Low Density Lipoprotein Receptor-related Protein (LRP) Interacts with Presenilin 1 and Is a Competitive Substrate of the Amyloid Precursor Protein (APP) for \(\gamma\)-Secretase*

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Presenilin 1 (PS1) is a critical component of the \(\gamma\)-secretase complex, which is involved in the cleavage of several substrates including the amyloid precursor protein (APP) and the Notch receptor. Recently, the low density receptor-related protein (LRP) has been shown to be cleaved by a \(\gamma\)-secretase-like activity. We postulated that LRP may interact with PS1 and tested its role as a competitive substrate for \(\gamma\)-secretase. In this report we show that LRP colocalizes and interacts with endogenous PS1 using coimmunoprecipitation and fluorescence lifetime imaging microscopy. In addition, we found that \(\gamma\)-secretase active site inhibitors do not disrupt the interaction between LRP and PS1, suggesting that the substrate associates with a \(\gamma\)-secretase docking site located in close proximity to PS1. This is analogous to APP-\(\gamma\)-secretase interactions. Finally, we show that LRP competes with APP for \(\gamma\)-secretase activity. Overexpression of a truncated LRP construct consisting of the C terminus, the transmembrane domain, and a short extracellular portion leads to a reduction in the levels of the \(\beta\)-amyloid \(\beta\)42, and \(\beta\)3 peptides without changing the total level of APP expression. In addition, transfection with the \(\beta\)-chain of LRP causes an increase in uncleaved APP C-terminal fragments and a concomitant decrease in the signaling effects of the APP intracellular domain. In conclusion, LRP is a PS1 interactor and can compete with APP for \(\gamma\)-secretase enzymatic activity.

The low density lipoprotein receptor-related protein (LRP)\(^1\) is a \(-600\) kDa type I integral membrane protein that belongs to the ancient low density lipoprotein receptor gene family (1). LRP interacts with at least 30 different ligands that bind to the extracellular domain, including \(\alpha\)-2-macroglobulin (2, 3), lipases, plasminogen activators (4–6), apolipoprotein E (7, 8), and the amyloid precursor protein (APP) (9, 10). The cytoplasmic tail of LRP contains two NPXY motifs that serve as docking sites for endocytosis machinery, scaffold proteins, and cytoplasmic adaptors involved in signaling events (11, 12). These include the mammalian Disabled-1 (Dab1) (12), the postsynaptic density protein (PSD-95) (13), and the scaffold protein Fe65, which interacts with LRP through its phosphotyrosine binding domain (12).

LRP is cleaved by furin in the trans-Golgi network, generating a \(515\)-kDa \(\alpha\)-subunit and a \(85\) kDa \(\beta\)-subunit that remain co- and post-translationally associated as they are transported to the cell surface (14). LRP also undergoes proteolytic shedding of the extracellular domain by a metalloproteinase (15). It has recently been shown that the cytoplasmic tail of LRP can be processed intramembranously by a \(\gamma\)-secretase activity that releases its intracellular domain (16). The \(\gamma\)-secretase complex is a multiprotein complex that is composed of at least four members, namely presenilin 1 (PS1), which is believed to contain the catalytic site, nicastrin, Pen-2, and Aph1 (17). This complex is responsible for cleavage of at least 15 substrates, including APP and the Notch receptor. APP is a type-I transmembrane protein that is cleaved by \(\alpha\)- or \(\beta\)-secretase to remove its large extracellular domain. The remaining \(83\)-residue (APPC83) or \(99\)-residue (APPC99) fragments are cleaved intramembranously by \(\gamma\)-secretase to release the fragment \(\beta3\) or the amyloid-\(\beta\) peptide (\(\beta\)P), respectively (18). \(\gamma\)-Secretase also cleaves near the inner leaflet to generate an APP intracellular domain (AICD), which may have signaling properties (19, 20). LRP has a complex set of interactions with APP. LRP interacts both at the extracellular domain of the Kunitz protease inhibitor-containing isoform of APP (APP770 and APP751) and with

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1. The abbreviations used are: LRP, low density lipoprotein receptor-related protein; \(\beta\)P, amyloid-\(\beta\) peptide; APP, amyloid precursor protein; AICD, APP intracellular domain; CHO, Chinese hamster ovary; CT, C terminus; CTF, C-terminal fragment; DAPT, N-(\(\alpha\)-3,5-difluorophenacyl)-\(\alpha\)-ethyl-L-alanyl)\(-\)phenylglycine \(t\)-butyl ester; FITC, fluorescein isothiocyanate; FLIM, fluorescence lifetime imaging microscopy; FRET, fluorescence resonance energy transfer; LC, \(\beta\)-subunit of LRP; PS1, presenilin 1; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine.
the intracellular domain of all isoforms of APP via adaptor proteins such as Fe65 (9, 10, 21, 22). LRP has also been shown to modulate Aβ clearance and APP processing (23–27).

In this study we explore whether LRP directly interacts with PS1 (28), and acts as a competitive substrate for γ-secretase. We (29, 30) and others (31, 32) have shown previously that Notch and APP can compete for γ-secretase activity. Here we extend the study to test whether LRP is also a competitive substrate of APP for γ-secretase. We show that LRP interacts with PS1, binds to a docking site on PS1/γ-secretase, and can compete with APP for the enzymatic activity.

MATERIALS AND METHODS

Plasmids, Cell Lines, and Transfection Protocol—A summary of the LRP constructs used in this study is presented in Fig. 1. The LC construct contains the β-subunit of LRP with a Myc tag at the N terminus. The muLC construct consists of the β-subunit of LRP with a Myc tag at the N terminus in which asparagines and the tyrosine in both NPXY motifs have been substituted by alamines (34). The LRP-CT construct, consisting of the last 370 amino acids of the 601-amino acid LRP β-subunit, was subcloned into the pLHCX retroviral expression vector and transferred into the 293 GP packaging cell line (27).

Chinese hamster ovary (CHO) cells, human neuroblastoma (H4) cells, human embryonic kidney cells, and mouse embryonic fibroblasts lacking PS1 (MEF PS1+/−) or MEF PS1−/−—stably expressing human wild-type PS1 (35) were used in this study. Cells were cultured in OPTI-MEM with 5% fetal bovine serum at 37 °C with 5% CO2 in a wild-type PS1 (35) were used in this study. Cells were cultured in

FIG. 1. Summary of the constructs used in this study. Because most γ-secretase substrates require shedding of their extracellular domain, we used LC, a short form of LRP that contains the β-chain of LRP, and LRP-CT, the LRP C-terminal fragment that contains the last 370 amino acids of the β-chain of LRP. We used two antibodies to detect LRP, 11H4, and 1704, both directed against the C terminus of LRP. mutLC, mutant LC.

Enzyme-linked Immunosorbent Assay Measurements and APP Internalization Assay—Substrates of APP for Notch and APP can compete for the enzymatic activity. We (29, 30) and others (31, 32) have shown previously that LRP interacts with Presenilin 1 (35) were used in this study. Cells were cultured in

Metabolic Labeling—Confluent cultures of CHO 7WD10 and CHO 7WD10 LRP-CT cells were incubated in methionine-free Dulbecco’s modified Eagle’s medium supplemented with 150 μCi/ml [35S]methionine/cysteine for 1 h. Media were collected after 12 h, and total Aβ was measured as described (24). To measure internalization of cell surface pools of APP, respectively. The ratio of acid-resistant to acid-labile counts, therefore, provided a measure of the internalized versus cell surface pools of APP. The experiments were repeated three times. Fe65-dependent APP Luciferase Transactivation Assay—Human embryonic kidney cells were cotransfected with the APP-Gal4 construct (a gift from Dr. Thomas Südhof, University of Texas Southwestern Medical Center, which was cloned from APP 695 with the Gal4 sequence spliced into the cytoplasmic tail), pG5E1B-Luc (a luciferase reporter plasmid with a Gal4 activation-dependent binding domain), Fe65, and a β-galactosidase plasmid (19). The generation of Fe65 with a C-terminal Myc tag has been reported elsewhere (42). Relative luciferase activity was measured and normalized to β-galactosidase activity.

Immunocytochemistry and Antibodies—Cells were fixed and immunostained 24 after transfection as described (43). Briefly, cells were permeabilized with 0.5% Triton X-100 for 10 min, washed in PBS (pH 7.3), permeabilized by 0.5% Triton X-100 for 20 min, and blocked with 1.5% normal goat serum for 1 h. We used an antibody directed against the N terminus of PS1 (X81) and a mouse monoclonal antibody, 11H4, against the LRP C-terminal fragment (a hybridoma secreting 11H4 was obtained from the American Type Culture Collection, Manassas, VA). For the fluorescence lifetime imaging microscopy (FLIM) assays a FITC-labeled secondary antibody was used as a donor fluorophore, and a Cy3-labeled secondary antibody was used as an acceptor fluorophore.

Fluorescence Lifetime Imaging Microscopy Assay—FLIM has been recently described as a novel technique for the analysis of protein proximity (44–46). The technique is based on the observation that fluorescence lifetimes of a donor fluorophore shorten in the presence of a FRET acceptor in close proximity (<10 nm). The decrease in lifetime is proportional to the distance between the fluorophores at R6. A mode-locked titanium-sapphire laser (Spectra-Physics, Fremont, CA) emits a femtosecond pulse every 12 ns to excite the fluorophore. A high-speed Hamamatsu (Bridgewater, NJ) detector and hardware/software (SPC-730, Hamamatsu) were used to measure fluorescence lifetimes on a pixel-by-pixel basis. Donor fluorophore (FITC) lifetimes were fitted to two exponential decay curves as described (44, 45). All samples were compared with a negative control in which the donor fluorophore (FITC) fluorescence lifetime was measured in the absence of the acceptor (no FRET) and was ~2300 ps. As a positive control, FITC lifetime was measured in the presence of a FRET acceptor (Cy3) in close proximity. In the positive control, FITC lifetime is shortened to <1000 ps.

Statistical Analysis—One-way analysis of variance (ANOVA) was performed to analyze differences in lifetime followed by least significant difference post hoc analysis. Levene’s test was also performed to determine whether variances were equal.

RESULTS

Colocalization, FRET, and Immunoprecipitation Show That LRP Interacts with Presenilin 1—Because LRP is cleaved by a γ-secretase activity and PS1 contains the catalytic site of the multi-protein γ-secretase complex (28), we first tested whether LRP interacts directly with PS1. We transfected CHO cells with wild-type PS1 and wild-type LRP/LC and double immunostained the cells with antibodies against PS1 and the C terminus of LRP. Using confocal microscopy, we observed that wild-type LRP/LC colocalized with PS1 mainly in the endoplasmic reticulum (Fig. 2). However, because colocalization has a spatial resolution of >250 nm, we used FLIM to test whether
PS1 and LRP interact more closely. FLIM is based on the observation that the lifetime of a donor fluorophore is shorter in the presence of a FRET acceptor in close proximity. We transfected CHO cells with wild-type PS1 and wild-type LC and immunostained the cells with a FITC-labeled antibody against the N terminus of PS1 and a Cy3-labeled antibody against the C terminus of LRP. In the absence of an acceptor, FITC lifetime was ~2300 ps. However, immunostaining of LRP with a Cy3-labeled antibody shortened the FITC lifetime markedly, indicating that the fluorophores are in close proximity (Table I). This shortening in FITC lifetime was specific, because immunostaining Bip, an endoplasmic reticulum-resident protein that colocalizes with PS1, with a Cy3-labeled antibody did not alter FITC lifetime. In cells transfected with PS1 and LRP/LC we observed that the strongest interactions occur close to the cell membrane (Fig. 3) in the same type of spatial patterns that we have observed previously for two other γ-secretase substrates, APP and Notch (44, 47). As expected, the lifetime pattern in cells stained for Bip did not differ from that of the negative control. Next, we examined whether the NPXY domains of LRP are necessary for the interaction of LRP with PS1, which might be the case if the interaction was mediated by an adaptor protein. We transfected cells with wild-type PS1 and a mutant LRP/LC construct in which both NPXY domains have been mutated and immunostained the cells for PS1 and LRP. Cells transfected with mutant LC showed similar patterns of expression and colocalization with PS1 as wild-type LC (Table I). The main interacting molecules were localized close to the cell membrane, similar to what was observed in wild-type LC-expressing cells (Fig. 3).

These data suggest that both wild-type LC and mutant LC interact with PS1 and that the NPXY domains of LRP are not essential for PS1-LRP interactions. The observed interaction between LRP and PS1 in transfected cells was also confirmed at the endogenous level in primary cultured neurons, as reflected by a significant shortening in donor lifetime when cells were double immunostained for LRP and PS1.

To further confirm this interaction, we performed communoprecipitation experiments using an LRP-specific antibody (1704) for immunoprecipitation of an endogenous LRP and the N-terminal PS1 antibody (PSN2) for the subsequent Western blotting. These experiments were performed in mouse embryonic fibroblasts PS1−/− cells expressing wild-type human PS1 and as control in PS1−/− cells. In cells expressing PS1, but not in PS1−/− cells, complexes of N-terminal fragments of PS1 can be recovered by immunoprecipitation with an LRP antibody (Fig. 4). Importantly, we did not detect any interaction of full-length PS1 with LRP (Fig. 4), suggesting that LRP interacts primarily with the active heterodimeric form of PS1. We then performed a reverse communoprecipitation experiment using PS1 N-terminal (X81) or PS1 C-terminal (4627) antibodies for immunoprecipitation of the endogenous PS1 from a normal rat brain and the LRP-CT antibody (11H4) for Western blotting. We found two LRP-positive bands representing LRP full-length β-chain and a C-terminal fragment (Fig. 4) in a PS1-immunoprecipitated (right lane) sample, but not in a no-antibody immunoprecipitated (left lane) sample. We interpret this as an indication of a direct interaction between PS1 and LRP in mammalian cells.

\[ \text{TABLE I} \]

| Condition       | No. of Cells Analyzed | FRET Acceptor | FITC Lifetime | Probability |
|-----------------|-----------------------|---------------|---------------|-------------|
| No acceptor     | 6                     | None          | 2274 ± 39 ps  | p > 0.001   |
| LRP/LC          | 6                     | LRP C terminus| 1934 ± 81 ps  | p < 0.001   |
| LRP/mutLC       | 6                     | LRP C terminus| 1966 ± 116 ps | p < 0.001   |
| Bip             | 10                    | Bip           | 2409 ± 115 ps | NS          |

- **a** Number of cells analyzed.
- **b** Cy3-labeled.
- **c** Mean ± S.E.
- **d** Compared to FITC without acceptor.
- **e** Not significant.
control the FITC lifetime was shortened, indicating that both fluorophores are in close proximity (Table II). Treatment with either DAPT or WPE-III-31C did not significantly change FITC lifetime as compared with a vehicle treatment, suggesting that the availability of the active site is not critical for the LRP-PS1 interactions observed. This result is analogous to observations with APP-PS1 in which a stable docking site interaction can be detected when the active site is occupied (44).

Both Wild-type LRP/LC and mutant LRP/LC are Competitive Substrates for γ-Secretase—Because both APP and LRP are substrates of γ-secretase, we asked whether LRP overexpression could affect the γ-secretase cleavage of APP. We metabolically labeled CHO cells stably transfected with APP751 and with LRP-CT (7WD10-CT) or without LRP-CT (7WD10) and measured the Aβ and p3 peptides, the two γ-cleaved products of APP. We observed that the levels of Aβ and p3 were markedly reduced in cells overexpressing LRP-CT as measured by immunoprecipitation (Fig. 5a). Interestingly, when we analyzed different clones of 7WD10-CT cells we observed an inverse relation between the levels of expression of LRP-CT and the levels of Aβ secretion (Fig. 5b, and c). We also measured Aβ40 and Aβ42 using an enzyme-linked immunosorbent assay and observed that both Aβ species were decreased when LRP-CT was overexpressed (Fig. 5d). Next, we analyzed whether the difference in Aβ secretion could be due to different rates of APP internalization by using radiolabeled APP antibodies. We did not find differences in APP internalization rates between cells coexpressing APP751 and those overexpressing APP751 and LRP-CT (Fig. 5e). We also examined whether transfection with LRP could alter the levels of APP C-terminal fragments (CTFs), the direct substrates of γ-secretase. We observed that, in CHO cells transfected with LRP/CLC and APP770, a small increase in the levels of APP CTFs could be seen compared with cells transfected with an empty vector and APP770 (Fig. 5f). It has been shown that γ-secretase also cleaves APP in the inner leaflet of the membrane to release the AICD (19). We asked whether transfection with LRP would have any impact on AICD generation. We postulated that, if LRP and APP competed for γ-secretase, we should observe a reduction in AICD generation after transfection with LRP. To measure AICD generation, we measured the downstream signaling effects of APP-Gal4 (19). Human embryonic kidney cells were cotransfected with APP-Gal4, pG5E1B-Luc, Fe65, and either wild-type LC, mutant LC, or an empty vector. An expected, an increase in the ability of APP-Gal4 to stimulate transcription was observed when Fe65 was cotransfected (Fig. 6). In addition, we found that both wild-type LRP/CLC and mutant LRP/CLC both attenuated this phenomenon, decreasing the signaling effects of the Fe65-stabilized AICD generated from the Gal4-dependent luciferase reported plasmid. The attenuation was not due to differences in the levels of expression of APP or Fe65 (Fig. 6).

Table II

γ-Secretase inhibitors do not disrupt the interaction between LRP/LC and presenilin 1

We transfected H4 cells with wild-type LRP/LC and PS1 and treated them with a vehicle, 1 μM DAPT, or 1 μM WPE-III-31C (36, 37). After 24 h we immunostained for LRP and PS1 with a FITC-labeled antibody and a Cy3-labeled antibody, respectively. We measured FITC lifetime in the absence or presence of a FRET acceptor. As expected, in the absence of an acceptor FITC lifetime was ~2300 ps, but in the presence of LRP/LC the FITC lifetime was markedly shortened. Treatment with γ-secretase inhibitors did not significantly change FITC lifetime. Values from a representative experiment are presented. Similar results were observed in four separate experiments (9–10 cells per condition).

DISCUSSION

Since the making of the initial discovery that APP is cleaved by PS1-dependent γ-secretase (28, 48–50), multiple other substrates that undergo γ-secretase cleavage have been identified, including Notch, Erb-B4, E- and N-cadherins, CD44, nectin-1, the Notch ligands Delta and Jagged, and LRP (17, 51). It is not clear whether these substrates are all cleaved by the same PS1-dependent activity or by the same population of enzyme complexes. We have now examined this issue with regard to LRP; we find that LRP interacts with PS1 in distal cell compartments as assessed by FLIM and coimmunoprecipitation experiments and that presentation of a truncated form of LRP can inhibit Aβ production, increase APP CTFs, and decrease AICD generation.

We interpret these data to be consistent with the idea that LRP is a competitive inhibitor of APP, implying that the two are cleaved by the same population of γ-secretase complexes.

LRP is a transmembrane glycoprotein implicated in diverse biological processes both as an endocytic receptor and as a signaling molecule. Recent reports show that LRP undergoes cleavage by a γ-secretase activity that results in the release of the LRP cytoplasmic domain (16). This LRP cytoplasmic domain also can interact with Fe65 and has been shown to translocate to the nucleus where it can interact with the transcription modulator Tip60 (52). Notch is also a single transmembrane domain protein whose γ-secretase-dependent cleavage initiates a transcriptional cascade. We and others have demonstrated previously that Notch and APP compete for the same PS1-dependent γ-secretase activity (29–32). Our current data on LRP support the possibility that multiple substrates can compete for γ-secretase activity.

Accumulating evidence suggests that γ-secretase is an aspartyl protease and that two conserved aspartates in presenilins are catalytic residues (28, 53). The direct binding of transition-state analogue γ-secretase inhibitors to PS1 fragments strongly suggest that the active site is at the interface between the N- and C-terminal fragments and that each subunit contributes one of the two critical aspartates (37, 53). In addition,
recent biochemical evidence points to the existence of an initial substrate binding site in γ-secretase distinct from the active site. Isolation of the γ-secretase complex with an immobilized transition state analogue co-purifies with an endogenous APP substrate, suggesting that the substrate can bind at some other site (54). Moreover, APP and PS1 remain in close proximity in the presence of γ-secretase transition state analogues as measured by FRET (44). In this study we found that LRP behaves much like APP in terms of its interactions with γ-secretase; we can detect strong FRET between LRP and PS1, and this close proximity persists after treatment with γ-secretase active site inhibitors. These data are consistent with the notion of an initial substrate binding site in or in close proximity to PS1. Because most γ-secretase substrates require a prior shedding of the extracellular domain, in this study we used short forms of LRP, namely LC, which contains the entire γ-chain of LRP, and the C-terminal fragment of LRP (LRP-CT) (27, 34). First, we show that overexpressing LRP-CT reduces Aβ40, Aβ42, and the p3 peptide and that this effect was not due to a change in APP expression level or APP internalization. In these cell lines we observed an inverse relation between the levels of expression of LRP-CT and the levels of Aβ secretion. We also measured Aβ40 and Aβ42 using an enzyme-linked immunosorbent assay and observed that both were decreased when overexpressing LRP-CT as compared with cells overexpressing only APP. The difference in Aβ secretion is not explained by different rates of APP internalization. We did not find differences in APP internalization between cells overexpressing APP751 and those overexpressing APP751 and LRP-CT using radiolabeled APP antibodies. f, CHO cells were transfected with APP770 and LC or an empty vector. In a Western blot, we observed a small increase in APP C-terminal fragments when we transfected with LC compared with an empty vector. No differences were seen in full-length (FL) APP. A representative blot is shown. Similar results were obtained in three separate experiments.

**FIG. 5. Overexpression of LRP-CT leads to a reduction in Aβ40, Aβ42, and p3 and an increase in APP C-terminal fragments. a,** we measured the Aβ and p3 peptides, the two γ-cleaved products of APP, in CHO cells stably transfected with APP751 and with LRP-CT (7.WD10-CT) or without LRP-CT (7.WD10) by immunoprecipitation. We observed that the levels of Aβ and p3 were markedly reduced in cells overexpressing LRP-CT. MW, molecular weight. **b** and **c,** we analyzed four different clones of 7W10-CT cells and observed an inverse relation between the levels of expression of LRP-CT and the levels of Aβ secretion. **d,** we measured Aβ40 and Aβ42 as explained by different rates of APP internalization. We did not find differences in APP internalization between cells overexpressing APP751 and those overexpressing APP751 and LRP-CT using radiolabeled APP antibodies. **e,** we also measured Aβ40 and Aβ42 using an enzyme-linked immunosorbent assay and observed that both were decreased when overexpressing LRP-CT as compared with cells overexpressing only APP. The difference in Aβ secretion is not explained by different rates of APP internalization. We did not find differences in APP internalization between cells overexpressing APP751 and those overexpressing APP751 and LRP-CT using radiolabeled APP antibodies. **f,** CHO cells were transfected with APP770 and LC or an empty vector. In a Western blot, we observed a small increase in APP C-terminal fragments when we transfected with LC compared with an empty vector. No differences were seen in full-length (FL) APP. A representative blot is shown. Similar results were obtained in three separate experiments.
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LRP-CT and the levels of Aβ. Next, we found that transfection with LRP/TC, which contains the β-chain of LRP, or with a mutant form of LRP/TC impairs the transcriptional activity of the APP-Gal4 construct. Of note, the fact that the mutant form of LRP/TC also decreased the transcriptional activity of the APP-Gal4 construct suggests that APP and LRP do not compete for binding to the adaptor Fe65 or for binding to Tip60, which is Fe65-dependent. Finally, we also observed that co-transfection of CHO cells with LC could have been able to increase the levels of APP CTFs when compared with cells transfected with an empty vector. However, we cannot rule out the possibility that this increase is due to the effects of LRP on APP CTF stability rather than competition for γ-secretase (27).

APP is also a transmembrane type I protein, which is processed by α-, β-, and γ-secretase, and cleavage by the latter generates the Aβ and the p3 peptides. In addition to intramembranous cleavage, γ-secretase also cleaves APP at the inner leaflet of the membrane to generate AICD. The released AICD is thought to form a complex with the adaptor protein Fe65 and the histone acetyltransferase Tip60, which might have a role in transcriptional regulation (19, 20, 22). APP and LRP have been shown to display a complex set of interactions. In addition to Kunitz protease inhibitor-containing isoforms of APP, and the critical region that modulates APP processing was mapped to a seven-peptide domain around the second NPYX domain of LRP (27).

Our current observations show yet another level of interaction between APP and LRP as competitive substrates for γ-secretase. Interestingly, the APP-LRP interactions noted above depend in great part on adaptor proteins like Fe65 forming a heterodimeric complex, and these interactions are blocked by NPYX mutants. By contrast, the interaction of LRP with γ-secretase is not dependent on the NPYX domains. It will be of interest to further examine the structural correlates of LRP-PS1/γ-secretase interactions.

In summary, we show that LRP colocalizes directly interacts with PS1 by using confocal microscopy, FLIM, and coimmunoprecipitation assays. This result suggests that LRP γ-secretase cleavage occurs via the same PS1-containing complex that is responsible for APP cleavage. We also found that the mutant LRP/TC, in which both NPYX domains have been substituted, colocalized and interacted with PS1 as assessed by confocal microscopy and FLIM. These data indicate that the NPYX domains are not required for the PS1-LRP interactions.

Finally, under some circumstances LRP is able to reduce the γ-secretase-dependent cleavage of APP that generates the Aβ peptide, p3, and AICD. Taken together, these data support a model in which LRP and APP are competitive γ-secretase substrates.

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Low Density Lipoprotein Receptor-related Protein (LRP) Interacts with Presenilin 1 and Is a Competitive Substrate of the Amyloid Precursor Protein (APP) for \( \gamma \)-Secretase

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