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Functional Homology of Mammalian and Yeast *RAS* Genes

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Summary

Yeast spores lacking endogenous *RAS* genes will not germinate. If such spores contain chimeric mammalian/yeast *RAS* genes or even the mammalian H-*ras* gene under the control of the galactose-inducible *GAL10* promoter, they will germinate in the presence of galactose and produce viable haploid progeny dependent on galactose for continued growth and viability. These results indicate that the biochemical function of *RAS* proteins is essential for vegetative haploid yeast and that this function has been conserved in evolution since the progenitors of yeast and mammals diverged.

Introduction

The *ras* genes were first discovered as the oncogenes contained in the Harvey and Kirsten rat sarcoma viruses (Ellis et al., 1981). The H-, K-, and N-*ras* genes comprise a family of conserved mammalian genes that encode proteins of 188–189 amino acids (Taparowsky et al., 1983). A number of laboratories, using DNA-mediated gene transfer, have demonstrated the presence of "activated" *ras* genes in the DNA of many tumor cells. These *ras* genes contain missense mutations which render them capable of morphological and tumorigenic transformation of NIH3T3 cells, an established cell line of murine origin (Tabin et al., 1982; Reddy et al., 1982; Taparowsky et al., 1982, 1983; Shimizu et al., 1983; Yuasa et al., 1983; Capon et al., 1983). The mammalian *ras* proteins are synthesized initially as cytoplasmic precursors that undergo a processing event while becoming localized to the inner surface of the plasma membrane (Shih et al., 1982b). Both mutant and normal *ras* proteins bind guanine nucleotides with high affinity (Shih et al., 1980, 1982a; Papageorge et al., 1982). The normal *ras* proteins, but not the mutant proteins, also have weak GTPase activity (Sweet et al., 1984; McGrath et al., 1984). Little else is known about the biochemical function of the mammalian *ras* proteins.

The yeast Saccharomyces cerevisiae contains two genes, *RAS1* and *RAS2*, which encode proteins that are highly homologous to the mammalian *ras* proteins, particularly in their N-terminal domains (Defeo-Jones et al., 1983; Powers et al., 1984; Dhar et al., 1984). Although neither *RAS1* nor *RAS2* are by themselves essential genes, at least one functional *RAS* gene is required for the resumption of vegetative growth by haploid yeast spores (Kataoka et al., 1984; Tatchell et al., 1984). This observation has allowed us to test for functional homology between the mammalian and yeast *RAS* proteins. To this end, we have made several mammalian/yeast *RAS* gene constructions in vitro and tested them for their ability to complement the loss of endogenous *RAS* function in yeast.

Results

An N-Terminal Chimeric Gene Complements *ras1⁻ ras2⁻* Yeast

The proteins encoded by the yeast *RAS2* and the human H-*ras* genes are most similar in their N-terminal domains (Defeo-Jones et al., 1983; Powers et al., 1984; Dhar et al., 1984). The yeast *RAS2* protein contains seven initial amino acids not present in the H-*ras* protein. After that, there is close to 90% homology between the proteins for the next 80 amino acid positions. There is nearly 50% homology for the 80 amino acid positions which follow that, and then homology breaks down in what we have called the variable domain. Finally, there is homology again in the four C-terminal amino acid positions. The yeast *RAS2* protein is predicted to be 322 amino acids, much larger than the 189 amino acid H-*ras* protein, mainly because of a larger variable region.

There is a conserved cleavage site for the restriction endonuclease Mst I in both the H-*ras* and *RAS2* genes, corresponding to the 73rd and 80th amino acid positions of the respective proteins. We have utilized this site to create a chimeric gene that encodes the first 73 amino acids of H-*ras* and the remaining 242 amino acids of *RAS2*. The resulting protein differs from the first 79 positions of the H-*ras* at only one position, encoding asparagine instead of threonine at position 74. It differs from *RAS2* at 17 positions in this region. This chimeric protein resembles the normal mammalian protein at all positions at which amino acid substitutions are known to activate the transforming potential of the mammalian protein. These coding regions have been arranged as part of a transcription unit that utilizes the *GAL10* galactose-inducible promoter (St. John and Davis, 1981; Broach et al., 1983) and *RAS2* termination sequences. The entire transcription unit is contained on the plasmid pTKJB-2(gly), which, in addition, contains the origin of replication and the β-lactamase gene of pBR322, and the *LEU2* gene of yeast. The details of this construction are given in Figure 1. A similar plasmid, pTKJB-2(val), was constructed differing only in encoding valine at position 12 of the hybrid H-*ras*/*RAS2* protein. We also constructed plasmid pTKJB-3(gly) which places the entire yeast *RAS2* coding sequence under the control of the GAL10 promoter (see Figure 1).

DNAs from pTKJB-2(gly) and pTKJB-2(val) were cleaved with Eco RI, which cuts within the *LEU2* gene, and used

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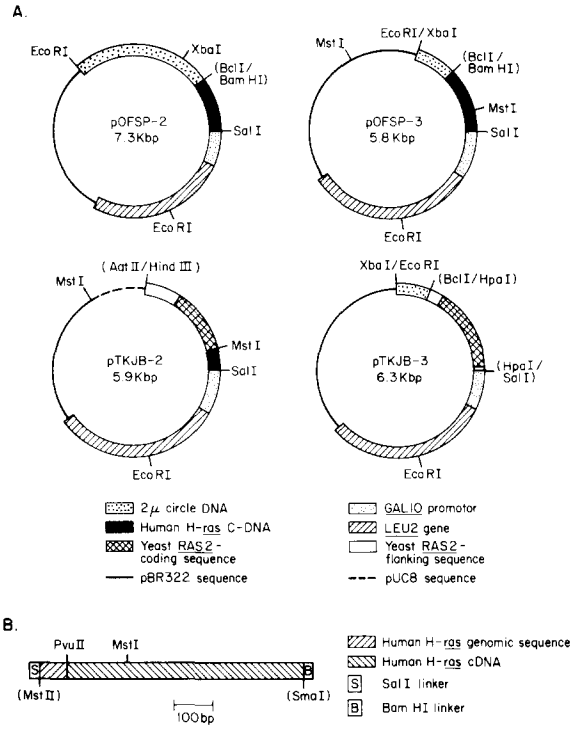

Figure 1. Construction of Plasmids Used in This Study

(A) The restriction maps of all plasmids are shown above. X/Y indicates that the restriction endonuclease site X was filled in with E. coli polymerase I and blunt-end ligated to the restriction endonuclease site Y, also filled in with E. coli polymerase I. Parentheses indicate when the restriction sites are lost by this process. pOFSP-2 was constructed by joining the 6.6 kb Bcl I/Sal I fragment of YEP51 (Broach et al., 1983) with the 0.7 kb Sal I/BAM HI fragment of the human H-*ras* DNA (see B, below). The 2μ circle origin of replication of pOFSP-2 was removed by deletion of sequences within the indicated Xba I/Eco RI fragment, creating pOFSP-3. pTKJB-3 was constructed by joining the 1.2 kb Hpa I fragment of *RAS2* with the 6.6 kb Bcl I/Sal I fragment of YEP51 and by subsequent deletion of the Xba I/Eco RI fragment containing the 2μ replication origin. pTKJB-2 was constructed as indicated by joining the following restriction endonuclease fragments: the 3.7 kb Mst I/Sal I fragment of YEP51, the 0.23 kb Sal I/Mst I fragment of H-*ras* (see below), the 1.04 kb Mst I/Hind III fragment of *RAS2*, and the 0.7 kb Aat II/Mst I fragment of pUC8 (Messing and Vieira, 1982). When H-*ras*^{gly12} or *RAS2*^{gly19} DNA are used, the plasmids are designated "(gly)"; when H-*ras*^{val12} or *RAS2*^{val19} are used, the plasmids are designated "(val)." (B) The H-*ras* sequences used in these constructions are a combination of genomic and cDNA sequences to which were added Sal I linkers (GGTCGACC) and Bam HI linkers (CGGGTACCCG) at the 5′ and 3′ ends of the gene, respectively, by blunt-end ligation. The 5′ boundary of the H-*ras* genomic DNA is an Mst II site, filled in by E. coli polymerase I, 9 bp 5′ to the H-*ras* ATG start codon. The 75 bp Mst II/Pvu II fragment of genomic DNA was blunt-end ligated to the 623 bp Pvu II/Sma I fragment of the H-*ras* cDNA plasmid, RS6 (Fasano et al., 1983). The Sma I site of RS6, 119 bp 3′ to the H-*ras* termination codon, was ligated to the Bam HI linker. The Sal I/Bam HI fragment thus created was used in the H-*ras* plasmid constructions described above.

to transform the haploid strain JR29-1B which is *leu2 ura3 his3 ras2*::*URA3* (see Table 1 for detailed strain description). Strains were isolated containing a single copy of the particular plasmid integrated at the *LEU2* locus. These were mated with the strain JR28-1D which is *his3 leu2 ura3 ras1*::*HIS3* to produce diploid strains JR32 and JR31, respectively. pTKJB-3 plasmid was transformed into JR28-1D after partial cleavage with Eco RI, and Leu$^+$ transformants were mated with JR29-1B to produce a diploid strain, JR33. These diploid strains were therefore doubly heterozygous at each *RAS* locus with one *RAS1* allele disrupted by the *HIS3* marker and one *RAS2* allele disrupted by the *URA3* marker and, additionally, heterozygous for either yeast *RAS2* or the chimeric human/yeast *RAS* gene inserted at one *leu2* allele. These genotypic assignments were confirmed by Southern hybridization analysis as shown in Figure 2. The diploid strains were sporulated, and tetrads dissected. Spores were germinated on agar plates containing either rich galactose medium (YPGal), or rich glucose medium (YPD) and the resulting spores scored for the resumption of vegetative growth. The genotypes of viable spores were determined by the presence of leucine, histidine, and uracil auxotrophies. The genotypes of nonviable spores were determined where possible from the genotypes of viable spores within tetrads assuming Mendelian segregation. The results of these analyses are given in Tables 2, 3, and 4.

Analysis of tetrads from JR33 (*RAS1/ras1*::*HIS3 RAS2/ras2*::*URA3 leu2/leu2*::pTKJB-3(gly)) clearly indicates that the *RAS2* gene of pTKJB-3(gly) is under the control of the galactose-inducible GAL10 promoter (Table 2). Spores containing pTKJB-3(gly) and disrupted at both endogenous *RAS* loci do not germinate on glucose medium, but do germinate on galactose medium. Similar results were obtained with JR32 (*RAS1/ras1*::*HIS3 RAS2/ras2*::*URA3 leu2/leu2*::pTKJB-2(gly)) which contains the chimeric H-*ras*^{gly12}/*RAS2* under *GAL10* control (Table 3). Our genetic assignments of *ras1*::*HIS3 ras2*::*URA3 leu2*::pTKJB-2(gly) haploid progenies were confirmed by Southern analysis (see Figure 2, lanes g and h). These results clearly indicate that expression of the chimeric human H-*ras*^{gly12}/yeast *RAS2* gene can complement the loss of endogenous *RAS* function, and that the chimeric gene is under the control of the galactose-inducible promoter.

Colonies arising on YPGal from *ras1*$^-$ *ras2*$^-$ spores containing the chimeric gene were not macroscopically distinct from colonies arising from spores with endogenous *RAS* genes, either in size, color, or shape. Moreover, in a rich liquid medium containing galactose as the primary carbon source (YPGal), the doubling times for both kinds of cells were equivalent (see Table 5). However, cells with only the chimeric gene did not grow in rich medium containing glucose as a carbon source (YPD). After continued growth for several generations in the absence of galactose, they arrested predominantly in the unbudded G1 phase and began to lose viability (see Table 6). Similar results were obtained for strains disrupted at their endogenous *RAS* loci but expressing yeast *RAS2* under *GAL10* control. These results confirm our conclusion, based on tetrad analysis, that the chimeric H-*ras*^{gly12}/*RAS2* gene complements loss of endogenous *RAS* function. These results also indicate that *RAS* function is required not only for the resumption of vegetative growth by spores, but also for the continued growth and viability of haploid cells.

Quite different results were obtained upon analysis of tetrads from JR31. JR31 contains the plasmid pTKJB-2(val),

Table 1. Yeast Strains Used in This Study

Strain	Genotype
JR25-3C[a]	*MATα leu2 ura3 trp1 his3*
TS-1[b]	*MATa leu2 ura3 trp1 his3 ade8 can1 gal2 ras1::HIS3*
KP-1[b]	*MATa leu2 ura3 trp1 his3 ade8 can1 gal2 ras2::URA3*
JR28-1D[c]	*MATa leu2 ura3 trp1 his3 can1 ras1::HIS3*
JR29-1B[c]	*MATα leu2 ura3 trp1 his3 can1 ras2::URA3*
JR29-1B-1[d]	*MATα leu2*::pTKJB-2(val) *ura3 trp1 his3 can1 ras2::URA3*
JR29-1B-2[d]	*MATα leu2*::pTKJB-2(gly) *ura3 trp1 his3 can1 ras2::URA3*
JR28-1D-3[d]	*MATa leu2*::pTKJB-3(gly) *ura3 trp1 his3 can1 ras1::HIS3*
JR28-1D-4[d]	*MATa leu2*::pOFSP-3(gly) *ura3 trp1 his3 can1 ras1::HIS3*
JR28-1D-5[d]	*MATa leu2*::pOFSP-3(val) *ura3 trp1 his3 can1 ras1::HIS3*
JR31[e]	*MATa/MATα leu2/leu2*::pTKJB-2(val) *ura3/ura3 trp1/trp1 his3/his3 ras1::HIS3/RAS1 ras2::URA3/RAS2*
JR32[e]	*MATa/MATα leu2/leu2*::pTKJB-2(gly) *ura3/ura3 trp1/trp1 his3/his3 ras1::HIS3/RAS1 ras2::URA3/RAS2*
JR33[e]	*MATa/MATα leu2/leu2*::pTKJB-3(gly) *ura3/ura3 trp1/trp1 his3/his3 ras1::HIS3/RAS1 ras2::URA3/RAS2*
JR34[e]	*MATa/MATα leu2/leu2*::pOFSP-3(gly) *ura3/ura3 trp1/trp1 his3/his3 ras1::HIS3/RAS1 ras2::URA3/RAS2*
JR35[e]	*MATa/MATα leu2/leu2*::pOFSP-3(val) *ura3/ura3 trp1/trp1 his3/his3 ras1::HIS3/RAS1 ras2::URA3/RAS2*
JR32-3D[f]	*MATa leu2*::pTKJB-2(gly) *ura3 trp1 his3 ras1::HIS3 ras2::URA3*
JR32-4D[f]	*MATa leu2*::pTKJB-2(gly) *ura3 trp1 his3 ras1::HIS3 ras2::URA3*
JR33-2B[g]	*MATa leu2*::pTKJB-3(gly) *ura3 trp1 his3 ras1::HIS3 ras2::URA3*
JR33-7B[g]	*MATa leu2*::pTKJB-3(gly) *ura3 trp1 his3 ras1::HIS3 ras2::URA3*
JR33-9A[g]	*MATα leu2*::pTKJB-3(gly) *ura3 trp1 his3 ras1::HIS3 ras2::URA3*
JR31-1A[h]	*MATa leu2*::pTKJB-2(val) *ura3 trp1 his3 ras1::HIS3*
JR31-4D[h]	*MATa leu2*::pTKJB-2(val) *ura3 trp1 his3*
JR31-6A[h]	*MATα leu2*::pTKJB-2(val) *ura3 trp1 his3 ras2::URA3*
JR31-8A[h]	*MATa leu2 ura3 trp1 his3*
JR34-3B[i]	*MATα leu2*::pOFSP-3(gly) *ura3 trp1 his3 ras1::HIS3 ras2::URA3*
JR34-11C[i]	*MATα leu2*::pOFSP-3(gly) *ura3 trp1 his3 ras1::HIS3 ras2::URA3*
JR34-14A[i]	*MATa leu2*::pOFSP-3(gly) *ura3 trp1 his3 ras1::HIS3 ras2::URA3*
KPPK-3A[j]	*MATα leu2 ura3 trp1 his3 ade8 can1 ras1::HIS3*

[a] This strain is from the collection of J. Broach.
[b] These strains were produced by transformation of the *ras1::HIS3* or *ras2::URA3* fragment into a yeast strain SP1 (Kataoka et al., 1984).
[c] These strains are segregants of diploid strains produced by mating JR25-3C with TS-1 or KP-1, respectively.
[d] These strains were produced by transformation of the linearized plasmids indicated into JR29-1B or JR28-1D and by selecting Leu$^+$ prototrophs.
[e] These strains were prepared by mating JR28-1D with JR29-1B-1, or JR29-1B-2, or by mating JR29-1B with JR28-1D-3, JR28-1D-4 or JR28-1D-5, respectively, and auxotrophic selection of diploid cells.
[f] These strains are haploid segregants of the diploid strain JR32 and were identified by their Leu$^+$ Ura$^+$ His$^+$ phenotypes and by Southern blot hybridization shown in Figure 2.
[g] These strains are haploid segregants of the diploid strain JR33 and were identified as described in the previous footnote.
[h] These strains are haploid segregants of the diploid strain JR31 and were identified by their Leu Ura His phenotypes.
[i] These strains are haploid segregants of the diploid strain JR34 and identified as described in footnote "f."
[j] This strain is a haploid segregant of a diploid strain KPPK-1, which was produced by mating KP-1 with a strain PK-1 (*MATα leu2 ura3 trp1 his3 ras1::HIS3*). PK-1 was produced by transformation of the *ras1::HIS3* fragment into SX50-1C (Kataoka et al., 1984).

integrated at the *leu2* locus. pTKJB-2(val) contains the chimeric H-*ras*^{val12}/*RAS2* gene under GAL10 control. pTKJB-2(val) therefore encodes a *RAS* protein with one of the amino acid substitutions that activates the transforming potential of the human H-*ras* gene, analogous to the substitution that alters the functional properties of yeast *RAS2* protein. The diploid JR31 was additionally doubly heterozygous at each *RAS* locus. Tetrad analysis (Table 4) indicated that, in contrast to previous results, the chimeric gene appeared unable to complement loss of endogenous *RAS* function. Indeed, spores containing the chimeric gene were unable to germinate on galactose-containing medium even when the endogenous *RAS* genes were intact. To confirm these results, all colonies arising from spores germinated on YPD were replica plated onto YPGal agar plates. Those colonies containing the human/yeast *RAS*^{val12} gene were unable to grow on YPGal. Some colonies containing this chimeric gene were placed into liquid YPGal medium and their cell number and cell viability measured (Table 6). In the presence of galactose, such cells rapidly lost viability. Thus, either expression of high levels of the human/yeast *RAS*^{val12} gene is lethal in general or lethal for cells incubated in medium containing galactose as the primary carbon source.

Expression of the Human H-*ras* Gene Complements *ras1*$^-$ *ras2*$^-$ Yeast

We next tested whether expression of the intact human H-*ras* protein could complement the loss of endogenous *RAS* function in yeast. To this end we again utilized the *GAL10* promoter. We used the entire coding sequence of the human H-*ras* gene lacking introns, starting 9 bp before the initiating ATG and 120 bp after the termination codon, to construct the extrachromosomally replicating plasmids pOFSP-2(gly) and pOFSP-2(val). Details of this construction are given in Figure 1. The (gly) variety expressed the normal human H-*ras* protein and the (val) variety expressed a human H-*ras* protein with valine in position 12 instead of glycine. These plasmids also contained the β-lactamase gene and origin of replication of pBR322, the yeast LEU2

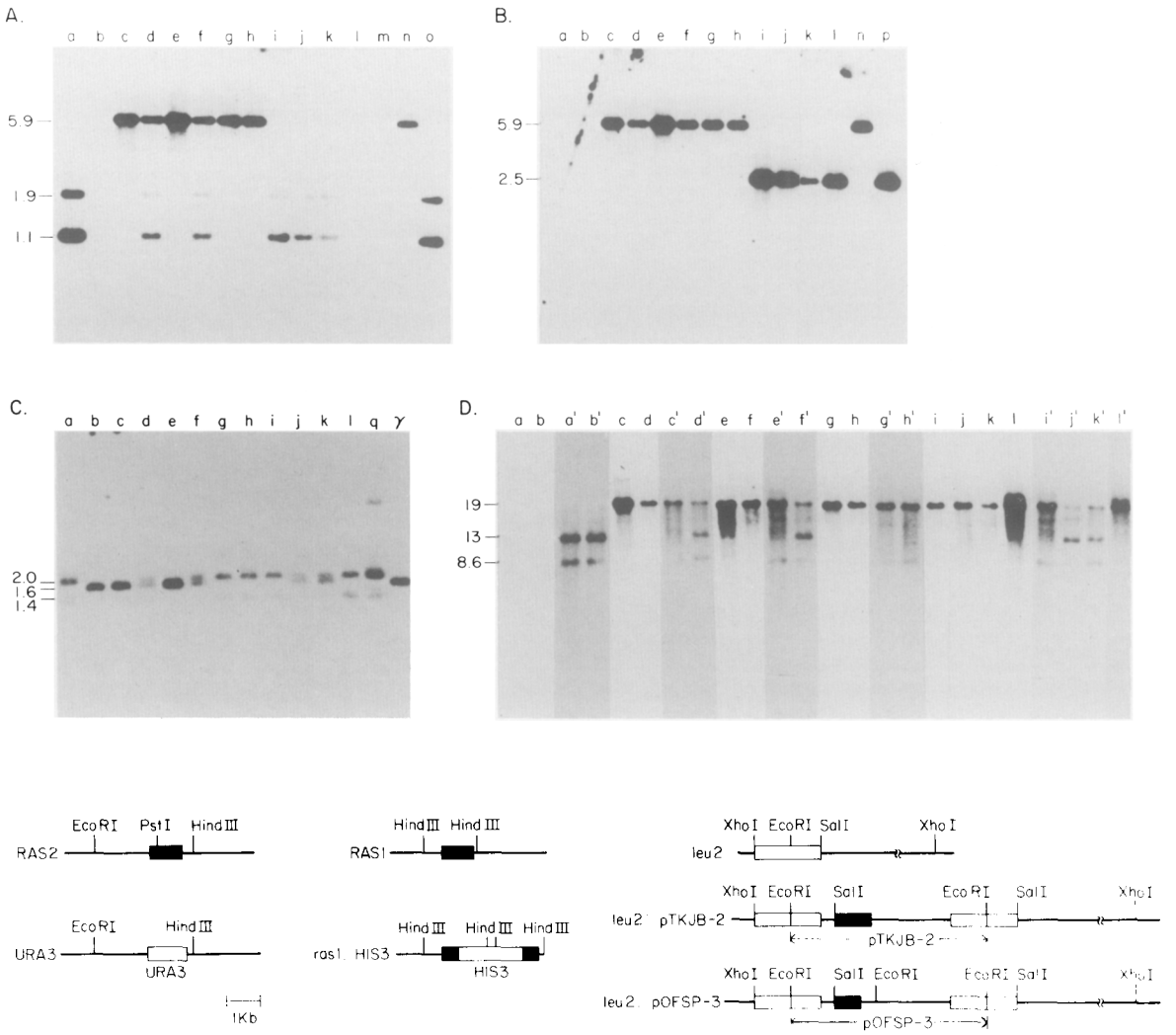

Figure 2. Southern Blot Analysis of Haploid and Diploid Yeast Strains

One tenth to 0.2 μg of various yeast strain DNAs or approximately 100 pg of plasmid DNAs were cleaved with restriction endonuclease, electrophoresed in 1.2% (for A, B, and C), or 0.5% (for D) agarose gels, blotted to nitrocellulose filters, and hybridized with nick-translated DNA fragments as described in Experimental Procedures.

(A) Cleaved with Eco RI, Pst I, and Hind III and hybridized with the 1.2 kb Hpa I *RAS2* gene fragment probe.

(B) Cleaved with Eco RI, Pst I, and Hind III and hybridized with the 0.7 kp Mst II/Sma I fragment of the H-*ras* gene.

(C) Cleaved with Hind III and hybridized with the 1.6 kb Hind III fragment of the *RAS1* gene.

(D) Cleaved with Xho I and hybridized with the H-*ras* (lanes a–l), or the 2.0 kb Xho I/Sal I *LEU2* (lanes a'–l') gene fragments. Yeast strains used as DNA sources are JR28-1D, lanes a and a'; JR29-1B, lanes b and b'; JR 29-1B-1, lanes c and c'; JR31, lanes d and d'; JR29-1B-2, lanes e and e'; JR32, lanes f and f'; JR32-3D, lanes g and g'; JR32-4D, lanes h and h'; JR28-1D-4, lanes i and i'; JR34, lanes j and j'; JR35, lanes k and k'; JR34-11C, lanes l and l'; p*ras2*::*URA3*, lane m; pTKJB-2, lane n; p*RAS2*, lane o; pOFSP-3, lane p; p*ras1*::*HIS3*, lane q; p*RAS1*, lane r. See Table 1 for a description of the yeast strains, and see Figure 1 for a description of plasmids. The structures of p*RAS2*, p*ras2*::*URA3*, p*RAS1* and p*ras1*::*HIS3* were described previously (Kataoka et al., 1984). Molecular sizes of the bands are indicated in kilobases. (E) Schematic representations of the *RAS1*, *RAS2*, and *leu2* genetic loci in their various configurations. Filled bars indicate structural sequences of the *RAS1*, *RAS2*, chimeric *RAS*, or H-*ras* gene, while open bars indicate the gene fragments containing the *URA3*, *HIS3*, *LEU2*, or *leu2* gene. Bands of 5.9 kb, 1.9 kb, and 1.1 kb in (A) indicate an Eco RI fragment of pTKJB-2, an Eco RI/Pst I fragment, and a Pst I/Hind III fragment of the intact *RAS2* gene, respectively. Since the *URA3* gene fragment was inserted between two Hpa I sites of the *RAS2* gene, deleting its entire structural sequence, no band corresponding to the disrupted *ras2*::*URA3* gene can be observed using the *RAS2* gene probe. Bands of 5.9 kb and 2.5 kb in (B) indicate an Eco RI fragment of pTKJB-2 and an Eco RI fragment of pOFSP-3, respectively. A 1.6 kb band in (C) represents a Hind III fragment of the intact *RAS1* gene, while 2.0 kb and 1.4 kb bands are derived from the *ras1*::*HIS3* gene as illustrated in (E). Bands of 13 kb and 19 kb in (D) indicate an intact Xho I fragment of the *leu2* gene and a rearranged Xho I fragment of the *leu2* gene produced by insertion of one copy of pTKJB-2 or pOFSP-3 at their Eco RI cleavage sites. (As expected, the 19 kb band hybridizes with the H-*ras* probe.) The Xho I cleavage site of the *LEU2* gene in YEP51 was lost by ligation with a Pvu II site. An 8.6 kb band in (C) seems to be derived from cross-hybridization of the *LEU2* gene probe with an unknown gene and is present in all of the lanes.

gene, and the 2μ circle origin of replication. The human H-*ras* genes were under the transcriptional control of the galactose-inducible *GAL10* promoter.

First we tested whether yeast containing high copy numbers of the extrachromosomally replicating plasmids expressed H-*ras* protein. *leu2*⁻ haploid cells DC04 were transformed with these plasmids. The resulting Leu⁺ cells were grown in synthetic medium with appropriate auxo-

Table 2. Spore Viability in Tetrads from JR33

Spore Genotype			Germination on YPD		Germination on YPGal	
RAS1	*RAS2*	*GAL10-RAS2*	Viable	Non-viable	Viable	Non-viable
+	+	+	7	0	10	0
+	+	–	9	1	13	1
+	–	+	9	0	10	1
+	–	–	6	2	9	0
–	+	+	9	0	9	2
–	+	–	7	0	10	0
–	–	+	0	6	13	1
–	–	–	0	7	0	10

The genotype of JR33 is given in Table 1. It contains one copy of plasmid pTKJB-3(gly), inserted at one *leu2* allele. This plasmid contains the coding sequences of *RAS2* under the control of the galactose-inducible *GAL10* promoter. The genotypes of spores were determined as described in the text.

Table 3. Spore Viability in Tetrads from JR32

Spore Genotype			Germination on YPD		Germination on YPGal	
RAS1	*RAS2*	*GAL10*-chimeric *RAS*(gly12)	Viable	Non-viable	Viable	Non-viable
+	+	+	7	0	12	0
+	+	–	6	0	10	1
+	–	+	13	0	15	0
+	–	–	8	1	20	0
–	+	+	16	1	15	3
–	+	–	12	2	19	1
–	–	+	0	6	13	4
–	–	–	0	8	0	8

The genotype of JR32 is given in Table 1. It contains one copy of plasmid pTKJB-2(gly) inserted at one *leu2* allele. This plasmid contains the chimeric H-*ras*^{gly12}/*RAS2* described in the text under the control of the galactose-inducible *GAL10* promoter.

Table 4. Spore Viability in Tetrads from JR31

Spore Genotype			Germination on YPD		Germination on YPGal
RAS1	*RAS2*	*GAL10*-chimeric *RAS*(val12)	Viable	Non-viable	Viable
+	+	+	6	0	0
+	+	–	10	0	10
+	–	+	9	1	0
+	–	–	9	0	11
–	+	+	11	0	0
–	+	–	9	0	9
–	–	+	0	7	0
–	–	–	0	7	0

The genotype of JR31 is given in Table 1. It contains one copy of plasmid pTKJB-2(val) inserted at one *leu2* allele. This plasmid contains the chimeric H-*ras*^{val12}/*RAS2* gene, described in the text, under the control of the galactose-inducible *GAL10* promoter. The genotypes of nonviable spores germinated on YPGal could not be determined in most cases since there were insufficient numbers of viable spores within most tetrads.

trophic additions and 5% galactose, and labeled with ^{35}S-methionine. Cellular extracts were prepared after varying labeling conditions, and immunoprecipitated with the monoclonal antibody Y13-259, which has a broad cross-reactivity to *ras* proteins (Furth et al., 1982). Immunoprecipitates were then analyzed by polyacrylamide gel electrophoresis and compared to immunoprecipitates from mammalian cells containing high concentrations of the normal human H-*ras* protein or the transforming H-*ras* protein with valine at position 12. Yeast cells containing pOFSP-2 plasmids express two protein species that immunoprecipitate with monoclonal antibody and comigrate with the precursor and mature human H-*ras* proteins (data not shown). Pulse-chase experiments indicated that the slower migrating yeast species is the precursor for the faster migrating species. It therefore appears that cells containing pOFSP-2 plasmids not only express the H-*ras* protein, but that this protein undergoes a processing event which resembles the processing event that occurs in mammalian cells (Shih et al., 1982b).

Next we tested whether the normal human H-*ras* protein would complement yeast lacking intact endogenous *RAS* proteins. For this purpose, we removed the 2μ circle origin of pOFSP-2 to create pOFSP-3 (see Figure 1). We then utilized the same experimental scheme described in the previous section to create a diploid (JR34) that was heterozygous at each *RAS* locus (disrupted by auxotrophic *HIS3* and *URA3* markers) and which contained the normal H-*ras* gene under *GAL10* control together with *LEU2* integrated at one *leu2* allele. Tetrad analysis (Table 7) of this diploid was performed as for earlier tetrads. No *ras1*$^-$ *ras2*$^-$ spores germinated on rich glucose medium (YPD). However, when spores were germinated on galactose (YPGal) agar, greater than one third of the *ras1*$^-$ *ras2*$^-$ spores that contained the H-*ras* gene gave rise to colonies. These colonies grew more slowly than other colonies, indicating that their growth was impaired. One of these colonies was picked, grown into mass culture in YPGal, and DNA prepared. Analysis of this DNA utilizing *RAS1*, *RAS2*, *LEU2*, and H-*ras* probes (Figure 2, lanes 1 and 1′) indicated that it had the predicted genotype *ras1*::*HIS3 ras2*::*URA3 leu2*::pOFSP-3(gly). When these cells were transferred to YPD, which lacks the transcriptional inducer galactose, they did not grow, but arrested predominantly in an unbudded state and subsequently lost viability (Table 6). These results indicate that the normal human H-*ras* protein can complement yeast lacking endogenous *RAS* function, and that it is under the control of the *GAL10* promoter. However, unlike spores containing only the chimeric human/yeast *RAS* gene, spores containing only the H-*ras* gene did not germinate efficiently. This result suggests that the H-*ras* protein does not function as well as the chimeric protein in yeast cells. Moreover, cells expressing only the H-*ras* protein did not grow as rapidly as wild-type cells. The doubling time of these cells in YPGal was from 5 to 6 hr, compared to about 2.3 hr for wild-type cells (Table 5). The proportion of budded cells was measured, and from this we calculated that the increase in doubling time was due almost entirely to an increase in the duration of the G1 phase of the cell cycle.

These same experiments were repeated using the plasmid pOFSP-3(val). JR35, a diploid doubly heterozygous for the endogenous *RAS* genes and containing the galactose-inducible H-*ras*^{val12} gene, was analyzed. This analysis (Table 8) clearly indicated that yeast cells lacking endogenous

Table 5. Doubling Time and Duration of G1 in Various Strains

Strain	Genotype[a]			Doubling Time, D[b]	Fraction Unbudded, F[b]	G1[b]	D-G1[b]
	RAS1	*RAS2*	*leu2*::[a]				
KPPK-3A	–	+	–	2.5 hr	.482	0.99 hr	1.51 hr
JR32-3D	–	–	pTKJB-2(gly)	2.3	.455	0.86	1.44
JR32-4D	–	–	pTKJB-2(gly)	2.6	.538	1.18	1.42
JR33-2B	–	–	pTKJB-3(gly)	2.3	.427	0.80	1.50
JR33-7B	–	–	pTKJB-3(gly)	2.6	.485	1.04	1.56
JR33-9A	–	–	pTKJB-3(gly)	2.3	.412	0.77	1.53
JR34-14A	–	–	pOFSP-3(gly)	4.8	.73	3.14	1.66
JR34-11C	–	–	pOFSP-3(gly)	5.6	.74	3.73	1.87
JR34-3B	–	–	pOFSP-3(gly)	6.0	.75	4.07	1.93

[a] The full genotypes of the indicated strains are given in Table 1. The plasmid integrated at the *leu2* allele is indicated. pTKJB-3(gly) contains the wild-type yeast *RAS2* under the transcriptional control of the galactose inducible *GAL10* promoter; pTKJB-2(gly) contains the chimeric H-*ras*^{gly12}/*RAS2* under *GAL10*; pOFSP-3(gly) contains the H-*ras*^{gly12} under *GAL10*.

[b] The doubling time, D, of cells inoculated in rich medium containing galactose (YPGal) was measured during exponential growth. The fraction, F, of unbudded cells (cells in G1) during exponential growth was determined by the microscopic examination of at least 100 cells. The duration of G1 was determined using the formula G1 = D(1-log(2-F)/log2) (Rivin and Fangman, 1980). The difference between D and G1 (D-G1) represents the duration of the cell cycle excluding G1.

Table 6. Viability of Various Yeast Strains

Strain	Genotype[a]			Viability[b] after Incubation in YPD for					
	RAS1	*RAS2*	*leu2*::	0 hr	2.5 hr	8.7 hr	16 hr	33.5 hr	50 hr
KPPK-3A	–	+	–	76	76	101	104	90	78
JR32-3D	–	–	pTKJB-2(gly)	78	76	95	52	3.2	1.6
JR32-4D	–	–	pTKJB-2(gly)	86	85	96	62	8.9	2.2
				0 hr	1 hr	4 hr	12 hr	23 hr	42 hr
JR34-14A	–	–	pOFSP-3(gly)	55	52	65	38	19	5.7
JR34-11C	–	–	pOFSP-3(gly)	52	51	50	37	25	3.3
JR34-3B	–	–	pOFSP-3(gly)	56	53	54	48	25	4.0
				Viability[c] after Incubation in YPGal for					
				0 hr	1 hr	4 hr	12 hr	23 hr	42 hr
JR28-1D	–	+	–	83	78	NT	NT	76	79
JR31-8A	+	+	–	NT	83	83	90	79	83
JR31-1A	–	+	pTKJB-2(val)	NT	90	80	51	24	5.1
JR31-4D	+	+	pTKJB-2(val)	NT	81	94	69	22	6.6
JR31-6A	+	–	pTKJB-2(val)	NT	80	93	53	17	5.1

[a] See footnote (a) Table 5. pTKJB-2(val) contains the H-*ras*^{val12}/*RAS2* chimeric gene under the control of the galactose-inducible *GAL10* promoter.

[b] Exponentially growing cells in YPGal were washed with YPD and then inoculated into YPD and incubated for the indicated periods. For each time point, the viability of cultures was determined by their plating efficiency on YPGal plates. JR34-3B, 11C, and 14A had only 50%–60% viability when they were cultured in YPGal for the same time.

[c] Exponentially growing cells in YPD were washed with YPGal and then inoculated into YPGal and incubated for the indicated times. For each time point, the viability of cultures was determined by measuring their plating efficiency on YPD plates. NT means not tested.

RAS function could not be successfully complemented by this gene when grown in galactose medium. In contrast to cells containing the chimeric H-*ras*^{val12}/*RAS2* gene, cells containing the H-*ras*^{val12} gene could grow in galactose medium when at least one endogenous *RAS* gene was intact. Possible explanations for these puzzling observations are discussed below.

Discussion

We and others have found genes in yeast homologous to the mammalian *ras* genes (Defeo-Jones et al., 1983; Powers et al., 1984; Gallwitz et al., 1983). The two closest homologs are *RAS1* and *RAS2*, two closely related genes with complementary functions (Kataoka et al., 1984; Tatchell et al., 1984). The protein encoded by *RAS2* shares antigenic sites with the mammalian H-*ras* protein (Powers et al., 1984; Papageorge et al., 1984) and, like the mammalian *ras* proteins, binds guanine nucleotides (Tamanoi et al., 1984). The strongest region of homology between the yeast *RAS* and mammalian *ras* proteins is in the N-terminal domain. There is identity at all positions where amino acid substitutions are known to activate the transforming potential of the mammalian *ras* proteins. *RAS2*^{val19}, a mutant *RAS2* encoding a protein with an amino acid substitution identical to one that activates H-*ras*, can dramatically alter the growth properties of haploid and diploid cells (Kataoka et al., 1984; Toda et al., 1985). These results suggest that the yeast *RAS* proteins are a good model for understanding the mammalian *ras* proteins.

Table 7. Spore Viability in Tetrads from JR34

Spore Genotype			Germination on YPD		Germination on YPGal	
RAS1	*RAS2*	*GAL10*-H-*ras*^{gly12}	Viable	Non-viable	Viable	Non-viable
+	+	+	6	0	15	0
+	+	–	7	0	29	0
+	–	+	6	0	20	0
+	–	–	4	0	18	1
–	+	+	3	0	22	0
–	+	–	6	2	17	2
–	–	+	0	10	10	16
–	–	–	0	4	0	16

The genotype of JR34 is given in Table 1. It contains one copy of plasmid pOFSP-3(gly) inserted at one *leu2* allele. This plasmid contains the human H-*ras*^{gly12} gene under the control of the galactose-inducible *GAL10* promoter. The genotypes of spores were determined as described in the text.

Table 8. Spore Viability in Tetrads from JR35

Spore Genotype			Germination on YPD		Germination on YPGal	
RAS1	*RAS2*	*GAL10*-H-*ras*^{val12}	Viable	Non-viable	Viable	Non-viable
+	+	+	5	0	9	0
+	+	–	9	0	11	0
+	–	+	8	0	11	0
+	–	–	9	0	14	1
–	+	+	7	1	15	1
–	+	–	8	0	13	0
–	–	+	0	9	0	10
–	–	–	0	5	0	8

The genotype of JR35 is given in Table 1. It contains one copy of plasmid pOFSP-3(val) inserted at one *leu2* allele. This plasmid contains the human H-*ras*^{val12} gene under the control of the *GAL10* promoter.

Since one functional *RAS1* or *RAS2* gene is required for the resumption of growth by haploid spores, we have been able to test whether there is functional analogy between the mammalian and yeast *RAS* proteins. We find that a chimeric gene encoding the mammalian H-*ras* N-terminal domain and the yeast *RAS2* C-terminal domain functions well as a substitute for endogenous *RAS* genes even though the chimeric gene differs from *RAS2* at 17 of the first N-terminal 79 positions. Not only do haploid spores containing this gene resume growth, but they subsequently grow in rich medium at rates comparable to wild-type cells. This is strong evidence that there has been conservation of the function of the N-terminal domain from the time the progenitors of yeast and mammals diverged. Amazingly, we find that even a gene specifying the intact human H-*ras* gene can complement the loss of endogenous *RAS* genes in yeast. These results imply that the biochemical effector function of *ras* has been conserved in evolution, and that this function in yeast will be homologous to the function of *ras* in mammalian cells. These results also indicate that while the variable regions near the carboxy terminus of yeast *RAS* proteins may be important in regulating *RAS* function, they do not contain essential functions.

Our present work again confirms that *RAS* function is required for the resumption of growth by haploid spores, as we and others have previously shown, and also demonstrates that *RAS* is required for the maintenance of growth and cell viability. Yeast without intact endogenous *RAS* loci but expressing either the yeast *RAS2* or the chimeric human/yeast *RAS* or the intact H-*ras* gene under *GAL10* control cease growth in the absence of galactose after several generations, accumulate predominantly in an unbudded state, and eventually lose viability. Yeast cells lacking endogenous *RAS* function but expressing the intact human H-*ras* protein grow with a prolonged G1 period. These results suggest that endogenous *RAS* function is not efficiently replaced by the H-*ras* protein and further suggest that *RAS* function is required for traversing G1. We cannot eliminate the possibility that *RAS* function may also be required for traversing other stages of the cell cycle.

We cannot presently explain two, perhaps related, experimental observations. First of all, we have found that cells with a chimeric human/yeast *RAS2*^{val12} gene under the control of the *GAL10* promoter cannot grow and lose viability when incubated in a medium with galactose as the primary carbon source even when such cells contain intact endogenous *RAS* genes. We have observed similar results (unpublished) when the yeast *RAS2*^{val19} gene is placed under *GAL10* control. Second, we find that, unlike the human H-*ras*^{gly12} gene, the human H-*ras*^{val12} gene, when under the transcriptional control of the *GAL10* promoter, is not able to complement yeast cells lacking their endogenous *RAS* genes in the presence of galactose. In contrast to the previous results, the expression of the H-*ras*^{val12} gene does not prevent cells from growing on galactose when endogenous *RAS* protein is present. Possibly, the H-*ras*^{val12} protein does not provide the critical *RAS* function as efficiently as the wild-type human H-*ras* protein. Alternatively, the H-*ras*^{val12} protein behaves like the chimeric H-*ras*^{val12}/*RAS2* or *RAS2*^{val19} gene when there is no available endogenous *RAS1* or *RAS2* protein to act as a competitor. We do not know why a cell expressing high levels of *RAS2*^{val19} or the chimeric H-*ras*^{val12}/*RAS2* protein fails to grow on a rich medium containing galactose. This question is under continuing investigation.

Experimental Procedures

Yeast Strains, Growth Media, and Transformation

General genetic manipulation of yeast cells was carried out essentially as previously described (Mortimer and Hawthorne, 1969). Nomenclature of the genotypes and phenotypes were as described previously (Powers et al., 1984, Kataoka et al., 1984). A rich galactose medium, YPGal (2% Bacto-peptone, 1% yeast extract, and 5% galactose) or a glucose medium, YPD (2% Bacto-peptone, 1% yeast extract, and 2% glucose) were used for growth of yeast strains and for tetrad dissections. The Leu, His, Ura auxotrophies of haploid segregants were determined by replica-plating onto synthetic media plates (0.7% yeast nitrogen based without amino acids, and 2% galactose or glucose) supplemented with appropriate amino acids and nucleic acid bases. The synthetic media were also used for ^{35}S-methionine labeling and selection and maintenance of transformants. Transformation into yeast cells was performed according to the method of Beggs (1978). Detailed methods for the growth profile and viability experiments are described in the legends to Tables 5 and 6.

Other Methods

Yeast DNA was prepared essentially as described previously (Struhl et al., 1979). Southern blot hybridization was performed as described

previously (Southern, 1975). The probes used for the hybridization were a 1.2 kb Hpa I fragment of the *RAS2* gene, a 1.6 kb Hind III fragment of the *RAS1* gene, a 0.7 kb Mst II–Sma I fragment of the H-*ras* cDNA plasmid (see Figure 1), and a 2.0 kb Xho I–Sal I fragment of the *LEU2* gene. They were labeled by nick translation (Maniatis et al., 1975). Labeling with ^{35}S-methionine and immunoprecipitation were performed as described previously (Powers et al., 1984). Sources of all the reagents and enzymes were described previously (Powers et al., 1984; Kataoka et al., 1984).

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