Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 6824–6828, August 1991
Biochemistry

The C-terminal half of UvrC protein is sufficient to reconstitute (A)BC excinuclease

(DNA repair/truncated protein/active site)

JING-JER LIN AND AZIZ SANCAR

Department of Biochemistry and Biophysics, University of North Carolina School of Medicine, Chapel Hill, NC 27599

Communicated by Mary Ellen Jones, April 22, 1991 (received for review March 4, 1991)

ABSTRACT The UvrC protein is one of three subunits of the *Escherichia coli* repair enzyme (A)BC excinuclease. This subunit is thought to have at least one of the active sites for nucleophilic attack on the phosphodiester bonds of damaged DNA. To localize the active site, mutant UvrC proteins were constructed by linker-scanning and deletion mutagenesis. *In vivo* studies revealed that the C-terminal 314 amino acids of the 610-amino acid UvrC protein were sufficient to confer UV resistance to cells lacking the *uvrC* gene. The portion of the *uvrC* gene encoding the C-terminal half of the protein was fused to the 3′ end of the *E. coli malE* gene (which encodes maltose binding protein), and the fusion protein MBP–C314C was purified and characterized. The fusion protein, in combination with UvrA and UvrB subunits, reconstituted the excinuclease activity that incised the eighth phosphodiester bond 5′ and the fourth phosphodiester bond 3′ to a psoralen-thymine adduct. These results suggest that the C-terminal 314 amino acids of UvrC constitute a functional domain capable of interacting with the UvrB-damaged DNA complex and of inducing the two phosphodiester bond incisions characteristic of (A)BC excinuclease.

(A)BC excinuclease is the collective activity of UvrA, UvrB, and UvrC proteins of *Escherichia coli* (1, 2). The enzyme initiates nucleotide excision repair of DNA damage ranging from covalently modified bases (2) to noncovalent drug-nucleotide complexes (3, 4) to abasic sites generated by oxidative metabolism or ionizing radiation (5, 6). This type of enzymatic activity appears to be widespread in both pro- and eukaryotes (1, 7). Therefore, understanding the details of nucleotide excision repair in *E. coli* is likely to contribute to studies of DNA repair in other biological systems as well.

The reaction mechanism of (A)BC excinuclease has been investigated in some detail (8, 9). These studies have revealed a unique mechanism for high-affinity binding to damaged DNA and specific removal of modified nucleotides. UvrA, which has a moderate affinity to damaged DNA compared to unmodified DNA, makes a $(UvrA)_2(UvrB)_1$ complex and this complex delivers UvrB to the damaged site; a very stable UvrB–DNA complex is formed and UvrA dissociates from the site. UvrC, which has no affinity for free UvrB, binds to the UvrB–DNA complex with high affinity and specificity and induces the cleavage of the eighth phosphodiester bond 5′ and the fourth or fifth phosphodiester bond 3′ to the damaged nucleotide. Since both UvrB and UvrC are present in the incision complex, it is unclear at present which of the two subunits is responsible for the dual nucleophilic attack on the phosphodiester backbone. It has been reported that UvrC has a weak nonspecific nuclease activity (10), which would suggest that at least one of the specific incisions is made by UvrC.

The *uvrC* gene of *E. coli* encodes a protein of 610 amino acids and a molecular weight of 68,510 (7, 11). To define the role of UvrC in excision repair more specifically and to identify the active site(s) within the protein, we mutagenized the gene by linker-scanning mutagenesis. The results obtained by this method led us to make plasmid constructs that expressed only parts of the gene from the N or C termini. We found that a plasmid expressing the 314 C-terminal amino acids of the protein complemented $uvrC^-$ mutations *in vivo*. A fusion protein (MBP–C314C) was constructed in which the sequence for the maltose binding protein (MBP) was fused to the sequence for the C-terminal 314 amino acids of UvrC. The fusion protein was purified and was found to produce the two incisions typical of (A)BC excinuclease when mixed with UvrA and UvrB proteins. We conclude that the C-terminal half of UvrC carries all the structural requirements necessary for (A)BC excinuclease activity.

MATERIALS AND METHODS

Materials. The *E. coli* K-12 DR1984 (*uvrC34 recA1*) was used as a host for initial testing of the complementing activities of various plasmids carrying mutant *uvrC* genes (11). The mutant genes that were studied in more detail were also tested in *E. coli* N3024 (*uvrC 279*::Tn*10*), a strain with a Tn*10* insertion in the 3′ half of *uvrC* (12). Finally, by "curing" N3024 of the Tn*10* insertion, we obtained UNC3024(Δ*uvrC*) in which at least 60% of the 5′ terminal half of the *uvrC* gene has been deleted, as determined by Southern blot analysis using the 1.2-kilobase-pair *Bgl* I–*Nco* I fragment of *uvrC* as the probe (11). The S1 nuclease, T4 DNA polymerase, and restriction enzymes were purchased from Bethesda Research Laboratories. *E. coli* endonuclease IV (13) was kindly provided by B. Demple (Harvard University). UvrA, UvrB, and UvrC proteins were purified according to Thomas *et al.* (14). The pMAL-c plasmid used for gene fusions was obtained from New England Biolabs.

Construction of Linker-Scanning and Deletion Mutants. Linker-scanning mutants of *uvrC* (Fig. 1) were generated in the UvrC-overproducing plasmid pDR3274 (11) as described (15). Briefly, an average of one apurinic site per plasmid was introduced by heating pDR3274 at 65°C at pH 5.2 for 30 min (5). The plasmid was then treated with endonuclease IV and S1 nuclease to induce a double-strand break. The linearized DNA was then ligated to the 8-base-pair (bp) *Sst* I linker CGAGCTCG. The plasmids were transformed into DR1984 and tested for phenotypic complementation by UV survival and the sites of linker insertion were mapped by restriction analysis. The exact locations of the inserts in plasmids of interest were determined by double-stranded DNA sequencing.

Plasmids expressing the N-terminal fragments of UvrC (1–182 and 1–404 amino acids with internal deletions of

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: MBP, maltose binding protein.

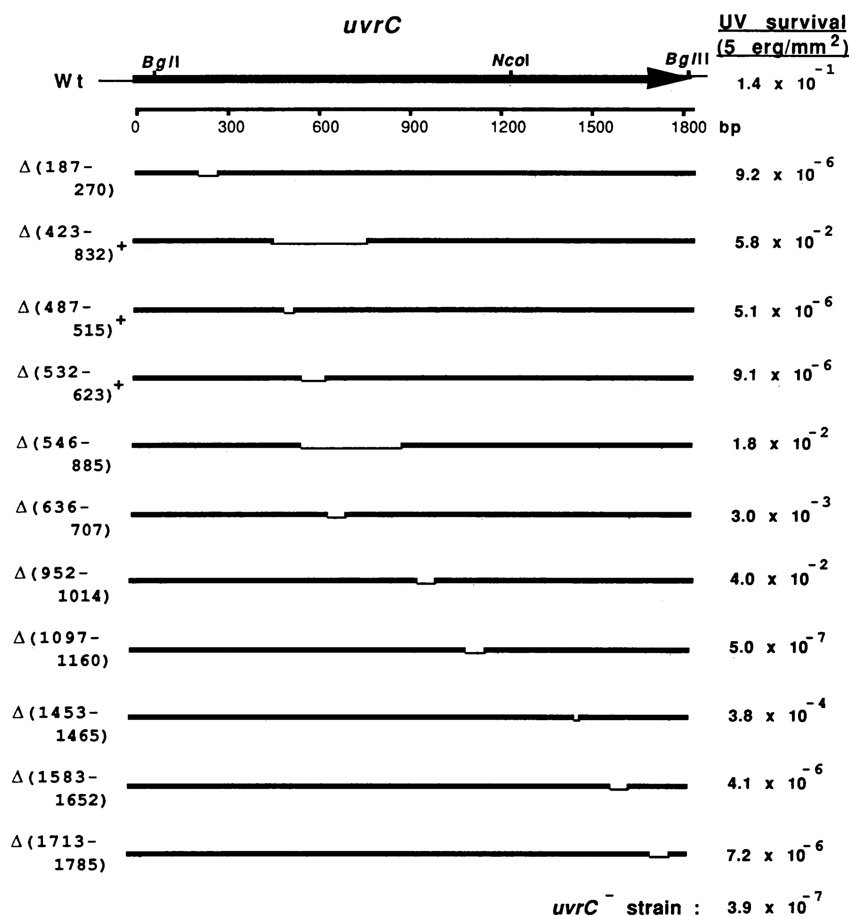

FIG. 1. Schematic representation of *uvrC* linker-scanning mutants. All 11 mutants shown had deletions (Δ) of the indicated base pairs and an insertion of an 8-bp *Sst* I linker. Thick lines, coding sequences; thin lines, internal deletions. On the right are the survivals of DR1984 (*uvrC34 recA1*) carrying the appropriate constructs after 254-nm irradiation (5 erg/mm^2; 1 erg = 0.1 μJ) from a germicidal lamp. Because of the extreme sensitivity of this strain to UV, minor differences in the UV dose delivered can cause survival differences of up to 10-fold with a given strain. Therefore, we do not consider the survivals from 4×10^{-6} to 4×10^{-7} to be significantly different. The higher survivals 10^{-2}–10^{-5} have been obtained reproducibly. +, In-frame deletion fusion. These experiments were repeated in a *uvrC* null background and identical results were obtained (data not shown).

various lengths) were constructed (Fig. 2) by digesting the appropriate linker-scanning mutants with *Eco*RI and *Sst* I or *Nco* I and inserting the resulting *uvrC* fragment into pBR328 (Boehringer Mannheim). Plasmids expressing C-terminal fragments of UvrC were constructed as follows. First, the linker-scanning plasmids were digested with *Sst* I and the sticky ends were "blunted" with T4 DNA polymerase. Then, *Nco* I linkers of appropriate lengths (for in-frame fusion with ATG at the *Nco* I site) were ligated to the blunt termini to generate plasmids in which the *Sst* I sites were replaced with *Nco* I sites. These latter plasmids were then digested with *Nco* I and *Pst* I to obtain fragments carrying the 3′-terminal regions of *uvrC*. These fragments were inserted into the expression vector pKK233-2 (Pharmacia LKB) to obtain

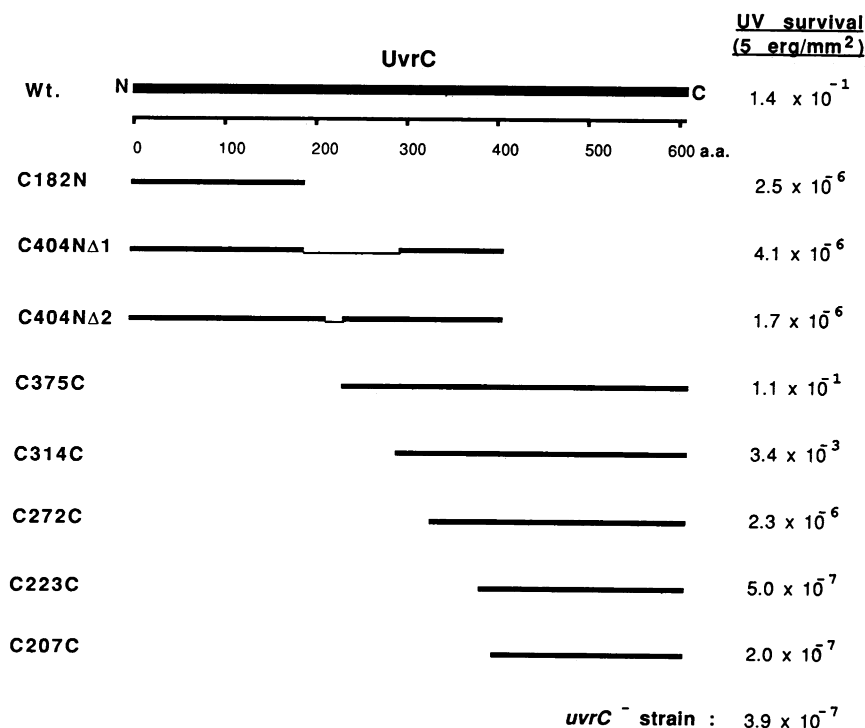

FIG. 2. UvrC C- and N-terminal deletions. The wild-type protein is 610 amino acids long. The deletion mutants are indicated by the number and relative locations of the amino acids they are predicted to contain. The constructs that encode N-terminal peptides C404NΔ1 and C404NΔ2 carry the first 1212-bp coding sequences of *uvrC* with internal deletions of 340 bp (Δ1) and 72 bp (Δ2), respectively, corresponding to deletions in constructs Δ(546–885) and Δ(636–707), respectively, in Fig. 1. Both of these deletions shift the reading frame. The constructs expressing C-terminal peptides were fused in-frame to a *tac* promoter with an initiation codon to express the indicated number of C-terminal amino acids of UvrC protein. The survivals of DR1984 (*recA1 uvrC34*) without plasmids or with wild-type or mutant plasmids to 254-nm irradiation (5 ergs/mm^2) are listed. Survivals within an order of magnitude of one another are not considered to be significantly different. Identical results were obtained in the Δ*uvrC* null background (data not shown).

plasmids expressing the C-terminal fragments of UvrC from the *tac* promoter on the expression vector.

UV Survival. Cells harboring the appropriate plasmids were grown in Luria broth containing the appropriate antibiotics. The cells were diluted in phosphate-buffered saline, plated on Luria broth agar plates, and irradiated at 254 nm with a General Electric germicidal lamp at a fluence rate of 5 $\mu W/cm^2$. After irradiation the plates were incubated at 37°C for 24 hr before counting colonies to measure survival relative to unirradiated cells.

Construction of *malE–uvrC* Fusion. Plasmid pMAL-C314C carrying the coding region of the C-terminal 314 amino acids of the UvrC protein fused to the gene for the MBP was constructed as follows. The linker-scanning mutant plasmid pUvrCΔ(546-885), which contains an *Sst* I insert at the 546- to 885-bp deletion site, was digested with *Sst* I, and the site was "blunted" with T4 DNA polymerase and ligated with the 8-bp *Xba* I linker GTCTAGAC. The DNA was then digested with *Xba* I and *Pst* I and the fragment carrying the 942-bp 3′-terminal part of the *uvrC* gene was purified. The purified fragment was then inserted into pMAL-c plasmid (New England Biolabs) that had been digested with *Xba* I and *Pst* I. The resulting construct pMAL-C314C contains the 942-bp 3′ terminal base pairs of *uvrC* linked to the 3′ terminus of the *malE* gene by a linker region that encodes a factor Xa cleavage site.

Purification of the MBP–C314C Fusion Protein. *E. coli* DR1984/pMAL-C314C cells were grown and induced with isopropyl β-D-thiogalactopyranoside according to the manufacturer's manual. The fusion protein that constituted ≈40% of total cellular proteins was purified by chromatography on single-stranded DNA-cellulose and phosphocellulose as described for the *Bacillus subtilis* UvrC protein (7). The fraction from the phosphocellulose column was subjected to affinity chromatography on an amylose-cellulose column to remove minor contaminants that remain after the first two purification steps.

Repair Assay. This assay measures the excision of a 12-mer containing a nucleotide adduct by (A)BC excinuclease. The substrate used was a 137-mer duplex with a centrally located furan-side thymine-psoralen monoadduct that had ^{32}P-label at the sixth phosphate 5′ to the adduct; thus incisions by (A)BC excinuclease result in the release of a radiolabeled 12-mer. The 137-mer substrate was constructed as described (16) using the psoralen-adduct-containing dodecanucleotide kindly provided by J. E. Hearst (University of California, Berkeley). The (A)BC excinuclease reactions were performed in a 25-μl reaction mixture containing 50 mM Tris·HCl (pH 7.5), 100 mM KCl, 10 mM $MgCl_2$, 2 mM ATP, 5 mM dithiothreitol, bovine serum albumin (50 μg/ml), 5 nM UvrA, 65 nM UvrB, and ≈2000 cpm of ^{32}P-labeled 137-mer. The reaction mixtures were incubated at 37°C for 30 min, then the indicated amounts of UvrC or MBP–C314C were added, incubation at 37°C was continued for another 30 min, and the reactions were stopped by adding 1 μl of oyster glycogen (10 mg/ml) and 60 μl of ice-cold ethanol. The precipitated DNA was collected by centrifugation, dried, resuspended in a formamide/dye mixture (16), and analyzed on a 12% sequencing gel. The autoradiograms were scanned with a densitometer (Zeineh Softlaser densitometer model SLR 2D/1D) to quantify excision.

RESULTS

Linker-Scanning Mutants of UvrC. In linker-scanning mutagenesis, short "linker" oligonucleotides are inserted randomly into a gene to identify the important regions for the structural–functional integrity of a protein. Using this methodology, we isolated 200 mutants in pDR3274(*uvrC*). Many of these were characterized by restriction mapping and 11 mutants, which were of particular interest, were further characterized by double-stranded DNA sequencing. All our mutants had extensive deletions from 134 bp Δ(1453–1465) to 340 bp Δ(546–885), presumably as a result of overdigestion by S1 nuclease during linearization of nicked DNA. Nevertheless, we were able to draw some conclusions regarding the structural organization of UvrC from the *in vivo* properties of these mutants.

Fig. 1 shows a schematic map of linker-scanning deletion mutants investigated and the phenotypic effect of these mutations. Sequence analysis shows that constructs Δ(423–832), Δ(487–515), and Δ(532–623) are in-frame fusions after the deletion–insertion reactions used to generate the mutants. With this fact in mind then and by assuming equal abundance of mutant proteins, the phenotypes of the mutants shown in Fig. 1 give a conflicting picture. Thus, loss of function in Δ(487–515) would indicate that the 10 amino acids deleted in this mutant are essential for function and, therefore, the N-terminal part of UvrC is vital for its complementing activity. Yet the mutant Δ(423–832) results in deletion of 137 amino acids including those deleted in Δ(487–515) but the resulting protein complements the $uvrC^-$ mutation almost to the wild-type level. In contrast, construct Δ(532–623), which is missing only a subset of the amino acids deleted in Δ(423–832), is also deficient in complementation. The out-of-frame deletion–insertion mutants Δ(546–855), Δ(636–707), and Δ(952–1014) complement the $UvrC^-$ phenotype, which implies that the region of *uvrC* beyond bp 635 (212 amino acids) is not required for activity. However, the last four deletion–insertion constructs that express more than the first 317 N-terminal amino acids of UvrC are defective in complementation, indicating that the C-terminal half is required for function.

Deletion Mutants of UvrC. To reconcile the seemingly contradictory data emerging from the deletion–insertion mutants, we constructed plasmids that expressed regions either from the N-terminal or C-terminal halves of UvrC protein. Fig. 2 is a schematic representation of the deletion mutants tested. C182N and C404NΔ1 were derived from the complementing Δ(546–885) and C404NΔ2 was derived from the complementing Δ(636–707) deletion–insertion mutants. These constructs, which express only N-terminal portions of UvrC, failed to complement $uvrC^-$ mutations. In contrast, constructs C375C and C314C complemented the $UvrC^-$ phenotype either fully or partially. The last three constructs shown in Fig. 2, which express the C-terminal 272, 223, or 207 amino acids, failed to complement the $UvrC^-$ phenotype.

As these results were somewhat contradictory to those obtained with the deletion–insertion mutants, we considered the possibility of an "α-complementation" type of an interaction between the mutant chromosomal protein (the nature of the mutation in *uvrC34* is not known) and the truncated proteins expressed from the plasmid constructs. Therefore, we tested the constructs in UNC3024(Δ*uvrC*) in which the region of *uvrC* spanning at least *Bgl* I–*Nco* I fragment (Fig. 1) has been deleted; the complementation pattern was the same (data not shown) as in DR1984 (*uvrC34 recA1*). Therefore, we conclude that the C-terminal 314 amino acids of UvrC protein are sufficient for UvrC activity.

Construction and Properties of MBP–C314C. Although the deletion mutants provided strong evidence for functional complementation by the C-terminal half of UvrC, the truncated proteins were not overproduced even when joined to the artificial *tac* promoter and, therefore, were not amenable to biochemical analysis. Thus we could not ascertain from the *in vivo* data whether the excinuclease reconstituted with UvrC–314C incised on both sides of the damage as the normal enzyme does or nicked only 5′ to the DNA adduct. It is known that the 5′ incision to pyrimidine dimers results in the eventual removal of the photodimers by the 5′ → 3′ exonu-

clease activity of DNA polymerase I (1). Lack of overproduction of truncated proteins is usually due to decreased stability of the mRNA or of the truncated peptide. The stability is often increased by fusing to another gene. It has been shown that the MBP of *E. coli* stabilizes a protein joined to its C terminus (17). Furthermore, the high affinity of MBP to amylose offers a convenient method for purifying the fusion proteins by affinity chromatography.

We joined the 3′ terminal 942 bp of *uvrC* to the polylinker region (which encodes factor Xa recognition site) at the 3′ terminus of *malE* in pMAL-c to obtain a *malE–uvrC* fusion under the control of a *tac* promoter (17). The resulting plasmid pMAL-C314C complemented the UV-sensitive phenotype of DR1984 (Fig. 3) as well as that of the *uvrC* deletion mutant UNC3024 whereas a plasmid expressing the N-terminal polypeptide of MBP had no effect on survival (data not shown). Thus, the fusion of the MBP to the C-terminal 314 amino acids of UvrC does not interfere with its complementing activity. Note, however, that at all doses tested the complementation was incomplete. Since even under noninducing conditions the fusion protein is overproduced compared to the 10 molecules of wild-type UvrC present in the control cells (1), it is clear that the fusion protein has reduced activity compared to wild type. However, no quantitative statement can be made from this data regarding the activity of the fusion protein.

Purification of the MBP–C314C Fusion Protein. The fusion protein was purified from a 500-ml culture of isopropyl β-D-thiogalactopyranoside-induced DR1984 F$lacI^Q$/pMAL-C314C. The cell-free extract, in which 40% of soluble protein was MBP–C314C, was loaded directly onto a single-stranded DNA-cellulose column. The fusion protein bound to this column with nearly the same affinity as wild-type UvrC and was further purified by successive chromatography on phosphocellulose and amylose resins. The final product was a 76-kDa protein at >99% purity, as determined by SDS/PAGE (Fig. 4). The fact that the fusion protein bound to single-stranded DNA and phosphocellulose resins with the same affinity as wild-type UvrC would indicate that the overall physical properties of MBP–C314C are similar to UvrC and in particular that the DNA binding site is located within the C-terminal 314 amino acids of UvrC (MBP has no affinity to DNA). Digestion of the purified fusion protein with factor Xa under optimal conditions yielded the C-terminal domain of UvrC with ≈50% yield (data not shown).

Reconstitution of (A)BC Excinuclease with MBP–C314C. A plasmid-nicking assay that measures the conversion of superhelical DNA to open circles (5), with wild-type UvrA and UvrB proteins and MBP–C314C gave an unexpected result.

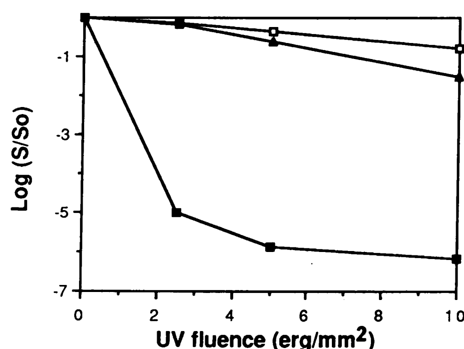

FIG. 3. UV survival of DR1984 (*recA1 uvrC34*) containing various plasmids. ■, DR1984 with no plasmid; □, DR1984/pDR3274 ($uvrC^+$); ▲, DR1984/pMAL-C314C (tac-malE-uvrC). Cultures were grown to stationary phase in Luria broth, and dilutions were plated on Luria agar plates and irradiated (254 nm) at a fluence rate of 5 $\mu W/cm^2$. S/S_0, relative survival.

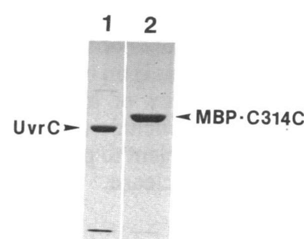

FIG. 4. Purified MBP–C314C fusion protein. Lanes: 1, UvrC protein (2 μg); 2, MBP–C314C (6 μg). UvrC is 69 kDa; the predicted molecular mass of the fusion protein is 76 kDa. This is a 10% polyacrylamide gel containing SDS stained with Coomassie blue.

In contrast to the near-wild-type level of complementation *in vivo*, the nicking activity with enzyme reconstituted with the fusion protein was barely detectable above background nicking (data not shown). We reasoned that the poor complementation *in vitro* might be due to the MBP part of the fusion that might be cleaved off *in vivo* by nonspecific proteases. Therefore, we conducted a plasmid-nicking assay with an enzyme reconstituted with a factor Xa-cleaved fusion protein. We saw no improvement over what could be achieved with the fusion protein alone and concluded that such a drastic reduction in activity is the result of deleting the N-terminal 295 amino acids of UvrC.

The low level of nicking activity observed with the enzyme reconstituted with the fusion protein precluded any firm conclusion regarding the specificity of the nicking. We decided to ascertain the specificity by conducting an "excision assay" (7). (A)BC excinuclease incises damaged DNA in a unique manner: it hydrolyzes the eighth phosphodiester bond 5′ and the fourth or fifth phosphodiester bond 3′ to the modified nucleotide resulting in the release of a 12- or a 13-mer (22). Therefore, if the low-level nuclease activity observed with the enzyme reconstituted with MBP–C314C is true (A)BC excinuclease activity, we would expect to see such an excision fragment with this system.

The experiment was conducted with an internally labeled 137-bp duplex containing a psoralen monoadduct near the internal label. Fig. 5 shows the incision products of (A)BC excinuclease reconstituted with 5 nM UvrA, 65 nM UvrB, and either 40 nM UvrC or 320 nM MBP–C314C fusion protein. A 12-mer carrying the psoralen adduct was excised by both systems. However, the level of excision, determined by densitometric scanning of the excised band, was 10 times more efficient in the reaction mixture containing wild-type protein and, considering that we used 8 times less wild-type UvrC than fusion protein, we calculate the fusion protein to have ≈1.25% the activity of wild-type UvrC. Although this activity is sufficient to achieve near-normal *in vivo* complementation when the protein is overproduced, it is too low to be detected by the less-specific plasmid-nicking assay. As in the nicking assay, cleavage of the fusion protein by factor Xa

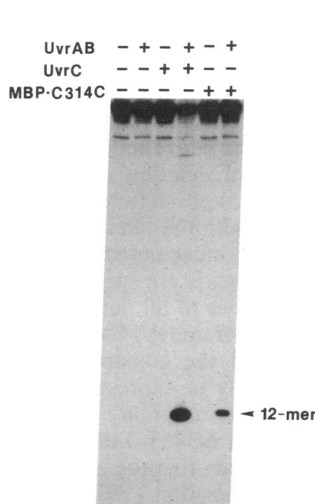

FIG. 5. Excision of psoralen monoadduct by (A)BC excinuclease reconstituted with the MBP–C314C fusion protein. A 137-mer duplex containing ^{32}P-label at the sixth phosphodiester bond 5′ to the adduct was incubated with the indicated proteins (+, protein present; −, protein absent) at 37°C for 30 min and the products were analyzed on a 12% polyacrylamide sequencing gel. The protein concentrations were 5 nM UvrA, 65 nM UvrB, and either 40 nM UvrC or 320 nM MBP–C314C fusion product. The excised 12-mer carrying the psoralen adduct comigrates with an unmodified 13-mer size marker.

did not improve the efficiency of excision by the enzyme reconstituted with the C terminus of UvrC (data not shown). Thus, it appears that low efficiency of excision is an intrinsic property of this system. This low efficiency of incision, notwithstanding our results, enables us to conclude that the C-terminal 314 amino acids of UvrC are sufficient to produce the unique incision pattern of (A)BC excinuclease.

DISCUSSION

UvrC binds specifically to UvrB-damaged DNA complexes and induces the dual incision characteristic of (A)BC excinuclease. There are three formal possibilities as to how the incisions are made. (*i*) UvrB may simply target UvrC to the damage site and UvrC makes both incisions. (*ii*) UvrC may bind to the UvrB–DNA complex and induce a conformational change in one or both components of this complex to position the two target phosphodiester bonds in proximity of the nucleophiles in UvrB. (*iii*) Finally, UvrC may bind to the UvrB–DNA complex, make one of the incisions, and induce a conformational change in the complex to enable UvrB to make the second incision, not necessarily in this temporal order. Some indirect evidence such as the weak nonspecific nuclease activity detected in UvrC preparations (10), the preferential inhibition of 5′ incision by either high pH or "aged" UvrC samples (1, 2), and the absolute lack of incision by DNA-bound UvrB alone lead us to favor the third alternative—that is, that one of the nucleophilic sites is on UvrB and the other is on UvrC. Based on this premise, we have proceeded herein to localize the active site of UvrC and have narrowed down the region to the C-terminal 314 amino acids of the 610-amino acid protein.

The approach we have used, generation of out-of-frame fusions and of internal and N- and C-terminal deletions and testing of these constructs by *in vivo* complementation is subject to potential pitfalls (18): recombination with the chromosomal gene, restoration of lost function by noncovalent interaction of the chromosome-encoded and plasmid-encoded peptides, and the loss of activity in a fragment possessing the active site by "tail" amino acids that may interfere with proper folding or substrate binding. Finally, translational frameshift and translational reinitiation and misreading may generate proteins not predicted from DNA sequence at low frequency (18, 19). When expressed from a strong promoter such as *tac*, the levels of such proteins may be sufficiently high to achieve full complementation. We have eliminated the two most serious sources of error, recombination and interpeptide complementation, by using strains with a RecA$^-$ phenotype or a strain deleted in about two-thirds of the 5′ region of *uvrC* gene. Therefore, we feel that our main conclusion that the C-terminal 314 amino acids of UvrC carry the active site of this protein is justified.

However, the results obtained with the deletion–insertion mutants further underscores the "hazards in the application of molecular biology to structure–function relationship" amply documented by Schimmel (18). Without the knowledge gathered from the N- and C-terminal deletion constructs shown in Fig. 2, it would have been impossible to propose structure–function model consistent with the phenotypes of all mutants shown in Fig. 1. Having independently established that the C-terminal 314 amino acids are sufficient for activity, we can explain the phenotypes of all out-of-frame mutants shown in Fig. 1. There are in-frame ATG codons at positions 930 and 1185 in *uvrC* and the constructs with complementing activity are more likely to express proteins from these restart sites. With this assumption, then, the complementing activity observed with Δ(1453–1465) out-of-frame fusion would lead one to conclude that the active site is located between amino acids 395 (position 1185) and 484 even though the possibility of translational frameshift in Δ(1453–1465) cannot be discounted.

The in-frame deletion fusions reveal another interesting aspect of this type of analysis. Thus, construct Δ(423–832), which encodes a UvrC protein with an internal deletion of 137 amino acids, complements *uvrC*$^-$ mutations whereas the two other in-frame constructs that are missing subsets of either 10 or 37 amino acids of the 137 amino acids deleted in Δ(423–832) do not complement. This is perhaps due to the dampening effect of the N-terminal "tails" on the function of mutant proteins as has been observed with glycine tRNA synthetase (20): a deletion of N-terminal 178 amino acids of the β chain results in an inactive synthetase; however, deletion of the N-terminal 306 amino acids yields a fully active β subunit, apparently because of removal of "activity-masking residues."

The data from in- and out-of-frame fusions, N- and C-terminal deletions, and finally the MBP fusion protein are consistent with the C-terminal 314 amino acids containing the active site of UvrC. It might be asked then what roles the N- and C-terminal halves of UvrC play in the incision reactions. We offer the following model: the C-terminal 314 amino acids carry the nucleophile responsible for one of the incisions, perhaps the 5′ incision as (A)BC excinuclease reconstituted with oxidized UvrC is defective in 5′ incision (unpublished observation). The function of the N-terminal half is mainly to make specific protein–protein contacts with UvrB and thus help form the UvrB–UvrC heterodimer, which makes the dual incisions. By deleting the N-terminal half of UvrC, we have created a protein with ≈100 times less affinity for the UvrB–DNA complex resulting in a 100 times diminished excinuclease activity.

It is interesting that *B. subtilis* UvrC (21), which can substitute for *E. coli* UvrC *in vivo* and *in vitro* (7), has a higher level of homology to *E. coli* UvrC in the N-terminal half (44%) compared to the C-terminal half (30%). This is consistent with our model that the N-terminal half is involved mainly in protein–protein interaction. Such interactions impose stringent selection on types of substitutions that can be made without significantly altering function.

We thank Dave Orren for technical help and Chris Selby for critical reading of the manuscript. This work was supported by National Institutes of Health Grant GM32833.

1. Sancar, A. & Sancar, G. B. (1988) *Annu. Rev. Biochem.* **57**, 29–67.
2. Selby, C. P. & Sancar, A. (1990) *Mutat. Res.* **236**, 203–211.
3. Lambert, B., Jones, B. K., Roques, B. P., Le Pecq, J.-B. & Yeung, A. T. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 6557–6561.
4. Selby, C. P. & Sancar, A. (1991) *Biochemistry* **30**, 3841–3849.
5. Lin, J.-J. & Sancar, A. (1989) *Biochemistry* **28**, 7979–7984.
6. Snowden, A., Kow, Y. W. & Van Houten, B. (1990) *Biochemistry* **29**, 7251–7259.
7. Lin, J.-J. & Sancar, A. (1990) *J. Biol. Chem.* **265**, 21337–21341.
8. Orren, D. K. & Sancar, A. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 5237–5241.
9. Orren, D. K. & Sancar, A. (1990) *J. Biol. Chem.* **265**, 15796–15803.
10. Caron, P. R. & Grossman, L. (1988) *Nucleic Acids Res.* **16**, 7855–7865.
11. Sancar, G. B., Sancar, A. & Rupp, W. D. (1984) *Nucleic Acids Res.* **12**, 4593–4608.
12. Maloy, S. R. & Nunn, W. D. (1981) *J. Bacteriol.* **145**, 1110–1112.
13. Levin, J. D., Johnson, A. W. & Demple, B. (1988) *J. Biol. Chem.* **263**, 8066–8071.
14. Thomas, D. C., Levy, M. & Sancar, A. (1985) *J. Biol. Chem.* **260**, 9875–9883.
15. Luckow, B., Renkawitz, R. & Schutz, G. (1987) *Nucleic Acids Res.* **15**, 417–429.
16. Van Houten, B., Gamper, H., Sancar, A. & Hearst, J. E. (1987) *J. Biol. Chem.* **262**, 13180–13187.
17. Guan, C., Li, P., Riggs, P. D. & Inouye, H. (1987) *Gene* **67**, 21–30.
18. Schimmel, P. (1990) *Biochemistry* **29**, 9495–9502.
19. Gold, L. (1988) *Annu. Rev. Biochem.* **57**, 199–233.
20. Toth, M. J. & Schimmel, P. (1990) *J. Biol. Chem.* **265**, 1000–1004.
21. Chen, N. Y., Zhang, J. J. & Paulus, H. (1989) *J. Gen. Microbiol.* **135**, 2931–2340.
22. Sancar, A. & Rupp, W. D. (1983) *Cell* **33**, 249–260.