Receptor-Ligand Interaction between Vitellogenin Receptor (VtgR) and Vitellogenin (Vtg), Implications on Low Density Lipoprotein Receptor and Apolipoprotein B/E

THE FIRST THREE LIGAND-BINDING REPEATS OF VTGR INTERACT WITH THE AMINO-TERMINAL REGION OF VTG*

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The vitellogenin receptor (VtgR) belongs to the low density lipoprotein receptor (LDLR) gene family. It mediates the uptake of vitellogenin (Vtg) in oocyte development of oviparous animals. In this study, we cloned and characterized two forms of Oreochromis aureus VtgR. Northern analysis showed that VtgR was specifically expressed in ovarian tissues. However, reverse transcription-PCR indicates that either there are trace levels of expression of VtgR or a homolog of LDLR exists in nonovarian tissues. The VtgR is highly homologous to the very low density lipoprotein receptor. To better understand the mechanism by which similar structural modules in the ligand-binding domain bind different ligands, we used the yeast two-hybrid system to screen for the minimal interaction motifs in Vtg and VtgR. The amino-terminal region of the lipovitellin I domain of Vtg interacts with the ligand-binding domain of VtgR. The first three ligand-binding repeats of the receptor were found to be essential for ligand binding. Computational analysis of the binding sequence indicates that Vtg has a similar receptor-binding region to apolipoprotein (apo) E and apoB. Site-directed mutagenesis of this region indicates electrostatic interaction between Vtg and its receptor. Sequence analysis suggests the coevolution of receptor-ligand pairs for the LDLR/apo superfamily and suggests that the mode of binding of LDLR/very low density lipoprotein receptor to apoB and apoE is inherited from the electrostatic attraction of VtgR and Vtg.

During vitellogenesis, vitellogenin (Vtg), a lipophosphoglycoprotein, is synthesized in the liver and transported to the ovary via blood circulation. This major yolk precursor protein binds to vitellogenin receptor (VtgR) on the surface of oocytes and is taken up by receptor-mediated endocytosis (1). A large amount of Vtg accumulates in the oocytes within a relatively short time. Once in the oocytes, Vtg is cleaved into yolk proteins, namely, lipovitellin (LV) and phosvitin, which are stored as nutrients for the developing embryo (2). Sequence analysis showed that the amino-terminal 700 amino acids of Vtg and apolipoprotein (apo) B-100 are homologous, although the similarity is limited (3). Coincidentally, Vtg also binds lipids and transports them into the oocytes. The sequence and functional relationship of these two proteins support the idea that they have a common ancestor.

VtgR belongs to the low density lipoprotein receptor (LDLR) family (4). The members of this family bind to various ligands and are involved in lipid metabolism in both vertebrates and invertebrates. These receptors have common structural features (5, 6) including (i) cysteine-rich ligand-binding repeats (LBRs), (ii) cysteine-rich epidermal growth factor precursor (EGFP)-like repeats spaced by cysteine-poor spacer regions, (iii) a single transmembrane domain, and (iv) a short carboxy-terminal cytoplasmic tail. In addition, a short region highly enriched in serine and threonine residues may exist in some receptors. The number of LBRs varies among different receptors. LDLR contains seven LBRs, whereas very low density lipoprotein receptor (VLDLR) and VtgR in vertebrates have eight LBRs. Larger receptors such as LDLR-related protein and megalin have more than 30 LBRs in several clusters (7, 8). Each LBR consists of about 40 amino acids including 6 cysteine residues, participating in the formation of three disulfide bonds, which are crucial for its proper folding (9). At the carboxy terminus of each LBR, there is a consensus acyclic tripeptide, Ser-Asp-Glu (SDE). Recent structural study by NMR and x-ray diffraction analysis of LBRs 1, 2, 5, and 6 from LDLR have revealed that the side chains of many of the aspartate and glutamate residues in the consensus peptides are involved in coordinating the calcium ion into a folded calcium cage (10–15).

The binding sites of Vtg for VtgR were presumed to be located on the lipovitellin I domain, LV1 (16). Residue modification studies showed that lysine and arginine residues were important for binding with the acidic clusters in LBR of VtgR through ionic interactions (17). However, new structural studies of LBR (10–15) indicate that those acidic residues might not be accessible to Vtg. This necessitates a reassessment of cur-
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experimental procedures

Cloning of VtgR cDNA—mRNA was purified from previtellogenic ovaries of Oreochromis aureus using Oligotex Direct mRNA kit (Qiagen). Reverse transcription was performed with oligo(dT) using the Thermostart RT-PCR system (Invitrogen). Two primers (A1, 5′-GGTTCGCAAGCGGTIG3′-A2; 5′-AAGTCGGCATCTCCTC3′-I = osine) designed from the consensus sequence of Vtg/VLDDL and other species such as mouse, rabbit, rat, chicken, and Xenopus were used for PCR using platinum Taq polymerase (Invitrogen) to obtain fragment A in the EGF-like domain. Two other fragments, fragments B and C, adjacent to and overlapping the 5′ and 3′ ends of fragment A, were obtained by PCR amplification using B1 (5′-GGACAGTGIGGIQIGCACCC3′-A2) and B2 (5′-GGTGTCCTCTGCGTCG3′-C1) or (5′-GGTGTCCTCAGTCTCGAG3′-C2) and C2 (5′-ATGCGIGTAIGTG3′-GTG3′), respectively, as primers. The 5′- and 3′-RACE system (Roche Molecular Biochemicals) was used to obtain full-length VtgR cDNA. The primer for 3′-RACE was C1. The primers for 5′-RACE were D (5′-GCAAGTGCAAGCGGTIGTATATG3′-E) and E (5′-GGTGTCCTCAGTCTCGAG3′-C2). All PCR products were cloned into pGEM-T easy vector (Promega) and sequenced (Fig. 1). Full-length VtgR cDNA was obtained by PCR using high-fidelity platinum Pfx polymerase (Invitrogen).

Northern Analysis—The total RNAs from ovary, liver, muscle, brain, spleen, and intestine were purified using Trizol reagent (Invitrogen). Aliquots of 20 μg of total RNAs were resolved in 1% agarose formaldehyde gel. The separated RNAs were transferred onto a nylon membrane (GeneScreenPlus; PerkinElmer Life Sciences) using capillary transfer. The membrane was UV-cross-linked, prehybridized in DIG Easy Hyb with DIG-labeled PCR fragment B as probe. Hybridization was performed at 42 °C for 18 h. The membrane was washed twice in 2× SSC, 0.1% SDS for 15 min each at room temperature and twice in 0.5× SSC, 0.1% SDS for 15 min each at 65°C. The membrane was washed overnight at 50°C in Easy Hyb with DIG-labeled PCR fragment B as probe. After hybridization, the membrane was washed twice in 2× SSC, 0.1% SDS for 15 min each at room temperature and twice in 0.5× SSC, 0.1% SDS for 15 min each at 65°C. The membrane was washed with 0.1× SSC, 0.1% SDS for 30 min at room temperature and twice in 0.1× SSC, 0.1% SDS for 15 min each at 65°C. The membrane was washed in blocking solution followed by a 30-min incubation in anti-DIG-AP (Roche Molecular Biochemicals) diluted 1:10,000 in blocking solution. After incubation, the membrane was washed twice in washing buffer (100 mM maleic acid, 150 mM NaCl, 0.3% Tween 20, pH 7.5), and the hybridized band was detected by chemiluminescence (CSPD reagent; Roche Molecular Biochemicals). The X-ray film was also labeled with DIG and used as a normalization control.

RT-PCR Analysis—Approximately 500 ng each of mRNA from ovary, liver, muscle, brain, spleen, and intestine, isolated using the Oligotex Direct mRNA kit, was reverse transcribed with oligo(dT) and Thermostart reverse transcriptase. The cDNAs were amplified by PCR using 0.2 μM concentrations of each of the following primers: C1 (5′-GGTGTCCTCAGTCTCGAG3′-G) and G (5′-ATGCCGGGTACCGTGTG-3′) primers that are specific for VtgR, in a final volume of 50 μl containing 2 units of platinum Taq DNA polymerase, 20 mM Tris-HCl (pH 8.4), 50 mM KCl, and 1.7 mM MgCl2. The PCR reaction was carried out by incubating the samples at 94°C for 1 min, followed by 35 cycles of 94°C for 30 s, 52°C for 30 s, and 72°C for 1 min.

Two Forms of VtgR cDNA Were Identified—To clone VtgR cDNA, RT-PCR was performed based on primers designed to obtain a 688-bp fragment A in the EGFP-like domain (Fig. 1).
The sequence information was used to design another two pairs of primers. By PCR, two fragments (fragments B and C) overlapping fragment A were cloned and sequenced. Fragment B was 734 bp, encompassing LBRs 5–8. Two forms of fragment C, of 580 and 520 bp, were observed in the PCR product. The corresponding amino acid sequence shows that the 580-bp cDNA has the O-linked sugar domain, which is a threonine-serine-rich region. The 520-bp fragment lacks this region. The primers for 5′ and 3′-RACE were used to obtain the full-length cDNA sequence (GenBank™ accession number AF514281). The analysis of cDNA sequences revealed two open reading frames of 2500 and 2560 bp, encoding 800 and 820 amino acids, respectively. The alignment of the amino acid sequences of VtgR to the VLDLR and VtgR from other species showed high homology.

**Differential Expression of VtgR in Various Tissues**—To examine the transcription of the VtgR gene, RT-PCR was carried out with C1 and G primers flanking the O-linked sugar domain. All the tissues consistently exhibited two forms of VtgR mRNA of 420 and 360 nucleotides (Fig. 2A). The size difference between these two forms was probably attributable to the differential splicing of a short region, as observed in the comparison of the sequences of these two PCR products. By aligning with VtgR and VLDLR genes of other species, this region was found to be located in the O-linked sugar domain. Interestingly, when Northern analysis was performed using fragment B (containing LBRs 5–8) as the probe, only the ovary exhibited one transcript of VtgR mRNA of 3.3-kb nucleotides (Fig. 2B). These data indicate that the VtgR gene was transcribed in both ovarian and nonovarian tissues. However, only the ovary VtgR mRNA is sufficiently abundant to be detectable by Northern analysis. In our study, all the RT-PCR products from different tissues share the same sequence. This result excludes the possibility that the RT-PCR amplified the LDLR that is homologous to VtgR. When fragment C was obtained from ovarian mRNA by RT-PCR, the dominant product was the one lacking the O-linked sugar domain. Additionally, in 3′-RACE, the same product was obtained (data not shown). These data indicate that the major form of VtgR in the ovary lacks the O-linked sugar domain. In chicken, the VtgR was reported to function as VLDLR or VtgR in different piscine tissues.

**The LBR of VtgR Is Sufficient for Vtg Binding**—In an effort to locate the important interactive domains between VtgR and Vtg, we first examined the interactions between full-length VtgR and different domains of VtgR using yeast two-hybrid assays to confirm that the yeast two-hybrid assay is suitable for the study of interaction between Vtg and VtgR. Although two forms of VtgR were identified in the ovary, the results indicate that the major form lacks the O-linked sugar domain. Therefore, the VtgR constructs used in the interaction studies were from the VtgR lacking O-linked sugar domain. Three constructs of VtgR, full-length VtgR, the extracellular part of VtgR, and the LBR of VtgR, were tested for binding to Vtg. The quantitative β-galactosidase assay confirms the binding of the ligand-binding domain of VtgR or extracellular part of VtgR to full-length Vtg in vivo (Fig. 3). However, full-length VtgR did not show binding with full-length Vtg. This may be due to hindrance by the transmembrane domain on the transportation of VtgR fusion protein into the nucleus, where the interaction-dependent activation of reporter gene transcription occurs in the yeast two-hybrid assay. Although some earlier studies have indicated that the EGF-like domain might be required for ligand binding (21, 22), our results clearly confirm that deletion of other parts of VtgR did not disrupt the binding between LBRs and Vtg. The controls showed that the interactions were not within the fusion proteins and the activation domain or DNA-binding domain encoded by the pGADT7 or pGBK77 vector. These data suggest that the LBR itself is sufficient for binding Vtg.
EGFP homology domain is not necessary for the receptor-ligand interaction. Therefore, further studies on the receptor focused on the LBD.

**LBRs 1–3 of VtgR Are Involved in the Interaction with Vtg**—To determine which subdomains of the receptor are important to Vtg binding, 25 different fragments of the VtgR ligand-binding domain were cloned into pGBKT7. The interaction between the pGBK7-VtgR deletion constructs and pGAD-Vtg was tested by the yeast two-hybrid assay. All the conditions used in the transformation of yeast and the enzymatic assays for all the constructs were the same. This made the yeast two-hybrid assay semiquantitative. The difference in the β-galactosidase activities most probably arose from the difference in the interaction.

Five carboxyl-terminal deletion constructs of LBD, including LBR1–7, LBR1–6, LBR1–5, LBR1–4, and LBR1–3, did not disrupt the binding to Vtg. The two carboxyl-terminal deletion constructs of LBD, namely, LBR1–2 and LBR1, lost binding to Vtg (Fig. 4). When the first LBR was deleted in constructs LBR2–8, LBR 2–7, LBR 2–6, and LBR2–3, dramatic drops in the β-galactosidase activities were observed. LBR2 alone did not show significant binding to Vtg. When both the first and second LBRS were deleted, all 11 constructs lost binding of Vtg. It is therefore clear that the first three LBRS are most important for binding Vtg. Without LBR3, the construct LBR1–2 exhibited weaker interaction with Vtg. On the other hand, constructs containing only LBR3 did not bind Vtg. Similarly, the deletion of LBR1 from various combinations of LBD (viz.,

![Graph showing interaction between Vtg and different domains of VtgR](image)

![Graph showing interactions between LBD of VtgR and different deletion constructs of Vtg](image)
constructs LBR2–8, LBR2–7, LBR2–6, LBR2–4, and LBR2–3) attenuated but did not abolish the interaction with Vtg. These data indicate that the interaction between LBD and Vtg utilizes more than one LBR in the LBD. There may be individual binding sites in the first three LBRs. However, LBRs 1–3 are not mutually exclusive in their interaction with Vtg. It is possible that one single binding site is insufficient to stabilize the interaction. Thus, the minimum ligand-binding region is a combination between either LBR1 or LBR2 or LBR3 or LBR1 and LBR3. Thus, the region required for maximal binding of Vtg is LBRs 1, 2, and 3.

The LBR of VtgR Interacts with the Amino-terminal Fragment of Vtg between Ala162 and Ile246—To define the actual binding sites in Vtg for VtgR, seven 3’ deletion constructs of Vtg together with full-length Vtg were tested for interaction with the VtgR LBRs (Fig. 5). The LBRs interacted with full-length Vtg and interacted to different extents with the following deletion constructs: pGADVtg-ClaI (1584 amino acids), pGADVtg-XmaI (1505 amino acids), pGADLV1 (1089 amino acids), pGADVtg-BamHI (286 amino acids), and pGADVtg-EcoRI (246 amino acids). No interaction was observed with pGADVtg-SacI (162 amino acids) and pGADVtg-XhoI (52 amino acids). Deletion of Vtg upstream of 246 amino acids completely abolished its binding to VtgR. Thus, the Vtg binding site is either between amino acids 162 and 246 or between amino acids 1 and 162. The deletion from 162 to the carboxyl terminus may cause a drastic change in the configuration of the upstream region that is necessary for binding with VtgR. Therefore, to delineate the actual binding site, the amino-terminal 84-amino acid fragment (flanked by SacI and EcoRI), VtgSE, containing amino acids 162–246 was subcloned into the pGAD vector to examine its interaction with LBR. The β-galactosidase assay shows that this VtgSE fragment has a binding capacity similar to that of full-length Vtg (Fig. 6A), thus indicating that this region contains the crucial binding site for VtgR and that deletion of other parts of Vtg does not seem to affect its binding to receptor.

Direct Binding of LBR and Vtg in Vitro—To test whether the interaction between LBD and the short fragment VtgSE is direct and to confirm the results of the yeast two-hybrid assay, the LBD and VtgSE were tested by in vitro pull-down assay. LBR and VtgSE were expressed as GST fusion protein and His fusion protein, respectively, in E. coli. The GST and GST-LBR proteins were immobilized on the glutathione-Sepharose beads and incubated with VtgSE cell lysate. After extensive washing, the elution of GST-LBR contained VtgSE, whereas the elution of GST did not (Fig. 6B). Thus, it is clear that the LBR and VtgSE interact directly in vitro. In the presence of DDT, the GST-LBR did not bind VtgSE. This suggests that the interaction was dependent not only on the charged residues in LBRs but also on the integrity of the disulfide bonds, which are crucial for the three-dimensional structure to confer the functional LBD.

Site-directed Mutagenesis of Vtg Reveals the Importance of Lys185 for Interaction with VtgR—By aligning the sequence of the binding site in Vtg with the apoB major binding site and the apoE binding site for LDLR (23, 24), a short motif in Vtg with basic residues was observed (Fig. 7A). This suggests that vitellogenin may utilize the same mechanism as apoB and apoE to bind the VgR. To test this hypothesis, we mutated the positively charged amino acids at His132, Lys185, and Lys187 in VtgSE into
alnine. These residues are correspondingly important in apoB and apoE for binding to their respective receptors. We examined the effect on binding of VtgR by yeast two-hybrid assay. The mutation constructs VtgSE(H182A) and VtgSE(K187A) showed similar binding of LBD and LBR1–3. However, the binding between the mutation construct VtgSE(K185A) and either LBD or LBR1–3 is attenuated (Fig. 8). This result highlights the importance of the residue Lys185 in the interaction between Vtg and VtgR.

When the sequences of different VtgR LBRs were aligned (Fig. 7C), the signature sequences of the LDLR superfamily emerged as well conserved. In LBR3, the three conserved acidic residues are EDE, whereas in all other LBRs, the sequence is SDE. Bajari et al. (25) have searched for the minimal binding site in chicken VtgR for receptor-associated protein (RAP) and proposed that the EDE region in LBR3 might be important for ligand binding because it has the highest negative charge density. Consistently, our study has empirically confirmed that LBR3 is critical to the interaction with Vtg. To demonstrate a potential relationship between this subtle difference in the acidic residues in LBR3 and its affinity for Vtg, we mutated the E144S in both the LBD and LBR1–3 and tested their interactions with Vtg and VtgSE. However, this mutation did not affect the binding between LBD/LBR1–3 and Vtg/VtgSE. Furthermore, the mutation of SDE to EDE in LBR6 of LBR4 did not show any gain of function for binding Vtg (data not shown). Hence, contrary to chicken VtgR, the increased negative charge density in EDE of piscine VtgR is not related to ligand binding. Instead, we propose that the EDE region in LBR3 is more likely to play a role in the formation of the calcium cage, which is also found in other LBRs of LDLR (9).

**DISCUSSION**

In this study, using RT-PCR and 5’- and 3’-RACE, we cloned a piscine VtgR, a member of the LDLR family. The VtgR contains eight low density lipoprotein complement type A ligand-binding repeats. Northern analysis indicates that the VtgR transcript. This may be attributable to the difference between O-linked sugar domain, was also reported in other species and in other tissues (28). The existence of two forms of mRNA, which is subtle and very short (60 bp),
compared with full-length VtgR mRNA (~3.5 kb). Previous studies indicated that lack of this 60-bp region would not affect the binding of the receptors to their ligands. The presence of VtgR transcript in the nonovarian tissues suggests that there are piscine versions of VLDLR/LDLR, which have distinct functions from VtgR in the ovarian tissues. However, the mRNA levels of both forms of VtgR, with or without O-linked sugar domain, in nonovarian tissues are very low. It is still not clear whether this trace amount of transcription has any distinct biological significance.

The ligand-binding domains in members of the LDLR family have been studied extensively. It is interesting that the highly conserved ligand-binding domains can form promiscuous interactions with various ligands. Analysis of the naturally occurring and engineered mutations in LDLRs has shown that LBRs 2–6 are required for the binding of apoB, whereas the binding of apoE depends critically on LBR5. Previous efforts to search for the minimal binding domain in chicken VtgR indicated that LBR3 is important for binding of RAP (25). It was reported that the minimal binding unit in LDLR for RAP binding was any two adjacent repeats (33). There is, however, no report on the participation of LBRs in the interaction with Vtg. Using the yeast two-hybrid system, we show that binding to Vtg requires LBRs 1, 2, and 3. Savonen et al. (34) reported that RAP interacts equally well with repeats 1–3 and 1–5, but not with repeats 6–8 in VLDLR. An analysis by Mikhailenko et al. (35) suggested that the RAP binding site of VLDLR is located within the amino-terminal four repeats and also suggested that the first three repeats are especially important for RAP binding. Consistent with these reports, we also observed the importance of the first three LBRs to binding Vtg. LBR3 is the only repeat that contains EDE instead of the consensus sequence, SDE. In chicken VtgR/VLDLR, this acidic region has been proposed to bind the basic residues on RAP because it has the highest negative charge density (25). However, according to the known structures of LBRs 1, 2, 5, and 6, many residues in these regions are involved in the formation of calcium cage and are most likely not accessible to the ligand. In our study, site-directed mutagenesis in LBR3 affirmed that the change of EDE sequence to SDE did not affect the binding of Vtg. In addition, mutagenesis of SDE to EDE in LBR6 failed to gain the function of binding Vtg. This also indicates that EDE sequence alone is insufficient for Vtg binding. The structural determinants in different LBRs, viz., three disulfide bonds and calcium cage-forming amino acids, are highly conserved. The backbone structures of those LBRs are expected to be similar. Thus the specificity of the LBRs to different ligands may be attributable to the nonconserved acidic groups, which are still available for interaction. The GST pull-down assay in the presence of dithiothreitol confirms the importance of disulfide bonds toward the nonconserved acidic regions, which are still available for interaction. The GST pull-down assay in the presence of dithiothreitol confirms the importance of disulfide bonds toward an appropriate architecture of LBRs for effective binding of Vtg (Fig. 6D). This clearly demonstrates that the primary sequence of LBD is not sufficient for binding Vtg. The three-dimensional structure of LBD must constitute the correct surface patch, which can recognize Vtg.

Both Vtg and apolipoprotein belong to the large lipid transfer protein, and they were found to be evolutionarily related not only in function but also in sequence (36). In our study, we found that the binding region, VtgSE, showed a pattern similar to the binding sites of apoB and apoE. The site-directed mutagenesis of the basic residues in VtgSE further confirmed that the positively charged residue, Lys185, plays a crucial role in receptor binding. Lys185 is highly conserved in Vtgs. In the single deviant case in chicken Vtg, the lysine residue is also substituted with a basic residue, arginine (Fig. 7B). These observations strongly suggest that electrostatic interaction is involved. The existence of common basic residues in the receptor-binding region of Vtg, apoB and apoE on one hand, and the inaccessibility of the conserved acidic triplets in LBRs on the other, is apparently contradictory to expectations for electrostatic interactions. Nevertheless, it is still possible that electrostatic interactions between receptors and ligands exist via other negatively charged residues present within LBRs. This assumption is supported by reports that all the LBRs in LDLR have negatively charged surface patch (37).

The crystal structure of lamprey Vtg is already known (38, 39). By alignment of the amino acid sequence, the receptor-binding region, VtgSE, was located to the LV1n part of lamprey Vtg. LV1n contains 4 α helices and 12 β strands, 11 of which form a barrel-like conformation. It is not clear whether this VtgSE receptor site is in the β strands or the α helices. Using the secondary structure prediction program, we predict that VtgSE is located in the α helices (data not shown). In both apoB and apoE, the receptor-binding region is in the α helices conformation, thus suggesting that they may utilize a similar mechanism for binding.

In nature, Vtg exists as a dimer containing symmetric binding sites (40). In our study, the receptor-binding site was found to be located in the VtgSE region. Comparable activities in the yeast two-hybrid assays observed with VtgSE and full-length Vtg indicate that the VtgSE region might be the only binding site in Vtg. The early study indicated that Vtg dimer and VtgR interact in a 1:1 stoichiometry (14). Therefore, to bind the Vtg dimer, VtgR must contain two Vtg-binding sites. This was supported by the results of our studies in deletion constructs of VtgR. LBRs 1–3 may contain more than one binding site for Vtg. Fig. 9 illustrates our proposed model of Vtg-VtgR interaction. Two molecules of Vtg dimerize through the dimerization domain in LV1 (39). The symmetric receptor binding sites in Vtg bind to two sites in the VtgR LBRs 1–3. The carboxyl-terminal of Vtg will form the lipid-binding cavity to transport lipid into the oocytes. Thus transportation of 2 Vtgs/VtgR molecule into the oocytes presents an efficient mechanism to meet
the temporal demands of oogenesis.

The receptor-ligand pairs of VtgR-Vtg and LDLR/VLDLR-apolipoprotein have existed together in fish, amphibians, reptiles, and birds for millions of years (42, 43). In the lower species, including insects and nematode, VtgR was the predominant form of receptor. The existence of VtgR in more ancient species such as Caenorhabditis elegans indicates that the LDLR and VLDLR might have evolved from VtgR by mutation and gene shuffling, and Vtg is the ancestor of apolipoprotein. It is accepted that apoB and Vtg share a common ancestor. However, the amino-terminal location of the receptor-binding site in Vtg in contrast to apoB, which contains receptor-binding sites in the carboxyl-terminal. This difference may arise from the structural change for better adaptation to the function. The mode of binding of LDLR/VLDLR to apolipoprotein is inherited from the electrostatic attraction of VtgR-Vtg. However, the sequence changes in apoB, especially in the region for lipid binding, probably facilitate the specific function of lipid transportation (39, 44). Responsively, the mutation in LDLR/VLDLR in different LBRs accumulates for the improved specific binding to apoB, thus LDLR and VLDLR utilize different LBRs for apoB binding. This coevolution of receptor-ligand pairs probably creates the current functionally and structurally distinct receptors and ligands. The receptor-binding sites in many ligands of LDLR family members are still unknown. We predict that they also contain domains rich in basic residues for binding electrostatically with their cognate receptors.

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