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# A Suppressor of Temperature-Sensitive *rna* Mutations that Affect mRNA Metabolism in *Saccharomyces cerevisiae*

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We have isolated a dominant suppressor of *rna* mutations (*SRN1*) that relieves the temperature-sensitive inhibition of mRNA synthesis of ribosomal protein genes in the yeast *Saccharomyces cerevisiae*. The suppressor was selected for its ability to alleviate simultaneously the temperature-sensitive growth phenotypes of *rna2* and *rna6*. Several independently isolated suppressors appeared to be recessive lethal mutations. One suppressor, *SRN1*, was recovered as viable in haploid strains. *SRN1* can suppress *rna2*, *rna3*, *rna4*, *rna5*, *rna6*, and *rna8* singly or in pairs, although some combinations of *rna* mutations are less well suppressed than others. The suppressor allows strains with *rna* mutations to grow at 34°C but is unable to suppress at 37°C; however, *SRN1* does not, by itself, prevent growth at 37°C. In addition, *SRN1* suppresses the *rna1* mutation which affects general mRNA levels and also leads to the accumulation of precursor tRNA for those tRNAs that have intervening sequences. *SRN1* can suppress the *rna1* mutation as well as the *rna1 rna2* double mutation at 34°C. The suppressor does not affect the temperature-sensitive growth of two unrelated temperature-sensitive mutations, *cdc4* and *cdc7*.

In *Saccharomyces cerevisiae*, the synthesis of ribosomal proteins (r-proteins) is coordinately regulated under a variety of physiological conditions (4, 13, 18). In addition, the relative rates of synthesis of the majority of r-proteins are coordinately depressed under the influence of several temperature-sensitive *rna* mutations (3). There are at least 10 different unlinked complementation groups of these recessive, temperature-sensitive *rna* mutations (*rna2* to *rna11*) that inhibit ribosome biosynthesis at the restrictive temperature (5). When shifted from 25 to 34°C, cells carrying one of these mutations grow for about one generation and then are arrested (12, 20). All of the strains with *rna* mutations studied in detail show a selective inhibition of r-protein synthesis and a coordinate depression in the relative abundance of translatable r-protein mRNA (17). More recently, Rosbash et al. (14, 21) used two cloned r-protein genes to show that at the restrictive temperature, strains with *rna2* mutations accumulate RNA transcripts that appear to be higher-molecular-weight precursors of r-protein mRNAs. In one case, the r-protein gene *rp51* has been shown to contain an intervening sequence which is not spliced out of the RNA transcript in *rna2* strains at 37°C (14). The accumulation of a precursor mRNA also was found with strains carrying *rna6* or *rna8* at the restrictive temperature (14). In addition, a survey of 13 other cloned r-protein genes by Fried et al. (2) has shown that at the restrictive temperature in strains with *rna2* mutations, most of the genes are also transcribed into apparent precursor mRNAs. Because similar phenotypes are found with all of the *rna* complementation groups, it is possible that all of these *RNA* genes may be involved in the processing of r-protein precursor mRNAs.

General mRNA levels are affected by another temperature-sensitive mutation, *rna1*. This mutation appears to prevent the synthesis or processing and transport of most mRNAs from the nucleus (7, 16). More recently, Rosbash et al. (14) showed that mRNA levels are significantly reduced in *rna1* strains at the restrictive temperature. In the case of r-protein mRNAs, no higher-molecular-weight precursors accumulate, and these mRNAs also decrease in concentration. The *rna1* mutation also causes an accumulation of precursor tRNA for those tRNAs that contain an intervening sequence (6, 8).

Despite the difference in phenotypes between *rna1* and *rna2* to *rna11*, there appears to be some connection among these mutations. Studies of mRNA levels with cloned genes have suggested that *rna2* is epistatic to *rna1* (14). An *rna1 rna2* double mutant has the phenotype of an *RNA1 rna2* strain, namely, general mRNA levels do

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not decrease at the restrictive temperature, and r-protein mRNAs accumulate as higher-molecular-weight precursor forms.

To determine whether the *rna* mutations are functionally related and to better understand the regulation of r-proteins in *S. cerevisiae*, we have isolated a suppressor that simultaneously relieves the temperature-sensitive growth phenotypes of several of these mutations. This paper describes the genetic and preliminary biochemical properties of a dominant suppressor (*SRN1*; suppressor of *rna* mutations) that suppresses the r-protein coordinate control mutations *rna2*, *rna3*, *rna4*, *rna6*, and *rna8*. *SRN1* also suppresses *rna1*, which affects general mRNA levels and the processing of tRNA.

## MATERIALS AND METHODS

**Strains.** The original strains from which diploids and derivative haploids were constructed came from the Berkeley Stock Collection (University of California at Berkeley). Haploid strains containing the mutations *rna2* to *rna11* were obtained from C. McLaughlin (University of California, Irvine). The strains used in these experiments are listed in Table 1.

**Media and cultivation.** Cells were grown vegetatively in YEPA (1% yeast extract–2% peptone [Difco Laboratories]–1% potassium acetate) or YEPD (1% yeast extract–2% peptone [Difco]–2% dextrose). To induce sporulation, diploids were grown to the midlog phase in YEPA (Klett reading, 90), washed two times with sporulation media, and then diluted 1:4 into sporulation medium (1% potassium acetate buffered to pH 5.5 with 0.2 M succinate) (10). Radioactive labeling experiments were done either in minimal medium (0.67% yeast nitrogen base without amino acids, 2% dextrose) or complete defined medium which lacked leucine or methionine, depending on the radioactive amino acid added. The generation time was approximately the same (3 h) in all three of these growth media.

Cell growth was monitored directly by a Klett colorimeter with a red filter. Cell numbers were estimated with a hemacytometer. The percentage of sporulation was estimated by direct count in a phase microscope in a field of approximately 100 cells at magnification ×400.

**Genetic techniques.** Diploids were selected, sporulated, and dissected by the method of Mortimer and Hawthorne (11). Temperature-sensitive revertants for growth were selected by plating approximately $10^7$ cells per plate, incubating them at 25°C for 48 h, replica plating them to 34 and 37°C, and then incubating them for 15 days. Colonies that grew were isolated, and a new single-colony isolate was picked for further analysis.

**Quantitation of r-proteins.** To quantitate r-proteins, 5 ml of cells ($10^7$ cells per ml) was pulse-labeled with [$^3$H]leucine (100 Ci/mmol; Amersham Corp.) for 5 min at 100 μCi/ml. Cells were then broken by blending in a Vortex mixer with glass beads. The proteins were acid extracted and mixed with a sample of [$^{14}$C]leucine-labeled protein extracted from logarithmically growing cells labeled for 6 h with [$^{14}$C]leucine (>170 mCi/mmol., New England Nuclear Corp.) at 10 μCi/ml. The proteins were then separated by electrophoresis according to the method of Warner and Gorenstein (19). r-Proteins were identified and quantitated by their procedures. The relative rates of synthesis of r-proteins (*Ai*) were expressed as follows: $Ai = (^3H/^{14}C$ for each r-protein spot)/ ($^3H/^{14}C$ for total protein).

TABLE 1. Genotypes of strains used

| Strain | Genotype |
|---|---|
| NP15 | *MATa rna2-1 rna6 ade1 + + +*<br>*MATα rna2-1 rna6 + thr4 ura1 his7* |
| NP16 | *MATa rna2-1 rna6 ade1 + +*<br>*MATα rna2-1 rna6 + thr4 ura1* |
| NP17 | *MATa rna2-1 rna6 ade1 + +*<br>*MATα rna2-1 tna6 + thr4 his7* |
| NP18 | *MATa rna2-1 rna6 SRN1 ura1* |
| NP21 | *MATa rna2-1 SRN1 leu2* |
| NP32 | *MATα rna8 ade1 ura1* |
| A203 | *MATα leu2 trp1 arg9 his6 ilv3 met14 pet8 pet19 rad2* |
| *ts*353 | *MATa rna2-2 trp1 arg9 his6 ilv3 met14 pet8 pet19 rad2* |
| *ts*125 | *MATa rna3 ade1 ade2 ura1 his7 lys2 gal1* |
| *ts*339 | *MATa rna4 ade1 ade2 ura1 his7 lys2 gal1* |
| *ts*108 | *MATa rna5 ade1 ade2 ura1 his7 lys2 gal1* |
| *ts*124 | *MATa cdc7 ade1 ade2 ura1 his7 lys2 gal1* |
| *ts*198 | *MATa cdc4 ade1 ade2 ura1 his7 lys2 gal1* |
| *ts*136 | *MATa rna1 ade1 ade2 ura1 his7 lys2 gal1* |
| NP36 | *MATα rna1 rna2 ade1 ade2 ura1 his7 lys2 thr4* |
| NP23 | *MATa rna2-1 SRN1 ade1 ura1*<br>*MATα rna2-1 SRN1 + ura1* |
| NP1 | *MATa rna2-1 tyr1 ura1 + + + + +*<br>*MATα rna2-1 + + ade1 ade2 lys2 his7 thr4* |
| NP2 | *MATa rna2-1 ade1 ade2 ura1 his7 lys2 tyr1 gal1 +*<br>*MATα + + + + + + + thr4* |

## RESULTS

**Attempts to select suppressors in *rna2 rna6* haploids.** To look for suppressors that would affect several *rna* mutants, we constructed haploid strains containing both *rna2* (*ts*368) and *rna6* (*ts*166). Approximately $10^7$ cells of these haploid strains were spread on YEPD plates at 25°C and allowed to grow overnight. They were then replica plated to YEPD plates and incubated at 34 and 37°C to find temperature-independent colonies. None of the five strains tested showed any colonies, even after 15 days at 34 or 37°C. UV mutagenesis also failed to produce any temperature-independent revertants.

**Selection of suppressors in *rna2/rna2 rna6/rna6* diploids.** The initial failure to find temperature-sensitive revertants in haploids suggested that such suppressors were either very rare or were lethal. We therefore attempted to select revertants in diploids homozygous for *rna2* and *rna6*, to look for dominant suppressors that were heterozygous with a wild-type allele. All three diploids yielded several colonies able to grow at 34°C. Since the exact number of cells on the YEPD plates was not known after the cells were replica-plated to 34°C, an accurate estimate of reversion frequency could not be calculated, however, it is obvious that revertants appear in diploids much more frequently than in haploids. It should be noted that revertants only appeared at 34°C and were unable to grow at 37°C.

Diploids homozygous for *rna2* and *rna6* are unable to sporulate when shifted from 25 to 34°C (12, 13). We therefore tested whether the revertants could sporulate, as well as grow, at 34°C. About half of these revertants could sporulate at both temperatures.

Because spontaneous revertants were readily selected in diploids but not in haploids, we suspected that the mutations which suppressed *rna2* and *rna6* might have a recessive lethal phenotype. Thus, haploids carrying only the suppressor mutation could not survive, but diploids which had one mutant gene and one wild-type gene could grow. If this were the case, one would expect that when revertant diploids were sporulated and dissected, only two of four spores would be viable, and that none of the viable spores would carry the suppressor. This is what we found when three different revertants were sporulated and dissected at 25°C (Table 2). No tetrad had more than two viable spores. The fact that many tetrads had only one viable spore may be explained by the generally poor spore viability found even in the parent diploid NP15, in which only about 80% of all spores were viable (data not shown). The lack of viability of segregants carrying the suppressor mutation was demonstrated by the fact that none of the viable spores from these 46 tetrads (Table 2) could grow at 34°C. All spores still contained both *rna2* and *rna6* (as shown by complementation testing at 37°C). These results indicated that two haploid spores of each tetrad that contained a suppressor of the *rna* mutations were unable to grow even at 25°C. We concluded that revertants contained a dominant suppressor of *rna* mutations (*SRN*) that was also a recessive lethal mutation.

TABLE 2. Properties of meiotic segregants from *rna2/rna2 rna6/rna6* diploids selected for growth at 34°C[a]

| Revertant | No. of tetrads with indicated no. of viable spores | | | |
|---|---|---|---|---|
| | 4 | 3 | 2 | 1 |
| NP15-5 | 0 | 0 | 10 | 25 |
| NP16-3 | 0 | 0 | 2 | 5 |
| NP17-6 | 0 | 0 | 3 | 1 |

[a] Purified revertants were sporulated at 25°C and dissected. All segregants which germinated at 25°C were unable to grow at 34°C and contained both *rna2* and *rna6* by complementation testing.

**Selection of a haploid carrying *SRN*.** Because this suppressor (*SRN1*) existed in a diploid and did not appear to be viable in a haploid, it was difficult to analyze its segregation or to test its ability to suppress other *rna* mutations. One possible solution to this problem was to select a haploid-viable strain carrying the suppressor. A similar procedure had been used to analyze a recessive lethal tRNA gene (1).

Revertant NP15-5, with the assumed genotype *rna2/rna2 rna6/rna6 SRN1/+*, was grown on a YEPD plate at 25°C for 2 days and then replica-plated to a sporulation plate at 25°C. After cells had sporulated, asci were scraped from the plate, digested with 1% Glusulase, and then sonicated to separate spores. The separated spores were spread on YEPD plates at 34°C. Colonies which grew at 34°C and expressed recessive markers heterozygous in the diploid were selected. Thirty-six haploid segregants able to grow at 34°C were isolated by this method. As in the parent diploid carrying the suppressor, none of these haploids could grow at 37°C. This suggests that the original suppressor mutation had been recovered in an apparently haploid segregant, but does not completely rule out the possibility that the haploid-viable suppressor arose spontaneously during spore germination.

One of the isolates that grew at 34°C, designated NP18, was selected for further study. This strain was mated with strain A203, which was wild type for both *RNA2* and *RNA6*. The resulting diploid was sporulated and dissected. Of 40 tetrads resulting from this cross, 15 had four

viable spores, and 10 had three viable spores. Because the suppressor was not effective at 37°C, we could complementation test each segregant for *rna2* or *rna6* even in the presence of the suppressor. Both of the *rna* mutations segregated 2+:2− in the complete tetrads and were unlinked to each other. All segregants that proved to have either *rna2* or *rna6* were then tested for the presence of the suppressor by their ability to grow at 34°C but not at 37°C. Of 63 *rna*-containing segregants from tetrads having at least three viable spores, 26 (approximately half) appeared to carry the suppressor. Thus, the *SRN1* mutation appeared to segregate as a single gene. To confirm that *SRN1* now segregated as a single allele, we crossed one segregant (designated NP21) carrying *rna2-1* and the suppressor with another strain containing the *rna2* mutation (*ts*353). This second strain (NP32) carried a second, independently isolated temperature-sensitive allele, *rna2-2*. From the tetrads summarized in Table 3, it is clear that *SRN1* segregated 2+:2−, allowing *rna2* segregants to grow at 34°C but not at 37°C. Furthermore, *SRN1* could suppress either *rna2* allele.

It should be noted that when haploid strains carrying the suppressor were grown at 25°C and then subcloned, about 20 to 30% of the subclones were unable to grow at 34°C. Thus, *SRN1* appears to be mitotically unstable.

***SRN1* suppression of other *rna* mutations.** The suppressor of *rna2* and *rna6* also suppressed a number of other *rna* mutations. We constructed a variety of diploids heterozygous for *SRN1* and homozygous for one or more *rna* mutations. As shown in Table 3, *SRN1* allowed the growth at 34°C of strains carrying *rna2*, *rna3*, *rna4*, *rna5*, and *rna8*. We did not retest *rna6*. None of the segregants could grow at 37°C. In the diploid PT10, which was homozygous for *rna2* and heterozygous for *SRN1* and *rna4*, it was evident that only about half (6 of 16) of the *rna2 rna4 SRN1* segregants could grow at 34°C. This was also true for segregants from another diploid, PT58, which was homozygous for *rna4* and heterozygous for *rna2* and *SRN1*. In both of these crosses, however, it was evident that *SRN1* could suppress *rna4* alone as efficiently as it suppressed other single *rna* mutations. A similar result was found with *rna2 rna3 SRN1* and *rna2 rna8 SRN1* segregants (data not shown). Some segregants with these presumed genotypes showed weak growth at 34°C after 2 to 3 days, whereas others grew well after the 1 day at 34°C usually used in testing suppression. It is possible that there is some other variation in the genetic backgrounds of some of these strains that results in lower penetrance when *SRN1* must suppress two *rna* mutations. Nevertheless, it is clear that *SRN1* can suppress any of the *rna* mutations tested.

TABLE 3. Suppression of various *rna* mutations by *SRN1*

| Diploid | Genotype | | | No. of tetrads with indicated segregation of growth at 34°C | | | |
|---|---|---|---|---|---|---|---|
| | | | | 2+:2− | 1+:3− | 0+:4− | 3+:1− |
| NP32 | *rna2-1* | *SRN1* | | 12 | 0 | 0 | 0 |
| | *rna2-2* | + | | | | | |
| PT8 | *rna3* | *SRN1* | | 14 | 0 | 0 | 0 |
| | *rna3* | + | | | | | |
| PT9 | *rna5* | *SRN1* | | 6 | 0 | 0 | 0 |
| | *rna5* | + | | | | | |
| PT6 | *rna2* | *rna8* | *SRN1* | 10 | 1 | 0 | 0 |
| | *rna2* | *rna8* | + | | | | |
| PT7 | *rna8* | *SRN1* | | 15 | 1 | 0 | 0 |
| | *rna8* | + | | | | | |
| PT10 | *rna2* | *rna4* | *SRN1* | 6 | 9 | 0 | 1 |
| | *rna2* | + | + | | | | |
| PT58 | *rna2* | *rna4* | *SRN1* | 6 | 9 | 2 | 0 |
| | + | *rna4* | + | | | | |

*SRN1* did not suppress the lack of growth at 34°C of two unrelated temperature-sensitive mutations, *cdc4* and *cdc7*. Strains carrying *cdc4 rna2* or *cdc7 rna2* were crossed with an *rna2 SRN1* strain (NP21). We analyzed 15 meiotic tetrads from the *cdc4*/+ *rna2*/*rna2 SRN1*/+ diploid. Whereas half (16 of 30) of the segregants carrying *rna2* but wild type for *CDC4* grew well at 34°C, none of the 30 *rna2 cdc4* segregants grew well at 35°C. One *rna2 cdc4* segregant did grow slowly at 34°C. We would have expected seven *rna2 cdc4* segregants to grow at 34°C if *SRN1* could suppress both mutations and if *SRN1* and *cdc4* were not closely linked. (Because *cdc4* is located close to the centromere of chromosome VI and *SRN1* is not apparently centromere linked, it is unlikely that these mutations are linked.) Similar experiments showed that none of eight *cdc7 rna2* segregants could grow at 34°C. *SRN1* also did not suppress *cdc7* in the absence of *rna2* (data not shown). We conclude that *SRN1* cannot suppress *cdc4* or *cdc7*.

**Effect of *SRN1* on *rna1*.** In view of the observation that *rna2* was epistatic to *rna1* (14), we tested whether *SRN1* could also suppress *rna1*. We crossed an *rna2 SRN1* strain (NP21) with an *rna2 rna1* strain (NP36). When we dissected tetrads from this diploid (PT11) we found that two of the four segregants could grow at 34°C but not at 37°C (Table 4). Complementation testing at 37°C confirmed that about half of the *SRN1* segregants carried *rna1*. We also backcrossed several *SRN1 rna2 rna1* strains with strains containing the *rna1* mutation to show that *SRN1* can also suppress *rna1* in the absence of *rna2*. The data from these tetrads are also

TABLE 4. *SRN1* suppression of *rna1* and *rna2*

| Diploid | Genotype | | | Fraction of tetrads containing two spores that grew at 34°C |
|---|---|---|---|---|
| PT11 | *rna2*/*rna2* | *rna1*/+ | +/*SRN1* | 6/6 |
| PT12 | *rna2*/+ | *rna1*/*rna1* | *SRN1*/+ | 4/4 |
| PT13 | *rna2*/+ | *rna1*/*rna1* | *SRN1*/+ | 5/5 |
| PT14 | *rna2*/+ | *rna1*/*rna1* | *SRN1*/+ | 7/7 |
| PT15 | *rna2*/+ | *rna1*/*rna1* | *SRN1*/+ | 5/5 |

included in Table 4. *SRN1* segregated 2+:2− and allowed strains carrying either *rna1* alone or both *rna1* and *rna2* to grow at 34°C. Again there was no suppression at 37°C. Thus it appears that *SRN1* can suppress *rna1*, a mutation that interferes with general mRNA metabolism, as well as it suppresses *rna2*, *rna3*, etc., which primarily affect r-protein mRNA metabolism.

**Effect of *SRN1* on the synthesis of r-proteins.** Since *SRN1* suppressed the temperature sensitivity of several *rna* mutations, we wished to know how it affected the coordinate regulation of r-proteins. In these experiments, strains which contained both *SRN1* and *rna2* were used. This allowed us to check for the presence of *SRN1* at any time by demonstrating that the strain could grow at 34°C but not at 37°C. Since the suppressor phenotype of this mutation has been shown to be unstable and since no other identifying phenotype for *SRN1* has been found, this test assures that the suppressor is still present.

Figure 1 illustrates the effect of *SRN1* on vegetative cells when the temperature was shifted from 25 to 34°C during growth in complete medium lacking leucine. We compared the relative rates of r-protein synthesis in an *rna2*/*rna2* *SRN1*/*SRN1* (NP23) diploid to the rates of r-protein synthesis in a wild-type *rna2*/+ (NP2) diploid lacking *SRN1*. In addition, we measured r-protein levels in an *rna2* *rna2* diploid strain (NP1) lacking *SRN1*. In the *rna2* homozygote shifted to 34°C, r-protein synthesis declined and reached a relative rate of synthesis of about 0.1 by 60 min. As expected, *SRN1* prevented the *rna2*-induced depression of r-protein synthesis and allowed cells to continue growing at the restrictive temperature. More unexpectedly, *SRN1* also appeared to abolish the transient depression of r-protein synthesis that has been shown to occur even in wild-type strains (17) shifted from 25 to 34°C. Thus, whereas an *rna2*/+ diploid showed a depression in r-protein synthesis at 10 to 20 min similar to that observed with other wild-type strains (Fig. 1B), there was no such effect on the *rna2*/*rna2* *SRN1*/*SRN1* diploid.

## DISCUSSION

We have used a genetic approach to examine the regulatory pathways which control the synthesis of r-proteins in *S. cerevisiae*. Several temperature-sensitive mutations (*rna2* to *rna11*) which selectively inhibit the synthesis of r-proteins have been isolated. The gene products of these mutations are not known, and their mechanisms of action are not understood; however, they are provisionally grouped together by the fact that they all have a similar biochemical phenotype. We have searched for mutations that suppress more than one of these *rna* mutations with the hope of genetically identifying other components which may be part of the same regulatory pathway. A similar approach, the genetic analysis of tRNA suppressors and antisuppressors, has been useful in defining components required for the synthesis and functioning of tRNA in yeasts (9). In this report we have described the isolation and genetic analysis of a dominant suppressor (*SRN1*) of several *rna* mutations. The fact that such a suppressor can be found demonstrates that these temperature-sensitive mutations are functionally related, as suggested by their similar phenotypes. Although we have not rigorously ruled out the possibility that *SRN1* is a translational suppressor, we think this is highly unlikely. It seems improbable that a single suppressor mutation could suppress two independently isolated alleles of *rna2*, plus mutations in *rna3*, *rna4*, *rna6*, *rna8*, and *rna1*. Furthermore, we are certain from previous work that the *rna6* mutation (strain *ts*166) is neither an amber nor an ochre mutation since it is not suppressed by amber (*SUP51*) or ochre (*SUP4-1*) suppressors and does not corevert with other known amber or ochre mutations (N. J. Pearson, Ph.D. thesis, Brandeis University, Waltham, Mass., 1980). We cannot totally rule out the possibility that *SRN1* is a suppressor of missense mutations, although we have not yet found any other mutations that are suppressed by *SRN1*. We have shown that *SRN1* does not suppress two unrelated temperature-sensitive mutations, *cdc7* and *cdc4*, at 34°C, in addition to a variety of nutritional mutations.

One interesting and unexplained observation is that none of the original revertants of *rna2* *rna6* diploids could grow at 37°C, although we found many diploids able to grow at 34°C. We have also tried to select a further mutation in a

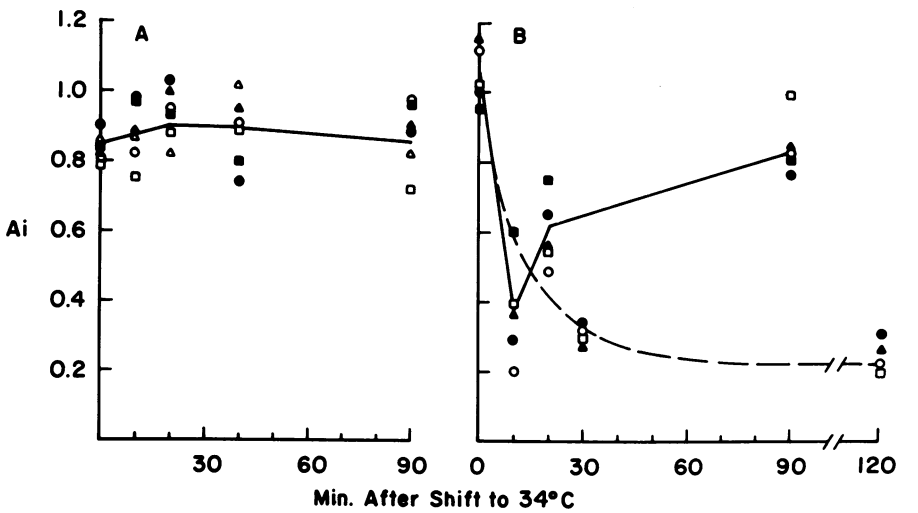


FIG. 1. Effect of *SRN1* on the transient inhibition of r-protein synthesis in cells shifted from 25 to 34°C. (A) An *SRN1*-containing strain (NP23) homozygous for *rna2* and *SRN1* was grown at 25°C and shifted to 34°C. At the times indicated, cells were labeled for 5 min with [$^3$H]leucine, and the r-proteins were extracted and analyzed as described in the text. The data for r-proteins 2 (▲), 6 (●), 8 (○), 25 (△), 39 (■), and 62 (□) are shown. The r-proteins are numbered according to the system used by Gorenstein and Warner (3) and Warner and Gorenstein (19). The relative rate of synthesis of an individual r-protein is designated *Ai*. (B) Similar data are shown for the same proteins in an *rna2*/+ heterozygote (NP2) (solid line) and a diploid homozygous for *rna2* (NP1) (broken line), both of which lack *SRN1*. Only the data for the endpoint of the experiment involving strain NP1 are shown.

haploid *SRN1* strain to allow it to grow at 37°C. Neither spontaneous nor UV-induced mutations have appeared. The failure of *SRN1* to suppress at 37°C does not result, however, from a direct effect of *SRN1* on cell growth. Meiotic segregants carrying *SRN1* but no *rna* mutations grew well at 37°C (data not shown).

The temperature-sensitive *rna1* mutation apparently prevents the accumulation of most mRNAs (7, 14, 16). In addition, strains containing the *rna1* mutation accumulate precursor tRNA for those tRNAs containing an intervening sequence (6, 8). Our finding that *SRN1* will suppress *rna1* as well as the mutations *rna2* to *rna8* suggests that these mutations indeed share some common features. It may be that all of these *rna* mutations, including *rna1*, may act indirectly on the mRNA processing or transport machinery. In the case of *rna1*, in vitro studies of pre-tRNA processing have suggested that the *rna1*-bearing mutant, *ts*136, does not confer a temperature-sensitive phenotype on any of the proteins required for removing the intervening sequence; thus, *rna1* must indirectly regulate the process (J. Abelson, personal communication). The same inference may be extended to the 10 *rna* complementation groups affecting r-protein mRNA; it is unlikely that most of these mutations affect processing directly. It is possible that all of these mutations alter normal pathways used to regulate the level of these mRNAs and that *SRN1* does not respond to such changes in regulatory signals. On the other hand, Sharp (15) has speculated that mRNA processing may occur sequentially along an RNA strand mediated by a ribosome-like particle. If this were true one could account for the direct involvement of 10 genes in the mRNA processing. In either case, the fact that *SRN1* suppresses six different *rna* mutations indicates that these gene products must interact in some common process.

When first isolated, three independent suppressor mutations in different *rna2*/*rna2 rna6*/*rna6* diploids all appeared to be recessive lethal mutations, as only two spores from any tetrad were viable, and none contained the suppressor. Subsequently we isolated a nonlethal variant of *SRN1* by selecting for a viable haploid meiotic segregant. The viable meiotic *rna2 rna6 SRN1* haploids were mitotically unstable under nonselective conditions. We do not know the nature of the event which produced viable, but unstable, variants. It does not seem that the viable haploid strain is disomic for the chromosome carrying *SRN1*. If the haploid *SRN1* segregants we analyzed (NP18 and NP21) were disomic and if the suppressor was still a recessive lethal mutation (as it was in the original diploid), we would have expected only approximately one-fourth of the tetrads dissected to have four viable spores. This was not the case. For example, in the diploid heterozygous for *SRN1* and homozygous

for *rna2* (Table 3), 12 of 27 tetrads had four viable spores, and 14 tetrads had three viable spores. Furthermore, in diploids heterozygous for *SRN1* and homozygous for one *rna* mutation (Table 3), 36 of 58 tetrads had four viable spores. Segregation analysis also indicated that *SRN1* was not centromere-linked or linked to any of the *rna* mutations tested. For example, when compared with the centromere-linked marker *ade1*, *SRN1* segregated with 4 parental ditypes, 5 nonparental ditypes, and 20 tetratypes. It is possible that the genetic event which resulted in the recovery of the viable haploid NP18 carrying *SRN1* resulted from a second site mutation closely linked to *SRN1*, or alternatively, arose by a duplication of part of the chromosome carrying the *SRN1* mutation so that it then carried both *SRN1* and its wild-type allele. The mitotic instability of *SRN1* may reflect the presence of a chromosome rearrangement or duplication. The recessive lethal phenotype of the original suppressor mutations suggests that *SRN1* mutations may eliminate or interface with an essential cellular function.

We have begun to use the *SRN1* mutation to determine whether regulation of r-proteins is affected by *SRN1* under other cellular states. Our initial experiments with *SRN1 rna2* strains demonstrate that none of the 40 r-proteins measured shows a decline in r-protein synthesis when the temperature is raised from 25 to 34°C. The suppressor apparently prevents the transient decline in r-proteins that is seen even in wild-type strains (17).

Further characterization of this mutation may lead to an understanding of the relationship between the *rna* genes and the role that all of these gene products play in the processing of r-protein mRNA and the regulation of ribosome synthesis in the cell.

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