RADIATION-INDUCED GENETIC SEGREGATIONS IN VEGETATIVE CELLS OF DIPLOID YEAST

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ULTRAVIOLET irradiation frequently induces homozygosis at one or more loci in heterozygous vegetative cells of *Saccharomyces cerevisiae*. For a particular allelic pair, the observed frequency of such inductions can approach two percent among survivors under conditions where fewer than fifty percent of the irradiated cells are killed. These alterations are almost certainly due to genetic segregation rather than to gene mutation, since: (1) complementary types (homozygous recessive and homozygous dominant) often appear as different lines of descent from a single irradiated heterozygote, (2) irradiated haploids show only rare changes from dominant to recessive, and (3) irradiated homozygous recessive cells show only rare changes to heterozygous (JAMES 1953; 1955). However, the observed homozygosis could result either from independent segregations of individual loci or from linked segregations involving whole chromosomes or parts of chromosomes. The present investigation was undertaken to obtain information on the mechanism by which the segregants are produced.

MATERIAL AND METHODS

Allelic gene pairs at five loci were used in this study. Of these, two were concerned with galactose utilization; one recessive allele (g_1) has been described previously (JAMES 1955), the other (g_2) was obtained by ultraviolet irradiation of a haploid strain. Absence of the dominant allele at either locus results in the recessive phenotype. Genes at the other three loci were associated with sex (*A*, *a*), and with non-requirement vs. requirement for uracil (*Ur*, *ur*) and for tryptophan (*Tr*, *tr*). The latter were obtained through the courtesy of DR. S. SPIEGELMAN.

The genes affecting galactose utilization were used for the initial detection of induced homozygotes. The isolation of variant cells homozygous recessive for galactose was easily accomplished, because dominant and recessive phenotypes differ in colony morphology on a galactose-EMB indicator medium; homozygous dominant or heterozygous cells form black (positive) colonies, whereas homozygous recessive cells form white (negative) colonies. The variant colonies that appear when heterozygous cells are irradiated and plated on this medium are either wholly white or sectored for black and white.

Isolates for genetic analysis were obtained by picking, dilution, and replating of individual colonies, followed by single colony isolation. Segregation analyses were confined to those isolates that did not contain radiation-induced recessive lethals. The segregants (either 3 or 4) from a single four-spored ascus were used to determine genotype if these indicated heterozygosity at the loci under study. This number was considered adequate in view of the consistent results of previous individual analyses

(JAMES 1955). However, in the case of those isolates where radiation-induced homozygosis was indicated, all four segregants from each of a minimum of two asci were characterized in order to eliminate possible erroneous classifications resulting from occasional irregular segregations.

Tryptophan and uracil requirements were determined by plating several hundred cells in the presence and absence of these compounds; recessive genotypes were indicated by the complete absence of visible colonies on deficient media. Galactose and sex reactions were determined in the manner already described (JAMES 1955). Tests for induced homozygosity for sex were performed in the following manner. Ability of isolates to sporulate was determined. Inability to sporulate was considered an initial indication of homozygosity for sex. Such isolates were tested for mating reaction, and those that showed a positive reaction were considered to be diploids homozygous for sex. Diploidy of these isolates, rather than haploidy, was inferred from large cell size and from the fact that, with each of four such variants isolated previously, the zygotic cultures produced by matings with haploids sporulated abundantly but exhibited a low frequency of spore germination. The frequencies as estimated by the above procedure are suggestive only, since they are subject to errors of unknown magnitude.

Media and methods of irradiation were similar to those used previously (JAMES 1954). All crosses were made by the technique of mating single cells and isolating the resultant zygote (CHEN 1950).

RESULTS AND DISCUSSION

Information on the mechanism by which radiation-induced genetic segregations occur in vegetative cells can be obtained by comparison of the behaviour of linked and unlinked marker genes.

Using tetrad analysis, a survey of linkage relationships indicated that the five loci *A*, G_1, G_2, *Ur*, and *Tr*, together with centromeres, comprise three linkage groups and one unlinked locus (table 1). These are: (1) *A*—centromere, (2) G_1—centromere, (3) (G_2—*Tr*)—centromere, and (4) *Ur* independent. With the evidence that the locus of *Tr* is on the same chromosome as that of G_2 (no crossover segregants in 91 segregations) but on a different chromosome from that of G_1, it is possible to test whether the radiation-induced segregations involve (1) each locus independently of the others, or (2) linked (chromosomal) segregations.

Results of such a test (summarized in table 2) indicated that the segregations are chromosomal in nature; G_2 and *Tr* show linkage in induced somatic segregations whereas G_1 and *Tr* do not. Among g_2g_2 variant isolates (meiotic linkage with tryptophan) seven of eight were homozygous for tryptophan (*TrTr*), whereas among g_1g_1 variants (no meiotic linkage with tryptophan) none of 16 were homozygous for tryptophan. The data also suggest that groups of linked genes do not segregate simply as units (whole chromosome segregation) since the isolate from one negative (g_2g_2) variant colony of the eight studied was not homozygous for tryptophan.

Further evidence of the chromosomal nature of the somatic segregations was obtained from the positive portions of sectored colonies. The genotypes of positive isolates associated with six of the g_2g_2 variant colonies of table 2 were determined

TABLE 1

Linkage relationships between five loci in yeast as determined by tetrad analysis

Two-point segregations	Genotypes segregated*						Significant evidence of linkage‡
	Aa G_1g_1 G_2G_2 *Urur Trtr*			*Aa* G_1G_1 G_2g_2 *Urur Trtr*			
	Tetrad types†			Tetrad types			
	PD	NPD	T	PD	NPD	T	
G_1-*A*	10	13	19	—	—	—	G_1 to centromere; *A* to centromere
G_1-*Ur*	3	9	30	—	—	—	
G_1-*Tr*	15	18	9	—	—	—	G_1 to centromere; *Tr* to centromere
G_2-*A*	—	—	—	29	25	37	G_2 to centromere; *A* to centromere
G_2-*Ur*	—	—	—	18	12	61	
G_2-*Tr*	—	—	—	91	0	0	G_2 to *Tr*
A-*Ur*	6	9	27	13	14	64	
A-*Tr*	10	16	16	29	25	37	*A* to centromere; *Tr* to centromere
Ur-*Tr*	6	7	29	18	12	61	

* *Aa*—sex; G_1g_1, G_2g_2—galactose; *Urur*—uracil; *Trtr*—tryptophan.

† PD—parental ditype; NPD—non-parental ditype; T—tetratype. The genotype of the fourth segregant was inferred in 45 of the 133 segregations. Eleven irregular segregations were omitted from the data.

‡ Evidence of linkage to centromere is based on the hypothesis that a significant deviation of the ratio of non-parental ditype to tetratype segregations in excess of 1:4 indicates that the two loci are on different chromosomes and within 33 units of their respective centromeres (PERKINS 1953).

TABLE 2

Induced homozygosis in isolates from galactose negative variant colonies formed by irradiated cells of two genotypes, as determined by segregation analysis. Dose: 200 ergs/mm²

Pre-irradiation genotype: $\frac{A}{a}\ \frac{g_1}{G_1}\ \frac{G_2}{G_2}\ \frac{Tr}{tr}\ \frac{Ur}{ur}$		Pre-irradiation genotype: $\frac{a}{A}\ \frac{G_1}{G_1}\ \frac{g_2}{G_2}\ \frac{Tr}{tr}\ \frac{Ur}{ur}$	
No. colonies	Induced homozygosity	No. colonies	Induced homozygosity
13	g_1 only	5	g_2 and *Tr*
2	g_1 and *Ur*	2	g_2, *Tr* and *ur**
1	g_1 and *ur*	1	g_2 and *ur*

* The asci from one colony segregated 2:2 for growth vs. no growth on galactose. For this colony, genotype at uracil and tryptophan loci was determined on dextrose medium. The proposed genotype at the galactose locus was based on absence of galactose positive segregants in three complete segregations.

by tetrad analysis. Here again, linkage of the galactose and tryptophan loci was evident in the somatic segregants. In three of the six positive isolates the genotypes were entirely complementary to those of the negative isolates. In two of these, homozygosity for galactose and tryptophan (G_2G_2 *trtr*) in positive isolates matched the homozygosity (g_2g_2 *TrTr*) of the negative isolates. With the third colony, complementary homozygosity was extended to a third locus, that for uracil. In this instance the genotype of the positive isolate was *Aa* G_2G_2 *UrUr trtr*; that of the negative was *Aa* g_2g_2 *urur TrTr*. The positive clones from the other three colonies were heterozygous at all four loci.

As might be expected, phenotypically normal colonies from irradiated cells are unlikely to contain homozygous dominant cells. This has already been shown for G_1 (JAMES 1955), and was shown for G_2 in the present study. Here tryptophan requirement was used as an indication of homozygosity in galactose positive is lates; in G_2tr/g_2Tr diploids, any induced *trtr* homozygotes would tend also to be G_2G_2 because of the close linkage between loci (table 1). Of the positive isolates from 24 sectored colonies, results indicated that 7 contained homozygous cells (mixed with heterozygous cells in 3 cases), whereas none of the isolates from 27 wholly positive colonies gave evidence of being homozygous. The assumed genotypes of the positive sectors were confirmed by tetrad analysis in six of the sectored colonies that were free of recessive lethals. (These six colonies provided the data on complementary homozygosis in the preceding paragraph.)

A hypothesis of genetic segregation requires an explanation of the presence of positive cells (in sectored colonies) whose genotype is non-complementary to that of the negative cells. In some instances their presence might be attributed to the plating of budding cells. However, the distribution of recessive lethals (JAMES 1955) precludes this as an overall explanation. Consequently, in at least some sectored colonies, the presence of noncomplementary positive cells must be attributed to a delay in genetic segregations until one or more mitotic divisions have occurred.

The absence of positive segregants (in wholly negative and many sectored colonies) also requires an explanation. There is evidence (JAMES 1954) that the occurrence of wholly negative colonies is due to the elimination of positive cells during early colony growth. Such eliminations would also account for the frequent absence of positive segregant cells in sectored colonies. Finally, it is of course likely that some fraction of radiation-induced variants results from mutation; in such cases one does not expect complementary genotypes to be present.

Any of several mechanisms can be involved in the production of chromosomal segregants. In artificially-synthesized diploid fungi spontaneous somatic segregations can result from mitotic crossing over and from haploid formation (PONTECORVO, GLOOR and FORBES 1954). Segregations in exceptional heterozygotes of *E. coli* apparently involve crossing over and haploid formation (LEDERBERG 1949). In Drosophila, both spontaneous (STERN 1936) and radiation-induced (WHITTINGHILL 1951) somatic segregations are attributable to mitotic crossing over. The possible importance of four mechanisms will be considered here. These are (1) reciprocal translocation between homologous chromosomes as a result of chromatid breakage, (2) induced meiosis with subsequent fusion to restore the diploid condition, (3) first meiotic division (disjunction of homologous centromeres), and (4) mitotic crossing over (disjunction of sister centromeres).

Chromatid breakage resulting in reciprocal translocations between homologous chromosomes could give rise to genetic segregations. However, any lack of precision in the exchange of chromosomal segments would produce a duplication in one segregant and a corresponding deficiency in the other The latter would be evident as a recessive lethal in one of the two lines of descent. Actually, no such condition has been found; of nine paired (i.e. complementary) segregants studied so far, recessive lethals have either been present in both lines of descent or in neither.

TABLE 3

*Recessive homozygosis for uracil and tryptophan, and homozygosis for sex, in isolates from induced galactose negative colonies**

Pre-irradiation genotype	Negative isolates from wholly negative or sectored colonies					Positive isolates from sectored colonies		
	No. of isolates	*ur*	*A*	*a*	*tr*	No. of isolates	*ur*	*tr*
a G_1 G_2 *tr ur*/*A* g_1 G_2 *Tr Ur*	197	9	3	4	3	67	3	2
a G_1 G_2 *Tr Ur*/*A* g_1 G_2 *tr ur*	281	11	17	6	6	121	8	3
	478	20	20	10	9	188	11	5

* Recessive homozygosity at loci for uracil and tryptophan was determined by appropriate platings. Homozygosity for sex in positive isolates was not determined. The analysis of one exceptional sectored colony has been omitted from the data. Isolates from both positive and negative sectors of this colony were uracil and tryptophan requiring and of sex *A*. Cell size suggested that they were not haploids but no successful matings were accomplished.

This suggests an exactness of exchange unexpected of radiation-induced chromatid breakage.

Induced meiosis with restoration of the diploid condition by fusion has seemed unlikely as the principal cause of induced homozygosis (JAMES 1955), and the following considerations provide further evidence against such a mechanism. If haploidy with later fusion occurred, then all heterozygous loci unlinked to sex would have an equal opportunity to effect an exchange. Under these circumstances it can be expected that homozygosis will be found at any such locus in the progeny of half the affected cells. A suitable sample of affected cells with which to test this hypothesis is available in galactose negative variant clones, providing the loci to be surveyed are unlinked to those for either galactose or sex. These requirements are fulfilled by the tryptophan locus in g_1g_1 variants (see table one). The frequency of *tr*/*tr* genotypes within g_1g_1 variant isolates is shown in table 3; only nine of 478 isolates were homozygous for tryptophan. Evidence against a hypothesis of meiosis is also provided by the data of table 3 on frequencies of homozygotes for uracil and sex. Recessive homozygosity for uracil, whose locus is independent of those for both sex and galactose, was found in only 20 isolates, and homozygosity for sex, unexpected under a hypothesis of meiosis and fusion, was found in 30 (20 *AA*, 10 *aa*) of the isolates. Thus, haploid formation plays a negligible part, if any, in the radiation-induced segregations under study here.

Evidence concerning the relative importance of mitotic crossing over as contrasted with first meiotic division can be obtained by a comparison of the characteristic frequencies of induced homozygosis at the loci for uracil, sex, and tryptophan. The information from such a comparison is not likely to be misleading unless the chromosomes involved are affected differently by radiation. In the case of mitotic crossing over one would expect a positive correlation between frequency of induced homozygosis and distance from centromere; in the case of first meiotic division a negative correlation (if crossovers occur) or no correlation (in the absence of crossing over) would be expected. The locations of the three loci in order of increasing proxim-

ity to their centromeres, are *Ur*, *A*, and *Tr*, as implied by proportions of tetratypes in G_2-*Ur*, G_2-*A*, and G_2-*Tr* segregations (data of table one). The data of table 3 show that the frequencies of homozygotes among galactose negative isolates were 20 *ur ur*, 20 *AA*, 10 *aa*, and 9 *trtr*. If the information from positive sectors is included, then the frequencies of recessive homozygotes for uracil and tryptophan (31 *urur*, 14 *trtr*) differ significantly. There is thus a positive correlation between frequency of induced homozygosis and distance from centromere, a fact which suggests that mitotic crossing over, rather than first meiotic division, is the mechanism by which the segregants are produced.

SUMMARY

Ultraviolet-induced chromosomal segregations in vegetative cells of diploid *Saccharomyces cerevisiae* have been indicated by a study of the behaviour of linked and unlinked marker genes in the formation of induced homozygotes for galactose utilization. The relative importance of (1) reciprocal translocation, (2) induced meiosis with subsequent fusion of haploid products, (3) first meiotic division, and (4) mitotic crossing over has been estimated. Results have suggested that mitotic crossing over is the mechanism by which the segregants are produced.

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