

## ***RAS2* of *Saccharomyces cerevisiae* is required for gluconeogenic growth and proper response to nutrient limitation**

(yeast/oncogenes/sporulation/storage carbohydrates)

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**ABSTRACT** *Saccharomyces cerevisiae* contains two genes with remarkable homology to members of the *ras* oncogene family. These two genes, *RAS1* and *RAS2*, constitute an essential gene family since spores with disruptions of both genes fail to grow. We report here that strains containing *RAS2* disruptions have three distinct phenotypes. First, they fail to grow efficiently on nonfermentable carbon sources. Second, they hyperaccumulate the storage carbohydrates glycogen and trehalose. Third, diploid cells homozygous for the *RAS2* disruptions sporulate on rich media. Extragenic suppressors have been isolated that suppress the gluconeogenic defect. These suppressors fall into at least three complementation groups, mutations in two of which bypass the normal requirement of *RAS* for cell viability, allowing cells containing neither *RAS* gene to grow. The phenotype of the *RAS2* mutant and extragenic suppressors implicate *RAS* with some function in the normal response to nutrient limitation.

*Saccharomyces cerevisiae* contains three genes whose protein products share homology with the mammalian *ras* oncogene products. *YP2*, located on chromosome VI (1), shares about 40% homology in the NH<sub>2</sub>-terminal 160 amino acids with mammalian *ras*, whereas *RAS1* and *RAS2* (2), located on chromosome XV (3, 4) and XIV (3), respectively are >60% homologous with mammalian *ras* in the same region (5, 6). *RAS1* and *RAS2* constitute an essential gene family. Cells containing disruptions of either *RAS* gene alone are viable, but haploid spores containing disruptions of both *RAS1* and *RAS2* fail to grow (3, 4). *YP2* fails to complement the double disruption and therefore encodes a separate function.

*RAS1* and *RAS2* code for proteins of 309 and 322 amino acids, respectively, which can be immunoprecipitated by antibodies directed against mammalian p21 (7, 8). The larger size of these proteins relative to the 21-kDa mammalian p21 is the result of a 7-amino acid extension on the NH<sub>2</sub>-terminal end of both yeast proteins and a 120- to 130-amino acid insertion in the COOH-terminal portion of the proteins. The COOH-terminal insertions are not homologous to each other and their location is analogous to the position at which the mammalian proteins show greatest divergence (amino acids 165-185).

Like their mammalian counterparts, the yeast *RAS* proteins bind GTP (7, 8) and, at least in the case of *RAS1*, have an intrinsic GTPase activity similar to that found in p21 (9). This activity is decreased or absent in *RAS1* proteins containing missense mutations at amino acid positions analogous to transforming mutations in p21 (9). Functional similarities also exist between p21 and yeast *RAS*. The normal mammalian Harvey *ras* gene (10) or the viral Harvey *ras* (11) can

substitute for both of the normal *RAS* genes in yeast and a modified *RAS1* gene can transform NIH-3T3 cells (11).

Given the functional and biochemical similarities between mammalian and yeast *RAS* proteins, it is likely that knowledge about *RAS* function in yeast may have direct implications for *RAS* function in mammals. We report here that yeast strains lacking *RAS2* function have a defect in gluconeogenic growth, accumulate excessive levels of storage carbohydrates, and sporulate prematurely. We have identified mutations in three additional genes that suppress the gluconeogenic defect. Two of these mutations allow strains without *RAS* to grow.

### MATERIALS AND METHODS

**Yeast Strains.** TX2-530.1.1C ( $\alpha$  *leu2 ura3 trp1 lys1 lys2 his3 ras2-530*) (4) was backcrossed to MCY638 ( $\alpha$  *his4-539 lys2-801 ura3-52*). MCY638 and MCY317 ( $\alpha$  *ade2-101 his4-539*), derived from strain S288C, were kindly provided by Marian Carlson. Meiotic segregants of EG81, the second backcross to MCY638, were used for quantitative measurements and revertant analysis. Although backcrosses were performed to show linkage between specific phenotypes and *ras2*, mendelian segregation patterns for the gluconeogenic defect and glycogen hyperaccumulation were clearly observed in original transformed diploid TX2-530. Specific strains used include EG81-40A ( $\alpha$  *ura3 his4 lys2*), EG81-40B ( $\alpha$  *ras2-530 leu2 ura3 his4 lys2 lys1*), EG81-40C ( $\alpha$  *ras2-530 leu2 ura3 his4 lys2 his3 lys1?*), EG81-40D ( $\alpha$  *ura3 his4 lys2 his3 lys1?*), EG81-22B ( $\alpha$  *ras2-530 his4-539 lys2-801 ura3*), and 112-699 [ $\alpha$  *leu2 ura3 can1-100 ade2-1 his3 ras2-699* (*His*<sup>+</sup>)].

The gluconeogenic revertants described below were derived from EG81-22B. Revertants were crossed to EG72-16C ( $\alpha$  *ras2-530 leu2 trp1 lys2*) and all subsequent crosses between the strains containing *sra* mutations were done with meiotic progeny from these crosses. EG73-14D ( $\alpha$  *ras1-545 leu2 ura3 lys1 his3*) and EG87-2B ( $\alpha$  *ras1-545 ura3 his4 lys2*) were used to test if the *sra* mutations bypass *RAS*. XCO262-485 [ $\alpha$  *ras1-485* (*Trp*<sup>+</sup>) *stel1<sup>ts</sup> leu2 his4-580 his3 trp1*] has been described elsewhere (7).

**Media and Genetic Techniques.** Unless otherwise stated, yeast cells were grown on 1% yeast extract and 2% peptone (YEP medium) containing 2% of the designated carbon source; either glucose (YEPD medium), ethanol (YEPE medium), glycerol (YEPG medium), or acetate (YEPA medium). Sporulation medium is described elsewhere (13). Genetic techniques are described by Mortimer and Hawthorne (14). Cell number determinations for the doubling time calculations of cells grown in liquid culture were made using a Klett colorimeter.

**Glycogen and Trehalose Determinations.** Qualitative glycogen determinations were routinely made by inverting a plate

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of cells over iodine vapors. Cells that accumulate glycogen stain brown, whereas those that don't remain yellow. Quantitative trehalose and glycogen determinations were made according to Lillie and Pringle (15).

**DNA Isolation and Southern Analysis.** Genomic yeast DNA was isolated according to Winston *et al.* (16). Gel electrophoresis, DNA blotting, and hybridization analysis are described elsewhere (4).

## RESULTS

**RAS2 Is Required for Efficient Gluconeogenic Growth.** *RAS1* and *RAS2* have been disrupted in diploid strains by inserting genes for selectable auxotrophic markers, *URA3* and *LEU2*, respectively, into the coding sequences of the cloned genes and replacing the normal genes with the disrupted copies (3, 4). No phenotypic change was observed in haploid meiotic products that contained disruptions of either *RAS1* or *RAS2* alone, but we have now observed that yeast cells containing disruptions of *RAS2* (allele *ras2-530*) have an impaired ability to grow on nonfermentable carbon sources such as ethanol, glycerol, acetate, and pyruvate. Five independent *ras2-530* mutants showed this growth defect. The defect segregated 2:2 through meiosis with *LEU2* (*ras2-530*) in all 52 four-spored asci tested and remained associated with *ras2* through three backcrosses to a wild-type strain (MCY638). Strain 112-699 contains a *RAS2* gene disrupted by the *HIS3* gene and had the same defect found in *ras2-530* strains (11). The congenic *RAS2* parent had no defect. No phenotypic changes have yet been associated with disruptions of *RAS1*, the other yeast *ras* homolog.

Growth on ethanol or glycerol was not completely eliminated in strains that contain *ras2-530*. Although they failed to grow on nonfermentable carbon sources at 37°C, significant growth was observed at lower temperatures. Doubling times for representative *RAS2* and *ras2-530* strains are presented in Table 1. Sister spores from tetrad EG81-40, which contained *ras2-530* (EG81-40B and -40C), had longer doubling times than the wild-type spores (EG81-40A and -40D) in YEPE medium (ethanol) at 21°C and 30°C and failed to grow at 37°C (doubling time >24 hr). No difference in growth rate between the *RAS2* and *ras2-530* strains was observed in YEPD medium (glucose) at 21°C or 30°C, although a difference was observed at 37°C (Table 1). Transfer of EG81-40B from glucose to ethanol medium resulted in a population of largely unbudded cells, which indicated cell cycle arrest in G<sub>1</sub>. EG81-40B also failed to grow on glycerol, acetate, or pyruvate but did grow on the fermentable sugars sucrose and raffinose. The growth defect was recessive since diploids heterozygous for *ras2-530* grew well on ethanol or glycerol.

***ras2-530* Causes Derepression of Sporulation and Carbohydrate Accumulation.** Yeast strains containing *ras2-530* consistently stained darker with iodine than wild-type strains, which suggested that *ras2-530* was responsible for the increased accumulation of glycogen. Like the gluconeogenic

defect, this glycogen phenotype segregated with *LEU2* through meiosis in 52 tetrads and remained associated with *ras2-530* through three backcrosses to a wild-type strain. Strain 112-699 (*ras2-699*) also stained darker than its congenic *RAS2* parent. Confirmation that the iodine stain reflected the levels of glycogen was accomplished by measuring the glycogen levels (15). EG81-40B and -40C, which contained *ras2-530*, had significantly higher levels of glycogen than the wild-type spore clones 40A and 40D (Table 1). Quantitative measurements of another storage carbohydrate, trehalose, showed the same trend.

We observed that diploids homozygous for *ras2* sporulated on rich medium. Most strains of *Saccharomyces cerevisiae* only undergo the meiotic cell cycle (referred to as sporulation) on medium deficient in reduced nitrogen and containing a nonfermentable carbon source such as acetate. A series of strains, homozygous or heterozygous for either the wild-type or mutant *RAS2* gene, were tested for sporulation on different media. Only the *ras2* homozygous diploids (EG81-40B/EG81-40C and EG81-44A/EG81-44B) sporulated on YEPD or YEPE medium (5–25%). Heterozygous and the homozygous wild-type strains (nine closely related strains) showed no sporulation on these media after 96 hr. Because these strains were not isogenic, the data were not sufficient to prove genetic linkage between the derepressed sporulation phenotype and *ras2*. However, Toda *et al.* (17) have recently observed a similar phenotype from independently constructed *ras2* mutations.

**Increased Gene Dosage of *RAS1* Can Suppress the Growth Defect of *ras2-530*.** Strain XCO262-485 contains multiple copies of the *RAS1* gene integrated at the *RAS1* locus (allele *RAS1-485*) and has increased levels of *RAS1* mRNA and protein (7). Strains containing both *ras2-530* and *RAS1-485* had no growth defect on ethanol (YEPE medium). Tetrad analysis on two strains heterozygous for both *ras2-530* and *RAS1-485* revealed that all spore clones containing *ras2-530* and *RAS1-485* (*Leu*<sup>+</sup>, *Trp*<sup>+</sup>) grew on YEPE medium (total of 35), whereas all spore clones containing *ras2-530* and *RAS1* (total of 27) failed to grow. The glycogen phenotype of *ras2-530* was also suppressed by the *RAS1-485* allele. All *Leu*<sup>+</sup>, *Trp*<sup>+</sup> spore clones stained to the same extent with iodine as the wild-type (*Leu*<sup>-</sup>) clones (data not shown).

**The Gluconeogenic Defect Reverts at a High Frequency.** Numerous growing clones arose when strains containing *ras2-530* were cultured on YEPE or YEPG medium. In order to characterize the events responsible for the reversions, individual colonies of strain EG81-22B (a *ras2-530*) were grown to stationary phase in YEPD medium and plated on YEPG medium. After 7 days at 37°C, growing colonies were observed at a frequency of one colony per 10<sup>4</sup>–10<sup>5</sup> cells plated. When the revertants were crossed to EG73-14D (*α ras2-530*), some of the resulting diploids could utilize ethanol or glycerol, whereas others could not. Thus, the mutations responsible for the reversions were either dominant or

Table 1. Doubling times and carbohydrate levels in *ras2-530* strains

Strain	Relevant genotype	Doubling time, min						Glycogen, $\mu\text{g}/\text{mg}$ of dry weight	Trehalose, $\mu\text{g}/\text{mg}$ of dry weight
		21°C		30°C		37°C			
		YEPD medium	YEPG medium	YEPD medium	YEPE medium	YEPD medium	YEPE medium		
EG81-40A	<i>RAS2</i>	240	408	90	198	117	480	9	1
EG81-40B	<i>ras2</i>	215	1269	90	600	235	>2400*	93	87
EG81-40C	<i>ras2</i>	240	1231	90	855	213	>2400	106	99
EG81-40D	<i>RAS2</i>	240	426	96	198	153	450	18	2

Glycogen and trehalose measurements were made on early stationary-phase cultures.

\*After an initial period of growth, the *ras2* strains stopped growing at 37°C. The same growth pattern was found on glycerol-, acetate-, or pyruvate-containing medium.

Table 2. Genetic analysis of *sra* mutants

Cross	Parental strains	Relevant genotype*	Ethanol utilizing/ethanol nonutilizing†					Total‡	% ethanol positive
			Four-spored asci						
			4:0	3:1	2:2	1:3	0:4		
RX14	R12b/EG72-16C	<i>sra1-1/+</i>	0	0	11	1	0	33/42	44
RX1	R1a/EG72-16C	<i>sra1-2/+</i>	0	0	4	0	0	14/18	44
RX12	R2b/EG72-16C	<i>sra2/+</i>	0	0	4	1	0	17/18	49
RX8	R6e/EG72-16C	<i>sra3/+</i>	0	0	4	0	1	12/19	39
RX26	RX14-7B/RX14-4D	<i>sra1-1/sra1-1</i>	Sporulation negative						
RX15	RX1-1B/RX1-6C	<i>sra1-2/sra1-2</i>	16	0	0	0	0	77/0	100
RX16	RX14-7B/RX1-1B	<i>sra1-1/sra1-2</i>	2	0	0	0	0	38/0	100
RX19	RX12-1B/RX12-3D	<i>sra2/sra2</i>	5	0	0	0	0	29/0	100
RX20	RX8-9B/RX8-9C	<i>sra3/sra3</i>	10	0	0	0	0	69/0	100
RX45	RX31-12B/RX12-4A	<i>sra1-1/sra2</i>	0	3	2	0	0	38/23	62
RX18	RX1-2D/RX12-1B	<i>sra1-2/sra2</i>	2	7	2	0	0	54/18	75
RX58	RX14-1C/RX8-9C	<i>sra1-1/sra3</i>	1	3	1	0	0	44/16	73
RX21	RX1-1B/RX8-9B	<i>sra1-2/sra3</i>	2	14	2	0	0	58/22	72
RX43	RX12-4A/RX8-7B	<i>sra2/sra3</i>	1	0	1	0	0	23/10	70

\*All strains contain the *ras2-530* mutation.

†Growth on ethanol was scored after 48 hr at 30°C on YEPE medium.

‡A high percentage of the spores failed to germinate (>40%) for some crosses (RX16 and RX43, for example) and therefore the data from tetrad analysis were not significant. For this reason, data from all the germinating spores were also included.

recessive. In this preliminary characterization we chose to look at only four recessive revertants.

The mutations responsible for the reversion of the gluconeogenic deficiency segregated as mendelian genes, as shown by the 2:2 segregation for ethanol growth in tetrads from strains heterozygous for the suppressors (Table 2). We have placed the mutations responsible for the suppression (designated as *sra* for "suppressor of *ras*") into complementation groups by crossing different suppressors and observing the ability of meiotic progeny to utilize ethanol or glycerol. The summary of these crosses is presented in Table 2. The suppressors in revertants R12b and R1a were in the same complementation group, whereas the suppressors in the two other revertants, R2b and R6e, fell into different complementation groups. Crosses between the suppressors in R2b, R6e, and either R12b or R1a gave rise to ≈25% YEPE<sup>-</sup> progeny (original *SRA ras2* phenotype). These data are presented in Table 2 in crosses RX45, RX18, RX58, RX21, and RX43. We designated the suppressors in R12b, R1a, R2b, and R6e as *sra1-1*, *sra1-2*, *sra2*, and *sra3*, respectively.

The revertants had other phenotypes in addition to the reversion of the gluconeogenic defect. Some of these are documented in Fig. 1, in which the parent and congeneric revertants were spotted onto YEPD medium and then replica-plated onto different media. As determined by iodine staining, R12b and R1a accumulated less glycogen than the parent. R6e, however, accumulated as high or higher levels than the parent. Although all revertants grew better than the parent on YEPE or YEPG at 30°C, R12b failed to grow on YEPE medium at 37°C. This growth defect was not observed on YEPG (Fig. 1) or YEP medium (data not shown). Another phenotype specific to R12b and R1a was decreased viability when grown on medium deficient in (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>. This was observed when the strains were replica-plated onto (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>-deficient medium, incubated for 48 hr and then plated back onto rich medium (YEPD medium). As seen in the last panel of Fig. 1, R12b and R1a, both of which contain alleles of *sra1*, showed a decreased plating efficiency compared to the other strains. R12b and all its meiotic progeny containing *sra1-1* accumulated a brown pigment when incubated for extended periods of time. Spectroscopic examination of the strain (data not shown) revealed an absorption band at 580–590 nm, indicative of a porphyrin compound (18).

The pleiotropic phenotypes observed for strains that contain *sra1* were for the most part independent of *ras2-530*. We obtained *sra1-1* strains that were wild type for *RAS2* and observed the same phenotypes found in the original revertant (R12b)—namely, accumulation of the brown pigment, lack of glycogen, and loss of viability on nitrogen-deficient media. The inability for R12b to grow on ethanol at 37°C was also shared by *sra1-1* strains wild type for *RAS2*. The failure of R12b to grow on ethanol at 37°C was not the failure of *sra1-1* to suppress the gluconeogenic defect of *ras2-530* but was rather a phenotype of *sra1-1* itself.

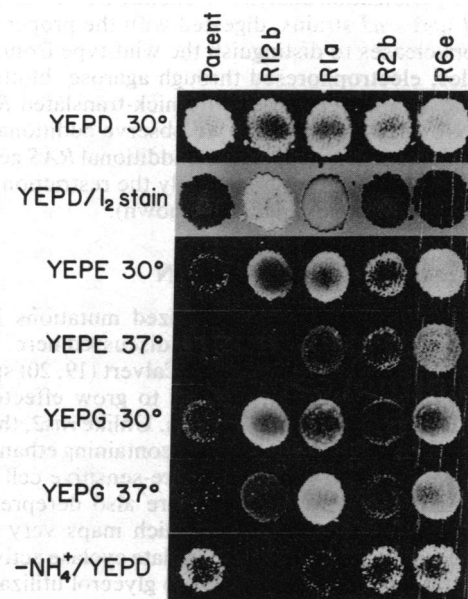


Fig. 1. Pleiotropic phenotypes of the *sra* mutations. Strain EG81-22B (parent) and four revertants of the *ras2* gluconeogenic defect were spotted onto a YEPD plate, allowed to grow for 24 hr at 30°C, replica-plated onto the media listed to the left, and photographed after 48 hr. For the second row, glycogen content was estimated by staining the cells with iodine vapor. In the last row, colonies were incubated on minimal medium lacking (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> for 48 hr, replica-plated onto YEPD medium, and photographed after 24 hr at 30°C. Most of the cells in R12b and R1a were nonviable after incubation on the (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>-deficient media, as seen by the decreased plating efficiency.

We observed that strains homozygous for *sral-1* failed to sporulate. However, *sral-2*, which appeared to be allelic to *sral-1* and shared many of the same phenotypes, sporulated when homozygous or when heteroallelic with *sral-1*. This was consistent with the generally weaker phenotype of *sral-2*. Strains containing *sral-2* accumulated some glycogen, did not turn as brown upon storage as *sral-1*, and showed slightly increased viability over *sral-1* on  $(\text{NH}_4)_2\text{SO}_4$ -deficient media (Fig. 1). We have not observed a sporulation defect in the other suppressors.

**Two Extragenic Revertants Bypass the Requirement for *ras*.** Haploid spores containing disruptions in both *ras1* and *ras2* either fail to germinate or arrest early in the cell cycle prior to bud emergence (3, 4). Since the *sra* mutations suppressed the gluconeogenic defect of *ras2*, we asked if they could bypass the requirement for *RAS* altogether. This was done by crossing strains containing *ras2-530* (marked by *LEU2*) and *sra* with strains containing the *ras1-545* disruption (marked by *URA3*). Haploid meiotic progeny containing both *ras2* and *ras1* ( $\text{Leu}^+$ ,  $\text{Ura}^+$ ) will be found among the viable spore clones if the *sra* mutation eliminates or bypasses the requirement for both *RAS* genes. This was found to be the case for both *sral-1* and *sra3*. *ras1*, *ras2* double mutants ( $\text{Leu}^+$ ,  $\text{Ura}^+$ ) were frequently found when either *sral-1* or *sra3* was present, which indicates that these mutations eliminated the requirement for the normally essential *RAS* genes. All  $\text{Ura}^+$ ,  $\text{Leu}^+$  spore clones from the *sral-1* crosses had phenotypes indicative of *sral-1* (glycogen deficiency, pigment accumulation, and decreased viability on  $(\text{NH}_4)_2\text{SO}_4$ -deficient media). They also had a cold-sensitive phenotype, growing well above  $30^\circ\text{C}$  but growing poorly at or below  $25^\circ\text{C}$ . All putative *ras1 ras2 sra3* strains grew poorly at all temperatures.  $\text{Ura}^+$ ,  $\text{Leu}^+$  spores were not observed when either *sral-2* or *sra2* was present.

We have ruled out a duplication of *RAS1* as an explanation for the apparent bypass phenotype of *sral-1* and *sra3* by Southern hybridization analysis. Genomic DNA was isolated from *sral* and *sra3* strains, digested with the proper restriction endonucleases to distinguish the wild-type from mutant *RAS* alleles, electrophoresed through agarose, blotted onto nitrocellulose, and hybridized with nick-translated *RAS1* or *RAS2* DNA (4). In no case did we observe additional bands of *ras* hybridization indicative of an additional *RAS* gene. The *ras1*, *ras2 sra* mutants contained only the restriction pattern of the disruption alleles (data not shown).

## DISCUSSION

A number of previously characterized mutations in yeast have phenotypes similar to the ones discussed here. The *spd* mutations described by Dawes and Calvert (19, 20) sporulate efficiently on rich medium and fail to grow effectively on most nonfermentable carbon sources. Unlike *ras2*, they grow well and fail to sporulate on medium containing ethanol as the sole carbon source. Two temperature-sensitive cell division cycle mutants, *cdc25* and *cdc35*, are also derepressed for sporulation (21) as is *cyr1* (22), which maps very close to *cdc35* and results in the loss of adenylate cyclase activity (23).

*spd* mutations, like *ras2*, revert to glycerol utilization at a high frequency, again via extragenic suppressors. The suppressors as well share some similarities to *sral-1* (24). They have a recessive asporogenous phenotype, decreased viability, and accumulate a brown pigment when incubated for extended periods. Unlike *sral1*, however, the *spd* suppressors are dominant for suppression of the respiratory defect. Two additional mutations have similar phenotypes to *sral-1*. *bcy1*, a mutation leading to a loss of cAMP dependence in the normal cAMP-dependent protein kinase (23), shares a number of phenotypes with *sral-1*. *bcy1* has a recessive sporulation defect (22), fails to accumulate trehalose (25), and

has been shown recently to bypass the requirement for *RAS* (17). We do not know if *bcy1* is allelic to *sral1*. *glc1*, a mutation originally characterized for its glycogen deficiency (12), like *sral1*, also has a recessive sporulation defect and accumulates a brown porphyrin pigment (26) but does not appear to be allelic to *sral1* (unpublished data).

Yeast cells normally grow unrestrained unless deprived of essential nutrients. Even in situations in which the cell cycle is inhibited, such as the response of haploid cells to mating hormones, cell growth continues (27). One of the first responses to deprivation of nutrients is the accumulation of the storage carbohydrates, glycogen and trehalose. In the case of glucose limitation, this accumulation begins before the consumption of all of the glucose from the growth medium, while the cells are still growing logarithmically (15). When the carbon source is finally depleted the cells arrest in  $G_1$  of the cell cycle, giving rise to a population of largely unbudded cells. Deprivation of reduced nitrogen, phosphate, and sulfate also elicits a similar response (15). In diploid cells, the induction of meiotic cell cycle, sporulation, is also governed by nutrient levels. With many strains, nitrogen and glucose must be eliminated before sporulation can occur. Optimal sporulation occurs in an acetate buffer. Some strains can be induced to sporulate by limiting phosphate or sulfate instead of nitrogen (28).

The increased glycogen levels and derepressed sporulation in the *ras2-530* strains might be taken as evidence that *RAS* has a function in nutritional assessment or, more specifically, that the lack of *RAS2* results in a premature starvation response. If decreased *RAS* expression leads to a premature starvation response, overexpression might decrease or eliminate the response. Strains carrying a *RAS2* missense mutation, resulting in the substitution of valine-19 for glycine-19, have a dominant phenotype consistent with an inability to respond to starvation conditions; these strains (*RAS2<sup>val19</sup>*) fail to sporulate (3), fail to accumulate glycogen and trehalose, and have decreased viability (17). We have confirmed these observations with a strain containing *RAS2<sup>ala16val19</sup>* (unpublished data). If these mutations result in a decrease in the intrinsic GTPase activity of the *RAS2* protein without affecting GTP binding, as they do in the *RAS1* gene product (9), the net result should be analogous to overexpression of *RAS*. The similarities between the *sral-1* and *RAS2<sup>val19</sup>* phenotypes, together with our observation that *sral-1* bypasses the requirement for *RAS*, suggest that the *sral* gene products may be acting downstream of *RAS* in the same pathway.

While the phenotypes described here implicate *RAS2* with a function in response to nutrient levels, the mechanism of signaling is not clear. However, cAMP metabolism appears to be involved. Strains with mutations in *CYR1*, which are deficient in adenylate cyclase activity, hypersporulate, whereas *bcy1* mutants are asporogenous (22). There is also evidence that enzymes responsible for the synthesis and degradation of glycogen and trehalose are regulated by phosphorylation (25, 29–33). cAMP-dependent protein kinase is directly implicated in the stimulation of trehalase and phosphorylase (25, 33). Taken together, these results suggest the testable hypothesis that *RAS* functions as part of the mechanism regulating protein kinases.

We do not yet understand why strains lacking *RAS2* have a defect in gluconeogenic growth. However, the defect has allowed us to isolate suppressors that bypass the normal requirement for *RAS*. Judging from the similarities in phenotypes between *sral-1* and a dominant *RAS2* mutation, it is likely that *sral-1* may function downstream in the same pathway. The recent observations that the mammalian (10) and viral Harvey *ras* (11) can substitute for both of the normal *RAS* genes in yeast and that a modified *RAS1* gene can transform NIH-3T3 cells (11) support the hypothesis that *RAS* has equivalent functions in both organisms. If this is the

case, the *sra* bypass mutants described above may have functional equivalents in mammals. Molecular cloning of these suppressors may allow the isolation of their mammalian counterparts and lead to further understanding of *RAS* function.

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