The C-terminal Region of the UvrB Protein of Escherichia coli Contains an Important Determinant for UvrC Binding to the Preincision Complex but Not the Catalytic Site for 3′-Incision*

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The UvrABC endonuclease from Escherichia coli repairs damage in the DNA by dual incision of the damaged strand on both sides of the lesion. The incisions are in an ordered fashion, first on the 3′-side and next on the 5′-side of the damage, and they are the result of binding of UvrC to the UvrB-DNA preincision complex. In this paper, we show that at least the C-terminal 24 amino acids of UvrB are involved in interaction with UvrC and that this binding is important for the 3′-incision. The C-terminal region of UvrB, which shows homology with a domain of the UvrC protein, is part of a region that is predicted to be able to form a coiled-coil. We therefore propose that UvrB and UvrC interact through the formation of such a structure. The C-terminal region of UvrB only interacts with UvrC when present in the preincision complex, indicating that the conformational change in UvrB accompanying the formation of this complex exposes the UvrC binding domain. Binding of UvrC to the C-terminal region of UvrB is not important for the 5′-incision, suggesting that for this incision a different interaction of UvrC with the UvrB-DNA complex is required. Truncated UvrB mutants that lack up to 99 amino acids from the C terminus still give rise to significant incision (1-2%), indicating that this C-terminal region of UvrB does not participate in the formation of the active site for 3′-incision. This region, however, contains the residue (Glu-640) that was proposed to be involved in 3′-catalysis, since a mutation of the residue (E640A) fails to promote 3′-incision (Lin, J. J., Phillips, A. M., Hearst, J. E., and Sancar, A. (1992) J. Biol. Chem. 267, 17693-17700). We have isolated a mutant UvrB with the same E640A substitution, but this protein shows normal UvrC binding and incision in vitro and also results in normal survival after UV irradiation in vivo. As a consequence of these results, it is still an open question as to whether the catalytic site for 3′-incision is located in UvrB, in UvrC, or is formed by both proteins.

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**EXPERIMENTAL PROCEDURES**

Construction of UvrB Mutants—The truncated UvrB mutants UvrB630 and UvrB649 were constructed by introducing a stop codon in the coding sequence using polymerase chain reaction. For mutant UvrB630, a stop codon was introduced at position 631 using primer CGGGAGACAGGCCTATTATTCGGGACATTC, and for B649, a stop codon was introduced at position 650 using primer CGGGAGACAGGCTTAATTCTGGCGGTGTG. The second primer used for both polymerase chain reaction reactions (CGCGTACTGGTCAACCACCTGAC—CAAG) hybridizes just 5’ of the BglII site of the uvrB gene. The polymerase chain reaction product was digested with BglII and Stul (which is present in the primers containing the stop codon) and subsequently inserted into pNP83, which contains the uvrB gene under control of the trc promoter (4). The E640A mutation was introduced into pNP83 according to the method described by Landt et al. (17) using primer CATGAGTGGCAAGGGTTGATG. Helicase mutants E514K, G509S, and R544H and mutant UvrB574 (Gln-575 amber) were isolated from a collection of random UvrB mutants and have been described previously (4). For complementation studies, the mutated uvrB genes were inserted into pNP77 (4). All constructs were checked by DNA sequencing.

Proteins and DNA Substrates—The wild type UvrA, UvrB, and UvrC proteins were purified as described by Visse et al. (18). The UvrB mutants UvrB630, UvrB649, and E640A were purified from strain CS5018 (UvrA,ΔuvrB) as described by Moolenaar et al. (4). UvrB* was purified from CS5018 containing pNP83. To increase the amount of proteolytic UvrB* fragment, the crude cell lysate was incubated at 37°C for 45 min. Purification was achieved using the same procedure as for wild type UvrB. Proteins were analyzed by SDS-gel electrophoresis (19) and Western blotting using the ECL system (Amersham Life Science). Proteins were analyzed by SDS-gel electrophoresis (19) and Western blotting using the ECL system (Amersham Life Science) and the supplemented procedures.

The 96-bp DNA fragment containing a single cis-Pt-GG adduct (Fig. 8) was constructed from six oligonucleotide fragments as described (11). Construction of the DNA substrate containing a nick on the 3’-side of the lesion was carried out using the same oligonucleotide fragments, but the 3’-oligonucleotide in the upper strand was not phosphorylated to prevent ligation (see Fig. 8).

Analysis of Protein-DNA Complexes and DNA Incision Assay—The interaction of UvrA and UvrB with the cis-Pt DNA fragment was analyzed by mobility shift gel electrophoresis as described (18). Binding of UvrC to the UvrB-DNA complex was analyzed in a mobility shift assay, either in the absence or in the presence of UvrC antiserum as described (4). UvrC binding assay. Mutants G509S and R544H, which have amino acid substitutions in one of the helicase motifs (motif V or VI) present in UvrB. These mutants can still form a specific UvrB-DNA preincision complex. This specificity can also be shown when UvrB mutants impaired in preincision complex formation are used. We have previously described such UvrB mutants, which have amino acid substitutions in one of the helicase motifs (motif V or VI) present in UvrB. These mutants can still form a specific UvrB-DNA complex at the site of DNA damage, but as a consequence of the impaired helicase activity, the UvrB-DNA preincision complex formation is also impaired (4). We have tested three of these helicase mutants (E514K, G509S, and R544H) in the UvrC binding assay. Mutants G509S and R544H, which...
have no helicase activity and as a consequence make no UvrB-DNA complex, are not capable of binding UvrC (Fig. 1B, lanes 5–8). Mutant E514K has partial helicase activity (4), resulting in a reduced formation of the preincision complex compared to wild type. As a consequence, the UvrC binding is also reduced (Fig. 1B, lanes 3 and 4). Apparently, UvrC recognizes a specific structure in the preincision complex, which is exposed during the conformational change that accompanies the formation of this complex.

Predicted Coiled-coil Motifs in UvrB and UvrC—Analysis of the amino acid sequence of the UvrB protein with the program COILS (21) revealed two α-helical regions with a high probability score for formation of a coiled-coil motif (Fig. 2). These two regions, located in the center of the protein between residues 240 and 300 and in the C-terminal region between residues 620 and 673 are conserved in all UvrB proteins that have been identified in other bacteria (Fig. 3 and not shown), suggesting that they are functionally important. The coiled-coil plot of the C-terminal region shows two domains having a high probability score, which are separated by a stretch of five amino acids with a very low score (Fig. 3). The second domain of the C-terminal region of UvrB contains homology with the UvrC protein (Fig. 3). The coiled-coil plot of the UvrC protein also predicts the presence of a coiled-coil motif in this homologous region from residues 200–240 (Fig. 3), and again this predicted coiled-coil domain in UvrC is conserved in the UvrC proteins from other bacteria. This analysis prompted us to explore the possibility that the C-terminal part of UvrB interacts with the homologous domain in UvrC to form the UvrBC-DNA incision complex.

The UvrB* Proteolytic Fragment of UvrB Lacks the UvrC Binding Domain—UvrB* is a proteolytic product generated during the lysis of E. coli cells by the OmpT protease and which is therefore missing the C-terminal part of the protein (22). From homology with one of the OmpT cleavage sites of the Ada protein, the cleavage of UvrB was proposed to occur between residues Lys-630 and Ala-631 (22). It has been shown (23) that the UvrB* protein is still able to form the preincision complex, but that incision is severely impaired (16, 22). Since the UvrB* protein lacks the predicted coiled-coil motif, this impaired incision could be the consequence of the absence of the UvrC binding domain. We therefore tested the binding of UvrC to the UvrB*-DNA preincision complex. For this purpose, we isolated the UvrB* protein in two different ways: by OmpT cleavage of the wild type protein and by construction of a uvrB deletion mutant (UvrB630) that encodes residues 1–630 (see “Experimental Procedures”). The deletion mutant was purified from a ΔuvrB strain to avoid contamination with the wild type protein. After purification of the two proteins, it appeared that they migrate at different positions on a protein gel (Fig. 4), indicating that the OmpT cleavage site is located at a different position to that previously proposed. Electrospray ionization mass spectrometric analysis of the UvrB* protein allowed identification of two series of ions of approximately equal intensity, which correspond to two species having M, values of 68,567 and 68,789 (results not shown). It has been reported that the N-formylmethionine of UvrB is cleaved off (22), but it is not known whether upon overproduction of the protein this processing is complete. Therefore, the two species might result from the same cleavage of OmpT in UvrB without the N-formylmethionine and of UvrB still carrying this first amino acid. On the other hand, it is also possible that the two protein fragments are the result of the presence of multiple cleavage sites for OmpT. In either case, the cleavage(s) by OmpT are expected to occur in the region encompassing residues 607–610. Since OmpT is known to cleave after a basic residue, this leaves us two possibilities for the UvrB* cleavage site(s), between Lys-607 and Ala-608 and between Lys-609 and Gly-610 (Fig. 3).

Both the UvrB* and UvrB630 proteins were tested for their ability to form UvrBC-DNA complexes in the mobility shift assay with UvrC antibodies. Fig. 5 (lanes 1–4) shows that neither of the two truncated UvrB proteins is capable of forming such a complex. DNase I footprinting of the mutant UvrA6B complexes on a DNA fragment containing a cis-Pt-GG adduct confirmed that both truncated proteins do form normal preincision complexes (results not shown), as was shown before for the UvrB* protein (5, 23). Therefore, we conclude that the C-terminal 43 amino acids of UvrB contain an important determinant for UvrC binding to the preincision complex.

The UvrC Homology in UvrB Is Important for UvrC Binding—A uvrB deletion mutant was constructed that encodes the truncated UvrB649 protein lacking only the second domain of the predicted coiled-coil region in the C-terminal region (Fig. 3). This mutant protein forms a normal preincision complex, but again, specific UvrC binding is not observed (Fig. 5, lanes 5 and 6). Apparently the C-terminal 24 amino acids, which contain the homology with the UvrC protein, are involved in UvrC binding.
Incision in the presence of UvrB*, UvrB630, and UvrB649

It has been described before (16) that the incision activity in the presence of UvrB* on a DNA fragment containing a thymine-psoralene adduct is ~0.1% of the activity in the presence of wild type UvrB. When we analyzed the 5'-incision (Fig. 6) or the 3'-incision (not shown) of the cis-Pt-containing DNA in the presence of UvrB*, UvrB630, or UvrB649, the activities appear to be about 1 and 2% of the wild type level in the presence of 20 and 40 nM UvrC, respectively, which is significantly higher than the activity reported for the psoralene adduct. The increase in incision activity with increasing concentration of UvrC supports the suggestion that the UvrB mutants are affected in their UvrC binding.

Residue Glu-640 of UvrB Is Not Involved in Catalysis

It has been postulated by Lin et al. (16) that residue Glu-640 of UvrB (indicated as Glu-639 in these studies, as these authors do not count the first methionine residue) is directly involved in making the 3'-incision, since a mutation of this amino acid (E640A) reduces the incision activity to ~0.1%. Residue Glu-640, however, is located in the domain that, according to the results described above, could be involved in the binding of UvrC. An alternative explanation for the effect of the E640A substitution might therefore be that it affects the UvrC binding. We therefore constructed the UvrB(E640A) mutant by site-directed mutagenesis and tested the mutant protein in the UvrC binding assay. Surprisingly, however, the mutant not only showed a normal UvrC binding (results not shown), but it also resulted in incision with the same efficiency as the wild type protein (Fig. 7). Moreover, in our hands, UvrB(E640A), when inserted into a low copy number plasmid (see “Experimental Procedures”), could fully complement a ΔuvrB strain for UV survival (not shown), although it was described (16) that this complementation is only partial. We do not understand this discrepancy, but our results show that residue Glu-640 cannot be an essential part of the catalytic site. The fact that the truncated UvrB mutants disturbed in UvrC binding still have a residual incision activity of 1–2% indicates that these mutants do not lack residues that are essential for catalytic activity. Moreover, a truncated UvrB mutant (UvrB574), which lacks 99 C-terminal amino acids, results in the same amount of incision as the other truncated mutants (Fig. 6, lane 8). This means that at least the 99 C-terminal amino acids of UvrB can be excluded from being involved in the formation of the active site for catalysis.

### Figure 3
Alignment of the C-terminal regions of different UvrB proteins and the homologous regions of different UvrC proteins. The C-terminal regions of the UvrB proteins from E. coli (Ec-B, Ref. 24), Neisseria gonorhoeae (Ng-B, Ref. 34), Streptococcus pneumoniae (Sp-B, Ref. 35), Micrococcus luteus (Ml-B, Ref. 36), and Zymomonas mobilis (Zm-B, K.K. Reuter and R. Ficner, unpublished data) are aligned with the homologous regions of the UvrC proteins from E. coli (Ec-C, Ref. 37), Pseudomonas fluorescens (Pf-C, Ref. 38), and Bacillus subtilis (Bs-C, Ref. 39). The conserved amino acids are in bold. The heptad repeats that are characteristic for the formation of a coiled-coil motif (abcdefg), with hydrophobic amino acids at the a and d positions are indicated. The endpoint of the truncated E. coli UvrB proteins used, the position of the two possible OmpT cleavage sites leading to UvrB* as determined in this paper, and the position of the mutation E640A are shown.

### Figure 4
Size comparison of UvrB* and UvrB630. Wild type UvrB (lane 1), UvrB* (lane 2), and UvrB630 (lane 3) were loaded on a protein gel, after which they were visualized by ECL-Western blotting.

### Figure 5
UvrC binding in the presence of truncated UvrB proteins. The cis-Pt-containing fragment was incubated with 10 nM UvrA, 20 nM UvrC, and 100 nM of wt UvrB (lanes 1, 3, and 5), UvrB* (lane 2), UvrB630 (lane 4), or UvrB649 (lane 6), after which UvrC antiserum was added. The positions of the UvrA2B- and UvrB-DNA complexes are indicated. The UvrBC-DNA complexes that are bound by UvrC antiserum remain in the slot of the gel.

### Incision in the Presence of UvrB*, UvrB630, and UvrB649—It has been described before (16) that the incision activity in the presence of UvrB* on a DNA fragment containing a thymine-psoralene adduct is ~0.1% of the activity in the presence of wild type UvrB. When we analyzed the 5'-incision (Fig. 6) or the 3'-incision (not shown) of the cis-Pt-containing DNA in the presence of UvrB*, UvrB630, or UvrB649, the activities appear to be about 1 and 2% of the wild type level in the presence of 20 and 40 nM UvrC, respectively, which is significantly higher than the activity reported for the psoralene adduct. The increase in incision activity with increasing concentration of UvrC supports the suggestion that the UvrB mutants are affected in their UvrC binding. We therefore constructed the UvrB(E640A) mutant by site-directed mutagenesis and tested the mutant protein in the UvrC binding assay. Surprisingly, however, the mutant not only showed a normal UvrC binding (results not shown), but it also resulted in incision with the same efficiency as the wild type protein (Fig. 7). Moreover, in our hands, UvrB(E640A), when inserted into a low copy number plasmid (see “Experimental Procedures”), could fully complement a ΔuvrB strain for UV survival (not shown), although it was described (16) that this complementation is only partial. We do not understand this discrepancy, but our results show that residue Glu-640 cannot be an essential part of the catalytic site. The fact that the truncated UvrB mutants disturbed in UvrC binding still have a residual incision activity of 1–2% indicates that these mutants do not lack residues that are essential for catalytic activity. Moreover, a truncated UvrB mutant (UvrB574), which lacks 99 C-terminal amino acids, results in the same amount of incision as the other truncated mutants (Fig. 6, lane 8). This means that at least the 99 C-terminal amino acids of UvrB can be excluded from being involved in the formation of the active site for catalysis.

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In conclusion, the presence of UvrB*, UvrB630, and UvrB649—It has been described before (16) that the incision activity in the presence of UvrB* on a DNA fragment containing a thymine-psoralene adduct is ~0.1% of the activity in the presence of wild type UvrB. When we analyzed the 5'-incision (Fig. 6) or the 3'-incision (not shown) of the cis-Pt-containing DNA in the presence of UvrB*, UvrB630, or UvrB649, the activities appear to be about 1 and 2% of the wild type level in the presence of 20 and 40 nM UvrC, respectively, which is significantly higher than the activity reported for the psoralene adduct. The increase in incision activity with increasing concentration of UvrC supports the suggestion that the UvrB mutants are affected in their UvrC binding. We therefore constructed the UvrB(E640A) mutant by site-directed mutagenesis and tested the mutant protein in the UvrC binding assay. Surprisingly, however, the mutant not only showed a normal UvrC binding (results not shown), but it also resulted in incision with the same efficiency as the wild type protein (Fig. 7). Moreover, in our hands, UvrB(E640A), when inserted into a low copy number plasmid (see “Experimental Procedures”), could fully complement a ΔuvrB strain for UV survival (not shown), although it was described (16) that this complementation is only partial. We do not understand this discrepancy, but our results show that residue Glu-640 cannot be an essential part of the catalytic site. The fact that the truncated UvrB mutants disturbed in UvrC binding still have a residual incision activity of 1–2% indicates that these mutants do not lack residues that are essential for catalytic activity. Moreover, a truncated UvrB mutant (UvrB574), which lacks 99 C-terminal amino acids, results in the same amount of incision as the other truncated mutants (Fig. 6, lane 8). This means that at least the 99 C-terminal amino acids of UvrB can be excluded from being involved in the formation of the active site for catalysis.
3'-incision, but it remains to be determined whether UvrB is directly involved in 3'-incision at all.

UvrC Binding Assay on a 3'-Nicked Substrate—It has been shown (16) that UvrB* is as efficient as wild type UvrB in promoting the 5'-incision when using a substrate that is preincised at the 3'-position. In the light of the experiments described above, the binding of UvrC to the C-terminal region of UvrB is apparently not essential for this second incision event. To examine the binding of UvrC to a preincised UvrB-DNA complex, we constructed the cis-Pt-containing DNA substrate with a 3'-nick by using a non-phosphorylated 39-mer in the top strand (Fig. 8). In this construct, the 3'-nick is at the 7th phosphodiester bond 3'-side of the DNA adduct, which is three nucleotides further away from the damage than the normal 3'-incision. First, we tested whether the aberrant position of the 3'-incision influences the 5'-incision event. Fig. 9 shows that both wild type UvrB and UvrB630 promote 5'-incision at the same position as in the normal substrate. Apparently, the exact position of the 3'-incision is not important for the 5'-incision event. The shortest UvrB mutant used (UvrB574) also promoted efficient 5'-incision of the nicked substrate (results not shown). It is also clear from Fig. 9 that the 5'-incision of the nicked substrate for wild type and mutant proteins is much faster than of the normal substrate, suggesting that now UvrC binds as efficiently to the mutant as to the wild type complex. However, when we tested the binding of UvrC to the preincised UvrB-DNA complex with the gel retardation assay (Fig. 10), no specific UvrC binding to the mutant UvrB complexes could be observed. Apparently, for the second incision event, UvrC needs to interact only transiently with the UvrB-DNA complex, which would be consistent with the very fast incision, or this interaction is too weak to be detected in a gel retardation assay. Since the 5'-incision in the presence of the mutant UvrB protein is comparable to that given by wild type UvrB, this second incision event does not seem to require the C-terminal region of UvrB.

We therefore propose two binding modes for the UvrC protein; for the 3'-incision, stable protein-protein interaction between UvrB and UvrC is required. This 3'-incision results in a conformational change in the complex, which is subsequently bound by another domain of UvrC, resulting in 5'-incision. The second interaction of UvrC, with the DNA and/or with another domain of UvrB, could possibly be accomplished by the UvrC molecule that is already present in the complex, but alternatively it might result from the binding of a second UvrC molecule.
DISCUSSION

The UvrB protein plays a key role in the nucleotide excision repair by the UvrABC endonuclease. First, UvrB interacts with the UvrA dimer, which subsequently helps it to bind to the DNA lesion where it finally interacts with the UvrC protein to promote incision. In this paper, we have shown that at least the 24-amino acid C-terminal region of UvrB is required for the interaction of the preincision complex with UvrC. One interpretation of this observation might be that the C-terminal region of UvrB induces a conformational change in the DNA of the preincision complex, which is subsequently recognized by UvrC. DNase I footprint analysis of preincision complexes formed by wild type UvrB or the truncated UvrB* protein, however, displays the same pattern of protected and enhanced cleavage sites, indicating that the DNA conformations in these complexes are the same (16). We favor the hypothesis that the C-terminal region of UvrB is directly involved in the binding of UvrC, since it contains homology with a central domain of UvrC (22, 24) and forms part of a longer region encompassing residues 629–670 that is predicted to be able to form a coiled-coil motif (Fig. 3). Coiled-coils are formed by two or more amphiphilic α helices, which display a pattern of hydrophilic and hydrophobic residues in a 7-residue repeat (25). They have been found to be implicated in protein-protein interactions such as the multimerization of myosins and keratins (25) or the interaction between Fos and Jun (26). The binding of UvrC to the preincision complex might well therefore be stabilized by the formation of such a coiled-coil structure. Interestingly in this respect, the C-terminal regions of all UvrB proteins identified as well as the homologous regions of all the known UvrC proteins show the same high probability of coiled-coil formation (Fig. 3). These regions also have in common the fact that they contain a discontinuity in the heptad repeats. Such discontinuities are often found in proteins with coiled-coils, and they are thought to contribute to protein function either by creating fixed bends or by providing flexibility to the protein interface (27). Flexibility of the UvrB-UvrC interacting domain might be important for the positioning of the catalytic site of the UvrBC complex, since it is likely that different types of damage impose structural variation upon the UvrBC-DNA complexes. It has been shown for cis-Pt-containing DNA that the binding of UvrC to the preincision complex is much faster than the incision (20), indicating that at least for this lesion the binding of UvrC does not directly position the catalytic site at the scissile phosphodiester bond.

The protein region preceding the postulated coiled-coil motif might also contribute to flexibility of the UvrBC interacting domain. This region is non-conserved and has a variable length in the UvrB proteins from different organisms (Fig. 3). From the molecular mass determination carried out on the UvrB* protein, we suggest that the cleavage site(s) of OmpT are between Lys-607 and Ala-608 and/or between Lys-609 and Gly-610, which is in this non-conserved region. In addition, cleavage by a number of other proteases also results in UvrB* like fragments (33). These properties indicate that the UvrC binding domain of UvrB is connected to the rest of the protein by a flexible hinge. Such a hinge might allow the positioning of the active site in different preincision complexes formed on different lesions.

The stable interaction between the C terminus of UvrB and UvrC that can be shown in a band shift assay seems only to occur after the preincision complex is formed. This is illustrated by the fact that helicase mutants of UvrB that are able to recognize the DNA lesion in the A2B complex but are impairs in preincision complex formation are also impaired in UvrC binding (Fig. 1B). Moreover, no specific UvrBC complexes can be observed when a mixture of both proteins is run on a
native gel in the absence of DNA (results not shown). Possibly, the stable binding of the UvrC protein not only requires interaction with UvrB but also with the DNA, and the latter might be dependent on the specific conformation that accompanies the formation of the preincision complex. On the other hand, it is also possible that formation of the preincision complex is needed to either expose the C-terminal domain of UvrB or to induce the transition to the proper α-helical structure to allow coiled-coil formation with UvrC. Such a “spring-loaded” mechanism has also been described for the hemagglutinin protein, where conformational changes upon membrane fusion result in the transition of a loop region to a coiled-coil conformation (28).

The stable interaction between UvrC and the C-terminal part of UvrB appears to be important for 3'-incision, although even without this stable interaction 1-2% incision can still occur. This low amount of incision is comparable to the amount of incision observed when a maltose binding protein-UvrC fusion protein was used, which contains only the C-terminal 314 amino acids of UvrC (14). Since this UvrC fusion protein lacks the UvrB homology, the impaired incision in the presence of this protein might be due to the inability to stably bind the preincision complex. The residual incisions that are observed with UvrB and UvrC proteins lacking their postulated coiled-coil motif do, however, imply that in the UvrBC-DNA complex, there are additional weaker interactions between the two proteins or between UvrC and the DNA. Recently, a weak interaction between UvrB and UvrC in the absence of DNA has been demonstrated using affinity chromatography (29). The domain of UvrB involved in this interaction could be defined as lying in the region encompassing residues 547-673. It is possible that this weak interaction between UvrB and UvrC is responsible for the residual incision that occurs when the stable interaction domain is absent. It is also conceivable that the UvrBC interaction that was observed in solution is not involved in 3'- but in 5'-incision. It was shown (16) that the UvrB* protein gives rise to a normal 5'-incision using a substrate that is pre-incised at the 3'-position. In this paper, we have shown that truncated UvrB proteins that lack up to 99 C-terminal amino acids are also capable of efficient 5'-incision of a substrate that contains a nick near the 3'-incision site. Apparently, the stable UvrBC interaction that is important for 3'-incision is not needed for the 5'-incision event. The inability to detect specific UvrBC-DNA complexes with a truncated UvrB protein in the presence of a nicked substrate in a band shift assay suggests that the UvrBC interaction involved in 5'-incision is very weak. Therefore, the observed UvrBC interaction in solution might represent the interaction that induces 5'-incision. If indeed the region from residues 547 to 673 represents a functional binding domain for 3'- and/or 5'-incision, this region can be narrowed down to residues 547-574 regarding the incision efficiency of the UvrB574 mutant.

The truncated UvrB mutants used in this study not only allow possible UvrC binding domain(s) to be assigned but they also yield information concerning the UvrA binding domain. It was shown that the UvrB* protein can still bind UvrA (5). Here, we show that the incision efficiencies in the presence of UvrB574 and UvrB* are the same, implying that UvrB574 normally binds UvrA. This allows the UvrA binding domain to be assigned to the N-terminal 574 amino acids of the UvrB protein. This is in agreement with the earlier observation that the UvrB574 mutant has a dominant phenotype in vivo (4).

It has been postulated that the C-terminal domain of UvrB is directly involved in catalysis of the 3'-incision (16). This suggestion derived from the observations that the UvrB* protein and a UvrB mutant carrying a E640A substitution are both unable to incise the DNA at the 3'-position. In this paper, we show that UvrB* has a residual 3'-incision activity (1-2%) on a cis-Pt-containing substrate, and we present evidence that the impaired incision efficiency is the result of impaired UvrC binding. Furthermore, we found that introduction of the E640A substitution in UvrB resulted in a protein with identical properties to those of the wild type protein in vivo as well as in vitro. It is not clear why our E640A mutant is different from the one isolated by Lin et al. (16). However, the fact that the mutant protein isolated by these authors is proficient in preincision complex formation and deficient in 3'-incision does not necessarily mean that it is affected in catalytic activity since, like UvrB*, it could also be deficient in UvrC binding. In any case, since UvrB574 is still capable of promoting incision, it can be concluded that the C-terminal 99 amino acids of UvrB do not directly participate in the catalysis of phosphodiester bond cleavage. Although the active site for 3'-incision might be located in the remainder of the UvrB molecule, it seems equally possible that it is entirely located in UvrC or that it is formed at the interface between the UvrB and UvrC proteins. Such an interface could be formed by the postulated additional weak interaction between UvrB and UvrC, which might be responsible for the residual 3'-incision with the truncated UvrB proteins.

Our model for the UvrABC incision reaction can be summarized as follows. Loading of UvrB onto the site of DNA damage results in local unwinding of the DNA and a conformational change in the protein that exposes the UvrC binding domain. This domain interacts with the homologous region of UvrC through formation of a coiled-coil, which together with possible UvrC-DNA interactions leads to a very stable complex. In this complex the active site for 3'-incision, formed by UvrB, UvrC, or both proteins, needs to be positioned, which, in the case of a cis-Pt lesion is a relatively slow process. Flexibility in the UvrBC-interacting domain might facilitate this positioning. Introduction of a nick at the 3'-site induces a conformational change in the UvrB-DNA complex, which subsequently leads to a very fast 5'-incision. Since the exact position of the 3'-nick does not seem important, the conformational change induced by the nick might be the mere release of stress in the DNA molecule, possibly leading to further unwinding and the generation of a double stranded-single stranded DNA junction near the site of the 5'-incision. Such a junction might be the determinant for 5'-incision by UvrC. A similar mechanism has been proposed for the 5'-incision by the Rad1-Rad10 complex in the nucleotide excision repair of Saccharomyces cerevisiae (30, 31) and for the 3'-incision by XP-C in human excision repair (32). The 5'-incision might be catalyzed by the same UvrC that is already bound to the complex and that has induced 3'-incision. Alternatively, a second UvrC molecule might be responsible for the cleavage at the 5'-site. The fact that the stable UvrBC interaction that is involved in 3'-incision does not seem to contribute much to the efficiency of 5'-incision argues for the latter.

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Addendum—After submission of this manuscript, D. S. Hsu and A. Sancar analyzed the uvrB gene of the E640A mutant that was used in the studies of Lin et al. (16). The mutant turned out to contain a frameshift resulting in a truncated UvrB protein that lacks the C-terminal 33 amino acids, which explains that it shows the same properties with respect to 3'- and 5'-incision as the truncated UvrB proteins used in our study.

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The C-terminal Region of the UvrB Protein of *Escherichia coli* Contains an Important Determinant for UvrC Binding to the Preincision Complex but Not the Catalytic Site for 3'-Incision

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