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Genetics

Endonuclease III (*nth*) mutants of *Escherichia coli*

(apurinic sites/thymine glycol-DNA glycosylase)

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ABSTRACT **Two strains that overproduce endonuclease III were found in a colony bank containing hybrid ColE1-*Escherichia coli* plasmids. The enzyme was identified in crude extracts by the degradation of partially depyrimidinated DNA in the presence of EDTA, by its sedimentation velocity, and by its associated thymine glycol-DNA glycosylase activity. An insertion mutation was produced by cloning the kanamycin-resistance gene of Tn*5* into the plasmid copy of the *nth* gene. The mutation was then transferred to the chromosome in the following steps: (*i*) selection for chromosomal integration of the plasmid at 42°C in a temperature-sensitive *polA* strain, (*ii*) curing via temperature shifts, and (*iii*) phage P1-mediated transduction of a new host. The insertion mutant, as well as a separately isolated deletion mutant, had no measurable glycosylase activity for DNA containing thymine glycol. Although such residues are common lesions in oxidized or irradiated DNA, the mutants were not unusually sensitive to H_2O_2 or γ-rays. The insertion mutation had a mutator effect (4- to 22-fold enhancement) on one tested allele.**

The thymine residues of DNA are especially susceptible to damage by oxidizing agents and by the oxygen-containing free radicals generated by normal aerobic metabolism or by ionizing radiation (1–7). The major detectable product is thymine glycol (*cis*-5,6-dihydroxy-5,6-dihydrothymine). This compound, as well as its decomposition products, can be hydrolyzed from DNA by the glycosylase activity associated with endonuclease III of *Escherichia coli*, leaving behind an apyrimidinic site. Endonuclease III is also an apurinic/apyrimidinic (AP) endonuclease—i.e., a DNase specific for AP sites in DNA. It can cleave the phosphodiester bond on the 3′ side of a base-free sugar that it generates via its glycosylase activity. It thus appears to be a bifunctional enzyme, catalyzing two sequential steps in a base-excision repair pathway (5, 8–11).

As reviewed by Lindahl (12), *E. coli* contains several other AP endonucleases. Exonuclease III has an associated AP endonuclease activity that accounts for about 85% of that found in cell-free extracts. Endonuclease IV is a monofunctional enzyme typical of that found throughout nature. Endonuclease V recognizes many types of lesions, and it cleaves even undamaged single-stranded DNA. Endonuclease VII is specific for AP sites in single-stranded DNA. By isolating and studying mutants, we may determine to what extent the individual enzymes function uniquely in specific repair pathways and to what extent are they redundant. Only exonuclease III (*xth*) mutants have been isolated (13). They are sensitive to H_2O_2, and they do not tolerate *dut* (deoxyuridine triphosphatase) mutations, both of which are expected to generate AP sites via base-excision repair. Under normal conditions, however, *xth* mutants display no defect except for a high frequency of intrachromosomal recombination (13).

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Mutants for exonuclease III were isolated mainly by the mass screening of mutagenized clones by semiquantitative enzymatic assay (14). This approach, however, was not feasible for other AP endonucleases because of their relatively low activity in crude lysates and their mutual interference. To circumvent these problems, we searched for overproducers instead of for enzyme-deficient mutants. We shall describe how we identified a plasmid containing the gene for endonuclease III and how we used it to construct a tight chromosomal insertion mutation that was marked by drug resistance and therefore readily transferrable.

MATERIALS AND METHODS

Genetic Nomenclature. *nth* is a gene affecting endonuclease III. *nth-1*::*kan* or *nth*::*kan* is a mutation created by the insertion of a fragment of Tn*5*, containing a gene for kanamycin resistance, into the *nth* gene. Deletion mutations are designated as follows: Δ*nth* = Δ(*manA-nth*)*84*; Δ*xth* = Δ(*xth-pncA*)*90*.

DNAs. T4·U DNA (uracil-containing T4 DNA) was obtained from phage T4 amE51 nd28 rIIH28 alc10 (56⁻ denA denB alc) (15) grown in a *dut ung* host, BW313. M9 medium was used for ^3H labeling and 3-(*N*-morpholino)propanesulfonic acid (Mops) medium was used for ^{32}P. Cells that were grown to 2×10^8 per ml in the presence of 100 μg of thymidine per ml were centrifuged and resuspended in a thymidine-free medium to which were added tryptophan (100 μg/ml), 2′-deoxyadenosine (400 μg/ml), and carrier-free [*methyl*-^3H]thymidine (or $^{32}P_i$) at 5 μCi/ml (1 Ci = 37 GBq). The cells were infected at a multiplicity of 0.2. After 3.5 hr at 37°C, $CHCl_3$ was added and the phages were purified by two cycles of differential centrifugation (16) followed by gel filtration chromatography with Bio-Gel A-5M (Bio-Rad). The DNA was extracted with phenol (16). T4·AP DNA (DNA containing apyrimidinic sites) was made by treating T4·U DNA with an excess of *E. coli* uracil-DNA glycosylase (17) (gift of B. Duncan) in a reaction mixture (1 ml) containing 40 μM radiolabeled T4·U DNA, 70 mM Hepes·KOH (pH 8.2), 1 mM dithiothreitol, 1 mM EDTA, and 4000 units of enzyme. The mixture was incubated at 37°C for 2 hr and stored up to several months at 4°C. The substrate was tested by incubating a sample in 0.15 M NaOH for 20 min at 37°C; 45–50% became acid-soluble.

λ*c*I857*S7* DNA and λ*b*221*c*I857*ral*::Tn*5* DNA (18) were prepared by phenol extraction of CsCl-purified phages (19). The latter DNA was the source for a *Xho* I fragment containing the kanamycin-resistance gene. λ·AP DNA was λ*c*I857*S7* DNA that had been partially depurinated (20). Plasmid DNA was amplified (19) and purified (21) as described.

Abbreviations: AP, apurinic/apyrimidinic; T4·U DNA, uracil-containing T4 DNA; T4·AP DNA, DNA containing apyrimidinic sites; λ·AP DNA, λ*c*I857*S7* DNA that has been partially depurinated; Mops, 3-(*N*-morpholino)propanesulfonic acid.

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Poly(dA-[^{3}H]dT) was synthesized in a reaction mixture (1.0 ml) containing 0.55 mM dATP, 0.50 mM [*methyl*-^{3}H]dTTP (Amersham, 1 mCi/μmol), 50 mM Tris·HCl (pH 7.4), 0.01 mM unlabeled sonicated d(A-T)$_n$ primer (14), 10 mM Mg(OAc)$_2$, 2 mM 2-mercaptoethanol, and 40 units of the Klenow fragment of *E. coli* DNA polymerase I (New England Nuclear). The reaction reached an apparent limit of 90% completion in 2.5 hr at 25°C ($\Delta A_{260} = -0.44$ in a 1-mm path length cuvette). NaCl was added to 0.1 M, and the DNA was precipitated in EtOH (19), redissolved in 100 mM NaCl/10 mM Na_3EDTA, extracted with phenol (19), reprecipitated in 90-nmol aliquots, and dried *in vacuo*.

OsO_4-treated poly(AT)$_n$ was made in a reaction mixture (0.6 ml) containing 90 nmol of poly(dA-[^{3}H]dT), 0.6 mM potassium phosphate (pH 7.4), and 16 mM OsO_4. The copolymer was dissolved in the phosphate buffer at 80°C for 5 min and cooled to 55°C before the OsO_4 was added from a 64 mM solution ($A_{260} = 16$). After 20 min at 55°C, the mixture was chilled and extracted three times with 5 vol of cold ethanol-free ether. Excess ether was evaporated under an air stream, and the mixture was dialyzed at 4°C against 1 M NaCl/10 mM Tris·HCl, pH 7.0, followed by 0.1 M NaCl/10 mM Tris·HCl, pH 7.0/1 mM Na_3EDTA.

Mass Assays for EDTA-Resistant AP Endonuclease Activity. Procedures for the growth and assay of colonies in microwell plates were as described (14). Cells grown in 50 μl of TY broth at 37°C were pelleted and lysed with lysozyme at 0.1 mg/ml in 33 mM Hepes·KOH, pH 8.2/2.5 mM EDTA. To each lysate was added 50 μl of a reaction mixture containing 100 mM Hepes·KOH (pH 8.2), 400 mM NaCl, 2.5 mM EDTA, 0.2 mM dithiothreitol, 100 μM λ·AP DNA, and 5 μM T4·AP [^{3}H]DNA (1.5×10^4 cpm/nmol). The concentration of the carrier λ·AP DNA had been adjusted such that wild-type cells hydrolyzed 2–4% of the radiolabeled substrate in 30 min at 37°C.

AP Endonuclease Assay for Endonuclease III. Extracts were prepared as described (22), except that the sonication buffer was 50 mM Tris·HCl, pH 8.0/50 mM KCl/0.1 mM dithiothreitol. The enzyme dilution buffer contained 200 mM NaCl, 50 mM potassium phosphate (pH 7.4), 1 mM dithiothreitol, and bovine serum albumin (50 μg/ml). The reaction mixture (0.2 ml) contained 1 μM T4·AP [^{3}H]DNA (1.5×10^4 cpm/nmol), 50 mM Hepes·KOH (pH 8.2), 200 mM NaCl, 1 mM EDTA, 0.1 mM dithiothreitol, yeast tRNA at 50 μg/ml, bovine serum albumin at 50 μg/ml, 0.005% Triton X-100, and 0.01–0.05 unit of enzyme. After 30 min at 37°C, the tube was chilled, and 0.1 ml of salmon sperm DNA (2.5 mg/ml) and 0.3 ml of cold 10% trichloroacetic acid were added. After centrifugation at 12,000 × *g* for 10 min, radioactivity was measured in 0.5 ml of the supernatant. One unit of enzyme released 1 nmol of nucleotide.

Thymine Glycol Glycosylase Assay. Preparation and dilution of extracts and reaction conditions were those of the AP endonuclease assay except that the substrate was 400 pmol of OsO_4-treated d(AT)$_n$ (350 cpm/pmol). Reactions were stopped by the addition of 0.5 ml of cold (−20°C) ethanol and 50 μl of bovine serum albumin (1 mg/ml). The mixtures were centrifuged for 10 min at 12,000 × *g*. Disposable columns were made by cutting 1 cm off each end of 1-ml polyethylene droppers (Samco no. 222) that were then plugged with glass wool. Each column was packed with 0.5 ml of a suspension of Dowex (Bio-Rad AG1-X8 formate, 100–200 mesh), containing 20 g of resin in 28 ml of 10 mM NH_4COOH buffer (pH 4.2), and equilibrated with 1 ml of the same buffer. An ethanol-soluble reaction supernatant (0.5 ml) was applied to each column, followed by 1 ml of 50 mM NH_4COOH (pH 4.2). The 1.5 ml of displaced buffer and eluate were combined for radioactivity measurement. Assays were linear to at least 2.5 pmol of base released. In the absence of enzyme, <0.05 pmol was Dowex-nonadsorbable. One unit of glycosylase releases 1 pmol of oxidized thymine.

Microbiological Methods. M9 medium (19) was supplemented with 0.4 glucose, 1% vitamin-free Casamino acids (Difco), and thiamine at 1 μg/ml. Mops medium was that of Rhoades *et al.* (23) in which Tris was replaced with sodium 3-(*N*-morpholino)propanesulfonate buffer. The following were as described: TY (nutrient) medium (24), transductions with phage P1 vira (24), bacterial transformations (19), and selections for colicin-resistance (25). Antibiotics were filter-sterilized and used at the following final concentrations: kanamycin, 50 μg/ml; ampicillin, 100 μg/ml; and kasugamycin, 400 μg/ml.

Concentrated Extracts for Sedimentation Analysis. Cells were grown in TY broth to 5×10^8 per ml, harvested by centrifugation, and sonicated in 5 vol of 50 mM KCl/50 mM Tris·HCl, pH 8.0/1 mM dithiothreitol/1 mM phenylmethylsulfonyl fluoride. A 0.9% streptomycin supernatant (26) was treated with $(NH_4)_2SO_4$ at 4°C. The material precipitating between 40% and 70% saturation was dissolved in 0.2 M KCl/50 mM potassium phosphate (pH 7.4)/1 mM dithiothreitol (0.5 ml per g of cells) and dialyzed against the same buffer.

Other Methods and Materials. The following methods were as described: restriction endonuclease and DNA ligase reactions (19), agarose gel electrophoresis in Tris/acetate buffer (19), protein determinations (27), sedimentation rate analysis (28), and carbonic anhydrase assays (29). Restriction endonucleases and T4 DNA ligase were obtained from New England Biolabs and bovine carbonic anhydrase B was from Sigma. [^{3}H]Thymine glycol and [^{3}H]thymidine glycol (TLC markers) were synthesized by treatment of [*methyl*-^{3}H]thymine or [*methyl*-^{3}H]thymidine with OsO_4 (1). The unlabeled glycols were prepared by $KMnO_4$ oxidation and purified by gel filtration (3). They were authenticated by their absorption spectra in alkali (30). Radioactivity was measured by liquid scintillation in 5 ml of a Triton X-100/toluene-based medium.

RESULTS

Isolation of *nth* Plasmids. Strains of the Clarke–Carbon colony bank (25) bear the ColE1 plasmid into which random pieces of the *E. coli* chromosome have been cloned. These strains were assayed for AP endonuclease activity in the presence of EDTA, a unique property of endonucleases III and IV (12). The activity was elevated in stains carrying plasmids pLC9-9 and pLC28-48, which contain overlapping regions of *E. coli* DNA (Fig. 1). By subcloning restriction fragments (Fig. 1), we found that a region near one end of the cloned inserts, between the *Eco*RI and the *Bgl* II site, was

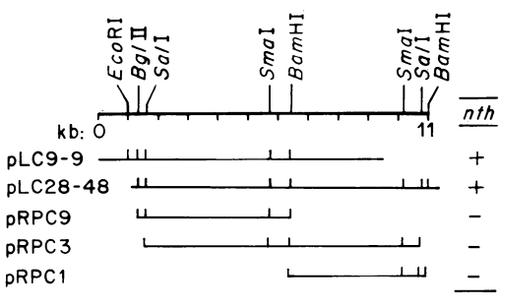

FIG. 1. Restriction map of the *nth* region in recombinant plasmids. Vector DNA is not shown. A *Sma* I site in the colicin gene was used to locate the cloning junctions in pLC9-9 and pLC28-48. pRPC9, pRPC3, and pRPC1 represent fragments of pLC9-9 subcloned in pBR322 (19). *nth* was scored by the AP endonuclease assay. Strains carrying *nth*$^-$ plasmids had from 0.8 to 1.2 times the specific enzymatic activity of the plasmid-free host JA200 (25), whereas *nth*$^+$ plasmids caused a >5-fold increase. kb, Kilobases.

needed for expression of the plasmid gene. Into the nearby *Sal* I site, we ligated a *Xho* I fragment of Tn*5* that contains a gene for kanamycin resistance (31). Drug-resistant, colicin-resistant transformants lacked an elevated AP endonuclease activity, indicating that we had interrupted the plasmid gene (or possibly an operon), producing a mutation that contained a kanamycin-resistance marker.

Isolation of *nth* Mutants. A chromosomal *nth* insertion mutation was obtained through genetic recombination with the plasmid. Strain MM383 (*polA12*), which contains a temperature-sensitive DNA polymerase I (32), was transformed to kanamycin resistance with the mutant plasmid at 32°C and then grown at 42°C in the presence of kanamycin. Because ColE1 plasmids require DNA polymerase I to replicate, this procedure should select for chromosomal integration of the plasmid. The recombination should occur mostly via the homologous chromosomal and plasmid *nth* regions, such that the integrated ColE1 DNA is bracketed by two copies of the *nth* region, containing an nth^+ and an *nth*::*kan* gene, respectively (33). To select for haploid (cured) *nth*::*kan* segregants, the cells were grown at 32°C and then at 42°C, both in the presence of kanamycin. The rationale for this step was that when DNA polymerase I resumed functioning at low temperature, the resulting autonomous replication of intrachromosomal ColE1 replicons would be lethal (33). Over 99% of the survivors were cured (i.e., colicin-sensitive). The *nth*::*kan* insertion mutation was then transduced into other strains via selection for kanamycin resistance.

The drug-resistance marker facilitated subsequent genetic mapping. In studies to be separately described, the *nth* gene was located near *manA* at 36 min. Strain SØ200, a *manA-add* deletion mutant (34), was found to be endonuclease-deficient as well. The deletion was transduced into other strains after linkage to *ksgB1*, which specifies kasugamycin resistance (35).

Identification of Endonuclease III. The following preliminary experiments (data not shown) confirmed that, in crude extracts, our DNase assay primarily detected AP endonucleases that were active in the presence of EDTA: (*i*) assay results were unaffected by a mutation in the gene for exonuclease III, the major AP endonuclease, which is EDTA-sensitive; (*ii*) although an extract from an *ung* (uracil-DNA glycosylase) mutant readily degraded the assay substrate (T4·AP DNA), it did not detectably degrade the uracil-containing DNA (T4·U DNA) from which the substrate was prepared; (*iii*) activities were not enhanced by using a sonicated (sheared) substrate, indicating minimal interference by exonucleases.

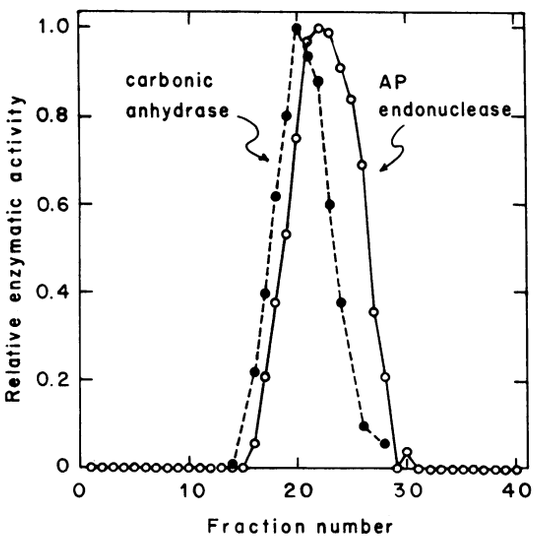

FIG. 2. Sedimentation profile of AP endonuclease activity. A mixture consisting of 10 μl of concentrated extract of JA200/pLC9-9 (0.4 mg of protein, 10.4 units/mg) and 100 μl of carbonic anhydrase B (5.0 mg/ml) was layered on a linear gradient of 5–20% sucrose in 200 mM NaCl/50 mM potassium phosphate, pH 7.4/10 mM Na_3EDTA and centrifuged in a Beckman SW 50.1 rotor at 49,000 rpm for 22 hr at 4°C. Fractions were collected from the bottom. Recovery of AP endonuclease activity was 67%.

Table 1. Enzymatic activities of mutants and overproducers

Strain†	Relevant genotype	Relative activity*	
		AP endonuclease	Glycosylase
AB1157	Wild type	(1.0)	(1.0)
BW372	*nth-1*::*kan*	0.20	<0.03
BW415	*Δnth*	0.34	<0.03
BW410	pLC9-9 (nth^+)	8.2	9.9
BW411	pLC28-48 (nth^+)	5.4	6.5

*Relative activity = ratio of the specific enzymatic activity of a strain to that of AB1157, which had 2.03 units of endonuclease and 37.9 units of thymine glycol-DNA glycosylase per mg of protein.
†The mutants are transductants, and the plasmid-bearing strains are transformants, of AB1157 (37).

Concentrated protein fractions were prepared for sedimentation analysis (see *Materials and Methods*). The specific activity of AP endonuclease in both the crude extracts and $(NH_4)_2SO_4$ fractions was 5 times higher for strain JA200/pLC9-9 than for JA200. The recovery of activity was 72% for both strains. The sedimentation coefficient of the overproduced enzyme was 2.7 S (Fig. 2) relative to that of carbonic anhydrase (3.06 S) and agreed with values of 2.6–2.7 S reported for endonuclease III (8, 36), but not with that for endonuclease IV (3.4 S) (26). The enzyme was further identified by its glycosylase activity.

Thymine Glycol Glycosylase Activity. The *nth* mutants still had about 20–34% of the parental level of EDTA-resistant AP endonuclease activity (Table 1), which may be due mainly to endonuclease IV, but they had no measurable thymine glycol glycosylase activity. Furthermore, the plasmid-bearing strains had elevated glycosylase levels. The glycosylase

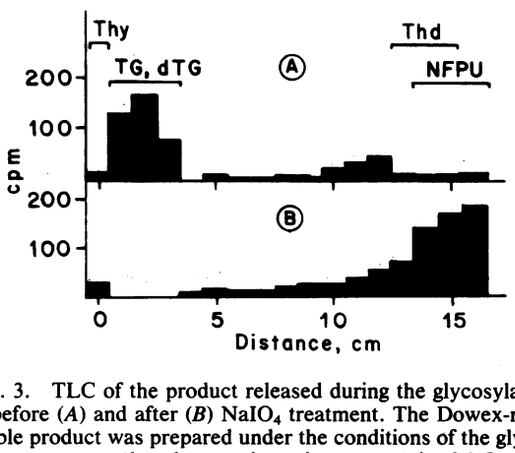

FIG. 3. TLC of the product released during the glycosylase assay, before (*A*) and after (*B*) $NaIO_4$ treatment. The Dowex-nonadsorbable product was prepared under the conditions of the glycosylase assay, except that the reaction mixture contained 1.5 nmol of OsO_4-treated poly(dA-[^{3}H]dT) and 10 μl of a sonicate of strain AB1157/pLC9-9 (20 μg of protein). The column eluate was dried *in vacuo* and a portion was treated with $NaIO_4$ (38). TLC was performed on methanol-soluble material, which contained 70% of the Dowex-nonadsorbable radioactivity. Samples were applied to 1.5 × 10 cm lanes on cellulose sheets (Eastman) and developed with *n*-butanol/H_2O (86:14). The lanes were cut into 5-mm strips for liquid scintillation spectroscopy. Radioactive markers were run in parallel, and their positions are indicated. TG, thymine glycol; dTG, thymidine glycol; NFPU, *N*-formyl-*N'*-pyruvylurea ($NaIO_4$-treated thymine glycol).

assay measured the release of ethanol-soluble, Dowex-non-adsorbable (i.e., relatively uncharged) residues derived from [^{3}H]thymine in OsO_4-treated DNA. The assay was authenticated by identification of the reaction product, >80% of which had the mobility of thymine glycol by TLC (Fig. 3*A*). Its mobility changed after mild periodate treatment (Fig. 3*B*), confirming that it is a *cis*-glycol. However, several TLC systems were unable to adequately separate thymine glycol from thymidine glycol, a compound that might be produced by the combined action of DNases and phosphomonoesterases in crude extracts. HPLC was therefore used (Fig. 4).

Preliminary Characterization of the *nth* Mutants. Unexpectedly, the *nth* mutants were about as sensitive as wild-type cells to ionizing radiation and to H_2O_2 (Fig. 5). Although exonuclease III (*xth*) mutants are extremely sensitive to H_2O_2 (39), an *nth* mutation did not enhance that sensitivity but appeared to offer a small protective effect (Fig. 5*B*). An *nth* mutation had a mutator effect on some alleles. In the example shown in Table 2, it caused a 4- to 22-fold increased reversion of *argE3* (ochre) mutants.

DISCUSSION

The method we used to obtain *nth* mutants resulted in a tight insertion mutation that was easily scored in recombinants because it also specified drug resistance. By screening for overproducers rather than mutants, we compensated for the relative insensitivity of the assay and for the effects of competing enzymes. The cloned gene and the overproducing strains were useful by-products of our search. Until we analyze the plasmid-specified polypeptides, however, we cannot be sure that the insertion is in the structural gene for endonuclease III. It may be exerting a polar effect or interrupting a regulatory gene.

To avoid purifying and identifying the wrong enzyme, we chose to characterize the overproduced DNase in relatively crude extracts. This approach led to the development of a thymine glycol-DNA glycosylase assay that is highly specific for endonuclease III in cell extracts and that may be used for studies on possible gene regulation.

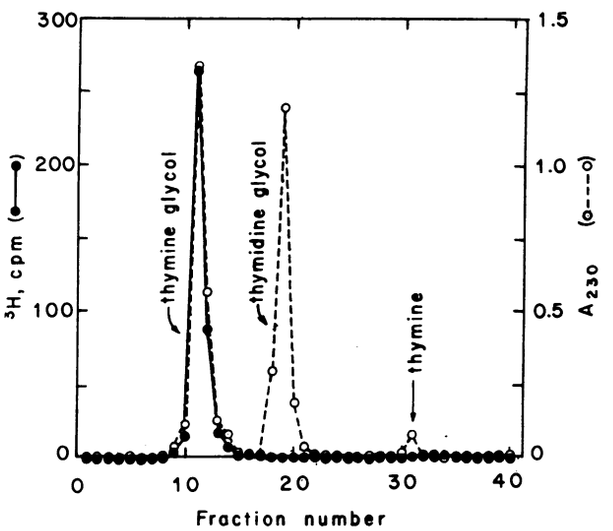

FIG. 4. HPLC of the glycosylase product. Radioactivity is that of the reaction product, and the absorbance is due to the unlabeled markers. The sample (100 μl) contained 1.4 pmol (500 cpm) of ^{3}H-labeled reaction product (see Fig. 3), 100 nmol each of thymine glycol and thymidine glycol, and 40 nmol of thymine. Reversed phase HPLC was performed with a Waters C_{18} column (0.39 × 30 cm) equilibrated with 2.5% methanol/2.5 mM potassium phosphate, pH 3.0. Flow rate was 1 ml/min at 1150 psi (1 psi = 6895 Pa). A_{230} was measured immediately after the addition of 40 μl of 0.1 M NaOH to each 0.37-ml fraction, and then 50 μl of 0.1 M HCl was added before liquid scintillation spectroscopy. Recovery of radioactive material was 79%. In separate runs monitored by A_{260}, dTMP was eluted 0.5 min after thymine glycol, and deoxythymidine was eluted 17 min after thymine.

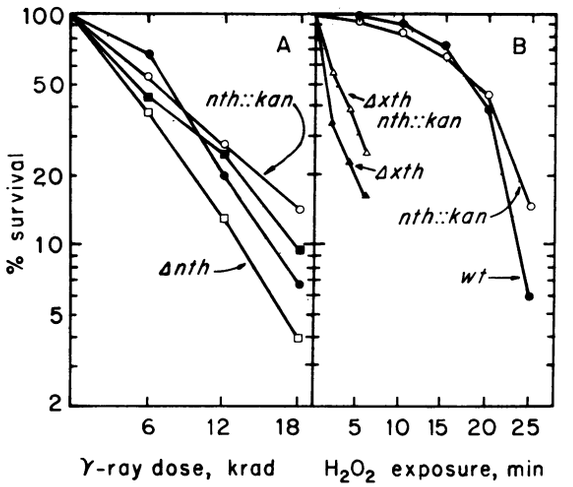

FIG. 5. Sensitivity of mutants to γ-rays (*A*) and to H_2O_2 (*B*). The strains and their relevant genotypes were as follows: AB1157, *nth*$^+$ (●); BW372, *nth-1*::*kan* (○); SØ333, *nth*$^+$ (■); SØ200, Δ*nth* (□); BW9109, Δ(*xth-pncA*)90 (▲); and BW434, Δ*nth* Δ(*xth-pncA*)90 (△). SØ200 is congenic with SØ333 (34). Other mutants were congenic transductants of AB1157. *wt*, Wild type. For γ-irradiation (*A*), fresh cultures, grown overnight with aeration, were kept in an ice bath during treatment. Prior to irradiation, the cultures were diluted 1:10,000 in 50 mM potassium phosphate (pH 7.6) and divided into samples that were bubbled with O_2 for 1 min. The tubes were sealed, exposed to 1200 rad/min in a ^{137}Cs irradiator (1 rad = 0.01 gray), and removed at 5-min intervals. Diluted samples were plated in duplicate on TY agar at 37°C for the determination of viable cells. Peroxide sensitivity (*B*) was measured by treating logarithmic phase cultures with 10 mM H_2O_2 at 37°C as described (39).

Because endonuclease III attacks oxidized thymine residues in DNA, we studied the reversion of an ochre mutation, which contains three A·T base pairs. In other studies (data not shown), however, we have found that many reversions of another ochre allele were due to suppressor mutations and may therefore not represent alterations of A·T pairs. Moreover, we do not yet know if the mutations are produced directly or are promoted by the inducible repair (SOS) system (42). In experiments to be separately reported, we found that the *nth* mutants were not unusually susceptible to the mutagenic effects of oxidizing agents.

The traits of the *xth* and *nth* mutants violate some of our preconceptions. Although endonuclease III is specific for oxidized DNA, it is the exonuclease III mutants that are sensitive to H_2O_2, and, whereas oxidizing agents and ionizing radiation are believed to produce similar types of damaged bases in DNA (1–5), *xth* mutants are unusually sensitive only to the former agent (13, 39). These results suggest that the predominant forms of damaged bases, such as thymine glycol, do not represent the most lethal lesions produced by these agents. We may be in a better position to delineate

Table 2. Mutation frequencies of *nth*$^+$ and *nth*$^-$ strains

Strain	Arg$^+$ revertants per 10^8 cells		
	Exp. 1	Exp. 2	Exp. 3
Wild type	3	4	4
nth-1::*kan*	14	24	88

Congenic strains were those of Table 1. Spontaneous revertants were scored after 4 days of growth on a limiting medium (40). Each value is the median for 10 independent cultures (41).

alternative repair pathways when we obtain mutations for endonuclease IV and combine them with the others.

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