Characterization of the Action of *Escherichia coli* DNA Polymerase I at Incisions Produced by Repair Endodeoxyribonucleases*

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The utilization for DNA synthesis by *Escherichia coli* DNA polymerase I of damaged circular duplex DNAs which had been incised by various endodeoxyribonucleases was studied. *E. coli* endonuclease III cleaves at apurinic/apyrimidinic (AP) lesions, generating 3'-deoxyribose and 5'-phosphomonoester termini. Whereas these 3' termini did not efficiently prime DNA synthesis, they could be activated to support efficient DNA synthesis by incubation with *E. coli* DNA polymerase I in the absence of deoxyribonucleoside triphosphates. Such maximally activated termini achieved a similar rate of DNA synthesis as termini from which the 3'-AP site had been removed by prior exposure to human fibroblast AP endonuclease II. In all cases, the product of DNA synthesis was covalently attached to the primer and stable to alkali, indicating that during the preincubation, *E. coli* DNA polymerase I could remove the 3'-deoxyribose termini. Indeed, deoxyribose 5-phosphate was observed after such a reaction. Activation by the DNA polymerase also was detected with W-irradiated DNA which had been incised by *E. coli* DNA polymerase I.

The two exonuclease functions of *E. coli* DNA polymerase I are distinct and separable from one another by proteolytic cleavage of the intact enzyme to produce a large fragment (78,000 daltons) containing the polymerase, and 3' to 5' exonuclease activities and a small fragment (36,000 daltons) which contains the 5' to 3' exonuclease activity (7-10). The 3' to 5' exonuclease has been suggested to increase the fidelity of DNA synthesis through a "proofreading" function which removes misincorporated nucleotides (5). The 5' to 3' exonuclease hydrolyzes DNA from a 5' terminus to produce 5'-mononucleotides and oligonucleotides (11). Deoxyribonucleoside triphosphates stimulate this exonuclease so as to coordinate polymerization and 5' to 3' exonuclease activity to result in "nick translation" (11). The excision of thymine dimers by the 5' to 3' exonuclease from UV-irradiated homopolymers (3) and from UV-irradiated DNA incised by T4 UV endonuclease (12) has been reported. Repair synthesis via nick translation might thus be initiated by a repair endonuclease cleaving one strand of a DNA duplex at or near a DNA lesion.

One type of DNA damage, apurinic or apyrimidinic (AP) lesions, accounts for a substantial amount of DNA damage, even under normal growth conditions (13, 14). AP lesions arise either spontaneously or by virtue of the action of specific DNA N-glycosylases which initiate repair of certain types of DNA damage by base removal to initiate "base excision repair" (14). Two classes of endonucleases have been described which recognize AP sites. Class I AP endonucleases cleave on the 3'-side of the AP site forming 3'-deoxyribose and 5'-phosphomonoester termini (Fig. 1) which are not efficient primers for DNA polymerase I (15). Class I AP endonucleases include human fibroblast AP endonuclease I (15), *E. coli* endonuclease III, and *T4* UV endonuclease (16-18). The T4 UV endonuclease and the analogous *Micrococcus luteus* UV endonuclease also incise DNA at pyrimidine dimers by the combined action of a pyrimidine dimer DNA N-glycosylase and a Class I AP endonuclease (17-19) to generate 3'-deoxyribose and 5'-phosphomonoester thymine dimer termini (Fig. 1). Incisions that contain 3'-AP termini are generally poor primers for DNA polymerase I (15, 16).

Class II AP endonucleases cleave on the 5'-side of AP lesions producing 3'-hydroxyl nucleotide and deoxyribose 5'-phosphate termini (Fig. 1). Such enzymes include human fibroblast AP endonuclease II (19), *HeLa* AP endonuclease (20), and *E. coli* endonucleases I V and VI (16). Incisions made by Class II AP endonucleases are efficient primers for DNA polymerase I. In addition, Class II AP endonucleases can activate Class I endonuclease incisions to support efficient DNA synthesis by DNA polymerase I through the removal of deoxyribose 5-phosphate from the AP site to produce a one...

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1 The abbreviations used are: AP, apurinic/apyrimidinic; dNTP, deoxyribonucleoside triphosphates; dNMP, deoxyribonucleoside monophosphates; pAp(d-ribose), a dinucleotide composed of a baseless sugar-phosphate at the 3' side and 5'dAMP at the 5' side.
nucleotide gap containing 3'-hydroxyl- and 5'-phosphomonoester termini (Fig. 1). When T4 UV endonuclease incisions are activated by a Class II AP endonuclease, an analogous reaction occurs (Fig. 1).

In the present report, the ability of DNA polymerase I to activate Class I AP endonuclease incisions by virtue of its 3'→5' exonuclease activity is examined. Whether DNA synthesis occurs via nick translation or strand displacement from various types of incisions made into damaged DNA is also investigated. Finally, the ability of the DNA polymerase I large fragment to substitute for the intact enzymes in reactions carried out on various incised circular duplex DNA primer/templates is investigated.

EXPERIMENTAL PROCEDURES

Materials

Supercoiled PM2 [H]HDNA (93% Form I) was isolated from phage grown on Alteromonas espejiana thymidine auxotroph Bal 31-14 as described by Espejo and Canelo (21) and modified by Kuhnlein et al. (22). Nonradioactive PM2 DNA was isolated similarly except that A. espejiana wild type Bal 31 was used. Activated salmon sperm DNA was prepared according to the procedure of Schlauder and Kornberg et al. (23); poly(dA-[^32]P)-dTdT was synthesized as described by Schachman et al. (24), and unlabeled poly(dA-dT) and its tri- and tetra-oligosomers were obtained from P-L Laboratories. Unlabeled deoxyribonucleoside triphosphates and monophosphates were purchased from Sigma. Deoxy[^3H]-uridine 5'-monophosphate and [5'[^3H]]dTTTP and [5'-[^3H]]dCTTP were from Amersham; [meth-[^3H]]thymidine, [5'[^3H]]dTTP, and [[^3H]]dATP came from Schwarz/Mann.

E. coli endonuclease III (Fraction VI) (25), uracil DNA N-glycosylase (Fraction VI) (26), homogeneous HeLa AP endonuclease (20), and human fibroblast AP endonuclease II (15) were prepared as previously described. Neurospora crassa endonuclease (Fraction IX) was isolated from Neurospora crassa endonuclease (Fraction IV) was as described by Friedberg and King (28).

DNA Synthesis Reactions—Reaction mixtures (450 pl) contained 70 mM potassium phosphate buffer (pH 7.5), 1 mM 2-mercaptoethanol, 7 mM MgCl2, 0.09 nM each of [3H]dUTP, [5'[^3H]]dTTP, and [[^3H]]dATP, and 1 pmol of activated salmon sperm DNA were substituted. The activated DNA was subjected to a sham incision reaction to which buffer was added instead of endonuclease prior to being used for DNA synthesis. Alkaline Sulfate Gradient Centrifugation—Reaction samples (200 pl) were placed on preformed agarose (3%) gel, which was stained with 40 mg/ml of 0.5, 10, and 30 mM Tris-HCl (pH 8.0), 250 mM NaCl, 900 mM NaCl, and 5 mM EDTA which had been formed over a 300-1 filter (Whatman GF/C filters) and washed with 1 ml 10% trichloroacetic acid. Precipitates were collected on filter Whatman GF/C filters, washed with 15 ml of 1 N HCl in 0.1 mM sodium pyrophosphate, dehydrated with 95% ethanol, and dried under a heat lamp. Acid-insoluble radioactivity was measured by liquid scintillation counting using double isotope counting.

DeAE-cellulose Chromatography of Reaction Products of [3P, uracil-[^3H]]poly(dA-dT)—Reactions (300 pl) contained 50 mM Tris-HCl (pH 8.2), 4 mM EDTA, 38 nM of [3P, uracil-[^3H]]poly(dA-dT), and 12 units of uracil DNA N-glycosylase. After incubation for 30 min at 37°C, the reactions were adjusted to 10 mM MgCl2, and 0.04 unit of E. coli endonuclease III was added. Following a second incubation for 90 min at 30°C, reactions were placed on ice, adjusted to DNA synthesis reaction conditions, and 20 units of DNA polymerase I or sodium citrate to the final reaction), and AP endonuclease as indicated by incubation with Neurospora crassa endonuclease was under identical conditions except that nondepurinated DNA was substrate. Incision with T4 UV endonuclease was carried out similarly in 25 mM potassium phosphate buffer (pH 7.5), 200 mM NaCl, 80 ng/ml of acetylated bovine serum albumin, 0.1 mM UV-irradiated DNA, and T4 UV endonuclease as described. Incubation was for 10 min at 37°C, followed by 3 min at 70°C to inactivate endonuclease. Samples containing approximately 2 nM of DNA were removed and the extent of endonuclease incision determined by the endonuclease assay. In some cases, excess AP endonuclease was then added during a subsequent 10-min incubation at 37°C. To determine whether DNA polymerase I would receive a second endonuclease treatment, an equal volume of 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, and 0.1 mg/ml of acetylated bovine serum albumin was added and incubation carried out as above. Following incubation, the reactions were heated for 3 min at 70°C. Endonuclease assays were also performed after the second reaction.
E. coli DNA polymerase I activates 3′-deoxyribose termini so as to prime DNA synthesis efficiently—When partially depurinated PM2 [3H]DNA is cleaved to a limit with E. coli endonuclease III, a Class I AP endonuclease, the resulting incisions contain 3′-deoxyribose and 5′-phosphomonoester termini (16) (Fig. 1). Such termini do not efficiently support DNA synthesis by DNA polymerase I (16). However, some incorporation of dNMPs (up to twice that seen for untreated PM2 DNA) was typically observed, and the rate of DNA synthesis during these reactions tended to increase as the reaction progressed (Fig. 2). Such a concave curve would be expected if 3′-deoxyribose termini were being activated in some manner during the DNA synthesis reaction so as to serve as efficient primers if primer activation was the rate-limiting step for subsequent DNA elongation.

In order to test the possibility that DNA polymerase I could activate 3′-deoxyribose termini, partially depurinated PM2 DNA incised with an excess of endonuclease III was preincubated with DNA polymerase I in the absence of dNTPs and then dNTPs were added and DNA synthesis was carried out. The results show an increase in the rate of DNA synthesis with increasing time of preincubation (Fig. 2). The same constant rate of DNA synthesis could be achieved after 2 h preincubation as was found for synthesis after a treatment with human fibroblast AP endonuclease II (Fig. 2). Human fibroblast AP endonuclease II, a Class II AP endonuclease, removed deoxyribose 5-phosphate from the 3′ termini formed by the E. coli endonuclease III to form a single nucleotide gap containing a 3′-hydroxyl nucleotide primer (15) (Fig. 1). Activation was dependent on DNA polymerase I or upon a second incision by a Class II AP endonuclease. Preincubation for 120 min in the absence of DNA polymerase I resulted in an 8.9% increased rate of DNA synthesis relative to that observed when the substrate was maximally activated with a Class II AP endonuclease. Thus, the majority of the activation of DNA synthesis required preincubation with DNA polymerase I and was not a result of spontaneous hydrolysis of the 3′-deoxyribose termini. It should be pointed out that the incubation with AP endonuclease II or DNA polymerase I treatment did not cause increased nicking; thus, the stimulation of DNA synthesis observed most likely resulted from modification of the endonuclease III incision sites.

**Activation of 3′-Deoxyribose Termini by DNA Polymerase**

![Fig. 1. Structure of the incision made by various endonucleases. Only one strand of the duplex DNA is shown.](http://www.jbc.org/)

![Fig. 2. Activation of E. coli endonuclease III incision sites by DNA polymerase I to support DNA synthesis. A 900-μl AP DNA incision reaction mixture containing 0.34 unit of endonuclease III was prepared and incubated for 10 min at 37 °C as described under “Experimental Procedures.” After heat inactivation of the endonuclease, a 240-μl sample was removed and 0.7 unit of human fibroblast AP endonuclease II was added (○—○). The original reaction mixture received an equal concentration of buffer and both reactions were incubated for an additional 10 min at 37 °C. Samples (20 μl) were removed at each reaction to assay for the extent of endonuclease activity. After heat inactivation, the original reaction was divided into three aliquots (△—△, ○—○, and Δ—Δ). Standard DNA synthesis reaction components were added to each reaction, except that dNTPs were omitted in two cases (○—○ and △—△) for the times indicated at 37 °C. Reactions from which dNTPs were omitted were preincubated at 37 °C for 80 min (△—△) or 120 min (○—○) with DNA polymerase I before dNTPs were added and DNA synthesis was carried out as above. The average number of incisions per DNA genome after endonuclease exposure is given in parentheses. PM2 [3H]DNA had been partially depurinated to contain about 0.7 AP site/DNA genome. The amount of DNA synthesis observed in reactions from which endonuclease III had been omitted was subtracted.](http://www.jbc.org/)
I to Support DNA Synthesis Involves the Removal of the 3'-AP Site—Activation of endonuclease III incisions by DNA polymerase I in the absence of dNTPs could occur by one of two mechanisms. Preincubation with DNA polymerase I could lead to an increase in utilization of baseless 3'-deoxyribose termini as primers, or the polymerase could remove the 3'-AP site to expose an internal 3'-hydroxyl nucleotide as primer. In order to distinguish between these two alternatives, partially depurinated PM2 [3H]DNA containing 0.7 AP site/duplex circle was incubated with an excess of endonuclease III and then the product was used as a substrate for DNA synthesis and analyzed after alkaline treatment by alkaline sucrose gradient centrifugation. If DNA polymerase I was capable of utilizing a 3'-deoxyribose terminus as a primer, then the initiation point of DNA synthesis should be alkali labile. Following alkaline hydrolysis of this 3'-deoxyribose moiety, single-stranded PM2 [3H]DNA should then be separable by alkali sucrose gradient sedimentation from the short [3'PP]DNA product of DNA synthesis. However, if the 3'-AP site was removed prior to DNA synthesis, then the H- and 32P-labels would remain covalently attached after alkali treatment and sediment near full length single-stranded linear DNA. Indeed, when an average of 145 [32P]nucleotides was incorporated per nick using the DNA treated with endonuclease III as primer-template, both labels co-sedimented as full length, linear single-stranded PM2 DNA (Fig. 3A). Likewise, after a 60-min preincubation of the substrate with DNA polymerase I in the absence of dNTPs, a 58% stimulation of DNA synthesis resulted in the incorporation of approximately 250 nucleotides/nick, and as before, the 32P product remained covalently attached to the single-stranded, linear PM2 [3H]DNA during alkali sucrose gradient sedimentation (Fig. 3B). In particular, no 32P was observed sedimenting at a position expected for a 250-nucleotide fragment. As a control, the substrate was totally activated by the addition of an excess of human fibroblast AP endonuclease I which removed the 3'-AP termini. After this removal of deoxyribose 5-phosphate from the 3' termini, DNA synthesis resulted in 420 nucleotides being incorporated/nick and, as expected, the product of this reaction was also alkali stable and both the H- and 32P-labels co-sedimented as before (Fig. 3C). Finally, when the unpolymerized, partially depurinated Form I PM2 [3H]DNA was subjected to alkaline hydrolysis followed by alkaline sucrose gradient sedimentation, similar [3H]DNA profiles were obtained as above, indicating that hydrolysis of the AP sites had occurred under these conditions (data not shown). The hydrolysis was dependent upon the alkali treatment since 0.17 nick/degurinated PM2 [3H]DNA molecule was observed prior to alkali treatment, whereas after exposure, 0.69 nicks/molecule was detected by the nitrocellulose filter endonuclease assay. The amount of single-stranded linear molecules produced by this treatment was directly proportional to the number of AP sites, assuming that the distribution of an AP site followed a Poisson distribution.

The above results argue that the 3'-deoxyribose moiety was removed by DNA polymerase I during the preincubation reaction such that the termini could efficiently prime DNA synthesis. The removal of the 3'-AP site was not due to contaminating AP endonuclease in the DNA polymerase I preparation since no endonuclease (less than 6.6 X 10^-3 units/unit of polymerase) was detected on partially depurinated PM2 [3H]DNA by the nitrocellulose filter endonuclease assay. In addition, preincubation of partially depurinated PM2 [3H]DNA with DNA polymerase I did not activate the substrate to support additional DNA synthesis unless the substrate had previously been cleaved by endonuclease III (Table I). The incorporation observed on partially depurinated PM2 DNA which was not treated with endonuclease most likely resulted from nicks which originate spontaneously in depurinated DNA during incubation, since this material typically contains roughly 8% Form II DNA above the 7% background.

It is unlikely that such an activity could account for the 38% stimulation of DNA synthesis relative to that observed when all 110 fmol of AP sites were cleaved with human fibroblast AP endonuclease II. However, we cannot discount the possibility that such a putative contaminating activity could cleave 3'-deoxyribose termini considerably more efficiently than internal AP sites.
observed for nondepurinated PM2 DNA.) Reactions carried out in the absence of PM2 DNA showed no significant DNA synthesis.

Nature of the Product Removed by DNA Polymerase I from 3'-Deoxyribose Termini—In order to elucidate the action of DNA polymerase 1 at 3'-AP sites, the hydrolysis products were isolated. [32P, uracil-3H]poly(dA-dT) (an alternating dA-dT polymer containing an occasional dUMP residue in place of dTMP) was depyrimidinated with uracil DNA N-glycosylase to release 3.6 pmol of uracil or 65% of the uracil content of the polymer. After treatment with endonuclease III and DNA polymerase I, the hydrolysis products were analyzed by urea-DEAE-cellulose chromatography (Table II). Two peaks of material containing only 32P were observed. One peak, containing 1.4 pmol of 32P-label, eluted coincidentally with deoxyribose 5-phosphate, while the other contained 0.34 pmol of 32P-label and eluted between the mono- and dinucleotide markers. The latter compound was not specifically identified, but migrated as though it were pAp(d-ribose).

The remaining 37% of the uracil that had not been removed by the uracil DNA N-glycosylase was observed after treatment with the endonuclease and polymerase to be dUMP, as evidenced by a 1:1 ratio of 3H to 32P and co-elution with a dUMP marker. We suspect that dUMP originated from terminal dUMP residues which were not internalized by DNA polymerase I. In support of this interpretation, we observed that other samples of [32P, uracil-3H]poly(dA-dT) which had been extended by DNA polymerase I were >85% sensitive to uracil DNA N-glycosylase.

E. coli DNA Polymerase I Activates T4 UV Endonuclease Incision Sites to Prime DNA Synthesis Efficiently—When UV-irradiated PM2 DNA is treated with T4 UV endonuclease, phosphodiester cleavage results in a 3'-deoxyribose juxtaposed to a thymine/thymidylate cyclodibutane dimer (18) (Fig. 1), and such termini do not efficiently support DNA synthesis by DNA polymerase I (16). However, upon incubation with the polymerase in the absence of dNTPs, these termini become activated to support DNA synthesis (Fig. 4). The level of activation achieved is virtually that observed by pretreatment with human fibroblast AP endonuclease II, a Class II enzyme that can remove 3'-deoxyribose termini (15) (Fig. 1). Presumably, the mechanism of activation with this substrate is similar to that described above, involving a removal of the deoxyribose 5-phosphate.

Mode of DNA Synthesis by E. coli DNA Polymerase I from Various Incision Sites—When undamaged Form I PM2 DNA is cleaved with N. crassa endonuclease, which cleaves Form I DNA to produce a single, 3'-hydroxyl- and 5'-phosphoryl-terminated nick (32, 33) (Fig. 1), the incision sites support efficient DNA synthesis by DNA polymerase I (15). After

| Table I | DNA Polymerase I Action at Repair Incisions |
| --- | --- |
| **E. coli endonuclease III** | **E. coli DNA polymerase I present during preincubation** |
|  | ([32P]dTMP incorporated into unit length single-stranded DNA) |
|  | cpm |
| - | - | 1600 |
| - | + | 2120 |
| + | - | 2960 |
| + | + | 7310 |

| Table II | DEAE-cellulose chromatography of products from partially depyrimidinated [32P, uracil-3H]poly(dA-dT) that had been incised with E. coli endonuclease III and exposed to DNA polymerase I |
| --- | --- |
| **Product recovered** | **DNA Polymerase I** |
| ([32P]deoxyribose 5-phosphate) | ([3P]Ap(d-ribose)) |
| pmol | | |
| 0.34 | 0.15 |
| 1.40 | 0.32 |

![FIG. 4. Activation of T4 UV endonuclease incision sites by DNA polymerase I to support DNA synthesis. UV-irradiated DNA was incubated in a 900-μl reaction mixture containing 0.25 unit of T4 UV endonuclease for 30 min at 37°C as described under "Experimental Procedures." After heat inactivation of the endonuclease, DNA synthesis was carried out for 30 min at 37°C. DNA synthesis reaction components were added and 20 units of DNA polymerase I or buffer was included as indicated. After 60 min at 30°C, reactions were chilled, added to 12 ml of distilled water, and applied to a DEAE-cellulose column (0.28 cm x 9 cm) equilibrated in water. The column was washed and developed as described under "Experimental Procedures." Fractions (1.8 ml) were collected.](http://www.jbc.org/)

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such synthesis with an average incorporation of 1550 nucleotides/nick, the product was observed by electron microscopy. Ninety-four per cent of the Form II DNA molecules observed were double-stranded DNA circles without tails, while 6% of the Form II molecules had tails containing an average of 300 nucleotides/tail (Table III). Synthesis was carried out with about 1 DNA polymerase I molecule/10 incision sites, and evidently under these conditions, DNA synthesis occurs primarily by nick translation.

When UV-irradiated PM2 DNA containing about one pyrimidine dimer per duplex circle was incised with an excess of T4 UV endonuclease and then incised with human fibroblast AP endonuclease II, the DNA substrate became an efficient primer for DNA synthesis (15). This DNA is thought to contain a one-nucleotide gap with a 3'-hydroxyl nucleotide and a 5'-pyrimidine dimer nucleotide terminus (15) (Fig. 1). After DNA synthesis using this substrate, 753 nucleotides were incorporated/nick and 87% of the Form II DNA molecules did not contain tails (Table III), suggesting, again, that nick translational synthesis had predominated, i.e. that the 5' → 3' exonuclease of polymerase I appears to be able to remove efficiently the pyrimidine dimers from this substrate under these conditions.

Partially depurinated PM2 DNA (0.8 AP site/duplex circle) was cleaved with the Class II human fibroblast AP endonuclease II so as to form a 3' terminus with a 3'-hydroxyl nucleotide and a 5' terminus with deoxyribose 5-phosphate.

**TABLE III**

| Substrate/enzyme                                      | Total number of Form II DNA molecules containing |
|-------------------------------------------------------|-----------------------------------------------|
|                                                       | No. of Form II molecules observed |
|                                                       | No tail | Tail | 2 Tails | 3 Tails |
| Undamaged PM2 DNA + N. crassa endonuclease            | 191      | 110  | 6       | 1       | 0       |
| UV-irradiated PM2 DNA +T4 UV endonuclease and fibroblast AP endonuclease II | 438      | 240  | 30      | 2       | 0       |
| Partially depurinated PM2 DNA + Fibroblast AP endonuclease II | 211      | 60   | 34      | 8       | 2       |
| + HeLa AP endonuclease                                | 211      | 62   | 52      | 5       | 2       |
| + E. coli endonuclease IV                             | 213      | 30   | 57      | 31      | 5       |
| + E. coli endonuclease III and fibroblast AP endonuclease II | 176      | 104  | 6       | 0       | 0       |
| + HeLa AP endonuclease III                            | 189      | 102  | 6       | 1       | 0       |

Two other Class II AP endonucleases, HeLa AP endonuclease and E. coli endonuclease IV, were also used to incise partially depurinated PM2 DNA and then DNA synthesis was carried out to an average of 1180 and 1320 nucleotides/nick, respectively. A preponderance of tails was similarly observed (Table III); 51% and 76% of the Form II molecules had one or more tails, respectively. The distribution of lengths of Form II DNA circles and tails produced from HeLa AP endonuclease is shown in Fig. 6, C and D. The average tail length calculated from the microscopy was 780. Thus, the presence

![Fig. 5](http://www.jbc.org/)
of a 5'-AP terminus at a nick dramatically increases the frequency of strand displacement regardless of the Class II AP endonuclease used to incise the DNA.

As a final substrate, partially depurinated PM2 DNA was incised to a limit with a Class I AP endonuclease, either human fibroblast AP endonuclease II or HeLa AP endonuclease. Treatment with the second enzyme resulted in no further nicking, assuring that all AP sites were cleaved during the first incubation. The combined treatment resulted in a substrate effectively containing a gap of one nucleotide where the AP sites had been (15, 20) (Fig. 1). With this molecule as a polymerase substrate, about 95% of the Form II DNA product lacked tail structures after the incorporation of 930 nucleotides/nick (Table III and Fig. 5B). These results are similar to those observed for DNA incised by the *N. crassa* endonuclease and indicate a strong preference for nick translational synthesis with these small gaps. They also show that exposure of DNA to a Class II AP endonuclease per se does result in increased strand displacement synthesis.

Potential of Various Incision Sites to Support DNA Synthesis by *E. coli* DNA Polymerase I Large Fragment—Proteolytic cleavage of DNA polymerase I with subtilisin yields a larger polypeptide fragment (molecular weight 76,000) which retains both the polymerase and 3'→5' exonuclease activities, but lacks the 5'→3' exonuclease which is present on the smaller fragment (molecular weight 36,000). One might thus expect the larger fragment to be capable only of strand displacement synthesis from primer termini located at a nick. When large fragment was used for DNA synthesis on PM2 DNA cleaved by *N. crassa* endonuclease, approximately 10% of the rate of intact DNA polymerase I was observed (Fig. 5B). The amounts of enzyme were chosen to be equal when each was used with "activated" (gapped) salmon sperm DNA primer/template (Fig. 7A). A similarly decreased rate of DNA synthesis by large fragment relative to intact DNA polymerase I was also observed when partially depurinated PM2 DNA that had been incised with human fibroblast AP endonuclease II was used as the substrate (Fig. 7B). These data suggest that unlike the intact DNA polymerase, the large fragment does not efficiently carry out synthesis from either of these two types of incision sites.

Since DNA polymerase I large fragment retains 3'→5' exonuclease activity which will act on both single-stranded DNA and unpaired termini in duplex DNA (36), we tested its ability to catalyze synthesis from partially depurinated PM2 DNA cleaved with *E. coli* endonuclease III. However, little if any DNA synthesis was observed under conditions wherein an equal amount of intact DNA polymerase could efficiently activate 3'-deoxyribose termini to support DNA synthesis (Fig. 8). Moreover, unlike the intact polymerase, the large fragment was also incapable of using a 3'-hydroxyl located at a one-nucleotide gap (formed by the combined action of a Class I and Class II AP endonuclease) to prime efficiently DNA synthesis (Fig. 8).

While the large fragment was unable to utilize endonuclease III incision sites for DNA synthesis, the enzyme might still be able to remove a 3'-AP terminus by virtue of its 3'→5' exonuclease. Hence, partially depurinated PM2 DNA was incubated with endonuclease III, then with large fragment for sufficient time to act on the 3'-deoxyribose termini, after which dNTPs were then added and DNA synthesis reactions

![Fig. 6. Distribution of lengths of Form II PM2 DNA and tails obtained after DNA synthesis. AP DNA incision reactions, DNA synthesis, and sample preparation for electron microscopy were as indicated in the legend to Fig. 5. Circle lengths (A) and tail lengths (B) of Form II DNA cleaved by human fibroblast AP endonuclease II, circle lengths (C) and tail lengths (D) of Form II DNA cleaved by HeLa AP endonuclease. *n* is the number of molecules measured. Tail lengths were calculated from the mean circle lengths, assuming that PM2 DNA contains 9500 base pairs.](http://www.jbc.org/)

![Fig. 7. *E. coli* DNA polymerase I large fragment does not efficiently carry out DNA synthesis from nicks. A, DNA synthesis reactions using activated salmon sperm DNA and 1.5 units of DNA polymerase I (○) or 1.6 units of DNA polymerase I large fragment (■) were as described under "Experimental Procedures." B, two incision reactions (260 pl) were prepared. One contained 26 nmol of partially depurinated PM2 DNA (0.6 AP site/duplex DNA circle) and 1.4 units of human fibroblast AP endonuclease. The other reaction contained 26 nmol of undamaged DNA and 0.09 unit of *N. crassa* DNase. Incubation was for 10 min at 37 °C. After heat inactivation of the endonuclease, 20-pl samples were removed to measure the degree of nicking. DNA synthesis reactions (250 pl) were at 37 °C using 0.9 unit of DNA polymerase I or large fragment and 10 nmol of DNA treated either with *N. crassa* endonuclease (○, ○) or AP endonuclease II (■, ■) and ■, ■. The average number of incisions per DNA genome is given in parentheses.](http://www.jbc.org/)
were prepared using 0.9 unit of DNA polymerase I and endonucleases indicated at erase I efficiently support DNA synthesis from one-nucleotide gaps. An AP DNA incision reaction contained 58 nmol of partially depurinated PM2 [3H]DNA (0.5 AP site/duplex DNA circle) and 0.22 unit of DNA polymerase I large fragment and PM2 DNA treated either with the endonuclease I11 alone or with both AP endonuclease I and I11. Similar reactions were also prepared using 0.9 unit of DNA polymerase I large fragment and PM2 DNA treated with endonuclease III alone or with both AP endonucleases DNA synthesis was at 37 °C. The average number of incisions per DNA genome is given in parentheses.

![Graph](image)

**Fig. 8.** E. coli DNA polymerase I large fragment does not efficiently support DNA synthesis from one-nucleotide gaps. An AP DNA incision reaction contained 58 nmol of partially depurinated PM2 [3H]DNA (0.5 AP site/duplex DNA circle) and 0.22 unit of endonuclease III. After 10 min at 37 °C and heat inactivation of the endonuclease, two samples were removed, each containing 26 nmol of DNA, and 0.5 unit of human fibroblast AP endonuclease II was added to one and buffer to the other. After 10 min at 37 °C and heat inactivation, the extent of nicking was measured as described under "Experimental Procedures." Two DNA synthesis reactions (250 μl) were prepared using 0.9 unit of DNA polymerase I and 10 nmol of PM2 DNA treated either with the endonuclease III alone or with both AP endonucleases I and II. Similar reactions were also prepared using 0.9 unit of DNA polymerase I large fragment and PM2 DNA treated with endonuclease III alone or with both AP endonucleases DNA synthesis was at 37 °C. The average number of incisions per DNA genome is given in parentheses.

![Graph](image)

**Fig. 9.** Apparent inability of E. coli DNA polymerase I large fragment to remove a 3'-deoxyribose terminus. Three AP DNA incision reactions (240 μl) were prepared containing 24 nmol of partially depurinated PM2 [3H]DNA (0.7 AP site/duplex DNA circle) and 0.09 unit of endonuclease III as described in the legend to Fig. 2. After 10 min at 37 °C and heat inactivation of the endonuclease, DNA synthesis components were added. Two of the reactions received 1.8 units of DNA polymerase I large fragment and were incubated for 60 min at 37 °C in the absence of dNTPs, after which dNTPs and 1.7 units of DNA polymerase I or buffer were added. The third reaction received an equal volume of buffer and was preincubated as above before dNTPs and 1.7 units of DNA polymerase I were added. All reactions were then incubated as indicated at 37 °C.

**DISCUSSION**

UV endonuclease activities have been reported from a variety of cell types, but the exact mechanism of incision has been described only for the M. luteus and T4 UV endonucleases which incise on the 5'-side of pyrimidine dimers by the joint action of a pyrimidine dimer DNA N-glycosylase and Class I AP endonuclease (17-19). Whether other UV endonucleases cleave in a similar manner remains to be determined directly, although it appears that pyrimidine dimers which are excised in vivo from UV-irradiated E. coli DNA may not be repaired by a pyrimidine dimer DNA N-glycosylase. Nevertheless, it is desirable to understand the mechanism of action of E. coli DNA polymerase I at the coliphage T4 UV endonuclease incisions, as such action presumably applies to infected cells. It is also desirable to study the action at Class I AP endonuclease incisions since UV and γ radiation results in the production of 5,6-dihydroxydihydrothymine residues (36, 37), and in E. coli these lesions are probably repaired by endonuclease III through the combined action of a DNA N-glycosylase and Class I AP endonuclease (18, 25). Endonuclease III also cleaves simple AP lesions by a Class I AP endonucleolytic incision.

The removal of 3'-AP termini by polymerase I was relatively slow but was apparently a property of that enzyme, not of a contaminating AP endonuclease. AP endonuclease activity upon partially depurinated circles could not be detected in any of our polymerase preparations, and the exoexonuclease removal was equally efficient with apparently homogeneous enzyme. The 3'-5' exoexonuclease associated with DNA polymerase I is an obvious candidate for this exoexonuclease activity, particularly since mismatched bases are normally removed by this activity (5). However, we were surprised to note that the polymerase appears also to release some pAp(d-ribose) as well as d-ribose 5-phosphate since the 3'-5' exoexonuclease normally forms only dNMP residues. An alternative and perhaps more efficient method for activating the 3'-AP sites in vivo might be the utilization of a Class II AP endonuclease to remove the deoxyribose 5-phosphate.

Under our reaction conditions, DNA polymerase I will catalyze predominantly coordinated polymerization and 5'→3' exoexonucleolytic degradation ("nick translation") from T4 UV endonuclease incision sites as well as from incisions generated by N. crassa endonuclease. These results might seem to contradict those reported by Masamune and Richardson (35) for DNA polymerase I using PM2 DNA incised by pancreatic DNase. In the latter, an initial phase of nick translational DNA synthesis for only about 50 nucleotides was followed by strand displacement. However, in those experiments, DNA polymerase I was in vast excess over the number of 3'-hydroxyl primers termini (about 30 molecules of DNA polymerase I/terminus, whereas under conditions used here, about 1 molecule of DNA polymerase I was used/1 to 10 termini). Indeed, when we carried out DNA synthesis using similar excesses of...
the polymerase, strand displacement was observed by electron microscopy from *N. crassa* endonuclease incisions. Thus, the level of DNA polymerase I seems to influence the mode of DNA synthesis. An excess of the polymerase tends to promote strand displacement. While many other factors probably influence the absolute level of strand displacement versus nick translational DNA synthesis in *vitro*, it is still reasonable to assume that a qualitative correlation between the observations in *vitro* and events in *vitro* may exist.

Whereas strand displacement predominated from nicks containing normal 5'-nucleotides or 5'-pyrimidine dimer nucleotides, we were surprised to observe that nicks containing 5'-deoxyribosyl phosphate termini promoted predominantly strand displacement synthesis. The tails thus produced were double-stranded and, hence, could have come about either by template switching by the polymerase or by a branch migration reaction. Strand displacement DNA synthesis may have been preferred on this substrate (i) because of a reluctance of the 5' → 3' exonuclease to hydrolyze a deoxyribosyl 5'-phosphate terminus, (ii) because of an increased frequency or extent of fraying of a terminus carrying a baseless site, thus allowing polymerization to supersede exonuclease hydrolysis; or (iii) by a physical blockage of the 5' → 3' exonuclease activity by an association of the AP endonuclease with the 5' termini. With respect to the latter, covalent linkage between polypeptides and 5' termini has been described for proteins involved in DNA synthesis (38, 39); however, since free deoxyribosyl 5'phosphate is formed by treatment of Class II, then Class I AP endonuclease (15), this association, if occurring, is weak. In addition, incisions made by three different Class II AP endonuclease preparations (human fibroblast AP endonuclease II, HeLa AP endonuclease, and *E. coli* endonuclease IV) each showed similar results.

As to the first two possibilities, one might have expected that the larger pyrimidine dimer would be more refractory to hydrolysis or more frayed than the baseless sugar. Whatever the cause of the increased strand displacement with 5'-terminal baseless sites, it is not unreasonable to assume that the production of strand-displaced DNA in response to incisions made at such damage may be one of the driving forces for DNA recombination or gene duplication which have been observed to be associated with DNA repair. It may be noteworthy as well that xeroderma pigmentosum fibroblasts of complementation Group D lack a Class I AP endonuclease which might normally remove deoxyribosyl 5-phosphate from 5' termini. The absence of such an enzyme might, by analogy, increase the frequency of strand displacement during repair in these human cells.

When DNA polymerase I large fragment was used in reactions primed by a nick, only about 10% the rate of DNA synthesis was observed compared to the intact enzyme. Since the DNA polymerase I large fragment lacks 5' → 3' hydrolytic activity, this synthesis presumably proceeds by a strand displacement mechanism. Such a product would not be expected to be substrate for DNA ligation and this expectation has been verified (35). In general, the large fragment appeared to be either reluctant or incapable of activity at any of the nicked substrates, so it might be worthwhile to measure the affinity of the fragment versus that of intact polymerase for nicks.

In conclusion, the availability of purified, well-characterized repair endonucleases now makes it possible to begin to characterize enzymatically the subsequent events of excision and repair synthesis. This study with *E. coli* DNA polymerase I is a beginning toward that aim.

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D W Mosbaugh and S Linn

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