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RRP1, a *Saccharomyces cerevisiae* Gene Affecting rRNA Processing and Production of Mature Ribosomal Subunits

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The *Saccharomyces cerevisiae* mutant ts351 had been shown to affect processing of 27S pre-rRNA to mature 25S and 5.8S rRNAs (C. Andrew, A. K. Hopper, and B. D. Hall, Mol. Gen. Genet. 144:29–37, 1976). We showed that this strain contains two mutations leading to temperature-sensitive lethality. The rRNA-processing defect, however, is a result of only one of the two mutations. We designated the lesion responsible for the rRNA-processing defect *rrp1* and showed that it is located on the right arm of chromosome IV either allelic to or tightly linked to *mak21*. This *rrp1* lesion also results in hypersensitivity to aminoglycoside antibiotics and a reduced 25S/18S rRNA ratio at semipermissive temperatures. We cloned the *RRP1* gene and provide evidence that it encodes a moderately abundant mRNA which is in lower abundance and larger than most mRNAs encoding ribosomal proteins.

In many respects, rRNA processing in the yeast *Saccharomyces cerevisiae* is similar to what has been observed in higher eucaryotes (51). The primary pre-rRNA (35S) transcript is not normally processed before transcription termination (33). Before association of proteins with the primary pre-rRNA transcript, the latter is extensively modified (for a review, see reference 38). These modifications are, for the most part, at positions destined to become mature rRNAs (27). Undermethylation of the pre-rRNAs leads to poor processing (5, 53). The general pathway of the processing steps for yeast rRNAs is as follows. The primary 35S pre-rRNA is cleaved to produce 20S and 27S pre-rRNAs. These pre-rRNAs are then processed to produce, respectively, the mature 18S rRNA and the 25S and 5.8S rRNAs (19, 43, 45). The enzymes catalyzing these reactions are not well defined.

Normally, processing of the rRNAs requires assembly of the 35S pre-rRNA into a ribonucleoprotein particle (RNP) (44; for reviews, see references 18, 19, and 51). The pre-rRNP particle that contains the 35S pre-rRNA (the 90S RNP) is converted to 43S and 66S RNPs containing the 20S pre-rRNA and associated proteins. The 43S RNP moves from the nucleus to the cytoplasm, where the 20S pre-rRNA is processed to the 18S mature rRNA and the 43S RNP is converted to the mature 40S ribosomal subunit. Conversion of the 66S RNP (27S pre-rRNA) to the mature 60S ribosomal subunit containing the 25S and 5.8S rRNAs is a nuclear rather than a cytoplasmic event (44, 46; for a review, see reference 18).

Present on the precursor ribosomal 90S particle are many proteins which are present on the mature 60S ribosomal subunits, some ribosomal proteins present on mature 40S subunits, and so-called nucleolus-limited proteins. Nucleolus-limited proteins are thought to be structural proteins involved in the assembly of preribosomal particles and the enzymes involved in rRNA processing and modification (for a review, see reference 18). Ribosomal proteins are known to be phosphorylated and methylated, but the timing of these modifications is not known (for reviews, see references 38 and 51). Proteins associated with the ribosomal precursor RNPs are also thought to be modified (for a review, see reference 18).

A number of yeast mutants affected in rRNA processing have been identified (1, 2, 9, 17, 20, 31, 41, 48–50). Among these mutants, however, only a single temperature-sensitive (ts) lethal mutant, ts351, was found to be defective specifically in the conversion of 27S pre-rRNA to mature 25S and 5.8S rRNAs (1). Gorenstein and Warner (16) further demonstrated that the 27S pre-rRNA was relatively unstable in ts351 cells and that the proteins normally associated with the 60S subunit were rapidly degraded at the nonpermissive temperature.

The phenotype of ts351 suggested that the mutation responsible for the rRNA processing defect may affect any of the following: (i) a protein component of the mature ribosome, (ii) a nuclease responsible for conversion of 27S pre-rRNA to 25S and 5.8S rRNAs, (iii) an enzymatic activity required for modification of pre-RNA or proteins associated with the preribosomal particle, and (iv) a protein component of a precursor ribosomal particle.

To begin to understand the basis of the rRNA-processing defect of ts351, we performed further genetic characterization of the original mutant strain and initial molecular characterization of the corresponding wild-type gene and its product.

MATERIALS AND METHODS

Yeast strains and methods. The *S. cerevisiae* strains used in this study are listed in Table 1. YEPD, complete synthetic medium, and sporulation medium were prepared as previously described (22); MB was prepared as described by Wickner (54). Antibiotic-containing medium was prepared by addition of the antibiotic to YEPD at given concentrations.

Selection of diploid yeast strains was accomplished by either prototroph selection or zygote isolation by micromanipulation. Standard methods for sporulation, ascus dissection, and tetrad analysis were used (32).

Yeast was transformed by either the glusulase method of Hinnen et al. (21) or the lithium acetate method described by

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TABLE 1. Yeast strains

Strain	Genotype	Source
A364a	a *his7 lys2 ura1 ade2 ade1 tyr1 gal1*	Lee Hartwell
ts351	a *his7 lys2 ura1 ade2 ade1 tyr1 gal1 tsm⁻ rrp1*	This study
20B-12	α *trp1 gal2 pep4-3*	Elizabeth Jones
GRF123	α *trp1 ade1 rrp1 lys1 gal1*	Derived from 20B-12 × ts351
G1/7-5-13a	α *his7 leu1 ade2 trp1 lys2 tyr1 gal7 gal2*	This study
G1/7-5-13c	α *leu1 ade2 trp1 lys2 tyr1 gal7 gal2 rrp1*	This study
HIS4B/C-1-5b	α *ade⁻ trp1 lys2 his4b*	This study
HIS4C-2-5c	a *trp1 tyr1 ade2 ura1 lys2 his4c rrp1*	This study
4cTSF	α *trp1 ura3-52 leu2-3 leu2-112 rrp1 his⁻*	This study
4cTSF/int7	α *trp1 ura3-52 leu2-3 leu2-112 his⁻ rrp1::RRP1⁺ URA3⁺*	Plasmid integrant in 4cTSF
dds5	a *dds5-1 ade1 his1 trp2*	Lee Johnson
555	a *ura1 leu2 mak21-1*	Reed Wickner
1088	a/α *ade2/ade2 ura1/+ lys2/+ rna1-1/+ +/his8 ++/trp1 mak21*	Reed Wickner

Ito et al. (24). Plasmids were extracted from yeast cells by the method described by Zakian and Scott (57).

Assay for the presence of the yeast killer virus. The plate assay used to detect the presence of the yeast killer virus was performed by the procedure of Ridley et al. (37). The (Kil-o) diploid strain used was 5×47.

Bacterial strains and culture conditions. The *Escherichia coli* strains used in thus study were RRI (F^- *pro leu thi lacY* Str^r r_k^- m_k^- endoI$^-$) and MC1066 (*leuB trpC pyrF*::Tn*5* r_k^- m_k^- *araT* Δ*lacX74 rpsL*). The mutations *leuB*, *trpC*, and *pyrF* in strain MC1066 can be complemented by the wild-type yeast genes *LEU2*, *TRP1*, and *URA3*, respectively. Bacterial strains were grown at 37°C in L broth or on YT or M9 plates (29) supplemented as required with ampicillin (30 μg/ml), tetracycline (20 μg/ml), or chloramphenicol (20 μg/ml) and leucine, tryptophan, or uracil.

Bacterial transformation and plasmid isolation. *E. coli* strains were transformed with plasmid DNA by the calcium chloride procedure (29). Plasmid DNA was isolated from bacteria by a modification of the procedure of Birnboim and Doly (4) in which the clarified cell lysates were treated with diethylpyrocarbonate.

DNA manipulations. Restriction enzymes were obtained from a variety of manufacturers, and digestion with these enzymes was per manufacturer instructions. Bacterial alkaline phosphatase was obtained from Bethesda Research Laboratories, and treatment of restriction enzyme-digested DNA was as described by Ullrich et al. (47). T4 DNA ligase was obtained from Bethesda Research Laboratories, and ligation of restriction enzyme-digested DNA was performed as described by Lathe et al. (28). Specific DNA fragments derived from restriction enzyme-digested plasmids were purified as described by Benson (3).

DNA probes were radiolabeled with a Bethesda Research Laboratories Nick-Translation Reagent Kit with [^{32}P]dCTP or [^{32}P]TTP (Amersham Corp.). Labeled DNA was enriched by the spun-column procedure (29) with Sephadex G50 medium beads.

Gel electrophoresis of DNA, transfer to nitrocellulose, and hybridization. Typically, 0.7% agarose gels were used for separation of restriction enzyme-digested DNA. Electrophoresis, transfer to nitrocellulose, prehybridization, and hybridization were performed as previously described (29, 42).

Preparation of radioactively labeled RNA from yeast. RNA was prepared by modification of the method described by Hopper et al. (23). Overnight cultures of yeast cells grown in either complete synthetic medium or complete synthetic medium minus uracil were used to inoculate fresh cultures in the same media at a cell density of 1×10^7 to 1.5×10^7 cells per ml. These cultures were then grown for 90 min under permissive conditions before a shift to nonpermissive growth conditions. Cultures were grown under nonpermissive conditions for various periods before addition of 10 μCi of [5,6-^{3}H]uracil per ml (see Results). Samples of each culture were taken immediately before the addition of label, and cell densities (cells per milliliter) were determined. For steady-state labeling of RNA, cells were then grown for 1 h. For pulse-chase labeling of RNA, cells were grown in the presence of [5,6-^{3}H]uracil for 6 min before addition of a 100-fold excess of cold uracil. Cells were quickly cooled, harvested, washed once, and suspended in 0.2 ml of RNA extraction buffer (60 mM Tris hydrochloride, 150 mM NaCl, 5 mM EDTA, 5% sodium dodecyl sulfate, pH 7.4). The cells were broken by vortexing with glass beads and phenol and chloroform extracted, and the RNA was precipitated with ethanol.

Electrophoresis and fluorography of labeled RNA. RNA species were resolved on 1% agarose gels in 0.1 M $NaPO_4$ buffer, pH 6.5, after denaturation of the RNA with glyoxal (29). Gels were stained with methylene blue, destained, and impregnated with sodium salicylate (10). The dried gels were exposed to XAR-5 X-ray film at −20 or −70°C.

Laser scanning densitometry. We scanned autoradiogram exposures, for which the intensity of the bands had not exhausted the exposable silver grains, with a Zeineh Laser Scanning Densitometer with a wedge filter to produce a narrow scanning beam.

Preparation of total and poly(A)$^+$ RNAs for Northern hybridization. Cultures were inoculated at low density in YEPD and grown to a density of 2×10^7 to 4×10^7 cells per ml. Poly(A)$^+$ RNA was selected by a single round of oligo(dT)-cellulose chromatography as described by Maniatis et al. (29). Electrophoresis and transfer to nitrocellulose of RNA for Northern blots was as described by Maniatis et al. (29) and Thomas (42), using 1% agarose–formaldehyde gels.

RESULTS

Genetic characterization of the lesion in ts351. Initial investigation into the genetic basis of the rRNA-processing defect of strain ts351 revealed that the strain carried two loosely linked (44 centimorgans) ts mutations. One ts lesion was tightly linked (7.5 centimorgans) to the centromere marker *trp1*, and the second ts lesion showed weak linkage (37.9 centimorgans) to *trp1*. Only the *trp1* distal ts lesion was responsible for the rRNA-processing defect of ts351. This locus was named *RRP1* (ribosomal RNA processing). The rRNA-processing defect cosegregated with the *rrp1* mutation (data not shown). This processing defect of the single lesion appeared identical to the defect in strain ts351 in that the 35S pre-rRNA was made, 20S to 18S processing proceeded, and 27S pre-rRNA was produced but rapidly degraded (Fig. 1).

The *rrp1* lesion showed no linkage to the *dds5* mutation in crosses to strain dds5 (25), indicating that it does not map to

the left arm of chromosome IV. The genetic marker *mak21* is located on the right arm of chromosome IV approximately 43 centimorgans from *trp1* (55). *MAK21* is necessary for maintenance of the killer (kil$^+$) phenotype. In an attempt to construct an *rrp1* (kil$^+$) strain necessary to map *mak21* with respect to *rrp1*, GRF123 (*rrp1* [kil$^-$]) was mated to A364a (*RRP1* [kil$^+$]), resulting in a diploid which was kil$^+$. Progeny of this cross, however, showed 2:2 segregation for the kil$^+$:kil$^-$ phenotype (23 tetrads) with a single aberrant tetrad which, based on the segregation of other markers in this cross, was a real tetrad. The kil$^-$ phenotype always cosegregated with the ts phenotype of *rrp1*. The degree of the kil$^-$ phenotype of *rrp1* appears to be dependent on the genetic background. These data showed that the *rrp1* strains also carried a *mak* lesion tightly linked to *rrp1*, which suggested that *rrp1* is tightly linked or allelic to *mak21*.

The effects of aminoglycoside antibiotics on *rrp1* strains. Many mutations in bacteria and yeast which affect ribosome structure, function, or both have enhanced resistance or sensitivity to a variety of antibiotics (36, 40; for reviews, see references 34 and 51). Since *rrp1* affects production of mature ribosomes, we investigated the possibility of a drug-sensitive or -resistant phenotype of *rrp1* strains. Spores derived by dissection of the diploid HIS4B/C-1-5b (*RRP1*$^+$) × HIS4C-2-5c (*rrp1*) were replica plated to YEPD plates supplemented with cryptopleurine, trichodermin, cycloheximide, or paromomycin at a variety of concentrations. Sensitivity to paromomycin at 30°C segregated 2:2 and cosegregated in 8/8 tetrads with the ts phenotype of *rrp1*; the other drugs showed no effects. We then tested sensitivity to other aminoglycoside antibiotics; sensitivity to hygromycin B and neomycin B, and less severe sensitivity to streptomycin also cosegregated with the *rrp1* mutation in 32/32 tetrads. Sensitivity to the antibiotics was much clearer at 30°C than at 23°C. *rrp1*-bearing strains grow at 30°C but at a reduced rate, suggesting partial impairment of the *rrp1* product at this temperature. Table 2 summarizes the thresholds of aminoglycoside antibiotic growth inhibition for *rrp1* and wild-type strains. The data indicated that *rrp1* is at least 100 times more sensitive to neomycin and at least 10 times more sensitive to hygromycin than is the wild type.

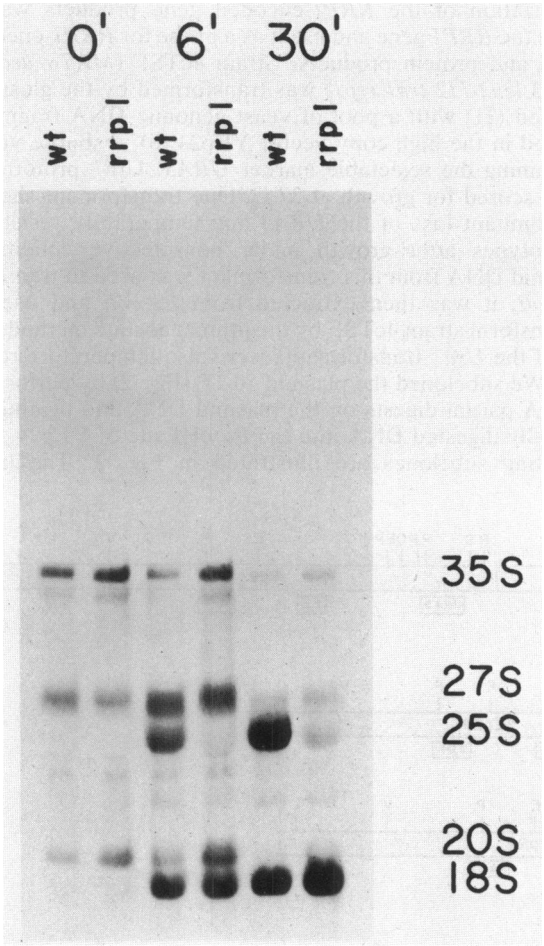

FIG. 1. The *rrp1* mutation blocks 27S pre-rRNA processing. RNAs of strains A364a (wt) and GRF123 (*rrp1*) were pulse labeled for 6 min with [^{3}H]uracil 30 min after a shift to 37°C and then chased with a 100-fold excess of unlabeled uracil as described in Materials and Methods. Samples were taken at the time of addition of unlabeled uracil (0') and at 6 min (6') and 30 min (30'). RNA (1.5×10^5 cpm) was denatured with glyoxal and resolved on a 1.0% agarose gel run in 0.1 M $NaPO_4$ buffer, pH 6.5, at 90 V for 6 h followed by fluorography.

TABLE 2. Thresholds of aminoglycoside antibiotic growth inhibition[a]

Antibiotic and concn (mg/ml)	Final OD_{650} for duplicate cultures of strain:	
	G1/7-5-13c (*rrp1*)	G1/7-5-13a (*RRP1*)
Neomycin		
10.00	0.24/0.30	1.80/1.69
1.00	0.80/0.96	2.22/1.98
0.10	1.75/1.90	2.71/2.80
0.01	2.27/2.40	2.73/2.78
0	2.35/2.38	2.90/>3.0
Hygromycin B		
1.000	0.12/0.09	0.08/0.11
0.100	0.09/0.11	0.12/0.06
0.010	0.10/0.09	2.43/2.71
0.001	2.72/2.85	2.96/>3.0
0	2.21/2.90	>3.0/2.88

[a] Duplicate cultures of 5 ml of YEPD were inoculated with approximately 10^6 cells per ml, each resulting in an initial optical density at 650 nm (OD_{650}) of less than 0.20. Cultures were then grown at 30°C for 3 days with aeration, and the final OD_{650} was read.

The *mak21* strain 555 and the *mak21*/*MAK21* heterozygous diploid (strain 1088) also showed a reduced growth rate in the presence of neomycin and hygromycin B. These data further implied a relationship between *rrp1* and *mak21*. This result is interesting in light of the report that at least one *MAK* locus, *MAK8*, encodes a ribosomal protein (56).

Aminoglycoside antibiotics, suspected to block protein synthesis, are known to cause misreading in vitro (12, 13) and suppression of nonsense and missense alleles in vivo (11, 39). Masurekar et al. (30) showed that omnipotent suppressor *SUP46*, together with paromomycin, resulted in lethality in vivo and enhanced misreading in vitro. *rrp1* segregants were examined for the ability to suppress a variety of missense and nonsense alleles, including *his7*, *leu1*, *gal1*, *gal7*, *lys2*, *tyr1*, *ade2-1*, *trp1-1*, *gal2*, *met8-1*, *leu2-1*, *ilv1-1*, and *lys1-1*. No evidence of suppressor activity of these mutations was evidenced. Thus, it appears unlikely that the mutation produces enough misreading to cause lethality in vivo by synergistic interaction with neomycin.

Effect of *rrp1* on processing of rRNA at 30°C. The rRNA-processing defect of *rrp1*-bearing strains grown at 37°C is nearly complete: greater than 95% inhibition of conversion

TABLE 3. Comparison of levels of rRNAs in *rrp1* and wild-type strains

Strain	*RRP1*	Trichloroacetic acid-precipitable count/cell at[a]:			Sp act of labeled RNA (cpm/μg)	% RNA[b]	RNA ratio[c]			Band density[d]		
		0 min	30 min	60 min			25S/18S	25S/4S	18S/4S	25S	18S	25S/18S ratio
G1/7-5-13a	+	0.001	0.050	0.081	11,136	44	1.62	9.56	5.89	82	52	1.58
G1/7-5-13c	−	0.001	0.060	0.088	12,057	57	0.98	7.25	7.36	64	74	0.86

[a] Samples of each culture were taken immediately before the addition of label, and cell densities were determined. Duplicate 50-μl samples were taken at 0, 30, and 60 min and added to cold 5% trichloroacetic acid. Average counts per minute per cell were then determined.

[b] Percentage of the initial counts per minute recovered from solubilized gel slices.

[c] Labeled RNAs were separated electrophoretically in a 1.5% agarose–6 M urea gel run in 0.025 M citric acid buffer, pH 3.5, and prepared for autoradiography as described in Materials and Methods. The autoradiogram and gel were then aligned, and slices covering each sample lane of the gel were excised. We placed these gel fragments in test tubes with 2 ml of water and heated them to 95°C for 30 min to solubilize the contents. Ratios of counts per minute corresponding to bands associated with the various RNA species were determined. The 5S and 5.8S rRNAs were not separated under these experimental conditions.

[d] Band densities are based on laser densitometer scans of autoradiograms.

of 27S to 25S and 5.8S rRNAs (16). The growth rate of *rrp1* strains at 23°C is approximately equal to that of wild-type strains, but at 30°C *rrp1* strains grow more slowly than do wild-type strains. Given the slowed growth rate of *rrp1* strains and the severe inhibition of growth caused by neomycin at 30°C, we compared rRNA synthesis in *rrp1* cells grown at 30°C with that of wild-type cells. Wild-type and mutant strains had approximately equal levels of [^{3}H]uracil incorporation, in a 1-h steady-state label, into total cellular RNA based on trichloroacetic acid-precipitable counts per cell (Table 3). The specific activities of the purified RNAs were also approximately equal for both strains. Thus, there did not appear to be a large difference in the total amount of rRNA present in each strain. The labeled RNAs were resolved on agarose gels, and the ratio of 25S to 18S rRNA was determined by elution and counting of [^{3}H]uracil in individual RNA species. The ratio of radioactivity in 25S as compared with 18S rRNA in *rrp1* strains was less than that in wild-type strains (Table 3). Comparison of the rRNA levels with the 4S band in each lane also supported the conclusion that the rRNA ratio is skewed in mutant strains. These conclusions were supported by laser scanning of autoradiograms. Thus, it is possible that there is a reduction in the number of functional ribosomes in the *rrp1* strain grown at 30°C relative to the wild-type strain under similar conditions. None of these observations were changed in the presence of neomycin (data not shown).

***RRP1* gene.** The most straightforward approach for characterization of the *RRP1*-encoded gene products was to clone the *RRP1* gene and use it as a probe for *RRP1*-encoded RNA and protein products. Strain 4cTSF (*MATα ura3-52 leu2-3 leu2-112 trp1 rrp1*) was transformed by the glusulase method (21) with a pool of yeast genomic DNA fragments carried in the high-copy vector YEp24 (8), a shuttle vector containing the selectable marker *URA3*. Ura$^+$ prototrophs were scored for growth at 37°C. One transformant showed concomitant loss of the *URA3* and temperature resistance phenotypes after growth under nonselective conditions. Plasmid DNA from this transformant was used to transform *E. coli*; it was then extracted from *E. coli* and used to retransform strain 4cTSF by the lithium acetate method (24). All of the *Ura*$^+$ transformants were also temperature resistant. We subcloned the plasmid 50-1F (Fig. 2) by performing *Sau*3A partial digests on the plasmid DNA and ligating the partially digested DNA into the *Bam*HI site of YEp24. Two resulting subclones are illustrated in Fig. 2. The larger

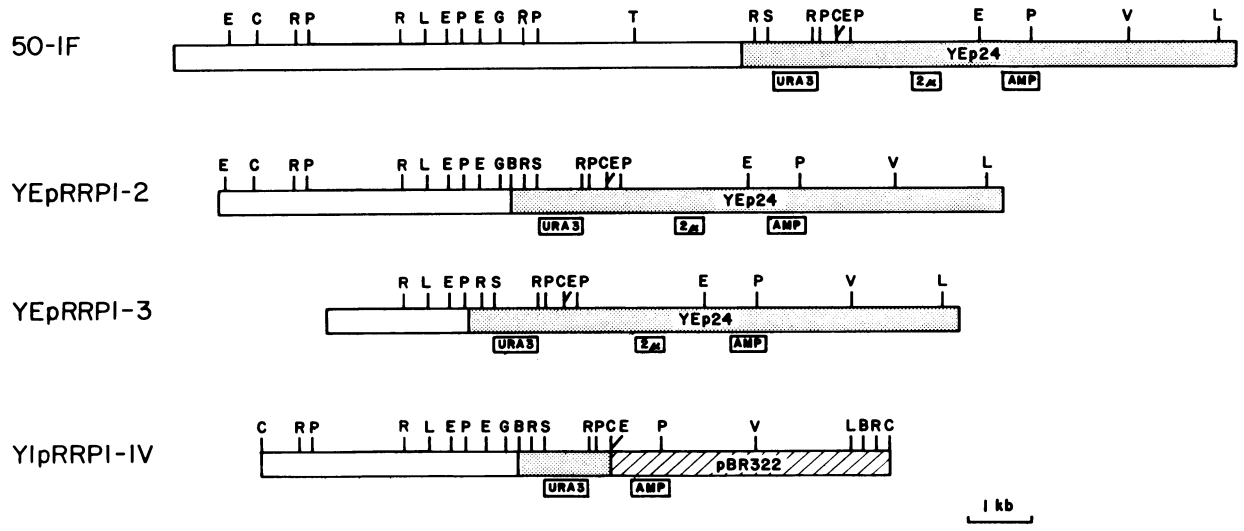

FIG. 2. Restriction maps of plasmids 50-1F, YEpRRP1-2, YEpRRP1-3, and YIpRRP1-IV. The restriction sites are as follows: B, *Bam*HI; C, *Cla*I; E, *Eco*RI; G, *Bgl*II; P, *Pst*I; L, *Sal*I; R, *Eco*RV; S, *Sma*I; T, *Sst*I; and V, *Pvu*II. The approximate locations of the sequences for the *URA3* gene, the *AMP* gene, and yeast 2μm are shown in blocks below the restriction maps. Genomic sequences are open boxes, YEp24 vector sequences are stippled, and pBR322 sequences are slashed. The size of 1 kb is shown as a bar for reference.

subclone, YEpRRP1-2, has a genomic insert of approximately 4.5 kilobases (kb), and a *Bam*HI site was fortuitously created upon ligation into the vector. The smaller subclone, YEpRRP1-3, has a genomic insert of approximately 2.3 kb.

To verify that the authentic *RRP1* locus had been cloned, we constructed an integrating vector not containing 2μm sequences. The approximately 5.5-kb *Cla*I fragment of YEpRRP1-2 that includes the *URA3* gene was ligated into vector pBR322, which had been cleaved with the same restriction enzyme. This integrating vector, YIpRRP1-IV (Fig. 2), was then used to transform strain 4cTSF, and *URA3* transformants were selected. To verify that the putative integrant 4cTSF/int7 carried plasmid sequences, we compared the restriction digestion patterns of genomic DNAs from 4cTSF/int7 and wild-type A364a. This analysis confirmed that integration of the cloned sequence was directed to the homologous chromosomal locus and showed that the coding sequences of *RRP1* were present in a single copy per genome (data not shown). We then crossed strain 4cTSF/int7 to the *RRP1* wild-type strain A364a to verify the integration genetically. In eight of eight tetrads the expected results were obtained.

RNA product of *RRP1*. Fragments of the insert in YEpRRP1-3 were used to examine total and poly(A)$^+$ RNAs purified from strain A364a. The 0.7-kb *Eco*RI-*Eco*RV fragment hybridized to a 1.3-kb RNA (not shown); the 1.8-kb

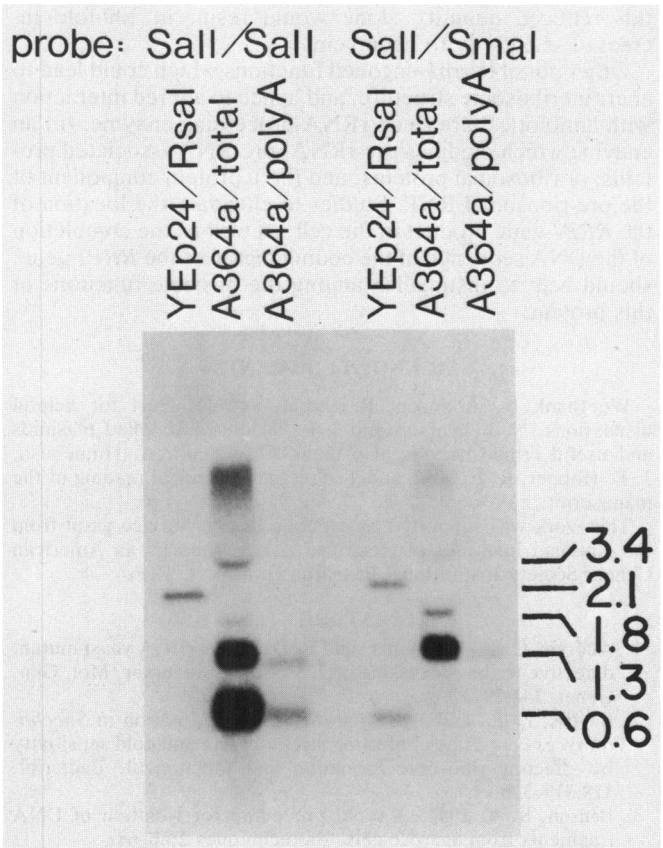

FIG. 3. Analysis of RNAs hybridizing to YEpRRP1-3. Total RNA (10 μg; A364a/total) and poly(A)$^+$ RNA [2 μg; A364a/polyA], both isolated from the wild-type strain A364a, were loaded in each lane. Electrophoresis, transfer, and hybridizations were done as described in Materials and Methods. The nick-translated probes used were the 1.8-kb *Sal*I-*Sal*I and 1.0-kb *Sal*I-*Sma*I fragments of YEpRRP1-3. YEp24, digested with the restiction enzyme *Rsa*I, was used as a size standard.

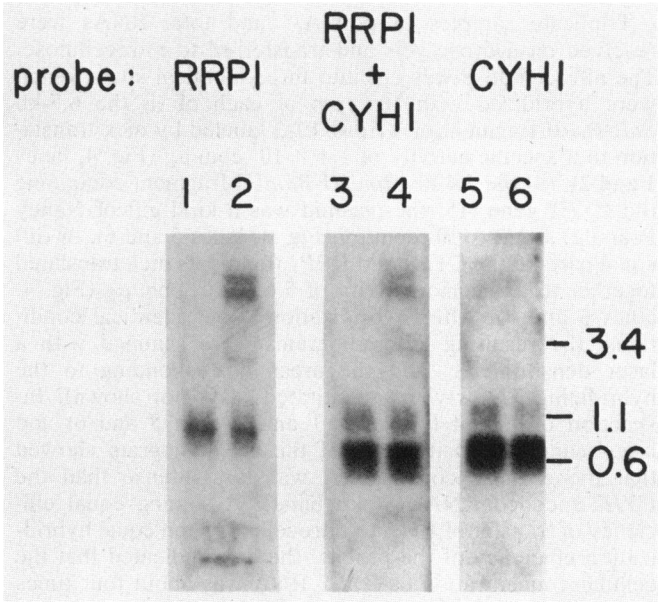

FIG. 4. Quantitation of the level of *RRP1* RNA relative to that of *CYH2* mRNA. RNAs were isolated from A364a, resolved on agarose gels, transferred to nitrocellulose, and hybridized with nick-translated DNA probes. Total RNA (10 μg; lanes 2, 4, and 6) or poly(A)$^+$ RNA (2 μg; lanes 1, 3, and 5) was loaded in each lane. Lanes 1 and 2 were probed with the 6.7-kb *Sal*I-*Pvu*II fragment of YEpRRP1-3 (Fig. 2); specific activity, 1.4×10^7 cpm/μg. Lanes 5 and 6 were probed with the 5.4-kb *Hin*dIII-*Bam*HI fragment containing the *CYH2* gene; specific activity, 1.2×10^7 cpm/μg. Lanes 3 and 4 were probed with a combination of equal parts of 5.4-kb *Hin*dIII-*Bam*HI *CYH2* mRNA and 6.7-kb *Sal*I-*Pvu*II *RRP1* RNA co-nick translated (specific activity, 5.4×10^6 cpm/μg). Migration of size standards is indicated on the right in kilobases.

*Sal*I-*Sal*I fragment of YEpRRP1-3 hybridized to two RNAs, 0.6 and 1.3 kb; and the 1.0-kb *Sal*I-*Sma*I fragment hybridized to two RNAs, 1.3 and 2.0 kb (Fig. 3). The 2-kb band was not reproducible and did not appear on subsequent RNA blots. Both the 1.8-kb *Sal*I-*Sal*I and 1.0-kb *Sal*I-*Sma*I fragments were individually subcloned into YEp24; neither of the resulting plasmids could complement the *rrp1* mutation. The data from the RNA analysis, taken in conjunction with the complementation data, indicated that the 1.3-kb RNA is the *RRP1* gene product and that the *Sal*I site interrupts the *RRP1* coding sequence.

To determine whether disruption of the *Sal*I site would destroy the complementing ability of the YEpRRP1-3 plasmid, we partially digested the plasmid with the enzyme *Sal*I and ligated it with the 2.5-kb *Xho*I-*Sal*I fragment of Yep13, which contains the *LEU2* gene (6) selected for in *E. coli* MC1066. A plasmid was isolated that had the *LEU2*-containing fragment inserted into the *RRP1 Sal*I site. This plasmid, when transformed into strain 4cTSF, gave only Ura$^+$ Leu$^+$ transformants that were ts. This result supports the assignment of the 1.3-kb RNA as the *RRP1*-encoded gene product.

Level of *RRP1*-encoded RNA relative to CYH2 mRNA. Since yeast normally maintains equimolar amounts of ribosomal protein mRNAs (26), it was of interest to compare the level of *RRP1*-encoded RNA with that of a ribosomal protein. We attempted to determine the relative levels of the 1.3-kb *RRP1*-encoded RNA and the 0.6-kb *CYH2*-encoded RNA (which codes for ribosomal protein L29) by Northern analyses.

Triplicate samples of poly(A)$^+$ and total RNAs were resolved on agarose gels and transferred to nitrocellulose. The nitrocellulose was cut into three identical strips which were hybridized with 10^7 cpm of each of (i) the 6.7-kb *Sal*I-*Pvu*II fragment of YEpRRP1-3 labeled by nick translation to a specific activity of 1.4×10^7 cpm/μg (Fig. 4, lanes 1 and 2), (ii) the 5.4-kb *Hin*dIII-*Bam*HI fragment containing the *CYH2* gene (15; the plasmid was a kind gift of Nancy Pearson) at 1.2×10^7 cpm/μg (Fig. 4, lanes 5 and 6), or (iii) equal parts of the *CYH2* and *RRP1* fragments nick translated together to a specific activity of 5.6×10^6 cpm/μg (Fig. 4, lanes 3 and 4). After hybridization under identical conditions, the resulting autoradiograms were scanned with a laser densitometer, and the areas corresponding to the hybridizing RNAs were quantitated (data not shown). Inspection of Fig. 4 (cf. lanes 1 and 2 with 5 and 6) and laser-scanning densitometry of the autoradiogram showed that the *RRP1*-encoded RNA was less intense than the *CYH2*-encoded RNA (0.6-kb band). Assuming equal efficiency of transfer of RNA to nitrocellulose and equal hybridization efficiency of the probes, the data indicated that the cellular concentration of *CYH2* RNA was about four times greater than that of *RRP1* RNA. This was further substantiated by the results of the hybridization in which *RRP1* and *CYH2* probes were colabeled (Fig. 4, lanes 3 and 4). However, this later analysis was complicated by the fact that the *CYH2* pre-mRNA (1.1 kb) comigrated on this gel with the 1.3-kb *RRP1* RNA. Correcting for the amount of 1.1-kb pre-mRNA expected and assuming that the two probes were nick translated with equal efficiency, it appeared that the level of *RRP1* RNA was approximately 4.5-fold less than the level of *CYH2* mature RNA. Both of these comparisons are minimum estimates of the *RRP1* RNA levels since the *RRP1* probe is about 24% larger than the *CYH2* probe. Thus, the concentration of *RRP1* mRNA appears to be substantially lower than that of the *CYH2* ribosomal protein mRNA.

DISCUSSION

Andrew et al. (1) showed that strain ts351 was defective in processing of 27S pre-rRNA to 25S and 5.8S mature rRNAs. Our genetic studies showed that ts351 contained two linked ts mutations. The mutation responsible for the rRNA processing defect was named *rrp1*.

We showed that the mutation in *RRP1* results in sensitivity to aminoglycoside antibiotics. Although interactions of the aminoglycoside antibiotics have been observed with the large and small bacterial ribosomal subunits (7), the primary interactions of the aminoglycoside antibiotics appear to be with the small subunit (for a review, see reference 35). The major defect of *rrp1* strains appears to be the block in conversion of 27S to 25S and 5.8S rRNAs; however, given the slowed conversion of 20S to 18S rRNA and the aminoglycoside sensitivity, the possibility that the *rrp1* lesion affects production of normal small subunits must be considered.

There are several possible explanations for the drug sensitivity. One possibility is that, according to the conventional means by which aminoglycoside antibiotics affect ribosomes, we would have expected the hypersensitivity of the *rrp1* mutant to these drugs to be due to an altered ribosomal protein. Preliminary DNA sequence analysis showed that *RRP1* does contain an open reading frame (R. J. Crouch and L. J. Lempereur, personal communication). However, two indirect lines of evidence suggest that this protein is not present on mature cytoplasmic ribosomes.

(i) Assuming a linear correlation between the size of mature mRNA and the translated product, it appears that the *RRP1*-encoded protein is larger than the vast majority of ribosomal proteins. Only a few of the ribosomal proteins are greater than 30 kb (51). Provided that a smaller protein does not arise through mRNA or protein processing, the size of the *RRP1* mRNA suggests that it encodes a protein of a size approximately equal to that of L3, the largest known ribosomal protein (14, 51). L3, encoded by *TCM1*, has a 1.3-kb mRNA and produces an approximately 50-kilodalton protein (14). *RRP1* and *TCM1* are not alleles since they are located on different chromosomes and each is present in only single copy based on genomic DNA analysis (14; this study). (ii) The mRNAs of ribosomal proteins are maintained in equimolar quantities (26, 52), and the abundance of the *RRP1* RNA is about 4.5-fold less than that of *CYH2* mRNA. Although neither of these data proves that *RRP1* does not encode a ribosomal protein, both argue that it does not.

An alternative explanation is that the hypersensitivity of *rrp1* cells to antibiotics could be due to an unconventional mechanism such as slower ribosome assembly at semipermissive temperatures or a decreased number of ribosomes. Analysis of the RNA species synthesized at 30°C showed that *rrp1* cells contained approximately half as much 25S rRNA relative to 18S rRNA as did wild-type cells. Thus, *rrp1* cells may contain half as many functional ribosomes as do wild-type cells. However, it is difficult to imagine how this reduced quantity alone would result in 100-fold increased sensitivity to neomycin.

Other possible *rrp1*-encoded functions which could lead to aberrant ribosome structure, and hence to altered interaction with antibiotics, are (i) an rRNA-processing enzyme; (ii) an enzyme which modifies pre-rRNA, pre-RNP-associated proteins, or ribosomal proteins; and (iii) a protein component of the pre-ribosomal RNP. Studies to elucidate the location of the *RRP1* gene product in the cell, as well as the completion of the DNA sequence of the coding region of the *RRP1* gene, should help to distinguish among the possible functions of this protein.

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