A NEW MITOCHONDRIAL MUTATION IN SACCHAROMYCES CEREVISIAE*

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SUMMARY

The genetic analysis of a yeast mutant resistant to D(+) glucosamine on glycerol medium is reported. The mutant phenotype segregates in vegetative diploids, can be enriched for in test medium and shows non-mendelian inheritance patterns. No cross resistance to several inhibitors of mitochondriogenesis was observed. The new locus is designated as [cry 1-r]; catabolite repression resistant yeast.

INTRODUCTION

Wild type S. cerevisiae are poisoned by glucose analogues, such as D (+) glucosamine and 2 deoxyglucose (1). Such yeast, when exposed to these analogues on non-fermentable carbon sources are unable to grow (2). This communication reports the genetic analysis of a UV induced mutant (GR10) which grows on glycerol medium in the presence of glucosamine.

METHODS

Yeast strains: D587-4B (α, his_1 [rho^+]) and D585-11C (α, lys_1 [rho^+]) were a gift from Dr. Fred Sherman, Department of Genetics, Rochester University. Back crosses were made to strain 4BL (α, lys_1 [rho^+]) a derivative of a cross between these parental strains. The mutant, GR10 was derived from 4B2 (α, his_1 [rho^+]) also derived from the original parental strain cross.
Media: YPD, 1% yeast extract, 2% peptone, 3% dextrose. YPG; substitute glycerol for dextrose in YPD. GGM; the glucosamine resistance test medium which consists of YPG + 0.05% glucosamine HCl.
Mating: was according to the procedure of Montenecourt et al. (3)
Ascus dissection: was according to the technique of Johnston and Mortimer (4). The mutant isolation will be described in detail elsewhere.

RESULTS AND DISCUSSION

Strain GR10 is one of several isolates which showed variable degrees of resistance when back crossed to the parental strain 4BL. This variability

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TABLE I

Vegetative Segregation of Glucosamine Resistance (G^R)

Strain number		% glucosamine resistant clones	
4BL and 4B2	(parentals)	0	(400)[a]
GR10	(mutant)	100	(375)
GR10/4BL	(diploid	100[m]	(410)
(GR10/4BL)s	(diploid derivative)	54[m]	(264)
(GR10/4BL)r	(diploid derivative)	100	(358)
(GR10/4BL)R	(enriched diploid)	100[c]	(306)
10P3r	(enriched haploid)	100[c]	(296)

[a] numbers in brackets are the total YPG^+clones scored: [c] strong resistance and rapid growth in all replicates: [m] colonies show micro-colony resistance only.

took the form of micro-colonies (1-20/replicate) within the imprint of diploid, YPD grown colonies replicated (5) to GGM test medium. Such micro-colonies were usually developed 72 hours after replication.

Table I shows the results of growing various haploid and diploid derivatives of GR10 in YPD broth, plating to YPD agar to give 150-200 colonies/plate and then testing these colonies for glucosamine resistance (G^R). Petite colonies (YPG^-) were excluded from these totals. Any clone which showed micro-colony resistance was scored as glucosamine resistant.

It can be seen that the diploid, GR10/4BL is less resistant than the haploid GR10 which is in turn far more resistant than the parental strains 4BL and 4B2. Also, that subsequent vegetative growth (in YPD broth) of sensitive (GR10/4BL)s or resistant (GR10/4BL)r clones selected from the testing of GR10/4BL show an enrichment for sensitivity (G^S) and resistance (G^R) respectively.

TABLE II

Segregation of G^R factors During Sporulation

Cross	4:0 r:s	3:1 r:s	2:2 r:s	1:3 r:s	0:4 r:s	Total[a] tetrads
GR10 vs 4BL; (G^R x G^S) [b]	0	1	1	7	1	10
(GR10/4BL)R; (G^R x G^S)	12	0	0	0	0	12
10P3rρ^- vs 4BL; (G^O x G^S)	0	0	0	0	15	15
10P3r vs 4BLρ^-; (G^R x G^O)	1	6	6	2	1	16

[a] all tetrads showed 2:2 segregation for the chromosomal markers his_1 and lys_1

[b] superscripts, R = resistant, S = Sensitive, 0 = eliminated.

The effect of selective enrichment (6) is also shown in Table I. The diploid GR10/4BL and a haploid derivative 10P3 were grown for 4 days in GGM broth and then plated on to YPD agar. Derivative clones (GR10/4BL)R and 10P3r respectively, were then subcultured to YPD broth and tested for G^R clones as described above. Both strains showed 100% resistance and in addition, all clones showed confluent growth on GGM agar, i.e., micro-colony resistance had been converted to confluent resistance by growth in selective medium.

The results of Table I, non-Mendelian segregation of mutant factors in vegetative clones (haploid and diploid) (7, 8) together with the enrichment effects of selective media (6) are strong indicators that the G^R factor is cytoplasmically inherited.

Confirmation of this hypothesis comes from the meiotic segregation data shown in Table II. All of the tetrads reported in Table II showed Mendelian (2:2) segregation for the chromosomal markers his_1 and lys_1.

Tetrads derived from diploid GR10/4BL showed a spectrum of segregant classes together with a spectrum of micro-colony numbers (3-30) in resistant clones. When the enriched diploid (GR10/4BL)R was sporulated all of the tetrads obtained showed 4:0, resistant:sensitive segregation, and all replicates showed confluent growth on GGM plates. These results also indicate cytoplasmic or mitochondrial inheritance of G^R. These results are not compatible with any simple form of Mendelian inheritance.

Our results do not prove that the locus of G^R is on mitochondrial DNA (mt-DNA). Treatment of yeast with ethidium bromide eliminates mtDNA (9) and petite [rho^o] derivatives of 4BL and 10P3r were made by growing these strains in YPD broth + 5 μg/ml ethidium bromide for 48 hours. This should produce petite cells which are devoid of mtDNA. The result of crossing 10P3rρ^- to 4BL and 10P3r to 4BLρ^- is shown in Table II.

When compared to the two [G^R rho^+] vs [G^S rho^+] crosses reported in Table II, it is clear that elimination of mtDNA from 10P3r has eliminated the transmission of G^R and the reciprocal is true for G^S in the 4BLρ^- cross.

The cumulative evidences of Tables I and II make it fairly certain that glucosamine resistance (G^R factor) is mitochondrially inherited but does not establish its uniqueness. Strain 10P3r was tested for cross resistance to erythromycin (5mg/ml), chloramphenicol (5 mg/ml) and oligomycin (5 μg/ml) and found to be sensitive to these drugs. Known mitochondrial resistant strains were used as controls and these proved to be sensitive to glucosamine poisoning. Strains used were: E^R, strain L411, Linnane (10), OL_I and OL_{II} strains D22-A16 and D22-A13, Avner and Griffiths (11) and C^R, strain 44-5a, Rank (12).

One can therefore designate the genotype of GR10 as α, his_1 [rho^+, cry 1-r].

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