Golgi matrix proteins interact with p24 cargo receptors and aid their efficient retention in the Golgi apparatus

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The Golgi apparatus is a highly complex organelle comprised of a stack of cisternal membranes on the secretory pathway from the ER to the cell surface. This structure is maintained by an exoskeleton or Golgi matrix constructed from a family of coiled-coil proteins, the golgins, and other peripheral membrane components such as GRASP55 and GRASP65. Here we find that TMP21, p24a, and gp25L, members of the p24 cargo receptor family, are present in complexes with GRASP55 and GRASP65 in vivo. GRASPs interact directly with the cytoplasmic domains of specific p24 cargo receptors depending on their oligomeric state, and mutation of the GRASP binding site in the cytoplasmic tail of one of these, p24a, results in it being transported to the cell surface. These results suggest that one function of the Golgi matrix is to aid efficient retention or sequestration of p24 cargo receptors and other membrane proteins in the Golgi apparatus.

Introduction

The Golgi apparatus is an organelle on the secretory pathway required for the processing of complex sugar structures on many proteins and lipids, and for the sorting of these proteins and lipids to their correct subcellular destinations (Farquhar and Palade, 1998). It is comprised of a series of cisternal membranes organized into a stacked structure, the first or cis-face receives material from the ER, whereas the final or trans-cisternae packages it for delivery to the plasma membrane and endocytic pathways (Farquhar and Palade, 1998). This structure is maintained by an exoskeleton or Golgi matrix which has been proposed to be required for linking cisternae together into the characteristic Golgi stack structure, and also for vesicle recognition during membrane traffic (Warren and Malhotra, 1998; Seeman et al., 2000).

The most studied components of the Golgi matrix are p115, the GRASP65–GM130 complex, and an integral membrane protein, giantin (Linstedt and Hauri, 1993; Nakamura et al., 1995; Sapperstein et al., 1995; Barr et al., 1997; Shorter and Warren, 1999). GRASP65 was identified in a screen for factors involved in the stacking of cisternae, and later shown to be a specific binding partner of GM130

required to target it to the Golgi (Barr et al., 1998). GM130 in turn is a receptor for p115, required for tethering vesicles to their target membrane (Barroso et al., 1995; Nakamura et al., 1997). More recently, the formation of Golgi stacks from cisternae in vitro was found to require p115, discrete from its membrane fusion function, and giantin (Shorter and Warren, 1999). GM130 and p115 also make interactions with giantin during vesicle docking, and potentially during cisternal stacking (Shorter and Warren, 1999; Dirac-Svejstrup et al., 2000; Lesa et al., 2000). The network of interactions between these proteins may be regulated by the rab GTPases, as both p115 and GM130 have been shown to interact with the active or GTP form of rab1 (Allan et al., 2000; Moyer et al., 2001; Weide et al., 2001). Recruitment of p115 to vesicles destined to fuse with the cis-Golgi is mediated via the interaction with rab1 (Allan et al., 2000), whereas the consequences of rab1 binding to GM130 are unknown. A second GRASP complex, containing GRASP55 and the coiled-coil protein golgin-45, exists in Golgi membranes (Shorter et al., 1999; Short et al., 2001). Like the GRASP65–GM130 complex, this complex also binds a rab GTPase and is required for the maintenance of normal Golgi structure and protein transport (Short et al., 2001).

In addition to the Golgi matrix proteins discussed above, some membrane proteins are of potential importance to Golgi structure. One such group is the medial-Golgi enzymes, found to specifically bind to the Golgi matrix although the mechanism remains uncharacterized (Slusarewicz et al.,

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Figure 1. GRASPs and p24 proteins form a complex in Golgi membranes. (A) GRASP55 complexes were isolated from detergent extracts of Golgi membranes. A 10% Coomassie blue–stained minigel run under nonreducing conditions is shown; loadings corresponding to 1/30 and 29/30 of the total isolated material. Bands corresponding to the specifically interacting proteins seen in all experiments performed ($n = 5$) were excised (arrowheads and bracket), and tryptic digests of the proteins contained therein analyzed by mass spectrometry. Asterisks indicate rat serum albumin confirmed by mass spectrometry and sequencing of tryptic peptides. The total eluted material from a minus antibody control is also shown. (B) Immunoprecipitations (IPs) were performed from 200 μg Golgi membranes with the following antibodies: sheep anti-GRASP55, mouse anti-GRASP65, rabbit anti-golgin-45, and sheep anti-GM130. All IPs were blotted with rabbit antibodies to p24a and gp25L. (C) Blots with the following antibodies were performed as controls: GRASP55 and GRASP65 IPs with rabbit anti-GM130, and rabbit anti–golgin-45; GM130 IPs with sheep anti-GRASP55 and mouse anti-GRASP65; golgin-45 IPs with sheep anti-GRASP55 and mouse anti-GRASP65. The asterisk indicates cross-reactivity to the heavy chain of the sheep anti-GM130 antibody.
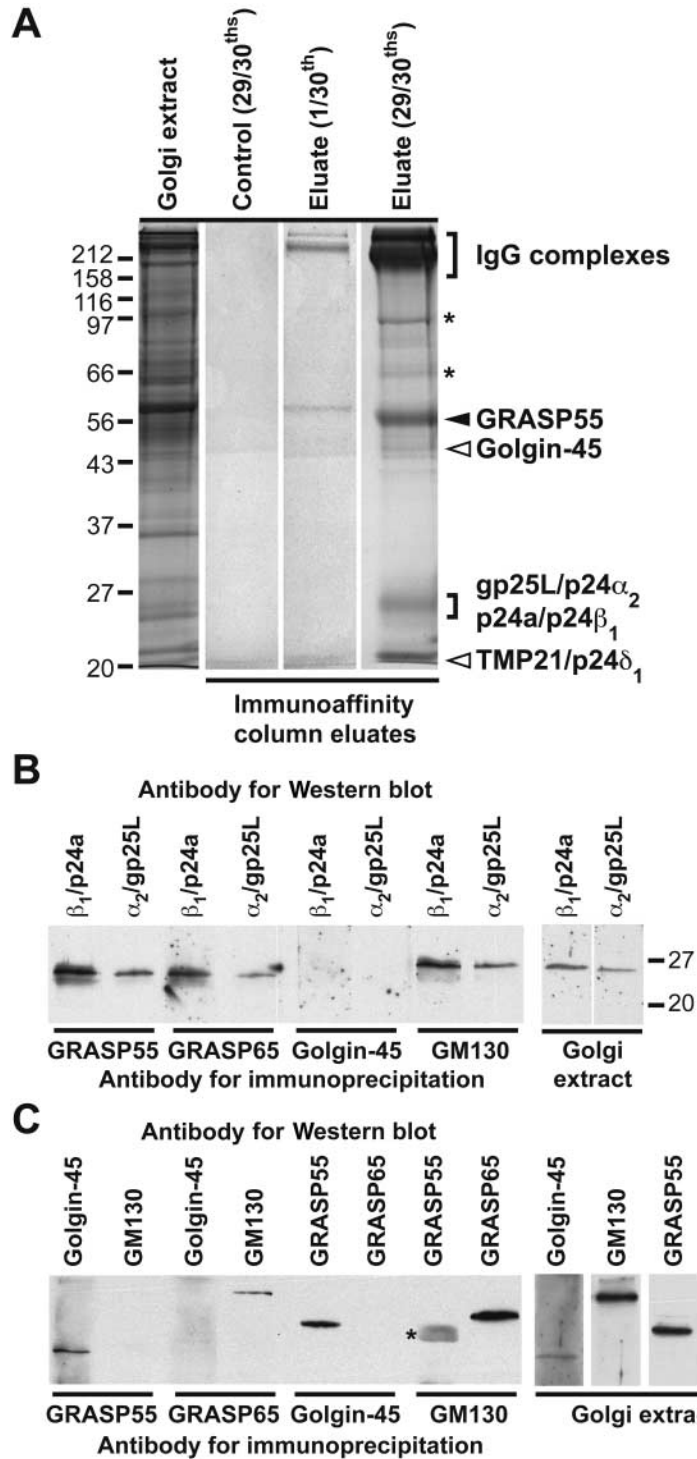
1994). Another is the p24 family of cargo receptors, identified as major transmembrane components of vesicles recycling between the ER and Golgi complex, and as potential structural Golgi proteins in mammalian cells (Wada et al., 1991; Stamnes et al., 1995; Rojo et al., 1997). All p24 proteins share a common structure, with a short cytoplasmic domain containing binding signals for the COP-I and COP-II vesicle coat complexes, plus a lumenal domain with potential secretory cargo binding capabilities (Fiedler et al., 1996; Sohn et al., 1996; Dominguez et al., 1998; Muniz et al., 2000). At least one p24 protein, TMP21, is an essential

gene in mammals, and a heterozygous deletion shows reduced levels of the protein and a partially disrupted Golgi apparatus (Denzel et al., 2000). However, in yeast, all p24 family members can be deleted with little or no effect on protein transport or secretory pathway morphology (Springer et al., 2000).

The drug brefeldin A disrupts the Golgi apparatus and causes Golgi enzymes, but not putative structural components such as the GRASPs and GM130, to be relocated to the ER (Nakamura et al., 1995; Seeman et al., 2000). Under conditions in which the Golgi is first disrupted with brefel-
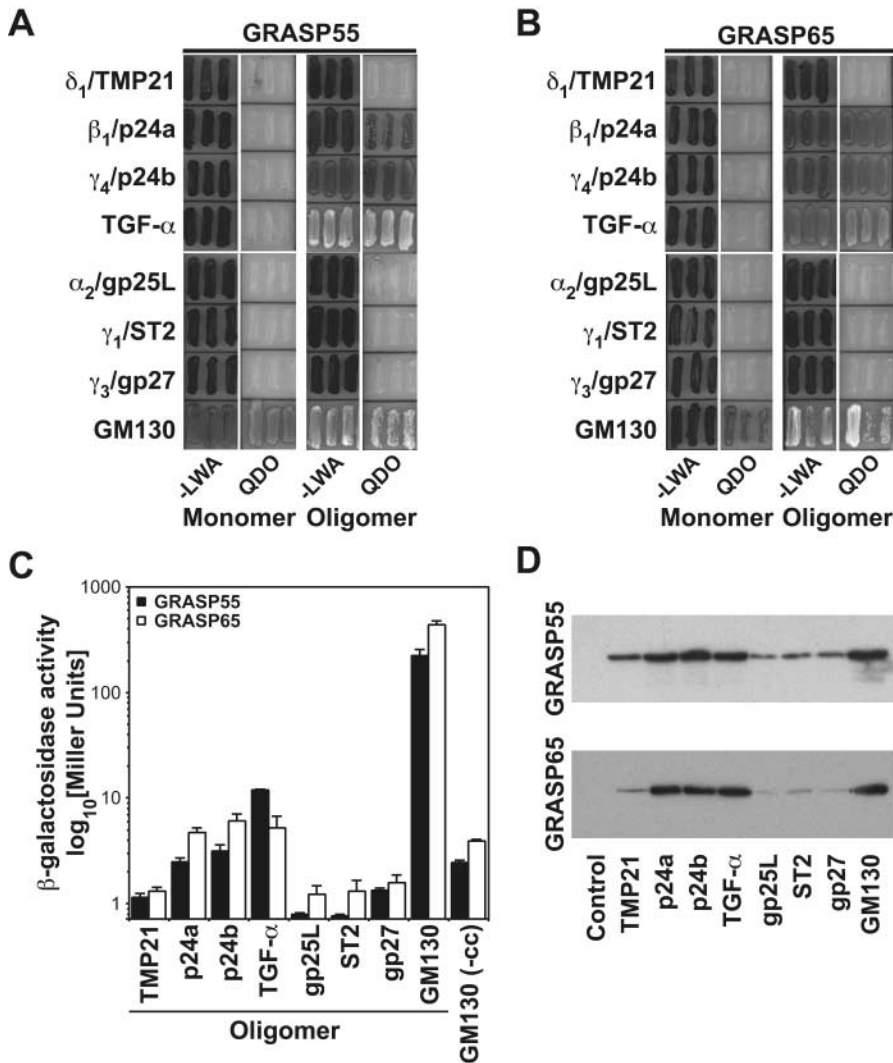
Figure 2. **A direct interaction between some p24 cytoplasmic tails and GRASPs.** (A) GRASP55 and (B) GRASP65 were screened against the cytoplasmic tails of the p24 proteins indicated, TGF-α, and GM130 either with or without a coiled-coil sequence to mediate oligomerization. Interactions were selected by the ability to grow on media lacking leucine, tryptophan, histidine, and adenine (QDO) after initial growth on minimal media lacking leucine, tryptophan and adenine (-LWA). (C) GRASP55 and GRASP65 two-hybrid interactions were measured using the lac-Z reporter gene. GM130 (-cc) is the monomeric form of the signal lacking the coiled coil. (D) Binding of purified GRASP55 and GRASP65 to GST fusion proteins bearing the cytoplasmic tails of the various p24 proteins, TGF-α, and GM130. Background binding of GRASPs to the beads is also shown (control).

din A and then allowed to recover while protein transport from the ER is blocked, a Golgi-like structure forms that lacks Golgi enzymes but contains Golgi matrix proteins (Seeman et al., 2000). However, under the electron microscope, these structures lack some key features of normal Golgi membranes, such as well-defined stacked cisternae. Therefore, the Golgi matrix must interact with additional factors, either integral membrane proteins or specific lipids to organize Golgi membranes. Therefore, we decided to investigate whether GRASPs could provide a link between Golgi matrix components and transported integral membrane proteins.

Results and discussion

Analysis of purified GRASP55 complexes by electrophoresis on nonreducing polyacrylamide gels showed that in addition to GRASP55 and golgin-45, distinct protein bands at 21 and 25 kD were visible (Fig. 1 A). Mass spectrometric tryptic fingerprinting revealed these proteins to be members of the p24 cargo receptor family (Fig. 1 A). Comparing the intensity of the Coomassie blue–stained GRASP55 and p24 protein bands suggests a stoichiometric complex of GRASP55 and TMP21, assuming equal staining efficiency

and allowing for the difference in molecular masses. Western blotting of GRASP55 and GRASP65 immunoprecipitates with antibodies specific to p24a and gp25L showed that they are in complexes with both GRASPs (Fig. 1 B). These are specific complexes, as GRASP55 immunoprecipitates did not contain GRASP65, and vice versa (Fig. 1 C). To find out if GRASPs are present in complexes containing both golgins and p24 proteins, immunoprecipitates of golgin-45 and GM130 were Western blotted for p24a, gp25L, and the GRASPs. This revealed that GM130 immunoprecipitates contained both p24 proteins tested for, whereas golgin-45 immunoprecipitates did not (Fig. 1 B), despite the fact that GRASP55 was easily detected (Fig. 1 C). In summary, GRASP65 is found in a complex containing both GM130 and p24 proteins, whereas GRASP55 may exist in two discrete complexes containing either golgin-45 or p24 proteins.

If the presence of p24 proteins in the purified GRASP complexes is relevant for GRASP function, there should be an interaction between the cytoplasmic domains of these proteins with GRASP55 and GRASP65. To investigate this we used the yeast two-hybrid system, fusing the cytoplasmic domains of different p24 proteins as baits, and full-length GRASP55 and GRASP65 as prey. This screen revealed no

Figure 3.   A GRASP binding motif exists in the p24a cytoplasmic tail. (A) GRASP55 and (B) GRASP65 were screened against sequences corresponding to the cytoplasmic tail of wild-type and point mutant forms of p24a either without (monomer) or with (oligomer) a coiled-coil sequence to mediate oligomerization. Interactions were tested for by selection on QDO after initial growth on minimal media lacking leucine and tryptophan (-LW). (C) GRASP55 and GRASP65 two-hybrid interactions were quantitated using the lac-Z reporter gene. (D) Binding of purified GRASP55 and GRASP65 to GST fusion proteins bearing the wild-type and point mutant forms of the p24 cytoplasmic tail, and GM130. Background binding of GRASPs to the beads is also shown (control).
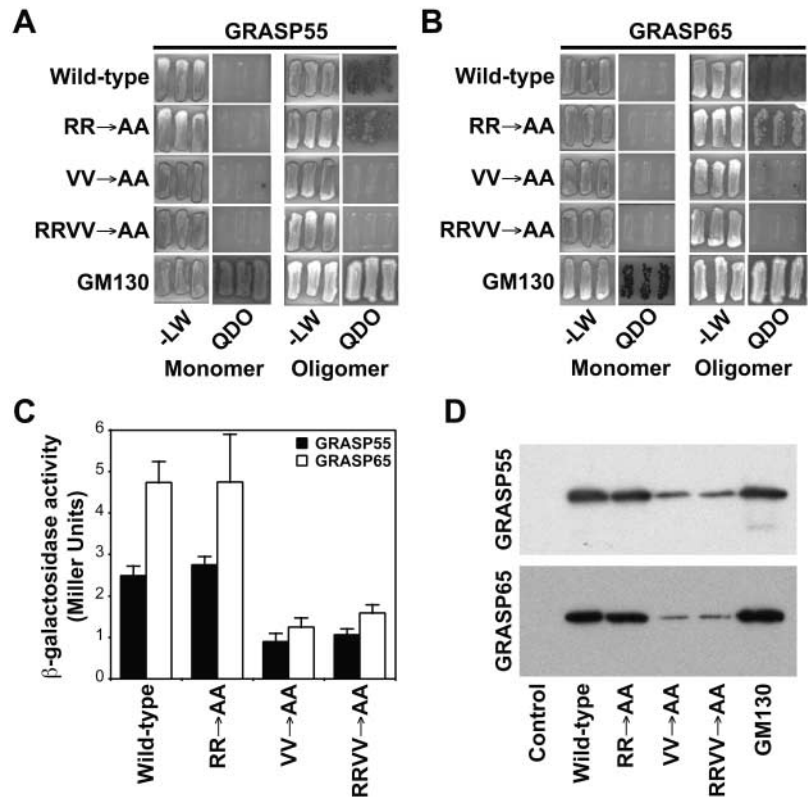
interaction between any of the p24 proteins tested or TGF-α with either GRASP, despite the fact that GM130 showed an interaction with the GRASPs (Fig. 2, A and B). One property these proteins share is that they exist as dimers or oligomers within the cell (Dominguez et al., 1998; Kuo et al., 2000), suggesting that oligomeric state might be important for their recognition by GRASPs. To oligomerize the cytoplasmic domains, a coiled coil was added to the two-hybrid bait constructs. This resulted in positive signals being obtained for both GRASPs with p24a, p24b, and TGF-α, in addition to GM130 in the growth (Fig. 2, A and B) and lacZ reporter gene assays (Fig. 2 C). The coiled-coil domain used gave no interaction with GRASPs on its own (unpublished data), and a number of the p24 proteins tested failed to interact with GRASPs, even with the coiled coil. Purified GRASP55 and GRASP65 were able to bind directly to the cytoplasmic domains of p24a, p24b, TGF-α, and the GM130 COOH terminus immobilized at high density on the surface of agarose beads (Fig. 2 D), consistent with the two-hybrid results. No binding was observed to a glutathione S-transferase (GST)* control, and only weak binding to other p24 proteins (Fig. 2 D). The only discrepancy between the two approaches was that the TMP21 cytoplasmic tail, which did not show an interaction in two-hybrid system bound to GRASP55 in the protein-binding assay. Therefore, GRASP55 and GRASP65 directly interact with the p24a and p24b cytoplasmic domains, and this recognition is apparently dependent on the oligomeric state of the signal. GRASP55 may also be able to bind to the TMP21 cytoplasmic domain.

We then sought to define the nature of the GRASP binding site in the cytoplasmic domain of the p24a protein. Visual inspection of the p24a sequence revealed that it ends with two valine residues, identical to TGF-α and similar to GM130. Mutation of these residues to alanine reduced the interaction of p24a with GRASP55 and GRASP65 in the growth (Fig. 3, A and B) and lacZ reporter two-hybrid assays (Fig. 3, C and D), reminiscent of previous observations that a hydrophobic signal at the extreme COOH terminus of GM130 is needed for it to bind GRASP65 (Barr et al., 1998). Alteration of the adjacent arginine residues had no effect on recognition of the signal by either GRASP, whereas a double mutant behaved identically to the double valine to alanine mutation (Fig. 3, A–C). Interactions were again only observed with oligomeric p24a tail constructs. Protein binding assays confirmed that point mutations reducing the two-hybrid interaction between the GRASPs and the p24a cytoplasmic tail also decrease the interaction between the respective proteins (Fig. 3 D). Therefore, GRASP55 and GRASP65 directly recognize a signal in the cytoplasmic tail of p24a comprised of the two valines at its extreme COOH terminus, and whereas the adjacent arginines are not important, the contribution of other residues to this signal cannot be excluded.

If the interaction of p24 proteins with GRASPs is important for their function, disruption of the GRASP binding signal should perturb their localization or transport. Mutation of the GRASP binding site in p24a caused the protein to appear at the cell surface even at low expression levels, whereas the wild-type protein was efficiently retained inside the cell (Fig. 4 A). Quantitation revealed that surface levels of p24a increased fourfold after inactivation of the GRASP binding site (Fig. 4 B). A double mutation in which the

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*Abbreviations used in this paper: GFP, green fluorescent protein; GST, glutathione S-transferase.

A

Total Surface

Wild-type

FF→AA

RR→AA

VV→AA
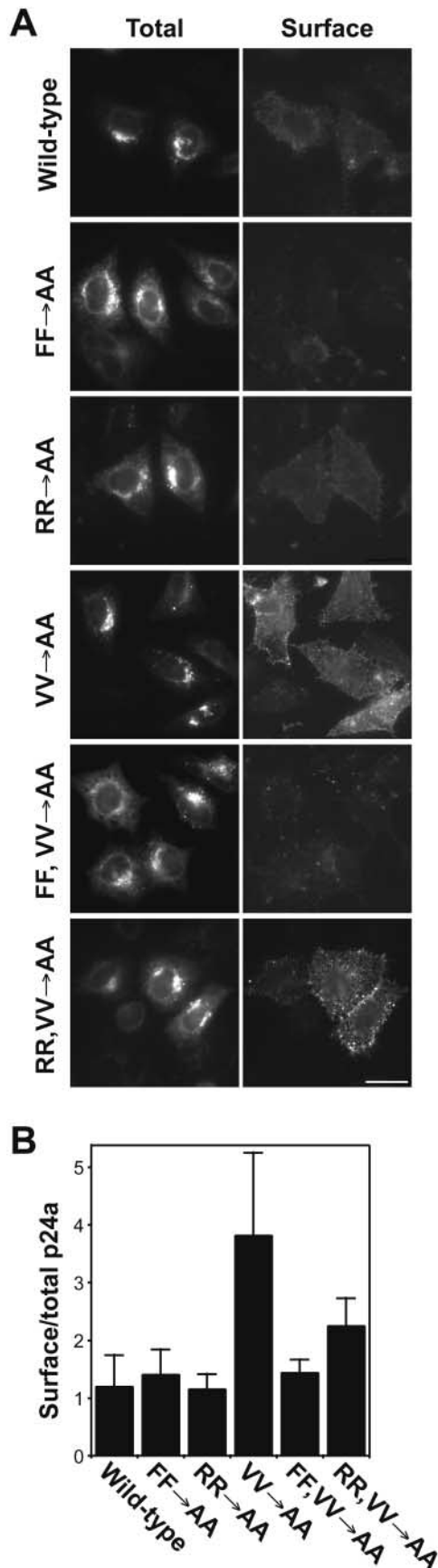
FF, VV→AA

RR, VV→AA

B

Figure 4. **The GRASP binding motif is needed for efficient Golgi retention of p24a.** (A) Cells were transfected for 18 h with plasmids encoding GFP-tagged p24a with either the wild-type cytoplasmic

COP II transport signal was abolished by mutating the two phenylalanines in the p24a cytoplasmic tail to alanine reverted this effect (Fig. 4 A). The COP II binding mutation alone was trapped in the ER and some adjacent vesicular structures as expected (Fig. 4 A) (Fiedler et al., 1996). Mutating the two arginines alone had little effect on the transport of p24a to the cell surface, whereas combining the GRASP binding site and arginine motif mutations gave a reduced level of transport to the cell surface to half that of the GRASP binding mutant alone (Fig. 4, A and B), indicating a transport rather than a retention defect. Therefore, the GRASP binding site in p24a is required for its efficient retention in the Golgi apparatus, but not for p24a transport to the Golgi or retention in the ER. This signal acts at the level of the Golgi, as a COP II transport signal mutant is trapped in the ER, and combining this with the GRASP binding mutant reduces cell surface levels of p24a.

We initially purified p24 proteins in a complex with GRASP55 from Golgi membranes, and subsequent analysis revealed that GRASP65 also binds to p24 proteins. It is known that four p24 proteins, gp25L, p24a, TMP21, and p27, form a stoichiometric complex (Füllekrug et al., 1999), consistent with our observation that three of these p24 proteins are found in association with GRASP55 and GRASP65. The basis for this association appears to be an interaction between the cytoplasmic tail of p24a and the GRASPs. We also found that p26/p24b, which is not present in the gp25L-containing complex, can interact with GRASPs in the two-hybrid system. Interestingly, the GRASP-binding p24 proteins, p24a and p24b, have been shown previously not to interact with COP I (Dominguez et al., 1998). Therefore, our observations may help to explain how complexes containing these two p24 proteins are retained in the Golgi, as GRASP interaction was only seen when the p24 cytoplasmic tail sequences were oligomerized, and no binding was observed with monomeric p24 tails. This may be a mechanism by which GRASPs can distinguish p24 protein complexes in the Golgi apparatus from those present in the ER. A relevant observation in this regard is that complexes containing up to 50 copies of the p24 proteins exist in Golgi membranes (Dominguez et al., 1998). Therefore, specific interaction of Golgi matrix proteins with p24 proteins in post-ER membranes could be an avidity effect, mediated by virtue of the approximately tenfold higher concentration (compared with the ER) of transported proteins, and presumably cargo receptors, in the Golgi apparatus (Quinn et al., 1984).

The transmembrane precursor of TGF-α also binds to GRASP55 in the Golgi, an interaction necessary for its correct localization and transport (Kuo et al., 2000). We show that GRASP65 can also bind to the TGF-α cytoplasmic do-

tail, or mutants abolishing the GRASP binding site, VV→AA, the phenylalanine and arginine motifs, FF→AA and RR→AA, or combinations thereof, FFVV→AA and RRVV→AA. Total fluorescence was visualized with GFP, and cell-surface fluorescence by an antibody to GFP detected with a CY3-coupled secondary antibody. (B) Extent of p24a transport to the cell surface was measured for wild-type and mutant p24a tails constructs. This ratio does not approach unity due to the different dyes used to measure surface and total fluorescence. Mean and standard deviation are plotted, $n = 20$. Bar, 20 μm

main, raising the possibility that GRASPs could bind to a variety of transmembrane cargo proteins via their cytoplasmic domains. Therefore, interaction with Golgi matrix proteins may be a general sequestration mechanism to regulate the transport and retention of integral membrane proteins within the Golgi apparatus.

Materials and methods

Materials
Antibodies to GRASP55, GRASP65, GM130, and golgin-45 were described previously (Barr et al., 1997; Shorter et al., 1999; Short et al., 2001); antibodies were raised in rabbits against the peptides ECFFERVTS-GTKMG from p24a, and KCFIEEIPDETMVI from gp25L. The plasmid encoding green fluorescent protein (GFP)-tagged p24a was a gift from Dr. R. Blum (Institut für Physiologie, Ludwig Maximilians Universität, München, Germany). The Max-Planck-Institute of Biochemistry's Oligonucleotide Synthesis Laboratory (Martinsried, Germany) provided DNA oligonucleotides.

Purification and analysis of GRASP55 complexes
GRASP55 complexes were purified as described previously (Short et al., 2001). Aliquots of total extract and bound protein complexes were analyzed by SDS-PAGE on 10% minigels under nonreducing conditions. Proteins were extracted from Coomassie blue–stained gel slices and digested with sequencing-grade trypsin (Promega) for analysis of the peptide fragments by MALDI-TOF (Reflex III; Bruker) and probability-based database searching (Perkins et al., 1999).

Yeast two-hybrid and protein binding assays
Standard molecular biology techniques were used for the construction of all constructs, and all constructs were confirmed by DNA sequencing (Medigenomix). Double-stranded oligonucleotides corresponding to the cytoplasmic domains of the p24 proteins, TGF-α, and the COOH terminus of GM130 were inserted into the EcoRI and BamHI sites of pGBT9. Coiled coil–containing constructs were made by inserting a PCR-amplified fragment corresponding to amino acids 755–974 of GM130 into the EcoRI site of pGBT9. The full-length rat GRASP55 and GRASP65 constructs in pACT2 were described previously (Shorter et al., 1999). Yeast two-hybrid assays were performed as described previously (Short et al., 2001). Measurements of lacZ reporter gene expression were performed according to the CLONTECH Yeast Protocol handbook, and the results are expressed in Miller units (Miller, 1992). Bacterial hexahistidine–tagged GST fusion protein constructs for p24, TGF-α, and GM130 were constructed by transferring the EcoRI and HindIII fragment containing the various tail sequences from pGBT9 to pGAT2. Hemaglutinin epitope GRASP55 and untagged GRASP65 were cloned into pQE32 for bacterial expression. Proteins were expressed in BL21(DE3) for pGAT2 constructs at 37°C for 3 h, or JM109 for pQE32 constructs at 23°C for 18 h. Fusion proteins were purified on nitrilotriacetic acid–nickel agarose according to the manufacturer's instructions (QIAGEN), dialyzed against PBS, and then stored at −80°C. For each binding assay, 5 µg of GST fusion protein was bound to 10 µl glutathione-Sepharose (Amersham-Pharmacia Biotech). These beads were incubated in the presence of 10 ng/µl GRASP55 or GRASP65 in a total volume of 200 µl PNT (25 mM sodium phosphate, pH 7.2, 100 mM NaCl, 0.1% TX-100) for 1 h at 4°C on a rotating wheel. Beads were washed three times with 400 µl PNT, and then bound proteins eluted with sample buffer for analysis by SDS-PAGE and Western blotting. Bound GRASP proteins were detected using a monoclonal antibody to the hemaglutinin epitope or the 7E10 monoclonal antibody to GRASP65, and GST fusion proteins with a polyclonal antibody to GST.

Microscopy and protein transport assays
GFP-tagged p24a expression constructs were made by amplification of the p24a lumenal and transmembrane domains using primers that replaced the cytoplasmic domain with the required mutant sequence. These fragments were then cloned back into the GFP-p24 expression vector (Blum et al., 2000). HeLa cells plated on glass coverslips were transfected with a plasmid encoding GFP-tagged p24a, or mutants thereof, for 18 h at 37°C. The cells were then fixed with 4% paraformaldehyde in PBS. Cell surface p24a was detected with a sheep antibody to GFP attached to the lumenal domain and a donkey anti–sheep secondary coupled to CY3 (Jackson ImmunoResearch Laboratories), and total p24a by GFP fluorescence. Images were collected using a Zeiss Axioskop-2 with 63× Plan Apochromat objective equipped with a 1300 by 1030 pixel cooled-CCD camera (Princeton Instruments), and Metaview software (Universal Imaging Corp.). The ratio of surface to total measured fluorescence was used to normalize the amount of p24a at the cell surface.

References

Allan, B.B., B.D. Moyer, and W.E. Balch. 2000. Rab1 recruitment of p115 into a cis-SNARE complex: programming budding COPII vesicles for fusion. *Science.* 289:444–448.

Barr, F.A., M. Puype, J. Vandekerckhove, and G. Warren. 1997. GRASP65, a protein involved in the stacking of Golgi cisternae. *Cell.* 91:253–262.

Barr, F.A., N. Nakamura, and G. Warren. 1998. Mapping the interaction between GRASP65 and GM130, components of a protein complex involved in the stacking of Golgi cisternae. *EMBO J.* 17:3258–3268.

Barroso, M., D.S. Nelson, and E. Sztul. 1995. Transcytosis-associated protein (TAP)/p115 is a general fusion factor required for binding of vesicles to acceptor membranes. *Proc. Natl. Acad. Sci. USA.* 192:527–531.

Blum, R., D.J. Stephens, and I. Schultz. 2000. Lumenal targeted GFP, used as a marker of soluble cargo, visualises rapid ERGIC to Golgi traffic by a tubulo-vesicular network. *J. Cell Sci.* 113:3151–3159.

Denzel, A., F. Otto, A. Girod, R. Pepperkok, R. Watson, I. Rosewell, J.J. Bergeron, R.C. Solari, and M.J. Owen. 2000. The p24 family member p23 is required for early embryonic development. *Curr. Biol.* 10:55–58.

Dirac-Svejstrup, A.B., J. Shorter, M.G. Waters, and G. Warren. 2000. Phosphorylation of the vesicle-tethering protein p115 by a casein kinase II-like enzyme is required for Golgi reassembly from isolated mitotic fragments. *J. Cell Biol.* 150:475–488.

Dominguez, M., K. Dejgard, J. Füllekrug, S. Dahan, A. Fazel, J.-P. Paccaud, D.Y. Thomas, J.J.M. Bergeron, and T. Nilsson. 1998. gp25L/emp24/p24 protein family members of the cis-Golgi network bind both COP I and II coatomer. *J. Cell Biol.* 140:751–765.

Farquhar, M.G., and G.E. Palade. 1998. The Golgi apparatus—100 years of progress and controversy. *Trends Cell Biol.* 8:2–10.

Fiedler K., M. Veit, M.A. Stamnes, and J.E. Rothman. 1996. Bimodal interaction of coatomer with the p24 family of putative cargo receptors. *Science.* 273:1396–1399.

Füllekrug, J., T. Suganuma, B.L. Tang, W. Hong, B. Storrie, and T. Nilsson. 1999. Localisation and recycling of gp27 (hp24γ₃): complex formation with other p24 family members. *Mol. Biol. Cell* 10:1939–1955.

Kuo, A., C. Zhong, W.S. Lane, and R. Derynck. 2000. Transmembrane transforming growth factor-α tethers to the PDZ domain-containing, Golgi membrane-associated protein p59/GRASP55. *EMBO J.* 19:6427–6439.

Lesa, G.M., J. Seemann, J. Shorter, J. Vandekerckhove, and G. Warren. 2000. The amino-terminal domain of the Golgi protein giantin interacts directly with the vesicle-tethering protein p115. *J. Biol. Chem.* 275:2831–2836.

Linstedt, A.D., and H.-P. Hauri. 1993. Giantin, a novel conserved Golgi membrane protein containing a cytoplasmic domain of at least 350 kDa. *Mol. Biol. Cell.* 7:679–693.

Miller, J.H. 1992. In a Short Course in Bacterial Genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. 74 pp.

Moyer, B.D., B.B. Allan, and W.E. Balch. 2001. Rab1 Interaction with a GM130 effector complex regulates COPII vesicle cis-Golgi tethering. *Traffic.* 2:268–276.

Muniz, M., C. Nuoffer, H.P. Hauri, and H. Riezman. 2000. The Emp24 complex recruits a specific cargo molecule into endoplasmic reticulum-derived vesicles. *J. Cell Biol.* 148:925–930.

Nakamura, N., C. Rabouille, R. Watson, T. Nilsson, N. Hui, P. Slusarewicz, T.E. Kreis, and G. Warren. 1995. Characterisation of a cis-Golgi matrix protein, GM130. *J. Cell Biol.* 131:1715–1726.

Nakamura, N., M. Lowe, T.P. Levine, C. Rabouille, and G. Warren. 1997. The

vesicle docking protein p115 binds GM130, a cis-Golgi matrix protein, in a mitotically regulated manner. *Cell.* 89:445–455.

Perkins, D.N., D.J. Pappin, D.M. Creasy, and J.S. Cottrell. 1999. Probability-based protein identification by searching sequence databases using mass spectrometry data. *Electrophoresis.* 20:3551–3567.

Quinn, P., G. Griffiths, and G. Warren. 1984. Density of newly synthesized plasma membrane proteins in intracellular membranes II. Biochemical studies. *J. Cell Biol.* 98:2142–2147.

Rojo, M., R. Pepperkok, G. Emery, R. Kellner, E. Stang, R.G. Parton, and J. Gruenberg. 1997. Involvement of the transmembrane protein p23 in biosynthetic protein transport. *J. Cell Biol.* 139:1119–1135.

Sapperstein, S.K., D.M. Walter, A.R. Grosvenor, J.E. Heuser, and M.G. Waters. 1995. p115 is a general vesicular transport factor related to the yeast endoplasmic reticulum to Golgi transport factor Uso1p. *Proc. Natl. Acad. Sci. USA.* 92:522–526.

Seeman, J., E. Jokitalo, M. Pypaert, and G. Warren. 2000. Matrix proteins can generate the higher order architecture of the Golgi apparatus. *Nature.* 407:1022–1026.

Short, B., C. Preisinger, R. Körner, R. Kopajtich, O. Byron, and F.A. Barr. 2001. A GRASP55-rab2 effector complex linking Golgi structure to membrane traffic. *J. Cell Biol.* 155:877–883.

Shorter, J., and G. Warren. 1999. A role for the vesicle tethering protein, p115, in the post-mitotic stacking of reassembling Golgi cisternae in a cell-free system. *J. Cell Biol.* 146:57–70.

Shorter, J., R. Watson, M.E. Giannakou, M. Clarke, G. Warren, and F.A. Barr.

1999. GRASP55, a second mammalian GRASP protein involved in the stacking of Golgi cisternae in a cell-free system. *EMBO J.* 18:4949–4960.

Slusarewicz, P., T. Nilsson, N. Hui, R. Watson, and G. Warren. 1994. Isolation of a matrix that binds medial Golgi enzymes. *J. Cell Biol.* 124:405–413.

Sohn, K., L. Orci, M. Ravazzola, M. Amherdt, M. Bremser, F. Lottspeich, K. Fiedler, J.B. Helms, and F.T. Wieland. 1996. A major transmembrane protein of Golgi-derived COPI-coated vesicles involved in coatomer binding. *J. Cell Biol.* 135:1239–1248.

Springer, S., E. Chen, R. Duden, M. Marzioch, A. Rowley, S. Hamamoto, S. Merchant, and R. Schekman. 2000. The p24 proteins are not essential for vesicular transport in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA.* 97:4034–4039.

Stamnes, M.A., M.W. Craighead, M.H. Hoe, N. Lampen, S. Geromanos, P. Tempst, and J.E. Rothman. 1995. An integral membrane component of coatomer-coated transport vesicles defines a family of proteins involved in budding. *Proc. Natl. Acad. Sci. USA.* 92:8011–8015.

Wada, I., D. Rindress, P.H. Cameron, W.J. Ou, J.J. Doherty 2nd, D. Louvard, A.W. Bell, D. Dignard, D.Y. Thomas, and J.J. Bergeron. 1991. SSR alpha and associated calnexin are major calcium binding proteins of the endoplasmic reticulum membrane. *J. Biol. Chem.* 266:19599–19610.

Warren, G., and V. Malhotra. 1998. The organisation of the Golgi apparatus. *Curr. Opin. Cell Biol.* 10:493–498.

Weide, T., M. Bayer, M. Koster, J.P. Siebrasse, R. Peters, and A. Barnekow. 2001. The Golgi matrix protein GM130: a specific interacting partner of the small GTPase rab1b. *EMBO Rep.* 2:336–341.