ERD2 proteins mediate ER retention of the HNEL signal of LRP’s receptor-associated protein (RAP)

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SUMMARY

The 39 kDa receptor-associated protein (RAP) is a receptor antagonist that interacts with several members of the low density lipoprotein (LDL) receptor gene family. Upon binding to these receptors, RAP inhibits all ligand interactions with the receptors. Our recent studies have demonstrated that RAP is an endoplasmic reticulum (ER) resident protein and an intracellular chaperone for the LDL receptor-related protein (LRP). The HNEL sequence at the carboxyl terminus of RAP represents a novel ER retention signal that shares homology with the well-characterized KDEL signal. In the present study, using immunoelectron microscopy we demonstrate that cells stably transfected with human growth hormone (GH) tagged with either KDEL (GH+KDEL) or HNEL (GH+HNEL) signals exhibit ER and cis-Golgi localization typical of ER-retained proteins. Overexpression of not only GH+HNEL but also GH+KDEL cDNA in transfected cells results in saturation of ER retention receptors and secretion of endogenous RAP indicating that the two signals interact with the same ER retention receptor(s). The role of RAP in the maturation of LRP is further supported by the observation that functional LRP is reduced about 60% as a result of decreased intracellular RAP. Pulse-chase labeling and immunolocalization studies of ERD2.1 and ERD2.2 proteins in transfected cells demonstrate a long half-life and Golgi localization for both receptors. Finally, overexpression of either ERD2.1 or ERD2.2 proteins significantly increases the capacity of cells to retain both KDEL and HNEL-containing proteins. Taken together, our results thus demonstrate that ERD2 proteins are capable of retaining the novel ER retention signal associated with RAP.

Key words: ERD2 protein, ER retention signal, Chaperone, Endocytic receptor

INTRODUCTION

The 39 kDa receptor-associated protein (RAP) is a unique regulator of receptors which belong to the low density lipoprotein (LDL) receptor gene family. These endocytic receptors include the LDL receptor (Yamamoto et al., 1984), the LDL receptor-related protein LRP (Herz et al., 1988), the kidney membrane protein gp330 (Raychowdhury et al., 1989), and the VLDL receptor (Takakashi et al., 1992). Among these receptors, LRP and gp330 are large multifunctional receptors each of which can bind and endocytose several structurally and functionally distinct ligands (Krieger and Herz, 1994; Kounnas et al., 1994). Upon binding to these receptors, RAP inhibits the binding and/or endocytosis of all the ligands by the receptors in vitro. This unique feature of RAP has allowed its extensive use in the study of receptor biology including the identification of new ligands and antagonizing receptors in vitro and in vivo (see Krieger and Herz, 1994).

The physiological function of RAP is not clear. The possibility that RAP may regulate LRP activity at the cell surface is confounded by the fact that, although the RAP sequence includes a putative signal peptide (Strickland et al., 1991), little of this protein is present at the cell surface or secreted into the extracellular fluid. Using colloidal gold immunoelectron microscopy and human glioblastoma U87 cells which express substantial amounts of LRP and RAP, RAP was localized most abundantly within the endoplasmic reticulum (ER, 70%) and Golgi compartments (24%) with only 2% of the total RAP found at the cell surface and 4% in the endosomes (Bu et al., 1994a). These observations suggest that the normal physiological function of RAP may be intracellular. Our recent studies have shown that the carboxyl-terminal tetrapeptide, HNEL, is responsible for the ER localization and retention of RAP (Bu et al., 1994). RAP retained within the ER functions as a molecular chaperone for LRP by transiently interacting with LRP and maintaining LRP in an inactive ligand-binding state. As RAP dissociates from LRP in response to the lower pH within the Golgi, LRP becomes active as it transits to the cell surface. The role of RAP as a molecular chaperone is also supported by the gene-knockout studies of Willnow et al. (1995) which demonstrate that cells lacking RAP exhibited 75% reduction of functional LRP.

Proteins containing hydrophobic signal peptides are cotranslationally transported to the ER lumen and subsequently targeted to various subcelular organelles or secreted to the extracellular fluid (Rothman and Orci, 1992). A selective group of proteins are retained and function within the ER. For example, immunoglob-
U87 cells were transfected with various plasmids at about 40% confluency using the calcium phosphate precipitation method (Chen and Okayama, 1987). For 10 cm dishes of U87 cells, 25 μg of DNA were used in a total volume of 10 ml medium. Sixteen hours after transfection, cells were washed twice with culture medium and cultured continuously for an additional 24 hours. For transient transfection, cells were used directly without trypsinization. The efficiency of transient transfection in these studies and subsequent studies was consistently between 30 and 50% as assessed by immunofluorescence staining. For stable transfection, cells were trypsinized and plated to low densities in medium containing G418 (0.4 mg/ml). Individual colonies were selected and assayed for the target protein by western blotting and immunofluorescence staining. Positive clones were grown as individual cell lines. The purity of each stable cell line was confirmed by immunofluorescence evaluation of the transfected protein. In general, five clones for each transfection were established before a representative one was selected for further analysis.

**Immunoprecipitation and SDS-PAGE**

Immunoprecipitations were carried out essentially as described before (Bu et al., 1993) using rabbit polyclonal antibodies. Preliminary experiments were performed to ensure that primary antibody used in each immunoprecipitation was in excess. Protein A agarose beads were used to precipitate protein-IgG complexes. The immunoprecipitated materials were released from the beads by boiling each sample for 5 minutes in reducing SDS sample buffer (62.5 mM Tris-HCl, pH 6.8, 2% (w/v) SDS, 10% (v/v) glycerol, 5% (v/v) β-mercaptoethanol; Laemmli, 1970). The percentage of SDS-polyacrylamide gels is indicated in each figure legend. Rainbow molecular mass markers (Bio-Rad) were used as the molecular mass standards.

**Western blot analysis**

U87 cells stably transfected with GH, GH+KDEL, or GH+HNEL were cultured in 10 cm dishes. After two days, medium and cell lysate were harvested for each cell line and immunoprecipitated with either anti-GH antiserum or anti-RAP IgG as described above (see Results). After SDS-PAGE and transferring to nitrocellulose, samples were blotted with the corresponding antibody used for immunoprecipitation. Horseradish peroxidase-conjugated Protein A was used as the secondary antibody.

**Immunofluorescence and immunoelectron microscopy**

U87 cells stably transfected with GH, GH+KDEL, or GH+HNEL, or HA-ERD2 cDNAs were fixed overnight in a mixture of 2% (w/v) paraformaldehyde and 0.5% (w/v) glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.4, and kept in 1% paraformaldehyde in the same buffer until further processing. Cell samples were embedded in 2.3 M sucrose in phosphate buffer prior to cryosectioning. Ultrathin cryosections were picked up from the knife and were immunolabeled with either anti-GH antibody or anti-HA antibody, followed with 10 nm Protein A-conjugated colloidal gold probes (Slot and Geuze, 1985). The labeled sections were contrasted and embedded as described previously (Slot et al., 1988). For immunofluorescence labeling with anti-HA antibody, experiments were carried out essentially as described previously (Bu et al., 1994a) using Texas red-conjugated goat anti-mouse IgG as the secondary antibody.

**Pulse-chase labeling**

For pulse-chase experiments, cells were pulse-labeled with [35S]methionine for 30 minutes (Bu et al., 1995), and chased with serum-containing medium for different periods of time as specified in each experiment. The total amount of labeled protein for each chase period was normalized by applying equal amounts of TCA-precipitable radioactivity in cell lysates for immunoprecipitation.

**Ligand binding analysis**

Binding of 125I-αM* (100 pM) to U87 cells was performed essen-
RESULTS

HNEL sequence at the carboxy terminus of RAP mediates ER retention

To investigate whether the HNEL signal present at the carboxy terminus of RAP can function similarly to the KDEL signal and mediate ER retention independently, we generated stably transfected cell lines using cDNA constructs of human growth hormone (GH) with or without the attachment of these signals (Bu et al., 1995). Human glioblastoma U87 cells were used as host cells for stable transfection since these cells do not express endogenous GH (Bu et al., 1994a). The GH cDNA constructs include GH in its native form (GH), with the KDEL sequence attached to its carboxy terminus (GH+KDEL), and GH with the HNEL sequence attached to its carboxy terminus (GH+HNEL). For each cDNA construct, 5-10 stable cell lines from individual colonies were selected and characterized for the presence of full length protein and comparable levels of GH expression. For each construct one representative cell line was selected and used for the subsequent experiments.

To analyze the effects of KDEL or HNEL addition to GH, we compare the steady-state levels of GH present in tissue culture medium and cell lysates of stable cell lines via immunoprecipitation and subsequent western blot analysis (Fig. 1). To ensure complete immunoprecipitation, excess anti-GH antibody was used in these experiments. As seen in the figure, for cells stably transfected with GH, the vast majority of GH is present in the medium with little protein identified in the cell lysate. However, for cells stably transfected with GH+KDEL, most of the GH was detected in the cell lysate. An almost identical distribution was seen for cells stably transfected with GH+HNEL (data not shown). The presence of GH+HNEL and GH+KDEL in the intermediate compartment is confirmed by their colocalization with the intermediate compartment marker p53 (Schweizer et al., 1988, data not shown). These results indicate that, at the subcellular level, addition of either KDEL or HNEL signals at the carboxy terminus of a secretory protein resulted in an ER retention. The fact that proteins tagged with either signal exhibit similar intracellular localizations suggests that the KDEL and the HNEL signals may exhibit a similar affinity to ER retention receptors (see below).

KDEL and HNEL sequences attached to GH compete with endogenous RAP for ER retention

Having demonstrated that both KDEL and HNEL sequences are sufficient to mediate ER retention, we then analyzed whether the two signals interact with the same ER retention receptor(s). Since we have detected secretion of GH+KDEL and GH+HNEL from cells stably transfected with these constructs, it is likely that the ER retention receptor(s) in these cells have been saturated. If the two signals interact with the same ER retention receptor(s), endogenous RAP (Bu et al., 1992) should be competed for its interaction with the ER retention machinery following the overexpression of both GH+HNEL as well as GH+KDEL. The results of this competition should result in RAP secretion. Thus, we examined the culture medium of cells transfected with GH constructs via immunoprecipitation followed by western blot analysis using anti-RAP antibody. As seen in Fig. 3, no detectable RAP is present in the medium of cells transfected with GH, consistent with previous observations that RAP is not secreted under normal conditions (Bu et al., 1994a). However, RAP is readily detectable in the medium of cells transfected with either GH+KDEL or GH+HNEL. The secreted RAP exhibits slightly slower migration on SDS-PAGE due to the addition of complex carbohydrates within the Golgi compartments (Bu et al., 1995). The fact that overexpression of GH+KDEL resulted in secretion of endogenous RAP suggests that the KDEL signal interacts with the same ER retention receptor(s) as does the HNEL signal of RAP. As a result of the continuous uptake of RAP via cell surface LRP (Bu et al., 1994a), the amount of RAP seen in the medium represents a steady-state level rather than its total accumulation. Consistent with the secretion of RAP, cell-associated RAP in GH+KDEL or GH+HNEL-transfected cells decreased significantly (60-70%) as compared to that observed in GH-transfected cells.

Fig. 1. Cellular retention of GH by KDEL and HNEL signals. U87 cells stably transfected with GH, GH+KDEL, or GH+HNEL cDNAs were cultured for two days. Medium and cell lysates from each cell line were immunoprecipitated and western blotted with anti-GH antibody. The amount of cell lysates and corresponding medium used for each precipitation was normalized by total cellular protein. Samples were analyzed on 15% SDS-PAGE under reducing conditions. The position of GH is indicated by an arrow.
Decrease of intracellular RAP results in a reduction of functional LRP on the cell surface

Our previous studies have suggested a role for RAP in the intracellular trafficking of LRP (Bu et al., 1995). To analyze the effect of decreased endogenous RAP on the expression of functional LRP at the cell surface, we examined the ligand-binding activity of LRP using cells transfected with GH constructs and methylamine-activated $\alpha_2$-macroglobulin ($\alpha_2$M) as the test ligand (Bu et al., 1994b). The binding of $^{125}$I-$\alpha_2$M to GH-transfected cells is indistinguishable from that of untransfected U87 cells (data not shown). However, we observed a significant reduction (~60%) in $^{125}$I-$\alpha_2$M-binding activity of LRP in cells transfected with either GH+KDEL or GH+HNEL when compared to that of GH-transfected cells (Fig. 4). The total cellular content of LRP in GH+KDEL- and GH+HNEL-transfected cells was also reduced to a similar extent when analyzed via western blotting (data not shown). These results show that RAP is required for normal levels of LRP, a finding consistent with our hypothesis that RAP acts as a molecular chaperone for LRP (Bu et al., 1995).

ERD2 proteins exhibit long half-lives and are localized primarily within the Golgi compartments in U87 cells

To examine whether the previously identified ERD2 proteins mediate the ER retention of the HNEL signal associated with RAP, we generated cDNA constructs of human ERD2.1 and ERD2.2. To facilitate the detection of expressed ERD2 proteins, the cDNAs were tagged with hemaglutinin (HA).
ERD2 proteins mediate ER retention of HNEL/RAP

To analyze whether ERD2 proteins are capable of retaining the HNEL sequence, we performed cotransfection experiments with ERD2 proteins as well as reporter proteins. GH, GH+KDEL, or GH+HNEL cDNAs were cotransfected into U87 cells with vector pcDNA alone, or vector containing ERD2.1 or ERD2.2 cDNAs. By double immunofluorescence labeling analysis (Bu et al., 1994b), we found that >80% of transfected cells received cDNAs of both GH and ERD2 proteins. The capacity of the cells to retain various GH constructs was examined for each combination of cotransfections (see Fig. 8) via pulse-chase analysis. Cells were pulse-labeled with [35S]methionine for 30 minutes and chased with complete medium containing serum for 2 hours. At the end of the chase period, both medium and cell lysates from each transfection were immunoprecipitated with anti-GH antibody and analyzed via SDS-PAGE. Transient transfection, followed with pulse-chase analyses in these experiments were designed to generate high base line secretion even for GH+KDEL and GH+HNEL constructs, so that the potential effects of ERD2 protein coexpression on the secretion of GH constructs can be assessed by the extent of their cellular retention. Thus, the band intensity for both cell lysate and the medium in each cotransfection was quantitated and plotted as the % of the total chase. As seen in the figure, cells transfected with GH exhibit a similar distribution of GH in the cell lysate/medium following cotransfection with pcDNA (10%/90%), ERD2.1 (14%/86%), or ERD2.2 (17%/83%). These results suggest that overexpression of ERD2 proteins has no apparent effect on the secretion of wild-type GH. However, when cells transfected with GH+KDEL labeling was found in the ER or elsewhere in the cell. Upon overexpression of ERD2, however, there was abundant labeling in extended rough ER cisternae, where the Golgi stacks showed labeling throughout (Fig. 7B). These cells showed little or no ERD2 labeling beyond the level of the trans-Golgi. Similar ERD2 overexpression cells with strong ER staining patterns were also observed using immunofluorescence labeling techniques (data not shown). Such staining was found more commonly for ERD2.1-transfected cells than ERD2.2-transfected cells.

**Overexpression of ERD2 proteins increases the capacity of cells to retain KDEL- and HNEL-containing proteins**

To directly analyze whether ERD2 proteins are capable of retaining the HNEL sequence, we performed cotransfection experiments with ERD2 proteins as well as reporter proteins. GH, GH+KDEL, or GH+HNEL cDNAs were cotransfected into U87 cells with vector pcDNA alone, or vector containing ERD2.1 or ERD2.2 cDNAs. By double immunofluorescence labeling analysis (Bu et al., 1994b), we found that >80% of transfected cells received cDNAs of both GH and ERD2 proteins. The capacity of the cells to retain various GH constructs was examined for each combination of cotransfections (see Fig. 8) via pulse-chase analysis. Cells were pulse-labeled with [35S]methionine for 30 minutes and chased with complete medium containing serum for 2 hours. At the end of the chase period, both medium and cell lysates from each transfection were immunoprecipitated with anti-GH antibody and analyzed via SDS-PAGE. Transient transfection, followed with pulse-chase analyses in these experiments were designed to generate high base line secretion even for GH+KDEL and GH+HNEL constructs, so that the potential effects of ERD2 protein coexpression on the secretion of GH constructs can be assessed by the extent of their cellular retention. Thus, the band intensity for both cell lysate and the medium in each cotransfection was quantitated and plotted as the % of the total chase. As seen in the figure, cells transfected with GH exhibit a similar distribution of GH in the cell lysate/medium following cotransfection with pcDNA (10%/90%), ERD2.1 (14%/86%), or ERD2.2 (17%/83%). These results suggest that overexpression of ERD2 proteins has no apparent effect on the secretion of wild-type GH. However, when cells transfected with GH+KDEL...
were examined, a significant decrease of GH+KDEL secretion was observed following cotransfection with either ERD2.1 (75%/25%) or ERD2.2 (82%/18%), compared to that seen with pcDNA (14%/86%). These results indicate that overexpression of ERD2 protein increases the capacity of the cells to retain KDEL-containing proteins. Similarly, increased cellular retention of GH+HNEL was observed following cotransfection with ERD2.1 (72%/28%), or ERD2.2 (81%/19%), compared to vector pcDNA alone (20%/80%). Thus, the ERD2 proteins are capable of retaining HNEL-containing proteins in addition to KDEL-containing proteins. The higher levels of GH+KDEL and GH+HNEL secretion seen in these experiments (co-transfected with pcDNA3) when compared to those in Fig. 1 were likely due to differential levels of protein expression. The levels of GH+KDEL and GH+HNEL expression in the transiently transfected cells (Fig. 8) were likely higher than in the

**Fig. 6.** Immunofluorescence localization of ERD2 proteins. U87 cells transiently transfected with HA-tagged ERD2.1 (A) or ERD2.2 (B) cDNA were immunolabeled with anti-HA antibody and detected with Texas red-conjugated goat anti-mouse IgG. The stained cells were examined using a Zeiss Axioskop microscope (×630).

**Fig. 7.** Immunogold labeling of ERD2 proteins in U87 cells. U87 cells were transiently transfected with ERD2 cDNA containing an HA epitope. Cells were stained with anti-HA antibody and 10 nm Protein A-gold. (A) Moderate expressor showing ERD2 in intermediate compartments (IC) and the cis-elements of the Golgi stacks (G), but not in the rough ER (ER). (B) Overexpressing cell with ERD2 present in extended rough ER cisternae (ER) and in the cisternae of the Golgi complex (G). Bars, 0.1 μm.
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stably transfected cells (Fig. 1), which would lead to saturation of endogenous ERD2 protein(s) and the higher level of secretion. To confirm that the effects of ERD2 coexpression on the ER retention of GH+KDEL and GH+HNEL is not due to an indirect effects (e.g. by altering the overall environment of the ER), we also performed the above experiments with 5 hours of chase time. The differences between GH and GH+KDEL (or GH+HNEL) were similar to those with 2 hours chase shown above. To ensure that expression of ERD2 proteins in U87 cells did not affect the normal secretory pathway, after anti-GH immunoprecipitation we reimmunoprecipitated the medium and cell lysates from each transfection with antibody to plasminogen activator inhibitor type-1 (PAI-1, Bu et al., 1992). As seen in Fig. 9, the amounts of PAI-1 secreted after 2 hours of chase are similar following all of the various transfections, indicating that the secretion of endogenous proteins is not affected by the overexpression of ERD2 proteins in transfected cells (30-50% of total cells following transient transfection). In experiments not shown here, we have also analyzed the effects of coexpression of ERD2 proteins on the ER retention of full length RAP. We found that both ERD2.1 and ERD2.2 were capable of mediating ER retention of RAP to a similar extent for those of GH+HNEL (data not shown).

To further confirm that the KDEL and HNEL signals are universal and not a consequence of the GH carrier, we tagged these sequences onto another reporter protein, hen egg lysozyme (HEL), and analyzed the effects of cotransfection of ERD2 proteins with various constructs of HEL. Results with these HEL constructs are virtually identical to those obtained with the GH constructs (data not shown).

DISCUSSION

The function of an intracellular protein is dependent upon the fidelity of its targeting following biosynthesis. Recent studies from our laboratory (Bu et al., 1995) have demonstrated that RAP is an ER resident protein and functions along the early secretory pathway as a chaperone for LRP. In the present report, we demonstrate that the HNEL sequence present at the carboxyl terminus of RAP is a novel mammalian ER retention signal. In addition, we have shown that the ERD2 proteins (i.e. KDEL receptors) are also capable of retaining HNEL-containing proteins. These results thus demonstrate that mammalian ER proteins are retained by diverse signals and that the ERD2 proteins may function as general ER retention receptors.

In mammalian cells ER retention is mediated by the KDEL sequence present at the carboxyl termini of ER resident proteins. This signal, first described by Munro and Pelham (1987), has been found in a number of mammalian ER resident proteins (Pelham, 1990). The HDEL sequence, on the other hand, serves as the ER retention signal in yeast (Pelham et al., 1988) and in one case, in mammalian cells (human calcium-binding protein; Weis et al., 1994). The HNEL sequence present in RAP is a ‘non-classical’ tetrapeptide which functions as an ER retention signal. A sequence search of GenBank has identified only one other potential protein that contains the HNEL signal at the carboxyl terminus of the protein (capripoxvirus; Gershon and Black, 1989). Whether the HNEL signal in this virus protein is expressed and functional is unknown at present.

The HNEL retention signal appears to function similarly to KDEL as: (1) KDEL- and HNEL-tagged proteins exhibit similar intracellular localization by immunoelectron microscopy; (2) overexpression of both HNEL- and KDEL-containing proteins results in secretion of endogenous RAP suggesting that the two signals are likely to interact with the same ER retention receptor(s); (3) ERD2 proteins retain HNEL-tagged proteins to a similar extent as the KDEL-tagged proteins. Therefore, the HNEL signal associated with RAP represents an alternative targeting signal for ER retention in mammalian cells.
Although they function similarly, it is not clear whether the KDEL and HNEL signals always utilize the same ER retention receptor(s). For example, certain receptors may more specifically interact with one signal relative to the other. Since the two ERD2 proteins are the only mammalian ER retention receptors identified to date, we tested the ability of each of these receptors to retain either KDEL- or HNEL-containing proteins. Our results indicate that the two signals interact similarly with either ERD2 protein. Using RT-PCR techniques (data not shown), we have detected the expression of both ERD2.1 and ERD2.2 proteins in U87 cells, as well as in human hepatoma HepG2 cells (Bu et al., 1993). Similarly, Lewis and Pelham (1992) have detected the expression of both ERD2 proteins in a number of mammalian tissues. It is not clear at present why cells simultaneously express both ER retention receptors. It is possible that the expression of the two receptors is differentially regulated under various physiological conditions. In addition, the two ERD2 proteins may display differential substrate specificity to signals yet unidentified, or each may function as the primary receptor for a subclass of proteins retained in the ER.

The biosynthesis and intracellular itinerary of ER resident proteins require their continuous retrieval from post ER compartments (cis- and medial-Golgi compartments) back to the ER (Rothman and Orci, 1992). This may result in a dynamic flux of ERD2 proteins between the Golgi and the ER compartments. Interestingly, Hsu and his colleagues (1992) reported a brefeldin A-like phenotype induced by the overexpression of ERD2 proteins in COS cells. Their studies suggested that the trafficking of ERD2 proteins is associated with membrane redistribution. We also show strong apparent ER staining of ERD2 proteins in some U87 cells following transient transfection with ERD2 cDNAs. However, in contrast to brefeldin A treated cells, the overexpression of ERD2 protein retains Golgi complexes with ERD2 present throughout. The normal morphology of Golgi complexes in ERD2-overexpressing cells is consistent with the fact that the trafficking of normally secreted protein (e.g. PAI-1) is not affected by the overexpression of ERD2 proteins. When cells expressing the two ERD2 proteins were compared, we found that cells transfected with ERD2.1 are more likely to exhibit apparent ER staining than those transfected with ERD2.2.

Molecular chaperones generally refer to those proteins involved in protein folding and/or the retention of unassembled proteins within the ER (Gething and Sambrook, 1992). However, the concept of a molecular chaperone is expanding and now includes roles for such proteins in the transport of substrate to a particular subcellular compartment as well as the modulation of substrate activity (Hendrick and Hartl, 1993). The function of RAP as a molecular chaperone has been demonstrated in our recent studies which show that RAP associates with LRP and regulates LRP activity and trafficking along the secretory pathway (Bu et al., 1995). The notion that RAP functions as a molecular chaperone for LRP trafficking is also supported by the studies of Willnow et al. (1995) who demonstrate that cells lacking RAP exhibit a ~75% reduction of functional LRP. Thus, these results further establish the role of RAP in LRP trafficking. The molecular mechanism(s) responsible for the effects of RAP on the trafficking of LRP is not clear at present. Based upon our previous studies (Bu et al., 1995), it is possible that RAP stabilizes LRP by maintaining the receptor in an aggregated state prior to its arrival at the cell surface. Without this stabilization, LRP may be more likely to interact with its other ligands (e.g. t-PA, PAI-1) within the ER and be misrouted and/or subject to rapid degradation. Elucidation of the mechanism by which RAP chaperones LRP along the secretory pathway should enhance our understanding of the ways in which a multifunctional receptor trafficks along the secretory pathway in the presence of its ligands.

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