ACCELERATED PUBLICATION

The low-density lipoprotein receptor-related protein 1 (LRP1) mediates the endocytosis of the cellular prion protein

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PrP^C (cellular prion protein) is located at the surface of neuronal cells in detergent-insoluble lipid rafts, yet is internalized by clathrin-dependent endocytosis. As PrP^C is glycosyl-phosphatidylinositol-anchored, it requires a transmembrane adaptor protein to connect it to the clathrin endocytosis machinery. Using receptor-associated protein and small interfering RNA against particular LDL (low-density lipoprotein) family members, in combination with immunofluorescence microscopy and surface biotinylation assays, we show that the transmembrane LRP1 (LDL receptor-related protein 1) is required for the Cu²⁺-mediated

endocytosis of PrP^C in neuronal cells. We show also that another LRP1 ligand that can cause neurodegenerative disease, the Alzheimer's amyloid precursor protein, does not modulate the endocytosis of PrP^C.

Key words: amyloid precursor protein (APP), clathrin, Cu²⁺-dependent endocytosis, low-density lipoprotein receptor-related protein-1 (LRP1), prion, receptor-associated protein (RAP).

INTRODUCTION

The prion protein (PrP) is the principal agent responsible for the transmissible spongiform encephalopathies, a group of fatal neurodegenerative diseases including Creutzfeldt–Jakob disease in humans, scrapie in sheep and bovine spongiform encephalopathy in cattle [1]. In these prion diseases the normal cellular form of the prion protein, PrP^C, undergoes a conformational change to the infectious form, PrP^{Sc}. PrP^C is transported through the secretory pathway to the cell surface, where it is attached to the outer leaflet of the plasma membrane via a GPI (glycosyl-phosphatidylinositol) anchor and localized in cholesterol- and glycosphingolipid-rich lipid rafts [2,3]. The N-terminal half of PrP^C contains four octapeptide repeats (PHGG(G/S)WGQ; residues 59–90) that bind Cu²⁺ ions. The physiological importance of Cu²⁺ binding to the octapeptides is evidenced by the finding that exposure of neuronal cells to concentrations (50–100 µM) of Cu²⁺, similar to that in the extracellular spaces of the brain [4,5], results in the rapid internalization of PrP^C [6–8]. This metal-dependent endocytosis of PrP^C was abrogated by deletion of the octapeptide repeats or by an insertional mutation within the repeats, which is associated with an inherited form of human prion disease [7].

Electron microscopy studies [9,10], as well as selective pharmacological and molecular disruption experiments [11], have shown that in neuronal cells PrP^C is endocytosed by a clathrin-mediated mechanism. PrP^C moves laterally out of detergent-insoluble lipid rafts into detergent-soluble regions of the plasma membrane prior to its endocytosis via clathrin-coated pits [10,11]. As PrP^C is GPI-anchored and lacks a cytoplasmic domain, it cannot interact

directly with the clathrin endocytic machinery on the cytoplasmic face of the plasma membrane. Rather, its internalization requires it to 'piggy-back' on an integral transmembrane protein. The existence of such a transmembrane adaptor protein was first postulated over 10 years ago [9], yet its identity remains unknown. Using mutants of PrP^C that lacked either the octapeptide repeats or the polybasic KKRP tetrapeptide at the N-terminus of the mature protein [11], we showed that copper binding to the octapeptide repeats promotes dissociation of PrP^C from lipid rafts, whereas the N-terminal polybasic region mediated its interaction with a transmembrane adaptor protein which engages the clathrin endocytic machinery [11].

The LDL (low-density lipoprotein) receptor family is a group of cell-surface transmembrane proteins that bind a variety of ligands and internalize via clathrin-coated pits [12–14]. LRP1 (LDL receptor-related protein 1), along with LRP1B and LRP2 (megalin) are the largest members of this family of endocytic receptors with multiple ligand binding sites, although only LRP1 and LRP1B are highly expressed in neuronal cells [13]. LRP1 is a 600 kDa transmembrane glycoprotein that is cleaved in the *trans*-Golgi network by furin to generate a 515 kDa α - and an 85 kDa β -subunit, which remain non-covalently associated. Several ligands, including apolipoprotein E, APP (amyloid precursor protein) and α_2 -macroglobulin, bind to the α -subunit of LRP1 [15,16].

We hypothesized that LRP1 or LRP1B may be candidates for the transmembrane adaptor protein required by PrP^C to endocytose via clathrin-coated pits in neuronal cells. By selectively blocking the interaction of LDL family members with their ligands using soluble RAP (receptor-associated protein) and by the use of siRNA (small interfering RNA), we show that LRP1, but not

Abbreviations used: APP, amyloid precursor protein; GPI, glycosyl-phosphatidylinositol; HRP, horseradish peroxidase; HSPG, heparin sulfate proteoglycan; KPI, Kunitz-type protease inhibitor; LDL, low-density lipoprotein; LRP, LDL receptor-related protein; PAI-1, plasminogen activator inhibitor-1; PrP^C, cellular form of the prion protein; PrP^{Sc}, infectious form of the prion protein; RAP, receptor-associated protein; siRNA, small interfering RNA; uPA, urokinase-type plasminogen activator; uPAR, uPA receptor.

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LRP1B, is required to mediate the Cu^{2+} -stimulated endocytosis of PrP^{C} in human neuroblastoma SH-SY5Y cells.

EXPERIMENTAL

PrP constructs and cell culture

Insertion of the coding sequence of murine PrP containing a 3F4 epitope tag into pIRESneo (BD Biosciences) and the generation of SH-SY5Y cells stably expressing the protein have been reported previously [7]. SH-SY5Y cells expressing PrP^{C} were also stably transfected with a pIREShyg vector containing the cDNA encoding the KPI (Kunitz-type protease inhibitor) domain containing isoform of APP, APP₇₅₁. Cells were cultured in Dulbecco's modified Eagle medium supplemented with 10 % (v/v) foetal bovine serum, 50 units/ml penicillin and 0.1 mg/ml streptomycin. Cells were maintained in a humidified incubator at 37 °C in a 5 % CO_2 /95 % air atmosphere.

RNA interference studies

SH-SY5Y cells expressing PrP^{C} were seeded into T25 flasks at 70 % confluence and incubated with 500 pmol of a 2 μM Smartpool siRNA solution against LRP1, LRP1B or APP (Dharmacon) in complex with DharmaFECT-1 transfection reagent (Dharmacon) in serum-free medium. Mock-transfectants were incubated in the presence of DharmaFECT-1 only. After 2 h, serum was added to 10 % (v/v). Cells were incubated for a further 28 h (LRP1 and LRP1B) or 46 h (APP) prior to experimentation.

Cell-surface biotinylation endocytosis assay and immunoprecipitation

The cell-surface biotinylation endocytosis assay was performed as described previously [7,11]. Cells were co-incubated with 20 $\mu\text{g}/\text{ml}$ recombinant RAP (Merck Biosciences) during copper treatment, where indicated. Immunoprecipitation was carried out as described previously [11].

SDS/PAGE and Western blot analysis

Immunoprecipitated biotinylated complexes were mixed with dissociation buffer [125 mM Tris/HCl, pH 6.8, 2 % (w/v) SDS, 20 % (v/v) glycerol, 100 mM dithiothreitol and 0.02 % Bromophenol Blue] and boiled for 5 min. Proteins were resolved by SDS/PAGE [14.5 % (v/v) gels] and then transferred to Hybond-P PVDF membranes. The membranes were blocked for 1 h in PBS (1.5 mM KH_2PO_4 , 2.7 mM Na_2HPO_4 , 150 mM NaCl, pH 7.4) containing 5 % (w/v) dried milk powder and 0.1 % Tween-20, followed by incubation with HRP (horseradish peroxidase)-conjugated streptavidin (1:1000 dilution in PBS containing 0.1 % Tween-20) for 1 h. Bound HRP conjugates were visualized using the ECL[®] detection system (Amersham Biosciences). PrP^{C} , APP and β -actin were detected using antibodies 3F4, 22C11 and AC15 respectively, with an HRP-conjugated rabbit anti-mouse secondary antibody (Sigma–Aldrich). For detection of LRP1 and LRP1B, cell lysate samples were prepared in non-reducing dissociation buffer. LRP1 was detected with a monoclonal antibody 5A6 (Merck Biosciences) and LRP1B was detected using a rabbit polyclonal antibody (kindly given by Dr Guojun Bu, Washington University School of Medicine, St. Louis, U.S.A.).

Immunofluorescence microscopy

Cells were seeded on to coverslips and grown to 50 % confluence. The fate of cell-surface PrP^{C} was monitored by pre-labelling cells

Figure 1 Cu^{2+} -stimulated endocytosis of PrP^{C} is inhibited by RAP

(A) SH-SY5Y cells expressing PrP^{C} were surface biotinylated and then either untreated or treated with 20 $\mu\text{g}/\text{ml}$ recombinant RAP in the presence or absence of 100 μM Cu^{2+} for 20 min at 37 °C. Prior to lysis, the cells were, where indicated, incubated with trypsin to digest cell-surface PrP^{C} . Cells were then lysed and total PrP^{C} immunoprecipitated from the sample using antibody 3F4 and then subjected to Western blot analysis. The biotin-labelled PrP^{C} fraction was detected with HRP-conjugated streptavidin. (B) Densitometric analysis (means $\pm$ S.E.M.) for multiple blots (lanes 4 and 5 in A) from three separate experiments.

with antibody 3F4 for 30 min at 4 °C. For endocytosis experiments, cells were incubated for 20 min at 37 °C in OptiMEM in the presence or absence of 100 μM CuSO_4 presented as a histidine chelate [7]. Cells were then fixed with 4 % (v/v) paraformaldehyde/0.1 % (v/v) glutaraldehyde in PBS for 15 min, permeabilized in PBS containing 0.1 % Triton X-100, and then blocked for 1 h in PBS containing 3 % (v/v) goat serum or 5 % (v/v) fish skin gelatin (Sigma–Aldrich). Coverslips were then incubated overnight at 4 °C with either a goat polyclonal antibody directed against LRP1 (N-20; Santa Cruz Biotechnology) or the rabbit polyclonal antibody against LRP1B. Finally, coverslips were incubated with the appropriate fluorescent probe-conjugated secondary antibodies (Molecular Probes) for 1 h and mounted on slides using fluoromount G mounting medium (SouthernBiotech). Cells were visualized using a DeltaVision Optical Restoration Microscopy System (Applied Precision). Data were collected from 30–40 0.1 μm -thick optical sections, and three-dimensional datasets were deconvolved using the softWoRx program (Applied Precision). The presented images represent individual z-slices taken from the middle of the cell. Co-localization of endocytosed PrP^{C} with LDL-family members was performed using Imaris 4.0 (Bitplane AG).

Figure 2 Endocytosis of PrP^C is blocked by siRNA directed against LRP-1, but not LRP-1B

SH-SY5Y cells expressing PrP^C were incubated with a 2 μ M solution of the indicated LRP siRNA prepared with DharmaFECT-1 transfection reagent in OptiMEM for 30 h. **(A)** Cell lysates were immunoblotted for LRP1 expression using Ab 5A6 and LRP1B expression using a goat polyclonal antibody, along with PrP^C and β -actin. **(B)** Cells were surface biotinylated and treated with or without 100 μ M Cu²⁺ for 20 min at 37 °C. Prior to lysis, the cells were, where indicated, incubated with trypsin to digest cell-surface PrP^C. Cells were then lysed and total PrP^C immunoprecipitated from the sample using antibody 3F4. Samples were then subjected to Western blot analysis. The biotin-labelled PrP^C fraction was detected with HRP-conjugated streptavidin. **(C)** Densitometric analysis (means $\pm$ S.E.M.) for multiple blots (lanes 5 and 6 in **B**) from three separate experiments. *, Students *t*-test with values of $P < 0.05$ were taken as statistically significant.

RESULTS

RAP reduces the Cu²⁺-mediated endocytosis of PrP^C

To initially test the hypothesis that members of the LDL family of endocytic receptors may act as transmembrane adaptors in the Cu²⁺-mediated endocytosis of PrP^C, potential PrP^C–LDL family interactions were inhibited using RAP. RAP is a specialized chaperone that binds tightly to LDL family members in the early secretory pathway to assist correct folding and disulfide bond formation, and to prevent premature association of family members with their ligands [17]. Exogenously applied RAP has been used to universally antagonize binding of ligands to LDL family members [17]. Incubation of SH-SY5Y cells expressing murine PrP^C with the 3F4 epitope tag with 100 μ M Cu²⁺ resulted in the rapid endocytosis of cell surface PrP^C, as shown using surface biotinylation and subsequent trypsin digestion of biotinylated material remaining at the cell surface after 20 min at 37 °C [7,11]. However, in the presence of 20 μ g/ml recombinant human RAP, a concentration used in previous studies [18], the endocytosis of PrP^C was reduced significantly (Figure 1A). Densitometric analysis of multiple blots from three separate experiments revealed that 44 ± 8 % of biotin-labelled PrP^C was endocytosed in response to 100 μ M Cu²⁺. However, in cells incubated with RAP, only

10 ± 6 % of PrP^C was endocytosed in response to 100 μ M Cu²⁺ (Figure 1B), indicating a role for LDL family members in the Cu²⁺-mediated endocytosis of PrP^C.

Knockdown of LRP1, but not LRP1B, by siRNA inhibits the endocytosis of PrP^C

Owing to its ability to bind to a wide variety of ligands and its neuronal expression, LRP1 was selected as a likely candidate of the LDL family to be involved in the endocytosis of PrP^C, along with the more recently discovered LRP1 splice-variant, LRP1B, which is suggested to share a number of ligands with LRP1, but to internalize them at a lower rate [19]. To determine which is involved in the Cu²⁺-mediated endocytosis of PrP^C, SH-SY5Y cells expressing PrP^C were incubated with siRNAs directed against either LRP1 or LRP1B for 30 h. The cells were then surface biotinylated, incubated with 100 μ M Cu²⁺ followed by trypsin digestion. LRP1 protein expression was absent in cells incubated with the siRNA against LRP1, whereas the siRNA against LRP1B had no effect on LRP1 expression (Figure 2A). Similarly, the siRNA against LRP1B reduced LRP1B expression significantly, but had no effect on the expression of LRP1

(Figure 2A). The amount of PrP^C in the cell lysates was not altered by the knockdown of either LRP1 or LRP1B (Figure 2A). Although prior incubation with siRNA directed against LRP1B had no effect on the Cu²⁺-mediated endocytosis of PrP^C, knockdown of LRP1 reduced the Cu²⁺-mediated endocytosis of PrP^C significantly (Figure 2B). Densitometric analysis of multiple blots from three separate experiments revealed that only $10 \pm 3\%$ of cell-surface PrP^C was endocytosed in the cells incubated with the siRNA against LRP1 compared with $44 \pm 4\%$ in the untreated cells (Figure 2C). There was no significant difference in the amount of PrP^C endocytosed in the cells incubated with siRNA directed against LRP1B as compared with the untreated cells (Figure 2C).

The subcellular localization of LRP1 and LRP1B relative to that of PrP^C was also examined. In order to visualize the fate of cell-surface PrP^C only, cells were pre-labelled with antibody 3F4 prior to incubation in the presence or absence of Cu²⁺. The cells were then fixed and permeabilized before incubation with antibodies against either LRP1 or LRP1B. Both LRP1 and LRP1B were localized at the cell surface, and in intracellular compartments in both Cu²⁺ and non-Cu²⁺-treated cells (Figures 3A and 3B). In cells incubated in the absence of Cu²⁺, 3F4-labelled PrP^C was located exclusively at the cell surface, with little co-localization with either LRP1 or LRP1B. After Cu²⁺ treatment, a portion of the 3F4-labelled PrP^C was endocytosed into intracellular compartments. In these Cu²⁺-treated cells, co-localization of PrP^C with both LRP1 and LRP1B was seen to increase at the cell surface. However, only LRP1, but not LRP1B, co-localized with intracellular PrP^C in Cu²⁺-treated cells (Figure 3C), consistent with a role for LRP1 in the Cu²⁺-mediated endocytosis of PrP^C.

Overexpression or knockdown of APP has no effect on the endocytosis of PrP^C

As the neuronal protein APP is also endocytosed by LRP1 [20], we examined whether APP might modulate the endocytosis of PrP^C. SH-SY5Y cells expressing PrP^C were also stably transfected with a pIREShyg vector containing the cDNA encoding APP₇₅₁. Immunoblotting of cell lysates revealed that there was an approx. 2-fold increase in APP₇₅₁ expression in these cells above endogenous levels (Figure 4A). When lysate samples from mock- and APP₇₅₁-transfected cells were immunoblotted for PrP^C the levels of PrP^C expression were identical (Figure 4A). The Cu²⁺-mediated endocytosis of PrP^C in the APP₇₅₁ transfected cells was then examined. There was no difference in the amount of cell-surface PrP^C endocytosed in the APP₇₅₁ transfected cells as compared with cells transfected with the empty pIREShyg vector (Figures 4B and 4E). Similarly, there was no change in the amount of PrP^C endocytosed when the non-KPI-containing isoform of APP, APP₆₉₅, was overexpressed in the SH-SY5Y cells (results not shown).

To confirm that APP does not interfere with the endocytosis of PrP^C, the expression of APP in the SH-SY5Y cells was knocked down using siRNA. A significant reduction in APP expression ($87 \pm 6\%$) was observed in the cells incubated with the siRNA against APP, with no effect on total PrP^C levels (Figure 4C). Cells were then surface biotinylated and incubated in the presence or absence of 100 μ M Cu²⁺ for 20 min followed by trypsin digestion. There was no significant difference in the amount of cell-surface PrP^C endocytosed in the absence of APP siRNA as compared with the presence of the APP siRNA ($43 \pm 4\%$ and $41 \pm 5\%$ of cell surface PrP^C was endocytosed respectively; Figures 4D and 4E). Together these data indicate that APP does not modulate the endocytosis of PrP^C by LRP1.

Figure 3 LRP1 but not LRP1B co-localizes with PrP^C after its Cu²⁺-mediated endocytosis

SH-SY5Y cells expressing PrP^C were seeded on to glass coverslips and grown to 50% confluence. Cells were then pre-incubated with antibody 3F4 at a dilution of 1:1000 in PBS for 30 min at 4 °C, washed three times in PBS and then incubated for 20 min at 37 °C in OptiMEM in either the absence or the presence of 100 μ M Cu²⁺. Cells were fixed, permeabilized and then incubated with either (A) a goat polyclonal antibody against LRP1 or (B) a rabbit polyclonal antibody against LRP1B overnight at 4 °C. Cells were then incubated with AlexaFluor[®] 488 conjugated rabbit anti-mouse antibody and then with either AlexaFluor[®] 594 conjugated rabbit anti-goat (for LRP1) or AlexaFluor[®] 594 conjugated goat anti-rabbit (for LRP1B). Nuclei were stained using DAPI (4',6-diamidino-2-phenylindole). Cells were viewed using a DeltaVision Optical Restoration Microscopy system. Images are representative of three individual experiments. Scale bar = 10 μ m. (C) The co-localization of intracellular PrP^C, internalized in response to Cu²⁺, with LRP1 or LRP1B was quantified using Imaris 4. The data represent the percentage of intracellular PrP^C-containing vesicles that also stained positive for LRP1 or LRP1B ($\pm$ S.E.M.); $n \geq 10$ cells.

DISCUSSION

To date, PrP^C has been reported to bind to several proteins present at the cell surface, including stress-inducible protein 1 [21], neural cell-adhesion molecules [22,23] and the 37 kDa/67 kDa laminin receptor [24]. Of these proteins, only the 37 kDa/67 kDa laminin receptor has been directly implicated in the internalization

Figure 4 Cu^{2+} -stimulated endocytosis of PrP^{C} is unaltered either by overexpression or knockdown of APP

(A) SH-SY5Y cells expressing PrP^{C} were stably transfected with a pIRESHyg vector containing the cDNA encoding APP₇₅₁. Mock transfectants were created by transfection of an empty pIRESHyg vector. Lysates were immunoblotted for APP, PrP^{C} and β -actin. (B) Cells stably expressing APP₇₅₁ were surface biotinylated and incubated with or without 100 μM Cu^{2+} for 20 min at 37 °C. Prior to lysis, the cells were incubated with trypsin to digest cell-surface PrP^{C} . Cells were then lysed and total PrP^{C} immunoprecipitated from the sample using antibody 3F4 and then subjected to Western blot analysis. The biotin-labelled PrP^{C} fraction was detected with peroxidase-conjugated streptavidin. (C) SH-SY5Y cells expressing PrP^{C} were incubated for 48 h with a 2 μM solution of the siRNA against APP, prepared with DharmaFECT-1 transfection reagent in OptiMEM. Mock-transfectants were incubated in the presence of DharmaFECT-1 only. Lysates were immunoblotted for APP, PrP^{C} and β -actin. (D) Endocytosis experiment as described in (B) using the cells incubated in the presence or absence of APP siRNA. (E) Densitometric analysis (means $\pm$ S.E.M.) for multiple blots from three separate experiments performed in (B) and (D).

of PrP^{C} [25]. However, the 37 kDa/67 kDa laminin receptor was responsible for the internalization of only 25–50 % of membrane-bound recombinant PrP^{C} [25] and the binding of PrP^{C} to all these proteins involves regions located C-terminally to residue 90, making them unlikely candidates to act as transmembrane adaptors in the Cu^{2+} -mediated endocytosis of PrP^{C} , which is dependent on the polybasic region at the extreme N-terminus of the protein [11]. Consistent with the existence of other transmembrane receptors for PrP^{C} , we show in the present study, for the first time, that LRP1 is required for the Cu^{2+} -mediated endocytosis of PrP^{C} . By exploiting the fact that ligand binding to LDL-family members can be universally antagonized by soluble RAP [17], we have shown that soluble RAP reduces significantly the Cu^{2+} -

mediated endocytosis of PrP^{C} in SH-SY5Y cells. More selective targeting of individual LDL-family members with siRNAs revealed that LRP1, but not LRP1B, was responsible for facilitating the Cu^{2+} -mediated endocytosis of PrP^{C} in SH-SY5Y cells. We cannot rule out the involvement of other LDL-family members or other, as yet unidentified, proteins in the endocytosis of PrP^{C} in other cell types.

The involvement of LRP1 in PrP^{C} endocytosis may not be entirely surprising, as over 40 ligands have been reported to bind to this scavenger receptor [14]. For example, LRP1 is an endocytic receptor for another GPI-anchored protein, uPAR (urokinase-type plasminogen activator receptor). LRP1 internalizes uPAR after uPAR has bound to a complex of uPA and PAI-1 (plasminogen

activator inhibitor-1) [26,27]. Like PrP^C, uPAR has been localized to lipid rafts [28] and its endocytosis is inhibited by both RAP- and LRP1-specific antibodies [13]. Although Cu²⁺ causes PrP^C to dissociate from detergent-insoluble lipid rafts [11], it is not known where PrP^C initially interacts with LRP1. A recent study demonstrated that LRP1 is capable of associating transiently with lipid rafts [29], thus the initial interaction between PrP^C and LRP1 may occur within lipid rafts after the octapeptide repeats become fully loaded with Cu²⁺, and a conformational change in the N-terminus of PrP^C [30] brings the polybasic region, critical for the clathrin-dependent endocytosis of PrP^C, [10,11] into an orientation that favours interaction either directly or indirectly with the ligand-binding domains of LRP1. A recent study suggested that clusters of basic residues are also critical in mediating the interaction between the uPA-PAI-1 complex and LRP1 [31]. It has been postulated that endogenous HSPGs (heparan sulfate proteoglycans) may be involved in the clathrin-dependent endocytosis of PrP^C [10]. Interestingly, HSPGs have been implicated in facilitating the binding of many ligands to LRP1 [13], and thus it is possible that cell-surface HSPGs may be involved in the LRP1-dependent endocytosis of PrP^C, perhaps through the formation of large multimeric protein complexes.

LRP1 mediates the endocytosis of another protein involved in neurodegenerative diseases, the Alzheimer's APP, from which the neurotoxic amyloid- β peptide is proteolytically cleaved [32]. LRP1 has been shown to associate directly with the ectodomain of APP isoforms containing the KPI-domain [33]. When cells were cultured in the presence of RAP, the level of cell-surface APP was shown to increase, and blocking the interaction of APP with LRP1 reduced the production of the amyloid- β peptide [18]. The interaction of both the KPI-containing and non-KPI-containing isoforms of APP with LRP1 can also occur via the cytoplasmic adaptor protein Fe65, which bridges the cytoplasmic domains of APP and LRP1 [34,35]. Neither overexpression of KPI-containing or non-KPI-containing APP isoforms nor knockdown of APP by siRNA affected the Cu²⁺-mediated endocytosis of PrP^C. This may be due to PrP^C and APP binding to different regions in the ectodomain of LRP1 and/or that, as a scavenger receptor, LRP1 is not limiting in the cell.

In conclusion, we have shown for the first time that LRP1 mediates the endocytosis of PrP^C in neuronal cells. Further studies will be required to elucidate whether interaction with LRP1 has a role to play in the conversion of PrP^C into PrP^{Sc}.

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