The mitochondrial inner membrane protein Lpe10p, a homologue of Mrs2p, is essential for magnesium homeostasis and group II intron splicing in yeast

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Abstract The yeast ORF YPL060w/LPE10 encodes a homologue of the mitochondrial protein Mrs2p. These two proteins are 32% identical, and have two transmembrane domains in their C-terminal regions and a putative magnesium transporter signature, Y/F-G-M-N, at the end of one of these domains. Data presented here indicate that Lpe10p is inserted into the inner mitochondrial membrane with both termini oriented towards the matrix space. Disruption of the LPE10 gene results in a growth defect on non-fermentable substrates (petite phenotype) and a marked defect in group II intron splicing. The fact that in intron-less strains lpe10 disruptants also exhibit a petite phenotype indicates that functions other than RNA splicing are affected by the absence of Lpe10p. In the mitochondria, concentrations of magnesium, but not of several other divalent metal ions, are increased when Lpe10p is overexpressed and reduced when it is absent. Magnesium concentrations are raised to normal levels and growth on non-fermentable substrates is partially restored by the expression of CorA, the bacterial magnesium transporter, in the lpe10 disruptant. These features are similar to those previously reported for Mrs2p, suggesting that Lpe10p and Mrs2p are functional homologues. However, they cannot easily substitute for each other. Their roles in magnesium homeostasis and, possibly as a secondary effect, in RNA splicing are discussed.

Key words Mitochondria · Magnesium homeostasis · Group II introns · Protein topology · CorA protein family

Introduction

Expression and assembly of the few structural genes encoded by mitochondrial DNA requires an unexpectedly large number of nuclear gene products. These products fulfill various functions in RNA modification and processing, particularly splicing of group I and group II introns, protein synthesis, assembly, stabilization and degradation of protein complexes of the respiratory chain (for review, see Costanzo and Fox 1990; Grivell 1995).

Information on nuclear gene products that facilitate group II intron excision from pre-mRNA is still scarce. One such factor in yeast, Mss116p, shows similarity to RNA helicases of the DEAD-box family (Seraphin et al. 1989), and extracts from cells overexpressing this protein exhibit an increase in ATP-dependent splicing of group II intron RNA in vitro (Niemer et al. 1995). Genes encoding several other factors have been selected as multicopy suppressors of a mitochondrial group II intron mutation in yeast (Koll et al. 1987; Waldherr et al. 1993). One of these is Mrs2p, a 56-kDa integral protein of the inner mitochondrial membrane, expression of which is essential for splicing of all four group II introns in yeast mitochondria. Yet, Mrs2p has an essential function in intronless yeast mitochondria which appears to be related to cytochrome biogenesis. It remains an open question whether Mrs2p is bifunctional or its effect on splicing is secondary to a more general function in mitochondrial biogenesis (Wiesenberger et al. 1992; Schmidt et al. 1998; Bui et al. 1999). The latter has been assumed to be the case for five other Mrs proteins, three of which are integral proteins of the mitochondrial inner membrane, related to the soluble carrier family (Mrs3p, Mrs4p, Mrs12p), while the other two are located in the intermembrane space proteins and constitute components of a specific import pathway for carrier proteins (Wiesenberger et al. 1991;
Van Dyck et al. 1995; Jarosch et al. 1996, 1997; Koehler et al. 1998). In fact, recent data from this laboratory indicate that Mr2p is a member of a large, poorly conserved family of magnesium transporters (Bui et al. 1999), the first member of which, CorA, was identified in bacteria (Smith et al. 1993). Characteristic features of these proteins are two or three transmembrane domains in their C-terminal segments, one of which terminates with a conserved motif, F/Y-M-N-G (Smith and Maguire 1998; Bui et al. 1999; Szegedy and Maguire 1999).

Here we report on a homologue of the MRS2 gene found during sequencing of the Saccharomyces cerevisiae genome (YPL060W; working name LPE10). We present data on its subcellular location and mutant phenotypes, particularly those affecting group II intron splicing. Furthermore, we show that Lpe10p is essential for the maintenance of intramitochondrial magnesium concentrations. The data support the notion that Lpe10p and its homologue Mr2p are functionally related and involved in mitochondrial metal ion homeostasis.

**Materials and methods**

Strains, plasmids, media and general procedures

If not stated otherwise, bacterial strains, yeast strains and plasmids, media and growth conditions, as well as the genetic methods used here, have been described previously (Jarosch et al. 1996; Bui et al. 1999).

**Gene disruption**

Plasmid pJ244 was digested with PvuII and a fragment containing the URA3 gene was filled in with the Klenow polymerase. Plasmid pBS-LPE10 was digested with SalI and NdeI, filled in with Klenow polymerase and ligated to the URA3 cassette, resulting in the construct pBS-LPE10:URA3. The oligonucleotide primers LPE10-BamHI-5' and LPE10-BamHI-3' were used to amplify the disruption cassette from the pBS-LPE10:URA3 construct by PCR. This PCR product was used for one-step gene disruption (Wach et al. 1994) in the strain DBY747, giving raise to the lpe10-1 allele. Disruption of the LPE10 gene was confirmed by Southern analysis.

In order to construct the mrs2-2 lpe10-2 double disruptant, MRS2::HIS and LPE10::URA constructs were generated. The HIS3 cassette was amplified from the plasmid pFA6aHISMX(6) (Wach et al. 1994) using oligonucleotide primers containing sequences flanking the MRS2 gene. HIS-MRS2-5' and HIS-MRS2-3', introduced mismatched into the pBS-LPE10:URA3 construct by PCR. This PCR product was used for one-step gene disruption (Wach et al. 1994) in the strain DBY747, giving raise to the lpe10-1 allele. Disruption of the LPE10 gene was confirmed by Southern analysis.

**Plasmid constructs**

The LPE10 gene was amplified from yeast chromosomal DNA (S. cerevisiae DBY747) using the oligonucleotide primers LPE10-

**BamHI-5' and LPE10-BamHI-3'.** The PCR product was digested with BamHI and cloned into the BamHI site of the vectors pBS-SK + and YEp351 to generate constructs pBS-LPE10 and YEP-LPE10, respectively. The oligonucleotide primers LPE10-BamHI-5' and LPE10-NotI/BamHI were used to amplify the LPE10 gene from the plasmid YEp-LPE10. The PCR product was digested with BamHI and cloned into 25°C in the buffer of the YEp351 vector, creating YEp-LPE10(NotI). A cassette coding for the triple hemagglutinin (HA) epitope tag (Tyers et al. 1993) was cloned in frame with the LPE10 gene at the NotI site of the plasmid YEp-LPE10(NotI), resulting in the construct YEp-LPE10-HA.

To create an in-frame deletion in the LPE10 gene, plasmid YEp-LPE10-HA was digested with BglII and the vector was ligated, resulting in YEp-LPE10-HAΔgll. The BamHI fragment of the YEp-LPE10-HA construct containing LPE10-ΔHA was cloned into BamHI site of the plasmid YCplac22, resulting in the construct YCP-LPE10-HA. Both YEp-LPE10-HA and YCP-LPE10-HA were able to complement the pet phenotype of the strain DBY747 lpe10-1.

For in vitro transcription and translation of Lpe10p and Msr2p, the respective coding sequences were amplified from yeast chromosomal DNA of strain FY1679 using the following oligonucleotide primers containing Kozak consensus sequences (indicated in bold) for initiation of translation (Kozak 1999): MRS2-5' (AAGCCCAATGGAATCCGCGTCCTCCTGTA-3'), MRS2-3' (TTCCTGACGCTCTTCTTCTTCG-3'), LPE10-5' (GAACCAATCGAATTCGAGTCCTCCC-3') and LPE10-3' (CCGAAATCGATTTGACCTGCCTTCTGGATA-3'). The PCR products were cloned into the pGEM-T vector (Promega), bringing them under the control of the phage SP6 promoter.

The second methionine codon of the LPE10 gene was changed to alanine (codon 40, AUG→GCG) by site-directed PCR mutagenesis using the primers LPE10MUT-5' and LPE10MUT-3' (AGCCAAATGGAATCCGCGTCCTCCTGTA-3'). The full-length mutant product was amplified with the primers LPE10-R and LPE10-F, and cloned to the pGEM-T vector in the appropriate orientation. The introduced mutations were verified by sequencing and the construct was named LPE10-M-A.

**Subcellular fractionation of mitochondria**

Isolation and subfractionation of mitochondria were performed as described in Bui et al. (1999). Alkaline extraction with sodium carbonate was done as described by Fujiki et al. (1982). SDS-polyacrylamide gels were loaded with approximately 30 µg of protein per lane. Antibodies used for immunodetection were described previously (Jarosch et al. 1996).

In vitro import of proteins into mitochondria

The LPE10, LPE10-M-A and MRS2 genes were transcribed in vitro using SP6 RNA polymerase. Translation of the mRNA was carried out in a rabbit reticulocyte lysate system (Promega) in the presence of [35S]methionine.

Mitochondrial import assays were performed essentially as described by Glick (1991). Radiolabelled protein was incubated with mitochondria for 20 min at 25°C in the import buffer (600 mM sorbitol, 1 mg/ml bovine serum albumin, 2 mM KH2PO4, 50 mM KCl, 10 mM MgCl2, 2.5 mM EDTA, 50 mM HEPES-KOH, pH 7.1, 2 mM NADH, 2 mM ATP). The mitochondrial membrane potential was dissipated in an aliquot of the import mixture by preincubation with 1 µM valinomycin. After import, mitochondria were resiolated and selected samples were treated with 200 µg/ml protease K to remove non-imported precursors.