GENETIC ANALYSIS OF A RESPIRATION-DEFICIENT MUTANT OF *SACCHAROMYCES CEREVISIAE* LACKING ALL CYTOCHROMES AND ACCUMULATING COPROPORPHYRIN

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THE cytoplasmic factor (ρ) and many nonallelic chromosomal genes determine the presence of cytochromes in yeast (EPHRUSSI 1953; GUNGE 1966; SHERMAN 1963). Ordinarily, a respiration-deficient (RD) mutant defective in any of these determinants lacks cytochromes a and b, but possesses cytochrome c at a normal or increased level, when grown aerobically. Recently, some mutant yeasts have been reported which have variously altered cytochrome patterns such as decreased amounts of cytochrome c with the normal presence of cytochromes a and b, deficiency in cytochrome a with the presence of cytochromes b and c, etc. (SHERMAN 1964; SHERMAN and SLONIMSKI 1964; SHERMAN 1965). Some pigment presumed to be porphyrin has been noticed.

We have previously reported an RD mutant, 5b-1, which lacks all cytochromes and other heme-proteins and accumulates coproporphyrin III as the predominant porphyrin, when grown aerobically in yeast extract-peptone glucose medium (SUGIMURA, OKABE, NAGAO and GUNGE 1966). A defect in the conversion of coproporphyrin to protoporphyrin was considered to be the most probable lesion.

Strain 5b-1 originated from a ρ^- culture of a segregational RD yeast, having the mutant gene p governing inability to grow on nonfermentable carbon source. The P/p corresponds to R_1/r_1 in a previous paper (GUNGE 1966). The question remained unsettled whether the simultaneous loss of all cytochromes was caused by mutations other than that of the chromosomal gene P and the ρ factor. It was also uncertain whether the loss of all cytochromes and the accumulation of coproporphyrin were coincidental.

The present study was designed to clarify these points. The results led to the conclusion that the loss of all cytochromes and the coproporphyrin-accumulation both resulted from a mutation in a single chromosomal gene. The problem of phenotypic reversion to wild type in this mutant is also dealt with, and discussed in relation to the temperature sensitivity of phenotypic expression.

MATERIALS AND METHODS

Nomenclature: The genetic symbols used are mating type (a/α), histidine (his) and lysine

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(*lys*) requirements, and respiratory competence (*P/p*). ρ^+ and ρ^- represent the presence and the absence of the cytoplasmic factor necessary for the development of respiration activity. "cyt" type yeast denotes an RD mutant which is characterized by a deficiency in all cytochromes and the accumulation of coproporphyrin in aerobically grown cells.

Strains: Strain 5b-1, a cyt-type mutant deficient for all cytochromes and accumulating coproporphyrin (ρ^-), was discovered as a secondary mutant from a haploid RD yeast 5b (α , his^+ , lys^+ , p , ρ^+). The respiring haploid strain 605 (α , his^- , lys^- , P), kindly provided by Dr. HIUGA SAITO, carried the stable ρ factor. Spontaneous occurrence of ρ^- cells in this strain was 0.5% or less.

Media: Yeast extract-peptone-glucose medium (YEPG) consisted of proteose peptone No. 3 (Difco) 20 g, yeast-extract (Difco) 10 g, glucose 20 g and water 1000 ml. Solid medium was prepared by the addition of agar at the concentration of 2.5%. Lactate agar medium consisted of peptone (Difco) 3.5 g, yeast-extract (Difco) 3.0 g, KH_2PO_4 2.0 g, $MgSO_4 \cdot 7H_2O$ 1.0 g, $(NH_4)_2SO_4$ 1.0 g, sodium lactate 10 g, agar 30 g and water 1000 ml.

Diagnosis of RD mutants: RD mutants were diagnosed by growth on lactate agar medium (OGURA and ST. JOHN 1956), TTC test (NAGAI 1959), and polarographic assay of oxygen consumption with 1% glucose as substrate.

The ρ factor in RD mutant: The presence of the ρ factor in RD mutants was proved by the restoration of the ability to assimilate lactate in a cross of the mutant tested by a ρ^- mutant of opposite mating type, which has been produced from a respiring haploid yeast by means of acriflavin treatment.

Spectrophotometric assay: The absorption spectrum of a suspension of cells grown aerobically in YEPG was determined with a Cary recording spectrophotometer, Model 14, according to the method described by SUGIMURA, OKABE, NAGAO and GUNGE (1966). Cytochromes a, b and c were identified by a peak at 602 m μ , a shoulder at 563 m μ and a peak at 550 m μ , respectively. The coproporphyrin accumulation was diagnosed by absorption maxima at 538 and 575 m μ , which have been proved to be due to the intracellularly accumulated coproporphyrin (SUGIMURA, OKABE, NAGAO and GUNGE 1966).

Measurement of colony-size: Cells grown on YEPG slants were inoculated so as to produce about 300 colonies on lactate agar plate, and were incubated at 30°. At appropriate intervals of time, diameters of several colonies were measured under a microscope equipped with an ocular micrometer and then averaged. Measurement was continued for seven days or more.

Temperature: All experiments were carried out at 30°C unless stated otherwise.

RESULTS

Segregation of cyt-type mutants: With the intention of disclosing the genesis of the complete cytochrome-deficiency in strain 5b-1 and of inquiring into its relation to the accumulation of coproporphyrin, genetic analysis was carried out with four-spored asci from a cross of strain 5b-1 $\times$ respiring yeast 605 (denoted as cross 215) (Table 1). All marker genes (α/α), (his^+/his^-) and (lys^+/lys^-) segregated in a ratio of 2 : 2 in all of 15 asci, indicating normal diploid meiosis. With respect to the ability to grow on lactate agar, however, there was a segregation of 2 wilds : 2 no-colony-type mutants in seven asci, and a segregation of 1 wild : 1 scanty colony : 2 no-colony-type mutants in eight asci. The possibility that a change of $\rho^+ \rightarrow \rho^-$ was responsible for these irregular segregations was ruled out, because these mutant segregants (except for 215-4B and 215-9A) contained ρ^+ cells. "No-colony-type mutant" refers to yeast which never produced any colony on lactate agar; this was characteristic of ordinary RD mutants. Yeast of the scanty-colony type usually formed barely visible colonies after three to four days incubation, and thereafter grew very slowly. In general, the maximum

TABLE 1

Segregation data of cross 215, 5b-1 × 605

$$\begin{array}{c} \alpha \text{ his}^+ \text{ lys}^+ \text{ p } \rho^- \\ \hline \alpha \text{ his}^- \text{ lys}^- \text{ P } \rho^+ \end{array}$$

Segregant	Mating type	<i>his</i>	<i>lys</i>	<i>P/p</i>	QO ₂ *	TTC†	Growth on lactate‡	Spectrum			
								a	b	c	p
1A	α	—	+	<i>p</i>	0	W	—	—	—	—	+
1B	α	+	+	<i>P</i>	14.6	R	+	+	+	+	—
1C	α	+	—	<i>P</i>	20.2	R	+	+	+	+	—
1D	α	—	—	<i>p</i>	0	W	—	—	—	—	+
2A	α	—	—	<i>p</i>	0	W	—	—	—	+	—
2B	α	+	+	<i>P</i>	4.3	R	+	±	±	+	—
2C	α	—	+	<i>p</i>	0	W	—	—	—	—	+
2D	α	+	—	<i>P</i>	0.2	W	±	—	—	—	+
3A	α	—	+	<i>P</i>	8.9	R	+	+	+	+	—
3B	α	+	—	<i>p</i>	0	W	—	—	—	+	—
3C	α	—	—	<i>p</i>	0	W	—	—	—	+	—
3D	α	+	+	<i>P</i>	2.4	W	±	—	—	—	+
4A	α	—	+	<i>P</i>	12.3	R	+	+	+	+	—
4B	α	+	+	<i>p</i>	0	W	—	—	—	+	—
4C	α	+	—	<i>p</i>	0	W	—	—	—	—	+
4D	α	—	—	<i>P</i>	0	P	±	—	—	—	+
5A	α	—	+	<i>p</i>	0	W	—	—	—	—	+
5B	α	+	—	<i>P</i>		W	±	—	—	—	+
5C	α	+	—	<i>p</i>	0	W	—	—	—	+	—
5D	α	—	+	<i>P</i>	9.3	R	+	+	+	+	—
6A	α	—	+	<i>p</i>	0.9	W	—	—	—	—	+
6B	α	—	—	<i>P</i>	36.8	R	+	+	+	+	—
6C	α	+	—	<i>p</i>	0	W	—	—	—	—	+
6D	α	+	+	<i>P</i>	42.0	R	+	+	+	+	—
7A	α	—	+	<i>p</i>	0.3	W	—	—	—	+	—
7B	α	+	—	<i>p</i>	0.9	W	—	—	—	—	+
7C	α	—	—	<i>P</i>	0.5	W	±	—	—	—	+
7D	α	+	+	<i>P</i>	21.8	R	+	+	+	+	—
8A	α	+	+	<i>P</i>	5.7	R	+	±	±	+	—
8B	α	+	—	<i>P</i>	0	W	±	—	—	—	+
8C	α	—	+	<i>p</i>	0	W	—	—	—	—	+
8D	α	—	—	<i>p</i>	0.3	W	—	—	—	+	—
9A	α	+	+	<i>p</i>	0	W	—	—	—	+	—
9B	α	—	+	<i>p</i>	0	W	—	—	—	+	—
9C	α	—	—	<i>P</i>	12.3	R	+	+	+	±	±
9D	α	+	—	<i>P</i>	40.3	R	+	+	+	+	—
10A	α	—	+	<i>P</i>	28.1	R	+	+	+	+	—
10B	α	—	+	<i>P</i>	30.3	R	+	+	+	+	—
10C	α	+	—	<i>p</i>	0	W	—	—	—	—	+
10D	α	+	—	<i>p</i>	0	W	—	—	—	—	+

TABLE 1—Continued

Segregant	Mating type	<i>his</i>	<i>lys</i>	<i>P/p</i>	QO ₂ *	TTC†	Growth on lactate‡	Spectrum			
								a	b	c	p
11A	α	+	+	<i>P</i>	8.4	R	+	+	+	±	±
11B	α	—	—	<i>P</i>	25.7	R	+	+	+	+	—
11C	<i>a</i>	—	+	<i>p</i>	0	W	—	—	—	—	+
11D	<i>a</i>	+	—	<i>p</i>	0	W	—	—	—	+	—
12A	α	—	—	<i>P</i>	25.6	R	+	+	+	+	—
12B	<i>a</i>	+	+	<i>p</i>	0	W	—	—	—	—	+
12C	<i>a</i>	+	+	<i>p</i>	0	W	—	—	—	—	+
12D	α	—	—	<i>P</i>	23.7	R	+	+	+	+	—
13A	<i>a</i>	+	+	<i>p</i>	0	P	—	—	—	+	—
13B	<i>a</i>	+	—	<i>p</i>	0	W	—	—	—	—	+
13C	α	—	+	<i>P</i>	7.6	R	+	+	+	+	—
13D	α	—	—	<i>P</i>	0.2	W	±	—	—	—	+
14A	α	—	—	<i>P</i>	9.2	R	+	+	+	+	—
14B	α	+	+	<i>P</i>	0.4	W	±	—	—	—	+
14C	<i>a</i>	—	+	<i>p</i>	0	W	—	—	—	—	+
14D	<i>a</i>	+	—	<i>p</i>	0	W	—	—	—	+	—
15A	<i>a</i>	+	+	<i>p</i>	0	W	—	—	—	+	—
15B	α	+	+	<i>P</i>	22.0	R	+	+	+	+	—
15C	α	—	—	<i>P</i>	18.6	R	+	+	+	+	—
15D	<i>a</i>	—	—	<i>p</i>	0	W	—	—	—	—	+

* μ mole/mg wet weight/minute.

† W=white, P=pink, R=red.

‡ +=confluent growth, ±=scanty growth, —=no growth.

diameter of the colony of scanty types was about one-fifth that of the wild type as shown in Figure 1. It was difficult to distinguish no-colony-type mutants from scanty-colony-type mutants by means of either TTC test or polarographic assay.

Spectrophotometric assays indicated that wild-type yeasts possess all cytochromes and show no sign of coproporphyrin accumulation (abbreviated as a⁺

FIGURE 1.—Growth curves on lactate agar in terms of average colony size. ○ Wild type, ● Scanty colony type, △ No colony type.

$b^+ c^+ p^-$), and that no-colony-type mutants have two different kinds of spectra: one, the absence of cytochromes a and b, the presence of cytochrome c and the nonaccumulation of coproporphyrin (abbreviated as $a^- b^- c^+ p^-$, an ordinary RD mutant type); the other, the absence of all cytochromes with the accumulation of coproporphyrin (abbreviated as $a^- b^- c^- p^+$, the cyt type). All scanty-colony-type mutants carry the spectrum of the cyt type. Spectrum patterns are shown in Figure 2.

FIGURE 2.—Absorption spectrum of aerobically cultured cell suspension. A, Wild type; B, Ordinary RD type; C, cyt type; D, Transitional type.

SUGIMURA, OKABE, NAGAO and GUNGE (1966) have suggested that cyt-type mutants seem to owe their respiration-deficiency to a defect in the heme-protein synthesis at the step of protoporphyrin formation. Based on the above consideration, it was anticipated that cyt mutants having the normal gene *P* would recover complementarily the ability to produce all cytochromes and to grow rapidly on lactate agar, when combined with an ordinary RD mutant to form a heterozygous *P/p* diploid. The genotype (*P/p*) of cyt-type mutants in cross 215, was determined by the above complementary growth test on lactate, and is listed in Table 1, where *P/p* shows 2 : 2 segregation. The no-colony-type mutant apparently carried *p*, and the scanty-colony-type mutants carried *P*.

No mutant characterized by normal cytochrome a and b content, and lack of cytochrome c, was found among these segregants, excluding the possibility that coincidental changes of *Cy* → *cy*, (described by SHERMAN 1964) and of *P* → *p* or $\rho^+ \rightarrow \rho^-$, had caused the deficiency in all cytochromes. From these facts it is obvious that the cyt-phenotype segregated independently of *P/p* as well as being independent of $\rho^+ \rightarrow \rho^-$ change.

As shown in Table 1, the ratios of coproporphyrin accumulation to nonaccumulation were 2 : 2 in 12 out of 15 asci, as though controlled by a single gene which is linked with *p* (evidenced by the absence of nonparental ditypes), whereas they were 1 : 3 in three asci. Two of these coproporphyrin accumulating segregants, 215-9C and 215-11A, exhibited a phenotype (abbreviated as $a^+ b^+ c^\pm p^\pm$) transitional between the cyt type and the wild type, as illustrated in Figure 2. Mutants with spectra of the transitional type grew slowly on lactate agar, and colonies of this mutant reduced TTC on glucose agar.

It is hard to explain the occurrence of respiring segregants (*P*) containing cytochromes a and b at the decreased level (segregants 215-2B and 215-8A), because these strains were observed to carry the stable ρ factor by TTC test.

Various phenotypes ranging from cyt-type to wild-type: In the preceding section, genetic analysis of the cyt-phenotype was complicated by the simultaneous involvement of the *P* → *p* change in 5b-1. In order to avoid this complication, spores from a cross homozygous for the normal gene *P* were analyzed (Table 2).

In dissection of 18 asci, the spectrophotometric assay revealed segregations of 2 wild : 2 cyt types in ten asci, 2 wild : 1 transitional type ($a^\pm b^\pm c^- p^\pm$ or $a^+ b^+ c^+ p^\pm$); 1 cyt in three asci, and 3 wild; 1 cyt in five asci, whereas (*a/a*), (*his*⁺/*his*⁻) and (*lys*⁺/*lys*⁻) segregated with a ratio of 2 : 2 in every ascus. All cyt-type segregants grew on lactate agar as scanty as those from cross 215. One quarter of these cyt mutants produced only TTC-unstainable colonies, but the others contained colonies faintly stainable with TTC. Absorption spectra of isolates from the latter show various patterns ranging from cyt-type to transitional-type (partial presence of cytochromes and slight accumulation of coproporphyrin). Cells of mutants faintly stainable with TTC were heterogeneous in this way, but they seem to have been mainly composed of the cyt mutant type, because the absorption spectrum of the transitional type was indiscernible on examination of the original culture. These results, together with those obtained in the previous section, show that a cross of the cyt mutant to wild type yields segregants with di-

TABLE 2

Segregation data of cross 217 (7C × 1B from cross 215)

$\frac{\alpha \text{ his}^- \text{ lys}^- P \rho^+}{\alpha \text{ his}^+ \text{ lys}^+ P \rho^+}$			
Ascus	TTC Red:Mixture of red and white:White	Growth on lactate - : ±	Spectrum* Wild:Transitional:cyt
1	3 : 1 : 0	3 : 1	3 : 0 : 1
2	3 : 1 : 0	3 : 1	2 : 1 ^a : 1
3	2 : 2 : 0	2 : 2	2 : 0 : 2
4	3 : 1 : 0	3 : 1	2 : 1 ^b : 1
5	2 : 1 : 1	2 : 2	2 : 0 : 2
6	2 : 0 : 2	2 : 2	2 : 0 : 2
7	2 : 1 : 1	2 : 2	2 : 0 : 2
8	3 : 1 : 0	3 : 1	3 : 0 : 1
9	2 : 2 : 0	2 : 2	2 : 0 : 2
10	2 : 2 : 0	2 : 2	2 : 0 : 2
11	2 : 2 : 0	2 : 2	2 : 0 : 2
12	2 : 1 : 1	3 : 1	3 : 0 : 1
13	2 : 1 : 1	2 : 2	2 : 0 : 2
14	2 : 1 : 1	2 : 2	2 : 0 : 2
15	3 : 1 : 0	3 : 1	3 : 0 : 1
16	2 : 2 : 0	3 : 1	3 : 0 : 1
17	2 : 1 : 1	2 : 2	2 : 0 : 2
18	3 : 0 : 1	3 : 1	2 : 1 ^c : 1

(α/α), (his^+/his^-), and (lys^+/lys^-) segregated in a 2:2 ratio in all asci.

* (a) $a^\pm b^\pm c^- p^\pm$ (Segregant 217-2D); (b) $a^+ b^+ c^+ p^\pm$ (Segregant 217-4A); (c) $a^\pm b^\pm c^- p^\pm$ (Segregant 217-18B).

verse patterns of absorption spectra varying between the cyt type and wild type, and frequently undergoes irregular segregation.

Normal segregation of cyt phenotype: The frequent occurrence of irregular segregation from a cross, $cyt \times wild$, could be explained if one assumes a suppressor gene which works by restoring the effect of mutation. Appearance of a transitional type may be caused by partial restoration. If the above assumption is correct, it would be expected that only normal segregation of 2 wild : 2 cyt-type would result when the assumed gene was eliminated by chance. With this in mind, several crosses (218, 223, 224, 301, 305, 310 and 311) were made between TTC-unstainable P cyt-type mutants and wild type. These are successive descendants from the cross 215, $5b-1 \times 605$. The results are shown in Table 3. Some crosses (218, 224 and 305) gave only normal segregation, suggesting that the assumed gene had been lost. All mutants from cross 305 were completely TTC unstainable, although some cyt-type segregants from crosses 218 and 224 still contained colonies faintly stainable with TTC. Detailed evidence for the existence of the assumed suppressor gene will be presented in a future study.

Temperature sensitivity of the suppressed mutant: A revertant 301-2C (L-1) was isolated from a wild-type-sized colony which appeared when cells of a faintly TTC stainable cyt mutant, (301-2C, one of segregants from cross 301) were

TABLE 3

Spore assay from crosses of cyt × wild (Normal and irregular segregation with respect to the cyt character)

Cross	Mating partners cyt × wild	No. of asci dissected	Absorption spectrum wild:transitional*:cyt			
			2:0:2	2:1:1	3:0:1	3:1:0
218	215-2D × 215-6B <i>a his⁺ lys⁻</i> × <i>a his⁻ lys⁻</i>	6	6	0	0	0
223	217-6D × 217-8C <i>a his⁻ lys⁻</i> × <i>a his⁻ lys⁺</i>	13	11	1 ^a	1	0
224	217-6D × 217-11B <i>a his⁻ lys⁻</i> × <i>a his⁻ lys⁺</i>	6	6	0	0	0
301	223-1D × 217-11C <i>a his⁻ lys⁻</i> × <i>a his⁺ lys⁺</i>	7	5	1 ^b	1	0
305	224-6D × 223-14A <i>a his⁻ lys⁺</i> × <i>a his⁻ lys⁻</i>	7	7	0	0	0
310	223-1C × 217-6B <i>a his⁻ lys⁺</i> × <i>a his⁺ lys⁺</i>	6	3	1 ^c	2	0
311	223-4C × 217-6C <i>a his⁻ lys⁻</i> × <i>a his⁺ lys⁺</i>	8	5	2 ^{d,e}	0	1 ^f

(*a/a*), (*his⁺/his⁻*) and (*lys⁺/lys⁻*) segregated as expected.

* Spectra of transitional type: (a) *a⁻ b⁺ c[±] p[±]* (Segregant 223-12B); (b) *a⁻ b⁺ c⁺ p⁺* (Segregant 301-7C); (c) *a⁺ b⁺ c⁺ p[±]* (Segregant 310-3C); (d) *a⁺ b⁺ c⁺ p⁺* (Segregant 311-4C); (e) *a[±] b[±] c⁺ p⁺* (Segregant 311-7D); (f) *a[±] b[±] c⁺ p⁺* (Segregant 311-8C).

FIGURE 3.—Absorption spectrum of aerobically cultured cell suspension of a revertant, 301-2C (L-1). A, Culture at 25°; B, Culture at 30°; C, Culture at 35°.

spread on lactate agar. Such wild-type sized colonies appeared with a frequency of approximately 1 per 10^5 – 10^6 plated cells. Cells of the revertant grew on lactate agar fairly rapidly and reduced TTC intensively when grown at 30° and 25°, but at 35° they showed the character of cyt mutant, that is inability to reduce TTC and scanty growth on lactate agar. These results agree with the data of spectrophotometric findings: nearly wild-type (the presence of all cytochromes, although somewhat decreased, and a trace amount of coproporphyrin) at 25°; transitional-type ($a^+ b^+ c^+ p^+$) at 30°; and cyt-type at 35°. The absorption spectra were given in Figure 3.

This revertant was crossed with wild type 223–1A, and six asci from the resultant diploid (cross 308) were dissected. The results of spectrophotometric assays (Table 4) revealed that each ascus gave normal segregation of 2 wild : 2 cyt types on cultivation at 35°. However these cyt mutants were wild- or nearly wild-type when grown at 25°. On 30° incubation, they were TTC stainable and able to assimilate lactate, but some of them varied between the cyt type and the transitional type spectrophotometrically. It should be noted that two wild-type segregants in each ascus never accumulated any coproporphyrin even when cultivated at a temperature above 35°. It is concluded that the revertant 301–2C (L-1) is a temperature sensitive mutant caused by a single-gene mutation, which varies phenotypically between the cyt type and wild or nearly wild type, depending on the growth temperature.

Different degrees of temperature sensitiveness: The discovery of the revertant, 301–2C (L-1), raised the question whether the expression of the cyt character may be generally influenced by growth temperature. In order to clarify this point, a spectrophotometric assay was carried out with various strains after growth at 25°, 30° and 35° (Table 5). In many cases, one of three wild-type segregants from asci showing segregation of 3 wild type : 1 cyt type, or segregation of 3 wild type : 1 transitional type at 30°, exhibited the cyt or transitional type at 35°. Transitional-type mutants at 30° showed the cyt type at 35° and were wild or nearly wild-type at 25°. Only some of those mutants that were cyt at 30° appeared transitional or nearly wild-type at 25°; these included mutant 301–2C, from which came the revertant 301–2C (L-1). Other strains which retained the cyt character spectrophotometrically at 25° (including mutants from cross 305) were also more or less restored in their ability to reduce TTC and their ability to grow on lactate at 25°. Mutants that were cyt at 30° usually failed to produce any colonies on lactate agar at 35°.

Thus, cyt mutants so far tested could be tentatively classified according to the degree of temperature sensitiveness as follows: (1) cyt or transitional-type at 35°, and nearly wild or wild-type at 30° and below; (2) cyt-type at 35°, transitional-type at 30° and nearly wild or wild-type at 25°; (3) cyt-type at 30° and above, and transitional or nearly wild-type at 25°, and (4) cyt-type at 25° and above.

In general, the mutant segregants from crosses 215, 217, 223, 301, 310 and 311 cover all of these types; those from crosses 218 and 224 include (3) and (4); and

TABLE 4
Segregation data of cross 308, 301-2C(L-1) $\times$ 223-1A

α his⁺ lys⁺ P p⁺
a his⁻ lys⁻ P p⁺

Segregant	Q ₀₂				TTC*				Growth on lactate†				Spectrum											
	25°	30°	35°		25°	30°	35°		25°	30°	35°		a	b	c	p	a	b	c	p	a	b	c	p
1A	8.6	5.3	12.0		R	R	R		0.46	1.03	0.74		+	+	+	—	+	+	+	—	+	+	+	—
1B	..	7.0	2.6		R	R	R		0.46	1.12	0.57		+	+	+	—	+	+	+	—	+	+	+	—
1C	5.6	1.2	0.5		R	R	W		0.44	1.07	0.21		+	+	+	±	+	+	±	+	+	±	+	+
1D	4.4	2.3	0.7		R	R	P		0.44	0.95	0.20		+	+	+	±	—	—	±	+	—	—	—	+
2A	..	6.8	14.3		R	R	R		0.73	1.32	0.76		+	+	+	—	+	+	+	—	+	+	+	—
2B	..	9.3	3.7		R	R	R		0.90	1.15	0.61		+	+	+	—	+	+	+	—	+	+	+	—
2C	2.8	1.6	0.6		R	R	W		0.70	0.85	0.14		+	+	+	±	—	—	±	+	—	—	+	+
2D	5.3	2.3	0.7		R	R	W		0.73	0.80	0.14		+	+	+	±	+	+	±	+	—	—	—	+
3A	..	4.9	0.3		R	R	R		0.63	1.21	0.66		+	+	+	—	+	+	+	—	+	+	+	—
3B	5.3	2.4	0.6		R	R	W		0.68	0.92	0.17		+	+	+	±	+	+	±	+	—	—	—	+
3C	5.2	1.5	0.0		R	R	W		0.53	0.88	0.07		+	+	+	±	—	—	±	+	—	—	—	+
3D	..	6.7	1.4		R	R	R		0.47	0.95	0.51		+	+	+	—	+	+	+	—	+	+	+	—
4A	..	6.0	0.6		R	R	R		0.65	1.10	0.51		+	+	+	—	+	+	+	—	+	+	+	—
4B	..	6.1	0.5		R	R	R		0.70	1.32	0.61		+	+	+	—	+	+	+	—	+	+	+	—
4C	4.3	1.9	1.0		R	R	P		0.77	1.32	0.17		+	+	+	±	+	+	±	+	—	—	+	+
4D	5.9	2.3	0.5		R	R	W		0.71	1.18	0.19		+	+	+	±	+	+	±	±	—	—	—	+
5A	..	10.4	13.7		R	R	R		0.71	1.25	0.55		+	+	+	—	+	+	+	—	+	+	+	—
5B	..	5.8	0.5		R	R	R		0.97	1.47	0.63		+	+	+	—	+	+	+	—	+	+	+	—
5C	2.1	2.3	0.5		R	R	P		0.69	0.70	0.07		+	+	+	±	—	—	±	+	—	—	—	+
5D	1.0	1.8	0.5		R	R	W		0.41	1.25	0.07		+	+	+	±	—	—	±	+	—	—	—	+
6A	..	8.3	13.9		R	R	R		0.52	1.40	0.57		+	+	+	—	+	+	+	—	+	+	+	—
6B	..	5.9	1.7		R	R	R		0.47	1.50	0.68		+	+	+	—	+	+	+	—	+	+	+	—
6C	6.5	2.4	0.8		R	R	W		0.48	0.80	0.15		+	+	+	±	—	—	±	—	—	—	—	+
6D	1.9	2.9	0.2		R	R	P		0.51	1.28	0.19		+	+	+	±	—	—	±	—	—	—	—	+

Q₀₂ = μ mole/mg wet weight/minute.
(a/a), (his⁺/his⁻) and (lys⁺/lys⁻) segregated as expected.
* R = red, W = white, P = pink.
† Diameter (mm) of colony on lactate (3 days culture).

TABLE 5
Various types of temperature sensitivity

Segregants	Spectrum											
	25°				30°				35°			
	a	b	c	p	a	b	c	p	a	b	c	p
215	4A	+	+	+	+	+	+	+	2A	+	+	+
	4B	—	+	—	—	+	—	—	2B	—	+	—
	4C	—	—	+	—	—	—	—	2C	—	+	—
	4D	+	+	—	—	+	—	—	2D	—	+	—
215	15A	—	+	—	—	+	—	—	301	—	+	—
	15B	—	+	—	—	+	—	—	4A	—	+	—
	15C	+	+	—	—	+	—	—	4B	—	+	—
	15D	—	—	+	—	—	—	—	4C	—	+	—
217	1A	+	+	—	—	+	—	—	4D	—	+	—
	1B	+	+	—	—	+	—	—	6A	—	+	—
	1C	+	+	—	—	+	—	—	6B	—	+	—
	1D	+	+	—	—	+	—	—	6C	—	+	—
217	2A	—	—	+	—	—	—	—	6D	—	+	—
	2B	+	+	—	—	+	—	—	2A	—	+	—
	2C	+	+	—	—	+	—	—	2B	—	+	—
	2D	+	+	—	—	+	—	—	2C	—	+	—
217	4A	+	+	—	—	+	—	—	2D	—	+	—
	4B	+	+	—	—	+	—	—	3A	—	+	—
	4C	+	+	—	—	+	—	—	3B	—	+	—
	4D	—	—	+	—	—	—	—	3C	—	+	—
217	8A	+	+	—	—	+	—	—	3D	—	+	—
	8B	—	—	+	—	—	—	—	5A	—	+	—
	8C	+	+	—	—	+	—	—	5B	—	+	—
	8D	+	+	—	—	+	—	—	5C	—	+	—
217	15A	+	+	—	—	+	—	—	5D	—	+	—
	15B	+	+	—	—	+	—	—	1A	—	+	—
	15C	+	+	—	—	+	—	—	1B	—	+	—
	15D	+	+	—	—	+	—	—	1C	—	+	—
223	2A	—	—	+	—	—	—	—	1D	—	+	—
	2B	—	—	+	—	—	—	—	8A	—	+	—
	2C	—	—	+	—	—	—	—	8B	—	+	—
	2D	—	—	+	—	—	—	—	8C	—	+	—
		—	—	+	—	—	—	—	8D	—	+	—

* No growth.

those from cross 305, only (4). Considering these facts, it seems that deviations from normal 2 : 2 segregations with a cross of cyt $\times$ wild, were caused by differences in temperature sensitivity of the cyt mutant segregants, in expression of the mutant phenotype.

DISCUSSION

The two unique properties of strain 5b-1—deficiency of all cytochromes, and coproporphyrin accumulation—segregate together as if controlled by a single gene. In spite of the fact that all cytochromes were invisible spectrophotometrically, cyt-type mutants were able to grow extremely slowly on lactate agar. Formation of scanty colonies on lactate probably means the presence of a slightly functioning terminal electron-transport system in cyt mutant cells grown on lactate agar. The invisibility of the absorption spectrum of cytochromes may have resulted from the glucose-repression of cytochrome synthesis in cyt mutants, because in this experiment, the spectrophotometric assay was carried out on cells grown in yeast extract-peptone glucose liquid medium containing 2% glucose. Glucose and related catabolites at such a concentration are known to repress extremely the synthesis of cytochrome *a* in yeast that bears an extensive lesion in the respiratory system (REILLEY and SHERMAN 1965). The existence among some of TTC stainable and lactate assimilable strains of types that were cyt spectrophotometrically, might be ascribed to a much accentuated sensitivity to glucose-repression of cytochrome-synthesis.

The existence of a suppressor gene responsible for the phenotypic reversion of cyt-type to wild-type was assumed. An attempt to separate such an assumed gene from the gene controlling the cyt character was promising, since only the segregation of 2 wild : 2 cyt types has been obtained from certain crosses of wild $\times$ cyt. The mechanism of such phenotypic reversion is not fully understood; reversions may be due partly to a difference in temperature sensitivity of cyt mutants, because some asci showing 3 : 1 or irregular segregation with transitional phenotype at 30°, gave 2 : 2 segregation when grown at 35°. Asci showing 3 : 1 segregation even at 35° have not been explained; possibly these asci also might give 2 : 2 segregation at higher temperatures, or by changing growth conditions other than temperature. This suggestion is based on the assumption that the mutationally altered enzyme may be protected from inactivation at 35° under some cellular conditions. Some auxotrophic mutants can be made to express the wild phenotype by either elevating the osmotic pressure of the medium, lowering the temperature (HAWTHORNE and FRIIS 1964), or changing the pH (POMPER 1952).

The above discussion has assumed that the temperature sensitive principle was an enzyme. However, it is possible that an enzyme-forming system instead of an enzyme is temperature sensitive.

An enzymatic study of the site of lesion in cyt mutants is under way. At present, a defect in the step of conversion of coproporphyrin to protoporphyrin seems to be the most probable. This was supported by the absence of any heme-proteins, the accumulation of coproporphyrin (SUGIMURA, OKABE, NAGAO and

GUNGE 1966) and the restoration of ability to reduce TTC when cultivated on medium supplemented by protoporphyrin or hemin (SUGIMURA, NAGAO, IWASAKI, MIYAKE and GUNGE 1966). It appears highly probable that cyt mutants produce a mutationally altered enzyme, coproporphyrinogen decarboxylase, catalyzing the step of conversion of coproporphyrin to protoporphyrin, which is more or less functional at low temperature, but not at high temperature. Additional details of genetic and biochemical studies on phenotypic reversion will be reported elsewhere in relation to the temperature sensitivity of cyt type mutants.

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SUMMARY

A deficiency in cytochromes a, b and c and an accumulation of coproporphyrin in aerobically grown cells of a mutant strain tentatively denoted as cyt type is due to a single gene mutation. This mutant produces barely visible colonies on lactate agar. Irregular segregation and transitional-type mutants occurred frequently in some crosses of cyt mutants $\times$ wild. The cyt-type mutant is temperature sensitive. Cytochromes a, b and c are absent and coproporphyrin is accumulated at high temperature, but ability to synthesize all cytochromes is restored at low temperature (although the degree of temperature sensitiveness varied among segregants). Deviations from a segregation ratio of 2 wild type : 2 cyt type are probably due to differences in temperature sensitivity of expression of the cyt character.

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