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Ovarian yolk formation in fishes: Molecular mechanisms underlying formation of lipid droplets and vitellogenin-derived yolk proteins

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ABSTRACT

Fish egg yolk is largely derived from vitellogenins, which are synthesized in the liver, taken up from the maternal circulation by growing oocytes *via* receptor-mediated endocytosis and enzymatically processed into yolk proteins that are stored in the ooplasm. Lipid droplets are another major component of fish egg yolk, and these are mainly composed of neutral lipids that may originate from maternal plasma lipoproteins. This review aims to briefly summarize our current understanding of the molecular mechanisms underlying yolk formation in fishes. A hypothetical model of oocyte growth is proposed based on recent advances in our knowledge of fish yolk formation.

1. Introduction

Teleost eggs contain a substantial yolk mass that serves as a protein- and lipid-rich source of nutrients for embryonic development and larval growth. This review summarizes our current understanding of molecular mechanisms underlying accumulation of the major nutritional components, yolk proteins and neutral lipids, in teleost oocytes (see Figs. 1 and 2 for graphical depiction).

A large portion of the yolk mass is derived from vitellogenin (Vtg), which is taken up from the maternal circulation by growing oocytes *via* endocytosis mediated by Vtg receptor(s) and is deposited in yolk granules or other inclusions in the ooplasm (Stifani et al., 1990; Mizuta et al., 2013). Widespread multiplicity of Vtg gene transcripts (*vtg*) and their protein products (Vtg) has become evident in fishes (Hiramatsu et al., 2005, 2006; Finn and Kristoffersen, 2007) and other oviparous vertebrates. An ovarian gene transcript encodes a lipoprotein receptor with a single ligand binding (LB) domain consisting of 8 low-density lipoprotein receptor (LDLR) class-A LB repeats (named LR8-; Bujo et al., 1995) that has been characterized and revealed to be the functional Vtg receptor (designated as *vtgr*/Vtgr in this review) in a number of oviparous vertebrates, including teleosts (Davail et al., 1998; Mizuta et al., 2013).

Recent studies of fishes have provided evidence of a system of multiple ovarian lipoprotein receptors, including the LR8-type Vtgr, that mediate Vtg-derived yolk formation (reviewed by Hiramatsu et al., 2013). Multiple ovarian membrane proteins that specifically bind Vtg have been discovered in salmonids (Tyler and Lubberink, 1996; Hiramatsu et al., 2013) and in perciforms (Reading et al., 2011). Employing proteomic and transcriptomic techniques in conjunction with biochemical and molecular biological analyses, several candidate gene transcripts possibly encoding some of these Vtg-binding proteins (other than the Vtgr) were partially characterized in cutthroat trout (*Oncorhynchus clarki*) and in *Morone* species (Hiramatsu et al., 2013; Reading et al., 2014). One of these novel putative Vtg receptors was initially called "LR*X* + 1" due to its unique structural properties (*X* = number of N-terminal class-A LB repeats). Briefly, the cloned cutthroat trout LR*X* + 1 cDNA contained a complete open reading frame encoding

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a protein with an expected mass of ~163 kDa. The deduced amino acid sequence of this protein included several domains conserved in sequences of LDLR family gene members, including an N-terminal LB domain consisting of 13 LDLR class-A LB repeats and a C-terminal LB domain consisting of one LDLR class-A LB repeat flanked by two epidermal growth factor precursor homology domains, and the protein was thus designated as cutthroat trout LR13 + 1. Similarly, a cDNA encoding an LR7 + 1 type receptor was cloned with full-length coding sequence from the ovaries of *Morone* species, striped bass (*Morone saxatilis*) and white perch (*Morone americana*). These trout and *Morone* LR*X* + 1 type receptors formed a cluster distinct from LR8-type Vtgrs in a phylogenetic analysis of LDLR family genes, and they were thus newly designated as "LDLR related protein 13: *lrp13*/Lrp13" (Reading et al., 2014).

Presently, at least two receptors (e.g., *vtgr*/Vtgr and *lrp13*/Lrp13), but possibly more, appear to be involved in yolk granule/globule formation in both salmonids and perciforms. The two teleost groups differ in composition of their multiple *vtg*/Vtg subtypes (Hiramatsu et al., 2002a; Buisine et al., 2002; Finn and Kristoffersen, 2007; Amano et al., 2008; Reading et al., 2009; Mushirobira et al., 2013; Williams et al., 2014b; Schilling et al., 2014), and they also generally differ in the prevalence of certain reproductive modes (e.g., demersal *versus* floating eggs, freshwater *versus* marine spawning, large *versus* small egg size, and long- *versus* short-term embryonic development, respectively). Therefore, their Vtg-derived yolk protein products are expected to differ in mechanisms of deposition into oocytes and the resulting yolk composition, as well as in their modes of proteolysis during oocyte growth and maturation and subsequent utilization by developing embryos.

A holistic understanding of yolk formation based on general principles will require that we verify how different types of yolk are formed and utilized in fishes with such divergent lineages and reproductive life histories. The first half of this review develops a comparative model of the deposition of Vtg-derived yolk proteins utilizing current data obtained for two distantly related representative research models, the cutthroat trout (salmonid) and the white perch (perciform) (Fig. 1). Background information based on earlier studies characterizing involvement of Vtg-derived yolk proteins in oocyte growth and maturation of salmonid and perciform fishes is summarized in prior reviews (Hiramatsu et al., 2002b, 2013; Reading and Sullivan, 2011), which include hypothetical descriptions and/or diagrams indicating the physiological significance of multiple Vtg systems in relation to the acquisition of egg buoyancy and the provision of embryonic and larval nutrition.

In some teleosts, the egg yolk contains a large mass of neutral lipids present as oil/lipid droplets in addition to the Vtg-associated polar lipids. The process involved in this neutral lipid accumulation is called oocyte lipidation. In Japanese eels (*Anguilla japonica*), the source(s) of ovarian neutral lipids appears to be a triacylglyceride (TAG)-rich serum lipoprotein, such as very-low-density lipoprotein, VLDL (Endo et al., 2011; see also Damsteegt et al., 2015), although the pathway of ovarian TAG accumulation has not yet been elucidated. Results of previous studies suggested two possible pathways for ovarian TAG accumulation (reviewed by Hiramatsu et al., 2013): (1) circulating VLDL may be processed by ovarian lipoprotein lipase (Lpl) into low-density lipoprotein (LDL) and the resulting free fatty acids (FFAs) released from TAG moieties are incorporated into oocytes and regenerated as lipid droplets; (2) VLDL may bind to one or more ovarian lipoprotein receptors belonging to the LDL receptor (Ldlr) family and be endocytosed into the oocyte before being stripped of FFAs, which are then utilized for lipid droplet formation. Fish oocyte lipidation has been visualized directly by tracing the fate of potential maternal precursor lipoproteins whose lipid and protein (apolipoprotein) moieties were separately labeled (discussed below). However, there is currently little information available on mechanisms underlying transportation of resulting hydrolyzed FFAs and their utilization to synthesize neutral lipids for formation of ooplasm lipid droplets. The second part of this review develops a model of molecular mechanisms underlying the oocyte neutral lipid formation that is mainly based on data recently obtained for our salmonid research model, the cutthroat trout (Fig. 2).

2. Molecular mechanisms underlying formation of vitellogenin-derived yolk proteins

Recent findings indicate that vitellogenesis varies among fishes with regard to the rates of production and deposition into oocytes of multiple subtypes of Vtg, as well as to the course of proteolysis of the Vtg-derived yolk proteins, which may especially tailor the

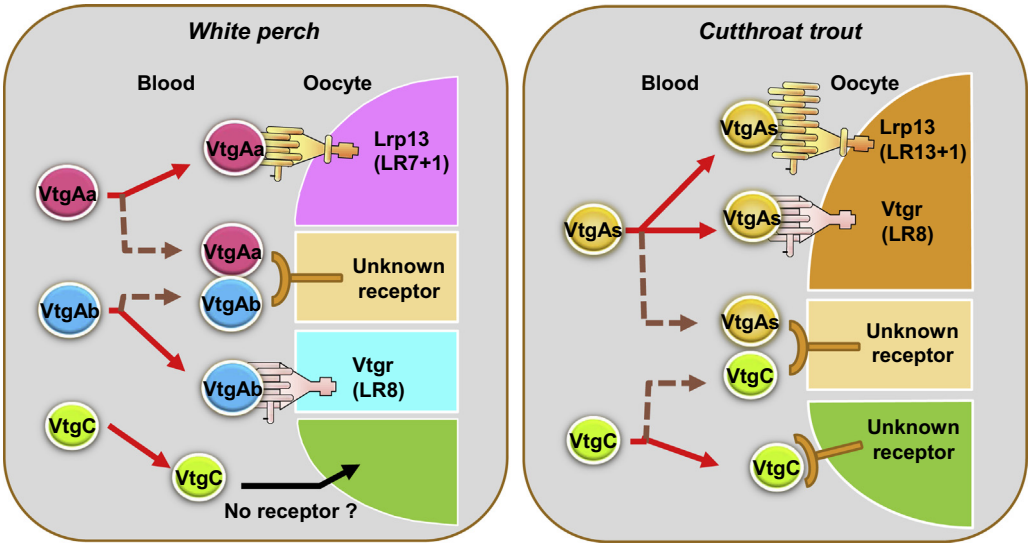

Fig. 1. Multiple vitellogenins (Vtg) and their receptors involved in Vtg-derived yolk formation of white perch and cutthroat trout. The A-type Vtgs (VtgAa, VtgAb and VtgAs) bind the 'classical' LR8-type Vtg receptor (Vtgr) and/or low-density lipoprotein receptor related protein 13 (Lrp13). Receptor proteins for C-type Vtg are detected in ligand blots of trout ovarian membrane, but not in white perch. Receptor proteins that universally bind multiple Vtg subtypes are also detected in the ovarian membrane preparations of both species. LR8: lipoprotein receptor (LR) with 8 ligand binding (LB) repeats; LR7 + 1: LR with 7 + 1 LB repeats; LR13 + 1: LR with 13 + 1 LB repeats.