Activities and Incision Patterns of ABC Excinuclease on Modified DNA Containing Single-base Mismatches and Extrahelical Bases*

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ABC excision nuclease of Escherichia coli is a DNA repair enzyme that recognizes major helical distortions caused by bulky base adducts and incises on both sides of the adduct, thus removing the modified nucleotides in the form of a 12-13-base long oligomer. We tested the enzyme with substrates that contained unusual helical structures caused by single-base mismatches or one, three, or four extrahelical bases (loops). We find that the enzyme does not cut DNAs containing helical perturbations caused by these structures. However, when the mismatched or extrahelical bases are modified with 1-cyclohexyl-3-(2-morpholinoethyl) carbodiimide, a reagent specific for unpaired G and T residues, the enzyme incises at the modified nucleotides in the regular manner. In addition, we find that when mismatches and loops are located near pyrimidine dimers and (6-4) photoproducts they do not inhibit incision at the photoproducts by the excinuclease but sometimes affect the incision pattern. Our results indicate that ABC excinuclease may be a useful enzymatic reagent to probe the structural changes caused by mismatches and deletions in DNA and provide additional information on the requirements for incision by this repair enzyme.

Mutations are caused either by synthesis errors by DNA polymerases of normal templates or by modification of DNA by physical and chemical agents followed by processing of the modified (damaged) nucleotides by cellular enzymes. Spontaneous mutations in vivo occur at about $10^{-7}$ to $10^{-11}$ errors/nucleotide incorporated (Drake, 1969), and this rate can be increased by numerous and diverse environmental agents. In Escherichia coli there are repair mechanisms that remove the mismatched or modified bases, thus maintaining the mutation rate at a tolerable level. The main mismatch repair mechanism in E. coli is the methyl-directed mismatch repair which is controlled by the Dam methylase and the products of mutH, mutL, and mutS genes (Marinus and Morris, 1973; Lu et al., 1983). There are several repair mechanisms in E. coli that eliminate damaged nucleotides from the chromosome, thus preventing deleterious effects such as mutagenesis and killing (see Lindahl, 1982; Friedberg, 1985; Walker, 1985). Of these repair mechanisms nucleotide excision repair is of particular interest because it acts on bases damaged by many agents.

Nucleotide excision repair is mediated by the UvRA

Experimental Procedures

Materials—E. coli strain NR9099 (Δ(pro-lac) recA– ara- thi- ΔM15) was provided by Roeland Schaaper (National Institute of Environmental Health Sciences, Research Triangle Park, NC). Wild-type bacteriophage M13mp2 was obtained from J. Eugene LeClerc (University of Rochester, Rochester, NY). Mutant M13mp2 derivatives were from our collection described previously (Kunkel, 1985a, 1985b; Kunkel and Alexander, 1986). Restriction enzymes were obtained from New England Biolabs or Bethesda Research Laboratories, and radiochemicals were purchased from New England Nuclear or Amersham Corp. 1-cyclohexyl-3-(2-morpholinoethyl) carbodiimide metho-p-toluenesulfonate (CMC1) was purchased from Aldrich.

Preparation and Purification of Heteroduplex Molecules—Double-stranded M13mp2 DNA molecules containing defined mismatches or extrahelical bases at known positions were constructed using appropriate combinations of wild-type and mutant M13mp2 derivatives, as follows. Each bacteriophage M13mp2 derivative was plated on minimal plates as described (Kunkel, 1985a, 1985b), using E. coli NR9099 as a host strain. A single plaque was added to a 4-liter flask containing 1 liter of 2XYT medium (containing, per liter, 16 g of Bacto Tryptone, 10 g of yeast extract, 5 g of NaCl, pH 7.4) and 10 ml of an overnight culture of E. coli NR9099. M13mp2-infected cells were grown overnight at 37°C with vigorous shaking. Cells were harvested by centrifugation at 5000 x g for 30 min. Replicative form DNA was

The abbreviations used are: CMC, 1-cyclohexyl-3-(2-morpholinoethyl) carbodiimide metho-p-toluenesulfonate; bp, base pairs; RF, replicative form.

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RESULTS

Construction of Heteroduplexes with Mismatches and Loops

A 268-bp DNA fragment carrying mismatches or loops at defined positions was generated by hybridizing the appropriate M13mp2 single-stranded viral DNA with the denatured RF form of either mutant or wild-type DNA and digesting the heteroduplexes with the restriction enzyme PvuII. A description of each hybridization and the resulting heteroduplex is presented in Table 1, and the sequence of the wild-type fragment used in our studies is shown in Fig. 1. During the construction of the heteroduplex forms of this fragment homoduplexes (wild-type) also were formed. Heteroduplexes migrate slower on polyacrylamide gels, making it possible to purify them away from homoduplexes. The differential migration of heteroduplexes with either a one-base pair mismatch or a one-base loop was not great enough to obtain these substrates in a pure form. However, the heteroduplexes with three- or four-base loops were considerably retarded in our gel system compared to homoduplexes, thus enabling us to obtain these substrates in greater than 90% purity (Fig. 2). In addition, the one-base loop consisting of an extrahelical T residue was present on the same 268-bp heteroduplex containing the four-base loop and was thus obtained at the same high level of purity.

Effect of ABC Excinuclease on DNA Containing Mismatches

Base mismatches cause helical deformations of varying severities. Although theoretical (Ninio, 1979; Chuprina and Polter, 1985) and experimental (Fresco and Alberts, 1960; Dodgson and Wells, 1977; Patel et al., 1982a; Aboul-ela et al., 1985) studies have provided valuable data for the structures of various base mismatches, only the crystal structure of a G:T base pair is known (Brown et al., 1985; Kneale et al., 1985). It is generally accepted that the order of stability of mismatches is G:T > C:C, T:T, G:A, A:A > T:C > A:C (Ninio, 1979; Aboul-ela et al., 1985). Thus, an A:C opposition destabilizes the helix by about 2 kcal/mol which is likely to result in significant helical deformity. ABC excinuclease of E. coli is a DNA repair enzyme which recognizes helical deformities formed by bulky DNA adducts and removes the adducts in the form of a 12-13-base long oligomer. Since the enzyme apparently recognizes gross structural deformities in DNA, we reasoned that the enzyme may act on mismatches that change the helical parameters significantly but not others which are known or presumed to have little effect on the canonical B-DNA structure (Wing et al., 1980). We constructed DNA fragments containing G:C (low destabilizing effect), T:C (moderately destabilizing), or C:A (highly destabilizing) mismatches, terminally labeled them, treated them with ABC excinuclease, and analyzed the reaction products on DNA sequencing gels. In all three cases we failed to detect any ABC excinuclease incision that could be ascribed to the mismatch. In Fig. 3 we present the result obtained with the substrate containing the T:C mismatch. The fragment is not incised by the ABC excinuclease in either strand but is cut at specific sites if it was irradiated with 254 nm UV light prior to the enzyme treatment. By comparing the intensity of the incision bands caused by the UV photoproducts (pyrimidine dimers and 6-4 photoproducts) to that of the “background bands” seen in nonirradiated DNA, we can state that none of the three mismatches that we examined act as a substrate for the excision nuclease, within the detection limit (0.5-1.0%) of our assay.
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TABLE I

Composition of heteroduplex molecules used

| Mutant used | Composition of resulting heteroduplex |
|-------------|--------------------------------------|
| C → A at -57 for (-) strand | G → A at 89 for (+) strand |
| G → C at -66 Minus T at 103 | G at -66 (wild-type) Minus G at -89 |
| Minus T at 103 | Minus GCT at -13 → -15 |
| Minus T at 103 | Minus ACTC at -56 → -59 |

For consistency with previous publications from which these mutants were derived (Kunkel, 1985a, 1985b; Kunkel and Alexander, 1986), the mutants are described with reference to the viral (+) strand, with position number 1 representing the first transcribed base of the lacZa gene.

Since this four-base loss occurs at two consecutive ACTC sequences, the actual loss could also have been -52 → -55.

Effect of ABC Excinuclease on Mismatched Bases Modified by Carbodiimide

CARBODIIMIDE reacts with the imino groups of unpaired G and T residues in DNA (Gilham, 1962; Metz and Brown, 1969) and has been used extensively as a probe for unpaired regions in nucleic acids (Lebowitz et al., 1976, 1977; Kelly and Maden, 1980; Novack et al., 1986). Having established that ABC excinuclease does not incise at mismatched bases, we wished to further probe the enzyme’s recognition mechanism by determining if the enzyme would recognize carbodiimide-modified bases. The DNA fragment carrying the G:G mismatch was treated with CMC and then incubated with ABC excinuclease. The results, presented in Figs. 4 and 9a, show that the enzyme incises at a carbodiimide-modified G in a mismatch. The enzyme removes the modified nucleotide in the usual manner, that is, by incising on either side of the modified base. We presume that the cuts are at unique sites, as is observed with other nucleotide monoadducts, i.e. the 8th phosphodiester bond 5' and the 5th phosphodiester bond 3' to the modified nucleotide. Indeed, the most prominent cut with the 5' labeled DNA is the one at the 8th phosphodiester bond. However, with both 5' labeled and 3' labeled fragments we observe multiple incisions either 5' or 3' to the modified nucleotides at varying distances from the modified base.
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Fig. 3. **Effect of ABC excinuclease on a C:T mismatch.** The (PvuII-EcoRI) fragment carrying the C:T mismatch and 5' 32P label at the PvuII terminus was digested with ABC excinuclease and the reaction products were analyzed on an 8% DNA sequencing gel followed by autoradiography. The lanes contained the following: lane 1, DNA alone; lane 2, DNA treated by ABC excinuclease; lane 3, UV-irradiated DNA; and lane 4, UV-irradiated DNA treated with ABC excinuclease. The background bands seen in all lanes in this gel as well as the gels in subsequent figures are probably the result of depurination/depyrimidination at specific sites. The broken arrow indicates the expected position of a fragment resulting from an incision by the excinuclease 7 bases 5' to the mismatch. The limit of detection by this assay is 0.5-1.0% as determined by densitometric scanning. The numbers refer to base pair markers.

suspect that the major factor contributing to this seemingly significant staggering by the enzyme in removing CMC-modified bases is that the G:G mismatch, by destabilizing the helix, makes the neighboring bases also susceptible to modification and these modifications give rise to incision by the excinuclease. Even though CMC is specific for non-hydrogen bonded bases, it reacts slightly with the termini of blunt-ended linear DNAs (Novack et al., 1986) and to a certain extent with bases in the duplex, presumably during "breathing." However, based on control gels, this modification does not appear to give rise to a heterogeneous population of fragments that carry CMC-modified nucleotides which might produce fragments of different mobilities when incised at unique sites. That there is slight modification internally, and probably at the termini, is apparent from the facts that CMC-modified fragments migrate slower on our sequencing gels compared to unmodified fragments and that ABC excinuclease produces incisions throughout the fragment in addition to the main incision caused by the modified Gs at the mismatch. That these incisions are specific is evident from the observation that they are 7 bases 5' to a G or a T in the 5' labeled fragment and 5 bases 3' to a G or a T in the 3' labeled fragment.

Since the mismatch we used in this experiment has two potentially reactive bases, it was of interest to determine whether ABC excinuclease preferentially incised one strand, incised either strand with about equal efficiency, or incised both strands in cases where both Gs in the mismatch were modified. We find that the enzyme cuts either strand with about equal efficiency (data not shown) but that the enzyme does not cut both strands simultaneously at a detectable level. In Fig. 5 we present the result of an experiment in which a fragment with a G:G mismatch was modified with CMC, treated with ABC excinuclease, and then analyzed on a non-denaturing gel. The following conclusions can be made from an inspection of this figure. First, the CMC-treated DNA (lanes 3–6) migrates as a discrete band and slower than nontreated DNA (lanes 1 and 2), indicating that the majority of the molecules were modified to the same extent and that this modification caused retardation of the fragment in the acrylamide gel (Novack et al., 1986). Second, treatment of the modified DNA with ABC excinuclease produced a band that is further retarded in the gel (lane 4) compared to the CMC-modified but undigested DNA (lane 3). This is due to generation by ABC excinuclease of DNA fragments containing a 12-nucleotide gap and we observe this phenomenon with DNA fragments damaged by other agents (UV and psoralen) and then digested by ABC excinuclease. The amount of the slow migrating species (~10% of total) is a measure of the fraction of the molecules that were digested with ABC excinuclease.

Third, ABC excinuclease does not produce double-strand breaks in a CMC-modified G:G mismatch-containing substrate. Thus, in such a mismatch when both Gs are potentially modified in the same molecule (we have been unsuccessful demonstrating this as yet), the enzyme cuts one strand or the other but never both strands. Alternatively, it is conceivable that the enzyme incises only those molecules that have a single G of the mismatch modified. In any event our data clearly show that the enzyme does not recognize mismatches but recognizes mismatched bases that have been chemically modified, and it does not produce double-strand breaks with CMC-modified substrate when both bases in the mismatch are potential candidates for CMC modification. We have also not observed double-strand breaks with psoralen-cross-linked DNA.²

² B. Van Houten and A. Sancar, unpublished results.
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**Fig. 4.** Effect of ABC excinuclease on a G:G mismatch before or after treatment with carbodiimide. The 268-bp PvuII fragment was either 5' labeled and cut with EcoRI or cut with EcoRI and 3' labeled. The subfragments carrying the mismatch were purified, modified with carbodiimide, and treated with ABC excinuclease. This figure shows autoradiograms of the sequencing gels used for analyzing the reaction products. a, 5'-labeled DNA; b, 3'-labeled DNA. The Maxam-Gilbert sequencing ladders A + G, T + C, and A, G, T, and C. Lane 1, carbodiimide-modified DNA; lane 2, carbodiimide-modified DNA digested with ABC excinuclease. Note that, in addition to the main cut(s) due to the mismatched G (circled), there are fainter bands throughout this lane. These cuts are always 7 bases 5' to either a T or G residue, suggesting specific modification by carbodiimide and removal by the excinuclease of Ts and Gs in a duplex. The arrows indicate the expected sites of incision by the nuclease, 7 bases 5' to the mismatched G in a, and 4 bases 3' to the mismatched G in b.

**Fig. 5.** ABC excinuclease does not make double-strand breaks on carbodiimide-modified G:G mismatch-containing substrate. The 268-bp PvuII fragment carrying the mismatch was terminally labeled, subjected to various treatments, and then separated on a 5% nondenaturing polyacrylamide gel. This is an autoradiogram of the gel. Lane 1, unmodified DNA; lane 2, unmodified DNA plus ABC excinuclease; lane 3, carbodiimide-modifiedDNA; lane 4, carbodiimide-modified DNA digested with ABC excinuclease; lane 5, carbodiimide-modified DNA treated with the A and B subunits; lane 6, treated with the B and C subunits. The solid arrow indicates the position of the digested DNA and the broken arrow the position expected for a fragment resulting from a double-strand break by the excinuclease. Shorter exposures more clearly demonstrate the shift in migration due to carbodiimide, though the longer exposure was shown here to observe the ABC excinuclease-digested DNA.

**Effect of ABC Excinuclease on DNA Containing Loops (Deletions)**

Having failed to detect the removal of unmodified mispaired bases by ABC excinuclease, we wanted to find out if greater structural deformities in the form of one to four extrahelical bases would create a recognition site for the enzyme. Towards this end we treated terminally labeled heteroduplex DNAs constructed with each of the deletions shown in Table I with ABC excinuclease and analyzed the reaction products on sequencing gels. By labeling the proper strands we were able to examine the effect of the enzyme on both strands of the heteroduplex, i.e. the “deletion”-containing strand or the “loop”-containing strand. The results obtained with heteroduplexes containing one-, three-, and four-base deletions and loops are presented in Figs. 6 and 7, respectively. In all three instances the enzyme does not cut the deleted strand or the “looped-out” strand, leading us to conclude that the helical deformities created by these structures are dissimilar to those generated by a number of DNA adducts that are removed by ABC excinuclease. As in the case of mismatched bases, CMC modification of the looped-out base makes it a substrate for ABC excinuclease. In Figs. 8 and 9, we show that when the looped-out T (in a one-nucleotide loop) is modified by CMC it is recognized for efficient incision by the enzyme. We have obtained similar results with bases in three- and four-nucleo-
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While investigating the effect of the excinuclease on these anomalous DNA structures, we used the same fragments that had been irradiated with UV in our reactions as an internal control. As expected, UV irradiation created substrate for the enzyme and thus enabled us to state with some certainty that the lack of incision by the enzyme of the nondamaged DNA is the result of nonrecognition by the enzyme of the helical deformities caused by mismatches, loops, and deletions. In addition, a careful inspection of the ABC excinuclease incision patterns of these various substrates revealed some noteworthy effects of the mismatches and loops on the removal of the UV photoproducts in their vicinity, by ABC excinuclease. The incision sites of ABC excinuclease determined from Figs. 4, and 6–8, as well as other gels not presented in this paper, are summarized in Fig. 9 and are briefly discussed below.

G:G Mismatch—A G:G mismatch does not change the cutting mode of ABC excinuclease when the enzyme removes a photoproduct by cutting on both sides of the mismatch. If the G in the mismatch happens to be the 8th nucleotide 5’ to a photoproduct the enzyme cuts the phosphodiester bond be-
between the base-paired nucleotide and the looped-out G as it does when the G is correctly paired (Fig. 9b). However, it appears that the G:G mismatch somewhat inhibits the removal of photoproducts nearby as the intensity of excinuclease bands produced by cuts near the G:G mismatch appears to be less than the intensity of bands due to cuts in other parts of the fragment (Fig. 4).

One-nucleotide Loop—We used duplexes with a T or a C loop in our experiments. The fragment containing the T loop was essentially pure, but that with the C loop was contaminated with about 30% of homoduplex. The most interesting observation with the heteroduplex containing the T loop is that a pyrimidine dimer is formed between the looped-out T and an adjacent base-paired T and that this dimer is removed by the excinuclease in the normal fashion, by incision of the 8th phosphodiester bond 5' to the pyrimidine dimer (Fig. 9g). When the enzyme cuts on both sides of a photoproduct such that the two cuts are on either side of the C loop, the looped-out base contributes to the cutting distance from the dimer as though it was fully base paired. This effect is seen in both the strand having the “insertion” (Fig. 9c, top strand) and the strand with the deletion (Fig. 9, c–e, bottom strand).

Three-nucleotide Loop—In the looped-out strand there is only one potential site for UV photoproducts in the vicinity of the loop, a CC sequence 1 base pair 3' to the loop. The excinuclease removes the photoproducts formed at this sequence by incising the 8th phosphodiester bond of double-stranded DNA 5' to the CC photoproduct and the 5th phosphodiester bond 3' to the lesion (Fig. 9h). Thus, it seems that
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**Fig. 8.** Removal of a carbodiimide-modified looped-out T residue by ABC excinuclease. The (PvuII-EcoRI) fragment containing the T loop at position 103 (bottom strand, Fig. 1) or 50 nucleotides from the 5′ EcoRI terminus was labeled at the EcoRI site by kinasing and then treated with carbodiimide before treating with ABC excinuclease and analyzed on a sequencing gel. Lane 1, carbodiimide-treated DNA; lane 2, carbodiimide-treated DNA digested with excinuclease; lane 3, DNA treated with carbodiimide, irradiated with UV, and then digested with ABC excinuclease; lane 4, irradiated DNA digested with the nuclease; lane 5, nonirradiated DNA incubated with the enzyme; lane 6, nontreated DNA. A, G, T, and C are the Maxam-Gilbert ladder. The looped-out T is circled and the 8th phosphodiester bond to this T is indicated by a perpendicular arrow. Note, however, that due to modification of the single-stranded tail of the EcoRI site the labeled terminus is modified by carbodiimide, causing slow migration of the full-length fragment as well as those produced by ABC excinuclease. Though it is impossible to determine the exact number of base equivalents of retardation resulting from carbodiimide treatment, by making approximate measurements from the center of the modified full-length fragment to the center of the unmodified full-length and assuming normal enzyme cutting, the darken band in the ABC excinuclease lane appears to be the one caused by cutting at the 8th phosphodiester bond 5′ to the looped-out T, even though there is considerable staggering in either direction. The strong inhibition of cutting of UV-irradiated DNA that is also modified by carbodiimide seen in lane 3 is apparently caused by interference with binding of the enzyme to photoproducts by carbodiimide bound to this T, indicated by a perpendicular arrow.

In “measuring” the distance between the incision sites and the photoproduct the enzyme “ignores” the three-nucleotide loop that is spanned by the two cutting sites. On the deletion strand of the three-nucleotide loop substrate, there are three potential sites for UV photoproducts in the vicinity of the deletion. Of these, the TT, 4 bp 3′ to the deletion, is not a hot spot for photoproduct formation even in the control duplexes and therefore no conclusion can be made regarding the effect of the deletion on the removal of this photoproduct. On the other hand, the 3′-CTT-5′ sequence, 4 bp 5′ to the loop, is a hot spot for photoproduct formation and these photoproducts are removed in the normal manner in homoduplexes, as seen on control gels. However, such a removal would have required the incision on the 3′ side of the photoproducts by the excinuclease to be “across from the loop” or 1 bp 5′ to it. Neither these incisions nor the ones on the 5′ side corresponding to the same photoproducts are observed (Fig. 9h). We, therefore, conclude that, if there is a three-nucleotide loop on the complementary strand across from the 3′ cutting site of ABC excinuclease, the cutting by the enzyme (both 3′ and 5′ to the damaged nucleotide) is inhibited. When the 3′ cutting site is removed from the deletion by 5 bp or more, the loop does not affect the activity of the enzyme.

**Fig. 9.** Summary of the effects of mismatches, deletions and, loops on ABC excinuclease. The incision sites of ABC excinuclease were determined from Figs. 4 and 6–8 as well as from other experiments where 3′-labeled DNA was used as a substrate. In the figure we only show (with the exception of e) those incisions caused by photoproducts that are removed by incising on both sides of the mismatches or loops. With carbodiimide-modified DNA the main incisions are shown with solid arrows and the potential staggered cutting sites by broken arrows. Only 5′ cutting data is shown for the CMC-modified T loop (f). A indicates a UV photoproduct and two arrows connected with a solid line represent the two incisions made by ABC excinuclease to remove the bracketed photoproduct whereas the dashed line with arrows indicate that the bracketed photoproduct was not removed in that particular DNA structure but was removed in the control. The numbering system is that used in Fig. 1.
Four-nucleotide Loop—The loop consists of the sequence 5'-TGAG-3' and since this sequence is repeated in tandem the loop can be drawn in two different ways. It is likely that the two forms are present in nearly equal amounts. Keeping this in mind the following points can be made. In the looped-out strand, when the incision sites are on both sides of the loop, just as with the three-base loop, the enzyme seems to ignore the loop in measuring the distance; in one case the 5' cut is one nucleotide closer to the photoproduct compared to the cutting with a fully duplex molecule (Fig. 9i); in another, the 5' cutting site is 7 base-paired nucleotides away from the photoproduct and the loop is totally ignored (Fig. 9j). If the loop is 3 bp away 5' from a 5' cutting site by ABC excinuclease, it does not affect its cutting efficiency nor its cutting site. In the deletion strand, a 5' TC photoprotect immediately 3' to the deletion is cut out normally by the enzyme (Fig. 9i); however, a TT photoprotect 4 bp on the 3' side of the deletion results in cutting of the 5th phosphodiester bond by the enzyme instead of the 8th while the incision on the 3' side of this adduct occurs at the normal place, the 5th phosphodiester bond (Fig. 9j). Photoprotects 10 (or 6) bp 3' and 11 (or 15 bp) 5' to the deletion (depending on the conformation of the loop) are removed by the excinuclease efficiently by the conventional incision mechanism (the 8th and 4th or 5th phosphodiester bonds 5' and 3', respectively, to the photoprotects are incised).

**DISCUSSION**

Three mechanisms, base selection, proofreading, and mismatch repair, ensure replication fidelity from one generation to another with around \( 10^{-11} \) errors/base pair formed (see Loeb and Kunkel, 1982). In *E. coli* postreplication mismatch repair is mostly if not exclusively mediated by methyl-directed mismatch repair: the A residue in the GATC sequence is methylated by Dam methylase, and during replication this sequence in the newly synthesized strand is transiently non-methylated, thus enabling the gene products of *mut H, L, and S* to recognize the parental strand and to correct any error introduced by the polymerase in the newly synthesized strand. The methyl-directed mismatch repair is well characterized genetically (Siegel and Kamel, 1974; Wagner and Meselson, 1976; Glickman, 1979; Pukkila et al., 1983; Kramer et al., 1984) and its enzymology is currently under intensive study (Lu et al., 1983; Pang et al., 1985). This repair system is more efficient in correcting certain mismatches than others and it is not clear whether it corrects pyrimidine:pyrimidine mismatches (Kramer et al., 1984) or loops and deletions that are produced at biologically relevant frequencies during replication (Fresco and Alberts, 1960; Streisinger et al., 1966; Kunkel and Alexander, 1986). Since nucleotide excision repair mediated by ABC excinuclease removes bases that cause major helical distortions in DNA and since pyrimidine:pyrimidine mismatches or single-strand loops are likely to induce major changes in the DNA helix, we were interested in finding out whether the enzyme acts on such structures. We find that the enzyme does not incise DNA containing mismatches or one-, three- or four-nucleotide loops and therefore conclude that it does not participate in mismatch repair. This conclusion is in agreement with an earlier report demonstrating that mutations in any of the *uvr* genes had no effect on correcting mispairs in λ phage heteroduplexes (Doerfler and Hgness, 1968). Thus, we conclude that mispairs and small frameshifts that are not corrected by methylation-directed mismatch repair are not corrected by the ABC excinuclease-initiated nucleotide excision repair pathway either and may be repaired by an, as yet, unidentified repair pathway. However, ABC excinuclease does remove mispaired nucleotides when the mispaired base is also modified (either as a pyrimidine dimer or by carbodiimide). We believe this finding is of physiological significance, since unpaired bases are more reactive with the many metabolites present in the cellular milieu (Morita et al., 1985), and once chemically modified a mismatched nucleotide may no longer be a substrate for methyl-directed mismatch repair. In this regard it is of interest to note that D-glucose and glucose 6-phosphate inactivate single-stranded DNA orders of magnitude more rapidly than double-stranded DNA (Bucala et al., 1984) and that ABC excinuclease is involved in repairing the glucose 6-phosphate DNA adducts produced in double-stranded DNA (Bucala et al., 1985). We are currently investigating the effect of ABC excinuclease in vitro on DNA modified by reducing sugars.

Based on the incision pattern by ABC excinuclease of a modified mismatch, it appears that this enzyme, in combination with carbodiimide, may be a useful tool in probing the distortions caused by mismatches, as well as the molecular structure of the mismatch itself. A G/G mismatch is thought to be relatively stable compared to other mismatches (Ninio, 1979; Aboul-els et al., 1985), yet it appears that at least two bases on either side of a GTTTGTTTTG base are susceptible to carbodiimide and ABC excinuclease digestion (Fig. 4). It will be of interest to probe other "less stable" mismatches in this manner. The hydrogen bonding of mismatches involving G and/or T residues that is proposed by crystallographic studies may also be tested using the methods described here. Other structures that are suspected of causing localized unpairing such as pyrimidine diners or chemically induced lesions can similarly be probed, as well as localized regions of a duplex that are easily melted out, such as transcriptional control sequences. These studies are currently in progress.

The second aim of the studies reported in this paper was to extend our repertoire of substrates recognized by the ABC excinuclease in hope of finding a common structural motif for binding (or assembly) of the enzyme. We found that the mismatches and loops tested in this study were not substrates for ABC excinuclease. However, the lack of action of the enzyme on these structures contributes to our understanding of the extent and form of helical deformities caused by mismatches and loops. Of particular importance is the observation that, when three- and four-base loops are in proximity of a base damage that is substrate for the excinuclease, the enzyme removes the damaged nucleotide as usual, ignoring the loops in measuring the distance between its two incision sites. This observation suggests that "noncomplementary nucleotide residues could in some manner rotate out of the helix to allow a continuity of complementary base pairs along its entire length" (Fresco and Alberts, 1960) and supports the helix "with loops" model of these authors. However, there is evidence that one extra base stack into the helix (Patel et al., 1982b), which supports our observations that the excinuclease "counts" one extra base in measuring the cutting distance. Such an action mechanism in turn should be taken into account in proposing models for damage recognition and removal by the ABC excinuclease. That mismatches and loops are not recognized by the enzyme suggests that these structures do not distort the helix in the same manner as bulky chemical adducts, which are substrates for the excinuclease. Because modification of the looped-out base(s) with CMC renders that site a substrate for the enzyme, the bulky CMC may be inserted into the helix and cause a "bending," as other adducts excised by the enzyme are thought to produce.

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