We have used specific inhibitors for farnesyltransferase (FTase) and geranylgeranyltransferase (GGTase) I as well as combinations of lovastatin with geranylgeraniol (GGOH) or farnesol (FOH) to investigate the role of protein prenylation in platelet-derived growth factor (PDGF)-induced PDGF receptor tyrosine phosphorylation. NIH-3T3 cells treated with the highly specific FTase inhibitor FTI-277 had no effect on PDGF receptor tyrosine phosphorylation, or PDGF activation of mitogen-activated protein kinase (MAPK) at doses that completely inhibit FTase-dependent processing. In contrast, treatment of these cells with GGTase I inhibitor GGTI-298 strongly inhibited receptor tyrosine phosphorylation, and co-treatment with FTI-277 had no additional effect. Interestingly, the inhibitory effect of GGTI-298 on PDGF activation of MAPK was only partial. Furthermore, although lovastatin, which inhibits both protein geranylgeranylation and protein farnesylation, blocked PDGF receptor tyrosine phosphorylation, co-treatment with GGOH, but not FOH, reversed the lovastatin block. In addition, although lovastatin was observed to block MAPK activation by PDGF, co-treatment with GGOH, but not FOH, restored its activation. Further investigations indicated that inhibition of receptor tyrosine phosphorylation was not due to decreased expression of the receptor or to inhibition of GGTase II. Thus, these results demonstrate that PDGF receptor tyrosine phosphorylation requires protein geranylgeranylation but not protein farnesylation and that the tyrosine phosphorylation levels of the receptor are modulated by a protein that is a substrate for GGTase I.

Prenylation is the process by which proteins are lipid-modified by the cholesterol biosynthesis intermediates farnesylpyrophosphate and geranylgeranylpyrophosphate (1). Several proteins, notably small G-proteins, require prenylation for proper cellular localization and function. These modifications are accomplished by enzymes capable of covalently attaching a farnesyl or a geranylgeranyl group to cysteine sulphydryls at the protein carboxyl terminal. Farnesyltransferase (FTase) attaches farnesyl to proteins that end with the tetrapeptide consensus sequence CAAX, in which C is cysteine, A is an aliphatic amino acid, and X is any amino acid except leucine or isoleucine, preferably methionine or serine (2). There are two geranylgeranyltransferases; GGTase I transfers geranylgeranyl to proteins containing a CAAX sequence in which X is leucine or isoleucine, whereas GGTase II transfers geranylgeranyl to proteins terminating in CC or CXC (3, 4). FTase and GGTase I are α/β heterodimers that share the α subunit. In vitro studies have shown that both enzymes are capable of binding farnesylpyrophosphate and geranylgeranylpyrophosphate, but only GGTase I can use both isoprenoids as substrates for protein prenylation (3, 5).

Ras, Rho, and Rac are small G-proteins that are prenylated and that play a pivotal role in cell growth regulation. For example, Rho and Rac have been recently shown to be implicated in the G1 to S phase transition of the cell cycle (6). Ras proteins cycle between their GDP (inactive) and GTP (active) states to transduce growth factor signals from cell surface receptor tyrosine kinases to the nucleus. This pivotal role in cell growth regulation requires plasma membrane localization of Ras, which is mediated by prenylation (7).

Mammalian cells express three different ras genes that encode four Ras proteins (Ha-Ras, N-Ras, KB-Ras, and Kα-Ras). Early in vitro work indicated that the CAAX sequence for all Ras isoforms displays a preference for farnesylation (2). Furthermore, farnesylation of oncogenic Ras has been shown to be required for its transforming activity (8, 9). This has led to a major effort aimed at designing inhibitors of FTase as potential anticancer drugs. Over the last 3 years, several groups have shown that CAAX peptidomimetic inhibitors of FTase can selectively inhibit Ha-Ras processing as well as oncogenic Ha-Ras-dependent signaling, transformation in vitro, and tumor growth in vivo (10–20). However, further investigation revealed that, in contrast to Ha-Ras, K-Ras in B is less sensitive to FTase inhibitors (11, 21). This was an important finding, as K-Ras in B is encoded by the most frequently mutated ras gene in human cancers. Recently, we have demonstrated that a potent GGTase I inhibitor can block K-Ras in B processing and oncogenic signaling (22). This has suggested that K-Ras in B may be prenylated by GGTase I as well as FTase. Thus, a combination of both GGTase I and FTase inhibitors may be required for inhibition of K-Ras in B-dependent tumor growth.

The mechanism by which prenylation inhibitors antagonize oncogenic signaling is ill understood (11, 19). Furthermore, very little is known about the effects of these inhibitors on growth factor signaling in normal cells. Although the involvement of prenylated small G-proteins in cell cycle progression and cell division has been suggested, the respective contributions of farnesylated and/or geranylgeranylated proteins is not...
known. We, therefore, used FTase and GGTase I inhibitors to determine the effects of prenylation on one of the earliest steps in growth factor signaling, that of tyrosine phosphorylation of the growth factor receptor itself.

Binding of growth factors such as platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) to their respective receptor tyrosine kinases results in autophosphorylation of the receptors. The resulting phosphoryrinosines recruit Src homology 2-containing signaling molecules. Among these, the growth factor receptor binding protein Grb-2 is recruited as a complex with a nucleotide exchange factor mSOS-1, which activates Ras by exchanging GDP for GTP. GTP-bound Ras then activates a cascade of MAPKs by first recruiting to the plasma membrane a Ser-Thr kinase, c-Raf-1 (23). c-Raf-1 activates MAPK kinase, which activates MAPK, which in turn can translocate to the nucleus and phosphorylate transcription factors (23). Although the Ras-MAPK pathway is the most thoroughly studied, several other pathways are also activated when Ras homology 2-containing proteins such as phospholipase C and phosphatidylinositol-3-kinase bind to distinct phosphoryrinosines on the receptors (24; reviewed in Ref. 25).

The only two studies that have investigated the effects of prenylation inhibitors on growth factor signaling in normal cells revealed that FTase inhibitors did not affect EGF- and PDGF-induced MAPK activation in Rat-1 and NIH-3T3 cells, respectively (17, 26). Recently, we found that lovastatin, a cholesterol biosynthesis inhibitor known to lower the levels of protein farnesylation and protein geranylgeranylation, decreases the levels of ligand-induced, tyrosine-phosphorylated PDGF and insulin receptors (27, 28). Similar effects were also observed when an FTase inhibitor, FTI-244, was used (26). However, FTI-244 is not highly selective for FTase over GGTase I (IC50 values in vitro, 0.2 and 2 μM, respectively), and whether its ability to decrease receptor tyrosine phosphorylation levels was due to inhibition of farnesylation and/or geranylgeranylation was not clear. In the present report, we have designed and synthesized a modestly potent and selective FTase and GGTase I inhibitor (GGTI-297) and used it along with a highly selective FTase inhibitor (FTI-277; see Ref. 11), as well as lovastatin, geranylgeraniol (GGOH), and farnesol (FOH) treatments, to demonstrate that protein geranylgeranylation, but not farnesylation, is required for proper PDGF-induced PDGF receptor tyrosine phosphorylation.

MATERIALS AND METHODS

Synthesis of CAAAX Peptidomimetics—The FTase-specific peptidomimetic FTI-276 and its methyl ester counterpart FTI-277 were synthesized by the routes described previously (11). The GGTase I-specific peptidomimetic GGTI-298 was synthesized as follows. Methyl 2-bromo-4-nitrobenzoate was coupled with a-α-naphthylboronic acid to form 2-naphthyl-4-amino benzoic acid. The ability of GGTI-297 to inhibit FTase and GGTase I was evaluated as described under “Materials and Methods.” Table I shows that GGTI-297 inhibits FTase (1 IC50, 0.5 nM) compared with GGTase I (IC50, 50 nM) (Table I). Thus, in vitro, FTI-276 is 100-fold more selective toward FTase, whereas GGTI-297 is 4-fold more selective.
PDGFR Tyr Phosphorylation and Protein Prenylation

\[ R = H, \quad \text{FTI-276} \]
\[ R = \text{CH}_3, \quad \text{FTI-277} \]

**Fig. 1. Structures of the FTase and GGTase I CAAX peptidomimetic inhibitors.** The inhibitors as their free acids (R = H), FTI-276 and GGTI-297, represent the active forms of the compounds in vitro toward FTase and GGTase I, respectively, whereas the methyl ester analogues (R = CH_3) are the prodrug forms used to inhibit protein farnesylation and geranylgeranylation, respectively, in vivo.

**TABLE I**

| In vitro and in vivo inhibition of GGTase I and FTase | \( IC_{50} \) | \( IC_{50} \) |
|-------------------------------------------------------|----------------|----------------|
| In vitro                                             | GGTase I       | FTase          | GGTase/FTase |
| FTI-276                                              | 50             | 0.5            | 100          |
| GGTI-297                                              | 50             | 200            | 0.25         |
| FTI-277                                               | ND             | ND             | 30           |
| GGTI-298                                              | ND             | ND             | 3            |

*ND, not determined.

Inhibition of both Rap1A (lower panel, lane 5) and PDGFR tyrosine phosphorylation (upper panel, lane 6) was observed. This effect was not limited to PDGFR but was also apparent with another receptor tyrosine kinase, epidermal growth factor receptor. Indeed, when Rat-1 cells were treated 2 days with GGTI-298 and then stimulated with EGF, levels of tyrosine-phosphorylated EGF receptor were also significantly reduced compared with vehicle-treated cells stimulated with EGF (data not shown). Furthermore, in time course studies, no effect of GGTI-298 (20 \( \mu \text{M} \)) was observed on PDGFR tyrosine phosphorylation or Rap1A processing after 1 or 3.5 h of treatment, but significant inhibition of both was observed after 22 h of treatment (data not shown). Interestingly, however, the inhibitory effect of 10 \( \mu \text{M} \) GGTI-298 on PDGF activation of MAPK was only partial (Fig. 2, middle panel, lane 6) and, thus, did not correlate with the strong inhibition observed at the level of receptor tyrosine phosphorylation. We then used an alternative approach to assess the role of protein geranylgeranylation in receptor tyrosine phosphorylation. Cells were treated with 30 \( \mu \text{M} \) lovastatin in the absence and presence of 15 \( \mu \text{M} \) GGOH. This co-treatment protocol was used, since we have recently demonstrated that although lovastatin treatment alone inhibits both protein geranylgeranylation and protein farnesylation, co-treatment with GGOH not only selectively restores protein geranylgeranylation but also enhances the ability of lovastatin to inhibit protein farnesylation.\(^a\) Fig. 2 shows that although lovastatin treatment alone blocked PDGFR-induced PDGFR tyrosine phosphorylation levels and activation of MAPK as well as inhibited the processing of Rap1A, co-treatment with GGOH restored all of these (upper and middle panels, lanes 7–9; lower panel, lanes 6–8).

PDGFR Receptor Tyrosine Phosphorylation Does Not Require Protein Farnesylation—The above results suggest that intact protein geranylgeranylation is important for proper PDGFR-induced PDGFR tyrosine phosphorylation. We next determined whether protein farnesylation also plays a role in PDGFR receptor tyrosine phosphorylation. In an effort to address this question, cells were treated with 10 \( \mu \text{M} \) GGTI-298 and 3 \( \mu \text{M} \) FTI-277 alone and in combination. Treatment of cells with FTI-277 (3 \( \mu \text{M} \)) alone inhibited Ras processing potently but had no effect on Rap1A processing or PDGFR-stimulated PDGFR tyrosine phosphorylation levels and activation of MAPK (Fig. 3, A, lanes 1–3, and B, lanes 1 and 2). (Similar results were also obtained with the EGF receptor when Rat-1 cells were treated with FTI-277 and subsequently stimulated with EGF (data not shown)). Although FTI-277 treatment alone had no effect on PDGFR tyrosine phosphorylation or MAPK activation, it is

\(^a\) McGuire, T. F., and Sebti, S. M. (1996) Oncogene, in press.
PDGF Receptor Tyrosine Phosphorylation and Protein Prenylation

FIG. 2. PDGF receptor tyrosine phosphorylation is dependent on protein geranylgeranylation. NIH-3T3 cells were plated on day 0 (750,000 cells/100-mm plate) and treated with the inhibitors on days 1 and 2. On day 3, the medium with inhibitors was removed, and the cells were stimulated with vehicle (lane 1) or PDGF (30 ng/ml, 10 min, 37 °C) and then harvested and lysed as described in "Materials and Methods." The levels of tyrosine phosphorylated PDGF receptor (PDGF-R-P), the activation of MAPK (MAPK, inactive, hypophosphorylated form; MAPK-P, active, hyperphosphorylated form), and the extent of inhibition of Rap1A processing (U, unprocessed; P, processed) were determined by electrophoresis equivalent amounts of protein (20 μg for PDGF-R-P and MAPK and 75 μg for Rap1A) and treating with appropriate antibodies as described in "Materials and Methods." Lanes 1 and 2, vehicle; lanes 3–6, 0.3, 1, 3, and 10 μM GGTI-298, respectively; lanes 7 and 9, 30 μM lovastatin; and lanes 8 and 10, 30 μM geranylgeraniol. Rap1A processing results (lower panel, lanes 1–8) correspond to lanes 2–9 of upper and middle panels.

FIG. 3. PDGF receptor tyrosine phosphorylation is not inhibited by FTI-277. NIH-3T3 cells were plated on day 0 and treated with the inhibitors on days 1 and 2. On day 3, the cells were stimulated with vehicle (lane 1) or PDGF and lysed as described in "Materials and Methods." A, levels of tyrosine-phosphorylated PDGF receptor (PDGF-R-P) and the activation of MAPK (MAPK, inactive form; MAPK-P, the active form). Lanes 1 and 2, vehicle; lanes 3 and 5, 3 μM FTI-277; and lanes 4 and 5, 10 μM GGTI-298. B, inhibition of Ras and Rap1A processing (U, unprocessed; P, processed). Lane 1, vehicle; lanes 2 and 4, 3 μM FTI-277; and lanes 3 and 4, 10 μM GGTI-298.

possible that a protein that is farnesylated under normal conditions might become geranylgeranylated if farnesylation is blocked. If this were the case, then co-treatment of cells with 3 μM FTI-277 and 10 μM GGTI-298 would be expected to produce a synergistic effect. However, as shown in Fig. 3A (lanes 4 and 5), no additional inhibition of receptor tyrosine phosphorylation or MAPK activation was observed with FTI-277 and GGTI-298 co-treatment above that which was observed for GGTI-298 treatment alone. To confirm that the various inhibitor treatments had the expected effect on protein prenylation, the processing of Rap1A and Ras was examined in these samples. As shown in Fig. 3B (lane 2), treatment of cells with 3 μM FTI-277 alone had no effect on Rap1A processing but significantly inhibited Ras processing. In contrast, 10 μM GGTI-298 nearly completely inhibited the processing of Rap1A but had no effect on that of Ras (Fig. 3B, lane 3). When cells were co-treated with 3 μM FTI-277 and 10 μM GGTI-298, the inhibitory effects on the processing of Ras and Rap1A were observed to be the same as for the individual compounds when used alone (Fig. 3B, lane 4). Furthermore, since some farnesylated proteins might exhibit higher affinity toward FTase than that of endogenous Ras, cells were treated with a high concentration (30 μM) of FTI-277, and its effect on PDGF-stimulated PDGF tyrosine phosphorylation levels was assessed. Fig. 4A, (lane 3) shows that, under this treatment condition, endogenous Ras processing was completely inhibited, Rap1A processing was slightly inhibited, but PDGF tyrosine phosphorylation was completely unaffected.

Finally, as an alternative approach to investigate a potential role for protein farnesylation in this system, cells were treated with lovastatin and FOH, previously shown to act as a metabolic precursor for protein farnesylation (34), to selectively preserve farnesylation while protein geranylgeranylation is inhibited. Using this co-treatment, recovery of Ras processing was observed, but no restoration of either Rap1A processing or receptor tyrosine phosphorylation was demonstrated (Fig. 4A, lanes 4 and 5). Furthermore, FOH co-treatment was ineffective at preventing the lovastatin block of PDGF activation of MAPK (data not shown). With lovastatin-treated cells, co-treatment with farnesylpyrophosphate or geranylgeranylpyprophosphate achieved essentially the same effects on PDGF-induced PDGF tyrosine phosphorylation and MAPK activation, as well as Ras and Rap1A processing, as was observed for co-treatments with FOH or GGOH, respectively (data not shown). Thus, under various treatment conditions that clearly showed differential effects on Rap1A and Ras processing, it can be concluded that only protein geranylgeranylation, and not protein farnesylation, is required for proper regulation of PDGF-stimulated PDGF tyrosine phosphorylation levels.

The results presented above using GGTI-298 strongly suggest that the effects of this inhibitor on receptor tyrosine phosphorylation are solely due to the inhibition of one or more protein substrates for GTTase I. To establish that its effects on PDGF early signaling events are not due to an overlapping inhibitory activity of GGTI-298 toward GTTase II, the processing of Rab5, a GTTase II protein substrate, was assessed. Fig. 4B shows that, although lovastatin treatment achieved significant inhibition of Rab5 processing (lane 5), no inhibition was detected from treatment of cells with GGTI-298 (15 μM) (lane 4). In addition, high concentrations of FTI-277 (30 μM) had no inhibitory effect on the processing of Rab5 (lane 3), demonstrating its selectivity as a FTase inhibitor. As expected, GGOH but not FOH co-treatment reversed lovastatin's inhibition of Rab5 processing (Fig. 4B, lanes 6 and 7).

Inhibition of PDGF Tyrosine Phosphorylation Is Not Due to Reduced PDGFR Expression—In an effort to investigate the
mechanism by which GGTI-298 and lovastatin inhibit PDGFR tyrosine phosphorylation levels, we assessed whether these compounds might decrease the expression of the PDGFR receptor in NIH-3T3 cells. The identical lysates used to demonstrate the inhibitory effects of GGTI-298 and lovastatin on receptor tyrosine phosphorylation (Fig. 2) were electrophoresed and immunoblotted with an anti-PDGFR antibody (s). As shown in Fig. 5, no effect on PDGFR expression was observed for either GGTI-298 or lovastatin that could account for the strong inhibition that these inhibitors display on receptor tyrosine phosphorylation.

DISCUSSION

Previously we had demonstrated that lovastatin (27) as well as the CAAX peptidomimetic FTI-244 (26) inhibited PDGF-dependent PDGFR tyrosine phosphorylation and its subsequent activation of phosphatidylinositol 3-kinase. We concluded from this work that protein prenylation was critical for proper regulation of PDGFR receptor early signaling events. However, due to a lack of selectivity with these two agents, we were unable to clearly determine whether inhibition of protein farnesylation and/or protein geranylgeranylation was responsible for their effects. In this report, we have used potent and selective CAAX peptidomimetic inhibitors of FTase and GGTase I as well as selective manipulations of lovastatin treatment to unambiguously determine whether geranylgeranylated and/or farnesylated proteins are essential for regulating the levels of PDGF-stimulated, tyrosine-phosphorylated PDGFR levels. We found that the GGTase I-selective peptidomimetic GGTI-298 strongly inhibited tyrosine phosphorylation levels of the receptor at a concentration that had no effect on protein farnesylation. This was not limited to the PDGFR receptor, since another receptor tyrosine kinase, the EGF receptor, was affected in a similar manner (data not shown). In addition, treatment of cells with the highly selective FTase inhibitor FTI-277 demonstrated no effect on PDGF-induced tyrosine phosphorylation levels and did not enhance the inhibition observed with GGTI-298. This was an important result, since it was possible that the processing of farnesylated protein(s) inhibited by FTI-277 might be recovered by geranylgeranylation (or possibly farnesylation) via GGTase I, thus “masking” a potential role for protein farnesylation in PDGFR receptor tyrosine phosphorylation. Furthermore, when protein geranylgeranylation or protein farnesylation were selectively replenished in lovastatin-treated cells by co-treating with either GGOH or FOH, respectively, we found that levels of tyrosine-phosphorylated PDGFR were significantly recovered by GGOH but remained completely blocked by FOH. Results obtained with lovastatin and GGOH co-treatment were particularly important, since we had recently shown that this is a very efficient and selective means of not only restoring the processing of geranylgeranylated proteins but also enhancing lovastatin’s ability to inhibit the processing of farnesylated proteins. Evidence for the efficacy of the co-treatments used was demonstrated by the differential effects of the inhibitor treatments on processing of the endogenous Rap1A, Ras, and Rab5 proteins. Thus, taking all of these results together, we conclude that proper control of PDGFR receptor tyrosine phosphorylation requires intact processing of protein substrates for GGTase I but not those for FTase. Furthermore, activation of MAPK by PDGF does not require protein farnesylation. This is consistent with previous work done with Rat-1 cells demonstrating that inhibition of Ras processing by FTase inhibitors does not affect the ability of EGF to stimulate MAPK (17). Thus, these findings suggest that activation of MAPK by growth factor receptor tyrosine kinases may not depend exclusively on Ras and provide a possible explanation for the lack of toxicity of FTase inhibitors to normal cells.

It is interesting to note that although strong inhibition of tyrosine-phosphorylated PDGFR receptor levels occurred after treatment of cells with 10 μM GGTI-298, only partial inhibition was observed at the level of MAPK activation in these same cells. Moreover, co-treatment of cells with 10 μM GGTI-298 and 3 μM FTI-277 failed to show any synergistic inhibitory effect on MAPK activation by PDGF. In contrast to results obtained with peptidomimetics, lovastatin blocked PDGF stimulation of MAPK, whereas co-treatment with GGOH, but not FOH, restored its stimulation. The different effects of GGTI-298 and lovastatin on MAPK activation may be due to the fact that lovastatin inhibits the processing of protein substrates for not only GGTase I and FTase but also GGTase II, which is not affected by GGTI-298. Thus, one interpretation of the results is that PDGF stimulation of MAPK requires intact processing of protein substrates for both GGTase I and GGTase II. If this is the case, lovastatin would be expected to have a more potent effect at the level of PDGF activation of MAPK than GGTI-298. Exactly how PDGF receptor tyrosine phosphorylation levels can be inhibited without affecting MAPK activation is not clear. If the only pathway(s) possible for MAPK activation by the PDGF receptor are those that are dependent on receptor tyrosine phosphorylation, then perhaps only a low level of phosphorylated receptor is needed to obtain full activation of MAPK. It is possible that very low levels of tyrosine-phosphorylated PDGFR receptor may still be present in PDGF-stimulated, GGTI-298-pretreated cells and that this is all that may be required for MAPK activation. Alternatively, it may be possible that the protein tyrosine kinase activity of the PDGF receptor is not necessary for its ability to stimulate MAPK. Precedence for a tyrosine phosphorylation-independent mechanism of MAPK activation has been found with EGF-stimulated fibroblasts (35).

The mechanism by which GGTI-298 and lovastatin inhibit PDGF-dependent receptor tyrosine phosphorylation cannot be accounted for by a reduction in expression of total cellular PDGFR. However, since geranylgeranylated protein(s) may be involved in the transport of the receptor to the plasma membrane, the reduction in phosphorylation may still be a reflection of the amount of receptor on the cell surface. Alternatively, and perhaps more intriguing, mechanism would be the involvement of a geranylgeranylated protein in the regulation of either the intrinsic receptor tyrosine kinase activity or a protein tyrosine phosphatase activity. Further investigation is needed to address these issues.

The results presented in this article point to an important role for geranylgeranylated protein(s) in the regulation of PDGF and EGF receptor tyrosine phosphorylation levels and allow us to assess specific proteins that appear as likely candidates. One of these is the geranylgeranylated protein Rho, which has recently been found to associate with the PDGF type B receptor (33). Rac, also a geranylgeranylated small G-protein, has been demonstrated previously to play an essential role, along with Rho, in regulating cell cycle progression from G0 to S phase. The possibility of K-Ras4B being a candidate is unlikely, since high concentrations of FTI-277 (30 μM), that
showed no effect on receptor tyrosine phosphorylation, nearly completely inhibit K-Ras4B processing (11).

REFERENCES

1. Casey, P. J. (1992) J. Lipid Res. 33, 1731–1740
2. Reiss, Y., Stradley, S. J., Gierasch, L. M., Brown, M. S., and Goldstein, J. L. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 732–736
3. Yokoyama, K., McGeady, P., and Gelb, M. H. (1995) Biochemistry 34, 1344–1354
4. Seabra, M. C., Goldstein, J. L., Sudhof, T. C., and Brown, M. S. (1992) J. Biol. Chem. 267, 14497–14503
5. Reiss, Y., Brown, M. S., and Goldstein, J. L. (1992) J. Biol. Chem. 267, 6403–6408
6. Olson, M. F., Ashworth, A., and Hall, A. (1995) Science 269, 1270–1272
7. Williams, B. M., Christensen, A., Hubbert, N. C., Papageorge, A. C., and Lowy, D. R. (1984) Nature 310, 583–586
8. Jackson, J. H., Cochrane, C. G., Bourne, J. R., Solski, P. A., Buss, J. E., and Der, C. J. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 3042–3046
9. Kato, K., Cox, A. D., Hisaka, M. M., Graham, S. M., Buss, J. E., and Der, C. J. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 6403–6407
10. Qian, Y., Blaskovich, M. A., Saleem, M., Seong, C.-M., Wathen, S. P., Hamilton, A. D., and Sebti, S. M. (1994) J. Biol. Chem. 269, 12410–12413
11. Lerner, E. C., Qian, Y., Blaskovich, M. A., Fassum, R. D., Vogt, A., Sun, J., Cox, A. D., Der, C. J., Hamilton, A. D., and Sebti, S. M. (1995) J. Biol. Chem. 270, 26807–26810
12. James, G. L., Goldstein, J. L., Brown, M. S., Rawson, T. E., Sun, J., Kohl, N. E., Wilson, F. R., Mosser, S. D., Giuliani, E. A., Graham, S. L., Hartman, G. D., and Gibbs, J. B. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 9141–9145
13. Graham, S. L., de Solms, S. J., Giuliani, E. A., Conner, M. W., Ankley, N. J., Holtz, W. J., Gomez, R. P., Smith, R. L., Graham, S. L., Hartman, G. D., and Gibbs, J. B. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 9141–9145
14. Garcia, A. M., Rowell, C., Ackermann, K., Kowalczyk, J. J., and Lewis, M. D. (1995) J. Biol. Chem. 270, 6221–6226
15. McGuire, T. F., Qian, Y., Blaskovich, M. A., Fassum, R. D., Sun, J., Marlowe, T., Corey, S. J., Wathen, S. P., Vogt, A., Hamilton, A. D., and Sebti, S. M. (1995) J. Biol. Chem. 270, 26770–26773
16. McCormick, F. (1995) Nature 363, 15–16
17. Prendergast, G. C., Davide, J. P., de Solms, S. J., Giurioli, E. A., Graham, S. L., Gibbs, J. B., Oliff, A., and Kohl, N. E. (1994) Mol. Cell. Biol. 14, 4193–4202
18. Kohl, N. E., Wilson, F. R., Mosser, S. D., Giurioli, E., de Solms, S. J., Conner, M. W., Anthon, N. J., Holtz, W. J., Gomez, R. P., Cox, A. D., Oliff, A., and Kohl, N. E. (1994) Mol. Cell. Biol. 14, 4193–4202
19. Feldman, S. B., Parquette, D. A., Wang, X., and Sebti, S. M. (1995) J. Biol. Chem. 270, 17221–17228
20. Armstrong, S. A., Hannah, V. C., Goldstein, J. L., and Brown, M. S. (1995) J. Biol. Chem. 270, 7964–7968
21. Seabra, M. C., Reiss, Y., Case, A. D., Hamilton, A. D., and Sebti, S. M. (1995) J. Biol. Chem. 270, 26807–26810
22. Lerner, E. C., Qian, Y., Blaskovich, M. A., Fassum, R. D., Vogt, A., Sun, J., Cox, A. D., Der, C. J., Hamilton, A. D., and Sebti, S. M. (1995) J. Biol. Chem. 270, 26647–26650
23. Armstrong, S. A., Hannah, V. C., Goldstein, J. L., and Brown, M. S. (1995) J. Biol. Chem. 270, 7964–7968
24. Sanford, M., Sanche, J., Terhorst, C., and Faller, D. (1995) J. Biol. Chem. 270, 17221–17228
25. J. Biol. Chem. 270, 7964–7968
26. Zoubi, M., Sanche, J., Terhorst, C., and Faller, D. (1995) J. Biol. Chem. 270, 17221–17228
27. Crik, D. C., Andres, D. A., and Waechter, C. J. (1995) Biochem. Biophys. Res. Commun. 211, 590–595
28. Gotsh, N., Tojo, A., Muroya, K., Hashimoto, Y., Hattori, S., Nakamura, S., Takenawa, T., Yanaka, Y., and Shibuya, M. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 167–171