Original Article

Differential expression of two gonadotropin (GTH) \( \beta \) subunit genes during ovarian maturation induced by repeated injection of salmon GTH in the Japanese eel *Anguilla japonica*

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**ABSTRACT:** Repeated injection of salmon gonadotropin (sGTH) can induce ovarian maturation in the female Japanese eel *Anguilla japonica*. The participation of endogenous GTH in this process remains unclear. The steady-state mRNA levels of pituitary GTH I\( \beta \) and GTH II\( \beta \) subunits in individual fish was investigated using a real-time quantitative reverse transcription-polymerase chain reaction assay based on the TaqMan™ fluorogenic detection system. Pituitary GTH I\( \beta \) mRNA levels were high when the fish were immature, particularly before being injected with sGTH, but levels decreased with the progression of ovarian development and remained very low from the late vitellogenic stage until postovulation. In contrast, mRNA levels of GTH II\( \beta \) were very low until the previtellogenic stage after injection with sGTH, and increased markedly in accordance with ovarian maturation and ovulation. These results suggest that sGTH injection inhibits GTH I synthesis, whereas it induces ovarian maturation and stimulates GTH II synthesis. Thus, the differential expression of GTH I\( \beta \) and GTH II\( \beta \) genes during artificial maturation suggests that the two GTH are synthesized sequentially and have separate functional roles relating to reproductive events in the Japanese eel.

**KEY WORDS:** gonadotropin I\( \beta \), gonadotropin II\( \beta \), mRNA levels, Japanese eel, vitellogenesis.

INTRODUCTION

Female Japanese eels *Anguilla japonica* cultivated in freshwater ponds possess immature ovaries in which most developed oocytes are at the previtellogenic stage.1 Silver eels migrating in the river or coastal areas toward their spawning ground1 and eels reared for long-term periods in seawater ponds normally exhibit oocytes at the early vitellogenic stage,2 but under ordinary rearing conditions, the further development of oocytes is not observed in the aforementioned eel species. The fact that eels do not mature under captive conditions is considered to be related to a deficiency of pituitary gonadotropic function.3 Therefore, repeated gonadotropin treatment, such as the injection of salmon or carp pituitary extract, is required for the artificial induction of ovarian maturation. Along these lines, exogenous gonadotropin (GTH) can induce ovarian maturation in eels, but the role of endogenous GTH in ovarian maturation remains unclear.

Gonadotropin is a pituitary glycoprotein hormone that regulates development of the gonads in vertebrates. Teleosts possess two distinct GTH, GTH I and GTH II, which are considered to be homologous to follicle-stimulating hormone and luteinizing hormone in higher vertebrates, respectively. The function of each GTH has been elucidated in salmonid species, whereby GTH I mediates vitellogenesis and spermatogenesis, and GTH II regulates oocyte maturation and spermiation.4–8 However, most physiological studies on
Telost GTH have been conducted on GTH II, and the functioning of GTH I in fishes has not been fully elucidated.

Each GTH is a heterodimer composed of non-covalently linked α and β subunits. The α subunit is identical, whereas the β subunit is structurally distinct and confers specific hormonal functions. GTH Iβ and GTH IIβ subunit cDNA have been isolated and characterized in some teleost species including the Japanese eel. Recently, the steady-state mRNA levels of GTH β subunits were monitored during the female reproductive cycle in rainbow trout Oncorhynchus mykiss, coho salmon Oncorhynchus kisutch, gilthead sea bream Sparus aurata, goldfish Carassius auratus, striped bass Morone saxatilis, blue gourami Trichogaster trichopterus, and red sea bream Pagrus major. In salmonids, which show synchronous development of oocytes, GTH Iβ gene expression is predominant during the early stages of oogenesis, whereas GTH IIβ gene expression becomes elevated at the late stages of oogenesis. In contrast, in multiple spawners such as sea bream, goldfish, and gourami, which show asynchronous development of oocytes, the expression of both GTH β subunit genes increases with the progression of ovarian maturity and peaks during the spawning season.

In the Japanese eel, stimulation of GTH II synthesis can be induced by treatment with salmon pituitary homogenate, salmon GTH (sGTH), and sex steroids. A preliminary investigation we have done previously has revealed that the GTH Iβ gene is expressed mainly in immature fish, whereas the GTH IIβ gene is expressed predominantly in ovulated fish. However, there is little information on the detailed profile of GTH I synthesis in the Japanese eel during induced ovarian maturation. Therefore, in the present study, we monitored steady-state mRNA levels of GTH Iβ and compared them with those of GTH IIβ and plasma steroid hormone levels during ovarian maturation induced by repeated sGTH injections, as a first step in understanding the contribution of endogenous GTH to induced oogenesis in female Japanese eels.

MATERIALS AND METHODS

Animals

Pond-cultivated immature female Japanese eels (range, 557–1084 g bodyweight; n = 61) were kindly supplied by a local aquaculture farm. The eels were maintained in circulated freshwater tanks of 3.4 metric tonnes at 20°C, and were gradually acclimated to seawater in the same tank at 20°C. Three metric tonnes at 20°C, and were gradually acclimated to seawater in the same tank at 20°C. Thereafter, the eels received a weekly intramuscular injection of sGTH (2 mg/kg bodyweight) emulsified with lipophilized gelatin (LG). Salmon GTH was extracted from chum salmon pituitaries by the methods of Suzuki et al. Fish were sampled in freshwater and seawater, or weekly after 1–15 sGTH injections at various oocyte developmental stages. Following repeated sGTH treatment, fish that were confirmed to have oocytes at the migratory nucleus stage were injected with LG emulsion containing sGTH and 17,20β-dihydroxy-4-pregnene-3-one (2 mg/kg bodyweight, respectively). These fish were anesthetized with 2-phenoxyethanol (1200 p.p.m) and blood samples were taken from the caudal vessels with a heparinized syringe and needle. After decapitation, the pituitaries were dissected immediately, frozen in liquid nitrogen, and stored at –80°C until RNA extraction was performed.

RNA preparation and first-strand cDNA synthesis

Total RNA was isolated from individual pituitaries using RNA extraction solution (Isogen; Nippon Gene, Toyama, Japan) according to the manufacturer’s protocol, and the extracted total RNA was then treated with deoxyribonuclease I (DNase I; Promega, Madison, WI, USA). DNase I treatment was carried out in 20 μL of reaction mixture containing 5 μg of total RNA, DNase I buffer (Promega), 10 mM dithiothreitol (DTT), 10 U of ribonuclease inhibitor (Nippon Gene), and 10 U of DNase I. The mixture was then incubated at 37°C for 1 h. After treatment with DNase I, the mixture was incubated at 95°C for 10 min, and total RNA was obtained by phenol–chloroform extraction and ethanol precipitation, and resuspended in sterile distilled water. Total RNA concentrations were measured using a spectrophotometer (Hitachi, Tokyo, Japan). First-strand cDNA was synthesized from 1 μg of total RNA from the individual pituitary in reaction mixture containing 20 μL of 1× first-strand synthesis buffer (Gibco/BRL, Gaithersburg, MD, USA), 10 mM DTT, 500 nM dNTP 200 U of SuperscriptII reverse transcriptase (Gibco/BRL), 10 U of ribonuclease inhibitor, and 50 nM of specific primers for GTH Iβ (I-RT) or GTH IIβ (II-RT) (Table 1). The mixture was incubated at 42°C for 1 h and 51°C for 30 min.

Quantitative polymerase chain reaction

Quantitative polymerase chain reaction (PCR) for eel GTH β subunit cDNAs was performed using real
The linear range of the curves fell within 10^2–10^7 copies of GTH I using six concentrations, respectively. \( b \) ranged from 10^2 to 10^7 copies of GTH I were made to produce standard curves that were quantified by measuring the threshold cycle value. This threshold cycle occurs at the most linear portion of the logarithmic phase of the PCR curve and allows for the creation of standard curves based on threshold cycle and starting concentration. Serial dilutions of plasmid DNA were produced triplicate PCR assessments. Primers and probes were chosen with the assistance of the computer program PrimerExpress (Applied Biosystems). Polymerase chain reaction was conducting using TaqMan™ technology and analysed with a Model 7700 Sequence Detector (Applied Biosystems). GTH \( \beta \) subunit cDNA were quantified by measuring the threshold cycle value. This threshold cycle occurs at the most linear portion of the logarithmic phase of the PCR curve and allows for the creation of standard curves based on threshold cycle and starting concentration. Serial dilutions of plasmid DNA were made to produce standard curves that ranged from 10^2 to 10^7 copies of GTH I \( \beta \) and GTH II \( \beta \) cDNA using six concentrations, respectively. The linear range of the curves fell within 10^2–10^7 copies and their correlation coefficients were more than 0.998 for all curves. Reproducibility of the quantitative measurements was evaluated by conducting triplicate PCR assessments. Primers and probes were chosen with the assistance of the computer program PrimerExpress (Applied Biosystems). Polymerase chain reaction was performed in 25\( \mu \)L of reaction mixture containing 1× TaqMan™ buffer (Applied Biosystems); 5 mM MgCl2; 200 \( \mu \)M each of dATP, dCTP, and dGTP; 400 \( \mu \)M of dUTP; 300 nM of each primer; 150 nM of TaqMan probe and 0.625 U of AmpliTaq Gold DNA polymerase (Applied Biosystems); and a 1/40 aliquot of first-strand cDNA solution synthesized from 25 ng of total RNA. The amplification procedure consisted of 94°C for 10 min followed by 35 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s. Table 1 shows the oligonucleotide sequences for the primers and TaqMan™ probes used in GTH I \( \beta \) (a primer pair, IF and IR; probe, I-TaqMan) and GTH II \( \beta \) (a primer pair, IIIF and IIR; probe, II-TaqMan).

<table>
<thead>
<tr>
<th>Primer and probe</th>
<th>Oligonucleotide sequence</th>
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<tbody>
<tr>
<td>I-RT</td>
<td>5’-AACAGCCAGCCAGC-3’</td>
</tr>
<tr>
<td>IF</td>
<td>5’-TCTGCACAACTTTCATCTC-3’</td>
</tr>
<tr>
<td>IR</td>
<td>5’-AGGCGGGTGTTGTAAGGT-3’</td>
</tr>
<tr>
<td>I-TaqMan</td>
<td>5’-TGGAGAATGAAATCAGGCTGT-3’</td>
</tr>
<tr>
<td>II-RT</td>
<td>5’-TGGGCTCAGACTCTG-3’</td>
</tr>
<tr>
<td>IIIF</td>
<td>5’-GGTCACGCATCAAGGA-3’</td>
</tr>
<tr>
<td>IIR</td>
<td>5’-CACAGCGCTGGTACACC-3’</td>
</tr>
<tr>
<td>II-TaqMan</td>
<td>5’-CCAAGCTACAAGAAGCCCGCTGAT-3’</td>
</tr>
</tbody>
</table>

time TaqMan™ technology and analysed with a Model 7700 Sequence Detector (Applied Biosystems, Foster City, CA, USA). GTH \( \beta \) subunit cDNA were quantified by measuring the threshold cycle value. This threshold cycle occurs at the most linear portion of the logarithmic phase of the PCR curve and allows for the creation of standard curves based on threshold cycle and starting concentration. Serial dilutions of plasmid DNA were made to produce standard curves that ranged from 10^2 to 10^7 copies of GTH I \( \beta \) and GTH II \( \beta \) cDNA using six concentrations, respectively. The linear range of the curves fell within 10^2–10^7 copies and their correlation coefficients were more than 0.998 for all curves. Reproducibility of the quantitative measurements was evaluated by conducting triplicate PCR assessments. Primers and probes were chosen with the assistance of the computer program PrimerExpress (Applied Biosystems). Polymerase chain reaction was performed in 25\( \mu \)L of reaction mixture containing 1× TaqMan™ buffer (Applied Biosystems); 5 mM MgCl2; 200 \( \mu \)M each of dATP, dCTP, and dGTP; 400 \( \mu \)M of dUTP; 300 nM of each primer; 150 nM of TaqMan probe and 0.625 U of AmpliTaq Gold DNA polymerase (Applied Biosystems); and a 1/40 aliquot of first-strand cDNA solution synthesized from 25 ng of total RNA. The amplification procedure consisted of 94°C for 10 min followed by 35 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s. Table 1 shows the oligonucleotide sequences for the primers and TaqMan™ probes used in GTH I \( \beta \) (a primer pair, IF and IR; probe, I-TaqMan) and GTH II \( \beta \) (a primer pair, IIIF and IIR; probe, II-TaqMan).

The intra-assay and the interassay coefficients of variation were 5.7% and 9.4% in the quantification of GTH I \( \beta \) mRNA levels and those were 3.7% and 2.9% in the quantification of GTH II \( \beta \) mRNA levels. Scheffe’s multiple comparisons test was used for statistical analysis. Data are presented as the mean ± standard error (SEM). Significance was set at \( P<0.05 \).

Radioimmunoassay

Testosterone (T) and estradiol-17\( \beta \) (E2) levels in the plasma of Japanese eel were measured using radioimmunoassay according to the methods of Aida et al. The intra-assay and the interassay coefficients of variation were 5.7% and 9.4% in the quantification of GTH II mRNA levels. The intra-assay and the interassay coefficients of variation were 5.7% and 9.4% in the quantification of GTH II mRNA levels. Scheffe’s multiple comparisons test was used for statistical analysis. Significance was set at \( P<0.05 \).

Histological procedures

Ovarian tissues were fixed with 10% neutral formalin, embedded in paraffin, sectioned to 8 \( \mu \)m thicknesses, and stained with hematoxylin–eosin for histological identification of the oocytes’ developmental stages.

RESULTS

Changes in gonadosomatic index during induced ovarian maturation

Experimental fish could be categorized into the following seven stages according to histological observation, gonadosomatic index (GSI), and rearing conditions: (i) previtellogenic stage in freshwater control fish before sGTH injection, GSI 0.98 ± 0.10 (\( n=5 \)); (ii) previtellogenic stage in seawater-acclimated fish before sGTH injection, GSI 0.95 ± 0.04 (\( n=5 \)); (iii) previtellogenic stage after sGTH injections, GSI 1.54 ± 0.23 (\( n=4 \)); (iv) early vitellogenic stage, GSI 5.35 ± 0.47 (\( n=22 \)); (v) late vitellogenic stage, GSI 14.91 ± 0.82 (\( n=9 \)); (vi) migratory nucleus stage, GSI 35.35 ± 1.12 (\( n=4 \)); and (vii) ovulated, GSI 42.86 ± 1.46 (\( n=12 \)) (Fig. 1).

Changes in GTH I \( \beta \) and GTH II \( \beta \) mRNA levels during induced ovarian maturation

Total RNA was extracted from individual pituitaries and results were expressed as the number of copies per 25 ng total RNA. Steady-state mRNA levels of pituitary GTH I \( \beta \) were high at the early stages of gonadal development, particularly before the injection of sGTH. They reached peak levels in seawater-acclimated fish after sGTH injection, but decreased with ovarian development and remained very low from the late vitellogenic stage to postovulation (Fig. 2a,3a). In contrast, GTH II \( \beta \)
mRNA levels were very low until the previtellogenic stage, increased at the early vitellogenic stage, and became significantly elevated at the late vitellogenic stage. High levels were maintained at the migratory nucleus stage and after ovulation (Figs 2b,3b).

The number of copies of GTH β subunit mRNA per pituitary are shown in Table 2. The presence of GTH Iβ subunit mRNA was dominant during the previtellogenic stages compared with GTH IIβ subunit mRNA. From the vitellogenic stage to postovulation, the GTH II β subunit mRNA became dominant compared with the GTH I β subunit mRNA. Of note, these results were normalized based on contents of total RNA in the pituitary according to the methods of Bustin.29

**Fig. 1** Changes in gonadosomatic index (GSI) during induced ovarian maturation in Japanese eel. F, Previtellogenic fish maintained in freshwater before salmon gonadotropin (sGTH) injection; S, previtellogenic fish acclimated in seawater before sGTH injection; PV, previtellogenic fish after sGTH injection; EV, early vitellogenic fish after sGTH injection; LV, late vitellogenic fish; MN, fish at the migratory nucleus stage; OV, ovulated fish. Values represent the mean ± SEM. Statistically significant differences between groups are indicated by differing letters.

**Table 2** Changes in number of copies of gonadotropin (GTH) β subunit mRNA per pituitary

<table>
<thead>
<tr>
<th>Group</th>
<th>No. fish used</th>
<th>sGTH injection</th>
<th>Rearing water</th>
<th>GTH Iβ mRNA copy numbers/pit.</th>
<th>GTH IIβ mRNA copy numbers/pit.</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>5</td>
<td>Before</td>
<td>FW</td>
<td>1.05 ± 0.35 × 10^6</td>
<td>9.58 ± 3.53 × 10^6</td>
</tr>
<tr>
<td>S</td>
<td>5</td>
<td>Before</td>
<td>SW</td>
<td>1.15 ± 0.25 × 10^6</td>
<td>3.20 ± 1.80 × 10^6</td>
</tr>
<tr>
<td>PV</td>
<td>4</td>
<td>After</td>
<td>SW</td>
<td>7.83 ± 1.03 × 10^7</td>
<td>4.05 ± 2.54 × 10^6</td>
</tr>
<tr>
<td>EV</td>
<td>22</td>
<td>After</td>
<td>SW</td>
<td>6.34 ± 0.97 × 10^7</td>
<td>3.91 ± 1.32 × 10^6</td>
</tr>
<tr>
<td>LV</td>
<td>9</td>
<td>After</td>
<td>SW</td>
<td>1.52 ± 0.66 × 10^7</td>
<td>2.32 ± 3.17 × 10^6</td>
</tr>
<tr>
<td>MN</td>
<td>4</td>
<td>After</td>
<td>SW</td>
<td>1.69 ± 1.28 × 10^6</td>
<td>3.14 ± 0.81 × 10^9</td>
</tr>
<tr>
<td>OV</td>
<td>12</td>
<td>After</td>
<td>SW</td>
<td>5.93 ± 0.95 × 10^5</td>
<td>2.42 ± 0.30 × 10^9</td>
</tr>
</tbody>
</table>

F, Previtellogenic fish maintained in freshwater (FW) before salmon gonadotropin (sGTH) injection; S, previtellogenic fish acclimated in seawater (SW) before sGTH injection; PV, previtellogenic fish after sGTH injection; EV, early vitellogenic fish after sGTH injection; LV, late vitellogenic fish; MN, fish at the migratory nucleus stage; OV, ovulated fish.
Changes in plasma sex steroid levels during induced ovarian maturation

Plasma profiles of T and E2 during ovarian maturation induced by sGTH injection are shown in Fig. 4. Plasma T levels were low before sGTH injection and increased after sGTH injection. Plasma T levels further increased at the migratory nucleus stage and high levels were maintained following ovulation (Fig. 4a). Plasma E2 levels remained low until the late vitellogenic stage, although a tendency of gradual increase was observed. Thereafter, plasma E2 levels increased markedly at the migratory nucleus stage and after ovulation (Fig. 4b).

Relationship between gonadotropin β subunit mRNA levels and plasma sex steroid levels

The relationship between GTH β subunit mRNA levels and plasma sex steroid levels are shown in Figs 5 and 6. When levels of plasma T (>3 ng/mL) and E2 (>1 ng/mL) were high, GTH Iβ gene expression was suppressed, whereas GTH IIβ gene expression was stimulated (Figs 5,6). These high levels of T and E2 were observed particularly at the migratory nucleus stage and during postovulation. During vitellogenesis, levels of plasma sex steroids were lower (T < 3 ng/mL; E2 < 1 ng/mL) and showed little change when examined 1 week after the sGTH injection, whereas GTH β mRNA levels started to
show remarkable changes; that is, increases in GTH Iβ mRNA levels and decreases in GTH IIβ mRNA levels.

**DISCUSSION**

A real-time quantitative RT-PCR assay based on the TaqMan™ fluorogenic detection system was established to quantify GTH β subunit mRNA levels. Detectable ranges were very wide (10^2–10^7 copies) and the intra-assay and the interassay coefficient of variations were low. These results indicate that this quantitative RT-PCR system is sensitive and precise. In the present study, we investigated the changes in steady-state mRNA levels of GTH Iβ and IIβ subunits in the pituitary of individual Japanese eels during artificial maturation using this real-time quantitative RT-PCR assay in order to understand the effect of endogenous GTH on the induction of ovarian development.

In a previous report, using northern blot analysis we succeeded in cloning GTH Iβ cDNA and revealed the presence of GTH Iβ mRNA, but the data obtained was limited to immature and ovulated fish. The present study is the first to investi-
igate the levels of GTH Iβ mRNA in Japanese eel during ovarian maturation induced by sGTH injection. Levels of GTH Iβ mRNA were high in immature fish in freshwater and immature fish acclimated to seawater before being injected with sGTH. After sGTH injections, GTH Iβ mRNA levels decreased following artificial ovarian development and reached a minimum level after ovulation. In salmonids, plasma GTH I levels are high during the vitellogenic stages, whereby GTH I is involved in vitellogenesis and the uptake of vitellogenin.4–8 In the common Japanese conger Conger myriaster, GTH Iβ mRNA levels increase from the previtellogenic stage to the early vitellogenic stage and start to decrease at the mid-vitellogenic stage,9 suggesting that, similar to salmonid fishes, GTH I synthesis in anguilliformes also increases up to the early vitellogenic stage. However, in the Japanese eel, levels of GTH Iβ started to decrease with the commencement of sGTH injection, and nearly ceased at the migratory nucleus stage. Just after the injection of sGTH, acute increases in plasma sex steroids were observed in previtellogenic and vitellogenic Japanese eels within a day, although plasma sex steroid levels returned to their initial levels after 1 week of sGTH injection.10 Temporary high levels of plasma sex steroids induced by sGTH injection were considered to inhibit the expression of GTH Iβ gene at the early vitellogenic stage. In juvenile goldfish, it is known that treatment with T or E2 exerts an inhibitory effect on GTH Iβ gene expression.11 Recently, an in vitro study has demonstrated that sex steroid treatment (T, E2, and 11-ketotestosterone) suppresses GTH Iβ subunit mRNA levels in cultured eel pituitary.12 Conversely, plasma sex steroid levels at the migratory nucleus and postovulation stages were still high after 1 week of sGTH injection, and GTH Iβ subunit mRNA levels were very low. It could be concluded that sustained high plasma sex steroid levels strongly inhibit GTH Iβ gene expression during the later stages of oogenesis in eels.

In the present study, the highest levels of GTH Iβ mRNA were observed in seawater-acclimated fish without hormone treatment. Higher levels of plasma E2 were observed in seawater-acclimated fish compared with fish kept in freshwater. In the New Zealand short-finned eel, levels of plasma E2 in migratory fish with early vitellogenic oocytes were somewhat higher than those in non-migratory fish.13 Kagawa et al. have reported that rearing the Japanese eel in seawater induces vitellogenesis in cultured immature females.5 It is considered that certain physiological factors are induced by seawater acclimation which stimulate GTH Iβ gene expression in the eel and that, subsequently, GTH I induces vitellogenesis via the actions of E2 and other factors. This hypothesis would explain the mechanisms for induction of vitellogenesis in eels living in natural seawater conditions.

In the present study, a wide range in the levels of GTH Iβ mRNA before sGTH injection was observed (Fig. 3). This may be due to individual differences in stress sensitivity, because sensitivity to stress by the eel gonadotropin-releasing hormone (GnRH) system has been observed in a preliminary study we have conducted.14 Further studies are required to understand the relationship between the GnRH system and stress.

In the present investigation, GTH I/β mRNA levels increased markedly in accordance with ovarian maturation and ovulation. Gonadotropin treatment is known to stimulate the production of the endogenous sex steroids, androgens and estrogens, in female eels.11,26 In the Japanese and the European eel, an increase in GTH I/β gene expression is considered to be the result of the positive feedback effects of T and E2.11,22,36,37 Nagae et al. have reported that repeated injections of salmon pituitary homogenate in female Japanese eels resulted in an abnormally high level of GTH Iβ transcription.11 In the present study, very high levels of GTH I/β gene expression were also observed, particularly during the later phases of ovarian maturation when plasma sex steroid levels showed rapid increases. It is considered that the increase in plasma sex steroids by sGTH injection induces the overexpression of the endogenous GTH Iβ gene.

Thus, the differential expression of GTH Iβ and GTH I/β genes during artificial maturation in artificially matured eels suggests that the two GTH genes are also expressed differentially and have separate functional roles in their reproductive events in the wild Japanese eel. Previous studies,10–12 the present study, and our unpublished results of western blot analysis of eel pituitary also suggest that the synthesis of the GTH I molecule in female Japanese eel is suppressed during ovarian development induced by repeated injection of sGTH, whereas that of GTH II molecule was activated. However, it is unknown whether eel GTH I and GTH II are secreted from the pituitary during induced ovarian development, because a specific radioimmunoassay for plasma GTH I in the Japanese eel has not yet been established.

In the present study, the Japanese eel showed a sequential pattern for GTH Iβ and GTH I/β gene expression during artificial maturation. This pattern is fundamentally similar to that found in salmonids,8,13,14 which are annual spawners and possess an ovary which shows synchronous oocyte development. This sequential pattern was also
observed until the mid-vitellogenic stage in the common Japanese conger. However, we observed that GTH Iβ gene expression started to decrease earlier in Japanese eel compared with salmonids and Japanese common conger, probably as a result of the sGTH injection. In salmonids, GTH Iβ mRNA levels increased during vitellogenesis, whereas that of GTH Iiβ predominated during the periovulatory period. Therefore, it is now considered that GTH I mediates vitellogenesis, whereas GTH II regulates final oocyte maturation in salmonids. 4–8 In anguilliformes, GTH I was involved during the early stages of ovarian development, and GTH II during the latter stages. Conversely, high mRNA levels for both GTH Iβ and GTH Iiβ have been demonstrated during gonadal maturation and spawning in fishes such as sea bream,17,21 goldfish,38 and blue gourami,20 demonstrating asynchronous development of oocytes throughout their spawning season. Artificially matured Japanese eels show asynchronous development of oocytes. It is possible that wild Japanese eel exhibit a similar GTH profile as that of the common Japanese conger and that oocyte development in wild eels differs from that in female eels induced to mature via hormone treatment. It is necessary to elucidate further the profiles of GTH synthesis and oocyte development throughout the reproduction stages of wild Japanese eel.

For artificial maturation in the Japanese eel, injection of sGTH is not sufficient because sGTH is composed mainly of salmon GTH II. GTH I and GTH II probably have distinct physiological functions in the gonadal development of Japanese eel, and both GTH types are considered to be essential for the induction of ovarian maturation and for obtaining higher quality eggs. To improve artificial maturation methods, we need to understand the control mechanisms of gonadal development via endogenous GTH synthesis in wild Japanese eel and to reproduce such conditions in an artificial environment. Further understanding of the reproductive physiology of the Japanese eel will lead to the establishment of a seed production technique for eel culture.

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