EXTRACHROMOSOMAL INHERITANCE OF OLIGOMYCIN RESISTANCE IN YEAST

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1. Introduction

Yeast mitochondria have several features in common with bacteria [1–3] including similar sensitivity of their protein synthesis to antibiotics [1, 4]. These antibiotics inhibit the growth of yeast when non-fermentable carbon sources are used. Erythromycin resistant mutants were isolated by use of this property [5]. The resistance was inherited in non-Mendelian way and was linked to ρ [6, 7], in contrast to chromosomal inheritance of resistance to other drugs such as actidione [8–10]. Chromosomal inheritance of oligomycin resistance was recently reported [11]. We have isolated a mutant resistant to oligomycin using 2,6-diaminopurine; this resistance was inherited cytoplasmically but was not removed by acriflavine nor by introduction of a nuclear gene which changed ρ^+ to ρ^-. Therefore the gene involved in this resistance was cytoplasmic but was not linked to ρ.

2. Experimental

Media: YPG medium was the same as that used in the previous study [12] except that a glucose concentration of 1% was used. For YPY medium, 1% glycerol was used instead of 1% glucose. Minimal medium was similar to the modified medium of Burkholder without adenine, arginine, histidine, leucine and lysine.

Strains: Haploids 102D (α, ade, ρ^+) and 706 (a, his, leu, thr, ρ^+) were sensitive to oligomycin. A haploid 101D (α, arg, lys, p, ρ^-) carried the p respiratory gene which caused the change from ρ^+ to ρ^-, previously described as the r_2 gene [13]. 102D and 101D were segregants from the same cross. All the strains used were heterothallic.

102D was inoculated into liquid YPG medium containing 2,6-diaminopurine (500 μg/ml) and was incubated at 30°C overnight. Cells were transferred to liquid YPG medium at 30°C and were then selected on a YPY plate containing oligomycin. 102P7 thus isolated was resistant to oligomycin at 1 mg per plate whereas the original 102D was inhibited by oligomycin at 25 μg per plate. The resistance was stable throughout successive subculture for a year. 102P7 was a deep red color. Petite mutants, 102P7SP, 102P7AC, 706AC and 102AC were obtained from 102P7, 706 and 102D, respectively, either by a spontaneous mutation or by acriflavine treatments as described previously [12].

Assay of resistance: 100 μg of oligomycin in ethanol were spread and dried on a YPY plate. About 10^4 cells to be tested were then spotted on assay plates and the plates were incubated at 30°C. Growth was examined after 3 days.

Preparation of mitochondria: The remove yeast cell walls, combined methods of reduction of disulphide groups by 2-mercaptoethanol and of digestion of cell wall with snail gut enzyme were used. The preparation of mitochondria from protoplasts was similar to a method described by Ohnishi et al. [14].

ATPase assay: The components of reaction mixture are given in table 3. The reaction was run at 30°C for 10 min and was stopped by the addition of silicotungstate. Determination of inorganic phosphate was per-

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Table 1
Resistance of dipoids from crosses between oligomycin-resistant and -sensitive cells.

Cross no.		Number of diploids examined	Oligomycin resistance	
			Resistant	Sensitive
1a	102P7 X 706	16	16	0
1b	102P7 X 706	32	32	0
2a	102P7SP X 706	16	16	0
2b	102P7SP X 706	32	32	0
3	102P7AC X 706	32	32	0
4	102D X 706	16	0	16
5	102AC X 706	16	0	16

Table 2
Tetrad analysis of a diploid from a cross between 102P7 and 706.

	Number of tetrads	Oligomycin resistance		Tolerance
		Resistant	Sensitive	
Complete	3	4	0	all >1 mg
Incomplete	10	3	0	all >1 mg

Table 3
Oligomycin sensitive ATPase of mutant yeast.

	-Oligomycin (μmoles/min/mg)	+Oligomycin (μmoles/min/mg)	Inhibition %
102D	2.7	0.33	88
102P7	4.3	0.34	92

The reaction mixture contained 50 μmoles of tris buffer pH 7.4, 1 μmole of $MgSO_4$, 5 μ moles of phosphoenolpyruvate, 30 μg of pyruvate kinase, 2 μmoles of ATP and about 30 μg of mitochondria with and without 10 μg of oligomycin in a total volume of 1 ml.

formed according to Martin et al. [15] and protein was determined according to Lowry et al. [16].

3. Results and discussion

Crosses were performed between 102P7 and 706 by mass mating technique and the resulting zygotes were selected on minimal media. Cells from each colony were then spotted on assay plates. Table 1 showed that

the zygotes were resistant. No growth of zygotes was observed in crosses of sensitive cells. The zygote derived from cross no. 1a was then subjected to tetrad analysis. Due to bad spore viability, 3 complete tetrads were obtained from 30 asci dissected. All these complete tetrads showed 4:0 (resistant to sensitive) segregation. Furthermore every three spores which survived from ten sets of incomplete tetrads were all resistant. All 42 spores isolated showed full resistance to oligomycin up to 1 mg per plate (table 2). No sensitive spore was found in those so far examined while nuclear markers segregated 2:2 in complete tetrads. These results support the existence of a cytoplasmic gene concerning oligomycin resistance.

Concerning the reported chromosomal inheritance of oligomycin resistance in UV induced mutants [11], dual resistance to streptomycin in Chlamydomonas might be a similar case. In the latter instance the presence of both nuclear and cytoplasmic inheritance of streptomycin resistance was reported [17].

In spite of the cytoplasmic inheritance of the resistance studied here, it was not possible to eliminate this resistance by a change from ρ^+ to ρ^-. As shown in table 1, petite mutants of 102P7 were crossed with 706 (cross no. 2a, 2b, 3) and the resulting zygotes were tested for oligomycin resistance in the same way. Again the zygotes showed resistance. Results were the same for both spontaneous and acriflavine induced mutants. Cross no. 5 was performed to exclude eventual introduction of resistance during acriflavine treatment. That no resistant colony was found in the zygotes supported the idea that resistance had persisted in petite mutants of 102P7. In contrast to the oligomycin resistance, resistance to erythromycin was reported to be eliminated by acriflavine treatment with the exception of strain L300 ρ^- w [7].

When mitochondrial ATPase was measured directly, mitochondrial ATPase of 102P7 and 102D were equally sensitive to oligomycin (table 3). This suggested that the mechanism of resistance was related to a change in permeability or induced detoxification. This data agreed well with the above findings that resistance was not linked to ρ, since oligomycin does not inhibit mitochondrial ATPase of ρ^- mutants i.e. cytoplasmic petite ($P\rho^-$) [18]. The presence of oligomycin sensitivity in 102P7 agreed with the conclusion that the mutation occurred at a site other than ρ.

An attempt was made to eliminate ρ by introduction of a p gene epistatic to ρ, into an oligomycin resistant cell to see whether resistance could be removed together with ρ. An oligomycin resistant haploid 321C derived from a complete tetrad in table 2 was crossed with 101D. The spores which received the p gene were isolated from the zygote by tetrad analysis. A resistance test, performed in the same way, showed that 6 spores examined retained resistance in all cases.

These results support the hypothesis that the genetic determinant involved in oligomycin resistance is different from ρ, although both are inherited cytoplasmically. This does not necessarily indicate that the genetic material involved in this resistance differs from mitochondrial DNA, since satellite DNA still remained in acriflavine induced mutants [19]. However the presence of a specific genetic determinant such as an R factor in bacteria remains a possibility.

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