Amino acid substitutions in mitochondrial ATPase subunit 6 of *Saccharomyces cerevisiae* leading to oligomycin resistance

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The amino acid substitutions in subunit 6 of the mitochondrial ATPase complex have been determined for 4 oligomycin resistant mutants of *Saccharomyces cerevisiae*. The data were obtained for each mutant by nucleotide sequence analysis of the mitochondrial *oli2* gene. Amino acid substitutions conferring oligomycin resistance in subunit 6 are located in two conserved regions that are thought to form domains which span the inner mitochondrial membrane. The disposition of these amino acid substitutions is consistent with the view that these two membrane-spanning domains interact structurally and functionally with the DCCD-binding proteolipid subunit 9 in the F_0-sector.

Mitochondrial ATPase complex *Amino acid substitution* *Oligomycin* *Drug-resistance domain* oli2 *gene* *(Yeast)* *mtDNA*

1. INTRODUCTION

The *oli2* gene in mitochondrial DNA (mtDNA) of *Saccharomyces cerevisiae* codes for subunit 6 (predicted length 259 amino acids) of the proton translocating mitochondrial ATPase complex. Together with subunit 6, subunits 8 and 9 (encoded by the mitochondrial *aap1* and *oli1* genes, respectively) constitute the membrane-bound F_0-sector of the mtATPase complex [1]. Subunit 6 has been implicated [2] in the coupling of proton translocation to ATP synthesis catalyzed by the soluble F_1-sector of the complex. The translocation of protons is generally considered [3] to be primarily effected by subunit 9, the 76 amino acid DCCD-binding proteolipid; it has further been suggested [4,5] that subunit 6 may itself play a role in this process.

From a genetic perspective, the *oli2* gene was initially identified as one of the loci in mtDNA at which resistance to oligomycin was determined [6]. This locus was shown to be separate from the other such mitochondrial locus, now known as *oli1* [7], on the basis of genetic recombination studies [8,9] and physical mapping by petite deletion analysis [10]. The existence of a further locus determining an oli^R phenotype, designated *oli4*, was proposed [11] on the basis of the observed low, but significant recombination frequency with a reference *oli2* mutant.

In contrast to the situation with the *oli1* gene, where more than fifteen oli^R mutants have been subjected to amino acid or nucleotide sequence analysis [7,12,13], only three oli^R mutants mapping in the *oli2* region have been analyzed at the DNA sequence level. Each such mutation leads to

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Abbreviations: DCCD, dicyclohexyl carbodiimide; mtATPase, mitochondrial proton-translocating ATPase; oli^R, oligomycin-resistant (in reference to phenotype)

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a single amino substitution near the C-terminus of subunit 6, at residues 171, 175 and 232, respectively [14,15]. The substitution at residue 232 was identified in a strain reportedly carrying an allele of the *oli4* locus [14].

In this communication we report the changes in the *oli2* gene of 4 further oli^R mutants. The data extend the information on amino acid substitutions in subunit 6 conferring oligomycin resistance. The disposition of these amino acid substitutions provides evidence for interactions between membrane-spanning domains of subunit 6 and the subunit 9 proteolipid in the F_0-sector. In addition, one of these strains which suffers a concomitant impairment in mtATPase function provides further insight into regions of subunit 6 critical to energy transduction in the F_0-sector.

2. MATERIALS AND METHODS

2.1. *Strains*

The strains analyzed here are listed in table 1, which shows the original denotation of the mitochondrial mutation leading to oligomycin resistance. In addition, a more systematic designation of the particular allele number is given for each mutation, in line with current usage.

2.2. *Methods*

Growth of strains, mtDNA extraction [16] and nucleotide sequence determination of the *oli2* gene [17] were carried out as described.

3. RESULTS AND DISCUSSION

3.1. *Strategy for characterization of oli^R mutants*

Most of the *oli2* mutants analyzed in this work come from the same collection of oli^R mutants from which Nagley et al. [13] have previously selected strains characterized for their *oli1* mutations. Briefly, this collection was derived by manganese mutagenesis [18] of the parent *rho*$^+$ strain J69-1B. Those oli^R isolates showing mitochondrial inheritance were subsequently classified as *oli2* mutants on the basis of low recombination frequencies (<1%) in crosses with the definitive *oli2* oli^R strain D22/A15 [8]. This reference strain D22/A15 was also subjected to nucleotide sequence analysis in this work.

The complete nucleotide sequence of the *oli2* gene in each of the 4 mutant strains (table 1) was determined as it was for *oli2 mit*$^-$ strains [17]. The entire sequence of 780 nucleotides of the *oli2* gene was obtained by the dideoxy method using the appropriate set of three *oli2*-specific synthetic primers on M13-derived single-stranded templates [17].

3.2. *Sequence changes in oli^R mutants*

The single nucleotide changes observed relative to the J69-1B wild-type sequence [15], together with the consequent amino acid substitutions, are shown in table 1. Isolate 0817 has an amino acid substitution ($Ile_{171} \longrightarrow Met$) identical to the one previously identified in the independently derived strain D273-10B/A48 [14] (table 2). However,

Table 1

Nucleotide sequence changes in the *oli2* gene and resulting amino acid substitutions in oligomycin resistant strains analyzed in this work

Strain	Allele designation		Nucleotide change[a]	Codon change[b]	Amino acid residue change	Source	Comment
	Previous	Current					
0817	–	*oli2-17*	T→G (513)	ATT→ATG (171)	Ile→Met	Monash[c]	Reduced growth rate
0836	–	*oli2-36*	T→A (523)	TCT→ACT (175)	Ser→Thr	Monash[c]	
0858	–	*oli2-58*	C→A (524)	TCT→TAT (175)	Ser→Tyr	Monash[c]	Heat-sensitive strain C58 [2]
D22/A15	O^R_{144}	*oli2-144*	C→G (524)	TCT→TGT (175)	Ser→Cys	[6]	Denoted O_{11} in [8]

[a] Nucleotide sequence numbers are specified with reference to fig.3 of [15]

[b] Codon numbers are specified with reference to fig.5 of [14]

[c] The strains are from a collection of oli^R mutants derived by $MnCl_2$ mutagenesis of strain J69-1B [13]

Table 2

Nucleotide sequence changes in the *oli2* gene and resulting amino acid substitutions in oligomycin resistant strains previously analyzed

Strain	Allele designation		Nucleotide change	Codon change	Amino acid residue change	Reference	Comment
	Previous	Current					
D273-10B/A48	O^{R}_{118}	*oli2-118*	T→G (513)	ATT→ATG (171)	Ile→Met	[14]	Denoted *oli-2* in [14]
D273-10B/A16	O^{R}_{622}	*oli2-622*	A→T (696)	TTA→TTT (232)	Leu→Phe	[14]	Denoted *oli-4* in [14]
70M	*oli2-23r*	*oli2-23*	T→A (523)	TCT→ACT (175)	Ser→Thr	[15]	Denoted *oli23-r* in [19]

Nucleotide and codon numbering as in table 1

unlike the latter strain, 0817 suffers a concomitant impairment in mitochondrial function manifested in its ability to grow only slowly on non-fermentable substrates such as ethanol. This observation may reflect a specific interaction between the nuclear genome of J69-1B and this particular mitochondrial allele (of the type described [13]), or alternatively there may be a second mutation in either the mtDNA or nuclear genomes of this isolate. This aspect has not been further investigated.

The other substitutions in oli^{R} mutants were all found to occur at the same amino acid residue Ser_{175}. Isolate 0836 carries a threonine residue at this position, which arises from precisely the same nucleotide substitution as previously observed in strain 70M [15] (table 2). The allele in strain 70M, *oli2-23*, originates from an oli^{R} isolate of J69-1B (0823) (cf. [19,20]); the nucleotide change in the *oli2* gene of isolate 0823 has also been directly confirmed by the methods reported in this paper (not shown).

On the other hand, in isolate 0858 the substitution $Ser_{175} \longrightarrow$ Tyr confers oligomycin resistance, with a concomitant reduced growth on the substrate ethanol at 28°C and an even more marked heat sensitivity (no growth at 36°C on ethanol media). This impairment in respiratory growth has been shown to result from an inefficient coupling of respiration to ATP synthesis in mitochondria [2]. A further isolate, 0803, which has almost identical genetic and biochemical properties to those of 0858 [2,15], was shown to carry precisely the same mutation as 0858 (not shown). These strains may not, however, have been independent isolates (R.M. Hall, personal communication).

Finally, the definitive *oli2* mutant D22/A15 was found to carry the substitution $Ser_{175} \longrightarrow$ Cys. This mutation is 10 nucleotides away from the mutation denoted *oli-2* by Macino and Tzagoloff [14] (table 2), and would be consistent with the assignation of both mutations to the same locus, due to the failure to observe recombinants between them ([12]; cf. [15]).

The only other *oli2* oli^{R} mutation for which sequence data are available (table 2) is that denoted *oli-4* by Macino and Tzagoloff [14] ($Leu_{232} \longrightarrow$ Phe) which lies some 180 bases away from the cluster of other mutations considered here. Unfortunately, no sequence data are available on the reference *oli4* strain characterized genetically by Clavilier [11].

Since the mutations described here in molecular terms clearly all lie within the *oli2* gene, there seems no compelling reason not to denote each of them alleles of the *oli2* locus, as suggested in tables 1 and 2. This is consistent with the unified nomenclature proposed for oli^{R} mutations in the *oli1* gene [7]. It would also seem desirable to consider renaming other mutations conferring antibiotic resistance reportedly closely linked to the so-called *oli2* and *oli4* loci, such as *oss1* [21], if nucleotide sequence analysis demonstrates the mutational site to lie within the *oli2* gene.

3.3. *Delineation of domains in subunit 6 involved in oligomycin resistance*

The known amino acid substitutions in subunit 6 leading to the oli^{R} phenotype (tables 1 and 2) are confined to two previously identified [15] evolutionarily conserved regions of this protein (fig.1). These two relatively short regions of amino acid se-

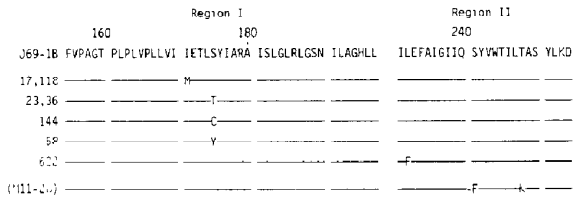

Fig.1. Disposition of substitutions conferring oligomycin resistance in two evolutionarily conserved regions of subunit 6. Regions I and II, delineated in [15] extend from amino acid residues 155–197, and 231–254, respectively. Allele numbers of *oli2* oli^R mutants are indicated at the left. The relevant amino acid substitutions (tables 1 and 2) are indicated within the horizontal lines representing wild-type sequence. Allele *oli2-58* confers a partial *mit*$^-$ phenotype (see text). The substitutions [17] in a *mit*$^-$ strain M11-28 are also indicated.

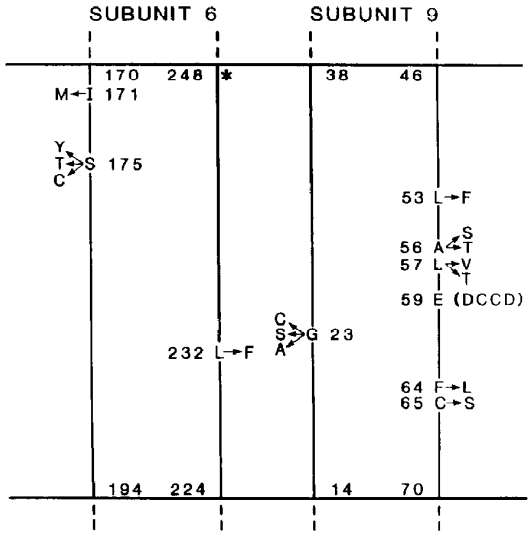

Fig.2. Location of amino acid substitutions conferring oligomycin resistance in membrane-spanning domains of mtATPase subunit 6 and 9. Vertical lines represent the 4th and 5th transmembrane helices [4] of subunit 6, and the two transmembrane helices of subunit 9 [3]. The length of a transmembrane span is taken to be 25 amino acid residues [4]. Data for amino acid substitutions leading to the oli^R phenotype are compiled for subunit 6 (tables 1 and 2), and subunit 9 ([7,12,13]; T.A. Wilson and P. Nagley, unpublished). The position of the DCCD-binding residue Glu_{59} is shown. Asterisk indicates the location of the critical amino acid substitution (Thr_{248} → Lys) in the *mit*$^-$ strain M11-28 (see text and fig.1).

quence correspond closely to the 4th and 5th transmembrane α-helices in a structure proposed for subunit 6, in which 5 transmembrane spans are predicted [4] (see fig.2). In this folding scheme the amino acid substitutions conferring the oli^R phenotype occur exclusively in these adjacent membrane spanning domains (fig.2).

3.4. *Evidence for structural and functional interaction of subunits 6 and 9*

Oligomycin is an antibiotic which inhibits mtATPase function by preventing the translocation of protons through the F_0-sector. Evidence from radiochemical labelling studies is interpreted to suggest that the primary binding site for oligomycin lies within subunit 9 [22,23]. This raises the question of how amino acid substitutions in subunit 6 confer resistance to oligomycin, if indeed oligomycin does not bind to subunit 6 directly. The occurrence of *oli2* oli^R mutations can be rationalised if subunits 6 and 9 are intimately associated in the membrane. The altered subunit 6 structure in the oli^R mutants could then allosterically alter the oligomycin binding site of subunit 9 (cf. [5,7]). This could be mediated, for example, by direct interaction of the transmembrane domains represented in fig.2.

Current concepts of the topological organization [4,5] of the F_0-sector of the *E. coli* proton-translocating ATPase complex place emphasis on dynamic structural and functional interactions between subunits *a* and *c* (homologous to yeast subunit 6 and 9, respectively), although the precise relative orientation of these subunits is still controversial. In one such proposal [4] the amphipathic 4th transmembrane helix of subunit *a* is juxtaposed with the C-terminal stem of subunit *c* and may constitute the proton pore. Applying this scheme to the yeast F_0-sector, the translocation of protons could involve an array of polar amino acids in region I (fig.1) of subunit 6 (the left-hand stem of subunit 6, fig.2) and the DCCD-binding Glu_{59} of subunit 9 (in its right-hand stem, fig.2). Evidence that the two transmembrane helices of subunit 6 (fig.2) may indeed play a direct role in energy transduction arises from the identification, in each of these helices, of amino acid substitutions that compromise mtATPase function. In con-

served region I the deleterious effects of the substitution $Ser_{175} \longrightarrow$ Tyr in isolate 0858 have already been considered above. In conserved region II (right-hand stem of subunit 6, fig.2) we have identified [17] the critical mutation ($Thr_{248} \longrightarrow$ Lys) (cf. figs 1 and 2) in a *mit*$^-$ mutant M11-28, that is unable to synthesize ATP intramitochondrially. Further work will be required to establish the role of this 5th transmembrane helix in energy transduction and its interaction with other membrane domains in the F_0-sector.

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