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STRUCTURAL, BIOCHEMICAL AND SIGNALING PROPERTIES OF THE LOW-DENSITY LIPOPROTEIN RECEPTOR GENE FAMILY

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1. ABSTRACT

The low-density lipoprotein (LDL) receptor (LDL-R) family members (LDL-R, LRP, megalin, VLDL-R, apoER2) bind several extra-cellular structurally dissimilar ligands and internalize them for degradation by lysosomes by a process called receptor-mediated endocytosis. The receptor-mediated endocytosis involves immobilization of circulating ligands onto the cell-surface followed by their internalization and degradation. All the receptors can perform both of these functions. However, in the majority of the cases, other proteins immobilize ligands on to the cell-surface and subsequent internalization is mediated by these receptors. The LDL-R and LRP play important roles in plasma cholesterol homeostasis and fetal development. Megalin is an antigenic determinant for Heymann nephritis in rats and may be important for re-absorption of various molecules by the kidney. VLDL-R homologue in chicken is essential for female fertility. This receptor and apoER2 are critical for the proper development of the brain in mice. The members of the LDL-R gene family contain several complement-type and EGF precursor-like repeats, and single transmembrane and cytoplasmic domain. Cysteine-rich complement-type repeats containing DxSDE sequences at the C-termini constitute ligand-binding domains. In contrast to the ligand binding domains, receptor-binding domains in different ligands do not share sequence homology. It has been proposed that positive electrostatic surface potentials, not the primary sequences, in different ligands constitute receptor-binding domains. The EGF precursor homology repeats in receptors are important for the dissociation of ligands from receptors in endocytic vesicles. The transmembrane domain is necessary for anchoring to membranes and the cytoplasmic domain is required for their targeting to coated pits and subsequent internalization. The receptor-mediated endocytosis involves recognition of the NPXY motif by clathrin. Recently, this motif has also been implicated in signaling pathways that are crucial in brain development. The signaling process involves the recognition of the NPXY motif by Disabled-1 protein and possibly other proteins involved in intracellular signaling cascade. The LDL-R gene family has provided important insights into the mechanisms of ligand catabolism and may serve as new targets for the treatment of different cardiovascular and neuronal disorders. In the future, their

Table 1. Characteristic features of the LDL-R family

1.	Cell-surface expression
2.	Extracellular ligand binding domain consisting of complement-type repeats containing DxSDE residues.
3.	Requirement of Ca^{+2} for ligand binding
4.	Recognition of RAP and apoE
5.	EGF precursor homology domain containing YWTD repeats
6.	Single membrane spanning region
7.	Presence of internalization signal (FDNPXY) in the cytoplasmic domain
8.	Receptor mediated endocytosis of various ligands

role in signaling may provide novel insights into brain development and neuronal layering.

2. INTRODUCTION

The LDL-R gene family consists of trans-membrane receptors that reside on the cell-surface and require Ca^{+2} for ligand binding (table 1). All the members recognize two structurally dissimilar proteins; receptor associated protein (RAP) and apolipoprotein E (apo E). These receptors share several structural motifs and functional properties and interact with a diverse group of ligands. In contrast to the signaling receptors that contain large intracellular domains, all the members of the family are trans-membrane glycoproteins that contain large extracellular and comparatively shorter intracellular domains, and lack enzymatic activities. The receptors bring various ligands from the extracellular environment into the cell for degradation and thus potentially control cellular phenotypes by modulating extracellular levels of various proteases, protease inhibitors, and growth factors. The knowledge concerning structural and functional domains, regulation of expression in various tissues, and diseases that arise due to receptor dysfunction has been discussed and reviewed (1). Here, I will summarize current understanding about structural motifs, biochemical features, endocytic properties, and their role in the signaling during brain development.

3. STRUCTURAL MOTIFS AND FUNCTION

Different receptors were placed in this family because of the sequence homologies in the four characteristic structural and functional motifs: complement-type cysteine-rich repeats, EGF precursor-like repeats, membrane-spanning region, and intracellular domain containing at least one internalization signal sequence (FDNPXY). Each complement-type repeat consists of ≈ 40 amino acids and contains six cysteine residues and one DxSDE sequence at the C-terminal end. Different receptors contain different numbers of these repeats (table 2), and a single repeat or several repeats constitute a ligand-binding domain. All the cysteine residues within a repeat are disulfide linked forming stable and compact, functionally independent motifs. It has been speculated that differential clustering of these repeats within a domain may be responsible for the specificity toward different ligands (1). The EGF precursor homology domains consist of cysteine-rich growth factor and YWTD repeats. The negatively charged residues (DxSDE) found in ligand binding domains are not present in the cysteine-rich EGF precursor domain. The growth hormone repeats of ≈ 50 amino acids are usually separated by a group of five YWTD repeats. The EGF precursor homology domains are necessary for the dissociation of ligands from the receptor in endosomes (2-4). In addition to these two cysteine-rich regions, all the members contain a single transmembrane domain of hydrophobic amino acid residues that help them anchor into membranes. The cytoplasmic domains contain at least one FDNPXY sequence necessary for the targeting of receptors to coated pits and subsequent internalization. In the brain, this sequence recruits adapter proteins involved in signal transduction.

3.1. LDL receptor

The LDL-R is a cell-surface, transmembrane glycoprotein of 839 amino acids. It controls plasma cholesterol by mediating uptake and catabolism of apoB100- and apoE-containing lipoproteins from plasma (table 3). The N-terminal ligand-binding domain consists of seven cysteine-rich, complement-type repeats (table 2). It also contains three EGF precursor homology domains and one O-linked glycosylated domain. The membrane-spanning region is rich in hydrophobic amino acid residues and is necessary for membrane anchoring. This region is not well conserved within different species. The cytoplasmic domain is highly conserved and contains an internalization signal. Different structural and functional domains of the receptors are derived from different exons. Exons 2-6, 7-14, 15, and 16-17 code for complement-type ligand binding domain, EGF precursor homology domain, O-linked glycosylation domain, and membrane spanning and cytoplasmic domains, respectively. Deletion of individual repeats and site-directed mutagenesis causing amino acid substitutions showed that complement-type repeats 3-7 and EGF precursor repeat A were required for the optimal binding of apoB, whereas only complement-type repeat 5 was crucial for apoE binding (5,6) . The three-dimensional structures of recombinant complement repeat 1 and 2 have been solved by NMR spectroscopy and complement repeat 5 by X-ray crystallography. These studies indicate that these repeats form a beta-hairpin structure exposing highly conserved acidic residues on one side of the molecule (7,8). The X-ray structural studies have indicated that conserved acidic residues were not exposed to the surface but were involved in Ca^{+2} binding and raised doubts about the mechanism of interactions between the receptors and various ligands (8). Nonetheless, the majority of biochemical and molecular evidence suggests that various repeats containing a cluster of negative charges, alone or in combination, contribute to specific, high affinity binding of apoE and apoB (for reviews, (2-4).

The major function of the LDL-R is to control plasma cholesterol levels. Mutations in the LDL-R gene cause familial hypercholesterolemia and it is the most common monogenic human disease with a heterozygous frequency of 1 in 500. The homozygotes, expressing nonfunctional receptors, have dramatically increased plasma cholesterol levels, and develop cutaneous

Table 2. Structural and functional motifs in the LDL-R gene family

Receptors	Complement-type repeats	EGF precursor-like repeats	Trans-membrane domains	NPXY sequences
LDL-R	7	3	1	1
VLDL-R	8	3	1	1
ApoE receptor 2	7	3	1	1
LRP	31	21	1	2
Megalin	36	16	1	2
LR11	11		1	1

xanthomas and atherosclerotic plaques at a very early age. The heterozygotes express half of the normal levels of the receptor, have twice the normal plasma cholesterol levels, and also develop tendon xanthomas and atherosclerotic plaques. Mutations in the LDL-R gene have been classified into five classes that affect the synthesis of the receptor, transport to cell surfaces, binding to ligands, clustering of the receptors in the endocytic vesicles, and recycling to the cell surface (for reviews, (2-4)).

3.2. LDL receptor-related protein (LRP)

LRP was the second member of the family identified by Herz et al. (9). It is synthesized as a single polypeptide of 4525 amino acids and is cleaved in the Golgi compartment by furin to produce two subunits of 515- and 85-kDa that remain non-covalently attached to each other (10,11). The larger subunit contains 31 acidic, cysteine-rich complement-type repeats clustered as 2, 8, 10 and 11 repeats in four domains (table 2). This subunit also contains 21 cysteine-rich EGF precursor-like repeats and O-linked glycosylation sites. The 85-kDa subunit is made up of a trans-membrane and a cytoplasmic domain containing two copies of the NPXY sequence that is important for endocytosis. LRP is involved in the metabolism of many structurally diverse proteins, proteinases, protein-lipid complexes, and proteinase-inhibitor complexes (table 3). Several experimental approaches have been used to identify regions in LRP that bind to various ligands (for a detailed discussion and references see (1)). These studies showed that domain 1 does not bind ligands. Domain 2 binds almost all the ligands such as $alpha_2$-M*, uPA:PAI-1, lipoprotein lipase, tPA:PAI-1, apoE, factor VIII, and RAP. Within this domain sub-domains C3-C7 may be important for ligand binding (12). Recently, it has been shown that domain 4 shares ligand-binding properties with domain 2 (12). Thus, different cyteine-rich complement-type repeats act independently and bind different ligands simultaneously and that a complete receptor is unnecessary for ligand binding.

In contrast to the LDL-R, mutations in LRP causing human disorders have not been described. It may indicate that it is essential for fetal development and that mutations may be lethal. In mice, LRP is required for the development of the fetus since deletion of the LRP gene results in the death of fetuses around day 10 (13). The physiological and biochemical reasons for early fetal death are not known. In addition to an important role in development, evidence suggests that LRP and a number of its ligands contribute to the development of Alzheimer's disease. LRP binds and internalizes proteins genetically associated with Alzheimer? s disease, such as certain secreted forms of amyloid precursor protein, apoE (14) and the $alpha_2$-macroglobulin (15,16). Recent genetic evidence suggests that a polymorphism within LRP itself may be associated with the expression of late-onset Alzheimer? s disease. However, molecular basis for the association between LRP polymorphism and Alzheimer? s disease is not known.

3.3. Megalin

It is a single polypeptide of 4,660 amino acids that is primarily expressed in the absorptive epithelial cells (17). The extracellular domain contains 36 cysteine-rich ligand binding and 16 EGF-precursor homology domains containing 40 YWTD repeats in (table 2). There are four ligand binding domains consisting of 7, 8, 10 and 11 complement-type repeats in the molecule that recognize several ligands (table 2). The intracellular domain contains two internalization signal sequences. In addition, it contains Src-homology binding regions, casein kinase II sites and protein kinase phosphorylation sites (18). The cellular expression pattern of the receptor indicates that it plays an important role in the absorption of some molecules by the intestine, re-absorption of proteins and vitamins by the kidney, and transport across the blood-brain barrier (for reviews, (1,18-21)).

Mice lacking megalin die within 2-3 h after birth due to defects in pulmonary inflation and alveolar expansion (22). These mice develop the holoprosencephalic syndrome (abnormal forebrain development, absence of olfactory apparatus etc.) indicating that megalin may be important in the development of the brain. Heymann nephritis in rats is caused by the formation of subepithelial immune deposits in glomerular basement membrane. It occurs due to the binding of circulating antibodies to two antigens, a cell-surface receptor megalin and an endoplasmic reticulum-resident RAP. Binding of antibodies to these antigenic determinants results in intracellular immune-deposits in the glomerular basement membrane, damage to glomerular capillary wall, and proteinuria (for reviews, (18-21).

3.4. VLDL receptor

The VLDL-R is very similar to the LDL-R (23). It contains one extra complement-type repeat than the LDL-R at the N-terminus in the ligand-binding domain (table 2). Not only the structural motifs but also the gene structures of these two receptors are very similar (24). The different functional motifs in the VLDL-R are encoded by different exons similar to that of the LDL-R. Two variants

Table 3. Binding of different ligands to the LDL-R family members

Ligands	LDL-R	LRP	Megalin	VLDL-R	ER-2	LR11	References
Lipoproteins							
Beta-VLDL	Yes	Yes	Yes	Yes	Yes	Yes	(23,35,58.79.81,82)
Chylomicron remnants	Yes	Yes		Yes			(76-80)
IDL	Yes			Yes			(2,23)
Lp(a)				Yes			(86)
VLDL	Yes			Yes	Yes		(2,23,35,83-85)
Apolipoproteins							
ApoB100	Yes		Yes				
ApoE	Yes	Yes	Yes	Yes	Yes	Yes	
Lipases							
Hepatic lipase		Yes					(87,88)
Lipoprotein lipase	Yes	Yes	Yes	Yes			(82,84,89-95)
Protease inhibitors							
Aprotinin		Yes	Yes				(96)
PAI-1		Yes	Yes				(97,98)
Plasminogen			Yes				(99)
Pro-Upa		Yes	Yes	Yes			(90,98,100)
Tissue factor inhibitor		Yes					(101)
TPA		Yes	Yes				(82,102-106)
Protease/inhibitor complexes							
Activated $alpha_2$-macroglobulin		Yes	No				(15,16,58,82,95,107)
$Alpha_1$-chymotrypsin/cathepsin G		No	Yes				(108)
Protease/protein C inhibitor		Yes		Yes			(109,110)
Others							
Albumin			Yes				(111)
ApoJ/clusterin		No	Yes				(94,112)
ApoJ/beta-amyloid peptide			Yes				(112)
Beta-Amyloid precursor protein				Yes			(113)
Circumsporozite protein			Yes	Yes			(114)
Cubulin							(115)
Gentamicin		Yes	Yes				(96)
Lactoferrin		Yes	Yes				(82,116)
Polymyxin B		Yes	Yes				(96)
Pseudomonas exotoxin A		Yes	No				(95,117)
RAP	Yes	Yes	Yes	Yes	Yes	Yes	(15,26,41,58,82,83,85,86,94,105,109,118-122)
Reelin				Yes	Yes		(38,39)
Saposin		Yes					(123)
Seminal vesicle secretory protein II		Yes	Yes				(124)
Thyroglobulin			Yes				(125)
Thrombospondin-1		Yes		Yes			(126,127)

of the receptor differing in molecular weight due to the presence or absence of the serine/threonine-rich O-linked glycosylation motif have been described (24-26). The presence or absence of this O-linked glycosylation site affects the size of the protein but has no effect on ligand binding properties (26). The tissue expression of the VLDL-R is very different from that of the expression of the LDL-R (table 3). 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, (1,27-29)).

Disruption of the VLDL-R gene in mice has no significant effect on fertility and lipoprotein catabolism in mice (30). However, it results in modest decrease in the whole body and adipose tissue mass. In contrast, mutations in the chicken homologue of this receptor result in female sterility. Chickens do not lay eggs because of the failure in the deposition of egg yolk proteins. These birds also develop severe hyperlipidemia associated with premature atherosclerosis (31). VLDL-R is a good candidate for hepatic gene therapy to control hyperlipidemias because it is not expressed in the liver and is not expected to be immunogenic. 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 (32,33). Similarly, VLDL-R expression in the livers of apoE deficient mice expressing E2 and apoE-Leiden resulted in decreased plasma cholesterol levels (34). Thus, hepatic expression of this receptor may be useful in controlling hyperlipidemias.

Table 4. Expression of LDL-R family members in different tissues

Tissues	LDL-R	LRP	Megalin	VLDL-R	ER2	LR11
Liver	+++	+++	_	_	_	++
Brain	+	+++	_	+	+++	+++
Heart	+		_	+++	_	
Intestine	+	+				
Kidney	+	+	+++	+	_	
Muscle	+		_	+++		
Adipose				+++		
Adrenal	+++	++	_	+		+
Lung	+	+++	++	+	_	
Placenta	+	+	+	+	++	
Ovary	+	+	+	++	+	++
Testis	+	+	_	++	+	++
References	(25,35,128)	(9,129-131)	(17,131)	(23,25,35,128)	(35,36)	(40,41)

The relative abundance (+++>++>+) of different receptor mRNAs. Absence of detection is represented by "_".

3.5. Apo E receptor 2 (ER2)

The human apoE receptor 2 (apoER2) contains seven ligand-binding repeats and is mainly found in the brain (35,36). The ligand binding complement-type cysteine-rich repeats are grouped very similar to that observed in the VLDL-R and are different from those observed in the LDL-R. Furthermore, the ER2 is closer to VLDL-R than LDL-R with respect to sequence homology and restricted tissue expression (table 4). Thus, it may bind several ligands as the VLDL-R (table 3). The EGF precursor homology with YWTD repeats, O-linked glycosylation motif, membrane anchoring region, and cytoplasmic domain with one internalization signal are similar to those present in the LDL-R and VLDL-R.

Recently, VLDL-R and apoER2 have been shown to play an important role in developmental neurobiology. Reelin is a large extracellular protein produced by the Cajal-Retzius cells in the developing brain. It is produced in specific regions that control neuronal migration and positioning in the cerebral cortex and cerebellum (37). Reelin has recently been shown to bind both the VLDL-R and apoER2 (38,39). This binding results in increased intracellular phosphorylation of Disabled-1 protein in cultured primary neurons (38). Phosphorylated Disabled-1 is known to interact with non-receptor tyrosine kinases of the Abl and Src family. Thus, reelin may signal via VLDL-R and apoER2 to control neuronal migration and positioning during development by recruiting intracellular Disabled-1 to these receptors and initiating a signaling cascade.

3.6. LR11

RAP-affinity chromatography and homology cloning resulted in the identification of LR11 or SORLA-1 (40,41). It is a hybrid receptor consisting of a ligand-binding domain of 11 complement-type repeats, 5 YWTD repeats, 1 membrane spanning region, and 1 cytoplasmic domain containing an internalization signal. It lacks EGF precursor homology repeats, but instead contains domains homologous to the vacuolar sorting receptor of yeast and fibronectin type III repeats observed in adhesion molecules. The presence of fibronectin type III repeats indicate that it may play a role in cell-cell interactions especially between neurons (40-43).

3.7. Other receptors

Some other related receptors that have been shown to contain ligand binding repeats are LRP3, LRP6, and LRP7 (44-48) . Petersen *et al* have isolated and characterized a novel receptor that binds to RAP but does not appear to share sequence homology with the LDL-R family members (49). The receptor has been called Sortilin and appears to bind RAP, lipoprotein lipase and neurotensin (49,50).

4. ENDOCYTIC PROPERTIES OF THE LDL-R GENE FAMILY

4.1. Ligands of the LDL-R gene family

Different receptors of the LDL-R gene family recognize several ligands. Similarly, different ligands are recognized by multiple receptors (table 3). However, each receptor appears to have a unique ligand (table 3). For example, alpha$_2$-macroglobulin only binds to LRP and this binding is inhibited by nickel (51-53). ApoJ may be a specific ligand for megalin. So far, apoB100 has been shown to bind to the LDL-R and megalin only (2,54). Molecular and biochemical bases for these specific interactions are not known.

RAP and apoE are very dissimilar in their amino acid sequences and yet both of these proteins are recognized by all of the family members (tables 1 & 3). RAP is a chaperone that assists in the proper folding of LRP and prevents premature intracellular binding of ligands to the receptor (55-57). Remarkably, it binds to all the receptors and acts as a potent inhibitor for the binding of all ligands to all the receptors probably because of its small size, compact structure and its ability to bind receptors at multiple sites (58,59). On the other hand, apoE is a secreted protein that acts as a ligand (tables 1 & 3) after associating with lipids or lipoproteins (60). It has a single receptor-binding domain and does not compete very well for the binding of other ligands. There are three apoE alleles that give rise to three different charged isoforms. Only the LDL-R distinguishes between these different isoforms. The binding affinities of the receptor for these isoforms are in the order of apoE4>apoE3>>apoE2 (60). The poor binding of apoE2 to the LDL-R results in type III hyperlipoproteinemia (61). Other receptors in the family do

not differentiate between these isoforms and bind all the isoforms.

Most of the ligands recognized by the receptors exist in at least two structural conformations, and only one of these conformations is recognized by the receptors. This is especially true for apoE, alpha$_2$-macroglobulin, and serine proteinase inhibitors (serpins). ApoE exists either free or associated with lipoproteins in plasma. The LDL-R family members recognize apoE that is bound to lipids or lipoproteins and do not recognize lipid-free apoE (60). Alpha$_2$-macroglobulin is a large plasma proteinase inhibitor that reacts with all major classes of proteinases (for reviews, (62,63)). The native form of alpha$_2$-macroglobulin is not recognized by LRP. When native alpha$_2$-macroglobulin interacts with different proteinases, these proteinases cleave the ?bait? region in the native alpha$_2$-macroglobulin molecule and induce a conformational change that results in the exposure of binding sites recognizable by LRP. Like alpha$_2$-macroglobulin, the serpins are substrates for various proteinases and various proteinases cleave an exposed loop region in these proteins. The LDL-R gene family members recognize serpin complexes containing inactive proteinases. PAI-1 differs from the other serpins and exists in four different forms: native or active, latent, cleaved, and the proteinase complexed form. The LDL-R family recognizes PAI-1 complexed to a proteinase, such as the uPA:PAI-1 complex. Thus, the receptor-binding domain in the native ligands is either buried or does not exist. Conformational changes in these ligands help expose a site on the surface of the molecule that is recognized by these receptors and these conformational changes signal for their removal from plasma.

Different ligands that bind to the LDL-R family members do not share significant sequence homology. However, all the ligands bind to heparin and contain basic domains rich in arginine and lysine residues. Several mutagenesis experiments have shown that positively charged basic amino acids are critical for receptor binding. Attempts have been made to derive consensus primary sequences that might constitute receptor-binding domains (1,64). There is very little sequence homology between different receptor-binding domains. Thus, it has been proposed that secondary or tertiary structural motifs rich in positive electrostatic surface potential may constitute a receptor-binding domain in different ligands (1). Proteins of different primary sequences may fold to expose a similar secondary or tertiary cluster of charges. The clusters of the basic amino acids may contribute to the positive electrostatic potential on the surface of these molecules and these positive potentials can form various contacts with an area of negative electrostatic potential present on the surface of receptor molecules.

4.2. Endocytosis of different ligands

Receptors and ligands bind to each other with high affinity. High affinity binding indicates that receptors can recognize ligands present at low concentrations (# 10^{-8} M). All the LDL-R family members display negative charges while ligands display positive charges on their surfaces. Thus, high affinity binding may occur due to multiple ionic interactions between receptors and ligands. Members of the LDL-R family recognize several structurally dissimilar ligands (table 3). Since the VLDL-R binds to a variety of ligands (table 3), a single ligand-binding domain of 8 complement-type repeats found in this receptor may be sufficient for binding to different ligands.

LDL-R family members are recycled whether or not they are associated with ligands. If different proteins, lipoproteins, or protein-protein complexes are bound to these receptors then these molecules are taken into cells by a process called "receptor-mediated endocytosis? (table 1). In intracellular compartments, ligands dissociate from receptors and ligands are transferred to lysosomes for degradation whereas receptors are recycled back to cell-surface. The process of receptor-mediated endocytosis involves the extraction of ligands from circulation onto the cell-surface and subsequent internalization of receptor-ligand complexes. LDL-R family members can perform both of these functions. For example, LDL-R extracts LDL from plasma, immobilizes it at least transiently onto cell-surface, and subsequently internalizes it for degradation. Similarly, LRP binds alpha$_2$-macroglobulin/proteinase complexes and internalizes them for intracellular degradation. In other instances, family members cooperate with other cell-surface proteins in the endocytic process. In these instances, other cell-surface proteins extract ligands and immobilize them onto the cell-surfaces. This allows further modification of the ligands on the cell-surface. Subsequently, these ligands are delivered to the LDL-R family members for the final internalization and delivery to lysosomes. It is known that remnant lipoproteins bind proteoglycans. The proteoglycan-immobilized remnant lipoproteins are probably further enriched with apoE. The apoE-enriched remnant lipoproteins are then transferred to LRP for internalization and degradation (for reviews, (65)).

An endocytic process originates at specialized regions in the inner surface of the plasma membrane called coated pits. The assembly of coated pits occurs by the binding of dephosphorylated AP-2 (adaptor protein) complexes to the plasma membrane, recruitment of clathrin from the cytosol, and formation of clathrin-coated vesicles. Different receptors from other regions of the plasma membrane are recruited to the coated region for internalization. The recruitment of LDL-R family members involves the binding of the FDNPXY motifs with the terminal domains of the clathrin heavy chain (66). The coated pits are pinched off from the membrane as coated vesicles in an energy-dependent process mediated by dynamin. The coated vesicles undergo rapid uncoating in the cytoplasm and contents from these uncoated vesicles are delivered to endosomes by a docking and fusing mechanism involving v- and t-SNAREs. In the endosomes, slightly acidic pH facilitates the dissociation of ligands from receptors, and receptors and ligands are separated into two different compartments. In this process, lumenal contents accumulate in vesicular regions and receptors accumulate in the tubular extensions of endosomes. The tubular extensions with receptors are recycled to the plasma membrane and vesicles fuse with late endosomes or

prelysosomes containing enzymatically active lysosomal hydrolases that degrade the ligands (for reviews, (67-71)).

5. SIGNALING BY THE LDL-R GENE FAMILY IN BRAIN

As discussed above an important function of the LDL-R gene family is to bring extracellular ligands into cells for degradation. The NPXY motif present in the intracellular domains of these receptors is critical for the endocytic function. The NPXY motif is also found in other receptors that are not involved in endocytosis but are involved in signaling. For example, epidermal growth factor receptor contains NPXY sequence in which the tyrosine residue is phosphorylated after the receptor binds to its ligand. The phosphorylated NPXY motif then binds to protein interacting domains present in *Shc* protein. *Shc* is an adapter protein that interacts with *ras* signaling pathway.

Trammsdorf *et al.* (72) have demonstrated that the unphosphorylated NPXY motifs of the LDL-R gene family interact with protein interacting domains in FE65 and mammalian Diabled-1 proteins present in the neurons. FE65 contains two protein interacting domains: FE65N and FE65C. The FE65N interacts with the NPXY motif in the LDL-R gene family but the FE65C does not. FE65C, however, interacts with other proteins. In fact, it has been shown to interact with the beta-amyloid precursor protein. Thus, it is possible that the binding of FE65 with the LDL-R family members and other proteins may facilitate the endocytosis of other proteins along with the endocytic receptors.

The mammalian Disabled-1 is a cytoplasmic protein that contains one protein interacting domain and acts as an adapter protein in phosphorylation dependent intracellular signal transduction (73). The protein interacting domain of Disabled-1 interacts with NPXY motif in the LDL-R gene family (72). Disbaled-1 is known to undergo phosphorylation and several proteins that contain SH2 domains bind to the phosphorylated Disabled-1. This binding plays an important role in the amplification of the signals. Mutations in Disabled-1 result in the disruption of the migration of neurons during development in scrambler mice (73). The extracellular signal for the activation of Disabled-1 appears to be the binding of reelin to cell surface receptors (37). Reelin is a large extracellular matrix protein synthesized and secreted by Cajal-Retzius cells in the forebrain and by granule neurons in the cerebellum (37,73). Autosomal recessive mutations in reelin in reeler mice result in widespread disruption of laminar structure throughout the brain. The phenoype is very similar to that observed in scrambler mice with defects in Disabled-1. Surprisingly, similar phenotype is also observed in mice that lack functional VLDL-R and apoER2 receptors (74). Furthermore, reelin has been shown to bind VLDL-R and apoER2 (38,75). It is conceivable that reelin binds to the extracellular ligand binding domains of the VLDL-R and apoER2 receptors and this binding result in the recruitment of Disabled-1 to the NPXY motifs in the intracellular domains of these receptors. The NPXY-anchored Disbaled-1 is then phosphorylated and interacts with other proteins that contain SH2 domains. These interactions may amplify signaling pathways that are crucial for the proper migration and growth of neurons. Thus, reelin, Disbaled-1, VLDL-R and apoER2 may form an integrated signaling cascade that is crucial for the proper migration and layering of the neurons during brain development.

6. CONCLUDING REMARKS

Significant information concerning the structural and functional motifs in different receptors of the LDL-R family has been obtained. Since, these receptors recognize structurally dissimilar ligands they play significant and very diverse physiological processes such as fetal development, brain development, cholesterol homeostasis, cellular migration, immune response, and protease clearance from the plasma. Furthermore, they have been implicated in atherosclerosis and neurodegenerative diseases. Studies with these receptors have helped understand receptor-ligand binding and endocytic processes. New studies are providing fascinating new information about the role of these receptors in signaling and fetal development. It is anticipated that the knowledge may be useful in the control of atherosclerosis and neurologic disorders.

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8. REFERENCES

1. Hussain MM, DK Strickland and A Bakillah:The mammalian low-density lipoprotein receptor family. *Annu Rev Nutr* 19, 141-172 (1999)
2. Brown MS and JL Goldstein: A receptor-mediated pathway for cholesterol homeostasis. *Science* 232, 34-47 (1986)
3. Hobbs HH, DW Russell, MS Brown and JL Goldstein: The LDL receptor locus in familial hypercholesterolemia: mutational analysis of a membrane protein. *Annu Rev Genet* 24, 133-170 (1990)
4. Goldstein JL, HH Hobbs and MS Brown: Familial hypercholesterolemia. In The metabolic and molecular bases of inherited disorders. Eds: Scriver CR, Beaudet AL, Sly WS and Valle D, McGraw-Hill, Inc., New York (1995)
5. Esser V, LE Limbird, MS Brown, JL Goldstein and DW Russell: Mutational analysis of the ligand binding domain of the low density lipoprotein receptor. *J Biol Chem* 263, 13282-13290 (1988)
6. Russell DW, MS Brown and JL Goldstein: Different combinations of cysteine-rich repeats mediate binding of low density lipoprotein receptor to two different proteins. *J Biol Chem* 264, 21682-21688 (1989)
7. Daly NL, MJ Scanlon, JT Djordjevic, PA Kroon and R Smith: Three-dimensional structure of a cysteine-rich repeat

from the low-density lipoprotein receptor. *Proc Natl Acad Sci U.S.A.* 92, 6334-6338 (1995)
8. Fass D, S Blacklow, PS Kim and JM Berger: Molecular basis of familial hypercholesterolaemia from structure of LDL receptor module. *Nature* 388, 691-693 (1997)
9. Herz J, U Hamann, S Rogne, O Myklebost, H Gausepohl and KK Stanley: Surface location and high affinity for calcium of a 500-kd liver membrane protein closely related to the LDL-receptor suggest a physiological role as lipoprotein receptor. *EMBO J* 7, 4119-4127 (1988)
10. Herz J, RC Kowal, JL Goldstein and MS Brown: Proteolytic processing of the 600 kd low density lipoprotein receptor-related protein (LRP) occurs in a trans-Golgi compartment. *EMBO J.* 9, 1769-1776 (1990)
11. Willnow TE, JM Moehring, NM Inocencio, TJ Moehring and J Herz: The low-density-lipoprotein receptor-related protein (LRP) is processed by furin *in vivo* and *in vitro*. *Biochem J.* 313, 71-76 (1996)
12. Neels JG, BM van Den Berg, A Lookene, G Olivecrona, H Pannekoek and AJ van Zonneveld: The second and fourth cluster of class A cysteine-rich repeats of the low density lipoprotein receptor-related protein share ligand-binding properties. *J Biol Chem* 274, 31305-31311 (1999)
13. Herz J, DE Clouthier and RE Hammer: LDL receptor-related protein internalizes and degrades uPA-PAI-1 complexes and is essential for embryo implantation. *Cell* 71, 411-421 (1992)
14. Beisiegel U, W Weber, G Ihrke, J Herz and KK Stanley: The LDL-receptor-related protein, LRP, is an apolipoprotein E-binding protein. *Nature* 341, 162-164 (1989)
15. Strickland DK, JD Ashcom, S Williams, WH Burgess, M Migliorini and WS Argraves: Sequence identity between the a_2-macroglobulin receptor and low density lipoprotein receptor-related protein suggests that this protein is a multifunctional receptor. *J Biol Chem* 265, 17401-17404 (1990)
16. Kristensen T, SK Moestrup, J Gliemann, L Bendtsen, O Sand and L Sottrup-Jensen: Evidence that the newly cloned low-density-lipoprotein receptor related protein (LRP) is the a_2-macroglobulin receptor. *FEBS Lett.* 276, 151-155 (1990)
17. Saito A, S Pietromonaco, AKC Loo and MG Farquhar: Complete cloning and sequencing of rat gp330/"megalin," a distinctive member of the low density lipoprotein receptor gene family. *Proc Natl Acad Sci USA* 91, 9725-9729 (1994)
18. Christensen EI, H Birn, P Verroust and SK Moestrup: Membrane receptors for endocytosis in the renal proximal tubule. *Int Rev Cytol* 180, 237-284 (1998)
19. Farquhar MG, A Saito, D Kerjaschki and RA Orlando: The Heymann nephritis antigenic complex: megalin (gp330) and RAP. *J Am Soc Nephrol* 6, 35-47 (1995)
20. Kerjaschki D and TJ Neale: Molecular mechanisms of glomerular injury in rat experimental membranous nephropathy (Heymann nephritis) *J Am Soc Nephrol* 7, 2518-2526 (1996)
21. Farquhar MG, D Kerjaschki, M Lundstrom and RA Orlando: gp330 and RAP: The Heymann nephritis antigenic complex: *Ann NY Acad Sci* 737, 96-113 (1994)
22. Willnow TE, J Hilpert, SA Armstrong, A Rohlmann, RE Hammer, DK Burns and J Herz: Defective forebrain development in mice lacking gp330/megalin. *Proc Natl Acad Sci USA* 93, 8460-8464 (1996)
23. Takahashi S, Y Kawarabayasi, T Nakai, J Sakai and T Yamamoto: Rabbit very low density lipoprotein receptor: A low density lipoprotein receptor-like protein with distinct ligand specificity. *Proc Natl Acad Sci USA* 899, 9252-9256 (1992)
24. Sakai J, A Hoshino, S Takahashi, Y Miura, H Ishii, H Suzuki, Y Kawarabayasi and T Yamamoto: Structure, chromosome location, and expression of the human very low density lipoprotein receptor gene. *J Biol Chem* 269, 2173-2182 (1994)
25. Webb JC, DD Patel, MD Jones, BL Knight and AK Soutar: Characterization and tissue-specific expression of the human `very low density lipoprotein (VLDL) receptor' mRNA. *Hum Mol Genet* 3, 531-537 (1994)
26. Martensen PM, K Oka, L Christensen, PM Rettenberger, HH Petersen, A Christensen, L Chan, CW Heegaard and PA Andreasen: Breast carcinoma epithelial cells express a very low-density lipoprotein receptor variant lacking the O-linked glycosylation domain encoded by exon 16, but with full binding activity for serine proteinase serpin complexes and M_r-40000 receptor- associated protein. *Eur J Biochem* 248, 583-591 (1997)
27. Yamamoto T, S Takahashi, J Sakai and Y Kawarabayasi: The very low density lipoprotein receptor: A second lipoprotein receptor that may mediate uptake of fatty acids into muscle and fat cells. *Trends Cardiovas Med* 3, 144-148 (1993)
28. Jingami H and T Yamamoto: The VLDL receptor: wayward brother of the LDL receptor. *Curr Opin Lipidol* 6, 104-108 (1995)
29. Yamamoto T and H Bujo: Close encounters with apolipoprotein E receptors. *Curr Opin Lipidol* 7, 298-302 (1996)
30. Frykman PK, MS Brown, T Yamamoto, JL Goldstein and J Herz: Normal plasma lipoproteins and fertility in gene-targeted mice homozygous for a disruption in the gene encoding very low density lipoprotein receptor. *Proc Natl Acad Sci USA* 92, 8453-8457 (1995)
31. Bujo H, T Yamamoto, K Hayashi, M Hermann, J Nimpf and WJ Schneider: Mutant oocytic low density lipoprotein receptor gene family member causes atherosclerosis and female sterility. *Proc Natl Acad Sci USA* 92, 9905-9909 (1995)
32. Kobayashi K, K Oka, T Forte, B Ishida, BB Teng, K Ishimura-Oka, M Nakamuta and L Chan: Reversal of hypercholesterolemia in low density lipoprotein receptor knockout mice by adenovirus-mediated gene transfer of the very low density lipoprotein receptor. *J Biol Chem* 271, 6852-6860 (1996)
33. Kozarsky KF, K Jooss, M Donahee, JF Strauss and JM Wilson: Effective treatment of familial hypercholesterolaemia in the mouse model using adenovirus-mediated transfer of the VLDL receptor gene. *Nat Genet* 13, 54-62 (1996)
34. Dijk KW Van, BJ van Vlijmen, A van der Zee, B van't Hof, H van der Boom, K Kobayashi, L Chan, LM Havekes and MH Hofker: Reversal of hypercholesterolemia in apolipoprotein E2 and apolipoprotein E3-Leiden transgenic mice by adenovirus-mediated gene transfer of the VLDL receptor. *Arterioscler Thromb Vasc Biol* 18, 7-12 (1998)
35. Kim DH, H Iijima, K Goto, J Sakai, H Ishii, HJ Kim, H Suzuki, H Kondo, S Saeki and T Yamamoto: Human apolipoprotein E receptor 2 - A novel lipoprotein receptor of the low density lipoprotein receptor family predominantly expressed in brain. *J Biol Chem* 271, 8373-8380 (1996)

36. Novak S, T Hiesberger, WJ Schneider and J Nimpf: A new low density lipoprotein receptor homologue with 8 ligand binding repeats in brain of chicken and mouse. *J Biol Chem* 271, 11732-11736 (1996)
37. Curran T and G D'Arcangelo: Role of reelin in the control of brain development. *Brain Res Brain Res Rev* 26, 285-294 (1998)
38. Hiesberger T, M Trommsdorff, BW Howell, A Goffinet, MC Mumby, JA Cooper and J Herz: Direct binding of Reelin to VLDL receptor and ApoE receptor 2 induces tyrosine phosphorylation of disabled-1 and modulates tau phosphorylation. *Neuron* 24, 481-489 (1999)
39. D'Arcangelo G, R Homayouni, L Keshvara, DS Rice, M Sheldon and T Curran: Reelin is a ligand for lipoprotein receptors. *Neuron* 24, 471-479 (1999)
40. Yamazaki H, H Bujo, J Kusunoki, K Seimiya, T Kanaki, N Morisaki, WJ Schneider and Y Saito: Elements of neural adhesion molecules and a yeast vacuolar protein sorting receptor are present in a novel mammalian low density lipoprotein receptor family member. *J Biol Chem* 271, 24761-24768 (1996)
41. Jacobsen L, P Madsen, SK Moestrup, AH Lund, N Tommerup, A Nykjaer, L Sottrup-Jensen, J Gliemann and CM Petersen: Molecular characterization of a novel human hybrid-type receptor that binds the alpha2-macroglobulin receptor-associated protein. *J Biol Chem* 271, 31379-31383 (1996)
42. Morwald S, H Yamazaki, H Bujo, J Kusunoki, T Kanaki, K Seimiya, N Morisaki, J Nimpf, WJ Schneider and Y Saito: A novel mosaic protein containing LDL receptor elements is highly conserved in humans and chickens. *Arterioscler Thromb Vasc Biol* 17, 996-1002 (1997)
43. Schneider WJ, J Nimpf and H.Bujo: Novel members of the low density lipoprotein receptor superfamily and their potential roles in lipid metabolism. *Curr Opin Lipidol* 8, 315-319 (1997)
44. Ishii H, DH Kim, T Fujita, Y Endo, S Saeki and TT Yamamoto: cDNA cloning of a new low-density lipoprotein receptor-related protein and mapping of its gene (LRP3) to chromosome bands 19q12-q13.2. *Genomics* 51, 132-135 (1998)
45. Brown SD, RCJ Twells, PJ Hey, RD Cox, ER Levy, AR Soderman, ML Metzker, CT Caskey, JA Todd and JF Hess: Isolation and characterization of *LRP6*, a novel member of the low density lipoprotein receptor gene family. *Biochem Biophys Res Commun* 248, 879-888 (1998)
46. Hey PJ, RCJ Twells, MS Philips, Y Nakagawa, SD Brown, Y Kawaguchi, R Cox, G Xie, V Dugan, H Hammond, ML. Metzker, JA Todd and JF Hess: Cloning of a novel member of the low-density lipoprotein receptor family. *Gene* 216, 103-111 (1998)
47. Dong Y, W Lathrop, D Weaver, Q Qiu, J Cini, D Bertolini and D Chen: Molecular cloning and characterization of LR3, a novel LDL receptor family protein with mitogenic activity. *Biochem Biophys Res Commun* 251, 784-790 (1998)
48. Chen D, W Lathrop and Y Dong: Molecular cloning of mouse Lrp7(Lr3) cDNA and chromosomal mapping of orthologous genes in mouse and human. *Genomics* 55, 314-321 (1999)
49. Petersen CM, MS Nielsen, A Nykjaer, L Jacobsen, N Tommerup, HH Rasmussen, H Roigaard, J Gliemann, P Madsen and SK Moestrup: Molecular identification of a novel candidate sorting receptor purified from human brain by receptor-associated protein affinity chromatography. *J Biol Chem* 272, 3599-3605 (1997)
50. Nielsen MS, C Jacobsen, G Olivecrona, J Gliemann and CM Petersen: Sortilin/neurotensin receptor-3 binds and mediates degradation of lipoprotein lipase. *J Biol Chem* 274, 8832-8836 (1999)
51. Hussain MM, RK. Kancha and TN Tulenko: Nickel is a specific inhibitor for the binding of activated a $_2$-macroglobulin to the low density lipoprotein receptor-related protein/a_2-macroglobulin receptor. *Biochemistry* 34, 16074-16081 (1995)
52. Kancha RK and MM Hussain: Nickel is a specific antagonist for the catabolism of activated a_2-macroglobulin. *Biochim Biophys Acta* 1355, 231-240 (1997)
53. Odom AR, UK Misra and SV Pizzo: Nickel inhibits binding of a_2-macroglobulin-methylamine to the low-density lipoprotein receptor-related protein/a_2- macroglobulin receptor but not the a_2-macroglobulin signaling receptor. *Biochemistry* 36, 12395-12399 (1997)
54. Stefansson S, DA Chappell, KM Argraves, DK Strickland and WS Argraves: Glycoprotein 330/low density lipoprotein receptor-related protein-2 mediates endocytosis of low density lipoproteins via interaction with apolipoprotein B100. *J Biol Chem* 270, 19417-19421 (1995)
55. Bu G, HJ Geuze, GJ Strous and AL Schwartz: 39 kDa receptor-associated protein is an ER resident protein and molecular chaperone for LDL receptor-related protein. *EMBO J* 14, 2269-2280 (1995)
56. Willnow TE, A Rohlmann, J Horton, H Otani, JR Braun, RE Hammer and J Herz: RAP, a specialized chaperone, prevents ligand-induced ER retention and degradation of LDL receptor-related endocytic receptors. *EMBO J* 15, 2632-2639 (1996)
57. Bu G and S Rennke: Receptor-associated protein is a folding chaperone for low density lipoprotein receptor-related protein. *J Biol Chem* 271, 22218-22224 (1996)
58. Herz J, JL Goldstein, DK Strickland, YK Ho and MS Brown: 39-kDa protein modulates binding of ligands to low density lipoprotein receptor-related protein/a_2-macroglobulin receptor. *J Biol Chem* 266, 21232-21238 (1991)
59. Bu G: Receptor-associated protein: a specialized chaperone and antagonist for members of the LDL receptor gene family. *Curr Opin Lipidol* 9, 149-155 (1998)
60. Mahley RW: Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science* 240, 622-630 (1988)
61. Mahley RW and CR Stanley, Jr.: Type III hyperlipoproteinemia (dysbetalipoproteinemia): The role of apolipoprotein E in normal and abnormal lipoprotein metabolism. In: The metabolic and molecular bases of inherited disease. Eds: Scriver CR, Beaudet AL, Sly WS and Valle D, McGraw-Hill, Inc, New York (1995)
62. Chu CT and SV Pizzo: alpha(2)-Macroglobulin, complement, and biologic defense: Antigens, growth factors, microbial proteases, and receptor ligation. *Lab Invest* 71, 792-812 (1994)
63. Sottrup-Jensen L: a-Macroglobulins: Structure, shape, and mechanism of proteinase complex formation. *J Biol Chem* 264, 11539-11542 (1989)
64. Nielsen KL, TL Holtet, M Etzerodt, SK Moestrup, J Gliemann, L Sottrupjensen and HC Thogersen: Identification of residues in alpha-macroglobulins important for binding to the alpha(2)-macroglobulin receptor low density lipoprotein

receptor-related protein. *J Biol Chem* 271, 12909-12912 (1996)
65. Mahley RW and ZS Ji: Remnant lipoprotein metabolism: Key pathways involving cell-surface heparan sulfate proteoglycans and apolipoprotein E. *J Lipid Res* 40, 1-16 (1999)
66. Kibbey RG, J Rizo, LM Gierasch and RGW Anderson: The LDL receptor clustering motif interacts with the clathrin terminal domain in a reverse turn conformation. *J Cell Biol* 142, 59-67 (1998)
67. Schmid SL: Clathrin-coated vesicle formation and protein sorting: an integrated process. *Annu Rev Biochem* 66, 511-548 (1997)
68. Mellman I: Endocytosis and molecular sorting. *Ann Rev Cell Dev Biol* 12, 575-625 (1996)
69. Smythe E: Endocytosis. *Sub-Cellular Biochemistry* 27, 51-92 (1996)
70. Mukherjee S, RN Ghosh and FR Maxfield: Endocytosis. *Physiol Rev* 77, 759-803 (1997)
71. Rothman JE and FT Wieland: Protein sorting by transport vesicles. *Science* 272 , 227-234 (1996)
72. Trommsdorff M, JP. Borg, B Margolis and J Herz: Interaction of cytosolic adaptor proteins with neuronal apolipoprotein E receptors and the amyloid precursor protein. *J Biol Chem* 273, 33556-33560 (1998)
73. Rice DS, M Sheldon, G D'Arcangelo, K Nakajima, D Goldowitz and T Curran: Disabled-1 acts downstream of Reelin in a signaling pathway that controls laminar organization in the mammalian brain. *Development* 125, 3719-3729 (1998)
74. Trommsdorff M, M Gotthardt, T Hiesberger, J Shelton, W Stockinger, J Nimpf, RE Hammer, JA Richardson and J Herz: Reeler/Disabled-like disruption of neuronal migration in knockout mice lacking the VLDL receptor and ApoE receptor 2. *Cell* 97, 689-701 (1999)
75. Gotthardt M, M Trommsdorff, MF Nevitt, J Shelton, JA Richardson, W Stockinger, J Nimpf and J Herz: Interactions of the low density lipoprotein (LDL) receptor gene family with cytosolic adaptor and scaffold proteins suggest diverse biological functions in cellular communication and signal transduction. *J Biol Chem* (2000)
76. Hussain MM, FR Maxfield, J Mas-Oliva, I Tabas, J Zhong-sheng, TL Innerarity and RW Mahley: Clearance of chylomicron remnants by the low density lipoprotein receptor-related protein/a_2-macroglobulin receptor. *J Biol Chem* 266, 13936-13940 (1991)
77. Mokuno H, S Brady, L Kotite, J Herz and RJ Havel: Effect of the 39-kDa receptor-associated protein on the hepatic uptake and endocytosis of chylomicron remnants and low density lipoproteins in the rat. *J Biol Chem* 269, 13238-13243 (1994)
78. Willnow TE, ZQ Sheng, S Ishibashi and J Herz: Inhibition of hepatic chylomicron remnant uptake by gene transfer of a receptor antagonist. *Science* 264, 1471-1474 (1994)
79. Hussain MM, RK Kancha, Z Zhou, J Luchoomun, H Zu and A Bakillah: Chylomicron assembly and catabolism: role of apolipoproteins and receptors. *Biochim Biophys Acta* 1300, 151-170 (1996)
80. Niemeier A, M Gåfvels, J Heeren, N Meyer, B Angelin and U Beisiegel: VLDL receptor mediates the uptake of human chylomicron remnants in vitro. *J Lipid Res* 37, 1733-1742 (1996)
81. Kowal RC, J Herz, JL Goldstein, v Esser and MS Brownz: Low density receptor-related protein mediates uptake of cholesteryl esters derived from apolipoprotein E-enriched lipoproteins. *Proc Natl Acad Sci USA* 86, 5810-5814 (1989)
82. Willnow TE, JL Goldstein, K Orth, MS Brown and J Herz: Low density lipoprotein receptor-related protein and gp330 bind similar ligands, including plasminogen activator-inhibitor complexes and lactoferrin, an inhibitor of chylomicron remnant clearance. *J Biol Chem* 267, 26172-26180 (1992)
83. Battey FD, ME Gafvels, DJ Fitzgerald, WS Argraves, DA Chappell, JF Strauss and DK Strickland: The 39-kDa receptor-associated protein regulates ligand binding by the very low density lipoprotein receptor. *J Biol Chem* 269, 23268-23273 (1994)
84. Takahashi S, J Suzuki, M Kohno, K Oida, T Tamai, S Miyabo, T Yamamoto and T Nakai: Enhancement of the binding of triglyceride-rich lipoproteins to the very low density lipoprotein receptor by apolipoprotein E and lipoprotein lipase. *J Biol Chem* 270, 15747-15754 (1995)
85. Medh JD, GL Fry, SL Bowen, MW Pladet, DK Strickland and DA Chappell: The 39-kDa receptor-associated protein modulates lipoprotein catabolism by binding to LDL receptors. *J Biol Chem* 270, 536-540 (1995)
86. Argraves KM, KF Kozarsky, JT Fallon, PC Harpel and DK Strickland: The atherogenic lipoprotein Lp(a) is internalized and degraded in a process mediated by the VLDL receptor. *J Clin Invest* 100, 2170-2181 (1997)
87. Kounnas MZ, DA Chappell, H Wong, WS Argraves and DK Strickland: The cellular internalization and degradation of hepatic lipase is mediated by low density lipoprotein receptor- related protein and requires cell surface proteoglycans. *J Biol Chem* 270, 9307-9312 (1995)
88. Krapp A, S Ahle, S Kersting, Y Hua, K Kneser, M Nielsen, J Gliemann and U Beisiegel: Hepatic lipase mediates the uptake of chylomicrons and beta-VLDL into cells via the LDL receptor-related protein (LRP) *J Lipid Res* 37, 926-936 (1996)
89. Medh JD, SL Bowen, GL Fry, S Ruben, M Andracki, I Inoue, JM Lalouel, DK Strickland and DA Chappell: Lipoprotein lipase binds to low density lipoprotein receptors and induces receptor-mediated catabolism of very low density lipoproteins in vitro. *J Biol Chem* 271, 17073-17080 (1996)
90. Argraves KM, FD Battey, CD Maccalman, KR McCrae, ME Gafvels, KF Kozarsky, DA Chappell, JF Strauss and DK Strickland: The very low density lipoprotein receptor mediates the cellular catabolism of lipoprotein lipase and urokinase- plasminogen activator inhibitor type I complexes. *J Biol Chem* 270, 26550-26557 (1995)
91. Beisiegel U, W Weber and G Bengtsson-Olivecrona: Lipoprotein lipase enhances the binding of chylomicrons to low density lipoprotein receptor-related protein. *Proc Natl Acad Sci USA* 88, 8342-8346 (1991)
92. Nykjær A, G Bengtsson-Olivecrona, A Lookene, SK Moestrup, CM Petersen, W Weber, U Beisiegel and J Gliemann: The a_2-microglobulin receptor/low density lipoprotein receptor-related protein binds lipoprotein lipase

and b- migrating very low density lipoprotein associated with the lipase. *J Biol Chem* 268, 15048-15055 (1993)
93. Chappell DA, GL Fry, MA Waknitz, PH Iverius, SE Williams and DK Strickland: The low density lipoprotein receptor-related protein/a_2-macroglobulin receptor binds and mediates catabolism of bovine milk lipoprotein lipase. *J Biol Chem* 267, 25764-25767 (1992)
94. Kounnas MZ, EB Loukinova, S Stefansson, JAK Harmony, BH Brewer, DK Strickland and WS Argraves: Identification of glycoprotein 330 as an endocytic receptor for apolipoprotein J/clusterin. *J Biol Chem* 270, 13070-13075 (1995)
95. Kounnas MZ, DA Chappell, DK Strickland and WS Argraves: Glycoprotein 330, a member of the low density lipoprotein receptor family, binds lipoprotein lipase in vitro. *J Biol Chem* 268, 14176-14181 (1993)
96. Moestrup SK, SY Cui, H Vorum, C Bregengård, SE Bjorn, K Norris, J Gliemann and EI Christensen: Evidence that epithelial glycoprotein 330/megalin mediates uptake of polybasic drugs. *J Clin Invest* 96, 1404-1413 (1995)
97. Nykjær A, L Kjoller, RL Cohen, DA Lawrence, BA Garni-Wagner, RF Todd, III, A-J Van Zonneveld, J Gliemann and PA Andreasen: Regions involved in binding of urokinase-type-1 inhibitor complex and pro-urokinase to the endocytic a_2-macroglobulin receptor/low density lipoprotein receptor-related protein. Evidence that the urokinase receptor protects pro-urokinase against binding to the endocytic receptor. *J Biol Chem* 269, 25668-25676 (1994)
98. Stefansson S, MZ Kounnas, J Henkin, RK Mallampalli, DA Chappell, DK Strickland and WS Argraves: gp330 on type II pneumocytes mediates endocytosis leading to degradation of pro-urokinase, plasminogen activator inhibitor-1 and urokinase-plasminogen activator inhibitor- 1 complex. *J Cell Sci* 108, 2361-2368 (1995)
99. Kanalas JJ and SP Makker: Identification of the rat Heymann nephritis autoantigen (GP330) as a receptor site for plasminogen. *J Biol Chem* 266, 10825-10829 (1991)
100. Kounnas MZ, J Henkin, WS Argraves and DK Strickland: Low Density Lipoprotein Receptor-Related Protein/a_2- Macroglobulin Receptor Mediates Cellular Uptake of Pro-Urokinase. *J Biol Chem* 268, 21862-21867 (1993)
101. Warshawsky I, GJ Broze, Jr. and AL Schwartz: The low density lipoprotein receptor-related protein mediates the cellular degradation of tissue factor pathway inhibitor. *Proc Natl Acad Sci USA* 91, 6664-6668 (1994)
102. Bu G, S Williams, DK Strickland and AL Schwartz: Low density lipoprotein receptor-related protein/a $_2$-macroglobulin receptor is a hepatic receptor for tissue-type plasminogen activator. *Proc Natl Acad Sci USA* 89, 7427-7431 (1992)
103. Orth K, EL Madison, M-J Gething, JF Sambrook and J Herz: Complexes of tissue-type plasminogen activator and its serpin inhibitor plasminogen-activator inhibitor type 1 are internalized by means of the low density lipoprotein receptor-related protein/a_2-macroglobulin receptor. *Proc Natl Acad Sci USA* 89, 7422-7426 (1992)
104. Orth K, TE Willnow, J Herz, MJ Gething and J Sambrook: Low density lipoprotein receptor-related protein is necessary for the internalization of both tissue-type plasminogen activator- inhibitor complexes and free tissue-type plasminogen activator. *J Biol Chem* 269, 21117-21122 (1994)
105. Bu G, A Maksymovitch and AL Schwartz: Receptor-mediated endocytosis of tissue-type plasminogen activator by low density lipoprotein receptor-related protein on human hepatoma HepG2 cells. *J Biol Chem* 268, 13002-13009 (1993)
106. Willnow TE, K Orth and J Herz: Molecular dissection of ligand binding sites on the low density lipoprotein receptor-related protein. *J Biol Chem* 269, 15827-15832 (1994)
107. Moestrup SK, S Nielsen, P Andreasen, KE Jorgensen, A Nykjaer, H Roigaard, J Gliemann and EI Christensen: Epithelial glycoprotein-330 mediates endocytosis of plasminogen activator-plasminogen activator inhibitor type-1 complexes. *J Biol Chem* 268, 16564-16570 (1993)
108. Poller W, TE Willnow, J Hilpert and J Herz: Differential recognition of alpha(1)-antitrypsin-elastase and alpha(1)-antichymotrypsin-cathepsin G complexes by the low density lipoprotein receptor-related protein. *J Biol Chem* 270, 2841-2845 (1995)
109. Kasza A, HH Petersen, CW Heegaard, K Oka, A Christensen, A Dubin, L Chan and PA Andreasen: Specificity of serine proteinase serpin complex binding to very-low-density lipoprotein receptor and a_2-macroglobulin receptor low-density-lipoprotein-receptor-related protein. *Eur J Biochem* 248, 270-281 (1997)
110. Storm D, J Herz, P Trinder and M Loos: C1 inhibitor-C1s complexes are internalized and degraded by the low density lipoprotein receptor-related protein. *J Biol Chem* 272, 31043-31050 (1997)
111. Cui S, PJ Verroust, SK Moestrup and EI Christensen: Megalin/gp330 mediates uptake of albumin in renal proximal tubule. *Am J Physiol* 271, F900-F907 (1996)
112. Hammad SM, S Ranganathan, E Loukinova, WO Twal and WS Argraves: Interaction of apolipoprotein J-amyloid b-peptide complex with low density lipoprotein receptor-related protein-2 megalin - A mechanism to prevent pathological accumulation of amyloid b-peptide. *J Biol Chem* 272, 18644-18649 (1997)
113. Kounnas MZ, RD Moir, GW Rebeck, AI Bush, WS Argraves, RE Tanzi, BT Hyman and DK. Strickland: LDL receptor-related protein, a multifunctional ApoE receptor, binds secreted beta-amyloid precursor protein and mediates its degradation. *Cell* 82, 331-340 (1995)
114. Shakibaei M and U Frevert: Dual interaction of the malaria circumsporozoite protein with the low density lipoprotein receptor-related protein (LRP) and heparan sulfate proteoglycans. *J Exp Med* 184, 1699-1711 (1996)
115. Moestrup SK, R Kozyraki, M Kristiansen, JH Kaysen, HH Rasmussen, D Brault, F Pontillon, FO Goda, EI Christensen, TG Hammond and PJ Verroust: The intrinsic factor-vitamin B12 receptor and target of teratogenic antibodies is a megalin-binding peripheral membrane protein with homology to developmental proteins. *J Biol Chem* 273, 5235-5242 (1998)
116. Meilinger M, M Haumer, KA Szakmary, F Steinböck, B Scheiber, H Goldenberg and M Huettinger: Removal of lactoferrin from plasma is mediated by binding to low density lipoprotein receptor-related protein/a_2- macroglobulin receptor and transport to endosomes. *FEBS Lett* 360 , 70-74 (1995)

117. Kounnas MZ, RE Morris, MR Thompson, DJ Fitzgerald, DK Strickland and CB Saelinger: The a_2-macroglobulin receptor/low density lipoprotein receptor-related protein binds and internalizes Pseudomonas exotoxin A. *J Biol Chem* 267, 12420-12423 (1992)
118. Simonsen ACW, C. W Heegaard, LK Rasmussen, L Ellgaard, L Kjoller, A Christensen, M Etzerodt and PA Andreasen: Very low density lipoprotein receptor from mammary gland and mammary epithelial cell lines binds and mediates endocytosis of M(r) 40,000 receptor associated protein. *FEBS Lett* 354, 279-283 (1994)
119. Moestrup SK and J Gliemann: Analysis of ligand recognition by the purified a_2-macroglobulin receptor (low density lipoprotein receptor-related protein): Evidence that high affinity of $_2$-macroglobulin-proteinase complex is achieved by binding to adjacent receptors. *J Biol Chem* 266, 14011-14017 (1991)
120. Kounnas MZ, WS Argraves and DK Strickland: The 39-kDa receptor-associated protein interacts with two members of the low density lipoprotein receptor family, a_2-macroglobulin receptor and glycoprotein 330. *J Biol Chem* 267, 21162-21166 (1992)
121. Conese M, D Olson and F Blasi: Protease nexin-1-urokinase complexes are internalized and degraded through a mechanism that requires both urokinase receptor and a_2-macroglobulin receptor. *J Biol Chem* 269, 17886-17892 (1994)
122. Orlando RA, D Kerjaschki, H Kurihara, D Biemesderfer and MG Farquhar: gp330 associates with a 44-kDa protein in the rat kidney to form the Heymann nephritis antigenic complex. *Proc Natl Acad Sci USA* 89, 6698-6702 (1992)
123. Hiesberger T, S Huttler, A Rohlmann, WJ Schneider, K Sandhoff and J Herz: Cellular uptake of saposin (SAP) precursor and lysosomal delivery by the low density lipoprotein receptor-related protein (LRP) *EMBO J* 17, 4617-4625 (1998)
124. Ranganathan S, C Knaak, CR Morales and WS Argraves: Identification of low density lipoprotein receptor-related protein- 2/megalin as an endocytic receptor for seminal vesicle secretory protein II. *J Biol Chem* 274, 5557-5563 (1999)
125. Zheng G, M Marino, J Zhao and RT McCluskey: Megalin (gp330): a putative endocytic receptor for thyroglobulin (Tg) *Endocrinology* 139, 1462-1465 (1998)
126. Mikhailenko I, D Krylov, KM Argraves, DD Roberts, G Liau and DK Strickland: Cellular internalization and degradation of thrombospondin-1 is mediated by the amino-terminal heparin binding domain (HBD) High affinity interaction of dimeric HBD with the low density lipoprotein receptor-related protein. *J Biol Chem* 272, 6784-6791 (1997)
127. Mikhailenko I, MZ Kounnas and DK Strickland: Low density lipoprotein receptor-related protein alpha 2-macroglobulin receptor mediates the cellular internalization and degradation of thrombospondin - A process facilitated by cell- surface proteoglycans. *J Biol Chem* 270, 9543-9549 (1995)
128. Jokinen EV, KT Landschulz, KL Wyne, YK Ho, PK Frykman and HH Hobbs: Regulation of the very low density lipoprotein receptor by thyroid hormone in rat skeletal muscle. *J Biol Chem* 269, 26411-26418 (1994)
129. Lorent K, L Overbergh, J Delabie, F Vanleuven and H Vandenberghe: Distribution of mRNA coding for alpha-2-macroglobulin, the murinoglobulins, the alpha-2-macroglobulin receptor and the alpha- 2-macroglobulin receptor associated protein during mouse embryogenesis and in adult tissues. *Differentiation* 55, 213-223 (1994)
130. Moestrup SK, J Gliemann and G Pallesen: Distribution of the a_2-macroglobulin recptor/low density lipoprotein receptor-related protein in human tissues. *Cell Tissue Res* 269, 375-382 (1992)
131. Zheng G, DR Bachinsky, I Stamenkovic, DK Strickland, D Brown, G Andres and RT McCluskey: Organ distribution in rats of two members of the low-density lipoprotein receptor gene family, gp330 and LRP/alpa 2MR, and the receptor-associated protein (RAP) *J Histochem Cytochem* 42, 531-542 (1994)

Abbreviations: Apo, Apolipoprotein; EGF, Epidermal growth factor; ER2, apoE receptor 2; LDL, Low density lipoprotein; LDL-R, LDL receptor; LRP, LDL-R related protein; VLDL-R, Very low density lipoprotein receptor; PAI-1, Plasminogen activator inhibitor-I; RAP, Receptor associated protein, Review

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