Genet. Res., Camb. (1976), **27**, *pp.* 89–93
Printed in Great Britain

SHORT PAPER

Mitochondrial factors in the utilization of sugars in *Saccharomyces cerevisiae*

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(*Received* 15 *December* 1975)

SUMMARY

Cytoplasmic petite mutants, spontaneous and induced, show various patterns of ability *v.* inability to utilize the sugars galactose, α-methylglucoside and maltose, depending on the strain from which they were isolated. Petites recombine and segregate their different sugar deficiencies among vegetative diploid progeny when crossed, indicating mitochondrial control. Crosses between petites and wild-type further indicate that mitochondrial factors may be interacting with nuclear factors in a complex regulatory system.

1. INTRODUCTION

There are a number of reports in the literature that cytoplasmic petites mutants (ρ^-) may or may not be able to utilize certain fermentable sugars other than glucose, for growth (see Puglisi & Algeri, 1974). Little has been done by way of genetic analysis of such deficiencies but Schamhart, ten Berge & van de Poll (1975) describe a mutant of *Saccharomyces cerevisiae* which has impaired maltose growth when in the petite condition and show that the mutant character may be cytoplasmically inherited.

We have classified various petite mutants of different genetic backgrounds with respect to their abilities to utilize the sugars galactose (GA), α-methylglucoside (MG) and maltose (MA) and report on the results of a series of crosses involving petites with various sugar deficiencies.

2. MATERIALS AND METHODS

Haploid strains of *S. cervisiae* of this laboratory were used. Growth medium contained Difco yeast extract (1%) supplemented with 2% of one or other of the sugars glucose, GA, MG and MA or with 4% of the non-fermentable carbon source glycerol. Standard techniques of crossing, sporulation and tetrad analysis were employed except that zygotes in most crosses were obtained by micromanipulation from mating mixtures. Analysis of diploid progeny of zygotes was carried out on colonies from random platings, initially by velvet pad replication but also by a drop-inoculum method for more detailed analysis of growth on solid medium (Wilkie, 1972). Petite colony was scored on the basis of small size and colour on glucose medium and inability to grow on glycerol medium. Acriflavine (20 μg/ml) was added to glucose medium to induce petite colony. Each petite colony was sampled and plated and a pure isolate obtained before proceeding to studies of growth characteristics and genetics.

3. RESULTS

All parental (ρ^+) strains were able to utilize all carbon sources. All individual petite mutants isolated were unable to grow or had significantly altered growth characteristics (except those of strain 45B) on one or more of the sugars other than glucose. A particular pattern of sugar utilization characterized the petites of different strains (Table 1). For the most part, petites either grew or failed to grow on the test sugar, but occasionally petites within a strain varied in ability to utilize a sugar. This variation among petites of the same strain emphasized the fact that respiratory deficiency *per se* was not responsible for inability to utilize the sugars. Variation was also seen among the growers, since some petites grew poorly and others grew almost as well as on glucose medium. This variation was particularly evident for MG utilization. These features applied to both induced and spontaneous petites. In the latter, there was no reason to suppose that any genetic change had occurred in the cell other than that affecting mitochondrial DNA. In other words, the loss of ability to use a sugar could be directly related to lesion in mitochondrial DNA.

Table 1. *Growth of acriflavine-induced petite mutants of various haploid strains of yeast supplied with different sugars in yeast extract medium*

Parent(ρ^+) strain	Galactose	Methyl glucoside	Maltose	Number of petites tested
A15	−	−	+	97
B41*	−	−	−	15
A285*	−	−	+	56
D6	−	+/−†	−	55
D18*	−	+	−	76
188	−	+	−	16
D22	+/−‡	−	+	38
D6R3	+	−	+	75
D4	+	−	+	30
D11	+	−	+	83
D26*	−	+	+	50
45B	+	+	+	104

* Spontaneous petites isolated from these strains were tested and showed the same sugar growth characteristics as those induced by acriflavine.

† Of petites examined, 10% did not grow on methyl glucoside.

‡ Of petites examined, 95% did not grow on galactose.

(i) *Genetic analysis*

(a) *Petite × petite crosses*

Random diploid cells were sampled from these crosses, plated, and resulting colonies, all of which were petite, were scored for ability to grow on the various sugars. Results are shown in Table 2. It is clear that the factors controlling utilization of sugars recombine and segregate among the diploid progeny. Not only are recombination frequencies high but preferential transmission and/or replication of particular recombinant genomes (usually the reconstituted + + +) seems to occur. In crosses 3, 4 and 5, in which parental strains are GA^-, presumably the postulated deletions are non-overlapping and recombine to give GA^+. The main difficulty is in trying to interpret recombination data where respective genomes have various deletions which may be extensive.

(*b*) *Petite* × *wild-type crosses.*

The following crosses were made: D6R3 × D18 ρ^-A; 6-81 (E^R) × A15 ρ^-A; 22–701 (E^R) × D6 ρ^-A; A285 × D18 ρ^-A. In each of these crosses, the ρ^- parent showed suppressive activity ranging from 3 to 21 – that is, the frequency of ρ^- diploid cells in random samples was 3–21 times that of the corresponding $\rho^+ \times \rho^+$ crosses. Petite diploid colonies that were further analysed were mainly recombinant with respect to the utilization of sugars and again the predominant phenotype was + + + (20 out of a total of 28 analysed). Presumably, ρ^- segregants originated by recombination of ρ^+ and ρ^- genomes in zygotes although reciprocal products (ρ^+ segregants with deficiencies in sugar utilization) were not detected among a total of 80 ρ^+ diploids analysed from the four crosses. Thus ρ^+

Table 2. *Growth on various sugars of diploid segregants from petite* × *petite crosses*

Cross	Growth of diploid segregants: GA	MG	MA	Numbers
1. D6 ρ^-4 (GA$^-$MG$^+$MA$^-$) × D4 ρ^-A (GA$^+$MG$^-$MA$^+$)	+	+	+	79
2. D6 ρ^-4 × 45B ρ^-A (GA$^+$MG$^+$MA$^+$)	+	+	+	78
	−	+	+	1
3. A285 ρ^-J (GA$^-$MG$^-$MA$^+$) × D18 ρ^-K (GA$^-$MG$^+$MA$^-$)	+	+	+	54
4. D18 ρ^-F (GA$^-$MG$^+$MA$^-$) × A285 ρ^-J	+	+	+	48
	+	−	+	6
5. A285 ρ^-R (GA$^-$MG$^-$MA$^+$) × D18 ρ^-F	+	+	+	22 (9)*
	+	−	+	82 (56)
	+	+	−	23 (8)
	+	−	−	3 (10)
6. A285ρ^-A (GA$^-$MG$^-$MA$^+$) × D18 ρ^-A (GA$^-$MG$^+$MA$^-$)	.	+	−	40
	.	−	+	57
	.	+	+	21
	.	−	−	25
7. B21 ρ^-A (GA$^+$MG$^+$MA$^+$) × A15 ρ^-A (GA$^-$MG$^-$MA$^+$)	+	−	+	81

* Results of repeat experiment.

diploids showed the same mitochondrial phenotype as the ρ^+ parent and this included the mitochondrially transmitted erythromycin resistance (E^R) (Thomas & Wilkie, 1968). It is possible that recombinational events between ρ^+ and ρ^- genomes result here in ρ^- genomes, a mechanism already proposed to explain suppressivity. Perhaps it is not possible to transfer a segment carrying a deletion into the ρ^+ genome without its becoming ρ^-. These speculations again emphasize the lack of knowledge of the mechanism of the petite mutation and of the structure of petite genomes (see Bernardi, 1975).

(ii) *Inheritance of the sugar-utilization patterns of petites*

A random sample of diploid cells from the cross D6R3 × D18 ρ^-A were sporulated and meiotic tetrads analysed. Sporulating cells were necessarily ρ^+ since ρ^- diploids are incapable of meoisis. Cells were then sampled from ascospore cultures and plated on acriflavine medium to induce petites. The ability of these petites to utilize the sugars was tested (Table 3). Six out of the 7 tetrads segregated in the non-mendelian ratio of 4:0 for ability *v.* inability to use GA and 5 out of 7 with respect to MA. In other words, there was every indication of control by cytoplasmic factors in the capabilities of the petites of D6R3 and D18 to use GA and MA. On the other hand, the tetrad data for MG utilization by petites indicated segregation of two unlinked nuclear genes the presence of either one of which permitted metabolism of MG in cells with petite mitochondria. These genes were

Table 3. *Tetrad analysis of ascospore cultures with respect to sugar utilization of petites* in the cross D6R3 × D18-p-A*

Tetrad types			
GA	MG	MA	No.
4+:0−	4+:0−	4+:0−	1
4+:0−	3+:1−	4+:0−	1
4+:0−	2+:2−	4+:0−	2
4+:0−	3+:1−	3+:1−	2
3+:1−	2+:2−	4+:0−	1

* Average number of petites tested from each ascospore culture = 24.

presumably present in the nucleus of strain D18, the petites of which utilized MG (Table 1). These genes appeared to be recessive since a good proportion of the ρ^- diploid segregants in crosses involving D18 were MG$^-$ (Table 2). The hypothesis of nuclear genes insensitive to mitochondrial deletions was substantiated by subjecting a petite of D18 to further treatment with 20 μg/ml acriflavine. After about ten cell generations, when it was expected that any mitochondrial DNA present to begin with would have been further degraded or eliminated, cells were sampled and plated. Twelve of the resulting colonies were tested and all had retained the ability to grow on MG medium. On the other hand, a petite of strain 188 similarly treated with acriflavine, resulted in a high proportion (71 out of 110 tested) of cells having lost the ability to grow on MG medium. In this latter case, it was concluded that the ability or inability of petite cells to utilize MG depended solely on the state of the mitochondrial DNA.

A further point may be made regarding the phenotypes of the petites of the ascospore cultures. Although these are listed in Table 3 as though all petites from any one culture were similar, this is not strictly the case. Approximately one petite in each batch of about 24 analysed, differed from the others with respect to the pattern of sugar utilization. Such rates of change are too high to be attributable to nuclear factors, but probably relate to the state of mitochondrial DNA in these cases.

4. DISCUSSION

As in the loss of erythromycin resistance (Thomas & Wilkie, 1968), loss of ability to utilize a sugar concomitantly with loss of the ρ factor provides evidence of deletion of a controlling factor in mitochondrial DNA. If this is so, the pattern of deletions in petites is characteristic of the strain from which they were derived. The genetic basis of strain differences in this respect is not known but may be a feature of the mitochondrial DNA

itself. As expected, recombination between genomes of different petites in zygotes occurred in that new patterns of sugar utilization emerged and segregated among diploid cells in zygote clones. We conclude that there are factors in mitochondrial DNA which regulate those genes in the nucleus involved in sugar metabolism. In some strains, the nuclear genes may have mutated to insensitivity to mitochondrial regulation as in MG utilization by strain D18.

Aspects of the mechanism of mitochondrial control will be discussed elsewhere but we have recently presented evidence that transcription products of mitochondrial DNA can control cellular characteristics (Evans & Wilkie, 1975). Whatever the mechanism, the results described here add a new parameter to yeast mitochondrial genetics allowing phenotypic effects of different petites, other than respiratory deficiency, to be directly assessed. Experiments to locate the postulated mitochondrial factors in sugar utilization relative to known mitochondrial markers are underway.

D.W. is grateful to the Cancer Research Campaign for a grant in support of this work. We are grateful to Mr Robert Hyams for help in screening strains for sugar utilization.

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