Mrs5p, an Essential Protein of the Mitochondrial Intermembrane Space, Affects Protein Import into Yeast Mitochondria*

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Mitochondria make a major contribution to the energy production and conservation in all eukaryotic cells. Unlike other eukaryotic organisms, where oxidative phosphorylation is essential for viability, the yeast Saccharomyces cerevisiae can switch to fermentative growth when suitable carbon sources are provided. Under these conditions yeast cells tolerate mutations in a large number of nuclear and mitochondrial genes, whose products are involved directly or indirectly in the formation of respiration competence and energy conservation. However, a series of nuclear encoded mitochondrial proteins are essential for viability of yeast cells under all growth conditions, indicating that certain functions of mitochondria are indispensable. Some of these proteins participate in the translocation of cytoplasmically translated proteins into mitochondria and their correct folding (1-6). Another set of recently isolated essential genes influences the morphology of mitochondria and their inheritance during cell division (7, 8). A third class of mitochondrial proteins has been shown to be essential only in cells with defects in the mitochondrial genome: the mitochondrial ATP/ADP carrier encoded by the AAC2 gene (9), a putative phosphatidylserine synthase encoded by the PEL1 gene (10, 11), the constitutive alcohol dehydrogenase encoded by the ADC1 gene (12), and a protein encoded by YME1, whose inactivation causes increased escape of DNA from mitochondria to the nucleus (13).

A useful method of isolating yeast genes involved in key steps of mitochondrial biogenesis is the search for multicopy suppressors of mitochondrial defects. The mrs2-1 mutation, a disruption of the unique nuclear MRS2 gene, causes a pleiotropic phenotype depending on the mitochondrial background (14), which makes mrs2-1 an interesting mutation for the investigation of nucleo-mitochondrial interactions. On one hand, splicing of mitochondrial group II introns is impaired, whereas other RNA processing events do not appear to be affected. On the other hand, mrs2-1 cells devoid of mitochondrial introns still show a respiratory-deficient (pet) phenotype. Although mitochondrial macromolecule synthesis appears to be unaffected, photometrically detectable amounts of cytochrome aa3 are lacking, and the amount of cytochrome b is reduced. Therefore the MRS2 gene product has been assumed to be bifunctional, being involved in the biogenesis of mitochondrial protein complexes and in RNA splicing. Alternatively, its effects on splicing may be the consequence of a function in membrane biogenesis (14).

In previous papers we reported the isolation from a yeast genomic library of several nuclear genes that suppress the respiration-deficient phenotype of an mrs2-1 mutant strain when present on a high copy number vector (15-17). The MRS5 gene isolated on the plasmid YEpMW5 suppresses the pet phenotype of the mrs2-1 mutation in a yeast strain containing a full set of mitochondrial introns (16). Interestingly, the same plasmid is also capable of suppressing a mitochondrial group II intron mutation M1301, which blocks the excision of the intron in vivo, although the intron is still capable of self-splicing in vitro (18). Here we report the characterization of the MRS5 gene as an essential factor of mitochondrial biogenesis. Mrs5p is the first essential protein isolated so far which is associated with the inner mitochondrial membrane and protrudes into the intermembrane space. Depletion of yeast cells from Mrs5p is lethal by causing severe defects in mitochondrial functions, such as accumulation of mitochondrial precursor proteins, the inability to form functional cytochrome complexes and changes in mitochondrial morphology. These data suggest an involvement of the Mrs5 protein in fundamental processes of mitochondrial biogenesis. The possible functions of Mrs5p and its suppressor activity on mitochondrial RNA processing defects will be discussed.

EXPERIMENTAL PROCEDURES

Strains and Media—The diploid yeast strain GA74D (16) was used to construct the mrs5-disrupted strain EJ 74/gd5. Strains DBY747/M1301...
Characterization of the Essential Yeast Gene MRS5

(19) and GW7/gd2-21.2 (ATTC 90737) (15) were used to test the suppressor activity of various subclones of plasmid YEpMWS. Standard yeast genetic methods and media (16, 17) were used throughout this study. YPG medium was enriched with 0.05% glucose (YPDg) where indicated to facilitate initial growth of cells with poor respiratory capacity. Esherichia coli strain XL1blue (Stratagene) and the following plasmids were used for subcloning: Bluestipit (Stratagene); pUC18 (20); YEp351 (21); and YEpplac181, YCplac111, and YIpplac121 (22).

Disruption of the MRS5 Gene—A 3.8-kb hisG-URA3 cassette was isolated after digesting plasmid pNKY51 (23) with BglII and BamHI and filling in the ends with Klenow polymerase. Plasmid pE/5/60, which harbors a 1.2-kb CiaI/Sphl MRS5 fragment inserted in pUC18, was digested with NruI/Sad, filled in with Klenow polymerase, and ligated with the hisG-URA3 fragment. The resulting plasmid pE/5/68 was digested with EcoRI/Sall, and the 5.8-kb fragment containing the mrs5-URA3 construct was used for one-step gene disruption in yeast (24).

Production of Anti-Mrs5p Serum—Plasmid PETKH-1 allows the production of 6xHis-tagged heterologous proteins under the control of the T7 promoter in E. coli. A 1-kb Sad/BamHl fragment of the MRS5 gene was cloned in frame into the Smal/BamHl site of pETKH-1, blunt ending the Sad site with Klenow enzyme, to generate plasmid pE/5/76. After transformation in E. coli strain DE3 (25), cells were grown to an O.D.~0.1 in LB + 500 mg//l ampicillin, and expression of recombinant MRS5 fusion protein was induced for 3 h by the addition of isopropyl 1-thio-β-D-galactopyranoside to a final concentration of 1 mm. 6xHis-Mrs5 protein from E. coli lysates was purified upon its ability to bind specifically to nickel-nitro-triacetic acid Sepharose according the manufacturer’s protocol (Quilagen) and used for the immunization of rabbits.

Isolation and Subfractionation of Mitochondria—Yeast mitochondria were isolated from spheroplasts as described by Daum et al. (26) and suspended in breaking buffer (0.6 M sorbitol, 10 mm Tris-Cl, pH 7.4). To disrupt the outer mitochondrial membrane, 5 volumes of 10 mm Tris-Cl, pH 7.4, were added to yield a concentration of 0.1 M sorbitol. After incubation on ice for 30 min samples were centrifuged at 40,000 g. The pellet-containing mitoplasts (mitochondria with disrupted outer membrane but without proteins of the intermembrane space) were suspended in a small volume of breaking buffer.

For sidedness studies Mrs5p mitochondria and mitoplasts at a concentration of 1 mg/ml, respectively, were incubated for 20 min on ice in 0.6 M sorbitol, 10 mm Tris-Cl, pH 7.4, in the presence or absence of 0.1 mg/ml proteinase K and 1% Triton X-100 as indicated.

Integral and peripheral membrane proteins were separated by incubating mitochondria at a concentration of 1 mg/ml in 0.1 M Na2CO3, pH 11.5, for 20 min on ice. The pellet (insoluble integral proteins) and supernatant (soluble peripheral proteins) were separated by centrifugation at 100,000 × g for 1 h and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blotting.

A mitochondrial membrane fraction was prepared by sonification of mitochondria (1 mg/ml) in breaking buffer for 1 min with intermitotic treatment using a Braun Labsonic 2000 sonificator. Membranes and soluble proteins were separated by centrifugation at 100,000 × g for 1 h. Subfractionation of mitochondrial membranes was performed as described by Dorn et al. (27).

Deletion of Mrs5p—Plasmid YIpGAL181 was constructed by inserting a 630-bp BamHl/EcoRI fragment of the GAL1/10 promoter into the BamHl/EcoRI sites of plasmid YIpplac181 (21). The MRS5 ORF was polymerase chain reaction amplified from plasmid pE/5/60 using the primers 5'-AAAGGATCCATGTCGTTCTTTTTAAATAG and M13 Universal, thereby truncating the MRS5 ORF by 39 bp. Plasmid YIpGAL181 was digested with Psfl, and the 3-protruding ends were removed by the 3′-5′ exonuclease activity of Klenow enzyme and redigested with BamHl. After ligation of vector and insert, the resulting construct p5/87 was linearized with EcoRV and integrated into the yeast genomic LEU2 locus.

Miscellaneous—The following procedures were performed essentially according to published methods: manipulation of nucleic acids (28), DNA sequencing (29), preparation of yeast protein extracts (30), separation of proteins on sodium dodecyl sulfate-polyacrylamide gels (31, 32), immunoblotting and immunodetection with the Enhanced Chemi

1 The abbreviations used are: kb, kilobase; bp, base pair(s); ORF, open reading frame; DASPMI, 2-(4-dimethylaminostyryl)-1-methylpyridinium iodide; Wt, wild type; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine.

FIG. 1. Restriction map and subcloning of MRS5. Panel A, subclones of the MRS5 gene. Plasmid YEpMWS, which contains a 7.7-kb yeast genomic insert, was digested with various restriction enzymes. The resulting fragments were cloned into plasmid YEp351 and tested for their ability to suppress the respiration-deficient phenotype of mutations mrs2-1 and M301. Fragments that restored respiration competence were marked with +, fragments that failed to do so with −. Panel B, relative position of the 1-kb CiaI/Sphl MRS5 subclone on yeast chromosome II. The accession numbers of overlapping sequences are given.

Luminiscence System (Amersham), low temperature cytochrome spectra (15). Computational analysis was performed using the Genetics Computer Group software package (33).

RESULTS

Subcloning and Chromosomal Localization of MRS5—Various restriction fragments of the 7.7-kb yeast genomic insert of the previously isolated plasmid YEpMWS (1) were subcloned into plasmid YEp351 and tested for their ability to suppress the respiration deficiency of strains GW7/gd2-21.2 and DBY M301. A 0.8-kb Sad/Sphl fragment restored the respiration competence of mutations mrs2-1 and M301 to an extend comparable with the original clone (Fig. 1A). Fig. 2 shows the nucleotide sequence of the 1-kb CiaI/Sphl subclone. Upon comparison of this sequence with the content of the EMBL data base, overlaps of the flanking regions with the sequence entries X01080, representing the PHO3 gene for the constitutive acidic phosphatase (34), and X15318, coding for NHP6B, a homolog of a DNA-binding protein (35), respectively, were observed (Fig. 1B). As the location of the PHO3 gene has been established on yeast chromosome I, the isolated DNA fragment on plasmid YEpMWS maps to this chromosome. These data were confirmed in the course of the yeast genome sequencing program (36).

Sequence analysis of the 0.8-kb Sad/Sphl fragment revealed a single ORF with a coding capacity for a protein of 109 amino acids (Fig. 2). Interestingly, this subclone contains only 30 bp of the original MRS5 promoter sequence with a putative TATA element at position −28 relative to the proposed start codon. Larger subclones containing longer MRS5 upstream regions did not show increased suppressor activity in mrs2-1 and M301 cells, respectively (data not shown), indicating that this 30-bp promoter is sufficient for MRS5 transcription. Investigation of the upstream region of the MRS5 gene revealed no sequences related to known binding motifs for transcription
Characterization of the Essential Yeast Gene MRS5

Factors. The predicted Mrs5 protein has a relatively poor codon adaptation index of 0.067, indicating that the gene is expressed weakly (37). The protein is rather hydrophilic and does not contain putative membrane spanning regions. Many nuclear proteins that are directed to the mitochondria contain amino-terminal leader sequences necessary for efficient import into mitochondria. These leader sequences are assumed to form amphiphilic helices and are cleaved off during the import into mitochondria (38). No such leader sequence can be deduced from the primary sequence of the Mrs5 protein. The amino acid sequence of the Mrs5 protein was compared with sequence entries of the SwissProt, release 31, and the PIR, release 46, data bases using the FASTA (39) and BLAST (40) algorithms. No convincing similarities of the Mrs5 protein with sequences in these libraries could be detected nor with any conserved motifs, which might represent domains with known enzymatic function.

MRS5 Is an Essential Gene—The chromosomal copy of the MRS5 gene was disrupted with the URS3 gene in the diploid yeast strain GA74D via gene replacement (see "Experimental Procedures"), and the disruption event in stable haploid cells showed significantly reduced amounts of cytochromes, including cytochrome c, which indicates a major defect in the formation of the corresponding protein complexes (Fig. 4C). To analyze further the growth defect of mrs5-disrupted cells the MRS5 gene was placed under the control of the GAL1 promoter in a yeast integrative plasmid. As a GAL1-regulated full-length MRS5 ORF complemented the mrs5 disruption not only on galactose- but also on glucose-containing medium where GAL1-regulated expression is repressed, the GAL1 promoter was placed in front of a shortened version of MRS5 (plasmid p5/87, see " Experimental Procedures"). This construct lacks the last 39 bp of the MRS5 ORF and gives rise to a truncated Mrs5 protein that is still able to complement a mrs5-deleted strain on galactose-containing medium. Haploid mrs5-disrupted cells harboring the GAL1-regulated MRS5 gene ectopically integrated in the LEU2 locus were obtained upon transformation of strain EJ 74/gd5 with plasmid pEJ 5/87, 5/87, spore progeny harboring the mrs5 disruption and the galactose-driven construct (strain EJ 75/gd5-2C) were viable on galactose medium but failed to grow on glucose. This indicates an essential function of the MRS5 gene for the vegetative growth of yeast cells.

Depletion of Mrs5p Blocks the Import of Hsp60—Overnight cultures of strain EJ 75/gd5-2C and an isogenic wild type strain (strain EJ 75/gd5-2D) grown in galactose were diluted in fresh glucose medium 1:1,000 and incubated at 28 °C. Hsp60 became assembled into large precursor molecules with an amino-terminal leader sequence, which is cleaved off during import. After 16 h of incubation both strains had a comparable protein pattern on Western blot experiments with antibodies against hsp60. Protein samples from both strains were taken in regular intervals and probed in Western blot experiments with antibodies against hsp60. Many nuclear encoded mitochondrial proteins are synthesized as large precursor molecules with an amino-terminal leader sequence, which is cleaved off during import. A block in mitochondrial import can be detected upon the accumulation of unprocessed mitochondrial precursor proteins that still contain their leader sequences. 16 h after the glucose shift of strain EJ 75/gd5-2C a precursor form of hsp60 was detected in a Western blot experiment, which indicates a severe block in the import of this protein into mitochondria (Fig. 4B). To confirm the effects of Mrs5p depletion on mitochondrial functions we analyzed the presence of cytochrome complexes by cytochrome absorption spectra. 16–20 h after shift to YPD, Mrs5p-depleted cells showed significantly reduced amounts of cytochromes, including cytochrome c1, which indicates a major defect in the formation of the corresponding protein complexes (Fig. 4C). To
Characterization of the Essential Yeast Gene MRS5

Control the mitochondrial membrane potential, cells were stained with DASPM1, a fluorescent dye that accumulates in mitochondria in a membrane potential-dependent manner (7). Mitochondria from strain E75 gd5-2C could be stained with DASPM1 even 36 h after the transfer to glucose, indicating that the mrs5 mutation did not directly affect the membrane potential across the mitochondrial inner membrane. However, the morphology of mitochondria in the mrs5 disruptant was changed, and overall mitochondrial mass was reduced strongly compared with the wild type (data not shown).

To control overall metabolic activity wild type and mrs5 mutant cells were stained with trypan blue, a dye that accumulates in cells with a disrupted membrane potential across the plasma membrane. Accumulation of trypan blue was not observed in wild type as well as in mrs5-depleted cells even 1 week after the shift to glucose-containing medium, indicating that the membrane potential across the plasma membrane of the mutant was still intact (data not shown). Thus, the growth defect after MRS5 depletion, the accumulation of hsp60 precursor, and the lack of photometrically detectable cytochromes are not the result of a general cytoplasmic defect. The fact that E75 gd5-2C cells shifted to glucose rapidly lost the ability to form colonies on both YPD and YPGal plates indicates an irreversible defect due to MRS5 depletion.

MRS5 is a Mitochondrial Protein—On Western blots of total yeast cell extracts an antiserum raised against the MRS5 protein (see "Experimental Procedures") detects a protein of about 13 kDa, which is consistent with the predicted molecular mass of MRS5 (12283.8 Da). The identity of this 13-kDa protein as MRS5 was confirmed by testing protein extracts of yeast strains harboring various MRS5 alleles. Plasmid p5/87 carries a 3' -shortened version of the MRS5 gene under control of the GAL1 promoter. In protein extracts of a haploid mrs5 disruptant transformed with plasmid p5/87 (MRS5 + p5/87) and grown on galactose the anti-MRS5 serum recognized a protein of about 10 kDa, which could not be detected in wild type cell extracts, but correlates with the predicted size of the truncated MRS5 protein expressed from plasmid p5/87 (Fig. 5). In extracts of a wild type strain transformed with plasmid p5/87 (Wt + p5/87), a 10- and a 13-kDa protein were recognized by the anti-MRS5 serum, thus verifying the specificity of this antibody.

When yeast cells were fractionated, a strong signal with the anti-MRS5 serum was observed in the mitochondrial fraction, paralleled by the enrichment of the ATP/ADP carrier and cytochrome b2. In the postmitochondrial supernatant with glyceraldehyde phosphate dehydrogenase as a marker, MRS5 was not detected (Fig. 6). The presence of a small amount of glyceraldehyde phosphate dehydrogenase in the mitochondrial fraction is possibly the result of a contamination caused by the unspecific association of this protein with membranes.

MRS5 is Located on the Intermembrane Space Leaflet of the Inner Mitochondrial Membrane—Proteinase K treatment of intact mitochondria did not digest the MRS5 protein, indicating that this protein is located within the mitochondrion (data not shown).

FIG. 4. Depletion of MRS5 leads to a growth defect, accumulation of a mitochondrial precursor protein, and loss of cytochromes. Panel A, strains E75 gd5-2C (ΔMRS5) and E75 gd5-2D (WT) were grown overnight in YPGal and diluted 1:1,000 in fresh YPD. Growth was determined by measuring the optical density at 600 nm of both cultures. Time units of the x axis refer to hours after the transfer to glycerol-containing medium; time points at which samples were taken for protein analysis and measurement of cytochrome spectra are marked with arrows. Panel B, MRS5-depleted cells accumulate a mitochondrial precursor protein in vivo. At the indicated time points protein samples were prepared from both cultures, and the steady-state levels of mature hsp60 (m) and precursor hsp60 (p) were determined by immunoblotting with an anti-hsp60 serum. Panel C, depletion of MRS5 causes loss of all cytochromes. Cytochrome spectra of both shifted cultures were measured at the given time points as described under "Experimental Procedures."
Characterization of the Essential Yeast Gene MRS5

17223

The MRS5 gene has been isolated as one of several multicopy suppressors of a respiration-deficient mutant, mrs2-1 (16). MRS5 is of particular interest because (i) it is located in the mitochondrial intermembrane space, a poorly characterized compartment; and (ii) it is essential for yeast cell viability. Depletion of MRS5 causes severe defects in mitochondrial biogenesis, whereas cytoplasmic functions are not affected. Thus, the MRS5 gene product seems to play a fundamental role in mitochondrial physiology.

Table I

| Enrichment factor | Mitochondria | OM | CS | IM | Sol |
|-------------------|--------------|----|----|----|-----|
| MRS5              | 1            | 0.05 | 0.5 | 1.5 | 0.05 |
| Porin             | 1            | 5.5 | 0.7 | 0.3 | 0.3 |
| AAC               | 1            | 0.1 | 1.5 | 2.0 | 0.01 |
| Cytb2             | 1            | 0.1 | 0.1 | 0.3 | 3.0 |

Sonification of mitochondria and subsequent centrifugation at 100,000 × g for 1 h results in the separation of membrane-bound and soluble mitochondrial proteins. MRS5, ATP/ADP carrier, and porin were recovered quantitatively in the membrane pellet (Fig. 8B). The partial removal of the F_{1α}-ATPase from the membrane fraction may be caused by its labile association with the tightly membrane-bound F_{0}ATPase. Cytochrome b_{2} as a marker for soluble mitochondrial proteins was released completely into the supernatant.

So far our data suggest a localization of the MRS5p in the intermembrane space with association with one of the mitochondrial membranes. Subfractionation of mitochondrial membranes (27) revealed that MRS5p cofractionates with the inner membrane marker ATP/ATP carrier (Table I). Porin was enriched selectively in the outer membrane fraction, and cytochrome b_{2} was found almost exclusively in the soluble fraction. Detection of MRS5p in the contact site fraction is because contact sites consist in part of components of the inner mitochondrial membrane.

DISCUSSION

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Fig. 7. Sub mitochondrial localization of MRS5p. Panel A, mitochondria (MI, left lane) were shocked osmotically (see “Experimental Procedures”), and the released proteins of the intermembrane space (IS, center lane) and recovered mitoplasts (MP, right lane) were tested for the presence of various mitochondrial proteins by Western blot analysis. Panel B, mitoplasts (lane 1) were incubated with proteinase K (0.1 mg/ml, lane 2) and proteinase K plus Triton X-100 (1%; lane 3). For abbreviations see Fig. 6. F_{1α} = F_{1α}-ATPase.

Fig. 8. Association of MRS5p with mitochondrial membranes. Panel A, peripheral membrane proteins were extracted from isolated mitochondria with alkaline carbonate as described under “Experimental Procedures” and subjected to Western blot analysis. Left lane, isolated mitochondria (MI); center lane, supernatant after Na_{2}CO_{3} extraction (Sn); right lane, pellet fraction (P). Panel B, mitochondria were sonified, and mitochondrial membranes were isolated as described under “Experimental Procedures.” Total mitochondrial proteins (MI, left lane), soluble proteins (Sn, center lane), and the mitochondrial membrane fraction (P, right lane) were subjected to Western blot analysis with various antisera directed against mitochondrial proteins. For abbreviations see Figs. 6 and 7.

Table I

Distribution of MRS5p between mitochondrial membranes

| MRS5p | Porin | AAC | Cytb2 | Enrichment factor |
|-------|-------|-----|-------|-------------------|
| Mitochondria | 1 | 0.05 | 0.5 | 1.5 | 0.05 |
| OM | 5.5 | 0.7 | 0.3 | 0.3 |
| CS | 0.1 | 1.5 | 2.0 | 0.01 |
| IM | 0.1 | 0.1 | 0.3 | 3.0 |

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Fig. 6. Mitochondrial localization of MRS5p. Equal amounts (80 μg) of total cellular extract (T, left lane), postmitochondrial supernatant (PS, center lane), and purified mitochondria (MI, right lane) were subjected to immunodecoration with antisera against yeast Mrs5p, ADP/ATP carrier (AAC, inner membrane), cytochrome b_{2} (Cytb2, intermembrane space), and glyceraldehyde phosphate dehydrogenase (GAPDH, cytoplasm).
Characterization of the Essential Yeast Gene MRS5

17224

mitochondrial function and/or biogenesis.

Most essential mitochondrial proteins characterized so far can be assigned to two classes: (i) proteins that are involved in mitochondrial protein import and correct folding of imported proteins (2, 7); and (ii) gene products that are needed for correct inheritance of mitochondria during cell division (8). Depletion of proteins essential for the import of proteins into mitochondria results in the accumulation of unprocessed mitochondrial precursor proteins in the cytoplasm. In our experiments, accumulation of hsp60 precursor molecules was observed as an early consequence of Mrs5p depletion, whereas inheritance of organelles during mitosis was unaffected (data not shown). As a result of the Mrs5p depletion mitochondria undergo dramatic changes in morphology (data not shown) and are unable to assemble cytochrome complexes. We cannot judge from the experiments presented here if Mrs5p is a general import factor and responsible for the translocation of most mitochondrial proteins, or if it is only required for the import of a small subset of proteins. Even if only a small number of mitochondrial proteins, including hsp60, is affected by Mrs5p depletion, lack of functional cytochrome complexes and morphological changes of mitochondria could still be the result because hsp60 is an essential factor of mitochondrial biogenesis as it governs the correct folding of newly imported proteins (41, 42).

The protein translocation machineries of the outer and inner mitochondrial membrane seem to act independently, only forming a transient complex during the process of protein import (43, 44). Although individual steps leading to the transport of proteins across both mitochondrial membranes have been well characterized, little is known about the interaction between outer and inner membrane and the formation of contact sites. As a hypothesis, Mrs5p may interact with both the outer and the inner membrane protein import machineries, thereby linking both complexes during protein translocation and possibly participating in the formation of contact sites. Studying components of the inner membrane protein import machinery Berthold et al. (45) discovered the existence of a 14-kDa protein, which was coinmunoprecipitated with antibodies directed against MIM23. Under conditions described by these authors, Mrs5p may be associated with the inner mitochondrial membrane and even with components of the mitochondrial protein translocation machinery. However, there is no further evidence for the identity of Mrs5p and the 14-kDa protein.

The relation between MRS5 and mitochondrial RNA splicing is only speculation at present. Although overexpression of MRS5 suppresses defects in the mitochondrial group II intron processing, it seems very unlikely that Mrs5p is a mitochondrial splicing factor. The following observations may support this notion.

1. The suppressor activity on the mrs2-1 and M1301 mutation is rather weak compared with other suppressor genes. Northern blot experiments of mitochondrial RNA preparations from mutant strains transformed with a multicopy MRS5 plasmid exhibited only a weak increase in splicing efficiency compared with the splicing-deficient mutants (data not shown).

2. Yeast strains with defects in mitochondrial RNA processing are respiration-deficient but viable on fermentable carbon sources. By contrast, Mrs5s is also essential for growth on fermentable carbon sources. MRS5 dysfunction affects mitochondria in a dramatic way, which suggests a more general role of the MRS5 gene in mitochondrial biogenesis.

3. Mrs5p is a peripheral protein of the inner mitochondrial membrane and protrudes into the intermembrane space. The overexpression of Mrs5p has no major effects on its localization and topology as determined by subtraction of a yeast strain transformed with the MRS5 gene on a multicopy plasmid (data not shown). Since mitochondrial splicing is supposed to occur in the mitochondrial matrix, a direct interaction of Mrs5p with mitochondrial RNA, as proposed for other proteins involved in mitochondrial RNA maturation, is very unlikely.

We conclude from our data that suppression of defects in mitochondrial RNA processing by elevated gene dosages of MRS5 occurs in a rather indirect manner. As shown for the MRS3, MRS4, and MRS12/RIM2 genes, overexpression of mitochondrial solute carrier proteins can suppress defects in mitochondrial RNA splicing and DNA replication (17, 46). The nature of this suppressor activity is possibly the modulation of the intramitochondrial environment via enhanced transport of solutes, although the substrates of these carriers have not yet been identified. On the assumption that Mrs5p is involved in mitochondrial protein import, overexpression of MRS5 might stimulate import or modification of mitochondrial proteins, which in turn could enhance mitochondrial transcription, RNA processing, and translation. As an example, increased amount of transcribed RNA would overcome poor processing of splicing-deficient mutants and provide enough mature mRNA to restore respiratory competence. Membrane association of Mrs5p and its possible involvement in the formation of contact sites might also cause another mechanism of suppression. Overexpression of MRS5 could result in changes in the membrane topology and thereby affect mitochondrial RNA processing, which is assumed to occur in association with the inner mitochondrial membrane (47, 48).

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