A NOVEL CLASS OF OVARIAN LIPOPROTEIN RECEPTOR IN CUTTHROAT TROUT: MOLECULAR CLONING AND EXPRESSION ANALYSIS

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Introduction:

Teleost eggs contain a substantial yolk mass, which serves as a protein- and lipid-rich nutritional source for embryonic development and larval growth. A large portion of the yolk mass is derived from multiple types of vitellogenins (Vgs), which are incorporated into the oocytes from the maternal circulation *via* endocytosis mediated by Vg receptors (VgRs) [1, 2]. A member of the low-density lipoprotein receptor (LDLR) gene family, which contains a single ligand binding (LB) domain consisting of 8 LB repeats has previously been identified and designated as the VgR. This VgR also has been termed 'lipoprotein receptor with 8 ligand repeats' (LR8) and 'very low-density lipoprotein receptor' (VLDLR). Our recent findings, however, have revealed multiplicity in ovarian membrane proteins that specifically bind Vg in perciforms, indicating that Vg is possibly incorporated by other oocyte LDLR family receptors [3]. With the exception of this classical LR8-type VgR, no prior characterization has been performed on other ovarian Vg-binding receptors in terms of their structures, expression profiles, or ligand specificities. As an initial step to understanding the physiological significance of multiple ovarian receptors during teleost yolk formation, we aimed to clone and characterize a novel LDLR family receptor (LR) from the ovary of cutthroat trout (*Oncorhynchus clarki*).

Methods:

A full-length cDNA encoding a novel LR was cloned from previtellogenic ovary of cutthroat trout by RT-PCR and TA cloning using degenerate PCR primers designed based on nucleotide sequences of a novel ovary LR in striped bass (*Morone saxatilis*) and white perch (*M. americana*) (Reading and Sullivan, *unpublished data*) and other mammalian LDLR family genes. The full-length sequence was obtained by 3' and 5' rapid amplification of cDNA ends (RACE). Expression levels of the LR mRNA in female cutthroat trout were determined using quantitative real-time reverse transcription PCR assay (qRT-PCR). *In situ* hybridization was performed by a routine method using specific probes labeled with digoxigenin.

Results and Discussion:

The cDNA cloned in this study contained a complete coding sequence (4,500 bp), encoding a protein with an expected mass of ~163 kDa (1,500 amino acid residues). The deduced amino acid sequence of this cDNA clone included several domains that are conserved in sequences of LDLR gene family members, including (from the N-terminus): an N-terminal LB domain consisting of 13 LDLR class-A LB repeats, an epidermal growth factor (EGF) precursor homology domain (A, B, C and D), five LDLR class B repeats flanked by EGF repeats A and B, three LDLR class B repeats flanked by EGF repeats B and C, a C-terminal LB domain consisting of one LDLR class-A LB repeat flanked by EGF repeats C and D, a transmembrane domain, and a cytoplasmic domain. A phylogenetic analysis placed this LR sequence into a new LR cluster consisting of several unclassified LR sequences predicted from genomes of various animals, and which included the novel ovary LR of *Morone* species ([3]; Reading and Sullivan, *unpublished data*). This novel receptor cluster (designated herein as LRX+1 class, where X = number of N-terminal LB repeats) was more similar to the insect VgR cluster than to the clusters of vertebrate LR7s (LDLR), LR8s (VgR, VLDLR), and LDLR-related proteins (LRPs). Expression of cutthroat trout LRX+1 (CtLRX+1) mRNA was exclusively observed in the ovary when various tissues were examined by qRT-PCR. The CtLRX+1 mRNA expression was high in the previtellogenic ovaries and gradually decreased during the vitellogenic phase, followed by a slight increase in ovaries at ovarian follicle maturation and in the post-ovulatory follicles. *In-situ* hybridization revealed an intense localization of CtLRX+1 mRNA expression in the cytoplasm of previtellogenic oocytes, while no detectable signal was observed in vitellogenic oocytes.

Conclusion:

In summary, a full-length cDNA encoding a novel ovarian LR was cloned from the ovary of cutthroat trout. Structural analyses suggest that this sequence is a new class of LR in the LDLR gene family (tentatively called LRX+1 class) and should be designated as cutthroat trout LR13+1 (CtLR13+1). The CtLR13+1 might function as a VgR, since the patterns of distribution in somatic and ovarian tissues, as well as the patterns of expression during oogenesis, were found to be similar to those of VgR in fishes. The present study provides further evidence for a system of yolk formation mediated by multiple ovarian receptors in teleosts.

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