OLIGOMYCIN RESISTANCE IN YEAST. LINKAGE OF THE MITOCHONDRIAL DRUG RESISTANCE

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1. Introduction

The presence of the extrachromosomal genetic element in yeast was reported in connection with the oligomycin resistance [1–3]. Erythromycin resistance has also been demonstrated to be associated with the extrachromosomal genetic element [4] and to give rise to a change in mitochondrial ribosomes [5]. It was shown in the previous paper that one of the oligomycin-resistant mutants possessed the altered function of the mitochondrial enzyme [3]. Thus, the recombination of these two mitochondrial genes was studied [3]. Further investigations revealed the presence of two incompatible plasmids in the yeast cells. The recombination of these plasmids is reported in this paper.

2. Materials and methods

2.1. Media

YPG media, YPY media, and YPY media with antibiotics were the same as those described in the previous papers [1, 3], except that the amount of oligomycin used was changed to 80 μg per plate. The YPY media with erythromycin or oligomycin or both are designated YE, YO, or YEO media, respectively, in this paper. For the minimal medium, yeast nitrogen base was replaced with yeast extract plus peptone in the YPG medium.

2.2. Strains

A double-resistant diploid, obtained from the cross 102E × 706R1 described in the previous paper [3], was plated on a YEO plate, and a single colony, EG9R, was isolated for the tetrad analysis. A double-resistant haploid R8 (a, ade1, leu2) was obtained by tetrad analysis. R8 was treated with 2×10^{-5} M ethidium bromide and a petite R8ET was isolated. Twenty six [rho]$^-$ mutants obtained by the spontaneous mutation from R8 were denoted R8SP. 1586-2B (α, leu1, met13, lys5, his1) was kindly donated by Dr. S. Nakai in National Institute of Radiological Sciences.

2.3. Genetic markers

The oligomycin resistance and the erythromycin resistance are denoted [oli] and [ery], respectively, in this paper.

2.4. Crosses and the assay of resistances in the diploids

Cells of R8 and 1586-2B were preincubated and crossed in YPG medium for 3.5 hr at 30°. A portion of the zygotes formed were spread on the plates with minimal media after suitable dilutions. The remaining zygotes were removed from the YPG medium and kept grown in the minimal media. After 24 hr and 72 hr, portions of the cells were removed and spread on plates with minimal media after suitable dilutions. The cells in each colony on the minimal media were suspended in a saline solution and about 10^4 cells were spotted on YPY, YE, YO, and YEO plates. The growth of the resistant cells was determined after 3 to 4 days.

For detection of the resistances in [rho]$^-$ cells, cells

of R8SP and 1586-2B were crossed and then spotted on plates with minimal media. A mixture of diploid cells grown in the spot was taken, and the cells were assayed on YPY, YE, YO, and YEO plates in the same way. For the analysis of each diploid cell, cells were spread on the minimal media after 3.5 hr and 24 hr and the tests were performed on $[rho]^+$ diploids.

3. Results and discussion

The double-resistant diploid EG9R was subjected to tetrad analysis. On 12 asci dissected, the oligomycin and erythromycin resistances showed segregation of 4:0 (R:S), while the nuclear markers segregated 2:2. The fact that all 48 spores were double resistant not only provided evidence for the extrachromosomal inheritance of these genes but also suggested the association of these genes. No loss of either of these resistances was observed in 416 colonies of a double-resistant haploid R8, when investigated by the replica method. Thus the dissociation of these genes in R8 was less than 0.25%. When cells of R8 were crossed with the double-sensitive cells of 1586-2B, the sensitive factors in 1586-2B segregated jointly in diploids. Table 1 shows that the double-resistant diploids and the double-sensitive diploids were found in almost equal numbers at 24 hr. The fact that both genes behaved jointly indicates the presence of two incompatible plasmids harboring both resistances or both sensitivenesses. They are probably mitochondria, in view of the reports showing that these genes are related to mitochondria.

Table 1

Segregation of resistances in a cross between R8 and 1586-2B.

	Number of colonies				
(hr)	type E	type O	type E+O	type EO	type SS
3.5	0	10	2	89	1
24	4	19	0	43	36
72	10	14	0	32	45

Type E or O or EO indicated the type of the colonies able to grow on YE or YO or YEO medium. Colonies of type E+O were able to grow on YE and YO medium but unable to grow on YEO medium. Colonies of type SS were unable to grow on YE and YO medium.

These two resistances in R8 were removed simultaneously with ethidium bromide. When R8ET was crossed with 1586-2B, the resulting diploids were found to be devoid of both resistances. This is explained by the withdrawal of the plasmids with which both resistances are associated.

Table 2

Resistances of diploids obtained from crosses R8SP9 × 1586-2B and R8SP2 × 1586-2B.

		Number of colonies				
Crosses	(hr)	type E	type O	type E+O	type EO	type SS
R8SP9 × 1586-2B	3.5	3	48	2	12	32
	24	4	22	0	2	72
R8SP2 × 1586-2B	3.5	4	89	1	0	5
	24	5	71	2	0	22

Explanations of the type E, O, EO, E+O and SS were given in table 1.

These plasmids seemed to be mixed in zygotes formed within 3.5 hr. Accordingly, almost all of the colonies formed from these zygotes possessed both resistances and yielded double-resistant cells, as shown in table 1. The formation of oligomycin-resistant diploids suggests recombination within 3.5 hr. Apart from the parental-type diploids, some recombinant-type diploids were found after 24 hr. Based on the absence of segregation of the single-resistant haploid in the cells of R8, the transfer of one of these resistances to another plasmid is indicated.

However, the partial loss of these resistances was found in some spontaneous $[rho]^-$ mutants. When 26 spontaneous $[rho]^-$ mutants were tested for the resistances after crossing with 1586-2B, five types were distinguished. The absence of both resistances (type SS) or of erythromycin resistance (type O) or of oligomycin resistance (type E) was found. Thus the elimination of the genetic material was in some cases restricted to a part of the mitochondrial genes.

R8SP9, which was a suppressive petite, was crossed with 1586-2B and the mixture of $[rho]^+$ diploids formed after 24 hr was tested for the resistances. The cells were capable of growing on YEO plates (type EO). On testing 97 colonies of these diploids taken after 3.5 hr, a high number of diploids resistant to oligomycin (type O) were found as shown in table 2. This can be explained by the fact that the linkage between [rho] and [ery] is closer than that between [ery] and [oli]. $[ery]^r$, which was not transferred to $[rho]^+$ plasmids, seemed to be linked with $[rho]^-$ and was probably

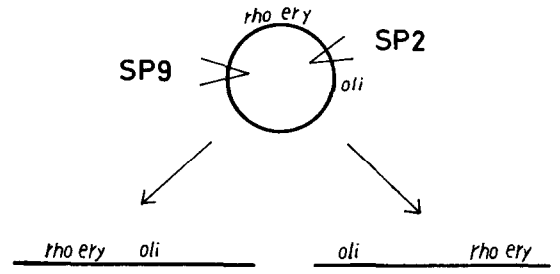

Fig.1. A model for the occurrence of two types of the mitochondrial genes.

present in the $[rho]^-$ diploids that segregated at high rates (54%). The formation of double-resistant diploids (type EO) showed recombination of the $[rho]^+$ gene with the $[ery]^r[oli]^r$ gene, indicating the sequential localization of these genes in the order [rho] [ery] [oli]. The preferential growth of diploids in the minimal media after mating resulted in the increase of the parental SS type and the decrease of the recombinant type. This is explained by the fact that the segregation of the sensitive plasmids and the recombinant-type plasmids occurred, both of which were present in the zygotes.

R8SP2, which was also a suppressive petite, gave rise to diploids, that grew on plates E and O but grew very poorly on plate EO (type E+O). From the investigation of each $[rho]^+$ diploid colony, it was found that diploids of type EO were absent, and diploids of type O were numerous. The process of recombination thus seemed to differ from that of R8SP9. Based on the high rate of formation of type O, very loose linkage between $[oli]^r$ and $[rho]^-$ was postulated. The lack of type EO in the case of R8SP2 in contrast to R8SP9, is best explained by the sequential localization of three genes in the order [oli] [rho] [ery], giving rise to the double-resistant diploids at very low rates due to the double crossing over required.

Two postulated forms of the mitochondrial genes, [rho] [ery] [oli] and [oli] [rho] [ery] can be explained without difficulty by the intermediary circular form of the mitochondrial genes. This model also offers a good explanation for the high rate of transfer of [oli] gene in R8SP2, assuming that the linkage between [ery] and [oli] is closer than the linkage between [rho] and [oli], as depicted in fig.1. The partial loss of the mitochondrial gene by the $[rho]^-$ mutation seems to render its linear form stable.

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