Parallel Changes in Catabolite Repression of Haem Biosynthesis and Cytochromes in Repression-resistant Mutants of *Saccharomyces cerevisiae*

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(Received 19 July 1988; revised 12 January 1989; accepted 26 January 1989)

Effects of three mutant genes, *CAT1-2^d*, *cat2-1* and *hex2-3*, on catabolite repression of mitochondrial cytochromes and the first two enzymes of haem biosynthesis were compared. The *CAT1-2^d* mutation gave no resistance to glucose, whereas *cat2-1* endowed both cytochromes and 5-aminolaevulinate dehydratase with resistance, but did not alter the effect of glucose on 5-aminolaevulinate synthase. The *hex2-3* mutation caused repression resistance of cytochromes and of the two haem biosynthetic enzymes. *hex2-3* strains also accumulated intracellular 5-aminolaevulinate. Co-inheritance of the latter traits, sensitivity to maltose inhibition and ability to grow on raffinose in the presence of 2-deoxyglucose, demonstrated that the pleiotropic phenotype is a function of the single gene *hex2-3*. Revertants which grew on maltose regained sensitivity to deoxyglucose and exhibited normal sensitivity of cytochromes and haem biosynthesis enzymes to repression. Addition of the *hex1-18* mutation, which renders cytochromes resistant to repression, to a *cat2-1* strain did not produce the same effect on 5-aminolaevulinate synthase as *hex2-3*. It is concluded that repression patterns of haem and cytochrome biosynthesis are substantially affected by *hex2-3* and *cat2-1* but not by *CAT1-2^d*.

INTRODUCTION

The aerobic respiratory system of the facultative yeast *Saccharomyces cerevisiae* is subject to extensive catabolite repression. When cells are grown on glucose medium the formation of mitochondrial cytochromes and other respiratory enzymes is drastically decreased (Polakis & Bartley, 1965; Perlman & Mahler, 1974; Mattoon *et al.*, 1979). As expected, restriction of cytochrome production is accompanied by a coordinate drop in haem biosynthesis. Preliminary studies (Jayaraman *et al.*, 1971) have indicated that 5-aminolaevulinate dehydratase (ALA-D), the second enzyme in the haem biosynthetic pathway, is sensitive to catabolite repression. Mahler & Lin (1978) proposed that this enzyme is rate-controlling for cytochrome synthesis during derepression.

Catabolite repression in yeast does not appear to be directly mediated by 3',5'-cyclic AMP as it is in prokaryotes (Matsumoto *et al.*, 1982). Genetic studies of yeast mutants with altered sensitivity to catabolite repression have revealed the existence of a regulatory network having branches controlling sets of enzymes: (a) enzymes of gluconeogenesis (Gancedo & Gancedo, 1971), (b) mitochondrial enzymes (Böker-Schmitt *et al.*, 1982; Ciriacy, 1978) and (c) enzymes required for assimilation of carbohydrate carbon sources other than glucose (Zimmermann & Eaton, 1974; Carlson *et al.*, 1981; Matsumoto *et al.*, 1983). Most of the known altered repression mutants are pleiotropic and may be affected in one or more of these enzyme sets (Zimmermann

Abbreviations: ALA, 5-aminolaevulinic acid; ALA-D, ALA dehydratase; ALA-S, ALA synthase; I-ALA, intracellular ALA.

& Scheel, 1977; Entian *et al.*, 1977; Entian & Zimmermann, 1980; Bailey & Woodward, 1984). Mutants have also been found in which smaller subsets of enzymes within one of the three general sets are affected as described above. For example, Szekely & Montgomery (1984) described a mutant in which α and β subunits of F1 ATPase and the ADP/ATP translocator, but not cytochrome *c*, are repression-resistant.

Various mutants have been examined for altered repression of representative enzymes from the three general sets, but in only a few cases have all mitochondrial cytochromes been studied (Borralho *et al.*, 1983; Mattoon *et al.*, 1979; Böker-Schmitt *et al.*, 1982). Moreover, the question of coordinate repression-derepression of biosynthesis of both mitochondrial cytochromes and their haem prosthetic groups has not been studied in detail. The present investigation compares the effects of several different repression-resistance mutations on cytochromes and the first two enzymes of haem biosynthesis.

METHODS

Yeast strains. The genotypes and sources of the strains used in this study are given in Table 1.

Gene symbols. *his4* and *leu1* are mutant alleles causing nutritional requirements for histidine and leucine respectively. *MAL2-8^c* is a dominant mutant allele which causes a constitutive (not requiring maltose as an inducer) but still glucose-repressible maltase synthesis (Zimmermann & Eaton, 1974). *MAL3* is one of the inducible maltose fermentation genes which is tightly linked to *SUC3*, one of the genes for invertase formation.

CAT1-2^a is a dominant mutant gene, and *cat2-1* is a recessive mutant gene, unlinked to *CAT1*, that allows for a rapid derepression of various enzymes after growth on glucose medium (Zimmermann *et al.*, 1977). *hex2-3* is a mutant gene that causes lack of repression of maltase, invertase and malate dehydrogenase. The presence of this gene causes inhibition of growth by maltose (Zimmermann & Scheel, 1977; Entian & Zimmermann, 1980; Entian, 1980). *hex1-18* is a mutant allele which causes production of non-repressible maltase, invertase and malate dehydrogenase (Zimmermann & Scheel, 1977; Entian *et al.*, 1977). The *gal2* mutation prevents fermentation of galactose, and the *mal* gene permits growth on maltose, but not the fermentation of this sugar. α and α indicate mating type.

Growth conditions. For growth, the medium contained 1.3% (w/v) yeast extract (Difco), 0.2% (NH₄)₂SO₄, 0.2% KH₂PO₄ and 2.0% (w/v) glucose; the pH was adjusted to 5.2. Erlenmeyer flasks (2 l) containing 400 ml of medium were shaken on a rotary shaker at 160 r.p.m. and 28°C. Growth was monitored as the optical density at 570 nm.

Depending upon the generation time of each strain, different inocula were used, and cells were collected before the external glucose concentration fell below 0.4% to avoid derepression (Perlman & Mahler, 1974).

Genetic analysis. Standard genetic procedures of crossing, sporulation and tetrad analysis were used (Mortimer & Hawthorne, 1969). Analysis of *hex2-3* segregants was made in medium containing (w/v) 1% yeast extract, 2% peptone, 2% 2-deoxyglucose, 4% raffinose and 2.0% Bactoagar (Zimmermann & Scheel, 1977). Analysis of growth was made on YPM (composition, w/v: 1% yeast extract, 2% peptone, 2% maltose and 2% Bactoagar).

Isolation of revertants of SMC-1B/3. Revertants of SMC-1B/3 were isolated 72 h after plating a heavy lawn of cells on YPM agar plates. Colonies were tested for loss of ability to grow on raffinose medium containing 2-deoxyglucose.

Enzyme activities. Activities were measured using *in situ* assays, with cells permeabilized by cycles of freezing and thawing. The assay of 5-aminolaevulinic acid synthase (ALA-S) (EC 2.3.1.37) was described by Arrese *et al.* (1983), and the assay of 5-aminolaevulinic acid dehydratase (ALA-D) (EC 2.4.1.24) was described by Borralho *et al.* (1983).

Determination of intracellular 5-aminolaevulinic acid (I-ALA). This was done as described by Malamud *et al.* (1979), using the modified Ehrlich reaction developed by Mauzerall & Granick (1956).

Cytochrome spectra. Cells were collected by centrifugation, washed twice with distilled water and suspended in 50 mM-potassium phosphate buffer, pH 6.0, to give a suspension of 25 mg dry wt ml⁻¹. The cytochrome spectrum of each suspension was measured with a double-beam Cary 17 spectrophotometer using dilute, reconstituted skimmed milk as a reference sample. Cytochrome reduction was accomplished by adding a few milligrams of dithionite to the sample cuvette.

Derepression of cytochromes was qualitatively assessed from spectra. Derepressed cells displayed resolved peaks for cytochrome *b* and cytochromes *c* + *c*₁, at 562 and 550 nm respectively. Frequently they also displayed a peak for cytochrome *aa*₃ (see Figs 3, 4 and 5*b*). Spectra of repressed cells always contained a band in the region 540–560 nm which was not resolved into individual *b* and *c* + *c*₁ peaks. Spectra of repressed cells commonly display a second band (labelled 'porphyrins' in the Figures). Pretlow & Sherman (1967) showed that many yeast strains accumulate one or more types of porphyrin in different relative concentrations. Zn porphyrins produce a two-banded visible spectrum with bands of similar intensity at about 540 nm and 575–585 nm. The exact positions

Table 1. *Yeast strains used in this study*

Source of strains. (1) K. Entian, Physiologisch-chemisches Institut der Universität Tübingen, Hoppe-Seyler-Str.1, D-7400 Tübingen, FRG; derived from strains from the Department of Genetics, University of Washington, Seattle, USA. (2) E. Cabib, National Institutes of Health, Bethesda, Md., USA. (3) Prepared at the Federal University of Rio de Janeiro by crossing strain SMC-1B/3 with strain S288c.

Strain	Genotype	Source
cat1.S3-14A	a <i>his4 MAL2-8^c MAL3 SUC3</i>	(1)
SMC-1B	a <i>his4 MAL2-8^c MAL3 SUC3 CAT1-2^d</i>	(1)
SMC-25A	α <i>leu1 MAL2-8^c MAL3 SUC3 CAT1-2^d</i>	(1)
SMC1B/3	a <i>his4 MAL2-8^c MAL3 SUC3 CAT1-2^d hex2-3</i>	(1)
cat2.3-2A	a <i>his4 MAL2-8^c MAL3 SUC3 cat2-1</i>	(1)
cat2.3-2A/18	a <i>his4 MAL2-8^c MAL3 SUC3 cat2-1 hex1-18</i>	(1)
S288c	α <i>gal2 mal</i>	(2)
DRM2	a <i>his4 MAL2-8^c MAL3 SUC3 CAT1-2^d hex2-3</i>	(3)
	α + <i>mal</i> <i>mal</i> * <i>CAT1</i> +	

* Not determined.

of the bands depends upon the predominant porphyrins in the mixture, and can vary from strain to strain. In repressed strains absorption in the 540–560 nm region of the spectrum represents a composite of Zn porphyrin (540 nm peak) and relatively small amounts of *b*- and *c*-type cytochromes. Because the relative amounts of these various species in repressed cells varies with genetic background, it was not possible to utilize difference spectra (mutant minus wild-type) to obtain quantitative values for cytochromes in repressed cells. Figs 1 and 2 illustrate various types of spectra obtained with different repressed strains. Fig. 4 shows that the 575–585 nm Zn porphyrin band sometimes appears in the spectrum of derepressed cells as well.

Chemicals. 5-Aminolaevulinic acid, raffinose, 2-deoxyglucose, maltose, acetylacetone, coenzyme A, NAD and *p*-dimethylaminobenzaldehyde were from Sigma. Medium constituents were from Difco.

RESULTS

The effects of three different catabolite-repression-resistance mutations on production of cytochromes and the activities of the haem biosynthetic enzymes ALA-S and ALA-D were studied.

Effects of the CAT1-2^d gene on cytochromes and haem-synthesis enzymes

A mutant containing a repression-resistant allele of the normal gene *CAT1* was isolated by Zimmermann *et al.* (1977) as a revertant of a permanently repressed *cat1* mutant. The *cat1* mutant could not grow on glycerol medium nor produce maltase, whereas the revertant, bearing the dominant allele *CAT1-2^d*, grew on glycerol medium and showed precocious derepression of maltase in an appropriate genetic background. The effects of *CAT1-2^d* on repression of cytochrome synthesis and haem synthesis enzymes are shown in Figs 1 and 2. Although the *CAT1-2^d* strain SMC-25A (Fig. 1*a*) exhibits somewhat greater ALA-D activity than the control strain (Fig. 1*b*), the same mutant gene in another strain, SMC-1B (Fig. 2), causes no significant difference relative to the wild-type. In both *CAT1-2^d* strains, cytochromes remain largely repressed. Therefore, even though the reversion of *cat1* to *CAT1-2^d* restored glycerol assimilation, this mutation does not render cytochromes repression resistant.

Derepression effects of the cat2-1 mutation

In contrast to the *CAT1-2^d* mutation, the *cat2-1* mutation, which suppresses the *cat1* defect (Zimmermann *et al.*, 1977) has a pronounced effect on the catabolite repression of both cytochromes and haem synthesis. As shown in Fig. 3, the specific activity of ALA-D (54 units) is substantially higher in mutant cells than in wild-type cells (20 units) (Fig. 1*b*) harvested in the same physiological condition (0.4% residual glucose). Moreover, the whole-cell spectrum clearly

Fig. 1. Comparison of cytochrome formation, I-ALA content, and ALA-S and ALA-D activities in a *CAT1-2^d* mutant (strain SMC-25A) (a) and the wild-type (strain cat1.S3-14A) (b). ■, I-ALA content [nmol ALA (g dry wt cells)⁻¹]; □, ALA-S specific activity [nmol ALA formed min⁻¹ (g dry wt cells)⁻¹]; ▨, ALA-D specific activity [nmol porphobilinogen formed min⁻¹ (g dry wt cells)⁻¹].

Fig. 2. Effect of the *CAT1-2^d* mutation (strain SMC-1B) on the parameters described in Fig. 1. Symbols, as Fig. 1.

Fig. 3. Effect of the *cat2-1* mutation (strain cat2.3-2A) on the parameters described in Fig. 1. Symbols, as Fig. 1.

Fig. 4. Effect of the *hex2-3* mutation (strain SMC-1B/3) on the parameters described in Fig. 1. Symbols, as Fig. 1.

shows the presence of all the cytochrome bands – aa_3 , b and $c + c_1$ – indicating repression resistance.

Repression-resistant phenotype of hex2-3

Like *cat2*, the *hex2-3* gene in strain SMC-1B/3 causes both cytochromes and ALA-D to become resistant to catabolite repression (Fig. 4). However, unlike *cat2-1*, *hex2-3* has an additional effect on the haem biosynthetic pathway; namely, it causes a substantial increase in ALA-S activity. This, in turn, causes a 2.5-fold elevation in intracellular 5-aminolaevulinate (I-ALA) concentration relative to that of the isogenic parent, SMC-1B (Fig. 2).

Inheritance of traits linked to hex2-3

The phenotype of *hex2-3* strain SMC-1B/3 is highly pleiotropic. The original mutant was selected for its ability to grow on raffinose in the presence of 2-deoxyglucose, a gratuitous repressor. The SMC-1B background includes the constitutive maltose fermentation gene *MAL2-8^c*. However, mutant SMC-1B/3 is inhibited by maltose. In addition, the *hex2-3* phenotype is characterized by repression resistance of respiratory enzymes and invertase, which liberates fructose from raffinose (Zimmermann & Scheel, 1977).

Table 2. *Growth of segregants of the diploid strain DRM2 (SMC1B/3 × S288c) on 2-deoxyglucose + raffinose (2-DG + Raf) and on maltose*

Segregant	Growth on:*		Repression resistance of cytochromes†
	2-DG + Raf	Maltose	
SMC-1B/3	++	0	+
S288c	0	++	0
DRM2-1A	0	++	0
DRM2-1B	0	++	0
DRM2-1C	+ -	++	0
DRM2-1D	++	0	+
DRM2-2A	0	++	0
DRM2-2B	++	0	+
DRM2-2C	0	+	0
DRM2-2D	++	0	+
DRM2-3A	0	++	0
DRM2-3B	0	++	0
DRM2-3C	+ -	++	0
DRM2-3D	+	0	+
DRM2-4A	0	++	0
DRM2-4B	++	0	+
DRM2-4C	0	+ -	(0)‡
DRM2-4D	++	0	+
DRM2-5A	0	++	0
DRM2-5B	0	++	0
DRM2-5C	++	+ -	+
DRM2-5D	++	0	+

* ++, Normal growth; +, slow growth; + -, weak growth; 0, no detectable growth.

† ++, Presence of distinct spectral peaks for cytochromes *b* and *c* + *c*₁; cytochrome *aa*₃ peak may or may not be evident. 0, No resolution of *b* and *c* + *c*₁ peaks and no cytochrome *aa*₃ peak.

‡ Cytochrome *b* and *c* + *c*₁ peaks barely detectable.

To determine whether or not these various traits and the repression resistance of cytochromes and haem biosynthetic enzymes are all linked to the single gene *hex2-3*, strain SMC-1B/3 was crossed with strain S288c, and tetrad analysis was done on spores produced by the resulting diploid, DRM2. Although tester strain S288c does not ferment maltose, it is capable of growing aerobically on maltose medium. Results obtained with five DRM2 tetrads are shown in Table 2. The *hex2-3* gene segregated 2:2, as indicated by growth of segregants on raffinose medium containing 2-deoxyglucose. All of the segregants sensitive to 2-deoxyglucose grew on maltose, whereas the defective growth on maltose was associated with 2-deoxyglucose resistance in only seven of ten segregants. Segregant 5C grew very poorly on maltose, whereas segregants 1C and 3C grew well on maltose, but they appeared to be partially inhibited by 2-deoxyglucose. It appears, therefore, that sensitivity of *hex2-3* segregants to maltose inhibition is subject to epistatic modification in these three segregants. The possibility exists that the *CAT1-2^d* allele is absent from these strains, and is necessary for expression of this trait.

When cytochrome spectra of these tetrads were made, repression resistance was found to segregate with deoxyglucose resistance in eight out of ten cases. Examples of two tetrads are shown in Fig. 5. Although segregant 4C shows indications of two peaks in the *b*-*c* region of the spectrum, segregants 4B and 4D are clearly less repressed. When tetrad 2 was subjected to complete analysis of the haem synthesis parameters, the results shown in Table 3 were obtained. Both *hex2-3* segregants, 2B and 2D, exhibit the phenotype of parental strain SMC-1B/3. It is noteworthy that the elevated ALA-S activity and high I-ALA are inherited together with 2-deoxyglucose resistance. The two 2-deoxyglucose-resistant segregants which did not exhibit derepressed cytochromes (1C and 3C) were also not inhibited by maltose.

Fig. 5. Cytochrome spectra of tetrads obtained from the diploid DRM2 (SMC-1B/3 $\times$ S288c). (a) Tetrad 4; (b) tetrad 5. Segregants 4B, 4D, 5C and 5D were scored 'repression resistant'.

Table 3. *ALA-S* and *ALA-D* specific activities, *I-ALA* content and cytochromes of a tetrad from the diploid strain DRM2

Segregants or parents	ALA-S [nmol ALA formed min ⁻¹ (g dry wt cells) ⁻¹]	ALA-D [nmol porphobilinogen formed min ⁻¹ (g dry wt cells) ⁻¹]	I-ALA [nmol ALA (g dry wt cells) ⁻¹]	Cytochromes*		
				a	b	c
SMC-1B/3	55.30 $\pm$ 3.91	78.55 $\pm$ 7.52	1757.9 $\pm$ 58.6	++	++	++
S288c	17.66 $\pm$ 3.77	26.77 $\pm$ 1.93	494.7 $\pm$ 77.7	—	tr.	tr.
DRM2-2A	23.73 $\pm$ 2.95	16.98 $\pm$ 2.55	550.5 $\pm$ 28.7	—	tr.	tr.
DRM2-2B	40.23 $\pm$ 1.95	77.74 $\pm$ 18.85	2245.4 $\pm$ 82.7	+	++	++
DRM2-2C	22.03 $\pm$ 0.83	37.34 $\pm$ 4.06	924.9 $\pm$ 42.7	—	tr.	tr.
DRM2-2D	37.65 $\pm$ 0.85	53.13 $\pm$ 2.64	1509.0 $\pm$ 56.7	+	++	++

* ++, Sharp cytochrome peak in spectrum; +, detectable cytochrome peak; tr., absorption in *b/c* region of spectrum, but no resolution of peaks; —, no detectable absorption peak in spectrum.

Restoration of repression sensitivity by reversion of *hex2-3*

Because strain SMC-1B/3 contains two maltose fermentation genes, revertants of *hex2-3* can be obtained readily by selecting colonies which grow on maltose medium. When two maltose-positive revertants were analysed it was found that the reversion had restored the repression sensitivity of cytochromes and ALA-D, and that the ALA-S activity and I-ALA returned to wild-type levels. (Compare Fig. 6a, b with Fig. 2.) Neither of the revertants could grow on raffinose medium containing 2-deoxyglucose.

Lack of additivity of *cat2* and *hex1*

The *hex1* mutation and its alleles *glr1* and *hxx2* (Lobo & Maitra, 1977; Zimmermann & Scheel, 1977; Michels & Romanowski, 1980) have already been shown to cause resistance to catabolite repression. Both cytochrome oxidase and NADH-dependent cytochrome *c* reductase are

Fig. 6. Effect of reverting the *hex2-3* mutation on the parameters described in Fig. 1. (a) and (b) show results from two different, independent revertants. Symbols, as Fig. 1.

resistant in strains containing the *glr1-1* allele. To determine whether the simultaneous presence of *cat2-1* and *hex1* exhibited any additive effects, a double mutant, strain *cat2.3-2A/18* was tested for effects of glucose on cytochromes and haem synthesis enzymes.

The results for this double mutant (*cat2-1 hex1*) are shown in Fig. 7, and may be compared to those for the isogenic *cat2-1 HEX1* strain presented in Fig. 3. Clearly, the presence of the *hex1* gene does not further enhance repression resistance, nor does it alter *ALA-S* activity like *hex2-3* does.

DISCUSSION

Various investigations have indicated that the catabolite repression system of yeast consists of several circuits or branches, each of which controls a different set of enzymes. The set comprised of respiratory enzymes includes cytochromes, enzymes of the citric acid cycle and other mitochondrial enzymes. A number of mutants resistant to catabolite repression of respiratory enzymes have been described (reviewed by Gancedo & Gancedo, 1986). The phenotypes of the various mutant genes exhibit different patterns of pleiotropy which can be described in terms of the spectrum of different repressible enzyme sets affected. For example, the *hex1* and *hex2* mutations described by Entian *et al.* (1977), Zimmermann & Scheel (1977) and Entian (1980) affect not only enzymes of carbohydrate catabolism, but several respiratory enzymes as well. On

Fig. 7. Effect of the *hex1* mutation in a *cat2-1* background (strain *cat2.3-2A/18(cat2 hex1)*) on the parameters described in Fig. 1. Symbols, as Fig. 1.

the other hand, these mutations do not render gluconeogenic enzymes non-repressible. In contrast, the action spectrum of the *cat80* mutation appears to be more or less limited to the carbohydrate catabolic enzymes (Entian & Zimmermann, 1980). *HEX2* and *CAT80* have been defined as members of the glucose repression cascade triggered by hexokinase PII (Entian, 1986).

The present investigation analyses the effects of three non-repressible mutations, *CAT1-2^d*, *cat2-1* and *hex2-3* on two subsets of respiratory enzymes, (i) the mitochondrial cytochromes and (ii) two enzymes of haem biosynthesis, ALA-S and ALA-D. Except for our preliminary report concerning the effect of *hex2-3* on ALA-D repression (Borrallho *et al.*, 1983), haem biosynthesis enzymes have not been included in studies of the action spectra of non-repressible mutants. The present study shows that whereas both the *hex2-3* and *cat2-1* mutations render ALA-D and cytochromes repression-resistant, the *CAT1-2^d* mutation, acting alone, produces little or no effect on these enzymes.

It is worth noting that neither of the 'repression resistance' genes *cat2-1* and *hex2-3* renders cytochromes of a given strain fully resistant to catabolite repression. When a wild-type yeast strain is allowed to grow for several hours beyond the point at which glucose is fully consumed, the absorption band for cytochrome *aa3* is substantially more intense than the comparable bands observed in any of the spectra reported here (Mattoon *et al.*, 1979). Moreover, in some genetic backgrounds the presence of one of these genes permits visualization of only cytochromes *b* and *c + c1*.

The repression resistance of ALA-D in both *hex2-3* and *cat2-1* is consistent with the earlier suggestion that ALA-D is rate-determining for haem biosynthesis (Jayaraman *et al.*, 1971), and therefore for cytochrome formation. This finding does not exclude the possibility that biosynthesis of some apocytochromes or subunits of cytochrome oxidase are independently controlled by catabolite repression.

It must not be concluded, however, that ALA-D is the sole target of glucose repression in the haem synthesis pathway in *S. cerevisiae*. Although ALA-S activity is not affected significantly by the *cat2-1* mutation, it is clearly increased by *hex2-3*. The relative increase in ALA-S activity is greater than that in ALA-D activity, so that the I-ALA levels of *hex2-3* strains are elevated compared to either wild-type or *cat2-1* strains. That the elevation of ALA-S and I-ALA is in fact caused by the *hex2-3* mutation and not by another gene is demonstrated by the coincident inheritance of 2-deoxyglucose resistance with high ALA-S and I-ALA. Moreover, reversion of *hex2-3* is accompanied by a return of ALA-S activity and I-ALA, as well as ALA-D activity, to wild-type levels. Although these results indicate that glucose exerts regulatory control on ALA-S activity, they do not necessarily imply that this control determines mitochondrial cytochrome repression.

Changes in ALA synthesis under different conditions of catabolite repression have been described by Labbe-Bois & Volland (1977), Malamud *et al.* (1983) and by Mattoon *et al.* (1978). In the late-exponential phase in glucose medium, ALA-S activity is quite high, so that I-ALA accumulates. Then, as cells progress through diauxie (derepression) and begin to grow on accumulated ethanol, ALA-S activity drops to low level. The molecular bases for these controls are unknown. Conceivably, the *hex2-3* mutation alters one of them.

It is possible that *hex2-3* alters not only catabolite repression but also catabolite inactivation. The original *hex2-3* mutant, SMC-1B/3, and most of the *hex2-3* segregants of the heterozygous diploid DRM2 are inhibited by maltose, which accumulates in the cells (Paschoalin *et al.*, 1986; Entian, 1980). This result indicates that maltose permease, which is normally inactivated by glucose (van Rijn & van Wijk, 1972), must retain activity in strains bearing the *hex2-3* mutation. It would be of interest to determine whether or not ALA-S is also sensitive to catabolite inactivation.

Unlike *hex2-3* mutants, strains containing the *cat2-1* gene are neither inhibited by maltose, nor does the response of ALA-S to glucose differ appreciably from that of wild-type cells.

Several laboratories have reported that mutations in the structural gene for hexokinase PII (hexokinase B) endow two enzyme sets with resistance to catabolite effects. Entian (1977) reported that the *hex1* mutation decreased catabolite inactivation of fructose-1,6-bisphosphatase and isocitrate lyase. However, the specificity of this effect is open to question because the mutant used also contained the *cat2-1* gene. Moreover, no maltose inhibition was reported for *hex1* mutants.

In a subsequent report (Entian & Zimmermann, 1980) the *hex1-18* allele was shown to endow cells with partial repression resistance of the respiratory enzymes succinate dehydrogenase and NADH dehydrogenase. Michels & Romanowski (1980) reported mutations which bestowed repression resistance on NADH:cytochrome *c* reductase and cytochrome oxidase. These mutant genes, designated *glr1*, were shown to be *hex1* alleles (Michels *et al.*, 1983). They are pleiotropic because they also alter repression of maltase and other carbohydrate catabolic enzymes. The latter studies do not clearly differentiate between resistance to catabolite repression and resistance to catabolite inactivation. The present investigation shows that the derepression pattern of cytochromes and ALA-D caused by *cat2-1* is not altered significantly by the added presence of *hex1*. It is particularly noteworthy that the phenotype of this double mutant does not mimic that of a *hex2-3* strain: ALA-S and I-ALA remain at wild-type levels.

This work was supported by NIH grant GM 27860 and by grants from FINEP, CNPq and CEPEG from Brazil. The authors thank Edison Bernardes for technical assistance and Georgia LeBeau for preparation of the manuscript.

REFERENCES

- ARRESE, M., CARVAJAL, E., ROBISON, S., SAMBUNARIS, A., PANEK, A. & MATTOON, J. (1983). Cloning of the δ -aminolevulinic acid synthase structural gene in yeast. *Current Genetics* **7**, 175-183.
- BAILEY, R. B. & WOODWARD, A. (1984). Isolation and characterization of a pleiotropic glucose repression resistant mutant of *Saccharomyces cerevisiae*. *Molecular and General Genetics* **193**, 507-512.
- BÖKER-SCHMITT, E., FRANCISCI, S. & SCHWEYEN, R. J. (1982). Mutations releasing mitochondrial biogenesis from glucose repression in *Saccharomyces cerevisiae*. *Journal of Bacteriology* **151**, 303-310.
- BORRALHO, L. M., PANEK, A. D., MALAMUD, D. R., SANDERS, H. K. & MATTOON, J. R. (1983). In situ assay for 5-aminolevulinic acid dehydratase and application to the study of a catabolite repression-resistant *Saccharomyces cerevisiae* mutant. *Journal of Bacteriology* **156**, 141-147.
- CARLSON, M., OSMOND, B. C. & BOTSTEIN, D. (1981). Mutants of yeast defective in sucrose utilization. *Genetics* **98**, 25-40.
- CIRIACY, M. (1978). A yeast mutant with glucose resistant formation of mitochondrial enzymes. *Molecular and General Genetics* **159**, 329-335.
- ENTIAN, K.-D. (1977). Lack of carbon catabolite inactivation in a mutant of *Saccharomyces cerevisiae* with reduced hexokinase activity. *Molecular and General Genetics* **158**, 201-210.
- ENTIAN, K.-D. (1980). A defect in carbon catabolite repression associated with uncontrollable and excessive maltose uptake. *Molecular and General Genetics* **179**, 169-175.
- ENTIAN, K.-D. (1986). Glucose repression: a complex regulatory system in yeast. *Microbiological Sciences* **3**, 366-371.
- ENTIAN, K.-D. & ZIMMERMANN, F. K. (1980). Glycoly-

- tic enzymes and intermediates in carbon catabolite repression mutants of *Saccharomyces cerevisiae*. *Molecular and General Genetics* **177**, 345–350.
- ENTIAN, K.-D., ZIMMERMANN, F. K. & SCHEEL, I. (1977). A partial defect in carbon catabolite repression in mutants of *Saccharomyces cerevisiae* with reduced hexose phosphorylation. *Molecular and General Genetics* **156**, 99–105.
- GANCEDO, J. M. & GANCEDO, C. (1971). Fructose-1,6-diphosphatase, phosphofructokinase and glucose-6-phosphate dehydrogenase from fermenting and nonfermenting yeasts. *Archives of Microbiology* **76**, 132–138.
- GANCEDO, J. M. & GANCEDO, C. (1986). Catabolite repression mutants of yeast. *FEMS Microbiology Reviews* **32**, 179–187.
- JAYARAMAN, J., PADMANABAN, G., MALATHI, K. & SARMA, P. S. (1971). Haem synthesis during mitochondrialogenesis in yeast. *Biochemical Journal* **121**, 531–535.
- LABBE-BOIS, R. & VOLLAND, C. (1977). Changes in the activities of the protoheme-synthesizing systems during growth of yeast under different conditions. *Archives of Biochemistry and Biophysics* **179**, 565–577.
- LOBO, Z. & MAITRA, P. K. (1977). Genetics of yeast hexokinase. *Genetics* **86**, 726–744.
- MAHLER, H. R. & LIN, C. C. (1978). Exogenous adenosine 3',5'-monophosphate can release yeast from catabolite repression. *Biochemical and Biophysical Research Communications* **83**, 1039–1047.
- MALAMUD, D. R., BORRALHO, L. M., PANEK, A. D. & MATTOON, J. R. (1979). Modulation of cytochrome biosynthesis in yeast by antimetabolite action of levulinic acid. *Journal of Bacteriology* **138**, 799–804.
- MALAMUD, D. R., PADRÃO, G. R. B., BORRALHO, L. M., ARRESE, M., PANEK, A. D. & MATTOON, J. R. (1983). Regulation of porphyrin biosynthesis in yeast. Use of δ -aminolevulinic acid in characterizing *in vivo* effects of mutation. *Brazilian Journal of Medical and Biological Research* **16**, 203–213.
- MATSUMOTO, K., UNO, I., TOH-E, A., ISHIKAWA, T. & OSHIMA, Y. (1982). Cyclic AMP may not be involved in catabolite repression in *Saccharomyces cerevisiae*. Evidence from mutants capable of utilizing it as an adenine source. *Journal of Bacteriology* **150**, 277–285.
- MATSUMOTO, K., YOSHIMATSU, T. & OSHIMA, Y. (1983). Recessive mutations conferring resistance to carbon catabolite repression of galactokinase synthesis in *Saccharomyces cerevisiae*. *Journal of Bacteriology* **153**, 1405–1414.
- MATTOON, J. R., MALAMUD, D. R., BRUNNER, A., BRAZ, G., CARVAJAL, E., LANCASHIRE, W. E. & PANEK, A. D. (1978). Regulation of heme formation and cytochrome biosynthesis in normal and mutant yeasts. In *Biochemistry and Genetics of Yeast, Pure and Applied Aspects*, pp. 145–160. Edited by M. Bacila, B. Horecker & A. O. M. Stoppani. New York: Academic Press.
- MATTOON, J. R., LANCASHIRE, W. E., SANDERS, H. K., CARVAJAL, E., MALAMUD, D. R., BRAZ, G. R. C. & PANEK, A. D. (1979). Oxygen and catabolite regulation of hemoprotein biosynthesis in yeast. In *Biochemical and Clinical Aspects of Oxygen*, pp. 421–435. Edited by W. Caughey. New York: Academic Press.
- MAUZERALL, D. & GRANICK, S. (1956). The occurrence and determination of δ -aminolevulinic acid and porphobilinogen in urine. *Journal of Biological Chemistry* **219**, 435–446.
- MICHELS, C. A. & ROMANOWSKI, A. (1980). Pleiotropic glucose repression-resistant mutation in *Saccharomyces carlsbergensis*. *Journal of Bacteriology* **143**, 674–679.
- MICHELS, C. A., HAHNENBERGER, K. M. & SYLVESTRE, Y. (1983). Pleiotropic mutations regulating resistance to glucose repression in *Saccharomyces carlsbergensis* are allelic to the structural gene for hexokinase B. *Journal of Bacteriology* **153**, 574–578.
- MORTIMER, R. K. & HAWTHORNE, D. C. (1969). Yeast genetics. In *The Yeasts 1*, pp. 385–460. Edited by A. H. Rose & J. S. Harrison. New York: Academic Press.
- PASCHOALIN, V. M. F., COSTA-CARVALHO, V. L. A. & PANEK, A. D. (1986). Further evidence for the alternative pathway of trehalose synthesis linked to maltose utilization in *Saccharomyces*. *Current Genetics* **10**, 725–731.
- PERLMAN, P. S. & MAHLER, H. R. (1974). Derepression of mitochondria and their enzymes in yeast: regulatory aspects. *Archives of Biochemistry and Biophysics* **162**, 248–271.
- POLAKIS, E. S. & BARTLEY, W. (1965). Changes in the enzyme activities of *Saccharomyces cerevisiae* during aerobic growth on different carbon sources. *Biochemical Journal* **97**, 284–297.
- PRETLOW, T. P. & SHERMAN, F. (1967). Porphyrins and zinc porphyrins in normal and mutant strains of yeast. *Biochimica et biophysica acta* **148**, 629–644.
- VAN RIJN, J. & VAN WIJK, R. (1972). Differential sensitivities of the two malate dehydrogenases and the maltose permease to the effect of glucose in *Saccharomyces carlsbergensis*. *Journal of Bacteriology* **110**, 477–484.
- SZEKELY, E. & MONTGOMERY, D. (1984). Glucose represses genes that encode mitochondrial components. *Molecular and Cellular Biology* **4**, 939–946.
- ZIMMERMANN, F. K., KAUFMANN, I., RASENBERGER, H. & HAUSSMAN, P. (1977). Genetics of carbon catabolite repression in *Saccharomyces cerevisiae*: genes involved in the derepression process. *Molecular and General Genetics* **151**, 95–103.
- ZIMMERMANN, F. K. & SCHEEL, I. (1977). Mutants of *Saccharomyces cerevisiae* resistant to carbon catabolite repression. *Molecular and General Genetics* **154**, 75–82.
- ZIMMERMANN, F. K. & EATON, N. R. (1974). Genetics of induction and catabolite repression of maltase synthesis in *Saccharomyces cerevisiae*. *Molecular and General Genetics* **134**, 261–272.