THE RELATIONSHIP BETWEEN RESPIRATORY DEFICIENCY AND SUPPRESSIVENESS IN YEAST AS DETERMINED WITH SEGREGATIONAL MUTANTS¹

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THE development of a normal respiratory phenotype in yeast requires the presence of an autoreproducing and presumably particulate cytoplasmic factor present in normal yeast and lost or functionally inactivated in the so-called respiration-deficient vegetative mutants ("vegetative petites"). This was inferred from the nontransmission of the respiratory deficiency to the sexual progeny in crosses of the neutral vegetative mutants with normal yeast (EPHRUSSI, HOTTINGUER and TAVLITZKI 1949; EPHRUSSI, DE MARGERIE-HOTTINGUER and ROMAN 1955) and confirmed by the study of heterokaryons (WRIGHT and LEDERBERG 1957).

It is now known that the majority of cytoplasmic mutants are suppressive, i.e., when crossed with normal yeast, they impose their respiratory phenotype on a fraction of the zygotes and/or the vegetative progeny of the zygotes (EPHRUSSI, DE MARGERIE-HOTTINGUER and ROMAN 1955). Vegetative mutants of independent origin differ in the degree of this interference with the maintenance or development of the respiratory ability: they are said to possess different "degrees of suppressiveness," reflected in, and measured by, the fraction of zygotes which give rise to respiration-deficient clones. The various degrees of suppressiveness exhibit considerable stability during the vegetative proliferation of cytoplasmic mutants (EPHRUSSI and GRANDCHAMP 1962). While the transmission of the respiratory deficiency of vegetative mutants is always cytoplasmic, little is known about the inheritance of the different degrees of suppressiveness. In other words, the genetic determination of suppressiveness and of its variations is unknown. One may wonder whether the suppressive property of the mutants under consideration is a consequence of (1) the lack of respiration, or (2) the "loss" of the normal cytoplasmic factor, or (3) the presence of a competing factor which, on the simplest hypothesis, could be the functionally inactive normal factor itself.

Some of these questions can be answered by the study of the suppressiveness of mutants which owe their respiratory deficiency to gene mutations and which do or do not contain the normal cytoplasmic factor (referred to as "segregational")

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and "double" mutants, respectively) (CHEN, EPHRUSSI and HOTTINGUER 1950). Such study could also reveal whether genetic blocks of respiration affect suppressiveness. The present report describes the results of the study of several segregational and double mutants from this point of view.

MATERIALS AND METHODS

Strains: The symbols P and p are used below to designate, respectively, the wild-type and mutant gene which control the ability to grow on nonfermentable substrates. ρ^+ and ρ^- denote, respectively, the presence or "absence" of the cytoplasmic factor required for the synthesis of respiratory enzymes. Combinations of these symbols designate the four cell types encountered (CHEN, EPHRUSSI and HOTTINGUER 1950; SHERMAN 1962); (1) wild type, $P \rho^+$; (2) vegetative mutant, $P \rho^-$; (3) segregational mutant, $p \rho^+$; and (4) double mutant, $p \rho^-$.

Three nonallelic segregational mutants ($p_5 \rho^+$, $p_6 \rho^+$, and $p_7 \rho^+$) were chosen from our yeast stocks (SHERMAN 1962) for (1) appropriate markers (as indicated in Table 2 and 3), (2) low frequency of doubles, and (3) difference in color exhibited by $p \rho^+ ad_1$ and $p \rho^- ad_1$ colonies (see below). Each of these strains was plated on complete medium and several subclones were isolated.

Several double mutants $p_5 \rho^-$, $p_6 \rho^-$ and $p_7 \rho^-$ were isolated from each of the above strains, each mutant coming from an independent $p \rho^+$ clone and therefore of independent origin. The double mutants $p_1 \rho^-$ and $p_3 \rho^-$ were isolated from strains 1275-2B ($p_1 \rho^+$) and 1334-6C ($p_3 \rho^-$), respectively, obtained from Dr. D. HAWTHORNE.

$p_6 \rho^+$ and $p_7 \rho^+$ and all $p \rho^-$ strains are similar to vegetative mutants ($P \rho^-$) in that they lack both cytochromes a and b, while $p_5 \rho^+$ lacks only cytochrome a (SHERMAN and SLONIMSKI 1962), and $p_6 \rho^+$ differs from $P \rho^-$ in that a small fraction of cells is capable of growing on nonfermentable carbon sources without obvious change in genotype.

The haploid strains, normal with respect to respiratory characteristics, used for the test of suppressiveness of the above mutants were 18609-1, $\alpha P \rho^+ hi_1$ (obtained from Dr. M. OGUR), containing approximately one percent vegetative mutants, and 1268-2B, $\alpha P \rho^+ hi_2 ad_6$ (obtained from Dr. D. HAWTHORNE), containing approximately five percent vegetative mutants. The former was used with p_6 , p_5 , and p_7 strains and the latter with p_1 and p_3 strains.

Frequency of $p \rho^-$ cells. In order to determine quantitatively the degree of suppressiveness of $p \rho^+$ cells of the various strains, it was necessary to find the percent of $p \rho^-$ cells in the mutant cultures. This was greatly facilitated by the use of the ad_1 gene (adenine dependence, color marker). This technique described by PITTMAN, WEBB, ROSHANMANESH and COKER (1960) is based on the observation that the pigment of ad_1 mutants is affected by the respiratory genotype as shown in Table 1. Thus the frequency of $p \rho^-$ cells in a $p \rho^+ ad_1$ culture can be inferred from the proportion of white colonies following plating. It should be noted that not all strains of the above genotypes react in this manner, for many $p \rho^+ ad_1$

TABLE 1

Alteration of the pigment produced by ad_1 with different respiratory genotypes
(TAVLITZKI 1951; PITTMAN, WEBB, ROSHANMANESH and COKER 1960)

Genotype	Color
$P \rho^+ ad_1$	pink
$P \rho^- ad_1$	white
$p \rho^+ ad_1$	brown
$p \rho^- ad_1$	white

strains are very faintly brown or white. This is not too surprising, since many $P \rho^+ ad_1$ strains are also faintly colored.

The percentage of $p_6 \rho^-$ cells was also inferred from the number of small colonies following plating on low glucose (0.1%) medium as described by SHERMAN and SLONIMSKI (1962).

In addition, the percent of $p \rho^-$ cells in a $p \rho^+$ population was determined by plating zygotes from a cross of the $p \rho^+$ strain with the neutral vegetative mutant, C982-19dA₁ ($\alpha P \rho^- hi_1 tr_1$; EPHRUSSI, DE MARGERIE-HOTTINGUER and ROMAN 1954). In such a cross presumably only the $p \rho^-$ cells form ρ^- hybrids.

Although a number of $p_6 \rho^+$ strains were examined for a low frequency of $p_6 \rho^-$ cells, no strain was found to contain under 40 percent of $p_6 \rho^-$ cells after prolonged cultivation. Therefore the "adaptability" of p_6 (SHERMAN and SLONIMSKI 1962) was taken advantage of in order to select against the ρ^- cells as follows: the $p_6 \rho^+$ strain was streaked on plates of 0.1 percent glucose medium and incubated for four to five days. At this time most of the colonies form readily distinguishable papillae, consisting of "adapted" cells and containing a low percentage of ρ^- cells. These papillae are picked and incubated in a three percent glucose medium until "deadaptation" occurs at around five to six generations. Such a culture, containing a low percentage of $p \rho^-$ cells, is then used for the determination of suppressiveness.

Determination of the degree of suppressiveness: The experimental procedure for the determination of the degree of suppressiveness of p mutants is similar to that employed for vegetative mutants (JACOB, EPHRUSSI and GRANDCHAMP 1962). This consists in plating on minimal synthetic medium (containing 0.25 percent glucose and three percent glycerol) the mass-mating of the normal and mutant cells in which zygotes have been formed but have not yet budded. After four to five days of incubation at 30°C, the prototrophic colonies are stained with tetrazolium chloride in order to determine the percent of zygotes giving rise to respiration-deficient clones.

Parallel to the zygote platings, the haploid parental strains were plated for the percent of ρ^- cells as described above. In addition, in the crosses involving $p \rho^+$ strains, the percent of back mutants ($P \rho^+$) and the percent of $p_6 \rho^+$ cells capable of growth on glycerol was determined by plating on glycerol medium.

Calculation of the degree of suppressiveness: If we define the degree of suppres-

siveness of a mutant as the probability that a zygote, resulting from the cross of the mutant with a normal strain, will give rise to a respiration-deficient clone, then the percent suppressiveness (%S) of a vegetative mutant ($P \rho^-$) or of a double mutant ($p \rho^-$) is:

$$\%S = \frac{X - Y}{1 - Y} 100 \quad (1)$$

where X is the fraction of ρ^- colonies produced by the zygotes and Y the small fraction of ρ^- cells in the normal haploid tester strain. The %S of a $p \rho^+$ strain, containing a fraction Z of $p \rho^-$ cells and a fraction W of $P \rho^+$ cells, is limited by the values:

$$\frac{X - YZ}{(1 - Y)(1 - Z - W)} 100 \quad (2)$$

and,

$$\frac{X - Y - Z + YZ}{(1 - Y)(1 - Z - W)} 100 \quad (3)$$

Formula (2) is based on the assumption that the degree of suppressiveness of $P \rho^-$ cells in a tester strain and of the $p \rho^-$ cells are equal to zero, while formula (3) assumes that they are 100 percent.

RESULTS AND DISCUSSION

The results of the crosses of $p_5 \rho^+$, $p_6 \rho^+$, and $p_7 \rho^+$ and of the subclones of these strains are given in Table 2 along with the results of the platings of the haploid cultures for the percent of $P \rho^+$ (growth on glycerol medium) and the percent of $p \rho^-$ (by colony color). Columns $\times P \rho^-$ and $\times P \rho^+$ give the percent of ρ^- in the crosses with the neutral vegetative mutant and the normal tester strains, respectively. Application of formulas (2) and (3) to the data of Table 2 leads to %S values of zero (formula 3) or nearly zero (formula 2). The values given in

TABLE 2

The percent of respiration-deficient hybrids (% ρ^-) obtained when segregational mutants ($p \rho^+$) are crossed to a neutral vegetative strain ($\times P \rho^-$) and a normal tester strain ($\times P \rho^+$).

The percent of colonies of the parental mutant growing on glycerol (% $P \rho^+$) and the percent of doubles (% $p \rho^-$) obtained by colony color is also shown

Strain	Genotype	Percent $P \rho^+$	Percent $p \rho^-$	Percent ρ^-	
				$\times P \rho^-$	$\times P \rho^+$
D213-9B	$a p_5 \rho^+ ad_1 ly_2$	0	3.2 ± 0.6	3.2 ± 0.6	0.8 ± 0.3
D213-9B-4		0	3.7 ± 0.6	3.6 ± 0.7	0.3 ± 0.2
D213-9B-5		0	4.1 ± 0.7	4.4 ± 0.8	0.7 ± 0.3
D225-1C-11	$a p_6 \rho^+ ad_1 ly_2$	6.1	1.1 ± 0.5	2.5 ± 0.6	0.7 ± 0.2
D225-1C-12		5.8	0.8 ± 0.3	0.2 ± 0.2	0.4 ± 0.1
D225-1C-13		8.9	1.3 ± 0.5	0.9 ± 0.4	0.4 ± 0.1
D243-2B	$a p_7 \rho^+ ad_1 ly_2$	0	8.5 ± 1.1	4.4 ± 0.6	0.8 ± 0.3
D243-2B-1		1.5	14.4 ± 2.3	9.9 ± 0.6	2.5 ± 0.5
D243-2B-2		0	9.5 ± 1.1	6.2 ± 0.6	1.3 ± 0.4

the last column of Table 2 are approximately equal to the highest theoretical values for %S (formula 2). In other words the degree of suppressiveness of $p\rho^+$ cells may be considered as equal to zero.

The situation is very different in the case of $p\rho^-$ strains. It can be seen in Table 3 that these strains exhibit a variety of degrees of suppressiveness, and may

TABLE 3

The percent suppressiveness (%S) of various double mutants ($p\rho^-$)

Strain	Genotype	%S
1275-2B-1	$a p_1 \rho^- ad_8$	34.8
-2		48.6
-3		41.4
-4		47.5
-5		32.6
-6		47.0
-7		23.4
-8		18.7
-9		31.4
1334-6C	$a p_3 \rho^- tr_1 me_2 ly_2$	3.4
-1		5.7
-2		-0.6 ± 1.0
-3		18.4
-4		0.2 ± 1.3
-5		23.2
-6		9.8
-7		83.2
-8		4.4
-9		61.6
D213-9B-8	$a p_5 \rho^- ad_1 ly_2$	15.1
-10		1.7 ± 0.9
-11		0.3 ± 0.6
-12		2.0 ± 0.7
D225-1C-4	$a p_6 \rho^- ad_1 ly_2$	66.6
-9		29.4
-10		22.0
D243-2B-3	$a p_7 \rho^- ad_1 ly_2$	82.2
-4		70.8
-6		59.5

not differ in this respect from vegetative mutants ($P\rho^-$). Although the present data do not permit a definite conclusion, $p_5\rho^-$ may represent an exception inasmuch as all clones thus far isolated from strain D213-9B exhibit a low degree of suppressiveness.

It can therefore be assumed that, with respect to suppressiveness, $p\rho^+$ and $p\rho^-$ cells behave as $P\rho^+$ and $P\rho^-$, respectively, and that respiration-deficiency does not in itself cause suppressiveness. In other words, suppressiveness is manifested only after the "loss" of the cytoplasmic factor. The nonfunctional cytoplasmic factor present in $p\rho^+$ cells does not appear to alter or compete with the normal factor.

SUMMARY

Several respiration-deficient yeast strains, which owe this deficiency to gene mutations at different loci, have been examined with respect to their suppressiveness. They were found to be neutral so long as they possess the cytoplasmic factor required for the synthesis of respiratory enzymes, and to become suppressive when they "lose" it. In this respect they are comparable therefore to normal yeast. Suppressiveness results from the loss of the cytoplasmic factor, not from that of respiration.

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