Chicken Oocytes and Somatic Cells Express Different Splice Variants of a Multifunctional Receptor*

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An abundant 95-kDa protein belonging to the low density lipoprotein receptor supergene family is essential for chicken oocyte growth by mediating the uptake of multiple plasma-borne yolk precursors. This receptor harbors at the amino terminus a cluster of eight tandemly arranged repeats typical of the ligand binding domains of members of this family and is designated low density lipoprotein receptor relative with 8 repeats (LR8). Here, we demonstrate by reverse transcriptase-polymerase chain reaction, Northern, and Western blot analyses that the chicken expresses two forms of LR8, which are generated by differential splicing of an exon encoding a serine- and threonine-rich region characteristic of LR8s, termed O-linked sugar domain. The female germ cell of the chicken expresses extremely high levels of the short form of LR8 (LR8−), i.e. the 95-kDa protein; in contrast, somatic cells express lower but detectable levels of the form containing the O-linked sugar domain (LR8+). The main sites of LR8+ expression in the chicken are the heart and skeletal muscle, i.e. the same tissues where LR8 mRNAs predominate in mammals; in addition, in situ hybridization demonstrates that a significant amount of LR8+ is produced in the hen’s ovarian follicular granulosa cells. We found no apparent functional difference between the two receptor forms; however, cell type-specific targeting of the multiple ligands of these receptors possibly relates to their respective expression on the cell surface.

Receptors belonging to the low density lipoprotein receptor (LDLR)1 supergene family play key roles in both systemic transport processes and oocyte growth in oviparous species (Schneider and Nimpf, 1993; Schneider, 1995). One of the recently characterized receptors in this group is a chicken protein whose established major functions are the transport of very low density lipoprotein (VLDL), vitellogenin, α2-macroglobulin, and lactoferrin into rapidly growing oocytes (Bujo et al., 1994, Jacobsen et al., 1995; Hiesberger et al., 1995). This chicken receptor, as well as its mammalian homologues (the so-called VLDL receptors), possesses in its ligand binding domain eight tandemly arranged complement-type repeats, each consisting of approximately 40 residues, that display a triple-disulfide bond-stabilized negatively charged surface. Receptors with eight ligand-binding repeats have been shown to interact with apolipoprotein (apo)B (George et al., 1987; Nimpf et al., 1988), apoE-rich VLDL and so-called β-migrating VLDL (Hayashi et al., 1989; Takahashi et al., 1992, Sakai et al., 1994), and the 39-kDa receptor-associated protein (RAP) (Battey et al., 1994; Wiborg Simonsen et al., 1994; Hiesberger et al., 1995). Because this broad ligand specificity does not allow for unambiguous nomenclature based on ligand definition, we here designate them LDLR relatives with eight repeats, LR8 in short, to distinguish them from the classical LDLRs (LR7), which harbor a cluster of seven such repeats.

Analysis of the mutant “restricted ovulator” hen, characterized by lack of oocyte growth that results in severe hyperlipidemia and sterility (Ho et al., 1974; J ones et al., 1975), revealed that its oocytes neither express normal LR8 nor bind VLDL or vitellogenin (Nimpf et al., 1989; Stifani et al., 1990a). These results indicate that LR8 is essential for yolk lipoprotein deposition into oocytes, a key requirement for reproduction. In this context, the expression of LR8 in mammals is of interest. In contrast to the major site of expression in oviparous species, the ovary, mammalian LR8s are found in tissues with active metabolism of fatty acids, such as skeletal muscle, heart, adipose tissue, and brain (Takahashi et al., 1992; Gåfvels et al., 1993, 1994; Oka et al., 1994b; Webb et al., 1994; J okinen et al., 1994). The chicken oocyte, on the other hand, avidly takes up yolk proteins including VLDL, not for immediate catabolism but rather for storage and later use as energy supply for the developing embryo.

Previously, we have identified by Northern blotting transcripts of a size similar to that of the oocyte LR8 in tissues of the chicken other than the ovary (Bujo et al., 1994). In particular, low levels of cross-reactive mRNA species were found in heart and skeletal muscle, i.e. the major sites of expression of mammalian LR8s (Takahashi et al., 1992; Gåfvels et al., 1993, 1994; Oka et al., 1994b; Webb et al., 1994; J okinen et al., 1994). Mammalian LR8 transcripts exist in splice variant forms: the major form is a mRNA that contains an exon coding for the so-called clustered O-linked sugar domain, but mRNAs coding for LR8 lacking that domain have also been identified (Sakai et al., 1994; Webb et al., 1994; J okinen et al., 1994). The sites of expression of the different forms in mammals have not been studied in detail. Since lack of the O-linked sugar domain is one

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LR8 causing the restricted ovulator phenotype, we have identified the chicken may produce low levels of a differently spliced LR8 transcript. Recently, in the course of studies on the mutation in LR8 causing the restricted ovulator phenotype, we have identified such a transcript because of its enhanced expression in mutant ovaries. Here, we demonstrate that chickens indeed produce these two differently spliced LR8 mRNAs, differing by 90 nucleotides, and the corresponding proteins in cell type-specific fashion. Interestingly, we could not detect a functional difference between the two receptor forms in vitro. However, since only rapidly growing oocytes express on their surface high levels of LR8 lacking the O-linked sugar domain, cell type-specific receptor production may be important for ligand targeting in vivo.

MATERIALS AND METHODS

RT-PCR Amplification of LR8 cDNA—Total RNA was extracted from adult (≥6 months old) female chicken tissues, and poly(A)⁺ RNA was isolated as described previously (Bujo et al., 1994). Single-stranded cDNA was synthesized from poly(A)⁺ RNA using SuperScript™ reverse transcriptase (Life Technologies, Inc.) and random primers. Four oligonucleotides (P1, -2, -3, and -4) were synthesized for use as PCR primers: P1, 5'-CCACCGGATATCCAGGAAGATTGGCC-3'; P2, 5'-AGGAGAAACCAGATGGCTCTG-3'; P3, 5'-ACCTGTAAGAACAACCTTGATAGCCTG-3'; and P4, 5'-TGGGAGAGATCTTCCAGACAC-3'. The nucleotide sequences of the primers were corresponding to nucleotide numbers 1396–1420 (sense), 2358–2382 (antisense), 2098–2122 (sense), and 2656–2680 (antisense) of chicken LR8—(see Fig. 1A). One-fourth of the synthesized cDNA was used for subsequent PCR amplifications. These were performed with the three pairs of primers, P1/P2, P3/P4, or P3/P2, respectively, using the GeneAmp PCR kit (Perkin-Elmer) on a Perkin-Elmer Thermal Cycler 480. PCR parameters were 94°C for 1 min, 60°C for 1 min, and 72°C for 2 min for 30 cycles. The amplified products were subjected to agarose gel electrophoresis and stained with ethidium bromide. The resulting amplified fragments were subcloned into the pGEM-T vector (Promega), and several clones from each fragment were isolated and sequenced using Sequenase (U.S. Biochemical Corp.).

Northern Blot Analysis of LR8 mRNA—Poly(A)⁺ RNA prepared from Northern Blot Analysis of LR8 mRNA was denatured using glyoxal dimethyl sulfoxide, separated by electrophoresis on agarose gels, and blotted onto Hybond C Extra membranes (Amer sham) using standard methods (Sambrook et al., 1989). Two independent cDNA probes for hybridization were prepared: 1) a mixture of two cDNA fragments; 2) two cDNA fragments containing the entire sequence of the previously characterized LR8 cDNA, corresponding to a site between the 5'-side (sense) and the 3'-side (antisense) of the 90-bp fragment, respectively. The two oligonucleotides were annealed, and the resulting DNA fragment was labeled with [³²P]UTP using the Klenow fragment of DNA polymerase (Sambrook et al., 1989). Northern blot analysis was performed as previously described (Bujo et al., 1994). Membranes were exposed to Fuji RX film with intensifying screens.

Western and Ligand Blot Analysis of LR8—Membrane fractions of the indicated tissues from adult chickens were prepared as described previously (Bujo et al., 1994). Samples were applied without heating or adding dithiothreitol (non-reducing conditions) to 4.5–18% gradient SDS-polyacrylamide gels according to Laemmli (1970). Electrophoresis, transfer to nitrocellulose membranes, and immunodetection were performed as described (Bujo et al., 1994). The rabbit IgG used for immunological analysis was prepared against a synthetic peptide corresponding to the last 14 amino acids of the deduced amino acid sequence of chicken LR8 (Bujo et al., 1994). Immunoblots were exposed to Hyperfilm™ ECL (Amer sham) for 10 s. In preparation for ligand

Results

Molecular Characterization of Splice Variant Forms of Chicken LR8—To test in chicken for the expression of splice variants of LR8 possibly including the O-linked sugar domain, RT-PCR was performed with two pairs of oligonucleotide primers. These (P1/P2, and P3/P4 in Fig. 1A) encompassed the region corresponding to the alternative splice site in human LR8 (Sakai et al., 1994; Webb et al., 1994). The products amplified with the primer pair P3/P4 from ovarian cDNA showed, in addition to the expected 583-bp band, a larger and much less abundant band (Fig. 1B, filled and open arrows, respectively). Sequence analysis of the amplified fragments revealed that the unexpected larger product contained an insert of 90 nucleotides between positions 2311 and 2312 of the previously characterized LR8-cDNA, corresponding to a site between the deduced amino acid sequence of chicken LR8 (Bujo et al., 1994). Immunoblots were exposed to Hyperfilm™ ECL (Amer sham) for 10 s. In preparation for ligand binding, nonspecific binding sites were blocked by incubating the membrane overnight at +4°C in buffer A (90 mM NaCl, 20 mM Tris, pH 7.4, 2 mM CaCl₂) containing 5% bovine serum albumin (BSA). Incubation with ligand were for 2 h at 23°C with labeled RAP (Hiesberger et al., 1995) (250 cpnm; 1 × 10⁶ cpnm) in buffer A containing 5% BSA. The membrane was then washed extensively with buffer A containing 1% BSA and 0.1% Tween 20, and subjected to autoradiography using Reflection™ film (DuPont NEN) with intensifying screens at –80°C for the times indicated.

In Situ Hybridization—Follicles from the ovaries of adult hens were dissected in ice-cold PBS, embedded in Tissue-Tek OCT compound (Miles) and immediately frozen with 2-methylbutane precooled in liquid nitrogen. Cryostat sections of 10-µm thickness were prepared, freeze-thawed onto glass slides pretreated with 2% 3-aminopropyltriethoxysilane, and stored at –70°C. For in situ hybridization, the sections were dried, washed twice with PBS for 5 min each, and treated with 1 µg/ml proteinase K for 5 min at 37°C. The slides were washed twice with PBS for 3 min, treated with 50 µM triethyamine and 0.2% acetic anhydride in PBS for 2 min at 23°C, and then washed twice for 2 min in 2 × SSC (Sambrook et al., 1989). Prehybridization was then carried out for 5 h at room temperature in a solution containing 5 × SSC, 0.1% SDS, 2% Denhardt’s solution, 250 µg/ml salmon sperm DNA, 250 µg/ml RNA, and 50% formamide. Digoxigenin-labeled antisense or sense RNA probes against the O-linked sugar region were prepared as follows. First, cDNA was prepared from heart mRNA by RT-PCR using two primers: 5'-TGGAGGAAGTCTTTCAGCCACAAGC-3' and 5'-CTCCAGGAGATCTTCCAGACAC-3'. annealing to the ends of the 90 nucleotides coding for the O-linked sugar domain. The amplified 90-nucleotide fragment was then separated on a 1.5% agarose gel, purified using the QIAEX DNA gel extraction kit (QIAGEN), and subcloned into the pGEM-T vector. The purified plasmids were then linearized for run-off RNA polymerase transcription in both directions with the DIG RNA labeling kit (SP6/T7, Boehringer Mannheim, no. 1175 025). Hybridization was performed overnight at 45°C with prehybridization solution containing 10% dextran sulfate and 300 ng/ml of the sense or antisense RNAs, and the slides were washed 3 × 10 min with 0.2 × SSC and 2 × 10 min with 0.1 × SSC at 50°C. The following operations were performed at 23°C. The slides were hybridized for immunodetection with biotin–streptavidin alkaline phosphatase Fab fragments (1:1000 dilution) in the same buffer for 2 h, and extensively washed first with buffer B and then with 100 mM NaCl, 100 mM Tris-HCl, pH 7.5 (buffer B), containing 3% normal goat serum and 1% BSA for 30 min, then exposed to anti-DIG-alkaline phosphatase Fab fragments (1:1000 dilution) in the same buffer for 2 h, and extensively washed first with buffer B and then with 100 mM NaCl, 100 mM Tris-HCl, 50 mM MgCl₂, pH 9.5. The alkaline phosphatase–tagged antibody was then detected by incubating the slides overnight with a precipitating purple alkaline phosphatase substrate (Boehringer Mannheim, no. 1442 074). The reaction was stopped by incubating the slides in 10 mM Tris, 1 mM EDTA, pH 8.0, and mounting them in Aquamount (BDH, Poole). Photographs were taken with a Zeiss Axiosvert 10 light microscope.

of the characteristics of the well-characterized chicken oocyte LR8, we considered the possibility that other somatic tissues of the chicken may produce low levels of a differently spliced LR8 transcript. Recently, in the course of studies on the mutation in LR8 causing the restricted ovulator phenotype, we have identified such a transcript because of its enhanced expression in mutant ovaries. Here, we demonstrate that chickens indeed produce these two differently spliced LR8 mRNAs, differing by 90 nucleotides, and the corresponding proteins in cell type-specific fashion. Interestingly, we could not detect a functional difference between the two receptor forms in vitro. However, since only rapidly growing oocytes express on their surface high levels of LR8 lacking the O-linked sugar domain, cell type-specific receptor production may be important for ligand targeting in vivo.
Sakai et al., 1994; Oka et al., 1994a, 1994b; Webb et al., 1994; Jokinen et al., 1994) but also in the chicken. Alignment with the known O-linked sugar domains of mammalian LR8s showed extensive identity with rabbit (70%) (Takahashi et al., 1992); human (67% according to Gåfvels et al., 1993), Sakai et al. (1994), and Webb et al. (1994); 63% according to Oka et al. (1994a)), mouse (57% according to Gåfvels et al. (1994); 53% according to Oka et al. (1994b)), and rat (53%) (Jokinen et al., 1994), respectively. In the following, LR8 lacking the O-linked sugar domain is designated LR8−, and the longer form is designated LR8+. 

Expression of Splice Variant mRNAs—We have already shown that chicken LR8− is the predominant, if not the only, form of the receptor present in oocytes (Nimpf et al., 1989; Barber et al., 1991; Bujo et al., 1994). However, Northern blot analysis revealed that muscle and heart, and possibly the ovary, express very low levels (0.5–1% of LR8− in the ovary) of a cross-reactive slightly larger mRNA (Bujo et al., 1994). In this context, in rabbit, mouse, and rat, LR8 mRNAs are abundant in heart, muscle, adipose tissue, and brain (Takahashi et al., 1992; Gåfvels et al., 1994; Oka et al., 1994b; Jokinen et al., 1994), and human receptor transcripts (Gåfvels et al., 1993; Webb et al., 1994) and rat protein (Jokinen et al., 1994) were reported to be expressed at high levels in ovary, heart, and muscle. However, it has not been determined which of the variant forms contribute to expression in the individual tissues. Our previous studies at the protein level (George et al., 1989; Hayashi et al., 1989; Barber et al., 1991; Bujo et al., 1994), which suggested exclusive expression of chicken LR8 in ovary, muscle, and heart of the variants described in Fig. 1 were significantly different (Fig. 2). The product corresponding to the amplified region in LR8+ (open arrow, of the expected size (375 bp)) predominates in muscle and heart (lanes 2 and 3) in distinct contrast to the ovary, where LR8− (closed arrow, product of the expected size (285 bp)) is by far the major form. In fact, the ratios of intensities of the two amplified fragments (LR8−/LR8+) were similar in muscle and heart but opposite to that in ovary. These results indicate that the splice variants are expressed at vastly different levels in these major sites of LR8 expression in the chicken. Only very few, if any, RT-PCR amplifiable transcripts were found in brain, kidney, and liver (data not shown; see below).

To obtain further evidence for the production of differently spliced transcripts, we next directly identified LR8− and LR8+ mRNAs in chicken tissues by Northern blot analysis. Previously, chicken LR8 transcripts in ovary, heart, and muscle have been shown, with probes corresponding to regions outside the O-linked domain, to have a size of ~3.5 kb (Bujo et al., 1994). However, the current results suggest that these ~3.5-kb
signals might actually represent both splice variant forms of chicken LR8. Therefore, we analyzed the original Northern blot with the 90-bp probe specifying exactly the O-linked sugar domain in chicken LR8+. With this probe, an expression pattern very different from that obtained with the probe corresponding to the common domains (Bujo et al., 1994) was obtained (Fig. 3). Under these conditions, the −3.5-kb signal, representing exclusively LR8+ transcripts, was detected only in muscle and heart but not in the ovary (upon prolonged exposure, there was a faint signal in ovary). These results reveal that there exist two forms of LR8 mRNA in the chicken, characterized by possessing or lacking a region coding for an O-linked sugar domain, and that their relative levels of expression vary greatly among different tissues and/or cells.

Variant Transcripts and Their Protein Products Are Expressed in Cell Typespecific Fashion—Next, we wanted to identify the protein products of the two LR8 transcripts. We have already shown that the cloned oocyte LR8 cDNA specifies a 95-kDa protein, characterized its ligand binding functions, and established its localization in oocytes (George et al., 1987; Nimpf et al., 1989; Steyer et al., 1990; Barber et al., 1991; Shen et al., 1993; Bujo et al., 1994; Jacobson et al., 1995; Hiesberger et al., 1995). Here, based on the results of RT-PCR and Northern blotting, we used an antibody directed against the C-terminal 34 residues of the receptor, which are common to LR8− and LR8+, to analyze the immunoreactive proteins in hearts of roosters and laying hens, and in ovarian follicles (Fig. 4). In the follicles, a very strong 95-kDa band was visualized in only 0.2 μg of total protein (lane 1, filled arrow). In both heart samples (lanes 2 and 3), a weak band was visible only upon analysis of at least 100-fold more protein than of follicle extract, comigrated with the major follicle protein, and a stronger band migrated slower (open arrow, approximate M0, 105,000). Taken together with the above results at the transcriptional level, these data lead us to conclude that the two immunoreactive proteins represent the splice variant forms of chicken LR8.

To gain insight into possible functional differences between the two receptor forms, we expressed them in heterologous somatic cell systems (for the expression of LR8−, cf. Bujo et al. (1994) and Jacobson et al. (1995)) and attempted to study their properties in cell surface binding studies. However, we consistently observed vastly different degrees of surface expression of the two forms, rendering such an approach inappropriate. Furthermore, the very low expression of LR8+ in tissues (cf. Fig. 4) precluded analysis by binding studies with physiological ligands. However, we already know that the best characterized non-lipoprotein ligand of the LR family, i.e. RAP, binds to LR8− with higher affinity than lipoproteins (Hiesberger et al., 1995). Thus, having identified the heart as a site of co-expression of LR8− and LR8+, we used 125I-RAP in ligand blots (Fig. 5) on extracts from this tissue; follicle extract served as positive control for the binding of RAP to LR8−. Clearly, LR8+ binds RAP as well; importantly, the ratio of intensities of the bands visualized via binding of 125I-RAP (Fig. 5) and 125I-VLDL (not shown) is equal to that detected by Western blotting (Fig. 4) or at the transcript level (Figs. 2 and 3).

The major site of expression of chicken LR8− is the growing oocyte (George et al., 1987; Hayashi et al., 1989; Barber et al., 1991; Shen et al., 1993; Bujo et al., 1994). Interestingly, previous results with binding studies indicated that receptor mRNA is concentrated in a narrow zone underlying the plasma membrane of vitellogenic oocytes (Bujo et al., 1994). The ovarian follicle preparations analyzed contained no hybridization signal (Fig. 6d) that receptor mRNA is concentrated under the plasma membrane of vitellogenic oocytes (Bujo et al., 1994). The ovarian follicle preparations analyzed consisted of the giant oocyte as well as somatic cells, in particular granulosa cells and theca cells that surround the oocyte. To determine whether the small amount of LR8+ transcript detected in follicles (Figs. 2 and 3) was derived from any or all of the somatic cells, we performed the following experiments (Figs. 5 and 6). For analysis of cell-specific mRNAs, the follicle was mechanically dissected (Gilbert et al., 1977) to isolate the granulosa cell monolayer (lanes 1) and the theca cells (lanes 2). We also analyzed mRNA isolated from undissected follicles containing oocytes at different growth stages (lanes 3 and 4). The mRNAs were hybridized with the probes reacting with both LR8− and LR8+ transcripts or with the O-linked sugar domain-specific probe, respectively. The results of Fig. 6 demonstrate that granulosa cells, as well as follicles (both 0.3 and 2 cm in diameter), contain detectable levels of LR8− transcripts; the oocyte-containing follicles clearly express high levels of LR8−, and the somatic cells express LR8+.

Since granulosa cells are present in the follicle preparations (see above), these results suggested that the minute amounts of LR8+ transcript seen in lanes 3 and 4, right panel (better visualized by prolonged exposure), are derived from these somatic cells. Direct evidence for exclusive expression of LR8+ in granulosa cells was obtained by in situ hybridization analysis of a vitellogenic follicle (Fig. 7). To this end, we used a 90-nucleotide probe corresponding exactly to the O-linked sugar domain of LR8+; the oocyte showed no reactivity with this probe. These findings are in accordance with our previous conclusion (George et al., 1987; Hayashi et al., 1989; Barber et al., 1991; Shen et al., 1993; Bujo et al., 1994) that within the follicle, it is the male germ cell that expresses LR8−.

**DISCUSSION**

LDL receptor family members containing a single cluster of eight complement-type repeats co-exist with other relatives in the same organism (Takahashi et al., 1992; Gáfvels et al., 1993, 1994; Sakai et al., 1994; Oka et al., 1994a, 1994b; Webb et al., 1994; Bujo et al., 1994; Jokinen et al., 1994). Of particular...
Binding of 125I-RAP to both LR8 splice variants. Chicken ovarian follicle membrane extract (lane 1) and laying hen heart extract (lane 2) were subjected to SDS-polyacrylamide gel electrophoresis and ligand blot analysis using 125I-labeled RAP as described under “Materials and Methods.” The binding of 125I-RAP to LR8+ (open arrowhead) and LR8− (closed arrowhead) was visualized by autoradiography for 24 h. Numbers on the left correspond to the molecular masses (kDa) of marker proteins.

Localization of splice variant forms of the LR8 mRNA in chicken ovarian follicles. 2 μg of poly(A)+RNA isolated from granulosa cell sheets (lane 1), the connective tissue surrounding the follicles (thecal layers) (lane 2), and from two undissected follicles containing oocytes of different sizes (lane 3, 0.3 cm, and lane 4, 2 cm in diameter) were used for Northern blot analysis on a 1.0% agarose gel.

Localization in a chicken follicle of the LR8+ mRNA. Cryostat sections of a chicken follicle (diameter, 6 mm) were subjected to in situ hybridization with an antisense (A) and a sense (B) digoxigenin-labeled RNA probe corresponding to the O-linked sugar domain of LR8+. The hybridized probe was detected using alkaline phosphatase-coupled goat anti-digoxigenin IgG as described under “Materials and Methods.” The horizontal cell layer, distantly stained in the middle part of panel A, represents the granulosa cells (g), which separate the theca cell layer (t) from the oocyte layer (o). Bar, 10 μm.

β-migrating VLDL, and RAP are ligands for all, and others, such as LDL and chylomicon remnants, for more than one of the known members of the family. The family thus far includes the classical LDLR, the so-called VLDL receptor, and the ambiguously named LDLR-related protein/α2-macroglobulin receptor(s) (LRP/α2MR) as well as two other members: SR-BI and LRP1b. Indeed, the ligand recognition spectra of these receptors preclude a useful nomenclature based on ligand designation; rather, we suggest to use the distinct structural features of these molecules (in particular the number of complement-type repeats) for their identification. Thus, here we designate these LDLR relatives (LRs) as LR7 and LR8 and propose the designations LR31 to LR36 for the large members of the family.

Mammalian LR8s are most abundantly expressed in heart, skeletal muscle, brain, and adipose tissue, but not in liver; one of the major sites of expression of LR7 and LR31 (Takahashi et al., 1992; Gåfvels et al., 1993, 1994; Oka et al., 1994b; Webb et al., 1994; J oikinen et al., 1994). Recently, it has been reported that the human ovary also expresses LR8 (Webb et al., 1994); this is of potential significance in the light of our finding that in the chicken, LR8 expression is by far the highest in oocytes (George et al., 1987; Hayashi et al., 1989; Barber et al., 1991; Shen et al., 1993; Bujo et al., 1994 and present data).

We have now discovered that those tissues which express LR8 in mammals also express this receptor in chicken, albeit at very low levels compared to the oocytes. In the chicken, the role of LR8 as an important mediator of oocyte growth via yolk deposition has been established both by biochemical and genetic evidence (George et al., 1987; Nimpf et al., 1989; Barber et al., 1991; Stifani et al., 1990b; Shen et al., 1993). However, in mammals neither the function nor the exact site of expression of LR8 in the ovary is known.

One difference in the structures of the major LR8s in mammals and the chicken oocyte LR8, i.e. the presence and absence of the O-linked sugar domain, respectively, prompted us to investigate in detail the expression of LR8 splice variants in the egg-laying animal. The results strongly suggest that somatic cells and tissues, in particular granulosa cells, heart, and skeletal muscle express predominantly LR8+, while the oocyte is by far the major site of LR8− expression. In the context of findings in the rat (J oikinen et al., 1994), which are compatible with a role of LR8 other than in lipoprotein metabolism, we interpret the results in the laying hen as follows. Oocytic LR8− is a multifunctional receptor, which transports lipoproteins and other components (Mac Lachlan et al., 1994; Jacobsen et al., 1995) required for embryonic growth; with the exception of unique yolk precursors, this may hold true for other tissues, including the mammalian ovary. However, a definitive answer to the physiological role of LR8− in mammals cannot be provided at present. LR8+, on the other hand, is likely to perform similar functions in mammals and aviparous species, as they express this receptor in the same tissues. Again, a physiological role of LR8−, not directly related to the transport of lipoproteins, has been suggested (J oikinen et al., 1994).

It is noteworthy that for rat and mouse LR8+, possible alternative splicing outside the region considered here has been discovered during splicing studies (Gåfvels et al., 1994; J oikinen et al., 1994). Northern blot analysis in rabbit, rat, mouse, and human tissues revealed, in addition to the transcripts corresponding to LR8−/LR8+, transcripts of 9.5 kb in rabbit (Takahashi et al., 1992), 9.1 kb in rat (J oikinen et al., 1994), 4.5 and 7.9 kb (Oka et al., 1994b) or 8 kb (Gåfvels et al., 1994) in mouse, and 6.0 kb (Gåfvels et al., 1993) or 5.2 kb (Webb et al., 1994) in human. The exact nature of these transcripts has not been delineated; in rats, all transcripts are
coordinated regulation in response to changes in the thyroid status (Jokinen et al., 1994). We have not detected any other but the 3.5-kb LR8 transcripts in the chicken.

In the light of our limited knowledge of the true physiological ligand(s) of LR8s, we can only speculate about the functional role of their O-linked sugar domain. In this context, preliminary experiments indicate that LR8- expressed in COS-7 or LR7-negative Chinese hamster ovary cells leads to detectable but poor surface activity (Bujo et al., 1994), while LR8+ is much more efficiently presented at the cell surface (data not shown). Thus, due to the very low levels of LR8+ protein in tissues, and as a result of these vastly different levels of surface expression of LR8+ versus LR8- in transfected cells, we could only analyze binding of the ligand with the highest affinity for the receptors (Fig. 5). Based on current results, we consider it unlikely, but nevertheless possible, that the ligand specificities of the two variant receptors differ sufficiently to direct different ligands to oocytes and somatic cells, respectively. Rather, it appears that regulation of surface expression of the variants in different cells or tissues holds the key to ligand targeting (Schneider, 1995). In strong support of this notion, deletion of the two variant receptors differs sufficiently to direct different ligands to oocytes and somatic cells, respectively. Rather, it appears that regulation of surface expression of the variants in different cells or tissues holds the key to ligand targeting (Schneider, 1995).

3Thus, due to the very low levels of LR8+ protein in tissues, and as a result of these vastly different levels of surface expression of LR8+ versus LR8- in transfected cells, we could only analyze binding of the ligand with the highest affinity for the receptors (Fig. 5). Based on current results, we consider it unlikely, but nevertheless possible, that the ligand specificities of the two variant receptors differ sufficiently to direct different ligands to oocytes and somatic cells, respectively. Rather, it appears that regulation of surface expression of the variants in different cells or tissues holds the key to ligand targeting (Schneider, 1995).

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