



Title	Immunobiochemical, molecular biological, and reverse genetical studies on vitellogenin receptor candidates in medaka, <i>Oryzias latipes</i> [an abstract of dissertation and a summary of dissertation review]
Author(s)	NAMGUNG, JIN
Citation	北海道大学. 博士(水産科学) 甲第14729号
Issue Date	2021-12-24
Doc URL	http://hdl.handle.net/2115/84446
Rights(URL)	https://creativecommons.org/licenses/by/4.0/
Type	theses (doctoral - abstract and summary of review)
Additional Information	There are other files related to this item in HUSCAP. Check the above URL.
File Information	Jin Namgung_abstract.pdf (論文内容の要旨)



[Instructions for use](#)

学位論文内容の要旨

博士の専攻分野の名称：博士（水産科学）

氏名： 南 宮 眞

学 位 論 文 題 目

**Immunobiochemical, molecular biological, and reverse genetical studies
on vitellogenin receptor candidates in medaka, *Oryzias latipes***

（メダカ *Oryzias latipes* におけるビテロジェニン受容体候補に関する
免疫生化学的、分子生物学的、及び逆遺伝学的研究）

Producing high-quality eggs and larvae is an essential issue in the establishment of a fish farming business. Elucidation of mechanisms concerning the egg yolk formation (also termed vitellogenesis), which appears to relate egg quality, provides the basis for that issue. The final goal of this study is to elucidate molecular mechanisms underlying fish egg yolk formation based on a hypothetical concept concerning multiplicity of the yolk protein precursors (vitellogenin: Vtg) and their receptors (Vtg receptor), which has recently been proposed as “multiple Vtg/multiple Vtg receptor model”, for better understanding on the commonality and variety of the mechanism among various fishes, as well as the corresponding biological and physiological significance. Toward this goal, this study aimed to accumulate basic knowledge on the "multiple Vtg/multiple Vtg receptor model" in medaka (*Oryzias latipes*), a typical basic research model of Acanthopterygii fish.

In Chapter 2, molecular biological and biochemical analyses concerning properties and localization of Vtg receptor candidates, i.e., low-density lipoprotein receptor (LDLR) relative with 8 ligand binding repeats (*lr8/Lr8*) and LDLR related protein 13 (*lrp13/Lrp13*), were conducted using the wild-type (WT) medaka. The mRNA and protein expression patterns of these receptors, as well as their localization patterns, appeared to be similar to those of the corresponding receptors in the cutthroat trout (*Oncorhynchus clarki*) of Protacanthopterygii and the white perch (*Morone americana*) of Acanthopterygii, strongly suggesting that these medaka receptors function as Vtg receptors. This is the second report in Acanthopterygii of which confirmed presence of multiple Vtg receptor candidates in one species, suggesting that the above multiple receptor model seems to be a norm in this fish lineage.

In Chapter 3, the *lr8* gene was chosen as the main Vtg receptor candidate for performing a reverse genetic functional analysis; the CRISPR/Cas9 system was used to introduce mutations into the medaka genome and produce the *lr8* gene knockout (*lr8*-KO) strain. In this study, two gRNAs targeting at the exon 1 of the *lr8* gene, as well as Cas9 mRNA, were simultaneously injected into one cell medaka embryos, resulting in a success in designing gRNAs and developing a protocol which can efficiently introduce mutations into the gene. The homozygous *lr8*-KO strain could be produced in F3 generation using an efficient mating strategy which selected mutants based on PCR amplification for the target DNA region followed by agarose gel electrophoresis and/or hetero duplex mobility assay.

In Chapter 4, changes in phenotypes following the *lr8* gene KO were confirmed to elucidate functional involvement of Lr8 in the molecular mechanism underlying the "multiple Vtg/multiple Vtg receptor model". First, the lack of Lr8 protein was confirmed in the ovaries of *lr8*-KO medaka using Western blot and immunohistochemical analysis. This loss of Lr8 function in *lr8*-KO strain did not significantly affect the egg size (i.e., diameter and weight) nor the total yolk protein contents, but caused a significant decrease in the larval survival, when comparing to those in WT medaka. In addition, among the four Vtg subtypes (VtgAa1, Aa2, Ab, and C), significant decrease in amount of VtgAb-derived egg yolk proteins (YPAb) was evident in the ovarian extracts of *lr8*-KO medaka; this demonstrated that the Lr8 is the major receptor for VtgAb. A significant increase in amount of VtgAa1-derived yolk proteins (YPAa1) compensated for the decrease in YPAb amount. However, a disruption in yolk protein composition (YPAa1:YPAb) was evident in *lr8*-KO medaka; this was considered to be one of potential causes for a significant decrease in survival rate of *lr8*-KO strain larvae. Meanwhile, the YPAb did not completely disappear, although the Lr8 production was completely lost. This was perhaps due to a possible presence of an unknown receptor that partially substitutes the function of Lr8. Such compensation and substitution described above appeared to be a possible reason why the *lr8*-KO strain larvae were not completely lethal.

In summary, expression/localization profiles of two Vtg receptor candidates, *lr8*/Lr8 and *lrp13*/Lrp13, were observed in the ovary of medaka; the results proposed a model of Vtg uptake *via* both receptors. Meanwhile, this study reverse genetically demonstrated that Lr8 functions as the major receptor for VtgAb; this revealed a part of "multiple Vtg/multiple Vtg receptor" system in medaka. Among findings, it was verified for the first time that composition of Vtg subtypes in the egg yolk can be a potential factor determining the egg/larval quality. It was also shown that various compensation/substitution mechanisms driven by multiple Vtg receptors were involved in the molecular mechanism underlying the Vtg uptake. Further understanding on the relationship between "multiple Vtg/multiple Vtg receptor" and egg quality in this model species will lead to the elucidation of the complicated mechanism determining quality of egg/larvae in fish; such studies will also provide the basis for the establishment of efficient seed production technology.