

# The murine macrophage apoB-48 receptor gene (*ApoB-48r*): homology to the human receptor<sup>1</sup>

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**Abstract** Previously we cloned the human macrophage apolipoprotein B-48 receptor (ApoB-48R) and documented its expression in human atherosclerotic foam cells (1). Now we have identified and characterized the murine macrophage apoB-48r cDNA gene sequence and its chromosomal location. The cDNA (3,615 bp)-deduced amino acid (aa) sequence (942 aa) is ~45% identical to the human macrophage APOB-48R, but not to other known gene families. The murine *ApoB-48r* gene, like the human *APOB-48R* gene, consists of four exons interrupted by three small introns and is syntenically located on chromosome 7. Functionally significant conserved domains include an N-terminal hydrophobic domain, a glycosaminoglycan attachment site, an N-glycosylation site, and an ExxxLL internalization motif C-terminal to the putative internal transmembrane domain. Two conserved coiled-coil domains are likely involved in the spontaneous homodimerization that generates the active dimeric ligand binding species (mouse, ~190 kDa; human, ~200 kDa). Transfection of the murine apoB-48R into Chinese hamster ovary cells (CHOs) confers apoB-48R function: rapid, high-affinity, specific uptake of known triglyceride-rich lipoprotein ligands of the apoB-48R and, of note, uptake of the cholesteryl ester-rich apoB-48-containing very low density lipoproteins that accumulate in atherosclerosis-prone apoE-deficient mice. Uptake of these ligands by murine apoB-48R-transfected CHOs causes saturable, visible cellular triglyceride and cholesterol accumulation in vitro that resemble foam cells of atherosclerotic lesions. In aggregate, the data presented here and that previously published suggest that the apoE-independent murine apoB-48R pathway may contribute to the spontaneous development of atherosclerotic lesions rich in macrophage-derived foam cells observed in apoE-deficient mice, a murine model of human atherosclerosis.—Brown, M. L., K. Yui, J. D. Smith, R. C. LeBoeuf, W. Weng, P. K. Umeda, R.

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**Supplementary key words** atherosclerosis • chylomicrons • hypertriglyceridemia • postprandial lipoproteins

Recently we cloned and characterized the human macrophage apolipoprotein (apo)B-48 receptor (apoB-48R) cDNA (accession #AF141332), which did not belong to any known protein families, and mapped the *APOB-48R* gene to chromosome 16p11 (1). The apoB-48R is implicated in atherogenesis because it specifically binds certain atherogenic lipoproteins and the protein is expressed by atherosclerotic foam cells. This receptor binds to apoB-48 of chylomicrons, the intestinally derived triglyceride-rich lipoproteins (TRL) that transport essential dietary lipids and lipid-soluble nutrients. The apoB-48R also binds to VLDL from hypertriglyceridemic (HTG) humans, TRL, that contain both apoB-48 and hepatically-derived apoB-100 (2). In vitro, TRL uptake by the apoB-48R converts macrophages and apoB-48R-transfected Chinese hamster ovary cells (CHOs) into lipid-engorged cells (1), similar to the macrophage-derived foam cells characteristic of

Abbreviations: apo, apolipoprotein; apoB-48R, apolipoprotein B-48 receptor; CHO, Chinese hamster ovary cell; GPDH, glyceraldehyde-3-phosphate dehydrogenase; HTG, hypertriglyceridemic; TRL, triglyceride-rich lipoproteins.

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atherosclerotic lesions and of eruptive xanthomas in diabetic subjects with fasting chylomicronemia (3). In humans, the apoB-48R is expressed by blood-borne monocytes, endothelial cells, monocyte-macrophages of the spleen, bone marrow, and other immune tissues, and the placenta. Of note, foam cells in human atherosclerotic arterial lesions also express the apoB-48R protein, as determined immunohistochemically (1). Its tissue distribution and ligand specificity suggest that the apoB-48R normally may function as a nutritional receptor to provide essential dietary lipids and lipid-soluble vitamins to cells of the immune system and the placenta. In some disease states, such as elevated plasma triglycerides or diabetes, the pathway, which is not regulated by sterol, appears to be overwhelmed and possibly contributes to the pathological formation of foam cells in the artery wall, the spleen, bone marrow, skin, and elsewhere observed in these diseases.

Previously published cell and ligand blotting studies documented the presence of the apoB-48R pathway in murine macrophages, albeit by different names (abnormal TG-rich lipoprotein, abnormal VLDL, or  $\beta$ -VLDL receptor) (4, 5, 6), as these studies were done prior to identification of the receptor's primary ligand, apoB-48 (7). The murine apoB-48R is considered by other investigators to be a likely candidate for the macrophage uptake of atherogenic apoB-48-containing lipoproteins, especially in apoE deficient (apoE<sup>-/-</sup>) mice (2, 8, 9). The apoE<sup>-/-</sup> mouse is an established murine model of human atherosclerosis in which atherosclerotic lesions with numerous macrophage-derived foam cells spontaneously develop on a chow diet (this effect is thought to be due to the apoB-48-containing lipoproteins that accumulate in plasma) (10, 11). Atherogenesis in apoE<sup>-/-</sup> mice is accelerated and exacerbated by an atherogenic Western diet that further elevates the plasma levels of apoB-48-containing lipoproteins (10). Monocyte-macrophages are primary cells involved in murine atherosclerosis. The op mutation in macrophage colony stimulating factor leads to decreased numbers of monocytes and macrophages, and in the apoE<sup>-/-</sup> background, the op mutation leads to a 2.5-fold increase in plasma cholesterol but a 90% reduction in lesions (12). These results support the role of macrophages in the uptake of apoE<sup>-/-</sup>  $\beta$ -VLDL, in which apoB-48 is the primary apoB species, and also supports the concept that the macrophage apoB-48R may contribute to atherosclerosis. Other macrophage lipoprotein receptors implicated in foam cell formation require apoB-100 or apoE as ligands, with or without oxidation, modification, lipoprotein lipase, and/or heparan sulfate proteoglycans. In contrast, the apoB-48R activity is independent of these factors and therefore could participate directly in foam cell formation in apoE<sup>-/-</sup> mice (13).

Here we present the murine apoB-48R cDNA and gene sequences, chromosomal location, the deduced primary protein sequence, predicted conserved secondary and tertiary structures, and functional characteristics of the transfected receptor. The murine apoB-48R is homologous to only two sequences in GenBank, the human *APOB-48R* on chromosome 16p11 (accession no. AF141332) (1) and a

recently submitted (July 12, 2001) human 1623 bp cDNA sequence called "*Homo sapiens* similar to apoB-48R" (NCBI accession XM\_051120) that is identical to the C-terminal (about) half of the human apoB-48R cDNA and also on chromosome 16. Thus, as we previously suggested (14), the *APOB-48R* appears to belong to a family of its own.

## MATERIALS AND METHODS

### Cloning of the apoB-48R

Standard molecular biology techniques were used throughout (15), as previously published (1). DNA sequencing was done in the automated sequencing cores at the University of Alabama at Birmingham. Initially, PCR primers based on the human apoB-48R sequence were used to screen murine libraries, but to no avail; the human sequences complimentary to these primers were not present in the murine apoB-48R sequence. Next, a full-length human apoB-48R cDNA clone (AF 141332) was used to screen a murine macrophage cDNA library (Stratagene). Four apoB-48R cDNA clones were identified and sequenced. Introns were identified by PCR using primers based on the murine cDNA sequence and the organization of the human *APOB-48R* gene (AF141333), with murine macrophage P388D<sub>1</sub> genomic DNA and murine 129 genomic tail tip DNA as templates, as previously described (1). Both sources of genomic DNA gave identical sequences.

### Transfection studies

The murine apoB-48R cDNA was removed from pBluescriptII KS (-) by *EcoRI/NotI* digestion and ligated into pcDNA 3.1 (-) with Ready-to-go T4 ligase (Amersham) per the manufacturer's protocol. Clones were isolated by standard procedures and verified by partial DNA sequencing.

CHO-K1 cells were from the American Type Culture Collection and ldl A-7 CHOs that lack the LDLR were kindly provided by Monty Krieger (16). These cells were transfected using lipofectamine (Life Technologies) per the manufacturer's protocol and selected in medium containing G418 (0.5 mg/ml; Agri-Bio) for 2–4 weeks, as previously described (1). Cells were trypsinized, subcultured, and grown for  $\geq 24$  h on coverslips for visualization after oil red O staining, or into 6-well plates for quantification of cellular TG or cholesterol after incubation with specified lipoproteins as described previously (1). In most studies with apoE<sup>-/-</sup> VLDL (total VLDL), the VLDL from apoE-deficient mice was isolated by overlaying plasma with an equal volume of PBS followed by ultracentrifugation for 16 h at 40,000 rpm in a 40.3 rotor (Beckman). The cloudy VLDL layer at the top of the tube was removed with a syringe. Because total VLDL is extremely heterogeneous, we also isolated murine VLDL ( $S_f$  100–400) from total VLDL and plasma of apoE<sup>-/-</sup> mice and human VLDL by cumulative flotation into the same flotation class normally used for human VLDL ( $S_f$  100–400) to compare lipoproteins of similar size and density in selected ligand blotting experiments; the  $S_f$  100–400 fraction was comparable to total apoE<sup>-/-</sup>  $\beta$ -VLDL binding to the isolated apoB-48R. Human VLDLs ( $S_f$  100–400) were trypsinized as described (17). Ligand blotting and Western blotting were done as described previously (7, 18). The antibody used for Western analyses was produced against a synthetic peptide representing the N-terminal 15 residues of the human apoB-48R (MDFLRLYLPLGLHQAL) coupled to keyhole limpet hemocyanin. The mouse and human sequences in this N-terminal domain are identical, except that mouse aa 7 is R instead of Y. Unlike other

anti-human apoB-48R antibodies (1), this anti-peptide antibody recognizes both the murine and the human apoB-48R.

### ApoB-48R mRNA characterization

For Fig. 3, oligonucleotide primers corresponding to -2 to +16 bp (sense) and +530 to +547 (antisense) of the cDNA sequence were used to label the probe using digoxigenin-modified UTP incorporation by PCR (Roche) with hybridization, wash, and detection per Genius kit instructions. Chemiluminescence was recorded on Hyperfilm™ ECL™ (Amersham Pharmacia) and digitized by a Hewlett Packard optical scanner. Probes for glyceraldehyde-3-phosphate dehydrogenase (GPDH) mRNA were prepared as just described for the apoB-48r probe except that the PCR primers listed below were used to generate the 983 bp digoxigenin-modified UTP cDNA probe used as control. For Fig. 4, a multiple tissue cDNA (MTC™) panel was purchased from Clontech (Mouse panel #1; K1423-1; Palo Alto, CA). Each panel is a set of first-strand cDNA normalized to five housekeeping genes. We used a mouse gene-specific set of primers for apoB-48r that include two small introns. If genomic DNA was amplified, a 1,035 bp product is generated. If a cDNA is amplified reflecting the mRNA, then a 860 bp product is found. The apoB-48r primers were: sense, 5'-gagctggaggactaca-3'; antisense, 5'-gtaaccagggtccagaag-3'. The mouse GPDH primers were: sense, 5'-gaaagacaggctgacaaggc-3'; and antisense, 5'-acacaaagcaagggatgg-3'. PCR was carried out on equal aliquots (2.5 µl) from the MTC panel for 21, 24, 27, and 30 cycles, analyzed on 1.5% agarose gels after electrophoresis for 1.5 h at room temperature, and visualized by ethidium bromide staining. GPDH results indicated that equal aliquots had been analyzed (data not shown).

### Chromosomal mapping

The mouse chromosomal location of *ApoB-48r* was determined by linkage analysis of restriction fragment length variants in progeny derived from matings of [(C57BL/6J)Ei × SPRET/Ei]F1 × SPRET/Ei mice (19). PCR amplification of genomic DNA from parental mice was performed using the primer set 5'-cgagagcagaaggtccaca and 3'-tgaccgcacccttgataa. The 966 bp products were digested with *Pst*I, which revealed restriction fragment length variants. The parental strain C57BL/6 exhibited bands of 750 and 275 bp, and the parental strain *Mus spretus* exhibited bands of 760 and 325 kb. The segregation of the 275 kb band representing the C57BL/6J allele was scored in 94 progeny from the backcross to SPRET/Ei. Data were sent to The Jackson Laboratory for pedigree analysis.

## RESULTS

### ApoB-48R cDNA and deduced protein sequence

The murine apoB-48R cDNA (GenBank accession no. AF141335) is 3,615 bp with a Kozac start site (bp -4 to +4), a 2,826 bp open reading frame beginning at bp +1, and a stop codon (TGA) at bp 2,827–2,829 (Fig. 1). The 3' untranslated region contains two overlapping polyadenylation signals (AATAAA) and a poly(A) tail. Sequencing tail tip genomic DNA (129) and genomic DNA isolated from cultured murine P388D<sub>1</sub> macrophages, previously shown to express this receptor (5, 6), gave identical sequences. The coding sequences of the two genomic clones were identical to the cDNA sequence.

### Conserved protein sequence

The murine apoB-48R amino acid (aa) sequence is homologous to the human apoB-48R. To date, it is not related to any other gene family (Fig. 2). The murine apoB-48R, like the human sequence, does contain several small and common functional motifs described below. The murine apoB-48R protein was originally identified in P388D<sub>1</sub> macrophage extracts by ligand blotting and has an apparent molecular mass of ~190 kDa on SDS-PAGE (6), while the major human monocyte apoB-48R ligand binding species has an apparent M<sub>r</sub> of ~200 kDa (20). The mouse apoB-48r cDNA (3,615 bp) predicts a protein of 942 aa and ~103 kDa mass, while the human cDNA (3744 bp) predicts a protein of 1,088 aa and ~115 kDa. The human apoB-48R appears to exist in human monocytes and macrophages and in apoB-48R-transfected CHOs as a homodimer of apparent M<sub>r</sub> ~200 kDa that cannot be dissociated by boiling and/or treatment with reducing agents, detergents, or denaturing reagents such as urea and guanidine (1). The dimerization of the receptor protein was supported by studies with GST-apoB-48R (human) fragment fusion proteins that spontaneously dimerize when the conserved coiled-coil protein-interacting domains are expressed (1). Likewise, the murine apoB-48R previously identified in murine P388D<sub>1</sub> macrophages (6) and in RAW 264.7 macrophages and apoB-48R-transfected CHOs described below has an apparent M<sub>r</sub> by ligand blotting of ~190 kDa and thus also appears to be a dimer.

The deduced protein sequences of the murine apoB-48R and the human THP-1 monocyte apoB-48R were aligned using the pairwise "Blast 2-sequences" program (NCBI, NIH) and were found to have an overall identity of ~45%, but were more conserved at the N-terminus and C-terminus (≥80% homology). The N-terminal ~75 aa conserved domain is encoded by the first exon (aa 1–18) and part of the second exon. The conserved 36 aa C-terminal domain is encoded by the 3' end of the third exon and the 5' end of the fourth exon. Other conserved protein homologies with likely functional significance include a potential glycosaminoglycan attachment site (aa 112 murine, 118 human), a conserved N-glycosylation site (aa 510 murine, aa 617 human), and two potential coiled-coil domains that appear to be involved in the dimerization of the receptor protein in macrophages and in apoB-48R-transfected CHOs. There are relatively few cysteines in either species (eight in human, six in mouse), and both receptors are not reduced in size and are active after reduction, indicating that the cysteines are not involved in dimerization or in ligand binding, in contrast to the members of the LDLR family that contain cysteine-rich ligand binding domains that are inactivated by reduction. The cysteines appear to produce the microheterogeneity sometimes seen in unreduced human apoB-48R preparations by forming mixed disulfides (13) and in the murine apoB-48R; these do not affect ligand binding. The mouse apoB-48R, like the human receptor, is highly polar, with ~22% acidic and ~10% basic residues and only two relatively hydrophobic domains, the N-terminal 30 aa and an internal 24 aa sequence, at aa 635–658, analogous to the

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| <pre> v          v          v          v          v          v          CAGG ATGGATTTCTCCGGCTTCGTCTCCCTGGGCTACATCAGGCTTTGAGGGGAGACTGGAC M D F L R L R L R L P G L H Q A L R G A C L D D v          v          v          v          v          v          v TCCTTCAGTGCCTTTGTGTCTTACCTCCTAGGACACACAGTCCCACAGTAGAGAGCAG S F S A F V S Y L V G D T V P T V E R Q v          v          v          v          v          v          v ACACAGCAGCTGAGAACTGGAGAGGTTGACTGAGGGGAAGGTTGTAGAGAGGAAGCC T Q A A E E L G E V T E G K V V G E E A v          v          v          v          v          v          v CAGGAGTACTGGAGGACTTAGAAGTGCCAGAGTGGGGGTTAGAAGCACCAGGAG Q E V L E G L R S G Q S E G V E A P E E v          v          v          v          v          v          v ACTAGAGATGCCAAGAAGAGTTTAGTGTGAAAGACACTGGGGTGGAGAGCAGAT T R R C Q E G S L A G E Q T W G W R A D v          v          v          v          v          v          v AGCTCTCAAGGCCCAAGCAAAAGCAGGACTGGTCTCGAAGGAGCAGTGGAT S S A R P Q A E R Q D T G S W K A A E D v          v          v          v          v          v          v GCCAGGGCCAGGAGCAAGTTCCTCACTGAAGCCTGAGCAGAGCTGGGACTCCAGCA A R G Q E P S V P L K P E A E P G T H R v          v          v          v          v          v          v GACAGGACAGTAATACAGCCAGGAGTCTGGGAGCAGGTGAAGAGGAAAGCAGT D R S S N T A Q E I W E H G E E A S S v          v          v          v          v          v          v GGGAGCCTCTGAGAACTGTGAACAGAGAAAGAGGAGGAGTGGTTCAGAGCAGCA G E P L R T C E Q K E E E E E V V R A A v          v          v          v          v          v          v GAGTCAAGGATGGCCAGGGGGTGGAGTCAACAGCACTGGCACAGGACCTGGGGGG E S G M A E G V E S Q P T W H S E P G G v          v          v          v          v          v          v AATGCTGGCCTGAGGGGCAGTGTGACAGAGGACCAAAAGATGACTGGGTGGCC N A G T E G Q H V T E D S K K E I D W V A v          v          v          v          v          v          v AAAGACATGGTTGCAGAAATCGAATGGTTTGGTCCAAAGGATTGACAAGGAAGAGGAG K D M V A E I E W F G A K G I D K E E E v          v          v          v          v          v          v AGGATGGTCCCAATGAGGATGGTGAAGGGCAAGGGACAGGGGACAAATGCCCGGG R M V P M R D G E R A R A Q G T Q C P G v          v          v          v          v          v          v GCCAGTCTGAGGACAGGCCATGTTGAGTAGAGAGCCTGGACAGTCTCAGACAGGGAG A E S E D Q A M L S R E A W T V S D R E v          v          v          v          v          v          v GGGCTGATAGCCTAGGAGTTGAGGAAAGAAATACGGGTGAGACCCAGGAGCAACTTC G A D S L G V Q G T E Y G S D P G D N F v          v          v          v          v          v          v CCAGGAAGTACTGGGAGTCTGGGTCTGAGAAGAGCTGACAAGGGAGACCAGCAGGAT P G T T G R V W V L E E A D K G D Q Q D v          v          v          v          v          v          v GAGTCTGAGCAGGAGAGAGGCTGAAGTTAGGTTCCGATCCAGACCTGGAGGCTGAG E V D E K R E A E A V R F P I Q T L E A E v          v          v          v          v          v          v AGAAGTGGAGAGATGACTGAAGGCCACATAGCTGAAGAGGAGGCTATGGGAGAACAGGAA R T G E M T E G H I A E E E A M G E Q E v          v     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        v          v          v CCAGACTGAGCCAGGCGGCTGCTAAGCTTGAAGGATATCCTGGGCTGTGGGGTTT P D L S P E R V L S L K E Y P G P V G F v          v          v          v          v          v          v GCAGGCCCTGAGTAGAAGCCTGGGAAACTGGATAGGGGTTGTGACCCAGAAATAGC A G P E L E A W G N W S R G V D R R N S v          v          v          v          v          v          v CAGGAGTAAAGCAGATGCTGAAGCAGGAAAGGAAAGACTGCAACCGAGCAGGAGTG Q E V K A D A E A G K E Q T A T E Q A V v          v          v          v          v          v          v GAGATCCGGGCTGAAGGAGGCCAGGAGGCCAACAGCCAGGTTCTTCGGGTCAGGAGGA E I R A E G G Q E A Q Q P E V F G S G G v          v          v          v          v          v          v GAGGAGCACTGACTCCGTAGCACTTAACCCGAGCTGGAGGAAAGCAAGGAGCAGAG E E A L T S V A L N P E L E G S Q G A E v          v          v          v          v          v          v GCGGGACAGAAGAGTCACTGGGAGGATCCAGCCACAGAAAACGAGGCTGCTGAGGAG A G T E E S V E E S K P T E N E A A E E v          v          v          v          v          v          v GAGGCTGAGTGCCTGGGAGGAGTGGAACTGCAGGAAGAGGAGGCTGGAGGAGTG 1864 </pre> | <p>-4<br/>60<br/>120<br/>180<br/>240<br/>300<br/>360<br/>420<br/>480<br/>540<br/>600<br/>660<br/>720<br/>780<br/>840<br/>900<br/>960<br/>1020<br/>1080<br/>1140<br/>1200<br/>1260<br/>1320<br/>1380<br/>1440<br/>1500<br/>1560<br/>1620<br/>1684<br/>1744<br/>1804<br/>600<br/>1864</p> | <pre> E A V V P W E A D G T C R K R R L E E V v          v          v          v          v          v          v ACTCTGAGCCTCAAGACAGTGGAGACAGAGACCAAGTTATTTGGCTGAGGATTAT T L S L Q D S E D T E T S Y L A E E I I v          v          v          v          v          v          v GTGGTATCAGGGCCGTGGATACCAGGAAGGGCCAAATGGAGCTGGGTTGGCTCCA V G I R A V D T E E G P K W E A G L A P v          v          v          v          v          v          v GAAACAGAGCTGGAAAGCCTGGTGTCCGAGGGCAGAGGGAGGCTGGGAGAGCAGC 2040 E T E L G K A W C S E G R G E A G R G T 680 v          v          v          v          v          v          v 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920 v          v          v          v          v          v          v TTTGGCTTTGTCTATCTGGCTGATGACAGAGTCAAGCCGACTGAGCCAGCAAG 2820 F G F A H P G M M Q E L Q A R L S Q P K 940 v          v          v          v          v          v          v CCACAGTCCAGGGCTAGAGCCCTAGAGAGAGACCTCCCAAGAGAGTCCGAAGGCTC 2880 P Q &lt; 942 v          v          v          v          v          v          v TTCTGGAACCTGGTACTCTTGAAGTCCCTGGCCACTGGAATATATCTTTTCACTCC 2940 v          v          v          v          v          v          v GGCCTCAGTAACTGGATTTTATGAAGCCTTGAATCTAGGAGTCTGATTAGCCAGT 3000 v          v          v          v          v          v          v GAGGAACCTGCTCCATGGCATCGATCATGATTCTTTCCCGGTCGCCCTTTCACATCTG 3060 v          v          v          v          v          v          v TCTCAGTACTCGGAAGGCTACCCGGAAGACTGGGCTGGGAAACTATTTTCCCCTAAAT 3120 v          v          v          v          v          v          v TTCCCAAGTATGCTCACTGCCCAATGTGAGATAGACCAGCAAGTGAAGTGTGCT 3180 v          v          v          v          v          v          v TGCGTGTAGATAGCACACTCTGGGTTGAAACCGCCCAAGGAAGAGGTTTCAAGG 3240 v          v          v          v          v          v          v GCTGAGCAAGGACACCCTGGAGTCCAGGCCACCGCCACAGATCAGTTGCTTTGAG 3300 v          v          v          v          v          v          v TTTCCAAATGACGGCTCGGTATCACAGCTCTGAGGTTACTATGTAACTCCATGCCA 3360 v          v          v          v          v          v          v GAGTTGGGGAACGTTCCCGGAAGCTCAAGCACATAGGATAGAGGGGTTCTGTGTG 3420 v          v          v          v          v          v          v GGTGAAGTTCCTCCCTCCCAACTCCATCCCAACCGGCCCTGTTCTTGGGTGTGAC 3480 v          v          v          v          v          v          v ACCTTCTGTGAAAAGATGTGGAACACAAACCGGGCTGGATGGCTTTATTAGGCCCA 3540 v          v          v          v          v          v          v GCATGGTGCCTAACTCAAGAGTGAACAATAATAAACATTTCTTAAAAATAAAAAAA 3600 AAAAAATAAAA 3611 </pre> |
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**Fig. 1.** The murine macrophage apoB-48R cDNA sequence and the deduced protein sequence. The 3,615 bp cDNA sequence has a 2,826 bp ORF beginning at bp +1 and ending at the stop codon (2,827–2,829). A Kozac start site is at –4 to +4. The 3' untranslated region contains 2 polyadenylation signals and a poly (A) tail. The cDNA encodes a 942 aa protein sequence. Both the cDNA and the protein are homologous to the human apoB-48R cDNA and protein sequence. The conserved N-glycosylation signal is in bold (aa 510–513); the conserved putative transmembrane domain is underlined; the conserved dileucine internalization signal (ExxxLL) is double underlined; and the two conserved coiled-coil protein interacting domains are bold and italicized.

HUMAN: 1 MDFLRRLYLPLGLHQALRGALDSLGT FVSYLLGDAVPTVEREAQAEEELGVVAVGKTGKIVE 60  
 MOUSE: 1 MDFLRRLYLPLGLHQALRGALDS FVSYL+GD VPTVER+ QAEEELG V GK+V  
 MDFLRRLYLPLGLHQALRGALDSFSAFVSYLVGDTVPTVERQTQAEEELGEVTV---EGKVVG 57

HUMAN: 61 EEAQEDLEGLRGSQNEGAGRLRGPDDRHEVGSVAEQTWGWDGSSHGSAQERQDSGA 120  
 MOUSE: 58 EEAQE LEGLR Q+EG + P + RR + GS A EQTWGM SS QAERQD+G+  
 EEAQVLEGLRSGQSEG---VEAPEETRRQCQESLAGEQTWGRADSSARPAERQDTGS 114

HUMAN: 121 GETAKAARCQEPSAHLEARKKSKAGSGACQDRSGQ-AQERQESHEQEVNREERLSWEQE 179  
 + A+ AR QEPS L K +A G +DRS AQE E E+E + E LR+ EQ+  
 MOUSE: 115 WKAAEDARGQEPSVPL----KPEAEPGTHRDRSSNTAQE IWEHGEEEAASSGEPLRTCEQK 170

HUMAN: 180 EEEEEV-RAREPGMARGAESWETWHGETEGKAGAVGPKAAGDNREMEQGVREADAGETEE 238  
 EEEEEV RA E GMA G ES+ TWH E G AG + + + + +E D  
 MOUSE: 171 EEEEEVRAAESGMAEGVESQPTWHSEPGGNAGT-----EGQHVTEDSKEID----- 217

HUMAN: 239 PGAEGAGKGEVTVVEKACESTRAWGTWGPAGPEPDWILG--REEARTTPGEEARAIL 296  
 W AE E+G G +EE R P R  
 MOUSE: 218 -----WVAKDMVAEIEWFGAKGIDKEERVMVPMR----- 246

HUMAN: 297 DGEARTISGGEEAETASGGEEAETASGGEEAGTASGGEEAGISSGGEEAGTASGGEEAGT 356  
 DGE AR + G E  
 MOUSE: 247 DGERARA-----QGTQCPGAESEDO 266

HUMAN: 357 ASGGDEAWTSGKEEADLLGVRQTYGAVPGERLLEATGKVVVLEE-----EGDEER 408  
 A EAWT S +E AD LGV+ T+YG+ PG+ TG+VWVLEE E DE+R  
 MOUSE: 267 AMLSREAWTSDREGADSLGVQGTQTEYGSDFGNFFGTTGRVWVLEEADKGDQDEVDEKR 326

HUMAN: 409 EAEVSPFPKQAVLGTERTEEAAESQTAGREAVGQEQEAGESFEGQVDLRGKEAMRQDLG 468  
 EAEV FP Q L ERT E E A EA+G QE SFE E RQDL  
 MOUSE: 327 EAEVR-PP--IQTLEAERTGEMTEGHIAEEEEAMQEETEGSFED-----EERQDLA 374

HUMAN: 469 IRADRARMEELVQAAEQEERSSRDPAELPSDGEAEGTADLEATPEARPEEELTGEES 528  
 IR + +EE V+AE+ E +S L D EA+ D E +PE EE GE S  
 MOUSE: 375 IRDNGVSLSEEVRAEESREKNSWATEPTLVLDTEAKDEPDEDSPEVSTEEELFVGEERS 434

HUMAN: 529 EAAQTSCLLGVVEGGLTHSVTKGGQPELMGGAQPTTKQPEEREAGEVELMGLVLSKEE 588  
 EAAQ + +L V+ VT+GQ PEL+ +Q TKQ EE + G+ E G LS E  
 MOUSE: 435 EAAQMTPEVLRVK-----VTEGQPELVRSQALTKLEEKGQKEETSGAPDLSPE- 486

HUMAN: 589 QERSLEAGPRHAGSVKPEASEAFPAGWENRTRKDMERGNQTQEDAADGEQREEEETAGGQT 648  
 + SL+ P G PE AW N +R ++R N+QE AD E +E+ TA Q  
 MOUSE: 487 RVLSLKEYPGPVGFAGPELE----AWGNWSR-GVDRRNSQEVKADAEAGKEQ-TATEQA 539

HUMAN: 649 LAEAEGDRESELSEVPEAGGELTTQDAGCTGTEEGEASVSENQELDGSTGADAGPCPSL 708  
 + AEG +E++ EV +CGE E SV+ N EL+GS GA+AG S+  
 MOUSE: 540 VEIRAEGGQEAQQPEVFGSGE-----EALTSVALNPELEGSQGAAGTEESV 587

HUMAN: 709 GEAYARETE--DEEA---EADRTSRGRWRLQAVAVGLPDREDAQTGSVAAGIMGGDVVP 762  
 E+ E E +EEA EAD T R+ RL+ V + L D ED +T +A I+ G  
 MOUSE: 588 EESKPTNEAAEEAEAVPWEADGTCRKR-RLEEVTLSLQDSEDTETSYLEEILV GIRAV 646

HUMAN: 763 HISAAG---AGEALEGALGQWDSKEKEEAAAG-----EHAGGQEFGLGSAEVEEVTG 812  
 AG A E LG+ W S+ + EA G E GQE GL GSAB+  
 MOUSE: 647 DTEEGPKWEAGLAPETELGKAWCSEGRGEARGTELEETTEKQSGQEVGLVGSAEKV--- 703

HUMAN: 813 RGSQVEAFESREGGFWGGRRVEAEE SAGAEDSCGLDPA--GSQTARAEGMGAMVEAGLLE 870  
 G ++ + E G + E E S AED G D GSQ RAEG +E GLL  
 MOUSE: 704 SGYDIQEI DGT EGE---QAEMETSVMAEDIRGTDGVTLSGQAERAEGSITPMTEGLLR 760

HUMAN: 871 KWTLLLEEAVGQEREQR-EDSEGRGQDYHPEGEAPRLDAGELMVTGRRRAEAKETEPE 929  
 LLEEEA G Q REQ+ +SEG E +  
 MOUSE: 761 DQMLLEEEAGGQSRQKQVHNSG-----EIQ 787

HUMAN: 930 SLEHVRGQEEQPTHQAPAEAPESVGEAETAEMAGSARGGAANSWSEAPLPGSLLDVSVF 989  
 +L+ QE Q THQ P A P G E+AEA A G ++W+EA LPSG LDVSVF  
 MOUSE: 788 TLDDSSDQEQQTHQIPTVAVP---GPLESAEATAGAPGDVHNSWNEALLPGSRLDVSVF 844

HUMAN: 990 RSRVHLSRSSSQRSSRPSFRPTPAWEQQEPPFAPNPPEEELSAPEQRPLQLEEPLEPSPL 1049  
 RSRV LSRSSS+RRSRPSF R E Q +PP+P P + E PEQ LQLEE E S  
 MOUSE: 845 RSRVLLSRSSSRRSRPSFHRISVPEPQCDPPSPQP-QAERFPVPEQSSQLQLEETPELSAT 903

HUMAN: 1050 RHDGTPVPARRRPLGHGFLAHPGMMOELQARLGRPKPQ 1088  
 + +GTPVPARR+ LG GFG AHPGMMOELQARL +PKPQ  
 MOUSE: 904 KPEGTPVPARRKMLGRGFGFAHPGMMOELQARLSQPKPQ 942

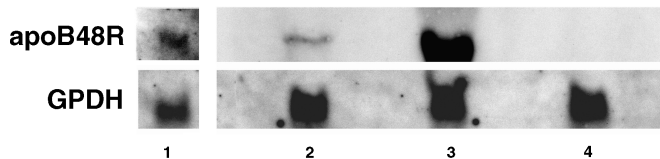
**Fig. 2.** Homology of the murine apoB-48R and the human apoB-48R protein sequences. The sequences were aligned using the pairwise Blast 2 sequences program (NCBI, NIH). The deduced mouse apoB-48R is 942 aa and the human apoB-48R is 1,088 aa. The overall identity is ~45%, but the conservation is higher in the N-terminal and C-terminal regions, suggesting these regions are functionally important. The center sequence represents perfect match and conserved changes (+) in the pairwise gap-aligned mouse and human sequences.

putative transmembrane domain in the human apoB-48R. Other predicted secondary structures and calculated pIs are similar and given in **Table 1**. Of note, a conserved dileucine internalization motif (ExxxLL; aa 754–759) is C-terminal to the conserved putative transmembrane domains, suggesting that the C-terminus is cytoplasmic. The murine apoB-48R has one nine residue sequence homologous to the human repeating sequence (GGEEAETAS) that is between the conserved coiled-coil domains in the human apoB-48R. This indicates that extensive repetition of this

**TABLE 1.** Similarities of the amino acid compositions and predicted secondary structures of the human and mouse apoB-48R

|  | Murine | Human |
|--|--------|-------|
| Acidic                                 | ~22%   | ~22%  |
| Basic residues                         | ~10%   | ~10%  |
| Alpha helix                            | ~42%   | ~45%  |
| Beta sheet                             | ~15%   | ~7%   |
| Random sheet                           | ~49%   | ~44%  |
| pI (calculated)                        | 4.18   | 4.13  |
| MW (kDa calculated)                    | 102.7  | 114.8 |
| M <sub>r</sub> apparent SDS-PAGE (kDa) | ~190   | ~200  |

MW, molecular weight; kDa, kilodalton.

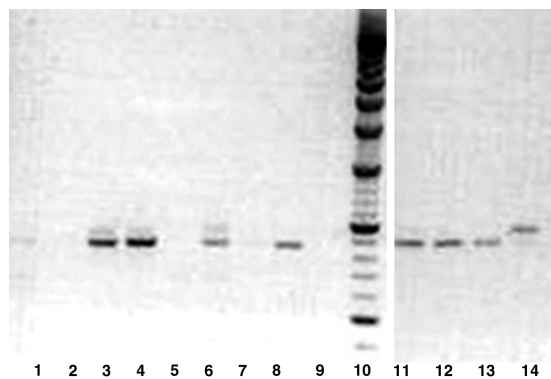


**Fig. 3.** Murine apoB-48R mRNA characterization by Northern analyses was performed as described in Materials and Methods. RNA from the murine macrophage line RAW 264.7, lane 1; CHO-K1s transfected with the murine apoB-48R cDNA in pcDNA 3.1 vector, lane 2; CHO-K1s transfected with an apoB-48R minigene that contains the three introns, lane 3; and CHO-K1s transfected at the same time with empty pcDNA vector, lane 4. GPDH was used as a control. The apoB-48R mRNA was detected with a DIG-labeled probe derived from the murine apoB-48R cDNA.

element is not necessary for ligand binding or other activities required for receptor function, because the human and murine apoB-48Rs are functionally identical (identical ligand specificities, binding kinetics, lack of regulation by sterol or state of differentiation, and other conserved functions).

#### ApoB-48R mRNA size and tissue distribution

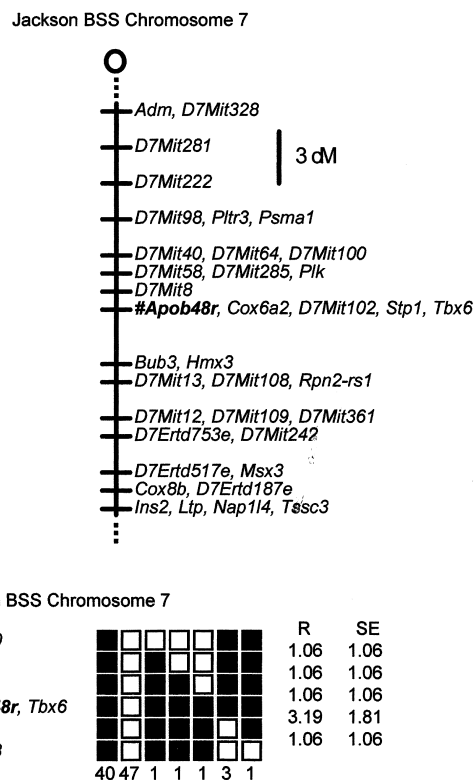
Consistent with the cDNA size, Northern analyses of murine macrophage RNA (RAW 264.7) (Fig. 3, lane 1), CHO-K1s transfected with murine apoB-48R-cDNA (lane 2) and apoB-48r minigene with four exons and three introns



**Fig. 4.** Mouse MTC panel used for tissue distribution of murine apoB-48 receptor. A mouse MTC cDNA panel that is normalized for five housekeeping genes was purchased from Clontech, and PCR was performed using gene specific primers that include two small introns; specific conditions are found in Materials and Methods. After 21, 24, 27, and 30 cycles, aliquots of each reaction mixture were loaded onto 1.5% agarose gels, electrophoresed for 1.5 h, and visualized by ethidium bromide staining. Shown here is a representative 30 cycle result, which is reflective of the shorter cycling results and three separate experiments; GPDH PCR analyses of these tissues indicated that they were indeed equal loadings (data not shown). The product size is 860 bp, expected for the apoB-48r mRNA transcript. Genomic contamination produces a 1,035 bp band under these identical conditions. Lane 1, heart; lane 2, brain; lane 3, spleen; lane 4, lung; lane 5, liver; lane 6, skeletal muscle; lane 7, kidney; lane 8, testis; lane 9, negative control (no DNA); lane 10, markers; lane 11, 7 day embryo; lane 12, 11 day embryo; lane 13, 15 day embryo; lane 14, 17 day embryo.

(lane 3) indicate the apoB-48R mRNAs from all sources are the same size, ~3.8 kb, similar to the human mRNA. The control CHO-K1s transfected with pcDNA vector had no detectable apoB-48R mRNA (lane 4), consistent with previous studies of CHO-K1s transfected with the human apoB-48R and empty vector controls (1).

To determine if the tissue distribution in mice is similar to that in humans, we used PCR and a commercial mouse multiple-tissue cDNA panel (Clontech), derived by first strand synthesis from poly(A)<sup>+</sup> RNA and normalized to five housekeeping genes. The apoB-48R mRNA (Fig. 4) was found at highest levels in spleen and lung (Fig. 4, lanes 3 and 4), then skeletal muscle (lane 6), and at low levels in brain, heart, kidney, and testis (lanes 2, 1, 7, 8). Surprisingly, it was not detected in mouse liver (lane 5), even with extended cycling. A similar human tissue cDNA panel detected mRNA in liver, albeit at low levels (1). Re-



**Fig. 5.** Chromosome location of the mouse *Apob-48r* gene. Upper panel: linkage map of mouse Chr 7 around the *Apob-48r* locus. A 3 cM scale bar is shown to the right of the figure. Loci mapping to the same position are listed in alphabetical order. Lower panel: the segregation pattern of *Apob-48r* and flanking markers. Loci are listed in order, with the most proximal at the top. The black boxes represent the C57BL/JEi allele, and the white boxes represent the SPRET/Ei allele. The number of offspring inheriting each type of chromosome is given at the bottom of each column. The percentage recombination (R) between adjacent loci is given to the right of the figure in centimorgans (cM), with the standard error (SE) for each recombination. Missing typings were inferred from surrounding data where assignment was unambiguous. Data for mouse markers were collected through the Mouse Genome Database maintained by The Jackson Laboratory Mouse Genome Database (<http://www.informatics.jax.org>).

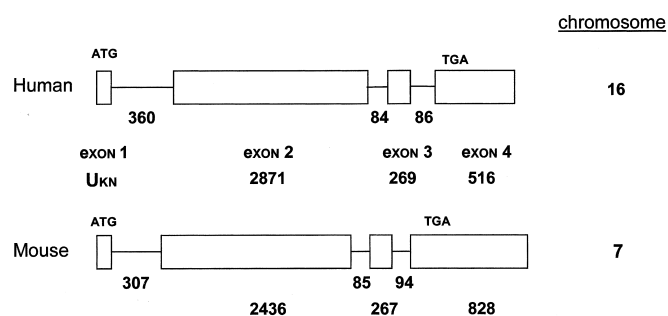
peated Northern blots of murine liver RNA from C57BL/6 fed chow or a high fat, high sucrose diet (21), apoE<sup>-/-</sup>, and LDLR<sup>-/-</sup> also did not reveal any apoB-48R mRNA, when the control Northern blots for actin and GPDH were strongly positive and, at long exposure, rRNAs were detected (data not shown). The mouse tissue distribution is generally consistent with the low levels of the mRNA in human liver and brain and high levels in monocytes and spleen (1). The apoB-48R mRNA may well be expressed in mouse liver at a much lower level and therefore not detectable even by RT-PCR. The only other apparent species difference is that the apoB-48R mRNA is expressed in mouse skeletal muscle but was not detectable in human skeletal muscle (1).

In addition, the apob-48r mRNA was found in mouse embryos, at the highest levels in early embryonic development (7 days, Fig. 4, lane 11), then progressively lower at 11 (lane 12) and 15 days, (lane 13) and finally very diminished at the day 17 (lane 14, and a potential new transcript expressed), suggesting a role in fetal development. Perhaps pertinent to this, the apoB-48R mRNA was also highly expressed in human placenta (1).

#### Conserved genomic localization and organization

The mapping data indicate that *Apob-48r* is located in the distal region of mouse chromosome 7, approximately 60–61 cM from the centromere (Fig. 5). Confirmation that *Apob-48r* is the actual structural gene was obtained by searching the genome for related sequences (none found). Further support is based on the homology with the human gene, which is located on chromosome 16p11 (near the centromere) (1).

The mouse *Apob-48r* gene (GenBank Accession No. AF141336) organization is strikingly similar to that of the human *APOB-48R* gene (GenBank Accession No. AF141333) (Fig. 6), with four exons and three small introns of similar sizes and locations. The three introns



**Fig. 6.** Conserved genomic organization of the apoB-48R. The murine apoB-48R is organized like the human gene, with four exons and three introns of comparable size and location. The first intron occurs after the first 18 aa in each species and is the largest intron (307 bp mouse; 360 bp human). The second exon is the largest (2,871 bp human, 2,436 bp mouse) and encodes the conserved N-glycosylation site, coiled-coil domains, the putative transmembrane, and the dileucine internalization signal. The number of base pairs of each intron and exon are as indicated, except for Exon 1, where the beginning is not yet certain.

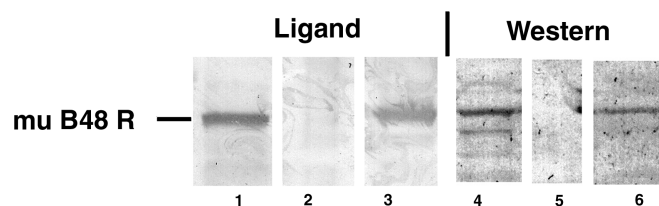
within the coding sequence were identified by PCR of genomic DNA (mouse strain 129 tailtip DNA and P388D<sub>1</sub> macrophage DNA) using primers based on the murine cDNA sequence and the exon/intron organization of the human *APOB-48R* gene. Sequence analysis of the 5' upstream region of murine *Apob-48r* indicates that it is located ~1.4 kb from the *Cln3* gene (accession No. U47106) on chromosome 7, syntenic to its location and organization on human chromosome 16. No other gene appears to be between *Apob-48r* and *Cln3*, and they have opposite orientations.

#### Structural characteristics of the transfected apoB-48R protein

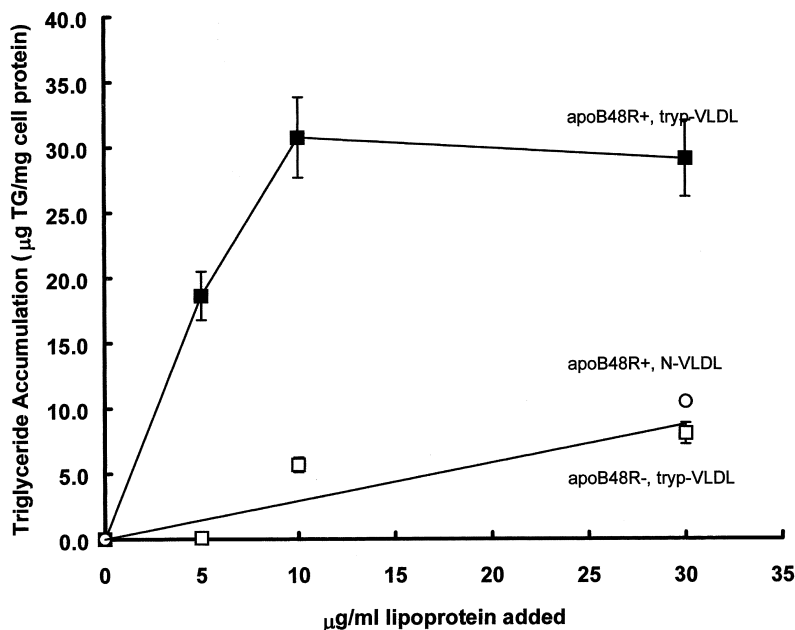
The murine full-length "minigene," consisting of the four exons and three introns of the apoB-48R, was transfected into CHO-K1s and tested for protein characteristics and receptor function. Figure 7 is a combined ligand blot (left panel) and Western blot (right panel) of extracts of CHO-K1s transfected with the murine apoB-48R minigene (lanes 3 and 4) or the empty vector (lanes 2 and 5). These were compared with extracts of murine RAW 264.7 macrophages (lanes 1 and 6). The cells transfected with the apoB-48R minigene, but not the CHO cells transfected with empty vector, contained apoB-48R similar in size (~190 kDa) to that of the murine RAW macrophages shown here by both ligand and immunochemical blotting and as previously reported for murine P388D<sub>1</sub>s macrophages (6).

#### Functional characteristics of the transfected murine apoB-48R

Two functional endpoints were used to determine whether the murine apoB-48R cDNA is sufficient to confer full receptor activity. First, quantification of TRL- or apoE<sup>-/-</sup> β-VLDL-induced cellular triglyceride or chole-



**Fig. 7.** Ligand and Western blots of native and transfected murine apoB-48 receptor. Whole-cell extracts were prepared from murine macrophages (RAW), vector-only transfected CHO-K1s, and apoB-48r-transfected CHO-K1s. Lanes 1 and 6, RAW macrophages; lanes 2 and 5, pcDNA-CHO (vector); lanes 3 and 4, apoB-48r minigene-transfected CHO cells. Extracts were loaded at equal protein concentration (30 μg/lane), electrophoresed on 7% acrylamide, transferred to nitrocellulose, and either blotted with an N-terminal antibody for the receptor (Western; right panel) or initially incubated with 10 μg/ml tryp-VLDL<sub>1</sub> for 1 h, and after thorough washing, the bound ligand was detected with an anti-apoB antibody and visualized with an alkaline phosphatase-conjugated second Ab and chromogenic substrate (Ligand; left panel). The occurrence of smaller bands in the Western blot could represent nonligand binding degradation products and/or possibly a precursor. The main receptor ligand binding activity is associated with the most prominent protein species detected by the anti-receptor antibody.



**Fig. 8.** Quantification of TG accumulation in apoB-48R-transfected and vector only-transfected CHOs. G418-selected, stable apoB-48R-transfected and vector-transfected CHO ldl A-7 cells, which lack the LDLR, were incubated with tryp-VLDL ( $S_f$  100–400) at the concentrations indicated for 4 h at 37°C, and the cells were processed to measure TG mass as described in Materials and Methods and as previously reported (6). The upper curve (closed squares, apoB-48R-transfected) quantifies the rapid, curvilinear accumulation of TG in apoB-48R-transfected CHOs exposed to increasing levels of tryp-VLDL. The lower curve (open squares, cells transfected with empty vector) shows the linear TG accumulation in CHOs without the apoB-48R, which represents low-affinity, nonspecific uptake. Normal VLDL uptake by apoB-48R-positive cells is indicated at the highest incubation level (30 µg/ml) as a single point (open circle); it resulted in uptake at levels seen in the receptor negative (vector only) transfected cells. Values (µg TG/mg cell protein) are averages from duplicate dishes, which differed by <10%, after correction for basal level of cellular TG (which averages 10–12 µg/mg). This experiment is representative of three different experiments with the CHO ldl A7 cells. Transfection of CHO-K1s gave identical results.

terol mass accumulation, respectively, and second, visualization of lipid droplets in response to incubation with a TRL known to bind to the mouse apoB-48R or apoE<sup>-/-</sup> β-VLDL. As shown in **Fig. 8**, ldl A-7 CHO cells that lack LDL receptors (16) transfected with the murine apoB-48R cDNA rapidly ( $\leq 4$  h) accumulate triglycerides in a saturable, high-affinity (i.e., receptor-mediated) manner when exposed to the apoB-48R-specific model TRL ligand, trypsinized VLDL, whereas control cells exhibit only low level, linear, i.e., nonspecific, triglyceride accumulation, similar to the apoB-48R-transfected cells incubated with normal human VLDL  $S_f$  100–400 (**Fig. 8**, apoB-48R+, N-VLDL). Trypsinized VLDL is used as a ligand for the apoB-48R because multiple previous publications document that this TRL, which is devoid of apoE and binds to the apoB-48R with high affinity, is not a ligand for the LDLR or scavenger receptor, thus reducing ambiguities (6, 20, 22, 23). The same results were obtained with apoB-48R-transfected CHO-K1s (data not shown).

The second line of evidence that transfection of the murine apoB-48R cDNA confers apoB-48R activity comes from histochemical studies with the neutral lipid stain oil red O. Lipid accumulation in apoB-48R-transfected CHO-K1s, but not in vector-transfected control CHOs, is visible after incubations as short as 1 h with large TRL ( $S_f > 100$ ) prior to staining with oil red O (**Fig. 9A, B**). ApoB-48R-transfected CHOs exposed to murine apoB-48-containing

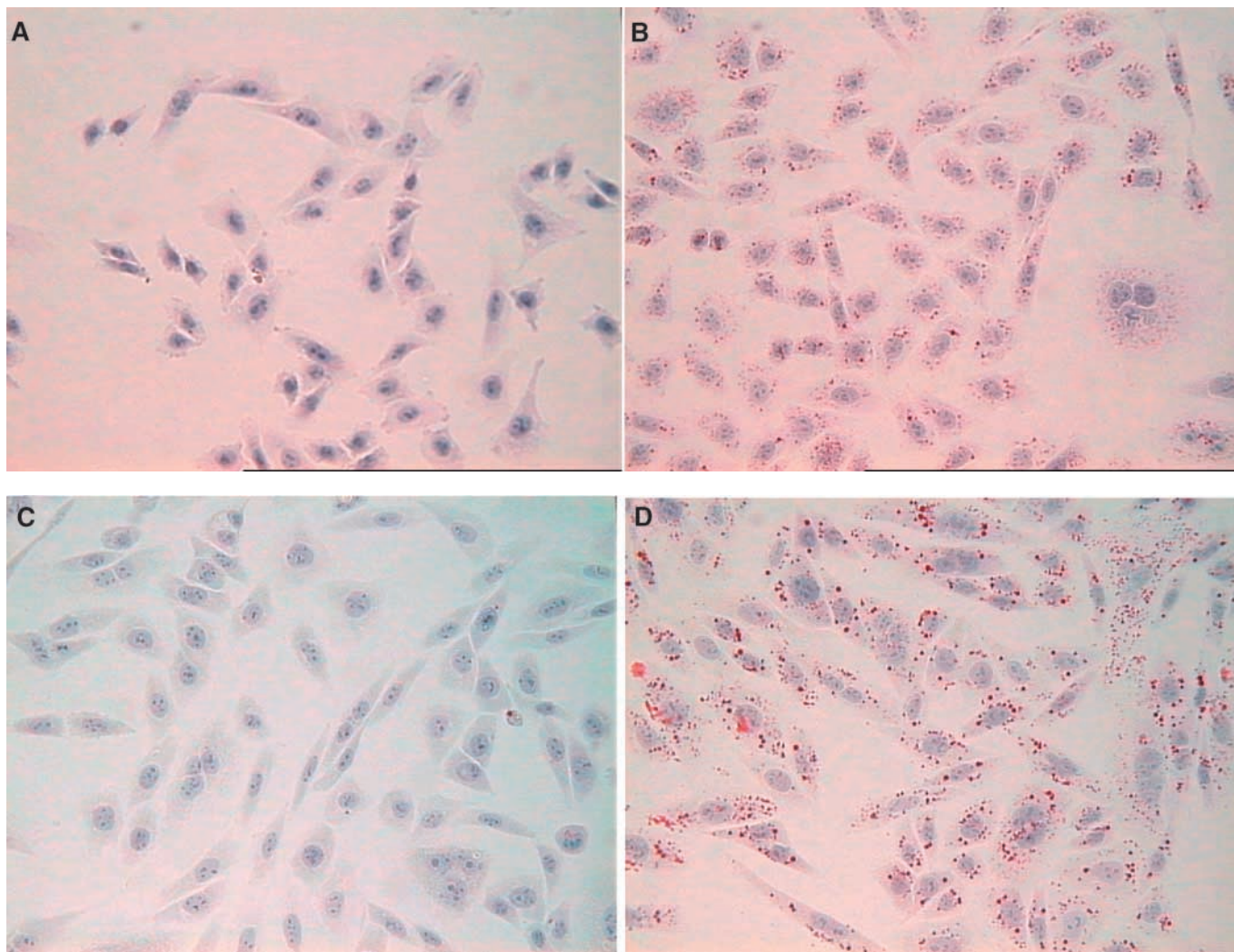
β-VLDL from apoE<sup>-/-</sup> mice also accumulate numerous cytoplasmic lipid droplets visible after staining with oil red O (**Fig. 9D**). In the absence of lipoproteins, neither the apoB-48R-transfected (**Fig. 9C**) nor the control transfected CHO-K1s (data not shown) contained significant lipid in 16 h incubations.

The timeframe for visible lipid droplet production or quantifiable mass increases depends on the relative amount of neutral lipid carried by the particle. Thus, short incubations (1–4 h) with large ( $S_f > 100$ ) TRL ligands are sufficient (**Fig. 9B**), whereas the smaller apoE<sup>-/-</sup> β-VLDL ( $d < 1.006$ ) requires 16–18 h incubations to induce similarly extensive lipid droplet formation (**Fig. 9D**) in apoB-48R-transfected CHOs. Thus, transfection of CHOs with the murine apoB-48R minigene reported here, like transfection of CHOs with the human apoB-48R reported previously (1), is sufficient to confer characteristic apoB-48R receptor activity upon CHOs.

## DISCUSSION

The murine apoB-48r cDNA and gene reported here encode a receptor protein that has identical ligand binding specificities and kinetics as the apoB-48R previously reported in murine and human macrophages (6, 20). Several lines of evidence indicate that this cDNA and gene





**Fig. 9.** Visualization of lipid accumulation in CHO cells transfected with the murine apoB-48R (B, C, and D) but not in cells transfected with empty vector (A). G418-selected, stable transfected CHO cells were grown on glass coverslips and incubated with 10 µg/ml tryptophan-VLDL S<sub>f</sub> 100–400 for 1 h at 37°C (A and B). ApoB-48R-transfected CHO cells were incubated with buffer control (C) or with murine apoE<sup>-/-</sup> β-VLDL 50 µg/ml (D) for 18 h at 37°C prior to staining with the neutral lipid stain oil red O, as described in Materials and Methods.

encode the murine apoB-48 receptor and that transfection of the murine apoB-48R cDNA or minigene into cells that do not normally express this receptor (CHO-K1s and CHO 1d1A7s) confers full receptor activity. First, CHO cells transfected with the murine *apoB-48r* cDNA, like RAW macrophages, express an mRNA of appropriate size (~3.8 kDa) that is lacking in CHO cells transfected with empty vector, as determined by Northern analyses. Second, ligand blotting studies identified an ~190 kDa protein in murine RAW macrophages and in murine apoB-48r-transfected CHO cells that was absent in the pcDNA empty vector-transfected CHO cells. The apparent M<sub>r</sub> of the translated murine apoB-48r was ~190 kDa, identical to that previously reported in murine P388D<sub>1</sub> macrophages (6). Further, the ligand specificity of the transfected apoB-48r was identical to that previously reported for the murine P388D<sub>1</sub> and the human blood-borne and THP-1 monocytes and macrophages (7, 20).

Third, murine apoB-48R-transfected CHO cells, but not vector-transfected controls, expressed ~190 kDa proteins detected with an anti-peptide antibody that recognizes both the human and the murine apoB-48R, providing immunochemical identifications of appropriately sized protein. Because the antibody is against the N-terminal 15 aa, this provides evidence that the murine receptor retains at least a portion of the N-terminal 15 aa (Fig. 7, lane 4). Of note, the apparent size of the active transfected apoB-48R protein on SDS-PAGE is close to twice that predicted by the aa sequence and is the same as that previously reported in murine P388D<sub>1</sub>s (~190 kDa) (6). Thus, the murine apoB-48R, like the human receptor, appears to be a dimer. The presence of two conserved coiled-coil domains coupled with the near doubling in M<sub>r</sub> of the active receptor compared with the predicted protein, in both transfected CHO cells and native macrophages, lends support to the previously proposed dimerization of the receptor protein (1).

Fourth, functional studies in apoB-48R-transfected CHOs and in apoB-48R-transfected CHO ldlA7s that lack the LDL receptor showed similar quantitative mass increases in cellular TG or cholesterol when exposed to TRL ligands or apoE<sup>-/-</sup>  $\beta$ -VLDL, respectively, as do human THP-1 macrophages. This suggests that the presence or absence of the LDLR does not affect apoB-48R activity.

Fifth, apoB-48R-transfected CHO-K1s and CHO ldlA7s exhibited visible accumulation of cytoplasmic lipid droplets when exposed to TRLs known to be high-affinity ligands or to apoE<sup>-/-</sup>  $\beta$ -VLDL. Further, normal VLDL, which is not a high-affinity ligand for either the murine apoB-48R (6) or the human apoB-48R (1, 7, 20), does not cause significant increases in TG mass (Fig. 8) or visible lipid loading in murine apoB-48R-transfected CHOs reported here, consistent with all of our studies of this receptor's ligand specificities in murine peritoneal and P388D<sub>1</sub> macrophages published previously (4, 6) when the apoB-48R positive control ligands, human chylomicrons, or trypsinized VLDL, cause lipid accumulation (Fig. 8). This is consistent with the previously published studies documenting the inability of normal VLDL to bind to the apoB-48R in ligand blots (6, 20) or to induce TG accumulation in murine or human macrophages (6, 7, 20) or in human apoB-48R-transfected CHOs (1).

Moreover, we show for the first time specific uptake of mouse apoE<sup>-/-</sup>  $\beta$ -VLDL by apoB-48R-transfected CHOs but not by control CHOs. This suggests that the apoB-48R could contribute to the spontaneous generation of macrophage-derived foam cells in atherosclerotic lesions of apoE-deficient mice, as has been suggested by others (2, 8, 9). Immunohistochemical studies revealed the presence of the apoB-48R in foam cells of human atherosclerotic lesions (1), suggesting that the apoB-48R may have a conserved role in mammalian atherosclerosis.

The nearly superimposable genomic organization, syntenic chromosomal localization, homologous protein sequences, similar predicted secondary and tertiary structures, and similar tissue distribution reported here for the murine apoB-48R and the human counterpart and the many shared functional characteristics of the murine and human macrophage apoB-48R pathways, previously published, including identical ligand specificities and similar binding kinetics (6, 13, 20), indicate that this gene has been conserved evolutionarily, at least in mammals. This conservation of the genomic structure and syntenic location, virtually identical predicted secondary structure (Table 1) and tertiary structure (dimer), and function of the apoB-48 receptor protein in murine and human macrophages implies that it may serve an important function in mammals in health (nutrition of monocyte-macrophages of the immune system and in embryologic development) and in disease (formation of macrophage-derived foam cells). ■

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