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Biochimica et Biophysica Acta 1228 (1995) 95–98

BBA Biochimica et Biophysica Acta

Short Sequence-Paper

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

Michel Simon, Bertrand Séraphin, Gérard Faye

Institut Curie-Biologie, Bât. 110, Centre Universitaire, 91405 Orsay Cédex, France

Received 14 November 1994; accepted 13 December 1994

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*

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 ^{35}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 *Hin*dIII and the 1.65 kb *Hin*dIII-*Sal*I 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 *Sal*I site, was found (Fig. 3). It codes for a 291 amino acid long protein (M_r 34 617) which does not

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

SSDI 0005-2728(94)00198-7

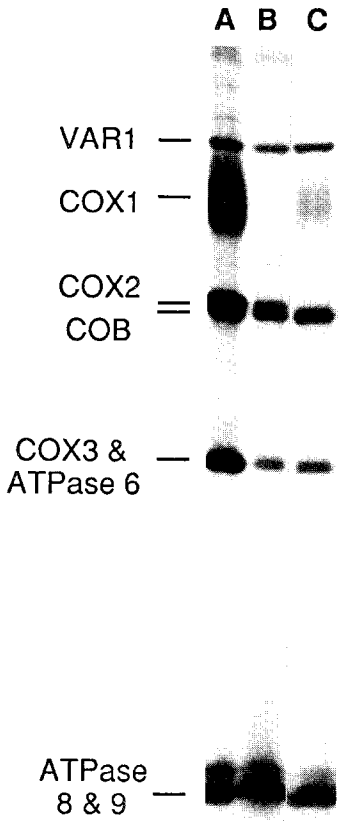

Fig. 1. ^{35}S-labelled mitochondrial translation products of wild-type strain D273-10B (A), strain E4-218 (B), containing the mss51 mutation [3,17], used as a control, and strain BSA-1D (C) containing the mss2-1 mutation. The positions of proteins synthesized on mitochondrial ribosomes are indicated: 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 *Hin*dIII fragment was cleaved within the *MSS2* ORF at a unique *Hpa*I site where the 2.4 kb *Hpa*I fragment bearing the yeast *LEU2* gene was inserted. The linear *Hin*dIII fragment containing the disrupted ORF was used to transform diploid strain DG109 [11]. Disruptants were forced to sporulate. Each Leu$^+$ 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].

We would like to thank A. Tzagoloff for strains and A. Leray for excellent secretarial assistance. This work was supported by the Association pour la Recherche sur le Cancer (Contract number, 6087) and by the European Communities (contract number, SC1-0479C).

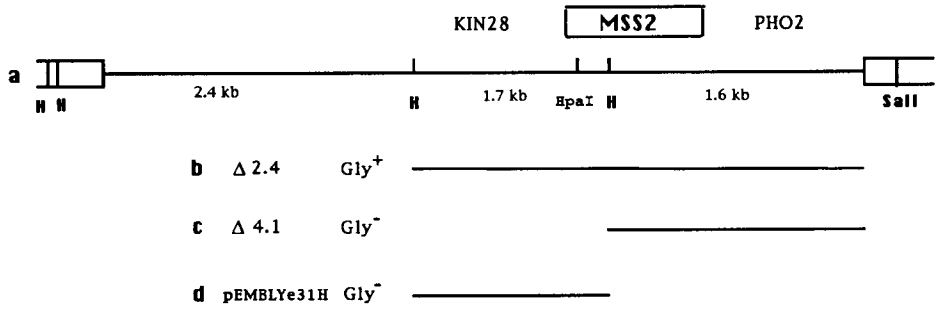

Fig. 2. Localization of gene *MSS2*. Plasmid pD6 was partially digested with *Hin*dIII, self-ligated and amplified in *E. coli*. Two deleted plasmids, D 2.4 and D 4.1, were obtained. Furthermore, the 1.7 kb *Hin*dIII fragment was cloned in pEMBLYe31 [13]. The resulting plasmid, pEMBLYe31H, as well as plasmids D 2.4 and D 4.1, were used to transform strain BSA1-3B and respiring transformants were screened for. Gly$^+$ indicates that transformants can use glycerol as a carbon source; gly$^-$ that they cannot. H: *Hin*dIII.

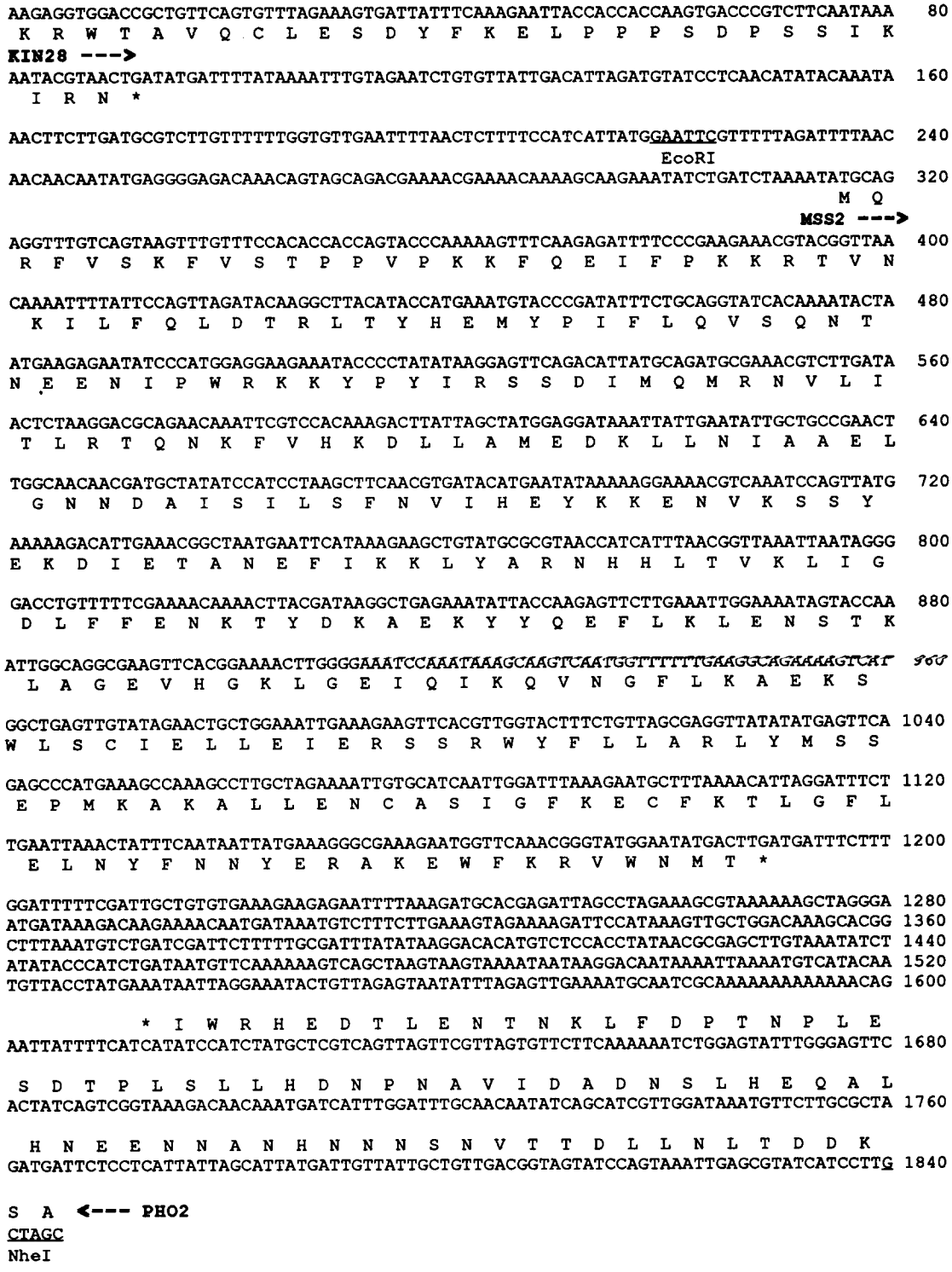

Fig. 3. Nucleotide sequence of the region analyzed. The deduced amino acid sequences of *KIN28* [14], *MSS2* and *PHO2* [15,16] is indicated above or below the nucleotide sequence depending on the direction of their transcription, indicated by arrows. Some restriction sites are underlined.

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