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[REVIEW]

**Molecular Regulation of Gonadotropin Secretion by Gonadotropin-Releasing Hormone
in Salmonid Fishes**

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Short title: GnRH Regulation of GTH Secretion in Salmonids

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ABSTRACT

Gonadotropin-releasing hormone (GnRH) plays a central role in the control of reproductive function in vertebrates. In salmonids, salmon GnRH (sGnRH) secreted by preoptic GnRH neurons regulates gonadal maturation through stimulation of synthesis and release of pituitary gonadotropins (GTHs). In addition, several lines of our evidence indicate that sGnRH is involved in spawning behavior, and serves to integrate the gonadal maturation with the reproductive behavior. A growing number of studies show that the effects of GnRH are mediated by multiple subtypes of GnRH receptors, successive multiple signaling pathways, and finally multiple transcription factors which act cooperatively to stimulate transcription of GTH subunit genes. This complex regulatory system of the action of GnRH may serve as a molecular basis of divergent physiological strategies of reproductive success in various vertebrate species. In this article, recent data on the molecular mechanisms of action of GnRH are reviewed with special reference to the regulation of synthesis and release of GTHs in the pituitary of salmonids to elucidate the multifunctional action of GnRH.

Key words: GnRH, GnRH receptor, GTH, Gene expression, Signal transduction pathway, Salmon, Seasonal reproduction, Spawning migration

1. Introduction

Gonadotropin-releasing hormone (GnRH) is a decapeptide that regulates synthesis and release of two gonadotropins (GTHs), follicle-stimulating hormone (FSH) previously referred to as GTH I and luteinizing hormone (LH) previously as GTH II, and thereby serves as a principal neuroendocrine mediator which controls reproductive function in a wide-range of vertebrate species. Since the first isolation of GnRH from pig and sheep hypothalami (Matsuo *et al.*, 1971; Amoss *et al.*, 1971), the number of GnRH forms rapidly increased, so that at least 14 different molecular forms are now known in vertebrates (Vickers *et al.*, 2004). Furthermore, unique GnRH forms were found in procordates (Powell *et al.*, 1996; Adams *et al.*, 2003) and in octopus (Iwakoshi *et al.*, 2002).

It is now evident that there are two or three GnRH forms in a single vertebrate species. One form of GnRH, so-called hypothalamic GnRH, regulates gonadal maturation through stimulation of pituitary gonadotropes. The second form of GnRH is chicken GnRH-II (cGnRH-II), which is highly conserved from fish through mammals. cGnRH-II neurons are localized in the midbrain tegmentum and send their axons widely throughout the central nervous system. cGnRH modulates sexual behavior in some vertebrate species (Millar, 2003). In modern teleosts such as perciformes and pleuronociformes, salmon GnRH (sGnRH) is produced as the third form of GnRH in the neuronal groups that are localized rostrally along the terminal nerve. These neurons project their axons throughout the various brain loci, and are shown to have neuromodulatory functions (Oka, 2002; Saito *et al.*, 2003).

There are two GnRH forms, sGnRH and cGnRH-II, in the brains of salmonid fishes. We have focused on the neuroendocrine regulation of spawning migration in salmonids, in particular the biological significance of sGnRH in control of homing behavior as well as sexual maturation. A number of studies conducted in salmonids showed that sGnRH has a central role in the regulation of synthesis and release of GTHs, and thus promotes the gonadal

maturation (Amano *et al.*, 1997).

In addition to the hypophysiotropic action, recent studies of pre-spawning salmonids that were captured on the route of homing migration indicated that sGnRH accelerates homing behavior. Administration of GnRH analog (GnRH_a) shortened the duration of homing behavior in sockeye salmon (Sato *et al.*, 1997; Kitahashi *et al.*, 1998b) and chum salmon (Kitahashi *et al.*, 2001). It is thus conceivable that sGnRH regulates both homing behavior and gonadal maturation, and synchronizes these functions to accomplish the reproductive success (Onuma *et al.*, 2005).

As described above, GnRH is the principal regulator for both the reproductive behavior and sexual maturation in many vertebrate species including salmonids, although molecular mechanisms of the multifunctional action of GnRH remain to be elucidated. Recent reports showed that multiple genes encoding GnRH receptors (GnRH-Rs) are present in a single species (Lethimonier *et al.*, 2004; Millar *et al.*, 2004). Several GnRH-R subtypes with different structural characteristics are expressed in the brain, pituitary and various peripheral organs. It has become increasingly more obvious that multiple signal transduction pathways are activated inside the target cells by binding of GnRH to its receptors, indicating that the complex GnRH signals in the target cells are responsible for the multiple functions of GnRH in reproduction. These intracellular processes that mediate the action of GnRH are, however, largely unknown at present, particularly in the brain. Since there were abundant studies that focused on the action of GnRH in the pituitary, that is, control of GTH secretion in mammals (Kaiser *et al.*, 1997) and teleosts (Ando *et al.*, 2001a; Yaron *et al.*, 2003), we intend to review recent data on the molecular mechanisms of the action of GnRH with special reference to the regulation of GTH secretion in the pituitary of salmonids.

2. GnRH and GTH during reproductive cycle

2-1. Seasonal activation of sGnRH gene expression

Two genes (sGnRH-I and -II) encode sGnRH precursors in salmonids (Higa *et al.*, 1997). They are co-expressed in almost all sGnRH neurons in the forebrain (Amano *et al.*, 1998). However, the expression level of sGnRH-II gene is much higher than that of sGnRH-I gene (Ando *et al.*, 2001b; Kitahashi *et al.*, 2004; Onuma *et al.*, 2005).

In masu salmon, the amounts of sGnRH mRNAs are high during winter through spring in the prepubertal stage, and decline toward summer, and then increase again in the spawning period (Ando *et al.*, 2001b; Kitahashi *et al.*, 2004). These changes correspond well with the content of sGnRH in the forebrain (Amano *et al.*, 1992, 1993). In contrast, the amount of sGnRH in the pituitary gradually increases with sexual maturation and reaches its maximum in the spawning period. The augmented expression of sGnRH genes in the prepubertal stage suggests that there is a neuromodulatory action of sGnRH that is involved in homing migration, because masu salmon initiate homing migration at this stage.

The expression of gene encoding prolactin (PRL), which is involved in teleostean adaptation to fresh water, was stimulated by GnRH α treatment in masu salmon pituitaries at the prepubertal stage in early spring (Bhandari *et al.*, 2003). This temporal stimulation of PRL gene expression by GnRH α may relate to the physiological action of sGnRH involved in the initiation of upstream migration mentioned above.

The coincidental elevation of the amounts of sGnRH and GTH subunit mRNAs in the pre-spawning and spawning periods is certainly important for the final sexual maturation and upstream migration to the spawning ground. In homing chum salmon, expression of sGnRH genes was elevated in almost all forebrain loci during the last phase of spawning migration (Fig. 1) (Onuma *et al.*, 2005). These results support the notion that sGnRH neurons are activated during spawning migration, and coordinately regulate migratory behavior and the

final sexual maturation. Furthermore, we hypothesized that sGnRH is involved in the onset of the motivated state of homing chum salmon in addition to the promotion of gonadal maturation at the beginning of spawning migration. By use of a quantitative real-time PCR technique, we are now examining whether the expression of sGnRH genes is activated in the brain of chum salmon when they leave the Bering Sea for their natal river.

2-2. Differential secretion of FSH and LH

FSH and LH are produced in independent adenohypophysial cells in salmonids (Nozaki *et al.*, 1990a; Naito *et al.*, 1991), and are differentially released during the reproductive cycle. Many studies on pituitary contents (Suzuki *et al.*, 1988; Sumpter and Scott, 1989; Nozaki *et al.*, 1990a, b; Naito *et al.*, 1991) and plasma levels (Suzuki *et al.*, 1988; Swanson *et al.*, 1989; Sumpter and Scott, 1989; Oppen-Berntsen *et al.*, 1994; Slater *et al.*, 1994; Prat *et al.*, 1996; Saligaut *et al.*, 1998; Gomez *et al.*, 1999) of GTHs showed that the FSH levels are elevated during spermatogenesis and vitellogenesis, whereas the LH levels increase during the final sexual maturation.

In masu salmon, we recently examined GTH release activity of primary pituitary cell cultures at four reproductive stages in March (initiation of sexual maturation), May (early maturation), July (pre-spawning), and September (spawning period) (Ando *et al.*, 2004). FSH levels in the culture medium increased with sexual maturation and peaked in September, whereas LH release remained low until July and considerably increased in September. These results indicate that the differential regulation of releases of two GTHs can be achieved in part *in vitro* condition without hypophysiotropic stimuli and neuronal inputs such as dopaminergic and γ -aminobutyric acid (GABA)-ergic (Yaron *et al.*, 2003). It thus appears that two different types of gonadotropes (FSH and LH cells) release GTH autonomously in different manners.

Recent studies on the temporal expression of GTH subunit genes during sexual maturation showed somewhat different patterns from that of release activity. The amounts of FSH β and LH β mRNAs increase with sexual maturation, and reach considerably high values at spawning. Nevertheless, the increase in the content of FSH β mRNA is initiated at early stage of gametogenesis and the elevation of that of LH β mRNA is initiated later during gametogenesis (Gomez *et al.*, 1999; Kitahashi *et al.*, 2004). In the primary pituitary cells of masu salmon, the amounts of $\alpha 2$, FSH β , and LH β mRNAs increased from March through May and reached their maximum at the pre-spawning stage in July (Ando *et al.*, 2004). The amounts of $\alpha 2$ and FSH β mRNAs then declined in September, while that of LH β mRNA remained at high levels.

The changes in the GTH synthesis and release activities apparently showed differential GTH secretion: FSH production is initiated at the early stage of gametogenesis and augmented in parallel with sexual maturation, and FSH release follows this change. In contrast, LH production is initiated at the early stage of gonadal maturation, but most of newly synthesized LH is accumulated in the adenohypophysis during the pre-spawning period for the massive release at the spawning.

There were many previous studies that focused on the mechanisms controlling the differential secretion of FSH and LH. It is well established in mammals that GTH synthesis and release are regulated by multiple factors including GnRH, sex steroids and gonadal peptides such as activin and inhibin. In fish, however, much less is known about this process. The fish, particularly seasonal spawner, provide an interesting model for investigation of the mechanism of differential GTH secretion, because their sexual maturation progress slowly in concert with environmental cues, such as day length and water temperature. In most salmonid species, gonadal maturation is initiated in spring and accomplished in autumn, and is accelerated by exposure to short photoperiod (Amano *et al.*, 1995). A use of

fish at various reproductive stages allows us to examine different regulatory mechanisms that depend on a certain reproductive stage. The salmonid fishes thus provide a good model for investigation of the regulatory mechanisms of differential synthesis and release of the two GTHs.

2-3. Effects of GnRH on GTH secretion

The role of GnRH as an inducer of LH release was established in fish (Trudeau, 1997; Yaron et al., 2003), whereas less attention was paid to the regulation of FSH release. In salmonids, GnRH α selectively stimulated FSH release from the pituitary of immature rainbow trout, but it stimulated LH release in mature fish (Kawauchi et al., 1989; Breton et al., 1998). sGnRH-induced releases of FSH and LH from primary pituitary cells were reported in maturing coho salmon (Dickey and Swanson, 2000) and in pre-spawning masu salmon (Ando et al., 2004). In the primary pituitary cells from spawning masu salmon, sGnRH stimulated release of LH but not FSH (Ando et al., 2004). It therefore appears that sGnRH stimulates releases of FSH and LH at different maturational stages (Table 1).

The stimulatory role of GnRH in GTH subunit gene expression was shown in various fish species including salmonids (Khakoo et al., 1994; Xiong et al., 1994a; Melamed et al., 1996, 2002; Ando et al., 1999; Hassin et al., 1998, 2000; Gur et al., 2001; Rosenfeld et al., 2001; Sohn et al., 2001; Kandel-Kfir et al., 2002; Klausen et al., 2002a; Chong et al., 2004). In salmonids, GnRH α that was implanted into the dorsal muscle increased the amount of α 2 and LH β mRNAs, but not FSH β mRNA in the pre-spawning sockeye salmon (Kitahashi et al., 1998a) and masu salmon (Kitahashi et al., 2004). However, in the primary pituitary cells prepared from the pre-spawning female masu salmon, sGnRH increased the amount of α 2 and FSH β mRNAs but not LH β mRNA (Ando et al., 2004). Furthermore, sGnRH increased only the levels of FSH β mRNA in the primary pituitary cells of maturing coho salmon

(Dickey and Swanson, 2000). These results indicate that the action of GnRH on expression of GTH subunit gene depends on the subunit gene and reproductive stage (Table 1).

The effects of GnRH on GTH gene expression were different between *in vivo* and *in vitro* conditions, indicating that there are additional endogenous factors that modulate the action of GnRH on GTH gene expression. Considering that the responsiveness to GnRH of gonadotropes is highly dependent on the reproductive stage, gonadal sex steroid hormones are strong candidates for the modulation of the action of GnRH.

2-4. Modulation of the action of GnRH by E2

Sex steroid hormones have both positive and negative effects on GTH secretion, depending on the mode of administration and the reproductive stage of animal. In immature and maturing salmonids, aromatizable androgens and estradiol-17 β (E2) stimulate LH synthesis (Crim and Evans, 1979; Crim *et al.*, 1981; Trinh *et al.*, 1986; Xiong *et al.*, 1994b; Borg *et al.*, 1998; Dickey and Swanson, 1998). In contrast, negative or no effects on LH synthesis of sex steroids were reported in mature fish. A gonadoectomy of spermiating coho salmon resulted in an increase in the plasma LH levels, suggesting a negative feedback control of LH secretion by sex steroids (Larsen and Swanson, 1997). The treatment of pituitary cells from masu salmon with E2 and testosterone (T) significantly increased the amount of LH β mRNA in the maturing and the pre-spawning stages, but significant changes were not observed in the spawning period (Ando *et al.*, 2004). In the same fish samples, E2 showed a weak stimulatory activity of LH release in the pre-spawning and the spawning stages (Table 1).

Beside many studies on the regulation of LH secretion by GnRH or sex steroids, a few studies investigated effects of a co-treatment with GnRH and sex steroid to clarify functional interaction between these factors. Pre- or co-treatment with E2 or T potentiated the action of

GnRH to induce LH release in rainbow trout (Crim and Evans, 1983; Weil and Marcuzzi, 1990). Similarly, sGnRH-induced LH release was synergistically stimulated by a co-treatment with E2 in the primary pituitary cells of masu salmon in all stages during sexual maturation (Ando *et al.*, 2004). The amount of LH β mRNA was also extensively elevated by the co-treatment with E2 in the maturing stage, although sGnRH alone did not have any effects on the amount of LH β mRNA. Therefore, the synergistic stimulation by the combination of GnRH and E2 seems to be evident in the reproductive stage-specific LH synthesis and release in the intact pituitary (Table 1), in addition to the stimulatory role of GnRH in LH release and of E2 in LH synthesis.

The effects of sex steroids on secretion of FSH received much less attention. It seems to be more complex. In maturing coho salmon, *in vivo* treatment with E2 or T had little effects on the pituitary contents of FSH and FSH β mRNA, whereas the plasma FSH level was markedly decreased by the treatment, suggesting a negative feedback by sex steroids on FSH synthesis at the translational level (Dickey and Swanson, 1998). Gonadoectomy of pre-spawning and spermiating coho salmon resulted in increases in the plasma FSH levels, supporting the negative feedback control on FSH mentioned just above (Larsen and Swanson, 1997). However, in Atlantic salmon, the pituitary and plasma levels of FSH in castrated fish at the spawning stage were lower than those in sham-operated fish, and a treatment with T increased the FSH levels, indicating a positive feedback effect of sex steroid on FSH secretion (Borg *et al.*, 1998). E2 and T did not have any effects on the amount of FSH β mRNA, regardless of coexistence of sGnRH, in the primary pituitary cells of masu salmon (Ando *et al.*, 2004). These results suggest that other factors are involved in stimulation of FSH synthesis in the pituitary (Table 1). Nevertheless, sGnRH stimulated FSH release in synergism with E2 in the maturing and pre-spawning stages, indicating that sGnRH and E2 participate in part in the release of FSH during sexual maturation (Table 1).

3. GnRH receptors

The action of GnRH is mediated through binding of GnRH to a single class of G protein-coupled membrane receptors in the target cells. Since the first molecular characterization of mouse GnRH-R (Tsutsumi *et al.*, 1992), cDNAs encoding GnRH-R were cloned in various vertebrate species. A phylogenetic analysis of these GnRH-R sequences indicates the presence of three main types, which were termed as type I, type II, and type III receptors (Fig. 2) (Millar *et al.*, 2004). Among the three types, fish GnRH-R belongs to the type I and type III. It is now evident that multiple GnRH-R types present in single species. In mammals, type I and type II GnRH-Rs have distinct ligand selectivity. The type I receptor is highly sensitive to mammalian GnRH, whereas the type II receptor shows a clear preference for cGnRH-II. In non-mammalian vertebrates, multiple GnRH-Rs in a single species, however, do not have such clear different selectivity for native GnRH ligands. All types of GnRH-Rs in teleosts have a particular preference to cGnRH-II, followed by sGnRH and then the third endogenous GnRH when identified (Lethimonier *et al.*, 2004). Therefore, ligand-receptor relationships of the multiple GnRH-Rs remain unclear in teleosts. Information on distribution and expression levels of GnRH-Rs in target organs is required to determine if the multiple GnRH-R types have different functions in terms of the action of GnRH.

In salmonids, only type I GnRH-R was first identified in the brain of rainbow trout (rtGnRH-R) (Madigou *et al.*, 2000). Thereafter, the second mRNA isoform, which is generated by alternative promoter usage and splicing, was characterized (Madigou *et al.*, 2002). We recently demonstrated in masu salmon that five different GnRH-R genes, termed as msGnRH-R1, R2, R3, R4, and R5, are expressed in the brain, the pituitary and other peripheral tissues with different patterns (Jodo *et al.*, 2003). A splicing variant of msGnRH-R1 (R1-v) is also expressed in these tissues. All these receptors are type I receptors, but are

divided into two subtypes, one subtype (type Ia) includes R1, R2, and R3 and the other (type Ib) includes R4 and R5 (Fig. 2). The identity of nucleotide sequences among R1, R2 and R3 is 96-99%, while that of R4 and R5 is 81%. The identity between the two subtypes decreases to 59-71%.

Ia subtype includes the goldfish GnRH-R (GfA, Illing *et al.*, 1999) and catfish GnRH-R (cfGnRH-R2, Bogerd *et al.*, 2002), while Ib includes GfB and cfGnRH-R1. Because salmonids, goldfish and catfish are rather ancient tetraploid teleosts, the two subtypes may arise from a genomic duplication. Recently, another type of GnRH-R that belongs to type III receptor was identified in rainbow trout (Lethimonier *et al.*, 2004). It is most probable that there is also a type III GnRH-R in masu salmon. If so, there are at least six different subtypes of GnRH-R in masu salmon. It is of considerable interest and importance to determine how these multiple GnRH-R subtypes mediate GnRH signals to different action during reproductive cycle as discussed above, and whether they have different roles in terms of the action of GnRH.

The different effects of GnRH on synthesis and release of two GTHs may be attributed in part to changes in expression of GnRH-Rs in FSH and LH cells. We therefore examined by real-time PCR seasonal variations in expression of the five msGnRH-R genes in the pituitary of masu salmon during the reproductive cycle. All five subtypes of msGnRH-R genes were expressed in the pituitary, although R4 mRNA was dominant. Interestingly, the expression patterns of five msGnRH-R genes differed among different subtypes. Among them, R4 mRNA increased only in the pre-spawning period, when expression of LH subunit genes was stimulated by GnRH_a, suggesting that the R4 subtype is involved in the GnRH-induced LH synthesis (Jodo *et al.*, in preparation). Other subtype mRNAs increased in different periods, so that they can be involved in other action of GnRH in the pituitary, such as GnRH-induced PRL gene expression as described previously. To obtain more accurate

information on the function of the multiple GnRH-R subtypes, cell type specific distribution of the GnRH-Rs in the pituitary is currently under investigation.

4. Signal transduction pathway

GnRH activates multiple signal transduction pathways such as Ca^{2+} and cAMP signaling through binding to GnRH-R, which is able to couple to multiple G proteins ($\text{G}_{q/11}$, G_s and $\text{G}_{i/o}$) (Stanislaus *et al.*, 1998; Ruf *et al.*, 2003; Millar *et al.*, 2004). In general, regulation of GTH secretion by GnRH is primarily mediated by Ca^{2+} signaling via $\text{G}_{q/11}$ (Grosse *et al.*, 2000). Activation of $\text{G}_{q/11}$ proteins stimulates phospholipase C to generate inositol trisphosphate and diacylglycerol. Increases of these signaling messengers lead to activation of protein kinase C (PKC) and also an increase in intracellular Ca^{2+} concentration. These two secondary signal mediators are involved in GnRH-induced GTH release and synthesis (Klausen *et al.*, 2002b). Afterwards, mitogen-activated protein kinase (MAPK) that locates downstream of PKC plays a role in the regulation of GTH subunit gene expression in response to GnRH. PKC/MAPK and Ca^{2+} influx differentially control expression of three GTH subunit genes (Ando *et al.*, 2001a). Recent studies indicated that Ca/calmodulin-dependent kinase II (Ca/CaMKII) pathway is also involved in the GnRH-induced GTH subunit gene expression (Haisenleder *et al.*, 2003).

In fish, GnRH is also involved in the regulation of GTH release and synthesis through multiple signaling pathways including PKC, Ca^{2+} , and PKA (Ando *et al.*, 2001a; Klausen *et al.*, 2002b; Yaron *et al.*, 2003). Most of these studies were conducted in goldfish and tilapia, and much less are known in salmonids. We examined whether the PKC/MAPK and Ca^{2+} influx signaling are involved in the GnRH-stimulated LH β gene expression using a gonadotrope-derived cell line, $\alpha\text{T3-1}$ (Ando *et al.*, 1999, 2001a).

GnRH α specifically stimulated the activity of promoter of chinook salmon LH β gene

when transfected in α T3-1 cells. A specific L-channel agonist, Bay K8644 did not stimulate LH β promoter activity, and an L-channel antagonist, nimodipine, did not block the GnRH-stimulated LH β gene expression. Furthermore, a MAPK inhibitor, PD098059, almost eliminated this stimulation. These results suggest that PKC/MAPK pathway but not Ca²⁺ influx mediates the stimulation of LH β gene expression by GnRH. However, it is possible that signal transduction pathways from GnRH-R to LH β gene are not identical to that in intact gonadotropes in a salmon pituitary. Further studies are necessary to define the significance of PKC/MAPK pathway in the GnRH-induced LH β gene expression in salmonids. Nevertheless, the transfection experiment using the heterologous cell system provided important information on molecular mechanism of the action of GnRH: GnRH can stimulate salmon LH β gene transcription by activation of DNA-binding transcription factors through PKC/MAPK pathway.

5. Transcriptional regulation of GTH gene

5-1. Transcription factors involved in the action of GnRH

Extensive studies in mammals showed that transcription factors which are involved in the stimulation by GnRH of GTH subunit genes are different among three GTH subunit genes (Ando *et al.*, 2001a; Ruf *et al.*, 2003). These include a LIM-homeodomain protein, an Ets-related transcription factor and cAMP response element binding protein (CREB) for α gene, Sp1, early growth response protein 1 (Egr1) and pituitary homeobox 1 (Ptx1) for LH β gene, and activating protein-1 (AP-1) and nuclear factor-Y (NFY) for FSH β gene. GnRH thus regulates GTH subunit genes by activating different sets of transcription factors through multiple signal transduction cascades.

Several additional transcription factors act in concert to mediate GnRH signals to LH β gene, with Egr1 serving an essential function. Egr1 stimulates LH β gene transcription in

response to GnRH in the synergism with Ptx1 and a nuclear receptor, steroidogenic factor-1 (SF-1), which is a mammalian homolog of *Drosophila fushi tarazu* factor 1 α (dFTZ-F1 α). When GnRH signals come into the nucleus, expression of Egr1 gene, which is an immediately early gene, is rapidly increased (Fig. 3), and Egr1 synergizes with Ptx1 and SF-1 to stimulate LH β gene. *Cis*-acting DNA elements corresponding to these factors are conserved in mammalian LH β genes.

In contrast to mammals, there is a paucity of information concerning transcription factors involved in the regulation of fish GTH subunit genes by GnRH (Ando *et al.*, 2001a; Yaron *et al.*, 2003). Although genomic sequences of fish GTH subunit genes have been determined from several fish species including chinook salmon (α gene, Suzuki *et al.*, 1995; LH β gene, Xiong and Hew, 1991; FSH β gene, Chong *et al.*, 2004), masu salmon (α gene, Gen *et al.*, 1997), carp (α gene, Huang *et al.*, 1992; LH β gene, Chang *et al.*, 1992), goldfish (LH β gene, Sohn *et al.*, 1999; FSH β gene, Sohn *et al.*, 1998), tilapia (LH β and FSH β genes, Rosenfeld *et al.*, 1997, 2001), and African catfish (FSH β gene, Vischer *et al.*, 2003), *cis*-acting elements and transcription factors required for GnRH induction were determined only in the chinook salmon LH β (Ando *et al.*, 1999, 2001; Melamed *et al.*, 2002) and the tilapia FSH β genes (Rosenfeld *et al.*, 2001; Yaron *et al.*, 2003).

Using murine cell lines such as α T3-1 and L β T2 as models, GnRH responsive regions were determined in the LH β gene promoter of chinook salmon. A proximal region (-258 to -199) that contains a binding site for Ptx1 was important in the GnRH stimulation (Ando *et al.*, 1999). Furthermore, other distal Ptx1 sites mediated GnRH-induced LH β gene transcription (Melamed *et al.*, 2002). It should be noted that there is no functional binding sites for Egr1 in the promoter of LH β gene, and Egr1 is ineffective to stimulate transcription of LH β gene (Le Drean *et al.*, 1997).

Interestingly, in the proximal region of salmon LH β promoter, there are two GC rich

sequences (-41 to -33 and -85 to -77) that are similar to a common consensus binding sites of Egr1 and Sp1. Gel retardation analysis using the proximal GC element as a probe showed that Sp1 but not Egr1 bound to this element (Fig. 4). Site-directed mutagenesis of the two GC rich Sp1 binding sites and also two proximal Ptx1 binding sites (-133 to -127 and -258 to -199) decreased the induction by GnRH, indicating that these Sp1 and Ptx1 sites are involved in stimulation of LH β gene by GnRH. Since Sp1 binding activity increased after GnRH α treatment in α T3-1 cells (Fig. 3), Sp1 is capable to serve as a direct molecular target of GnRH signals like Egr1. It is highly conceivable that the stimulation of LH β gene by GnRH is primary mediated by Sp1, which act in synergism with Ptx1 and other transcription factors, such as SF-1 and estrogen receptor (ER) as discussed below.

5-2. Regulation of LH β gene transcription

GnRH synergizes with E2 to stimulate LH synthesis in the pituitary of salmonids. The effects of E2 are mainly mediated by ER, a transcription factor which regulates target genes through binding to estrogen responsive elements (EREs) in their promoters. The presence of two functional EREs, pERE (-274 to -261) and dERE (-2736 to -2655), were reported in the chinook salmon LH β promoter (Liu *et al.*, 1995; Xiong *et al.*, 1994b). They cooperatively stimulate LH β gene transcription. In addition, SF-1 synergized with the ER through binding to a proximal SF-1 binding site (gonadotrope-specific element, GSE2, Fig. 5), and then stimulated LH β gene transcription dramatically (Le Drean *et al.*, 1996). Since SF-1 was reported to interact with Sp1 (Kaiser *et al.*, 2000; Sugawara *et al.*, 2000), protein-protein interactions among ER, SF-1, Sp1, and Ptx1 is most probably important for the functional synergism between GnRH and E2 in the stimulation of LH β gene expression (Fig. 5). The expression of two salmon FTZ-F1 homolog genes, sFF1-I and sFF1-II, was augmented in the pituitaries of chum salmon and sockeye salmon at the late stage of sexual maturation (Higa *et*

al., 2000). These results support the physiological significance of SF-1 in the regulation of LH β gene expression.

5-3. Regulation of FSH β gene transcription

Much less is known about GnRH regulation of FSH β gene expression. In the ovine FSH β gene, GnRH activated transcription through two binding sites for activating protein-1 (AP-1). These binding sites are well conserved in mammalian FSH β gene promoters (Strahl *et al.*, 1997; 1998). Furthermore, a novel AP-1 site, which binds AP-1 and NFY, was important for the induction of the mouse FSH β gene by GnRH (Coss *et al.*, 2004).

In tilapia, FSH β promoter activity was stimulated by GnRH when transfected to the primary pituitary cells (Rosenfeld *et al.*, 2001), and a GnRH responsive region (-1211 to -821) that contains an AP-1 binding site was determined (Yaron *et al.*, 2003). In chinook salmon, the 5' upstream region of FSH β gene was recently isolated and was shown to be induced by GnRH when transfected into L β T2 cells (Chong *et al.*, 2004). *Cis*-acting elements that mediate the response to GnRH remain to be determined.

6. Conclusions and perspectives

In the present article, we focused on the molecular mechanisms of the action of GnRH, in particular regulation of GTH subunit gene expression. The data presented in this review indicate that the action of GnRH is mediated by multiple subtypes of GnRH-Rs, successive multiple signaling pathways, and finally multiple transcription factors that act cooperatively to stimulate transcription of GTH subunit genes. During sexual maturation, GnRH synergize with sex steroid hormones to regulate synthesis and release of GTHs, thus increasing the complexity of mediation of GnRH signals. As a result of this complex molecular function stimulated by the action of GnRH, FSH and LH are differentially secreted by pituitary

gonadotropes in concert with developmental and environmental stimuli to accomplish the reproductive success. However, it should be noted that the data concerning the GnRH signals presented in our article is only “the tip of the iceberg” of more diverged molecular mechanisms of the action of GnRH. Indeed, we do not know how GnRH regulates α subunit gene in addition to FSH β gene. The mechanisms of differential regulation of synthesis and release of GTH by GnRH are almost not known.

GnRH in cooperation with E2 stimulates only synthesis of LH in the early stage of sexual maturation, but it plays as a major secretagogue of LH in the spawning period. The effects on synthesis and release by GnRH are probably mediated in part through a common signal transduction pathway where Ca²⁺ serves as a central mediator. Nevertheless, the successive two distinct pathways, one leading to secretory granules and the other leading to nucleus remain unclear. The two pathways may interact with each other, and are most probably balanced to achieve the reproductive stage-specific LH secretion.

There is little information on the molecular mechanisms of the action of GnRH in the brain. GnRH has neuromodulatory roles involved in the regulation of reproductive behavior. Several lines of evidence in our research indicate that this is true in the upstream migration of salmonids. GnRH may regulate neuronal excitability and release of neurotransmitters in target neurons involved in reproductive behavior. However, the target sites and also its molecular action have not yet been determined. These actions may be mediated in part by GnRH-R through a common pathway with that in the gonadotropes. In our current research about expression of the five msGnRH-R genes in the brain, their expression patterns and also regulation was different between the brain and the pituitary. It is thus important to determine accurate distribution of target sites and molecular events in response to GnRH.

Our study in salmonids and other studies in mammals suggest that some transcription factors, such as Ptx1, Sp1, and SF-1 are commonly utilized by LH β genes in both salmon and

mammals, indicating that molecular mechanisms of stimulation of LH β gene by GnRH are partially conserved across vertebrate evolution. Nevertheless, in the pituitary of salmon, GnRH alone does not stimulate LH β gene, whereas it does in mammals. Furthermore, E2 has a negative effect on GTH subunit gene expression in most mammalian cases, whereas it has a stimulatory effect in the fish pituitary. Despite a use of similar sets of transcription factors which play a central role in GTH genes in response to GnRH, their functional interaction is shown to be different between fishes and mammals. These different regulatory mechanisms of the action of GnRH may serve as a molecular basis of divergent physiological strategies of reproductive success in various vertebrate species. Thus, it is of considerable interest and importance to determine a regulatory network of transcription factors regulating GTH gene expression by GnRH in various species of vertebrates, particularly seasonal breeders like salmonids.

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REFERENCES

- Adams BA, Tello JA, Erchegeyi J, Warby C, Hong DJ, Akinsanya KO, Mackie GO, Vale W, Rivier JE, Sherwood NM (2003) Six novel gonadotropin-releasing hormones are encoded as triplets on each of two genes in the protochordate, *Ciona intestinalis*. *Endocrinology* 144: 1907-1919
- Amano M, Aida K, Okumoto N, Hasegawa Y (1992) Changes in salmon GnRH and chicken GnRH-II contents in the brain and pituitary, and GTH contents in the pituitary in female masu salmon, *Oncorhynchus masou*, from hatching through ovulation. *Zool Sci* 9: 375-386
- Amano M, Aida K, Okumoto N, Hasegawa Y (1993) Changes in levels of GnRH in the brain and pituitary and GTH in the pituitary in male masu salmon, *Oncorhynchus masou*, from hatching to maturation. *Fish Physiol Biochem* 11: 233-240
- Amano M, Hyodo S, Kitamura S, Ikuta K, Suzuki Y, Urano A, Aida K (1995) Short photoperiod accelerates preoptic and ventral telencephalic salmon GnRH synthesis and precocious maturation in underyearling male masu salmon. *Gen Comp Endocrinol* 99: 22-27
- Amano M, Urano A, Aida K (1997) Distribution and function of gonadotropin-releasing hormone (GnRH) in the teleost brain. *Zool Sci* 14: 1-11
- Amano M, Ashihara M, Yoshiura Y, Kitamura S, Ikuta K, Aida K (1998) Two differing salmon GnRH precursor mRNAs are co-expressed in the brain of sockeye salmon. *Cell Tissue Res* 292: 267-273
- Amoss M, Burgus R, Blackwell R, Vale W, Fellows R, Guillemin R (1971) Purification, amino acid composition and N-terminus of the hypothalamic luteinizing hormone releasing factor (LRF) of ovine origin. *Biochem Biophys Res Commun* 44: 205-210
- Ando H, Ando J, Le Drean Y, Liu D, Xiong F, Hew CL (1999) Salmon gonadotropin II β subunit promoter contains multiple DNA elements responsible for stimulation by

- gonadotropin-releasing hormone through protein kinase C-dependent and -independent pathways. *Mol Cell Endocrinol* 157: 143-152
- Ando H, Hew CL, Urano A (2001a) Signal transduction pathways and transcription factors involved in the gonadotropin-releasing hormone-stimulated gonadotropin subunit gene expression. *Comp Biochem Physiol Part B* 129: 525-532
- Ando H, Sasaki Y, Okada H, Urano A (2001b) Prepubertal increases in the levels of two salmon gonadotropin-releasing hormone mRNAs in the ventral telencephalon and preoptic area of masu salmon. *Neurosci Lett* 307: 93-96
- Ando H, Swanson P, Kitani T, Koide N, Okada H, Ueda H, Urano A (2004) Synergistic effects of salmon gonadotropin-releasing hormone and estradiol-17 β on gonadotropin subunit gene expression and release in masu salmon pituitary cells *in vitro*. *Gen Comp Endocrinol* 137: 109-121
- Bhandari RK, Taniyama S, Kitahashi T, Ando H, Yamauchi K, Zohar Y, Ueda H, Urano A (2003) Seasonal changes of responses to gonadotropin-releasing hormone analog in expression of growth hormone/prolactin/somatolactin genes in the pituitary of masu salmon. *Gen Comp Endocrinol* 130: 55-63
- Borg B, Antonopoulou E, Mayer I, Andersson E, Berglund I, Swanson P (1998) Effects of gonadoectomy and androgen treatments on pituitary and plasma levels of gonadotropins in mature male Atlantic salmon, *Salmo salar*, parr-positive feedback control of both gonadotropins. *Biol Reprod* 58: 814-820
- Bogerd J, Diepenbroek WB, Hund E, Van Oosterhout F, Teves ACC, Leurs R, Blomenröhr M (2002) Two gonadotropin-releasing hormone receptors in the African catfish: no differences in ligand selectivity, but differences in tissue distribution. *Endocrinology* 143: 4673-4682
- Breton B, Govoroun M, Mikolajczyk T (1998) GTH I and GTH II secretion profiles during the reproductive cycle in female rainbow trout: relationship with pituitary responsiveness to

- GnRH-A stimulation. *Gen Comp Endocrinol* 111: 38-50
- Chang YS, Huang FL, Lo TB (1992) Isolation and sequence analysis of carp gonadotropin β -subunit gene. *Mol Mar Biol Biotech* 1: 97-105
- Chong KL, Wang S, Melamed P (2004) Isolation and characterization of the follicle-stimulating hormone β subunit gene and 5' flanking region of the Chinook salmon. *Neuroendocrinology* 80:158-170
- Coss D, Jacobs SB, Bender CE, Mellon PL (2004) A novel AP-1 site is critical for maximal induction of the follicle-stimulating hormone β gene by gonadotropin-releasing hormone. *J Biol Chem* 279: 152-162
- Crim LW, Evans DM (1979) Stimulation of pituitary gonadotropin by testosterone in juvenile rainbow trout (*Salmo gairdneri*). *Gen Comp Endocrinol* 37: 192-196
- Crim LW, Evans DM (1983) Influence of testosterone and/or luteinizing hormone releasing hormone analogue on precocious sexual development in the juvenile rainbow trout. *Biol Reprod* 29: 137-142
- Crim LW, Peter RE, Billard R (1981) Onset of gonadotropic hormone accumulation in the immature trout pituitary gland in response to estrogen or aromatizable androgen steroid hormones. *Gen Comp Endocrinol* 44: 374-381
- Dickey JT, Swanson P (1998) Effects of sex steroids on gonadotropin (FSH and LH) regulation in coho salmon (*Oncorhynchus kisutch*). *J Mol Endocrinol* 21: 291-306
- Dickey JT, Swanson P (2000) Effects of salmon gonadotropin-releasing hormone on follicle stimulating hormone secretion and subunit gene expression in coho salmon (*Oncorhynchus kisutch*). *Gen Comp Endocrinol* 118: 436-449
- Gen K, Okuzawa K, Aida K, Kagawa H (1997) Isolation and characterization of genes encoding two types of α - glycoprotein subunits from pituitary of masu salmon (*Oncorhynchus masou*). In "Advances in Comparative Endocrinology, XIII International

- Congress of Comparative Endocrinology” Ed by Kawashima S, Kikuyama S, Monduzzi Editore, Bologna, pp 861-865
- Gomez JM, Weil C, Ollitrault M, Le Bail P-Y, Breton B, Le Gac FL (1999) Growth hormone (GH) and gonadotropin subunit gene expression and pituitary and plasma changes during spermatogenesis and oogenesis in rainbow trout (*Oncorhynchus mykiss*). *Gen Comp Endocrinol* 113: 413-428
- Grosse R, Schmid A, Schoneberg T, Herrlich A, Muhn P, Schultz G, Gudermann T (2000) Gonadotropin-releasing hormone receptor initiates multiple signaling pathways by exclusively coupling to Gq/11 proteins. *J Biol Chem* 275: 9193-9200
- Gur G, Banfil D, Safarian H, Naor Z, Yaron Z (2001) GnRH receptor signaling in tilapia pituitary cells: role of mitogen-activated protein kinase (MAPK). *Comp Biochem Physiol* 129: 517-524
- Haisenleder DJ, Burger LL, Aylor KW, Dalkin AC, Marshall JC (2003) Gonadotropin-releasing hormone stimulation of gonadotropin subunit transcription: evidence for the involvement of calcium/calmodulin-dependent kinase II (Ca/CAMK II) activation in rat pituitaries. *Endocrinology* 144: 2768-2774
- Hassin S, Gothilf Y, Blaise O, Zohar Y (1998) Gonadotropin-I and -II subunit gene expression of male striped bass (*Morone saxatilis*) after gonadotropin-releasing hormone analogue injection: quantification using an optimized ribonuclease protection assay. *Biol Reprod* 58: 1233-1240
- Hassin S, Claire M, Holland H, Zohar Y (2000) Early maturity in the male stripes bass, *Morone saxatilis*: follicle-stimulating hormone and luteinizing hormone gene expression and their regulation by gonadotropin-releasing hormone analogue and testosterone. *Biol Reprod* 63: 1691-1697
- Higa M, Kitahashi T, Sasaki Y, Okada H, Ando H (1997) Distinct promoter sequences of two

- precursor genes for salmon gonadotropin-releasing hormone in masu salmon. *J Mol Endocrinol* 19: 149-161
- Higa M, Kanda H, Kitahashi T, Ito M, Shiba T, Ando H (2000) Quantitative analysis of *fushi tarazu* factor 1 homolog messenger ribonucleic acids in the pituitary of salmon at different prespawning stages. *Biol Reprod* 63: 1756-1763
- Huang CJ, Huang FL, Wang YC, Chang YS, Lo TB (1992) Organization and nucleotide sequence of carp gonadotropin α subunit genes. *Biochim Biophys Acta* 1129: 239-242
- Illing N, Troskie BE, Nahorniak CS, Hapgood JP, Peter RE, Millar RP (1999) Two gonadotropin-releasing hormone receptor subtypes with distinct ligand selectivity and differential distribution in brain and pituitary in the goldfish (*Carassius auratus*). *Proc Natl Acad Sci USA* 96: 2526-2531
- Iwakoshi E, Takuwa-Kuroda K, Fujisawa Y, Hisada M, Ukena K, Tsutsui K, Minakata H (2002) Isolation and characterization of a GnRH-like peptide from *Octopus vulgaris*. *Biochem Biophys Res Commun* 291: 1187-1193
- Jodo A, Ando H, Urano A (2003) Five different types of putative GnRH receptor gene are expressed in the brain of masu salmon (*Oncorhynchus masou*). *Zool Sci* 20: 1117-1125
- Kaiser UB, Conn PM, Chin WW (1997) Studies of gonadotropin-releasing hormone (GnRH) action using GnRH receptor-expressing pituitary cell lines. *Endocr Rev* 18: 46-70
- Kaiser UB, Halvorson LM, Chen MT (2000) Sp1, steroidogenic factor 1 (SF-1), and early growth response protein 1 (egr-1) binding sites form a tripartite gonadotropin-releasing hormone response element in the rat luteinizing hormone- β gene promoter: an integral role for SF-1. *Mol Endocrinol* 14: 1235-1245
- Kandel-Kfir M, Gur G, Melamed P, Zilberstein Y, Cohen Y, Zmora N, Kobayashi M, Elizur A, Yaron Z (2002) Gonadotropin response to GnRH during sexual ontogeny in the common carp, *Cyprinus carpio*. *Comp Biochem Physiol* 132: 17-26

- Kawauchi H, Suzuki K, Itoh H, Swanson P, Naito N, Nagahama Y, Nakai Y, Itoh S (1989) The duality of teleost gonadotropins. *Fish Physiol Biochem* 7: 29-38
- Khakoo Z, Bhatia A, Gedamu L, Habibi HR (1994) Functional specificity for salmon gonadotropin-releasing hormone (GnRH) and chicken GnRH-II coupled to the gonadotropin release and subunit messenger ribonucleic acid level in the goldfish pituitary. *Endocrinology* 134: 838-847
- Kitahashi T, Alok D, Ando H, Kaeriyama M, Zohar Y, Ueda H, Urano A (1998a) GnRH analog stimulates gonadotropin II gene expression in maturing sockeye salmon. *Zool Sci* 15: 761-765
- Kitahashi T, Sato A, Alok D, Kaeriyama M, Zohar Y, Yamauchi K, Urano A, Ueda H (1998b) Gonadotropin-releasing hormone analog and sex steroids shorten homing duration of sockeye salmon in Lake Shikotsu. *Zool Sci* 15: 767-771
- Kitahashi T, Takagi Y, Ban M, Ando H, Ueda H, Urano A (2001) Effects of GnRH α administration on upstream migration of homing chum salmon. *Proc Japan Soc Comp Endocrinol* 16: 11
- Kitahashi T, Bhandari RK, Taniyama S, Ando H, Ueda H, Urano A (2004) Changes in expression of salmon GnRH genes and responsiveness of pituitary hormone genes to GnRH analog during growth and sexual maturation in masu salmon. In "Trends in Comparative Endocrinology, Proceedings of the Fifth Congress of the Asia and Oceania Society for Comparative Endocrinology" Ed by Oishi T, Tsutsui K, Tanaka S, Kikuyama S, AOSCE, pp 92-94
- Klausen C, Chang JP, Habibi HR (2002a) Time- and dose-related effects of gonadotropin-releasing hormone on growth and gonadotropin subunit gene expression in the goldfish pituitary. *Can J Pharmacol* 80: 915-924
- Klausen C, Chang JP, Habibi HR (2002b) Multiplicity of gonadotropin-releasing hormone

- signaling: a comparative perspective. *Prog Brain Res* 141: 111-128
- Lamonerie T, Tremblay JJ, Lanctot C, Therrien M, Gauthier Y, Drouin J (1996) Ptx1, a bicoid-related homeo box transcription factor involved in transcription of the pro-opiomelanocortin gene. *Genes Dev* 10: 1284-1295
- Larsen D, Swanson P (1997) Effects of gonadoectomy on plasma gonadotropins I and II in coho salmon, *Oncorhynchus kisutch*. *Gen Comp Endocrinol* 108: 152-160
- Le Drean Y, Liu D, Wong AOL, Xiong F, Hew CL (1996) Steroidogenic factor 1 and estradiol receptor act in synergism to regulate the expression of the salmon gonadotropin II β subunit gene. *Mol Endocrinol* 10: 217-229
- Le Drean Y, Liu D, Xiong F, Hew CL (1997) Presence of distinct *cis*-acting elements on gonadotropin gene promoters in diverse species dictates the selective recruitment of different transcription factors by steroidogenic factor-1. *Mol Cell Endocrinol* 135: 31-40
- Lethimonier C, Madigou T, Muñoz-Cueto JA, Lareyre JJ, Kah O (2004) Evolutionary aspects of GnRHs, GnRH neuronal systems and GnRH receptors in teleost fish. *Gen Comp Endocrinol* 135: 1-16
- Liu D, Xiong F, Hew CL (1995) Functional analysis of estrogen-responsive elements in chinook salmon (*Oncorhynchus tshawytscha*) gonadotropin II β subunit gene. *Endocrinology* 136: 3486-3493
- Madigou T, Mañanos-Sanchez E, Hulshof S, Anglade I, Zanuy S, Kah O (2000) Cloning, tissue distribution, and central expression of the gonadotropin-releasing hormone receptor in the rainbow trout (*Oncorhynchus mykiss*). *Biol Reprod* 63: 1857-1866
- Madigou T, Uzbekova S, Lareyre J-J, Kah O (2002) Two messenger RNA isoforms of the gonadotrophin-releasing hormone receptor, generated by alternative splicing and/or promoter usage, are differentially expressed in rainbow trout gonads during gametogenesis. *Mol Reprod Dev* 63: 151-160

- Matsuo H, Baba Y, Nair RM, Arimura A, Schally AV (1971) Structure of the porcine LH- and FSH-releasing hormone. I. The proposed amino acid sequence. *Biochem Biophys Res Commun* 43: 1334-1339
- Melamed P, Gur G, Elizur A, Rosenfeld H, Sivan B, Rentier-Delrue F, Yaron Z (1996) Differential effects of gonadotropin-releasing hormone, dopamine and somatostatin and their second messengers on the mRNA levels of gonadotropin II β subunit and growth hormone in the teleost fish, tilapia. *Neuroendocrinology* 64: 320-328
- Melamed P, Koh M, Preklathan P, Bei L, Hew C (2002) Multiple mechanisms for Pitx-1 transactivation of a luteinizing hormone β subunit gene. *J Biol Chem* 277: 26200-26207
- Millar RP (2003) GnRH II and type II GnRH receptors. *Trends Endocrinol Metab* 14: 35-43
- Millar RP, Lu ZL, Pawson AJ, Flanagan CA, Morgan K, Maudsley SR (2004) Gonadotropin-releasing hormone receptors. *Endocr Rev* 25: 235-275
- Naito N, Hyodo S, Okumoto N, Urano A, 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
- Nozaki M, Naito N, Swanson P, Miyata K, Nakai Y, Oota Y, Suzuki K, Kawauchi H (1990a) Salmonid pituitary gonadotrophs. I. Distinct cellular distributions of two gonadotropins, GTH I and GTH II. *Gen Comp Endocrinol* 77: 348-357
- Nozaki M, Naito N, Swanson P, Dickhoff WW, Nakai Y, Suzuki K, Kawauchi H (1990b) Salmonid pituitary gonadotrophs. II. Ontogeny of GTH I and GTH II cells in the rainbow trout (*Salmo gairdneri irideus*). *Gen Comp Endocrinol* 77: 358-367
- Oka Y (2002) Physiology and release activity of GnRH neurons. *Prog Brain Res* 141: 259-281
- Onuma T, Higa M, Ando H, Ban M, Urano A (2005) Elevation of gene expression for salmon gonadotropin-releasing hormone in discrete brain loci of prespawning chum salmon during upstream migration. *J Neurobiol* (in press)

- Oppen-Berntsen DO, Olsen SO, Rong CJ, Taranger GL, Swanson P, Walther BT (1994) Plasma levels of eggshell zr-proteins, estradiol-17 β , and gonadotropins during an annual reproductive cycle of Atlantic salmon (*Salmo salar*). *J Exp Zool* 268: 59-70
- Powell JF, Reska-Skinner SM, Prakash MO, Fischer WH, Park M, Rivier JE, Craig AG, Mackie GO, Sherwood NM (1996) Two new forms of gonadotropin-releasing hormone in a protochordate and the evolutionary implications. *Proc Natl Acad Sci USA* 93: 10461-10464
- Prat F, Sumpter JP, Tyler CR (1996) Validation of radioimmunoassays for two salmon gonadotropins (GTH I and GTH II) and their plasma concentrations throughout the reproductive cycle in male and female rainbow trout (*Oncorhynchus mykiss*). *Biol Reprod* 44: 29-38
- Rosenfeld H, Levavisivan B, Melamed P, Yaron Z, Elizur A (1997) The GTH β subunits of tilapia: gene cloning and expression. *Fish Physiol Biochem* 17: 85-92
- Rosenfeld H, Levavi-Sivan B, Gur G, Melamed P, Meiri I, Yaron Z, Elizur A (2001) Characterization of tilapia FSH β gene and analysis of its 5' flanking region. *Comp Biochem Physiol* 129: 389-398
- Ruf F, Fink MY, Sealfon SC (2003) Structure of the GnRH receptor-stimulated signaling network: insights from genomics. *Front Neuroendocrinol* 24: 181-199
- Saito D, Hasegawa Y, Urano A (2003) Gonadotropin-releasing hormones modulate electrical activity of vasotocin and isotocin neurons in the brain of rainbow trout. *Neurosci Lett* 351: 107-110
- Saligaut C, Linard B, Mananos EL, Kah O, Breton B, Govoroun M (1998) Release of pituitary gonadotropins GTH-I and GTH-II in the rainbow trout (*Oncorhynchus mykiss*): modulation by estradiol and catecholamines. *Gen Comp Endocrinol* 109: 302-309
- Sato A, Ueda H, Fukaya M, Kaeriyama M, Zohar Y, Urano A (1997) Sexual differences in homing profiles and shortening of homing duration by gonadotropin-releasing hormone

- analog implantation in lacustrine sockeye salmon (*Oncorhynchus nerka*) in Lake Shikotsu. Zool Sci 14: 1009-1014
- Slater CH, Schreck CB, Swanson P (1994) Plasma profiles of the sex steroids and gonadotropins in maturing female spring chinook salmon (*Oncorhynchus tshawytscha*). Comp Biochem Physiol 109A: 167-175
- Sohn YC, Suetake H, Yoshiura Y, Kobayashi M, Aida K (1998) Structural and expression analyses of gonadotropin I β subunit genes in goldfish (*Carassius auratus*). Gene 222: 257-267
- Sohn YC, Yoshiura Y, Suetake H, Kobayashi M, Aida K (1999) Nucleotide sequence of gonadotropin II β subunit gene in goldfish. Fisheries Sci 65: 800-801
- Sohn YG, Kobayashi M, Aida K (2001) Regulation of gonadotropin β subunit gene expression by testosterone and gonadotropin-releasing hormones in the goldfish, *Carassius auratus*. Comp Biochem Physiol 129: 419-426
- Stanislaus D, Pinter JH, Janovick JA, Conn PJ (1998) Mechanisms mediating multiple physiological responses to gonadotropin-releasing hormone. Mol Cell Endocrinol 144: 1-10
- Strahl BD, Huang HJ, Pedersen NR, Wu JC, Ghosh BR, Miller WL (1997) Two proximal activating protein-1-binding sites are sufficient to stimulate transcription of the ovine follicle-stimulating hormone- β gene. Endocrinology 138: 2621-2631
- Strahl BD, Huang HJ, Sebastian J, Ghosh BR, Miller WL (1998) Transcriptional activation of the ovine follicle-stimulating hormone β -subunit gene by gonadotropin-releasing hormone: involvement of two activating protein-1-binding sites and protein kinase C. Endocrinology 139: 4455-4465
- Sugawara T, Saito M, Fujimoto S (2000) Sp1 and SF-1 interact and cooperate in the regulation of human steroidogenic acute regulatory protein gene expression. Endocrinology 141: 2895-2903

- Sumpter JP, Scott AP (1989) Seasonal variations in plasma and pituitary levels of gonadotrophin in males and females of two strains of rainbow trout (*Salmo gairdneri*). Gen Comp Endocrinol 75: 376-388
- Suzuki K, Kanamori A, Kawauchi H, Nagahama Y (1988) Development of salmon GTH I and GTH II radioimmunoassays. Gen Comp Endocrinol 71: 459-467
- Suzuki K, Dong L, Hew CL (1995) A gene encoding chinook salmon (*Oncorhynchus schawytscha*) gonadotropin α subunit: gene structure and promoter analysis in primary pituitary cells. Mol Mar Biol Biotech 4: 10-19
- Swanson P, Bernard M, Nozaki M, Suzuki K, Kawauchi H, Dickhoff WW (1989) Gonadotropins I and II in juvenile coho salmon. Fish Physiol Biochem 7: 169-176
- Trinh KY, Wang NC, Hew CL, Crim LW (1986) Molecular cloning and sequencing of salmon gonadotropin β subunit. Eur J Biochem 159: 619-624
- Trudeau VL (1997) Neuroendocrine regulation of gonadotrophin II release and gonadal growth in the goldfish, *Carassius auratus*. Rev Reprod 2: 55-68
- Tsutsumi M, Zhou W, Millar RP, Mellon PL, Roberts JL, Flanagan CA, Dong K, Gillo B, Sealfon SC (1992) Cloning and functional expression of a mouse gonadotropin-releasing hormone receptor. Mol Endocrinol 6: 1163-1169
- Vickers ED, Laberge F, Adams BA, Hara TJ, Sherwood NM (2004) Cloning and localization of three forms of gonadotropin-releasing hormone, including the novel whitefish form, in a salmonid, *Coregonus clupeaformis*. Biol Reprod 70: 1136-1146
- Vischer HF, Teves AC, Ackermans JC, van Dijk W, Schulz RW, Bogerd J (2003) Cloning and spatiotemporal expression of the follicle-stimulating hormone beta subunit complementary DNA in the African catfish (*Clarias gariepinus*). Biol Reprod 68: 1324-1332
- Weil C, Marcuzzi O (1990) Cultured pituitary cell GtH response to GnRH at different stages of rainbow trout oogenesis and influence of steroid hormones. Gen Comp Endocrinol 79:

483-491

Xiong F, Hew CL (1991) Chinook salmon (*Oncorhynchus tshawytscha*) gonadotropin II β subunit gene encodes multiple messenger ribonucleic acids. *Can J Zool* 69: 2572-2578

Xiong F, Liu D, Elsholtz HP, Hew CL (1994a) The chinook salmon gonadotropin II β subunit gene contains a strong minimal promoter with a proximal negative element. *Mol Endocrinol* 8: 771-781

Xiong F, Liu D, Le Drean Y, Elsholtz HP, Hew CL (1994b) Differential recruitment of steroid response elements may dictate the expression of the pituitary gonadotropin II β subunit gene during salmon reproduction. *Mol Endocrinol* 8: 782-793

Yaron Z, Gur G, Melamed P, Rosenfeld H, Elizur A, Levavi-Sivan B (2003) Regulation of fish gonadotropins. *Int Rev Cytol* 225: 131-185

FIGURE LEGENDS

Fig. 1. Activation of sGnRH gene expression in the brain of female chum salmon during upstream migration. Fish were sampled in three points on the route of upstream migration to the hatchery in 1997: Atsuta, coastal area; Ebetsu, midway of the river; Chitose, the Chitose Salmon Hatchery. The contents of sGnRH-I and -II mRNAs were determined in ten different loci in the brain by real-time PCR assays (see detail in Onuma *et al.*, 2005). The data are represented by the circle size. OB, olfactory bulb; T, telencephalon; POA, preoptic area.

Fig. 2. Phylogenetic analysis of GnRH-R in vertebrates. A phylogenetic tree was generated by the neighbor-joining method using the partial amino acid sequences of GnRH-Rs, spanning from the extracellular N terminal domain to the third transmembrane domain. The *Drosophila* GnRH-R homolog was used as an outgroup.

Fig. 3. Transient increases in the binding activities of Egr1 and Sp1 in response to GnRH α in α T3-1 cells. Nuclear extracts of α T3-1 cells were prepared at different time after the treatment with 100nM GnRH α (des-Gly¹⁰, [D-Ala⁶]-GnRH ethylamide). The binding activities of Egr1, Ptx1, and Sp1 were examined by gel mobility shift assays using CE3 (a Ptx1 binding element in the rat preopiomelanocortin gene promoter, Lamonerie *et al.*, 1996) and rE/S (A binding site for Egr1 and Sp1 in the rat LH β gene promoter, -55 to -35) as probes. Asterisks denote significant difference with respect to the binding intensity at time 0 (* P<0.05, ** P<0.01, *** P<0.001).

Fig. 4. Binding of Sp1 to a GC-rich sequence (sSp1) in the proximal region of the chinook salmon LH β gene. (A) Gel shift analysis of the GC-rich element in the salmon LH β gene (-

55 to -35). Nuclear extracts of α T3-1 cells were prepared at 1 hr after the treatment with 100nM GnRHa (des-Gly¹⁰, [D-Ala⁶]-GnRH ethylamide). Gel shift analysis was performed using the sSp1 and the rE/S (see Fig. 3). A band corresponding to Sp1 but not Egr1 was shifted using the sSp1 as a probe. (B) Supershift experiment of the sSp1. The nuclear extract was pre-incubated with antiserum against Sp1 or Egr1. The intensity of the shifted band of sSp1 was decreased by anti-Sp1 antiserum.

Fig. 5. Proposed model of molecular mechanisms of cooperative regulation of salmon LH β gene by GnRH and E2. GnRH signals activate Sp1 to interact with Ptx1 and SF-1. Furthermore, E2 stimulates LH β gene transcription through pERE. ER synergizes with SF-1, facilitating the functional interaction between the GnRH and E2 signals to enhance LH β gene transcription.

Table 1. Effects of GnRH and E2 on release and synthesis of GTH in salmonids at three different reproductive stages*.

	Release			Synthesis		
	GnRH	GnRH & E2	E2	GnRH	GnRH & E2	E2
FSH						
Maturing	+	++	-	+	-	-
Pre-spawning	+	++	-	+	-	-
Spawning	-	-	-	-	-	-
LH						
Maturing	+	+	-	-	+++	++
Pre-spawning	+	++	+	-	++	++
Spawning	+++	+++	+	-	-	-

+++ strong stimulation, ++ moderate stimulation, + weak stimulation, - no effect

* It should be noted that the relative intensities of effects are reliable only within the same hormone and function.









