

## Somatic Segregation, Recombination and Elimination of Mitochondrial Genes in *Saccharomyces cerevisiae*

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**ABSTRACT** To investigate the mitochondrial genome, the genetic analyses were performed using a strain with the three mitochondrial genes, (*OLG<sup>r</sup>*), (*ERY<sup>r</sup>*) and (*CHL<sup>r</sup>*), conferring resistance to oligomycin, erythromycin and chloramphenicol, respectively.

1. The rate of somatic segregation of the mitochondrial genes was examined analysing zygotic clones obtained from the various growth phases of zygotes. Segregation rate was the highest with (*CHL*), followed by (*ERY*) and (*OLG*), in the order.

2. Analysis of the zygote progeny from three-factor crosses showed that the averages of recombination frequencies were 0.19, 0.10 and 0.19, respectively, between (*OLG*) and (*ERY*), between (*ERY*) and (*CHL*), and between (*OLG*) and (*CHL*).

3. The frequency of loss of each mitochondrial gene in the petites induced by ethidium bromide treatment was examined. The results indicated that the frequency of loss of the genes was in the order of (*CHL*), (*ERY*) and (*OLG*), and that (*ERY*) was retained or lost together with (*CHL*) with a high frequency, but (*OLG*) was readily separable from either (*ERY*) or (*CHL*).

4. From these results, the gene arrangement in mitochondrial genome is in the order of (*OLG*), (*ERY*) and (*CHL*). Two genes, (*ERY*) and (*CHL*) are closely linked together.

### Introduction

Cytoplasmically inheritable drug-resistance factors of yeast (*S. cerevisiae*) have been generally regarded as the genes lying in mitochondrial genome and as a useful tool for studying mitochondria on the genetic bases. However many technical difficulties were underlaid and hindered prompt progress. It is quite recent that plausible experimental results have become available (1~12).

The present paper will describe some results of experiments using a haploid strain which possesses the three mitochondrial genes, oligomycin-resistance (*OLG<sup>r</sup>*),

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Abbreviations: ( ), cytoplasmically inheritable gene; *OLG*, oligomycin; *ERY*, erythromycin; *CHL*, chloramphenicol; *r*, resistance; *s*, sensitivity; *o*, loss of the gene.

erythromycin-resistance (*ERY<sup>r</sup>*) and chloramphenicol-resistance (*CHL<sup>r</sup>*). The experiments were conducted in three different ways; 1) the rate of somatic segregation of each resistance factor during the vegetative growth of zygotes, 2) recombination analysis by the three-factor crosses, and 3) the rate of loss of the resistance factors by the treatment of ethidium bromide.

The results indicated that the three genes were arranged in the order of (*OLG*), (*ERY*) and (*CHL*).

### Materials and Methods

*Strains.* OEC1222 ( $\alpha$ , *ade1*, *his4*, *thr4*, (*OLG<sup>r</sup>*, *ERY<sup>r</sup>*, *CHL<sup>r</sup>*)) which carries the three cytoplasmic resistance factors to oligomycin, erythromycin and chloramphenicol was obtained as previously described (5). S11-17R (*a*, *ura3*) and DB51 (*a*, *ura3*) are sensitive strains to the three drugs.

*Media, mating procedure, determination of drug-resistances, ethidium bromide treatment and determination of retention or loss of the drug-resistance factors* were described previously (5, 8, 12).

*Synchronous zygote formation.* Cells of the parental haploid strains were grown in the complete glucose medium for 1 day at 30°C. One tenth ml of culture of OEC1222 was mixed with 0.9 ml of the culture of S11-17R, and 0.05 ml of 50 % glucose was added, and centrifuged. The supernatant was discarded and the cells precipitated at the bottom of the tube were allowed to stand for 1 day at 30°C to form zygotes.

### Results

#### 1. *Somatic segregation of the mitochondrial genes.*

Zygotes formed as described in Materials and Methods were inoculated in the liquid minimal glucose medium and incubated at 30°C with shaking. A typical growth curve of the zygotes is shown in Fig. 1. At appropriate times cells were sampled and plated on the minimal glucose plates. Thirty colonies derived from 0 hr-, 6 hr- and 9 hr-incubation were picked up and respread on the minimal glucose plates. Twenty six subclones grown on the plates were isolated and analysed for drug resistance or sensitivity by spotting on the minimal glycerol and drug plates, which contained 20  $\mu$ g oligomycin, 1 mg erythromycin or 4 mg chloramphenicol per ml. The results were shown in Table 1-A (0 hr), B (6 hr) and C (9 hr).

When a clone gives rise to mixed subclones resistant and sensitive to the drug, the clone should be heterozygous for the gene. On the other hand a clone yields solely resistant or sensitive subclones, the clone should be homozygous. Numbers

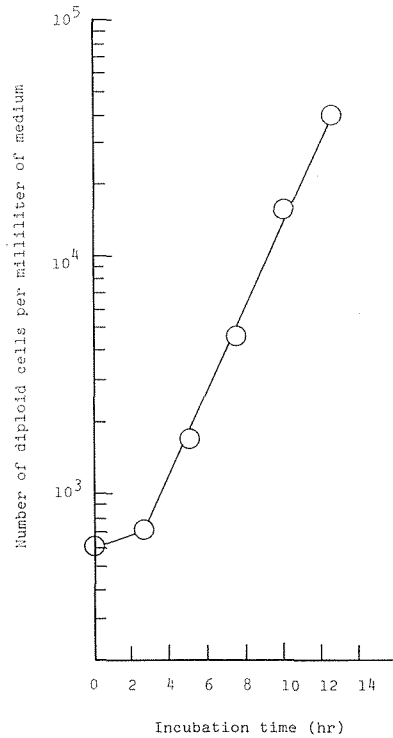


Fig. 1. Growth curve of the zygotes. Synchronous zygotes formed by crossing, OEC1222  $\times$  S11-17R, were transferred in the liquid minimal glucose medium and incubated at 30°C with shaking. At given times of incubation, the samples were diluted appropriately and spread on the minimal glucose plates. After a few day's incubation, diploid colonies formed were scored.

and fractions of heterozygous clones are shown in Table 2. One can see that the almost of primary zygotes were heterozygous for the three mitochondrial genes, but that the fractions of heterozygous clones decreased with the subsequent growth. The rate of somatic segregation of (*CHL*) is the highest and that of (*OLG*) is the lowest. The all three genes are segregated with a considerably high rate.

## 2. Recombination of the mitochondrial genes.

Three-factor crosses were performed using the strain, OEC1222 ( $\alpha$ , (*OLG*<sup>r</sup>, *ERY*<sup>r</sup>, *CHL*<sup>r</sup>)), and the strains sensitive to the drugs, as described previously (5, 12). In the previous section it was mentioned that the almost of primary zygotes are heterozygous for the drug-resistance factors and the fractions of heterozygotes decrease after their growth. To complete the segregation, zygotes were grown in the minimal glucose medium over 20 generations, after which isolated progeny cells were proved to be homozygous for resistance and sensitive factors to each drugs,

Table 1 Phenotypes of colonics isolated at the various growth periods of zygotes (OEC1222×S11-17R)

A. 0 hr-incubation		Zygotic clone	
(OLG)	r	A-1	2
(ERY)	r	A-2	6
(CHL)	r	A-3	19
(OLG)	r	A-4	0
(ERY)	r	A-5	6
(CHL)	r	A-6	19
(OLG)	r	A-7	0
(ERY)	r	A-8	1
(CHL)	r	A-9	17
(OLG)	r	A-10	9
(ERY)	r	A-11	12
(CHL)	r	A-12	2
(OLG)	r	A-13	2
(ERY)	r	A-14	23
(CHL)	r	A-15	6
(OLG)	r	A-16	14
(ERY)	r	A-17	0
(CHL)	r	A-18	4
(OLG)	r	A-19	1
(ERY)	r	A-20	0
(CHL)	r	A-21	14
(OLG)	r	A-22	26
(ERY)	r	A-23	0
(CHL)	r	A-24	1
(OLG)	r	A-25	11
(ERY)	r	A-26	0
(CHL)	r	A-27	1
(OLG)	r	A-28	0
(ERY)	r	A-29	3
(CHL)	r	A-30	6

B. 6 hr-incubation		Zygotic clone	
(OLG)	r	B-1	0
(ERY)	r	B-2	0
(CHL)	r	B-3	0
(OLG)	r	B-4	26
(ERY)	r	B-5	1
(CHL)	r	B-6	0
(OLG)	r	B-7	0
(ERY)	r	B-8	0
(CHL)	r	B-9	6
(OLG)	r	B-10	8
(ERY)	r	B-11	0
(CHL)	r	B-12	0
(OLG)	r	B-13	26
(ERY)	r	B-14	0
(CHL)	r	B-15	0
(OLG)	r	B-16	0
(ERY)	r	B-17	0
(CHL)	r	B-18	26
(OLG)	r	B-19	0
(ERY)	r	B-20	26
(CHL)	r	B-21	26
(OLG)	r	B-22	0
(ERY)	r	B-23	0
(CHL)	r	B-24	19
(OLG)	r	B-25	0
(ERY)	r	B-26	0
(CHL)	r	B-27	26
(OLG)	r	B-28	0
(ERY)	r	B-29	0
(CHL)	r	B-30	14

C. 9 hr-incubation		Zygotic clone	
(OLG)	r	C-1	0
(ERY)	r	C-2	0
(CHL)	r	C-3	26
(OLG)	r	C-4	26
(ERY)	r	C-5	0
(CHL)	r	C-6	0
(OLG)	r	C-7	0
(ERY)	r	C-8	2
(CHL)	r	C-9	0
(OLG)	r	C-10	0
(ERY)	r	C-11	0
(CHL)	r	C-12	0
(OLG)	r	C-13	0
(ERY)	r	C-14	26
(CHL)	r	C-15	0
(OLG)	r	C-16	0
(ERY)	r	C-17	26
(CHL)	r	C-18	26
(OLG)	r	C-19	26
(ERY)	r	C-20	0
(CHL)	r	C-21	0
(OLG)	r	C-22	12
(ERY)	r	C-23	0
(CHL)	r	C-24	26
(OLG)	r	C-25	0
(ERY)	r	C-26	0
(CHL)	r	C-27	0
(OLG)	r	C-28	0
(ERY)	r	C-29	0
(CHL)	r	C-30	0

Table 2 Change of numbers of heterozygous clones after the growth of zygotes.

	Experiment		
	A	B	C
( <i>OLG</i> )	24 (80)	11 (37)	7 (23)
( <i>ERY</i> )	23 (77)	10 (33)	4 (13)
( <i>CHL</i> )	23 (77)	6 (20)	2 ( 7)

Data is taken from experiments shown in Table 1 and numbers in brackets denote frequencies in per cent.

Table 3 Phenotypes of the zygote progeny from three-factor crosses.

Phenotype			Cross	
( <i>OLG</i> )	( <i>ERY</i> )	( <i>CHL</i> )	A	B
<i>r</i>	<i>r</i>	<i>r</i>	65 (17.0)	94 (24.6)
<i>r</i>	<i>r</i>	<i>s</i>	10 ( 2.6)	11 ( 2.9)
<i>r</i>	<i>s</i>	<i>r</i>	11 ( 2.9)	10 ( 2.6)
<i>r</i>	<i>s</i>	<i>s</i>	21 ( 5.5)	19 ( 5.0)
<i>s</i>	<i>r</i>	<i>r</i>	29 ( 7.6)	31 ( 8.1)
<i>s</i>	<i>r</i>	<i>s</i>	6 ( 1.6)	13 ( 3.4)
<i>s</i>	<i>s</i>	<i>r</i>	9 ( 2.4)	13 ( 3.4)
<i>s</i>	<i>s</i>	<i>s</i>	230 (60.4)	193 (50.5)
Total			381	384

OEC1222 (*OLG'*, *ERY'*, *CHL'*) was crossed to the different sensitive strains, S11-17R (A) and DB51 (B). Numbers in brackets denote frequencies in per cent.

so far tested. Parental and recombinant phenotypes of the progeny are shown in Table 3. Although the sensitive strains used were different in the experiment A B, the recombination frequencies of two experiments are very close. The recombination frequencies were 0.18 between (*OLG*) and (*ERY*), 0.09 between (*ERY*) and (*CHL*), and 0.18 between (*OLG*) and (*CHL*) in the experiment A, and 0.19, 0.12 and 0.19, respectively in the experiment B. The averages of recombination frequencies between (*OLG*) and (*ERY*), between (*ERY*) and (*CHL*), and between (*OLG*) and (*CHL*) are 0.19, 0.10 and 0.19, respectively. From the data in Table 3, frequencies of the mitochondrial genes recovered from the  $\alpha$  parent were calculated; they were 0.28, 0.29 and 0.30 for (*OLG*), (*ERY*) and (*CHL*), respectively, in the experiment A, and 0.35, 0.39 and 0.39, respectively, in the experiment B. Thus, these frequencies deviated from 0.5 and were characteristic of each cross employed. But, irrespective of the crosses, the recovery of (*OLG*) of the  $\alpha$  parent more deviated from 0.5 than those of (*ERY*) and (*CHL*), while the deviation of recovery of (*CHL*)

was less than or equal to that of (*ERY*). The ordered frequencies of recoveries of the genes would be suggestive of the arrangement of the genes concerned (5).

### 3. Retention or loss of the mitochondrial genes by the treatment of ethidium bromide.

Ethidium bromide is known as a potential inducer of cytoplasmic petite mutation (13). Petite induced by the treatment of the dye had a decreased molecular size of mitochondrial DNA (14), and longer treatment lead to eventual loss of mitochondrial DNA (15, 16).

The effect of ethidium bromide was examined on the loss of the three mitochondrial genes in the petites of the strain, OEC1222 (*OLG<sup>r</sup>*, *ERY<sup>r</sup>*, *CHL<sup>r</sup>*). Cells were treated by ethidium bromide as described for legend to Table 4 and plated on the complete glucose plates. Petite colonies were scored by colony formation according to the *ade1* marker (17), and non sectored colored colonies were isolated. The higher the doses of ethidium bromide, the more petites and loss of drug resistances were induced, so far as the same experimental conditions concerned. To analyse drug resistance of the petites, they were crossed to respiratory sufficient, drug sensitive cells, S11-17R or DB51. The resultant zygotes were further grown in the liquid minimal glucose medium for over 10 generations and zygote suspension

Table 4 Genetic constitutions of the petites induced by ethidium bromide treatment.

Genetic constitution			Experiment				
( <i>OLG</i> )	( <i>ERY</i> )	( <i>CHL</i> )	A	B	C	D	E
<i>r</i>	<i>r</i>	<i>r</i>	28 (23.3)	24 (12.3)	17 (11.3)	17 (17.3)	38 (12.8)
<i>r</i>	<i>r</i>	<i>o</i>	0 (0.0)	1 (0.5)	0 (0.0)	3 (3.1)	5 (1.7)
<i>r</i>	<i>o</i>	<i>r</i>	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)	0 (0.0)
<i>r</i>	<i>o</i>	<i>o</i>	24 (20.0)	29 (14.9)	20 (13.3)	12 (12.5)	41 (13.8)
<i>o</i>	<i>r</i>	<i>r</i>	6 (5.0)	16 (8.2)	11 (7.3)	7 (7.3)	9 (3.0)
<i>o</i>	<i>r</i>	<i>o</i>	0 (0.0)	2 (1.0)	0 (0.0)	0 (0.0)	6 (2.0)
<i>o</i>	<i>o</i>	<i>r</i>	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)
<i>o</i>	<i>o</i>	<i>o</i>	62 (51.7)	123 (63.0)	102 (68.0)	56 (58.3)	197 (66.3)
Total			120	195	150	96	297
Fraction of respiratory sufficient cells			0.82	0.51	0.41	0.40	0.18

Cells of OEC1222 were precultured in the complete medium containing 1% glucose for 1 day under the static condition for A, D and E, in the complete medium containing 2.5% glucose for 1 day under the static condition for B, and in the complete medium containing 1% glucose for 2 days with shaking for C. Cells of OEC1222 were treated by ethidium bromide; 1 $\mu$ g per ml for 15 min (A and B), 5 $\mu$ g per ml for 15 min (C), 5 $\mu$ g per ml for 30 min (D), and 10 $\mu$ g per ml for 15 min (E). Genetic constitutions were determined after the petites were crossed to respiratory sufficient, drug sensitive haploid strain, DB51 or S11-17R. Numbers in brackets denote frequencies in per cent.

was spotted on the glycerol drug plates. The ability of the zygote progeny to grow on the glycerol drug plate indicates that the corresponding drug-resistance factor was retained in the petite clone (18).

Table 4 shows genetic constitutions of each petite isolated. (*OLG*<sup>r</sup>), (*ERY*<sup>r</sup>) and (*CHL*<sup>r</sup>) in the table denote the retention of the resistance factors to oligomycin, erythromycin and chloramphenicol, respectively, whereas (*OLG*<sup>o</sup>), (*ERY*<sup>o</sup>) and (*CHL*<sup>o</sup>) denote the loss of the corresponding resistance factor. The drug resistances were lost in various combination, the most frequent of those combinations was the one that lost all three resistances, the second was the one that retained all three resistances or only oligomycin resistance, and the third was the one that lost only oligomycin resistance. There seems to be a tendency that loss of chloramphenicol resistance is a little higher than that of erythromycin resistance, though experimental numbers may not be sufficient to conclude at present time. This experiment also showed that (*ERY*) and (*CHL*) is closely linked and those are easily separable from (*OLG*). The three genes are lost by ethidium bromide in the order of (*CHL*), (*ERY*) and (*OLG*).

### Discussion

Present experiment indicates that almost of primary zygotes are heterozygous for the mitochondrial genes, (*OLG*), (*ERY*) and (*CHL*), and segregation starts shortly after beginning of their growth. The rate of segregation differs from gene to gene; the highest is (*CHL*) and the lowest is (*OLG*). Similar attempt had been done with TTC overlay method (19). However this method could not be applied for (*OLG*), since colonies sensitive as well as resistant to oligomycin were stained on the glucose plates containing oligomycin by TTC. So far to two genes, (*CHL*) segregated faster than (*ERY*) (20).

The recombination analysis indicates that the (*ERY*)-(*CHL*) is more closely linked than either (*OLG*)-(*ERY*) or (*OLG*)-(*CHL*). The slightest difference was observed between the recovery of (*ERY*) and (*CHL*) from the  $\alpha$  parent, it might be some meaning when experiments with ethidium bromide is considered.

A similar linkage relationship of the three mitochondrial genes are also revealed from the results of retention or loss of the genes in the petites induced by ethidium bromide. In most case, (*ERY*) and (*CHL*) are coordinately retained or lost in the petites induced by ethidium bromide treatment and are easily separable from (*OLG*). Resistance factors are lost accordingly to the dose of ethidium bromide. The rate of loss of the resistance factors were again in the order of (*CHL*), (*ERY*) and (*OLG*). The same order of the mitochondrial genes in the spontaneous petites of OEC1222 is also observed (Suda *et al.*, in preparation).

In conclusion, the three different experiments indicate the consistent arrangement of the three genes in the mitochondrial genome that is in the order of (*OLG*)-(*ERY*)-(*CHL*). And yet (*ERY*) and (*CHL*) are more closely linked than either (*OLG*) and (*ERY*) or (*OLG*) and (*CHL*).

The recombination analysis concerning oligomycin, erythromycin and chloramphenicol resistances has been recently described by Avner *et al.* (10) and Wolf *et al.* (11). In some points the present results do not conflict with their results; *i.e.*, (*ERY*) and (*CHL*) are linked and separable from (*OLG*). However relative position of (*ERY*) and (*CHL*) are confusing in those results including ours. The interesting is that relative position of the three mitochondrial genes is the same with experiments by them and those of ours but the direction is reversed. Those discrepancies may be caused by different strains and crosses used and are left to be elucidated by further investigations.

The rate of somatic segregation of the mitochondrial genes were considerably high, suggesting that the number of heritable mitochondrial genomes in a cell is small. This may be supported by the results of the petite induction by ultraviolet irradiation (21, 22) and ethidium bromide (10). In these experiments number of mitochondrial genomes in a cell was very small. Furthermore, the number of mitochondria in a diploid cell was only one by the electron microscopic observation (23).

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