MUTANTS OF YEAST DEFICIENT IN CYTOCHROME C

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CYTOCHROME C is an ideal protein for biochemical studies because of its ease of isolation and low molecular weight. Its primary structure from a number of species is entirely (cf. SMITH, MATSUBARA, MCDOWALL and ROTHFUS 1963) or partially (cf. TUPPY 1958) known. Therefore, any system exhibiting genetic control of cytochrome c would be of considerable interest for investigating gene-protein relationships.

In a previous investigation, a mutant of yeast was shown to have a decreased concentration of cytochrome c controlled by a single gene. The cytochrome c isolated from this mutant was found to differ from wild-type cytochrome c by a number of criteria indicating differences in primary structure (SLONIMSKI and SHERMAN, in preparation). The investigation reported herein deals primarily with the isolation and genetic analyses of other cytochrome c deficient mutants.

Nomenclature: The symbol *cy* will generally be used to denote single-gene mutants having abnormal absorption spectra, while retaining the ability to grow on nonfermentable substrates. However, in this paper *cy* will be used exclusively to describe mutants partially deficient in cytochrome c and having normal or near normal amounts of cytochromes a and b. Nonallelic mutants will be designated by different subscripts, i.e., cy_1, cy_2, etc. and independent mutants at the same locus will be designated by a second number, i.e., cy_{1-1}, cy_{1-2}, etc. cy_{1-1} will be reserved for the original cytochrome c deficient mutant (SLONIMSKI and SHERMAN, in preparation). The symbol *CY* will denote the wild type, and if no subscript is used, will imply the presence of all wild-type genes controlling the formation of cytochrome c. $\rho+$ and $\rho-$ (SHERMAN 1963) will designate, respectively, wild types and cytoplasmic mutants which are deficient in cytochromes a and b (cf. EPHRUSSI 1953). Other symbols used to denote mating type (a/α), nutritional requirements (ad_1, hi_1, tr_1, ly_2) and maltose fermentation (ma/MA) have been described elsewhere (cf. HAWTHORNE and MORTIMER 1960).

MATERIALS AND METHODS

Strains: The principal haploid strains of *Saccharomyces cerevisiae* employed in this investigation, D273–10B ($\alpha\, CY\, \rho+\, ma$) and D276–1D ($a\, CY\, \rho+\, ma\, tr_1\, hi_1\, ad_1\, ly_2$), were obtained from crosses involving strains with low frequencies of $\rho-$ cells. The use of strains having low frequencies of $\rho-$ cells (approximately one percent) is desirable, since previous studies of mutants with altered cytochrome systems were sometimes hampered by the occurrence of high proportion of $\rho-$ cells in the cultures (SHERMAN 1963). Strains carrying the mutant gene cy_{1-1} have been described elsewhere (SLONIMSKI and SHERMAN, in preparation).

Mutagenic treatments: Mutants were induced in cultures of D273–10B by using various doses of ultraviolet light (UV) and nitrous acid. In most UV experiments, cultures were grown for one day on YPAD (1 percent Bacto-yeast extract, 2 percent Bacto-peptone, 2 percent dextrose, 40 mg/l adenine sulfate, 2 percent agar), suspended in 20 ml water, and transferred to a petri

dish. The suspension was vibrated with a Thomas Shaking Apparatus and irradiated with 2500 ergs mm^{-2} UV by means of a germicidal lamp. Controls and irradiated samples were diluted when necessary and appropriate aliquots were streaked on the surfaces of YPD (1 percent Bacto-yeast extract, 2 percent Bacto-peptone, 2 percent dextrose, 2 percent agar) plates. After three days incubation at 30°C there was approximately 0.5 percent survival.

Nitrous acid treatments were usually performed by incubating 2×10^6 cells/ml in 0.2 M $NaNO_2$ plus 0.25 M sodium acetate buffer having a final pH of 4.8. After treating for 5 min at 30°C the cells were quickly diluted in one percent yeast extract and plated on YPD medium. In most experiments there was approximately 0.5 percent survival after four days of incubation.

Isolation of mutants deficient in cytochrome c: Two methods were used to detect mutant strains which were cytochrome c deficient using (1) a 546.07mμ lamp and (2) a Hartree microspectroscope (Beck Limited, London). Yeast strains were examined at —190°C with both methods.

Since the method using the 546.07mμ lamp was not too successful, it will not be described in detail. Low-pressure mercury lamps were fitted in a housing and covered with a 4-inch square Wratten 77A filter, which passed only the 546.07mμ band of Hg. When plastic petri dishes containing both *CY* and *cy* colonies were frozen in liquid nitrogen and placed over the light, a faint difference was observed in the light transmitted through the two types of colonies. At —190°C the α-band of cytochrome c is observed at 546.5mμ and the extinction coefficient is greater than at room temperature. Plates containing colonies derived from UV-irradiated cells were examined in this manner.

Most *cy* mutants described in this paper were isolated by the rapid examination of each clone with a spectroscope. This consisted of reinoculating the edge of each colony on a square plastic petri dish (Falcon Plastics, Los Angeles) containing YPD medium. After three days incubation, the square plates containing 36 clones per dish were frozen in liquid nitrogen and placed on a plastic block mounted under a Hartree microspectroscope (Figure 1). The plastic block contained a number of grooves in order that the square dish could be quickly moved by hand to center each strain in the light path. In this manner, it is possible to examine over 2000 strains a day. After a mutant was detected, the plate was defrosted and the strain reinoculated on a slant culture.

Genetic analyses: The general procedures employed in this investigation for the genetic analysis of yeast have been described by HAWTHORNE and MORTIMER (1960) and SHERMAN (1963). Strains were routinely tested for the ability to grow on glycerol medium (1 percent Bacto-yeast extract, 2 percent Bacto-peptone, 3 percent [v/v] glycerol) in order to determine whether the electron transport system is functional (cf. SHERMAN 1963 and references therein).

Low-temperature spectroscopy: 1 mm thick samples of all mutants, hybrids, and segregants were examined at —190°C with a Hartree spectroscope by the method described by SHERMAN and SLONIMSKI (in preparation). It should be stressed that this procedure results in better clarity compared with the rapid method using agar plates. (The rapid method was used *only* for the initial isolation of the mutants.)

Since it was not feasible to make quantitative measurements of cytochrome c concentration in the 800 strains encountered in this investigation, estimated concentrations were obtained by comparing the intensity of the α-band of cytochrome c to the α-band of cytochromes b and c_1. In normal yeast the relative order of absorption of the cytochromes is: cytochrome c > cytochrome b > cytochrome c_1. The units of cytochrome c assigned are given in Table 1, and do not necessarily imply linearity. Quantitative measurements, along with low-temperature spectrophotometric recordings, will be presented in a subsequent publication. For the present it is sufficient to say that 2 units correspond to approximately 20 percent of normal cytochrome c concentration.

RESULTS

Cytochrome mutants: Approximately 98 percent of the clones examined by the rapid spectroscopic method had cytochrome spectra of either wild types or typical ρ^- strains which are deficient in cytochromes a and b. In some UV experiments, as high as 50 percent of the surviving cells gave rise to ρ^- colonies, as observed by

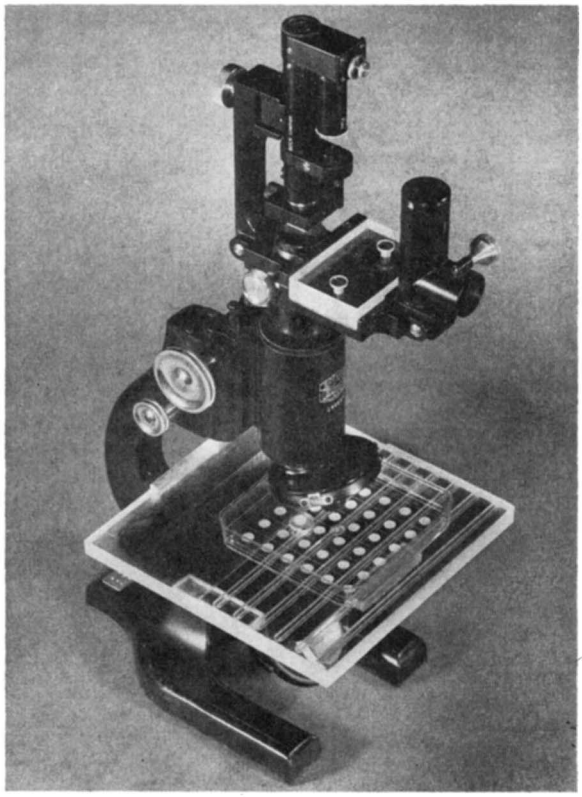

FIGURE 1.—Apparatus used for the rapid examination of absorption spectra at low temperature.

TABLE 1

Numbers used to denote the estimated concentration of cytochrome c in cy *mutants, segregants and hybrids*

Units		Intensity of cytochrome c band
Mutant types	0	Not visible
	1	Barely visible
	2	Visible but less than cytochrome c_1
	3	Equal to normal cytochrome c_1
	4	Greater than cytochrome c_1, but less than cytochrome b
	5	Equal to cytochrome b
	6	Greater than cytochrome b, but less than most wild-type amounts
Wild types	7	Equal to cytochrome c in the majority of wild types

RAUT (1954) and others. The remaining clones (approximately two percent) showed a variety of abnormal spectra involving deficiences and/or the presence of additional pigments. We will be concerned here with only the *cy* ρ^+ (deficient in cytochrome c) and *cy* ρ^- (deficient in all cytochromes) phenotypes.

TABLE 2

The number of cy *mutants obtained under various conditions*

Method	Mutagen	Number examined	Number of mutants	
			cy $\rho+$	*cy* $\rho-$
546.1 mμ light	UV	25,120	1	0
Spectroscope	UV	10,940	3	2
Spectroscope	HNO_2	3,850	6	0

Ten independent *cy* ρ^+ mutants obtained by various methods (Table 2) were streaked, and one subclone isolated for further studies. The units of cytochrome c in these strains (Table 3) were found to be reproducible to ± 1 units with repeated examinations during a period of more than one year.

The *cy* ρ^- mutants will be discussed below.

Single-gene segregations: The ten *cy* ρ^+ strains were crossed to D276–1D (α *CY* ρ^+ tr_1 hi_1 ad_1 ly_2) and the asci analyzed. 2:2 segregations, indicative of single genes, were observed for cytochrome c deficiencies in almost all tetrads (Table 4). The approximate frequencies of second division segregation obtained with the centromere marker tr_1 (HAWTHORNE and MORTIMER 1960) are included in Table 4. No linkage was observed with the markers α, tr_1, hi_1, ad_1 and ly_2.

The irregular segregation in one tetrad of cy_{1-2} was probably due to gene conversion (LINDEGREN 1953) since all other markers segregated in a normal 2:2. The 3:1 segregation of $CY:cy_{3-1}$ may have been due either to gene conversion or a back mutation.

The units of cytochrome c in the *cy* segregants corresponded to the parental *cy* mutants (Table 3) within ± 1 unit except for cy_{3-2} segregants which differed by ± 2 units. Although there was no ambiguity in differentiating mutants from wild

TABLE 3

Properties of the cy *mutants*

Symbol	Strain No.	Mutagen	Units of cytochrome c	
CY $\rho+$			7	
cy_{1-1} $\rho+$		?	2	
cy_{1-2} $\rho+$	B-295	HNO_2	2	
cy_{2-1} $\rho+$	B-204	UV	2	
cy_{2-2} $\rho+$	B-240	UV	3	
cy_{2-3} $\rho+$	B-268	HNO_2	2	
cy_{2-4} $\rho+$	B-318	HNO_2	3	
cy_{2-5} $\rho-$	B-326	UV		
cy_{3-1} $\rho+$	B-254	UV	3	(no cytochrome a with modifying gene)
cy_{3-2} $\rho+$	B-274	HNO_2	4	
cy_{3-3} $\rho-$	B-207	UV	2	
cy_{4-1} $\rho+$	B-271	HNO_2	5	
cy_{5-1} $\rho+$	B-277	UV	5	(decreased growth on glycerol medium)
cy_{6-1} $\rho+$	B-320	HNO_2	4	(presence of ~570 mμ band)

TABLE 4

Segregation from hybrid crosses of the cy *mutants with wild type* ($CY \times cy_x$) *and* cy_{1-1} ($cy_{1-1} \times cy_x$)

	$CY \times cy_n$					$cy_{1-1} \times cy_n$		
	$CY:cy_n$			Second division segregation				
	1:3	2:2	3:1	frequency	percent	PD	NPD	T
cy_{1-2}	1	11	0	11/11	100	11	0	0
cy_{2-1}	0	7	0	2/7	29	0	1	4
cy_{2-2}	0	5	0	0/5	0	0	0	5
cy_{2-3}	0	5	0	2/5	40	0	1	4
cy_{2-4}	0	5	0	2/5	40	1	1	3
cy_{3-1}	0	9	1	7/9	78	2	0	3
cy_{3-2}	0	10	0	8/10	80	2	0	3
cy_{3-3}	0	9	0	7/9	78	1	1	4
cy_{4-1}	0	10	0	6/10	60	0	0	5
cy_{5-1}	0	5	0	4/5	80	1	2	2
cy_{6-1}	0	5	0	5/5	100	0	0	5

PD = parental ditype tetrad; NPD = nonparental ditype; T = tetratype.

type, some segregants containing genes cy_{3-2}, cy_{4-1}, cy_{5-1} or cy_{6-1} had concentrations of cytochrome c (6 units) approaching typical wild types.

Other properties, beside cytochrome c deficiency, segregated with some *cy* genes (Table 3). Strains containing the cy_{5-1} gene were found to have decreased ability to utilize glycerol, probably indicating a partially defective electron transport system. A faint absorption band at about 570mμ was observed in all cy_{6-1} strains. A modifying gene caused approximately one half of the cy_{3-1} segregants to be deficient in cytochrome a. The details will be presented below.

Tests of allelism and recombination with cy_{1-1}: Segregants were intercrossed to provide diploid strains containing all pairwise combinations of the *cy* genes, and these hybrids were examined for cytochrome c content (Table 5). If one excludes the two mutants cy_{1-1} and cy_{1-2}, an unambiguous assignment can be made to five loci (cy_2 . . . . . cy_6) so that all heterozygous strains are cytochrome c sufficient and all homozygous strains are cytochrome c deficient, indicating recessive mutations. However, crosses involving cy_{1-1} and cy_{1-2} were not as informative since these genes are semidominant. Therefore in order to ascertain if any of the *cy* genes were at the cy_1 locus, all ten *cy* ρ^+ strains were crossed to cy_{1-1} and the asci analyzed. The parental ditype (PD), nonparental ditype (NPD) and tetratype (T) asci frequencies given in Table 4 clearly indicate that there is no close linkage between cy_{1-1} and any other mutant except, as expected, with cy_{1-2}. cy_{1-1} and cy_{1-2} hybrids, heterozygous with all other genes including *CY*, had five $\pm$ 1 units, while homoallelic (cy_{1-1}/cy_{1-1}, cy_{1-2}/cy_{1-2}) and heteroallelic (cy_{1-1}/cy_{1-2}) strains had concentrations of cytochrome c corresponding to the parental haploids (about 2 units).

In summary, by tests of recombination with cy_{1-1} and of allelism it was determined that six different loci controlled cytochrome c concentration, with all genes recessive except cy_1, which is semidominant. cy_2 . . . . cy_6 genes are not closely

TABLE 5

Units of cytochrome c in diploid strains constructed from pairwise combinations of the cy *mutants and wild type*

	CY	cy_{1-1}	cy_{1-2}	cy_{2-1}	cy_{2-2}	cy_{2-3}	cy_{2-4}	cy_{2-5}	cy_{3-1}	cy_{3-2}	cy_{3-3}	cy_{4-1}	cy_{5-1}	cy_{6-1}
CY	7	5	5	7	7	7	7	7	7	7	7	7	7	7
cy_{1-1}		2	2	5	5	5	5	6	5	5	5	5	4	4
cy_{1-2}			1	5	5	5	5	5	5	5	5	5	5	5
cy_{2-1}				3	4	3	2	2	7	7	7	7	7	7
cy_{2-2}					4	3	3		6	7	6	7	7	7
cy_{2-3}						2	2		7	7	6	7	7	6
cy_{2-4}							3		7	7	7	7	7	7
cy_{2-5}											7			
cy_{3-1}									3	3	2	7	7	7
cy_{3-2}										4	4	7	7	7
cy_{3-3}											1	7	7	7
cy_{4-1}												4	7	6
cy_{5-1}													4	6
cy_{6-1}														4

Seven units are found in most wild type strains while 1 to 5 units are considered below normal. Six units may be either normal or mutant type (see Table 1).

linked to cy_1. In this investigation, the only linkage that was observed was cy_2 with its centromere (Table 4) by presenting a second division segregation frequency significantly less than 0.667, with a level of significance less than 0.1 percent. Interallelic complementation has not been excluded among cy_3, cy_4, cy_5, and cy_6.

cy ρ^- *strains:* Thirteen strains obtained from UV experiments were observed to be devoid of cytochromes a and b, and to have concentrations of cytochrome c lower than *CY* ρ^- strains. This phenotype is characteristic of *cy* ρ^- variants isolated from *cy* ρ^+ strains. The 11 of the 13 strains that proved to be ρ^-, were crossed to cy_1 ρ^+, cy_2 ρ^+ and cy_3 ρ^+ for tests of allelism. Only two mutants (Table 2), cy_{2-5} ρ^- and cy_{3-3} ρ^-, were established to have *cy* genes (Table 5). cy_{3-3}, which was studied in more detail, showed 2:2 segregation (Table 4); ρ^+ strains of cy_{3-3} had a typical *cy* ρ^+ phenotype (Table 3). However, a hybrid cross with cy_{3-3} ρ^- was highly "unstable," resulting in abnormal segregation of cytochromes a and b (see below).

The *CY* strains exhibiting low cytochrome c content are reminiscent of the mutant isolated by RAUT (1953) having a protoporphyrin requirement (YČAS and STARR 1953).

md-cy_{3-1}: As mentioned above, approximately one half (11/29) of the segregants containing the cy_{3-1} gene were completely deficient in cytochrome a and could not utilize glycerol for growth. The simplest explanation is that a single modifying gene (*md-*cy_{3-1}) was present in the parental *CY* strain (D276–1D). The possibility of *md-*cy_{3-1} acting on a gene other than cy_{3-1} has not been excluded. However, this is highly unlikely, for the hypothetical gene would have to be closely linked to cy_{3-1}, and induced simultaneously with cy_{3-1}. By examining

a number of hybrids homozygous for cy_{3-1} and heterozygous for *md*-cy_{3-1}, it was established that the modifying gene was recessive.

In addition to the loss of cytochrome a, *some* cy_{3-1} *md*-cy_{3-1} segregants had a pigment absorbing at approximately 575mμ, and showed a high rate of reversion on glycerol medium. The revertant strains of cy_{3-1} *md*-cy_{3-1} had spectra similar to cy_{3-1} *md*$^+$-cy_{3-1}, indicating the instability of *md*-cy_{3-1} in the presence of cy_{3-1}, and not cy_{3-1} in the presence of *md*-cy_{3-1}.

The modifying gene was not closely linked to cy_{3-1} (PD:NPD:T frequencies were 4:1:9). An example of a tetratype ascus is given in Table 6.

The remarkable property of *md*-cy_{3-1} is its specificity. It does not modify the action of any of the *cy* genes listed in Table 4, including cy_{1-1} and *CY*. (Its effect on cy_{3-3} could have escaped detection due to the high frequency of ρ^- segregants; see below.) In other words, it shows specificity not only for a particular locus, but also for a particular allele.

"Unstable" strains: An unexpected result was obtained after analyzing asci from the hybrid cross involving cy_{3-3} ρ^-: most segregants were ρ^- (Tables 7 and 9). It should be pointed out that sporulation in an aerobic process and ρ^- diploids do not sporulate. It is believed that the occurrence of high frequencies of ρ^- segregants is not related to the cy_{3-3} mutation.

B-207 (cy_{3-3} ρ^-) was crossed with D276–1D (*CY* ρ^+) and a single hybrid clone

TABLE 6

An example of segregation in a tetrad from a cross CY md-cy_{3-1} × cy_{3-1} md$^+$-cy_{3-1}

Segregant	Cytochromes a	b	c	c_1	Growth on glycerol medium	Genotype
A	+	+	+	+	+	*CY md*-cy_{3-1} ρ+
B	+	+	±	+	+	cy_{3-1} *md*$^+$-cy_{3-1} ρ+
C	+	+	+	+	+	*CY md*$^+$-cy_{3-1} ρ+
D	−	+	±	+	−	cy_{3-1} *md*-cy_{3-1} ρ+

TABLE 7

Segregation of unstable hybrids

Strain no.	Frequency of asci	Genotypes of segregants A	B	C	D
D-285	3	*CY* ρ^-	*CY* ρ^-	cy_{3-3} ρ^-	cy_{3-3} ρ^-
(*CY*/cy_{3-3} ρ+)	1	*CY* ρ^-	*CY* ρ^-	cy_{3-3} ρ+ (D285–1C)	cy_{3-3} ρ^-
D-304	3	*CY* ρ^-	*CY* ρ^-	cy_{3-3} ρ^-	cy_{3-3} ρ^-
(*CY*/cy_{3-3} ρ+)	2	*CY* ρ+	*CY* ρ+	cy_{3-3} ρ+	cy_{3-3} ρ+
D-297	4	*CY* ρ+	cy_{3-3} ρ+	cy_{1-1} ρ+	cy_{1-1} cy_{3-3} ρ+
(cy_{1-1}/cy_{3-3} ρ+)	1	cy_{1-1} ρ+	cy_{1-1} ρ+	cy_{3-3} ρ+	cy_{3-3} ρ+
	1	*CY* ρ+	*CY* ρ^-	cy_{1-1} cy_{3-3} ρ^-	cy_{1-1} cy_{3-3} ρ+

(D-285) was grown on glycerol medium in order to select against ρ^- cells. However, after growth on glucose medium, D-285 gave rise to a high frequency of ρ^- cells during vegetative growth (83 percent, Table 8). After sporulation, D-285 also gave rise to a high frequency of ρ^- segregants (94 percent, Tables 7 and 9). The one ρ^+ segregant from the cross, D285–1C (Table 7), which also had a high mutation rate to ρ^- (Table 8), was crossed with D273–10B($CY\rho^+$) and D240–1D ($cy_{1-1}\ \rho^+$). Also, these two hybrids, D-297 and D-304, gave rise to higher than expected frequencies of ρ^- cells during vegetative growth (Table 8) and meiosis (Tables 7 and 9).

This occurrence of high frequencies of ρ^- cells during vegetative growth and after sporulation is probably an extreme case of a phenomenon reported by CHEN, EPHRUSSI and HOTTINGUER (1950) and EPHRUSSI and HOTTINGUER (1951). In a "control" experiment, a hybrid (cross F, CHEN, *et al.* 1950) was found to give rise to 25 percent of ρ^- segregants. One of the parent strains of this cross contained a gene which caused a high rate of spontaneous mutation to ρ^- (EPHRUSSI and HOTTINGUER 1951)

The occurrence of high frequencies of ρ^- cells in "unstable" strains should not be confused with "suppressiveness" which denotes the process resulting in ρ^- hybrids from zygotes of a $\rho^+ \times \rho^-$ cross (EPHRUSSI, DEMARGERIE-HOTTINGUER and ROMAN 1955). Although ρ^- segregants can be obtained from asci which are formed directly from such zygotes, ρ^+ segregants are mainly obtained if the zygotes are allowed to proliferate prior to sporulation.

It is known that unstable strains exhibit phenotypic lags during vegetative

TABLE 8

The percent of ρ^- cells in various stable and unstable strains

Strain no.	Genotype	Percent ρ^-
D273–10B	$\alpha\ CY\ \rho+$	1 ± 1
D276–1D	a $CY\ \rho+\ tr_1\ hi_1\ ad_1\ ly_2$	1 ± 1
D240–1D	$\alpha\ cy_{1-1}\ \rho+\ ad_1$	6.5 ± 1
B-207	$\alpha\ cy_{3-3}\ \rho^-$	100
D285–1C	a $cy_{3-3}\ \rho+\ hi_1\ tr_1$	80 ± 4
D-285	B-207 × D276–1D	83 ± 4
D-304	D285–1C × D273–10B	12 ± 2
D-297	D285–1C × D240–1D	17 ± 2

TABLE 9

The percent of ρ^- segregants

Strain no.	Frequency	Percent
D-285	15/16	94
D-297	2/24	8.3
D-304	12/20	60
All others	0/528	0

growth, such that the potential ρ^- cells have residual respiratory enzymes for several divisions (OGUR, ST. JOHN and OGUR 1959). Although the datum is not sufficient to establish any quantitative relationships, the occurrence of ρ^- segregants from unstable ρ^+ cultures may be simply the result of sporulation of ρ^+ cells undergoing mutations to ρ^-. Under such a condition, the phenotype is ρ^+ during the sporulation process, but is transformed to ρ^- after germination and vegetative growth. In this respect it is similar to the sporulation of zygotes from suppressive crosses (EPHRUSSI *et al.* 1955). In both cases a phenotypic lag may be present, and the potential ρ^- cell has the necessary apparatus for sporulation.

DISCUSSION

Most genetic investigations deal with auxotrophic mutants having losses of enzymic activities which result in the inability of the organism to utilize nutrilites. In contradistinction, this investigation has considered mutants which are partially deficient in the protein cytochrome c. Mutants having a defective cytochrome c that was present at normal concentrations would not have been detected by the methods employed herein.

It is of interest to note that no *cy* ρ^+ mutant was completely devoid of cytochrome c. Only cy_{5-1} ρ^+ (and cy_{3-1} *md*-cy_{3-1} ρ^+ due to the deficiency of cytochrome a) was found to have a decreased ability to utilize glycerol for growth.

The determination of the nature of these mutants awaits chemical analysis of cytochrome c. However, with the information at hand, it appears that the cy_1 locus controls the structural protein, while the other loci are concerned with regulation. The action of these many regulatory genes is uncertain, but cytochrome c content is known to vary with the growth phase of a culture (EPHRUSSI, SLONIMSKI, YOTSUYANAGI and TAVLITZKI 1956) and the degree of aeration (cf. YČAS and DRABKIN 1957).

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SUMMARY

A method is described for the detection of cytochrome mutants of yeast. Thirteen independent mutants (*cy*) partially deficient in cytochrome c were found to be due to single-gene mutations at six different loci. Mutant genes at the cy_1 locus were semidominant, while all others were recessive. A modifying gene (*md*-cy_{3-1}) was shown to cause a cytochrome a deficiency when in combination with the allele cy_{3-1}. Hybrids, which were obtained from crosses with the mutant B-207, gave rise to a high frequency of cytoplasmic mutants (ρ^-) during vegetative growth and meiosis.

ADDENDUM: It has recently been found that there are two types of cytochromes c in normal Bakers' yeast: iso-1-cytochrome c and iso-2-cytochrome c (P. P. SLONIMSKI, *et al.* 1964. Elements du systeme respiratoire et leur regulation: cytochromes et isocytochromes. *International Symposium on Mechanisms of Regulation of Cellular Activities in Microorganisms.* Gordon and

Breach Ltd., New York, in press). cy_{1-1} strains are completely deficient in iso-1-cytochrome c, while retaining iso-2-cytochrome c.

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