GLUCOSAMINE RESISTANCE IN YEAST. I. A PRELIMINARY GENETIC ANALYSIS[1]

A. J. S. BALL,[2] D. K. WONG AND J. J. ELLIOTT[3]

Department of Biological Sciences, Brock University
St. Catharines, Ontario, Canada L2S 3A1

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ABSTRACT

Mutants of the yeast *Saccaromyces cerevisiae* which can grow on glycerol medium in the presence of 0.05% D (+) glucosamine have been isolated. Genetic analysis of 13 of these glucosamine resistant (GR) mutants demonstrated two modes of inheritance. One group of mutants (GR 5, 6, 7, 8, 9 and 10) gave results characteristic of non-Mendelian inheritance and it is suggested that these mutants represent one or more new mitochondial loci. Four of the remaining mutants showed clear-cut Mendelian inheritance. These mutants fell into two complementation groups and subsequent mapping experiments demonstrated that two independent loci, *gay 1* and *gay 2*, unlinked to each other or to the centromeres of chromosomes *I, II, IV, VIII or IX*, were responsible for conferring glucosamine resistance in these mutants.

IT has been known for some time that glucose analogs inhibit growth and respiration in yeast (for review, HOCHESTER and QUASTEL 1963). These analogs apparently mimic the Crabtree Effect (CRABTREE 1929) i.e., repress mitochondrial respiration by disrupting inorganic phosphate and/or adenine nucleotide metabolism in the cell (RACKER 1974).

Although the Crabtree Effect is a transient phenomenon, sustained inhibition of respiration is produced by glucose analogs (HOCHESTER and QUASTEL 1963). Prolonged repression of mitochondrial biosynthesis by fermentation of sugars is also well documented (SCHATZ and MASON 1974) and it was thought that there might be some connection between these two phenomena.

The intent of this work was to use non-metabolizable glucose analogs as gratuitous repressors of mitochondrial function in the hope that strains resistant to such repression might also show resistance to the repression of mitochondriogenesis by glucose. Such mutants would lead to a better understanding of the Crabtree and Pasteur Effects as well as contribute to unravelling the mechanism(s) by which mitochondriogenesis is controlled in faculative eukaryotes.

The present paper describes the isolation and genetic analysis of some glucosamine-resistant mutants.

[1] Supported by NRC grant #A6795
[2] To whom all correspondence should be directed
[3] Present address: c/o Medical Sciences, McMaster University, Hamilton, Ontario

METHODS

Media and Growth Conditions

All cultures were grown at 30°.

YPD=1% yeast extract, 2% peptone, 3% Dextrose; *YPG*=substitute glycerol for dextrose above; *GGM*=YPG+0.05% D (+) glucosamine HCl; *SD*=Difco Yeast Nitrogen Base w/o Amino Acids+3% dextrose; lysine and histidine were added at 40 mg/l as appropriate. Agar to 2% was added for solid medium.

Chemicals: Yeast extract, Peptone, Yeast Nitrogen Base (Difco) and all other chemicals were obtained from BDH (Canada), Toronto, Ontario.

Mutant Isolation was carried out in the following way. The parental strains 4B2 (*α his1*) or 4BL (**a** *lys1*) derived from a cross of D587-4B (*α his1*) and D585-11C (**a** *lys1*) were treated with UV or EMS to 1% survival and plated to YPG medium. After 48–72 hours such plates were replica-plated (LEDERBERG and LEDERBERG 1952) to GGM medium. Clones which showed growth after 72–96 hours were subsequently streaked to fresh GGM medium before storing on YPD slants at 2°.

Mutant strains used were GR 5, 6, 7, 8, 9, 10 and 133 which were derived from 4B2 and were α, *his1* GGM^R together with strains GR 71, 81, 90, 91, 108 and 130 derived from 4BL which were **a**, *lys1*, GGM^R. Two other strains were used in mapping studies: X3382–3A, (**a**, *ade1*, *gal1*, *trp1*, *his2*, *his6*, *arg4*, *arg5*, *pet17*, *tyr7*, *cdc14* and X6–14 (**a**, *ade1*, *arg4*, *asp5*, *gal1*, *trp1*, *his6*) which was derived from a cross between X3382–3A and 4B2.

Haploid stability was tested by subculturing glucosamine-resistant isolates (GR strains) to YPD broth (24 hour) plating to YPD to get 100–150 clones/plate followed by replicating to YPG and GGM. Only YPG$^+$ clones were scored on the GGM plates.

Diploid stability and *Diploid isolation* were carried out in similar manners. Haploid strains were grown up overnight in YPD broth, mixed and then re-incubated for 3–5 hours. Subsequently, a small aliquot (<0.1 ml) was transferred to SD broth and grown for 24 hours. These SD broths were then plated to SD solid medium (100–150 clones/plate) and diploids for sporulation were isolated from these plates. For diploid stability tests these same SD plates were replicated after 48 hours to YPG and GGM media for scoring as described for hapoid stability tests.

Mating was carried out according to the procedure of MONTENECOURT, KUO and LAMPEN (1972).

Tetrad Dissection was performed according to the method of JOHNSON and MORTIMER (1959).

All other genetic procedures followed the methods of SHERMAN, FINK and LUKINS 1970.

Ethidium bromide treatment was for 48 hr in YPD broth at a concentration of 50 μg/ml ethidium bromide.

RESULTS

Our initial screening process (plating to YPG) eliminated respiratory deficient *petite* (or ρ^-) survivors, thus ensuring that all the clones tested on GGM could assemble effective mitochondria. Subsequent replicates to YPD, SD, SD+ his and SD+lys ensured that the GGM^R isolates (GR strains) were suitable for subsequent genetic analysis. As we had a number of isolates which were either *α*, *his1*, GGM^R or **a**, *lys1*, GGM^R we decided to do a complementation test on these strains. The diploids constructed from 8 *α* strains and 8 **a** strains did not give clear cut results. Most diploid colonies gave heterogeneous responses (see below) when YPD master plates were replicated to GGM but homogeneous responses (uniform growth of inoculum) on YPG. The heterogeneity took the form of micro-colonies growing in the imprint. Subsequent replication of imprints containing micro-colonies after 48 hr growth on GGM to YPG medium

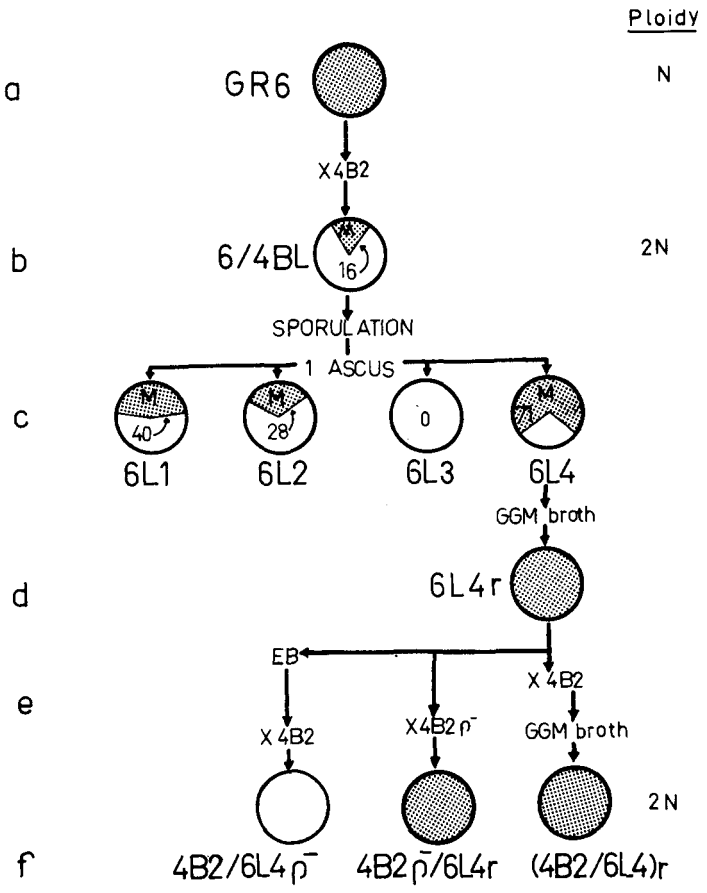

FIGURE 1.—The behavior of the glucosamine resistance factor in strain GR6.

(a) haploid GR6, 100% resistant clones
(b) diploid: back crossed to parental strain 4BL
(c) *glucosamine resistance in haploid populations derived from four sister spores* in a 4:0, GGM^R:GGM^S ascus
(d) selective enrichment by passage through GGM broth
(e) the various processes to which this enriched haploid was subjected. Details are given in the text, X indicates a cross
(f) the frequency of glucosamine resistance in diploid populations resulting from the processes outlined above.
stipled segments = % glucosamine resistance
M indicates micro colonies in resistant replicates.

failed to resuscitate the background inoculum. The speed with which these micro-colonies appeared (24–48 hr) and the variable numbers (3–confluent) make it unlikely that they were spontaneous mutants induced on the GGM plates after replication (BIRKY 1973). We therefore subjected all of our haploid isolates to the following test. Clones were subcultured to YPD broth for 24 hr, plated to YPD (100–150 colonies/plate) and replicated after 48 hr to YPG and GGM media. The resultant clones were then scored for growth and the %

resistant clones calculated, (number of YPG^+, GGM^R clones/total YPG^+ clones x 100. Similar experiments were conducted with diploids formed by back-crossing the GR strains to 4B2 and 4BL as appropriate and also with sister spores derived from sporulating such back-cross diploids.

Figure 1 shows the results of a series of such experiments performed on mutant GR6. The pie-dish circles indicate the % GGM^R colonies formed after growth in YPD broth followed by replica-plate testing on YPG and GGM media. (See METHODS). In this example GR6 gave 100% resistance (replicates with microcolonies=GGM^R) and the backcross diploid (4BL/6) gave 16% resistance. Sporulation of 4BL/6 produced a full spectrum of tetrads from 4:0−0:4 (GGM^R:GGM^S). Figure 1 (c) shows the results of subculturing four sister spores to YPD broth to determine % resistance. Even though this was a 4:0 (GGM^R:GGM^S) spore, one can see that the % resistance varies amongst the derived populations. Subsequent tests were enrichment subculture in GGM broth (Figure 1 (d)) 6L4r to 4B2 gave rise to ten 4:0 (GGM^R:GGM^S) tetrads out of 10 (Figure 1, (e)) and all spores were highly resistant. Petites derived from 6L4r with ethidium bromide, when back-crossed to 4B2, showed complete loss of glucosamine resistance (Figure 1, (f)). The reciprocal cross, 4BL ρ^- vs 6L4r resulted in a complete absence of GGM^S clones in the progeny (Figure 1, (e) (f)). In the last test, diploid 4B2/6L4 was subcultured to GGM broth to give a highly resistant diploid (4B2/6L4)r, again showing selective enrichment for (cytoplasmic) GGM^R factors. Similar results were obtained for strains GR5, 7, 8, 9 and 10. Lack of cross resistance to known mitochondrial inhibitors (chloramphenicol, erthromycin and oligomycin) in strains GR5–10 suggests that the GGM^R factor may be a unique mitochondrial allele (ELLIOT and BALL 1975). This locus was originally designated [cry-1] although we have since discovered that *cry* has been used previously (PLISCKE *et al.* 1975). We therefore suggest that this putative mitochondrial locus be renamed GGM1.

Although a large number of our isolates showed haploid and diploid segregation of glucosamine resistance, eight strains were 100% resistant as haploids and 100% sensitive as back-cross diploids. These suspected chromosomal mutants were therefore sporulated and the segregation data for true tetrads is shown

TABLE 1

Segregation of glucosamine resistance during sporulation

Diploid	No. of true* tetrands	Number of Tetrands of Type GGM^R:GGMS 1:3	2:2	3:1	4:0
4B2/71	15	1	4	10	0
4B2/81	18	1	15	2	0
4B2/90	14	2	9	2	1
4B2/91	7	2	4	1	0
4B2/92	1	0	1	0	0
4B2/108	16	0	16	0	0
4B2/130	10	0	10	0	0
4BL/133	12	0	12	0	0

* Tetrads which showed 2:2 segreation for both *his1* and *lys1*

TABLE 2

Establishing the genotype of a typical 3:1 (GGM^R:GGM^S) ascus from the cross of GR81 versus GR108 using complementation tests.

	GR81	GR108	Deduced Genotype of Spore	
Spore 1 (GGM^R)	R	S	81	+
Spore 2 (GGM^R)	S	R	+	108
Spore 3 (GGM^R)	R	R	81	108
Spore 4 (GGM^S)	S	S	+	+

R Growth on GGM, noncomplementation
S No growth on GGM, complementation

in Table 1. Only strains GR81, 108, 130 and 133 showed clear-cut Mendelian inheritance. Spores derived from the 4B2/71 cross showed variability and microcolony formation in subsequent testing and GR71 was deemed to be a cytoplasmic mutant. While the other strains were not pursued, a complementation test was set up for all combinations of strains GR81, 108, 130 and 133.

The results were clear-cut and the strains fell into two complementation groups: I=GR81 and GR133; II=GR108 and GR130. The next step was to test for inter- or intracistronic complementation by crossing GR81 x GR108. Of twelve true tetrads tested, ten were 3:1 (resistant:sensitive) and two were 4:0 (resistant:sensitive). A 3:1 ascus could be tetratype (T) derived from two independently assorting genes and a 4:0 would be a non-parental ditype (NPD). In order to confirm our hypothesis that the 3:1 asci were tetratypes, complementation testing was used to identify the glucosamine-resistance-conferring locus in the four sister spores of a 3:1 ascus. The results of Table 2 confirm our conclusion: the isolates GR81 and GR108 carry different mutations, probably located on different chromosomes.

TABLE 3

Data for total spores germinated for the crosses GR81 x X6-14 and GR108 x X6-14.

Gene pair*	No. of Parentals	No. of recombinants
gay1–arg4	20	19
gay1–lys1	16	21
gay1–his6	18	20
gay1–gal1	20	18
gay1–trp1	22	15
gay1–ade1	19	20
gay2–arg4	79	59
gay2–lys1	62	76
gay2–his6	64	74
gay2–gal1	73	60
gay2–trp1	64	74
gay2–ade1	74	60

**gay1*=GR81; *gay2*=GR108

We have made one attempt to map these two loci, using a multiple marker strain, X6–14. The strain was derived by crossing strain X3382–3A to 4B2 in order to remove the *pet17* locus which would interfere with the scoring of glucosamine resistance. Tetrad analysis of the cross X6–14 x GR108 showed a predominance of tetratype asci which suggests that this locus is not linked to any of the six centromeres tested. Due to poor germination, equivalent data were not available for GR81. Table 3 shows the ratio of parental to recombinant spores for the six loci and both strains. That these two mutations are not linked to any of the loci tested is fairly obvious and this was confirmed using the χ^2 test.

DISCUSSION

Of the 13 glucosamine-resistant mutants that were tested 7 showed characteristics similar to GR6 (Figure 1) and were tentatively designated as mitochondrial mutants (BOLOTIN, *et al.* 1971).

Segregation of resistance is not an exclusive property of cytoplasmically inherited traits. Aneuploidy or mitotic recombination in diploids can also lead to segregation of resistance in a way which mimics mitochondrial inheritance (MORTIMER and HAWTHORNE 1969). However if one works out the possible meiotic products produced from the sporulation of mutants carrying two independent chromosomal genes (segregating via some mitotic process) one can derive only three tetrad classes 4:0, 3:1 and 2:2. One cannot simultaneously derive 4:0 and 0:4 tetrads unless either some form of non-Mendelian process or some very complicated mitotic system is involved.

This, together with the ethidium bromide experiments (Figure 1) and the phenotype of the mutants leads us to suppose that the GGM1 type mutants are mitochondrial in nature. We are currently attempting to confirm this hypothesis and determine the number of alleles involved through mapping studies against established mitochondrial genes.

Of the remaining strains, 4 showed clear cut Mendelian inheritance (Table 1) and the remainder gave ambiguous results. The former mutants fell into two complementation groups and a cross between GR81 and GR108, followed by locus identification (Table 2) showed that these loci behaved as independently assorting alleles exhibiting inter-genic complementation. We have decided to call these loci *gay1* (Group I, GR81 and GR133) and *gay2* (Group II, GR108 and 130).

The crosses to the centromere marker strain X6–14 showed that GR108 is definitely not centromere linked. The *trp1* locus shows a second division segregation frequency of only 1% (MORTIMER and HAWTHORNE 1969) therefore the high frequency of tetratypes (8/12), *trp1* versus *gay2* must be due to cross overs between *gay2* and its centromere. The mapping data (Table 3) are largely negative but one can conclude that these two loci, represented by GR81 and GR108 are probably not centromere linked and do not map close to the centromeres of chromosomes *I*, *II*, *IV*, *VIII* or *IX*.

The catabolite repression-resistant mutants described by MONTENCOURT KUO and LAMPEN (1972) are not glucosamine-resistant although we have induced

GGM1 type resistance in one of these strains (#1710, ELLIOT, unpublished results).

As these strains grow relatively normally on YPD and YPG it seems that these mutants (*gay1*, *gay2* and GGM1) represent unique loci which may provide further insight into the mechanism whereby glycolysis represses mitochondriogenesis and/or respiration in yeast.

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Corresponding editor: F. SHERMAN