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 **Figures 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 “LRX+1” due to its unique structural properties (X = number of N-terminal class-A LB repeats). Briefly, the cloned cutthroat trout LRX+1 cDNA contained a complete open reading frame encoding 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 (*M. saxatilis*) and white perch (*M. americana*). These trout and *Morone* LRX+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

81 their modes of proteolysis during oocyte growth and maturation and subsequent utilization by
82 developing embryos.

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

94 In some teleosts, the egg yolk contains a large mass of neutral lipids present as oil/lipid
95 droplets in addition to the Vtg-associated polar lipids. The process involved in this neutral lipid
96 accumulation is called oocyte lipidation. In Japanese eels (*Anguilla japonica*), the source(s) of
97 ovarian neutral lipids appears to be a triacylglyceride (TAG)-rich serum lipoprotein, such as
98 very-low-density lipoprotein, VLDL (Endo et al., 2011; see also Damsteegt et al., *this volume*),
99 although the pathway of ovarian TAG accumulation has not yet been elucidated. Results of
100 previous studies suggested two possible pathways for ovarian TAG accumulation (reviewed by
101 Hiramatsu et al., 2013): 1) circulating VLDL may be processed by ovarian lipoprotein lipase
102 (Lpl) into low-density lipoprotein (LDL) and the resulting free fatty acids (FFAs) released from
103 TAG moieties are incorporated into oocytes and regenerated as lipid droplets; 2) VLDL may bind
104 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 (**Figure 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 resulting yolk to each particular species. In many species, the circulating Vtg ratios do not always correspond to their ratios of deposition into egg yolk (**Table 1** and references therein). This selective deposition may be regulated by a system of multiple ovarian receptors engaged in endocytosis of the different types of circulating Vtg. The first direct evidence of this was provided by Reading et al. (2011) for the white perch and indicated the presence of a selective regulatory system for the controlled accumulation of multiple Vtgs (designated as VtgAa, VtgAb, and VtgC subtypes in this species) by growing oocytes. This system appears to be driven by distinct Vtg-binding membrane proteins (i.e., receptors) with different affinities for VtgAa and VtgAb and none for VtgC. Specifically, one receptor protein, Lrp13 (a LR7+1 type), preferentially binds VtgAa,

while another receptor protein, Vtgr (the LR8-type Vtgr), preferentially binds VtgAb (Reading et al., 2014). Western blotting using bi-specific polyclonal antibodies raised against synthetic peptides specific to either Lrp13 or Vtgr were recently used to verify the identities of these Vtg-binding proteins as VtgAa and VtgAb receptors, respectively (Reading et al., 2014). Immunohistochemistry using these antisera showed that the Vtgr appears to localize at the zona radiata, whereas Lrp13 localizes to both the zona radiata and to the follicle cell layer in white perch vitellogenic ovary (Schilling J. and Reading B.J., *unpublished*). Another Vtg-binding protein from white perch ovary membranes that weakly binds both VtgAa and VtgAb has not yet been conclusively identified. The apparent molecular weight (~150 kDa) of this protein suggests that it may possibly be Ldlr (LR7) or Lrp13 monomer (Reading et al., 2014). Further investigation will be required to confirm the identity of this protein.

Ovary membrane protein ligand blotting and receptor binding assays were used to show that VtgC does not bind any ovary membrane proteins in the white perch (Reading et al., 2011). The results of a recent study of multiple Vtgs in a closely related member of the family *Moronidae*, the European sea bass (*Dicentrarchus labrax*), suggest that differences in receptor binding between the A-type and C-type Vtgs may have evolved with extensive changes to the 3-dimensional distribution of positively and negatively charged amino acid residues across the receptor binding surface on the N-sheet of the lipovitellin heavy chain of these proteins (see Yilmaz et al., *this volume*). Most recently, protein affinity purification coupled to tandem mass spectrometry was used to analyze the white perch VtgC-ovary membrane interactome (Schilling J. and Reading B.J., *unpublished*). The results of this work also indicate that white perch VtgC does not bind to any lipoprotein receptors in the ovary membrane. Therefore, the mechanisms by which VtgC is taken up by oocytes in this species remain to be discovered.

The results of a recent study of cutthroat trout (Mushirobira et al., *unpublished*; 10th ISRPF abstract) revealed that multiple Vtgs designated as being salmonid A-type Vtgs (VtgAs) and C-type Vtg (VtgC) seem to be endocytosed into the trout oocytes *via* their interactions with at least four distinct Vtg-binding membrane proteins with different affinities to VtgAs and VtgC. Briefly, two kinds of proteins that specifically bind VtgAs were visualized by ligand blotting and immunologically identified as Lrp13 (LR13+1-type: ~210 kDa) and Vtgr (LR8-type: 95~110 kDa). The trout VtgC did not bind to either of these receptors for VtgAs, but it did specifically bind an unidentified receptor protein. In addition, another Vtg-binding protein that commonly bound both VtgAs and VtgC was evident in the trout ovarian membrane preparation. Thus, unlike VtgC in white perch, at least two unidentified receptor proteins may be involved in VtgC endocytosis into trout oocytes.

Our current understanding of the molecular mechanisms underlying Vtg-derived yolk formation is summarized in **Figure 1** as a comparative model proposed for salmonid and perciform fishes. Subtypes of Vtg appear to differ in a species-specific manner with respect to their receptor interactions, while both Vtgr and Lrp13 are responsible for specific binding of A-type Vtgs (i.e., trout VtgAs; perch VtgAa and VtgAb), but not VtgC, in both fish groups. The Lrp13 differs between salmonids and perciforms in the number of N-terminal class-A LB repeats. The reduced number of class-A LB repeats present in highly evolved perciform fishes may relate to the loss of VtgAb-binding properties (or, conversely, to the gain of selective VtgAa-binding *via* Lrp13), providing for precise regulation of the deposition ratio of the two Vtg subtypes into oocytes, although this hypothesis remains to be verified. Conclusive identification of a VtgC-specific receptor and another non-specific or 'universal' Vtg receptor, as well as a possible pathway of VtgC uptake that is not based on receptor-mediated endocytosis, also remains to be verified in perciforms.

Thus far, in addition to the relative abundance of circulating multiple Vtgs, their selective uptake by multiple ovary receptors seems to control the proportional accumulation of specific yolk proteins, which appears to vary between species (Table 1). During the vitellogenic phase of oocyte growth, such mechanisms result in formation of the initial yolk composition required by each species. Previous studies have demonstrated that, following initial yolk formation during vitellogenesis, a species-specific secondary proteolysis of yolk proteins creates the final ‘tailor-made’ yolk derived from multiple Vtgs in each species. Specifically, during oocyte maturation, a large portion of VtgAa-derived yolk proteins may undergo extensive degradation into free amino acids that are osmotically active and promote oocyte hydration, leading to proper buoyancy of the eggs in marine pelagic-egg spawners, such as barfin flounder (Matsubara et al., 1999), red seabream (Sawaguchi et al., 2006), Atlantic halibut (Finn et al., 2002), and grey mullet (Amano et al., 2008). Partial degradation or a limited proteolysis (e.g., polypeptide nicking) of VtgAa-derived yolk proteins occurs in some fresh water spawners such as striped bass (Williams et al., 2014a). In some cases, the greater degree of degradation of Vtg-derived yolk proteins in marine teleosts spawning pelagic eggs seems to reflect a physiological need for more extensive oocyte hydration (Finn et al., 2002; Kolarevic et al., 2008).

Collectively, teleosts appear to have evolved mechanisms for regulating oocyte hydration, in part, *via* the selective synthesis (i.e., species-specific Vtg-subtypes), accumulation (i.e., Vtg-subtype specific ovary receptors), and degradation (i.e., Vtg-subtype specific processing) of yolk proteins and their precursors. This complex system of multiple Vtgs and their derivative yolk proteins may enable highly evolved teleost species to more precisely control oocyte hydration and, subsequently, egg buoyancy. This would be an especially important adaptation for fishes spawning in estuaries or brackish water, where salinity fluctuations can influence buoyancy of the eggs.

Additional studies are needed to identify and fully characterize the multiple ovarian receptors responsible for Vtg uptake, the endocrine control of the expression of such receptor transcripts and proteins including *vtgr/Vtgr* and *lrp13/Lrp13*, and the acquisition by ovarian follicles of competency for Vtg uptake (i.e., initiation of vitellogenesis). As mentioned earlier, further investigation also is required to elucidate the molecular mechanisms of VtgC-endocytosis, in conjunction with identification of VtgC-specific and 'universal' Vtg receptors. The endocrine control of vitellogenesis has received considerable attention (Polzonetti-Magni et al., 2004), but only one report on possible endocrine factors regulating *vtgr* expression is currently available (Dominguez et al., 2012). Based on studies of *vtgr* expression in cultured ovarian follicles of largemouth bass (*Micropterus salmoides*), the authors suggest that insulin, estradiol and 11-ketotestosterone are involved in the complex regulation of *vtgr* expression. Such experiments need to be extended to other fish species to verify whether or not this scenario generally applies to teleosts. In terms of follicular competency for Vtg-uptake, our recent histological studies indicate Vtgr (Mizuta et al., 2013) and Lrp13 (Reading et al., 2014) proteins are already translated and translocated near the oolemma of pre-vitellogenic oocytes (lipidic stage) in cutthroat trout and striped bass, respectively. These observations support results of a previous study that confirmed functional Vtg-binding by ovarian follicles at early growth stages (Lancaster and Tyler, 1994), indicating that the pre-vitellogenic follicles are primed for receptor-binding of Vtg by the time the liver initiates Vtg synthesis and secretion. However, for reasons unknown at this time, pre-vitellogenic follicles are not competent to internalize Vtg *in vivo* and *in vitro* (Hiramatsu N. et al., *unpublished*), which leads us to hypothesize that activation of certain molecular components of endocytosis aside from receptor-binding may be required for acquisition of the competency to internalize Vtg, although these components remain to be identified.

3. Molecular mechanisms underlying oocyte lipidation

As noted above, previous studies have suggested two possible pathways for oocyte neutral lipid droplet formation (reviewed by Hiramatsu et al., 2013): 1) the lipase-dependent pathway without receptor-mediated endocytosis of lipoproteins (termed the lipase-dependent, non-endocytotic pathway in this review) and 2) the lipoprotein receptor-mediated endocytotic pathway. With regard to the source of lipids in the ooplasm droplets, Endo et al. (2011) first demonstrated that plasma VLDL appears to be the primary candidate in Japanese eel; 11-ketotestosterone also seems to be an essential factor for the promotion of lipid droplet formation in cultured eel ovaries (Divers et al., 2010). In the endocytotic pathway, our previous review (Hiramatsu et al., 2013) proposed Ldlr (LR7) to be a possible receptor for ovarian VLDL-binding and -endocytosis based on its expression profiles in cutthroat trout ovaries (Luo et al., 2013) as well as its general ligand-binding properties in several vertebrates, including fishes (Tyler and Lubberink, 1996; Hussain et al., 1999). However, no significant receptor binding was observed in trout ovarian membrane preparations when ligand blots were probed with labeled plasma lipoproteins including VLDL, LDL, and high-density lipoprotein (HDL), although both LDL and VLDL appeared to bind a putative Ldlr protein in some somatic tissues (Tyler and Lubberink, 1996; Mushirobira Y. et al., *unpublished*: 10th ISRPF abstract). These findings led us to perform direct tracing experiments using fluorescent-labeled plasma lipoproteins in order to conclusively confirm whether endocytosis of lipoprotein particles is responsible for neutral lipid accumulation in fish oocytes.

Three classes of lipoproteins (VLDL, LDL and HDL) were isolated from plasma of cutthroat trout, labeled with a fluorescent fatty acid analogue, and added to cultures of trout ovarian

follicles (Ryu Y.-W. et al., *unpublished*). Among the three classes of lipoproteins, VLDL showed the fastest and highest rate of uptake of fluorescent FFA into trout follicles, with numerous, intensely fluorescent lipid droplets evident in the ooplasm. This result indicates that trout (this study) and eel (Endo et al., 2011) share a common system in terms of the origin of oocyte neutral lipids: maternal circulating VLDL is a major contributor to neutral lipids in oocyte lipid droplets. Subsequently, the VLDL was dually-labeled in its lipid and protein moieties with fluorescent fatty acids and fluorescent dye, respectively, and then cultured with ovarian follicles isolated from cutthroat trout (Ryu Y.-W. et al., *unpublished*: 10th ISRPF abstract). Fluorescent labeled VLDL lipid moieties were incorporated into the ooplasm and present as intense fluorescent lipid droplets, while fluorescent labeled VLDL protein moieties were restricted to the follicle layers and vitelline envelope and did not appear in the ooplasm. Similar results also were obtained in treatments of ovarian follicles of other teleosts with dually-labeled fluorescent trout VLDL *in vivo* (medaka, *Oryzias latipes*) and *in vitro* (black skipjack tuna, *Euthynnus lineatus*). Thus far, both *in vivo* and *in vitro* experiments demonstrate that the fluorescent labeled neutral lipid moieties of VLDL enter oocytes, while the protein moieties remain extracellular. These results do not support the translocation of intact VLDL particles across the oolemma by an endocytotic pathway during oocyte lipidation, at least in the species mentioned above.

The proposed lipase-dependent, non-endocytotic pathway of oocyte lipidation includes Lpl-dependent lipolysis of VLDL that may occur outside of the oocyte. The resulting FFAs are expected to cross the oolemma either by simple diffusion or *via* the action of fatty acid transporters or binding proteins, and are then used as substrates for *de novo* synthesis by the oocyte of neutral lipids that are deposited as droplets in the ooplasm. We recently cloned and characterized cDNAs encoding four types of membrane-bound, long-chain fatty acid transporters (scavenger receptor class B member 1: *srb1*; fatty acid transfer protein 1: *fatp1*; plasma

membrane fatty acid binding protein: *fabpm*; cluster differentiation 36: *cd36*) and three kinds of cytosolic fatty acid binding proteins (*fabp1*, *fabp3* and *fabp11*) from the ovaries of cutthroat trout (Saito K., Hiramatsu N., and Todo T., *unpublished*). Among these, *srb1*, *fatp1* and *fabp1* gene transcripts were dominantly expressed in the ovaries when 10 tissues were tested. In addition, these three gene transcripts were highly expressed in pre-vitellogenic (i.e., lipidic and yolk vesicle stage) trout ovaries. These observations suggest that *fatp1* and *fabp1* may be involved in active translocation of FFAs across the oolemma or in cytosolic transport of FFAs, possibly playing a significant role in uptake of FFAs as substrates for *de novo* synthesis of neutral lipids by the oocyte. In mammals, SR-BI (mammalian orthologue of fish *Srb1*) appears to be involved in the selective uptake of HDL-bound cholesteryl ester into cells without endocytosis and degradation of the HDL particle (review: Williams et al., 1999). Therefore, high expression of *srb1* in the trout ovary may relate to uptake of cholesterol ester; HDL possibly binds *Srb1* on the surface of the oocyte membrane with its constituent cholesterol ester being incorporated into the oocyte *via* *Srb1*, followed by inclusion into the oocyte lipid droplets.

Based on the collective findings of the research discussed above, our previous “two-pathways hypothesis” concerning mechanisms for teleost oocyte lipidation can be revised as shown in **Figure 2**. This revised model is primarily based on our findings obtained for cutthroat trout, in conjunction with the supplemental findings obtained for medaka and black skipjack tuna. We have confirmed that maternal plasma VLDL makes the major contribution to ooplasm lipid droplets by supplying triacylglyceride as a source of FFAs, while LDL and HDL may contribute to a lesser extent by supplying cholesteryl ester. In trout and some other species (e.g., medaka and black skipjack tuna), the *Lpl*-dependent, non-endocytotic pathway appears to be the major route for oocyte lipidation. Gene expression patterns of lipoprotein lipases (Kwon et al., 2001; Ibanez et al., 2008; Luckenbach et al., 2008; Ryu et al., 2013) and some fatty acid transporters

(this report) suggest their involvement in hydrolysis of VLDL-associated lipids followed by active transport of the resulting FFAs into the oocytes. In addition, LDL- and HDL-associated lipids may be taken into oocytes by transporters such as Srb1.

It should be noted that there are some apparent differences between cutthroat trout and eel in endocrine control of oocyte lipidation and in the possible roles of lipoprotein receptors in this process. We found that VLDL alone can induce active oocyte lipidation in cultured trout follicles, whereas administration of both VLDL and 11-ketotestosterone is required to induce oocyte lipidation in cultured eel follicles (Endo et al., 2011). Furthermore, addition of an antibody against Ldlr (LR7) to the cultures significantly reduced accumulation of lipid droplets by short-fin eel ovarian follicles in the presence of VLDL and 11-ketotestosterone (Damsteegt et al., *this volume*), indicating that Ldlr is somehow involved in the processes of lipid droplet formation in this species. In mammals, the LDL receptor is primarily responsible for the endocytosis of cholesterol-rich LDL into cells (Goldstein et al., 1985; Hussain et al., 1999). In the eel, after conversion of VLDL to LDL through Lpl-dependent lipolysis, ovarian Ldlr possibly interacts with LDL prior to delivering LDL-associated lipids (e.g., cholesteryl ester) into the oocyte, although whether or not receptor-mediated endocytosis of lipoproteins other than Vtgs occurs in this species remains to be verified.

4. Conclusion

It is evermore apparent that each distinct fish species exhibits a unique composition of Vtg-derived proteins deposited in the egg yolk. Such tailor-made yolk seems to be manufactured in a species-specific manner *via* a combination of complex regulatory systems including differential expression of multiple *vtg* genes followed by translation and disparate deposition of the resulting multiple Vtg proteins *via* subtype-specific and/or 'universal' Vtg receptors, followed

by selective processing of the Vtgs and their yolk protein products during oocyte growth and maturation. It is likely that the diversity and complexity of these mechanisms underlying yolk formation (i.e., vitellogenesis) support the taxonomic diversity and complexity of reproductive strategies found amongst fishes in order to maximize their reproductive success in a broad range of environments. Among recent findings, the discovery of multiple Vtg receptors (LR8-type Vtgr, Lrp13, others) as well as knowledge of the phylogenic distribution of multiple Vtg subtypes among teleosts, set the stage for elucidation of molecular mechanisms by which Vtg-derived yolk products are properly accumulated and processed in a species-specific manner. Further research should be directed at identifying remaining unknown Vtg receptors and at elucidating the molecular mechanisms regulating Vtg receptor gene expression. Likewise, regulation of the translation, translocation and activation of Vtg receptor proteins begs further study, including investigation of the endocrine control of these processes (e.g., by sex steroids and gonadotropins). Additionally, the process of receptor-mediated endocytosis of Vtg needs scrutiny at a finer level of molecular detail, to reveal actions and interactions of other receptor-associated and/or -adapter proteins that may be needed for the oocyte to acquire competence for Vtg endocytosis.

Our comparable knowledge of the molecular mechanisms of oocyte lipidation is only rudimentary but is now evolving rapidly. The results of *in vivo* and *in vitro* experiments performed in salmonids and to a lesser extent in some other marine and freshwater species, indicate that 1) maternal VLDL is a major source of ooplasm lipids, and 2) the lipase-dependent, non-endocytotic pathway is the major route for the uptake of VLDL-associated lipids. Although the lipoprotein receptor-mediated pathway does not seem to be involved in the process of ooplasm lipid droplet formation, a possible association of Ldlr with this process still remains for some species, such as anguillid eels. Studies on oocyte lipid transporters have just begun and the expression profiles of several lipid transporter genes suggest their possible association with

formation of ooplasm lipid droplets. The novel discoveries described here set the stage for elucidation of the molecular details of neutral lipid accumulation in fish oocytes, a research area that until now has been largely unexplored. A useful general model of teleost oocyte lipidation must be applicable to species with or without ooplasm lipid droplets and will likely include considerable diversity in molecular mechanisms for acquisition of maternal lipoprotein-associated lipids, as already evidenced by comparisons of trout and eels. Comparative studies of fish species with few or no ooplasm lipid droplets (e.g., some flounder species and primitive fishes), perhaps involving experiments tracking the fate of fluorescent labeled-lipoproteins, will be especially useful as we seek to define unifying principles broadly applicable to teleost fishes.

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Figure legends

Figure 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.

Figure 2. A hypothetical model of oocyte neutral lipid droplet formation in cutthroat trout. Neutral lipid moieties of circulating very-low-density lipoprotein (VLDL) are hydrolyzed by lipoprotein lipase (Lpl) that is predominantly synthesized in granulosa cells and acts locally and/or distantly (e.g., peripheral vascular endothelial cells, perivitellin space, and/or elsewhere in ovarian tissues external to the oocyte) presumably associating with heparin sulphate proteoglycan (Hspg; Mahley and Ji, 1999). Following the hydrolysis, liberated free-fatty acids (FFAs) are subsequently incorporated into the oocyte, traversing the oolemma by diffusion and/or *via* putative membrane FFA transporters (e.g., fatty acid transfer protein 1: Fatp1). The FFA may then bind to cytoplasmic fatty acid binding proteins (e.g., Fabp1) and will finally be used as a substrate for synthesis of the neutral lipids stored in ooplasm droplets. Molecular pathways that occur before and after the VLDL hydrolysis are indicated by uniformly dashed lines and solid lines, respectively, with an arrowhead. In addition, cholesteryl ester (CE) moieties of circulating low-density lipoprotein (LDL) and high-density lipoprotein (HDL) may be incorporated into the

533 oocyte *via* their interaction with some membrane lipid transporters (e.g., scavenger receptor class
534 B type 1: Srb1, and/or others); these hypothetical pathways are indicated by lines made up of
535 alternating long and short dashes.

Table 1. Relative proportions of circulating multiple vitellogenin (Vtg) subtypes and their derived ovarian yolk proteins (Yp) in some teleost species

Species	VtgAa : VtgAb : VtgC	YpAa : YpAb : YpC
Striped bass ¹	3 : 34 : 1 to 3 : 25 : 1	1.43 : 1.4 : 1
White perch ²	<i>no data</i>	7.7: 16 : 1
European sea bass ³	7.6 : 22.6 : 1	1.5 : 3 : 1
Barfin flounder ⁴	13 : 18 : 1 to 32 : 10 : 1	9 : 15 : 1
Grey mullet ⁵	1 : 11.9 : 1 to 1 : 10 : 1	4 : 13.4 : 1
Species	VtgAs : VtgC	YpAs : YpC
Sakhalin taimen ⁶	21.5 : 1	22 : 1

¹Williams et al. (2014b), Vtg proportions: values for midvitellogenic- and postvitellogenic-stage females, Yp proportion: value for postvitellogenic stage females;

²Schilling et al. (2014), value for postvitellogenic stage females; ³Yilmaz et al. (*accepted*, this volume), values for postvitellogenic-stage females; ⁴Sawaguchi et al. (2008), Vtg proportions: values for midvitellogenic- and postvitellogenic-stage females, Yp proportion: value for postvitellogenic stage females; ⁵Amano et al. (2008), Vtg proportions: values for midvitellogenic- and latetvitellogenic-stage females, Yp proportion: value for midvitellogenic stage females; ⁶Amano et al. (2010), values for postvitellogenic stage females.

Fig. 1

Fig. 2