was modulating the response of the mice to the parasite.
11. S. Mellouk, S. J. Green, C. A. Nacy, S. L. Hoffman, *J. Immunol.* **146**, 3971 (1991).
12. A. Nussler *et al.*, *Eur. J. Immunol* **21**, 227 (1991).
13. K. A. Rockett, M. M. Awburn, W. B. Cowden, I. A. Clark, *Infect. Immun.* **59**, 3280 (1991).
14. M. A. Marletta, P. S. Yoon, R. Iyengar, C. D. Leaf, J. S. Wishnok, *Biochemistry* **27**, 8706 (1988); D. J. Stuehr and C. F. Nathan, *J. Exp. Med.* **169**, 1543 (1989).
15. R. G. Knowles and S. Moncada, *Trends Biochem. Sci.* **17**, 399 (1992).
16. F. Y. Liew *et al.*, *Eur. J. Immunol.* **21**, 2489 (1991).
17. T. R. Mosmann and R. L. Coffman, *Immunol. Today* **8**, 223 (1987).
18. D. W. Taylor, in *Malaria: Host Responses to Infection*, M. M. Stevenson, Ed. (CRC Press, Boca Raton, FL, 1990), pp. 1–35.
19. S. A. McLean, C. D. Pearson, R. S. Phillips, *Exp. Parasitol.* **54**, 296 (1982).
20. M. M. Stevenson and E. Skamene, *Infect. Immun.* **54**, 600 (1986).
21. K. A. Rockett, M. M. Awburn, B. B. Aggarwal, W. B. Cowden, I. A. Clark, *ibid.* **60**, 3725 (1992).
22. Mice (six per group) were CD4-depleted (*8*). Twelve days after the last antibody treatment, they were injected intravenously with $4 \times 10^7$ cells per mouse, or not reconstituted, and then infected intravenously immediately with $10^5$ *P. c. chabaudi* pRBC. The preparation of the T cell clone has been described in detail (*6*). We enriched naïve splenic T cells for $CD4^+$ cells using a mouse T cell separation kit (Pierce). Some of the mice reconstituted with $T_H1$ or $T_H2$ were injected intraperitoneally with L-NMMA (250 mg/kg per day) dissolved in sterile PBS. Courses of infection and nitrate levels in the sera were monitored as described (*4, 5*). Data shown are pooled from three separate experiments.
23. We assayed parasite-specific antibody in the serum by an indirect fluorescent antibody test [S. A. McLean, C. D. Pearson, R. S. Phillips, *Exp. Parasitol.* **54**, 213 (1982)], using trophozoite-schizont–infected RBC as the target antigen. Isotype-specific antibodies were obtained from serum separated by affinity chromatography on protein A–Sepharose CL-4B into fractions containing IgG1, IgG2a, IgG2b, or IgG3 [I. Seppälä, H. Sarvar, F. Peterfy, O. Mäkelä, *Scand. J. Immunol.* **14**, 335 (1981)]. Immunoglobulin M was isolated by Sepharose 6B gel filtration from nonbinding material that contained a pool of IgA and IgE. We confirmed the purity of each fraction by double radial immunodiffusion, using antibodies specific to each Ig isotype.
24. We thank H. Hodson for L-NMMA and D-NMMA and E. Galloway and D. McLaughlin for technical support. We thank The Wellcome Trust for financial support.

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# Selective Inhibition of *ras*-Dependent Transformation by a Farnesyltransferase Inhibitor

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**To acquire transforming potential, the precursor of the Ras oncoprotein must undergo farnesylation of the cysteine residue located in a carboxyl-terminal tetrapeptide. Inhibitors of the enzyme that catalyzes this modification, farnesyl protein transferase (FPTase), have therefore been suggested as anticancer agents for tumors in which Ras contributes to transformation. The tetrapeptide analog L-731,735 is a potent and selective inhibitor of FPTase in vitro. A prodrug of this compound, L-731,734, inhibited Ras processing in cells transformed with v-*ras*. L-731,734 decreased the ability of v-*ras*–transformed cells to form colonies in soft agar but had no effect on the efficiency of colony formation of cells transformed by either the v-*raf* or v-*mos* oncogenes. The results demonstrate selective inhibition of *ras*-dependent cell transformation with a synthetic organic inhibitor of FPTase.**

The mammalian *ras* genes encode guanosine triphosphate (GTP)–binding proteins that can acquire the potential to transform mammalian cells as a result of point mutations in codons 12, 13, or 61 (*1*). Mutated, oncogenic forms of *ras* are frequently found in many human cancers, most notably in more than 50% of colon and pancreatic carcinomas (*1, 2*). These observations indicate that Ras functions in the pathogenesis of human cancers and emphasize the potential broad utility of anticancer agents directed against *ras*-induced cell transformation.

Ras is synthesized as a cytosolic precursor that ultimately localizes to the cytoplasmic face of the plasma membrane after a series of posttranslational modifications (*3*). The first and obligatory step in this series is the addition of a farnesyl moiety to the cysteine residue of the COOH-terminal CAAX motif (C, cysteine; A, usually aliphatic residue; X, any other amino acid) in a reaction catalyzed by farnesyl protein transferase (FPTase). This modification is essential for Ras function, as demonstrated by the inability of Ras mutants lacking the COOH-terminal cysteine to be farnesylated, to localize to the plasma membrane, and to transform mammalian cells in culture (*4, 5*). Moreover, strains of *Saccharomyces cerevisiae* having a mutation in *RAM1*, a gene that encodes one of the structural polypeptides of the yeast FPTase, are resistant to the biological effects of oncogenic *ras* (*6*). The subsequent posttranslational modifications—cleavage of the AAX residues, carboxyl methylation of the farnesylated cysteine, and palmitoylation of cysteines located upstream of the CAAX motif—are not obligatory for Ras membrane association or cell-transforming activity (*5, 7*). Thus, FPTase appears to be an appropriate biochemical target for the development of inhibitors of posttranslational processing of Ras that might be expected to interfere with Ras-mediated cellular transformation.

The substrates of the farnesylation reaction, farnesyl diphosphate (FPP) and polypeptides containing a CAAX motif, can be used as a starting point for the design of FPTase inhibitors. Several analogs of FPP are potent and selective inhibitors of FPTase in vitro (*8, 9*), and one has shown activity in cells (*9*). The CAAX tetrapeptide is the minimal sequence required for the interaction of Ras with FPTase (*10, 11*). Thus, tetrapeptides with amino acid sequences identical to the COOH-terminal sequences of protein substrates for FPTase compete with Ras for farnesylation by acting as alternative substrates (*12, 13*). CAAX derivatives have also been identified that are not substrates for farnesylation and therefore behave as pure inhibitors of FPTase (*8, 13, 14*).

Although other cellular proteins besides Ras are in vivo substrates for farnesylation, most isoprenylated proteins are modified by the 20-carbon geranylgeranyl moiety (*15*). Two classes of enzymes that catalyze the addition of a geranylgeranyl group to proteins have been identified in mammalian cells. One class consists of geranylgeranyl protein transferase–I (GGPTase-I), which modifies proteins having a COOH-terminal CAAX sequence where X is a Leu residue (*12, 16*). The other class consists of one or more enzymes (for example, GGPTase-II) that catalyze the modification of proteins terminating in Cys-Cys or Cys-X-Cys (*12, 17*). Comparison of the activity of various CAAX tetrapeptides suggests that it may be possible to design a specific inhibitor of FPTase that does not affect the GGPTases. For example, CAAX tetrapeptides terminating in Ser or Met are potent inhibitors of FPTase but are less active as inhibitors of GGPTase-I or GGPTase-II (*12*). Conversely, CAAX tetrapeptides terminating in Leu, which act as effective inhibitors of

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GGPTase-I, are relatively poor inhibitors of FPTase (*12*, *18*).

To obtain selective antagonists of FPTase, we examined CAAX tetrapeptides as models for the development of inhibitors. The COOH-terminal tetrapeptide of human K4A-Ras, CIIM, was used as a template to design the tetrapeptide analogs L-731,734, which is *N*-{2(*S*)-[2(*R*)-amino-3-mercaptopropylamino]-3(*S*)-methylpentyl}isoleucyl-homoserine lactone, and L-731,735, which is *N*-{2(*S*)-[2(*R*)-amino-3-mercaptopropylamino]-3(*S*)-methylpentyl}isoleucyl-homoserine (Fig. 1). These compounds differ from CIIM in two respects. First, in both compounds, the two $NH_2$-terminal peptide bonds were reduced. Second, either homoserine lactone or homoserine was substituted for methionine. The $NH_2$-terminal peptide bonds were reduced in order to confer resistance to hydrolysis by aminopeptidases, enzymes commonly found in mammalian cell extracts (*19*). Homoserine was anticipated to be tolerated as a replacement for methionine because of its structural similarity to serine, which is found in the X position of the Ha-Ras (*20*) and *S. cerevisiae* RAS2 (*21*) CAAX motifs. Although this compound (homoserine in the X position) was anticipated to be an effective inhibitor in vitro, the charge on the COOH-terminal carboxylate was perceived as a possible impediment to the entry of CAAX tetrapeptides into whole cells. Cyclization of homoserine to a lactone might facilitate cell penetrability by masking the anionic carboxylate and increasing the solubility of the compound in lipid.

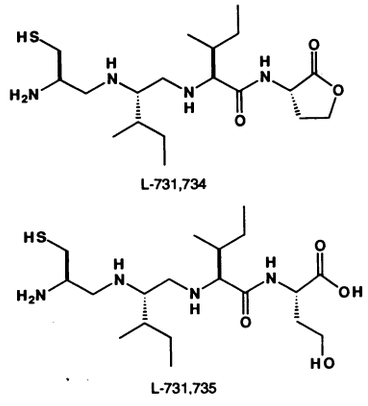


**Fig. 1.** Structure of L-731,734, which is *N*-{2(*S*)-[2(*R*)-amino-3-mercaptopropylamino]-3(*S*)-methylpentyl}isoleucyl-homoserine lactone, and L-731,735, which is *N*-{2(*S*)-[2(*R*)-amino-3-mercaptopropylamino]-3(*S*)-methylpentyl}isoleucyl-homoserine.

**Table 1.** Selective inhibition of farnesyl protein transferase. Prenyl protein transferase assays were done essentially as described (*12*) and contained the following: FPTase, 100 nM [$^3$H]FPP and 650 nM *E. coli*–produced Ras-CVLS; GGPTase-I, 100 nM [$^3$H]GGPP and 500 nM *E. coli*–produced Ras-CAIL (I, Ile); GGPTase-II, 100 nM [$^3$H]GGPP and 500 nM *E. coli*–produced YPT1. All enzymes were partially purified from bovine brain cytosol as described (*12*). FPTase values are the average of four separate determinations ± SEM. GGPTase values represent three determinations. $IC_{50}$, 50% inhibitory concentration.

| Compound | $IC_{50}$ (nM) | | |
|---|---|---|---|
| | FPTase | GGPTase-I | GGPTase-II |
| L-731,734 | 282 ± 41 | >100,000 | >100,000 |
| L-731,735 | 18 ± 6 | >100,000 | >100,000 |

L-731,735 exhibited potent inhibition of FPTase in vitro. In assays containing [$^3$H]farnesyl diphosphate and Ras produced in *Escherichia coli* (Ras-CVLS: V, Val; L, Leu; S, Ser), 50% inhibition ($IC_{50}$) of partially purified FPTase from bovine brain was observed at a concentration of 18 nM L-731,735 (Table 1). The related lactone compound, L-731,734, was less potent in inhibiting FPTase ($IC_{50}$ = 282 nM). These results suggest that the COOH-terminal carboxylate is an important determinant of intrinsic FPTase inhibitory potency and are consistent with our observation that amidation of the COOH-terminal carboxyl group of the tetrapeptide CVLS similarly decreased FPTase inhibitory activity (*8*). When evaluated as inhibitors of homogeneous recombinant human FPTase, $IC_{50}$ values for L-731,734 and L-731,735 were comparable to those observed with the bovine enzyme (*22*). L-731,735 is a nonsubstrate inhibitor that is competitive with respect to Ras [inhibition constant ($K_i$) = 20 ± 6 nM] and noncompetitive with respect to FPP in the FPTase reaction. Although L-731,735 was a potent inhibitor of FPTase, it was a less effective (>5000 times) inhibitor of the type I and type II GGPTases (Table 1).

NIH 3T3 cells transformed by v-*ras* were used to evaluate the effect of these compounds on the posttranslational processing of Ras in intact cells. The cells were incubated in the presence of the indicated compound for 24 hours and were labeled with [$^{35}$S]methionine during the final 20 hours. Ras was immunoprecipitated from detergent lysates of cell extracts with the monoclonal antibody to Ras, Y13-259 (*23*). Viral Ha-Ras contains a threonine at amino acid position 59 that is a substrate for autophosphorylation (*24*). The viral Ras in the transfected cells consists of a mixture of phosphorylated and unphosphorylated protein with approximately 25% being phosphorylated (*25*). Farnesylation and phosphorylation both alter the migration of viral Ha-Ras during SDS–polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 2A). In the presence of lovastatin, a compound that blocks Ras processing in cells by inhibiting a rate-limiting step in the isoprenoid biosynthetic pathway (*5*, *26*, *27*), the phosphorylated and unphosphorylated forms of Ras migrated more slowly (Fig. 2A), indicating a lack of posttranslational processing. A similar pattern of migration was observed for cells incubated in the presence of 250 μM L-731,734 (Fig. 2A), demonstrating that this compound similarly blocked processing of Ras in cells. Further inhibition of Ras processing was not observed at higher concentrations of L-731,734 (up to 1 mM) (*28*). Titration of

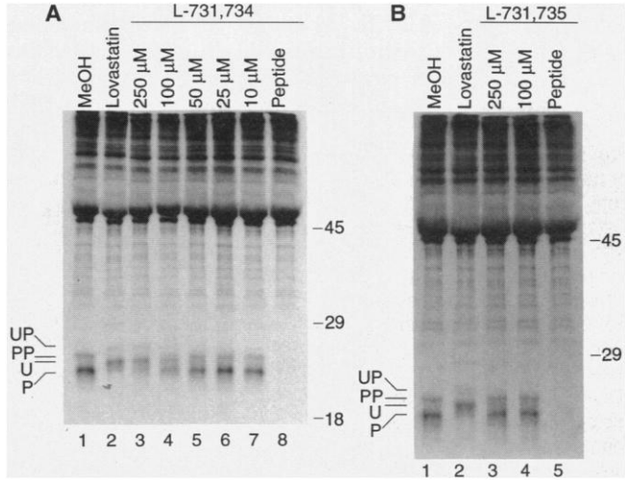


**Fig. 2.** Inhibition of Ras processing by L-731,734. Metabolic labeling and immunoprecipitation of Ras protein was done essentially as described (*26*). Briefly, NIH 3T3 cells transformed by viral H-*ras* were incubated with the indicated concentration of L-731,734 (**A**) or L-731,735 (**B**) (dissolved in methanol; the final concentration of methanol in the assay was 0.1%) for 4 hours, at which time fresh compound was added together with [$^{35}$S]methionine (133 μCi/ml) (Amersham). After incubation for another 20 hours, Ras was immunoprecipitated from detergent lysates of cell extracts with monoclonal antibody to Ras Y13-259 (10 μg), resolved by SDS-PAGE (13% gels), and detected by fluorography. MeOH, 0.1% methanol; Lovastatin, lovastatin (15 μM); Peptide, competition of the antibody with an excess of the peptide spanning the epitope (Ras 60–76; ~100-fold molar excess); P, processed, unphosphorylated Ras; U, unprocessed, unphosphorylated Ras; PP, processed, phosphorylated Ras; and UP, unprocessed, phosphorylated Ras. Molecular sizes of the protein standards are indicated on the right (in kilodaltons).

L-731,734 in this assay indicated that the compound inhibited Ras processing with an $IC_{50}$ of ~100 μM (Fig. 2A). L-731,734 (up to 1 mM) did not inhibit the processing of a chimeric Ras protein (Ras-CVLL) that is prenylated by a geranylgeranyl moiety (*28, 29*). This result indicates the specificity of L-731,734 for the FPTase reaction in cells.

To demonstrate that the change in Ras mobility on SDS-PAGE correlated with an inability of Ras to localize to the plasma membrane, we subjected v-*ras* cells treated with solvent alone or L-731,734 to cell fractionation. Most of the Ras in the solvent-treated cells was associated with the membrane, whereas the unprocessed Ras in cells treated with L-731,734 was found exclusively in the soluble fraction (*28*). Furthermore, we were unable to radiolabel the soluble Ras with [$^3$H]mevalonate (*30*), which serves as a metabolic precursor of FPP in these cells, suggesting that L-731,734 blocked at least one step in the biochemical pathway leading from mevalonate to farnesylation of Ras. These results indicate that L-731,734 inhibited Ras farnesylation in vivo.

In contrast to L-731,734, the pattern of migration of Ras in cells incubated in the presence of L-731,735 (250 μM) closely resembled that seen with the solvent alone (Fig. 2B). The ability of L-731,734 to efficiently inhibit Ras processing in cells appears, therefore, to be dependent on the presence of the lactone, which was incorporated into the molecule to enhance cell penetrability. Indeed, using $^3$H-labeled CAAX tetrapeptide analogs similar to L-731,734 and L-731,735, we found that the homoserine lactone form accumulated in v-*ras* cells whereas the homoserine form of the compound did not (*31*). Given the moderate potency of L-731,734 relative to that of L-731,735 in vitro, it seems likely that the active form of the inhibitor is generated in vivo after penetration of the drug into the cells. This process may involve cleavage of the lactone by intracellular esterases to yield L-731,735. L-731,735 is approximately 5000 times more potent at blocking FPTase activity in vitro ($IC_{50}$ = 18 nM) than L-731,734 is at blocking Ras processing in cells ($IC_{50}$ = 100 μM). This difference may result from inefficient cell penetration of L-731,734 or incomplete hydrolysis of the lactone (or both). Chemical or metabolic instability of either form of the compound may also limit activity in cell culture.

To determine the biological consequences of FPTase inhibition, we assayed the effect of L-731,734 on the anchorage-independent growth of Rat1 cells transformed with either a v-*ras*, v-*raf*, or v-*mos* oncogene. Cells transformed by v-Raf and v-Mos were included in the analysis to evaluate the specificity of L-731,734 for Ras-induced cell transformation. Neither Raf nor Mos requires farnesylation to achieve full biological activity. Moreover, Raf and Mos appear to act independently of Ras because dominant negative Ras mutants (*29, 32*) and neutralizing antibodies against Ras (*33*) do not interfere with Raf- or Mos-induced cell transformation. In the presence of 1 mM L-731,734, the v-*ras*–transformed cells did not form the multiple, large colonies that grew in the presence of solvent alone (Fig. 3). The minute colonies observed for these cells in the presence of L-731,734 were identical to those seen for the untransformed parental Rat1 cells plated under the same conditions (*28*). The inhibition of Ras transformation by L-731,734 was dose-dependent (Fig. 3). In contrast, 1 mM L-731,734 had no effect on the anchorage-independent growth of Rat1 cells transformed by either v-*raf* or v-*mos* (Fig. 3). Thus, the biological effects of L-731,734 in these experiments appear to be specific for *ras*-transformed cells. Moreover, the resistance of cells transformed with v-*raf* and v-*mos* to the effects of L-731,734 suggests that the effect seen with the v-*ras*–transformed cells is not due to general cytotoxicity. Compounds related to L-731,734 that inhibited the anchorage-independent growth of Ras- but not Raf-transformed cells also inhibited the anchorage-independent growth of cells transformed with v-*src* (*31*). Although Src does not require prenylation to achieve biological activity, its ability to transform cells has been shown to proceed by a *ras*-dependent pathway (*33*). This result is consistent with the specificity of our compounds for the Ras pathway. Furthermore, these same compounds caused growth arrest and morphological reversion of Ras- but not Raf-transformed cells in monolayer culture (*34*).

In this report, we have shown that a rationally designed, cell-active inhibitor of FPTase specifically inhibited Ras-dependent transformation. This demonstration of specificity is important because other farnesylated cellular proteins, including lamin B (*35*), which are critical components of normal cell physiology, appear to be substrates for the same prenylation enzyme that modifies Ras (*11*). The specificity of L-731,734 for Ras-transformed cells contrasts with the lack of specificity observed with lovastatin. Although lovastatin and L-731,734 are similar in their ability to block Ras processing, lovastatin also inhibits the growth of cells transformed by v-*raf* (*26*). Apparently, lovastatin's growth-inhibitory effects involve multiple mevalonate-dependent pathways that are required for all cells to grow and divide. The specificity observed with L-731,734 likely results from inhibition of a single enzyme that is directly responsible for promoting Ras biological activity. In this regard, neither L-731,734 nor L-731,735 (50 μM) had any effect on the conversion of mevalonate into squalene, an intermediate in the cholesterol biosynthesis pathway, in an in vitro assay (*36*).

Studies on the posttranslational modifications of Ras have suggested that pharmacological inhibition of FPTase would block *ras* functions. Our findings provide experimental evidence that FPTase is an appropriate biochemical target to inhibit *ras*-dependent cell transformation. L-731,734 is now a prototype for compounds that target a specific biochemical reaction that governs cell growth and mitogenesis.

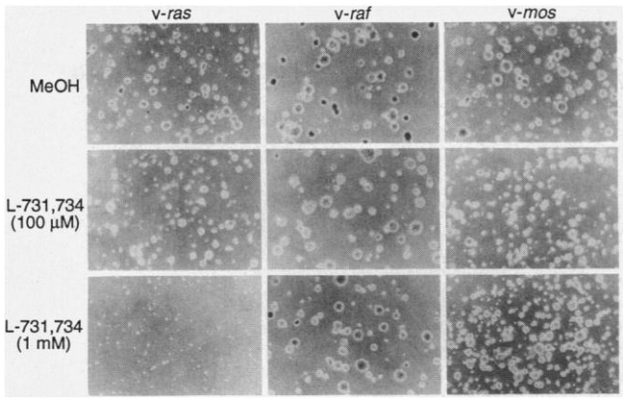


**Fig. 3.** Inhibition of growth of *ras*-transformed cells in soft agar by L-731,734. Rat1 cells transformed with either v-*ras*, v-*raf*, or v-*mos* were seeded at a density of 1 × $10^4$ cells per plate (35 mm in diameter) in a 0.3% top agarose layer in medium A (Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum) over a bottom agarose layer (0.6%). Both layers contained 0.1% methanol or the indicated concentration of L-731,734 (dissolved in methanol at 1000 times the final concentration used in the assay). The cells were fed twice weekly with 0.5 ml of medium A containing 0.1% methanol or the indicated concentration of L-731,734. Photomicrographs were taken 16 days after the cultures were seeded.

## REFERENCES AND NOTES


1. M. Barbacid, *Annu. Rev. Biochem.* **56**, 779 (1987).
2. J. L. Bos, *Cancer Res.* **49**, 4682 (1989).
3. J. B. Gibbs, *Cell* **65**, 1 (1991); A. D. Cox and C. J. Der, *Crit. Rev. Oncogenesis* **3**, 365 (1992).

4. B. M. Willumsen, K. Norris, A. G. Papageorge, N. L. Hubbert, D. R. Lowy, *EMBO J.* **3**, 2581 (1984); J. H. Jackson *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 3042 (1990).
5. J. F. Hancock, A. I. Magee, J. E. Childs, C. J. Marshall, *Cell* **57**, 1167 (1989).
6. S. Powers *et al.*, *ibid.* **47**, 413 (1986); A. Fujiyama, K. Matsumoto, F. Tamanoi, *EMBO J.* **6**, 223 (1987).
7. K. Kato *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 6403 (1992).
8. D. L. Pompliano *et al.*, *Biochemistry* **31**, 3800 (1992).
9. J. B. Gibbs *et al.*, *J. Biol. Chem.* **268**, 7617 (1993).
10. M. D. Schaber *et al.*, *ibid.* **265**, 14701 (1990).
11. Y. Reiss, J. L. Goldstein, M. C. Seabra, P. J. Casey, M. S. Brown, *Cell* **62**, 81 (1990).
12. S. L. Moores *et al.*, *J. Biol. Chem.* **266**, 14603 (1991).
13. J. L. Goldstein, M. S. Brown, S. J. Stradley, Y. Reiss, L. M. Gierasch, *ibid.*, p. 15575.
14. M. S. Brown, J. L. Goldstein, K. J. Paris, J. P. Burnier, J. C. Marsters, Jr., *Proc. Natl. Acad. Sci. U.S.A.* **89**, 8313 (1992).
15. H. C. Rilling, E. Breunger, W. W. Epstein, P. F. Crain, *Science* **247**, 318 (1990); C. C. Farnsworth, M. H. Gelb, J. A. Glomset, *ibid.*, p. 320.
16. P. J. Casey, J. A. Thissen, J. F. Moomaw, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 8631 (1991); A. Joly, G. Popjak, P. A. Edwards, *J. Biol. Chem.* **266**, 13495 (1991); M. C. Seabra, Y. Reiss, P. J. Casey, M. S. Brown, J. L. Goldstein, *Cell* **65**, 429 (1991); K. Yokoyama, G. W. Goodwin, F. Ghomashchi, J. A. Glomset, M. H. Gelb, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 5302 (1991); Y. Yoshida *et al.*, *Biochem. Biophys. Res. Commun.* **175**, 720 (1991).
17. M. C. Seabra, J. L. Goldstein, T. C. Südhof, M. S. Brown, *J. Biol. Chem.* **267**, 14497 (1992); H. Horiuchi *et al.*, *ibid.* **266**, 16981 (1991).
18. Y. Reiss, S. J. Stradley, L. M. Gierasch, M. S. Brown, J. L. Goldstein, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 732 (1991).
19. D. Heimbrook and J. B. Gibbs, unpublished data.
20. D. J. Capon, E. Y. Chen, A. D. Levinson, P. H. Seeburg, D. V. Goeddel, *Nature* **302**, 33 (1983).
21. R. Dhar, A. Nieto, R. Koller, D. DeFeo-Jones, E. M. Scolnick, *Nucleic Acids Res.* **12**, 3611 (1984); S. Powers *et al.*, *Cell* **36**, 607 (1984).
22. S. D. Mosser, J. B. Gibbs, C. A. Omer, unpublished data.
23. M. E. Furth, L. J. Davis, B. Fleurdelys, E. M. Scolnick, *J. Virol.* **43**, 294 (1982).
24. T. Y. Shih, P. E. Stokes, G. W. Smythers, R. Dhar, S. Oroszlan, *J. Biol. Chem.* **257**, 11767 (1982).
25. J. B. Gibbs, R. W. Ellis, E. M. Scolnick, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 2674 (1984).
26. J. E. DeClue, W. C. Vass, A. G. Papageorge, D. R. Lowy, B. M. Willumsen, *Cancer Res.* **51**, 712 (1991).
27. M. Sinensky, L. A. Beck, S. Leonard, R. Evans, *J. Biol. Chem.* **265**, 19937 (1990).
28. N. E. Kohl, unpublished data.
29. A. D. Cox, M. M. Hisaka, J. E. Buss, C. J. Der, *Mol. Cell. Biol.* **12**, 2606 (1992).
30. N. E. Kohl and J. B. Gibbs, unpublished data.
31. F. R. Wilson and N. E. Kohl, unpublished data.
32. L. A. Feig and G. M. Cooper, *Mol. Cell. Biol.* **8**, 3235 (1988); D. W. Stacey *et al.*, *Oncogene* **6**, 2297 (1991).
33. M. R. Smith, S. J. DeGudicibus, D. W. Stacey, *Nature* **320**, 540 (1986).
34. G. C. Prendergast and N. E. Kohl, unpublished data.
35. C. C. Farnsworth, S. L. Wolda, M. H. Gelb, J. A. Glomset, *J. Biol. Chem.* **264**, 20422 (1989).
36. J. Karkas, J. Bergstrom, J. B. Gibbs, unpublished data.
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# Benzodiazepine Peptidomimetics: Potent Inhibitors of Ras Farnesylation in Animal Cells

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**Oncogenic Ras proteins transform animal cells to a malignant phenotype only when modified by farnesyl residues attached to cysteines near their carboxyl termini. The farnesyltransferase that catalyzes this reaction recognizes tetrapeptides of the sequence *CAAX*, where C is cysteine, *A* is an aliphatic amino acid, and *X* is a carboxyl-terminal methionine or serine. Replacement of the two aliphatic residues with a benzodiazepine-based mimic of a peptide turn generated potent inhibitors of farnesyltransferase [50 percent inhibitory concentration ($IC_{50}$) < 1 nM]. Unlike tetrapeptides, the benzodiazepine peptidomimetics enter cells and block attachment of farnesyl to Ras, nuclear lamins, and several other proteins. At micromolar concentrations, these inhibitors restored a normal growth pattern to Ras-transformed cells. The benzodiazepine peptidomimetics may be useful in the design of treatments for tumors in which oncogenic Ras proteins contribute to abnormal growth, such as that of the colon, lung, and pancreas.**

Oncogenic Ras proteins are causally implicated in certain human malignancies (*1*). Poised at the inner surface of the plasma membrane, Ras proteins normally respond to growth stimuli like epidermal and platelet-derived growth factors by exchanging guanosine triphosphate (GTP) for constitutively bound guanosine diphosphate (GDP), thereby triggering cell division. The signal is terminated when the Ras protein hydrolyzes its bound GTP to GDP in a reaction that is stimulated by a guanosine triphosphatase (GTPase) activating protein (GAP) (*2*). About 50% of human colon carcinomas and 90% of pancreatic carcinomas produce mutant Ras proteins that bind GTP but cannot hydrolyze it (*1*). The mutant proteins are constitutively active, and this constant signal, coupled with other regulatory abnormalities, leads to malignant transformation.

The function of normal and oncogenic Ras proteins is absolutely dependent on the posttranslational attachment of a 15-carbon isoprenoid moiety, farnesyl, through a thioether linkage to a cysteine near the COOH-terminus of the protein (*3*). This modification is catalyzed by a heterodimeric $Zn^{2+}$-dependent enzyme designated CAAX farnesyltransferase (*4*, *5*). The enzyme uses farnesyl pyrophosphate as a donor and attaches a farnesyl group to the cysteine residue at the fourth position from the COOH-terminus of various proteins, including all four Ras proteins, nuclear lamins A and B, skeletal muscle phosphorylase kinase, and three retinal proteins (the $\gamma$ subunit of transducin, the $\alpha$ subunit of cyclic guanosine monophosphate phosphodiesterase, and rhodopsin kinase) (*6*). The COOH-termini of all of these substrates share the tetrapeptide sequence CAAX, where C is cysteine, A stands for aliphatic residues, and X is methionine or serine (*7*). This CAAX motif appears to be the sole recognition site for the enzyme; hence, addition of CAAX sequences to the COOH-termini of other proteins renders them substrates for farnesylation (*8*). Moreover, in vitro the enzyme attaches a farnesyl group to tetrapeptides that conform to the CAAX consensus (*7*, *9*).

In the cell, farnesylation is the first step in a sequence of modifications that renders the COOH-terminus of the Ras protein hydrophobic. Farnesylation is followed by proteolytic removal of the terminal three amino acids and methylation of the free COOH group on the farnesylated cysteine (*3*). These reactions are necessary for Ras to become attached to the inner surface of the plasma membrane.

Oncogenic Ras proteins lose their transforming ability when farnesylation is prevented, either by mutation of the CAAX sequence or by blocking synthesis of farnesyl pyrophosphate with an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase (*3*, *10*). Under some circumstances the cytosolic nonprenylated form of Ras may

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