Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 2913–2917, April 1991
Genetics

Expression of three mammalian cDNAs that interfere with RAS function in *Saccharomyces cerevisiae*

(RAS suppressors/oncogenes/yeast expression vector/mammalian cDNA cloning)

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ABSTRACT **_Saccharomyces cerevisiae_ strains expressing the activated *RAS2*^{Val19} gene or lacking both cAMP phosphodiesterase genes, *PDE1* and *PDE2*, have impaired growth control and display an acute sensitivity to heat shock. We have isolated two classes of mammalian cDNAs from yeast expression libraries that suppress the heat shock-sensitive phenotype of a *RAS2*^{Val19} strain. Members of the first class of cDNAs also suppress the heat shock-sensitive phenotype of *pde1*$^-$ *pde2*$^-$ strains and encode cAMP phosphodiesterases. Members of the second class fail to suppress the phenotype of *pde1*$^-$ *pde2*$^-$ strains and therefore are candidate cDNAs encoding proteins that interact with RAS proteins. We report the nucleotide sequence of three members of this class. Two of these cDNAs share considerable sequence similarity, but none are clearly similar to previously isolated genes.**

The mammalian *RAS* genes were first discovered as homologs of retroviral oncogenes (1). Activated, mutant *RAS* alleles are frequently found in human tumors (2). *RAS* homologs have been found and described in many eukaryotic organisms (3–7). In the yeast *Saccharomyces cerevisiae* products of the two *RAS* homologs, *RAS1* and *RAS2*, activate adenylyl cyclase (8, 9). Although mammalian RAS proteins can function in this way when expressed in yeast (10, 11), the function of mammalian RAS in mammalian cells is still unknown. One candidate target of mammalian RAS action is GAP, the GTPase-activating protein, which also has been proposed as a regulator of RAS action (12, 13).

Like mammalian *RAS*, the yeast *RAS2* gene can be activated by point mutation (14). Yeast containing *RAS2*^{Val19} have multiple defects. They are sensitive to heat shock and cannot survive prolonged nutrient deprivation (8, 15). The same phenotypes are also seen in *pde1*$^-$ *pde2*$^-$ yeast, which lack cAMP phosphodiesterases (16). The aberrant phenotypes of both *RAS2*^{Val19} and *pde1*$^-$ *pde2*$^-$ strains can be suppressed by overexpression of yeast or mammalian phosphodiesterases (16, 17). Expression of enzymatically inactive adenylyl cyclase, the target of RAS action in yeast, can also suppress the phenotype of *RAS2*^{Val19} strains but cannot suppress the phenotype of *pde1*$^-$ *pde2*$^-$ strains (18). The ability of truncated adenylyl cyclase to interfere with RAS function probably reflects its nonproductive interactions with RAS.

We have screened libraries of mammalian cDNAs, cloned into yeast expression vectors, for those cDNAs capable of suppressing the phenotype of *RAS2*^{Val19} yeast. We previously reported isolating in this manner a rat gene encoding a cAMP phosphodiesterase homologous to the *Drosophila melanogaster* dunce protein (17). The rat cDNA also suppresses the phenotype of *pde1*$^-$ *pde2*$^-$ yeast and presumably works by lowering intracellular cAMP concentrations. We now report three new human cDNAs§ that suppress the phenotypes of *RAS2*^{Val19} yeast but fail to suppress the phenotypes of *pde1*$^-$ *pde2*$^-$ yeast. These cDNAs therefore may encode mammalian proteins that interact directly with RAS proteins.

MATERIALS AND METHODS

Strains, Growth Conditions, Heat Shock Assays, and Segregation Analysis. Plasmids were propagated in *Escherichia coli* strains HB101 or SCS1 (Stratagene). *S. cerevisiae* strains TK161RV (*MATa RAS2*^{Val19} *leu2 his3 ura3 ade8 trp1*) (8) and 10DAB (*MATα leu2 his3 ura3 ade8 pde1::ADE8 pde2::URA3 ras1::HIS3*) (17) were grown in rich medium [yeast extract/peptone/dextrose (YPD)] or synthetic medium (SC) with appropriate supplements. Heat shock assays were performed as described (17). Yeast transformants were plated at ≈10^3 colonies per plate on selective medium. Colonies were allowed to grow for 3 days and then were replica-plated onto preheated plates. Heat shocks were carried out at 55°C for 10 min and followed by 2–3 days of recovery at 30°C. Surviving colonies were picked, restreaked on synthetic medium plates for colony purification, and then cultured in rich medium for 2–3 days to allow for plasmid loss from some cells. Of these cultures, 1 μl was plated onto YPD plates. After 2–3 days of growth, colonies were replica-plated onto synthetic medium plates (Leu$^+$ selection), YPD plates, and YPD heat shock plates. Colonies were scored to ascertain if the observed heat shock resistance was plasmid dependent.

Vector Construction and Cloning. The expression vector pADNS has been described (17). pADNS contains the alcohol dehydrogenase gene *ADH1* promoter immediately followed by unique *Hin*dIII and *Not* I cloning sites. pADANS was constructed as follows. A polymerase chain reaction (PCR) was carried out on the yeast *ADH1* gene in pJD14 (19). The first oligonucleotide primer (5′-TCTAAACCGTGGAATATT) was placed within the promoter region of the gene and upstream of the *Eco*RV site within the promoter that is also contained in pADNS. The second primer (5′-GTCAAAGCTTCGTAGAAGATAACACC) is complementary to the coding stand of the *ADH1* gene. This primer included additional sequences that incorporate a new *Hin*dIII endonuclease recognition site. The PCR product was then purified, digested with *Eco*RV and *Hin*dIII, and ligated together with the 8.0-kilobase (kb) *Eco*RV/*Hin*dIII-digested

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Abbreviation: GAP, GTPase-activating protein.

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§The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M37190, M37191, and M37192).

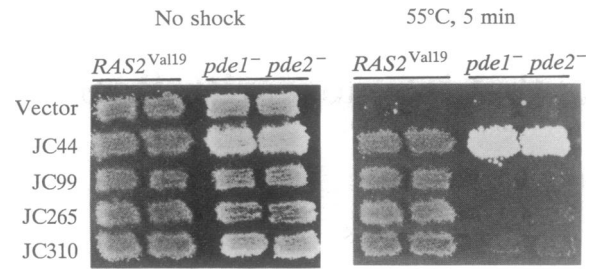

FIG. 1. The heat shock-sensitive strains TK161-R2V (*RAS2*^{Val19}) and 10DAB (*pde1*$^-$ *pde2*$^-$) were transformed with the cloning vector pADANS or with plasmids expressing each of the cDNA isolates. Transformants were patched in duplicate on synthetic medium plates and replica-plated onto fresh plates without heat shock (*Left*) or with heat shock (*Right*). All plates were then incubated at 30°C for 48 hr. Longer duration of the heat shock (20 min) indicated that JC310 is a weaker suppressor than the others (data not shown).

fragment of pADNS. (Since the *Eco*RV site is not unique in pADNS, the 8.0-kb fragment was obtained by partial digestion.) The resulting plasmid, pADANS, thus contains the entire *ADH1* promoter and the first 14 amino acid codons of the *ADH1* gene followed by the *Hin*dIII and *Not* I restriction endonuclease sites.

cDNA was prepared from poly(A)$^+$ mRNA isolated from the human glioblastoma cell line U118-MG (Human Tumor Cell Line Bank, Human Tumor Cell Laboratory, Memorial Sloan–Kettering Cancer Institute) (20) by standard procedures (21). cDNAs were ligated to *Not* I linkers as described (17) and cloned into λ ZAP (Stratagene). Approximately 1.5 × 10^6 recombinant phage were obtained, and the average insert size was 1.5 kilobase pairs (kbp). The phage library DNA (10 mg) was digested with *Not* I, and insert fragments were purified and size-selected on a 10–40% sucrose gradient (22). Insert DNA fragments were then ligated with *Not* I-cleaved, phosphatase-treated pADANS. SCS1 cells were used as transformation recipients. One-liter cultures were prepared for transformation by standard procedures (23). Transformation efficiency was monitored by plating a small portion of these cells onto ampicillin plates. The remainder of the culture was selected for ampicillin-resistant transformants in liquid culture, and plasmid DNA was prepared by CsCl gradient purification (24). The library DNA thus obtained was used to transform TKRV-161 yeast cells by the lithium acetate method (25).

DNA Sequence Analysis. DNA sequencing was performed by the dideoxynucleotide chain-termination method (26, 27). GENALIGN was used for pairwise amino acid sequence alignments (GENALIGN is a copyrighted software product of IntelliGenetics, developed by Hugo Martinez). Pairwise homology search programs used were FASTA 28 and FASTDB (IntelliGenetics). GenBank, European Molecular Biology Laboratory, Swiss-Prot, and PIR (Protein Information Resource) data bases were searched. We used the MACAW program for multiple sequences-alignment searches (29).

RESULTS AND DISCUSSION

Isolation and Genetic Characterization of cDNA Suppressors. In our previous work we utilized the yeast expression vector pADNS (17). This vector supplied a strong yeast transcriptional promoter, the *ADH1* promoter, and *ADH1* transcription termination sequences. Initiation codons were provided by cDNA inserts. We have used a modified vector, pADANS, to express cDNA inserts as fusion proteins containing the first 14 amino acids of the ADH1 protein. For expression in pADANS, cDNA inserts do not need an in-frame methionine codon. In addition, all expressed gene products will now carry an amino-terminal fusion peptide

JC99

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ATTGGGTACCGCGTGCCAGCAGGCAGTGGCCCTAGCCTTCCGCCTATGCCCTCCCTCCAAGAGGTGGACTGCGGCTCCCCCAGCAGCTCCGAGGAGGAGGGGGTGCCAGGGTCCCGGGGG  120
IleGlyTyrArgValProAlaGlySerGlyProSerLeuProProMetProSerLeuGlnGluValAspCysGlySerProSerSerSerGluGluGluGlyValProGlySerArgGly   40

AGCCCAGCGACCTCACCCCACCTGGGCCGCCGACGACCTCTGCTTCGGTCCATGAGCGCCGCCTTCTGCTCCCTACTGGCACCGGAGCGGCAGGTGGGCCGGGCTGCGGCAGCACTGATG  240
SerProAlaThrSerProHisLeuGlyArgArgArgProLeuLeuArgSerMetSerAlaAlaPheCysSerLeuLeuAlaProGluArgGlnValGlyArgAlaAlaAlaAlaLeuMet   80

CAGGACCGACACACAGCCGCGGGCCAGCTGGTGCAGGACCTACTGACCCAGGTGCGGGATGGGCAGAGGCCCCAGGAGCTCGAGGGCATCCGTCAGGCGCTGAGCCGGGCCCGGGCCATG  360
GlnAspArgHisThrAlaAlaGlyGlnLeuValGlnAspLeuLeuThrGlnValArgAspGlyGlnArgProGlnGluLeuGluGlyIleArgGlnAlaLeuSerArgAlaArgAlaMet  120

CTGAGTGCGGAGCTGGGCCCTGAGAAGCTCGTGTCGCCTAAGAGGCTGGAACATGTCCTGGAGAAGTCATTGCATTGCTCTGTGCTCAAGCCTCTCCGGCCCATCCTGGCAGCCCGCCTG  480
LeuSerAlaGluLeuGlyProGluLysLeuValSerProLysArgLeuGluHisValLeuGluLysSerLeuHisCysSerValLeuLysProLeuArgProIleLeuAlaAlaArgLeu  160

CGGCGCCGGCTTGCCGCAGACGGCTCCCTGGGCCGCCTAGCTGAGGGCCTCCGCCTGGCCCGGGCCCAGGGCCCCGGAGCCTTCGGGTCCCACCTGAGCCTGCCCTCCCCAGTAGAGTTG  600
ArgArgArgLeuAlaAlaAspGlySerLeuGlyArgLeuAlaGluGlyLeuArgLeuAlaArgAlaGlnGlyProGlyAlaPheGlySerHisLeuSerLeuProSerProValGluLeu  200

GAGCAAGTGCGCCAGAAGCTGCTGCAGCTCGTCCGCACCTACTCACCCAGCGCCCAGGTCAAGCGGCTCCTGCAGGCCTGCAAGCTGCTCTACATGGCCCTGAGGACCCAGGAAGGGGAG  720
GluGlnValArgGlnLysLeuLeuGlnLeuValArgThrTyrSerProSerAlaGlnValLysArgLeuLeuGlnAlaCysLysLeuLeuTyrMetAlaLeuArgThrGlnGluGlyGlu  240

GGCTCGGGTGCCGACGGGTTCCTGCCTCTGCTGAGCCTCGTCTTGGCCCACTGTGACCTTCCTGAGCTGCTGCTGGAGGCCGAGTACATGTCGGAGCTGCTGGAGCCCAGCCTGCTTACT  840
GlySerGlyAlaAspGlyPheLeuProLeuLeuSerLeuValLeuAlaHisCysAspLeuProGluLeuLeuLeuGluAlaGluTyrMetSerGluLeuLeuGluProSerLeuLeuThr  280

GGAGAGGGTGGCTACTACCTGACCAGCCTCTCTGCCAGCCTGGCCCTGCTGAGTGGCCTGGGTCAGGCCCACACCCTCCCACTGAGCCCCGTGCAGGAGCTACGGCGCTCCCTCAGCCTC  960
GlyGluGlyGlyTyrTyrLeuThrSerLeuSerAlaSerLeuAlaLeuLeuSerGlyLeuGlyGlnAlaHisThrLeuProLeuSerProValGlnGluLeuArgArgSerLeuSerLeu  320

TGGGAGCAGCGCCGCCTGCCTGCCACCCACTGCTTCCAGCACCTCCTCCGAGTAGCCTATCAGGATCCCAGCAGTGGCTGCACCTCCAAGACCCTGGCCGTGCCCCCAGAGGCCTCGATT 1080
TrpGluGlnArgArgLeuProAlaThrHisCysPheGlnHisLeuLeuArgValAlaTyrGlnAspProSerSerGlyCysThrSerLysThrLeuAlaValProProGluAlaSerIle  360

GCCACCCTGAACCAGCTCTGTGCCACCAAGTTCCGAGTGACCCAGCCCAACACTTTTGGCCTCTTCCTGTACAAGGAGCAGGGCTACCACCGCCTGCCCCCTGGGCCCTGGCCCACAGGC 1200
AlaThrLeuAsnGlnLeuCysAlaThrLysPheArgValThrGlnProAsnThrPheGlyLeuPheLeuTyrLysGluGlnGlyTyrHisArgLeuProProGlyProTrpProThrGly  400

TGCCCACCACTGGCTACCTCGTCTACCGCCGGGCAGAGTGGCCTGAGACCCAGGGGGCTGTGACAGAGGAGGAGGGCAGTGGGCAGTCAGAGGCAAGAAGCAGAGGGGAGGAGCAAGGGT 1320
CysProProLeuAlaThrSerSerThrAlaGlyGlnSerGlyLeuArgProArgGlyLeuTER                                                              420

GCCAGGGAGATGGGGATGCTGGGGTCAAAGCCAGCCCCAGGGACATTCGGGAACAGTCTGAGACAACTGCTGAAGGGGGCCAGGGTCAAGCCCAGGAAGGCCCTGCTCAGCCAGGGGAAC 1440
CAGAGGCAGAGGGAAGCCGGGCAGCAGAGGAGTAGCTTGAAGTGGCCAGAAGGGTCATTCGGGGCGGGAGACCCTGAGCCTGCTGAGAAATCCTTTTAGCGCCAGCAAGCCCCACCCAGG 1560
GCCCTGTCCTGTGTCTGCCACCACCTTTGTCTGATACTTGTTTCCAGGGAAGCTGGGGGAACTGCCACATCTGAGGAACTGGAATAAAGATGAGGGGCCTTCGGGGGCCAAT          1672
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FIG. 2. The cDNA sequence and predicted amino acid sequence of JC99 are presented. Translation initiation is at the *ADH1* methionine codon located 54 nucleotides upstream of the sequence presented. The amino acids encoded by the *ADH1* sequence and the adjacent *Hin*dIII and *Not* I sites of the polylinker are: Met-Ser-Ile-Pro-Glu-Thr-Gln-Lys-Gly-Val-Ile-Phe-Tyr-Glu-Ala-Cys-Gly-Arg. Nucleotide and amino acid coordinates are given in the right margin. Numbering begins with the cDNA sequence and does not include the fusion sequences described above. The calculated molecular mass of the predicted polypeptide shown is 45 kDa.

derived from an endogenous, stable, cytoplasmically expressed yeast protein. In our previous work we utilized cDNAs obtained from poly(A)$^+$ rat brain mRNA. In this work, we synthesized cDNAs from poly(A)$^+$ mRNA prepared from the human glioblastoma cell line U118-MG. These cDNAs were first cloned as *Not* I fragments into λ ZAP and subsequently transferred into pADANS. A library of complexity 1.5×10^6 was obtained.

The *RAS2*^{Val19} yeast strain, TK-161R2V, was transformed with library DNA, and 5×10^5 transformants were screened for sensitivity to heat shock by a replica plate method. Twenty-eight colonies were found that contained plasmid DNAs capable of suppressing the heat shock-sensitive phenotype of TK-161R2V. These plasmids contained four groups of related cDNAs. The largest group, with 22 members, is represented by cDNA JC99. The next largest, with 4 members, is represented by cDNA JC44. JC265 and JC310 were found once each.

Plasmids containing representative cDNAs were tested for their effects on the *pde1*$^-$ *pde2*$^-$ strain, 10DAB (see Fig. 1). Although all plasmids could suppress the heat shock-sensitive phenotype of TK-161R2V, only the plasmid containing JC44 could suppress the heat shock phenotype of 10DAB. The amino-terminal amino acid sequences derived from the expression vector (see the legend of Fig. 2) are not required for this assay, since when these sequences were replaced with a different amino-terminal epitope fusion (9), the same results were obtained (data not shown).

Sequence of cDNA Suppressors. The cDNAs corresponding to JC44, JC99, JC265, and JC310 were sequenced. JC44, the cDNA capable of suppressing *pde1*$^-$ *pde2*$^-$ yeast, is a human homolog of the rat dunce protein-like phosphodiesterase, which we isolated in a previous screen. Its sequence appears to be identical to a recently reported human phosphodiesterase gene (30) and is not reported here. The entire nucleotide sequences of the other cDNAs are shown in Figs. 2, 3, and 4. JC99 contains an open reading frame of 420 amino acids. JC265 has an open reading frame of 471 amino acids. JC310 has an open reading frame of 429 amino acids. All open reading frames are in-frame with the leader peptides of the expression vector. The open reading frames of JC99 and JC265 terminate with stop codons. The open reading frame of JC310 continues into the vector.

We searched existing data banks for nucleotide or amino acid sequences similar to JC99, JC265, and JC310. No striking similarities were found to other genes in the pairwise searches of sequence data bases. However, JC99 and JC265 share extensive amino acid sequence similarity with each other (see Fig. 5*A*). Overall there is 38% sequence identity between the products of these two genes. In one stretch of 47 amino acids, there is 70% identity and about 80% similarity when one includes conservative amino acid changes. Neither gene shows significant similarity to JC310.

Despite the failure of computer searches to uncover similarities to the predicted protein products of JC99, JC265, and JC310 cDNAs in pairwise searches of large data bases, we performed multiple sequence alignment searches with amino acid sequences of proteins known or thought to interact with RAS. These sequences included human GAP (32), the yeast IRA1 and IRA2 proteins (33, 34), which are functionally homologous to GAP (35), NF1 (36, 37), which is the protein encoded by the von Recklinghausen neurofibromatosis locus that is related to both GAP and the IRA proteins (inhibitory regulators of the RAS–cAMP pathway) (38–40), and sar1, which is a protein in *Schizosaccharomyces pombe* that is also related to GAP, NF1, and the IRA proteins (Y. Wang and M.W., unpublished data). Marginal similarities were found between JC99, JC265, and subsets of the above se-

JC265

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GGCCGGCAGCGGCTGAGCGACATGAGCATTTCTACTTCCTCCTCCGACTCGCTGGAGTTCGACCGGAGCATGCCTCTGTTTGGCTACGAGGCGGACACCAACAGCAGCCTGGAGGACTAC  120
GlyArgGlnArgLeuSerAspMetSerIleSerThrSerSerSerAspSerLeuGluPheAspArgSerMetProLeuPheGlyTyrGluAlaAspThrAsnSerSerLeuGluAspTyr   40

GAGGGGGAAAGTGACCAAGAGACCATGGCGCCCCCCATCAAGTCCAAAAAGAAAAGGAGCAGCTCCTTCGTGCTGCCCAAGCTCGTCAAGTCCCAGCTGCAGAAGGTGAGCGGGGTGTTC  240
GluGlyGluSerAspGlnGluThrMetAlaProProIleLysSerLysLysLysArgSerSerSerPheValLeuProLysLeuValLysSerGlnLeuGlnLysValSerGlyValPhe   80

AGCTCCTTCATGACCCCGGAGAAGCGGATGGTCCGCAGGATCGCCGAGCTTTCCCGGGACAAATGCACCTACTTCGGGTGCTTAGTGCAGGACTACGTGAGCTTCCTGCAGGAGAACAAG  360
SerSerPheMetThrProGluLysArgMetValArgArgIleAlaGluLeuSerArgAspLysCysThrTyrPheGlyCysLeuValGlnAspTyrValSerPheLeuGlnGluAsnLys  120

GAGTGCCACGTGTCCAGCACCGACATGCTGCAGACCATCCGGCAGTTCATGACCCAGGTCAAGAACTATTTGTCTCAGAGCTCGGAGCTGGACCCCCCCATCGAGTCGCTGATCCCTGAA  480
GluCysHisValSerSerThrAspMetLeuGlnThrIleArgGlnPheMetThrGlnValLysAsnTyrLeuSerGlnSerSerGluLeuAspProProIleGluSerLeuIleProGlu  160

GACCAAATAGATGTGGTGCTGGAAAAAGCCATGCACAAGTGCATCTTGAAGCCCCTCAAGGGGCACGTGGAGGCCATGCTGAAGGACTTTCACATGGCCGATGGCTCATGGAAGCAACTC  600
AspGlnIleAspValValLeuGluLysAlaMetHisLysCysIleLeuLysProLeuLysGlyHisValGluAlaMetLeuLysAspPheHisMetAlaAspGlySerTrpLysGlnLeu  200

AAGGAGAACCTGCAGCTTGTGCGGCAGAGGAATCCGCAGGAGCTGGGGGTCTTCGCCCCGACCCCTGATTTTGTGGATGTGGAGAAAATCAAAGTCAAGTTCATGACCATGCAGAAGATG  720
LysGluAsnLeuGlnLeuValArgGlnArgAsnProGlnGluLeuGlyValPheAlaProThrProAspPheValAspValGluLysIleLysValLysPheMetThrMetGlnLysMet  240

TATTCGCCGGAAAAGAAGGTCATGCTGCTGCTGCGGGTCTGCAAGCTCATTTACACGGTCATGGAGAACAACTCAGGGAGGATGTATGGCGCTGATGACTTCTTGCCAGTCCTGACCTAT  840
TyrSerProGluLysLysValMetLeuLeuLeuArgValCysLysLeuIleTyrThrValMetGluAsnAsnSerGlyArgMetTyrGlyAlaAspAspPheLeuProValLeuThrTyr  280

GTCATAGCCCAGTGTGACATGCTTGAATTGGACACTGAAATCGAGTACATGATGGAGCTCCTAGACCCATCGCTGTTACATGGAGAAGGAGGCTATTACTTGACAAGCGCATATGGAGCA  960
ValIleAlaGlnCysAspMetLeuGluLeuAspThrGluIleGluTyrMetMetGluLeuLeuAspProSerLeuLeuHisGlyGluGlyGlyTyrTyrLeuThrSerAlaTyrGlyAla  320

CTTTCTCTGATAAAGAATTTCCAAGAAGAACAAGCAGCGCGACTGCTCAGCTCAGAAACCAGAGACACCCTGAGGCAGTGGCACAAACGGAGAACCACCAACCGGACCATCCCCTCTGTG 1080
LeuSerLeuIleLysAsnPheGlnGluGluGlnAlaAlaArgLeuLeuSerSerGluThrArgAspThrLeuArgGlnTrpHisLysArgArgThrThrAsnArgThrIleProSerVal  360

GACGACTTCCAGAATTACCTCCGAGTTGCATTTCAGGAGGTCAACAGTGGTTGCACAGGAAAGACCCTCCTTGTGAGACCTTACATCACCACTGAGGATGTGTGTCAGATCTGCGCTGAG 1200
AspAspPheGlnAsnTyrLeuArgValAlaPheGlnGluValAsnSerGlyCysThrGlyLysThrLeuLeuValArgProTyrIleThrThrGluAspValCysGlnIleCysAlaGlu  400

AAGTTCAAGGTGGGGGACCCTGAGGAGTACAGCCTCTTTCTCTTCGTTGACGAGACATGGCAGCAGCTGGCAGAGGACACTTACCCTCAAAAAATCAAGGCGGAGCTGCACAGCCGACCA 1320
LysPheLysValGlyAspProGluGluTyrSerLeuPheLeuPheValAspGluThrTrpGlnGlnLeuAlaGluAspThrTyrProGlnLysIleLysAlaGluLeuHisSerArgPro  440

CAGCCCCACATCTTCCACTTTGTCTACAAACGCATCAAGAACGATCCTTATGGCATCATTTTCCAGAACGGGGAAGAAGACCTCACCACCTCCTAGAAGACAGGCGGGACTTCCCAGTGG 1440
GlnProHisIlePheHisPheValTyrLysArgIleLysAsnAspProTyrGlyIleIlePheGlnAsnGlyGluGluAspLeuThrThrSerTER                          471

TGCATCCAAAGGGGAGCTGGAAGCCTTGCCTTCCCGCTTCTACATGCTTGAGCTTGAAAAGCAGTCACCTCCTCGGGGACCCCTCAGTGTAGTGACTAAGCCATCCACAGGCCAACTCGG 1560
CCAAGGGCAACTTTAGCCACGCAAGGTAGCTGAGGTTTGTGAAACAGTAGGATTCTCTTTTGGCAATGGAGAATTGCATCTGATGGTTCAAGTGTCCTGAGATTGTTTGCTACCTACCCC 1680
CAGTCAGGTTCTAGGTTGGCTTACAGGTATGTATATGTGCAGAAGAAACACTTAAGATACAAGTTCTTTTGAATTCAACAGCAGATGCTTGCGATGCAGTGCGTCAGGTGATTCTCACTC 1800
CTGTGGATGGCTTCATCCCTG                                                                                                    1821
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FIG. 3. The cDNA sequence and predicted amino acid sequence of JC265 are presented. The calculated molecular mass of the predicted polypeptide shown is 54 kDa. See also comments in the legend to Fig. 2.

JC310

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ATTGCCGACCCGGCCCGTAGTGTGGAAGCAGCTTCAGCTCAAAGATTAGAACGACTCCGAAAAGAGAGACAAAACCAGATCAAATGCAAAAATATTCAGTGGAAAGAAAGAAATTCTAAG  120
IleAlaAspProAlaArgSerValGluAlaAlaSerAlaGlnArgLeuGluArgLeuArgLysGluArgGlnAsnGlnIleLysCysLysAsnIleGlnTrpLysGluArgAsnSerLys   40

CAATCAGCCCAGGAGTTAAAGTCACTGTTTGAAAAAAAATCTCTCAAAGAGAAGCCTCCAATTTCTGGGAAGCAGTCGATATTATCTGTACGCCTAGAACAGTGCCCTCTGCAGCTGAAT  240
GlnSerAlaGlnGluLeuLysSerLeuPheGluLysLysSerLeuLysGluLysProProIleSerGlyLysGlnSerIleLeuSerValArgLeuGluGlnCysProLeuGlnLeuAsn   80

AACCCTTTTAACGAGTATTCCAAATTTGATGGCAAGGGTCATGTAGGTACAACAGCAACCAAGAAGATCGATGTCTACCTCCCTCTGCACTCGAGCCAGGACAGACTGCTGCCAATGACC  360
AsnProPheAsnGluTyrSerLysPheAspGlyLysGlyHisValGlyThrThrAlaThrLysLysIleAspValTyrLeuProLeuHisSerSerGlnAspArgLeuLeuProMetThr  120

GTGGTGACAATGGCCAGCGCCAGGGTGCAGGACCTGATCGGGCTCATCTGCTGGCAGTATACAAGCGAAGGACGGGAGCCGAAGCTCAATGACAATGTCAGTGCCTACTGCCTGCATATT  480
ValValThrMetAlaSerAlaArgValGlnAspLeuIleGlyLeuIleCysTrpGlnTyrThrSerGluGlyArgGluProLysLeuAsnAspAsnValSerAlaTyrCysLeuHisIle  160

GCTGAGGATGATGGGGAGGTGGACACCGATTTCCCCCCGCTGGATTCCAATGAGCCCATTCATAAGTTTGGCTTCAGTACTTTGGCCCTGGTTGAAAAGTACTCATCTCCTGGTCTGACA  600
AlaGluAspAspGlyGluValAspThrAspPheProProLeuAspSerAsnGluProIleHisLysPheGlyPheSerThrLeuAlaLeuValGluLysTyrSerSerProGlyLeuThr  200

TCCAAAGAGTCACTCTTTGTTCGAATAAATGCTGCTCATGGATTCTCCCTTATTCAGGTGGACAACACAAAGGTTACCATGAAGGAAATCTTACTGAAGGCAGTGAAGCGAAGAAAAGGA  720
SerLysGluSerLeuPheValArgIleAsnAlaAlaHisGlyPheSerLeuIleGlnValAspAsnThrLysValThrMetLysGluIleLeuLeuLysAlaValLysArgArgLysGly  240

TCCCAGAAAGTTTCAGGCCCTCAGTACCGCCTGGAGAAGCAGAGCGAGCCCAATGTCGCCGTTGACCTGGACAGCACTTTGGAGAGCCAGAGCGCATGGGAGTTCTGCCTGGTCCGCGAG  840
SerGlnLysValSerGlyProGlnTyrArgLeuGluLysGlnSerGluProAsnValAlaValAspLeuAspSerThrLeuGluSerGlnSerAlaTrpGluPheCysLeuValArgGlu  280

AACAGTTCAAGGGCAGACGGGGTTTTTGAGGAGGATTCGCAAATTGACATAGCCACAGTACAGGATATGCTTAGCAGCCACCATTACAAGTCATTCAAAGTCAGCATGATCCACAGACTG  960
AsnSerSerArgAlaAspGlyValPheGluGluAspSerGlnIleAspIleAlaThrValGlnAspMetLeuSerSerHisHisTyrLysSerPheLysValSerMetIleHisArgLeu  320

CGATTCACAACCGACGTACAGCTAGGTATCTCTGGAGACAAAGTAGAGATAGACCCTGTTACGAATCAGAAAGCCAGCACTAAGTTTTGGATTAAGCAGAAACCCATCTCAATCGATTCC 1080
ArgPheThrThrAspValGlnLeuGlyIleSerGlyAspLysValGluIleAspProValThrAsnGlnLysAlaSerThrLysPheTrpIleLysGlnLysProIleSerIleAspSer  360

GACCTGCTCTGTGCCTGTGACCTTGCTGAAGAGAAAAGCCCCAGTCACGCAATATTTAAACTCACGTATCTAAGCAATCACGACTATAAACACCTCTACTTTGAATCGGACGCTGCTACC 1200
AspLeuLeuCysAlaCysAspLeuAlaGluGluLysSerProSerHisAlaIlePheLysLeuThrTyrLeuSerAsnHisAspTyrLysHisLeuTyrPheGluSerAspAlaAlaThr  400

GTCAATGAAATTGTGCTCAAGGTTAACTACATCCTGGAATCGCGAGCTAGCACTGCCCGGGCTGACTACTTTGCTCAAAAAAAAA                                    1285
ValAsnGluIleValLeuLysValAsnTyrIleLeuGluSerArgAlaSerThrAlaArgAlaAspTyrPheAlaGlnLysLys                                       428
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FIG. 4. The cDNA sequence and predicted amino acid sequence of JC310 are presented. The calculated molecular mass of the predicted polypeptide shown is 48 kDa. No stop codon was found in the correct reading frame. See also comments in the legend to Fig. 2.

quences. The only similarity between all of the sequences occurred within the region common to all GAP-like molecules. This alignment is presented in Fig. 5*B*. Similar searches were conducted with the sequences of CDC25 (41) and SCD25 (42), proteins from *S. cerevisiae* that are believed to activate RAS by accelerating nucleotide exchange, and ste6 (43), a related protein from *Sch. pombe*. The most significant common alignment was to JC310 in a region of the CDC25-like molecules thought to be essential for function (44). This alignment is shown in Fig. 5*C*. No significant similarities with yeast adenylyl cyclase or RAS proteins themselves were found.

Functional Significance. We have isolated several mammalian genes that interfere with the function of the RAS pathway in the yeast *S. cerevisiae*. Three of these genes do not suppress the phenotypes of yeast cells lacking phosphodiesterases, and since RAS simulates adenylyl cyclase, we infer that these three genes encode proteins that interfere with the function of yeast RAS itself. In each case we appear to have isolated truncated cDNAs. We have previously demonstrated that expression of a truncated form of adenylyl cyclase, normally a RAS effector in yeast, can result in a potent disruption of RAS function (22). Nevertheless, we cannot infer from our experiments the true functions of these

A

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JC99   IGYRVPAGSGPSLPPMPSLQEVDCGSPSSSEEEGVPGSRGSPATSPHLGRRRPLLRSMSAAFCSLLAPERQVGRAAAALMQDRHTAAGQLVQD---------LLTQVRDGQRP  104
JC265  EFDRSMPLFGYEADTNSSLEDYEGESDQETMAPPIKSKKKRSSSFVLPKLVKSQLQKVSGVFSSFMTPEKRMVRRIAELSRDKCTYFGCLVQDYVSFLQENKECHVSSTDMLQ  131

JC99   QELEGIRQALSRARAMLSAELGPEKLVSPKRLEHVLEKSLHCSVLKPLRPILAARLRRRLAADGSLGRLAEGLRLARAQGPGAFGSHLSLPSPVELEQVRQKLLQLVRTYSPS  217
JC265  TIRQFMTQVKNYLSQSSELDPPIESLIPEDQIDVVLEKAMHKCILKPLKGHVEAMLKDFHMADGSWKQLKENLQLVRQRNPQELGVFAPTPDFVDVEKIKVKFMTMQKMYSPE  244

JC99   AQVKRLLQACKLLYMALRTQEGEGSGADGFLPLLSLVLAHCDLPELLLEAEYMSELLEPSLLTGEGGYYLTSLSASLALLSGLGQAHTLPL----SPVQELRRSLSLWEQRRL  326
JC265  KKVMLLLRVCKLIYTVMENNSGRMYGADDFLPVLTYVIAQCDMLELDTEIEYMMELLDPSLLHGEGGYYLTSAYGALSLIKNFQEEQAARLLSSETRDTLRQWHKRRTTNRTI  357

JC99   PATHCFQHLLRVAYQDPSSGCTSKTLAVPPEASIATLNQLCATKFRVTQPNTFGLFLYKEQGYHRLPPGPWPTGCPPLATSSTAGQSGLRPRGL                      420
JC265  PSVDDFQNYLRVAFQEVNSGCTGKTLLVRPYITTEDVCQICAEKFKVGDPEEYSLFLFVDETWQQLAEDTYPQKIKAELHSRPQPHIFHFVYKR                      451
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B

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IRA1   E Y A Y I A V G S F V F L R F I G P A L V S P D S E N I I I V T  (1724)
IRA2   D H A I I A A G S F V F L R F F C P A L V S P D S E N I I D I S  (1870)
NF1    Q N S I G A V G S A M F L R F I N P A I V S P Y E A G I L D K K  (1074)
SAR1   S T I C S L I G G F F F L R F V N P A I I S P Q T S M L L D S C  (358)
HGAP   T M R T R V V S G F V F L R L I C P A I L N P R M F N I I S D S  (921)
JC99   T Q E G E G S G A D G F L P L L S L V L A H C D L P E L L L E A  (271)
JC265  N N S G R M Y G A D D F L P V L T Y V I A Q C D M L E L D T E I  (296)
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C

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JC310:  S Q K V S G P Q Y R L E K Q S E P N V A  (334)
CDC25:  S A L Y S S P I Y R L E K T W D L V S T  (1421)
SCD25:  S A L Y S S P I Y R L E K T W Q A V I P  (399)
STE6:   S A L N S S P I H R L R K T W A N L N S  (138)
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FIG. 5. Sequence alignments of predicted amino acid sequences. (*A*) The alignment of JC99 and JC265. In each pair of lines, the upper line shows the complete sequence of JC99, and the lower line shows the sequence of JC265 beginning at amino acid residue 18 and ending at residue 451. The area of greatest sequence identity lies within residues 243–289 of JC99. Identities are marked with vertical lines, and conservative amino acid differences are marked with dots. (*B*) The alignment of JC99 and JC265 with GTPase-activating protein (GAP)-like molecules. The coordinates of the sequences are shown at the right margin. Regions of similarity are boxed. (*C*) The alignment of JC310 with CDC25-like molecules. Coordinates are at the right, and similar residues are boxed. In *A*, *B*, and *C*, the grouping of similar amino acids follows the system of Jiminez-Montano and Zamora-Cortina (31). Groupings of similar amino acids are: (V, L, I, M), (F, Y, W), (K, R), (E, D), (Q, N), (S, T), and (A, G).

proteins in mammalian cells. One possibility is that these proteins interact with yeast RAS proteins fortuitously. A second possibility is that the genes encode physiological inhibitors of mammalian RAS-like proteins. A third possibility is that the proteins are components of mammalian RAS targets that can compete for interaction with RAS in yeast. Many additional experiments are required to resolve these possibilities. In any event, we have demonstrated that genetic screening of cDNAs in yeast may prove to be a powerful tool for the isolation of novel components of mammalian signal transduction pathways.

We thank Ira Herskowitz for a critical reading of our manuscript, Kenneth Ferguson for advice and assistance, and Patricia Bird for help in the preparation of this manuscript. This work was supported by the National Cancer Institute, the American Cancer Society, and the Pfizer Biomedical Award. M.W. is an American Cancer Society Research Professor.

1. Ellis, R. W., DeFeo, D., Shih, T. Y., Gonda, M., Young, H. A., Tsuchida, N., Lowy, D. R. & Scolnick, E. M. (1981) *Nature (London)* **292,** 506–511.
2. Barbacid, M. (1987) *Annu. Rev. Biochem.* **56,** 779–827.
3. Fukui, Y. & Kaziro, Y. (1985) *EMBO J.* **4,** 687–691.
4. Reymond, C. D., Gomer, R. H., Mehdy, M. C. & Firtel, R. A. (1984) *Cell* **39,** 141–148.
5. Powers, S., Kataoka, T., Fasano, O., Goldfarb, M., Strathern, J., Broach, J. & Wigler, M. (1984) *Cell* **36,** 607–612.
6. DeFeo-Jones, D., Scolnick, E. M., Koller, R. & Dhar, R. (1983) *Nature (London)* **306,** 707–709.
7. Shilo, B.-Z. & Weinberg, R. A. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 6789–6792.
8. Toda, T., Uno, I., Ishikawa, T., Powers, S., Kataoka, T., Broek, D., Cameron, S., Broach, J., Matsumoto, K. & Wigler, M. (1985) *Cell* **40,** 27–36.
9. Field, J., Nikawa, J., Broek, D., MacDonald, B., Rodgers, L., Wilson, I. A., Lerner, R. A. & Wigler, M. (1988) *Mol. Cell. Biol.* **8,** 2159–2165.
10. Kataoka, T., Powers, S., Cameron, S., Fasano, O., Goldfarb, M., Broach, H. & Wigler, M. (1985) *Cell* **40,** 19–26.
11. DeFeo-Jones, D., Tatchell, K., Robinson, L. C., Sigal, I., Vass, W., Lowy, D. R. & Scolnick, E. M. (1985) *Science* **228,** 179–184.
12. Trahey, M. & McCormick, F. (1987) *Science* **238,** 542–545.
13. McCormick, F. (1989) *Cell* **56,** 5–8.
14. Kataoka, T., Powers, S., McGill, C., Fasano, O., Strathern, J., Broach, J. & Wigler, M. (1984) *Cell* **37,** 437–445.
15. Sass, P., Field, J., Nikawa, J., Toda, T. & Wigler, M. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 9303–9307.
16. Nikawa, J., Sass, P. & Wigler, M. (1987) *Mol. Cell. Biol.* **7,** 3629–3636.
17. Colicelli, J., Birchmeier, C., Michaeli, T., O'Neill, K., Riggs, M. & Wigler, M. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 3599–3603.
18. Field, J., Xu, H.-P., Michaeli, T., Ballester, R., Sass, P., Wigler, M. & Colicelli, J. (1990) *Science* **247,** 464–467.
19. Bennetzen, J. L. & Hall, B. D. (1982) *J. Biol. Chem.* **257,** 3018–3025.
20. Birchmeier, C., Sharma, S. & Wigler, M. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 9270–9274.
21. Watson, C. J. & Jackson, J. F. (1984) in *DNA Cloning: A Practical Approach*, ed. Glover, D. (IRL, Oxford), pp. 53–70.
22. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
23. Hanahan, D. (1983) *J. Mol. Biol.* **166,** 557–580.
24. Katz, L., Kingsbury, D. T. & Helinski, D. E. (1973) *J. Bacteriol.* **114,** 447–485.
25. Ito, H., Fukuda, Y., Murata, K. & Kimura, A. (1983) *J. Bacteriol.* **153,** 163–168.
26. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
27. Biggin, M. D., Gibson, T. J. & Hong, G. F. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 3963–3965.
28. Pearson, W. R. & Lipman, D. J. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 2444–2448.
29. Schuler, G., Altschul, S. & Lipman, D. (1991) *Protein Struct. Funct. Genet.*, **9,** 180–190.
30. Livi, G. P., Kmetz, P., McHale, M. M., Cieslinski, L. B., Sathe, G. M., Taylor, D. P., Davis, R. L., Torphy, T. J. & Balcarek, J. M. (1990) *Mol. Cell. Biol.* **10,** 2678–2686.
31. Jimenez-Montano, M. & Zamora-Cortina, L. (1981) Proceedings of the VII International Biophysics Congress, Aug. 23–28, 1981, Mexico City.
32. Trahey, M., Wong, G., Halenbeck, R., Rubinfeld, B., Martin, G., Ladner, M., Long, C., Crosier, W., Watt, K., Koths, K. & McCormick, F. (1988) *Science* **242,** 1697–1700.
33. Tanaka, K., Matsumoto, K. & Toh-e, A. (1989) *Mol. Cell. Biol.* **9,** 757–768.
34. Tanaka, K., Nakafuku, M., Tamanoi, F., Kaziro, Y., Matsumoto, K. & Toh-e, A. (1990) *Mol. Cell. Biol.* **10,** 4303–4313.
35. Ballester, R., Michaeli, T., Ferguson, K., Xu, H.-P., McCormick, F. & Wigler, M. (1989) *Cell* **59,** 681–686.
36. Xu, G., O'Connell, P., Viskochil, D., Cawthon, R., Robertson, M., Culver, M., Dunn, D., Stevens, J., Gesterland, R., White, R. & Weiss, R. (1990) *Cell* **62,** 599–608.
37. Buchberg, A., Cleveland, L., Jenkins, N. & Copeland, N. (1990) *Nature (London)* **347,** 291–294.
38. Ballester, R., Marchuk, D., Boguski, M., Saulino, A., Letcher, R., Wigler, M. & Collins, F. (1990) *Cell* **63,** 851–859.
39. Martin, G. A., Viskochil, D., Bollag, G., McCabe, P. C., Crosier, W. J., Haubruck, H., Conroy, L., Clark, R., O'Connell, P., Cawthon, R. M., Innis, M. A. & McCormick, F. (1990) *Cell* **63,** 843–849.
40. Xu, G., Lin, B., Tanaka, K., Dunn, D., Wood, D., Gesteland, R., White, R., Weiss, R. & Tamanoi, F. (1990) *Cell* **63,** 835–841.
41. Broek, D., Toda, T., Michaeli, T., Levin, L., Birchmeier, C., Zoller, M., Powers, S. & Wigler, M. (1987) *Cell* **48,** 789–799.
42. Boy-Marcotte, E., Damak, F., Camonis, J., Garreau, H. & Jacquet, M. (1989) *Gene* **77,** 21–30.
43. Hughes, D. A., Fukui, Y. & Yamamoto, M. (1990) *Nature (London)* **344,** 355–357.
44. Petitjean, A., Hilger, F. & Tatchell, K. (1990) *Genetics* **124,** 797–806.