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Rabbit very low density lipoprotein receptor: A low density lipoprotein receptor-like protein with distinct ligand specificity

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ABSTRACT **A cDNA that expresses a receptor for very low density lipoprotein (VLDL) was isolated from a rabbit heart cDNA library and characterized. The deduced amino acid sequence of the cDNA revealed that the cDNA encodes a protein with striking homology to the low density lipoprotein (LDL) receptor. Like the LDL receptor, the mature protein consists of the following five domains spanning 846 amino acids: 328 N-terminal amino acids including an 8-fold repeat of 40 amino acids homologous to the ligand binding repeat of the LDL receptor; 396 amino acid residues homologous to the epidermal growth factor precursor including three cysteine-rich repeats; a region immediately outside of the plasma membrane rich in serines and threonines; 22 amino acids traversing the plasma membrane; and 54 amino acids including the NPVY sequence that is required for clustering of the LDL receptor in coated pits and that projects into the cytoplasm. LDL-receptor-deficient Chinese hamster ovary cells transfected with the cDNA bound and internalized VLDL, β-migrating VLDL, and intermediate density lipoprotein but did not bind LDL with high affinity. The 3.6- and 9.5-kilobase mRNAs for the VLDL receptor are highly abundant in heart, muscle, and adipose tissue. Barely detectable amounts of the mRNAs were present in liver. Based on the structural features, ligand specificity, and tissue expression of the mRNAs, we suggest that this VLDL receptor may mediate uptake of apolipoprotein E-containing lipoproteins enriched with triglyceride in nonhepatic tissues that are active in fatty acid metabolism.**

Receptors for plasma lipoproteins play crucial roles in the metabolism of fats and cholesterol. The low density lipoprotein (LDL) receptor, the most extensively characterized lipoprotein receptor, binds apolipoprotein (apo) B-100-containing LDL and carries it into cells by receptor-mediated endocytosis (for review, see ref. 1). The LDL receptor also binds and internalizes apoE-containing lipoproteins, β-migrating very low density lipoprotein (β-VLDL), and a cholesterol-induced high density lipoprotein particle that contains apoE as its sole apo, with high affinity (2–5). The mature LDL receptor consists of five domains (5–8): (*i*) an N-terminal ligand-binding domain composed of seven cysteine-rich repeats (2, 3), (*ii*) an epidermal growth factor precursor homology domain that mediates the acid-dependent dissociation of ligands (9), (*iii*) an O-linked sugar domain of unknown function, (*iv*) a transmembrane domain, and (*v*) a cytoplasmic domain that mediates the clustering of the receptor into coated pits via an NPXY amino acid sequence (10).

Although the LDL receptor binds apoE-containing lipoproteins and is responsible for their plasma clearance, the presence of distinct apoE receptors has been suggested. In the current investigation, we demonstrate the presence of a specific receptor for apoE-containing lipoproteins and describe the structure,§ ligand specificity, and tissue distribution of this apoE lipoprotein binding receptor.

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MATERIALS AND METHODS

Standard Procedures. Unless otherwise indicated, all molecular biological techniques were performed essentially as described by Sambrook *et al.* (11).

LDL-Receptor-Subtracted cDNA Library. A cDNA library was constructed in an Okayama–Berg vector (12) with poly(A)$^+$ RNA isolated from normal rabbit heart. To exclude the rabbit LDL receptor, the entire pooled cDNA library was digested with *Sal* I and recircularized with T4 DNA ligase. The presence of a unique *Sal* I site in the rabbit LDL receptor cDNA (7) and the vector results in loss of any LDL receptor cDNAs after recircularization and retransformation. The resulting LDL-receptor-subtracted cDNA library was screened with the 1.9-kilobase *Sma* I–*Sal* I fragment from the rabbit LDL receptor cDNA (7) under low-stringency hybridization conditions.

Lipoprotein Preparation. Rabbit LDL, VLDL, and intermediate density lipoprotein (IDL) were prepared from WHHL rabbits as described (13). Rabbit β-VLDL was prepared from plasma of 0.5% cholesterol-fed animals (14). Recombinant human apoE (isoform E-3), obtained from *Escherichia coli* (15), was provided by Satoru Murayama (Mitsubishi Kasei, Kanagawa, Japan). ApoE-containing liposomes were prepared by the procedure of Innerarity *et al.* (16). Fluorescently labeled lipoproteins were prepared by incorporating 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate into lipoproteins as described (17). ^{125}I-labeled lipoproteins were prepared by the Bolton and Hunter method (18).

Isolation of Transfected Cell Lines. Plasmids (5.4 μg) containing the human LDL receptor and the rabbit VLDL receptor were introduced into *ldlA*-7 cells (19), a mutant Chinese hamster ovary cell line lacking LDL receptors (provided by Monty Krieger, Department of Biology, Massachusetts Institute of Technology, Cambridge, MA), by transfection with 0.6 μg of pSV2-Neo (20) using Lipofectin (BRL) (21).

Ligand Binding Assays. Receptor-mediated endocytosis of lipoproteins by transfected cells was visualized with fluorescently labeled lipoproteins at 37°C as described (17). Binding and internalization of ^{125}I-labeled lipoproteins by transfected cells were assayed as described (2, 3, 22).

RESULTS AND DISCUSSION

Screening of 5×10^5 colonies from an LDL-receptor-subtracted cDNA library constructed from poly(A)$^+$ RNA of rabbit heart under low-stringency hybridization conditions

Abbreviations: VLDL, very low density lipoprotein; LDL, low density lipoprotein; β-VLDL, β-migrating very low density lipoprotein; IDL, intermediate density lipoprotein; apo, apolipoprotein.

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§The sequence reported in this paper has been deposited in the GenBank data base (accession no. D11100).

yielded five positive clones that cross-hybridized with an LDL receptor probe. These five positive clones were independently introduced into LDL-receptor-deficient *ldlA*-7 cells by cotransfection with pSV2-Neo. G418-resistant cells were isolated and lipoprotein binding activities were visualized by using fluorescently labeled rabbit LDL, VLDL, and β-VLDL. One of the cDNA clones (designated pVLDLR1) conferred VLDL and β-VLDL binding activities on the cells (Fig. 1 *E* and *F*). The intense localization of fluorescence within the cells suggests that fluorescent VLDL and β-VLDL were taken up by receptor-mediated endocytosis. Although LDL-receptor-transfected cells bound LDL, VLDL, and β-VLDL (Fig. 1 *A–C*), pVLDLR1-transfected cells did not bind LDL efficiently (Fig. 1*D*). Control cells transfected with pSV2-Neo did not take up any ligands (Fig. 1 *G–I*). The ligand specificity of the VLDL receptor expressed in *ldlA*-7 cells was also analyzed by using ^{125}I-labeled rabbit VLDL, β-VLDL, IDL, and LDL. CHO cells transfected with the rabbit VLDL receptor cDNA bound apoE-containing lipoproteins including VLDL, β-VLDL, and IDL with high affinity but did not bind LDL with high affinity, whereas CHO cells transfected with the human LDL receptor cDNA bound both apoB- and apoE-containing lipoproteins with high affinity (data not shown). The ^{125}I-labeled β-VLDL binding to the transfected VLDL receptor was inhibited by unlabeled apoE liposomes (data not shown), indicating that the receptor recognizes apoE.

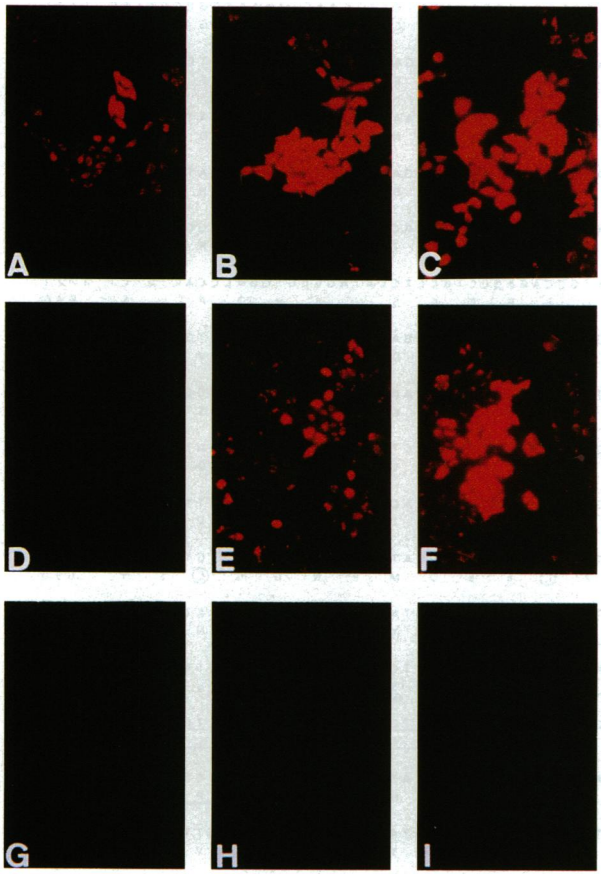

FIG. 1. Visualization of receptor-mediated uptake of fluorescent VLDL and β-VLDL by VLDL-receptor-transfected CHO cells. The human LDL receptor cDNA (pLDLR2) (*A–C*) or the rabbit VLDL receptor cDNA (pVLDLR1) (*D–F*) were introduced into LDL-receptor-deficient *ldlA*-7 cells together with pSV2-Neo. Control cells (*G–I*) were transfected with pSV2-Neo alone. G418-resistant cells were incubated with fluorescent LDL (*A*, *D*, and *G*), fluorescent VLDL (*B*, *E*, and *H*), or fluorescent β-VLDL (*C*, *F*, and *I*) for 4 h at 37°C. (×200.)

The insert of pVLDLR1 includes a 2619-base-pair open reading frame encoding a protein of 873 amino acids with a calculated molecular weight of 96,279 (Fig. 2). The N-terminal 27-amino acid (residues −27 to −1) sequence has the characteristics of a classic signal sequence. The amino acid sequence after the 27th residue (threonine) contains cysteine-rich sequence that is homologous to the N terminus of the mature LDL receptor. Therefore, we tentatively assigned residue 28 (Gly-1 in Figs. 2 and 3) to be the N terminus of the mature protein. The mature rabbit receptor would then consist of 846 amino acids with a calculated molecular weight of 93,377.

A striking feature of the VLDL receptor is the structural similarity to the LDL receptor (5, 6, 7, 8) (Fig. 3). The two receptors consist of cysteine-rich repetitive sequences similar to complement proteins C8 and C9, epidermal growth factor precursor homology domains with three growth factor repeats, O-linked sugar domains with clusters of serines and threonines, transmembrane domains, and cytoplasmic domains. Structural features of each domain are highly conserved between the two receptors. The spacing of the cysteines is highly conserved among the repeats of the ligand binding domains of the two receptors (Fig. 3*A*). Moreover, the amino acid sequence SDE, which forms part of the ligand binding site of the LDL receptor, is also conserved in each of the repeats of the VLDL receptor. Within the cytoplasmic domains of the two receptors, amino acids surrounding the NPVY sequence (10) are highly conserved (Fig. 3*A*). A key structural difference is the number of cysteine-rich repeat sequences at the N terminus: the VLDL receptor contains an 8-fold repeat whereas the LDL receptor consists of a 7-fold repeat. The extensive similarity of the VLDL receptor with the LDL receptor suggests that the two perform similar functions in lipoprotein metabolism.

Consistent with this hypothesis is our finding that the VLDL receptor binds apoE-containing lipoproteins, VLDL, β-VLDL, and IDL with high affinity but does not bind LDL with high affinity. The LDL binding function of the LDL receptor requires contributions from repeats 3–7, whereas β-VLDL binding was insensitive to the loss of any single repeat with the exception of repeat 5 (2, 3). The additional ligand binding repeat in the VLDL receptor may inhibit the binding of LDL to this receptor.

Northern blot analysis of RNA prepared from various rabbit tissues revealed hybridization to a major transcript of 3.6 kilobases (Fig. 4*A*). A minor transcript of ≈9.5 kilobases was also detected. This minor transcript may be a consequence of alternative splicing or polyadenylylation. The mRNAs encoding the VLDL receptor are most abundant in heart, muscle, and adipose tissue. The mRNAs are also present in spleen, lung, brain, kidney, adrenal, testis, and small intestine. Barely detectable amounts of the mRNAs were present in liver. The high levels of the VLDL receptor mRNA in tissues active in fatty acid metabolism suggest that this receptor may mediate the uptake of triglyceride-rich lipoproteins such as apoE-containing VLDL and chylomicrons.

In the current studies, we demonstrate the presence of a VLDL receptor for apoE-containing lipoproteins. The presence of a putative second lipoprotein receptor may be of considerable physiological and pathological importance. Although the exact physiological function of this receptor remains to be elucidated, the availability of a cDNA will promote studies that define the metabolism and regulation of lipoproteins containing apoE by this protein.

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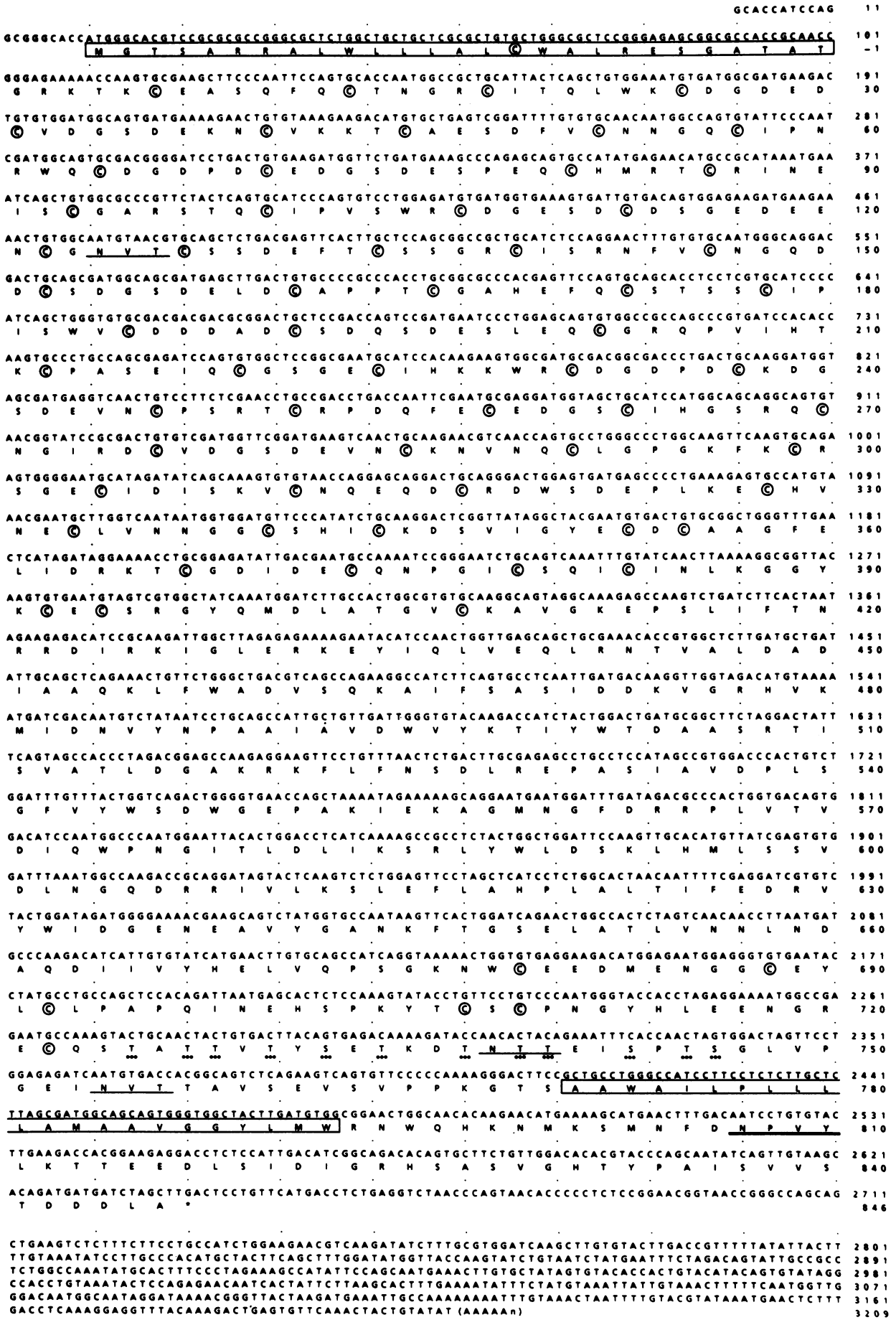

FIG. 2. (*Legend appears at the bottom of the opposite page.*)

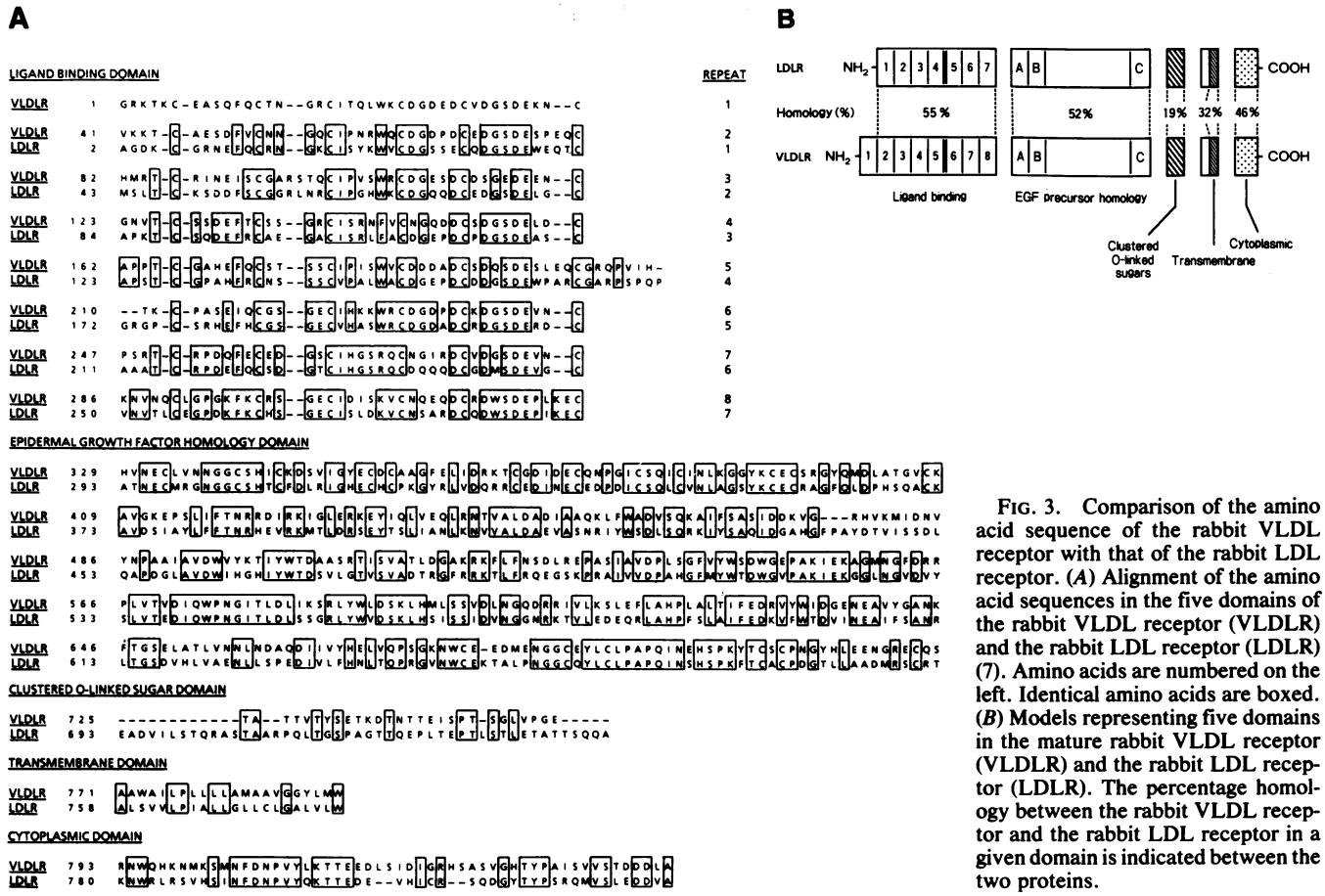

FIG. 3. Comparison of the amino acid sequence of the rabbit VLDL receptor with that of the rabbit LDL receptor. (*A*) Alignment of the amino acid sequences in the five domains of the rabbit VLDL receptor (VLDLR) and the rabbit LDL receptor (LDLR) (7). Amino acids are numbered on the left. Identical amino acids are boxed. (*B*) Models representing five domains in the mature rabbit VLDL receptor (VLDLR) and the rabbit LDL receptor (LDLR). The percentage homology between the rabbit VLDL receptor and the rabbit LDL receptor in a given domain is indicated between the two proteins.

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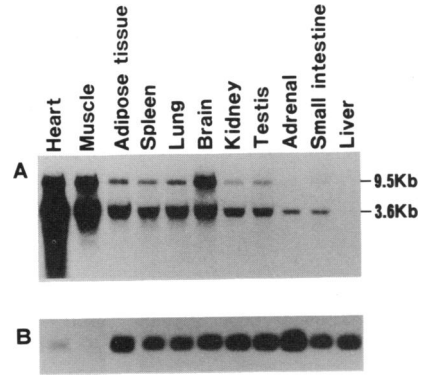

FIG. 4. Tissue expression of the VLDL receptor mRNAs. (*A*) Total RNA (20 μg) from various rabbit tissues was electrophoresed on an agarose gel, transferred to a nylon membrane, and hybridized with ^{32}P-labeled rabbit VLDL receptor cDNA by random priming. (*B*) The same filter was then reprobed with a ^{32}P-labeled oligonucleotide corresponding to rat cyclophilin cDNA (23).

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FIG. 2 (*on opposite page*). Nucleotide and predicted amino acid sequences of the cDNA encoding the rabbit VLDL receptor. Residue 1 is the glycine that is believed to constitute the N terminus of the mature protein. Negative numbers refer to the cleaved signal sequence (boxed at the N terminus). Cysteine residues are circled. Three potential sites of N-linked glycosylation (Asn-Xaa-Ser or Asn-Xaa-Thr) are indicated by solid underlines. Serine and threonine residues in a region that corresponds to the O-linked sugar domain of the rabbit LDL receptor are indicated by dotted underlines. The 22-residue transmembrane segment located toward the C terminus of the protein is boxed. The NPXY sequence (10) required for clustering of the LDL receptor in coated pits is indicated by the heavy underline. A stop codon is indicated with an asterisk.

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