Yeast mitochondrial RNase P, RNase Z and the RNA degradosome are part of a stable supercomplex

Rachid Daoud, Lise Forget and B. Franz Lang*

Robert-Cedergren Centre for Bioinformatics and Genomics, Department of Biochemistry, Université de Montréal, 2900 Edouard-Montpetit, Montreal, Quebec, H3T 1J4 Canada

Received August 19, 2011; Revised September 30, 2011; Accepted October 12, 2011

ABSTRACT

Initial steps in the synthesis of functional tRNAs require 5'- and 3'-processing of precursor tRNAs (pre-tRNAs), which in yeast mitochondria are achieved by two endonucleases, RNase P and RNase Z. In this study, using a combination of detergent-free Blue Native Gel Electrophoresis, proteomics and in vitro testing of pre-tRNA maturation, we reveal the physical association of these plus other mitochondrial activities in a large, stable complex of 136 proteins. It contains a total of seven proteins involved in RNA processing including RNase P and RNase Z, five out of six subunits of the mitochondrial RNA degradosome, components of the fatty acid synthesis pathway, translation, metabolism and protein folding. At the RNA level, there are the small and large rRNA subunits and RNase P RNA. Surprisingly, this complex is absent in an oar1Δ deletion mutant of the type II fatty acid synthesis pathway, supporting a recently published functional link between pre-tRNA processing and the FAS II pathway—apparently by integration into a large complex as we demonstrate here. Finally, the question of mt-RNase P localization within mitochondria was investigated, by GFP-tracing of a known protein subunit (Rpm2p). We find that about equal fractions of RNase P are soluble versus membrane-attached.

INTRODUCTION

To synthesize functional tRNAs, precursor tRNAs (pre-tRNA) need to be processed at their 5'- and 3'-termini. An almost ubiquitous RNase P is responsible for endonucleolytic 5'-processing (1). Until the discovery that human and Arabidopsis mitochondrial RNase P are protein-only enzymes (2,3), these activities were thought to be always ribonucleo-proteins, composed of a catalytic RNA plus one or several proteins, throughout Archaea, Bacteria and eukaryotes (4–9). Processing of tRNA 3'-termini is achieved by exonucleolytic and/or endonucleolytic activities, depending on the organism and the cellular compartment (10–14). In Escherichia coli, processing of 3'-termini requires a multi-step process initiated by an endonucleolytic cleavage, followed by exonucleolytic trimming (11). In other bacteria and most eukaryotes (including nuclear, mitochondria, and plastid) and in all Archaea, 3'-tRNA processing is an endonucleolytic cleavage catalyzed by tRNase Z that exists in two forms, a long form of 750–930 amino acids only present in Eukarya (tRNase ZL), and a short form of 280–360 amino acids (tRNase ZS) (15).

Most information on mitochondrial (mt) pre-tRNA processing comes from studies of Saccharomyces cerevisiae. A highly purified form of mitochondrial RNase P (mt-RNase P) has been obtained by lysis of mitochondria in the presence of detergent and high salt, and a series of chromatographic steps. It contains the nucleus-encoded Rpm2p protein (Rpm2p; 119 kDa), and only an incomplete set of RNA fragments covering the mtDNA-encoded RPM1 RNA subunit (mt-P RNA) of 427 nt (16–18). Yet, given that yeast mt-P RNA has a highly reduced RNA structure compared to its cytosolic counterpart (19), we strongly expect that native mt-RNase P contains more than just one protein, to compensate for the lack of RNA structure [for comparison, cytosolic RNase P contains nine proteins; (20)]. Previous studies revealed that Rpm2p is involved not only in mt-RNase P activity, but also effects mitochondrial import (21), fermentative growth (22), and transcriptional activation of several nucleus-encoded mitochondrial components (23). In addition (and most curiously), tRNA processing intersects with the type II fatty acid synthesis pathway (24). Disruption of any enzyme in the FAS II pathway leads to a defective mt-RNase P (24). One possibility to explain

*To whom correspondence should be addressed. Tel: 514 343 5842; Fax: 514 343 2210; Email: Franz.Lang@Umontreal.ca

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this observation is that there is a structural association between mt-RNase P and FAS II that is impaired with deletion of the FAS II subunits. In fact, this led us to the hypothesis that a super-structure may combine some or all of the above-mentioned mitochondrial functions into a large physical unit. Indications that RNase P may be associated with other functions also come from mt-RNase P purification experiments. In early isolation steps, its activity co-fractionates with the tRNA 3’-processing activity (18), and purification to homogeneity is achieved at an extremely low yield, despite the use of high salt and detergent. In yeast mitochondria, 3’-tRNA processing is an endonucleolytic cleavage (25), which is accomplished by a multifunctional RNase Z (11,26,27). In yeast, both the nuclear and mitochondrial forms of RNase Z are encoded by a nuclear gene (TRZ1) (15). Again, this enzyme is also implicated in ribosomal RNA maturation (26).

Controlling RNA levels (balance between RNA synthesis and degradation) is vital for both the regulation and functioning of the mitochondrial system (28–30). RNA degradation is mediated principally by (arguably) small multiprotein complexes, like the exosome in the cytoplasm (33), which is organized in an RNA degradosome (31) or the degradosome in bacteria (32). The activity responsible for RNA turnover in mitochondria is a 3’- to 5’-processive exoribonuclease (33), which is organized in an RNA degradosome complex (mtEXO), as first described in S. cerevisiae (34). The highly purified mtEXO complex is composed of only two protein subunits, the exoribonuclease (35), and an NTP-dependent RNA helicase (related to the DExH superfamily) (36). Yet, the degradosome apparently associates with the mitochondrial ribosome, as mtEXO co-purifies with ribosomal proteins that are difficult to remove (34). These observations reinforce the hypothesis that the RNA degradosome is part of a large superstructure that associates with a variety of mitochondrial functions.

In this study, we demonstrate that mt-RNase P and tRNA Z activities are part of a large ribo-nucleoprotein complex, which also includes the RNA degradosome, five additional RNA processing proteins, plus other mitochondrial functions. We further show that the biogenesis of this complex is impaired in a mutant that is deficient in type II fatty acid synthesis, rationalizing its known pleiotropic tRNA processing phenotype discussed above.

**MATERIALS AND METHODS**

**Cell culture and mitochondrial isolation**

*Saccharomyces cerevisiae* (BY 4743) was kindly provided by Dr S. Michnick (Université de Montréal), and yeast GFP (Green Fluorescent Protein) constructs used in this study (MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 RPM2-GFP::HIS3) by Dr J. Vogel (McGill). Cells were grown to an optical density of 1.5–2.5 in a medium containing 1% yeast extract, 2% peptone, 3% glycerol and 1% galactose, pH 5.0 (YEPlgal). Mitochondrial purification followed previously published procedures with slight modifications (37). Yeast cell walls were removed by digestion with glucanase, spheroplasts were disrupted by osmotic shock, and a crude mitochondrial fraction was isolated by differential centrifugation. Mitochondria were resuspended in ‘washing buffer’ (600 mM sucrose, 0.2 mM PMSF and 10 mM MOPS/KOH, pH 7.2) and further purified by centrifugation (60 min at 134000g) through a discontinuous sucrose gradient (concentrations from top to bottom, 25%, 36% and 60%; 5 ml each). For a second purification step, the mitochondrial fraction was collected and mixed with about four times its volume of 80% sucrose, layered at the bottom of a discontinuous sucrose gradients, and purified by centrifuged at 134000g for 120 min. Intact mitochondria move upwards (‘floation’), and accumulate at the interface between the 36% and 60% sucrose layers. For enhanced purity, this two-step purification of the mitochondria was repeated twice. Purified mitochondria were resuspended in buffer (600 mM sucrose, 0.2 mM PMSF and 10 mM Tricine/KOH, pH 7.2) and pelleted at 14000g for 15 min. Usually, mitochondria were directly processed in further steps, but they may also be shock-frozen and kept at −80°C until use.

**Extraction of mitochondria soluble matrix proteins and membrane fractions**

For the extraction of matrix proteins, we adapted a previously published procedure (37,38). Mitochondria were re-suspended at 10 mg/ml in breaking buffer (600 mM sucrose, 20 mM HEPES–KOH, pH 7.4, 10 mM EDTA) and incubated for 30 min on ice in nine volumes of 20 mM HEPES–KOH, pH 7.4, 0.5 mM EDTA and 1 mM PMSF. We used three alternative ways for rupturing mitochondria, which only differ in yield but not in banding pattern or protein composition of supercomplexes. The gentlest method is to burst mitochondria through osmotic shock, by abruptly adding 10 volumes buffer. Alternatively, the mitochondrial suspension is adjusted to a final sucrose concentration of 0.45 M, incubated for 30 min on ice, and then homogenized in a Potter homogenizer for 90 s. The third method, for maximum yield of matrix proteins, involves mechanical disruption of mitochondria by shaking with an equal ratio of 125–212 μ and 425–600 μ glass beads (Sigma), in three rounds of 20 s each at 4°C. After a clarifying centrifugation at 12000g for 15 min, the supernatant was subjected to centrifugation at 100000g, for 2 h at 4°C. The pellet containing mitochondrial membranes was discarded, and the supernatant was subjected to a second centrifugation at 120000g, for 30 min at 4°C. The matrix protein fraction (supernatant) was mostly used immediately, but may be stored at −80°C after shock-freezing. Protein concentrations were determined with the Bradford protein assay. A mitochondrial membrane fraction was prepared as described previously (39,40).

**Tracking of GFP-tagged marker proteins**

Soluble matrix protein complexes (1.5 mg) isolated from an Rpm2-GFP construct were separated by ultracentrifugation (16 h at 20000g, Beckman SW41 rotor) on
discontinuous glycerol gradients (10–70% glycerol, in a
buffer containing 20 mM HEPES–KOH pH 7.9, 50 mM
KCl, 1.5 mM MgCl₂ and 1 mM DTT). 0.7 ml fractions
were collected and analyzed for protein concentration
(Bradford protein assay) and relative GFP fluorescence
intensity (excitation at 488 nm, light emission between
500 and 540 nm).

Purification of mt-RNase P activity
To purify the native RNase P complex, we developed a
preparative Blue Native Column Electrophoresis (NEC),
which permits large-scale purification of native protein
complexes. It is based on the regular blue native gel elec-
phoresis (BN–PAGE) protocol (41–44), using instead
of a slab gel a cylindrical running chamber filled with
polyacrylamide gel (e.g. 7%). The different concentrations
of native polyacrylamide gel were prepared as previously
published (41–44). The mitochondrial extracts (~250 µg)
were separated at constant voltage (140 V and 9 mA,
overnight, in a cold room 4°C), using the same electrophoresis
buffers as in regular BN–PAGE. Samples were collected
every 30 min from a 200 µl dialysis-cup placed at the outlet
of the column. Collected fractions were assayed for
mt-RNase P activity and analyzed for complexes com-
pared with BN–PAGE.

In vitro preparation and radio-labeling of pre-tRNAproline
The mitochondrial Reclinomonas americana
pre-tRNAproline substrate for the RNase P assays was
prepared in vitro transcription and purified as described
previously (45). The tRNAproline DNA ligated into pFBS/
EcoRV (2.9 kb) vector with T4 DNA ligase (Roche) and
amplified by PCR using 5′-GAAATTTAATACGACTCTAC
TATAGGTTAACCAGTATTTAAGGTGTT-3′,
5′-TGGTCCGGATGACGTATTGGACAC-3′ and
5′-TCATACAAAGGAACAAAGCTGCTGGT-3′ primers
produce respectively 5′-leader pre-tRNAproline and,
5′-leader and 3′-termini pre-tRNAproline The two amplified
mRNAproline DNAs were used separately for two in vitro radio-labeled transcriptions. Two micrograms
of each amplified mRNAproline DNA were used for
RNAproline transcription with Invitrogen T7 RNA poly-
merase (2 µl) and α-32P ATP (10 mCi/ml) (Perkin Elmer)
as previously described (45). After an overnight incuba-
tion at 37°C, loading buffer was added and the samples
were heated at 75°C for 2 min before loading on a 9%
polyacrylamide/8 M urea gel (4 h, 200 V at room tempera-
ture). The 117 nt (5′-leader pre-tRNAproline) and 148 nt
(5′-leader and 3′-termini pre-tRNAproline) RNA bands
were cut out of the gel and incubated overnight at 37°C
in 300 µl buffer extraction (30 µl of 1% SDS and 270 µl
of H2O2). After phenol–chloroform extraction and ethanol
precipitation, the precursor RNA was 5′-labeled with
α-ATP32.

Activity assay of mt-RNase P
To test the mt-RNase P activity we used the same proced-
ure as described previously (46). Radio-labeled
pre-tRNAproline (2000 cpm) was dissolved in 1× PA
buffer (50 mM Tris–HCl, pH 7.5, 100 mM NH4Cl,
10 mM MgCl₂) and incubated 30 min at 37°C, in a 15-µl
mixture reaction. After incubation, 10 µL of loading buffer
was added, and the sample was heated at 75°C for 2 min
before loading on a 9% polyacrylamide/8 M urea gel (4 h,
200 V at room temperature). The gels were then either
exposed to a Kodak film (~12 h) or a Biorad molecular
imaging screen K (2 h).

The M1 RNA ribozyme was used as positive control for
5′-tRNA processing. The expression plasmid carrying the
E. coli M1 RNA gene (provided by Sidney Altman) was
used for in vitro transcription. For all experimental infor-
mation for M1 RNA transcription and purification see
(45). The primers for amplification of the rnpB gene
were 5′-GAAATTTAATACGACTCTACATAGGGAAG
CTGACCAGACGTGC-3′ and 5′-AGTGGAACCTG
ACCGATAAGCC 3′. The M1 RNA was activated
before use, by heating (65°C for 5 min), slow
cooling to room temperature in 1× PA buffer (50 mM
Tris–HCl, pH 7.5, 100 mM NH4Cl, 10 mM MgCl₂).
Radio-labeled pre-tRNAproline (2000 cpm) was incubated
at 37°C in 10 mM Tris–HCl, pH 7.5, 100 mM MgCl₂,
100 mM NH4Cl, 4% PEG, in the presence of ~10 nM
M1 RNA. The total volume of the reaction is 15 µl.
After 30 min, 10 µL of loading buffer was added, and the
sample was heated at 75°C for 2 min before loading on
a 9% polyacrylamide/8 M urea gel.

Identification of mt-RNase P RNA (RPM1) by RT–PCR
Endogenous RNA from the identified MRT complex was
purified using the ‘RNeasy plus’ kit by QIAGEN, and
RT–PCR assays were performed on 10 ng RNA with
AMV reverse transcriptase (cDNA synthesis). The
cDNA was amplified with the Expand High Fidelity
PCR system provided by Roche. The PCR primers for
RPM1 RNA amplification are 5′-TAAATAGGGAAATCT
AAATAAT-3′, 5′-GTATATATATATATATATTG
GAATAG-3′ and 5′-TTATATTATTACGAA
ATA-3′. For RT–PCR the same primers of the PCR
were used in addition to 5′-AGAGAATATATATATAT
AAATATATAT-3′, 5′-GGATATATATTATAAGC
A-3′ and 5′-AAGCATATTTCGTATAAA-3′. The
expected PCR products have lengths of 51, 71, 52, 387
and 400 nt.

Blue native gel electrophoresis
Preparation of samples and of 4–14% BN–PAGE gel
followed previously published procedures (41–44), except
that detergents were omitted (their effect was only tested
in supercomplex stability tests). Approximately 150 µg
protein was loaded per well, and electrophoretic separa-
tion was performed in a Höfer (18 x 16 cm) electrophor-
esis chamber, at 140 V and 9 mA, overnight at 4°C.

Analysis of supercomplex composition by mass
spectrometry
To identify protein complexes that form discrete bands in
BN–PAGE, complex bands were cut from the gel and
submitted to liquid chromatography tandem mass spec-
trometry (LC–MS/MS) analysis (47,48). In-gel tryptic
digestion and LC–MS/MS analysis was performed by a
service at the Université de Montréal (IRIC), including functional annotation by Mascot (49).

RESULTS AND DISCUSSION

Purification of mt-RNase P without the use of detergents

To preserve the integrity of a postulated large complex that combines mt-RNase P with other mitochondrial functions, we have developed purification procedures that do not make use of detergents or other highly disruptive conditions as previously applied [such as detergent, high salt, EDTA, heat shock etc; (16–18)]. For this we start with highly purified yeast mitochondria, gently disrupt the organelles and extract a soluble matrix fraction by centrifugation, which represents ~57% of total mitochondrial proteins. When separating the soluble extract on a glycerol gradient (10–70%; for details see ‘Materials and Methods’ section), most of the material moves into the glycerol phase, in support of the idea that most soluble proteins are organized in large complexes. In fact, tracing of the protein subunit of mt-RNase P (Rpm2p) by in vivo GFP fluorescence labeling reveals that about half (54%) of the GFP-Rpm2p is in the soluble mitochondrial matrix fraction, and that most of it (81%) is associated with a high-molecular-weight complex as it migrates far into the glycerol gradient (35–40%; Figure 1). This gradient fraction also contains the bulk of RNase P activity (Supplementary Figure S1). GFP-labeled cells grow normally on non-fermentable substrates, indicating that the GFP fusion does not lead to functional disturbances.

As gradient procedures have only limited resolution, we set out to find an alternative, non-disruptive and preparative purification procedure for large protein complexes. This led us to develop a variant of BN–PAGE (50), in which all steps are performed under physiological conditions (e.g. pH 7), and in the absence of detergents (named non-denaturing polyacrylamide gel electrophoresis column, or NEC; for details see ‘Materials and Methods’ section). Instead of using a slab gel, preparative NEC separates in a large column with a continuous polyacrylamide gel, and fractions are continuously recovered in a dialysis cup as they leave the column. Tracking of mt-RNase P by in vitro cleavage of a mitochondrial pre-tRNAproline precursor identifies ~70% of the total mt-RNase P activity in fractions 7–9 (with a total of 20 fractions; Figure 2A). To investigate the purity of fractions 7–9, they were separated by a second, regular slab-gel NEC that has more resolution than preparative NEC, and at a gel concentration that is optimal for separation close to the complex’s size range. Several complexes were identified in all three fractions, but only one of them occurs regularly (Figure 3A), which is the putative mt-RNase P-containing complex. Therefore, for further large-scale purification, fractions 7, 8 and 9 were pooled and processed on a second preparative NEC column at a higher polyacrylamide concentration (7.5%). Under this condition, mt-RNase P activity elutes exclusively in fraction 38 (for a total of 50 samples; Figure 2B and Supplementary Figure S2). Slab-gel NEC analysis confirms that it contains a single complex (Figure 3A), which remains stable following treatments with DNase, RNase and to some degree (~50%) with a mild detergent (digitonin; Figure 3B). Taken together, we argue that this complex closely represents the in vivo organization of a set of soluble mitochondrial matrix proteins, combining a variety of RNA maturation and other functions as shown in the following experiments.

Association of 5’- and 3’-tRNA processing activities

The processing activity of the purified complex was subsequently tested in an in vitro assay, with a different pre-tRNAproline substrate that has both 5’-leader and 3’-trailer extensions. We find essentially two forms of processed RNAs; the mature tRNAproline and an intermediate pre-tRNAproline with a 5’-leader (Figure 4), revealing the presence of a 3’-tRNA processing that is somewhat more effective than RNase P. This result is consistent with the previously observed co-fractionation of RNase P and RNase Z in early steps of yeast mt-RNase P purification (18).

Presence of RPM1 RNA and Rpm2p protein in the complex

To further characterize the homogeneity and composition of this tRNA processing complex, we tracked the presence of the known yeast mt-RNase P core RNA and protein components (i.e. the RPM1 RNA subunit and Rpm2p). Proteomic analysis by mass spectrometry (LC–MS/MS; see ‘Materials and Methods’ section) shows that the Rpm2p is present only in this complex, together with a variety of other proteins (see below). Likewise, an extraction and analysis of the endogenous RNA of the purified complex demonstrate the presence of the two (un-degraded) small and large subunit tRNAs, as well as several RNAs of ~70–90 nt (Figure 5A). As expected, an RNA with the predicted size of an intact RPM1 RNA (427 nt) is absent, which is in agreement with previous investigations on purified yeast mt-RNase P [e.g. (16)], in which only fragments of RPM1 RNA are detected. In fact, consistent with these studies, we find by RT–PCR and sequencing the same two RPM1 RNA fragments of 52, 89 and 71 nt (Figure 5B and Supplementary Figure S3). Our results thus confirm that an intact RPM1 RNA subunit is not required for RNase P activity in vitro (16), and that the mt-RNase P core subunits are integrated within a large complex when isolated under non-denaturing conditions.

Protein composition of the mt-RNase P (RMT) complex

Proteomic analysis with liquid chromatography and tandem mass spectrometry (LC–MS/MS) reveals the presence of an unexpected high number of proteins (130) in the purified ‘native’ RNase P/RNase Z complex (confirmed by three independent analyses; Supplementary Table S1). These proteins are implicated in numerous functions including (as expected) RNA processing, but also metabolism (e.g. TCA cycle), translation (two aminocyl–tRNA synthetases; two translation elongation factors; an incomplete set of ribosomal proteins but...
Figure 1. Density-based fractionation of mitochondrial GFP-Rpm2p. After fractionation on a discontinuous 10–70% glycerol gradient, relative GFP fluorescence absorbance/fraction identify most Rpm2-GFP with high-molecular-weight complexes.

Figure 2. Purification of mt-RNase P from the mitochondrial matrix extract. Purification and assays conditions are given in ‘Materials and Methods’ section. Mt-RNase P activities in (A) correspond to a separation on a 5.5% NEC column and (B) on 7.5% NEC slab gel. Aliquots from each fraction were assayed for RNase P activity. The negative control (–) is without and the positive control (+M1) with E. coli M1 RNA. The RNA corresponding to 5′-leader pre-tRNAproline (117 nt), tRNAproline (78 nt) and the removed 5′-leader sequence (39 nt) are indicated. Mt-RNase P activity was found in fractions 7, 8 and 9 in preparative NEC (A), and in fraction 38 only in a subsequent regular NEC separation (B). Note that the activity test of fraction 38 (B) was performed with more material, leading to almost complete processing of the tRNA precursor, but also to partial, unspecific cleavage of the mature tRNA into smaller fragments. With less material, the processing pattern is similar to that in (A) (Supplementary Figure S2).
un-degraded forms of the large and small rRNA subunits (Figure 5A), and others (e.g. mitochondrial genome maintenance and chaperons) (Supplementary Table S1). In the following we will refer to this complex as the ‘RMT complex’ (for RNA processing, metabolism, translation complex). A total of 564 physical protein–protein interactions are listed among proteins of the RMT complex in the BioGRID and SGD databases, in support of their organization in a complex.

Besides Rpm2p and tRNA Z that are involved in 5’- and 3’-tRNA maturation, we find five out of six subunits (Mtr4p; Dead-box family of ATP dependent helicase; ribosomal proteins Mrp1p, Mrpl3p, Mrpl35p and Mrpl40) of the RNA degradosome complex, as previously characterized by others (34). The remaining second catalytic subunit of the RNA degradosome (3’–5’ exoribonuclease, Dss1p) was identified only once in the three LC-MS/MS experiments, which is consistent with its low level of cellular expression [only about 1000 molecules/cell (51)]. The RNA degradosome is responsible for RNA turnover (34), but loss of function of any of its subunits also results in accumulation of RNA precursors with abnormal 5’- and 3’-termini, and stalled mitochondrial translation (33,34). Finally, the presence of five further proteins involved in rRNA and mRNA processing and RNA modification demonstrates a structural integration of most mitochondrial RNA processing activities (Nop7p; involved in rRNA processing, Mrm1p; ribose methyltransferase of the mitochondrial 21S rRNA, Hsh155p; mRNA-binding protein, Ngl1p; putative endonuclease and Prp22p; DEAH-box RNA-dependent ATPase/ATP-dependent RNA helicase).

That the RMT complex contains 22 ribosomal proteins of the large subunit and 13 of the small subunit is in agreement with the existing data that show an association of a highly purified RNA degradosome complex with four ribosomal proteins (Mrpl3p, Mrpl35p, Mrpl40 and Mrp1p) (34). Depending on the adopted experimental
procedure, the mitochondrial ribosome contains about 70 proteins (52,53). The presence of only 35 mito-ribosomal proteins in the RMT complex falls short of about one half of the protein components, yet contains the un-degraded large and small subunit rRNAs (Figure 5A). This may be interpreted in two ways: (i) the RMT complex results from dissociation of a large membrane-attached complex that contains the intact ribosome. The dissociation may be caused by experimental manipulation (e.g. mechanical extraction procedure). It has been reported that the mitochondrial ribosome is attached to the inner membrane (40), which would be consistent with our result that mt-RNase P and the RMT complex is partially membrane-attached. Alternatively, (ii) the RMT complex represents a biogenesis intermediate, with an incomplete set of ribosomal proteins that are being added to the rRNA subunits.

The finding of metabolic proteins (e.g. the complete TCA cycle) and chaperons in this complex is consistent with the observed co-purification of metabolic (TCA cycle) and ribosomal proteins in enriched human mt-RNase P fractions (2). Interestingly, the RMT complex also contains several chaperons that are involved in protein maturation, modification, targeting and assembly steps.

Lack of RMT complex in a FAS II deletion mutant

A recent investigation of yeast mutants demonstrates that deficiency in any enzyme of the mitochondrial fatty acid type II biosynthetic pathway (FASII) leads to impaired 5'-tRNA processing in yeast mitochondria and an overall pleiotropic phenotype (24). Our data reveal the presence of one regular component of the pathway (Oar1p; 3-Oxoaeryl-[acyl-carrier-protein] reductase; FASII biosynthesis) in the RMT complex. Two other FAS II enzymes (Htd2p and Mct1p) were also identified, but only once in the three LC-MS/MS experiments. This is probably due to their relatively low level of cellular expression (51). Consistent with the above observations, the RMT complex is no longer discernable in an Oar1 deletion mutant, and the amount of other complexes is strongly reduced (Figure 6). Our data support the interpretation that lack of FAS II enzymes indirectly affects RNAse P assembly and its activity (24), as well as enzymes in the TCA cycle (24,54). As a consequence of lacking mature
tRNAs and strongly reduced mitochondrial translation, FAS 2 mutants have a pleiotropic phenotype.

A membrane-attached form of mt-RNase P?

Previous publications suggest that yeast mt-RNase P is difficult to purify without the aid of detergents (16–18), and at least partially membrane-bound. Unfortunately, measuring mt-RNase P activity of mitochondrial membrane fractions is difficult because of rapid unspecific degradation of the pre-tRNA. We have therefore tracked mt-RNase P quantities by localization of its known GFP-labeled protein subunit (Rpm2p), and find that ~54% of GFP-Rpm2p is in the soluble mitochondria matrix fraction. With a mild detergent (1% digitonin) the amount of soluble GFP-Rpm2p increases to only ~68%, suggesting that the RMT complex has indeed a strong tendency for membrane attachment, but we clearly confirm that more than half is soluble, integrated with the RMT complex.

CONCLUSION

Here we show that native mt-RNase P and RNase Z activities are organized in a stable RMT supercomplex in yeast mitochondria, associated with the RNA degradosome, other RNA processing activities and several other mitochondrial functions including fatty acid synthesis, the complete TCA cycle and components of the translation machinery including a ribosome with both rRNAs but a reduced set of ribosomal proteins. Our results rationalize and further extend the surprising finding by others, that a deficiency in any component of the fatty acid type II biosynthetic pathway leads to impaired tRNA processing (24), and that deletion of genes coding for components of the RNA degradosome lead to pleiotropic defects in RNA processing and protein translation (33,34). In addition, our demonstration of stable (detergent-resistant), structural organization of metabolic activities in the RMT complex signals an end to the common belief that most metabolic enzymes are small separate enzymes that interact only casually (by diffusion) from within a pool.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online: Supplementary Table S1, Supplementary Figures S1–S3.

ACKNOWLEDGEMENTS

The authors thank Nihade El Kraimi for discussions and valuable comments on the manuscript.

FUNDING

National Science and Engineering Research Council (NSERC) of Canada; Funding for open access charge: NSERC Canada.

Conflict of interest statement. None declared.

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