Differential Functions of Triplicated Repeats Suggest Two Independent Roles for the Receptor-associated Protein as a Molecular Chaperone*

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The 39-kDa receptor-associated protein (RAP)1 is a molecular chaperone for the low density lipoprotein receptor-related protein (LRP), a large endocytic receptor that binds multiple ligands. The primary function of RAP has been defined as promotion of the correct folding of LRP, and prevention of premature interaction of ligands with LRP within the early secretory pathway. Previous examination of the RAP sequence revealed an internal triplication. However, the functional implication of the triplicated repeat was unknown. In the current study using various RAP and LRP domain constructs, we found that the carboxyl-terminal repeat of RAP possesses high affinities to each of the three ligand-binding domains on LRP, whereas the amino-terminal and central repeats of RAP exhibit only low affinity to the second and the fourth ligand-binding domains of LRP, respectively. Using truncated soluble minireceptors of LRP, we identified five independent RAP-binding sites, two on each of the second and fourth, and one on the third ligand-binding domain of LRP. By coexpressing soluble LRP minireceptors and RAP repeat constructs, we found that only the carboxyl-terminal repeat of RAP was able to promote the folding and subsequent secretion of the soluble LRP minireceptors. However, when the ability of each RAP repeat to inhibit ligand interactions with LRP was examined, differential effects were observed for individual LRP ligands. Most striking, both the amino-terminal and central repeats, but not the carboxyl-terminal repeat, of RAP inhibited the interaction of α2-macroglobulin with LRP. These differential functions of the RAP repeats suggest that the roles of RAP in the folding of LRP and in the prevention of premature interaction of ligand with the receptor are independent.

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1 The abbreviations used are: RAP, receptor-associated protein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; LRP, LDL receptor-related protein; SLRP, soluble LRP; ER, endoplasmic reticulum; PCR, polymerase chain reaction; GST, glutathione S-transferase; t-PA, tissue-type plasminogen activator; αM*, protease- or methylamine-activated α2-macroglobulin; EGF, epidermal growth factor; HA, hemagglutinin.

2 The 39-kDa receptor-associated protein (RAP)1 is an unique receptor antagonist. The target receptors for RAP are cysteine-rich endocytic receptors that belong to the low density lipoprotein (LDL) receptor family (1). The four representative receptors in this family are the LDL receptor (2), the LDL receptor-related protein (LRP, Ref. 3), glycoprotein gp330/megalin (4), and the VLDL receptor (5). Among these receptors, LRP and gp330/megalin are large multifunctional receptors with multiple ligand-binding domains, which bind several structurally and functionally distinct ligands (for reviews, see Refs. 1 and 6). While RAP exhibits high affinities for LRP, gp330/megalin, and the VLDL receptor, it binds only weakly to the LDL receptor (7). Upon binding to these receptors, RAP inhibits the binding and/or endocytosis of all the ligands by the receptors. This unique feature of RAP has allowed its extensive use in biological studies of these endocytic receptors. Recent evidence has suggested that, under normal physiological conditions, RAP is an endoplasmic reticulum (ER) resident protein and functions within the early secretory pathway (8–10). Using LRP as the target protein, it was found that RAP retained within the ER functions as a regulator of LRP activity 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 (9). The role of RAP in the maturation and trafficking of LRP is further supported by gene-knockout studies (11), which demonstrate that cells lacking RAP exhibit a 75% reduction in functional LRP.

LRP is the largest endocytic receptor identified to date (~600 kDa). It is synthesized as a single polypeptide chain and cleaved in the trans-Golgi into two subunits (12, 13). The 515-kDa extracellular subunit contains 31 copies of complement-type ligand-binding domains arranged in four clusters with 2, 8, 10, and 11 repeats, respectively (1, 3). Also present in this subunit are 22 copies of cysteine-rich epidermal growth factor (EGF) precursor-type repeats which flank the ligand-binding domains. The complement-type repeats in LRP are similar to those in the LDL receptor in which the 40-residue-long cysteine-rich repeats exhibit a highly conserved spacing pattern of six cysteine residues that form three intramolecular disulfide bonds (14). The disulfide bonds are believed to be important for the stability of the ligand-binding sites on the receptor. The complexity of LRP’s structure, largely due to the extensive intradomain disulfide bonds, presents a challenging task for proper folding during its biosynthesis. This process may well be assisted by molecular chaperone(s) within the ER. Indeed, using anchor-free, soluble mini-receptors that represent each of the four putative ligand-binding domains of LRP (SLRPs), our most recent studies (15) showed that coexpression of RAP is both necessary and sufficient for the correct folding and subsequent secretion of the SLRPs. Without the coexpression of RAP, SLRPs are misfolded due to the formation of intermolecu-
ular disulfide bonds and are retained within the ER with little secretion. It is not known at present whether the role of RAP in the receptor’s folding is independent from its function in preventing premature ligand interaction with the receptor.

The HNEL tetrapeptide at the carboxyl terminus of RAP has been shown to mediate its ER localization and retention (9). In addition to this ER-retention signal, examination of the RAP sequence also identified an internal triplication (9). In the present study, using various molecular and cellular approaches, we have analyzed the function of each of the three repeats of RAP. We found that while the carboxyl-terminal repeat of RAP functions similarly to the full-length RAP in terms of interacting with the receptor and in assisting the receptor to fold, inhibition of at least one LRP ligand, α2-macroglobulin, can only be achieved with the amino-terminal or the central repeats of RAP. These differential functions of the RAP repeats suggest that the functions of RAP in receptor folding and inhibition of ligand interactions are independent.

MATERIALS AND METHODS

Construction of cDNAs for SLRPs—Construction of cDNAs for SLRP1, SLRP2, and SLRP4 was essentially as described previously (15). The reverse primer used for generating additional soluble minireceptors of LRP with an HA epitope tag inserted after the signal cleavage site. All oligonucleotides were synthesized in the Washington University School of Medicine Protein Chemistry Laboratory. The regions represented in the new SLRPs are illustrated in Fig. 4, and the following amino acid sequences are shown.

Sequence Analysis for RAP—In our previous studies (9), we noted a possible internal triplication in the primary structure of RAP. However, the boundaries and the relationship among the three repeats were not clear. In the present studies, we used several DNA computer analysis programs to examine the RAP sequence. Shown in Fig. 1 is the sequence analysis using the GCG package (Program Manual for the Wisconsin Package, version 8, Genetics Computer Group). The programs Compare and Dotplot examine the sequence homology by identifying both identical and similar residues along the sequence. When RAP was compared with itself using a window of 30 residues and a stringency of 15 residues, we found significant homology between regions within 1–100 and 201–300, as well as between regions 101–200 and 201–300 (Fig. 1A). Only scattered homology was found between regions of 1–100 and 101–200. Thus, we assigned the RAP sequence into three repeats approximately equal in sizes (repeat 1 = 1–100; repeat 2 = 101–200; repeat 3 = 201–323). Using these boundaries, we compared the sequence homology among the three repeats. The lower homology between repeat 1 and repeat 2 prompted us to compare each of the two repeats separately, using the Gap program of the GCG package. We found that both repeat 1 and repeat 2 have high homology with repeat 3 (46.4% and 45.5% similarity, respectively), whereas repeat 1 and repeat 2 have relatively low homology (38.9% similarity). Thus, we speculate that if the three repeats of RAP were derived from the same ancestral sequence, repeat 1 and repeat 2 may have been derived from repeat 3 separately during evolution. Two regions between repeat 1 and 3 were further identified for high homology, which are represented by two columns of amino acid sequence lines in Fig. 1A. Only one (albeit longer) region was found between repeat 2 and repeat 3 that shares high homology. These homologous regions are graphically illustrated in Fig. 1B.
ing GST/RAP constructs representing each of the three repeats (9). In addition to the full-length RAP (GST/RAP-(1–323)), three GST/RAP repeat constructs were made, which slightly overlapped at the assigned boundaries (i.e. GST/RAP-(1–110), GST/RAP-(91–210), and GST/RAP-(191–323), see Fig. 2A). When these GST fusion proteins were tested for interaction with LRP via ligand blotting (Fig. 2B), we found that repeat 3 possessed similar affinity for LRP as full-length RAP. Although they have much lower affinities, repeat 1 and repeat 2 of RAP also appear to be capable of interacting with LRP. Due to low specific radioactivity following iodination of repeats 1 and 2, we were unable to conclusively compare the affinities of RAP repeats with LRP on the cell surface directly. However, to examine the relationships of these interactions at the cell surface, we performed competition analysis for the binding of full-length RAP to U87 cells (8, 9) by each of the RAP repeat constructs. To eliminate potential steric hindrance effects of GST on ligand inhibition, we removed the GST portion from each of the fusion constructs, and thereafter repurified the resulting RAP fragments. Shown in Fig. 2C are the binding of either 125I-RAP-(1–323) or 125I-RAP-(191–323) to U87 cells in the absence or the presence of excess unlabeled competitors (100-fold excess). As seen in the figure, in addition to the full-length RAP, only repeat 3, but not repeats 1 and 2, demonstrated inhibition of the binding of 125I-RAP-(1–323). For binding of 125I-RAP-(191–323), both unlabeled full-length RAP and repeat 3 maximally inhibited, while repeat 1 and repeat 2 inhibited binding slightly. These results suggest that, among the three repeats of RAP, the third repeat possesses the highest affinity for LRP, consistent with the results obtained from the ligand blotting analysis.

Differential Interactions of RAP Repeats with Ligand-binding Domains of LRP—In our previous studies, we have shown that RAP binds to the second, third, and fourth ligand-binding domains of LRP (15). To analyze to which of the ligand-binding domains each of the RAP repeat binds, we performed experiments in which potential interactions between a given RAP repeat and a given ligand-binding domain of LRP were examined. Ligand-binding domains of LRP were represented by SLRPs, which upon coexpression with RAP, are secreted into the media (15) (also see illustrations in Fig. 4 for schematic representations of SLRPs). GST/RAP constructs, separated via SDS-polyacrylamide gel electrophoresis, were transferred onto nitrocellulose and incubated with conditioned media containing SLRP2 (Fig. 3A), SLRP3 (Fig. 3B), or SLRP4 (Fig. 3C). The SLRPs that interacted with GST/RAP constructs were then detected using anti-HA antibody (15). As seen in the figures, no interaction of the negative control, GST, with any SLRP was detected. Both full-length RAP (GST/RAP-(1–323)) and repeat 3 of RAP (GST/RAP-(191–323)) interacted with each of the three SLRPs, suggesting that repeat 3 of RAP contains the binding determinants for interacting with each of the three ligand-binding domains on LRP. Weak interaction of repeat 1 was detected only with SLRP2, whereas weak interaction of repeat 2 of RAP was seen with only SLRP4. These weaker interactions were enhanced when the amounts of GST/RAP fusion protein were increased by 10-folds (as is the case presented in Fig. 3). These results suggest differential interac-
tions of RAP repeats with ligand-binding domains on LRP. Two additional RAP repeat constructs were tested in these experiments, one with the ER-retention signal HNEL deleted (GST/RAP-(1–319)), and one containing repeats 1 and 2, and part of repeat 3 of RAP (GST/RAP-(1–250)). As shown in Fig. 3, GST/RAP-(1–319) interacted similarly to full-length RAP with LRP, indicating that the HNEL signal is not important for RAP interaction with LRP. The fact that GST/RAP-(1–250) does not interact with SLRP3 is consistent with the notion that neither repeat 1, nor repeat 2 of RAP interacts with the third ligand-binding domain of LRP, and suggests residue 250–323 are important for interacting with SLRP3.

Our previous cell surface saturation binding analyses have suggested that each LRP molecule contains 5–7 binding sites for RAP (22). Thus, to further define the binding sites of RAP on the subdomains of LRP, we prepared constructs representing approximately each half of the three ligand-binding domains, with or without the flanking EGF precursor type repeats (see “Materials and Methods”). These regions are graphically illustrated in Fig. 4. Using these SLRPs and the coexpression of RAP, we have analyzed the interaction with each of the repeats within RAP, as well as the full-length RAP. The results are summarized in Table I. As seen in the table, the amino-terminal (SLRP2N) and carboxyl-terminal (SLRP4C) cDNA-transfected U87 cells. Each transfection was carried out with the cotransfection of cDNA for RAP. The bound SLRPs were then detected with anti-HA antibody and ECL detection method.

Fig. 2. Interaction of triplicated repeats with LRP. A, schematic representation of GST/RAP constructs. Numbers represent amino acid residue positions. B, ligand blotting of GST/RAP constructs to LRP. Purified human LRP was electrophoresed on 5% SDS gel and transferred to nitrocellulose membrane. Strips of membrane were then incubated with GST or GST/RAP constructs, and bound proteins were detected using anti-GST antibody and enhanced chemiluminescence (ECL, Amersham) detection method. C, competition of 125I-RAP-(1–323) or 125I-RAP-(191–323) binding to U87 cells by RAP repeat constructs. Binding of 125I-RAP-(1–323) or 125I-RAP-(191–323) (4 nM) to U87 cells was performed in the absence or presence of unlabeled competitors (400 nM) as labeled in the figure. The GST portion had been cleaved off from each of the proteins used in these experiments.

Fig. 3. Interaction of SLRPs with repeat constructs of RAP. GST (10 μg), GST/RAP-(1–323) (1 μg), GST/RAP-(1–110) (10 μg), GST/RAP-(91–210) (10 μg), GST/RAP-(191–323) (1 μg), GST/RAP-(1–319) (1 μg), or GST/RAP-(1–250) (10 μg) were electrophoresed on 10% SDS gels and transferred to nitrocellulose membrane. After blocking, membranes were incubated with conditioned media harvested from SLRP2 (A), SLRP3 (B), or SLRP4 (C) cDNA-transfected U87 cells. Each transfection was carried out with the cotransfection of cDNA for RAP. The bound SLRPs were then detected with anti-HA antibody and ECL detection method.
To examine the ability of RAP repeats to promote the folding process of LRP, we constructed cDNAs corresponding to the full-length RAP and each of its three repeats of RAP. An HA epitope and five methionine residues were included in the construct to monitor the expression of these proteins after cell transfection. When these cDNAs were transfected into U87 cells (8), we found abundant expression for each of them following metabolic labeling with $[^{35}S]$methionine and immunoprecipitation with anti-HA antibody (Fig. 5A). The expression levels of RAP and its individual repeats after transfection were approximately 50-fold higher when compared with the endogenous RAP (data not shown, see Ref. 15). In our previous studies, we developed a system by which the folding of LRP can be evaluated using SLRPs (15). Correct folding and the subsequent secretion of each of the three ligand-binding domains of LRP (SLRP2, SLRP3, and SLRP4) require the coexpression of RAP. Shown in Fig. 5B is an experiment in which SLRP2 is expressed in U87 cells without or with coexpression of full-length RAP (RAP-(1–323)). The secretion level of SLRP2 was monitored by analyzing radiolabeled SLRP2 in the media and cell lysates after pulse labeling with $[^{35}S]$cysteine for 1 h and chase for 3 h. As seen in the figure, without coexpression of RAP-(1–323), little secretion (~8% of total radioactivity) of SLRP2 was seen, whereas when RAP-(1–323) was coexpressed, about 50% of total $[^{35}S]$-SLRP2 was secreted. Using the same assay, we analyzed the ability of each RAP repeat construct in the secretion of SLRPs. Shown in Fig. 5C are results from a representative experiment of the four performed. The percent of SLRP secretion after pulse-chase labeling was plotted against individual constructs. As seen in the figure, repeat 3 of RAP (RAP-(191–323)) functioned at least as well (SLRP4) if not better (SLRP2 and SLRP3) than the full-length RAP in its ability to assist the folding and secretion of each of the three SLRPs. Both repeat 1 and 2 of RAP had little effect on the secretion of SLRPs, possibly due to their lower affinity for the SLRPs. Also examined in these experiments was RAP-(1–250), which functioned similarly to the full-length RAP in the secretion of SLRP2 and SLRP3, but not SLRP4, consistent with its ability to bind LRP (see Fig. 3).

Role of RAP Repeat Constructs in the Inhibition of Ligand Interactions with LRP—RAP has been used extensively in the study of LRP ligands due to its ability to antagonize ligand interactions with the receptor (1). To examine the ability of each of the RAP repeats to inhibit LRP ligands, we analyzed the interaction of several ligands with LRP in the absence or presence of either the full-length RAP or each of the repeat constructs of RAP. To exclude any possible steric hindrance by GST, we proteolytically removed it from each of the fusion constructs of LRP. Figure is a schematic representation of domain and subdomain constructs of SLRPs used in our studies.

![Schematic representation of domain and subdomain constructs of SLRPs](image)

**Table I** Interaction of SLRPs with GST/RAP constructs

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Differential Functions of RAP Repeats

Role of RAP Repeats in the Folding and the Secretion of SLRPs—To examine the ability of RAP repeats to promote the folding process of LRP, we constructed cDNAs corresponding to the full-length RAP and each of its three repeats of RAP. An HA epitope and five methionine residues were included in the constructs to monitor the expression of these proteins after cell transfection. When these cDNAs were transfected into U87 cells (8), we found abundant expression for each of them following metabolic labeling with $[^{35}S]$methionine and immunoprecipitation with anti-HA antibody (Fig. 5A). The expression levels of RAP and its individual repeats after transfection were approximately 50-fold higher when compared with the endogenous RAP (data not shown, see Ref. 15). In our previous studies, we developed a system by which the folding of LRP can be evaluated using SLRPs (15). Correct folding and the subsequent secretion of each of the three ligand-binding domains of LRP (SLRP2, SLRP3, and SLRP4) require the coexpression of RAP. Shown in Fig. 5B is an experiment in which SLRP2 is expressed in U87 cells without or with coexpression of full-length RAP (RAP-(1–323)). The secretion level of SLRP2 was monitored by analyzing radiolabeled SLRP2 in the media and cell lysates after pulse labeling with $[^{35}S]$cysteine for 1 h and chase for 3 h. As seen in the figure, without coexpression of RAP-(1–323), little secretion (~8% of total radioactivity) of SLRP2 was seen, whereas when RAP-(1–323) was coexpressed, about 50% of total $[^{35}S]$-SLRP2 was secreted. Using the same assay, we analyzed the ability of each RAP repeat construct in the secretion of SLRPs. Shown in Fig. 5C are results from a representative experiment of the four performed. The percent of SLRP secretion after pulse-chase labeling was plotted against individual constructs. As seen in the figure, repeat 3 of RAP (RAP-(191–323)) functioned at least as well (SLRP4) if not better (SLRP2 and SLRP3) than the full-length RAP in its ability to assist the folding and secretion of each of the three SLRPs. Both repeat 1 and 2 of RAP had little effect on the secretion of SLRPs, possibly due to their lower affinity for the SLRPs. Also examined in these experiments was RAP-(1–250), which functioned similarly to the full-length RAP in the secretion of SLRP2 and SLRP3, but not SLRP4, consistent with its ability to bind LRP (see Fig. 3).
proteins and repurified the RAP constructs. Since the primary binding sites on the cell surface of some LRP ligands are not on LRP (e.g., tissue factor pathway inhibitor, or TFPI; see Ref. 23), but subsequent internalization and degradation is, we performed ligand degradation assays using U87 cells. Each of the ligands examined was radioiodinated and incubated with U87 cells for 4 h in the absence or the presence of RAP constructs. Shown in Fig. 6 are summaries for four LRP ligands: t-PA (24), α2M* (25), antithrombin III-thrombin complex (26), and TFPI (23). As seen in the figure, full-length RAP inhibited the degradation of each of the ligands with LRP. Repeat 3 of RAP inhibited the degradation of 125I-t-PA, 125I-antithrombin III-thrombin complex, and 125I-TFPI, but not 125I-α2M*. Interestingly, while both repeat 1 and repeat 2 of RAP were generally inefficient in inhibiting other ligands, these repeats were very effective in inhibiting the interaction of α2M* with LRP. Thus, LRP ligands were differentially inhibited by RAP repeat constructs, consistent with the hypothesis that LRP ligands bind to different sites on the receptor.

DISCUSSION

Despite being widely used as an antagonist to LRP on the cell surface, RAP has recently been defined as a specialized ER chaperone and functions during LRP’s folding and subsequent trafficking along the early compartments of the secretory pathway (9, 10, 15). Previously it had been noted that RAP contained a triplicated repeat within its sequence (9). However, the functional aspects of these repeats have not been defined. In the current report, we have re-examined the sequence of RAP using several sequence analysis programs. Although no clear boundaries can be defined among the three repeats, we found that the homologous regions are retained within each boundary if RAP is divided into three approximately equally sized regions (i.e. repeat 1, amino acids 1–100; repeat 2, amino acids 101–200; and repeat 3, amino acids 201–323). Several studies have suggested that there are multiple RAP-binding sites on each LRP molecule. However, the exact number of RAP-binding sites on LRP is unknown. Using cell surface binding analyses, we have previously shown that there were approximately 5–7 times more RAP-binding sites on hepatocytes when compared with t-PA binding sites (i.e. repeat 1, amino acids 1–100; repeat 2, amino acids 101–200; and repeat 3, amino acids 201–323). Several studies have suggested that there are multiple RAP-binding sites on each LRP molecule. However, the exact number of RAP-binding sites on LRP is unknown. Using cell surface binding analyses, we have previously shown that there were approximately 5–7 times more RAP-binding sites on hepatocytes when compared with t-PA binding sites (i.e. functional LRP molecules, see ref. 22). However, other reported binding studies of RAP to purified LRP concluded that two RAP-binding sites are present on each LRP molecule (27). Using the soluble LRP minireceptors, we have recently reported that each of the three putative ligand-binding domains of LRP is capable of interacting with RAP (15). In the current report, we have further analyzed the RAP-binding sites on LRP using domain and subdomain constructs of LRP. We found that there were at
least two RAP-binding sites on the second and fourth, and one on the third ligand-binding domains of LRP, yielding a total of at least five RAP-binding sites on the receptor. It should be pointed out that, given the fact that each half of the second and fourth ligand-binding domains of LRP is capable of binding RAP, these repeats may contain more than two RAP binding sites. The exact number of RAP-binding sites, as well as the regions where RAP binds, will require further investigation.

The physiological function of RAP has become more clear during the past 2 years. The fact that RAP is localized primarily within the ER with little or no secretion suggests an intracellular role for this protein. It appears that RAP associates with LRP during or immediately after the biosynthesis of the receptor (9). Association of RAP with LRP would ensure that LRP remains in an inactive ligand-binding state during its trafficking along the early secretory pathway. This appears to be important for receptor trafficking as LRP ligands are secreted proteins and often expressed in the same cells as the receptor. By associating with the receptor, RAP prevents premature interaction of ligands with the receptor (9, 10). This function of RAP resembles that of the invariant chain in preventing premature binding of peptides with the MHC class II molecules during their trafficking along the secretory pathway (28). Interestingly, using the soluble minireceptor system, our recent studies also suggest that RAP is required for the folding process of LRP by preventing the formation of intermolecular disulfide bonds (15). It is not clear at present whether the function of RAP in the folding of the receptor and in preventing premature ligand interaction with the receptor are related. For example, if a ligand interacts with LRP before the receptor completes its folding, the folding process might be impaired. In this case, preventing ligand interaction with the receptor ensures the proper folding of LRP. On the other hand, it is possible that RAP is independently involved in the folding, but remains associated with the receptor after the folding. Thereafter, premature ligand interaction with the receptor may be prevented. The precise role of RAP as a chaperone for LRP, as well as the mechanisms involved, await further definition.

The differential functions of the RAP repeats may also explain their roles and appearance during evolution. Since repeat 3 of RAP is capable of assisting the folding of each of the ligand-binding domains of LRP and of inhibiting most of the ligand interactions with the receptor, it is likely that this repeat is essential for RAP’s function and may be the ancestral region for the whole molecule; consistent with the indications from sequence alignment. However, the fact that interaction of some of the LRP ligands (e.g., α2-macroglobulin, shown in this study) is not inhibited by repeat 3 of RAP suggests the need for the first two repeats, which do inhibit these ligand interactions with the receptor. It is interesting to note that LRP is present in an organism as primitive as the nematode Caenorhabditis elegans (29). Examination of the C. elegans gene bank also identifies a 290-amino acid protein sequence (gene accession no. Z75527) that shares high sequence homology with human RAP, particularly in the portion where repeat 2 and repeat 3 of human RAP share most homology. However, no ER-retention signal is present at the carboxyl terminus of this sequence. Whether this protein is the nematode equivalent of RAP requires functional studies. It will be interesting in future studies to examine the appearance of LRP ligands during evolution. For example, α2-macroglobulin has been described in the horseshoe crab (30). If most LRP ligands are absent in the nematode,
the primary function of RAP may be to aid in receptor folding. The role of RAP in inhibiting ligand interaction with the receptor may have evolved only after LRP expression became high in tissues (e.g., liver and brain) of higher organisms and with the appearance of the diverse array of LRP ligands.

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