Genet. Res., Camb. (1976), **26**, *pp.* 319–325
Printed in Great Britain

Relative retention of mitochondrial markers in petite mutants: mitochondrially determined differences between *RHO*(+) strains

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(*Received* 30 *September* 1975)

SUMMARY

The relative frequency of retention of two mitochondrial loci, determining resistance to oligomycin (*oli1*) and erythromycin (*ery1*), has been analysed in petite (*rho*(−)) mutants derived from a number of unrelated strains of *Saccharomyces cerevisiae*. The frequency of retention of one marker relative to the other in spontaneous petites showed marked variation dependent on the strain of origin. The differences between strains in this characteristic were shown to be mitochondrially determined. Further, for individual strains, the relative retention of the markers in petites derived after UV-irradiation varied considerably in several cases from that observed with spontaneous petites. The observations on relative marker retention and the varied effects of UV-irradiation are discussed in terms of possible structural differences in the mitochondrial genomes of the various strains.

1. INTRODUCTION

The characterization in recent years of antibiotic-resistance mutations encoded on the mitochondrial genome of *Saccharomyces cerevisiae* has promoted intensive investigation of the mitochondrial genetic system. Proceeding in parallel with this work have been extensive studies of the physical structure of the mitochondrial genome. Recently both physical and genetic studies have suggested that differences exist between the genomes of wild-type strains. For instance, in a series of crosses between strains of diverse genetic background, Howell *et al.* (1973) observed a wide variation in parameters relating to mitochondrial gene recombination, namely, the frequency of mitochondrial gene transmission, frequency of recombination and polarity of recombination. The determination of some of these differences, other than those attributable to the polarity alleles (ω^+/ω^-) described by Bolotin *et al.* (1971), have been shown to be encoded in the mitochondrial genome (Linnane, Howell & Lukins, 1974). Direct physical studies by Bernardi, Prunell & Kopecka (1975) with mitochondrial DNA (mtDNA) and restriction enzymes, have also indicated that there are considerable differences in the primary nucleotide sequences of *rho*(+) strains.

In this communication we report on the determination by the mitochondrial genome of some further parameters of mitochondrial function and discuss the differences observed in relation to a possible wide divergence of structure in the mitochondrial genomes of strains of *S. cerevisiae*.

RESULTS

(a) *The relative frequency of retention of different mitochondrial markers in petite isolates*

It has been previously reported by Molloy, Linnane & Lukins (1975) that the relative frequency of retention of different mitochondrial markers among petite isolates was dependent on the parental *rho*(+) strain. These observations have been extended by an analysis of relative gene retention among petite mutants derived from a number of unrelated strains. These strains all carry mutations to resistance to erythromycin and oligomycin at loci shown to be allelic with the previously described *oli1* and *ery1* mutations. The relative retention of these markers in petites derived from seven strains is shown in Table 1; the frequency of petite types retaining one but not the other of these two markers ranged from 11 to 30% among petite isolates from the different strains.

Table 1. *Retention of the* oli1 *and* ery1 *loci in petite mutants from a series of strains*

Strain	Petite frequency (%)	Number of petites analysed	Frequency of petite genotype (%)				Ratio: $\frac{oli1\text{-}0\ ery1\text{-}r}{oli1\text{-}r\ ery1\text{-}0}$
			oli1-r ery1-r	*oli1-0 ery1-r*	*oli1-r ery1-0*	*oli1-0 ery1-0*	
761-7A	0·4	226	35	8	9	48	0·9
770-7B	0·8	303	15	12	8	65	1·4
863-2C	3·0	427	21	6	5	68	1·3
750.17-2C	1·1	211	15	14	6	65	2·4
413.16	2·5	436	11	4	26	59	0·14
652.1.2	11	364	60	14	6	20	2·2
D515-1B.47.3	1·5	328	12	6	20	62	0·3

The genotypes of the strains used are as follows: 761-7A, *a arg4-17 lys2 ura* [ω^+ *oli1-r ery1-r cap1-r*]; 770-7B, *a arg4-17 lys2 trp1* [ω^+ *mik1-r oli1-r ery1-r cap1-r*]; 863-2C, *a ura* [ω^+ *par1-r mik1-r oli1-r ery1-r cap1-r*]; 750.17-2C, *a his trp1 ura* [ω^+ *oli1-r ery1-r cap2-r*]; 413.16, *a ade1 lys2* [ω^- *oli1-r* ery1-r cap1-r*]; 652.1.2, *a ilv met trp* [ω^- *oli1-r* ery1-r**]; D515-1B.47.3, *a his2* [ω^- *oli1-r* ery1-r**]. Markers indicated with an asterisk were isolated independently from other mutations conferring the same phenotype and shown to be allelic by recombination.

The retention of markers in petite mutants was determined as described in Molloy *et al.* (1975) by crossing with *rho*(+) antibiotic-sensitive tester strains of the same polarity type and analysing the diploid progeny for transmission of resistance determinants.

The relative frequency of the two gene types *oli1-0 ery1-r* and *oli1-r ery1-0* among these petites showed considerable variability, the ratio of the frequencies of the two genotypes varying over a twentyfold range from 0·14 to 2·4. Thus, it is evident that the probability of retention of a particular region of the mitochondrial genome relative to another region in a petite mutant is dependent on the parental *rho*(+) strain.

In order to determine whether these differences were inherent to the mitochondrial genome or determined by nuclear factors, the two strains, 413.16 and 750.17-2C, yielding the most extreme ratios of the petite types *oli1-0 ery1-r* and *oli1-r ery1-0*, were analysed further. Isonuclear diploids were constructed by crossing each strain with the *rho*(*0*) derivative of the other strain, the latter being produced by treatment with ethidium bromide under conditions giving rise to elimination of all mtDNA (Nagley & Linnane, 1970). These diploids containing one or other mitochondrial genome were sporulated, and petites were then isolated from the four meiotic products of a tetrad from each cross. The petite mutants were spontaneous isolates, with the exception of those obtained from spores 855-5A and 855-5D, which were induced with ethidium bromide because of the very low spontaneous petite frequency of these strains. The relative retention of the *oli1* and *ery1* loci among these petites was analysed and the results are presented in Table 2.

Table 2. *Analysis of the relative gene retention among the meiotic products of diploids carrying different mitochondrial genomes*

Strain	Petite frequency (%)	Number of petites of genotype: *oli1-r ery1-r*	*oli1-0 ery1-r*	*oli1-r ery1-0*	*oli1-0 ery1-0*	Ratio: $\frac{oli1\text{-}0\ ery1\text{-}r}{oli1\text{-}r\ ery1\text{-}0}$	Probability that difference from mean ratio significant*: A	B
750.17-2C	1·1	32	29	12	138	2·4	0·2	<0·005
854-1A	0·4	13	10	1	36	10	0·7	<0·005
854-1B	1·0	36	61	17	140	3·6	0·8	<0·005
854-1C	3·3	47	37	6	106	6·2	0·4	<0·005
854-1D	0·5	20	15	2	124	7·5	0·65	<0·005
413.16	2·5	48	16	114	258	0·14	<0·005	0·96
855-5A	41†	11	3	54	138	0·06	<0·005	0·12
855-5B	0·8	28	7	15	75	0·5	<0·005	0·02
855-5C	2·3	84	10	47	104	0·21	<0·005	0·35
855-5D	7†	42	4	42	18	0·1	<0·005	0·55

The spores 854-1A to -1D carry the mitochondrial genome of strain 750.17-2C, i.e. they are of mitochondrial genotype ω^+ *oli1-r ery1-r cap2-r*. The spores 855-5A to -5D, which carry the mitochondrial genome of strain 413.16, are of mitochondrial genotype ω^- *oli1-r ery1-r cap1-r*. Gene retention in petite mutants isolated from the spores was determined in crosses with antibiotic-sensitive strains of the same polarity type as described in Molloy *et al.* (1975).

* Petites of the genotypes *oli1-r ery1-0* and *oli1-0 ery1-r* were summed for the upper five (A) and lower five (B) strains, and the mean ratio calculated for each set of strains. A χ^2 test was then performed to determine the probability that the ratio for each strain was not significantly different from each mean.

† Petite mutants from these strains were induced with ethidium bromide.

The mean ratios A and B were 4·0 and 0·15 respectively.

The relative retention of the *oli1* and *ery1* loci in petite isolates from the spore products was similar in all cases to that seen for the parent strain possessing the same mitochondrial genome. Statistical analysis was performed by grouping the two sets of five strains containing the same mitochondrial genome. The number of petites of the different genotypes were summed and the mean ratio of genotypes *oli1-0 ery1-r/oli1-r ery1-0* calculated for each set. *Chi*-square tests of each spore with these two mean ratios revealed that the two groups of strains were different with very high probability. The group of strains possessing the mitochondrial genome of strain 750.17-2C was fairly homogeneous and there was little evidence for the segregation of any nuclear genes affecting the relative retention of the *oli1* and *ery1* loci. By contrast, the relative retention of the *oli1* and *ery1* loci among petite isolates from the two spores 855-5A and 855-5B differed significantly from the mean ratio for the group of strains with the 413.16 mitochondrial genome. It would appear, therefore, that the major determinant of the relative frequency of retention of the *oli1* and *ery1* loci was the mitochondrial genome, although nuclear genes may also influence the relative frequency of gene retention.

(*b*) *Ultraviolet light induction of petite mutants*

Ultraviolet light is a potent inducer of the petite mutation in yeast (Raut & Simpson, 1955) and can also exert a marked effect on the transmission and recombination of mitochondrial genes when a strain is irradiated prior to crossing (Bolotin *et al.* 1971). Because of these effects of UV irradiation on mitochondrial genomes an investigation of the effects of UV irradiation on gene retention among petite isolates from a number of strains was undertaken. An analysis of this type involving a few related strains has been reported previously (Molloy *et al.* 1975), and even among these strains marked differences in the response of different strains to UV irradiation were seen.

As shown in Table 3, the effects of UV irradiation on relative gene retention were found to be critically strain-dependent, the ratio of petite types *oli1-0 ery1-r/oli1-r ery1-0* ranging from 17·5 to 0·03. For some strains, 863-2C and 413-16, relative retention of the *ery1* and *oli1* loci was similar among spontaneous and UV induced mutants. However, for other strains, UV irradiation markedly effected the outcome of petite mutagenic events, the ratio of petite types *oli1-0 ery1-r/oli1-r ery1-0* being increased relative to that observed among spontaneous petites for one strain (770-7B) and decreased for others (829-5B,

Table 3. *Retention of the* olil *and* eryl *loci in UV-induced petite mutants isolated from a number of strains*

Strain	Petite frequency (%)*	Number analysed	Ratio of *oli1-0 ery1-r/oli1-r ery1-0* UV-induced mutants	Ratio of *oli1-0 ery1-r/oli1-r ery1-0* Spontaneous mutants†
761-7A	45	670	6·8	0·9
770-7B	25	521	17·5	1·4
413.16	24	279	0·08	0·14
652.1.2	35	243	0·66	2·2
D515–1B.47.3	33	164	0·025	0·32

* Induction of petite mutants with UV-light was performed as described in Molloy *et al.* (1975).

† Data was taken from Table 1.

652.1.2 and D515-1B.47.3). The changes do not merely represent an accentuation of the bias in gene retention seen among spontaneous mutants, since for strain 652.1.2, the preferentially retained locus was reversed. In addition, the response observed here to UV irradiation is clearly not related to the effects of UV irradiation on recombination

as reported by Bolotin *et al.* (1971) and Avner *et al.* (1973), since strains 770-7B, 829-5B, 863-2C and D515-1B.47.3 are ω^+ and 413.16 and 652.1.2, ω^-.

(c) *Rate of growth on non-fermentable substrate*

It was noted in the course of these experiments that some derived strains, carrying two or more mitochondrial antibiotic-resistance alleles, grew at much slower rates than other laboratory strains on non-fermentable substrate.

Two of these strains, 761-7A and 770-7B, were taken for further analysis. The generation times (6·7 and 5·7 h respectively) of these strains on enriched medium with 1% (v/v) ethanol as substrate were significantly longer than those of the standard laboratory strains, L2000 (3·5 h) and D253-3C (3·8 h). Biochemical studies indicated that the efficiency of substrate utilization was not impaired, the final growth yield being the same for all strains; the rate of protein synthesis *in vitro* was also found to be similar in all strains. However, the level of cytochromes and respiration rates of these slow-growing strains were lowered considerably compared with wild-type strains. The nature of the primary lesion has not, however, been characterized.

Table 4. *Analysis of mode of inheritance of 'slow-growth' characteristic*

Strain	Generation time (h)
761-7A	6·7
L2000	3·5
D253-3C	3·8
770-7B	5·7
761-7A *rho(0)* × D253-3C *rho(+)*	3·5
761-7A *rho(+)* × D253-3C *rho(0)*	5·4
761-7A *rho(0)* × L2000 *rho(+)*	3·4
761-7A *rho(+)* × L2000 *rho(0)*	4·1
770-7B *rho(0)* × D253-3C *rho(+)*	3·6
770-7B *rho(+)* × D253-3C *rho(0)*	5·3
770-7B *rho(0)* × L2000 *rho(+)*	3·6
770-7B *rho(+)* × L2000 *rho(0)*	6·0

The media used for determination of growth rates contained 1% (w/v) yeast extract (Difco), 2% (w/v) peptone (Bacto) and ethanol, 1% (v/v). The media was filter sterilized. If glucose, 1% (w/v) was used in place of ethanol as carbon source generation times of all strains lay within the range 1·3–1·7 h.

The genotypes of the antibiotic-sensitive strains L2000 and D253-3C are *α ade1 trp1* and *α his1 trp1* respectively; those of strains 761-7A and 770-7B are given in the legend to Table 1.

To establish whether the slow growth-rate was primarily determined by the nuclear or mitochondrial genome, or resulted from nuclear-cytoplasmic interaction, isonuclear diploid strains carrying different mitochondrial genomes were constructed, as shown in Table 4. For example, *rho(0)* derivatives of strains 761-7A and D253-3C were prepared by ethidium bromide treatment and were then crossed with the *rho(+)* strains D253-3C and 761-7A respectively. Diploids carrying the mitochondrial genome of strain 761-7A were found to grow more slowly (generation time, 5·4 h) than those containing the mitochondrial genome of strain D253-3C (3·5 h). This pattern was repeated for the four pairs of isonuclear diploids studied, the presence of the mitochondrial genome from a slow-growing strain always resulting in a slow growth rate with ethanol as substrate. The extent of the difference varied, being least for the pair of strains 761-7A and L2000. It was evident, however, that the mitochondrial genome was the major determinant of these differences in growth rate.

These differences could be caused by a direct phenotypic interaction of the gene products of the mutant (antibiotic-resistance) alleles carried by these strains, but as this

phenomenon has not been observed for other strains carrying similar combinations of antibiotic-resistance markers, it appears likely that the observed slow growth may be dependant on other properties of the mitochondrial genomes of the strains studied. Further work is in progress to define the nature of the differences between these strains.

2. DISCUSSION

In this paper we have demonstrated the importance of the mitochondrial genome in determining the relative frequency of retention of different loci (*ery1* and *oli1*) among petite mutants. The variations observed must derive from differences in the mitochondrial genomes of the strains studied other than the presence of different antibiotic-resistance mutations or ω^+/ω^- alleles. Further genetic evidence for the presence of widespread divergence among yeast mitochondrial genomes is the wide variability in polarities of recombination and transmission of mitochondrial genes observed in crosses of a number of strains by Howell *et al.* (1973), the determination of which appears to be partly mitochondrial (Linnane *et al.* 1974).

Bernardi *et al.* (1975) have presented direct physical evidence for such divergence. Comparison of the restriction fragments obtained following digestion of mtDNA from different strains with the HpaII restriction endonuclease showed the presence of widespread differences between wild-type strains. It was concluded that these differences were too great to be accounted for by a small frequency of point mutations.

The presence in the mitochondrial DNA of 'spacer' regions of high A.T content, interspersed with G.C-rich informational regions (Prunell and Bernardi, 1974) provides a possible explanation for the extent of divergence. As the A.T-rich regions are presumably not translated into protein products the selection pressure in these regions must not be based on the function of gene products but on the function of these regions, as yet undefined. The pressure for conservation of sequences in these regions is probably much less, therefore, than for other regions of the genome.

It has been proposed by Prunell & Bernardi (1974) and Clark-Walker & Miklos (1974) that petite mutagenesis may proceed via internal recombination events between A.T-rich regions. The probability of such events occurring in A.T-rich rather than G.C-rich regions is very high; on the assumption that a sequence of eight paired bases was required to initiate an illegitimate recombination event, the probability of such sequences existing in an A.T-rich region is about thirty times greater than in a G.C-rich region. It is possible that our observation of differences in relative gene retention between strains may reflect differences in these A.T-rich spacer regions. The marked effects of UV-irradiation, which would be expected to preferentially affect A.T-rich regions, on relative retention of the *ery1* and *oli1* loci provides initial evidence supporting the concept that divergence in the A.T-rich regions of the mitochondrial genome could be a major cause of differences in the genetic behaviour of strains.

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