A GENETIC ANALYSIS OF SECTORING IN ULTRAVIOLET-INDUCED VARIANT COLONIES OF YEAST

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A VARIATION from galactose positive to galactose negative has been found in *Saccharomyces cerevisiae* that is exceptional in that it can be induced with very high frequency by ultraviolet irradiation (JAMES 1954). The variant lines often occur as sectors in otherwise phenotypically normal colonies grown from irradiated vegetative cells on galactose-EMB indicator plates. The nature of the induced change has been studied by determining the genotypes of (a) the parental strain, (b) isolates from negative (variant) sectors, (c) the associated positive sectors, and (d) phenotypically unaffected colonies occurring in the same plates.

Previous breeding tests indicated that the parent line is heterozygous for the galactose character (positive being dominant), and that the induced variant is homozygous recessive. These facts suggested that the variant arose through mutation of the dominant gene in the heterozygote (see also WINGE and ROBERTS 1950). The sectoring, which there is reason to believe is more frequent than would appear from gross examination of the colonies (JAMES 1954), could be the result of delayed mutation. It would be possible, however, to account for both the high frequency of the change and the sectoring on the hypothesis that radiation induces genetic segregations in vegetative cells. Evidence for this is presented in the following account.

MATERIAL AND METHODS

Strain 562 of *Saccharomyces cerevisiae*, used in these investigations, is a descendant of CRI which has been described elsewhere (JAMES 1954). This strain shows a lower induced variant frequency than CRI, but has been used because of the low frequency of ascospore germination in the latter. The pedigree of strain 562 will be given later in a discussion of its genetic constitution.

The galactose-EMB indicator medium and the method of irradiation were similar to those used previously in this laboratory (JAMES 1954). An ultraviolet dose of 200 ergs/mm^2 was used in all irradiations.

Determinations of genetic constitutions of variant and non-variant colonies were made with clones derived by picking, dilution and replating, followed by single colony isolation. Sex was determined by incubating with two tester stocks of opposite sex. Cultures were also incubated alone to detect any anomalous behaviour. Galactose reaction was determined by plating 50 to 200 cells on indicator medium and observing the resultant colonies.

Two mating systems were used. A mass mating technique with isolation of apparent diploids (FOWELL 1951) was used in the series of crosses leading to strain 562. All other crosses were made with the technique of mating individual cells and isolating resultant zygotes (CHEN 1950).

RESULTS

Variant cells are induced in strain 562 with an estimated frequency of 1.7 percent among cells surviving an ultraviolet dose of 200 ergs/mm^2. With this dose the proportion of variant colonies that are sectored is approximately 67 percent. A vast majority of the variant cells are stable on transfer.

The investigation consisted primarily of a comparison of the genetic constitutions of 12 sectored colonies and 12 phenotypically normal colonies produced by irradiated cells. Each sectored colony was represented by a positive (b) clone and a negative (w) clone, and each wholly positive colony by a single positive clone. The genetic constitutions of these were determined in regard to galactose, sex and lethal genes. The results obtained are summarized in table 1.

The clones were found to have a variety of aberrant genotypes; in addition to those 12 negative clones that were homozygous recessive for galactose, 11 of the 36 were heterozygous for recessive lethals, 3 were homozygous for sex, 9 were homozygous for dominant galactose genes, and one was tetraploid. Further, the positive clones from sectored colonies showed a greater number of aberrations for the galactose character than did positive clones derived from phenotypically normal colonies; seven of the clones from positive portions of sectored colonies were homozygous dominant, whereas only two of the clones from phenotypically normal colonies were aberrant. The correlation in appearance of aberrant positive and negative variant cells suggests that genetic exchange, rather than gene mutation, is the mechanism producing the variant cells. In subsequent sections this possibility is examined critically.

Heterozygous origin of sectored colonies

The classification of strain 562 as a diploid heterozygous for a single gene pair controlling the galactose character was based on its ancestry and on the results of progeny tests. The strain was obtained from a negative segregant (385) of a tenth generation inbred line of CRI. No. 385 was crossed to the positive haploid ATCC 10275. This mating was followed by first and second generation backcrosses of negative segregants to ATCC 10275, the latter resulting in strain 562. Each zygotic generation was galactose positive, and heterozygosity throughout the pedigree was inferred when segregants from three asci of the F_1 generation and from three asci of the first generation backcross produced regular 2:2 ratios for galactose and for sex.

In a progeny test of strain 562, the four segregants from each of 20 asci were tested for galactose reaction, and mating reaction was determined in the segregants from 14 of the same asci. All the segregations were completely regular.

Genetic analyses of isolates

Of the 36 clones representing the twelve sectored and twelve phenotypically normal colonies in table 1, three (2w, 5w and 18) failed to sporulate and one (2b) produced irregular segregation ratios. The analyses of these four clones will be considered later. The analyses of the remaining 32 clones were made primarily with data obtained from ascus dissections, and they will be considered as a unit. Segregation data for sex and galactose genes were obtained only from those asci that produced four spore colonies unless the clones contained recessive lethals In these cases, only two spore

TABLE 1

*Genetic constitutions of isolates from twelve sectored and twelve phenotypically normal colonies formed by irradiated vegetative cells of genotype $AaLLL_1L_1Gg$**

(Dose: 200 ergs per mm.^{2})

Genetic constitution†		No. of colonies observed	Colony number (in order of picking)
Positive sector	Negative sector		
Sectored colonies			
Aa LL Gg	*Aa LL gg*	2	9, 11
Aa Ll Gg	*Aa Ll gg*	2	1, 6
Aa LL GG	*Aa LL gg*	5	4, 7, 8, 10, 12
Aa Ll GG	*Aa Ll gg*	1	3
Aa Ll L_1L_1 GG	*aa L- L_1- gg*	1	5
AA aa LL LL GG gg	*aa L- gg*	1	2
Positive (phenotypically wild type) colonies			
Aa LL Gg	—	6	13, 15, 17, 20, 23, 24
Aa Ll Gg	—	4	14, 16, 19, 22
AA L- GG	—	1	18
Aa LL GG	—	1	21

* *A*, *a*:—sex, *L*, *l* and L_1 l_1:—lethals, *G*, *g*:—galactose.

† The genetic constitutions are from tests of a single isolate from each sector or colony and may or may not apply to the whole of the sector (or colony).

colonies per ascus could be examined. Ten asci were studied from each of the positive clones, with the exception of clone 20 which sporulated so poorly that only two complete asci could be obtained from it. Negative clones were represented by two asci if lethals were absent or by four if lethals were present.

Eleven of the 32 clones were classified in table 1 as having originated from isolates containing recessive lethals. This classification was based on the frequencies with which dissected spores produced colonies; in a total of 239 asci, not fewer than five from any one clone, none produced more than two viable spore colonies. On the other hand, 76 percent of 363 asci from clones designated free of recessive lethals produced either three or four spore colonies. One clone, 5b, was classified as heterozygous for two recessive lethals because the distribution of asci producing 0, 1, and 2 spore colonies was 19, 30, and 11.

Data on the segregation of sex genes were in close accord with the proposition that all 32 clones were normal diploids. Of the 146 tetrad analyses, 125 were completely orthodox. Of the 92 diad analyses, 84 were apparently orthodox. Only one type of deviation from regularity occurred. This was displayed by those spore cultures that exhibited a mating reaction with both tester strains. This occurred in 43 of the 768 spore cultures tested. In 40 of these a mating reaction was also observed in the unmated control cultures. Such anomalous behaviour has been attributed to spontaneous mutations for sex prior to testing (AHMAD 1952; POMPER and MCKEE 1953). Consistent haploidy of the segregants was further indicated when attempts to sporulate 428 of the spore cultures were unsuccessful in all cases.

TABLE 2

Details of segregation of galactose character in isolates of colonies formed by irradiated cells

	Genotype	No. of colonies	Type of analysis	Ratio pos.:neg. spores in ascus	No. of asci	Colony number
Sectored colonies						
Negative sectors	*gg*	7	tetrad	0:4	14	4, 7, 8, 9, 10, 11, 12
		3	diad	0:2	12	1, 3, 6
Positive sectors	*Gg*	2	tetrad	2:2	20	9, 11
		2	diad	2:0	4	1, 6
				1:1	13	
				0:2	3	
	GG	5	tetrad	4:0	50	4, 7, 8, 10, 12
		2	diad	2:0	20	3, 5
Phenotypically normal colonies	*Gg*	6	tetrad	2:2	52	13, 15, 17, 20, 23, 24
		4	diad	2:0	13	14, 16, 19, 22
				1:1	22	
				0:2	5	
	GG	1	tetrad	4:0	10	21

Scoring of the galactose character was unequivocal on the indicator medium. There was no evidence of heterogeneity in galactose reaction among the colonies of negative segregants, and among positive segregants heterogeneity was limited to three spore cultures. Even in these three instances the heterogeneity was slight, consisting of a maximum of two negative colonies among the several hundred positive colonies on test plates.

The galactose segregation data summarized in table 2 indicate that a single locus is concerned in the galactose reaction. The ten negative clones gave no indication of being other than homozygous recessive, since all 80 segregants from 26 asci were negative. Further evidence for the homozygous recessive condition of negative clones was obtained when the four negative spore cultures of an ascus of 4w were mated to positive haploids (progeny of strain 562, unirradiated). All the resultant cultures were positive.

It seems unlikely that the negative character is due to the complete loss of a locus in view of the existence of reversions which can occur, if not at the original locus, then close to it. Such reversions appear as papillae on negative colonies. Eleven reversions of independent origin segregated as heterozygotes. One of the positive segregants from a reversion of 7w was mated to a positive segregant of strain 562 (unirradiated). None of the spore cultures from eleven segregations of the resultant diploids (four from one mating, seven from the other) was negative.

The 22 positive clones fell into either of two distinct classes with respect to their segregations for galactose, one indicating heterozygosity (*Gg*), the other indicating homozygosity (*GG*). That any of the clones in this latter class were dihybrids for galactose seems most unlikely in view of the complete absence of negative spore cultures among 280 segregants.

Confirmation of the genetic difference between those clones classified as heterozygous and those classified as homozygous dominant was found in a comparison of in-

TABLE 3

Induced variant frequencies of cultures of different genotype

(Dose: 200 ergs/mm²)

Genotype	No. clones	No. irradiations	Colonies scored	Percent induced variants*
Gg	14	56	98,431	1.8
GG	8	32	74,326	0.2
G	5	17	42,178	0.1
gg	4	8	15,550	0.03

* Combined data. Percent variants by individual clones:

Gg:—1.7, 1.6, 1.9, 1.3, 1.9, 2.4, 1.7, 1.7, 1.6, 2.2, 3.4, 1.6, 2.2, 2.0

GG:—0.1, 0.4, 0.3, 0.2, 1.0, 0.2, 0.1, 0.2

G:—0.4, 0.2, 0.1, 0.1, 0.04

gg:—0.00, 0.04, 0.04, 0.02

duced variant frequencies (see table 3). The 14 assumed heterozygotes have induced frequencies similar to that of strain 562. The frequencies of the eight assumed homozygotes ranged between 0.1 and 0.4 percent with the exception of clone 3b which had an average induced variant frequency of 1.0 percent. This high frequency is unexplained although it might be attributed to the spontaneous appearance of heterozygous cells within the clone.

The actual difference between the two genotypes in induced variant frequency is undoubtedly greater than the data imply. These data are uncorrected for those colonies that were scored as variants but which were not stable on transfer. Such colonies, when replated, produce either typically positive or brownish colonies. Although the number of such colonies is low, in those cultures where the total variant count is also low their contribution to the estimated variant frequency is large. In later irradiations of homozygous diploids all colonies scored as variant were tested by replating. In these tests, of 16 variants scored among 13,120 colonies of 4b, 12b and 21, only two were stable, indicating a corrected frequency of 0.02 percent.

Data concerning the linkage relationship of sex and galactose are shown in table 4. There is no indication of linkage, the numbers of the two ditypes being 27 and 22. Linkage of lethals with sex and with galactose genes would be indicated for individual clones by inequality of 2:0 and 0:2 diad segregations. No significant indication of linkage with galactose was found. Linkage with sex was indicated in one clone (19) where the ratio of 2:0 to 0:2 segregations was 7 to 0 (significant at 1 percent level).

Clone 2b produced segregation ratios typical of a tetraploid heterozygous for galactose. Data were obtained from ten asci. Of the segregations for the galactose character, seven were 4:0, one was 3:1, and two were 2:2, positive to negative. Irregularities in the segregation of sex genes were demonstrated by the fact that 16 of the 40 spore cultures failed to give any mating reaction. Because of this, all 40 spore cultures were tested for ability to sporulate. With one exception, positive mating reactions were accompanied by failure to sporulate, whereas negative mating reactions were coincident with an ability to sporulate. These results are consistent with the supposition that 2b was tetraploid, since non-mating spore cultures with ability

TABLE 4

Absence of linkage between sex and galactose

Clone	No. of asci tested	Distribution of ascus types		
		Parental ditype (*aG, aG, Ag, Ag*)	Non-parental ditype (*AG, AG, ag, ag*)	Tetratype (*AG, Ag, aG, ag*)
Parental strain	14	3	5	6
Sectored colonies				
9b	10	1	3	6
11b	10	3	3	4
Phenotypically normal colonies				
13	10	6	2	2
15	10	4	2	4
17	10	4	2	4
20	1	0	1	0
23	10	4	1	5
24	10	2	3	5
Total	85	27	22	36

to sporulate are suggestive of heterozygosity for sex, and segregants with ability to mate could be homozygous for sex. The four non-mating segregants of one ascus were sporulated. Two asci from each segregant, when tested for galactose reaction, were found to have segregated 2:2, confirming the conclusion that 2b was a tetraploid heterozygous for galactose and demonstrating that it was free of recessive lethals.

The genetic analyses of the two negative clones 2w and 5w and of the positive clone 18 were less complete than those of the other clones because they could not be induced to sporulate. On testing for sex, 2w and 5w gave reactions indicative of sex "a" and 18 gave a reaction indicative of sex "A". In an attempt to determine the ploidy of these clones, they were mated to haploid segregants of strain 562 (unirradiated) of opposite galactose reaction. The three resulting zygotic cultures were galactose positive and sporulated abundantly. Ten asci from each mating were dissected. The matings of 5w and 18 produced no viable segregants; the mating of 2w produced 2, 1, and 0 spore colonies per ascus with frequencies 3, 1, and 6. On the basis of these results, indicating triploidy, the three original clones were classified as diploids homozygous for sex. The classification of clone 18 as homozygous for galactose was based on its induced variant frequency (0.2 percent of 9,310 colonies) which was closely comparable to the low frequency of clones known to be of that genotype.

The uninuclear origin of sectored colonies

Evidence that the sectored colonies under study were derived from single irradiated nuclei was obtained from the distribution of lethals within these colonies.

That the majority of these lethals were of irradiation-induced origin was demonstrated in a test utilizing a single culture of 9b. Twenty clones from positive portions of sectored colonies and 20 clones from phenotypically normal colonies were obtained from platings of irradiated cells. Twenty control clones were also obtained from platings of non-irradiated cells. These 60 clones were sporulated and spore germination

tests were made. A clone was designated free of recessive lethals if any dissected ascus produced three or four spore colonies, but was designated heterozygous for a recessive lethal if not more than two spore colonies could be obtained from any ascus with a minimum of eight dissections. No lethals were found in the controls, seven lethals were found in the 20 clones from sectored colonies, and seven lethals were found in 19 of the clones from normal colonies, one clone failing to sporulate. It is apparent that these lethals were irradiation-induced. And since the proportion of colonies which contained lethals was almost identical to that of the previous test (36 percent as compared to 35 percent in table 1) there can be little doubt that the lethals of that test were also irradiation-induced.

With this information, the distribution of lethals within sectored colonies (see table 1) provides good evidence that the majority of these sectored colonies were derived from single irradiated nuclei. Of the ten sectored colonies (omitting colonies 2 and 5 which could not be completely analyzed), presence or absence of a lethal in the positive portion was consistently accompanied by a corresponding presence or absence in the negative portion. If all these colonies had been formed by the seeding of two nuclei at one locality, the probability of obtaining such a distribution is less than one in 1000.

Correlated induction of homozygous dominant and homozygous recessive genotypes

The analyses of colonies from irradiated cells of strain 562 showed that more sectored colonies than phenotypically normal colonies contained homozygous dominant cells. However, the proportions (7/12 and 2/12) do not differ significantly. It was consequently considered necessary to determine whether the observed difference in proportions could be attributed to sampling error.

This was accomplished by determining the genotypes in regard to galactose, of the 60 isolates from 9b that had already been analysed for lethal genes. The isolates were tested only to provide evidence of homozygosity or heterozygosity, and the validity of the proposed genotypes was based wholly on the consistency of the segregations in more thoroughly analyzed clones. With those clones that did not contain a recessive lethal, tests were confined to segregants (either three or four) from a single ascus. With those clones that contained a lethal, paired segregants were tested. If no negative spore cultures appeared among the paired segregants of four asci, the clone concerned was classified as homozygous. If fewer than four paired segregants were available and heterozygosity remained in doubt, genotype was inferred from induced variant frequency. Ten homozygous dominant clones were found among the 20 representatives of sectored colonies, whereas none was found among the 20 representatives of control platings or among the 20 normal colonies from platings of irradiated cells. Although the tests contained no analysis for the presence of occasional tetraploids, there can be little doubt that the occurrence of homozygous dominant cells within colonies is correlated with that of homozygous recessive cells. The combined results of the two sets of analyses (involving strain 562 and clone 9b) indicate that 17 of 32 clones from positive sectors were homozygous dominant while only two of 32 clones from phenotypically normal colonies were homozygous dominant. The difference is highly significant.

Induced variant frequency of positive haploids and homozygous recessive diploids

The correlated appearance of homozygous dominant and homozygous recessive cells prompted a determination of the induced variant frequencies of positive haploids, and homozygous recessive diploids. In the case of positive haploids, ATCC 10275 (the source of the positive character in strain 562) and four segregants from an ascus of 7b were irradiated and plated. The results are included in table 3. The variant frequencies of the haploids are significantly lower than those of heterozygotes, averaging 0.1 percent. All colonies scored as variant were picked and replated. Of the 38 colonies so tested, 23 proved to be stable, indicating a corrected frequency of 0.05 percent.

In tests of the induced variant frequencies of homozygous recessive diploids, cultures of 4w, 7w, 9w, and 12w were irradiated as above. Counts were made of induced reversions among the resultant colonies. The results of these tests (see table 3) indicate that the frequency of induced reversions in homozygous recessive diploids is much lower than the frequency of homozygous dominants which appear after irradiation of heterozygotes.

DISCUSSION

The present study indicates that genetic segregations occur in yeast as a result of ultraviolet irradiation of diploid vegetative cells. It should be noted that both spontaneous and irradiation-induced segregations have been observed in the somatic cells of Drosophila (STERN 1936; WHITTINGHILL 1950), and that spontaneous segregations also occur in Penicillium and Aspergillus (PONTECORVO *et al.* 1954). Segregations, both spontaneous and irradiation induced, that may be somatic, have also been observed in exceptional heterozygotes of *E. coli* (LEDERBERG 1949; LEDERBERG *et al.* 1951).

In the present material, not only does gene reassortment supply a simple explanation of both the high frequency of variants and the high frequency of sectoring, but it also accounts for the correlated appearance of homozygous dominant and homozygous recessive cells. Further, the occurrence of a high induced frequency of homozygous recessive and homozygous dominant variants in heterozygous diploids, a low induced frequency of negative variants in positive haploids, and a low induced frequency of positive variants in homozygous recessive diploids is to be expected on such a hypothesis.

The occurrence of wholly negative colonies, and of wholly positive colonies that contain homozygous dominant cells (18 and 21), is not necessarily contrary to a hypothesis of gene segregation since the presence of such colonies can be attributed to elimination of some lines of descent from irradiated cells (JAMES 1954). Similarly, the failure to isolate complementary homozygous dominant genotypes from some sectored colonies is not critical since analyses were confined to single isolations from each sector and it may well be that some positive sectors contained a mixture of homozygous and heterozygous cells. The presence of heterozygous cells in sectored colonies does, however, imply that such exchanges sometimes occur subsequent to at least one normal mitotic division.

Although these studies have not excluded gene mutation, they have supplied information that is difficult to interpret on such a basis. Explanations for the corre-

lated appearance of homozygous dominant and homozygous recessive cells might be found in the assumptions that (1) delayed dominant mutations actually occur independently of negative variants but that selection pressures tend to eliminate them in colonies that do not contain homozygous recessive cells, or that (2) an extreme heterogeneity in sensitivity to irradiation exists between cells. The first assumption demands that dominant mutations be induced with a frequency of at least 50 percent. Evidence unfavourable to the second assumption is found in that data on induced recessive lethals. These were distributed independently of sectoring; 11 of 31 sectored colonies and 11 of 31 phenotypically normal colonies contained such lethals. Further, to explain the correlation of induced variant frequency with the heterozygous condition of cells, it would be necessary to assume either that mutation is dependent on the presence of an allele identical to the mutant allele, or that reproduction of the mutant cell is dependent on the presence of such an allele. Gene segregation, however, seems to involve less complicated assumptions.

There are several means by which reassortment could be induced. At this time the most likely of these appears to be (1) somatic crossing over or (2) disjunction of homologous chromosomes. Reciprocal chromatid translocation seems unlikely as a mechanism because those derived from random chromatid breaks would be expected to produce a heterozygous lethal condition in one sector of the colony, whereas such lethals as were found affected both sectors.

Induced meiosis also appears to be an unlikely mechanism. (1) If meiosis contributes to variant production one would expect clones of phenotype *AAGg* or *aaGg* to exhibit a low induced variant frequency since these do not ordinarily undergo sporulation. Tests with two such clones have shown that this is not the case; both exhibited variant frequencies similar to that shown by clones of genotype *AaGg*. (2) Although aberrations in cell morphology are frequent following irradiation, nothing resembling an ascus has been noted. (3) If meiosis were a factor, one might expect the occurrence of many haploid clones, and these have not been found. (4) It seems unlikely that each of the three clones, 2w, 5w, and 18, which were homozygous for sex, resulted from the fusion of haploids.

At this time it seems likely that irradiation-induced variations of high frequency are not peculiar to the locus under investigation here or to the particular strain of yeast. This is suggested by the high frequency of maltose variants in hybrids of *S. cerevisiae* and *S. Chevalieri* (WINGE and ROBERTS 1950). Also, we have noted a high frequency of induced galactose negative variants (greater than 2 per cent) in a strain of *S. cerevisiae* unrelated to that used in these studies.

SUMMARY

Evidence that irradiation of vegetative diploid cells of yeast induces a reassortment of genetic loci has been obtained using a galactose positive strain of *Saccharomyces cerevisiae*. Variant negative colonies are produced in this strain with a high frequency approaching 2 percent of survivors when cells are irradiated with an ultraviolet dose of 200 ergs/mm^2 previous to plating. With this dose, approximately 67 percent of these are sectored, containing both phenotypically normal and variant cells. Investigations were centered on a genetic analysis of sectored colonies.

Results have indicated that (1) parental cells are heterozygous for a single gene pair controlling the galactose character, (2) variant negative cells are homozygous recessive, (3) sectored colonies are derived from single irradiated nuclei, (4) irradiation of heterozygotes induces homozygosity for dominant genes as well as for recessive genes, (5) the appearance of homozygous dominant cells is correlated with that of homozygous recessive cells, although induced recessive lethals are distributed independently of these, (6) a much higher frequency of variant negative colonies results from irradiation of heterozygotes ($Gg \rightarrow gg$) than from irradiation of positive haploids ($G \rightarrow g$), and (7) the estimated frequency of induced homozygous dominant variants produced by heterozygotes ($Gg \rightarrow GG$) is higher than the frequency of induced positive reversions produced by homozygous recessive diploids ($gg \rightarrow Gg$).

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