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# Genes in S. cerevisiae Encoding Proteins with Domains Homologous to the Mammalian *ras* Proteins

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## Summary

**The *ras* genes, which were first identified by their presence in RNA tumor viruses and which belong to a highly conserved gene family in vertebrates, have two close homologs in yeast, detectable by Southern blotting. We have cloned both genes (*RAS1* and *RAS2*) from plasmid libraries and determined the complete nucleotide sequence of their coding regions. They encode proteins with nearly 90% homology to the first 80 positions of the mammalian *ras* proteins, and nearly 50% homology to the next 80 amino acids. Yeast *RAS1* and *RAS2* proteins are more homologous to each other, with about 90% homology for the first 180 positions. After this, at nearly the same position that the mammalian *ras* proteins begin to diverge from each other, the two yeast *ras* proteins diverge radically. The yeast *ras* proteins, like the proteins encoded by the mammalian genes, terminate with the sequence cysAAX, where A is an aliphatic amino acid. Thus the yeast *ras* proteins have the same overall structure and interrelationship as the family of mammalian *ras* proteins. The domains of divergence may correspond to functional domains of the *ras* proteins. Monoclonal antibody directed against mammalian *ras* proteins immunoprecipitates protein in yeast cells containing high copy numbers of the yeast *RAS2* gene.**

## Introduction

The *ras* genes were first identified as the oncogenes of Harvey (v-H-*ras*) and Kirsten (v-K-*ras*) sarcoma viruses (Ellis et al., 1981). Certain tumor cells contain structurally mutated *ras* genes, which are capable of the tumorigenic transformation of NIH3T3 cells upon DNA-mediated gene transfer (Tabin et al., 1982; Reddy et al., 1982; Taparowsky et al., 1982; Yuasa et al., 1983; Taparowsky et al., 1983; Shimizu et al., 1983a; Capon et al., 1983b). At least three *ras* genes exist in mammals, the H-, K- and N-*ras*, which encode highly related proteins of 188–189 amino acids (Capon et al., 1983a; Shimizu et al., 1983; McGrath et al., 1983; Taparowsky et al., 1983). Homologous genes have been identified in Drosophila (Shilo and Weinberg, 1981). Since little is known about the normal biochemical or physiological function of the *ras* proteins, or the consequence of mutations upon these functions, we sought evidence for the existence of homologous genes in yeast Saccharomyces cerevisiae, a simple eucaryotic organism that can be subjected to powerful genetic analysis. Recently, Defeo-Jones et al. (1983) published the predicted partial amino acid sequence of a yeast gene that has considerable homology to mammalian *ras* proteins. Gallwitz et al. (1983) have also recently published the complete nucleotide and predicted amino acid sequence of another yeast gene, called *YP2*. The *YP2*-encoded protein has much weaker yet significant homology to the mammalian *ras* proteins. We demonstrate here two yeast genes that encode proteins with amino acid sequences highly related to the mammalian *ras* proteins. The gene that Defeo-Jones et al. have called c-ras$^{sc}$-1 corresponds to the gene we have called yeast *RAS1*.

We present here the complete nucleotide and predicted amino acid sequence of this gene and that of a highly related companion gene, called yeast *RAS2*. We have tentatively identified the yeast *RAS2* protein by immunoprecipitation with an anti-mammalian *ras* protein monoclonal antibody from extracts of yeast cells harboring *RAS2* on a high copy number plasmid. Comparison of the encoded amino acid sequences of the known *ras* genes suggests the existence of functional domains of the encoded proteins.

## Results

### Hybridization of Yeast DNA with Mammalian *ras* Probes

DNA was prepared from yeast, digested with various restriction endonucleases, electrophoresed in agarose gels in triplicate, transferred to nitrocellulose filter paper, and hybridized according to the method of Southern using three different nick-translated probes: DNA fragments from the viral H-*ras*, the viral K-*ras*, and the human N-*ras* genes. Two yeast DNA restriction fragments with strong homology to all three probes were observed. Additional DNA fragments displayed a weak hybridization signal. Results with the K-*ras* probe are shown in Figure 1a.

### Cloning Yeast Homologs to Mammalian *ras* Genes

The complete yeast S. cerevisiae genome has been cloned into bacterial/yeast shuttle vectors as Sau 3A partial digests (Broach et al., 1979; Nasmyth and Reed, 1980). This library was screened on nitrocellulose filters using as probes fragments of the mammalian *ras* genes. A number of candidate plasmid clones were isolated and analyzed by restriction endonuclease digestion and Southern blotting. Each of these plasmids contained one of two nonoverlapping regions of DNA. The composite restriction endonuclease maps of these regions are shown in Figure 2. We call the homologous genes in yeast *RAS1* and *RAS2*. The restriction endonuclease sites present on these genes identify them as the two major bands seen on Southern blot analysis. We verified this for yeast *RAS2* by using it as a probe in Southern hybridization with nitrocel-

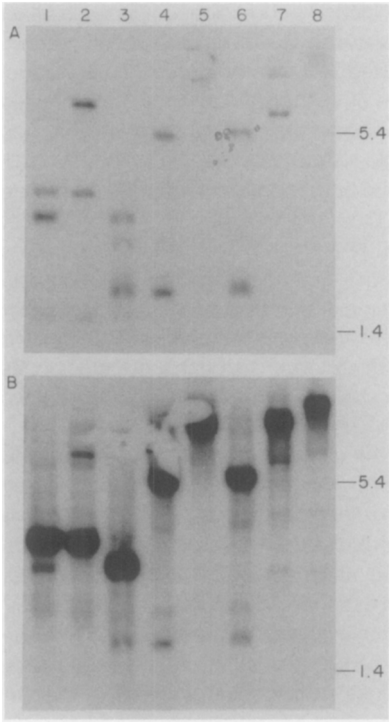


Figure 1. Blot Hybridization Analysis of the Yeast Genome with *ras* Probes.

Yeast DNA from the DC5 haploid strain was digested with the indicated restriction endonucleases, electrophoresed in 1.0% agarose, transferred to nitrocellulose, and hybridized with $^{32}$P-labeled probes. (A) Nitrocellulose filter hybridized with the 1.0 kb Hinc II fragment of the viral K-*ras* gene nick-translated to 1.0 × $10^8$ cpm per microgram. Exposure time was 24 h. (B) The identical filter used above was rehybridized with the 3.0 kb Eco RI–Hind III fragment of yeast *RAS2*, nick-translated to 1.0 × $10^8$ cpm per microgram. Exposure time was also 24 hr. Restriction endonuclease digestions were: 1) Eco RI–Bam HI; 2) Eco RI; 3) Eco RI–Hind III; 4) Hind III; 5) Bam HI; 6) Bam HI–Hind III; 7) Bam HI–Sal I; 8) Sal I. Numbers on the right are size markers in kb.

lulose filters previously hybridized with the K-*ras* probe (Figure 1b).

## DNA Sequence Analysis of Yeast *ras* Genes

To determine the precise relatedness of the yeast and mammalian *ras* genes, we sequenced the yeast genes as shown in Figure 2. The nucleotide sequences and predicted amino acid sequences are shown in Figure 3. There is an open reading frame encoding exactly 309 amino acids for yeast *RAS1* and exactly 322 amino acids for yeast *RAS2*. The initial methionine shown is the first methionine in the open reading frame. The predicted amino acid sequences show striking homology to each other, through position 180. After this they diverge radically, until homology is observed again for the last eight amino acids.

For purposes of comparison to the yeast *ras* proteins, the amino acid sequence of the human H-*ras* protein is also shown in Figure 3. Homology between the mammalian and yeast *ras* proteins begins at position 10 for the yeast and position 3 for the human H-*ras* protein. For the next 80 amino acids, there is nearly 90% homology between

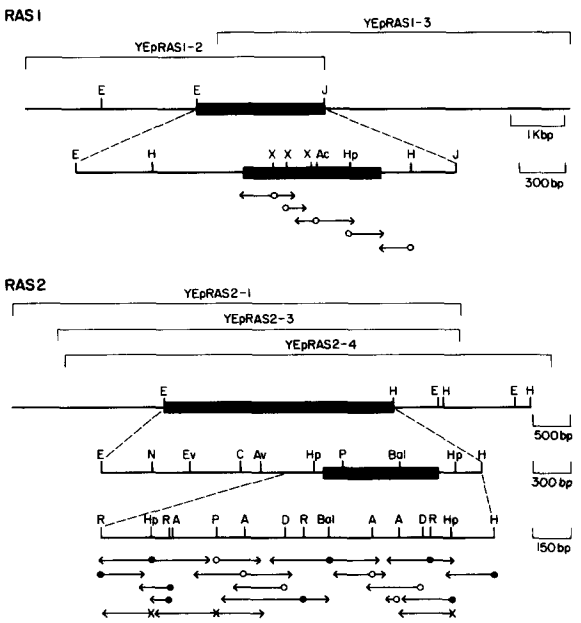


Figure 2. Composite Restriction Endonuclease and Sequencing Maps of the *RAS1* and *RAS2* Loci

Above each map are shown the Sau 3A inserts of the indicated plasmids that covered the *ras* loci. Within each map, all sites for a given restriction endonuclease are indicated. Arrows indicate regions sequenced. (O) and (●) indicate sequencing by the Maxam/Gilbert method from 3′-OH- and 5′-OH-labeled restriction endonuclease fragments, respectively; and (×) indicates dideoxy sequencing. Restriction endonucleases used were: E, Eco RI; H, Hind III; X, Xba I; Ac, Acc I; Hp, Hpa I; Bal, Bal I, P, Pst I; N, Nco I; C, Cla I, A, Alu I; R, Rsa I; D, Dde I; Ev, Eco RV; Av, Ava II. J indicates a *joint* restriction endonuclease site between Sau 3A and Bam HI.

any pair of the three. Patchy homology to H-*ras* is observed thereafter. Homology to H-*ras* is increased if we introduce an insertion at position 128 of the human H-*ras* protein. Significantly, the yeast *RAS1* and *RAS2* proteins show homology to positions 12, 13, 59, 61, and 63 of the H-*ras* protein. It is amino acid substitutions at these positions that activate the transforming potential of the mammalian *ras* proteins (Tabin et al., 1982; Reddy et al., 1982; Taparowsky et al., 1982, 1983; Yuasa et al., 1983; Fasano et al., submitted). By contrast, the *YP2* protein of Gallwitz et al. (1983) is not homologous to positions 12 or 63 of the mammalian *ras* proteins.

To visualize better the relation of the three proteins, we have plotted in Figure 4 three cumulative difference functions, D(i), where D(i) = D(i−1) if two proteins under comparison are identical at the equivalent $i^{th}$ position; D(i) = D(i−1) + 1/2 if there is a conservative amino acid difference (glycine and alanine; glutamic and aspartic acid; lysine and arginine; or leucine, valine, and isoleucine); and D(i) = D(i−1) + 1 if there is no homology at this position or a frame-shift. For further comparison, we have plotted a modified difference function, D′(i), of the three human *ras* genes. D′(i) = D′(i−1) + 1 when there is no amino acid consensus at the $i^{th}$ position, D′(i) = D′(i−1) otherwise. For ease of comparison, the D′(i) function is appro-

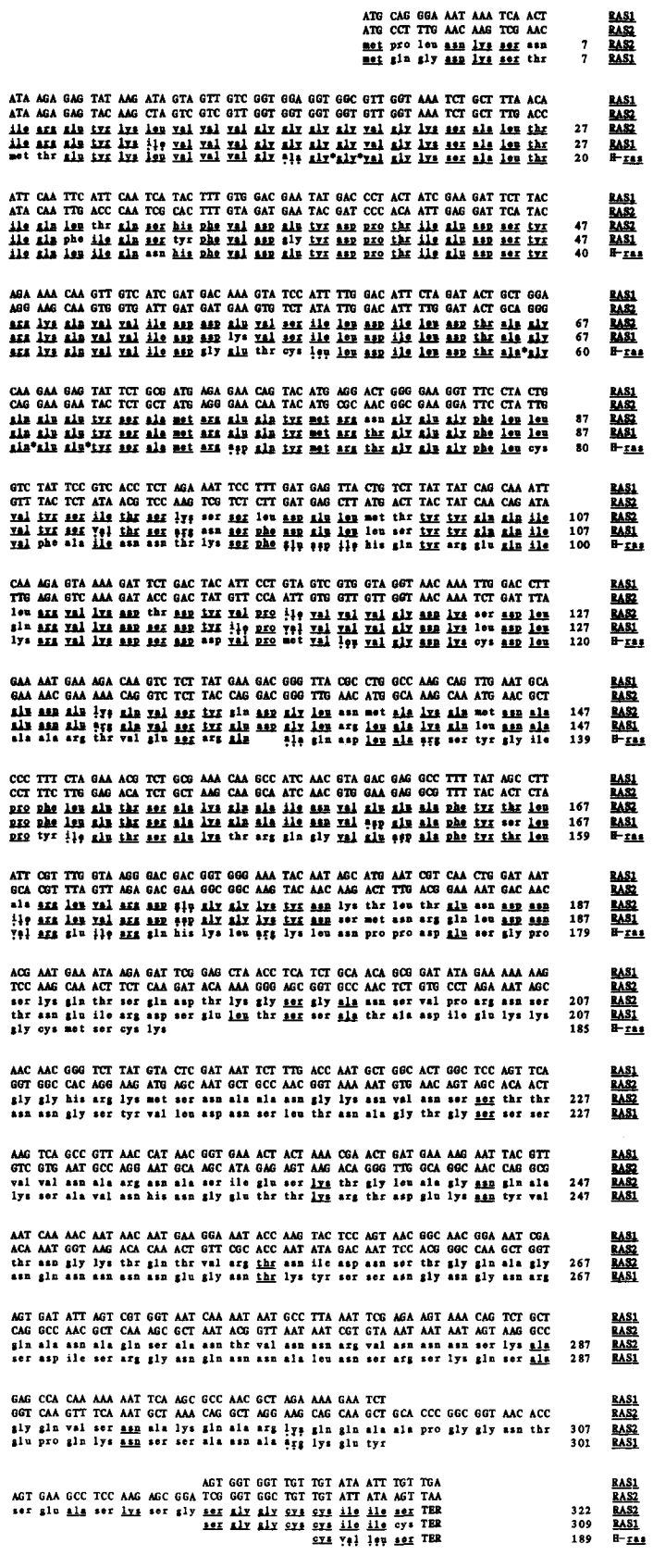

Figure 3. Yeast *ras* Sequence Comparisons

Shown at left are the nucleotide and predicted amino acid sequences of the yeast *RAS1* and *RAS2* genes. For comparison, the predicted amino acid sequence of the human H-*ras* gene is shown. The numbers in the right margin indicate the position of the last amino acid in each respective line. Positions are underlined when identical amino acids are encoded and dotted when similar amino acids are encoded (see text). Gaps in nucleotide and amino acid sequence reflect attempts to align better the respective proteins. Asterisks in the H-*ras* amino acid sequence indicate positions where amino acid substitutions are known to activate the transforming potential of the H-*ras* protein.

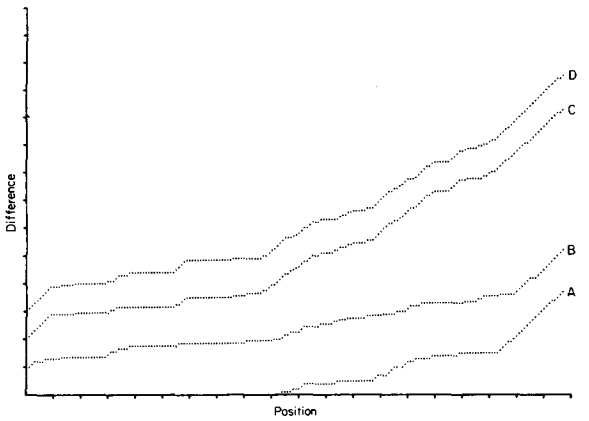


Figure 4. Cumulative Difference Functions of the Various *ras* Genes

The cumulative difference functions D(i) are plotted for (A) H-, K- and N-*ras*; (B) *RAS1* vs *RAS2*; (C) H-*ras* vs *RAS2*; and (D) H-*ras* vs *RAS1*. For ease of visual comparison, the initial values, D(O), are set to 0.0 for (A); 10.0 for (B); 20.0 for (C); and 30.0 for (D). Tick marks on abscissa (amino acid position, yeast *ras* coordinates) and on ordinate (cumulative difference values) are in units of 10. Functions are plotted up to position 197 in the yeast coordinates. See text for an explanation of the difference functions.

priately frame-shifted to align with yeast *ras* sequences. In this representation, regions of amino acid homology are indicated by plateaus, regions of divergence by increasing slopes. At the amino acid level, yeast *RAS1* and yeast *RAS2* are equally diverged from H-*ras*. Although the data is not represented, the yeast *ras* proteins show no more homology to H-*ras* than to any of the other known mammalian *ras* proteins. *RAS1* and *RAS2* are clearly more closely related to each other than they are to mammalian *ras*. Our data also indicate that the yeast *ras* proteins diverge from the human *ras* proteins in roughly the same regions as the human *ras* proteins have begun to diverge from each other. Significantly, the yeast *ras* proteins diverge radically from each other in the domain corresponding to the C-terminal variable domain of the mammalian *ras* proteins (see below).

### Immunoprecipitation of Yeast *ras2*

The extraordinary conservation of the yeast and human *ras* genes prompted us to attempt immunoprecipitation of the yeast products with a monoclonal antibody (Y13-259) raised initially to the Harvey sarcoma virus *ras* protein, but which has broad reactivity with mammalian *ras* proteins (Furth et al., 1982). For this purpose we performed immunoprecipitations and mock immunoprecipitations on $^{35}$S-methionine-labeled extracts from yeast cells containing high copy number, autonomously replicating plasmids containing the yeast *ras* genes (Figure 5). Elevated amounts of an immunoprecipitable 42 kd protein are seen in yeast cells harboring high copy numbers of the plasmid YEp-*RAS2*-1 containing *RAS2* (Figure 2). A lower molecular weight (30 kd) protein is also seen. Since the molecular weight of yeast *RAS2*, calculated from DNA sequence analysis, is 35 kd, these immunoprecipitable proteins may have undergone post-translational modifications. These

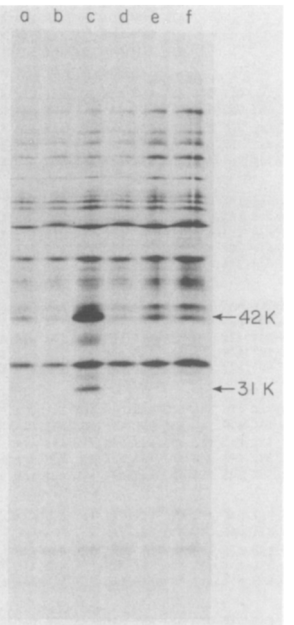


Figure 5. Immunoprecipitation of Extracts from Yeast with High Copy Number Plasmids

DC5 strains carrying the high copy number plasmids YEp*RAS1*-2 (lanes a and b); YEp*RAS2*-1 (lanes c and d), and the parental plasmid YEp13 with no insert (lanes e and f) were labeled with $^{35}$S and total-cell extracts prepared. Immunoprecipitates with (lanes b, d, f) or without (lanes a, c, e) monoclonal antibody Y13-259 were obtained and electrophoresed in SDS polyacrylamide gels. See Experimental Procedures for details. Numbers and arrows on right indicate molecular weights in kilodaltons of specific immunoprecipitated protein, calculated from molecular weight markers.

results suggest that we have cloned a complete copy of the *RAS2* gene and that it is expressed. We have not observed detectable amounts of immunoprecipitable protein in yeast cells with high copy numbers of the cloned yeast *RAS1*, either because *RAS1* protein does not cross-react with monoclonal Y13-259 or because YEp*RAS1*-2 (see Figure 2) does not contain the complete *RAS1* gene, or because the *RAS1* gene is not expressed. See Figure 5 for more experimental details.

## Discussion

We have demonstrated the presence in yeast of two genes with striking homology to the mammalian *ras* genes. Our results are in agreement with those of Defeo-Jones et al. (1983), who also found two genes in yeast closely homologous to mammalian *ras* genes. Our predicted amino acid sequence for *RAS1* is in accord with the partial amino acid sequence predicted for the gene they called c-*ras*$^{sc}$-1. Our data indicates that the *RAS1* and *RAS2* proteins are equidistant from the three known mammalian *ras* proteins but are more closely related to each other than to their mammalian homologs. A third gene, *YP2*, has been described in Saccharomyces cereviseae by Gallwitz et al. (1983), which can encode a protein with significant but much weaker homology to the mammalian *ras* proteins.

Our data indicate that the protein encoded by *YP2* is also quite diverged from both yeast *ras* proteins.

Little is known about the functional domains of the *ras* proteins. Nevertheless, we can clearly speak of domains of divergence. The first domain comprises the first 80 or so N-terminal amino acids. This domain is the most highly conserved. The three human *ras* genes, H-, K-, and N-*ras*, encode proteins with identical amino acids sequence in this region. It is within this region that homology between mammalian and yeast *ras* proteins is highest. The second domain comprises the next 80 amino acid positions. In this domain the three human *ras* proteins have begun to diverge from each other, with about 85% homology between any pair (Taparowsky et al., 1983). The yeast *ras* proteins are also highly homologous to each other in this second domain, but are not as homologous as in the previous domain. We observe only patchy homology between yeast and mammalian *ras* proteins here. The third domain we call the variable domain. In mammalian *ras* it is short, comprising 15 amino acids near the C terminus. In mammals, this domain varies radically among different members of the *ras* gene family, but it is highly conserved for the same member in different species and is therefore under evolutionary constraint (Shimizu et al., 1983a; Taparowsky et al., 1983). In yeast *ras* proteins we may also speak of a C-terminal variable domain since we can observe no clear homology between yeast *RAS1* and yeast *RAS2* proteins in this region. However, the variable domain in yeast *ras* proteins is much larger than the corresponding domain in mammalian *ras* proteins. Finally, at the very carboxy terminus of *ras* proteins there is again a small conserved domain. As we noted previously, all the mammalian *ras* proteins terminate with the sequence cysAAX, where A is an aliphatic amino acid and X is the terminal amino acid (Shimizu et al., 1983a; Taparowsky et al., 1983). Interestingly, both the yeast *ras* proteins have this terminal peptide. However, the yeast *RAS1* and *RAS2* proteins contain a larger common terminus: seven of the last eight amino acids are identical.

We think these domains of divergence correspond to functional domains of the *ras* proteins as well. We suggest that the N-terminal domain is the effector region of the *ras* proteins, involved in interactions of a catalytic or regulatory nature that have been conserved in evolution. It is in this region where certain amino acid substitutions can activate the transforming potential of the mammalian *ras* proteins. On the other hand, we believe the C-terminal variable domain contains the determinants of physiological specificity. Through this region the *ras* proteins may receive their normal physiologic signals, which are then transduced or mediated to the N-terminal domain by way of the intervening, semiconserved domain. In this view of things, the yeast *RAS1* and *RAS2* proteins would have the same effector functions, but would carry out these functions in response to different stimuli.

We have demonstrated that the cloned yeast *RAS2* gene expresses a protein that is immunoprecipitable with a monoclonal antibody raised against the mammalian H-*ras* protein. We are currently investigating whether overexpression or disruption of either of the yeast *ras* genes results in a discernible phenotype. Study of the yeast *ras* genes may greatly accelerate our understanding of the normal and transforming mammalian *ras* genes at three levels. First of all, the high degree of conservation at the N terminus suggests to us that the biochemical effector function of the *ras* genes may have been conserved in evolution. (For example, yeast and mammalian *ras* genes may be regulatory components of a homologous catalytic system.) This hypothesis can be put to a rigorous test by examining the function of mammalian/yeast *ras* chimeric genes. Second, the yeast *ras* proteins are homologous to the mammalian proteins about amino acid positions where amino acid substitutions lead to activation of the transforming potential of the mammalian protein. By examining the consequences of similar amino acid substitutions on the function of the yeast *ras* proteins, we may gain valuable insights into the molecular mechanism of *ras* activation. Third, what we learn about the physiologic function of the yeast *ras* proteins may provide tantalizing clues to the physiologic role of the *ras* proteins in mammalian cells.

## Experimental Procedures

### Yeast Strains, Media, and Transformation

General procedures for genetic manipulation of yeast were performed as described by Mortimer and Hawthorne (1969). DNA from strain DC5 (*MATa his3⁻ leu2⁻*) was used for Southern blot analysis. This strain was also used for transformations with the high copy number plasmid clones of *RAS1* and *RAS2* (see below). Yeast transformations were done according to the method of Beggs (1978). Cells were grown either in rich medium (2% Bacto-peptone, 1% yeast extract, and 2% glucose) or synthetic medium (0.7% yeast nitrogen base without amino acids [Difco], supplemented with appropriate amino acids and nucleic acid bases, and 2% glucose). Synthetic media was used for both $^{35}$S-methionine labeling and for selection and maintenance of transformants.

### Nomenclature

Consonant with standard yeast nomenclature we have designated the *ras* homologous genes in yeast as *RAS1* and *RAS2*. These correspond respectively to c-*ras*$^{sc}$-1 and c-*ras*$^{sc}$-2 of Defeo-Jones et al. (1983).

### Southern Analysis

Yeast DNA was prepared essentially as described (Struhl et al., 1979). DNAs were digested with restriction endonucleases (suppliers New England Biolabs or Bethesda Research), and 5 μg was loaded onto agarose gels for electrophoresis and blotting as described (Shimizu et al., 1983b). Low-stringency hybridizations were in aqueous 6× SSC at 55°C with a final blot wash in 2× SSC at 55°C. The *ras* probes used were the 2.2 kb Bam HI–Eco R1 fragment of the viral H-*ras* plasmid clone pHB-11 (Ellis et al., 1981), the 1.0 kb Hinc II fragment of the viral K-*ras* plasmid clone pKBE-2 (Ellis et al., 1981), and the 0.5 kb Nco I–Sal I fragment of the N-*ras* cDNA plasmid clone p6al (Taparowsky et al., 1983).

### Screening Plasmid Libraries

The Grunstein and Hogness procedure (1975) was used to screen a genomic library that had been constructed in the plasmid vector YEp13 from yeast DNA partially digested with Sau 3A restriction endonuclease (Broach et al., 1979; Nasmyth and Reed, 1980). Low-stringency hybridizations were performed as described above. For our initial screening we used a 0.6 kb Pvu II–Sma I fragment from the H-*ras* cDNA plasmid clone RS6 (Fasano et al., 1983). Six candidates that gave relatively strong signals on duplicate filters were purified and further analyzed by restriction endonucle-

ase digestion and Southern blotting. Three of these plasmid clones were distinct and contained in common a 3.6 kb Eco RI fragment that hybridized to mammalian *ras*. Next, the library was screened with the 0.6 kb Nco I–Sal I fragment of the N-*ras* cDNA plasmid clone p6al. We obtained three candidates, two of which contained a 1.7 kb Hind III fragment that hybridized to mammalian *ras*. See Figure 2 for more experimental details.

### Immunoprecipitations

The plasmid library clone YEp*RAS1*-2 and YEp*RAS2*-1 (see Figure 2) were used to transform DC 5 to obtain yeast with high copy number of either *RAS1* or *RAS2* genes, respectively. Extracts of these transformants, along with DC 5 transformed with YEp13 (parental plasmid without any insert; Broach et al., 1979) were prepared from logarithmically growing cultures ($10^8$ total cells per extract) labeled with 250 $\mu$Ci $^{35}$S-methionine (Amersham) for 90 min. The cells were lysed in 200 $\mu$l PBS containing 1% Triton X-100, 0.5% deoxycholate, 1 mM PMSF, and 0.1 mg/ml aprotinin (Sigma) by vortexing with glass beads on ice. This crude extract was clarified, immunoprecipitated with monoclonal antibody Y13-259 (Furth et al., 1982), and analyzed by SDS-PAGE electrophoresis as described (Shimizu et al., 1983b).

### Sequencing

Restriction endonuclease fragments were separated by gel electrophoresis and sequenced by the method of Maxam and Gilbert (1980) after 3′-OH end-labeling with E. coli polymerase I large fragment (Bethesda Research Labs) or 5′-OH-labeling with T4 polynucleotide kinase (Miles Labs). Confirmatory sequence data was obtained by the dideoxy method of Sanger et al. (1977) as modified by Biggin et al. (1983). Restriction endonuclease fragments were cloned into either M13 mp8 or M13 mp9 (Messing and Vieira, 1982). See Figure 2 for a detailed description.

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### References

Beggs, J. D. (1978). Transformation of yeast by a hybrid replicating plasmid. Nature *275*, 104–109.

Biggin, M. D., Gibson, T. J., and Hong, G. F. (1983). Buffer gradient gels and $^{35}$S label as an aid to rapid DNA sequence determination. Proc. Nat. Acad. Sci. USA *80*, 3963–3965.

Broach, J. R., Strathern, J. N., and Hicks, J. B. (1979). Transformation in yeast: development of a hybrid cloning vector and isolation of the CAN1 gene. Gene *8*, 121–133.

Capon, D., Ellson, Y., Levinson, A., Seeburg, P., and Goeddel, D. (1983a). Complete nucleotide sequences of the T24 human bladder carcinoma oncogene and its normal homologue. Nature *302*, 33–37.

Capon, D., Seeburg, P., McGrath, J., Hayflick, J., Edman, U., Levinson, A., and Goeddel, D. (1983b). Activation of Ki-*ras2* gene in human colon and lung carcinoma by different point putations. Nature *304*, 507–513.

DeFeo-Jones, D., Scolnick, E., Koller, R., and Dhar, R. (1983). *ras*-related gene sequences identified and isolated from *Saccharomyces cerevisiae*. Nature *306*, 707–709.

Ellis, R. W., Defeo, D., Shih, T. Y., Gonda, M. A., Young, H. A., Tsuchida, N., Lowy, D. R., and Scolnick, E. M. (1981). The p21 *src* genes of Harvey and Kirsten sarcoma viruses originate from divergent members of a family of normal vertebrate genes. Nature *292*, 506–511.

Fasano, O., Taparowsky, E., Fiddes, J., Wigler, M., and Goldfarb, M. (1983). Sequence and structure of the coding region of the human H-*ras*-1 gene from T24 bladder carcinoma cells. J. Mol. App. Genet. *2*, 173–180.

Furth, M. E., Davis, L. J., Fleurdelys, B., and Scolnick, E. M. (1982). Monoclonal antibodies to the p21 products of the transforming gene of Harvey sarcoma virus and of the cellular *ras* family. J. Virol. *43*, 294–304.

Gallwitz, D., Dorath, C., and Sander, C. (1983). A yeast gene encoding a protein homologous to the human C-*has*/*bas* proto-oncogene product. Nature *306*, 704–707.

Grunstein, M., and Hogness, D. (1975). Colony hybridization: a method for the isolation of cloned DNAs that contain a specific gene. Proc. Nat. Acad. Sci. USA *72*, 3961–3965.

McGrath, J. P., Capon, D. J., Smith, D. H., Chen, E. Y., Seeburg, P. H., Goeddel, D. V., and Levinson, A. D. (1983). Structure and organization of the human Ki-*ras* proto-oncogene and a related processed pseudogene. Nature *304*, 501–506.

Messing, J., and Vieira, J. (1982). A new pair of M13 vectors for selecting either DNA strand of double-digest restriction fragments. Gene *19*, 269–276.

Mortimer, R. K., and Hawthorne, D. C. (1969). In The Yeasts, A. H. Rose, and J. S. Harrison, eds. Vol. 1, (New York: Academic Press Inc.), pp. 385–460.

Nasymth, K. A., and Reed, S. I. (1980). Isolation of genes by complementation in yeast. Proc. Nat. Acad. Sci. USA *77*, 2119–2123.

Reddy, E., Reynolds, R., Santos, E., and Barbacid, M. (1982). A point mutation is responsible for the acquisition of transforming properties by the T24 human bladder carcinoma oncogene. Nature *300*, 149–152.

Sanger, F., Nicklen, S., and Coulson, A. R. (1977). DNA sequencing with chain terminating inhibitors. Proc. Nat. Acad. Sci. USA *74*, 5463–5467.

Shilo, B., and Weinberg, R. (1981). DNA sequences homologous to vertebrate oncogenes are conserved in *Drosophila melanogaster*. Proc. Nat. Acad. Sci. USA *78*, 6789–6792.

Shimizu, K., Birnbaum, D., Ruley, M., Fasano, O., Suard, Y., Edlund, L., Taparowsky, E., Goldfarb, M., and Wigler, M. (1983a). The structure of the K-*ras* gene of the human lung carcinoma cell line Calu-1. Nature *304*, 497–500.

Shimizu, K., Goldfarb, M., Suard, Y., Perucho, M., Li, Y., Kamata, T., Feramisco, J., Stravnezer, E., Fogh, J., and Wigler, M. (1983b). Three human transforming genes are related to the viral *ras* oncogenes. Proc. Nat. Acad. Sci. USA *80*, 2112–2116.

Struhl, K., Stinchcomb, D. T., Scherer, S., and Davis, R. W. (1979). High-frequency transformation of yeast: autonomous replication of hybrid DNA molecules. Proc. Nat. Acad. Sci. USA *76*, 1035–1039.

Tabin, C., Bradley, S., Bargmann, C., Weinberg, R., Papageorge, A., Scolnick, E., Dhar, R., Lowy, D., and Chang, E. (1982). Mechanism of activation of a human oncogene. Nature *300*, 143–148.

Taparowsky, E., Suard, Y., Fasano, O., Shimizu, K., Goldfarb, M., and Wigler, M. (1982). Activation of the T24 bladder carcinoma transforming gene is linked to a single amino acid change. Nature *300*, 762–765.

Taparowsky, E., Shimizu, K., Goldfarb, M., and Wigler, M. (1983). Structure and activation of the human N-*ras* gene. Cell *34*, 581–586.

Yuasa, Y., Srivastava, S., Dunn, C., Rhim, J., Reddy, E., and Aaronson, S. (1983). Acquisition of transforming properties by alternative point mutations within C-*bas*/*has* human proto-oncogene. Nature *303*, 775–779.