A human homolog of the mitochondrial protein import receptor Mom19 can assemble with the yeast mitochondrial receptor complex

Naohiko Seki[a], Martin Moczko[b], Takahiro Nagase[a], Nicole Zufall[b], Bruno Ehmann[c], Klaus Dietmeier[b], Eberhard Schäfer[c], Nobuo Nomura[a], Nikolaus Pfanner[b,*]

[a]*Kazusa DNA Research Institute, Chiba 292, Japan*
[b]*Biochemisches Institut, Universität Freiburg, Hermann-Herder-Straße 7, D-79104 Freiburg, Germany*
[c]*Institut für Biologie II, Universität Freiburg, D-79104 Freiburg, Germany*

Received 13 October 1995

Abstract Import of preproteins into mitochondria requires transport machineries in both mitochondrial membranes that have been characterized in *Saccharomyces cerevisiae* and *Neurospora crassa*. By cDNA analysis, we identified a human protein of 16 kDa with significant overall homology to the fungal mitochondrial import receptor Mom19, including the three typical characteristics: a hydrophobic N-terminal segment, a tetratrico peptide motif in the middle and a negatively charged C-terminus. The human Mom19 homolog is expressed in all tissues analyzed. When synthesized in vitro, the human Mom19 homolog is targeted to isolated yeast mitochondria and specifically associates with the outer membrane receptor complex, suggesting that indeed a mitochondrial import receptor was identified.

Key words: Mitochondrion; Human Mom19; Protein sorting; Import receptor

1. Introduction

The mitochondrial genome codes only for a small number of proteins. Over 95% of mitochondrial proteins are encoded by nuclear genes and synthesized on cytosolic polysomes. The mitochondrial machinery for import of preproteins has been mainly characterized with mitochondria from *S. cerevisiae* and *N. crassa* (summarized in [1]). This led to the identification of at least eight outer membrane proteins that form a dynamic protein complex, termed the mitochondrial receptor complex [2,3]. Four of them, termed Mom19 (Mas20), Mom22 (Mas22), Mas37 and Mom72 (Mas70), are involved in recognition of preproteins on the mitochondrial surface (import receptors). Other Mom proteins participate in formation of a general insertion pore with Mom38 (Isp42) as central component. The preprotein translocase of the mitochondrial inner membrane contains at least three essential proteins, Mim17 (Sms1), Mim23 Mas6) and Mim44 (Isp45), that cooperate with the heat shock protein Hsp70 of the mitochondrial matrix [4–7]. While some of the soluble components involved in mitochondrial protein import, such as cytosolic and mitochondrial matrix chaperones [8–10] and matrix processing enzymes [11] have been described for mammalian cells, none of the membrane components of the mitochondrial import machinery has been identified in mammalian cells so far.

By sequencing of human cDNAs [12] we identified an open reading frame coding for a protein with significant homology to Mom19 from *S. cerevisiae* and *N. crassa*. We report that the human protein shows the typical characteristics of this mitochondrial import receptor and is expressed in all tissues analyzed. When synthesized in rabbit reticulocyte lysates, the human Mom19 homolog was targeted to isolated *S. cerevisiae* mitochondria and assembled with the outer membrane receptor complex. This suggests that we have identified the first mammalian mitochondrial membrane protein of the preprotein import machinery.

2. Materials and methods

2.1. cDNA analysis and Northern hybridization

The human immature myeloid cell line KG-1 (ATCC CCL246) [13] was used as a source of mRNA to construct size-fractionated cDNA libraries, and the cDNA inserts were sonified as described [12]. Fragmented DNAs of 500–1,000 bp were cloned into the *Sma*I site of M13mp19, and the nucleotide sequence was determined by the dideoxy-method [14] using the ABI 373A sequencer and ABI sequence analysis system, INHERIT 670. Sequence data representing about 10 times the total length of the cDNA insert were recorded from which the entire sequence was reconstructed. Gaps were filled by dye-terminator sequencing using flanking sequences as primers. Computer analyses were carried out with the GCG software package [15] and the DNASIS system (Hitachi Co.).

For Northern analysis, the poly(A)$^+$ RNA (2 μg) of KG-1 was incubated in 1 M glyoxal, 50% DMSO and 10 mM sodium phosphate (pH 7.0) at 50°C for 60 min (20 μl final volume) [12]. The glyoxylated RNA was fractionated on 0.7% agarose gel and transferred to a Biodyne A filter (Pall Corp., USA) [16]. Human multiple tissue Northern blots were purchased from Clontech laboratories.

2.2. Synthesis of Mom19 in vitro and import into isolated yeast mitochondria

The coding region of hMOM19 was cloned into the *Sal*I-site of the pGEM4-Z vector. By in vitro transcription (SP6 RNA polymerase) and translation in rabbit reticulocyte lysate in the presence of [^{35}S]methionine and [^{35}S]cysteine [17], hMom19 was synthesized. Similarly, the coding region of *S. cerevisiae* (y) MOM19 [18] was cloned into pGEM4-Z, and yMom19 was synthesized and rabiolabeled in rabbit reticulocyte lysate. Mitochondria were isolated [19] from the haploid *S. cerevisiae* strain YPH500 (*MATα, ade2-101, his3-Δ200, leu2-Δ1, ura3-52, trp1-Δ63, lys2-801*). Mitochondria (150 μg mitochondrial protein) were incubated with reticulocyte lysate (10 μl) containing radiolabeled Mom19 in the presence of 2 mM ATP and 2 mM NADH in BSA-containing buffer [17] for 20 min at 25°C (final volume 200 μl). The mitochondria were reisolated by centrifugation and lysed in 0.5% digitonin, 250 mM sucrose, 100 mM NaCl, 3% BSA, 1 mM EDTA, 10 mM MOPS (pH 7.2), followed by immunoprecipitation with protein A-Sepharose and antibodies directed against yeast mitochondrial outer membrane

*Corresponding author. Fax (49) (761) 203-5261.

Abbreviations: Mom19, mitochondrial outer membrane protein 19; *N. crassa, Neurospora crassa*; *S. cerevisiae, Saccharomyces cerevisiae*; TPR, tetratrico peptide repeat.

SSDI 0014-5793(95)01229-X

proteins or preimmune serum under non-denaturing conditions as described [20,21]. The samples were analyzed by SDS-PAGE and autoradiography using the storage phosphor imaging system (Molecular Dynamics).

3. Results and discussion

We established a strategy for the rapid determination of the sequences of full-length cDNAs. By applying the protocol, cDNA clones with inserts longer than 2 kb were isolated from a cDNA library of human immature myeloid cell line KG-1, and the sequences of more than 200 cDNAs were determined [12,22–24]. During the course of this work, we identified a cDNA with an open reading frame encoding a putative protein of 145 amino acids (16.3 kDa) (Fig. 1). The predicted protein shows 49% similarity, including 29% identical amino acid residues, to *S. cerevisiae* Mom19 and 60% similarity, including 29%

```
   1  GGCCGTCGGGTGTGAGCTGCGCCGACCGCTCTGAGGGTTCGTGGCCCACCGCTCCTTCGC
  61  GGTCCCTGCCGCCACCGTCCACGCTCAGCGTTGTAGAGAAGATGGTGGGTCGGAACAGCG
                                          M  V  G  R  N  S  A
 121  CCATCGCCGCCGGTGTATGCGGGGCCCTTTTCATTGGGTACTGCATCTACTTCGACCGCA
        I  A  A  G  V  C  G  A  L  F  I  G  Y  C  I  Y  F  D  R  K
 181  AAAGACGAAGTGACCCCAACTTCAAGAACAGGCTTCGAGAACGAAGAAAGAAACAGAAGC
        R  R  S  D  P  N  F  K  N  R  L  R  E  R  R  K  K  Q  K  L
 241  TTGCCAAGGAGAGAGCTGGGCTTTCCAAGTTACCTGACCTTAAAGATGCTGAAGCTGTTC
        A  K  E  R  A  G  L  S  K  L  P  D  L  K  D  A  E  A  V  Q
 301  AGAAGTTCTTCCTTGAAGAAATACAGCTTGGTGAAGAGTTACTAGCTCAAGGTGAATATG
        K  F  F  L  E  E  I  Q  L  G  E  E  L  L  A  Q  G  E  Y  E
 361  AGAAGGGCGTAGACCATCTGACAAATGCAATTGCTGTGTGTGGACAGCCACAGCAGTTAC
        K  G  V  D  H  L  T  N  A  I  A  V  C  G  Q  P  Q  Q  L  L
 421  TGCAGGTCTTACAGCAAACTCTTCCACCACCAGTGTTCCAGATGCTTCTGACTAAGCTCC
        Q  V  L  Q  Q  T  L  P  P  P  V  F  Q  M  L  L  T  K  L  P
 481  CAACAATTAGTCAGAGAATTGTAAGTGCTCAGAGCTTGGCTGAAGATGATGTGGAATGAG
        T  I  S  Q  R  I  V  S  A  Q  S  L  A  E  D  D  V  E  *
 541  AAACAAATGTCAACATAATAAAATCTCAGTTAAAAATATTTTAAAAATTCTTGGTAGTTG
 601  AGCAGCTCTGGGGGAATAAGGGCAAATATGCTTGTTATGAACTACACTGAAATCTACCAA
 661  AGTTAATGTTTACTTTGTGTAGATCCATTTGTCTATTTATTTATTTTTCCCAGTGAAAA
 721  GTGTATTTTGATAGAGAACTTTTCATTCTATAAATACACTATGAGTTACTAAAATATCAT
 781  GGATTTTGTTTATTCCTGAAACATAGTTACATAGTTAAACTGTACATATGACATGGCTTA
 841  TGTTAAAAATACCCAGTGCTCAGTTTTGAAAGATAGGCAAAAAAAAAAAAAGTATAGGAG
 901  AAACTGAAGAATGTACACTTTTTTAGAGGGCACATTTTGCTGTAAATCTGGAAATTTGAT
 961  AGACTTGACTGTGTTTGTGAAAACTGAGCATTAAAGGTTTTGATTGATCCTTTCTTTCCA
1021  TTTAATCTCTGAGACGTAAATATGTGAGGTGTGCTGCTGTGCTGGGTTAACAGCTTCCTT
1081  CCCTTTCTGTGTAGCAGTCTTGAAATGTTCTGTTTAAATCAGTAGGCTTAATGTGTTCTG
1141  GGTATTTATCTCCTTGTATTTTAAATATATGTAGTTGCAAATAGCACCAGGAATTAGATT
1201  TCTGTACACCCCTAATCTAGCCTTGTGAGCTTCGCTAGTTAATGTGTGCTCACTTTCCCT
1261  CCATTTGTTACGTGAGAGAATGCGTCTGCTGATCACTGAAGTGTCCCTTTTAGCTTCTGA
1321  TTCATTGGGTTCTGTTGGGCATCTTTAAATCCACCTTAACCTGAGGAATGTATGTGGGCA
1381  ACCAGGCCCTGCATTTTTTTATATTCTGAATTTTGCATGCTTGCCTGACTTAGTATTTCT
1441  GAATTGATGTTTTTTTTAATGGTATAACTATCTTGATTTTCACTGAAATTATATGGTTCT
1501  GTCACTACTCTGTAAATTAATCCGAAACTTTTAAGGTAACTGGGATGATCTGCTTGTAAA
1561  AATGCTTGTTGCCTTTTGCTTTATCTTCAGTGTACCTCCTTAATCCTGCTTCAACTTGAT
1621  TATCTTGTGAAACGATGAGAGTAAGTTGCAACCTTGTGACTGAAAACTTGAAAAGAGTGG
1681  AGCAGGTGGGACCTCTTATTCTCAAATAGTGACATATTCTCCGTAGTCACAGTTTCAGAA
1741  CTGAGTAAGGATCCTTGGTACTTGGTGGCATCTGTTGAACTGAGGAGCATTTCTCATTGT
1801  AAAGATTGCCTTTGTTCTGTCTAAAAGTCTGGAGAAATCCCAAAGACTTTTCCTATGTAC
1861  TAGGCATTTTATTTTGATTGACTTACAAACTCTTCTTAATCATTATCAATCTCGGTTTTT
1921  TTGTGGTGCAGTGGAAGGAGAAATAGGTCTAGTTTCTGCCTCTGATTAGCCGCACAGCCT
1981  TGAACAAATCACATTTCATCTTTGAACTTACCTCTACTGTTAGACTAGGCGACTCACATT
2041  TGAGGACTTTTCTCGGGTATCTTGAGGGTTTGTGATCCTGAACCCTTAAACAGTGCTTTT
2101  TTGTTACACAGGAGGGCTTTTTTGGGGGGATGACCAGTACAGACATGCCAGTTAGTTTTA
2161  CTAGTGGGATCCCAAATCCAAAGCAGTGTAGTGGTGATTGGTCAGTGACTAACCAGGCAG
2221  CTAAGAAGTCTTAGGCAGCAGCCCAGACATGTATAGAGGGGCAGTTAGAGGGAGAACAGG
2281  GGTGGGAAAGGGAGCAAGGGGCAGATAGCTCAGCAAGGAAAGAATGGGCTCAGAAAAAGG
2341  AGGGCTGGCTGGAGGAGTGAGGGGCAGCTTAAGTTTGGGGAGGGTAGAAACGCCGTTTCC
2401  TTGGGAACTGGAGTGCAGTATGAGCTGGGTGTCACTTGGCTCTGAACATACTGGCTTTGC
2461  TGTAATGCTTGAAAAGGCGTTGGTATCTTCATTTTACAGTTCATTAACCCAAGTACGTTT
2521  TCTTATTTAAATGACAACTTTGGTGCTTTAAAATGAGGTACCACTTTTTAAAGCTAGCTG
2581  TGTCGAGTTAAAGAAAAAATCAGCAGTTTTTTCTCCCAGAAATGTAATTGCCAAACACTT
2641  TTCATCCCCATCTTAAGTTTTACAAGGTGATGTAATCAGCTTGTTGTAGTGATGCTGGCC
2701  AAATGGTGCTCAGCAGGTGAGAACAAAAAAACCCCAGATTTCAGTGAACTAATACACAGC
2761  TTGAGCGTTTCCATGTGCTAATGTTGCACACTTACTAAAAAACTTTGGAAATGGAAAATA
2821  ATGTATTAGTGCAACAGTTGATGTGCTTCTTTGGGCAAAGATATAGTTTTGTTCCACAAT
2881  TTGTACTTAAAAGCGAAAGAACATTGAAAACATAGACTTACTGGCTGTAGCAATGCTGGC
2941  CTGTTAACTGATAACTAGAACTTAGGTTCACGTTTATGTAAAGTGTGTAAAACCTAGTAG
3001  AGCTTGCATAGTCGGCACTCAGTAAATGTTTGGTTCCTTTTGCCCCTTGGTAAGTTTATT
3061  TTACCATCCTCCCACCTGCCATTCTGACTTTATTAAATCAACATGTGGACCAGAGTGTTA
3121  ATGAGATGTTATTGCAGAAGAGATTGAGAAAATTGGTATATCATGCAGATAACATACAAA
3181  ATCTTTTTGTAACGTAAAAAATGCAGTTTTATTATTGCTTGTGCCTCAACTGTTTAAGTG
3241  AATATTAAAGGGCTTGGAG  3259
```

Fig. 1. Nucleotide sequence of cDNA KIAA0016 and derived amino acid sequence of hMom19. The amino acids are given in the one letter code. The accession number for the sequence reported here is D13641 in the EMBL/GenBank/DDBJ databanks.

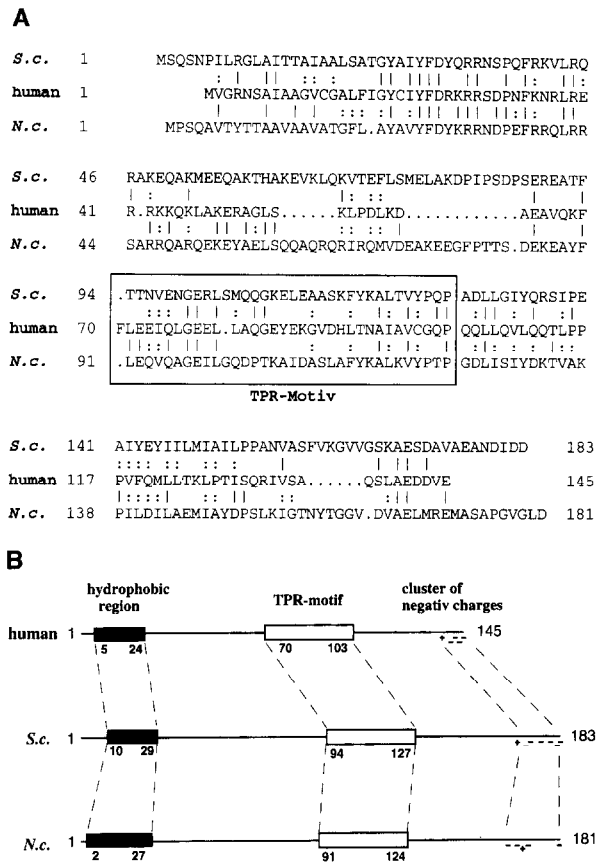

Fig. 2. Sequence similarities and motifs of Mom19 from human, *S. cerevisiae* and *N. crassa*. (A) Comparison of the predicted primary sequences of *S. cerevisiae* (*S.c.*), human and *N. crassa* (*N.c.*) Mom19. Identical residues are indicated by vertical lines. Similar residues are indicated by double dots. The TPR-motif is boxed. (B) Characteristic segments of Mom19. The charged residues are given for the C-terminal 20 amino acids of each Mom19 protein. Numbers indicate the beginning and end of a segment.

identical amino acid residues, to *N. crassa* Mom19 (Fig. 2A). The degree of similarity of the human sequence to the fungal sequences is remarkable since the similarity between *S. cerevisiae* and *N. crassa* Mom19 is 56%, including 34% identical amino acid residues [18,25].

By analysis of the fungal Mom19 we previously suggested three characteristics of this mitochondrial protein import receptor [18]: (i) a hydrophobic segment at the N-terminus with sufficient length to function as membrane anchor; (ii) a tetratrico peptide motif (TPR-motif) in the middle of the protein; and (iii) a C-terminal segment with a net negative charge. All three motifs are present in the predicted human Mom19 homolog (now referred to as hMom19) (Fig. 2B). TPR-motifs are loosely conserved segments of 34 amino acids with a predicted amphipathic α-helical structure and are thought to be involved in dynamic protein–protein interactions [26]. Interestingly, Mom72 contains several TPR motifs [27,28] and thus is a possible binding partner of Mom19. The C-terminal 20 amino acid residues of all three Mom19 proteins contain 4–6 negatively charged residues and only one positively charged residue (Fig. 2B). This segment may be involved in the interaction of Mom19 with the positively charged targeting sequences (presequences) of mitochondrial preproteins.

Using PCR in panels of human–rodent hybrids, we determined the location of the *hMOM19* gene to chromosome 1 [12]. By fluorescence in situ hybridization (FISH) combined with detection of replication R-bands, *hMOM19* was found to be localized at 1q42 (N. Seki, K. Ishikawa, T. Nagase, N. Nomura, unpublished). During the course of the mapping studies we noticed the presence of at least one sequence-related pseudogene of *hMOM19*.

In case hMom19 functions as a protein import receptor of human mitochondria, one would expect an expression in practically all tissues. We therefore analyzed mRNA expression by Northern hybridization [12] and found that the *hMOM19* gene was expressed ubiquitously in all the 16 tissues studied, including heart, brain, liver and so on (Fig. 3A). The expression of β-actin mRNA was used as a control (Fig. 3B). In addition to the major transcript of 3.6 kb that corresponds to the cDNA of *hMOM19* shown in Fig. 1, three small transcripts of 1.3, 1.0 and 0.8 kb were observed (Fig. 3A). A short cDNA which was terminated at position 578 in Fig. 1 should be derived from one of these small mRNAs.

The results obtained so far raised the possibility that hMom19 represents a component of the outer membrane import machinery of mammalian mitochondria. Since no other component of the preprotein import apparatus of mammalian mitochondrial membranes is known, we asked if hMom19 could assemble with the well-characterized receptor complex of *N. crassa* mitochondria [20,29,30]. The coding region of hMom19 was cloned into the pGEM4-Z vector. By transcription/translation, hMom19 was synthesized and ^{35}S-labeled in rabbit reticulocyte lysate. The apparent molecular mass of the protein was 16 kDa as expected from the primary sequence. After addition of isolated *S. cerevisiae* mitochondria, hMom19 associated with the mitochondria (Fig. 4, lane 2). As control we used the authentic precursor of yeast Mom19 (Fig. 4, lane 1). The mitochondria were reisolated and lysed with digitonin under conditions that do not dissociate the outer membrane receptor complex [20,31]. With antibodies directed against Mom38 or Mom22, the complex was then co-precipitated under non-denaturing conditions [20,21,30], evidenced by the co-precipitation of in vitro imported yeast Mom19 (Fig. 4, lanes 3 and 7). The specificity of the co-precipitation was demonstrated by the lack of precipitation of yeast Mom19 with antibodies from preimmune serum (Fig. 4, lane 5) or antibodies directed against the most abundant outer membrane protein porin (Fig. 4, lane 9). hMom19 was co-precipitated by both anti-Mom38 antibodies and anti-Mom22 antibodies (Fig. 4, lanes 4 and 8), but not by preimmune antibodies or anti-porin antibodies (Fig. 4, lanes 6 and 10). This demonstrates that hMom19 is specifically associated with the yeast mitochondrial receptor complex.

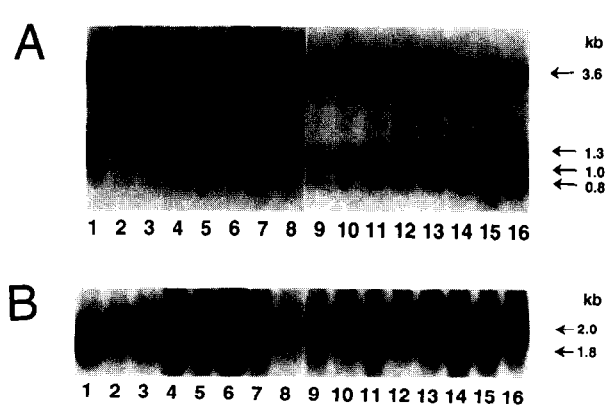

Fig. 3. mRNA expression patterns of (A) *hMOM19* and (B) β-actin. cDNA fragments were randomly labeled, and Northern hybridization was performed as described in section 2. Lanes 1 = heart; 2 = brain; 3 = placenta; 4 = lung; 5 = liver; 6 = skeletal muscle; 7 = kidney; 8 = pancreas; 9 = spleen; 10 = thymus; 11 = prostate; 12 = testis; 13 = ovary; 14 = small intestine; 15 = colon; 16 = peripheral blood leukocyte.

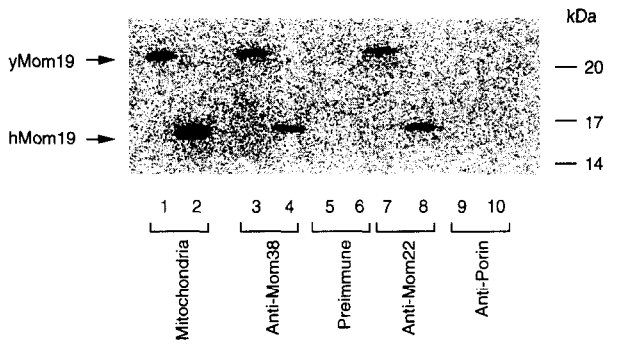

Fig. 4. Targeting of hMom19 to the yeast mitochondrial outer membrane. Reticulocyte lysate containing ^{35}S-labeled *S. cerevisiae* Mom19 (yMom19, samples 1, 3, 5, 7 and 9) or ^{35}S-labeled human Mom19 (hMom19, samples 2, 4, 6, 8 and 10) was incubated with isolated *S. cerevisiae* mitochondria for 20 min at 25°C as described in section 2. The mitochondria were reisolated, washed, and either dissociated in sample buffer (samples 1 and 2) or lysed in digitonin-containing buffer, followed by immunoprecipitation with the indicated antibodies (samples 3–10). The immunoprecipitates were dissolved in sample buffer. Analysis was by SDS-PAGE and digital autoradiography. Samples 1 and 2 represent one-ninth of the import reaction used for samples 3–10, respectively.

In conclusion, hMom19 shows significant sequence homology to the mitochondrial protein import receptor Mom19 from *S. cerevisiae* and *N. crassa*, including the three typical characteristics of Mom19 (N-terminal hydrophobic segment, TPR-motif in the middle, C-terminal negatively charged segment). hMom19 is expressed in probably all tissues. It can be targeted to yeast mitochondria and specifically associates with the outer membrane receptor complex. A databank search revealed an unpublished rat sequence (S.D. Nuttall, J. Sinding, B. Hanson and N.J. Hoogenraad, submitted to EMBL/GenBank with accession number U21871) with striking similarity to hMom19, only three amino acids of 145 residues were different. We suggest that both hMom19 and the highly homologous rat protein are the first mammalian components of the protein import machinery of the mitochondrial membranes. These findings open a broad new area of research on mitochondrial protein import, in particular the search for and characterization of other components of an outer membrane receptor complex and the analysis of a potential involvement of the protein import machinery in human diseases with defects of mitochondrial function [32].

Acknowledgements: We are grateful to Dr. A. Schapira and Dr. M. Takanami for discussion. This work was supported by the Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 388) and the Fonds der Chemischen Industrie.

References

[1] Kübrich, M., Dietmeier, K. and Pfanner, N. (1995) Curr. Genet. 27, 393–403.
[2] Pfanner, N., Söllner, T. and Neupert, W. (1991). Trends Biochem. Sci. 16, 63–67.
[3] Lithgow, T., Glick, B.S. and Schatz, G. (1995) Trends Biochem. Sci. 20, 98–101.
[4] Kronidou, N.G., Oppliger, W., Bolliger, L., Hannavy, K., Glick, B.S., Schatz, G. and Horst, M. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 12818–12822.
[5] Rassow, J., Maarse, A.C., Krainer, E., Kübrich, M., Müller, H., Meijer, M., Craig, E.A. and Pfanner, N. (1994) J. Cell Biol. 127, 1547–1556.
[6] Schneider, H.C., Berthold, J., Bauer, M.F., Dietmeier, K., Guiard, B., Brunner, M. and Neupert, W. (1994) Nature 371, 768–774.
[7] Pfanner, N., Craig, E. A. and Meijer, M. (1994) Trends Biochem. Sci. 19, 368–372.
[8] Beckmann, R.P., Mizzen, L.A. and Welch, W.J. (1990) Science 248, 850–854.
[9] Ryan, M.T., Hoogenraad, N.J. and Hoj, P.B. (1994) FEBS Lett. 337, 152–156.
[10] Hachiya, N., Mihara, K., Suda, K., Horst, M., Schatz, G. and Lithgow, T. (1995) Nature 376, 705–709.
[11] Kalousek, F., Neupert, W., Omura, T., Schatz, G. and Schmitz, U.K. (1993) Trends Biochem. Sci. 18, 249.
[12] Nomura, N., Miyajima, N., Sazuka, T., Tanaka, A., Kawarabayasi, Y., Sato, S., Nagase, T., Seki, N., Ishikawa, K. and Tabata, S. (1994) DNA Res. 1, 27–35.
[13] Koeffler, H.P. and Golde, D.W. (1978) Science 200, 1153–1154.
[14] Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463–5467.
[15] Devereux, J., Haeberli, P. and Smithies, O. (1984) Nucleic Acids Res. 12, 387–395.
[16] Thomas, P.S. (1983) Meth. Enzymol. 100, 255–266.
[17] Söllner, T., Rassow, J. and Pfanner, N. (1991) Meth. Cell Biol. 34, 345–358.
[18] Moczko, M., Ehmann, B., Gärtner, F., Hönlinger, A., Schäfer, E. and Pfanner, N. (1994) J. Biol. Chem. 269, 9045–9051.
[19] Daum, G., Böhni, P.C. and Schatz, G. (1982) J. Biol. Chem. 257, 13028–13033.
[20] Moczko, M., Dietmeier, K., Söllner, T., Segui, B., Steger, H.F., Neupert, W., and Pfanner, N. (1992) FEBS Lett. 310, 265–268.
[21] Alconada, A., Gärtner, F., Hönlinger, A., Kübrich, M. and Pfanner, N. (1995) Methods Enzymol. 260, 263–286.
[22] Nomura, N., Nagase, T., Miyajima, N., Sazuka, T., Tanaka, A., Sato, S., Seki, N., Kawarabayasi, Y., Ishikawa, K. and Tabata, S. (1994) DNA Res. 1, 223–229.
[23] Nagase, T., Miyajima, N., Tanaka, A., Sazuka, T., Seki, N., Sato, S., Tabata, S., Ishikawa, K., Kawarabayasi, Y., Kotani, H. and Nomura, N., (1995) DNA Res. 2, 37–43.
[24] Nagase, T., Seki, N., Tanaka, A., Ishikawa, K. and Nomura, N. (1995) DNA Res. 2, 167–174.
[25] Ramage, L., Junne, T., Hahne, K., Lithgow, T. and Schatz, G. (1993) EMBO J. 12, 4115–4123.
[26] Lamb, J.R., Tugendreich, S. and Hieter, P. (1995) Trends Biochem. Sci. 20, 257–259
[27] Hines, V., Brandt, A., Griffiths, G., Horstmann, H., Brütsch, H. and Schatz, G. (1990) EMBO J. 9, 3191–3200.
[28] Steger, H.F., Söllner, T., Kiebler, M., Dietmeier, K.A., Pfaller, R., Trülzsch, K.S., Tropschug, M., Neupert, W. and Pfanner, N. (1990) J. Cell Biol. 111, 2353–2363.
[29] Alconada, A., Kübrich, M., Moczko, M., Hönlinger, A. and Pfanner, N. (1995) Mol. Cell. Biol. 15 (in press).
[30] Hönlinger, A., Kübrich, M., Moczko, M., Gärtner, F., Mallet, L., Bussereau, F., Eckerskorn, C., Lottspeich, F., Dietmeier, K., Jacquet M. and Pfanner, N. (1995) Mol. Cell. Biol. 15, 3382–3389.
[31] Kiebler, M., Pfaller, R., Söllner, T., Griffiths, G., Horstmann, H., Pfanner, N. and Neupert, W. (1990) Nature 348, 610–616.
[32] Darley-Usmar, V. and Schapira, A.H.V. (eds.) (1994) Mitochondria: DNA, Proteins and Disease, Portland Press, London.