Two mutant forms of the S1/TPR-containing protein Rrp5p affect the 18S rRNA synthesis in Saccharomyces cerevisiae

C. Torchet, C. Jacq and S. Hermann-Le Denmat

RNA 1998 4: 1636-1652

RNA (1998), *4*:1636–1652. Cambridge University Press. Printed in the USA.

Two mutant forms of the S1/TPR-containing protein Rrp5p affect the 18S rRNA synthesis in *Saccharomyces cerevisiae*

CLAIRE TORCHET, CLAUDE JACQ, and SYLVIE HERMANN-LE DENMAT

Laboratoire de Génétique Moléculaire, Ecole Normale Supérieure, URA C.N.R.S. 1302,
46 rue d'Ulm F-75230 (Cedex 05) Paris, France

ABSTRACT

The genetic depletion of yeast Rrp5p results in a synthesis defect of both 18S and 5.8S ribosomal RNAs (Venema J, Tollervey D. 1996. *EMBO J 15*:5701–5714). We have isolated the *RRP5* gene in a genetic approach aimed to select for yeast factors interfering with protein import into mitochondria. We describe here a striking feature of Rrp5p amino acid sequence, namely the presence of twelve putative S1 RNA-binding motifs and seven tetratricopeptide repeats (TPR) motifs. We have constructed two conditional temperature-sensitive alleles of *RRP5* gene and analyzed them for associated rRNA-processing defects. First, a functional "bipartite gene" was generated revealing that the S1 and TPR parts of the protein can act independently of each other. We also generated a two amino acid deletion in TPR unit 1 (*rrp5Δ6* allele). The two mutant forms of Rrp5p were shown to cause a defect in 18S rRNA synthesis with no detectable effects on 5.8S rRNA production. However, the rRNA processing pathway was differently affected in each case. Interestingly, the *ROK1* gene which, like *RRP5*, was previously isolated in a screen for synthetic lethal mutations with snR10 deletion, was here identified as a high copy suppressor of the *rrp5Δ6* temperature-sensitive allele. *ROK1* also acts as a low copy suppressor but cannot bypass the cellular requirement for *RRP5*. Furthermore, we show that suppression by the Rok1p putative RNA helicase rescues the 18S rRNA synthesis defect caused by the *rrp5Δ6* mutation.

Keywords: ribosomal protein S1; ribosome; RNA helicase; rRNA processing; yeast

INTRODUCTION

The nucleolus compartment of eukaryotic cells is devoted to the assembly of large and small ribosomal subunits (reviewed by Mélèse & Xue, 1995). The association of almost 80 ribosomal proteins into 60S and 40S subunits occurs concomitantly with the maturation of ribosomal RNA (rRNA) molecules. The rRNAs have a mixed transcriptional origin. The nucleolar RNA polymerase I transcribes a single, large rRNA precursor 35S which is processed into three mature rRNAs (18S, 5.8S and 25S). The fourth eukaryotic rRNA molecule (5S) is transcribed independently by RNA polymerase III. The 5S, 5.8S and 25S rRNA species constitute the RNA molecules of mature 60S subunits whereas mature 40S subunits contain only 18S rRNA. The maturation of the pre-rRNA 35S polymerase I transcript is largely studied in yeast and numerous small nucleolar RNAs (snoRNAs) and protein components involved in this process have been characterized (Eichler & Craig, 1994; Venema & Tollervey, 1995; Tollervey & Kiss, 1997). The 35S primary transcript is successively matured by both endonucleolytic and exonucleolytic cleavages that generate stable rRNA precursors whose identification by in vivo labeling or by Northern hybridization has led to the processing pattern drawn in Figure 1. The nucleic cleavages contribute to a precise excision of both external (5′ and 3′ ETS) and internal transcribed spacers (ITS1 and ITS2) that interrupt the 35S rRNA sequence. Aberrations in any of the cleavage steps result in a defective maturation that modifies the reference pattern shown in Figure 1. In such cases, an increase or decrease in intermediate precursors is evident whereas some aberrant and sometimes dead-end pre-rRNA species are detected. This observation of altered profiles is classically used to determine at which step either a given mutation or the depletion of a cellular component will affect the rRNA maturation process. These approaches have indeed revealed that a large number of trans-acting factors are required for

Reprint requests to: Sylvie Hermann-Le Denmat, Laboratoire de Génétique Moléculaire, Ecole Normale Supérieure, URA C.N.R.S. 1302, 46 rue d'Ulm F-75230 (Cedex 05) Paris, France; e-mail: shermann@biologie.ens.fr.

the same processing step (Venema & Tollervey, 1995). For example, the snoRNAs U3, U14, snR30, and snR10 are all essential (or at least important in the case of snR10) for efficient cleavage at sites A_0, A_1, and A_2 (Tollervey, 1987; Li et al., 1990; Hughes & Ares, 1991; Morrissey & Tollervey, 1993; see Fig. 1B). The snoRNAs are believed to function as small nucleolar ribonucleoprotein (snoRNP) complexes rather than as free RNAs (Maxwell & Fournier, 1995) and some nucleolar proteins were shown to be physically associated with snoRNAs (Maxwell & Fournier, 1995; Smith & Steitz, 1997). Two RNP particles involved in the yeast rRNA processing are considered in this article: the above-mentioned snoRNP complex (Venema & Tollervey, 1995) and the RNase MRP complex (Schmitt & Clayton, 1993; Morrissey & Tollervey, 1995), which are required for cleavage at sites A_0/A_1/A_2 and A_3 respectively (see Fig. 1B). An alteration of any early cleavages at sites A_0, A_1, or A_2 prevents synthesis of the mature 18S rRNA whereas the inhibition of cleavage at A_3 affects synthesis of the 5.8S rRNA.

It was found that *cis*-mutations at site A_2 can inhibit cleavage at site A_3 and reciprocally that a small deletion at A_3 inhibits cleavage at A_2 (Allmang et al., 1996). These results reveal that the processing events at sites A_2 and A_3 are not fully independent and suggest that the two above-mentioned RNP complexes may interact during the maturation process. Moreover, Venema & Tollervey (1996) have identified the essential yeast *RRP5* gene as the first rRNA processing component required for the synthesis of both 18S and 5.8S rRNAs. It was proposed that the product of the *RRP5* gene could indeed act as a "bridging" factor between the two RNP complexes.

We have independently isolated *RRP5* in a genetic screen aimed at selecting genes interfering with mitochondrial import (Claros et al., 1996; and unpubl. results). We present here the striking features of the Rrp5p protein which consists almost entirely of two kinds of repeated motifs: a large amino-terminal part of the protein contains twelve putative S1 RNA-binding motifs while the carboxy-terminal part is composed of seven tetratricopeptide repeats (TPR) motifs (see Fig. 2). Two mutant alleles of *RRP5* have been generated. Based on the particular repeat-arranged structure of Rrp5p, we have constructed the hereafter named "bipartite allele," that results in the independent co-expressions of the first nine S1 motifs and of the seven TPR units. This bipartite allele was unexpectedly functional. We have obtained a temperature-sensitive allele (*rrp5Δ6*) by deleting two amino acids in TPR unit 1. We show here that the two mutant alleles of *RRP5* cause a defect only in 18S rRNA synthesis without affecting the 5.8S rRNA formation, thus showing that the two rRNA-processing functions of Rrp5p can be separated. Finally, we have isolated the *ROK1* gene as a high copy and also low copy acting suppressor of the temperature-sensitive *rrp5Δ6* mutation and shown that the Rok1p putative RNA helicase can rescue the 18S rRNA synthesis defect caused by the temperature-sensitive *rrp5Δ6* allele.

RESULTS

RRP5 overexpression modifies mitochondrial import

The *RRP5* gene was isolated by two independent genetic approaches. Venema & Tollervey (1996) have identified *RRP5* as a new gene implicated in rRNA processing in a synthetic lethality screen with the snR10 deletion (see below). We have independently isolated *RRP5* as a gene whose overexpression interferes with mitochondrial import in a genetic screen based on respiratory growth rescue of the yeast strain CW02/S678M (Table 1). Briefly, the strain CW02 (Table 1) contains a mitochondrial deficiency for the essential bI4 maturase activity (Labouesse & Slonimski, 1983) that can be complemented by a cytoplasmic translated bI4 maturase (Banroques et al., 1986). Secondly, the strain CW02/S678M contains a cytoplasmic version of the bI4 maturase within the previously described S678M chimeric protein (Claros et al., 1995) that harbors hydrophobic sequences preventing its importation into mitochondria. The resulting CW02/S678M strain cannot grow on glycerol. Starting from this strain, we have isolated multicopy suppressors that restore growth on glycerol at 30 °C by transforming the yeast strain with a wild-type genomic library borne on the multicopy vector pFL44L (Claros et al., 1996; unpubl. results). For each transformant, we confirmed that plasmid retransformation of the original strain CW02/S678M allowed growth on glycerol while transformation of strain CW02 (Table 1) did not allow growth on glycerol, thus eliminating genes whose overexpression restored respiratory growth independently of the presence of the chimeric protein into the cell (Dujardin et al., 1983; Labouesse et al., 1985; see Materials and Methods). Finally, we confirmed that removing the suppressor plasmid restored the respiratory defect of the original strain CW02/S768M (data not shown).

Restriction mapping showed that five glycerol growing transformants contained plasmids with overlapping inserts. One of these plasmids (p44-*RRP5*) was partially sequenced and shown to contain a fragment from chromosome XIII comprising a unique 5,190 open reading frame (ORF) first named *FMI1* for Facilitator of Mitochondrial Import and hereafter named *RRP5* according to Venema & Tollervey (1996; accession number Z49939). The putative mechanism(s) by which *RRP5* could interfere with mitochondrial import are currently being studied (unpubl. results) and will be discussed below.

Northern analysis has revealed a single ~5.5-kb mRNA that is strongly overexpressed in strain CW02/

S678M containing the p44-*RRP5* multicopy plasmid (data not shown). The *RRP5* promoter region contained in the 6.6-kb insert of the isolated p44-*RRP5* plasmid is 804 bp long and encloses an ABF-I box (at position −198 to −210; Della Seta et al., 1990) and three HAP2,3,4 binding consensus sequences (at positions −223 to −229, −380 to −373, and −497 to −504; Rosenkrantz et al., 1994) that might be relevant

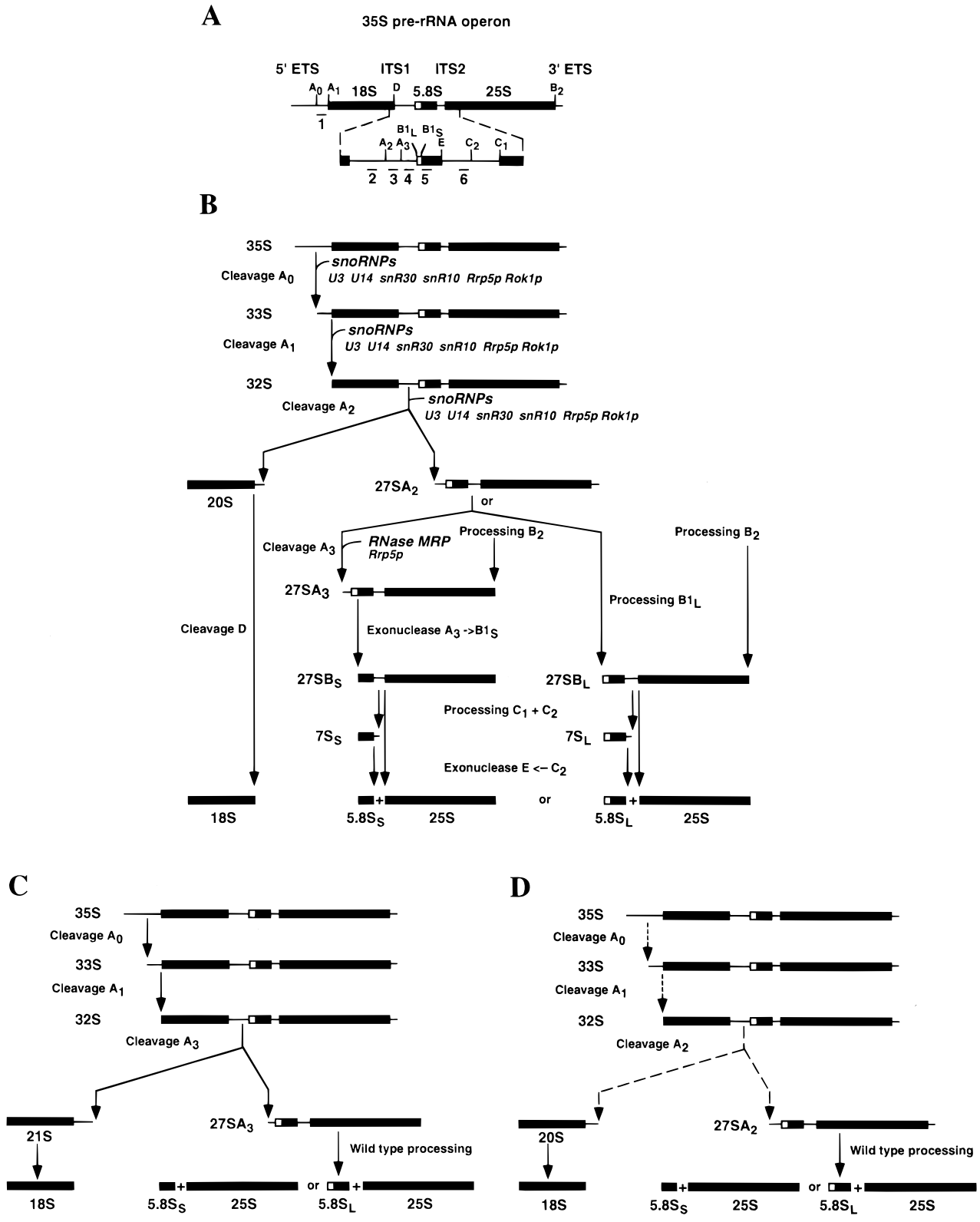

FIGURE 1. (*Legend on facing page.*)

to gene regulation. At the chromosomal location, the *RRP5* gene shares a divergent promoting region of 1,290 bp with the ORF YMR230w that codes for a predicted protein of 105 amino acids sharing similarity with the rat 40S ribosomal S10 protein (accession number S57957).

RRP5 gene codes for a conserved TPR and S1 motifs containing protein

The *RRP5* gene codes for a polypeptide of 1,729 amino acids (193 kDa) whose unique modular structure is described here and consists almost exclusively of repetitions of two kinds of motifs (Fig. 2A). Database searches first revealed significant homology to the 30S ribosomal protein S1 of bacteria (Schnier et al., 1982; Subramanian, 1983), which plays an essential role in the translation initiation in prokaryotes by both interacting with the small 30S ribosomal subunit and sequences in the 5′ ends of mRNAs (Subramanian, 1983; Boni et al., 1991; Voorma, 1996). The ribosomal protein S1 consists of tandem repeats of a stretch of 70 amino acids that were later identified in both prokaryotic and eukaryotic RNA-associated proteins (Company et al., 1991; Cormack et al., 1993; Bycroft et al., 1997). This domain was named the S1 RNA-binding motif or S1 motif. Whereas bacterial ribosomal protein S1 contains several S1 motifs (six for the Rps1p protein of *Escherichia coli*, see Fig. 2A,B), the other described S1-containing proteins possess only one S1 motif (Company et al., 1991; Bycroft et al., 1997). Since no S1-like ribosomal protein has been described in any eukaryote except human (Eklund et al., 1995), it is worth noting that the FASTA program gives a one-stretch alignment of the whole amino acid sequence of the *E. coli* Rps1p protein to an internal part of the yeast Rrp5p protein that encompasses residues 812 to 1373 (Fig. 2A,B). Over these 540 amino acids, the yeast and *E. coli* proteins share 21% identity and 55% similarity. Yeast Rrp5p contains six additional amino-terminal S1 motifs which are more divergent from the amino acid sequence of the *E. coli* Rps1p and cannot be aligned with the whole bacterial sequence. The twelve S1 motifs of Rrp5p protein extend over 62% of the length of the sequence (Fig. 2A). Rrp5p S1 motifs have been aligned with each other and a consensus sequence has been inferred from the alignment (Fig. 2C). The secondary structure of the S1 motif was recently resolved by Bycroft et al. (1997) and is shown in Figure 2C. This structural analysis has revealed well conserved residues which were also found in the Rrp5p S1 consensus: the glycine residues located in β strands 1, 2, and 3 or at the beginning of strand 4 and the valine/isoleucine residue located in strand 1 (Fig. 2C). Moreover, gaps or insertions are limited to regions outside the β strands except for strands 5 of repeats S1-3 and S1-4.

The carboxy-terminal part of Rrp5p contains seven tandem repeats of another motif: the tetratricopeptide repeat or TPR motif (Fig. 2A,D) that is involved in protein–protein interactions in a large array of structurally and functionally diverse proteins (Lamb et al., 1995). The TPR units are degenerated 34-amino-acid sequences that share a minimal homology limited to a few consensus residues (Sikorski et al., 1990). Higher degrees of sequence conservation can be found outside the consensus residues, thus defining TPR subfamilies. Database searches have revealed that the TPR domain of yeast Rrp5p is close to a part of the TPR domain of the *Drosophila crooked neck* (*crn*) protein that contains the longest tandem array of TPR units described to date (16 repeats; Zhang et al., 1991). Figure 2D shows an alignment of the seven TPR units of the Rrp5p protein along with a consensus sequence that is compared to the consensus given for the *cnr* protein (Zhang et al., 1991). The Rrp5p and *cnr* consensus share 41% identity and 68% similarity.

As previously reported (Venema & Tollervey, 1996), Rrp5p has homologues in human (accession number D80007) and *Caenorhabditis elegans* (accession num-

FIGURE 1. Scheme of pre-rRNA processing in *S. cerevisiae*. **A**: Structure and processing sites of the 35S pre-rRNA. The 35S operon contains the sequences for the mature 18S, 5.8S, and 25S rRNAs separated by the two internal transcribed spacers ITS1 and ITS2. Two external transcribed spacers, the 5′ ETS and the 3′ ETS, are present at either end. The locations of the different oligonucleotide probes (numbered from 1 to 6) used in this study are indicated by small lines. Black and white boxes represent mature rRNA species, and thin lines represent transcribed spacers. Cleavage sites are indicated by vertical lines. **B**: Pre-rRNA processing pathway. The 35S primary transcript is cleaved at site A_0 to give the 33S pre-rRNA. This molecule is rapidly processed at sites A_1 and A_2, giving rise successively to the 32S pre-rRNA and to the 20S and $27SA_2$ precursors. Cleavage at A_2 separates the pre-rRNAs destined for the small and large ribosomal subunits. The 20S precursor is matured by an endonucleolytic cleavage at site D to yield 18S rRNA. The $27SA_2$ precursor is processed by two alternative pathways, forming the mature 5.8S and 25S rRNAs. In the major processing pathway cleavage at site A_3 occurs, giving the $27SA_3$ species, which is then rapidly processed to site $B1_S$ by an exonuclease activity, generating the $27SB_S$ precursor. A minor pathway involves direct cleavage of the $27SA_2$ molecule at site $B1_L$, producing the $27SB_L$ pre-rRNA. At the same time as processing at B_1 is completed, the 3′ end of mature 25S rRNA is generated by processing at site B_2. The subsequent processing of both 27SB species appears to be identical. Cleavage at sites C_1 and C_2 releases the mature 25S rRNA and 7S pre-rRNAs. The 7S pre-rRNA undergoes rapid 3′ $\rightarrow$ 5′ exonuclease digestion to the 3′ end of mature 5.8S rRNA (site E). **C**: rRNA processing associated with the bipartite allele. Cleavage at site A_2 is inhibited and the 32S pre-rRNA is cleaved directly at site A_3 generating the aberrant 21S intermediate as well as the normal $27SA_3$ precursor. **D**: rRNA processing defects generated by the temperature-sensitive *rrp5Δ6* allele. Cleavage at sites A_0, A_1, and A_2 are altered as indicated by dotted lines.

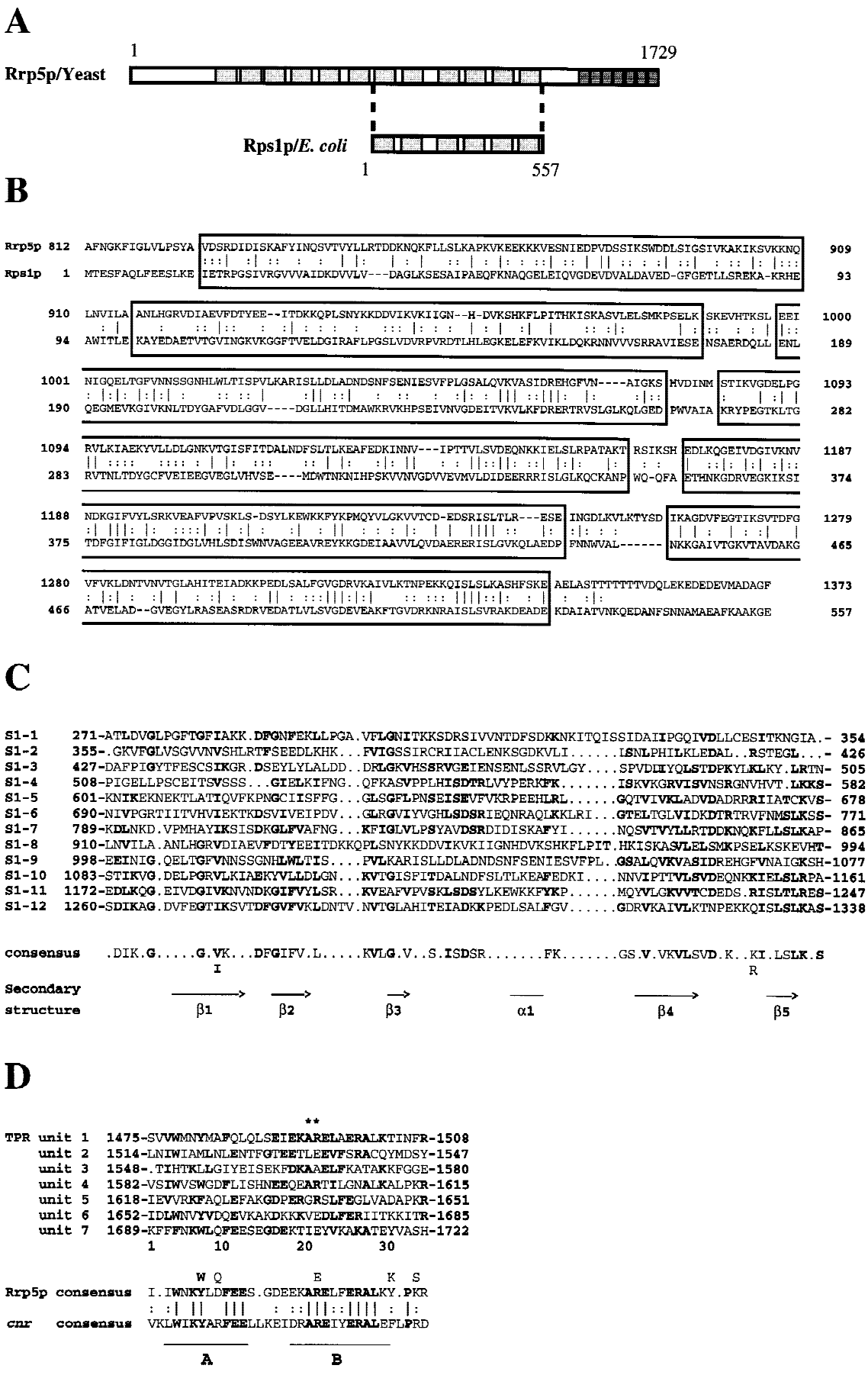

FIGURE 2. (*Legend on facing page.*)

TABLE 1. Yeast strains used in this study.

Strain	Relevant genotype	Reference
BMA41	*MAT**a**/MATα ura3-1/ ura3-1 ade2-1/ade2-1 leu2-3,112/leu2-3,112 his3-11,15/his3-11,15 Δtrp1/Δtrp1*	Baudin et al., 1993
CW02	*MATα ura3-1 ade2-1 leu2-3 his3-11 trp1-1 can1-100*	Labouesse & Slonimski, 1983
CW02/S678M	*MATα ura3-1 ade2-1 leu2-3 his3-11 trp1-1 can1-100* (pS678M *LEU2* 2μ)	Claros et al., 1995
YCJL1	*MAT**a**/MATα ura3-1/ ura3-1 ade2-1/ade2-1 leu2-3,112/leu2-3,112 his3-11,15/his3-11,15 Δtrp1/Δtrp1 rrp5Δ1::TRP1/RRP5*	This work
YCJL2	*MAT**a** ura3-1 ade2-1 leu2-3,112 his3-11,15 Δtrp1 rrp5Δ1::TRP1* (p36-*RRP5 LEU2* CEN)	This work
YCJL3	*MAT**a** ura3-1 ade2-1 leu2-3,112 his3-11,15 Δtrp1 rrp5Δ1::TRP1* (p38-*RRP5 URA3* CEN)	This work
YCJL4	*MAT**a** ura3-1 ade2-1 leu2-3,112 his3-11,15 Δtrp1 rrp5Δ1::TRP1* (p36-*rrp5Δ6 LEU2* CEN)	This work
YCJL5	*MATα ura3-1 ade2-1 leu2-3,112 his3-11,15 Δtrp1 rrp5Δ1::TRP1* (p44-*RRP5 URA3* 2μ)	This work
YCJL7	*MAT**a** ura3-1 ade2-1 leu2-3,112 his3-11,15 Δtrp1 rrp5Δ1::TRP1* (p44-*RRP5-N2 URA3* 2μ) (BFG1-*RRP5-C1 LEU2* 2μ)	This work
YCJL9	YCJL2 transformed with pFL44L	This work
YCJL10	YCJL4 transformed with pFL44L	This work
YCJL11	YCJL4 transformed with p44CT4	This work
YCJL12	YCJL4 transformed with p44-*ROK1*	This work
YCJL13	YCJL4 transformed with p38-*ROK1*	This work

ber U41534). These polypeptides present the same modular arrangement of S1 and TPR repeats (data not shown). The strongest similarity lies between each TPR domain of the three proteins. TPR domains of yeast and human proteins share 47% identity and 66% similarity over a range of 254 amino acids. In spite of the high degree of similarity, we could not observe any heterologous complementation when expressing the entire human cDNA in yeast. This was either assayed by suppression of the temperature sensitivity of the *rrp5Δ6* strain (Table 1) or by suppression of the lethality of a *RRP5* deleted strain (data not shown).

RRP5 is an essential gene that can be functionally divided in two parts

RRP5 was inactivated in the diploid strain BMA41 (Table 1) by a one-step disruption method (see Materials and Methods) and, as previously observed (Venema & Tollervey, 1996), tetrad analysis showed a 2:2 segregation for cell viability indicating that the *RRP5* deletion is lethal (data not shown). Microscopic observation showed that the two nonviable spores from each tetrad were able to germinate and gave three to four cells before ceasing to divide. The heterozygous diploid strain YCJL1 (Table 1) was used for subsequent work. First, we confirmed that p36-*RRP5* (*LEU2* CEN), p38-*RRP5* (*URA3* CEN), or p44-*RRP5* (*URA3* 2μ), vectors that all express the wild-type *RRP5* allele, restore viability of the haploid-deleted strains (strains YCJL2, YCJL3, and YCJL5, respectively; Table 1). The heterozygous diploid strain YCJL1 was also transformed with each of three carboxy-terminal-truncated versions of *RRP5* borne on the multicopy plasmid pFL44L and schematically drawn in Figure 3A (alleles *RRP5-N1*, *RRP5-N2,* and *RRP5-N3*; see Materials and Methods). Tetrad analysis showed a 2:2 segregation indicating that none of these truncated alleles restored viability. Nevertheless, microscopic observations showed that the shortened *RRP5-N1* and *RRP5-N2* constructions have

FIGURE 2. S1 and TPR motifs of the yeast protein Rrp5p. **A**: Schematic representation of Rrp5p with the location of the S1 and TPR motifs. The S1 stretches of 70 residues are indicated by grey boxes for yeast and *Escherichia coli* proteins. The seven carboxy-terminal tetratricopeptide (TPR) motifs are shown in darker boxes. **B**: Amino acids alignment of *E. coli* Rps1p with an internal part of yeast Rrp5p. The alignment was obtained with the FASTA program. Identical residues are indicated by vertical lines whereas conserved substitutions are marked by dots. The six S1 motifs of *E. coli* protein are boxed together with the homologous region of yeast Rrp5p. In each boxed region the percentage of identity are (in order): 19-11, 5-23-23-29, and 28%. The positions of residues are given for each protein. **C**: Alignment of the Rrp5p S1 motifs. The 12 repeats are aligned with a consensus sequence. In the alignment, the residues conserved in at least 3 of 12 positions are written in bold as are the conservative substitutions often found at the same position. The substitutions are based on the following groupings: (I, L, V); (K, R); (D, E); and (W, Y, F). For the consensus sequence only residues found in at least 42% (5 of 12) of the repeats are written in bold. The positions of residues at the N and C termini of the S1 motif are given. The secondary structure is indicated according to Bycroft et al. (1997). The five β strands are shown as arrows and the α-helix as a line. **D**: Alignment of Rrp5p TPR units. The 7 copies are aligned and residues conserved in at least 3 of 7 positions are written in bold, as are the conservative substitutions. The substitutions are based on the groups mentioned above. The positions of residues at the N and C termini of the TPR motif are given. A Rrp5p consensus sequence which identifies the most frequent residue found at each position (dot indicates 7 of 7 different residues) is compared to the *cnr* consensus sequence of the *Drosophila crooked neck* protein. The highly conserved residues fall into the two antiparallel α-helices termed A and B. Asterisks indicate the two residues of TPR unit 1 (alanine 1494 and arginine 1495) which were deleted in the *rrp5Δ6* allele.

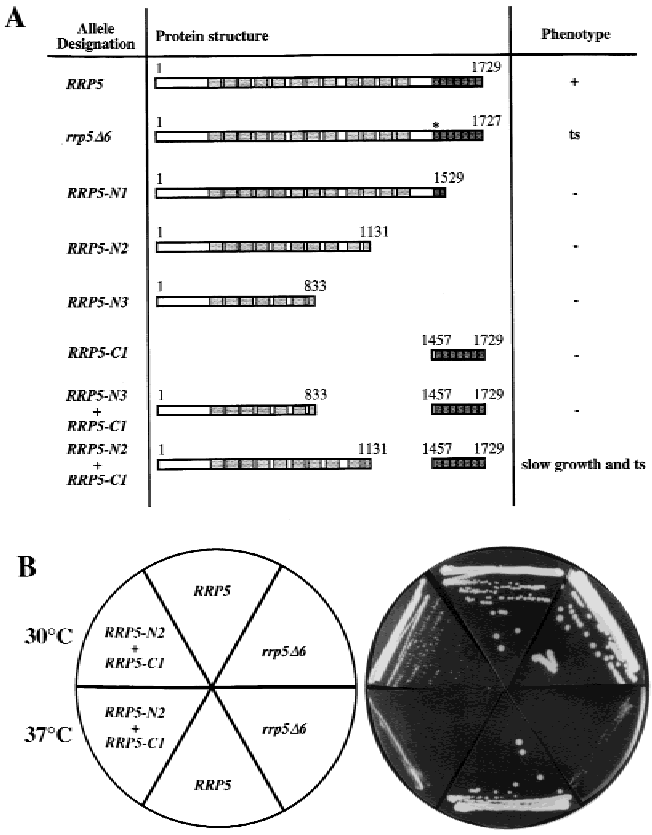

FIGURE 3. Schematic representation of the mutant forms of Rrp5p. **A**: The wild-type and mutant forms of yeast Rrp5p are drawn according to the scheme of Figure 2A. The phenotype symbols are +: wild-type growth; −: lethality; ts: temperature sensitivity. The asterisk indicates the mutated TPR unit 1 (*rrp5Δ6*). **B**: Growth on glucose minimum medium at 30 °C or 37 °C for the following strains: *RRP5*: wild-type control strain YCJL2; *rrp5Δ6*: mutant strain YCJL4; *RRP5-N2 + RRP5-C1*: bipartite-allele-containing strain YCJL7. Strains were streaked and plates were incubated at the indicated temperature for 3 days.

allowed the deleted *rrp5Δ1::TRP1* spores to divide further. A cluster of about 30 cells was reproducibly observed for the two nonviable spores descended from the sporulation of the heterozygous diploids overexpressing either the *RRP5-N1* or *RRP5-N2* alleles (data not shown). These 30 cells-clusters were not observed for the shortest *RRP5-N3* form.

These observations have prompted us to assay a "trans-complementation" by transforming the heterozygous diploids that expressed the *RRP5-N2* or *RRP5-N3* alleles with a second plasmid harboring the missing carboxy-terminal TPR domain of Rrp5p (*RRP5-C1* allele) and allowing them to sporulate (see Materials and Methods). Very surprisingly, we recovered viable [Trp$^+$, Ura$^+$, Leu$^+$] spores when the p44-*RRP5-N2* (*URA3* 2μ) plasmid is associated with the BFG1-*RRP5-C1* (*LEU2* 2μ) plasmid in the cell. These data indicate that the viability of the *rrp5Δ1::TRP1*-deleted spores can be restored by the co-expression of the *RRP5-N2* and *RRP5-C1* alleles (Fig. 3A,B and data not shown). Neither [Trp$^+$, Leu$^+$] nor [Trp$^+$, Ura$^+$] spores were recovered and we have confirmed that the overexpression of the TPR domain alone was not able to suppress the lethality of the *RRP5*-deleted strain (Fig. 3A and data not shown). The relevant YCJL7 strain (Table 1) harbored a reduced growth rate at 30 °C and was temperature sensitive at 37 °C (*RRP5-N2* + *RRP5-C1*; Fig. 3B). In glucose-rich medium at 30 °C, the YCJL7 strain had a doubling time of 330 min versus 90 min for the wild-type strain. The YCJL7 strain was hereafter reported to contain the bipartite allele. This allele corresponds to the conjugate expression of two independent and nonoverlapping parts of Rrp5p: the first 1,131 amino-terminal residues encompassing nine S1 motifs (*RRP5-N2* allele) and the last 273 carboxy-terminal residues encompassing all TPR repeats (*RRP5-C1* allele; Fig. 3A). Viable [Trp$^+$, Ura$^+$, Leu$^+$] spores were also recovered when the *RRP5-N1* and *RRP5-C1* alleles were combined, but in this case we observed an unstable, slow growing phenotype because of recombination between the homologous overlapping sequences shared by the two alleles (data not shown).

The *RRP5* bipartite allele inhibits rRNA cleavage at site A_2

Using an in vivo depletion approach, Venema & Tollervey (1996) showed that the *RRP5* gene is the first rRNA processing component found to be required for early cleavages at sites A_0, A_1, A_2, and A_3. We performed Northern analyses to study whether rRNA processing defects could be associated with the bipartite allele. Total RNAs were isolated from wild-type BMA41 (WT) and mutant YCJL7 (*RRP5-N2* + *RRP5-C1*) strains after growth at 30 °C or following a shift to 37 °C for 8 or 16 h, separated on agarose gel (see Materials and Methods) and analyzed by methylene blue staining (Herrin & Schmidt, 1988; Sambrook et al., 1989) to visualize the 25S and 18S rRNA products and by Northern hybridization to visualize the other rRNAs species. The yeast pre-rRNA processing steps are schematically drawn in Figure 1, together with the location of the oligonucleotide probes used in this study. Results are presented in Figure 4. Methylene blue staining shows a decrease in 18S rRNA steady-state level for YCJL7 strain at 30 °C (Fig. 4A). This defect is increased after shifting to the restrictive temperature (compare Fig. 4A, lanes 5 and 6 to lane 4). Figure 4B–G represent the same membrane shown in panel A following hybridization with probes complementary to sequences 5′ and 3′ of the A_2 site (probes 2 and 3 respectively; Fig. 4B,C), sequence 3′ of the A_3 site (probe 4; Fig. 4D), 5.8S (probe 5; Fig. 4E), ITS2 (probe 6; Fig. 4F) and the 5′ ETS (probe 1; Fig. 4G). The bipartite allele gives rise to a large accumulation of the pre-rRNA 32S species that can be detected with all probes (Fig. 4B–F) except probe 1 (Fig. 4G) which allows for discrimination between 33S and 32S pre-rRNA species (see Fig. 1B). The 32S accumulation observed at both temperatures

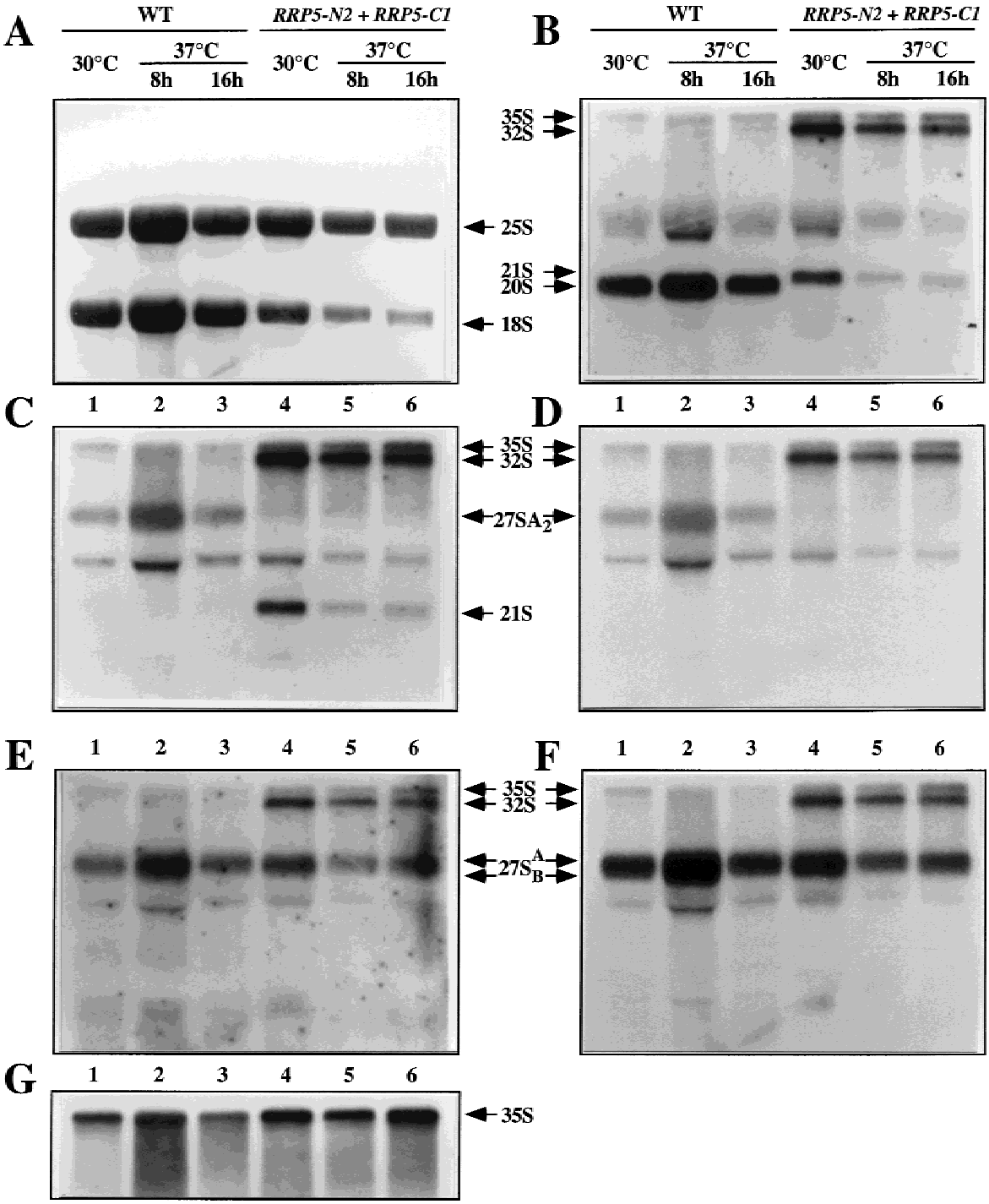

FIGURE 4. Effect of the bipartite allele on pre-rRNA processing. Strains BMA41 (WT [lanes 1 to 3]) and YCJL7 (*RRP5-N2* + *RRP5-C1* [lanes 4 to 6]) were grown in glucose-rich medium at 30 °C (lanes 1 and 4) or shifted at 37 °C during 8 h or 16 h (lanes 2 and 5 or 3 and 6, respectively). Total RNA was extracted and subjected to Northern analyses. About 10 μg of total RNAs were loaded in each lane. The panels represent consecutive hybridizations of the same membrane with the different probes drawn in Figure 1A. **A**: Methylene blue staining of mature 18S and 25S rRNA. **B**: Hybridization with oligonucleotide 2, complementary to site within the ITS1 upstream of site A_2. **C**: Oligonucleotide 3 in ITS1 downstream of site A_2. **D**: Oligonucleotide 4 in ITS1 downstream of site A_3. **E**: Oligonucleotide 5 in 5.8S rRNA. **F**: Oligonucleotide 6 in ITS2 upstream of site C_2. **G**: Oligonucleotide 1 in the 5′ ETS. The arrowheads indicate the different pre-rRNAs and mature rRNAs. Note that a cross hybridization to the mature 25S rRNA is observed in all lanes which each probe.

indicates that cleavage at site A_2 is strongly inhibited. Consequently, the products of the A_2 cleavage (pre-rRNA 20S and $27SA_2$ species) were not detected in that strain (Fig. 4B and 4C–D, respectively; lanes 4 to 6). Instead of the 20S rRNA, the YCJL7 strain grown at 30 °C contains large amounts of the higher molecular weight rRNA 21S species which is detected with probes both complementary to sequences 5′ and 3′ of the A_2 site

(Fig. 4B,C, lane 4) but not with probes in the 5′ ETS (data not shown) or downstream of the A_3 site (Fig. 4D). This aberrant pre-rRNA species is therefore predicted to result from the direct cleavage of the accumulated 32S precursor at site A_3, thus producing a 20S precursor extended at its 3′ end (Fig. 1C). The 21S accumulation is more modest when YCJL7 strain is grown at 37 °C (Fig. 4B,C, lanes 4 to 6) although 32S accumulation appears nearly equivalent at both temperatures (Fig. 4B–F, lanes 4 to 6). This could either suggest that the direct cleavage of the 32S pre-rRNA at site A_3 might be a temperature-sensitive event or that the 21S species is unstable at 37 °C. We favor the latter hypothesis since the direct cleavage of 32S species at site A_3 also produces the $27SA_3$ species (Fig. 1C) whose specific derivative product $5.8S_S$ rRNA accumulates normally (Fig. 5A; see below). The 21S pre-rRNA was first reported as accumulating in a yeast strain deleted for the snoRNA snR10 (Tollervey, 1987; see Fig. 1B) and was also detected in three *rrp5* mutant strains, but not in the Rrp5p-depleted strain (Venema & Tollervey, 1996). The fact that the *RRP5* bipartite-allele-expressing strain does not contain detectable 20S precursor but does contain mature 18S rRNA strongly suggests that the aberrant 21S species can be used as a precursor for 18S synthesis and that this 21S pre-rRNA can be cleaved at site D (Fig. 1C).

There is a modest accumulation of the 35S rRNA in the mutant YCJL7 strain (Fig. 4B–G) indicating that the early cleavages at sites A_0 and A_1 are weakly affected. Nevertheless, the excised A_0A_1 fragment accumulates at a wild-type level as shown in Figure 5B, thus indicating that the cleavages occur in the YCJL7 strain. Furthermore, hybridization with probe in 5′ ETS failed to reveal any accumulation of 33S pre-rRNA species (Fig. 4G).

While the 27SB pre-rRNAs were found to be strongly reduced in a Rrp5p-depleted strain (Venema & Tollervey, 1996), these species accumulate near a wild-type level in the bipartite-allele-containing strain (Fig. 4E,F). Consistently, the mature 25S and 5.8S derivative products were normally detected in that strain (Fig. 4A and Fig. 5A). The apparent lower level of 27SB and 25S rRNAs detected at 37 °C (Fig. 4A and 4E–F, lanes 5 and 6) is rather because of a lower loading of the corresponding lanes than to a real accumulation defect. Moreover, the two forms of 5.8S rRNA, the major short form ($5.8S_S$) and the minor long form ($5.8S_L$; see Henry et al., 1994), were both detected at normal levels in YCJL7 strain (Fig. 5A) and wild-type reported ratios ranging from 1:7 to 1:10 ($5.8S_L$:$5.8S_S$) were determined by densitometry analyses (Eichler & Craig, 1994; Allmang et al., 1996). Venema & Tollervey (1996) have shown that the Rrp5p-depleted strain strongly underaccumulated the major short form $5.8S_S$ rRNA due to an inhibition of the A_3 cleavage in that strain. No such defect was observed in the YCJL7 strain which is consistent with an efficient cleavage event occurring at site A_3.

We conclude that despite its unusual genetic organization the so-called *RRP5* bipartite allele causes only one of the two rRNA maturation defects found to be associated with the in vivo depletion of Rrp5p (Venema & Tollervey, 1996). The 18S rRNA formation alone is found to be affected because of a strong inhibition of the cleavage at site A_2 (Fig. 4A–D; summarized in Fig. 1C). The other three early cleavages (A_0, A_1, and A_3) are not or only weakly affected and 5.8S rRNA accumulates normally (Fig. 5A).

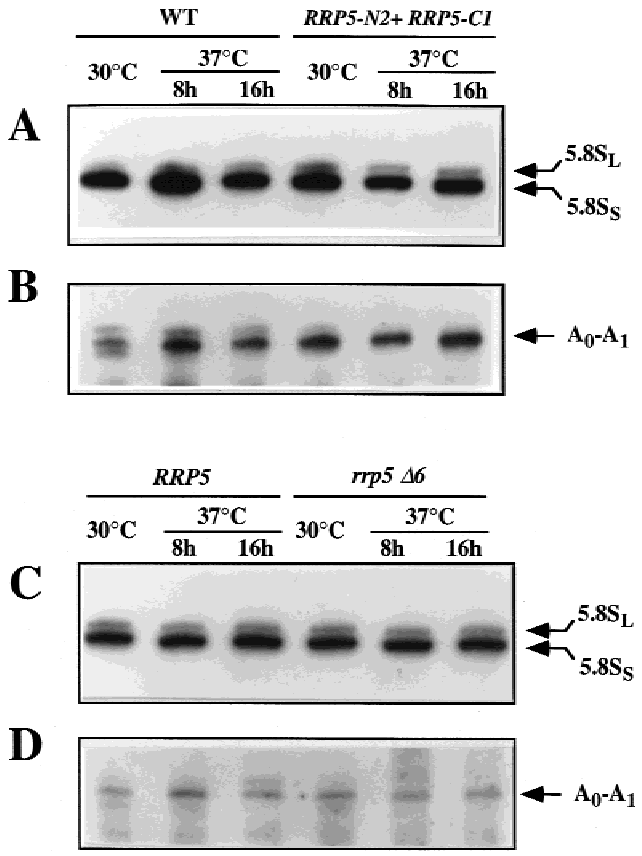

FIGURE 5. Effect of the two mutant alleles on the processing of low-molecular-weight pre-RNAs. **A**,**B**: yeast YCJL7 strain expressing the bipartite allele together with the wild-type strain BMA41. **C**,**D**: temperature-sensitive YCJL10 strain together with the wild-type strain YCJL9. **A** and **B** or **C** and **D** represent hybridizations of the same membrane. About 3 μg of total RNAs were loaded in each lane. **A**,**C**: oligonucleotide 5 hybridizing within 5.8S rRNA. **B**,**D**: oligonucleotide 1 in the 5′ ETS that is complementary to the sequence between the A_0 and A_1 sites (see Fig. 1A). The positions of the different pre-rRNA species are indicated by arrowheads.

Mutation in TPR unit 1 affects pre-rRNA cleavages at sites A_0, A_1, and A_2

We obtained an in-frame deletion of six base pairs in the *RRP5* gene (nt 4480–4485; see Materials and Methods) that leads to the deletion of conserved amino acids A1494 and R1495 in TPR unit 1 of the protein (see Fig. 2D). The resulting *rrp5Δ6* allele was functionally tested by plasmid shuffling (Mann et al., 1987) in

strain YCJL3 (Table 1) which contains the resident p38-*RRP5* (*URA3* CEN) plasmid. The YCJL3 strain was transformed by the p36-*rrp5Δ6* (*LEU2* CEN) plasmid and [Leu$^+$] transformants were streaked on 5-fluoroorotic-acid- (5-FOA) containing medium (Boeke et al., 1984). Viable 5-FOA resistant cells carrying the *rrp5Δ6* allele were obtained at 30 °C and the resulting YCJL4 strain (Table 1) was tested for growth at low and high temperatures on glucose-rich medium. As shown in Figure 3B, the *rrp5Δ6* mutation causes temperature sensitivity at 37 °C.

To study the effects of the *rrp5Δ6* mutated allele on pre-rRNA processing, we performed Northern analyses using the same experimental conditions as those described above for the bipartite allele. Total RNAs were isolated from the wild-type strain YCJL9 (Table 1) and the *rrp5Δ6* mutant strain YCJL10 after a growth at 30 °C or a shift at 37 °C for 8 or 16 h. Results are presented in Figure 6 (lanes 1–6). As previously observed for the bipartite-allele-containing strain, the mutant strain YCJL10 (*rrp5Δ6*) underaccumulates mature 18S rRNA when shifted to the restrictive temperature (compare lanes 5 and 6 to lanes 2 and 3 in Fig. 6A). Nevertheless, the rRNA processing pathway was found to be altered in a different way for the *rrp5Δ6* strain. At 37 °C, the mutant *rrp5Δ6* strain underaccumulates the two by-products of 32S pre-rRNA cleaved at A_2 (the 20S and 27SA_2 pre-rRNAs; see Fig. 6B and C–D, respectively), indicating that A_2 cleavage does occur in that strain but is partially inhibited. While the 32S pre-rRNA was found to strongly accumulate in the bipartite-allele-containing strain (Fig. 4B–F), it is only weakly observed in the mutant strain at 37 °C (Fig. 6B–F). In contrast, large amounts of the primary 35S precursor accumulate, indicating that the early cleavages at sites A_0 and A_1 are strongly delayed in that strain (Fig. 6B–G). However, both cleavages take place, because the small, excised A_0A_1 fragment accumulates at equivalent levels in the wild-type and mutant strains (Fig. 5D) and furthermore, no accumulation of 33S pre-rRNA species was detected (Fig. 6G). As already observed for the bipartite-allele-containing strain, the 27SB pre-rRNAs are present at a wild-type level in the mutant strain *rrp5Δ6* (Fig. 6E,F) indicating that cleavages subsequent to A_2 are not detectably affected. Consistently, there is no defect in 5.8S rRNA synthesis and a usual ratio of 1:7 ($5.8S_L$:$5.8S_S$) was determined by densitometry analysis for both the wild-type and the mutant strain (Fig. 5C).

An aberrant RNA of ~900 nt in length is accumulated in the mutant strain shifted to the restrictive temperature (Fig. 6B–E; species noted X). This species is detected with probes complementary to ITS1 (probe 2, 3, and 4; Fig. 6B–D) and the 5.8S rRNA (probe 5; Fig. 6E) but not to the 5′ ETS or ITS2 (Fig. 6F and data not shown). Since Figure 6E was obtained with a second membrane (see Fig. 6 legend), we have checked by hybridization that the X signal of Figure 6E did correspond to the X species detected in Figure 6B–D (data not shown). Interestingly, an aberrant rRNA species of equivalent size was similarly detected in Rrp5p-deleted strain and named 12S′ (Venema & Tollervey, 1996). Precise mapping of the 5′ and 3′ ends of the 12S′ aberrant intermediate were not determined but according to its length, it was proposed to extend from sites within the 18S rRNA sequence to the 3′ end of 5.8S rRNA (Venema & Tollervey, 1996).

In conclusion, the *rrp5Δ6* mutation causes an inhibition of the synthesis of 18S rRNA with no detectable effects on 5.8S rRNA accumulation as previously observed for the bipartite allele. Nevertheless, the rRNA processing pathway is differently affected. The bipartite allele causes a strong defect at cleavage site A_2, whereas early cleavages at sites A_0, A_1, and A_2 were all found to be altered in the *rrp5Δ6* mutant strain shifted to restrictive temperature (summarized in Fig. 1C,D).

Additional copies of *ROK1* suppress the 18S rRNA synthesis defect caused by the *rrp5Δ6* mutation

To gain further information concerning *RRP5* function we looked for genes that, when overexpressed, suppress the temperature-sensitive phenotype generated by the *rrp5Δ6* mutation. Multicopy suppressors were isolated at 37 °C by transforming the *rrp5Δ6* mutant strain YCJL4 with a wild-type genomic library borne on the multicopy vector pFL44L (*URA3* 2μ) as described in Materials and Methods. Fast-growing transformants were retained for further analysis. For these transformants, we confirmed that plasmid retransformation of the original YCJL4 strain suppressed its temperature sensitivity whereas removal of the suppressor plasmid by 5-FOA selection (Boeke et al., 1984) restored mutant phenotype. Seven transformants had a wild-type growth rate at 37 °C and harbored plasmids with *RRP5* insert as shown by restriction-mapping analysis and partial sequencing. Two other fast-growing transformants were shown to contain plasmids with overlapping inserts. The genomic fragment encoding suppressor function was mapped by subcloning (see Materials and Methods). It contained the unique *ROK1* gene which encodes a predicted DEAD-box, ATP-dependent RNA helicase shown to be involved in 18S rRNA processing (Venema et al., 1997). As shown in Figure 7, the overexpression of *ROK1* efficiently suppresses the temperature sensitivity of the *rrp5Δ6* mutant strain. Strikingly, when assayed as a low copy number suppressor, the *ROK1* gene was also able to weakly suppress the mutant phenotype of the *rrp5Δ6* strain (Fig. 7). We then tested whether the *ROK1* gene could bypass the cellular requirement for *RRP5* gene when expressed on a high or low copy number vector. This was assayed either by plasmid shuffling in YCJL2 strain (*rrp5Δ1::TRP1*, p36-*RRP5*) or by transformation and sporulation of the heterozygous diploid strain YCJL1

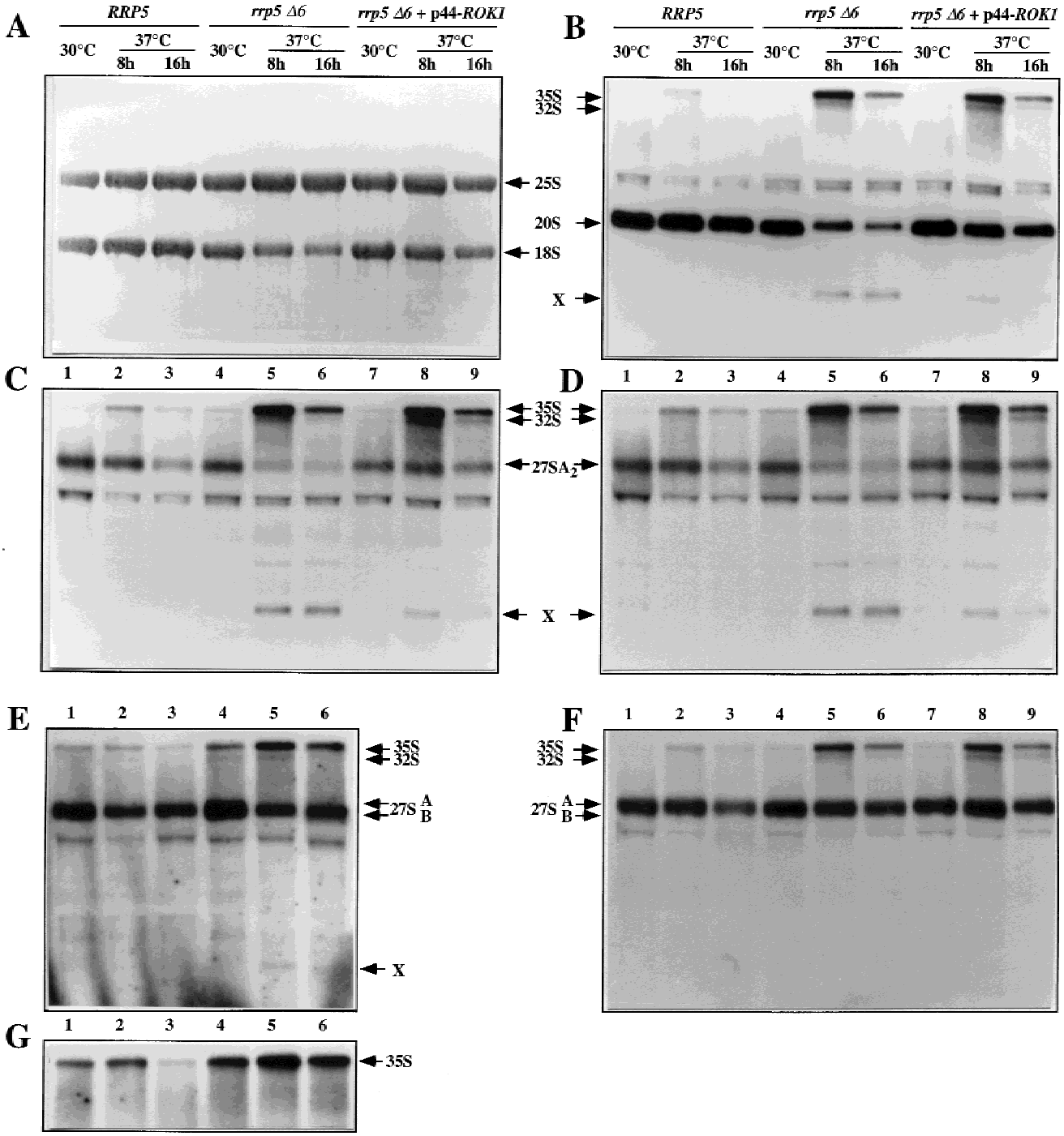

FIGURE 6. Additional copies of *ROK1* suppress the rRNA processing defects of the *rrp5Δ6* mutant strain. Strains YCJL9 (*RRP5* [lanes 1–3]), YCJL10 (*rrp5Δ6* [lanes 4–6]) and YCJL12 (*rrp5Δ6* + p44-*ROK1*) were grown in glucose-minimum medium at 30 °C (lanes 1, 4, and 7) or shifted at 37 °C during 8 h or 16 h (lanes 2, 5, and 8, and 3, 6, and 9, respectively). Total RNA was extracted and subjected to Northern analyses. About 10 μg of total RNAs were loaded in each lane. **B**, **C**, **D**, and **F** represent the same membrane shown in **A** hybridized with probes 2, 3, 4, and 6, respectively. **E** and **G** represents a second membrane loaded with the same RNA samples from lanes 1–6 and hybridized with probe 5 and 1, respectively. **A**: Methylene blue staining reveals the mature 18S and 25S rRNAs. **B**: Hybridization with oligonucleotide 2, complementary to site within the ITS1 upstream of site A_2. **C**: Oligonucleotide 3 in ITS1 downstream of site A_2. **D**: Oligonucleotide 4 in ITS1 downstream of site A_3. **E**: Oligonucleotide 5 in 5.8S rRNA. **F**: Oligonucleotide 6 in ITS2 upstream of site C_2. **G**: Oligonucleotide 1 in the 5′ ETS. The positions of the different pre-rRNAs and mature rRNAs are indicated by arrowheads. X indicates an aberrant species that is about 900 nt long. Note that each oligonucleotide gives a cross hybridization to the mature 25S rRNA that is equivalent for all loaded lanes. This nonspecific signal appears like a doublet in **B** and **C**.

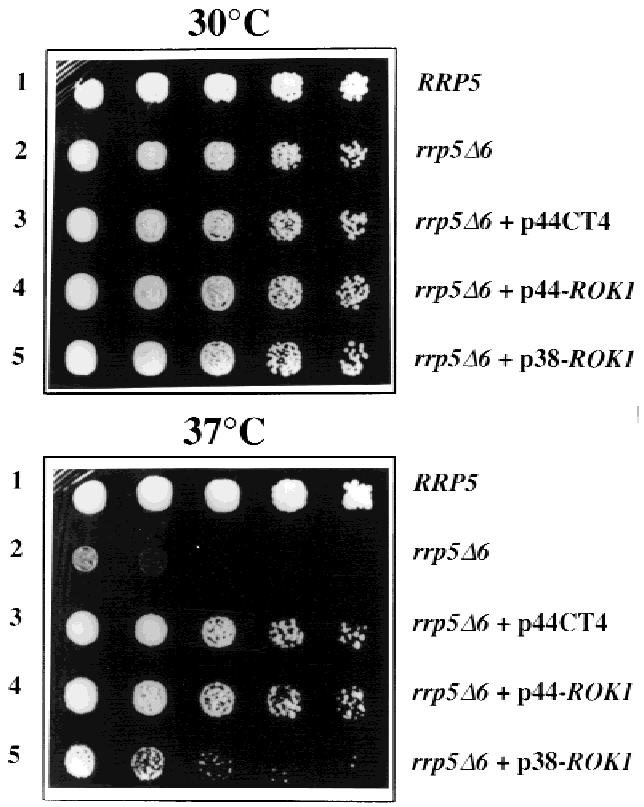

FIGURE 7. High and low expression of *ROK1* suppresses the temperature sensitivity of *rrp5Δ6* mutant strain. *RRP5*-deleted strain YCJL2 containing the p36-*RRP5* plasmid was transformed with the pFL44L vector yielding positive control strain YCJL9 (*RRP5*, lane 1). *RRP5*-deleted strain YCJL4 containing the p36-*rrp5Δ6* plasmid was transformed with the following plasmids: pFL44L vector yielding mutant strain YCJL10 (*rrp5Δ6*, lane 2); p44CT4 plasmid isolated from multicopy library yielding suppressive strain YCJL11 (*rrp5Δ6* + p44CT4, lane 3); high-copy p44-*ROK1* plasmid yielding suppressive strain YCJL12 (*rrp5Δ6* + p44-*ROK1*, lane 4); low-copy p38-*ROK1* plasmid yielding suppressive strain YCJL13 (*rrp5Δ6* + p38-*ROK1*, lane 5). Successive tenth dilutions of each liquid culture were spotted on glucose-selective medium at 30°C or 37°C and incubated for 3 days.

(*RRP5/rrp5Δ1::TRP1*). In both cases, negative results were obtained, indicating that *ROK1* cannot fulfill all of the cellular function(s) of *RRP5*.

The suppressive strain YCJL12 (Table 1) that overexpresses the *ROK1* gene was tested in Northern analyses (see Fig. 6, lanes 7–9) to determine whether additional copies of *ROK1* could rescue the pre-rRNA processing defects associated with the *rrp5Δ6* mutated allele. Growth and Northern hybridizations were performed under the same conditions as those described above for the *rrp5Δ6* strain. As seen in Figure 6A, the suppressive strain (noted *rrp5Δ6* + p44-*ROK1*) contains wild-type level of 18S rRNA after shift to 37°C (compare lanes 8 and 9 to lanes 2 and 3). Furthermore, the overexpression of the Rok1p RNA helicase almost totally cancels the A_2 cleavage defect of *rrp5Δ6* strain since 20S and 27SA_2 pre-rRNAs (which both result from the A_2 cleavage) were detected near wild-type levels in the suppressive strain (Fig. 6B and C–D, compare lanes 8 and 9 to lanes 2 and 3). In contrast, the 35S rRNA precursor accumulates to the same extent in the suppressive and mutant strains (Fig. 6B–E, compare lanes 5 and 6 to lanes 8 and 9) indicating that cleavage at site A_0 is still delayed in the *rrp5Δ6* strain overexpressing the *ROK1* gene. Finally, a lesser amount of RNA X is detected in the suppressive strain (Fig. 6B–D, compare lanes 5 and 6 to lanes 8 and 9) suggesting that the overexpression of the *ROK1* gene could alleviate the processing defect leading to the accumulation of this aberrant species.

It is worth noting that the overexpression of the *ROK1* gene alleviates only some of the processing defects generated by the *rrp5Δ6* mutation. Nevertheless, it is sufficient to rescue a normal accumulation of 18S rRNA in the suppressive strain.

DISCUSSION

RRP5 and mitochondrial import

We have isolated *RRP5* in a genetic screen based on the retargeting of a cytoplasmic reporter protein to the mitochondria (Claros et al., 1995, 1996). The overexpression of the *RRP5* gene was thus found to modify mitochondrial import (S. Hermann-Le Denmat, C. Torchet, C. Jacq, in prep.). Yeast protein retargeting has been successfully used as a tool to identify factors implicated in sorting process into the cell (Garret et al., 1991; Bossie et al., 1992; Ellis & Reid, 1993; Zoladek et al., 1995). Interestingly, previous genetic screens of retargeting of yeast mitochondrial polypeptides have uncovered proteins that, like Rrp5p, contain RNA-binding motifs: the known mRNA-binding protein Npl3p (Ellis & Reid, 1993) and the putative mRNA-binding protein Pan1p (Zoladek et al., 1995). The mechanism by which such proteins interfere with the targeting of mitochondrial proteins is not well characterized but it was proposed that, according to their RNA-binding potential, they could participate in a pathway that would address mitochondrial proteins by the prime localization of the mRNAs close to the organelle (Lithgow et al., 1997). We can therefore speculate that Rrp5p could similarly be able, when overexpressed, to participate in such a mechanism. While the role of *RRP5* in rRNA processing is established (Venema & Tollervey, 1996; this work), its link with mitochondrial targeting remains to be further characterized and is currently under study.

Rrp5p remains partially functional when S1 and TPR domains are physically separated

We present here a detailed analysis of the Rrp5p amino acid sequence that reveals a unique modular structure of repetitions of twelve putative S1 RNA-binding motifs (encompassing 62% of the amino acid sequence) and seven carboxy-terminal tetratricopeptide repeats (about

15% of the protein length). Rrp5p is the first functional eukaryotic protein described to contain so many repeated S1 motifs and might therefore be a S1-like protein in yeast. We have physically separated the S1 and TPR domains of the protein by expressing independently the first nine S1 motifs and the TPR motifs. Strikingly, the resulting so-called "bipartite gene" can rescue the lethality of a *RRP5*-deleted strain whereas each single part cannot. This shows that the putative RNA-binding and protein-binding domains of the protein can act independently of each other to fulfill most of the cellular functions of Rrp5p. This result implies that the bipartite gene encodes two stable polypeptides that are correctly targeted to their final compartment, the nucleolus (Venema & Tollervey, 1996). It can be proposed that each independent part of the protein may either reach the nucleolar compartment by itself (especially because they are expressed from multicopy plasmids) or by being associated with other nucleolar components. Considering that neither the shortened S1 domain nor the TPR domain of Rrp5p contains a recognizable nuclear localization signal consensus sequence, the second hypothesis may be more probable. We believe that the functionality of the "bipartite protein" might be due to its capacity to contact various partners into the cell. Such components could act as linkers to reassociate the carboxy- and amino-terminal parts of the protein, thus reconstituting a functional factor.

18S and 5.8S rRNA-processing functions of Rrp5p can be separated

The early pre-rRNA cleavages at sites A_0, A_1, and A_2 are known to be required for the synthesis of 18S rRNA (Venema & Tollervey, 1995) whereas cleavage at site A_3 is required for the normal formation of 5.8S rRNA (Henry et al., 1994). Venema & Tollervey (1996) have recently demonstrated that Rrp5p is the first rRNA-processing component required for both snoRNP-dependent cleavages $A_0/A_1/A_2$ and RNase MRP cleavage A_3 (see Fig. 1B). This was shown by genetic depletion of Rrp5p, which results in a synthesis defect of both 18S and 5.8S rRNAs. We show here that two conditional alleles of *RRP5* cause a defect only in 18S rRNA formation without altering the 5.8S rRNA synthesis. These results indicate that the Rrp5p functions in 18S and 5.8S rRNA-processing can be separated. It also suggest that our modified forms of Rrp5p could be affected for relationship with the snoRNP complex while putative functions within the RNase MRP complex could take place normally.

The bipartite protein is defective for the pre-rRNA cleavage at site A_2

Although the bipartite allele and *rrp5Δ6* allele cause an equivalent defect in steady-state level of 18S rRNA, they differently affect the pre-rRNA processing pathway. The *rrp5Δ6* allele has a rather classical defect by affecting cleavages at sites A_0, A_1, and A_2 that is indeed similar to the phenotype of the *rrp5* synthetic lethal mutants reported by Venema & Tollervey (1996). In comparison with rRNA-processing defects described to date (reviewed by Venema & Tollervey, 1995), the bipartite allele generates an unusually simple rRNA processing defect: the A_2 cleavage does not detectably occur, whereas preceding and subsequent cleavages take place almost normally. Therefore, the bipartite protein is primarily affected in an important role fulfilled by the wild-type Rrp5p at processing step A_2, whereas other rRNA processing functions of Rrp5p are not deeply altered. The genetic structure of the bipartite allele may be considered to predict functional defects potentially associated with this allele. As mentioned, the bipartite allele codes for two parts of Rrp5p that, once "brought together," correspond to a polypeptide deleted for three of its twelve putative S1 RNA-binding motifs (Fig. 3A). Thus, it can be roughly considered that the bipartite protein might be a defective RNA-binding factor that would be affected, for example, in the correct assembly of RNA component(s) required for the A_2 cleavage reaction. As a RNA-binding protein, wild-type Rrp5p could either be involved in interactions with snoRNAs of the RNP complex and/or with pre-rRNA molecules themselves. Since *RRP5* has been identified in a screen for synthetic lethality with a deletion of snR10 (Venema & Tollervey, 1996) the latter is an attractive candidate. In addition, snR10 is known to be required for cleavage at site A_2 (Tollervey, 1987). Nevertheless, neither snR10, nor U14 (Li et al., 1990), nor U24 (Kiss-Laszlo et al., 1996) were detectably coprecipitated with Rrp5p (Venema & Tollervey, 1996).

Rrp5Δ6p may be a defective protein-binding polypeptide

Rrp5p TPR motifs could be implicated in protein–protein interactions (Lamb et al., 1995). The crystal structure of the TPR repeats was recently resolved (Das et al., 1998) and revealed a spatial arrangement of one TPR unit in two antiparallel α-helices encompassing the A and B domains reported in Figure 2D. The α-helices A and B were shown to constitute the inside and outside faces of the TPR repeats respectively, thereby indicating that the conserved residues of the B domain are probably essential residues to mediate protein–protein interactions (Das et al., 1998). The *rrp5Δ6* allele encodes a polypeptide deleted for two amino acids in TPR unit 1 (alanine 1494 and arginine 1495) which are conserved residues of the B domain of the TPR motif (Fig. 2D). Therefore, the *rrp5Δ6* allele could encode a defective protein-binding factor at restrictive temperature (37 °C) that would be impaired in the recruitment of important component(s) of the maturation process. As

already mentioned, a large number of *trans*-acting factors other than Rrp5p are required for the same rRNA-processing step (Venema & Tollervey, 1995). The Rok1p protein is an attractive candidate to interact with Rrp5p since it was isolated in this study as an extragenic multicopy and low-copy suppressor of the *rrp5Δ6* temperature-sensitive mutation, and was also previously isolated with the same synthetic lethality screening used to isolate *RRP5* (Venema & Tollervey, 1996; Venema et al., 1997).

Rrp5p and Rok1p tightly participate in rRNA processing at step A_2

The *ROK1* gene encodes a putative ATP-dependent RNA helicase of the DEAD-box family (Song et al., 1995) that is required for pre-rRNA cleavages at sites A_0, A_1, and A_2 (Venema et al., 1997). During the rRNA maturation process, it is likely that RNA helicase activities are required to unwind secondary structures in pre-rRNA molecules inside the RNP complexes (Fuller-Pace, 1994), and several RNA helicases implicated in 18S rRNA maturation have been identified in yeast (O'Day et al., 1996; Kressler et al., 1997; Liang et al., 1997). It could be assumed that Rrp5p might play a role in the recruitment of the Rok1p RNA helicase within the snoRNP complex and that alterations in Rrp5p TPR unit 1 might have affected this function. Indeed, the rRNA-processing defects generated by the *rrp5Δ6* allele are very similar to the ones described for the in vivo depletion of Rok1p: a reduction in the levels of the pre-rRNAs 20S and $27SA_2$ and a strong accumulation of the 35S precursor (Venema et al., 1997). In addition, we show here that the overexpression of *ROK1* can rescue some of the rRNA-processing defects of the *rrp5Δ6* mutant. The *ROK1* gene mainly acts by restoring a near wild-type level of the pre-rRNA species 20S and $27SA_2$, whereas 35S remains accumulated (Fig. 6). These results indicate that the overexpression of *ROK1* is sufficient to alleviate the *rrp5Δ6*-defect at cleavage site A_2 but not at cleavage site A_0. These data also suggest that Rrp5p and Rok1p could tightly participate in the A_2 processing step but not in the A_0 processing step, although they are both required for this cleavage event. The Rrp5p and Rok1p proteins seem to be differently required during the 18S rRNA maturation process which is consistent with the inability of the *ROK1* gene to bypass the cellular requirement for the *RRP5* gene (data not shown).

The yeast Rrp5p protein presents a particular amino-acid structure that allows various interactions with rRNA-processing partners during the maturation process. The Rok1p RNA helicase might be an essential Rrp5p-interacting protein during the snoRNP-dependent cleavage at site A_2. Identification of other Rrp5p-interacting components will be hepful to clarify the rRNA-processing role(s) of Rrp5p. Moreover, since we have shown that the 18S and 5.8S rRNA-processing functions of Rrp5p can be separated, we can assume that *RRP5* mutations specifically defective in RNase MRP-dependent A_3 cleavage could be obtained. Finally, we have used here a new approach to perform the functional analysis of the yeast *RRP5* gene that consists of the construction of "trans-complementary" alleles. This gene partition could be a useful genetic tool in dissecting the functions of genes encoding proteins having distinguishable domains.

MATERIALS AND METHODS

Strains and media

Escherichia coli strain DH5α [*supE44* Δ*lacU169* (Φ80 *lacZ*Δ*M15*) *hsdR17 recA1 endA1 gyrA96 thi-1 relA1*] was used for cloning and for propagation of plasmids. Yeast strains used and constructed in this study are listed in Table 1. *Saccharomyces cerevisiae* genetic techniques and media were as described by Sherman (1991) and Boeke et al. (1984). Strains were grown either in 2% glucose-rich medium, 2% glucose-minimum medium supplemented with the appropriate nutrients for plasmid maintenance, or 2% glycerol-rich medium when screened for their ability to respire.

Isolation of the *RRP5* gene

Multicopy-acting genes were isolated by transformation of the respiratory deficient CW02/S678M strain (Claros et al., 1995; see Table 1) with a wild-type genomic library of *S. cerevisiae* FL100 strain (Stettler et al., 1993) carried in the 2-μm vector pFL44L (*URA3* 2μ) (Bonneaud et al., 1991). After growth for 3 days at 30 °C, ~100,000 uracil prototroph transformants were obtained and replicated twice on glycerol-rich medium to test for respiratory growth. Seventy seven respiratory colonies were isolated, and plasmids were extracted and used to transform the CW02 maturase deficient strain (Table 1) to eliminate candidates that contained inserts allowing a complete bypass of the requirement for bI4 maturase. After retransformation of the CW02/S678M recipient strain with the remaining plasmids and testing on glycerol, 20 independent candidates were retained for further analyses. Restriction mapping showed that five of them contained inserts that overlapped for a length of 6.6 kb. This smallest fragment encoded a unique 5,190 bp ORF designated YMR229c in the yeast genome sequencing project (accession number Z49939) and first named *FMI1* for Facilitating Mitochondrial Import and renamed *RRP5* according to the designation given by Venema & Tollervey (1996). The plasmid that contained the smallest genomic insert was named p44-*RRP5* (*URA3* 2μ) and further used for analysis. The *Xba*I-*Pvu*II fragment of this plasmid was cloned at the corresponding sites of the pFL36 or pFL38 centromeric plasmids (Bonneaud et al., 1991) yielding plasmids p36-*RRP5* (*LEU2* CEN) or p38-*RRP5* (*URA3* CEN), respectively. Homology searches were performed on the EMBL (release 53.0), GenBank (release 105.0), SWISS-PROT (release 35.0) and PIR-Protein (release 55.0) data banks with the FASTA program (Pearson & Lipman, 1988).

RRP5 gene disruption

The inactivation of the *RRP5* gene was performed as described by Baudin et al. (1993). The *rrp5Δ1::TRP1* deleted allele was constructed by one step PCR amplification using two oligonucleotides designed with one part homologous to the *RRP5* locus and a second part complementary to the *TRP1* cassette, so that the total *RRP5* ORF was deleted and replaced by the *TRP1* gene. The nucleotide sequence of the primers was: (TOM3) 5′-GCTATTAGTATCTACTACTACAGT CTGAATCAAGGACCGTTggccaagagggagggc-3′; (TOM4) 5′-GAGATTCATGGCTAGCGACATACTCAGTAGCTTTAGCTTT AACcttaaataaatactactcag-3′ (upper and lower case letters represent the *RRP5* or *TRP1* sequence respectively; see also below). The PCR was performed with the pUC19-*TRP1* plasmid as a template. The BMA41 diploid strain was transformed with this PCR amplification product after Gene Clean (BIO 101, Inc.) purification, and transformants were selected for tryptophan prototrophy. The genetic structure of the heterozygous *rrp5Δ1::TRP1/RRP5* strains was confirmed by PCR analysis on isolated genomic DNA (Hoffman & Winston, 1987). The following three pairs of oligonucleotides were used: set A: (TOM2-*RRP5*) 5′-CGGGATCCCGCCATCAGGAATTA TTCGTC-3′ and (TOM6-*TRP1*) 5′-gatatcgtccaactgcatgg-3′; set B: (TOM5-*RRP5*) 5′-GATGAGATGAGCTAAACCC-3′ and (OL07-*TRP1*) 5′-gcatcggaatctagagcacattctg-3′; set C: (TOM2-*RRP5*) and (TOM5-*RRP5*).

Construction of carboxy-terminal deleted versions of *RRP5*

The three carboxy-terminal truncated versions of *RRP5* were constructed as follows. The *RRP5-N1* allele (encoding amino acids 1–1,529; see Fig. 3A) was obtained by deleting the 1.2-kb *Kpn*I fragment of the p44-*RRP5* plasmid yielding p44-*RRP5-N1* plasmid. For the *RRP5-N2* construction, the 4.2-kb *Hind*III fragment of the p44-*RRP5* plasmid was first cloned at the corresponding site in a Bluescript plasmid. The yeast genomic insert was then isolated as a *Not*I–*Xho*I fragment and cloned at *Not*I–*Sal*I sites of the pFL44L vector yielding plasmid p44-*RRP5-N2*, where the truncated *RRP5* gene is 3,393 bp long (amino acids 1–1,131). The *RRP5-N3* allele was constructed by subcloning the 3.3-kb *Eco*RV fragment of p44-*RRP5-N2* at the *Sma*I site of pFL44L yielding p44-*RRP5-N3* plasmid.

Construction of the bipartite *RRP5* gene

The bipartite allele is borne by two multicopy plasmids that expressed an amino-terminal part and a carboxy-terminal part respectively of the wild-type gene (Fig. 3A). The p44-*RRP5-N2* plasmid was described above and encodes the first 1,131 residues of the protein (Fig. 3A). The carboxy-terminal part of the *RRP5* gene that encompasses the seven TPR motifs (nt 4,368–5,190; amino acids 1457–1729; Fig. 3A) was cloned into the expression vector TL38 (*LEU2* 2μ, *PGK* promoter) (Chardin et al., 1993) that contains triple repeats of the nine amino acids epitope (YPYDVPDYA) of influenza virus hemagglutinin. A PCR fragment was generated with *BamH*I cloning sites at the 5′ and 3′ end using primers (TOM1) 5′-CGGGATCCCGATGGCACCAGAATCTG TTG-3′ and (TOM2) (see above). The amplified fragment was first inserted at the *BamH*I site of the vector pGAD424 (accession number U07647). The TPR sequence was then recovered as an *Eco*RI–*Sal*I fragment and cloned at the corresponding sites of TL38 plasmid yielding plasmid BFG1-*RRP5-C1* (*LEU2* 2μ). The sequence of the fusion joint was determined and the expression of the tagged TPR domain was confirmed by Western analysis.

Construction of temperature-sensitive mutant

The *rrp5Δ6* allele was obtained by deleting 6 bp close to the unique *Xho*I restriction site present in the p44-*RRP5* plasmid and located in the carboxy-terminal part of *RRP5* ORF (see Figs. 2D and 3A). The deletion was produced by a Mung Bean Nuclease digestion of plasmid p44-*RRP5* first opened at the *Xho*I site. After phenol-chloroform extraction and ethanol precipitation, the extremities were filled in with the Klenow enzyme and the plasmid was religated in the presence of *Xho*I enzyme in order to amplify the ligation mixture with molecules deleted for the restriction site. Twelve plasmids were recovered after *E. coli* transformation, analyzed for the presence of the *Xho*I restriction site, and sequenced. Most of the plasmids contained a 4-bp deletion corresponding to the 5′ overhangs of the *Xho*I site. One plasmid, named p44-*rrp5Δ6* (*URA3* 2μ), contained a 6-bp deletion leading to an in frame deletion of amino acids 1494 and 1495 of the protein (Fig. 2D). The *Xba*I–*Pvu*II fragment of this plasmid was cloned at the corresponding sites of the pFL36 plasmid (Bonneaud et al., 1991) yielding plasmid p36-*rrp5Δ6* (*LEU2* CEN).

Selection of multicopy suppressors and subcloning of the *ROK1* gene

Extragenic suppressor genes were identified by transformation of the temperature-sensitive *rrp5Δ6* mutant strain YCJL4 by a wild-type genomic library of *S. cerevisiae* FL100 (Stettler et al., 1993) on the high copy vector pFL44L (Bonneaud et al., 1991). After one night at the permissive temperature (30 °C), the plates were incubated at the restrictive temperature (37 °C) and observed for 6 days. Twenty-six transformants growing at 37 °C were obtained among ~21,000 uracil prototroph transformants. After plasmid extraction and YCJL4 retransformation, 23 temperature-resistant transformants were retained for further analysis. A set of the seven fastest growing transformants (those appearing after 2 days at 37 °C) were shown by restriction analysis and partial sequencing to contain the wild-type *RRP5* gene. Two other fast-growing transformants (those appearing after 4 days) contained plasmids harboring overlapping genomic inserts of 4.2 kb and 6.3 kb that were subjected to further analysis. The remaining transformants corresponded to weaker suppressors and were not analyzed in this study. The two overlapping inserts contained two ORF in their 4.2-kb common region: the YGL170c ORF and the previously identified *ROK1* gene (Song et al., 1995). The plasmid p44CT4, isolated from the high copy library, contained the genomic insert of 4.2 kb that was subcloned by deleting a 1.2-kb *Nhe*I fragment. The resulting p44-*ROK1* (*URA3* 2μ) plasmid contained a single ORF, the *ROK1* gene. The 3-kb *Pvu*II–*Bgl*II fragment of the p44-*ROK1* plasmid was cloned into the *Pvu*II–*BamH*I sites of the pFL38 plasmid

(Bonneaud et al., 1991) yielding plasmid p38-*ROK1* (*URA3* CEN).

Northern analyses

Cells were grown to an OD_{580} of 1–2 in either rich medium or minimum-selective medium as indicated. RNA extractions were done as described by Schmitt et al. (1990). Small RNAs (3 μg of total RNAs) were separated on a 6% acrylamide gel containing 7 M urea and transferred to a positively charged nylon membrane by electro-transfer in 1× TAE (1×X TAE is 0.04 M Tris-acetate plus 1 mM EDTA [pH 8.0]) as transfer buffer. The membrane was crosslinked by using a UV Stratalinker (Stratagene) prior to hybridization. High-molecular-weight RNAs (10 μg of total RNAs) were analyzed on a 1% agarose-formamide containing gel as described by Sambrook et al. (1989). After electrophoresis, RNAs were transferred overnight to a positively charged nylon membrane by capillary elution with 20× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) as transfer buffer. The membrane was crosslinked in a UV Stratalinker, stained with methylene blue to reveal the 25S and 18S rRNA species (Herrin & Schmidt, 1988; Sambrook et al., 1989), and used for successive hybridizations. The following oligonucleotides were used for Northern hybridization: (1) 5′-TTCCCACCTATTCCCTC-3′; (2): identical to the oligonucleotide 5 reported by Venema & Tollervey (1996); (3) 5′-TGTTACCTCTGGGCCC-3′; (4) 5′-CCAGTTACGAAAATTCTTG-3′; (5) 5′-TTTCGCTGCGTTCTTCATC-3′; and (6) 5′-GGCCAGCAATTTCAAGTTA-3′ (Fig. 1A). All oligonucleotides were kinased with [γ^{32}P]-labeled ATP and purified on Nickspin columns (Pharmacia) before use. Prehybridization and hybridization were performed at 45 °C in 0.5 M sodium phosphate (pH 7.0), 10 mM EDTA, 7% SDS. Filters were washed twice for 10 min in 2× SSC–0.1% SDS at room temperature and autoradiographed at −70 °C. When necessary, additional washes in a more stringent buffer (2× SSC–0.5% SDS) were performed. Membranes were subjected to several autoradiographies and the signal intensity was measured by using a Fuji PhosphorImager for densitometry analysis.

ACKNOWLEDGMENTS

We thank Francois Lacroute (CNRS, Gif-sur-Yvette, France) for the *S. cerevisiae* multicopy library; Manuel Claros (Malaga University, Spain) for having initiated the study of yeast genes interfering with mitochondrial import; Jean-Paul di Rago (CNRS, Gif-sur-Yvette, France) for the BMA41 strain and the pUC19-*TRP1* plasmid; and Jacques Camonis (Institute Curie, Paris, France) for the TL38 vector. We are very grateful to Agnès Delahodde, Valerie Goguel, and Isabelle Iost for critical reading of the manuscript and to Susan Joyce for checking the English language. Special thanks go to Sylvie Camier for fruitful and friendly discussions during this work. C.T. held fellowships from the French Ministère de la Recherche et des Technologies. This work was supported by a grant from the French Association against Myopathies (AFM).

Received August 27, 1998; returned for revision September 9, 1998; revised manuscript received September 21, 1998

REFERENCES

Allmang C, Henry Y, Morrissey JP, Wood H, Petfalski E, Tollervey D. 1996. Processing of the yeast pre-rRNA at sites A_2 and A_3 is linked. *RNA 2*:63–73.

Banroques J, Delahodde A, Jacq C. 1986. A mitochondrial RNA maturase gene transferred to the yeast nucleus can control mitochondrial mRNA splicing. *Cell 46*:837–844.

Baudin A, Ozier-Kalogeropoulos O, Denouel A, Lacroute F, Cullin C. 1993. A simple and efficient method for direct gene deletion in *Saccharomyces cerevisiae*. *Nucleic Acids Res 21*:3329–3330.

Boeke JD, Lacroute F, Fink GR. 1984. A positive selection for mutants lacking orotidine-5′-phosphate decarboxylase activity in yeast: 5-fluoro-orotic acid resistance. *Mol Gen Genet 197*:345–346.

Boni IV, Isaeva DM, Musychenko ML, Tzareva NV. 1991. Ribosome-messenger recognition: mRNA target sites for ribosomal protein S1. *Nucleic Acids Res 19*:155–162.

Bonneaud N, Ozier-Kalogeropoulos O, Li G, Labouesse M, Minvielle-Sebastia L, Lacroute F. 1991. A family of low and high copy replicative, integrative and single-stranded *S. cerevisiae/E. coli* shuttle vectors. *Yeast 7*:609–615.

Bossie MA, DeHoratius C, Barcelo G, Silver P. 1992. A mutant nuclear protein with similarity to RNA binding proteins interferes with nuclear import in yeast. *Mol Biol Cell 3*:875–893.

Bycroft M, Hubbard TJP, Proctor M, Freund SVM, Murzin AG. 1997. The solution structure of the S1 RNA binding domain: A member of an ancient nucleic acid-binding fold. *Cell 88*:235–242.

Chardin P, Camonis JH, Gale NW, Van Aelst L, Schlessinger J, Wigler M, Bar-Sagi D. 1993. Human Sos1: A guanine nucleotide exchange factor for ras binds to Grb2. *Science 260*:1338–1343.

Claros MG, Perea J, Jacq C. 1996. Allotopic expression of yeast mitochondrial maturase to study mitochondrial import of hydrophobic proteins. *Methods Enzymol 264*:389–403.

Claros MG, Perea J, Shu Y, Samatey FA, Popot JL, Jacq C. 1995. Limitations to in vivo import of hydrophobic proteins into yeast mitochondria. The case of a cytoplasmically synthesized apocytochrome *b*. *Eur J Biochem 228*:762–771.

Company W, Arenas J, Abelson J. 1991. Requirement of the RNA helicase-like protein PRP22 for release of messenger RNA from spliceosomes. *Nature 349*:487–493.

Cormack RS, Genereaux JL, Mackie GA. 1993. RNase E activity is conferred by a single polypeptide: Overexpression, purification, and properties of the *ams/rne/hmp1* gene product. *Proc Natl Acad Sci USA 90*:9006–9010.

Das AK, Cohen PTW, Barford D. 1998. The structure of the tetratricopeptide repeats of protein phosphatase 5: Implications for TPR-mediated protein-protein interactions. *EMBO J 17*:1192–1199.

Della Seta F, Treich I, Buhler JM, Sentenac A. 1990. ABF1 binding sites in yeast RNA polymerase genes. *J Biol Chem 265*:15168–15175.

Dujardin G, Labouesse M, Netter P, Slonimski PP. 1983. Genetic and biochemical studies of the nuclear suppressor *NAM2*: Extraneous activation of the latent pleiotropic maturase. In: Schweyen RJ, Wolf K, Kaudewitz F, eds. *Mitochondria*. Berlin: Walter de Gruyter. pp 233–250.

Eichler DC, Craig N. 1994. Processing of eukaryotic ribosomal RNA. *Prog Nucleic Acid Res Mol Biol 49*:197–239.

Eklund EA, Lee SW, Skalnik DG. 1995. Cloning of a cDNA encoding a human DNA-binding protein similar to ribosomal protein S1. *Gene 155*:231–235.

Ellis EM, Reid GA. 1993. The *Saccharomyces cerevisiae MTS1* gene encodes a putative RNA-binding protein involved in mitochondrial protein targeting. *Gene 132*:175–183.

Fuller-Pace FV. 1994. RNA helicases: Modulators of RNA structure. *Trends Cell Biol 4*:271–274.

Garrett JM, Singh KK, von der Haar RA, Emr SD. 1991. Mitochondrial protein import: Isolation and characterization of the *Saccharomyces cerevisiae MFT1* gene. *Mol Gen Genet 225*:483–489.

Henry Y, Wood H, Morrissey JP, Petfalski E, Kearsey S, Tollervey D. 1994. The 5′ end of yeast 5.8S rRNA is generated by exonucleases from an upstream cleavage site. *EMBO J 13*:2452–2463.

Herrin DL, Schmidt GW. 1988. Rapid, reversible staining of Northern blots prior to hybridization. *Biotechniques 6*:196–197.

Hoffman CS, Winston F. 1987. A ten-minute DNA preparation from

yeast efficiently releases autonomous plasmids for transformation of *Escherichia coli*. *Gene 57*:267–272.
Hughes JMX, Ares M Jr. 1991. Depletion of U3 small nucleolar RNA inhibits cleavage in the 5′ external transcribed spacer of yeast pre-ribosomal RNA and impairs formation of 18S ribosomal RNA. *EMBO J 10*:4231–4239.
Kiss-Laszlo Z, Henry Y, Bachellerie JP, Caizergues-Ferrer M, Kiss T. 1996. Site-specific ribose methylation of preribosomal RNA: A novel function for small nucleolar RNAs. *Cell 85*:1077–1088.
Kressler D, de la Cruz J, Rojo M, Linder P. 1997. Fal1p is an essential DEAD-box protein involved in 40S-ribosomal-subunit biogenesis in *Saccharomyces cerevisiae*. *Mol Cell Biol 17*:7283–7294.
Labouesse M, Dujardin G, Slonimski PP. 1985. The yeast nuclear gene *NAM2* is essential for mitochondrial DNA integrity and can cure a mitochondrial RNA-maturase deficiency. *Cell 41*:133–143.
Labouesse M, Slonimski PP. 1983. Construction of novel cytochrome *b* genes in yeast mitochondria by subtraction or addition of introns. *EMBO J 2*:269–276.
Lamb JR, Tugendreich S, Hieter P. 1995. Tetratrico peptide repeat interactions: To TPR or not to TPR? *Trends Biochem Sci 20*:257–259.
Li HV, Zagorski J, Fournier MJ. 1990. Depletion of U14 small nuclear RNA (snR128) disrupts production of 18S rRNA in *Saccharomyces cerevisiae*. *Mol Cell Biol 10*:1145–1152.
Liang WQ, Clark JA, Fournier MJ. 1997. The rRNA-processing function of the yeast U14 small nucleolar RNA can be rescued by a conserved RNA helicase-like protein. *Mol Cell Biol 17*:4124–4132.
Lithgow T, Cuezva JM, Silver PA. 1997. Highways for protein delivery to the mitochondria. *Trends Biochem Sci 22*:110–113.
Mann C, Buhler JM, Treich I, Sentenac A. 1987. *RPC40*, a unique gene for a subunit shared between yeast RNA polymerases A and C. *Cell 48*:627–637.
Maxwell ES, Fournier MJ. 1995. The small nucleolar RNAs. *Annu Rev Biochem 35*:897–934.
Mélèse T, Xue Z. 1995. The nucleolus: An organelle formed by the act of building a ribosome. *Curr Opin Cell Biol 7*:319–324.
Morrissey JP, Tollervey D. 1993. Yeast snR30 is a small nucleolar RNA required for 18S rRNA synthesis. *Mol Cell Biol 13*:2469–2477.
Morrissey JP, Tollervey D. 1995. Birth of the snoRNPs: The evolution of RNase MRP and the eukaryotic pre-rRNA-processing system. *Trends Biochem Sci 20*:78–82.
O'Day CL, Chavanikamannil F, Abelson J. 1996. 18S rRNA processing requires the RNA helicase-like protein Rrp3. *Nucleic Acids Res 16*:3201–3207.
Pearson WR, Lipman DJ. 1988. Improved tools for biological sequence comparison. *Proc Natl Acad Sci USA 85*:2444–2448.
Rosenkrantz M, Kell CS, Pennell EA, Devenish LJ. 1994. The HAP2,3,4 transcriptional activator is required for derepression of the yeast citrate synthase gene, *CIT1*. *Mol Microbiol 13*:119–131.
Sambrook J, Fritsch EF, Maniatis T. 1989. *Molecular cloning: A laboratory manual*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.
Schmitt ME, Brown TA, Trumpower BL. 1990. A rapid and simple method for preparation of RNA from *Saccharomyces cerevisiae*. *Nucleic Acids Res 18*:3091–3092.
Schmitt ME, Clayton DA. 1993. Nuclear RNase MRP is required for correct processing of pre-5.8S rRNA in *Saccharomyces cerevisiae*. *Mol Cell Biol 13*:7935–7941.
Schnier J, Kimura M, Foulaki K, Subramanian AR, Isono K, Wittman-Liebold B. 1982. Primary structure of *Escherichia coli* ribosomal protein S1 and of its gene *rpsA*. *Proc Natl Acad Sci USA 79*:1008–1011.
Sherman F. 1991. Getting started with yeast. *Methods Enzymol 194*:3–20.
Sikorski RS, Boguski MS, Goebl M, Hieter P. 1990. A repeating amino acid motif in *CDC23* defines a family of proteins and a new relationship among genes required for mitosis and RNA synthesis. *Cell 60*:307–317.
Smith CM, Steitz JA. 1997. Sno storm in the nucleolus: New roles for myriad small RNPs. *Cell 89*:669–672.
Song Y, Kim S, Kim J. 1995. *ROK1*, a high-copy-number plasmid suppressor of *kem1*, encodes a putative ATP-dependent RNA helicase in *Saccharomyces cerevisiae*. *Gene 166*:151–154.
Stettler S, Chiannilkulchai N, Hermann-Le Denmat S, Lalo D, Lacroute F, Thuriaux P. 1993. A general suppressor of RNA polymerase I, II, and III mutations in *Saccharomyces cerevisiae*. *Mol Gen Genet 239*:169–176.
Subramanian AR. 1983. Structure and functions of ribosomal protein S1. *Prog Nucleic Acid Res Mol Biol 28*:101–142.
Tollervey D. 1987. A yeast small nuclear RNA is required for normal processing of pre-ribosomal RNA. *EMBO J 6*:4169–4175.
Tollervey D, Kiss T. 1997. Function and synthesis of small nucleolar RNAs. *Curr Opin Cell Biol 9*:337–342.
Venema J, Bousquet-Antonelli C, Gelugne JP, Caizergues-Ferrer M, Tollervey D. 1997. Rok1p is a putative RNA helicase required for rRNA processing. *Mol Cell Biol 17*: 3398–3407.
Venema J, Tollervey D. 1995. Processing of pre-ribosomal RNA in *Saccharomyces cerevisiae*. *Yeast 11*:1629–1650.
Venema J, Tollervey D. 1996. *RRP5* is required for formation of both 18S and 5.8S rRNA in yeast. *EMBO J 15*:5701–5714.
Voorma HO. 1996. *Control of translation initiation in Prokaryotes: Translational control*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press. pp 759–777.
Zhang K, Smouse D, Perrimon N. 1991. The *crooked neck* gene of *Drosophila* contains a motif found in a family of yeast cell cycle genes. *Genes & Dev 5*:1080–1091.
Zoladek T, Vaduva G, Hunter LA, Boguta M, Go BD, Martin NC, Hopper AK. 1995. Mutations altering the mitochondrial-cytoplasmic distribution of Mod5p implicate the actin cytoskeleton and mRNA 3′ ends and/or protein synthesis in mitochondrial delivery. *Mol Cell Biol 15*:6884–6894.