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The post-incision steps of the DNA base excision repair pathway in *Escherichia coli*: studies with a closed circular DNA substrate containing a single U:G base pair

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ABSTRACT

The DNA base excision repair pathway is responsible for removal of oxidative and endogenous DNA base damage in both prokaryotes and eukaryotes. This pathway involves formation of an apurinic/apyrimidinic (AP) site in the DNA, which is further processed to restore the integrity of the DNA. In *Escherichia coli* it has been suggested that the major mode of repair involves replacement of a single nucleotide at the AP site, based on repair synthesis studies using oligonucleotide substrates containing a unique uracil base. The mechanism of the post-incision steps of the bacterial base excision repair pathway was examined using a DNA plasmid substrate containing a single U:G base pair. Repair synthesis carried out by repair-proficient *ung*, *recJ* and *xon E.coli* cell extracts was analyzed by restriction endonuclease cleavage of the DNA containing the uracil lesion. It was found that replacement of the uracil base was always accompanied by replacement of several nucleotides (~15) 3′ of the uracil and this process was absolutely dependent on initial removal of the uracil base by the action of uracil-DNA glycosylase. In contrast to findings with oligonucleotide substrates, replacement of just a single nucleotide at the lesion site was not detected. These results suggest that repair patch length may be substrate dependent and a re-evaluation of the post-incision steps of base excision repair is suggested.

INTRODUCTION

The major pathway for the removal of oxidative base damage is the DNA base excision repair pathway, found in prokaryotes and eukaryotes (1). In this pathway oxidized DNA bases are removed by specific DNA glycosylases, leaving apurinic/apyrimidinic (AP) sites in the DNA (1,2). These lesions can also arise spontaneously in DNA through depurination (1,3) and, being devoid of genetic information, are both cytotoxic and mutagenic lesions (4–6). Several DNA glycosylases have been found that convert a variety of damaged nucleotide residues to AP sites by removing deaminated, oxidized or alkylated bases from DNA. Uracil, either misincorporated in place of thymine or resulting from deamination of cytosine, is removed by a specific glycosylase, uracil-DNA glycosylase, found in prokaryotes and eukaryotes (1,4). A single-strand break is then introduced on the 5′-side of the base-free deoxyribose phosphate moiety at the AP site through hydrolysis of the phosphodiester bond by an AP endonuclease. In *Escherichia coli* the reaction is catalyzed by the AP endonuclease activity of exonuclease III, which accounts for nearly 90% of the activity detected in the bacteria (2,7,8), or by the inducible endonuclease IV (2,9). The major AP endonucleases isolated from mammalian cells seem to act in a similar fashion (2). Following cleavage of the DNA the resulting sugar phosphate residue is then excised and repair proceeds by the action of a DNA polymerase to replace the missing nucleotide(s), followed by subsequent rejoining of the phosphodiester backbone by a DNA ligase.

In previous studies using *E.coli* cellular extracts it has been demonstrated that removal of the deoxyribose phosphate residue seems to involve generation of a single nucleotide gap (10,11). These studies were performed with short oligonucleotide substrates that contained a single U:G or U:A base pair. In enzyme reconstitution experiments it was shown that in the absence of a deoxyribophosphodiesterase (dRpase) activity associated with the RecJ protein, a longer repair patch was generated at the AP site (11). This second pathway was considered to be minor compared with the pathway where just one nucleotide was replaced.

We wished to examine the post-incision steps of the DNA pathway using a closed circular DNA substrate. Unlike short linear oligonucleotide substrates, this DNA substrate would not be subject to degradation by single- and double-stranded exonucleases present in cell extracts. We have synthesized a plasmid substrate containing a single U:G base pair and have examined replacement of the uracil residue by the action of enzymes present in *E.coli* cell extracts. We observed that

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replacement of the uracil was always accompanied by replacement of additional nucleotides 3′ of uracil and generation of a single nucleotide gap was not seen. The implications of these findings in defining the pathway for the post-incision steps of base excision repair are discussed.

MATERIALS AND METHODS

Reagents and enzymes

Escherichia coli uracil-DNA glycosylase was obtained from US Biochemical. T4 DNA ligase and T4 polynucleotide kinase were obtained from New England Biolabs. DNA polymerase I was obtained from Boehringer Mannheim. Endonuclease IV was prepared as previously described (12). Creatine phosphokinase and diTris-phosphocreatine were obtained from Sigma. Oligonucleotide markers (8–32 nt) were purchased from Pharmacia.

Construction of a plasmid substrate containing a single U:G site

An oligonucleotide (12mer) containing a single uracil (5′-ACCG-GTACUGGC-3′) and a complementary oligonucleotide (20mer) (5′-ACGTGCCGGTACCGGTCTAG-3′) were prepared by automated DNA synthesis (see Fig. 1). The 12mer (10 µg) was phosphorylated at the 5′-end in a reaction (50 µl) containing 50 mM Tris–HCl, pH 7.5, 10 mM $MgCl_2$, 5 mM dithiothreitol (DTT), 1 mM ATP, 35 U T4 polynucleotide kinase. Following incubation at 37°C for 70 min the reaction was stopped by addition of 1.1 µl 0.5 M Na_2EDTA, followed by precipitation with 3 vol ethanol and lyophilization. The 5′-phosphorylated 12mer (18 µg) was annealed to 30 µg 20mer (equimolar quantities) in a reaction (30 µl) containing 250 mM NaCl, 10 mM Tris–HCl, pH 8.0, 1 mM Na_2EDTA. After incubation for 2.5 min at 68°C the reaction was allowed to cool slowly to 25°C. The double-stranded (ds) oligonucleotide was precipitated with 3 vol ethanol and resuspended in 35 µl TE buffer, pH 8.0. An aliquot of the duplex DNA was electrophoresed in a non-denaturing 20% polyacrylamide gel to confirm that annealing had occurred. This ds oligonucleotide contains *Pst*I and *Bgl*II sticky ends.

Plasmid pUC119 (13) (120 mg) was linearized to completion with 150 U *Pst*I in a 3 h reaction at 37°C. Agarose gel electrophoresis was used to confirm complete linearization. Linearized pUC119 (60 pmol) was ligated to the ds oligonucleotide (160 pmol) in a reaction (400 µl) containing 50 mM Tris–HCl, pH 7.5, 10 mM $MgCl_2$, 100 mM NaCl, 1 mM DTT, 1.5 mM ATP, 60 U *Pst*I, 50 U (Weiss) T4 DNA ligase. Following incubation overnight at 16°C, phenol/chloroform extraction and isopropanol precipitation, the DNA was lyophilized and subjected to 5′-phosphorylation and digestion with *Bam*HI in a reaction (200 µl) containing 10 mM Tris–HCl, pH 8.0, 5 mM $MgCl_2$, 100 mM NaCl, 1 mM 2-mercaptoethanol, 2.5 mM ATP, 50 U T4 polynucleotide kinase, 120 U *Bam*HI. Following incubation at 37°C for 3.5 h, phenol/chloroform extraction, ethanol precipitation and lyophilization, the DNA was suspended in 100 µl H_2O. To remove a 34mer restriction fragment the plasmid DNA was eluted from a MicroSpin S-400 HR column (Pharmacia). The plasmid DNA was circularized in a reaction (1 ml) containing 50 mM Tris–HCl, pH 7.5, 10 mM $MgCl_2$, 100 mM NaCl, 1 mM DTT, 1 mM ATP, 60 U T4 DNA ligase, 150 U *Bam*HI, 150 U *Bgl*II. Following incubation overnight at 16°C the DNA was precipitated with ethanol, lyophilized and suspended in 65 µl H_2O. The closed

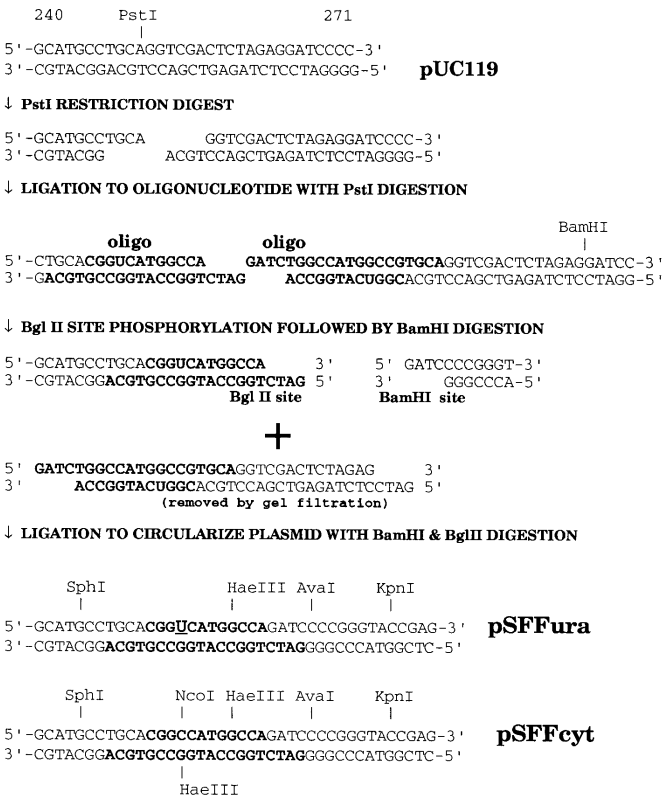

Figure 1. Synthesis of the DNA plasmid substrate pSFFura containing a single U:G base pair.

circular form of the uracil-containing plasmid, designated pSFFura, was isolated following agarose gel electrophoresis. An analogous plasmid containing cytosine in place of uracil, designated pSFFcyt, was isolated following transformation of *E.coli* DH5α competent cells (Gibco BRL) with pSFFura. The sequence of pSFFcyt was confirmed by DNA sequencing. Both plasmids were treated with uracil-DNA glycosylase and endonuclease IV and analyzed by agarose gel electrophoresis as described previously (14,15).

Escherichia coli extracts

The following *E.coli* strains were obtained from the *E.coli* Genetic Stock Center (Yale University): AB1157 [*thr-1*, *ara-14*, *leuB6*, Δ(*gpt-proA*)*62*, *lacY1*, *tsx-33*, *gsr′-0*, *glnV44*, *galK2*, *LAM*$^-$, *rac-0*, *hisG4*, *rfbD1*, *mgl-51*, *rpoS396*, *rpsL31*, *kdgK51*, *xylA5*, *mtl-1*, *argE3*, *thi-1*], KL148 [Δ(*sbcB-his17*)], BW310 (*ung-1*) (16) and JC13031 (*recJ153*) (17). Strain BS101 (*recJ284*::Tn*10* *fpg-1*::*Kan*^{r}) (18) was a gift of Dr Barbara Sedgwick (Imperial Cancer Research Fund, Clare Hall Laboratories. South Mimms, UK). Bacteria were grown in Luria broth with corresponding antibiotics and extracts were prepared by lysozyme/EDTA treatment as described previously (19).

In vitro repair synthesis reactions

In vitro repair synthesis reactions with plasmid DNA and *E.coli* cell extracts were analyzed as described previously (10) with

modifications. Reactions (50 µl) contained either 0.16 pmol pSFFura or 0.3 pmol pSFFcyt DNA, 80 µg protein extract, 100 mM Tris–HCl, pH 7.5, 5 mM $MgCl_2$, 1 mM DTT, 0.1 mM Na_2EDTA, 2 mM ATP, 0.5 mM NAD, 5 mM diTris-phosphocreatine, 10 U creatine phosphokinase, 20 µM dATP, 20 µM dGTP. Reactions also contained either 12–15 µCi [α-^{32}P]dCTP (3000 Ci/mmol; Amersham), 20 µM dTTP, 5 µM dCTP or 12–15 µCi [α-^{32}P]dTTP (3000 Ci/mmol; Amersham), 20 µM dCTP, 5 µM dTTP. Following incubation for 30 min at 37°C reactions were stopped by addition of 6 µl 0.5 M Na_2EDTA, followed by phenol/chloroform extraction, elution from a MicroSpin S-400 HR column, ethanol precipitation and lyophilization. The DNA was suspended in H_2O and divided into three samples. One sample was digested with 10 U *Sph*I for 1 h at 37°C, followed by digestion with *Nco*I for 1 h at 37°C. The second sample was digested with 10 U *Ava*I, followed by digestion with *Hae*III for 1 h at 37°C. The third sample was digested with 10 U *Hae*III for 2 h at 37°C. The digestion products were resolved on 20% polyacrylamide gels containing 7 M urea. The gels were exposed to Kodak XAR film with intensifying screens at –70°C. To determine the limit of repair synthesis DNA was also digested with *Kpn*I for 1 h at 37°C, followed by digestion with *Ava*I for 1 h at 37°C.

RESULTS

DNA plasmid substrate containing a single U:G site

A closed circular DNA plasmid containing a single U:G base pair was synthesized as shown in Figure 1 and described in Materials and Methods. This substrate, designated pSFFura, contains the U:G base pair at a *Nco*I/*Hae*III restriction site; replacement of the U:G base pair with a C:G base pair restores these sites. Other restriction sites flanking the U:G base pair permit an analysis of the extent of repair synthesis following incorporation of radiolabelled nucleoside triphosphates, either [^{32}P]dCTP or [^{32}P]dTTP. Cleavage of the plasmid with *Sph*I and *Nco*I yields a 10mer with sequence 5′-CCTGCACGG<u>C</u>-3′ with a terminal dCMP at the site of the U:G base pair; this fragment should be labelled with [^{32}P]dCTP and not with [^{32}P]dTTP, assuming that repair occurs in a 5′→3′ direction. Cleavage with *Hae*III yields a 6mer with sequence 5′-<u>C</u>CATGG-3′, with the first cytosine being at the site of the U:G base pair. This fragment should be labelled with [^{32}P]dCTP following repair, but will only be labelled with [^{32}P]dTTP if repair occurs 3 nt downstream of the site of the uracil. Cleavage with *Hae*III and *Ava*I yields the same 6mer fragment as above and an additional 8mer fragment with sequence 5′-CCAGATCC-3′. This second fragment will be labelled with [^{32}P]dCTP only if repair occurs at least 6 nt downstream of the uracil and will be labelled with [^{32}P]dTTP only if repair occurs at least 11 nt downstream of the uracil.

To demonstrate the presence of the U:G base pair in pSFFura the plasmid was digested with uracil-DNA glycosylase and endonuclease IV of *E.coli*, which cleaves AP sites. As seen in Figure 2, cleavage of the plasmid with both enzymes resulted in conversion of closed circular DNA to nicked circles. Treatment of the analogous plasmid pSFFcyt, which contains a normal C:G base pair in place of the U:G base pair, with the enzymes did not result in cleavage.

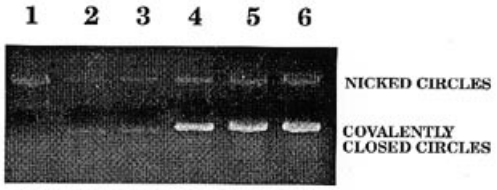

Figure 2. Enzymes acting on a DNA plasmid (pSFFura) containing a single U:G base pair. Lane 1, pSFFura treated with uracil-DNA glycosylase and endonuclease IV; lane 2, pSFFura treated with uracil-DNA glycosylase; lane 3, pSFFura without enzyme treatment; lane 4, pSFFcyt treated with uracil-DNA glycosylase and endonuclease IV; lane 5, pSFFcyt treated with uracil-DNA glycosylase; lane 6, pSFFcyt without enzyme treatment. Plasmids were separated on a 0.8% agarose gel containing ethidium bromide (14).

Repair of a single U:G base pair in *E.coli* extracts

To examine the extent of repair synthesis at a single U:G either plasmid pSFFura or plasmid pSFFcyt was treated with *E.coli* cell extracts containing either [^{32}P]dCTP or [^{32}P]dTTP in an *in vitro* reaction. After incubation plasmid DNA was then subjected to cleavage with three combinations of restriction enzymes as described above to determine the extent of repair synthesis at and around the site of the uracil base. In Figure 3 the extent of repair synthesis with an extract derived from repair-proficient strain AB1157 cells is shown. Figure 3A shows repair synthesis occurring at the U:G site as determined by incorporation of [^{32}P]dCTP. As seen in lanes 1–3, no labelled fragments were released following restriction enzyme digestion of plasmid pSFFcyt containing a normal C:G base pair. In lane 4 a 10mer labelled fragment was released following cleavage with *Sph*I/*Nco*I, indicating replacement of the uracil with cytosine. In lane 5 a 6mer labelled fragment was released following cleavage with *Hae*III, again confirming replacement of uracil with cytosine. In lane 6 cleavage with *Hae*III/*Ava*I released a 6mer labelled fragment as well an 8mer labelled fragment. The presence of this second 8mer labelled fragment suggests that a dCMP residue was replaced at least 6 nt downstream of the site of the U:G base pair. A second band in this lane that migrates with a length of ~14 nt is most likely a fragment resulting from incomplete digestion at the second *Hae*III site downstream of the U:G base pair. The slower migrating bands seen in the gel result from incorporation of [^{32}P]dCTP at random nicks that are present in the plasmid DNA, as has been shown previously (20,21).

To further determine the extent of repair synthesis at the U:G base pair incorporation of [^{32}P]dTTP into plasmid DNA was also determined, as shown in Figure 3B. As seen in lanes 1–3, no release of labelled fragments above background was detected following restriction enzyme digestion of plasmid pSFFcyt containing a normal C:G base pair. In lane 4 cleavage with *Sph*I/*Nco*I did not release a fragment labelled with [^{32}P]dTMP; this is expected as the 3′-terminal nucleotide is at the site of the uracil. In lane 5 a 6mer labelled fragment was released following cleavage with *Hae*III, suggesting replacement with [^{32}P]dTMP 3 nt downstream of the U:G base pair. In lane 6 cleavage with *Hae*III/*Ava*I released a 6mer labelled fragment as well as an 8mer labelled fragment. The presence of this second 8mer labelled

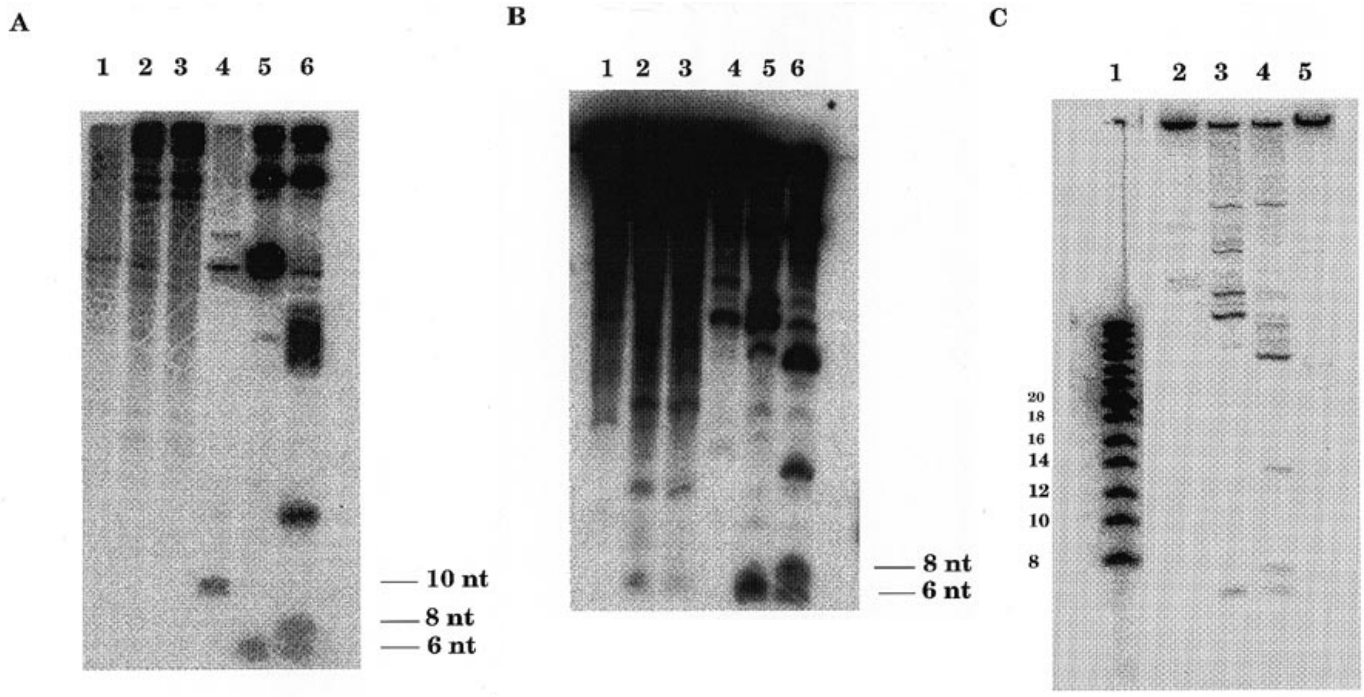

Figure 3. Repair synthesis at a U:G or C:G base pair in a DNA plasmid substrate incubated with a repair-proficient *E.coli* extract. Plasmid pSFFura or pSFFcyt was incubated with an AB1157 extract in a reaction containing either [^{32}P]dCTP (**A**) or [^{32}P]dTTP (**B**) and subsequently digested with restriction endonucleases. Lane 1, pSFFcyt cleaved with *Sph*I/*Nco*I; lane 2, pSFFcyt cleaved with *Hae*III; lane 3, pSFFcyt cleaved with *Hae*III/*Ava*I; lane 4, pSFFura cleaved with *Sph*I/*Nco*I; lane 5, pSFFura cleaved with *Hae*III; lane 6, pSFFcyt cleaved with *Hae*III/*Ava*I. (**C**) Plasmid pSFFura was incubated with an AB1157 extract in a reaction containing [^{32}P]dTTP and subsequently digested with restriction endonucleases. Lane 1, 5′-^{32}P-end-labeled oligonucleotide markers (8–32 nt); lane 2, pSFFura cleaved with *Sph*I/*Nco*I; lane 3, pSFFura cleaved with *Hae*III; lane 4, pSFFura cleaved with *Hae*III/*Ava*I; lane 5, pSFFura cleaved with *Kpn*I/*Ava*I.

fragment suggests that a dTMP residue was replaced at least 11 nt downstream of the site of the U:G base pair.

In order to define a limit on the size of the repair patch following treatment of pSFFura with the AB1157 cell extract and incorporation of [^{32}P]dTTP the plasmid was digested with *Ava*I and *Kpn*I, which will release an 8mer labelled fragment starting 13 nt downstream of the U:G base pair. As seen in Figure 3C, no fragment was evident, even though this fragment contains a dTMP residue located 19 nt downstream of the uracil. This establishes that the repair patch size is <19 nt and >11 nt, or ~15 nt long.

Repair of a U:G base pair requires uracil–DNA glycosylase

The first step in repair of DNA containing uracil is removal of the uracil base by the action of uracil-DNA glycosylase (1). It has been shown previously with oligonucleotide substrates that this enzyme is required for repair of the lesion (10). The DNA plasmid substrate containing the U:G base pair was reacted with an *E.coli* extract derived from a strain deficient in uracil-DNA glycosylase activity (BW310). As seen in Figure 4, no incorporation of [^{32}P]dCMP was seen in restriction fragments flanking the U:G base pair. Likewise, no incorporation of [^{32}P]dTMP was found when [^{32}P]dTTP was present in the reaction mixtures (data not shown). These results suggest that uracil-DNA glycosylase is absolutely required for initiation of DNA base excision repair for removal of uracil in these substrates.

Repair of a single U:G base pair in *E.coli* extracts lacking DNA dRpase activities

Enzymatic activities, termed deoxyribophosphodiesterases or dRpases, have been described in *E.coli* that remove 5′-deoxyribose phosphate residues at an incised AP site. These activities have been found to associate with the enzymes exonuclease I and RecJ (15,18). We examined the extent of repair synthesis at the U:G base pair in the plasmid substrate using cell extracts derived from strains deficient in each of these activities. Figure 5A shows incorporation of [^{32}P]dCTP during repair synthesis at the U:G base pair utilizing an extract derived from an exonuclease I-deficient cell line (KL148). As seen in lanes 4–6, restriction enzyme digestion of the plasmid substrate containing the U:G base pair yielded the identical set of labelled fragments as seen with AB1157. Figure 5B shows incorporation of [^{32}P]dTTP during repair synthesis at the site of the U:G base pair. Again, the identical set of labelled fragments were released following restriction enzyme digestion of the plasmid substrate as seen with the repair-proficient AB1157 cell extract.

We also examined the extent of repair synthesis with an *E.coli* strain deficient in either RecJ alone (JC13031) or in both RecJ and Fpg protein (BS101). An activity associated with the Fpg protein of *E.coli*, the product of the *mutM* gene, has been demonstrated to remove 5′-deoxyribose phosphate groups at an incised AP site via a β-elimination mechanism (22). Again, no difference was seen in the pattern of labelled fragments released by restriction

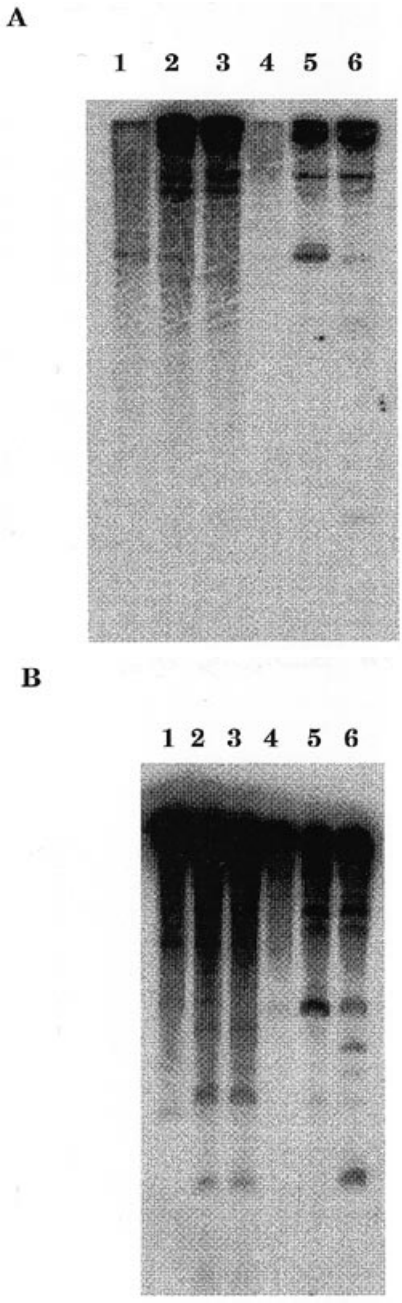

Figure 4. Repair synthesis at a U:G or C:G base pair in a DNA plasmid substrate incubated with a bacterial extract prepared from an *E.coli* strain (BW310) deficient in uracil-DNA glycosylase. Plasmid DNA substrates following incubation with bacterial extracts containing either [^{32}P]dCTP (**A**) or [^{32}P]dTTP (**B**). Lane 1, pSFFcyt cleaved with *Sph*I/*Nco*I; lane 2, pSFFcyt cleaved with *Hae*III; lane 3, pSFFcyt cleaved with *Hae*III/*Ava*I; lane 4, pSFFura cleaved with *Sph*I/*Nco*I; lane 5, pSFFura cleaved with *Hae*III; lane 6, pSFFcyt cleaved with *Hae*III/*Ava*I.

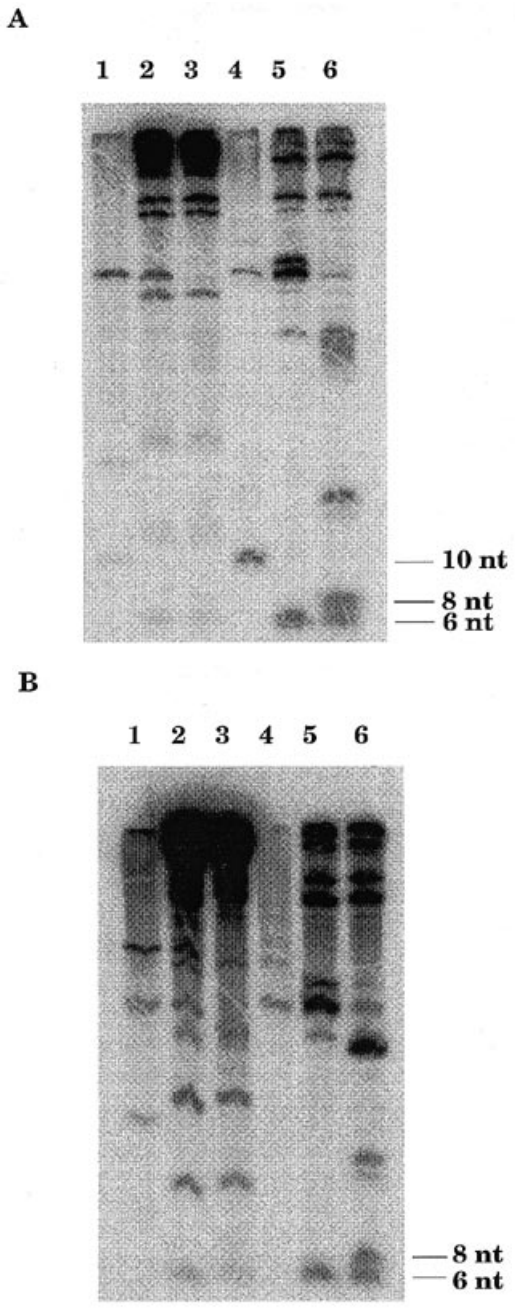

Figure 5. Repair synthesis at a U:G or C:G base pair in a DNA plasmid substrate incubated with a bacterial extract prepared from an *E.coli* strain (KL148) deficient in exonuclease I. Plasmid DNA substrates following incubation with bacterial extracts containing either [^{32}P]dCTP (**A**) or [^{32}P]dTTP (**B**). Lane 1, pSFFcyt cleaved with *Sph*I/*Nco*I; lane 2, pSFFcyt cleaved with *Hae*III; lane 3, pSFFcyt cleaved with *Hae*III/*Ava*I; lane 4, pSFFura cleaved with *Sph*I/*Nco*I; lane 5, pSFFura cleaved with *Hae*III; lane 6, pSFFcyt cleaved with *Hae*III/*Ava*I.

digestion of the plasmid containing the single U:G base pair incorporating either [^{32}P]dCTP or [^{32}P]dTTP (data not shown).

DISCUSSION

Using a closed circular plasmid DNA substrate containing a single U:G base pair we have demonstrated that repair of the U:G base pair does not result in replacement of just the single dUMP nucleotide, but is accompanied by replacement of several (~15) nucleotides downstream of the uracil. This pattern of nucleotide replacement was seen in repair-proficient cells as well as in cells deficient in exonucleases that have associated dRpase activities (exonuclease I and RecJ) and in a strain deficient in RecJ and Fpg, a protein shown to catalyze removal of 5′-terminal deoxyribose

phosphate groups by β-elimination. It was not possible to obtain a strain deficient in all three dRpase-containing activities, as apparently this combination bestows lethality (Barbara Sedgwick, personal communication). Initiation of repair of the U:G base pair absolutely required the presence of uracil-DNA glycosylase. A second class of enzymes in *E.coli* that remove uracil opposite guanine residues has been reported (23) that are related to human thymine-DNA glycosylases, but this activity does not seem to be involved in removal of uracil as seen in the system studied here. However, we have not demonstrated explicitly the presence or absence of these activities in extracts prepared from the uracil-DNA glycosylase mutant (BW310).

Previous studies with oligonucleotide substrates have suggested that most of the repair of uracil is accompanied by replacement of just a single nucleotide, with a secondary pathway resulting in formation of a longer repair patch (10,11). The results with the plasmid DNA substrates suggest that most of the repair results in non-single nucleotide repair patches and we believe this difference is due to the nature of the DNA substrate. The short (~30mer) oligonucleotides used in previous studies may not allow extension of the repair patch due to the short length of DNA able to interact with DNA polymerase and other accessory proteins involved in the post-incision reactions of DNA base excision repair. Furthermore, these short DNA molecules are very susceptible to exonuclease digestion.

What is the role of dRpase activity in the post-incision step of DNA base excision repair of DNA containing a U:G base pair? Our findings suggest that in the absence of either RecJ or exonuclease I, repair occurs with formation of a repair patch at least 11 nt in length. Since it was not possible to obtain a strain deficient in all three known dRpase activities it is possible that there may be substitution of one activity for another in removal of the 5′-terminal deoxyribose phosphate present in DNA following AP endonuclease cleavage. It may be possible that the dRpase functions *in vivo* to improve the efficiency of repair by allowing a possible replacement of just one nucleotide or by easing the reaction for removal of an oligonucleotide by an activity such as the 5′→3′ exonuclease of DNA polymerase I.

Repair of an AP site in a plasmid DNA substrate has been measured using extracts derived from hamster cells (20). As was shown in that study, the size of the repair patch seemed to be 7 nt long and was dependent on the presence of proliferating cell nuclear antigen (PCNA). The authors suggested that in mammalian cell extracts the relative contributions of the pathway to replace just a single nucleotide at the AP site and the pathway to replace a longer patch (PCNA-dependent) remain an open question. In that study replacement of the AP site by just a single nucleotide patch was not seen unless an anti-PCNA polyclonal antibody was added to the reaction. Removal of just a single nucleotide could occur by the action of eukaryotic dRpase enzymes associated with enzymes such as DNA polymerase β (24) and the ribosomal S3 protein (25). It appears that the 5′→3′ exonuclease activity associated with the enzyme flap endonuclease-1 (FEN-1 or DNase IV) (26,27) may be responsible for the longer patch repair.

Studies with reconstituted systems using purified enzymes from both bacteria and human cells have demonstrated that it is possible to manipulate post-incision repair favoring single nucleotide replacement over a longer repair patch (10,11,28), but it is still not clear which additional cellular factors control the length of the repair patch. Studies that begin to examine the interaction between the proteins involved in DNA base excision repair may begin to answer this question. For example, it has been shown that in *E.coli* a protein–protein interaction occurs between exonuclease I and the single-stranded DNA binding protein which stimulates the dRpase activity associated with exonuclease I (29,30) and in human cells an interaction occurs between DNA polymerase β and the XRCC1 protein (31). Further studies that examine the role of these and other factors that interact with the catalytic activities will give insights into problems such as how the length of the repair patch is determined during the post-incision steps of the DNA base excision repair pathway.

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