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Relationship between a subunit of NADH dehydrogenase (complex I) and a protein family including subunits of cytochrome reductase and processing protease of mitochondria

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The primary structure of a 40 kDa subunit of the respiratory chain NADH:ubiquinone reductase from *Neurospora crassa* was determined by sequencing cDNA, genomic DNA and the N-terminus of the mature protein. The gene which is interrupted by 7 introns encodes a preprotein consisting of 375 amino acids with a 26 amino acid long presequence typical for a mitochondrial targeting signal. The sequence of the mature subunit shows conspicuous similarities to the recently [(1989) Nature 339, 147–149] discovered protein family which includes subunits I and II of the ubiquinol:cytochrome *c* reductase, and the processing proteins, matrix processing peptidase and processing enhancing protein, of mitochondria. The possible role of the subunit is discussed.

NADH:ubiquinone reductase; Ubiquinol:cytochrome *c* reductase; Processing protease; Mitochondria

1. INTRODUCTION

NADH:ubiquinone reductase (NADH dehydrogenase or complex I, EC 1.6.99.3) is the first of the 3 proton pumping electron transfer complexes of mitochondria. The complex is an assembly of some 30 different subunits of which 7 (in *N. crassa* at least 6) are of mitochondrial genetic origin and the remaining ones are imported from the cytoplasm. Only a few subunits which carry the FMN and the 4 or 5 iron–sulfur clusters partake directly in electron transport (for a recent review see [1]). The functions of the many additional subunits which do not contain prosthetic groups are largely unknown. In this paper we report the primary structure of a 40 kDa subunit of the NADH dehydrogenase from *N. crassa* determined by sequencing of the genomic DNA, the cDNA and the N-terminus of the mature protein. We compare the protein sequence with the sequences of members of a recently discovered mitochondrial protein family including the subunits I and II of ubiquinol:cytochrome *c* reductase, the matrix processing peptidase, and the processing enhancing protein from *N. crassa* and yeast [2,3]. Significant sequence similarities suggest that the 40 kDa subunit of the NADH dehydrogenase also belongs to this protein family.

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2. MATERIALS AND METHODS

The cloning and sequencing strategy is described elsewhere [4]. *N. crassa* cDNA libraries were created in λgt 11 and λgt 10. Antiserum against the 40 kDa subunit was used to screen 2×10^5 plaques of the λgt 11 library. Inserts of 4 positive clones were excised with *Eco*RI and subcloned into the plasmid pT7T3-18U (Pharmacia) yielding the plasmids pNUO40-2,4,5,10. One of the inserts was labeled with [α-^{32}P]dATP by random priming and used to screen the λgt 10 library. Among 5×10^4 plaques one positive clone was found containing an insert of 1246 bp (pNUO40-18). It contained the entire cDNA of the subunit except 54 bp in the 3′ region. This missing region was found in pNUO40-10 together with the termination codon, a 135 nucleotide 3′-untranslated region and 10 adenine residues as part of the poly(A) tail. Genomic clones [4] containing parts of the entire gene were identified by Southern blotting and hybridization with the [α-^{32}P]dATP-labeled insert of plasmid pNUO40-18. Restriction fragments generated by digestion with *Bam*HI and *Cla*I and containing overlapping fragments at the sizes of 2.0, 1.8 and 0.5 kbp were subcloned in pT7T3-18U or -19U. Both strands were sequenced twice by the dideoxynucleotide chain termination procedure using [5′-α-^{35}S]thio-dATP (Amersham) and T7-polymerase (Pharmacia). Regions difficult to sequence were established by using c^7-deaza-GTP. For Edman degradation, the 40 kDa subunit was transferred to a polyvinylidene difluoride membrane and sequenced in an Applied Biosystems 477A instrument. Sequences were analysed using PCGENE and Heidelberg Unix Sequence Analysis Resources (HUSAR).

3. RESULTS AND DISCUSSION

The sequencing strategy is shown in Fig. 1 and the primary structures of the gene and the protein are shown in Fig. 2. The coding region of the gene is divided by seven introns evenly distributed in the gene and ranging in size from 57 to 93 bp. The length and the 5′- and 3′-boundaries of the intervening sequences are

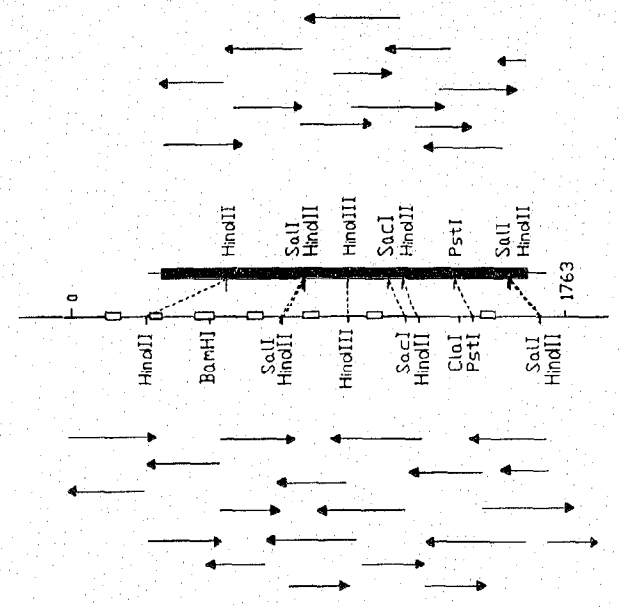

Fig. 1. Partial restriction maps of the combined cDNA inserts and the genomic fragments. The sequencing strategy is indicated by arrows. Introns are indicated by boxes.

similar to introns of *N. crassa* genes. The consensus sequence for lariat formation PyGCTAACN [5] is found in intron 1, the other introns show sequence stretches which correspond to the consensus sequence at equivalent points. The isolated 40 kDa subunit was submitted to 19 cycles of Edman degradation and the amino acids determined were in accordance with position 27–45 of the sequence deduced from the cDNA. The mature subunit therefore is preceded by a sequence of 26 residues starting with methionine. The subunit consists of 349 amino acids and its calculated molecular mass is 40109 Da. (A value of 42 kDa resulted from SDS gel electrophoresis.) The N-terminal presequence of the subunit has the characteristic features of a mitochondrial import sequence, a net positive charge, no acidic residues and is able to form an amphipathic helix [6]. The processing site shows the typical motif of 2 arginines at positions −3 and −2, alanine at −1 and serine at +2 [7].

Fig. 3 shows the sequence alignment of the 40 kDa NADH dehydrogenase subunit, the matrix processing peptidase (MPP_N) [8], the processing enhancing protein (PEP_N) which is identical to the subunit I of cytochrome reductase [2], all 3 from *N. crassa*, and MPP_Y [9] and PEP_Y [10] from *Saccharomyces cerevisiae*. Significant similarity in the N-terminal moieties of the 5 proteins exist. Direct comparison of the region from residues 27 to 188 (encoded by the first 4 exons of the NADH dehydrogenase subunit) with the corresponding stretches of MPP_N, PEP_N, MPP_Y, and PEP_Y gave sequence identities of 10%, 18%, 19% and 15%. Comparison of this region of the NADH

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-30  GGCCAGCCCTGCCACCCCCCAACTCACAAG  ATGGCTCCCTTGACGGCCGCGATGCGTCGGACTCCCAGAATTATCGTTTCCAATGCCTTTGGCTTTCAGCGACGAGCTATCTCCGACGTG
                                     M  A  P  L  T  A  A  M  R  R  T  P  R  I  I  V  S  N  A  F  G  F  Q  R  R  A  I  S  D  V
 91  ACCATCACCCGCACCGGCAAGCCCATCATCCGTAACCAGGGTGGCC GTAATTGTTGCTATATCTCTGCTTGGGTAGAATGGGCGATTGCTAACACGTCGTCTACCTCCACAG  GCTCCTCC
      T  I  T  R  T  G  K  P  I  I  R  N  Q  G  G                                                                    R  S  S
211  CTCGGTGGACATACGGCGACAGTCTTCGGTGCGACCGGCCAACTCGGCCGCTACATCGTCAACAGACTAG  GTACGATCTGGCGACTTTGTGGCGTAGGCCTCTATAGAGATCACCATGGCT
      L  G  G  H  T  A  T  V  F  G  A  T  G  Q  L  G  R  Y  I  V  N  R  L
362  TATATCAAGAACATTTGCAG  CGAGACAAGGATGCACTGTCGTCATTCCCTTCCGCGACGAGTACAACAAGCGCCATCTGAAAGTTACGGGTGATTTGGGCAAGGTGGTCATGATT GTGA
                           A  R  Q  G  C  T  V  V  I  P  F  R  D  E  Y  N  K  R  H  L  K  V  T  G  D  L  G  K  V  V  M  I
451  GCTTTTCCCCCACAACAAGAGTCATGTCCACACAGTCGAGGCCCACGTACGCGCAAGGATCCAGCTACTAAGCATAACCACCACTCCAG  GAGTTCGACTTGCGCAACACCCAATCCATCGAG
                                                                                                 E  F  D  L  R  N  T  Q  S  I  E
573  GAGAGCGTCCGACACTCCGATGTCGTCTACAACCTGATCGGTCGCGACTACCCGACCAA  GTGAGTCTGGATTTACTGGGAAAGGTTGTGGTGGAGATGCACGAGTATTGACCTGGGTCCCA
      E  S  V  R  H  S  D  V  V  Y  N  L  I  G  R  D  Y  P  T  K
694  G GAACTTCTCGTTCGAGGACGTCCATATCGAGGGTGCTGAGCGCATCGCCGAACGTGTCGCCAAATACGATGTCGACCGCTTCATCCACGTCTCGTCTTACAATGCAGACCCCAACTCGGAG
       N  F  S  F  E  D  V  H  I  E  G  A  E  R  I  A  E  R  V  A  K  Y  D  V  D  R  F  I  H  V  S  S  Y  N  A  D  P  N  S  E
816  TGCGAGTTCTTCGCCACCAA GTACGATTACAAGACAGCCTCTTCCAGCGAGTCGCATGGTTATGGGTTTCTGATTGGATCTGTAG  GGCGCGCGGTGAGCAAGTAGTCAGGAGCATTTTC
      C  E  F  F  A  T  K                                                                    A  R  G  E  Q  V  V  R  S  I  F
935  CCCGAAACCACCATTGTCCGGCCAGCTCCCATGTTTGGCTTCGAGGACAGGTTGCTTCACAAGCTTGCCAGCGTCAAGAATATCCTGACATCCAATGGGATGCAAGAGAAGTACAACCCTGTC
      P  E  T  T  I  V  R  P  A  P  M  F  G  F  E  D  R  L  L  H  K  L  A  S  V  K  N  I  L  T  S  N  G  M  Q  E  K  Y  N  P  V
1058 CAC GTCTTCCCCCACGATATAACCCTTTGCGCGCGGACGGTAGCATCTGACAGCCGTTTTAG  GTGATTGATGTTGGCCAGGCTCTTGAACAGATGCTCTGGGACGACAACACTGCTTCG
      H                                                                 V  I  D  V  G  Q  A  L  E  Q  M  L  W  D  D  N  T  A  S
1177 GAGACTTTTGAGCTCTACGGTCCCAAGACCTACACTACCGCTGAGATCTCCGAGATGGTTGACCGCGAAATCTACAAGAGGCGGCGCCACGTGAATGTGCCCAAGAAGATCCTCAAGCCGATC
      E  T  F  E  L  Y  G  P  K  T  Y  T  T  A  E  I  S  E  M  V  D  R  E  I  Y  K  R  R  R  H  V  N  V  P  K  K  I  L  K  P  I
1300 GCCGGTGTGCTGAACAAGGCCCTCTGGTGGCCCATCATGTCTGCCGATGAGATTGAGCGTGAGTTCCACGACCAGGTCATCGATCCGGAGGCCAAGACCTTCAAGGACCTCGGCATCGAGCCT
      A  G  V  L  N  K  A  L  W  W  P  I  M  S  A  D  E  I  E  R  E  F  H  D  Q  V  I  D  P  E  A  K  T  F  K  D  L  G  I  E  P
1423 GCAGATATCGGCCAACTTCACCTACCATTACTT GTAAAGTTGCCCACATCACATTCGAGTGCAAAAGTATGATGACTGATTCCCATGCAG  GCAAAGTTATCGCAGCAATGCCTACTACGAC
      A  D  I  A  N  F  T  Y  H  Y  L                                                                Q  S  Y  R  S  N  A  Y  Y  D
1543 CTTCCGCCAGCGACCGAAAAGGAGAGGAGGGAGGACAGGGAGTACATTCACATGCTGTGA GTATCTTTGGGGTTGCACAGGGATGCATGTAGATTGTAGAAATCCACCGTAGAGCATTTGGT
      L  P  P  A  T  E  K  E  R  R  E  D  R  E  Y  I  H  M  L  *
1665 GAGTCGACGGAACACAGAACGTCCAGACTGGATGCTGTGGTGAGAATTAGAGCAATCAAGAGATTTGCGCAACGTTTGAACGATCTGGGGTGAAATTGAAGCCTTCAACTCGACGGGGTTGTG
1788 TTTACTGGTATGATGCGACCAAAAAGGGGAACGGATAAGGGAAATGGCATCAATGCATCCACAAATTTTT
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Fig. 2. Sequence of the gene and the precursor protein of the 40 kDa subunit of NADH dehydrogenase. The N-terminus of the mature protein is indicated by boldface type. Transcription termination is marked by an asterisk.

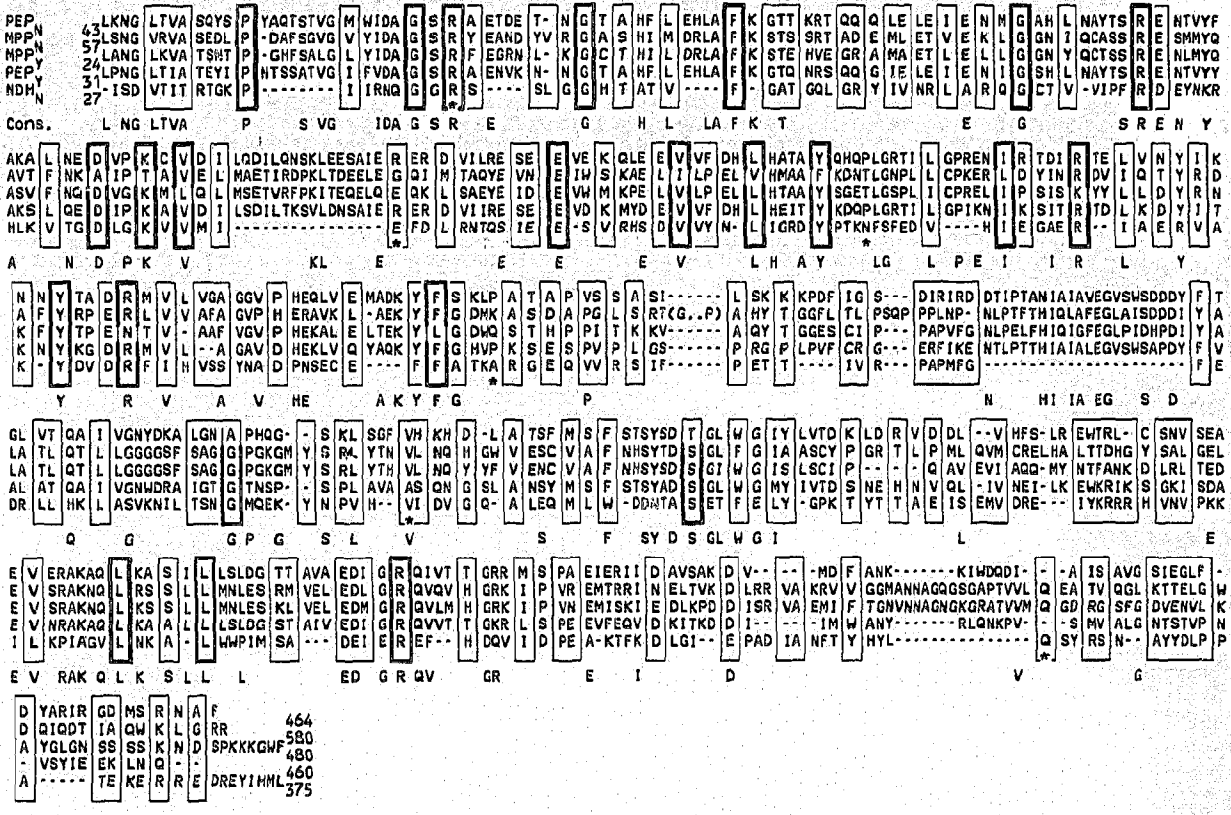

Fig. 3. Alignment of the amino acid sequences of the processing enhancing protein and the matrix processing peptidase of *N. crassa* (PEP_N, MPP_N) and yeast (PEP_Y, MPP_Y) and the 40 kDa subunit of the *N. crassa* NADH dehydrogenase (NDH_N). The region between residues 271 and 333 in the sequence of MPP_N is left out. The simple blocked areas show similarities while the double blocked areas show identities. The boundaries of the exons are marked by asterisks. Cons. means the consensus sequence deduced from the sequences of PEP and MPP of both *N. crassa* and yeast. PEP_N is identical to subunit I of cytochrome reductase.

dehydrogenase subunit with a consensus sequence derived from MPP and PEP of *N. crassa* and yeast showed a degree of identity of 28%. The sequence comparison was also extended to the subunits I and II of the yeast cytochrome reductase [11,12], but less similarity was found.

As with the other members of this protein family, the 40 kDa NADH dehydrogenase subunit contains no hydrophobic region long enough to span the membrane. The subunit is also a constituent part of a smaller form of the NADH dehydrogenase which is made in *N. crassa* in place of the large form when the mitochondrial protein synthesis is inhibited by chloramphenicol [13]. Electron microscopic studies clearly showed, that this small form of NADH dehydrogenase is located at the matrix side of the inner mitochondrial membrane and protrudes deeply into the aqueous phase [14].

Why should mitochondrial proteins that can be grouped as members of the same protein family be involved with such different functions as electron transfer linking to proton translocation and processing of imported polypeptides. Common to these proteins is their location in the matrix space of the mitochondrion, either tightly bound to a respiratory chain complex, such as PEP_N=subunit I_N of cytochrome reductase, subunit II of cytochrome reductase and the 40 kDa subunit of NADH dehydrogenase, or in free form such as $MPP_{N,Y}$ and PEP_Y. The common denominator with their function might be that protein–protein contact must occur. MPP has a proteolytic activity, and PEP is required for this reaction by interacting with MPP and the precursor protein. Subunits I and II of cytochrome reductase, like the 40 kDa subunit of NADH dehydrogenase, might function as a control unit, sensing what is going on inside the mitochondria. Regulatory effects of the membrane potential on the H^+/e^- stoichiometry have been demonstrated for the *N. crassa* cytochrome reductase [15]. Alternatively, the 40 kDa NADH dehydrogenase subunit might make contact with matrix dehydrogenases. In mammalian mitochondria substrate channeling of NADH from matrix dehydrogenases to NADH dehydrogenase has been reported to occur by transient enzyme–enzyme binding [16]. The function of the subunit might thus be to facilitate ordered sequences of reaction.

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REFERENCES

[1] Weiss, H., Friedrich, T., Hofhaus, G. and Preis, D. (1990) Eur. J. Biochem., submitted for publication.
[2] Schulte, U., Arretz, M., Schneider, H., Tropschug, M., Wachter, E., Neupert, W. and Weiss, H. (1989) Nature 339, 147–149.
[3] Weiss, H., Leonard, K. and Neupert, W. (1990) Trends Biochem. Sci. 15, 178–180.
[4] Preis, D., Van der Pas, J.C., Nehls, U., Röhlen, D.A., Sackmann, U., Jahnke, U. and Weiss, H. (1990) Curr. Genet. 18, 59–64.
[5] Gurr, S.J., Unkles, S.E. and Kinghorn, J.R. (1987) in: Gene Structure in Eucaryotic Microbes (Kinghorn, J.R. ed.) pp.93–162, IRL Press, Oxford.
[6] Von Heijne, G. (1986) EMBO J. 5, 1335–1342.
[7] Hartl, F.U., Pfanner, N., Nicholson, D.W. and Neupert, W. (1989) Biochim. Biophys. Acta 988, 1–45.
[8] Schneider, H., Arretz, M., Wachter, E. and Neupert, W. (1990) J. Biol. Chem. 265, 9881–9887.
[9] Jensen, R.E. and Yaffe, M.P. (1988) EMBO J. 7, 3863–3871.
[10] Witte, C., Jensen, R.E., Yaffe, M.P. and Schatz, G. (1988) EMBO J. 7, 1439–1444.
[11] Tzagoloff, A., Wu, M. and Crivellone, M. (1986) J. Biol. Chem. 261, 17163–17169.
[12] Oudshoorn, P., Van Steeg, H., Swinkels, B.W., Schoppink, P. and Grivell, L.A. (1987) Eur. J. Biochem. 163, 97–103.
[13] Friedrich, T., Hofhaus, G., Ise, W., Nehls, U., Schmitz, B. and Weiss, H. (1989) Eur. J. Biochem. 180, 173–180.
[14] Hofhaus, G., Weiss, H. and Leonard, K. (1990) J. Mol. Biol., submitted for publication.
[15] Bechmann, G. and Weiss, H. (1990) Eur. J. Biochem., in press.
[16] Fukushima, T., Decker, R.V., Anderson, W.M. and Spivey, H.O. (1989) J. Biol. Chem. 264, 16483–16488.