LACK OF CHEMICALLY INDUCED MUTATION IN REPAIR-DEFICIENT MUTANTS OF YEAST

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ABSTRACT

Two genes, rad6 and rad9, that confer radiation sensitivity in the yeast Saccharomyces cerevisiae also greatly reduce the frequency of chemically-induced reversions of a tester mutant cyc1-131, which is a chain initiation mutant in the structural gene determining iso-l-cytochrome c. Mutations induced by ethyl methanesulfonate (EMS), diethyl sulfate (DES), methyl methanesulfonate (MMS), dimethyl sulfate (DMS), nitroquinoline oxide (NQO), nitrosoguanidine (NTG), nitrogen mustard (HN2), β-propiolactone, and tritiated uridine, as well as mutations induced by ultraviolet light (UV) and ionizing radiation were greatly diminished in strains homozygous for either the rad6 or rad9 gene. Nitrous acid and nitrosoimidazolidone (NIL), on the other hand, were highly mutagenic in these repair-deficient mutants, and at low doses, these mutagens acted with about the same efficiency as in the normal RAD strain. At high doses of either nitrous acid or NIL, however, reversion frequencies were significantly reduced in the two rad mutants compared to normal strains. Although both rad mutants are immutable to about the same extent, the rad9 strains tend to be less sensitive to the lethal effect of chemical mutagens than rad6 strains. It is concluded that yeast requires a functional repair system for mutation induction by chemical agents.

THE repair capacity of organisms not only determines their survival after exposure to agents which damage their DNA, but also influences their response to the mutagenic effects of these agents. In prokaryotic and eukaryotic micro-organisms, radiation-induced mutations have been shown to depend in part on a functional repair system. Escherichia coli strains carrying mutations at the uvrA or uvrB loci, i.e., defective in the ability to excise ultraviolet light (UV)-induced pyrimidine dimers, give much higher yields of mutations at doses which cause no detectable effect in the corresponding wild-type strains (Hill 1965; Witkin 1966; Bridges and Munson 1968; Kondo et al. 1970). As in E. coli, excision-defective mutants of the yeast Saccharomyces cerevisiae show greatly increased UV-induced reversion frequencies for several different loci tested (Nakai and Yamaguchi 1969; Resnick 1969; Zakharov, Kozina and Fedorova 1970; Averbeck et al. 1970; Lawrence et al. 1974). Recombination-deficient strains of E. coli having a mutation at either the exr (or lex) locus (Witkin 1967; Bridges, Law and Munson 1968) or the recA locus (Witkin 1969a; Kondo et al. 1970), on the other hand, are refractory to UV-induced mutation. UV mutagenesis is now thought to result from errors in post-replication repair,

via recombinational repair, of gaps occurring opposite unexcised pyrimidine dimers (Witkin 1967; Bridges, Dennis and Munson 1967; Rupp et al. 1971).

Strains of yeast which do not respond to UV-induced mutation (rev) are also known (Lemontt 1971). Two of the three rev loci, rev1 and rev3, reduce UV-induced mutability at all loci tested (Lemontt 1972). Although the rev mutants are somewhat sensitive to UV and X-ray, they show normal frequencies of meiotic recombination (Lemontt 1971). They are therefore not recombination-deficient mutants and are not comparable to the recA mutants of E. coli. Nevertheless, a recombinational type of repair process may still be involved in UV mutagenesis in eukaryotic organisms.

Although a functional repair system is required for radiation mutagenesis, the role of repair in chemically induced mutations has not been clearly defined. Mutations induced by the so-called "radiomimetic" chemical mutagens such as methyl methanesulfonate (MMS) and nitroquinoline oxide (NQO), seem to depend on the same repair systems required for radiation-induced mutations. Excision-defective E. coli strains show increased sensitivity and increased mutability to both UV and NQO (Kondo et al. 1970). E. coli rec mutants show increased sensitivity and decreased mutability to UV, NQO, X-ray and MMS (Witkin 1969a,b; Kondo et al. 1970). However, none of the known repair-deficient E. coli mutants, including the rec and uvr mutants, exhibit altered responses to mutation induction by non-radiomimetic chemical agents such as nitrosoguanidine (NTG) and ethyl methanesulfonate (EMS) (Kondo et al. 1970).

In this paper, we provide evidence that a functional repair system is indeed required for the induction of mutations by non-radiomimetic chemical mutagens, including EMS and NTG. We find that strains of the yeast S. cerevisiae carrying either the mutant genes rad6 or rad9, two genes conferring radiation sensitivity (Cox and Parry 1968), show greatly reduced frequencies of induced mutation compared to the normal RAD strain. Both the rad6 and rad9 genes are involved in chemically-induced mutation since there was a great diminution in the reversion of a tester mutant, cyl-131, a chain initiation mutant in the structural gene determining iso-1-cytochrome c (Stewart et al. 1971), after treatment with most of the chemical mutagens tested, including EMS, NTG, and NQO. Of the eleven chemical mutagens tested, only nitrous acid and nitrosoimidazolidone (NIL) under some conditions, had the same effect on the rad6, rad9 and the normal RAD strain; at low doses, revertibility in all three strains was virtually the same, while at high doses, mutability of the rad6 or rad9 strains was greatly reduced compared to the normal RAD strain. Thus, these two rad genes, which are not comparable to any of the genes described so far in E. coli, are required for chemically-induced mutations.

MATERIALS AND METHODS

Strains: The cyl1–131 mutant was isolated by the chlorolactate procedure (Sherman et al. 1974) and contains an alteration of the initiation codon of the structural gene determining iso-1-cytochrome c (Stewart et al. 1971). The cyl1–131 mutant requires a G:C to A:T transition in
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order to yield true revertants and has been shown to revert with high frequencies with mutagens that show specificity for inducing G:C to A:T transitions as well as with moderate frequencies with non-specific mutagens (PRAKASH and SHERMAN 1973). The rad6 and rad9 mutations were isolated by Cox and PARRY (1968). The rad6 gene maps near ole1 on chromosome VII (LAWRENCE, unpublished results.

Diploid strains used in these experiments were constructed from haploid stocks by standard procedures of yeast genetics. Meiotic segregants from the cross B-651 (a cycl1-131 lys2 his1 trp2 RAD6+) × CL21-4A (a CYC1 rad6) were used to generate the diploids LC-0 (cycl1-131/cycl1-131 RAD6+/RAD6+ RAD9+/RAD9+) and LC-6 (cycl1-131/cycl1-131 rad6/rad6 RAD9+/RAD9+). B-651 canR (a canavanine-resistant derivative of B-651) was crossed to α rad9 ade2-1, kindly provided by B. S. Cox. Haploid segregants obtained from random spores were used to generate diploid LC-9 (cycl1-131/cycl1-131 RAD6+/RAD6+ rad9/rad9). The three diploids thus were derived from segregants of related pedigrees. The diploid LC-9A was constructed by using the same a (LP23-90) as was used in the cross to generate LC-9. The a parent was obtained by crossing LP23-90 to α cycl1-131 ilv3 (LP74-12), to yield the strain α cycl1-131 ilv3 rad9 (LP118-1D). The full genotype of heterozygous markers in these strains is given in Table 1. The diploid strains are each homozygous for the cycl1-131 allele, which is the site of reversion in the mutation experiments that follow. LC-0 is homozygous for the wild-type alleles of rad6 and rad9 and will be referred to as the RAD diploid, for the sake of brevity. LC-6 is homozygous for the rad6 mutation but wild-type at the RAD9 locus and will be referred to as the rad6 diploid. Likewise, LC-9 and LC-9A, both homozygous for the rad9 mutation but wild-type at the RAD6 locus, will be referred to as the rad9 diploids (Table 1).

Source of chemical mutagens: The mutagens were obtained from the following sources: methyl methanesulfonate (MMS) and ethyl methanesulfonate (EMS), Eastman Organic Chemicals; diethyl sulfate (DES); Fisher Scientific Co.; dimethyl sulfate (DMS) and N-methyl-N-nitro-N-nitrosoguanidine (NTG), Aldrich Chemical Co.; β-propiolactone (β-PL), trade name "Betaprone", Fellows Medical Mfg. Co., Oak Park, Mich.; nitrogen mustard (HN2), trade name "Mustargen", Merck, Sharp and Dohme; 1-nitrosomimidazolidone-2 (NIL), DRS. F. K. ZIMMERMANN (Technische Hochschule, Darmstadt, Germany) and R. PREUSSMAN (Forschegruppe Präventivmedizin, Freiburg, Germany); [5-3H] uridine ([3H]-U), Schwarz-Mann; 4-nitroquinoline-1-oxide (NQO), DRS. Y. KAWAZOE and T. SUSUMURA (National Cancer Center, Tokyo, Japan) and was purchased from T. Schuchardt GMBH and Co. (Munich, Germany).

Since commercially available preparations of the monofunctional alkylating agents EMS, MMS, DES and DMS often contain impurities which increase toxicity but do not affect mutagenicity (LOPPES 1968; STRAUSS, personal communication), these chemical agents were repurified by vacuum distillation at 10 to 15 mm Hg before use.

Media and quantitative reversion frequencies: The selective media and methods used for determining reversion frequencies of cycl1 mutants have been described in detail by PRAKASH and

<table>
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<th>Strain numbers and genotypes</th>
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<tr>
<td>Common name</td>
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<td>--------------------------</td>
</tr>
<tr>
<td>RAD</td>
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<td></td>
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<td>rad6</td>
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SHERMAN (1973) and by PRakash, Stewart and Sherman (1974). Mutagenized and untreated cells were plated on lactate medium and the revertants were scored after 7 to 14 days of incubation at 30°C. Viability before and after mutagenic treatment was determined by plating cells on glycerol medium and scoring after three days of incubation at 30°C. A solution of filter-sterilized 10% sodium thiosulfate was used to inactivate the chemical mutagens at the end of the desired time, except in the cases described below. It should be pointed out that p- strains (cytoplasmic "petites"), which may be induced by certain mutagens, do not grow on either glycerol medium or lactate medium, while the cyc1 strains grow on glycerol medium but not on lactate medium.

Mutagenic treatments

1) Diethyl sulfate (DES): cells suspended in 0.05 M potassium phosphate buffer, pH 7.1 (phosphate buffer) were incubated for 20 min at 30°C with 0.1 to 1.0% (v/v) DES.

2) Dimethyl sulfate (DMS): cells suspended in phosphate buffer were incubated for 12 min at 30°C with 0.01 to 0.05% DMS (v/v).

3) Ethyl methanesulfonate (EMS): cells suspended in phosphate buffer were incubated for up to 5 hr at 30°C with 0.5% EMS which was prepared by adding 0.1 ml EMS to 20 ml cells.

4) Methyl methanesulfonate (MMS): cells suspended in phosphate buffer were incubated for up to 40 min at 30°C with 0.1% MMS (v/v).

5) Nitrogen mustard: (di-(2-chloroethyl)methylamine) (HN2); HN2 solutions were made just before use by dissolving the 10 mg content of a vial of "Mustargen" in phosphate buffer and diluting in the same buffer to the desired concentrations. Cells suspended in phosphate buffer were treated for 1 hr at 30°C with 0.02 to 0.2 mg HN2/ml.

6) 4-nitroquinoline-1-oxide (NQO): NQO solutions were made just before use by dissolving to 1 mg NQO/ml in acetone and then diluting in phosphate buffer to the desired concentrations. Cells suspended in phosphate buffer were treated for 30 min at 30°C with 0.1 to 1.0 μg NQO/ml.

7) Nitrosomimidazole (NIL): NIL solutions were made just before use by dissolving NIL as 10 mg/ml in phosphate buffer and diluting in the same buffer to the desired concentrations. Cells suspended in phosphate buffer were incubated with 0.05 to 0.4 mg NIL/ml for 30 min at 30°C.

8) N-methyl-N'-nitro-N-nitrosoguanidine (NTG): NTG solutions were made just before use by dissolving to 10 mg/ml in acetone and then diluting in phosphate buffer to the desired concentrations. Cells suspended in phosphate buffer were treated for 40 min at 30°C with 10 to 40 μg NTG/ml.

9) Nitrous acid (HNO2): in order to obtain more reproducible results, the cells were starved by suspending in sterile distilled water and storing at 4°C for 1 to 3 days. Starved cells were washed with sterile distilled water and resuspended in 0.5 M sodium acetate buffer, pH 4.8 (acetate buffer). HNO2 was made just before use by dissolving sodium nitrite in acetate buffer to the desired concentrations. Cells suspended in acetate buffer was treated for 20 min at 30°C with 0.1 to 0.5 mg HNO2/ml. The reaction was terminated by the addition of an equal volume of a solution of 2.7% Na2HPO4·7H2O/1% yeast extract.

10) β-propiolactone (β-PL): cells suspended in phosphate buffer were incubated at 30°C for 1 hr with 0.004 to 0.02% β-PL (v/v) prepared just before use in sterile water. The reaction was terminated by centrifugation and washing the cells with water.

11) Tritiated-uridine([5-3H]-U): described in detail in Prakash and Sherman (1973). Cells were grown for 16 hours in a synthetic glucose medium containing 50 μCi[5-3H]-U/ml. The next day, cells were washed several times in distilled water, resuspended in 0.05 M KH2PO4 and stored at 4°C. Cells were plated for viability and number of revertants both immediately and after 10 days of storage. A control culture was treated as described above, except that the [3H]-U was omitted from the medium.

RESULTS

A special set of mutants containing identified altered codons in the iso-1-cytochrome c gene of yeast has been used as tester strains for determining mutagenic
specificities (Prakash and Sherman 1973). One of the mutants, cyc1–131, contains a GUG codon in place of the normal chain initiation codon AUG and requires a G:C to A:T transition to yield the normal protein (Stewart et al. 1971). This mutant was reverted preferentially and with a high frequency by EMS, DES, HNO₂, NTG, NIL, [³H]-U, NQO and β-PL and it was therefore concluded that these agents, to varying degrees, selectively induce G:C to A:T transitions, at least at the cyc1–131 site (Prakash and Sherman 1973; Prakash, Stewart and Sherman 1974). In addition, UV, X-rays, HN2, MMS and DMS were found to revert all the tester strains with about the same efficiency or without any dependence on simple types of base-pair changes and it was concluded that these mutagens were relatively non-specific.

Since the cyc1–131 tester reverted well with all eleven chemical mutagens and with ionizing and UV radiation, it was used in studies designed to determine the genetic control of mutation induction using a wide variety of mutagens (Prakash, unpublished results). This tester was coupled to over 20 different rad genes and diploids were constructed which were homozygous for cyc1–131 as well as a particular rad gene. Initial studies concerning the effect of these rad genes on chemically-induced reversion of cyc1–131 (Prakash, in preparation) indicated that both the rad6 and rad9 diploids were relatively refractory to mutation induc-

![Figure 1](image_url)
tion. These strains were therefore further characterized by testing a wide variety of mutagens. Complete dose response curves were obtained for the RAD and rad6 diploids with each mutagen.

The potent mutagen EMS reverts the RAD diploid very efficiently over a dose range resulting in virtually 100% survival (Figure 1). However, the presence of either the rad6 gene or the rad9 gene greatly reduces EMS-induced reversion frequencies (Figure 1). Even at non-lethal doses of EMS, the reversion frequency is about 10 times greater in the RAD diploid than in the rad6 diploid. Survival in the rad6 diploid decreases with increasing time of exposure to EMS, whereas in the rad9 diploid, there is no loss in viability over the dose range tested. Nevertheless, EMS mutability in the rad9 diploid is quite low.

The reduced reversion frequencies in the rad6 and rad9 diploids extend to other chemical mutagens including NQO, NTG, MMS, DMS, HN2, β-PL, [3H]-U, and DES (Table 2). NQO and NTG mutability are reduced 50 to 100-fold compared to the normal RAD diploid (Figures 2 and 3). The reduction occurs even at doses resulting in high survival in the rad6 and rad9 diploids, as

<table>
<thead>
<tr>
<th>Mutagenic treatment</th>
<th>Strains</th>
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<tr>
<td></td>
<td>RAD</td>
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<tr>
<td>HNO₂ (0.35 mg/ml, 20 min)</td>
<td>142 (100%)</td>
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<tr>
<td>NIL (0.4 mg/ml, 30 min)</td>
<td>1588 (89%)</td>
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<tr>
<td>EMS (0.5%, 5 hr)</td>
<td>1124 (80%)</td>
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<tr>
<td>DES (0.5%, 15 min)</td>
<td>226 (94%)</td>
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<tr>
<td>MMS (0.1%, 40 min)</td>
<td>5 (94%)</td>
</tr>
<tr>
<td>DMS (0.05%, 12 min)</td>
<td>24 (89%)</td>
</tr>
<tr>
<td>HN2 (0.2 mg/ml, 1 hr)</td>
<td>10 (80%)</td>
</tr>
<tr>
<td>NQO (1 μg/ml, 1 hr)</td>
<td>395 (78%)</td>
</tr>
<tr>
<td>NTG (40 μg/ml, 40 min)</td>
<td>1506 (76%)</td>
</tr>
<tr>
<td>β-PL (0.02%, 1 hr)</td>
<td>16 (100%)</td>
</tr>
<tr>
<td>[3H]-U (10 days decay)</td>
<td>14 (98%)</td>
</tr>
<tr>
<td>UV (25 Joules/m²)‡</td>
<td>15 (96%)</td>
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Numbers in parenthesis represent percent survival.
Reversion frequencies are expressed as the number of revertants obtained after treating cells minus those that occurred spontaneously, except for treatments with [3H]-U. Reversion frequencies for cells grown in the presence of [3H]-U are expressed as the number of revertants obtained after 10 days of storage at 4°C, minus the number obtained after no storage. In most experiments, spontaneous reversion frequencies were less than 1 per 10⁷ cells. An entry of 0 indicates that no revertants were found out of 2 to 8 x 10⁷ total cells plated, or that the difference between the induced and spontaneous reversion frequency was 0.
* Reversion rates decrease at higher doses. See Figures 4 and 5.
† LC-9A.
‡ The UV survival and reversion data were kindly provided by Dr. C. Lawrence.
No entry indicates that the strain was not tested with the mutagen.
was observed for EMS-induced reversions. The three mutagens EMS, NQO and NTG, which are efficient in reverting \textit{cycl-131} in the \textit{RAD} diploid, essentially lose their mutagenicity for that site in the \textit{rad6} or \textit{rad9} diploids.

The reduced mutability of \textit{cycl-131} observed in the \textit{rad6} diploid is due to the \textit{rad6} gene and not to variation in genetic background of the \textit{RAD} and \textit{rad6} diploids. Three other diploids homozygous for both \textit{cycl-131} and \textit{rad6} were constructed by crossing meiotic segregants from the same cross used to generate LC-6. The four \textit{rad6} diploids, representing six independent tetrads, were tested for revertibility of \textit{cycl-131} by EMS and DES by placing 20 µ liter of each mutagen on a filter disc placed in the center of a petri plate seeded with the appropriate strain. All four diploids homozygous for \textit{rad6} showed reduced reversion with EMS and DES, whereas the \textit{RAD} diploid and three diploids heterozygous for \textit{rad6} showed normal reversion. The reduction in mutability is thus a property of the \textit{rad6} gene.

Of the eleven chemical mutagens tested, only HNO₂ and NIL reverted the \textit{rad6} and \textit{rad6} diploids (Table 2). In fact, reversion frequencies are virtually the same in the normal \textit{RAD} diploid strain as in the radiation-sensitive mutants.
Figure 3.—Survival and cyc1-131 reversion dose-response curves for LC-0 (○), LC-6 (●), and LC-9 (□) treated with NTG.

when low doses of either HNO₂ or NIL are used (Figures 4 and 5). NIL-induced revertants arise initially with the same kinetics in the RAD and the rad6 diploid for concentrations up to 0.2 to 0.3 mg/ml. However, as the dose increases, the frequency of induced revertants rises in the RAD diploid and falls in the rad6 diploid. It should be noted that since there is no loss in viability in either strain over the dose range used, the decreased frequency of revertants observed in the rad6 diploid is a reflection of an absolute decrease in the number of revertants. In the presence of the rad9 gene, the frequency of NIL-induced revertants increases with increasing dose, as it does in the RAD diploid, but at a reduced rate (Figure 4). The kinetics of HNO₂-induced mutation in the rad6 diploid (Figure 5) is similar to the kinetics of NIL-induced mutation in that strain (Figure 4). At low doses, there is an increase in reversion frequency, a leveling off and then a decrease in frequency with increasing dose. In the rad9 diploids, on the other hand, the frequency of revertants increases with increasing HNO₂ concentration, within the dose ranges used (Figure 5). There is no apparent difference in reversion frequency at low doses of HNO₂ in RAD, rad6 and rad9
diploids. In the rad6 diploid, however, there is a decrease in viability at higher HNO₂ doses and a corresponding decrease in numbers of revertants.

All or almost all of the large revertant colonies on lactate medium are the result of intragenic mutations and not the result of extragenic suppressors. Intragenic and extragenic reversion of cycl mutants can be differentiated on the basis of colony size and cytochrome c content (see Sherman et al., 1974). Most intragenic revertants have normal or near normal amounts of cytochrome c and have normal colony sizes on lactate medium, while nearly all of the extragenic revertants have below normal amounts of cytochrome c and form smaller colonies on lactate medium. Extragenic revertants of cycl-131 can be due to suppressors that usually cause increased levels of iso-2-cytochrome c. These can be distinguished clearly from intragenic revertants, since most of them still have far below the normal amounts of total cytochrome c. Even the rare revertants having normal amounts of cytochrome c that is entirely iso-2-cytochrome c can be conveniently identified by the slightly different spectral properties of the cₐ-band. Over 50 nitrous acid- and NIL-induced revertants of the RAD, rad6 and rad9 diploids were picked and subcloned, and the resulting strains were examined for cytochrome c content and growth on lactate medium. From these results, we are confident that the colonies scored on lactate medium are truly the result of intragenic mutations. Thus, most of the HNO₂- and NIL-induced revertants expressed in the results of Figures 4 and 5 represent intragenic revertants. Extragenic sup-
pressors occurred with very low frequencies. It should be stressed that all of the HNO₂- and NIL-induced revertants of the rad6 diploid retained their UV sensitivity and could therefore not have arisen as a result of simultaneous reversion of the rad6 locus.

In summary, both rad6 and rad9 are similar in their response to mutability with chemical agents. Both rad6 and rad9 greatly reduce reversion of cyc1–131 with all chemical agents tested, except for NIL and HNO₂, when low doses of these agents are used. At high doses, however, reversion frequencies are reduced in rad6 and rad9 strains, even with these agents (see Figures 4 and 5). Both rad6 and rad9 were originally selected for on the basis of their sensitivity to the lethal effects of UV (Cox and Parry 1968); however, rad6 is much more sensitive to UV and x-ray than is rad9. Perhaps correlated with this observation is the fact that the UV mutability of cyc1–131 is reduced to a much greater extent in rad6 than it is in rad9 (Table 2; Lawrence, personal communication).

It is evident from the results presented here that in the rad6 or rad9 diploids, chemically-induced and radiation-induced reversion of cyc1–131 is greatly diminished compared to the RAD strain carrying the wild-type allele of both of those genes.
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DISCUSSION

It is now fairly well-established that mutations induced by both ionizing radiation (such as X-ray and γ-ray) and UV depend on genes conferring sensitivity to these agents and involved in repair of damage caused by these agents (Bridges 1969; Bridges, Law and Munson 1968; Kondo et al. 1970; Witkin 1969). Correlations exist between sensitivity of organisms to radiation and sensitivity to certain chemical agents. Excision-defective, UV-sensitive strains of E. coli are also sensitive to bifunctional alkylating agents such as nitrogen and sulfur mustards (Bridges and Munson 1966). In addition, such strains are also sensitive to the lethal effects of NQO (Kondo et al. 1970). The correlation between a defect in excision-repair and sensitivity to UV and NQO is also found in Bacillus subtilis (Felkner and Kadlubar 1968; Lumback and Felkner 1972; Tanooka and Takahashi 1972), Salmonella typhimurium (Yamamoto, Fukada and Takebe 1970) and cultured cells from patients with xeroderma pigmentosum (Takebe et al. 1972; Stich, San and Kawazoe 1973. Increased rates of mutation caused by both UV and NQO occur in E. coli mutants unable to excise pyrimidine dimers. Such strains are not sensitive to the lethal effects of X-ray or the monofunctional alkylating agent MMS and show normal responses to mutation induction by X-ray and MMS (Kondo et al., 1970). E. coli strains carrying a mutation at the recA or exr locus show greatly reduced mutability induced by X-ray and MMS (Kondo et al. 1970) as well as UV and NQO (Kondo et al. 1970). In yeast correlations have been found for sensitivity to UV and HN2 on the one hand and X-ray and MMS on the other hand (Brendel, Khan and Haynes 1970; Brendel and Haynes 1973). These findings certainly suggest that mutation induction by at least the “radiomimetic” chemical agents such as NQO and MMS should depend partly on the same genes which control UV and X-ray mutagenesis.

The results presented in this paper indicate that mutations induced by the non-radiomimetic chemical agents also depend on genes involved in repair. A mutation at either the RAD6 or RAD9 locus substantially reduces the frequency of reversion of cycl-131 induced by a wide variety of chemical agents as well as with UV (Table 2). The reduced mutability is reflected over a wide range of concentrations in the dose-response curves (Figures 1 to 5). Even at doses resulting in comparable survival in all three strains, very low reversion frequencies were obtained in the radiation-sensitive mutants with EMS, NQO, NTG, HN2, β-PL, [3H]-U decay and DES. NIL and HNO₂ were the only chemical mutagens tested which, at low doses, induced similar reversion frequencies in all three strains (Table 2; Figures 4 and 5). It is not readily apparent why the frequencies of NIL- and HNO₂-induced revertants should decrease in the rad6 diploid and increase in the RAD diploid at doses resulting in such high survival in both strains. Since the rad6 mutant is an amber-containing nonsense mutant (Lawrence et al. 1974; B. S. Cox, personal communication), one possible explanation for the observed revertibility of the rad6 diploid with nitrous acid and NIL is that these two mutagens are efficient inducers of amber suppressors. However, over 50 NIL- and nitrous acid-induced CYCl revertants of the rad6 diploid were...
tested to determine the nature of the revertants. All of them were shown by
spectroscopic examination of intact cells to be intragenic revertants which
retained their UV sensitivity. Therefore, the high frequency of reversions induced
by low doses of NIL and HNO₂ in the rad6 diploid cannot be accounted for by
simultaneous reversion of rad6 to RAD6⁺ or by the induction of an extragenic
suppressor of the rad6 amber site. In addition, the synthetic lactate medium used
to score for cycl revertants tends to inhibit the appearance of amber suppressors
(SHERMAN et al. 1973).

The rad6 mutation has been shown to decrease the reversion of other cycl alleles
and its effect is therefore not restricted to the cycl-131 allele. A diploid
hozygous for cycl-115, a missense mutant which reverts by G:C transitions
and transversions, shows high revertibility induced by NTG, whereas NTG-
duced reversion of this same site is barely detectable when the strain is simul-
taneously homozygous for the rad6 mutation (PRAKASH, unpublished results).

While both cycl alleles, cycl-131 and cycl-115, revert by an alteration of a
G:C base-pair, the effect of rad6 is not restricted to G:C sites. The UAA (ochre)-
containing nonsense mutant cycl-9 (STEWART et al. 1972) is highly UV-revert-
ible (STEWART et al. 1971; SHERMAN and STEWART 1973; LAWRENCE et al. 1974).
Although any amino acid whose mRNA codon differs from UAA by a single base
change is compatible with function at that position, UV induces predominantly
A:T to G:C changes of the first base in the UAA codon, resulting in insertion of
 glutamine (STEWART et al. 1972; SHERMAN and STEWART 1974). This change is
obviously not a change involving a G:C base-pair, nor are any of the other rever-
sional events, since the UAA codon lacks G:C base-pairs. This highly UV-revert-
able ochre mutant also shows a drastic reduction in UV-induced revertibility
when coupled with the rad6 gene (LAWRENCE et al. 1974). The specificity of UV-
duced reversion of cycl-9 is changed by the rad6 mutation. Glutamine is no
longer the predominant amino acid found in intragenic revertants of cycl-9
when the rad6 mutation is present; instead, various amino acid replacements
occur (LAWRENCE et al. 1974). When the rad6 mutant is reverted either by intra-
genic reversion or by a suppressor mutation, then the reversion frequencies in
these strains approach the values obtained in the corresponding RAD strain
(LAWRENCE et al. 1974). Also, rad6 reduces γ-ray-induced reversion of cycl-9
(LAWRENCE et al. 1974).

In addition, the effect of the rad6 gene extends to all loci tested so far. In the
presence of rad6, UV-induced back mutation of hist1 to HIS is reduced (MOUS-
TAGCHI 1972) as are UV-induced back reversions of arg4-17 to ARG and lys2-1
to LYS, as well as forward mutation of CAN⁸ to can⁸ (LAWRENCE et al. 1974).
Similar results have been obtained by B. S. Cox (personal communication).

The reduction in mutability observed in the presence of the rad9 gene is also
likely to be due to the rad9 gene and not to variation in genetic background. Both
rad9 diploids (LC-9 and LC-9A) show similar responses to the lethal effect of
HNO₂ and to mutability induced by HNO₂ (Figure 5). In addition, both rad9
diploids show similar mutagenicity in response to NQO, EMS and NIL.
Although perhaps more studies involving the influence of repair defects on mutation-induction have been done in *E. coli* than in any other single organism thus far, none of the repair-deficient mutants of *E. coli* described so far are comparable in terms of their mutagenic response to either the *rad6* or *rad9* mutants in yeast (see Table 3). *RecA* mutants are perhaps most similar to *rad6* in terms of their mutability; however, EMS mutability is normal and NTG mutability only slightly reduced (Kondo et al. 1970).

In *B. subtilis*, an MMS-sensitive, UV-sensitive mutant was refractory to his reversions induced by MMS, UV and NTG, even though spontaneous revertants occurred with a normal frequency (Hill, Prakash and Strauss 1972). It was concluded that the establishment of induced mutations, including those produced by NTG, require repair functions but that different mechanisms are involved in the production of spontaneous and induced mutations. A mutant which does not respond to NTG mutagenesis has also been described in *Haemophilus influenzae* (Kimball, Setlow and Liu 1971). However, neither of these two mutants were tested for revertibility with a wide variety of chemical mutagens.

None of the bacterial mutants described so far are as non-responsive to mutations induced by as wide a variety of mutagens as are the *rad6* and *rad9* mutants described in this paper. The *rad6* strain shows greater UV sensitivity than the *rad9* strain, although both are UV-sensitive (Cox and Parry 1968; Lawrence, 1974).

### TABLE 3

**Comparison of mutability in normal and radiation-sensitive strains of** *Saccharomyces cerevisiae* and *Escherichia coli*

<table>
<thead>
<tr>
<th>Mutagen</th>
<th><em>S. cerevisiae</em></th>
<th><em>E. coli</em>&lt;sup&gt;+&lt;/sup&gt;</th>
<th>E. coli*&lt;sup&gt;+&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>RAD</strong> (normal)</td>
<td><em>rad6</em></td>
<td><em>rad9</em></td>
</tr>
<tr>
<td>HNO₂</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>NIL</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>EMS</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DES</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MMS</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DMS</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HN2</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NQO</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NTG</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>β-PL</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>[³H]-U</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>UV</td>
<td>+</td>
<td>-‡</td>
<td>±§</td>
</tr>
</tbody>
</table>

Ionizing radiation

+ = revertible.
± = revertibility somewhat lower than normal strain.
− = very low or barely detectable revertibility.
+ + + + + = revertibility higher than in normal strain.
No entry indicates that the strain was not tested.

From *Kondo et al. 1970*, except where indicated.
† *Within 1967*.
‡ *Lawrence et al. 1974*.
§ *Lawrence, personal communication*. 

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personal communication). The rad9 gene reduces the frequency of UV reversion of cycl-131 about twofold over a wide range of doses whereas the rad6 reduction factor is substantially greater (LAWRENCE et al. 1974 and LAWRENCE, unpublished results). Although rad6 and rad9 strains show essentially similar patterns of response in terms of mutability by chemical agents, i.e., both are fairly immutable strains, the rad9 diploid tends to be more resistant to the lethal effect of all agents tested than is the rad6 diploid (Tables 2 and 4). The rad6 and rad9 sites complement each other (Cox and PARRY 1968; PRAKASH, unpublished results) and recombine with each other (Cox and PARRY 1968; LAWRENCE, personal communication). Thus, they represent mutations at different genetic loci.

The precise nature of the biochemical step(s) specified by the rad6 or rad9 mutants has not yet been identified. Diploids homozygous for rad9 sporulate normally, whereas diploids homozygous for rad6 do not sporulate (COX and PARRY 1968). Therefore, it is not possible to determine whether the RAD6 locus has any effect on meiotic recombination. UV-induced mitotic intragenic recombination is normal in a rad6 diploid but has not been tested in a rad9 diploid (HUNNABLE and Cox 1971). Also, excision of UV-induced primidine dimers

### Table 4

**Sensitivity to the lethal effect of various mutagens in normal and radiation-sensitive strains of Saccharomyces cerevisiae and Escherichia coli**

<table>
<thead>
<tr>
<th>Mutagen</th>
<th>S. cerevisiae</th>
<th>E. coli*</th>
<th>Normal</th>
<th>uvrA</th>
<th>polA</th>
<th>recA</th>
<th>lex (or exr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RAD-normal</td>
<td>rad6</td>
<td>rad9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HNO₂</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NIL</td>
<td>+</td>
<td>±</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>EMS</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>DES</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>MMS</td>
<td>+</td>
<td>-</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMS</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HN₂</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NQO</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>NTG</td>
<td>+</td>
<td>-</td>
<td></td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>β-PL</td>
<td>+</td>
<td>-</td>
<td></td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>[³H]-U</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Ionizing</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

+ = same sensitivity as the normal RAD strain.
± = same sensitivity as the normal RAD strain at low doses, more sensitive than RAD at high doses.
- = greater sensitivity than the normal RAD strain.

No entry indicates that the strain was not tested. From KONDO et al. 1970, except where indicated.
† HOWARD-FLANDERS and BOYCE 1966.
‡ WITKIN 1967.
§ ZIMMERMANN 1968.
* BRIDGES and MUNSON 1966.
** MOUNT, LOW and EDMISTON 1972.
*** LAWRENCE et al. 1974.
++ COX and PARRY 1968.
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is known to occur in rad6 strains, although at a somewhat slower rate than in normal RAD strains (B. S. Cox, personal communication).

In order to explain the reduction in mutagenic potential observed in strains defective in either the RAD6 or RAD9 loci, one has to invoke some step which is common to the pathways of mutation induction by all the diverse chemical agents tested. Although the initial lesions produced by these agents are certainly different with each mutagen, there is likely to be a mechanism common in the repair of the damage induced by the various agents. The pathway(s) of error-prone repair for mutations induced by UV, ionizing radiation, and the various chemical agents probably share some common steps. On the other hand it is possible that HNO₂ and NIL produce mutations via different error-prone repair pathway(s). These two agents revert the wild type and both radiation-sensitive mutants at low doses to about the same degree. It should be stressed that it is only at increasing doses that differences in both lethality and mutations become apparent in these three strains (Figures 4 and 5). In the rad6 diploid, the decrease in NIL-induced revertants does not coincide with decreased survival (Figure 4). In fact, within the dose-range used, viability in all three diploids is the same. On the other hand, the reduced frequency of HNO₂ induced revertants coincides with a loss of viability in the rad6 diploid (Figure 5). These results suggest that the pathway(s) of repair of HNO₂- and NIL-induced mutations may be different from the pathways of repair of mutation induced by the rest of the chemical agents. A common step of repair may involve some kind of repair synthesis dependent on a “repair” DNA polymerase. According to this view, a strain deficient in the hypothetical repair replication enzyme would not undergo repair synthesis, and might be fairly immutable as well as sensitive to various agents. However, preliminary results (Prakash, unpublished results) indicate that there is no difference in DNA polymerase activity in mitochondria-free crude cell extracts of the RAD and rad6 diploids.

Our results lead us to agree with the conclusion reached by Lawrence et al. (1974), namely, that the RAD6 locus is involved in error-prone DNA repair. It would also seem that the RAD9 locus is involved in error-prone DNA repair. Whatever the nature of the defect in the rad6 and rad9 mutants, it is clear that both loci are intimately concerned with induced mutations. Whether these two loci affect the same pathway or not has not yet been determined.

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LITERATURE CITED


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