Prenylated Rab Acceptor Protein Is a Receptor for Prenylated Small GTPases*

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Localization of Ras and Ras-like proteins to the correct subcellular compartment is essential for these proteins to mediate their biological effects. Many members of the Ras superfamily (Ha-Ras, N-Ras, TC21, and RhoA) are prenylated in the cytoplasm and then transit through the endomembrane system on their way to the plasma membrane. The proteins that aid in the trafficking of the small GTPases have not been well characterized. We report here that prenylated Rab acceptor protein (PRA1), which others previously identified as a prenylation-dependent receptor for Rab proteins, also interacts with Ha-Ras, RhoA, TC21, and Rap1a. The interaction of these small GTPases with PRA1 requires their post-translational modification by prenylation. The prenylation-dependent association of PRA1 with multiple GTPases is conserved in evolution; the yeast PRA1 protein associates with both Ha-Ras and RhoA. Earlier studies reported the presence of PRA1 in the Golgi, and we show here that PRA1 co-localizes with Ha-Ras and RhoA in the Golgi compartment. We suggest that PRA1 acts as an escort protein for small GTPases by binding to the hydrophobic isoprenoid moieties of the small GTPases and facilitates their trafficking through the endomembrane system.

Ras proteins (Ha-Ras, K-Ras, N-Ras, TC21, and Ras1/2) regulate cell growth in eukaryotic cells, and perturbation of signaling pathways by mutation and constitutive activation of Ras proteins is a common occurrence in a wide spectrum of human tumors (1). In addition to regulating cell proliferation, Ras proteins also regulate differentiation, cell death, and cell survival (1). The Ras proteins are members of a large superfamily of low molecular weight GTP-binding proteins, which include members that regulate the actin cytoskeleton (Rho and Rac), vesicle trafficking (Rabs), and nuclear transport (Ran).

The biological activity of Ras proteins is controlled by a regulated GTP/GDP cycle (2). The GTP-bound, or active, Ras relays signals to downstream effector proteins. In the case of Ha-Ras, numerous effector proteins have been described, including serine/threonine kinases (c-Raf, A-Raf, and B-Raf), lipid kinases (type I phosphatidylinositol 3-kinase), and guanine nucleotide dissociation stimulators for Ral (Ral GDS1 and RGL) (1). Activation of Ras effector pathways leads to proliferation, differentiation, cell death, and cell survival. Which biological outcome predominates is somewhat of a mystery but seems to depend on cell type and the coordinate integration of signaling pathways activated and/or inhibited within a cell.

Members of the Ras superfamily are subject to post-translational modifications. The spectrum of modifications depends on the composition of the carboxyl terminus. In the case of Ha-Ras, the protein is subject to prenylation, proteolysis, carboxyl-methylation, and S-acylation/palmitoylation (3). Prenylation is the covalent attachment of farnesyl or geranylgeranyl isoprenoids at or near the carboxyl terminus of the GTPase. For Ras family members, prenylation occurs at a conserved cysteine in the carboxyl-terminal motif termed the CAAX box, where C represents cysteine, A is an aliphatic amino acid, and X is usually Ser, Met, Gln, or Leu (3, 4). Whereas Ha-Ras, N-Ras, K-Ras4B, and yeast Ras2 are farnesylated, most other small GTPases are geranylgeranylated (5). RhoB can be either farnesylated or geranylgeranylated (5). The enzymes that catalyze prenylation, farnesyltransferase and geranylgeranyltransferase-I and II, reside in the cytoplasm.

Prenylation is followed by proteolysis of the carboxyl-terminal tripeptide and methylation of the newly generated carboxyl-terminal amino acid in the endoplasmic reticulum (ER) (3, 4). Some Ras proteins, including Ha-Ras, N-Ras, yeast Ras2, and Rac1 are further modified by S-acylation (palmitoylation) of cysteine(s) located near the carboxyl terminus of the protein (3, 4). S-acylation is likely to occur in the ER, since Erf2, a novel integral membrane protein required for palmitoylation of Ras in yeast, is localized to the ER (6). Other proteins, such as K-Ras4B, that are not subject to S-acylation have a polybasic stretch of amino acids, also located near the CAAX box, within the 20-amino acid hypervariable domain of the Ras proteins. The combination of two signals, prenylation and S-acylation or prenylation and a polybasic stretch of amino acids, are required for plasma membrane targeting of Ras proteins. Ras proteins (Ha-Ras, N-Ras) transit through the ER and Golgi and finally reach the plasma membrane via exocytic transport vesicles (7, 8). The trafficking of K-Ras4B, however, is unlikely to involve the Golgi compartment; rather, once prenylated and further modified in the ER, the trafficking of K-Ras4B to the plasma membrane involves a microtubule network (9, 10).

As a consequence of the post-translational modifications, Ras is membrane-localized. In mammalian cells, membrane localization of Ras is necessary for transformation and differentiation. Membrane localization of Ras creates a docking site for effectors, including the Raf kinases and phosphatidylinositol

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1 The abbreviations used are: GDS, guanine nucleotide dissociation stimulator; ER, endoplasmic reticulum; GDI1, guanine nucleotide dissociation inhibitor 1; GST, glutathione S-transferase; PRA1, prenylated Rab acceptor protein; SOS, son of sevenless; GFP, green fluorescent protein; eGFP, enhanced GFP; PCR, polymerase chain reaction; IP, immunoprecipitation.
3-kinase. Membrane localization of Raf may also facilitate
and/or enable the subsequent events needed for activation of its
catalytic activity, including interactions with phospholipids
and phosphorylation by serine/threonine and tyrosine kinases.
Membrane localization of phosphatidylinositol 3-kinase may
accelerate its enzymatic activity by localizing the enzyme to a
site rich in substrate lipids. In Saccharomyces cerevisiae, mem-
brane localization of Ras is required for the transient increase
in CAMP in response to glucose addition but is not required for
Ras to fulfill its essential function in the cell (11).

A number of studies have demonstrated that prenylation of
Ras is a critical determinant in initiating membrane attach-
ment. Without prenylation, the subsequent processing events
do not take place, and the Ras proteins remain soluble (3, 4).
In addition to initiating the process that ultimately leads to
membrane attachment of the small GTPases, farnesylation may also
contribute to protein-protein interactions. Studies using an
activated Ras2 protein mutated at the farnesylation site may have
reported a decreased interaction between the mutant Ras2 and
its effector in yeast, adenylcyclase, suggesting that the far-
nesyl moiety itself or the conformation imposed on the protein
by modification may play a role in mediating productive Ras-
effectors interactions (12). In addition, in vitro studies have
suggested that farnesylation of Ras may be needed for SOS to
promote nucleotide exchange (13). Finally, although farnesyla-
tion by itself is not sufficient to target proteins to the plasma
membrane, this modification may contribute to stable mem-
brane binding (23). Taken together, these observations suggest
that prenylation is crucial for Ras function.

Using a yeast two-hybrid screen, we identified Ha-Ras-inter-
acting proteins (14). We demonstrate in this report that one of
these proteins, Rip69, interacts with multiple members of the
Ras superfamily, including Ha-Ras, RhoA, and TC21, and that
the interaction of Rip69 with the small GTPases requires their
post-translational modification by prenylation. Rip69 encodes
residues 20–186 of PRA1, prenylated Rab acceptor protein.
PRA1 was identified as a Rab-interacting protein in yeast
two-hybrid screens, and PRA1 was reported to interact with
Rab proteins but not other members of the Ras superfamily
(15–17). In addition to binding Rab proteins, PRA1 also asso-
ciates with GDP dissociation inhibitor 1 (GD11) and the v-
SNARE VAMP2 (15, 16). In vitro PRA1 can block the ability of
GD11 to extract Rab3A from membranes, and therefore the op-
posing actions of GD11 and PRA1 on Rab proteins may influ-
ence the membrane localization of the Rab proteins (16). Further,
Rab3A can displace VAMP2 from VAMP2-PRA1 complexes, so
displacement of PRA1 from VAMP2 by Rab may also facilitate
v-SNARE/t-SNARE interactions and vesicle fusion (15).

With this report, we demonstrate that PRA1 is not a specific
partner for Rab proteins but also partners with other members
of the Ras superfamily. The association of Ras family members
with PRA1 requires their post-translational modification by
prenylation. Further, our results suggest that an isoprenoid
moiety, either farnesyl or geranylgeranyl, is the critical recog-
nition target for PRA1. The interaction between Ha-Ras and
PRA1 and between RhoA and PRA1 is observed in vivo by
co-precipitation experiments and co-localization in mamma-
lian cells. The co-localization of Ha-Ras and PRA1 and RhoA
and PRA1 to the Golgi compartment suggests that PRA1 may play
a role in facilitating the trafficking of small GTPases through
the endomembrane system. In addition, we have cloned the yeast
PRA1 gene and demonstrate that, like its higher eukaryotic
counterpart, the yeast PRA1 protein also interacts with multiple
small GTPases, and the interaction requires prenylation of the
small GTPase. Thus, PRA1 is predicted to play a conserved role
in the biology of small GTPases in all eukaryotic cells.

EXPERIMENTAL PROCEDURES

Plasmids—Rip69 was identified in a yeast two-hybrid screen of a 9.5- and
10.5-day short insert site, random primed mouse embryo library using
LexA-Ha-RasV12 as bait (14). Rip69 encodes residues 20–186 of
PRA1 fused in frame to a nuclear localized VP16 protein in pVP16.
pleXa-Ha-Ras V12, pLexA-RhoA63, pLexA-TC21, and pLexA-Rap1a
have been described (14, 19); these plasmids all express the full-length
small GTPases fused in frame to the LexA DNA binding domain
in pLexA or pLexA-Adh. pLexA-Ha-RasV12K6 and pLexA-Ha-RasV12
S181 S184 were constructed by inserting a BamHI–EcoRI fragment
generated by polymerase chain reaction (PCR) using a Ha-RasV12
template, Expand polymerase (BM), and the following forward and
reverse primers, respectively: 5′-CGGAATTCATGCAGGATATAAACG-
CAGCGAG-3′ and 5′-CGGATCCCTACTCTCTCTCTTCCTTTGAGCAC-
CATTTCGCGC-3′ or 5′-CGGAATTCTGGCGCGGCGCAATTATCCACCGCCCTT-
AGCTCATGAGCGGCCGGCCAC-3′. pLexA-GFP and pLexA-GFP-
CAAX were generated by PCR using pcS2 + eGFP BIV as template and
the following forward and two reverse oligonucleotide primers:
5′-CGGATCCCGCACAAGGGCGAGGCGGAG3′ and 5′-CGGAT-
CTTGGCGCGCGCAATTATCCACCGCCCTTGGTGAGCAAGGGCGAG-
CACTTGCAGCT-3′. pLexA-GFP-Cter and pLexA-GFP-Nter were gener-
ated after PCR was sequenced after cloning. Ha-RasV12 and RhoA63 were expressed as fusion proteins
in HEK293 cells to glutathione S-transferase (GST) in the expression
vector pEG-BX. Full-length yeast PRA1 was isolated from yeast
genomic DNA using PCR with gene-specific oligonucleotide primers
with appropriate restriction sites for subcloning into pCS3 +
MT and sequenced.

Yeast Two-hybrid Assays—The S. cerevisiae strain L40 was trans-
formed with plasmids expressing fusions to the LexA DNA binding
domain in pLexAde or pLexAdeNot and with plasmids expressing fusions
to a nuclear localized VP16 acidic activation domain in pVP16
in parallel to the LexA reporter. The activation of the LexA reporter
was assessed after PCR was sequenced after cloning the yeast
DNA. Insertions generated after PCR were screened after
cloning. Ha-RasV12 and RhoA63 were expressed as fusion proteins
in HEK293 cells to glutathione S-transferase (GST) in the expression
vector pEG-BX. Full-length yeast PRA1 was isolated from yeast
DNA using PCR with gene-specific oligonucleotide primers
with appropriate restriction sites for subcloning into pVP16. Full-
length mouse PRA1 was isolated from yeast DNA as described
using gene-specific oligonucleotide primers. The full-length mouse PRA1 DNA was subcloned into pcS3 +
MT and sequenced.

In Vitro Protein-Protein Interactions—HEK293 cells were trans-
formed with expression vectors for GST-Ha-RasV12 and GST, pEBG3X-
Ha-RasV12, or pEBG, respectively. Processed and unprocessed GST-
Ha-Ras proteins were prepared by Triton X-114 partitioning essentially
as described (20) followed by purification of the processed and unproc-
essed GST-Ras proteins on glutathione-Sepharose resin. VP16-Rip69
and VP16 proteins were prepared in the presence of [35S]methionine by
coupled in vitro transcription-translation (TNT, Promega) of the appro-
ropriate expression vector, pRip69 or pVP16. The labeled Rip69 and VP16
proteins were incubated with glutathione-Sepharose bound processed
GST-Ras protein, unprocessed GST-Ha-Ras protein, or control GST
protein. After incubation for 1 h at 4 °C, the resin was washed three
times in Triton IP buffer. The GST-fusion proteins bound to glutathione-
Sepharose beads were analyzed by Western blotting with anti-GST
and anti-Myc tag epitope antibodies.
The clonal hybridoma supernatant harvested from 9E10 cells (My epi
tag antibody) or with anti-GST antisera, washed in phosphate-buffered
saline, and then incubated with Cy3-conjugated secondary antibodies
(Jackson ImmunoResearch Laboratories, Inc.). After washing, the cov-
slips were mounted on glass slides with ProLong (Molecular Probes,
Inc., Eugene, OR). The cells were viewed on a Noran OZ laser-scanning
confocal microscope, and the data were collected in a UNIX-based
Silicon Graphics INDY R5000 work station or viewed on a Nikon
deconvolution microscope. The figures were prepared using Photoshop
5.5 (Adobe), and the relative intensities have been adjusted to visualize
spatial overlap.

RESULTS

Rip69 was identified as a Ha-Ras-interacting protein in a yeast
two-hybrid screen of a mouse embryo CDNA library (14). Among the
clones recovered in this screen were those that required an intact Ras
effector domain for the Ras-Rip interaction. The proteins encoded by
these isolates, which include Raf family members (14), the catalytic subunit of phosphati-
dylinositol 3-kinase (21), and guanine nucleotide exchange fac-
tors for Rap, were subsequently shown to be direct downstream
targets of Ras. In addition, isolates were recovered that associ-
ated with Ras proteins mutated in the effector domain; among these was Rip69. The lack of a requirement for an intact
Ras effector domain for the Ras-Rip protein-protein interaction suggests that the Rip69 isolate does not encode an effector
of Ras function but is more likely to be either a regulator of Ras or a protein involved in the trafficking of Ras to the plasma
membrane. Rip69 encodes residues 20–186 of prenylated Rab
acceptor protein PRA1.

Three published reports demonstrate that PRA1 interacts
with members of the Rab family of GTPases, including Rab1,
Rab3A, Rab5, and Rab6 (15, 17, 22). Among small GTPases,
PRA1 is thought to be a relatively specific partner for Rab pro-
teins because, in two reports, PRA1 failed to interact with other
members of the Ras superfamily, Ras, Rhe, and Rac (15, 17).

Because we had identified PRA1 in a two-hybrid screen with
Ha-Ras, we investigated the ability of the full-length mouse
PRA protein, PRA1, to interact with RhoA and TC21. In addition
to interacting with Ha-Ras, PRA1 interacts with TC21 and
RhoA, Fig. 1. These results indicate that the interaction of
PRA1 with small GTPases is not specific to Rab proteins; in-
stead, PRA1 interacts with multiple members of the Ras super-
family, including Ha-Ras, RhoA, and TC21. Although Rap1a
does not interact with PRA1 in a yeast two-hybrid experiment,
Rap1a does interact with PRA1 in vivo in mammalian cells (as described below and in Fig. 2). Perhaps the
LexA-Rap1a fusion protein is not prenylated efficiently in
yeast, or it may not localize to the nucleus as efficiently as the
unprenylated form.

To confirm our yeast two-hybrid experiments, we examined
the association of Ha-Ras and RhoA with PRA1 in vivo. As
shown in Fig. 2, A and B, Ha-Ras and PRA1 and RhoA and
PRA1 associate in HEK293 cells overexpressing these proteins.
In addition, because we did not observe an association of PRA1
with Rap1a in yeast, we examined the interaction in mammalian
cells. As shown in Fig. 2C, Rap1a and PRA1 co-associate in vivo in
HEK293 cells. Thus, PRA1 associates with multiple members
of the Ras superfamily in vivo.

Ha-Ras proteins undergo a series of post-translational modifi-
cations: 1) attachment of an isoprenoid farnesyl moiety to a
cysteine located 4 residues from the carboxyl terminus of the
protein; 2) removal of the carboxyl-terminal tripeptide; 3)
methylation of the carboxyl group of the farnesylated cysteine; and,
4) palmitoylation of two cysteines near the carboxyl termi-
minus of the protein, Cys181 and Cys184 (3, 4). To investigate
the requirement for post-translational modification of Ha-Ras
on the association of PRA1 with Ha-Ras, we examined the
association of PRA1 with mutant Ras proteins missing key
post-translational modifications (Fig. 3). Ha-RasV12CAAX is
an activated Ras that lacks all post-translational modifica-
tions. Ha-RasV12K6 is an activated Ras with polylysine sub-
stituted for Ser189, the last residue of the CAAX box; this
mutant Ras is palmitoylated but not farnesylated (23). Ha-
RasV12 S181 S184 is an activated Ras that is farnesylated,
proteolyzed, and methylated but not palmitoylated (23).
PRA1 associates with Ha-RasV12 S181 S184 but not Ha-
RasV12CAAX or Ha-RasV12K6 (Fig. 3). Thus, Ras proteins
that are not palmitoylated are still capable of associating with
PRA1, indicating that palmitoylation of Ras is not required for
the association of the proteins. Ras proteins that are not far-
essylated or are not modified in any way do not interact with
PRA1, suggesting that farnesylated Ras is the binding partner
for PRA1.

In order to determine whether the modified carboxyl-termi-
nal tetrapeptide of Ha-Ras is both necessary and sufficient for
recognition by PRA1, we assessed the association of PRA1 with
GFP-CAAX, a GFP protein to which only the last 4 amino acids
of Ha-Ras, CVLS, were added. This tetrapeptide is both neces-
sary and sufficient for farnesyltransferase and geranylgeranyl
transferase I to recognize and prenylate Protein A and G 

a, which have been mutated to end in a CAAX sequence (24, 25).
Likewise, we expect that in yeast this tetrapeptide will be
recognized and prenylated by yeast farnesyltransferase and probably proteolyzed and methylated because mammalian Ha-Ras when expressed in yeast is appropriately modified. As shown in Fig. 4, PRA1 interacts with GFP-CAAX but not GFP. This result suggests that the molecular determinants for the binding of PRA1 reside within the CAAX box of Ha-Ras and that a modified tetrapeptide (farnesylated, proteolyzed, and/or methylated) is both necessary and sufficient to mediate the protein-protein interaction.

The sole post-translational modification in common among the PRA1 interacting proteins, Rabs, Ras, Rho, and TC21, is isoprenylation, the attachment of farnesyl or geranylgeranyl moieties to the proteins (Table I). To demonstrate that post-translational processing events subsequent to prenylation (proteolysis and/or methylation) are not essential for PRA1 binding, we examined the association of PRA1 with GFP-CVYS. GFP-CVYS contains a substitution of Tyr for Leu at the A2 position of the CAAX box. A K-Ras Tyr187 CAAX box mutant protein is prenylated but not further processed (33). As shown in Fig. 4B, PRA1 interacts with GFP-CVYS. Taken together, our observations suggest that an isoprenoid moiety, either farnesyl or geranylgeranyl, is the critical recognition target for PRA1.

To confirm the prenylation dependence observed in yeast two-hybrid experiments, modified and unmodified GST-Ha-Ras was purified from mammalian cells after Triton X-114

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**Fig. 2.** Ha-Ras, RhoA, and Rap1a interact with PRA1 in vivo. GST or GST fusion proteins were purified from lysates prepared from HEK293 cells transfected with the indicated constructs using glutathione-Sepharose. The pull-downs were subject to SDS-PAGE followed by Western blotting with antibodies directed against the epitope tags on Ha-Ras, RhoA, or Rap1a (GST) or PRA1 (Myc). A, GST-Ha-RasV12, but not GST or GST-Ha-RasV12CAAX, associates with PRA1. B, GST-RhoAL63 but not GST associates with PRA1. C, GST-Rap1a but not GST associates with PRA1.

**Fig. 3.** Farnesylation of Ha-Ras but not palmitoylation is required for the Ha-Ras/PRA1 association. The yeast L40 strain was transformed with the various plasmid combinations, and the activation of the HIS3 reporter was assessed. pLexA-GFP CAAX expresses the LexA DNA binding domain fused to a GFP to which the carboxyl-terminal Ha-Ras tetrapeptide CVLS has been added. pLexA-GFP expresses the LexA DNA binding domain fused to GFP. pLexA-GFP-CVYS expresses the LexA DNA binding domain fused to GFP to which a mutant Ha-Ras CAAX box, CVYS, has been added. LexA-GFP-CVYS is prenylated but not further processed (methylated and/or proteolyzed). pVP16-PRA1 expresses the VP16 activation domain fused to full-length PRA1. pVP16-PRA1 expresses the VP16 activation domain fused to full-length mouse PRA1. VP16, vector control. Both yeast and mouse PRA1 associate with GFP CAAX and with GFP-CVYS but not GFP.

**Fig. 4.** A, the addition of the Ha-Ras CAAX box to GFP is necessary and sufficient to mediate the association of PRA1 with GFP. B, post-translational events subsequent to prenylation are not essential for PRA1 binding. The yeast L40 strain was transformed with the various plasmid combinations, and the activation of the HIS3 reporter was assessed. pLexA-GFP CAAX expresses the LexA DNA binding domain fused to a GFP to which the carboxyl-terminal Ha-Ras tetrapeptide CVLS has been added. pLexA-GFP expresses the LexA DNA binding domain fused to GFP. pLexA-GFP-CVYS expresses the LexA DNA binding domain fused to GFP to which a mutant Ha-Ras CAAX box, CVYS, has been added. LexA-GFP-CVYS is prenylated but not further processed (methylated and/or proteolyzed). pVP16-PRA1 expresses the VP16 activation domain fused to full-length yeast PRA1. pVP16-PRA1 expresses the VP16 activation domain fused to full-length mouse PRA1. VP16, vector control. Both yeast and mouse PRA1 associate with GFP CAAX and with GFP-CVYS but not GFP.
PRA1 Associates with Multiple Ras Family Proteins

Table I

<table>
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<th>Methylation</th>
<th>Palmitoylation</th>
<th>Two-hybrid interaction</th>
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<td>Y</td>
<td>Y</td>
<td>++</td>
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<tr>
<td>TC21</td>
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<td>G</td>
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<td>+</td>
</tr>
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</table>

Although Rap1A fails to interact with PRA1 in a yeast two-hybrid test, Rap1A interacts with PRA1 in vivo in mammalian cells (see Fig. 2 and “Results”).

Fig. 5. Post-translationally processed Ha-Ras associates with PRA1. GST-Ha-Ras or GST was transiently expressed in HEK293 cells, and processed and unprocessed proteins were prepared by a Triton X-114 partitioning assay. Hydrophobic proteins partition into the detergent phase, whereas hydrophilic proteins partition into the aqueous phase of Triton X-114. The processed Ha-Ras partitions into the detergent phase, whereas the unprocessed Ha-Ras is found in the aqueous phase. GST, a soluble protein, partitions exclusively into the aqueous phase of Triton X-114. The processed Ha-Ras partitions into the detergent phase, whereas hydrophilic proteins partition into the aqueous phase of Triton X-114. The processed Ha-Ras partitions into the detergent phase, whereas hydrophilic proteins partition into the aqueous phase of Triton X-114.

Fig. 6. The association of PRA1 with Ha-Ras and RhoA is conserved in evolution. The yeast L40 strain was transformed with the various plasmid combinations, and the activation of the HIS3 reporter was assessed. The yeast homolog of PRA1, yPRA1, associates with both Ha-Ras and RhoA. The association of Ha-Ras with yPRA1 is dependent on the post-translational modification of Ras because Ha-RasCAAX is unable to bind to yPRA1 (Fig. 6). Thus, the interaction of PRA1 with Ras family members is conserved in evolution.

Recent studies have demonstrated an association of Ha- and N-Ras with endomembranes (8, 26). Ha- and N-Ras are prenylated in the cytoplasm and then undergo additional modifications as they transit through the ER and then Golgi. RhoA also is present in the ER and/or endosomal vesicles (27, 28). The prenylation-dependent association of small GTPases with PRA1, together with the ability of PRA1 to interact with small GTPases with effector domain mutations (a result that strongly suggests that PRA1 is not an effector of the small GTPases with which it interacts) suggested to us that PRA1 may function in an endomembrane compartment. Therefore, we compared the spatial distribution of PRA1 and Ha-Ras and RhoA in COS1 cells. Both Ha-Ras and PRA1 exhibit perinuclear staining, indicative of Golgi localization, and Ha-Ras and PRA1 co-localize in this perinuclear region, Fig. 7A. In addition, RhoA and PRA1 also co-localize in the Golgi compartment (Fig. 7B). Recent studies have shown that PRA1 is present in the Golgi compartment (16), and our studies using Golgi markers confirm this (data not shown).

The 15 carbon farnesyl and 20 carbon geranylgeranyl isoprenoid groups are hydrophobic, although the hydrophobicity of
these groups do not support stable membrane localization. Since PRA1 specifically recognizes the isoprenoid moieties of small GTPases, the binding of PRA1 to prenylated small GTPases may facilitate their trafficking through the endomembrane system by masking the hydrophobicity of the isoprenoid groups.

**DISCUSSION**

With this report, we demonstrate that PRA1 associates with multiple members of the Ras superfamily. We show that PRA1 associates with Ha-Ras, RhoA, TC21, and Rap1a. Others have shown that PRA1 associates with Rab family members (15–17). The sole feature in common among these small GTPases is the presence of farnesyl or geranylgeranyl isoprenoid moieties covalently linked to cysteines located at or near the carboxyl terminus of the small GTPase (Table I). Our Ras CAAX box mutant studies (Fig. 3), Triton-X114 partitioning studies (Fig. 5), and GFP-CAAX/PRA1 protein-protein interaction studies (Fig. 4) are consistent with the farnesyl or geranylgeranyl moiety of the small GTPase as being the key recognition target for PRA1.

Small GTPases are not the only proteins subject to modification by prenylation. Prelamin A, lamin B, γ subunits of heterotrimeric G proteins, and serine/threonine kinases such as LKB1 are prenylated (3, 29). It will be interesting to determine whether PRA1 associates with proteins outside the Ras superfamily.

A search of the data bases reveals the presence of PRA1 homologs in many organisms. PRA1 homologs are present in both budding and fission yeast, in *A. thaliana*, *C. elegans*, *D. melanogaster*, and *Mus musculus*. γPRA1, the PRA1 homolog from budding yeast, interacts with multiple prenylated small GTPases (Fig. 6). Thus, the interaction of PRA1 with multiple small GTPases is conserved in evolution, and this conservation of the association suggests that the function of the PRA1-small GTPase interaction may also be conserved in evolution. Experiments are in progress to genetically decipher the physiological role of PRA1 in yeast.

The association of PRA1 with multiple small GTPases *in vitro* and *in vivo* suggests that PRA1 has a role that is held in common with all small GTPases that it interacts with. The feature in common among the small GTPases that PRA1 interacts with is their post-translational modification by farnesyl or geranylgeranyl isoprenoid moieties. We suggest that PRA1 binds prenylated GTPases to act as an escort protein for the GTPases. Biochemical fractionation and localization experiments show that PRA1 is found both in the cytoplasm and in the Golgi (16). PRA1 may associate with small GTPases after prenylation occurs in the cytoplasm to “solubilize” the hydrophobic prenyl motifs and thus facilitate trafficking through the endomembrane system. Alternatively, PRA1 may function in the Golgi to facilitate the entry of small GTPases into vesicles for transport to cellular compartments. PRA1 is found in synaptic vesicle membranes, so PRA1 may cycle with the Ras proteins in the exocytic vesicles out to the plasma membrane (30).

PRA1 also associates with GDI1 (16). The addition of recombinant GDI1 to membranes prepared from PC12 cells was effective at removing Rab3A from the membrane; the addition of PRA1 blocked the ability of GDI to extract Rab3A (16). These observations suggest that the opposing actions of GDI1 and PRA1 may influence the membrane localization of the Rab proteins. GDIs have been described that solubilize membrane-associated Rho proteins (31). Possibly, the opposing action of GDI and PRA1 is a general mechanism for regulating the membrane status of small GTPases that are acted upon by GDIs.

The prevalence of Ras mutations in human tumors and the requirement of plasma membrane localization of Ras for cellular function suggested early on that inhibitors of farnesyltransferase might be a promising class of cancer therapeutics (5). Some of these inhibitors of farnesyltransferase are presently being evaluated in phase II clinical trials. However, small GTPases, such as RhoC, which has been recently associated with promotion of metastasis (32), are geranylgeranylated, so inhibitors of geranylgeranyl transferase may also prove to be a promising class of cancer therapeutics. In addition, some GTPases are “switch-hitters,” subject to either farnesylation or geranylgeranylation. Therefore, inhibiting one pathway may not necessarily prove efficacious in cancer treatment. PRA1 binds both farnesylated and geranylgeranylated small GTPases and may act as a receptor or escort protein for Ras superfamily members. Thus, inhibitors directed against PRA1 may provide a means to pharmacologically intervene to inhibit the signaling pathways activated aberrantly by both farnesylated and geranylgeranylated small GTPases, which can result in sustained cell growth (tumorigenesis) and metastasis.

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