Mutations in MTO1 express a respiratory defect only in the context of a mitochondrial genome with a paromomycin-resistance allele. This phenotype is similar to that described previously for mss1 mutants by Decoster, E., Vassal, A., and Faye, G. (1993) J. Mol. Biol. 232, 79–88. We present evidence that Mto1p and Mss1p are mitochondrial proteins and that they form a heterodimer complex.

In a paromomycin-resistant background, mss1 and mto1 mutants are inefficient in processing the mitochondrial COX1 transcript for subunit 1 of cytochrome oxidase. The mutants also fail to synthesize subunit 1 and show a pleiotropic absence of cytochromes a, a3, and b. In vivo pulse labeling of an mto1 mutant, however, indicate increased rates of synthesis of other mitochondrial translation products. The respiratory defective phenotype of mto1 and mss1 mutants is not seen in a paromomycin-sensitive genetic background. The visible absorption spectra of such strains indicate a higher ratio of cytochromes b/a and elevated NADH- and succinate-cytochrome c reductase activities. To explain these phenotypic characteristics, we proposed that the Mto1p-Mss1p complex plays a role in optimizing mitochondrial protein synthesis in yeast, possibly by a proof-reading mechanism.

Respiratory competence in Saccharomyces cerevisiae is controlled by upward of several hundred nuclear genes referred to as PET genes (1). Mutations in PET genes are generally recessive and are recognized by an inability of the mutant to utilize nonfermentable carbon sources. In some instances, loss of mitochondrial functions depends on the genotype of mitochondrial DNA (mtDNA). For example, lesions in gene products that promote splicing of specific mitochondrial introns elicit a respiratory defect only in strains whose mitochondrial genome contains the cognate intron (2, 3). Another interesting situation has been reported in mss1 mutants which become respiratory-deficient only when their mtDNA has a point mutation in the 15 S rRNA gene further suggesting that Mss1p may be necessary for optimization of mitochondrial translation but is not essential for this activity (4).

According to this interpretation, the processing block is secondary to a translational defect. The absence in mss1 mutants of an obvious phenotype in the context of a wild type 15 S rRNA gene further suggests that Mss1p may be necessary for optimization of mitochondrial translation but is not essential for this activity (4).

The pet phenotype expressed in the presence of the paromomycin-resistance allele is not unique to mss1 mutants. In the present communication, we show that mutations in another gene, here designated as MTO1 (mitochondrial translation optimization), result in properties similar to those described for mss1 mutants. Evidence is also presented that Mto1p and Mss1p are complexed to one another and therefore are likely to carry out a common function.

**MATERIALS AND METHODS**

**Yeast Strains and Media**—The genotypes and sources of the strains of S. cerevisiae used in this study are listed in Table I. The media used to grow yeast have been described elsewhere (7).

**Cloning of MTO1 and MSS1**—MTO1 was cloned by transformation of E221/L1 (α leu2-3,112 mss1) with a yeast genomic plasmid library by the method of Schiestl and Gietz (8). The library used for the transformation was made from partial Sac3A fragments of nuclear DNA (averaging 7–15 kb) cloned into the BamHI site of the shuttle vector YEp24 (9). This library was kindly provided by Dr. Marian Carlson, Department of Genetics and Development, Columbia University. Approximately 1 × 10⁸ cells were transformed with 50 μg of plasmid DNA. The transformation mixtures were plated on minimal glucose medium to select Ura⁻ clones. After 3 days, the transformation plates containing 10⁴ colonies were replicated to YEPMG medium to identify respira-tory-competent transformants.

**Preparation of Yeast Mitochondria and Enzyme Assays**—Wild type and mutant yeast were grown to stationary phase in YPgal (2% galactose, 1% yeast extract, and 2% peptone), and mitochondria were prepared by the procedure of Faye et al. (12), except that Glusulase was replaced by Zymolase 20,000 (ICN Biomedicals, Inc.). Mitochondria were assayed for NADH-cytochrome c reductase, succinate-cytochrome c reductase, and cytochrome oxidase as described previously (13).

**Construction of W303MTO1 and W303MSS1**—A null allele of MTO1 was constructed by polymerase chain reaction amplification of the 5' and 3' sequences adjacent to the gene using the divergent primers 5’-gcggtgctagctgtaacatacaatactg and 5’-gcggtgctagcttgggtgggt. The template for the amplification consisted of the 3.5-kb BamHI-SacI fragment cloned in pUC18. The polymerase chain reaction product was digested with KpnI and ligated to a 1.1-kb KpnI fragment containing the yeast URA3 gene.

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* This work was supported by Research Grant HL22174 from the National Institutes of Health, United States Public Health Service. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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‡ The abbreviations used are: kb, kilobase pair; pet mutant, nuclear respiratory-deficient mutant of yeast; ρ⁻ mutant, cytoplasmic petite mutant lacking mitochondrial DNA; YEPG, yeast extract-peptone-glyceral.
The null allele, recovered as a linear BamHI-SacI fragment, was introduced into the diploid strain W303 by the one-step gene replacement procedure (14). Ura+ transformants were sporulated and subjected to tetrad dissections. Four independent Ura+ spores were verified by Southern analysis of their genomic DNA to harbor the null allele. The disrupted allele of MSS1 was obtained by insertion of a 1.8-kb BamHI fragment with HIS3 at the BglII site inside the gene. The linear KpnI-EcoRI fragment containing the disrupted allele was substituted for the wild type gene in the respiratory-competent haploid strain W303–1A. Transformation with the linear fragment yielded seven His+ transformants of which one (aW303D273–10B/A21) was respiratory-deficient and was not complemented by a ρ0 tester, indicating that it lacked or had a deletion in mitochondrial DNA.

Preparation of Antibodies to Mto1p and Ms1p—Antibodies against Mto1p were obtained by immunizing rabbits with a fusion protein expressed in Escherichia coli. The gene for the fusion protein was made by ligating the 900-base pair PstI fragment expressing in Mto1p were obtained by immunizing rabbits with a fusion protein construct. The insoluble protein fraction was dissolved in a 10 mM reduced glutathione, and used to raise antibodies in rabbits.

Fractions enriched for the fusion protein were pooled, concentrated by acetone precipitation, and used to raise antibodies in rabbits.

The above method was also used to obtain an antibody against an Ms1p fusion protein. The gene was made by ligating the 1.1-kb BglII fragment containing the disrupted allele was substi-
tuted for the wild type gene in the respiratory-competent haploid strain W303–1A. Transformation with the linear fragment yielded seven His+ transfor-
mants of which one (aW303ΔMSS1) was respiratory-deficient and was not complemented by a ρ0 tester, indicating that it lacked or had a deletion in mitochondrial DNA.

Miscellaneous Procedures—Standard procedures were used for the preparation and ligation of DNA fragments and for transformation and recovery of plasmid DNA from E. coli (16). The preparation of yeast nuclear DNA and the conditions for the Southern hybridizations, were as described by Myers et al. (7). DNA probes were labeled by random priming (17). DNA was sequenced either by the method of Maxam and Gilbert (18) or Sanger et al. (19) with T7 sequencing kit (United States Biochemical). Proteins were separated by polyacrylamide gel electrophoresis in the buffer system of Laemmli (20), and Western blots were treated with antibodies against the fusion proteins followed by a second reaction with 125I-protein A (21). Protein concentrations were determined by the method of Lowry et al. (22).

RESULTS

Phenotype of Mutants from Complementation Group G158—E39 is one of two independent isolates assigned to complementation group G158 of a pet mutant collection (1). Both mutants were derived from the respiratory-competent haploid strain E39 is one of two independent isolates assigned to complementation group G158 of a pet mutant collection (1). Both mutants were derived from the respiratory-competent haploid strain

| Strain | Genotype | Source |
|--------|----------|--------|
| CB11   | ade1     | Ref. 5 |
| D273–10B/A1 | met6   | Ref. 6 |
| D273–10B/A21 | met6 [oliP eryR parF] | Ref. 6 |
| W303–1A | ade2–1 his3–1,15 leu2–3,112 trp1–1 ura3–1 | W303–1A × W303–1B |
| W303–1B | ade2–1 his3–1,15 leu2–3,112 trp1–1 ura3–1 | aW303ΔMTO1 × D273–10B/A21 |
| W303   | ade2–1 his3–1,15 his3–1,115 leu2–3,112 leu2–3,112 trp1–1 ura3–1 ura3–1 | aW303ΔMSS1 |
| E39    | met6 mto1–1 | E39 × W303–1A |
| E39U1  | ura3–1 mto1–1 | E39 × W303–1A |
| E221   | met6 mss1 | E221 × W303–1A |
| E221/I | leu2–3,112 mss1 | W303–1A |
| W3033MTO1(PF) | ade2–1 his3–1,15 leu2–3,112 trp1–1 ura3–1 mto1–1:URA3 | This study |
| aW3033MTO1(PF) | ade2–1 his3–1,15 leu2–3,112 trp1–1 ura3–1 mto1–1:URA3 | This study |
| aW3033MTO1/P(M) | ade2–1 his3–1,15 leu2–3,112 trp1–1 ura3–1 mto1–1:URA3 | This study |
| aW3033ΔMSS1(PF) | ade2–1 his3–1,15 leu2–3,112 trp1–1 ura3–1 mto1–1:URA3 | aW3033MTO1(PF) × W3033ΔMSS1(PF) |
| aW3033MTO1/MSS1(PF) | ade2–1 his3–1,15 leu2–3,112 trp1–1 ura3–1 mto1–1:URA3 | aW3033MTO1(PF) × W3033ΔMSS1(PF) |

* Dr. Rodney Rothstein, Department of Human Genetics, Columbia University.

![Fig. 1. Spectra of mitochondrial cytochromes in wild type and mutant yeast.](image-url)
tion is subunit 1 of cytochrome oxidase that is not detected among the labeled proteins. It is interesting that incorporation of [³⁵S]methionine is more efficient in the mutant than in wild type yeast. The exact increase is difficult to quantitate but must be more than indicated by the autoradiogram in view of the fact that the mutant culture used in the experiment consisted of 50% r− and r+ cells which do not contribute to the activity.

The deficit in the cytochrome oxidase subunit 1 was consistent with the results of Northern blot analysis of the mitochondrial COXI transcripts for cytochrome oxidase subunit 1. Probes specific for exon regions of these RNAs indicated lower concentrations of the mature mRNAs and accumulation of partially processed precursors (Fig. 2B). Processing of cytochrome b is also retarded in the mutant. It is not clear from the gel of the radioactively labeled proteins whether synthesis of this protein is affected in the mutant because of its comigration with subunit 2 of cytochrome oxidase (Fig. 1).

Cloning of MTO1 and MSS1—A gene capable of complementing the respiratory defect of E39/U1 was cloned by transformation of the mutant with a yeast genomic library based in the episomal plasmid YE24 (9). The transformation yielded five respiratory-competent and uracil-independent clones. The plasmids were isolated from the transformants and amplified in E. coli, and their nuclear DNA inserts were characterized. Restriction mapping indicated that the nuclear DNA inserts of the different plasmids were related. The end points of one of the plasmids (pG158/T1) were sequenced and located in the genomic sequence of chromosome VII. This plasmid was also used to subclone the complementing gene by transferring different regions of the insert to the yeast/E. coli shuttle vector YEp352 (24) and testing the ability of the new constructs to confer respiration in E39/U1 (Fig. 3). The results of these transformations indicated that the gene is located between the sites for BamHI (B), SacI (S), and EcoRI (E) are shown above the nuclear DNA insert in pG158/T1. The unique SphI site in the vector is marked for orientation purposes. The regions of pG158/T1 subcloned in YEp352 are represented by the bars in the top part of the figure. The plus and minus signs enclosed by the brackets indicate complementation and lack thereof, respectively, of E39/U1. The reading frames corresponding to MTO1 and the flanking HAP2 and ADE5,7 genes are indicated by the solid arrows.
coding for a 74-kDa protein of unknown function. Neither pG158/ST3 or pG158/ST2 containing the entire HAP2 gene restored respiration, indicating that the complementing gene is YGL23C. We propose MTO1 (mitochondrial translation optimization) as a name for this gene for reasons discussed later in this paper. The sequence of Mto1p is homologous to the gdiA proteins encoded in the genomes of various bacteria (26, 27) and of Caenorhabditis elegans.2 The sequence similarity extends from approximately residue 40 to the very carboxyl terminus of the protein (Fig. 4). The function of this highly conserved protein is not known.

MSS1 was cloned by transformation of E221/L1, a representative of complementation group G122, with a YEp13 library. This mutant is phenotypically similar to E39/U1. A single respiratory-competent clone was obtained in the transformant. Respiration of the transformant was found to be dependent on a segregating plasmid (pG122/T1) whose nuclear DNA insert was determined to span the region of chromosome XIII from nucleotides 314,900 to 321,400. The smallest subclone (pG122/ST4) capable of complementing E221/L1 contained MSS1 (Fig. 5).

Properties of mto1 Null Mutants—To determine whether E39/U1 has a mutation in MTO1, a null mutation (Δmto1::URA3) was introduced in the respiratory-competent diploid strain W303. The construction of the null allele was described under “Materials and Methods” and depicted in Fig. 6. Uracil-independent clones obtained from a transformation of the diploid strain W303 with the null allele on a linear fragment of DNA were subjected to tetrad dissections. The auxotrophic requirement of the meiotic spore progeny indicated a 2:2 segregation of the URA3 disrupter. The Uracil− haploid strains were confirmed by Southern analysis of their genomic DNA to harbor the Δmto1::URA3 null mutation in their chromosomal DNA (Fig. 6). Unlike the original mutant, however, the Uracil− clones (W303ΔMTO1(P8)) were respiratory-competent as judged by growth on glycerol (Fig. 7). The presence of a functional respiratory chain in the null mutant was also evident from the visible absorption spectrum of mitochondria, which indicated the presence of cytochromes b and cytochrome oxidase (Fig. 8). The transformants, however, have a higher concentration of cytochrome b relative to cytochrome a. The aberrant ratio of the two cytochromes is also reflected in the increased NADH-cytochrome c reductase and reduced cytochrome oxidase activity of mitochondria from the transformant (Fig. 9). The increased ratio of cytochromes b/a is also seen in an mto1/mss1 double mutant (W303ΔMTO1/MSS1(P8)) (Fig. 7).

The ability of the mto1 null mutant, W303ΔMTO1(P8), to respire could indicate either that MTO1 is a suppressor of E39 or that the respiratory defect of mto1 mutants is affected by the mitochondrial genetic background. The mitochondrial genomes of W303 and D273–10B/A21 used to obtain E39 differ in their intron composition and in the presence in the latter of mutations in the OL1, ERY, and PAR loci conferring resistance to these antibiotics (6). To test the possible influence of mitochondrial genetic background on the phenotype of mto1 mutants, W303ΔMTO1(P8) was cured of mtDNA by treatment with ethidium bromide. The ρ− derivative was crossed to D273–10B/A21, whose mtDNA carries the drug-resistance mutations oli1R, eryR, and parR, and to D273–10B/A1, which has an identical genome except for the absence of the drug-resistant mutations. Diploid cells were sporulated for tetrad analysis. Meiotic progeny issued from the cross to D273–10B/A21 showed a 2:2 segregation of the respiratory-competent phenotype. In all cases, the respiratory-defective phenotype coincided with the uracil-independence. In contrast, all four meiotic progeny from the cross to the drug-sensitive strain D273–10B/A1 were respiratory-competent even though the Uracil− phenotype showed the expected 2:2 segregation patterns. These results excluded

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2 A. Gardner (1995), GenBank™ accession number Z66512.
an effect of intron composition difference on the phenotype but rather suggested that expression of respiratory deficiency in the mto1 mutant was dependent on one of the drug-resistance mutations (Fig. 5).

In a previous study mutations in MSS1 were shown to elicit respiratory deficiency only in strains carrying the paromomycin-resistance allele in mitochondrial DNA (4). To determine whether this was also true of the mto1 mutant, meiotic progeny were analyzed from a cross of the ro derivative of W303–D MTO1(PS) to a strain containing only the paromomycin-resistance marker. Tetrad analysis of meiotic products of this cross confirmed that the presence of mtDNA with the paromomycin-resistance allele was sufficient for the expression of respiratory deficiency in the mto1 mutant.

In addition to its respiratory deficiency, W303–D MTO1(PR) containing the paromomycin-resistance marker displayed the same pleiotropic absence of cytochromes b, a, and a3 as E39 (Fig. 1). The similar phenotypes of W303–D MTO1(PR) and E39 suggested linkage of the two mutations. This was confirmed genetically. For these allelism tests, MTO1 was transferred to the integrative vector YIp352 (24) yielding pG158/ST5. This plasmid was linearized at the unique Sma I site of MTO1. The linear plasmid was used to transform E39/U1. Ura+ clones obtained from the transformation of the mutants were respiratory-competent, suggesting that the integration had occurred at the locus of the mutation. This was verified by crosses of a respiratory-competent clone to the wild type haploid strain W303–1B and to W303ΔMTO1(PR) containing the Δmto1::URA3 allele. Diploid cells issued from each cross.
were sporulated for tetrad analysis. The four meiotic products from the back-cross to the wild type W303 strain were respiratory-competent (nine complete tetrads). Analysis of the tetrads obtained from the cross to the mto1 null mutant showed a 2:2 segregation of the respiratory-competent phenotype (nine complete tetrads). In all cases, respiratory competence cosegregated with uracil independence. These results provide direct evidence of genetic linkage of the E39 and mto1 null allele.

Mto1p Is a Mitochondrial Protein—To facilitate the localization of Mto1p, part of the gene was fused in-frame to a segment of trpE coding for the amino-terminal half of anthranilate synthase component I. Antibodies raised against the fusion protein were used to test for the presence of Mto1p in the mitochondrial and postmitochondrial supernatant fractions of wild type yeast, a mutant transformant with the gene on a high copy plasmid, and the mto1 null mutant. The antibody detected a protein of the expected size (approximately 70 kDa) in mitochondria of wild type and of the transformant but not in mitochondria from the null mutant. The signal was weak in wild type mitochondria and was greatly enhanced in the transformant (Fig. 10).

Antibodies were also raised against an Mss1p fusion protein. This antibody was used to probe for Mss1p in the mitochondria and postmitochondrial supernatant fractions from wild type and mutant yeast strains. The results of the Western blot analysis confirmed the presence of a protein of approximately 55 kDa in the mitochondrial but not in the soluble fraction of wild type yeast, a mutant transformant with the gene on a high copy plasmid, and the mto1 null mutant. The antibody detected a protein of the expected size (approximately 70 kDa) in mitochondria of wild type and of the transformant but not in mitochondria from the null mutant. The signal was weak in wild type mitochondria and was greatly enhanced in the transformant (Fig. 10).

Do Mto1p and Mss1p Form a Complex?—The similarity in the phenotypes of mto1 and mss1 mutants raised the possibility that the two proteins are functionally related and therefore may exist in a physical complex. This was tested by sedimentation analysis of a yeast transformed with a multicopy plasmid (pG158/ST12) containing a copy of MTO1 and MSS1 in a tandem orientation. An extract obtained by sonic disruption of mitochondria was applied to a 5–20% sucrose gradient. Hemoglobin and lactate dehydrogenase were added to the extract as molecular weight standards. The gradient was analyzed for the distribution of Mto1p, Mss1p, and for the two molecular weight standards, hemoglobin and lactate dehydrogenase. The sedimentation properties of Mto1p and Mss1p are consistent with their existence in a complex. Mto1p and Mss1p coseiment and peak in a region of the gradient closer to lactate dehydrogenase than to hemoglobin (Fig. 11). Based on its position relative to the two marker proteins, the average molecular weight of the complex was estimated as 124,000. This would suggest a heterodimer consisting of a single copy of each protein.

DISCUSSION

The respiratory deficiency of mto1 mutants is contingent on the presence in mitochondrial DNA of a single base change at nucleotide 1477 of the 15 S ribosomal RNA gene (28, 29). The mutation, a C → G transposition, confers resistance to paromomycin, an inhibitor of procaryotic protein synthesis. This unusual phenotype has also been reported for mss1 mutants.
MTO1 Codes for Mitochondrial Protein Needed for Respiration

FIG. 10. Mitochondrial localization of Mto1p and Mss1p. Top panel, mitochondria (M) and the postmitochondrial supernatant fraction (S) from the respiratory-competent strain W303-1A, the mto1 mutant aW303A/MSS1(pPH1), and from the transformant E39/U1/T1 were separated on a 9% polyacrylamide gel. In each case, 20 μg of protein was applied per lane. Following transfer to nitrocellulose, the blot was incubated with antiserum against the trpE/MTO1 fusion protein followed by a second reaction with 125I-protein A. The migration of the bands seen in the mitochondrial lanes of wild type and of E39/U1/T1 are consistent with the expected size of Mto1p. Bottom panel, total mitochondrial and postmitochondrial supernatant proteins were separated and analyzed with antibody against a trpE/Mss1p fusion protein. The weak band seen in the wild type mitochondrial lane and the prominent band seen in the comparable lane of the transformant E221/L1/ST1 migrates in the region expected for Mss1p.

P. G. F. and J. V. (4). These and other properties shared by mto1 and mss1 mutants prompted us to examine if the products of the two genes might be subunits of a single complex.

As expected, immunochemical studies confirmed Mto1p and Mss1p to be constituents of mitochondria. When a mitochondrial extract obtained from a yeast transformant overexpressing Mto1p and Mss1p was centrifuged through a sucrose gradient, however, failed to reveal any association of either protein with the large or small subunit of the ribosomes. Even though this constitutes strong evidence that neither protein is a subunit of mitochondrial ribosomes, it does not exclude a transient interaction of the Mto1p-Mss1p complex with ribosomes during translation.

The properties of the mutants are indicative of a role of Mss1p and Mto1p in some aspect of mitochondrial translation. In a paromomycin-resistant background, mutations in MSS1 result in an increase of the steady-state concentration of partially spliced COX1 transcripts (4). Decoster et al. (4) concluded that the processing block was a consequence of the failure of the mutant to translate the maturases encoded in some of the intronic regions of the COX1 pre-mRNA, thereby causing accumulation of incompletely spliced transcripts. Another hallmark of mss1 mutants is the complete absence of subunit 1 of cytochrome oxidase, the product of COX1. Both of the above properties are also true of mto1 mutants. The results of in vivo labeling of mitochondrial translation products indicate that other translation products including subunits 2, ATP6, and subunit 9 (the status of cytochrome b is difficult to ascertain from the radioautogram because of its comigration with subunit 2 of cytochrome oxidase) are more efficiently translated in the mto1 mutant. A possible explanation for this observation is that the translation rate of the mutant is less regulated. Although we have not examined mitochondrial translation in mto1 mutants with a paromomycin-sensitive genome, their spectra are also indicative of an unbalanced production of the respiratory chain components. The mutants have more cytochrome b relative to cytochromes a and a₃ and higher NADH- and succinate-cytochrome c reductase activity. The latter is probably a consequence of an increase in coenzyme QH₂-cyto-

FIG. 11. Sedimentation behavior of Mto1p and Mss1p. Mitochondria were isolated from W303-1A transformed with pG158/ST12. This plasmid contains MTO1 and MSS1 in a tandem arrangement in YEp352. The transformant was ascertained to overexpress both gene products. Mitochondria (6 mg) were sonically irradiated and centrifuged at 220,000 × g for 15 min. The clear supernatant (0.3 ml) was mixed with 2 mg of hemoglobin and 0.15 mg of canine muscle lactate dehydrogenase and layered on a 7–20% sucrose gradient containing 10 mM Tris-HCl, pH 7.5, 0.5 mM EDTA, and 0.05% Triton X-100. The gradient was centrifuged at 64,000 rpm for 7 h in a Beckman SW 65 rotor. The gradient was collected by gravity in 15 fractions. Each fraction was assayed for hemoglobin by absorption at 410 nm (a–a) and for lactate dehydrogenase (●●●) by measuring NADH-dependent conversion of pyruvate to lactate. The sedimentation of Mto1p and Mss1p in the gradient was determined by Western blot analysis. Proteins were separated on a 9% polyacrylamide gel, electrophoretically transferred to nitrocellulose, and probed for Mto1p. The same blot was subsequently treated with antibody to Mss1p.
chrome c reductase, which contains cytochrome b as one of its electron carriers.

The absence of subunit 1 in mto1 and mss1 mutants, despite 30–50% of mature COXI mRNA, could be because of a defect in initiation of translation. Although the involvement of the Mto1p-Mss1p complex in translational initiation cannot be excluded, it seems unlikely for the following reasons. With the exception of the COXI, the mutants are able to translate most other mRNAs even more efficiently than the wild type strain. The argument would therefore need to be made that only translational initiation of selected transcripts such as COXI depend on Mto1p-Mss1p. This is difficult to reconcile with the widespread occurrence of homologous proteins in bacteria, which implies general rather than a specialized function of the proteins. A requirement in initiation of translation also fails to explain the almost 2-fold increase in the coenzyme QH2-cytochrome c reductase relative to wild type in the context of the paromomycin-sensitive allele.

An alternative explanation is that the Mto1p-Mss1p complex has a proofreading function in mitochondrial and perhaps more generally in eucaryotic protein synthesis. According to this interpretation, Mto1p-Mss1p interact with the small ribosomal subunit probably at a site in the 15 S rRNA close to or inclusive of the paromomycin locus. In the presence of the paromomycin-resistance mutation, this interaction is preempted, resulting in a high rate of mutations in mitochondrial translated proteins. As a result, a large percentage of the proteins are no longer functional. The accumulation of partially spliced COXI and COB intermediates in the mutant according to this interpretation is a consequence not of a defect in translation of the intron-encoded maturases but rather of mutations in proteins of this class that impair their functions. The complete absence of the subunit 1 despite the presence of some COXI mRNA protein may be explained by a more rapid turnover of mutant copies of subunit 1. Although the other proteins are present in higher than normal concentrations, the steady-state levels may give a false impression of the actual amounts synthesized. Thus, depending on their intrinsic stability there may be differences in the rates of degradation. It is interesting to note that there appears to be an inverse relationship between the size of the protein and the apparent increase in its concentration in the mutant. For example, Atp9, the smallest product of the paromomycin locus. In the presence of the paromomycin-resistant allele.

MTO1 Codes for Mitochondrial Protein Needed for Respiration

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