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Insight into the interactions between subunits I and II of the cytochrome *c* oxidase of the yeast *Saccharomyces cerevisiae* by means of extragenic complementation

Brigitte Meunier**, Anne-Marie Colson*

Universite Catholique de Louvain, Laboratoire de Genetique Microbienne, Place Croix du Sud 4, 1348 Louvain-la-Neuve, Belgium

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In yeast, revertants were selected from two respiratory deficient mutants carrying mutations in the catalytic subunits of cytochrome oxidase. From a mutant carrying a double mutation in the vicinity of the copper binding pocket in subunit II, two genetically independent revertants were obtained in which the same extragenic reversion mutation was observed, A147V, in the putative helix 4 of subunit I. A comparison with revertants derived from the second deficient mutant, carrying the deficiency mutation, S140L, in the loop 3–4 of subunit I, provides additional data in favour of an interaction between helix 4 of subunit I and subunit II.

Cytochrome oxidase; Yeast; Extragenic complementation

1. INTRODUCTION

Mitochondrial cytochrome *c* oxidase is the major terminal oxidase in eukaryotes. It is a member of a large family of protonmotive oxidases [1]. The mitochondrial enzyme has up to 13 subunits [2] but only the three mitochondrially-encoded subunits (I, II and III) form the essential catalytic unit which is common to all superfamily members.

Most, if not all, of the prokaryotic enzymes in this superfamily have subunits which are homologues of the three mitochondrially encoded subunits (I, II and III) of the eukaryotic oxidase. The bacterial systems have allowed the application of site-directed mutagenesis and this has provided a major contribution to the significant advances in understanding of their structure which have been made recently [3–5].

In the eukaryotic system, in contrast to the bacterial system, subunits I, II and III which are directly involved in the catalytic activity are encoded by the mitochondrial DNA and thus are not easily manipulated by site-directed mutagenesis. Nevertheless, the eukaryotic systems (and yeast in particular) have complementary information to offer. The characterisation of random mutants and their revertants may allow otherwise unsuspected important regions of the protein to be identified, and provides a means of determining parts of the protein which may be close to each other [6–9].

*Corresponding author.

***Present address:* Glynn Research Institute, Bodmin, Cornwall, PL30 4AU, UK.

This approach also provides a means of obtaining information on the interactions between subunits I, II and III. Starting from a deficient mutant L3, carrying a double mutation in the COX 2 gene, spontaneous revertants were selected. In addition to the intragenic revertants, two genetically independent but identical extragenic revertants have been obtained. The extragenic mutation responsible for the restoration of the respiratory growth ability was located in the COX 1 gene.

2. MATERIALS AND METHODS

The strains, media and selection of the revertants were described in [9]. The ethidium bromide mutagenesis was described in [10].

3. RESULTS AND DISCUSSION

3.1. *Revertants derived from the subunit II deficient mutant*

The deficiency mutation L3 has been mapped in the COX 2 gene. The mutation leads to an inability to grow on glycerol medium even although the mutant contains optically-detectable oxidase [6,7].

The sequencing of the COX 2 gene of the mutant has been performed as described in [6]. Two mutations were observed, A189V and V220V. The double mutation is located in the vicinity of the predicted copper binding pocket of the subunit II [11–13].

Revertants have been selected. The mitochondrial heredity of the reversion was determined before sequencing as described in [9,10]. Mutations have been sequenced directly on DNA segments amplified by polymerase chain reaction [6]. The results are presented

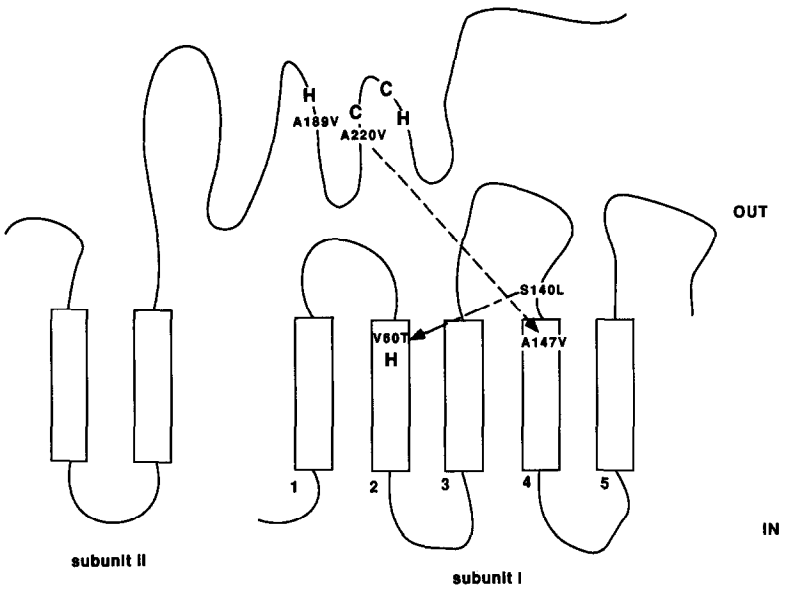

Fig. 1. Localisation of the amino acid replacements in the folding model of the cytochrome oxidase subunits I and II. Only helices 1 to 5 of the subunit I are represented. H is histidine 62, one of the two ligands of haem *a*. In the subunit II model, adapted from [11], the ligands of Cu_A are noted in bold letters, H186, C221, C225 and H229. The deficiency mutations in subunits I and II and the second-site reversions are in bold letters. The arrows point to the second-site reversions.

in Table I. Wild type reversion was not found. However reversions at each mutated site were identified suggesting that each mutation alone is responsible for a deficiency phenotype and each reversion compensates the other deficiency mutation. In addition, a distant reversion was found in the COX 1 gene. The amino acid replacement, A147V, is located in the putative helix 4 of the model (Fig. 1). This extragenic reversion was observed in two genetically independent revertants and the sequencing data were confirmed by the genetic localisation of the reversion. As described in [10], a rho$^-$ clone (large deletion mutant) able to suppress the subunit II deficiency mutation was produced by ethidium bromide treatment. The mitochondrial genes retained by this rho$^-$ clone were determined by crossing with COX 1, 2 and 3 deficiency mutations (data not shown). We observed that this rho$^-$ genome had lost the COX 2 and COX 3 genes but retained the COX 1 gene. The sequencing of the COX 1 gene retained by the rho$^-$ clone has been performed and only the A147V mutation was observed. Therefore, the A147V mutation is considered as responsible for the restoration of the respiratory competence. This result suggests a functional and maybe topological interaction between the copper binding pocket of subunit II and helix 4 of the subunit I (Fig. 1).

Table I

Codon and amino acid replacements in the subunit II mutant, L3 and the revertants

Strains	Initial mutations loci in subunit II		Distant reversion locus in subunit	Number of revertants
Wild type	A189 GCT	A220 GCA	A147 GCA	
L3	A189V GCT > GTT	A220V GCA > GTA		
Revertant	V189F GTT > TTT	A220V		11
Revertant	A189V	V220M GTA > ATA		11
Revertant	A189V	V220G GTA > GGA		1
Revertant	A189V	A220V	A147V GCA > GTA	2

The revertants are selected and sequenced as described in [9]. They are spontaneous and genetically independent.

3.2. *Revertants derived from the subunit I deficient mutant*

The study of revertants selected from another deficient mutant (L176) has provided additional evidence in favour of the interaction. The L176 deficiency mutation has been mapped in a subregion of the COX 1 gene. This region was sequenced and an amino acid replacement, S140L, has been observed. This change is located in the putative loop between helices 3 and 4, seven amino acids before the reversion mutation A147V (Fig. 1). Revertants have been selected and sequenced as described [9]. The results are shown in Table II. Wild type reversion and non wild type reversion at the same locus

Table II

Codon and amino acid replacement in the subunit I mutant, L176, and the revertants

Strains	Initital mutation locus	Distant reversion locus	Number of revertants
Wild type	S140 TCA	V60 GTT	
L176	S140L TCA > TTA		
Revertant	Wild type		4
Revertant	L140T TTA > CTA		3
Revertant	S140L	V60T GTT > CTT	3

The revertants are selected and sequenced as described in [9]. They are spontaneous and genetically independent.

(L140T) were observed. Three genetically independent revertants were found to carry a second-site reversion of V60T, two amino acids before H62 which is likely to be a ligand of the haem *a*. This result suggests a functional interaction and maybe topological proximity between the loop 3–4 and the helix 2 which carries one of the ligands of haem *a*.

As shown in Fig. 1, if residues 60 (helix 2) and 140 (loop 3–4) are close in the three-dimensional structure, then the mutation A147V is also likely to be in the vicinity of the H62. Since this suppresses the subunit II mutation (A189V + A220V), the results overall indicate that there may be proximity of the Cu_A ligands of subunit II and the haem *a* ligands of subunit I. This seems logical if we consider that the electrons are transferred from Cu_A of subunit II to the binuclear centre via the haem *a* [14].

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