OLIGOMYCIN RESISTANCE IN YEAST. II

CHANGE IN MITOCHONDRIAL ATPase OF A MUTANT AND ITS GENETIC CHARACTER

Sir:

Studies on the cytoplasmic drug resistance in yeast were introduced by LINNANE *et al.*[1] and SLONIMSKI and his associates[2]. Despite these well elaborated genetic investigations over four years, gene action of these resistances were still poorly correlated with the function of mitochondria. The mechanism of cytoplasmic erythromycin resistance was reported to be due to a change of mitochondrial ribosomes[3]. Several nuclear and cytoplasmic oligomycin resistant mutants of yeast were isolated[4~7], but the action of these resistance genes are still under discussion. To obtain a good comprehension of the mitochondrial gene, efforts were made in this laboratory to obtain an antibiotic resistant mutant, whose gene action was based on the function of mitochondria.

An oligomycin resistant mutant of *Saccharomyces cerevisiae* was isolated in order to obtain a mutant carrying a genetic marker of mitochondrial protein, in this case an oligomycin-rutamycin sensitivity conferring protein. A mutant 706 Rl was obtained from the yeast strain 706 (*a*, his, leu, thr) by use of 2, 6-diaminopurine as described previously[5]. It was anticipated from the enzymic property of this protein, that this mutant would be cross resistant to rutamycin. Mutant cells were tested for growth on yeast extract-peptone-2 % glycerol medium on which 500 μg of rutamycin was spread as described in the previous paper[5]. This mutant was resistant to rutamycin but sensitive to other drugs such as erythromycin (3 mg/ml), spiramycin (4 mg/ml), and antimycin (20 μg/ml). These facts rendered the above assumption plausible. It was rather difficult to explain the cross resistance to rutamycin by the reduced permeability of the membrane of mitochondria or cells.

For the rigorous chemical proof of this assumption, the mitochondria were isolated from the sensitive and resistant cells, and the sensitivity of mitochondrial ATPase was assayed with rutamycin. Rutamycin was used throughout the study on ATPase on account of its availability. Resistant or sensitive cells were grown in 1 liter of liquid YPY medium (yeast extract-peptone-10 % glycerol) supplemented with histidine, leucine, and threonine at 1×10^{-4} M at 30°C for 20 hours with or without rutamycin. Cells were first precultured in YPG medium (yeast extract-peptone-glucose), washed well with saline and suspended in 1 liter of YPY medium to give the absorbance of 0.5. Rutamycin was added to a concentration of 50 μg/ml. The mitochondria were prepared as reported in the previous paper[5] in the presence of 2×10^{-3} M cycloheximide during whole operation. Fig. 1 showed that the mitochondrial ATPase in the resistant cells was less inhibited by rutamycin than in the sensitive cells. Furthermore the insensitivity of the mitochondrial ATPase in the resisiant cells was enhanced when the cells were grown in the presence of rutamycin. The isolated mitochondria possessed ATPase,

Fig. 1. Sensitivity of the mitochondrial ATPase of the oligomycin resistant and sensitive cells to rutamycin.
■—■ sensitive cells 706; ○—○ resistant cells 706 R1 grown under the absence of rutamycin; •—• same cells grown under the presence of rutamycin. About 30 μg of mitochondria was used.

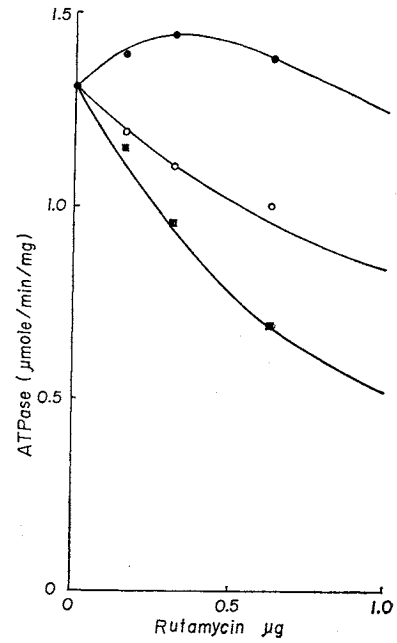

Table 1. Clonal segregation of two oligomycin resistance factors

Cross no.		Oligomycin resistance	
		Resistant	Sensitive
1 706R1×102E	ERY^R	27	27
	ERY^S	14	1
2 102P7×706E		24	0

Diploids were obtained by mass mating, followed by plating on minimum media. The suspension of cells were dropped out on the test medium having each or both of oligomycin and erythromycin. ERY denotes the erythromycin resistance.

which was free from inhibition by rutamycin within 1 μg/ml. Thus the action of the resistance gene was associated with the mitochondrial component.

These changes in the sensitivity of ATPase of strain 706 R1 were in contrast to the absence of the change of mitochondrial ATPase in strain 102 P 7 reported in a previous paper[5]. Genetic characters of these two mutants were also different in two points. (1) The resistance in 706 R1 was removed by the treatment with ethidium bromide at 2×10^{-5} M. A petite 706 R 1-ET thus obtained was crossed with 102 D (α, ade) and the resulting diploids were all sensitive in contrast to 102 P 7. (2) Strain 706 R1 or 102 P 7 was crossed with erythromycin resistant mutants 102 E or 706 E. As shown in Table 1, cross 1 gave segregation of resistant and sensitive diploids while cross 2 gave all resistant diploids. The presence of all four types of diploids with respect to both resistances in cross 1 showed the recombination of two drug resistance factors.

Thus two types of oligomycin resistance were distinguished. The first type reported in 706 R1 in this paper possessed a change which rendered its mitochondrial ATPase insensitive. The mitochondrial component carrying the insensitivity was induced. The genetic character of this mutant was similar to that of cytoplasmic oligomycin resistant mutant[6] and erythromycin resistant mutant[1]. This mutant differed from the second type reported on 102 P 7 previously[5] in terms of mitochondrial ATPase and of genetic characters.

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