Molecular structure of the *uvrC* gene of *Escherichia coli*: identification of DNA sequences required for transcription of the *uvrC* gene

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ABSTRACT

We have carried out experiments to identify the regulatory regions of the *uvrC* gene of *Escherichia coli*. A *uvrC*⁺ plasmid, pUV7, containing the intact transcriptional unit for the *uvrC* gene, was used to subclone either the structural gene or combinations of the structural gene and 5'-flanking sequences. The plasmids so constructed were tested for ability to restore UV-resistant phenotype to *uvrC*⁻ cells as an indication of expression of the *uvrC* gene. The chromosomal DNA in plasmid pUV7 was probed for strong binding with *E. coli* RNA polymerase in an attempt to identify a restriction fragment which bears the regulatory sequences for the *uvrC* transcriptional unit. The results indicate that DNA sequences at least 0.9 Kb upstream from the structural gene, but not the 5'-proximal sequences, regulate expression of the *uvrC* gene. Analysis of protein synthesis encoded by plasmid pUV7 and its derivatives suggest that there may be another gene that lies between the promoter and the *uvrC* gene and codes for a 27,000-Mr protein. The relation of this gene to *uvrC* function is not clear.

INTRODUCTION

The genetics and biochemistry of *uvr* mutants of *Escherichia coli* suggest that the enzymes encoded by the *uvrA*, *uvrB* and *uvrC* genes are required for the excision repair of UV-induced damage in DNA (1,2). The excision repair mechanism involves multienzymatic events including strand-scission at the site of damage, excision of damage, repair synthesis, and ligation. Recent studies on the initial step of strand-scission have raised the possibility that the *uvrA*, *uvrB* and *uvrC* gene products may function in the form of a complex (3, 4). The individual components of this complex may also be involved in the events following strand-scission, notably the *uvrC* component as suggested by our studies (5). Recent evidence indicates that the *uvrA* and *uvrB* genes may be induced by the agents which damage DNA (6,7,8,9). These studies also suggest that regulation of the *uvrA* and *uvrB* genes is modulated by the *recA-lexA* control system. Whether the *uvrC* gene follows the same mechanism of induction and expression as do the *uvrA* and *uvrB* genes is unknown.

We (10) and others (11, 12, 13) have recently cloned the uvrC gene using the multicopy plasmid pBR322. Analysis of our uvrC⁺ hybrid plasmids (10) shows that the uvrC structural gene is located on a 1.9 Kb DNA fragment, coding for a protein of approximately 66,000 monomer molecular weight. It is not clear, however, whether the 1.9 Kb DNA fragment also contains the uvrC gene promoter, since this fragment was fused to the tetracycline gene (tet) promoter of the vector.

We have carried out a structural analysis of the uvrC gene region to determine which DNA sequences are required for transcription of the uvrC gene. In this work we have analyzed DNA sequences upstream from the probable start point of the gene and have looked for genetic complementation of uvrC⁻ cells by recombinant plasmids containing these sequences in addition to the structural gene. A strong binding site for RNA polymerase is identified far upstream from the structural gene. The data suggest that the uvrC gene is part of a cluster of genes.

MATERIALS AND METHODS

Bacterial strains and plasmids:

The host strains used in these experiments were AB1884 uvrC⁻ (1), AB1157 wild-type (1) and CSR603 uvrA⁻, recA1, phr-1 (14). CSR603 was used for the identification of plasmid encoded proteins in maxi-cells. The uvrC⁺ plasmid pUV7 has been previously described (10).

Growth conditions and bacterial transformation:

Bacterial growth conditions and CaCl₂ mediated transformation were as described (10). In brief, cultures were either grown in L-broth or K9 medium (15), containing ampicillin (30 mg/liter), tetracycline (25 mg/liter) or streptomycin (100 mg/liter) respectively, when required. Initial selection for plasmid-containing transformants was done by growth on L-broth agar plates with the appropriate antibiotic. Complementation screening for hybrid-plasmid-containing transformants was done by streaking on L-broth agar plates followed by UV-irradiation (20-30 J/m²). For quantitative UV-survival curves, cells transformed with hybrid plasmids were grown to an O.D.₆₀₀ of approximately 1 in M9 medium (16). The cells were irradiated at different UV doses at a cell density of 1 x 10⁷ cells/ml, and portions were diluted. These dilutions were grown on LB agar plates for 24 hours and surviving colonies counted.

All cultures were stored at -80°C in 40% glycerol and checked for the presence of plasmids before use.

Purification of DNA and restriction enzyme analysis:

A rapid method for plasmid DNA preparation was used to determine relative DNA sizes in purified single colonies. Circular plasmid DNA was isolated either by microscale technique (17) or by lysozyme/Triton X-100 lysis and CsCl/ethidium bromide gradient centrifugation (18).

We followed conditions for restriction enzyme digestions as suggested by suppliers. λ -HindIII DNA fragments and ϕ X-174 HaeIII fragments were used as standard markers.

Analysis of polypeptides encoded by plasmids in maxi-cells:

Strain CSR603 was transformed with plasmids and maxi-cells were prepared as described (19) with some modifications. We used 20 J/m² UV-dose to irradiate bacteria, and after incubation for 1 hour at 37°C, added D-cycloserine (100 μ g/ml) and again at 5 hours. Proteins were labeled with 10 μ Ci/ml of ³⁵S-methionine. Preparation of cell extract and fluorography were performed as described (10). Plasmid-encoded proteins were analyzed on 11% polyacrylamide/0.1% sodium dodecyl sulfate gels.

Reaction conditions for E. coli RNA polymerase binding with DNA fragments:

RNA polymerase-DNA complexes were assayed according to procedures described by Jones and Reznikoff (20) and Strauss *et al.* (21). Plasmid pUV7 (4 μ g) digested with BamHI, HindIII and BglII was mixed with 500 μ l of RNA polymerase binding buffer (20 mM Tris-HCl pH 7.1, 10 mM MgCl₂, 0.1 mM DTT, 0.1 mM EDTA, 0.1 M NaCl, and 50 μ g/ml BSA) and incubated with various amounts of *E. coli* RNA polymerase holoenzyme for 30 minutes at 37°C. Weak RNA polymerase-DNA complexes were further chased by continued incubation for 10 minutes with heparin (200 μ g). The reaction samples were passed through nitrocellulose filters at a rate of 5 ml/min and elution of DNA was conducted as described (21). The DNA was precipitated with 2 volumes of isopropyl alcohol at -20°C overnight and dissolved in 20 μ l of water. The products were separated on 7.5% polyacrylamide gel and visualized by ethidium bromide staining.

Materials:

Phage T₄ ligase, *E. coli* RNA polymerase holoenzyme, and restriction endonucleases were purchased from Bethesda Research Laboratories (Rockville, MD), New England Biolabs, and Boehringer Biochemicals. Growth media were purchased from Difco. Ampicillin, tetracycline, D-cycloserine, chloramphenicol, heparin, and dimethylsulfoxide, were from Sigma. Ethidium bromide, agarose, and acrylamide were obtained from Bio-Rad. 2,5-Diphenylosazole for fluorography was purchased from New England Nuclear.

RESULTS

a. Location of E. coli regulatory sequences for *uvrC* gene transcription:

We have recently reported construction of hybrid plasmids that confer UV-resistance to *uvrC*⁻ cells (10). The analysis of these plasmids indicated that we had isolated two types of plasmids: (1) plasmids containing the structural gene, but not the regulatory regions; (2) plasmids containing both of these elements. Plasmids without the regulatory regions, but *uvrC*-complementing, always had the *tet* promoter of pBR322 fused to the structural gene, suggesting the probable expression of the *uvrC* gene under the control of that vector promoter.

To locate the *E. coli* regulatory sequences responsible for expression of the *uvrC* gene, we have subcloned DNA fragments containing both the structural gene and/or 5'-flanking sequences. The construction of new plasmids began with the *uvrC*⁺ hybrid plasmid, pUV7, which contained a 4.3 Kb *Bam*HI-*Bgl*II uninterrupted DNA fragment inserted at the *Bam*HI site of pBR322. A physical map of pUV7 is shown in Fig. 1. We have made use of the *Bam*HI, *Bgl*II and *Hind*III restriction sites on pUV7 for subcloning. The 4.3 Kb DNA fragment encompasses the *uvrC* structural gene (1.9 Kb, *Bgl*II-*Bam*HI/*Bgl*II) and the 2.4 Kb DNA fragment flanking the 5'-terminus of the gene. The direction of *uvrC* gene transcription has been defined (10, 12, 13), establishing the probable start point for mRNA biosynthesis in the vicinity of the *Bgl*II restriction site on pUV7.

Fig. 1 shows the cloning strategy used to construct plasmids containing different fragment(s) of pUV7. The critical steps were (a) deletion of the *Bam*HI-*Bgl*II fragment (2.4 Kb) and sealing of the remaining DNA fragment with T₄ DNA ligase; (b) isolation of the *Bam*HI-*Bgl*II fragment and its insertion at the *Bam*HI site of pBR322; and, (c) isolation of the *Hind*III-*Hind*III fragment (3 Kb) and its insertion at the *Hind*III site of pBR322. The DNA from these reactions was used to transform strain AB1884 (*uvrC*⁻). Ap^R colonies were streak-tested for UV-resistant phenotype (UV^R). No UV^R transformants were observed. The transformants from each reaction were purified and screened for relative plasmid DNA size as described in Materials and Methods. We obtained three types of plasmids in addition to pBR322: pUV704, pUV11 and pUV301 (Fig. 1). The structure of these plasmids was confirmed by restriction enzyme analysis with respect to restriction sites present on pUV7.

Fig. 2 shows the quantitative UV survival of AB1884 when transformed with either pUV7, pUV11, pUV301, pUV704 or pBR322. For control, a wild-type

Figure 1: Schematic representation of *uvrC*⁺ plasmid pUV7 and its derivatives, pUV704, pUV11 and pUV301. Double lines represent cloning vector pBR322 regions and single lines represent *E. coli* chromosomal DNA. Tc^R and Ap^R are for the tetracycline resistance gene promoter and ampicillin-resistance gene, respectively, in pBR322. Plasmid pUV7 consists of vector pBR322 and chromosomal DNA (4.3 Kb) harboring the *uvrC* gene (1.9 Kb) and 5'-flanking sequences (2.4 Kb). Plasmid pUV7 was subjected to BamHI, BglII and HindIII digestions, respectively. The resulting DNA fragments were separated on 1% agarose gel. BamHI-BglII (2.4 Kb) and HindIII-HindIII (3 Kb) DNA fragments isolated by electrophoretic elution were ligated at the BamHI and HindIII sites of pBR322 with T₄ ligase in separate reactions (b and c above). In another reaction, pUV7 was digested with BamHI and BglII, and the reaction mixture was directly subjected to DNA ligation (a above). The DNA ligation mixture from these three reactions was used to transform AB1884 (*uvrC*⁻) as described in Materials and Methods. Transformants were selected on ampicillin-L broth agar plates and streak-tested for UV^R phenotype. The first two reactions gave rise to only UV^S colonies. In the last reaction we observed both UV^R and UV^S colonies. Plasmid sizes were measured by microcolony assay. UV^R colonies contained the original plasmid, pUV7, while UV^S colonies contained plasmid pUV11 (b above), plasmid pUV301, (c above), and pUV704 (a above). The dotted line represents the direction of transcription for respective genes on plasmids.

strain AB1157 transformed with pBR322 was also included in the UV test, giving a typical UV survival. It is clear from Fig. 2 that a transformant containing pUV7 does not differ much from a wild-type strain. However, plas-

Figure 2: Gene complementation in *uvrC*⁻ cells by hybrid plasmids. Wild-type and *uvrC*⁻ cells were grown to 8×10^8 cells/ml in M9 medium, centrifuged and washed with M9 buffer. Cells were resuspended in M9 buffer and subjected to various UV-doses as described in Materials and Methods. No survivors were observed at 10 J/m² for the strains containing noncomplementing plasmids. The symbols represent: o, AB1157 wild-type/pBR322; Δ, AB1884 *uvrC*⁻/pUV7; □, AB1884/pUV11; ●, AB1884/pUV704; Δ, AB1884/pUV301; ■, AB1884/pBR322.

mids pUV11, pUV301 or pUV704 do not confer UV resistance even at low UV doses. The failure of pUV704 to complement is striking since we have reported that the 1.9 kb fragment alone can complement a *uvrC* defect (10). This suggests that the orientation of *uvrC* with regard to the *tet* promoter is incorrect for its expression in pUV704. The inability of plasmid pUV11 to restore the UV-resistant phenotype to AB1884 can be attributed to the absence of the *uvrC* structural gene (as shown in Fig. 1) and to the absence of factors which can independently complement the defect in AB1884. In the case of plasmid pUV301 the sequences upstream from those cloned may be involved in positive regulation of expression of the *uvrC* gene, or the functional promoter may lie within these upstream sequences.

b. The tet promoter permits expression of the *uvrC* structural gene

We have constructed a *uvrC*⁺ plasmid, pUV18, that contains the *Bgl*II-*Bgl*II fragment (1.9 Kb) which was isolated from a larger *uvrC*⁺ plasmid, pPG1 (22), and inserted into the *Bam*HI site of pBR322. The resulting plas-

mid, pUV18, was selected by screening for complementation of the defect in uvrC⁻ cells. From this same experiment we also isolated uvrC⁻ cells carrying plasmids of similar size.

Plasmid pUV18 (Fig. 3) carries the uvrC structural gene fused to the tet promoter of vector pBR322 and complements the defect in uvrC⁻ cells. However, plasmid pUV704 (Fig. 1) having the same structural features does not complement the uvrC defect, suggesting lack of expression of the gene. We suspected that the orientation of the structural gene in pUV704 and pUV18 was reversed and that the uvrC non-complementing or complementing nature of these plasmids was caused by the placement of the tet promoter at 3' or 5'.

Figure 3: Location of the tet gene promoter with respect to the uvrC structural gene in pUV18 (uvrC⁺) and pUV704 (uvrC⁻). (A) Plasmids are represented in linear forms emphasizing the uvrC gene (1.9 Kb). Tc^P represents the tet promoter. (B) Samples of DNA (2 μ g) were digested with SphI (6 units) for 1 hr at 37°C. The reactions were stopped by heating at 65°C for 5 min. The digestion products were separated by electrophoresis in 1% agarose gel and stained with ethidium bromide. Lanes: a, λ DNA HindIII + ϕ X174 DNA HaeIII; b, pUV18 (undigested); c, pUV18 + SphI; d, pUV704 (undigested); e, pUV704 + SphI. Sizes of standard markers in lane a are in Kilo-base pairs.

terminus of the gene, respectively.

Fig. 3(A) shows a physical map of the region encompassing the uvrC gene in pUV18 and pUV704. We have made use of a single restriction site for SphI located in the structural gene 0.4 Kb downstream from the BglII site on pUV7, the probable beginning of the gene (Fig. 1), to determine structural gene orientation. The vector pBR322 also contains a unique SphI site at 0.566 Kb from the EcoRI site in the tet gene region (23). The SphI digestion of pUV704 and pUV18 should produce asymmetric DNA fragments depending on orientation of the uvrC structural gene. The SphI digestion pattern is shown in Fig. 3(B). Lanes b and d represent undigested circular pUV18 and pUV704 respectively, which are of the same size. Lanes c and e show digestion patterns of pUV18 and pUV704, respectively. Lane a shows λ HindIII and ϕ X174 HaeIII DNA size markers. The sizes of DNA fragments generated by SphI agree with the sizes predicted in Fig. 3A, supporting the conclusion that the tet promoter is fused to the uvrC structural gene at the 5'-terminus in the case of pUV18 and at the 3'-terminus in the case of pUV704, respectively. The same conclusion was reached from partial restriction enzyme digestion of reference-end labeled HindIII-SalI fragments generated from pUV18 and pUV704 (data not shown). In addition the noncomplementing plasmids isolated during the construction of pUV18 were found to have the same structure as pUV704.

c. Analysis of polypeptides synthesized by pUV7 and its derivatives:

The maxi-cell method was used to study expression of polypeptides encoded by the uvrC complementing or non-complementing plasmids to correlate the inability of plasmids pUV11, pUV301 and pUV704 to complement the uvrC defect with failure in the synthesis of the uvrC gene product by these plasmids. Our previous results (10) have demonstrated that uvrC complementing plasmids, like pUV18, code for a polypeptide of approximately 66,000 monomer molecular weight. Fig. 4 shows the autoradiogram of a polyacrylamide/sodium dodecyl sulfate gel. Lane a represents host strain CSR603 by itself, showing no incorporation of ^{35}S -methionine into any major polypeptide. Lane g shows labeling of polypeptides stimulated by pBR322. The expression of a 66,000-Mr protein by pUV18 confirms our previous results (lane f).

When the proteins synthesized by plasmids pUV11 (lane b), pUV301 (lane d) or pUV704 (lane e) are compared to lane f, it is clear that a 66,000-Mr protein band is absent. In several lanes, particularly b and d, we observe a few extra bands besides the β -lactamase band (30,000-Mr), most likely representing host specific proteins since these bands appear in lane a also,

Figure 4: Analysis of plasmid-encoded proteins in maxi-cells. CSR603 was transformed with various plasmids and processed for maxi-cell preparation as described in Materials and Methods. A 6 ml culture was grown for each clone (plasmid), irradiated, labeled with ^{35}S -methionine (10 $\mu\text{Ci/ml}$), and prepared for SDS/polyacrylamide gel electrophoresis. A 50 μg protein sample for each was loaded onto a 11% polyacrylamide/0.1% SDS gel. The labeled protein bands were detected by fluorography. Lane a, CSR603; lane b, CSR603/pUV11; lane c, CSR603/pUV7; lane d, CSR603/pUV301; lane e, CSR603/pUV704; lane f, CSR603/pUV18; lane g, CSR603/pBR322; lane h, CSR603/pUV7 (150 μg protein). Molecular weights of standard protein markers are: phosphorylase b 92,000; bovine serum albumin, 67,000; ovalbumin, 45,000; carbonic anhydrase, 31,000; soybean trypsin inhibitor 21,000. The unlabeled arrow indicates the band for 27,000-Mr protein.

on overexposure of the x-ray film. However, plasmid pUV11 (lane b) codes for an additional protein of 27,000-Mr, which does not appear in any other lane even on over-exposure.

It is interesting to note that there is no apparent stimulation of ^{35}S -methionine incorporation by pUV7 (lane c), although this plasmid confers maximum UV-resistance to $uvrC^-$ cells (Fig. 2). Even the band for β -lactamase protein is very weak, which is not normal, since there is usually a heavy ^{35}S -methionine incorporation because of the high number of methionine residues present in β -lactamase protein (24). Applying 2- to 3-fold more total protein or exposing the film longer makes it possible to detect β -lactamase (30,000-Mr) and 27,000-Mr protein bands (lane h), but not the $uvrC$ protein band (66,000-Mr).

d. Analysis of RNA polymerase binding sites on pUV7:

Selective binding of RNA polymerase holoenzyme to DNA fragments has been shown to be an effective approach to determine promoter and operator sequences. Preferential and strong binding to such sequences is resistant to heparin, and complexes of RNA polymerase-promoter sequences can be retained on nitrocellulose filters (21). We have used this approach to iden-

tify regulatory regions for the *uvrC* gene.

Plasmid pUV7 was digested with *Bam*HI, *Hind*III and *Bgl*II and the resulting fragments were assayed for heparin-resistant complexes with various amounts of RNA polymerase as described in Materials and Methods. Figure 5 shows the restriction fragments which exhibited binding to RNA polymerase. Digestion of pUV7 with *Bam*HI, *Hind*III and *Bgl*II produced four fragments including three from the insert (lane b): pBR322 DNA, 4.05 Kb; insert DNA, 1.5 Kb (*Bam*HI-*Hind*III), 0.9 Kb (*Hind*III-*Bgl*II), and 2.2 Kb (*Bgl*II-*Hind*III). The 2.2 Kb fragment contains part of the *tet* promoter. Without RNA polymerase, no DNA fragment is retained on the nitrocellulose filter (lane c). As expected, the 4.05 Kb fragment containing the promoters pertinent to pBR322 showed strongest binding even at the lowest RNA polymerase/pUV7 molar ratio

Figure 5: Filter binding assay for RNA polymerase-DNA complexes. (A) Plasmid pUV7 is shown in linear form emphasizing the *uvrC* structural gene and its upstream region. Promoter-X gene-structural gene organization is assumed on the basis of results presented in Figs. 1, 2 and 4. X gene represents the gene for 27,000-Mr protein. (B) Plasmid pUV7 DNA (5 μ g) was digested with *Bam*HI, *Bgl*II and *Hind*III. The resulting fragments were mixed with various amounts of RNA polymerase, passed through nitrocellulose filters and then eluted from the filters as described in Materials and Methods. The eluted fragments were separated on 7.5% acrylamide gel and stained with ethidium bromide. Lanes: a, ϕ X174 DNA *Hae*III markers; b, pUV7 DNA + *Bam*HI + *Hind*III + *Bgl*II loaded directly; c, lane b products + no RNA polymerase; d, lane b products + 1:1 RNA polymerase; e, lane b products + 2:1 RNA polymerase; f, lane b products + 4:1 RNA polymerase; g, lane b products + 8:1 RNA polymerase; h, λ DNA *Hind*III markers.

(lane d). In addition to the 4.05 Kb fragment, only the 1.5 Kb fragment of insert DNA showed strong RNA polymerase binding site(s) at all RNA polymerase/pUV7 molar ratios used in this study. The 0.9 Kb fragment exhibited weak binding at 8:1 RNA polymerase/pUV7 molar ratio (lane g) implying that a weak promoter(s) may be present in this region. These results agree with the results presented in Figs. 1 and 2 indicating that the regulatory sequences for the uvrC gene transcription are separated at least by 0.9 Kb from the structural gene.

DISCUSSION

The experiments presented here enable us to study the structural organization of the uvrC gene, including its transcriptional regulatory sequences. Our previous results suggested that the uvrC structural gene fused to the tet promoter of pBR322 was a transcriptionally active unit and complemented the defect(s) in uvrC⁻ cells (10); this observation is further supported by the properties of pUV18. We now show that DNA sequences at least 0.9 Kb upstream from the uvrC structural gene are responsible for its expression. For example, derivatives of uvrC⁺ plasmid pUV7 containing only the structural gene (pUV704) failed to restore the UV^R phenotype of uvrC⁻ cells (Fig. 1) and failed to stimulate the synthesis of the uvrC protein *in vivo* (Fig. 4). Furthermore, plasmids containing the 0.9 Kb extra sequences and lacking upstream regions which exhibited preference for RNA polymerase binding (Fig. 5) also failed to complement uvrC⁻ cells (Fig. 2) and did not stimulate synthesis of the uvrC protein *in vivo* (Fig. 4). Our results cannot rule out the presence of internal promoters within the 0.9 Kb fragment, not normally stimulating efficient transcription. The presence of such promoters is suggested by weak binding of the 0.9 Kb fragment to RNA polymerase.

The inability to detect the uvrC protein in maxicells containing the uvrC⁺ plasmid pUV7 (Fig. 4) may be due to the overall suppression of protein synthesis by this plasmid. It should be pointed out that normal cellular levels of uvrC protein sufficient for repairing UV-induced damage are lower than the sensitivity of the method used in Fig. 4. .

Synthesis of a 27,000-Mr protein is stimulated by pUV7. This 27,000-Mr protein is also encoded by plasmid pUV11 which harbors a 2.4 Kb DNA fragment upstream from the uvrC gene on pUV7. Assuming that the uvrC protein is synthesized by pUV7, though at low levels, the synthesis of the 27,000-Mr protein by both pUV7 and pUV11 raises the question of whether the uvrC locus is

multicistronic. Although our results do not permit the exact location of coding sequences for the 27,000-Mr protein on pUV11, it is possible to consider that genes for the uvrC and 27,000-Mr proteins lie in a co-transcribed unit, since pUV301 does not code for either the uvrC protein or the 27,000-Mr protein (Fig. 4). Plasmid pUV11 fails to complement the defects in uvrC⁻ cells, suggesting that the upstream region from the structural gene does not carry sequences that can independently perform the role of the uvrC gene. This region may still be related to the uvrC gene if it codes for regulatory factors. The analysis of a variety of regulatory units in *E. coli* has shown that many promoters require protein factors to control transcription (25). Expression of the uvrC protein may be limited by the levels of 27,000-Mr protein. In the case of plasmid pUV18 that codes for the uvrC protein, the levels of 27,000-Mr protein would be derived from the host or since expression is under tet promoter control, the 27,000 Mr protein may not be involved. It is clear that production of the 66,000-Mr protein alone is adequate to complement the defect in the uvrC mutants we have investigated.

Two other repair genes, uvrA and uvrB, do not show such a complicated structure. In these cases, the promoter and the structural gene are proximal (26). Recently, the uvrB gene has been shown to contain two promoters, and both the promoters seem to be functional in vivo (27). However, the uvrB gene does not seem to belong to an operon unit. The biological significance of the complex control of the uvrC gene still remains to be seen.

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