



Molecular function of gonadotrophins and their receptors in the ovarian development of turbot (*Scophthalmus maximus*)

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ABSTRACT

Gonadotropins (GtHs) and their receptors (follicle-stimulating hormone receptor, FSHR; luteinizing hormone receptor, LHR) are involved in the regulation of gametogenesis and play important roles during the reproductive cycles in vertebrate species, including fish. This minireview focuses on the molecular characterization and quantification of GtHs (common glycoprotein α subunit CG α , FSH β , and LH β) and their receptors (FSHR and LHR) throughout the reproductive cycle of female turbot *Scophthalmus maximus*. Information about GtHs, FSHR, LHR as well as other ligand-receptors interaction from different teleosts are also included in this review for the implications they may have on the functions of GtHs, FSHR and LHR in the reproductive development of turbot. These findings may enhance our understanding of the physiological roles of the GtHs, FSHR and LHR in controlling of flatfish ovarian development during the reproductive cycle and contributing to the improvement of management strategies for turbot in captivity.

1. Introduction

Turbot (*Scophthalmus maximus*) is an economically relevant flatfish species belonging to the genus *Scophthalmidae* (order Pleuronectiformes) and naturally distributed from Norway to the Mediterranean and the Black Sea (Nielsen, 1986, Daniels and Watanabe, 2001). Turbot production was first conducted in the United Kingdom (UK) during the 1970s. Since then, turbot aquaculture has quickly expanded to Spain and France because of its enormous economic market value. Owing to the technical and biological improvements that considerably increased the rearing capacities of turbot in captivity, turbot production was initiated in other European countries, including Portugal, Denmark, Germany, Iceland, Ireland, Italy, Norway, and Wales. Turbot was first introduced to China from the UK in 1992, and the first batch of turbot larvae was produced in 1995 by Lei Jilin and his colleagues (Lei and Liu, 2010). Over the past decade, the annual production of turbot in China has been maintained at approximately 50,000–60,000 tons accounting for approximately 80% of the world's total output of aquacultured turbot (FAO, 2013). Turbot culture has become the largest marine industry in the north coast of China and prompted the development of other marine fish culture.

Production of sufficient viable eggs with high survival rates is a key

factor of intensive production in aquaculture. Turbot is oviparous and produces yolks containing eggs. Mature female turbot spawn multiple times and group-asynchronous development of multiple batches of oocytes has been observed during the reproductive season. However, the natural breeding behavior of turbot and its spontaneous spawning is lost in captivity. Thus, turbot eggs must be hand-stripped from ripe fish and artificially fertilized. Each female can spawn 8–12 times at 3–5 days intervals throughout the spawning season because of their 70–90 h ovulatory cycles (Mugnier et al., 2000). In teleosts, the major regulators during vitellogenesis and oocyte maturation are the pituitary gonadotropins and sex steroids (Mylonas et al., 2010), which exert control via specific receptors (follicle-stimulating hormone receptor, FSHR; luteinizing hormone receptor, LHR). Thus, the hormonal manipulations of the reproductive function in economically reared fish mainly focus on the use of either exogenous luteinizing hormone (LH) preparations that act directly at the level of the gonad or synthetic agonists of gonadotropin-releasing hormone (GnRH α) that acts on the pituitary to induce the release of endogenous LH, which in turn acts on the gonad to induce steroidogenesis and oocyte maturation. However, knowledge on the molecular mechanisms regulating the group-asynchronous development of multiple batches of oocytes in turbot and information about the functional characterization of GtHs and their receptors during the

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reproductive cycle are limited. Therefore, this minireview focuses on the molecular characterization and quantification of GtHs (common glycoprotein α subunit CG α , FSH β , and LH β) and their receptors (FSHR, and LHR) during the ovarian development of turbot.

2. Gonadotrophins

The first gonadotropic hormone of fish was identified from the common carp pituitary in the early 1970s (Burzawa-Gerard, 1971). The duality of the gonadotropic activity was later identified from chum salmon pituitary extracts (Kawauchi et al., 1986) and two bioactive gonadotropic hormones, named GtH1 and GtH2 were purified subsequently in different fish species (Suzuki et al., 1988; Swanson, 1991). Fish gonadotropins are structurally heterodimeric glycoproteins formed by CG α that is noncovalently linked to the hormone-specific β subunit (FSH β or LH β), which determines the biological activity and specificity of the hormone. Analysis of the nucleotide and deduced amino acid sequences of the turbot FSH β (KP658394.1), CG α (MK234904) and LH β (MK290836) revealed that these three genes are highly homologous to fish species and has the typical structural features of heterodimeric glycoproteins. The basic structures and characteristics of turbot CG α , FSH β and LH β are similar to fish and mammalian species, and significantly homologous (90%) to that of the Atlantic halibut (*H. hippoglossus*) (Supplemental data, S1).

Several studies already identified that GtHs are mainly located in pituitary cells and are involved in the regulation of folliculogenesis, ovulation, spermatogenesis and steroidogenesis (Plansa et al., 2000; Yaron et al., 2003). However, GtHs have also been observed in extra-gonadal tissues (including the brain, kidney, ovary, and liver), and the putative roles in these tissues are likely related to the binding of their receptors (So et al., 2005). As expected, the highest mRNA levels of *cg α* , *lh β* and *fs β* were found in the pituitary, with less transcript being observed in other tissues (Supplemental data, S2). Additionally, both *lh β* and *fs β* manifested different expression manners during ovarian development, the highest values being observed at the migratory nucleus stage and late vitellogenic stage, respectively (Supplemental data, S3). These findings are in agreement with similar reports in mammals and other fish species (Parhar et al., 2003; Levavi-Sivan et al., 2010).

2.1. Gonadotrophin receptors

Fish gonadotrophin receptors (GtHRs) were first observed from binding studies via mammalian gonadotropin (human chorionic gonadotrophin, hCG) and purified hypophysial glycoproteins in the early 1970s (Breton et al., 1973). Purification of salmon gonadotropin led to the discovery of two distinct receptors in the early 1992s (Yan et al., 1992; Miwa et al., 1994). The presence of two distinct receptors (FSHR, and LHR) in a single fish species were confirmed by the molecular cloning of two different cDNAs in several fish species (Levavi-Sivan et al., 2010). Meanwhile, two *lhr* genes are present in eel and orange-spotted grouper (Maugars et al 2015; Peng et al., 2018). Turbot has two distinct receptors FSHR and LHR. These two receptors were highly homologous to known vertebrate sequences and have the typical structural features of glycoprotein receptors (Jia et al., 2014; Jia et al., 2016). Briefly, a relatively long extracellular domain followed by seven transmembrane (TM) helices and a carboxy-terminal intracellular tail. In the extracellular domains of turbot FSHR and LHR, the nine-imperfect leucine-rich repeats were observed and thought to be as the ligand-binding domain. The extracellular loops between TM II-III and TM IV-V contained cysteine residues that were assumed to link the extracellular loops via a disulfide bridge. Potential N-linked glycosylation sites and phosphorylation sites were identified in the N-terminal and C-terminal regions of turbot FSHR and LHR, respectively. These structural features of turbot FSHR and LHR are crucial determinants of ligand binding affinity and signal transduction. Phylogenetic analysis revealed that turbot FSHR and LHR are significantly homologous to the

Atlantic halibut (*H. hippoglossus*) FSHR and LHR.

FSHR and LHR are highly expressed in the ovaries and less expressed in other tissues of turbot similar to those of in mammals and other fish species (Levavi-Sivan et al., 2010). However, the extra-gonadal expression of FSHR and LHR in turbot is different from that observed in other fish species (Vischer and bogerd, 2003; Maugars and Schmitz, 2006; Rocha et al., 2007; Kobayashi et al., 2008). The difference may be species-specific and should be investigated via *in vivo* experiments to explore their functionalities. Histological observations of the turbot ovary indicated that oocyte maturation includes both vitellogenesis and ovulation stages (Jia et al., 2014). The mRNA levels of *fs β* and *lhr* were found to increase from the previtellogenic to the migratory nucleus stages, with the highest values being observed at the late vitellogenic stage and migratory nucleus stage, respectively. Studies on two multiple spawners Nile tilapia and zebrafish demonstrated that FSHR is predominantly associated with vitellogenesis and LHR plays a key role in final oocyte maturation and ovulation (Hirai et al., 2002; Kwok et al., 2005). Similar results were also observed in European sea bass (Rocha et al., 2009). These results are in agreement with the timeline observed in turbot. Furthermore, turbot hepatic vitellogenin (*v β*) expression showed the similar trend like *fs β* during the reproductive cycle in our recent study (Hu et al., 2018). Overall, FSHR seems to stimulate vitellogenesis and oocyte maturation and LHR promotes ovulation, which indicates that both are involved in mediating the ovarian development of turbot during reproductive cycle.

3. Interactions between gonadotropin and gonadotropin receptors

In mammals, gonadotropin hormone/receptor interactions are specific, FSHR and LHR bind to their respective ligands specifically and show minimal cross-activation (0.01–0.1%) with no cross-stimulation occurs under physiological conditions. Strict ligand selectivity of GtHRs was reported in some fish species such as sea bass (Molés et al., 2011), and mummichog (Ohkubo et al., 2013). However, promiscuous activation of fish GtHRs was described in salmonids and other fish species (Oba et al., 1999; Vischer and Bogerd, 2003; Kwok et al., 2005; Levavi-Sivan et al., 2010). We used a mammalian gonadotropin to detect turbot GtHR selectivity because of the lack of a specific homologous gonadotropin for turbot. Functional analysis with HEK293T cells continually expressing FSHR demonstrated that FSHR is specifically stimulated by ovine FSH, but not ovine LH (Jia et al., 2016). By contrast, turbot LHR was activated by ovine FSH and ovine LH. Similar results were observed in zebrafish and amago salmon (Kwok et al., 2005; Oba et al., 1999). The difference in ligand selectivity suggests that the action of GtHRs in teleosts does not overlap fully with that of their mammalian counterparts and underscores the need for further investigation.

4. Conclusions and perspectives

The structural characterization of turbot CG α , FSH β , LH β , FSHR and LHR provides valuable information about the conservation of glycoprotein hormones and their receptors among vertebrates. The distributions and expression profiles of these genes confirm their involvement in the regulation of ovarian development and their different roles throughout the reproductive cycle of turbot. Previous studies suggested that the ligand selectivity/promiscuity observed in other teleosts may be both present in turbot, but the specific turbot gonadotropins that can be for properly testing this hypothesis are current unavailable. Therefore, additional experiments are needed to i) obtain recombinant turbot gonadotropins (FSH β , LH β) and identify ligand specificity of LHR and FSHR; ii) improve the efficacy of the recombinant gonadotropin and build standard enzyme linked immunosorbent assay method for the broodstock management of turbot, and iii) investigate the interaction of GtHs/GtHRs with other neuroendocrine hormones and local endocrine factors via gene editing tools (CRISPR/Cas9 or TALENs) during gonadal development. Future

studies may provide further insights into the roles of GtHs and GtHRs in the regulation of reproductive development in turbot.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ygcen.2019.02.014>.

References

- Breton, B., Billard, R., Jalabert, B., 1973. Spécificité d'action et relations immunologiques des hormones gonadotropes de quelques téléostéens. *Ann. Biol. Anim. Biochem. Biophys.* 13, 347–362.
- Burzawa-Gerard, E., 1971. Purification d'une hormone gonadotrope hypophysaire de poisson téléostéen la carpe (*Cyprinus carpio* L.). *Biochimie* 53, 545–552.
- Daniels, H.V., Watanabe, W.O., 2001. *Practical Flatfish Culture and Stock Enhancement*. Wiley-Blackwell, Oxford, UK.
- FAO, 2013. *Statistical Yearbook 2013. World Food and Agriculture*.
- Hirai, T., Oba, Y., Nagahama, Y., 2002. Fish gonadotropin receptors: molecular characterization and expression during gametogenesis. *Fish. Sci. Suppl.* 68, 675–678.
- Hu, P., Meng, Z., Jia, Y.D., 2018. Molecular characterization and quantification of estrogen receptors in turbot (*Scophthalmus maximus*). *Gen. Comp. Endocrinol.* 257, 38–49.
- Jia, Y.D., Meng, Z., Niu, H.X., Hu, P., Lei, J.L., 2014. Molecular cloning, characterization, and expression analysis of luteinizing hormone receptor gene in turbot (*Scophthalmus maximus*). *Fish. Physiol. Biochem.* 40, 1639–1650.
- Jia, Y.D., Sun, A., Meng, Z., Liu, B.L., Lei, J.L., 2016. Molecular characterization and quantification of the follicle stimulating hormone receptor in turbot (*Scophthalmus maximus*). *Fish. Physiol. Biochem.* 42, 179–191.
- Kawauchi, H., Suzuki, K., Nagahama, Y., Adachi, N., Naito, N., Nakai, Y., Yoshimura, F., Gorbman, A., 1986. Occurrence of two distinct gonadotropins in chum salmon pituitary. In: *Pars Distalis of the Pituitary Gland: Structure Function and Regulation*. Elsevier, Amsterdam, pp. 383–390.
- Kobayashi, T., Pakarinen, P., Torgersen, J., Huhtaniemi, I., Andersen, Ø., 2008. The gonadotropin receptors FSH-R and LH-R of Atlantic halibut (*Hippoglossus hippoglossus*)-2. Differential follicle expression and asynchronous oogenesis. *Gen. Comp. Endocrinol.* 156, 595–602.
- Kwok, H.F., So, W.K., Wang, Y., Ge, W., 2005. Zebrafish gonadotropins and their receptors: I. Cloning and characterization of zebrafish follicle-stimulating hormone and luteinizing hormone receptors-evidence for their distinct functions in follicle development. *Biol. Reprod.* 72, 1370–1381.
- Lei, J., Liu, X., 2010. Chapter 11 Culture of turbot: Chinese perspective. In: Daniels, H.V., Watanabe, W.O. (Eds.), *Practical Flatfish Culture and Stock Enhancement*. Blackwell Publishing, Ames, pp. 185–202.
- Levavi-Sivan, B., Bogerd, J., Mañanós, E.L., Gómez, A., Lareyre, J.J., 2010. Perspectives on fish gonadotropins and their receptors. *Gen. Comp. Endocrinol.* 165, 412–437.
- Maugars, G., Schmitz, M., 2006. Molecular cloning and characterization of FSH and LH receptors in Atlantic salmon (*Salmo salar* L.). *Gen. Comp. Endocrinol.* 149, 108–117.
- Maugars, G., Dufour, S., Vaudry, H., 2015. Demonstration of the coexistence of duplicated LH receptors in teleosts, and their origin in ancestral actinopterygians. *PLoS One* 10, 1–29.
- Miwa, S., Yan, L.G., Swanson, P., 1994. Localization of two gonadotropin receptors in the salmon gonad by in vitro ligand autoradiography. *Biol. Reprod.* 50, 629–642.
- Molás, G., Gómez, A., Carrillo, M., Rocha, A., Mylonas, C.C., Zanuy, S., 2011. Determination of Fish quantity and bioactivity during sex differentiation and oogenesis in European sea bass. *Biol. Reprod.* 85, 848–857.
- Mugnier, C., Guennoc, M., Lebegue, E., Fostier, A., Breton, B., 2000. Induction and synchronisation of spawning in cultivated turbot (*Scophthalmus maximus* L.) broodstock by implantation of a sustained-release GnRH-a pellet. *Aquaculture* 181, 241–255.
- Mylonas, C.C., Fostier, A., Zanuy, S., 2010. Broodstock management and hormonal manipulations of fish reproduction. *Gen. Comp. Endocrinol.* 165, 516–534.
- Nielsen, J.G., 1986. *Scophthalmidae*. In: Whitehead, P.J.P., Bauchot, M.L., Hureau, J.C. (Eds.), *Fishes of the North-eastern Atlantic and the Mediterranean*.
- Oba, Y., Hirai, T., Yoshiura, Y., Yoshikuni, M., Kawauchi, H., Nagahama, Y., 1999. Cloning, functional characterization, and expression of a gonadotropin receptor cDNA in the ovary and testis of Amago Salmon (*Oncorhynchus rhodurus*). *Biochem. Biophys. Res. Commun.* 263, 584–590.
- Ohkubo, M., Yabu, T., Yamashita, M., Shimizu, A., 2013. Molecular cloning of two gonadotropin receptors in mummichog *Fundulus heteroclitus* and their gene expression during follicular development and maturation. *Gen. Comp. Endocrinol.* 184, 75–86.
- Parhar, I.S., Soga, T., Ogawa, S., Sakuma, Y., 2003. FSH and LH-beta subunits in the preoptic nucleus: ontogenic expression in teleost. *Gen. Comp. Endocrinol.* 132, 369–378.
- Peng, C., Xiao, L., Chen, H., Han, Y., Huang, M., Zhao, M., et al., 2018. Cloning, expression and functional characterization of a novel luteinizing hormone receptor in the orange-spotted grouper, *Epinephelus coioides*. *Gen. Comp. Endocrinol.* 267, 90–97.
- Rocha, A., Gómez, A., Zanuy, S., Cerdá-Reverter, J.M., Carrillo, M., 2007. Molecular characterization of two sea bass gonadotropin receptors: cDNA cloning, expression analysis, and functional activity. *Mol. Cell. Endocrinol.* 272, 63–76.
- Rocha, A., Zanuy, S., Carrillo, M., Gmez, A., 2009. Seasonal changes in gonadal expression of gonadotropin receptors, steroidogenic acute regulatory protein and steroidogenic enzymes in the European sea bass. *Gen. Comp. Endocrinol.* 162, 265–275.
- Suzuki, K., Kawauchi, H., Nagahama, Y., 1988. Isolation and characterization of subunits from two distinct salmon gonadotropins. *Gen. Comp. Endocrinol.* 71, 302–306.
- So, W.K., Kwok, H.F., Ge, W., 2005. Zebrafish gonadotropins and their receptors: II. Cloning and characterization of zebrafish follicle-stimulating hormone and luteinizing hormone subunits-their spatial-temporal expression patterns and receptor specificity. *Biol. Reprod.* 72, 1382–1396.
- Swanson, P., 1991. Salmon gonadotropins: reconciling old and new ideas. In: *Proceedings of the 4th International Symposium on the Reproductive Physiology of Fish*, pp. 2–7.
- Vischer, H.F., Bogerd, J., 2003. Cloning and functional characterization of a gonadal luteinizing hormone receptor complementary DNA from the African Catfish (*Clarias gariepinus*). *Biol. Reprod.* 68, 262–271.
- Yan, L., Swanson, P., Dickhoff, W.W., 1992. A two-receptor model for salmon gonadotropins (GTH I and GTH II). *Biol. Reprod.* 47, 418–427.
- Yaron, Z., Gur, G., Melamed, P., Rosenfeld, H., Elizur, A., Levavivian, B., 2003. Regulation of fish gonadotropins. *Int. Rev. Cytol.* 225, 131–185.