

## 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<sup>§</sup> 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 (*MATa 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  $\approx 10^3$  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  $\mu$ l was plated onto YPD plates. After 2–3 days of growth, colonies were replica-plated onto synthetic medium plates (Leu<sup>+</sup> 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 *HindIII* and *Not I* cloning sites. pADNS 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'-TCTAAACCGTG-GAATATT) was placed within the promoter region of the gene and upstream of the *EcoRV* 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 *HindIII* endonuclease recognition site. The PCR product was then purified, digested with *EcoRV* and *HindIII*, and ligated together with the 8.0-kilobase (kb) *EcoRV/HindIII*-digested

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).

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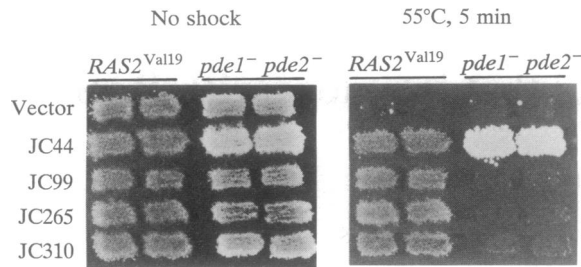


FIG. 1. The heat shock-sensitive strains TK161-R2V (*RAS2*<sup>Val19</sup>) and 10DAB (*pde1*<sup>-</sup> *pde2*<sup>-</sup>) were transformed with the cloning vector pADANS or with plasmids expressing each of the cDNA isolates. Transformants were patched in duplicate on synthetic medium plates and replica-plated 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 *EcoRV* 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 *HindIII* and *Not I* restriction endonuclease sites.

cDNA was prepared from poly(A)<sup>+</sup> 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<sup>6</sup> 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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ATTGGGTACCGCGTGCCAGCAGGCGAGTGGCCCTAGCCTTCCGCCTATGCCCTCCCTCCAAGAGGTGGACTGCGGCTCCCCAGCAGCTCCGAGGAGGAGGGGTGCCAGGGTCCCAGGGG 120
IleGlyTyrArgValProAlaGlySerGlyProSerLeuProMetProSerLeuGlnGluValAspCysGlySerProSerSerSerGluGluGluGlyValProGlySerArgGly 40

AGCCAGCGACCTCACCCACCTGGGCCCGACGACCTCTGCTTCGGTCCATGAGCGCCCTTCTGCTCCCTACTGGCACCGGAGCGGAGGTCGGCCGGCTCGCCGAGCACTGATG 240
SerProAlaThrSerProHisLeuGlyArgArgArgProLeuLeuArgSerMetSerAlaAlaPheCysSerLeuLeuAlaProGluArgGlnValGlyArgAlaAlaAlaAlaLeuMet 80

CAGGACCGACACACAGCCGCGCCAGCTGGTGCAGGACCTACTGACCCAGGTGCGGGATGGGAGAGGCCAGGAGCTCGAGGGCATCCGTCAGGCGCTGAGCCGGCCCGGCCATG 360
GlnAspArgHisThrAlaAlaGlyGlnLeuValGlnAspLeuLeuThrGlnValArgAspGlyGlnArgProGlnGluLeuGluGlyIleArgGlnAlaLeuSerArgAlaArgAlaMet 120

CTGAGTGGCGAGCTGGCCCTGAGAAGCTCGTGTGCCTAAGAGGCTGGAACATGCTCGGAGAAGTCAATGGCATTTGCTCTGTGCTCAAGCCTCTCCGGCCCTCTGGCAGCCCGCCTG 480
LeuSerAlaGluLeuGlyProGluLysLeuValSerProLysArgLeuGluHisValLeuGluLysSerLeuHisCysSerValLeuLysProLeuArgProIleLeuAlaAlaArgLeu 160

CGGCGCCGGCTTCCGCGAGCGCTCCCTGGGCCCGCTAGCTGAGGGCTCCGCTGGCCCGGCCAGGCCCGGAGCCTTCGGGTCCACCTGAGCCTGCCCTCCCCAGTAGAGTTG 600
ArgArgArgLeuAlaAlaAspGlySerLeuGlyArgLeuAlaGluGlyLeuArgLeuAlaArgAlaGlnGlyProGlyAlaPheGlySerHisLeuSerLeuProSerProValGluLeu 200

GAGCAAGTGCAGCAGAAGCTGCTGCAGCTCGTCCGACCTACTCACCCAGCGCCAGGTCAAGCGGCTCTGCAGGCTGCAAGCTGTCTACATGGCCCTGAGGACCCAGGAAGGGGAG 720
GluGlnValArgGlnLysLeuLeuGlnLeuValArgThrTyrSerProSerAlaGlnValLysArgLeuLeuGlnAlaCysLysLeuLeuTyrMetAlaLeuArgThrGlnGluGlyGlu 240

GGCTCGGGTCCGCGAGGGTTCCCTGCTCTGCTGAGCCTCGTCTGGCCCACTGTGACCTTCTGAGCTGTGCTGGAGGCCGAGTACATGTCGGAGCTGTGGAGCCAGCCTGCTTACT 840
GlySerGlyAlaAspGlyPheLeuProLeuLeuValLeuAlaHisCysAspLeuProGluLeuLeuGluAlaGluTyrMetSerGluLeuLeuGluProSerLeuLeuThr 280

GGAGAGGTGGCTACTACCTGACCAGCCTCTCTGCAGCCTGGCCCTGCTGAGTGGCCTGGTTCAGGCCACACCTCCCACTGAGCCCGTGCAGGAGCTACGGCGCTCCCTCAGCCTC 960
GlyGluGlyGlyTyrTyrLeuThrSerLeuSerAlaSerLeuAlaLeuLeuSerGlyLeuGlyGlnAlaHisThrLeuProLeuSerProValGlnGluLeuArgArgSerLeuSerLeu 320

TGGGAGCAGCGCCGCTGCCTGCCACCCACTGCTTCCAGCACCTCCCGAGTAGCCTATCAGGATCCAGCAGTGGCTGCACCTCCAAGACCTGGCCGTGCCCCAGAGGCCCTCGATT 1080
TrpGluGlnArgArgLeuProAlaThrHisCysPheGlnHisLeuLeuArgValAlaTyrGlnAspProSerSerGlyCysThrSerLysThrLeuAlaValProProGluAlaSerIle 360

GCCACCCTGAACAGCTCTGTGCCACCAAGTTCCGAGTGACCCAGCCCAACTTTTGGCCCTTCTCCTGTACAAGGAGCAGGGCTACCACCGCTGCCCCCTGGGCCCTGGCCACAGGC 1200
AlaThrLeuAsnGlnLeuCysAlaThrLysPheArgValThrGlnProAsnThrPheGlyLeuPheLeuTyrLysGluGlnGlyTyrHisArgLeuProProGlyProTrpProThrGly 400

TGCCACCAGCTGGTACTCTGCTACCGCGGGCAGAGTGGCCTGAGACCCAGGGGGCTGTGACAGAGGAGGAGGCGAGTGGCGAGTGCAGAGGCAAGAAGCAGAGGGGAGGAGCAAGGGT 1320
CysProProLeuAlaThrSerSerThrAlaGlyGlnSerGlyLeuArgProArgGlyLeuTER 420

GCCAGGGAGATGGGGATGCTGGGGTCAAAGCCAGCCCAAGGACATTCGGGAACAGTCTGAGACAACTGCTGAAGGGGGCCAGGGTCAAGCCAGGAAGGCCCTGCTCAGCCAGGGGAAC 1440
CAGAGGCAGAGGGAGCCGGCAGCAGAGGAGTAGCTTGAAGTGGCCAGAGGGTCAATTCGGGGCGGGAGACCTGAGCCTGTGAGAAATCCTTTTAGCGCCAGCAAGCCCAAGCCAGG 1560
GCCCTGCTGTGTCTGCCACCACCTTTGTCTGATACTGTTTCCAGGGAAGCTGGGGAACTGCCACATCTGAGGAATGGAATAAAGATGAGGGGCCCTTCGGGGCCCAAT 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 *HindIII* 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)<sup>+</sup> rat brain mRNA. In this work, we synthesized cDNAs from poly(A)<sup>+</sup> mRNA prepared from the human glioblastoma cell line U118-MG. These cDNAs were first cloned as *Not* I fragments into  $\lambda$  ZAP and subsequently transferred into pADANS. A library of complexity  $1.5 \times 10^6$  was obtained.

The *RAS2*<sup>Val19</sup> yeast strain, TK-161R2V, was transformed with library DNA, and  $5 \times 10^5$  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*<sup>-</sup> *pde2*<sup>-</sup> 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*<sup>-</sup> *pde2*<sup>-</sup> 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 phosphodi-

esterase 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. 5A). 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

GGCGGCGAGCGCTGAGCGACATGAGCATTCTACTTCTCTCGACTCGTGGAGTTCGACCGGAGCATGCCTCTGTTGGCTACGAGGCGGACACCAACAGCAGCCTGGAGGACTAC	120
GlyArgGlnArgLeuSerAspMetSerIleSerThrSerSerSerAspSerLeuGluPheAspArgSerMetProLeuPheGlyTyrGluAlaAspThrAsnSerSerLeuGluAspTyr	40
GAGGGGAAAGTGACCAAGAGACCATGGCCCGCCCATCAAGTCCAAAAAGAAAGGAGCAGCTCCTTCGTGCTGCCAAGCTCGTCAAGTCCCAGCTGCAGAAGGTGAGCGGGTGTTC	240
GluGlyGluSerAspGlnThrMetAlaProProIleLysSerLysLysLysArgSerSerSerPheValLeuProLysLeuValLysSerGlnLeuGlnLysValSerGlyValPhe	80
AGTCTCTTATGACCCCGGAGAAGCGGATGGTCCCGAGGATCGCCGAGCTTCCCGGGACAAATGCACCTACTTCGGGTGCTTAGTGCAGGACTACGTGAGCTTCTGCAGGAGAACAAG	360
SerSerPheMetThrProGluLysArgMetValArgArgIleAlaGluLeuSerArgAspLysCysThrTyrPheGlyCysLeuValGlnAspTyrValSerPheLeuGlnGluAsnLys	120
GAGTGCACGTGTCCAGCCAGCAGATGTCGACACCATCCCGCAGTTCATGACCCAGGTCAGAAGTATTTGTCTCAGAGCTCGGAGCTGGACCCCGCATCGAGTTCGTGATCCCTGAA	480
GluCysHisValSerSerThrAspMetLeuGlnThrIleArgGlnPheMetThrGlnValLysAsnTyrLeuSerGlnSerSerGluLeuAspProProIleGluSerLeuIleProGlu	160
GACCAAAATAGATGTGGTGTGGAAAAAGCCATGCACAAAGTGCATCTTGAAGCCCTCAAGGGGACGTTGGAGGCCATGTGAAGGACTTTCACATGGCCGATGGCTCATGGAAGCACTC	600
AspGlnIleAspValValLeuGluLysAlaMetHisLysCysIleLeuLysProLeuLysGlyHisValGluAlaMetLeuLysAspPheHisMetAlaAspGlySerTrpLysGlnLeu	200
AAGGAGAACCCTGCAGCTGTGCGGCAGAGAAATCCGCAGGAGCTGGGGTCTTCGCCCCGACCCCTGATTTTGTGGATGTGGAGAAAATCAAAGTCAAAGTTCATGACCATGCGAAGATG	720
LysGluAsnLeuGlnLeuValArgGlnArgAsnProGlnGluLeuGlyValPheAlaProThrProAspPheValAspValGluLysIleLysValLysPheMetThrMetGlnLysMet	240
TATTGCGCGAAAAGAGGTCATGCTGCTGCTGCGGGTCTGCAAGCTCATTACACGGTTCATGGAGAACAACCTCAGGAGGATGTATGGCGCTGATGACTTCTTGCAGTCTCGACCTAT	840
TyrSerProGluLysLysValMetLeuLeuLeuArgValCysLysLeuIleTyrThrValMetGluAsnAsnSerGlyArgMetTyrGlyAlaAspAspPheLeuProValLeuThrTyr	280
GTATAGCCAGTGTGACATGCTTGAATTTGACACTGAAATCGAGTACATGATGGAGCTCCTAGACCCATCGTGTTCATATGAGAGAAGGAGGCTATTACTTGCACAGCGCATATGGAGCA	960
ValIleAlaGlnCysAspMetLeuGluLeuAspThrGluIleGluTyrMetMetGluLeuLeuAspProSerLeuLeuHisGlyGluGlyGlyTyrTyrLeuThrSerAlaTyrGlyAla	320
CTTCTCTGATAAAGAATTTCCAAGAAGAACAAGCAGCGGACTGCTCAGCTCAGAAACCAGAGACACCCCTGAGGCGAGTGGCACAAACGGAGAACCACCAACCGGACCATCCCCCTGTG	1080
LeuSerLeuIleLysAsnPheGlnGluGlnAlaAlaArgLeuLysSerSerGluThrArgAspThrLeuArgGlnTrpHisLysArgArgThrThrAsnArgThrIleProSerVal	360
GACGACTTCCAGAATTACCTCCGAGTTGCAITTCAGGAGTCAACAGTGGTTCACAGGAAAAGACCCTCTGTGAGACCTTACATCACCCTGAGGATGTGTGTCAGATCTGCGCTGAG	1200
AspAspPheGlnAsnTyrLeuArgValAlaPheGlnGluValAsnSerGlyCysThrGlyLysThrLeuLeuValArgProTyrIleThrThrGluAspValCysGlnIleCysAlaGlu	400
AAGTTCAAGGTGGGGACCCCTGAGGAGTACAGCCTCTTCTCTTCGTTGACAGACATGGCAGCAGCTGGCAGAGGACTTACCCCTCAAAAAATCAAAGCGGAGCTGCACAGCCGACCA	1320
LysPheLysValGlyAspProGluGluTyrSerLeuPheLeuPheValAspGluThrTrpGlnGlnLeuAlaGluAspThrTyrProGlnLysIleLysAlaGluLeuHisSerArgPro	440
CAGCCCCACATCTTCCACTTTGTCTACAAACGCATCAAGAAGCATCTTATGGCATCATTTTCCAGAACGGGAAGAAGACCTCACACCTCTAGAAGACAGGCGGACTTCCAGTGG	1440
GlnProHisIlePheHisPheValTyrLysArgIleLysAsnAspProTyrGlyIleIlePheGlnAsnGlyGluGluAspLeuThrThrSerTER	471
TGCATCCAAAGGGAGCTGGAAGCCTTCCCTTCCCGTCTTACATGCTTGAGCTTGAAGCAGTCACTCCTCGGGACCCCTCAGTGTAGTACTAAGCCATCCACAGGCCAACTCGG	1560
CCAAGGGCACTTTAGCCACGCAAGGTAGCTGAGGTTTGTGAAACAGTAGGATTCCTTTTGGCAATGGAGAATTCATCTGATGGTCAAGTCTCTGAGATTTGTTGCTACCTACCCC	1680
CAGTCAGTTCTAGGTTGGCTTACAGGTATGTATATGTCGACAAGAAACACTTAAGATACAAGTTCTTTTGAATTCACAGCAGATGCTTGGATGTCAGTGCCTCAGTGTATCTCACTC	1800
CTGTGGATGGCTTACCTCCCT	1821

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

ATTGCCGACCCGGCCCGTAGTGTGGAAGCAGCTTCAGCTCAAAGATTAGAACGACTCCGAAAAGAGACAAAACAGATCAAATGCAAAAATATTCAGTGGAAGAAAAGAAATTTCTAAG 120  
 IleAlaAspProAlaArgSerValGluAlaAlaSerAlaGlnArgLeuGluArgLeuArgLysGluArgGlnAsnGlnIleLysCysLysAsnIleGlnTrpLysGluArgAsnSerLys 40

CAATCAGCCAGGAGTTAAAGTCACTGTTTGAATAAAATCTCTCAAAGAGAAGCCTCCAATTTCTGGGAAGCAGTCGATATTATCTGTACGCCTAGAACAGTGCCTCTGCAGCTGAAT 240  
 GlnSerAlaGlnGluLeuLysSerLeuPheGluLysLysSerLeuLysGluLysProProIleSerGlyLysGlnSerIleLeuSerValArgLeuGluGlnCysProLeuGlnLeuAsn 80

AACCCTTTTAAACAGTATTCCAATTTGATGGCAAGGGTCATGTAGGTACAACAGCAACCAAGAAGATCGATGTCTACCTCCCTGCACTCGAGCCAGGACAGACTGTGCCAATGACC 360  
 AsnProPheAsnGluTyrSerLysPheAspGlyLysGlyHisValGlyThrThrAlaThrLysLysIleAspValTyrLeuProLeuHisSerSerGlnAspArgLeuLeuProMetThr 120

GTGGTGACAATGCCAGCCAGGGTGCAGGACCTGATCGGGCTCATCTGCTGGCAGTATACAAGCGAAGAGCGGAGCCGAAGCTCAATGACAATGCAGTGCCTACTGCCTGCATATT 480  
 ValValThrMetAlaSerAlaArgValGlnAspLeuIleGlyLeuIleCysTrpGlnTyrThrSerGluGlyArgGluProLysLeuAsnAspAsnValSerAlaTyrCysLeuHisIle 160

GCTGAGGATGATGGGAGGTGGACACCGATTTCCCCCGCTGGATTCCAATGAGCCATTCATAAGTTTGGCTTCACTTTGGCCCTGGTTGAAAAGTACTCATCTCTGGTCTGACA 600  
 AlaGluAspAspGlyGluValAspThrAspPheProProLeuAspSerAsnGluProIleHisLysPheGlyPheSerThrLeuAlaLeuValGluLysTyrSerSerProGlyLeuThr 200

TCCAAAGAGTCACTCTTGTTCGAATAAATGCTGCTCATGGATTCTCCCTTATTCAGGTGGACAACAAAAGGTTACCATGAAGGAAATCTTACTGAAGGCAGTGAAGCGAAGAAAAGGA 720  
 SerLysGluSerLeuPheValArgIleAsnAlaAlaHisGlyPheSerLeuIleGlnValAspAsnThrLysValThrMetLysGluIleLeuLeuLysAlaValLysArgArgLysGly 240

TCCAGAAAGTTTTCAGGCCCTCAGTACCGCCTGGAGAAGCAGAGCGAGCCCAATGTCGCCCTGACCTGGACAGCAGCTTTGGAGAGCCAGAGCGCATGGGAGTTCTGCCTGGTCCGCGAG 840  
 SerGlnLysValSerGlyProGlnTyrArgLeuGluLysGlnSerGluProAsnValAlaValAspLeuAspSerThrLeuGluSerGlnSerAlaTrpGluPheCysLeuValArgGlu 280

AACAGTCAAGGCAGAGCGGGTTTTTGGAGGAGATTTCGAAATTCAGATAGCCACAGTACAGGATATGCTTAGCAGCCACCATTACAAGTCATTCAAAGTCAGCATGATCCACAGACTG 960  
 AsnSerSerArgAlaAspGlyValIlePheGluGluAspSerGlnIleAspIleAlaThrValGlnAspMetLeuSerHisHisTyrLysSerPheLysValSerMetIleHisArgLeu 320

CGATTCAACCGAGTACAGCTAGGTATCTCTGGAGACAAAGTAGAGATAGACCCTGTACGAAATCAGAAAGCCAGCAGTAAAGTTTGGATTAGCAGAAACCCATCTCAATCGATTCC 1080  
 ArgPheThrThrAspValGlnLeuGlyIleSerGlyAspLysValGluIleAspProValThrAsnGlnLysAlaSerThrLysPheTrpIleLysGlnLysProIleSerIleAspSer 360

GACCTGCTCTGCTGCTGACCTTGTGTAAGAGAAAAGCCCGCAGTACGCAATATTTAAACTACGATCTAAGCAATCAGACTATAAACACCTCTACTTTGAATCGGACGCTGCTACC 1200  
 AspLeuLeuCysAlaCysAspLeuAlaGluGluLysSerProSerHisAlaIlePheLysLeuThrTyrLeuSerAsnHisAspTyrLysHisLeuTyrPheGluSerAspAlaAlaThr 400

GTCATGAAATGTGCTCAAGTTAACTACATCTGGAATCGGAGCTAGCAGTCCCGGGCTGACTACTTTGCTCAAAAAAAA 1285  
 ValAsnGluIleValLeuLysValAsnTyrIleLeuGluSerArgAlaSerThrAlaArgAlaAspTyrPheAlaGlnLysLys 428

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. 5B. 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. 5C. 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

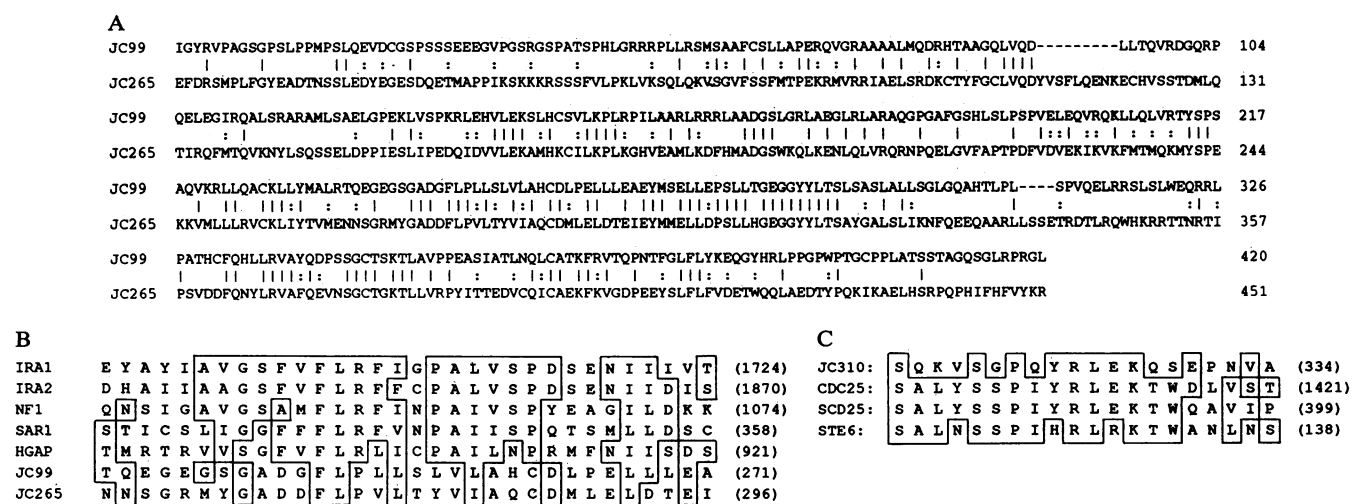


FIG. 5. Sequence alignments of predicted amino acid sequences. (A) The alignment of JC99 and JC265. In each pair of lines, the upper line shows the complete sequence of JC99, and the lower line shows the sequence of JC265 beginning at amino acid residue 18 and ending at residue 451. The area of greatest 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 Jimenez-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.

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