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Mitochondrial instability in a strain of *Saccharomyces cerevisiae*

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SUMMARY

A haploid strain of *Saccharomyces cerevisiae* has been described which, on glucose medium, segregates vegetatively a high frequency of mutants with different degrees of respiratory impairment. The range of mutants seemingly encompasses both non-revertible ρ- petites and revertible point mutations resembling leaky *mit*- mutations. The segregants have aberrant cytochrome contents and reduced growth capabilities on fermentable sugars other than glucose; these defects apparently correlate with the degree of respiratory impairment. Genetic analysis of this mutator phenomenon has implicated a nuclear gene which appears to show specificity of interaction with the mitochondrial genome as well as a requirement for glucose repression. The mutator effect seems to extend also to the loci in mitochondrial DNA for resistance to the antibiotics erythromycin and oligomycin.

1. INTRODUCTION

Extensive deletions in the mitochondrial DNA of yeast cells occur spontaneously with high frequency giving rise to the irreversible respiratory mutant known as *petite* or ρ-. Treatment with ethidium bromide results in up to 100% conversion to ρ- and extensive treatment can eliminate the mitochondrial genome altogether. Reversible, point mutations of comparatively low frequency also occur in the mitochondrial DNA and include drug resistance (Thomas & Wilkie, 1968) and respiratory deficiency of the class now know as *mit*$^-$ (Flury, Mahler & Feldman, 1974; Tzagaloff, Akai & Needleman, 1975). The induction of reversible mutants can be facilitated by the use of manganese chloride as a selective mutagen (Putrement, Baranowska & Prazmo, 1973).

In this communication we describe a strain of *S. cerevisiae* which generates spontaneously a wide range of mitochondrial genetic lesions both reversible and irreversible.

2. METHODS

Strains D22 and A285 were taken from the stock collection of this laboratory and carried the respective nuclear markers: ade_2, a; *his try* α. Strains 5178B and 2180 were prototrophic strains of mating type *a*, kindly supplied by Dr Bruce Mackler, University of Washington, U.S.A.

Culture media contained yeast extract (YE, 1 %) and one or other of the carbon sources, glucose (2 %, YED), glycerol (4 %, YEG), galactose (2 %), α-methyl-D-glucoside (2 %) and maltose (2 %). When required, chlorimipramine (CI, Geigy) and cycloheximide (CHI, Sigma) were added to give concentrations of 70 μg/ml and 1 μg/ml respectively in YED medium. Assessment of growth in a series of different media was carried out in the first instance by drop-inoculation onto agar media using a multiple inoculator device (Wilkie, 1972). Growth curves were obtained from shake culture in flasks with side arms to allow optical density readings to be made in an EEL colorimeter. In mutation studies, erythromycin as the lactobionate (Abbott, Chigao) and oligomycin (Sigma) were added to YEG medium to give concentrations of 2 mg/ml and 2 μg/ml respectively.

For absorption spectra, cells were grown to stationary phase in YED, washed with distilled H_2O and scanned in an SP 1800 recording spectrophotometer, using a tissue paper blank. Standard techniques were used in crossing, sporulation and tetrad analysis. Microdissection was carried out with the DeFonbrune micromanipulator. Incubation temperature was 30° throughout.

3. RESULTS

Strain A285 is a respiratory competent strain capable of growing in media containing the non-fermentable carbon source, glycerol (YEG). Cells plated on YEG agar gave rise to colonies of normal morphology, that is, uniformly round (Fig. 1*b*). When YEG-grown cells were plated on glucose (YED) medium less than 1 % gave petite colonies, and in this respect A285 was similar to most other strains. However, all other colonies that appeared on YED grew unevenly giving a scalloped morphology. After about 3 days incubation, outgrowths arose which could overgrow the scalloped base of the colony (Fig. 1*a*). When cells were sampled from the scalloped base and plated on YED they gave rise mainly to smaller colonies which tended to have normal morphology. Cells sampled from the outgrowths (papillae) gave, on the other hand, almost entirely scalloped colonies once more when plated.

Sixty-eight of the smaller colonies from the original plating of the scalloped base were isolated and growth capability tested by drop inoculation onto the drug/sugar series. It has been established that ability to utilize the sugars galactose, methylglucoside and maltose is related to respiratory capacity; the typical petite mutant of A285, for example, cannot utilize galactose and α-methylglucoside (Evans & Wilkie, 1976; Mahler & Wilkie, 1978). The tests here similarly showed a correlation between growth characteristics on YEG (impaired in nearly all isolates) and utilization of the two sugars. From the growth curves in Fig. 2, it is evident that there is a wide variation in growth in the various media. Growth curves of an authentic petite, obtained by treatment of segregant 25 with ethidium bromide (20 μg/ml), are presented in Fig. 3 for comparison.

The cytochrome spectra of the segregants and the petite (Fig. 4) indicate some correlation between the relative amounts of cytochromes synthesized and growth in the various carbon sources. Where cytochromic aa_3 and *b* are absent as in

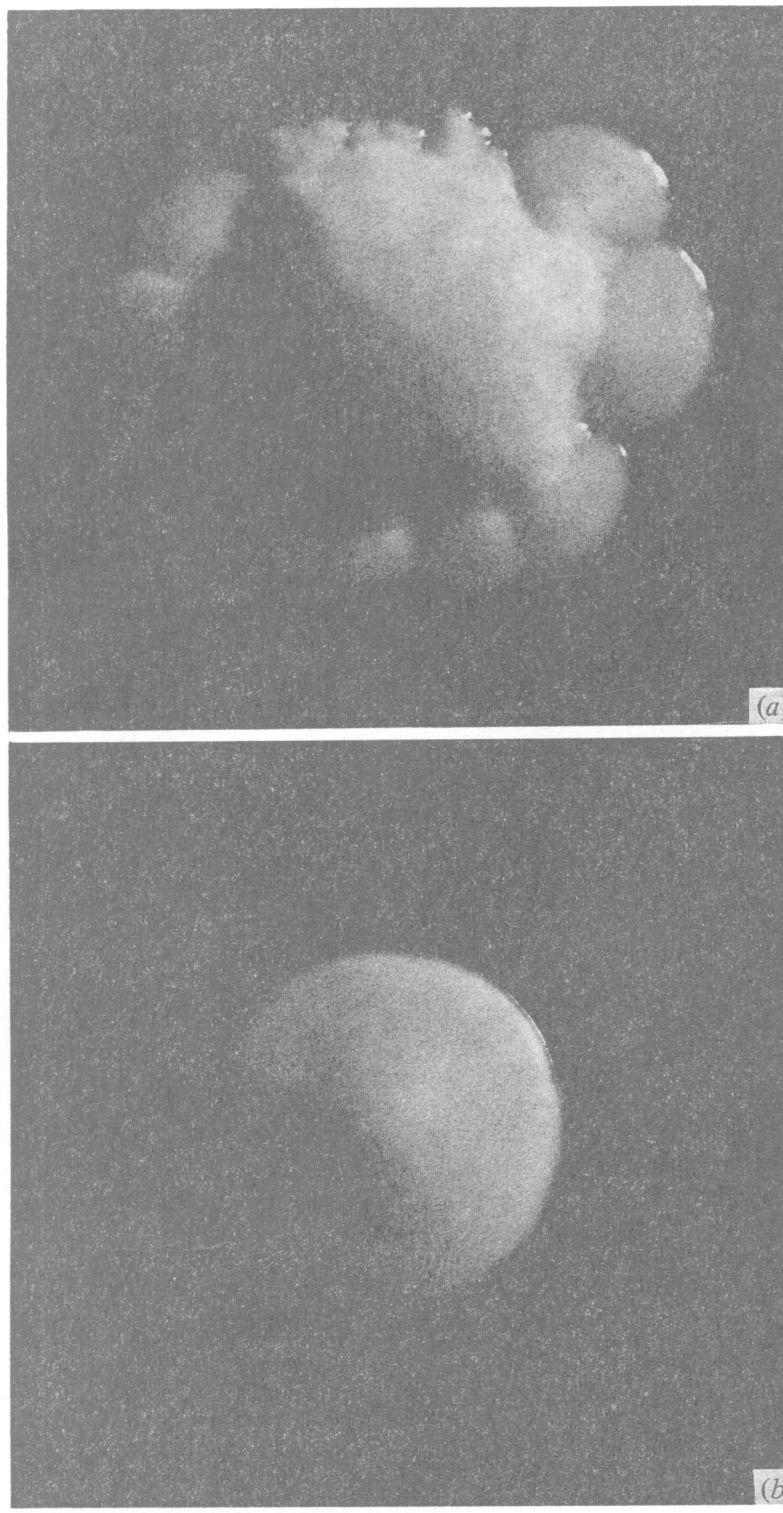

Fig. 1. (*a*) Mature colony of A285 on solid glucose medium. × 15. (*b*) As (*a*) but on glycerol medium.

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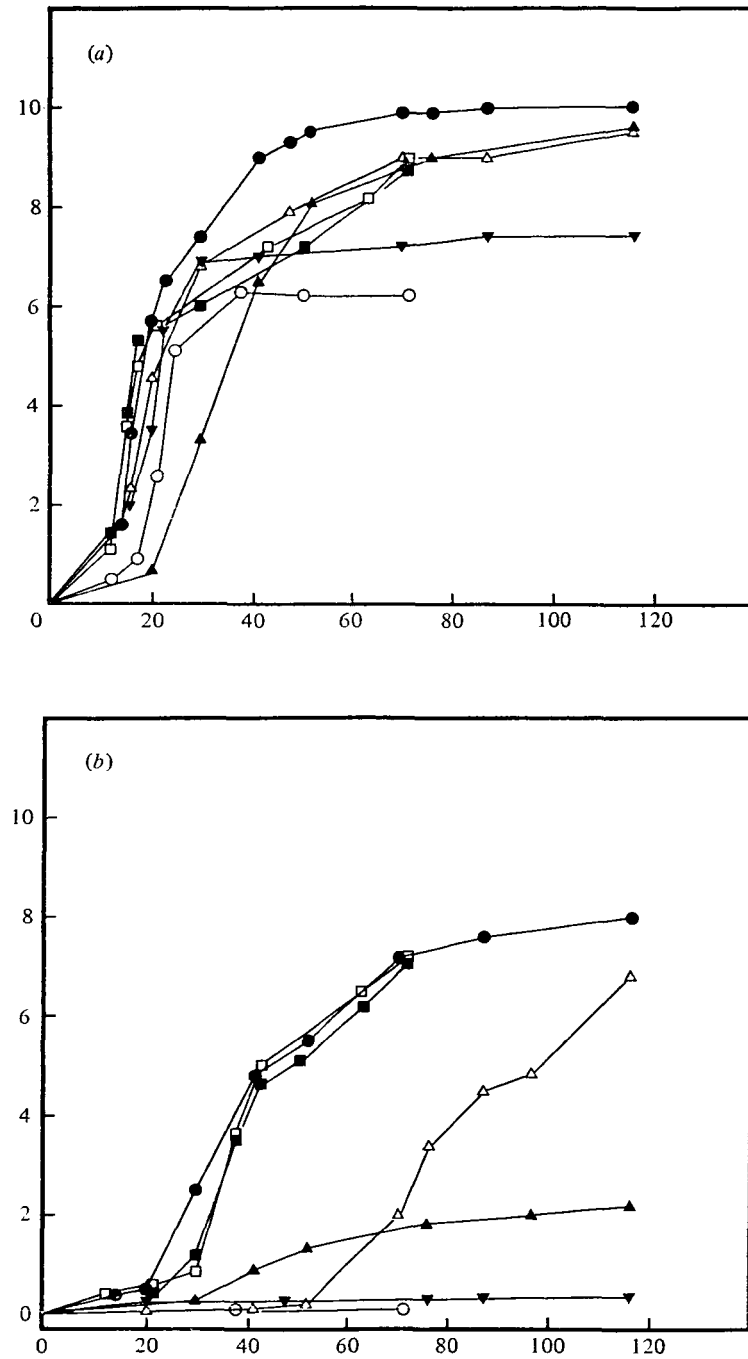

Fig. 2. Growth curves of A285 and various numbered segregants in liquid media containing different carbon sources: ●, A285; ○, 1; □, 5; ■, 8; ▼, 9; ▲, 16; △, 25. Abscissa: time in hours. Ordinate: optical density. (*a*), glucose; (*b*), glycerol; (*c*), galactose; (*d*), α methylglucoside; (*e*), maltose.

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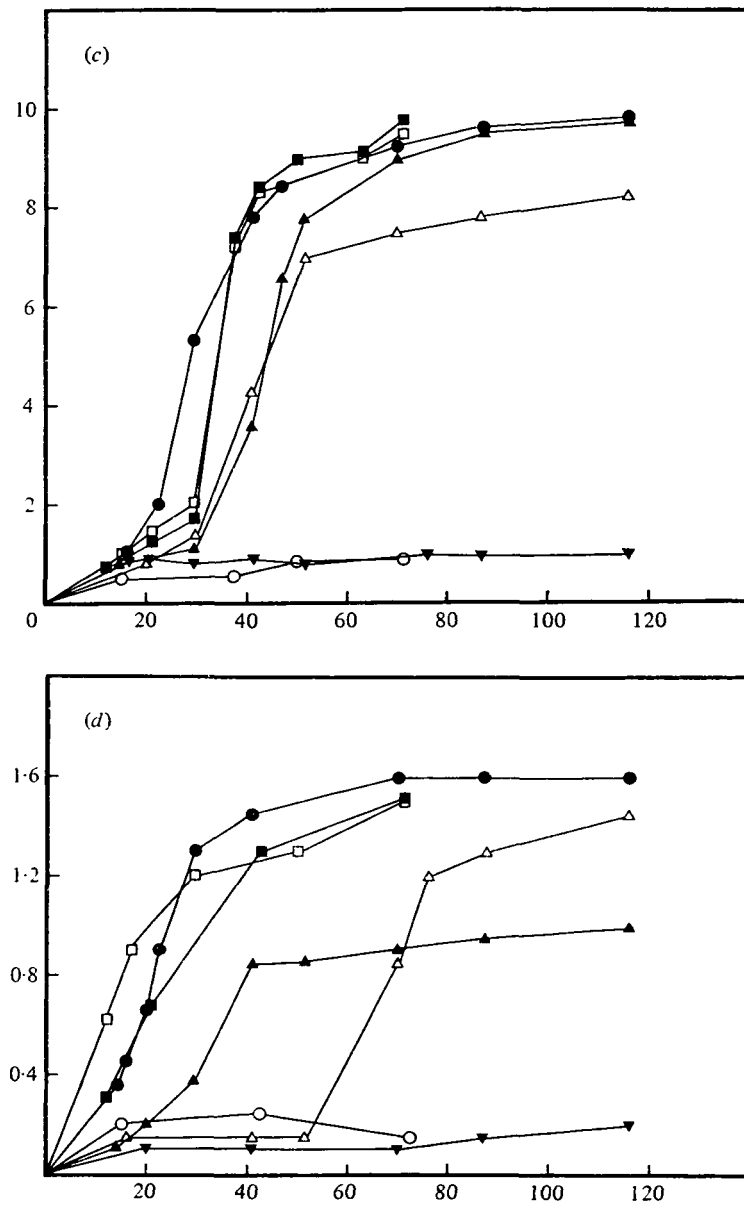

Fig. 2. (continued)

segregants 1, 9 and in 25EB, no growth occurs either in YEG or in medium containing the two sugars. Apart from loss of ability to utilize the two sugars, a change in cellular tolerance to CI is also indicative of mitochondrial damage (Linstead, Evans & Wilkie, 1974). In a later analysis of 20 other segregants, as well as deficiency in respiration and sugar utilization, it was noted that two had acquired resistance to CI and cycloheximide (CHI) and six others showed resistance to CHI alone.

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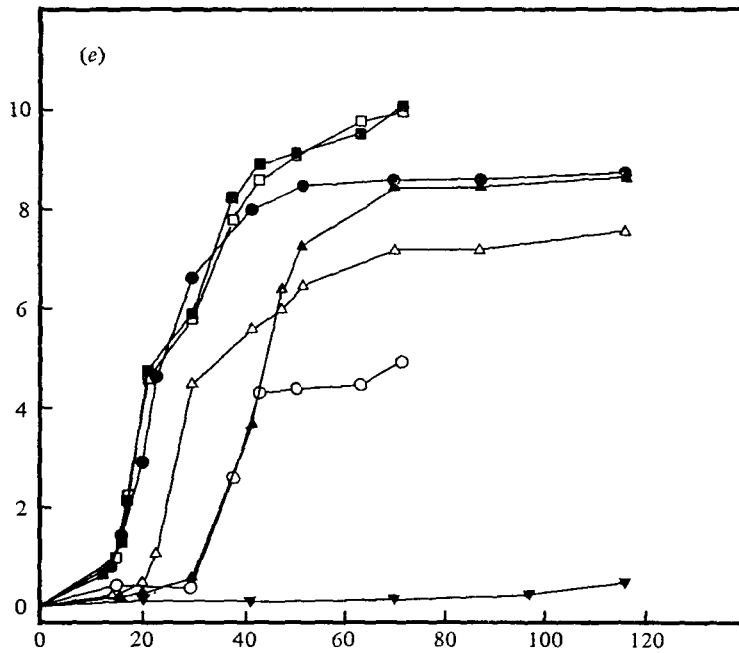

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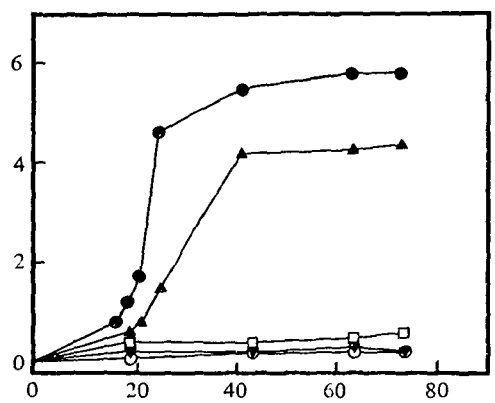

Fig. 3. Growth curves for different carbon sources of a petite induced in segregant 25 by ethidium bromide treatment: ●, glucose; ○, glycerol; □, galactose; ▼, α methylglucoside; ▲, maltose. Abscissa: time in hours. Ordinate: optical density.

Stability of segregants

It was noticed in the drop-inoculation tests that, against the background of slow-growing cells of some of the segregants on YEG and sugar media, papillae of faster growing cells (revertants) came up. This apparent revertibility was also seen in some segregants which did not grow on YEG, a condition characteristic of some of the respiratory mutants in the class know as *mit-*. One of the reverting types was segregant 25 while segregant 9 came into the category of apparent irreversibility.

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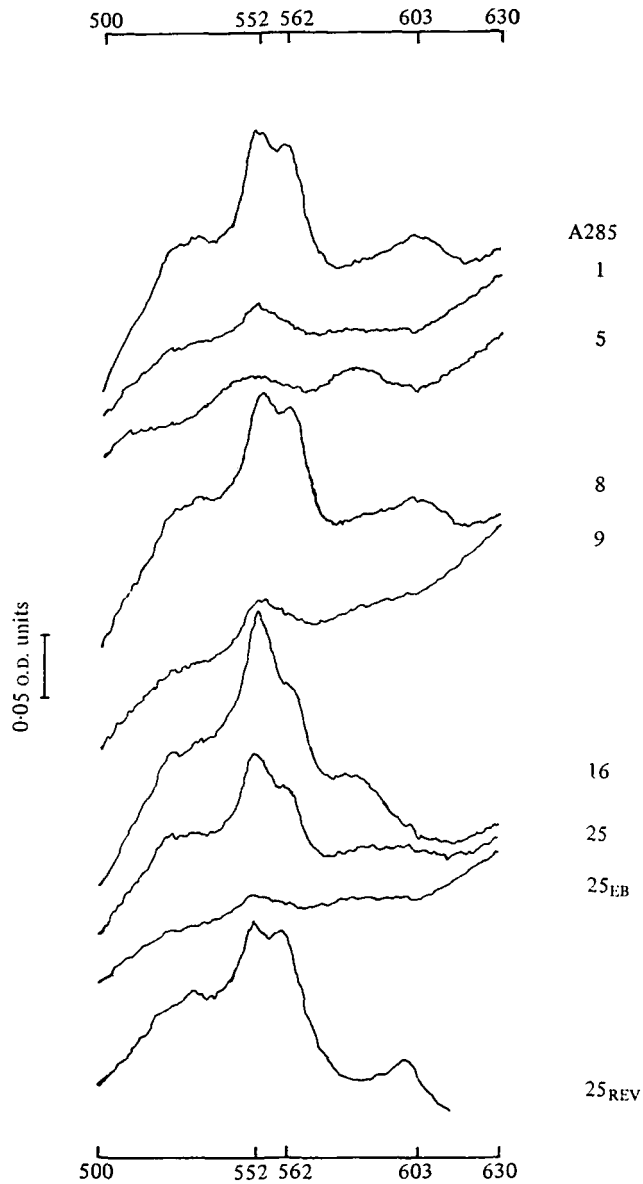

Fig. 4. Cytochrome spectra of A285 and various numbered segregants. Abscissa: wavelength in nanometres. Ordinate: optical density. The absorption peaks of various component cytochromes are indicated on the abscissa: 522, *c*; 562, *b* and 603, $a + a_3$.

Cells of segregants 9 and 25, pregrown on YED agar overnight, were plated onto YEG medium. From a total of 10^8 cells plated of segregant 9, no revertant was seen while 93 faster growing colonies came up from the plating of 2×10^6 cells of segregant 25. One of these revertants when tested showed a normal cytochrome spectrum (Fig. 4).

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Mutations to erythromycin and oligomycin resistance

The above results indicated that strain A285 was spontaneously generating a range of respiratory deficient mutants with high frequency but only on YED medium and not on YEG medium. In other words, the instability of the mitochondrial genome was apparent under conditions of glucose repression. This is not unprecedented and a mutant described by Wilkie & Negrotti (1968) buds off daughter cells which are almost entirely petite in a glucose medium but gives normal daughter cells in glycerol medium. Maltose (and to a lesser extent α-methyl-glucoside) had a similar effect on colony morphology to glucose in the case of A285, but colonies developing on galactose (which is a non-repressing sugar) were normal. Using glycerol-grown cells as a control, an initial experiment was undertaken to ascertain whether A285 was generating mitochondrial mutations other than those associated with respiratory deficiency. Since erythromycin and oligomycin appear to be specific mitochondrial inhibitors manifesting their effects only in non-fermentable medium, mutants isolated as resistant to these drugs must obviously be respiratory competent.

Cells of A285 were pregrown for 24 h on YEG and YED agar respectively, and plated on erythromycin- and oligomycin-containing YEG medium. At the same time appropriate aliquots were plated on YEG and YED alone to obtain an estimate of the percentage respiratory deficient cells in these suspensions. It was found as expected that YEG-grown cells were comprised of approximately 100% respiratory competent cells while only about 55% of the YED-grown cells gave colonies on YEG. After 10 days incubation, colonies were scored on the drug plates. The frequency of resistant mutants in YEG-grown cells was $4{\cdot}3/10^6$ for erythromycin and $54/10^6$ for oligomycin. In the case of YED-grown cells these figures were respectively $18/10^6$ and $206/10^6$. These results indicated that antibiotic resistant mutants were arising at a higher rate under the conditions of mitochondrial instability. Further experiments were performed with diploid cells to establish this point and will be described below.

Genetics

A285 was crossed to strains of 5178B and 2180 respectively by setting up mating mixtures on YED medium. Since 5178B and 2180 are prototrophic, it was necessary to isolate zygotes by micromanipulation in crosses with these strains. This was done after 2–3 h incubation of the mating mixtures. In a third cross (to D22), mating mixtures were set up on minimal medium and incubated for 3 days. Samples of colonies that came up on the minimal medium were shown to be diploid by the sporulation test.

Crosses of A285 to 5178B and 2180 were analysed by plating cell samples from zygote clones on YED as a first step. It was noted before plating that all zygote clones (12 in each cross) showed the scalloped morphology and, when sub-cultured onto sporulation medium, produced asci. Colonies that came up after plating were mainly scalloped with a range of smaller colonies similar to platings of A285. When

isolated and tested, the latter colonies showed the same range of respiratory deficiency as the A285 segregants. It was concluded that the scallop phenotype was dominant in these crosses.

The asci from the cross A285 × 5178B were microdissected for tetrad analysis, but no complete tetrad was found in 24 dissected. Viability of ascospores was poor and only 14 ascospore cultures were obtained. Of these, 10 showed the scalloped morphology and 4 developed normal colonies when plated on YED. These results are not inconsistent with a single nuclear gene control of the scalloped condition. In the cross A285 × D22, scalloping was evident in some of the diploid colonies (zygote clones) on minimal medium. These were mainly smaller colonies and the larger colonies showed little or no evidence of scalloping. When samples of the different colonies were plated on YED, the colonies that came up segregated for scalloped and normal phenotype. The frequencies of the two classes correlated with the size and morphology of the colony on minimal medium from which the cells were sampled; large, round colonies giving mainly round colonies on plating and smaller colonies giving mainly scalloped. These phenotypes were stable on further subculture and plating. Segregation of this type during vegetative growth is indicative of cytoplasmic control (Thomas & Wilkie, 1968). It was tentatively concluded that the activity of the hypothetical scallop gene in the nucleus depended on the state of a cytoplasmic factor, presumably the mitochondrial genome, and this state was such in the case of D22 that the mitochondrial system was largely unaffected by the scallop gene, whereas A285, 5178B and 2180 were all affected. In the cross between A285 and D22 it would be expected that mitochondrial type would segregate among the diploid progeny. A scalloped diploid segregant was sporulated and asci dissected. Again, there was poor viability and only 2 complete tetrads were obtained in over 30 asci dissected. These segregated 2 scalloped to 2 normal. The tetrads also segregated 2:2 for the red (*ade*$_2$) marker which recombined with the scalloped phenotype. Other individual ascospores which gave cultures numbered 15 and these segregated 6 normal: 9 scalloped. These results indicated once again that a nuclear gene was controlling the scalloped phenotype.

When a normal segregant was sporulated and dissected, there was again poor ascospore viability. Of 30 asci dissected, only 15 individual spores gave cultures. The phenotypes of all 15 were normal viewed both as ascospore clones and as plating of these on YED. The ascospores segregated 5 red: 10 white. These results supported the hypothesis that the expression of the nuclear gene for scalloped morphology depended on the type of the mitochondrion; if diploid progeny inherited the A285 type, they developed scalloped colonies; if the D22 type was inherited, the gene had little or no effect on morphology.

The hypothesis was tested in another way by inducing petite in the two strains with ethidium bromide (48 h treatment) and carrying out the reciprocal crosses A285$\rho+$ × D22$\rho-$ and A285$\rho-$ × D22$\rho+$. When random diploid cells from these crosses were plated, the first cross gave all scalloped colonies and the second all normal except for a few colonies which showed a marginal scalloped condition. These results provide good evidence that the mitochondrial genome is involved in

the expression of the scalloped phenotype. Finally, a backcross was made between an ascospore culture which showed the scalloped morphology and strain 5178B. Platings of random diploid cells from the cross gave scalloped colonies on YED medium as expected.

Mutation rates in diploid segregants

A scalloped diploid and a normal diploid from the cross A285 × D22 were pre-grown in YED medium for 24 h and then plated on YEG medium containing erythromycin. After 7 days incubation the number of resistant colonies that came up from the scalloped type was 62 and that from the normal was 24 out of 10^6 cells plated in each case. This was a preliminary test and no account was taken of the

Table 1. *Frequency of erythromycin (ER) and oligomycin (OL) resistant mutants in scalloped and normal diploid segregants from the cross A285 × D22*

Segregant	Total cells plated	Respiratory competent (%)	Resistant colonies*	
			ER	OL
Scalloped	$0{\cdot}9 \times 10^6$	47 (247/520)	28 (64)	59 (134)
Normal	$2{\cdot}4 \times 10^6$	98 (280/285)	8 (3·4)	7 (3)

* Figures in parentheses are per 10^6 respiratory competent cells.

proportion of cells from the scalloped culture which were respiratory deficient and so unable to give colonies on YEG medium. In a more detailed experiment, different scalloped and normal segregants were used from those above and the results are given in Table 1. Cultures were pre-grown in YED for 48 h then plated on drug media with control platings on YEG and YED. After 7 days incubation resistant colonies were scored.

The results indicate that the scalloped segregant generates significantly more antibiotic resistant mutants than normal segregant.

4. DISCUSSION

A strain of yeast spontaneously yielding high frequencies of ρ- mutations was first described by Ephrussi & Hottinguer in 1951; their genetic analysis attributed responsibility to a single nuclear gene. Similar 'unstable' strains have been encountered subsequently (Nagai, Yanagashima & Nagai, 1961; Sherman, 1963) and it has been found in some cases that the mutator activity of the nuclear gene is manifested only under glucose repression (Wilkie & Negrotti, 1968). More recently an account has been given of a *ts* mutant in which there is virtually complete conversion to petite (ρ-) cells during growth at an elevated temperature; the mutation maps in mitochondrial DNA between paromomycin resistance and chloramphenical resistance and it too demands glucose repression for activity (Bandlow, 1976). Assuming nuclear control in A285, then our strain apparently contrasts with these cases in two respects. Firstly, the nuclear allele primarily

responsible for the mutagenic effect is inactive in combination with some types of mitochondrial genome, though it is possible that further crosses with 'scallop' genes reported in the past could have revealed a similar kind of specificity; this observation is reminiscent of the situation in maize, where the recessive nuclear gene *iojap* seems to generate a high frequency of heritable defect in some but not all types of chloroplast genome (Rhoades, 1946). Secondly, and more importantly, the proposed scallop gene described here appears to cause so-called point mutations in the mitochondrial DNA such as *mit-* and antibiotic resistance, as well as non-revertible lesions of the ρ- class, in agreement with the possibility that these two types of mutation when occurring spontaneously, share a common 'premutational' origin.

The postulated A285 scallop gene would apparently have a similar requirement for the presence of catabolite repression as the mitochondrial *tsm* 8 gene studied by Bandlow (1976). This could be a general feature of mutator genes affecting the mitochondrial genome. In this respect it is interesting to recall that caffeine, which has been reported to antagonize glucose repression of sporulation in yeast (Tsubio & Yanagishima, 1976), has also been shown to suppress the high rate of petite production in an 'unstable' strain of yeast (Nagai, 1962), though caffeine's well publicized property of inhibiting error-prone DNA repair (Timpson, 1977) may also be relevant here. In a very recent experiment we have found that our strain A285 is also 'stabilized' by caffeine: colonies in glucose medium in the presence of 1·6–2·0 mg/ml caffeine are smooth and round.

It is possible that point mutations of the mitochondrial DNA were a feature of previously described mutator genes but were simply overlooked because of the predominance of the classical ρ- mutation. In A285 the heterogeneity of mutation type was immediately apparent in the striking variation in growth competence on different carbon sources. As we have already reported cytoplasmic petites display strain dependent patterns of ability to grow on the three fermentable sugars galactose, α methylglucoside and maltose (Evans & Wilkie, 1976), ethidium bromide induced petites of A285 being defective on the two first mentioned sugars. Many A285 segregants though deficient in respiration (glycerol growth, cytochrome spectra) contrasted strongly with expectation in showing clear growth on, for example, galactose (see Fig. 2). A segregant of particular interest is No. 9; this strain is severely impaired in growth on all three fermentable sugars (other than glucose) considered here, and so displays a more profound 'weakness' in this area of metabolism than petites isolated in the conventional manner by ethidium bromide mutagenesis.

The results show a mutator influence on the mitochondrial genome of A285. Whether or not there is a similar effect on the nucleus has yet to be investigated.

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