THE TIMING OF UV MUTAGENESIS IN YEAST: A PEDIGREE ANALYSIS OF INDUCED RECESSIVE MUTATION

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ABSTRACT

The mechanism of UV-induced mutation in eukaryotes was studied in individual yeast cells by a procedure that combined pedigree analysis and tetrad analysis. The technique involved the induction of recessive lethals and semilethals in G1 diploid cells. Induced frequencies were 25 and 61 percent at survival levels of 90 and 77 percent, respectively. No evidence of gross chromosome aberrations was detected. Recessive mutations that affect only one strand or that affect both strands of the DNA molecule are induced much at random among a population of cells, and both types can occur within the same cell. However, the data confirm that two-strand mutations are in the majority after a low level of irradiation. The simplest explanation involves a mechanism whereby most mutations are fixed in both strands prior to the first round of post-irradiation DNA replication. The recessive mutational consequences of irradiation are exhausted at the conclusion of the first post-irradiation cell division, although dominant-lethal sectoring continues at a high level through the second post-irradiation division. It is concluded that pyrimidine dimers that persist to the second round of DNA replication are rare or ineffective.

THE mechanisms by which UV-induced mutations are produced in bacteria are not yet clear, but are thought to include either error-prone replication past a pyrimidine dimer or misrepair of single strand gaps in DNA, or both. (WITKIN 1975). Gaps are known to arise in a number of ways, *e.g.*, as discontinuities in daughter strands after a dimer-containing duplex is replicated, or as gaps that result from excision of pyrimidine dimers.

In contrast to the rapidly developing picture in bacteria, our understanding of eukaryotic UV mutagenesis is still rudimentary. Pyrimidine dimers have been clearly implicated in the mutation process by the use of photoreactivation, but their precise role is at present unknown (KILBEY and DE SERRES 1967). Both the quantitative and the qualitative patterns of UV mutagenesis have been shown to be dependent upon alterations in repair proficiency (LAWRENCE *et al.* 1974). However, we remain largely ignorant of the mechanisms involved and, although it is tempting to anticipate a model for UV mutagenesis closely based on bac-

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terial models, further evidence is essential either to support this view or to provide an alternative.

The present experiments were undertaken with yeast to provide evidence concerning the timing of mutation in relation to post-irradiational DNA synthesis and to obtain direct information concerning the involvement of one or both DNA strands in the fixation of new UV-induced mutations. Investigations with this organism have usually utilized the red-white adenine system, whereby simple visual inspection of the colonies produced by irradiated haploid cells is sufficient to permit them to be classified as wholly nonmutant, wholly mutant (indicative of two-strand mutations) or mosaic (indicative of one-strand mutations). However, the procedure requires separate determinations of the extent to which several well-recognized phenomena are likely to distort the data. These include lethal sectoring at the first post-irradiation cell division, clustering of irradiated cells at the time of plating (NASIM and AUERBACH 1967), and replicating instability, a phenomenon known to affect adenine loci (NASIM 1967; JAMES, NASIM and MCCULLOUGH 1972).

The means chosen for this study was pedigree analysis of individual cells of *S. cerevisiae* in G1, a procedure which permits the virtually error-free detection of one-strand, two-strand, and delayed mutations. A high frequency of mutation at high survival is essential if this technique is to be feasible, and these conditions were achieved by using recessive lethal and semilethal mutations. In turn, to utilize these mutants successfully, it must be possible to discriminate between recessive lethal mutations and other kinds of induced lethality. This was accomplished by the irradiation of diploid rather than haploid cells and by the subsequent detection of recessive mutations through tetrad analysis. A recessive that is confined to one of the two products of the first post-irradiation mitotic division indicates a one-strand mutation, whereas a recessive that appears in both products is indicative of a two-strand mutation.

MATERIALS AND METHODS

Strain

A diploid strain of *Saccharomyces cerevisiae*, designated C 107, was synthesized with the following composition:

a/α, *phr/ phr*, *ADE2/ade2*.

The homozygous mutation for the lack of photoreactivating enzyme was included to reduce the possibility of photoreactivation of UV damage during the early stages of micromanipulation. The heterozygous adenine mutation is noncentromere linked and was included as a monitor of mitotic recombination.

Experimental method

The experimental procedure is outlined in Figure 1. Cells from a culture of C 107, maintained in stationary phase for 48 hr, were thinly streaked on YEPD agar slabs and immediately exposed to UV doses of either 15 (Exp. 1) or 20 (Exp. 2) seconds of UV at a fluence rate of approximately 0.8 J m^{-2} sec^{-1}. The UV source was a Hanovia Vicor mercury vapor lamp, emitting mainly at 253.7 nm. Individual cells were then removed to specific locations and incubated at 30° until their mitotic products could be separated and relocated. For the lower dose, separations were confined to the two products of the first post-irradition division. For the higher dose, separations included the products of the second post-irradiation division, yielding four cells.

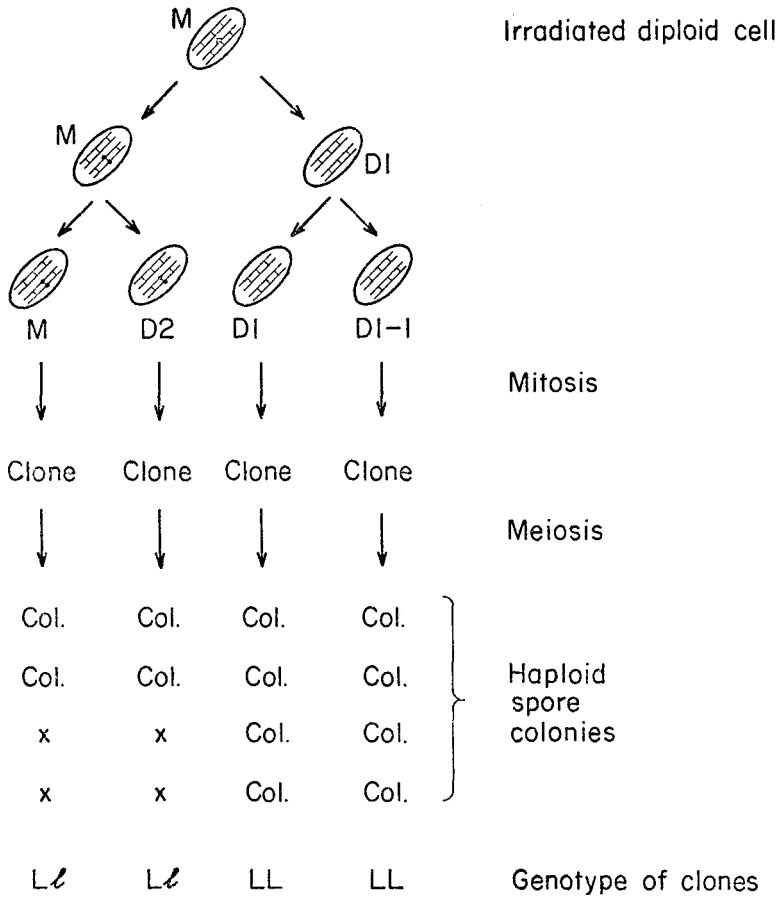

FIGURE 1.—The general procedure, involving pedigree analysis and subsequent tetrad analysis. The segregations pictured are those expected of a one-strand recessive mutation. In the case of a two-strand mutation, all four clones would segregate as heterozygotes for the same mutation.

These cells were then incubated and the resultant clones picked and streaked on agar plates for subsequent genetic analysis. Pedigrees were discarded if it was found that lethal sectoring had eliminated one of the products of the first post-irradiation division.

The genetic constitution of individual clones was determined by tetrad analysis. Some clones failed to sporulate, a circumstance which led to further reduction in the number of pedigrees available for analysis. Segregant spores were incubated at 23°, inspected microscopically at both two and three days, and then replica-plated. The replicas were incubated at 23° and 33° in order to detect temperature-sensitive mutants and to provide information concerning the segregation of the adenine marker.

Heterozygosity for a mutant producing recessive lethality or impaired growth was indicated by a 2:2 segregation of normal to abortive or mutant meiotic products. At least four tetrads from each clone were analyzed, a procedure that prevented misclassification due to a low background frequency of abortive spores. In those clones in which the presence of a single recessive was not clearly indicated, tetrad analysis was continued until a distinctive segregational pattern could be described. Such patterns invariably indicated either that (1) the clones contained a few reverted cells of pre- or post-meiotic origin (yielding occasional 4:0 and 3:1 segregations, respec-

tively) and that (2) individual clones were heterozygous for more than one mutant (yielding frequent 1:3 and 0:4 segregations).

The occurrence of radiation-induced mitotic crossing over was indicated by the appearance of sister clones, one of which was homozygous wild-type for adenine requirement, while the other was homozygous for the recessive mutation. Other deviations from 2:2 for adenine were attributable either to gene conversion, to premeiotic mitotic crossing over, or to back mutation.

It was important to know the background frequency of recessive lethals in the cell populations prior to irradiation, because cells bearing pre-existing recessive lethal mutations will produce pedigrees that are indistinguishable from those produced by new mutations affecting both DNA strands. The procedure described above (repeated dissection within clones derived from individual cells) was not appropriate because of the low frequency of mutants expected in the untreated populations. Therefore, an upper limit for the frequency of pre-existing mutants was obtained by a different method: tetrad analysis of a random sample of sporulated cells from the pre-irradiated cultures. About 7 percent of the spores from these asci failed to germinate or produced abortive colonies. This level of inviability is not unusual in yeast, and although there may be a genetic component, it is clear that such spore death is not always due to the presence of a heterozygous lethal in the parental cell. This is certainly true of 3:1 (live to dead) and 0:4 segregations. The latter constitute a unique but fairly common class, all four spores being dead at the time of dissection. The upper limit for pre-existing mutations was thus based on the frequency of 2:2 and 1:3 segregations.

RESULTS

1. *Background mutations*

The results of ascus dissections made on untreated populations of C 107 to establish an upper level of recessive lethality or semilethality are shown in Table 1. In all, 586 tetrads were analyzed and, based on the numbers of 2:2 and 1:3 tetrads (see the previous section), a frequency of about 5 percent was found. This frequency is significantly lower than that of the "whole-colony" mutants later detected after irradiation with either dose, but it is clear that the actual frequency of mutants in the control was negligible for the purpose of this investigation. For instance, the manner of spore death in the controls was different from that of the established mutants in the irradiated population: 72 percent of the former failed to germinate at all, whereas only 13 percent of the latter died in this way.

2. *Survival and dominant effects after UV*

The two different exposures to UV gave 90 percent and 77 percent survival (see Table 2). These low levels of killing were accompanied by a high frequency

TABLE 1

Tetrad analysis of unirradiated control cells

	Alive: dead or abnormal				
	4:0	3:1	2:2	1:3	0:4
Control for 15″ UV	242	27	11	2	4
	(0.846)	(0.094)	(0.038)	(0.007)	(0.014)
Control for 20″ UV	247	33	16	1	3
	(0.823)	(0.110)	(0.053)	(0.003)	(0.010)

TABLE 2

Dominant effects of irradiation

UV	Dom. lethality	Leth. sectoring Gen. 1	Leth. sectoring Gen. 2	Nonsporulation Gen. 0	Nonsporulation Gen. 1	Nonsporulation Gen. 2
0	0/120	0/120	—	—	—	—
	(0.0)	(0.0)	—	—	—	—
15 sec	23/239	28/216	—	6/188	3/182	—
	(0.10)	(0.13)	—	(0.03)	(0.02)	—
20 sec	64/280	61/205	51/309	14/143	12/130	8/205
	(0.23)	(0.30)	(0.17)	(0.10)	(0.09)	(0.04)

of dominant-lethal sectoring among the survivors at the first post-irradiation division. The frequency of lethal sectoring was 13 percent at the lower dose and 30 percent at the higher. With the higher dose, the frequency was 17 percent at the second post-irradiation division. It is notable that lethal sectoring is also apparent in subsequent divisions, though at progressively lower frequencies.

3. *Induced mitotic crossing over and irregularities at the adenine locus*

Data concerned with the incidence of mitotic crossing over after UV are presented in Table 3. The lower of the two exposures, 15 seconds, gave 1 percent mitotic crossing over, whereas the higher exposure, 20 seconds, gave 9 percent. The extension of the pedigree analysis in the second experiment allowed the observation to be made that crossing over declined to zero, or near zero, after the first post-irradiation division. The precipitous decline is in sharp contrast to the more gradual decline in lethal sectoring. It is clear that the role of mitotic crossing over in lethal sectoring is minor.

Five pedigrees produced irregular segregations at the *ade2* locus attributable to mechanisms other than induced mitotic crossing over. All may have resulted from gene conversion (see WILDENBERG 1970). Otherwise, one was indicative of a pre-irradiation mitotic crossing over or of an induced two-strand mutation (−/−, −/−, −/−, −/−), whereas three were indicative of induced one-strand mutations (+/−, +/− −/−, −/−, or +/−, +/−, +/+, +/+). The remaining pedigree provided the only instance of a possible second generation mutation found in this study: the genotypes of the four mitotic products of the test cell were +/−, −/−, −/−, and −/−. This pedigree is indicative of two mutational events and is thus suggestive of replicating instability.

TABLE 3

Induced mitotic crossing over

UV	Generation 1		Generation 2	
0	0/120*	(0.0)	—	
15 sec	2/179	(0.01)	—	
20 sec	11/126	(0.09)	0/173	(0.0)

* Based on absence of pink mitotic segregant colonies after separation of unirradiated control cells.

TABLE 4

Segregations among test clones classified as mutant-free

UV	No. of clones	Normal: dead or aberrant 4:0	3:1	2:2	1:3	0:4	Total
15 sec	294	989	116	44	10	40	1199
		(0.825)	(0.097)	(0.037)	(0.008)	(0.033)	
20 sec	264	898	141	51	13	34	1137
		(0.790)	(0.124)	(0.045)	(0.011)	(0.030)	

Isolated instances of 3:1 or 1:3 segregations for adenine among sporulated clones were attributable to meiotic gene conversion and have been ignored in these data.

4. *Induced mutations*

802 clones derived from 296 pedigrees were analyzed. Of these, 218 were found to be heterozygous for single recessive mutations, whereas 26 were heterozygous for two mutations. The data have been summarized in Tables 4, 5, 6 and 7. It is noteworthy that the frequencies of 2:2 and 1:3 segregations in the clones classified as mutant-free (Table 4) correspond closely to those of the control populations (Table 1). This similarity provides additional support for the supposition that the great majority of 2:2 and 1:3 segregations in the controls were not a consequence of recessive lethal mutations present in the cells before irradiation.

The data for clones in which a single mutant appears to be present are shown in Table 5. The data were strikingly free of discrepant 4:0 and 3:1 segregations. Where a clone did produce this type of discrepant result, an extended analysis was made, and the clone, eventually characterized unambiguously. In practice, these deviant asci were rare and could have arisen in part by dissection of "false" asci. However the 4:0 segregations could also be attributed either to back mutation or to mitotic crossing over prior to sporulation, and the 3:1 segregations to reversion within individual spore colonies after dissection.

The recessive mutations recovered by tetrad analysis produced a surprisingly wide range of well-defined phenotypes. Different mutants were easily distin-

TABLE 5

Segregations among test clones classified as bearing one lethal or semilethal

UV	No. of clones	Normal: mutant 4:0*	3:1†	2:2	1:3	0:4	Total
15 sec	61	5	5	325	39	32	406
		(0.012)	(0.012)	(0.800)	(0.096)	(0.079)	
20 sec	157	5	12	588	82	28	715
		(0.007)	(0.017)	(0.822)	(0.115)	(0.039)	

* The ten 4:0 segregations were distributed among the 92 segregations of 6 clones.
† The 17 3:1 segregations were distributed among the 264 segregations of 13 clones.

TABLE 6

Phenotypes of recessive mutants

Lethal					
1 cell	>1 cell	Semi-lethal	Morphological	Temperature-sens.	Total
17	65	21	22	1	126
(0.13)	(0.52)	(0.17)	(0.17)	(0.01)	

guishable on the basis of their cell size and shape and the extent of their growth. They have been arranged into four loose categories in Table 6. The spore colonies produced by outright lethals contained only morbund cells after incubation for three days, the number of these varying from a single ungerminated spore to several scores of cells. Semilethals produced visible "pin-point" colonies at 3 days, whereas morphological mutants produced colonies that, though small, were sufficiently large to permit successful replica plating. Because of the distinctive phenotypes of the mutants, it was possible to discriminate reliably between mutations affecting one locus and those affecting another in the same pedigree. Although many of the mutants showed a degree of sensitivity to the higher or the lower temperature of incubation, only one was classified as temperature sensitive. This mutant went undetected at 23°, but was completely lethal at 33°.

The segregation patterns that led us to conclude that certain clones contained two independent mutations are shown in Table 7. In these cases, a clear 2:2 segregation of the more extreme mutation could be observed together with a 2:2 segregation of the less extreme mutant within the limits of epistasis. The types of asci found in repeated analysis of particular pedigrees indicate an inde-

TABLE 7

Segregations of clones classified as dihybrid

UV	Pedigree	No. clones	Coupling ditype	Repulsion ditype	Tetratype	Omitted*
15 sec	1–6	1	1	1	13	3
	1–14	2	0	14	7	5
	1–17	2	2	5	17	2
	6–11	2	1	3	20	0
	8–17	1	7	1	10	0
20 sec	4–12	4	6	7	19	0
	7–8	3	5	1	20	2
	10–12	2	2	2	6	6
	11–11	2	3	4	14	4
	12–10a	1	2	7	7	0
	12–10b	2	10	3	6	0
	12–14	2	3	3	10	0
	12–16	1	2	1	12	0
	13–14	1	4	4	6	3

* Omissions were those tetrads in which the occurrence of one or more nongerminating spores precluded classification.

TABLE 8

*Genotypes of the two products of the first post-irradiation division**

UV	LL LL	LL Ll	LL Ll L'l' L'L'	Ll Ll L'l' L'L' L"l" L"L"	Ll Ll L'L' L'l'	Ll LL L'l' L'l' L"L" L"l"	Ll Ll	Ll Ll L'l' L'l'	Total
15 sec	139	12	1	0	1	0	23	3	179
20 sec	58	21	2	1	4	1	28	2	117

* Multiple mutations in the same cell or pedigree are indicated by primes.

pendent assortment of most pairs of recessive lethals in these cases, although linked mutations were occasionally found. These findings, and the absence of 4:0 and 3:1 segregations, make it unlikely that any of these complex patterns of segregation was caused by chromosomal aberration (see PERKINS 1974).

5. *Genetic heterogeneity*

It was possible to construct a genetic pedigree for each of 296 of the irradiated cells from the data of Tables 4, 5, and 7. Table 8 gives a summary of the data from these pedigrees in terms of the genotypes of the daughter cells M and D1, the two products of the first post-irradiation division. Information relating to the second post-irradiation division is not included, since no new mutations appeared at this division. Occurrence of multiple mutations in the same pedigree or in the same cell are shown by the insertion of primes. From these data, it is possible to determine whether any individual mutation was the result of a one-strand (*LL*, *Ll*) or a two-strand (*Ll*, *Ll*) event, and this information is summarized in Table 9.

Several facts relating to mutation induction by UV light are evident:

(a) The dose-effect curve is nonlinear; the percentage of mutations scored rose by a factor of more than two (from 25 percent to 61 percent) when the dose was increased by a factor of only one-third.

(b) Two-strand mutations are very frequent; at the lower dose (90 percent survival) they were twice as frequent as one-strand mutations.

(c) There is a strong suggestion that the relative frequency of one-strand mutations increases with dose, although the change was not statistically significant here ($P = 0.1$).

(d) Both one-strand and two-strand mutations can occur in the same cell, an indication that cell phase (G1 and G2) cannot always be a decisive factor.

(e) The distributions of pedigrees containing 0, 1, 2, or 3 mutations at each

TABLE 9

The frequencies of one- and two-strand mutations induced by ultraviolet light in yeast

UV	No. pedigrees	2-strand	1-strand	Total No.	Total Freq.
15 sec	179	30	15	45	0.25
20 sec	117	37	34	71	0.61

dose and for either one-strand or two-strand mutations are, with one exception, those expected of random induction. The exception is that of two-strand mutations at the higher dose. Here the data deviated ($P = 0.05$) from that expected of a Poisson series in such a way as to suggest that the preirradiated population of cells may have contained a few spontaneous mutations.

(f) The induction of recessive mutations is strongly correlated with the induction of dominant-lethal sectoring (Table 10). The mean frequency of mutations among pedigrees that produced lethal sectors at the second post-irradiation division was about one (22/21), whereas the frequency among pedigrees free of lethal sectors was only 0.5 (59/108). The difference is statistically significant. Comparable information relating to lethal sectoring at the first division is not available because these pedigrees were discarded. It is perhaps noteworthy that no correlation was evident between mutation and nonsporulating pedigrees.

(g) The mutational consequences of UV irradiation are effectively exhausted at the conclusion of the first post-irradiation mitosis; the genotypes of cells M and D2 in Figure 1 were always identical, as were the genotypes of cells D1 and D1–1. This induced frequency of zero (0/199, Exp. 2) was thus in sharp contrast to the frequency of 61 percent (71/117) for mutations occurring prior to this division.

DISCUSSION

Two-thirds of the mutations found in this study after exposure of cells to very low doses of ultraviolet light affected both strands of the DNA molecules. We consider this to be a nearly exact estimate, within the limits of normal sampling error. Distortion of the estimate due to the presence of mutants of spontaneous origin prior to irradiation must have been negligible, and the influence of induced mitotic crossing over must likewise have been small and in the opposite direction. The influence of the latter can be estimated from the behavior of the adenine marker that was included in the test strain. The data of Table 3 indicate that mutations induced at the lower dose had a one percent probability of being involved with a mitotic crossover, if the former was far removed from its centromere. One-strand mutations are unaffected by their location relative to a crossover. However, there is a 50 percent probability that a two-strand mutation distal to a crossover will be either eliminated as a lethal sector or will appear as a homozygote for a semilethal or morphological mutant. It is evident, then, that the influence of mitotic crossing over on the data of Table 9 is negligible; even after the higher dose, the number of two-strand mutations (37) is probably

TABLE 10

Correlation of recessive mutation with lethal sectoring in the second post-irradiation cell division

Pedigrees	Mutations per cell 0	1	2	3	$\bar{x}$
Lethal sectoring	7	8	4	2	$22/21 = 1.05 \pm 0.21$
Lethal sector-free	56	45	7	0	$59/108 = 0.55 \pm 0.06$

underestimated by fewer than 2. It may be noted here, also, that mitotic crossing over cannot contribute in a major way to the phenomenon of lethal sectoring; the frequency of lethal sectoring was 17 percent at a time when the frequency of mitotic crossing over was zero.

The results confirm recent indirect evidence in yeast (ABBONDANDALO and SIMI 1971; HANNAN, DUCK and NASIM 1976; ECKARDT and HAYNES 1977) and much earlier evidence in *Ustilago maydis* (HOLLIDAY 1962) that two-strand mutations are common in lower eukaryotes. At the same time they emphasize that full information about lethal sectoring is essential, at least in diploid yeast, to prevent misinterpretation of data. Not only is the frequency of lethal sectoring high, but mutations are twice as frequent in pedigrees prone to lethal sectoring.

The best current explanation for two-strand mutations is probably that based on an error-correction procedure that locates and corrects mismatched bases, either in the direction of the mutated sequence or back to the unmutated sequence (HOLLIDAY 1962). Direct evidence for this type of error correction has been obtained in bacteria (WILDENBERG and MESELSON 1975). It is also possible that a two-strand mutation is a consequence of two-hit events, a theory proposed by BRESLER (1975). This seems unlikely, however, in view of the fact that the proportion of two-strand mutations does not increase with dose, and may very well decrease.

Of particular significance is the fact that the segregation of lethal-bearing and nonlethal-bearing strands is complete by the end of the first post-irradiation division. The frequency of induced recessives was 61 percent at the conclusion of this division; it was 0 percent in the interval between this and the conclusion of the succeeding division. This one finding disposes of a number of uncertainties: (1) it supports the assumption that the cells were in G1 at the time of irradiation, since second generation mutations have been detected in *S. pombe* irradiated in G2 (HAEFNER 1967), (2) it suggests that a single double helix of DNA is present in each of the irradiated chromosomes. A multistranded condition might be expected to yield, in some pedigrees at least, some evidence of delayed mutation, (3) it suggests that spontaneous mitotic crossing over in post-irradiation generations has little influence on the data, since these would yield mixed clones of genotype *Ll* and *LL* and few such clones were found (Table 5), (4) it eliminates the possibility that replicating instability common to the adenine loci is sufficiently frequent among the recessive mutations to influence the data, (5) it demonstrates that the mechanisms responsible for lethal sectoring and recessive mutation are not identical, since the frequency of the former remained high in the second generation, and (6) most importantly, it demonstrates that, in wild-type yeast, dimers that persist to the second post-irradiation division are either rare or ineffective.

Are UV-induced two-strand mutations in yeast pre- or post-replicational in origin? It is difficult to construct simple post-replicational models of mutation that do not result in mosaics, and for this reason it seems likely that two-strand mutations are fixed prior to the first post-irradiation DNA synthesis. Current information from *E. coli* (WITKIN 1975) suggests that most UV-induced muta-

tions in that organism are post-replicational in origin as a consequence of *recA*$^+$ *exr*$^+$-dependent, error-prone repair of daughter-strand gaps. If this is confirmed, it would seem that lower eukaryotes and prokaryotes differ dramatically in their mutational mechanisms.

The experimental technique applied in this investigation permits one-strand mutations to be detected with the same high degree of certainty as are two-strand mutations. These may be post-replicational in nature. However, the apparent increase in their frequency at higher doses suggests, as does the data of ABBONDANDALO and SIMI (1971) and HANNAN, DUCK and NASIM (1976), a saturation or inactivation of the misrepair system responsible for two-strand mutations, and thus provides some support for the supposition that *both* one-strand and two-strand mutations are pre-replicational. Further support of this interpretation is to be found in recent evidence that caffeine, a repair inhibitor, can alter the relative frequencies of one- and two-strand mutations (DEVIN 1976).

The results of this investigation may be compared with those of a related study (JAMES and WERNER 1966) of X-ray-induced recessive lethals in diploid *S. cerevisiae*. The results were remarkably similar, though in the case of X irradiation the preponderance of two-strand mutations was attributed to the supposed prevalence of two-strand breaks.

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