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Isolation and Characterization of the *RNA2*$^+$, *RNA4*$^+$, and *RNA11*$^+$ Genes of *Saccharomyces cerevisiae*

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We used genetic complementation to isolate DNA fragments that encode the *Saccharomyces cerevisiae* genes *RNA2*$^+$, *RNA4*$^+$, and *RNA11*$^+$ and to localize the genes on the cloned DNA fragments. RNA blot-hybridization analyses coupled with genetic analyses indicated that *RNA2*$^+$ is coded by a 3.0-kilobase (kb) transcript, *RNA4*$^+$ is coded by a 1.6-kb transcript, and *RNA11*$^+$ is coded by a 1.3-kb or a 1.7-kb transcript or both; none of the cloned genes contains detectable introns. All three genes were transcribed into messages of very low abundance (~20 times lower than a ribosomal protein message). DNA blot-hybridization revealed that all cloned genes are represented only once in the yeast chromosome. mRNA for *RNA2*$^+$ and *RNA4*$^+$ is produced in approximate proportion to gene dosage, whereas *RNA11*$^+$ transcription appears to be not nearly so dependent on gene dosage. On a medium-copy plasmid (5 to 10 copies per cell), each cloned gene complemented mutations only in its own gene, indicating that each gene encodes a unique function. Genetic analysis by integrative transformation indicated that we cloned the *RNA2*$^+$, *RNA4*$^+$, and *RNA11*$^+$ structural genes and not second-site suppressors.

Hartwell and co-workers (19, 20) have identified temperature-sensitive mutations in *Saccharomyces cerevisiae*, called *rna2* through *rna11*, which fall into 10 distinct complementation groups. Originally it was found that at an elevated temperature (36°C) *rna* mutants exhibit inhibition of ribosomal subunit formation (20) and that the 35S rRNA precursor was not properly processed but was subject to increased degradation (20, 50). Furthermore, it was found that at 36°C there was coordinate and preferential inhibition of ribosomal protein biosynthesis which was distinct from the transient inhibition of ribosomal protein synthesis in wild-type cells (12, 56). This inhibition was shown to result from a depletion of the mRNA for ribosomal proteins. Even more recently, RNA blot-hybridization analyses has shown that the decrease in the amount of mRNA for several individual ribosomal proteins was accompanied by the accumulation of larger RNA species (10, 46). Of the 14 ribosomal protein genes examined, a longer transcript appeared in 9 of them. DNA sequence analysis has now confirmed that at least 10 ribosomal protein genes contain introns (27, 31, 53; J. Teem, personal communication), and thus the deficiency of ribosomal protein mRNA in the *rna* mutants at the restrictive temperature can be attributed to a defect in RNA splicing. In contrast, tRNA synthesis remains unaffected at the restrictive temperature, and total protein synthesis remains unchanged, at least initially (20). This final point is consistent with the fact that although to date over 60 nuclear genes have been isolated from *S. cerevisiae*, only 3 genes, actin (11, 39), *MAT*a (36), and *INO1* (L. S. Klig and S. A. Henry, J. Biol. Chem., in press), in addition to most ribosomal protein genes, show a precursor mRNA which contains an intervening sequence. Thus, as the evidence now stands, *rna2* through *rna11* appear to be implicated directly or indirectly in mRNA processing (7, 53).

Results of previously published studies have suggested that mutants *rna2* through *rna11* all have similar phenotypes, although they have not all been studied in sufficient detail to allow this statement to be made unequivocally. However, it is interesting that Pearson et al. (41) have isolated a mutation (*SRN1*) that suppresses several *rna2* through *rna11* mutations, which further suggests that the *rna2* through *rna11* genes are functionally related. Beyond this, virtually nothing is known at the molecular level about the normal function of the *RNA* gene products. We undertook the isolation of the wild-type counterpart of three *rna* genes as a first step toward the characterization of their products and mechanisms of action.

MATERIALS AND METHODS

Microbial strains and plasmids. The *S. cerevisiae* strains used in this study are described in Table 1. The *Escherichia coli* strain used was JF1754 (*hsdR* Lac$^-$ Gal$^-$ *metB leuB hisB*) and was grown in Luria broth. *S. cerevisiae* transformation was carried out as described previously (35). *E. coli* transformation was performed by the method of Mandel and Higa (33). The shuttle vectors used for DNA cloning were pYF91 (51) or pJZ1 (47). pJH18 (supplied by Jim Haber) is a pBR322 derivative containing a 1.1-kilobase *Hin*dIII DNA fragment containing the *URA3*$^+$ gene. Temperature-sensitive strains were grown at room temperature (23°C) or as otherwise indicated. Other strains were grown at 30°C. Growth media used were YPD medium (1% yeast extract [Difco Laboratories, Detroit, Mich.], 2% peptone [Difco], 2% glucose) or SD medium (0.67% yeast nitrogen base without amino acids [Difco] buffered to pH 5.8 with sodium succinate) plus 2% glucose and 50 μg of required amino acids per ml (48).

Biochemical procedures. Enzymes and reagents were purchased from Boehringer-Mannheim, Canada; Bethesda Research Laboratories; New England Nuclear Corp.; and New England Biolabs. Enzyme reactions were carried out by the procedures supplied by the manufacturers. DNA manipulations were carried out by standard methods (34). Plasmid DNA was extracted by the alkaline method (4) and purified by cesium chloride-ethidium bromide gradient centrifugation. Yeast DNA for blot-hybridization analyses was purified as described previously (8). RNA for blot-hybridization analyses was extracted from exponentially growing yeast

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TABLE 1. *S. cerevisiae* strain used in this study

Strain	Genotype	Source
A364A	**a** *ade1 ade2 his7 lys2 tyr1 ura1 gal1*	YGSC[a] (20, 21, 36)
ts368	**a** *ade1 ade2 his7 lys2 tyr1 ura1 gal1 rna2-1*	YGSC (20, 21, 36)
ts339	**a** *ade1 ade2 his7 lys2 tyr1 ura1 gal1 rna4-1*	YGSC (20, 21, 36)
ts382	**a** *ade1 ade2 his7 lys2 tyr1 ura1 gal1 rna11-1*	YGSC (20, 21, 36)
YR02-92	α *ade1 ade2 leu2-3,112 lys2 tyr1 ura1 rna2-1*	ts368 × LL20 segregant
YR04-304	α *ade1 his3-11,15 his7 leu2-3,112 lys2 tyr1 rna4-1*	ts339 × LL20 segregant
YR11-182	α *ade1 leu2-3,112 lys2 tyr1 ura1 rna11-1*	ts382 × LL20 segregant
YR2-172	α *his3-11,15 his7 leu2-3,112 lys2 tyr7 ura3-52 rna2-1*	ts368 × LL20 × SR25-1A
YR4-8	α *ade2 tyr1 ura3-52 rna4-1 can1*	ts339 × A192 × CG378
YR11-5	**a** *his2 ura3-52 trp1 rna11-1*	ts382 × YF1 × SR25-1A
YR11-A	α *ura3-52 rna11-1*	ts382 × YF1 × SR25-1A
LL20	α *his3-11,15 leu2-3,112 can1*	Lester Lau
Sc252	α *ade1 leu2-3,112 ura3-52*	David Thomas
SR25-1A	**a** *his4-912 ura3-52*	Shirleen Roeder
YF1	α *ade1 his2 leu2 trp1 gal1*	
CG378	**a** *ade5 leu2-3,112 ura3-52 trp1-289 can1*	C. N. Giroux
A192	α *trp1*	James Haber

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cells as described elsewhere (15). In some instances, precipitation of RNA with 2 M LiCl (1, 26) was included to eliminate contamination with DNA. DNA blotting, RNA blotting, nick-translation, and nucleic acid hybridization have been described previously (34). Transcript sizes were determined relative to the sizes of *S. cerevisiae* and *E. coli* rRNA stained with ethidium bromide.

Genetic mapping. Genetic crosses, sporulation, and tetrad dissection were carried out as described previously (48).

RESULTS

Cloning *RNA2*$^+$, *RNA4*$^+$, and *RNA11*$^+$. We used genetic complementation of the temperature-sensitive phenotype of the *rna* mutants to isolate three of the *S. cerevisiae* *RNA*$^+$ genes. Mutants of *S. cerevisiae* carrying the *leu2* mutation in addition to the temperature-sensitive mutation *rna2*, *rna4*, or *rna11* (strains YR02-92, YRO4-304, YR11-182, respectively; Table 1) were transformed with one of two libraries of DNA fragments prepared from *S. cerevisiae*. One of these libraries (from which *RNA2*$^+$ was isolated) was from a total *Bam*HI digest of strain CLP1 DNA (13) carried on pJZ1 (47), and the other library (from which *RNA4*$^+$ and *RNA11*$^+$ were isolated) was a partial *Hin*dIII digestion of strain S288C DNA carried on pYF91 (51) (the cloning vectors and typical shuttle vectors containing a 2 μm plasmid replication origin, the *LEU2*$^+$ *S. cerevisiae* gene, and pBR322 sequences). Transformants which were both Leu$^+$ and temperature resistant when grown at 37°C were selected. An 18-h period of growth at 23°C was included before growth at 37°C to allow expression of the cloned wild-type alleles of the *rna* temperature-sensitive genes. Since yeast cells usually take up more than one plasmid during the transformation procedure, several transformant colonies were isolated, crude DNA extracts were prepared from them, and the DNA was used to transform *E. coli*. In comparison with *S. cerevisiae*, the probability of multiple plasmid uptake by *E. coli* is much lower. Thus, this step results in plasmid segregation. DNA was isolated from several ampicillin-resistant *E. coli* colonies and was used for retransformation of the appropriate *leu2 rna S. cerevisiae* mutant. About 20% of the crude *E. coli* DNA preparations tested in the second round of yeast transformations was able to complement both the *leu2* and *rna* mutations. These plasmids were purified by cesium chloride-ethidium bromide isopycnic gradient centrifugation. Portions of the *S. cerevisiae* DNA carried on these plasmids were subcloned to pBR322 or pBR325 for more detailed restriction endonuclease analysis. Restriction maps of the cloned genes are shown in Fig. 1. DNA blot-hybridization analyses with DNA digested with six different restriction endonucleases (*Hin*dIII, *Bam*HI, *Pst*I, *Pvu*I, *Eco*RI, *Xba*I) and probes comprising the entire cloned DNA fragments showed that each of the three cloned genes was present as only a single copy on the haploid chromosome (unpublished data).

Localizing the RNA genes. Genetic methods for localizing a gene on a cloned DNA fragment rely on either replicating or nonreplicating plasmids. In the former case, one depends on complementation in *trans* of a chromosomal mutation by an intact, fully functional wild-type copy of the gene that is carried on the replicating plasmid. In the second case, one relies on recombination to regenerate a functional copy of the gene at the genetic locus on the chromosome. Directed integration of nonreplicating plasmids into the *S. cerevisiae* genome has been described previously (16, 40). Normally the efficiency of transformation with a nonreplicating plasmid in *S. cerevisiae* is several orders of magnitude lower than for autonomously replicating plasmids (2, 16, 52). This efficiency can be greatly enhanced by cutting the plasmid with restriction endonucleases in a region of homology to chromosomal DNA. The free DNA ends enhance recombination, and the plasmid is directed into the chromosomal site that is homologous to the cloned DNA fragment (40). For mapping purposes, the recombination event can be detected by including a *URA3*$^+$-containing DNA fragment on the plasmid and transforming a nonreverting *ura3*$^-$ host strain (*ura3-52* [5]).

(i) ***RNA2*$^+$.** Fragments were subcloned in pBR325 and used for transformation of *S. cerevisiae* YRO2-92 (Fig. 2A). There was a significant increase in the efficiency of complementation of the *rna2* mutation after restriction endonuclease digestion when plasmids carrying fragments A, B, and C were used (Fig. 2A). Only the largest of these three fragments (C) complemented *rna2* when introduced into cells on a replicating plasmid (Fig. 1A). (Note that, as discussed above in the case of expression from a replicating plasmid, the entire functional gene is needed for complementation in

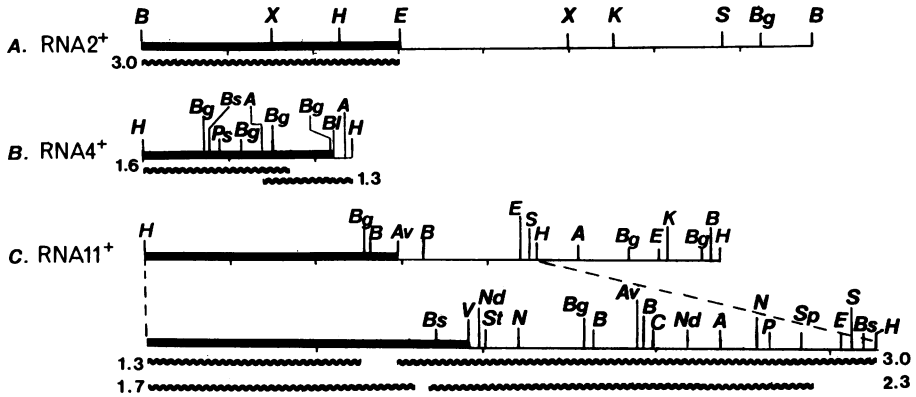

FIG. 1. Restriction endonuclease maps of DNA fragments carrying the *RNA2*$^+$, *RNA4*$^+$, and *RNA11*$^+$ genes. Thick lines represent the smallest fragments that are sufficient for complementation of the relevant mutated gene when carried on autonomously replicating vectors. Wavy lines represent approximate positions of the transcripts detected by RNA blot-hybridization. Numbers indicate the transcript sizes in kb. Downward pointing marks are 1 kb apart. Restriction endonuclease abbreviations: A, *Acc*I; Av, *Ava*I; B, *Bam*HI; Bl, *Bal*I; Bg, *Bgl*II; Bs, *Bst*XI; C, *Cla*I; E, *Eco*RI; H, *Hin*dIII; K, *Kpn*I; N, *Nco*I; Nd, *Nde*I; P, *Pvu*I; Ps, *Pst*I; S, *Sal*I; Sp, *Sph*I; St, *Stu*I; V, *Eco*RV; X, *Xba*I.

trans.) From these data we conclude that wild-type sequences corresponding to the mutated site of the *rna2* gene are located on the smallest of the fragments (A) and that the intact functional gene is carried completely on fragment C.

(ii) ***RNA4***$^+$. The smallest subcloned DNA fragment capable of complementing the *rna4* mutation in *trans* was a 2-kb *Hin*dIII-*Bal*I fragment (Fig. 1B). In view of the transcript analysis discussed below, further localization was not necessary.

(iii) ***RNA11***$^+$. The data from integrative transformation of subcloned DNA fragments indicate that the *rna11* mutation lies between the leftward *Hin*dIII site and the *Eco*RV site of

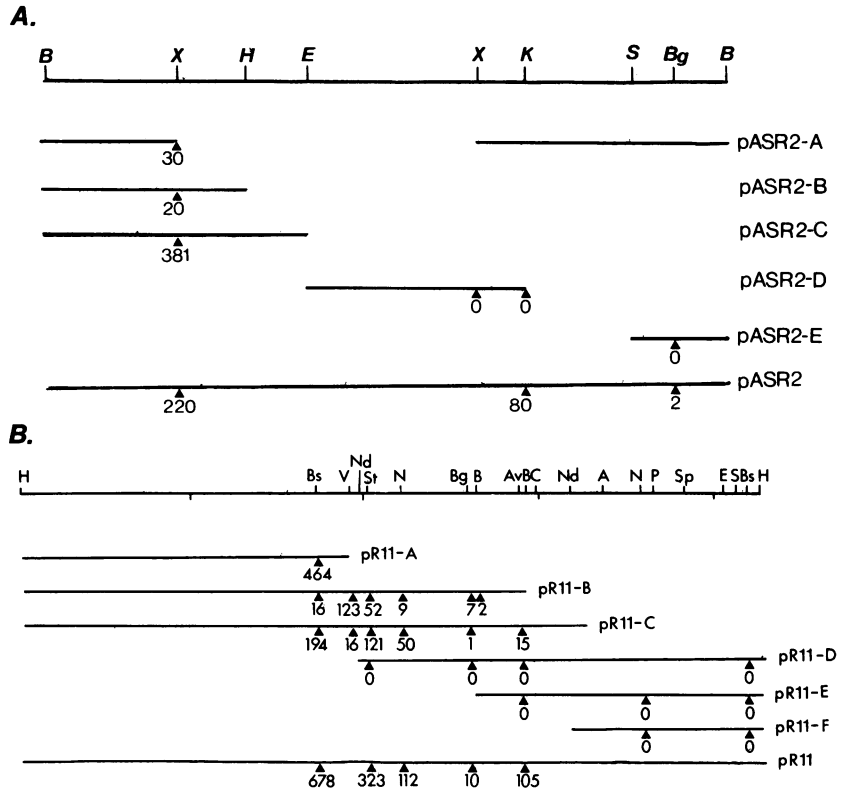

FIG. 2. Localization of *RNA2*$^+$ (A) and *RNA11*$^+$ (B) genes on the *S. cerevisiae* DNA insert. DNA fragments that complemented *rna2* or *rna11* mutations were transferred to pBR325 (pASR2 series) or pBR322 (pR11 series), respectively. DNA fragments (horizontal lines) were subcloned by complete or partial (as in the case of pR11-B) restriction endonuclease digestion and ligation of plasmids pASR2 or pR11. Triangles indicate restriction sites used to direct integrative transformation (39). Numbers below each triangle indicate the number of integrative temperature-resistant transformants per microgram of DNA when the plasmid was cut at the indicated site. The number of transformants obtained with the intact plasmids was 1 to 6 per microgram. Restriction endonuclease abbreviations are as given in the legend to Fig. 1.

the DNA fragment shown in Fig. 2B, since a plasmid that carries this fragment (pR11-A) can correct the *rna11* defect by integration. In addition, when the leftward *Hin*dIII to *Eco*RV fragment carried on pR11-A was transferred to a replicating plasmid, pYF91, this complemented *rna11* in *trans* (data not shown). From this, we conclude that *RNA11*$^+$ lies between the leftward *Hin*dIII and the *Eco*RV sites on the DNA fragment shown in Fig. 1 and 2.

Transcript analysis. The sizes and locations of transcripts from the three cloned genes were determined by blot-hybridization analyses of total RNA extracted from exponentially growing cultures; DNA probes derived from different segments of the cloned genes were used.

(i) *RNA2*$^+$. Only one transcript of 3.0 kb was found (Fig. 3A, lanes a and b), using as a probe the *Bam*HI-*Hin*dIII fragment (ASR2-B; Fig. 2A). We semiquantitatively determined the relative abundance of *RNA2*$^+$ mRNA in comparison with *tcm*, a ribosomal protein mRNA of middle-abundance (21); by inspection we estimated that the ratio of *RNA2*$^+$ mRNA to *tcm* RNA is about 1:20 (Fig. 3A, lanes a and d). When *RNA2*$^+$ was transferred to a typical replicating plasmid (pJZ1) that is present in 5 to 10 copies per cell (9, 52), the mRNA level increased approximately fivefold (Fig. 3A, lanes a and b), indicating the absence of strict dosage compensation. The size of the *rna2* mRNA isolated from an *rna2* mutant at either the permissive or the restrictive temperature was 3.0 kb (Fig. 3B, lanes c and d), and there was no decrease in the amount of this transcript. Since it has been shown that the *rna2* mutants grown at the restrictive temperature fail to process mRNA introns (see above), the lack of effect on its own transcript is consistent with the possibility that this gene itself has no, or an undetectably small, intron. Under the same conditions, the presence of the intron in the *S. cerevisiae* actin mRNA (11, 39, 53) was detected, as was the typical reduction in the abundance of mature mRNA (Fig. 3B, lanes a and b).

(ii) *RNA4*$^+$. The 2.3-kb *Hin*dIII DNA fragment that carries the *RNA4*$^+$ gene was found to hybridize to two RNA species, 1.3 and 1.6 kb, of approximately equal intensity (Fig. 3C). In a strain that carries the 2.3-kb *Hin*dIII fragment on a 5- to 10-copy replicating plasmid, both transcripts are overproduced (Fig. 3C; cf. lanes a and b with lanes c and d). Thus, both transcripts probably initiate and terminate in this *Hin*dIII fragment. Since the sum of the two transcripts (2.9 kb) is somewhat greater than the size of the *Hin*dIII fragment (2.3 kb), it is possible that these two transcripts overlap. When isolated fragments of the *RNA4*$^+$ region were used as probes in a more detailed RNA blot-hybridization experiment (Fig. 4), it was clear that the 1.6-kb transcript lies to the left and center of the *RNA4*$^+$ region, and the 1.3-kb transcript lies to the center and right of this region (Fig. 4). Results of this experiment also support the suggestion that the two transcripts overlap. Since the subcloned *Hin*dIII-*Bal*I fragment carries the functional *RNA4*$^+$ gene (see above), it is most probable that the 1.6-kb transcript encodes *RNA4*$^+$. The experiment presented in Fig. 3B (lane e) yields no evidence that the *RNA4*$^+$ gene has an intron.

(iii) *RNA11*$^+$. The *RNA11*$^+$ clone hybridizes to four transcripts of 1.3, 1.7, 2.3, and 3.0 kb (Fig. 5A, lane a). None of the transcripts was overproduced significantly when the *RNA11*$^+$ region was carried on a 5- to 10-copy replicating plasmid (Fig. 5, lane b). Experiments with isolated DNA fragments used as probes indicated that the 1.3- and 1.7-kb transcripts lie to the left and the 2.3- and 3.0-kb transcripts lie to the middle and right on the cloned fragment (Fig. 5B); these transcripts are indicated in Fig. 1 and 5. When com-

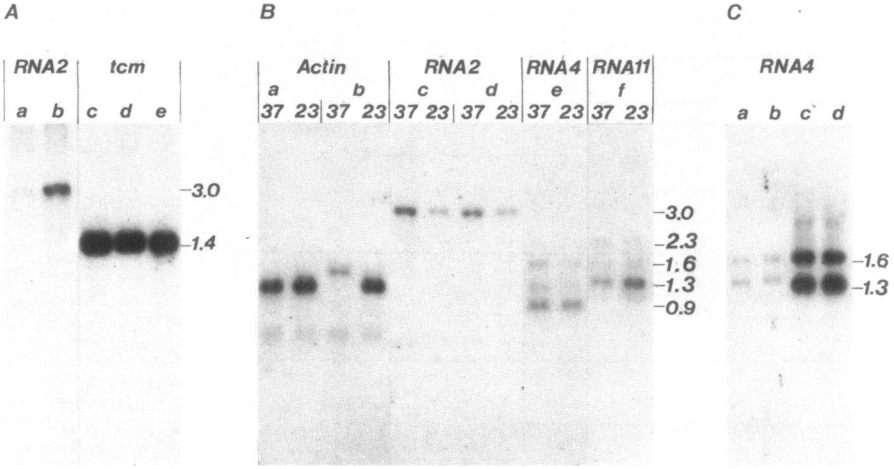

FIG. 3. Characterization of transcripts coded by cloned DNA fragments. (A) Blot-hybridization analysis of total RNA isolated from strains YR02-92 (*rna2*) (lanes a and d), A364A (*RNA*$^+$) (lane c), or YR02-92 transformed with plasmid pJZ1-R2-C (a replicating plasmid carrying the functional *RNA2*$^+$ *Hin*dIII-*Eco*RI gene derived from pASR2-C) (lanes b and e). Lanes a and b were probed with a purified *Hin*dIII-*Eco*RI DNA fragment carrying the *RNA2*$^+$ gene (Fig. 1); lanes c through e were probed with a DNA fragment coding for ribosomal protein L3 (*tcm*) (from plasmid pLS5T [46]). The *RNA2*$^+$ transcript is 3.0 kb, and the *tcm* transcript is 1.4 kb. (B) Blot-hybridization of total RNA isolated from strain A364A (*RNA*$^+$) (lanes a and c) or strain YR02-92 (*rna2*) (lanes b, d, e, and f) grown at the permissive temperature (23°C) or after the transfer of cells to the restrictive temperature (37°C) for 2 h. Blots were probed with plasmid pYactI, a pBR322 derivative carrying the actin gene (12, 38) (obtained from R. Ng) (lanes a and b), or purified DNA fragments carrying the *RNA2*$^+$ gene *Hin*dIII-*Eco*RI DNA fragment (lanes c and d), the *RNA4*$^+$ gene (*Hin*dIII-*Hin*dIII DNA fragment) (lane e), or the *RNA11*$^+$ gene (*Hin*dIII-*Eco*RV DNA fragment) (lane f). Lane e was also probed with DNA carrying the *URA3*$^+$ gene to observe that equivalent amounts of RNA were loaded in each lane (0.9-kb bands). (C) Total RNA isolated from strain A364A (*RNA*$^+$) (lane a) or YR04-304 (*rna4*) (lane b) or YR04-304 transformed with plasmid pYF91 carrying the DNA fragment coding for the *RNA4*$^+$ gene (pYF91-R4) (lanes c and d). The blot was probed with the isolated *Hin*dIII DNA fragment carrying the *RNA4*$^+$ gene. Lanes c and d show RNA from duplicate independent transformants. Equal amounts (20 μg) of total RNA were loaded onto each lane as judged by optical density and by probing a parallel blot with a DNA fragment carrying the *URA3*$^+$ gene.

bined with the genetic data presented above, the results shown in Fig. 5B suggest that either the 1.3- or 1.7-kb transcript encodes *RNA11*$^+$. Neither the 1.3- nor the 1.7-kb transcript from the *RNA11*$^+$ region appears to contain an intron (Fig. 3B, lanes f).

Genetic analysis. (i) Specificity of complementation. Each of the mutants *rna2*, *rna4*, *rna11* was transformed with a 5- to 10-copy replicating vector carrying the *RNA2*$^+$, *RNA4*$^+$, or *RNA11*$^+$ gene. For transformation we used temperature-sensitive *rna* strains (YR02-92, YR04-304, YR11-182), which were constructed in our laboratory, as well as those obtained from the Yeast Genetic Stock Center, University of California; Berkeley. With these low-copy-number plasmids, each cloned gene complements mutations only in its own gene, indicating that each gene encodes a separate function, as indicated by an earlier complementation analysis (20) (Table 2). We did not test cross-complementation with high-copy-number plasmids that overproduce the *RNA*$^+$ gene products to a high degree.

(ii) Genetic mapping. Using integrative transformation, we mapped the cloned DNA fragments to show that we had isolated the actual structural genes and not a second site suppressor. This mapping was done by constructing nonreplicating plasmids which carry both the cloned *RNA*$^+$ and *URA3*$^+$. Mapping of the *RNA2*$^+$ DNA fragment illustrates the method (Fig. 6). The nonreplicating plasmid pASU12 carries a portion of *RNA2*$^+$ that is capable of correcting the chromosomal *rna2* mutation; this was determined from

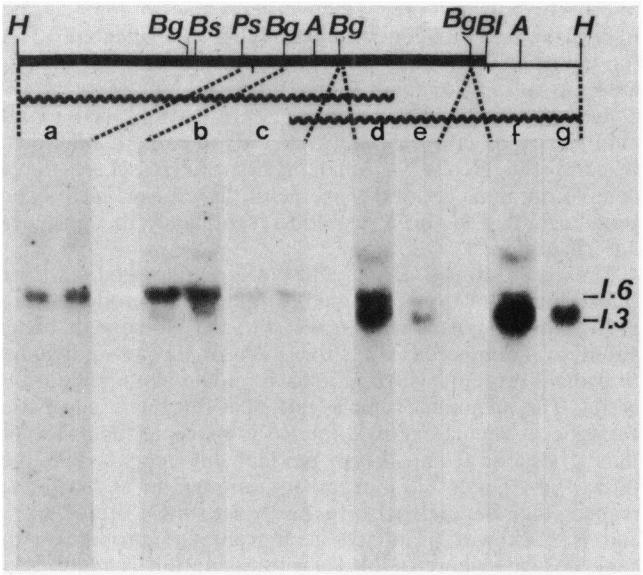

FIG. 4. Analysis of transcripts coded by DNA fragments containing small regions of the *RNA4*$^+$ insert. Total RNA was isolated from strain YR04-304 (*rna4*) transformed with pFY91 carrying the entire *RNA4*$^+$ DNA fragment and was probed with the isolated DNA fragments indicated by the stippled lines (from left to right): *Hin*dIII-*Pst*I, 0.9 kb; *Bgl*II-*Bgl*II, 0.26 kb; *Bgl*II-*Bgl*II, 0.75 kb; *Bgl*II-*Hin*dIII, 0.3 kb. The data in lanes a through c are duplicates of RNA extracted from two independent colonies. Lane c is a shorter exposure of lane b, lane e is a shorter exposure of lane d, and lane g is a shorter exposure of lane f. The wavy lines represent the two transcripts from the *RNA4*$^+$ region. The upper faint band (in lanes d and f) results from transcription of the vector portion of the plasmid since it does not appear in RNA that is isolated from untransformed cells. Restriction endonuclease abbreviations are as given in the legend to Fig. 1.

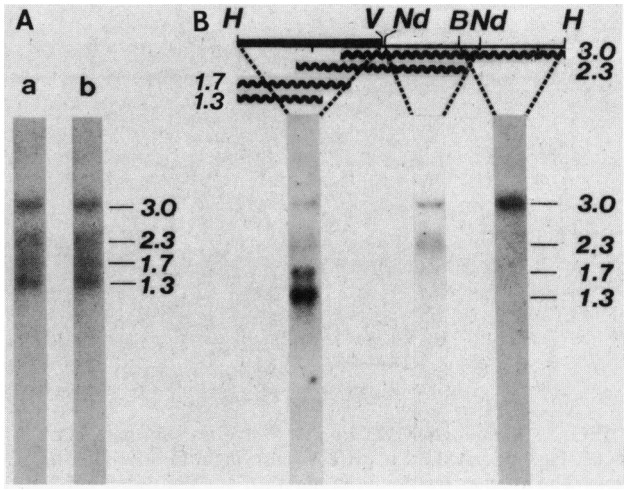

FIG. 5. Analysis of transcripts in the *RNA11*$^+$ region. (A) Total RNA was isolated from strain YR11-182 (*rna11*) (lane a) or strain YR11-182 (lane b) transformed with a replicating plasmid that carries the *Hin*dIII DNA fragment with the entire *RNA11*$^+$ (Fig. 1). The probe was the purified *Hin*dIII fragment. Equal amounts (20 μg) of total RNA were loaded in both lanes as indicated by optical density and ethidium bromide staining. (B) Total RNA was isolated from strain YR11-182 and probed with the isolated DNA fragments indicated by the stippled lines (from left to right): *Hin*dIII-*Eco*RV, 1.8 kb; *Nde*I-*Nde*I, 1.1 kb; *Bam*HI-*Hin*dIII, 1.2 kb. The wavy lines represent the approximate placement of the transcripts. The numbers indicate the size of transcripts in kb. Restriction endonuclease abbreviations are as given in the legend to Fig. 1.

previous experiments (Fig. 2A). This plasmid also carries the *URA3*$^+$ gene. Plasmid pASU12 was used to transform strain YR2-172 (α *ura3-2 rna2*; for other markers, see Table 1) by directed integration with a restriction endonuclease (*Xba*I) that cuts in *RNA2*$^+$; the resulting transformant was Ura$^+$ and temperature resistant. DNA blot-hybridization analyses showed that the plasmid had become integrated into the chromosomal site that we identified as *rna2* (Fig. 6B). Integrative transformants were crossed with strain CG378 (**a** *ura3-52 RNA2*$^+$). Integration of the plasmid into an unlinked suppressor site should result in the recovery of haploid spores with the *rna2 ura3* phenotypes. In the population one would expect the following progeny: *RNA2*$^+$ *URA3*$^+$/*RNA2*$^+$ *ura3*/*rna2 ura3* in a ratio of 2:1:1. However, if plasmid pASU12 were integrated into the chromosomal *rna2* gene, the expected segregation ratio of *RNA2*$^+$

TABLE 2. Complementation of *rna* temperature-sensitive mutations by cloned genes

Host strain (genotype)	No. of temperature-resistant transformants per μg of DNA for the following strains[a]:		
	pJZ1-R2	pYF91-R4	pYF91-R11
ts368[b] (*rna2*)	1,200	0	0
YR02-92 (*rna2*)	4,400	0	0
YR04-304 (*rna4*)		294	0
ts382 (*rna11*)	0	0	495
YR011-182 (*rna11*)	0	0	6,620

[a] Replicating plasmids carrying the *RNA2*$^+$ (pJZ1-R2), *RNA4*$^+$ (pYF91-R4), or *RNA11*$^+$ (pYF91-R11) genes were used to transform the indicated host strains. Temperature-resistant colonies appearing at 37°C were scored after 4 days.

[b] ts, Temperature sensitive.

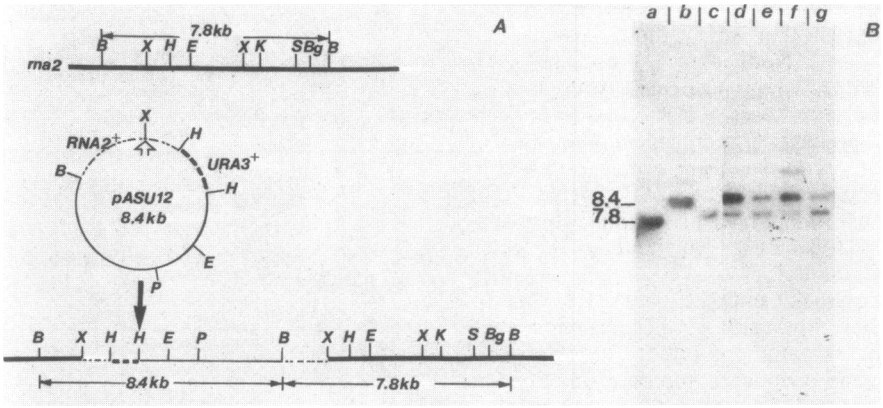

FIG. 6. Mapping *RNA2*$^+$ by integrative transformation. (A) The upper part of the diagram is a restriction map of the chromosomal *rna2* locus. Plasmid pASU12 is pBR322 carrying a *Bam*HI-*Hin*dIII DNA fragment with a terminal portion of the *RNA2*$^+$ and the entire *URA3*$^+$ gene. The open arrow points to the *Xba*I site that was used to direct plasmid recombination into the chromosome (39). The lower portion of the diagram represents the expected result of the integration event. Restriction endonuclease abbreviations: B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; H, *Hin*dIII; K, *Kpn*I; S, *Sal*I; X, *Xba*I. (B) DNA blot-hybridization analysis. The *Bam*HI DNA fragment from plasmid pASR2 (lane a), DNA from plasmid pASU12 linearized with *Xba*I (lane b), and DNA extracted from strain YR2-172 (*rna2*) (lane c) or from strain YR2-172 integratively transformed with pASU12 (lanes d through g) were digested with *Bam*HI and electrophoresed on a 0.8% agarose gel. The blot was probed with nick-translated pASR2-C plasmid (Fig. 2A).

URA3$^+$/*RNA2*$^+$ *ura3* would be 2:2, behaving as a two-factor cross. The latter was found to be the case in 23 of 23 tetrads analyzed.

An analogous experiment was carried out with the cloned *RNA4*$^+$ DNA fragment. Strain SR25-1A (**a** *ura3-52 RNA4*$^+$) was transformed with a plasmid consisting of pJH18 (carrying the *URA3*$^+$ gene) and a *Hin*dIII-*Bal*I DNA fragment (Fig. 1) with the entire *RNA4*$^+$ gene. The *Bst*XI site was used to direct integration. Resulting Ura$^+$ transformants were crossed with strain YR4-8 (α *rna4 ura3-52*). In the case of integration into an unlinked suppressor locus, one would expect (as described above) the following progeny: *RNA4*$^+$ *URA3*$^+$/*RNA4*$^+$ *ura3*/*rna4 ura3* in a ratio of 2:1:1. On the other hand, integration of the plasmid into the chromosomal *rna4* gene should result in segregation of *RNA4*$^+$ *URA3*$^+$/*rna4 ura3* of 2:2. From 20 tetrads dissected, all 20 exhibited the latter pattern of segregation.

A mapping experiment was also carried out with the *RNA11*$^+$ gene, although in this case linkage to *trp1* was measured. Strain YR11-5 (**a** *rna11 trp1 ura3-52*) was transformed with plasmid pJH18 (containing the *URA3*$^+$ gene) carrying the *Hin*dIII-*Eco*RV DNA fragment of the *RNA11*$^+$ gene. Integration was directed to the *rna11* locus by digesting the plasmid with *Bst*XI. Transformants that were both *URA3*$^+$ and *RNA11*$^+$ were crossed with strain YR11-A (α *rna11 TRP1*$^+$ *ura3-52*) Tetrad analysis of 49 tetrads showed 32 parental ditypes, 17 tetratypes, and 0 nonparental ditypes. This result indicated that *URA3*$^+$ (tightly linked to *RNA11*$^+$ by integration of the *URA3*$^+$ *RNA11*$^+$ plasmid into the *rna11* locus) and *trp1* were 17.3 ± 0.07 centiMorgans apart, which is in excellent agreement with the published linkage data of 17.2 centiMorgans between *rna11* and *trp1* (37, 38).

DISCUSSION

We isolated and made an initial characterization of 3 (*RNA2*$^+$, *RNA4*$^+$, and *RNA11*$^+$) of a set of 10 (*rna2* through *rna11*) temperature-sensitive genes that have a pleiotropic effect in *S. cerevisiae* (see above). As far as can be ascertained from the available data, all 10 genes have a very similar phenotype (12, 19, 20). This phenotype has evolved as information has accumulated (see above) but has most recently centered on a defect in mRNA intron processing (10, 29, 46, 53). One cannot be certain that even this is the last word in *rna2* through *rna11* phenotype.

The three genes that we isolated most likely code for proteins, rather than, for example, small nuclear RNA species (32), because their transcripts are in the range of 1.3 to 3.0 kb. Furthermore, we have been able to demonstrate hybridization to oligodeoxythymidylic acid-selected RNA (unpublished data), and thus the transcripts are polyadenylated. According to their transcript sizes, the proteins encoded by *RNA2*$^+$, *RNA4*$^+$, and *RNA11*$^+$ would have maximum sizes of approximately 90, 50, and 40 kilodaltons, respectively. Genes for small nuclear RNA species have been found in *S. cerevisiae*; some of these genes have been isolated (57), and these bear no resemblance to the genes described here.

The three cloned *RNA* genes are similar in that their transcripts, and thus presumably their gene products, are present in low abundance in the cell; we estimate that it is about 5% compared with the mRNA for an individual ribosomal protein, which is itself a middle-abundance transcript. The meaning of this is not clear but might suggest a catalytic or regulatory role for *RNA* gene products, rather than a structural one. From our data, it appears that the three *RNA* genes differ from one another in at least one respect: their dependence on gene dosage. mRNA for *RNA2*$^+$ and *RNA4*$^+$ was produced in approximate proportion to gene dosage, whereas *RNA11*$^+$ transcription is much less dependent on gene dosage. This might be a reflection of differences in biosynthetic regulation: *RNA2*$^+$ and *RNA4*$^+$ might be transcribed from constitutive promoters, or perhaps regulated post-transcriptionally, and *RNA11*$^+$ might be subjected to direct transcriptional regulation. The availability of the cloned genes will enable clarification of this question. The transcripts from the *RNA4*$^+$ and *RNA11*$^+$ genes are unexpectedly complex; both genes appear to have double transcripts. One possibility is that in each case the shorter transcript is a processing product of the larger; if so, this is probably not due to intron processing because we could find no evidence for this. A second possibility is the existence of multiple promoter sites. A third possibility is overlapping transcripts from opposite DNA strands.

There are at least three classes of function that *RNA* genes could encode. The first is a direct catalytic or stabilizing involvement in mRNA splicing. Since very little is known about the biochemical mechanism of mRNA splicing in *S. cerevisiae*, it is difficult to assess whether it might be so complex as to require 10 (or more) components. One can imagine one or several maturase-like proteins (30) that might act in concert with nucleotide sequences to bring the splice junctions into register, as well as scission and ligation functions such as have been demonstrated for tRNA (14, 43). A second possible class of functions for the *RNA* genes is to encode structural components of some basic cellular function, the integrity of which is essential for mRNA processing. Examples of this could be the protein components of heterogenous nuclear RNA particles (3, 25, 42, 45) or components of the nuclear matrix (18, 45, 45a) or nuclear pore (6, 45). If processing of mRNA were obligatorily coupled to its translocation through the nuclear envelope (17, 55), then any perturbation of that structure would inhibit translocation and thus, indirectly, mRNA splicing. A third possible class of functions for *RNA* genes could be components of a complex regulatory pathway for genes involved in mRNA processing. We cannot evaluate this possibility any further, except to note that at least one multistage regulatory cascade has been described in *S. cerevisiae* (22).

Southern blot analysis of genomic digests probed with *RNA2*$^+$, *RNA4*$^+$, and *RNA11*$^+$ cloned genes indicates that each is a single copy in a haploid genome (data not shown). Thus, by extrapolation it is likely that, although all 10 *rna* genes have a similar phenotype, they encode different functions. An apparently unrelated mutant, *rna1* (24) has been reported to affect the transport of all RNA species from the nucleus to the cytoplasm (24, 48). This mutant fails to process tRNA introns (23, 28), suggesting a relationship between transport and processing of tRNA. In an *rna1* mutant, mRNA processing can still occur, albeit with reduced efficiency (46). On the other hand, in *rna2* through *rna11* mutants tRNA synthesis is normal (20), but mRNA processing (10, 46) is absent. These observations suggest that tRNA and mRNA processing involve different functions in vivo. This is underlined by the fact that an *S. cerevisiae* in vitro tRNA processing system has been described previously (28, 43, 44), but one for mRNA has not. On the other hand, the *SRN1* mutation (41; see above) suppresses the phenotype of both *rna2* through *rna11* and *mal* mutations, suggesting that some functions are shared between the two classes of genes. One possible function for *SRN1* is as a regulatory gene, the alteration of which by mutation results in overproduction of the *RNA* gene products, thus compensating for the relative inactivity of any mutant gene product. This idea can be tested with the aid of the cloned *RNA* genes.

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