Ras-related proteins are small GTPases that are posttranslationally modified with mevalonate-derived isoprenoids. Although the effects of inhibition of isoprenylation on protein function have been examined, the consequences of depletion of isoprenoid pools on regulation of expression of isoprenylated proteins have yet to be investigated. In these studies we have shown that depletion of mevalonate results in increased total levels of Ras, Rap1a, RhoA, and RhoB in K562 cells. Cycloheximide and [35S]methionine pulse/pulse-chase experiments reveal that mevalonate depletion increases the \textit{de novo} synthesis of Ras and RhoA and decreases the degradation of existing Ras and RhoA protein. Pretreatment with actinomycin D completely prevents the induced up-regulation of RhoB and only partially prevents the up-regulation of Ras, Rap1a, and RhoA. Although depletion of mevalonate does not alter steady state levels of Ras mRNA, there is an increase in RhoB mRNA. Our results are the first to demonstrate that mevalonate depletion induces up-regulation of Ras and Ras-related proteins by discrete mechanisms that include modulation of transcriptional, translational, and post-translational processes.

Members of the Ras protein superfamily, including Ras, Rap1a, and the Rho proteins, are membrane-bound small GTPases that cycle between an active GTP-bound state and an inactive GDP-bound state. These proteins influence fundamental cellular processes. For example, Ras plays a central role in signal transduction pathways regulating cell survival, proliferation, and differentiation (1). Rap1a has been shown to act as a negative regulator of Ras by binding to Ras effector proteins such as Raf-1, thus preventing Ras-induced Raf-1 activation (2). RhoA has been implicated as having a key role in regulating cytoskeletal organization (3, 4). The physiological function of RhoB remains largely unclear. RhoB knock-out mice do not exhibit developmental defects, diminished fertility, or impaired wound healing; however, there is abnormality in fibroblast motility (5). For these proteins, proper membrane association is believed to be necessary for normal function (6–8).

The Ras-related proteins become membrane-associated after undergoing a series of post-translational modifications, the first of which involves the addition of a 15-carbon farnesyl or a 20-carbon geranylgeranyl chain to a cysteine residue at the carboxyl terminus. Ras proteins are generally farnesylated (9), whereas Rap1a and RhoA are geranylgeranylated (10, 11). RhoB may be either farnesylated or geranylgeranylated (12, 13). These isoprenylation reactions are catalyzed by the enzymes farnesyl transferase and geranylgeranyl transferase 1, and the isoprenoid substrates in these reactions are derived from mevalonate. Addition of the lipid chain to the protein serves to anchor the protein to the membrane (6). It is generally believed that isoprenylation is required for the proteins to exert their biological effects; however, there is evidence to suggest that unmodified versions may also have functional effects (14, 15). These effects were observed in studies involving overexpression of mutant Rho proteins that could not be isoprenylated. Although there is substantial understanding of the results of inhibition of post-translational modification of these proteins, little is known of the effects of altered levels of these proteins on cell processes.

Statins competitively inhibit HMG-CoA reductase, the enzyme that converts HMG-CoA to mevalonate (16). Depletion of mevalonate is known to alter the expression of key proteins involved in isoprene metabolism, most notably HMG-CoA reductase. The level of HMG-CoA reductase is under multivalent control at both transcriptional and post-transcriptional sites, and this regulation is dependent on both sterol and non-sterol components of the cholesterol biosynthetic pathway (17–20). We and others have used statins as a tool to impair protein isoprenylation because depletion of mevalonate results in depletion of farnesyl pyrophosphate and geranylgeranyl pyrophosphate (21–23). The consequences of this impairment, or perhaps more appropriately, of mevalonate depletion on isoprenylated protein expression have not previously been investigated. To further understand the consequences of mevalonate depletion on expression of the small GTPases that are normally isoprenylated we have investigated the effects of mevalonate depletion on Ras, Rap1a, RhoA, and RhoB expression.

**EXPERIMENTAL PROCEDURES**

**Cell Cultures and Reagents**—The K562 cell line was purchased from the American Type Culture Collection (Manassas, VA). The K562 cell line is a human erythroleukemia line that was established from a patient with chronic myelogenous leukemia (24). K562 cells were grown in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, penicillin/streptomycin, amphotericin (2.5 μg/ml), and glutamine (2 mM). Cells were grown at 37 °C and 5% CO2 in T-75 culture flasks. Anti-RhoA, anti-RhoB, anti-Rap1A, anti-β-tubulin, and anti-goat IgG horseradish peroxidase antibodies as well as agarose-conjugated RhoA and actin antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The NCC-004 anti-pan Ras antibody

---

* This project was supported by the Leukemia and Lymphoma Society in the form of a translational research grant, the Roy J. Carver Charitable Trust, and the Roland W. Holden Family Program for Experimental Cancer Therapeutics. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1744 solely to indicate this fact.

† To whom correspondence should be addressed: Dept. of Internal Medicine, C32 GH, University of Iowa, Iowa City, IA 52242. Tel.: 319-356-8110; Fax: 319-353-8383; E-mail: raymond-hohl@uiowa.edu.
Ras, Rap1a, RhoA, and RhoB was examined via Western blot analysis. K562 cells were incubated with 10 μM lovastatin for 0–24 h with cells collected for Western blot analysis at 2-h intervals. As shown in Fig. 1, there was an increase in the levels of Ras, RhoA, RhoB, and Rap1a over time. For Ras this effect could be observed after only 2 h of incubation with lovastatin, as indicated by the appearance of the more slowly migrating band in the Ras immunoblot, suggesting unmodified Ras protein. By 24 h there was a 2-fold increase in the total amount of Ras present. RhoA levels increased to approximately 4-fold by 24 h. Although unmodified Rap1a could not be detected under control conditions, it was detectable after 2 h of lovastatin treatment, and levels increased over 24 h. RhoB levels increased significantly from 10 to 24 h (at least 5-fold) compared with control. As a control, it was shown that lovastatin did not alter β-tubulin levels.

Role of New Protein Synthesis in the Up-regulation of Ras and Ras-related Proteins—To determine whether the increases in Ras, RhoA, RhoB, and Rap1a levels induced by mevalonate depletion were dependent on the synthesis of new protein, cells were pretreated with the protein synthesis inhibitor cycloheximide (28) prior to incubation with lovastatin. As shown in Fig. 2, pretreatment with cycloheximide (1.4 μg/ml) completely blocked mevalonate depletion-induced up-regulation of RhoA and RhoB and partially blocked the increase in Ras and Rap1a. Higher concentrations of cycloheximide (10 μg/ml or 20 μg/ml) further reduced the increase in Ras and Rap1a levels (data not shown). Treatment with cycloheximide alone did not significantly alter the levels of Ras, RhoA, or tubulin. As with untreated cells, unmodified Rap1a was not detected in cells treated with cycloheximide, and RhoB was minimally detected.

To more carefully examine the effects of mevalonate depletion on de novo synthesis of isoprenylated proteins, experiments were performed in which cells were labeled with [35S]methionine. Cells were incubated with or without 10 μM lovastatin for 4, 8, 12, and 24 h. Cells were pulsed with [35S]methionine (120 μCi/ml) for 4 h. Cells were then washed with complete RPMI medium plus 10% FBS, 3% cysteine, and 10% fetal calf serum and incubated for 0–24 h in the presence or absence of lovastatin and/or mevalonate. Cells were lysed in RIPA buffer, and following pre-clearing, 200 μg of whole cell lysate was diluted in RIPA + 1% bovine serum albumin and incubated with agaroose-conjugated antibodies at 4 °C. Ras, RhoA and actin immunocomplexes were obtained per the manufacturer’s protocol. Immunocomplexes were washed with RIPA + 1% bovine serum albumin and 1× phosphate-buffered saline + 1% bovine serum albumin. The pellets were fractionated by SDS-PAGE, and dried gels were exposed to film at −70 °C. The radioactivity of excised bands was determined using liquid scintillation counting.

Preparation and Synthesis of RNA Probe Templates—All RNA probes used in this study were generated in our laboratory using reverse transcriptase-polymersase chain reaction with the addition of an RNA polymerase site (LigT'scribe, Ambion, Austin, TX) to the RT-PCR product. A 580-bp human RhoB cDNA was generated using the following primer sets: 5′-GCC TCA TGG GGA GCA GGT AGC TGT-3′ and 5′-GTT TAC GAT TTT TGG GAG GCC ATT-3′. A 194-bp human Ha-Ras cDNA was generated using the following primer sets: 5′-ACC TAC TAC TGC CTA TCA TCC GAG TCT AC AAT TTA GGG-3′ and 5′-GGG AGA AGA GTA AGA GCT ATC GGT TGG TCA AT-3′. An additional 60 bp was added to all of the above templates when the RT reaction polymerase site was added to the cDNA using the LigT'scribe RNA Polymerase Promoter Addition Kit (Ambion). Each probe template was sequenced using a ABI Prism Genetic Analyzer (PerkinElmer Life Sciences) prior to use.

Preparation of RNA and Northern Blot Analysis—Total RNA was isolated from cells using the single-step method (27), lysing the cells in 1.2 ml of Trizol Reagent (Invitrogen). Chloroform was added and the total RNA precipitated from the aqueous phase by the addition of isopropyl alcohol, the RNA pellet washed with ethanol and solubilized in RNase-free water. The yield and purity of the total RNA were quantitated by measuring the ratio of the absorbance at 260 and 280 nm. RNA integrity was determined by examining the 28 and 18 S RNA bands on a 1.2% agarose, 2.2M M formaldehyde gel, transferred to Hybond-N+ (Amersham Life Sciences, Inc.) membrane by capillary action overnight and UV-crosslinked. The RNA containing membranes were prehybridized at 68 °C for 5 h in UltraHyb Hybridization Buffer (Ambion). The 32P-labeled antisense riboprobe, at a concentration of 1 × 106 cpm/ml, was added to the hybridization buffer, and the blots were hybridized overnight at 68 °C. The blots were then washed twice in 2× SSC, 0.1% SDS at 68 °C for 5 min each followed by two washes in 0.1× SSC, 0.1% SDS at 68 °C. The blots were then exposed to x-ray film at −70 °C.

RESULTS

Mevalonate Depletion Increases Ras and Ras-related Protein Levels—The effect of mevalonate depletion on total levels of Ras, Rap1a, RhoA, and RhoB was examined by Western blot analysis. K562 cells were incubated with 10 μM lovastatin for 0–24 h with cells collected for Western blot analysis at 2-h intervals. As shown in Fig. 1, there was an increase in the levels of Ras, RhoA, RhoB, and Rap1a over time. For Ras this effect could be observed after only 2 h of incubation with lovastatin, as indicated by the appearance of the more slowly migrating band in the Ras immunoblot, suggesting unmodified Ras protein. By 24 h there was a 2-fold increase in the total amount of Ras present. RhoA levels increased to approximately 4-fold by 24 h. Although unmodified Rap1a could not be detected under control conditions, it was detectable after 2 h of lovastatin treatment, and levels increased over 24 h. RhoB levels increased significantly from 10 to 24 h (at least 5-fold) as compared with control. As a control, it was shown that lovastatin did not alter β-tubulin levels.

Role of New Protein Synthesis in the Up-regulation of Ras and Ras-related Proteins—To determine whether the increases in Ras, RhoA, RhoB, and Rap1a levels induced by mevalonate depletion were dependent on the synthesis of new protein, cells were pretreated with the protein synthesis inhibitor cycloheximide (28) prior to incubation with lovastatin. As shown in Fig. 2, pretreatment with cycloheximide (1.4 μg/ml) completely blocked mevalonate depletion-induced up-regulation of RhoA and RhoB and partially blocked the increase in Ras and Rap1a. Higher concentrations of cycloheximide (10 μg/ml or 20 μg/ml) further reduced the increase in Ras and Rap1a levels (data not shown). Treatment with cycloheximide alone did not significantly alter the levels of Ras, RhoA, or tubulin. As with untreated cells, unmodified Rap1a was not detected in cells treated with cycloheximide, and RhoB was minimally detected.

To more carefully examine the effects of mevalonate depletion on de novo synthesis of isoprenylated proteins, experiments were performed in which cells were labeled with [35S]methionine. Cells were incubated with or without 10 μM lovastatin for 4, 8, 12, and 24 h. Cells were pulsed with [35S]methionine (120 μCi/10 × 106 cells) during the last 4 h of each incubation, and Ras, RhoA, and actin were immunoprecipitated. As shown in Fig. 3, A and B, incubation with lovastatin increased the amount of newly synthesized Ras and RhoA at each time point compared with control cells. In addition, as seen by the presence of the more slowly migrating band, the 35S-labeled Ras population in mevalonate-depleted cells was composed solely of unmodified Ras. As a control, synthesis of actin was examined, and it was found that depletion of mevalonate did not alter the level of 35S-labeled actin (Fig. 3C). Trichloroacetic acid precipitation studies revealed that the incorporation of [35S]methionine into total protein pools was not significantly altered by treatment with lovastatin (data not shown).

Role of Protein Turnover in the Up-regulation of Ras and Ras-related Proteins—Pulse-chase experiments were per-
FIG. 2. Effects of pretreatment with cycloheximide on up-regulation of Ras and Ras-related proteins induced by mevalonate depletion. K562 cells were pretreated with cycloheximide (1.4 μg/ml) for 1 h prior to the addition of lovastatin. Cells were collected every 2 h. These immunoblots were developed as described under “Experimental Procedures.” Each lane contains an equivalent amount of protein from cell lysate. The results are representative of two independent experiments.

FIG. 3. Mevalonate depletion increases de novo synthesis of Ras and RhoA. K562 cells were incubated with or without lovastatin (10 μM) for 4, 8, 12, and 24 h. Cells were pulsed with [35S]methionine (120 μCi/10 × 10⁶ cells) during the last 4 h of each incubation. Ras (A), RhoA (B), and actin (C) were immunoprecipitated, fractionated on SDS-PAGE, and exposed to film at −70 °C for 2–5 days. Representative gels from duplicate experiments are displayed. Bands were subsequently excised and radiolabel counted via liquid scintillation counting. For Ras and RhoA the counts reflect radiolabel from cells incubated withLovastatin.

FIG. 4. Mevalonate depletion decreases the rate of Ras and RhoA degradation. Cells were pulse-chased for 24 h in the presence of lovastatin and/or mevalonate. As shown in Fig. 4A, there was a decline in labeled Ras over 24 h. Mevalonate depletion decreased the degradation of labeled Ras. On the basis of these experiments, the half-life of Ras was estimated to be 19 h under control conditions and 30 h in lovastatin-treated cells. Depletion of mevalonate also decreased the rate of degradation of RhoA (Fig. 4B). The half-life of RhoA was also found to be prolonged from 24 h in control cells to 34 h inLovastatin-treated cells. As a control, the degradation of actin was examined, and it was found that mevalonate depletion did not alter the half-life of actin (Fig. 4C). The loss of 35S from total protein pools over time in the pulse-chase experiments was not affected by incubation withLovastatin.

Reversal of Lovastatin-induced Changes by Mevalonate—To verify that the effects of lovastatin on protein synthesis and degradation were due to depletion of mevalonate, pulse and pulse-chase experiments were performed with or without the addition of mevalonate. For the pulse experiments, cells were incubated for 24 h withlovastatin (10 μM) and/or mevalonate (5 μM) and pulsed with [35S]methionine during the last 4 h of the incubation. As shown in Fig. 5A, coincubation of mevalonate prevented theLovastatin-induced increase in [35S]methionine incorporation into newly synthesized Ras and RhoA. Treatment with mevalonate alone did not alter the level of labeled Ras or RhoA. Pulse-chase experiments were also performed in which cells were pulsed for 4 h with [35S]methionine and then chased for 24 h in the presence oflovastatin and/or mevalonate. Coincubation with mevalonate prevented theLovastatin-induced alteration in Ras and RhoA degradation (Fig. 5B). As a control, the effects ofLovastatin and/or mevalonate on actin synthesis and degradation were also examined, and no differences were observed.

Dependence of Protein Up-regulation on Pretranslational Events—To determine whether protein up-regulation by mevalonate depletion requires new mRNA synthesis, cells were pretreated with actinomycin D, an inhibitor of transcription. As shown in Fig. 6, pretreatment with actinomycin D (0.5 μg/ml) prevented the increase inRhoB levels and partially blocked the up-regulation of Ras, Rap1a, and RhoA induced by mevalonate depletion. As a control, cells were also incubated with actinomycin D alone, and no changes were observed.

To further examine the effects of mevalonate depletion on transcription, Northern blot analyses were performed. As shown in Fig. 7, incubation with Lovastatin for 0–24 h did not alter the steady state levels of Ha-Ras or N-Ras mRNA. Unlike Ha-Ras and N-Ras, however, depletion of mevalonate did alter RhoB mRNA levels. As demonstrated in Fig. 7, there was a progressive increase in RhoB mRNA levels with this depletion. The increase inRhoB message was completely blocked by pretreatment with actinomycin D (data not shown).

DISCUSSION

For many years it has been recognized that depletion of mevalonate by inhibition of HMG-CoA reductase alters the post-translational processing of CAAX-containing small GTPase proteins (21, 22). We have provided the first evidence demonstrating that mevalonate depletion alters the regulation of expression of CAAX proteins. In this context, there are relatively limited prior publications with which to integrate our findings. Fig. 1 clearly...
Effects of Mevalonate Depletion on Ras, Rap1a, RhoA, and RhoB

For these proteins the $t_{1/2}$ was similarly prolonged by $-58\%$ for Ras and $42\%$ for RhoA. Because base-line levels of RhoB are only minimally-detectable, it is not possible to determine the effects of mevalonate depletion on protein half-life in this system. There has been very limited data published on the $t_{1/2}$ or regulation of production/degradation of these small GTPases. That the $t_{1/2}$ for Ras in K562 cells is estimated at $19\ h$ (Fig. 4A) is in agreement with the $t_{1/2}$ of $20\ h$ for Ras described in transfected NIH-3T3 cells (29). In these latter cells the $t_{1/2}$ of Ras may have been in part correlated with its phosphorylation state. Similarly, the $t_{1/2}$ for RhoA in K562 cells is estimated at $24\ h$ (Fig. 4B). This is in relative agreement with the reported $t_{1/2}$ for RhoA of $31\ h$, albeit in RAW264 cells (30). In these cells the $t_{1/2}$ for RhoA appeared to be decreased to $12\ h$ with carboxyl methylation inhibition. Although the prior studies describing the $t_{1/2}$ for Ras and RhoA implicated phosphorylation and carboxyl methylation as being important, the basis for the $t_{1/2}$ regulation was not explored. Our studies of K562 cells implicate the mevalonate-derived isoprenoid pool as also contributing to the degradation of these proteins. It is of interest that the reduction of these isoprenoids decreases the degradation of already isoprenylated Ras and RhoA. This finding suggests regulatory pathways to sustain levels of isoprenylated Ras and RhoA under conditions that would otherwise limit protein isoprenylation.

Recent studies have indicated that lovastatin may have additional functions independent of its inhibition of HMG-CoA reductase (31, 32). For example, lovastatin binds to the I domain of $\alpha_{i}\beta_{2}$ integrin and thus blocks the interaction of this integrin with ICAM-1 (intercellular adhesion molecule 1) (33, 34). This process was not reversed by the addition of mevalonate. Fig. 5 demonstrates that the effects of lovastatin on Ras and RhoA synthesis and degradation are due to inhibition of HMG-CoA reductase. Coincubation of cells with both lovastatin and mevalonate negates the effects of lovastatin.

To further dissect the mechanisms for mevalonate depletion-induced up-regulation of proteins, studies were performed utilizing actinomycin D. Fig. 6 displays that inhibition of DNA-dependent RNA polymerase completely abrogates the increase in RhoB protein observed with lovastatin (Fig. 1). For Ras, Rap1a, and RhoA, pretreatment with actinomycin D diminishes lovastatin-induced up-regulation. Message levels of Ras and RhoB were examined because of the differential responses of Ras and RhoB with regard to timing of up-regulation and effect of actinomycin D. Fig. 7 demonstrates that mevalonate depletion does not significantly alter steady state mRNA levels of Ha-Ras or N-Ras but does induce a progressive increase in

---

**Fig. 4.** The rate of degradation of Ras and RhoA is decreased by mevalonate depletion. K562 cells were pulsed with $^{[35]S}$methionine (120 $\mu$Ci/ml) for 4 h and then chased in the absence or presence of lovastatin (10 $\mu$m). Ras (A), RhoA (B), and actin (C) were immunoprecipitated, fractionated on SDS-PAGE, and exposed to film at $-70\ ^\circ C$ for 3-5 days. Bands were subsequently excised and counted via liquid scintillation counting. For Ras and RhoA the counts are expressed as a percentage of the radioactivity at the conclusion of the pulse and are displayed in semi-log plots. The results are representative of two independent experiments.

**Fig. 5.** Coincubation with mevalonate prevents lovastatin-induced changes in Ras and RhoA synthesis and degradation. A. K562 cells were incubated with 10 $\mu$m lovastatin (Lov) and/or 5 mM mevalonate (Mev) for 24 h and pulsed with $^{[35]S}$methionine during the last 4 h. B, cells were pulsed with $^{[35]S}$methionine for 4 h and then chased for 24 h in the presence of lovastatin and/or mevalonate. Ras, RhoA, and actin were immunoprecipitated as described under “Experimental Procedures.” Control cells were incubated without drugs. The results are representative of two independent experiments.

For these proteins the $t_{1/2}$ was similarly prolonged by $-58\%$ for Ras and $42\%$ for RhoA. Because base-line levels of RhoB are only minimally-detectable, it is not possible to determine the effects of mevalonate depletion on protein half-life in this system. There has been very limited data published on the $t_{1/2}$ or regulation of production/degradation of these small GTPases. That the $t_{1/2}$ for Ras in K562 cells is estimated at $19\ h$ (Fig. 4A) is in agreement with the $t_{1/2}$ of $20\ h$ for Ras described in transfected NIH-3T3 cells (29). In these latter cells the $t_{1/2}$ of Ras may have been in part correlated with its phosphorylation state. Similarly, the $t_{1/2}$ for RhoA in K562 cells is estimated at $24\ h$ (Fig. 4B). This is in relative agreement with the reported $t_{1/2}$ for RhoA of $31\ h$, albeit in RAW264 cells (30). In these cells the $t_{1/2}$ for RhoA appeared to be decreased to $12\ h$ with carboxyl methylation inhibition. Although the prior studies describing the $t_{1/2}$ for Ras and RhoA implicated phosphorylation and carboxyl methylation as being important, the basis for the $t_{1/2}$ regulation was not explored. Our studies of K562 cells implicate the mevalonate-derived isoprenoid pool as also contributing to the degradation of these proteins. It is of interest that the reduction of these isoprenoids decreases the degradation of already isoprenylated Ras and RhoA. This finding suggests regulatory pathways to sustain levels of isoprenylated Ras and RhoA under conditions that would otherwise limit protein isoprenylation.

Recent studies have indicated that lovastatin may have additional functions independent of its inhibition of HMG-CoA reductase (31, 32). For example, lovastatin binds to the I domain of $\alpha_{i}\beta_{2}$ integrin and thus blocks the interaction of this integrin with ICAM-1 (intercellular adhesion molecule 1) (33, 34). This process was not reversed by the addition of mevalonate. Fig. 5 demonstrates that the effects of lovastatin on Ras and RhoA synthesis and degradation are due to inhibition of HMG-CoA reductase. Coincubation of cells with both lovastatin and mevalonate negates the effects of lovastatin.

To further dissect the mechanisms for mevalonate depletion-induced up-regulation of proteins, studies were performed utilizing actinomycin D. Fig. 6 displays that inhibition of DNA-dependent RNA polymerase completely abrogates the increase in RhoB protein observed with lovastatin (Fig. 1). For Ras, Rap1a, and RhoA, pretreatment with actinomycin D diminishes lovastatin-induced up-regulation. Message levels of Ras and RhoB were examined because of the differential responses of Ras and RhoB with regard to timing of up-regulation and effect of actinomycin D. Fig. 7 demonstrates that mevalonate depletion does not significantly alter steady state mRNA levels of Ha-Ras or N-Ras but does induce a progressive increase in
Ha-Ras-, N-Ras-, or RhoB-specific riboprobes as described under "Experimental Procedures." Each lane contains an equivalent amount of protein from cell lysate. Representative gels from duplicate experiments are displayed.

RhoB mRNA. These results suggest an explanation for why the increase in total Ras levels occurs earlier than that of RhoB. The early up-regulation of Ras is less dependent on production of mRNA than is the later occurring up-regulation of RhoB. The timing of the increase in RhoB mRNA coincides with the increase in RhoB protein level. Heretofore relatively little work has been published on the transcriptional regulation of Ras and Ras-related small GTPases. RhoB has been shown to be transcriptionally activated by genotoxic stress mediated via a CCAAT element (35). In addition, sequence analysis of the rhoB promoter identifies TATA, Sp1, and CAAT box elements and AP-2, AP-4, and p53 consensus sequences (36). The interaction of mevalonate-derived isoprenoids with these binding sequences or with their associated transcription factors has yet to be investigated. MAST er transcriptional regulators of cholesterol biosynthesis influence HMG-CoA reductase expression (37). These studies provide potential targets for additional investigation directed toward understanding the influence of mevalonate depletion on expression of Ras-related proteins.

In summary, while mevalonate depletion is known to up-regulate some proteins such as HMG-CoA reductase, our studies are the first to reveal that such depletion induces the up-regulation of Ras, Rap1α, RhoA, and RhoB. Mechanisms underlying this up-regulation are shown to be increased mRNA synthesis, increased protein synthesis, and decreased protein degradation. Interestingly, the relative contribution of these discrete mechanisms to the up-regulation differs among these Ras-related proteins. Because mevalonate depletion results in a decrease in the levels of farnesyl pyrophosphate and geranylgeranylated pyrophosphate, one might hypothesize that this depletion might similarly alter the levels of farnesylated and geranylgeranylated proteins. Although there is similar up-regulation of farnesylated (Ras, RhoB) and geranylgeranylated (Rap1α, RhoA, RhoB) proteins, the mechanism for the up-regulation differs for proteins both within and between these two groups. Future studies of the effects of mevalonate-derived isoprenoids will further advance the understanding of the regulatory mechanisms identified by our studies that influence expression of these isoprenylated proteins.

REFERENCES
1. Vojtek, A. B., and Der, C. J. (1998) J. Biol. Chem. 273, 19925–19928
2. Hu, C. D., Kariya, K., Kotani, G., Shirouzu, M., Yokoyama, S., and Kataoka, T. (1997) J. Biol. Chem. 272, 11702–11705
3. Takai, Y., Sasaki, T., Tanaka, K., and Nakashiki, H. (1995) Trends Biochem. Sci. 20, 227–231
4. Van Aelst, L., and D’Souza-Schorey, C. (1997) Genes Dev. 11, 2295–2322
5. Liu, A.-X., Rane, N., Liu, J.-H., and Prendergast, G. C. (2001) Mol. Cell. Biol. 21, 6906–6912
6. Hancock, J. F., Magee, A. I., Childs, J. E., and Marshall, C. J. (1989) Cell 57, 1167–1177
7. Kato, K., Casev, P. J., Soksi, P. A., Der, C. J., and Buss, J. E. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 8323–8327
8. Buss, J. E., Quilliam, L. A., Kato, K., Casey, P. J., Solski, P. A., Wong, G., Clark, R., McCormick, P., Bokoch, G. M., and Der, C. J. (1991) Mol. Cell. Biol. 11, 1523–1530
9. Katayama, M., Kawata, M., Yoshida, Y., Horuchi, H., Yamamoto, T., Matsuzo, Y., and Takai, Y. (1991) J. Biol. Chem. 266, 12639–12645
10. Adamson, P., Marshall, C. J., Hall, A., and Tilburn, P. A. (1992) J. Biol. Chem. 267, 20033–20038
11. Lebowitz, P. F., Casey, P. J., Prendergast, G. C., and Thissen, J. A. (1997) J. Biol. Chem. 272, 15591–15594
12. Lebowitz, P. F., Du, W., and Prendergast, G. C. (1997) J. Biol. Chem. 272, 16093–16095
13. Allal, C., Favre, G., Courderc, B., Salicio, S., Sixou, S., Hamilton, A. D., Sebti, S., Lajus-Mazenc, I., and Pradines, A. (2000) J. Biol. Chem. 275, 31001–31008
14. Grundy, S. M. (1988) N. Engl. J. Med. 319, 24–33
15. Goldstein, J. L., and Brown, M. S. (1990) Nature 343, 425–430
16. Nakashiki, M., Goldstein, J. L., and Brown, M. S. (1988) J. Biol. Chem. 263, 8929–8937
17. Roitelman, J., and Simoni, R. D. (1992) J. Biol. Chem. 267, 25264–25273
18. Straka, M. S., and Panini, S. R. (1995) Arch. Biochem. Biophys. 317, 235–243
19. Schaffer, W. R., Kim, R., Sterne, R., Thorner, J., Kim, S. H., and Rine, J. (1989) Science 245, 379–385
20. Leonard, S., Beck, L., and Sinensky, M. (1990) J. Biol. Chem. 265, 5157–5160
21. Hoh, R. J., and Lewis, K. (1995) J. Biol. Chem. 270, 17508–17512
22. Lopizzo, C. B., and Loizzo, B. B. (1975) J. Biol. Chem. 250, 321–334
23. Kanai, T., Hirohashi, S., Noguchi, M., Shimoyama, Y., Shimosato, Y., Noguchi, S., Nishimura, S., and Abe, O. (1997) Jpn. J. Cancer Res. 88, 1314–1318
24. Lowry, O. H., Rosenbrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
25. Chomczynski, P., and Sacchi, N. (1987) Anal. Biochem. 162, 156–159
26. Ohara, T., Gelp, W. J., McKeegan, W. L., and Hardesty, B. (1971) J. Biol. Chem. 246, 174–181
27. Ulsh, L. S., and Shih, Y. T. (1984) Mol. Cell. Biol. 4, 1647–1652
28. Backlund, P. S., Jr. (1997) J. Biol. Chem. 272, 31375–31380
29. Bellotta, S., Ferri, N., Bernini, F., Paolotti, R., and Corini, A. (2000) Ann. Med. 32, 164–176
30. Frenette, P. S. (2001) N. Engl. J. Med. 345, 1419–1421
31. Kallen, J., Welzenbach, K., Ramage, P., Geyl, D., Kriwacki, R., Legge, G., Cottens, S., Weitz-Schmidt, G., and Hommel, U. (1999) J. Mol. Biol. 292, 1–9
32. Weitz-Schmidt, G., Welzenbach, K., Brinkmann, V., Kamata, T., Kallen, J., Bruns, C., Cottens, S., Takada, Y., and Hommel, U. (2001) Nat. Med. 7, 687–692
33. Fritz, G., and Kaina, B. (2001) Nucleic Acids Res. 29, 792–798
34. Fritz, G., and Kaina, B. (1997) J. Biol. Chem. 272, 30637–30644
35. Edwards, P. A., Tabor, D., Kast, H. R., and Venkatesswaran, A. (2000) Biochem. Biophys. Acts 1529, 105–113

FIG. 6. Effects of pretreatment with actinomycin D on up-regulation of Ras and Ras-related proteins induced by mevalonate depletion. K562 cells were pretreated with actinomycin D (0.5 μg/ml) for 1 h prior to addition of lovastatin. Cells were collected every 2 h. These immunoblots were developed as described under “Experimental Procedures.” Each lane contains an equivalent amount of protein from cell lysate. Representative gels from duplicate experiments are displayed.

FIG. 7. Effects of mevalonate depletion on Ha-Ras, N-Ras, and RhoB mRNA levels. K562 cells were incubated with 10 μM lovastatin for up to 24 h. Total RNA was isolated, fractionated on a 1.2% agarose, 2.2 M formaldehyde gel, transferred to membrane, and probed with Ha-Ras-, N-Ras-, or RhoB-specific riboprobes as described under “Experimental Procedures.” The lower panel depicts an ethidium bromide-stained gel.