RESPIRATION-DEFICIENT MUTANTS OF YEAST. I. GENETICS[1]

FRED SHERMAN[2]

Laboratoire de Génétique physiologique du CNRS
Gif-sur-Yvette (Seine et Oise), France

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IN most growing yeast cultures, there are under most conditions, approximately one percent of respiration-deficient cells ("vegetative petites") which are known to be due to a cytoplasmic mutation probably arising from the loss or irreversible inactivation of autoreproducing cytoplasmic units necessary for the synthesis of respiratory enzymes (cf. EPHRUSSI 1953). Proof of non-Mendelian inheritance of these vegetative mutants was provided by ascus (EPHRUSSI, HOTTINGUER and TAVLITZKI 1949) and heterokaryon analyses (WRIGHT and LEDERBERG 1957). One manifestation of the respiratory deficiency of these variants is the inability to utilize nonfermentable carbon sources such as glycerol, lactate, acetate and ethanol (TAVLITZKI 1949; SLONIMSKI 1953; OGUR and ST. JOHN 1956; YANAGISHIMA 1956). Spectroscopic examinations of intact cells of vegetative mutants have revealed the absence of cytochromes a, a_3, and b (cf. SLONIMSKI 1953), which can account for the absence of a number of enzymatic activities involved in the electron transport system such as cytochrome oxidase (cytochromes a and a_3), and succinic-cytochrome c reductase and α-glycerophosphate-cytochrome c reductase (cytochrome b).

In addition to the vegetative mutants, there exist phenotypically similar mutants which result from single-gene mutations, and therefore show Mendelian (2:2) segregation following crosses with normal strains (CHEN, EPHRUSSI and HOTTINGUER 1950; RAUT 1953; PITTMAN 1959; PITTMAN, WEBB, ROSCHANMANESH and COKER 1960). It was first shown by CHEN, *et al.* (1950) that these "segregational mutants" can exist in two states, which either can or cannot be complemented by vegetative mutants. It was inferred that both nuclear and cytoplasmic determinants are necessary for normal respiration and that the segregational mutants can exist with or without the cytoplasmic factor.

The purpose of the present investigations (see also SHERMAN and SLONIMSKI 1963; SLONIMSKI and SHERMAN 1963; YOTSUYANAGAI 1962; SHERMAN and EPHRUSSI 1962) was to study the genetics and biochemistry of a series of non-allelic segregational mutants and of their relationships to vegetative mutants. The investigation presented herein deals primarily with the genetics of these variants.

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[2] Public Health Service Postdoctoral Fellow of the Naitonal Cancer Institute. Present address: Department of Radiation Biology, University of Rochester School of Medicine and Dentistry, Rochester, New York.

Nomenclature: The following nomenclature (similar to that of PITTMAN, *et al.* 1960; MARQUARDT 1952) will be adopted in order to distinguish the various mutants dealt with in these investigations. The symbol *p* will denote mutants carrying a single recessive allele of a gene resulting in the loss of ability to grow on nonfermentable substrates such as lactate, glycerol, acetate, ethanol, etc. Nonallelic mutants will be designated by different subscripts—i.e., p_1, p_2, etc. However, if a symbol has been previously employed to denote a gene which is associated with the p phenotype, the original symbol will be retained, as in the case of ly_6 and ly_8. *P* will denote the wild type, and if no subscript is utilized, will imply the presence of all wild-type genes necessary for the growth on nonfermentable substrates. The symbols ρ^+ and ρ^- will designate the presence or "absence," respectively, of the cytoplasmic factor required for the synthesis of respiratory enzymes as determined by complementation with known vegetative mutants. Thus the four cell types that will be encountered are: 1) normal (wild type), $P\ \rho^+$; 2) vegetative mutant, $P\ \rho^-$; 3) segregational mutant, $p\ \rho^+$; and 4) double mutant, $p\ \rho^-$.

MATERIALS AND METHODS

Mutant strains of yeast which were shown or suspected to contain *p* genes were received from DRS. HAWTHORNE, MORTIMER, and PITTMAN as shown in Table 1.

The general procedure for genetic studies of yeast has been previously described by HAWTHORNE and MORTIMER (1960) and is as follows: Diploid hybrid clones, obtained by isolating single zygotes from a mating mixture or by prototroph selection, are grown for 24 hours on YPAD medium (one percent yeast extract, one percent peptone, two percent glucose, and 40 mg/l of adenine sulfate), and inoculated on sporulation medium of MCCLARY, NULTY and MILLER (1959) (0.98 percent potassium acetate, 0.1 percent glucose, 0.25 percent yeast extract, and two percent agar). After three to five days of incubation at 25°C, the sporulated culture is treated with commercial snail juice (L'Industrie Biologique Française, Génévilliers, France) to which 15 mg/ml of cysteine-HCl is

TABLE 1

Origin of genes

Gene symbol	Strain	Reference
p_1	several strains	CHEN, *et al.* 1950 (used symbol *r*) HAWTHORNE and MORTIMER 1960
p_2	several strains	HAWTHORNE and MORTIMER 1960
p_3	several strains	HAWTHORNE and MORTIMER (unpublished)
p_4	several strains	SLONIMSKI and SHERMAN 1963
p_5	8256.27	PITTMAN, *et al.* 1960
p_6	8256.27	PITTMAN, *et al.* 1960
p_7	468.5	PITTMAN (unpublished)
ly_6	S2106A	MORTIMER (unpublished)
ly_8	S2531C	MORTIMER (unpublished)

added (SLONIMSKI, private communication), and four-spored asci are dissected. Complete tetrads are then tested for nutritional requirements, mating type (a/α), ability to grow on glycerol medium (one percent yeast extract and three percent glycerol), and staining with tetrazolium (OGUR, ST. JOHN, and NAGAI 1957; YANAGISHIMA 1956). Complementation tests of respiration-deficient strains were performed by incubating a mixture of two strains of opposite mating-type for 24 hours on YPAD and testing on glycerol medium for growth.

In all the hybrids analyzed, various nutritional markers, such as genes governing requirements for tryptophan, lysine, adenine, etc. (tr_1, ly_2, ad_1, etc; HAWTHORNE and MORTIMER 1960), were introduced to ascertain normal diploid meioses—i.e., 2:2 segregations. In the present investigation, approximately one percent of the tetrads analyzed gave irregular segregation for some of the markers.

RESULTS

Single-gene segregations: If an inability to grow on glycerol medium were due to a single gene, then one would expect to observe a 2:2 segregation of this character in asci of a heterozygous diploid hybrid. This condition has previously been found with p_1, p_2, p_3, p_4, ly_6 and ly_8 by the investigators cited in Table 1, and was confirmed in the present investigation. However, since other mutant strains did not fullfill this expectation, a more detailed presentation is necessary.

p_5, p_6 *and* p_7: Irregular segregations for the ability to grow on glycerol medium were obtained with several mutant strains received from DR. PITTMAN (1959), PITTMAN, *et al.* 1960). A complete analysis of two mutants lead to the conclusion that multiple gene segregation was involved.

In the first hybrid, D-213, the mutant strain 8256.27 was crossed to a respiration-sufficient haploid D202-2C(α ad_1 tr_1 ly_2). Ten analyzed tetrads gave 2:2 segregations for mating type and other markers, and a more complex segregation for the ability to grow on glycerol medium. When a number of glycerol negative segregants of opposite mating type were tested to determine allelism, a clear two gene segregation was obtained, as illustrated in Table 2 for a tetratype ascus. The demonstration of a two-gene segregation became possible by selecting four segregants which had the following complementation properties on glycerol: $a\ p_5 \times \alpha\ p_5 = -$; $a\ p_6 \times \alpha\ p_6 = -$; $a\ p_5 \times \alpha\ p_6 = +$; $a\ p_6 \times \alpha\ p_5 = +$. Each segregant was

TABLE 2

An example of segregation in a tetrad from cross D-213

Segregant designation	Growth on glycerol medium*				Genotype
	Segregant	$\times$ $P\ p^-$	$\times$ $p_5\ p^+$	$\times$ $p_6\ p^+$	
A	+	+	+	+	$P_5\ P_6\ p^+$
B	$-\Delta$	+	+	$-\Delta$	$P_5\ p_6\ p^+$
C	−	+	−	+	$p_5\ P_6\ p^+$
D	−	+	−	$-\Delta$	$p_5\ p_6\ p^+$

* + = confluent growth after 24 hours. − = no growth. $-\Delta$ = revertant colonies after one week incubation.

tested against *a* p_5 or α p_5, and *a* p_6 or α p_6. All ten tetrads gave 2:2 segregations for p_5 and p_6 with parental ditype (PD), nonparental ditype (NPD), and tetratype (T) asci frequencies of 4:0:6. The genotype *a* p_5 p_6 ad_1, as inferred from the results of tetrad analyses and by direct tests of allelism, was ascribed to the original variant, 8256.27.

The two genes p_5 and p_6 have distinct phenotypes in that, after prolonged incubation on glycerol, revertant colonies appear only with cultures of p_6. The double mutant p_5 p_6 does not revert on glycerol medium (Table 2). Other methods of differentiating these two variants will be described in a subsequent publication (SHERMAN and SLONIMSKI 1963). One exceptional segregant, D213-2A, which is phenotypically different, will be discussed below.

The two-gene segregation was again verified by back-crossing two segregants, *a* p_5 ad_1 and *a* p_6 ad_1, to the original respiration-sufficient haploid strain D202-2C. In the eight asci analyzed in each of the two hybrids, only 2:2 segregations were obtained.

Identical analyses of a second respiration-deficient variant (486.5) of this series led to a similar two-gene segregation with PD:NPD:T frequencies of 1:0:10. One of these two genes was found to be allelic with p_6, and therefore the genotype of 468.5 was established as *a* p_6 p_7 ad_1. Like the previously described genes, p_5 and p_6, p_7 segregated 2:2 when backcrossed to D202-2C.

When p_5, p_6, and p_7 were employed in tests of allelism with three other variants obtained from DR. PITTMAN (strain numbers 360.5, 368.6 and 466.1), it was found that these variants contained *at least* the following mutant genes: α p_5 p_6 ad_1; α p_5 p_6 ad_1; and *a* p_5 p_6 p_7 ad_1. The reason for the simultaneous presence of more than on *p* gene in each of the five mutants of this series remains obscure.

md_1 -p: In the analysis of the hybrid cross D-213 described above, one exceptional segregant, D213-2A, had an altered phenotype. If one assumes a 2:2 segregation, the genotype of this strain should have been *a* p_6 ad_1, but in actuality it deviated from other p_6 segregants by confluent growth on glycerol medium after three days incubation, and moreover, by a more intense cytochrome c band (SHERMAN and SLONIMSKI 1963). The presence of p_6 in D213-2A was demonstrated by crossing to a typical p_6 segregant and examining the hybrid, which proved to have the phenotype of a p_6/p_6 homozygous diploid. That the anomalous segregant of p_6 arose with a second recessive gene mutation is consistent with the analysis of the hybrid backcross of D213-2A with the respiration-sufficient parent D202-2C, since three examined tetrads gave 2:2 segregations for growth on glycerol medium after one day incubation, with one negative segregant in each tetrad behaving like the parent D213-2A by growing on glycerol medium after three days of incubation (and by having an altered cytochrome pattern, SHERMAN and SLONIMSKI 1963). The simplest hypothesis is that the genotype of D213-2A is *a* p_6 md_1 -*p* ad_1 (where md_1 -*p* is a single gene modifying the phenotype of p_6), and that the three analyzed tetrads are tetratype asci.

Complementation with vegetative mutants: A culture of a segregational strain would not be expected to be composed entirely of *p* ρ^+ cells, for as in the case of

the wild type, a small fraction of ρ^- cells are always observed. Furthermore, since $p\ \rho^+$ cells probably do not have as great a selective advantage over $p\ \rho^-$ as P strains do, one might expect a higher fraction of ρ^- cells to occur in segregational mutants.

By the use of the routine test of complementation with vegetative mutants (as described above), one cannot discriminate between populations containing high or low proportions of $p\ \rho^-$ cells. Even when a culture contains 99 percent $p\ \rho^-$ cells, it will be scored as $p\ \rho^+$, since the remaining one percent of $p\ \rho^+$ cells are sufficient to produce a confluent growth on glycerol medium when crossed to the $P\ \rho^-$ tester strain. However, if $p\ \rho^+$ cells are extremely rare, they produce discrete colonies in a mixture with vegetative mutants, when replicated on glycerol medium. There are, however, methods by which one can determine the exact frequency of ρ^- cells in a $p\ \rho^+$ culture: 1) when zygotes from a cross of the segregational mutant with a neutral vegetative mutant are plated, only the $p\ \rho^-$ cells form ρ^- hybrids (SHERMAN and EPHRUSSI 1962); 2) sometimes the pigment produced by ad_1 (adenine dependence) mutants is affected by the respiratory genotype so that $p\ \rho^+$ and $p\ \rho^-$ colonies exhibit distinct colors (PITTMAN, *et al.* 1960; SHERMAN and EPHRUSSI 1962); 3) most segregants of p_4 give rise to $p_4\ \rho^+$ and $p_4\ \rho^-$ colonies which are and are not stained, respectively, by tetrazolium chloride (SHERMAN and SLONIMSKI 1963); and 4) following plating on low glucose medium, $p_6\ \rho^+$ cells give rise to tetrazolium-stainable large colonies, as compared with tetrazolium-unstainable small colonies formed by $p_6\ \rho^-$ cells (SHERMAN and SLONIMSKI 1963).

A large number of segregants from various hybrid crosses involving the nine p genes have been examined for the frequencies of ρ^- cells. These will be considered in two classes: 1) all segregants containing a specific p gene which under ordinary conditions have never been observed to be complemented by vegetative mutants. Such mutants may be due to a gene controlling the retention or synthesis of the cytoplasmic factor; 2) segregants with different degrees of stability, but where at least some stable $p\ \rho^+$ strains have been observed.

p_3, ly_6, and ly_8 segregants belong to the first class inasmuch as no stable ρ^+ strains were found in the examination of 16, 10 and 8 tetrads, respectively. Other properties of these three genes will be discussed below.

Segregants containing the mutant genes, p_1, p_2, p_4, p_5, p_6, and p_7 have been found to exhibit various degrees of stability in retaining the cytoplasmic factor as measured by the frequencies of $p\ \rho^-$ cells. Some segregants of all these mutants were completely ρ^- or were transformed to ρ^- cultures after one transfer. The most unstable of these six segregational mutants was p_2, since only three out of 22 p_2 segregants from a hybrid were found to retain the cytoplasmic factor. The frequency of ρ^+ cells in the most stable p_2 culture was about 0.1 percent. In the analysis of 20 p_2 segregants from another hybrid, no ρ^+ culture was observed. Segregational mutants derived from several hybrids heterozygous for p_1 were also found to be unstable since about one-half of the p_1 segregants were ρ^- and many of the ρ^+ segregants showed a high frequency of ρ^- cells. However, most segregants carrying p_4, p_5, p_6, and p_7 were ρ^+. The frequencies

of ρ^- cells in the most stable segregants of each of the nine segregational mutants are given in Table 3.

No systematic investigation was undertaken to determine the genetics of this instability since only diploids heterozygous for the p genes can be employed: respiration-deficient diploids do not sporulate.

A study was undertaken to determine if the inability of p_3 strains to retain the cytoplasmic factor is a property of the locus in question, or due to unspecific, unlinked genes. A hybrid, $p_3\ P_5\ \rho^- \times P_3\ p_5\ \rho^+$, was constructed in order to test this by determining if the loss of the cytoplasmic factor would segregate with p_3. The results clearly indicated that ρ^- segregates with p_3 and that $p_3\rho^-$ is epistatic to $p_5\ \rho^+$. (The six tetrads analyzed gave PD:NPD:T frequencies of 0:1:5, with five $p_3\ P_5\ \rho^-$, five $P_3\ p_5\ \rho^+$, and seven $p_3\ p_5\ \rho^-$ segregants.)

The inability of p_3 strains to be complemented by vegetative mutants was further investigated to determine if this property is absent early in the formation of the strain, or is a result of an early loss during the growth of the germinated spore. In this experiment, a heterozygous diploid strain, p_3/P, was sporulated and several asci were dissected. Each spore was observed microscopically until it gave rise to a clone of about 15 to 20 cells. At this time the clone was divided into three groups of about six cells with the aid of a micromanipulator; the first and second groups were mixed with several a and α neutral vegetative cells respectively, and the last group was allowed to grow in order to test its genotype. Thus complementation would be said to occur when any respiration-sufficient diploid cells arose in the first or second group, if the original segregant was determined to be p_3. The three asci studied in this manner were found to contain two p_3 segregants each, all complementing vegetative mutants. After the p_3 segregants had grown sufficiently to be tested in a mass culture (about 25 generations), the p_3 segregants could no longer be complemented. These three asci also showed 2:2 segregation for mating type,

TABLE 3

Properties of the nine segregational mutants

	Percent p^- cells*	Centromere linkage data			
		Number of tetrads analyzed	Percent second-division segregation	Centromere linkage	Chromosome†
p_1	55	18	16.7	+	VIII
p_2	99.9	22	68.2	—	X
p_3	100	6	50	—	VIII
p_4	40	41	51.2	+	
p_5	1	36	63.9	—	
p_6	1	115	81.6	—	
p_7	5	38	58.0	—	
ly_6	100	9	89	—	
ly_8	100	8	88	—	

* The approximate percent of p^- cells (doubles) in the most stable segregant of each segregation mutant.

† p_1, p_2, and p_3 localized by HAWTHORNE and MORTIMER (1960 and private communication).

and other markers. One can therefore say that the p_3 strains lose their cytoplasmic factor after five to 25 generations.

Lysine-requiring segregational mutants (ly_6 *and* ly_8): Two mutants were observed by DR. R. K. MORTIMER (private communication) to have both a requirement for lysine and an inability to grow on glycerol medium. This pleiotropic phenotype segregated 2:2 in about 50 asci. These results were confirmed in the present investigation.

The ly_6 and ly_8 genes proved to be nonallelic for their lysine requirements, but as expected, could not complement their respiratory deficiencies, since as mentioned above, they were always observed to be ρ^-.

Are the inability to grow on glycerol medium and the lysine requirement two properties of a single gene, or properties of closely linked genes? If the cytoplasmic factor were not involved, this question could be answered simply by determining if both properties revert simultaneously in back-mutants for one of the characters. Thus a revertant of ly_8 from lysineless medium could be represented as (1) $LY_8\ p\ \rho^-$ or (2) $LY_8\ (P)\ \rho^-$, where (1) indicates two genes and (2) a single gene. In both cases, the revertant would be respiration- deficient, inasmuch as they are ρ^-. However, the two cases can be distinguished by crossing the revertant to wild type and determining if p segregates. The analysis of 12 tetrads from a hybrid cross of a lysine-independent ρ^- strain obtained from ly_8, showed all 48 segregants to be $P\ \rho^+$. Five of these tetrads were further analyzed and proved to be LY, and to show 2:2 segregation for several markers. The simplest conclusion is that the ly_8 gene controls both the lysine requirement and the p phenotype.

A similar study with ly_6 was complicated by the fact that no true back-mutant for lysine independence was found. When a cross involving a lysine revertant from ly_6 was analyzed, irregular segregation for lysine (LY:ly) was observed with the frequency of: two, 2:2, seven, 3:1; and one, 4:0. All 29 LY segregants were P, and all 11 ly_6 segregants were simultaneously p. One can therefore conclude that the lysine "revertant" was probably due to a mutation of an unlinked suppressor gene which segregated with the PD:NPD:T frequencies of 1:2:7, and that this suppressor acts simultaneously on ly_6 and p. This also suggests, as in the case of ly_8, that the lysine requirement and the p phenotype is under the control of a single ly_6 gene. Since ly_6 and ly_8 have always been observed to be ρ^-, it may be that a specific lysine requirement causes a high rate of mutation to ρ^-. However, other lysine loci have not been observed to have this effect.

Tests of allelism of p *segregants, and linkage:* Various segregational and vegetative mutants of opposite mating types were intercrossed to provide diploid strains containing all pairwise combinations of the nine p genes. The diploid cultures were subsequently tested for growth on glycerol medium in order to determine allelism and the presence of the cytoplasmic factor. The results, given in Table 4, clearly show that each $p\ \rho^+$ strain complements every other $p\ \rho^+$ and $p\ \rho^-$ strain, and therefore, indicate nonallelism. Tests of allelism performed among p_3, ly_6 and ly_8 strains were not informative inasmuch as these strains

TABLE 4

Tests of "allelism" of the nine segregational mutants by growth of diploids on glycerol medium. No combinations of genes tested were found to be closely linked. The symbol ⊕ in the upper right-hand grid indicates the combination tested either directly by recombination or indirectly by linkage to other genes

	Mating type α									
Mating type a	$P\ \rho^-$	$p_1\ \rho^+$	$p_2\ \rho^+$	$p_3\ \rho^-$	$p_4\ \rho^+$	$p_5\ \rho^+$	$p_6\ \rho^+$	$p_7\ \rho^+$	$ly_6\ \rho^-$	$ly_8\ \rho^-$
$P\ \rho^-$	—	+	+	—	+	+	+	+	—	—
$p_1\ \rho^+$	+	—	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕
$p_2\ \rho^+$	+	+	—	⊕	+	+	+	+	+	+
$p_3\ \rho^-$	—	+	+	—	+	⊕	+	+	—	—
$p_4\ \rho^+$	+	+	+	+	—	⊕	⊕	⊕	+	+
$p_5\ \rho^+$	+	+	+	+	+	—	⊕	⊕	+	+
$p_6\ \rho^+$	+	+	+	+	+	+	—	⊕	+	+
$p_7\ \rho^+$	+	+	+	+	+	+	+	—	+	+
$ly_6\ \rho^-$	—	+	+	—	+	+	+	+	—	—
$ly_8\ \rho^-$	—	+	+	—	+	+	+	+	—	—

lack the cytoplasmic factor necessary for growth on glycerol medium. However, it has been shown that $ly_6\ \rho^-$ and $ly_8\ \rho^-$ strains are nonallelic for their lysine requirement. Moreover, the p_3 mutant, since it is lysine independent, possibly also contains a mutant gene which differs from that of ly_6 and ly_8. It can therefore be said that (with the possible exception of p_3, ly_6 and ly_8), the nine segregational mutants are nonallelic.

As many recent investigations have shown, nonallelism, as determined by complementation, is not necessarily indicative of different gene loci. Although no complete study was undertaken to determine if any of the nine *p* genes were indeed complementing alleles of the same locus, the analysis of crosses indicated no close linkage between p_3 and p_5, or between p_4, p_5, p_6, and p_7 in all pairwise combinations. There is also indirect evidence for nonlinkage of other *p* genes by the localization of these genes with respect to a known marker or centromere.

HAWTHORNE and MORTIMER (1960 and private communication) have shown that p_1 and p_3 are on chromosome VIII and that p_2 is on chromosome X, and that only p_1 is closely linked to its centromere. In the present investigation, the results of tetrad analyses with segregational mutants were examined in order to determine possible linkage. For this study, centromere linkage was determined from second division segregation frequencies which were approximated by the use of markers known to be closely linked to the centromere. The approximate second division segregation frequencies of the nine *p* genes, obtained with the centromere marker tr_1 and/or other centromere markers (HAWTHORNE and MORTIMER 1960), are given in Table 3. One can see that p_1 and p_4 are the only genes which show centromere linkage by presenting a second division

segregation frequency significantly less than 0.667 with a level of significance less than 0.1 percent and 4 percent respectively. Also p_6 shows a second division segregation frequency significantly *greater* than 0.667, indicating that the gene may be separated from its centromere by an interval of about 45 to 75 units (cf. HAWTHORNE and MORTIMER 1960). A summary of the linkage thus far found for the nine *p* genes from the present investigation and from the investigations of HAWTHORNE and MORTIMER (1960 and private communication) is given in Table 3.

These linkage results exclude the possibility that complementation among some of the *p* genes is due to interallelic complementation. For example, p_1, since it has been found to be closely linked to its centromere, probably occupies a locus different from other *p* genes. The combinations of genes which are excluded from being complementing alleles, either directly by recombination, or indirectly by linkage relationships, are given in Table 4.

DISCUSSION

Most likely, a number of unrelated lesions cause yeast to be respiration deficient and unable to utilize nonfermentable carbon sources. The large number of such variants encountered in the present investigation may reflect this complexity.

The simplest explanation for respiratory deficiency in strains containing the genes p_3, ly_6 and ly_8 is that these genes control the retention or synthesis of the cytoplasmic factor. However, the situation may be more complicated in view of the results with p_2 in which most, but not all segregants were ρ^-. It is possible that conditions or modifying genes can be found in which these three genes will give rise to respiration-deficient mutants which still retain the cytoplasmic factor.

Since p_3 strains do not differ from vegetative mutants with respect to their "suppressiveness" (SHERMAN and EPHRUSSI 1962)—i.e., they do not have a higher degree of suppressing and cytoplasmic factor when crossed to normal strains—one cannot *simply* say that the p_3 gene is responsible for the active destruction of the cytoplasmic factor. A possibility is that gene products are necessary for the development of this factor, with some of these products being closely associated with lysine metabolism.

Although unrelated, the genes which control the retention or synthesis of the cytoplasmic factor are reminiscent of the *k* gene which controls the maintenance of kappa in Paramecium (cf. SONNEBORN 1959).

The respiration-deficient variants which still retain the cytoplasmic factor most likely have their lesions at other sites. A more complete discussion will be given in a subsequent publication, in the light of the physiological and biochemical differences found among these variants (SHERMAN and SLONIMSKI 1963).

SUMMARY

A genetic investigation was undertaken of nine nonallelic genes which control the ability of yeast to grow on nonfermentable carbon sources. No close linkage was found among all combinations of genes that were tested. Some of the variants (p_3, ly_6, ly_8) can be considered to have genes controlling the retention or synthesis of the cytoplasmic factor. Two of these (ly_6 and ly_8) have a simultaneous requirement for lysine.

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