Ribosome-binding Proteins Mdm38 and Mba1 Display Overlapping Functions for Regulation of Mitochondrial Translation

Heike Bauerschmitt,*‡ David U. Mick,†§‡ Markus Deckers,¶ Christine Vollmer,‖ Soledad Funes,*† Kirsten Kehrein,* Martin Ott,* Peter Rehling,‖ and Johannes M. Herrmann*

*Zellbiologie, Universität Kaiserslautern, 67663 Kaiserslautern, Germany; †Institut für Physiologische Chemie, Universität München, 81377 München, Germany; §Institut für Biochemie und Molekularbiologie, ZBMZ, Universität Freiburg, 79104 Freiburg, Germany; ‡Fakultät für Biologie, Universität Freiburg, 79104 Freiburg, Germany; †Abteilung Biochemie II, Universität Göttingen, 37073 Göttingen, Germany; and ‖Departamento de Genética Molecular, Instituto de Fisiología Celular, Circuito Exterior s/n, Ciudad Universitaria, Universidad Nacional Autónoma de México, Mexico, Distrito Federal, 04510, Mexico

Submitted February 5, 2010; Revised April 12, 2010; Accepted April 19, 2010

Monitoring Editor: Thomas D. Fox

Biogenesis of respiratory chain complexes depends on the expression of mitochondrial-encoded subunits. Their synthesis occurs on membrane-associated ribosomes and is probably coupled to their membrane insertion. Defects in expression of mitochondrial translation products are among the major causes of mitochondrial disorders. Mdm38 is related to Letm1, a protein affected in Wolf-Hirschhorn syndrome patients. Like Mba1 and Oxa1, Mdm38 is an inner membrane protein that interacts with ribosomes and is involved in respiratory chain biogenesis. We find that simultaneous loss of Mba1 and Mdm38 causes severe synthetic defects in the biogenesis of cytochrome reductase and cytochrome oxidase. These defects are not due to a compromised membrane binding of ribosomes but the consequence of a mis-regulation in the synthesis of Cox1 and cytochrome b. Cox1 expression is restored by replacing Cox1-specific regulatory regions in the mRNA. We conclude, that Mdm38 and Mba1 exhibit overlapping regulatory functions in translation of selected mitochondrial mRNAs.

INTRODUCTION

Cells of animals or fungi contain two translation machineries, one in the cytosol and one in mitochondria. Whereas the cytosolic translation machinery is well characterized, the process by which mitochondrial ribosomes synthesize proteins is still ill-defined. In Saccharomyces cerevisiae, only eight proteins are encoded by the mitochondrial genome: subunits 1–3 of cytochrome oxidase (Cox1, Cox2, and Cox3); cytochrome b (Cyt b) of cytochrome reductase; subunits 6, 8, and 9 of the F_F-ATPase (Atp6, Atp8, and Atp9); and the ribosomal subunit Varl. The specialization on the synthesis of a small number of hydrophobic membrane proteins might explain why the mitochondrial translation system—in contrast to that of the cytosol—is intimately associated with the inner membrane. In mitochondria, even mRNAs are bound to the inner membrane because of the presence of membrane-associated translational activators, which bind to 5' untranslated regions of the mRNAs (Michaelis et al., 1991; Fox, 1996). Each gene appears to have at least one specific translational activator. Similarly, mitochondrial ribosomes are localized to the membrane through interactions with ribosome-binding membrane proteins. In particular, the Oxa1 insertase binds to the ribosome, presumably in order to couple protein synthesis physically and functionally to membrane integration (Jia et al., 2003; Szrych et al., 2003). Cotranslational insertion of nascent chains is facilitated by a second mitochondrial membrane protein, Mba1. Like Oxa1, Mba1 binds to the large subunit of the mitochondrial ribosome (Preuss et al., 2001; Ott et al., 2006) and functions as ribosome receptor critical for coordination of protein synthesis. However, its molecular function in translation is unclear (Ott et al., 2006).

Recently, we identified Mdm38 as a third integral membrane protein, which binds to mitochondrial ribosomes (Frazier et al., 2006). Mdm38 and its homolog Ylh47 are the yeast paralogs of the human protein Letm1. Deletions of the LETM1 gene are associated with Wolf-Hirschhorn syndrome (Endele et al., 1999), a disorder characterized by severe growth and mental retardation, microcephaly, seizures, and hypotonia. Yeast mutants lacking Mdm38 show an altered mitochondrial morphology and defects in potassium homoeostasis (Dimmer et al., 2002; Nowikovsky et al., 2004). Because nigericin, a K+/H+ exchanger, mitigates the defects of ∆mdm38 mutants, it was suggested that Mdm38 might be critical, directly or indirectly, for the K+/H+ exchange across the inner membrane of mitochondria (Nowikovsky et al., 2007). In agreement with a role of Mdm38 in ion transport,
a recent study by Jiang et al. (2009) reported that *Drosophila* Let-1 mediates Ca\(^{2+}\)/H\(^+\) exchange in mitochondria.

For both proteins, Mba1 and Mdm38, a function as membrane-associated ribosome receptors was postulated. The results shown in this study strongly support this idea because the simultaneous deletion of both proteins leads to severe synthetic defects in the biogenesis of mitochondrial translation products. We show that Mdm38 and Mba1 play a critical and selective role in the regulation of mitochondrial translation of COX1 and CYTB mRNA. Moreover, we provide evidence that the defect in respiratory chain biogenesis is distinct from the postulated role of Mdm38 in K\(^+\)/H\(^+\) homeostasis.

**MATERIALS AND METHODS**

**Yeast Strains and Growth Media**

Yeast strains used in this study are derivatives of W303 except for strains generated from XPM171 (Perez-Martinez et al., 2003) and S88 (Tavarez-Carreón et al., 2008; Table 1). The deletions of MDM38, MBA1, and the sequence corresponding to the C-terminus of Oxa1 were carried out as described (Preuss et al., 2001; Frazier et al., 2006; Ott et al., 2006). For the deletions of MDM38 and MBA1 in the XPM171 background a Cre-LoxP-system for integrating and removing a *kanMX* marker was used (Guldener et al., 1996). To generate *rho*\(^0\) derivatives of the strains AFY25, XPM171, DaMY33, DaMY34, and DaMY48, cells were grown on ethidium bromide-containing media. To generate mutant strains that lack mitochondrial introns, DaMY49, DaMY50, DaMY51, and DaMY52, were mated with the strain XPM72 containing intronless mtDNA (X. Perez-Martinez) derived from CK520 (Labouesse, 1990). Cytoductants were selected by their ability to respire and to grow on media lacking adenine. Fused cells containing two nuclei were identified by their growth on media without leucine and discarded. Yeast cultures were grown at 30°C in 1% yeast extract, 2% peptone, 15 mM KH\(_2\)PO\(_4\), 13 mM MgSO\(_4\), 0.15 mg/ml all amino acids except methionine, 4 mM ATP, 0.5 mM GTP, 5 mM α-ketoglutarate, 5 mM phosphate, 0.7 mg/ml fatty acid-free bovine serum albumin, 20 mM Tris/HCl, pH 7.4) containing 0.6 U/ml pyruvate kinase and 10 μM [\(^{35}\)S]methionine. Samples were incubated for indicated time points at 25°C, and labeling was stopped by addition of 25 mM unlabeled methionine. The samples were further incubated for 5 min to complete synthesis of nascent chains. Mitochondria were isolated by centrifugation, washed in 1 ml 0.6 M sorbitol, 20 mM HEPES/HCl, pH 7.4, lysed in 25 μl sample buffer (2% SDS, 10% glycerol, 2.5% β-mercaptoethanol, 0.02% bromphenol blue, 60 mM Tris/HCl, pH 6.8), and subjected to SDS-PAGE. In vivo labeling of mitochondrial translation products was performed in whole cells in the presence of cycloheximide essentially as described (Barrientos et al., 2002) with the difference that cells were grown on YP medium containing 2% galactose. Proteins were precipitated in the presence of 10% trichloroacetic acid, and precipitates were washed with ice-cold acetone. For quantification of Cox1 by digital autoradiography the amount of Cox1 was standardized to that of Cox3. Quantification was performed by using ImageQuant TL software (GE Healthcare, Munich, Germany).

**Table 1. Yeast strains used in this study**

| Strain       | Genotype                        | Source                                      |
|--------------|---------------------------------|---------------------------------------------|
| W303-1A (wt) | MATa ade2-1 his-3-11,15 leu2-3,112 ura3-1 trp1-1 | Thomas and Rothstein (1989)                 |
| HHY029 (Δmba1) | MATa ade2-1 his-3-11,15 leu2-3,112 ura3-1 trp1-1 oxal-1-332* | Szyrach et al. (2003)                       |
| DaMY15 (Δmba1 Δmdm38) | MATa ade2-1 his-3-11,15 leu2-3,112 ura3-1 trp1-1 mb1::HIS5MX6 | This study                                  |
| DaMY17 (Δmdm38) | MATa ade2-1 his-3-11,15 leu2-3,112 ura3-1 trp1-1 mb1::HIS5MX6 mdm38::TRPI | This study                                  |
| DaMY18 (Δmdm38 Δmba1) | MATa ade2-1 his-3-11,15 leu2-3,112 ura3-1 trp1-1 mb1::HIS5MX6 mdm38::TRPI | Perez-Martinez et al. (2003)                |
| XPM171 (wt) | MATa leu2-3,112 ura3-52 lys2 arg8::hisG [cox1::ARG8m cox2::COX1 COX2] | This study                                  |
| DaMY33 (Δmba1) | MATa leu2-3,112 ura3-52 lys2 arg8::hisG mb1::kanMX4 [cox1::ARG8m cox2::COX1 COX2] | This study                                  |
| DaMY34 (Δmdm38) | MATa leu2-3,112 ura3-52 lys2 arg8::hisG mdm38::kanMX4 [cox1::ARG8m cox2::COX1 COX2] | This study                                  |
| DaMY48 (Δmdm38/Δmba1) | MATa leu2-3,112 ura3-52 lys2 arg8::hisG mdm38Δ mb1::kanMX4 [cox1::ARG8m cox2::COX1 COX2] | This study                                  |
| DaMY49 (wt) | MATa leu2-3,112 ura3-52 lys2 arg8::hisG [rho0] | This study                                  |
| DaMY50 (Δmba1) | MATa leu2-3,112 ura3-52 lys2 arg8::hisG mb1::kanMX4 [rho0] | This study                                  |
| DaMY51 (Δmdm38) | MATa leu2-3,112 ura3-52 lys2 arg8::hisG mb1:kanMX4 [rho0] | This study                                  |
| DaMY52 (Δmdm38/Δmba1) | MATa leu2-3,112 ura3-52 lys2 arg8::hisG mdm38Δ mb1::kanMX4 [rho0] | This study                                  |
| XPM72 | MATa ade2-1 ade2-1 ura3-52 lys2 arg8::hisG kar1-1 [rho0 ΔΔαl ΔΔβl] | X. Perez-Martinez                           |
| SFMEY01 (wt) | MATa leu2-3,112 ura3-52 lys2 arg8::hisG [rho0 ΔΔαl ΔΔβl] | This study                                  |
| SFMEY02 (Δmba1) | MATa leu2-3,112 ura3-52 lys2 arg8::hisG mb1::kanMX4 [rho0 ΔΔαl ΔΔβl] | This study                                  |
| SFMEY03 (Δmdm38) | MATa leu2-3,112 ura3-52 lys2 arg8::hisG mb1::kanMX4 [rho0 ΔΔαl ΔΔβl] | This study                                  |
| SFMEY04 (Δmdm38/Δmba1) | MATa leu2-3,112 ura3-52 lys2 arg8::hisG mdm38Δ mb1::kanMX4 [rho0 ΔΔαl ΔΔβl] | This study                                  |
| SB5 | MATa ade2 ura3Δ PET30a::PET30a::ura3Δ | Tavarez-Carreón et al. (2008)               |
| YPH499 (wt) | MATa ade2-101 his3-Δ200 leu2-Δ1 ura3-52 trp1-Δ63 lys2-801 | Sikorski and Hieter (1989)                  |
| AFY8 (Δmba1) | MATa ade2-101 his3-Δ200 leu2-Δ1 ura3-52 trp1-Δ63 lys2-801 mb1::HIS3MX6 | This study                                  |
| AFY25 (mdm38protA) | MATa ade2-101 his3-Δ200 leu2-Δ1 ura3-52 trp1-Δ63 lys2-801 mdm38::MDM38protA-HIS3MX6 | This study                                  |
| DaMY14 (rho0/Δmdm38protA) | MATa ade2-101 his3-Δ200 leu2-Δ1 ura3-52 trp1-Δ63 lys2-801 mdm38::MDM38protA-HIS3MX6 [rho0] | This study                                  |

wt, wild-type strains.
In Vitro Binding Experiments and Immunoprecipitation

For expression of a glutathione S-transferase (GST)-Mdm38 fusion protein, the open reading frame of MDM38 (encoding amino acids 159-573) was cloned into the pET11d vector (Novagen, Madison, Wisconsin, USA). After expression in the E. coli BL21 (DE3) strain (Stratagene, La Jolla, CA), the fusion proteins were purified according to published procedures (Eisenbrand et al., 2004). Purified GST-Mdm38 or GST was immobilized on glutathione Sepharose and incubated with mitochondrial extracts essentially as described (Heiskel et al., 2002). In brief, purified mitochondria were solubilized in lysis buffer (20 mM Tris, pH 7.4, 50 mM NaCl, 5 mM EDTA, 1% glycerol, and 2 mM PMSF) containing 1% digitonin. After binding, the column material was extensively washed in lysis buffer containing 0.5% Triton X-100 and eluted with SDS sample buffer. Samples were processed for SDS-PAGE and Western blotting. Antibodies and precipitates using anti-hemagglutinin (HA; Roche, Mannheim, Germany) or anti-FLAG (Sigma, Munich, Germany) antibodies as a control. Antibodies and bound proteins were depleted from the lysate with protein G Sepharose, and beads were washed extensively. Bound material was eluted with SDS sample buffer. Samples were analyzed by SDS-PAGE and Western blotting.

Membrane Floatation Assay

Mitochondria (400 μg) were disintegrated by freeze thawing and sonified 20 times for 30 s in a sonifying bath in 50 mM KCl, 10 mM Mes/Tris, and 20 mM Tris/HC1, pH 7.4. The suspension was adjusted to 1.6 M sucrose, and layers of 1.4 M sucrose and 0.25 M sucrose were placed on top. After centrifugation at 255,000 g for 2 h at 2°C the gradient was separated into a top (membranes) and a bottom (soluble proteins) fraction. Proteins in the fractions were precipitated by the addition of 12% trichloroacetic acid and analyzed by Western blotting.

Northern Blotting

RNA was isolated from purified mitochondria as described (Schmitt et al., 1990), resolved on a agarose gel, and transferred to a nylon membrane. The membrane was hybridized with 32P-labeled DNA probes according to published procedures (Sambrook et al., 1989).

Miscellaneous

Enzyme activities were analyzed as described (Tzagoloff et al., 1975). Purification of protein A fusion proteins from solubilized mitochondria was performed as reported (Frazier et al., 2006), with the exception that bound proteins were released by TEV (tobacco etch virus) protease treatment. Blue native PAGE analyses were performed essentially as described (Dekker et al., 1997).

RESULTS

Loss of Mdm38 and Mba1 Leads to a Synthetic Respiration Defect

Oxa1, Mba1, and Mdm38 are ribosome-associated membrane proteins. Oxa1 and Mba1 cooperate in the coordination of mitochondrial protein insertion (Peterson et al., 2006). In contrast to Dmba1 and Doxa1 mutants, Dmdm38 cells show only minor defects in the membrane insertion of mitochondrial translation products (Frazier et al., 2006). To assess whether this mild phenotype is due to a functional overlap of Mdm38 with Oxa1 or Mba1, we constructed deletion mutants lacking Mdm38 and the C-terminal 71 residues of Oxa1 (Oxa11–331 or oxa1ΔC) as well as Mdm38 and Mba1. Simultaneous deletion of Mdm38 and the C-terminus of Oxa1 did not aggravate the growth defect of the single mutants on nonfermentable carbon sources (Figure 1A). In contrast, Dmba1/Dmdm38 double mutant cells displayed a respiratory-deficient phenotype. Even in the presence of low concentrations of galactose, which partially rescued the single mutants, the Dmba1/Dmdm38 double mutant was unable to grow on glycerol at all tested temperatures (Figure 1B). Because Mdm38 and Mba1 display a genetic interaction, we conclude that Mba1 and Mdm38 have overlapping important roles in the assembly, the maintenance, or the function of the respiratory chain.

Δmdm38/Δmba1 Mutants Lack Complex III and IV of the Respiratory Chain

To identify the molecular basis for the synthetic growth defect of Δmba1/Δmdm38 mutants, we isolated mitochondria from wild-type and mutant cells and measured cytochrome oxidase (complex III) and cytochrome oxidase (complex IV) activity. Although Δmba1 and Δmdm38 mitochondria displayed reduced activities for both enzymes, Δmba1/Δmdm38 mitochondria exhibited severe synthetic enzyme deficiencies. Only ~14% of cytochrome oxidase activity was detected. As a control, we measured the activity of malate dehydrogenase (MDH, Figure 2C), which was only slightly reduced in the mutants. This finding is in agreement with the observed growth phenotypes and with previous studies, which reported that even a reduction of respiratory chain activity to <10% did not lead to a full block of cell growth on nonfermentable medium (LaMarche et al., 1992; Bauerschmitt et al., 2008; Prestele et al., 2009).

Defects in complexes III and IV were confirmed by Blue Native PAGE (BN-PAGE). Both complex III and complex IV were reduced in Δmba1 and Δmdm38 single mutants (Figure 2D). In the double mutant mitochondria, no complexes were detected. As a control we analyzed the levels of the inner membrane TIM22 translocase and the F1F0-ATPase, which were not or only slightly reduced in the mutants. In contrast, Western blotting revealed significantly reduced steady-state levels of subunits of complex III (Cyt b, Rip1, Qcr10) and complex IV (Cox2) in the double mutant (Figure 2E). Moreover, we recognized a slight increase of the amount of Mdm38 in Δmba1 mitochondria and of Mba1 in Δmdm38 mitochondria possibly as a compensatory effect.
We conclude that Mba1 and Mdm38 are essential for the biogenesis or stability of complex III and complex IV.

**Mba1 and Mdm38 Bind to Mitochondrial Ribosomes**

Because Mdm38 and Mba1 displayed a genetic interaction, we analyzed if the lack of Mba1 affects ribosome binding to Mdm38. The purified soluble C-terminal domain of Mdm38 fused to GST or GST were immobilized and incubated with mitochondrial extracts from wild-type and Δmba1 mitochondria. Both from wild-type and Δmba1 mitochondria, ribosomes specifically bound to GST-Mdm38 (Figure 3A). Positions of molecular-weight markers in kDa are indicated. Mitochondria (50 μg) of the indicated strains were analyzed by Western blotting using antibodies against the indicated proteins. Cyt b, cytochrome b; Cyt c1, cytochrome c1; Flα, α subunit of the F,F1-ATPase. The arrows indicate proteins that show diminished levels in the double mutant.

To test if Mba1 and Mdm38 were present in a common complex, we isolated Mdm38ProtA from solubilized mitochondria under conditions that maintained the Mdm38-ribosome interaction (Frazier et al., 2006). The extracts were incubated with IgG Sepharose. The resin was washed and bound proteins were eluted and analyzed by Western blotting. Four percent of the total sample and 100% of the eluate were loaded on the gels. (C) Mitochondria of the indicated strains were fractionated into membrane (M) and soluble (S) fractions by freeze-thawing and floatation. Proteins of these fractions were analyzed by Western blotting.

**Figure 2.** Δmba1/Δmdm38 mitochondria show severe defects in complexes III and IV of the respiratory chain. (A–C). Complex III, complex IV, and malate dehydrogenase activities were measured in isolated mitochondria from the strains indicated. SEs were calculated from three independent experiments. (D) Mitochondrial protein complexes were resolved by BN-PAGE, transferred to PVDF membranes, and probed with antibodies against Rip1 (complex III), Tim54 (TIM22 complex), and Atp5 (F,F1-ATPase). Positions of molecular-weight markers in kDa are indicated. (E) Mitochondria (50 μg) of the indicated strains were analyzed by Western blotting with antibodies against the indicated proteins. Cyt b, cytochrome b; Cyt c1, cytochrome c1; F1α, α subunit of the F,F1-ATPase. The arrows indicate proteins that show diminished levels in the double mutant.

**Figure 3.** Mba1 and Mdm38 physically interact with each other and with mitochondrial ribosomes. (A) Wild-type (wt) and Δmba1 mitochondria were lysed and incubated with purified GST or GST-Mdm38 bound to glutathione Sepharose. After extensive washing, bound proteins were eluted and visualized by Western blotting using antibodies against the indicated proteins. Control lanes show an aliquot of the mitochondrial extracts. (B) Wild-type or Mdm38ProtA-expressing mitochondria that contain or lack a mitochondrial genome were lysed with buffer containing 1% digitonin. The extracts were incubated with IgG Sepharose. The resin was washed and bound proteins were eluted and analyzed by Western blotting.

To test if Mba1 and Mdm38 were present in a common complex, we isolated Mdm38ProtA from solubilized mitochondria under conditions that maintained the Mdm38-ribosome interaction (Frazier et al., 2006). The native Mdm38 complexes were released from the affinity matrix by TEV-protease treatment (Figure 3B). In addition to mitochondrial
ribosomes, indicated by the presence of Mrpl39 in the eluate, Ylh47 and Mba1 were specifically recovered in complex with Mdm38. Because the observed interaction of Mba1 with Mdm38 could be indirectly mediated through the mitochondrial ribosome, we analyzed if Mba1 could be purified together with Mdm38ProtA from ribosome-deficient rho0-mitochondria. Even in the absence of intact ribosomes we observed copurification of Mba1 with Mdm38, indicating that complex formation between these proteins was not dependent on the presence of ribosomes. Thus, we conclude that by direct or indirect means Mba1 and Mdm38 form a complex in mitochondria.

Are Mba1 and Mdm38 critical for membrane localization of mitochondrial ribosomes? When mitochondria were separated into membranes and soluble proteins by floatation centrifugation, about half of the ribosomes were recovered with the membrane fraction (Figure 3C). This ratio was not significantly altered in the Δmba1 or Δmdm38 single or Δmba1/Δmdm38 double mutant mitochondria. Accordingly, we conclude that membrane association of ribosomes is not compromised in the absence of Mba1 or Mdm38.

**Mba1 and Mdm38 Are Critical for Cox1 and Cyt b Synthesis**

Because enzyme complexes with mitochondrially encoded subunits were specifically affected in the Δmba1/Δmdm38 double mutant, but coupling of ribosomes to the inner membrane was not, we monitored the synthesis of translation products in mitochondria of the Δmba1 and Δmdm38 mutants. Surprisingly, the simultaneous deletion of Mba1 and MDM38 selectively prevented the synthesis of Cox1 and Cyt b (Figure 4A, arrows). In contrast, some translation products (in particular, Atp6 and Atp9) appeared to be synthesized at increased rates in this mutant. This points to a mis-regulation of mitochondrial translation in the double mutant, which could explain the absence of complex III and complex IV in this strain. Apparently, Mba1 and Mdm38 possess overlapping functions and can thus partially substitute for each other in Cox1 and Cyt b expression. However, upon loss of both proteins the complete loss of Cox1 and Cyt b expression becomes evident.

The inability of the double mutant to synthesize Cox1 and Cyt b might be due to a transcriptional defect, instability of the respective mRNAs, or a specific defect in their translation. To distinguish between both possibilities, we isolated RNA from yeast mitochondria and assessed the levels of different mRNAs by Northern blotting (Figure 4B). In yeast mitochondria the COX1 and CYTB transcripts undergo splicing. The Δmba1/Δmdm38 double mutant showed severely reduced mRNA levels of COX1 and CYTB, whereas other mRNAs were not affected or even increased (COX3). However, in the mutant mitochondria we detected higher molecular weight COX1 and CYTB transcript species (data not shown), indicating that transcription occurred in the mutant mitochondria.

Because mitochondrial RNA splicing has been found to be affected by defects in mitochondrial ion homeostasis, we introduced intron-less mitochondrial DNA into cells carrying deletions of MBA1, MDM38, or MBA1/MDM38 by cytoduction. These mutant strains displayed similar growth defects on nonfermentable carbon sources as seen for the intron-containing mutant strains (Figure 4C). Thus, the observed growth defect of the mutant strains under conditions that require respiration cannot be attributed to defective RNA splicing. In agreement with this, when we analyzed mitochondrial translation in these strains in vivo or in organello, the previously observed defect in Cox1 synthesis was not cured. We therefore conclude that the translational defects observed in Δmba1/Δmdm38 double mutant mitochondria are independent of RNA splicing.

Mitochondrial protein synthesis is tightly regulated by translational activators, which bind to the 5’-UTRs of mRNAs (Michaelis et al., 1991; Fox, 1996). To assess a func-
tion of Mba1 and Mdm38 in translational regulation, we tested for Cox1 expression in cells in which the reading frame of COX1 was flanked by 5′- and 3′-UTRs of COX2. Yeast cells were grown in the presence of cycloheximide to inhibit cytoplasmic translation, and mitochondrial translation products were labeled with [35S]methionine. From the wild-type mitochondrial genome, Cox1 was not expressed in Δmba1/Δmdm38 mutant cells (Figure 4D). In contrast, Cox1 synthesis was independent of Mba1 and Mdm38 when the COX1 reading frame was flanked by the UTRs of COX2 (Figure 4E). Thus, by changing the COX1-specific UTR into a COX2-specific UTR (Perez-Martinez et al., 2003) we were able to render Cox1 expression independent of Mba1 and Mdm38. A quantification of the translation products revealed that compared with the wild-type control ~10% of Cox1 was synthesized in Δmba1/Δmdm38 mutant cells. We therefore conclude that Mba1 and Mdm38 are specifically involved in translational regulation of Cox1 and Cytb.

The Δmba1/Δmdm38 Growth Defect Cannot Be Rescued by Nigericin Treatment

Mdm38 was proposed to be critical for mitochondrial potassium homeostasis because the growth of Δmdm38 mutant cells on glycerol-containing media is improved by nigericin, a K+/-H+ antiporter (Nowikovsky et al., 2007). We therefore analyzed if the growth defect of Δmba1/Δmdm38 mutants could be similarly rescued by nigericin (Figure 5A). Although the growth defect of Δmdm38 cells on nonfermentable medium could be compensated by nigericin as reported, the growth defect of the Δmba1/Δmdm38 double mutants was not improved. Accordingly, nigericin was unable to substitute for the lack of Mba1 and Mdm38. Thus, the respiratory chain assembly defect, which is already apparent in Δmdm38 mutant mitochondria but aggravated in Δmba1/Δmdm38 double mutant cells, cannot be suppressed by nigericin and thus is unrelated to a role of Mdm38 in K+/-H+ homeostasis.

**DISCUSSION**

Here we show that Mba1 and Mdm38 have an overlapping function in mitochondrial protein synthesis. Double mutants lacking both proteins display synthetic growth defects on nonfermentable carbon sources. The levels of COXI and CYTB mRNAs are significantly reduced in these strains and the corresponding proteins are therefore not synthesized. This causes specific defects in complexes III and IV of the respiratory chain explaining the respiration deficiency.

In addition to the genetic interaction between Mba1 and Mdm38, coisolation experiments suggest a physical association of both proteins. This interaction does not require the presence of ribosomes. However, ribosomes apparently stimulate or stabilize the binding because only reduced amounts of Mba1 were found in association with Mdm38 in rho0 cells. Our observations are consistent with a role of Mba1 and Mdm38 in recruiting components to the ribosome, which are critical for the stabilization and translation of specific mRNAs (Figure 5B). Recently, a 900-kDa multisubunit complex was identified that contains Cbp1 and Pet309 as well as several nonidentified subunits (Krause et al., 2004).
Cbp1 is a factor that is specifically required for the translation and stability of the mRNA of CYTB (Dieckmann et al., 1984). Pet309 plays a comparable role for COX1 transcripts (Manthey and McEwen, 1995). Interestingly, Cbp1 and Pet309 were identified together in a genetic screen for components critical for the cotranslational translocation of protein domains across the inner membrane (Saracco and Fox, 2002). Here we show that Pet309, the translational activator for COX1 mRNA, is present in a complex with Mdm38 as well as with mitochondrial ribosomes in agreement with a function of Mdm38 in mitochondrial translation.

Although our results demonstrate a role of Mdm38 and Mba1 in translation, they do not rule out a role of Mdm38 in K* homeostasis. Because nigericin did not rescue the double mutant, it is likely that Mdm38 is involved in two processes, regulation of translation and, indirectly or directly, ion transport. We report that the defects found here for mitochondrial translation are not indirectly caused by defective splicing. In agreement with this is the fact that Mdm38 is conserved in human although mitochondrial RNAs do not undergo splicing in human mitochondria.

A recent study showed that, in human cells, the Mdm38 homolog Letm1 is associated with mitochondrial ribosomes (Piao et al., 2009). Similar to the situation in yeast, a knock-down of Letm1 by RNA interference leads to defects in respiratory chain biogenesis (Tamai et al., 2008). Interestingly, also overexpression of Letm1 caused a problem in the biogenesis of respiratory chain complexes from which it was concluded that Letm1 could function as a regulator in translation (Piao et al., 2009). It is tempting to speculate that loss of Letm1 in Wolf-Hirschhorn syndrome patients leads to a mis-regulated translation, which contributes to the pathological phenotype. It will be exciting to explore the potential role of Mba1 and Mdm38 in ribosome binding and their interaction with translational activators in further detail in the future.

ACKNOWLEDGMENTS

We thank S. Knaus and I. Perschil for excellent technical assistance, A. E. Frazier for providing yeast strains, T. Fox for anti-Mrp51 antiserum, and X. Perez-Martinez for strains and helpful discussion. This work was supported by the Deutsche Forschungsgemeinschaft and the Stiftung für Innovation in Rheinland-Pfalz.

REFERENCES

Altmann, K., Durr, M., and Westermann, B. (2007). Saccharomyces cerevisiae as a model organism to study mitochondrial biology: general considerations and basic procedures. Methods Mol. Biol. 372, 81–90.

Barrientos, A., Korr, D., and Tzagoloff, A. (2002). Shy1p is necessary for full mitochondrial function and stability of the mRNA of COX1 in the yeast model of Leigh’s syndrome. EMBO J. 21, 43–52.

Bauerschmitt, H., Funes, S., Herrmann, J. M. (2008). The membrane-bound GTPase Guf1 promotes mitochondrial protein synthesis under suboptimal conditions. J. Biol. Chem. 283, 17139–17146.

Ditmer, K. S., Fritz, S., Fuchs, F., Messerschmitt, M., Weinbach, N., Neupert, W., and Westermann, B. (2002). Genetic basis of mitochondrial function and morphology in Saccharomyces cerevisiae. Mol. Biol. Cell. 13, 847–853.

Dekker, P. J., Martin, F., Maarse, A. C., Gümer, H., Güldener, U., Meier, M., Rassow, J., and Pfanner, N. (1997). The Tim core complex defines components critical for the cotranslational translocation of protein domains across the inner membrane (Saracco and Fox, 1996). Regulation of mitochondrial protein expression. In: Translational Control, ed. J.W.B. Hershey, M. B. Matthews, and N. Sonnenberg, Cold Spring Harbor, NY: Cold Spring Harbor Press, 733–758.

Frazier, A. E., Taylor, R. D., Mick, D. U., Warscheid, B., Stoopel, N., Meyer, H. E., Ryan, M. T., Guiraud, B., and Relling, P. (2006). Mdm38 interacts with ribosomes and is a component of the mitochondrial protein export machinery. J. Cell Biol. 172, 553–564.

Funes, S., and Herrmann, J. M. (2007). Analysis of mitochondrial protein synthesis in yeast. Methods Mol. Biol. 372, 255–263.

Geissler, A., Chacinska, A., Truscott, K. N., Wiedemann, N., Brandner, K., Sickmann, A., Meyer, H. E., Messinger, C., Pfanner, N., and Relling, P. (2002). The mitochondrial presequence translocase. An essential role of Tim50 in directing preproteins to the import channel. Cell 111, 507–518.

Guldener, U., Heck, S., Fielder, T., Behnhauser, J., and Hegemann, J. H. (1996). A new efficient gene disruption cassette for repeated use in budding yeast. Nucleic Acids Res. 24, 2519–2524.

Jia, L., Dienhart, M., Schramm, M., McCauley, M., Hell, K., and Stuart, R. A. (2003). Yeast Oxl1 interacts with mitochondrial ribosomes: the importance of the C-terminal hydrophilic region of Oxl1. EMBO J. 22, 6438–6447.

Jiang, D., Zhao, L., and Clapham, D. E. (2009). Genome-wide RNAi screen identifies Letm1 as a mitochondrial Ca2+/H* antiporter. Science 326, 144–147.

Krause, K., Lopes de Souza, R., Roberts, D. G., and Dieckmann, C. L. (2004). The mitochondrial message-specific mRNA protectors Cbp1 and Pet309 are associated in a high-molecular-weight complex. Mol. Biol. Cell. 15, 2674–2683.

Labouesse, M. (1990). The yeast mitochondrial leucyl-tRNA synthetase is a splicing factor for the excision of several introns I introns. Mol. Gen. Genet. 224, 249–251.

LaMarche, A.E.P., Abate, M. I., Chant, S.H.P., and Trumpower, B. L. (1992). Isolation and characterization of COX12, the nuclear gene for a previously unrecognized subunit of Saccharomyces cerevisiae cytochrome c oxidase. J. Biol. Chem. 267, 22473–22480.

Manthey, G. M., and McEwen, J. E. (1995). The product of the nuclear gene PET309 is required for translation of mature mRNA and stability or production of intron-containing RNAs derived from the mitochondrial COX1 locus of Saccharomyces cerevisiae. EMBO J. 14, 4031–4043.

Manthey, G. M., Przybyla-Zawislak, B. D., and McEwen, J. E. (1998). The Saccharomyces cerevisiae Pet309 protein is embedded in the mitochondrial inner membrane. Eur. J. Biochem. 255, 156–161.

Michaelis, U., Körte, A., and Rödel, G. (1991). Association of cytochrome b translational activator proteins with the mitochondrial membrane: implications for cytochrome b expression in yeast. Mol. Gen. Genet. 230, 177–185.

Naithani, S., Saracco, S. A., Butler, C. A., and Fox, T. D. (2003). Interactions among COX1, COX2, and COX3 mRNA-specific translational activator proteins on the inner surface of the mitochondrial inner membrane of Saccharomyces cerevisiae. Mol. Biol. Cell. 14, 324–333.

Nowikovsky, K., Froschauer, E. M., Zsurka, G., Samaj, J., Reipert, S., Kolisek, M., Wiesenberger, and G., and Schweyen, R. J. (2004). The LETM1/YOL027 gene family encodes a factor of the mitochondrial K+ exchange activity, osmotic swelling and morphology. Cell Death Differ. 14, 1647–1656.

Ott, M., Prestele, M., Bauerschmitt, H., Funes, S., Bonnefoy, N., and Herrmann, J. M. (2006). Mba1, a membrane-associated ribosome receptor in mitochondria. EMBO J. 25, 1603–1610.

Perez-Martinez, X., Broadley, S. A., and Fox, T. D. (2003). Mss51p promotes mitochondrial Cox1p synthesis and interacts with newly synthesized Cox1p. EMBO J. 22, 5951–5961.

Piao, L., et al. (2009). Association of LETM1 and MRPL36 contributes to the regulation of mitochondrial ATP production and necrotic cell death. Cancer Res. 69, 3397–3404.

Prestele, M., Vogel, F., Reichert, A. S., Herrmann, J. M., and Ott, M. (2009). Mrp36 is important for generation of assembly competent proteins during mitochondrial translation. Mol. Cell. Biol. 20, 2615–2625.

Preuss, M., Leonhard, K., Hell, K., Stuart, R. A., Neupert, W., and Herrmann, J. M. (2001). Mba1, a novel component of the mitochondrial protein export machinery of the yeast Saccharomyces cerevisiae. J. Cell Biol. 153, 1085–1096.

Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989). Molecular cloning, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
Saracco, S. A., and Fox, T. D. (2002). Cox18p is required for export of the mitochondrially encoded *Saccharomyces cerevisiae* Cox2p C-tail and interacts with Pnt1p and Mss2p in the inner membrane. Mol. Biol. Cell 13, 1122–1131.

Schmitt, M. E., Brown, T. A., and Trumpower, B. L. (1990). A rapid and simple method for preparation of RNA from *Saccharomyces cerevisiae*. Nucleic Acids Res. 18, 3091–3092.

Sikorski, R. S., and Hieter, P. (1989). A system of shuttle vectors and host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics 122, 19–27.

Szyrach, G., Ott, M., Bonnefoy, N., Neupert, W., and Herrmann, J. M. (2003). Ribosome binding to the Oxa1 complex facilitates cotranslational protein insertion in mitochondria. EMBO J. 22, 6448–6457.

Tamai, S., Iida, H., Yokota, S., Sayano, T., Kiguchiya, S., Ishihara, N., Hayashi, J., Miyara, K., and Oka, T. (2008). Characterization of the mitochondrial protein LETM1, which maintains the mitochondrial tubular shapes and interacts with the AAA-ATPase BCS1L. J. Cell Sci. 121, 2588–2600.

Tavares-Carreón, F., Camacho-Villasana, Y., Zamudio-Ochoa, A., Shingú-Vázquez, M., Torres-Larios, A., and Pérez-Martínez, X. (2008). The pentatricopeptide repeats present in Pet309 are necessary for translation but not for stability of the mitochondrial *COX1* mRNA in yeast. J. Biol. Chem. 283, 1472–1479.

Thomas, B. J., and Rothstein, R. (1989). Elevated recombination rates in transcriptionally active DNA. Cell 56, 619–630.

Truscott, K. N., *et al.* (2003). A J-protein is an essential subunit of the presequence translocase-associated protein import motor of mitochondria. J. Cell Biol. 163, 707–713.

Tzagoloff, A., Akai, A., and Needleman, R. B. (1975). Assembly of the mitochondrial membrane system. Characterization of nuclear mutants of *Saccharomyces cerevisiae* with defects in mitochondrial ATPase and respiratory enzymes. J. Biol. Chem. 250, 8228–8235.