OLIGOMYCIN RESISTANCE IN YEAST. IV. LOSS OF MITOCHONDRIAL GENOME IN A NUCLEAR PETITE

Sir:

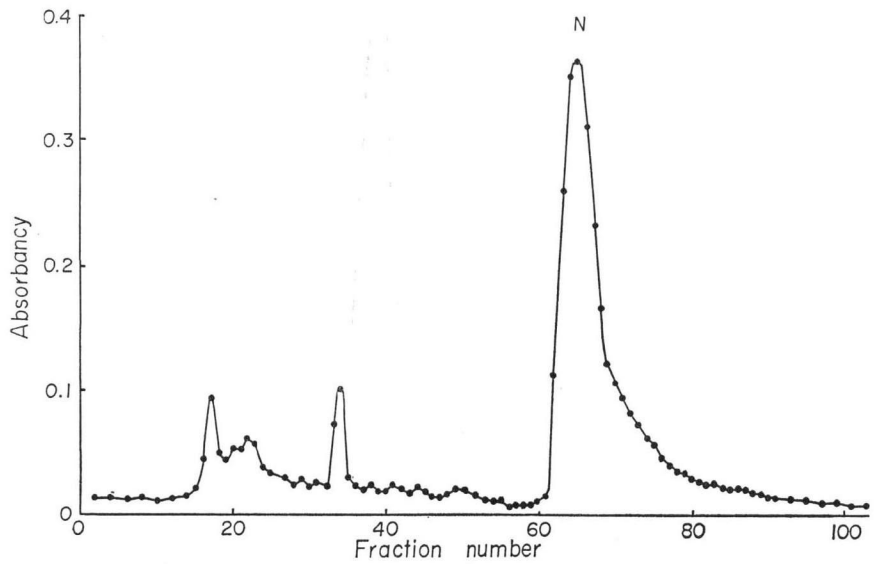
Studies have been done in this laboratory on the linkage of the mitochondrial genes.[1,2,3] Yeast cells which had mitochondrial drug resistance for erythromycin and oligomycin, did not segregate singly-resistant or sensitive cells.[2] However, rho-minus mutants obtained from doubly resistant cells lost both or either one of the resistance characters.[2,4] In the present work, a nuclear petite was studied, in which rho gene was completely lost. The loss of erythromycin resistance and oligomycin resistance was measured and was compared with the loss in a spontaneous rho-minus mutant.

A nuclear petite 101D (α, arg, lys, pet) was crossed with a doubly resistant mutant R8 (a, ade, leu, [oli ery])*. The diploids formed were then tested for the resistances. All of 100 diploids tested showed double resistance, and no sensitive diploids were found. This showed the absence of sensitivity in the cells of 101D. When DNA was isolated from 101D and was chromatographed on a hydroxyapatite column, no peak of mitochondrial DNA was found (Fig. 1). By comparison with the mitochondrial DNA from cells of the wild type, 106A(α, arg), the amount of mitochondrial DNA in mutant 101D is small, if it is present at all (Fig. 2).

The doubly-resistant diploids thus obtained were then subjected to tetrad analysis. All nuclear auxotrophic markers as well as the pet gene segregated 2:2. All 80 haploids from 20 sets of tetrads isolated were found to possess double resistance. Since resistance in haploids having pet gene could not be tested directly on yeast extract-peptone-glycerol medium supplemented with drugs, on account of the respiratory deficiency, these haploids were crossed with tester sensitive rho-plus cells. All these haploids with the pet gene were found to possess double resistance.

This is apparently inconsistent with the absence of sensitiveness in 101D. Accordingly, stability of the resistance in this nuclear petite was examined carefully. A doubly-resistant haploid with pet gene, 801A, (a, leu, pet) was plated and tested by clonal analysis. Cells in each colony were crossed with tester

Fig. 1. Chromatography of DNA from a nuclear petite, 101D.
Hydroxyapatite column was used. DNA was eluted by the linear gradient of phosphate buffer (pH 6.8) from 0.01 M to 0.5 M according to BERNARDI *et al.*[5] N indicates nuclear DNA.



* pet denotes a chromosomal gene of inability to utilize a carbon source for growth. [oli ery] denotes mitochondrial genes for oligomycin resistance and erythromycin resistance.

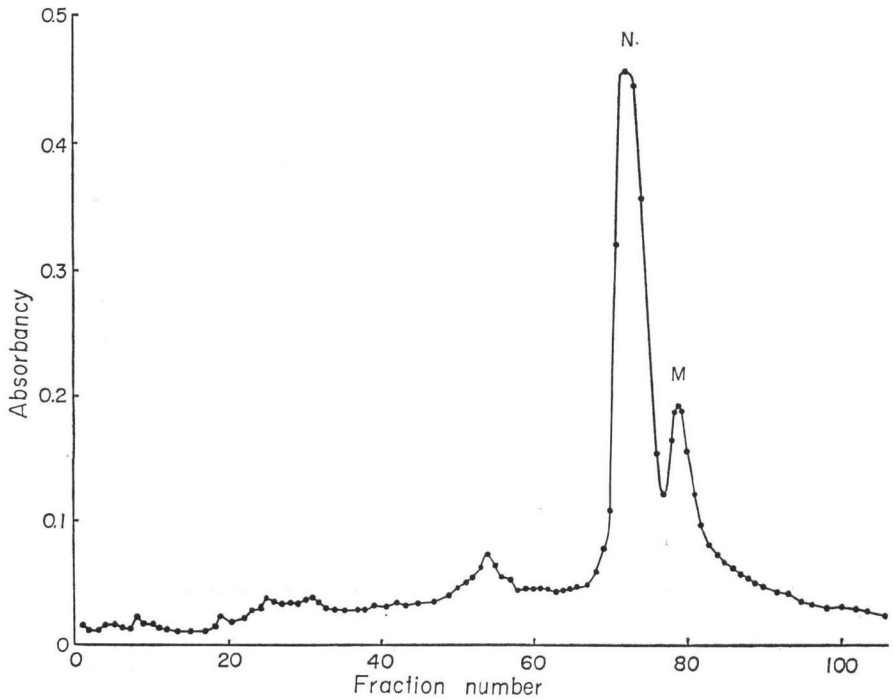
rho-plus cells and the diploids were tested for resistance. It was found that only 7 % of the total colonies were of the doubly-resistant type, 61 % showed oligomycin resistance and 28 % were devoid of resistance as shown in Table 1. When haploid cells in a doubly-resistant colony of the original plate were again plated and subjected to clonal analysis, 75 colonies of a total 90 colonies were of the doubly-resistant type. When cells from the doubly-resistant colonies were then grown for about 30 generations and replated for a second clonal analysis, it was found that only 5 % of the colonies were of the doubly-resistant type. Almost all of the other colonies were devoid of resistances. This low rate of double-resistant cells was almost the same (6 %) after about 40 generations. The doubly-resistant cells were then selected and tested by clonal analysis. It showed that 90 % of the cells were doubly-resistant. However the instability of this double resistance was again demonstrated after growth of the cells for 30 generations. Clonal analysis was carried out and revealed that the rate of the doubly-resistant colonies was again decreased to 45 %. Forty % of the colonies lost resistance to both antibiotics. This high rate of loss of resistance implies that a rapid loss of mitochondrial DNA takes place during the growth of the cells. Thus the cells of 101D, used in the cross 101D× R8 seemed to have lost sensitiveness during

Table 1. Loss of resistances in the progeny of a nuclear petite, 801A.

Progeny	Number of colonies					Total number of colonies
	E	O	E+O	EO	SS	
801A	2	61	2	7	28	100
801A-E0	1	0	0	75	14	90
801A-E030	1	5	0	5	89	100
801A-E040	2	3	0	6	89	100

E, O, E+O, EO and SS denote the type of cells capable of growing on the media with antibiotics. E and O indicate the growth on plate with erythromycin or oligomycin. E+O indicates the growth on both plates but not on plates with both drugs, while EO indicates the growth on all these plates. SS indicates the absence of growth on all these plates.

Fig. 2. Chromatography of DNA from wild type cells, 106A.
Legend is the same as in Fig. 1. M indicates mitochondrial DNA.



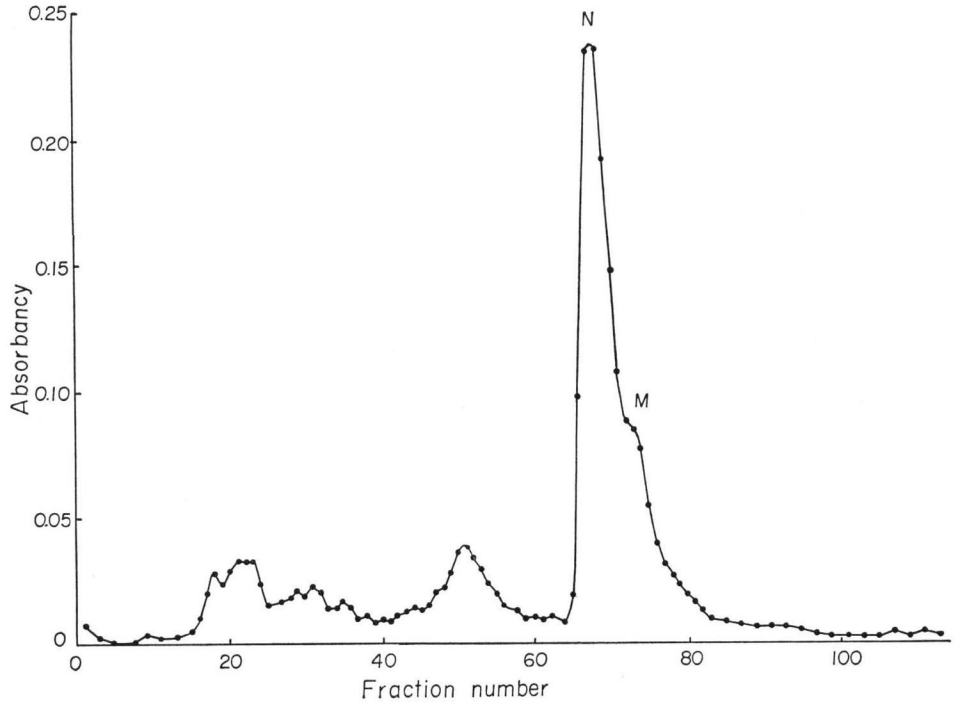
subculture, and explaining the absence of formation of sensitive diploids in the cross 101D ×R8.

R8SP9, a doubly-resistant, spontaneous rho-minus mutant of R8 gave, however, different results. After plating cells of R8SP9 and carrying out the clonal analysis in the same way, it was found that only 8 % of the total colonies were doubly-resistant. When the cells in a doubly-resistant colony were replated, 80 % of the total colonies were found to be of the doubly-resistant type. This high rate of doubly-resistant cells was not altered after subculture and a second clonal analysis showed that 75 % of total colonies were doubly-resistant.

When DNA was extracted from this mutant and was chromatographed on a hydroxyapatite column, a large peak of nuclear DNA was associated with a shoulder, which was eluted at the same concentration of salt, as that for mitochondrial DNA (Fig. 3). The persistence of resistance in this mutant was in agreement with the presence of mitochondrial DNA. This spontaneous petite thus differed from the nuclear petite studied in this paper.

Thus rho-minus mutation in the nuclear petite studied gave rise to a rapid loss of mitochondrial genome, while the mitochondrial genome was retained in the cytoplasmic petite. A difference between the mechanism involved in rho minus mutation in both mutants was suggested by studies on mitochondrial genes and DNA.

Fig. 3. Chromatography of DNA from a cytoplasmic petite, R8SP9.
Legend is the same as in Fig. 1. M indicates mitochondrial DNA.



KAZUHIKO WAKABAYASHI
SACHIKO KAMEI

Department of Biochemistry,
Faculty of Medicine,
University of Tokyo, Tokyo, Japan

(Received May 2, 1974)

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