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Review

Molecular mechanisms of lipoprotein receptor signalling

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Abstract. The low-density lipoprotein (LDL) receptor is the prototype of a classical endocytosis receptor that mediates the uptake of extracellular ligands. Other members of the LDL receptor gene family, on the other hand, have been shown to regulate intracellular signalling cascades. Among these are the LDL receptor-related protein 1, LRP1, a promiscuous and ubiquitously expressed receptor which is critically involved in a multitude of diverse physiological processes; the Reelin receptors ApoER2 and VLDL receptor, which participate in neuronal development; and megalin, a multifunctional receptor expressed in various epithelia. In this review, we focus on recent developments that highlight similarities and differences between these related receptors and their biological function, and discuss open questions as to the underlying molecular mechanisms.

Key words. LRP; ApoER2; VLDLR; megalin; PDGF; Reelin; Dab1; MAP kinase; signal transduction; endocytosis; atherosclerosis; development; neuronal migration; synapse; gamma-secretase; apolipoprotein E; Alzheimer's disease

Introduction

The LDL receptor gene family consists of seven structurally closely related transmembrane proteins that participate in a wide range of biological processes, including the regulation of lipid metabolism, protection against atherosclerosis, neurodevelopment, and transport of nutrients and vitamins (table 1). The LDL (low-density lipoprotein) receptor, the founding member of the family, regulates cholesterol homeostasis by receptor-mediated endocytosis of lipoprotein particles, and mutations of the encoding gene cause familial hypercholesterolemia. While currently available data suggest that the role of the LDL receptor is limited to the uptake of lipoproteins, growing experimental evidence suggests that the other members of the gene family have additional functions as signal transducers [1–3]. LRP (LDL receptor-related protein, LRP1) was cloned as the second and one of the most versatile members of this ancient gene family. It binds more than 40 different ligands ranging from lipoproteins, protease/protease inhibitor complexes, extracellular matrix proteins and viruses to growth factors and cytokines [4, 5]. Recent insights into its atheroprotective functions in the vessel wall as well as its role in the adult brain will be reviewed in this article. Two other members of the family, apoER2 (apoE receptor 2) and VLDL receptor (very low density lipoprotein receptor, VLDLR), have been recognized as signal transducers for the secreted glycoprotein Reelin, which regulates neuronal positioning in laminated structures of the developing brain. Novel developments with regard to the underlying signal trans-

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Table 1. Ligands and functions of the mammalian core LDL receptor family members. The table highlights only a selection of biological functions and ligands of the listed receptors.

Receptor	Expression	Biological functions	Ligands
LDLR	ubiquitous, e.g. hepatocytes, macrophages, central nervous system	cholesterol homeostasis	apolipoprotein E, apolipoprotein B, LDL
LRP1 (LRP)	expression by a wide range of cell types and tissues, e.g. hepatocytes, neurons, vascular smooth muscle cells, macrophages, trophoblast, embryonic tissues	endocytosis of a broad range of ligands, including protease/protease inhibitor complexes etc. (see ligands), chylomicron remnant receptor, regulation of PDGF receptor signaling, regulation of calcium currents, phagocytosis of apoptotic cells, embryonic development	apolipoprotein E, chylomicron remnants, α2-macroglobulin, amyloid precursor protein (APP), tPA, protease/protease inhibitor complexes, lipoprotein lipase, PDGF, TGFβ
VLDLR	developing and adult brain, heart and endothelial cells, adipose tissue	neuronal migration; synaptic transmission	apolipoprotein E, Reelin, lipoprotein lipase, tissue factor pathway inhibitor
ApoER2	Developing and adult brain, testis	neuronal migration, synaptic transmission, male fertility	apolipoprotein E, Reelin
Megalin	apical plasma membrane of absorptive and secretory epithelia (e.g. renal proximal tubule), thyroid and parathyroid gland, tropho-ectoderm, visceral yolk sac, neuroectoderm	vitamin/nutrient supply; calcium homeostasis, recovery of excreted low-mole-cular weight proteins and vitamin D/vitamin D binding protein complexes, uptake and transcytosis of thyroglobulin; PTH internalization, regulation of BMP-4 signaling	apolipoprotein B, Apolipoprotein E, Apolipoprotein J, Apolipoprotein H, albumin, cubilin, retinol-binding protein, Vitamin D-binding protein, sonic hedgehog, BMP-4
LRP1b	restricted expression pattern (central nervous system)	unknown	synaptotagmin, laminin receptor precursors
MEGF7	restricted expression pattern; embryogenesis, adult CNS	unknown	unknown

duction mechanisms will be another focus of this review. Finally, we will discuss recent work which suggests a role for megalin, the largest member of the LDL receptor family, at the crossroads of signal transduction and endocytosis in forebrain development where it regulates the availability of the morphogen BMP4. The function and cell biology of LRP1b and MEGF7, the remaining two members of the gene family, are still largely unknown to date and will not be covered in this review. For excellent summaries of LDL receptor structure and function and of the signaling activities of the more distantly related receptors LRP5/6, we refer the reader to [6, 7].

LRP1

LRP1 was the second member of the LDLR-family to be identified [8]. It is a ubiquitously expressed protein that has a dual role in endocytosis and signal transduction [4]. First, its function as an endocytic receptor was recognized and a wide variety of ligands for this receptor have been identified up to now ([2], see also table 1).

In an initial study by Gotthardt et al., which identified the interaction of lipoprotein receptor intracellular domains with cytoplasmic signaling and adaptor molecules, LRP1 was found to have binding partners unrelated to the endocytosis machinery [9]. This suggested that LRP1, like ApoER2 and VLDLR [10], might have a role in the regulation of cellular signal transduction processes – a novel function that might help explain the early embryonic lethality of the conventional *LRP1* gene knockout in mice [11], which had been difficult to understand on the basis of defective ligand-uptake alone. The early embryonic death of homozygous *LRP1*-deficient mice had hindered the molecular analysis of LRP1-regulated processes, but the advent of tissue-specific *LRP1* knockout animals as well as the in vivo and in vitro use of neutralizing receptor antibodies helped to elucidate its functions in different organs as well as the mechanisms of its actions, including the interaction with different signaling pathways (summa-

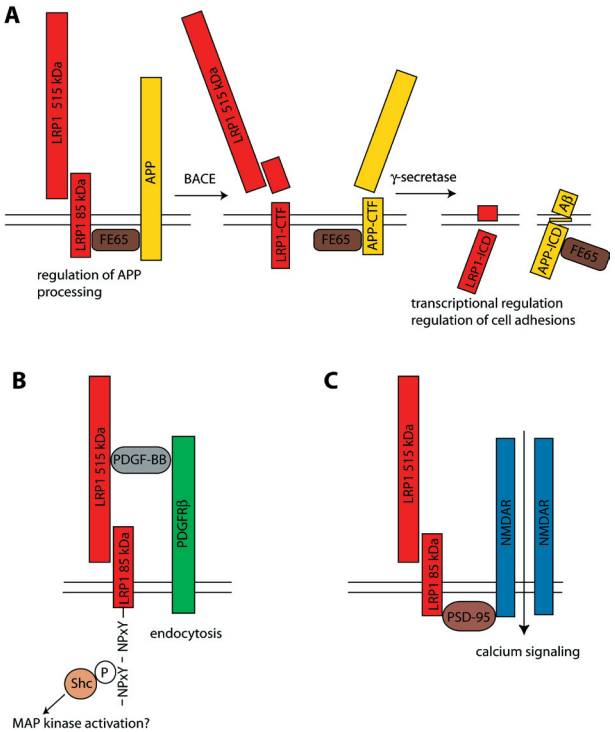

Figure 1. (A) LRP1 undergoes proteolytic processing. After furin cleavage in the trans-Golgi the mature LRP1 consists of two non-covalently linked subunits. The membrane-bound fragment can be cleaved extracellularly so that most of the receptor's extracellular domain is shed. Recently, BACE was identified as a protease that can execute this processing step. Following the shedding of the extracellular domain, the intracytoplasmic tail of LRP1 is released by an additional proteolytic cleavage event. This allows translocation of the ICD and interacting proteins to other subcellular localizations. Processing of LRP1 is similar to that of the transmembrane amyloid precursor protein APP, which it can interact with through the cytoplasmic adaptor protein FE65. LRP1 influences APP processing in that it increases BACE-dependent extracellular and subsequent γ-secretase-mediated cleavage of APP. This effect is dependent both on the LRP1 cytoplasmic domain and the presence of FE65. (B) LRP1 regulates PDGF receptor signaling. LRP1 directly binds to PDGF-BB and is phosphorylated in response to PDGF treatment. Tyrosine-phosphorylated LRP1 can interact with the cytoplasmic adaptor protein Shc, providing a possible link to the intracellular effectors of PDGF receptor activation. Besides, LRP1 regulates the surface expression and half-life of PDGFRβ via endocytosis and regulation of ubiquitination of the PDGFR. (C) LRP1 and calcium signaling. LRP1 binds to the postsynaptic density protein PSD-95, which in turn interacts with NMDAR subunits. Consequently, NMDAR subunits can be coprecipitated with LRP1 from neuronal lysates. This interaction may underlie the previously observed influence of LRP1 on NMDA-dependent calcium currents.

rized in fig. 1). The tissues that have been studied include blood vessels, where the interaction with growth factors and tissue plasminogen activator (tPA) influence vessel development and integrity, tone and grade of permeability [12–14], neuronal tissues, where ion channel function and APP processing are influenced, and reticuloendothelial cells, where phagocytosis and inflammatory signaling pathways are partly dependent on LRP1 [15, 16].

LRP1 in the vessel wall

The study of conditionally gene-targeted mice that lack *LRP1* in smooth muscle cells, including those of the tunica media of blood vessels, show that this receptor has a previously unappreciated role in the formation and maintenance of the wall of medium- and large-size arteries. Loss of *LRP1* in smooth muscle cells leads to vessels with a hugely hypertrophic media and disrupted lamina elastica as well as increased susceptibility to atherosclerosis [12]. Hyperactive PDGF signaling likely contributes to the observed phenotype, as increased steady-state levels of PDGF receptor β and augmented phosphorylation of Erk1/2 were observed in the aortic walls of the conditionally *LRP1*-defective animals [12]. PDGF is a growth factor that stimulates smooth muscle cell proliferation and migration in the arterial wall both during development [17] and in pathological states such as atherogenesis (reviewed in [18]). It could be shown by in vitro studies that LRP1 binds directly to PDGF BB and is phosphorylated in response to this interaction [19, 20]. Phosphorylated LRP1 can interact with Shc [2, 21], which might constitute a link to downstream PDGF-dependent signaling events such as MAP kinase activation. Furthermore, it could be shown in a tissue culture model that LRP1 contributes to the regulation of cell surface levels of PDGF receptor β [22], providing an additional mechanism for the LRP1-dependent control of PDGF signaling pathways.

Additional interactions between LRP1 and growth factors that could contribute to the vessel pathology described above include a link to transforming growth factor- (TGFβ) signaling. Indeed, LRP1 is identical to TGFβ receptor V, which mediates a growth inhibitory response to TGFβ [23]. In what way this ligand-receptor interaction influences downstream TGFβ-dependent signaling events is unknown, but a dysregulation of cell proliferation and inflammatory reactions during vessel formation and after vessel injury in conditionally *LRP1*-deficient mice would be compatible with their vessel hypertrophy and predisposition to atherosclerosis.

Another aspect of LRP1 function in the vessel wall that has been studied intensely recently concerns the interaction of LRP1 with tPA-related signaling pathways. Independent of its function as a plasminogen activator, tPA regulates the integrity of the blood-brain barrier [14] and vessel tone [13]. LRP1 is necessary for these actions, although it is not known how it mediates tPA effects. tPA is an LRP1 ligand [24], but a direct interaction between LRP1 and the specific plasminogen activator receptor has not yet been demonstrated. Independent of the receptor interaction, ligand-induced activation of LRP1-dependent signaling pathways could also take place, although, again, this has not been demonstrated so far.

LRP1 undergoes furin-mediated processing of its extracellular domain similar to that of Notch receptors [25]: the

mature receptor dimer, generated from a large precursor protein [26], can be cleaved again extracellularly close to the transmembrane region, resulting in the shedding of the bulk of its extracellular domain [27]. Subsequently, the receptor's intracellular domain is released from the plasma membrane by a third proteolytic cleavage [25]. This enables a change of subcellular localization of this part of the receptor and of proteins that bind to the LRP1 cytoplasmic tail, including the adaptor proteins that link LRP1 to different signaling pathways. Stimuli that regulate the rate of LRP1 tail release include those that influence the shedding of the LRP1 extracellular domain [25, 28]. It seems that removal of the receptors' extracellular domain triggers activation of the enzymatic activity, the presenilin-containing γ-secretase complex, that mediates the proteolytic release of the LRP1 intracellular tail [25]. Whether ligand binding can induce cleavage of the LRP1 extracellular domain, as is the case for Notch receptors, remains to be shown.

For tPA-dependent signaling it is conceivable, however, that tPA induces the extracellular cleavage of LRP1, either in its function as an LRP1 ligand or by proteolytic activation of the shedding protease.

Other aspects of LRP1 function that might contribute to its role in the vessel wall include its involvement in the formation and disassembly of focal adhesions. The LRP1 ligand thrombospondin induces the disassembly of these cellular connections through a calreticulin LRP1 receptor complex and Erk as well as phosphatidylinositol-3 kinase (PI3K) activation [29]. The change in cellular adhesion contributes to regulation of cellular migration [30], a function that might be of importance in the LRP1-dependent atherosclerosis phenotype, and possibly to the regulation of endothelial permeability, which might contribute to LRP1-dependent influence on the blood-brain barrier.

Recent experimental studies brought to our knowledge another mechanism through which LRP1 could contribute to the maintenance of vascular integrity. It was shown that LRP1 is involved in the regulation of certain inflammatory processes that play a role in the activation of endothelial cells and regulate their interaction with and recruitment of leukocytes to the vessel wall [31]. Namely, the induction of nuclear factor kappa B (NF-B)-regulated leukocyte adhesion factor E-selectin by the chemokine platelet factor 4 is partly dependent on LRP1 [31]. The dysregulated expression of inflammatory mediators such as E-selectin could lead to inadequate infiltration by inflammatory cells and thereby increased risk of proatherogenic reactions to noxious stimuli.

LRP1 in neurons

It was shown early on that LRP1 is ubiquitously expressed in the central nervous system [32–34], and several studies demonstrated its interaction with neuronal proteins such as APP, FE65 and PSD-95 [9, 35–39]. Furthermore, regulation of calcium currents by LRP1 was shown to occur in in vitro models [40]. The generation of genetically engineered mice that lack LRP1 in differentiated neurons proved the in vivo importance of LRP1 functions in this cell type, as LRP1-defective animals exhibit severe neurological disease with tremor, ataxia, hyperactivity and finally cachexia and premature death [36]. Conventional histological examination of the central nervous system of these animals did not reveal any structural abnormalities, indicating that a functional defect in LRP1-deficient neurons rather than gross neurodegeneration underlies their disease.

It could be shown that LRP1 co-localizes with postsynaptic proteins in neurons and can be co-precipitated both with the postsynaptic density protein 95 and NMDA receptor subunits [36]. Together with the earlier findings that LRP1 regulates calcium flux through NMDA receptor channels [40], these findings may partly explain in what way LRP1 is necessary for neuronal functioning.

Other findings include the notion that LRP1 functions as an endocytic receptor in neurons and mediates the uptake of certain neural proteases. Namely, neuroserpin uptake is disturbed in the absence of LRP1, leading to increased synaptic levels of this protein and possibly to synaptic dysfunction due to imbalance between proteases and protease inhibitors at synaptic regions [41].

In addition, LRP1 has been shown to regulate the metabolism of the amyloid precursor protein, an enigmatic molecule that is centrally involved in the pathogenesis of Alzheimer's disease. The physiological role of this transmembrane protein is still only incompletely understood, with synapse formation [42, 43] and transcriptional regulation [44] being some central functions that have been ascribed to it. In the pathological state, the amyloid peptide Aβ, which is derived from its precursor APP by proteolytical processing, aggregates to insoluble fibrils and accumulates extracellularly in amyloid plaques, one of the histological characteristics of Alzheimer's disease (reviewed in [45]).

LRP1 has been shown to influence the process of Aβ generation: in the absence of LRP1 Aβ generation is reduced, and an alternative proteolytic processing of APP, where a soluble APP extracellular fragment is generated, takes place [37]. The intracellular domain of LRP1 is necessary for this interaction to occur, and a ternary complex between APP, FE65 and LRP1 is formed [38]. Whether the proteolytical processing of LRP1 itself plays a role in this regulatory process has not been examined so far. Recently, however, it was found that the protease BACE1, which generates the Aβ N-terminus through the cleavage of APP, is also able to exert the extracellular cleavage of LRP1, which further links the processing of the two proteins. More insight into the physiological role of APP will surely help to understand new aspects of the

function of neuronal LRP1 and its function as a modulator of cellular signaling.

LRP1 in the lung

Recently, a new function for LRP1 has been suggested in the regulation of the inflammatory response of the lung. It was reported that LRP1 modulates the reaction of alveolar macrophages to foreign or damaged cells when these are opsonized by surfactant proteins. Aggregation of the surfactant molecules leads to their binding to a calreticulin/LRP1 receptor complex and subsequently phagocytosis, and the production of proinflammatory cytokines are initiated [15]. The mechanism of the proinflammatory reaction may involve the regulation of p38 by the activated receptor complex, but the details are still unknown. LRP1-dependent phagocytosis, on the other hand, has been linked to intracellular interaction with CED-6/GULP and may take place in an analogous way to CED-1/CED-6-dependent cell corpse removal in *Caenorhabditis elegans* [46]. LRP1-dependent phagocytosis is probably a more widespread mechanism that does not take place only in pulmonary macrophages but also in phagocytic cells of other tissues, e.g. in the vessel wall [47].

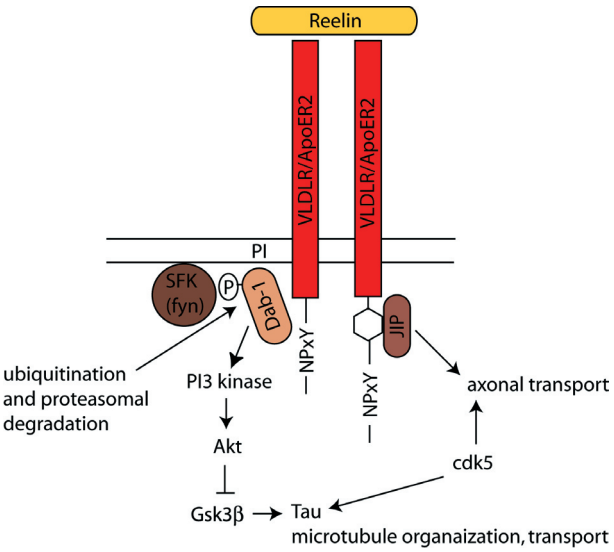

Figure 2. Reelin binding to ApoER2 or VLDLR leads to the clustering of receptor molecules and subsequent phosphorylation of the intracellular adaptor protein Disabled-1 by kinases of the Src family. Phosphorylated Disabled-1 binds to the regulatory subunit p85, which leads to the activation of PI3 kinase. PI3 kinase in turn activates Akt, which inhibits the Tau kinase Gsk3β. In addition, ApoER2 binds the adaptor proteins JIP1/2 through an alternatively spliced cytoplasmic insert, which might also mediate the coupling of the receptor to axonal transport mechanisms.

ApoER2 and VLDLR: two LDL receptor family members with roles in neurodevelopment and beyond

Both the very low density lipoprotein receptor (VLDLR) and the apolipoprotein E receptor 2 (apoER2) display a close structural similarity to the LDL receptor (see fig. 2). Their predominant expression in the central nervous system suggests that they do not function primarily as regulators of systemic lipoprotein metabolism. Indeed, inactivation of either gene in the mouse by homologous recombination did not result in disturbances of lipid homeostasis, whereas compond knockout mice display a neurodevelopmental defect which is indistinguishable from the reeler mouse [48, 49]. In this mouse mutant, autosomal-recessive mutations result in neuronal positioning defects in laminated structures of the central nervous system, which lead to tremor, ataxia and coordination defects. The mutated gene encodes a glycoprotein called Reelin, which is secreted by specialized Cajal-Retzius neurons in the developing neocortex and hippocampus during embryonic development and by populations of interneurons in the adult brain [50].

The NPXY tetra-amino acid motif, which is present in the intracellular tails of all LDL receptor family members, was originally identified as an endocytosis motif that regulates the internalization of the LDL receptor [51], and interacts with a range of different cytoplasmic proteins [9, 52, 53]. Many of these proteins are characterized by an amino terminal phosphotyrosine binding (PTB) domain, a protein interaction domain which recognizes amino acid sequences containing the NPXY consensus sequences as well as different phospholipids [54].

One of these intracellular adaptor molecules, Disabled-1 (Dab1), is tyrosine-phosphorylated upon Reelin binding to neuronal apoER2 or VLDLR [55–57]. Remarkably, mice with spontaneous or targeted mutations of the *dab1* gene display a phenotype, which is indistinguishable from *reeler* or vldlr/apoer2 compound knockout mice [58, 59]. Since Reelin is secreted by pioneer neurons in the developing brain, whereas apoER2, VLDLR and Dab1 are coexpressed in Reelin-responsive migrating postmitotic neurons, it was hypothesized that the apoE receptors convey the Reelin signal from the extracellular space into the cell through Dab1 in a linear pathway to regulate brain development. This assumption was verified by demonstrating that Reelin binds directly to apoER2 and VLDLR, which in turn interacts physically with Dab1 [49, 55, 56]. Other putative Reelin receptors include members of the integrin and cadherin-related neuronal receptor (CNR) gene families [60, 61], although a role of the latter as Reelin receptors has been disputed [62]. Dab1 consists of an aminoterminal PTB domain with a preference for the unphosphorylated form of the NPXY motif [52] in lipoprotein receptor tails, followed by a stretch of phosphorylatable tyrosine residues and a poorly structured carboxylterminal domain [63]. Mice that express a mutated full-length form of Dab1 which

cannot be tyrosine-phosphorylated display a reeler-like phenotype, indicating that its tyrosine phosphorylation is essential for proper transmission of the Reelin signal [64]. On the other hand, a mutated Dab1 protein including the PTB domain and the adjacent tyrosine cluster but lacking the carboxylterminal part can rescue its function, although haploinsufficiency of the truncated Dab1 gene displays subtle neuronal positioning defects in the neocortex and hippocampus [65].

Structural insights into apoE receptor interactions at the plasma membrane

Although the sequence of Dab1 does not predict a membrane targeting motif, it is tightly attached to the particulate fraction in neurons [66]. The structural requirements for the interaction of Dab1 with the intracellular apoE receptor domain and the inner leaflet of the plasma membrane were recently elucidated. Solution of the Dab1 PTB domain in complex with a 14-amino acid peptide corresponding to the ApoER2 tail and the membrane phospholipid phosphatidylinositol-4,5-bisphosphate (PI-4,5P2) revealed not only the structural basis for the unusual requirement of unphosphorylated tyrosine within the NPXY motif of the tail, but also explained how the PTB domain can simultaneously, and noncooperatively [67], bind to PI-4,5P2 [68]. Both binding sites lie on opposite sites of the PTB domain. Identification and mutation of the residues involved in peptide and phospholipid binding allowed functional studies aimed at identifying their relative contribution to membrane localization of Dab1 and transmission of a Reelin signal [68, 69]. By reexpressing lentiviral vectors encoding the different Dab1 mutants in cultured cortical neurons, it was shown that interaction with PI-4,5P2 is sufficient to direct Dab1 to the plasma membrane, whereas binding of both phospholipids and the receptor tails is necessary for Reelin-induced tyrosine phosphorylation of Dab1 [70]. In line with these data, mice expressing Dab1 protein with a point mutation that strongly decreases Dab1 binding in vitro to peptides containing the ApoER2 or VLDLR cytoplasmic regions do not have a reeler phenotype [71]. These observations are consistent with a model where Dab1 is concentrated in PI-4,5P2-enriched plasma membrane domains, which facilitates the subsequent recruitment to and modification by the Reelin signaling machinery, including apoE receptors and Src family tyrosine kinases (SFKs) [68–70]. This model is supported by the reported localization of ApoER2 to cholesterol- and phospholipid-enriched raft-like membrane compartments [72] and the disruption of Reelin signaling by cholesterol-depleting reagents in neurons [66]. Interestingly, it has been reported that Reelin induces recruitment of Dab1 to its receptors [73], which is in line with the suggested two-step model of apoE receptor-mediated Dab1 activation. Alternatively, or additionally, phospholipid binding might promote Reelin signaling by ensuring that Dab1 is integrated into the signaling complex in a proper orientation.

The ApoER2 binding site of Reelin to the complement-type repeat domains 1 and 3 [74] overlaps with the binding site for receptor-associated protein (RAP), a universal chaperone for all LDL receptor gene family members that blocks Reelin signaling in cultured neurons when added as a recombinant protein [56, 75, 76]. Binding data are consistent with the formation of a multimeric Reelin-receptor complex [74], which supports observations that dimerization or oligomerization of Reelin receptors is required for initiation of the signaling cascade [77]. Specifically, induced dimerization of Dab1 is sufficient to induce phosphorylation of the Reelin-responsive tyrosine residues even in the absence of Reelin and apoE receptors [77]. Mapping studies of the regions within the Reelin molecule that are necessary for its biological functions indicate that an aminoterminal region adjacent to the signal peptide sequence, which is recognized by the function-blocking monoclonal antibody CR-50 [78], mediates oligomerization of Reelin [79]. The central fragment of Reelin has been reported to bind to the apoER2 and VLDL receptors. Surprisingly, the middle fragment, which is generated by the action of metalloproteinases in vivo [80], was not only able to induce Dab1 tyrosine phosphorylation but also to partially rescue the malformation in reeler cortical slice cultures, whereas divalent monoclonal antibodies against the reeptor ectodomains did not prevent the reeler phenotype [62].

The function of another Reelin-binding receptor complex, $\alpha3$-$\beta1$ integrin [60, 81], in the Reelin signaling cascade remains elusive. Lack of alpha3 integrin causes a downregulation of Dab1 [60], and tyrosine phosphorylation of Dab1 is not affected in deficient mice, which do not display a reeler-like phenotype [82]. The binding site for the integrin receptor complex was mapped to the aminoterminal fragment containing the CR-50 epitope [82]. To date, one report has described a physiological function for $\alpha3$ integrin in Reelin signaling, i.e. the rescue of abnormal neuronal-glial interaction in cortical slices of Dab1-deficient *scrambler* mice by a function-blocking antibody against $\alpha3$ integrin [83]. Also of interest, a neuronal migration defect in several hindbrain nuclei of reeler mice was recently reported. This defect was not found in apoER2/VLDLR compound knockout mice, suggesting the requirement for another or additional receptor system in subsets of neuronal precursor cells [84]. Other important yet unresolved issues include the importance of Reelin as a positional cue for migrating neurons and the relative contribution of Reelin signaling to radial glial cells in the developing brain. For reasons that are not completely understood, Reelin must

be presented in an oriented fashion to rescue the migratory defects of dentate granule cells in the postnatal developing hippocampus of reeler slice cultures [85], whereas addition of recombinant Reelin is sufficient to prevent the reeler phenotype in embryonic neocortical cultures [86].

A lipoprotein receptor-mediated signaling cascade

Apolipoprotein E receptors have no intrinsic tyrosine kinase activity. Since Dab1 was originally identified as a Src-interacting protein in a yeast-two hybrid screen [63], it was reasonable to assume that members of the Src family of SFKs might phosphorylate Dab1 in vivo. Using genetic and pharmacological approaches, it could be demonstrated that the major Dab1 kinase in neurons is Fyn, a Src family member [76, 87]. Fyn-deficient mice have only subtle positioning defects of late-born cortical neurons and subtle positioning defects in the hippocampal region but do not display a reeler phenotype due to compensation by other Src family members [88–90]. However, these mice exhibit increased levels of neuronal Dab1 protein [76, 87], which is a characteristic feature of mutant mice lacking components of the Reelin signaling cascade, including reeler mice, VLDLR/apoER2 compound knockout mice, and mice expressing the nonphosphorylatable form of Dab1 [49, 64, 91]. Unfortunately, compound knockout mice lacking multiple SFKs are not viable, but pharmacological inhibition of Src family kinases in an embryonic tissue culture model of neocortical development resulted in a reeler-like morphology and Dab1 upregulation, underlining the importance of SFKs in cortical plate formation [86].

In addition to being a substrate for SFKs, Dab1 is also an activator of this family of non-receptor tyrosine kinases [76], and both functions require the presence of least one of the Reelin receptors, apoER2 or VLDLR [76]. The observation that prolonged Reelin stimulation induces apoE receptor-dependent ubiquitination, endocytosis and subsequent proteasomal degradation of tyrosine-phosphorylated Dab1 offers a simple self-regulatory mechanism that limits the mutual activation of Dab1 and SFKs, which would otherwise render the signaling cascade insensitive to external regulation [92, 93]. Interestingly, treatment with low doses of the proteasome inhibitor epoxomicin prevents the formation of proper cortical plate-like structures in cultured embryonic brain slices, indicating a role for the proteasome in brain development [93]. The proto-oncogene c-Cbl, which is abundantly expressed in postmitotic neurons and regulates cellular signaling cascades by targeting tyrosine kinases for degradation, has been shown to ubiquitinate phosphorylated Dab1 in cotransfected HEK-293T cells [94], but it remains to be established whether this E3 ligase is responsible for the proteasomal targeting of Dab1 in vivo. Tyrosine phosphorylation of c-Cbl, a prerequisite for its activation, was not observed in Reelin-treated neurons [92]. Neuronal migration defects in c-Cbl-deficient mice have not been described [95], although this could be due to the activity of related family members, similar to the compensation of the Dab1 kinase Fyn by other SFKs. Unfortunately, mice lacking both c-Cbl and Cbl-b, which is expressed in fetal brain, die early during development [96].

Effectors of the Reelin-Dab1 signaling cascade

Since tyrosine phosphorylation of the adaptor molecule Dab1 is essential for mediating the biological effects of Reelin during cortical development [64], it seemed likely that this Reelin-inducible covalent modification provides docking sites for phosphotyrosine-interacting downstream signaling molecules which affect pathways involved in cellular migration or adhesive properties. Indeed, the sequences surrounding the three Dab1 tyrosine residues at positions 198, 220 and 232 that are phosphorylated by Reelin resemble SH2 consensus sequences, and tyrosine-phosphorylated Dab1 binds to the SH2 domains of SFKs and the related nonreceptor tyrosine kinase Abl in vitro [63, 64, 97]. Mutation of the phosphorylation site at position 198 abolished activation of SFKs in transfected retinal cultures [98].

Using affinity chromatography purification followed by mass spectrometry, several proteins were isolated from embryonic brain lysates, which specifically bind to the tyrosine-phosphorylated form of Dab1 [99–101]. Among these were members of the Crk family of adaptor proteins, which function in the relocation and activation of small GTPases and guanine nucleotide exchange factors (GEFs), thereby modulating cell adhesion and migration [102]. Binding of Crk proteins involved both tyrosine phosphorylation sites at positions 220 and 232, and resulted in the Reelin-induced activation of the GTPase Rap1 and tyrosine phosphorylation of its GEF C3G [99]. This pathway is known to be involved in integrin-mediated regulation of cell adhesion and motility [103]. Intriguingly, tyrosine to phenylalanine exchanges at the positions 220 or 232, but not 198, prevented the rescue of the *scrambler* phenotype by reexpressed wild-type Dab1. In scrambler mice, a naturally occuring dysfunctional *dab1* mutant, radially migrating neurons remain attached to their parental radial glial fibers, a phenotype which seems to be mediated by alpha3 integrin, a Reelin-binding receptor [83].

Another interaction that depends on the tyrosine phosphorylation of Dab1 involves Nckβ [104], an adaptor protein that plays a role in actin cytoskeleton remodeling [105]. Reelin stimulation of cultured neurons induced relocation of Nckβ from the soma into neuronal processes [104], the predominant sites of Reelin action [52, 75, 93]. In conclusion, tyrosine-phosphorylated Dab1 initi-

ates the formation of a multiprotein signaling complex which is able to recruit or sequester adaptor and signaling molecules that interact only indirectly with Dab1, such as Dock1 through CrkII.

Further support for a link between Reelin signaling and the actin cytoskeleton came from the observation that the neuronal Wiscott-Aldrich syndrome protein N-WASP, which regulates actin polymerization through the Arp2/3 complex, is activated by direct interaction with the PTB domain of Dab1 [94]. Interestingly, filopodia formation in cotransfected Cos-7 cells was interrupted in the presence of activated Fyn, which phosphorylates Dab1, presumably due to the proteasomal degradation of Dab1 [94]. Direct evidence for Reelin-induced actin polymerization in vivo or in cultured neurons is still lacking, however.

Activation of PI3 kinase and modulation of Tau phosphorylation – a common pathway in brain development and neurodegeneration ?

Hyperphosphorylation of the microtubule-associated protein Tau, a component found in the neurofibrillary tangles that are pathological hallmarks of several neurodegenerative diseases, is a common feature observed in brains of mice deficient in components of the Reelin signaling cascade [56]. Recently, a molecular pathway was delineated which directly links Reelin to the phosphorylation state of Tau [75]. It was found that Reelin inhibits the activity of glycogen synthase kinase 3ß (GSK3ß) in cultured neurons, a serine/threonine kinase that phosphorylates tau, but not of Cdk5 [75], another Tau kinase that regulates neuronal positioning in laminated brain structures, or of MAP kinases [106]. Reduced activity of phosphatases does not seem to play a role in the observed Tau hyperphosphorylation [107]. Inhibition of GSK3ß by Reelin required the apoER2/VLDLR receptor and Dab1-dependent activation of PI3-kinase and AKT (also known as protein kinase B/PKB), which phosphorylates the inhibitory serine 9 residue of GSK3ß. Reelin-inducible activation of PI3K involves the interaction of its regulatory subunit p85 with tyrosine-phosphorylated Dab1 [66]. Although knockout animal models of the different PI3K isoforms are not informative due to compensatory mechanisms or early embryonic lethality in compound mutants [108], treatment of embryonic brain slices with low doses of the PI3K-inhibitor LY294002 caused a reeler-like neuroanatomy [66], supporting the conclusion that activation of this pathway plays an important role during brain development.

It is not known whether apoE receptor-dependent modulation of this pathway also plays a role in the adult brain. Reelin modulates long-term potentiation, an electrophysiological correlate of memory formation, in hippocampal brain slices, and mice lacking apoER2 or VLDLR display contextual fear conditioning deficits in addition to long-term potentiation defects [109]. Since apoE binds to both receptors, it is tempting to speculate that the apoe4 polymorphism, which is genetically linked to neurodegenerative disorders, including Alzheimer's disease, affects the activation of downstream signaling cascades by differentially binding to the receptors and competing with other ligands such as Reelin, as recently reviewed by Beffert et al. [110]. Remarkably, tyrosine phosphorylation of Dab1 as well as SFK and AKT activation by different apoE isoforms in cultured primary neurons was described [111], although apoE3 decreased Reelin-induced Dab1 tyrosine phosphorylation in an earlier report [55]. Both studies used recombinant apoE; however, apoE likely exists only as a component of larger lipoprotein particles in the brain. Interestingly, two modifier loci which affect the degree of Tau hyperphosphorylation in Dab1-deficient mice [112] lie within the chromosomal regions of amyloid precursor protein (APP) and presenilin-1, two genes affected in familiar forms of Alzheimer's disease. Notably, Dab1 interacts with the NPXY motif in the intracellular APP domain [52, 53] and ApoER2 has been shown to be a substrate of the γ-secretase complex [28], which contains presenilin as the enzymatically active component. These biochemical observations strengthen the plausible yet circumstantial connections between apoE receptor signaling and neurodegenerative disorders.

Crosstalk with other neurodevelopmental signaling pathways

Epistatic interactions between *lis1*, the gene defective in the human neuronal migration disorder lissencephaly, and genes encoding for components of the Reelin pathway hint at a level of coordination between different signaling cascades which act in concert to regulate neuronal positioning and lamination in the mammalian brain. A Reelin-dependent direct interaction between Dab1 which requires phosphorylation of both tyrosine residues at positions 198 and 220 and Lis1 was demonstrated, yet it is currently unknown whether Lis1 is an obligate mediator of Reelin signaling in the developing brain [113].

Mice carrying a deletion of the *cdk5* gene display defects that appear phenotypically similar, but not identical to reeler animals, although they also show an inversion of the normal inside-out layering of cortical neurons [114]. Cdk5 is a serine/threonine kinase that is activated by two other kinases, p35 and p39, which are almost exclusively expressed in the brain [115]. Importantly, proper splitting of the cortical preplate into a marginal zone and a subplate, which is disrupted in the reeler mutant, takes place in Cdk5- and p35- or p35/p39-deficient mice [114, 116, 117]. Using time-lapse recordings of cultured wild-type and p35-deficient cortical slices, it was demonstrated that this difference is attributable to a novel migration mode

named branched migration in the mutant slices, which replaces the normal translocation mode that is predominant during early cortical migration. In contrast, branched migration was rare in Dab1-deficient mice [118]. These functional observations help delineating the Reelin and Cdk5 signaling pathways, which seem to act in a parallel rather than in a linear fashion, a conclusion which is further supported by biochemical and genetic experiments [75, 119, 120]. A potential site of crosstalk between both pathways is the carboxylterminal domain of the Dab1 molecule, which contains consensus Cdk5 phosphorylation sites that are phosphorylated in vitro and in vivo [121]. Although Reelin does not directly modulate Dab1 phosphorylation by Cdk5 [121], it is conceivable that modification at this site fine-tunes and coordinates Dab1-dependent signaling with other neuronal migration signaling pathways in response to environmental cues. This would be consistent with the described haploinsufficiency of mice expressing a carboxylterminally truncated form of Dab1 [65].

The role of the alternatively spliced intracellular apoER2 exon

The *apoer2* gene exists in several splice variants, which are spatially and temporally differentially expressed [7]. The most interesting variant, which is expressed in the brain, includes an additional exon that encodes a proline-rich insertion of 59 amino acids in the intracellular domain of the receptor [122]. This insert interacts with the scaffolding proteins JIP1 and 2 (JNK-interacting proteins 1 and 2) [9, 123], which aggregate components of a MAP kinase signaling module [124]. Moreover, JIP proteins, which are concentrated in nerve terminals, are found in a complex with the molecular motor protein kinesin that also contains apoER2, suggesting a function for these scaffolding proteins in the transport of apoER2 by the microtubule-dependent kinesin motor [125]. Since VLDLR does not interact with JIP proteins and lack of apoER2 is not sufficient to copy the reeler phenotype, it seems likely that the insert is important for apoER2 functions in the adult brain. Interestingly, JIP1 also interacts with the intracellular tail of APP [126, 127], and a JIP1 promoter variant has been reported to be genetically linked to Alzheimer's disease [128].

Another interesting feature of ApoER2 is its cleavage by the γ-secretase complex [28]. Pharmacological inhibition of γ-secretase activity does not influence the Reelin-inducible tyrosine phosphorylation of Dab1. However, it was recently reported that apoE reduces the activation of JNK and that this effect can be blocked by γ-secretase inhibitors [111]. The physiological consequences of these effects, which are specific for the apoER2 variant containg the intracellular insert, remain to be established.

Another putative function of the apoER2 insert has been highlighted by a study which found that the proline-rich sequence, together with the transmembrane region of apoER2, excluded receptor chimeras containing the extracellular domain of the LDL receptor from clustering into coated pits, thereby preventing endocytosis [129]. A low rate of RAP internalization by apoER2, as compared with other members of the LDLR gene family, had been previously described, although it was not mentioned whether the apoER2 construct used in this study contained the insert or not [130]. Together, these in vitro studies underline the need for mouse models in which either spliced form is exclusively expressed from the endogenous *apoer2* locus to study their contributions to normal physiology.

Megalin

Megalin (a.k.a gp330, LRP2) is another multifunctional member of the LDL receptor gene family with a size of approximately 600 kDa. The expression of megalin by many resorptive epithelia points to a predominant role in endocytosis and transport. Like LRP1, megalin has been designated a scavenger receptor due to its multiligand binding properties. Among its ligands are lipoproteins, vitamin-binding and carrier proteins, drugs, hormones and enzymes as well as signaling molecules. In addition, the extracellular domain of megalin interacts with the large glycoprotein cubilin in a partially RAP-inhibitable manner, which is important for the megalin- and cubilin-dependent uptake of the vitamins B12 and D [131]. The intracellular domain of megalin contains three NPXY motifs, which have been shown to interact with signaling adapter molecules and proteins known to be involved in the regulation of endocytosis, including Dab2, ARH, PSD-95 and megalin binding protein (MegBP) [9, 132–135]. A γ-secretase-mediated cleavage of the intracellular domain has been described as well [136].

One of the best-characterized physiological functions of megalin is the proximal-tubular reuptake of low molecular weight proteins, including vitamin D-binding protein from the glomuerular filtrate, resulting in proteinuria and vitamin D deficiency in megalin-deficient mice [137]. Another prominent feature of megalin knockout mice is a defect in forebrain development, which results in a fusion of forebrain hemispheres known as holoprosencephaly [138]. Loss of the secreted morphogen sonic hedgehog (Shh), which is activated by a proteolytic cleavage step followed by covalent attachment of a cholesterol moiety [139], also leads to a holoprosencephalic syndrome [140–142]. Since megalin binds to sonic hedgehog and mediates its endocytic uptake [143], it was speculated that megalin deficiency in the yolk sac might limit the cholesterol supply in the developing embryo [144], or alternatively, that megalin might function as signal transduction receptor for Shh, in a way analogous to the apoE

receptor-dependent Reelin signaling cascade [10, 145]. By conditionally deleting megalin in the epiblast but not in the yolk sac, it was shown that megalin expression in the neuroepithelium of the embryo is required for proper forebrain development [146]. The authors found that absence of megalin in the neuroepithelium led to a loss of sonic hedgehog expression in the ventral forebrain, which they traced back to an increase in Bmp4 signaling, a negative regulator of Shh. Interestingly, this could be attributed to the direct binding of Bmp4 to megalin, followed by its endocytic uptake and degradation [146]. Indeed, ectopic Bmp4 in the chicken forebrain induces holoprosencephaly [147]. The effect of megalin on forebrain development highlights an additional aspect of lipoprotein receptor function at the crossroads of endocytosis and cellular signal transduction, i.e. the regulation of availability of secreted signaling molecules by endocytosis. It remains to be tested whether megalin also participates more directly in cellular signaling cascades, by transducing extracellular signals to intracellular binding partners.

Taken together, our understanding of lipoprotein receptor functions has broadened considerably during the last few years. The discovery of receptor-mediated endocytosis of the LDL receptor first led to all lipoprotein receptors being considered as pure cargo receptors only. It is now clear, however, that some of them have additional functions in the regulation of cellular signaling. A direct relationship between endocytosis and the modulation of signal transduction processes has been shown for megalin, while there are receptors such as ApoER2 and VLDL receptor whose main functions seem to lie in the transmission of extracellular signals with only a limited role for endocytosis. Future studies will help to further elucidate the molecular mechanisms by which lipoprotein receptors fulfill their roles in the development and functioning of different tissues in health and disease.

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