ABSTRACT  Yolk represents the last growth stage of a single cell, the oocyte, which contains, besides bona fide cyttoplasm, endocytosed serum-derived lipoproteins and minor components essential for normal embryo development. Transport of bulk lipoproteins, micronutrients, and morphogens to oocytes in parallel with maintenance of somatic homeostasis is achieved by ligand targeting via cell-specific expression of receptors and subtle differences in ligand structure. Lipoprotein metabolism is the prime example of these regulatory principles, in which receptors belonging to the low density lipoprotein receptor gene family play key roles. Here, we present the laying hen’s features that make it an attractive model system to dissect macromolecular transport processes at the molecular level. In addition to the characterization of a family of yolk precursor receptors, studies on systemic vs. oocyte-directed transport have uncovered new aspects of the biological rationale for simultaneous expression of closely related genes in a single organism. J. Nutr. 127: 801S–804S, 1997.

KEY WORDS: • receptors • lipoproteins • yolk • gene family • chicken

OCYTE DEVELOPMENT

Normal growth and development of the female germ cell is not only of paramount biological importance but also is the macroscopically most astounding event in the reproduction of oviparous species. In the female chicken (Gallus gallus domesticus), fertilization-competent oocytes develop continuously in follicles of the left ovary (the right ovary regresses during embryogenesis). The final stage of this development is characterized by a dramatic 7-d growth spurt during which the next ovulating oocyte extracts from the circulation up to 14 mL of yolk. More than 30% of the yolk weight is composed of lipid imported in the form of serum-borne lipoproteins, mainly VLDL and vitellogenin (VTG), which together contribute to the yolk mass on average approximately 4 g of triglycerides and approximately 220 mg of cholesterol. The lipoproteins and other, minor, yolk precursors are synthesized in and secreted from the liver and taken up by the growing oocytes via (receptor-mediated) endocytosis; endogenous synthesis of yolk proteins by the oocyte has never been demonstrated beyond doubt. A particularly intriguing feature of the laying hen model is the necessity to simultaneously achieve massive lipid transport into the oocyte and regulation of homeostasis in somatic cells, a metabolic dichotomy mirrored in the cell-specific expression of relevant genes. Since the last report in this series (Nimpf and Schneider 1991), our knowledge about transport of yolk precursors and formation of yolk has widened considerably, as outlined below. Here we shall emphasize the transport of lipoproteins, but will also consider minor constituents and aspects of the oocyte/somatic tissue dichotomy where appropriate.

Chicken oocytes develop in three phases: 1) over several months, numerous oocytes increase in size from 60-μm diameter to 2–3-mm diameter, characterized by the absence of typical “yellow” yolk; 2) several of these oocytes enter a slow growth phase; and 3) upon reaching a size of 6–7 mm, a decision is made as to which of these oocytes (an estimated 50%) are destined for atresia (resorption) and which ones enter the last phase (7 d) during which they will reach a diameter of 35 mm and undergo ovulation. Ovulation occurs approximately every 25 h and triggers the beginning of the rapid growth phase of the next oocyte (laid as egg 7 d later), establishing a hierarchy of preovulatory follicles. Thus, at any time of the reproductive period, the ovary contains five to eight follicles of decreasing size (the five largest are designated F1 to F5) together with rapidly growing oocytes. The largest follicle (F1) is the next one to be released into the oviduct.

If the hen has been inseminated, fertilization takes place in the uppermost portion of the oviduct. During the next 25 h in the reproductive tract, albumen, shell membranes, and the
shell are deposited around the oocyte to complete the process of egg formation.

The yolk itself appears homogeneous but actually is quite complex and precisely structured. From the germinal vesicle (the visible white spot on the oocyte surface), a pear-shaped yolk-free cytoplasmic region, the latebra, extends radially into the center of the oocyte. A medial cross-section reveals concentric shells of yellow yolk (seven on average, possibly related to the 7 d of massive yolk deposition), separated by bona fide cytoplasm (<200-μm thick), which is in continuity with the latebra (in older accounts termed “white”) yolk). The cytoskeletal and regulatory machinery generating this structural organization, which probably is unique to avian oocytes, is not known in any detail; it is noteworthy and significant, however, that it is maintained for several months upon storage at 4°C, or during rapid heat denaturation.

The yellow yolk of chicken oocytes consists of organellar compartments. The most clear-cut distinction can be made based on the biphasic electron microscopical appearance of deep yolk shells. Electron-lucent membrane-enclosed endosomes termed yolk spheres reach a diameter of 140 μm, contain electron-dense spherical structures (“granules”) that represent insoluble yolk components formed by proteolysis of soluble macromolecules. As described below, most of the contents of the yolk spheres are imported by the action of a multifunctional oocyte-specific receptor belonging to the LDL receptor family gene.

RECEPTOR-MEDIATED OOCYTE GROWTH: PARADIGM OF CELLULAR ECONOMY

Up to 1986, molecular information on any of the proteins involved in oocyte growth and systemic lipoprotein transport in laying hens was lacking. It has since been discovered that a single 95-kDa protein in the plasma membrane of the oocyte binds both major yolk lipoproteins, VLDL and VTG (Barber et al. 1991, George et al. 1987, Nimpf and Schneider 1991, Stifani et al. 1990). This work was the starting point for investigations of receptor-mediated lipoprotein metabolism in laying hens, previously summarized in this series (Nimpf and Schneider 1991). It was shown that the 95-kDa protein reacts with antibodies to mammalian LDL receptors (LDLR) (Hayashi et al. 1989) and surprisingly, recognizes apolipoprotein (apo) E (Steyrer et al. 1990), an apolipoprotein not known to be produced in birds. These hallmark properties predicted that the oocyte receptor, termed oocyte vitellogenesis receptor (OVR), would be a homologue of mammalian LDL receptors. The LDLR are characterized by the presence of seven complement-type repeats that make up the ligand-binding domain. In contrast, the so-called VLDL receptors (VLDLR), first described by the laboratory of Yamamoto (Takahashi et al. 1992), are characterized by a cluster of eight such repeats (Giffels et al. 1993 and 1994, Oka et al. 1994a, 1994b, Sakai et al. 1994, Webb et al. 1994). Molecular cloning revealed that OVR has such an eight-repeat ligand-binding domain (Bujo et al. 1994). The amazing high degree of sequence identity between OVR and VLDLR (84% overall, which is greater than that among LDLR) suggests an important and conserved function of this protein.

However, to date the physiological function of mammalian VLDLR is not clearly established; in contrast, the role of OVR is documented by biochemical as well as genetic evidence (Nimpf et al. 1989): it mediates a key step in the reproductive effort of hens, i.e., normal oocyte growth. This conclusion is based on the findings in a mutant non-laying chicken strain. This strain (the “restricted ovulator,” R/O, strain) carries a single mutation at the ovr locus and cannot lay eggs, i.e., displays female sterility (Nimpf et al. 1989). The underlying mutation has been delineated (Bujo et al. 1995b): a single base exchange leads to the replacement of a cysteine residue in the extracellular domain of OVR with a serine (Bujo et al. 1995b), rendering the mutant receptor unable to reach the cell surface normally. As a consequence of the failure to deposit into their oocytes VLDL and VTG, which are produced at normal levels, the mutant females develop severe hyperlipidemia and features of atherosclerosis. The gene for OVR is located on the sex chromosome, Z, in concordance with the results of breeding studies (male birds are specified by ZZ, and hens by WZ) (Bujo et al. 1994); ovr−/ovr− (carrier) roosters and regulatory machinery generating this structural organization, which probably is unique to avian oocytes, is not known in any detail; it is noteworthy and significant, however, that it is maintained for several months upon storage at 4°C, or during rapid heat denaturation.

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Oocyte vitellogenesis receptor expressed in COS-7 cells binds VLDL and VTG with high affinity (Bujo et al. 1995a). Mammalian VLDLR bind VLDL and/or chylomicron remnants, particles that contain apo E, which is not synthesized by birds. In this context, VTG, which is absent from mammals, and apo E have certain common biochemical properties and regions of sequence similarities and have been suggested to be functional analogues (Steyrer et al. 1990). Thus, if triglyceriderich particles indeed prove to be the physiological substrate for mammalian VLDLR, they could transport triglycerides into metabolically active tissues (such as muscle, where receptors are abundant), whereas in avian oocytes VTG and VLDL are taken up to provide nutrients and energy for the developing embryo. Furthermore, there is extensive overlap in ligand recognition patterns with other lipoprotein receptors, in particular with LDLR-related proteins/α2-macroglobulin (α2-M) receptors (LRP) (Schneider and Nimpf 1993).

The OVR transports not only major yolk components but also non-lipoproteins. Riboflavin-binding protein (ribBP) is a 29-kDa phosphoglycoprotein that is synthesized under the influence of estrogen (MacLachlan et al. 1993) in liver and oviduct. Riboflavin-binding protein produced in the liver is the major carrier of the vitamin, via the serum, to the oocyte (future egg yolk), whereas ribBP produced in the oviduct transports riboflavin into the albumen (egg white). Serum ribBP associates with VTG in the circulation, and this complex is recognized, via VTG, by the OVR (MacLachlan et al. 1994). Thus, riboflavin is incorporated into yolk by a piggy-back mechanism whose key component is OVR; similar to the accumulation of VTG, this transport system achieves a roughly ninefold higher concentration of the vitamin complex in yolk than in serum.

The most recently discovered trick mastered by OVR is the transport of α2-M. There’s a special twist again: whereas LRP are known to recognize α2-M only following so-called activation (i.e., production of α2-M* through cleavage by proteases in vivo), OVR interacts with the native ligand as well (Jacobson et al. 1995). This finding emphasizes one of the principles of metabolic dichotomy in laying hens, that is, one form of the ligand (α2-M*) is destined for systemic (hepatic) clearance, whereas the other form (native α2-M) is targeted for uptake into oocytes, where it can function in embryogenesis. In addition to the α2-M/α2-M* metabolic dichotomy, receptor dichotomy, a second principle that involves ligand targeting via cell-type specific expression of receptor genes, also occurs in laying hens and is described below.
PARTNERS: SOMATIC RECEPTORS

In laying hens, LDLR homologues come at least in pairs and sometimes in groups. They are products of genes expressed in mutually exclusive fashion in somatic cells and germ cells, respectively. One biological explanation for this is that oocyte growth must not be feedback-inhibited by intracellular accumulation of ligands, as is the case in somatic cells, and thus the regulation of the two receptor gene groups must be different. The functional counterpart of OVR in somatic cells is a 130-kDa sterol-regulated LDLR (Schneider and Nimpf 1993), structural details of which are yet unknown. The chicken LDL(apo B)R cannot bind apo E or VTG; it is in fact the only known LDLR that interacts with apo B but not apo E. It will be interesting to determine the structural element(s) responsible for this unique property.

Oocyte vitellogenesis receptor itself seems to share one additional feature with its mammalian VLDLR relatives: the expression of splice variant forms, i.e., the production of mRNA with or without the exon coding for a serine- and threonine-rich domain (see Bujo et al. 1995a). As stated above, the oocyte-specific receptor lacks this region, but analysis by PCR of mRNA from several somatic tissues, in particular ovarian granulosa cells, has revealed a longer mature transcript that contains an O-linked sugar domain (Bujo et al. 1995a). Such a somatic cell–specific receptor seems to be expressed in addition to the LDLR but, as a member of the VLDLR group, is not sterol-regulated (Gäfvels et al. 1993 and 1994, Oka et al. 1994a, 1994b, Sakai et al. 1994, Webb et al. 1994).

There is also at least one pair of LRP, one germ cell–specific and one found only in somatic cells, mainly the liver. Compared with human LRP, 83% of the amino acids in the somatic cell–specific chicken protein (4,522 residues) are identical (Nimpf et al. 1994). Thus, somatic LRP from different animal classes have common structures; they also may share physiological roles. The current list of proposed functions, including transport of lipoproteins, α2-M, proteinase/proteinase inhibitor complexes, toxins, etc. (reviewed by Krieger and Herz 1994), can be expected to grow.

Less is known about the oocyte-specific LRP (Nimpf et al. 1994, Stefani et al. 1991). It is smaller and has not yet been characterized at the molecular level. Because it is a major component of the oocyte’s plasma membrane and its ligand-binding properties seem to overlap with those of other LDLR family members, it might serve a backup role for OVR. However, the fact that it cannot compensate for the lack of OVR in R/O hens speaks against this hypothesis, unless there is a conditional order of expression such that the absence of OVR-mediated early growth precludes subsequent expression of the germ cell–specific LRP. Current studies address this interesting possibility.

TRANSPORT OF HDL AND RETINOL ARE DIFFERENT

Small amounts of HDL particles, undistinguishable from serum lipoproteins, can be isolated from yolk, but they are not imported via OVR (Vieira et al. 1994). High density lipoprotein production, in contrast to that of major yolk precursors, is not induced by estrogen; rather, upon onset of laying production of apo AI seems reduced. This property indicates a metabolic fate of HDL different from that of the major, estrogen-inducible yolk precursors. As a matter of fact, a receptor-independent mechanism such as bulk-phase uptake via the general endocytotic activity of the oocyte surface could explain the small amount of bona fide HDL found in yolk. An interesting aspect of the presence of HDL in yolk will be to determine its role in lipid mobilization during embryogenesis.

The major serum carrier of vitamin A (lipid-soluble retinol) is the 21-kDa retinol-binding protein (RBP), which is complexed to the 54-kDa homotetrameric protein transthyretin (TTR). The holo-RBP/TTR complex has been found to accumulate in yolk (Vieira and Schneider 1993, Vieira et al. 1995) and specifically in coated vesicles, suggesting a receptor-mediated uptake mechanism. Almost unexpectedly, however, the complex is not recognized by OVR but, as recent results indicate, by an oocyte membrane protein (~115 kDa) (Vieira et al. 1995). Apparently TTR is the determining factor in this interaction, but rigorous demonstration of specificity of the putative receptor is still awaited. As is the case for HDL proteins, the production of neither TTR nor RBP is subject to specific expression of the receptors, as described here. For other components, we cannot exclude a similar role of receptors, because lipoprotein receptors are, at least under certain physiological conditions, capable of transporting additional ligands quite different from lipoproteins. Even certain cold viruses can gain cellular entry via LDLR family members (Hofer et al. 1994). As an alternative to receptor distribution, subtle structural differences in ligands could direct them to selected target cells, as is the case for α2-M and α2-M*.

We must learn more about the regulation of the individual genes to be able to understand exactly why several similar gene products are expressed in a single organism. Specifically, do common regulatory elements exist in genes of receptors that trigger growth of the germ cell? Do posttranslational modifications of ligands other than α2-M determine their cellular fate (that is, receptor recognition)?

We anticipate that answers to these questions emerging from studies in the laying hen will continue to produce novel insights into general aspects of lipoprotein metabolism, nutrition (at both the cellular and whole-animal levels), controlled cell growth and reproduction.

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