Transcriptional activities in the pituitaries of channel catfish before and after induced ovulation by injection of carp pituitary extract as revealed by expressed sequence tag analysis

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ABSTRACT

Expressed sequence tag (EST) analysis was adopted to address physiological changes after injection of carp pituitary extract for induction of ovulation. ESTs were analyzed from cDNA libraries constructed from mRNA isolated from channel catfish (*Ictalurus punctatus*) pituitaries before and after induction of ovulation by injection of carp pituitary extract. One hundred randomly picked clones were analyzed. Of the sequences generated, a large percentage (59%) of ESTs were identified as known genes by identity comparisons. These 59 clones of known gene products represent transcriptional products of 30 genes. The 41 clones of unknown gene products represent 33 genes. Expression of gonadotropin (GtH) α-subunit (149%) and prolactin (176%) was slightly enhanced as a result of induced ovulation. Large increases in frequencies of several peptide hormones were observed as a result of induced ovulation: GtH β-I, 486%; GtH β-II, 933%; growth hormone, 393%; proopiomelanocortin (POMC), 345%. POMC represented about 21% of all transcriptional activity in the pituitaries after induced ovulation. This is the first study addressing physiological changes after injection of carp pituitary extract, a procedure widely used in catfish hatcheries.

Journal of Molecular Endocrinology (1998) **21**, 121–129

INTRODUCTION

Channel catfish is the most important aquaculture species in the United States. The fish spawn once a year in the spring when water temperature is between 24 and 30 °C. Artificial spawning is often used to induce synchronized ovulation. Several ovulating agents were tested for catfish, such as carp pituitary, catfish pituitary, clomiphene citrate, human chorionic gonadotropin, Ovaprim, luteinizing hormone-releasing hormone, and pregnant mare serum (Sneed & Clemens 1960, Sneed 1962, Busch 1978, Bidwell *et al.* 1985, Busch & Steeby 1990, Goudie *et al.* 1992, Kelly & Kohler 1996). All are effective, although carp pituitary is the most potent with 70–100% of females ovulating after injection (Dunham 1993, Kim 1996). However, no information is available concerning changes in physiological processes after injection of carp pituitary extract. The approach of expressed sequence tag (EST) analysis is useful for characterization of gene expression. It is particularly valuable for characterizing genes with low levels of expression and for systems, such as pituitaries, where biological samples are limited.

ESTs are partial cDNA sequences corresponding to mRNAs and are generated from randomly selected library clones (Adams *et al.* 1991). Characterization of ESTs is a convenient and rapid way for identification of new genes in various organisms. The EST approach is being used extensively to analyze expressed genes from various organisms such as humans (Adams *et al.* 1991, Wolfsberg & Landsman 1997), *Caenorhabditis elegans* (Waterston *et al.* 1992), *Arabidopsis thaliana* (Hofte 1993), *Plasmodium falciparum* (Chakrabarty *et al.* 1994), *Schitosoma mansoni* (Franco *et al.* 1995), *Entamoeba histolytica* (Azam *et al.* 1996), and rice (Aliyeva *et al.* 1996). EST is generated by a single pass sequencing at both the upstream and the

downstream of cDNAs using arbitrarily selected cDNA libraries. The EST approach is very important for generating type I (expressed sequence) markers for gene mapping. The generation of human ESTs, for example, was a crucial step in the progress of the human genome project (Wolfsberg & Landsman 1997). The availability of these human ESTs enabled the recent mapping of 16 000 genes in the human genome (Schuler *et al.* 1996). Although many other types of molecular markers are currently available for use in genome mapping, ESTs represent real genes. Because DNA sequences are more conserved in genes than in non-expressed sequences, it is possible to construct comparative anchorage maps using ESTs.

In an effort at genome mapping for catfish, we have initiated the development of EST markers for mapping using a channel catfish (*Ictalurus punctatus*) and blue catfish (*I. furcatus*) hybrid system. Availability of EST markers will facilitate catfish genome mapping. More importantly, by using ESTs derived from pituitary cDNA libraries constructed before and after induction of ovulation, it is possible to examine some of the important hormonal changes resulting from induced ovulation and spawning. Such information should be useful in the consideration and design of artificial spawning induction. In this report, we present data from EST analysis of the channel catfish pituitary cDNA library and from analysis of changes in expression of some hormonal genes using the cDNA libraries constructed from mRNA isolated before and after induced ovulation by injection of carp pituitary extract.

MATERIALS AND METHODS

Animals and treatments

Channel catfish were obtained from the Fish Genetics Research Unit of Auburn University. To gain initial understanding of some physiological changes after injection of carp pituitary extract for induced ovulation, gravid fish were selected for experiments, randomized in seven groups of five fish each, and placed in indoor tanks. Water temperature was kept at 27–28 °C. Pituitaries from fish in each treatment were collected at different times to isolate RNA. Carp pituitary extract (Stollers, Spirit Lake, Iowa, USA) was weighed into 15 ml plastic test tubes, then saline solution was added to give a concentration of 4 mg/ml. The carp pituitaries were homogenized by using a tissue tearer. The homogenates were centrifuged in an SS-34 rotor for 10 min at 3000 r.p.m. The supernatant was collected for injection.

At the beginning of the experiment, tissues from the first group of fish were harvested as the 0 h control. Carp pituitary extract was injected intraperitoneally into the fish in the remaining six groups at a priming dose of 2 mg/kg body weight, and at a resolving dose of 8 mg/kg body weight after 12 h where appropriate. Pituitaries were collected 4 h, 8 h, 12 h (before the second injection), 16 h (4 h after the second injection), 20 h, and 36 h after the initial injection. Pituitaries from different time points after injection were pooled to obtain enough tissue samples for preparation of RNA. All fish were treated with MS-222 at 300 p.p.m. to anesthetize the fish before harvesting the pituitaries.

RNA isolation

Five pituitaries from non-injected fish were pooled, and 30 pituitaries from fish after injection of carp pituitary extract were pooled prior to RNA extraction. The 30 pituitaries included 5 pituitaries from each time point: 4, 8, 12, 16, 20, and 36 h after the initial injection. The pituitary glands were harvested from freshly killed fish, frozen in liquid nitrogen and then transferred for long-term storage at −80 °C in a freezer. Pituitaries were frozen in liquid nitrogen again for grinding with a mortar/pestle and then homogenized with a hand-held tissue tearer (Model 985-370, Biospec Products, Inc., Dremel, WI, USA) in RNA extraction buffer following the guanidinium thiocyanate method (Chomczynski & Sacchi 1987). Poly (A)$^+$ RNA was then purified by using the Oligotex Spin Column Kit from Qiagen according to the manufacturer's instructions (Qiagen Inc., Chatsworth, CA, USA).

Preparation of λ Unizap cDNA libraries

Two Unizap λ cDNA libraries were prepared from the poly (A)$^+$ RNA according to the manufacturer's instructions (Stratagene, La Jolla, CA, USA). The procedures for construction of the two cDNA libraries have previously been reported (Liu *et al.* 1997) and used the standard protocols of oligo dT priming for first strand cDNA synthesis. The primary cDNA libraries had a titer of two million recombinant clones with a blue background of less than 0·4%. The primary cDNA libraries were then amplified once.

Mass *in vivo* excision

The cDNA libraries prepared in λ Unizap vectors allow the conversion of the λ libraries into plasmid libraries by using the mass *in vivo* excision procedure (Stratagene). When the cDNA inserts

were all in a plasmid vector, the procedures for DNA preparation and handling, sequencing, and EST analysis were simplified. The *in vivo* excision process was conducted according to the manufacturer's instructions. Briefly, 20 million plaque-forming units (p.f.u.) were used for each library representing clones equivalent to 10 initial cDNA libraries before amplification (primary recombinant clones). The XL1-blue plating bacteria were infected with the 20 million phages (10 cells : 1 phage), and also infected with the ExAssist helper phage at 1:1 helper phage-to-cell ratio. Cells were incubated at 37 °C for phage absorption. The infected cells were then grown in 20 ml LB broth for 3 h with shaking at 37 °C. During this time, phagemids were excised and secreted into the medium. The cells were then heated to 70 °C for 20 min and removed by centrifugation. The excised phagemids in the supernatant were titered. Any given number of clones can be grown on plates using the titer of the phagemids and the SOLR strain of *E. coli* (Stratagene), which can only be infected by the phagemids but not by the λ phage. SOLR cells were infected with the desired numbers of phagemids and incubated at 37 °C for 15 min. The cells were then plated onto LB plates containing ampicillin at 50 μg/ml. Colonies were picked and cultures were grown in LB liquid culture for plasmid preparation.

Plasmid preparation and sequencing analysis

Plasmid DNA was prepared by using the alkaline lysis procedure (Sambrook *et al*. 1989). Since the direction of cDNAs in the Unizap vector is fixed, the direction of cDNAs in the plasmid vector is also fixed, so that one knows which portion of the cDNA is sequenced with both the universal and reverse sequencing primers. All cDNA clones were manually sequenced using the Sanger's dideoxy termination method (Sanger *et al*. 1977) with a cycle sequencing kit (Applied Biosystems, Inc., Foster City, CA, USA). Mini-preparation plasmid DNA (1 μl, about 200–500 ng) was used for all sequencing reactions. The profiles for cycling were: 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min for 30 cycles. An initial 2 min of extra denaturation at 94 °C was always used. DNA sequences were read and analyzed by using the micro-computer software packages DNASIS (Hitachi, version 2·0) and DNASTAR (DNA Star Inc., Madison, WI, USA). For most clones, readable sequences of about 350 bases were generated from a single pass sequencing. Because of the specialized function of the pituitary, we expected that some genes were expressed at high levels and, therefore, would be detected at high frequencies. DNA dot blot analysis was used to detect repeated clones after a second detection of the cDNA from sequencing. Vector sequences were removed manually before searching for homologies using BLASTN and BLASTX servers, through Internet (NCBI, Bethesda, MD, USA). Matches were considered to be significant only when the probability (P) was less than 0·0001 and scores were greater than 160 for BLASTN and greater than 80 for BLASTX. The 100 pituitary cDNA clones used in this study were from the pituitary cDNA library constructed with mRNA isolated from pituitaries after induction of ovulation using carp pituitary extracts.

Validation of quantitative data

Validation of the quantitative data depends on the assumption that the frequency of detecting cDNAs from the pituitary cDNA library approximately reflects the abundance of a particular gene product which is a result of gene transcriptional activities from the catfish pituitary, and that differences before and after induction of ovulation with carp pituitary extract indicate gene expression changes as a result of induction of ovulation by injection of carp pituitary extract. This assumption is generally valid since there is no discrimination mechanism in the reverse transcription procedure for construction of cDNA libraries, although differential amplifications of specific clones are possible after the primary cDNA library is made. To exclude the possibility of differential amplification of certain clones during library amplification, RNA dot blot analysis was conducted. No preferential amplification of clones was observed. The assumption was thus validated by comparing dot blot hybridization data with the frequencies detected with library screening. This was accomplished with β-actin probe (Liu *et al*. 1990, screening data not shown), gonadotropin (GtH) α-subunit probe (Liu *et al*. 1997), growth hormone (GH) probe, GtH β-I probe, GtH β-II probe, and pro-opiomelanocortin (POMC) probe. Analysis of EST rather than direct analysis of RNA is important in this case because of the limited biological samples of the pituitaries. The killing of large numbers of brood fish was not possible or not desirable.

Plaque and colony hybridization

Plaque hybridization or colony hybridization was used to compare frequencies of certain hormone genes: GH, prolactin, POMC, GtH α-subunit, GtH β-I, and GtH β-II. Lambda phage or plasmid libraries (converted from the λ libraries) were

TABLE 1. Genes identified by high similarity with sequences known from channel catfish (*Ictalurus punctatus*)

Clone	Accession number	Putative identification	Overlap (bp)	Score	% identity	Probability	No. of ESTs
Ip097	S69215	Growth hormone	145	721	100	2.3e-83	4
Ip063	U33273	Ictacalcin	147	443	100	1.9e-43	1
Ip095	*	Gonadotropin α	189	530	100	1.2e-75	1

*Liu *et al*. (1997).

grown and transferred to nitrocellulose filter and hybridized to specific probes following standard protocols (Sambrook *et al*. 1989). Probes were labeled using [^{33}P]dATP or [^{32}P]dCTP and the random primer labeling kit (Boehringer Mannheim, Indianapolis, IN, USA). In each case, probes were isolated from the cDNA clones that harbor the insert as revealed by the EST sequencing analysis. The cDNA fragments were isolated by digesting the corresponding clones with *Eco*RI and *Xho*I restriction endonucleases and gel-purified (Sambrook *et al*. 1989). Five hundred to two thousand five hundred plaques or colonies were used per plate for accurate counting and calculation of frequencies of clones. The frequencies were expressed as the percentage of positive clones of the total clones examined.

RESULTS AND DISCUSSION

Known genes and novel genes from the catfish expressed sequence tags

Of the 100 clones sequenced, 59 clones were found to represent known genes and 41 clones were found to be unknown genes. The sequences of the ESTs will be available from our web site http://www.ag.auburn.edu/genemap and will also be deposited to the Genbank. The proportion (59%) of known genes is slightly higher than was found from ESTs of *Entamoeba histolytica* (Azam *et al*. 1996), probably because: (1) the pituitary is more specialized than other tissues in gene expression; thus a fewer total number of genes are expressed in the pituitary than in other tissues, and (2) perhaps more genes are known from the pituitary because of its importance in hormone production and regulation.

Of the 59 clones, multiple clones were encountered for several genes including the ribosomal protein L41, cyclic adenosine monophosphate (cAMP)-responsive element modulator, POMC, prolactin, GH, and β-globin. Among these genes, the POMC appears to be the most abundant RNA species in the pituitary of catfish during the spawning season, and accounted for 21% of the 100 clones sequenced (Tables 1 and 2). Thus the 59 clones of known genes represented gene products from 30 known genes. Three of the thirty genes were identified by high similarity with known catfish genes (Table 1). These are GH (Tang *et al*. 1993), GtH α-subunit (Liu *et al*. 1997), and ictacalcin (Porta *et al*. 1996). The rest of the 30 genes were identified by high similarities with known genes from other organisms (Table 2).

Multiple clones were also encountered among the 41 clones representing unknown genes. Of the 41 clones, 29 clones were unique and 4 clones were encountered twice. Four clones did not generate readable sequences and were eliminated from further analysis. Thus the 41 unknown clones represent gene products of at least 33 genes. Along with the 30 genes from the 59 clones representing known genes, a total of 63 gene products was partially sequenced and characterized for the EST study.

Hormones and other regulators are the major transcriptional products in the catfish pituitary during spawning

Among the 100 clones sequenced, only the 59 clones representing products of 30 known genes were further analyzed. These 59 clones can be grouped into three major groups: the hormones and other cell cycle regulators, the translational proteins, and several enzymes (Table 3). Among the 59 known gene product clones, hormonal clones accounted for 32 clones or 54·2%: POMC (21/59 or 35·6% of known genes), GH (4/59), prolactin (3/59), GtHs (3/59), and somatolactin (1/59). In addition to the hormonal clones, several other cellular regulators were also among the known gene product clones: RAP1A (1/59), cAMP-responsive element modulator (2/59), cyclin 2b (2/59), and GTP-binding protein (rap 7, 1/59). Overall, 37 out of 59 clones were hormonal or other cellular regulators (62·7%).

TABLE 2. Genes identified by high similarity with sequences known from other organisms

Clone	Accession number	Putative identification	Overlap (bp)	Score	% identity	Probability	No. of ESTs
Ip009	M27010	Prolactin	68	232	82	2.4e-09	3
Ip032	X84195	Acylphosphatase	172	226	58	7.5e-09	1
Ip046	D14521	Ribosomal protein L30	87	332	86	4.9e-63	1
Ip057	X62640	Ribosomal protein L7a	70	270	92	4.9e-28	1
Ip030	X87106	Ribosomal protein L10	74	231	78	3.6e-29	1
Ip043	X52238	Ribosomal protein L18	149	313	67	3.3e-33	1
Ip049	U30454	Ribosomal protein S2	144	482	81	1.7e-40	1
Ip027	X06617	Ribosomal protein S11	101	379	86	5.8e-24	1
Ip035	X60289	Ribosomal protein S24	168	557	81	2.8e-46	1
Ip039	L31609	Ribosomal protein S29	70	215	78	4.1e-08	1
Ip061	X51705	Ribosomal protein L35	80	283	83	5.1e-40	1
Ip095	U93862	Ribosomal protein L41	80	269	87	2.6e-16	2
Ip016	X85755	RAP1A	82	284	82	4.5e-31	1
Ip093	X92411	MHR23B	127	415	80	2.8e-25	1
Ip076	U10900	Alcohol dehydrogenase	80	260	81	1.7e-14	1
Ip021	X69808	Pro-opiomelanocortin	137	352	84	1.5e-21	21
Ip044	D88023	Gonadotropin β-I	223	654	74	1.7e-71	1
Ip094	X97761	Gonadotropin β-II	100	250	73	6.8e-34	1
Ip024	K02064	Cytochrome c oxidase	160	395	89	2.4e-25	1
Ip034	M24193	MHC B complex protein	199	725	84	9.8e-53	1
Ip067	U06753	Somatolactin	101	197	80	7.2e-16	1
Ip064	S67786	cAMP-responsive element modulator	93	232	93	4.2e-15	2
Ip058	U13842	Vascular adenosine triphosphatase subunit c	153	261	86	6.4e-31	1
Ip029	L14945	Mitochondrial cytochrome oxidase 1	120	223	71	2.1e-20	1
Ip001	Z31401	Cyclin 2b	100	221	97	7.6e-24	2
Ip099	S83540	β-globin	195	326	81	6.4e-31	2
Ip068	M17886	Ribosomal phosphoprotein P1	96	137	94	3.4e-08	1
Ip015	M35522	GTP-binding protein	47	208	93	4.7e-08	1

TABLE 3. Major transcriptional activities of the channel catfish (*Ictalurus punctatus*) pituitaries during induced ovulation with carp pituitary extract

Gene products	No. of clones analyzed	% of known gene product clones
Hormones	32	53·3
Cellular regulators	4	6·7
Ribosomal proteins	11	18·3
Enzymes	4	6·7
Other	9	15·0

The second major group included translational machinery proteins such as large and small ribosomal proteins. Among these were 7 clones of large ribosomal proteins representing six proteins: L7a, L10, L18, L30, L35, and L41 (two clones). Similarly, three small ribosomal protein clones were sequenced: ribosomal protein S11, S24, and S29. One clone represented the ribosomal phosphoprotein P1. Altogether, translational proteins represented 11 clones out of 59 clones of known gene product clones (18·6%).

The third group included several enzyme RNAs: alcohol dehydrogenase, cytochrome c oxidase, vascular adenosine triphosphatase, and acylphosphatase (Table 2). In addition, two clones were found to be β-globin cDNA; one clone to be MHC B complex protein, one clone as ictacalcin. Clearly, the major transcriptional activities in the pituitary are to maintain proper hormonal and other cellular regulation. All of the other gene products are probably 'supporting facilities' for the hormonal and regulatory functions of the pituitary. Because we used pituitaries from the spawning season and the catfish were actually injected with carp pituitary extract to induce ovulation, the pituitary may be more polarized than in non-spawning seasons. Some of cDNA clones such as the two β-globin cDNA clones may actually represent contamination of pituitaries with blood cells.

Changes of transcriptinal activities in the catfish pituitary as a function of induced ovulation

To gain basic understanding of transcriptional activities during ovulation, especially after induced

TABLE 4. Increased expression of several hormone genes during induction of ovulation

	Before ovulation		After ovulation		
	Observed positives	% of total expression	Observed positives	% of total expression	% induction
Genes					
Pro-opiomelanocortin	35/641	5·5	470/2476	19	345
Prolactin	27/641	4·2	182/2476	7·4	176
Growth hormone	9/641	1·4	137/2476	5·5	393
GtH α-subunit	8/641	1·2	46/2476	1·9	149
GtH β-I	7/1385	0·5	46/1894	2·4	486
GtH β-II	5/2283	0·2	40/1957	2·0	933

ovulation, pituitaries were harvested before and after induction of ovulation. Because of the limitation of the small amount of biological material available with pituitaries, we decided to characterize ESTs from cDNA libraries that were constructed from mRNA isolated from these pituitaries. A comparison of hormone gene expression by analyzing randomly selected EST clones using plaque hybridization (Sambrook *et al*. 1989) allowed us to compare gene expression activities of several of the pituitary hormones before and after induction of ovulation by injection of carp pituitary extract.

In general, all hormone cDNAs analyzed were detected at higher frequencies after induction of ovulation by injection of carp pituitary extract than before injection. However, there was only a slight increase in the GtH α-subunit and prolactin (only 49–76% increase) (Table 4). In contrast, there was a major increase for gene products representing the GtH β-I subunit, GtH β-II subunit, GH, and the POMC (345–933% increase in abundance) (Table 4). The GtH β-II subunit expression was induced almost 10-fold.

Although induction of ovulation for artificial spawning is an important process that is routinely used by fisheries and aquaculture biologists, the exact mechanism of induction is not completely understood. It was assumed that GtHs from the carp pituitary extract used for induction of ovulation may directly elicit ovulation at the level of the ovarian follicle, as pituitary extract can induce oocyte maturation and ovulation *in vitro* (Kime & Dolben 1985, Kime *et al*. 1989). However, an indirect effect from GtH-releasing hormones (GnRH) may increase endogenous gene expression of certain hormones as well, which in turn, can induce ovulation (Liu *et al*. 1997). It has long been speculated that GtH-II is involved in final oocyte maturation and it has been referred to as the maturational GtH. However, the major body of literature describing the role of the maturational GtH in fish reproductive physiology was derived

from studies using immunoassays to measure pituitary and plasma levels of GtH during sexual maturation and the subsequent reproductive cycles (Hassin *et al*. 1995). As pointed out by Hassin *et al*. (1995), many data obtained in studies prior to 1986 are hard to interpret since the cross-reactivity of the antisera used in these assays with GtH-I was not known. Additionally, most research of this kind was carried out with salmonids (reviewed by Swanson 1991), which respond more to photoperiod for their reproductive cycles, whereas temperature is the more important natural regulator of catfish reproductive cycles.

Using EST analysis, our results here clearly indicated differential regulation of GtH α and GtH β. The large increase in gene expression from GtH β after induced ovulation suggests a significant role of GtH β in final oocyte maturation and ovulation in catfish, similar to the situation in salmonids and other fishes (Swanson 1991, Prat *et al*. 1996, Zohar 1996). GtH β-II was also induced to a much greater extent than GtH β-I (933% vs 486%), indicating a crucial role for GtH-II in ovulation and final oocyte maturation.

A large increase in GH expression (393%) after injection was observed. The role of GH in teleost ovulation is, to our knowledge, largely unknown. The large increase in GH expression after injection of carp pituitary extract suggests that research in this area should be conducted with fish. In mammals, GH has been used together with GtHs for induction of ovulation (Artini *et al*. 1996). It was suggested that GH acts as a co-GtH to augment the actions of follicle-stimulating hormone and luteinizing hormone (LH) on estradiol and progesterone production (Sharara & Giudice 1997). It has been demonstrated that GH significantly accelerates bovine oocyte maturation *in vitro* (Izadyar *et al*. 1996). Significantly enhanced expression of GH and prolactin has been reported from chicken during embryogenesis (Kansaku *et al*. 1994, Karatzas *et al*. 1997). In catfish, however, prolactin was only

modestly induced by injection of carp pituitary extract.

POMC was observed to be the most abundant gene product in the pituitaries of spawning catfish. An increase of 345% was observed after injection. POMC is well known for its involvement in stress in mammals (Hollt *et al*. 1986, Wu & Childs 1991, Larsen & Mau 1994). In general, a similar involvement of POMC in the stress response of teleost fish was also found (Balm *et al*. 1994, reviewed by Bonga *et al*. 1995). Whether the increase in POMC RNA expression is related to stress response in catfish after injection is not known at present. It is possible that increased POMC expression is simply a result of stress caused by injection and/or it may be up-regulated to cope with the overwhelming increase in GtH β expression during induced ovulation. POMC is the common precursor of adrenocorticotropin (ACTH) and ACTH-related peptides, β-endorphin (Eipper & Mains 1980), and multiple copies of the melanotropins (Nakanishi *et al*. 1979). In relation to reproduction, transcriptional activation and/or repression of POMC have been reported in several studies in late gestation and during active labor in mammals (McMillen *et al*. 1988, Yang *et al*. 1991, Myers *et al*. 1993, Matthews *et al*. 1994, Matthews & Challis 1995). It is generally recognized that the opioid peptides inhibit GnRH-mediated LH release and interfere with the GtH stimulatory effect on gonadal sex steroid production (Fabbri *et al*. 1989).

Genes important for biotechnology

Several cDNA clones identified in this study may be important for application in biotechnology, for example for gene transfer studies in catfish. Among these clones, the channel catfish GH cDNA is useful for gene transfer using mini-gene constructs rather than the complete gene. Although the channel catfish GH gene was cloned several years ago (Tang *et al*. 1993), its cDNA has not yet been isolated. Thus the availability of the cDNA clones will be useful for construction of expression vectors used for production of transgenic fish for enhancing growth (for review, see Hackett 1993, Iyengar *et al*. 1996). Channel catfish prolactin has not been reported. We isolated three complete clones of prolactin cDNA in this study. They should also be useful for use in growth promotion studies of transgenic fish (B Moav, personal communication).

Artificial spawning is very important in catfish for production of channel catfish female × blue catfish male hybrids, which are superior in many ways to either of the channel catfish or the blue catfish

parents in economic traits. However, artificial spawning is extremely difficult for mass production of hybrids because induction of ovulation in catfish using currently available reagents such as carp pituitary extract is extremely inefficient. A 10% hatching rate using eggs ovulated by using carp pituitary extracts is not unusual. Thus, it is desirable to develop alternative ovulation-inducing reagents using the homologous catfish GtHs. In this study, we isolated one clone of GtH β-I and one clone of GtH β-II. Along with previously reported GtH α glycoprotein (Liu *et al*. 1997), it is possible to develop bioactive recombinant catfish GtHs, which could potentially be used as an alternative for induction of ovulation in catfish. POMC is expressed as the most abundant gene product after injection of carp pituitary extract for induction of ovulation. Indeed, it was expressed at high levels before injection in catfish in captivity. It would be interesting to compare expression of POMC of wild fish with that of fish in confinement. In addition to other physiological indications, POMC may be useful as an indicator for stress in catfish.

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