CMLS, Cell. Mol. Life Sci. 60 (2003) 892–903
1420-682X/03/050892-12
DOI 10.1007/s00018-003-2183-z


CMLS Cellular and Molecular Life Sciences

Review

# LDL receptor relatives at the crossroad of endocytosis and signaling

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Received 17 July 2002; received after revision 8 October 2002; accepted 9 October 2002

**Abstract**. For many years, the low-density lipoprotein (LDL) receptor and the LDL receptor-related protein (LRP) have been considered to be prototypes of cargo receptors which deliver, via endocytosis, macromolecules into cells. However, the recent identification of additional members of this gene family and examination of their biology has revealed that at least some of these proteins are also signaling receptors. Very low density lipoprotein receptor and ApoER2 transmit the extracellular reelin signal into migrating neurons, and thus are key components of the reelin pathway which governs neuronal layering of the forebrain during embryonic brain development. LRP5 and LRP6 are integral components of the Wnt signaling pathway which is central to many processes of metazoan development, cell proliferation, and tumor formation. Adaptor proteins interacting with the cytosolic domains of these receptors might orchestrate their ability to deliver their cargo or a signal.

**Key words.** LDL receptor relatives; endocytosis; signaling; adaptor protein.

## Introduction

The low-density lipoprotein (LDL) receptor (LDLR) family comprises a large number of genes, whose products contain a characteristic set of structural domains. Here, we refer to the members of this family as LDLR relatives; several of the receptors are still better known under their originally proposed names and, where appropriate, these will also be indicated. LDLR relatives comprise composite membrane proteins engaged (i) in receptor-mediated endocytosis of a broad variety of ligands, the list of which now reaches far beyond lipoproteins and (ii) also, where indicated, in signal transduction pathways. The most prominent members of the family are, listed in the order of their discovery: LDLR; LDLR-related protein (LRP, LRP-1); megalin (LRP-2, originally called gp330); very low-density lipoprotein (VLDL) receptor (VLDLR; in chicken termed LR8); LR11 (also named sorLA); apolipoprotein E (apoE) receptor type 2 (apoER2, LRP-8, also known as LR7/8B); LRP-3, -4, -5, and -6, and LR32 (also termed LRP-1B) [1–3].

Common features of these proteins are structurally and functionally defined modules, which are frequently specified by distinct exons in the corresponding genes. These modules are (i) head-to-tail-arranged 'type A-binding repeats' (LA repeats) of ~40 residues harboring six paired cysteines in identical positions; (ii) 'type B repeats,' also containing six cysteines each; (iii) modules of ~50 residues with a consensus tetrapeptide, Tyr-Trp-Thr-Asp (YWTD); together with the type B repeats, five of these modules, tandemly arranged, constitute the so-called epidermal growth factor (EGF) precursor homology domain of LDLR relatives; (iv) a short stretch rich in serines and threonines carrying O-linked sugars, the so-called O-linked sugar domain; (v) a single transmembrane domain of approximately 20 amino acids, and (vi) the cytoplas-

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mic region with one or more short signals for receptor internalization via coated pits (e.g., FDNPVY in the LDLR).

In this review, we will deal in detail with the VLDLR, ApoER2, LRP, and LRP-5/6 (fig. 1), which have received attention either because of their involvement in signal transduction pathways, or, as is the case for LDLR, because novel interacting proteins modulate their endocytosis.

## LDLR

### The LDLR pathway

The LDLR is the key component in the feedback-regulated maintenance of cholesterol homeostasis in the body [4]. As an active interface between extra- and intracellular cholesterol pools, it is itself subject to regulation at the cellular level. LDL-derived cholesterol (generated by hydrolysis of LDL-borne cholesteryl esters) and its intracellularly generated oxidated derivatives mediate a complex series of feedback control mechanisms that protect the cell from overaccumulation of cholesterol. The key features of this pathway can be summarized as follows. First, (oxy)sterols suppress the activities of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase and HMG-CoA reductase, two key enzymes in cellular cholesterol biosynthesis. Second, the cholesterol activates the cytoplasmic enzyme acyl-CoA: cholesterol acyltransferase (ACAT; E.C. 2.3.1.26) which allows the cells to store excess cholesterol in re-esterified form. Third, the synthesis of new LDLRs is suppressed, preventing further cellular entry of LDL and thus cholesterol overloading. The overall benefits from, and consequences of, this LDLR-mediated regulatory system are coordination of the utilization of intra- and extracellular sources of cholesterol at the systemic level. Mammalian cells are able to subsist in the absence of lipoproteins because they can synthesize cholesterol from acetyl-CoA. When LDL is available, however, most cells primarily use the LDLR to import LDL cholesterol and keep their own synthetic activity suppressed. Thus, a constant level of cholesterol is maintained within the cell, while the external supply in the form of lipoproteins can undergo large fluctuation.

These concepts have arisen from detailed studies on cultured fibroblasts from normal subjects and patients with the disease familial hypercholesterolemia (FH). Lack of the above-described regulatory features in FH fibroblasts

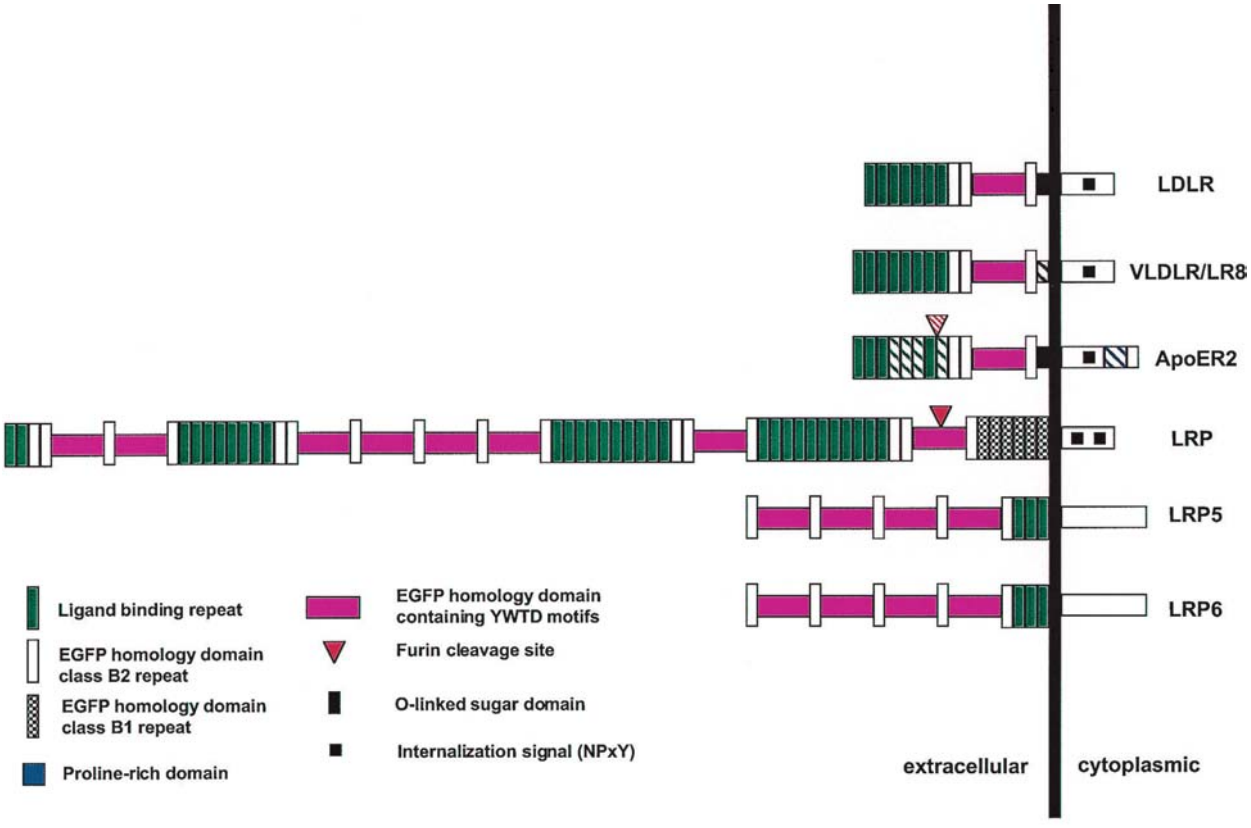


Figure 1. Structural organization of members of the LDLR family described in detail in this review. Distinct domains presented in the figure are described in the text. Hatched elements are facultatively present in variants of the corresponding receptors. Domains are not drawn to scale.

led to the conclusion that the abnormal phenotype is caused by lack of LDLR function and, thus, disruption of the LDLR pathway. In particular, the balance between extracellular and intracellular cholesterol pools is disturbed. Clinically, the most important effect of LDLR deficiency is hypercholesterolemia with ensuing accelerated development of atherosclerosis and its complications. In the following sections, a detailed description of the LDLR is provided, with emphasis on the impact of mutations on its structure and function.

## Relationships between structure and function of the LDLR

Studies at the levels of protein chemistry, molecular biology, and cell biology have led to a detailed understanding of the biology of the LDLR. The mature receptor is a highly conserved integral membrane glycoprotein consisting of five domains. As indicated in the Introduction, in order of appearance from the amino terminus these domains are: (i) the ligand-binding domain; (ii) a domain that has a high degree of homology with the EGF precursor (EGFP); (iii) a domain that contains a cluster of O-linked carbohydrate chains; (iv) a transmembrane domain, and (v) a short cytoplasmic region. Each of these is described briefly below.

The ligand-binding domain mediates the interaction between the receptor and lipoproteins containing apoB100 and/or apoE [5]. The function is localized to a region at the amino terminus of the receptor, comprised of seven repeats of approximately 40 residues each. These seven repeats each have six cysteines, which presumably mediate the folding of the domain into a rigid structure with clusters of negatively charged residues on its surface (with the signature tripeptide Ser-Asp-Glu, SDE). These clusters are thought to participate in the binding of lipoprotein(s) via positively charged residues on apoB100 or apoE.

The EGFP homology domain of the LDLR lies adjacent to the ligand-binding site and is comprised of approximately 400 amino acids; its outstanding feature is the sequence similarity to parts of the EGFP, i.e., three regions termed 'growth factor repeats.' Two of these are located in tandem at the amino terminus, while the other is at the carboxy terminus of the precursor homology region of the LDLR. The remainder consists of five ~50-residue stretches that contain tetrapeptide sequences with a consensus of Tyr-Trp-Thr-Asp. Experimental evidence suggests involvement of this region in the acid-dependent dissociation of the receptor from LDL, and its subsequent recycling.

The O-linked sugar domain of the human LDLR is a 58-amino acid stretch highly enriched in serine and threonine residues, located just outside the plasma membrane. Most, if not all, of the 18 hydroxylated amino acid side chains are glycosylated. The O-linked oligosaccharides undergo elongation in the course of receptor synthesis and maturation: when leaving the endoplasmic reticulum, N-acetylgalactosamine is the sole O-linked sugar present, and upon processing in the Golgi, galactosyl and sialyl residues are added. Despite detailed knowledge about the structure of this region, its functional importance remains unclear.

The membrane-anchoring domain lies carboxy terminally to the O-linked carbohydrate cluster. It consists of 22–25 hydrophobic amino acids; as expected, the deletion of this domain in certain naturally occurring mutations, or by site-directed mutagenesis, leads to secretion of truncated receptors from the cells.

The cytoplasmic tail of the LDLR constitutes a short stretch of 50 amino acid residues involved in the targeting of LDLRs to coated pits. Naturally occurring mutations and site-specific mutagenesis [6] have identified an 'internalization signal,' Asn-Pro-Xxx-Tyr (NPxY; where x denotes any amino acid). Recently, the cytoplasmic domains of the LDLR and structural relatives have come into new focus, since they hold the key to the involvement of these receptors in signal transduction, as indicated below.

There is a strong correlation between the functional domains of the protein and the exon/intron organization in the gene. The ~48-kb human LDLR gene contains 18 exons and is localized on the distal short arm of chromosome 19. For example, the seven cysteine-rich repeats of the ligand-binding domain are encoded by exons 2 (repeat 1), 3 (repeat 2), 4 (repeats 3, 4, and 5), 5 (repeat 6), and 6 (repeat 7). The EGFP homology domain is encoded by eight exons, organized in a manner very similar to the gene for the EGFP itself. The third domain is translated from a single exon between introns 14 and 15. Thus, the LDLR gene is a compound of shared coding sequences; in fact, many more molecules containing all or some of these elements have been discovered and likely will continue to be found.

Molecular genetic studies in FH patients have identified over 600 different mutations in the LDLR gene. A list of all mutations with their original literature citations can be found at http://www.ucl.ac.uk/fh/.

## Modulation of LDLR endocytosis activity

### Autosomal recessive hypercholesterolemia

Recently, a phenotype analogous to FH has been shown to be associated with a defect other than in the LDLR gene [7]. This defect has subsequently been determined to involve a novel gene on the short arm of chromosome 1, ARH (autosomal recessive hypercholesterolemia) [8–10]. The product of the gene has been invoked as a putative adaptor protein for the LDLR, as it contains a phos-

photyrosine-binding (PTB) domain. PTB domains have been reported to bind NPxY motifs in the cytoplasmic tails of cellsurface receptors; the selectivity of this binding may be responsible for the specificity of the biological response. For example, although the *Drosophila* SHC adaptor protein binds to several receptor tyrosine kinases, different combinations of SHC with other cytoplasmic adaptor proteins trigger the activation of different downstream targets [11]. In human fibroblasts, the levels of ARH mRNA, in contrast to LDLR mRNA, were not affected by the addition of sterols to the medium, and in the fibroblasts of ARH patients, LDLR function was unchanged [10]. Thus, at least in this cell type, the function of the protein may not be important in the endocytic pathway of the LDLR. A different adaptor protein may be more important in fibroblasts, or may compensate for the mutated ARH gene. On the other hand, the product of the ARH gene could be important in a step of the LDLR pathway that is specific to polarized cells like hepatocytes [12]; for example, it may be required for the trafficking of LDLR to the basolateral surface. Alternatively, it may target the LDLR to coated pits after the receptor binds LDL, because, in contrast to fibroblasts, LDLRs in hepatocytes do not cluster in coated pits in the absence of ligands [13]. Another possibility is that it is involved in LDLR recycling from the lysosome to the basolateral surface after dissociation from LDL [10].

The lipid abnormalities in ARH appear to be a close phenocopy of homozygous FH, which suggests that all clinical sequelae of ARH gene mutations are attributable to defective LDLR activity, and this in turn has been the main argument for the suggestion that the ARH protein specifically binds to the LDLR [10]. However, although both ARH and LDLR genes appear to be nearly ubiquitously expressed, LDLR expression is relatively low in some of the tissues that express high levels of ARH (e.g., kidney and placenta), raising the possibility that this protein may (also) be involved in other receptor pathways [10].

Although the specific role and the importance of the ARH protein in the function of the LDLR in polarized cells and fibroblasts remains to be defined, it obviously has at least partly different functions, or interacts with different components of the endocytic machinery in the two cell types. In any case, the crucial role of this protein is revealed by the profound hypercholesterolemia that occurs in ARH patients and, thus, it will be extensively investigated in the future.

### SNX17 modulates LDLR endocytosis

The sorting nexin (SNX) family of proteins is characterized by the presence of a phox homology domain [14]. This domain mediates the association of SNX proteins with phosphoinositides and recruits them to specific membranes or vesicular structures within cells. Although only limited information about SNXs and their functions is available, they seem to be involved in membrane trafficking and sorting processes by directly binding to target proteins including certain growth factor receptors such as EGFR, platelet-derived growth factor receptor (PDGFR), and transforming growth factor (TGF)-$\beta$ receptors. SNX1, the best studied mammalian member of this family, interacts with the lysosomal targeting signal of EGFR, thereby enhancing the rate of degradation of this receptor [15]. In a screen for candidate interaction partners of apoER2, we recently identified SNX17 as an adaptor protein which interacts with the intracellular domains of the LDLR, VLDLR, apoER2, and LRP [16]. SNX17 resides on distinct vesicular structures partially overlapping with endosomal compartments characterized by the presence of EEA1 and rab4. Use of rhodamine-labeled LDL demonstrated that during endocytosis, LDL passes through SNX17-positive compartments of the endosomal machinery. Functional studies of the LDLR pathway showed that SNX17 enhances the endocytosis rate of this receptor [16].

## VLDLR and apoER2

### VLDLR in mammals

The overall modular structure of the VLDLR [17] is virtually superimposable with that of the LDLR, except that the ligand-binding domain contains eight, rather than seven, LA repeats. The two receptors contain an 8- to 10-residue so-called linker region, in the LDLR between repeats 4 and 5, and in the VLDLR between repeats 5 and 6; furthermore, repeats 3–5 in the LDLR and 4–6 in the VLDLR are encoded by single exons, suggesting that repeat 1 is the 'additional' repeat in the VLDLR [18]. Amazingly, the VLDLR gene shows almost complete conservation of its exon/intron organization when compared to the LDLR gene [18]. Analysis of approximately 1200 bp of the 5′-flanking region of the human VLDLR gene revealed an inverted CCAAT box, an Sp1 site, half sites for binding of glucocorticoid and estrogen receptors and, most notably, two copies of a potential sterol regulatory element (SRE-1), which mediates the downregulation of LDLR expression in response to rising intracellular cholesterol levels [19]. In the promoter of the murine vldlr gene, nine half-sites for progesterone and glucocorticoid receptors are also present in addition to other potential sites for Jun/Ap-1, Sp1, AP-1, GATA-1, and NF-1 [20]. Whether the half-sites for glucocorticoid receptors are responsible for the strong induction of VLDLR expression in differentiating 3T3-L1 cells by dexamethasone remains to be established. Other expression studies in different cell types and organisms have not yet resulted in a clear understanding of the regulation of this gene [reviewed in ref. 21].

The VLDLR shows a very high degree of conservation among different species, e. g., 95% identity within mammals. Even the proteins of more distant species such as the chicken [22] and *Xenopus laevis* [23] share about 80% identical residues with the human VLDLR [18]. The VLDLR exists in variant forms arising from differential splicing [18, 24–27]. The longer and predominant splice form contains a stretch of 84 nt (exon 16 in the human gene) specifying an O-linked sugar domain similar to that always present in LDLRs. In rat and human brain, an additional variant lacking exon 4, which encodes LA repeat 3, has been detected [25, 28].

**VLDLR and mammalian lipoprotein metabolism**

The function of the mammalian VLDLR in relation to lipoprotein metabolism is still not completely resolved. Due to its expression in adipose tissue, striated muscle, and brain, but not in the liver [27, 29, 30] and due to its high affinity for apoE [17, 31], this receptor has been implicated in the extrahepatic metabolism of triglyceride-rich lipoproteins. Indeed, the VLDLR mediates uptake of chylomicron remnants in overexpressing cells [32] and hypercholesterolemia in LDLR knock-out mice could be reversed by adenovirus-mediated transfer of the vldlr gene leading to high-level hepatic expression of the receptor [33, 34]. In addition, adenovirus-mediated VLDLR expression in livers of apoE2- and apoE3-Leiden mice lowered cholesterol levels, showing that the VLDLR, in contrast to the LDLR, binds these apoE variants avidly [35]. Taken together, these results demonstrate that the VLDLR is competent in binding and internalization of apoE-containing lipoproteins in vitro and in vivo. Close inspection of the expression sites in the relevant tissues (muscle, heart, and adipose tissue) revealed that the VLDLR resides on endothelial cells of capillaries and small arterioles rather than on parenchymal cells of these organs [36]. The capillary endothelium in muscle and adipose tissue is continuous and therefore impermeable for particles with the size of lipoproteins. This is consistent with a scenario where TG-rich lipoproteins are taken up directly by endothelial cells and triglycerides or free fatty acids subsequently delivered to adipocytes or myocytes. Upregulation of VLDLR in heart and downregulation in adipose tissue in fasting rats [37] would be in agreement with such a model. Another possibility is that triglyceride-rich particles are trapped on the surface of endothelial cells by the VLDLR and, subsequently, are lipolyzed by lipoprotein lipase (LPL) which also resides on endothelial cells. Furthermore, LPL might tether triglyceride-rich particles to the cell surface by bridging them to proteoglycans [38, 39]. Subsequently, triglycerides would become mobilized by the lipolytic action of LPL and the resulting remnants taken up by the VLDLR. However, as demonstrated by Argraves et al. [40], the VLDLR is able to directly bind and catabolize LPL, suggesting that the VLDLR might serve as a regulator of cell surface-bound LPL.

In any case, all of these proposed function(s) would predict that the VLDLR plays a significant role in the metabolism of triglyceride-rich particles. However, homozygous disruption of the vldlr gene in mice did not alter the lipoprotein profile of these animals, nor did it impair the function of the heart or fertility [41]. A slightly reduced body mass index of these mice due to a reduction in adipose tissue mass nevertheless points to a reduced energy transport into adipocytes. Since the mouse is a 'high-density lipoprotein animal,' lack of the VLDLR might not lead to a dramatic phenotype under normal dietary conditions. Thus, VLDLR–/– mice have been cross-bred to LDLR–/– mice which show a 'humanized' lipoprotein profile [42], and their lipoprotein profiles were studied under various dietary conditions [43]. Under a high-fat diet as well as after prolonged fasting, lack of the VLDLR resulted in a significant increase in serum triglyceride levels in these animals, suggesting that the VLDLR is involved in peripheral triglyceride uptake. Furthermore, a detailed study of weight gain in VLDLR–/– mice under a high-fat, high-calorie diet and on an ob/ob background was conducted [44]. The most important finding of this study was that mice lacking a functional VLDLR were protected from obesity, obviously via a significant reduction in whole-body free fatty acid uptake. Taken together, the VLDLR seems to be part of a machinery transporting triglycerides or free fatty acids to peripheral cells, but the molecular details of the mechanism still remain to be elucidated.

Besides lipoproteins, the VLDLR also binds many other unrelated ligands, and as discussed below is involved in physiological processes other than lipid metabolism. These ligands include urokinase-type plasminogen activator-type-1/plasminogen activator inhibitor complexes [45], thrombospondin [46], and reelin [47, 48].

**VLDL/vitellogenin receptors in egg-laying species**

The biological role of the VLDLR homologue of the chicken (also termed LR with eight LA repeats, or LR8) is documented by both biochemical and genetic evidence: it mediates a key step in the reproductive effort of the hen, i. e., oocyte growth via yolk precursor uptake [22]. In the female chicken, large amounts of VLDL and vitellogenin (VTG) are synthesized in the liver and transported to the ovary by the general circulation. The specific uptake of these components into growing oocytes, termed vitellogenesis, is achieved by endocytosis mediated by LR8, as reviewed elsewhere [21, 49, 50]. Functional absence of LR8 leads to the inability of oocytes to enter the rapid growth phase and, consequently, failure to lay eggs, as observed in the 'restricted ovulator' (R/O) strain, which car-

ries a point mutation at the lr8 locus [51]. As a consequence of the inability to deposit VLDL and VTG, which are produced at normal levels in the liver, into their oocytes the mutant R/O females develop severe hyperlipidemia and features of atherosclerosis. Interestingly, LR8 expressed in chicken oocytes lacks the O-linked sugar domain; the physiological relevance of this, however, is still not resolved [24]. Homologues of LR8 in *Xenopus* [23], fish [52], mosquito [53], and *Caenorhabditis elegans* [54] have been characterized and support the hypothesis that this protein plays a key function in oocyte development in most if not all egg-laying species. This is surprising, since the major yolk components are quite different in these species, varying from lipophorin in mosquito to VTG in fish, and VTG and VLDL in birds. VLDLRs can apparently interact with many, if not all, ligands of younger relatives of the receptor family. In this context, VTG, absent from mammals, and apoE, not found in birds, but recognized by LR8 in vitro, have certain common biochemical properties and sequence similarities, and thus have been suggested to be functional analogues [55]. Even high-density lipophorin, an abundant lipoprotein in the circulation of insects, binds to an eight-repeat LR with very high similarity to LR8. In any case, the requirement for these receptors in oocyte development, at least in oviparous species, suggests that the VLDLR/LR8 family branch is indeed among the oldest in the line of LDLR relatives. VLDLR homologues are not the only yolk receptors however, since in *Drosophila* [56] and mosquito [57], another member of the LDLR family takes part in VTG transport into the oocyte. These proteins are significantly larger than VLDLRs and, harboring a second cluster of five ligand-binding repeats and additional EGFP repeats, appear to have amino-terminal expansions of the VLDLR, but are still smaller than LRP or megalin.

**apoER2**

apoER2 [58] and its avian homologue LR7/8B [59] are made up of exactly the same domains in the same order as in the LDLR and VLDLR. However, the occurrence of tissue- and species-specific splice variants adds a level of complexity to this sub-family of proteins. In chicken, where the prevalent site of expression is the brain, two distinct variants of the ligand-binding domain harboring seven or eight LA repeats exist [60]. The mouse, however, produces three major transcripts varying in their ligand-binding domain, none of which contains repeats 4–6 [61]. Analysis of the murine gene demonstrated almost complete conservation of the exon/intron organization when compared to the vldlr gene, suggesting that the ldlr, vldlr, and apoer2 genes are the most closely related members of the family. This analysis also demonstrated that the exon coding for repeats 4–6 exists in the murine gene, but is constitutively deleted by differential splicing in mice. However, these results have to be taken with some caution, since for human transcripts of apoER2, due to the strong secondary structure in the mRNA, RT-PCR in some cases preferentially amplifies variants lacking exon 5 (coding for repeats 4–6) [62]. There is also an additional small exon following that for LA repeat 8, which gives rise to a variant harboring a furin consensus cleavage site at the carboxyl-terminal end of the ligand-binding domain. This leads to the secretion of a soluble receptor fragment containing the entire ligand-binding domain [63]. Most interestingly, this variant is the only one detectable in the placenta, showing that some of the splicing events are tissue specific. The situation in humans is not yet clear. Obviously, none of the characterized transcripts contain repeat 8 [58, 64]. The corresponding exon, however, seems to be present in the human gene, but due to a mutation in the 5′ splice donor site, it is constitutively skipped [65]. In addition to the murine variants described, a receptor transcript containing only repeats 1–3 has been reported to be present in humans. Although not described as part of the human gene [64], RT-PCR analysis of human transcripts [60] suggests the presence of a corresponding exon coding for a furin site at the carboxyl-terminal end of the ligand-binding domain, as in the murine gene. Analysis of apoER2 transcripts present in the vascular wall revealed an additional transcript which lacks repeats 4–6, but contains an additional LA repeat and a unique cysteine-rich domain with no homology to known modules in the receptor family [66]. Besides variations in the ligand-binding domain of apoER2, an interesting structural feature in the short intracellular domain was discovered [60]. In human and murine transcripts, an insertion of 177 bp is derived from a separate exon in the human and murine gene, absent from the chicken gene. It codes for a 59-amino-acid proline-rich insertion in the respective receptor tails. The protein sequence of the murine insert differs from that of the human in only five positions, all of them due to conservative substitutions. Homology searches revealed that the 59-amino-acid insert represents a unique sequence not found in any published protein so far.

Expression of apoER2 in 293 cells demonstrated that it is endocytosis competent [61]. However, direct comparison of the cytoplasmic domains of LRP, LDLR, VLDLR, and apoER2 demonstrated that the cytoplasmic tail of apoER2 exhibited the lowest endocytosis rate [67]. These results are supported by the finding that in contrast to LDLR, apoER2 overexpressed in CHO cells is localized to caveolae, suggesting a role in signaling rather than in endocytosis [68].

**VLDLR and apoER2 mediate reelin signaling**

An exciting breakthrough in understanding the biology of the VLDLR and apoER2 was made by analyzing mice

lacking both of these receptors by targeted disruption of the respective genes [69]. Absence of both apoER2 and the VLDLR, leads to an inversion of cortical layers and absence of cerebellar foliation. This phenotype is indistinguishable from that seen in animals carrying either a mutation in the reelin gene (reeler mouse) [70] or in the disabled-1 gene [71]. During mammalian brain development, layer I (marginal zone) and the sub-plate (layer VII) of the neocortex evolve first from the primordial plexiform neuropil [for reviews see refs 72, 73]. At this stage of development (E11), both layers together form the preplate. Then (E13), newborn neurons migrate along radial glial fibers from their birthplace in the ventricular zone until they reach the marginal zone, thereby separating the marginal zone from the subplate. These early neurons establish the first neuronal layer of the forming cortical plate as they detach from the fibers, fully differentiate, and form dendrites anchoring these cells in the marginal zone. Neurons generated later bypass the first layer of the cortical plate until they again reach the marginal zone where they establish the next neuronal layer of the cortical plate. In humans, this process is repeated five times, establishing the cortical plate with a characteristic pattern of six neuronal layers in an inside-out orientation where the youngest layer is located directly underneath the pial surface and the marginal zone. In the reeler mouse, however, sub-plate neurons invade the marginal zone forming a densely packed preplate together with the cells present in the marginal zone [for reviews see refs 74, 75]. Cortical plate neurons cannot invade this layer, thus the preplate does not split, and consecutive waves of cortical neurons accumulate underneath it in the sequence of their generation, giving rise to an inverted layering of the cortical plate neurons. Correct positioning of the neurons of the sub-plate and cortical plate neurons depends on reelin, an extracellular matrix protein secreted from Cajal-Retius cells [76], and on the intracellular adaptor protein disabled-1 (Dab1) which is expressed by cortical plate neurons [77]. As mentioned above, mutations in these genes, i.e., the reelin gene [70] and the Dab1 gene [71], have the same cortical layering defect, suggesting that both genes are coding for proteins which are part of the same signaling pathway. Homologous proteins have been presumably present in stem amniotes and the corresponding signaling pathway (‘reelin pathway’) played an important role in the architectonic evolution of the mammalian brain [for a review see ref. 78].

The current working model proposes that reelin binds directly to apoER2 and VLDLR expressed by cortical plate neurons [47, 48]. Subsequent phosphorylation of Dab1 is a key event leading to the ultimate cell responses required for correct positioning of newly generated neurons [80–82]. Dab1 was originally identified as an interaction partner of Src, Fyn, and Abl [83] and contains a phosphotyrosine-binding (PTB) domain which interacts with the unphosphorylated NPxY motif present in the cytoplasmic domains of LDLR reltives [84, 85]. Despite genetic evidence that cdk5/p35 might be part of the reelin pathway [86, 87], very little is known about the signaling cascade downstream of Dab1, nor is the kinase known which phosphorylates Dab1. A first step toward identifying mechanisms modulating the reelin signal was recently achieved in our laboratory. As mentioned above, neurons express an apoER2 variant which contains a furin cleavage site at the end of the ligand-binding domain. This receptor variant is indeed cleaved, and the secreted soluble ligand-binding domain interacts with reelin, thereby preventing reelin-induced signaling [63]. Whether phosphokinase C-dependent phosphorylation of the intracellular domain of the VLDLR plays a role in reelin signaling remains to be established [88].

Unraveling parts of the reelin pathway clearly showed for the first time that members of the LDLR family are part of a signal transduction cascade. Since Dab1 not only binds to the intracellular domains of the VLDLR and apoER2, but also to those of the LDLR and LRP [69], signal transduction could be a general function besides endocytosis of macromolecules common to many members of the LDLR family. Adaptor molecules like Dab1 might be part of a machinery which defines the actual function of a particular member of the receptor family. Recent screens for such adaptors have led to a list of potential interacting partners including Jip-1 and -2, PSD-95, CAPON, and SNX17, to name a few [16, 89, 90]; for a comprehensive list of potential interaction partners see Nykjaer an Willnow [2]. Proteins like Jip (JNK-interacting protein) might link the receptors to signal cascades, while others like SNX17 and ARH [10] modulate the endocytic function of the receptors (see above).

**LRP, more than a receptor for ‘everything’?**

As discussed above, LRP is one of the largest members of the LDLR family. It might be its sheer size which enables LRP to recognize more than 30 different ligands [for review see ref. 91]. For most of these ligands, LRP acts as a cargo receptor removing the proteins from the surface of a variety of cells. In contrast to the role of apoER2, endocytosis is an undisputed and probably the most important function of LRP. LRP has an intracellular tail which is significantly longer than those of the VLDLR and LDLR, and which mediates the highest endocytosis rate in comparison to other members of the family [67]. The intracellular domain of LRP contains two NPxY motifs and one YxxL motif which is not present in LDLR, VLDLR, or apoER2. Interestingly, the YxxL motif serves as the dominant signal for endocytosis of LRP [92]. In addition, cAMP-dependent phosphorylation of serine 76 within the cytoplasmic tail of LRP modulates the efficiency of endocytosis, suggesting a possible regulation of

LRP-mediated endocytosis by external signals [93]. Tyrosine phosphorylation of the intracellular domain, however, might be involved in cellular transformation by v-Src [94]. In these cells, v-Src present at the cell membrane phosphorylates the tail of LRP, which then provides a binding site for the PTB domain of Shc. This interaction brings Shc in close proximity to v-Src, which then also becomes tyrosine phosphorylated. In such a scenario, LRP acts as an anchor rather than as bona fide signaling receptor. Recent results, however, demonstrated that PDGF-BB directly binds to LRP and together with the PDGFR induces tyrosine phosphorylation of the intracellular domain of LRP [95, 96]. The target tyrosine is located in the second NPxY motif and once phosphorylated generates a binding site for Shc as demonstrated in v-Src-transformed cells [94]. Interestingly, PDGF-BB induced phosphorylation of LRP present in the caveolae fraction, but LRP present in the non-caveolae fraction remained unphosphorylated [95]. These data suggest that PDGF-BB might heterodimerize LRP and the PDGFR, both of which are present in the caveolae fraction of the cell membrane. Whether the PDGFR kinase directly phosphorylates LRP, or if another kinase present in the PDGFR/LRP signalosome is necessary is not yet resolved. In any case, identification of LRP as part of a signaling pathway involving the PDGFR confirms results that show that apo-E inhibits PDGF-induced cell migration in an LRP-dependent manner [97, 98]. This may also involve plasminogen activator inhibitor-1/urokinase-type plasminogen activator rceceptor metabolism by LRP, modulated by LR11 [99].

Another strong indication that LRP is both an endocytic cargo receptor and a signaling receptor came from studies on neuronal calcium signaling via N-methyl-D-aspartate receptors [100] and from studies demonstrating that urokinase regulates vascular smooth muscle cell contraction via an LRP-dependent signaling pathway [101]. Activated $\alpha_2$-macroglobulin, which is present in the cerebrospinal fluid [102] and is a ligand for LRP [103], induces calcium influx in cultured primary neurons. This effect is mediated by the N-methyl-D-aspartate receptor, and apparently involves LRP dimerization. Whether this signaling effect also involves phosphorylation of the intracellular domain of LRP is not known. PSD-95, which binds to the LRP tail [89], might be a candidate adaptor protein mediating the interaction of LRP with the N-methyl-D-aspartate receptor as suggested by Herz and Strickland [91]. On the other hand, blood pressure regulation by urokinase in mice is lost in vivo by the administration of receptor-associated protein and an antibody against LRP but not by an antibody against the LDLR [101].

A recent publication from the laboratory of G. Baier demonstrated that a fusion protein of the intracellular domain of LRP and the transmembrane and extracellular domains of IgG-Fc sequesters activated JNK to the plasma membrane, thereby inhibiting JNK-dependent activation of Elk-1 and c-Jun [104].

Finally, recent work by the group of J. Herz also suggests that LRP not only internalizes 'everything,' but might also signal in almost every possible way [105]. In analogy to members of the Notch family, LRP can be proteolytically processed within the transmembrane domain leading to the release of the entire cytoplasmic domain of the receptor into the cytosol. Cleavage is at least in part mediated by a $\gamma$-secretase-like activity and can be modulated by phorbol esters. Whether the released LRP tail acts as a cofactor in a putative transcription complex or as a modulator for the localization of adaptor proteins which could bind to the intracellular domains of LRP and other signaling members of the family can be expected to be answered in the near future.

## LRP-5 and LRP-6 in Wnt signaling

LRP-5 and LRP-6 are closely related type I membrane proteins, approximately 1600 residues long (about twice as large as the LDLR), and their extracellular domains are organized exactly as a portion of LRP [106, 107]. The cytoplasmic domains of LRP-5 and LRP-6 contain motifs (dileucine, and aromatic-X-X-aromatic/large hydrophobic) similar to those known to be functional in endocytosis of other receptors. Importantly, they harbor serine- and proline-rich stretches that may serve as ligands for Src homology 3 (SH3) and WW (a variant of SH3) domains, properties that relate these receptors to signal transduction pathways different from those of apoER2 and the VLDLR described above. Indeed, LRP-6 has been shown to be an indispensable element of the canonical Wnt pathway [108] which has been implicated in many processes of metazoan development, cell proliferation, and tumor formation [for a review see ref. 109]. Wnts are secreted glycoproteins which bind to seven-transmembrane receptors of the frizzled family. These are linked via dishevelled to a multiprotein complex consisting of axin, GSK3$\beta$, and others. In the absence of Wnts, $\beta$-catenin is phophorylated by GSK3$\beta$, which leads to its ubiquitination and subsequent degradation by proteasomes. In the presence of Wnts, the activity of GSK3$\beta$ is blocked, $\beta$-catenin is not degraded and is translocated into the nucleus where it assembles with TCF/LEFs to a transcription complex regulating the expression of Wnt target genes. Experiments performed in *Drosophila* [110], *Xenopus* [111], and mice [112] demonstrated that LRP-5 and LRP-6 act as coreceptors for Wnts, which have to bind to both frizzled and LRP-5 or LRP-6 in order to turn on the canonical Wnt pathway. As demonstrated for LRP-5, these receptors seem to transduce the Wnt signal by binding and recruiting axin to the cell membrane [113]. As mentioned above, axin, which inter-

acts with the tumor suppressor APC, is an important component of the complex regulating the phosporylation status of $\beta$-catenin. Recently, LRP6 has been demonstrated to interact with proteins called Dickkopf (DKK) [114]. DKKs inhibit Wnt signaling by releasing receptor-bound Wnt from LRP6 and bridging LRP-6 to Kremen 1 and 2 [115]. The Kremen/DKK/LRP6 complex is then rapidly removed from the cell surface by endocytosis. Thus, the inhibitory action of DKK is not restricted to disruption of the Wnt signaling complex, but is even potentiated by removing one player from the place of action.

As demonstrated by the identification of LRP-5 as the affected gene in the autosomal recessive disorder osteoporosis-pseudoglioma syndrome [116], the interplay of Wnt, Dickkopf and Axin associations with LRP-5/6 may hold the key to important developmental signals, similar to the role of the VLDLR and apoER2 in neuronal migration.

*Acknowledgements.* Experiments by the authors cited in this article were supported by the Austrian Science Foundation Grants P11694-MOB, P13940-MOB, and F608 (to W. J. S.) and P11692, P13931-MOB, and F606 (to J. N.).

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