Volume 330, number 1, 66–70 FEBS 12935 September 1993

Identification of MIM23, a putative component of the protein import machinery of the mitochondrial inner membrane

Peter J.T. Dekker[a], Petra Keil[b], Joachim Rassow[b], Ammy C. Maarse[a], Nikolaus Pfanner[b,*], Michiel Meijer[a]

[a]*Institute for Molecular Cell Biology, BioCentrum Amsterdam, Kruislaan 318, 1098 SM Amsterdam, The Netherlands*
[b]*Biochemisches Institut, Universität Freiburg, Hermann-Herder-Strasse 7, D-79104 Freiburg, Germany*

Received 12 July 1993

A screening for yeast mutants impaired in mitochondrial protein import led to the identification of two genes (*MPI1* and *MPI2*) encoding the essential components MIM44 and MIM17 of the inner membrane import machinery. We analyzed twelve additional mutants obtained in the screening and found two further complementation groups. One group represents mutants of *SSC1*, the gene encoding mitochondrial hsp70, an essential matrix protein required for protein import across the inner membrane. The second complementation group represents mutants of a new gene (*MPI3*) encoding a 23 kDa integral inner membrane protein (MIM23). MIM23 is synthesized without a presequence, and its import to the inner membrane requires a membrane potential. MIM23 contains a domain homologous to half of MIM17. We speculate that MIM23 is a new member of the protein import machinery of the mitochondrial inner membrane.

Mitochondrial inner membrane; MIM23; Protein translocation

1. INTRODUCTION

In recent years, a number of components of the mitochondrial machinery for the import of preproteins have been identified. These are, in particular, the cytosolic heat shock protein hsp70 and further cytosolic cofactors [1–3], seven mitochondrial outer membrane proteins (MOMs), including two import receptors and a general insertion pore [4–6], and mitochondrial matrix proteins, including the heat shock proteins mitochondrial hsp70 [7], hsp60 [8,9], and processing enzymes [5,10,11].

Little was known about the protein import machinery of the mitochondrial inner membrane. A genetic screening procedure for *Saccharomyces cerevisiae* mutants with defects in mitochondrial protein import (based on the mislocalization of a cytosolic enzyme) enabled us to identify the genes *MPI1* and *MPI2*, encoding the first two essential mitochondrial inner membrane proteins (MIM44 and MIM17) required for the import of preproteins [12,13] (Maarse et al., in preparation). Furthermore, Scherer et al. [14] identified an inner membrane protein of about 45 kDa (ISP45) by crosslinking to a preprotein in transit, however, the protein sequence or gene of ISP45 are not yet available.

In this study, we analyzed twelve additional *S. cerevisiae* mutants that were selected by the genetic screening described above [12]. The mutants were classified in four complementation groups, two of them represented by *mpi1* and *mpi2* mutants. Another complementation group represented mutants of *SSC1*, encoding mitochondrial hsp70 [7,15,16]. The last complementation group contains mutants of a new gene (*MPI3*) that is shown to code for a mitochondrial inner membrane protein of 23 kDa (MIM23). MIM23 contains a domain of significant homology to MIM17 and we speculate that MIM23 is a further component of the mitochondrial inner membrane import machinery.

*Corresponding author. Fax: (49) (761) 203 3331 (from 11 October 1993: (49) (761) 203 5253).

Abbreviations: MIMx, mitochondrial inner membrane protein of x kDa; MOMy, mitochondrial outer membrane protein of y kDa; *S. cerevisiae*, *Saccharomyces cerevisiae*.

2. MATERIALS AND METHODS

To complement the mitochondrial import defect of *S. cerevisiae* mutants obtained by Maarse et al. [12], mutants harbouring the SOD-URA test plasmid (encoding a chimeric protein with a mitochondrial targeting sequence and the *URA3* gene product) were transformed by the method of Klebe et al. [17] with libraries of yeast genomic DNA fragments in the shuttle plasmid vectors YEp13 [18] or p366 (constructed by Spencer and Hieter). Complementing clones were selected by plating cells on minimal medium supplemented with uracil and 5-fluoro-orotic acid [19]. *MPI3* was cloned by complementation-transformation of MB3–46 with a partial *Sau*3A digest of genomic DNA in the vector YEp13 (kindly supplied by M. Bolotin-Fukuhara). Sequence analysis was performed on subclones in bacteriophage M13 with the method of Sanger [20]. Both strands of the *Sph*I fragment were sequenced. Standard molecular biological procedures were according to Sambrook et al. [21].

A *Sph*I–*Bst*1107I fragment of the *MPI3* gene containing the complete coding region for MIM23 was cloned into a pGEM4 transcription vector (Promega) cut with *Sph*I and *Sma*I. By in vitro transcription and translation, MIM23 was synthesized in rabbit reticulocyte lysate in the presence of [^{35}S]methionine [22]. The reticulocyte lysate was incubated with isolated *S. cerevisiae* wild-type mitochondria in BSA-containing import buffer as described [22]. Treatment with pro-

Published by Elsevier Science Publishers B V

teinase K or trypsin, reisolation of mitochondria, and analysis by SDS-PAGE, fluorography, immunodecoration and laser densitometry were performed as published [22]. Treatment of mitochondria with 100 mM Na_2CO_3 (pH 11.5) and separation of pellet and supernatant were performed as described [23,24].

3. RESULTS AND DISCUSSION

Our screening for the isolation of *S. cerevisiae* mutants with defects in mitochondrial protein uptake was based on a mislocalization approach described in Maarse et al. [12]. Briefly, the yeast strain MB3 with a deleted *URA3* gene cannot grow on minimal medium lacking uracil. A chimeric protein that consists of a mitochondrial targeting sequence and the cytosolic gene product of *URA3* was expressed in the strain and efficiently targeted into mitochondria. Growth of transformed MB3 cells was therefore still dependent on exogenously added uracil. Mutations that inhibited mitochondrial import of the chimeric protein allowed growth of the strain in the absence of added uracil. Mutants with *trans*-acting recessive nuclear mutations were selected and analyzed by genetic crosses and complementation transformation with yeast nuclear DNA banks. Besides the previously characterized mutants MB3–52, MB3–68 and MB3–75 (all defective in *MPI1*) [12] and MB3–33 (defective in *MPI2*) (Maarse et al., in preparation), twelve other mutants were analyzed (Table I). These mutants could be classified into four complementation groups. Two mutants were defective in *MPI1*, and three others in *MPI2*. Five mutants appeared to carry a mutation in *SSC1*, an essential gene encoding mitochondrial hsp70 [7,15]. The last two mutants (complementation group IV) could only be complemented by a new gene, *MPI3*.

The *MPI3* gene was obtained by complementation transformation of mutant MB3–46 with a yeast genomic library in YEp13. A *Sph*I fragment of the original complementing clone, containing 1.6 kb of genomic sequence, rescued the genetic defect of mutants MB3–45 and MB3–46 not only on the high copy number vector YEplac181, but also on the centromeric vector YCplac111 [25]. The gene contained on this fragment was sequenced. Fig. 1 shows the nucleotide sequence of *MPI3* and the deduced amino acid sequence. A protein

```
   1                GCATGCCGGGTTCGTTGCGTTCAGGCCTGACGGGCGATTCCCCACCAAGTATAAGTGTCATGATA  65
  66 TACGCGGCACCTGAGCAGCACATTAGGTCCCAATTCAACCCAACATATATAATGGTTCTGTATGGGTATTTTAAGTGTGC  145
 146 AAACAAAATGTGAGTTACCCGGCTGTTTGCTAGTCGCTGCGTACCAACTGGCTTGCTAAAGCTCGGTAAAAATAATTTAA  225
 226 TCGCAAAAGGAAGAAAAGGAAATCGACGATTCATTGAAAAAAAGAGAAAATACTGAAAAAAAAAAGACACCGACAAAAAA  305
 306 AAGAGAGAAAGGAACCTTTACGTAGAGCAAAAGGGAAACATTTGTTGCGTTTAATTAATAGAAATAATAGGTTATTGCAT  385
 386 TCGCCCTCCATTGCAGAAAAAAAAAAAAAAAAAGACCATTTTCCTCTTCTACTCTCTTTGCCTGTACATACTACACGTTAT  465
 466 AGCGTTAACAAAAGCAGATAGAAAAAAAAAAAATAACCAAGATAATATAGGTATACTTGTTTAACAGATCACACACAATC  545

 546 ATG TCG TGG CTT TTT GGA GAT AAG ACA CCT ACC GAT GAT GCG AAT GCT GCC GTG GGC GGC  605
   1 M   S   W   L   F   G   D   K   T   P   T   D   D   A   N   A   A   V   G   G   20

 606 CAA GAT ACA ACC AAG CCT AAG GAA CTA TCG TTG AAG CAG AGT TTA GGT TTC GAG CCA AAC  665
  21 Q   D   T   T   K   P   K   E   L   S   L   K   Q   S   L   G   F   E   P   N   40

 666 ATC AAT AAC ATA ATA TCA GGT CCT GGT GGA ATG CAT GTC GAC ACC GCT AGG CTG CAT CCT  725
  41 I   N   N   I   I   S   G   P   G   G   M   H   V   D   T   A   R   L   H   P   60

 726 TTG GCT GGT CTA GAC AAG GGT GTG GAG TAT TTA GAT CTG GAA GAA GAA CAA CTA TCC TCG  785
  61 L   A   G   L   D   K   G   V   E   Y   L   D   L   E   E   E   Q   L   S   S   80

 786 TTA GAA GGC TCA CAG GGT CTG ATC CCT TCC CGT GGG TGG ACC GAT GAC CTA TGT TAC GGT  845
  81 L   E   G   S   Q   G   L   I   P   S   R   G   W   T   D   D   L   C   Y   G   100

 846 ACC GGT GCC GTC TAC CTG CTG GGA CTT GGT ATC GGA GGG TTT TCT GGT ATG ATG CAG GGT  905
 101 T   G   A   V   Y   L   L   G   L   G   I   G   G   F   S   G   M   M   Q   G   120

 906 CTG CAG AAT ATT CCG CCC AAT AGT CCC GGA AAA TTG CAA TTG AAC ACC GTC CTG AAT CAC  965
 121 L   Q   N   I   P   P   N   S   P   G   K   L   Q   L   N   T   V   L   N   H   140

 966 ATT ACT AAG AGA GGT CCC TTC TTA GGT AAT AAT GCG GGG ATT CTC GCG TTG AGC TAC AAT 1025
 141 I   T   K   R   G   P   F   L   G   N   N   A   G   I   L   A   L   S   Y   N   160

1026 ATC ATC AAT TCT ACA ATA GAT GCA CTA AGA GGC AAA CAT GAC ACC GCG GGC TCC ATT GGC 1085
 161 I   I   N   S   T   I   D   A   L   R   G   K   H   D   T   A   G   S   I   G   180

1086 GCT GGG GCC CTC ACG GGC GCT TTG TTC AAG TCT TCA AAA GGT TTG AAA CCC ATG GGT TAT 1145
 181 A   G   A   L   T   G   A   L   F   K   S   S   K   G   L   K   P   M   G   Y   200

1146 TCC TCG GCA ATG GTG GCC GCT GCG TGC GCC GTC TGG TGT AGT GTC AAG AAA AGA CTA CTT 1205
 201 S   S   A   M   V   A   A   A   C   A   V   W   C   S   V   K   K   R   L   L   220

1206 GAA AAA TGA GCAACACAAGAACCTACTCTCTCTCTCTCTTTCTCTCTCTCTCCCTCTTCGCTTTTCCCCCATGCA 1282
 221 E   K   *                                                                   222

1283 CTGATGGCGCTTGTATATAGCATTTGAAAAATAATAGTACGTAACGCAGAAAACAAACCAATGAAAGTAGAAACCCGGAG 1362
1363 AAAAGATCTAAAAAAACAAAAAAAAAAAGATGGAAAGAGCGCATGTATGTGTAGATATGTACATATATACAACTACTTGTA 1442
1443 TGTTATTTGCCTTTTGTACTGTTAAGCTATTGTTTTCGATGGCCAATTAAAGTAAAACAAACCGCTCACTCTCGGGTATT 1522
1523 TCCCGAAACTGTCAGAGCCGTCCC                                                          1546
```

Fig. 1. Nucleotide sequence of the *MPI3* gene and deduced amino acid sequence. Hydrophobic sequences are underlined. The sequence has been deposited in the EMBL data bank (accession number X74161).

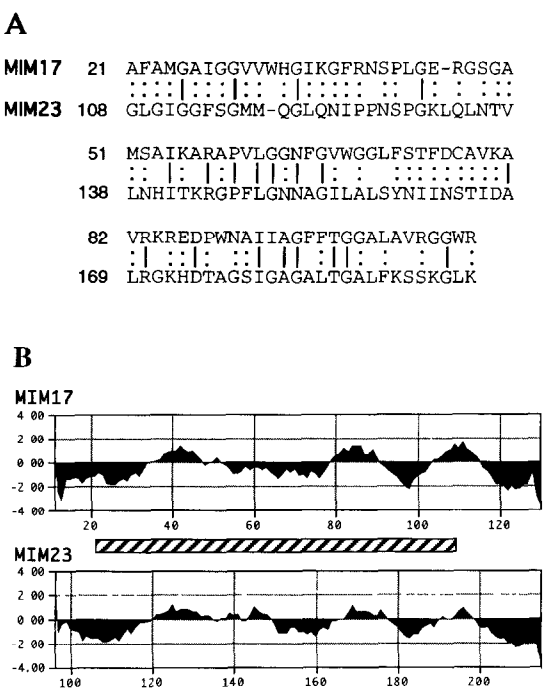

Fig. 2. MIM23 and MIM17 share a domain of homology. (A) Comparison of the amino acid sequences of MIM23 and MIM17. Identical residues are represented by vertical lines, similarities according to the FASTA comparison [27] by double dots. (B) Hydrophilicity plots of MIM23 and MIM17 were created using the algorithm of Kyte and Doolittle [32] with a window size of 12. Hydrophobic segments have negative values. The homologous region between the two proteins is represented by the hatched bar

of 222 amino acid residues and 23.2 kDa is predicted. The low codon bias index of *MPI3* (0.118) [26] suggests that, like the previously identified *MPI1* gene [12], the in vivo expression of *MPI3* is low. Since we show below that the gene product is a mitochondrial inner membrane protein (as is the case with the *MPI1* and *MPI2* gene products), it was termed MIM23. The amino-terminal half of MIM23 has an overall hydrophilic character, while the carboxy-terminal half contains four hydrophobic segments (underlined in Fig. 1). At least two of the hydrophobic sequences (amino acid residues 97–119 and 145–166) would be of sufficient length to function as membrane anchor sequences. This suggests that MIM23 may be an integral membrane protein. The amino-terminal sequence of MIM23 contains more acidic than basic residues and thus does not exhibit the typical characteristics of the positively charged mitochondrial presequences.

A computer search did not reveal a significant homology of MIM23 to any known protein sequence with the exception of MIM17. A FASTA comparison [27] between the amino acid residues 108–196 of MIM23 and residues 21–109 of MIM17 revealed a similarity of 77.5% (22.5% identical residues) (Fig. 2A). The statistical significance of the alignment was tested by the use of the programme RDF2 (ktup = 1; 200 shuffles) leading to an optimized score of 10.81 S.D. above mean, which is significant by the criteria suggested by Pearson and Lipman [27]. Furthermore, the hydrophilicity profiles of both proteins are similar to each other in this region (Fig. 2B). This domain contains the potential membrane spanning sequences of both MIM23 and MIM17.

To analyze the biogenesis and submitochondrial location of MIM23, the nucleotide region coding for MIM23 was cloned into the vector pGEM4. By in vitro transcription and translation, the precursor of MIM23 was synthesized in rabbit reticulocyte lysate in the presence of [^{35}S]methionine (Fig. 3A, lane 1). The precursor with a size of 23 kDa was incubated with isolated energized yeast mitochondria at 25°C and then the mitochondria were reisolated. The precursor of MIM23 efficiently associated with the mitochondria and was thereby protected against digestion by proteinase K or trypsin even at high concentrations (Fig. 3A, lanes 4 and 6). The precursor in the reticulocyte lysate was digested at low concentrations of protease (Fig. 3A, lanes 2 and 3) as was the imported protein upon lysis of the mitochondria with detergent (Fig. 3A, lane 8), indicating that the protease-resistant MIM23 was protected by the mitochondrial membranes. The imported MIM23 had the same apparent size as the precursor. Thus MIM23 is apparently not proteolytically processed upon import into mitochondria in agreement with the primary sequence which does not show the presence of a typical mitochondrial presequence. In lanes 5 and 7 of Fig. 3A, the membrane potential $\Delta\Psi$ across the mitochondrial inner membrane was dissipated by the addition of the potassium ionophore valinomycin. Thereby, the import of MIM23 was strongly inhibited, demonstrating that the import depended on the presence of $\Delta\Psi$.

To analyze if MIM23 is a mitochondrial membrane protein, mitochondria containing imported MIM23 were treated with sodium carbonate at pH 11.5. At pH 11.5 soluble proteins and peripheral membrane proteins are extracted and integral membrane proteins remain in the membrane sheets [23,24]. MIM23 remained largely in the membrane pellet (Fig. 3B, column 1) as was the

Table I

Classification of yeast mitochondrial protein import mutants found in a mislocalization approach

Group	I	II	III	IV
Mutant	MB3-17 MB3-20 MB3-27 MB3-36 MB3-43	MB3- 4 MB3-42 MB3-52 MB3-68 MB3-75	MB3-31 MB3-33 MB3-56 MB3-81	MB3-45 MB3-46
Gene	*SSC1*	*MPI1*	*MPI2*	*MPI3*
Protein	Mt-hsp70	MIM44	MIM17	MIM23

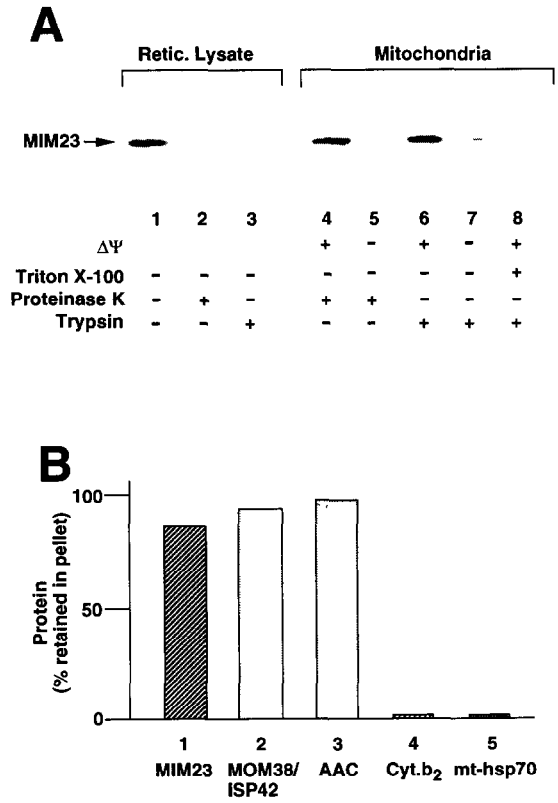

Fig. 3. MIM23 is an integral protein of the mitochondrial inner membrane. (A) The precursor of MIM23 is synthesized without a presequence and its import into mitochondria requires a membrane potential $\Delta\Psi$. Reticulocyte lysate containing the ^{35}S-labeled precursor of MIM23 (0.5 μl; lane 1) was treated with proteinase K (25 μg/ml; lane 2) or trypsin (25 μg/ml; lane 3) under import buffer conditions [22]. Isolated *S. cerevisiae* wild-type mitochondria (25 μg protein) were incubated with the reticulocyte lysate (2 μl per lane) in the presence of a membrane potential $\Delta\Psi$ (lanes 4, 6 and 8) or in the absence of $\Delta\Psi$ (addition of 0.5 μM valinomycin and 20 μM oligomycin; lanes 5 and 7) for 30 min at 25°C. Then the samples were made chemically identical by addition of valinomycin and oligomycin to the samples 4, 6 and 8. The mitochondria (samples 4–8) were reisolated. Sample 8 received 1% Triton X-100. Samples 4 and 5 were treated with proteinase K (200 μg/ml) and samples 6–8 with trypsin (300 μg/ml for samples 6 and 7; 100 μg/ml for sample 8). The samples were analyzed by SDS-PAGE and fluorography. (B) Imported MIM23 is not extracted from the membranes at alkaline pH. MIM23 was imported into isolated yeast mitochondria as described for sample 4 of A. The mitochondria were reisolated and incubated in 100 mM Na_2CO_3. Pellet (membranes) and supernatant were separated and analyzed by SDS-PAGE, fluorography, immunodecoration with antibodies directed against MOM38/ISP42, ADP/ATP carrier (AAC), cytochrome b_2 (Cyt. b_2), mt-hsp70, and laser densitometry. The total amount of protein in pellet plus supernatant was set to 100%

case with the integral outer membrane protein MOM38/ISP42 and the integral inner membrane protein ADP/ATP carrier (Fig. 3B, columns 2 and 3). Soluble proteins, such as cytochrome b_2 of the intermembrane space and mt-hsp70, were completely extracted (Fig. 3B, columns 4 and 5). This indicates that MIM23 imported into mitochondria behaves as an integral membrane protein. The import of mitochondrial outer membrane proteins is independent of the membrane potential $\Delta\Psi$, while the import of inner membrane proteins strictly depends on $\Delta\Psi$ [4,5,28]. We thus conclude that MIM23 is an integral protein of the mitochondrial inner membrane.

In summary, we found that MIM23 is synthesized in the cytosol without a cleavable presequence and imported into the mitochondrial inner membrane in a $\Delta\Psi$-dependent manner. MIM23 contains several hydrophobic segments that can mediate its anchoring in the inner membrane. Since the amino-terminal region of MIM23 does not show the typical properties of mitochondrial targeting sequences (i.e. prevalence of positive charges and nearly complete absence of negative charges), the mechanism of targeting of MIM23 into the inner membrane is unusual. However, this is not unprecedented. Members of the inner membrane family of metabolite carriers, such as the ADP/ATP carrier and the uncoupling protein, are usually synthesized without a presequence and appear to contain their targeting sequences within mature portions that are not directly located at the amino-terminus [29–31]. Two results suggest that MIM23 may represent another component of the mitochondrial protein import machinery. (i) The *mpi3* mutant represents one complementation group of the genetic screening for mutants in mitochondrial protein import. The other three complementation groups obtained in the screening were all shown to represent mutants of essential components of the protein import machinery of the inner membrane (mt-hsp70, MIM44 and MIM17). (ii) MIM23 has a domain with significant similarity to a domain in MIM17. It is tempting to speculate that the integral membrane proteins MIM23 and MIM17 participate in the formation of a specific channel for the translocation of preproteins across the inner membrane, whereas the peripheral membrane protein MIM44 and the soluble protein mt-hsp70 are required in the initiation and completion of membrane translocation of preproteins.

Acknowledgements We thank Dr. B. Guiard for the antiserum against cytochrome b_2 and H. Müller for expert technical assistance. This study was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie.

REFERENCES

[1] Murakami, H., Pain, D. and Blobel, G. (1988) J. Cell Biol. 107, 2051–2057.
[2] Murakami, K. and Mori, M. (1990) EMBO J. 9, 3201–3208.
[3] Hachiya, N., Alam, R., Sakasegawa, Y., Sakaguchi, M., Mihara, K. and Omura, T. (1993) EMBO J. 12, 1579–1586.
[4] Pfanner, N., Söllner, T. and Neupert, W. (1991) Trends Biochem. Sci. 16, 63–67
[5] Baker, K.P. and Schatz, G. (1991) Nature 349, 205–208.
[6] Söllner, T., Rassow, J., Wiedmann, M., Schlossmann, J., Keil, P., Neupert, W. and Pfanner, N. (1992) Nature 355, 84–87.
[7] Kang, P.J., Ostermann, J., Shilling, J., Neupert, W., Craig, E.A. and Pfanner, N. (1990) Nature 348, 137–143.

[8] Cheng, M.Y., Hartl, F.-U., Martin, J., Pollock, R.A., Kalousek, F., Neupert, W., Hallberg, E.M., Hallberg, R.L. and Horwich, A.L. (1989) Nature 337, 620–625.
[9] Ostermann, J., Horwich, A.L., Neupert, W. and Hartl, F.-U. (1989) Nature 341, 125–130.
[10] Arretz, M., Schneider, H., Wienhues, U. and Neupert, W. (1991) Biomed. Biochim. Acta 50, 403–412.
[11] Braun, H.-P., Emmermann, M., Kruft, V. and Schmitz, U.K. (1992) EMBO J. 11, 3219–3227.
[12] Maarse, A.C., Blom, J., Grivell, L.A. and Meijer, M. (1992) EMBO J. 11, 3619–3628.
[13] Blom, J., Kübrich, M., Rassow, J., Voos, W., Dekker, P.J.T., Maarse, A.C., Meijer, M. and Pfanner, N. (1993) Mol. Cell. Biol., in press.
[14] Scherer, P.E., Manning-Krieg, U.C., Jenö, P., Schatz, G. and Horst, M. (1992) Proc. Natl. Acad. Sci. USA 89, 11930–11934.
[15] Craig, E.A., Kramer, J. and Kosic-Smithers, J. (1987) Proc Natl. Acad. Sci. USA 84, 4156–4160.
[16] Craig, E.A., Kramer, J., Shilling, J., Werner-Washburne, M., Holmes, S., Kosic-Smithers, J. and Nicolet, C.M. (1989) Mol. Cell. Biol. 9, 3000–3008.
[17] Klebe, R.J., Harriss, J.V., Sharp, Z.D. and Douglas, M.G. (1983) Gene 25, 333–341.
[18] Nasmyth, K.A. and Tatchell, K. (1980) Cell 19, 753–764.
[19] Boeke, J.D., LaCroute, F. and Fink, G R. (1984) Mol. Gen. Genet. 197, 345–346.
[20] Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463–5467.
[21] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning, A Laboratory Manual, 2nd Edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[22] Söllner, T., Rassow, J. and Pfanner, N. (1991) Methods Cell Biol. 34, 345–358.
[23] Fujiki, Y., Hubbard, A L., Fowler, S. and Lazarow, P.B. (1982) J. Cell Biol. 93, 97–102.
[24] Pfanner, N., Hartl, F.U., Guiard, B. and Neupert, W. (1987) Eur. J. Biochem. 169, 289–293
[25] Gietz, R.D. and Sugino, A. (1988) Gene 74, 527–534.
[26] Sharp, P.M. and Li, W.-H. (1987) Nucleic Acids Res. 15, 1281–1295.
[27] Pearson, W.R. and Lipman, D.J. (1988) Proc. Natl. Acad. Sci. USA 85, 2444–2448.
[28] Hartl, F.-U., Pfanner, N., Nicholson, D.W. and Neupert, W. (1989) Biochim. Biophys. Acta 988, 1–45.
[29] Pfanner, N., Hoeben, P., Tropschug, M. and Neupert, W. (1987) J. Biol. Chem. 262, 14851–14854.
[30] Smagula, C.S. and Douglas, M.G. (1988) J. Cell. Biochem. 36, 323–327.
[31] Liu, X., Bell, A.W., Freeman, K.B. and Shore, G.C. (1988) J. Cell Biol. 107, 503–509.
[32] Kyte, J. and Doolittle, R.F. (1982) J. Mol. Biol. 157, 105–132.

NOTE ADDED IN PROOF

Emtage and Jensen (J. Cell Biol., in press) showed that the yeast mutant *mas6* is defective in mitochondrial protein import and identified the essential gene *MAS6*. We found that *MAS6* is identical to *MP13*. The results of Emtage and Jensen support the proposal that MIM23 is a component of the protein import machinery of the mitochondrial inner membrane.