MOLECULAR CLONING AND LOCALIZATION OF TWO CLASSICAL OVARIAN LIPOPROTEIN RECEPTORS IN CUTTHROAT TROUT *ONCORHYNCHUS CLARKI*

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Introduction:

Eggs of teleost fishes contain a substantial yolk mass, which serves as a nutrition source (e.g., proteins and lipids) for embryonic development and larval growth. Various types of ovarian lipoprotein receptors, which belong to the low-density lipoprotein receptor (LDLR) gene family, may be involved in the process of ovarian yolk formation. In teleosts, an ovarian lipoprotein receptor that binds to and regulates uptake of, a major yolk precursor, vitellogenin (vitellogenin receptor, VgR), has been extensively characterized [1, 2, 3]. However, no additional receptors of the LDLR family have been characterized in the ovary of teleosts. In order to understand the physiological significance of multiple ovarian receptors during teleost yolk formation, we aimed to confirm the expression and localization of two classical lipoprotein receptors (i.e., VgR and LDLR) in developing ovarian follicles of cutthroat trout, *Oncorhynchus clarki*.

Methods:

Molecular cloning of cDNAs encoding VgR and LDLR was performed by RT-PCR and TA-cloning using trout ovary cDNA as template. Recombinant receptor proteins targeting the ligand binding domains of each receptor were prepared for use as antigens to produce specific polyclonal antibodies (a-rVgR and a-rLDLR). Expressed recombinant receptor proteins were purified, emulsified with Freund's complete adjuvant, and injected into rabbits. Using these antibodies, both receptor proteins were detected by Western blot analyses of ovarian membrane preparations, as well as by immunohistochemistry of ovarian follicles. Localization of each corresponding *vgr* and *ldlr* mRNA was confirmed by *in situ* hybridization.

Results and discussion:

Two cDNAs were isolated and each appeared to encode either full-length VgR (2529 bp) or LDLR (2625 bp) orthogues in the trout; predicted masses of their translated products were ~93 kDa and ~96 kDa, respectively. Phylogenetic analysis placed the trout VgR and LDLR into two separate branches: the trout VgR peptide sequence clustered with other vertebrate lipoprotein receptors with 8 ligand repeats (LR8 type receptors; i.e., VgR or very low density lipoprotein receptor, VLDLR), while the trout LDLR sequence clustered with other vertebrate LR7 receptors (i.e., LDLR). The a-rVgR and a-rLDLR used in Western blot analyses of ovarian membrane preparations detected major bands with apparent masses of ~105 kDa and ~230 kDa, respectively. Immunohistochemistry using these antibodies revealed that the receptors were uniformly distributed throughout the ooplasm of early perinucleolus stage oocytes. Both receptors appear to migrate toward the oocyte periphery and become localized near the oocyte membrane during the oil droplet stage. In addition, *in situ* hybridization using antisense probes for *vgr* and *ldlr* revealed a strong signal, uniformly distributed in the ooplasm of perinucleolus stage oocytes, which became barely detectable in lipidic and vitellogenic stage oocytes.

Conclusion:

The present study confirmed the patterns of expression and localization of two classical ovarian lipoprotein receptor genes and proteins (i.e., VgR and LDLR) throughout oogenesis of cutthroat trout. Characterization of LDLR was conducted for the first time in teleosts and patterns of LDLR expression and localization appeared similar to those observed for VgR, indicating possible functional similarities in terms of ovarian yolk formation *via* receptor-mediated endocytosis of lipoproteins (e.g., vitellogenin and/or other plasma lipoproteins). The results also support a "translocation-dependent" model for initiation of ovarian receptor-mediated endocytosis. Although *vgr* and *ldlr* transcripts and their protein products are expressed during early oogenesis (i.e., perinucleolus stage), the VgR and LDLR likely begin to function only after they localize near the oocyte membrane during the later stages of oogenesis that are characterized by active endocytosis (e.g., lipidic and vitellogenic stages).

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