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Import of proteins into mitochondria: nucleotide sequence of the gene for a 70-kd protein of the yeast mitochondrial outer membrane

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The nucleotide sequence of the yeast chromosomal gene coding for the 70-kd protein of the mitochondrial outer membrane was determined. The deduced amino acid sequence of the protein agrees with the experimentally determined size and amino acid composition of the purified protein and correctly predicts the fragments obtained by cleaving the protein at its single tryptophan residue. The deduced NH_2-terminal sequence features an uninterrupted stretch of 28 uncharged amino acids flanked on both sides by basic amino acids. By sequencing a truncated version of the gene it was found that the corresponding polypeptide product lacks the 203 carboxy-terminal amino acids of the authentic 70-kd protein. As shown in the accompanying paper, this protein fragment still becomes attached to the mitochondrial outer membrane *in vivo*.

Key words: mitochondrial protein import/nucleotide sequence/mitochondrial outer membrane/addressing signals/membrane anchor

Introduction

To understand the molecular mechanisms governing the import of proteins into mitochondria, one must know the primary structure of the imported proteins. This is now most conveniently achieved by sequencing the nuclear genes or the mRNAs coding for imported mitochondrial proteins. During the past year, three such sequences have been published (Viebrock *et al.*, 1982; Faye and Simon, 1982; Kaput *et al.*, 1982); all these sequences were from proteins imported into internal mitochondrial compartments.

We have focussed our attention on the import of proteins into the mitochondrial outer membrane since this mitochondrial import route appears to be the simplest of those discovered so far: it requires neither an energized inner membrane nor proteolytic removal of transient NH_2-terminal extensions (Freitag *et al.*, 1982; Mihara *et al.*, 1982; Gasser and Schatz, 1983) and can be studied *in vitro* with purified outer membrane vesicles (Gasser and Schatz, 1983). So far, however, no amino acid sequence of a mitochondrial outer membrane protein has been reported.

We now describe the nucleotide sequence of the gene coding for the major 70-kd protein of the yeast mitochondrial outer membrane. The deduced amino acid sequence of the protein as well as previous data (Reizman *et al.*, 1983a, 1983b) indicate that the NH_2 terminus functions as a membrane anchor and suggest that it may also participate in targeting the protein to the mitochondrial outer membrane.

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Results

Nucleotide sequence of the gene coding for the 70-kd outer membrane protein

As described in the accompanying report (Riezman *et al.*, 1983b) the complete gene of the 70-kd protein was obtained as a 4.1-kb *Bam*HI fragment in the 'shuttle vector' YEp13. The nucleotide sequence of this fragment was determined mainly by random sequencing of smaller fragments prepared by sonication. In order to connect non-overlapping regions, several specific restriction fragments were also sequenced (Figure 1).

Two long continuous sequences (2630 and 1445 bp) could be determined, starting from each end of the 4.1-kb *Bam*HI fragment. The 2630-bp nucleotide sequence (Figure 2, upper panel) reveals an open reading frame of 1851 nucleotides corresponding to 617 amino acids or ~70-kd of protein. As discussed below, this sequence proved to be the whole gene for the 70-kd outer membrane protein. No other long open reading frame was found in the 4.1-kb *Bam*HI fragment.

A truncated copy of the gene was also sequenced. This copy had been obtained as a 2.5-kb *Hind*III fragment in the pFL-1 derivative B-26 (Riezman *et al.*, 1983b). The first 345 bp between the *Hind*III and *Bam*HI sites were derived from the pBR322 portion of the vector pFL-1 (Sutcliffe, 1979); the sequence downstream from the *Bam*HI site was completely identical with that of the whole gene up to nucleotide 1243 of the open reading frame (Figure 2, lower panel). From that point, the sequence was different: an open reading frame continued for 74 nucleotides and then terminated with a TAA stop codon. This interruption of the normal sequence coincides with a *Sau*3A site in the whole gene; since the clone bank had been prepared by partial digestion of yeast DNA with *Sau*3A, the truncated gene is almost certainly a cloning artefact. This is further supported by the finding that the 52-kd protein product of the truncated gene cannot be detected in wild-type yeast, whereas it is readily found in cells transformed with plasmids carrying the truncated gene (Riezman *et al.*, 1983b).

The DNA sequence agrees with the structure of the 70-kd protein

The following observations indicate that the cloned gene codes for the 70-kd outer membrane protein. (i) The deduced mol. wts. of the protein products (70 216 for the full gene, 50 098 for the fragment) agree well with the apparent mol. wts. determined by SDS-polyacrylamide gel electrophoresis (70 000 and 52 000, respectively). (ii) The deduced amino acid composition of the 70-kd protein is in good agreement with the composition determined by direct amino acid analysis (Figure 3). The only significant discrepancy is the higher content of glycine found by direct analysis; however, glycine is a commonly encountered contaminant, particularly if small amounts of a protein are recovered from SDS-polyacrylamide gels. (iii) The DNA sequence predicts a single tryptophan residue at position 124. Cleavage of the protein at this residue should thus yield two fragments of 14 and 56 kd, respectively. The actual experiment yields two fragments

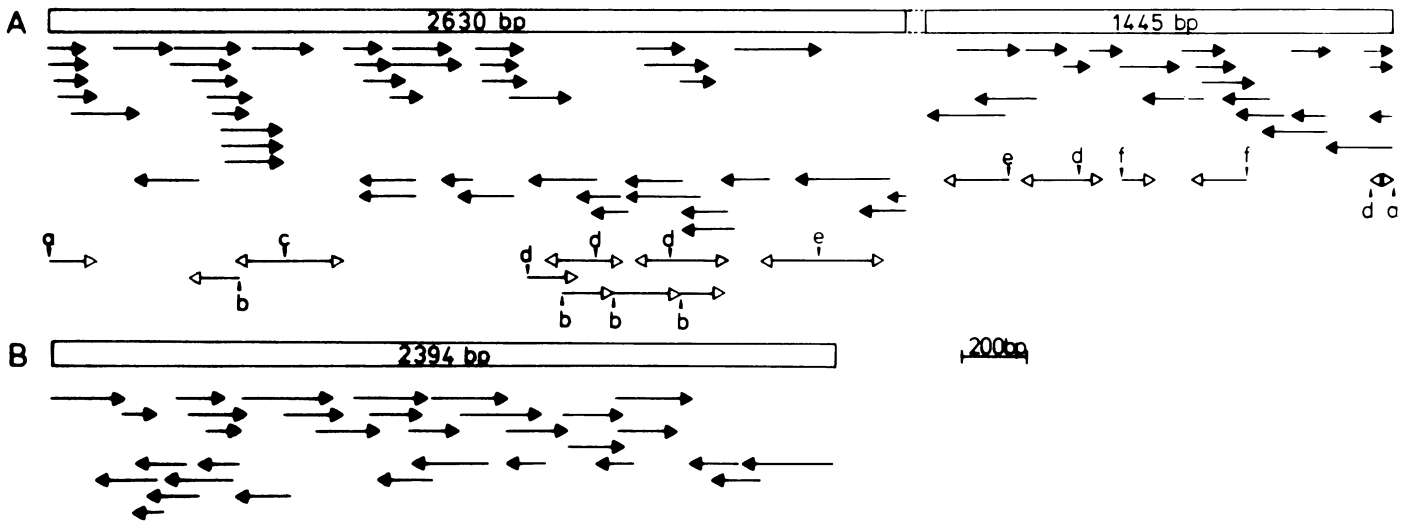


Fig. 1. Strategy for sequencing the whole and the truncated gene for the 70-kd outer membrane protein. **A**: 4.1-kb *Bam*HI fragment containing the whole gene. **B**: 2.5-kb *Hind*III fragment containing the truncated gene. The fragment has a *Bam*HI site at position 345; the 345-bp segment between the *Hind*III site and the *Bam*HI site is derived from the cloning vector pFL-1 (Sutcliffe, 1979). Solid and open arrows: direction and extent of sequences determined on fragments prepared by sonication and restriction endonucleases, respectively. a, *Bam*HI; b, *Sau*3A; c, *Bgl*I; d, *Hae*III; e, *Cla*I; f, *Taq*I.

whose apparent masses (as determined by SDS-polyacrylamide gel electrophoresis) are 14 and 58 kd (Figure 4). (The experiment also yields some uncleaved molecules of slightly lower mobility; this may reflect covalent modification of some of the 70-kd protein molecules by the cleaving reagent.)

The excellent agreement between the predicted and experimentally-determined size of the 14-kd cleavage product makes it very unlikely that translation starts at another ATG initiation codon: there is no other ATG codon between the first ATG of the open reading frame and the single tryptophan codon.

These data, together with the genetic experiments described in the accompanying paper (Riezman *et al.*, 1983b) leave little doubt that the DNA sequence shown in Figure 2A is that of the gene coding for the 70-kd outer membrane protein.

Discussion

The deduced sequence of 617 amino acids shown in Figure 2 is the first sequence of a mitochondrial outer membrane protein. Its most interesting feature is found near the NH_2 terminus: within the first 46 amino acids there is a continuous stretch of 28 uncharged amino acids (residues 10–37) flanked on both sides by a total of seven basic amino acids. Since this region lacks any acidic amino acids, it is very basic. This region displays many features found in other 'membrane anchoring' domains of viral- and plasmid-membrane proteins (Kreil, 1981) and may thus serve to anchor the 70-kd protein to the outer membrane. This is also supported by the observations that the membrane anchor of the 70-kd protein has a mol. wt. of 10 000 or less and is at the extreme NH_2 terminus (Riezman *et al.*, 1983a, 1983b and this work).

There are three additional, but shorter stretches of uncharged amino acids in other parts of the molecule (residues 65–80, 132–146 and 250–264). About 30% of the total residues are charged amino acids (46 Asp, 55 Glu, 68 Lys, 3 His and 21 Arg) which are distributed almost uniformly over the molecule (except for the NH_2-terminal region discussed above). The overall polarity index (53%) is within the normal range for soluble proteins (Capaldi and Vanderkooi, 1972). This agrees with the fact that most of the protein can be released as a soluble, 60-kd fragment upon light trypsin-treatment of the mitochondrial surface (Riezman *et al.*, 1983a).

Materials and methods

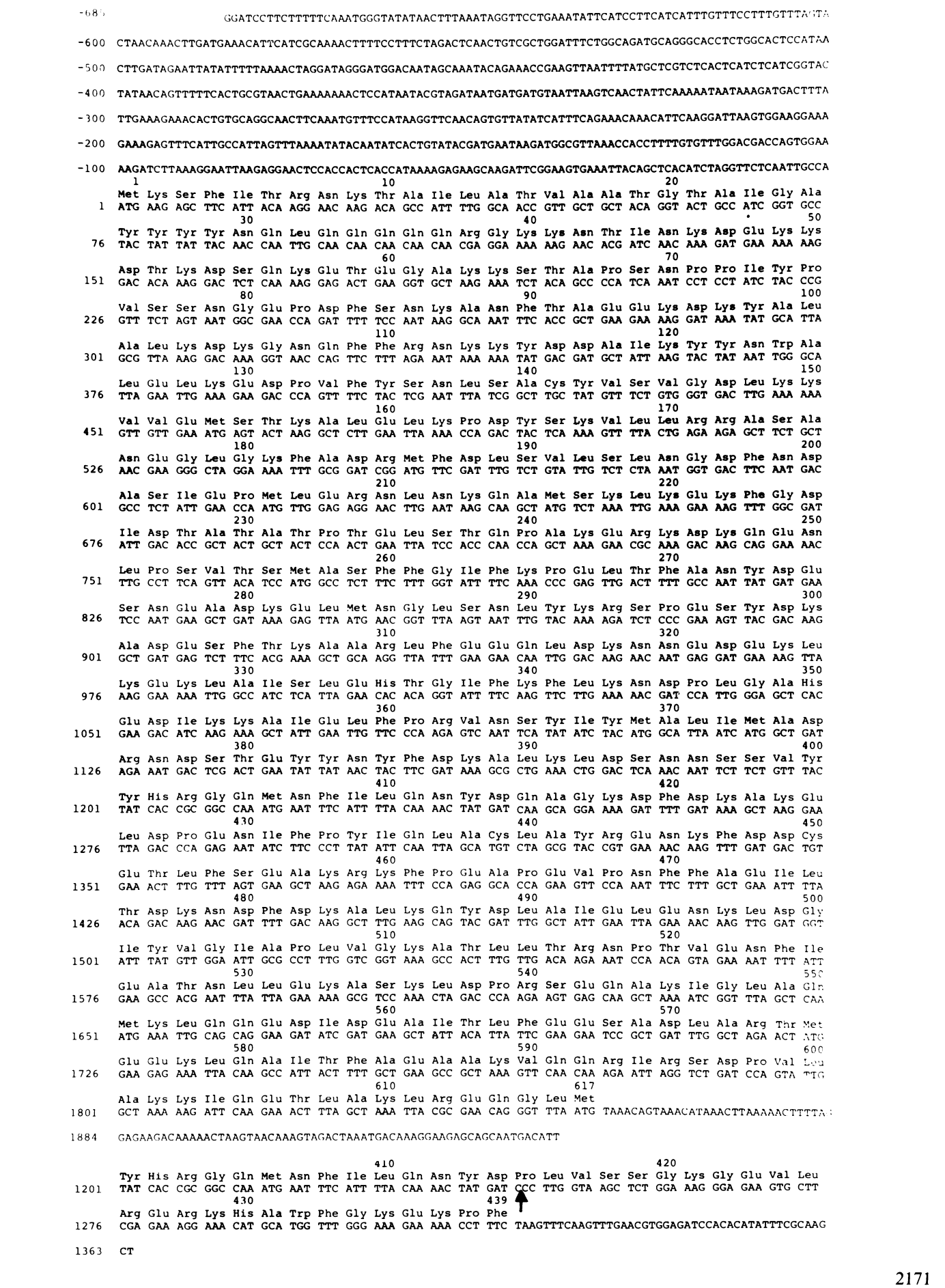
DNA sequencing

Sequencing was performed by the dideoxy method (Sanger *et al.*, 1977) using single-strand templates prepared by cloning in the M13 derivative mp8 (Sanger *et al.*, 1980; Messing *et al.*, 1981). The 4.1-kb *Bam*HI- and the 2.5-kb *Hind*III fragments (see Results) were converted to random 200–500 bp fragments by circularization, sonication and sizing on gels. The ends of the small fragments were filled in with T4 DNA polymerase and the blunt-ended fragments inserted into the *Sma*I site of M13 mp8 (Messing and Vieira, 1982). Specific DNA subfragments for the analysis of the 4.1-kb *Bam*HI fragment were also prepared by digestion with the restriction nucleases *Cla*I, *Bgl*I, *Hae*III, *Sau*3A, or *Taq*I.

Purification of the 70-kd protein

Mitochondria were prepared from wild-type cells of *Saccharomyces cerevisiae* strain D-273-10B (ATCC 25657) that had been grown on a semi-synthetic medium containing 2% lactate and 0.1% glucose (Daum *et al.*, 1982). Mitochondrial membranes were separated from soluble components by treating the mitochondria with 100 mM Na_2CO_3, pH 11.3 (Fujiki *et al.*, 1982). Almost all proteins, including the 70-kd protein, were then solubilized by suspending the membranes in 10 mM Tris-HCl, pH 8.0, 10 mM KCl, 2 mM dithiothreitol (DTT), 1 mM EDTA, 1 mM phenylmethyl sulfonyl fluoride and 0.3% Triton X-100. Solubilized proteins were fractionated on a DEAE-cellulose column (Whatman DE-52) developed with a linear gradient of NaCl (from 0 to 400 mM) in the same solution as above. The fractions containing the 70-kd protein were pooled, adsorbed onto a small DEAE-cellulose column and the column was washed with 50 mM CH_3COONH_4, 2 mM DTT and 8 M urea; the 70-kd protein was eluted with 250 mM NaCl in the buffer mentioned above, further purified by SDS-(10%) polyacrylamide gel electrophoreis and eluted from the gel electrophoretically. Detection of the 70-kd

Fig. 2. Nucleotide sequence of the gene for the 70-kd outer membrane protein. **Upper panel**: the complete gene with flanking regions. **Lower panel**: 3′ end region of the truncated gene. The site of rearrangement is indicated by the arrow. The sequence preceding the rearrangement is the same as that of the complete gene (see Figure 1 and Results). The deduced amino acid sequence is superimposed on the coding sequence.


-685 GGATCCTTCTTTTTCAAATGGGTATATAACTTTAAATAGGTTCCTGAAATATTCATCCTTCATCATTTGTTTCCTTTGTTTAGTA
-600 CTAACAAACTTGATGAAACATTCATCGCAAAACTTTTCCTTTCTAGACTCAACTGTCGCTGGATTTCTGGCAGATGCAGGGCACCTCTGGCACTCCATAA
-500 CTTGATAGAATTATATTTTTAAAACTAGGATAGGGATGGACAATAGCAAATACAGAAACCGAAGTTAATTTTATGCTCGTCTCACTCATCTCATCGGTAC
-400 TATAACAGTTTTTCACTGCGTAACTGAAAAAAACTCCATAATACGTAGATAATGATGATGTAATTAAGTCAACTATTCAAAAATAATAAAGATGACTTTA
-300 TTGAAAGAAACACTGTGCAGGCAACTTCAAATGTTTCCATAAGGTTCAACAGTGTTATATCATTTCAGAAACAAACATTCAAGGATTAAGTGGAAGGAAA
-200 GAAAGAGTTTCATTGCCATTAGTTTAAAATATACAATATCACTGTATACGATGAATAAGATGGCGTTAAACCACCTTTTGTGTTTGGACGACCAGTGGAA
-100 AAGATCTTAAAGGAATTAAGAGGAACTCCACCACTCACCATAAAAGAGAAGCAAGATTCGGAAGTGAAATTACAGCTCACATCTAGGTTCTCAATTGCCA
1 10 20
Met Lys Ser Phe Ile Thr Arg Asn Lys Thr Ala Ile Leu Ala Thr Val Ala Ala Thr Gly Thr Ala Ile Gly Ala
1 ATG AAG AGC TTC ATT ACA AGG AAC AAG ACA GCC ATT TTG GCA ACC GTT GCT GCT ACA GGT ACT GCC ATC GGT GCC
30 40 50
Tyr Tyr Tyr Tyr Asn Gln Leu Gln Gln Gln Gln Gln Arg Gly Lys Lys Asn Thr Ile Asn Lys Asp Glu Lys Lys
76 TAC TAT TAT TAC AAC CAA TTG CAA CAA CAA CAA CAA CGA GGA AAA AAG AAC ACG ATC AAC AAA GAT GAA AAA AAG
60 70
Asp Thr Lys Asp Ser Gln Lys Glu Thr Glu Gly Ala Lys Lys Ser Thr Ala Pro Ser Asn Pro Pro Ile Tyr Pro
151 GAC ACA AAG GAC TCT CAA AAG GAG ACT GAA GGT GCT AAG AAA TCT ACA GCC CCA TCA AAT CCT CCT ATC TAC CCG
80 90 100
Val Ser Ser Asn Gly Glu Pro Asp Phe Ser Asn Lys Ala Asn Phe Thr Ala Glu Glu Lys Asp Lys Tyr Ala Leu
226 GTT TCT AGT AAT GGC GAA CCA GAT TTT TCC AAT AAG GCA AAT TTC ACC GCT GAA GAA AAG GAT AAA TAT GCA TTA
110 120
Ala Leu Lys Asp Lys Gly Asn Gln Phe Phe Arg Asn Lys Lys Tyr Asp Asp Ala Ile Lys Tyr Tyr Asn Trp Ala
301 GCG TTA AAG GAC AAA GGT AAC CAG TTC TTT AGA AAT AAA AAA TAT GAC GAT GCT ATT AAG TAC TAT AAT TGG GCA
130 140 150
Leu Glu Leu Lys Glu Asp Pro Val Phe Tyr Ser Asn Leu Ser Ala Cys Tyr Val Ser Val Gly Asp Leu Lys Lys
376 TTA GAA TTG AAA GAA GAC CCA GTT TTC TAC TCG AAT TTA TCG GCT TGC TAT GTT TCT GTG GGT GAC TTG AAA AAA
160 170
Val Val Glu Met Ser Thr Lys Ala Leu Glu Leu Lys Pro Asp Tyr Ser Lys Val Leu Leu Arg Arg Ala Ser Ala
451 GTT GTT GAA ATG AGT ACT AAG GCT CTT GAA TTA AAA CCA GAC TAC TCA AAA GTT TTA CTG AGA AGA GCT TCT GCT
180 190 200
Asn Glu Gly Leu Gly Lys Phe Ala Asp Arg Met Phe Asp Leu Ser Val Leu Ser Leu Asn Gly Asp Phe Asn Asp
526 AAC GAA GGG CTA GGA AAA TTT GCG GAT CGG ATG TTC GAT TTG TCT GTA TTG TCT CTA AAT GGT GAC TTC AAT GAC
210 220
Ala Ser Ile Glu Pro Met Leu Glu Arg Asn Leu Asn Lys Gln Ala Met Ser Lys Leu Lys Glu Lys Phe Gly Asp
601 GCC TCT ATT GAA CCA ATG TTG GAG AGG AAC TTG AAT AAG CAA GCT ATG TCT AAA TTG AAA GAA AAG TTT GGC GAT
230 240 250
Ile Asp Thr Ala Thr Ala Thr Pro Thr Glu Leu Ser Thr Gln Pro Ala Lys Glu Arg Lys Asp Lys Gln Glu Asn
676 ATT GAC ACC GCT ACT GCT ACT CCA ACT GAA TTA TCC ACC CAA CCA GCT AAA GAA CGC AAA GAC AAG CAG GAA AAC
260 270
Leu Pro Ser Val Thr Ser Met Ala Ser Phe Phe Gly Ile Phe Lys Pro Glu Leu Thr Phe Ala Asn Tyr Asp Glu
751 TTG CCT TCA GTT ACA TCC ATG GCC TCT TTC TTT GGT ATT TTC AAA CCC GAG TTG ACT TTT GCC AAT TAT GAT GAA
280 290 300
Ser Asn Glu Ala Asp Lys Glu Leu Met Asn Gly Leu Ser Asn Leu Tyr Lys Arg Ser Pro Glu Ser Tyr Asp Lys
826 TCC AAT GAA GCT GAT AAA GAG TTA ATG AAC GGT TTA AGT AAT TTG TAC AAA AGA TCT CCC GAA AGT TAC GAC AAG
310 320
Ala Asp Glu Ser Phe Thr Lys Ala Ala Arg Leu Phe Glu Glu Gln Leu Asp Lys Asn Asn Glu Asp Glu Lys Leu
901 GCT GAT GAG TCT TTC ACG AAA GCT GCA AGG TTA TTT GAA GAA CAA TTG GAC AAG AAC AAT GAG GAT GAA AAG TTA
330 340 350
Lys Glu Lys Leu Ala Ile Ser Leu Glu His Thr Gly Ile Phe Lys Phe Leu Lys Asn Asp Pro Leu Gly Ala His
976 AAG GAA AAA TTG GCC ATC TCA TTA GAA CAC ACA GGT ATT TTC AAG TTC TTG AAA AAC GAT CCA TTG GGA GCT CAC
360 370
Glu Asp Ile Lys Lys Ala Ile Glu Leu Phe Pro Arg Val Asn Ser Tyr Ile Tyr Met Ala Leu Ile Met Ala Asp
1051 GAA GAC ATC AAG AAA GCT ATT GAA TTG TTC CCA AGA GTC AAT TCA TAT ATC TAC ATG GCA TTA ATC ATG GCT GAT
380 390 400
Arg Asn Asp Ser Thr Glu Tyr Tyr Asn Tyr Phe Asp Lys Ala Leu Lys Leu Asp Ser Asn Asn Ser Ser Val Tyr
1126 AGA AAT GAC TCG ACT GAA TAT TAT AAC TAC TTC GAT AAA GCG CTG AAA CTG GAC TCA AAC AAT TCT TCT GTT TAC
410 420
Tyr His Arg Gly Gln Met Asn Phe Ile Leu Gln Asn Tyr Asp Gln Ala Gly Lys Asp Phe Asp Lys Ala Lys Glu
1201 TAT CAC CGC GGC CAA ATG AAT TTC ATT TTA CAA AAC TAT GAT CAA GCA GGA AAA GAT TTT GAT AAA GCT AAG GAA
430 440 450
Leu Asp Pro Glu Asn Ile Phe Pro Tyr Ile Gln Leu Ala Cys Leu Ala Tyr Arg Glu Asn Lys Phe Asp Asp Cys
1276 TTA GAC CCA GAG AAT ATC TTC CCT TAT ATT CAA TTA GCA TGT CTA GCG TAC CGT GAA AAC AAG TTT GAT GAC TGT
460 470
Glu Thr Leu Phe Ser Glu Ala Lys Arg Lys Phe Pro Glu Ala Pro Glu Val Pro Asn Phe Phe Ala Glu Ile Leu
1351 GAA ACT TTG TTT AGT GAA GCT AAG AGA AAA TTT CCA GAG GCA CCA GAA GTT CCA AAT TTC TTT GCT GAA ATT TTA
480 490 500
Thr Asp Lys Asn Asp Phe Asp Lys Ala Leu Lys Gln Tyr Asp Leu Ala Ile Glu Leu Glu Asn Lys Leu Asp Gly
1426 ACA GAC AAG AAC GAT TTT GAC AAG GCT TTG AAG CAG TAC GAT TTG GCT ATT GAA TTA GAA AAC AAG TTG GAT GGT
510 520
Ile Tyr Val Gly Ile Ala Pro Leu Val Gly Lys Ala Thr Leu Leu Thr Arg Asn Pro Thr Val Glu Asn Phe Ile
1501 ATT TAT GTT GGA ATT GCG CCT TTG GTC GGT AAA GCC ACT TTG TTG ACA AGA AAT CCA ACA GTA GAA AAT TTT ATT
530 540 550
Glu Ala Thr Asn Leu Leu Glu Lys Ala Ser Lys Leu Asp Pro Arg Ser Glu Gln Ala Lys Ile Gly Leu Ala Gln
1576 GAA GCC ACG AAT TTA TTA GAA AAA GCG TCC AAA CTA GAC CCA AGA AGT GAG CAA GCT AAA ATC GGT TTA GCT CAA
560 570
Met Lys Leu Gln Gln Glu Asp Ile Asp Glu Ala Ile Thr Leu Phe Glu Glu Ser Ala Asp Leu Ala Arg Thr Met
1651 ATG AAA TTG CAG CAG GAA GAT ATC GAT GAA GCT ATT ACA TTA TTC GAA GAA TCC GCT GAT TTG GCT AGA ACT ATG
580 590 600
Glu Glu Lys Leu Gln Ala Ile Thr Phe Ala Glu Ala Ala Lys Val Gln Gln Arg Ile Arg Ser Asp Pro Val Leu
1726 GAA GAG AAA TTA CAA GCC ATT ACT TTT GCT GAA GCC GCT AAA GTT CAA CAA AGA ATT AGG TCT GAT CCA GTA TTG
610 617
Ala Lys Lys Ile Gln Glu Thr Leu Ala Lys Leu Arg Glu Gln Gly Leu Met
1801 GCT AAA AAG ATT CAA GAA ACT TTA GCT AAA TTA CGC GAA CAG GGT TTA ATG TAAACAGTAAACATAAACTTAAAAACTTTTA
1884 GAGAAGACAAAAACTAAGTAACAAAGTAGACTAAATGACAAAGGAAGAGCAGCAATGACATT
410 420
Tyr His Arg Gly Gln Met Asn Phe Ile Leu Gln Asn Tyr Asp Pro Leu Val Ser Ser Gly Lys Gly Glu Val Leu
1201 TAT CAC CGC GGC CAA ATG AAT TTC ATT TTA CAA AAC TAT GAT CCC TTG GTA AGC TCT GGA AAG GGA GAA GTG CTT
430 439
Arg Glu Arg Lys His Ala Trp Phe Gly Lys Glu Lys Pro Phe
1276 CGA GAA AGG AAA CAT GCA TGG TTT GGG AAA GAA AAA CCT TTC TAAGTTTCAAGTTTGAACGTGGAGATCCACACATATTTCGCAAG
1363 CT

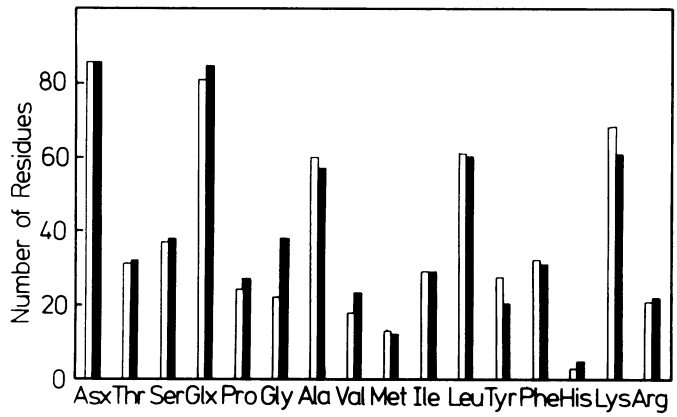


Fig. 3. The deduced and the experimentally determined amino acid composition of the 70-kd outer membrane protein are virtually identical. Open bars: values deduced from the nucleotide sequence (Figure 2, upper); closed bars; values determined by analyzing the purified 70-kd protein.

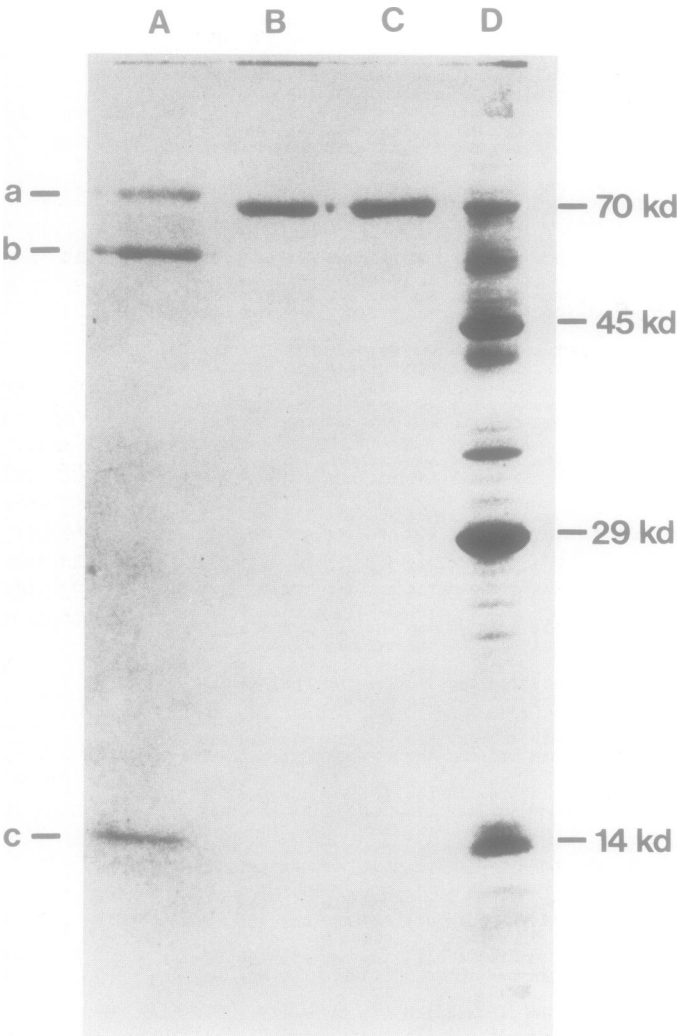


Fig. 4. Cleavage of the 70-kd protein at its single tryptophan residue generates the fragments predicted by the nucleotide sequence of the gene. Purified 70-kd protein, untreated, or after cleavage by BNPS-skatole, was analyzed by SDS-polyacrylamide gel electrophoresis (Materials and methods). **A**: products generated upon cleavage; **B**: protein incubated in reaction buffer, but without BNPS-skatole; **C**: untreated protein; **D**: mitochondrial outer membrane. a, b and c indicate the positions of the uncleaved (but modified) protein, the 58-kd fragment, and the 14-kd fragment, respectively. The apparent mol. wts. of the major outer membrane proteins are given in the right-hand margin in kd.

protein at each step was carried out by immunoblotting (Towbin *et al.*, 1979) using a monoclonal antibody against this protein (Riezman *et al.*, 1983a).

Determination of amino acid composition

Amino acid composition of the 70-kd protein was determined with a Durrum amino acid analyzer after hydrolysis of the protein with 6 N HCl in an evacuated sealed tube at 105°C for 24 h.

Cleavage of the tryptophanyl peptide bond

Cleavage of the protein with 2-(2-nitrophenylsulfenyl)-3-methyl-3-bromoindolenine (BNPS-skatole) was carried out as described (Fontana, 1972) and the sizes of the resulting peptides were determined by SDS-(15%) polyacrylamide gel electrophoresis with the following mol. wt. standards: bovine serum albumin (68 kd), ovalbumin (45 kd), human erythrocyte carbonic anhydrase (29 kd), bovine β-lactalbumin (18.4 kd), egg white lysozyme (14.3 kd) and beef heart cytochrome *c* (12 kd). Outer membrane of yeast mitochondria was prepared as described (Riezman *et al.*, 1983a).

Miscellaneous

All other procedures, as well as the plasmids carrying the cloned genes, are described in the accompanying paper (Reizman *et al.*, 1983b).

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References

Capaldi,R.A. and Vanderkooi,G. (1972) *Proc. Natl. Acad. Sci. USA*, **69**, 930-932.
Daum,G., Böhni,P.C. and Schatz,G. (1982) *J. Biol. Chem.*, **257**, 13028-13033.
Faye,G. and Simon,M. (1982) *Cell*, **32**, 77-87.
Fontana,A. (1972) *Methods Enzymol.*, **25**, 419-423.
Freitag,H., Janes,M. and Neupert,W. (1982) *Eur. J. Biochem.*, **126**, 197-202.
Fujiki,Y., Fowler,S., Shio,H., Hubbard,A.L. and Lazarow,P.B. (1982) *J. Cell Biol.*, **93**, 103-110.
Gasser,S. and Schatz,G. (1983) *J. Biol. Chem.*, **258**, 3427-3430.
Kaput,J., Goltz,S. and Blobel,G. (1982) *J. Biol. Chem.*, **257**, 15054-15057.
Kreil,G. (1981) *Annu. Rev. Biochem.*, **50**, 317-348.
Messing,J., Crea,R. and Seeburg,P.H. (1981) *Nucleic Acids Res.*, **9**, 309-321.
Messing,J. and Vieira,J. (1982) *Gene*, **19**, 269-276.
Mihara,K., Blobel,G. and Sato,R. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 7102-7106.
Riezman,H., Hay,R., Gasser,S., Daum,G., Schneider,G., Witte,C. and Schatz,G. (1983a) *EMBO J.*, **2**, 1105-1111.
Riezman,H., Hase,T., van Loon,A.P.G.M., Grivell,L.A., Suda,K. and Schatz,G. (1983b) *EMBO J.*, **2**, 2161-2168.
Sanger,F., Nicklen,S. and Coulson,A.R. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 5463-5467.
Sanger,F., Coulson,A.R., Barrell,B.F., Smith,A.Z.H. and Roe,B. (1980) *J. Mol. Biol.*, **143**, 161-178.
Sutcliffe,J.G. (1979) *Cold Spring Harbor Symp. Quant. Biol.*, **43**, 77-90.
Towbin,H., Staehelin,T. and Gordon,J. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 4350-4354.
Viebrock,A. Perz,A. and Sebald,W. (1982) *EMBO J.*, **1**, 565-571.