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Isoprenoid modification of rab proteins terminating in CC or CXC motifs

(ras-related proteins/membrane association/vesicular transport)

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ABSTRACT **Mevalonate starvation of hamster fibroblasts resulted in a shift of rab1b from the membrane to the cytosolic fraction, suggesting that rab1b depends upon an isoprenoid modification for its membrane localization. rab1b and rab3a proteins expressed in insect cells incorporated a product of [^{3}H]mevalonate, and gas chromatography analysis of material released by Raney nickel cleavage demonstrated that rab1b and rab3a are modified by geranylgeranyl groups. Additionally, *in vitro* prenylation analysis demonstrated farnesyl modification of H-ras but geranylgeranyl modification of five rab proteins (1a, 1b, 2, 3a, and 6). Together, these results suggest that the carboxyl-terminal CC/CXC motifs (X = any amino acid) specifically signal for addition of geranylgeranyl, but not farnesyl, groups. A rab1b mutant protein lacking the two carboxyl-terminal cysteine residues was not prenylated *in vitro*. However, since a mutant H-ras protein that terminates with tandem cysteine residues was also not modified, the CC motif may be essential, but not sufficient, to signal prenylation of rab1b. Finally, rab1b and rab3a proteins were not efficient substrates for either farnesyl- or geranylgeranyltransferase activities that modify CAAX-containing proteins (A = any aliphatic amino acid). Therefore, rab proteins may be modified by a prenyltransferase(s) distinct from the prenyltransferases that modify carboxyl-terminal CAAX proteins.**

An essential requirement for *ras* transforming activity is the localization of oncogenic ras proteins to the inner surface of the plasma membrane (1, 2). This localization is promoted by a series of posttranslational modifications signaled by the carboxyl-terminal consensus CAAX motif (C = cysteine, A = any aliphatic amino acid, and X = any amino acid), which is present at the carboxyl termini of all ras proteins. Processing includes the addition of the isoprenoid farnesyl group to the cysteine residue of the CAAX motif (3–5), proteolytic removal of the AAX residues (6, 7), and methylation of the carboxyl group of the now-terminal cysteine (6, 8, 9). Substitution of the cysteine of the CAAX motif, or the absence of the AAX residues, abolishes processing and renders the proteins cytosolic and nontransforming (2, 3, 10).

While ras proteins are prenylated with the C_{15} farnesyl group (5), the majority of prenylated proteins in mammalian cells are modified by a C_{20} geranylgeranyl group (11, 12). Such proteins include the γ subunits of G proteins (13–15) and the ras-related rap1b (16), G25K (17), and Krev-1/rap1a (18) proteins. To date, all identified prenylated proteins contain the conserved CAAX motif at their carboxyl termini, and the addition of either the farnesyl or the geranylgeranyl group apparently occurs on the cysteine of the CAAX sequence (3, 5, 16–18).

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rab proteins constitute a distinct branch of the ras-related protein superfamily (19–21). At least 19 members of the mammalian *rab* gene family have been identified. Although the function of the mammalian rab proteins is not presently known, the observation that the *Saccharomyces cerevisiae* homologs of rab (YPT1 and SEC4) are involved in intracellular transport of proteins in the secretory pathway suggests that rab proteins may play an analogous role in mammalian transport processes (22, 23).

A striking structural distinction between rab and ras proteins is the absence of a carboxyl-terminal CAAX motif. Instead, most rab proteins terminate in either CC or CXC motifs. However, there is evidence that rab proteins do undergo hydrophobic carboxyl-terminal posttranslational modifications that promote their membrane association (24, 25). Thus, the rab CC/CXC motifs may be functionally analogous to the CAAX motif of the other members of the ras superfamily. Using both *in vivo* and *in vitro* approaches, we have determined that rab proteins are modified by geranylgeranyl groups, that the carboxyl-terminal CC motif of rab1b is essential, but not sufficient, to signal prenylation, and that rab proteins may be modified by a prenyltransferase(s) that is distinct from the farnesyl- and geranylgeranyltransferases that prenylate CAAX-containing proteins.

MATERIALS AND METHODS

rab1b Protein–Membrane Association in Mammalian Cells. To compare the effects of mevalonic acid (MVA) starvation on ras and rab1b proteins, Mev-1 hamster fibroblasts, which are MVA auxotrophs (26), were incubated in growth medium in either the presence (40 μg/ml) or absence of MVA for 16 hr. For fractionation analysis, control and MVA-starved Mev-1 cells were lysed in hypotonic buffer, broken in a Dounce homogenizer, and separated into crude membrane- and cytosol-containing fractions by centrifugation (100,000 × *g*, 30 min) as described previously (5). The cytosolic (S100) and membrane-containing (P100) fractions were then resolved by SDS/polyacrylamide gel electrophoresis (SDS/PAGE) and subjected to Western blot analysis with the p68 anti-rab1b rabbit polyclonal antiserum, the m5C6b rab1b-specific mouse monoclonal antibody, or the 142-24E5 anti-ras mouse monoclonal antibody (18). The generation and characterization of the rab antibodies will be described elsewhere (41).

Expression of rab Proteins in *Escherichia coli*. Bacterial, insect cell, and mammalian expression vectors were con-

Abbreviations: CAAX, protein sequence, in which C = cysteine, A = any aliphatic amino acid, and X = any amino acid; MVA, mevalonic acid; FPP, farnesyl pyrophosphate; GGPP, geranylgeranyl pyrophosphate; ER, endoplasmic reticulum.

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structed from canine rab1a (24), rat rab1b (27), and human rab2, rab3a, and rab6 (28) cDNA sequences that have been generously provided by M. Zerial (European Molecular Biology Laboratory) or A. Tavitian (Institut National de la Santé et de la Recherche Médicale). Each rab cDNA sequence was ligated into the pAR3040 (pET3a) bacterial expression vector, then introduced into the BL21 DE3 *E. coli* strain, and rab proteins were purified as described previously (24, 27, 29) for use in the prenylation assays described below.

Generation of rab1b and H-ras Carboxyl-Terminal Mutants. A rab1b mutant that lacks the two codons encoding the two carboxyl-terminal cysteine residues [designated rab1b(ΔCC)] was generated by oligonucleotide-directed mutagenesis to change codon 200 from TGC (Cys) to TGA (termination codon) and then introduced into pAR3040. A mutant H-*ras*(61-Leu) gene that encodes an H-ras protein that lacks the carboxyl-terminal CVLS motif, and instead terminates with two cysteines [CMSSKCC; designated H-ras(CC)], was generated by polymerase chain reaction and introduced into the pAT-rasH bacterial expression vector (30).

Expression of rab1b and rab3a in Sf9 Insect Cells. Recombinant rab baculovirus vectors were constructed by ligating either the 1.1-kilobase (kb) (rab1b) or the 0.6-kb (rab3a) *Nde* I–*Bam*HI fragments and an *Eco*RI–*Nde* I linker into the *Eco*RI/*Bam*HI sites of the pAcC12 transfer vector (30). Recombinant viruses were then isolated by procedures described previously (31). For radiolabeling of rab proteins in insect cells, *Spodoptera frugiperda* Sf9 cells were infected with recombinant baculovirus, then incubated with growth medium containing [5-^{3}H]MVA (lactone form; New England Nuclear) at 200 μCi/ml (1 Ci = 37 GBq). Triton X-114 partitioning of radiolabeled cells was done as described previously (32). The detergent-depleted aqueous and detergent-enriched phases were analyzed by SDS/PAGE (3).

Chemical Analysis of the Isoprenoid Group. For gas chromatography (GC) analysis, [^{3}H]MVA-labeled rab1b and rab3a proteins expressed in Sf9 cells were resolved by SDS/PAGE and recovered by electroelution. Raney nickel treatment and pentane extraction were conducted according to Kawata *et al.* (16). GC analysis was performed on a Perkin–Elmer model 3920 gas chromatograph with a flame ionization detector and a flow-through gas proportional radiodetector (IN/US). A Dexsil 300 packed column (Supelco) was used with a temperature program starting at 100°C and increasing to 340°C at 16°C/min. Peaks were identified by comparison of t_R (retention time) values with the products generated from synthetic farnesyl- and geranylgeranylcysteine methyl esters.

***In Vitro* Protein Prenylation Assays.** *In vitro* prenylation of bacterially expressed rab proteins was analyzed by three approaches. First, specific prenylation of H-ras and rab proteins was determined in a reticulocyte lysate system (33). Purified proteins (1.2 μM) were incubated in a final volume of 50 μl with untreated reticulocyte lysate (Promega) in the presence of 1 mM $MgCl_2$, 1 mM dithiothreitol, and 5 μM ^{3}H-labeled farnesyl pyrophosphate (FPP) (2.5 Ci/mmol), 5 μM ^{3}H-labeled geranylgeranyl pyrophosphate (GGPP) (2.5 Ci/mmol), or 40 μM [^{3}H]MVA (24 Ci/mmol); [^{3}H]FPP and [^{3}H]GGPP were prepared as described in ref. 42. Incubations were conducted at 37°C for 1 hr and the reaction was stopped by the addition of trichloroacetic acid and quantitation of the precipitated radioactive material as described previously (34) or by the addition of an equal volume of 2× SDS/PAGE sample buffer for SDS/PAGE analysis. Proteins labeled with [^{3}H]MVA were also excised from the gel and subjected to GC analysis to determine the nature of the attached isoprenoid. Second, rab proteins were incubated with partially purified farnesyltransferase (provided by Y. Reiss, University of Texas Southwestern Medical Center, Dallas) and the amount of [^{3}H]farnesyl incorporation into protein from [^{3}H]FPP was determined by using the assay conditions described previously (34). Finally, purified geranylgeranyltransferase activity derived from bovine brain cytosol (P. Casey, personal communication) was incubated with [^{3}H]GGPP and rab proteins, and the amount of [^{3}H]geranylgeranyl moieties transferred to the rab proteins was determined as described above.

EXPERIMENTAL RESULTS

Prenylation of rab1b Protein Is Critical for Membrane Association. To determine if rab1b protein undergoes an MVA-dependent processing step, Mev-1 cells (which require exogenous MVA for growth) were grown in either the presence or the absence of MVA, then lysed and separated into cytosolic (S100) and membrane-containing (P100) fractions, resolved by SDS/PAGE, and analyzed by Western blotting with anti-rab1b antibodies. In the presence of MVA, both ras (data not shown) and rab1b proteins were >95% membrane-associated, with very little detected in the soluble fraction (Fig. 1). However, Mev-1 cells starved for MVA displayed a shift of rab1b from the membrane-containing fraction to the cytosolic fraction. Thus, rab1b protein undergoes an MVA-dependent processing step, presumably prenylation, which is important for membrane association. Interestingly, in contrast to the different mobilities observed for the soluble and membrane-associated forms of ras (3, 5, 10), the cytosolic and membrane-associated forms of rab1b displayed indistinguishable mobilities on SDS/PAGE.

Hydrophobic Modification of Recombinant rab1b and rab3a Expressed in Sf9 Insect Cells. Since Sf9 insect cells can specifically modify proteins with farnesyl or geranylgeranyl moieties (18, 35), we have utilized recombinant rab baculovirus-infected Sf9 cells to further characterize the isoprenoid modification of rab proteins. Consistent with the analysis of Mev-1 cells, insect cell-expressed rab1b and rab3a proteins incorporated a product of [^{3}H]MVA (Fig. 2*A*). Single bands of either ≈22 kDa or ≈23 kDa were observed on SDS/PAGE of labeled rab1b and rab3a, respectively. To determine if isoprenoid modification was associated with an increase in hydrophobicity, bacterially and insect cell-expressed proteins were characterized by partitioning into Triton X-114 (6, 32). While the unprocessed H-ras (3), rab1b, and rab3a (25) proteins expressed in *E. coli* partitioned exclusively into the aqueous phase (data not shown), [^{3}H]MVA-labeled H-ras, rab1b, and rab3a proteins expressed in insect cells were found predominantly in the detergent phase (Fig. 2*A*). This

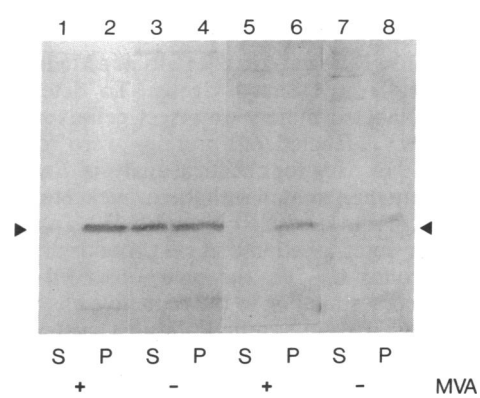

FIG. 1. Prenylation of rab1b protein contributes to membrane association. Mev-1 cells were grown in the presence (+) or absence (−) of exogenous MVA for 16 hr, then separated into crude cytosolic (S) and membrane-containing (P) fractions. Fractions were then analyzed by SDS/PAGE and Western blot analysis with either p68 anti-rab1b rabbit polyclonal antiserum (lanes 1–4) or m5C6b anti-rab1b mouse monoclonal antibody (lanes 5–8). Arrowheads indicate location of rab1b.

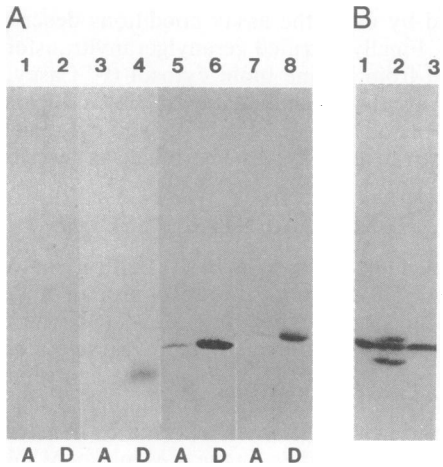

FIG. 2. rab1b protein is posttranslationally modified in Sf9 insect cells. (*A*) Sf9 cells infected with wild-type baculovirus (lanes 1 and 2) or recombinant baculovirus expressing H-ras (lanes 3 and 4), rab1b (lanes 5 and 6), or rab3a (lanes 7 and 8) were labeled with [^{3}H]MVA, lysed in 1% Triton X-114, then partitioned into detergent-depleted aqueous (A) and detergent-enriched (D) phases. (*B*) Purified bacterially expressed protein (lane 1), total infected insect cell lysate (lane 2), and total NIH(rab1b) cell lysate (lane 3) were resolved on SDS/PAGE and analyzed by Western blotting with p68 anti-rab1b polyclonal antiserum. NIH 3T3 cells were stably transfected with an expression vector (pSUZ-rab1b) to establish NIH(rab1b), which expresses rab1b protein ≈10-fold higher than endogenous levels.

increased hydrophobicity is consistent with a role for isoprenoid modification in facilitating membrane association.

Since posttranslational modification of ras proteins results in altered mobilities on SDS/PAGE (3, 5, 10), rab1b proteins expressed in *E. coli*, insect cells, and NIH 3T3 mouse fibroblasts were compared. Interestingly, while the major insect cell-expressed rab1b band comigrated with the unprocessed, bacterially expressed protein, two additional bands were also observed (Fig. 2*B*). Since neither of these two bands was observed in [^{3}H]MVA-labeled insect cells (Fig. 2*A*), and they did not fractionate into the Triton X-110 detergent-enriched phase (data not shown), their altered mobilities were apparently not due to isoprenoid addition. rab1b expressed in NIH 3T3 cells comigrated with the bacterially expressed protein and the isoprenylated form in insect cells. In contrast to rab1b, the isoprenylated form of insect cell-expressed rab3a displayed a distinctly faster electrophoretic mobility than the bacterially expressed rab3a protein (data not shown).

rab1b and rab3a Expressed in Sf9 Cells Are Modified by the C_{20} Isoprenoid Geranylgeranyl Group. To determine the identity of the labeled moiety on insect cell-expressed rab proteins, [^{3}H]MVA-labeled rab proteins were resolved on SDS/PAGE and excised for chemical analysis. Radiolabeled rab proteins were then treated with Raney nickel to cleave the thioether linkage and analyzed by radio-GC. In agreement with our earlier determination that ras proteins are modified by farnesyl groups (5, 18), the peak observed for H-ras displayed a t_R corresponding to the reductive cleavage product that is derived from synthetic farnesylcysteine methyl ester (presumably 2,6,10-trimethyl-2,6,10-dodecatriene; see ref. 16) (data not shown). In contrast, the peak observed after reductive cleavage of both rab1b and rab3a corresponded to that of geranylgeranylcysteine methyl ester (presumably 2,6,10,14-hexadecatetraene; see ref. 16) (Fig. 3 *Upper*).

Specific *in Vitro* Modification of rab Proteins with Geranylgeranyl Groups. To confirm and extend the *in vivo* insect cell studies, the specific isoprenoid modification of bacterially expressed H-ras, rab1a, rab1b, rab2, rab3a, and rab6 proteins was also addressed by using *in vitro* prenylation in

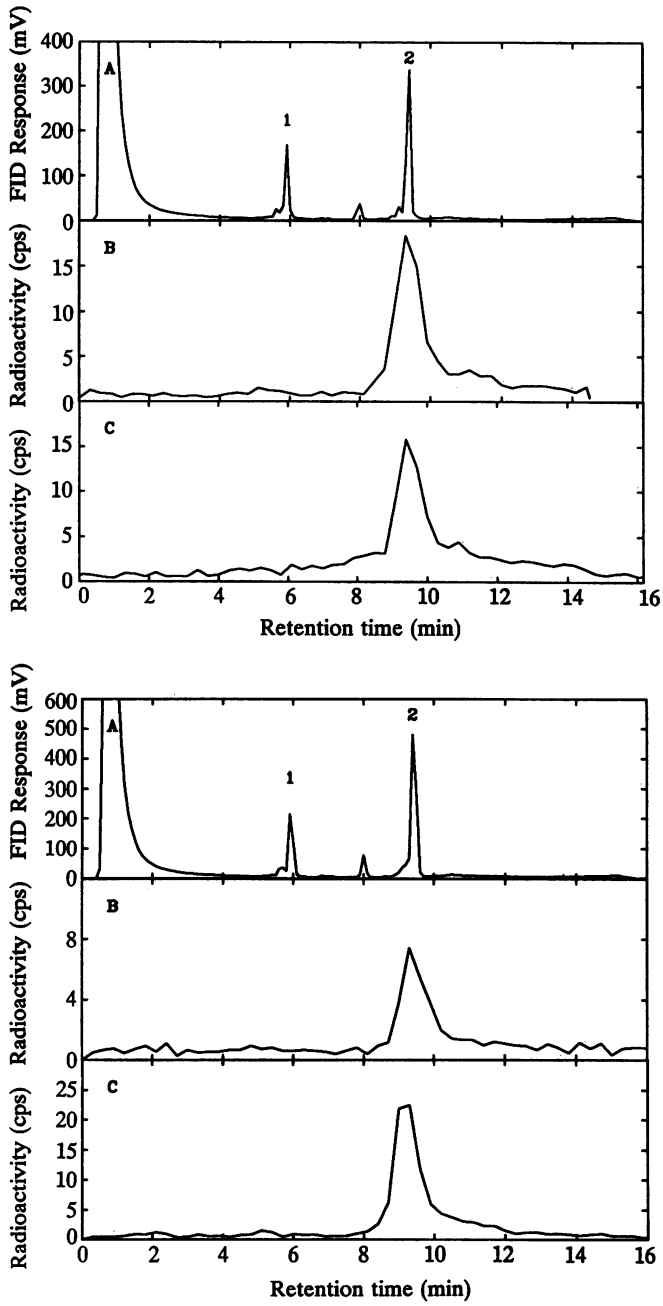

FIG. 3. Geranylgeranyl modification of rab proteins *in vivo* and *in vitro*. (*Upper*) Gel-purified rab1b and rab3a proteins were isolated from [^{3}H]MVA-labeled Sf9 cells and analyzed by radio-GC with flame ionization detection (FID) (*A*) or radioactivity measurement in counts/sec (cps) (*B* and *C*). (*A*) The products of treatment of synthetic farnesyl- (peak 1) and geranylgeranyl- (peak 2) cysteine methyl ester are shown. Peaks were identified by chromatography of the individual products (data not shown). (*B*) The product of treatment of [^{3}H]MVA-labeled rab1b. (*C*) The product of treatment of [^{3}H]MVA-labeled rab3a. Peaks were identified by comparison of t_R values with internal standards derived from synthetic farnesyl- (peak 1) and geranylgeranyl- (peak 2) cysteine methyl ester (see *A*). (*Lower*) Gel-purified rab proteins incubated with [^{3}H]MVA in the reticulocyte lysate assay were processed and subjected to GC analysis. (*A*) Standards. (*B*) rab1b. (*C*) rab3a.

a rabbit reticulocyte lysate assay (33). Consistent with the chemical analysis of insect cell-expressed proteins, radio-GC analysis of *in vitro* [^{3}H]MVA-labeled proteins also identified geranylgeranyl addition to rab1b and rab3a proteins (Fig. 3 *Lower*), and farnesyl addition to H-ras protein (data not shown). When farnesyl and geranylgeranyl substrates were

used instead of the MVA precursor, the same specificity of prenylation was observed. H-ras protein was efficiently labeled with [^{3}H]FPP but not [^{3}H]GGPP. In contrast, all five rab proteins were efficiently labeled with [^{3}H]GGPP but not [^{3}H]FPP (Fig. 4 *A* and *B*). These results are consistent with the prenylation specificity observed in Sf9 cells and show that all five rab proteins are modified by geranylgeranyl rather than by the farnesyl substituent found in ras proteins. A rab1b mutant that lacks the carboxyl-terminal double cysteines [rab1b(ΔCC)] did not incorporate either [^{3}H]GGPP or [^{3}H]MVA (Fig. 4*B*), suggesting that the CC motif is an essential component of the signal for prenylation of rab1b, and it may be the site for this geranylgeranyl addition. However, an H-ras mutant [H-ras(CC)] that terminates with two cysteine residues (carboxyl-terminal sequence CMSSKCC) was also not modified (data not shown). Thus, additional sequences upstream of the rab1b CC motif may be required to signal prenylation.

To address the possibility that rab proteins are also modified by the same prenyltransferases that modify proteins containing carboxyl-terminal CAAX motifs, *in vitro* prenylation assays were done with partially purified farnesyl- (34) or geranylgeranyltransferase isolated from rat or bovine brain cytosol, respectively. While H-ras incubated with farnesyltransferase efficiently incorporated label from [^{3}H]FPP, neither rab1b nor rab3a proteins incorporated significant radioactivity (Fig. 4*C*). Similarly, while the geranylgeranyltransferase efficiently transferred [^{3}H]GG onto a mutant H-ras chimeric protein with a carboxyl-terminal sequence (CVLL) derived from a geranylgeranyl-modified protein (A.D.C. and C.J.D., unpublished results), only 1–5% incorporation was observed with rab1b and rab3a proteins (data not shown).

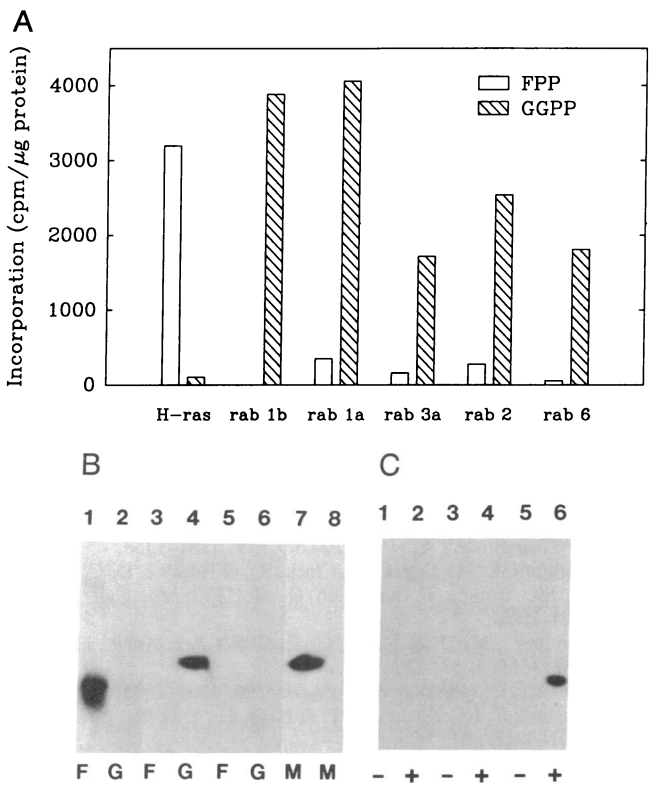

FIG. 4. *In vitro* prenylation of bacterially expressed rab proteins with geranylgeranyl but not farnesyl groups. (*A*) Bacterially expressed proteins were incubated with untreated reticulocyte lysate containing 5 μM [^{3}H]FPP (2.5 Ci/mmol) or 5 μM [^{3}H]GGPP (2.5 Ci/mmol). Incorporation of [^{3}H]FPP (empty bars) and [^{3}H]GGPP (hatched bars) was calculated from radioactivity of trichloroacetic acid-precipitable material after subtraction of values from blank incubations in which no rab or ras protein was added. (*B*) Purified H-ras (lanes 1 and 2), rab1b (lanes 3, 4, and 7), and rab1b(ΔCC) (lanes 5, 6, and 8) were incubated with [^{3}H]FPP (F) (lanes 1, 3, and 5) or [^{3}H]GGPP (G) (lanes 2, 4, and 6), or [^{3}H]MVA (M) (lanes 7 and 8). Labeled protein samples were prepared as described for *A* and were resolved on SDS/PAGE. (*C*) Gel analysis of proteins incubated with farnesyltransferase. Bacterially expressed proteins were incubated with (+) or without (−) partially purified farnesyltransferase and [^{3}H]FPP to measure incorporation of [^{3}H]farnesyl into rab1b (lanes 1 and 2), rab3a (lanes 3 and 4), or H-ras (lanes 5 and 6), using the assay conditions described in the text, and resolved by SDS/PAGE.

DISCUSSION

Mutant ras proteins lacking either the cysteine or the three terminal amino acids of the CAAX motif do not undergo farnesylation. Furthermore, all proteins previously identified to be modified by either the C_{15} farnesyl or C_{20} geranylgeranyl isoprenoid possess carboxyl-terminal CAAX motifs (36). Thus, the CAAX motif is an essential structural component to signal protein prenylation. However, the results described here demonstrate that rab proteins, which lack a CAAX motif, and instead terminate with either a CC or CXC motif, can also be modified by isoprenylation. Macara and colleagues have also independently identified the same modification for rab2 and rab3a (M.S. and I. Macara, unpublished results). Our observation that rab1a (carboxyl-terminal sequence CC), rab1b (CC), rab2 (CC), rab3a (CAC), and rab6 (CSC) proteins are substrates for addition of geranylgeranyl, but not farnesyl, moieties suggests that all rab proteins with CC or CXC motifs may be modified by geranylgeranyl moieties. Finally, the demonstration that a mutant rab1b protein that lacks the carboxyl-terminal CC sequence is not a substrate for isoprenylation suggests that these tandem cysteines are an essential component of the prenylation signal sequence, and, like the cysteine of the CAAX motif, may also be the site(s) for geranylgeranyl addition.

The minimal sequence required to signal isoprenoid modification of ras proteins has been determined to entail only the tetrapeptide CAAX motif. First, the introduction of the H-ras carboxyl-terminal four amino acids (CVLS) onto either protein A (3) or the α subunit of the inhibitory G protein (39) resulted in efficient prenylation of these normally nonprenylated proteins. Second, synthetic CAAX tetrapeptides corresponding to the carboxyl-terminal sequences of ras were efficient competitors for a farnesyltransferase that modifies ras proteins (34). Thus, sequences upstream of the CAAX motif are apparently not required for either farnesyl modification or geranylgeranyl modification (A.D.C. and C.J.D., unpublished results). In contrast, the inability of the H-ras mutant protein that terminates with tandem cysteines to serve as an efficient substrate for *in vitro* prenylation suggests that the CC motif alone is an insufficient signal and that additional rab1b sequences upstream of CC are essential components of the prenylation signal sequence.

Interestingly, while rab proteins incubated with [^{3}H]MVA in the reticulocyte assay incorporated [^{3}H]GG, no significant incorporation was observed when the proteins were incubated with [^{3}H]FPP. Analysis of the conversion of radioactive MVA to water-soluble isoprenyl pyrophosphates suggests that there are substantial pools of FPP in these lysates but little else. Thus, although high specific activity [^{3}H]MVA can apparently be converted to [^{3}H]isopentenyl pyrophosphate (IPP), which can react with FPP to form [^{3}H]GGPP, there is insufficient endogenous IPP to react with the exogenously added [^{3}H]FPP. We have found that this results in no detectable conversion of exogenously added [^{3}H]FPP to [^{3}H]GGPP.

Our studies indicate that rab1b and the rab3a proteins are not efficiently modified by the farnesyl- (34) or geranylgeranyltransferase enzymes that prenylate ras proteins and other proteins with CAAX motifs, and they suggest the

existence of a different prenyltransferase(s) for modification of rab proteins that terminate with CC/CXC sequences. Thus, the farnesyltransferase enzyme responsible for isoprenoid modification of ras proteins is apparently distinct from the prenyltransferases that produce geranylgeranyl-modified proteins. Therefore, pharmacologic approaches aimed at inhibiting ras farnesyltransferase activity may not have the broad and nonspecific effects observed for hydroxymethylglutaryl-CoA reductase inhibitors such as lovastatin or compactin (40), which inhibit MVA biosynthesis and prevent all protein prenylation.

We have recently completed studies demonstrating that the rab1b protein is localized to the endoplasmic reticulum (ER) and Golgi complex and that rab1b regulates vesicular transport between the ER and successive Golgi compartments (41). Since isoprenoid modification is important for both the plasma membrane localization and transforming activity of ras proteins, geranylgeranyl modification is also anticipated to play an important role in rab1b function. Our observation that rab1b membrane association is dependent on isoprenoid modification supports this role. However, while rab1b membrane association requires modification by geranylgeranyl groups, this modification alone is not likely to be sufficient to promote the precise association of rab1b specifically with the ER and Golgi complex. For example, while rab2, rab3a, and rab6 proteins are all modified by geranylgeranylation, rab2 is present in an intermediate compartment between the ER and Golgi complex (24), rab3a is present in synaptic vesicles (25), and rab6 has been localized to the medial and trans Golgi compartments (37). Since rab proteins exhibit their greatest amino acid divergence in their carboxyl-terminal sequences (24), it is likely that residues upstream of the CC/CXC carboxyl-terminal motifs determine the specific localization of each rab protein to the various compartments of the exocytic and endocytic pathways.

Isoprenoid modification is believed to stabilize ras proteins at the plasma membrane. If rab proteins function in promoting vesicular trafficking between discrete compartments of the intracellular pathway (23), then a more dynamic interaction with membranes would be required (22). For example, to regulate vesicular trafficking between the ER and Golgi complex, rab1b must cycle between two distinct membrane compartments, and it should reversibly cycle between cytosolic and membrane fractions. The availability of an *in vitro* assay for rab1b function in ER to Golgi vesicular transport (41) should allow us to determine the precise contribution of isoprenoid modification to rab1b function.

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