MUTANTS OF YEAST DEFECTIVE IN MUTATION INDUCED BY ULTRAVIOLET LIGHT[1,2]

JEFFREY F. LEMONTT[3]

Donner Laboratory, University of California, Berkeley, California 94720

Received December 14, 1970

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 *E. coli* mutations are produced during postreplication repair of lethal damage induced by ultraviolet light (UV) and controlled by *rec*$^+$ and *exr*$^+$ (or *lex*$^+$) genes. Strains carrying *rec* or *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 *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) *arg4-17* 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

Yeast strains: Heterothallic strains of *Saccharomyces cerevisiae* were obtained from Dr. ROBERT K. MORTIMER. Reversionless mutants were induced in X1687-16C (*a arg4-17 his5-2 trp5-48 lys1-1 ade2-1 leu1-12 met1-1*), while X1687-12B (*α arg4-17 his5-2 trp5-48 lys1-1 ade2-1*) and S288C (*α* 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).

[1] Part of a dissertation submitted for the Ph.D. degree.

[2] Supported by U.S. Public Health Service Fellowship 5-F01-GM41059-02. Facilities provided by U.S. Atomic Energy Commission.

[3] Present address: Division of Biology, National Research Council of Canada, Ottawa 7, Ontario, Canada.

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/mm^2/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 revertant 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/mm^2.

To induce reversionless mutants, cells of strain X1687-16C were treated with 3% ethyl methanesulfonate (EMS) for 1 hr according to the method of LINDEGREN *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 10^8 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 asci 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/mm ^{2}), 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-

TABLE 1

Numbers of PD, NPD, and T asci in crosses involving* rev *and* leu1

rev gene	Number of asci			T (percent)	SDS* frequency
	PD	NPD	T		
rev1	15	11	53	67.0	0.670
rev2	17	31	26	35.1	0.324
rev3	11	16	51	65.4	0.654

* PD (parental ditype), NPD (nonparental ditype), T (tetratype), and SDS (second-division segregation).

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 *rev1-1*, two failed to complement *rev2-1*, and 11 failed to complement *rev3-1*. 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 *rev1-1*, *rev2-1*, and *rev3-1* 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):

$$\text{gene–gene distance (cM)} = 50(\text{T} + 6\text{NPD}) / (\text{PD} + \text{NPD} + \text{T}).$$

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/mm^2, locus reversion frequencies in *rev1-1* and *rev3-1* strains are about 1/30 that in wild type (X1687-16C). The effect of *rev2-1* is less (about ⅓ wild type). The three *rev3* alleles result in different reversion phenotypes, spanning the entire range given above. Induced suppressor reversion frequencies in *rev1-1*, *rev2-1*, 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

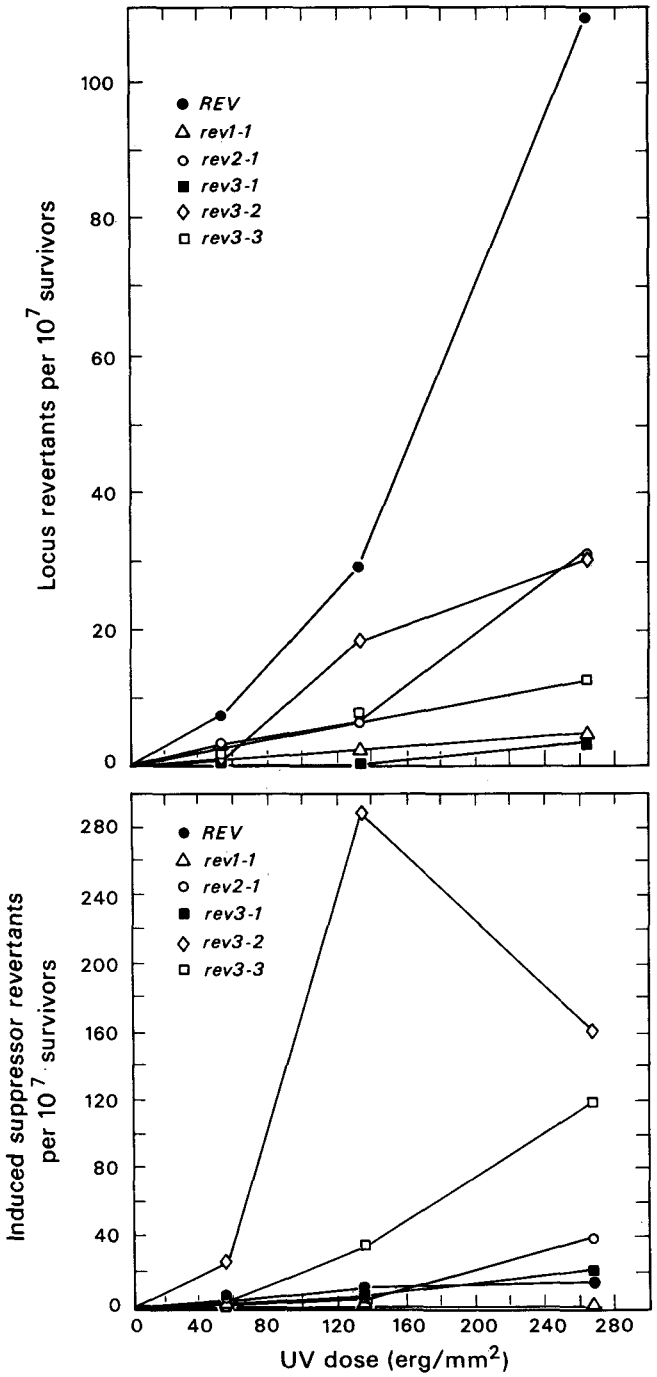


FIGURE 1.—Locus reversion (top) and induced suppressor reversion (bottom) of *arg4-17* vs. UV dose in *REV* and *rev* haploid strains.

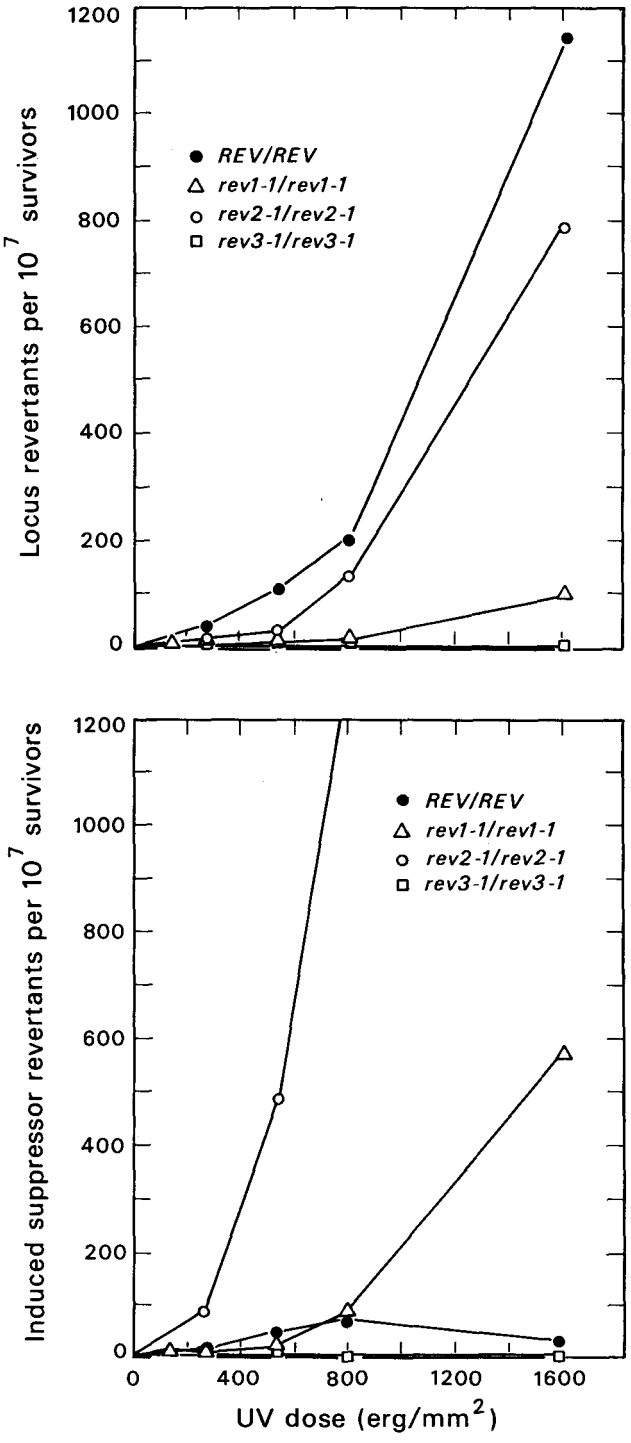


FIGURE 2.—Locus reversion (top) and induced suppressor reversion (bottom) of *arg4-17* vs. UV dose in *REV/REV* and *rev/rev* diploid strains.

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

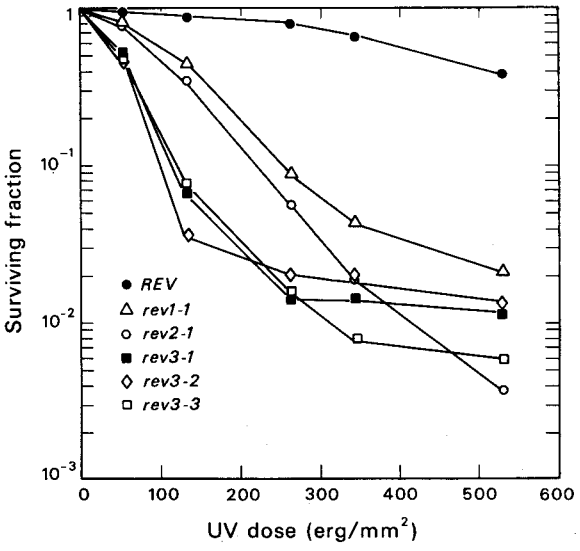


FIGURE 3.—UV survival curves of *REV* and *rev* haploids.

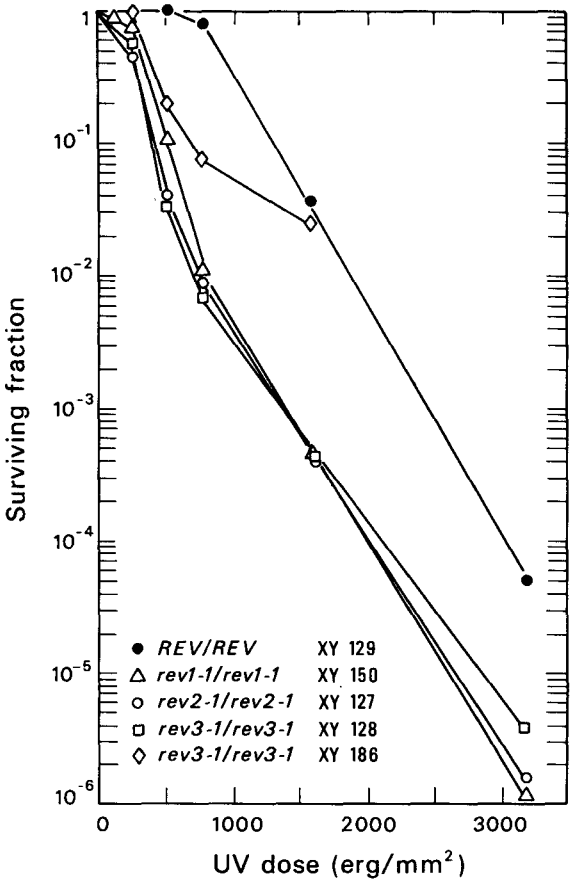


FIGURE 4.—UV survival curves of *REV/REV* and *rev/rev* diploids.

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, *rev1-1* and *rev3-1* severely reduce UV-locus reversion of the ochre-suppressible (GILMORE 1967; HAWTHORNE 1969; HAWTHORNE and MORTIMER 1963) *lys1-1* allele, whereas *rev2-1* 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

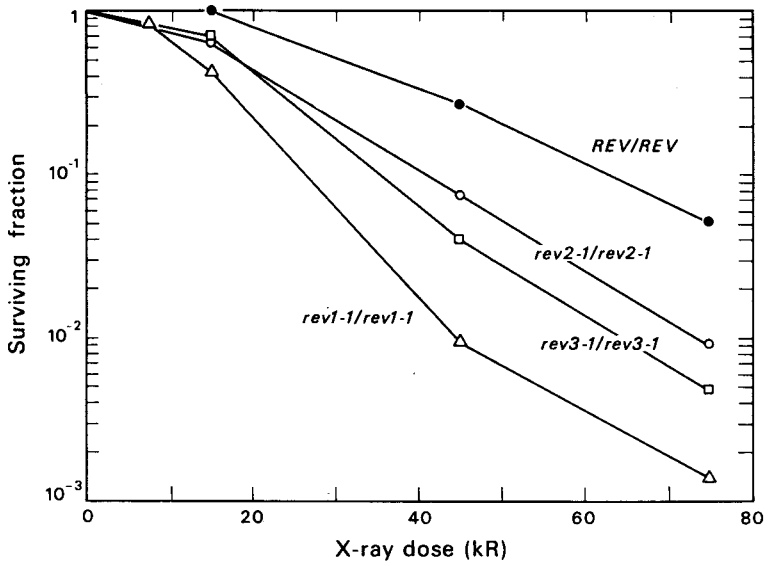


FIGURE 5.—X-ray survival curves of *REV/REV* and *rev/rev* diploids.

it is not ochre suppressible and it complements other *arg4* alleles (MORTIMER, personal communication). A strain carrying *arg4-6* was crossed to *rev arg4-17* strains. After tetrad analysis, *arg4-6* and *arg4-17* were identified among the spores using X-ray-induced heteroallelic mitotic recombination (MANNEY and

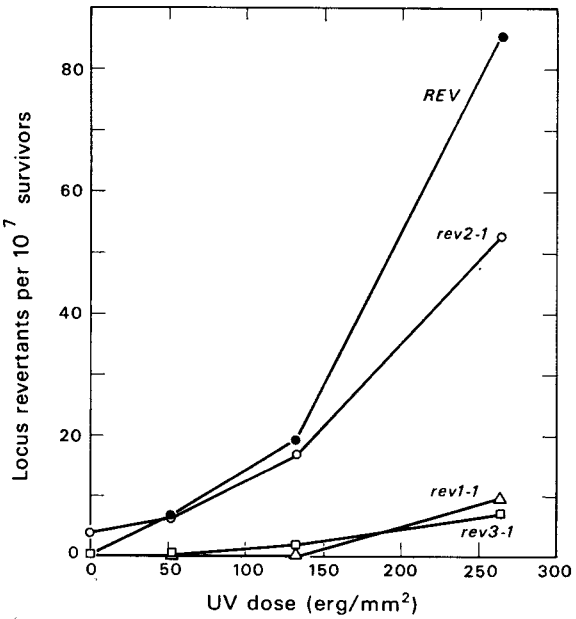
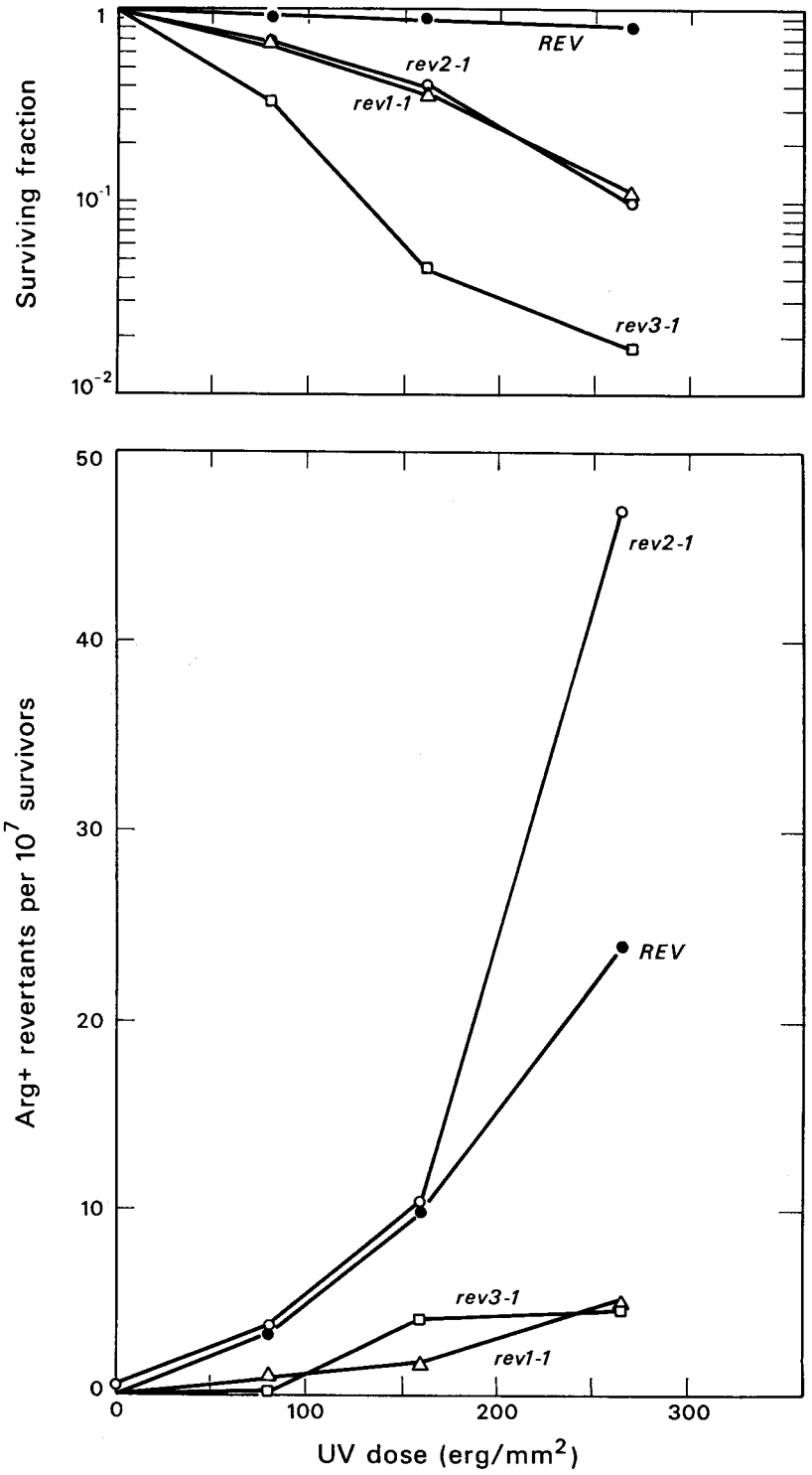


FIGURE 6.—Locus reversion of *lys1-1* vs. UV dose in *REV* and *rev* haploid strains.


Surviving fraction
1
10^{-1}
10^{-2}
REV
rev2-1
rev1-1
rev3-1
Arg+ revertants per 10^7 survivors
50
40
30
20
10
0
rev2-1
REV
rev3-1
rev1-1
0
100
200
300
UV dose (erg/mm^2)

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/mm^2, 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

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.

I am grateful to Dr. ROBERT K. MORTIMER for his advice and support during the course of this study. I also wish to thank Dr. ALLEN P. JAMES for criticism offered in the preparation of this manuscript.

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/mm^2), but also of the ochre-suppressible *lys1-1* allele (1/10 wild type). The effect of *rev2-1* is only ⅓ and ½ wild type, respectively. UV reversion of the missense allele *arg4-6* is also reduced by *rev1-1* and *rev3-1* to ⅕ the wild-type frequency at 265 erg/mm^2, but is unaffected by *rev2-1* at lower UV doses. At 265 erg/mm^2 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

AVERBECK, D., W. LASKOWSKI, F. ECKARDT and E. LEHMANN-BRAUNS, 1970 Four radiation-sensitive mutants of Saccharomyces: Survival after UV and X-ray irradiation as well as

UV-induced reversion rates from isoleucine–valine dependence to independence. Mol. Gen. Genet. **107**: 117–127.

BRIDGES, B. A., 1969 Mechanisms of radiation mutagenesis in cellular and subcellular systems. Ann. Rev. Nuclear Sci. **19**: 139–178.

CHANG, L., J. E. LENNOX and R. W. TUVESON, 1968 Induced mutation in UV-sensitive mutants of *Aspergillus nidulans* and *Neurospora crassa*. Mutation Res. **5**: 217–224.

COX, B. S. and J. M. PARRY, 1968 The isolation, genetics and survival characteristics of ultraviolet light-sensitive mutants in yeast. Mutation Res. **6**: 37–55.

GILMORE, R. A., 1967 Super suppressors in *S. cerevisiae*. Genetics **56**: 641–658.

HAWTHORNE, D. C., 1969 Identification of nonsense codons in yeast. J. Mol. Biol. **43**: 71–75.

HAWTHORNE, D. C. and R. K. MORTIMER, 1960 Chromosome mapping in Saccharomyces: Centromere-linked genes. Genetics **45**: 1085–1110. ——, 1963 Super suppressors in yeast. Genetics **48**: 617–620.

JOHNSTON, J. R. and R. K. MORTIMER, 1959 Use of snail digestive juice in isolation of yeast spore tetrads. J. Bacteriol. **78**: 292.

LEMONTT, J. F., 1970 Genetic control of mutation induction in *Saccharomyces cerevisiae*. Ph.D. thesis. University of California Lawrence Radiation Laboratory Report UCLRL-20115.

LEMONTT, J. F. and R. K. MORTIMER, 1970 The selection of mutants of yeast that are defective in ultraviolet light induced mutation. (Abstr.) Genetics **64**: s37.

LINDEGREN, G., Y. L. HWANG, Y. OSHIMA and C. C. LINDEGREN, 1965 Genetical mutants induced by ethyl methanesulfonate in Saccharomyces. Canad. J. Genet. Cytol. **7**: 491–499.

MAGNI, G. E. and P. O. PUGLISI, 1966 Mutagenesis of super suppressors in yeast. Cold Spring Harbor Symp. Quant. Biol. **31**: 699–704.

MANNEY, T. R. and R. K. MORTIMER, 1964 Allelic mapping in yeast by X-ray-induced mitotic reversion. Science **143**: 581–582.

MCCLARY, D. O., W. L. NULTY and G. R. MILLER, 1959 Effect of potassium *versus* sodium in the sporulation of Saccharomyces. J. Bacteriol. **78**: 362–368.

MORTIMER, R. K. and R. A. GILMORE, 1968 Suppressors and suppressible mutations in yeast. Advan. Biol. Med. Physics **12**: 319–331.

MORTIMER, R. K. and D. C. HAWTHORNE, 1966 Genetic mapping in Saccharomyces. Genetics **53**: 165–173.

MORTIMER, R. K., F. SHERMAN and R. C. VON BORSTEL, 1969 Proposal for a new system for genetic nomenclature for use in yeast genetics research. Microbial Genet. Bull. no. 31, Yeast Genetics Suppl.

MOUSTACCHI, E., 1969 Cytoplasmic and nuclear genetic events induced by UV light in strains of *S. cerevisiae* with different UV sensitivities. Mutation Res. **7**: 171–185.

NASIM, A., 1968 Repair mechanisms and radiation-induced mutations in fission yeast. Genetics **59**: 327–333.

PERKINS, D. D., 1949 Biochemical mutants in the smut fungus *Ustilago maydis*. Genetics **34**: 607–626.

RESNICK, M. A., 1968 Genetic control of lethality and mutation in *Saccharomyces cerevisiae*. Ph.D. thesis. U.S. AEC Doc. UCLRL-18404. ——, 1969 Induction of mutations in *Saccharomyces cerevisiae* by ultraviolet light. Mutation Res. **7**: 315–332.

WITKIN, E. M., 1969 UV-induced mutation and DNA repair. Ann. Rev. Genet. **3**: 525–552.

WOHLRAB, G. and R. W. TUVESON, 1969 Effects of liquid holding on the induction of mutations in an ultraviolet light sensitive strain of *Aspergillus nidulans*. Mutation Res. **8**: 265–275.

ZAKHAROV, I. A., T. N. KOZINA and I. V. FEDOROVA, 1970 Effets des mutations vers la sensibilité rayonnement ultraviolet chez la levure. Mutation Res. **9**: 31–39.