Analysis of Sequential Steps of Nucleotide Excision Repair in *Escherichia coli* Using Synthetic Substrates Containing Single Psoralen Adducts*

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*Nucleotide excision repair in *Escherichia coli* can be viewed as proceeding in five interdependent steps: 1) damage recognition; 2) incision; 3) excision; 4) gap filling; and 5) nick sealing (see Weiss and Grossman, 1987; Sancar and Sancar, 1988 for recent reviews). Damage recognition, DNA incision, and nucleotide excision are mediated by ABC excinuclease.

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to move in a 3' to 5' direction (on the single strand) and unwind the two DNA strands, the enzyme may not participate in turnover of ABC excinuclease by DNA unwinding but may act by direct protein-protein interactions (Matson, 1986).

We have recently reported the use of DNase I footprinting to follow the formation of the active ABC excinuclease complex on a DNA fragment containing a site-specific psoralen adduct (Van Houten et al., 1987). In the present study we use this technique to investigate the role of ATP in nucleotide excision as well as the interactions of the six proteins (UvrA, -B, and -C, helicase II, Pol I, DNA ligase), which are thought to be necessary and sufficient for nucleotide excision repair. In addition, using a double-stranded M13 DNA substrate carrying a single psoralen adduct at a defined position, we have measured the size of the repair patch made by Pol I in the presence or absence of helicase II.

**MATERIALS AND METHODS**

**Chemicals and Enzymes**—DNase I, restriction endonucleases, T4 polynucleotide kinase, E. coli DNA ligase, and T4 DNA ligase were purchased from Bethesda Research Laboratories. ATP, ATP+, and ADP (less than 0.1% ATP) were purchased from Boehringer Mannheim, and NAD and NMN were from Sigma. Radiotopes were obtained from Du Pont-New England Nuclear or ICN Radiochemicals.

The UvrA, UvrB, and UvrC subunits of ABC excinuclease were prepared and stored as described previously (Thomas et al., 1985). Helicase II was a kind gift of S. Matson (University of North Carolina).

**DNA Substrates**—A 137-bp-long DNA fragment containing a psoralen adduct was constructed as described elsewhere (Van Houten et al., 1987). Briefly, a 12-mer containing a 4'-hydroxymethyl-4,5,8-trimethylpsoralen (HMT)-furan side monoadducted thymine was ligated to five other properly kinased oligomers of overlapping complementarity to obtain the desired duplex. To ensure full-length duplex DNA without internal nicks, the ligation products must be purified using successive denaturing and nondenaturing 8% polyacrylamide gels. M13mp19 replicative form containing a HMT-furan side monoadducted thymine at the KpnI site of the polylinker was synthesized by the method of Kodadek and Gamper (1988). ABC Excinuclease Assay—The reaction buffer contained 50 mM Tris-HCl, 50 mM KCl, 10 mM MgCl₂, 2 mM ATP, 2 mM dithiothreitol, and 100 μg/ml bovine serum albumin. The UvrA, UvrB, and UvrC subunits of ABC excinuclease were synthesized with the method of Kodadek and Gamper (1988).

**DNase I Footprinting**—DNase I footprinting with the ABC excinuclease alone was performed as described previously (Van Houten et al., 1987). The following modifications were made for footprinting experiments which were done using ABC excinuclease under turnover conditions. The Uvr subunits (5-40 nm) were incubated with the HMT-modified DNA (0.5-2.0 nm) for 20 min at 37°C, and then all 4 dNTPs (40 μM each), helicase II (5-20 nm), and Pol I (0.5 units) were added, and the reaction mixture was incubated for an additional 10 min at 22°C. In reactions containing ligase the mixtures were first incubated with E. coli DNA ligase (0.5 units) and NAD at 2 nm, and then NMN was added to 10 mM before adding CaCl₂ (2 mM) and DNase I (0.5 units). This order of addition was to ensure the sealing of the repair gaps but not the nicks of DNase I and thus enabled us to obtain a footprint in the presence of ligase. Following DNase I treatment the samples were made 15 mM in EDTA, frozen in dry ethanol, and processed as described previously (Van Houten et al., 1987).

**Repair Patch Assay**—The size of the nucleotide excision repair patches was determined as follows. The UvrA (0.5 pmol), UvrB (1.3 pmol), and UvrC (1.5 pmol) subunits were added to 50 μl of reaction buffer, and after 5 min at 37°C, modified or unmodified M13mp19 (8.3 fmol) was added to the enzyme. The mixture was incubated at 37°C for 15 min, and then dATF, dGTP, and TTP (40 μM each) plus 30 μCi of [α-32P]dCTP (6000 Ci/mmol), Pol I (2 units), helicase II (0.43 pmol), and T4 DNA ligase (2 units) were added, and the mixture was incubated for an additional 10 min at 37°C. The reaction was terminated by the addition of EDTA to 15 mM followed by extraction with an equal volume of phenol. The aqueous phase was extracted with ether and the DNA precipitated with ethanol. The DNA was then resuspended in buffer and digested with PvuII and one of the enzymes that cuts in the polylinker region, thus liberating two labeled DNA fragments. The fragments were separated on an 8% polyacrylamide gel. After autoradiography the bands corresponding to each fragment were cut out and the radioactivity measured by Cerenkov counting. From the distribution of label in the two restriction fragments the patch size was estimated. The amount of radioactivity incorporated 3' to the 3'-incision site of ABC excinuclease was taken to be a measure of nick translation.

**RESULTS**

The Role of ATP in Formation of UvrA-DNA or UvrA-UvrB-DNA Complexes—ABC excinuclease is an ATP-dependent nuclease, but the exact role of ATP in the nuclease function is not known. Filter binding studies showed that ATP stimulated specific binding of UvrA, the ATPase subunit of the enzyme, to UV-irradiated DNA while ADP inhibited specific binding, and ATP-S abolished specific binding by increasing nonspecific binding of the protein (Seeberg and Steinum, 1982). To gain an insight into the role of ATP hydrolysis in the formation of specific complexes we conducted DNase I footprinting with various combinations of ABC excinuclease subunits.

The results are shown in Fig. 1. UvrA binds specifically to the UvrC subunits, either in the absence or presence of ATP. The bottom and top strands refer to the nonadducted and adducted strands, respectively. The extent of the UvrA and UvrA-UvrB footprints is shown by bracketed arrows. The positions of the HMT-modified thymidine and the complementary adenine are indicated by a circled T and A, respectively. The bands marked by 5' and 3' are due to the 5'- and 3'-incisions of ABC excision nucleases. The asterisk indicates the position of the DNA I-hypersensitive site, which is diagnostic for the binding of both the UvrA and UvrB subunits. Note the top strand labeled DNA used in these experiments contained some 136 nucleotide (n-1) fragment, which resulted in the formation of doublet bands. The faint bands seen below the 5'-incision band in lane 22 are due to incision by ABC excinuclease 5' to G residues that were damaged during DNA synthesis by the photophotriester method (Van Houten et al., 1987).
the psoralen-adducted region even in the absence of ATP. In the presence of ATP, the affinity is increased about 4-fold without any change in the quality of the footprint. This suggests that the UvrA-DNA complex does not undergo a significant conformational change during ATP hydrolysis. As reported previously (Van Houten et al., 1987) when UvrB is added to UvrA in the presence of ATP, two characteristic changes are seen: the footprint shrinks from 33 to 19 bp and a DNase I-hypersensitive site appears at the 11th phosphodiester bond 5′ to the HMT-modified thymine (indicated by an asterisk). In the absence of ATP, the UvrA-UvrB footprint was quantitatively as well as qualitatively different. The UvrA-UvrB footprint is smaller than the UvrA footprint, but this shrinkage is not as prominent as in the presence of ATP. Perhaps more significantly, the hypersensitive site produced in the presence of ATP is never observed in the absence of ATP, even at very high UvrB concentrations (compare lanes 17 and 21, and data not shown). As expected, addition of UvrC to the UvrA·UvrB·DNA ternary complex results in cleavage of the DNA only when ATP is present (compare lanes 18 and 22). This subunit had no discernible effect on the UvrA-UvrB footprint in the absence of ATP (compare lanes 6 and 7). It thus appears that UvrC does not associate with the UvrA·UvrB·DNA complex in the absence of ATP.

To discern whether the binding of ATP, its hydrolysis, or the binding of ADP is responsible for formation of the productive UvrA·UvrB·DNA complex (defined as the complex which upon addition of the UvrC subunit results in bi-incision) we performed footprinting experiments in the presence of ADP and the nonhydrolyzable ATP analog, ATPγS. The results of these experiments are shown in Fig. 2. UvrA binds specifically to the HMT-modified DNA with about 2-fold less affinity compared to its binding in the absence of any cofactor (compare lane 4 with lane 7).

As UvrA has a 10-fold higher affinity for ADP (Kd = 20 µM) compared to its affinity for ATP (Kacinski et al., 1981; Seeberg and Steinum, 1982), we conclude that under our experimental conditions all UvrA is bound to ADP and that the ADP-bound form of UvrA makes essentially the same contacts with substrate DNA (no qualitative change in the footprint) but with somewhat lower affinity. ADP also lowers the affinity to nonsubstrate DNA (compare lanes 6 and 9). A UvrA·UvrB·DNA complex also formed in the presence of ADP (Fig. 2, compare lanes 20 and 21); however, this complex lacks the specific hypersensitive bond that is formed in the presence of ATP and thus is a nonproductive complex similar to the one formed in the absence of any cofactor. ATPγS inhibited specific binding of UvrA at low concentrations of the protein (compare lanes 7 and 14) and at high protein concentrations caused tight nonspecific binding as evidenced by near total lack of DNase I digestion of the DNA (lane 8 versus lane 15). Similarly, no productive UvrA·UvrB·DNA complexes were formed in the presence of ATPγS (lane 23 versus lane 25). Thus, we conclude that the binding of ATP is not sufficient to confer specificity upon UvrA or the UvrA and UvrB subunits and that the hydrolysis product, ADP, lowers both the specific and nonspecific binding. Since ADP does not promote formation of the productive UvrA·UvrB·DNA complex (even though it does not abolish the specific UvrA·DNA or UvrA·UvrB·DNA complexes) it is concluded...
DNase I + + + + + + - -
Poi I - - - - - + + +
Hel II - - - - - + + +

Fig. 4. Effect of Pol I on ABC excinuclease footprint in the absence of dNTPs. The substrate (~0.5 nM) was incubated with the indicated repair proteins (UvrA, 40 nM; UvrB, 50 nM; UvrC, 50 nM; Pol I, 1 unit; helicase II, 20 nM) for 10 min at 37°C and then digested with 2 ng of DNase I for 2 min at 20°C. The UvrAB(C) footprint is bracketed, and the position of the psoralen-adducted thymine is indicated. In the lanes containing ABC excinuclease about 40% of the substrate was incised as determined by comparing the intensities of the 5'-incision band and that of full-length DNA in a sample without DNase I treatment. ABC, ABC excinuclease; Hel II, helicase II.

that ATP hydrolysis is necessary for the formation of the productive complex.

Dissociation of the Postincision Complex—Earlier studies indicated that ABC excinuclease remains bound to DNA following incision and that the three subunits along with the excised oligomer are released by the joint action of DNA polymerase I and helicase II (Husain et al., 1985; Caron et al. 1985). We analyzed the possible protein-protein and DNA-protein interactions during this process.

As a first step, the effect of helicase II on the ABC excinuclease complex was investigated. It has been suggested that helicase II alone may interact with the complex and release the UvrC subunit (Caron et al., 1985). Fig. 3 shows the results of our footprinting studies with helicase II. As is apparent from this figure helicase II does not bind to HMT-modified DNA specifically (lanes 5 and 10) nor does it have any effect on the UvrA or UvrA-UvrB footprint (lanes 2 and 3 versus lanes 6 and 7). It seems, however, that helicase II has a minor but reproducible effect on the footprint of the postincision complex: a new DNase I-sensitive site is produced on the nonadducted strand across from the 5'-incision site of the adducted strand (lane 8, arrow). This may be taken as evidence for (but not as a proof of) binding of helicase II near the 5'-incision site as has been proposed by Matson (1986).

The binding of Pol I to the postincision complex was similarly investigated by DNase I footprinting. The results are shown in Fig. 4. Pol I in the absence (lane 5) or presence
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FIG. 6. Uniquely modified M13mp19 substrate containing a HMT-thymine adduct at the polylinker region and its utilization for measuring repair patch size. The M13mp19 DNA contains a HMT-thymine adduct at the central TA sequence of the KpnI recognition sequence, located in the multiple restriction site polylinker region. Digestion of this DNA with PvuII produces three fragments; the KpnI site is contained in the 222-bp fragment. Complete repair of the HMT adduct by the excision of the damage containing dodecamer by ABC excinuclease followed by repair synthesis of DNA by Pol I generates the KpnI site. Digestion of the 322-bp fragment with KpnI results in the formation of a 102- and a 220-bp fragment.

(lane 6) of helicase II does not appreciably change the postincision ABC excinuclease footprint (lane 4). Minor differences seen between Pol I + ABC excinuclease and ABC excinuclease lanes are due to the 3′→5′ exonucleolytic action of Pol I on DNase I-generated nicks. This is evident from a comparison of lane 1 which contained Pol I but no ABC excinuclease with the other lanes in this figure. Thus, it appears that under our experimental conditions Pol I does not bind stably to the proteins in the postincision complex nor to the two nicks produced by ABC excinuclease. Under similar experimental conditions it has been shown that Pol I produces a 20-bp-long footprint around a nonprotected nick at a defined location (Joyce et al., 1986). An attempt to obtain such a complex by including into the reaction mixture the first nucleotide incorporated into the excision gap (dCTP) also failed to elicit any Pol I footprint even though the nucleotide was efficiently incorporated into the gap (data not shown). Similarly, in the presence of all 4 dNTPs Pol I binds to the 5′ nick and fills in the gap (see below).

To study the interactions of all six proteins thought to be necessary for complete nucleotide excision repair, Pol I, helicase II, and ligase were added to the postincision ABC excinuclease complex separately or in combination (under resynthesis conditions), and the resulting changes in the two nuclease incisions and the DNase I footprints were examined. By following the fate of the bands which result from the “normal” 5′-incision and the “uncoupled” 3′-incision, (with 5′-terminally labeled DNA a band corresponding to the 3′-incision is seen only when this incision is not coupled to the incision on the 5′ side), the accessibility of these two cleavage sites in the postincision complex can be inferred (Fig. 5A, lanes 1–9). Pol I, when added to the postincision complex in the presence of dNTPs, reduces the intensity of the 5′-incision band by filling in the gap. During this repair synthesis there is a significant level of premature terminations resulting in a “ladder” within the repair patch (Fig. 5A, lane 4). Ligase when added alone to the postincision complex reduces the intensity of the normal 5′-incision band and increases the intensity of the uncleaved 3′-incision band (Fig. 5A, lane 5). This result suggests that the 5′-incision site in the postincision complex is more accessible to DNA ligase than the 3′-incision site. However, the intensity of the uncleaved 3′-incision band does not increase proportionally to the decrease of the 5′-incision band suggesting that the 3′-incision site is also accessible, yet is more sterically hindered than the 5′ site, to ligase. When helicase II and ligase are added together to the postincision complex the decrease in intensity of the 5′-incision band is less compared to that obtained with ligase alone (lane 5 versus lane 7). This observation is consistent with the suggestion that helicase II binds near the 5′-incision site (Matson, 1986) partly blocking accessibility of this nick to ligase. The Pol I + helicase II and Pol I + ligase combinations were not significantly different from Pol I alone with regard to filling in the gap in the postincision complex. However, the addition of helicase II and ligase together with Pol I decreases the intensity of the gap-filling ladder generated by the stalling of Pol I (compare the band intensities in lanes 9 versus lanes 4, 6, and 8), suggesting that helicase II increases the processivity of Pol I in this reaction. DNase I footprinting of the top strand in the presence of various protein combinations was not very informative because it is not possible to examine the region 3′ to the 5′-incision site of ABC excinuclease under our experimental conditions (lanes 10–20) where more than half of the substrate has been incised by the ABC excinuclease. In contrast, the bottom strand footprint was quite informative regarding the fate of the postincision complex. Helicase alone resulted in the appearance of an additional DNase I band as noted above (Fig. 5B, lane 6). Ligase alone had no discernible effect (data not shown). Pol I + ligase and especially Pol I + helicase II + ligase combinations resulted in partial disappearance of the ABC excinuclease footprint (lanes 7 and 8) suggesting that under these conditions the excision gap was filled in and ligated resulting in release of the ABC excinuclease complex from a fraction of the molecules. That Pol I could perform repair synthesis in the absence of helicase II and in so doing displace the excised oligomer and ABC excinuclease was unexpected in view of the fact that it had been reported (Husain et al., 1985) that the joint action of Pol I + helicase II was necessary for turnover of ABC excinuclease. We, therefore, conducted the experiments described in the following section to examine the repair synthesis in the presence and absence of helicase II in more detail.

Repair Synthesis Reaction—We conducted these studies with a circular substrate because the linear substrate used in the previous experiments is only 50–80% repaired under optimal conditions, presumably because the ABC excinuclease makes nonspecific complexes at the termini which interfere
Fig. 7. Patch size measurements. Panel A, the HMT-adducted M13mp19 DNA (8.3 fmol) was incubated with the complete repair system which contained UvrA (0.5 pmol), UvrB (1.3 pmol), UvrC (1.5 pmol), Pol I (1 unit), helicase I1 (0.4 pmol), T4-DNA ligase (2 units), dATP, dGTP, dTTP (40 μM), and [α-32P]dCTP (30 μCi, 6000 Ci/mmol). Following removal of unincorporated radioactivity the DNA was divided into 10 aliquots which were digested with the indicated restriction enzymes. The resultant products were analyzed by separation on a nondenaturing 8% polyacrylamide gel followed by autoradiography. Panel B, the amount of radioactivity incorporated into each band in panel A was determined by scanning properly exposed autoradiographs and/or directly counting the excised bands. These values were used to generate the physical map of the distribution of label incorporated into the repair patches. The given percentages (mean of four experiments) represent the distribution of label in the repair patches and were calculated as follows: 83% of the label was incorporated in the region 5' to the SmaI incision site, 93% into the region 5' to the XbaI site, 97% 5' to the SalI site, 99% 5' to the PstI site, and 100% into the region 5' to the HindIII site. These percentages are not weighted for the relative frequencies of Cs at the corresponding intervals.

Fig. 8. Repair synthesis in the absence of helicase I1. Pol I-mediated repair synthesis was examined using the M13mp19 DNA substrate and the complete repair system as described in Fig. 5. Lane 1, ABC excinuclease + Pol I + ligase; lane 2, ABC excinuclease + Pol I + helicase II + ligase; lane 3, helicase II + Pol I + ligase. In the absence of ABC excinuclease, Pol I removes the psoralen adduct and incorporates label into the substrate by nick translation, starting at nicks generated during preparation of the substrate. This gives rise to the low level of background "repair" seen in lane 3.

Fig. 9. Complete repair of HMT-modified M13mp19 DNA. The HMT-modified M13mp19 DNA was repaired as described in Fig. 7, except all four dNTPs were cold and 100 ng of M13mp19 DNA was used per reaction. Following repair reaction the DNA was digested with the indicated restriction enzymes and analyzed by electrophoresis on a 0.8% agarose gel. Lanes 1 and 7 contain unmodified M13mp19 digested with ClaI; lanes 2 and 8 contain unmodified M13mp19 digested with ClaI and KpnI; lanes 3 and 4 contain HMT-modified M13mp19 digested with ClaI or ClaI and KpnI, respectively (see Fig. 6). Lanes 5 and 6 contain HMT-modified M13mp19 DNA which was digested with ClaI and KpnI followed treatment with ABC excinuclease, Pol I, and ligase in the absence or presence of helicase II, respectively.
7A. As is apparent from this figure nearly all the label incorporated into the M13 substrate is within the PvuII fragment carrying the HMT-adducted T. Also, nearly all of the radio-labeled PvuII fragment has become sensitive to KpnI suggesting almost complete restoration of this site. Digestion with Smal, which incises at the 3'-incision site of ABC excinuclease, reveals that 83% of the label is incorporated into the 12-nucleotide gap of ABC excinuclease. Digestion with other enzymes gave the results shown in Fig. 7A and summarized in Fig. 7B. From this data it appears that under the conditions used here, Pol I simply fills in the gap to produce a dodecanucleotide patch; only in 10% of the molecules the repair patch is 12-20 nucleotides, and in 4%, 20-38 nucleotides. No molecules were detected with patches longer than 45 nucleotides.

The role of helicase II in the gap filling reaction was studied by conducting similar experiments with Pol I plus ligase alone. The results of such an experiment are shown in Fig. 8. Nearly the same level of repair synthesis is obtained with and without helicase, supporting the conclusion arrived at earlier with linear substrates that helicase II is not essential for repair synthesis and ligation (see also Fig. 5).

To demonstrate that the repair synthesis observed in the previous experiments in the presence or absence of helicase II was not due to repair in only a fraction of the molecules, we conducted similar experiments to regenerate the KpnI site and analyzed the reaction products by ethidium bromide staining instead of autoradiography (Fig. 9). Polymerase I restores the KpnI site nearly quantitatively even in the absence of helicase II (lane 5). However, a subtle helicase effect was apparent, as even under these experimental conditions where ABC excinuclease was in molar excess over substrate, helicase II increased the efficiency of overall repair (compare lanes 5 and 6). Attempts at conducting these experiments with substantially lower concentrations of ABC excinuclease in order to see a more pronounced stimulation of repair by helicase II were unsuccessful. We believe this to be due to inactivation of the enzyme by the large dilutions of the ABC excinuclease subunits which were necessary to achieve sub-stoichiometric concentrations (10-25 pm). However, in assays with randomly damaged DNA where these experiments are conducted with higher concentrations of substrate and ABC excinuclease, helicase II has a dramatic effect on incision (Husain et al., 1985).

**DISCUSSION**

In this paper we have addressed three issues regarding the mechanism of nucleotide excision in *E. coli*: the function of ATP in the assembly and function of ABC excinuclease, the role of helicase in repair synthesis, and the exact size of the repair patch.

Our finding that ATP is not necessary for formation of specific UvrA-DNA complexes is in agreement with the conclusion of Seeberg and Steinum (1982) based on experiments with UV-irradiated plasmid. Our study reveals that the UvrA-DNA complexes formed in the absence of ATP or in the presence of ADP are qualitatively similar to those formed in the presence of ATP. We find that ATP hydrolysis by UvrA molecules bound to DNA does not induce a significant conformational change (unwinding, bending) in the DNA substrate. In contrast, ATP hydrolysis makes a drastic difference in the conformation of DNA in the UvrA-UvrB-DNA complex as evidenced by the appearance of a diagnostic DNase I-hypersensitive bond. Recent experiments indicate that UvrA and UvrB are in the form of a A2B1 complex under physiological conditions and that the formation of the A2B1 complex requires ATP hydrolysis (Orren and Sancar, 1988). The fact that we obtain what appears to be an UvrA-UvrB footprint even when ATP is absent suggests that there are three different types of UvrA-UvrB complexes: 1) the complex formed in the presence of ATP and absence of DNA; 2) that formed in the presence of DNA and absence of ATP; and 3) the complex formed in the presence of both. What is the role of ATP in the action mechanism of ABC excision nucleases? We know that ATP hydrolysis (not just binding) is required for the formation of A2B1, and productive UvrA-UvrB-DNA com-
plexes, and it has also been reported that the UvA-UvrB complex acts as a helicase to dissociate small oligonucleotides from DNA duplexes (Oh and Grossman, 1987). It is therefore possible that the energy of ATP hydrolysis is used in the formation of a UvA-UvrB-DNA ternary complex in which the DNA is in an altered, possibly strained, conformation, such that the addition of the UvrC subunit results in the hydrolysis of the 5th phosphodiester bond 5' and the 5th phosphodiester bond 3' to the damaged nucleotide.

We also attempted to uncover the mechanism by which Pol I and helicase II participate in the catalytic turnover of ABC excinuclease. Based on our data, we consider the following three models (Fig. 10). 1) Pol I alone fills in the gap and ligase seals the resulting nick but ABC excinuclease remains attached to this postrepair 3-stranded structure; helicase II releases the ABC excinuclease and the excised oligomer. 2) Pol I fills in the gap and releases the ABC excinuclease (still attached to the excised oligomer); helicase II dissociates the excision oligomer from the enzyme, allowing the Uvr proteins to enter new rounds of repair. 3) Pol I and helicase II bind to the 5'-incision site and move in the same direction, polymerase synthesizing the incised strand 5' to while helicase II moves 3' to 5' on the complementary single strand (Matson, 1985) displacing the excised oligomer and ABC excinuclease. The net effect is filling in the gap and releasing the ABC excinuclease subunits and the excised dodecamer. Although model 3 seems attractive and it is supported by the evidence that helicase II appears to bind to the 5'-incision site in our footprints, the fact that we obtain complete gap filling and unsealing in the absence of helicase II suggests that this model cannot be entirely correct. Model 1 predicts that the postincision footprint of ABC excinuclease + helicase II and ABC excinuclease + helicase II + Pol I should be different having a distinct footprint in the former but not in the latter case. We do not see such a difference and therefore think this model highly unlikely. Model 2 is consistent with nearly all the data in this paper and with the previously published accounts of helicase II effects. However, it is inconsistent with the evidence for the apparent binding of helicase II to the postincision complex. Perhaps, a hybrid of Models 2 and 3 would account for all the observation made so far; helicase II acts in a secondary role during the filling in of the gap by Pol I and then releases the components of the ABC excinuclease-excised oligomer complex that has been displaced as a result of gap filling. However, it must be pointed out that even though our data suggest that Pol I and helicase II bind to the 5'-incision site and travel in the same direction during resynthesis, we have failed to obtain any evidence for the presence of a supramolecular "repairosome" complex consisting of the incision and resynthesis-ligation proteins. It appears that the interaction of postincision proteins with the incision complex is too transient for detection by DNase I footprinting.

Finally, in this paper we have measured the size of the repair patch made at a defined adduct site and obtained values for the relative frequencies of the different size classes. We have found that Pol I can carry out repair synthesis even in the absence of helicase II and that 80-90% of the gaps are filled in without any nick translation. No patches as long as 50 nucleotides were detected. Thus, it appears that the primary repair process in nucleotide excision repair generates only short patches and that the long patches observed in vivo (Hanawalt et al., 1979) may be the result of joint excision-recombination repair processes.

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