EVIDENCE FOR THE GENETIC CONTROL OF PHOTOREACTIVATION¹

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PHOTOREACTIVATION is the reversal by near-ultraviolet or visible light of ultraviolet radiation damage to biological systems (JAGGER 1958). This phenomenon has been demonstrated in the intact cell in a spectrum of organisms (extending from bacteria to mammals) (JAGGER 1958) and *in vitro* with ultraviolet-inactivated transforming factor (deoxyribose nucleic acid) of *Hemophilus influenzae* (GOODGAL, RUPERT, and HERRIOTT 1957; RUPERT, GOODGAL, and HERRIOTT 1958). Photoreactivation of the above factor following ultraviolet treatment proceeds only in the presence of cell-free extracts which thus far may be obtained from either *Escherichia coli* (RUPERT *et al.* 1958) or baker's yeast (RUPERT, *in press*). The activity of these extracts resides in a cellular component, probably an enzyme, present in some organisms while absent in others. One implication of the above *in vitro* experiments, when coupled with the observed variability or lack of photoreactivation within species or families of microorganisms (BELLAMY and GERMAIN 1955; STUY 1956; GOUCHER and KOCHOLATY 1957; GOODGAL *et al.* 1957), is that the photoreactivation mechanism in the intact organism is under genetic control. Direct evidence for the genetic control of photoreactivation is presented in the present study on the photoreactivation of ultraviolet-induced extrachromosomal mutations (producing respiratory deficiency) in yeast.

Genetic nomenclature of ultraviolet-induced respiration deficient mutants: The induction of respiration deficient mutants of yeast by ultraviolet and the photoreactivation of this mutation have been previously described (RAUT 1954; PITTMAN 1956; SARACHEK 1958; PITTMAN, RANGANATHAN, and WILSON 1959). These mutants are phenotypically similar to spontaneously derived respiration deficient variants in their inability to (1) consume significant quantities of oxygen (WHELTON and PHAFF 1947), (2) grow on an obligate aerobic substrate such as lactate (OGUR and ST. JOHN 1956), and (3) reduce triphenyl tetrazolium chloride (OGUR, ST. JOHN, and NAGAI 1957). Genetic analyses (RAUT 1954; PITTMAN 1959) show that the majority (about 95 percent) of respiration deficient mutants derived from ultraviolet irradiated haploid stocks fall into three classes, each having been previously described by EPHRUSSI and co-workers (CHEN, EPHRUSSI, and HOTTINGUER 1950; EPHRUSSI 1953): (1) petite mutants, the principal class, which result from the loss or functional inactivation of extrachromosomal determinants; (2) genic (or segregational) mutants which carry the recessive allele of a gene controlling respiration; and (3) double mutants

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which carry both of the above defects. Genic and double mutants are rarely encountered (less than one percent) among irradiated survivors and only after high doses of radiation; hence, their frequency does not significantly bias the mutation-dose curves (Figure 3a) generally used to describe the induction of petite mutants. Double mutants have not been detected spontaneously in our normal stocks over a period of three years, although they occur with a spontaneous frequency of about five percent in most genic stocks. The remaining (about five percent) mutants induced by ultraviolet consist of heterogeneous colonies which usually revert to wild type on subculturing (PITTMAN 1959).

Descriptive genetic analyses of the petite, genic, and double mutants are presented elsewhere (CHEN *et al.* 1950; RAUT 1954; PITTMAN 1959), and only the genetic nomenclature employed in the present study need be considered: Normal stocks and the three mutant types are designated: $r^+\gamma^+$ (normal); $r\gamma^+$ (genic); $r^+\gamma$ (petite); and $r\gamma$ (doubly defective), where r^+/r denote the gene pair controlling respiration, and γ^+/γ denote the presence (absence) of the extrachromosomal factor. Use of the latter symbols is justified on the grounds that firstly, the extrachromosomal factors appear to carry their own genetic information (EPH-RUSSI 1958; PITTMAN 1959), and secondly, the defect may reflect a functional inactivation, rather than physical loss, of the extrachromosomal factor.

Method for distinguishing the three types of respiration deficient mutants: The above three mutant types are phenotypically indistinguishable when oxygen consumption, growth on lactate, and reduction of tetrazolium chloride are employed as the diagnostic tests for respiratory deficiency (PITTMAN 1959). The mutants can be readily identified, however, by the method outlined in Figure 1. This method utilizes the fact that when the three mutant types are crossed in all combinations on glucose nutrient agar, only diploids of $r^+\gamma \times r\gamma^+$ constitution are respiration sufficient and capable of growth on lactate, the hybrids of the remaining combinations being respiration deficient and incapable of growth on

FIGURE 1.—Method for identifying the different respiration deficient mutants. α/α denote the mating type gene pair; r^+/r , the gene pair controlling respiratory competence; and γ^+/γ , the presence (absence) of the functional extrachromosomal factor. Dotted lines designate the absence of growth; closed lines, luxuriant growth.

lactate. Thus, to classify mutants of unknown constitution, a sample of each mutant is first "spotted" on glucose nutrient agar and then mixed individually with $r^+\gamma$, $r\gamma^+$, and $r\gamma$ tester strains of appropriate mating type, as shown in Figure 1. After 24 hours, the mating mixtures (containing about 30 percent diploids) are replica plated, along with the parents, on lactate nutrient agar. A mutant may be classified on the basis of which (if any) hybrid grows on lactate (for example, mutant 30.3—"x" in Figure 1—was diagnosed as $r\gamma^+$). Alternatively, the above glucose plates may be overlaid with triphenyl tetrazolium agar to determine the presence of respiration sufficient hybrids. A team of two workers can classify several hundred mutants within a week by this procedure. It is evident that the method also provides a means whereby new mutants may be uncovered, and we have thus far uncovered two nonallelic mutants in some 3,000 colonies analyzed. (The general application of this method and its limitations with respect to "suppressive" mutants will be discussed elsewhere).

Origin of extrachromosomal mutants: Respiration deficient genic mutants are of singular interest since it can be shown with these stocks that the extrachromosomal factor associated with normal cells is transmitted in the presence of the recessive allele of the r gene and, consequently, independently of the inherent ability (inability) of the cell to respire. In 17 parallel plating experiments of a normal culture (8256) and a genic mutant (8256.27), 5.3 percent (181/3398) of the colonies arising from the former culture were $r^+\gamma$ and 8.3 percent (277/3328) of the colonies arising from the latter culture were $r\gamma$. These results and those obtained with 20 of 22 additional ultraviolet induced mutants indicate that the mutant frequency is slightly higher in genic strains than in the respective parental stocks. Transmission of the extrachromosomal trait in genic stocks has been previously noted by CHEN *et al.* (1950), and our studies simply serve to extend their findings. The essential point is that extrachromosomal mutants may be either $r^+\gamma$ or $r\gamma$, depending on whether they originate from normal or genic stocks respectively.

Preliminary experiments indicated that extrachromosomal mutants were induced with comparable frequencies in normal and genic stocks irradiated with intermediate doses of ultraviolet (20 percent survival). However, in contrast to previous results with normal stocks, the mutation in genic stocks was nonphoto-reactivable. This phenomenon was studied quantitatively by a method permitting a direct enumeration of extrachromosomal mutants in both normal and genic stocks.

MATERIALS AND METHODS

Stocks: The principal cultures employed were adenine-dependent (*ad-1*) haploids 8256 ($r^+\gamma^+$), 8282 ($r^+\gamma^+$), 8256.1, 8256.27, 8256.30, 8282.1 (all $r\gamma^+$ mutants derived by ultraviolet irradiation of the preceding parental stocks); adenine-independent (*AD*) haploids 18971 ($r^+\gamma^+$) and 18971.2 ($r\gamma^+$); and $r^+\gamma$, $r\gamma^+$, and $r\gamma$ haploid "tester" strains of α and a mating types. The spontaneous frequency of extrachromosomal mutants in the above stocks never exceeded eight percent in experiments carried out over a two-year period.

Media: Glucose nutrient agar contained: peptone, 3.5 gm; KH_2PO_4 , 1 gm; $(\text{NH}_4)_2\text{SO}_4$, 1 gm; $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$, 1 gm; Difco dried yeast extract, 5 gm; dextrose, 10 gm; agar, 25 gm; and distilled water, 1000 ml. Lactate nutrient agar was prepared by substituting 18 ml of 50 percent sodium lactate for the glucose in the above recipe. Tetrazolium overlay agar was prepared as previously described (OGUR *et al.* 1957).

Irradiation and plating procedure: Cultures were grown aerobically for five days on glucose nutrient agar, harvested, washed three times in M/15 KH_2PO_4 , and resuspended in this menstruum to a concentration of 10^6 cells/ml. Thirty ml of the suspension were transferred to a petri dish bottom and exposed to ultraviolet radiation (95 percent 2537 Å) delivered from a Hanovia quartz envelope lamp housed in a dark chamber and centered 56 cm above the surface of the cell suspension. Radiation was delivered at a rate of 5.1 ergs/mm²/sec at the surface of the cell suspension, as determined by a Westinghouse photometer. A "Solar" constant voltage transformer was employed to minimize fluctuation in the line voltage. A magnetic stirrer was employed to insure homogeneity of cell suspensions during irradiation. Control and irradiated samples were diluted when necessary, and appropriate aliquots (100–125 viable cells) spread on the surfaces of glucose nutrient agar plates. From ten to 20 plates were employed in each experiment at each dosage level. Plates were incubated for 3–4 days, after which colony counts were made and the number of the mutants scored. In photoreactivation (PR) experiments, dilution tubes (200 mm × 150 mm) containing control and irradiated suspensions were slanted on a rotating platform centered nine inches below a 500 watt (3200 K) projection bulb in a Fresnel photospot lamp. A glass trough containing one inch of 0.2 N CuSO_4 was interposed four inches below the spot lamp. The temperature during this treatment was maintained at 30°C ($\pm 1^\circ\text{C}$) by the addition of 0.2 N CuSO_4 ice cubes to the trough and by an electric fan. Yellow light was employed throughout the experiments to facilitate pipetting and plating. Under the above conditions, maximum reversal of both killing and mutation was obtained with 60 min of "reactivating light" (hereafter designated as "PR light").

Mutation frequencies and survival ratios are based on from three to six independent experiments involving from 1000 to 5000 colonies at each dosage level. Approximately 95 percent of the mutants derived from irradiated normal stocks exhibited the stable respiration deficient phenotype on successive transfers on glucose; the remainder produced variegated or sectorial colonies when overlaid with tetrazolium agar and were not included in calculation of mutant frequencies.

Conventional manometric procedures were employed for the determination of respiration and aerobic fermentation of cells. The reaction vessels contained 2.2 ml of yeast suspension (5×10^7 cells/ml); 0.5 ml of 6 percent glucose (or phosphate buffer) in the side arm; and 0.3 ml of 10 percent KOH (or buffer) in the center well.

Direct enumeration of extrachromosomal mutants in normal and genic stocks: The rationale of the method for scoring extrachromosomal mutants in normal and genic respiration deficient stocks is based on our own and previous observa-

tions (REAUME and TATUM 1949; TAVLITZKI 1951) that adenine-dependent (*ad-1* or *ad-2*) respiration sufficient yeast form a pink-red pigment when streaked on one percent glucose nutrient agar, whereas the petite ($r^+ \gamma$) mutants derived from these strains form little or no pigment on the same medium. In the present studies, we observed that genic ($r \gamma^+$) mutants derived from pink *ad-1* stocks consistently produce a light brown pigment on one (or two) percent glucose nutrient agar. When normal and genic stocks were plated on the above medium, from one to eight percent of the colonies were white. Although phenotypically indistinguishable, the white colonies derived from normal and genic stocks were genetically different, as shown by crossing each with the three respiration deficient "tester" strains (Table 1). Of 842 mutants tested, those derived from normal stocks produced wild type diploids when crossed to $r \gamma^+$ mutants *only*, whereas those mutants derived from genic stocks produced no respiration deficient diploids when crossed to each of the three tester strains. The white colony mutants derived from normal and genic stocks were therefore $r^+ \gamma$ and $r \gamma$, respectively. The genetic constitution of the white mutants from each culture was confirmed by tetrad analysis (PITTMAN 1959), the results of which are in accord with previous genetic studies (CHEN *et al.* 1950) of petite and genic mutants.

The possibility that the white mutants carried not only an extrachromosomal defect but also one or more recessive alleles of adenine genes (other than *ad-1*) controlling pigmentation (ROMAN 1956) was tested by crossing each of ROMAN's adenine (*ad-1-ad-7*) mutants to the following cultures: parental stocks 8256 *ad-1* and 8282 *ad-1*; ten spontaneous and ten ultraviolet derived white isolates of the preceding parental stocks; ten spontaneous and ten ultraviolet derived white isolates of genic mutants 8256.27 and 8282.3. On the basis of ROMAN's "spot" test for allelism (ROMAN 1956), all of the 80 white mutants and their respective parents were found to carry a single recessive gene allelic to the *ad-1* mutant of ROMAN's strains. Consequently, it may be inferred that the majority of

TABLE 1

The respiratory phenotype of diploids derived by crossing spontaneous and ultraviolet derived white colony isolates of normal ($r^+ \gamma^+$) and genic ($r \gamma^+$) stocks to respiration deficient tester strains

Number of isolates tested	Origin	Crossed to tester strains:			Inferred genetic constitution of isolates
		$r \gamma$	$r \gamma^+$	$r \gamma$	
26 white	8256 ($r^+ \gamma^+$)-s	—	+	—	$r^+ \gamma$
425 white	8256 ($r^+ \gamma^+$)-UV	—	+	—	$r^+ \gamma$
29 white	8256.27 ($r \gamma^+$)-s	—	—	—	$r \gamma$
183 white	8256.27 ($r \gamma^+$)-UV	—	—	—	$r \gamma$
78 white	8282.3 ($r \gamma^+$)-UV	—	—	—	$r \gamma$
101 brown*	8256.27 ($r \gamma^+$)-stock	+	—	—	$r \gamma^+$

+ (—) = Respiration sufficient (respiration deficient).

s = Spontaneous.

UV = Ultraviolet derived.

* Parental type (brown) colonies of 8256.27 $r \gamma^+$ picked at random.

white mutants arising spontaneously and following ultraviolet radiation carried the *ad-1* genotype of their respective parents.

Back mutants at the *ad-1* locus form white rather than pigmented colonies; however, their frequency was less than one per 10^6 *ad-1* cells plated (with or without radiation) on adenineless-synthetic agar. These back mutants would be expected to comprise a negligible proportion of the number of spontaneous and induced white colony mutants, and experimentally no back mutants were detected in over 4000 ultraviolet derived white colony mutants replica plated on adenineless-synthetic agar.

The concomitant loss of pigmentation and the extrachromosomal factor in the above adenine-dependent cultures permitted a direct enumeration of extrachromosomal mutants present in normal and respiration deficient genic stocks. In most experiments, however, the genetic constitution of the white mutants was confirmed by crossing from 30 to 100 white isolates to each of the three respiration deficient tester strains.

RESULTS

Figures 2a and 2b present the ultraviolet survival curves of normal ($r^+\gamma^+$) and mutant ($r^+\gamma$, $r\gamma^+$, and $r\gamma$) cultures plated without photoreactivation (D) and after maximum photoreactivation (PR). The percentage PR was approximately the same for each culture, although the mutants were generally more sensitive to killing than normal cells when plated in the dark. From these and previous results (PITTMAN and PEDIGO 1959), it would appear that the respira-

FIGURE 2.—Ultraviolet survival curves of normal ($r^+\gamma^+$) yeast cells and of the three respiration deficient mutants, with and without maximum photoreactivation. (2a) normal ($r^+\gamma^+$) and petite ($r^+\gamma$) stocks; (2b) genic ($r\gamma^+$) and double mutant ($r\gamma$) stocks.

tory enzymes and cytochrome complement present in normal cells but lacking in the mutant type (SLONIMSKI and EPHRUSSI 1949) play no obligate role in photoreactivation of killing in yeast. Similar studies (LATARJET and BELJANSKI 1956) have shown that ultraviolet inactivation of a cytochrome and porphyrin deficient mutant of *E. coli* is photoreactivated to the same degree as that of the normal, wild type strain.

Characteristic dose-response curves for extrachromosomal (r^+y) mutants induced in normal cultures plated without photoreactivation and after maximum photoreactivation are shown in Figure 3a. The mutant frequency was reduced by roughly one half by PR light for doses greater than 100 sec. Since inactivation is photoreactivated to the same degree in both normal and r^+y cultures (Figure 2a), and since the r^+y mutant is not differentially killed by PR light (PITTMAN *et al.* 1959), the reduction in mutant frequency by PR light can be ascribed to the reversal of the mutational process producing the extrachromosomal defect.

The corresponding dose-response curves for extrachromosomal (ry) mutants induced in the genic stock plated without PR and after maximum PR are shown in Figure 3b. No significant decrease in mutant frequency in genic stocks occurred following PR light treatment. The mutant frequency (with or without PR) increased, however, more rapidly after 300 sec of ultraviolet than did the mutant frequency in normal stocks (Figure 3b). The similarity of the curves in Figure 3b cannot be ascribed to differential PR of killing in genic and ry cells since the survival curves for both types following PR were essentially the same

FIGURE 3.—Ultraviolet mutation-dose response curves for extrachromosomal mutations induced in normal (r^+y^+) and genic (ry^+) stocks, with and without maximum photoreactivation.

(Figure 2b). Similar results (summarized in Table 2) were obtained with four additional genic mutants, including one adenine-independent culture (18971.2 $r^+ \gamma^+$). The mutant frequencies given for the latter culture and its parents (18971 $r^+ \gamma^+$) were determined by crossing a minimum of 250 mutants to each of the three respiration deficient tester strains. It is evident that lack of photoreactivation of the extrachromosomal mutation is a characteristic of $r^+ \gamma^+$ stocks, whether genotypically *AD* or *ad-1*.

Previous experiments had shown that PR mutation curves, obtained with 0.2 N, 0.4 N, and 0.8 N CuSO_4 as the filter, were not statistically different. PR survival curves, however, were slightly higher with 0.2 N CuSO_4 , although this concentration permits passage of considerable infrared radiation. A repeat of the above experiments with $r^+ \gamma^+$ and $r \gamma^+$ stocks confirmed the above results and show that the difference in the photoreactivable trait of the strains cannot be ascribed to the 0.2 N CuSO_4 filter routinely used. Although the shape of the mutation curves, with and without PR, changes with ageing of cultures (PITTMAN 1959), we observed no photoreactivation of mutation in $r \gamma^+$ stocks grown aerobically for 1, 3, 6 and 8 days prior to irradiation. (The authors are indebted to DR. B. RANGANATHAN for aid in many of the above experiments which, although not tabulated here, are available on request.)

The possibility that the observed lack of photoreactivation of the extrachromosomal mutations in genic mutants reflected a differential killing by PR light of genic and $r \gamma$ mutant cells could not be demonstrated with singly or artificial mixtures of the two cell types exposed to PR light for as long as 110 minutes. Furthermore, no significant decrease in mutant frequency in irradiated genic stocks was noted in photoreactivation dose-rate experiments (Figure 4) extending over a period of 100 minutes. Under similar conditions, the mutant frequency in normal stocks, after a short lag, dropped sharply with increasing dose of light until maximum PR was obtained (Figure 4). It is concluded, therefore, that the

TABLE 2

Photoreactivability of the mutational damage producing extrachromosomal mutants in three normal ($r^+ \gamma^+$) haploid stocks and four ultraviolet derived genic ($r \gamma^+$) mutants

Culture	Mutant induced	Percent mutants* Dose†			
		Zero		300 sec.	
		D	PR light	D	PR light
8256 $r^+ \gamma^+$	$r^+ \gamma$	0.66	1.98	36.1	15.1
8282 $r^+ \gamma^+$	$r^+ \gamma$	0.47	0.45	39.5	12.5
8256.1 $r \gamma^+$	$r \gamma$	0.93	0.92	41.9	36.3
8256.30 $r \gamma^+$	$r \gamma$	2.57	2.72	45.6	58.2
8282.3 $r \gamma^+$	$r \gamma$	0.81	0.70	48.5	52.5
18971 $r^+ \gamma^+$	$r^+ \gamma$	1.01	1.21	39.2	13.6
18971.2 $r \gamma^+$	$r \gamma$	0.69	0.66	34.1	38.5

* Mutant frequencies for each culture are based on a minimum of 2200 colonies, except for stocks 18971 and 18971.2 (see text).

† Ultraviolet dose, 1.5×10^8 ergs/mm²; D, without photoreactivation; PR light, after exposure to photoreactivating light for 60 min.

FIGURE 4.—Photoreactivation of extrachromosomal mutations induced in normal (r^+y^+) and genic ($r y^+$) stocks. Cells were irradiated for 400 sec and then exposed to PR light for the time intervals shown.

extrachromosomal mutation is photoreactivated in normal stocks but not in the genic mutant.

Photoreactivation of the extrachromosomal mutation in diploids homozygous dominant, heterozygous, and homozygous recessive for the r gene pair is shown in Table 3. In these experiments, four day-old cultures were harvested, washed, and irradiated with 300 (or 400) sec of ultraviolet and plated with and without exposure to PR light (60 min). PR light reduced the mutant frequency in the homozygous dominant (A) and heterozygous (B) diploids, but had no effect on the mutant frequency in the homozygous recessive (C) diploids. The genetic constitution of the mutants derived from the above diploids could not be determined since they were incapable of either sporulation or mating, the latter defect presumably due to heterozygosity for the mating type gene. However, similar studies (PITTMAN, COKER and PERRY, unpublished data) with diploid yeasts (strains 11294 and 11296) capable of mating with the three respiration deficient tester strains indicate that the majority of respiration deficient mutants induced by ultraviolet in diploid and polyploid yeasts result from extrachromosomal

TABLE 3

Photoreactivability of the ultraviolet damage producing extrachromosomal mutations in diploids homozygous dominant (r⁺r⁺), heterozygous (r⁺r), and homozygous recessive (r r) for the gene controlling respiration and of the segregants of four tetrads of heterozygote (B) and one tetrad of the dominant homozygote (A)

Culture	Mutant induced	Dose*							
		Dark		Zero		PR light		300 sec	
Diploids	Exp:	1	2	1	2	1	2	1	2
(A) 8256 r ⁺ γ ⁺	r ⁺ r ⁺ γ ⁺ †	2.5	1.4	1.9	1.0	24.9	26.9	11.0	8.6
×									
8282 r ⁺ γ ⁺									
(B) 8282 r ⁺ γ ⁺	r ⁺ r γ ⁺ †	1.4	1.7	0.7	1.3	40.0	37.6	12.1	15.1
×									
8256.27 r γ ⁺									
(C) 8282.3 r γ ⁺	r r γ ⁺ †	2.6	1.2	2.9	2.7	48.8	45.2	49.8	47.5
×									
8256.27 r γ ⁺									
Ascospore segregants of diploid (B)‡									
1a r γ ⁺	r γ	1.0	2.6	1.4	2.8	38.1	34.0	38.2	39.3
1b r ⁺ γ ⁺	r ⁺ γ	1.9	1.2	1.1	1.6	37.7	32.1	12.5	13.8
1c r ⁺ γ ⁺	r ⁺ γ	1.6	0.9	1.2	1.2	34.2	36.3	15.1	15.9
1d r γ ⁺	r γ	0.8	1.0	0.5	0.8	36.6	40.2	43.3	39.0
2a r γ ⁺	r γ	1.7	1.5	1.4	2.0	55.5	48.0	65.0	58.3
2b r ⁺ γ ⁺	r ⁺ γ	5.5	4.7	6.6	5.7	34.4	31.5	13.1	11.0
2c r ⁺ γ ⁺	r ⁺ γ	1.4	1.6	1.4	2.2	36.2	33.3	10.4	13.3
2d r γ ⁺	r γ	2.2	1.2	1.3	1.0	45.9	50.0	44.0	46.7
3a r γ ⁺	r γ	8.2	7.1	6.5	4.4	57.1	64.2	61.0	52.0
3b r γ ⁺	r γ	5.6	6.0	3.9	5.5	58.7	55.8	64.8	57.7
3c r ⁺ γ ⁺	r ⁺ γ	5.5	4.8	4.1	3.4	40.4	44.4	18.1	17.7
3d r ⁺ γ ⁺	r ⁺ γ	3.9	3.4	3.7	2.2	42.3	37.7	15.9	14.1
5a r ⁺ γ ⁺	r ⁺ γ	2.5	1.2	2.0	1.9	44.8	41.4	26.2	16.6
5b r γ ⁺	r γ	0.9	1.5	0.4	1.2	49.0	43.9	52.4	55.1
5c r γ ⁺	r γ	0.8	1.8	0.7	1.5	51.6	47.6	48.8	49.9
5d r ⁺ γ ⁺	r ⁺ γ	1.2	1.8	1.1	1.4	39.2	38.8	13.0	11.8
Ascospore segregants of diploid (A)									
1a r ⁺ γ ⁺	r ⁺ γ	1.3	...	0.9	...	44.0	...	16.1	...
1b r ⁺ γ ⁺	r ⁺ γ	0.4	2.6	0.9	2.8	33.2	37.7	16.6	18.1
1c r ⁺ γ ⁺	r ⁺ γ	6.0	...	5.9	...	38.8	...	14.8	...
1d r ⁺ γ ⁺	r ⁺ γ	3.9	2.4	4.6	3.0	42.2	36.3	19.2	15.5

* Ultraviolet dose, 1.5 × 10³ ergs/nm²; Dark, without photoreactivation; PR light, after exposure to photoreactivating light for 60 min.
† Inferred.
‡ Genetic constitution of segregants determined by crossing each to respiration deficient tester strains.

rather than gene mutations. We conclude, therefore, that the lack of photoreactivation of the mutation in $r\gamma^+$ mutants is expressed as a recessive trait in the heterozygote.

Each of four asci of heterozygote (B) segregated $2 r^+ \gamma^+ : 2 r \gamma^+$ (Table 4), in accord with previous genetic studies on the segregation of normal by genic hybrids (CHEN *et al.* 1950). As shown in Table 3, PR light reduced the ultraviolet-induced frequency of mutants in each of the eight $r^+ \gamma^+$ segregants, but had no effect on the induced mutation frequency in any of the eight $r \gamma^+$ segregants. Eight tetrads of a similar heterozygote ($8282 r^+ \gamma^+ \times 8256.30 r \gamma^+$) yielded results qualitatively similar to those shown in Table 3; namely, that the mutation is photoreactivable in $r^+ \gamma^+$ segregants but nonphotoreactivable in the $r \gamma^+$ segregants. Four segregants of a tetrad of homozygous dominant diploid (A) segregated $4 r^+ \gamma^+ : 0 r \gamma$. As shown in Table 3, the mutation induced in each segregant was photoreactivable. The above results show that the photoreactivable *vs.* nonphotoreactivable character of the mutation in question segregates in typical Mendelian fashion and as if under control of the r gene pair.

No unequivocal case of back mutation to wild type ($r^+ \gamma^+$) has been observed in mutants carrying the extrachromosomal defect, and repeated attempts by the senior worker to induce such mutations (detectable at a frequency of one per 10^9) by either X-rays or ultraviolet have met with failure. In contrast, 20 of 22 genic stocks thus far studied show a low spontaneous reversion frequency (about one per 10^8) to wild type (PITTMAN 1959). To determine whether the extrachromosomal mutations induced in the back mutant is photoreactivable, a back mutant ($8256.27\text{-B } r^+ \gamma^+$) of stock $8256.27 r \gamma^+$ was subjected to 400 sec of ultraviolet and then plated with and without exposure to PR light. The results (Table 4) show that the mutation induced in the back mutant is photoreactivated and to the same degree as that in the normal stock, indicating that the photoreactivable *vs.* nonphotoreactivable character associated with the r gene pair is an inherent trait of this gene rather than a closely linked gene.

GIESE and SWANSON (1947) observed that ultraviolet radiation suppressed exogenous respiration and fermentation in yeast. In the present study, restriction of the photoreactivable character of the extrachromosomal damage to respi-

TABLE 4

Photoreactivability of extrachromosomal mutations induced in a respiration-deficient genic ($r \gamma^+$) stock and a respiration sufficient back mutant ($r^+ \gamma^+$) of this culture

Culture	Exp.	Percent mutants							
		Dose							
		D*	Zero PR light	300 sec PR light	400 sec PR light	D	300 sec PR light	D	400 sec PR light
8256.27 $r \gamma^+$ (genic)	1	1.63	1.99	36.6	41.0	71.2	70.3		
	2	2.60	2.71	38.7	38.0	78.9	78.1		
8256.27-B $r^+ \gamma^+$ (back mutant)	1	0.70	1.12	32.2	14.4	48.1	21.4		
	2	1.20	1.03	36.1	15.2	46.2	22.5		

* D, plated without photoreactivation; PR, plated after exposure to 60 min of PR light.

ration sufficient stocks suggested that the photoreactivable site is the intact respiratory mechanism. However, as shown in Figure 5, suppression of either exogenous respiration or aerobic fermentation by ultraviolet is nonphotoreactivable, excluding, thereby, this effect as a plausible candidate for the photoreactivable site in question.

DISCUSSION

Interpretation of the genetic control of the above photoreactivation process at the cellular level hinges on what we know of the site and nature of ultraviolet damage producing the extrachromosomal defect. Inhibition of the intact respiratory mechanism falls short as an attractive candidate for the photoreactivable

FIGURE 5.—The effect of ultraviolet radiation on respiration (top) and aerobic fermentation (bottom) of normal ($r^+\gamma^+$) cells with and without treatment with photoreactivating light. Cells were irradiated for 4.6×10^3 ergs/mm² and equivalent aliquots (a) held in the dark for 60 min and (b) exposed to PR light for 60 min. Final substrate concentration, one percent glucose; abscissa, incubation time in Warburg reaction vessel.

site(s), since this effect was nonphotoreactivable. The ultraviolet action-spectrum curve for induction of the extrachromosomal mutants (RAUT and SIMPSON 1955) suggests that nucleic acids are some way involved in the mutational process, but whether the photoreactivable site is nuclear or cytoplasmic remains obscure. It would appear that the r^+ allele is not the photoreactivable site, since $r^+\gamma$ rather than $r\gamma^+$ (or $r\gamma$) mutants were obtained from irradiated normal cells plated without photoreactivation. Disruption, directly or indirectly, of the normal replication (or synthesis) of the extrachromosomal determinants has been postulated as the ultraviolet process subject to photoreactivation (PITTMAN *et al.* 1959). On this assumption, the following plausible hypothesis is formulated to account for the present results: Ultraviolet radiation effects (directly or indirectly) loss of the extrachromosomal factor, that damage being potentially photoreactivable. The r gene pair controls the synthesis of a cellular component, either a chromophore or one of several components (enzymes) necessary in the transfer of PR energy to the photoreactivable site. In the presence of the r^+ allele, the energy absorbed by the chromophore is transferred by a sequence of enzymatically controlled reactions to the photoreactivable site, which is subsequently "repaired." In the presence of the r allele, PR energy transfer fails to occur since the chromophore (or enzyme) is lacking, and the lesion is expressed at a rate comparable to that in cells plated in the dark. Back mutation of the r allele results in the restoration of the ability of the cell to synthesize the component necessary for PR energy transfer. Implicit in this hypothesis is the assumption that there are several steps in PR energy transfer, each under genetic control and, therefore, each presenting a potential block in the photoreactivation process. Indeed, a study of 61 ultraviolet derived genic mutants has shown that at least two genetic blocks occur to prevent photoreactivation of the extrachromosomal mutation, each compensated for in the heterozygote to restore the photoreactivable character of the mutational damage.

The relation between photoreactivation of the extrachromosomal mutation and the inherent ability of the cell to respire suggests that the cellular component necessary for photoreactivation is associated with the aerobic mechanism. This possibility is further strengthened by the fact that the extrachromosomal mutation is photoreactivable in aerobically but not anaerobically grown cells (SARACHEK 1958). Since respiration in yeast is an adaptive process (SLONIMSKI 1953), the postulated component may be adaptively synthesized in aerobically grown, normal cells but absent either in anaerobically grown, normal cells or in mutants carrying the recessive allele of the gene controlling respiration.

An obvious attack on the above hypothesis is to determine the effect, if any, of extracts of respiration sufficient stocks on photoreactivation of extrachromosomal mutations in the genic mutant. Preliminary experiments with $10,000 \times G$ supernatants of normal cell extracts have yielded promising results.

Although our experiments raise the possibility that photoreactivation mechanisms in general are under genetic control, we have shown it for a specific case only. As clearly shown here, photoreactivation of killing is not under direct control of the r gene pair. However, along this line, it is interesting to note that

several years ago DR. A. KELNER failed to show photoreactivation of killing in certain yeast strains obtained from DR. C. C. LINDEGREN. While these strains probably were not respiration deficient, it is possible they were genetically non-photoreactivable (DR. A. KELNER, personal communication).

SUMMARY

Extrachromosomal mutations in yeast affecting respiratory ability are induced by ultraviolet radiation in haploid stocks carrying either the dominant or recessive allele of a gene (*r*) controlling respiratory competence. Photoreactivation of the mutation depends on the allelic state of the gene: In stocks carrying the dominant allele *r*⁺, the mutation is photoreactivable; in stocks carrying the recessive allele *r*, the mutation is nonphotoreactivable. The photoreactivable *vs.* non-photoreactivable character of the mutation in question segregates in typical Mendelian fashion and as if controlled by the *r* gene pair. The photoreactivable trait is expressed as a dominant character in the heterozygote.

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