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Molecular Cloning of a Lipolysis-stimulated Remnant Receptor Expressed in the Liver*

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The lipolysis-stimulated receptor (LSR) is a lipoprotein receptor primarily expressed in the liver and activated by free fatty acids. Antibodies inhibiting LSR functions showed that the receptor is a heterotrimer or tetramer consisting of 68-kDa (α) and 56-kDa (β) subunits associated through disulfide bridges. Screening of expression libraries with these antibodies led to identification of mRNAs derived by alternate splicing from a single gene and coding for proteins with molecular masses matching that of LSR α and β. Antibodies directed against a synthetic peptide of LSR α and β putative ligand binding domains inhibited LSR activity. Western blotting identified two liver proteins with the same apparent molecular mass as that of LSR α and β. Transient transfections of LSR α alone in Chinese hamster ovary cells increased oleate-induced binding and uptake of lipoproteins, while cotransfection of both LSR α and β increased oleate-induced proteolytic degradation of the particles. The ligand specificity of LSR expressed in cotransfected Chinese hamster ovary cells closely matched that previously described using fibroblasts from subjects lacking the low density lipoprotein receptor. LSR affinity is highest for the triglyceride-rich lipoproteins, chylomicrons, and very low density lipoprotein. We speculate that LSR is a rate-limiting step for the clearance of dietary triglycerides and plays a role in determining their partitioning between the liver and peripheral tissues.

Chylomicrons transport, in plasma, dietary triglycerides (TG)[1] and liposoluble vitamins absorbed by the intestine after a meal (1). Lipoprotein lipase (LPL), which is anchored to the surface of capillary endothelium, hydrolyzes chylomicron TG into free fatty acids (FFA) that are targeted to the underlying muscles and adipose tissue. The residues of chylomicrons are then released from the endothelium and taken up by the liver. Both the low density lipoprotein (LDL) receptor and the LDL receptor-related protein (LRP) contribute to this process (2, 3). Studies using anti-LDL receptor antibodies or mice with a deficiency of the apoE gene suggest that the LDL receptor accounts for up to half of the clearance of chylomicrons (4, 5). However, human subjects deficient for the LDL receptor clear chylomicron remnants normally (6). In addition, mice with CRE-loxP-mediated selective disruption of the LRP gene in the liver are not hyperlipidemic (7). If LRP-deficient mice are crossbred with LDL receptor-deficient mice, apoB48, the main chylomicron apolipoprotein, accumulates in the plasma (7), but plasma TG concentrations in these mice are not dramatically increased. This is in contrast with the effect of the 39-kDa receptor-associated protein, a known inhibitor of LRP activity, which induces a massive increase of plasma TG and cholesterol when overexpressed in mice (8).

We have reported the characterization of a lipoprotein receptor that is inhibited by receptor-associated protein at concentrations similar to those achieved in the receptor-associated protein overexpression study (9). This receptor was originally identified by its binding of LDL in the presence of FFA and is hereafter referred to as the lipolysis-stimulated receptor (LSR). LSR binds apoB and apoE, displays the greatest affinity for TG-rich lipoprotein (chylomicrons and very low density lipoprotein (VLDL)), and does not bind β-VLDL isolated from subjects with type III hyperlipidemia (10, 11). Several characteristics of LSR suggest that it represents an important step for the clearance of chylomicrons. Indeed, LSR is expressed in the liver, and its activity is markedly increased in endocytic vesicles (10). LSR is inhibited by lactoferrin, a milk protein that, when injected intravenously, inhibits the uptake of chylomicrons by the liver (10, 12, 13). Also, apoCIII inhibits the binding of triglyceride-rich lipoprotein chylomicrons and VLDL but not that of LDL to LSR (14), while apoCIII overexpression in mice induces profound hypertriglyceridemic effects (15). Finally, in rats, the apparent numbers of LSR expressed at the surface of hepatocytes correlate strongly and negatively with plasma TG levels measured in the postprandial stage (12). The limitations of this model are 2-fold. First, maximal activation of the receptor requires FFA at concentrations that exceed albumin-binding capacity. It is our hypothesis, as yet unproven that large amounts of FFA are released by hepatic lipase acting upon chylomicrons and VLDL directly in the environment that bathes the receptors (16). Second, the molecular characterization of the receptor remained incomplete and relied entirely on the identification of candidate proteins by ligand blotting in the presence of oleate (10, 12).

We now report the cloning and characterization of a new gene, primarily expressed in the liver, which encodes a multimeric receptor that binds lipoproteins in the presence of FFA. We propose that LSR represents a rate-limiting step for the clearance of dietary TG from the circulation.

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[1] The abbreviations used are: TG, triglyceride(s); apo, apolipoprotein; BSA, bovine serum albumin; CHO, Chinese hamster ovary; DiI, 1,1′-dioctadecyl-3,3,3′,3′-tetramethyl indocarbocyanine perchlorate; DMEM, Dulbecco's modified Eagle's medium; FFA, free fatty acid(s); LSR, lipolysis-stimulated receptor; LPL, lipoprotein lipase; LDL, low density lipoprotein(s); LRP, low density lipoprotein receptor-related protein; PBS, phosphate-buffered saline; VLDL, very low density lipoprotein; PCR, polymerase chain reaction.

EXPERIMENTAL PROCEDURES

Materials

Na^{125}I and [^{35}S]methionine/cysteine (Promix) were obtained from Amersham Pharmacia Biotech (Les Ulis, France); [^{33}P]dCTP was purchased from NEN Life Science Products (Paris, France). Oleic acid, bovine serum albumin (A2153) (BSA), 1,2-cyclohexanedione, *n*-octyl glucopyranoside, and the 5′-nucleotidase kit were obtained from Sigma (St. Quentin, Fallavier, France). Sodium suramin was a generous gift from Bayer Pharmaceuticals (Puteaux, France), and sodium heparin was purchased from Choay Laboratories (Gentilly, France). Pronase and 1,1′-dioctadecyl-3, 3,3′,3′-tetramethyl indocarbocyanine perchlorate (DiI) were obtained from Calbiochem (Meudon, France) and Molecular Probes, Inc. (Eugene, OR), respectively. Fetal bovine serum, Dulbecco's modified Eagle's medium (DMEM), Ham's F-12 medium, trypsin, penicillin/streptomycin, and glutamine were purchased from Life Technologies, Inc. (Eragny, France). Methionine- and cysteine-free RPMI medium was obtained from BioWhittaker (Gagny, France). Secondary antibodies conjugated to alkaline phosphatase and transfection reagent Superfect were purchased from Immunotech (Marseille, France) and Qiagen (Courtaboeuf, France), respectively.

Animals

Male Sprague-Dawley rats were purchased from R. Janvier Breeding Center (Le Genest St. Isle, France) and housed in an animal care facility approved and monitored by the French Ministries of Health and Agriculture.

Methods

Purification of LSR—Rat liver total membranes were prepared from overnight-fasted 350-g Sprague-Dawley rats, followed by solubilization in 125 mM *n*-octyl glucoside (20 mg of membrane protein/ml of detergent) in 20 mM Tris-HCl containing 2 mM EDTA, pH 7.4, and proteolytic inhibitor mixture, as described previously (12, 17). Solubilized membrane protein was then separated under nondenaturing conditions on preparative 4–12% gradient polyacrylamide gels (35–40 mg/gel). A strip of gel was cut and transferred to nitrocellulose membrane, which was incubated with 0.8 mM oleate and 40 μg/ml ^{125}I-LDL and then washed extensively in phosphate-buffered saline (PBS) containing 0.5% (v/v) Triton X-100 (12). The 240-kDa band exhibiting the ability to bind ^{125}I-LDL in the presence of oleate was then excised from the remaining gel, electroeluted, and used for rabbit immunization.

Production of Polyclonal Anti-LSR Protein and Synthetic Peptide Antibodies—New Zealand rabbits were injected with partially purified LSR proteins emulsified with Freund's complete adjuvant and subsequently with incomplete adjuvant following the protocol described by Harlow and Lane (18). Sera were collected prior to injections (preimmune IgG) and at various times from 2 to 12 months after the injections. IgG were purified using protein A-Sepharose (Amersham Pharmacia Biotech) following the manufacturer's instructions.

Anti-LSR synthetic peptide with a sequence corresponding to LSR α residues 488–502 was obtained commercially. Polyclonal antibodies directed against this synthetic peptide conjugated to KLH were obtained, and the IgG fraction was purified as described above.

Preparation of Rat Liver Plasma Membranes—For isolation of livers, overnight-fasted animals were anesthetized with ether, and the livers were perfused through the portal vein with ice-cold Hepes-buffered saline solution (150 mM NaCl containing 5 mM Hepes and 2 mM EDTA, pH 7.4). The livers were then immediately excised, and plasma membranes were prepared according to the procedure described previously (19). Plasma membranes were stored at −80 °C in the presence of a proteolytic inhibitor mixture (19).

Measurement of LSR Activity in Rat Liver Plasma Membranes—Oleate-induced binding of ^{125}I-LDL to rat liver plasma membranes was measured as reported previously (9, 12) with some modification. Briefly, aliquots of membranes (100 μg of protein/tube) were incubated at 37 °C for 30 min in the absence or presence of the 0.8 mM oleate adjusted to a final volume of 250 μl with 0.1 M phosphate buffer containing 350 mM NaCl and 2 mM EDTA, pH 8.0 (buffer A). The membranes were then washed by six series of centrifugation (35,000 × *g*, 15 min, 4 °C) and resuspended into 250 μl of buffer A by brief sonication (Bioblock Scientific Vibracell, power 1.0, 90% pulse, 5 s). At the final wash, the membrane pellets were resuspended into 200 μl of buffer A, and the membranes were incubated at 4 °C for 60 min with the indicated concentrations of an irrelevant IgG or anti-LSR IgG. ^{125}I-LDL (5 μg/ml) was then added, and the membranes were further incubated for 1 h at 4 °C in a final volume of 250 μl. At the end of the incubation, 25 μl/tube of PBS containing 2% (w/v) BSA was added. Membrane-bound ^{125}I-LDL was separated from unbound ^{125}I-LDL by layering a 200-μl aliquot over a 600-μl cushion of 5% (w/v) BSA in buffer A and centrifuging (35,000 × *g*, 20 min, 4 °C). After careful aspiration of the supernatants, the bottoms of the tubes containing the membrane pellets were cut and counted in a γ-counter (Pharmacia 1470 Wizard).

Binding, Uptake, and Degradation Studies—For these studies, primary cultures of rat hepatocytes were used 48 h after plating (10). Oleate-induced ^{125}I-LDL binding, uptake, and degradation was measured as described previously with the following modifications (10). Hepatocytes were preincubated for 30 min at 37 °C with 20 ng/ml mouse recombinant leptin (20), followed by 30 min at room temperature with the indicated concentrations of IgG. ^{125}I-LDL was then added, and the cells were further incubated for 4 h at 37 °C in the presence or absence of 0.5 mM oleate, followed by analysis of the amount of ^{125}I-LDL bound, internalized, and degraded.

Immunoprecipitation—Primary cultures of rat hepatocytes (48 h after plating) were incubated with ^{35}S-Promix in methionine- and cysteine-free RPMI medium and then lysed in PBS containing 1% Triton X-100. Immunoprecipitates were prepared and separated on SDS-polyacrylamide gels as described by Oukka *et al.* (21).

Preparation of Lipoproteins—Human LDL (1.025 < density (*d*) < 1.055 g/ml) were purified from plasma obtained from the local blood bank exactly as described previously and stored under N_2 and in the dark for not more than 15 days prior to use (10, 11). Human VLDL ($d <$ 1.006) and high density lipoprotein (1.085 < d < 1.21) were obtained from overnight-fasted normolipidemic volunteers by sequential ultracentrifugation (11, 12). Chylomicrons were obtained by catheterization of the abdominal lymphatic duct of rats weighing between 150 and 200 g after force feeding of a fat meal with a composition similar to that described above for mice (11). The chylomicrons were separated from the lymph by two consecutive centrifugations (200,000 × *g*, 1 h, 15 °C, SW41 Beckman rotor) at d = 1.006 g/ml.

Pronase treatment and 1,2-cyclohexanedione modification were performed as described by Bihain and Yen (11) and Shepherd and Packard (22), respectively. The inability of cyclohexanedione-modified LDL to bind to the LDL receptor was verified in control experiments using normal human fibroblasts. LDL was labeled with DiI according to the method described by Via and Smith (23). Lipoprotein-deficient ($d >$ 1.21 g/ml) fetal bovine serum was used as a source of cholesterol ester transfer protein. Radioiodinations of LDL were based on the McFarlane procedure modified by Bilheimer *et al.* (24). Radiolabeled lipoproteins were filtered (0.2 μm) on the day of the experiment and used within 1 week of preparation.

Western Blotting—Nitrocellulose membranes were incubated 30 min at room temperature with PBS containing 3% (w/v) BSA and then washed three times for 10 min each in PBS containing 0.5% (v/v) Tween 20. The strips were incubated for 1 h at room temperature with a 1:400 dilution of anti-LSR serum or 75 μg/ml anti-LSR peptide 170 IgG (immunoglobulin was purified by protein A affinity column chromatography (Amersham Pharmacia Biotech)) in PBS containing 0.5% (v/v) Tween 20. After washing three times for 10 min in PBS containing 0.5% Tween 20, the membranes were incubated 1 h at room temperature with goat anti-rabbit IgG that was either 1) radiolabeled with ^{125}I at 20,000 cpm/ml (Iodobeads; Pierce) according to the manufacturer's instructions) (Fig. 1) or 2) conjugated to alkaline phosphatase (Fig. 6). Protein bands were revealed by 1) exposing on a phosphor screen and image analysis on a PhosphorImager (Molecular Dynamics, Inc., Sunnyvale, CA) or 2) coloration, using alkaline phosphatase substrates.

Library Screening—A λgt11 rat liver 5′ stretch plus cDNA expression library (CLONTECH, Ozyme, Montigny Le Bretonneux, France) was screened with polyclonal anti-LSR antibodies (10 μg/ml purified IgG, 10 mM Tris-HCl, pH 8, containing 150 mM NaCl, and 0.05% Tween 20 (TNT)) in the presence of 5% nonfat dry milk (25, 26). After washing with TNT, membranes were incubated with an alkaline phosphatase-conjugated affinity-purified F(ab′)$_2$ fragment goat anti-rabbit IgG (Immunotech). Positive clones were isolated and verified by secondary and tertiary screening. Sequences of LSR α, α', and β forms can be found in GenBank™ (accession numbers AF119667, AF119668, and AF119669, respectively).

5′ Rapid Amplification of cDNA Ends PCR—Rat liver mRNA was purified using Dynabeads oligo(dT)$_{25}$ (Dynal, Compiègne, France) following the manufacturer's instructions. cDNA were synthesized at 50 °C using Superscript II (Life Science Technologies) with primer T$_{12}$VN where V stands for A, C, or G and N for the four nucleotides according to the manufacturer's protocol. Double strand DNA was obtained by the replacement technique, and recessed termini were re-

paired by T4 DNA polymerase as described by Sambrook *et al.* (27). Double strand adapters included the *Not*I site and were prepared by hybridization of the two modified oligonucleotides (AD1, 5′-phosphate-GCGGCCGCAT-NH_2-3′; AD2, 5′-GCTATCTGAGCGATCGACATGCG-GCCGC-3′) and ligated to cDNA with T4 ligase (26). Nested PCRs were then performed with 5′-RA (5′-GCTATCTGAGCGATCGAC-3′) and LSR 10 (5′-TGGGTCACTGGCTGGAACAGTATCACTACG-3′) and LSR 12 (5′-CGATGAATTCGAGACACACGAAGACCACGGTA-3′), which introduced an *Eco*RI site. PCR products were cloned into the *Not*I and *Eco*RI sites of pBluescript (Stratagene, Ozyme).

Northern Blot Analysis—Rat multiple tissue Northern blots (CLONTECH) were hybridized at 42 °C for 16 h with a [^{33}P]dCTP-labeled *Xba*I–*Xba*I fragment from the LSR candidate cDNA and a [^{33}P]dCTP-labeled β-actin cDNA that was provided with the blot (CLONTECH). The hybridization buffer used contained 5× SSPE, 10× Denhardt's solution, 0.5% SDS, 100 μg of salmon sperm DNA, and 50% deionized formamide. The filters were washed in 2× SSC, 0.5% SDS at room temperature and in 1× SSC, 0.1% SDS at 65 °C.

Primers for RT-PCR—Primer sequences for the analysis of the mRNA by RT-PCR were as follows (Fig. 3*c*): a, 5′-GTTACAGAATTCG-CCGCGATGGCGCCGGCG-3′; b, 5′-GCCAGGACAGTGTACGCACT-3′; c, 5′-ACCTCAGGTGTCCCGAGCAT-3′; d, 5′-GAAGATGACTGGCGAT-CGAG-3′; e, 5′-ACCTCTATGACCCGGACGAT-3′; b′, 5′-CACCACCCT-GACAGTGCGTA-3′; c′, 5′- CTGGGGGCATAGATGCTCGG-3′; d′, 5′-G-CCCTGGAAGGCCTCGATCG-3′; e′, 5′-AAGTCCCTAGGATCGTCCG-3′; f′, 5′-CGTCACGAATTCCGTGGATCAGACGTC-3′. The complete coding sequences corresponding to LSR 1893 and LSR 2097 were obtained by RT-PCR using primers a and f′ and cloned in pcDNA3 (Invitrogen, Leek, The Netherlands).

Synthetic Peptide—A synthetic peptide, peptide 170, with the sequence EEGQYPPAPPPYSET was obtained commercially, conjugated to KLH, and used to immunize rabbits (Eurogentec, Seraing, Belgium).

Transient Transfection Studies—Chinese hamster ovary cells (CHO-K1, CCL-61, ATCC, Rockville, MD) were plated in six-well plates (Falcon) at 2.5–2.75 × 10^5 cells/well. After 24-h culture in Ham's F-12 medium containing 10% (v/v) fetal bovine serum, 2 mM glutamine, and 100 units/ml each of penicillin and streptomycin, 2 μg of plasmid/well were transfected using Superfect (Qiagen) according to the manufacturer's instructions (10 μl of Superfect/well, 2 h at 37 °C in serum-free Ham's F-12 medium). The plates were then washed in PBS to remove the transfection reagent, and the cells were further grown in serum-containing Ham's F-12 medium. LSR activity was then measured as described previously (10, 11) 48 h after transfection.

Preparation of Recombinant Mouse Leptin—The leptin cDNA was obtained from mouse C57BL/6J (R. Janvier Breeding Center) adipose tissue mRNAs by reverse transcription-PCR. The PCR 5′ primer introduced an initiation codon after the signal sequence, which was deleted, and a sequence coding a hexahistidine tag. The modified mouse leptin coding sequence was cloned into the pSE280 expression vector (Invitrogen, France) and expressed in *E. coli* (TG1). DNA sequencing of the plasmid confirmed the sequence of the coding open reading frame. Cells were grown at 37 °C, and the protein synthesis was induced by 1 mM isopropyl-β-D-thiogalactopyranoside. The cells were collected by low speed centrifugation and lysed by repeated freeze-thaw cycle and deoxyribonuclease I digestion. The cell membranes were extracted by detergent, and the inclusion bodies were pelleted. After three washes with 1% (w/v) sodium deoxycholate in PBS, the inclusion bodies were solubilized in 6 M guanidine-HCl. The renaturation of the recombinant protein was performed by a 100× dilution in PBS. The renatured protein was purified and concentrated by immobilized metal affinity chromatography with a nickel-ion affinity column (Probond, Invitrogen); protein was eluted with 300 mM imidazol. The purity of the recombinant leptin was determined by SDS-polyacrylamide gel electrophoresis to be >90%.

To test the activity of recombinant leptin, *ob*/*ob* and *db*/*db* C57BL/Ks (R. Janvier Breeding Center) mice were injected intraperitoneally with 25 μg of recombinant leptin or physiological saline ($n = 3$ for each condition). This caused a 32% ($p < 0.005$) decrease in the amount of food ingested by the *ob*/*ob* strain (6.9 ± 0.15 g (saline) *versus* 4.7 ± 0.87 g (leptin) food intake/24 h) but no change in the food intake of *db*/*db* mice (4.33 ± 0.15 g (saline) *versus* 4.5 ± 0.46 g (leptin) food intake/24 h). To rule out the possibility that the leptin-induced body weight reduction did not result from bacterial contaminants, lysates from *E. coli* transfected with empty plasmid were subjected to the same purification procedure. These preparations had no detectable effects on the body weight of *ob*/*ob* or *db*/*db* mice or on the LSR activity in cultured cells.

Protein Determinations—Protein concentrations were determined using a modified Lowry assay as described previously (11), using BSA as a standard.

Statistical Analysis—Results were analyzed using an unpaired Student's *t* test.

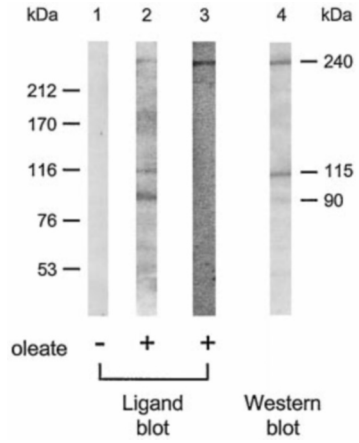

FIG. 1. **Identification of LSR elements by ligand and Western blotting.** Ligand blots were performed, as described previously (10, 12), on protein solubilized from rat liver membranes (400 μg/lane; *lanes 1*, *2*, and *4*) or the LSR 240-kDa band purified by preparative electrophoresis (80 μg/lane; *lane 3*). Nitrocellulose membranes were incubated with 40 μg/ml ^{125}I-LDL in the absence (*lane 1*) or presence (*lanes 2* and *3*) of 0.8 mM oleate or polyclonal anti-LSR antibodies prepared against the 240-kDa band (*lane 4*), as described under "Experimental Procedures."

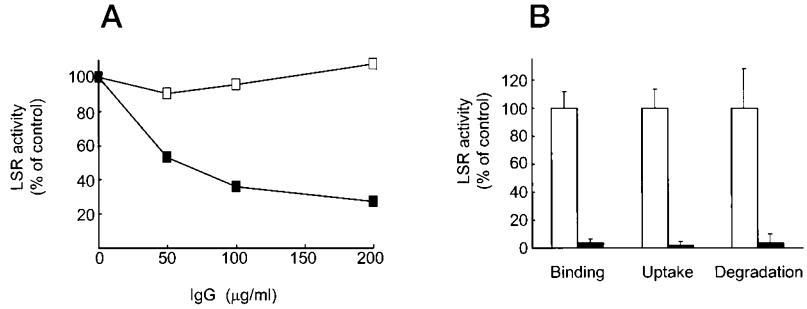

FIG. 2. **Inhibiting effect of anti-LSR antibody on its activity.** *A*, oleate-induced binding of ^{125}I-LDL (5 μg/ml) to rat liver plasma membranes was measured as reported previously (12), except that after preincubation with 0.8 mM oleate, membranes were washed six times and incubated for 1 h at 4 °C with increasing concentrations of an irrelevant IgG (□) or anti-LSR IgG (■) before the addition of ^{125}I-LDL. *B*, binding, uptake, and degradation of ^{125}I-LDL (20 μg/ml) was measured (triplicate determinations) in primary cultures of rat hepatocytes incubated 4 h at 37 °C in the presence or absence of 0.5 mM oleate and 200 μg/ml irrelevant IgG (*open bars*) or anti-LSR IgG (*closed bars*). In both *A* and *B*, results shown as mean ± S.D. are expressed as percentage of LSR activity (for *A*, the control value (100%) was 0.45 μg of ^{125}I-LDL bound per mg of membrane protein; for *B*, oleate-induced binding, uptake, and degradation of ^{125}I-LDL were 208 ± 24, 1215 ± 1.6, and 138 ± 38 ng/mg cell protein, respectively).

RESULTS

Ligand blotting experiments were performed to identify the protein responsible for LSR activity. As described previously, three main bands of apparent molecular mass ~240, 115, and 90 kDa bound ^{125}I-LDL after incubation with oleate (Fig. 1). Polyclonal antibodies were prepared against the ~240-kDa band obtained by preparative electrophoresis. This molecular mass corresponds to that of the representative example shown here in Fig. 1. However, the average molecular weight, determined on the basis of 48 ligand blots, was 230 ± 60 kDa with 3 peaks at 174 ± 16 (46%), 244 ± 20 (31%), and 314 ± 19 (23%), respectively. Despite the fact that the antigen preparation remained rather crude, the specificity of the antibodies was found adequate by Western blotting (Fig. 1, *lane 4*). After electrophoresis and transfer of total solubilized plasma membrane proteins, positive signals were detected only at bands of apparent molecular masses of 240, 115, and 90 kDa. We next tested the inhibitory effect of these antibodies on LSR activity using two independent assays. Purified IgG directed against the 240-kDa band inhibited oleate-induced binding of ^{125}I-LDL to rat liver plasma membranes (Fig. 2*A*) and the oleate-induced binding, uptake, and degradation of ^{125}I-LDL in primary cultures of rat hepatocytes (Fig. 2*B*) by ~60% and >90%, respectively.

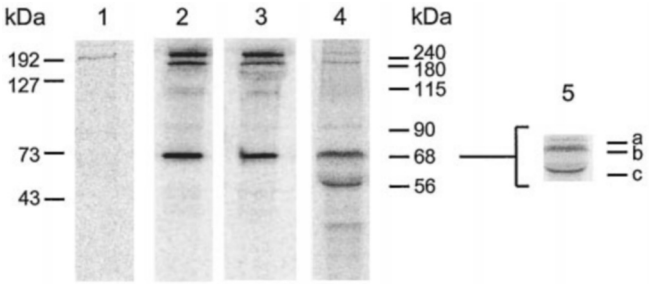

FIG. 3. **Immunoprecipitation studies in metabolically labeled rat hepatocytes using anti-LSR antibodies.** Immunoprecipitations with irrelevant rabbit (*lane 1*) or anti-LSR (*lanes 2–5*) antiserum were performed on lysates of primary cultures of rat hepatocytes metabolically labeled with ^{35}S-Promix for 2 h (22×10^6 cells/lane). Immunoprecipitate elution was performed either under nonreduced conditions without (*lane 2*) or with (*lane 3*) boiling for 5 min or under reduced conditions (5% β-mercaptoethanol, boiling for 5 min; *lanes 1*, *4*, and *5*). In some experiments, two protein bands were observed with apparent molecular masses of 66 and 64 kDa (*lane 5*, *bands a* and *b*).

Lysates of cultured hepatocytes labeled for 2 h with ^{35}S-Promix (methionine and cysteine) were obtained and immunoprecipitated with the same antibodies. After SDS-polyacrylamide gel electrophoresis under nonreduced conditions, three major bands (240, 180, and 70 kDa) and two minor bands (115 and 90 kDa) were identified (Fig. 3, *lanes 2* and *3*), while under stringent reducing conditions, two main bands of 68 and 56 kDa were detected (Fig. 3, *lane 4*). Analysis of gels run under

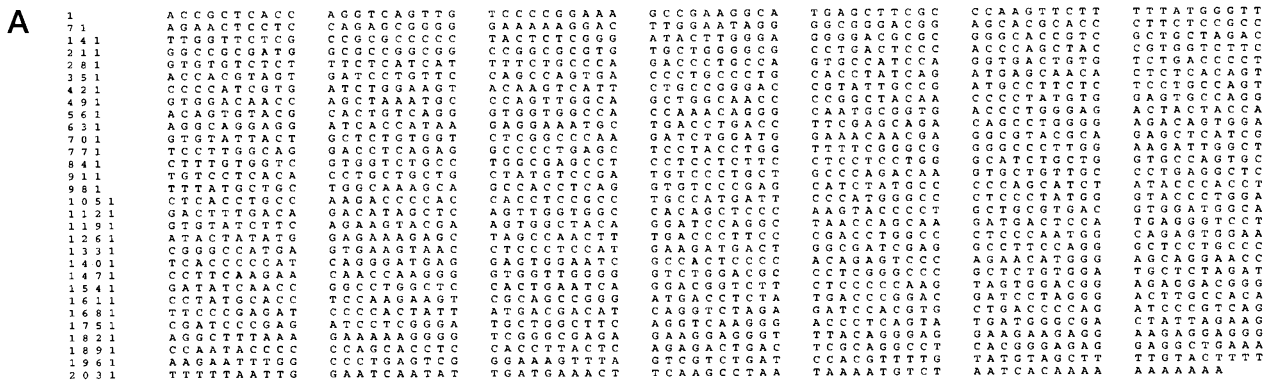

FIG. 4. **cDNA and corresponding amino acid sequence of rat LSR.** *A*, cDNA sequence of rat LSR clone. *B*, predicted amino acid sequence translated from the cDNA sequence of LSR 2097. The part of the LSR 2097 sequence in *italics* and *underlined* is absent in the LSR 1893; that which is in *boldface type* is absent in the LSR 2040. *C*, schematic representation of the domains of the sequences encoded by LSR 2097 (α), 2040 (α′), and 1893 (β). The location of the synthetic peptide 170 is also indicated (*C* is reprinted with permission (16)).

nonreduced conditions in the first dimension and reduced conditions in the second dimension, indicated that the 240-kDa band resolved into a 68-kDa (α) and 56-kDa (β) band (data not shown). Occasionally the 68-kDa band appeared as a doublet; a representative example is provided in Fig. 3, *lane 5*. After 30 min of labeling followed by a 60-min chase to allow the expression of newly synthesized protein on the cell surface, treatment of hepatocytes for 5 min with trypsin degraded most of the 68- and 56-kDa subunits (data not shown). This indicated that these proteins were primarily expressed on the cell surface.

Anti-LSR IgG was next used to screen a λGT11 expression library of rat liver cDNA. Positive clones with a 1.8-kilobase insert were obtained and found to have identical sequences. The full-length (2.1 kilobases) cDNA was obtained by 5′-rapid amplification of cDNA ends PCR, cloned, and sequenced (Fig. 4*A*). Analysis of this sequence indicated that it contained an open reading frame starting at base 218 within a Kozak consensus sequence (Fig. 4*A*) (28). The predicted protein sequence showed no homology with that of the LDL receptor or any of its related proteins (Fig. 4*B*) (2, 3). Nevertheless, the different putative domains of the candidate cDNA were compatible with a function as plasma membrane receptor (Fig. 4*C*). Indeed, a single cluster of hydrophobic residues of a length sufficient to constitute a potential transmembrane-spanning domain and several putative cellular routing motifs were present. Among those are a phosphorylation site that also corresponds to a partial clathrin binding site (29) and a dileucine-lysosomal targeting signal (30–32). All of these motifs were located toward the NH_2-terminal end, consistent with this domain being intracellular. A cysteine-rich domain that corresponded to a TNF-α receptor signature was found near the transmembrane-spanning domain. Such a motif is present in multiple copies on the extracellular domain of various cytokine receptors but was present only as a single copy on the putative LSR gene (33). Distal to this motif was a cluster of alternatively positively and negatively charged residues that provided a potential apolipoprotein-binding site.

Multiple-tissue Northern blot analysis pointed toward the liver as the primary site of LSR candidate gene expression (Fig. 5*A*); more detailed examination of blots obtained with different liver RNA extracts revealed two bands of ~2.1 and 1.9 kilobases, respectively (data not shown). We therefore sought by reverse transcription-PCR for other mRNA with overlapping sequences (Fig. 5*B*). A single band was obtained for all set of primers except for one (bc′), which gave three different products. Analysis of the sequence of the three products was consistent with a mechanism of alternate splicing from a single precursor.

The three mRNAs were designated after their base number as LSR 2097, 2040, and 1893. The predicted molecular mass of LSR 2097 translation product is 65.8 kDa and matched that of the α subunit (68 kDa) identified after immunoprecipitation (Fig. 3).

LSR 2040 encodes a 574 (63.8-kDa) amino acid α' subunit that is identical to the α except that it lacks the dileucine repeat and its second leucine-isoleucine is no longer properly positioned (Fig. 4*C*). Hence, the intracellular routing of the α' could markedly differ from the α (30–32).

LSR 1893 encodes a 525-amino acid protein with a predicted molecular mass of 58.3 kDa, which corresponded to LSR β (56 kDa). This protein lacks the putative lysosomal targeting signals, the transmembrane-spanning domain, and the cysteine-rich domain, but contains the putative FFA and ligand binding domains (Fig. 4*C*). We therefore speculate that the α or α' subunit provides the transmembrane spanning domain and that the β subunits are either located extra- or intracellularly.

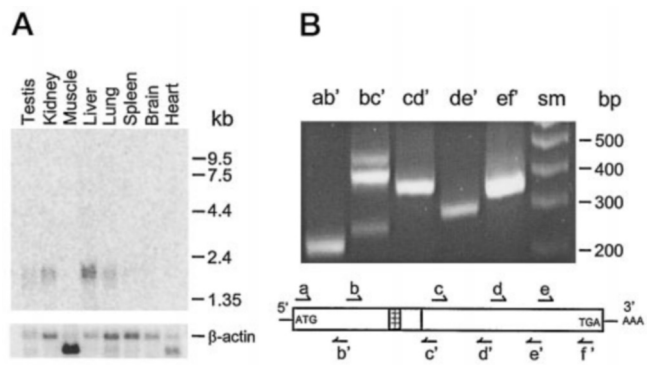

FIG. 5. **Cloning of LSR gene.** *A*, multiple tissue Northern blot analysis of LSR mRNA. A nylon membrane containing 2 μg of mRNA from different tissues (CLONTECH) was probed with a *Xba*I–*Xba*I fragment from the LSR cDNA and reprobed with β-actin as a control. *B*, reverse transcription-PCR analysis of LSR mRNA with sets of primers covering the full cDNA sequence. A single band was obtained with each set of primers except for one set (bc′), which gave three bands. PCR products from these three bands were cloned and sequenced. The schematic representation *below* summarizes the sequence analysis of the three different forms of cDNA of LSR 2097, 2040, and 1893; the *cross-hatched box* indicates the part of sequence absent in LSR 2040 and 1893; the *hatched box* indicates the part of the sequence absent only in the LSR 1893.

We next performed semiquantitative analysis of immunoprecipitation data, taking into account both the difference in molecular masses and the cysteine and methionine content of the α,α', and β subunits. This analysis showed that the β:α ratio was 2.9 ± 1 ($n = 29$). The histogram plot of the β:α ratios indicated that a 2:1 ratio was observed in 41%, a 3:1 ratio in 31%, and a 4:1 or 5:1 ratio in 20% of all experiments. The predicted molecular masses of the complexes were 185, 241, 297, and 353 kDa for the $\alpha_1\beta_2$, $\alpha_1\beta_3$, $\alpha_1\beta_4$, and $\alpha_1\beta_5$, respectively.

We next raised polyclonal antibodies against a 15-amino acid synthetic peptide with a sequence identical to that found within the highly charged putative apolipoprotein-binding domain *i.e.* on LSR α between residues 556 and 570 and on LSR β between residues 488 and 502 (peptide 170; see Fig. 4*C*). Western blotting with these anti-LSR peptide antibodies indicated that they recognized primarily two proteins with apparent molecular masses of 66 and 58 kDa, respectively (Fig. 6*A*). The signal is 2–3-fold more intense with the β band than with the α, consistent with the results of the stoichiometry analysis after immunoprecipitation. Interestingly, this antisynthetic peptide antibody also identified a low abundance band with an apparent molecular mass of ~75 kDa. The origin and function of this band are currently unclear. The antipeptide antibody significantly inhibited LDL binding to LSR in plasma membranes isolated from rat liver by ~40% and in primary cultures of rat hepatocytes by ~80% (Fig. 6, *B* and *C*). Similar to what was observed with polyclonal anti-240-kDa band (Fig. 2), the inhibitory effect was more pronounced in the binding assay that used intact cells than in that which relied on isolated plasma membranes. These data provided the first direct evidence that the products of the newly identified gene were responsible for LSR activity. Transfection experiments were then performed to further support this conclusion.

Transient transfection of LSR α into CHO-K1 cells increased the binding of ^{125}I-LDL after incubation with oleate (Fig. 7*A*, *open squares*) while leaving virtually unchanged LDL binding measured after incubations without oleate (Fig. 7*A*, *open circles*). Cotransfection of both the α and β plasmid consistently increased the oleate-induced binding of LDL (oleate (*closed squares*) *versus* no oleate (*closed circles*)). Besides the representative experiment shown in Fig. 7*A*, transient transfections

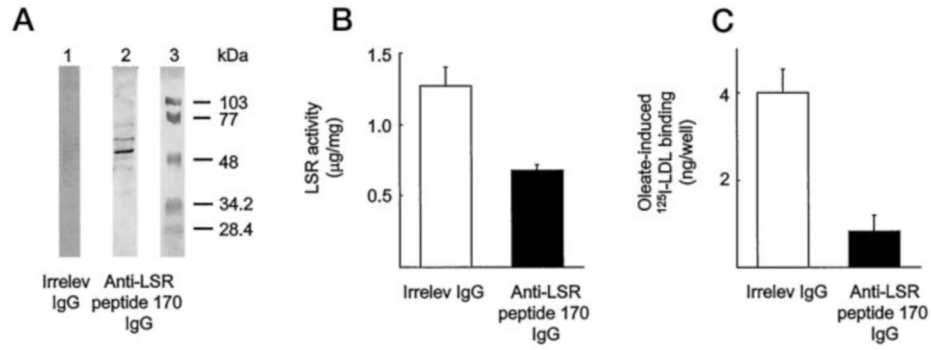

FIG. 6. *A*, Western blot of hepatocytes using anti-LSR peptide antibody 170. Primary cultures of rat hepatocytes were used at 48 h after plating (22 million cells/162-cm^2 flask). Cells were washed in PBS and then incubated 1 h at 4 °C with PBS containing 1% Triton X-100. The lysates were applied to a 10% SDS-PAGE gel under reducing conditions (2% SDS, 5% β-mercaptoethanol, and 20 mM dithiothreitol, 56 °C, 1 h). After transferring to nitrocellulose, Western blots were then performed using 75 μg/ml irrelevant IgG (*lane 1*), or anti-LSR peptide 170 antibody (*lane 2*). Bands were revealed by incubation with goat anti-rabbit IgG conjugated to alkaline phosphatase and subsequently alkaline phosphatase substrates and compared with different standards (*lane 3*). *B* and *C*, effect of anti-LSR peptide 170 antibody on LSR activity in rat liver plasma membranes (*B*) or primary cultures of rat hepatocytes (*C*). LSR activity was measured as for Fig. 2 in the presence of 200 μg/ml irrelevant (*open bars*) or anti-LSR peptide 170 IgG (*solid bars*). Results are shown as mean ± S.E. (n = 3).

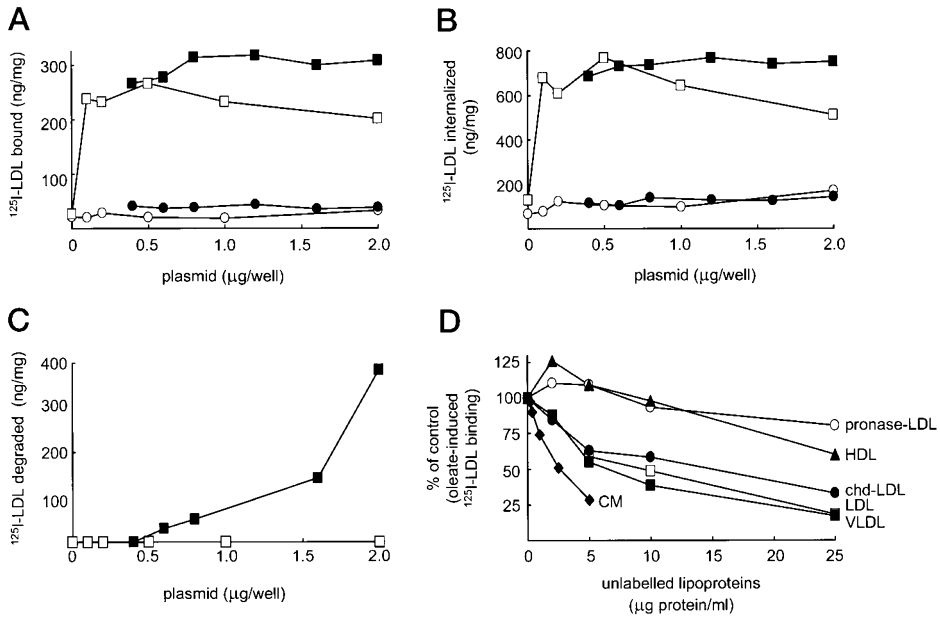

FIG. 7. **Effect of transient transfection of CHO-K1 with LSR α and β plasmid on the binding (*A*), uptake (*B*), and degradation (*C*) of LDL in the presence or absence of oleate.** *A* and *B*, CHO-K1 cells were transiently transfected with increasing concentrations of α alone (*open symbols*) or cotransfected with 0.4 μg of α and increasing concentrations of β plasmid (*closed symbols*). The plasmid amount indicated on the *x* axis represents the total amount of plasmid added. After a 48-h culture, the cells were washed once with PBS (2 ml/dish) and incubated 3 h at 37 °C with 20 μg/ml ^{125}I-LDL in the presence (*squares*) or absence (*circles*) of 1 mM oleate in DMEM containing 0.2% BSA, 5 mM Hepes, and 2 mM $CaCl_2$, pH 7.5. Following this, cells were washed as described before (11) and incubated at 4 °C for 1 h with 10 mM suramin in PBS. The medium was then recovered and counted in a γ-counter. This represents the amount of ^{125}I-LDL bound (*A*). Cells were lysed in 0.1 NaOH containing 0.24 mM EDTA and counted; this represents the amount of ^{125}I-LDL internalized (*B*). Results are shown as means of duplicate determinations. *C*, CHO-K1 cells were transiently transfected with increasing concentrations of α alone (*open squares*) or cotransfected with 0.4 μg of α and increasing concentrations of β plasmid (*closed squares*), as described for *A* and *B*. After 48 h of culture, the cells were washed once with PBS (2 ml/dish) and incubated for 3 h at 37 °C with 20 μg/ml ^{125}I-LDL in the presence or absence of 1 mM oleate in DMEM containing 0.2% BSA, 5 mM Hepes, and 2 mM $CaCl_2$, pH 7.5. Following this, cells were washed, and the amount of ^{125}I-LDL degraded was measured as described before (11). Results are shown as the amount of oleate-induced ^{125}I-LDL degraded. *D*, ligand specificity of LSR in CHO-K1 cells transfected with LSR α and β subunits (1:3, w/w). CHO-K1 cells were transfected with α and β plasmids (1:3, w/w; 2 μg of plasmid/well), followed by analysis of the ligand specificity of LSR. Binding of ^{125}I-LDL was measured as described for *A* in the presence of the indicated concentrations of unlabeled rat chylomicrons (♦), human VLDL (■), LDL (□), high density lipoprotein (▲), Pronase-treated LDL (○), or cyclohexanedione-modified LDL (*chd-LDL*, ●). All lipoproteins were prepared and modified as described previously (10, 11).

with the α plasmid alone increased oleate-induced binding by 146 ± 37% in three independent experiments. In the same experiment, cotransfections with the α and β plasmids increased binding by 560 ± 406% (data not shown). Uptake of LDL followed a pattern similar to that of binding (Fig. 7*B*). In nontransfected CHO cells, we were unable to detect the presence of oleate-induced LDL degradation products in the incubation medium. Transfection of the α plasmid alone failed to increase LDL degradation (Fig. 7*C*) despite causing a significant increase in oleate-induced LDL binding and uptake (Fig. 7, *A* and *B*). An increase in oleate-induced degradation of LDL was detected only after cotransfection with α and β plasmids (Fig. 7*C*, *closed squares*). The increase in oleate-induced binding of ^{125}I-LDL to CHO-K1 cotransfected with α and β was inhibited by more than 80% by polyclonal antibodies directed against the 240-kDa band (data not shown). The results of five different transient transfection experiments with α alone showed no detectable oleate-induced degradation products.

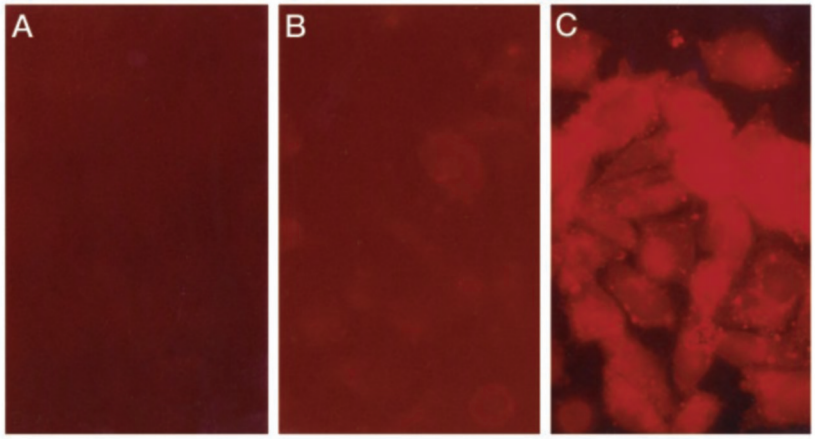

FIG. 8. **Fluorescent studies of the uptake of DiI/cyclohexanedione-modified LDL in CHO-K1 cells transfected with LSR α and β subunits.** CHO-K1 cells were plated in six-well plates containing a microscope coverslip at a density of 2.75×10^5 cells/well. After 24 h of culture, the cells were transfected with 2 μg/well of LSR α and β at a ratio of 1:3 (w/w) (*B*, *C*) in the presence of 10 μl of Superfect or with Superfect alone (*A*). After 48 h of culture, the plates were incubated with DMEM containing 0.2% BSA and supplemented (*C*) or not (*B*) with 0.5 mM oleate. After this, 5 μg/ml DiI-LDL treated with cyclohexanedione were added, and the incubation was continued for 15 min at the same temperature. The plates were then washed five times with PBS, and the cells were fixed by 20 min of incubation at room temperature with 4% paraformaldehyde. The fixative was removed, and the coverslips were recovered, rinsed in water, and mounted. The frames in *A* and *B* were obtained after a 10-min film exposure under green fluorescent light, while frame *C* was obtained after a 5-min exposure.

However, significant amounts of LDL degradation products were present after cotransfection of α and β in three out of five experiments. Transient transfections with α or with α and β did not modify the binding, uptake, or degradation of LDL in absence of oleate. Thus, transient transfection of LSR α and β reproducibly increased LSR activity in CHO-K1 cells. However, the divergence in the dose-response curves between binding/uptake and degradation after transient transfections is consistent with other variables affecting the functioning of LSR. We are therefore in need of a much deeper understanding of the biology of this gene and of its products and possibly of other genes that contribute to the regulation of this new pathway.

Competition experiments were performed next to test the ligand specificity of the LSR expressed in CHO-K1 cells cotransfected with α and β plasmid. As shown in Fig. 7*D*, chylomicrons were the most efficient ligand, while LDL and VLDL demonstrated similar affinity, with a slight but reproducible advantage to the latter. High density lipoproteins were 5–8-fold less efficient competitors than LDL and had an affinity similar to that of Pronase-treated LDL. However, in keeping with our previous observations, cyclohexanedione modifications of apoB arginine and lysine residues only slightly reduced LDL affinity for LSR (11). These data suggest that the apoB acidic residues interacting with positively charged residues of the LSR putative ligand binding domain are sufficient to allow the binding of the particles to the LSR. Together, these data indicated that the ligand specificity of the receptor expressed in transiently cotransfected CHO cells closely resembled that of native LSR present in rat liver plasma membranes and in normal human fibroblasts (10–12).

Because in transfection experiments the proteolytic degradation of LDL did not systematically follow the uptake of the particles, we sought to ascertain that LDL were indeed internalized. The fluorescent pattern of CHO-K1 cells incubated with DiI-LDL treated with cyclohexanedione to prevent LDL receptor-mediated binding and uptake were examined. Virtually no uptake of cyclohexanedione-LDL was detectable after incubation without oleate (Fig. 8, *left panel*). A faint but detectable pattern of fluorescence was found in nontransfected cells incubated with 0.5 mM oleate (Fig. 8, *middle panel*). This corresponded most likely to endogenous LSR activity. Cotransfection of LSR α and β plasmids dramatically increased the fluorescent pattern observed after incubations with oleate (Fig. 8, *right panel*) while leaving it unchanged after incubations without oleate (data not shown).

DISCUSSION

Since 1994, ligand blotting in the presence of oleate has led to identification of candidate LSR proteins. In fibroblasts isolated from a patient with familial hypercholesterolemia, we initially described two bands of 85 and 115 kDa (10). When solubilized rat liver membrane proteins were used (12), three bands of 90, 115, and 240 kDa were observed. Optimization of the experimental conditions to improve the efficiency of transfer of large molecular weight complexes and minimization of the storage of the sample to avoid proteolytic degradation indicated that the ~240-kDa band accounted for most of LSR activity.

Partial purification of the unstable LSR complexes allowed the production of polyclonal antibodies, which 1) established a link between the candidate proteins identified by ligand blotting and LSR function and 2) demonstrated immunological cross-reactivity between the 240-, 115-, and 90-kDa bands. These antibodies were then used to characterize LSR proteins endogenously labeled with [^{35}S]methionine and cysteine. Immunoprecipitation studies under nonreduced and reduced conditions revealed that the LSR complex was constituted of two main subunits designated α (68 kDa) and β (56 kDa). Occasionally, the α band appeared as a doublet, consistent with PCR analysis of LSR mRNA, which indicated that at least three mRNAs are produced. However, the probes currently available do not allow us to distinguish between the α and α'. Stoichiometry analysis was therefore performed with the assumption that the α subunit is primarily expressed. This analysis indicated that the most abundant (41%) LSR complexes were $\alpha_1\beta_2$ with predicted molecular masses of 180 kDa and $\alpha_1\beta_3$ with predicted molecular masses of 236 kDa (31%). Therefore, the results of immunoprecipitation experiments appeared to match

those of ligand blotting with respect to both the apparent molecular mass of the LSR complexes and the relative abundance of the $\alpha_1\beta_2$ (46 *versus* 41%) and $\alpha_1\beta_3$ (31 *versus* 31%) complexes. Clearly, the LSR β subunit is more abundant than the α or α'. Indeed, the β band was more pronounced in Western blots using antibodies directed against a synthetic peptide derived from a sequence common to α, α', and β subunits. Thus, three independent sets of experiments indicated that the LSR is a multimeric receptor consisting of two main subunits, organized either as a heterotrimer or tetramer and assembled through disulfide bridges. We hypothesize that it is because of the instability of these complexes that the breakdown products of 90 and 115 kDa were those initially identified.

Screening of expression libraries using polyclonal antibodies led to the cloning of a candidate gene. This gene was selected for further analysis because 1) the predicted molecular mass of its products matched that of the two main LSR subunits; 2) the sequence predicted by the open reading frame was compatible with the function of a receptor; and 3) the gene is primarily expressed in the liver, *i.e.* the tissue in which most LSR activity has been found. We initially relied upon the production of antisynthetic peptide antibodies to establish the link between the newly identified gene and the LSR function. The inhibitory effect of these antibodies was pronounced (40–80%), detected in two independent types of assays, and followed a pattern similar to that of polyclonal anti-240-kDa band antibodies. However, the inhibitory effect was less pronounced with the antisynthetic peptide. We cannot therefore completely rule out the possibility that another gene accounts for part of LSR activity.

Transient transfection of the LSR α subunit increased the oleate-induced binding and uptake of LDL but did not increase proteolytic degradation of the particle. This was only seen after cotransfection of α and β plasmids. Clearly, further studies using stably transfected cell lines are needed to define the biology of LSR and understand how the β subunit contributes to the regulation of its cellular routing. At this stage, however, transient transfection data reproducibly established that the products of the LSR gene functioned as lipoprotein receptors only when the cells were incubated with oleate.

The reasons for interexperimental variation in the efficiency of transient transfections are not clearly understood. The cell density and the time of plating appeared to be critical factors with optimum values being obtained with plating cell densities of 2.5–2.75 $\times$ 10^5 cells/well (six-well plates). The possibility that the assembly of LSR multimeric complex is under the regulation of molecular chaperons must also be considered. We are currently pursuing the identification of such a protein that coprecipitates with LSR and is visible as a 33-kDa band in Fig. 3, *lane 4*. The identification of this LSR-associated protein has been achieved through NH_2 terminus sequencing, and its role in the regulation of LSR assembly and function is currently being investigated.

The ligand specificity of the reconstituted receptor closely resembles that of the LSR expressed in fibroblasts and in hepatocytes; triglyceride-rich lipoproteins are those with optimal affinity for the LSR (10, 12). We nevertheless used LDL in most experiments mainly because of technical considerations. Indeed, LDL, unlike triglyceride-rich lipoprotein, contains a single nonexchangeable apolipoprotein (apoB), is easily prepared in large quantities, and is radiolabeled mostly on its protein moiety. Because of this, LDL binding studies provided a better signal:noise ratio than those using ^{125}I-VLDL or ^{125}I-chylomicrons (data not shown). We do not believe, however, that under normal conditions LDL is a physiological ligand of the LSR. Indeed, LDL contains mainly cholesterol and cannot, when acted upon by hepatic lipase, produce FFA in sufficient amounts to induce the conformational shift of the LSR complex that unmasks the lipoprotein binding site (12). It is possible, however, that a significant part of LDL are cleared through the LSR when the LDL receptor is defective and plasma LDL concentrations are therefore increased. Such a mechanism could explain that the bulk of LDL is cleared by hepatocytes, even in subjects lacking the LDL receptor (34).

LSR is capable of binding unmodified TG-rich chylomicrons, and its affinity is greatest for these as well as the larger of the VLDL particles (11). We therefore postulate that it provides a significant pathway for the clearance of chylomicrons. One would therefore anticipate that it binds apoE. Previous studies have shown that triolein phosphatidylcholine emulsions supplemented with apoE bind to LSR while the same emulsions not supplemented with apoE do not (10). Furthermore, VLDL isolated from subjects with the apoE 2/2 phenotype and with symptomatic type III hyperlipidemia does not bind to LSR (10). In addition, studies of subjects with type III hyperlipidemia have shown that this condition was markedly influenced not only by the presence of a specific apoE isoform (apoE 2/2) but also by yet unidentified nutritional factors (35). The characterization of the LSR gene also shed new light on the results of previous studies of the apoE receptor (36, 37). Indeed, the fraction of apoE binding proteins, initially found to be partially contaminated with F_1-ATPase, contained an unidentified 59-kDa apoE-binding protein (37). The molecular weight of this protein matches closely the predicted 58 kDa of the most abundant LSR β subunit. It is possible that prior to the assembly of the LSR complex, the most abundant β subunit is localized within the cell, *i.e.* the location of the previously identified putative apoE receptor (37).

In contrast with the previously described apoE receptor, the LSR complex also binds apoB. The apoB domain that binds to LSR appears to be different from that of the LDL receptor. Indeed, cyclohexanedione modification of apoB basic residues suppresses its ability to bind to the acidic residues of the LDL receptor, whereas apoB binding to LSR is slightly, but not markedly, decreased by cyclohexanedione. This suggests that apoB acidic residue, interacting with LSR positively charged residues, are sufficient to mediate the binding.

The finding of a second receptor that binds both apoB and apoE was rather unexpected. It is, however, not the first characterization of a receptor distinct from the LDL receptor but capable of binding unmodified LDL. Indeed, Hoeg *et al.* (38) have characterized hepatic membrane receptors capable of binding LDL and with apparent molecular masses of 270 and 320 kDa. These correspond reasonably well with those of the $\alpha_1\beta_3$ and $\alpha_1\beta_4$ LSR complexes. These binding sites were expressed in membranes from subjects homozygous for familial hypercholesterolemia, which eliminated the possibility that the LDL binding site corresponds to the di- or trimer of the LDL receptor (M_r ~130 kDa). Finally, similar to LSR, the LDL binding sites were independent of calcium. Thus, the apparent molecular mass of the most abundant LSR subunit matches that of the previously identified intracellular apoE-binding protein. However, because of the multimeric organization of the LSR complex, it may also account for the previously described paradoxal LDL binding site.

FFA are thus far the only molecular tools that cause a direct activation of LSR. The relative physiological importance of such mechanism, however, remains to be determined. Indeed, the concentrations of FFA that are needed to achieve such an effect are greater than those circulating in plasma bound to albumin, and it is currently unclear whether the concentrations generated at the site of lipolysis are sufficient to activate

LSR. The possibility that other as yet unknown mechanisms of acute activation intervene must also be considered. However, it is our current hypothesis that the activity of the lipolytic enzymes LPL and hepatic lipase, present in the space of Disse (39, 40), act upon TG-rich particles generating FFA that induce the conformational shift of LSR, leading to its activation and the internalization of the particles. A key issue is to determine whether the receptor or the enzymes represent the rate-limiting step. Because genetic defects in LPL or its activator, apoCII, lead to massive hypertriglyceridemia, it is widely accepted that LPL is responsible for the clearance of plasma TG. Lipoprotein receptors, on the other hand, account for the removal of small cholesterol-enriched and triglyceride-depleted chylomicron remnants. Indeed, this might very well be the function of LRP (7). It is, however, likely that LSR acts in concert with the lipase system and contributes to the clearance of a significant fraction of plasma TG. In support of this interpretation is the finding that the LSR binds with high affinity large TG-rich chylomicrons (10). Further, overexpression of receptor-associated protein up to concentrations that inhibit LSR activity caused hypertriglyceridemia (8, 9). Also, injections with the LSR inhibitor lactoferrin induce a massive postprandial hypertriglyceridemia (10).[2] In addition, LSR apparent number in the liver correlates strongly and negatively with postprandial plasma TG but not with plasma cholesterol. It is likely that the LSR represents a rate-limiting step for the removal of TG. We therefore speculate that the relative activity of LPL in peripheral tissues *versus* that of LSR in the liver plays a key role in determining the partitioning of dietary lipid between these different tissues.

Identification of the LSR gene will allow the production of transgenic mice overexpressing or deficient for this receptor. These animal models can be used to define the precise role of the liver in determining the ability of individuals to dispose of dietary lipid.

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