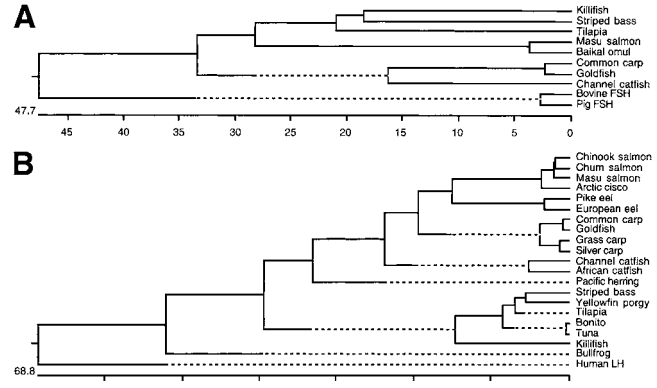

Figure 5. Cluster analysis of vertebrate pituitary glycoprotein FSH **(A)** and LH **(B).** The dendrograms were generated by the MEGALIGN program of the DNASTAR package using the CLUSTAL method. With the balanced display, MEGALIGN averages the distances between ancestors in the tree. The dotted lines indicate a negative branch length introduced by averaging the tree. The accession numbers were as described for Figures 3 and 4.

and LH from other organisms, in addition to the expected similarities to FSH (e.g., smallest accumulative probability for similarity between the channel catfish FSH and the African catfish LH is 1.7×10^{-29}). Thus, in terms of molecular evolution, it is likely that the FSH and LH of higher organisms both evolved from the teleost FSH (Power et al., 1997). This prompted us to compare the channel catfish FSH with LH. A comparison of the channel catfish FSH and LH amino acid sequences indicated 41% similarity between them. All 12 cysteine residues are conserved with fixed positions. Querat (1994) suggested that the cysteines are necessary to establish disulfide bonds with the α subunit and to determine tertiary structure essential for receptor binding. In addition to the 12 cysteine residues, a block of 10 amino acids located between the 7th cysteine and the 10th cysteine are identical between the channel catfish FSH and LH. This block of amino acids contain Pro-Val-Ala (PVA) sequences suggested for LH binding to the α subunit. In addition, all other key residues in LH suggested for binding to the α subunit (Querat, 1994; Power et al., 1997) are also shared by the FSH: the Cys (C) and Gly (G) in the teleost-specific sequence (CSGH), the N-glycosylation site, and the Glu (E) at the fourth position before the second cysteine. The histidine (H) residue in the so-called teleost-specific sequence (CSGH) is replaced by a tyrosine (Y) residue in killifish, frog, and human. Other identical blocks include the YXSP between the fifth and the sixth cysteine, the YET between the sixth and the seventh cysteine, and the GVD between

the seventh and the eighth cysteine residues. These similarities indicate 2 possibilities: the structural requirement or the functional requirement. The β subunits must couple to α subunits in the form of heterodimers to function. Thus, these shared blocks of amino acids may directly be involved in binding to the α subunit. However, extensive identity of amino acid sequences may suggest highly similar functions. In this view, most earlier studies concerning functions of FSH and LH were based on temporal difference in expression and perhaps also on inference from the mammalian FSH and LH. Systematic determination of FSH expression during ovulation was conducted in few teleosts. In fact, most studies used immunoassays to measure pituitary and plasma levels of GTH during sexual maturation and the subsequent reproductive cycles. The cross-reactivity of the antisera used in these studies is unknown (Hassin et al., 1995), especially considering the structural similarities between the FSH and LH. The possibility that FSH may also function for ovulation and spawning activities needs to be studied. In view of this notion, the channel catfish FSH was also activated during carp-pituitary-induced ovulation as discussed below.

Enhanced Expression of FSH and LH During Carp-Pituitary-Induced Ovulation

Expression of FSH and LH before and after injection of CPE was determined by Northern blot hybridization. Drastic increases in expression levels of the FSH and LH RNAs were observed during ovulation induced by injection of CPE (Figure 6). FSH and LH RNAs were expressed 4.3-fold and 10-fold, respectively, compared with those before CPE-induced ovulation. Although the enhanced gene expression was determined by one set of experiments in this research, similar levels of enhanced expression of FSH and LH were revealed by expressed sequence tag analysis (Karsi et al., 1998). Expression of the GTH β subunits was more dramatically activated during ovulation by CPE than the α subunit. We previously found only a 40% increase of the α-subunit RNA using the same animals and pituitaries (Liu et al., 1997). The absolute expression level of the α subunit, however, was much higher than the β subunits.

This research may have significant practical implications for manipulation of catfish spawning. Currently, CPE is the most frequently used reagent for induction of spawning in catfish hatcheries, but the level of success is inconsistent. CPE contains the full multiplicity of pituitary hormones that may directly work on the gonad. In addition to

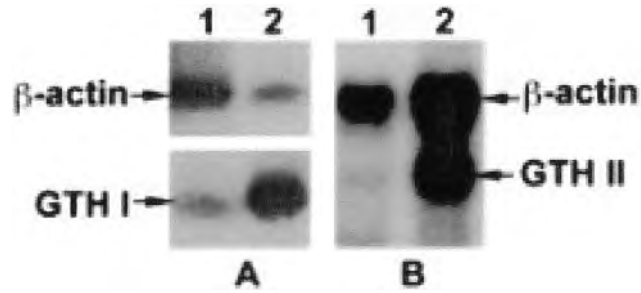

Figure 6. Expression of the channel catfish FSH and LH. Total RNA was used in the Northern hybridization analysis. For FSH **(A),** 2 separate gels with equal loading were run, transferred, and probed with β-actin probe (Liu et al., 1990) (top) and the FSH probe (bottom). For LH **(B),** the blot was simultaneously probed with both β-actin and LH probes. Pituitary RNA before injection with the carp pituitary extract (lane 1) and after induced ovulation with carp pituitary extract (lane 2) was used to determine activation of gene expression.

the direct roles of GTHs, growth hormone and IGF are known to be vital for gamete development and maturation (Kagawa et al., 1994; Izadyar et al. 1997; Maestro et al., 1997; Huang et al., 1998; Weil et al., 1999; Baker et al., 2000; Tacon et al., 2000). We also found significant induction of endogenous growth hormone gene expression during CPE-induced ovulation (Karsi et al., 1998). Furthermore, receptors for prolactin, TSH, and gonadotropin-releasing hormone have been recently reported to be present in the gonad tissues of fish, suggesting direct action of these hormones on the gonad (Alok et al., 2000; Kumar et al., 2000, 2001a, b). Endogenous GTH expression may also be involved in CPE-induced spawning as evidenced by the drastic activation of GTH gene expression. In a similar mechanism, luteinizing hormone–releasing hormone can effectively induce catfish spawning as well (Lin et al., 1988), although not as effectively as carp pituitary extract.

The drastic induction of FSH and LH, as well as growth hormone (Karsi et al., 1998), during CPE-induced spawning suggests that multiple pituitary hormones may be needed for optimal induction of catfish spawning activities. In a practical sense, this research suggests that a cocktail or sequential administration of FSH and LH, or perhaps growth hormone and other pituitary hormones may produce better results for artificially induced spawning. Even though CPE may contain all these hormones, the exact amount of the hormones contained within CPE may vary greatly from batch to batch. Therefore, the results of CPE-induced spawning may be unpredictable. As a matter of

fact, different preparations of CPE have produced highly variable results (unpublished results). It is, therefore, imperative to produce biologically active recombinant FSH and LH and other hormones for the study of reproductive physiology of catfish. The availability of the channel catfish FSH and LH cDNAs would make it possible to produce biologically active hormonal cocktails for studying regulation of spawning in channel catfish and for manipulation of their reproductive capacity. Such cocktails are particularly important for the production of interspecific hybrids of channel catfish females and blue catfish males, which exhibit superior aquaculture traits; however, mass production of their fingerlings is not yet feasible.

Acknowledgments

This project was supported by a USDA NRICGP grant to Z.L. and R.D. (98-35205-6738), by the Auburn University Competitive BioGrant (Biogrant J. Liu 99), and in part by a USDA BARD grant to Z.L., R.D., and B. Moav (US2954-97). S. Kim was supported by a graduate research assistantship from the Department of Fisheries and Allied Aquacultures. We thank Dr. Bernhart Kaltenback for allowing us to use his DNASTAR sequence analysis package and Dr. Skip Bartol for his GS-525 Molecular Imaging System to quantify our Northern blots.

References

Alok, D., Hassin, S., Kumar, R.S., Trant, J.M., Yu, K., and Zohar, Y. (2000). Characterization of a pituitary GnRH-receptor from a perciform fish, *Morone saxatilis:* functional expression in a fish cell line. *Mol Cell Endocrinol* 168:65–75.

Baker, D.M., Davies, B., Dickhoff, W.W., and Swanson, P. (2000). Insulin-like growth factor I increases follicle-stimulating hormone (FSH) content and gonadotropin-releasing hormone-stimulated FSH release from coho salmon pituitary cells *in vitro. Biol Reprod* 63:865–871.

Chang, Y.S., Huang, F.L., and Lo, T.B. (1992). Isolation and sequence analysis of carp gonadotropin beta-subunit gene. *Mol Mar Biol Biotechnol* 1:97–105.

Chomczynski, P., and Sacchi, N. (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. *Anal Biochem* 162:156–159.

Dunham, R.A. (1993). Observations on controlled artificial spawning of channel catfish. *Prog Fish Cult* 55:60–61.

Elizur, A., Zmora, N., Rosenfeld, H., Meiri, I., Hassin, S., Gordin, H., and Zohar, Y. (1996). Gonadotropins beta-GtHI and beta-GtHII from the gilthead seabream, *Sparus aurata. Gen Com Endocrinol* 102:39–46.

Esch, F.S., Mason, A.J., Cooksey, K., Mercado, M., and Shimasaki, S. (1986). Cloning and DNA sequence analysis of the cDNA for the precursor of the beta chain of bovine follicle stimulating hormone. *Proc Natl Acad Sci USA* 83:6618–6621.

Garcia-Hernandez, M.P., Koide, Y., Diaz, M.V., and Kawauchi, H. (1997). Isolation and characterization of two distinct gonadotropins from the pituitary gland of Mediterranean yellowtail, *Seriola dumerilii. Gen Comp Endocrinol* 106:389–399.

Hassin, S., Elizur, A., and Zohar, Y. (1995). Molecular cloning and sequence analysis of striped bass (*Morone saxatilis*) gonadotropins I and II subunits. *J Mol Endocrinol* 15:23–35.

Hein, J. (1990). Unified approach to alignment and phylogenies. *Methods Enzymol* 183:626–645.

Huang, Y.S., Rousseau, K., Le Belle, N., Vidal, B., Burzawa-Gerard, E., Marchelidon, J., and Dufour, S. (1998). Insulin-like growth factor-I stimulates gonadotrophin production from eel pituitary cells: a possible metabolic signal for induction of puberty. *J Endocrinol* 159:43–52.

Izadyar, F., Van Tol, H.T., Colenbrander, B., and Bevers, M.M. (1997). Stimulatory effect of growth hormone on in vitro maturation of bovine oocytes is exerted through cumulus cells and not mediated by IGF-I. *Mol Reprod Dev* 47:175–180.

Kagawa, H., Kobayashi, M., Hasegawa, Y., and Aida, K. (1994). Insulin and insulin-like growth factors I and II induce final maturation of oocytes of red seabream, *Pagrus major,* in vitro. *Gen Comp Endocrinol* 95:293–300.

Karsi, A., Li, P., Dunham, R.A., and Liu, Z.J. (1998). Transcriptional activities in the pituitaries of channel catfish (*Ictalurus punctatus*) before and after induced ovulation as revealed by expressed sequence tag analysis. *J Mol Endocrinol* 21:121–129.

Kato, Y. (1988). Cloning and DNA sequence analysis of the cDNA for the precursor of porcine follicle stimulating hormone (FSH) beta-subunit. *Mol Cell Endocrinol* 55:107–112.

Kato, Y., Gen, K., Maruyama, O., Tomizawa, K., and Kato, T. (1993). Molecular cloning of cDNAs encoding two gonadotrophin beta subunits (GTH-I beta and -II beta) from the masu salmon, *Oncorhynchus masou:* rapid divergence of the GTH-I beta gene. *J Mol Endocrinol* 11:275–282.

Kawauchi, H., Suzuki, K., Nagahama, Y., Adach, S., Naito, N., and Nakai, Y. (1986). Occurrence of two distinct gonadotropins in chum salmon pituitary. In: *Pars Distalis of the Pituitary Gland-Structure, Function, and Regulation,* F. Yoshimura, A., and Gorb-

man, A., (eds.). New York, N.Y.: Elsevier Science Publishers BV (Biomedical Division), 383–390.

Koide, Y., Itoh, H., and Kawauchi, H. (1993). Isolation and characterization of two distinct gonadotropins, GTH I and GTH II, from bonito (*Katsuwonus plelamis*) pituitary glands. *Int J Pept Protein Res* 41:52–65.

Kumar, R.S., Ijiri, S., Kight, K., Swanson, P., Dittman, A., Alok, D., Zohar, Y., and Trant, J.M. (2000). Cloning and functional expression of a thyrotropin receptor from the gonads of a vertebrate (bony fish): potential thyroid-independent role for thyrotropin in reproduction. *Mol Cell Endocrinol* 167:1–9.

Kumar, R.S., Ijiri, S., and Trant, J.M. (2001a). Molecular biology of channel catfish gonadotropin receptors, 1: cloning of a functional luteinizing hormone receptor and preovulatory induction of gene expression. *Biol Reprod* 64:1010–1018.

Kumar, R.S., Ijiri, S., and Trant, J.M. (2001b). Molecular biology of the channel catfish gonadotropin receptors, 2: complementary DNA cloning, functional expression, and seasonal gene expression of the follicle-stimulating hormone receptor. *Biol Reprod* 65:710–717.

Lambert, D., Argue, B., Liu, Z.J., and Dunham, R.A. (1999). Effects of seasonal variations, thyroid and steroid hormones, and carp pituitary extract on the artificial production of channel catfish *Ictalurus punctatus* × blue catfish *I. furcatus* hybrids. *J World Aquaculture Soc* 30:80–89.

Lin, H.R., Van der Kraak, G., Zhou, X.J., Liang, J.Y., Peter, R.E., Rivier J.E., and Vale, W.W. (1988). Effects of [D-Arg^6, Trp^7, Leu^8, Pro^{9}NEt]-luteinizing hormone-releasing hormone (sGnRH-A) and [D-Ala^6, Pro^{9}NEt]-luteinizing hormone-releasing hormone (LHRH-A), in combination with pimozide or domperidone, on gonadotropin release and ovulation in the Chinese loach and common carp. *Gen Comp Endocrinol* 69:31–40.

Lin, Y., Rupnow, B.A., Price, D.A., Greenberg, R.M., and Wallace R.A. (1992). *Fundulus heteroclitus* gonadotropin, 3: cloning and sequencing of gonadotropic hormone (GTH) I and II β-subunits using the polymerase chain reaction. *Mol Cell Endocrinol* 85:127–139.

Liu, Z.J., Zhu, Z., Roberg, K., Faras, A., Guise, K., Kapuscinski, A., and Hackett, P. (1990). Isolation and characterization of the β-actin gene of carp (*Cyprinus carpio*). *DNA Seq* 1:125–136.

Liu, Z.J., Li, P., Argue, B., and Dunham, R.A. (1997). Gonadotropin α subunit glycoprotein from channel catfish (*Ictalurus punctatus*) and its expression during hormone-induced ovulation. *Mol Mar Biol Biotechnol* 6:221–231.

Maestro, M.A., Planas, J.V., Moriyama, S., Gutierrez, J., Planas, J., and Swanson, P. (1997). Ovarian receptors for insulin and insulin-like growth factor I (IGF-I) and effects of IGF-I on steroid production by isolated follicular layers of the preovulatory coho salmon ovarian follicle. *Gen Comp Endocrinol* 106:189–201.

Nagae, M., Todo, T., Gen, K., Kato, Y., Young, G., Adachi, S., and Yamauchi, K. (1996). Molecular cloning of the cDNAs encoding pituitary glycoprotein hormone alpha- and gonadotropin II beta-subunits of the Japanese eel, *Anguilla japonica,* and increase in their mRNAs during ovarian development induced by injection of chum salmon pituitary homogenate. *J Mol Endocrinol* 16:171–181.

Naito, N., Hyodo, S., Okumoto, N., Urano, A., and Nakai, Y. (1991). Differential production and regulation of gonadotropins (GtH-I and GtH-II) in the pituitary gland of rainbow trout, *Oncorhynchus mykiss,* during ovarian development. *Cell Tissue Res* 266:457–467.

Naito, N., Suzuki, K., Swanson, P., Nozaki, M., Kawauchi, H., and Nakai, Y. (1993). Ultrastructural characteristics of two distinct gonadotrophs (GtH-I and GtH-II cells) in the pituitary of the rainbow trout (*Oncorhynchus mykiss*). *Fish Physiol Biochem* 11: 241–246.

Nozaki, M., Naito, N., Swanson, P., Dickhoff, W.W., Nakai, Y., Suzuki, K., and Kawauchi, H. (1990a). Salmonid pituitary gonadotropins, II: ontogeny of GtH-I and GtH-II cells in the rainbow trout (*Salmo gairdneri irideus*). *Gen Com Endocrinol* 77:358–367.

Nozaki, M., Naito, N., Swanson, P., Miyata, K., Nakai, Y., Oota, Y., Suzuki, K., and Kawauchi, H. (1990b). Salmonid pituitary gonadotropins, I: distinct cellular distributions of two gonadotropins, GtH I and II. *Gen Com Endocrinol* 77:348–357.

Okada, T., Kawazoe, I., Kimura, S., Sasamoto, Y., Aida, K., and Kawauchi, H. (1994). Purification and characterization of gonadotropin I and II from pituitary glands of tuna. *Int J Pept Protein Res* 43:69–80.

Patino, R. (1995). Mechanisms of maturational competence in fish gonads. In: *Proceedings of the Fifth International Symposium on the Reproductive Physiology of Fish.* Austin: University of Texas at Austin, 143–145.

Patino, R. (1997). Manipulations of the reproductive system of fishes by means of exogenous chemicals. *Prog Fish Cult* 59:118–128.

Pierce, J.G., and Parsons, T.F. (1981). Glycoprotein hormones: structure and function. *Annu Rev Biochem* 50:465–495.

Power, M.E., Carolsfeld, J., Wallis, G.P., and Sherwood, N.M. (1997). Isolation and characterization of a cDNA for gonadotropin II-β of Pacific herring, an ancient teleost. *J Fish Biol* 50:315–323.

Querat, B. (1994). Molecular evolution of the glycoprotein hormones in vertebrates. In: *Perspectives in Comparative Endocrinology,* Davey, K.G., Peter, R.E., and Tobe, S.S. (eds.). Ottawa: National Research Council of Canada, 27–35.

Querat, B., Moumni, M., Jutisz, M., Fontaine, Y.A., and Counis, R. (1990). Molecular cloning and sequence analysis of the cDNA for the putative beta subunit of the type-II gonadotrophin from the European eel. *J Endocrinol* 4:257–264.

Rebers, F.E.M., Tensen, C.P., Schulz, R.W., Goos, H.J.T., and Bogerd, J. (1997). Modulation of glycoprotein hormone alpha- and gonadotropin II beta-subunit mRNA levels in the pituitary gland of mature male African catfish, *Clarias gariepinus. Fish Physiol Biochem* 17:99–108.

Rosenfeld, H., Levavi-sivan, B., Melamed, P., Yaron, Z., and Elizur, A. (1997). The GTH β subunits of tilapia: gene cloning and expression. *Fish Physiol Biochem* 17:85–92.

Sambrook, J., Frisch, E.F., and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press.

Sanger, F., Nicklen, S., and Coulson, A.R. (1977). DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA* 74: 5463–5467.

Sekine, S., Saito, A., Itoh, H., Kawauchi, H., and Itoh, S. (1989). Molecular cloning and sequence analysis of chum salmon gonadotropin cDNAs. *Proc Natl Acad Sci USA* 86:8645–8649.

Tacon, P., Baroiller, J.F., Le Bail, P.Y., Prunet, P., Jalabert, B. (2000). Effect of egg deprivation on sex steroids, gonadotropin, prolactin, and growth hormone profiles during the reproductive cycle of the mouthbrooding cichlid fish *Oreochromis niloticus. Gen Comp Endocrinol* 117:54–65.

Talmadge, K., Vamvakopoulos, N.C., and Fiddes, J.C. (1984). Evolution of the genes for the beta subunits of human chorionic gonadotropin and luteinizing hormone. *Nature* 307:37–40.

Weil, C., Carre, F., Blaise, O., Breton, B., Le Bail, P.Y. (1999). Differential effect of insulin-like growth factor I on in vitro gonadotropin (I and II) and growth hormone secretions in rainbow trout (*Oncorhynchus mykiss*) at different stages of the reproductive cycle. *Endocrinology* 140:2054–2062.

Xiong, F., and Hew, C.L. (1993). Chinook salmon (*Oncorhynchus tschawytscha*) gonadotropin II beta-subunit gene encodes multiple messenger ribonucleic acids. *Can J Zool* 69:2572–2578.

Yoshiura, Y., Kobayashi, M., Kato, Y., and Aida, K. (1997). Molecular cloning of the cDNAs encoding two gonadotropin beta subunits (GTH-I beta and -II beta) from the goldfish, *Carassius auratus. Gen Comp Endocrinol* 105:379–389.