Characterization and Purification of *Saccharomyces cerevisiae* RNase MRP Reveals a New Unique Protein Component*

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In the yeast *Saccharomyces cerevisiae*, RNase mitochondrial RNA processing (MRP) is an essential endoribonuclease that consists of one RNA component and at least nine protein components. Characterization of the complex is complicated by the fact that eight of the known protein components are shared with a related endoribonuclease, RNase P. To fully characterize the RNase MRP complex, we purified it to apparent homogeneity in a highly active state using tandem affinity purification. In addition to the nine known protein components, both Rpr2p and a protein encoded by the essential gene *YLR145w* were present in our preparations of RNase MRP. Precipitation of a tagged version of Ylr145w brought with it the RNase MRP RNA, but not the RNase P RNA. A temperature-sensitive ylr145w mutant was generated and found to exhibit a RNA processing defect identical to that seen in other RNase MRP mutants, whereas no defect in tRNA processing was observed. Homologues of the Ylr145w protein were found in most yeasts, fungi, and *Arabidopsis*. Based on this evidence, we propose that *YLR145w* encodes a novel protein component of RNase MRP, but not RNase P. We recommend that this gene be designated *RMP1*, for RNase MRP protein 1.

RNase MRP\(^1\) is a highly conserved and essential ribonucleoprotein endoribonuclease that cleaves substrates in at least two intracellular compartments. Most RNase MRP is localized to the nucleolus (1), where a role in processing of rRNA precursors has been identified (2). RNase MRP-mediated cleavage at the A3 site of pre-rRNA ultimately leads to the generation of cursors has been identified (2). RNase MRP-mediated cleavage to the nucleolus (1), where a role in processing of rRNA precursors to generate mature 5′ termini. Eight of the proteins associated with RNase MRP (Pop1p, Pop3p, Pop4p, Pop5p, Pop6p, Pop7p, Pop8p, and Rpp1p) are also components of RNase P (11–14). An RNA-binding protein, encoded by the gene *SNM1*, is the only known protein component that associates with RNase MRP RNA but not RNase P RNA (15). Similarly, Rpr2p has been identified as a unique protein component of the RNase P complex (14).

The similarities between RNase MRP and RNase P extend beyond that of shared protein components. The RNA subunits of RNase MRP and RNase P are evolutionarily and structurally related (16, 17). They share only weak sequence homology, but they fold into similar cage-like secondary structures (16). In addition to subunit composition, both RNase MRP and RNase P localize to the nucleolus and the mitochondria and have been shown to cleave common substrates (14, 18).

Despite their similarities, RNase MRP and RNase P appear to assemble into separate catalytic complexes. Nuclear RNase P, purified to homogeneity by high-resolution anion exchange chromatography, retains tRNA processing activity independently of RNase MRP (5, 14). In this study, we outline a method for purifying nuclear RNase MRP in *S. cerevisiae* to apparent homogeneity using a tandem affinity purification system (19). Characterization of the purified complex confirms that the MRP RNA and nine previously identified proteins are components of the RNase MRP complex. In addition, we found the protein encoded by the essential gene *YLR145w* and the gene *RPR2* in preparations of purified and active RNase MRP. We demonstrate that the Ylr145w protein is indeed a protein component of RNase MRP essential for its activity but is not a component of RNase P. This will be the second unique protein component of RNase MRP. We recommend the new name for this gene be designated *RMP1*, for RNase MRP protein 1.

**MATERIALS AND METHODS**

*Strains and Media—* Yeast media and genetic manipulations have been described previously (19, 20). The *E. coli* strain used for cloning, DH5α, has the genotype *φ80dlacZAM15 endA1 recA1 hsdR17 (r− m−) supe44 thi-1 x-garA86 relA1 ΔlacZYA-argF-U169 F−*. Basic molecular techniques were performed as described previously (21). To purify RNase MRP, the YSW1 strain, which has the genotype *MATa POP4::TAPTAG:TRP1 his3::LEU2 ura3::LEU2 sep1::URA3 trp1::his3::11,15 can-100 ura3–1 leu2–3,112*, was used (10). This strain was constructed by using PCR to amplify the TAP fusion cassette from pBS1479 (a gift from Bertrand Séraphin) and then using PCR-based genomic TAP tagging to integrate the tag into the carboxy-terminal codon of the POP4 gene (19). LYS389–34A, which has the genotype *MATa sep1::URA3 pep4::LEU2 nuc1::LEU2 ade2–1 trp1–1 his3–11,15*
Whole-cell Western Blot Analysis—Whole-cell yeast protein extracts were prepared and described previously (20). Samples were denatured in 2% (w/v) SDS and 5% (v/v) 2-mercaptoethanol for 5 min at 95 °C and resolved on a 12% polyacrylamide gel (21). Separated proteins were blocked with 5% (w/v) milk-TBST (20 mM Tris/Cl (pH 7.6), 150 mM NaCl, 5 mM EDTA, 0.1% Triton X-100, 10% glycerol, 1 mM dithiothreitol, and 1 mM phenylmethylsulfonyl fluoride), was incubated with a primary antibody for 2 h; washed with 5% (w/v) milk-phosphate-buffered saline (pH 7.4; 137 mM NaCl, 2.7 mM KCl, 10 mM Na2PO4, and 1 mM EDTA) for one quick wash and three 10-min washes; blotted with an anti-rabbit IgG-POD (1:2500; Ref. 24). Protein A–coupled rabbit IgG agarose (1:1000) was used to amplify a 1.1-kb gene product that contained the YLR145w 3' gene. Error-prone Deep Vent (exo-) polymerase (New England Biolabs) was used to increase the mutation rate. The plasmid YCplac111 (LEU2 CEN) contains unique HindIII and BamHI restriction sites that were used to remove a 30-bp region surrounding the gene (25). Purified RNase MRP-containing extracts or mutational and/or genetic analysis were RNase MRP RNA (1,088-bp EcoRI fragment of pMES140 LEU2 CEN NME1) was used as a template for PCR mutagenesis (23). The oligonucleotides YLR145wFOR (5'–GTGACGGAGTAAACATTCTCACA-CAGGAAACAGCTATGACCATGATTACGCCAAGCTTCACCTGCAACATGG–3') and YLR145wREV (5'–GTTGAGCGGATAACAATTTCACAGTGGGAAACAGCTATGACCATGATTACGCCAAGCTTCACCTGCAACATGG–3') were used to amplify a 1.1-kb gene product that contained the YLR145w 3' gene. Precipitation of TAP-tagged Protein— Forty µl of rabbit IgG agarose (Sigma), which was equilibrated in ice-cold buffer A (20 mM Tris/Cl (pH 8.0), 150 mM KCl, 5 mM EDTA, 0.1% Triton X-100, 10% glycerol, 1 mM dithiothreitol, and 1 mM phenylmethylsulfonyl fluoride), was incubated with 40 µl of whole-cell yeast extract on ice for 1 h. The samples were washed three times with 0.5 ml of buffer A. The volume of each sample was brought up to 100 µl with buffer A. The samples were 2% (w/v) SDS and 5% (v/v) 2-mercaptoethanol and heated at 95 °C for 5 min. After the beads were removed by centrifugation at 16,000 × g for 5 min, the RNA was extracted with phenol/chloroform equilibrated to pH 5.3, precipitated with ethanol, and examined by Northern analysis. Purification of Nuclear RNA MRP Using Tandem Affinity Purification—RNase MRP was purified from strain YSW1 as described in detail previously (10). Several individual preparations were pooled for further analysis. Western Blot Analysis of RNase MRP Protein Components—For each antibody tested, 2 µg of purified nuclear RNase MRP was denatured as described above, resolved on a 15% polyacrylamide gel, and transferred to a nitrocellulose membrane (Scherle & Schuell, Inc., Keene, NH) by use of a Bio-Rad transfer apparatus and stained with Ponceau S solution (0.1% Ponceau S, 5% acetic acid) and washed with double distilled H2O to ensure proper transfer. The membrane was dried under vacuum. The gel pieces were then reduced at 56 °C for 30 min to remove contaminating gel pieces, and then dried under a vacuum. Each sample was resuspended in 8 µl of 0.1% trifluoroacetic acid and passed through a C18 microzip tip (Millipore, Bedford, MA) before MALDI-TOF mass spectrometry analysis. Mass spectrometry data were collected at the Proteomics Core Facility at State University of New York Upstate Medical University using a TOF Spec 2E mass spectrometer (Waters Corp., Beverly, MA). All samples were analyzed under identical conditions in reflection mode. The protein mass spectra generated were analyzed using ProFound (http://prowl.rockefeller.edu/profound_bin/WebProFound.exe). PCR Mutagenesis of YLR145w—Plasmid pKLS108 (UR3 CEN YLR145w) was used as a template for PCR mutagenesis (23). The oligonucleotides YLR145wFOR (5′–GTGACGGAGTAAACATTCTCACA-CAGGAAACAGCTATGACCATGATTACGCCAAGCTTCACCTGCAACATGG–3′) and YLR145wREV (5′–GTTGAGCGGATAACAATTTCACAGTGGGAAACAGCTATGACCATGATTACGCCAAGCTTCACCTGCAACATGG–3′) were used to amplify a 1.1-kb gene product that contained the YLR145w 3′ gene. Precipitation of TAP-tagged Protein— Forty µl of rabbit IgG agarose (Sigma), which was equilibrated in ice-cold buffer A (20 mM Tris/Cl (pH 8.0), 150 mM KCl, 5 mM EDTA, 0.1% Triton X-100, 10% glycerol, 1 mM dithiothreitol, and 1 mM phenylmethylsulfonyl fluoride), was incubated with 40 µl of whole-cell yeast extract on ice for 1 h. The samples were washed three times with 0.5 ml of buffer A. The volume of each sample was brought up to 100 µl with buffer A. The samples were 2% (w/v) SDS and 5% (v/v) 2-mercaptoethanol and heated at 95 °C for 5 min. After the beads were removed by centrifugation at 16,000 × g for 5 min, the RNA was extracted with phenol/chloroform equilibrated to pH 5.3, precipitated with ethanol, and examined by Northern analysis. Purification of Nuclear RNA MRP Using Tandem Affinity Purification—RNase MRP was purified from strain YSW1 as described in detail previously (10). Several individual preparations were pooled for further analysis. Western Blot Analysis of RNase MRP Protein Components—For each antibody tested, 2 µg of purified nuclear RNase MRP was denatured as described above, resolved on a 15% polyacrylamide gel, and transferred to a nitrocellulose membrane (Scherle & Schuell, Inc., Keene, NH) by use of a Bio-Rad transfer apparatus and stained with Ponceau S solution (0.1% Ponceau S, 5% acetic acid) and washed with double distilled H2O to ensure proper transfer. The membrane was dried under vacuum. The gel pieces were then reduced at 56 °C for 30 min to remove contaminating gel pieces, and then dried under a vacuum. Each sample was resuspended in 8 µl of 0.1% trifluoroacetic acid and passed through a C18 microzip tip (Millipore, Bedford, MA) before MALDI-TOF mass spectrometry analysis. Mass spectrometry data were collected at the Proteomics Core Facility at State University of New York Upstate Medical University using a TOF Spec 2E mass spectrometer (Waters Corp., Beverly, MA). All samples were analyzed under identical conditions in reflection mode. The protein mass spectra generated were analyzed using ProFound (http://prowl.rockefeller.edu/profound_bin/WebProFound.exe).
yeast TRP1 marker. This cassette was integrated into the carboxyl-terminal codon of the POP4 gene at its chromosomal locus (Fig. 1A). PCR amplification of the POP4 gene in the resulting YSW1 strain was done to confirm proper integration of the TAP cassette (Fig. 1B). YSW1 grew at rates comparable to wild type at all temperatures tested and maintained normal rRNA processing. The modified Pop4 was examined in whole-cell extracts to ensure that it was not rapidly degraded with addition of the tag or that the tag was being rapidly removed (Fig. 1C). In the YSW1 strain, a 54.3-kDa band is clearly visible, which corresponds to the expected size of the Pop4-TAP tag fusion protein. Immunoprecipitation of the Pop4 tag using rabbit IgG was also performed to confirm association of the modified Pop4 with the MRP RNA. As shown in the Northern analysis of the co-immunoprecipitation experiment (Fig. 1D), the wild-type control strain does not precipitate MRP RNA. However, the TAP fusion strain precipitated MRP RNA, indicating that the TAP tag effectively isolates RNA molecules associated with Pop4.

After confirming that the modified Pop4 protein was functioning comparable to the wild-type protein, we were able to purify the nuclear RNase MRP complex using tandem affinity purification. Because the Pop4 protein is also a component of the RNase P complex, we modified the standard purification procedure to maximize yields of RNase MRP while minimizing co-purification of RNase P. This was accomplished mainly by breaking and extracting cells in a low to moderate salt concentration. RNase MRP was readily released under these conditions, whereas a majority of the RNase P was retained in the cells. In addition, the RNase P complex was found to bind poorly to the calmodulin beads in the final stage of the purification. Samples were collected throughout the purification process, and both total RNA and protein were analyzed (Fig. 2). In the protein A elution, small amounts of the RNase P RNA were detected; however, this RNA was 1% of the total RNA after the final calmodulin step, as measured by fluorescence intensity of the ethidium bromide-stained RNA. The final elution from the calmodulin beads resulted in a single band consistent in size with NME1, the 340-nucleotide component of RNase MRP (Fig. 2A). The identity of this band was confirmed by Northern analysis (Fig. 2B). Indeed, very little breakdown product of the MRP RNA was detected, indicating the complex is whole. The enzyme complex was found to be highly active on both the rRNA A3 substrate and CLB2-5′-UTR substrate, indicating that all of the components required for RNase MRP function are present (10).

The complex was analyzed on a 15–30% glycerol gradient (37) to ensure it was a single complex. As can be seen in Fig. 3A, a single peak was present that contained all of the RNase MRP proteins. Spreading of a trace amount of the Pop4 protein and the Rpp1 protein indicates that a very small amount of a partial complex of these two proteins may be co-purifying with the complex. This may represent an intermediate during RNase MRP and RNase P assembly or may simply be a break-
down product. Negative staining electron microscopy of the purified complex indicated homogeneous size particles of between 25 and 30 nm across.\(^2\)

Identification of Purifying Proteins—Proteins isolated from many separate purifications were analyzed by SDS-PAGE. Consistently, there were at least 9–10 protein bands that separated between \(6 \text{ kDa}\) and 100 kDa, consistent with the size and number of proteins believed to be in the RNase MRP complex (Fig. 2). To identify which bands corresponded to known complex components, we took advantage of available antibodies. Western analyses were performed using antibodies for five of the established protein components of nuclear RNase MRP, namely, Snm1, Pop1, Pop3, Pop4, and Rpp1 (Fig. 3B). Proteins isolated from the final product of the TAP purification were run on SDS-PAGE and transferred to nitrocellulose. Each blot was incubated with an antibody corresponding to one of the individual protein components, probed with a secondary peroxidase-conjugated antibody, and visualized by chemiluminescence. In each case, the RNase MRP proteins were found in the final purified product, assisting in identifying the appropriate bands (Fig. 4). Antibodies against Pop1 identified a minor amount of breakdown product of this protein. These bands were absent in fresh preparations and increased in older preparations after several rounds of freezing and thawing. Antibodies to Snm1 also detected multiple bands near the same molecular mass. This is consistent with what is seen with these antibodies in whole-cell extracts (15). The multiple forms may be the result of proteolytic digestion or secondary modifications. We looked expressly for potential phosphorylation of this protein, but none was detected (data not shown).

Because antibodies are not available for all the known protein components of RNase MRP, we sought to identify the remaining components by mass spectrometry. Protein bands were excised from SDS-PAGE, digested with trypsin, and identified using MALDI-TOF mass spectroscopy (Table I). This identified with high confidence Pop1, Pop4, Snm1, Ylr145w, Pop3, Pop7, Pop5, Rpr2, Pop6, and Pop8 in the purified preparations of nuclear RNase MRP. All of these proteins were identified with a high degree of confidence in separate purifications. As a result, all the known protein components of RNase MRP, as well as Rpr2, a protein thought to be unique to the RNase P complex, were detected. Previous research suggested that RNase MRP contained only nine protein components. However, our analysis identified two additional proteins in the complex. Ylr145w is an essential gene that was recently identified in a genomic analysis as being part of the RNase P enzyme complex (30). Our data indicate that Rpr2p may also associate with RNase MRP. A summary of the MALDI-TOF mass spectroscopy data is shown in Table I.

**Determination of the Stoichiometry of the RNase MRP Complex**—In order to quantitate the relative amount of each of the proteins in the RNase MRP complex, we utilized the protein dye SPYRO Ruby. This fluorescent dye binds most proteins in a linear fashion independent of their amino acid composition. SDS-PAGE gels were stained with SPYRO Ruby and scanned using a Bio-Rad FluorS imager. Utilizing this approach we quantitated each of the identified bands relative to Pop1, which

\(^2\) M. E. Schmitt and D. R. Mitchell, unpublished observations.

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**Fig. 2. Tandem affinity purification profile of nuclear RNase MRP.** Aliquots of yeast extracts from the YSW1 strain were isolated from the final TAP fraction (Cal-Elution), the IgG affinity purification step (Prot A-Elution), unbound extracts (Prot A FT and Cal FT), and tightly bound extracts (Prot A-SDS and Cal-SDS) for step-by-step analysis of the TAP purification process. A, analysis of RNA collected from extracts. RNA was extracted from aliquots using phenol/chloroform and precipitated. Total RNA from the indicated aliquots was separated on a 6% acrylamide-7M urea gel and examined by staining with ethidium bromide. B, Northern analysis of the RNA purified from the Pop4-TAP fusion strain. RNA from whole-cell extracts was run on a 6% acrylamide-7M urea gel and transferred to a nylon membrane. The MRP RNA was visualized by hybridization with a \(32P\)-labeled probe containing the NME1 gene. C, analysis of proteins purified. Proteins isolated from aliquots were separated on 12% SDS-PAGE gel and visualized using silver stain. Standards are the Invitrogen Mark12 protein standard. The sizes of these standards are provided in Fig. 4. **nt**, nucleotides.
was given a value of 1. After imaging, the proteins were excised from gels, and their identity was confirmed using MALDI-TOF mass spectroscopy. These data are summarized in Table I.

**Ylr145w Is a Component of RNase MRP, but Not RNase P**—Our MALDI-TOF mass spectroscopy data indicate that the protein encoded by the uncharacterized open reading frame *YLR145w* may be a component of nuclear RNase MRP. In an attempt to confirm that this gene product associates with RNase MRP and P, we tried to precipitate the corresponding RNAs with a tagged version of the Ylr145w protein. The POP4::TAP-tagged strain was used as a positive control. Whole-cell lysates were incubated with IgG beads. RNAs extracted from the beads were separated on 6% acrylamide-7 M urea gels, transferred to nitrocellulose membranes and probed with polyclonal antibodies against the indicated components (see "Materials and Methods"). The blots were probed with a secondary peroxidase-conjugated anti-rabbit antibody and visualized with chemiluminescence.

**Homologues of the YLR145w Gene**—Nearly all of the RNase MRP components are highly conserved throughout eukaryotes. We examined whether this was also true of the Ylr145w protein. We did a BLAST search in an attempt to identify homologues of the Ylr145w protein. As shown in Fig. 6, we discovered that proteins in a wide variety of yeast and fungi species...
The TAP-tagged Pop4 strain was used as a positive control. Equal min and then washed several times. RNA was extracted from the beads.

strain in particular exhibited a strong temperature-sensitive growth phenotype of the rmp1-6 mutant. Random PCR mutagenesis of the gene was performed. Potential mutants were screened for a defect in the processing of 5.8S rRNA (2, 11, 15). Two species of 5.8S rRNA exist in yeast; they differ in length by only 7 nucleotides and are generated through independent processing pathways. The smaller species, which is generated in a MRP-dependent manner, is 8–10-fold more abundant than the larger species. Loss of function of components of the RNase MRP complex results in decreased processing of the smaller 5.8S rRNA and an increase in the larger RNase MRP-independent species. The rmp1-6 strain was grown at 30 °C until it reached stationary phase and then shifted to non-permissive temperature for 4 h. Total RNA was isolated from cells before and after the shift. As shown in Fig. 8, a defect in 5.8S rRNA processing was observed in the rmp1-6 mutant at both the permissive and non-permissive temperatures. This change in the ratio of 5.8S rRNA species correlates well with the phenotype seen in other RNase MRP mutants (2, 11, 20, 31). These results demonstrate that Rmp1p is required for the function of the RNase MRP enzyme in rRNA processing.

**The Stability of RNase MRP RNA in the rmp1-6 Mutant—**

The RNA product of the NME1 gene is essential and is required for the function of the RNase MRP complex. If Rmp1p is associated with the RNase MRP complex, then mutations in Rmp1p could compromise the stability of the RNase MRP RNA, leading to a defect in rRNA processing. To test this possibility, Northern analyses were performed on RNA isolated from the rmp1-6 mutant at both the permissive and non-permissive temperatures. As shown in Fig. 8, MRP RNA was stable in the rmp1-6 strain under all conditions. This result is similar to what is seen in mutations in the RNase MRP-specific protein Snm1p (20). It also indicates that Rmp1p is probably not a core protein subunit of the complex such as Pop1 that loses stability of the RNA rapidly upon depletion or mutation (11). In addition, the RNase P RNA was found to be stable in the rmp1-6 mutant strain (Fig. 8).

The Processing of tRNA in the rmp1-6 Mutant—

The RNase P complex is involved in the processing of the 5′ ends of tRNAs. Analysis of total steady-state levels of tRNAs indicated that there were no gross changes in the levels of tRNAs, as would be expected in an RNase P mutant (Fig. 8) (11). We also performed Northern analyses on RNA isolated from the rmp1-6 mutant to identify low abundant tRNA precursors. Blots were probed for the presence of a pre-tRNAArg. As shown in Fig. 8, only mature pre-tRNAArg is seen in the rmp1-6 mutant strain at both the permissive and non-permissive temperatures. There is no accumulation of tRNA precursors, as would be expected in an

| Component | Mass/subunit (kDa) | No. of peptides >500 Da identified | Theoretical no. of peptides between 500 Da and 4 kDa | Total mass coverage (%) | Stoichiometry |
|-----------|-------------------|-----------------------------------|-----------------------------------------------|-------------------------|--------------|
| Pop1      | 100.4             | 56                                | 71                                            | 57                      | 1            |
| Pop4+TAP | 38.8              | 23                                | 35                                            | 52                      | 3 (2.95)     |
| Rpp1      | 32.2              | 11                                | 22                                            | 28                      | 3 (3.33)     |
| Rmp1      | 23.6              | 5                                 | 14                                            | 18                      | 2 (1.73)     |
| Snm1      | 22.5              | 11                                | 15                                            | 27                      | 2 (2.38)     |
| Pop3      | 22.6              | 7                                 | 14                                            | 19                      | 2 (1.90)     |
| Pop5      | 19.6              | 7                                 | 15                                            | 28                      | 4 (4.26)     |
| Pop6      | 18.2              | 4                                 | 11                                            | 19                      | 4 (3.92)     |
| Rpr2      | 16.4              | 4                                 | 12                                            | 31                      | 2 (2.20)     |
| Pop7      | 15.8              | 2                                 | 12                                            | 14                      | 4 (3.84)     |
| Pop8      | 15.5              | 4                                 | 10                                            | 32                      | 4 (4.21)     |
| NME1 RNA  | 109.7             |                                   |                                               |                         |              |
DISCUSSION

We were able to purify RNase MRP to apparent homogeneity using tandem affinity purification of Pop4 and its associated proteins. The POP4 locus is an ideal candidate for integration of the TAP tag because Pop4 is an integral component of the RNase MRP complex. Depletion of Pop4 results in the loss of RNase MRP RNA. Not only does Pop4p interact with most of the shared protein components of RNase MRP and RNase P, but it has also been shown to interact with the only unique protein component of RNase MRP identified so far, Snm1p (15). In addition, Pop4p is a relatively stable protein. Although Pop1p is another integral protein component of the RNase MRP complex that could be considered a potential target for the TAP cassette, Pop1p is relatively unstable, and Western analysis using an anti-Pop1 antibody confirms that multiple breakdown products are present (14, 32, our study). In addition, carboxyl-terminal fusions to the Pop1p have been found to be lethal (11). Judging by RNA analysis of the purified RNase MRP, the intact complex was contaminated with 1% of RNase P. The use of mild salt conditions to obtain whole-cell yeast extracts creates an environment more favorable for the isolation of RNase MRP than RNase P. Previous work aimed at isolating RNase P has shown that high salt conditions are amenable for purification and extraction of this complex (14). Although it appears that some RNase P RNA co-purifies through the first column, the use of a second affinity purification matrix, calmodulin, eliminates detectable RNase P RNA. Purified RNase MRP is probably altered very little from its natural state. Pop4 isolated from the final TAP fraction only retains the calmodulin-binding portion of the TAP cassette. Previous studies using the TAP fusion cassette suggest that the calmodulin-binding domain does not affect the structure or function of most purified products (19). Indeed, our purified RNase MRP is fully active in cleaving in vitro the A3 site in a yeast rRNA and the
and Smn1p have been shown to interact strongly with themselves and with Pop4p, suggesting that they might associate as dimers (32). The two proteins share sequence similarity and are probably evolutionarily derived from the same gene. In addition, depletion of Rpr2p does result in a 2-fold reduction in RNase MRP RNA levels (14). The presence of Rpr2p in our purified RNase MRP could be explained by the existence of Smn1p-Rpr2p dimers. Based on the subunit quantitation, Smn1 is present in equal concentrations to Rpr2, and the two could form Smn1p-Rpr2p dimers that do not compromise the function of the RNase MRP complex. In the absence of Rpr2, the Smn1 protein can functionally replace the Rpr2 protein. Indeed, Rpr2 may be able to partially replace Smn1 because mutations in the SNM1 gene that produce very little protein are still viable (20).

We also provide evidence that a protein encoded by the uncharacterized open reading frame YLR145w is present in purified RNase MRP. MALDI-TOF mass spectrometry analysis of purified protein components revealed the new potential protein component. The fact that a TAP-tagged version of YIR145w precipitates RNase MRP RNA, but not RNase P RNA, suggests that this protein is unique to RNase MRP. In support, the rmp1-6 mutation confers a 5.8S rRNA processing defect, but not a tRNA processing defect. Previous research aimed at identifying the function of uncharacterized open reading frames showed that TAP-tagged YIR145w precipitated most of the shared protein components of the RNase MRP and RNase P, but no further analysis was performed (30). Based on our evidence, we believe that YLR145w encodes a new unique protein component of RNase MRP, Rmp1p.

We have uncovered putative homologues of Ylr145w. Nearly all of the MRP components including the RNA have conserved homologues in higher eukaryotes (38). More research will be required to further characterize the association of Rmp1p with the RNase MRP complex. Rmp1p is required for proper rRNA processing, but we have yet to determine whether Rmp1 is also required for other functions of RNase MRP. Indeed, the requirement of RNase MRP to perform multiple processing events may require specialized proteins for substrate recognition or regulation. RNase P may also contain other yet to be identified proteins that are required for its specific cellular processes.

The availability of large amounts of highly purified RNase MRP will open the door to structural analysis of this complex. The purification level is high enough to allow for both cryo-electron microscopy and crystallization of the complex for x-ray diffraction studies. In addition, it will allow for easy analysis of other potential substrates of the complex and for identification of post-translational modifications that may regulate the activity of RNase MRP.

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Purification of the Yeast RNase MRP

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