Near-Ultraviolet Mutagenesis in Superoxide Dismutase-deficient Strains of *Escherichia coli*

Rick L. Knowles¹ and Abraham Eisenstark²

¹Division of Biological Sciences, University of Missouri, Columbia, MO 65211 USA; ²Cancer Research Center, Columbia, MO 65201 USA

We compared mutagenic spectra induced by polychromatic near-ultraviolet radiation (near-UV; 300–400 nm) with superoxide anion (O₂⁻)–dependent mutagenesis using a set of *Escherichia coli* tester strains. Near-UV radiation produced increased frequencies of G:C to A:T transitions, G:C to T:A and A:T to T:A transversions, and small increases in frameshift mutations in wild-type cells. Tester strains lacking superoxide dismutase (SOD) activity (*sodA* or *sodB* double mutants) demonstrated high spontaneous mutation frequencies and increased near-UV sensitivity. The double mutants also showed increased mutations induced by near-UV compared to either isogenic wild type, *sodA* or *sodB* single mutants. Furthermore, these mutants had an unusual spontaneous mutation spectrum, with a predominance of A:T to T:A transversions, followed by G:C to T:A transversions and frameshifts generated in runs of adenines in both the +1 and -1 direction. Other frameshifts were detected to a lesser degree. The oxygen dependency and the type of mutations spontaneously induced in SOD-deficient cells indicated that this mutagenic spectrum was caused by oxidative DNA damage. However, no apparent synergistic action between near-UV radiation and an increased flux of O₂⁺ could be detected. From the frequency and types of mutations induced by the two agents, we speculate that near-UV-induced mutagenesis and O₂⁻–dependent mutagenesis involve, in part, different lesion(s) and/or mechanism(s). The nature and possible mutagenic pathways of each are discussed. Key words: A:T to T:A transversion, free radicals, hydroxyl radical, mutagenic specificity, near-UV radiation, superoxide anion, superoxide dismutase. *Environ Health Perspect* 102: 88–94 (1994)

Near-ultraviolet radiation (near-UV; 320–400 nm) has been shown to be cytotoxic, mutagenic, and weakly carcinogenic (1–3). Near-UV may be one of the most ubiquitous mutagens to which organisms are exposed. Furthermore, with the depletion of the ozone filter in the stratosphere, there is increasing concern that additional near-UV radiation may impinge on the earth’s surface.

Cellular death and DNA damage by near-UV occur mainly by indirect photo-sensitization pathways that produce reactive intermediates from intracellular chromophores such as porphyrin, flavins, and reduced nicotinamide coenzymes; however, near-UV may also damage DNA (4), key enzymes (5), and thiolated tRNA (7) directly. Near-UV generation of reactive oxygen species (e.g., hydroxyl radicals, hydrogen peroxide, superoxide anion, and singlet oxygen) kills cells; near-UV radiation in the absence of oxygen significantly reduces cell death (6). Although hydrogen peroxide (H₂O₂) resulting from normal metabolism may play an important role in spontaneous mutagenesis in *E. coli* (7,8), H₂O₂ generated by 365-nm near-UV radiation is not important in the death of some Chinese hamster ovary cell lines (9). It has been speculated that much of the toxicity of superoxide anion (O₂⁻) and H₂O₂ in cells is due to hydroxyl radical (OH⁻) or some other reactive species generated by a Haber-Weiss reaction, using iron as a catalyst (10). Increased intracellular O₂⁻ concentrations resulting from near-UV irradiation (11) may react in such a way with H₂O₂ as to produce highly reactive OH⁻ that damages DNA directly. On the other hand, while the reactivity of O₂⁻ has been questioned (12), there is increasing evidence that O₂⁻ can directly damage such molecules (13).

Compounds that produce oxygen free-radicals have been shown to be mutagenic (14,15), while mutagenesis by free-radical generators can be prevented by free-radical scavengers (8). Of particular interest, *E. coli* mutants completely lacking superoxide dismutase (SOD) have greatly enhanced mutation rates during aerobic growth, and treatments that increase the flux of O₂⁺ further stimulate mutagenesis in these strains (15). Further, near-UV radiation significantly increases the mutation frequency in SOD-deficient cells (16). This study (16) suggests that the synergistic action of near-UV and O₂⁺ induces premutational lesions and the enzyme exonuclease III converts these lesions to mutations. Further evidence of the importance of O₂⁺ in mutagenesis is provided, as an increase in the aerobic, spontaneous mutation rate has been reported in copper, zinc SOD mutants of *Saccharomyces cerevisiae* (17).

In *E. coli*, two forms of SOD are produced: manganese SOD (MnSOD) encoded by the *sodA* gene and inducible under increased levels of oxygen, and iron SOD (FeSOD) encoded by the *sodB* gene, which is not induced by oxidative stress but is synthesized constitutively (18). Despite the strong evidence of near-UV and O₂⁺ involvement in mutagenesis, little is known about the frequency and types of mutations induced by these agents, either individually or in some synergistic fashion.

Using a set of *E. coli* mutagenicity tester strains that can detect the six base substitutions and five specific frameshifts in the lacZ gene (19,20), we determined a mutagenic spectrum in cells under an increased flux of O₂⁺ radicals and the involvement of near-UV in this mutagenicity. Because reports vary on the mutagenicity of redox cycling compounds, such as plumbagin and pararquat [drugs that are highly toxic at the concentrations required to observe significant mutagenesis (21,22)], a more direct approach to analyzing O₂⁺ mutagenicity was taken. Additionally, because O₂⁺ is generated during normal aerobic metabolism, we assessed O₂⁺-dependent mutagenesis in tester strains completely lacking SOD.

**Materials and Methods**

The *E. coli* strains used in this study are described in Table 1. Briefly, the indicator or tester strains CC101–CC111 (kindly supplied by C. Cupples, Department of Biology, Concordia University) are derivatives of the strain P90C (ara Δ[lac pro]BIXIII) carrying an F’ lac I Z’ proB episome. Each strain carries a different lacZ mutation affecting one of two crucial active site residues of β-galactosidase, Glu-461 or Tyr-503. A brief description of each reversion event necessary to restore the Lac⁺ phenotype is listed in Table 2. A complete description of the nucleotide sequences involved in Lac⁺ reversion for each of the strains is given in Cupples and Miller (19) and Cupples et al. (20).

With respect to SOD phenotype, we studied four tester strains for each mutational event: wild-type Sod⁺, *sodA* and *sodB* single mutants, and *sodA*Δor *sodB* double mutants. The *sodA* and *sodB* mutations were introduced into strains CC101–CC111 by generalized transduction using wild-type phage P1 (23). P1 phage lysates were prepared from donor strains QC781 (*sodA*) and QC7673 (*sodB*) (obtained from D. Touati, Institut Jacques Monod, CNRS, Université Paris).

To further assess the Sod⁺ phenotypes in the constructed indicator strains, near-UV inactivation and mutagenesis were compared to other strains [J130 (*sodA*), J131 (*sodB*), J132 (*sodA*/*sodB* double]
Table 1. Bacterial strains

| Strain     | Genotype and description                                      | Reference or source |
|------------|---------------------------------------------------------------|---------------------|
| CC101–CC111| P90C [ara Δlac proB]XIII]                                    | (20)                |
| QC781      | K-12 F- Δlac U189 rpsL                                        | (24)                |
| QC773      | K-12 F- Δlac U189 rpsL                                        | (24)                |
| RKCC101–RKCC111 | As CC101–CC111 but                                        | This study          |
| AB1157     | F- thr-1 leuB proA2 his-4 thi-1 argE3                        | (7)                 |
| J1130      | As AB1157 but o sod:A- Mup PR1325 CmR                       | (7)                 |
| J1131      | As AB1157 but o sod:B-kan-1 Δ2 KmR                           | (7)                 |
| J1132      | As AB1157 but o sod:B-kan-1 Δ2 KmR                           | (7)                 |

*CM*, chloramphenicol resistance; *Km*, kanamycin resistance.

*P1.0C781 X CC101–CC111 select CmR and Lac' then P1.0C773 select KmR and Lac'.

Table 2. Reversion necessary to restore the Lac' phenotype in strains CC101–CC111

| Strain     | Reversion event (Lac → Lac' phenotype) |
|------------|----------------------------------------|
| CC101      | A.T → C.G                                   |
| CC102      | G.C → A.T                                  |
| CC103      | G.C → C.G                                  |
| CC104      | G.C → T.A                                  |
| CC105      | A.T → T.A                                  |
| CC106      | A.T → G.C                                  |
| CC107      | +1G                                       |
| CC108      | -1G                                       |
| CC109      | -1G (G+)                                   |
| CC110      | +1A                                       |
| CC111      | -1A                                       |

radiation was provided by eight GTE Sylvania F15T8/black-light blue-integral-filter light bulbs with emission in the 300–420 nm range and a peak at approximately 365 nm (GTE Sylvania Engineering Bulletin 0-306, GTE Sylvania, Danvers, Massachusetts). The ends of the bulbs were sealed with black tape to prevent any contamination from energy in the far-ultraviolet region. The eight bulbs were housed radially in a wooden box as described (4), equipped with a built-in fan for temperature regulation, a sample holder, and an air pump for mixing and aeration of cells. We irradiated the 10-ml cell suspensions for 35 min at ambient temperature in 15 × 100 mm Pyrex glass test tubes with gentle aeration. Fluence rates behind the Pyrex glass were determined to be 17.3 J/m²/sec at the 365 nm wavelength using a Spectrolite DM-365N ultraviolet meter.

To measure cell survival, 0.1-ml aliquots were removed every 5 min during irradiation, serially diluted in M9 buffer, appropriately plated onto LB medium, and incubated for 24 hr at 37°C before viable cell counts where made.

To monitor mutations occurring spontaneously as well as those induced by near-UV radiation in wild-type and SOD mutants, we used two assays. First, mutagenesis was inferred by measuring rifampicin-sensitive (Rif') to rifampicin-resistant (Rif') mutation frequency. The site of action of the antibiotic rifampicin is the β subunit of the RNA polymerase. Rifampicin-resistant mutants accomplish transcription with an altered β subunit due to mutations in the rpoB gene (27). It is considered that most of these mutations are base substitutions. Therefore, measuring the Rif' to Rif" mutation frequency is a sensitive assay for monitoring this class of mutational events. We also measured mutagenesis by assaying the frequency of thymine-requiring (Thy') mutants. Mutations in the thymidylate synthetase gene (thym) are resistant to the drug thymo-prim and can be selected from a Thy' population (23). Assaying mutagenesis in Sod' E. coli by monitoring Rif' to Rif" and Thy' to Thy" mutation frequencies was reported in Farr et al. (15), and the thymidylate synthetase gene has been reported to be a useful qualitative mutation marker in Chinese hamster cells (28).

At 5-min intervals throughout the 35 min of near-UV irradiation, 0.1-ml aliquots were removed from the treated cell suspensions and inoculated into 5 ml of fresh LB broth. After overnight growth at 37°C with shaking, 0.1-ml aliquots of each culture were plated in duplicate directly onto LB medium containing 100 μg/ml rifampicin (Sigma). Another 0.1-ml
aliquot of each culture was plated in duplicate onto LB medium containing 200 μg/ml thymine and 15 μg/ml trimethoprim. A final 0.1 ml aliquot was removed, diluted, and plated onto LB medium without trimethoprim and incubated 24 hr at 37°C to determine the titer of each overnight culture. We incubated plates containing rifampicin or thymine and trimethoprim for 36 hr at 37°C, at which time mutants were scored and the number of mutants per total viable cells were calculated for all tester strains. Strains AB1157, JJ130, JJ131, and JJ132 were handled in a similar manner.

To detect any mutagenic specificity occurring as a result of treatment with a mutagen, we assessed the Lac" reversion frequency or each indicator strain. For mutagenic specificity induced by near-UV treatment, 0.1-ml aliquots were taken from the overnight cultures described above and plated in duplicate directly onto lactose-minimal A medium (23). Another 0.1-ml aliquot was removed, diluted, and plated onto glucose-minimal A medium (same as above, except supplemented with 0.2% glucose instead of lactose) to again determine the titer of each overnight culture. Plates were incubated 48 hr at 37°C before viable cell counts were made and Lac" reversion frequencies calculated.

As a control, we tested the ability of the wild-type indicator strains to detect mutagenic specificity induced by other mutagens. Mutagenesis with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG; Aldrich Chemical Co., Inc., Cedar Knolls, New Jersey), ethyl methanesulphonate (EMS; Aldrich), and far-ultraviolet irradiation (far-UV; 254 nm) was essentially as outlined by Cupples and Miller (19). Handling of cultures, plating, and scoring of Lac" revertants, as well as monitoring mutagenic treatments, were as described above.

Results

Figure 1 presents the inactivation data expressed as percent survival during 35 min of near-UV irradiation for strains CC101–CC111 and the corresponding SOD mutants. Figures 2–5 represent the Rif" and Thy" mutation frequencies induced by near-UV for wild-type and SOD mutant tester strains. With respect to SOD phenotype, all the tester strains were qualitatively identical in response to near-UV treatment. The data represent an average of three to five individual experiments for each strain (two plates per time point) and then a final average for all 11 strains. The standard error was calculated for mutational frequency data and is so indicated. Identical experiments were carried out using the AB1157-derived strains, which produced near-UV survival and mutation frequencies similar to those of the mutant indicator strains (data not shown). In general, the near-UV survival curves for the wild-type, sodA, or sodB mutant indicator strains were qualitatively similar, while all of the sodA sodB double mutants were more sensitive to near-UV irradiation.

The spontaneous mutation frequency, as monitored by Rif", was approximately twofold greater in the sodA mutant indicator strains (19.2 versus 9.6) and sixfold greater in the sodAsodB double mutants (RKCC101-RKCC111) compared to the isogenic wild types (63 versus 9.6). A threefold increase in the spontaneous Thy" mutation frequency was seen in the sodA sodB double mutant tester strains (190 versus 63), whereas only a slight increase was seen in strains with the sodA mutation alone. Both Rif" and Thy" mutation frequencies increased by approximately twofold
in wild-type indicator strains after 20.8 and 26.0 kJ/m² (20 and 25 min, respectively) of near-UV exposure. However, mutations were not induced as a simple linear function of the time of irradiation. The numbers of Rif⁺ and Thy' mutants decreased below spontaneous mutation frequencies as the number of cells surviving near-UV treatment decreased. A similar pattern of increased mutation frequency was seen in the sodA mutants. However, the sodAsodB double mutants had even higher mutation frequencies induced by near-UV. Tester strains lacking both the MnSOD and the FeSOD had a fourfold increase in the Rif⁺ and almost a sixfold increase in the Thy' mutation frequencies as a result of near-UV irradiation. Although the calculated Rif⁺ and Thy' frequencies are estimates because they incorporate variations in titration of viable bacteria plated and titration of mutants among total bacteria plated, the data do suggest that there is an overall mutation increase of twofold induced by near-UV fluences between 20 and 25 kJ/m² in wild-type tester strains. In addition, near-UV induced a significant increase in mutations occurring in the sodAsodB double mutants.

Wild-type tester strains CC101–CC111 were treated with MNNG, EMS, and far-UV to confirm their sensitivity in detecting mutagenic specificity as a result of exposure to various mutagens. Several trials using these strains verified their sensitivity, providing results qualitatively similar to those reported in Cupples and Miller (19) and Cupples et al. (20) (data not shown).

Table 3 summarizes the Lac' reversion frequencies for all tester strains studied with standard error included. Data represent the average of three to five individual experiments for each strain. Strains CC101–CC111 containing either the single sodA or sodB mutations responded similarly to the wild-type strains, and the results were not tabulated. There was some preferential stimulation of strain CC102, the indicator for G:C to A:T transitions (33 versus 3.9), as well as of the A:T to G:C transition in strain CC106 (2.3 versus 0.3). Furthermore, tester CC104 detected some G:C to T:A transversions induced by near-UV (26.5 versus 4.8), with a weaker stimulation of the A:T to T:A transversion evidenced to a lesser extent in strain CC105 (9.1 versus 2.6). However, neither the A:T to C:G nor the G:C to C:G transversions were significantly induced by the near-UV treatment. All of the frameshift tester strains showed weak stimulation in the number of Lac' revertants upon exposure to near-UV. Again, the relationship between mutagenesis and near-UV irradiation was not completely linear. The number of mutants in most cases increased and then declined with increasing near-UV exposure. Spontaneous mutation frequencies for all wild-type indicator strains were similar to those in Cupples et al. (20).

Although Lac' reversion frequencies induced by near-UV irradiation in Sod' indicator strains were not significantly dif-
different from those seen in the wild types, spontaneous mutation rates in several of these strains did preferentially increase over isogenic wild types. A 20-fold increase in the number of Lac' spontaneous revertants was seen in strain RKCC105, the indicator for A:T to T:A transversions (59.8 versus 2.6). Also, a sixfold stimulation of the G:C to T:A transversion was detected in strain RKCC104 (30 versus 4.8). However, the spontaneous mutation rate for the other four base substitutions remained unchanged in the absence of SOD activity. In addition, the number of spontaneous Lac' revertants was elevated for all frameshift tester strains. Tester strains lacking superoxide dismutase had higher spontaneous rates of reversion in runs of adenines in both the +1 and -1 directions. Elevated reversion rates were stimulated in RKCC110 and RKCC111, the two strains that detect frameshifts in runs of adenes. A 15-fold increase in the spontaneous reversion rate was detected in RKCC110 (+1A) (74.2 versus 5.3), whereas a 5-fold increase was stimulated in RKCC111 (-1A) (300 versus 57). However, about a twofold stimulation occurred spontaneously in RKCC108 (-1G) and RKCC109 [-2(−C−G−)], but only a very weak stimulation was detected in RKCC107 (+1G). The Sod² phenotype of several randomly chosen colonies scored as Lac' revertants was confirmed by SOD activity gels (see Materials and Methods).

**Discussion**

Although the tester strains used only detect mutations occurring in two target codons of the lacZ gene and did not necessarily detect mutational hot spots or strand specificity, the assay allowed analysis of specific DNA mutations. While near-UV radiation was weakly mutagenic in wild-type cells and four- to sixfold more mutagenic in SOD-deficient cells, the frequency and type of mutations produced by near-UV radiation were different from those spontaneously occurring in cells assumed to have an increased flux of O₂⁻.

The polychromatic near-UV (300-420 nm) radiation studied preferentially induced G:C to A:T transitions most frequently, followed by the G:C to T:A transversion in wild-type *E. coli*. Furthermore, the A:T to T:A transversion was weakly stimulated along with all of the frameshifts analyzed. The finding that G:C to A:T transitions predominate near-UV-induced base substitutions correlates with other studies of ultraviolet radiation mutagenesis (29,30). Armstrong and Kunz (30) report-

---

**Table 3. Lac' revertants per 10⁸ cells induced by near-UV irradiation in Sod² and SodAB tester strains**

| Near-UV fluence (kJ/m²) | Strain and reversion event | CC107 (+1G) | CC108 (-1G) | CC109 [-2(−C−G−)] | CC110 (−1A) | CC111 (+1A) |
|-------------------------|---------------------------|-------------|-------------|-------------------|-------------|-------------|
| Sod²                    | Sod²                      | Sod²        | Sod²        | Sod²              | Sod²        | Sod²        |
| 0                       | 57.3 (4.2)                | 70.3 (4.2)  | 20.3 (3.2)  | 50.3 (4.1)        | 192.5 (4.5) | 510.3 (5.9) |
| 5.2                     | 50.4 (3.5)                | 75.2 (4.5)  | 25.0 (4.1)  | 47.3 (3.2)        | 201.3 (4.2) | 505.7 (6.1) |
| 10.4                    | 67.4 (4.9)                | 74.5 (4.2)  | 24.0 (3.2)  | 51.3 (3.5)        | 242.3 (4.5) | 472.5 (7.2) |
| 15.6                    | 51.7 (4.7)                | 76.5 (4.6)  | 28.8 (3.8)  | 52.0 (3.5)        | 200.5 (4.5) | 513.2 (7.3) |
| 20.8                    | 54.6 (4.4)                | 79.3 (4.4)  | 16.5 (3.5)  | 45.7 (5.2)        | 154.5 (5.3) | 422.6 (8.1) |
| 26.0                    | 47.4 (4.6)                | 87.0 (4.7)  | 24.1 (4.1)  | 42.5 (4.1)        | 88.7 (6.2)  | 405.3 (4.7) |
| 31.1                    | 44.9 (3.8)                | 69.6 (5.6)  | 26.7 (4.0)  | 36.5 (4.4)        | 155.2 (7.2) | 357.3 (4.6) |
| 36.3                    | 40.2 (3.7)                | 71.2 (5.2)  | 23.4 (3.5)  | 30.1 (4.5)        | 110.4 (5.1) | 274.8 (5.5) |
|                         |                           |             |             |                   | 5.5 (2.3)   | 75.4 (4.9)  | 32.1 (9.2)  | 284.8 (5.4) |

*Environmental Health Perspectives*
ed site and strand specificity in the SUP4-o gene in S. cerevisiae after UV-B (285–320 nm) mutagenesis. Their study further suggests that mutations induced by mid-UV or UV-B (290–320 nm) radiation and far-UV or UV-C (190–290 nm) radiation involve the same lesion(s) and/or mechanisms(s). Mutagenic spectra induced by both of these radiations include the predominance of G:C to A:T transitions and the preference for substitutions at the 3’ base of dipyrimidine sequences. This has been interpreted to indicate that cyclobutane pyrimidine dimers and pyrimidine- pyrimidone (6-4) photoproducts are the most important premutational lesions induced by far-UV radiation. However, cyclobutane dimers have recently been implicated as the major form of premutational DNA damage for both far-UV and mid-UV radiations (30). It should be noted that there is some region of overlap in the mid-UV (285–320 nm) radiation studied by Armstrong and Kunz (30) and the near-UV (300–420 nm) radiation used in these experiments. The involvement of cyclobutane dimer formation in this near-UV-induced mutagenesis remains a possibility. However, the prevalence of the G:C to T:A and A:T to T:A transversions suggests that other lesion(s) or mutagenic pathways may be involved in near-UV mutagenesis, as these transversions occurred infrequently in mid-UV mutagenesis. This is further supported by the noticeable absence of the other base substitutions apparently not stimulated by near-UV. Interestingly, the G:C to T:A transversion is the most common base substitution detected after the SOS system has been induced in the absence of DNA damage (31).

In comparison, the frequency and types of mutations occurring spontaneously in E. coli mutants lacking both MnSOD and FeSOD were different. Slightly higher spontaneous mutation frequencies for sodA mutants, but not sodB mutants, in agreement with other reports (15). Because the high spontaneous mutation rate in SOD-deficient cells is oxygen dependent (15), the mutagenic specificity detected in these mutants is likely due to oxidative DNA damage. This is also supported by the observation that the predominant base substitution induced in these mutants is the A:T to T:A transversion, the base-pair change most frequently caused by oxidative mutagens. The A:T to T:A transversion spontaneously occurred 20-fold over wild-type (Sod+) cells, followed by a 6-fold stimulation in G:C to T:A transversions. There was also significant increase of the +1A and -1A frameshift mutations in the Sod+ mutants. These results are consistent with Storz et al. (32), who found a similar mutation spectrum in Salmonella typhimurium strains containing deletions of oxyR, a gene that positively regulates cellular defenses against oxidative stress in both S. typhimurium and E. coli. However, Storz et al. (32) also reported a substantial increase in G:C to A:T transitions, a base change not detected in the sodA sodB double mutants of E. coli. Furthermore, the mutagenic spectrum was quite different from that produced by Fe2+-induced oxidative DNA damage in the M13mp2 forward mutation assay, which implicates the formation of 8-hydroxyguanines as the most frequently produced base modification, responsible for G:C to C:G transversions (33). However, others have found that the G:C to T:A transversion is predominantly induced (34).

There is evidence that mutations induced by H2O2 are not necessarily those induced during O2- dependent mutagenesis. First, O2- mutagenesis is independent of the SOS response (22), whereas the SOS response is induced by H2O2 (7). Second, although O2- induces synthesis of endonuclease IV, H2O2 does not; rather H2O2 mutagenesis depends heavily on the activity of exonuclease III (35). There is indirect proof that endonuclease IV and exonuclease III do not share the same substrate specificities (36). Results from this study support this conclusion. H2O2-dependent mutagenesis largely generates transitions, whereas the sodA sodB E. coli mutants examined here show elevated frequencies of two transversions, as well as frameshifts in runs of adenines in both the +1 and -1 direction. The three other frameshifts analyzed were also stimulated, although to a lesser extent.

Considering possible mutagenic pathways involved in the spontaneous mutagenesis seen in SOD mutants, it has been demonstrated that oxidizing agents can cause the disruption of the imidazole ring of purines, producing the formamidopyrimidine derivative of adenine and guanine (37). Generation of these derivatives in cells with increased O2- flux may explain, in part, some of the mutations induced. The E. coli formamidopyrimidine-DNA glycosylase, the mutM gene product, is not part of the SOS regulon, and its possible involvement in O2- mutagenicity should be analyzed. Alternatively, it has been shown that MutY, which is an adenine glycosylase specific for GA mispairs, has homology to endonuclease III and may be an (Fe-S)4- containing protein (38). Some (Fe-S)4- containing proteins have been shown to be sensitive to increased concentrations of O2- (39). Furthermore, E. coli mutY mutants demonstrate a high stimulation of transversions (40,41). We therefore suggest a possible role of MutY in O2- induced mutagenesis. It is possible that the MutY protein may become inactivated in SOD-deficient mutants and play some role in O2- dependent mutagenesis.

Curiously, while near-UV radiation induced up to a sixfold increase in the mutation frequency in the sodA sodB double mutants (Sod+), no similar increase in any of the specific mutations was detected. Specific mutations induced by near-UV were similar in both wild-type cells and SOD-less mutants. However, the different frequencies and types of mutations induced by near-UV and O2- suggests, in part, separate lesion(s) and/or mechanisms of mutagenesis. Although no apparent synergistic action could be interpreted from these data, further mutagenic analysis using complete target genes such as lacI (29) may resolve this issue.

References

1. Eisenstark, A. Bacterial genes involved in response to near-ultraviolet radiation. Adv Genet 26:99–147 (1989).
2. Peak MJ, Peak JG. Solar-ultraviolet-induced damage to DNA. Photodermatololgy 6:1–15 (1989).
3. Ananthaswamy HN, Piercell WE. Molecular mechanisms of ultraviolet radiation carcinogenesis. Photochem Photobiol 52:119–1136 (1990).
4. Ananthaswamy HN, Eisenstark A. Near-UV-induced breaks in phage DNA: sensitization by hydrogen peroxide (a trypropian photo product). Photochem Photobiol 24:439–442 (1976).
5. Smyk-Randall E, Brown OR, Wilke A, Eisenstark A, Flink DH. Near ultraviolet light inactivation of dihydroxyacid dehydratase in Escherichia coli. Free Rad Biol Med 14:609–613 (1993).
6. Danpure HJ, Tyrrell RM. Oxygen-dependent of near-UV (365nm) lethality and the interaction of near-UV and X-rays in two mammalian cell lines. Photochem Photobiol 25:171–177 (1976).
7. Imlay JA, Linn S. Mutagenesis and stress responses induced in Escherichia coli by hydrogen peroxide. J Bacteriol 169:2967–2976 (1987).
8. Greenberg JT, Dempie B. Overproduction of peroxide-scaping enzymes in Escherichia coli suppresses spontaneous mutagenesis and sensitivity to redox-cycling agents in oxyH mutants. EMBO J 7:2611–2617 (1988).
9. Peak MJ, Jones CA, Sedita B, Dukek EJ, Spirit DR, Peak JG. Evidence that hydrogen peroxide generated by 365-nm UVA radiation is not important in mammalian cell killing. Radiat Res 123:220–233 (1990).
10. Halliwell B. Oxidants and human disease: some new concepts. FASEB J 1:358–364 (1987).
11. Ahmad SI. Synergistic killing of coliphage T7 by near-ultraviolet radiation plus hydrogen peroxide: possible role of superoxide radicals. Photobiobiochem Photobiophys 2:170–180 (1981).
12. Fee JA. Is superoxide important in oxygen poisoning? Trends Biochem Sci 7:84–86 (1982).
13. Ewing D. Radiation sensitization of E. coli B/r by nitrous acid. Radiat Res 94:171–192 (1983).
14. Ames B. Dietary carcinogens and anti-carcino-
gens. Science 221:1256–1264 (1983).
15. Farr SB, D’Ari R, Touati D. Oxygen-dependent mutagenesis in Escherichia coli lacking superoxide dismutase. Proc Natl Acad Sci USA 83:8268–8272 (1986).
16. Hoeter JA, Eisenstark A, Touati D. Mutations by near-ultraviolet radiation in Escherichia coli strains lacking superoxide dismutase. Mutat Res 215:161–165 (1989).
17. Gralla EB, Valentine JS. Null mutants of Saccharomyces cerevisiae. Cu, Zn superoxide dismutase: characterization and spontaneous mutation rates. J Bacteriol 173:5918–5920 (1991).
18. Touati D. Molecular genetics of superoxide dis-
mutases. Free Rad Biol Med 5:393–402 (1988).
19. Cupples C, Miller JH. A set of lacZ mutations in Escherichia coli that allow rapid detection of each of the six base substitutions. Proc Natl Acad Sci USA 86:5345–5349 (1989).
20. Cupples C, Cabrera M, Cruz C, Miller JH. A set of lacZ mutations in Escherichia coli that allow rapid detection of specific frameshift mutations. Genetics 125:275–280 (1990).
21. Moody CS, Hasan HM. Mutagenicity of oxy-
gen free radicals. Proc Natl Acad Sci USA 79:2855–2859 (1982).
22. Farr SB, Narváez DO, Kogoma T. Toxicity and mutagenicity of plumbagin and the induction of a possible new DNA repair pathway in Escherichia coli. J Bacteriol 164:1309–1316 (1985).
23. Miller JH. Experiments in molecular genetics.

Cold Spring Harbor, NY: Cold Spring Harbor Labora-
tory Press, 1972.
24. Carltoz A, Touati D. Isolation of superoxide
dismutase mutants in Escherichia coli: super-
oxide dismutase necessary for aerobic life? EMBO J 5:623–630 (1986).
25. Beauchamp C, Fridovich I. Superoxide dismu-
tase: improved assays and an assay applicable to acrylamide gels. Anal Biochem 44:276–287 (1971).
26. Turner MA, Webb RB. Comparative mutagen-
esis and interaction between near-ultraviolet (313- to 405-nm) and far-ultraviolet (254-nm) radiation in Escherichia coli strains with differ-
ering repair capabilities. J Bacteriol 147:410–417 (1981).
27. Sarin PS, Gallo RC, eds. Inhibitors of DNA and RNA polymerases. New York: Pergamon Press, 1980.
28. Li I-Chan, Chang CC, Trosko JE. Thymidylate synthetase gene as a quantitative mutation marker in Chinese hamster cells. Mutat Res 243:233–239 (1990).
29. Miller JH. Mutagenic specificity of ultraviolet light. J Mol Biol 182:45–68 (1985).
30. Armstrong JD, Kuzn BA. Site and strand speci-
ficities of UVB mutagenesis in the SUP4-o gene of yeast. Proc Natl Acad Sci USA 87:9005–9009 (1990).
31. Miller JH, Low KB. Specificity of mutagenesis resulting from the induction of the SOS system in absence of mutagenic treatment. Cell 37:675–682 (1984).
32. Storz G, Christian MF, Sies H, Ames B. Spontaneous mutagenesis and oxidative damage to DNA in Salmonella typhimurium. Proc Natl Acad Sci USA 84:8917–8921 (1987).
33. McBride T, Preston BD, Loeb LA. Mutagenic spectrum resulting from DNA damage by oxygen radicals. Biochemistry 30:207–213 (1991).
34. Prieto-Alamo MJ, Abril N, Puyo C. Mutagenesis in Escherichia coli K-12 mutants defec-
tive in superoxide dismutase or catalase. Carcinogenesis 14:237–244 (1993).
35. Demple B, Hallbrook J, Linn S. Escherichia coli 4th mutants are hypersensitive to hydro-
gen peroxide. J Bacteriol 153:1079–1082 (1983).
36. Cunningham RP, Saporito SM, Spitzer SG, Wiess B. Endonuclease IV (nfo) mutant of Escherichia coli. J Bacteriol 168:1120–1127 (1986).
37. Farr SB, Kogoma T. Oxidative stress responses in Escherichia coli and Salmonella typhi-
murium. Microbiol Rev 55:561–585 (1991).
38. Michaels ML, Pham L, Nghiem Y, Cruz C, Miller JH. MutY, an adenine glycosylase active on GA mispairs, has homology to endonuclease III. Nucleic Acids Res 18: 3843–3845 (1990).
39. Gardner P, Fridovich I. Superoxide sensitivity of the Escherichia coli 6-phosphogluconate dehydrogenase. J Biol Chem 266:1478–1483 (1991).
40. Au KG, Cabrera M, Miller JH, Modivich P. Escherichia coli mutY gene product is required for specific A G→C G mismatch correction. Proc Natl Acad Sci USA 85:9163–9167 (1988).
41. Nghiem Y, Cabrera M, Cupples CG, Miller JH. The mutY gene: A mutator locus in Escherichia coli that generates G:C→T:A transversions. Proc Natl Acad Sci USA 85: 2709–2713 (1988).