MUTANTS OF YEAST DEFECTIVE IN MUTATION INDUCED BY ULTRAVIOLET LIGHT\textsuperscript{1,2}

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Induced mutations are thought to arise as a result of enzymatic processes utilizing DNA damage as a substrate (BRIDGES 1969). Although the molecular mechanism is not known, evidence reviewed by WITKIN (1969) suggests that in \textit{E. coli} mutations are produced during postreplication repair of lethal damage induced by ultraviolet light (UV) and controlled by \textit{rec}\textsuperscript{+} and \textit{exr}\textsuperscript{+} (or \textit{lex}\textsuperscript{+}) genes. Strains carrying \textit{rec} or \textit{exr}, although normal in excision repair, exhibit reduced or no UV mutability compared to wild type. In addition, such strains are UV sensitive, X-ray sensitive, and recombination deficient in varying degrees (WITKIN 1969).

In previous studies of UV-induced mutation in fungi, UV-sensitive strains have been selected on the assumption that UV mutagenesis might be related to dark repair of lethal damage. In some of these UV-sensitive strains, the frequencies of UV mutation are reduced compared to wild type at equal UV doses (CHANG, LENNOX and TUVESON 1968; NASIM 1968; WOHLRAB and TUVESON 1969), whereas in others these frequencies are enhanced (AVERBECK et al. 1970; RESNICK 1969; ZAKHAROV, KOZINA and FEDOROVA, 1970). To identify new genes controlling UV-induced mutation, it is desirable to select strains directly for defective mutation induction, thereby avoiding the prior condition that all such mutants be UV-sensitive.

Mutants of \textit{Saccharomyces cerevisiae} were selected (LEMONTT and MORTIMER 1970) for reduced ability to undergo UV-induced locus reversion of the ochre-suppressible (GILMORE 1967; HAWTHORNE 1969) \textit{argl-17} allele. This paper describes the isolation and some characteristics of these "reversionless" mutants, The results are discussed in relation to current ideas about induced mutagenesis.

MATERIALS AND METHODS

\textit{Yeast strains:} Heterothallic strains of \textit{Saccharomyces cerevisiae} were obtained from Dr. ROBERT K. MORTIMER. Reversionless mutants were induced in X1687-16C (\textit{a arg4-17 his5-2 trp5-48 lys1-1 ade2-1 leu1-12 met1-1}), while X1687-12B (\textit{a arg4-17 his5-2 trp5-48 lys1-1 ade2-1}) and S288C (\textit{a wild type}) were used in genetic testing. Strains denoted by XY were derived mainly from these three. Genetic symbols have been described (MORTIMER, SHERMAN and VON BORSTEL 1969).

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**Media:** YEPD contained 1% Difco yeast extract, 2% Difco Bacto-Peptone, 2% dextrose, and 2% Difco agar. Liquid YEPD contained no agar. Synthetic complete (C) contained 0.67% Difco yeast nitrogen base without amino acids, 2% agar, 2% dextrose, 20 mg/l each of arginine (AR), histidine (HI), tryptophan (TR), adenine (AD), methionine (ME), 30 mg/l each of leucine (LE) and lysine (LY), and 100 mg/l threonine (THR) after autoclaving. Omission media were prepared similarly but lacked one or more metabolites, e.g., C—AR, C—HI—TR. Presporulation medium (GNAP) contained 5% dextrose, 1% yeast extract, 2.3% Difco nutrient agar, 2% Bacto-Peptone, and 0.5% agar. Sporulation medium contained 1% potassium acetate, 0.1% dextrose, 0.25% yeast extract, and 2% agar (McClary, Nulty and Miller 1959).

**Radiation sources:** Cells on agar were exposed to X rays from a beryllium window X-ray tube (Machlett OEG 60) at 250 r/sec (50 KVp, 25 ma unfiltered). Three 8-watt UV germicidal lamps (General Electric G8T5, 90% intensity at 253.7 nm) delivered 26.5 erg/mm²/sec to cells on agar.

**Experimental procedures:** Mating was achieved in 3–4 hr after mixing overnight haploid cultures of opposite mating type on YEPD agar. Zygotes, isolated by micromanipulation, were cloned on YEPD, grown overnight on GNAP, and then replica-plated onto sporulation medium. Asci were treated with Glusulase (Endo Laboratories, Garden City, New York), and dissected by the method of Johnston and Mortimer (1959). Tetrad analysis (Mortimer and Hawthorne 1966; Hawthorne and Mortimer 1960) and random spore analysis (Gilmore 1967) have been described.

Survival and reversion induction curves were obtained by first growing the cells for 4–5 days at 30°C in liquid YEPD inoculated with a single-colony isolate culture. Cells were washed twice with distilled water and then placed on ice at the proper titer. For measurements of survival, cells were diluted in distilled water, plated on YEPD, and irradiated. To measure UV reversion of arg4-17, lys1-1, or arg4-6, cells were plated onto C—AR, C—LY, or C—AR, respectively, at densities needed to achieve reliable reversion counts. In both survival and reversion studies, multiple plates were employed at each radiation dose including zero. Revertants carrying ochre suppressors were identified by growth independence on other omission media corresponding to other ochre-suppressible alleles carried in the strain (Gilmore 1967). Revertants not scored as suppressors by this method were assumed to be site revertants (Magni and Puglisi 1966). Since suppressors were not classified (Gilmore 1967), some may have escaped detection (Mortimer and Gilmore 1968). Thus the estimate of site revertants represents an upper limit.

The high UV revertibility of the arg4-17 site (Resnick 1968) provided a convenient system for detecting mutants unable to undergo induced reversion. At the optimum inducing dose (OID) of UV, the frequency of revertants, uncorrected for killing, reaches a maximum value. The OID for arg4-17 reversion in X1687-16C was measured to be 345 erg/mm².

To induce reversionless mutants, cells of strain X1687-16C were treated with 3% ethyl methanesulfonate (EMS) for 1 hr according to the method of Lindgren et al. (1965). The surviving clones on YEPD were replica-plated onto two C—AR plates. One was irradiated with the OID, while the other served as a control of spontaneous reversion. After incubation at 30°C in the dark for at least 4 days, most colony replicas on the UV-treated plates exhibited between 10 and 20 arginine revertants per colony replica, compared to none or very few on the control plates. Clones whose replicas lacked UV-induced revertants were isolated and subjected to the exclusion tests.

**Exclusion tests:** Reversionless mutants were retested twice for the reversionless phenotype and once for growth on synthetic complete. On retesting, mutants exhibiting either UV revertants or lack of growth on complete were excluded from consideration. As a second screening procedure, colonies bearing spontaneous ochre suppressors selected in the remaining mutants by plating about 10⁶ cells on C—HI—TR were tested for suppression of the arginine requirement. Lack of growth on C—AR indicated the probable existence of another defective arginine locus or another defective arg4 allele. Such mutants were excluded.

The remaining mutants were crossed to S288C. After tetrad analysis, a ratio of two spores growing to two spores not growing on C—AR in all ascii indicated that the arginine requirement was confined to arg4. Spores with UV-reversion ability exhibited revertants on C—AR replicas,
after exposure of the replicas to the OID, whereas reversionless spores exhibited none. Random segregation of the reversionless phenotype relative to the arginine requirement indicated that UV reversion was blocked by a gene unlinked to arg4. Strains whose reversionless character was either allelic (or tightly linked) to arg4 or associated with two-gene arginine segregation were excluded.

The remaining strains were crossed to X1687-12B. After tetrad analysis, observation of a 2:2 segregation pattern (reversion : no reversion) in all asci verified that a single nuclear gene difference accounted for the reversionless phenotype. The second-division segregation frequency was also estimated for reversionless genes relative to the heterozygous centromere-linked marker leu1-12 (HAWTHORNE and MORTIMER 1960).

The entire isolation procedure was considered complete only when each tentative reversionless mutant was shown to exhibit a UV-reversion frequency less than that of wild type, on a per survivor basis for every UV dose. A UV-sensitive strain may produce many revertants, but a large fraction would be inviable after exposure to the OID used for UV-resistant strains. The OID for UV-sensitive strains would be smaller. UV-induction curves of locus reversion of arg4-17 were obtained for the remaining putative mutants. Mutants in which locus reversion frequencies were equal to or greater than control (X1687-16C) at every UV dose were excluded, since the apparent reversionless phenotype was due to mutation to UV sensitivity only. Those exhibiting frequencies less than the control were considered to be defective in UV-induced reversion.

RESULTS

Isolation and allelism: Mutants were isolated in two separate experiments. In the first, of approximately 28,000 clones surviving the EMS treatment (about 50% survival), 262 putative isolates were subjected to the exclusion tests. Five true mutants designated by 235, 63, 184, 255, and 10 remained. To determine whether or not these mutants represented five separate genetic loci, complementation tests for the UV-reversion function were performed. Diploid cultures of 25 pairwise matings involving meiotic segregants of these mutants were tested for UV-induced reversion of arg4-17 carried in homozygous condition. As described previously, one plate containing C—AR replicas was UV irradiated (530 erg/mm²), while another received no UV. Absence of induced revertants after incubation indicated noncomplementation. Mutants 184, 255, and 10 failed to complement one another but each did complement both 235 and 63. Each of these two complemented all of the other four. Thus 235 and 63 each represent different complementation groups, whereas mutants 184, 255, and 10 all represent a third group. After sporulation of the diploids from these pairwise matings, random spore analysis revealed that complementing diploids produced UV-reverting spores at frequencies significantly greater than zero. Tetrad analysis showed that noncomplementing diploids, however, produced only parental ditype asci (4 nonreverting spores per ascus). Thus the three complementation groups represent three unlinked loci. The genes blocking UV reversion in mutants 235, 63, 184, 255, and 10 were denoted as rev1-1, rev2-1, rev3-1, rev3-2, and rev3-3, respectively, where rev signifies the UV-reversionless phenotype.

In the second isolation experiment, a slightly different procedure was employed to facilitate the detection of new mutant loci. Among 9000 clones surviving the EMS treatment (about 30% survival), 330 putative isolates were subjected to preliminary screening tests (not including crosses or quantitative re-
version induction). All 30 mutants not excluded by these tests were crossed to three strains, each carrying one of the rev genes. Two failed to complement rev1-I, two failed to complement rev2-I, and 11 failed to complement rev3-I. Such noncomplementation was considered to be due to allelism. The remaining 15 mutants complemented all three rev testers and were crossed to S288C. Although three of these failed to exhibit a 2:2 segregation of the arginine requirement, the remaining 12 exhibited a reversionless character unlinked to arg4. These 12 mutants were UV sensitive but not blocked in reversion and thus were excluded. They were found to represent 11 complementation groups. In summary, the numbers of mutants isolated at the rev1, rev2, and rev3 loci were 3, 3, and 14, respectively. The results presented below concern further study mainly of the red-2, rev2-I, and rev3-I alleles, and to a lesser extent rev3-2 and rev3-3.

Centromere linkage: Pooled data from tetrad analyses of crosses involving rev and leu1 are shown in Table 1. Only rev2 is centromere linked, since its second-division segregation frequency is significantly less than 2/3 (HAWTHORNE and MORTIMER 1960). Crosses involving rev2 and other centromere-linked genes indicated that rev2 is linked to asp5 on linkage group XII. For PD:NPD:T values of 80:0:7 exhibited by the gene pair rev2-asp5, the distance between rev2 and asp5 is estimated to be about 4.0 centimorgans (cM) using the equation (PERKINS 1949):

\[ \text{gene-gene distance (cM)} = \frac{50(T^4 - 6NPD)}{(PD^4 - NPD - T)} \]

In all seven asci with an exchange between rev2 and asp5, asp5 exhibited first-division segregation. Thus the most likely arrangement of the two genes on linkage group XII is: centromere–asp5–rev2.

UV-induced reversion of arg4-17: Figure 1 shows the UV induction of both locus and suppressor revertants of arg4-17 in five rev haploid segregants. The frequencies of induced suppressors were calculated by subtracting the spontaneous value from the frequency at each dose. At 265 erg/mm², locus reversion frequencies in rev1-I and rev3-I strains are about 1/30 that in wild type (X1687-16C). The effect of rev2-I is less (about 1/3 wild type). The three rev3 alleles result in different reversion phenotypes, spanning the entire range given above. Induced suppressor reversion frequencies in rev1-2, rev2-I, and rev3-1 strains are approximately equivalent to that in wild type, but are greater in rev3-2 and rev3-3 strains. In both REV and rev strains spontaneous arginine revertants were
Figure 1.—Locus reversion (top) and induced suppressor reversion (bottom) of arg4-17 vs. UV dose in REV and rev haploid strains.
Figure 2.—Locus reversion (top) and induced suppressor reversion (bottom) of arg4-17 vs. UV dose in REV/REV and rev/rev diploid strains.
due almost entirely to suppressors. In the wild type, suppressors are induced by UV at much lower frequencies than are site revertants at equal UV doses. This was also observed by Resnick (1969). In rev strains, however, since locus reversion is significantly reduced, a large proportion of the total revertants carry ochre suppressors.

The UV-locus reversion phenotypes of homozygous rev diploids (Figure 2) are similar to those of rev haploids in that rev3-1 and rev1-1 homozygotes each exhibit severely reduced frequencies, while the rev2-1 homozygote is least affected compared to wild type. Suppressor induction in the rev3-1/rev3-1 strain is blocked, but the response in the rev1-1/rev1-1 strain is similar to wild type in the low dose region and greater than wild type at higher doses. Suppressor induction in the rev2-1/rev2-1 strain is much greater than in wild type. The effect of rev3-2 and rev3-3 in diploids was not studied.

Heterozygous rev diploids exhibit UV-reversion phenotypes similar to REV/REV diploids. Thus rev mutations are concluded to be recessive to their wild-type forms. The complementation pattern obtained for reversionless mutants supports this conclusion.

Radiation sensitivity: The three rev genes cause cells to be moderately UV sensitive. UV survival curves of the haploid rev strains discussed previously are shown in Figure 3. The dose reduction factors (DRF) at 40% survival for these strains compared to wild type (i.e., dose to wild type at 40% survival divided by the dose to rev strain at 40% survival) are 3.7, 4.3, 8.5, 8.5, and 8.5 for rev1-1, rev2-1, rev3-1, rev3-2, and rev3-3 strains, respectively. While the three mutants of rev3 exhibit different UV-reversion phenotypes, their UV sensitivities, however, are similar. UV sensitivities of homozygous rev diploids are approximately the same (Figure 4). The DRF at 10% survival is about 2 compared...
Figure 4.—UV survival curves of REV/REV and rev/rev diploids.

to wild type. The meaning of the resistant "tail" on the curve for XY186 at higher UV doses has not been investigated.

The rev genes also cause cells to be slightly X-ray sensitive. X-ray survival curves of homozygous rev diploids are shown in Figure 5. The DRF's vary between 1.5 and 2.4. Both UV sensitivity and X-ray sensitivity segregate with the reversionless phenotype in REV/rev crosses and are thus different expressions of a single rev mutation.

UV-induced reversion of lys1-1 and arg4-6: The frequencies of UV reversions of other alleles are also affected by rev genes. Similar to the effect on arg4-17 reversion, rev1-l and rev3-l severely reduce UV-locus reversion of the ochre-suppressible (Gilmore 1967; Hawthorne 1969; Hawthorne and Mortimer 1963) lys1-l allele, whereas rev2-l has the least effect (Figure 6).

To determine whether rev genes can interfere with UV reversion of missense as well as nonsense alleles, UV reversion of arg4-6 was measured in rev and REV strains. The osmotic remedial arg4-6 allele is probably a missense mutation since
it is not ochre suppressible and it complements other \( \text{arg4} \) alleles (Mortimer, personal communication). A strain carrying \( \text{arg4-6} \) was crossed to \( \text{rev arg4-17} \) strains. After tetrad analysis, \( \text{arg4-6} \) and \( \text{arg4-17} \) were identified among the spores using X-ray-induced heteroallelic mitotic recombination (Manney and

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**Figure 5.**—X-ray survival curves of \( \text{REV/REV} \) and \( \text{rev/rev} \) diploids.

**Figure 6.**—Locus reversion of \( \text{lys1-1} \) vs. UV dose in \( \text{REV} \) and \( \text{rev} \) haploid strains.
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MORTIMER 1964). REV segregants exhibited UV reversion of arg4-17. The rev arg4-6 segregants were deduced in asci containing two UV-reverting arg4-17 spores. UV induction of arg4-6 revertants (Figure 7) reveals that while the rev1-1 and rev3-1 genes significantly block reversion, rev2-1 has no effect. At 265 erg/mm², the reversion response in the rev2-1 strain is twice that in wild type. All three rev strains exhibit UV survival curves expected of rev segregants. These results, together with those for lys1-1 reversion, indicate that the effects of rev genes are not specific for UV-induced reversion of arg4-17 alone. Based on these few data, rev1-1 and rev3-1 may have a general nonspecific action in reducing UV-induced reversion, but rev2-1 might specifically block UV-locus reversion of only ochre-suppressible alleles.

DISCUSSION

The method developed for selecting yeast mutants defective in UV-induced mutation is useful because it does not involve the assumption that all such mutants are UV sensitive. The mutants obtained by this method allow at least three important questions to be answered: 1) Is mutation to defective UV mutagenesis always accompanied by an increase in UV sensitivity? 2) Are such mutants sensitive to other mutagenic agents? and 3) Is UV mutability controlled by a large or a small number of genes?

All reversionless mutants isolated are moderately UV and X-ray sensitive. This observation suggests that UV-induced mutations in yeast are produced by pathways that share common steps with pathways that repair lethal UV and X-ray damage. One attractive hypothesis is that UV-induced mutations are produced during the repair for which rev genes are defective. Loss of this repair then reduces not only UV resistance but also UV mutability. Such an hypothesis has been proposed by WITKIN (1969) to account for UV mutagenesis in E. coli.

Twenty reversionless mutants were found to represent only three genes. This suggests that in yeast, UV mutability is controlled by a small number of genes. Twelve UV-sensitive mutants exhibiting reversion ability were also isolated, but represented 11 complementation groups. Cox and PARRY (1968) found 96 uvs mutants occupying 22 different loci. Thus many UV-sensitive mutations either do not affect or else they enhance UV mutability (AVERBECK et al. 1970; MOUSTACCHI 1969; RESNICK 1969; ZAKHAROV, KOZINA and FEDOROVA 1970), whereas very few reduce UV mutation. Assuming that REV gene products are enzymes involved in UV mutagenesis, the unusually small number of rev loci identified suggests that UV-induced mutation occurs during a small number of enzymatic steps.

Are UV-induced mutations in yeast produced during a postreplication repair process analogous to that found in bacteria? Unfortunately, neither excision repair nor postreplication repair has been directly demonstrated in yeast. One indirect approach is to determine whether or not REV pathways are blocked by

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*Figure 7.—UV survival curves (top) and reversion of arg4-6 vs. UV dose (bottom) in REV and rev haploid strains.*
other UV-sensitive genes that exhibit phenotypes similar to excision-defective mutants of *E. coli*. For example, the extreme UV sensitivity of *uvs9* strains to both killing and induced mutation (Resnick 1969) suggests that they are blocked in excision repair. The UV-sensitivity and UV-reversion phenotypes of *rev uvs9* double mutant haploids indicate that *REV* pathways are not blocked by *uvs9*, but rather act on intermediates produced earlier by the *uvs9* pathway (Lemontt 1970). Another indirect approach is to determine whether or not genetic recombination is associated with UV mutagenesis. If so, one might expect that genes blocking UV mutation would also cause recombination deficiency, as observed in *E. coli*. Neither meiotic nor mitotic recombination (UV or X-ray induced) in diploids is reduced by homozygous *rev* mutations (Lemontt and Mortimer 1970; Lemontt 1970). This suggests that recombination events may not be essential for UV mutagenesis.

The characteristics of *rev* mutants isolated in this study suggest that UV-induced mutation in yeast, controlled by a small number of genes, occurs during repair of UV damage to DNA. Experiments are in progress to determine whether *rev* genes can suppress both forward and reverse mutations induced by UV and other mutagens.

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**SUMMARY**

Twenty mutants of *Saccharomyces cerevisiae*, selected for reduced UV-induced site reversion of the highly UV-revertible ochre-suppressible *arg4-17* allele, were found to represent only three unlinked recessive genes denoted by *rev1*, *rev2*, and *rev3*. The *rev2* locus is about 4.0 cM distal to *asp5* on linkage group XII, but neither *rev1* nor *rev3* is centromere linked. The *rev* genes confer moderate UV sensitivity and slight X-ray sensitivity. The data suggest that UV-induced mutation in yeast is controlled by a small number of genes, sharing common enzymatic steps with the repair of lethal UV and X-ray damage to DNA. Both *rev1-1* and *rev3-1* exert a strong effect on UV-induced locus reversion of not only *arg4-17* (1/30 the wild-type frequency at 265 erg/mm²), but also of the ochre-suppressible *lys1-1* allele (1/10 wild type). The effect of *rev2-1* is only 1/2 and 1/2 wild type, respectively. UV reversion of the missense allele *arg4-6* is also reduced by *rev1-1* and *rev3-1* to 1/4 the wild-type frequency at 265 erg/mm², but is unaffected by *rev2-1* at lower UV doses. At 265 erg/mm² the frequency of *arg4-6* reversion in the *rev2-1* strain is twice that in wild type. Based on these few data, *rev1-1* and *rev3-1* may have a general nonspecific action in reducing UV reversion, but *rev2-1* might specifically block UV-locus reversion of only ochre-suppressible alleles.

**LITERATURE CITED**

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