

Rpm2p: separate domains promote tRNA and Rpm1r maturation in *Saccharomyces cerevisiae* mitochondria

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ABSTRACT

Rpm2p is a protein subunit of yeast mitochondrial RNase P and is also required for the maturation of Rpm1r, the mitochondrially-encoded RNA subunit of the enzyme. Previous work demonstrated that an insertional disruption of *RPM2*, which produces the C-terminally truncated protein Rpm2- Δ Cp, supports growth on glucose but cells lose some or all of their mitochondrial genome and become petite. These petites, even if they retain the *RPM1* locus, lose their ability to process the 5'-ends of mitochondrial tRNA. We report here that if strains containing the truncated *RPM2* allele are created and maintained on respiratory carbon sources they have wild-type mitochondrial genomes, and a significant portion of tRNA transcripts are processed. In contrast, precursor Rpm1r transcripts accumulate and mature Rpm1r is not made. These data show that one function of the deleted C-terminal region is in the maturation of Rpm1r, and that this region and mature Rpm1r are not absolutely required for RNase P activity. Finally, we demonstrate that full activity can be restored if the N-terminal and C-terminal domains of Rpm2p are supplied *in trans*.

INTRODUCTION

Mitochondrial transfer RNAs (tRNAs) are synthesized as precursors that undergo a number of essential processing steps before assuming a functional role in mitochondrial protein synthesis (1). Ribonuclease P (RNase P) is the endoribonuclease that generates the mature 5'-ends of tRNA by removal of the 5'-leaders from precursor-tRNAs in all organisms (reviewed in 2). All RNase P enzymes described to date, with the exception of plant chloroplast RNase P (3,4), are established ribonucleoproteins, composed of at least one RNA and one protein (5,6). In Eubacteria and a subset of Archea, the RNA component of RNase P is catalytically active *in vitro* in the absence of proteins (7,8). In contrast, the RNase P RNAs of

the Eukarya are not catalytically active by themselves, and RNase P activity requires both RNA and protein subunits.

Yeast mitochondrial RNase P is essential for the production of mitochondrially-encoded tRNAs and, thus, is essential for growth on media containing non-fermentable carbon sources. These tRNAs translate a small number of proteins coded by mitochondrial DNA (mtDNA) that are necessary for electron transport and oxidative phosphorylation. Mitochondrial protein synthesis is also required for the maintenance of wild-type mtDNA in yeast (9), although the reason for this requirement is not known. Therefore, if cells cannot support mitochondrial tRNA processing, they should not be able to maintain the wild-type mitochondrial genome.

Subunits of mitochondrial RNase P are encoded in both mitochondrial and nuclear genomes. The RNA subunit of mitochondrial RNase P, Rpm1r, is encoded by mtDNA, whereas the protein subunit, Rpm2p, is encoded in the nuclear genome and imported into mitochondria (10,11). The gene coding for Rpm1r, *RPM1*, was originally identified by demonstrating that petite deletion mutants could only process tRNAs if they retained mtDNA located between the tRNA^{Met} and tRNA^{Pro} genes (10). Purification of yeast mitochondrial RNase P to near homogeneity revealed a 105 kDa protein named Rpm2p (12). The evidence that Rpm2p is a subunit of yeast mitochondrial RNase P is compelling. Rpm2p antibodies raised to a Trp-Rpm2p fusion protein immunoprecipitate all RNase P activity and all RNase P RNA from mitochondrial extracts (13). Moreover, an insertional disruption of the *RPM2* gene causes the accumulation of precursor tRNAs *in vivo* (13). While it is clear that Rpm2p is a subunit of a mitochondrial RNase P enzyme, the role that it plays in enzyme activity is not yet known.

Further studies revealed that *RPM2* is a complex, multi-functional gene. Experiments designed to delete the entire gene revealed, unexpectedly, that *RPM2* is essential for growth on all carbon sources (14). Since mitochondrial protein synthesis is required for respiratory but not fermentative growth, Rpm2p must have at least one other function in addition to its role as a subunit of mitochondrial RNase P. We do not yet know why *RPM2* is essential but cells containing the insertional disruption producing a protein with a C-terminal deletion of 468 amino acids sustain deletions in their mitochondrial DNA and

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do not process tRNA even if they retain the *RPM1* gene (14). These results were consistent with a model that placed RNase P function in the C-terminal region of Rpm2p and the essential function in the N-terminal portion of the protein.

We now report that the C-terminal deletion allele can support RNase P activity if diploid cells with this allele are sporulated and maintained on non-fermentable carbon sources. Therefore, RNase P activity does not, as previously thought, require the C-terminal region of *RPM2*. We show here that the C-terminal deletion allele of *RPM2* differentially affects processing of pre-tRNAs and Rpm1r. The C-terminal deletion mutant allele, *rpm2-734::LEU2*, produces Rpm2- Δ Cp, a protein that does not support Rpm1r processing, but does support pre-tRNA processing, albeit not as efficiently as does Rpm2p. These data show that the two RNA maturation functions of Rpm2p can be separated. Finally, we demonstrate that all functions of Rpm2p are provided if the N-terminal and C-terminal domains, both with mitochondrial targeting signals, are expressed *in trans*.

MATERIALS AND METHODS

Strains, media and reagents

Standard yeast manipulations were used (15). Yeast cells were transformed with plasmid DNA using a lithium acetate method (16). Rich media included 1% Bacto-yeast extract, 2% Bacto-peptone and 2% glucose (YPD); in other cases 3% glycerol and 2% ethanol (YPGE), 2% galactose or 2% raffinose were used instead of glucose. Synthetic complete (SC) media lacking appropriate amino acids for plasmid retention contained 0.67% Bacto-nitrogen base and 2% glucose. Solid media for plates included 2% Bacto-agar. SC medium containing 1 g/l of 5-fluoroorotic acid (5-FOA) was used to select *ura*⁻ yeast segregants. Culture media reagents were from Fisher Scientific (Pittsburgh, PA) or Difco (Detroit, MI). The haploid strain with chromosomal deletion of *RPM2* was created by sporulation of the diploid strain W303 (*ade2-1/ade2-1*, *his3-11,15/his3-11,15*, *leu2-3,112/leu2-3,112*, *ura3-1/ura3-1*, *trp1-1/trp1-1*, *can1-100/can1-100*, *RPM2/rpm2-144,1170::LEU2*) containing a deletion of 3080 bp of the chromosomal *RPM2* gene encoding amino acids 144–1170, replaced with the *LEU2* gene. Wild-type *RPM2* in this haploid strain is provided by the plasmid YEp352/*RPM2*. The C-terminally truncated *RPM2*, *rpm2-734::LEU2*, haploid strain was obtained by inserting the *LEU2* gene into *RPM2* at the unique *HpaI* site downstream of the codon for amino acid 734 as described by Morales *et al.* (12) into the diploid W303 strain. This strain was sporulated and dissected on glycerol/ethanol plates.

Plasmid construction

Standard procedures were used for the preparation and ligation of DNA fragments and recovery of plasmid DNA from *Escherichia coli* (17). Restriction and modification enzymes were used as recommended by the supplier (New England Biolabs, Beverly, MA). Plasmid DNA was isolated using Qiagen columns, and DNA fragments were isolated from agarose gels using a QIAEX II Extraction Kit (Qiagen, Chatsworth, CA). To construct plasmid pRS314/*rpm2- Δ 735–1180* (Δ C), plasmid pRS314/*RPM2* was cut with *HpaI* and *PpuMI*, filled in with Klenow fragment, gel-purified and religated. To

construct pRS314/*rpm2- Δ 714–1097* (Δ C-20), plasmid pRS314/*RPM2* was cut with *PstI*, gel-purified and religated. To construct pRS313/*rpm2- Δ 216–734* (Δ N), plasmid pRS314/*RPM2* was cut with *EcoRV* and *HpaI*, gel-purified and religated.

RNA isolation

Total RNA was isolated by hot phenol extraction (18). Mitochondrial RNA was purified by guanidinium extraction (19) of isolated mitochondria (20).

Northern analysis

Total RNA or mitochondrial RNA (25–30 μ g) was separated on a 6% polyacrylamide/8 M urea/Tris–borate/EDTA gel, transferred to a Zeta-Probe membrane, and processed as recommended by the manufacturer (Bio-Rad, Hercules, CA). A riboprobe complementary to the tRNA_f^{Met} was transcribed from *PstI*-digested pMM6 (20). A riboprobe complementary to Rpm1r was transcribed from *HindIII*-digested pMH777 (20). A riboprobe complementary to the tRNA^{Pro} was transcribed from *HindIII*-digested pT3/T7Pro. All plasmids were transcribed with T3 RNA polymerase in the presence of [α -³²P]UTP (800 Ci/mmol; Dupont NEN, Boston, MA) using an Ambion T7/T3 Maxiscript kit as specified by the supplier. Oligonucleotide probes 5'-TTTATCGCTTTACCAC-3' and 5'-CCATTAGACGATAAGGTC-3' complementary to tRNA^{Phe} and tRNA^{Glu}, respectively, were radiolabeled with [γ -³²P]ATP at the 5'-end as described (17). The signals were detected using a phosphorimager (PhosphorImager SF, Molecular Dynamics, Eugene, OR) or autoradiography.

RNA end mapping

Primer extension for the mapping of 5'-ends and RNA protection analysis for the 3'-end mapping of Rpm1r was performed as described by Stribinskis *et al.* (21).

Western analysis

Mitochondria were isolated as described by Fox *et al.* (22) and mitochondrial proteins were separated by 7% Tris–acetate polyacrylamide gel (Invitrogen, Carlsbad, CA), transferred to Immobilon-P membranes (Millipore, Bedford, MA) and treated with antibodies. The anti-Rpm2p antibodies were made against a peptide encoding amino acids 306–323 (QCB Inc., Hopkinton, MA) and were used at 1:200 dilution.

RESULTS

Yeast strains with a truncation of Rpm2p grow slowly on a non-fermentable carbon source

Dang *et al.* (13) demonstrated that deletion of the C-terminal end of Rpm2p by inserting the *LEU2* gene in *RPM2* downstream of the codon for amino acid 734 induced loss of wild-type mtDNA on medium containing 2% glucose. To determine the effect of the C-terminal deletion in cells grown on non-fermentable media, *RPM2/rpm2-734::LEU2* diploid cells (Fig. 1A) were sporulated, tetrads dissected and spores grown on plates containing glycerol and ethanol as carbon sources. We found that all four spores in each tetrad grew on glycerol/ethanol plates, but two spores formed smaller colonies (Fig. 1B). The slower growing cells carry the *rpm2-734::LEU2* allele, whereas cells from larger-sized colonies contained

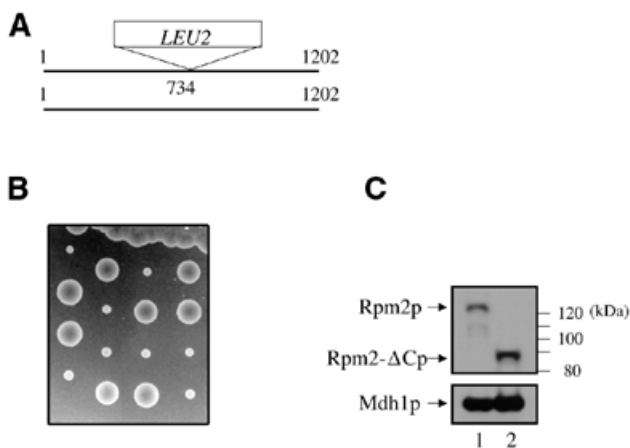


Figure 1. The *rpm2-734::LEU2* allele supports respiratory growth if spores are grown directly on medium with glycerol/ethanol. (A) A schematic representation of the diploid *RPM2/rpm2-734::LEU2*. Numbers indicate amino acid residues in Rpm2p. (B) Diploid cells were sporulated and tetrads were dissected on YPGE plates. The small colony size segregated with the *LEU2* allele. (C) Mitochondrial proteins were isolated from wild-type and *rpm2-734::LEU2* cells, separated by Tris-acetate-polyacrylamide gel electrophoresis, and analyzed by immunoblotting using anti-Rpm2p polyclonal antibodies. The nuclear-encoded mitochondrial protein Mdh1p was used as a loading control. Lane 1, *RPM2*; lane 2, *rpm2-734::LEU2*. Protein molecular mass standards are indicated on the right.

RPM2. This result predicts that the mutant protein is properly expressed, localized to mitochondria and provides enough RNase P activity to support slow growth on glycerol. To test this prediction, mitochondrial extracts were prepared from wild-type and mutant cells grown in glycerol/ethanol medium and analyzed by western blotting. Figure 1C shows that both wild-type and mutant *RPM2* alleles produce Rpm2 proteins that are localized to mitochondria. Thus, the deletion introduced into Rpm2p does not affect the stability and localization of the mutant protein.

One possible explanation for the decreased doubling time is that a percentage of the *rpm2-734::LEU2* cells lose their wild-type mitochondrial genome (become petite) and cannot continue to divide on non-fermentable carbon sources. Such cells should grow if plated to glucose medium but not if plated to glycerol/ethanol medium. Alternatively, the entire population of cells might maintain its wild-type mitochondrial DNA but grow slowly. To differentiate between these two possibilities, the frequency of respiratory deficient cells generated during growth of wild-type and *rpm2-734::LEU2* strains on glycerol/ethanol media was determined. In one approach, we removed aliquots from liquid cultures grown on glycerol/ethanol media and plated an equal number of cells to either glucose or glycerol/ethanol containing plates. In another, after 24 h of growth on glycerol/ethanol plates, colonies were dispersed by micromanipulation and the ability of single cells to multiply on glycerol/ethanol was monitored. Fewer than 3% of wild-type and mutant cells failed to divide under conditions that required respiration. Thus, we conclude that *rpm2-734::LEU2* cells maintain their wild-type mitochondrial DNA and the slow growth phenotype observed on glycerol/ethanol medium cannot be attributed to higher than normal rates of petite formation.

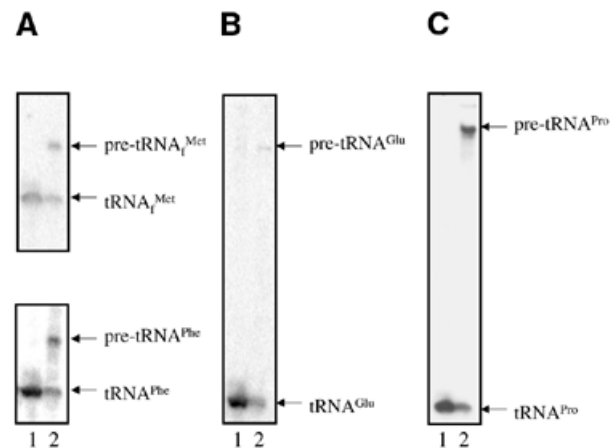


Figure 2. Processing of mitochondrial tRNAs in wild-type and *rpm2-734::LEU2* cells. Mitochondrial RNA was isolated from cells grown in glycerol/ethanol medium (YPGE). A northern blot was performed with probes to (A) $tRNA_{Met}$ and $tRNA_{Phe}$ (top and bottom, respectively), (B) $tRNA_{Glu}$ and (C) $tRNA_{Pro}$. Wild-type (lane 1) and *rpm2-734::LEU2* (lane 2). Pre-tRNA and tRNA mark the positions of precursor and mature tRNA, respectively.

C-terminal deletion of Rpm2p differentially affects processing of tRNA and Rpm1r

The tRNA processing capacity of the ΔC mutant grown in glycerol/ethanol liquid medium was assessed by northern analysis. Figure 2 demonstrates that while mature tRNAs are produced in the mutant, their steady-state levels are lower compared to wild-type and precursor tRNAs are detected. This occurs regardless of whether they have short ($tRNA_{Met}$ and $tRNA_{Phe}$, Fig. 2A) or long ($tRNA_{Glu}$ and $tRNA_{Pro}$, Fig. 2B and C, respectively) 5'-leader sequences.

Rpm2p is also required for maturation of the RNA subunit of mitochondrial RNase P, Rpm1r (21). To analyze the effect of the C-terminal deletion mutation on the synthesis of Rpm1r, steady-state levels of mature Rpm1r and pre-Rpm1r were assessed by northern analysis. The majority of *RPM1* transcripts were mature in wild-type cells with a small amount of an intermediate in the processing pathway detected (Fig. 3B, lane 1). In contrast, only a small amount of an intermediate and no mature Rpm1r was detected in the mutant and instead, high molecular weight primary transcripts accumulated (Fig. 3B, lane 2). Hybridization of the same filter with a probe complementary to $tRNA_{Pro}$ (Fig. 3C) identified the same major high molecular weight primary transcripts as those detected with the Rpm1r probe (Fig. 3B and C, compare lanes 2). There was no comparable transcript detected in wild-type RNA at this exposure (Fig. 3C, lane 1). These data suggest that Rpm1r processing is disrupted at both 5'- and 3'-ends in cells producing Rpm2- ΔC p.

Mature Rpm1r 5'- and 3'-ends are not made in *rpm2-734::LEU2* cells

The 5'-ends of transcripts from *RPM1* were mapped by primer extension. A labeled oligonucleotide complementary to Rpm1r was annealed to wild-type and mutant mitochondrial RNA and extended with reverse transcriptase. The extension products

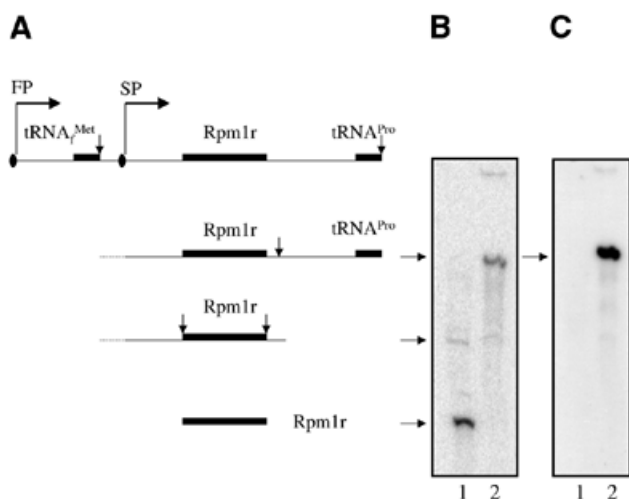


Figure 3. *rpm2-734::LEU2* cells do not make mature Rpm1r. (A) Rpm1r is transcribed either with tRNA^{Met} and tRNA^{Pro} from the FP promoter, or with only tRNA^{Pro} from the SP promoter (top). Vertical arrows correspond to processing events and putative processing intermediates are represented below. The dotted line corresponds to 5'-ends, which can be generated either by cleavage at the 3'-end of tRNA^{Met} or by transcription initiation at SP promoter. Mitochondrial RNA was isolated from cells grown in YPGE. A northern blot was performed with probes to Rpm1r (B) and tRNA^{Pro} (C). Lane 1, wild-type; lane 2, *rpm2-734::LEU2*.

were separated on a 6% denaturing polyacrylamide gel and compared to a DNA sequence generated with the same oligonucleotide (Fig. 4A). The most abundant termination product in wild-type RNA results from the mature Rpm1r (Fig. 4A, lanes 6 and 7). No corresponding extension product was present in the mutant (Fig. 4A, lane 8). Transcripts corresponding to Rpm1r precursors have the same 5'-ends, previously mapped at the nucleotide level to the SP promoter or to the 3'-end of the tRNA^{Met} gene (21), in RNA isolated from wild-type and mutant cells.

To determine if 3'-end maturation occurred, a DNA fragment spanning the mature 3'-end of Rpm1r was end-labeled, strand-separated, hybridized to mitochondrial RNA from wild-type and mutant cells, and digested with S1 nuclease as described (21). The major fragment protected by wild-type RNA corresponds to the mature 3'-end of Rpm1r and is labeled 3'-end (Fig. 4B, lane 3). Its identity was determined by sizing against a chemical sequencing ladder of the same fragment used in the protection experiment (data not shown). No corresponding reaction product was obtained from RNA isolated from the mutant (Fig. 4B, lanes 4–6). This result demonstrates that the 3'-end of mutant Rpm1r is extended relative to the wild-type Rpm1r 3'-end. There is a product formed in RNA isolated from both wild-type and mutant cells (Fig. 4B, lanes 3–6, Intermediate), that could have arisen from an RNA extended 80 nt downstream of the mature 3'-end of Rpm1r (21).

These results, together with those identifying the 5'-ends (Fig. 4A), predict discrete RNAs which were detected, but not separated, in the northern analysis shown in Figure 3B (lanes 1 and 2). The results reported above indicate that removal of the C-terminus of Rpm2p blocks processing of Rpm1r. Transfer RNA processing is only partially affected. This suggests that

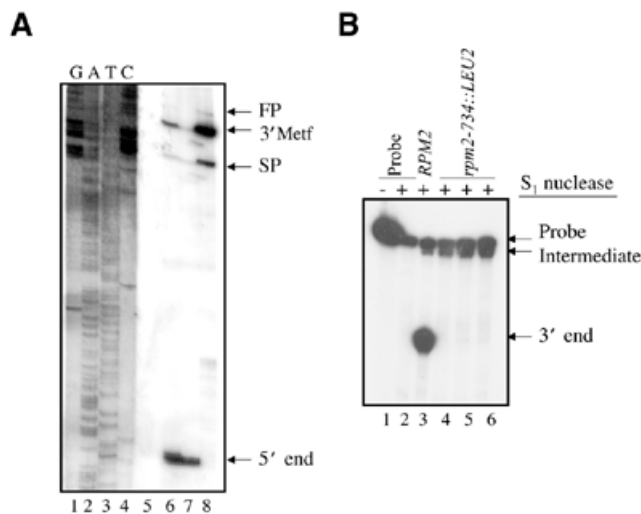


Figure 4. Mapping the 5'- and 3'-ends of *RPM1* transcripts. (A) 5'-ends were determined by extending a radiolabeled primer complementary to a sequence in Rpm1r. A dideoxy sequencing ladder (lanes 1–4) and extension products from wild-type (lanes 6 and 7, mitochondrial RNA isolated from YPD and YPGE grown cells, respectively), and *rpm2-734::LEU2* (lane 8, mitochondrial RNA isolated from YPGE grown cells) RNAs were separated on a 6% denaturing gel. 5' end represents the mature 5'-end of Rpm1r. SP and FP represent 5'-ends of primary transcripts, which originate at consensus promoter sequences downstream and upstream of the tRNA^{Met} gene, respectively. 3' Met₁ represents the 5'-end of a product that is likely formed by an endonuclease cleavage at the 3'-end of tRNA^{Met}. (B) A single-stranded DNA probe was hybridized with mitochondrial RNA isolated from YPGE grown cells. The samples were digested with S1 nuclease, and protected fragments were separated on a 6% polyacrylamide denaturing gel. Probe alone (lanes 1 and 2, in the absence or presence of S1 nuclease, respectively); probe plus S1 nuclease in the presence of wild-type RNA (lane 3) and increasing amounts of *rpm2-734::LEU2* RNA (10, 20 and 30 μ g in lanes 4, 5 and 6, respectively). 3' end represents the mature 3'-end of Rpm1r. Intermediate marks a putative processing intermediate discussed in the text.

the C-terminal domain of Rpm2p plays a more significant role in the processing of Rpm1r than it plays in tRNA processing.

N- and C-terminal truncated Rpm2 proteins function *in trans*

To determine if the C-terminal domain can act *in trans* to restore full activity of Rpm2- Δ Cp, we used three deletion mutants (Fig. 5A). The *RPM2* alleles *rpm2 Δ 735–1180* (Δ C) and *rpm2 Δ 216–734* (Δ N) together cover the entire *RPM2* sequence, while the *rpm2 Δ 715–1097* (Δ C-20) and *rpm2 Δ 216–734* (Δ N) pair is missing amino acids 715–734. All three Δ C, Δ C-20 and Δ N deletion mutants retain DNA coding for the putative mitochondrial targeting information at the N-terminus, so that the protein products they produce should be localized to mitochondria. We introduced the Δ C + Δ N, or Δ C-20 + Δ N genes, in combination on different low-copy plasmids, into Δ *rpm2* cells containing *RPM2* on a *URA3* plasmid. After selection for transformants, we then measured the ability of cells to grow on 5-FOA plates. Only cells that have lost the *URA3* plasmid and have forms of Rpm2p that provide the essential function can grow. *RPM2* and Δ C + Δ N containing cells appeared on 5-FOA plates at the same rate (Fig. 5B). However, the combination of Δ C(-20) and

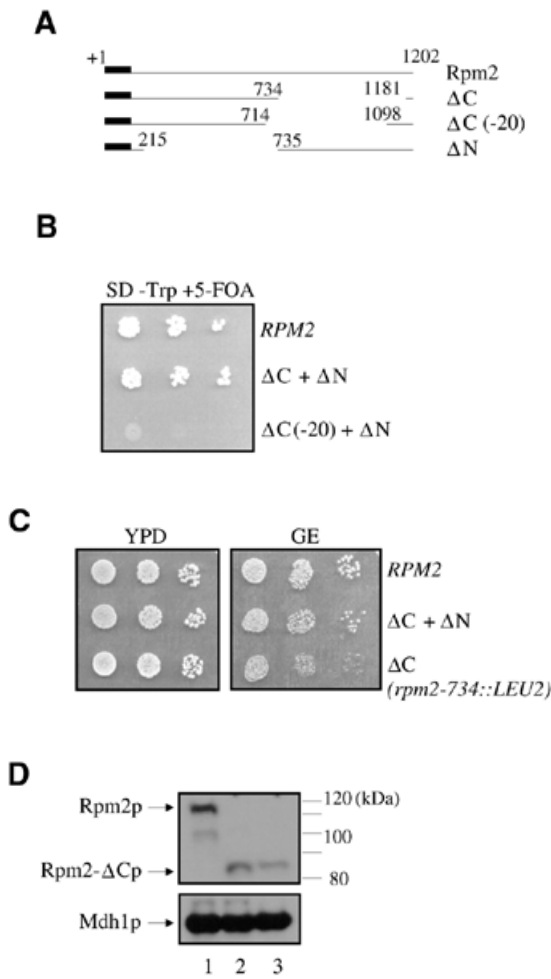


Figure 5. N- and C-terminal truncated Rpm2p proteins function *in trans*. (A) A schematic representation of Rpm2 proteins. Numbers indicate amino acid residues. The filled box represents a putative mitochondrial targeting sequence. (B) *RPM2*, $\Delta C + \Delta N$ or $\Delta C(-20) + \Delta N$ alleles on different low-copy plasmids were introduced into a $\Delta rpm2$ haploid strain containing *RPM2* on a *URA3* plasmid and serial dilutions of transformants plated onto 5-FOA plates to assess growth in the absence of wild-type *RPM2*. (C) Strains were grown overnight in liquid YPGE medium and serial dilutions plated on YPD and YPGE. Cells were incubated 2 and 4 days for YPD and YPGE plates, respectively. (D) Mitochondrial proteins were isolated from the glycerol/ethanol grown cells shown in (C), separated on Tris-acetate-polyacrylamide gel electrophoresis and analyzed by immunoblotting using anti-Rpm2p polyclonal antibodies. The nuclear-encoded mitochondrial protein Mdh1p was used as a loading control. Lane 1, *RPM2*; lane 2, $\Delta C + \Delta N$; lane 3, ΔC (*rpm2-734::LEU2*). Protein molecular mass standards are indicated on the right.

ΔN plasmids does not support growth on glucose medium. This indicates that amino acids 715–734 are essential for growth. Figure 5C demonstrates that cells expressing $\Delta C + \Delta N$ fragments *in trans* grow on the non-fermentable carbon sources glycerol/ethanol at a rate similar, but slightly slower, compared to cells expressing an intact, plasmid-encoded Rpm2p. To determine if ΔC and ΔN are maintained *in trans* during these experiments, mitochondrial extracts were prepared and western analysis with anti-Rpm2p antibodies was performed. Our anti-peptide antibodies do not recognize Rpm2- ΔN protein because the deletion includes the

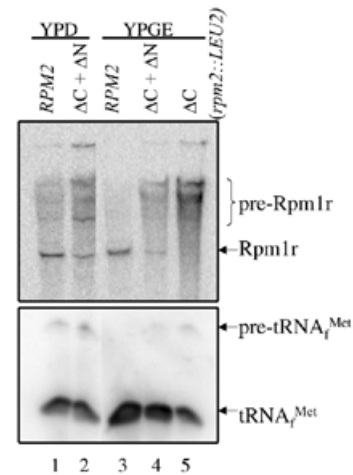


Figure 6. The C-terminal domain together with the N-terminal domain restores full activity of Rpm2p. Northern analysis of wild-type (lanes 1 and 3), $\Delta C + \Delta N$ (lanes 2 and 4) and *rpm2-734::LEU2* (lane 5) total RNAs. Upper panel shows the membrane probed with a riboprobe to Rpm1r; lower panel shows the membrane probed with a riboprobe to tRNA^{Met}.

epitope-coding region but it is clear from the phenotype that Rpm2- ΔN p is being produced. Figure 5D shows that Rpm2- ΔC protein from the *in trans* strain and Rpm2- ΔC p from the original disruption strain migrate identically. There was no full-length Rpm2p, which indicates that Rpm2- ΔC protein is maintained as a separate protein.

Cells containing both ΔC and ΔN also maintain the ability to be transferred from glucose to glycerol/ethanol media, indicating that these cells keep their wild-type mtDNA independent of growth on a particular carbon source. Indeed, Figure 6 shows that the ΔC and ΔN genes together support Rpm1r processing and consequently RNase P activity (lanes 2 and 4 in upper and lower panel, respectively) in both fermentative and respiratory conditions. The amount of mature Rpm1r, however, does appear lower than that found in wild-type cells. Together, the results indicate that the C-terminus harbors a domain that is required for Rpm1r processing and is capable of functioning to a significant degree *in trans*.

DISCUSSION

Petite deletion mutants result when *rpm2-734::LEU2* cells are grown on glucose (13) and even though Rpm1r and tRNA genes are retained, the tRNA gene transcripts are not processed. Providing *RPM2* by transformation restores the tRNA processing ability to these petites. We report here that the same *rpm2-734::LEU2* allele supports mitochondrial tRNA processing and maintenance of wild-type mtDNA if cells are grown on respiratory carbon sources. Therefore, under conditions that require respiration for growth, a truncated version of Rpm2p can support RNase P activity and provide enough tRNA for mitochondrial protein synthesis.

The observation that *rpm2-734::LEU2* cells grow on respiratory carbon sources clearly demonstrates that the entire *RPM2* open reading frame is not essential for mitochondrial RNase P activity. Nevertheless, the ratio of precursor to mature

mitochondrial tRNA is greater in these cells relative to wild-type, indicating that removal of the C-terminus of Rpm2p has a negative effect on RNase P activity. This effect may not, however, be a direct effect of the truncation on RNase P activity but instead may be related to the role of Rpm2p in the maturation of Rpm1r, the RNA subunit of mitochondrial RNase P.

In *Saccharomyces cerevisiae*, Rpm1r is made as a part of a polycistronic transcript, tRNA^{Met}-Rpm1r-tRNA^{Pro}, that undergoes a series of processing steps to release mature Rpm1r (23). Previous results have shown that neither 5'- nor 3'-ends of Rpm1r are matured when *rpm2-734::LEU2* cells are grown on glucose (21). Interestingly, the results shown here demonstrate that even though *rpm2-734::LEU2* cells grown on non-fermentable carbon sources are capable of processing mitochondrial tRNAs, Rpm1r processing is blocked. Thus, the C-terminal region of Rpm2p plays a more significant role in Rpm1r processing than tRNA processing. Moreover, the role of the C-terminal region in Rpm1r processing can be provided *in trans* suggesting that it may be a relatively autonomous domain. Together, these results indicate that the primary defect in *rpm2-734::LEU2* cells is in the processing of Rpm1r, and defects in tRNA processing may be linked to the absence of mature Rpm1r.

Rpm1r is necessary for mitochondrial RNase P activity both *in vivo* and *in vitro* (11,20). Therefore, in the absence of detectable mature Rpm1r, it is surprising to find mature tRNAs in *rpm2-734::LEU2* mitochondria. This observation suggests that pre-Rpm1r may be capable of functioning as a component of RNase P. If this is the case, pre-Rpm1r is not as efficient as mature Rpm1r in supporting tRNA processing since tRNA precursors accumulate. In this context, it has been shown that bacterial RNase P RNA precursors function in tRNA processing *in vitro* (24). Alternatively, levels of mature Rpm1r below our detection limit may be supporting RNase P activity in *rpm2-734::LEU2* cells grown on non-fermentable carbon sources.

The mechanism by which Rpm2p influences Rpm1r processing is unknown. There are several scenarios that could lead to the accumulation of Rpm1r precursors with both 5' and 3' extensions. First of all, the RNase P activity *per se* could be required for maturation of one, or both, ends of Rpm1r. In this context, addition of the *Bacillus subtilis* RNase P protein to a precursor form of the P RNA *in vitro* results in autolytic processing of the 5'- and 3'-ends of the precursor (25). Another possibility is that one or more additional nuclease activities necessary for Rpm1r maturation are encoded by *RPM2* and function independently of RNase P. Finally, Rpm2p could play a chaperone role, facilitating Rpm1r folding into a conformation recognized by 5'- and 3'-end processing enzymes.

It is clear that growth conditions have a profound effect on the ability of the C-terminally truncated form of Rpm2p to provide RNase P activity in mitochondria. One possibility is that there is more enzyme produced in respiring cells and an increase in amount could compensate for reduced activity caused by the truncation. Indeed, increased expression of nuclear-encoded mitochondrial proteins, as well as expression of genes encoded in mtDNA, does accompany growth on glycerol/ethanol (26). Similarly, expression of genes involved in mitochondrial biogenesis are repressed by glucose (catabolite

repression) and this decrease coupled with the truncation could cause RNase P activity to fall below the threshold required for mitochondrial protein synthesis necessary to maintain a wild-type mitochondrial genome. Cells with deletions of *ABF2* and *MMF1*, two other yeast genes encoding mitochondrial proteins, also maintain their wild-type mitochondrial genome if grown by respiration but lose it when grown by fermentation (27,28).

While *rpm2-734::LEU2* cells are capable of growth on non-fermentable carbon sources, their growth is slow. This reduction in growth rate could be a consequence of inefficient tRNA processing linked to truncated Rpm2p and/or inefficient Rpm1r processing. However, Cliften *et al.* (29) showed that there is normal respiratory growth if only 10–20% of wild-type mitochondrial transcripts are present. Clearly, at least 20% of tRNAs are processed in *rpm2-734::LEU2* mitochondria suggesting that tRNA maturation may not be limiting for growth on respiratory carbon sources. Therefore, it is possible that the slow respiratory growth of *rpm2-734::LEU2* might be a reflection of some other mitochondrial dysfunction. In this regard, we have recently demonstrated that Rpm2p, apart from its role in RNase P, has an additional function in the synthesis of mitochondrially-encoded subunits of cytochrome *c* oxidase (30). This function is also required for respiratory growth and may also be affected by the Rpm2p truncation.

An approach to understand why cells expressing the same Rpm2-ΔC protein display differences in their ability to maintain tRNA processing on different carbon sources would be to search for suppressors that allow maintenance of a wild-type mtDNA under fermentative growth. Preliminary results with this approach show promise and will be reported elsewhere.

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