UV MUTAGENESIS IN RADIATION-SENSITIVE STRAINS OF YEAST

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ABSTRACT

The yeast *Saccharomyces cerevisiae* appears to possess a single mutagenic or "error prone" pathway for the repair of UV damage, probably involving the functions of at least seven genes; *rev1, rev2, rev3* (LEMONTT 1971a), *rad6, rad8, rad9* and *rad18* (LAWRENCE *et al.* 1974; present results). Strains carrying *rad6* are the most sensitive to the lethal effects of UV light in this group and double mutants carrying *rad6* and either *rev1, rev3, rad9* or *rad18* are no more sensitive than this single mutant strain. *rev3 rad6* double mutant diploids failed to show any UV-induced reversion of the normally highly revertible ochre allele *cyc1-9*, even though a total of more than 2.5×10^9 viable cells was examined, suggesting that strains of this kind are entirely UV-immutable; spontaneous revertants could be recovered, however.—The *rad6* and *rev3* gene products would appear to be necessary for all kinds of mutagenic events at all sites within the genome, but the products of the other genes that act in the "error-prone" pathway have a more restricted role and are involved in the production of only some kinds of mutations. It is suggested that such selectivity arises from the interaction of some repair enzymes with specific nucleotide sequences.

IT has been suggested (LEMONTT 1971a; LAWRENCE *et al.* 1970; LAWRENCE *et al.* 1974) that UV mutagenesis in the eukaryote *Saccharomyces cerevisiae* is an enzymatic process, mutations arising as a result of errors made during the repair of damaged DNA in a manner similar to that first proposed by WITKIN (1967) for the prokaryote *Escherichia coli.* Such repair has been called "error prone" (WITKIN 1967) and the evidence for its occurrence in yeast comes from the discovery of mutations, specifically *rad6* and *rad18* (LAWRENCE *et al.* 1970; LAWRENCE *et al.* 1974), *rev1* and *rev3* (LEMONTT 1971a) whose phenotypic attributes include an increased sensitivity to the lethal effects of ultraviolet light and a marked reduction in UV-induced mutability at all test loci studied.

Although these nonallelic mutations exhibit similar phenotypes, at least in these respects, it is not known whether they are concerned with the same repair pathway or whether there is more than one mode of error-prone repair in yeast. We have attempted to answer this questions by means of experiments described in this report, and moreover to determine in general what other genes are involved in UV mutagenesis and whether all of them act in the same manner or possess the same phenotype.

That other genes with this phenotype exist is clearly shown by the recent work of LEMONTT (1974) who has isolated additional mutations, designated *umr*, that reduce UV mutagenesis, two of which complement all of the earlier set. Our approach has been to survey previously isolated *rad* (radiation-sensitive) mutations for their effects on UV mutagenesis, using reversion of defined mutations in the structural gene for iso-1-cytochrome *c* as test systems. In addition to the *rad* mutations, alleles of the three *rev* loci (LEMONTT 1971a) were also included in this survey. The relationship of genes which possess similar phenotypes with respect to mutagenesis has been examined by constructing double mutant strains in order to determine whether the two mutations show epistatic interaction with regard to survival following UV exposure. Mutations that interact epistatically are thought to interfere with the functioning of the same repair pathway (GAME and COX 1972, 1973; GAME and COX 1974; BRENDEL and HAYNES 1973). Finally, certain of the mutations that interfere with error-prone repair were examined for differential effects on the reversion of two well defined *cyc1* alleles.

Our results suggest that there is a single error-prone repair pathway in Saccharomyces involving the function of a minimum of perhaps seven genes (*rad6*, *rad9*, *rad18*, *rev1*, *rev3* and possibly *rev2* and *rad8*). The function of two of these (*rad6*, *rev3*) would seem to be essential for UV mutagenesis at all sites and of all types, while the others appear to act principally or exclusively during the production of only certain mutational events. In contrast, all mutations blocking excision repair exhibit enhanced mutability per unit dose, a result compatible with the idea that this process is largely error-free. Finally, we have evidence that suggests that the postulated third, minor, pathway for the repair of UV damage in yeast (COX and GAME 1974) may also be largely error-free.

MATERIALS AND METHODS

Strains: The series of diploid strains (LC1–23, 25–28) designed to survey the influence of radiation-sensitive (*rad* and *rev*) mutations on UV mutability was constructed in collaboration with DR. LOUISE PRAKASH, who has conducted a parallel study of chemical mutagenesis with this material (PRAKASH 1974; PRAKASH 1976). The strains were all homozygous for *cyc1–131*, a mutation of the structural gene for iso-1-cytochrome *c* containing a GUG triplet in place of the AUG initiation codon (STEWART *et al.* 1971), which was chosen as test allele for these studies because it reverts well with both chemical and physical mutagens (PRAKASH and SHERMAN 1973). The strains were also homozygous for one of a series of *rad* or *rev* alleles, including mutations at each of the *rad* loci identified by COX and PARRY (1968), with the exception of *rad7* (i.e. *rad1-rad6*, *rad8-rad17*, *rad19-rad22* and *rad50*, formerly called *uvs1-uvs22*), as well as *rad18–2* (formerly *uxs1–1*) and *rad52–1* (formerly *xs1–1*), both isolated by RESNICK (1969), and *rev1–1*, *rev2–1* (≡ *rad5–2*) and *rev3–1* isolated by LEMONTT (1971a). Strains carrying *cyc1–131* and *rad7* could not be easily obtained since these genes are extremely tightly linked (LAWRENCE *et al.* 1975). The source of the radiation-sensitive mutations used in the construction of the LC strains is given in Table 1. The complementation tests establishing the interlaboratory designations of the mutants were carried out by GAME and COX (1971).

The series of LC strains, which included a wild-type control strain (designated *RAD+* for convenience), were constructed by crossing the 27 strains carrying *rad* or *rev* mutations to B-651 (**a** *cyc1–131 his1 lys2 trp2 RAD+*), sporulating the resulting diploids and intercrossing segregants from each pedigree. In most cases the LC diploid were isolated by prototrophic selection

TABLE 1

Source of the rad *or* rev *mutations*

Mutation	Strain	From
rad1–rad17	mutant derivatives	B. S. Cox
rad19–rad22	of 197/2d	B. S. Cox
rad18	KC 372	R. C. von Borstel
rad52	KC 376	R. C. von Borstel
rev1	XY6–5A	J. F. Lemontt
rev2	XY36–3D	J. F. Lemontt
rev3	XY19–1D	J. F. Lemontt

and their diploid status verified by sporulation. Generally, a single strain homozygous for a given *rad* or *rev* mutation was constructed, but duplicate strains were made in the case of *rad8*, *rad10*, *rad13*, *rad20*, *rad21*, and *rad50*, while four replicate diploids homozygous for *rev1* were isolated.

Double mutant studies were for the most part carried out using sets of haploid segregants obtained by sporulating diploids heterozygous for the two radiation-sensitive mutations in question. The numbers and genotypes of these diploids are given in Table 2. For each pair of radiation-sensitive mutations, survival curves were always determined for wild type, each single mutant and double mutant segregants. At least three, and in almost all cases four, replicate strains of each of these four types were examined since background genotype can alter survival significantly. The set of haploids which included the double mutant *rad1 rad52* all carried *cyc1–9*, an ochre allele of the structural gene for iso-1-cytochrome *c* (Stewart *et al.* 1972) and this was used as test allele to study reversion as well as survival in these lines.

Survival and *cyc1–9* reversion were also examined in several sets of diploid strains. One set included a wild-type strain, a strain homozygous for *rad1*, a strain homozygous for *rad6*, and a strain homozygous for both of these mutants, while another set contained two wild-type strains, two *rev3* homozygotes, two *rad6* homozygotes and two strains homozygous for both *rev3* and *rad6* (see Table 2). Other sets included two diploid strains homozygous for either the *rev1–1*, *rad8–1*, *rad9–1* or *rad15–1* mutations, as well as *cyc1–9*, and also a pair of appropriate wild type (*REV*+, *RAD*+) controls (Table 2).

Finally, the possible influence of *rad7* on reversion was examined in diploid strains homozygous for this gene and either *cyc1–13* or *arg4–17*, together with wild-type controls. The *cyc1–13* allele contains an isoleucine codon, AUC, AUU or AUA) in place of the normal AUG initiation colon (Stewart *et al.* 1971).

Identification of radiation-sensitive segregants: With the exception of *rev* strains, radiation-sensitive segregants were identified by irradiating replicas of pedigree master plates with a series of UV, or in some cases gamma ray, doses. These replicas were made using a rod-type replicator that transfers drops of cell suspension, since this method allows a much clearer discrimination between radiation-resistant and-sensitive segregants than velveteen replication, particularly if care is taken to ensure that the cell concentration in the suspension is uniform. This procedure was found to be accurate and convenient with most pedigrees, though difficulty was encountered with some, mostly those in which at least sensitive mutations were segregating. In particular, clear cut segregation could not be found in pedigrees segregating for *rad8*, *rad11*, *rad19*, *rad20* and *rad21*. The additional use of nitrous acid, toward which both *rad20* and *rad21* strains were found to be sensitive (Zimmermann 1968), did not resolve the problem for these genes. This is not perhaps very surprising for the latter four mutations, since all are only marginally more sensitive to UV than the wild type from which they were derived (Cox and Parry 1968). As such, it seems doubtful that they are materially repair-deficient. The *rad8* strain, however, was appreciably UV- and gamma-ray-sensitive, and the inability to obtain a clear 2:2 segregation in tetrads must be due to other causes. Crosses between the most sensitive segregants in each pedigree gave

TABLE 2

Genotypes of diploid strains used in experiments or from which haploid segregants were derived

Strain no.	Genotype	
CL-110	α *cyc1–9 rad6–1*	× **a** *rev3–1 arg4–17 his5 ade1 lys1 leu1 trp5 met1*
CL-127	α *cyc1–131 arg4–17 his5 lys1 ade2 met1*	× **a** *arg4–17 ade2*
CL-128	α *cyc1–131 arg4–17 trp1 lys1*	× **a** *arg4–17 trp2 lys1 ade2*
CL-132	α *cyc1–9 rad6 arg4 ade2 lys1*	× **a** *cyc1–9 arg4 leu1 met1 lys1 trp5*
CL-134	α *cyc1–9 lys1*	× **a** *cyc1–9 rev3 rad6 arg4 leu1 his5 ade2*
CL-135	α *cyc1–9 rev3 leu1 ade2 lys1 trp5*	× **a** *cyc1–9 rev3 arg4 leu1 met1 his5 ade2 trp5*
CL-137	α *cyc1–9 rev3 met1 his5 lys1*	× **a** *cyc1–9 rev3 arg4 leu1 met1 lys1 trp5*
CL-138	α *cyc1–9 rad6 arg4 leu1 met1 ade2 trp5*	× **a** *cyc1–9 rad6 arg4 met1 his5 ade2*
CL-139	α *cyc1–9 rad6 arg4 his5 ade2 lys1*	× **a** *cyc1–9 rad6 arg4 met1 his5 ade2*
CL-141	α *cyc1–9 rev3 rad6 met1 his5 lys1*	× **a** *cyc1–9 rev3 rad6 leu1 his5 ade2*
CL-143	α *cyc1–9 rev3 rad6 leu1 met1 ade2 trp5*	× **a** *cyc1–9 rev3 rad6 leu1 his5 ade2*
CL-162	α *rad52 arg4 lys1*	× **a** *cyc1–9 rad1 his5 leu1*
CL-164	α *cyc1–9 rad6 leu1 met1 arg4 ade2 trp5*	× **a** *cyc1–131 rad9 lys2*
CL-167	α *cyc1–9 rad18 leu1*	× **a** *cyc1–9 rad6 met1 arg4 his5 ade2*
CL-168	α *cyc1 rad22 his5*	× **a** *cyc1–9 rad1 leu1*
CL-171	α *cyc1–9 rad1 rad6 met1 arg4*	× **a** *cyc1–9 rad1 rad6 met1 lys1*
CL-172	α *cyc1–9 rad1 rad6 met1 arg4*	× **a** *cyc1–9 rad1 his5 leu1*
CL-173	α *cyc1–9 rad1 rad6 met1 arg4*	× **a** *cyc1–9 rad6 leu1*
CL-174	α *cyc1–9 rad1 rad6 met1 arg4*	× **a** *cyc1–9 his5 leu1*
CL-185	α *cyc1–9 rev1 arg4–17 lys1 leu1 ura4 his5 ade2 trp5*	× **a** *cyc1–9 rev1 arg4–17 lys1 leu1 ura4 his5 met1*
CL-186	α *cyc1–9 rev1 arg4–17 lys1 leu1 his5 met1*	× **a** *cyc1–9 rev1 arg4–17 lys1 leu1 his5 trp5*
CL-189	α *cyc1–9 arg4–17 lys1 leu1 ura4 his5*	× **a** *cyc1–9 arg4–17 lys1 leu1 his5 ade2 trp5*
CL-190	α *cyc1–9 arg4–17 lys1 leu1 his5 ade2*	× **a** *cyc1–9 arg4–17 lys1 leu1 ura4 his5 trp5*
CL-194	α *cyc1–9 rad9 lys2*	× **a** *cyc1–9 rad9 met1 ade2*
CL-197	α *cyc1–9 rad9 ade2 trp5*	× **a** *cyc1–9 rad9 met1 lys2*
CL-198	α *cyc1–9 rad9 ade2 trp5*	× **a** *cyc1–9 met1 lys2*
CL-202	α *cyc1–131 arg4–17 trp5 met1*	× **a** *cyc1–131 rev1 arg4–17 his5 trp5 leu1*
CL-203	α *cyc1–131 rev1 arg4–17 lys1 ade2 met1*	× **a** *cyc1–131 arg4–17 his5 trp5 leu1*
CL-204	α *cyc1–9 rad6 arg4–17 ade2 leu1 met1 trp5*	× **a** *cyc1–9 rev1 arg4–17 lys1 leu1 his5 trp5*
CL-323	α *cyc1–13 his5 trp5 can^r ilv3 met1 ura4 leu1 tyr7*	× **a** *cyc1–1 his1 trp1 ade1*
CL-324	α *cyc1–13 his5 trp5 can^r ilv3 ura4*	× **a** *cyc1–1 his1 trp1 ade1*
CL-325	α *cyc1–13 rad7 his5 trp5 can^r met3 ura4*	× **a** *cyc1–1 his1 trp1 ade1*
CL-326	α *cyc1–13 rad7 his5 trp5 can^r met3 ura4 tyr7*	× **a** *cyc1–1 his1 trp1 ade1*
CL-327	α *cyc1–1 arg4–17 leu1 ura4 ade2 trp5*	× **a** *cyc1–1 arg4–17 lys1 ade2 trp5*
CL-328	α *cyc1–1 arg4–17 leu1 ura4 ade2 trp5*	× **a** *cyc1–1 arg4–17 rev1 leu1 his5 ade2 trp5*
CL-329	α *cyc1–1 arg4–17 leu1 ura4 ade2 trp5*	× **a** *arg4–17 lys1 ura4 his5 trp5*
CL-330	α *cyc1–1 arg4–17 leu1 ura4 ade2 trp5*	× **a** *arg4–17 rev1 lys1 his5 ade2 trp5*
CL-335	α *cyc1–9 rad15 lys1 leu1*	× **a** *cyc1–9 rad15 his1*
CL-336	α *cyc1–9 rad15 lys1*	× **a** *cyc1–9 rad15 his1*
CL-338	α *cyc1–9 arg4*	× **a** *cyc1–9 rad15 his1*
CL-339	α *cyc1–9 leu1*	× **a** *cyc1–9 rad15 his1*
CL-366	α *cyc1–9 rad9 his5 ade met1*	× **a** *cyc1–9 rad9 lys2 ade*
CL-367	α *cyc1–9 leu1 his5*	× **a** *cyc1–9 rad9 lys2 ade*

TABLE 2—Continued

Strain no.	Genotype	
CL-368	α *cyc1–9 leu1 his5 ade*	× **a** *cyc1–9 rad9 lys2 ade*
CL-369	α *cyc1–9 rad8 his5 ade2*	× **a** *cyc1–9 rad8 leu1 ade2 met1*
CL-370	α *cyc1–9 rad8 his5 ade2*	× **a** *cyc1–9 rad8 leu1 ade2 met1*
CL-371	α *cyc1–9 leu1 his5 ade2*	× **a** *cyc1–9 rad8 leu1 ade2 met1*
CL-372	α *cyc1–9 his5 ade2 met1*	× **a** *cyc1–9 rad8 leu1 ade2 met1*

diploids that were all UV-resistant, and these were used as additional controls (LC*, Tables 4 and 5). It has been shown recently (RODNEY ROTHSTEIN personal communication) that the parent strain (D311–3A) in which the *cyc1–131* mutation was induced is aneuploid for chromosome XI. It therefore appeared possible that *rad8* is located on this chromosome and that lack of 2:2 segregation was due to aneuploidy. Indirect support for this explanation comes from the observation that *rad8* segregated normally in a pedigree derived from a diploid heterozygous for this gene and also for *cyc1–9*. The parent containing the *cyc1–9* allele was selected from a pedigree in which the chromosome XI marker *met1* showed normal 2:2 segregation.

The three *rev* mutations were insufficiently radiation-sensitive to be identified reliably by the method outlined above. As a consequence, they were detected by their influence on the reversion of *arg4–17*, exposing approximately 10^7 cells plated on arginineless medium to 25 J m^{-2} of germicidal UV. After it had been demonstrated that *rev3* had a similar effect on the reversion of *cyc1–9*, this property was used in place of *arg4–17* reversion in a few instances. The genotype of diploid strains thought to be homozygous for one of the *rev* mutations was also verified by the *arg4–17* reversion test, appropriate *REV+* controls being constructed for this purpose.

The genotypes of spore clones in pedigrees segregating for mutations with similar or identical phenotypes were determined by crosses to suitable tester strains. This was necessary in the case of *rad1* and *rad6*, *rad1* and *rad22*, *rad6* and *rad9*, and *rad6* and *rad18*. It was also necessary for the *rad6/rev1* and *rad6/rev3* pedigrees since the *rev* alleles cannot be detected in the presence of the *rad6* mutation, which also reduces the reversion of *arg4–17*.

Determination of cyc1 *reversion frequency and surviving fraction*: All strains were grown for three days, that is to late stationary phase, in 10 mls of liquid YPD medium (Difco yeast extract, 1%; Bacto-peptone, 2%; dextrose, 2%) at 30° with vigorous shaking. Haploid cultures were briefly sonicated after harvesting to disrupt cell aggregates. The *cyc1* revertants were detected by spreading up to 1×10^7, or in a few cases up to 5×10^7, cells on plates containing a semi-synthetic lactate (SL) medium (Difco yeast nitrogen base without amino acids, 0.6%; Difco yeast extract, 0.05%; DL-lactate, 1%; Colab Ionagar no. 2S, 1.5%), supplemented with nutrilites where necessary. Colonies arising from revertants were counted after 5–7 days incubation at 30° and recounted after 10–14 days incubation. Viability was assessed on YPG medium, similar to YPD but containing 3% (v/v) glycerol in place of the dextrose and solidified with 1% Ionagar. Colonies growing on YPG medium were counted after 3–5 days incubation at 30°. Evidence supporting the validity and reliability of this procedure are given in LAWRENCE *et al.* (1974).

For the survey of mutability in the LC strain, a series of UV fluences comprising 0, 5, 10, 15 and 25 J m^{-2} was chosen to ensure that at least one comparison between radiation-sensitive strains and wild type could be made at a dose level which gave relatively high survival. This is important since artefacts can arise when survival is low. Some of the least sensitive strains were also examined after exposure to fluences of 50, 75 and 100 J m^{-2}. Induced reversion frequencies of *cyc1–131* were determined for groups of up to six strains at a time, each strain being examined at least twice on separate occasions, and the wild-type (*RAD+*) control strain (LC0) was always included in each group. Spontaneous reversion frequencies rarely exceeded 1×10^{-7} in most experiments and if higher than 5×10^{-7} the results were rejected and the experiment repeated. Standard errors were computed from the variation between replicate determinations or in some instances between both replicate strains and replicate determinations. Details regarding the UV radiation source and its dosimetry are given in LAWRENCE *et al.* (1974).

Samples of *cyc1* revertants induced in all haploid and most diploid strains were examined spectroscopically by the method of SHERMAN and SLONIMSKI (1964) to confirm that the scoring of intragenic revertants was accurate.

RESULTS

The rev3 rad6 *double mutant*: Although strains carrying either the *rev3–1* (LEMONTT 1971a, 1972) or *rad6–1* (LAWRENCE *et al.* 1974) alleles show very low frequencies of UV-induced mutations, in neither case is UV mutagenesis entirely eliminated. This might arise because some induced mutations are the consequence of a nonenzymatic process, because of the existence of more than one error-prone repair pathway, or because both the *rad6–1* and *rev3–1* alleles are leaky. A choice between these alternatives can be made by comparing the properties of the double mutant to those of either single mutant.

The results given in Figure 1, which shows survival curves for haploid strains, indicate that the *rev3 rad6* double mutant is no more sensitive to UV than the *rad6* single mutant, and this suggests that *rad6–1* and *rev3–1* block the same repair pathway. Each curve in Figure 1 is the average of results from at least three, and in some cases four, different segregants. A similar, though somewhat less clear cut, result was obtained with diploid strains (Figure 2). Each curve is the average of data from two independent strains. The slopes of the survival curves for the *rad6* and *rev3 rad6* diploids are virtually identical and differ only with respect to a larger inflection in the former case. The reason for the inflection

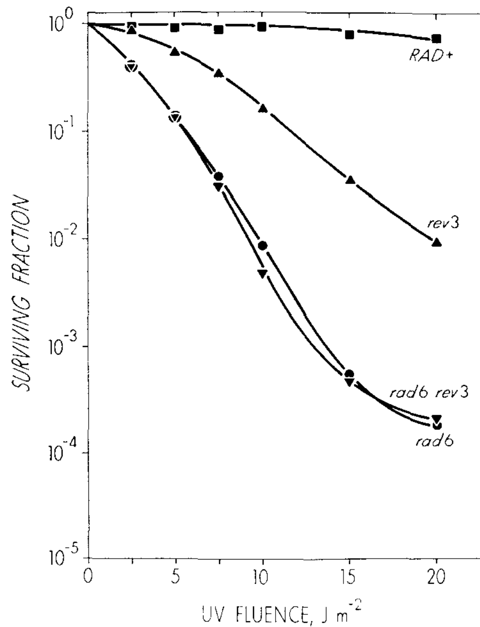

FIGURE 1.—Average survival curves for *RAD+* (CL110-2A, -4A, -14C), *rev3* (CL110-4D, -7A, -12A, -21A), *rad6* (CL110-7C, -8D, -13D, -18C) and *rev3 rad6* (CL110-3B, -3D, -19A) haploid strains.

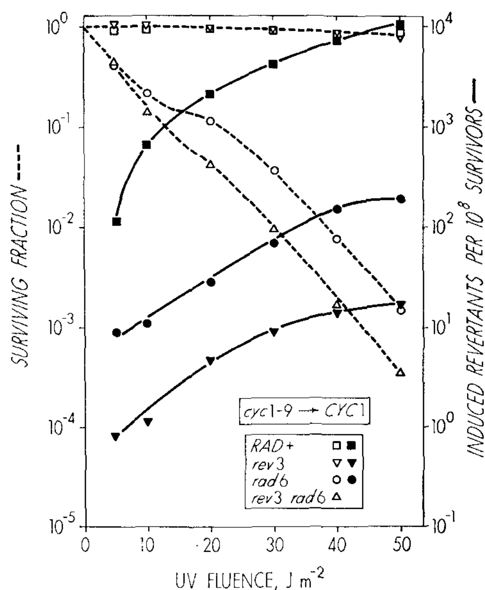

FIGURE 2.—Average survival and *cyc1–9* induced reversion dose-response curves for *RAD+* (CL-132, CL-134), *rev3* (CL-135, CL-137), *rad6* (CL-138, CL-139), and *rev3 rad6* (CL-141, CL-143) diploid strains.

is not known, though similar results have been obtained previously with other *rad6* strains. Figure 2 also gives the frequencies of *cyc1–9* revertants induced in these diploids and, as expected, shows that the reversion frequency is much reduced by the presence of the *rad6* or *rev3* mutations, though not eliminated. No revertants at all were found in the *rev3 rad6* double mutant strains, even though more than 6×10^8 cells were plated for each dose point. This suggests that UV mutagenesis is entirely absent in these strains, a conclusion which was further tested in a larger scale experiment using a single fluence of 15 J m^{-8} (Table 3). A significant, even if small, increase in the frequency of revertants was found in irradiated *rev3* diploids, but there was no evidence for the induction of revertants in the double mutant strains, even though a total of about 1×10^{10} cells from each of them was irradiated. These results suggest that the occurrence of reversion by

TABLE 3

cyc1–9 *reversion frequencies in two* rev3 *and two* rev3 rad6 *diploid strains exposed to a fluence of 15 J m^{-2} of 254 nm UV*

Strain	Revertants per 10^8 survivors		% Survival
	0 J m^{-2}	15 J m^{-2}	
CL-135 *rev3*	1.00 ± 0.32	2.41 ± 0.49	105
CL-137 *rev3*	0.25 ± 0.11	1.71 ± 0.33	110
CL-141 *rev3 rad6*	0.57 ± 0.10	0.70 ± 0.20	14
CL-143 *rev3 rad6*	0.04 ± 0.02	0.00	6

a nonenzymatic process is, at best, an extremely rare event. Viewed together, the results from the experiments with the *rev3 rad6* double mutant imply that there is a single error-prone repair pathway in yeast which can be substantially, though not entirely, blocked by either mutant alone. The reason for the "leakiness" of these mutations is not known, though since *rad6–1* is an amber mutation (LAWRENCE *et al.* 1974), it may be susceptible to some kind of transient or low level phenotypic suppression in these strains. *CYC1* revertants induced in *rad6* strains remain fully UV sensitive, however, and do not therefore arise in cells containing a spontaneously arising amber suppressor of the usual sort. The mutational alteration in the *rev3–1* allele is unknown.

Other radiation-sensitive mutations that reduce the mutagenic effectiveness of UV: A survey of the influence of other radiation-sensitive (*rad* or *rev*) genes on the process of UV mutagenesis was carried out using twenty diploid (LC) strains each homozygous for *cyc1–131* and for a different, nonallelic radiation-sensitive mutation. The frequency of *cyc1–131* revertants, measured after exposure of the strains to a range of UV fluences, was used to indicate whether

TABLE 4

UV-induced reversion frequencies of cyc1–131 *in diploid strains homozygous for various* rad *or* rev *mutations*

Strain		Induced revertants/10^8 survivors							
		5 J m^{-2}		10 J m^{-2}		15 J m^{-2}		25 J m^{-2}	
LC-0	*RAD+*	19 ± 3	(101)	51 ± 5	(101)	85 ± 7	(100)	156 ± 12	(98)
LC*	*RAD+*	11 ± 2	(105)	28 ± 5	(102)	52 ± 7	(100)	132 ± 8	(98)
LC-12	*rad1*	261 ± 61	(94)	463 ± 37	(95)	590 ± 72	(75)	846 ± 159	(30)
LC-2	*rad2*	367 ± 94	(47)	801 ± 72	(2.3)	2790 ± 330	(0.18)	4790 ± 957	(0.03)
LC-13	*rad3*	829 ± 302	(12)	2700 ± 1070	(0.09)	—	—	—	—
LC-4	*rad4*	467 ± 119	(74)	1010 ± 270	(24)	1810 ± 509	(6.2)	4560 ± 1550	(0.58)
LC-6	*rad6*	0	(45)	0	(5.0)	0	(1.0)	0	(0.09)
LC-9	*rad9*	9 ± 1	(102)	14 ± 1	(98)	41 ± 4	(79)	82 ± 1	(52)
LC-10	*rad10**	731 ± 74	(81)	1280 ± 246	(28)	1680 ± 624	(8.6)	2320 ± 753	(1.3)
LC-18	*rad12*	388 ± 73	(40)	537 ± 163	(0.57)	1150 ± 0	(0.01)	—	—
LC-3	*rad13*	0	(90)	5 ± 2	(77)	7 ± 4	(74)	39 ± 26	(60)
LC-14	*rad14*	59 ± 18	(103)	86 ± 1	(107)	101 ± 16	(103)	103 ± 17	(86)
LC-15	*rad15*	10 ± 1	(95)	25 ± 2	(80)	51 ± 7	(54)	56 ± 16	(16)
LC-16	*rad16*	7 ± 7	(96)	33 ± 11	(92)	50 ± 8	(93)	200 ± 98	(57)
LC-17	*rad17*	5 ± 3	(96)	30 ± 5	(94)	83 ± 25	(75)	138 ± 31	(40)
LC-23	*rad18*	6 ± 2	(64)	16 ± 4	(41)	39 ± 13	(24)	210 ± 52	(8.3)
LC-22	*rad22*	73 ± 55	(63)	208 ± 192	(18)	420 ± 178	(5.2)	1210 ± 230	(0.50)
LC-5	*rad50**	5 ± 2	(95)	18 ± 8	(89)	22 ± 9	(87)	66 ± 13	(82)
LC-25	*rad52**	29 ± 24	(89)	52 ± 21	(91)	103 ± 7	(77)	202 ± 102	(71)
LC-26	*rev1*†	3 ± 1	(103)	13 ± 4	(100)	27 ± 3	(97)	80 ± 11	(95)
LC-27	*rev2*	5 ± 1	(107)	13 ± 4	(97)	17 ± 6	(105)	105 ± 33	(76)
LC-28	*rev3*	0	(99)	0.3 ± 0.3	(98)	0.7 ± 0.7	(94)	2.1 ± 0.2	(91)

Each entry in the table is the average of at least two determinations. Percent survival in parentheses.
* Average of two replicate strains.
† Average of four replicate strains.

each strain was capable of normal UV mutagenesis. Diploids were used in preference to haploid strains because they give clearer results (PRAKASH and SHERMAN 1973). "Suppressor" mutations, not to be confused with nonsense or missense suppressors, which allow growth on lactate medium in the absence of appreciable amounts of iso-1-cytochrome *c* are much less frequent in diploids, no doubt because most are recessive. Spectroscopic examination of samples of revertants verified that they were indeed of negligible importance in the present results. It was expected that these results, given in Table 4, would confirm previous observations that the *rad6*, *rad18*, *rev1*, and *rev3* gene products were required for normal UV mutagenesis, and it was hoped that other genes involved in this process would also be uncovered. On the contrary, as can be seen from Table 4, only two strains, those homozygous for *rad6* and *rev3*, gave *cyc1–131* reversion frequencies substantially lower than the controls. LC-3, a strain homozygous for *rad13*, also gave *cyc1–131* reversion frequencies that were appreciably lower than the controls when exposed to this range of fluences, but was normal when exposed to higher fluences (Table 5). Since the revertants grew poorly on lactate medium, the results obtained with the lower fluences may be misleading. Data from two types of control are shown; LC-0 was the wild-type diploid included as standard in each group of strains examined, and LC* represents several strains supposedly homozygous for *rad5*, *rad8*, *rad11*, *rad19*, *rad20*, and *rad21* but which proved to be no more UV sensitive than LC-0. The difference between these two sets of data, which is not very great, provides an indication of the effect of variation in genetic background on the reversion frequency of *cyc1–131*.

TABLE 5

UV-induced reversion frequencies of cyc1–131 *in diploid strains homozygous for various* rad *or* rev *mutations*

Strain		Induced revertants/10^8 survivors		
		50 J m^{-2}	75 J m^{-2}	100 J m^{-2}
LC-0	*RAD+*	343 ± 37 (89)	574 ± 37 (56)	1260 ± 163 (26)
LC*	*RAD+*	375 ± 46 (93)	973 ± 123 (70)	2170 ± 306 (43)
LC-9	*rad9*	248 ± 44 (8.4)	444 ± 46 (2.3)	1030 ± 187 (0.62)
LC-3	*rad13*	219 ± 145 (15)	290 ± 120 (4.7)	1170 ± 831 (1.9)
LC-14	*rad14*	302 ± 33 (34)	1320 ± 81 (6.4)	6830 ± 440 (0.88)
LC-15	*rad15*	156 ± 42 (1.3)	945 ± 428 (0.22)	2190 ± 743 (0.09)
LC-16	*rad16*	814 ± 473 (3.9)	2940 ± 761 (0.62)	9230 ± 344 (0.11)
LC-17	*rad17*	243 ± 10 (3.3)	1390 ± 96 (0.23)	4930 ± 2610 (0.05)
LC-5	*rad50**	214 ± 2 (65)	595 ± 16 (47)	1790 ± 92 (22)
LC-25	*rad52*	453 ± 7 (50)	797 ± 383 (11)	1250 ± 649 (3.1)
LC-26	*rev1*†	290 ± 26 (72)	627 ± 64 (35)	1220 ± 221 (9.6)

Each entry in the table is the average of at least two determinations. Percent survival in parentheses.

* Average of two replicate strains.

† Average of four replicate strains.

The absence of any effect of the *rev1–1* mutation on *cyc1–131* reversion is particularly surprising since this radiation-sensitive mutation has been shown to substantially reduce forward mutation to auxotrophy (LEMONTT 1972). Since the results given in Table 4 represent the average of data taken from four independent *rev1* strains, it is unlikely that some unspecified gene is suppressing the *rev1–1* allele. Moreover, these strains, as well as those homozygous for *rec2–1* and *rev3–1*, all show the low *arg4–17* reversion frequencies characteristic of *rev* strains (Figure 3). Extending the fluence range also fails to reveal any difference between the *rev1* and *REV+* strains (Table 5).

It therefore seems likely that, as with the *rev2* gene (LEMONTT 1971a, 1972), the gene products of the *rev1* and *rad18* genes are required for the UV-induced reversion of only some alleles and not for others. It also seems likely that the mode of UV-induced reversion of *cyc1–131* is atypical of most induced mutational alterations and that as a consequence several radiation-sensitive mutations that, like *rev1–1* and *rad18–2*, reduce some modes of UV mutagenesis, may well have remained undetected in the survey.

To explore this situation further, and also to confirm that *rev1–1* can influence the reversion of a *cyc1* allele, a limited number of diploids were constructed that were homozygous for *cyc1–9* as well as a radiation-sensitive mutation. In addition to *rev1–1*, mutations at the *rad8*, *rad9*, and *rad15* loci were chosen for this study because some (*rad9* and *rad15*) had been shown to reduce the effectiveness of chemical mutagenesis (PRAKASH (1975) and also because strains carrying these mutations are, like those containing *rad6*, *rad18* or *rev* mutations, sensitive

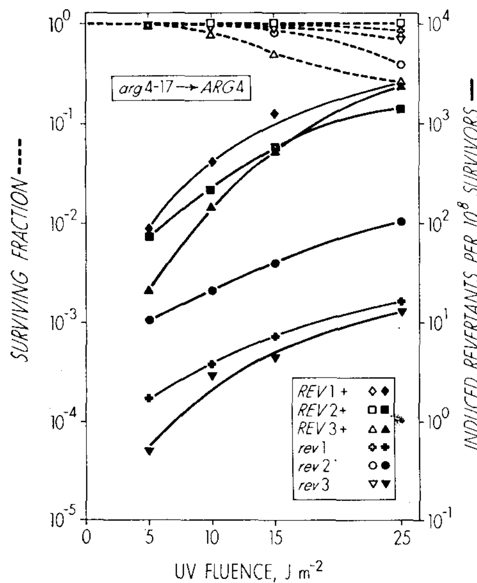

FIGURE 3.—*arg4–17* reversion dose-response curves for *REV1+* (CL-202, CL-203), *REV2+* (CL-127), *REV3+* (CL-128), *rev1* (LC-26, -26a, -26b, -26c), *rev2* (LC-27) and *rev3* (LC-28) diploid strains.

TABLE 6

Induced reversion frequencies of cyc1–9 *in diploid strains heterozygous or homozygous for* **rev1**

UV fluence J m^{-2}	Induced revertants/10^8 survivors and survival			
	rev1 cyc1–9		*REV1 cyc1–9*	
	CL-185	CL-186	CL-189	CL-190
5	3 (88)	0 (88)	141 (100)	143 (96)
10	12 (91)	7 (96)	781 (101)	393 (90)
15	12 (90)	8 (86)	1280 (110)	1350 (103)
25	33 (90)	22 (71)	3350 (98)	3940 (94)
50	188 (36)	118 (40)	9520 (82)	10400 (73)
75	847 (10)	887 (10)	15600 (54)	22700 (49)
100	866 (5.9)	2045 (1.9)	25100 (18)	35100 (26)

Percent survival in parentheses.

to X-rays as well as to UV light. In each case two diploid strains were constructed, together with two wild-type (*REV*+, *RAD*+) controls.

The results from *rev1* diploids are given in Table 6, and shown graphically in Figure 4. They clearly show that the *rev1–1* mutation has a large effect on the UV-induced reversion of *cyc1–9*, reducing the frequency to only a few percent of that found in the *REV*+ strains. This contrasts markedly with the data for *cyc1–131* reversion (Figure 5). The influence of *rad9* is less clear cut (Table 7). Although the average reversion frequency of *cyc1–9* is reduced more than ten-fold at the higher fluence levels (Figure 6), there is little difference at the lower

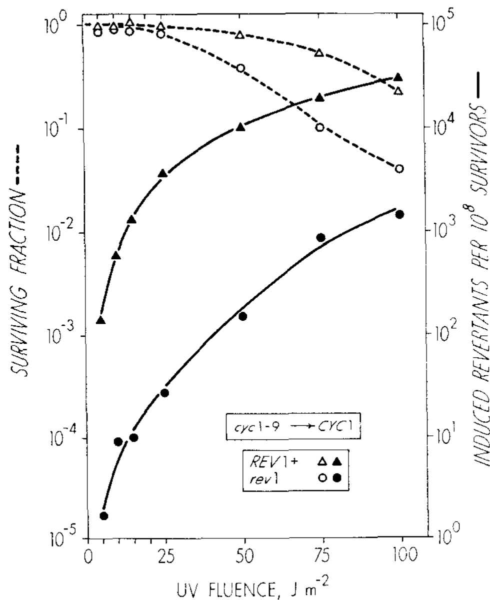

FIGURE 4.—Average *cyc1–9* reversion dose-response curves for *REV1*+ (CL-189, CL-190) and *rev1* (CL-185, CL-186) diploid strains.

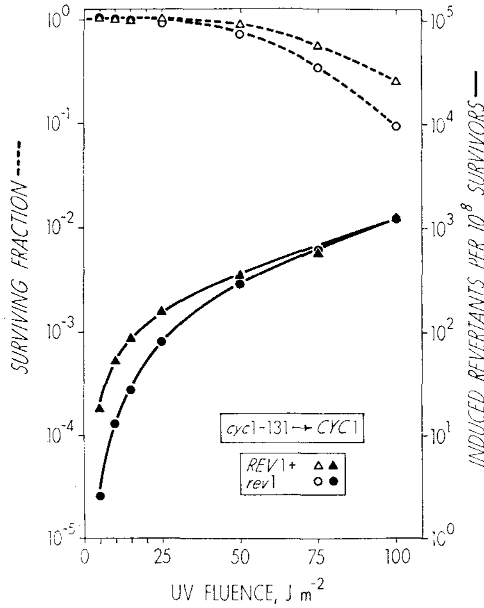

FIGURE 5.—*cyc1–131* reversion dose-response curves for *REV+* (LC-O) and *rev1* (LC-26, -26a, -26b, -26c) diploid strains.

levels. The first two *rad9* strains made were rather heterogeneous with respect to induced reversion frequency so a third *rad9* diploid was constructed, together with controls, from an independent pedigree. This set confirmed the earlier result, and statistical analysis of the total data showed that the difference in reversion frequency between *rad9* and *RAD+* strains was highly significant ($P < 0.001$). Such a result again contrasts with the lack of influence of *rad9* on the reversion of *cyc1–131* (Figure 7). The *rad15–1* mutation, on the other hand, has as little effect on the UV-induced reversion of *cyc1–9* (Table 8) as of *cyc1–131* (Tables 4 and 5). Finally, the *rad8–1* mutation, whose effect on

TABLE 7

Induced reversion frequencies for cyc1–9 *in diploid strains heterozygous or homozygous for* rad9

UV fluence J m^{-2}	Induced revertants/10^8 survivors and survival					
	rad9 cyc1–9			*RAD9 cyc1–9*		
	CL-194	CL-197	CL-366	CL-198	CL-367	CL-368
5	444 (111)	49 (105)	24 (110)	455 (97)	124 (94)	156 (91)
10	1040 (108)	148 (81)	125 (102)	1290 (103)	684 (95)	528 (102)
15	1820 (108)	346 (75)	370 (77)	2570 (100)	1516 (83)	1027 (107)
25	2230 (78)	912 (25)	448 (61)	7160 (106)	3621 (97)	2602 (104)
50	4700 (1.7)	901 (8.2)	382 (23)	22200 (87)	9427 (84)	8748 (91)
75	5410 (0.47)	1360 (1.7)	408 (7.1)	40800 (73)	12591 (86)	15877 (93)
100	3080 (0.11)	834 (0.59)	1481 (3.9)	40400 (66)	15834 (60)	24751 (76)

Percent survival in parentheses.

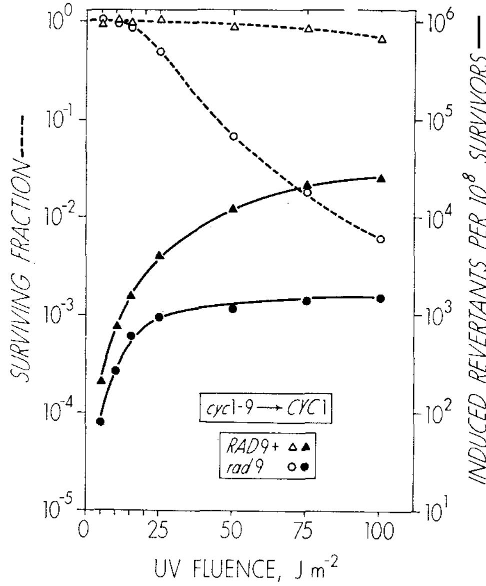

FIGURE 6.—Average *cyc1–9* reversion dose-response curves for *RAD9*+ (CL-198, CL-367, CL-368) and *rad9* (CL-194, CL-197, CL-366) diploid strains.

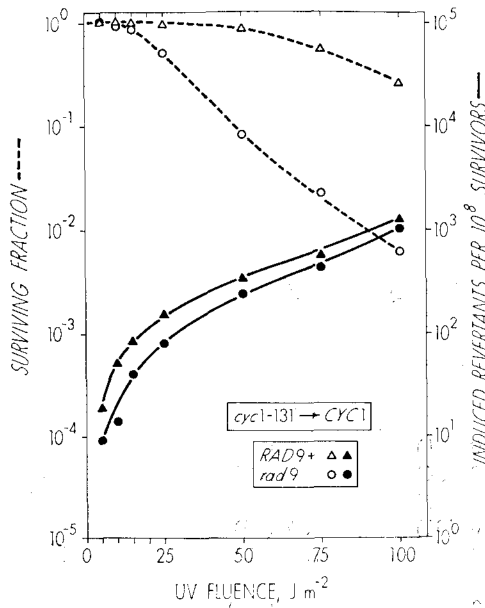

FIGURE 7.—*cyc1–131* reversion dose-response curves for *RAD9*+ (LC-O) and *rad9* (LC-9) diploid strains.

TABLE 8

Induced reversion frequencies of cyc1-9 *in diploid strains heterozygous and homozygous for* rad15

UV fluence J m^{-2}	Induced revertants/10^8 survivors and survival			
	rad15 cyc1-9		*RAD15 cyc1-9*	
	CL-335	CL-336	CL-338	CL-339
5	92 (103)	195 (90)	59 (96)	190 (88)
10	446 (85)	825 (84)	326 (91)	427 (98)
15	787 (70)	1266 (84)	797 (107)	1274 (89)
25	1068 (46)	3071 (56)	2119 (92)	2222 (96)
50	2458 (9.0)	5112 (12)	5893 (89)	6272 (95)
75	4939 (0.83)	16127 (0.75)	8759 (75)	11274 (80)
100	12779 (0.061)	23471 (0.094)	16129 (40)	15116 (67)

Percent survival in parentheses.

cyc1–131 reversion we were unable to test, appears to have a very substantial influence on *cyc1–9* reversion (Table 9, Figure 8); one *rad8* diploid failed to give any induced revertants at all, while revertants were induced in the other at a frequency over a hundred-fold smaller than in the wild-type controls.

It appears, therefore, that the products of at least seven genes are required for normal UV mutagenesis; *rev1*, *rev2* and *rev3* (LEMONTT 1971a), *rad6*, *rad8*, *rad9* and *rad18* (LAWRENCE *et al.* 1974; present results). Two of these genes, *rev3* and *rad6* would appear to be essential for the production of all kinds of mutations at many and possibly all sites in the genome, while the other have a more restricted activity. The reversion of *cyc1–131* appears to be unusual since it does not require the products of the *rev1*, *rad9*, and *rad18* genes.

The survival characteristics of double mutant strains carrying rad6–1 *and either* rad9–1, rad18–2, *or* rev1–1: Data have already been presented in an earlier section that show that *rev3 rad6* double mutant strains are no more sensitive to UV light than *rad6* haploids or diploids, a result consistent with the view

TABLE 9

Induced reversion frequencies of cyc1-9 *in diploid strains heterozygous and homozygous for* rad8

UV fluence J m^{-2}	Induced revertants/10^8 survivors and survival			
	rad8 cyc1-9		*RAD8 cyc1-9*	
	CL-369	CL-370	CL-371	CL-372
5	0 (91)	1 (91)	364 (100)	135 (95)
10	0 (94)	1 (87)	488 (113)	473 (101)
15	0 (95)	10 (85)	821 (115)	791 (98)
25	0 (91)	34 (89)	3392 (96)	2755 (92)
50	0 (63)	103 (49)	11944 (98)	7848 (95)
75	0 (27)	110 (18)	30802 (74)	21432 (82)
100	0 (7.6)	171 (7.3)	39273 (72)	35186 (61)

Percent survival in parentheses.

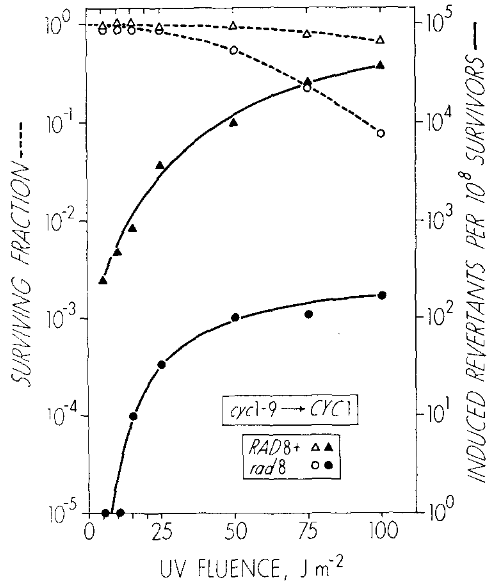

FIGURE 8.—Average *cyc1-9* reversion dose-response curves for *RAD8+* (CL-371, CL-372) and *rad8* (CL-369, CL-370) diploid strains.

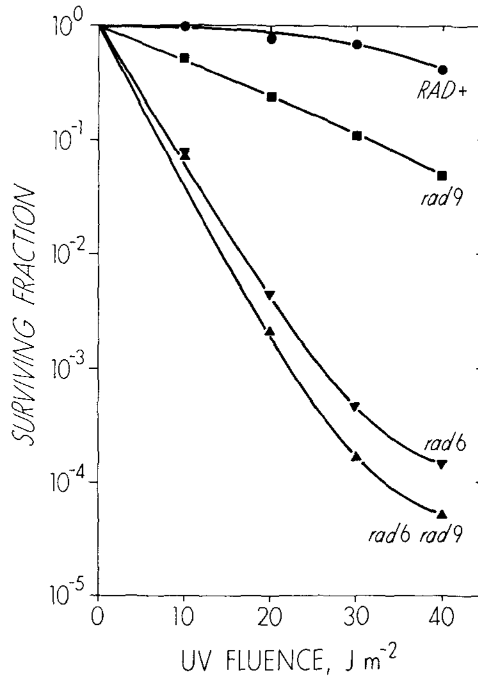

FIGURE 9.—Average survival curves for *RAD+* (CL-164-4A, -6B, -8A, -9B), *rad9* (CL-164-4B, -6A, -8C, -9A), *rad6* (CL164-4D, -6D, -8B, -9D) and *rad6 rad9* (CL164-4C, -6C, -8D, -9C) haploid strains.

that these two genes are concerned with the same repair process and act within the same repair pathway. It might be expected therefore that a similar result would be obtained with strains carrying *rad6* and any of the other mutations which influence UV-induced reversion frequencies. Sets of haploid strains were therefore constructed which contained either *rad6 rad9*, *rad6 rad18* or *rad6 rev1* double mutants, together with appropriate single-mutant and wild-type controls. Survival curves were obtained from four replicate strains of each type in every set in order to diminish the effects of variation in background genotype. It should be emphasized that data from single strains can be very misleading. The results, given in Figures 9, 10, and 11, show that the UV sensitivities of the double mutants are in all cases only slightly greater than those of the *rad6* strains. These survival curves are therefore consistent with the view that the *rad6*, *rad9*, *rad18*, and *rev1 loci* are each concerned with a single pathway for the repair of UV-damaged DNA.

Radiation-sensitive mutations that enhance UV mutagenesis: Inspection of Table 4 reveals that seven strains, those homozygous for *rad1*, *rad2*, *rad3*, *rad4*, *rad10*, *rad12*, and *rad22*, all show enhanced frequencies of *cyc1–131* reversion. Such a result with the first four strains is not unexpected, since the *rad1*, *rad2*, *rad3*, and *rad4* genes all appear to be concerned with excision repair (UNRAU, WHEATCROFT and COX 1971; RESNICK and SETLOW 1972; GAME and COX 1972). Excision defective strains of yeast, like those of *E. coli*, all appear to enhance UV-induced mutation rates (RESNICK 1969; MOUSTACCHI 1969, 1971; ZAKHAROV, KOZINA and FEDOROVA 1970; AVERBECK *et al.* 1970; LAWRENCE *et al.* 1974). It should be noted that the *rad1* allele in LC-12 is leaky, and the strain much less sensitive than those studied previously (LAWRENCE *et al.* 1974) which carried *rad1–2*. Studies involving liquid holding and photoreactivation (PARRY and PARRY 1969) provided no evidence, however, to indicate that *rad10*, *rad12*, or *rad22* mutant strains lack excision capabilities. During the course of a further examination of these genes, which involved the construction of strains simultaneously carrying *rad1–2* and mutations at one of the above three loci, it became apparent that the *rad12* mutation from our collection was an allele of the *rad1* gene. This prompted us to look at the relationship between *rad10* and *rad22* and the four genes concerned with excision. From this it emerged that the *rad10* mutation was an allele of the *rad4* locus but that the *rad22* mutation complemented mutations at all four loci. Strains carrying the *rad10* or *rad12* mutations are not as UV-sensitive as strains carrying other *rad4* or *rad1* alleles, suggesting that these mutations may be leaky. This may explain why photoreactivability was lost during liquid holding (PARRY and PARRY 1969). Except for the removal of a resistant tail, double mutant strains carrying *rad1–2* and *rad22* are no more sensitive than *rad1* single mutant strains (Figure 12), suggesting that the *rad22* gene product is required for excision repair. If so, at least five genes are required for excision repair, mutations in each of which increase sensitivity to both the mutagenic and lethal properties of UV light.

The remaining radiation-sensitive mutations: Six strains, those homozygous for *rad13*, *rad14*, *rad16*, *rad17*, *rad50*, and *rad52* remain unaccounted for by the

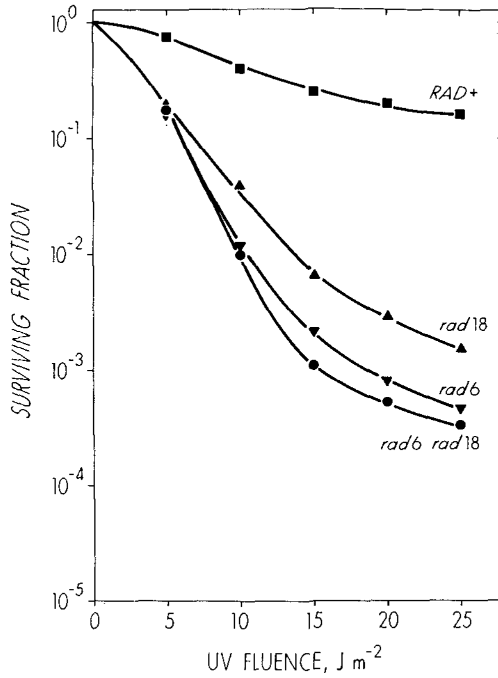

FIGURE 10.—Average survival curves for *RAD+* (CL167-1B, 4A, -5A, -6A), *rad18* (CL167-1A, -4B, -5D, -6D), *rad6* (CL167-1D, -4D, -5C, -6C), and *rad6 rad18* (CL167-1C, -4C, -5B, -6B) haploid strains.

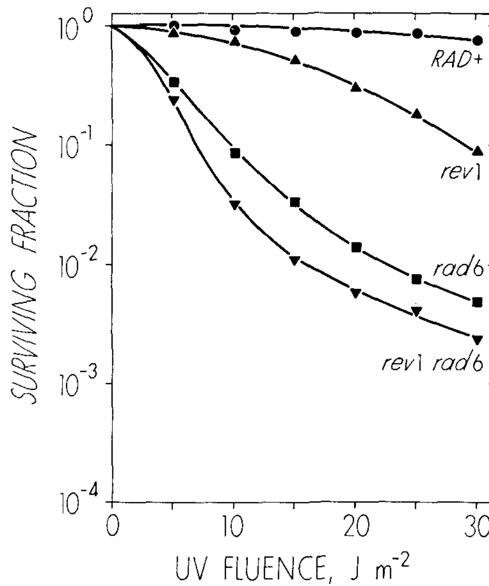

FIGURE 11.—Average survival curves for *RAD+* (CL204-1A, -4A, -5A, -8A), *rev1* (CL204-1B, -4B, -5B), *rad6* (CL204-1D, -4D, -5D, -8D) and *rev1 rad6* (CL204-1C, -4C, -5C, -8C) haploid strains.

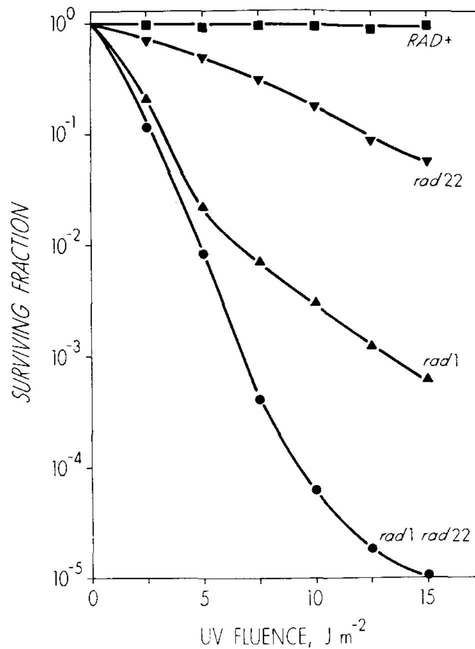

FIGURE 12.—Average survival curves for *RAD+* (CL168-17A, -17D, -19B, -19C), *rad22* (CL168-5A, -5B, -8B, -8D), *rad1* (CL168-5C, -5D, -8A, -8C) and *rad1 rad22* (CL168-17B, -17C, -19A, 19D) haploid strains.

two previous categories. None are very sensitive to UV light (Table 4), although the *rad50* and *rad52* strains are quite X-ray sensitive (COX and PARRY 1968; RESNICK 1969). Reversion of *cyc1–131* occurs at a normal frequency in all of these strains (Table 4), even when they are exposed to higher UV fluences (Table 5), thought the *rad14* and *rad16* strains may show marginally higher frequencies than the control. The *rad7* mutation could not be included easily in this survey because the *rad7* and *cyc1* loci are very tightly linked (LAWRENCE *et al.* 1975). Strains carrying *cyc1–13*, a mutation which contains an isoleucine codon in place of the initiation codon (STEWART *et al.* 1971), as well as *rad7*, were available from an earlier study, however, and two of these together with two *RAD+* segregants were crossed to a strain carrying *cyc1–1* (Table 2). The *cyc1–1* mutation is a deletion of the whole of the *cyc1* gene which extends into the *rad7* locus, and is phenotypically *rad7* (SINGH and SHERMAN, unpublished results). Since UV-induced reversion of *cyc1–13* occurs at comparable frequencies in each of these four diploid strains (Table 10), there is no evidence that the *rad7* locus is involved in mutagenic repair. Data regarding the reversion of *arg4–17* in diploid strains homozygous and heterozygous for *cyc1–1* also support this conclusion (Table 10).

It is not clear whether most of the *rad* mutations in this group have little effect on survival and mutability because they are "leaky" or because the genes are only remotely and indirectly involved in repair processes. The *rad50* and *rad52*

TABLE 10

Induced reversion frequencies of cyc1–13 *and* arg4–17 *in diploid strains heterozygous and homozygous for* rad7

UV fluence J m^{-2}	Induced revertants/10^8 survivors and survival			
	rad7 cyc1–13		*RAD7 cyc1–13*	
	CL-325	CL-326	CL-323	CL-324
25	24 (49)	30 (41)	54 (89)	52 (97)
50	314 (11)	250 (7.0)	135 (95)	168 (94)
	rad7 arg4–17		*RAD7 arg4–17*	
	CL-327	CL-328	CL-329	CL-330
20	16700 (62)	12843 (73)	3190 (97)	1669 (99)
40	34387 (12)	34331 (11)	9808 (85)	5316 (88)

Percent survival in parentheses.

mutants are substantially X-ray sensitive, however, and it has been postulated that these genes are concerned with a minor pathway for the repair of UV damage (Cox and GAME 1974).

The influence of rad52 *on UV mutagenesis in excision-defective strains*: If *rad50* and *rad52* are indeed concerned with a minor pathway distinct from excision or mutagenic repair (Cox and GAME 1974), it might be imagined that these mutations would lead to increased mutability per unit dose of UV like the excision-defective mutants, though to a lesser extent. Such an increase has in fact been observed in a *rad50* strain (Cox and GAME 1974) but was not found in the present results. This lack of an increase may occur because of a compensatory increase in the extent of excision repair in *rad50* and *rad52* strains, and if this is the case the influence of these mutations on mutability should become more obvious in excision-defective strains.

To test this prediction, *cyc1–9* reversion was studied in sixteen haploid strains, including four each that were either wild type, *rad1*, *rad52*, or *rad1 rad52* in constitution (Figure 13). As is commonly encountered, replicate strains gave rather variable results, particularly those that were wild-type, but it is clear that *cyc1–9* reversion frequencies are very similar in wild-type and *rad52* haploids. As expected, reversion frequencies are more than ten-fold higher in *rad1* strains but in *rad1 rad52* double mutant strains *cyc1–9* reverts at frequencies that are nearly ten-fold higher again. These results therefore support the view that the *rad52* gene product is concerned with error-free repair, but since the survival curve for *rad1* and *rad1 rad52* strains have almost identical slopes, differing only in the presence or absence of a shoulder, it is not clear whether these *rad* genes act in the same or different pathways.

The influence of rad6 *in excision-defective strains*: Previous results (LAWRENCE *et al.* 1974) from a set of haploid strains that included a wild-type, *rad1–2* and *rad6–1* single mutants and a *rad1–2 rad6–1* double mutant showed that the double mutant was "super sensitive" to the lethal effect of UV light. The data

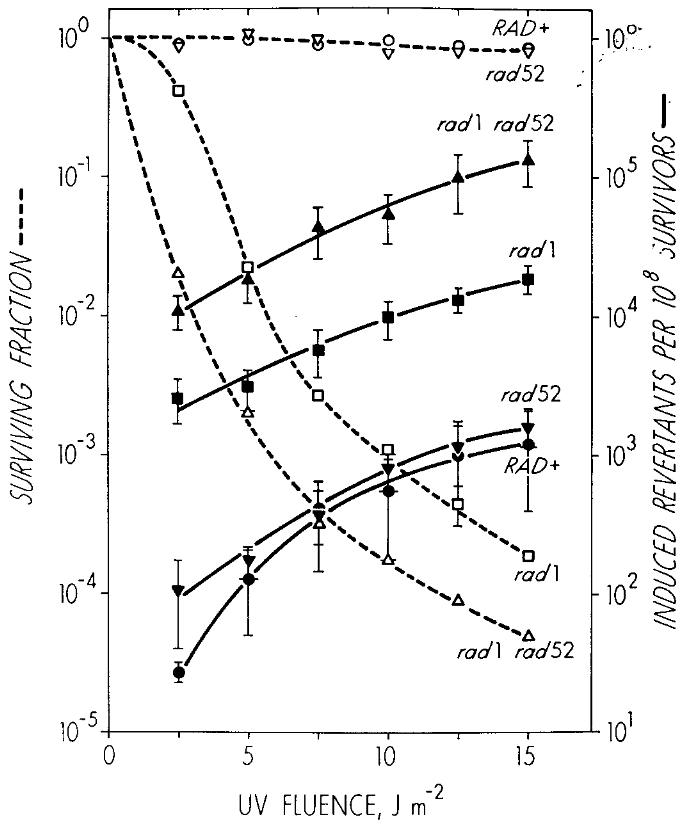

FIGURE 13.—Average survival and *cyc1–9* reversion dose-response curve for *RAD+* (CLC162-1B, -2C, -3B, -12B), *rad52* (CL162-4D, -6C, -9D, -10D), *rad1* (CL162-5A, -7B, -10A, -11B) and *rad1 rad52* (CL162-1D, -2B, -12C, -14C) haploid strains.

were also consistent with the view that the double mutant possessed the low level of UV mutagenesis characteristic of *rad6* strains, but the extreme sensitivity of the double mutant prevented a firm conclusion on this point. Attempts were therefore made to examine this point in comparable diploid strains, and the results from this experiment are shown in Figure 14. These results suggest that diploidy has increased the UV resistance of all but the double mutant strain. They also show that reversion of the ochre allele *cyc1–9* is enhanced about ten-fold in the *rad1* diploid, and diminished ten to a hundred-fold in the *rad6* strain, as expected. No UV-induced reversions were obtained in the double mutant strain, and sufficient viable cells were examined to establish that the level of reversion was significantly lower than in the wild-type control. It would therefore appear that, with regard to UV mutagenesis, *rad6* is epistatic to *rad1* in much the same way that the three *rev* mutants are epistatic to *rad2* (LEMONTT 1971b).

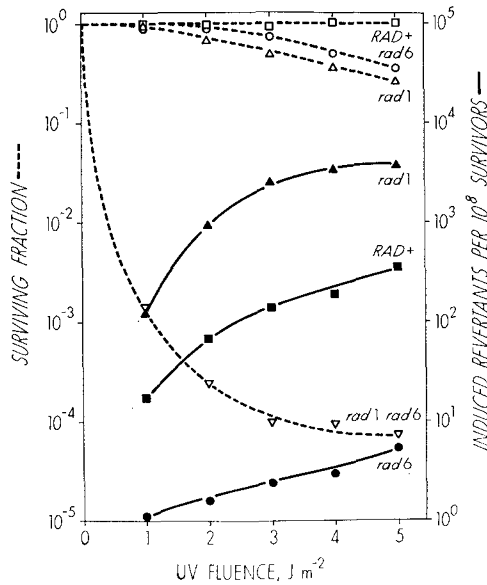

FIGURE 14.—Survival and *cyc1–9* reversion dose-response curves for *RAD+* (CL-174), *rad1* (CL-172), *rad6* (CL-173) and *rad1 rad6* (CL-171) diploid strains.

DISCUSSION

The aims of the experiments described in this report were to examine the relationship between the four mutations *rev1*, *rev3* (LEMONTT 1971a), *rad6* and *rad18* (LAWRENCE *et al.* 1974), each of which increases the lethal and decreases the mutagenic effectiveness of UV light, to determine the number of "error-prone" repair pathways in yeast, to search for other *rad* genes that act in the pathway(s) and to examine the properties of these *rad* genes.

Among the four mutations studied previously, *rev3* and *rad6* possess the most extreme phenotypes with respect to UV mutagenesis. Haploid and diploid strains that carry these mutations exhibit very low frequencies of forward or reverse mutations in all systems tested (LEMONTT 1971a, 1972; LAWRENCE *et al.* 1974), though in both cases the frequencies are significantly above the spontaneous level. This may be because some mutations arise by a nonenzymatic process because *rev3–1* and *rad6–1* each block a separate repair pathway or because both are slightly leaky mutations. Experiments described in the present report show that double mutant haploid or diploid strains carrying both *rev3–1* and *rad6–1* are no more sensitive to the lethal effect of UV light than strains which carry *rad6–1* alone, this being the more sensitive of the two single mutants. An epistatic relationship of this kind suggests that the products of these genes act within the same repair pathway. At the same time the double mutant strains showed no detectable frequency of induced reversions for the normally highly UV-revertible ochre allele *cyc1–9*, even though a total of more than 2.5×10^9 viable cells

were tested; spontaneous reversions could be recovered in these strains, however. The most simple interpretation of these results is that there exists a single mutagenic or "error-prone" repair pathway for UV damage in Saccharomyces, and that the *rev3–1* and *rad6–1* mutations are very slightly leaky. It should be emphasized that the level of leakiness envisaged is very low, far lower than that normally associated with the use of that term, since the detection of induced mutations provides a very sensitive test.

In addition to *rev3* and *rad6*, five other genes would appear to be involved in "error-prone" repair in yeast, including *rev1*, *rev2*, *rad8*, *rad9*, and *rad18*. Strains carrying mutations of any one of these genes are, like *rad6* and *rev3*, all more UV- and X-ray-sensitive than comparable wild type (LEMONTT 1971a; COX and PARRY 1968; RESNICK 1969), and all exhibit reduced levels of UV mutagenesis with respect to at least one of the test systems employed. *rev1* mutant strains show much reduced UV-induced reversion of *arg4–17*, *arg4–6*, *lys1–1* (LEMONTT 1971a), forward mutation to auxotrophy (LEMONTT 1972), as well as reversion of *cyc1–9* (present results) and other *cyc1* nonsense, missense and frameshift alleles (unpublished data); the *rev1–1* mutation does not, however, influence the UV-induced reversion of *cyc1–131*. Strains carrying *rev2–1* show reduced reversion of the ochre alleles *arg4–17*, *lys1–1* (LEMONTT 1971a) and *cyc1–91* (unpublished results), though not of *arg4–6* (LEMONTT 1971a) *cyc1–131* (present results), other ochre, amber or missense alleles (unpublished results), or forward mutation to auxotrophy (LEMONTT 1972). Finally, UV-induced reversion of *cyc1–9* is lower in strains carrying either *rad8*, *rad9* or *rad18*, but reversion of *cyc1–131* is unaltered in the latter two strains (LAWRENCE *et al.* 1974; present results). It is not clear why *rad9* influences reversion of *cyc1–9* only when cells are exposed to moderate or high UV fluences; possibly strains carrying this allele contain a low level of *RAD9+* activity sufficient to cope with small numbers of pyrimidine dimers. A different *rad9* allele has been shown to reduce the frequency of UV-induced reversion of *ade2*, *lys1*, and *his3* mutations in diploid, though not in haploid, strains (ECKARDT, KOWALSKI and LASKOWSKI 1975).

It would therefore appear that these mutations fall into two groups, the first of which comprises *rad6* and *rev3*, whose activities seem to be necessary for the production of UV-induced mutations of many and perhaps all kinds at perhaps all sites within the genome, while the second includes *rev1*, *rev2*, *rad9* and *rad18*, which show much more specific effects and whose products are required during the formation of only some mutational alterations. It is not yet clear to what group *rad8* belongs. The generalization concerning *rad6* and *rev3* must inevitably be regarded as provisional, since it cannot be excluded that future work will uncover a mutational event for which the *rad6* or *rev3* gene products are not required. A functional *rad6* gene has, however, been shown to be necessary for the reversion of several *cyc1* nonsense, missense and frameshift mutations as well as the reversion of less well characterized alleles at other loci and forward mutation to canavanine resistance (LAWRENCE *et al.* 1974; unpublished results). Similarly, the *rev3–1* allele has been shown to reduce the frequency of a variety of UV-induced mutations.

Possibly the best explanation of this situation is that the error-prone repair pathway, in addition to a number of steps in common which can be blocked by mutations like *rad6* or *rev3*, possesses separate branches for particular mutational events. A suggestion of this kind was made previously by LEMONTT (1971b) to explain the relationship of the three *rev* genes, and the present results support and extend this idea. It should be pointed out that experiments of the kind reported here cannot determine whether the steps that are required in common for the production of all mutation events precede or follow steps that are necessary for only certain specific alterations; the data are compatible with a repair pathway that proceeds in either direction. The nature of the differences between the kinds of mutational events mediated by the various branches is not yet understood, though recent work (LAWRENCE and CHRISTENSEN, unpublished) on the specific effects of *rev1* and *rev2*, in which these are analyzed using the reversion of well defined *cyc1* alleles, suggests that the differences reside in specific nucleotide sequence rather than in type of alteration (base pair substitution, frameshift) or other factors such as position of the mutational alteration within the gene.

Apart from the *rad* mutations that decrease UV mutability, a number were found that increased the frequency of *cyc1–131* revertants induced by UV light. As expected, they include mutations at the *rad1*, *rad2*, *rad3* and *rad4* loci all of which appear to be concerned with excision repair (UNRAU, WHEATCROFT and COX 1971; RESNICK and SETLOW 1972; GAME and COX 1972; PARRY and PARRY 1969). Excision-defective mutants of yeast, like those of *E. coli* (HILL 1965; WITKIN 1967) have been shown to give higher frequencies of UV-induced mutations per unit dose than wild-type strains by several workers (RESNICK 1969; ZAKHAROV, KOZINA and FEDEROVA 1970; AVERBECK *et al.* 1970; MOUSTACCHI 1969, 1971; LAWRENCE *et al.* 1974). In addition to these, *rad22*, a new mutation not previously thought to be concerned with excision repair, was also found to enhance the frequency of *cyc1–131* reversion. This mutation, which complements mutations at each of the other four loci, when combined with *rad1–2* gives a double mutant that is no more sensitive to the lethal effects of UV than a *rad1–2* single mutant strain, suggesting that the *rad22* gene is also involved in excision repair. UV mutability would therefore appear to be a useful means of characterizing unknown *rad* mutants.

It has been suggested that there is a third, minor, pathway for the repair of UV damage in Saccharomyces, in addition to the excision and error-prone pathways (COX and GAME 1974). A pathway of this kind is suspected because the D_0 slope of survival curves for double mutant strains carrying either *rad6–1* or *rad18–2* together with a clean excision-defective mutation indicate that a lethal hit corresponds to many dimers, rather than to a value of about one which is expected if repair is entirely absent. Sensitivity can be enhanced in triple mutants that also include *rad50*, *rad51* or *rad52* mutations (COX and GAME 1974; RESNICK personal communication), even though *rad50*, *rad51*, or *rad52* single mutants are only very slightly UV-sensitive.

Since the present results indicate that there is only a single error-prone pathway for the repair of UV damage in yeast, it can be predicted that mutations

which block this third minor repair pathway should enhance UV mutability like excision-defective mutants, though to a lesser extent. This was not found in the results given in Table 4, though Cox and GAME (1974) report such a result for a *rad50* mutant strain. A compensatory enhancement of excision repair may account for our result, a suggestion that is supported by the observation that UV-induced reversion of *cyc1–9* in some ten-fold higher in *rad1 rad52* double mutants than in *rad1* single mutant strains. This result also lends support to the view that the minor repair pathway is largely "error-free."

Viewed as a whole, our results are consistent with the scheme proposed by Cox and GAME (1974) in which three pathways for the repair of UV damage are outlined. Different mutants defective in the early stages of excision repair are all substantially UV, though not X-ray, sensitive, exhibit enhanced UV mutability and have no effect on recombination or sporulation. Mutants blocked in error-prone repair are both significantly UV- and X-ray sensitive, though the degree of sensitivity to these radiations varies considerably between extremes typified by *rad6* and *rad18* on the one hand and *rev1* on the other. Such mutants exhibit reduced UV mutagenesis for at least one reversion system, and most have no influence on spontaneous recombination or sporulation. Diploids homozygous for *rad6–1* fail to sporulate, however, and are defective in X-ray-induced mitotic crossing over (Cox and GAME 1974) while diploids homozygous for *rad9–4* show no spontaneous or UV-induced inter- or intragenic recombination (KOWALSKI and LASKOWSKI 1975). Finally, mutants blocking the suggested minor pathway are all substantially X-ray sensitive, though not very UV-sensitive, exhibit normal or only slightly enhanced levels of UV mutagenesis and are frequently defective in sporulation or spore viability (GAME and MORTIMER 1974).

The phenotypes of some of the mutations studied do not easily fit in with this scheme, however, and in the absence of double mutant analysis it is difficult to suggest the pathway to which they may belong. Strains carrying such mutations are among the least sensitive to UV light and some may have defects that are only remotely concerned with DNA repair. Alternatively, the mutations may be very leaky, and if so *rad7*, *rad14*, and *rad17* may be concerned with excision repair since strains carrying these mutations all show slightly higher reversion frequencies at high fluences, and none are X-ray sensitive. Strains carrying *rad15* on the other hand are both UV- and X-ray sensitive, a phenotype associated with a defect in error-prone repair, but the frequency of UV-induced reversion of *cyc1–131* and *cyc1–9* was normal in these strains.

Despite these minor problems, our data are reasonably consistent with the proposed three UV-repair pathway scheme (Cox and GAME 1974) and therefore lend support to it. At the same time, we have certain reservations concerning pathway analysis based on the phenotypes of double and single mutants, of both a practical and theoretical kind. Double mutant analysis, like other genetic tests of function such as complementation, provide information that is often useful but which on some occasions may be difficult to interpret without further data. UV-irradiated DNA in repair defective cells may offer unusual substrates for the the remaining repair enzymes or enzymes of other kinds and therefore provide

opportunities for interactions not normally found in wild-type cells. Similarly the regulation of enzymes, such as nucleases, may be disturbed. Where branched pathways are found or other anomalies such as regulatory links between pathways exist, the concept of a pathway itself becomes obscure. Problems of these kinds are underscored by recent findings with *E. coli* which suggest that *recA* and *lex* mediated functions are active in the repair of gaps generated both by replication of dimers and by defects in excision repair (WITKIN and GEORGE 1973).

On a practical level, background genotype can often significantly modify UV sensitivity and this requires comparisons to be made within isogenic series or between groups of replicate strains. Moreover, generally only a single mutant allele of each radiation-sensitive gene is examined, and different alleles may exhibit different properties. Finally, we should like to point out that our results pertain only to UV light; as shown by the work of PRAKASH (1976), the genetic control of chemical mutagenesis may be partially or totally independent.

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