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Low-Density Lipoprotein Receptor Family

Endocytosis and Signal Transduction

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Abstract

The low-density lipoprotein receptor (LDLR) family is composed of a class of single transmembrane glycoproteins, generally recognized as cell surface endocytic receptors, which bind and internalize extracellular ligands for degradation by lysosomes. Structurally, members of the LDLR family share homology within their extracellular domains, which are highlighted by the presence of clusters of ligand-binding repeats. Recently, information regarding the structural and functional elements within their cytoplasmic tails has begun to emerge, which suggests that members of the LDLR family function not only in receptor-mediated endocytosis, but also in transducing signals that are important during embryonic development and the pathogenesis of Alzheimer's disease. This review focuses on recent knowledge of the structural and functional aspects of LDLR family members in endocytosis and signal transduction. The relationship of these functions to the development of the neuronal system and in the pathogenesis of Alzheimer's disease is specifically discussed.

Index Entries: LDL receptor family; LRP; apoER2; VLDL receptor; LRP6; signaling; endocytosis; Alzheimer's disease.

LDLR Family

The low-density lipoprotein receptor (LDLR) family comprises at least 10 members, in mammals: the LDLR itself, the apolipoprotein E receptor 2 (apoER2), the very low density lipoprotein receptor (VLDLR), the LDLR-related protein (LRP), LRP1B, megalin, LRP3, LRP4, LRP5, and LRP6 *(1–8).* Receptors in this family are recognized by the presence of several structural modules that are present in their extracellular regions (Fig. 1). These modules include ligand-binding repeats of ~40 amino acids, which include six cysteine residues

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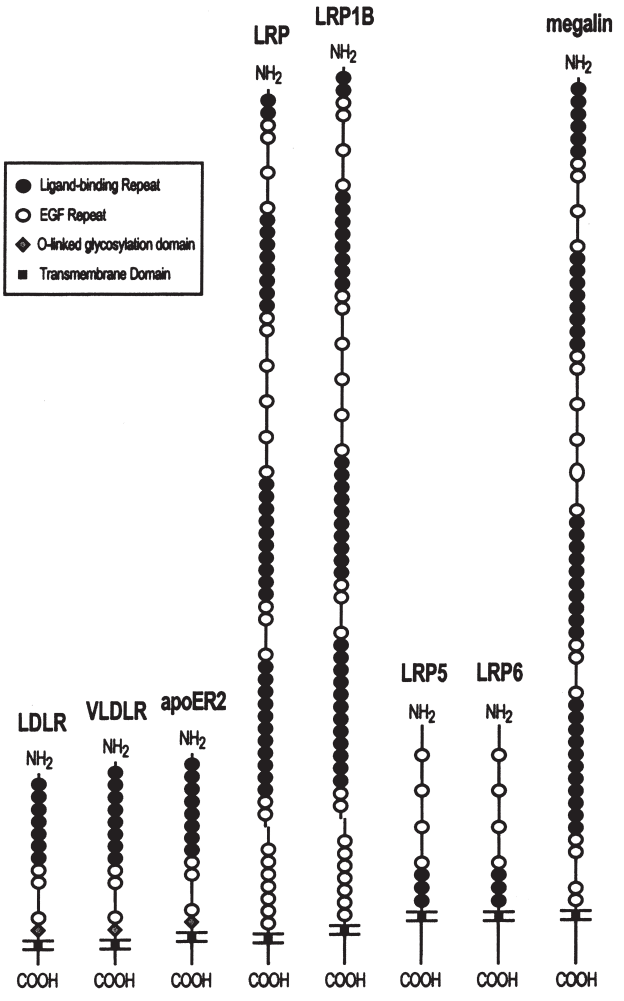

Fig. 1. Schematic representation of LDLR family members. Members of the LDLR family share common structural motifs, including ligand-binding repeats, epidermal growth factor precursor repeats, YMTD spacer domains, a single transmembrane domain, and a relatively short cytoplasmic tail. LRP and LRP1B are heterodimers produced by proteolytic cleavage of single-chain precursor molecules.

forming three disulfide bonds; epidermal growth factor (EGF) precursor repeats, which also contain six cysteines residues each; and modules of ~50 amino acids with a consensus tetrapeptide, Tyr-Trp-Thr-Asp (YWTD). In addition to these extracellular modules, each of these receptors also contains a single transmembrane domain and a relatively short cytoplasmic tail with endocytosis signals *(1–8)*.

Ligand-binding to members of the LDLR family is mediated by clusters of ligand-binding repeats, which form ligand-binding domains. Each of the three large members of the LDLR family (LRP, megalin, and LRP1B) contains four putative ligand-binding domains; other members generally contain only one. The organization of the ligand-binding repeats, EGF precursor repeats, and YWTD repeats in LRP5 and LRP6 is unique among the LDLR family members *(4,5)*, i.e., each contains only three ligand-binding repeats, and their positions are very close to the transmembrane domain. Thus, the arrangement of the EGR precursor repeats and the ligand-binding repeats in the extracellular regions of LRP5 and LRP6 is reversed, relative to other members of the LDLR family *(4,5)*. Finally, all members of the LDLR family but one are type I transmembrane receptors: The exception is LRP4, which appears to be a type II transmebrane receptor, with its N-terminal region located within the cytoplasmic side of the plasma membrane *(8)*. The significance of LRP4's unique structure is not clear, at present.

Although the molecular determinants in ligand recognition are still not clear, both the primary structure within individual ligand-binding repeats and the overall spatial arrangement of these repeats are generally believed to contribute to ligand specificity for each member of the LDLR family *(1–3)*. Within the endosomes, following endocytosis, the EGF precursor homology repeats and YWTD repeats are found to play roles in ligand dissociation from the receptors *(9,10)*. A unique feature, shared by members of the LDLR family, is that ligand interactions with these receptors can be antagonized by a 39-kDa receptor-associated protein (RAP), which functions intracellularly as a molecular chaperone, by facilitating receptor folding, and by preventing premature ligand interaction with the receptors during their trafficking within the early secretory pathway *(11)*. Because of its ability to

universally inhibit ligand interaction with the receptors, RAP has been used frequently as a tool in the study of ligand–receptor interaction *(11).*

Although the characteristics of the extracellular domains of the LDLR family members are well-documented, information regarding the structural and functional elements within their cytoplasmic tails has just begun to emerge. Here, this review centers on the structural and functional aspects of the cytoplasmic tails of the LDLR family members, and their relationship to endocytosis and signal transduction pathways, which plays a role in neuronal development and the pathegenesis of Alzheimer's disease (AD).

Cytoplasmic Tails of LDLR Family Members

Compared to "signaling" receptors, which often contain large intracellular domains with kinase activities, the cytoplasmic tails of the LDLR family members are relatively short, with no kinase domains. However, recent studies indicate that, in addition to the endocytosis signals, the cytoplasmic tails of these receptors contain critical elements for interaction with a set of cytoplasmic adaptor and scaffold proteins, and mediate signal transduction.

Endocytosis Signals

Cell surface receptors, which traffic between the plasma membrane and the endocytic compartments, contain signals within their cytoplasmic tails that allow their efficient recruitment into endocytic vesicles. To date, four classes of endocytosis signals have been identified, which target surface proteins to clathrin-coated vesicles. The two Tyr-based signals, NP*X*Y and Y*XX*Ø (where *X* can be any amino acid and ∅ is an amino acid with a bulky hydrophobic group), were initially identified within the cytoplasmic tails of the LDLR and transferrin receptor, respectively *(12,13).* In many cases, these signals are constitutively active, i.e., receptors undergo continuous rounds of endocytosis and recycling, independent of ligand binding. The di-leucine motif is another well-known endocytosis motif that is present within many transmembrane cell surface proteins *(14).* The third type of endocytosis signal is serine phosphorylation. For several members of the G-protein-coupled receptor family, ligand-induced phosphorylation of Ser residues serves as a signal for receptor endocytosis *(15).* In CD4 and CD3γ, the di-Leu motif acts in cooperation with a neighboring phosphorylated Ser residue, to mediate endocytosis *(16,17).* Finally, the attachment of ubiquitin moieties has recently been identified as another regulator of the endocytosis of several membrane receptors *(18–20).*

A common characteristic of most members of the LDLR family is that at least one copy of the NP*X*Y sequence is found within their cytoplasmic tails. For LDLR, this NP*X*Y motif serves as a signal for receptor endocytosis, through coated pits *(12).* Therefore, it is generally believed that the NP*X*Y sequences in these receptors categorically serve as endocytosis signals. However, the authors' recent study showed that the Y*XX*L motif, but not the two NP*X*Y sequences, within the cytoplasmic tail of LRP, serves as the dominant signal for receptor-mediated endocytosis *(21).* In addition, the authors showed that the distal di-Leu motif within the LRP tail contributes to receptor endocytosis *(21).* These results suggest that each member of the LDLR family may utilize different signal(s) within their cytoplasmic tails for receptor-mediated endocytosis.

The cytoplasmic tails of apoER2 and the VLDLR show high homology to that of the LDLR. Thus, it is possible that the NP*X*Y motifs within the VLDLR and apoER2 tails also function as their dominant endocytosis signals. The cytoplasmic tails of LRP5 and LRP6 are comprised of 207 and 218 amino acid residues, respectively. Unlike other members of the LDLR family, LRP5 and LRP6 lack the conserved NP*X*Y motif. However, these two receptors contain putative di-Leu motifs *(4,5),*

which may serve as endocytosis signals. Obviously, the exact definition of the endocytosis signals for these LDLR family members requires further experimental analysis.

Phosphorylation

Phosphorylation of cell surface receptors is one of the most important mechanisms by which receptor trafficking and/or signal transduction is regulated *(15,22,23).* Until recently, little was known regarding phosphorylation and its function for members of the LDLR gene family. Studies by Kishimoto et al. *(24)* demonstrated that the cytoplasmic tail of the LDLR could be phosphorylated on a Ser residue, by a LDLR kinase that was purified from the cytosol of bovine adrenal cortex, and shared several properties with casein kinase II. However, this phosphorylation event occurs only in vitro, because neither cultured human fibroblasts nor A431 carcinoma cells were able to incorporate [^{32}P]orthophosphate into the LDLR *(24).* Recently, Sakthivel et al. reported that the ligand-binding activity of the human VLDLR is regulated by protein kinase C (PKC)-dependent phosphorylation *(25).* The binding activity of the VLDLR in several cell types was diminished following treatment with the PKC stimulator, phorbol 12-myristate 13-acetate (PMA). Furthermore, the authors found that PKC inhibitors, including a specific inhibitor of the PKC βII isoform, blocked this response to PMA. Finally, it was shown that this VLDLR phosphorylation is associated with Ser residues within the cytoplasmic tail of the receptor *(25).*

LRP is another member of the LDLR family that can be phosphorylated *(26,27).* Recent in vitro and in vivo phosphorylation analyses from this laboratory have demonstrated that LRP is phosphorylated by protein kinase A (PKA) *(28).* By using specific protein kinase inhibitors, truncated LRP minireceptors, and site-directed mutagenesis techniques, the authors showed that Ser 76 (the first amino acid, following the transmembrane domain, is numbered 1) within the cytoplasmic tail of LRP is the major phosphorylation site. Mutation of Ser 76 to either alanine or Thr, which abolished LRP phosphorylation by PKA, resulted in a decrease in both the initial endocytosis rate of LRP and the efficiency of ligand delivery for degradation, which suggests that LRP phosphorylation regulates receptor-mediated endocytosis *(28).* These results are consistent with those from of Goretzki and Mueller *(29),* which demonstrated that LRP-mediated endocytosis is inhibited by specific PKA inhibitors, H-89 and PKI.

Interactions with Cytosolic Adaptor and Scaffold Proteins

A set of cytoplasmic adaptor and scaffold proteins containing protein interaction domain (PID) or PSD-95/Dlg/ZO-1 (PDZ) domains, including mammalian Disabled-1 (mDab1), mDab2, FE65, c-Jun N-terminal kinase-interacting proteins (JIP-1) and JIP-2, PSD-95, CAPON, and SEMCAP-1, bind to the cytoplasmic tails of members of the LDLR family *(30–35).* These new findings suggest that members of the LDLR family may participate in several signal transduction pathways, including the regulation of mitogen-activated protein kinases (MAPKs), cell adhesion, vesicle trafficking, neurotransmission, and neuronal migration. Many of these analyses were performed using apoER2 and the VLDLR. Although apoER2 shares high structural homology with both the LDLR and the VLDLR, including the positions of the exon/intron boundaries of the genes *(36),* the cytoplasmic tail of apoER2 contains an insertion of 59 amino acid residues encoded by a separate exon *(37).* This region of the apoER2 tail has recently been reported to interact with two members of the JUK-interacting protein family, JIP-1 and JIP-2, which belong to a group of MAPK scaffolding proteins *(33,34).* The interaction with JIPs is specific for apoER2, since neither the LDLR nor the VLDLR bind to these adaptor proteins *(34).* These results suggest that apoER2 may have unique roles in intracellular signal transduction that are not currently defined.

mDab1 is a cytosolic adaptor protein that binds to all members of the LDLR family that contain NYXP motif(s) within their cytoplasmic tails. Gotthardt et al. *(33)* have recently shown that binding of mDab1 to the cytoplasmic domain of the LDLR impedes its interaction with the endocytic machinery *(33).* Thus, the receptor endocytosis may be regulated by interactions between the receptor cytoplasmic tails and the cytosolic adaptor and scaffold proteins, via competition for common structural elements.

Signaling via Members of LDLR Family

Traditionally, all members of the LDLR family have been regarded as cell surface endocytosis receptors that function in delivering their ligands to lysosomes for degradation *(1–3).* However, recent studies have revealed new roles for these receptors in several signal transduction pathways.

Reelin/Disable Signaling Pathway

One molecular pathway that regulates the cortical layering and positioning of neurons involves a large extracellular protein, Reelin, which acts autonomously on the cell *(38),* and a cytoplasmic protein, mDab1 *(39).* Reelin, the product of the *reeler* gene *(40),* is synthesized and secreted in the cerebral cortex, predominantly by the Cajal-Retzius cell of the marginal zone, the outermost layer of the developing cortex *(40,41).* In *reeler* mice, in which the gene encoding Reelin is defective, the disorganized cortex is approximately inverted, with early-born neurons occupying abnormal superficial positions and later-born neurons adopting abnormal deep positions *(42).* The cytoplasmic adaptor protein, mDab1, is predominantly expressed in neurons, and has been shown to function downstream of Reelin. mDab1-deficient mice develop a phenotype that is indistinguishable from that of *reeler* mice *(43,44).* In addition, mDab1 accumulates in the absence of Reelin, suggesting that Reelin may promote degradation of Dab1 *(44,45).* Furthermore, addition of Reelin to primary neuronal cultures results in an increase of Tyr phosphorylation of mDab1 *(46).* Thus, mDab1 is thought to function downstream of Reelin in a signal transduction cascade that controls appropriate cell positioning during brain development.

The linkage between extracellular Reelin and cytosolic mDab1 was not clear until recently, when Trommsdorff et al. *(31)* showed that mice lacking both VLDLR and apoER2 precisely mimic the phenotype of those with Reelin or mDab1 deficiency. Subsequently, Reelin was demonstrated to be a ligand for both VLDLR and apoER2 *(47,48).* Additionally, in a yeast two-hybrid system, VLDLR and apoER2 were shown to interact with mDab1 through the NPXY motif of these receptors and the protein interaction/phosphotyrosine-binding (PI/PTB) domain of mDab1. Finally, binding of Reelin to its receptors was found to induce mDab1 phosphorylation and this phosphorylation was abolished when Reelin binding to its receptors on neurons was blocked by RAP or by apoE *(47,48).* Thus, these findings indicate that Reelin acts, via cell-surface VLDLR and apoER2, to induce mDab-1 Tyr phosphorylation, which in turn regulates neuronal migration.

Wnt Signaling Pathway

The Wnt family of secreted molecules functions in cell-fate determination and morphogenesis during development, in both vertebrates and invertebrates *(49).* Mutational analysis in mice has shown the importance of Wnts in controlling diverse developmental processes, such as patterning of the body axis, central nervous system, and limbs, and the regulation of inductive events during organ genesis. Although many components of the Wnt signaling pathway have been identified, little is known about how Wnts and their cognate Frizzled (Fz) receptors signal to downstream effector molecules. Recently, however,

studies from several labs have demonstrated that LRP6 is a critical component for Wnt signaling *(50–52).*

In *Drosophila, arrow* phenocopies the wingless (DWnt-1) phenotype, and encodes a transmembrane protein that is homologous to two members of the mammalian LDLR family, LRP5 and LRP6 *(52).* In mouse, embryos homozygous for an insertional mutation in the LRP6 gene, developmental defects are seen that display a striking composite of those caused by mutations in individual Wnt genes *(50).* In *Xenopus* embryos, LRP6 activates Wnt-Fz signaling, and induces Wnt-responsive genes, dorsal axis duplication, and neural crest formation *(51).* Biochemical studies showed that the extracellular domain of LRP6 bound Wnt-1, and associated with Fz in a Wnt-dependent manner. An LRP6 mutant, lacking the carboxyl intracellular domain, blocked signaling by Wnt or Wnt-Fz, and inhibited neural crest development *(51).* Together, these studies thus demonstrate that LRP6 functions as a co-receptor for Wnt signal transduction.

Signaling via LRP

LRP is a widely expressed endocytic receptor that is highly expressed in neurons, in hepatocytes, and in fibroblasts. LRP is regarded as a clearance receptor for more than 15 structurally and functionally distinct ligands, which include apoE/lipoprotein, β-amyloid precursor protein (APP), α_2-macroglobulin (α_2M), and tissue-type plasminogen activator (tPA) *(1–3).* In addition to receptor-mediated endocytosis, recent studies from several labs have implicated this receptor in direct transmission of extracellular signals across the plasma membrane.

Goretzki and Mueller *(53)* demonstrated that the cytoplasmic tail of LRP interacts with a guanosine triphosphate-binding protein, and that treatment of LRP-expressing cells with LRP ligands, lactoferrin and the urokinase/plasminogen activator inhibitor type-I complex, resulted in a significant increase in intracellular cyclic adenosine monophosphate (cAMP) level and PKA activity. More recently, Bacskai et al. *(54)* showed that LRP is a signaling receptor in neuronal calcium signaling via N-methyl-D-aspartate receptors (NMDAR). A robust, spatially and temporally discrete calcium signal is observed in neurons treated with ligand-competent α_2M. The calcium influx is blocked by RAP, and by an antagonist of the NMDAR *(54).* However, the physiological significance of these two signaling pathways via LRP is poorly understood, at present.

Recently, studies from this lab demonstrated that interactions between tPA and LRP are important for hippocampal long-term potentiation (LTP) *(55).* LTP is one of the best models for investigating cellular and molecular mechanisms for memory formation and storage *(56).* In the CA1 region of the hippocampus, LTP has two distinct phases: early-phase LTP and late-phase LTP. One unique feature, which makes late-phase LTP different from early-phase LTP, is that it requires new protein synthesis, activity of cAMP-dependent PKA, and transcription *(57).* It has been reported that induction of tPA expression may contribute to activity-dependent synaptic plasticity in the hippocampus and cerebellum, and that LTP is significantly decreased in mice lacking tPA *(58–60).* Metabolic labeling and ligand-binding analysis indicate that both tPA and LRP are synthesized by hippocampal neurons, and that LRP is the major cell surface receptor that binds tPA *(55).* Perfusion of hippocampal slices with RAP significantly reduced late-phase LTP. In addition, RAP also blocked the enhancing effect of synaptic potentiation, by exogenous tPA in hippocampal slices prepared from tPA knockout mice *(55).* tPA binding to LRP in hippocampal neurons was found to enhance the activity of PKA, a key molecule known to be involved in late-phase LTP *(55).* Taken together, interaction between tPA and cell surface LRP is likely to initiate intracellular signal transduction pathway(s), which include an increase in PKA activity, which in turn regulates late-phase LTP.

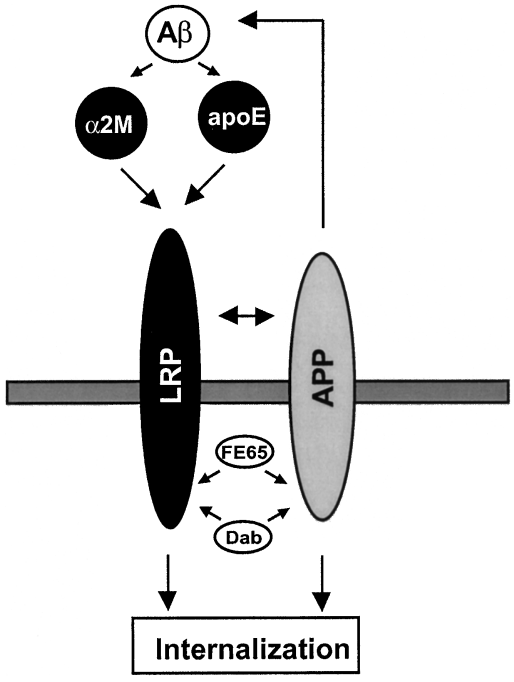

Fig. 2. Schematic representation of interactions between LRP and APP. LRP may associate with the full-length, membrane-anchored APP containing the Kunitz protease inhibitor domain. In addition, FE65 and Dab1 may bind simultaneously to the cytoplasmic tails of APP and LRP, then modulate APP trafficking, although such interaction remains to be demonstrated. Furthermore, LRP can regulate Aβ clearance via internalization of α_2M* – Aβ complex and apoE – Aβ complex.

LRP and AD

AD is a neurodegenerative disease that results in impaired memory and cognition. The main neuropathological hallmarks of AD are amyloid plaques and neurofibrillary tangles deposited throughout the brain. The major components of amyloid plaques are aggregates of the 40–42-amino-acid amyloid β-peptides (Aβ40 or Aβ42) *(61).* These are proteolytically derived from the APP. Although Aβ is generated as a normal product of cellular metabolism *(62),* substantial data indicate that progressive accumulation of Aβ plays a central role in AD pathogenesis *(63).* Thus, it has been hypothesized that the clearance of Aβ is decreased, and/or the processing of APP to Aβ is increased, in AD *(63).*

Several lines of evidence indicate that LRP may have an influential role in the pathogenesis of AD (Fig. 2). First, several LRP ligands, such as apoE, lactoferrin, and α_2M, have been shown to bind Aβ, and to mediate its clearance and degradation through LRP *(64–67).* LRP levels decrease with age, and also are drastically reduced in AD *(68).* Thus, the loss of LRP function to clear ligands complexed to Aβ may contribute to the emergence of amyloid plaques in AD and aging populations. Second, presenilin, another protein that is pathologically associated with AD, can also affect LRP expression; LRP levels are decreased in both presenilin transgenic mice and presenilin-transfected cells *(69).* These data suggest that presenilin may indirectly influence Aβ clearance through LRP. Third, LRP may also contribute to AD pathogenesis, by influencing the processing of APP to Aβ, through extracellular and intracellular association with APP. LRP can form extracellular complexes with the Kunitz protease inhibitor (KPI) domain-containing forms of APP *(70,71).* In addition, LRP may potentially affect APP processing, through intracellular interactions bridged by neuronal adaptor proteins, such as FE65 *(30).* Toward this hypothesis, it has recently been demonstrated that expression of LRP favors amyloidogenic processing of APP *(72).* Finally, a role of LRP in AD has been demonstrated in genetic association studies, as several groups have reported a genetic association between a silent polymorphism within the *LRP* gene and late-onset AD *(73–77).*

Role of LRP in Aβ Clearance

Carriers of the E4 isoform of apoE are at a greater risk for developing AD *(78,79),* compared to E2 and E3 carriers. ApoE has also been localized to amyloid plaques *(80).* Aβ has been hypothesized to form a complex with apoE, and thereafter is endocytosed via LRP, preventing the accumulation of amyloid.

Transgenic mouse models support the function apoE in Aβ clearance. Mice expressing a mutant form of APP, and lacking mouse apoE, have less early deposition of fibrillar Aβ, when crossed with human apoE-transgenic mice *(81).* possibly the isoform-related risk of AD results from differences in the binding affinity to Aβ. In support of this hypothesis, apoE4 isoforms have been shown to bind Aβ with lower affinity, compared to other apoE isoforms *(82).* Since LRP is the major apoE receptor in the brain *(83,84),* these data emphasize the important function LRP may play in Aβ clearance and AD pathogenesis.

Activated α_2M (α_2M*) also binds Aβ *(65–67),* and has been found in amyloid plaques *(85).* Blacker et al. *(86)* reported an increased risk of AD in carriers of an exon 18 splice-site deletion near the bait region of *α_2M*. A separate study by Liao et al. *(87)* examined a Val1000 (GTC)/lle1000(ATC) polymorphism within *α_2M*, near the active site of the molecule. Their study found the G/G genotype overrepresented in AD. These data suggest α_2M plays an influential role in AD pathophysiology. In support of this hypothesis, a study by Du et al. *(88)* demonstrated that α_2M could attenuate fibrillar Aβ formation and its toxicity toward neurons. Clearance of α_2M by LRP also appears to be important in mediating Aβ toxicity. Van Uden et al. *(89)* demonstrated that Aβ toxicity, and associated cell death, is decreased in the presence of α_2M*. More cell death was observed in the presence of RAP, and also in cells that have decreased expression of LRP, suggesting that the protective role of α_2M* is dependent on LRP.

Another LRP ligand, lactoferrin, was also able to reduce soluble Aβ levels in culture. This effect was also attenuated by RAP, indicating that both α_2M and lactoferrin clear Aβ via LRP *(67).* Although how these ligands act in concert to clear Aβ under normal circumstances, and in AD, is still not clear, LRP appears to be the common pathway by which these ligands regulate cellular degradation of Aβ. Possibly in AD, these ligands compete for LRP-mediated clearance, and are less successful at clearing their Aβ cargo. Recently, Kang et al. *(68)* quantified LRP expression in the midfrontal cortex of control and AD subjects, and found that LRP expression was decreased during aging in normal subjects, and was severely decreased in patients with AD. These data support the hypothesis that impeded clearance of LRP ligands, complexed with Aβ, may increase AD susceptibility.

LRP and Presenilin

Recent data suggest that presenilin may also function to regulate LRP expression, and thus LRP-mediated clearance of Aβ. Mutations within the presenilin-1 and 2 genes lead to an increase in Aβ42, but do not change levels of Aβ40 *(90,91).* Since the mechanism by which increased Aβ occurs is unknown, Van Uden et al. *(69)* explored whether LRP expression was affected by presenilin-1. In transgenic mice expressing the presenilin M146L mutation or L286V mutation, they found less LRP expression in neuronal populations. Furthermore, overexpression of wild-type or mutant presenilin-1 in cell culture resulted in decreased LRP mRNA and protein. Functionally, downregulation of LRP by presenilin-1 has been shown to influence the toxicity of Aβ toward cells in the presence of α_2M* *(89).* Cells with decreased expression of LRP exhibited more cell death in the presence of α_2M and Aβ.

LRP and APP Processing to Aβ

Kounnas et al. *(70)* first reported an association between LRP and APP. Their data demonstrated that a secreted form of APP751, which contains a KPI domain, is a ligand for LRP. They found that soluble APP751 was degraded by LRP-expressing, but not LRP-deficient, fibroblasts. The KPI domain was necessary for APP degradation, because non-KPI-containing APP was not degraded by these cells. The interaction between APP and LRP appears to be mediated in some way by cell-surface heparan sulfate proteoglycan, since both RAP

and heparin blocked the degradation of APP. Knauer et al. *(71)* later demonstrated that cell-surface KPI-containing APP can also complex to EGF-binding protein, and be internalized by LRP. The internalization of cell-surface APP was inhibited by RAP, indicating a common pathway of degradation with secreted APP through LRP. Since it had been established that cell surface APP could be processed to Aβ in the endocytic pathway *(92)*, these findings suggest that LRP may influence APP endocytic trafficking, and, thus, the processing of APP to Aβ. Such a hypothesis was tested recently by Ulery et al. *(72)*, who showed that long-term treatment of cells with the LRP antagonist, RAP, decreased Aβ production. In addition, they found that expression of LRP, via transfection in LRP-deficient cells, increased Aβ production.

Although KPI-containing species of APP are the major isoform of APP in nonneuronal cells *(93)*, non-KPI-containing APP is the predominant neuronal species *(94,95)*. The cytoplasmic domains of LRP and APP may interact through neuronal adaptor proteins, such as FE65. An intracellular interaction between LRP and APP would not require the presence of a KPI domain. Using GST-fusion protein pull-down experiments, Trommsdorff et al. *(30)* showed that the N-terminus of FE65 interacts with the cytoplasmic tail of LRP; the C-terminus of FE65 can bind to the cytoplasmic tail of APP. Thus, a potential interaction between APP and LRP tails may bridge non-KPI-containing APP with LRP, and may further strengthen the association between LRP and KPI-containing forms of APP. In this manner, LRP could influence the trafficking and processing of APP in both neuronal and nonneuronal cells.

Although LRP is primarily a neuronal receptor expressed in the cortex and hippocampus, it is also expressed in activated astrocytes *(85,96,97)*, glia *(98)*, and microglia *(99)*. Thus, increased LRP expression in these cell types could possibly lead to increased APP processing to Aβ in AD. Whether this occurs in vivo still remains to be determined.

Genetic Association of LRP with AD

Genetic studies have provided additional evidence that LRP is associated with AD. Kang et al. *(73)* first reported a silent polymorphism (C776T) within exon 3 of the *LRP* gene on chromosome 12. This polymorphism was underrepresented in AD, and associated with a later age of disease onset. This association was later confirmed in four independent studies *(74–77)*. Since this polymorphism does not appear to affect the protein itself, it is believed that the polymorphism may exist in linkage disequilbrium with another portion of the gene *(68)*. A polymorphism within exon 6 of the *LRP* gene (A216V) has also been reported *(100)*, although this polymorphism appears to be negatively associated with AD.

Genetic studies have identified the *LRP* gene as an important susceptibility locus for AD; however, how these LRP polymorphisms contribute to AD is not clear, at present. From biochemical and histological studies, LRP may possibly play a dual role in AD pathology. In neurons, LRP may function in clearing Aβ via its ligands, such as apoE and α_2M. In the diseased state, LRP expression may decrease *(68)*, which would in turn lead to reduced Aβ clearance, and eventually Aβ deposition. In activated astrocytes and microglia, LRP expression may increase, which would cause an increase in amyloidogenic processing of APP to Aβ. Thus, these pathways suggest that differential LRP expression in neurons and glia may well influence extracellular Aβ levels, which in turn may contribute to the pathogenesis of AD.

Concluding Remarks

Recent understanding of the functions of the LDLR family has been expanded from receptor-mediated endocytosis to signal transduction. Although these receptors contain various endocytosis signals that mediate their interactions with the endocytic machinery, the cytoplasmic tails of these receptors appear to bind many cytosolic adaptor and scaffold proteins

that contribute to signal transduction. The functions of these receptors in neuronal development are highlighted by recent studies on the apoER2 and the VLDLR in Reelin/mDab1 pathway, and by LDR6 in Wnt signaling. One common feature in the transmission of extracellular signals across the plasma membrane by the LDLR family members is that they may collaborate with other molecules on the cell surface. For example, LDR6 functions as a co-receptor for Wnt signaling *(50–52);* LRP is a signaling receptor in neuronal calcium signaling via NMDAR *(54).* In the Reelin signaling, other molecules, such as α3β1 integrin and members of cadherin-related neuronal receptor family, have recently been identified as Reelin-binding proteins *(101,102).* Studies on macrophages suggest that α_2M* binds two types of cell surface receptors, LRP and a α_2M signaling receptor. Stimulation of the latter triggers typical signaling cascades, which regulate cell proliferation *(103,104).* The expression and function of the α_2M signaling receptor in other types of cells is unclear.

Studies in the past few years have demonstrated that members in the LDLR family function from neuronal development to aging and AD. The roles of the LDLR family members in AD are best represented by LRP, which mediates the clearance of Aβ via its physiological ligands, and may potentially influence the endocytic trafficking of APP and its processing to Aβ. Future studies will be directed to understanding the molecular mechanisms underlying these functions, and identifying novel functions of these receptors in the neuronal system.

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Reference

1. Krieger M. and Herz J. (1994) Structures and functions of multiligand lipoprotein receptors: macrophage scavenger receptors and LDL receptor-related protein (LRP). *Annu. Rev. Biochem.* **63,** 601–637.
2. Strickland D. K., Kounnas M. Z., and Argraves W. S. (1995) LDL receptor-related protein: a multiligand receptor for lipoprotein and proteinase catabolism. *FASEB J.* **9,** 890–898.
3. Hussain M. M., Strickland D. K., and Bakillah A. (1999) The mammalian low-density lipoprotein receptor family. *Annu. Rev. Nutr.* **19,** 141–172.
4. Hey P. J., Twells R. C., Phillips M. S., Yusuke N., Brown S. D., Kawaguchi Y., et al. (1998) Cloning of a novel member of the low-density lipoprotein receptor family. *Gene.* **216,** 103–111.
5. Brown S. D., Twells R. C., Hey P. J., Cox R. D., Levy E. R., Soderman A. R., et al. (1998) Isolation and characterization of LRP6, a novel member of the low density lipoprotein receptor gene family. *Biochem. Biophys. Res. Commun.* **248,** 879–888.
6. Liu C. X., Musco S., Lisitsina N. M., Forgacs E., Minna J. D., Lisitsyn N. A. (2000) LRP-DIT, a putative endocytic receptor gene, is frequently inactivated in non-small cell lung cancer cell lines. *Cancer Res.* **60,** 1961–1967.
7. Ishii H., Kim D. H., Fujita T., Endo Y., Saeki S., and Yamamoto T. T. (1998) CDNA cloning of a new low-density lipoprotein receptor-related protein and mapping of its gene (LRP3) to chromosome bands 19q12-q13. 2. *Genomics* **51,** 132–135.
8. Tomita Y., Kim D. H., Magoori K., Fujino T., and Yamamoto T. T. (1998) A novel low-density lipoprotein receptor-related protein with type II membrane protein-like structure is abundant in heart. *J. Biochem.* **124,** 784–789.
9. Davis C. G., Goldstein J. L., Sudhof T. C., Anderson R. G., Russell D. W., and Brown M. S. (1987) Acid-dependent ligand dissociation and recycling of LDL receptor mediated by growth factor homology region. *Nature* **326,** 760–765.
10. Mikhailenko I., Considine W., Argraves K. M., Loukinov D., Hyman B. T., Strickland D. K. (1999) Functional domains of the very low density lipoprotein receptor: molecular analysis of ligand binding and acid-dependent lig-

and dissociation mechanisms. *J. Cell Sci.* **112,** 3269–3281.
11. Bu G. (1998) Receptor-associated protein: a specialized chaperone and antagonist for members of the LDL receptor gene family. *Curr. Opin. Lipidol.* **9,** 149–155.
12. Chen W. J., Goldstein J. L., and Brown M. S. (1990) NPXY, a sequence often found in cytoplasmic tails, is required for coated pit-mediated internalization of the low density lipoprotein receptor. *J. Biol. Chem.* **265,** 3116–3123.
13. Trowbridge I. S., Collawn J. F., and Hopkins C. R. (1993) Signal-dependent membrane protein trafficking in the endocytic pathway. *Annu. Rev. Cell Biol.* **9,** 129–161.
14. Hirst J. and Robinson M. S. (1998) Clathrin and adaptors. *Biochim. Biophys. Acta* **1404,** 173–193.
15. Lefkowitz R. J. (1998) New roles for receptor kinases and β-arrestins in receptor signaling and desensitization *J. Biol. Chem.* **273,** 18,677–18,680.
16. Dietrich J., Hou X., Wegener A. M., and Geisler C. (1994) CD3 gamma contains a phosphoserine-dependent di-leucine motif involved in down-regulation of the T cell receptor. *EMBO J.* **13,** 2156–2166.
17. Pitcher C., Honing S., Fingerhut A., Bowers K., and Marsh M. (1999) Cluster of differentiation antigen 4 (CD4) endocytosis and adaptor complex binding require activation of the CD4 endocytosis signal by serine phosphorylation. *Mol. Biol. Cell* **10,** 677–691.
18. Hershko A. and Ciechanover A. (1998) The ubiquitin system. *Annu. Rev. Biochem.* **67,** 425–479.
19. Strous G. J. and Govers R. (1999) The ubiquitin-proteasome system and endocytosis. *J. Cell Sci.* **112,** 1417–1423.
20. Shih S. C., Sloper-Mould K. E., and Hicke L. (2000) Monoubiquitin carries a novel internalization signal that is apeended to activated receptors. *EMBO J.* **19,** 187–198.
21. Li Y., Marzolo M. P., van Kerkhof P., Strous G. J., and Bu G. (2000) The YXXL motif, but not the two NPXY motifs, serves as the dominant endocytosis signal for low density lipoprotein receptor-related protein. *J. Biol. Chem.* **275,** 17,187–17,194.
22. Sibley D. R., Benovic J. L., Caron M. G., and Lefkowitz R. J. (1987) Regulation of transmembrane signaling by receptor phosphorylation. *Cell* **48,** 913–922.
23. Hausdorff W. P., Caron M. G., and Lefkowitz R. J. (1990) Turning off the signal:desensitization of beta-adrenergic receptor function. *FASEB J.* **4,** 2881–2889.
24. Kishimoto A., Brown M. S., Slaughter C. A., and Goldstein J. L. (1987) Phosphorylation of serine 833 in cytoplasmic domain of low density lipoprotein receptor by a high molecular weight enzyme resembling casein kinase II. *J. Biol. Chem.* **262,** 1344–1351.
25. Sakthivel R., Zhang J. C., Strickland D. K., Gafvels M., and McCrae K. R. (2001) Regulation of the ligand binding activity of the human very low density lipoprotein receptor by protein kinase-C dependent phosphorylation. *J. Biol. Chem.* **276,** 555–2001.
26. Bu G., Sun Y., Schwartz A. L., and Holtzman D. M. (1998) Nerve growth factor induces rapid increases in functional cell surface low density lipoprotein receptor-related protein. *J. Biol. Chem.* **273,** 13,359–13,365.
27. Djordjevic J. T., Waterkeyn J. G., Hennessy K. L., and Stanley K. K. (2000) Role of phosphorylation of the cytoplasmic domain of the α2-macroglobulin receptor. *Cell Biol. Int.* **24,** 599–610.
28. Li Y., van Kerkhof P., Marzolo M. P., Strous G. J., and Bu G. (2001) Identification of a major PKA phosphorylation site within the cytoplasmic tail of LRP: implication for receptor-mediated endocytosis. *Mol. Cell. Biol.* **21,** 1185–1195.
29. Goretzki L. and Mueller B. M. (1997) Receptor-mediated endocytosis of urokinase-type plasminogen activator is regulated by cAMP-dependent protein kinase. *J. Cell Sci.* **110,** 1395–1402.
30. Trommsdorff M., Borg J. P., Margolis B., and Herz J. (1998) Interaction of cytosolic adaptor proteins with neuronal apolipoprotein E receptors and the amyloid precursor protein. *J. Biol. Chem.* **273,** 33,556–33,560.
31. Trommsdorff M., Gotthardt M., Hiesberger T., Shelton J., Stockinger W., Nimpf J., et al. (1999) Reeler/Disabled-like disruption of neuronal migration in knockout mice lacking the VLDL receptor and ApoE receptor 2. *Cell* **97,** 689–701.
32. Howell B. W., Lanier L. M., Frank R., Gertler F. B., and Cooper J. A. (1999) The disabled 1 phosphotyrosine-binding domain binds to the internalization signals of transmembrane glycoproteins and to phospholipids. *Mol. Cell. Biol.* **19,** 5179–5188.

33. Gotthardt M., Trommsdorff M., Nevitt M. F., Shelton J., Richardson J. A., Stockinger W., Nimpf J., and Herz J.(2000) Interactions of the low density lipoprotein receptor gene family with cytosolic adaptor and scaffold proteins suggest diverse biological functions in cellular communication and signal transduction. *J. Biol. Chem.* **275,** 25,616–25,624.
34. Stockinger W., Brandes C., Fasching D., Hermann M., Gotthardt M., Herz J., Schneider W. J., and Nimpf J. (2000) The reelin receptor ApoER2 recruits JNK-interacting proteins-1 and -2. *J. Biol. Chem.* **275,** 25,625–25,632.
35. Oleinikov A. V., Zhao J., and Makker S. P. (2000) Cytosolic adaptor protein Dab2 is an intracellular ligand of endocytic receptor gp600/megalin. *Biochem. J.* **347,** 613–621.
36. Kim D. H., Magoori K., Inoue T. R., Mao C. C., Kim H. J., Suzuki H., et al. (1997) Exon/intron organization, chromosome localization, alternative splicing, and transcription units of the human apolipoprotein E receptor 2 gene. *J. Biol. Chem.* **272,** 8498–8504.
37. Kim D. H., Iijima H., Goto K., Sakai J., Ishii H., Kim H. J., et al. (1996) Human apolipoprotein E receptor 2. A novel lipoprotein receptor of the low density lipoprotein receptor family predominantly expressed in brain. *J. Biol. Chem.* **271,** 8373–8380.
38. Miyata T., Nakajima K., Mikoshiba K., and Ogawa M. (1997) Regulation of Purkinje cell alignment by Reelin as revealed with CR-50 antibody. *J. Neurosci.* **17,** 3599–3609.
39. Curran T. and D'Arcangelo G. (1998) Role of reelin in the control of brain development. *Brain Res. Brain Res. Rev.* **26,** 285–294.
40. D'Arcangelo G., Miao G. G., Chen S. C., Soares H. D., Morgan J. I., and Curran T. (1995) A protein related to extracellular matrix proteins deleted in the mouse mutant reeler. *Nature* **374,** 719–723.
41. Ogawa M., Miyata T., Nakajima K., Yagyu K., Seike M., Ikenaka K., Yamamoto H., and Mikoshiba K. (1995) The reeler gene-associated antigen on Cajal-Retzius neurons is a crucial molecule for laminar organization of cortical neurons. *Neuron* **14,** 899–912.
42. Caviness V. S., Jr. and Sidman R. L. (1973) Time of origin or corresponding cell classes in the cerebral cortex of normal and reeler mutant mice: an autoradiographic analysis.*J. Comp. Neurol.* **148,** 141–151.
43. Howell B. W., Hawkes R., Soriano P., and Cooper J. A. (1997) Neuronal position in the developing brain is regulated by mouse disabled-1. *Nature* **389,** 733–737.
44. Sheldon M., Rice D. S., D'Arcangelo G., Yoneshima H., Nakajima K., Mikoshiba K., et al. (1997) Scrambler and yotari disrupt the disabled gene and produce a reeler-like phenotype in mice. *Nature* **389,** 730–733.
45. Rice D. S., Sheldon M., D'Arcangelo G., Nakajima K., Goldowitz D., and Curran T. (1998) Disabled-1 acts downstream of Reelin in a signaling pathway that controls laminar organization in the mammalian brain. *Development* **125,** 3719–3729.
46. Howell B. W., Herrick T. M., and Cooper J. A. (1999) Reelin-induced tyrosine phosphorylation of disabled 1 during neuronal positioning. *Genes Dev.* **13,** 643–648.
47. D'Arcangelo G., Homayouni R., Keshvara L., Rice D. S., Sheldon M., and Curran T. (1999) Reelin is a ligand for lipoprotein receptors. *Neuron* **24,** 471–479.
48. Hiesberger T., Trommsdorff M., Howell B. W., Goffinet A., Mumby M. C., Cooper J. A., and Herz J. (1999) Direct binding of Reelin to VLDL receptor and ApoE receptor 2 induces tyrosine phosphorylation of disabled-1 and modulates tau phosphorylation. *Neuron,* **24,** 481–489.
49. Wodarz A. and Nusse R. (1998) Mechanisms of Wnt signaling in development. *Annu. Rev. Cell Dev. Biol.* **14,** 59–88.
50. Pinson K. I., Brennan J., Monkley S., Avery B. J., and Skarnes W. C. (2000) An LDL-receptor-related protein mediates Wnt signalling in mice. *Nature* **407,** 535–538.
51. Tamai K., Semenov M., Kato Y., Spokony R., Liu C., Katsuyama Y., et al. (2000) LDL-receptor-related proteins in Wnt signal transduction. *Nature,* **407,** 530–535.
52. Wehrli M., Dougan S. T., Caldwell K., O'Keefe L., Schwartz S., Vaizel-Ohayon D., et al. (2000) arrow encodes an LDL-receptor-related protein essential for Wingless signalling. *Nature,* **407,** 527–530.
53. Goretzki L. and Mueller B. M. (1998) Low-density-lipoprotein-receptor-related protein (LRP) interacts with a GTP-binding protein. *Biochem. J.* **336,** 381–386.
54. Bacskai B. J, Xia M. Q., Strickland D. K., Rebeck G. W., and Hyman B. T. (2000) The endocytic receptor protein LRP also mediates

neuronal calcium signaling via N-methyl-D-aspartate receptors. *Proc. Natl. Acad. Sci. USA* **97,** 11,551–11,556.
55. Zhuo M., Holtzman D. M., Li Y., Osaka H., DeMaro J., Jacquin M., and Bu G. (2000) Role of tissue plasminogen activator receptor LRP in hippocampal long-term potentiation. *J. Neurosci.* **20,** 542–549.
56. Nicoll R. A. and Malenka R. C. (1995) Contrasting properties of two forms of long-term potentiation in the hippocampus. *Nature* **377,** 115–118.
57. Schuman E. M. (1997) Synapse specificity and long-term information storage. *Neuron* **18,** 339–342.
58. Carmeliet P., Schoonjans L., Kiecken L., Ream B., Degen J., Bronson R., et al. (1994) Physiological consequences of loss of plasminogen activator gene function in mice. *Nature* **368,** 419–424.
59. Frey U., Muller M., and Kuhl D. (1996) A different form of long-lasting potentiation revealed in tissue plasminogen activator mutant mice. *J. Neurosci.* **16,** 2057–2063.
60. Huang Y. Y., Bach M. E., Lipp H. P., Zhuo M., Wolfer D. P., Hawkins R. D., et al. (1996) Mice lacking the gene encoding tissue-type plasminogen activator show a selective inference with late-phase long-term potentiation in both Schaffer collateral and mossy fiber pathways. *Proc. Natl. Acad. Sci. USA* **93,** 8699–8704.
61. Glenner G. G. and Wong C. W. (1984) Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochem. Biophys. Res. Commun.* **120,** 885–890.
62. Seubert P., Vigo-Pelfrey C., Esch F., Lee M., Dovey H., Davis D., et al. (1992) Isolation and quantification of soluble Alzheimer's beta-peptide from biological fluids. *Nature* **359,** 325–327.
63. Selkoe D. J. (1998) The cell biology of beta-amyloid precursor protein and presenilin in Alzheimer's disease. *Trends Cell Biol.* **8,** 447–453.
64. Jordan J., Galindo M. F., Miller R. J., Reardon C. A., Getz G. S., and LaDu M. J. (1998) Isoform-specific effect of apolipoprotein E on cell survival and beta-amyloid-induced toxicity in rat hippocampal pyramidal neuronal cultures. *J. Neurosci.* **18,** 195–204.
65. Qiu W. Q., Borth W., Ye Z., Haass C., Teplow D. B., and Selkoe D. J. (1996) Degradation of amyloid beta-protein by a serine protease-alpha2-macroglobulin complex. *J. Biol. Chem.* **271,** 8443–8451.
66. Narita M., Holtzman D. M., Schwartz A. L., and Bu G. (1997) Alpha2-macroglobulin complexes with and mediates the endocytosis of beta-amyloid peptide via cell surface low-density lipoprotein receptor-related protein. *J. Neurochem.* **69,** 1904–1911.
67. Qiu Z., Strickland D. K., Hyman B. T., and Rebeck G. W. (1999) Alpha2-macroglobulin enhances the clearance of endogenous soluble beta-amyloid peptide via low-density lipoprotein receptor-related protein in cortical neurons. *J. Neurochem.* **73,** 1393–1398.
68. Kang D. E., Pietrzik C. U., Baum L., Chevallier N., Merriam D. E., Kounnas M. Z., et al. (2000) Modulation of amyloid beta-protein clearance and Alzheimer's disease susceptibility by the LDL receptor-related protein pathway. *J. Clin. Invest.* **106,** 1159–1166.
69. Van Uden E., Carlson G., St George-Hyslop P., Westaway D., Orlando R., Mallory M., Rockenstein E., and Masliah E. (1999) Aberrant presenilin-1 expression downregulates LDL receptor-related protein (LRP): is LRP central to Alzheimer's disease pathogenesis? *Mol. Cell Neurosci.* **14,** 129–140.
70. Kounnas M. Z., Moir R. D., Rebeck G. W., Bush A. I., Argraves W. S., Tanzi R. E., Hyman B. T., and Strickland D. K. (1995) LDL receptor-related protein, a multifunctional ApoE receptor, binds secreted beta-amyloid precursor protein and mediates its degradation. *Cell* **82,** 331–340.
71. Knauer M. F., Orlando R. A., and Glabe C. G. (1996) Cell surface APP751 forms complexes with protease nexin 2 ligands and is internalized via the low density lipoprotein receptor-related protein (LRP). *Brain Res.* **740,** 6–14.
72. Ulery P. G., Beers J., Mikhailenko I., Tanzi R. E., Rebeck G. W., Hyman B. T., and Strickland D. K. (2000) Modulation of beta-amyloid precursor protein processing by the low density lipoprotein receptor-related protein (LRP). Evidence that LRP contributes to the pathogenesis of Alzheimer's disease. *J. Biol. Chem.* **275,** 7410–7415.
73. Kang D. E., Saitoh T., Chen X., Xia Y., Masliah E., Hansen L. A., et al. (1997) Genetic association of the low-density lipoprotein receptor-related protein gene (LRP), an apolipoprotein

E receptor, with late-onset Alzheimer's disease. *Neurology* **49,** 56–61.
74. Kamboh M. I., Ferrell R. E., and DeKosky S. T. (1998) Genetic association studies between Alzheimer's disease and two polymorphisms in the low density lipoprotein receptor-related protein gene. *Neurosci. Lett.* **244,** 65–68.
75. Baum L., Chen L., Ng H. K., Chan Y. S., Mak Y. T., Woo J., Chiu H. F., and Pang C. P. (1998) Low density lipoprotein receptor related protein gene exon 3 polymorphism association with Alzheimer's disease in Chinese. *Neurosci. Lett.* **247,** 33–36.
76. Hollenbach E., Ackermann S., Hyman B. T., and Rebeck G. W. (1998) Confirmation of an association between a polymorphism in exon 3 of the low-density lipoprotein receptor-related protein gene and Alzheimer's disease. *Neurology* **50,** 1905–1907.
77. Lambert J. C., Wavrant-De Vrieze F., Amouyel P., and Chartier-Harlin M. C. (1998) Association at LRP gene locus with sporadic late-onset Alzheimer's disease. *Lancet* **351,** 1787–1788.
78. Schmechel D. E., Saunders A. M., Strittmatter W. J., Crain B. J., Hulette C. M., Joo S. H., et al. (1993) Increased amyloid beta-peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer disease. *Proc. Natl. Acad. Sci. USA* **90,** 9649–9653.
79. Corder E. H., Saunders A. M., Strittmatter W. J., Schmechel D. E., Gaskell P. C., Small G. W., et al. (1993) Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* **261,** 921–923.
80. Namba Y., Tomonaga M., Kawasaki H., Otomo E., and Ikeda K. (1991) Apolipoprotein E immunoreactivity in cerebral amyloid deposits and neurofibrillary tangles in Alzheimer's disease and kuru plaque amyloid in Creutzfeldt-Jakob disease. *Brain Res.* **541,** 163–166.
81. Holtzman D. M., Bales K. R., Wu S., Bhat P., Parsadanian M., Fagan A. M., et al. (1999) Expression of human apolipoprotein E reduces amyloid-beta deposition in a mouse model of Alzheimer's disease. *J. Clin. Invest.* **103,** R15–R21.
82. LaDu M. J., Falduto M. T., Manelli A. M., Reardon C. A., Getz G. S., and Frail D. E. (1994) Isoform-specific binding of apolipoprotein E to beta-amyloid. *J. Biol. Chem.* **269,** 23,403–23,406.
83. Strickland D. K., Ashcom J. D., Williams S., Burgess W. H., Migliorini M., and Argraves W. S. (1990) Sequence identity between the alpha 2-macroglobulin receptor and low density lipoprotein receptor-related protein suggests that this molecule is a multifunctional receptor. *J. Biol. Chem.* **265,** 17,401–17,404.
84. Holtzman D. M., Pitas R. E., Kilbridge J., Nathan B., Mahley R. W., Bu G., and Schwartz A. L. (1995) Low density lipoprotein receptor-related protein mediates apolipoprotein E-dependent neurite outgrowth in a central nervous system-derived neuronal cell line. *Proc. Natl. Acad. Sci. USA* **92,** 9480–9484.
85. Rebeck G. W., Reiter J. S., Strickland D. K., and Hyman B. T. (1993) Apolipoprotein E in sporadic Alzheimer's disease: allelic variation and receptor interactions. *Neuron* **11,** 575–580.
86. Blacker D., Wilcox M. A., Laird N. M., Rodes L., Horvath S. M., Go R. C., et al. (1998) Alpha-2 macroglobulin is genetically associated with Alzheimer disease. *Nat. Genet.* **19,** 357–360.
87. Liao A., Nitsch R. M., Greenberg S. M., Finckh U., Blacker D., Albert M., et al. (1988) Genetic association of an aplha2-macroglobulin (Val1000lle) polymorphism and Alzheimer's disease. *Hum. Mol. Genet.* **7,** 1953–1956.
88. Du Y., Bales K. R., Dodel R. C., Liu X., Glinn M. A., Horn J. W., Little S. P., and Paul S. M. (1998) Alpha2-macroglobulin attenuates beta-amyloid peptide 1–40 fibril formation and associated neurotoxicity of cultured fetal rat cortical neurons. *J. Neurochem.* **70,** 1182–1188.
89. Van Uden E., Sagara Y., Van Uden J., Orlando R., Mallory M., Rockenstein E., and Masliah E. (2000) A protective role of the low density lipoprotein receptor-related protein against amyloid beta-protein toxicity. *J. Biol. Chem.* **275,** 30,525–30,430.
90. Scheuner D., Eckman C., Jensen M., Song X., Citron M., Suzuki N., et al. (1996) Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease [see comments]. *Nat. Med.* **2,** 864–870.
91. Borchelt D. R., Thinakaran G., Eckman C. B., Lee M. K., Davenport F., Ratovitsky T., et al. (1996) Familial Alzheimer's disease-linked presenilin 1 variants elevate Abeta1-42/1-40 ratio in vitro and in vivo. *Neuron* **17,** 1005–1013.

92. Koo E. H. and Squazzo S. L. (1994) Evidence that production and release of amyloid beta-protein involves the endocytic pathway. *J. Biol. Chem.* **269,** 17,386–17,389.
93. Van Nostrand W. E., Farrow J. S., Wagner S. L., Bhasin R., Goldgaber D., Cotman C. W., and Cunningham D. D. (1991) The predominant form of the amyloid beta-protein precursor in human brain is protease nexin 2. *Proc. Natl. Acad. Sci. USA* **88,** 10,302–10,306.
94. Kang J. and Muller-Hill B. (1990) Differential splicing of Alzheimer's disease amyloid A4 precursor RNA in rat tissues: PreA4(695) mRNA is predominantly produced in rat and human brain. *Biochem. Biophys. Res. Commun.* **166,** 1192–1200.
95. Wertkin A. M., Turner R. S., Pleasure S. J., Golde T. E., Younkin S. G., Trojanowski J. Q., and Lee V. M. (1993) Human neurons derived from a teratocarcinoma cell line express solely the 695-amino acid amyloid precursor protein and produce intracellular beta-amyloid or A4 peptides. *Proc. Natl. Acad. Sci. USA* **90,** 9513–9517.
96. Bu G., Maksymovitch E. A., Nerbonne J. M., and Schwartz A. L. (1994) Expression and function of the low density lipoprotein receptor-related protein (LRP) in mammalian central neurons. *J. Biol. Chem.* **269,** 18,521–18,528.
97. Rebeck G. W., Harr S. D., Strickland D. K., and Hyman B. T. (1995) Multiple, diverse senile plaque-associated proteins are ligands of an apolipoprotein E receptor, the alpha 2-macroglobulin receptor/low-density-lipoprotein receptor-related protein. *Ann. Neurol.* **37,** 211–217.
98. Lopes M. B., Bogaev C. A., Gonias S. L., and VandenBerg S. R. (1994) Expression of alpha 2-macroglobulin receptor/low density lipoprotein receptor-related protein is increased in reactive and neoplastic glial cells. *FEBS Lett.* **338,** 301–305.
99. Marzolo M. P., von Bernhardi R., Bu G., and Inestrosa N. C. (2000) Expression of alpha(2)-macroglobulin receptor/low density lipoprotein receptor-related protein (LRP) in rat microglial cells. *J. Neurosci. Res.* **60,** 401–411.
100. Wavrant-DeVrieze F., Lambert J. C., Stas L., Crook R., Cottel D., Pasquier F., et al. (1999) Association between coding variability in the LRP gene and the risk of late-onset Alzheimer's disease. *Hum. Genet.* **104,** 432–434.
101. Senzaki K., Ogawa M., and Yagi T. (1999) CNR Proteins of the CNR family are multiple receptors for Reelin. *Cell* **99,** 635–647.
102. Dulabon L., Olson E. C., Taglienti M. G., Eisenhuth S., McGrath B., Walsh C. A., Kreidberg J. A., and Anton E. S. (2000) Reelin binds $\alpha 3\beta 1$ integrin and inhibits neuronal migration. *Neuron* **27,** 33–44.
103. Misra U. K., Chu C. T., Gawdi G., and Pizzo S. V. (1994) Evidence for a second $\alpha 2$-macroglobulin receptor. *J. Biol. Chem.* **269,** 12,541–12,547.
104. Misra U. K. and Pizzo S. V. (1998) Ligation of the $\alpha 2$M signaling receptor with receptor-recognized forms of $\alpha 2$-macroglobulin initiates protein and DNA synthesis in macrophages. The effect of intracellular calcium. *Biochim. Biophys. Acta* **1401,** 121–128.