Rpp2, an essential protein subunit of nuclear RNase P, is required for processing of precursor tRNAs and 35S precursor rRNA in Saccharomyces cerevisiae

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ABSTRACT RPP2, an essential gene that encodes a 15.8-kDa protein subunit of nuclear RNase P, has been identified in the genome of Saccharomyces cerevisiae. Rpp2 was detected by sequence similarity with a human protein, Rpp20, which copurifies with human RNase P. Epiotype-tagged Rpp2 can be found in association with both RNase P and RNase mitochondrial RNA processing in immunoprecipitates from crude extracts of cells. Depletion of Rpp2 protein in vivo causes accumulation of precursor tRNAs with unprocessed introns and 5′ and 3′ termini, and leads to defects in the processing of the 35S precursor rRNA. Rpp2-depleted cells are defective in processing of the 5.8S rRNA. Rpp2 immunoprecipitates cleave both yeast precursor tRNAs and precursor rRNAs accurately at the expected sites and contain the Rpp1 protein orthologue of the human scleroderma autoimmune antigen, Rpp30. These results demonstrate that Rpp2 is a protein subunit of nuclear RNase P that is functionally conserved in eukaryotes from yeast to humans.

RNase P is a ubiquitous endoribonuclease that cleaves 5′ terminal leader sequences of precursor tRNAs to generate 5′ mature termini of tRNAs (1, 2). It is a ribonucleoprotein enzyme composed of RNA and protein subunits. Eubacterial RNase P consists of a single catalytic RNA subunit (3) and a single, very basic protein cofactor (~14 kDa) that increases the catalytic activity and substrate range of the enzyme. However, the exact identity of all the eukaryotic protein subunits has not yet been determined (4). At least seven proteins are associated with highly purified RNase P from human cells: Rpp14, Rpp20, Rpp25, Rpp29, Rpp30, Rpp38, and Rpp40 (10). Genetic and biochemical approaches in Saccharomyces cerevisiae identified four proteins—Pop1, Pop3, Pop4 (homologue of Rpp29), and Rpp1 (homologue of Rpp30)—that associate with RNase P; these proteins also associate with a related enzyme called RNase mitochondrial RNA processing (RNase MRP; refs. 11–14).

Yeast nuclear RNase P and RNase MRP are related ribonucleoprotein (RNP) enzymes essential for biosynthesis of tRNAs and rRNAs (15–18). The RNA component of RNase P is similar to that of RNase MRP, and the RNase P protein subunit (14). Rpp2-depleted cells are defective in processing of precursor rRNAs and 35S precursor rRNA. These results demonstrate that Rpp2 is a protein subunit of nuclear RNase P that is functionally conserved in eukaryotes from yeast to humans (10, 12, 14, 25).

MATERIALS AND METHODS


Abbreviations: Rpp2, RNase P protein 2; prRNA, precursor rRNA; prtRNA, precursor tRNA; Rpp2, RNase MRP; RNase mitochondrial RNA processing; ITS, internal transcribed sequences.

Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession no. AF055991).

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Gene Disruption. A genomic clone encoding the RPP2 locus was identified in the S. cerevisiae genome database (SGD) and obtained from the American Tissue Culture Collection (ATCC) as a cosmid (ATCC 71051), which was sequenced previously. A 2.2-kb AflII fragment that encodes RPP2 was subcloned into pBluescript (SK) vector to generate pRPP2SK. After this, oligonucleotides RPP2FH and RPP2J were used to amplify genomic DNA containing the RPP2 allele was verified by PCR digest analysis. Oligonucleotides used in this study are: RPP2HIS5 and RPP2HIS3, which were verified by sequencing. Oligonucleotides used in this study are: RPP2HIS5 and RPP2HIS3, which were verified by sequencing.

Construction of Plasmids. A plasmid encoding the FLAG-HIS epitope-tagged RPP2, pRS314FHRPP2(TRP1), was generated by PCR using oligonucleotides RPP2FH, RPP2J, RPP2T, and pRS314FHRPP2 as template. The PCR fragment was subcloned into pRS314(TRP1) plasmid to generate pRS314FHRPP2. To generate the GAL::RPP2 construct, the RPP2 coding sequence was subcloned from pRPP2SK as a 0.9-kb BsrNI fragment into pYCPGAL(URA3) to generate pYCPGAL::rpp2.

Strain Construction. RPP2-disrupted diploid strain VS200 was transformed with pRS314FHRPP2 and sporulated. The resulting spores were dissected, and spores that had a disrupted RPP2 gene but harbored pRS314FHRPP2 plasmid were viable (VS202A). The two haploids that depend on the plasmid pRS314FHRPP2 grew at rates identical to the wild-type haploids (VS211B). VS202A also was transformed with pRS316::3xmyc::RPP1 (14) and grown in media lacking uracil, to generate VS203. VS200 was transformed with pYCPGAL::rpp2 sporulated, and germinated on medium containing galactose. Viable spores were obtained from several independent tetrads. Haploids with a disrupted RPP2 allele that depend on pYCPGAL::rpp2 (VS301A) grew on galactose-containing plates but not on glucose-containing plates (data not shown).

Other Methods. Immunoprecipitations, RNase P and RNase MRP enzymatic assays, and RNA extraction and analysis were performed as described; immunoprecipitates were washed in IP150 buffer (14). Primer extension analysis of the cleavage of internal transcribed sequences 141 (ITS141) prRNA substrate at the A3 site was performed by using oligonucleotide 6 (14), according to manufacturer’s procedure for reverse transcriptase (Promega).

RESULTS
An Essential Yeast Gene Encodes a Homologue of Human Rpp20, a Protein Subunit of Nuclear RNase P. We identified the RPP2 gene by searching the SGD (26) with the protein sequence of the human Rpp20 protein, which copurifies with human nuclear RNase P (10, 25). We used a computational sequence search algorithm (BLASTP) (27) to identify a previ-

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**Fig. 1.** Yeast Rpp2 is homologous to the human Rpp20 protein. (A) The amino acid sequence of the yeast Rpp2 protein is aligned with the amino acid sequence of the human Rpp20 protein. The RPP2 gene is encoded on yeast chromosome II by the ORF, YBR167c. The amino acid sequences of both proteins are numbered from the N terminus. Shaded amino acids represent identical residues, and boxed amino acids represent similar residues. (B) The heterozygous diploid strain VS200, RPP2/rpp2::HIS3, is shown after segregation of four spores (A–D) from five independent tetrads (1–5). All spores segregated 2:2 for cell viability.
**Fig. 2.** Rpp2 protein coimmunoprecipitates with the Rpp1 protein and the RNase P and RNase MRP RNAs. (A) Immunoprecipitates derived from wild-type (untagged) cells (VS211B, lane 1), FLAGHIS-RPP2 and 5xmycRPP1 double epitope-tagged cells (VS203, lane 2), and FLAGHISRPP2-2-epitope-tagged cells (VS202A, lane 3) were denatured, resolved on a 12% SDS/PAGE gel, transferred to nitrocellulose membranes, and immunoblotted with polyclonal antibodies against the myc and his epitopes. The carboxy-terminal ends of the human Rpp20 and the yeast Rpp2 also show weak amino acid sequence similarity with a short segment of the yeast protein Rrp5 (28), which functions in rRNA processing and was shown to coimmunoprecipitate with the epitope-tagged Rpp1, RNase P, and RNase MRP proteins (lanes 1–5) and ptRNA (lanes 6–10) and incubated for 1 hr at 37°C (see Materials and Methods). Sub. (lane 1) is precursor RNA, ITS141, and sub. (lane 6) is precursor tRNA\(^{5\text{-oxo}}\). (B) The ITS141 RNA was transcribed in vitro and was incubated with immunoprecipitates derived from wild-type cells (lanes 1–5; VS211B, lane 2) and FLAGHISRPP2 cells (+; VS202A, lane 3). Sub. (lane 1) is ITS141. Accurate cleavage of the prRNA substrate at the A3 cleavage site was determined by primer extension analysis of the cleaved products. A 5′ end-labeled oligonucleotide (Oligo 6, which hybridized 40 nt 3′ to the A3 cleavage site; see ref. 14) was used in a primer extension reaction. Primer extension products were resolved on an 8% polyacrylamide/7 M urea gel. ITS141 was sequenced with the same oligonucleotide as was used for the primer extension reaction.

**Fig. 3.** RNase P and RNase MRP coprecipitate with Rpp2 and accurately cleave both prRNA and ptRNA substrates. (A) Immunoprecipitates derived from wild-type cells (lanes 1–5; VS211B) and individually epitope-tagged Rpp1 (+; VS162B, see ref. 14) and Rpp2 (+; VS202A) cells were resuspended with internally labeled prRNA (ITS141, see ref. 14) and ptRNA\(^{5\text{-oxo}}\) (lanes 6–10) and incubated for 1 hr at 37°C (see Materials and Methods). Sub. (lane 1) is precursor RNA, ITS141, and sub. (lane 6) is precursor tRNA\(^{5\text{-oxo}}\). (B) The ITS141 RNA was transcribed in vitro and was incubated with immunoprecipitates derived from wild-type cells (lanes 1–5; VS211B, lane 2) and FLAGHISRPP2 cells (+; VS202A, lane 3). Sub. (lane 1) is ITS141. Accurate cleavage of the prRNA substrate at the A3 cleavage site was determined by primer extension analysis of the cleaved products. A 5′ end-labeled oligonucleotide (Oligo 6, which hybridized 40 nt 3′ to the A3 cleavage site; see ref. 14) was used in a primer extension reaction. Primer extension products were resolved on an 8% polyacrylamide/7 M urea gel. ITS141 was sequenced with the same oligonucleotide as was used for the primer extension reaction.

**Construction of an Epitope-Tagged Allele of RPP2.** Previously we used a triple c-myc epitope to characterize the yeast RNase P protein subunit, Rpp1 (14). An epitope-tagged strain of RPP2 was constructed by inserting a DNA fragment that encodes both FLAG and HIS epitopes at the N terminus of the RPP2 gene by PCR amplification in a low-copy-number plasmid (pRS314) (see Materials and Methods). The resulting strain of S. cerevisiae (VS202A) grew at rates identical to wild-type cells, suggesting that the FLAG-HIS-RPP2 allele is fully functional (data not shown).

**Materials and Methods**

**Fig. 2**

**Fig. 3**
The association of Rpp2 protein with enzymatically active RNase P and RNase MRP holoenzymes was also demonstrated by immunoprecipitation. Immunoprecipitates derived from FLAG-HIS-tagged Rpp2 cells were resuspended with radiolabeled pRNA\textsubscript{SET} (29) to assay RNase P activity and with radiolabeled prRNA (ITS141) (see ref. 14) to assay rRNA processing at the A3 site. Both substrates were cleaved accurately at the appropriate cleavage sites (Fig. 3). We conclude that Rpp2 is a component of, or is tightly associated with, catalytically active RNase P and RNase MRP. These data also suggest that processing of tRNA and rRNA precursors may depend in part on Rpp2 function in vivo.

**Defects in tRNA and rRNA Processing of a Conditional Lethal Allele of RPP2 Show that Rpp2 Is Required for RNase P and RNase MRP Activities in Vivo.** The regulatable \textit{GAL1} promoter was used to construct a conditional lethal strain of \textit{RPP2} to investigate RNA processing events on depletion of Rpp2 \textit{in vivo}. The resulting strain, \textit{GAL::rpp2}, grew indistinguishably from wild-type cells in galactose-containing medium. However, the \textit{GAL::rpp2} strain slowed growth at 12 hr after a shift into glucose-containing medium, which represses the \textit{GAL1} promoter (Fig. 4A). Previously, we showed that depletion of Rpp1 affects both tRNA and 35S rRNA processing (14). Because the Rpp1 and Rpp2 proteins are found to communoprecipitate and their respective human homologues, Rpp30 and Rpp20, cofractionate biochemically (10, 25), we wanted to investigate whether Rpp2-depleted yeast cells would exhibit defects in RNA processing that are similar...
to Rpp1-depleted cells. Total RNA was extracted from the GAL::rpp2 strain at different times after a switch from galactose- to glucose-containing medium. This RNA was analyzed by ethidium bromide staining and by Northern blot analysis to detect RNA processing defects on depletion of the Rpp2 protein.

As observed in conditional mutants of RNase P RNA (24) and the Rpp1, Pop1, Pop3, and Pop4 proteins that associate with RNase P (11-14), depletion of the Rpp2 protein resulted in accumulation of precursor rRNAs (Fig. 4B). Rpp2 depletion also caused an increase in the abundance of the 5.8S rRNA (L) relative to the 5.8S rRNA (S), as judged by ethidium bromide staining (Fig. 4B). Because this phenotype is associated with depletion of the RNase P and RNase MRP RNAs (ref. 14; RPR1 and NME1, respectively), we used specific probes for these RNAs to determine their steady-state levels. The amount of Rpp2 protein correlates with the maturation and stability of both RNase P RNA (RPR1) and RNase MRP (NME1) RNA, as shown by the decreasing amount of both RNAs on depletion of Rpp2 (Fig. 4C). In contrast, depletion of Rpp2 does not affect steady-state levels of the snRNAs 190 (Fig. 4C) and U14 (data not shown). This may reflect a requirement for the intact structure of the RNase P and RNase MRP particles to prevent degradation of the RNA components.

Northern blot analysis of total RNA from Rpp2-depleted cells confirmed the defect in processing of ptRNAs, which accumulate with an unprocessed intron and both 5’ and 3’ extended forms (Fig. 5A). Defects in the processing of 35S rRNA were shown with oligonucleotides that hybridize to the ITS1 and ITS2 (see Fig. 6A). This analysis revealed defects in processing of the 5.8S rRNA (Fig. 6B and C). On depletion of Rpp2, an oligonucleotide that is complementary to the region between sites A2 and A3 (oligonucleotide a) detects an aberrant 5.8S rRNA that extends from site A2 to the 3’ end of 5.8S rRNA (Fig. 6B). This rRNA is expected to accumulate in the absence of cleavage at the A3 site. Additionally, Rpp2-depleted cells are depleted in 3’ extended 5.8S rRNA, 7S(S), which is detected with an oligonucleotide that hybridizes to ITS2 between site E and C2 (oligonucleotide b, Fig. 6A and C). This result suggests a defect in 5.8S rRNA processing at its 3’ terminus as a consequence of inhibition of processing at its 5’ terminus. Interestingly, as in Rpp1-depleted cells, 18S and 25S rRNA levels remain unaffected (data not shown). These results demonstrate the characteristic loss of both RNase P and RNase MRP activities in vivo as a result of Rpp2 depletion and suggest that Rpp2 is required for the function of both enzymes in vivo.

**DISCUSSION**

We have identified a gene, **RPP2**, which encodes a protein component of nuclear RNase P in *S. cerevisiae*. This gene was identified by searching the yeast genome database for protein homologues of the human Rpp2, one of seven proteins (Rpp14, Rpp20, Rpp25, Rpp29, Rpp30, Rpp38, and Rpp40) that copurified with human RNase P (10, 25). Rpp2 is a protein subunit of nuclear RNase P that is conserved from yeast to humans (10, 12, 14). The other human Rpp proteins do not show significant sequence similarity to any yeast genes (data not shown).

The Rpp2 protein is physically associated with both RNase P and RNase MRP enzymatic activities and affects both ptRNA and prRNA processing *in vivo*. This strongly indicates that Rpp2 is a component that is shared between RNase P and RNase MRP particles *in vivo*. It is also found in a complex with the Rpp1 protein, the yeast orthologue of the human scleroderma autoimmune antigen Rpp30. These findings further support the hypothesis that the two RNases are evolutionarily related. Which proteins contribute to the respective essential catalytic mechanisms of these enzymatic activities remains unknown. The relative contribution of these proteins to the RNase P and RNase MRP activities remains to be determined by biochemical fractionation and reconstitution of the separate enzymatic activities. By contrast, bacterial RNase P requires only a single protein component for its activity (1). Additional protein components of RNase P and RNase MRP that have been identified genetically in yeast (Pop1 and Pop3) show similar conditional defects in ptRNA and prRNA processing *in vivo* (11, 13).

As observed in other conditional mutants of RNase P and RNase MRP, the depletion of Rpp2 in *in vivo* has a characteristic RNA-processing phenotype (11-14). Interestingly, these mutant yeast strains accumulate not only 5’ terminal ptRNAs, as would be expected for a RNase P-defective phenotype, but also accumulate 3’ extended and intron-containing forms of prRNA and are deficient in processing of the 5.8S prRNA. What is the biochemical defect that accounts for the accumulation of both 5’ and 3’ extended forms of these RNAs? Recent findings suggest that yeast RNase MRP may collaborate with a 5’ to 3’ exonuclease, *Xrn1*, in processing of rRNA intermediates and, perhaps, mRNAs (32). Inhibition of this collaboration by an accumulation of a metabolite called adenosine 3', 5'-bisphosphate (pAp) may result in lethality (32). Additional defects in prRNA modification, such as pseudouridylation and/or methylation, may also account for the impaired processing of the 35S prRNA on depletion of the protein components of RNase P and RNase MRP. Such defects would
result from direct and/or indirect effects of RNase P or RNase MRP in snRNA processing. Whether such functions of RNase P exist in cells remains unknown.

None of the human or yeast protein subunits of RNase P exhibits significant sequence similarities to any predicted ORFs from eubacterial or archaeal genomes (data not shown). Unlike the RNA component, the protein subunits of eukaryotic RNase P do not appear to share common ancestry with any eubacterial or archaeal sequences.

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