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Carbon source regulation of *RAS1* expression in *Saccharomyces cerevisiae* and the phenotypes of *ras2*$^-$ cells

(oncogenes/transcription/carbon metabolism)

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ABSTRACT **Transcriptional analysis of the yeast *RAS* genes in different culture conditions suggests that the inability of *ras2* mutants to grow in nonfermentable carbon sources results from the regulation of *RAS1* mRNA expression. The amount of *RAS1* mRNA is significantly repressed in cultures grown on the nonfermentable carbon sources ethanol and acetate. As a result, low *RAS* function should be expressed under these conditions in a *ras2* mutant. This can explain the inability of *ras2*$^-$ cells to grow on nonfermentable carbon sources. This interpretation is supported by the finding that an extragenic suppressor of *ras2*$^-$ (*sra6-15*), which restores growth on ethanol or acetate, also leads to an increase in the amount of *RAS1* mRNA under these conditions. The *sra6-15* mutation does not alter the level of *RAS1* mRNA in cells grown on glucose. The pattern of transcriptional regulation described for the *RAS1* gene is not shared by *RAS2*, indicating differential control of the functionally homologous yeast *RAS* genes at the level of gene expression.**

Two unlinked genes in *Saccharomyces cerevisiae* have been cloned (1–3) on the basis of sequence homology with the *ras* oncogene found in Harvey and Kirsten rat sarcoma viruses (4). These genes, *RAS1* and *RAS2*, constitute an essential gene family. Yeast cells lacking either one of the two are viable, but spores lacking both fail to grow (5, 6). Casperson *et al.* (7) have shown that adenylate cyclase in yeast is stimulated by GTP, as is the adenylate cyclase from a number of metazoan systems (8). In yeast, RAS protein appears to mediate this stimulation (9). Yeast cell membranes prepared from cells lacking RAS function show no GTP stimulation of adenylate cyclase. The system can be reconstituted by addition of exogenous RAS protein (10). A primary function of cyclic AMP is to stimulate a cyclic AMP-dependent protein kinase (11). Consistent with the role of RAS in stimulating adenylate cyclase is the observation that *bcy1*, a mutation resulting in low levels of the regulatory subunit of the cyclic AMP-dependent protein kinase (12), suppresses the lethality of a *ras1 ras2* double mutant (9).

A human RAS protein can complement the inviability in yeast *ras1 ras2* mutants (13, 14), and a modified *RAS1* yeast gene can transform NIH-3T3 cells (13). This, together with other biochemical similarities (15, 16), suggests that RAS proteins might carry out similar functions in both of these eukaryotic cells.

An insertion mutation in only one of the two yeast *RAS* genes is not lethal, suggesting that the two RAS proteins can functionally substitute for one another (5, 6). While it is not unconditionally lethal, a single *ras2* mutation does produce three phenotypes: (*i*) failure to grow on nonfermentable carbon sources—e.g., ethanol; (*ii*) sporulation in the absence of nutrient deprivation; and (*iii*) hyperaccumulation of the storage carbohydrates glycogen and trehalose (9, 17, 18). Whereas human and viral RAS proteins can complement the carbon source defect in a *ras2* mutant (13), it is not yet known if they can complement the other two phenotypes. No phenotype has been associated with a *ras1* single mutation.

The phenotypes of *ras2* mutants could indicate that *RAS2* carries out specialized functions for which *RAS1* cannot substitute. However, it has been reported that the growth defect observed in *ras2*$^-$ *RAS1*$^+$ cells cultured on nonfermentable carbon sources can be suppressed by overproduction of the *RAS1* gene product (17). This would suggest that this phenotype of the *ras2*$^-$ *RAS1* mutants does not result from the absence of specialized functions carried out by *RAS2*, but rather is the result of insufficient *RAS* gene expression. This possibility led us to analyze the regulation of *RAS1* transcription under the culture conditions in which *ras2* mutant phenotypes are evident. Our results indicate that the level of *RAS1* mRNA is strongly diminished during growth in nonfermentable carbon sources. In addition, even when glucose is the carbon source, *RAS1* mRNA levels drop substantially during exponential growth. This result may explain the inability of *ras2*$^-$ cells to grow on media containing nonfermentable carbon sources since, under these conditions, *RAS1* expression is repressed. Additional support for this idea comes from the finding that an extragenic suppressor of this phenotype (*sra6-15*) leads to an increase in the level of *RAS1* mRNA when *ras2*$^-$ mutants are grown in media containing either acetate or ethanol. On the other hand, the facts that *sra6-15 ras2*$^-$ homozygous diploid cells still sporulate in rich media seems to suggest the possibility of some specific, noncomplementing role for the two *RAS* genes.

MATERIALS AND METHODS

Growth Conditions. Yeast cells were grown in minimal medium [yeast nitrogen base (Difco) plus ammonium sulfate; see ref. 19] containing the nutritional requirements and glucose (dextrose) (SD), acetate (SA), or ethanol (SE) as carbon sources at a final concentration of 2%. The acetate medium was buffered to pH 5 with phthalic acid. Growth at 30°C was monitored by measuring cell density at 600 nm and by direct counting in an hemocytometer. In the nutrient deprivation experiments previously published media and culture conditions were employed (20). Briefly, S288C cells were grown to early-exponential phase (>80% budded cells) in MIN medium (20) with 2% glucose at 30°C. At this point they were shifted to nitrogen-, sulfur-, or carbon (glucose)-deficient medium and cultured for 24 hr. At 2 and 6 hours after this transfer, the essential missing nutrient was added to half of the culture. Growth and terminal phenotype of the cells

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Abbreviations: bp, base pair(s); kb, kilobase(s).

were assessed, the latter by observation of briefly sonicated cells under a phase-contrast microscope at ×400 magnification. MIN medium differs from SD medium in that the basic ingredients (vitamins, trace elements, salts) are completely defined rather than prepared commercially as an undefined mixture. MIN medium is also buffered to pH 5 with succinic acid.

Yeast Strains. The genotypes of the strains employed are given in parentheses as follows: S288C (α, *gal2*), 112 (α, *leu2-3-112, ura3-1, can1-100, ade2-1, his3*), 112-699 (α, *ras2-699, leu2-3-112, ura3-1, can1-100, ade2-1, his3*), JC326-22B (α, *leu2, ura3-52, lys2-801*), JC303-16 (α, *leu2, ras2-530, ura3-52, his4-539, sra5-3*). *ras2-699* is a *HIS3* disruption of *RAS2* at the *RAS2 Pst* I site (13). *ras2-530* is a *LEU2* disruption of *RAS2* at the *RAS2 Pst* I site (17). The ascospore clones examined in Fig. 4 were meiotic segregants of a diploid produced by a cross between strain JC302-26D (**a**, *leu2, ras2-530, ade2, lys2, his4*) and strain JC303-46 (α, *leu2, ras2-530, ura3-52, his4-539, sra6-15*).

RNA Blot-Hybridization Analysis. Total RNA was extracted at different times during the growth period according to published procedures (21). Ten micrograms of each RNA sample was denatured and electrophoresed in the presence of formaldehyde according to Maniatis *et al.* (22). After electrophoresis, RNA species were blotted to a GeneScreen*Plus* membrane according to the supplier's directions (New England Nuclear). A 625-base-pair (bp) *Acc* I–*Hin*dIII fragment that corresponds to the 3′ end of the *RAS1* gene (3) was used to probe the RNA blots for *RAS1* mRNA. The purified fragment was radiolabeled with ^{32}P by nick-translation (Amersham nick-translation kit). Conditions for prehybridization, hybridization, and washing of the RNA blots were according to the New England Nuclear protocol for high stringency requirements. No cross-reactivity with *RAS2* mRNA was observed when this probe was hybridized with RNA extracted from *ras1*$^-$ cells (unpublished observations). A 498-bp *Rsa* I restriction fragment from the 3′ end of the *RAS2* gene (3) was used to detect *RAS2* mRNA in the same way described for RAS1. No cross-reactivity with *RAS1* mRNA was detected when this *RAS2* probe was hybridized with RNA extracted from *ras2*$^-$ cells (unpublished observations). For the control *Ty* element RNA hybridizations, plasmid S13 (23) was nick-translated and used as probe. Quantitation of the relative levels of hybridization was performed by scanning the autoradiographs with an LKB laser scanning densitometer. Hybridization to *Ty* RNA has been used to control for possible fluctuations in the amount of mRNA relative to total RNA. This choice relies on the fact that *Ty* mRNA is abundant in haploid cells (24) and is easily separated from the *RAS* mRNAs.

RESULTS

Carbon Source and Growth Modulation of the Amount of RAS1 mRNA. We first analyzed the level of *RAS1* mRNA at various times during growth of *RAS*$^+$ yeast cultures in minimal medium containing different carbon sources: glucose (SD), acetate (SA), or ethanol (SE) (Fig. 1*A*). In all three carbon sources *RAS1* mRNA expression is highest during the lag preceding logarithmic growth and drops dramatically as the culture proceeds through the exponential phase of growth. This is illustrated for growth in glucose-containing medium in Fig. 2*A*. In cells grown on glucose, the level of *RAS1* mRNA is uniformly higher during the preexponential phase than in acetate- or ethanol-grown cells (compare in Fig. 2*B* the 1- and 2-hr points in SD versus the 2- and 11-hr points in SA and SE). Moreover, *RAS1* mRNA is still detectable in glucose-grown cells in early exponential growth but it drops to almost undetectable levels (6–8% relative to the corresponding SD sample) in cells growing exponentially on

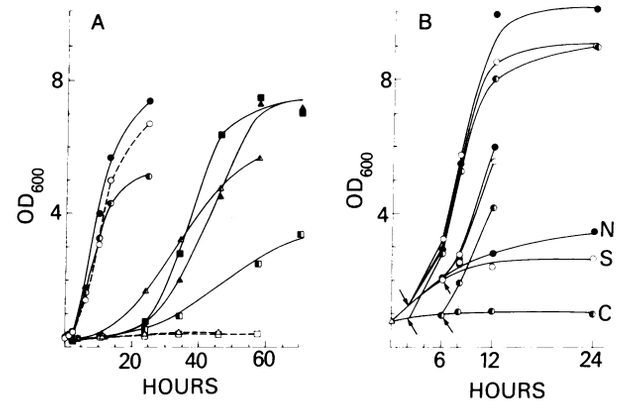

FIG. 1. Growth curves. (*A*) Growth in minimal media containing different carbon sources. Cells were inoculated into fresh media at an OD_{600} of 0.3 from an SD stationary-phase culture. Solid lines refer to wild-type cells and broken lines refer to the *ras2* mutant 112-699. Circles refer to media containing glucose as carbon source, triangles to ethanol, and squares to acetate. Solid symbols indicate strain S288C (*RAS2*$^+$), half-filled symbols strain 112 (*RAS2*$^+$), and open symbols strain 112-699 (*ras2*$^-$). (*B*) Nutritional deprivation. S288C were grown to early exponential phase (80% budded cells) in MIN medium and then transferred to the same medium lacking nitrogen (N, filled circles), sulfur (S, open circles), or glucose (C, half-filled circles). Arrows indicate the times (2 or 6 hr) at which the specific starvation regime was terminated by readdition of the appropriate requirements.

acetate or ethanol (compare in Fig. 2*B* the 6-hr SD point with the 33-hr point from the SA and SE cultures).

The reduction in the level of *RAS1* mRNA during exponential growth appears to be specific for the *RAS1* gene because the level of the RNA homologous to the yeast transposable element *Ty* remains constant throughout the growth period (Fig. 2*A*). In addition, unlike *RAS1* mRNA, the *RAS2* transcript is maintained at easily detectable levels throughout exponential growth in all three carbon sources (Fig. 2*B*). This distinction is most striking in SA and SE media. By the time cells have entered the exponential phase of growth in these media (33 hr), *RAS1* mRNA has dropped to a barely detectable level. By contrast, *RAS2* mRNA remains at a high level throughout exponential phase and is still evident when cells enter stationary phase. These results demonstrate differential transcriptional regulation of the two *RAS* genes.

Similar results were obtained when *RAS*$^+$ cells were grown to the beginning of the exponential phase in medium containing one carbon source and then shifted to medium containing another. One hour after a shift from SD to SE, we observed a decrease to 1/10th in the amount of *RAS1* mRNA (unpublished observations). Conversely, a 10-fold increase in the level of *RAS1* mRNA was observed 1 hr after shifting exponentially growing cells from SE to SD medium (unpublished observations). These results further confirm that the amount of *RAS1* mRNA is modulated by the carbon source. As above, the amount of *RAS2* mRNA in the same experiments was found to remain relatively unaffected by the changes in the carbon source (unpublished observations).

Interestingly, we failed to detect any induction of *RAS1* mRNA by adding glucose to cells approaching stationary phase, at which time the levels of *RAS1* mRNA are very low on any carbon source (unpublished observations). The inability to induce *RAS1* expression by the addition of glucose in this latter experiment indicates that the lack of *RAS1* mRNA late in exponential growth is not simply due to depletion of glucose in the medium. Rather, it suggests that two mechanisms may be operating to regulate *RAS1* expression, one by the carbon source and another that is dependent

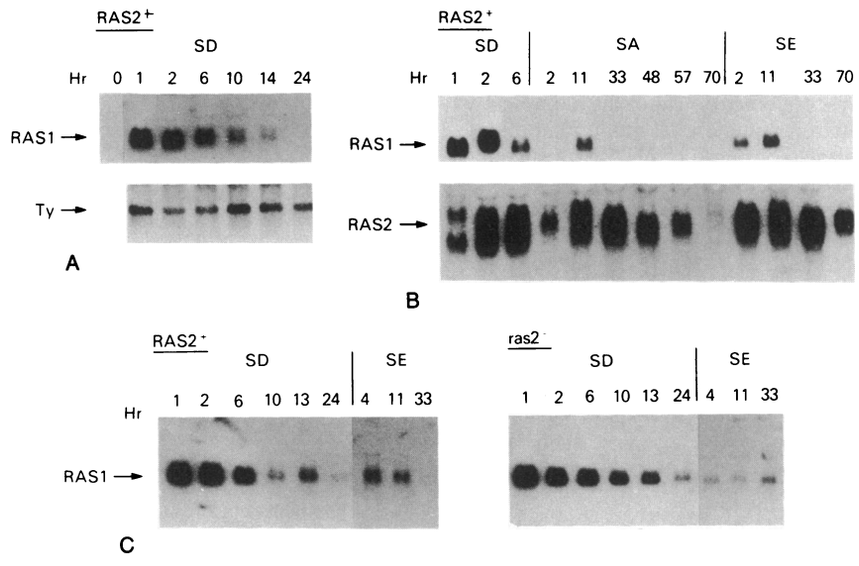

FIG. 2. RNA blot-hybridization analysis of *RAS1* and *RAS2* mRNA in cells grown on different carbon sources. (*A*) Analysis of *RAS1* and *Ty* mRNA in glucose-grown *RAS2*$^+$ cells. Total RNA was extracted at various times during the growth of strain S288C in SD. The time points shown correspond to the SD growth curve given in Fig. 1*A*. The sizes determined for both *RAS1* mRNA [approximately 1.2 kilobases (kb)] and *Ty* mRNA (5.7 kb) are consistent with previous reports (24, 25). (*B*) Analysis of *RAS1* and *RAS2* mRNA in *RAS2*$^+$ cells grown in three different carbon sources. Total RNA was extracted at various times during growth of strain S288C in SD, SA, and SE. These time points are from the growth curves given in Fig. 1*A*. The size heterogeneity observed for *RAS2* mRNA is the result of transcriptional regulation occurring at the 5′ end of the *RAS2* gene (unpublished data). (*C*) Analysis of *RAS1* mRNA in *RAS2*$^+$ and *ras2*$^-$ cells. Total RNA was extracted from *ras2*$^-$ mutant 112-699 and its isogenic wild-type strain 112 during growth in SD or SE. Time points are from the growth curves given in Fig. 1*A*.

upon the growth phase of a batch culture. Experiments with the *sra6-15* mutation further support this hypothesis (see below).

We next examined the response of *RAS1* mRNA synthesis to a complete removal of the carbon source from the medium. Wild-type cells were grown to early-exponential phase in a minimal medium (MIN) similar to SD (see *Materials and Methods*; ref. 20) and were transferred to a carbon starvation medium and incubated for 24 hr. In addition, cells from the same MIN preculture were held in the carbon starvation medium for only 2 or 6 hr and then glucose was added back to the cultures. As expected, the abrupt withdrawal of glucose led to a complete block in cell growth and division (26) and its readdition rapidly returned these cultures to active growth (Fig. 1*B*). The analysis of *RAS1* mRNA showed that its expression was dramatically reduced by carbon starvation (C in Fig. 3). Four hours after the removal of glucose from the medium the amount of *RAS1* transcript was less than 10% relative to zero time (similar results were also observed after 2 hr of carbon deprivation). This response could be reversed, and a high level of *RAS1* mRNA was restored by readdition of glucose to the medium. This behavior was not observed for the *Ty* transcript examined as a control in this experiment. Moreover, this high degree of repression of *RAS1* mRNA was not observed when cells were starved in the same fashion for nitrogen (Fig. 1*B* and 3*N*) or sulfur (Fig. 1*B* and 3*S*). Therefore, a severe reduction in the amount of *RAS1* mRNA is not a general response to starvation for an essential nutrient, but rather another manifestation of the positive effect of glucose on *RAS1* mRNA expression. If so, these results suggest that the reduced level of *RAS1* mRNA detected in media containing acetate or ethanol is not simply a consequence of the lower growth rate found in these nonfermentable carbon sources.

***RAS1* mRNA Expression Is Relatively Unaffected by a *ras2* Mutation.** Either *RAS1* or *RAS2* function is required for growth on glucose. If *RAS1* expression is reduced in the presence of nonfermentable carbon sources, then the growth defect observed in *ras2*$^-$ mutants in such conditions could be the result of an insufficient amount of any RAS product. However, the validity of this explanation requires that a *ras2* mutation does not lead to an increase in *RAS1* transcription when cells are cultured on nonfermentable carbon sources. To assess this possibility, we employed a strain containing a *ras2*$^-$ insertion mutation.

When inoculated from a stationary-phase SD culture, *ras2*$^-$ cells grow in SD but remain unbudded when introduced into SE or SA medium (Fig. 1*A*). The expression of *RAS1* mRNA observed in these conditions for the *ras2*$^-$ mutant is compared with the corresponding data from an isogenic

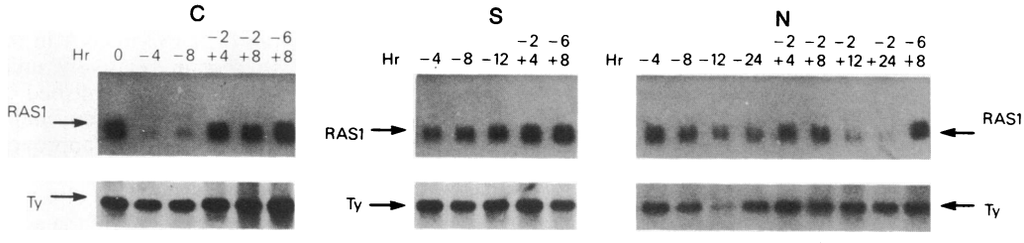

FIG. 3. Effect of nutrient deprivation on the level of *RAS1* mRNA. Total RNA was extracted in cells starved for glucose (*C*), sulfur (*S*), or nitrogen (*N*) and examined by blot-hybridization analysis for the levels of *RAS1* and *Ty* mRNA. Time points refer to Fig. 1*B*. Single numeration of the time points refers to total time of starvation for that particular nutrient, which corresponds to the time when the RNA was extracted. Double numeration indicates both the length of starvation for the particular nutrient (upper number with a minus) and the total elapsed time when the RNA was extracted (lower number with a plus).

wild-type strain in Fig. 2*C*. The overall pattern of expression for the two strains is very similar: the amount of *RAS1* mRNA is much lower in SE compared to SD medium in both *RAS2*$^+$ and *ras2*$^-$ cells. A reduction to approximately 1/10th is observed when corresponding points in the growth curves for the two media are compared (compare for both strains the 4-, 11-, and 33-hr points in SE with the 1-, 2-, and 6-hr points in SD). Similar reductions in the level of *RAS1* mRNA were also observed in both *RAS2*$^+$ and *RAS2*$^-$ cells when shifted from exponential growth in SD to SE medium (unpublished observations). Thus, we conclude that a *ras2* mutation does not lead to significant overexpression of *RAS1* mRNA. As a result, the growth defect exhibited by *ras2*$^-$ cells when cultured in nonfermentable carbon sources could indeed be the consequence of insufficient *RAS* gene product.

Although a *ras2*$^-$ mutation appears to have little effect on the level of *RAS1* mRNA, two additional observations regarding these experiments should be noted. First, the level of *RAS1* mRNA at 33 hr in SE medium is higher in the *ras2*$^-$ mutant than in wild-type cells. At 33 hr in SE, whereas *ras2*$^-$ cells are not dividing, wild-type cells are growing exponentially. Second, prolonged expression of *RAS1* mRNA can also be observed in the *ras2*$^-$ mutant compared to wild type during late-exponential growth in SD medium (Fig. 2*C*, 10- and 13-hr points). At present, the significance of these differences is not understood.

A Suppressor of *ras2*$^-$ Inviability on Nonfermentable Carbon Sources Increases the Amount of *RAS1* mRNA. If the inability of *ras2* mutants to grow on nonfermentable carbon sources is the result of insufficient *RAS1* expression in these conditions, it should be possible to revert this phenotype by elevating the level of *RAS1* mRNA. Among a set of extragenic suppressors of the *ras2* insertion mutation that restore growth on nonfermentable carbon sources to *RAS1*$^+$ *ras2*$^-$ strains (27) we found one, *sra6*, that appears to employ this mechanism of suppression. This is demonstrated by the data in Fig. 4*A*, which shows the amounts of *RAS1* mRNA present in the four ascospores of a tetrad obtained from a cross between a *ras2*$^-$ *sra6-15* suppressor strain and a closely related *ras2*$^-$ *SRA6*$^+$ strain. When grown in SD (Fig. 4*A*), all four spores exhibit the pattern of *RAS1* mRNA expression expected for *ras2*$^-$ cells shown above in Fig. 2*C*. This indicates that the *sra6-15* mutation has no effect on *RAS1* expression when glucose is the carbon source. In SA or SE medium, the two *ras2*$^-$ *SRA6*$^+$ segregants exhibit the expected low level of *RAS1* mRNA relative to the amount observed in the same strains during early exponential growth in SD medium. [Note, however, that, as mentioned earlier (Fig. 2*C*), *RAS1* transcript remains detectable in *ras2*$^-$ *SRA6*$^+$ segregants when cultured in SE medium.] By contrast, the *sra6-15* mutation leads to a significant increase in the level of *RAS1* mRNA in both SE and SA medium: in SA, a 5-fold increase can be observed even at very early times in the growth curve (Fig. 4*A*, 5-hr point). In SE, the increase in the amount of *RAS1* mRNA observed in the *sra6-15* spore clones is less dramatic (2- to 3-fold) early in the growth phase (Fig. 4*A*, SE); however, at later times, when cells are actively growing, a similar increase of 4- to 5-fold in the *sra6-15* versus the *SRA6*$^+$ segregants is observed (Fig. 4*B*). Fig. 4*B* also shows that the *sra6-15* mutation not only increases the level of *RAS1* mRNA but also sustains these higher levels throughout the growth period. A likely consequence of such sustained expression is that the proper amount of *RAS* gene product can in this way be provided to *ras2*$^-$ cells throughout their active phase of growth. (Note that *RAS1* expression is normally heavily reduced in wild-type *SRA6*$^+$ *RAS*$^+$ cells during exponential growth on ethanol as shown in Fig. 2 *B* and *C*, 33-hr point.)

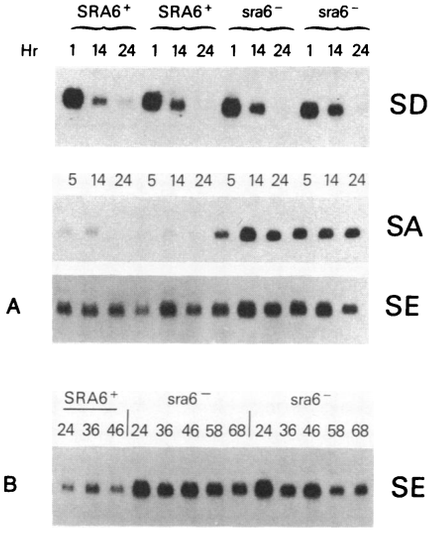

FIG. 4. RNA blot-hybridization analysis of the effect of the *sra6-15* suppressor mutation on the level of *RAS1* mRNA. (*A*) Analysis of *RAS1* mRNA for the four meiotic products of diploids produced by a cross between *SRA6*$^+$ strain JC302-26D and *sra6-15* strain JC303-46. Strains were cultured in SD, SA, or SE medium and RNA was extracted at the indicated times. (*B*) Analysis of *RAS1* mRNA at later time points for one of the *SRA6*$^+$ *ras2*$^-$ segregants and the two *sra6-15 ras2*$^-$ segregants from cross JC302-26D × JC303-46. The result shown here is typical of *SRA6*$^+$ segregants (data not shown). The beginning of stationary phase occurs at 60 hr for the *sra6-15* segregants. The growth curves pertaining to these experiments are not presented but are not significantly different from those shown in Fig. 1*A*.

These results suggest that the *sra6-15* mutation restores the ability of *RAS1*$^+$ *ras2*$^-$ strains to grow on nonfermentable carbon sources by increasing the levels of *RAS1* mRNA under these conditions. This explanation is consistent with the fact that *sra6-15* is unable to suppress the lethality associated with a *ras1 ras2* double mutation, as suppression by *sra6-15* requires a functional *RAS1* gene. A second extragenic suppressor of the *ras2*$^-$ growth defect, *sra5-3*, also requires a functional *RAS* gene (*RAS1* or *RAS2*) but does not lead to an increase in the level of *RAS1* mRNA (unpublished observations). This indicates that an increase in *RAS1* transcription is not the only mechanism to overcome the inability of *ras2*$^-$ cells to grow on nonfermentable carbon sources.

DISCUSSION

The results presented in this study show that the expression of *RAS1* mRNA is regulated by the carbon source and growth phase. The amount of *RAS1* mRNA is uniformly lower throughout the growth curve in cells grown on the nonfermentable carbon sources ethanol or acetate in comparison to growth on glucose. In addition, complete removal of the carbon source leads to a nearly complete disappearance of *RAS1* mRNA. This modulation is unlikely to be the simple result of lower growth rates in nonfermentable carbon sources, since *RAS1* mRNA levels are completely unaffected by cell-cycle arrest brought about by nitrogen or sulfur starvation. Carbon source regulation of *RAS1* mRNA level is a feature that distinguishes *RAS1* from *RAS2*. *RAS2* mRNA is expressed with roughly equal efficiency in any carbon source we have tested. Moreover, its level diminishes only at the very end of exponential growth, similar to other yeast transcripts we have assayed (unpublished observations).

The most interesting implication of our data is that they offer an explanation for the inability of *ras2* mutants to grow in media containing nonfermentable carbon sources. We suggest that this phenotype is the result of the low level of *RAS1* mRNA present in these culture conditions. In a *ras2*

mutant this leads to a situation in which neither RAS1 nor RAS2 protein can be efficiently expressed. The consequence of a complete lack of *RAS* function, even in glucose-containing media, has been shown to be cell-cycle arrest (14). Further support for this idea is provided by the fact that an extragenic suppressor of the inability of *ras2*$^-$ cells to grow on nonfermentable carbon sources, *sra6*, leads to a significant increase in the level of *RAS1* mRNA in cells grown in such conditions. In glucose-containing medium, in which *RAS1* is normally expressed at high levels, *sra6-15* has no effect on the amount of *RAS1* mRNA. We note that cells lacking *RAS2* function do accumulate some *RAS1* mRNA when shifted to media containing ethanol (Figs. 2*C* and 4*A*). We suggest that this relatively low level of expression is insufficient to support growth of *ras2*$^-$ cells. However, it is also possible that the low level of *RAS1* mRNA expression is only one factor in the growth defect of *ras2*$^-$ cells.

Since the *sra6-15* mutation is recessive, these findings suggest that *SRA6* encodes a negative effector of *RAS1* expression that operates strictly on nonfermentable carbon sources. The fact that the *sra6-15* mutation is not able to increase the level of *RAS1* mRNA in SD medium when cells approach stationary phase also indicates that there are at least two different mechanisms by which *RAS1* transcriptional regulation can occur. One is dependent upon the nature of the carbon source and the second seems to be associated with the growth phase, cell density, or both. It is interesting that in SA and SE, the *SRA6* product appears to mediate both aspects of *RAS1* regulation, whereas in SD medium, the growth phase regulation is independent of *SRA6* function.

A second phenotype of *RAS1*$^+$ *ras2*$^-$ mutants is the occurrence of sporulation on rich media, or hypersporulation (9, 17). It is of interest to consider whether this phenotype may also be the consequence of the negative regulation of *RAS1* expression, especially since *ras2*$^-$/*ras2*$^-$ diploids sporulate much more efficiently on rich media containing nonfermentable carbon sources compared to glucose (9, 17). An observation at odds with this idea is that the *sra6-15* mutation, which restores a high level of *RAS1* mRNA expression in media containing either acetate or ethanol as a carbon source, does not suppress the ability of *ras2*$^-$/*ras2*$^-$ diploids to sporulate on rich medium. In fact, we find that *ras2*$^-$ *sra6-15* homozygous diploids sporulate on YEP/acetate and YEP/ethanol media (17) with the same efficiency as congenic *ras2*$^-$/*ras2*$^-$ *SRA6*$^+$/*SRA6*$^+$ cells (50–60% of asci after 38 hr of incubation at 30°C). Since we have shown that the *sra6-15* mutation increases *RAS1* mRNA expression even in a saturated SE culture (Fig. 4*B*), this observation suggests that the hypersporulation phenotype of *ras2*$^-$ diploid cells can occur irrespective of the presence of *RAS1* gene product. This would imply a specific role for the RAS2 protein in modulating the proper response to conditions of starvation that is not replaceable by RAS1. Two observations are consistent with this hypothesis. First, we find that *RAS1-485*, an allele of *RAS1* that contains about 40 copies of the *RAS1* gene integrated at the *RAS1* locus and expresses large amounts of both *RAS1* mRNA and protein (25), suppresses the growth defect of strains lacking *RAS2* function but does not eliminate the hypersporulation phenotype exhibited by the same *ras2* mutants (unpublished observations). Second, a dominant missense mutation in *RAS2*, resulting in the substitution of valine for glycine at amino acid position 19, leads to low levels of sporulation (5). *RAS1* alleles containing the same mutation have no sporulation defect and fail to express any of the dominant phenotypes associated with *RAS2*$^{Val\text{-}19}$ (unpublished observations).

In summary, we report that in certain conditions, namely the presence of glucose in the medium and early in the growth phase, yeast cells express a large amount of *RAS1* mRNA. We also report the occurrence of differential transcriptional regulation of the two homologous *RAS* genes during growth on medium containing either ethanol or acetate. Such regulation may provide an explanation for the inability of *ras2*$^-$ cells to grow on nonfermentable carbon sources. Finally, we suggest the possibility that the *RAS2* gene product carries out a function in sporulation for which *RAS1* cannot substitute.

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