Cold Spring Harbor Symposia on Quantitative Biology

Ras Partners

L. Van Aelst, M.A. White and M.H. Wigler

Cold Spring Harb Symp Quant Biol 1994 59: 181-186
Access the most recent version at doi:10.1101/SQB.1994.059.01.022

Ras Partners

L. Van Aelst, M.A. White, and M.H. Wigler
Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724

THE RAS PROBLEM

The *RAS* oncogenes were first discovered as the transforming elements of acutely oncogenic retroviruses. Subsequently, cellular *RAS* genes were found to be frequently activated by mutation in a wide variety of human cancers, providing the first example of a common oncogenic mechanism. As a consequence, Ras proteins have been studied extensively (for review, see Barbacid 1987).

RAS genes are found ubiquitously in eukaryotic organisms. They encode low-molecular-weight guanine nucleotide-binding proteins that hydrolyze GTP and localize to the inner surface of the plasma membrane after undergoing elaborate carboxy-terminal processing. Proteins that are involved in processing Ras, or in regulating its activity, e.g., by accelerating guanine nucleotide hydrolysis or exchange, have been largely conserved in evolution (for review, see Wigler 1993).

Understanding the function of Ras has been more difficult. Genetic and biochemical studies point to the involvement of Ras in regulating protein kinases in vertebrate cells and in the yeasts, *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*. In *S. cerevisiae*, this occurs by the direct regulation of adenylyl cyclase (Wigler et al. 1988). This particular biochemical function is not observed in other organisms. In both *S. pombe* and vertebrate cells, Ras appears to activate a conserved protein kinase cascade that we call the MAP kinase module (Neiman et al. 1993). Parallels between these two systems indicated the possibility of a conserved biochemical function. There is strong evidence for other Ras functions in both *S. pombe* and *S. cerevisiae* (Toda et al. 1987; Wigler et al. 1988; Wang et al. 1991; Chang et al. 1994).

In this paper, we address the identification of *Ras b*inding (Rsb) proteins and experimental approaches to the analysis of their function. We have employed a powerful genetic tool, the "two-hybrid system" of Fields and Song, which enables the experimenter to determine when two proteins form complexes within yeast cells (Fields and Song 1989). The method comprises expressing the test proteins as "hybrid" proteins, fused to DNA-binding and transcription-activating proteins, such that when the test proteins interact, a reporter gene is transcribed. We describe the use of this system to discover new Ras-binding proteins and to analyze interactions between Ras and these candidate targets.

SEARCHING FOR RAS PARTNERS

S. pombe byr2 is a protein kinase homologous to the Ste11 protein kinase of *S. cerevisiae*. Both Byr2 and Ste11 function on the sexual differentiation pathway in these yeasts. Genetic analysis in *S. pombe* suggests that byr2 is a downstream target for ras1. Overexpression of *byr2* could bypass defects in $ras1^{null}$ cells; disruption of *byr2* led to defects common to $ras1^{null}$ cells; and dominant interfering mutants of *byr2* partially block the effects of activated *ras1* (Wang et al. 1991; Neiman et al. 1993; Xu et al. 1994). Similar analysis in mammalian cells suggested that Raf1 was a potential downstream target of Ras proteins. Raf1, like Byr2 and Ste11, is a protein kinase that participates in the conserved MAP kinase cascade (for review, see Blumer and Johnson 1994; Cook and McCormick 1994). Activation of Raf1 is oncogenic and bypasses the effects of blocking Ras function; moreover, interfering mutants of Raf1 block activated Ras mutants (Smith et al. 1986; Cai et al. 1990; Kolch et al. 1991). Although Raf1 and Byr2 share no significant homology outside of their kinase domains, they have a similar relationship to Ras (Van Aelst et al. 1993).

We thus sought genetic evidence, utilizing the two-hybrid system, that Ras can form complexes with either protein kinase (Table 1). As we reported previously (Van Aelst et al. 1993), Ras proteins can complex with both proteins, and this interaction appears to require an intact effector loop, that region of Ras that has been suggested, by both genetic and crystallographic studies, to be a domain required for effector interactions (for review, see Polakis and McCormick 1993). Mutants of Ras that affect guanine nucleotide binding also fail to interact with Raf1 and Byr2 (Van Aelst et al. 1993). Mutants lacking the Ras CAAX box, the carboxy-terminal residues that are required for Ras processing, still interact with Raf1 and Byr2. In contrast, whereas an intact effector loop and intact guanine nucleotide binding are also required for complex formation between Ras and its target in *S. cerevisiae*, adenylyl cyclase (Vojtek et al. 1993), this interaction does require an intact CAAX box (Table 1). This result is

Table 1. Interaction between Hras Proteins and Known Ras Targets

	Cyr1[a]	Byr2	Raf1
Hras	+	+	+
Hras(C186S)	−	+	+
Hras(G15A)	−	−	−
Hras(T35A)	−	−	−

Hras wild-type and mutant proteins were expressed as LexA-binding domain (LBD) fusions. Ras targets were expressed as GAL4 activation domain (GAD) fusions. Pairs were co-expressed in the yeast reporter strain L40 (Vojtek et al. 1993). β-Galactosidase was monitored using a filter assay (for description, see Van Aelst et al. 1993). (+) represents a positive induction of β-galactosidase activity. At least 4 independent transformants were tested for each pair. Previously we reported no interaction between $Hras^{wt}$ expressed as a GAL4 DNA-binding (GBD) fusion with Raf1 expressed as a GAD fusion using the YBP2 strain as reporter, and we only observed interaction with Hras proteins carrying the C186S mutation. However, using the LBD fusion and the yeast reporter strain L40, we do observe this interaction.

[a] *S. cerevisiae* adenylyl cyclase.

consistent with the biochemical experiments of Kataoka and coworkers showing that processed Ras is a more effective activator of adenylyl cyclase than is unprocessed Ras (Kurodi et al. 1993).

We next tested whether the two-hybrid system could be used to screen cDNA libraries for Ras binding partners. In particular, we screened libraries in which cDNA inserts were fused to the carboxyl terminus of the *trans*-activating domain of Gal4 to find genes encoding proteins that could interact with either Ras or Raf1 fused to the DNA-binding domain of Gal4 or to the DNA-binding domain of LexA (Fields and Song 1989; Vojtek et al. 1993). Using a variety of different cDNA libraries, on the order of 1 in 50,000 clones was found able to encode a protein that interacts with these targets. Some of the cDNAs we isolated encoded members of the Ras family (interacting with Raf1) or members of the Raf family (interacting with Ras). Some encoded new proteins. We discuss here only those putative proteins that interact with Ras.

Table 2 contains the results of screening several libraries, a total of about 10^7 cDNA clones. cDNAs encoding Raf1 or RafA were found multiple times. In addition, five other genes were identified, each multiple times. Among these were RalGDS, a protein that was previously identified as a guanine nucleotide exchange factor for Ral, a member of the RAS superfamily of guanine nucleotide binding proteins (Albright et al. 1993). The smallest binding fragment comprised amino acids 770 to the carboxy-terminal end, the region just carboxy-terminal to the conserved nucleotide exchange domain. In addition, another cDNA encoded a product, which we call Rsb3, that was homologous to RalGDS (67% homology between amino acids 580 and 768). Other workers have since reported finding these two targets for Ras (Hofer et al. 1994; Kikuchi et al. 1994). On the basis of these results, it is apparent that Rsb3 (called RGL by Kikuchi et al. 1994) is a global homolog of RalGDS. Therefore, interaction with Ras appears to be a conserved feature of these proteins. These results suggest the hypothesis that Ras can regulate Ral. Previous studies have indicated that members of the RAS superfamily can regulate each other (Chang et al. 1994; Horii et al. 1994).

Rsb1 was another peptide identified by Ras binding. Its sequence is completely contained within a protein called AF6, which has been previously described in a single example as a fusion partner for ALL-1 in acute lymphoblastic leukemias (Prasad et al. 1993). ALL-1 is a homolog of *Drosophila* trithorax and is found fused to various partners in acute lymphoblastic leukemia (Gu et al. 1992; Tkachuk et al. 1992; Domer et al. 1993; Morrissey et al. 1993; Nakamura et al. 1993). Rsb1 comprises amino acids 1–180 of AF6. These sequences overlap with the sequences of AF6 that are deleted when fused to ALL-1 (1–35). We do not know the biological significance, if any, of the interaction between AF6 and Ras, nor whether this relates to its involvement in leukemias.

All these targets, Byr2, Cyr1, Raf1, RafA, RalGDS, Rsb3, and Rsb1, share the property that they do not bind to Ras mutated in the effector loop Hras(T35A) or impaired in guanine nucleotide binding Hras(G15A). We infer from this that these proteins interact with Ras in its active, GTP-bound state. In contrast, two other proteins were found, Rsb2 and Rsb4, which putatively bind Ras containing these mutations, but not Ras with mutations in the -CAAX box. One of these, Rsb4, is identical to the catalytic (β) subunit of the Rab geranyl-geranyl transferase (Armstrong et al. 1993).

Table 2. Interaction between Hras Proteins and New Ras Binding Partners

	Raf1	RafA	Ral GDS	Rsb3	Rsb1	Rsb2	Rsb4
Hras	+	+	+	+	+	+	+
Hras(C186S)	+	+	+	+	+	−	−
Hras(G15A)	−	−	−	−	−	+	+
Hras(T35A)	−	−	−	−	−	+	+

Hras proteins were expressed as LBD fusions. Ras targets were expressed as GAD fusions. Pairs were co-expressed in the yeast reporter strain L40. (+) represents a positive induction of β-galactosidase activity using filter assays. The Ras binding partners were isolated by co-transforming mouse brain, rat embryonic, and Jurkat cDNA libraries in which cDNA inserts were fused to the carboxyl terminus of the *trans*-activating domain of GAL4 with either Hras(C186S) mutant fused to GBD or $Hras^{wt}$ fused to LBD. Raf1, RafA, Ral GDS, Rsb3, and Rsb1 were isolated several times both in the Gal4 and LexA systems. RSB2 and RSB4 were isolated only when $Hras^{wt}$ fused to LBD was used. The tester strain used for the screens was either L40 or HF7e (Vojtek et al. 1993; Feilotter et al. 1994). Primary positives were isolated by selecting for colonies that could grow in the absence of histidine and subsequently screened for β-galactosidase activity.

Table 3. Interaction between Ras-like Proteins and Ras Binding Partners

	Cyr1	Byr2	Raf1	RafA	RalGDS	Rsb3	Rsb1	Rsb2	Rsb4
Hras	+	+	+	+	+	+	+	+	+
Sc.Ras2		+	+	+	+	+	+	+	
Sp.Ras1		+	+	+	+	+	+	+	
Rap1A	–	+	+	+	+	+	+	–	–
R-Ras		+	+	+	+	+	+		
Rab6	–	–	–	–	–	–	–	–	–
Rho1A	–	–	–	–	–	–	–	–	–
Cdc42	–	–	–	–	–	–	–	–	–
Rac1	–	–	–	–	–	–	–	–	–

Ras and Ras-like proteins were expressed as LBD fusions. All proteins were wild type except for R-Ras(C215R). Ras targets were expressed as GAD fusions. (+) represents a positive induction of β-galactosidase activity using filter assays. At least 4 independent transformants were tested for each pair. Positive interactions in the two-hybrid system have been observed between Rab6, Rho1A, Cdc42, and Rac1 with other proteins (data not shown).

Rab proteins are members of the RAS superfamily. Although the βRabGGtase is homologous to the β subunit of the Ras farnesyl transferase, the carboxy-terminal consensus processing signal for Rab is -CXC or -CC, and the Rab geranyl-geranyl transferase does not process Ras (Khosravi-Far et al. 1992). These results raise fundamental questions about what features the transferases recognize in their substrates, and what their role is in cellular compartmentalization. Curiously, we do not detect interaction between the βRabGGtase and Rab proteins in the two-hybrid system (Table 3). We believe that the productive enzymatic interaction between βRabGGtase and Rab may lead to a very transient interaction.

Rsb2 has been sequenced and does not show homology with known proteins. It does have homology with a protein predicted from nucleotide sequencing of the *Caenorhabditis elegans* genome, and we have found a homolog in *S. cerevisiae*. Curiously, although Rsb2 will bind mammalian H-ras, *S. cerevisiae* Ras2, and *S. pombe* ras1, it does not bind other members of the Ras superfamily that we have tested, including Rap1a (Table 3), which is otherwise very homologous to Ras proteins.

EVALUATING PARTNERS

To evaluate the physiological role of candidate Hras targets, we next sought mutant Hras proteins that could discriminate among the candidates. Libraries of vectors expressing mutant Hras proteins fused to GBD were created by PCR mutagenesis (White et al. 1995) and screened against Raf1 and byr2 fused to GAD. Two mutants were identified. One, Hras(T35S), could bind Raf1 but not Byr2, and one, Hras(E37G), could bind Byr2 but not Raf1. These mutants contain mutations in the conserved effector loop of Hras.

In keeping with the hypothesis that Raf1 is a critical target for Hras, the Hras(G12V, E37G) mutant failed to induce transformed foci of NIH-3T3 cells (Fig. 1). To further test this hypothesis, we identified mutants in Raf1 that restore interaction with Hras(E37G). These mutant Raf1s can cooperate with Hras(G12V, E37G) in the induction of transformed foci (Fig. 1). Such results provide further genetic evidence for the physiological importance of Hras/Raf1 interactions. A more detailed description of these results can be found in White et al. (1995).

More puzzling is that the Hras(G12V, T35S) mutant is greatly attenuated in focus induction. Perhaps its interaction with Raf1 is altered in some way that is not reflected by the two-hybrid assay. Alternatively, or in addition, the interaction of Hras(G12V,T35S) with another effector, perhaps one homologous to Byr2, is adversely affected. In keeping with this idea, Hras(G12V,E37G) is capable of cooperating with Hras(G12V,T35S) in focus induction (White et al. 1995). These results suggest that two different effectors of Hras can contribute to mammalian cell transformation. Unfortunately, none of the candidate mammalian targets we have identified to date, except Raf1, discriminates between our Hras mutants. Continued screening of cDNAs in the two-hybrid system, using the mutant Hras proteins, may identify candidates for this putative additional effector of Hras transformation. Alternatively, this effector may be among our candi-

Table 4. Interaction between New Ras Mutants and Ras Binding Partners

	Cyr1	Byr2	Raf1	RalGDS	Rsb3	Rsb1	Rsb2	Rsb4
Ras(G12V)	+	+	+	+	+	+	+	+
Ras(G12V,E37G)	+	+	–	+	+	+	+	+
Ras(G12V,T35S)	–	–	+	+	+	+	+	+

The Ras mutants were expressed as LBD fusions. Isolation and characterization of these mutants is described elsewhere (White et al. 1995). Ras targets were expressed as GAD fusions. (+) represents a positive induction of β-galactosidase activity using filter assays. At least 4 independent transformants were tested.

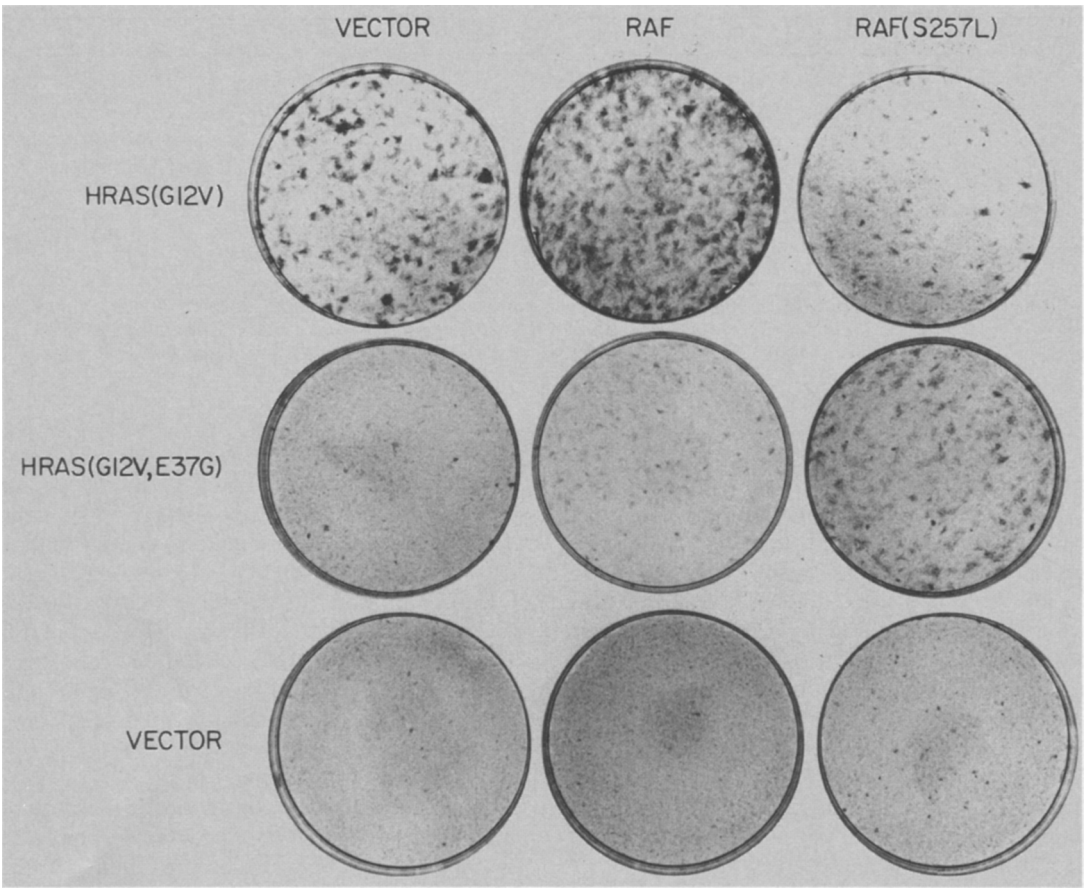

Figure 1. NIH-3T3 cells were cotransfected with the indicated genes or empty vectors (Shih et al. 1979; Wigler et al. 1979; Perucho et al. 1981). Transfected cells were grown in reduced serum for 14 days, then fixed in 10% formaldehyde and stained with Giemsa. Foci of transformed cells appear as diffuse darkly staining spots. Transfection efficiencies, as measured by growth in selective media, were similar for each transfection (White et al. 1995).

dates and interact in the two-hybrid system with Hras(T35S) but interact nonproductively with it in mammalian cells.

THE RAS PROBLEM REVISITED

From our work and that of others, we conclude that Raf1 is a direct mediator of Ras in mammalian cells (Moodie et al. 1993; Van Aelst et al. 1993; Vojtek et al. 1993; Warne et al. 1993; Zhang et al. 1993; White et al. 1995). From our genetic studies, we know that Ras has multiple functions in yeasts (Wigler et al. 1988; Chang et al. 1994) and appears to have multiple functions that can contribute to mammalian cell transformation. We do not know what these other functions may be, although we have defined approaches that may ultimately lead to their identification.

We do not know at the biochemical level how Ras activates effectors. Some data support the idea that Ras merely causes Raf1 to colocalize to the plasma membrane, where Raf1 can encounter other regulators or substrates. For example, Raf1 is activated by fusion to peptides that direct it to the plasma membrane (Leevers et al. 1994; Stokoe et al. 1994), and the function of Ras is severely impaired by mutations that block the processing events which direct it to the plasma membrane (Willumsen et al. 1984). Although Ras may function to cause protein localization, this is certainly not its only function in evolution. In *S. cerevisiae*, Ras activates the enzymatic activity of adenylyl cyclase in a cell-free system (Broek et al. 1987; Field et al. 1988), and in *S. pombe*, Ras can promote the cooperative binding of proteins involved in morphogenesis (Chang et al. 1994). None of the mechanisms of productive interaction between Ras and its effectors has been solved.

ACKNOWLEDGMENTS

We thank Anne Vojtek, Chaker N. Adra, and Isabelle Janoueix-lerosey for providing some of the plasmids and yeast strains used in this study. We thank Jacques Camonis for his help in constructing the cDNA libraries. We thank Linda Rodgers, Michael Riggs, Kim Farina, and Gigi Asouline for technical assistance; and Patricia Bird for preparing the manuscript. This work was supported by the American Cancer Society and by the National Cancer Institute of the National Institutes of Health. L.V.A. is a member of the Belgian National

Fund for Scientific Resarch. M.H.W. is an American Cancer Society Research Professor.

REFERENCES

Albright, C.F., B.W. Giddings, J. Liu, M. Vito, and R.A. Weinberg. 1993. Characterization of guanine nucleotide dissociation stimulator for a *ras*-related GTPase. *EMBO J.* **12:** 339.

Armstrong, S.A., M.C. Seabra, T.C. Sudhof, J.L. Goldstein, and M.S. Brown. 1993. cDNA cloning and expression of the α and β subunits of rat Rab geranylgeranyl transferase. *J. Biol. Chem.* **268:** 12221.

Barbacid, M. 1987. *ras* genes. *Annu. Rev. Biochem.* **56:** 779.

Blumer, K.J. and G.L. Johnson. 1994. Diversity in function and regulation of MAP kinase pathways. *Trends Biochem. Sci.* **19:** 236.

Broek, D., T. Toda, T. Michaeli, L. Levin, C. Birchmeier, M. Zoller, S. Powers, and M. Wigler. 1987. The *S. cerevisiae CDC25* gene product regulates the RAS/adenylate cyclase pathway. *Cell* **48:** 789.

Cai, H., J. Szeberenyi, and G.M. Cooper. 1990. Effect of a dominant inhibitory Ha-*ras* mutation on mitogenic signal transduction in NIH 3T3 cells. *Mol. Cell. Biol.* **10:** 5314.

Chang, E.C., M. Barr, and Y. Wang. 1994. Cooperative interaction of *S. pombe* proteins required for mating and morphogenesis. *Cell* **79:** 131.

Cook, S. and F. McCormick. 1994. Ras blooms on sterile ground. *Nature* **369:** 361.

Domer, P.H., S.S. Fakharzadeh, C.-S. Chen, J. Jockel, L. Johansen, G.A. Silverman, J.H. Kersey, and S.J. Korsmeyer. 1993. Acute mixed-lineage leukemia t(4;11)-(q21;23) generates an *MLL-AF4* fusion product. *Proc. Natl. Acad. Sci.* **90:** 7884.

Feilotter, H.E., G.J. Hannon, C.J. Ruddell, and D. Beach. 1994. Construction of an improved host strain for two hybrid screening. *Nucleic Acids Res.* **22:** 1502.

Field, J., J. Nikawa, D. Broek, B. MacDonald, L. Rodgers, I.A. Wilson, R.A. Lerner, and M. Wigler. 1988. Purification of a RAS-responsive adenylyl cyclase complex from *Saccharomyces cerevisiae* by use of an epitope addition method. *Mol. Cell. Biol.* **8:** 2159.

Fields, S. and O.-K. Song. 1989. A novel genetic system to detect protein-protein interactions. *Nature* **340:** 245.

Gu, Y., T. Nakamura, H. Alder, R. Prasad, O. Canaani, G. Cimino, C.M. Croce, and E. Canaani. 1992. The t(4:11) chromosome translocation of human acute leukemias fuses the *ALL-1* gene related to *Drosophila trithorax*, to the *AF-4* gene chromosome translocations in acute leukemias. *Cell* **71:** 701.

Hofer, F., S. Fields, C. Schneider, and G.S. Martin. 1994. Activated Ras interacts with the Ral guanine nucleotide dissociation stimulator. *Proc. Natl. Acad. Sci.* **91:** 11089.

Horii, Y., J.F. Beeler, K. Sakaguchi, M. Tachibana, and T. Miki. 1994. A novel oncogene, *ost*, encodes a guanine nucleotide exchange factor that potentially links Rho and Rac signaling pathways. *EMBO J.* **13:** 4776.

Khosravi-Far, R., G.J. Clark, K. Abe, A.D. Cox, T. McLain, R.J. Lutz, M. Sinensky, and C.J. Der. 1992. Ras (CXXX) and Rab (CC/CXC) prenylation signal sequences are unique and functionally distinct. *J. Biol. Chem.* **267:** 24363.

Kikuchi, A., S.D. Demo, Z.-H. Ye, Y.-W. Chen, and L.T. Williams. 1994. ralGDS family members interact with the effector loop of *ras* p21. *Mol. Cell. Biol.* **14:** 7483.

Kolch, W., G. Heidecker, P. Lloyd, and U. Rapp. 1991. Raf-1 protein kinases is required for growth of induced NIH/3T3 cells. *Nature* **349:** 426.

Kurodi, Y., N. Suzuki, and T. Kataoka. 1993. The effect of posttranslational modifications on the interaction of Ras2 with adenyl cyclase. *Science* **259:** 683.

Leevers, S.J., H.F. Paterson, and C.J. Marshall. 1994. Requirement for Ras in Raf activation is overcome by targeting Raf to the plasma membrane. *Nature* **369:** 411.

Moodie, S.A., B.M. Willumsen, M.J. Weber, and A. Wolfman. 1993. Complexes of Ras-GTP with Raf-1 and mitogen-activated protein kinase kinase. *Science* **260:** 1658.

Morrissey, J., D.C. Tkachuk, A. Milatovich, U. Francke, M. Link, and M.L. Cleary. 1993. A serine/proline-rich protein is fused to *HRX* in t(4;11) acute leukemias. *Blood* **81:** 1124.

Nakamura, T., H. Alder, Y. Gu, R. Prasad, O. Canaani, N. Kamada, R.P. Gale, B. Lange, W.M. Crist, P.C. Nowell, C.M. Croce, and E. Cannani. 1993. Genes on chromosome 4,9 and 19 involved in 11q23 abnormalities in acute leukemia share sequence homology and/or common motifs. *Proc. Natl. Acad. Sci.* **90:** 4631.

Neiman, A., B. Stevenson, H.-P. Xu, G.F. Sprague, Jr., I. Herskowitz, M. Wigler, and S. Marcus. 1993. Functional homology of protein kinases required for sexual differentiation in *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* suggests a conserved signal transduction module in eukaryotic organisms. *Mol. Biol. Cell* **4:** 107.

Perucho, M., M. Goldfarb, K. Shimizu, C. Lama, J. Fogh, and M. Wigler. 1981. Human-tumor-derived cell lines contain common and different transforming genes. *Cell* **27:** 467.

Polakis, P. and F. McCormick. 1993. Structural requirements for the interaction of $p21^{ras}$ with GAP, exchange factors, and its biological effector target. *J. Biol. Chem.* **268:** 9157.

Prasad, R., Y. Gu, H. Alder, T. Nakamura, O. Canaani, H. Saito, K. Huebner, R.P. Gale, K. Nowell, K. Kuriyama, Y. Miyazaki, C.M. Croce, and E. Canaani. 1993. Cloning of the *ALL-1* fusion partner, the *AF-6* gene, involved in acute myeloid leukemias with the t(6;11) chromosome translocation. *Cancer Res.* **53:** 5624.

Shih, C., B. Shilo, M. Goldfarb, A. Dannenberg, and R. Weinberg. 1979. Passage of phenotypes of chemically transformed cells via transfection of DNA and chromatin. *Proc. Natl. Acad. Sci.* **76:** 5714.

Smith, M.R., S.J. DeGudicibus, and D.W. Stacey. 1986. Requirement for c-*ras* proteins during viral oncogene transformation. *Nature* **320:** 540.

Stokoe, D., S.G. MacDonald, K. Caddwallader, M. Symons, and J.F. Hancock. 1994. Activation of Raf as a result of recruitment to the plasma membrane. *Science* **264:** 1463.

Tkachuk, D., S. Kohler, and M. Cleary. 1992. Involvement of a homolog of *Drosophila trithorax* by 11q23 chromosome translocations in acute leukemias. *Cell* **71:** 691.

Toda, T., S. Cameron, P. Sass, and M. Wigler. 1987. Three different genes in the yeast *Saccharomyces cerevisiae* encode the catalytic subunits of the cAMP dependent protein kinase. *Cell* **50:** 277.

Van Aelst, L., M. Barr, S. Marcus, A. Polverino, and M. Wigler. 1993. Complex formation between RAS and RAF and other protein kinases. *Proc. Natl. Acad. Sci.* **90:** 6213.

Vojtek, A., S.M. Hollenberg, and J.A. Cooper. 1993. Mammalian Ras interacts directly with the serine/threonine kinase Raf. *Cell* **74:** 205.

Wang, Y., H.-P. Xu, M. Riggs, L. Rodgers, and M. Wigler. 1991. *byr2*, an *S. pombe* gene encoding a protein kinase capable of partial suppression of *ras1* mutant phenotype. *Mol. Cell. Biol.* **11:** 3554.

Warne, P.H., P.R. Viciana, and J. Downward. 1993. Direct interaction of Ras and the amino-terminal region of Raf-1 *in vivo*. *Nature* **364:** 352.

White, M.A., C. Nicolette, A. Minden, A. Polverino, L. Van Aelst, M. Karin, and M. Wigler. 1995. Multiple RAS functions can contribute to cell transformation. *Cell* (in press).

Wigler, M.H. 1993. The Ras system in yeasts. In *The* ras *superfamily of GTPases* (ed. J.C. Lacal and F. McCormick), p. 155. CRC Press, Boca Raton, Florida.

Wigler, M., A. Pellicer, S. Silverstein, R. Axel, G. Urlaub, and L. Chasin. 1979. DNA mediated transfer of the *APRT* locus into mammalian cells. *Proc. Natl. Acad. Sci.* **76:** 1373.

Wigler, M., J. Field, S. Powers, D. Broek, T. Toda, S. Cameron, J. Nikawa, T. Michaeli, J. Colicelli, and K. Ferguson. 1988. Studies of RAS function in the yeast *Saccharomyces cerevisiae*. *Cold Spring Harbor Symp. Quant. Biol.* **53:** 649.

Willumsen, B.M., A. Christensen, N.L. Hubbert, A.G. Papageorge, and D.R. Lowy. 1984. The p21 *ras* C-terminus is required for transformation and membrane association. *Nature* **310:** 583.

Xu, H.-P., M. White, S. Marcus, and M. Wigler. 1994. Concerted action of RAS and G proteins in the sexual response pathways of *Schizosaccharomyces pombe*. *Mol. Cell. Biol.* **14:** 50.

Zhang, X.-F., J. Settleman, J.M. Kyriakis, E. Takeuchi-Suzuki, S.J. Elledge, M.S. Marshall, J.T. Bruder, U.R. Rapp, and J. Avruch. 1993. Normal and oncogenic $p21^{ras}$ proteins bind to the amino-terminal regulatory domain of c-raf-1. *Nature* **364:** 308.