Yeast Vps10p is a receptor for transport of the soluble vacuolar hydrolase carboxypeptidase Y to the lysosomelike vacuole. Its functional equivalents in mammalian cells are the mannose 6-phosphate receptors that mediate sorting to lysosomes of mannose 6-phosphate-containing lysosomal proteins. A chimeric receptor was constructed by substituting the cytoplasmic domain of M₄ 300,000 mannose 6-phosphate receptor with the Vps10p cytoplasmic tail. Expression of the chimer in cells lacking endogenous mannose 6-phosphate receptors resulted in a subcellular receptor distribution and an efficiency in sorting of lysosomal enzymes similar to that of the wild type M₄ 300,000 mannose 6-phosphate receptor. Moreover, the cytoplasmic tail of the Vps10p was found to interact with GGA1 and GGA2, two mammalian members of a recently discovered family of clathrin-binding cytosolic proteins that participate in trans-Golgi network-endosome trafficking in both mammals and yeast. Our findings suggest a conserved machinery for Golgi-endosome/vacuole sorting and may serve as a model for future studies of yeast proteins.

In mammalian cells, soluble lysosomal proenzymes are posttranslationally modified in the Golgi complex and acquire mannose 6-phosphate residues that function as lysosomal sorting signals. The two known mannose 6-phosphate receptors (MPRs),¹ the larger MPR300 and the smaller MPR46, both mediate the transport of newly synthesized enzymes from the trans-Golgi network (TGN) to a prelysosomal compartment (for review see Ref. 1). In contrast to their ligands, the MPRs do not reach the lysosomes but recycle between endosomes and the TGN and additionally between endosomes and the plasma membrane. However, only MPR300 binds and internalizes ligands at the plasma membrane (2). Exchange of domains between MPR300 and MPR46 results in functional proteins mediating lysosomal enzyme sorting (3).

Sorting and delivery of vacuolar hydrolases in yeast is very similar to that of lysosomal proteins in mammalian cells (4). The receptor for Golgi-vacuole transport of the soluble carboxypeptidase Y (CPY), i.e. the type I integral protein Vps10p, was discovered by the isolation of Saccharomyces cerevisiae mutants that missort CPY (5–9). Vps10p binds luminal ligands via its N-terminal domain, and although it bears no homology to MPRs and sorts CPY by recognition of a peptide and not a carbohydrate, its function in yeast corresponds to the function of MPRs in mammalian cells.

The cytoplasmic domains (cds) of various receptors have been extensively analyzed for signals regulating their interaction with cytosolic binding partners and their intracellular trafficking. Clathrin has been directly linked to the process of MPR sorting at the TGN (10). The ligand-MPR complex is transported in clathrin-coated vesicles to a prelysosomal compartment, and after dissociation of the complex, the ligands, i.e. lysosomal hydrolases, are delivered to the lysosomes, whereas the MPRs return to the TGN for another round of transport. Previous work has shown that MPRs are recruited into clathrin-coated vesicles at the TGN by a process involving specific signals in the receptor cds (11). Thus, a dileucine-based motif as well as a tyrosine-based motif is important for the efficient transport of MPRs from the TGN to prelysosomes and may represent binding sites for the Golgi-specific clathrin AP-1 complex (12–15). However, sorting of a mutant MPR in which the dileucine residues were substituted by two alanines was not affected at the TGN (16), and AP-1 knock-out appears to block the return of MPRs from the endosomes rather than their exit from Golgi (17), indicating that alternative or additional proteins partake in sorting at TGN. Two such proteins could be the recently described TIP47 (18) and PACS-1 (19). Both bind the cytoplasmic tail of MPR300, and TIP47 has been shown to play a role in the retrieval of MPR300 and MPR46 from late endosomes to the TGN (18). Similar studies show evidence that the Vps10p cd plays a corresponding role in the retrieval of Vps10p from the prevacuolar compartment and that transport from the TGN to the endosome is independent of direct interactions between the receptor and the clathrin coat (20). Thus, recycling of Vps10p to the TGN is conditioned by a tyrosine-based signal located in its cd (21, 22), and cells that lack clathrin grow poorly but are viable and do not missort CPY (23).

A novel family of ubiquitous coat proteins mediating the formation of intracellular transport-intermediates and selection of cargo are the GGAs (Golgi-localizing γ-adaptin ear homology domain ADP-ribosylation factor binding protein). The GGAs are characterized by an N-terminal VHS domain, and although they have a C-terminal domain with homology to the γ-adaptin “ear” domain, they are otherwise not related to the adaptor proteins. Disruption of the two GGA genes in S. cerevisiae results in missorting of CPY and in morphologically defective vacuoles (24–26). Also, overexpression of a GGA construct lacking the clathrin binding domains seems to block the clathrin-dependent transport of MPR300 from the TGN to en-
dosomes (27). These and other findings have strongly suggested that GGAs function to facilitate trafficking of proteins between TGN and the vacuole or its mammalian equivalent, the lysosome (for review see Ref. 28). Recent reports have shown that GGAs interact directly with sortilin, a multifigand Vps10p-like mammalian receptor, and with the two MPRs, and that the interaction is established between the GGA-VHS domain and a C-terminal segment in the receptor cdc (29–31). Thus, the picture emerges that the mammalian GGAs select receptors for TGN-endosome transport in clathrin-coated carriers and that the receptors are targeted on the basis of specific binding sites harbored within C-terminal acidic cluster-dileucine motifs of the receptor cd.

In the present study we have expressed a MPR300/Vps10p cd chimeric receptor in MPR-deficient cells and show that it is as efficient as the wt MPR300 for sorting of newly synthesized lysosomal enzymes. Moreover, we demonstrate for the first time that the Vps10p cd can bind (mammalian) GGAs that are new and potentially important participants in TGN-endosome transport. Our findings evidence a highly conserved sorting machinery and suggest that expression of Vps10p or other yeast receptors in similar mammalian cell models may provide new information on protein sorting and trafficking.

**EXPERIMENTAL PROCEDURES**

**Receptor Constructs**—The chimera was named corresponding to the origin of the luminal, transmembrane, and cytoplasmic domain of either the MPR300 (L) or the Vps10p(V) (compare Fig. 1). The chimera was constructed from the human cDNAs using the overlap extension PCR-based mutagenesis method as described (3). Fragments of the genes that are to be recombined are generated in separate PCR reactions. For expression all cDNAs were subcloned into the pMPVSVE or pMPSVHE vector (32). For subcloning into the MPR300, an MluI restriction site was introduced behind the stop codon at position 7626. Chimeras containing the luminal MPR300 portion were subcloned into a pMPSV-derived vector containing the MPR300 cdNA by an internal Ndel site at position 6724 and the recombinant MluI site.

MPR300-furin-tail, MPR300-TGN38-tail and MPR300-LDL-receptor tail constructs were constructed by the above mentioned procedure, using cDNAs from cow for furin (33), from rat for TGN38 (34), and from human LDL receptor (35). All constructs were synthesized in regions encompassing mutations using the ABI 310 system (Applied Biosystems). The sequences of the primers used in this study are available on request.

**Cells, Cell Culture, and Transfection**—Mouse embryonic fibroblasts (MEF) were grown in Dulbecco’s minimal essential medium and 10% fetal calf serum. Baby hamster kidney cells (BHK21) were grown in the media supplemented with 5% fetal calf serum. Transfection of BHK21 was performed as reported (36), and transfection of MEF deficient in MPR 46 and MPR300 (mpr—) MEF was performed as described (37).

**Expression Levels**—For determination of the relative amounts of wild type MPR300 and MPR300-Vps10p chimera, the monocalon mouse antibody 2C2 recognizing the luminal epitope of human MPR300 was used (38). The cells were seeded on 5.5-cm plates. The next day the plates were incubated with 5 μg of 2C2 antibody/ml of binding medium (Dulbecco’s minimal essential medium, 7.5% heat-inactivated fetal calf serum, and 20 μg Heps/KOH, pH 7.4) for 2 h at 4 °C in the presence of 0.5% saponin. For determination of surface-associated receptors, the incubation was performed in the absence of saponin. The cells were then washed twice in phosphate-buffered saline in the absence or presence of saponin, respectively, harvested by using a rubber maid, and then washed twice in phosphate-buffered saline in the absence or presence of 0.5% saponin. For determination of surface-associated receptors, the cells were metabolically labeled with [35S]methionine exactly as described. Immunoprecipitation was performed from cells and media as described (3). The human MPR300 was immunoprecipitated with an antisem directed against the human MPR300 (40). All immunoprecipitates were subjected to SDS-PAGE and fluorography. The radioactivity incorporated into the polypeptides was quantified by densitometry using the PhosphorImager “Storm” and the program Image Quant (Amersham Biosciences, Inc.).

**Western Blot Analysis**—Samples were prepared and subjected to Western blot analysis as described (40). For cathepsin D 25 μg of total cell protein were subjected to Western blot analysis. The plate was probed with antisem specific for cathepsin D. The blot was probed with an ECL light-based immunodetection system (Amersham Biosciences, Inc.).

**Primary Antibodies**—Mouse Lamp1 was detected using a monoclonal anti-mouse hybridoma medium (1D4B, Developmental Hybridoma Bank, Iowa City, IA), human MPR300 was detected using a mouse monoclonal antibody (2C2) recognizing a lumenal epitope (38), human MPR46 was detected with an antisem directed against the human MPR46 (41), and cathepsin D was detected using a rabbit antisem (42).

**Two-hybrid Analysis, GGA Constructs, and Coprecipitation Experiments**—Yeast two-hybrid analysis (Matchmaker LexA two-hybrid system; CLONTECH) of the various receptor cd constructs and generation of the GGA2 domain constructs were carried out as previously described (33). The GGA1-VHS (nucleotides 1429–1935) and GGA1-EAR (nucleotides 1429–1935) GGA1 cDNA kindly provided by L. Jacobsen. Glutathione S-transferase (GST) fusion proteins with GGA1 and GGA2 were constructed in the pGEXvector (29). For pull-down experiments mpr— MEF (80% confluent), wild type or transfected with full-length MPR300 and MPR46, MPR300-Vps10p cd, MPR300-Furin cd, MPR300-TGN38 cd, or MPR300-LDL receptor cd chimeric constructs, were lysed on ice in 1% Triton X-100 buffer either containing 300 mM KCl, 4 mM MgCl2, 0.2 mM EDTA, 1 mM dithioretritol, 20 mM Heps, pH 7.4 (buffer A), or containing 300 mM NaCl, 4 mM MgCl2, 3 mM CaCl2, 20 mM Heps, pH 7.4 (buffer B) and supplemented with proteinase inhibitors (CompleteMini; Roche Molecular Biochemicals). Debris was removed by pelleting, and 100 μl of lystate supernatant supplemented with 900 μl of buffer A or B (with proteinase inhibitors) prior to addition of 10 μg of GST fusion protein (GST-GGA1-FL, GST-GGA1-VHS, GST-GGA1-EAR, GST-GGA2-VHS, or GST alone) according to Ref. 29. Following overnight incubation (4 °C), the samples were supplemented with 50 μl of glutathione-Sepharose (Amersham Biosciences, Inc.) and incubated for an additional 4 h at 4 °C. The glutathione-Sepharose was pelleted, and the beads were washed (three washes were performed with 900 μl of buffer A or B with proteinase inhibitors) 0.1% Triton X-100 before resuspension in sample buffer and SDS-PAGE. For quantitation, the proteins precipitated from cells were dissociated from the Sepharose beads by the addition of surplus glutathione (Sigma) and subsequently subjected to Western blotting detecting MPR300. The amounts of MPR300 protein found in lysates prior to precipitation and the quantity isolated by affinity beads were finally compared by SDS-PAGE.
RESULTS

Construction and Expression of the MPR300/Vps10p cd Chimera—MPR300 and Vps10p are type I transmembrane proteins. For the construction of the receptor chimera, the cd of the MPR300 was replaced by that of the Vps10p receptor (Fig. 1). The domains were designated L or V, according to their origin from the MPR300 or Vps10p, respectively. In the three-letter code the first letter signifies the lumenal, the second signifies the transmembrane, and the third signifies the cytoplasmic domain, e.g. MPR300 is termed LLL, and the MPR300/Vps10p cd chimera is termed LLV. The receptors were expressed in mouse embryonic fibroblasts that lack endogenous MPR46 and MPR300 (mpr−/− ME) (42) and missort 98% of soluble lysosomal enzymes to the medium. In these cells, loss of lysosomal enzymes can be minimized by the expression of a receptor protein that re-establishes functional sorting. Stably transfected clones were selected, and the level of the chimera expression was quantified in permeabilized cells by measuring the binding of a monoclonal antibody directed against the lumenal domain of MPR300 (monoclonal antibody 2C2). The expression level of the chimeric receptor LLV was 0.7–1.9-fold that of endogenous MPR300 in normal MEF cells (Table I). Clones expressing similar levels of wt MPR300 were used for comparison (3). After expression, the subcellular distribution of the chimeric receptor, its ability to bind ligands, and its efficiency in sorting of lysosomal ligands was compared with that of the wild type MPR.

Localization and Stability of the Receptor Chimera LLV—The fraction of receptors accessible to the 2C2 antibody at the cell surface varied between 6 and 11% for the wt MPR300 as well as for the LLV chimera (Table I). Thus, the fraction of receptors present at the cell surface was essentially unaffected by substitution of the Vps10p cd for the MPR300 cd. Based on indirect immunofluorescence, the intracellular distribution of MPR300 and the LLV receptor was comparable. The bulk of receptors was concentrated in structures surrounding the nucleus and in vesicular structures that were scattered throughout the cytoplasm, with few toward the periphery of the cells. We saw no morphological signs indicating accumulation within the endoplasmic reticulum or within lysosomal structures (as defined by the lysosomal enzyme cathepsin D; Fig. 2).

The capacity for lysosomal proteolysis in the mpr−/− MEF depends on the sorting function of the expressed MPRs. The apparent stability of MPRs expressed in mpr−/− MEF is therefore linked to their targeting to lysosomes and their efficiency in sorting of lysosomal enzymes. For this reason, the stability of the chimera and of the wild type receptor was compared in BHK cells (described under “Experimental Procedures”). The BHK cells express endogenous MPR46 and MPR300, and the proteolytic capacity of lysosomes therefore depends less or not at all on the sorting function of the recombinant receptors. Similar half-lives were detected for wt MPR300 and LLV chimera (LLL, 11 h; LLV, 11 h).

The LLV Chimera Mediates Ligand Binding and Endocytosis—The ability of the LLV chimera and the wt MPR300 to bind ligands was analyzed by testing their binding to phosphomannan-Sepharose. Affinity chromatography of cell lysates was performed as described (3), and the amount of material that was eluted from the affinity matrix with 5 mM mannose 6-phosphate was found to be comparatively similar for the wt and chimeric MPR (LLL, 98%; LLV, 98%).

Receptor-mediated endocytosis was examined using the monoclonal antibody 2C2, which is directed against the lumenal MPR300 domain. Thus, mpr−/− MPR300 and mpr−/− LLV cells were incubated with the 2C2 antibody for 2–60 min at 37 °C and subsequently analyzed by indirect immunofluorescence. As seen in Fig. 3, the staining demonstrated that both receptors are subject to rapid internalization from the plasma membrane.

Sorting of Lysosomal Enzymes by the Receptor Chimera LLV—To assess the sorting function of the chimera and the wt MPR300, the accumulation of lysosomal enzymes in the medium was determined. When untransfected mpr−/− MEF are incubated for 24 h, about 90% of the total β-hexosaminidase activity contained in the culture is found in the medium, and only 10% is found in the cells. Transfection with wt MPR300 increases the cellular fraction of β-hexosaminidase to about 80% of total, depending on the level of receptor expression (37). The ability of the LLV chimera to increase the fraction of intracellularly retained β-hexosaminidase was comparable with that of wt MPR300 (91% for wt MPR300 and 82% for LLV).

It is known from earlier studies that in cells expressing only MPR300, part of the newly synthesized lysosomal enzymes are not transferred directly from the secretory route to endosomes/lysosomes but are first secreted and then recaptured by MPR300-mediated endocytosis (37). The extracellular activity of β-hexosaminidase was therefore measured in the presence and absence of 5 mM mannose 6-phosphate, which inhibits receptor-mediated uptake from the medium. As apparent from Fig. 4, the presence of mannose 6-phosphate in mpr−/− cultures increased the extracellular fraction of β-hexosaminidase from 18 to 38% in mpr−/− LLV cells and from 9 to 26% in wt MPR300 transfectants with a similar level of receptor expression. This indicates that the high sorting efficiency partly results from receptor-mediated binding and internalization of secreted lysosomal enzymes and that the secretion-recapture pathway known for MPR300 is also used by the LLV chimera.

Analysis of the lysosomal proteinase cathepsin D by Western blotting revealed that expression of MPR300 or LLV chimera induced normal intracellular steady state concentrations of cathepsin D. Moreover, the relative distribution of the cathepsin D precursor, the single chain intermediate and of the double chain mature form was as in control MEF cells (Fig. 5).
Because the proteolytic processing that leads to the intermediate and mature forms depends on the transport of the cathepsin D precursor to endosomes/lysosomes, these data further show evidence that cathepsin D is correctly targeted in cells expressing LLV chimera and that the endosomes/lysosomes in these cells contain the proteinases required for the full processing. This was supported by indirect immunofluorescence, showing colocalization of cathepsin D and the lysosomal marker Lamp1 in transfected as well as in MEF control cells (Fig. 2). Finally, no difference could be detected between wt MPR300 and LLV in the sorting of metabolically labeled newly synthesized H9252-glucuronidase (not shown).

The Vps10p Cytoplasmic Tail Mediates Lysosomal Sorting—The above results show that the Vps10p cd is an efficient substitute for the MPR300 cd and that it conveys Golgi-endosome transport in mammalian cells. This strongly suggests that the cytoplasmic domain of the yeast protein is capable of interacting not only with the yeast adaptor molecules involved in this type of sorting but also with their mammalian counterparts. The recently discovered GGAs contribute to Vps10p sorting in yeast and are believed to play an important role in the Golgi-endosome trafficking of the Vps10p-related receptor sortilin and the two MPRs. Additional experiments were therefore performed to clarify whether the Vps10p cd could in fact bind to human GGA1 and GGA2. A series of chimeras containing the luminal and the transmembrane part of the MPR300 in combination with the cds of various receptors were expressed in MEF cells and tested for interaction by pull-down experiments using GST-GGA fusion proteins comprising full-length GGA1 or the VHS or EAR domain of GGA1 and GGA2. As demonstrated in Fig. 6, the LLV chimera bound to full-length GGA1 as well as to the VHS domain of both GGA1 and GGA2, whereas pull-down experiments by GGA1-EAR (Fig. 6) and GGA2-EAR (not shown) were unproductive. The results were similar with wild

![Fig. 2. Morphology of late endosomes/lysosomes in normal MEF, in mpr− (MEF cells deficient in mannose 6-phosphate receptors), in mpr− cells expressing MPR300 (LLL) and MPR-Vps10p chimera (LLV).](image)

![Fig. 3. Internalization of MPR300-Vps10p chimeras in transfected mpr− MEF.](image)

![Fig. 4. Effect of mannose 6-phosphate on the secretion of lysosomal enzymes.](image)
type receptors (shown in Fig. 6 for MPR46 and MPR300 and for sortilin in Nielsen et al. (29)) and chimeras carrying the cd of receptors known to bind GGAs and to mediate Golgi-lysosome transport (MPR46, MPR300, and sortilin (data not shown)). The MPR46, in accordance with previous observations (43), did not react with GGAs. In contrast, none of the fusion protein constructs were able to precipitate chimeras containing the cd of the endocytic low density lipoprotein receptor or the Golgi-resident proteins Furin and TGN38.

Furthermore, evidence to an interaction between the Vps10p cd and GGAs was obtained in the yeast two-hybrid system. Initial analysis demonstrated that the wt sortilin tail interacted readily with VHS constructs of GGA1 (Fig. 7) and GGA2 (not shown), whereas the wt cds of Vps10p, MPR300, and MPR46 provided little or no response (not shown). Positive reactions, however, were obtained using truncated constructs constituting the C-terminal parts of the receptor cds. Thus, the GGA2-VHS produced a weak response with the truncated MPR46 cd and a strong response with the truncated MPR300 cd (not shown), and both MPR constructs interacted strongly with GGA1-VHS (Fig. 7). Moreover, the truncated Vps10p cd, which did not interact with the GGA2-VHS (not shown), gave rise to a comparatively weak but distinct and specific response in combination with GGA1-VHS (Fig. 7).

**DISCUSSION**

The function of MPRs in mammalian cells corresponds to the function of Vps10p in yeast. Both receptors are type I integral membrane proteins harboring signals for intracellular trafficking in their cds. This suggested that their cds might be exchangeable and that a chimeric MPR containing the Vps10p cd would target newly synthesized lysosomal enzymes to lysosomes in mammalian cells. The present study confirms this assumption and demonstrates that the chimeric receptor LLV, which combines the lumenal ligand binding and the transmembrane parts of human MPR300 with the yeast Vps10p cd, is functional in mammalian cells and corrects the missorting of mammalian lysosomal enzymes in MPR-deficient mouse embryonic fibroblasts.

The analysis demonstrates that the chimeric receptor can internalize mannose 6-phosphate-containing ligands, indicating that the yeast Vps10p cd contains endocytosis signals that are recognized by mammalian cells. Thus, LLV and MPR300 are both functional in endocytosis despite their lack of cd structural homology, and, to our surprise, the LLV chimera cycles via the plasma membrane, a characteristic not observed for the wt Vps10p cd in yeast (44). We cannot exclude the possibility that unknown determinants harbored in the MPR300 luminal domain may influence the subcellular receptor localization, but our findings strongly indicate that the yeast Vps10p cd contains sequence motifs that are recognized by the sorting machinery of mammalian cells.

Indirect immunofluorescence showed no difference in the intracellular distribution of wt MPR300 and the LLV chimera. The bulk of the receptors was concentrated in the perinuclear area and in vesicular, tubular, and cisternal structures. None...
of the receptors accumulated in the endoplasmic reticulum, nor did we see any accumulation of receptors in lysosome-like structures. The stability of MPR300 depends on its luminal domain because loss of the receptor mainly occurs through non-lysosomal proteolysis in this segment. The cleaved luminal domain is released into the extracellular fluid, indicating that proteolysis occurs at the cell surface or within the secretory route (45). Various combinations of the luminal, transmembrane, and cytoplasmic domains of MPR300 and MPR46 result in unstable receptors (3), and MPR chimeras containing the furin or the TGN38 cd likewise have short half-lives. It is therefore interesting that the LLV chimera and the wt MPR300 in this study exhibit similar stability, suggesting that they are exposed to similar environmental conditions and consequently may travel through the same compartments.

We furthermore present the first direct evidence of an interaction between the cytoplasmic domain of Vps10p and (mammalian) members of the recently described GGA family of cytosolic proteins, human GGA1 and GGA2, mediated by the VHS domains. This finding is in accordance with the present observations of LLV trafficking and with the concept that the GGAs are tail-binding proteins involved in receptor-mediated transport of lysosomal enzymes in mammalian and yeast cells. The pull-down experiments clearly demonstrated binding of the Vps10p cd to the receptor-binding VHS domains, in contrast to the EAR domains, of GGA1 as well as GGA2. However, in the two-hybrid analysis the interaction could only be confirmed by use of a truncated Vps10p cd construct. Because the cds of the two MPRs, but not the sortilin cd, also require truncation for a successful two-hybrid interaction, one explanation for this phenomenon could be that relatively large cytoplasmic domains have additional (structural) requirements not met by the two-hybrid system. The fact that the Vps10p cd only reacted with the GGA1 construct might in addition reflect that GGA1 have fewer requirements for binding than GGA2.2

The combined data support the hypothesis that adapter proteins other than AP-1 are involved in transport of transmembrane receptors and their cargo from the TGN to the vacuole/lysosome. It also suggests that the transport systems to the yeast vacuole and the mammalian lysosome may be more conserved than expected. Expression in yeast of a Vps10p chimera containing the MPR300 cd should demonstrate whether the mammalian receptor cd harbors functional motifs that can mediate sorting to the yeast vacuole.

In summary, our analysis shows that the LLV chimera is functional in terms of lysosomal enzyme sorting. The yeast Vps10p cd was as efficient as the human MPR300 cd for receptor-mediated transfer of lysosomal enzymes to lysosomes in mouse embryonic fibroblasts. Another construct comprising the Vps10p cd in combination with the luminal part of MPR46, the second MPR of mammalian cells, is currently under investigation.

The GGAs have differential but related/overlapping requirements for binding, and the Vps10p cd contains several segments similar to the acidic cluster-dileucine-like motifs that serve as GGA-binding sites in sortilin and the MPRs (compare Fig. 7 and Fig. 1 in Ref. 28). Future studies should identify the functional motifs for GGA1 and GGA2 interaction in the Vps10p cd and determine whether binding includes the third mammalian isoform, i.e. GGA3. Finally, our findings suggest that expression of MPR300/Vps10p cd (LLV) chimeras in mammalian cells may prove a useful tool in future attempts to discriminate between the functional roles of individual GGAs.

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André Dennes, Peder Madsen, Morten S. Nielsen, Claus M. Petersen and Regina Pohlmann

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