At a T_A of 0°C, however, the wing-beat frequency of the temperature-conforming geometrids should be decreased considerably. If the geometrid wing-beat frequency is halved at 0°C, it would be approximately 30 times lower than that of the noctuids.

Geometrids and other nocturnal nonfeeding Lepidoptera generally have large wings (*1*) relative to their mass (low wing loading), and this characteristic confers a low energetic cost of flight (*25*). The mean wing-loading in *Eupsilia* sp. was 43 mg/cm^2 (*24*). In the winter-flying *Operophtera* and *Alsophila*, however, wing-loading (3.20 mg/cm^2 and 3.90 mg/cm^2) is not only 10 to 14 times lower than in the noctuids but is also lower than in all other geometrid (and other moth) species from both tropical (*1, 26*) and temperate regions (*2*) that have been examined. Low wing-loading that decreases the cost of transport may be a preadaptation in these geometrids that has allowed them to fly at low ambient and muscle temperatures. Our findings suggest that although morphology affecting thermoregulation can vary radically, the adaptations at the enzyme level for temperature are apparently highly conservative.

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8. A solution containing sugar or honey and mashed fruit was painted on trees in the evening, and the moths that arrived to feed were brought into the laboratory and held in a refrigerator at −6°C until they were used.
9. Body temperatures during preflight warm-up were measured with 0.001-mm diameter copper-constantan thermocouples inserted approximately 1 mm into the thorax. Thoracic and ambient temperatures were each recorded at 10-second intervals with a Honeywell potentiometric recorder. All temperatures were referenced to a National Bureau of Standards thermometer. For moths in flight, thoracic temperature was measured after the animal had been in continuous flight for sufficiently long to ensure equilibration of body temperature (>1.5 minutes). Temperature measurements were made with a Bailey Bat-12 digital thermocouple thermometer [B. Heinrich and M. J. Heinrich, *Physiol. Zool.* **56**, 552 (1983)]. Measuring artifacts of temperature in the small moths were corrected as previously described by B. Heinrich and K. Pantle [*J. Exp. Biol.* **62**, 599 (1975)].
10. At a T_A of 20° and 0°C, for example, the moths shivered for 1.0 to 23 minutes, to achieve a flight temperature of 32° to 35°C.
11. During preflight warm-up abdominal temperature did not exceed 0.5°C above T_A, and during flight it did not exceed 3°C above T_A.
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13. After capture, moths were quick-frozen on dry ice and stored for a maximum of 4 weeks at −80°C. Tissues from three to six specimens of the geometrid moths (*Alsophila* or *Operophtera*) were pooled, although specimens of the other two species were analyzed individually. Thoraxes, thawed and carefully freed of legs, wings, and hair, were homogenized and sonicated extensively in 20 volumes of imidazole buffer (50 m*M* imidazole-HCl, containing 10 m*M* glycerol, *p*H 7.4 at 20°C). Homogenates, prepared on ice, were centrifuged at 6000*g* for 20 minutes at 0°C. Resulting supernatants were kept on ice and diluted with buffer. Enzyme activities were measured spectrophotometrically [T. P. Mommsen, C. J. French, P. W. Hochachka, *Can. J. Zool.* **58**, 1785 (1980)]. All assays were performed in duplicates or triplicates (temperatures below 10°C), and cuvette temperature was monitored before and after incubation. One unit is defined as the amount of enzyme forming 1 μmol of product per minute under saturating conditions.
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20. In vivo, however, secondary effects of changes in membrane fluidity with decreasing T_A may alter enzyme catalysis over and above direct thermodynamic considerations [M. Sinensky, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 522 (1974)]. Enzyme-level adaptation through a compensation in the entropy term is an interesting possibility (*17*), but one beyond the scope of our study.
21. Rates of heat production were calculated from passive rates of heat loss in dead animals and heat storage during T_{Th} increase in live animals during shivering (*5*).
22. Oxygen consumption of flying moths (*Eupsilia* spp.) was measured with an S-3A Applied Electrochemistry oxygen analyzer system measuring to 0.01 percent O_2 concentration. The moths were flown for timed durations (2 to 15 minutes) in a 1680-ml respirometer, and O_2 content of the air was determined immediately before and after each flight.
23. Solving the equation of Casey and Joos (*2*) for conductance in geometrids (log C = −0.61 log mass + 3.01) indicates that the *Operophtera* and *Alsophila* with mean mass of 9.8 mg and 8.6 mg should expend 3.68 and 3.95 cal per gram of body weight per minute, respectively, to maintain a T_{Th} of 0.5°C above T_A during flight at approximately 0.5 m/second.
24. The regression equation of Casey and Joos (*2*) for wing-beat frequency for geometrids is: log n = −0.025 + 0.492 log m + 0.133 log ℓ + 0.255 log $w\ell$, where n = wing-beat frequency per second, m = mass in milligrams, ℓ = wing length in centimeters, and $w\ell$ = wing loading in milligrams per square centimeter. Mean values ($n \geq 10$) for *Operophtera*: m = 9.8 mg, ℓ = 1.38 cm, and $w\ell$ = 3.20 mg/cm^2. Mean values for *Alsophila*: m = 8.64 mg, ℓ = 1.23 cm, and $w\ell$ = 3.90 mg/cm^2. The corresponding equation for noctuid moths is: log n = 1.224 + 0.849 log m − 1.39 log ℓ − 0.643 log $w\ell$; mean values for *Eupsilia* spp. were: m = 164 mg, ℓ = 1.58, and $w\ell$ = 43 mg/cm^2.
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27. We thank D. Schweitzer and J. A. Franklemont for valuable discussion and for identifying the moths. Supported by NSF grant DEB-816662 to B.H. and by an NSERC (Canada) operating grant to T.P.M.

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Mammalian and Yeast *ras* Gene Products: Biological Function in Their Heterologous Systems

Abstract. *Activated versions of* ras *genes have been found in various types of malignant tumors. The normal versions of these genes are found in organisms as diverse as mammals and yeasts. Yeast cells that lack their functional* ras *genes, RAS^{SC}-1 and RAS^{SC}-2, are ordinarily nonviable. They have now been shown to remain viable if they carry a mammalian* ras^H *gene. In addition, yeast-mammalian hybrid genes and a deletion mutant yeast RAS^{SC}-1 gene were shown to induce morphologic transformation of mouse NIH 3T3 cells when the genes had a point mutation analogous to one that increases the transforming activity of mammalian* ras *genes. The results establish the functional relevance of the yeast system to the genetics and biochemistry of cellular transformation induced by mammalian* ras *genes.*

The *ras* genes constitute a multigene family that is highly conserved among eukaryotes, including mammals and yeast. These genes and their 21-kD protein products (p21) were first identified as the viral oncogenes of Harvey and Kirsten murine sarcoma viruses (Ha-MuSV and Ki-MuSV, respectively) (*1*).

High level expression of a normal (nonmutated) mammalian p21 *ras* protein can induce tumorigenic transformation of established rodent cells. However, forms of the protein encoded by *ras* genes that contain certain point mutations can induce these tumorigenic changes with much greater efficiency. Mutated forms of cellular *ras* oncogenes have been implicated in the pathogenesis of several human cancers. Activating point mutations that lead to a more highly oncogenic form of mammalian p21 protein, which are present in v-*ras* (viral) and c-*ras* (cellular) oncogenes isolated from tumor cells, include those that substitute any one of several amino acids for Gly^{12} or Gln^{61} or that substitute Thr^{59} for the normal Ala^{59}. Addition of an enhancer element to these structurally altered *ras* genes can enable them to transform primary rodent cells directly (*1, 2*).

Relatively little is known about the normal function of p21 *ras* proteins or the mechanism by which they induce cellular transformation. It is known that they have guanosine triphosphate (GTP) binding activity as well as guanosine triphosphatase activity and that the hydrolysis of GTP is significantly impaired in mutated oncogenic *ras* proteins as compared with that in the normal p21 protein (*3*). It is also known that the mature p21 protein is located at the inner surface of the plasma membrane; this location probably represents the functionally active site, since mutant p21 proteins that do not migrate to the plasma membrane are transformation-defective (*4*).

The c-*ras* genes are also found among invertebrate eukaryotes. The yeast *Saccharomyces cerevisiae*, which contains at least two *ras* genes (RAS^{SC}-1 and RAS^{SC}-2), represents an organism in which eukaryotic *ras* gene function can be readily investigated (*5*). In this report, we discuss the potential relevance of this yeast-derived information to the function of mammalian *ras* genes.

The yeast and mammalian *ras* proteins share many structural, immunological, and biochemical features, although certain structural differences of unknown functional significance have also been noted (*6, 7*). Mammalian (*1, 8, 9*) and *Drosophila* (*10*) *ras* genes encode proteins consisting of 188 or 189 amino acids. By contrast, the yeast RAS^{SC}-1 and RAS^{SC}-2 genes have primary translation products of 309 and 322 amino acids, respectively. The yeast genes encode a stretch of 165 amino acids near their NH_2-terminus that is 65 percent homologous to the first 164 amino acids of the mammalian *ras* proteins; conservative changes in the yeast proteins account for about half of the divergent amino acids in this region.

Compared with the mammalian *ras* proteins, the yeast RAS^{SC}-1 and RAS^{SC}-2 proteins contain 112 and 125 additional amino acids, respectively, at the COOH-terminus, as well as seven additional amino acids at the extreme end of the NH_2-terminus and one additional amino acid farther downstream. Most of the sequences coding for the COOH-terminus are poorly conserved in all *ras* genes. However, the yeast RAS sequence encoding the extreme COOH-terminus shows homology with the corresponding mammalian *ras* sequence; the yeast RAS genes encode a cysteine residue comparable to Cys^{186}, which is encoded by all mammalian *ras* genes and which is absolutely required for cellular transformation and membrane localization of a mammalian *ras* protein (*11*). Although RAS^{SC}-1 and RAS^{SC}-2 encode proteins that are larger (34 and 35 kD, respectively) than the mammalian proteins, the yeast proteins share antigenic determinants with mammalian p21 proteins and have GTP binding and hydrolyzing activities comparable to those of the mammalian proteins (*6, 12*).

These common structural, immunological, and biochemical features of the yeast and mammalian *ras* proteins prompted us to investigate the possibility that the *ras* genes from these divergent

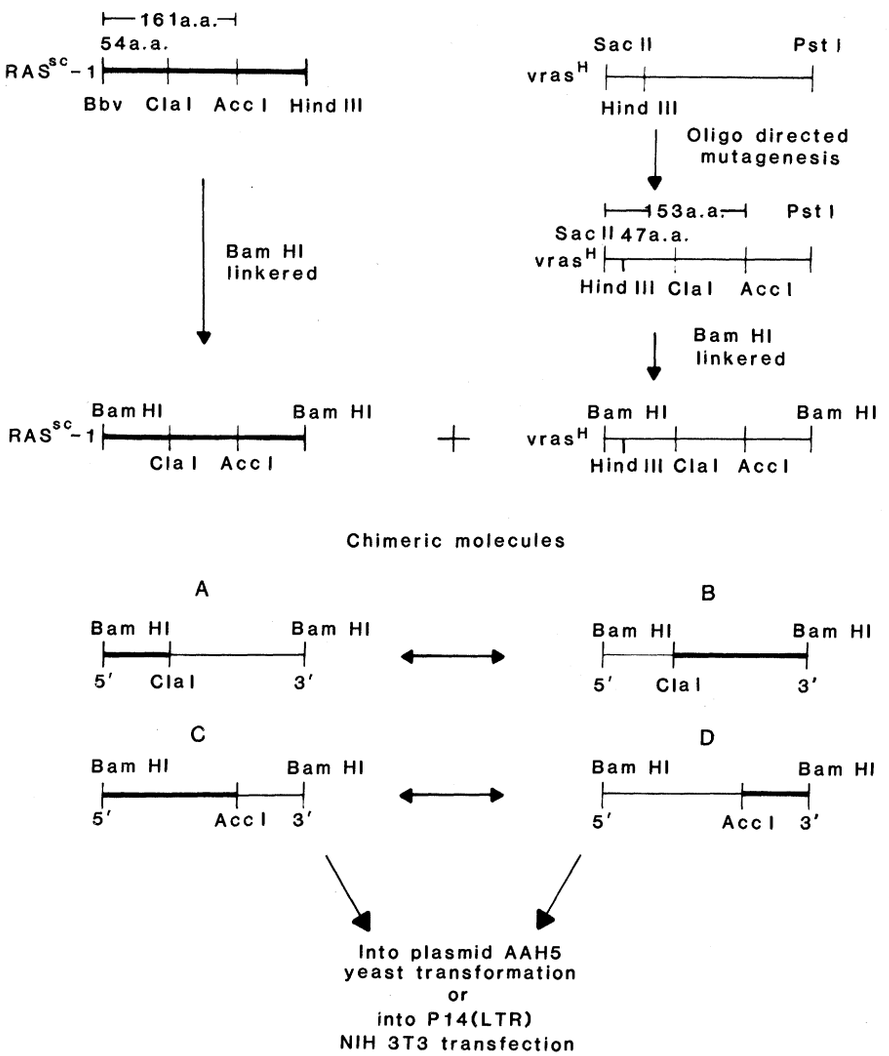

Fig. 1. Constructs of the genes used in both the yeast and mammalian biological assays. The heavy horizontal lines represent yeast RAS^{SC}-1 sequences; the light horizontal lines represent v-ras^H sequences. The yeast RAS^{SC}-1 gene (*5*) was cut with the restriction enzyme Bbv I to remove upstream yeast promotor signals that could interfere with transcription in mammalian cells. It was secondarily cut with the enzyme Hind III, the ends were made flush and ligated to Bam HI linkers. The final fragment was subcloned into the Bam HI site of pBR322. The v-ras^H gene was subcloned into M13mp8 as a 720-base-pair Bam HI fragment, and oligonucleotide-directed mutagenesis was performed (*12*) to introduce a Cla I site and a Acc I site at positions in frame and analogous to those sites found in yeast RAS^{SC}-1. The oligonucleotides used were synthesized by Biosearch Research Chemicals, San Rafael, California. The resulting DNA molecule was cut with Bam HI and subcloned into that site in pBR322. The chimeric molecules A, B, C, and D were made by switching regions between the yeast RAS^{SC}-1 gene and the v-ras^H gene as diagrammed; the Cla I and Acc I sites found in yeast RAS^{SC}-1 and substituted into v-ras^H break these *ras* genes into regions coding for amino acids 1 to 54, corresponding to amino acids 1 to 47 of mammalian *ras* (from the AUG start codon to the Cla I site); amino acids 55 to 161, corresponding to 48 to 153 of mammalian *ras* (from the Cla I site to the Acc I site); and amino acids 162 to 309, corresponding to 154 to 189 of mammalian *ras* (from the Acc I site to termination). Hybrid C was further modified by introducing potentially activating mutations into this clone, thus creating two variants (hybrid C^{Leu} and hybrid C^{Thr}). This was done by taking the Cla I–Acc I fragment of a RAS^{SC}-1 gene containing one or the other of two activating mutations (encoding Leu^{68} or Thr^{66}) (*12*) and substituting it for the same region in hybrid C. All molecules were secondarily subcloned into the Bam HI site of the Ha-MuSV–p14 molecule (*24*) or flush-ended and then blunt end–ligated into the AAH5 yeast shuttle vector (*18*).

eukaryotes could function biologically in the heterologous system. We report that the mammalian *ras* gene can substitute functionally in yeast for the yeast RAS genes and that a modified yeast RAS^{SC}-1 gene can be biologically active in mammalian cells. These data suggest that yeast and mammalian *ras* proteins function similarly. Therefore, results obtained with *ras* genes in any eukaryotic species will probably have relevance to the function of *ras* genes in all eukaryotes.

Assays and chimeric constructions. Functional assays have been developed to assess the biological activity of a given *ras* gene in mammalian and yeast cells, respectively. The capacity of these genes to induce transformation of NIH 3T3 cells is a sensitive mammalian cell assay (*1, 13*).

In yeast, cells containing disruptions of either RAS^{SC}-1 or RAS^{SC}-2 are viable; however, haploid yeast spores carrying disruptions of both genes fail to grow, demonstrating that the RAS genes provide some essential function in yeast (*14, 15*). We have also observed that haploid yeast strains bearing disruptions of RAS^{SC}-2 alone fail to grow on nonfermentable carbon sources (*16*). This defect can be eliminated by increasing the gene dosage of RAS^{SC}-1. Thus, the ability of any *ras* gene to function in yeast can be assayed by two criteria: (i) the ability to substitute for both yeast RAS genes by allowing haploid spores containing disruptions of both RAS^{SC}-1 and RAS^{SC}-2 to grow, and (ii) the ability to allow disrupted strains of RAS^{SC}-2 to grow on ethanol or glycerol.

Figure 1 shows the scheme for the construction of a series of molecules that have been used to determine if all or any part of the mammalian and yeast *ras* genes can function in the heterologous system. The yeast RAS^{SC}-1 gene has restriction endonuclease sites (Cla I and Acc I) that break the protein into convenient segments. The v-*ras*^{H} gene of Ha-MuSV was chosen because, like the yeast gene, it lacks introns, and it can induce transformation of NIH 3T3 cells with high efficiency. Oligonucleotide site-directed mutagenesis was used to introduce Cla I and Acc I sites (found in yeast RAS^{SC}-1 but absent from v-*ras*^{H}) in the homologous regions of v-*ras*^{H} (between amino acids 46 and 47 for Cla I and between 152 and 153 for Acc I). Using this modified v-*ras*^{H} gene (which retained its transforming activity on NIH 3T3 cells) and the RAS^{SC}-1 gene as parental molecules, we constructed chimeric molecules between the yeast and mammalian genes (see Fig. 1 and below).

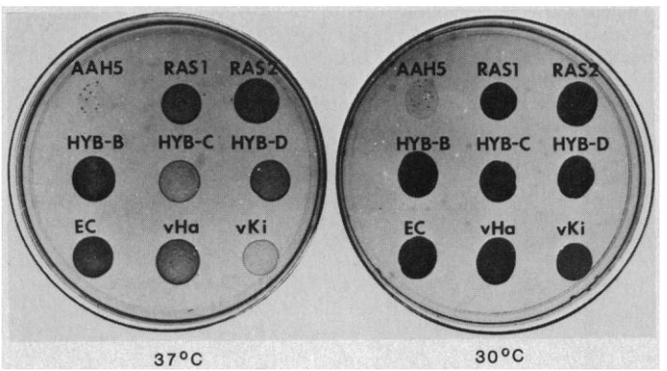

Fig. 2. *Saccharomyces cerevisiae* strain 112-699 [*aleu2, ura3, his3, ras2-699* (HIS3)] was transformed with plasmid AAH5 and the *ras*/AAH5 constructs outlined in Fig. 1. Transformants were grown in liquid culture containing glucose but lacking leucine, and equal numbers of cells were spotted onto the plates containing 2 percent peptone, 1 percent yeast extract, and 2 percent ethanol (YEPethanol) and incubated at either 30° (right plate) or 37°C (left plate). All *ras*/AAH5 plasmids complement the ethanol growth deficiency of strain 112-699. To construct the *HIS3*-associated RAS^{SC}-2 disruption in 112-699, the *LEU2* gene of pRA530 (*14*) was replaced with the *HIS3* gene from plasmid PYF88 (*19*). This was accomplished by excising the *HIS3* gene from PYF88 with the enzyme Bam HI, flush-ending the fragment, and ligating it into the flush-ended plasmid pRA530 cut with the enzymes Xho I and Kpn I to remove the *LEU2* marker. The resulting disrupted gene of RAS^{SC}-2 was transplaced into strain 112 (*14*).

Substitution of mammalian ras *for RAS^{SC}-1 and RAS^{SC}-2 in yeast.* In order to assay the various *ras* genes for biological activity in the yeast system, we subcloned the molecules RAS^{SC}-1, RAS^{SC}-2, v-*ras*^{H}, v-*ras*^{K} (from ki-MuSV) (*9*), EC (the normal human c-*ras*^{H} gene without intervening sequences) (*17*), and hybrids B, C, and D into the yeast shuttle vector AAH5. This vector is a high-copy, 2μ-containing vector. It makes use of the yeast alcohol dehydrogenase promotor and terminator and carries the auxotrophic marker for leucine (*LEU2*) (*18*). This plasmid allows for high expression of the subcloned fragments in yeast cells. The plasmids containing the subcloned fragments were assayed for their ability to function in the two yeast assays described above.

To test for growth on nonfermentable carbon sources, we first transformed the plasmids into a haploid yeast strain in which only the RAS^{SC}-2 gene had been disrupted (the disrupted gene is referred to as ras^{SC}-2) by inserting *HIS3* (*19*) into the RAS^{SC}-2 coding sequence (Fig. 2). This strain (designated 112-699) is viable, owing to the presence of wild-type RAS^{SC}-1, but it fails to grow efficiently on nonfermentable carbon sources. Transformed strains where therefore assayed for their ability to grow on ethanol or glycerol. When strain 112-699 is transformed with the shuttle vector AAH5 alone, it fails to grow on yeast extract, peptone, and ethanol (YEP-ethanol), as seen in the upper left-hand corner of each petri dish (Fig. 2). By contrast, all the *ras*-containing plasmids complement the defect, although to differing extents. The growth defect is more severe at 37°C. At this temperature, we see some variation in the degree to which the plasmids suppress the defect. In particular, hybrid C, EC, and v-*ras*^{K} complemented the defect to a lesser extent than did the other *ras* genes.

The capacity of the *ras*-containing plasmids to induce viability of haploid spores bearing disrupted RAS genes was tested by mating the 112-699 transformants described above to a haploid strain in which the RAS^{SC}-1 gene had been disrupted (ras^{SC}-1) by inserting *URA3* into the RAS^{SC}-1 coding sequence (strain 130) (*14, 15*). The resulting diploids, which were heterozygous for both ras^{SC}-1 and ras^{SC}-2, were sporulated, and tetrad analysis was performed. As expected, many spores from the untransformed parent were nonviable, and all spores containing both ras^{SC}-1 and ras^{SC}-2 failed to grow. The only tetrads in which all four spores germinated and grew into colonies were those with the parental genotype with respect to RAS^{SC}-1 and RAS^{SC}-2. Tetrads with the nonparental or tetratype configuration always had nonviable spores of the ras^{SC}-1 or ras^{SC}-2 genotypes. Unlike the parent strain, however, the strains containing many of the chimeric plasmids gave rise to spore clones with disruptions in both yeast RAS genes. The most striking result was obtained with the plasmid containing v-*ras*^{H}, which could substitute for yeast RAS, allowing the ras^{SC}-1, ras^{SC}-2 double mutant to grow. In the second column of Table 1 the number of viable ras^{SC}-1, ras^{SC}-2 meiotic segregants for each transformant are presented along with the total number of haploid progeny that contained the plasmid. All plasmids tested, except the vec-

Table 1. Tetrad analysis for diploid strain 112-699/130 transformed with AAH5-derived plasmids. Ura^+, His^+ spore clones define those with disruptions in both RAS^{SC}-1 and RAS^{SC}-2. All Ura^+, His^+ clones are also Leu^+, indicating that the plasmid containing yeast RAS or mammalian *ras* is required to maintain viability of the strain. The absence of double mutants for EC and v-ras^K could be the result of small sample size. PD designates parental ditype asci. Nonparental ditype (NPD) and tetratype (T) asci with four viable spores can arise only if the plasmid-borne yeast RAS or mammalian *ras* gene can substitute for both disrupted chromosomal genes.

Plasmids	Ratio of Ura^+, His^+ clones to Leu^+ clones	Leu^+ clones (%)	Four-spored asci		
			PD	NPD	T
RAS1/AAH5	8/39	59	2	1	5
RAS2/AAH5	21/80	70	6	1	9
HYB B/AAH5	17/70	43	7	1	7
HYB C/AAH5	2/10	5	9	1	0
HYB D/AAH5	10/42	35	7	2	4
EC/AAH5	0/3	5	2	0	0
vHa/AAH5	4/57	54	1	0	3
vKi/AAH5	0/3	8	2	0	0

tor alone (AAH5), v-ras^K, and EC gave rise to viable ras^{SC}-1, ras^{SC}-2 double mutants.

The absence of haploid meiotic progeny with plasmid EC substituting for the yeast *ras* genes should not be taken as evidence that this mammalian gene cannot function in yeast. For reasons we do not understand, the plasmids containing EC, v-ras^K, and hybrid C were transmitted inefficiently (5 to 8 percent) to the spores, whereas plasmids containing RAS^{SC}-1, RAS^{SC}-2, HYB-B, HYB-D, and v-ras^H were transferred to 35 to 70 percent of the haploid spores (Table 1).

Although the AAH5-derived plasmids bearing the *LEU2* marker gene were mitotically unstable, segregating Leu sectors at a high frequency, we found that the ras^{SC}-1, ras^{SC}-2 double mutants were stably Leu^+. Such a result would be predicted if the plasmid-associated *ras* gene is essential for cell viability. When the double mutants were mated to wild-type strains, the *LEU2* marker again becomes mitotically unstable. This result indicated that when functional RAS genes are again present, cells can eliminate the plasmid without losing viability.

We conclude that mammalian v-ras^H and chimeric *ras* genes can substitute functionally for the normal yeast RAS genes. The presence of the appropriate *ras*-containing plasmid has been confirmed by Southern blot analysis on the transformed strains. Haploid spore clones with the phenotype indicative of the ras^{SC}-1, ras^{SC}-2 genotype (HIS3, *URA3*) have chromosomal *ras* restriction endonuclease patterns consistent only with disrupted copies of yeast RAS. In addition, the v-ras^H gene product p21 has been identified as the sole *ras* protein in the v-ras^H transformants (data not shown).

Activity of yeast RAS^{SC}-1 in mammalian cells. Having demonstrated that a mammalian *ras* protein could substitute functionally in yeast for a yeast RAS protein, it was important to determine if the yeast RAS protein in mammalian cells might also have biological activity similar to that of mammalian *ras* proteins by testing the ability of RAS^{SC}-1 to induce cellular transformation in the NIH 3T3 cell assay. High-level expression of the full RAS^{SC}-1 gene in mammalian cells was achieved by placing it in a mammalian expression vector that contains the Ha-MuSV regulatory element [the long terminal repeat (LTR)], which has sequences for transcriptional enhancement, promotion, and initiation (see legend to Fig. 1). However, no focal transformation was obtained with the full RAS^{SC}-1 gene in this vector (clone RAS^{SC}-1/p14 in Table 2), although this vector enables the normal rat c-ras^H gene to induce focal transformation of NIH 3T3 cells (*20*). Given this negative result, we tested the capacity of portions of the yeast protein to substitute functionally for the homologous region of the mammalian protein by constructing chimeric molecules between the RAS^{SC}-1 and v-ras^H genes and placing these chimeric genes in the LTR vector.

Hybrid A/p14 contained the portion of yeast RAS^{SC}-1 coding for amino acids 1 to 54 (this corresponds to amino acids 1 to 47 in the mammalian *ras* genes because of the seven additional amino acids at the extreme NH_2-terminus encoded by the yeast RAS genes) and the portion of v-ras^H coding for amino acids 48 to 189 (which includes the highly transforming Thr^{59}). This recombinant efficiently transformed the NIH 3T3 cells, indicating a functional equivalence between the 47 amino acids at the NH_2-terminus of the mammalian gene product and the first 54 amino acids of the yeast gene product. The opposite hybrid (HYB B/p14), which contained amino acids 1 to 47 of the v-ras^H product (including the highly transforming Arg^{12} mutation) and amino acids 55 to 309 of the RAS^{SC}-1 product, did not transform the NIH 3T3 cells; this suggests that some yeast sequences downstream from the RAS^{SC}-1 codon for amino acid 54 did not function efficiently.

Recombinant HYB C/p14, contained

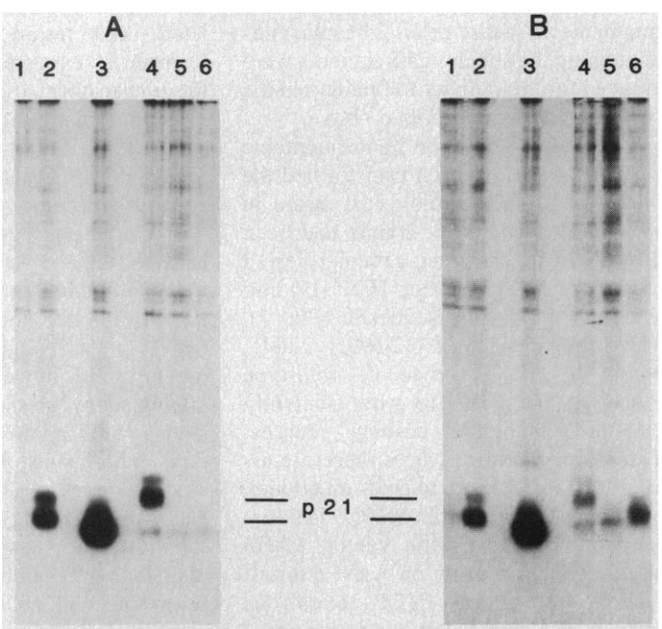

Fig. 3. Immunoprecipitation of yeast-mammalian hybrid proteins. NIH 3T3 cells morphologically transformed with the hybrids were metabolically labeled overnight with ^{35}S-methionine (250 μCi/ml) in methionine-free medium. Extracts of whole cells were prepared and precipitated with a p21 monoclonal antibody (*22*) which precipitates v-ras^H but not yeast RAS^{SC}-1 (Y13-238) or one which precipitates both v-ras^H and yeast RAS^{SC}-1 (Y13-259) (*11*). Immunoprecipitates were subjected to electrophoresis in 15 percent sodium dodecyl sulfate–polyacrylamide gels and autoradiographed. (A) Antibody Y13-238; (B) antibody Y13-259. (Lane 1) NIH 3T3 control cells; (lane 2) transformed by v-ras^H; (lane 3) transformed by the normal rat c-ras^H gene; (lane 4) transformed by HYB A/p14; (lane 5) transformed by HYB C^{Leu}/p14; and (lane 6) HYB C^{Thr}/p14. In those transformants that produce p21 doublets (lanes 2, 4, and 6), the slower migrating form is phosphorylated (due to the presence of Thr^{59}).

the portion of the yeast gene coding for amino acids 1 to 161 (corresponding to amino acids 1 to 153 in the mammalian *ras* protein) and the portion of v-*ras*^{H} coding for amino acids 154 to 189. Since an activating mutation might be required to render HYB C/p14 transforming, two potentially activating mutations were also introduced into the yeast portion of this clone, creating two variants (see legend to Fig. 1). One clone (HYB C^{Thr}/p14) coded for Thr^{66} (corresponding to Thr^{59} for the mammalian gene), and the other (HYB C^{Leu}/p14) coded for Leu^{68} (corresponding to Leu^{61} for the mammalian gene). While the "normal" clone (HYB C/p14) and the full-length yeast gene coding for Leu^{68} (RAS^{SC}-1^{Leu}/p14) both failed to induce foci, clones HYB C^{Thr}/p14 and HYB C^{Leu}/p14 each induced focal transformation of the NIH 3T3 cells. The transforming efficiency of HYB C^{Thr}/p14 was significantly less than that obtained with v-*ras*^{H}. Nevertheless, the results indicated that the 153 amino acids at the NH_2-terminal of the mammalian *ras* gene product can be replaced functionally by the corresponding region encoded by the yeast RAS^{SC}-1 gene. In addition, two structural mutations that convert mammalian *ras* genes to ones coding for the high-transformation phenotype have a similar effect when they are introduced in the yeast portion of the gene.

These results suggested further that sequences coding for the COOH-terminal half of the yeast RAS^{SC}-1 product had prevented the full-length gene from transforming the cells. The much larger size of this portion of the yeast gene is one obvious difference between the yeast and mammalian *ras* genes. Genetic analysis of v-*ras*^{H} has indicated that the protein encoded by this mammalian gene can transform 3T3 cells if sequences immediately upstream from the codon for the required Cys^{186} are deleted or duplicated, but insertion of enough additional nucleotides in this region to encode a v-*ras*^{H} protein whose length is comparable to that of yeast RAS^{SC}-1 prevents the protein from transforming the cells (*4, 21*). It seemed possible by analogy that yeast RAS^{SC}-1 might become transforming if the yeast gene were made similar in length to a mammalian gene by deleting sequences from this region upstream from the codon for the conserved cysteine residue. A RAS^{SC}-1 deletion mutant that differed in two respects from the normal gene was constructed; it contained the region coding for the activating Leu^{68} mutation (comparable to Leu^{61} for mammalian *ras*) and lacked the region coding for 117 amino acids located upstream from the conserved cysteine (see legend to Table 2 for its construction). This mutant (RAS^{SC}-1^{Leu} del/p14), which is composed entirely of yeast coding sequences, transformed the NIH 3T3 cells with an efficiency comparable to that of HYB C^{Thr}/p14.

Immunoprecipitation of metabolically labeled *ras* protein has been carried out for NIH 3T3 cells transformed by each of the three transforming yeast-mammalian hybrid genes (Fig. 3). After methionine labeling, specific precipitation was noted with each transformant, although very small amounts of *ras* protein were precipitated from the cells transformed by HYB C^{Leu}/p14 (Fig. 3B, lane 5). As anticipated, the migration rate of the hybrid A protein (lane 4) was significantly slower than that of the v-*ras*^{H} gene product, since it contained seven additional amino acids. On the other hand, the products of the two hybrid C genes (lanes 5 and 6), which presumably code for eight additional amino acids, displayed a migration rate that was similar to that of the v-*ras*^{H} product. We do not understand this proportionately faster mobility. These transformants do not, however, represent contaminants with a mammalian *ras* gene; the hybrid C gene products were not precipitated by a monoclonal antibody (Y13-238) (*22*) that specifically precipitates v-*ras*^{H} proteins [but not yeast RAS proteins (*6*)] (Fig. 3A), and the Thr^{59}-containing hybrid C protein was labeled metabolically with ^{32}P [Thr^{59} of v-*ras*^{H} unlike the normal Ala^{59}, is a phospho-acceptor (*3*)], whereas the Leu^{61}-containing hybrid C protein was not labeled with ^{32}P (data not shown for ^{32}P).

Significance. Our results demonstrate that individual mammalian and yeast *ras* genes can function biologically in the heterologous system. An intact mammalian *ras* gene can substitute in yeast cells for a defective yeast RAS gene in two different functional assays, although with less efficiency than the yeast RAS genes. A modified yeast RAS^{SC}-1 gene and yeast-mammalian hybrid genes can induce focal transformation of NIH 3T3 cells that is similar to that induced by mammalian *ras* genes. When point mutations that increase the transforming activity of the mammalian *ras* genes are introduced into the regions coding for the corresponding amino acids in the yeast sequences, they also increase the transforming activity of these genes. The yeast RAS genes are more than 50 percent larger than their mammalian counterparts principally because "additional" amino acids are present in the COOH-terminal portion upstream from the conserved cysteine residue. These extra sequences, probably only because they encode a protein that is so much larger in this region than that encoded by the mammalian gene, somehow prevent the yeast gene from transforming mammalian cells, but deletion of these "extra" sequences causes the yeast gene to be transforming.

We have shown for the first time that homologous yeast and mammalian gene products each have biological activity in the other's system. It has been reported that a gene cloned from *Drosophila* (ADE8) was able to compensate for the lack of that gene in yeast (*23*). However, the converse experiment was not performed. In addition, the thymidine kinase gene of herpes simplex virus has also been shown to complement the *CDC8* gene mutation in *Saccharomyces* (*23*).

The ability of v-*ras*^{H} to function in yeast also allows the biological effects of mutations in mammalian *ras* genes to be tested in these cells. There are significant differences in the capacity of the different hybrids and the various forms

Table 2. Transformation induced in NIH 3T3 cells by yeast-mammalian hybrid genes. Transformation is measured as focus forming units (ffu) per microgram of DNA. The transfection assay was performed as described (*11, 13*). Foci began to appear 5 to 6 days after addition of the DNA to the cells, except for HYB C^{Thr}/p14, which did not induce morphologic transformation until 9 to 10 days after addition of the DNA. The foci were counted 2 weeks after addition of the DNA. No foci were noted with HYB B/p14, HYB C/p14, or RAS^{SC}-1. In contrast to normal NIH 3T3 cells, representative transformants from each positive clone were able to form multicellular colonies in agar; colonies formed by HYB C^{Thr}/p14 were smaller than those formed by the other transformants.

Clone	Transformation (ffu/μg)
v-*ras*^{H} (clone H-1)	6.0×10^3
HYB A/p14	7.6×10^2
HYB B/p14	0.3×10^0
HYB C^{Leu}/p14	1.5×10^3
HYB C^{Thr}/p14	1.0×10^2
HYB C/p14	0.3×10^0
RAS^{SC}-1/p14	0.3×10^0
RAS^{SC}-1^{Leu}/p14*	0.3×10^0
RAS^{SC}-1^{Leu}del/p14†	2.1×10^2

*The coding sequences are the full-length yeast gene with the codon for Leu^{68}. †The coding sequences are the yeast gene with the Leu^{68} codon and from which sequences encoding amino acids 186 to 302 have been deleted. This mutant was constructed by preparing a Bam HI–Hinc II RAS^{SC}-1 amino terminus fragment that was intact through a Hinc II site located at amino acid 183 and ligating this fragment to synthetic complementary oligonucleotides that regenerated nine downstream amino acids (184, 185, and 303 to 309), a stop codon (TGA), and a Bam HI end. The resulting Bam HI fragment was ligated into the p14 vector.

of the mammalian *ras* genes to compensate for the gluconeogenic defect of yeast cells lacking RAS^{SC}-2 or the lethality associated with disruption of both yeast RAS^{SC}-1 and yeast RAS^{SC}-2.

We conclude that the capacity of the yeast RAS and mammalian *ras* genes to be biologically active in the heterologous system indicates that their associated functions may also be conserved. The ease with which second-site and pseudorevertants can be obtained in yeast and the facility with which the genes responsible for the reversion can be cloned has opened the way for determining the events in the normal function of the RAS genes. Our results suggest that such data obtained in yeast may have direct relevance to the pathway by which *ras* functions in mammalian cells.

Note added in proof: Kataoka *et al.* have found independently that yeast cells lacking functional RAS^{SC}-1 and RAS^{SC}-2 can remain viable if they carry a mammalian *ras* gene (*24*).

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A Region of the *Herpesvirus saimiri* Genome Required for Oncogenicity

Abstract. Herpesvirus saimiri *naturally infects squirrel monkeys* (Saimiri sciureus) *without producing signs of disease; infection of other New World primates, however, results in a rapidly progressing, malignant, T-cell lymphoma. Results described in this report identify a region of the viral genome that is required for oncogenicity in owl monkeys* (Aotus trivirgatus); *this region is not required for replication of the virus. This is believed to be the first such genomic region identified in a herpesvirus system.*

Unlike other model tumor virus systems, little is known at the molecular level about any herpesvirus-induced oncogenic transformation. The existence of a *Herpesvirus saimiri* variant (11att) that has lost its oncogenic potential provides an opportunity to investigate the basis for the oncogenicity of the parental, oncogenic virus (strain 11) (*1–6*). Previous results demonstrated a deletion of 2.3 kilobase pairs (kbp) from the strain 11 virus genome in the generation of 11att (*2*). Since the 11att variant arose in cell culture, it seemed possible that point mutations or other undetected changes might be responsible for or contribute to the loss of oncogenicity. We wished to determine if the deletion already identified was responsible for the loss of oncogenicity. To address this problem, we constructed replication-competent virus strains with deletions in the same region as the 11att deletion (*3*). In this report we show that the replication-competent deletion mutant S4 is also not oncogenic and that restoring the DNA sequences lost in the S4 and 11att deletion mutants also restores the oncogenicity.

The limits of virion DNA deletions in the S4 and 11att strains of *H. saimiri*, which were described previously, are shown in Fig. 1. The deletion in 11att (2.3 kbp) extends into repetitive H-DNA, leftward from the left H/L-DNA border (*2*). The S4 deletion (4.0 kbp) was constructed by eliminating DNA sequences between two sites cut by the restriction enzyme Sst I (*3*); this deletion includes only L-DNA sequences.

We constructed *H. saimiri* strains in which the sequences lost in the 11att and S4 deletion mutants were replaced. Virion DNA from non-oncogenic deletion mutants was introduced into permissive owl monkey kidney (OMK) cells together with a cloned DNA fragment from strain 11 spanning the deletion. We used the calcium precipitation procedure of Graham and van der Eb (*7*). Cloned DNA's spanning the deletions are indicated in Fig. 1. After viral-induced destruction of the cell monolayer was complete, cell lysates were screened for the presence of individual virus particles that had reacquired the appropriate DNA sequences. Recombinant viruses