

Amino acid substitutions in mitochondrial ATP synthase subunit 9 of *Saccharomyces cerevisiae* leading to venturicidin or ossamycin resistance

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A series of mitochondrially inherited mutants of yeast has been analysed, which were previously identified as showing resistance to the antibiotics venturicidin or ossamycin and whose mutations showed tight linkage to oligomycin-resistance alleles affecting subunit 9 of the mitochondrial ATP synthase. DNA sequence analysis of the *oli1* gene of these mutants has been used to define the nature of amino acid substitution in the subunit 9 protein. In the case of the two venturicidin-resistant mutants, mutations affect amino acids on the N-terminal stem of the protein, namely Gly25→Ser (*ven^R oss^S oli^R*) and Ala 27→Gly (*ven^R oss^S oli^S*). The mutations found in the two ossamycin-resistant mutants affect amino acids on the C-terminal stem of the protein, namely Leu53→Phe (*van^S oss^R oli^R*) and Leu57→Phe (*ven^S oss^R oli^S*). These results allow us to further develop a fine structure map of domains within the subunit 9 protein involved in antibiotic interaction.

ATPase complex, mitochondrial; Amino acid substitution; Venturicidin; Ossamycin; Drug-resistance domain; Gene, *oli1*; (*Saccharomyces cerevisiae*)

1. INTRODUCTION

The yeast mitochondrial *oli1* gene encodes the dicyclohexylcarbodiimide-binding proteolipid subunit 9 of the mitochondrial ATP synthase complex [1]. Subunit 9 is a 76 amino acid proteolipid which resides in the membrane F_0 sector of the mitochondrial ATP synthase (mtATPase) and plays a major role in the proton-translocation activity of the enzyme complex [2]. Subunit 9 adopts a hairpin structure in the inner mitochondrial membrane, with two transmembrane stems and a hydrophilic loop [3] which is thought to interact with the F_1

sector of the enzyme on which ATP synthesis occurs. The drugs venturicidin, ossamycin and oligomycin all act to block proton-translocation through the membrane-associated F_0 sector of the mtATPase [4], interacting particularly with subunit 9 [5]. In previous work, direct amino acid sequence analysis of the subunit 9 protein [6] and nucleotide sequence analysis of the *oli1* gene of oligomycin-resistant (*oli^R*) mutants [7] revealed amino acid substitutions on the C-terminal stem in the vicinity of Glu59 and on the N-terminal stem of the protein close to Gly23. Analysis of those *oli^R* mutants which showed cross-resistance to venturicidin suggested that the domain of resistance to venturicidin lies entirely within the oligomycin-resistance domain [8].

Another set of mutants, isolated primarily on the basis of their venturicidin-resistant (*ven^R*) or ossamycin-resistant (*oss^R*) phenotype, has been subjected to extensive genetic analysis (including petite deletion analysis and recombination studies

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Abbreviations: mtATPase, mitochondrial proton-translocating ATP synthase; mtDNA, mitochondrial DNA; *ven^R*, venturicidin-resistant; *oss^R*, ossamycin-resistant; *oli^R*, oligomycin-resistant (in reference to phenotype)

Table 1

Molecular genetic characteristics of drug-resistant strains affected in the *oli1* gene

Strain	Allele designation		Phenotype	DNA sequence number	Nucleotide change	Codon number	Codon change	Amino acid change
	Previous	Current						
D22/61	V ^R 61	<i>oli1-61</i>	ven ^R oli ^R	+ 73	G→A	25	GGU→AGU	Gly→Ser
D22/60	V ^R 60	<i>oli1-60</i>	ven ^R	+ 80	C→G	27	GCU→GGU	Ala→Gly
D27/110	oli ^R -110	<i>oli1-110</i>	oli ^R oss ^R	+ 159	A→T	53	UUA→UUU	Leu→Phe
D27/101	oss ^R -101	<i>oli1-111</i>	oss ^R	+ 171	A→C	57	UUA→UUC	Leu→Phe

[4,9]). Although these analyses showed that these mutations were mitochondrially inherited and tightly linked to representative alleles of the *oli1* locus, the definitive fine structure genetic mapping of this set of mutations was not possible [9].

In this work a series of four mutants in this group, resistant to venturicidin or ossamycin, have been studied by DNA sequence analysis of the *oli1* gene. The definition of the nucleotide substitutions allows correlations to be made with recombinational analyses; the consequent amino acid changes extend our insight into domains within subunit 9 involved in interactions with antibiotics.

2. MATERIALS AND METHODS

2.1. Strains

The strains of *Saccharomyces cerevisiae* analysed here (table 1) were isolated by W.E. Lancashire [4,9], and were available in the collection maintained in Colorado. These mutants were primarily selected for their resistance to venturicidin or ossamycin; relevant cross-resistance patterns are shown in table 1. A systematic designation of the allele number is given for each mutation, together with the original denotation (table 1).

2.2. Methods

Growth of strains, extraction of mtDNA and nucleotide sequence determination of the *oli1* gene were carried out as described [7].

3. RESULTS AND DISCUSSION

3.1. Nucleotide and amino acid sequence changes

The single nucleotide changes observed relative to the J69-1B wild-type sequence, together with the consequent amino acid substitutions, are shown in table 1. Strain D22/61 is resistant to both oligomycin and venturicidin. Its mutation leads to

the amino acid substitution Gly25 → Ser; it is thus distinct from previously characterised ven^Roli^R mutants substituted at Gly23 [8].

Strain D22/60 represents a class of mutant not previously analysed at the molecular level, resistant to venturicidin but sensitive to oligomycin. The mutation in this mutant leads to the amino acid substitution Ala27 → Gly. Thus, the mutations in both D22/61 and D22/60 lead to changes in subunit 9 located in the N-terminal stem very close to the previously recognised oligomycin-resistance substitutions at Ala22 [10] and Gly23 [7,8].

Two ossamycin-resistant mutants were also analysed. Strain D22/110 (oss^Roli^R) contains the amino acid substitution Leu53 → Phe. Precisely the same mutation affecting Leu53 has been shown in many cases to be associated with an oli^Rven^S phenotype [5]; presumably, in most instances resistance to ossamycin was not tested. Perhaps the oss^Roli^R phenotype of D27/110 is characteristic of the Leu53 → Phe substitution; alternatively, the oss^R phenotype may be elicited specifically in this strain by other as yet unidentified mitochondrial or nuclear factors. For example, when the mtDNA of a oli^Rven^R mutant (0851) carrying the subunit 9 substitution Phe64 → Leu, is transferred into different nuclear backgrounds, the resulting strains show varying resistance to venturicidin [8].

Strain D27/101, which is oss^R but showed no cross-resistance to other drugs tested, carries the amino acid substitution Leu57 → Phe. This particular mutation at Leu57 has not been observed before and is of interest because another substitution at the same position, Leu57 → Val, leads to the oli^Rven^R phenotype [6]. No data on the ossamycin-resistance of the latter strain (D273-10B/A68) have been reported.

3.2. Correlation of recombinational data with location of mutation

Whilst attempts at the fine-structure genetic mapping of drug-resistance mutations in the *oli1* gene using recombinational analysis yielded much data, no coherent mapping of alleles, or even of loci defined by phenotype, could be obtained until amino acid and DNA sequence analyses were applied [6,7].

These problems can be illustrated with reference to genetic recombinational data reported for the mutations analysed here. Recombinational analysis [4] of the mutations in the strains D22/60 and D22/61 yielded an observed recombinational frequency of 1.5%, suggesting that these mutations are some distance apart. Yet the DNA sequence analysis carried out in this work (table 1) shows that these mutations are only 7 base pairs apart in the *oli1* gene. By contrast, the mutation in D22/60 was found to yield a recombination frequency of <0.2% with the reference *oli1-1* allele [4]. This *oli1-1* mutation has been assigned by DNA sequence analysis to nucleotide +159, which is 86 base pairs away from the mutation in D22/61 (nucleotide +73; table 1). Moreover, the mutation in D22/61 was found to recombine with a frequency in the order of 0.1% when crossed to D273-10B/A68 carrying the *oli1-119* mutation (localised to nucleotide +170 [6]). Nevertheless, in a separate study the mutation in D22/61 was found to recombine with the *oli1-1* mutation (nucleotide +159) at a frequency of 3–4% [4]. The molecular rationalization of these erratic recombination frequencies is not understood.

On the other hand, similar analyses carried out for the mutations of D27/101 and D27/110 showed recombinational data to be consistent with the DNA sequence analysis. Thus, the mutation in D27/110 was not found to show detectable recombination with the *oli1-625* mutation (nucleotide +159) in strain CD9 [9], consistent with the present demonstration that the mutations are precisely allelic. Moreover, the mutation in D27/101 was found to recombine at a frequency of 0.3% with the *oli1-625* mutation [9] consistent with the demonstrated physical separation of their mutations by 12 base pairs on the *oli1* gene. It is clear that the most definitive way to map the mutations in such drug-resistant mutants is direct DNA sequence analysis of the *oli1* gene of these mutants.

Similar considerations apply to the *oli2* gene where several drug-resistance mutations are clustered within a 250 base pair block [11,12]. On the other hand the mapping of drug-resistant mutations on the yeast mitochondrial *COB* gene encoding cytochrome *b* is generally more reliable because of the much greater physical distance between alleles [13].

3.3. Domains of subunit 9 involved in inhibitor interaction

The amino acid substitutions in subunit 9 generated by the nucleotide sequence changes reported here give further insight into the domains of this protein involved in drug interaction. Together with all the amino acid substitutions thus far recognised in *S. cerevisiae* subunit 9, this new information not only allows for further expansion of the previously defined oligomycin- and venturicidin-binding domains of the protein but also allows for the definition of an ossamycin-resistance domain. The drug-resistance domains, within which the reported amino acid substitutions lie are shown in fig.1. The oligomycin- and the venturicidin-resistance domains are centred on amino acids 23 and 59 and encompass both the N-terminal and C-terminal stems of the protein. The venturicidin-resistance domain was previously thought to lie entirely within the oligomycin-binding domain [10]. Whilst the substitution at amino acid 25 in the *ven^Roli^R* mutant D22/61 is consistent with this generalisation and extends the common domain, the substitution at amino acid 27 arising in the *ven^R* mutant D22/60 indicates that the venturicidin-binding domain does not lie entirely within the oligomycin-binding domain at this point. There are thus two overlapping, but independent domains for resistance to these inhibitory drugs, which is not altogether surprising in terms of their very different chemical structures.

The amino acid substitutions leading to ossamycin resistance in the mutants D27/101 and D27/110 lie in close proximity to each other on the C-terminal stem of the protein at amino acids 57 and 53, respectively. As these amino acids also lie close to the boundaries of the oligomycin- and venturicidin-resistance domains it may be inferred that this region of the protein interacts in some way with all three inhibitors.

It is of interest that a further ossamycin-

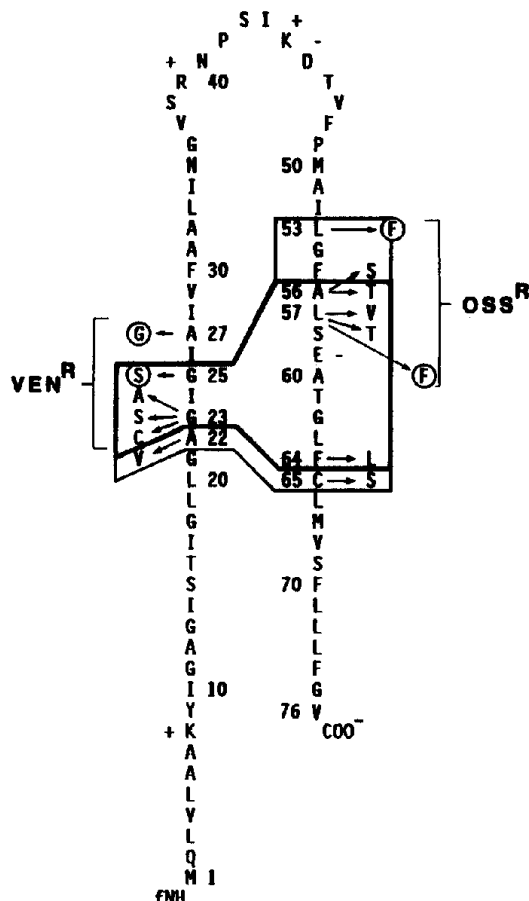


Fig.1. Intra-membrane domains of drug resistance of subunit 9 in yeast mtATPase. The subunit 9 polypeptide is represented in a hairpin configuration [2,5], spanning the membrane [3]. The membrane domains of resistance to either oligomycin (thin lines) or to both oligomycin and venturicidin (thick lines) in the membrane-internal portion of subunit 9 are indicated. Amino acid substitutions conferring ossamycin-resistance or venturicidin-resistance, identified in this paper, are circled. The ossamycin-resistance phenotype of all mutants carrying the substitution Leu53→Phe is not certain (see text).

resistance mutation in the yeast mitochondrial *oli2* gene (allele designated *Oss^r1-92*), has been shown to direct the substitution of an amino acid in subunit 6 of mtATPase (Asp254 → Asn) which is conjectured to lie outside the membrane [11]. The molecular details of the interaction of ossamycin with subunits 9 and 6 both within and outside the membrane remain to be established.

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