Table 2. Comparison of the production of different methyl halides by methyl chloride transferase from *E. muricata*. Crude extract, prepared as in Fig. 2, was incubated in 4-ml reaction volumes containing 250 mM of the appropriate potassium halide, 10 mM phosphate, pH 7.58, and 500 μM SAM. Reactions were incubated at 25°C for 6 hours. Gas samples were removed and analyzed as in Fig. 1 except the appropriate methyl halide was used as a standard. The synthesis of all methyl halides was confirmed by gas chromatography–mass spectroscopy.

Methyl halide	Rate of methyl halide production (pmol liter^{-1} min^{-1})
CH_3Cl	8
CH_3Br	16
CH_3I	2165

were identified as methyl chloride producers. Thus it is likely that the methyl transferase enzyme is a constitutive activity in a variety of microorganisms and marine algae. The presence of the enzyme in ice plant, a terrestrial plant which grows in great abundance in the California coastal soils, is an interesting observation that perhaps signals a need for a survey of methyl chloride transferase activity in other succulents that grow in saline-rich environments. Also noteworthy is the fact that ice plant has a wide global distribution. Although the production of 5×10^6 tons per year represents a prodigious rate of methyl chloride synthesis, this number may be quite understandable in terms of the large terrestrial and marine biomass that can contribute to its formation.

REFERENCES AND NOTES

1. R. A. Rasmussen, L. E. Rasmussen, M. A. K. Khalil, R. W. Dalluge, *J Geophys. Res.* **85**, 7350 (1980).
2. D. B. Harper, *Nature* **315**, 55 (1985).
3. P. M. Gschwend, J. K. MacFarlane, K. A. Newman, *Science* **227**, 1033 (1985); S. L. Manley and M. N. Dastoor, *Limnol. Oceanogr* **32**, 709 (1987); G. R. Harvey, quoted in W. L. Chameides and D. D. Davis, *J Geophys. Res.* **85**, 7383 (1980).
4. P. D. Shaw and L. P. Hager, *J Am. Chem. Soc.* **81**, 1011 (1959); D. R. Morris and L. P. Hager, *J. Biol. Chem* **241**, 1763 (1966); F. S. Brown and L. P. Hager, *ibid.* **242** (1967); J. Manthey and L. P. Hager, *ibid.* **256**, 11232 (1981); J. E. Harrison and J. Schultz, *ibid.* **251**, 1371 (1976); R. D. Libby, J. A. Thomas, L. W. Kaiser, L. P. Hager, *ibid.* **257**, 5030 (1982); M. Morrison and G. R. Schonbaum, *Annu. Rev. Biochem.* **45**, 861 (1976); H. Vilter, *Phytochemistry* **23**, 1387 (1984); S. L. Neidleman and J. Geigert, *Biohalogenation: Principles, Basic Roles and Applications* (Halstead, New York, 1986).
5. R. Theiler, J. C. Cook, L. P. Hager, J. F. Siuda, *Science* **202**, 1094 (1981).
6. R. S. Beissner, W. J. Guilford, R. M. Coates, L. P. Hager, *Biochemistry* **20**, 3724 (1981).
7. R. H. White, *Arch. Microbiol.* **132**, 100 (1982).
8. *Endocladia muricata* was collected from intertidal rocks in Monterey Bay, California, in June 1989. Ice plant cuttings were taken from plants growing on the grounds of the Hopkins Marine Station of Stanford University, Pacific Grove, California.
9. M. M. Hitt, T. Wu, G. Cohen, S. Linn, *J. Biol. Chem.* **263**, 4392 (1988); J. M. Glick and P. S. Leboy, *ibid.* **252**, 4790 (1977); D. C. Eicher, N. K. Raber, C. M. Shumard, S. J. Eales, *Biochemistry* **26**, 1639 (1987).
10. A. Streitweiser, *Chem Rev.* **56**, 571 (1956).
11. O. C. Zafirou, *J. Mar. Res.* **33**, 75 (1975).
12. A. Wuosmaa and L. P. Hager, unpublished observations.
13. We thank J. Jirell for generating the GC-MS data. We are also grateful for the use of facilities at the Hopkins Marine Station of Stanford University. Supported by NSF grant DMB 88-13243.

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Identification of Small Clusters of Divergent Amino Acids That Mediate the Opposing Effects of *ras* and K*rev*-1

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K*rev*-1 is an anti-oncogene that was originally identified by its ability to induce morphologic reversion of *ras*-transformed cells that continue to express the *ras* gene. The K*rev*-1–encoded protein is structurally related to Ras proteins. The biological activities of a series of *ras*–K*rev*-1 chimeras were studied to test the hypothesis that K*rev*-1 may directly interfere with a *ras* function. The *ras*-specific and K*rev*-1–specific amino acids immediately surrounding residues 32 to 44, which are identical between the two proteins, determined whether the protein induced cellular transformation or suppressed *ras* transformation. Because this region in Ras proteins has been implicated in effector function, the results suggest that K*rev*-1 suppresses *ras*-induced transformation by interfering with interaction of Ras with its effector.

INTERPLAY BETWEEN POSITIVE AND negative regulators determines whether a cell will grow and divide, with oncogenes stimulating and anti-oncogenes (tumor suppressor genes) inhibiting these processes. Considerable insight has been gained into the mechanism by which oncogenes stimulate cell proliferation; however, much less is known about mechanisms of anti-oncogene function (*1*). Although the proteins of some anti-oncogenes are structurally unrelated to those of oncogenes, the K*rev*-1 anti-oncogene (*2*) encodes a protein that is structurally related to the *ras* oncogene–encoded proteins (*3*), with which it shares about 50% sequence similarity (*4, 5*). As is true of *ras*-encoded proteins, the K*rev*-1–encoded protein binds guanine nucleotides and possesses a guanosine triphosphatase (GTPase) activity (*4*).

K*rev*-1 induces morphological reversion of a cell line transformed by a *ras* oncogene. Because the reverted line still expresses the transforming *ras* gene, K*rev*-1 may inhibit *ras* function by interfering with some aspect of the *ras* pathway (*2*). To test this hypothesis, we identified the sequences in *ras* and K*rev*-1 that account for their opposing biological activities. If the critical differences between the two proteins were limited to a few amino acids, it would focus attention on the function served by this region. Therefore we have made a series of *ras*–K*rev*-1 chimeric genes and studied their ability to induce cellular transformation and to suppress *ras*-induced transformation.

The proteins encoded by K*rev*-1 and *ras* are 184 and 189 amino acids, respectively (Fig. 1). Sequence alignment indicates that the two proteins are colinear, with K*rev*-1 encoding two additional amino acids between *ras* residues 120 to 136, and seven fewer amino acids between *ras* residues 161 to 186. Compared with K*rev*-1, 70% of the NH_2-terminal 60 amino acids encoded by ras^H are identical, 60% of the next 60 residues are identical, and 33% of the COOH-terminal residues are identical.

The highly transforming v-*ras*-AT gene that we used for our chimeric constructions was derived from the Harvey murine sarcoma virus oncogene (*6, 7*) (Fig. 1). The protein product of v-*ras*-AT differed from c-*ras* by only two amino acids, a highly activating Arg^{12} in place of Gly, and Thr^{61} in place of Gln (Fig. 1). To facilitate construction of *ras*–K*rev*-1 chimeric genes, we engineered restriction endonuclease cleavage sites at comparable regions of each gene. This change resulted in restriction endonuclease sites at nucleotides encoding amino acids 5 (Hind III), 60 (Pst I), 109 (Aat II), and 146 (Hae II). In *ras*, the Hind III site is present in the wild-type gene, and the mutations that created the Aat II and Hae II sites were silent. The mutations required to make the Pst I site changed Thr^{59}, which is specific to v-*ras*, to Ala, the amino acid encoded by c-

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ras. Gln^{61} was changed from Gln to Thr, which is partially activating in c-*ras* (*8*). K*rev*-1 was unchanged, as it normally encodes Thr^{61}.

To maximize the likelihood that the chimeric genes would be expressed at similar concentrations, we synthesized a synthetic K*rev*-1 gene that encoded the authentic protein, with codon usage similar to that of v-ras^H; the synthetic K*rev*-1 gene contained the same restriction endonuclease sites as v-*ras*-AT (Fig. 1). For the biological studies, the first five amino acids of the Krev-1 protein, as well as of all chimeric genes, were provided by v-*ras*. The protein encoded by this gene, K*rev*-1-T, differs from authentic K*rev*-1 by substituting Thr^2 for Arg. The K*rev*-1-T gene induced morphological reversion of *ras*-transformed cells, as does the authentic gene (Fig. 2). Using the common restriction endonuclease sites, we constructed chimeric v-*ras*-AT–K*rev*-1-T genes (Fig. 2) by dividing each gene into four comparable restriction endonuclease fragments (encoding amino acids 5–60, 61–108, 109–146, and 147–end, designated fragments A, B, C, and D, for v-*ras*-AT and a, b, c, and d for K*rev*-1-T) and ligating one member of each fragment in various combinations.

We identified three different classes of chimeric genes: (i) those that were transforming, (ii) those that suppressed, and (iii) those that were null for both biological activities. Each chimera encoded a stable protein.

Plasmid pK39, whose Ras-specific amino acids were limited to fragment A, was fully transforming. These results indicate (i) the divergent v-*ras*–encoded amino acids required for transformation are located NH_2-terminal to residue 55 (residues 55–60 are identical between the two genes), and (ii) the divergent K*rev*-1 amino acids located COOH-terminal to amino acid 54 can substitute functionally for those encoded by v-*ras*. pK50 contained K*rev*-1–specific amino acids located NH_2-terminal to amino acid

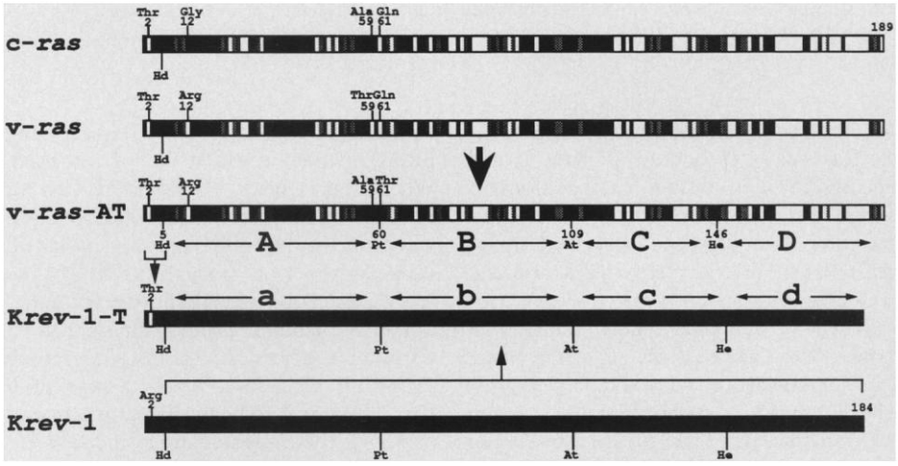

Fig. 1. Structure and origin of v-*ras*-AT and K*rev*-1-T genes used to generate the chimeric genes shown in Figs. 2 and 3. For amino acid sequence comparison in this and subsequent figures, K*rev*-1 is used as the standard. The three *ras* genes shown differ only at codons 12, 59, and 61. The first five amino acids of K*rev*-1-T are derived from *ras*. They are divergent only at codon 2, as shown. The vertical arrows indicate that v-*ras*-AT was derived from v-*ras* and K*rev*-1-T from K*rev*-1. Standard methods of molecular cloning were used throughout (*17*). Mutations were introduced into v-ras^H by oligonucleotide site-directed mutagenesis (*18*). Oligonucleotide sequences are available on request. A synthetic K*rev*-1 gene was constructed to have codon usage similar to v-ras^H. To construct K*rev*-1, we synthesized a series of nine pairs of complementary, single-stranded oligonucleotides, each 60 to 70 bases in length, so that when each pair was annealed to its complementary strand there would be a unique 7-bp single-stranded overhang complementary to the overhang of the adjacent pair of olignucleotides (sequences available on request). To facilitate construction of the chimeras shown in Fig. 2, we included an Xho I site (not shown) 114 nucleotides downstream from the K*rev*-1 stop codon. The synthetic gene was cloned in pUC18. After transfer to M13, the sequence was verified by dideoxy sequencing (*19*). Hd, Hind III; Pt, Pst I; At, Aat II; He, Hae II. Identical regions are shown in black, divergent regions are white, and conservative changes are mottled.

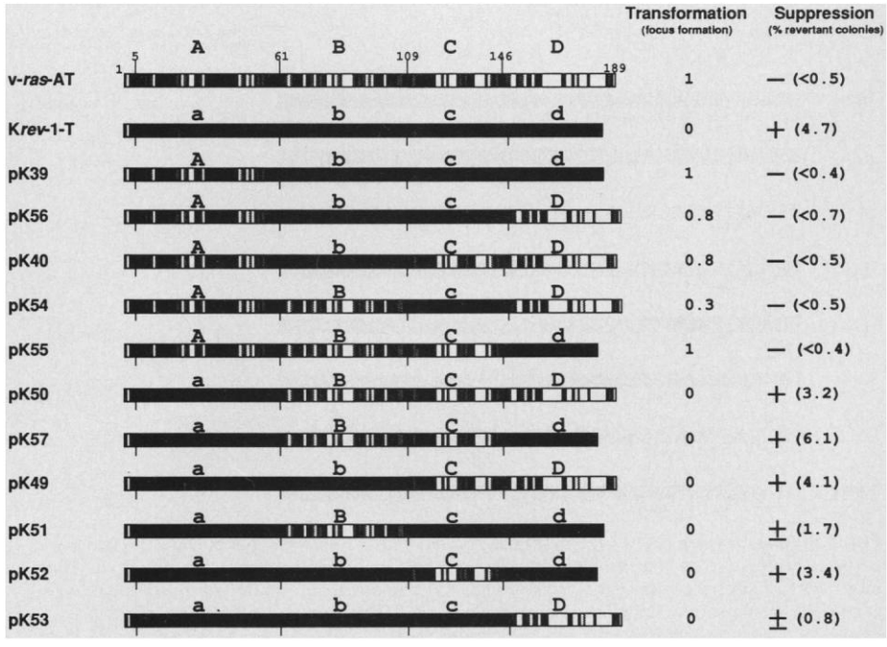

Fig. 2. Biological activities of v-*ras*-AT–K*rev*-1 chimeric genes. Chimeric genes were placed in a retroviral vector, pBW1423 (*7*), which contains authentic v-ras^H and a dominant selectable marker (neo^r), and has a unique Xho I site located downstream from the v-ras^H stop codon. The 3′ end of fragment D of v-*ras*-AT and K*rev*-1-T each terminate in an Xho I site downstream from the stop codon. The v-*ras*-AT, K*rev*-1-T, and the chimeric genes were each inserted in the vector as a Hind III–Xho I fragment (containing all coding sequences downstream from codon 5). Therefore the first five codons of each gene are derived from v-*ras*. K*rev*-1 encoded amino acids are in black; residues that are identical in v-*ras* are shown in black, divergent residues are white, and those with conservative changes are mottled. Ability to induce cellular transformation of mouse NIH 3T3 cells, as determined by focus formation, was assayed as previously described (*4*), by the calcium phosphate precipitation technique. The transforming activity of v-*ras*-AT ($\sim 10^4$ focus-forming units per milligram of DNA) was similar to that of wild-type v-ras^H. Suppression of transformation was tested, as previously described (*2*), with the highly transformed DT cell line, which is NIH 3T3 cells that have been nonproductively transformed by two copies of the v-ras^K gene of Kirsten murine sarcoma virus. Briefly, the DT cells were transfected with calcium phosphate–precipitated plasmid DNA, treated with geneticin (1 mg/ml), and the proportion of flat, geneticin-resistant colonies was determined without knowing which chimeric DNA had been placed on the cells.

55 and suppressed transformation by *ras*. Paradoxically, the regions of *Krev*-1 and *ras* required for suppression and transformation, respectively, shared the greatest similarity between the two genes. The v-*ras* sequences at the 3′ end of the gene can substitute functionally for *Krev*-1, although they shared only 46% identity.

Chimeras whose three COOH-terminal restriction endonuclease fragments (B, C, D or b, c, d) were derived from a combination of both genes had significantly reduced activity (Fig. 2). For example, pK54, which had only fragment c from *Krev*-1, was significantly less transforming than pK39; pK53 did not induce clear-cut suppression of transformation, although its fragments a, b, and c were from *Krev*-1. Therefore, although divergent sequences 3′ to those encoding amino acid residue 54 do not mediate the distinct biological activities of the two genes, the COOH-terminal two-thirds of the protein may act as a unit whose function is impaired by certain chimeric constructions. For example, some sequences from each fragment participate in guanine nucleotide binding (*3*, *7*).

Having localized the critical sequences for transformation by v-*ras* or suppression by *Krev*-1 to their NH_2-terminal 54 amino acids, we assessed the contribution of sequences within this segment by studying additional v-*ras*-AT–*Krev*-1-T chimeric genes (Fig. 3). These genes were constructed by dividing nucleotides encoding amino acids 5–60 of each gene into three fragments (amino acids 5–17, 18–40, and 41–60, designated respectively A_1, A_2, and A_3 for Ras and a_1, a_2, and a_3, for Krev-1) and ligating them in various combinations to amino acid 60 of *Krev*-1 (Fig. 3). Each of the three small segments contained amino acids that are divergent between *Krev*-1 and v-*ras* (Fig. 4).

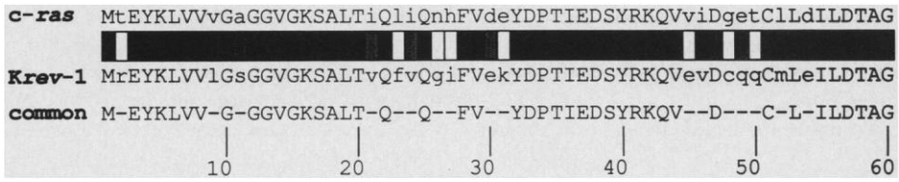

Fig. 4. Similarity between the NH_2-terminal 60 amino acid residues of c-*ras* and *Krev*-1. *Krev*-1–encoded amino acids are the standard; residues that are identical in *ras* are shown in black, divergent residues are white, and those with conservative changes are mottled.

When we examined cellular transformation, pK96, whose v-*ras* sequences were limited to nucleotides encoding amino acids 18–60, was found to induce transformation with an efficiency close to that of v-*ras*. In contrast, substitution of *Krev*-1 amino acids 18–40 (pK97 and pK81) or 41–60 (pK95 and pK63) severely impaired the transforming activity. Thus, transformation by the chimeras is determined by Ras amino acids 21–60. Because amino acids 18–20, 32–44, and 55–60 in Ras and Krev-1 proteins are identical (Fig. 4), our data imply that divergent amino acids within regions 21–31 and 45–54 are essential for *ras* transforming activity.

Analysis of the chimeras for their ability to suppress *ras*-mediated transformation indicated that substitution of *ras* sequences encoding amino acids 18–40 (pK95 and pK63) greatly impaired *Krev*-1 function. When amino acids 18–40 were derived from *Krev*-1, suppression of transformation was maintained whether amino acids 41–60 were derived from *Krev*-1 (pK64) or *ras* (pK97 and pK81). In addition, point mutation of nucleotides encoding amino acid 38 abolished suppression of transformation by *Krev*-1 (*9*). Therefore, biological activity of *Krev*-1 appeared to be mediated by a region of *Krev*-1 that is analogous to the segment of *ras* that controls effector function and cellular transformation. Divergent amino acids required for suppression by *Krev*-1 were restricted to residues 21–31, which is one of the two analogous segments of *ras* that specify transformation.

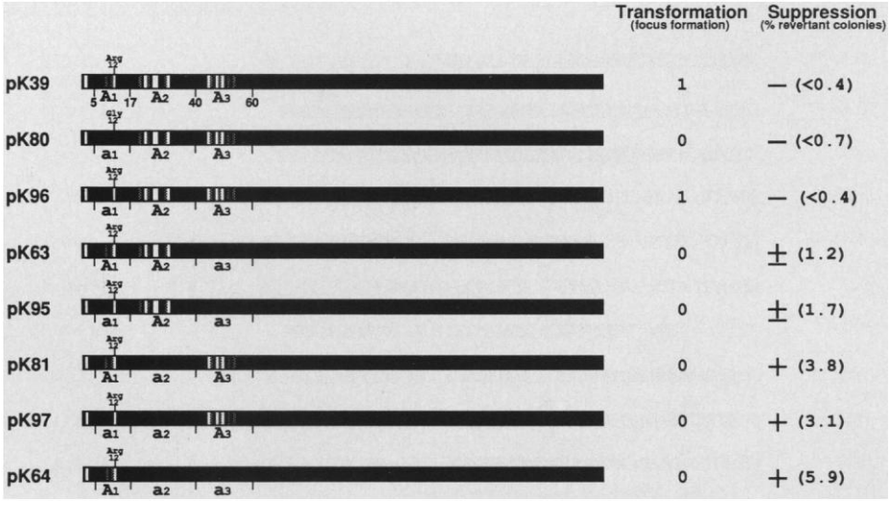

Fig. 3. Biological activities of NH_2-terminal v-*ras*-AT–*Krev*-1 chimeric genes. *Krev*-1–encoded amino acids are black; residues that are identical in v-*ras* are shown in black, divergent residues are white, and those with conservative changes are mottled. Combinations of double-stranded oligonucleotides composed of nucleotides encoding amino acids 5–17, 18–40, and 41–60 of v-*ras*-AT or *Krev*-1 were ligated to sequences encoding the first five amino acids of v-*ras* and to the 3′ residues of *Krev*-1, as shown, and studied in the expression vector described in Fig. 2. pK96, pK95, and pK97, whose nucleotides encoding amino acid residues 5–17 were derived from *Krev*-1, encoded Arg in place of Gly^{12}. Transformation and suppression assays were carried out as described in Fig. 2.

Previously, we thought the areas of sequence divergence between *ras* and *Krev*-1 that mediate their opposing biological effects might be distributed throughout the protein. However, we found that the specific function of each gene was limited to only a few divergent amino acids located within a limited, highly conserved region of the NH_2-termini. Because this region in Ras protein has been implicated in effector function (*7*, *10*), *Krev*-1 may antagonize *ras* by directly interfering with Ras effector function.

Previous analyses of *ras* genes have used mutation-induced loss of biological activity to define residues required for ras effector function (*7*, *10*). One study concluded that amino acids 32–40 were involved in this function (*10*). Independent analysis of several in-frame *ras* insertion-deletion mutants revealed that lesions involving amino acids 22–43 displayed a similar phenotype, suggesting that at least this segment might, in a genetic sense, be involved in effector function (*7*). Mutants in this region encode stable proteins that retained their membrane localization and ability to bind guanine nucleotides, but they are unable to induce transformation. Mutation of *ras* nucleotides encoding amino acids 32–40 (*10*) gives rise to mutants with a similar phenotype that, in addition, were shown to be defective in their ability to promote growth of yeast or to stimulate yeast adenylate cyclase (*11*).

By extending the analysis to *Krev*-1, we

examined sequences required for change of function (suppression of transformation). Given that amino acids 32–44 are identical in K*rev*-1 and *ras*, that amino acid 38 is required for K*rev*-1 suppression, and that K*rev*-1–specific amino acids between residues 21 and 31 are also essential for suppression (*9*), our results imply that suppression by K*rev*-1 is mediated by a region in K*rev*-1 that is analogous to the region of *ras* previously defined by the deletion mutants as the ras effector region.

Ras protein crystal structure indicates that residues 26–36 form an exterior loop (*12*), which suggests that this region is sterically available for interaction with Ras target molecules. Amino acid residues 26, 27, and 31, which lie within the NH_2-terminal half of the loop, are three of the four divergent amino acids within segment 21–31 that were essential for transformation (Fig. 4). This structure is also consistent with results from experiments with *ras* deletion mutants that implicated these residues in effector function (*7*). Because specific amino acids between residues 45–54 are required for efficient transformation by *ras*, amino acids within this segment may impose subtle alterations on the effector region that are critical for transformation but not for suppression.

Previous studies with a chimera formed between the α subunits of the stimulatory (G_s) and inhibitory (G_i) G proteins of adenylate cyclase, two larger guanine nucleotide binding proteins, suggested that COOH-terminal sequences contribute to their effector function (*13*). Another study showed that a chimeric gene formed between *ras* and the *ras*-related gene R-*ras* was transformation-defective when the NH_2-terminal 111 amino acids were encoded by *ras* and the COOH-terminal amino acids were encoded by R-*ras* (*14*). Although this loss of function suggests that residues located downstream from codon 111 might contribute to Ras effector function (*14, 15*), results obtained herein suggest an alternative explanation. We observed nearly full biological activity (determined by fragment A or a) when the COOH-terminal amino acids were derived from the same gene, but several chimeras whose COOH-terminal amino acids were derived from combinations of the two genes displayed anomalously low activity. Perhaps functional integrity of residues carboxyl to amino acid 61 must be maintained for biological activity, but these residues do not contribute to the specificity of the activity (transformation versus suppression). Indeed, our data indicate that the amino acids carboxyl to residue 61 in the Ras and Krev-1 protein can substitute functionally for each other, although fewer than 50% are identical. Because these COOH-terminal residues in Ras are known to be required for membrane association and guanine nucleotide binding, our results suggest that the corresponding region of the K*rev*-1–encoded protein mediates these same functions. We infer that other gene families encoding small guanine nucleotide binding proteins with similarity to Ras proteins, such as *rho*, *ral*, R-*ras*, and *rab* (*16*), also possess a similar functional organization.

Our results would be expected if K*rev*-1 suppressed *ras*-induced transformation by competing with Ras protein for a target molecule. Whereas our results are most consistent with such a model, they do not exclude the possibility that K*rev*-1 antagonizes *ras* function by transmitting its signal to distinct target molecules that in turn mediate the anti-*ras* activity of K*rev*-1. It will be difficult to distinguish unambiguously between these two possibilities until the target molecules are identified.

REFERENCES AND NOTES

1. J. M. Bishop, *Science* **235**, 305 (1987); G. Klein, *ibid* **238**, 1539 (1987); M. R. Green, *Cell* **56**, 1 (1989); R. Sager, *Science* **246**, 1406 (1989).
2. H. Kitayama, Y. Sugimoto, T. Matsuzaki, Y. Ikawa, M. Noda, *Cell* **56**, 77 (1989).
3. M. A. Barbacid, *Annu Rev Biochem.* **56**, 779 (1987); F. McCormick, *Cell* **56**, 5 (1989); J. B. Gibbs and M. S. Marshall, *Microbiol. Rev.* **53**, 171 (1989).
4. M. Kawata *et al.*, *J. Biol. Chem* **263**, 18965 (1988).
5. V. Pizon, P. Chardin, I. Lerosey, B. Olofsson, A. Tavitian, *Oncogene* **3**, 201 (1988).
6. R. Dhar *et al*, *Science* **217**, 934 (1982); S. Yasuda, M. Furuichini, E. Soeda, *Nucleic Acids Res* **12**, 5583 (1984).
7. B. M. Willumsen *et al*, *Mol. Cell Biol* 6, 2646 (1986).
8. C. J. Der, T. Finkel, G. M. Cooper, *Cell* **44**, 167 (1986).
9. H. Kitayama, T. Matsuzaki, Y. Ikawa, M. Noda, *Proc. Natl Acad. Sci. U.S.A* **87**, 4284 (1990).
10. I. S. Sigal, J. B. Gibbs, J. S. D'Alonzo, E. M. Scolnick, *ibid.* **83**, 4725 (1986).
11. J. Field *et al.*, *Mol Cell Biol* **8**, 2159 (1988).
12. A. M. de Vos *et al.*, *Science* **239**, 888 (1988); E. F. Pai *et al. Nature* **341**, 209 (1989).
13. S. B. Masters *et al.*, *Science* **241**, 448 (1988).
14. D. G. Lowe, M. Ricketts, A. D. Levinson, D. V. Goeddel, *Proc. Natl. Acad. Sci U.S.A.* **85**, 1015 (1988).
15. I. S. Sigal, *Nature* **332**, 485 (1988).
16. P. Madaule and R. Axel, *Cell* **41**, 31 (1985); P. Chardin and A Tavitian, *EMBO J.* **5**, 2203 (1986); D. Lowe *et al*, *Cell* **48**, 137 (1987); N. Touchot, P. Chardin, A. Tavitian, *Proc. Natl Acad. Sci. U.S.A.* **84**, 8210 (1987).
17. T. Maniatis, E. F. Fritsch, J. Sambrook, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982).
18. K. Nakamaye and F. Eckstein, *Nucleic Acids Res* **14**, 9679 (1986).
19. F. Sanger, S. Nicklen, A. R. Coulson, *Proc. Natl Acad Sci U.S.A.* **74**, 5463 (1977).

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Common Modifications of Trimeric G Proteins and ras Protein: Involvement of Polyisoprenylation

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The heterotrimeric guanine nucleotide–binding regulatory proteins act at the inner surface of the plasma membrane to relay information from cell surface receptors to effectors inside the cell. These G proteins are not integral membrane proteins, yet are membrane associated. The processing and function of the γ subunit of the yeast G protein involved in mating-pheromone signal transduction was found to be affected by the same mutations that block ras processing. The nature of these mutations implied that the γ subunit was polyisoprenylated and that this modification was necessary for membrane association and biological activity. A microbial screen was developed for pharmacological agents that inhibit polyisoprenylation and that have potential application in cancer therapy.

GUANINE NUCLEOTIDE BINDING proteins (G proteins) participate in eukaryotic signal transduction. Common characteristics of these proteins are guanosine triphosphate (GTP) binding and GTPase activity, which are central to the G protein's signal-transducing ability. G proteins are grouped into at least two classes: the hormone-linked trimeric G proteins (*1*) and the single-subunit G protein typified by the ras family (*2*). Trimeric G proteins are membrane-associated, consist of three different subunits (α, β, and γ), and transduce signals from cell surface receptors to downstream effector proteins on or near the inner surface of the plasma membrane. This class includes the G proteins G_s, recently identified as an oncogene (*3*), and G_t [which transduces a signal from adrenergic receptors to stimulate or inhibit cyclic adenosine