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A 105-kDa protein is required for yeast mitochondrial RNase P activity

MICHAEL J. MORALES*, YAN LI DANG, YAN CHUN LOU, PAVOL SULO, AND NANCY C. MARTIN†

Department of Biochemistry, University of Louisville, Louisville, KY 40292

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ABSTRACT **RNase P from the mitochondria of *Saccharomyces cerevisiae* was purified to near homogeneity >1800-fold with a yield of 1.6% from mitochondrial extracts. The most abundant protein in the purified fractions is, at 105 kDa, considerably larger than the 14-kDa bacterial RNase P protein subunits. Oligonucleotides designed from the amino-terminal sequence of the 105-kDa protein were used to identify and isolate the 105-kDa protein-encoding gene. Strains carrying a disruption of the gene for the 105-kDa protein are viable but respiratory deficient and accumulate mitochondrial tRNA precursors with 5′ extensions. As this is the second gene known to be necessary for yeast mitochondrial RNase P activity, we have named it *RPM2* (for RNase P mitochondrial).**

RNase P removes 5′ leaders from tRNA precursors and is an RNA–protein complex in bacteria (1–3), yeasts (4–6), and metazoans (7–9). The bacterial enzymes are the best characterized and consist of RNAs around 300 nucleotides and proteins in the 14-kDa size range (10). Genes for bacterial RNase P protein subunits from *Escherichia coli* (11), *Bacillus subtilis* (12), *Proteus mirabilis* (13), *Micrococcus luteus* (14), and *Streptomyces bikiniesis* (15) have been isolated and sequenced. They are all ≈14 kDa in size and have an abundance of charged amino acids. The buoyant densities of the *E. coli* enzyme at 1.55 g/cm^3 (16) and the *Haloferax volcanii* enzyme at 1.61 g/cm^3 (17) reflect their RNA–protein composition. In contrast, the RNase P from *Sulfolobus solfataricus* (18) appears more proteinaceous, with a buoyant density of 1.28 g/cm^3. Even though the primary structures of RNAs and proteins are known for some bacterial enzymes, neither the structure of the holoenzyme nor the function of the protein in bacteria is understood.

Although both the protein and RNA subunits of the bacterial enzymes are essential *in vivo, in vitro* the RNAs are catalytic (19). Comparisons of prokaryotic RNase P RNA genes (20, 21) have produced a secondary structural model that has been further refined by genetic analyses (22). The data base for eukaryotic RNase P RNAs that function to process nuclear-coded tRNAs is small compared with that of prokaryotes. RNase P RNA genes that remove 5′ leaders from nuclear-coded tRNAs in human (8), *Xenopus* (9), *Schizosaccharomyces pombe* (23), and *Saccharomyces cerevisiae* (6) have been sequenced. Although there have been models of eukaryotic RNase P RNAs proposed (6, 8, 23, 24), extensive phylogenetic comparisons are not yet possible. RNase P RNAs required for mitochondrial RNase P activity in yeast are coded by mitochondrial DNA (25), they are extremely A+U rich (26–28), and they vary in size from 140 to >490 nucleotides in different strains (29). Despite their A+U-rich nature, all contain two short blocks of sequence similarity found in other RNase P RNAs, suggesting the functional importance of these short regions (27–29). No eukaryotic RNase P RNAs have been shown to be catalytic in the absence of protein but other than that they are ribonucleoprotein complexes, nothing about their protein subunit(s) is known.

The buoyant density of the human enzyme (8) and the mitochondrial enzyme of yeast (30) is 1.28 g/cm^3, which makes them more like the enzyme from *Sulfolobus* than from *E. coli*. Why the RNA–protein composition of these enzymes is not reflected in their densities is unclear. Although the buoyant density of a yeast nuclear enzyme has not been reported, the size of the *Sc. pombe* enzyme is 450 kDa (5). A 40-kDa protein in Hela extracts is recognized by an antibody to the 14-kDa *E. coli* protein, and the same antibody precipitates human RNase P activity and the RNase P RNA (31). Thus, the human nuclear enzyme has an association with a 40-kDa protein, but whether this protein is required for activity *in vivo* or *in vitro* is unknown.

We have extended our analysis of the yeast mitochondrial RNase P to identify the protein(s) associated with the enzyme. Our interest in mitochondrial RNase P is several-fold. (*i*) The RNAs are unusual in sequence, and knowing the structure of the protein subunit(s) will probably help to understand structure–function relationships of RNase P enzymes in general. (*ii*) The biogenesis of this enzyme requires the assembly of an RNA–protein complex made from mitochondrial RNA and nuclear-coded protein and as such presents an interesting aspect of mitochondrial biogenesis and nuclear cytoplasmic interaction (4). (*iii*) There is more than one RNase P activity in eukaryotic cells, one necessary for the biosynthesis of nuclear-coded tRNAs and one necessary for each organelle that codes for tRNAs. Although it is clear that the RNA subunits of these enzymes differ in yeast (6), determining whether or not the proteins are the same or are distinct is of interest.

Our efforts to characterize the yeast mitochondrial RNase P have resulted in the purification of the enzyme to near homogeneity. A 105-kDa protein that fractionates with activity and remains in the most purified fractions has been identified, and the gene that codes for it has been isolated. The availability of the gene allowed us to take advantage of the techniques of yeast molecular biology to create a mutant 105-kDa protein-encoding gene. Mitochondrial tRNA precursors accumulate in the mutant strain, showing directly that the 105-kDa protein is required for mitochondrial RNase P activity.

MATERIALS AND METHODS

Yeast and *E. coli* Strains. *E. coli* strain JM101 [F$^-$, *hsdR514* (r_B^-, m_B^-), *supE44, supF58, lacY1, galK2, galT22, metB1, trpR55*, λ^- (32)] was used for DNA manipulations. *Sa. cerevisiae* strain CMY44 [*α, his4Δ29, pep4-3, prb1-1122, prc1-126* (33)] was used for enzyme isolation. Strain W3031A

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Abbreviation: FPLC, fast protein liquid chromatography.

*Present address: Department of Neurobiology and Anatomy, Washington University, St. Louis, MO 63110.
†To whom reprint requests should be addressed.

(a, *ade2-1, his3-11, 15, leu2-3,112, ura3-1, trp1-1, can1-100*) was used for the disruption experiment (34). Strain ND40 (a, *ade2, his1*) does not contain the mitochondrial RNase P RNA gene, accumulates 5'-extended tRNA precursors (35), and was used as a control for RNA analysis.

Enzyme Quantitation and Purification. Precursor tRNAs were prepared *in vitro*; enzyme activity was quantitated as described by Morales *et al.* (30). Protein was measured by the bicinchoninic acid assay (BCA) (36). Cells were lysed in a Parr bomb (30), and all subsequent steps were carried out at 4°C. Freshly isolated mitochondria were suspended in 1 ml (per g of cells) of 1.0 M $(NH_4)_2SO_4$/15 mM KCl/25 mM Hepes·KOH, pH 7.9/10 mM $MgCl_2$/1 mM EDTA/0.2 mM phenylmethylsulfonyl fluoride/0.2 mM benzamidine/2 mM dithiothreitol/1 μM leupeptin/1 μM pepstatin A. The mixture was stirred on ice for 30 min and centrifuged at 30,000 × *g* for 30 min. The supernatant was discarded, and the pellet was suspended in 0.2 ml (per g of cells) of 1 M KCl/25 mM Hepes·KOH, pH 7.9/5 mM $MgCl_2$/1 mM EDTA/0.2 mM phenylmethylsulfonyl fluoride/0.2 mM benzamidine/2 mM dithiothreitol/1 μM leupeptin/1 μM pepstatin A/10% (vol/vol) glycerol/1.2% Tween 20. This extract was stirred vigorously on ice for 30 min and centrifuged at 30,000 × *g* for 30 min. The supernatant (S30) was either used immediately or stored at −70°C.

The S30 was dialyzed vs. 4 liters of buffer A (25 mM Hepes·KOH, pH 7.9/5 mM $MgCl_2$/0.2 mM EDTA/0.2 mM phenylmethylsulfonyl fluoride/0.2 mM benzamidine/0.2 mM dithiothreitol/10% (vol/vol) glycerol/1% Tween 20) until the conductivity was below 0.15 M KCl plus buffer A. The solution was then applied at 7 ml per min to a 450-ml DE-52 cellulose column (Whatman) equilibrated in 0.1 M KCl plus buffer A, washed with 500 ml of 0.1 M KCl plus buffer A and with 5400 ml of 0.15 M KCl plus buffer A. The enzyme was eluted with 0.5 M KCl plus buffer A at 3 ml per min.

The peak A_{280} absorbing fractions were pooled, diluted 2-fold with buffer A, and applied to a 90-ml heparin-Ultrogel (IBF) column (equilibrated in 0.2 M KCl plus buffer A) at 2.5 ml/min. The column was washed with 270 ml of 0.2 M KCl plus buffer A at 0.25 ml/min. Enzyme was eluted with a 900-ml linear gradient from 0.2 M KCl to 1.25 M KCl plus buffer A at 1.5 ml/min. RNase P eluted at ≈0.75 M KCl.

The heparin pool was dialyzed vs. 4 liters of buffer A until the conductivity was less than that of 0.2 M KCl plus buffer A. The dialyzed pool was applied to a fast protein liquid chromatography (FPLC) Mono Q HR 10/10 (8 ml) column equilibrated in 0.15 M KCl plus buffer A at 2 ml/min. The column was washed with 40 ml of 0.2 M KCl plus buffer A and eluted with a 250-ml linear gradient from 0.2 M KCl to 0.75 M KCl plus buffer A at 0.25 ml/min. Enzyme eluted at ≈0.35 M KCl.

Active fractions from the Mono Q column were pooled and dialyzed vs. 2 liters of buffer A until the conductivity was less than that of 0.2 M KCl plus buffer A and diluted to 0.1 M KCl with buffer A. The extract was then applied, at 0.75 ml/min, to an FPLC Mono S HR 5/5 (0.5 cm × 5 cm, 1-ml) column equilibrated in 0.1 M KCl plus buffer A. The column was washed with 5 ml of 0.1 M KCl plus buffer A, and enzyme eluted with a 20-ml linear gradient from 0.1 M KCl to 0.6 M KCl. The flow rate was 0.5 ml/min; enzyme eluted at 0.3 M KCl.

Glycerol gradients were formed by putting 8 ml of 15% (vol/vol) glycerol in the tube and then adding from the bottom sequentially 9 ml, 9 ml, and 8 ml of 22%, 28%, and 35% glycerol solution, respectively, in buffer A. One inch × 3.5-inch Ultra-Clear (Beckman) tubes were used. The gradients were allowed to incubate 12–16 hr at 4°C. The Mono S pool was concentrated in a Centricon 30 microconcentrator (Amicon) at 4300 × *g* for ≈30 min and layered onto three preformed gradients in 0.9-ml aliquots. Centrifugation was at 141,000 × *g* in a Beckman SW28 rotor for 30 hr. Gradients were fractionated on an ISCO 185 gradient fractionator, from the bottom, using a Flourinert-40 chase at 3 ml/min. Absorbance was monitored with an ISCO UA-5 absorbance detector and type 10 optical unit.

SDS/PAGE Analysis of Proteins. Before SDS/PAGE, samples were concentrated by precipitation in $CHCl_3/CH_3OH$ (37). Protein was transferred to poly(vinylidene difluoride) membranes (Millipore) by electrophoresis in a cold room at 90 V for 1 hr (38).

Protein Sequencing and Oligonucleotide Design. Protein sequence information was obtained by the Protein Sequencing Facility at the University of Florida. The sequence was used to design an oligonucleotide mixture with the sequence 5'-ACTGGTCCAACTYTACCAACTAAYCARTAYGAY-CCAYTGAA-3'.

Isolation and Disruption of the 105-kDa Protein Gene. The oligonucleotide was used to screen a yeast genomic library (39) and to identify a 4.5-kilobase (kb) *Bgl* II fragment with an internal *Hpa* I site in the coding region for the 105-kDa protein. The 4.5-kb *Bgl* II fragment was subcloned into pBluescript II KS+ (Stratagene), the resulting recombinant DNA was cut with *Hpa* I, and a *Hpa* I fragment containing the *LEU2* gene from YEp13 (40) was inserted. The fragment used for the gene-disruption experiment was isolated by cutting the resultant plasmid with *Sph* I and *Sna*B1, introduced by electroporation (41), and LEU+ colonies were selected. Southern analysis with an oligonucleotide (5'-GTATGTAGATTTATTCAGG) complementary to the 5' end of the 105-kDa protein gene confirmed the disruption. Hybridization with an oligonucleotide specific for the mitochondrial RNase P RNA gene (*RMP1*) (5'-TATTTAT-GACTTTCCTATTA) confirmed the presence of the *RPM1* gene in the disrupted strains.

RNA Analyses. RNase P RNA fragments in gradient fractions were detected with mitochondrial RNase P RNA-specific oligonucleotides (30). tRNAs were separated on an 8% polyacrylamide gel, transferred to nylon membranes, and probed with a $tRNA_f^{Met}$ probe, as described in Shu *et al.* (27).

RESULTS

Isolation of Mitochondrial RNase P. Our previously reported partial purification of the RNase P activity from yeast mitochondria (30) did not lead to homogenous enzyme, and addition of further steps resulted in complete loss of activity. The protocol reported here differs in the replacement of deoxycholate with Tween 20 and utilization of buffers used successfully in the partial purification of the *Sa. cerevisiae* nuclear RNase P activity (6). The DEAE-cellulose chromatography separated 90% of the protein (Table 1) and much of the endogenous nuclease activity from the RNase P activity. As reported (30), the RPM1 RNA in active fractions is fragmented by endogenous nuclease, but the fragmentation itself does not affect activity measurably. Even though activity does not seem to be compromised by the fragmentation, it may account for the low yields we obtain. The

Table 1. Purification of RNase P

Sample	Protein, mg	Activity, nmol/min	Specific activity, nmol/min per mg	Yield, %	Purification, -fold
S30	3033	173	0.057	100	1
DEAE	345	73.8	0.214	42.7	3.7
Heparin	37.4	30.5	0.816	17.6	14.3
Mono Q	10.4	20.6	1.98	11.9	34.7
Mono S	1.6	7.6	4.75	4.4	83.3
Glycerol	0.025	2.7	108	1.6	1894.7

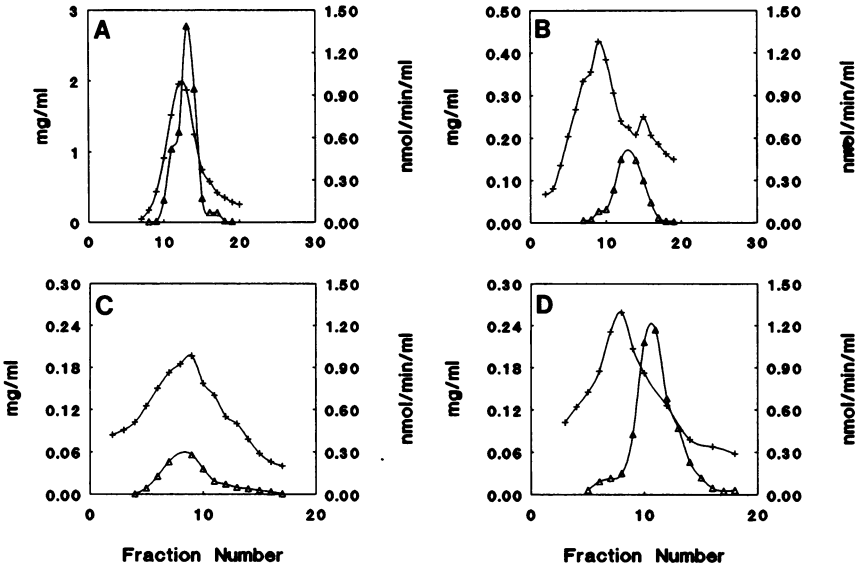

FIG. 1. Column chromatography of mitochondrial RNase P. (*A*) DEAE-cellulose column. (*B*) Heparin-Ultrogel column profile. (*C*) FPLC mono Q column. (*D*) FPLC mono S column profiles obtained as described. +, Concentration of protein in mg/ml; △, RNase P activity expressed as nmol of precursor processed per min.

heparin-Ultrogel, FPLC Mono Q, and FPLC Mono S columns that followed the DEAE column each increased purity several-fold (Fig. 1, Table 1). The most effective purification step was the glycerol gradient. The activity (Fig. 2 *A* and *D*) and the mitochondrial-coded RNA pieces from RPM1 RNA required for the activity (Fig. 2 *B* and *C*) were concentrated in a narrow peak at 22% glycerol.

Identification of the 105-kDa Protein. Fig. 3*A* shows SDS/PAGE analysis of pooled fractions obtained from the columns and glycerol gradient. Proteins in the 105-kDa range are enriched in the heparin pool, and all but one is removed in subsequent steps. Fig. 3*B* shows an SDS/PAGE analysis of gradient fractions from a different purification than that shown in *A*. It is clear from this, as well as from lane 7 in *A*, that the 105-kDa protein is the most prominent protein fractionating with activity. Minor proteins are hardly visible. Fractionation of the most abundant protein with activity is a biochemical criteria for assigning a protein as an enzyme subunit, but a less-abundant protein could account for the observed activity. To determine whether the 105-kDa protein plays a role in mitochondrial RNase P activity, a mutant allele of the 105-kDa gene was created.

Isolation of the 105-kDa Protein-Encoding Gene. The genetic analysis was initiated by isolating the gene coding for the protein of interest. The 105-kDa protein was isolated, separated from other proteins by electrophoresis, transferred to Immobilon paper (38), and sequenced directly. The amino-terminal sequence derived from this analysis was (S)TGPTLPTNQY(D or F)PLN(F)?N(R)N; uncertain assignments are indicated by parentheses. This information was used to design an oligonucleotide mixture following codon-usage rules for yeast (42). We used the oligonucleotides to screen a yeast genomic library (39), and one positive clone was analyzed in sufficient detail to confirm that it codes for the 105-kDa protein. Sequence obtained by priming from an oligonucleotide predicted from the amino acid sequence was used to design a primer of the opposite polarity but complementary to the upstream sequence obtained in the first sequencing run. The DNA sequence obtained from this experiment confirms that the gene codes for the 105-kDa protein. The actual amino-terminal sequence predicted from the DNA sequence is STGPTLPTNQYDPLNFSNRN.

The 105-kDa Protein Is Required for Mitochondrial RNase P Activity. The restriction mapping and DNA sequencing accomplished to date has not identified significant similarities to any other sequence. We have, however, gained enough information to allow design and execution of a disruption. A gene >3 kb is required to code for a 105-kDa protein, and introduction of foreign DNA downstream of the region coding for the amino-terminal sequence should impair function of

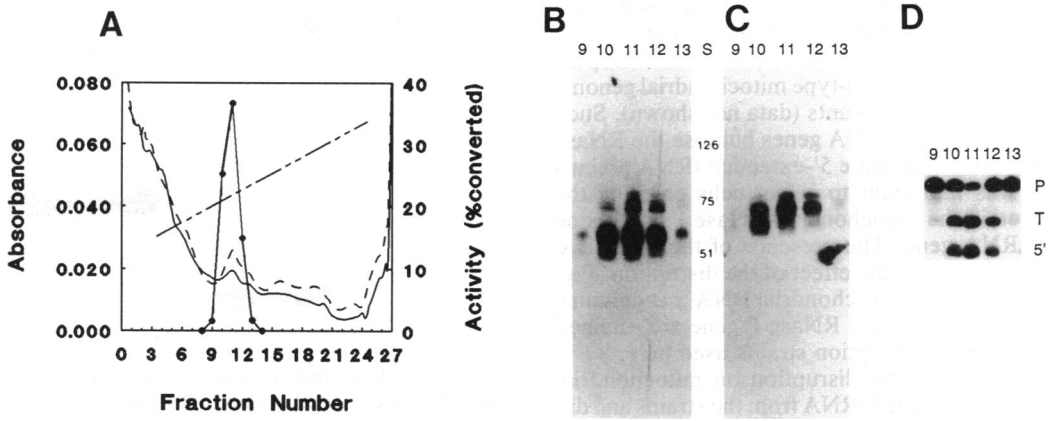

FIG. 2. Glycerol-gradient fractionation of FPLC mono S column fractions. (*A*) Mono S pool was layered on a 35-ml 15–35% glycerol gradient and centrifuged for 30 hr at 28,000 rpm in a Beckman SW28 rotor. - - - -, A_{254}; ——, A_{280}; —●—, activity; — - -, glycerol. (*B–D*) Activity and RPM1 RNA cofractionate on glycerol gradients. RNA was isolated from glycerol gradient fractions, transferred to paper, and probed with oligonucleotides complementary to the 3′ conserved sequence block (*B*), and the 5′ conserved sequence block (*C*) described in Morales *et al.* (30). (*D*) RNase P activity assays. Numbers across top refer to fractions shown in *A*; 126, 75, and 51 mark positions of migration of RNA-size standards. Positions of the precursor tRNA (P), tRNA product (T), and 5′ leader (5′) are indicated at right.

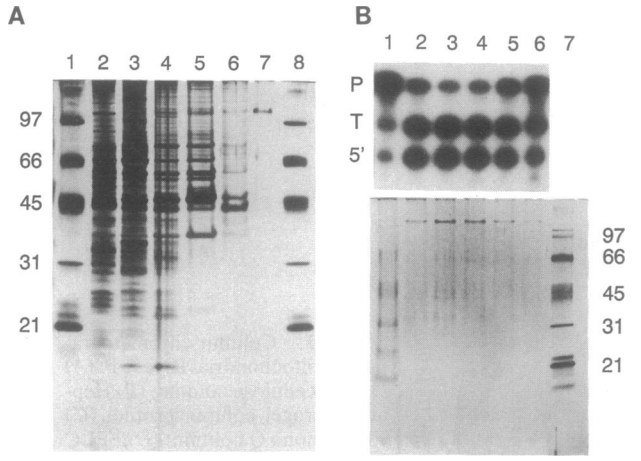

FIG. 3. PAGE analysis of column pools and glycerol-gradient fractions. (*A*) Lanes: 1 and 8, molecular weight ($\times 10^{-3}$) standards; 2, 4.3 μg of protein from mitochondrial extract; 3, 4.3 μg of protein from DEAE column pool; 4, 2.2 μg from the heparin-Ultrogel column pool; 5, 2.2 μg of protein from FPLC mono Q column; 6, 0.8 μg of protein from FPLC mono S column; 7, protein from glycerol-gradient fraction, amount unknown. (*B*) (*Top*) RNase P assays. Conversion of precursor tRNA (P) to 5′ leader (5′) and tRNA (T). (*Bottom*) SDS/PAGE of glycerol-gradient fractions. Lanes: 1, fraction 11; 2, fraction 12; 3, fraction 13; 4, fraction 14; 5, fraction 15; 6, fraction 16 from the gradient; 7, molecular weight size ($\times 10^{-3}$) standards.

the enzyme if the 105-kDa protein is, indeed, an RNase P subunit. An *Hpa* I site ≈1.8 kb downstream from the region coding for the amino-terminal sequence was used to insert a *LEU2* gene into the 105-kDa protein gene, and a fragment containing the *LEU2* gene flanked by 105-kDa gene sequences was used to transform haploid cells. The fact that many transformants able to grow on medium without leucine were recovered indicates that the 105-kDa protein gene is not essential, as most integration events are predicted to be in that gene. Approximately 95% of the transformants were unable to grow on glycerol media, indicating that they have a defect in respiratory metabolism. DNA from two transformants unable to grow on glycerol and one transformant able to do so were isolated. The *Bgl* II fragment of the 105-kDa protein gene obtained from the respiratory-deficient transformants analyzed here increases in size by 2 kb, as would be predicted were the *LEU2* gene inserted in the targeted 105-kDa protein gene (Fig. 4*A*). The transformant that retains respiratory metabolism sustains an integration somewhere other than the 105-kDa protein gene, as the size of the *Bgl* II fragment containing part of the 105-kDa protein gene is unchanged (Fig. 4*A*). Strains with the disrupted 105-kDa gene do not retain a wild-type mitochondrial genome and produce petite deletion mutants (data not shown). Such deletion mutants that retain tRNA genes but lose the RNase P RNA are known to accumulate 5′-extended tRNA precursors (35), so it was important to select cells carrying the disruption that retain the mitochondrial RNase P RNA gene and a reporter tRNA gene. The presence of the latter two genes is necessary to assess the effect of the disruption. Fig. 4*B* shows that the region of mitochondrial DNA that contains the reporter tRNA gene and the RNase P gene are retained in the two 105-kDa gene-disruption strains used here.

To assess the effect of the disruption on mitochondrial tRNA biosynthesis we isolated tRNA from the strains and did an RNA analysis. The $tRNA_f^{Met}$ was chosen as the reporter tRNA because its precursor and product are well characterized (35), and a petite deletion mutant that does not contain the RNase P RNA gene, *RPM1*, and thus accumulates the $tRNA_f^{Met}$ precursor could be used for comparison. Fig. 5 shows that RNA from the disrupted strains contains mitochondrial tRNA precursors identical to those that accumulate in the absence of the RNA subunit, proving that the 105-kDa protein is required for yeast mitochondrial RNase P activity.

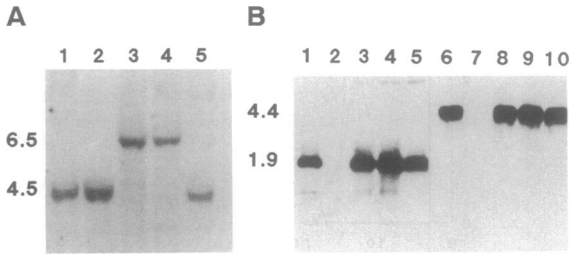

FIG. 4. Southern analysis of nuclear and mitochondrial DNA in wild-type and disrupted strains. (*A*) Total yeast DNA was cut with *Bgl* II and hybridized with a probe specific for the 105-kDa protein-encoding gene. Lanes: 1, W3031A wild-type DNA; 2, W3031A ρ° (contains no mitochondrial DNA); 3, W3031A *RPM2*::*LEU2-6*; 4, W3031A *RPM2*::*LEU2-10*; 5, W3031A ?::*LEU2*. (*B*) Total yeast DNA cut with *Nco* I and *Taq* I (lanes 1–5) or with *Sau*3A (lanes 6–10) and probed with an RNase P RNA probe. Lanes: 1 and 6, W3031A wild-type DNA; 2 and 7, W3031A ρ°; 3 and 8, W3031A *RPM2*::*LEU2-6*; 4 and 9, W3031A *RPM2*::*LEU2-10*; 5–10, W3031A ?::*LEU2*. Fragment sizes were determined relative to a λ *Hin*dIII digest.

DISCUSSION

We report the purification of yeast mitochondrial RNase P to near homogeneity and find the most abundant protein remaining is the 105-kDa protein. Partial purifications of other eukaryotic RNase P enzymes lead to the identification of RNA but not protein subunit(s) of these enzymes. The Hela cell nuclear RNase P was purified 1700-fold over a DEAE-Sepharose column, two glycerol gradients (both 15%–25%), and a Cs_2SO_4 gradient; the yield was 1.4% (8). *Sa. cerevisiae* nuclear enzyme was enriched 730-fold by chromatography of nuclear extract over BioRex 70, DEAE, hydroxylapatite, and a sucrose gradient (6). Purification of the *Sc. pombe* RNase P involved two DEAE columns followed by gel filtration on Sephacryl S-300 (5). *Xenopus* RNase P has been purified by similar methods to 2740-fold over total protein with an impressive yield of 25% (9). Mitochondrial RNase P activities have been described but not extensively purified in both plant (43) and animal mitochondria (44), and one would predict that the organelle enzymes would be less abundant than their nuclear counterparts. From our work, we estimate the yield of protein from 3 kg of cells at 5–15 μg. Given a yield of 1.6% and if the protein is directly proportional to activity, this

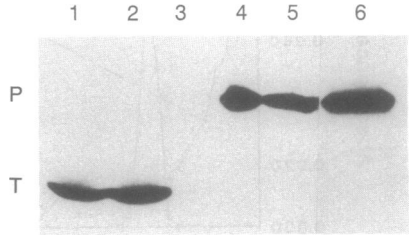

FIG. 5. RNA analysis of tRNA gene transcripts made in wild-type and *RPM2*-disrupted strains. Fifty micrograms of RNA was separated on an 8% polyacrylamide gel, transferred to paper, and probed with a probe to $tRNA_f^{Met}$. Lanes: 1, W3031A; 2, W3031a ?::*LEU2*; 3, W3031A ρ°; 4, W3031A *RPM2*::*LEU2-6*; 5, W3031A *RPM2*::*LEU2-10*; 6, ND40, a strain without the RNase P RNA gene that accumulates 5′-extended tRNA precursors (35); lane 6 has been deliberately underexposed to compensate for the increased $tRNA_f^{Met}$ copy number in strain ND40.

makes the 105-kDa protein only 0.001%–0.002% of the total cellular protein or between 100–200 molecules per cell.

The results of the genetic experiments show that the association of the 105-kDa protein with RNase P activity is not fortuitous. Precursor tRNAs accumulate in the disrupted strain (Fig. 5) and are the same size as those in strains with a wild-type 105-kDa protein gene but missing the RNase P RNA gene (35). The mitochondrial RNA gene has been referred to as the tRNA synthesis locus in the past (25), a name assigned to it before the knowledge that it coded for an RNase P RNA. We have registered the name *RPM* (RNase P mitochondrial) for these genes in yeast, and the RNA subunit is coded by the *RPM1* gene, whereas the 105-kDa protein will be called *RPM2*.

The consequences of disrupting the 105-kDa gene extend beyond the accumulation of mitochondrial tRNA precursors. As expected from a mutation with an effect on mitochondrial protein synthesis, these strains are respiratory deficient and unable to grow on nonfermentable carbon sources. Mutations that cause mitochondrial-protein-synthesis defects destabilize mitochondrial DNA, resulting in the production of petite deletion mutants (45). Disruption of the *RPM2* gene does lead to production of petite deletion mutants, but several that retained the *RPM1* gene and its flanking tRNAs were selected so the effect of the disruption could be assessed in their presence. Petiteness, *per se*, does not affect RNase P RNA processing, import of the protein subunit, or assembly of the enzyme, as many petites carry out all of these processes (46). The presence or absence of the *RPM1* or *RPM2* gene determines whether active mitochondrial RNase P is formed. No other phenotypes associated with the disruption are evident. Thus, the *RPM2* gene is not required for cytoplasmic tRNA synthesis and is not, like some tRNA modification enzymes (47, 48) and ATP (CTP): tRNA nucleotidyltransferase (49), shared between the mitochondria and the nucleus.

The most straightforward interpretation of the purification is that the 105-kDa protein is a subunit of RNase P. Alternatively, the 105-kDa protein could be necessary for producing RNase P activity but not be a *bona fide* subunit. This explanation is less likely, as it is the most abundant protein in purified fractions. As no other protein consistently fractionates with activity, and proteins other than the 105-kDa protein are only minor components of the near-homogeneous enzyme, we hypothesize that the *Sa. cerevisiae* mitochondrial RNase P consists of the 105-kDa protein we have identified and the previously identified 490-base RNA (26).

Until now, information about RNase P-associated proteins has been limited to the bacterial enzyme and to a 40-kDa Hela cell protein identified by crossreactivity to an antibody raised to the *E. coli* protein (31). Thus, the 40-kDa Hela and 105-kDa yeast mitochondrial RNase P-associated proteins are larger than the 14-kDa bacterial subunits. That the eukaryotic enzymes are larger and more proteinaceous suggested that their protein subunits might be larger than their bacterial counterparts. As no eukaryotic RNase P RNA has yet been shown to be catalytic on its own, it is possible that some functions carried out by the bacterial RNA have been relegated to the protein subunit of the eukaryotic enzymes, and this could explain the larger size. Alternatively, the very A+U-rich yeast mitochondrial RNAs may require a larger protein simply to keep them in an active conformation. Finally, we do not know the size of this protein subunit in any other yeast strain, but as the size of the RNA varies from yeast to yeast, the proteins may vary as well. As further proteins are added to the data base and additional analysis reveals their structures and role in enzyme activity, the similarities that account for their function should be revealed.

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