Elements of Neural Adhesion Molecules and a Yeast Vacuolar Protein Sorting Receptor Are Present in a Novel Mammalian Low Density Lipoprotein Receptor Family Member*

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Normal cell development depends to a large part on multifunctional proteins that have evolved by recombination of proven modular elements. We now have discovered and characterized in rabbit such a multi-domain protein, and classify it as novel member of the low density lipoprotein (LDL) receptor gene family. The extracellular portion of the ~250-kDa membrane protein, termed LR11, contains a cluster of 11 LDL receptor ligand binding repeats, a group of 5 LDL receptor "YWTD" repeats, a large hexarepeat domain of structural elements found in neural cell adhesion molecules, and a domain with similarity to a yeast receptor for vacuolar protein sorting, VPS10. The cytoplasmic domain exhibits features typical of endocytosis-competent coated-pit receptors. The mosaic, and presumably multifunctional, receptor is expressed abundantly in brain, in particular the hippocampus, dentate gyrus, and cerebral cortex, and is present at significant levels in liver, adrenal glands, and testes. Western blotting of tissues and ligand blotting of LR11-transfected cells demonstrated that the novel protein binds apolipoprotein E-containing lipoproteins. In contrast to the LDL receptor, hepatic expression of LR11 is unaffected by hyperlipidemia. The identification of this highly conserved and superbly complex protein offers the opportunity to gain new insights into the emergence of multifunctional mosaic proteins akin to the ever expanding LDL receptor gene family.

The discovery of the LDL receptor (LDLR)¹ and its functional and genetic characterization were hallmark in research on lipoprotein transport pathways and led to the molecular delineation of the common hereditary disease, familial hypercholesterolemia (Brown and Goldstein, 1986). In contrast to the clarity of our understanding of LDLR function, the physiological roles of new LDLR homologues, which have been identified in the past decade and form the ever expanding LDLR gene family, are far from being established. Their apparently much more diverse functions are indicated by a wide range of possible ligands, which include spent, biologically inactive and/or unwanted plasma carrier complexes and complex proteins, certain toxins, yolk precursors, as well as circulating plasma lipoproteins (reviewed in Krieger and Herz (1994), Schneider and Nimtz (1993), Moestrup (1994), and Schneider (1995)). The bewildering array of ligands they can bind, at least in vitro, is probably due to their highly variable content of different numbers and combinations of common structural elements.

To date, these identified common structural modules are (i) the so-called "LDLR ligand binding repeats," complement-type domains consisting of ~40 residues displaying a triple-disulfide bond-stabilized negatively charged surface; (ii) epidermal growth factor precursor-type repeats, also containing six cysteines each; (iii) modules of ~50 residues each, most often in groups of five, with a consensus tetrapeptide, Tyr-Try-Thr-Asp (LDLR "YWTD" repeats); and (iv) in the cytoplasmic region, signals for receptor internalization via coated pits, containing the consensus tetrapeptide Asn-Pro-Xaa-Tyr (NPXY) (Chen et al., 1990).

The best characterized binding domain is that of the LDLR, which consists of seven complement-type repeats and recognizes apolipoprotein (apo) B and apoE (Russel et al., 1989). LDLR family members harbor from 1 to 4 clusters with varying numbers of ligand binding repeats. To date, the family includes in addition to the LDLR, the very low density lipoprotein (VLDL) receptor (Takahashi et al., 1992; Bujo et al., 1994), Drosophila yolkless (Yl) (Schonbaum et al., 1995), LDLR related protein/α2-macroglobulin receptor (LRP) (Herz et al. 1988), the Caenorhabditis elegans LRP-like gene (Yochem and Greenwald, 1993), and gp330/megalin (Saito et al., 1994). Extensive studies on the ligand specificities of these proteins have shown that they likely are multifunctional receptors, cellular uptake of plasma lipoproteins being just one of their tasks. For instance, one of the functionally best documented receptor, the chicken oocytes' eight ligand binding repeat receptor, termed LR8 (Bujo et al., 1995b), takes up the yolk precursors, riboflavin binding protein (Mac Lachlan et al., 1994), α2-macroglobulin (Jacobsen et al., 1995), VLDL, and vitellogenin (Bujo et al., 1994). Chicken LR8 also recognizes the iron-binding protein, lactoferrin (Hiesberger et al., 1995). Importantly, a mutation in LR8 (ovr) lends genetic proof to LR8's function as yolk precursor transporter: it causes female sterility (non-laying) and...
severe hyperlipidemia with associated premature atheroscleroticosis (Bujo et al., 1995a).

Without question, the ligand binding repeats are the most characteristic structural modules of the LDLR gene family. However, in a wide variety of species these subdomains have been shuffled into proteins whose functions seem to be unrelated to lipoprotein metabolism. Among these are several proteins of the blood complement system (Haefliger et al., 1967), reviewed in Hobbs et al. (1980); a basement membrane heparan sulfate proteoglycan, perlecan (Noonan et al., 1991; Kallunki and Tryggvason, 1992); a rat apical early endosomal glycoprotein (Speelman et al., 1995); a cortical granule protein in sea urchin (Wessel, 1995); a linker chain of earthworm hemoglobin (Suzuki and Biggs, 1993); a G-protein coupled receptor in the ganglion of Lymnaea (Tensen et al., 1994); and a chicken rous sarcoma virus receptor (Bates et al., 1993). Thus, the complement-type repeats in the LDLR are shared by a number of proteins that participate in diverse biological processes. For instance, the occurrence of complement-type repeats together with EGF precursor homology domain(s) in some extracellular matrix proteins, such as perlecan, suggests that certain family members might be involved in cell growth and cellular attachment.

We now have discovered a novel and unusually complex member of the LDLR gene family from rabbit. The predominant domain of the type I membrane protein consists of a cluster of 11 LDLR ligand binding repeats; according to our preferred nomenclature which avoids confusing and/or proven ligand designations (Bujo et al., 1995b), the new receptor is termed LR11. It also contains sequences highly homologous to a yeast receptor for vacuolar protein sorting, and to cellular attachment molecules. Highly expressed in brain, LR11 and a 130-kDa receptor, LR8B (Novak et al., 1996) are proposed to be members of a hitherto unknown branch of the LDLR gene family which may participate in brain-specific physiological processes.

**EXPERIMENTAL PROCEDURES**

**Isolation of LR11 cDNAs and Northern Blotting**—Total RNA was extracted from the liver of cholesterol fed (0.5%; Kovanen et al. (1991)) adult Japanese White male rabbits, and poly(A)⁺ RNA was isolated as described previously (Bujo et al., 1994). Random hexamer, oligo(dT), and specific sequence-primed cDNA libraries in agt10 were constructed using poly(A)⁺ RNA as described (Sakamura et al., 1990). The random-primed cDNA library was first screened with a polymerase chain reaction-amplified 487-bp cDNA fragment comprising the ligand-binding domain of the rabbit LDL receptor (nucleotides 166-1012) (Yamamoto et al., 1986). Approximately 2 x 10⁵ recombinant plasmids were screened by hybridization with the 3²P-labeled probe (Megaprime DNA labeling kit, Amersham) in 5 x SSC, 5 x Denhardt’s solution, 1% SDS, 100 μg/ml salmon sperm DNA, and 30% formamide at 37°C for 20 h (Sambo et al., 1989). The filters were washed in 0.3 x SSC, 0.1% SDS at 45°C, positive clones were subcloned into pHsueScript II SK (Stratagene), and sequenced on both sides using Sequenase (U. S. Biochemical Corp.). One of them, JH-1 (open reading frame, 1548 bp), encoded a new sequence homologous to the binding regions of LDL receptor family members. To obtain 5′- and 3′-overlapping cDNAs, a random hexamer-primed cDNA library was screened with JH-1; the hybridization was performed in 50% formamide at 42°C, and washing in 0.1 x SSC, 0.1% SDS at 50°C. Polymerase chain reaction-amplified cDNA corresponding to regions at and beyond both ends of JH-1 were used for subsequent screening rounds of the random hexamer-, specific sequence-, or oligo(dT)-primed cDNA libraries to obtain extensively overlapping clones covering the complete LR11 coding sequence. Nucleotide sequences were analyzed by the DNASIS (Hitachi) and GeneWorks (IntelliGenetics Inc.) computer programs. Homology searches were performed using GeneBank R88.0 or SWISS-PROT R31.0.

For Northern blotting, poly(A)⁺ RNA prepared from various tissues of adult normal rabbits and the liver of WHHL rabbits was denatured using glyoxal/dimethyl sulfoxide, separated by electrophoresis on a 1.0% agarose gel, and blotted onto Hybond-N⁺ membrane (Amersham) using standard methods (Sambo et al., 1989). JH-1, and probes corresponding to regions ~1000 bp upstream and ~1800 bp downstream of the ends of JH-1 were used; hybridization was performed in 50% formamide, 5 x SSC, 5 x Denhardt’s solution, 0.1% SDS, 50 mM sodium phosphate (pH 7.0), 100 μg/ml salmon sperm DNA, and 3²P-labeled probe. Washing was performed in 0.1 x SSC, 0.1% SDS at 50°C. Filters were exposed to Reflection autoradiography film (DuPont NEN) with intensifying screens.

**Expression of LR11 cDNA in ldA-7 Cells**—The recombinant expression plasmid pBRCMLR11 was constructed by cloning a 7-kilobase EcoRI/EcoRI fragment carrying the entire protein coding sequence of the rabbit LR11 cDNA, obtained by multiple ligations, into the EcoRI site of the vector pBKCVM (Stratagene). All ligations were subjected to sequence analysis. Transfection into ldA-7 cells, an LDLR deficient Chinese hamster ovary cell line (a kind gift of Dr. M. Krieger, MIT), was carried out with 20 μg (per 6 x 10⁶ cells) of pBKCMLR11 or pBRCMV (control) by using the calcium phosphate method (Sambrook et al., 1989). Stable transfectants were selected in medium supplemented with 500 μg/ml G418 (Life Technologies, Inc.). The cells were cultured in Ham’s F-12 medium containing 5% fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, 500 mM glutamine, and 500 μg/ml G418.

**Monoclonal Antibody Production, Immuno-, and Ligand Blotting**—The synthetic peptide, GHQCRDGSDGDFAGCS, corresponding to residues 1297–1315 derived from the rabbit LR11 cDNA sequence, was coupled to maleimide-activated keyhole limpet hemocyanin (Pierce) according to the manufacturer’s instructions and used for immunization of Balb/c mice (Goding, 1986). P3U1 murine myeloma cells were used to produce hybrid cell, and anti-peptide antibody secreting cells were cloned by limiting dilution and one of the clones, 43405 (IgG3, κ), was used for the current experiments.

Membranes from rabbit brain, liver, and heart, and cultured cells were prepared and solubilized as described previously (Bujo et al., 1994). Protein concentrations were determined with the BCA Protein Assay Reagent (Pierce). 5% SDS-PAGE was performed according to Laemmli (1970) using a minigel system (Nihon Eido). Ethorectroosynthesis was performed at 30 mA for 60 min under reducing conditions (30 mm 2-mercaptoethanol) and following heating of the samples to 95°C for 5 min. Calibration was with Rainbow™ colored protein molecular weight markers (Amersham). Electrophoretic transfer of the proteins to polyvinylidine difluoride membranes (Millipore, pore size 0.45 mm) was performed in transfer buffer (100 mM Tris, 192 mM glycine, 20% methanol) for 1 h at room temperature and 110 mA, using the Atto Horizoblot System AE-6670. Western blotting was performed using the superantigen from the cultured 43405 cells at 1:2 dilution, or a control monoclonal antibody to glutathione S-transferase from Schistosoma japonicum (Sigma; 1 μg/ml), followed by peroxidase-conjugated antioimmise IgG (heavy and light chain, Promega) and the chemiluminescence detection method (ECL, Amersham). Membranes were exposed for 5–15 min on Hyperfilm™-ECL (Amersham). Ligand blotting with ³²P-labeled rabbit β-VLDL (Kovanen et al., 1981) following SDS-PAGE under nonreducing conditions was performed as reported (Bujo et al., 1994).

**Immunohistochemical Analysis**—Cerebrum from adult rabbit brain was dissected in ice-cold phosphate-buffered saline, embedded in Tissue-Tek OCT compound (Miles), and immediately frozen in liquid nitrogen. Cryostat (10 μm) were prepared, freeze-thawed onto glass slides pretreated with 2% 3-aminopropyltriethoxysilane, and stored at –70°C. For immunostaining, we used Pathostain ABC-POD (M) kit (Wako) according to the manufacturer’s instructions. Briefly, the sections were incubated at 95°C for 1 min, and then incubated for 5–10 min with 1% H₂O₂ to inactivate endogenous peroxidase. The monoclonal antibody as used for Western blot analysis was applied in immunostaining the heat-denatured and hydrated sections. The adjacent sections were used for control incubation with a monoclonal anti-glutathione S-transferase antibody; development was with peroxidase-labeled streptavidin and incubation for 30 min in the peroxidase substrate provided.

**RESULTS**

**A New Member of the LDLR Gene Family, LR11, Is a Type I Transmembrane Protein**—Fig. 1 presents the amino acid sequence deduced from the rabbit LR11 cDNA obtained by homology cloning based on LDLR ligand binding repeat sequences. Rabbit LR11 cDNA contains an open reading frame of 6639 bp (encoding 2213 amino acids) following a 177-bp 5′ untranslated region. A single ATG (methionine) codon is fol-
The LR11 Protein Contains Structural Modules of Several Proteins—
The predicted amino acid sequence suggests that mature LR11 has seven distinct domains (Fig. 2), briefly described as: domain I, the NH2-terminal; domain II, a segment related to a yeast receptor for vacuolar protein sorting; domain III, five tandem LDLR “YWTD” repeats; domain IV, 11 LDLR ligand binding repeats; domain V, 6 motifs related to the fibronectin-type (FN)III repeat; domain VI, a putative membrane spanning region; and domain VII, at the COOH terminus, 54 amino acids predicted to be cytoplasmic with an internalization signal.

Following the VPS10-like domain, domain III (residues 757–1019) contains five repeats of 50 residues each with a consensus tetrapeptide, F/YWX D (see text) found in the EGF-precursor. In LR11, these repeats are replaced by a single subdomain with 8 cysteines, which we have tentatively assigned to the amino terminus of domain VI (Fig. 2).

Amino acid residue numbers are shown on the right. Cysteine residues are shaded. Potential N-linked glycosylation sites are underlined. F/YWX D tetrapeptide motifs (see text) are in boldface, and a putative internalization sequence is indicated by a heavy underline. The RGD tripeptide is double underlined. The signal sequence and the presumptive transmembrane segment are boxed.
described subdomain containing 8 cysteine residues (residues 1020 to 1070). All 66 cysteines in the 11 ligand binding repeats as well as the typical Asp-Xaa-Ser-Asp-Glu motifs (DXSDE, residues 1020 to 1070). All 66 cysteines in the 11 ligand binding repeats are conserved in LR11 compared to LDLR family proteins in several species.

The immediately following domain V (residues 1549–2134) is composed of six putative FNIII repeats (Fig. 5A). The characteristic highly conserved tryptophan, tyrosine, and leucine residues, as well as numerous less well conserved amino acids (Patthy, 1990) are present in the large majority of the repeats in LR11. In fact, part of domain V is most similar to the FNIII domains in LDLRs and VLDL receptors (Fig. 4).

Amino acid residues conserved in more than 50% of the repeats are boxed. Domain VI, the putative membrane spanning region (a stretch of 25 mostly hydrophobic amino acids) is followed by 25 mostly hydrophobic amino acids.

The sequence of the binding repeats in LDLRs (Mehta et al., 1992) is similar to the internalization signal of coated pit receptors (Chen et al., 1990; Naim and Roth, 1994; and see “Discussion”); there are also positively and negatively charged residues near the transmembrane and the COOH terminus, respectively, equivalent to those in the similarly short cytoplasmic domains of three rabbit receptors belonging to the LDLR gene family (B). A, alignment was optimized and positively charged residues (boxed) are indicated. B, numbers on the left refer to the amino-terminal residue number indicated on the left. All conserved cysteine residues are boxed. Amino acid residues conserved in more than 50% of the repeats are shaded and shown below as consensus sequence. The consensus sequence of the binding repeats in LDLRs (Mehta et al., 1992) is also shown as a shaded sequence. The consensus sequence of the binding repeats in LDLRs (Mehta et al., 1992) is also shown as a shaded sequence.
the protein specified by LR11 cDNA, we generated stable LR11 expression in the LDLR-deficient CHO cell line, ldlA-7 (Kingsley and Krieger, 1984). Immunoblot analysis of the stably expressing cells revealed a single band with the same migration as that observed in brain (lane 3), whereas CHO cells transfected with the vector alone did not show any immunological signal (lane 4), even after prolonged exposure. We conclude that the LR11 cDNA-encoded protein corresponds to the 250-kDa protein in rabbit tissues.

These results prompted us to perform a detailed analysis of LR11 expression in rabbit brain. Using extracts from different portions of brain, immunoblotting revealed the presence of the protein predominantly in the cerebrum, but also in the cerebellum and brain stem (Fig. 8A). Finally, immunohistochemical analysis to determine LR11 localization in cerebrum (Fig. 8B) revealed the presence of LR11 mainly in the hippocampal CA regions (CA1 and CA3) and dentate gyrus, with low level signals in the thalamus (below the dentate gyrus), as well as the cerebral cortex ("Cx," and bottom of Fig. 8B).

LR11 Binds Apolipoprotein E-rich Lipoproteins—All members of the LDLR gene family reported to date bind the apoE-rich lipoprotein, rabbit \( \beta \)-migrating VLDL (\( \beta \)-VLDL) (Brown and Goldstein, 1986; Beisiegel et al., 1989; Hussain et al., 1991; Willnow et al., 1992; Takahashi et al., 1992). To test whether LR11 has retained this property, we examined the binding of \( \beta \)-VLDL to the LR11-transfected cells by ligand blot analysis (Fig. 9). A clear signal in the same...
position as that revealed by Western blot analysis (cf. Fig. 7) was obtained. The signals observed at and above the 97-kDa marker, as well as the ~250-kDa band seen in the control cells (lane 1), might result from a greater sensitivity of ligand blotting compared to Western blotting, and/or correspond to other members of the LDLR gene family, e.g. the VLDL receptor (Jokinen et al., 1994). However, under our conditions, LRP was not detected, in agreement with previous results (Takahashi et al., 1995).

**DISCUSSION**

Genetic and structural elements defining the LDLR supergene family are widespread. Family members identified so far are products of genes that are either entirely made up of elements found in the LDLR itself or in which the complement-type repeats contribute only a minor portion to the overall structure. It is believed that the wide variety of ligands that interact with LDLR family members do so via these complement-type repeats; information on the functional contribution of additional protein domains may be obtained from genes in which LDLR- and non-LDLR elements are present in novel combinations. In the present study, we have identified the product of exactly such a gene in rabbit, LR11, which is a striking mosaic protein containing several structural elements so far not found in LDLR gene family members.

The elements that put LR11 in the LDLR gene family, domains III and IV, are located in the center of the molecule and display three unusual features. First, they are separated by a hitherto unknown short domain containing 8 cysteine residues, rather than by 6-cysteine “EGF precursor type-B” repeats (Herz et al., 1988) typifying LDLR family members described to date. To our knowledge, entactin/nidogen (Durkin et al., 1988; Mann et al., 1989) and the sevenless protein (Norton et al., 1990) are the only other proteins with YWTD repeats that lack the typical flanking type-B repeats. In nidogen/entactin, a major component of basement membranes, the YWTD repeats are involved in binding to another protein in the basement membrane, laminin (Fox et al., 1991). In laminin, the site responsible for binding to nidogen/entactin is located to a single “EGF-like repeat” with 8 cysteines (Mayer et al., 1993). These findings raise the possibility that LR11 may interact via domain III with other cell surface (glyco)proteins.

Second, groups of five YWTD repeats (domain III in LR11) have been found so far either downstream, or downstream and upstream from the ligand binding repeat cluster (domain IV in

![Fig. 8. Localization of LR11 in rabbit brain.](image)
LR11. The presence of the YWTD repeats only at the aminoterminal side of the LR11 binding repeat cluster suggests, on the one hand, substantial domain shuffling, and on the other hand, that multi-repeat modules are conserved because they function as such.

Third, LRP (Herz et al., 1988), the C. elegans putative LRP like-gene product (Yochem and Greenwald, 1993), and gp330/megalin (Saito et al., 1994) are the only other proteins that contain clusters of 11 complement-type repeats. In the clusters of these receptors, certain repeats are separated from each other by amino acid stretches called “linker regions” (Südhof et al., 1985). LR11 appears to lack a typical linker region; there are, however, a few extra residues preceding repeat 9 (residues 1043–1048) and 10 (residues 1453–1960), respectively.

Of interest are also the features of domain V, which consists of 6 FNIII modules (Fig. 5A) originally defined as 90-residue units repeated 15 times in fibronectin (Kornbluh et al., 1985). Two FNIII repeats in LR11 are particularly similar to the repeats in N-CAMs, murine L1 and its homologues (Fig. 5B). A number of extracellular matrix proteins and receptors contain FNIII repeats, including neural cell adhesion molecules, insulin receptor family members, and several receptor tyrosine kinases and phosphatases (O’Byran et al., 1991). The presence of these repeats in cell adhesion molecules and receptors for cell growth and attachment further suggests a potential role of LR11 in cell-cell interaction.

The amino-terminal domain (domain I), to our knowledge, lacks homologous sequences in current data bases. It is notable, however, that the rabbit LR11 protein has an Arg-Gly-Asp (RGD) tripeptide in this domain (Fig. 1), as this tripeptide is also found in L1 and homologues (Moos et al., 1988; Ruppert et al., 1995). The presence of structural features of neural adhesion molecules, together with the high expression of the LR11 mRNA in brain, are compatible with an involvement of LR11 in neuron-neuron adhesion and/or outgrowth of neurites, as suggested for N-CAMs and L1s (Goridis and Brunet, 1992).

In the cytoplasmic domain, LR11 has a putative internalization signal, Phe-Ala-Asn-Ser-His-Tyr (FANSYH), highly reminiscent of the sequence FDNPXXY, the common internalization signal of LDLR gene family members (Chen et al., 1990). The Asn and Tyr residues, conserved in LR11, have been reported to be crucial for the formation of a β-turn structure required for internalization (Chen et al., 1990; Bansal and Gierasch, 1991). Furthermore, the hexapeptide signal in LR11 is similar to that in the macrophage mannose receptor (FENTLY). Taylor et al. (1990) and Naim and Roth (1994) reported that sequence requirements for internalization of receptors via tyrosine-containing signals are even more relaxed. Interestingly, similar motifs in the cytoplasmic regions of the β-subunits of integrins play key roles in the interaction between integrins and cytoskeletal proteins (Filardo et al., 1995; O’Toole et al., 1995). Although further studies are needed to determine the role of the consensus motif in LR11, the characteristic signature sequence, together with clusters of charged residues in the cytoplasmic domain also found in other LDLR gene family members (Fig. 4B), strongly suggest that the LR11 protein is an endocytosis-competent receptor. Notably, clustered negatively charged amino acids in context with a tyrosine residue, shown to be important for basolateral sorting of the LDLR (Matter et al., 1992), are absent from the cytoplasmic domain of LR11.

The yeast gene VPS10 encodes a sorting receptor for the proteinase carboxypeptidase Y. VPS10 is proposed to execute multiple rounds of targeting by cycling between the late Golgi and a prevacuolar endosome-like compartment (Marcussen et al., 1994). The luminal domain of VPS10 contains two imperfectly repeated sequences, each followed by a cysteine-rich motif (Horazdovsky et al., 1995). Surprisingly, domain II of LR11 shows striking similarities to both VPS10-repeats including their cysteine-rich regions. The sequence Phe-Tyr-Val-Phe in VPS10, which may function as Golgi retention/retrieval signal (Horazdovsky et al., 1995), is not found in LR11. Because the luminal region of the VPS10 protein is suggested to be involved in carboxypeptidase Y binding (Horazdovsky et al., 1995), the homologous region in LR11, proposed to be extracellular, may be a site for ligand-receptor interaction(s). We can only begin to speculate what such ligand(s), if they exist, might be. LRP, e.g. is able to bind soluble proteinase/proteinase inhibitor complexes (Krieger and Herz, 1994); domain II of LR11 could participate in (transient) binding to proteinases or proteinase-like molecules on the cell surface of adjacent cells. Although vacuolar protein sorting in yeast and lysosomal protein sorting in certain mammalian cell types seem to share similar mechanisms and proteins, any involvement of the VPS10 domain in LR11’s intracellular and/or vesicle-mediated routing must remain highly speculative.

Although the complex structure of LR11 and sterol-insensitivity suggest a function(s) other than in lipoprotein metabolism, its abundance in tissues with active cholesterol metabolism, such as brain, liver, and adrenal glands, in which the LDLR is also highly expressed (Yamamoto et al., 1986; Pitas et al., 1987) requires attention. In any case, the fact that β-VLDL, known to interact with LDLR relatives via its apoE moiety, binds to LR11 efficiently, allows for the first time to conclude that a type-B repeat downstream of an 11-ligand binding repeat cluster is not required for apoE-binding in vitro. Recently, Kounnas et al. (1995) reported that LRP binds, in addition to many other possible ligands, β-amyloid precursor protein and mediates its degradation in vitro. In the absence of the transgenic animal model, we cannot determine which ligand(s) may be the true physiological partner(s) of LR11. The localization of rabbit (Fig. 8) and human (data not shown) LR11 in the hippocampus, dentate gyrus, and cerebral cortex suggests functional significance in metabolically active brain regions. For instance, glutamate receptors, thought to play key roles in active brain functions such as learning (Bliss and Collingridge, 1993), are highly expressed in the same brain structures (Meguro et al., 1992).

Thus, LR11 in rabbit as well as in man and chicken, and the recently identified LR8B in chicken and mouse (Novak et al., 1996) appear to define a new group of LDLR family members with possible brain-specific functions. The molecular characterization of the amazingly complex LR11 opens new avenues toward the investigation of the biological significance of apparent redundancy in the expression of homologous functional units in different structural context. The extremely high degrees of similarity between the proteins from rabbit, man, and chicken suggests that the shuffling of evolutionarily mobile domains resulting in LR11 has not been totally random (Doolittle, 1995). Future studies will address the question whether LR11 participates in the regulation of cell growth, particularly that of neuronal cells, and/or mediates cell-cell adhesion processes, and will attempt to identify the ligands of this interesting protein.

**Acknowledgments**—We are grateful to Dr. H. Hashimoto (Daiichi Pure Chemicals Co., Ltd.) for antibody production. Drs. M. Otabe and I. Ishii for help with expression experiments, and Dr. K. Tanaka for help with immunohistochemical analysis is greatly appreciated. We also thank Drs. H. Tanii and M. Nakamura (Kowa Co., Ltd.) for support and advice during cDNA cloning.

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2 S. Möwald, H. Yamazaki, H. Bujo, J. Kusunoki, T. Kanaki, K. Seimiya, N. Morisaki, J. Nimpf, W. J. Schneider, and Y. Saito, manuscript in preparation.
