

## Short Sequence-Paper

The nuclear-encoded *MSS2* gene is involved in the expression of the mitochondrial cytochrome-c oxidase subunit 2 (Cox2) <sup>☆</sup>

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Abstract

*Saccharomyces cerevisiae* cells carrying the *mss2-1* pet mutation contain no Cox2 protein (cytochrome-c oxidase subunit 2), though COX2 transcripts are synthesized and processed normally. Gene *MSS2* was cloned and sequenced. It is localized on chromosome IV. The Mss2 protein does not show any significant homology with published sequences.

**Keywords:** (*S. cerevisiae*) ; Pet mutation ; Cytochrome-c oxidase ; COX2 ; COX1 ; *MSS2*

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Numerous nuclear genes inactivate respiration when they are altered (nuclear pet mutants). Among them, a large group acts on either the processing or the translation of specific mitochondrial messenger RNAs [1].

The E2-116 strain of *Saccharomyces cerevisiae* [2], which we have previously studied [3], harbors two pet mutations. The properties of one of them, named *mss116-1*, has been described in Séraphin et al. [4]. The second mutation, *mss2-1*, is the subject of the present study.

The yeast mitochondrial genome codes for one ribosomal protein, three subunits of the mitochondrial ATP synthetase and four components of the respiratory chain [5]. The possible interference of the *mss2-1* mutation with mitochondrial translation was examined by analyzing the mitochondrial translation products of a wild-type strain, D273-10B, and those of the isomitochondrial BSA-1D strain harboring the *mss2-1* mutation. Autoradiograms of <sup>35</sup>S labelled mitochondrial proteins [6] fractionated by SDS-PAGE [7] are shown in Fig. 1. It appears that strain BSA-1D contains no Cox2 protein and a reduced amount of protein Cox1.

COX2 belongs to a mitochondrial transcription unit composed of *COX2* and *ORF2* (*RF1*) [1]. Northern blot analysis of the mitochondrial RNAs encompassing the *COX2* locus showed that the processing pattern of the pre-mRNA as well as the amount of the COX2 mature mRNA were identical in the *mss2-1* mutant and a wild-type strain (data not shown). Therefore, we assume that the *MSS2* gene most probably affects the translation (or the stability) of the Cox2 subunit, though small alteration in post-transcriptional modification might have escaped our analysis.

Gene *MSS2* was cloned by functional complementation of mutant *mss2-1*. A yeast library constructed with the yeast/*E. coli* vector YEp13 and the whole fragmented genome of strain AB320 [8] was used to transform strain BSA1-3B. A glycerol positive transformant was obtained. It harbored a plasmid, named pD6, containing an insert of 5 kilobases. The complementing gene was localized more precisely as described in Fig. 2.

To determine the sequence of gene *MSS2*, the 1.7 kb *HindIII* and the 1.65 kb *HindIII-SalI* fragments were cloned in the M13 replicative form DNA and a set of DNaseI deleted subclones [9] was submitted to dideoxy sequence analysis. An open reading frame (ORF), extending towards the *SalI* site, was found (Fig. 3). It codes for a 291 amino acid long protein (*M<sub>r</sub>* 34 617) which does not

<sup>☆</sup> This sequence has been submitted to EMBL/Genbank Data Library under the accession number X81477.

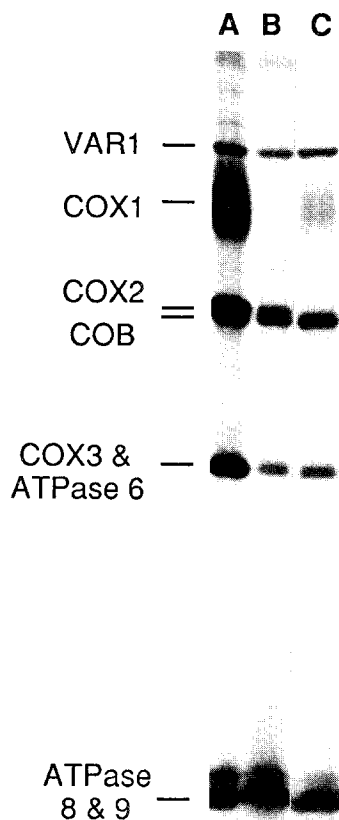


Fig. 1.  $^{35}$ S-labelled mitochondrial translation products of wild-type strain D273-10B (A), strain E4-218 (B), containing the *mss51* mutation [3,17], used as a control, and strain BSA1-1D (C) containing the *mss2-1* mutation. The positions of proteins synthesized on mitochondrial ribosomes are indicated: VAR1, var1 ribosomal protein; COX1, COX2 and COX3, subunits I, II and III of cytochrome *c* oxidase; COB, cytochrome *b*; ATPase 6, 8 and 9, subunits of mitochondrial oligomycin-sensitive ATPase.

show any significant homology with published sequences. However, the amino end of Mss2 presents features which are common to most presequences of imported mitochondrial proteins [5].

Gene *MSS2* is localized on chromosome IV between genes *KIN28* and *PHO2* [10].

To disrupt gene *MSS2*, plasmid pUC19K containing the 1.7 kb *HindIII* fragment was cleaved within the *MSS2* ORF at a unique *HpaI* site where the 2.4 kb *HpaI* fragment bearing the yeast *LEU2* gene was inserted. The linear *HindIII* fragment containing the disrupted ORF was used to transform diploid strain DG109 [11]. Disruptants were forced to sporulate. Each *Leu*<sup>+</sup> spore was respiratory deficient. Mutations *mss2-1* and *mss2::LEU2* are recessive. An *mss2::LEU2* strain was crossed with BSA1-3B. The diploid obtained was respiratory deficient proving thereby that we have really cloned the *MSS2* wild type allele.

Three nuclear genes, *PET111*, *PET112* and *SCO1* have been described as being required for the translation of the COX2 mRNA or the assembly of Cox2 in the cytochrome oxidase complex [5]. *MSS2* appears to be a new nuclear gene which is necessary for the accumulation of protein Cox2 and to a lesser extent that of Cox1. It remains to determine, in future experiments, whether protein Mss2 intervenes in the translation of COX2 mRNA, is necessary for some post-translational modification of protein Cox2 or is implicated in the assembly of protein Cox2 into the inner mitochondrial membrane [12].

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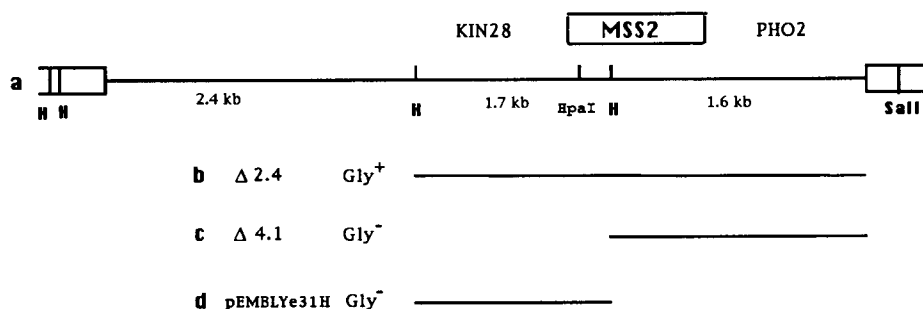


Fig. 2. Localization of gene *MSS2*. Plasmid pD6 was partially digested with *HindIII*, self-ligated and amplified in *E. coli*. Two deleted plasmids, D 2.4 and D 4.1, were obtained. Furthermore, the 1.7 kb *HindIII* fragment was cloned in pEMBLye31 [13]. The resulting plasmid, pEMBLye31H, as well as plasmids D 2.4 and D 4.1, were used to transform strain BSA1-3B and respiring transformants were screened for. Gly<sup>+</sup> indicates that transformants can use glycerol as a carbon source; gly<sup>-</sup> that they cannot. H: *HindIII*.

AAGAGGTGGACCGCTGTTCAAGTGTGTTAGAAAGTGAATTATTTCAAAGAATTACCACCACCAAGTGACCCGCTCTTCAATAAA 80  
 K R W T A V Q C L E S D Y F K E L P P P S D P S S I K  
**KIN28** ---->  
 AATACGTAACGTATGATATGATTTTATAAAATTTGTAGAATCTGTGTTATTGACATTAGATGTATCCTCAACATATACAAATA 160  
 I R N \*  
 AACTTCTTGATGCGTCTGTTTTTTGGTGTGAATTTTAACTCTTTTCCATCATTATGGAATTCGTTTTTAGATTTTAAC 240  
 EcoRI  
 AACACAATATGAGGGGAGACAAACAGTAGCAGACGAAAACGAAAACAAAAGCAAGAAATATCTGATCTAAAATATGCAG 320  
 M Q  
**MSS2** ---->  
 AGGTTTGTCAAGTTTGTGTTTCCACACCACCGTACCCAAAAGTTTCAAGAGATTTTCCCGAAGAAACGTACGGTTAA 400  
 R F V S K F V S T P P V P K K F Q E I F P K K R T V N  
 CAAAATTTTATCCAGTTAGATACAAGGCTTACATACCATGAAATGTACCCGATATTTCTGCAGGTATCACAAAATACTA 480  
 K I L F Q L D T R L T Y H E M Y P I F L Q V S Q N T  
 ATGAAGAGAATATCCCATGGAGGAAGAAATACCCCTATATAAGGAGTTCAGACATTATGCAGATGCGAAACGTCTTGATA 560  
 N E E N I P W R K K Y P Y I R S S D I M Q M R N V L I  
 ACTCTAAGGACGCGAACAATTCGTCCACAAAGACTTATTAGCTATGGAGGATAAATTATTGAATATTGCTGCCGAACT 640  
 T L R T Q N K F V H K D L L A M E D K L L N I A A E L  
 TGGCAACAACGATGCTATATCCATCCTAAGCTTCAACGTGATACATGAATATAAAAAGGAAAACGTCAATCCAGTTATG 720  
 G N N D A I S I L S F N V I H E Y K K E N V K S S Y  
 AAAAAGACATTGAAACGGCTAATGAATTCATAAAGAAGCTGTATGCGCGTAACCATCATTTAACGGTTAAATTAATAGGG 800  
 E K D I E T A N E F I K K L Y A R N H H L T V K L I G  
 GACCTGTTTTTCGAAAACAAAACCTTACGATAAGGCTGAGAAATATTACCAAGAGTTCTTGAAATTGAAAAATAGTACCAA 880  
 D L F F E N K T Y D K A E K Y Y Q E F L K L E N S T K  
 ATTGGCAGGCGAAGTTCACGGAAAACCTTGGGGAAATCCAAATAAAGCAAGTCAATGGTTTTTTGAAGGCAGAAAAGTCTAT 960  
 L A G E V H G K L G E I Q I K Q V N G F L K A E K S  
 GGCTGAGTTGTATAGAAGTGTGGAATTTGAAAGAAGTTCACGTTGGTACTTTCTGTAGCGAGGTTATATATGAGTTCA 1040  
 W L S C I E L L E I E R S S R W Y F L L A R L Y M S S  
 GAGCCCATGAAAGCCAAAGCCTTGCTAGAAAATTGTGCATCAATTGGATTTAAAGAATGCTTTAAACATTAGGATTTCT 1120  
 E P M K A K A L L E N C A S I G F K E C F K T L G F L  
 TGAATTAAACTATTTCAATAATTATGAAAGGCGAAAAGTGGTTCAACCGGTATGGAATATGACTTGATGATTTCTTT 1200  
 E L N Y F N N Y E R A K E W F K R V W N M T \*  
 GGATTTTTCGATTGCTGTGTGAAAGAAGAGAATTTTAAAGATGCACGAGATTAGCCTAGAAAGCGTAAAAAGCTAGGGA 1280  
 ATGATAAAGACAAGAAAACAATGATAAATGTCTTTCTTGAAAGTAGAAAAGATTCCATAAAGTTGCTGGACAAAGCACGG 1360  
 CTTTAAATGTCTGATCGATTCTTTTTCGATTATATAAGGACACATGTCTCCACCTATAACGCGAGCTTGTAATATCT 1440  
 ATATACCCATCTGATAATGTTCAAAAAGTCAGCTAAGTAAGTAAATAATAAGGACAATAAAATTAATATGTCATACAA 1520  
 TGTTACCTATGAAATAATTAGGAAATACTGTTAGAGTAATATTAGAGTTGAAAATGCAATCGCAAAAAAAAAAAAAACAG 1600  
 \* I W R H E D T L E N T N K L F D P T N P L E  
 AATTATTTTCATCATATCCATCTATGCTCGTCAGTTAGTTTCGTTAGTGTCTTCAAAAAATCTGGAGTATTTGGGAGTTC 1680  
 S D T P L S L L H D N P N A V I D A D N S L H E Q A L  
 ACTATCAGTCGGTAAGACAACAAATGATCATTGGATTGCAACAATATCAGCATCGTTGGATAAATGTTCTTGCCTA 1760  
 H N E E N N A N H N N N S N V T T D L L N L T D D K  
 GATGATTCTCCTCATTATTAGCATTATGATTGTTATTGCTGTTGACGGTAGTATCCAGTAAATTGAGCGTATCATCCTTG 1840  
 S A <---- **PHO2**  
 CTAGC  
 NheI

Fig. 3. Nucleotide sequence of the region analyzed. The deduced amino acid sequences of *KIN28* [14], *MSS2* and *PHO2* [15,16] is indicated above or below the nucleotide sequence depending on the direction of their transcription, indicated by arrows. Some restriction sites are underlined.

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