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Sporulation and *rna2* Lower Ribosomal Protein mRNA Levels by Different Mechanisms in *Saccharomyces cerevisiae*

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In *Saccharomyces cerevisiae*, the levels of ribosomal protein mRNAs are regulated coordinately. Vegetative strains carrying the temperature-sensitive *rna2* mutation exhibit a dramatic decrease in the levels of most ribosomal protein mRNAs at the restrictive temperature. Similarly, in wild-type cells induced to sporulate by nitrogen starvation, there is a fivefold reduction in the relative synthesis rate of ribosomal proteins. Using Northern gel analysis and cloned ribosomal protein genes, we compared the way in which ribosomal protein mRNA is affected under these two conditions. In vegetative *rna2* cells, incubation at 34°C led to the disappearance of ribosomal protein mRNAs and the accumulation of higher-molecular-weight precursor RNAs. A different phenotype was observed during sporulation. Although sporulating conditions led to a significant reduction in the relative abundance of ribosomal protein mRNA, there was no detectable accumulation of precursor RNAs even in *rna2/rna2* diploids at 34°C. A suppressor of *rna2* and of other *rna* mutations, *SRN1*, at least partially relieved the block in the splicing of the ribosomal protein 51 intron in vegetative *rna2* cells but did not detectably affect the level of ribosomal protein mRNA in sporulating cells. We concluded that the *rna2* mutation and sporulation conditions affected ribosomal protein mRNA metabolism in two quite different ways. In vegetative cells the mutant *rna2* effected a block which occurred primarily in post-transcriptional processing, whereas in sporulating cells the ribosomal protein mRNA levels were decreased by some other mechanism, presumably a change in the relative rate of transcription or mRNA turnover. Furthermore, the data suggest that the mutation *rna2* has no additional effect on ribosomal protein mRNA metabolism in sporulating cells.

Diploid cells of the yeast *Saccharomyces cerevisiae* can be induced to sporulate by nitrogen deprivation. Although RNA and protein synthesis continue under these conditions (8), a number of changes in macromolecular metabolism occur (10). Among these changes is a 5- to 10-fold decrease in the relative rate of synthesis of at least 40 ribosomal proteins (rp's) (16). The nitrogen starvation conditions used to induce sporulation cause the decrease in rp synthesis even in strains that are genetically unable to complete ascus formation (16). Therefore, this decreased synthesis may be analogous to the decrease in rp synthesis induced by deprivation of certain amino acids (20).

The synthesis of rp's is also decreased in a number of strains which carry one of ten *rna* mutations, *rna2* through *rna11*. These strains are temperature sensitive for both growth and rp synthesis; a shift to the nonpermissive temperature results in a rapid and dramatic decrease in the levels of most rp mRNAs (7, 19).

Although both nitrogen starvation and incubation of *rna* mutant strains at the restrictive temperature cause a decrease in the relative synthesis of most rp's, there is evidence that the mechanisms of regulation may be quite different (16). For example, the synthesis of one rp (rp39) is not coordinately depressed along with other rp's when *rna2* strains are exposed to 34°C; however, rp39 synthesis is decreased along with other rp's during sporulation (16). Furthermore, since incubation of homozygous *rna2/rna2* strains at 34°C during sporulation does not further reduce the level of rp synthesis, it has been suggested that the *rna* mutants have no effect on rp synthesis during sporulation (16). However, strains homozygous for the *rna2* mutation are unable to sporulate at 34°C (15).

Recently, another genetic tool for analyzing

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TABLE 1. Strains

Strain	Genotype												
AP-1	*MAT***a**/*MAT*α	*ade1*/+	*ade2*/*ade2*	*ura1*/+	*gal1*/+	*tyr1*/+	*lys2*/+	*his7*/+	+/*leu1*	+/*ura3*	+/*can1*	+/*cyh2*	*csp*/+
NP1	*MAT***a**/*MAT*α	*rna2*/*rna2*	*tyr1*/+	*ura1*/+	+/*ade1*	+/*ade2*	+/*lys2*	+/*his7*	+/*thr4*				
NP2	*MAT***a**/*MAT*α	*rna2*/+	*tyr1*/+	*ura1*/+	+/*ade1*	+/*ade2*	+/*lys2*	+/*his7*	+/*thr4*				
1B/10C	*MAT***a**/*MAT*α	*rna2*/*rna2*	*SRN1*/+	*ura1*/+	*leu2*/+	*ade2*/+	*thr4*/+						

the regulation of rp synthesis has become available. A suppressor of the *rna* mutations (*SRN1*) has been isolated (17). It allows strains carrying one or more of the *rna* mutations to grow at the nonpermissive temperature and also permits diploids homozygous for *rna2* to sporulate. It was therefore of interest to examine whether the presence of the suppressor alters the effects of either *rna2* or sporulation on the synthesis of rp's.

The availability of cloned yeast rp genes (2, 4, 21) has made it possible to examine in more detail the regulation of rp mRNA. Recently we used some of these cloned probes to examine the effect of the *rna* mutations on rp mRNA levels. In at least three of the ten *rna* strains, the decrease in the level of rp51 mRNA is accompanied by the accumulation of a larger intron-containing precursor RNA (18), providing an additional biochemical phenotype characteristic of the *rna* mutations. It appears that many rp transcripts respond to *rna2* as rp51 RNA does (4, 21; R. J. Planta, personal communication). These data and more recent data from our laboratory (3) and that of J. R. Warner (personal communication) make it likely that the *rna* mutations affect rp synthesis at a post-transcriptional step, presumably in the processing of precursor RNAs.

In this work, we used Northern blot analysis of RNA to ask a number of questions about the relationship of the genetic and physiological mechanisms (*rna2*, *SRN1*, and sporulation) involved in the regulation of rp synthesis. (i) Is the post-transcriptional processing of rp mRNA detectably altered during sporulation as compared with its processing during vegetative growth? (ii) Does the *rna2* mutation have any additional effect on the levels or species of rp mRNA sequences present during sporulation? (iii) What is the biochemical phenotype of *SRN1*, the genetic suppressor of *rna2* (17); i.e., does the suppressor modulate the effects of either *rna2* or sporulation on the levels of different rp mRNA sequences? The results reinforced the interpretation that *rna2* and sporulation decrease the levels of rp mRNA by different mechanisms.

MATERIALS AND METHODS

Strains. The complete genotypes of the strains used are listed in Table 1. Strain AP-1 was provided by Anita K. Hopper, Institution, and is described elsewhere (8). Strains NP1, NP2, and 1B/10C were constructed by Nancy J. Pearson (16) from strains originally provided by Calvin S. McLaughlin, University of California, Irvine.

Culture conditions. Cells were grown in AcII medium (1.0% potassium acetate, 0.6% yeast nitrogen base without amino acids [Difco], 0.5% peptone, 1.0% potassium biphthalate, 0.004% adenine, pH 5.5). Growth was monitored with a Klett-Summerson colorimeter equipped with a red filter. The culture doubled every 150 to 180 min. Vegetative cultures were always maintained below 5×10^7 cells per ml with maximum aeration.

For sporulation, cultures were harvested during exponential growth at a concentration of 1×10^7 to 2×10^7 cells per ml, washed, and suspended in 2 volumes of sporulation medium (1.0% potassium acetate, 0.2 M succinic acid, pH 5.5, with 10 M KOH) as previously described (12).

Isolation of RNA. RNA was isolated by the method of Zitomer et al. (23) as modified by Bromley et al. (3). Briefly, cells were suspended in cold extraction buffer (0.1 M Tris, 0.1 M LiCl, 0.1 mM EDTA, pH 7.4), glass beads were added, and the mixture was blended with a Vortex mixer. Sodium dodecyl sulfate was added to 0.5%. The RNA was extracted with phenol-chloroform and then recovered by ethanol precipitation. The RNA concentration was determined spectrophotometrically by absorbance at 260 nm.

DNA probes. The isolation and characterization of the relevant ribosomal protein genes have been previously described (18, 21). A genomic clone, pY13-86, contains the gene for rp52 and a non-rp gene as well. A gene encoding rp39 was isolated on plasmid pY11-40. A subclone of the gene for rp51 (subclone 2 [18]) was also used. A plasmid (pJH2) containing both the *MAT***a** region and the *URA3* gene has been described previously (6). This probe hybridizes with both *MAT***a** and *MAT*α transcripts due to flanking sequence homologies (9, 13). A *MAT*α probe, pJH14, consisting of a 3.5-

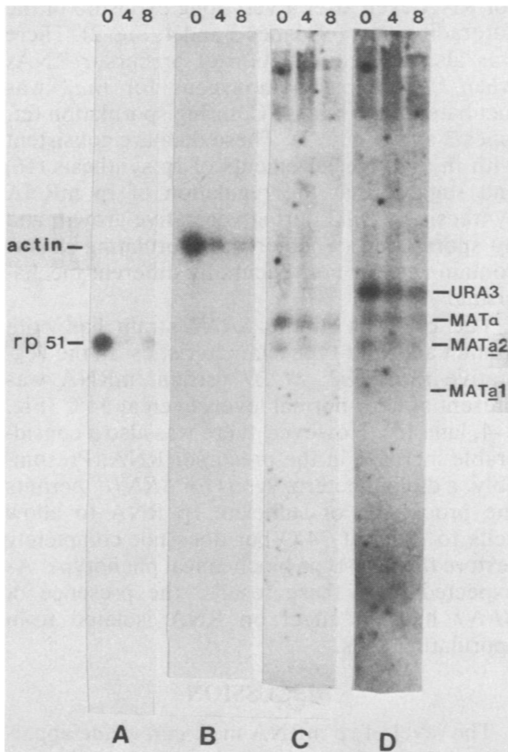

FIG. 1. Specific RNA transcripts during growth and sporulation of *S. cerevisiae*. RNA was extracted either from strain AP-1 vegetative cells (time zero; 0) or from cultures at hour 4 or 8 of sporulation. Total RNA (10 μg) was fractionated by size on a denaturing methylmercury gel and transferred to DBM paper. The blot was then hybridized sequentially with several different radioactive probes: (A) subclone HS-2, which hybridized to rp51 mRNA (600 b); (B) plasmid pRB111, which hybridized to action mRNA (1,400 b) and *URA3* mRNA (950 b); (C) plasmid pJH14, containing *MATα*, hybridized to *MATα* transcripts (800 b) and to the *MAT***a***2* transcript (600 b); (D) plasmid pJH2, containing *MATα* and *URA3*, hybridized to *MAT***a** transcripts of 600 and 450 b and to *MATα* (800 b) and *URA3* mRNAs (950 b).

kilobase *Eco*RI-*Hin*dIII fragment cloned into pBR322 (D. T. Rogers and J. E. Haber, unpublished data) was also used. This probe is not homologous to the *MAT***a***1* transcript. The actin gene probe (pRB111) (14) was a generous gift from D. Shortle and D. Botstein, Massachusetts Institute of Technology. It is present on the yeast vector YIP5 and thus has homology to *URA3* mRNA as well.

Northern gels. Total RNA was fractionated on methylmercury gels as described by Golden et al. (5). It was then transferred to diazobenzyloxymethyl (DBM) paper (1) and hybridized with nick-translated DNA probes (5, 11). After hybridization, the DBM paper was washed with 0.1× SSC–0.1% sodium dodecyl sulfate at 50°C and then exposed to Kodak XR5 film; an intensifying screen was used.

RESULTS

Control of rp mRNA during growth or sporulation. The metabolism of rp mRNA during growth and sporulation in wild-type cells was compared by extracting total RNA from the diploid strain AP-1 during vegetative growth and at 4 or 8 h into sporulation. The RNAs were fractionated by size, transferred to DBM paper, and hybridized sequentially with several different radioactive probes (Fig. 1). In the vegetative RNA sample (Fig. 1A), there was an mRNA species approximately 600 bases (b) in length which hybridized to the rp51 gene probe (18). However, RNA extracted from sporulating cells contained only 5 to 10% as much of this mRNA. Therefore, it is likely that the decrease in rp synthesis during sporulation (16) reflects a lower steady-state level of the mRNA. These data provided no evidence for the accumulation of a precursor RNA.

We also examined the effect of sporulation on the transcription of actin (Fig. 1B), *URA3* (1D), *MAT***a** (1C), and *MATα* (1D) mRNAs. The amounts of all of these transcripts decreased somewhat during sporulation. Although the differences between vegetative and sporulating cell RNAs in hybridizing to these non-rp probes was somewhat variable among the several *rna2* strains and AP-1 derivatives examined (data not shown), these differences were consistently much less than the changes in the level of rp mRNA.

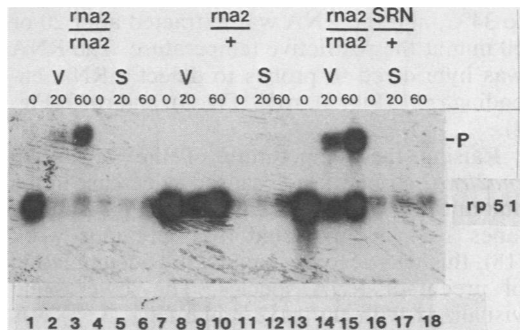

FIG. 2. rp51 RNAs during growth and sporulation of *rna2* strains. RNA was extracted from vegetative (V) and sporulating (S) cells grown at 23°C. The cultures were shifted to 34°C, and portions were removed after 20 or 60 min at the restrictive temperature for RNA extraction. Total RNA from each sample was fractionated on a methylmercury gel and blotted on DBM paper. The blot was hybridized with subclone HS-2 containing the rp51 gene. The strains examined include: *rna2*/*rna2* (lanes 1–6), *rna2*/+ (lanes 7–12), and *rna2*/*rna2 SRN1*/+ (lanes 13–18). Both vegetative and sporulating cultures were included. The duration of incubation at 34°C is indicated. P, Precursor RNA.

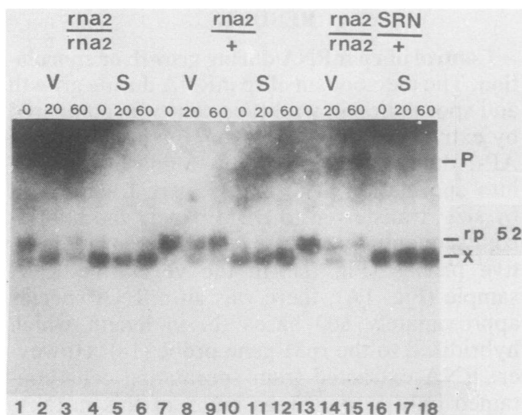

FIG. 3. rp52 RNAs during growth and sporulation of *rna2* strains. The blot described in the legend to Fig. 2 was hybridized with nick-translated DNA from pY13-86, a genomic clone containing the rp52 gene. Lanes and abbreviations are as described in the legend to Fig. 2, except P indicates putative precursor RNA. x, Non-rp gene mRNA (see text).

rp mRNA during growth and sporulation of *rna2* strains. We next compared the regulation of rp mRNA in *rna2* strains during growth and sporulation. We used three diploid strains: one heterozygous for *rna2*/+ and phenotypically similar to the wild type, one homozygous for *rna2*, and one heterozygous for the *SRN1* suppressor and homozygous for *rna2*. Since *rna2* is a temperature-sensitive lesion, all strains were grown at 23 to 25°C. Growing cells were shifted to 34°C, and the RNA was extracted after 20 or 60 min at the restrictive temperature. The RNA was hybridized to probes to detect mRNA encoding rp51 (Fig. 2), rp52 (Fig. 3), and rp39 (Fig. 4).

Raising the temperature of the vegetative *rna2*/*rna2* strain to 34°C caused a decrease in the amount of rp51 and rp52 mRNAs (Fig. 2 and 3, lanes 1–3). As expected from previous work (18), this was accompanied by the accumulation of precursor RNA species. The lower band visualized with the rp52 probe (Fig. 3, x) was caused by hybridization to a non-rp gene also present on the plasmid (22). This mRNA was far more abundant during sporulation (this work) and in *rna2* strains held at the restrictive temperature (22). In contrast, the level of rp39, which is not regulated by the *rna* mutations, was unaffected by a temperature shift for 60 min in either wild-type or mutant strains (Fig. 4, cf. lanes 1 and 3).

During sporulation of these strains at 23°C the level of all three rp mRNAs decreased significantly (cf. lanes 1 and 4, Fig. 2–4). Interestingly, there was no detectable accumulation of precursor RNA even after a very long exposure of the autoradiograms (cf. lanes 3 and 4, Fig. 2). There was also no accumulation of precursor RNAs when the diploid homozygous for *rna2* was incubated for 1 h at 34°C during sporulation (cf. lanes 3 and 6, Fig. 2). These data are consistent with in vivo measurements of rp synthesis (16) and suggest that the regulation of rp mRNA synthesis by *rna2* during vegetative growth and by sporulation (whether the sporulating strains contain *rna2* or not) occurs by different mechanisms.

The rp RNA in the *SRN1* strain had both mutant and wild-type characteristics. In the vegetative *rna2*/*rna2* *SRN1*/$^+$ strain, mRNA was present at near-normal levels even at 34°C (Fig. 2–4, lane 15). However, there was also a considerable increase in the precursor RNA. Presumably, a diploid heterozygous for *SRN1*/$^+$ permits the processing of sufficient rp RNA to allow cells to grow at 34°C but does not completely restore the wild-type biochemical phenotype. As expected from these results, the presence of *SRN1* had no effect on RNA isolated from sporulating cells.

DISCUSSION

The level of rp mRNA in *S. cerevisiae* apparently can be decreased in at least two distinct ways. In vegetative cells carrying one of the temperature-sensitive *rna* mutations, a decrease in mature rp mRNA is accompanied by a vastly increased abundance of precursor RNAs (4, 18; R. J. Planta, personal communication). Although there may also be some effect on transcription, the primary effect of the *rna* mutations on rp RNA seems to be post-transcriptional,

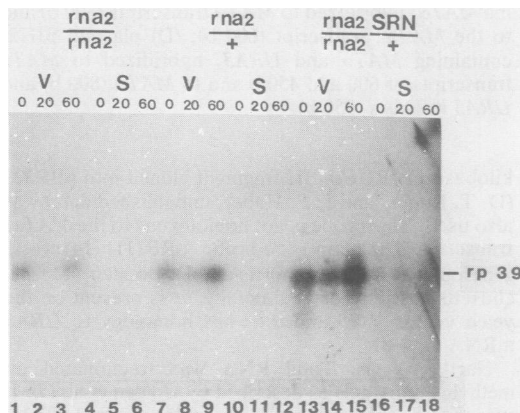

FIG. 4. rp39 RNAs during growth and sporulation of *rna2* strains. Experimental conditions, lanes, and abbreviations are described in the legend to Fig. 2. In this experiment, the blot was hybridized with a probe containing a copy of the gene encoding rp39.

i.e., it occurs in the processing of intervening sequences (3). In contrast, under the nitrogen starvation conditions that induce sporulation, there is no detectable accumulation of precursor RNA but an approximately equivalent ~90% decrease in mature rp mRNA (16; this work). The lack of any significant precursor induction under sporulating conditions makes it unlikely that sporulation, like incubation of *rna2* cells at the nonpermissive temperature, lowers rp levels by causing a block in mRNA processing. It is possible that during sporulation the low steady-state level of rp mRNA results from a low level of transcription of these genes, but our data do not distinguish between this and other mechanisms, such as an increased level of mRNA degradation.

The results presented here are consistent with previous data which suggested that the *rna2* mutation does not further affect the synthesis of rp's during sporulation (16). When diploids homozygous for *rna2* sporulated at the restrictive temperature, there was no significant accumulation of precursor mRNA for rp51 or rp52. Thus, if there is continued transcription (albeit perhaps at a low level) of rp mRNA during sporulation, the processing of the precursor RNA to mature mRNA species is relatively unaffected by *rna2*. The fact that *rna2* does not inhibit rp synthesis during the transition of sporulating cells back to vegetative growth is consistent with this interpretation (16).

Some additional insight into the control of rp synthesis has been gained from the characterization of the dominant suppressor of RNA mutations, *SRN1* (17). The fact that *SRN1* did not completely reverse the accumulation of precursor RNA (Fig. 2) may mean that mRNA processing was still somewhat defective. In any case, it is clear that during vegetative growth the suppressor reverses a major portion of the biochemical phenotype of *rna2*, but has little or no effect on the level of rp mRNA under sporulation conditions.

The data presented suggest that *rna2* and sporulation affect the expression of rp genes in two different ways. Indeed, it would appear that the *rna2* mutation has little or no effect on rp gene expression during sporulation, although, as discussed above, this conclusion is somewhat tentative. Further work will be required to define precisely the mechanisms by which the *rna2* mutation and sporulation act on rp gene expression.

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