Prenylated Rab acceptor (PRA1) is a protein that binds Rab GTPases and the v-SNARE VAMP2. The protein is localized to the Golgi complex and post-Golgi vesicles. To determine its functional role, we generated a number of point mutations and divided them into three classes based on cellular localization. Class A mutants were retained in the endoplasmic reticulum (ER) and exerted an inhibitory effect on transport of vesicular stomatitis virus envelope glycoprotein (VSVG) from the ER to Golgi as well as to the plasma membrane. Class B mutants exhibited a highly condensed Golgi complex and inhibited exit of anterograde cargo from this organelle. Class C mutants exhibited an intermediate phenotype with Golgi and ER localization along with extensive tubular structures emanating from the Golgi complex. There was a direct correlation between the cellular phenotype and binding to Rab and VAMP2. Class A and C mutants showed a significant decrease in Rab and VAMP2 binding, whereas an increase in binding was observed in the class B mutants. Thus, PRA1 is required for vesicle formation from the Golgi complex and might be involved in recruitment of Rab effectors and SNARE proteins during cargo sequestration.

Rab GTPases constitute the largest group within the Ras superfamily. They regulate vesicle trafficking by cycling through active membrane-bound GTP- and inactive cytosolic GDP-bound states. Membrane localization requires modification of the cysteine-containing motif at the carboxyl terminus by prenyl residues. Cycling between the membrane and cytosol is mediated by GDP dissociation inhibitor (GDI), which extracts GDP-bound Rab from the membrane. Activation through guanine nucleotide exchange at the membrane is catalyzed by a guanine nucleotide exchange factor, of which a number have been identified in mammals (1, 2).

Vesicular transport through the secretory pathway undergoes a number of discrete steps each involving budding, membrane remodeling, targeting, docking, and fusion. In ER to Golgi transport, anterograde cargo proteins such as VSVG are selectively transported to the Golgi along with resident ER proteins with the latter retrieved by a salvage process that recognizes distinct motifs within the protein (3). These transport vesicles contain an electron-dense coat assembled under the control of the small GTPase ARF (4). The fungal metabolite brefeldin A (BFA) inhibits ARF activation by stabilizing the inactive ARF-guanine nucleotide exchange factor (GEF) complex (5) resulting in retrograde transport of Golgi content to the ER. At the Golgi complex, cargo proteins destined for post-Golgi locations are sorted into distinct carriers upon exit from the trans face, whereas Golgi resident proteins such as mannosidase II (Man II) are selectively retrieved in COPI-coated vesicles and returned to the cis face (6).

Vesicle fusion is mediated by the core SNARE complex consisting of the vesicle protein VAMP, or syntaxin and SNAP-25 (7). Rab effectors play a regulatory role in this process either through direct interaction with t-SNAREs (8) or recruitment of other SNARE regulatory proteins (9–12). We have isolated previously (13) a Rab and VAMP2-interacting protein called prenylated Rab acceptor or PRA1. PRA1 inhibits the removal of Rab from the membrane by GDI (14) suggesting that recycling of Rab depends on the opposing action of PRA1 and GDI, with PRA1 favoring membrane retention and GDI favoring solubilization. PRA1 has also been shown to interact with a variety of proteins as follows: the presynaptic cytoskeletal matrix protein Piccolo (15); other Ras GTPases (16); the Epstein-Barr Bcl-2 homologue BHRF1 (17); and the SIV envelope protein gp41 (18). However, the functional significance of these interactions remains unclear. To determine the physiological function of PRA1, we generated a number of point mutations and examined their effect on cellular localization, organellar morphology, protein trafficking, and binding to Rab and VAMP2.

MATERIALS AND METHODS

PRA1 Mutagenesis—PRA1 mutations were generated by the PCR amplification using pQE11/HA-tagged PRA1 as template (13). For bacterial expression, the PCR products were inserted between the Aap1 and SpeI sites of a modified pQE10® vector (Qiagen). For mammalian expression, the PRA1 mutants, the constructs were subcloned into the pRESpuro vector (CLONTECH) between the ClaI and EcoRI sites.

Cell Culture and Immunocytochemistry—Chinese hamster ovary (CHO) cells were maintained in minimum Eagle’s medium α (Invitrogen) supplemented with 5% fetal bovine serum and 100 units/ml penicillin, 100 μg/ml streptomycin. For transient expression, 6 × 10⁴ CHO cells were seeded overnight on 12-mm coverslips. Cells were transfected with LipofectAMINE (Invitrogen) and fixed 36–48 h after transfection.
with 4% paraformaldehyde in phosphate-buffered saline for 1 h followed by incubation in blocking buffer (1% bovine serum albumin, 2% normal goat serum, and 0.4% saponin in phosphate-buffered saline) for 15 min. Mouse monoclonal anti-HA (Roche Molecular Biochemicals) and rabbit anti-Man II (a generous gift from Dr. M. Farquhar) antibodies were used as primary antibodies, and after washing washed with 100 mM glycine in phosphate-buffered saline, Alexa 488 or 595-labeled secondary antibodies (Molecular Probes) were used. Coverslips were mounted with SlowFade Light antifade (Molecular Probes). For sensitivity to BFA, cells were treated with 10 μg/ml BFA for 30 min before fixation. For stable transfection, CHO were transfected with pHRESpuRO/HA-PRA1 constructs and clonal lines selected for puromycin resistance. Several clonal lines were isolated, and positive clones were identified by immunocytochemistry.

To examine VSVG-GFP trafficking time course, CHO and PRA1 stable cell lines were seeded on glass coverslips, transfected with pHCMV.GLVSVGΔ448-GFP (19), and 18 h after transfection shifted to 42°C for 5 h. Cycloheximide (20 μg/ml) was added 10 min before shifting back to 37°C, and cells were then fixed after 0, 15, 30, 60, and 90 min. Cells were analyzed by confocal microscopy and were scored by phenotype (ER, Golgi-trans-Golgi network, and plasma membrane) with a minimum of 100 cells scored per coverslip. The values presented were from three independent experiments and verified on at least two clonal lines. For surface labeling, cells were chased for 0, 60, and 120 min and fixed with ice-cold 4% paraformaldehyde for 30 min. The cells were processed for immunocytochemistry as described above except in the absence of saponin, and all steps were performed at 4°C. The monoclonal antibody BWS6G5 directed against the extracellular domain of VSVG (a generous gift from Dr. M. Farquhar) was used to label cell surface-exposed VSVG followed by Alexa 594-labeled secondary antibodies.

**In Vitro Binding Assays**—The PRA1 mutants were subcloned into the modified pGAD424X prey vector (13) at the EcoRI and XhoI sites. They were then co-transformed as described (20) with Rab3A or VAMP2 bait plasmids (13) and grown on Trp and Leu drop-out plates for 3–5 days. The cells were patched onto filter paper, lysed by brief liquid nitrogen treatment, and incubated with 5-bromo-4-chloro-3-indolyl-β-d-galactoside (21). The intensity as well as the time of onset was used to assess the strength of the interactions.

To verify the yeast two-hybrid results, *in vitro* binding studies were performed with recombinant His$_{6}$-tagged Rab3A as described previously (14). GST-tagged VAMP2 (from W. S. Trimble) and His$_{6}$-HA-tagged PRA1 wild type and mutants were purified as described previously (13), except that the PRA1 used in the PRA1/Rab3A binding assay was eluted with 50 mM EDTA. All recombinant proteins were quantified by densitometric analysis of Coomassie Blue-stained gels using bovine serum albumin as a standard. His$_{6}$-tagged, purified PRA1s were covalently cross-linked to CNBr-activated Sepharose 4B (Amersham Biosciences). A typical PRA1-Rab3A binding assay contained 10.8 pmol of His$_{6}$-HAPRA1 cross-linked to CNBr-activated Sepharose 4B and 320 μl of clonal lines selected for puromycin resistance at 10 μg/ml. Several clonal lines were isolated, and positive clones were identified by immunocytochemistry.

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significant accumulation at the plasma membrane at 60 min release. Almost all of the cells exhibited VSVGts045-GFP at the plasma membrane after 90 min. Cells overexpressing the wild type PRA1 showed a slight delay in transport of VSVGts045-GFP to the Golgi complex such that a significant amount of the protein remained in the ER or in dispersed intermediate compartment at 30 min release. However, the bulk of the protein was transported to the Golgi complex at 60 min and eventually reached the plasma membrane after 90 min. In contrast there was a significant delay in transport of VSVGts045-GFP out of the ER in both the class A mutant N70T and C mutant H166A after 15 min release. The protein finally cleared the ER compartment after 30 min with some reaching the plasma membrane at 90 min in the class A mutant N70T. Finally, VSVGts045-GFP was transported rapidly from the ER to Golgi in the class B mutant S76A after 15 min release. However, the protein remained at the Golgi complex with little or no transport to the plasma membrane after 90 min.

We quantified the percentage of cells exhibiting the VSVGts045-GFP signal at the different intracellular compartments after release from the non-permissive temperature. In untransfected CHO, only 10% of the cells retained a detectable VSVGts045-GFP signal in the ER 15 min after release (Fig. 5A). VSVGts045-GFP rapidly exited the Golgi complex (Fig. 5B) and reached the plasma membrane at 90 min release (Fig. 5C). In cells transfected with the wild type PRA1, a significant percentage of the cells showed VSVGts045-GFP retention in the ER at 15 min release (Fig. 5A) and in the Golgi at 60–90 min (Fig. 5B). This resulted in a decrease in the number of cells with detectable VSVGts045-GFP at the plasma membrane at 90 min (Fig. 5C). An even larger fraction of the cells showed ER accumulation of VSVGts045-GFP at 15 min release in the class A mutant N70T. There was also decreased localization of VSG-
VGts045-GFP in the plasma membrane at 90 min. Retention of VSVGts045-GFP in the ER at 15 min release was less severe in the class B mutant S76A (Fig. 5A), but the protein failed to exit the Golgi complex (Fig. 5B) resulting in very low plasma membrane localization at 90 min release (Fig. 5C). This was also seen in the class C mutant H166A except that this mutant also exhibited extensive accumulation in the ER at 15 min release (Fig. 5A). Thus, overexpression of wild type PRA1 has a mild inhibitory effect on transport of the anterograde cargo VSVGts045-GFP to its final destination at the plasma membrane.

Transport out of the ER was significantly affected in the class A mutant N70T, although final localization to the plasma membrane was also affected. This also appeared to be the case for the class C mutant H166A. Finally, exit from the Golgi complex was the major defect in the class B mutant S76A.

Delayed Transport of VSVG to the Cell Surface—To verify that transport of VSVGts045-GFP to the plasma membrane was indeed delayed, we labeled cell surface-exposed VSVG with BW8G65, a monoclonal antibody against the extracellular domain, under non-permeabilized conditions. In all cases, VSVG was undetectable at the cell surface after 5 h of incubation at the non-permissive temperature, thus verifying that the luminal domain was indeed inaccessible to the antibody under our non-permeabilizing conditions (Fig. 6). VSVG was detectable in CHO as discrete patches on the cell surface at 60 min release, and the entire surface was labeled at 120 min release (Fig. 6). Patches of VSVG were detected on the plasma membrane in cells overexpressing wild type PRA1 at 60 and 90 min release. However, the overall intensities were significantly lower than the untransfected CHO at the corresponding time points. Even

![Image of confocal images showing transport of VSVGts045-GFP in CHO cells expressing wild type or mutant PRA1.](http://www.jbc.org/download/jbc.319.36411-20200407.trumbullfig4.jpg)

**Fig. 4.** Transport of VSVGts045-GFP in CHO and cells stably transfected with wild type or mutant PRA1. Representative confocal images of VSVGts045-GFP in CHO expressing wild type or mutant PRA1 (as indicated in left margin). Cells were incubated at the non-permissive temperature for 5 h and shifted to the permissive temperature for time intervals indicated at the top.

![Image of bar graphs showing percentage of cells with VSVGts045-GFP in intracellular compartments and on the cell surface.](http://www.jbc.org/download/jbc.319.36411-20200407.trumbullfig5.jpg)

**Fig. 5.** Percentage of cells with VSVGts045-GFP in intracellular compartments and on the cell surface. A, fraction of cells with VSVGts045-GFP in the ER at 15 min release. B, fraction of cells with VSVGts045-GFP in the Golgi complex at various times in the permissive temperature. C, fraction of cells with VSVGts045-GFP in the plasma membrane at various times in the permissive temperature. B and C, untransfected CHO (○), wild type PRA1 (■), class A mutant N70T (▲), class B mutant S76A (○), and class C mutant H166A (□).
less VSVG reached the cell surface in cells transfected with mutant PRA1s at both 60 and 120 min release with S76A being the worst (Fig. 6). The green fluorescence signal was quite strong in all mutants indicating extensive intracellular accumulation of VSVGts045-GFP. Taken together, the data indicate that overexpression of wild type PRA1 delayed the transport of VSVGts045-GFP to the cell surface. This effect was exacerbated by the mutant PRA1s with S76A showing the highest level of inhibition.

Mutation of PRA1 Affects Binding to Rab3A and VAMP2—We next examined the binding properties of the mutant PRA1s to determine whether this might be an underlying cause of the altered Golgi morphology and inhibition in transport of VSVGts045-GFP. We and others (13, 16, 22) have shown previously that PRA1 binds to Rab GTPases and VAMP2. We first screened the binding properties of the mutant PRA1s in the yeast two-hybrid system by subcloning representatives from each into the prey vector. The resulting vectors were co-transformed into the Y190 tester strain with either Rab3A as a representative Rab GTPase or VAMP2 bait, and the transformants were scored for $\beta$-galactosidase activity on X-gal filter paper and intensity as well as the time of onset used to assess the strength of the interactions.

The data presented in Table II demonstrate that while all class A mutants showed extremely weak or no interaction with either Rab3A or VAMP2, whereas the class B mutants showed increased interaction with both Rab3A and VAMP2 (Table II). The class C mutant H166A showed a weak interaction with VAMP2 but lost its interaction with Rab3A. Thus, the data suggest that interaction of the mutant PRA1 with Rab and VAMP2 may underlie mislocalization of the protein, the altered Golgi morphology, and defect in VSVGts045-GFP transport.

We verified the binding properties of the mutant PRA1s by in vitro pulldown assays using purified recombinant His$_6$-HA-tagged PRA1s, GST-VAMP2, and His$_6$-tagged Rab3A, with Rab3A expressed in yeast to ensure prenyl modification that is essential for PRA1 binding. Because both PRA1 and Rab3A were His$_6$-tagged, purified PRA1s were covalently attached to
Values represent mean and S.E. (type PRA1 (H), N70T (N), or H166A (S)). Immobilized PRA1s was used for Rab3A pulldown, and glutathione-agarose was used to recover GST-VAMP2. Control beads (C), wild type PRA1 (W), N70T (N), S76A (S), or H166A (H). B, binding of Rab3A and VAMP2 to the mutant PRA1s normalized to that of the wild type PRA1. Values represent mean and S.E. (n = 3 with each performed in triplicate).

CNBr-Sepharose beads. We first determined the saturating amount of wild type PRA1 needed to pulldown GDP-bound Rab3A. We then used the EC₅₀ value to determine the amount of Rab3A recovered with the mutant PRA1s by Western immunoblot (Fig. 7A), and we normalized this to the wild type PRA1 (Fig. 7B). In all cases, we detected two immunoreactive Rab3A (Fig. 7A), which probably represents mono- and di-geranylgeranylated species. A similar approach was used to determine VAMP2 binding using glutathione-agarose beads. The class A mutant N70T showed only residual binding to Rab3A and VAMP2 when compared with wild type PRA1 (Fig. 7B). In contrast, there was a 3-fold increase in Rab3A and a 6-fold increase in VAMP2 binding in the class B mutant S76A. A significant reduction in Rab3A and VAMP2 binding was observed in the class C mutant H166A. Thus, there was a direct correlation between cellular phenotype and ability of PRA1 to bind Rab and VAMP2. Loss of Rab and VAMP2 binding in the class A mutants correlated with retention of the mutant PRA1 in the ER, whereas enhanced binding to both in the class B mutants correlated with retention of the mutant protein to the ER, which interferes with proper functioning through oligomerization with endogenous protein. Defect at later transport steps might arise from reduced exit of endogenous PRA1 and other components of the secretory machinery from the ER. This is likely to be the same underlying mechanism in the class C mutants although at lower severity. In the class B mutants, the increased binding to Rab and VAMP2 by the class B mutant S76A might affect either recycling or functional interaction with Rab effector molecules that ultimately lead to decreased transport out of the Golgi apparatus.

Our data suggest a direct involvement of PRA1 in anterograde transport to and out of the Golgi complex. Aside from its interaction with Rab GTPases (13, 16, 22, 23, 28), PRA1 also binds GDI (14) and SNAREs (13, 30), which is consistent with its assigned transport function based on interaction of the yeast homologue Yip3p with proteins in the secretory pathway (31, 32). The ability of PRA1 to form at least a dimer (13, 32) suggests that it might recruit Rab GTPases and their effectors to membrane domains along with functional SNAREs to ensure proper sequestration in the budding vesicle. ER to Golgi transport was affected in the class A mutants possibly due to incorporation of the mutant protein to the ER, which interferes with proper functioning through oligomerization with endogenous protein. Defect at later transport steps might arise from reduced exit of endogenous PRA1 and other components of the secretory machinery from the ER. This is likely to be the same underlying mechanism in the class C mutants although at lower severity. In the class B mutants, the increased binding to Rab GTPases may either interfere with functional interaction with their effectors or their retrieval from membranes. Exit of proteins such as VAMP2 might also be inhibited thereby affecting the fusion of secretory vesicles with the plasma membrane.

The localization of the mutant PRA1 to the Golgi complex is consistent with its effect on exit of anterograde cargo from this compartment. The condensed Golgi morphology in the class B mutant S76A indicates that PRA1 is also involved in trafficking through this compartment. The mutation significantly inhibited transit of the anterograde cargo VSVG<sup>ts045</sup>-GFP through the Golgi and its subsequent incorporation into the plasma membrane. Because PRA1 is known to interact with multiple Rab GTPases (13, 16, 22, 23, 28), it is likely that Golgi-localized Rab GTPases, which in yeast are required for formation of transport vesicles out of the Golgi apparatus (29), are affected. We surmise that the increased binding of Rab and VAMP2 by the class B mutant S76A might affect either recycling or functional interaction with Rab effector molecules that ultimately lead to decreased transport out of the Golgi apparatus.

**DISCUSSION**

PRA1 is a protein that is localized primarily to the Golgi complex (14) and post-Golgi compartments (15). We have shown here that point mutations of PRA1 can alter its cellular localization as well as vesicle trafficking. The class A mutants displayed an ER phenotype along with a significant decrease in binding to both Rab3A and VAMP2. A number of factors are known to affect protein transport from the ER to Golgi complex. Efficient exit of PRA1 from the ER has been shown to be dependent on the DXEE motif at the carboxyl terminus (23). Because this motif remained intact in the class A mutants, it is unlikely that a defect in the DXEE-mediated mechanism is the underlying cause of ER retention. Protein folding or oligomerization is another factor. Although it is possible that all class A point mutations might bring about complete denaturation of the protein, we believe that it is more plausible that functional interaction of PRA1 with Rab is required for anterograde transport. A number of studies have shown involvement of Rab GTPases in vesicle formation at various stages of the secretory and endocytic pathways (24–27). Such loss of Rab binding would affect either membrane recruitment of Rab or Rab effectors leading to inhibition of transport. This would be consistent with both accumulation of recycling Man II-containing vesicles and delay in ER to Golgi transport of the anterograde cargo VSVG in these cells. Thus, the significant decrease in binding to Rab and VAMP2 implies that functional interaction of PRA1 with these proteins is tightly linked to vesicular transport. It remains to be seen whether the effect on ER to Golgi transport is solely due to PRA1 or interference of PRA2, which is the isoform localized exclusively to the ER compartment (23).

The condensed Golgi morphology in the class B mutant S76A is solely due to PRA1 or interference of PRA2, which is the isoform localized exclusively to the ER compartment (23).
4. Rothman, J. E., and Wieland, F. T. (1996) Science 272, 227–234
5. Peyroche, A., Antonny, B., Robineau, S., Acker, J., Cherfils, J., and Jackson, C. L. (1999) Mol. Cell 3, 275–285
6. Martinez-Menarguez, J. A., Prekeris, R., Oorschot, V. M., Scheller, R., Slot, J. W., Geuze, H. J., and Klumperman, J. (2001) J. Cell Biol. 155, 1213–1224
7. Chen, Y. A., and Scheller, R. H. (2001) Mol. Cell 3, 275–285
8. Martinez-Menarguez, J. A., Prekeris, R., Oorschot, V. M., Scheller, R., Slot, J. W., Geuze, H. J., and Klumperman, J. (2001) J. Cell Biol. 155, 1213–1224
9. Chen, Y. A., and Scheller, R. H. (2001) Nat. Rev. Mol. Cell. Biol. 2, 98–106
10. McBride, H. M., Rybin, V., Murphy, C., Giner, A., Teasdale, R., and Zerial, M. (1999) Cell 10, 1873–1889
11. Seals, D. F., Eitzen, G., Margolis, N., Wickner, W. T., and Price, A. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 9402–9407
12. Nielsen, E., Christoforidis, S., Uttenweiler-Joseph, S., Miaczynska, M., Dewitte, F., Wilm, M., Hoflack, B., and Zerial, M. (2000) J. Cell Biol. 151, 601–612
13. Martincic, I., Peralta, M. E., and Ngsee, J. K. (1997) J. Biol. Chem. 272, 26991–26998
14. Hutt, D. M., Da-Silva, L. F., Chang, L. H., Prosser, D. C., and Ngsee, J. K. (2000) J. Biol. Chem. 275, 18511–18519
15. Fenster, S. D., Chung, W. J., Zhai, R., Cases-Langhoff, C., Voss, B., Garner, A. M., Koempf, U., Kindler, S., Gundelfinger, E. D., and Garner, C. C. (2000) Neuron 35, 203–214
16. Figueroa, C., Taylor, J., and Vojtek, A. B. (2001) J. Biol. Chem. 276, 28219–28225
17. Li, Y. Y., Shih, H. M., Liu, M. Y., and Chen, J. Y. (2001) J. Biol. Chem. 276, 27354–27362
18. Evans, D. T., Tillman, K. C., and Desruessers, R. C. (2002) J. Virol. 76, 327–337
19. Presley, J. F., Cole, N. B., Schroer, T. A., Hirschberg, K., Zaal, K. J., and Lippincott-Schwartz, J. (1997) Nature 389, 81–85
20. Gietz, D., Steigerwald, G., and Schiestl, R. H. (1992) Nucleic Acids Res. 20, 425
21. Bartel, P., Chien, C. T., Sterngranz, R., and Fields, S. (1993) BioTechniques 14, 920–924
22. Bucci, C., Chiariello, M., Lattero, D., Maiorano, M., and Bruni, C. B. (1999) Biochem. Biophys. Res. Commun. 258, 657–662
23. Abdul-Ghani, M., Gougeon, P. Y., Prosser, D. C., Da-Silva, L. F., and Ngsee, J. K. (2001) J. Biol. Chem. 276, 6225–6233
24. Riederer, M. A., Soldati, T., Shapiro, A. D., Lin, J., and Pfeffer, S. R. (1994) J. Cell Biol. 125, 573–582
25. Wilson, B. S., Ndufer, C., Meinkoth, J. L., McCaffery, M., Feramisco, J. R., Balch, W. E., and Farquhar, M. G. (1994) J. Cell Biol. 125, 557–571
26. Mammoto, A., Newell, J., Merrice, N., Osborne, A., West, M., and Smythe, E. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 1257–1261
27. Bader, G. D., Donaldson, I., Wolting, C., Ouellette, B. F., Pawson, T., and Hogue, C. W. (2001) Mol. Cell. Biol. 21, 1982–1992
28. Ito, T., Tashiro, K., Muta, S., Ozawa, R., Chiba, T., Nishizawa, M., Yamamoto, K., Kuhara, S., and Sakaki, Y. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 1143–1147
29. Schwickowski, B., Uetz, P., and Fields, S. (2000) Nat. Biotechnol. 18, 1257–1261
30. Bader, G. D., Donaldson, I., Wolting, C., Ouellette, B. F., Pawson, T., and Hogue, C. W. (2001) Nucleic Acids Res. 29, 242–245
31. Cao, H., Thompson, H. M., Krueger, E. W., and McNiven, M. A. (2000) J. Cell Sci. 113, 1999–2002
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