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Multiple Pathways for Repair of Hydrogen Peroxide-Induced DNA Damage in *Escherichia coli*

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The repair response of *Escherichia coli* to hydrogen peroxide has been examined in mutants which show increased sensitivity to this agent. Four mutants were found to show increased in vivo sensitivity to hydrogen peroxide compared with wild type. These mutants, in order of increasing sensitivity, were *recA*, *polC*, *xthA*, and *polA*. The *polA* mutants were the most sensitive, implying that DNA polymerase I is required for any repair of hydrogen peroxide damage. Measurement of repair synthesis after hydrogen peroxide treatment demonstrated normal levels for *recA* mutants, a small amount for *xthA* mutants, and none for *polA* mutants. This is consistent with exonuclease III being required for part of the repair synthesis seen, while DNA polymerase I is strictly required for all repair synthesis. Sedimentation analysis of cellular DNA after hydrogen peroxide treatment showed that reformation was absent in *xthA*, *polA*, and *polC*(Ts) strains but normal in a *recA* cell line. By use of a λ phage carrying a *recA-lacZ* fusion, we found hydrogen peroxide does not induce the *recA* promoter. Our findings indicate two pathways of repair for hydrogen peroxide-induced DNA damage. One of these pathways would utilize exonuclease III, DNA polymerase III, and DNA polymerase I, while the other would be DNA polymerase I dependent. The RecA protein seems to have little or no direct function in either repair pathway.

Hydrogen peroxide is known to have several effects on DNA. These include inhibition of transforming activity, release of all four bases (12), and decreasing the melting temperature (12, 15). In addition, various mutants of *Escherichia coli* have been shown to be sensitive to this agent. These mutants include those deficient in RecA protein (*recA*) (1, 3), exonuclease III (*xthA*) (5), DNA polymerase I (*polA*) (1, 3, 8), and DNA polymerase III (*polC*) (7). The interaction of these enzymes in the repair of hydrogen peroxide damage has not been determined.

We have investigated the repair response to hydrogen peroxide-induced DNA damage in intact and toluene-treated wild-type *E. coli* (8) and shown that hydrogen peroxide causes strand cleavage of DNA directly and produces a repair response involving DNA polymerase I and DNA ligase. In this report, we demonstrate that there are at least two repair pathways for repair of hydrogen peroxide damage. Quantitative cell survival, repair synthesis, and DNA reformation indicate that both of these pathways require DNA polymerase I. However, one pathway also utilizes exonuclease III and DNA polymerase III. We found no direct role for the RecA protein in the repair of hydrogen peroxide damage as assessed by DNA synthesis after incision and reformation of DNA or as assessed by the ability of hydrogen peroxide to induce the *recA* gene.

MATERIALS AND METHODS

Bacterial strains and plasmids. The strains used are listed in Table 1. The construction of CSM61, ER11, and HM1 and their properties are detailed elsewhere (2). MH21 and MH86 were constructed from strain BW9116 containing an *xthA* deletion linked to Tn*10* (14). This strain was used for transduction of the *xthA* deletion into CSM61, producing MH21, and into HM1, producing MH86. These strains were verified by the 6-aminonicotinamide procedure (15). MH68 is a CSM61 derivative which contains the *recA* gene by transduction from SB229. The presence of the *recA* mutation was verified by sensitivity to UV irradiation. MH91 contains the *pcbA1* mutation in a wild-type background (7). The strain W3L is a λ lysogen with λGE190. This λ strain was provided by G. Weinstock and contains the *recA* promoter fused to the *lacZ* gene (13).

Materials. Growth media were purchased from Difco Laboratories. Hydrogen peroxide was obtained from Fisher Scientific Company. [^{3}H]thymidine was purchased from ICN Pharmaceuticals Inc.

Quantitative cell survival. Cells were grown in K medium (5) to an optical density at 600 nm of 0.6 (4×10^8 cells) at the desired temperature. A 3-ml sample was removed and treated with 15 mM hydrogen peroxide unless noted otherwise. These conditions were similar to those used by Demple et al. (5). After appropriate time points, samples were removed, diluted in M9 salts, and plated on L-broth plates. After overnight incubation at the desired temperature, the survivors were counted. Data were fit by least squares analysis.

Toluene treatment of cells, repair synthesis assay, and alkaline sucrose sedimentation. These procedures were performed as in Hagensee and Moses (8).

Measurement of the *recA* induction. Induction was measured by assaying β-galactosidase activity from a λ phage carrying a *lacZ-recA* fusion (λGE190) as a lysogen provided by G. Weinstock (13). This strain was grown in L broth at the desired temperature to an optical density at 600 nm of 0.5. The cells were either UV irradiated at 30 J/m^2, exposed to mitomycin C at a concentration of 2 μg/ml, or treated with hydrogen peroxide at a concentration of 117 mM and incubated at the desired temperature for 30 min to allow for induction of the SOS response. Samples were taken and β-galactosidase activity was measured and calculated as described by Miller (11).

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TABLE 1. Bacterial strains and bacteriophage used

Strain or phage	Genotype or phenotype	Source (reference)
Strains		
BW9109	Δ(*xth-pncA*)*90 leuB6 thr-1* Δ(*gpt-proA2*) *hisG4 argE3 lacY1 galK2 ara-14 mtl-1 xyl5 thi-1 tsx-33 rpsL31 supE44 rac*	B. Weiss
BW9116	Δ(*xth-pncA*)*90 zdh-201*::Tn*10 relA1 spoT1 thi-1* Tc^r HfrKL16 PO-45 (*lysA-serA*)	B. Weiss
CSM61	*polA1 polB100 polC1026 pcbA1 supE* Leu$^-$ His$^-$ (PolI$^+$) (Tr)[a]	This laboratory (2)
ER11	*polC486 pcbA1 zic-1*::Tn*10* Tc^r (Tr)	This laboratory (2)
HM1	*polA1 polB100 polC$^+$ pcbA1* Leu$^-$ His$^-$ (Tr)	This laboratory (2)
MH21	CSM61 with Δ(*xth-pncA*)*90 zdh-201*::Tn*10*	This laboratory P1(BW9116) × CSM61[b]
MH68	CSM61 with *recA56 srlC300*::Tn*10*	This laboratory P1(SB229) × CSM61
MH86	HM1 with Δ(*xth-pncA*)*90 zdh-201*::Tn*10*	This laboratory P1(BW9116) × HM1
MH91	*pcbA1 zic-1*::Tn*10* F$^-$ λ$^-$ Thy$^-$ Sm^r IN(*rrnD-rrnE*)*1*	This laboratory (11)
P3478	*polA1* Thy$^-$	J. Cairns
SB229	*recA56 srlC300*::Tn*10* Δ(*lac-pro*) *supE thi-1 rpsL endA sbcB15 hsdR4* Tc^r F′ *traD36 proAB lacI^q*M13	This laboratory
W3110	F$^-$ λ$^-$ Thy$^-$ Sm^r IN(*rrnD-rrnE*)*1*	J. Cairns
Phage		
GE190	*lac′ZY* Δ(*srI lacA-srI*λ2) *att$^+$ int$^+$ sr*Iλ3° *c*I857 *sr*Iλ4° *nin5 st*Iλ5° Sum100 Φ(*recA-lacA*)*1*(Hyb) *lacY$^+$ eI* Ind$^-$	G. Weinstock

[a] Tr, Strain grows at 43°C.
[b] P1 crosses listed as P1 (donor) × recipient.

RESULTS

Quantitative survival of mutants. To define the repair response, strains of *E. coli* were tested for sensitivity to a 15 mM dose of hydrogen peroxide. Strains which exhibited increased sensitivity to hydrogen peroxide are shown in Fig. 1. A *recA* derivative (data not shown) of CSM61 (MH68) was only slightly more sensitive to hydrogen peroxide than wild type was. We have previously described the *pcbA1* mutation which allows DNA polymerase I-dependent replication without a functional DNA polymerase III α subunit (2). Strains with a temperature-sensitive DNA polymerase III gene [*polC*(Ts)] and the *pcbA1* mutation survive at the restrictive temperature for DNA polymerase III, allowing study of DNA repair not dependent on DNA polymerase III. The action of the *pcbA1* allele is not understood, but it is very tightly linked to *gyrB*. These strains were significantly more sensitive to hydrogen peroxide at the restrictive temperature at which DNA polymerase III is inactive than at the permissive temperature (7). It is worth noting that the level of DNA polymerase I, whether normal or partial, does not affect the results of this comparison (7). These strains were also more hydrogen peroxide sensitive than MH91, which carries only *pcbA1* (Table 2). Cells lacking a functional exonuclease III also show increased sensitivity to hydrogen peroxide (5). Strains deficient in DNA polymerase I were the most sensitive of those tested (1). Strains deficient in *uvrA* or *uvrC* did not show increased sensitivity. We have compared relative

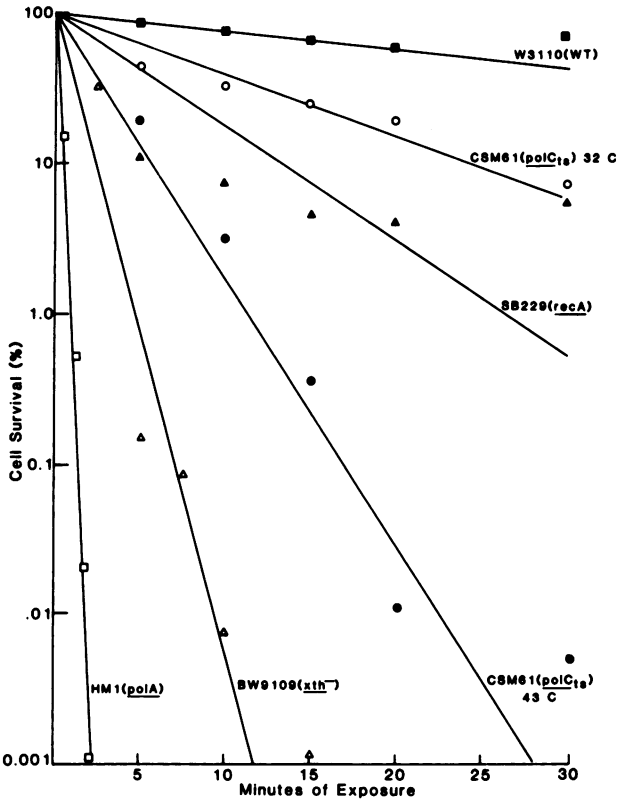

FIG. 1. Cell survival after 15 mM hydrogen peroxide treatment. Cells were exposed as described in text.

TABLE 2. Quantitative cell survival after exposure to hydrogen peroxide at 43°C

Strain	Relevant genotype	D_{37}[a]
MH91	*pcbA1*	21.1
BW9109	Δ*xthA*	1.1
CSM61	*polC1026 pcbA1*	2.5
ER11	*polC486 pcbA1*	2.7
MH21	*polC1026* Δ*xthA*	2.1
HM1	*polA1 pcbA1*	0.20
P3478	*polA1*	0.19
MH86[b]	*polA1* Δ*xthA*	0.18

[a] The time in min required to reduce the survivors to 37% of the original at 15 mM hydrogen peroxide at 43°C.
[b] The concentration of hydrogen peroxide used was 5 mM.

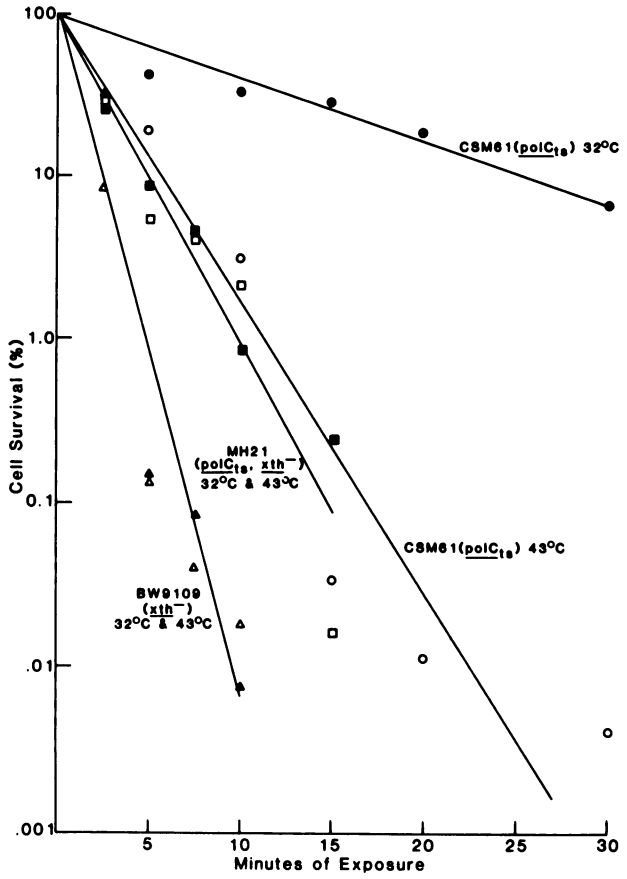

FIG. 2. Cell survival of *polC*(Ts)-*xthA* mutants after 15 mM hydrogen peroxide treatment. Cells were exposed as described in text. Symbols: ●, CSM61 [*polC*(Ts)] 32°C; ○, CSM61 [*polC*(Ts)] 43°C; ■, MH21 [*polC*(Ts) Δ*xthA*] 32°C; □, MH21 [*polC*(Ts) Δ*xthA*] 43°C; ▲, BW9109 (Δ*xthA*) 32°C; △, BW9109 (Δ*xthA*) 43°C.

survivals in other closely related strains with these mutations (7) with similar results.

There are at least two pathways of repair. Combinations were constructed by using the mutations which were the most sensitive to hydrogen peroxide, *polC*(Ts), *xthA*, and *polA*, to investigate how these three gene products interact in the repair of hydrogen peroxide damage. Strain BW9116 contains a deletion mutation in exonuclease III linked to the transposon Tn*10*. This mutation was moved into a *polC*(Ts) strain and a *polA* strain by P1 transduction, constructing strains MH21 and MH86. A *polC*(Ts) *polA* strain could not be constructed since the *pcbA1* mutation in the *polC*(Ts) strain requires a functional DNA polymerase I to survive at the restrictive temperature for DNA polymerase III (2).

No significant increase in sensitivity was noted in an *xthA1-polC1026* mutant, compared with the isogenic parent CSM61 or the more sensitive single mutant, BW9109 (Fig. 2; Table 2). It seems that these two enzymes function in the same pathway of repair for hydrogen peroxide damage. Under our conditions, we did not note a marked temperature effect on hydrogen peroxide killing (7).

The *xthA1* mutant and the *pcbA1-polA* mutant were shown to be more sensitive to hydrogen peroxide than the isogenic *polA* single mutant (Table 2). A *recA-polC*(Ts) mutant, MH68, showed no increased sensitivity to hydrogen peroxide compared with the *polC*(Ts) parent at the restrictive

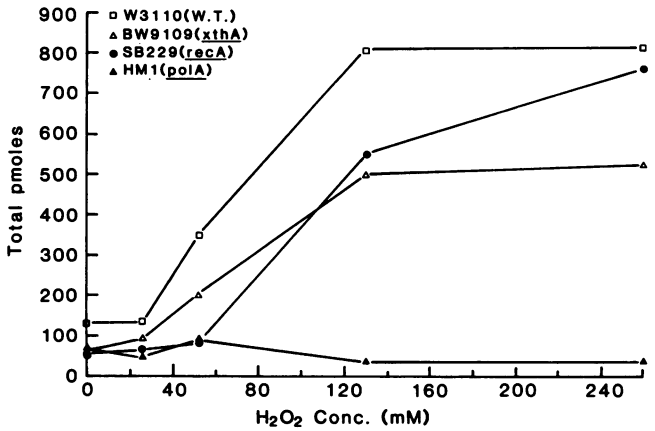

FIG. 3. DNA synthesis in toluene-treated cells after exposure to increasing concentrations of hydrogen peroxide. Reactions were without ATP present.

temperature for DNA polymerase III (data not shown). These data are consistent with *xthA*, *polC*, and *polA* functioning in the same repair pathway. However, the extreme sensitivity of the *polA* mutants to hydrogen peroxide suggests that this enzyme also functions in an additional repair pathway.

Repair synthesis in mutants. Toluene-treated wild-type cells show an ATP-independent repair synthesis (8). Cells were toluene treated and examined for repair synthesis after hydrogen peroxide treatment. *recA* mutant cell lines showed a normal repair synthesis response to hydrogen peroxide (Fig. 3). On the other hand, *polA* mutants showed no repair synthesis. A strain deficient in exonuclease III, BW9109, showed a lower level of synthesis. This intermediate level of synthesis was also seen in a second exonuclease III-deficient cell line, BW9116 (data not shown). Finally, *polC*(Ts) mutants showed little or no repair response to hydrogen peroxide even at the permissive temperature for DNA polymerase III (data not shown), and they also showed little or no replicative synthesis (2, 8). These experiments showed that exonuclease III is required for much of the DNA repair synthesis after hydrogen peroxide damage, whereas DNA polymerase I is required for all repair synthesis. DNA polymerase III apparently is required for synthesis also. The RecA protein appeared to have no direct role in the repair synthesis, although repair synthesis rose to full levels at slightly higher doses of hydrogen peroxide in *recA* mutants.

Incision and reformation in mutant cell lines. Cells were exposed to hydrogen peroxide, and incision and reformation of the DNA were followed by alkaline sucrose sedimentation as we have described (7). The *recA* strain SB229 and MH68 (data not shown) had normal responses, being able to repair DNA in 4 h after removal of the hydrogen peroxide (Table 3; Fig. 4). Cells with *xthA*, *polA*, and *polC*(Ts) (at the restrictive temperature for DNA polymerase III) mutations were all unable to reform their DNA after a dose of hydrogen peroxide. Thus, exonuclease III, DNA polymerase I, and DNA polymerase III appear required for reformation of high-molecular-weight DNA after hydrogen peroxide treatment.

Requirements for reformation in mutant cell lines. We have demonstrated (8) that toluene-treated *E. coli* can reform DNA in 60 min after a 10-min exposure to 70 mM hydrogen peroxide. This response is ATP-independent but requires magnesium. Toluene-treated *recA* cells showed a normal

TABLE 3. DNA reformation in whole cells

Strain	Relevant genotype	DNA reformed (temp)[a]
W3110	Wild type	+
SB229	*recA56*	+
BW9109	Δ*xthA*	0
CSM61	*polC1026*	+ (32°C)
		0 (43°C)
ER11	*polC486*	+ (32°C)
		0 (43°C)
P3478	*polA1*	0
HM1	*polA1*	0

[a] Values determined by alkaline sucrose sedimentation after exposure to 117 mM hydrogen peroxide for 10 min followed by removal of the agent and incubation for 4 h at 37° or the temperature indicated, as described (10). All strains showed incision of DNA after hydrogen peroxide treatment. + denotes >90% reformation to pretreatment high-molecular-weight DNA. 0 denotes no significant change of peak from incised control.

TABLE 4. DNA reformation in toluene-treated cells[a]

Strain	Relevant genotype	H_2O_2 concn (mM)	DNA reformed
W3110	Wild type	70	+
SB229	*recA56*	70	+
BW9109	Δ*xthA*	70	0
		70[b]	+
		7	+
HM1	*polA1*	70	0
		7	0
		0.7	0

[a] Values obtained from alkaline sucrose gradients on toluene-treated cells after hydrogen peroxide treatment at the indicated concentrations for 10 min followed by removal of the agent, addition of nucleotides, and incubation for 1 h at 37°C as described (10). All strains showed incision of their DNA after hydrogen peroxide treatment. + denotes >90% incision or reformation to pretreatment high-molecular-weight DNA. 0 denotes no significant change of peak from incised control.

[b] Incubation was for 6 h at 37°C.

response to hydrogen peroxide for both incision and reformation (Table 4), similar to that seen with intact cells. No reformation was seen in BW9109 (*xthA*) after 1 h (Table 4). Because of the decreased repair synthesis seen in the *xthA* mutants, we allowed more time for the reformation to occur.

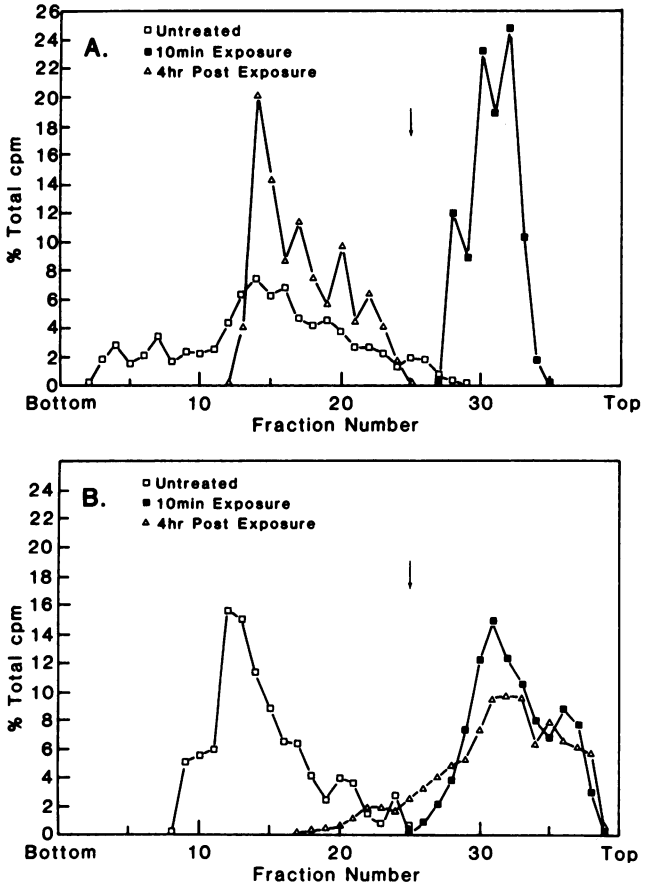

FIG. 4. Alkaline sucrose sedimentation of DNA in CSM61 after hydrogen peroxide damage. Cells were exposed to hydrogen peroxide for 10 min and then allowed to repair for 4 h (Materials and Methods). (A) Cells exposed to 117 mM hydrogen peroxide at 32°C. (B) Cells exposed to 117 mM hydrogen peroxide at 43°C. The arrow denotes the sedimentation of ϕX174 RFI (53S).

After 6 h of incubation (Table 4), the DNA reformed in the *xthA* cells. In addition, if the dose of hydrogen peroxide was decreased 10-fold, the DNA reformed within 1 h. Thus, it appears that cells deficient in exonuclease III can repair hydrogen peroxide-induced DNA damage at a reduced rate.

Similar studies were undertaken with the *polA* mutant, HM1 (derived from CSM61). HM1 did not reform DNA after a 10-min exposure to 70 mM hydrogen peroxide (Table 4). The dose of hydrogen peroxide was progressively reduced, but no reformation was ever detectable in this strain, implying that DNA polymerase I is required for repair of all damage produced by hydrogen peroxide.

Hydrogen peroxide does not induce the *recA* gene. Since the effect of a *recA* mutation on hydrogen peroxide repair appeared to be small but hydrogen peroxide damages DNA, we wondered whether hydrogen peroxide could induce the *recA* gene. This was investigated by using λGE190, which contains the *recA* promoter fused to a *lacZ* gene (13). The amount of β-galactosidase measured from this construct has been shown to be an indicator of induction of the *recA* gene. The amount of β-galactosidase activity measured with no treatment was 408 U. A 5- to 10-fold increase in β-galactosidase activity was found after treatment by the known inducers of the *recA* gene, UV light (1,486 U) and mitomycin C (2,506 U). However, no increase in β-galactosidase activity was seen with hydrogen peroxide (360 U). Thus, hydrogen peroxide did not induce the *recA* gene, and we could not demonstrate a definitive role for the RecA protein in the repair of hydrogen peroxide DNA damage. This observation is in contrast to others (9).

DISCUSSION

Quantitative kill curves demonstrated that four mutations, *recA*, *polC*, *xthA*, and *polA*, increase sensitivity to hydrogen peroxide compared with wild type. The *recA* strain was somewhat more sensitive than wild type. This result is similar to that seen by Ananthaswamy and Eisenstark (1), as well as Demple et al. (5). However, Carlsson and Carpenter (3) showed a much greater sensitivity of *recA* to hydrogen peroxide under anaerobic conditions. Ananthaswamy and Eisenstark (1) found that *recA* mutants have an increase in the number of unrepaired single-strand breaks after exposure to hydrogen peroxide as compared with wild type. However, their data also demonstrated a significant amount of repair in the *recA* strain after hydrogen peroxide treat-

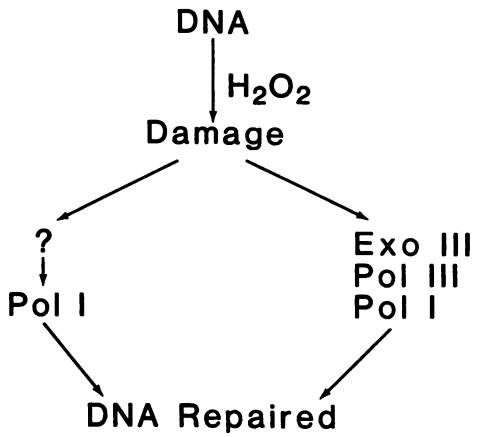

FIG. 5. Model of repair of hydrogen peroxide-induced DNA damage.

ment. Our conclusion is that the *recA*-dependent repair does not contribute to a great extent in the repair of hydrogen peroxide damage in the two backgrounds tested, a CSM61 derivative and an unrelated strain.

Cells which are deficient in exonuclease III are extremely sensitive to hydrogen peroxide (5). Exonuclease III represents approximately 90% of the apurinic (AP) endonuclease activity of *E. coli*, cleaving 5′ to an AP site. We have shown that *xthA* cells do show normal incision of DNA after exposure to hydrogen peroxide and that hydrogen peroxide can incise naked DNA directly in the presence of a low concentration of a metal (8). This implies that the AP endonucleolytic activity of exonuclease III may not be required for repair of hydrogen peroxide damage. One of the other functions of the enzyme may be necessary for the optimal repair of hydrogen peroxide damage. Perhaps this involves the 3′-phosphatase or exonuclease modification of termini to serve as suitable sites for repair synthesis. Demple et al. (6) concluded that this is the case.

Exonuclease III mutants do show an ATP-independent synthesis after exposure to hydrogen peroxide, but the amount of the synthesis seen was significantly less than that of wild type, implying that these mutants either repair hydrogen peroxide damage more slowly than wild type or that hydrogen peroxide produces multiple types of damage, some of which *xthA* mutants cannot repair. The former conclusion is supported by gradient analyses which showed that toluene-treated *xthA* mutants could fully reform their DNA after hydrogen peroxide treatment but needed an additional 5 h. However, in intact cells, the *xthA* mutant could not reform high-molecular-weight DNA after hydrogen peroxide treatment. The partial repair seen in *xthA* mutants may be due to other enzymes that substitute for exonuclease III in the repair process but are not as efficient as exonuclease III.

The recent development of an endonuclease IV (*nfo*) mutant (4) supports this hypothesis. Endonuclease IV, like exonuclease III, is a 5′-AP endonuclease accounting for approximately 10% of the total AP endonuclease activity found in wild-type *E. coli*. Unlike exonuclease III, it has no apparent associated activities (10). Cunningham et al. (4) have shown that *nfo-xthA* double mutants are more sensitive to hydrogen peroxide than either parent is. Endonuclease IV could substitute for exonuclease III in the repair of hydrogen peroxide damage, but may not be as efficient in the handling of this damage.

It appears that multiple pathways exist for the repair of hydrogen peroxide-induced DNA damage. A model to explain the results is given in Fig. 5. DNA damage produced by hydrogen peroxide can be repaired by two mechanisms: one pathway utilizes DNA polymerase I alone or with other components; the other pathway consists of exonuclease III, DNA polymerase III, and DNA polymerase I. The RecA protein may interact with these enzymes or other cellular components. It is interesting that two DNA polymerases are required for the optimal repair of hydrogen peroxide-induced DNA damage. These enzymes may be acting on different types of hydrogen peroxide-induced DNA damage. On the other hand, DNA polymerase III may have a nonsynthetic role in the repair of hydrogen peroxide damage and in this way prepare the DNA for subsequent DNA polymerase I synthesis.

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LITERATURE CITED

1. **Ananthaswamy, H. N., and A. Eisenstark.** 1977. Repair of hydrogen peroxide-induced single-strand breaks in *Escherichia coli* deoxyribonucleic acid. J. Bacteriol. **130**:187–191.
2. **Bryan, S. K., and R. E. Moses.** 1984. Map location of the *pcbA* mutation and physiology of the mutant. J. Bacteriol. **158**: 216–221.
3. **Carlsson, J., and V. S. Carpenter.** 1980. The *recA* gene product is more important than catalase and superoxide dismutase in protecting *Escherichia coli* against hydrogen peroxide toxicity. J. Bacteriol. **142**:319–321.
4. **Cunningham, R. P., S. M. Saponito, and B. Weiss.** 1986. Endonuclease IV (*nfo*) mutant of *Escherichia coli*. J. Bacteriol. **168**:1120–1127.
5. **Demple, B., J. Halbrook, and S. Linn.** 1983. *Escherichia coli xth* mutants are hypersensitive to hydrogen peroxide. J. Bacteriol. **153**:1079–1082.
6. **Demple, B., A. Johnson, and D. Fung.** 1986. Exonuclease III and endonuclease IV remove 3′ blocks from DNA synthesis primers in H202-damaged *Escherichia coli*. Proc. Natl. Acad. Sci. USA **83**:7731–7735.
7. **Hagensee, M., S. K. Bryan, and R. Moses.** 1987. DNA polymerase III requirement for repair of DNA damage caused by methylmethane sulfonate and hydrogen peroxide. J. Bacteriol. **169**:4608–4613.
8. **Hagensee, M., and R. Moses.** 1986. Repair response of *Escherichia coli* to hydrogen peroxide DNA damage. J. Bacteriol. **168**:1059–1065.
9. **Imlay, J., and S. Linn.** 1987. Mutagenesis and stress response induced in *Escherichia coli* by hydrogen peroxide. J. Bacteriol. **169**:2967–2976.
10. **Ljundquist, S.** 1977. A new endonuclease from *Escherichia coli* acting at apurinic sites in DNA. J. Biol. Chem. **252**:2808–2814.
11. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
12. **Rhaese, H., and E. Freese.** 1968. Chemical analysis of DNA alterations. I. Base liberation and backbone breakage of DNA and oligodeoxyadenylic acid induced by hydrogen peroxide and hydroxylamine. Biochim. Biophys. Acta **155**:476–490.
13. **Weisemann, J. M., C. Funk, and G. M. Weinstock.** 1984. Measurement of in vivo expression of the *recA* gene of *Escherichia coli* by using *lacZ* gene fusions. J. Bacteriol. **160**:112–121.
14. **White, B. J., S. J. Hochhauser, N. M. Cintrón, and B. Weiss.** 1976. Genetic mapping of *xthA*, the structural gene for exonuclease III in *Escherichia coli* K-12. J. Bacteriol. **126**:1082–1088.
15. **Yamamoto, N.** 1969. Damage, repair and recombination. II. Effect of hydrogen peroxide on the bacteriophage genome. Virology **38**:457–463.