The Zoological Society of Japan

ZOOLOGICAL SCIENCE 16: 139–146 (1999) © 1999 Zoological Society of Japan

Changes in Expression of Prolactin- and Cortisol-receptor Genes during Early-life Stages of Euryhaline Tilapia (*Oreochromis mossambicus*) in Fresh Water and Seawater

Kiyono Shiraishi[1,2], Manabu Matsuda[1], Takao Mori[1] and Tetsuya Hirano[2,3]

[1]*Department of Biological Science, Graduate School of Science, University of Tokyo, Bunkyo-ku, Tokyo 113-0033, Japan*
[2]*Ocean Research Institute, University of Tokyo, Nakano-ku, Tokyo 164-8639, Japan*

ABSTRACT—Expression of prolactin receptor (PRLR) and cortisol receptor (CR) mRNAs was examined during early-life stages of euryhaline Mozambique tilapia (*Oreochromis mossambicus*) by competitive reverse transcription-polymerase chain reaction (cRT-PCR). Concentration of prolactin receptor mRNA was higher in the gills of mature fish reared in fresh water (FW) than in those reared in seawater (SW), whereas no difference was seen in CR mRNA. Whole eggs just after fertilization contained the receptor mRNAs for both prolactin and cortisol. The concentration of PRLR mRNA increased gradually as the embryo grows both in FW and in SW. On the other hand, the concentration of CR mRNA was highest in the egg just after fertilization, decreased rapidly toward hatching, and increased slightly thereafter. When embryos 3 days before hatching were transferred to SW, the levels of PRLR mRNA were significantly lower at the time of hatching and also 3 days after hatching than in the embryo and larvae maintained in FW. Environmental salinity did not affect CR mRNA content at any stage examined. Both PRLR and CR mRNAs were identified in the yolk-sac membrane and in the embryonic body. Significantly more PRLR gene was expressed in the embryonic body developing in FW than in SW, whereas no difference was seen in the yolk-sac membrane. The greater expression of PRLR gene in embryos and larvae developing in FW than in those in SW clearly indicates the presence of regulatory mechanisms of gene expression in early-life stages of tilapia.

INTRODUCTION

In teleosts, prolactin and cortisol are central hormones in freshwater (FW) and seawater (SW) adaptation, respectively (see reviews; Hirano, 1986; Bern and Madsen, 1992; McCormick, 1995). Recently, prolactin receptor (PRLR) gene was cloned in Nile tilapia (Oreochromis niloticus) (Sandra *et al.*, 1995) and cortisol receptor (CR) gene in rainbow trout (*Oncorhynchus mykiss*) and Mozambique tilapia (*O. mossambicus*) (Ducouret *et al.*, 1995; Tagawa *et al.*, 1997). Osmoregulatory surfaces such as the gills, kidney and intestine are the target organs of prolactin and cortisol as indicated by hormone binding assay in several teleost species (for prolactin, Edery *et al.*, 1984; Dauder *et al.*, 1990; Auperin *et al.*, 1994a, 1995; for cortisol, Sandor *et al.*, 1984; Chakraborti *et al.*, 1987; Weisbart *et al.*, 1987; Maule and Shreck, 1990; Shrimpton and Randall, 1994; Shrimpton *et al.*, 1995). In agreement with the binding studies, the gills, kidney and intestine exhibit significant expression of PRLR and CR mRNAs (Ducouret *et al.*, 1995; Sandra *et al.*, 1995; Tagawa *et al.*, 1997; Uchida *et al.*, 1997).

Chloride cells or mitochondrion-rich (MR) cells in the gills and opercular membrane are the sites of ion transport particularly in fish in SW (Foskett and Scheffey, 1982; Zadunaisky, 1984; McCormick, 1995; Marshall, 1995). In the embryo and larva of euryhaline Mozambique tilapia, rich populations of chloride cells are found in the yolk-sac membranes prior to the development of the gills and opercular membrane (Ayson *et al.*, 1994a; Shiraishi *et al.*, 1997). When fish were transferred from FW to SW, there is a marked increase in the activity of chloride cells (Pisam, 1981; Foskett *et al.*, 1981, 1983; Pisam and Rambourg, 1991; Kültz *et al.*, 1992; Yoshikawa *et al.*, 1993; Ayson *et al.*, 1994a; Shiraishi *et al.*, 1997). Similar morphological changes in chloride cells have been seen in Mozambique tilapia after treatment of FW fish with cortisol (Foskett *et al.*, 1981; McCormick, 1990; Ayson *et al*, 1995). On the other hand, transfer of the fish from SW to FW, or prolactin treatment of the fish in SW elicited de-differentiation of chloride cells (Herndon *et al.*, 1991; Pisam *et al.*, 1993).

* Corresponding author: Tel. +81-3-3812-2111 ext. 4436;
FAX. +81-3-3816-1965.
[3] Present address: Hawaii Institute of Marine Biology, University of Hawaii, P.O.Box 1346, Coconut Island, Kaneohe, HI 96744

NII-Electronic Library Service

The Zoological Society of Japan

Little information is available on the endocrine control of hydromineral balance during early-life stages of fishes. In Mozambique tilapia, the beginning of embryonic synthesis of two forms of prolactins (PRL_{188} and PRL_{177}) was identified at the time of hatching (Ayson *et al.*, 1994b) and cortisol synthesis several days after hatching (Hwang *et al.*, 1992; Hwang and Wu, 1993; Ayson *et al.*, 1995). On the other hand, tilapia eggs are known to contain maternal cortisol in the yolk (Hwang *et al.*, 1992; Hwang and Wu, 1993; Ayson *et al.*, 1995). Tagawa *et al.* (1997) demonstrated CR gene expression in the whole eggs of Mozambique tilapia just after fertilization. These results suggest possible roles for prolactin and cortisol during early development, possibly in the functional differentiation of chloride cells. We have recently established competitive reverse transcription-polymerase chain reaction (cRT-PCR) assay, which enabled us to quantify the relative levels of PRLR and CR mRNAs simultaneously in small amounts of tissue such as developing tilapia embryos. The present study describes the presence of PRLR and CR mRNAs in tilapia embryos and yolk-sac membranes, and their developmental changes in relation to environmental salinity.

MATERIALS AND METHODS

Fish

Mature Mozambique tilapia (*O. mossambicus*) were collected from a pond in northern Okinawa Island, Japan, and were maintained in tanks with re-circulating FW (Tokyo tap water) or natural SW (Cl^-: 560 mM) at 25°C at the Ocean Research Institute, University of Tokyo. Fish were fed on artificial tilapia pellets ("Tilapia 41M", Shikoku Kumiai Shiryo, Tokushima, Japan) once a day. Male tilapia, weighing 30 to 40g, were used for cloning, and larger fish (100 to 150g) for quantification of PRLR and CR mRNAs. They were sacrificed after anesthesia with 0.1% 2-phenoxyethanol. Gills were immediately dissected, frozen in liquid nitrogen, and kept at –80°C until use.

Mature fish for breeding were kept in 200-l tanks, and mating behavior was checked hourly. The eggs just after fertilization were obtained from the mouth of brooding females. Otherwise, eggs were taken from the female 2 days after fertilization to minimize disturbance of the egg. Eggs were collected by keeping the mouth of the female open and flushing with water. Half of the eggs were transferred directly to natural SW 2 days after fertilization and the other half were maintained in FW. Two to three hundred eggs were incubated in each 10-l plastic tank at 25°C with aeration. The day of hatching was designated as day 0. Transfer of the egg to SW did not affect normal development of the embryos, and no mortality was seen after the transfer.

For examination of the developmental changes in the receptor mRNA, 10-30 embryos were collected on days -5 (fertilization), -3 (appearance of yolk-sac membrane), 0 (hatching) and 3 (early stages of gill development). At days -3, 0 and 3, yolk-sac membrane and embryonic or larval body were isolated. The embryo or larva was removed from the yolk sac, and the yolk-sac membrane was carefully peeled off the yolk in Ringer's solution (122 mM Na^+, 124.6 mM Cl^-, 3 mM K^+, 1.25 mM Mg^{2+}, 1 mM Ca^{2+}, 1.25 mM SO_4^{2-}, 0.4 mM PO_4^{3-}, 2 mM CO_3^{2-}, pH 7.4). They were frozen in liquid nitrogen and stored at –80°C.

RNA preparation

Total RNA was extracted from the frozen tissues by the acid guanidium-phenol-chloroform method (Chomczynski and Sacchi, 1987). In the extraction from the yolk-sac membrane and the isolated body, Ethachinmate (Nippon Gene, Tokyo), the carrier, was used to increase recovery rate of RNA. Concentrations of total RNA from the gills of adult fish and from whole embryos were quantified by spectrometric optical density at 260 nm.

Cloning

To establish a homologous cRT-PCR, a partial sequence of PRLR cDNA was first obtained. One μg of total RNA obtained from the gill of adult tilapia reared in FW was used to generate single-strand cDNA by Superscript™ reverse transcriptase (GIBCO BRL, Gaitherburg, MA) and oligo $d(T)_{12-18}$ as a primer. The cDNA was amplified by PCR using Takara Ex Taq polymerase (Takara Shuzo Co., Ltd., Tokyo), and 0.25 μM of each primers as follows: 5'-AATTCTGGATGTTGGCGATAACC-3' and 5'-CATAGCTTCCATGACCAGAGTCAC-3'. These primers were designed based on the sequence of PRLR cDNA of Nile tilapia (*O. niloticus*) (Sandra *et al.*, 1995) and mouse (Moor and Oka, 1993). The PCR was performed in triplicate.

The PCR products were fractionated by agarose gel electrophoresis, and subcloned into pT7Blue(R) T-vector (Novagen, Madison, WI). This plasmid was called $pT7_{PR}$ in this study. Nucleotide sequences of the insert was determined by the dideoxynucleotide chain-termination method (Sanger *et al.*, 1977), using the Thermo sequenase core sequencing kit (Amersham, Buckinghamshire) and the automated laser fluorescence sequencer (SQ-5500, Hitachi Electronics Engineering Co., Ltd., Tokyo).

Construction of competitor plasmids for cRT-PCR

The plasmid $pT7_{PR}$ was digested with SacI and BamHI sites, located at position 692 and 896 respectively, to delete a 204-bp-long fragment. Blunting and ligation followed to obtain the competitor plasmid ($pT7_{cPR}$).

Tilapia cDNA regions of CR and β-actin (BA) as the control were amplified by PCR using primers as follows: for CR, 5'-TTTCGGTAATTGGTTGCTGATGAT-3' and 5'-AGTGCTCCTGGCTGTT(C,T)CT-(A.C,G,T)AGT-3' (Tagawa *et al.*, 1997); for BA, 5'-CTACGAGCTGCCTGACGGACA-3' and 5'-CACATGTGCTGGAAGGTGGACA-3'. We referred to medaka (*Oryzias latipes*) and striped bass (*Morone saxatilis*) sequences for design of BA primers. PCR products were subcloned into pT7Blue(R) T-vector ($pT7_{CR}$ and $pT7_{BA}$), and the sequences were confirmed as described above. Using these plasmids, inverse PCR was performed to generate deletion mutants of each tilapia DNA using primers designed in juxtaposition to each other. Resulting amplification of the entire vector and insert produced 70- and 58-bp deletions, respectively. The primers were as follows: for CR, 5'-AGCAGTGTTTGATGAGATTCGC-3' and 5'-AGGTACTCATCGTGGGAAACCTG-3'; for BA, 5'ATGCAGAAGGAGATCACAGCCC-3' and 5'-GATGTCAACGGTCGCACTTCATG-3'. The amplicons were subjected to blunting and ligation, and named $pT7_{cCR}$ and $pT7_{cBA}$.

Synthesis of competitor RNA (cRNA)

Digestion of $pT7_{cPR}$, $pT7_{cCR}$ and $pT7_{cBA}$ with appropriate restriction enzymes resulted in linearized DNA fragments. Using these fragments as templates, sense-strand cRNAs were synthesized by T7 RNA polymerase (Stratagene, La Jolla, CA). The template DNA was subsequently removed by adding RNase-free DNase. The concentration of cRNA was quantified by absorbance at 260 nm. After adequate dilution, cRNA was stored at –80°C until use.

cRT-PCR

Our method of cRT-PCR was based on the procedure by Matsuda and Mori (1997) with some modification. The mixture of total RNA and adequate concentrations of the three cRNAs (for BA, PRLR and CR) were subjected to RT-PCR, using *Bca*BEST™ RNA PCR kit (Takara, Tokyo) and specific antisense primers. Reverse transcription was performed according to the recommended protocol of the kit with modification. After incubation at 55°C for 5 min, *Bca*BEST Polymerase was added to the reaction mixture. The temperature was then

NII-Electronic Library Service

The Zoological Society of Japan

maintained for 5 min prior to incubation at 65°C for 30 min. Subsequently, PCR was performed for 35 cycles (denaturation at 95°C for 30 sec, annealing at 59°C for 1 min, and extension at 72°C for 1 min) with 9.25 kBq of [α-^{32}P]-dCTP (Amersham, UK) and 0.25 μM of each primers (see Fig. 1). After PCR, the products were separated by electrophoresis on 3.5% (for BA and PRLR) or 8% (for CR) acrylamide gel. Figure 1 schematically shows the amplified region of target RNAs and cRNAs. Radioactive incorporation of the PCR products was quantified on a Bioimage analyzer (Fuji BAS 2500; Fuji Film, Tokyo). The value of intensity of each DNA band was divided by the number of deoxycytidine residues contained in cRNA and the target RNA (BA, 76 and 98; PRLR, 154 and 202; CR, 28 and 44, respectively).

To examine the relationship between the initial ratio of target/competitor and the PCR product ratio of target/competitor, cRT-PCR was performed with fixed amount of target RNA and varying amount of cRNA. Subsequently, the logarithms of the PCR product ratio of target/competitor was plotted against the logarithms of the initial ratio of target/competitor RNA (Fig. 2). The results indicate that the initial ratio was maintained after cRT-PCR.

Statistical analysis

Statistical significance of differences between means was assessed with Student's *t*-test.

RESULTS

To establish a homologous cRT-PCR in the Mozambique tilapia (*O. mossambicus*), partial cloning of PRLR cDNA in the gill was performed, and the nucleotide sequence consisting of 1222 bases has been registered in GenBank (accession number AF080247). The sequence exhibits a high homology (97.8%) with putative extracellular and transmembrane domains of PRLR of the Nile tilapia (*O. niloticus*).

The expression of PRLR and cortisol receptor (CR) mRNAs was quantified simultaneously using a newly developed cRT-PCR assay. Fig. 3 shows the effect of environmental salinity on the ratio of PRLR and CR mRNAs relative to BA mRNA in the gills of mature tilapia. In fish reared in FW, the expression of PRLR was significantly higher than that in SW-acclimated fish ($p<0.05$). Conversely, the environmental salinity did not significantly affect gill CR mRNA content.

In the embryo just after fertilization (day -5), PRLR and CR mRNAs were already detected (Fig. 4). The PRLR mRNA levels relative to total RNA increased gradually during development, and became significantly greater in the embryo maintained in FW compared to those transferred to SW at hatching (day 0). Interestingly, CR mRNA levels were highest just after fertilization (day -5), decreased markedly toward hatching, and increased slightly thereafter. There was no significant difference between the embryos kept in FW and those transferred to SW.

To determine the major sites of the receptor mRNA ex-

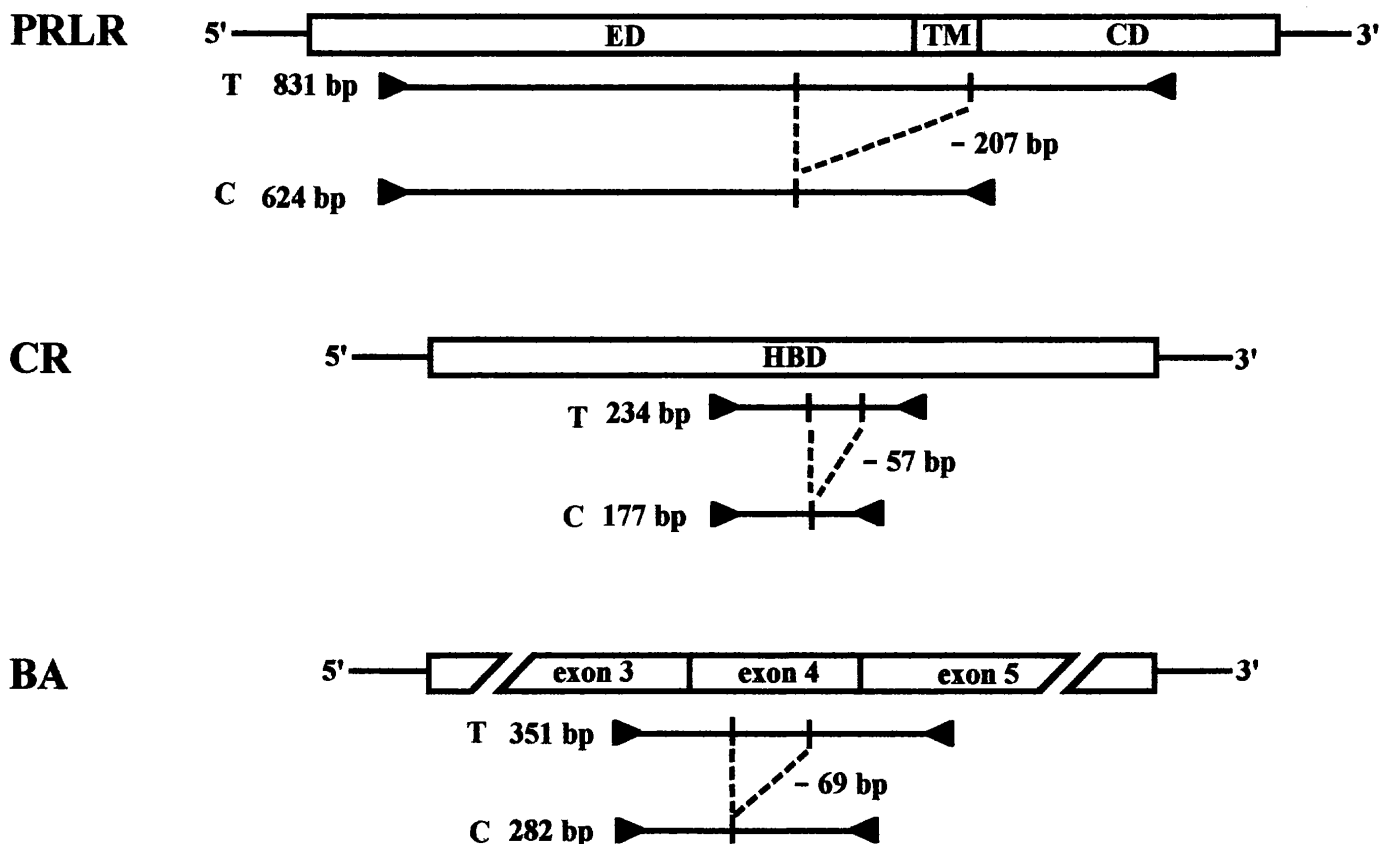

Fig. 1. Schematics of amplified regions from the target RNA (T) and competitor RNA (cRNA) (C) by competitive RT-PCR for prolactin receptor (PRLR), cortisol receptor (CR) and b-actin (BA). The depleted region in cRNA is indicated by broken lines. The sequences of primers (arrowheads) are as follows: for PRLR, 5'-CAGAGATCAAATGCCGTTCTCC-3' and 5'-CCTCATAGTTAGACGTGGTTGG-3'; for CR, 5'-AATGAGGAGAGGATGAAGCTGC-3' and 5'-GTTCTGCTCACGCTTCACAATG-3'; for BA, 5'-CTACGAGCTGGCCTGACGGACA-3' and 5'-CACATGTGCTGGAAGGTGGACA-3'. Abbreviations: EM, extracellular domain; TM, transmembrane domain; CD, cytoplasmic domain; HBD, hormone-binding domain.

NII-Electronic Library Service

The Zoological Society of Japan

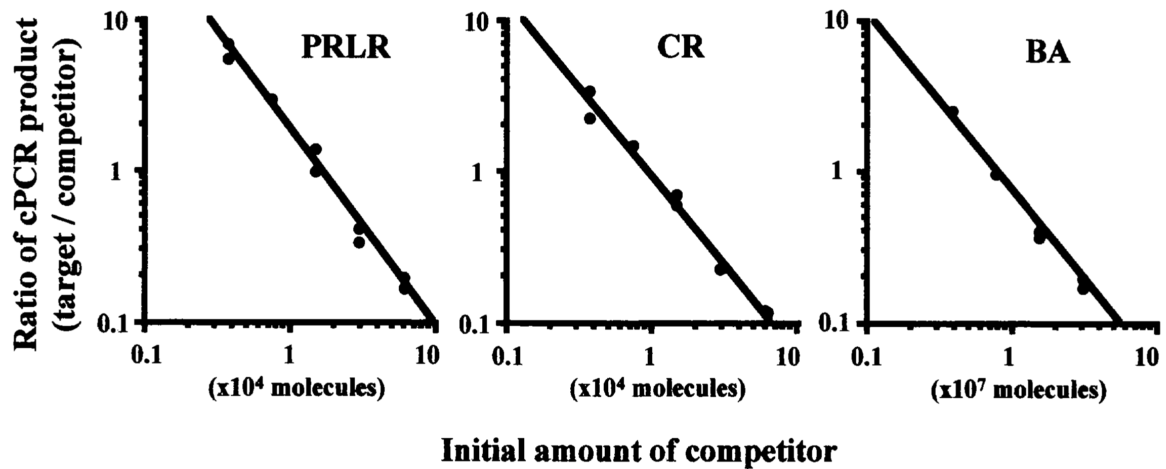

Fig. 2. Relationship between the initial ratio of target/competitor RNA and the PCR product ratio of target/competitor after competitive RT-PCR for prolactin receptor (PRLR), cortisol receptor (CR) and β-actin (BA).

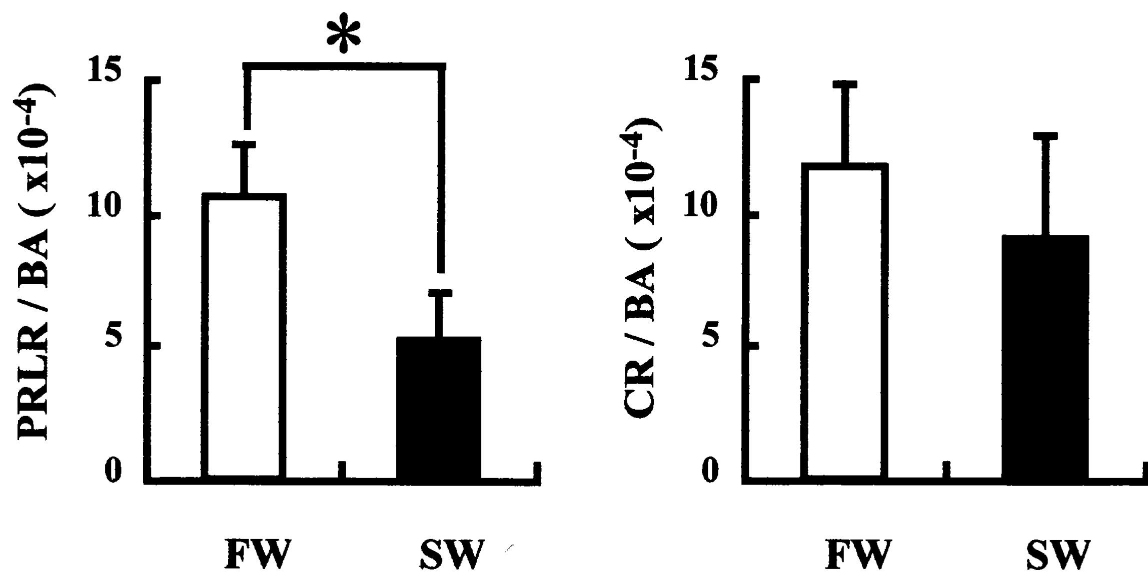

Fig. 3. Effect of environmental salinity on the ratio of PRLR mRNA and CR mRNAs to BA mRNA in the gills of mature tilapia. Vertical bars indicate S.E.M. (n = 4). *p<0.05.

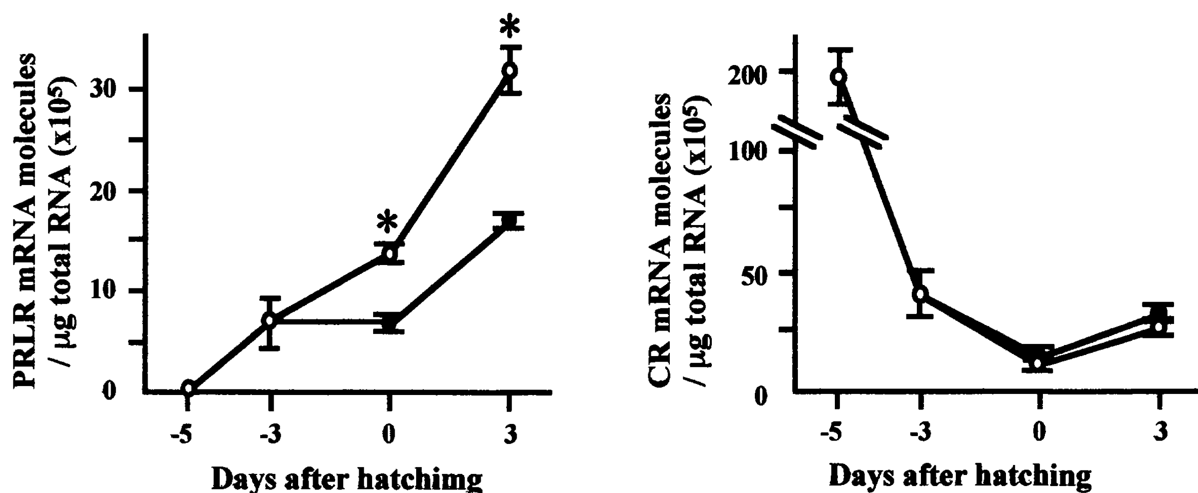

Fig. 4. Changes in the PRLR mRNA and CR mRNA in whole eggs during development. Half of the eggs were maintained in FW (○) and the other half were transferred to SW 3 days before hatching (●). Data are expressed as concentrations of the receptor mRNAs in 1 µg of total RNA. Eggs from 4 brooding females were used for the quantification. *p<0.05.

NII-Electronic Library Service

The Zoological Society of Japan

pression, cRT-PCR was also performed on the embryos or larvae and on the yolk-sac membrane separated from the yolk. As the sample size was too small to estimate total RNA concentrations, the ratios of PRLR/BA and CR/BA were adopted. As shown in Fig. 5, both PRLR and CR mRNAs were detected in the embryonic body and in the yolk-sac membrane as early as 3 days before hatching. Changes in salinity affected the ratio of PRLR/BA in the isolated body but not in the yolk-sac membrane. On day 3, the ratio of PRLR/BA in the larval body isolated from FW-reared larva was significantly greater than that in SW larvae. On the other hand, there was no difference in the level of PRLR mRNA in the yolk-sac membrane between the two groups. The CR mRNA concentration in the embryonic and larval body and in the yolk-sac membrane showed no detectable change following transfer from FW to SW.

DISCUSSION

In the present study, concentrations of PRLR and CR mRNAs were quantified by cRT-PCR assay. This method is useful for quantifying levels of mRNA in small samples such as tilapia embryos and yolk-sac membrane, tissues which express low levels of mRNA. The tilapia pituitary is known to produce and secrete two PRLs, PRL_{188} and PRL_{177} or PRL_{I} and PRL_{II} (Specker *et al.*, 1985; Yamaguchi *et al.*, 1988;

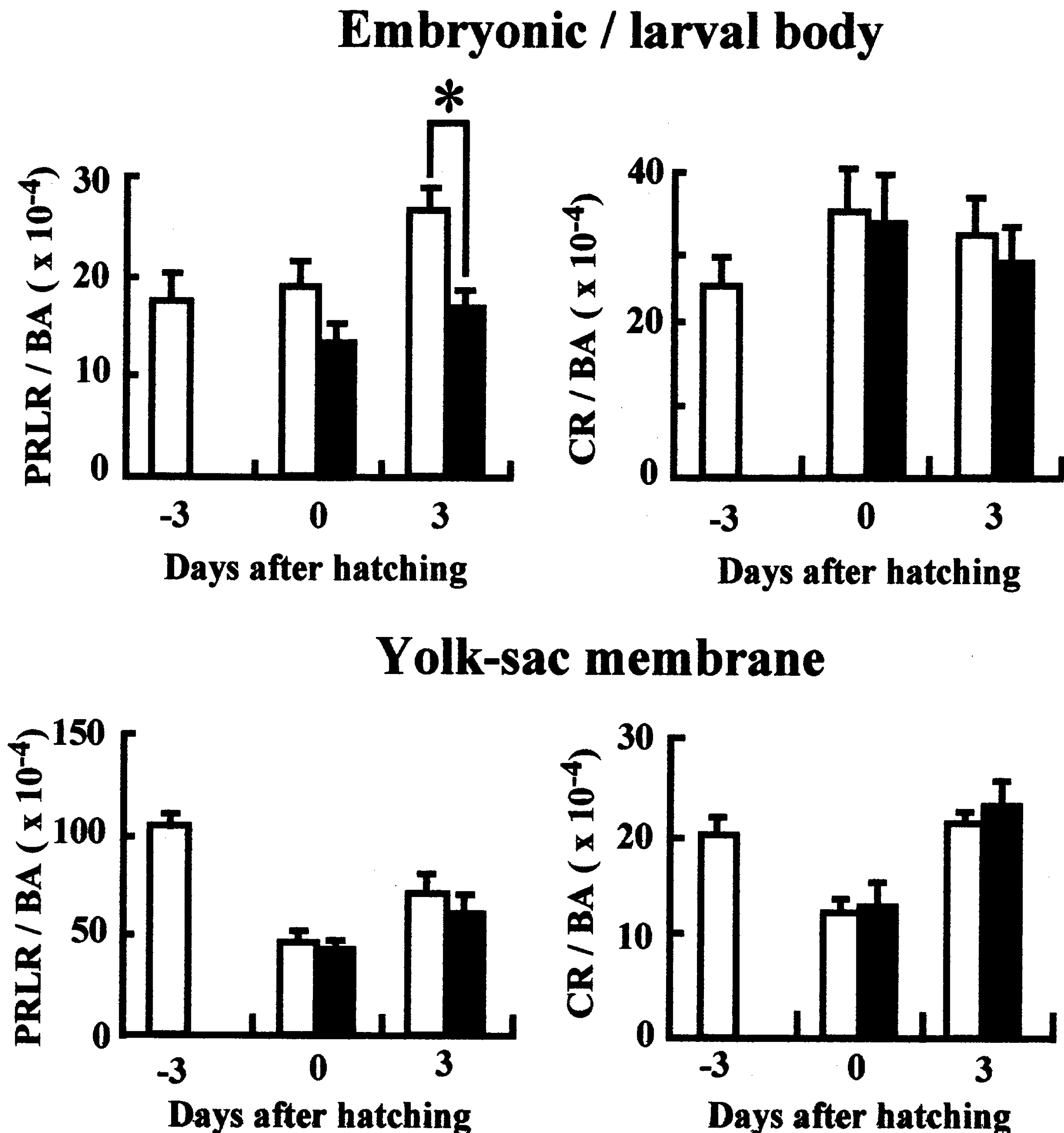

Fig. 5. Effect of environmental salinity on the ratio of PRLR mRNA and CR mRNAs to BA mRNA in the embryonic and larval body and in the yolk-sac membrane. Half of the eggs were maintained in FW (clear column) and the other half were transferred to SW 3 days before hatching (solid column). Vertical bars indicate S.E.M. (n = 4). *$p<0.05$.

NII-Electronic Library Service

The Zoological Society of Japan

Rentier-Delrue *et al.*, 1989). Auprin *et al.* (1994a) characterized a single PRLR in Nile tilapia (*O. niloticus*), which binds both PRLs. As shown in the present study, levels of PRLR mRNA relative to BA mRNA (PRLR/BA) in the gills of Mozambique tilapia (*O. mossambicus*) were significantly higher in fish kept in FW than in those in SW. This is consistent with the change in PRLR mRNA in the gills following transfer of Nile tilapia to blackish water (20‰), as measured by Northern-blot analysis (Sandra *et al.*, 1995). The expression of PRLR mRNA in the gills seems to be up-regulated by the higher levels of circulating PRLs present in FW than in SW or in blackish water (Ayson *et al.*, 1993; Auperin *et al.*, 1994b). According to Auperin *et al.* (1995), however, specific binding of PRLs to the gill membrane of Nile tilapia was significantly higher in the fish kept in blackish water than in FW. This suggests that the receptor protein is down-regulated at high concentrations of circulating PRLs in FW. Genty *et al.* (1994) reported that internalization of the cell surface PRLR was enhanced in the presence of PRL, leading to apparent down-regulation of the receptor. In mammals, the complexity of up- and down-regulation of PRLR gene expression has been well documented, depending on target organs, hormone concentrations and duration of the exposure to the hormone (Kelly *et al.*, 1991; Prunet and Auperin, 1995). Further studies are needed to elucidate the regulatory mechanisms of PRLR at transcript and protein levels in teleosts.

Ayson *et al.* (1994b) reported significantly higher expression of PRL_{188} mRNA in the pituitary of tilapia (*O. mossambicus*) larva reared in FW than those in SW at the time of hatching, and in PRL_{177} mRNA 2 days after hatching. In the present study, levels of PRLR mRNA were also higher in the whole embryo and larva kept in FW as well as in the embryonic body separated from the yolk at the time of hatching and in the larval body 3 days after hatching, as compared with those in SW. These results clearly indicate the presence of regulatory mechanisms of the hormone production in the pituitary and possibly its release into circulation as well as the expression of the PRLR gene in early-life stages of tilapia.

In higher vertebrates, functional versatility of PRL and adrenal corticoids extends to growth-promoting effects during early development. In cultured rat embryos, Karabulut and Pratten (1998) demonstrated the potency of PRL as a growth-promoting molecule during embryogenesis. On the other hand, Cole *et al.* (1995) showed that targeted disruption of the glucocorticoid receptor (GR) gene, causing lethal abnormalities of the lung and adrenal gland in the mice. Significant expression of PRLR and CR mRNAs in tilapia eggs as early as just after fertilization suggests important roles for these hormones during embryogenesis.

Hwang and Wu (1989) reported more advanced development of the kidney in Mozambique tilapia larva hatched in FW than in SW. According to Li *et al.* (1995), numerous functional chloride cells or MR cells are present in the newly developed gills of tilapia larvae as early as 3 days after hatching in FW. On the other hand, we have reported that chloride cells in the yolk-sac membrane are more developed in tilapia embryos and larvae in SW than those in FW (Ayson *et al.*, 1994a; Shiraishi *et al.*, 1997). Although chloride cells are well recognized as the site of ion extrusion in the fish in SW, their function in FW is still disputed (Goss *et al.*, 1995; Marshall, 1995). Thus, the greater expression of PRLR gene in whole larvae and in the isolated embryonic or larval body in FW implies a role for prolactin in differentiation of osmoregulatory surfaces including FW-type chloride cells. However, there was no significant change in the ratio of PRLR/BA in the yolk-sac membrane after transfer to SW, suggesting that prolactin may be involved in the maintenance and/or function of the yolk-sac membrane, in addition to differentiation of chloride cells.

Several steroid and thyroid hormones are present in the unfertilized egg of various teleost species (see a review by Tagawa, 1996). Recently, Green and Chen (1997) reported mRNA of insulin-like growth factors (IGFs) in unfertilized eggs of rainbow trout. Peptide hormones, including IGFs and prolactin, may also be among the maternal hormones/factors utilized by the fish embryo. Indeed, the present study demonstrates significant expression of PRLR and CR genes in the newly developed yolk-sac membrane (day -3). The organ culture of the yolk-sac membrane of the tilapia embryo 1 day before hatching resulted in a decrease in chloride cell density, and the decrease was countered by adding cortisol to the culture medium (Ayson *et al.*, 1995). Thus, cortisol is likely to play an important role in the differentiation and/or proliferation of chloride cells. Interestingly, the highest concentration of CR mRNA was found just after fertilization in this study. The result suggests that the maternal cortisol and the receptor are utilized in the developing embryo before the commencement of its own production.

Environmental salinity, however, did not affect the CR mRNA expression in any tissues at all stages examined in the present study. Although the level of CR expression is under control of various factors including steroids and neurotransmitters, cortisol itself seems to be the most important regulator (Bamberger *et al.*, 1996). In the gill of coho salmon (*O. kisutch*), cortisol treatment caused the down-regulation of the binding site (Maule and Schreck, 1991; Shrimpton and Randall, 1994). In Mozambique tilapia, however, there was no difference in the plasma cortisol level between fish in FW and those in SW, although cortisol production by the interrenal was greater in SW fish (Balm *et al.*, 1995). Similarly, cortisol contents in the whole embryo and larva of Mozambique tilapia were not affected by environmental salinity (Hwang and Wu, 1993: Ayson *et al.*, 1995). In chum salmon (*O. keta*) fry, Uchida *et al.* (1997) reported that CR gene expression, examined by in situ hybridization and immunostainig with anti-CR serum, was found in two types of chloride cells in the gill filaments and lamellae. However, there was no difference in plasma cortisol nor in the expression of CR gene. Cortisol sensitivity could be regulated by mRNA stability, translatability, and turnover of the receptor protein. Heat shock proteins and transcriptional factors may also modify the hormone-binding affinity to the receptor and the transcriptional activities of the target gene (Bamberger *et al.*, 1996). In fact, fluctuations of

NII-Electronic Library Service

The Zoological Society of Japan

the both were observed in the gills of a goby, *Gillichthys mirabilis*, following osmotic shock (Kültz, 1996).

The present study demonstrates the expression of PRLR and CR in the yolk-sac membrane and embryonic body of tilapia, suggesting that prolactin and cortisol may be involved in growth and development, and/or hydromineral balance of tilapia embryos, possibly by affecting functional differentiation of chloride cells. However, direct effect of the hormones or specific localization of their receptors on chloride cells or other cell types have not been demonstrated yet. Further studies by means of in situ hybridization will provide useful information on the sites of expression of PRLR and CR genes on specific cell types in the developing embryos

ACKNOWLEDGMENTS

We thank Dr. M. Tagawa, Kyoto University, Japan, for kindly providing the PCR primers for $pT7_{BA}$ construction and for valuable suggestions. We are grateful to Prof. H. A. Bern, University of California, Berkeley, and to Dr. Malia Chow, Hawaii Institute of Marine Biology, University of Hawaii, for critical reading of the manuscript. The present study was supported in part by grants-in-aid from the Ministry of Education and also for the Fisheries Agency of Japan.

REFERENCES

Auperin B, Rentier-Delrue F, Martial JA, Prunet P (1994a) Characterization of a single prolactin (PRL) receptor in tilapia (*Oreochromis niloticus*) which binds both PRL_I and PRL_{II}. J Mol Endocrinol 13: 241–251

Auperin B, Rentier-Delrue F, Martial JA, Prunet P (1994b) Evidence that two tilapia (*Oreochromis niloticus*) prolactins have different osmoregulatory functions during adaptation to a hyperosmotic environment. Gen Comp Endocrinol 12: 13–24

Auperin B, Rentier-Delrue F, Martial JA, Prunet P (1995) Regulation of gill prolactin receptors in tilapia (*Oreochromis niloticus*) after a change in salinity or hypophysectomy. J Endocrinol 145: 213–220

Ayson FG, Kaneko T, Tagawa M, Hasegawa S, Grau EG, Nishioka RS, King DS, Bern HA, Hirano T (1993) Effects of acclimation to hypertonic environment on plasma and pituitary levels of two prolactins and growth hormone in two species of tilapia, *Oreochromis mossambicus* and *Oreochromis niloticus*. Gen Comp Endocrinol 89: 138–148

Ayson FG, Kaneko T, Hasegawa S, Hirano T (1994a) Development of mitochondrion-rich cells in the yolk-sac membrane of embryos and larvae of tilapia, *Oreochromis mossambicus*, in fresh water and seawater. J Exp Zool 270: 129–135

Ayson FG, Kaneko T, Hasegawa S, Hirano T (1994b) Differential expression of two prolactin and growth hormone genes during early development of tilapia (*Oreochromis mossambicus*) in fresh water and seawater: implications for possible involvement in osmoregulation during early life stages. Gen Comp Endocrinol 95: 143–152

Ayson FG, Kaneko T, Hasegawa S, Hirano T (1995) Cortisol stimulates the size and number of mitochondrion-rich cells in the yolk-sac membrane of embryos and larvae of tilapia (*Oreochromis mossambicus*) *in vitro* and *in vivo*. J Exp Zool 272: 419–425

Balm PHM, Haenen HEMG, Wendelaar Bonga SE (1995) Regulation of interrenal function in freshwater and sea water adapted tilapia (*Oreochromis mossambicus*). Fish Physiol Biochem 14: 37–47

Bamberger CM, Schulte HM, Chrousos GP (1996) Molecular determinant of glucocorticoid receptor function and tissue sensitivity to glucocorticoids. Endocr Rev 17: 245–261

Bern HA, Madsen SS (1992) A selective survey of the endocrine system of the rainbow trout (*Oncorhyncus mykiss*) with emphasis on the hormonal regulation of ion balance. Aquaculture 100: 237–262

Chakraborti PK, Weisbart M, Chakraborti A (1987) The presence of corticosteroid receptor activity in the gills of brook trout, *Salvelinus fontinalis*. Gen Comp Endocrinol 66: 323–332

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

Cole TJ, Blendy JA, Monaghan AP, Krieglstein K, Schmid W, Aguzzi A, Fantuzzi G, Hummler E, Unsicker K, Schtz G (1995) Targeted disruption of the glucocorticoid receptor gene blocks adrenergic chromaffin cell development and severely retards lung maturation. Genes Dev 9: 1608–1621

Dauder S, Young G, Hass L, Bern HA (1990) Prolactin receptors in liver, kidney, and gill of the tilapia (*Oreochromis mossambicus*): characterization and effect of salinity on specific binding of iodinated ovine prolactin. Gen Comp Endocrinol 77: 368–377

Ducouret B, Tujague M, Ashra J, Mouchel N, Servel N, Valotaire Y, Thomson EB (1995) Cloning of a teleost fish glucocorticoid receptor shows that it contains a deoxyribonucleic acid-binding domain different from that of mammals. Endocrinol 136: 3774–3783

Edery M, Young G, Bern HA, Steiny S (1984) Prolactin receptors in tilapia (*Sarotherodon mossambicus*) tissues: binding studies using ^{125}I-labeled ovine prolactin. Gen Comp Endocrinol 56: 19–23

Foskett KJ, Schffey C (1982) The chloride cell: definitive identification as the salt secreting cell in teleosts. Science 215: 164–166

Foskett KJ, Logsdon CD, Turner T, Machen TE, Bern HA (1981) Differentiation of the chloride extrusion mechanism during seawater adaptation of a teleost fish, the cichlid *Sarotherodon mossambicus*. J Exp Biol 93: 209–224

Foskett KJ, Bern HA, Machen TE, Conner M (1983) Chloride cells and hormonal control of teleost fish osmoregulation. J Exp Biol 106: 255–281

Genty N, Paly J, Edery M, Kelly PA, Djiane J, Salesse R (1994) Endocytosis and degradation of prolactin and its receptor in Chinese hamster ovary cells stably transfected with prolactin receptor cDNA. Mol Cell Endocrinol 99: 221–228

Goss G, Perry S, Laurent P (1995) Ultrastructual and morphometric studies on ion and acid-base transport processes in freshwater fish. In "Cellular and Molecular Approaches to Fish Ionic Regulation" Ed by CM Wood and TJ Shut, Academic Press, London, pp 257–284

Green MW, Chen TT (1997) Temporal expression pattern of insulin-like growth factor mRNA during embryonic development in a teleost, rainbow trout (*Oncorhynchus mykiss*). Mol Mar Biol 6: 144–151

Hwang PP, Wu SM (1989) Morphological study on kidneys of tilapia larvae (*Oreochromis mossambicus*) hatched in freshwater and seawater. Bull Inst Zool, Academia Sinica 28: 73–80

Hwang PP, Wu SM (1993) Role of cortisol in hypoosmoregulatory in larvae of the tilapia (*Oreochromis mossambicus*). Gen Comp Endocrinol 92: 318–324

Hwang PP, Wu SM, Lin JH, Wu LS (1992) Cortisol content of eggs and larvae of teleosts. Gen Comp Endocrinol 86: 189–196

Herndon TM, McCormick SD, Bern HA (1991) Effect of prolactin on chloride cells in opercular membrane of seawater-adapted tilapia. Gen Comp Endocrinol 83: 283–289

Hirano T (1986) The spectrum of prolactin action in teleosts. In "Comparative Endocrinology: Development and Directions" Ed by CL Ralph and Alan R. Liss, NewYork. pp 53–74

Karabulut AK, Pratten MK (1998) Species-specificity of growth-promoting effects of prolactin during rat embryogenesis. J Anat 192: 1–12

NII-Electronic Library Service

The Zoological Society of Japan

Kelly PA, Djiane J, Postel-Vinay MC, Edery M (1991) The prolactin / growth hormone receptor family. Endocr Rev 12: 235–251

Kültz D (1996) Plasticity and stressor specificity of osmotic and heat shock responses of *Gillichthys mirabilis* gill cells. Am J Physiol 271: C1181–C1193

Kültz D, Bastrop R, Jrss K, Siebers D (1992) Mitochondria-rich (MR) cells and the activities of the Na^+/K^+-ATPase and carbonic anhydrase in the gill and opercular epithelium of *Oreochromis mossambicus* adapted to various salinities. Comp Biochem Physiol B 102: 293–301

Li J, Eygensteyn J, Lock RAC, Verbost PM, van der Heijden AJH, Wendelaar Bonga SE, Flik G (1995) Branchial chloride cells in larvae and juveniles of freshwater tilapia *Oreochromis mossambicus*. J Exp Biol 198: 2177–2184

Marshall WS (1995) Transport processes in isolated teleost epithelia: Opercular epithelium and urinary bladder. In "Cellular and Molecular Approaches to Fish Ionic Regulation" Ed by CM Wood and TJ Shut, Academic Press, London, pp 1–23

Maule AG, Shreck CB (1990) Glucocorticoid receptors in leukocytes and gill of juvenile coho salmon (*Oncorhynchus kisutch*). Gen Comp Endocrinol 77: 448–455

Maule AG, Schreck CB (1991) Stress and cortisol treatment changed affinity and number of glucocorticoid receptors in leukocytes and gill of coho salmon. Gen Comp Endocrinol 84: 83–93

McCormick SD (1990) Cortisol directly stimulates differentiation of chloride cells in tilapia opercular membrane. Am J Physiol 259: R857–863

McCormick SD (1995) Hormonnal control of gill Na^+, K^+-ATPase and chloride cell function. In "Cellular and Molecular Approaches to Fish Ionic Regulation" Ed by CM Wood and TJ Shut, Academic Press, London, pp 285–315

Moor RC, Oka T (1993) Cloning and sequencing of the cDNA encoding the murine mammary gland long-form prolactin receptor. Gene 134: 263–265

Pisam M (1981) Membranous systems in the "chloride cell" of teleostean fish gill; their modifications in response to the salinity of the environment. Anat Rec 200: 401–414

Pisam M, Rambourg A (1991) Mitochondria-rich cells in the gill epithelium of teleost fishes: an ultrastructual approach. Int Rev Cytol 130: 191–232

Pisam M, Auperin B, Prunet P, Rentier-Delrue F, Martial J, Rambourg A (1993) Effects of prolactin in α and β chloride cells in the gill epithelium of the saltwater adapted tilapia "*Oreochromis niloticus*". Anat Rec 235: 275–284

Prunet P, Auperin B (1995) Prolactin receptors. In "Fish Physiology Vol XIII" Ed by N Sherwood and CL Hew, Academic Press, London, pp 367–391

Rentier-Delrue F, Swennen D, Prunet P, Lion M, Martial JA (1989) Tilapia prolactin: Molecular cloning of cDNA and expression in *Escherichia coli*. DNA 8: 271–278

Sandor T, DiBattista JA, Mehdi, AZ (1984) Glucocorticoid receptors in the gill tissue of fish. Gen Comp Endocrinol 53: 353–364

Sandra O, Sohm F, de-Luze A, Prunet P, Edery M, Kelly PA (1995) Expression cloning of cDNA encoding a fish prolactin receptor. Proc Natl Acad Sci USA 92: 6037–6041

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

Shiraishi K, Kaneko T, Hasegawa S, Hirano T (1997) Development of multicellular complexes of chloride cells in the yolk-sac membrane of tilapia (*Oreochromis mossambicus*) embryos and larvae in seawater. Cell Tissue Res 288: 583–590

Shrimpton JK, Randall DJ (1994) Downregulation of corticosteroid receptors in the gills of coho salmon due to the stress and cortisol treatment. Am J Physiol 267: R432–R438

Shrimpton JK, Delvin RH, McLean E, Byatt JC, Donaldson EM, Randall DJ (1995) Increases in gill cytosolic corticosteroid receptor abundance and saltwater tolerance in juvenile coho salmon (*Oncorhynchus kisutch*) treated with growth hormone and placental lactogen. Gen Comp Endocrinol 98: 1–15

Specker JL, King DS, Nishioka RS, Shirahata K, Yamaguchi K, Bern HA (1985) Isolation and partial characterization of a pair of prolactins released *in vitro* by the pituitary of a cichlid fish, *Oreochromis mossambicus*. Proc Natl Acad Sci USA 82: 7490–7494

Tagawa M (1996) Current understanding of the presence of hormones in fish eggs. In "Survival Strategies in Early Life Stages of Marine Resources" Ed by Y Watanabe, Y Yamashita and Y Oozeki, AA Balkema, Rotterdam, pp 27–38

Tagawa M, Hagiwara H, Takemura A, Hirose S, and Hirano T (1997) Partial cloning of the hormone-binding domain of the cortisol receptor in tilapia, *Oreochromis mossambicus*, and change in the mRNA level during embryonic development. Gen Comp Endocrinol 108: 132–140

Uchida K, Kaneko T, Tagawa T, Hirano T (1997) Localization of cortisol receptor in branchial chloride cells in chum salmon fry. Gen comp Endocrinol 109: 175–185

Weisbart M, Chakraborti PK, Gillivan G, Eales JG (1987) Dynamics of cortisol receptor activity in the gills of the brook trout, *Salvelinus fontinalis*, during seawater adaptation. Gen Comp Endocrinol 68: 440–448

Yamaguchi K, Specker JL, King DS, Yokoo Y, Nishioka RS, Hirano T, Bern HA (1988) Complete amino acid sequences of a pair of fish (Tilapia) prolactins, $tPRL_{177}$ and $tPRL_{188}$. J Biol Chem 263: 9113–9121

Yoshikawa JSM, McCormick SD, Young G, Bern HA (1993) Effects of salinity on chloride cells and Na^+, K^+-ATPase activity in the teleost *Gillichthys mirabilis*. Comp Biochem Physiol A 105: 311–317

Zadunaisky JA (1984) The chloride cell: the active transport of chloride and the paracellular pathway. In "Fish Physiology Vol XB" Ed by WS Hoar and DJ Randall, Academic Press, London, pp 130–176

(Received September 2, 1998 / Accepted October 10, 1998)

NII-Electronic Library Service