Global genome removal of thymine glycol in *Escherichia coli* requires endonuclease III but the persistence of processed repair intermediates rather than thymine glycol correlates with cellular sensitivity to high doses of hydrogen peroxide

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ABSTRACT

Using a monoclonal antibody that specifically recognizes thymine glycol (Tg) in DNA, we measured the kinetics of the removal of Tg from the genomes of wild-type and repair gene mutant strains of *Escherichia coli* treated with hydrogen peroxide. Tg is rapidly and efficiently removed from the total genomes of repair-proficient cells *in vivo* and the removal of Tg is completely dependent on the *nth* gene that encodes the endonuclease III glycosylase. Hence, it appears that little redundancy in the repair of Tg occurs *in vivo*, at least under the conditions used here. Moreover, previous studies have found that *nth* mutants are not sensitive to killing by hydrogen peroxide but *xth* mutant strains (deficient in the major AP endonuclease, exonuclease III) are sensitive. We find that cell death correlates with the persistence of single-strand breaks rather than the persistence of Tg. We attempted to measure transcription-coupled removal of Tg in the lactose operon using the Tg-specific monoclonal antibody in an immunoprecipitation approach but were not successful in achieving reproducible results. Furthermore, the analysis of transcription-coupled repair in the lactose operon is complicated by potent inhibition of β-galactosidase expression by hydrogen peroxide.

INTRODUCTION

Reactive oxygen species are formed inside cells as a consequence of exposure to ionizing radiation and a variety of chemical agents and as by-products of normal cellular metabolism (1). Free radicals introduce a large number of modifications to DNA, including alterations of purine and pyrimidine bases, the deoxyribose sugar and cleavage of the phosphodiester backbone. Oxidative DNA damage has been implicated in the etiology of human diseases ranging from cancer (2) to aging (3).

One of the major stable modifications of thymine produced by oxidative agents is thymine glycol (Tg), which is formed by oxidation of the 5,6 double bond of thymine. It is primarily removed by base excision repair (BER) in *Escherichia coli*, yeast and mammalian systems (1,4–6). In *E.coli*, endonuclease III (endo III) is an oxidative damage DNA glycosylase with somewhat broad substrate specificity that includes Tg (7–9). In addition to its glycosylase activity, it has an associated lyase activity that cleaves the DNA backbone by β-elimination. This produces an α, β-unsaturated aldehyde attached to the 3’ end of a single-strand break that must be repaired prior to DNA polymerization. *Escherichia coli* possesses two 5’ AP endonucleases, exonuclease III (exo III) and endonuclease IV (exo IV). Exo III is the major AP activity and cleaves 5’ to the site of base loss producing the required 3′ OH for DNA repair synthesis. The single base gap is filled in by DNA polymerase I and sealed by DNA ligase. Endo IV also acts as a 5’ AP endonuclease and while it represents less than 10% of the constitutive AP endonuclease activity in the cell, it is induced 10- to 20-fold by paraquat (10) and nitric oxide (11).

Tg poses a strong block to *E.coli* Klenow DNA polymerase (12) and eukaryotic α DNA polymerase (13). Its presence in single-stranded phage reduces or inactivates the transfection efficiency of phage DNA into host cells (14). While Tg is only weakly mutagenic, this could be a consequence of accurate translesion synthesis by specialized DNA polymerases (15,16) or recombination. The ability of Tg to block DNA polymerases and inactivate phage DNA suggests that it can contribute to oxidative damage-induced lethality in cells.
pro®cient bacterial cells in vivo that speci®cally recognizes Tg in DNA. Tg is rapidly and hydrogen peroxide was measured using a monoclonal anti-

III glycosylase. Hence, it appears that little redundancy in the nth gene mutant strains indicates that the removal of Tg is

repair of Tg occurs in vivo. Previous studies have found that nth mutants are not hypersensitive to hydrogen peroxide and ionizing radiation (20). This could be explained by redundancy in the repair of Tg. An additional glycosylase that recognizes Tg, endonuclease VIII (endo VIII), has been identi®ed in E.coli and characterized (21–23). Endo VIII is encoded by the nei gene and nth nei double mutants are hypersensitive to hydrogen peroxide and ionizing radiation. Furthermore, Tg has been reported to be a substrate for the UvrABC-mediated nucleotide excision repair (NER) system in E.coli (24,25). Hence, there may be redundancy in the removal of Tg in cells.

Table 1. Bacterial strains used in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB1157</td>
<td>Wild type</td>
<td>R. Cunningham</td>
</tr>
<tr>
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<td>Δnth</td>
<td>R. Cunningham</td>
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<td>BW9109</td>
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<td>Δxth Δnth</td>
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<td>BW534</td>
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<tr>
<td>W3110</td>
<td>Wild type</td>
<td>R. Bockrath</td>
</tr>
<tr>
<td>MGM</td>
<td>uvrA277::Tn10</td>
<td>R. Bockrath</td>
</tr>
</tbody>
</table>

Strains with mutations in xth, de®cient in the major AP endonuclease, exo III, are very sensitive to treatment with hydrogen peroxide (26–29). As suggested by Demple et al. (26), the sensitivity of xth strains may, at least in part, be caused by the persistence of repair intermediates generated by the glycosylase and lyase activities of endo III or abnormal termini generated directly by hydrogen peroxide. This is supported by the observation that xth mutants exposed to hydrogen peroxide accumulate large numbers of single-strand breaks in their chromosomes (30).

Transcription-coupled repair (TCR) is a subpathway of repair whereby lesions are removed more rapidly or more ef®ciently from the transcribed strands of expressed genes than from the non-transcribed strands (31,32). This feature of repair has been clearly demonstrated to be a subpathway of NER in E.coli (33), yeast (34,35) and mammalian cells (36–38). It operates on a wide spectrum of bulky adducts that generally block transcription elongation. While the precise mechanism is unknown, an early event likely involves blockage of the RNA polymerase complex at lesions present in the transcribed strands of expressed genes.

It has been suggested that BER pathways can also be coupled to transcription and Tg has been found to be removed more rapidly from the transcribed strands of expressed genes in yeast (34) and mammalian cells (39,40). However, there has been no direct genetic demonstration of a role of BER in TCR. While it has been reported that 8-oxoguanine is a substrate for the glycosylase and lyase activities of endo III or abnormal termini generated directly by hydrogen peroxide. This is supported by the observation that Tg (43) are not ef®cient blocks to RNA polymerase progression. Hence, it is unclear if TCR is a bona ®de subpathway of a BER pathway or whether Tg is removed in a transcription-dependent manner in E.coli.

In the present study, the removal of Tg from the genomes of wild-type and repair gene mutant strains of E.coli treated with hydrogen peroxide was measured using a monoclonal antibody that speci®cally recognizes Tg in DNA. Tg is rapidly and ef®ciently removed from the total genomes of repair-proficient bacterial cells in vivo. Investigation of BER and NER gene mutant strains indicates that the removal of Tg is completely dependent on the nth gene that encodes the endo III glycosylase. Hence, it appears that little redundancy in the repair of Tg occurs in vivo, at least under the conditions used here.

Bacterial strains and growth conditions

The bacterial strains used in this work are derivatives of E.coli K-12 and are listed in Table 1. The MGM strain is a derivative of W3110. The other mutant strains are derivatives of AB1157. The AB1157 strain and its derivatives were grown at 37°C in Difco Bacto minimal broth Davis (MB) supplemented with 0.4% glucose, the appropriate antibiotic, 200 µg/ml each of arginine, histidine, leucine, proline and threonine and 10 µg/ml thiamine (complete medium). W3110 and MGM were grown at 37°C in MB supplemented with 0.4% glucose and 2 µg/ml thymine (complete medium).

Measurement of thymine glycol

Cultures were grown to saturation in complete medium, diluted 1:50 in complete medium and grown to an absorbance of 0.2 at 650 nm. To induce transcription of the lac operon, isopropyl β-d-thiogalactoside (IPTG) was added to a final concentration of 1 mM and growth was continued to an absorbance of 0.55. Hydrogen peroxide was added and the cultures were incubated on ice for 10 min. To terminate the hydrogen peroxide treatment, cells were collected by centrifugation on 0.45 µm Millipore membrane filters, washed with MB and resuspended at the same density in complete medium containing 1 mM IPTG. Cells were either lysed immediately or incubated in complete medium for increasing periods of time at 37°C to allow repair and then lysed. For each time point, 20 ml samples were collected on ice and quickly chilled by the addition of 20 ml of pre-chilled 2× NET (200 mM NaCl, 20 mM EDTA pH 8.0, 20 mM Tris pH 8.0). Cells were collected on 0.45 µm Millipore filters, resuspended in 700 µl TE (10 mM Tris pH 8.0, 1 mM EDTA pH 8.0) and lysed by the addition of 1 mg lysozyme for 30 min at 37°C. Samples were treated with RNase A at 200 µg/ml for 30 min, and then incubated overnight at 37°C with proteinase K at 100 µg/ml
and 0.5% Sarkosyl sulfate. Samples were extracted with phenol and DNA was precipitated with 2.5 M ammonium acetate and 0.6 vol isopropl alcohol. Purified DNA was resuspended in TE and incubated with ApoI and SstII (BRL Life Technologies, Grand Island, NY) overnight.

The introduction and removal of Tg was quantified using a monoclonal antibody that recognizes Tg in DNA in an enzyme-linked immunosorbent assay (ELISA) as described by Leadon et al. (44) as follows. Polystyrene microtitre plates were incubated with 20 μg/well protamine sulfate for 90 min at room temperature. Protamine sulfate was removed by washing the plates three times with distilled water. OsO4-modified DNA in 50 μl of phosphate-buffered saline (PBS) was adsorbed to each well by drying the DNA for 16 h at 37°C. The plates were then washed with PBS containing 0.05% Tween 20 and 1% heat-inactivated horse serum (PBS/Tween/hs) and incubated with 1% heat-inactivated horse serum for 4 h at room temperature. The solution was removed and plates were washed with PBS/Tween/hs. The monoclonal antibody with or without competitor DNA (DNA from bacterial cells were washed with PBS/Tween/hs) and incubated with 1% heat-inactivated horse serum for 4 h at room temperature. The amount of primary antibody bound in each well was determined by spectrophotometric analysis of the production of p-nitrophenol.

Clonogenic survival
Cultures were grown to saturation in complete medium, diluted 1:50 in complete medium and grown to an absorbance of 0.4 at 650 nm. Hydrogen peroxide was added and cells were incubated on ice or at 37°C. Samples (10 μl) were taken after cultures were incubated for 7.5 or 15 min, diluted in 1.8 ml of pre-chilled MB and placed on ice. Serial dilutions were spread on LB plates and incubated for 24 h at 37°C.

Measurement of β-galactosidase
Cultures were grown to saturation in complete medium, diluted 1:50 in complete medium, grown to an absorbance of 0.4 at 650 nm and incubated with hydrogen peroxide for 10 min on ice or at 37°C. Cells were then collected by filtration on 0.45 μm Millipore filters, washed with MB and resuspended in complete MB medium and 1 mM IPTG. Samples (500 μl) were taken after 30, 60, 90 and 120 min of incubation at 37°C. β-Galactosidase activities were determined as described by Miller (45) except that cells were permeabilized by freezing samples in a dry ice/ethanol bath and then quickly thawing them at 37°C (three cycles).

Examination of persisting strand breaks in wild-type and mutant strains treated with hydrogen peroxide
Cultures were expanded, treated with hydrogen peroxide, incubated for increasing times to allow repair and the DNA was purified and treated with restriction enzymes as described in the ‘Measurement of thymine glycol’ section. Two samples of DNA from each time point were examined for each experiment using wild-type, nfo, xth or nfo xth mutant strains. One sample from each time point was denatured for 3 min at 100°C and the other sample was not heat denatured. All samples were incubated on ice for 15 min and then electrophoresed overnight in non-denaturing 1% agarose gels. Gels were stained with either ethidium bromide or GelStar (FMC Bioproducts, Rockland, ME), photographed and the DNA from the gel transferred to a membrane, hybridized with 32P-labeled probe, washed and exposed to X-ray film. RNA probes were made using the pZH10 plasmid that contains a portion of the lactose operon (33).

RESULTS
Tg is induced in a linear, hydrogen peroxide dose-dependent manner and cell death is reduced by treatment of cells with the drug at 0°C
Since one of the goals of this study was to measure the removal of Tg from each strand of the lac operon, cells were treated with increasing concentrations of hydrogen peroxide to determine the concentration that would introduce approximately two Tg residues per 6.6 kb of DNA in each strand. This lesion frequency has proven optimal for the investigation of TCR of UV-induced damage in E.coli (33,46). Wild-type cells were exposed to increasing concentrations of hydrogen peroxide ranging from 25 to 200 mM, for 10 min at 0 or 37°C. DNA was isolated from cells, treated with restriction enzymes and the ELISA method was used to determine the frequency of Tg introduced by each dose of hydrogen peroxide. Treatment of cells with increasing concentrations of hydrogen peroxide resulted in a linear increase in the number of Tg adducts introduced into DNA and treatment of cells with 200 mM hydrogen peroxide resulted in the introduction of approximately two Tg lesions per 6.6 kb of DNA/strand (Fig. 1A). Similar results were obtained when cells were treated with hydrogen peroxide on ice or at 37°C.

Cell survival was compared after treatment of wild-type cells with 200 mM hydrogen peroxide on ice or at 37°C. Substantially more cell death was observed when cells were treated at 37°C (Fig. 1B). To minimize the lethal effects of the drug, subsequent experiments to examine the removal of Tg were carried out by exposing cells to hydrogen peroxide on ice.

Global genome removal of Tg is completely dependent on the nth gene
The removal of Tg from the total genomes of wild-type and various DNA repair mutant strains was measured. Cells were treated with hydrogen peroxide at 0°C and lysed immediately or after incubating for increasing periods of time at 37°C to allow repair. Results obtained using two wild-type strains, AB1157 and W3110, indicate that Tg is rapidly removed from total DNA; 50% of the initial Tg damage was removed within 10 min and 80% was removed within 40 min after hydrogen peroxide treatment (Fig. 2A). Repair was examined in three different single BER mutant strains. The nth mutant strain is deficient in the glycosylase, endo III. The xth mutant strain is deficient in exo III which provides the major AP endonuclease activity. The nfo mutant strain is defective in endo IV which provides a minor AP endonuclease activity. Only the nth mutant defective in endo III showed reduced repair of Tg (Fig. 2A). Moreover, no removal of Tg was observed in the
absence of endo III. Repair in the xth or nfo mutant strains resembled that found in wild-type strains, it was fast and very efficient. In addition, global genome repair was examined in several strains containing different combinations of double mutations (Fig. 2B). In the double AP endonuclease mutant strain, xth nfo, repair was very similar to that found in wild-type cells. In contrast, consistent with the single mutant data, double mutant strains containing a mutation in nth were completely deficient in the removal of Tg.

**Global genome removal of Tg is unaffected in a NER-deficient strain**

The UvrABC damage recognition and incision complex that functions in NER in *E.coli* has been found to recognize Tg in vitro (24,25). To test whether NER plays a major role in the removal of Tg from cells *in vivo*, repair was measured in a *uvrA* mutant strain. First, the UV sensitivity of the *uvrA* strain was tested and found to exhibit the expected UV-sensitive phenotype (data not shown). However, the removal of Tg was similar to that observed in the wild-type strains (Fig. 2A).

**Hydrogen peroxide inhibits induction of β-galactosidase**

Measurement of the removal of Tg from each strand of the lac operon was attempted using the Tg-specific monoclonal antibody in an immunoprecipitation assay. The method uses the Tg antibody to physically separate DNA fragments containing Tg from DNA fragments free of Tg. While this assay has been used to measure repair in the individual strands...
plates. As has been found previously, the treatment and the appropriate dilutions were plated on LB agar were taken at 7.5 and 15 min after hydrogen peroxide effects of hydrogen peroxide was examined (Fig. 4). Cells The sensitivity of wild-type and mutant strains to the killing below those observed with 40 J/m² UV light. Hence, it is likely that the concentrations of hydrogen peroxide required to measure TCR using established methods inactivate transcription of the operon.

The generation of repair intermediates by endo III and perhaps other glycosylases contributes to hydrogen peroxide-induced cell death

The sensitivity of wild-type and mutant strains to the killing effects of hydrogen peroxide was examined (Fig. 4). Cells were treated with 200 mM hydrogen peroxide at 0°C, aliquots were taken at 7.5 and 15 min after hydrogen peroxide treatment and the appropriate dilutions were plated on LB agar plates. As has been found previously, the nth mutant strain was not sensitive to killing by hydrogen peroxide (20) while the xth mutants deficient in the major AP endonuclease activity were very sensitive (26–29) (Fig. 4). Sensitivity was even greater in the double nfo xth mutant than in the single xth mutant strain, although the nfo mutation alone did not exhibit a sensitivity different from that of the wild-type. Hence, as previously shown (20,26–29), inactivation of the major AP endonuclease activity results in significant cell killing by treatment with hydrogen peroxide. Cell death is further increased by inactivation of both the major and minor AP endonuclease activities. In contrast, inactivation of the endo III glycosylase that we have shown is required for repair of Tg does not result in enhanced sensitivity to hydrogen peroxide.

The repair and cell survival data are consistent with a model that hydrogen peroxide-induced cell death is, at least in part, caused by the persistence of abnormal 3’ termini (repair intermediates) rather than the persistence of Tg. Abnormal 3’ termini can be generated by the direct action of oxidative agents or during the repair of Tg and other oxidative damage by bifunctional DNA glycosylases. To test the model, the effect of exo III and endo IV in processing single-strand termini was investigated by examining the integrity of DNA isolated from wild-type and mutant cells at different times after treatment with hydrogen peroxide. Two equivalent samples of DNA from each repair time point were examined: one was not denatured and the other one was heat denatured for 3 min at 95°C. The samples were electrophoresed in parallel using non-denaturing agarose gels, transferred to a membrane and probed with a 32P-labeled RNA probe specific for the lac operon. Single-strand DNA migrates with a faster mobility compared to duplex DNA under the conditions used in these experiments and DNA possessing single-strand breaks does not appear as full-length in the denatured samples. It appears, instead, as a smear due to fragmentation into smaller segments.
The DNA from wild-type cells and the nfo mutant strain had minimal single-strand breaks at each time point examined (Fig. 5). In contrast, denatured DNA from each time point for the xth mutant strain and the xth nfo double mutant strain possessed large numbers of breaks. This was observed as a conversion of the full-length fragment present in the non-denatured samples to a smear below the position of the full-length single-strand DNA in the denatured lanes at the different time points (Fig. 5). Hence, it appears that in the absence of exo III, strand breaks likely representing abnormal 3’ termini generated by glycosylase activities persist after treatment of cells with hydrogen peroxide. In the wild-type and nfo mutant strain, little smearing is observed below the full-length single-strand DNA even at the 0 time point and early repair time points. This likely reflects the coordination of glycosylase activity with post-incision events, otherwise smearing should have been observed at the early times that converts to full-length fragments at the later time points. Thus, the formation of strand breaks at this dose of hydrogen peroxide is more likely to be a consequence of repair rather than the direct action of reactive oxygen species.

**DISCUSSION**

Various aspects of the removal of Tg in E.coli were examined *in vivo*. The approach involved using a monoclonal antibody that specifically recognizes Tg in DNA. In establishing conditions for treating cells with hydrogen peroxide, we found that treating cells on ice resulted in a significant reduction in cell death compared with treating them at 37°C. This result is consistent with two previous reports that the cytotoxicity of hydrogen peroxide is diminished at low temperatures in yeast (47) and in mammalian cells (48). However, the levels of Tg introduced at the two different temperatures were comparable, results similar to those obtained in a previous study of yeast (47). Hence, the temperature-dependent difference in hydrogen peroxide-induced cytotoxicity in E.coli does not correlate with differences in the levels of Tg. This may reflect temperature-dependent differences in the direct introduction of other types of DNA damage by hydrogen peroxide, such as clustered DNA lesions, or differences in the production of repair intermediates that may result in a greater frequency of lethal double-strand breaks at 37°C.

The removal of Tg is rapid and efficient in wild-type strains even at the relatively high lesion frequency introduced in these experiments, approximately two Tg per 6.6 kb of DNA per strand. Previous studies of Tg removal in E.coli have used biochemical assays to measure repair *in vitro* (8,9) or have used more indirect approaches, such as measuring the transfection efficiency of oxidatively damaged phage DNA (19). Here, the introduction and removal of Tg was directly measured in cells. There are at least two mechanisms that produce damage in E.coli exposed to hydrogen peroxide and two modes of cell death have been observed (29). ‘Mode one’ killing occurs at low doses of hydrogen peroxide and reaches a maximum at 1–3 mM hydrogen peroxide. It requires active metabolism and has been shown to occur through the Fenton reaction *in vivo* and *in vitro* (28,49). ‘Mode two’ killing occurs at higher doses (>20 mM) and can occur in the absence of metabolism. The dose–response curve for the generation of lesions that result in ‘mode two’ killing is linear with respect to the concentration of hydrogen peroxide while the dose–response curve for ‘mode one’ killing is more complicated (28,50). The repair and survival data presented here likely relate to ‘mode two’ killing. However, it would be surprising if the removal of Tg from wild-type and repair gene mutant strains would differ at lower doses of hydrogen peroxide or

![Figure 5](https://example.com/figure5.png)

**Figure 5.** Single-strand breaks persist in xth strains after treatment with hydrogen peroxide. Cells were treated with 200 mM hydrogen peroxide at 0°C for 10 min, the drug was removed and cells were incubated at 37°C for the indicated times to allow repair. DNA was isolated, treated with restriction enzymes and purified. Two samples were taken at each time point. One sample was denatured by incubation at 100°C for 3 min and the other sample was not denatured. All samples were electrophoresed under non-denaturing conditions and the DNA was transferred to a membrane and the fragment containing the lac operon detected using radiolabeled probe. The arrows indicate the position of full-length single-stranded lac-containing fragment.
under conditions where cells are exposed to the drug at 37 rather than 0°C.

Biochemical studies have demonstrated that endo III acts as a glycosylase to recognize and remove Tg from DNA (7). However, many years ago, studies of nth mutants lacking endo III found that they are no more sensitive to treatment with oxidative agents than wild-type strains (20), while xth mutant strains were found to be very sensitive (26,27). These observations have provided an interesting conundrum. Either Tg is not a lethal lesion or back-up systems exist that can also remove Tg. The identification of an additional glycosylase, Tg is not a lethal lesion or back-up systems exist that can also remove Tg. This is followed by the AP lyase activity of endo III that produces a normal 3'-terminus and a gap of 1 nt. No significant removal of Tg is found in nth mutant strains but they survive exposure to high doses of hydrogen peroxide as well as wild-type cells. Hence, the presence of Tg in DNA does not correlate with cell death. In contrast, xth mutant strains efficiently remove Tg and the removal of Tg by endo III or the processing of other lesions induced by hydrogen peroxide results in the formation of strand breaks containing abnormal 3' termini. The sensitivity of xth mutants to high concentrations of hydrogen peroxide is likely a consequence of the inability to process repair intermediates.

This study indicates that Tg is not a lethal lesion in the E.coli genome in cells treated with high concentrations of hydrogen peroxide. A diagram illustrating how the processing of hydrogen peroxide-induced damage can contribute to cell death is presented (Fig. 6). The nth mutant cells are completely deficient in removing Tg from their genomes but they are not sensitive to doses of hydrogen peroxide that induce the formation of substantial levels of Tg. In contrast, previous studies (26–29) and ours find that xth mutant cells are highly sensitive to hydrogen peroxide. In addition, we find that the xth mutant strains are proficient in the removal of Tg from their genomes but are deficient in the processing of strand breaks or abnormal termini. These results indicate that the abnormal termini generated by endo III, other glycosylases or directly by reactive oxygen species represent lethal lesions induced by hydrogen peroxide. These results are consistent with other studies that have provided evidence that the lethal damage induced by hydrogen peroxide is 3'-blocked termini repair intermediates (30,51,52).

We were unable to measure TCR in the lac operon. The use of the Tg-specific monoclonal antibody in an immuno-precipitation approach to fractionate Tg-containing lac fragments from those free of Tg proved not to be amenable to these studies. The investigation of TCR was also complicated by the observation that treatment with hydrogen peroxide resulted in a significant reduction in β-galactosidase induction. Levels were significantly below those found after treatment with 40 J/m² UV light. Hence, even if the antibody fractionation approach had been successful, it is likely that

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**Figure 6.** Diagram of the initial steps of BER (reviewed in 5,6) to illustrate how the processing of different lesions influences cell death. One strand of DNA is represented containing a Tg. Endo III first catalyzes the breakage of the glycosyl bond between the base and the DNA sugar-phosphate backbone and releases Tg. This is followed by the AP lyase activity of endo III that produces a 5'-unsaturated aldehyde and a 5' phosphate. Exo III then cleaves the phosphodiester bond 5' to the AP site producing a normal 3' terminus and a gap of 1 nt. No significant removal of Tg is found in nth mutant strains but they survive exposure to high doses of hydrogen peroxide as well as wild-type cells. Hence, the presence of Tg in DNA does not correlate with cell death. In contrast, xth mutant strains efficiently remove Tg and the removal of Tg by endo III or the processing of other lesions induced by hydrogen peroxide results in the formation of strand breaks containing abnormal 3' termini. The sensitivity of xth mutants to high concentrations of hydrogen peroxide is likely a consequence of the inability to process repair intermediates.

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the levels of transcription inhibition would have precluded the ability to measure TCR. Given the genetic tractability of E.coli to study the specific involvement of BER in TCR of oxidative damage, future investigation is warranted to determine if oxidative damage is repaired by TCR in E.coli and, if so, what roles different repair pathways play in it.

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