STUDIES ON THE MITOCHONDRIAL GENE. I

THE RECOMBINATION OF MITOCHONDRIAL DRUG RESISTANCES

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Mutants of yeast with mitochondrial chloramphenicol-resistance, erythromycin-resistance and oligomycin-resistance were crossed and the recombination values were studied. Crosses were compared, which were performed between the cells of the same type and of different types (type I and type II). The recombination values between erythromycin-resistance and oligomycin-resistance did not show any significant change in both crosses, but increased rates of recombination between chloramphenicol-resistance and other two resistance markers were found in the latter crosses. It was also observed in these crosses that the recombination values for [cap]-[oli] was close to the addition of the values for [cap]-[ery] and for [ery]-[oli].

Extrachromosomal inheritance of oligomycin-resistance was first reported by WAKABAYASHI *et al.*[1] Mutants with cytoplasmically inherited oligomycin resistance were also reported by STUART[2] and both mutants differed in several genetic characters. Another oligomycin-resistant mutant, 706R1, which was isolated by WAKABAYASHI was proved to possess change(s) of mitochondrial ATPase[3]. Two different loci for oligomycin-resistance were reported by AVNER *et al*[4].

Crosses of chloramphenicol-resistants and erythromycin-resistants were studied by BOLOTIN *et al.* and asymmetrical formation of recombinants was reported[5]. However, no such data were obtained in a cross in which oligomycin-resistance and erythromycin-resistance were used[6]. Crosses were thus studied in this paper, in which these three mitochondrial drug resistances were involved.

Materials and Methods

Strains: Oligomycin-resistants, N11 and N15, were spontaneous mutants[7] obtained from 1586-2B (α, leu 1, met 13, lys 5, his 1). An oligomycin- and erythromycin-resistant R8 (a, ade 1, leu 2, [ery oli]), an oligomycin-resistant 711 (α, thr 4, [oli]) and a sensitive strain S22 were obtained by the dissection of asci, after sporulation of double-resistant, oligomycin-resistant, and sensitive diploids, derived from a cross 102E (α, ade 1, [ery]) and 706R1 (a, his 4, leu 2, thr 4, [oli]). Strains 1L126-1C (a, ura 1, [cap ery]) and 1L8-8D (a, ura 1, [cap ery]) were donated by P. P. SLONIMSKI. 1L1A was obtained from a cross of 1L8-8D and S22 after dissection of asci.

Media: YPG media consisted of 3.5 g of yeast extract, 3.5 g of peptone, 2 g of KH_2PO_4, 1 g of $(NH_4)_2SO_4$, 1 g of $MgSO_4$ and 10 g of glucose in 1 liter of water, and 2.5% agar was used for solid media. For minimal media 0.67% of yeast extract without amino acids (Difco) replaced the yeast extract and peptone of YPG media. Twenty g of glycerol replaced as nonfermentable carbon source the glucose used in YPG media (medium Y). For media with chloramphenicol and/or erythromycin a 60 mg/ml ethanol solution of chloramphenicol and/or

erythromycin was added to the sterilized medium Y prior to solidification to make the final concentration of each drug 3 mg/ml (medium YCE, YC and YE). Oligomycin media were prepared by spreading 0.2 ml of 0.4 mg/ml ethanol solution of oligomycin on the plates with medium Y with or without chloramphenicol and erythromycin (medium YCO, YEO and YO).

Crosses: Cells to be crossed were preincubated at 30°C in YPG media separately, then mixed and incubated at 30°C for 3.5 hours. The cells were then spun down, washed and the zygotes were further grown in minimal media. After 24 hours, a portion of the cells was transferred to new minimal media. The cells were spread on plates with minimal media at 48 hours.

Assay of drug resistances: The cells in each colony, which grew on plates with minimal media, were spotted on plates with minimal media in a regular pattern. These spots were replica plated to plates with or without antibiotics (plates YC, YE, YO, YCE, YCO, YEO and Y). The plates were incubated at 30°C for 3 days for media Y, YE, YO and YEO and from 5 to 6 days for media YC, YCO and YCE. The numbers of singly resistant recombinants were scored by comparing each spot on plates YC, YE and YO. A few colonies showed growth on plates with different drugs (*i.e.* on YC and YO) but no growth on plates with both drugs (*i.e.* on YCO). These were indicated as C+O *etc.*

Tetrad analysis: Dissection of asci was performed according to JOHNSTON *et al*[5].

Chemicals: Oligomycin, a mixture of 15% A and 85% B, was purchased from Sigma Chemical Co.

Genetic symbols: Genetic symbols are those proposed by VON BORSTEL[9]. Symbols for plasmagenes are enclosed in brackets and cap, ery, and oli indicate chloramphenicol-, erythromycin-, and oligomycin-resistance, respectively.

Results

An oligomycin-resistant mutant, N15, was crossed with an erythromycin- and chloramphenicol-resistant mutant 1L126-1C to investigate the recombination between mitochondrial genetic markers. The zygotes formed were grown in minimal media for 48 hours, followed by plating the diploids on the minimal media. The diploids were then replica plated to plates containing drugs (*i.e.* YC, YO, YE, YCO, *etc.*). After analyzing 247 colonies, it was found that the recombination values for [cap]-[oli] was 25.9%, and was larger than the value of 12.1% for [cap]-[ery] and of 17.0% for [ery]-[oli]. It was close to the addition of the latter two recombination values, but was 3.2% less (Table 1). No recombinant of [cap^R ery^S] type was found, although 30 recombinants of [cap^S ery^R] type were observed; 63 recombinants of type [cap^S oli^S] were scored, while only one recombinant of type [cap^R oli^R] was present.

Since two extrachromosomal oligomycin-resistant mutants with different loci were obtained[7], we tried to test N 11, another oligomycin resistant in the same way. After analyzing 247 colonies, it was found that the recombination values for [cap]-[oli] was 30.8%, while the recombination value was 16.6% for [cap]-[ery] and 23.1% for [ery]-[oli]. The addition of these two values was 39.7%. The difference of the value for [cap]-[oli] from this value was 8.9% and was larger than that in the first cross. It was again found that the recombinants of [cap^R ery^S] type was absent and the recombinants of [cap^R oli^R] tpye was very few, as in cross 1.

These two oligomycin-resistants were then crossed with another chloramphenicol- and erythromycin-resistant mutant 1L8-8D. As shown in Table 1, recombinants of both types were

Table 1. Recombination of chloramphenicol-, erythromycin- and oligomycin-resistance. I

Cross No.	Crosses		Recombinants						Total number of colonies
	Type of crosses	Cross	$cap^S\ ery^R$	$cap^R\ ery^S$	$ery^S\ oli^S$	$ery^R\ oli^R$	$cap^S\ oli^S$	$cap^R\ oli^R$	
1	I×II	N15×1L126-1C	30	0	37	5	63	1	247
2	I×II	N11×1L126-1C	41	0	45	12	71	5	247
3	I×I	N15×1L8-8D	14	5	15	22	16	17	248
4	I×I	N11×1L8-8D	22	4	21	28	29	27	248

Cross No.	Crosses	Recombination values (%)		
		[cap]-[ery]	[ery]-[oli]	[cap]-[oli]
1	N15×1L126-1C	12.1	17.0	25.9
2	N11×1L126-1C	16.6	23.1	30.8
3	N15×1L8-8D	7.7	14.9	13.3
4	N11×1L8-8D	10.5	19.8	22.6

Recombination value indicates the rate of recombinants for two markers to the total number of colonies examined.

found and no asymmetrical formation of recombinants occurred. The recombination value for [cap]-[ery] was 7.7% in the cross N15×1L8-8D and was less than that in cross 1. It was about half of the recombination value of 14.9% for [ery]-[oli] and of 13.3% for [cap]-[oli]. The value for [cap]-[oli] was close to the value for [ery]-[oli] and was not equal to the addition of the value for [cap]-[ery] and [ery]-[oli]. Similar results were also obtained in another cross N11×1L8-8D.

Since two oligomycin-resistants with different loci gave similar results in crosses with 1L126-1C and also with 1L8-8D, only the oligomycin-resistance of the first type was studied. Chloramphenicol- and erythromycin-resistant 1L126-1C and 1L8-8D were then crossed with an oligomycin-resistant 711, which possesses the same loci of oligomycin resistance as that of N15. Since N15 and 711 possess the same mating type and can not be crossed with each other, an oligomycin-resistant R8, having the opposite mating type was crossed with N15. Both resistants 711 and R8 were derived from the same cross and thus possessed the same oligomycin resistance. No sensitive recombinants were found among 300 diploids examined. When 711 was crossed with 1L126-1C, it was found that the recombination value for [cap]-[oli] and the value for [ery]-[oli] were close and the value for [cap]-[ery] was significantly smaller than these values (Table 2). The results were similar to the cross 3 and 4.

Then the mutant 711 was crossed with another chloramphenicol- and erythromycin-resistant 1L8-8D. As shown in cross 6 in Table 2, the cross gave the recombination value of 26.1% for [cap]-[oli] which was close to the addition of the values of 13.5% for [cap]-[ery] and of 16.8% for [ery]-[oli]. The difference was 4.2% and was very close to the difference found in cross 1. The predominant formation of the recombinants of [$cap^R\ ery^S$] and [$cap^R\ oli^R$] types was also found in contrast to the very few number of recombinants of [$cap^S\ ery^R$] and [$cap^S\ oli^S$] types. These results were similar to the results from the crosses 1 and 2.

Table 2. Recombination of chloramphenicol-, erythromycin- and oligomycin-resistance. II

Cross No.	Crosses		Recombinants						Total number of colonies
	Type of crosses	Crosses	$cap^R ery^S$	$cap^S ery^R$	$ery^R oli^R$	$ery^S oli^S$	$cap^R oli^R$	$cap^S oli^S$	
5	II×II	711×1L126-1C	7	10	34	19	32	20	304
6	II×I	711×1L8-8D	40	2	36	16	76	5	310
7	II×I	706R1×1L1A	37	1	34	9	65	4	248

Cross No.	Crosses	Recombination values (%)		
		[cap]-[ery]	[ery]-[oli]	[cap]-[oli]
5	711×1L126-1C	5.6	17.4	17.1
6	711×1L8-8D	13.5	16.8	26.1
7	706R1×1L1A	15.3	17.3	27.8

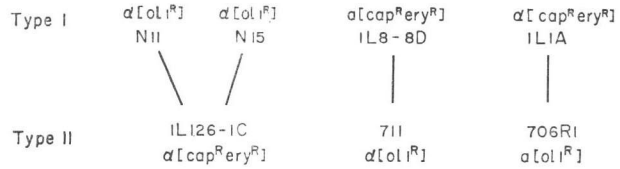
Recombination value indicates the rate of recombinants for two markers to the total number of colonies examined.

But cross 1 differed from cross 6 in that the [cap^S ery^R] or [cap^S oli^S] type recombinants were predominantly found in cross 1, while [cap^R ery^S] or [cap^R oli^R] recombinants were predominantly found in cross 6.

An oligomycin-resistant haploid, 706R1, was also studied. The 706R1 was the parent strain of 711, but possessed the opposite mating type to 711. In order to perform the cross, a chloramphenicol- and erythromycin-resistant haploid 1L1A was isolated which possessed the opposite mating type to 1L8-8D. The 1L1A was obtained from a cross between 1L8-8D and a sensitive haploid S 22 by a tetrad analysis of chloramphenicol- and erythromycin-resistant diploids formed from this cross. The results were shown in cross 7 in Table 2. The recombination value for [cap]-[ery] was 15.3% and the value for [ery]-[oli] was 17.3%. The addition of these values was close to the value of 27.8% for [cap]-[oli]. The asymmetrical formation of the recombinants having [cap^R ery^S] or [cap^R oli^R] type was again observed.

Different results were obtained in crosses 1, 2, 6 and 7 from crosses 3, 4 and 5. Thus the strains used can be grouped into two types as depicted in Fig. 1. The cross of the cells of different types gave rise to the recombinants of one of two types (*i.e.* [cap^S ery^R] in cross 1), and higher recombination values for [cap]-[ery] or for [cap]-[oli] than the cross between the cells of the same type. It also showed that the addition of the recombination values for [cap]-[ery] and [ery]-[oli] was close to the value for [cap]-[ery]. These two types for the recombination of mitochondrial genes were in agreement with the types, described as ω^+ and ω^- by other workers.[5] Based on the study on the three factor crosses, these types were

Fig. 1. Type of the strains



Lines indicate the crosses in which the recombination values for [cap]-[ery] increased and the asymmetrical formation of recombinants occurred.

described as type I and type II in this paper.

Since similar results were obtained from the crosses 1, 2 and 7, irrespective of the chromosomal genes in the cells used in these crosses, it was suspected that extrachromosomal genetic element is involved in these results obtained.

Discussions

Crosses, between cells of type I and type II were characterized with respect to several points: Recombinants of one of two types were predominantly found, which possessed chloramphenicol markers of the parent of type I. The recombination values for [cap]–[ery] and [cap]–[oli] were higher than those in the crosses between the same type. Two types of crosses have already been reported for the recombination of mitochondrial genes by BOLOTIN *et al.*[5] Based on the asymmetrical formation of [cap ery] recombinants, cells were grouped into two types, ω^+ and ω^- by these workers. However, crosses of the cells of the same type also gave asymmetrical formation of [cap ery] recombinants in some crosses and the type of the cells can not be tested simply. When a three-factor cross was carried out, the recombination values for [cap]–[ery] were larger in crosses of different types than for crosses of the same type. Based on the three-factor cross, cells were grouped into two types in this paper.

Table 3. Recombinants in three-point crosses

Type of recombinants			Cross 1		Cross 7	
[cap]	[ery]	[oli]	Number of colonies	%	Number of colonies	%
+	+	+	141	57.1	104	41.9
+	+	−	37	15.0	33	13.3
−	+	+	0	0	0	0
−	+	−	0	0	1	0.4
+	−	+	4	1.6	5	2.0
+	−	−	26*	10.5	32***	12.9
−	−	+	1	0.4	4	1.6
−	−	−	38**	15.4	69****	27.8
Total			247	100	248	100

+ indicates the resistance or sensitiveness in the parent of type I (*i.e.* [cap^S], [ery^S], [oli^R] in cross 1 and [cap^R], [ery^R], [oli^S] in cross 7). * 5 colonies were E+O type; ** 4 colonies were CE+O type; *** 2 colonies were CE+CO type; **** One colony was CE+O type.

In order to investigate the segregation of markers in crosses between type I and type II, the number of recombinants of all types were scored. It was shown in cross 1 in Table 3 that the cells of both parental types segregated and the cells with [cap^S] marker segregated predominantly, while the cells with [oli^R] marker and [oli^S] marker segregated almost equally. The number of recombinants with double crossing over was small. The results were the same in cross 7 except that the cells with [cap^R] markers segregated predominantly, which were derived from [cap^R] markers in the parent cells of type I.

The data on the recombination values obtained in the crosses between type I and type II were in accordance with the linear arrangement of the mitochondrial drug resistances. However an explanation for the formation of a very few or no recombinants of one of two types is still open. Postulation can be made that the gene of chloramphenicol-resistance was transferred, as in the case of donor to recipient in *Escherichia coli*.

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