EXPRESSION OF GENES INVOLVED IN OOCYTE LIPIDATION IN CUTTHROAT TROUT, *ONCORHYNCHUS CLARKI*

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Introduction:

In salmonid fishes, as in many other teleosts, high amounts of neutral lipids (NLs) are accumulated in ooplasm lipid droplets during oocyte growth; they are later utilized as an energy resource by developing embryos and larvae. However, little is known about the origin of such lipids and the mechanisms underlying their accumulation into oocytes. Triacylglycerol (TAG)-rich lipoproteins, such as very low-density lipoprotein (VLDL), are considered to be the primary carriers of the NLs to the cell [1]. Lipoprotein lipase (LPL) is an enzyme engaged in hydrolysis of TAG from VLDL and chylomicron, and the free fatty acids (FAs) released from TAG are transported into the cell through their receptors and transporters [2]. Thus, we have proposed a model for the oocyte lipidation as follows; VLDL are metabolized by LPL outside of the oocyte and resulting FAs then enter oocytes as a source for *de novo* biosynthesis of NLs. In order to verify this model, we cloned cDNAs encoding factors considered to be involved in oocyte lipidation, and analyzed their expression in ovary of the cutthroat trout, *Oncorhynchus clarki*.

Methods:

cDNAs encoding two kinds of lipase, LPL and endothelial lipase (EL), two membrane FA receptors (e.g., FA translocases), cluster of differentiation 36 (CD36) and scavenger receptor class B1 (SR-B1), and intercellular FA transporters, FA binding proteins (FABPs), were isolated from the cutthroat trout ovary using a polymerase chain reaction (PCR) based cloning strategy. Expression of these mRNAs in various tissues and in ovarian follicles was analyzed using simple reverse transcription-PCR (RT-PCR), real-time quantitative RT-PCR and *in situ* hybridization (ISH).

Results and Discussion:

Two types of cDNAs were obtained for both LPL (LPL1 and LPL2) and EL (EL1 and EL2). Both types of LPL mRNA were highly expressed in lipid storage tissues (e.g. adipose tissue, muscle and ovary) and predominantly expressed in granulosa cells of ovarian follicles. Ovarian LPL1 mRNA levels showed a remarkable peak in April (oocyte lipid droplet stage) and then decreased to low values sustained until November (mid-vitellogenesis) and followed by a small peak in LPL1 gene expression in December (late vitellogenesis). LPL2 mRNA levels did not show pronounced changes. In contrast, both ELs were highly expressed in ovary, and their expression was mainly observed in oocytes. Levels of both EL mRNAs were high in early vitellogenesis and otherwise sustained at low values. ISH analysis showed that both LPL mRNAs were strongly expressed in ovarian somatic cells, especially granulosa cells in pre-vitellogenic follicles, whereas expression of both ELs was restricted to oocytes. These results suggest that, in cutthroat trout, VLDL is metabolized by the action of LPLs in the granulosa cell layer to generate free FAs to be incorporated into growing oocytes. It has been shown that EL has significantly higher phospholipase activity than triglyceride activity [3] and, thus, the ELs may act as phospholipases for metabolizing polar lipids of vitellogenin-derived yolk proteins in trout oocytes.

A partial CD36 cDNA and a full length SR-BI cDNA were cloned from the trout ovary. CD36 mRNA was widely expressed in various tissues including ovary and, in ovarian follicles, its expression was found in both somatic cells and oocytes. SR-BI mRNA was highly expressed in ovary; the transcripts were highly expressed in granulosa cells and moderately expressed in oocytes. The expression of CD36 and SR-BI genes in oocytes suggests that they play important roles in uptake of FAs into oocytes.

Among 7 subtypes of FABP (FABP1, 2, 3, 6, 7, 10, 11) known to be present in teleost fishes [4], we found that FABP1, 3, 7 and 11 were expressed in ovary. In RT-PCR analysis of vitellogenic ovarian follicles, mRNAs of FABP1, 3 and 11 were expressed in both somatic cells and oocytes, whereas FABP7 mRNA was expressed very weakly and only in oocytes. ISH analyses using pre-vitellogenic ovary showed that FABP1 mRNA was strongly expressed in both somatic cells and oocytes, whereas FABP3 mRNA was weakly expressed only in somatic cells. Expression of FABP11 mRNA was strong in somatic cells but weak in oocytes. However, strong expression of FABP11 mRNA was found in the ooplasm of atretic follicles. These results suggest that FABP1 and

FABP11 are involved in FA transportation in the ooplasm of pre-vitellogenic ovarian follicles.

Conclusion:

In summary, we have cloned cDNAs encoding factors likely to be involved in oocyte lipidation (lipid droplet formation) from the ovary of cutthroat trout, and analyzed their expression in ovarian follicles. Results of the present study update our proposed model for oocyte lipidation, as follows: 1) VLDL is metabolized by the action of LPLs in the granulosa cell layer to generate free FAs, 2) the FAs are incorporated into growing oocytes through CD36 and/or SR-BI, 3) the FAs are then transferred to endoplasmic reticulum (ER) by FABP1 and/or FABP11 in ooplasm, and 4) NLs are synthesized from the FAs and lipid droplets are formed in the oocyte ER [5].

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