New Light on a Long-Known Protein Family**

Olav M. Andersen and Helle H. Petersen*[a]

1. Introduction

The scope of the present minireview is to give a short presentation of a family of membrane proteins that were first thought to function only as endocytic receptors regulating components of the extracellular fluids but that are now known to be important in a multitude of cellular events.

The low-density lipoprotein receptor (LDLR), the first to be identified, serves as the prototypical member of the LDLR family, which now includes nine members (Figure 1). The protein family members bind a diverse range of unrelated ligands. In this review, we will describe how the molecular basis for ligand recognition can be elegantly encoded by arrays and combinations of similar small domains that cooperate to generate high-affinity binding sites. We will also show how the use of transgenic mouse models has shed new light on the functions of this ancient protein family and has demonstrated that several of these proteins have essential roles in cellular signaling pathways, including the Reelin and Wnt signaling pathways.

Substantial knowledge of the molecular mechanisms underlying ligand recognition has now been obtained, which makes the receptors attractive targets in drug development for the medical treatment of defects arising from lack of function of single or multiple members of the receptor family.

2. Ligand Binding

The structural architecture of the prototypical LDLR includes several small, independent folding units that are found in a variety of extracellular proteins. The mosaic proteins are

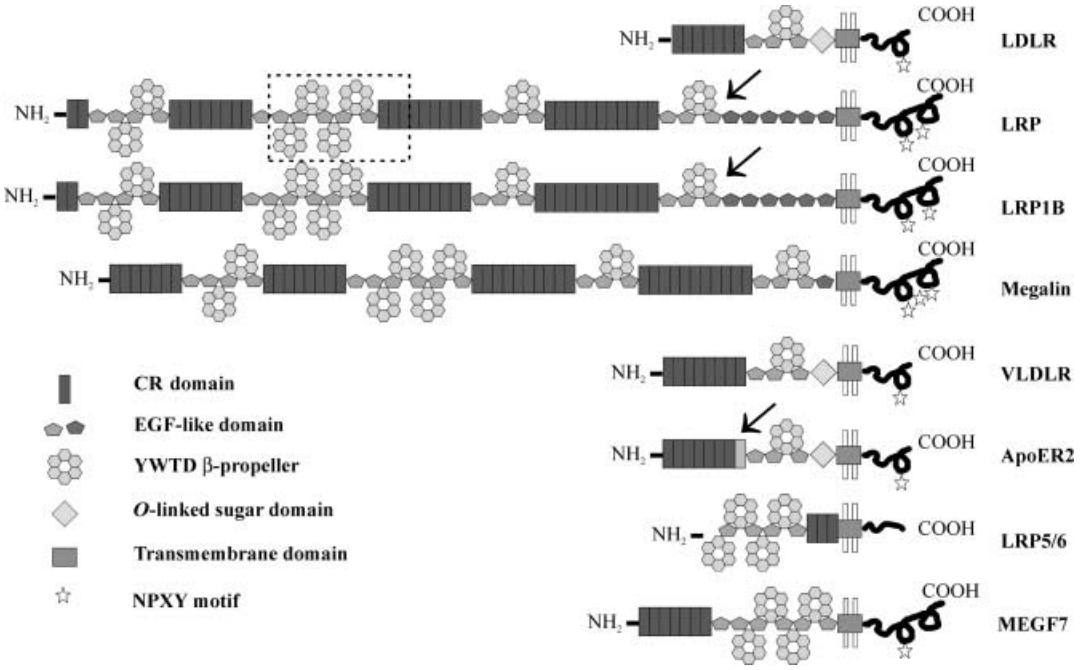

Figure 1. The LDLR gene superfamily. The figure shows the canonical members of the LDLR gene family identified to date. All receptors are anchored in the plasma membrane by a single membrane-spanning segment and contain a short cytoplasmic tail of 50–250 amino acids. Between one and three NPXY endocytosis motifs are present within most of the tail sequences. The extracellular domains consist of arrays of ligand-binding CR domains, the EGF-precursor homology domains, and sometimes an O-linked sugar domain. Arrows indicate putative furin cleavage sites, and the box surrounding part of the extracellular domain of LRP indicates a region very similar to that of LRP5/6.

[a] Dr. H. H. Petersen, Dr. O. M. Andersen
Max Delbrück Center for Molecular Medicine
Robert-Rössle-Strasse 10
13125 Berlin (Germany)
Fax: (+49) 30-9406-3382
E-mail: petersen@mdc-berlin.de

[**] A list of abbreviations can be found at the end of the text.

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composed of small, cysteine-rich, complement-type repeat (CR) domains, similar to those found in many components of the complement system, and parts analogous to the epidermal growth factor (EGF) precursor that consist of repeating EGF-like domains and a six-bladed β-propeller domain with multiple copies of a YWTD tetrapeptide consensus sequence (Figure 1). A highly diverse set of otherwise unrelated ligands have been shown to bind to one or more of the lipoprotein receptors. A subset of these is listed in Table 1. For more information, the reader may consult ref. [1]. Besides lipoproteins, proteinases in complex with their inhibitors, carrier proteins for lipophilic molecules, and proteins with a specific function in signaling pathways, such as the Sonic hedgehoc,[2] Dickkopf, and Reelin[3] proteins, are known to bind and enter cells by way of the lipoprotein receptors. The receptor-associated protein (RAP) has attracted special interest because it is a specific chaperone for several of the low-density lipoprotein receptor related proteins (LRPs) within the secretory pathway and, thus, binds every member of the LDLR family and functions as a universal inhibitor of ligand binding.

Table 1. Selection of identified ligands for the LDLR family.

Type of ligand	Identified ligands
Interaction with the extracellular receptor parts:	
apolipoproteins and lipases	apo(a), apoB, apoE, apoH, apoJ, lipoprotein lipase, hepatic lipase
proteinases, inhibitors, and their complexes	uPA, tPA, thrombin, elastase, Factors VIII, IX, and X, PAI-1, PN1, α_1-antitrypsin, α_2-macroglobulin (α_2M), tissue factor pathway inhibitor
vitamin/hormone-carrier complexes	vitamin A – retinol binding protein, 25-OH vitamin D_3 – vitamin D binding protein (DBP), vitamin B_{12} – transcobalamin, parathyroid hormone (PTH), thyroglobulin, transthyretin
others	Sonic hedgehoc, Reelin, HIV-1 transactivator, lactoferrin, Wnt proteins, Dickkopf, receptor-associated protein (RAP)
membrane proteins/coreceptors	cubilin, amyloid precursor protein (APP), uPAR
growth factors	epidermal growth factor (EGF), platelet-derived growth factor (PDGF), connective tissue growth factor, midkine
Interactions with the intracellular receptor parts:	JNK-interacting protein-1 (JIP-1), Disabled protein (Dab) 1/2, autosomal recessive hypercholesterolemia protein (ARH), axin, Shc, Fe65, megalin binding protein (MegBP), X11

Ligand binding extends to the entire luminal domain

The identification of ligand binding sites within the receptor structure has revealed sites for protein interaction throughout the entirety of the receptor ectodomains. Information on the different ligand binding sites has often been provided from studies of how inhibitors with known binding sites displace a given ligand and/or how a potential ligand influences the association of well-established ligands. For this purpose the use of specific antibodies with already known binding sites has turned out to be very useful, for example, in identifying the binding sites for apoE and lactoferrin within megalin.[4] In particular, the ligands that bind to LRP and megalin have been analyzed, since they are superior in the number of different ligands as well as in their molecular size. Some ligands bind to identical or partially overlapping sites, which results in competitive receptor binding, while other ligands have unique sites that are strongly suggestive of multiple (identical or discrete) ligand binding sites within the luminal domains. Molecular dissection of the large ectodomains has been used for the demonstration of direct ligand interactions with specific receptor fragments.[5, 6]

As LDLR was the first known member of the receptor family, the initial studies on ligand binding determinants have been extracted from the data of lipoprotein particle binding to LDLR. The presence of repetitive negative charges in the CR-domain sequences led to the hypothesis that these domains constitute the major sites for association of positively charged lipoprotein epitopes. Also, in the gigantic receptor molecules containing multiple clusters of CR domains (for example, LRP, LRP1B, and megalin; Figure 1), most, if not all, of the CR clusters are able to mediate ligand binding.[7]

For a long time it was not known whether the additional domains present in the extracellular domain of the lipoprotein receptors also participate in ligand binding. However, both the EGF-like repeats and the β propellers are well known to bind proteins when present in other molecules. For these domains new insight has recently been given by the newly identified receptors LRP5 and LRP6, which contain only a small CR cluster with three CR domains but have several EGF-precursor homology domains (Figure 1). Indeed, compelling evidence has been provided that two of the most important ligands hitherto identified, the Wnt proteins and Dickkopf proteins, bind directly to the EGF-precursor homology domains.[8] In addition, a functional mutation at the surface of the first β propeller within LRP5 has been described and is speculated to result in impaired ligand recognition.[9] From comparison of the domain architecture of LRP with LRP5/6, the similarity between the latter and the region just on the C-terminal side of CR cluster II in the former is intriguing (Figure 1). The resemblance between these regions and the presence of protein recognition sites within the EGF-precursor homology domains in LRP5/6 make it very likely that ligand binding in the whole family of LRPs is not solely made by the CR clusters and that the ligand binding sites are distributed along the entire luminal domain. This hypothesis is further underscored by the recent X-ray crystal structure data of the complete LDLR luminal domain. This structure was solved at the endosomal pH value to gain a better understanding of the

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underlying molecular details of ligand release from the endocytosis receptors. The lower pH value induces a specific protonation of two histidine residues within the β-propeller region; this region is then able to function as an intramolecular ligand for the CR cluster. The binding of the β-propeller domain displaces the already bound ligand within the endosome.[10] This demonstrates that also the EGF-precursor domain of LDLR is directly involved in protein – protein interactions, in a similar manner to the domains of LRP5/6.

Identification of specific determinants for protein recognition

The list of structurally different ligands has made it very interesting to analyze and to try to understand protein recognition by the lipoprotein receptors, especially because the information for binding so many unrelated and dissimilar protein structures seems to be encoded in part by arrays of highly similar CR domains.

It is of interest to establish how many CR domains contribute to the direct binding of any ligand. Analyses of LRP minireceptors comprising isolated CR clusters II or IV (8 and 11 CR domains, respectively), as well as the very low density lipoprotein receptor (VLDLR) and LDLR CR clusters (8 and 7 CR domains, respectively), demonstrate that a single CR cluster is sufficient for high-affinity ligand binding. From naturally occurring apoE receptor 2 (apoER2) variants, it is also evidenced that an apoER2 CR cluster comprising four CR domains is sufficient for ligand binding, and four adjoined LRP CR domains possess high-affinity ligand binding.[11] Further delineation of the minimal functional binding unit was provided by means of surface plasmon resonance techniques, with which it was demonstrated that three CR domains from either LRP or VLDLR are sufficient for RAP association,[6, 12] and recently we have provided evidence for CR-domain pairs being sufficient for the binding of several different ligands.[13, 14] Finally, interactions between ligands and single CR domains have been reported.[15] However, the reported affinity decreased in proportion with the number of CR domains, a fact suggestive of an avidity determinant in these receptor – ligand interactions.

Indeed, as demonstrated for the binding of a proteinase – inhibitor complex to CR-domain pairs from LRP, ligands exhibit preference for some sites.[14] However, binding is not restricted to a sole site, since the preferred site is not a unique binding site, so most ligands are able to bind through multiple receptor interactions. This is in accord with a hypothesis where several CR domains generate a high-affinity binding site because individual weaker binding sites reside in each CR domain and cooperate to generate the equivalent of a high-affinity site.[14]

Some of these isolated binding sites have now started to be uncovered by our studies using tandem CR-domain pairs from LRP and the introduction of specific, designed, single amino acid substitutions. These studies have demonstrated that a conserved acidic residue within the CR-domain sequences of the LDLR family contributes significantly to the direct interaction with several ligands. Furthermore, a partly conserved tryptophan residue is shown to be important for intermolecular ligand recognition.[13, 14, 16] Interestingly, tryptophan and aspartic acid residues at identical positions in the CR-domain sequences of the LDLR are also involved in binding to intramolecular receptor domains after protonation of histidine residues within the β propeller of the EGF-precursor domain at endosomal pH values.[10]

As a way to graft additional ligand binding sites, members of the LDLR family may interact in a dual-receptor complex. One example, which might also be the best characterized system, is the cooperation between megalin and cubilin. Cubilin binds several ligands that do not bind directly to megalin, for example, the vitamin-B_{12}-intrinsic factor complex, but cubilin is only peripherally attached to the cell membrane and is therefore unable to mediate endocytosis on its own. However, interaction of cubilin with megalin allows this protein to participate in the endocytosis machinery and thereby play a pivotal role in the uptake of several proteins within the kidney.[17] Hereby, a single binding site within the lipoprotein receptor that exhibits affinity toward cubilin significantly expands the number of ligands directed into the cell by way of megalin. In fact, cubilin deficiency mimics a lack of megalin and has been identified as a cause of abnormal metabolism of vitamin D_3 in the kidney.[18]

An essential role of megalin in steroid metabolism

Recent work from our group has shown that megalin and its coreceptor cubilin are vital for the retrieval of vitamin D_3 in complex with its carrier protein, vitamin D binding protein (DBP), from the glomerular filtrate of the kidney.[18, 19] Megalin is abundantly expressed at the apical surface of the proximal tubular cells facing the lumen of the kidney. In accordance with the proposed function of megalin in ligand internalization, the endocytic machinery in megalin-deficient mice is diminished in the proximal tubular cells and the animals loose low-molecular-weight ligands into the urine, where DBP has been identified.[19, 20] Due to the urinary loss of DBP, these mice suffer from vitamin D_3 deficiency and bone disease.[19] Subsequently, characterization of human patients (Imerslund – Grasbeck syndrome) and dogs with functional defects in cubilin also revealed urinary loss of vitamin D_3, an observation underscoring the importance of cubilin in the dual-receptor complex.[18] These findings were surprising because according to the "free-hormone hypothesis" steroid hormones and vitamin D_3 enter target cells primarily by passive diffusion over the cell membrane (Figure 2). Thus, the principle roles of the carrier proteins are to keep the steroids soluble in a biologically inactive form and to regulate the concentration of free hormone for passive diffusion. By identifying an endocytic pathway where direct binding of the vitamin D_3 – DBP complex to the receptors megalin and cubilin ensures retrieval of vitamin D_3 from the urine,[21] these results challenge the traditional concept of how steroid hormones enter cells. In keeping with our observations, reduced vitamin D_3 uptake has also been observed in DBP knockout mice.[22] This is the first example of an endocytic uptake pathway for steroid hormones that has been elucidated in molecular detail. However, reports exist of receptor-mediated uptake of carrier proteins in complex with testosterone and β-estradiol in epididymis and endometrium, respectively.[23] Intriguingly, high expression of megalin and

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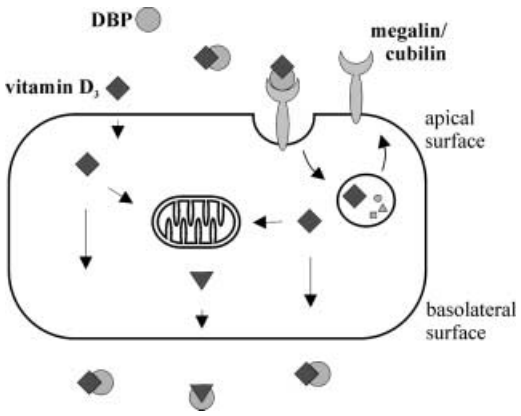

Figure 2. Hormone action. The classical "free-hormone hypothesis" pathway for cellular uptake of steroid hormones and vitamin D_3 is shown on the left. According to this hypothesis, after dissociation from its carrier protein, DBP, vitamin D_3 passively diffuses across the cell membrane into the cytosol and then into the mitochondria, where it is processed before release into the interstitial fluid. We have now demonstrated the importance of receptor-mediated uptake of vitamin D_3, as illustrated on the right. The DBP – vitamin D_3 complex binds to megalin and cubilin, and megalin mediates internalization and delivery of the DBP – vitamin D_3 complex to endosomal compartments. There, DBP is degraded, whereas vitamin D_3 is released into the cytosol where it possibly associates with intracellular carrier proteins, which shuttle the vitamin to the mitochondria for processing.

apoER2 in the genital system has been reported, a fact raising the possibility that receptor-mediated endocytosis might be a general mechanism for regulating hormone concentrations and, for example, for achieving a temporarily high concentration of steroid hormones in target tissues.

3. Prototypical Signaling Mechanisms

The prototypical function of the LDLR family has long been thought to be solely the endocytosis of the large multitude of different ligands that bind to the luminal domains. Recent research has now provided compelling evidence showing that the function of several members extends far beyond that of clearing proteins from the extracellular fluid. Members of the LDLR family might indirectly influence several important cellular signaling pathways. For example, megalin mediates the clearing of parathyroid hormone (PTH) from the extracellular space, whereby less PTH is available for binding to the original PTH signaling receptor. Accordingly, there is a decrease in PTH signal transduction and Ca^{2+} mobilization, and so megalin is linked indirectly to this pathway.[24] However, more direct actions of LDLR family members as signal transducers have now started to be elucidated; for example, it has been observed that the cortical developmental defects of mice deficient in apoER2 and VLDLR phenocopy the brain from Reeler mice. These receptors function directly in a linear signaling pathway within migrating neurons, as described in detail below.

Identification of cytoplasmic adaptors and scaffold complexes

Recognizing that the receptors of the LDLR family may be directly involved in signal transduction through interaction with cytoplasmic proteins has initiated an extensive search for scaffold proteins binding the intracellular domain of the family members. Here we will introduce a few of the newly identified interaction partners. (For further data, see ref. [25]). Several adaptors linking the receptors to the endocytic and sorting machinery have been identified. Notable is the discovery that the familiar disease autosomal recessive hypercholesterolemia is caused by mutations in the gene encoding ARH, an adaptor molecule binding to LDLR.[26] ARH contains a phosphotyrosine binding (PTB) domain found in several adaptor molecules including disabled protein (Dab) 1 and Shc.[26] PTB domains recognize a canonical NPXY motif, in a manner either dependent on tyrosine phosphorylation (for example, Shc) or independent thereof (for example, Dab1 and ARH). Through its PTB domain ARH binds the NPXY motif in the LDLR tail, which is required for LDLR-mediated endocytosis. Furthermore, ARH binds clathrin and the β2-adaptin subunit of adaptor protein 2 (AP-2), two key components of clathrin-coated pits, which strongly suggests that ARH is critical for linking LDLR to the endocytic machinery.[27] In line with this, LDLR-mediated endocytosis is blocked in hepatocytes and lymphocytes from autosomal recessive hypercholesterolemia patients despite normal receptor expression.[28]

Dab2, the second mammalian Dab homologue, was first identified as a putative tumor suppressor gene.[29] Dab2 comprises an N-terminal PTB domain, a proline-rich C-terminal domain, and several phosphorylation sites. Apart from being connected with several signaling pathways, Dab2 has been linked to the endocytic machinery. The PTB domain of Dab2 binds to the NPXY motif within the cytoplasmic tail of megalin, as well as to phosphoinositides.[30] The C-terminal part associates with AP-2 and clathrin. These observations suggest a role as a scaffold protein involved in cargo selection and clathrin lattice assembly, which places Dab2 in the same protein family of cargo-specific adaptor proteins as ARH. Also, analysis of Dab2 knockout mice suggests that Dab2 might be important for linking megalin to the endocytic machinery and to signaling pathways. Like the megalin knockout mice, the proximal tubule cells of mice deficient in Dab2 have reduced clathrin-coated pits and DBP is secreted into the urine. Furthermore, in one mouse model, Dab2 influences the structural organization of the visceral endoderm, perhaps functioning by a mechanism similar to Dab1 (see below).[31]

We have recently identified a novel scaffold protein binding the cytoplasmic tail of megalin, the megalin binding protein (MegBP).[32] The recognition site maps to an N-terminal region on the megalin tail comprising a proline-rich sequence. High expression levels of MegBP in cell lines with a high endogenous megalin expression result in cellular lethality. The identification of several transcriptional activators and components of signal transduction cascades as MegBP interaction partners has led to the proposal that megalin may regulate gene transcription through adaptors targeted to the megalin tail by MegBP.[32]

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Reeler mouse phenocopy by the apoER2/VLDLR double knockout mouse

Brain development is markedly altered in mice lacking both VLDLR and apoER2.[33] The double knockout mutants have obvious and highly characteristic defects in neuronal layering in the cerebral cortex, hippocampus, and cerebellum. The histological analysis shows that the VLDLR$^{-/-}$/apoER2$^{-/-}$ phenotype is virtually identical to that seen when either of the genes encoding the extracellular matrix protein Reelin or the intracellular adaptor protein Dab1 is mutated.

Reelin is secreted by the external layer of Cajal–Retzius neurons in the developing brain but is not expressed by those neurons that show a disrupted migration pattern, for example, cortical plate neurons or cerebellar Purkinje cells. This suggests that Reelin acts on target cells from a distance through the extracellular matrix. However, the interaction of specific anti-Reelin antibodies with the surface of Purkinje cells implies that Reelin is associated with cell surface receptors, and this has led to the identification of three different types of membrane-associated Reelin binding proteins, $\alpha_3\beta_1$ integrins,[34] members of the cadherin-related neuronal receptor family,[35] and VLDLR/apoER2[3, 36] (Figure 3).

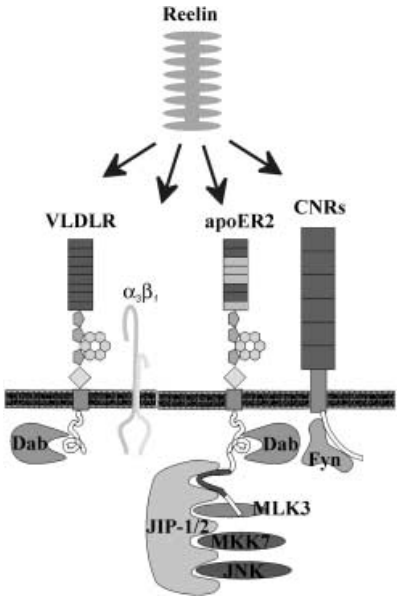

Figure 3. VLDLR and apoER2 are requisite components in Reelin/Dab1 signaling. Reelin is able to associate with several classes of cell surface proteins, including apoER2 and VLDLR, integrins, and members of the cadherin-like neuronal receptors (CNRs). Binding of Reelin causes apoER2/VLDLR to recruit the cytoplasmic adaptor Dab1, which becomes phosphorylated on tyrosine residues, perhaps mediated by Src, Abl, and/or Fyn. The apoER2 tail harbors an alternative spliced exon that binds members of the c-jun signaling pathway, the JIP-1/2 scaffolding proteins. JIPs organize mitogen-activated protein kinase kinases (for example, MLK3 and MKK7) into a complex, and these observations suggest that activation of the JNK pathway may function specifically through apoER2-mediated Reelin signaling.

The adaptor Dab1 is a phosphotyrosine protein that resides in the cytoplasm within the neurons that manifest the Reeler phenotype. Dab1-deficient mice develop a phenotype indistinguishable from the Reeler one, and several lines of evidence place Dab1 as a key element in the cellular response to the Reelin signal. Dab1 tyrosine phosphorylation is stimulated directly by Reelin in neuronal cultures, and Dab1 tyrosine phosphorylation is reduced in embryos that lack Reelin.[37, 38] The phosphorylated Dab1 protein is able to recruit nonreceptor tyrosine kinases like Fyn, Src, and Abl that are further implicated in diverse cellular signaling cascades. The importance of Dab1 phosphorylation is further underscored by the failure to rescue a Dab1-deficient phenotype with a Dab1 mutant that cannot undergo tyrosine phosphorylation, whereas wildtype Dab1 can.[38]

In conclusion, Reelin, VLDLR/apoER2, and Dab1 are working as components in a linear pathway, where the interaction of Reelin with the luminal receptor domain(s) results in phosphorylation of Dab1 because the adaptor is brought into the proximity of an as yet unidentified kinase by the direct binding of Dab1 to the NPXY motif within the receptor tail (Figure 3).

The signal affects the migration of postmitotic neurons along guidance fibers, where it is speculated that Reelin acts as a detachment signal, which allows the correct positioning of neurons in the developing as well as the postnatal brain.[39]

ApoER2, but not VLDLR, also binds the family of c-Jun N-terminal kinase (JNK) interacting proteins (JIPs) that act as molecular scaffolds for complexes working in the JNK signaling pathway.[40] The differential binding of JIPs presents a possible explanation for differences observed between mice lacking apoER2 and VLDLR-deficient mice.[33]

A function of the Reelin signaling pathway in man has also been suggested. The possible role of Reelin in human malformations and polygenic diseases was hypothesized after observation of decreased Reelin expression as a putative vulnerability factor in schizophrenia and the discovery of two cases of autosomal recessive lissencephaly with cerebellar hypoplasia associated with Reelin mutations.[41]

LRP5 and LRP6 are fundamental in the Wnt/β-catenin signaling pathway

Wingless signaling plays a critical role in various developmental processes and in tumor genesis. Wnt is a group of secreted proteins that initiates signaling by binding to seven-transmembrane receptors of the frizzled protein (Fz) family. Two wingless signaling pathways are known at present. In the canonical Wnt/β-catenin pathway, binding of Wnt to Fz results in stabilization of β-catenin, which, in complex with members of the lymphoid enhancer factor/T cell factor (LEF/TCF) family, initiates transcription of target genes (Figure 4). The alternative pathway does not involve β-catenin but leads to intracellular Ca^{2+} release and is important for cell polarity (reviewed in ref. [42]). Recently, it was shown that disruption of expression of functional LRP6 in mice and of the LRP5/6 homologue in fruitflies results in phenotypes reminiscent of some mutant Wnt phenotypes, whereas LRP6 overexpression in frogs activates Wnt–Fz signaling.[43] LRP5/6 binds Wnt and can form a Wnt-dependent ternary complex with Fz. Together these observations suggest that

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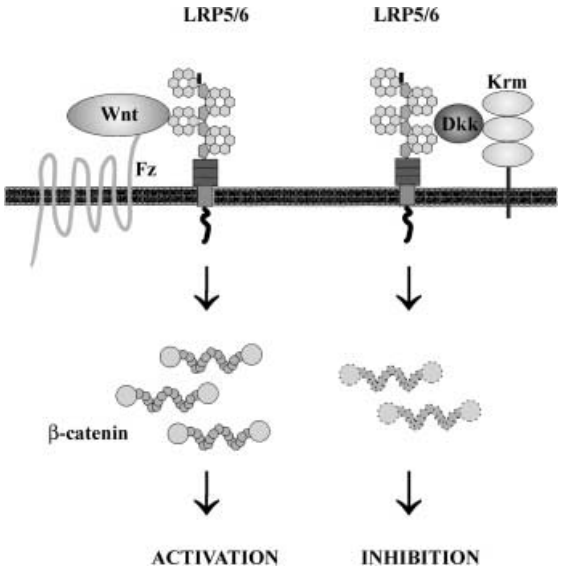

Figure 4. The canonical Wnt signaling pathway. Wnt binding to LRP5/6 and Fz results in stabilization and accumulation of β-catenin, which after entry into the nucleus activates target gene expression through complex formation with transcription factors of the TCF and LEF families. The Wnt signaling pathway is attenuated by Dickkopf1 (Dkk). Binding of Dickkopf1 to LRP5/6 inhibits ternary LRP5/6 – Wnt – Fz complex formation and thereby induces β-catenin degradation. Binding of Dickkopf1 to Kremen (Krm) does not inhibit its binding to LRP5/6, rather Kremen functions as a coreceptor.

interaction of Wnt with LRP5/6 is followed by β-catenin stabilization and initiation of transcription (Figure 4). In addition, the LRP5 tail binds directly to axin, an important component in the complex of adaptor molecules responsible for the phosphorylation and thus destabilization of β-catenin. Thus, by binding axin, LRP5 is able to remove axin from the phosphorylation complex, whereby reduced β-catenin degradation and enhanced target gene transcription is achieved.[44] Furthermore, LRP5/6 has proved to be critical for the regulation of Wnt signaling through the secreted inhibitor Dickkopf1, which binds directly to LRP5/6 and inhibits ternary LRP5/6 – Wnt – Fz complex formation.[8, 45] Dickkopf1 also binds to the cell surface receptors Kremen1/2, which function as a coreceptor concentrating Dickkopf1 at the cell surface.[46] The LRP5/6 – Dickkopf1 – Kremen1/2 complex can be removed from the cell surface by endocytosis, possibly mediated by internalization signals in the cytoplasmic domains of LRP5/6.

The physiological importance of LRP5 in Wnt signal transduction is underscored by the finding of an autosomal recessive defect in the LRP5 gene in patients with osteoporosis-pseudoglioma syndrome. Affected patients exhibit a low bone mass and suffer from recurring fractures and deformities. They lack functional LRP5, which suggests that loss of LRP5 results in defective bone formation as a result of impaired Wnt signal transduction.[47] Interestingly, analysis of LRP5 from patients with high bone mass identified a single amino acid substitution in the first β propeller. The substitution appears to inhibit binding of the inhibitor Dickkopf1, thereby leading to enhanced Wnt signaling.[9, 48]

4. Revised View on the Function of the LDLR Family Members

In recent years, the view of the LDLR family has shifted from a family of endocytosis receptors towards a class of membrane proteins participating in complex signaling pathways. The importance of apoER2/VLDLR and LRP5/6 in canonical signal transduction, where a direct role in a linear pathway has been characterized, was described above. The following section will focus on the potential involvement of other members of the LDLR family in signaling and their putative roles in regulating cellular events. Functional roles of LRPs in the pathological events that, for example, end with the accumulation of toxic peptides (Aβ) in some neurological diseases will also be suggested.

LRP in Alzheimer's Disease

Alzheimer's disease (AD) is a neurodegenerative syndrome characterized by the deposition of amyloid plaques and neurofibrillary tangles (reviewed in ref. [49]). Accumulating evidence suggests that the apoE receptors (see below) have pivotal functions in the biogenesis of the main constituent of the amyloid plaques, the Aβ peptide, which is derived from the amyloid precursor protein (APP) through sequential proteolytic cleavage.[50] LRP especially has been thoroughly investigated for its role in Aβ metabolism, since it was shown that LRP is able to mediate endocytosis of the secreted precursor[51] and that LRP, as well as several of its ligands, is found in the amyloid plaques. Furthermore, genetic linkage and association studies have linked polymorphisms in the genes for LRP and some ligands, for example, α_2-macroglobulin (α_2M), to late-onset AD.

The identification of an essential role of apoE for amyloid deposition[52] was provided by the lack of plaque formation in apoE-deficient mice transgenic for a plaque-inducing APP variant carrying a specific mutation identified in the APP gene from patients with AD. This observation suggests that the apoE receptors might be critical components for the pathogenesis of AD. Furthermore, the gene for apoE has three alleles, and to carry homozygously the apoE ε4 allele is one of the most significant risk factors for the late development of AD.[53] In addition, the demonstration of a protective effect of the ε2 allele[54] has further put focus on the LDLR family as potential candidates for pharmacological intervention in neurodegenerative disorders.

One possible mechanism by which LRP and the other members of the LDLR family can influence the pathogenesis of Aβ is by lowering the Aβ burden by clearance of Aβ-complexed ligands (Figure 5). Indeed, the role of complexing Aβ was demonstrated for α_2M, which is a well-established ligand for LRP, and α_2M can therefore exert protection against Aβ neuronal toxicity.[55] In parallel, it seems reasonable to expand this view for the additional ligands known to form complexes with secreted Aβ and thereby to present a way of Aβ clearance by endocytosis of the complex. Known ligands that are able to form complexes with Aβ include apoE, α_2M, apoJ, and lactoferrin (Table 1).

Another important functional role of LRP in the pathological accumulation of the amyloidegenic Aβ peptide relies on the

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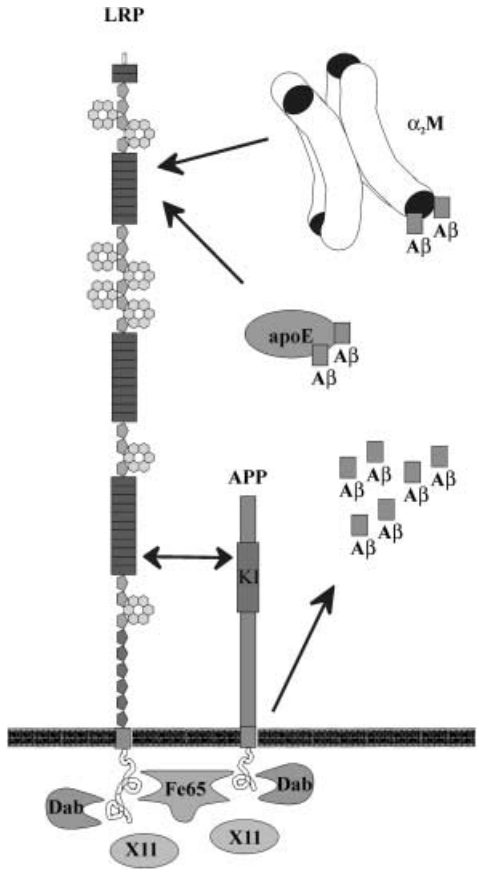

Figure 5. *Possible pathogenetic functions of LDLR-like proteins in Aβ production and clearance. Interactions with LRP might influence the intracellular routing of APP, for example, by direct binding between the luminal domains or by linkage through scaffold proteins like Fe65. Alternatively, the sharing of and/or competing for a set of cytoplasmic adaptor proteins, for example, Dab and X11, could further contribute to altered APP processing and Aβ production. Extracellular clearance of Aβ can occur after binding of the peptide to several LRP ligands, for example, apoE and α_2M, whereby Aβ is taken up into the cell and routed for lysosomal degradation.*

intracellular trafficking of the precursor, which can influence the secretion of Aβ. One approach to analyze the influence of lipoprotein receptors in the modulation of APP processing included the blocking of LRP-mediated internalization of APP from the cell surface by using RAP. This increased the levels of both cell surface APP and the nonamyloidogenic α-secretase processing and sAPPα liberation, with a significant decrease in Aβ production due to less efficient transport of APP from the cell surface into the endosomal compartment where Aβ can be generated.[56]

In addition to the interaction between APP and the LRP ectodomains, growing evidence for association of their intracellular tails has been obtained; for example, the APP tail was found to influence the association with LRP[57] and APP routing was reported mainly to be directed by the LRP tail.[58] The binding of cytoplasmic proteins has also been suggested to affect APP processing. One such adaptor molecule, Fe65, does in principal present the possibility for a direct link between the two receptors due to its two PTB domains (Figure 5), where the N-terminal PTB domain binds LRP and the C-terminal PTB domain interacts with APP.[59] Alternatively, however, scaffold proteins like Dab1 and X11, which have been shown to bind to both tails, could compete for the binding of other adaptors and thereby further contribute to the complexity of the interaction pattern. The importance of functional LDLR-like proteins for modulation of the Aβ level was recently underscored by the finding that transgenic mice that overexpress human APP show elevated levels of the amyloidogenic Aβ when crossed into a hypomorphic animal model of LRP and megalin deficiency.[60]

Growth factor binding and signaling through LDLR proteins

All members of the LDLR family are known to participate in receptor-mediated endocytosis and targeting of ligands for lysosomal degradation. In addition, LRP may participate in cellular signal transduction in a similar manner to VLDLR and apoER2, for example, by modulation of the JNK signaling cascade,[61] and recent studies have started to elucidate how the activity of these receptors can be controlled. In particular, the cytoplasmic tail of LRP can be phosphorylated on serine and tyrosine residues, which might influence the rate of endocytosis and divert signaling. The linkage between LRP and signaling was first provided by showing an association with a stimulatory heterotrimeric G-protein and activation of downstream cyclic adenosine monophosphate (cAMP) dependent protein kinase A (PKA) dependent pathways.[62] Work by Bu and colleagues has shown that phosphorylation on serine residues within LRP is mediated by PKA. More specifically, they have identified serine residue 76 within the cytoplasmic tail as the predominant phosphorylation site.[63] Tyrosine phosphorylation of the LRP tail by the Src kinase was first described by Barnes et al.[64] They further demonstrated that the phosphorylated LRP tail associates with the adaptor protein Shc, which in turn becomes highly phosphorylated, findings that support the proposal of a role for LRP in Src signaling.

Recently, two papers reported that platelet-derived growth factor (PDGF) is able rapidly and transiently to induce tyrosine phosphorylation of the intracellular part of LRP, and a mechanism involving the PDGF receptor as a coreceptor responsible for bridging to members of the Src kinase family and for subsequent phosphorylation events was suggested.[65, 66] Interestingly, only PDGF, not other closely related growth factors like EGF, basic-fibroblast growth factor, and insulin-like growth factor, is capable of LRP-mediated signaling initiation.[65] It will be of importance to establish whether additional growth factors also involve LRP to transduce signals, as well as to investigate whether the other members of the LDLR family play similar roles for other growth factors. Some examples thus far include reports of binding to LRP by connective-tissue growth factor[67] and midkine.[68] The latter is targeted to the nucleus by an LRP-dependent pathway after direct interaction with LRP at the cell surface. One possible mechanism for the nuclear translocation might involve processing of LRP that results in liberation of only the tail (see below).[69]

Another level of functional regulation by growth factors may rely on the induction of rapid increases in functional cell surface receptors. Indeed, nerve growth factor has been reported to induce an increase of LRP.[70]

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Proteolytic processing and ectodomain shedding of the LDLR family

In recent years, it has become evident that many membrane-bound receptors have soluble counterparts. These are generated by a number of different mechanisms, for example, alternative splicing, expression from two related genes, or proteolytic release from the plasma membrane.

Soluble counterparts of receptors of the LDLR family have also been observed. Accordingly, megalin fragments have been identified in culture media of a rat yolk sac carcinoma cell line (L2) and immortalized rat proximal tubule cells and also in urine.[71] However, no further characterization of the fragments or possible mechanism for their release has been provided.

Recently, a splice variant of apoER2 containing a furin recognition site in the extracellular domain close to the transmembrane region has been described (see Figure 1). The receptor variant is proteolytically processed in the trans-Golgi network. The two parts generated by the cleavage do not interact and a fragment comprising the CR cluster of apoER2 is released. This apoER2 fragment binds ligands with unchanged affinity and is speculated to function as a dominant-negative receptor in signal transduction.[72]

LRP and its closest relative LRP1b also contain furin cleavage sites in the plasma-membrane-proximal position of their extracellular domains.[73] However, contrary to the situation with apoER2, the two chains of LRP resulting from furin processing remain noncovalently attached. Nevertheless, a soluble LRP fragment comprising most of the luminal domain has been identified in human plasma.[74, 75] The receptor fragment is generated by proteolytic cleavage close to the cell membrane, possibly by a metalloproteinase.[69, 75] The sequential processing of LRP (furin cleavage, metalloproteinase-mediated shedding of the extracellular domain) and the ability of the cytoplasmic domain to interact with adaptor proteins are similar to the mechanisms involved in the processing of APP. These observations prompted Herz and co-workers to investigate if LRP is also subject to intramembranous or cytoplasmic cleavage.[69] Indeed, they found that the cytoplasmic tail could be released by a cleavage mechanism that may engage γ-secretases (also involved in APP processing), and the cytoplasmic tail is subsequently able to translocate to the nucleus (Figure 6). Phorbol esters, known to increase expression of certain metalloproteinases, enhance release of the LRP tail in cell lines, a fact indicating that shedding is necessary for intramembraneous/cytoplasmic processing. Tail processing does not appear to be influenced by the presence or absence of ligands.[69] The function of the released cytoplasmic fragment is not known at present, but its ability to translocate to the nucleus suggests a direct role for LRP in transcriptional regulation.

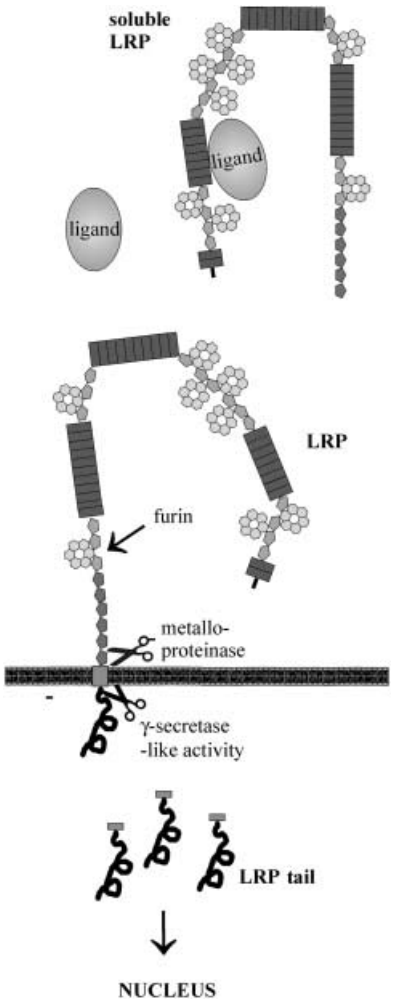

Figure 6. Ectodomain shedding and processing of LRP. LRP is processed by furin in the trans-Golgi network but the two resulting chains remain noncovalently associated. After reaching the cell surface, LRP can be further processed at a membrane-proximal position. The cleavage, probably carried out by a metalloproteinase, results in release of the luminal domain. Subsequently, the cytoplasmic domain may be released by γ-secretase-like activity and the LRP tail can translocate to the nucleus.

5. Perspectives

The essential role of these proteins in the control of several developmental and physiological processes, as well as their involvement in many pathological events including cancer, atherosclerosis, and Alzheimer's disease, makes them attractive drug targets. Knowledge of the molecular basis for ligand recognition and an appreciation of the many functions of these receptors will be key determinants for the development of drugs targeted at members of the LDLR family. In particular, focus could be put on the design of specific inhibitors of the interaction between receptors and ligands. For example, if receptor-mediated endocytosis is a general pathway for steroid hormones to be regulated and to enter target tissues, instead of using a nonselective steroid antagonist, the alternative would be to design a compound selectively antagonizing receptor-mediated uptake of carrier-bound steroids; this approach may be more efficient or cause fewer side effects.

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Abbreviations

α_2M	α_2-macroglobulin
AD	Alzheimer's disease
AP-2	adaptor protein 2
ApoER2	apoE receptor 2
APP	amyloid precursor protein
ARH	autosomal recessive hypercholesterolemia protein
cAMP	cyclic adenosine monophosphate
CR	complement-type repeat
Dab	disabled protein
DBP	vitamin D binding protein
EGF	epidermal growth factor
Fz	frizzled protein
JNK	c-Jun N-terminal kinase
JIP	JNK-interacting protein
LDLR	low-density lipoprotein receptor
LEF	lymphoid enhancer factor
LRP	low-density lipoprotein receptor related protein
MegBP	megalin-binding protein
PDGF	platelet-derived growth factor
PKA	cAMP-dependent protein kinase A
PTB	phosphotyrosine binding
PTH	parathyroid hormone
RAP	receptor-associated protein
TCF	T cell factor
VLDLR	very low density lipoprotein receptor

Keywords: Alzheimer's disease · low-density lipoprotein receptor · membrane proteins · signal transduction · steroid metabolism

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