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Recombination Among Three Mitochondrial Genes in Yeast (*Saccharomyces cerevisiae*)

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Eight crosses involving three different mitochondrial genes were made in *Saccharomyces cerevisiae*. All three genes were linked, but an unambiguous linkage map could not be constructed.

Coen et al. (2) have contributed significantly to the study of mitochondrial genetics by detailing the methods for analyzing crosses involving two genes located in mitochondrial deoxyribonucleic acid (mDNA) in yeast. In this paper we present, using methods of Coen et al. (2), the results of crosses involving mitochondrial genes for resistance to erythromycin [*ery1*], chloramphenicol [*cap1*], and paramomycin [*par1*].

Our wild-type strains, 37 (*α his3 try1*), 41 (*a leu*), and 48 (*a lys*), were sensitive to the three drugs, erythromycin, chloramphenicol, and paramomycin. Independent spontaneous mutants were obtained which were resistant to only one of the drugs. All mutant strains were crossed to sensitive strains by mixing the appropriate haploids on solid glucose minimal medium (1% glucose, 0.5% yeast nitrogen base without amino acids [Difco], 2% agar). After 48 hr at 30 C a random sample of diploid progeny from a random sample of zygotic clones was spread on glucose minimal medium. These plates were incubated for 48 hr at 30 C and then replica plated to glycerol complete medium (2% glycerol, 1% yeast extract, 1% Casamino Acids, 2% agar) containing 2 mg of antibiotic/ml, and to glycerol complete medium without antibiotic. The colonies which grew on the glycerol plate without antibiotic were scored for resistance or sensitivity on the antibiotic plate. If both kinds of colonies were found, one had evidence that the factor controlling resistance was probably carried in the cytoplasm since segregation occurred during mitotic divisions. In crosses with each mutant studied, this type of segregation was found. In addition, when an antibiotic-resistant diploid from each of the crosses was sporulated, the four spores of the tetrads segregated 4:0 (resistance to sensitivity) as expected with cytoplasmic inheritance. Mounolou et al. (3) have shown that cytoplasmically inherited petites (respiratory deficient) possess an altered mDNA. We have obtained cytoplasmic petites in our drug-resistant strains, and, when crossed to sensitive wild type, the drug resistance is not transmitted. This suggests the lesion in the mDNA giving rise to the petite also affected the drug resistance factor which is, therefore, apparently located on mDNA.

The resistant mutants, 37 [*cap1*] and 41 [*ery1*], were crossed and a double-resistant recombinant was obtained. This [*cap1*][*ery1*] strain was sporulated and a double-resistant spore of *α* mating type was obtained and crossed to mutant 48 [*par1*]. All of the triple-resistant strains and the [*cap1*][*par1*] and [*ery1*][*par1*] strains used in this study were recombinant types obtained from this initial cross involving three genes. Crosses were made giving all combinations of coupling and repulsion for these three drug-resistant genes.

The methods used are similar to what Coen et al. (2) describe as "quantitative replica plating of random diploid cells" and involved sampling a random number of diploid cells coming from a large number of different zygotic clones (*see below*). The clones are usually heterogeneous, and the distribution of genotypes within a zygotic clone varies considerably from clone to clone. Because of this variable, frequencies of transmission of a particular genotype or frequencies of recombination by sampling one clone cannot be defined for a particular cross. However, the frequencies are repeatable for the cross involved if large numbers of clones are sampled. (Thomas and Wilkie [4] have obtained data from a three-factor cross by scoring individual zygotic clones. They produced zygotes anaerobically which they report gave rise to pure clones.)

After zygote formation the crosses were incubated on solid glucose minimal medium at 30 C for 2 days. At this time the diploid cells were

pure with respect to their drug phenotype and no longer segregated during mitosis. A sample of cells was spread on glucose minimal medium without the drug, grown 2 days at 30 C, and then replica plated to each of three plates containing glycerol complete medium plus 2 mg of chloramphenicol or erythromycin or paramomycin per ml. Initially we used drug plates containing all two- and three-way combinations of the drugs. This allowed us to make certain that a colony which grew on chloramphenicol and erythromycin, e.g., also grew on chloramphenicol plus erythromycin, was in fact a recombinant colony which had resistance to both drugs and not a mixed colony. However, the frequency of these mixed colonies was well below 1%, so that the cost of using these additional combinations of drug plates caused us to eliminate them from most of the analyses.

The data from the crosses involving three genes are presented in Table 1. All eight possible combinations of genotypes are obtained in each cross. There is not an equal frequency of the reciprocal classes such as one might observe with Mendelian genetics. Rather these frequencies may be more reminiscent of some phage crosses. This characteristic has also been observed by Coen et al. (2) and Bolotin et al. (1) and is specific for the strains and the particular cross combinations involved.

Our first attempt to analyze these data was based on the assumption that the genes were in a linear array. It was apparent that [*eryl*] and [*capl*] were linked more tightly than [*eryl*] and [*parl*] or [*capl*] and [*parl*]. Identifying putative double-crossover classes was difficult since two pairs of reciprocal classes were about equally frequent. However, if the pair of classes of lowest frequency was assumed to be the double-crossover pair, then in six of the seven crosses studied the gene order [*eryl*]-[*capl*]-[*parl*] was obtained. In the other cross the gene order was [*capl*]-[*eryl*]-[*parl*].

Using [*eryl*]-[*capl*]-[*parl*] as the gene order, approximately twice as much recombination between [*capl*] and [*parl*] is found as between [*eryl*] and [*capl*] (Table 2). There is consistency among the seven crosses for this twofold increase in recombination in the [*capl*] to [*parl*] region as compared to the [*eryl*] to [*capl*] region. The weighted average recombination for [*eryl*] to [*capl*] was 9.1% and for [*capl*] to [*parl*] 18.6% ([*eryl*]-9.1-[*capl*]-18.6[*parl*]).

Either high negative interference or perhaps repeated rounds of recombination with alternating single crossovers in the two regions could give rise to a high frequency of apparent double-crossover genotypes. The coincidence values for the seven crosses ranged from 2.0 to 4.2. On the

TABLE 1. *Three-factor crosses involving cytoplasmically inherited genes for antibiotic resistance in yeast*

Genotype of progeny	[*capl*][*eryl*]+ × ++[*parl*]	[*capl*][*eryl*][*parl*]−1[a] × +++ (47)	[*capl*][*eryl*][*parl*]−2 × +++ (47)	[*capl*][*eryl*][*parl*]−3 × +++ (48)	[*capl*][*eryl*][*parl*]−4 × +++ (48)	+[*eryl*][*parl*] × [*capl*]++	[*capl*]+[*parl*] × +[*eryl*]+
[*capl*][*eryl*][*parl*]	138	266	257	998	1292	89	56
[*capl*][*eryl*]	902	110	138	247	269	95	51
[*capl*]+[*parl*]	26	17	21	37	47	119	899
[*capl*]++	29	36	43	60	71	803	236
+[*eryl*][*parl*]	36	17	19	51	53	1,165	168
+[*eryl*]+	35	45	77	89	75	234	708
++[*parl*]	539	82	148	150	205	78	77
+++	98	1,677	1,515	889	480	53	98
Total	1,803	2,250	2,218	2,521	2,492	2,636	2,293

[a] Four different triple-resistant recombinant spores were obtained from the original cross in the first column. [*capl*][*eryl*][*parl*]−1 and −2 are *a* mating type, whereas −3 and −4 are *α* mating type. Strains 47 and 48 are sensitive strains of *α* and *a* mating type, respectively. All four parents of the last two crosses are recombinant spores from the original cross.

TABLE 2. *Recombination values for each of the seven crosses in Table 1*[a]

Region	Recombination (%)						
[*eryl*] to [*capl*]	7.0	5.1	7.2	9.4	9.9	12.0	12.3
[*capl*] to [*parl*]	16.5	10.9	15.7	20.2	24.0	18.8	23.2

[a] They are arranged in the same order as the crosses appear in Table 1.

other hand, the linkage map may not be linear, but circular. If we disregard the double-crossover class and simply search for recombinants between [*eryl*]-[*cap1*], [*cap1*]-[*par1*], and [*eryl*]-[*par1*], recombination distances of 9.1, 18.6, and 19.4, respectively, are obtained. At this point we are not able to confirm whether the linkage map is circular or linear. Additional genes are needed to allow us to be precise in deciding which map is correct.

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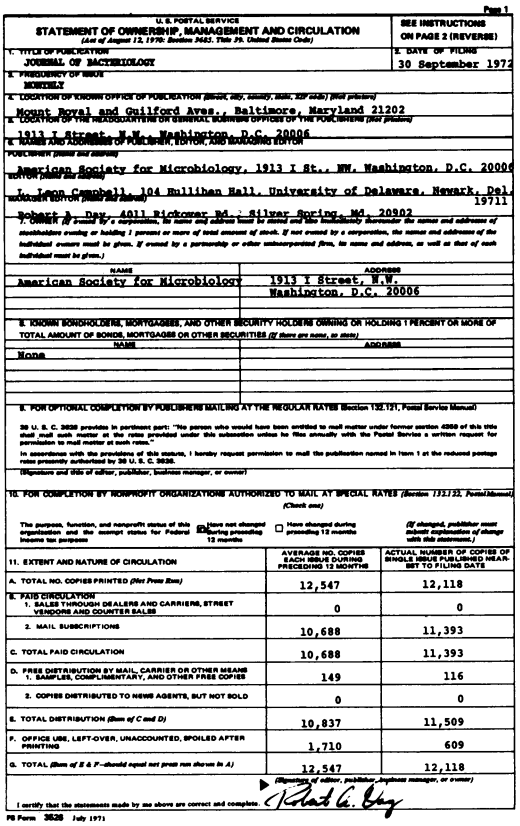

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