Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 8934–8938, October 1991
Biochemistry

Posttranslational modification of Ha-ras p21 by farnesyl versus geranylgeranyl isoprenoids is determined by the COOH-terminal amino acid

(mevalonate/isoprenylation/mutagenesis/CAAX motif/GTP-binding proteins)

B. THERESE KINSELLA, ROBERT A. ERDMAN, AND WILLIAM A. MALTESE*

Weis Center for Research, Geisinger Clinic, Danville, PA 17822

Communicated by Stephen J. Benkovic, July 22, 1991 (received for review April 29, 1991)

ABSTRACT **ras proteins undergo posttranslational modification by a 15-carbon farnesyl isoprenoid at a cysteine within a defined COOH-terminal amino acid motif; i.e., Cys-Ali-Ali-Ser/Met (where Ali represents an aliphatic residue). In other low molecular mass GTP-binding proteins, cysteines are modified by 20-carbon geranylgeranyl groups within a Cys-Ali-Ali-Leu motif. We changed the terminal Ser-189 of Ha-ras p21 to Leu-189 by site-directed mutagenesis and found that the protein was modified by [^{3}H]geranylgeranyl instead of [^{3}H]farnesyl in an *in vitro* assay. Gel-permeation chromatography of [^{3}H]mevalonate-labeled hydrocarbons released from immunoprecipitated ras proteins overexpressed in COS cells indicated that Ha-ras p21(Leu-189) was also a substrate for 20-carbon isoprenyl modification *in vivo*. Additional steps in Ha-ras p21 processing, normally initiated by farnesylation, appear to be supported by geranylgeranylation, based on metabolic labeling of Ha-ras p21(Leu-189) with [^{3}H]palmitate and its subcellular localization in a particulate fraction from COS cells. These observations indicate that the amino acid occupying the terminal position (Xaa) in the Cys-Ali-Ali-Xaa motif constitutes a key structural feature by which Ha-ras p21 and other proteins with ras-like COOH-terminal isoprenylation sites are distinguished as substrates for farnesyl- or geranylgeranyltransferases.**

A variety of proteins in eukaryotic cells contain cysteine residues that are modified posttranslationally by thioether linkage to isoprenyl groups (see refs. 1 and 2 for review). Of the isoprenylated proteins thus far identified, the p21 products of the *ras* genes have received particular attention because isoprenylation initiates a series of posttranslational processing events required for protein interaction with the cell membrane and expression of oncogenic activity (3–9). ras proteins undergo modification by a 15-carbon farnesyl group at a cysteine within a defined COOH-terminal amino acid motif; i.e., Cys-Ali-Ali-Ser or Cys-Ali-Ali-Met (where Ali represents an aliphatic amino acid) (3, 4, 7–13). Three amino acids distal to the farnesyl cysteine are subsequently removed (3, 4, 14) and the terminal COOH group is methylated (14, 15). In some cases (e.g., Ha-ras p21) cysteines upstream from the farnesylation site are palmitoylated, resulting in stable membrane association (3, 4).

Several proteins in mammalian cells contain cysteines that are modified by 20-carbon geranylgeranyl instead of 15-carbon farnesyl groups (16, 17). As in the case of ras p21, the modified cysteines are located in the fourth position from the COOH terminus in the predicted sequences of many geranylgeranylated proteins; e.g., rap1A (18), rap1B (19), G25K (20), rac1, rac2, ralA (21), and the γ_6 subunits of the heterotrimeric brain guanine nucleotide binding proteins (G proteins) (22, 23). Studies with purified ras p21 farnesyltransferase indicate that the minimal structural element recognized by the enzyme consists of the COOH-terminal Cys-Ali-Ali-Ser/Met tetrapeptide (10, 24). Consistent with this finding, farnesylation occurs in two proteins that are unrelated to ras p21 except for the presence of a COOH-terminal Cys-Ali-Ali-Met sequence; i.e., nuclear lamin B (25) and the γ subunit of transducin (26, 27). The existence of a separate protein geranylgeranyltransferase that recognizes a COOH-terminal structural element distinct from the Cys-Ali-Ali-Met/Ser farnesylation signal is suggested by three observations. First, discrete sets of farnesylated and geranylgeranylated proteins have been found to coexist within a single cell type (17, 28). Second, synthetic tetrapeptides based on the COOH-terminal sequences of ras proteins compete efficiently with Ha-ras p21 in a farnesylation assay, whereas a tetrapeptide based on the COOH-terminal sequence of a geranylgeranylated protein (the G-protein γ_6 subunit) does not (10, 24). Third, protein geranylgeranyltransferase activities have been described that are chromatographically separable from ras p21 farnesyltransferase (29, 30). In considering possible structural features that may serve to mark proteins as substrates for modification by 20-carbon versus 15-carbon isoprenyl groups, it is noteworthy that the predicted sequences of many geranylgeranyl-modified proteins contain leucine in the last position (Xaa) of the Cys-Ali-Ali-Xaa motif, instead of methionine or serine as found in the farnesylated proteins. In the present study, the potential significance of this difference was assessed by determining the effects of COOH-terminal serine to leucine mutation on the posttranslational isoprenoid modification of Ha-ras p21.

MATERIALS AND METHODS

Materials. [1-^{3}H(N)]Farnesyl pyrophosphate (FPP) triammonium salt (20 Ci/mmol; 1 Ci = 37 GBq), L-[^{35}S]methionine (1163 Ci/mmol), [9,10-^{3}H]palmitic acid (38 Ci/mmol), and the ras-11 (pan) antibody were obtained from DuPont/NEN. [1-^{3}H]Geranylgeranyl pyrophosphate (GGPP) (8 Ci/mmol) was purchased from the USF Research Foundation (Tampa, FL). [5-^{3}H]Mevalonolactone (MVA) (2.0 Ci/mmol) was prepared by reduction of mevaldic acid precursor (Sigma) with sodium boro[^{3}H]hydride (Amersham) as described by Keller (31). Lovastatin was provided by Alfred Alberts (Merck Sharp & Dohme). Human Ha-ras wild-type (wt) cDNA was provided by Channing Der (La Jolla Cancer Research Foundation). COS-M6 cells and the pCMV5 expression vector were supplied by David Russell (University of Texas Health Science Center).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: FPP, farnesyl pyrophosphate; GGPP, geranylgeranyl pyrophosphate; MVA, mevalonolactone; G protein, guanine nucleotide binding protein; wt, wild type.

*To whom reprint requests should be addressed.

Site-Directed Mutagenesis of Ha-*ras*. The plasmid pG-Hras was constructed by subcloning a 1.2-kilobase (kb) *Bam*HI/*Eco*RI Ha-*ras* cDNA fragment into pGEM4. Site-directed mutagenesis of the COOH-terminal Ser-189 codon (TCC) to a Leu-189 codon (CTC) was performed by polymerase chain reaction amplification of the Ha-*ras* cDNA from the plasmid template pG-Hras, using the T7 promoter primer and the mutator oligodeoxynucleotide (5'-ACGTGAATTCACCTGGTGTCAGAGCAGCACACACTTGCAGCT-3'), where the sequence complementary to the Leu-189 codon is underlined. After amplification, the DNA product was digested with *Bam*HI and *Eco*RI (the *Eco*RI site was introduced by the mutator oligonucleotide) and subcloned into pGEM4, generating the plasmid pG-Hras(Leu-189). The mutation in pG-Hras(Leu-189) was confirmed by DNA sequence analysis (32). Routine DNA manipulations, including restriction endonuclease digestions, DNA ligations, dephosphorylations, bacterial transformations, plasmid isolations, and agarose gel electrophoresis were carried out by standard procedures (33).

***In Vitro* Translation and Isoprenylation of ras p21.** pG-Hras and pG-Hras(Leu-189) plasmids were linearized and transcribed with T7 RNA polymerase (34). *In vitro* translations were carried out in a methionine-deficient rabbit reticulocyte lysate system (Promega) according to the manufacturer's instructions. Standard 25-μl reaction mixtures containing 2.5 μg of RNA were incubated for 1 h at 30°C. Translation was monitored by including 20 μCi of L-[^{35}S]methionine (1186 Ci/mmol) in the reaction mixture. Reaction mixtures without exogenous RNA served as controls for background incorporation. To monitor isoprenylation of the Ha-ras p21 proteins, translation was carried out for 1 h, using unlabeled methionine in place of radiolabeled methionine. The reaction mixture was then supplemented with [^{3}H]FPP (1.25 μCi), [^{3}H]GGPP (2.5 μCi), or [^{3}H](MVA) (25 μCi), and incubation was continued for 1 h at 37°C. Translation products were analyzed by SDS/PAGE and fluorography as described (34). For studies aimed at estimating the chain lengths of the radiolabeled protein-bound isoprenoids (see below), translation reaction mixtures containing [^{3}H]MVA were scaled up to a final vol of 137 μl.

Transient Expression and Metabolic Labeling of Ha-ras p21 in COS Cells. Simian COS cells were grown in 60-mm dishes in Dulbecco's medium (high glucose) supplemented with 10% fetal calf serum. Transfection was performed with 5 μg of plasmid DNA mixed with DEAE-dextran (35). Control cultures were transfected with the pCMV5 expression vector (39) without a ras insert. Parallel cultures were transfected with pCMV5-Hras, constructed by subcloning a 1.2-kb *Bam*HI Ha-ras(wt) fragment into the polylinker of pCMV5, or with pCMV5-Hras(Leu-189). The latter plasmid was constructed by cloning the *Eco*RI-digested, Klenow filled-in, *Bam*HI-digested Ha-ras(Leu-189) fragment from pG-Hras(Leu-189) into the *Bam*HI/*Sma* I sites of pCMV5. Isoprenylated proteins were labeled by incubating the transfected cells in medium containing [^{3}H]MVA (200 μCi per ml of medium) for 20 h, beginning 24 h after transfection. In separate cultures, proteins were also labeled with [^{35}S]methionine (50 μCi per ml of medium) or [^{3}H]palmitate (1 mCi per ml of medium) for the same period of time. For studies aimed at determining the chain lengths of the isoprenyl groups attached to Ha-ras p21, cells from two parallel cultures were pooled and incorporation of [^{3}H]MVA was enhanced by adding 25 μM lovastatin to the medium during the labeling period. To determine the subcellular distribution of ras p21, cells were harvested 48 h after transfection and fractionated as described (35). Proteins in the soluble (100,000 × *g* supernatant) and particulate (100,000 × *g* pellet) fractions were separated on SDS gels and immunoblotted with pan-reactive ras antibody as described in detail elsewhere (35).

Estimation of Isoprenoid Chain Length. [^{3}H]MVA-labeled Ha-ras proteins from *in vitro* translations or COS cell immunoprecipitates were electroeluted from SDS gels and reacted with Raney nickel catalyst (17, 25). Radiolabeled isoprenoids were extracted into pentane, reduced over platinum oxide, and subjected to high-performance gel-permeation chromatography as described (21, 28). Retention times of the radiolabeled hydrocarbons were determined in relation to hydrocarbons of defined chain length (mixed with samples prior to injection on the column); i.e., 2,6,10-trimethyldodecane (farnesane), 2,6,10,14-tetramethylhexadecane (phytane), and 2,6,10,15,19,23-hexamethyltetracosane (squalane).

Immunoprecipitation of ras p21. Cells were lysed by sonication in 0.4 ml of 0.5% SDS/2 mM EDTA/aprotinin (100 μg/ml)/1 mM phenylmethylsulfonyl fluoride/100 mM Tris·HCl, pH 8.3. The volume was adjusted to 0.5 ml by addition of solution containing 5% sodium deoxycholate, 5% Nonidet P-40, and aprotinin (1 mg/ml). Samples were then incubated with ras antibody (1:33 dilution) for 2 h at 4°C. Immune complexes were collected as recommended by the manufacturer (DuPont/NEN), using protein A-Sepharose beads coated with rabbit anti-mouse IgG. Proteins were released from the beads by incubation at 100°C for 5 min with 2% SDS, 10% 2-mercaptoethanol, 30% (vol/vol) glycerol, 0.02% bromphenol blue, 0.05 M Tris·HCl (pH 6.8), and SDS/PAGE was carried out as described (34, 35).

RESULTS

Isoprenylation of Ha-ras p21 Proteins *in Vitro*. Reticulocyte lysates contain enzymes capable of catalyzing the modification of proteins by both 15-carbon and 20-carbon isoprenyl groups (21, 34, 36, 37). Therefore, this system was used to determine whether changing the terminal amino acid in the sequence for Ha-ras p21 from serine to leucine altered its ability to undergo farnesylation. Based on incorporation of [^{35}S]methionine, the Ha-ras and Ha-ras(Leu-189) proteins were synthesized with comparable efficiencies (Fig. 1*A*). Whereas Ha-ras p21 was readily labeled by [^{3}H]FPP, incorporation of radioactivity into Ha-ras p21(Leu-189) was undetectable (Fig. 1*B*). When identical translations were performed with [^{3}H]GGPP instead of [^{3}H]FPP, radioactivity was incorporated only into the Leu-189 mutant protein and not the wild-type Ha-ras p21 (Fig. 1*C*). Recent studies indicate that protein geranylgeranyltransferase is structurally distinct from ras p21 farnesyltransferase (29, 30). Thus, it is possible that different specific activities of the two enzymes in the reticulocyte lysate may account for the lower incorporation of [^{3}H]geranylgeranyl into Ha-ras p21(Leu-189) compared to the incorporation of [^{3}H]farnesyl into Ha-ras p21.

Both the Ha-ras and Ha-ras(Leu-189) translation products were labeled when the isoprenoid precursor [^{3}H]MVA was added to the lysate (Fig. 1*D*). Chromatographic determinations of the relative sizes of the [^{3}H]MVA-derived isoprenyl groups released from each protein were consistent with the results of the [^{3}H]FPP and [^{3}H]GGPP labeling experiments; i.e., the [^{3}H]MVA-labeled hydrocarbons from Ha-ras p21 coeluted with the 15-carbon farnesane standard, whereas the [^{3}H]MVA-labeled hydrocarbons from Ha-ras p21(Leu-189) coeluted with the 20-carbon standard (Fig. 2). In contrast to the results obtained with the [^{3}H]FPP and [^{3}H]GGPP substrates (Fig. 1 *B* and *C*), the labeling of the Leu-189 protein with [^{3}H]MVA was better than that observed for Ha-ras p21(wt) (Fig. 1*D*). The explanation for this difference is not obvious, but one possibility is that the concentration of FPP generated from [^{3}H]MVA in the reticulocyte lysate is not optimal for the farnesyltransferase reaction.

Isoprenylation of Ha-ras Proteins in Cultured Cells. To determine whether changing the COOH-terminal amino acid of Ha-ras p21 from serine to leucine altered its modification

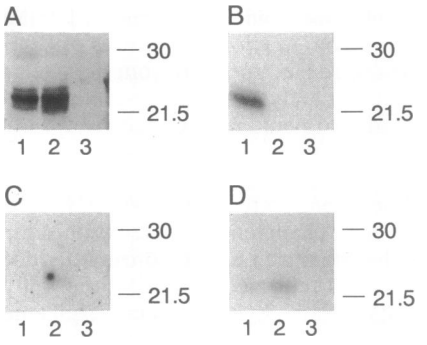

FIG. 1. Incorporation of radiolabeled isoprenoid precursors into Ha-ras proteins translated *in vitro*. Translation/isoprenylation reactions were carried out in reticulocyte lysates containing Ha-ras(wt) RNA (lane 1), Ha-ras(Leu-189) RNA (lane 2), or no exogenous RNA (lane 3). The following radiolabeled precursors were added to the translation reaction mixtures: [^{35}S]methionine (*A*), [^{3}H]FPP (*B*), [^{3}H]GGPP (*C*), and [^{3}H]MVA (*D*). Aliquots from each reaction (5 μl for ^{35}S-labeled samples, 12.5 μl for ^{3}H-labeled samples) were subjected to SDS/PAGE and autoradiography or fluorography. The autoradiogram in *A* was exposed for 3 days. All fluorograms (*B–D*) were exposed for 14 days. Molecular masses of marker proteins (kDa) are indicated on the right. Based on scintillation counting of [^{35}S]methionine in the dried gels (*A*), ≈0.169 pmol of Ha-ras p21 and 0.192 pmol of Ha-ras p21(Leu-189) were synthesized. Assuming comparable protein synthesis in the parallel reaction mixtures containing ^{3}H-labeled substrates, the stoichiometries of isoprenylation were 1.1 mol of [^{3}H]farnesyl per mol of Ha-ras p21 (*B*) and 0.2 mol of [^{3}H]geranylgeranyl per mol of Ha-ras p21(Leu-189) (*C*). [^{3}H]MVA incorporation values (*D*) were 1698 dpm (≈0.62 mol of 15-carbon prenyl group per mol of Ha-ras p21), and 3721 dpm [≈0.90 mol of 20-carbon prenyl group per mol of Ha-ras p21(Leu-189)].

from farnesylation to geranylgeranylation *in vivo*, COS cells were transfected with expression vectors containing either Ha-ras or Ha-ras(Leu-189) cDNA inserts. As indicated by incorporation of [^{35}S]methionine into immunoprecipitated ras p21, both the wild-type and Leu-189 Ha-ras proteins were overexpressed relative to endogenous ras proteins in the COS cells (Fig. 3*A*). The 23-kDa [^{35}S]methionine-labeled ras protein was assumed to represent unprocessed Ha-ras p21 that accumulated due to saturation of the normal posttranslational processing pathway. Consistent with this interpretation, [^{3}H]MVA was incorporated only into the faster-migrating forms of Ha-ras p21 and Ha-ras p21(Leu-189) (Fig. 3*A*). When the sizes of the [^{3}H]MVA-derived groups were estimated by gel-permeation chromatography, the radiolabeled hydrocarbon released from ras p21 immunoprecipitated from the Ha-ras-transfected cells coeluted exclusively with the 15-carbon standard (Fig. 3*B*). In contrast, most of the [^{3}H]MVA-labeled hydrocarbon extracted from ras p21 in the Ha-ras(Leu-189)-transfected cells coeluted with the 20-carbon standard (Fig. 3*B*). In the latter case, the small amount of radiolabeled material coeluting with the 15-carbon standard was comparable to that extracted from the endogenous ras proteins in control cells (Fig. 3*B*).

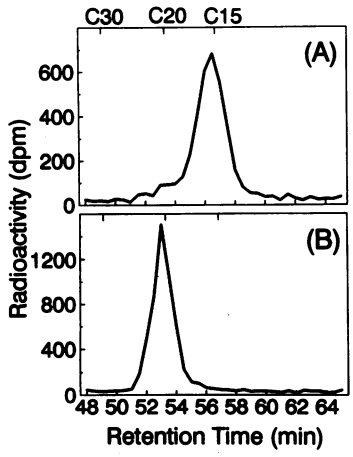

FIG. 2. Estimated sizes of [^{3}H]MVA-derived hydrocarbons released from Ha-ras translation products. [^{3}H]MVA-labeled isoprenyl groups released from Ha-ras(wt) (*A*) or Ha-ras(Leu-189) (*B*) translation products were extracted into pentane and reduced over platinum oxide. Recoveries of ^{3}H from the proteins were 45% (6380 dpm) for Ha-ras(wt) and 41% (12,400 dpm) for Ha-ras(Leu-189). Aliquots of the radiolabeled hydrocarbons [3301 dpm for Ha-ras(wt) and 5612 dpm for Ha-ras(Leu-189)] were subjected to gel-permeation chromatography along with standards of defined chain length. Elution of the radiolabeled hydrocarbons (94–100% recovery of dpm loaded) was monitored by liquid scintillation counting. Retention times of the squalane (C30), phytane (C20), and farnesane (C15) standards, which are marked at the top of the chromatogram, were determined with an on-line refractive index detector.

Palmitoylation and Subcellular Distribution of ras Proteins. A 21.5-kDa protein immunoprecipitated from COS cells overexpressing Ha-ras was metabolically labeled by [^{3}H]palmitate (Fig. 4*A*), as well as by [^{3}H]MVA (Fig. 4*B*). Moreover, immunoblots indicated that this 21.5-kDa ras protein was localized predominantly in the particulate frac-

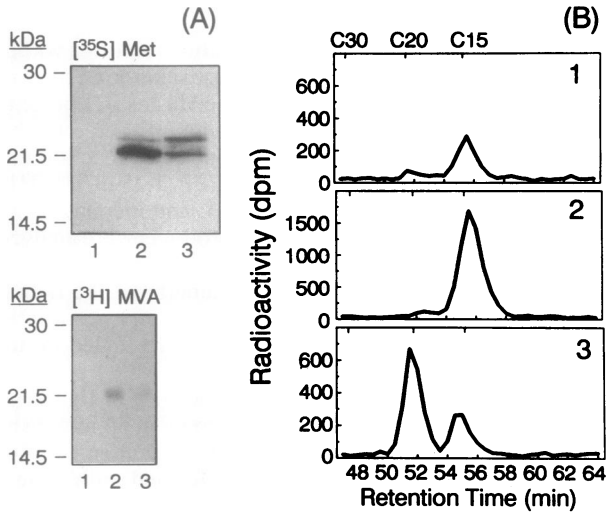

FIG. 3. Estimated sizes of [^{3}H]MVA-derived hydrocarbons released from Ha-ras proteins overexpressed in COS cells. COS cells were transfected with pCMV5 (no insert) (lanes 1), pCMV5-Hras(wt) (lanes 2), or pCMV5-Hras(Leu-189) (lanes 3). Twenty-four hours after transfection, cells were labeled for 18 h with either [^{35}S]methionine or [^{3}H]MVA, and ras proteins were recovered from cell lysates by immunoprecipitation. (*A*) (*Upper*) The entire [^{35}S]methionine-labeled immunoprecipitate from each culture was subjected to SDS/PAGE and fluorography (24-h exposure). Scintillation counting of gel slices indicated that the 21.5-kDa band contained 19,293 dpm (79% of total recovered radioactivity) in the case of the Ha-ras protein, and 8277 dpm (51% of the total recovered radioactivity) in the case of the Ha-ras(Leu-189) protein. (*Lower*) [^{3}H]MVA-labeled ras proteins were immunoprecipitated from parallel cultures and one-fifth of the protein was subjected to SDS/PAGE and fluorography (14 days of exposure). The remaining immunoprecipitated protein was eluted from adjacent lanes of the gel and the [^{3}H]MVA-derived isoprenyl group was released by reaction with Raney nickel. Recoveries were 86% (2816 dpm) for the control, 84% (36,547 dpm) for Ha-ras(wt), and 97% (15,652 dpm) for Ha-ras(Leu-189). (*B*) The size of the labeled isoprenyl group was then determined by subjecting aliquots of the saturated hydrocarbons to gel-permeation chromatography. Radioactivity loaded and column recoveries were as follows: control, 1642 dpm, 69%; Ha-ras(wt), 7354 dpm, 93%; Ha-ras(Leu-189), 2575 dpm, 100%. Retention times of the squalane (C30), phytane (C20), and farnesane (C15) standards are marked at the top of the chromatogram.

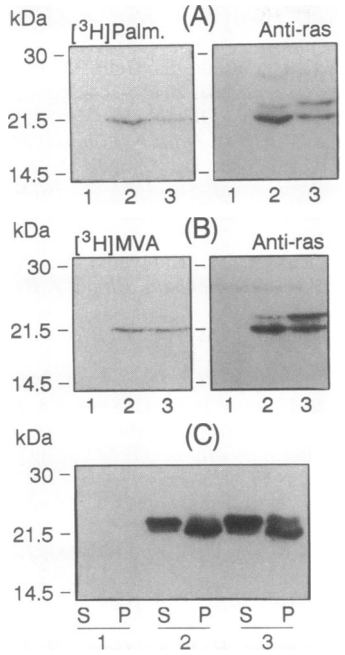

FIG. 4. [3H]Palmitate labeling and subcellular distribution of Ha-ras versus Ha-ras(Leu-189) proteins. COS cells were transfected with pCMV5 (no insert) (lanes 1), pCMV5-Hras(wt) (lanes 2), or pCMV5-Hras(Leu-189) (lanes 3). Cells were incubated for 18 h with [3H]palmitate (*A*) or [3H]MVA (*B*), beginning 24 h after transfection. Immunoprecipitated ras proteins were subjected to SDS/PAGE, transferred to nitrocellulose, and subjected to fluorography (17- and 13-day exposures for [3H]palmitate and [3H]MVA, respectively). After removing the fluor, the same blots were incubated with ras antibody, followed by ^{125}I-labeled goat anti-mouse IgG. (*C*) Cells from parallel cultures were fractionated into soluble (lanes S) and particulate (lanes P) components and the total protein in each fraction was subjected to SDS/PAGE and immunoblotting with ras antibody. The percentage of total ras protein in the particulate fraction, estimated by direct determination of bound ^{125}I was as follows: Ha-ras p21(wt), 57% (lanes 2); Ha-ras p21(Leu-189), 43% (lanes 3).

tion (Fig. 4*C*), suggesting that it was the fully processed form of Ha-ras p21 (3, 4). In cells overexpressing the mutant Ha-ras(Leu-189) protein, the 21.5-kDa ras protein was similarly localized in the particulate fraction and labeled with [3H]palmitate (Fig. 4 *A* and *C*). The [3H]palmitate signal in the immunoprecipitated Ha-ras p21(Leu-189), although weaker than in Ha-ras p21(wt), appeared to arise exclusively from overexpressed Ha-ras(Leu-189) protein, since palmitoylation of endogenous ras proteins in control COS cell cultures was not detected under identical conditions (Fig. 4*A*).

DISCUSSION

These studies establish that a single amino acid substitution (serine to leucine) at the COOH terminus of Ha-ras p21, a natural substrate for farnesyl modification, converts the protein to a substrate for geranylgeranyl modification *in vivo*, as well as *in vitro*. This observation supports the notion that occupancy of the terminal position (Xaa) of the Cys-Ali-Ali-Xaa motif by leucine in many of the known geranylgeranyl-modified proteins (18–21) constitutes a key structural element required for their recognition by a specific geranylgeranyltransferase. The importance of the terminal amino acid in specifying the isoprenyl modification is further underscored by recent studies in which synthetic variants of the Ki-rasB p21 COOH-terminal tetrapeptide Cys-Val-Ile-Met were evaluated as potential inhibitors of ras p21 farnesyltransferase. Most notably, substitutions in the terminal position normally occupied by methionine had the greatest effect on inhibitory activity, and peptides ending with leucine were generally poor inhibitors of farnesylation (10, 24).

Farnesylation is regarded as a prerequisite for palmitoylation and membrane localization of Ha-ras p21 (3–9). Geranylgeranylated proteins such as the brain G-protein γ_6 subunit (23), rap1B (19), and G25K (20) apparently undergo additional processing steps analogous to those described for Ha-ras p21; i.e., removal of three amino acids distal to the modified cysteine and methylation of the terminal COOH group. The studies described in Fig. 4 extend these observations insofar as they indicate that geranylgeranylated Ha-ras p21(Leu-189) can also undergo palmitoylation. The basis for the lower ratio of processed (21.5 kDa) to unprocessed (23 kDa) forms of ras protein observed in the cells overexpressing Ha-ras(Leu-189) (see Figs. 3 and 4) remains to be determined. Possibilities include a lower cellular activity of geranylgeranyltransferase relative to farnesyltransferase, or differences in the available pools of FPP versus GGPP isoprenyl donors. Although we did not assess the effect of a 20-carbon versus a 15-carbon modification on the transforming ability of ras, recent findings with a Ha-ras(Leu-61)/rap1A chimeric protein suggest that the transforming ability of oncogenic ras variants can be supported by a geranylgeranyl modification (18).

Mammalian cells contain many isoprenylated proteins whose identities have yet to be established (1, 2). In the case of proteins ending with the ras-like Cys-Ali-Ali-Met motif, the altered substrate specificity conferred by a single amino acid difference, coupled with the loss of isoprenylation observed when the position of the cysteine is changed or the three-amino acid extension (-Ali-Ali-Xaa) is deleted (10–12, 21, 24), implies that the structural elements recognized by the farnesyl- and geranylgeranyltransferases are quite stringent. On the other hand, our recent finding that rab1B and rab2 proteins can be modified by 20-carbon isoprenyl groups attached to cysteines within a very different COOH-terminal structural element (GGCC-COOH) (38) suggests that the enzymology of protein isoprenylation is more complex than heretofore suspected. It now seems probable that mammalian cells contain multiple protein:isoprenyltransferases, each recognizing a different class of target proteins with a distinct type of COOH-terminal motif.

This work was supported by Grant CA-34569 from the National Institutes of Health.

1. Glomset, J. A., Gelb, M. H. & Farnsworth, C. C. (1990) *Trends Biochem. Sci.* **15**, 139–142.
2. Maltese, W. A. (1990) *FASEB J.* **4**, 3319–3328.
3. Hancock, J. F., Magee, A. I., Childs, J. E. & Marshall, C. J. (1989) *Cell* **57**, 1167–1177.
4. Casey, P. J., Solski, P. A., Der, C. J. & Buss, J. E. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 8323–8327.
5. Schafer, W. R., Kim, R., Sterne, R., Thorner, J., Kim, S.-H. & Rine, J. (1989) *Science* **245**, 379–384.
6. Leonard, S., Beck, L. & Sinensky, M. (1990) *J. Biol. Chem.* **265**, 5157–5160.
7. Kim, R., Rine, J. & Kim, S.-H. (1990) *Mol. Cell. Biol.* **10**, 5945–5949.
8. Schaefer, W. R., Trueblood, C. E., Yang, C.-C., Mayer, M., Rosenberg, S., Poulter, C. D., Kim, S.-H. & Rine, J. (1990) *Science* **249**, 1133–1139.
9. Jackson, J. L., Cochrane, C. G., Bourne, J. R., Solski, P. A., Buss, J. E. & Der, C. J. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 3042–3046.
10. Reiss, Y., Goldstein, J. L., Seabra, M. C., Casey, P. J. & Brown, M. S. (1990) *Cell* **62**, 81–88.
11. Schaber, M. D., O'Hara, M. E., Garsky, V. M., Mosser, S. D., Bergstrom, J. D., Moores, S. L., Marshall, M. S., Friedman, P. A., Dixon, R. A. F. & Gibbs, J. B. (1990) *J. Biol. Chem.* **265**, 14701–14704.
12. Manne, V., Roberts, D., Tobin, A., O'Rourke, E., DeVirgilio,

M., Meyers, M., Meyers, C., Ahmed, N., Kurz, B., Resh, M., Kung, H.-F. & Barbacid, M. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 7541–7545.
13. Goodman, L. E., Judd, S. R., Farnsworth, C. C., Powers, S., Gelb, M. H., Glomset, J. A. & Tamanoi, F. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 9665–9669.
14. Clarke, S., Vogel, J. P., Deschenes, R. J. & Stock, J. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 4643–4647.
15. Stephenson, R. C. & Clarke, S. (1990) *J. Biol. Chem.* **265,** 16248–16254.
16. Rilling, H. C., Breunger, E., Epstein, W. W. & Crain, P. F. (1990) *Science* **247,** 318–319.
17. Farnsworth, C. C., Gelb, M. H. & Glomset, J. A. (1990) *Science* **247,** 320–322.
18. Buss, J. E., Quilliam, L. A., Kato, K., Casey, P. J., Solski, P. A., Wong, G., Clark, R., McCormick, F., Bokoch, G. M. & Der, C. J. (1991) *Mol. Cell. Biol.* **11,** 1523–1530.
19. Kawata, M., Farnsworth, C. C., Yoshida, Y., Gelb, M. H., Glomset, J. A. & Takai, Y. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 8960–8964.
20. Yamane, H. K., Farnsworth, C. C., Xie, H., Evans, T., Howald, W. N., Gelb, M. H., Glomset, J. A., Clarke, S. & Fung, B. K. K. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 286–290.
21. Kinsella, B. T., Erdman, R. A. & Maltese, W. A. (1991) *J. Biol. Chem.* **266,** 9786–9794.
22. Mumby, S. M., Casey, P. J., Gilman, A. G., Gutowski, S. & Sternweis, P. C. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 5873–5877.
23. Yamane, H. K., Farnsworth, C. C., Xie, H., Howald, W., Fung, B. K.-K., Clarke, S., Gelb, M. H. & Glomset, J. A. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 5868–5872.
24. Reiss, Y., Stradley, S. J., Gierasch, L. M., Brown, M. S. & Goldstein, J. L. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 732–736.
25. Farnsworth, C. C., Wolda, S., Gelb, M. H. & Glomset, J. A. (1989) *J. Biol. Chem.* **264,** 20422–20429.
26. Fukada, Y., Takao, T., Ohguro, H., Yoshizawa, T., Akino, T. & Shimonishi, Y. (1990) *Nature (London)* **346,** 658–660.
27. Lai, R. K., Perez-Sala, D., Canada, F. J. & Rando, R. R. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 7673–7677.
28. Reese, J. H. & Maltese, W. A. (1991) *Mol. Cell. Biochem.* **104,** 109–116.
29. Yoshida, Y., Kawata, M., Katayama, M., Horiuchi, H., Kita, Y. & Takai, Y. (1991) *Biochem. Biophys. Res. Commun.* **175,** 720–728.
30. Seabra, M., Reiss, Y., Casey, P. J., Brown, M. S. & Goldstein, J. L. (1991) *Cell* **65,** 429–434.
31. Keller, R. K. (1986) *J. Biol. Chem.* **261,** 12053–12059.
32. Chen, R. J. & Seeburg, P. H. (1985) *DNA* **4,** 165–170.
33. Sambrook, J., Fritsch, E. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
34. Maltese, W. A. & Robishaw, J. D. (1990) *J. Biol. Chem.* **265,** 18071–18074.
35. Maltese, W. A. & Sheridan, K. M. (1990) *J. Biol. Chem.* **265,** 17883–17890.
36. Vorburger, K., Kitten, G. T. & Nigg, E. A. (1989) *EMBO J.* **8,** 4007–4013.
37. Pollard, K. M., Chan, E. K. L., Grant, B. J., Sullivan, K. F., Tan, E. M. & Glass, C. A. (1990) *Mol. Cell. Biol.* **10,** 2164–2175.
38. Kinsella, B. T. & Maltese, W. A. (1991) *J. Biol. Chem.* **266,** 8540–8544.
39. Andersson, S., Davis, D. N., Dahlback, H., Hornwall, H. & Russell, D. W. (1989) *J. Biol. Chem.* **264,** 8222–8229.