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THE MAMMALIAN LOW-DENSITY LIPOPROTEIN RECEPTOR FAMILY

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ABSTRACT

The low-density lipoprotein (LDL) receptor (LDL-R) family consists of cell-surface receptors that recognize extracellular ligands and internalize them for degradation by lysosomes. The LDL-R is the prototype of this family, which also contains very-low-density lipoprotein receptors (VLDL-R), apolipoprotein E receptor 2, LRP, and megalin. The family members contain four major structural modules: the cysteine-rich complement-type repeats, epidermal growth factor precursor-like repeats, a transmembrane domain, and a cytoplasmic domain. Each structural module serves distinct and important functions. These receptors bind several structurally dissimilar ligands. It is proposed that instead of a primary sequence, positive electrostatic potential in different ligands constitutes a receptor binding domain. This family of receptors plays crucial roles in various physiologic functions. LDL-R plays an important role in cholesterol homeostasis. Mutations cause familial hypercholesterolemia and premature coronary artery disease. LDL-R–related protein plays an important role in the clearance of plasma-activated α_2-macroglobulin and apolipoprotein E–enriched lipoproteins. It is essential for fetal development and has been associated with Alzheimer's disease. Megalin is the major receptor in absorptive epithelial cells of the proximal tubules and an antigenic determinant for Heymann nephritis in rats. Mutations in a chicken homolog of VLDL-R cause female sterility and premature atherosclerosis. This receptor is not expressed in liver tissue; however, transgenic expression of VLDL-R in liver corrects hypercholesterolemia in experiment animals, which suggests that it can be a candidate for gene therapy for various hyperlipidemias. The functional importance of individual receptors may lie in

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their differential tissue expression. The regulation of expression of these receptors occurs at the transcriptional level. Expression of the LDL-R is regulated by intracellular sterol levels involving novel membrane-bound transcription factors. Other members of the family are not regulated by sterols. All the members are, however, regulated by hormones and growth factors, but the mechanisms of regulation by hormones have not been elucidated. Studies of these receptors have provided important insights into receptor structure-function and mechanisms of ligand removal and catabolism. It is anticipated that increased knowledge about the LDL-R family members will open new avenues for the treatment of many disorders.

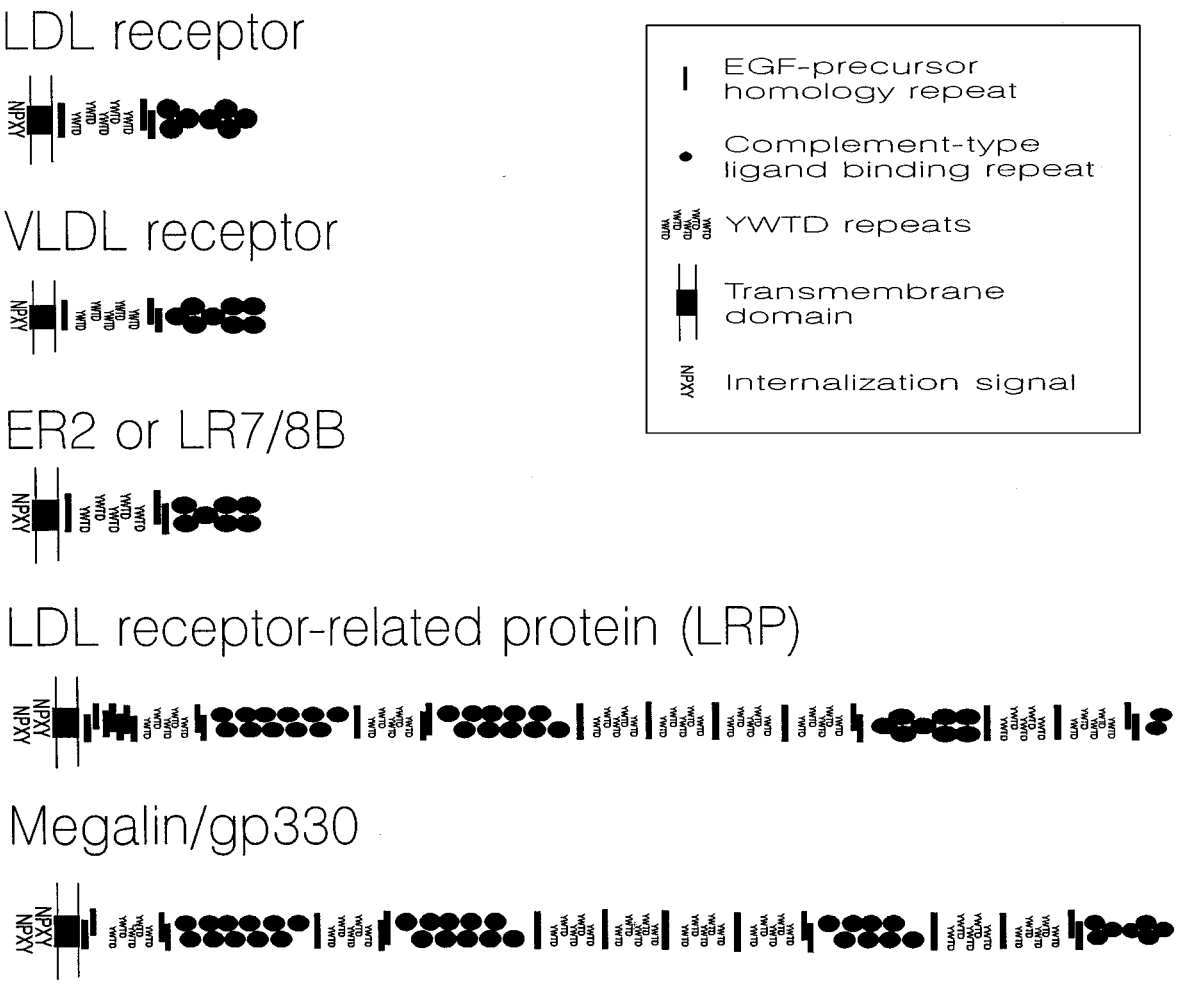
CONTENTS

INTRODUCTION

The low-density lipoprotein (LDL) receptor (LDL-R) is a prototype of a growing family of membrane-anchored, transmembrane receptors that reside on the cell surface. These receptors share structural and functional properties and interact with a diverse group of ligands, delivering them to lysosomes for degradation. There are seven characteristic features of the LDL-R family: cell-surface expression; extracellular ligand binding domain consisting of complement-type repeats; requirement of Ca^{2+} for ligand binding; recognition

of receptor-associated protein and apolipoprotein (apo) E; epidermal growth factor (EGF) precursor homology domain containing YWTD repeats; single membrane-spanning region; and receptor-mediated endocytosis of various ligands (Figure 1). The LDL-R family has been called group I in the LDL-R supergene family (59). The complement-type repeats of ~40 amino acids containing six cysteine residues per repeat constitute a ligand binding motif. All the cysteines within a repeat are disulfide linked to form a compact, stable, functionally independent motif. Each of these repeats contains DxSDE residues at their C-terminal ends. A cluster of several of these repeats constitutes a ligand binding domain, and differential clustering of these repeats within a domain may impart specificity with respect to ligand recognition. The EGF precursor homology domains consist of cysteine-rich growth factor and YWTD repeats. The cysteine-rich repeats in these domains lack the concentration of negatively charged residues found in ligand binding domains. These repeats have been further subdivided into an A group and a B group, based on the spacing of cysteine residues within each repeat. The growth hormone repeats are usually separated by the YWTD repeats that are usually found as a group of five in a module of ~50 amino acids. The EGF precursor homology domains are necessary for the dissociation of ligands from the receptor in endosomes (10, 46, 59). In most of the receptors, ligand binding and EGF precursor homology domains are separated from transmembrane anchoring domains by an O-linked glycosylation domain. The O-linked glycosylation domains are characterized by the preponderance of serine and threonine residues. These glycosylations are usually not required for receptor function, but they may serve to keep the ligand binding domains away from the cell surface. All the members contain a single transmembrane domain consisting of a stretch of hydrophobic residues that anchors them into membranes. The cytoplasmic domains contain at least one FDNPXY sequence necessary for the targeting of receptors to coated pits and for subsequent internalization.

All the members of the family are transmembrane glycoproteins that contain large extracellular and comparatively shorter intracellular domains, as opposed to signaling receptors that contain large intracellular domains. So far, no enzymatic activity has been ascribed to them. They bring various ligands from the extracellular environment into the cell for degradation, usually providing essential nutrients for cellular functions. In addition, they can potentially control cellular phenotypes by modulating extracellular levels of various proteases, protease inhibitors, and growth factors. In this review, we summarize knowledge concerning structural and functional domains, regulation of expression in various tissues, and diseases that arise in mammals that are due to receptor dysfunction.



THE LDL-R FAMILY MEMBERS

LDL-R

The pioneering work of Brown & Goldstein led to the identification and characterization of the LDL-R (10). It is a cell-surface, transmembrane glycoprotein of 839 amino acids that regulates plasma cholesterol by mediating uptake and catabolism of plasma LDL, the major carrier of plasma cholesterol. The main function of the receptor is to remove apoB100- and apoE-containing lipoproteins from plasma (Table 1). The N-terminal ligand binding domain is characterized by the presence of seven cysteine-rich, complement-type repeats (Figure 1). The receptor also contains three EGF precursor homology domains. The third domain is rich in serine and threonine residues that are O-linked glycosylated. This is the least conserved domain among different species and is not required for receptor function. The fourth domain is the membrane-spanning region characterized by a preponderance of hydrophobic residues. The final cytoplasmic domain is a highly conserved region and contains an internalization signal. The different domains of the receptors are derived from different exons. For example, the seven cysteine-rich ligand binding domains are encoded by five exons, 2–6. Similarly, the EGF precursor homology region is encoded by exons 7–14. The O-linked glycosylation region is encoded by one exon, 15. The membrane-spanning region and the cytoplasmic domains are derived from two exons, 16 and 17. This domain structure and exon relationship suggests that the functional receptor probably evolved by exon shuffling (for reviews see 10, 46, 59).

The role of individual complement-type and EGF precursor homology repeats of the LDL-R in the binding of apoE and apoB have been determined by deletion of individual repeats and site-directed mutagenesis causing amino acid substitutions (37, 156). Complement-type repeats 1 and 2 and EGF precursor repeat B were not required for the binding of either apoE or apoB to the LDL-R. Complement-type repeats 3–7 and EGF precursor repeat A were required for the optimal binding of apoB. In contrast, only complement-type repeat 5 was crucial for apoE binding. The three-dimensional structures of recombinant repeat 1 and 2 have been solved by nuclear magnetic resonance (NMR) spectroscopy and repeat 5 by X-ray crystallography (33, 40). The basic folding patterns of these repeats were similar and consisted of a β-hairpin structure followed by

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Figure 1 Schematic representation of low-density lipoprotein (LDL) receptor family members. The structure of different family members has been depicted to highlight the presence of ligand binding, epidermal growth factor (EGF) precursor homology, and YWTD repeats. In addition, the presence of a single transmembrane domain and internalization signals in the cytoplasmic domain have been emphasized (not to scale). LRP has not been depicted as a heterodimer. VLDL, very-low-density lipoproteins; ER2, apolipoprotein E–receptor 2.

Table 1 Different ligands recognized by the LDL-R family[a]

Determinants	LDL-R	VLDL-R	ER2	LRP	Megalin	LR11	References
Proteins involved in lipoprotein metabolism							
apoB100 LDL	+				+		8, 10, 167
apoE	+	+	+	+	+	+	6, 10, 65, 86, 90, 93, 96, 108, 175, 176, 191, 204
Chylomicron remnants	+	+		+			65, 68, 129, 136, 196
Hepatic lipase				+			90, 98
IDL	+	+					10, 175
Lipoprotein lipase	+	+		+	+		1, 5, 22, 89, 93, 115, 138, 176, 191
Lp(a)		+					2
VLDL	+	+	+				4, 10, 86, 116, 175, 176
β-VLDL	+	+	+	+	+		53, 65, 86, 96, 175, 191
Proteases and protease/inhibitor complexes							
Activated α_2-M*				+	−		53, 89, 100, 127, 172, 191
Aprotinin				+	+		122
α_1-Chymotrypsin/ cathepsin G				−	+		150
PAI-1				+	+		139, 168
Plasminogen					+		77
Protease/α_1-antitrypsin				+			91, 150
Protease/α_2-M* complexes				+			3, 53, 100, 123, 125, 172, 191
Protease/C1-inhibitor		+		+			83, 171
Protease/PAI-1 complexes		+		+	+		1, 2, 16, 19, 27, 50, 52, 83, 122, 127, 141, 146 147, 168, 169 191
Protease/protein C inhibitor		+		+			83, 171
Protease/protease nexin-1		+		+	+		27, 83, 112
Pro-uPA		+		+	+		1, 92, 168
Protease/antithrombin III		+		+			83, 91
Thrombin/heparin cofactor II		+		+			83, 91

Table 1 (*Continued*)

Determinants	LDL-R	VLDL-R	ER2	LRP	Megalin	LR11	References
Tissue factor inhibitor				+			182
tPA				+	+		16, 19, 146, 147, 191, 194
Ricin A and saponin				+			21
Others							
Albumin					+		31
β-Amyloid precursor protein				+			94
apoJ/clusterin				−	+		47, 93
apoJ/β-amyloid peptide					+		47
Circumsporozoite protein				+			161
Cubulin					+		126
Gentamicin				+	+		122
Lactoferrin				+	+		117, 191
Polymyxcin B				+	+		122
Pseudomonas exotoxin A				+	−		89, 95
RAP	+	+	+	+	+	+	2, 4, 16, 27, 53, 73, 83, 88, 93, 112, 116, 123, 145, 162, 172, 191
Saposin				+			57
Thyroglobulin					+		206
Thrombospondin-1		+		+			119, 120

[a]LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein; R, receptor; ER2, apolipoprotein E receptor 2; LRP, LDL-R–related proteins; apo, apolipoproteins; IDL, intermediate-density lipoproteins; Lp(a), lipoprotein a; α_2-M*, activated α_2-macroglobulin; PAI-1, plasminogen activator inhibitor 1; uPA, urokinase plasminogen activator; tPA, tissue plasminogen activator; RAP, receptor-associated protein. +, Ligands bind to receptors; −, ligands do not bind to receptors.

several β-turns (33). Furthermore, highly conserved acidic residues were clustered on one side of the molecule. Calcium ions had no significant effect on the structure solved by NMR spectroscopy. The X-ray structure indicated that conserved acidic residues were not exposed to the surface but were involved in Ca^{2+} binding (40). These data raise questions regarding the nature of interactions between the receptors and various ligands. However, evidence suggests that various repeats containing a cluster of negative charges, alone or in combination, contribute to specific, high-affinity binding of certain ligands (for reviews see 10, 46, 59).

Very-Low-Density Lipoprotein Receptor

The very-low-density lipoprotein (VLDL) receptor (VLDL-R) was first cloned by low-stringency hybridization of a normal rabbit heart LDL-R–subtracted cDNA library with the rabbit LDL-R probe (175). As expected, the cloned

receptor was remarkably similar to the LDL-R. The major difference was the presence of one extra complement-type repeat in the ligand binding domain present at the N terminus (Figure 1). Not only the cDNA but also the gene structures of these two receptors are remarkably similar (158). As observed in the LDL-R, different functional motifs in the VLDL-R are also encoded by different exons. Furthermore, the amino acid sequence of the receptor is highly conserved among different species, indicating its physiologic importance (188). Two variants of the receptor differing in molecular weight have been described (112, 158, 188). The polymorphism arises because of the presence or absence of 84 bp that code for the serine/threonine–rich O-linked glycosylation motif. In the normal receptor, the O-linked glycosylation motif is encoded by exon 16. In the variant, this exon is spliced out and the protein does not contain an O-linked glycosylation motif (112). The normal variant containing exon 16 is usually found in cells of fibroblastic origin, whereas epithelial cells express receptors that lack this exon. The presence or absence of this O-linked glycosylation site affects the size of the protein but has no effect on ligand binding (112). The tissue distribution of the VLDL-R is different from that of the LDL-R (Table 2). In contrast to the LDL-R, the majority of the VLDL-R is expressed in extrahepatic tissues, e.g. heart, muscle, and adipose tissue. Thus, the VLDL-R may function in the uptake of triglyceride-rich, apoE-containing lipoproteins in tissues that are active in fatty acid metabolism (for reviews see 74, 202, 203).

ApoE Receptor 2

ApoE receptor 2, or LR7/8B, was cloned simultaneously from brain cDNA libraries of humans, mice, and chickens (86, 137). The human receptor contains seven ligand binding repeats, whereas mouse and chicken receptors contain eight ligand binding repeats. The variants observed in different species probably arise because of differential splicing (9, 160). In humans, the ligand binding repeats are grouped as $5+2$ separated by a linker, whereas in mice and chickens they are clustered as $5+3$. The $5+2$ or 3 clustering is similar to that found in VLDL-R ($5+3$) and is different from that observed in LDL-R ($4+3$). Furthermore, with respect to sequence homology and restricted tissue expression, the apoE receptor 2 is closer to VLDL-R than to LDL-R. Thus, it is expected to have broad ligand binding ability, similar to that observed for VLDL-R, rather than the restricted specificity observed for LDL-R (Table 1). The EGF precursor homology and YWTD repeats, an O-linked glycosylation motif, a membrane-anchoring region, and cytoplasmic domains are similar to those of the LDL-R and VLDL-R. The cytoplasmic domain contains one internalization signal.

The LDL-R–Related Protein

LDL-R–related protein (LRP) was first cloned in 1988 (54) and was later discovered (100, 172) to be a receptor for activated α_2-macroglobulin (α_2-M*).

Table 2 Tissue expression of different LDL-R family members[a]

Receptors	Liver	Brain	Heart	Intestine	Kidney	Muscle	Adipose	Adrenal	Lung	Placenta	Ovary	Testis	References
LDL-R	+++	+	+	+	+	+		+++	+	+	+	+	75, 86, 188
VLDL-R	−	+	+++		+	+++	+++	+	+	+	++	++	75, 86, 175, 188
ER2	−	+++	−		−				−	++	+	+	86, 137
LRP	+++	+++		+	+			++	+++	+	+	+	54, 107, 124, 205
Megalin	−	−	−		+++	−		−	++	+	+	−	157, 205
LR11	++	+++						+			++	++	73, 204

[a]Relative abundance (+++ > ++ > +) of different receptor mRNAs in various tissues when equal amounts of RNA were used for hybridization. −, No detection in majority of the studies. VLDL, very-low-density lipoproteins; LDL, low-density lipoproteins; R, receptor; ER2, apolipoprotein receptor 2; LRP, LDL-R–related protein.

LRP is a hetero-dimeric protein consisting of 515- and 85-kDa subunits. It is synthesized as a single polypeptide of 4525 amino acids and is cleaved by furin in the Golgi compartment to produce two subunits of 3924 and 601 amino acids (55, 193). Both subunits remain noncovalently attached to each other and serve two independent functions. The 85-kDa subunit consists of a transmembrane and a cytoplasmic domain containing two copies of the NPXY sequence. The 515-kDa subunit contains 31 acidic, cysteine-rich, complement-type repeats that are similar to the repeats found in the ligand binding domain of the LDL-R. They are clustered as 2, 8, 10, and 11 repeats in four domains (Figure 1). This subunit also contains 21 cysteine-rich EGF precursor-like repeats and O-linked glycosylation sites (for reviews see 12, 14, 51, 65, 99, 121, 173, 174).

LRP is involved in the catabolism of several structurally unrelated proteins, proteinases, proteinase-inhibitor complexes, and protein-lipid complexes (Table 1). Four different approaches have been used to identify regions in LRP that bind to various ligands. First, purified LRP was cleaved by enzymatic or chemical proteolysis, and different fragments were transferred to nitrocellulose. Ligand blotting to these fragments indicated that amino acids 776–1399 containing ligand binding domain 2 of LRP-bound α_2-M*, urokinase plasminogen activator (uPA):plasminogen activator inhibitor-1 (PAI-1), and receptor-associated protein (RAP) (125). Second, a monoclonal antibody, A8, was identified that inhibited the binding of lipoprotein lipase, uPA:PAI-1, pro-uPA, and RAP to LRP but had no effect on the binding of α_2-M* and tissue plasminogen activator (tPA):PAI-1 complexes (61). It should be pointed out that lipoprotein lipase partially competes for the binding of α_2-M* to LRP (138). Thus, the antibody could distinguish between the binding sites of two competing ligands. A8 bound to amino acids 776–1399, indicating that this region might be involved in the binding of all four ligands. Third, ligand binding domains 2 and 4 were independently fused with membrane-spanning and cytoplasmic domains to construct minireceptors (194). These chimeric minireceptors were expressed in cells, and the binding, uptake, and degradation of various radiolabeled ligands were studied. Both minireceptors bound and degraded RAP. In contrast, only minireceptors expressing domain 2 bound and degraded tPA:PAI-1 complexes. Fourth, individual domains of LRP were expressed as secretory proteins (17). Secreted domains were immobilized on nitrocellulose membranes and used to study the binding of RAP. RAP bound strongly to domains 2 and 4 but only weakly to ligand binding domain 3. All these studies together indicate that a complete receptor is not required for ligand binding and that LRP has different domains that act independently and bind different ligands simultaneously. Furthermore, they provide compelling evidence for the involvement of domain 2 in the binding of α_2-M*, uPA:PAI-1, lipoprotein lipase, tPA:PAI-1, and RAP. Even though α_2-M* and tPA:PAI-1 bind to the same domain, they do not cross-compete for their binding to LRP (19, 141), indicating that

as-yet-unidentified subdomains within domain 2 determine the specific binding of these ligands.

Megalin

Megalin (also known as gp330 or LRP-2) was first identified as a target antigen for circulating antibodies in Heymann nephritis (reviewed in 24, 38, 39, 84). It is a single polypeptide of 4660 amino acids containing 36 cysteine-rich ligand binding domains, 16 EGF precursor homology domains, and 40 YWTD repeats in the extracellular domain (Figure 1). There are four clusters of ligand binding domains consisting of 7, 8, 10, and 11 complement-type repeats in the molecule that recognize several groups of ligands (Table 1). The intracellular domain contains two internalization signals, as observed in other members, but its overall sequence is different from that of other receptors. The intracellular domain of megalin contains Src-homology binding regions, casein kinase II sites, and protein kinase phosphorylation sites, indicating that it may be involved in signal transduction (24). In order to identify the binding site for various ligands, Orlando et al (143) identified a monoclonal antibody that inhibited the binding to megalin of RAP, apoE-β-VLDL, lipoprotein lipase, aprotinin, and lactoferrin. Because one antibody could inhibit the binding of four different ligands, it was suggested that these ligands bind to similar or juxtaposed binding sites. The monoclonal antibody recognized complement-type repeats 4 and 5 within domain 2 (amino acids 1111–1210), indicating that this contains a binding site for these four ligands. Further studies are required to identify repeats crucial for the binding of individual ligands.

Megalin is a large cell-surface receptor that is primarily expressed in the absorptive epithelial cells (157). The cellular expression pattern of the receptor indicates that it may play an important role in the absorption of some molecules by the intestine, in reabsorption by the kidney, and in transport across the blood-brain barrier (for reviews see 24, 38, 39, 84). For example, megalin may play a role in the reabsorption of Ca^{2+} by renal tissue and may be important for Ca^{2+} homeostasis in the parathyroid and cytotrophoblasts. Furthermore, it may play a role in the reabsorption by the kidney of various proteins that are recognized by this receptor (Table 1). Megalin appears to be involved in the reabsorption of vitamins in the kidney, and it has been shown to be involved in the binding and trafficking of cubilin, a 460-kDa protein that associates with intrinsic factor/vitamin B_{12} complexes (126). Because they can bind and take up several antibiotics, megalin and possibly other members may be responsible for toxic side effects observed in various tissues after the use of antibiotics (24, 122).

Related Members

LR11, or SORLA-1, was simultaneously identified by homology cloning and RAP-affinity purification (73, 204). It is highly conserved in humans and

chickens (130). This hybrid receptor contains one ligand binding domain, five YWTD repeats, one membrane-spanning region, and one cytoplasmic domain containing an internalization signal. In contrast to other members, it lacks EGF precursor homology repeats. Instead, it contains additional domains that are homologous to the vacuolar sorting receptor of yeast and fibronectin type III repeats observed in adhesion molecules. Because the ligand binding domain contains 11 complement-type repeats similar to those observed in LRP and megalin, it may be involved in the catabolism of diverse ligands. It has already been shown to bind apoE and RAP. The presence of fibronectin type III repeats indicates that it may play a role in cell-cell interactions, especially between neurons (73, 130, 160, 204). Some other related receptors that have been shown to contain ligand binding repeats are LRP3 and LRP6 (13, 56, 72).

CHARACTERISTIC FEATURES OF THE LDL-R FAMILY

Receptor-Mediated Endocytosis

LDL-R family members are constitutively recycling receptors, i.e. they are endocytosed whether or not they are associated with ligands. They import different proteins, lipoproteins, or protein-protein complexes into cells by a process called receptor-mediated endocytosis. An endocytic pathway consists of functionally distinct compartments that originate at the inner surface of the plasma membrane and end with lysosomes. Receptor-mediated endocytosis begins at specialized regions, called coated pits, in the plasma membranes. The assembly of coated pits is initiated by the binding of dephosphorylated adaptor protein (AP)-2 complexes to the plasma membrane. The AP-2 complexes consist of two (α and β) ~100-kDa, one (μ) ~50-kDa, and one (σ) ~20-kDa subunit. The binding of the AP-2 complex to the membrane results in the recruitment of clathrin from the cytosol to form clathrin-coated vesicles and of receptors from other regions of the plasma membrane to the coated region for internalization. The recruitment of clathrin involves binding of ~190-kDa clathrin subunits with the β subunit of the AP-2 complex. The recruitment of LDL-R family members may involve the binding of the FDNPXY motifs with the terminal domains of the clathrin heavy chain (85). Purified clathrin consists of three heavy chains (~190 kDa) and three light chains (~30 kDa) and has a three-legged appearance called a triskelion. The binding of clathrin triskelions with membrane-bound AP-2 results in the formation of lattices consisting of hexagons and pentagons. These lattices impart the characteristic coated appearance to the membranes when visualized under the electron microscope. The coated pits are pinched off from the membrane as coated vesicles in an energy-dependent process mediated by dynamin, a high-molecular-weight GTP binding protein. It is believed that GDP-bound dynamin interacts with clathrin lattices

through its C-terminal proline-rich domain. Exchange of GDP with GTP results in the redistribution of dynamin to form a collar around the neck of invaginated coated pits. GTP hydrolysis may induce a constriction of this collar and release of the pits as coated vesicles from the membrane. The coated vesicles undergo rapid uncoating in the cytoplasm, which involves an uncoating ATPase, hsc70. The hydrolysis of ATP by hsc70 results in the release of clathrin, but not AP-2 complexes, from coated vesicles. Mechanisms involved in the release of AP-2 have not yet been elucidated, but Rab-guanine nucleotide exchange factors may be involved. The uncoated vesicles deliver their contents to endosomes by a docking and fusing mechanism involving v- and t-SNAREs. The endosomes are a collection of vacuolar and reticular structures that play an important role in the dissociation of ligands and the sorting of receptors. Ligands are dissociated from receptors because of the slightly acidic PH of these endosomes. Next, physical separation of receptors from ligands occurs. In this process, receptors accumulate in the tubular extensions of endosomes, whereas the luminal contents accumulate in vesicular regions. The tubular extensions are pinched off and recycled back to the plasma membrane. The vesicles fuse with late endosomes or prelysosomes containing enzymatically active lysosomal hydrolases that degrade the ligands (for reviews see 118, 131, 154, 159, 163).

Recognition of Multiple Ligands

Receptors bind to their ligands with high affinity, i.e. receptors can recognize ligands present at low concentrations ($\leq 10^{-8}$ M). In the LDL-R family, receptors display negative charges on their surfaces, whereas ligands display positive charges. This is different from the scavenger receptor family, where receptors display positive charges and recognize negatively charged ligands (99). Multiple interactions between ionic residues of receptors and ligands result in high-affinity binding. Different cells have different capacities to bind and internalize different ligands. The binding capacity of a cell is a good indication of the number of receptors displayed on the cell surface.

Members of the LDL-R family recognize several structurally dissimilar ligands (Table 1). The smallest receptor that binds to a variety of ligands is the VLDL-R (Figure 1 and Table 1). Thus, a single ligand binding domain consisting of eight complement-type repeats found in the VLDL-R appears to be sufficient for the binding of different ligands.

Two ligands, RAP and apoE, of dissimilar amino acid sequences bind to all the members of the family (Table 1). There are differences with respect to the binding of RAP and apoE to different receptors. RAP, a chaperone present in the lumen of the endoplasmic reticulum, has been shown to assist in the proper folding of LRP and in preventing premature binding of ligands to the receptor (15, 17, 195). Very likely, it plays a similar function during the biosynthesis of

other members. A remarkable property of the protein is that it binds to all the members and acts as a universal antagonist for the binding of all the ligands to different receptors (14, 53). Small size, compact structure, and multiple binding sites on this protein may contribute to this unusual property of competing for the binding of various ligands to different receptors. On the other hand, apoE is a secretory protein and acts as a ligand for several receptors (Table 1) after its association with lipids or lipoproteins (108). It has a single receptor binding domain and does not compete well for the binding of other ligands. It exists in three different isoforms. The LDL-R can distinguish between these isoforms with binding affinities in the order of apoE4 > apoE3 ≫ apoE2 (108). However, other receptors in the family do not discriminate and bind all isoforms. The molecular basis for the differences between LDL-R and other members with respect to ligand binding and apoE isoform specificity is not known.

In addition to overlapping ligand specificities, each receptor appears to have a unique ligand (Table 1). For example, α_2-M* only binds to LRP and this binding is inhibited by nickel (64, 79, 142). Similarly, apoJ may be specific for megalin. ApoB100, the protein constituent of LDL, has been shown to bind to the LDL-R and to megalin (10, 167). The seven (4 + 3) complement-type repeats and one EGF precursor homology domains are required for the binding of LDL to the LDL-R (10, 59). A similar arrangement of seven complement repeats and EGF precursor regions is present in megalin. Thus, this arrangement may be essential for the binding of LDL. Other receptors contain eight complement-type repeats in their ligand binding domains. The presence of the eight repeats may prevent the binding of a larger LDL to these receptors. More studies are required to understand fully the molecular basis for the specificity of interactions between different ligands and receptors of this family.

It is important to highlight that many of the LDL-R family ligands undergo a conformational change before they are recognized by members of this receptor family. This is especially true of α_2-macroglobulin, serine proteinase inhibitors (serpins), and apoE. α_2-Macroglobulin is a large plasma proteinase inhibitor that exhibits broad specificity and reacts with proteinases from all major classes (for reviews see 25, 164). The native form of α_2-macroglobulin is not recognized by LRP. Proteinases cleave the bait region of α_2-macroglobulin and trigger a rapid conformational change in the inhibitor that traps the proteinase. This conformational alteration exposes sites on the inhibitor that are recognized by LRP. The conformational alteration can also be triggered by methylamine, a small nucleophile capable of attacking the internal thiolester bond in α_2-macroglobulin. Like α_2-macroglobulin, the serpins function as proteinase inhibitors of serine proteinases. The serpin family consists of antithrombin III, CI inhibitor, PAI-1 and -2, and α_1-antitrypsin. In their active state, these serpins are substrates for various proteinases. The proteinases cleave an exposed loop region (strained

loop). Serpins are thought to inhibit the target proteinase by a common mechanism involving formation of a Michaelis complex, nucleophilic attack by the proteinase on the serpins P_1-P'_1 reactive center peptide bond, and generation of a stable complex (for reviews see 44, 152, 165). The proteinase-inhibitor complex can be recognized by LDL-R family members. For most serpins, certain proteinases can cleave the exposed loop region without being inhibited, which results in the generation of a cleaved inhibitor. Like the native serpins, cleaved serpins do not bind to receptors. PAI-1 differs from the other serpins in that the native conformation is relatively unstable, and PAI-1 is rapidly converted to a latent form that is not capable of interacting with proteinases. Thus, PAI-1 exists in four different forms: native or active, latent, cleaved, and the proteinase complexed form (for reviews see 44, 152, 165, 180). The active form is a substrate for proteinases, whereas the latent form is a stable conformation of the molecule that is not recognized by proteinases. Of all these forms, the only form that binds with high affinity to members of the LDL-R family is the form complexed to a proteinase, such as the uPA:PAI-1 complex. Unlike α_2-macroglobulin and serpins, apoE is not a proteinase inhibitor. It is an exchangeable apolipoprotein that exists either free or associated with lipoproteins in plasma. apoE has to associate with lipids or lipoproteins before it is recognized by receptors (108). The acquisition of apoE by different lipoproteins results in their removal from plasma. Thus, the conformational changes in these ligands are signals for their removal from plasma.

Receptor Binding Motifs of Different Ligands

As opposed to the well-conserved, complement-type ligand binding repeats in receptors, different ligands do not share significant sequence homology (Table 3). However, it is important to note that most ligands are able to bind to heparin and the identified receptor binding domains in different ligands are rich in arginine and lysine residues (Table 3). Several mutagenesis experiments have shown that positively charged amino acids are crucial for receptor binding. Thus, primary sequences rich in basic amino acid residues may be critical for receptor binding. An attempt has been made to derive a consensus primary sequence that might constitute a receptor binding domain (133). We propose that instead of a short stretch of a primary sequence, a secondary or tertiary structural motif rich in positive electrostatic potential may constitute a receptor binding domain in different ligands. Proteins of different primary sequences may fold to expose a similar secondary or tertiary cluster of charges. The positive electrostatic potential is derived from the surface exposure of lysine and arginine side groups that are not involved in intramolecular salt bridges. The positive potential can form various contacts with an area of negative electrostatic potential present on the surface of receptor molecules. This proposal is

Table 3 Polypeptide sequences in various ligands critical for receptor binding[a]

Ligands (amino acids)	Sequences critical for receptor binding	References
apoE (134–150)	**R**V**R**LASHL**RK**L**RKR**LL**R**	108
apoB (3359–3369)	**R**LT**RKR**GL**K**LA	8
Aprotinin (38–47)	C**R**A**KR**NNF**K**SA	122
Lipoprotein lipase (380–384)	L**K**W**K**S	134
Lipoprotein lipase (403–425)	**K**I**R**V**K**AGETQ**KK**VIFCS**RE**K**V**SHL	134
α_2-Macroglobulin (1366–1392)	FIPL**K**PTV**K**MLE**R**SNHVS**R**TEVSSNHV	133
PAI-1 (69–87)	DKGMAPAL**R**HLY**K**ELMGPWN	170
Pseudomonas exotoxin A (54–67)	DAL**K**LAIDNALSIT	95

[a]apo, apolipoprotein; PAI-1, plasminogen activator inhibitor 1.

supported by data obtained from molecular mutagenesis and three-dimensional structural analysis of several ligands. X-ray diffraction analysis of apoE showed that positively charged residues critical for the LDL-R binding are clustered into a surface patch on one long helix (197). In the amino-terminal half of the helix 4 of apoE3, residues R134, R136, H140, R142, K143, R145, K146, R147, and R150 are solvent exposed and are not involved in intramolecular salt bridges. These residues create a positive electrostatic potential almost 15 Å away from the protein (197). In lipoprotein lipase, the C-terminal folding domain (amino acids 378–448) has been shown to be critical for LRP binding (134, 140, 190). Within this region, deletions of amino acids 380–384 and 404–430, and mutations of K407, significantly decrease receptor binding. Molecular modeling of lipoprotein lipase based on the X-ray crystallographic structure of pancreatic lipase showed that the enzyme contains four clusters of positive electrostatic potential away from the active site (179). Cluster 4 of lipoprotein lipase consists of residues K319, R405, K407, and K414, which have been implicated in receptor binding (134, 179). Thus, the positive electrostatic potential present in cluster 4 of lipoprotein lipase may constitute a receptor binding domain. Site-directed mutagenesis of aprotinin identified several positive charges critical for receptor binding (122). Residues K15, R17, and R42 are of particular interest because X-ray structural analysis of aprotinin indicated that K15, R17, R39, and R42 are surface exposed (199, 200) and may form a receptor binding domain. In the case of PAI-1, mutagenesis of R76, K80, R118, and K122 [numbered according to Pannekoek et al (149)] resulted in loss of receptor binding (153, 170). Most of these residues have been shown to be surface exposed and are localized to one face of the molecule (153). Although the X-ray crystallographic structure of apoB is not known, antibody binding studies have shown that the identified receptor binding sequences are exposed to the surface of the LDL (8, 23). Despite

numerous efforts, it has been difficult to identify a primary sequence in RAP that might be involved in receptor binding (36, 144, 183–185). This is due to the fact that RAP contains three internal repeats of ~100 amino acids that can individually bind to receptors (36, 185). A solution structure of the N-terminal repeat of RAP obtained by NMR spectroscopy consists of three helices arranged in up-down-up antiparallel topology (135). Nielsen et al (135) have proposed that a groove formed by these three helices might constitute a receptor binding domain. Thus, a positive electrostatic potential exposed on the surface of different ligands may constitute a domain that is recognized by various members of the LDL-R family.

RECEPTOR DYSFUNCTIONS AND DISEASES

LDL-R and Familial Hypercholesterolemia

The major function of the LDL-R is cholesterol homeostasis, which is accomplished by controlling plasma LDL levels. Mutations in the LDL-R gene cause familial hypercholesterolemia characterized by increased plasma cholesterol levels, arcus cornea, tendon xanthomas, and coronary heart disease. This is the most common monogenic human disease, with a heterozygous frequency of 1 in 500, and it is transmitted as an autosomal dominant trait. The heterozygotes express half of the normal levels of the receptor, have twice the normal plasma cholesterol levels, and develop tendon xanthomas and many atherosclerotic plaques. The homozygotes, expressing nonfunctional receptors, have dramatically increased plasma cholesterol levels and develop cutaneous xanthomas and atherosclerotic plaques at a very early age. Several mutations in the LDL-R, classified into classes 1–5, affect different steps in the life cycle of the receptor—synthesis, transport to cell surfaces, binding to ligands, clustering of the receptors in the endocytic vesicles, and recycling to the cell surface (for reviews see 10, 46, 59).

LRP in Fetal Development and Alzheimer's Disease

In contrast to the LDL-R, mutations in LRP causing human disorders have not been described, perhaps because LRP is essential for fetal development: Deletions in mice result in the death of the fetuses around day 10 (52). The reasons for early fetal death are not apparent. It is possible that either lack of uptake of yet-unknown ligands or lack of removal of potentially toxic proteins from its environment may lead to fetal death. Alternatively, LRP may contribute to fetal development by a mechanism not yet understood.

Evidence suggests that in addition to playing an important role in development, LRP and a number of its ligands contribute to the development of Alzheimer's disease (AD). LRP binds and internalizes proteins genetically associated with AD, such as certain secreted forms of amyloid precursor protein

(see below), apoE (6), and the α_2-M* (100, 172). Recent genetic evidence suggests that a polymorphism within LRP itself may be associated with the expression of late-onset AD (60, 76, 81, 103, 187). However, the molecular basis for the association between LRP polymorphism and AD is not known.

AD is a neurodegenerative disorder characterized by memory loss and distinct neuropathological hallmarks involving intracellular neurofibrillary tangles and extracellular β-amyloid plaques (30, 151). The tangles consist of paired helical filaments formed from the hyperphosphorylated microtubule-associated protein, tau, whereas the plaques consist of fibrillar assemblies of Aβ peptides of 39–43 amino acids derived from an integral protein termed amyloid precursor proteins (APP). APP exists in multiple isoforms resulting from alternate splicing of mRNAs (82, 177). Two of these isoforms, APP770 and APP751, contain a Kunitz proteinase-inhibitor domain. These isoforms of APP appear to be more amyloidogenic than the forms lacking the Kunitz proteinase-inhibitor domain and are also elevated in the brain of patients with Alzheimer's disease (58, 128).

The generation of Aβ peptides from APP occurs by multiple pathways. One of the pathways involves release of APP from cell surface, internalization, and targeting to lysosome (30). Cell surface proteolysis of APP occurs by the action of α-secretase within the Aβ peptide region of APP precluding the formation of β-amyloid plaques. This cleavage generates a secreted form of APP that is released from the cell. It has been shown that secreted isoforms of APP that contain the Kunitz proteinase-inhibitor domain are recognized, internalized, and degraded by the LRP (94). It is feasible that LRP can also associate with the full-length, membrane-anchored APP containing the proteinase-inhibitor domain and can influence the internalization and subsequent production of Aβ peptides. If this occurs, then molecules, such as RAP, that antagonize these interactions may be expected to influence the progression of the disease.

The molecular basis for the genetic association of α_2-macroglobulin and apoE with AD are not known. α_2-Macroglobulin has been reported to bind Aβ peptides and appears capable of mediating their removal via binding to LRP (35, 62, 132). Thus, LRP in conjunction with α_2-macroglobulin may reduce Aβ levels. AD has been associated with a deletion within an intron close to the bait region of α_2-macroglobulin (7). This deletion may preclude the binding of α_2-macroglobulin to proteases, prevent its activation, deter complexing with Aβ, and increase chances for plaque formation. In addition, AD has been positively correlated with apoE4, an important ligand of LRP involved in lipoprotein metabolism (29). ApoE interacts with Aβ peptides and apoE4 has been shown to be more effective than apoE3 in promoting in vitro Aβ fibril formation (189). It is likely that apoE/Aβ complexes may not be recognized and removed by LRP causing fibrillar depositions. In summary, LRP appears to play a central, but still undefined, role in AD by mediating the catabolism of three molecules that are genetically linked to this disease.

Megalin in Heymann Nephritis

Mice lacking megalin are defective in pulmonary inflation and alveolar expansion, develop the holoprosencephalic syndrome (abnormal forebrain development, absence of olfactory apparatus, etc), and die within 2–3 h of birth because of respiratory insufficiency (192). These studies indicate that megalin plays an essential role in the development of the brain. In brain development, megalin may be necessary for the delivery of essential nutrients, such as cholesterol.

Heymann nephritis in rats is caused by the formation of subepithelial immune deposits in glomerular basement membrane. It occurs because of the binding of circulating antibodies to two endogenous antigens, a cell-surface receptor megalin and an endoplasmic reticulum-resident RAP that acts as a chaperone during the biosynthesis of different receptors. Several antigenic determinants in megalin and RAP have been identified. Binding of antibodies to these antigenic determinants results in (*a*) intracellular immune deposits in the glomerular basement membrane, (*b*) damage to glomerular capillary wall, and (*c*) proteinuria (for reviews see 24, 38, 39, 84). The knowledge about the antigenic determinants should in the near future help in designing drugs that interfere with the binding of antibodies to these determinants and in preventing membranous nephropathy and proteinuria.

VLDL-R and Female Sterility in Chickens

Currently, no human diseases have been associated with the VLDL-R dysfunction. The function and importance of the receptor have been studied by disrupting the VLDL-R gene and by overexpressing the receptor in different knock-out mice. Disruption of the VLDL-R gene in mice had no significant effect on fertility and lipoprotein catabolism in mice (41). However, these mice had a modest decrease in the whole body and adipose tissue mass. In contrast, mutations in the chicken homolog of this receptor have been shown to result in female sterility (non–egg laying), mainly because of failure in the deposition of egg yolk proteins. These chickens also have severe hyperlipidemia associated with premature atherosclerosis (20).

VLDL-R may be a good candidate for gene therapy to control hyperlipidemias. The adenovirus-mediated overexpression of VLDL-R in the livers of LDL-R-deficient mice resulted in marked decrease in plasma apoE, apoB100, and cholesterol because of a significant decrease in VLDL/IDL (intermediate-density lipoproteins) (87, 97). Similarly, VLDL-R expression in the livers of apoE-deficient mice expressing E2 and apoE-Leiden resulted in decreased plasma cholesterol levels (178), indicating that gene therapy with DNA coding for the VLDL-R expression could be used to decrease plasma cholesterol levels in familial hypercholesterolemia and type III hyperlipidemia. The use of a receptor that is expressed by tissues other than liver is a good candidate for hepatic gene therapy because expression in the liver of an endogenous protein

may not be recognized as a foreign protein and would not be expected to elicit an immune response.

REGULATION OF RECEPTOR EXPRESSION

Most of the LDL-R family members are expressed in many tissues (Table 2). However, only a few tissues express significant amounts of individual receptors (Table 2). Thus, the functional importance of these receptors may lie in differential tissue expression. For example, LDL-R and LRP are mainly expressed in liver and are crucial for the removal of circulating ligands such as lipoproteins and α_2-M*. VLDL-R is mainly expressed in heart, muscle, and adipose tissue and may be important for lipoprotein uptake by these tissues. Megalin is mainly expressed in kidney and lungs and plays an important role in membranous nephropathy and respiration. Significant amounts of apoE receptor 2, LRP, and LR11 are expressed in brain and may be critical for the proper functioning of brain. *cis* elements controlling the tissue expression of these receptors have yet to be determined.

In contrast to the sequence homology in the coding region, these receptors do not share any significant homology in the untranslated regions of their genes, indicating that their expression is regulated differently. The regulation of cellular expression of all the family members occurs at the level of transcription. Thus, the presence or absence of different *cis* regulatory or enhancer elements in individual genes determines their regulation by several factors.

The LDL-R is mainly regulated by intracellular cholesterol (see below). In addition to sterols, it is also regulated by nonsterol factors, such as growth factors and hormones, but the mechanism of regulation by nonsterol factors is poorly understood (111, 148, 155, and references therein). The LDL-R has a simple promoter consisting of 200 base pairs upstream of the protein translation initiation methionine codon. The promoter region of the LDL-R contains two TATA boxes and two Sp1 binding sites. These sites confer the basal transcription of the LDL-R gene. A third sterol-regulatory enhancer 1 (SRE 1) is present between the two Sp1 binding sequences. In the absence of sterols, SRE binding proteins (SREBPs) bind to SRE 1 and act in synergy with the Sp1 to induce maximum induction in the transcription of the LDL-R gene. Sterols indirectly control the binding of SREBPs to SRE 1. SREBPs are membrane-bound transcription factors present in the endoplasmic reticulum. In the absence of sterols, the membrane-bound transcription factors become susceptible to proteolytic cleavages. The proteolytic cleavages result in the release of an N-terminal ~480–amino acid polypeptide that contains a basic-helix-loop-helix-leucine-zipper motif observed in other transcription factors. The released N-terminal transcription factor reaches the nucleus, binds to SRE 1 region, and enhances the transcription of the LDL-R and other genes that contain the SRE 1 binding

sequences. When cells have sufficient levels of sterols, the proteolysis of the membrane-bound SREBPs is inhibited and LDL-R transcription is repressed to basal levels (for reviews see 10, 11, 45, 46, 59).

The LRP promoter has no TATA box and SRE 1 binding sequence (42, 101). However, it contains Sp1 and NRF-1 binding sites (42, 101). In addition, an enhancer element containing a cluster of Sp1 sites has been identified (42). LRP is not regulated by factors that regulate LDL-R, such as high cholesterol, 25-hydroxycholesterol, and mevalonate (12, 101). It has, however, been shown to be up-regulated in macrophages by macrophage colony stimulating factor (70, 186) and down-regulated by interferon-γ, phorbol myristic acid esters, and lipopolysaccharides (26, 69, 102). In HepG2 cells, dexamethasone has a modest effect on receptor expression (78). The expression of this receptor is increased during differentiation of trophoblasts and suppressed in the presence of cAMP (43). In cultured adipocytes, insulin stimulates LRP activity (34). In neuronal cells, nerve growth factor increases cell-surface receptor and mRNA levels (18). Receptor levels have been shown to increase (201) or decrease (80, 104) in various tumor cell lines. Molecular mechanisms of the regulation of LRP have not been well characterized, mainly because receptor levels are only modestly affected by various manipulations.

During in vitro cellular differentiation, F9 cells preferentially induce the expression of megalin compared with LRP (32). F9 cells are embryonic carcinoma cell lines that differentiate into endoderm-like cells in the presence of retinoic acid and dibutyryl cyclic AMP, and this treatment specifically increases the expression of megalin (32, 93, 167). In addition, vitamin D induces megalin expression in various cell lines (106). F9 cells may be useful in understanding the molecular mechanisms involved in the induction of megalin expression.

The VLDL-R gene does not contain the classical TATA and CAAT boxes. However, the promoter region includes sites for the binding of Sp1, heat-shock transcription factor, estrogen, and glucocorticoid receptor binding site (158, 188). It has been shown to be up-regulated by granulocyte-macrophage colony stimulating factor, estradiol, and thyroid hormones in muscle and heart (71, 74, 75, 114) and down-regulated in heart during cardiac hypertrophy (113). The expression of the receptor is markedly reduced in muscle and heart during experimental renal failure and nephrosis (105, 181). In trophoblast-derived cell lines, it is up-regulated by insulin and down-regulated by cAMP (198).

ROLE IN DIETARY LIPID METABOLISM

Dietary lipids and lipid-soluble vitamins are packaged into chylomicrons and secreted into the lymph. In the circulation, triglycerides present in these lipoproteins are hydrolyzed by the endothelial-bound lipoprotein lipase, resulting in

the formation of remnant lipoproteins. The remnants are cleared by the liver and bone marrow (66, 67). ApoE plays a crucial role in the clearance of these particles by the liver. Enrichment of lipoproteins with apoE or infusion of apoE into plasma increases lipoprotein clearance by the liver (63, 66, 110). In the liver, remnants are either removed by the LDL-R or sequestered in the space of Disse. The sequestered molecules are enriched with apoE or hepatic lipase and are cleared by the LRP. Thus, the LDL-R and the LRP play significant roles in the uptake of remnant lipoproteins by hepatocytes. The role of these receptors in chylomicron catabolism has been recently reviewed (28, 49, 65, 109, 110a, 166).

CONCLUDING REMARKS

Significant information concerning the structural and functional motifs in different receptors of the LDL-R family has been obtained. These receptors have the remarkable property of recognizing structurally dissimilar molecules. Because of this property, members of the family play physiologically significant functions in many processes, such as fetal development, cholesterol homeostasis, cellular migration, immune response, and protease clearance from the blood. Furthermore, they have been implicated in atherosclerosis and neurodegenerative diseases. Because of the multifunctionality of these receptors, it is desirable neither to alter expression in all cells nor to inhibit the binding of all ligands. However, specific antagonists that inhibit specific ligands will be useful. For example, inhibition of the uptake of apoE-containing lipoproteins by foam cells is expected to be advantageous in controlling atherosclerosis. Each receptor has a unique tissue and cell expression pattern. More needs to be learned about the nonsterol regulation of these receptors at the cellular level. It may be possible to regulate the tissue expression of individual receptors and control progression of some diseases. For this purpose, we must learn more about the tissue expression of individual receptors and devise new strategies to control tissue-specific expression.

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