The YYXL Motif, but Not the Two NPYX Motifs, Serves as the Dominant Endocytosis Signal for Low Density Lipoprotein Receptor-related Protein

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All members of the low density lipoprotein (LDL) receptor family contain at least one copy of the NPYX sequence within their cytoplasmic tails. For the LDL receptor, it has been demonstrated that the NPYX motif serves as a signal for rapid endocytosis through coated pits. Thus, it is generally believed that the NPYX sequences function as endocytosis signals for all the LDL receptor family members. The primary aim of this study is to define the endocytosis signal(s) within the cytoplasmic tail of LDL receptor-related protein (LRP). By using LRP minireceptors, which mimic the function and trafficking of full-length endogenous LRP, we demonstrate that the YYXL motif, but not the two NPYX motifs, serves as the dominant signal for LRP endocytosis. We also found that the distal di-leucine motif within the LRP tail contributes to its endocytosis, and its function is independent of the YYXL motif. Although the proximal NPYX motif and the proximal di-leucine motif each play a limited role in LRP endocytosis in the context of the full-length tail, these motifs were functional within the truncated receptor tail. In addition, we show that LRP minireceptor mutants defective in endocytosis signal(s) accumulate at the cell surface and are less efficient in delivery of ligand for degradation.

The low density lipoprotein (LDL) receptor-related protein (LRP) is a member of the LDL receptor (LDLR) gene family, which consists of at least six known cell surface receptors: LDLR itself, LRP, the very low density lipoprotein receptor (VLDLR), megalin/LRP-2, apolipoprotein E receptor-2 (apoER2)/LR8B, and LR11 (1–4). LRP is synthesized as a 600-kDa single-chain precursor, which undergoes post-translational proteolytic processing within the trans-Golgi compart-

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§ The abbreviations used are: LDL, low density lipoprotein; CHO, Chinese hamster ovary; LDLR, low density lipoprotein receptor; LRP, low density lipoprotein receptor-related protein; RAP, receptor-associated protein; apoE, apolipoprotein E; apoER2, apolipoprotein E receptor-2; VLDLR, very low density lipoprotein receptor; scuPA, single chain urokinase; PAGE, polyacrylamide gel electrophoresis; HA, hemagglutinin; BSA, bovine serum albumin; ER, endoplasmic reticulum.

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within their cytoplasmic tails. For LDLR, it has been demonstrated that the NPXY motif serves as a signal for rapid endocytosis through coated pits (24). Therefore, it is generally believed that the NPXY sequences in these receptors categorically serve as endocytosis signals. More recently, Herz and colleagues (25–27) have demonstrated that two cytoplasmic adaptor proteins, mammalian Disabled-1 and FE65, interact with NPXY motifs in the cytoplasmic tails of LRP, LDLR, VLDLR, and ApoER2, and that VLDLR and ApoER2 function as obligate components in the Reelin/Disabled-mediated neuronal migration pathway. These data suggest that the NPXY motifs within the tails of the LDLR family members may function not only as endocytosis signals but also as binding motifs for cellular components, which are involved in signal transduction. The tail of LRP consists of 100 amino acid residues, and contains multiple potential endocytosis motifs including two NPXY motifs, one YXXØ motif, and two di-leucine motifs. In this study, the five potential endocytosis signals are termed, in their relationships to the transmembrane domain, as proximal NPXY, proximal di-leucine, distal NPXY, YXXXL, and distal di-leucine (see Fig. 1). The primary aim of this study is to define endocytosis signal(s) within the LRP tail. Here, we demonstrate that the YXXXL motif, but not the two NPXY motifs, serves as the dominant signal for LRP endocytosis. We also show that the distal di-leucine motif contributes to LRP endocytosis, and its function is independent of the YXXXL motif. These data suggest that each member of the LDLR family may utilize different potential signal(s) within their cytoplasmic tails for receptor-mediated endocytosis.

**EXPERIMENTAL PROCEDURES**

**Materials**—Human recombinant RAP was expressed in a glutathione S-transferase expression vector and isolated as described previously (28). scFv,PA was kindly provided by G. F. Vovis of Collaborative Research (29). All tissue culture media, serum, and plasticware were from Life Technologies, Inc. Non-enzymatic cell dissociation solution was from Sigma. Monoclonal anti-HA antibody has been described before (30). Goat anti-mouse IgFITC was from Becton Dickinson. Cy3-Goat anti-mouse IgG was from Sigma. Quantum Simply Cellular microbead kit scoring was purchased from Bio-Rad. Carrier-free Na125I was purchased from NEN Life Science Products. Anti-mouse IgG was from Sigma. Quantum Simply Cellular microbead standard was from Flow Cytometry Standards Corp., San Juan, Puerto Rico. Peroxidase-labeled anti-mouse antibody and ECL system were from Amersham Pharmacia Biotech. Immobilon-P transfer membrane was from Millipore. Rainbow molecular weight markers were from Bio-Rad. Carrier-free Na125I was purchased from NEN Life Science Products.

**Chemical Culture and Transfection**—The LRP-null Chinese hamster ovary (CHO) cell line (kindly provided by David F. Galthoff, National Institutes of Health, ref. 31) was cultured in Ham’s F-12 medium as described (31). Stable transfection into LRP-null CHO cells was achieved by transfection of 30 μg of plasmid DNA in 10-cm dishes by using a calcium phosphate precipitation method (32). Stable transfectants were selected using 700 μg/ml G418 and maintained with 350 μg/ml G418.

**Construction of LRP Minireceptor and Site-directed Mutagenesis**—The construction of the membrane-containing minireceptor of LRP (see Fig. 1, A and C) via polymerase chain reaction was performed essentially as described previously (30, 33). Site-directed mutagenesis was carried out using the QuickChange mutagenesis kit (Stratagene) according to the manufacturer’s instructions. All oligonucleotides were synthesized at the Washington University School of Medicine Protein Chemistry Laboratory.

**Western Blotting of LRP Minireceptors**—Stably transfected CHO cells were lysed with 0.5 ml of lysis buffer (phosphate-buffered saline containing 1% Triton X-100 and 1 mM phenylmethylsulfonyl fluoride) at 4 °C for 30 min. Equal quantities of protein were subjected to SDS-PAGE (6%) under reducing conditions. Following transfer to polyvinylidene difluoride membrane, successive incubations with anti-HA antibody and horseradish peroxidase-conjugated goat anti-mouse IgG were carried out for 60 min at room temperature. The immunoreactive proteins were then detected using the ECL system. Films showing immunoreactive bands were scanned using a Kodak Digital Science DC120 Zoom digital camera and analyzed with Kodak Digital Science ID image analysis software.

**Results**

**Flow Cytometric Analysis of Cell Surface LRP Minireceptors**—For cell surface LRP minireceptor analysis, living cells were used (34). Briefly, CHO cells were detached by incubation with non-enzymatic cell dissociation solution. Successive incubations with affinity-purified anti-HA IgG (25 μg/ml) and goat anti-mouse IgG(FITC) were carried out at 4 °C for 45 min. Background fluorescence intensity was assessed in the absence of primary monoclonal antibody. The antibody binding capacities were evaluated from the standardized Quantum Simply Cellular bead calibration plot (35). The bead standards consist of four populations of microbeads coated with goat anti-mouse antibody, which bind different numbers of mouse IgG monoclonal antibody molecules (5868, 18,329, 50,908, and 150,477 molecule binding capacities) in addition to a blank population. The beads were stained in the same way as the CHO cells.

**Protein Iodination**—RAP and scFvPA (50 μg) were iodinated by using the IODO-GEN method as described previously (36).

**Kinetic Analysis of Endocytosis**—Kinetic analysis of endocytosis was performed according to previously published methods (37, 38). Stably transfected CHO cells were plated in 12-well plates at a density of 2 × 105 cells/well and used after overnight culture. Cells were rinsed twice in ice-cold ligand binding buffer (minimal Eagle’s medium containing 0.6% BSA), and 125I-RAP was added at 5 nM final concentration in cold ligand binding buffer (0.5 ml/well). The binding of 125I-RAP was carried out at 4 °C for 30 min with gentle rocking. Binding of 125I-RAP was specific, i.e. the addition of 100-fold excess unlabeled RAP inhibited binding by >95%. Unbound ligand was removed by washing cell monolayers three times with cold binding buffer. Ice-cold stop/strip solution (0.2 M acetic acid, pH 2.6, 0.1 M NaCl) was added to one set of plates without warming up and kept on ice. The remaining plates were then placed in a 37 °C water bath, and 0.5 ml of ligand binding buffer prewarmed to 37 °C was quickly added to the well monolayers to initiate internalization. After each time point, the plates were quickly placed on ice and the ligand binding buffer was replaced with cold stop/strip solution. Ligand that remained on the cell surface was stripped by incubation of cell monolayers with cold stop/strip solution for a total of 20 min (0.75 ml for 10 min, twice) and counted. Cell monolayers were then solubilized with low SDS lysis buffer (62.5 mM Tris-HCl, pH 6.8, 0.2% SDS, 10% v/v glycerol) and counted. The sum of ligand that was internalized plus that which remained on the cell surface after each assay was used as the maximum potential internalization. The fraction of internalized ligand after each time point was calculated and plotted.

**Analyses of LRP Ligand Binding Activity and Ligand Degradation Efficiency**—Cells (2 × 105) were seeded into 12-well dishes 1 day prior to assays. Assay buffer (minimal Eagle’s medium containing 0.6% BSA and 0.4% bovine serum albumin) was added to each well for the absence or the presence of unlabeled 500 nM RAP, followed with incubation for 1 h at 4 °C. Thereafter, overlying buffer containing unbound ligand was removed, and cell monolayers were washed and lysed in low SDS lysis buffer and counted.

Ligand degradation efficiency was measured using the methods as described (24, 39). Briefly, 2 × 105 cells were seeded into 12-well dishes 1 day prior to assays. Pre-warmed assay buffer was added to cell monolayers, in the absence or the presence of unlabeled 500 nM RAP, followed with incubation for 4 h at 37 °C. Thereafter, the medium overlying the cell monolayers was removed and proteins were precipitated by addition of BSA to 10 mg/ml and trichloroacetic acid to 20%. Degradation of radioligand was defined as the appearance of radioactive degradation products in the overlying medium that were soluble in 20% trichloroacetic acid. The protein concentrations of each cell lysates were measured in parallel dishes that did not contain LRP ligands. The ligand degradation efficiency is the value of degraded ligand (cpm/mg cell protein) divided by the number of cell surface LRP minireceptors (as determined by flow cytometry, and calculated relative to wild type mLRP4T100).

**Immunofluorescence Microscopy**—Stably transfected CHO cells expressing various proteins were grown on glass coverslips, fixed in 4% paraformaldehyde in phosphate-buffered saline for 20 min, and permeabilized with 0.2% Triton X-100 for 10 min at room temperature. The cells were then incubated for 30 min with anti-HA antibody followed by a 30-min labeling with Cy3 goat anti-mouse IgG. Confocal microscopy was performed on a Bio-Rad MRC 1024 model using a Zeiss 63× (nominal aperture, 1.4) oil immersion lens.
**LRP Endocytosis Signals**

Fig. 1. Endocytosis rate of LRP minireceptors with truncated cytoplasmic tails. A, schematic representation of mLRP4T100. mLRP4T100 is depicted in comparison to the full-length LRP molecule. The four putative ligand-binding domains are labeled with Roman numerals I, II, III, and IV. B, the sequence of LRP cytoplasmic tail. The first amino acid following the transmembrane domain is numbered 1. C, schematic representation of potential endocytosis signals within the tails of mLRP4T100, and its deletion variants. D, effects of tail truncation on LRP minireceptor-mediated endocytosis. LRP-null CHO cells stably transfected with mLRP4T100, and its deletion variants were incubated with 5 nM 125I-RAP at 4 °C for 30 min, and then shifted to 37 °C for the indicated times. The amounts of ligand internalized as the fraction of the total cell-associated ligand (the sum of the internalized ligand plus the ligand remaining on the cell surface at the end of the assay; see “Experimental Procedures” for further explanation) are plotted against time. Values are the average of triple determinations with the S.E. indicated by error bars. This experiment is a representative of three such experiments performed with similar data.

The YXXL Motif, but Not the Two NPXY Motifs, Serves as the Dominant Endocytosis Signal for LRP—Based on the above results, we generated eight LRP minireceptors with specific mutations in the cytoplasmic tail based on mLRP4T100 (Fig. 2A). These mutations were targeted to each of the putative endocytosis signals, which allowed us to evaluate the contribution of these signals in LRP endocytosis. Fig. 2B shows the endocytosis rates of wild type mLRP4T100 and its proximal NPXY motif mutants. Alteration of the tyrosine to alanine resulted in only a slight decrease in RAP internalization, with the initial endocytosis rates being indistinguishable from that of mLRP4T100. These results indicate that the proximal NPXY motif is not important for LRP endocytosis. Fig. 2C shows the endocytosis rates of wild type mLRP4T100 and its distal NPXY motif mutants. As the phenylalanine is the important residue within the FXNFXY motif for LDLR endocytosis (24), we also mutated this residue to alanine. Replacement of the phenylalanine or the asparagine by alanine resulted in only a slight decrease in RAP internalization, with the initial endocytosis rates being indistinguishable from that of mLRP4T100. In contrast, alteration of the tyrosine to alanine resulted in a dramatic decrease in RAP internalization. At 15, 30, and 60 s, this mutant internalized 3%, 5%, and 12%, respectively, of the total cell-associated 125I-RAP, corresponding to a 80–90% impairment in endocytosis relative to wild type mLRP4T100. These results indicate that the tyrosine residue at position 63 is critical for LRP endocytosis. Because the tyrosine within the distal NPXY motif is also within the YXXL motif, the decreased endocytosis rate of mLRP4T100(Y63A) may have resulted from the mutation of the YXXL motif. Fig. 2D shows the endocytosis rates of wild type mLRP4T100 and its YXXL motif mutants. As shown in the figure, the endocytosis rate of mLRP4T100(L66A) was significantly decreased, and was indistinguishable from that of mLRP4T100(Y63A). Taken together, these results clearly demonstrate that the YXXL motif, but not the distal NPXY motif, serves as the major endocytosis signal for LRP.

For the complete text, see the original source.
Distal Di-leucine Motif, but Not the Proximal Di-leucine Motif, Contributes to LRP Endocytosis—The di-leucine motif is another well characterized endocytosis signal that is present in many transmembrane cell surface proteins. Thus, we next compared the endocytosis rate of mLRP4T100 with that of its di-leucine motif mutants (Fig. 2E). Mutation of both leucines to alanines in the proximal di-leucine motif resulted in little or no change in RAP internalization. However, the mLRP4T100(L86A,L87A) mutant clearly demonstrates a defect in internalization, although not as substantial as mLRP4T100(Y63A) or mLRP4T100(L66A) (Fig. 2, D and E). At 15, 30, and 60 s, mLRP4T100(L86A,L87A) internalized 18%, 30%, and 40%, respectively, of the total cell-associated 125I-RAP, corresponding to a 30–40% impairment in endocytosis relative to wild type mLRP4T100. These results clearly demonstrate that the distal di-leucine motif, but not the proximal di-leucine motif, contributes to LRP endocytosis.

The YXXL Motif and the Distal Di-leucine Motif Function Independently in LRP Endocytosis—To address whether the YXXL motif and the distal di-leucine motif function independently in LRP endocytosis, we generated a double mutant, mLRP4T100(Y63A,L86A,L87A). The endocytosis rate of this mutant receptor was compared with that for wild type mLRP4T100(Y63A) or mLRP4T100(L66A) (Fig. 2, D and E). At 15, 30, and 60 s, mLRP4T100(L66A,L86A,L87A) internalized 18%, 30%, and 40%, respectively, of the total cell-associated 125I-RAP, corresponding to a 30–40% impairment in endocytosis relative to wild type mLRP4T100. These results clearly demonstrate that the distal di-leucine motif, but not the proximal di-leucine motif, contributes to LRP endocytosis.

The Proximal NPXY Motif and the Proximal Di-leucine Motif Function in a Truncated Receptor Tail—In Fig. 2 (B and E), we demonstrated that the proximal NPXY motif and the distal di-leucine motif exhibit little or no role in LRP endocytosis within the full-length LRP tail. However, comparison of the
endocytosis rate of mLRP4T100 with that of mLRP4T59 indicated that mLRP4T59 is still capable of internalizing RAP (Fig. 1D). To address this question, we generated two mLRP4T100 mutants mLRP4T59(Y29A) and mLRP4T59(L43A, L44A) using mLRP4T59 as the template. Fig. 4 shows the endocytosis rates of the stably transfected CHO cells expressing mLRP4T100, mLRP4T59, mLRP4T59(Y29A), mLRP4T59(L43A, L44A), and mLRP4Ttailess. As shown in the figure, the endocytosis rates of mLRP4T59(Y29A) and mLRP4T59(L43A, L44A) were significantly lower than that of mLRP4T59, indicating that the proximal NPXY motif and the proximal di-leucine motif are functional within the truncated receptor tail.

mLRP4T100 Mutants Defective in Endocytosis Accumulate at the Cell Surface—We hypothesized that mLRP4T100 mutants that are defective in endocytosis signals would accumulate at the cell surface. We initially compared the ligand binding activity of wild type mLRP4T100 with those of endocytosis mutants expressed in stably transfected CHO cells. As shown in Fig. 5A, CHO cells expressing wild type mLRP4T100 exhibited a moderate level of cell surface RAP binding, while CHO cells transfected with the pcDNA3 vector exhibited only ~10% of RAP binding compared with those transfected with mLRP4T100. The residual RAP binding to pcDNA3-transfected cells is likely mediated by cell surface heparan sulfate proteoglycan (40). Interestingly, CHO cells expressing mLRP4T100 mutants defective in endocytosis signals exhibited significant increase in cell surface RAP binding activity. To analyze whether the increase of RAP binding to CHO cells expressing endocytosis mutants is due to an accumulation of mature minireceptor on the cell surface, we examined the steady-state distribution of various forms of minireceptors via Western blotting analysis using anti-HA antibody (Fig. 5B). For mLRP4T100 and its mutants, two distinct bands are seen on 6% SDS-PAGE gel under reducing conditions. The 120-kDa band represents the mature furin-processed minireceptor form that corresponds to the LRP-ligand binding domain 4 (see Fig. 1, and Ref. 30). Since the HA epitope is within the amino terminus of the LRP minireceptors, Western blot analyses with anti-HA antibody do not detect the LRP-85 band. However, the presence of this band was confirmed by metabolic labeling with [35S]cysteine and immunoprecipitation (data not shown). The upper band that migrates ~200 kDa represents the full-length ER precursor form that lacks complex sugar modification (see Ref. 30). mLRP4Ttailess exhibits a similar banding pattern on SDS-PAGE, except that the ER form migrates faster than that of mLRP4T100 due to the tail truncation. The results show that, although the ER forms of mLRP4T100 and those mutants defective in endocytosis as well as mLRP4Ttailess are expressed at a similar level, the furin-processed 120-kDa forms are detected at distinctly different levels, i.e. the 120-kDa forms of mLRP4T100(Y63A), mLRP4T100(L66A), mLRP4T100(Y63A,L86A,L87A), and mLRP4Ttailess are significantly increased, while the 120-kDa form of mLRP4T100(L86A,L86A) is only slightly greater than that of mLRP4T100 (Fig. 5B). The ratio of 120-kDa/ER of mLRP4T100 is 0.37, and the ratios of
FIG. 6. Flow cytometric analysis of cell surface LRP minireceptor expression in LRP-null CHO cells. 

A, histograms of LRP minireceptor cytofluorimetric analysis in LRP-null CHO cells. LRP-null CHO cells stably transfected with LRP minireceptors were labeled with anti-HA antibody and detected with goat anti-mouse IgFITC. Background fluorescence intensity was assessed in the absence of primary monoclonal antibody (thin line). The x axis represents relative fluorescence intensity, and the y axis represents relative cell number. 

B, the number of cell surface LRP minireceptors in LRP-null CHO cells. LRP-null CHO cells stably transfected with LRP minireceptors were stained as described above. The number of cell surface minireceptors per cell were determined by using Quantum Simply Cellular microbead standard as described under “Experimental Procedures.” Values are the average of triple determinations with the S.E. indicated by error bars. This experiment is a representative of two such experiments performed with similar data.

FIG. 7. Scanning confocal images of LRP minireceptors. LRP-null CHO cells stably transfected with mLRP4T100 (A), mLRP4T100(L86A,L87A) (B), mLRP4T100(Y63A) (C), mLRP4T100(L66A) (D), mLRP4T100(Y63A,L86A,L87A) (E), and mLRP4Ttailess (F) were immunostained with anti-HA antibody and detected with Cy3-Goat anti-mouse IgG. Images represent single 0.16-μm-thin midsections from scanned cells.

est numbers observed with mLRP4Ttailess and mLRP4T100-(Y63A,L86A,L87A). Taken together, these data clearly demonstrate that mLRP4T100 mutants defective in endocytosis accumulate at the cell surface.

To confirm the above results, we determined the steady-state distribution of LRP minireceptors stably transfected in CHO cells via confocal microscopy. As seen in Fig. 7A, wild type mLRP4T100 exhibits both cell surface and vesicular staining, similar to that seen with endogenous LRP (41). Compared with mLRP4T100, the change of cell surface mLRP4T100(L86A,L87A) expression is not significant (Fig. 7B). However, mutations in LRP dominant endocytosis motif YXXL resulted in high levels of expression at the cell surface (Fig. 7, C and D). mLRP4T100(Y63A,L86A,L87A) and mLRP4Ttailess also show markedly increased cell surface localization (Fig. 7, E and F). These observations of increased expression at the cell surface for the various endocytosis mutants support the role for the YXXL motif as a dominant signal in LRP endocytosis.

mLRP4T100 Mutants Defective in Endocytosis Exhibit Reduction in the Efficiency of Ligand Degradation—Having established that LRP endocytosis is mediated by the YXXL motif and the distal di-leucine motif, we then investigated the efficiency of LRP ligand degradation for wild type mLRP4T100 and its endocytosis mutants. Fig. 8A shows that the 125I-RAP degradation efficiency of the LRP endocytosis mutants decreased significantly with mLRP4Ttailess showing the lowest level of 125I-RAP degradation. As expected, mLRP4T100(L86A,L87A) shows partially reduced degradation, while mLRP4T100(Y63A), mLRP4T100(L66A), and mLRP4T100(Y63A,L86A,L87A) show only 12.9%, 8.8%, and 6% that of wild type mLRP4T100, respectively.

Our recent studies have shown that mLRP4T100 is able to degrade scuPA efficiently. Unlike RAP, scuPA is a physiological ligand for LRP. Thus, we utilized scuPA to measure the efficiency of ligand degradation for the LRP minireceptors. Similar to that seen with 125I-RAP, LRP endocytosis mutants exhibited impaired degradation of 125I-scuPA. mLRP4Ttailess showed lowest level of scuPA degradation. mLRP4T100(L86A,

2 L. M. Obermoeller, Y. Li, and G. Bu, unpublished results.
Although endocytosis of the wild type growth hormone receptor depends on the ubiquitin system, a truncated form of growth hormone receptor, which lacks the ubiquitin-dependent endocytosis motif, utilizes a di-leucine motif for its endocytosis (42). Alternatively, the function of these potential endocytosis motifs within wild type receptor tail may be overridden by the presence of dominant YXXL motif.

Recently, studies have revealed new roles of LDLR family members as transducers of extracellular signals. It has been demonstrated that VLDLR and apoER2 function as obligate components in the Reelin/Disabled-mediated neuronal migration pathway (25–27, 43). A signaling pathway involving the extracellular protein Reelin and the intracellular adaptor protein Disabled-1 is involved in the control of cell position during mammalian brain development (44–47). It has been shown that Disabled-1 interacts with the NPXY motifs within the tails of several members of the LDLR family (25, 26). Mice lacking the genes for both VLDLR and apoER2 demonstrate a neurological and neuroanatomical phenotype that is indistinguishable from animals deficient in either Reelin or Disabled-1 (26). Potential signaling functions for members of the LDLR family have also been suggested from other observations. For example, Goretzki and Mueller (48) have shown that the LRP tail interacts with a GTP-binding protein and induces cyclic-AMP-dependent protein kinase activity. Similar signal transduction event down stream from LRP was also implicated in hippocampal neurons (49). At present, it is still unclear whether the signaling event(s) initiated by lipoprotein receptors can be regulated by endocytosis.

Studies using different experimental systems have revealed that tyrosine-based as well as di-leucine-based sorting signals of membrane proteins can be recognized by adaptor complexes, which in turn associate with clathrin and other accessory molecules to generate clathrin coats and coated transport vesicles (18, 50). Adaptor complex AP-2 plays a critical role in two early steps of the endocytic pathway at the plasma membrane: the formation of the clathrin lattice and the selection of specific cargo proteins for internalization (18, 51). AP-2 interacts with clathrin through its β subunits and promotes coat formation. Interaction of the AP-2 β subunit with receptors containing tyrosine-based as well as di-leucine-based internalization motifs contributes to their localization to coated pits. In the present studies, we have shown that the YXXL motif serves as the dominant motif for LRP endocytosis, and that the distal di-leucine motif further contributes to LRP endocytosis. Thus, it will be interesting to examine whether AP-2 plays a major role in LRP endocytosis and whether it interacts directly with the receptor endocytosis signals.

In conclusion, our results show that the YXXL motif, but not the two NPXY motifs, serves as the dominant signal for LRP endocytosis. We have also defined that the distal di-leucine motif contributes to LRP endocytosis, and that its function is independent of the YXXL motif. Mutants defective in endocytosis accumulate at the cell surface, and exhibit reduced efficiency for ligand degradation. These data together with that of other’s, suggest that the YXXL motif and the distal di-leucine motif of the LRP tail serve as endocytosis signals, while the NPXY motifs serve as binding sites for cytosolic signaling proteins.

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