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Regulation of the *uvrC* gene of *Escherichia coli* K12: localization and characterization of a damage-inducible promoter

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Operon fusion and S1 nuclease mapping have been employed to locate a putative *uvrC* promoter, which is situated ~200 bp ahead of the *uvrC* structural gene. The promoter sustains transcription towards the *uvrC* coding sequence and is inducible by DNA damaging agents. The inducibility is dependent on the *Escherichia coli* LexA and RecA functions. Examination of the DNA sequence in the promoter region reveals the presence of a sequence similar to the consensus of a SOS box. In contrast to the related *uvrA* and *uvrB* genes, *uvrC* gene expression is characterized by a delayed onset of induction after DNA damaging treatment. Furthermore, no induction is observed with nalidixic acid.

Key words: mitomycin C/SOS induction/S1-mapping/*uvrC.galK* fusion

Introduction

The *Escherichia coli, uvrA, uvrB* and *uvrC* genes are jointly involved in the recognition and removal of a variety of damages from cellular DNA (Seeberg, 1981; Hanawalt *et al.*, 1979). Considerable insight has been obtained into the organization of these genes by molecular cloning (Sancar *et al.*, 1981a, 1981b, 1981c; Van den Berg *et al.*, 1981; Van Sluis and Brandsma, 1981), which also greatly facilitated the isolation of the respective gene products (Seeberg *et al.*, 1983; Sancar *et al.*, 1981c). The UvrA, UvrB and UvrC proteins are presently believed to catalyze a specific endonucleolytic reaction which removes a damaged oligonucleotide from irradiated DNA (Sancar and Rupp, 1983; Seeberg *et al.*, 1983).

Subsequently, studies have been carried out to establish the mode of regulation of the *uvr* genes. Using insertions of Mud::(Ap,*lac*) into the bacterial chromosome, Kenyon and Walker (1980) identified a number of *d*amage-*in*ducible *(din)* genes, all involved in either DNA replication, repair or mutagenesis (for a review, see Little and Mount, 1982). Both the *uvrA* and *uvrB* genes are inducible by several DNA damaging agents (Kenyon and Walker, 1981; Fogliano and Schendel, 1981) and in the regulatory regions of the *uvrA* and *uvrB* genes, a LexA repressor binding site (SOS box) has been identified (Van den Berg *et al.*, 1981; Sancar *et al.*, 1982a, 1982b). Since insertions of Mud::(Ap,*lac*) in the *uvrC* gene have not been found (Kenyon and Walker (1980), we used another approach, namely the fusion of the *uvrC* regulatory elements to the structural *galK* gene. In the case of *uvrA* this method was shown to give results comparable to those obtained with the Mud::(Ap,*lac*) insertions (Backendorf *et al.*, 1983). Consequently we employed *uvrC.galK* fusion plasmids to investigate the regulation of the *uvrC* gene.

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In previous work the major part of the *uvrC* structural gene has been assigned to a 1.9-kb *Bgl*II fragment (Sancar *et al.*, 1981c; Van Sluis and Brandsma, 1981; Yoakum and Grossman, 1981) (see Figure 1). The direction of transcription of the *uvrC* gene was established by transposon insertions and by determining the orientation of the 1.9-kb *Bgl*II fragment relative to external promoters used to express the *uvrC* gene. From these data (Van Sluis and Dubbeld, 1983) it was concluded that the sequences involved in expression of the *uvrC* gene are contained within a 3.4-kb *Pst*I fragment on which the UvrC gene region was originally cloned.

In the present study we describe the localization and characterization of the UvrC regulatory elements. The promoter is situated ~200 bp ahead of the *uvrC* structural gene and is shown to be damage-inducible.

Results

Construction of uvrC.galK fusion plasmids

*Taq*I fragments arising from the cloned UvrC region were inserted into the unique *Cla*I site of plasmid pCA95 and galactokinase-positive clones were selected. The characterization of recombinant plasmids showed that the GalK$^+$ plasmids pCA9505 and pCA9507 contained a 1300-bp and a 280-bp *Taq*I insert, respectively (see Figure 1A). The orientation could easily be determined as both inserts contained one of the two *Bgl*II sites situated on the cloned UvrC fragment (see Figure 1). The orientation of the promoter present on the 1300-bp fragment in pCA9505 was in accordance with the direction of transcription of the *uvrC* gene (Sancar *et al.*, 1981c; Van Sluis and Brandsma, 1981), whereas transcription mediated by the small 280-bp *Taq*I fragment in pCA9507 was directed away from the *uvrC* structural gene. To localize the promoter present on the 1300-bp *Taq*I fragment, we deleted the 300-bp *Eco*RI-*Pvu*II fragment from pCA9505 (Figure 1B). This deletion also removed the '-35' sequence from the pBR322 Tc^R gene (Sutcliffe, 1979) which might complicate the interpretation of expression studies with this plasmid. The derivative plasmid pCA95051 (Figure 1B) carrying 500 bp ahead of the *uvrC* structural gene, still directed the synthesis of galactokinase in GalK bacteria and was investigated in detail.

Expression of the pCA95051 uvrC.galK plasmid

To determine whether the promoter present on pCA95051 is inducible and regulated by the SOS system, we measured the intracellular level of galactokinase after treatment with mitomycin C, u.v. irradiation or nalidixic acid, which are inducers for a number of *din* genes (Little and Mount, 1982). The results given in Figure 2 show that the promoter is inducible by mitomycin C and u.v. irradiation but not by nalidixic acid (Figure 2C). The latter result is unexpected as nalidixic acid induces all other *din* genes, although this agent primarily inhibits DNA replication and does not directly cause chemical damage in DNA (Sugino *et al.*, 1977).

From the results given in Figure 2A and B it is evident that the increase of galactokinase activity in AB1157 is rather delayed both in response to mitomycin C treatment and u.v.

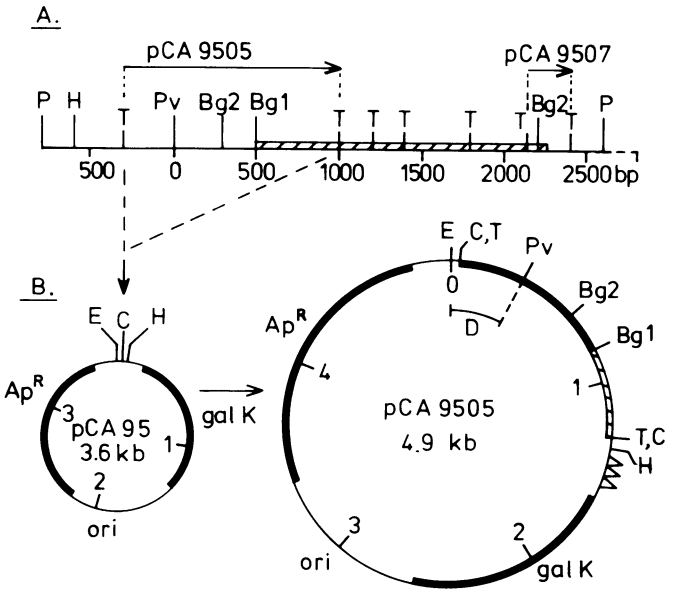

Fig. 1. Construction of *uvrC.galK* fusion plasmids. **(A)** Structure of the parental 3.4-kb *Pst*I DNA fragment carrying the functional *uvrC* gene. *Taq*I sites used for subcloning into pCA95 are indicated. Arrows mark the direction of transcription promoted by the cloned fragments. The hatched area denotes the coding sequence for the 68-kd UvrC protein. **(B)** Construction of pCA9505 by insertion of the 1.3-kb *Taq*I fragment in the *Cla*I site of vector pCA95 (for details see Materials and methods). Heavy lines represent the pBR322 derived β-lactamase gene (Ap^R) and the promoterless bacterial galactokinase gene *(galK*, preceded by translation stops (△△△). The deletion (D) of an *Eco*RI-*Pvu*II fragment yielding the derivative plasmid pCA95051, is also indicated. Restriction sites: Bg1 = *Bgl*I; Bg2 = *Bgl*II; C = *Cla*I; E = *Eco*RI; H = *Hind*III; P = *Pst*I; Pv = *Pvu*II; T = *Taq*I; C,T and T,C: hybrid *Cla*I-*Taq*I sites.

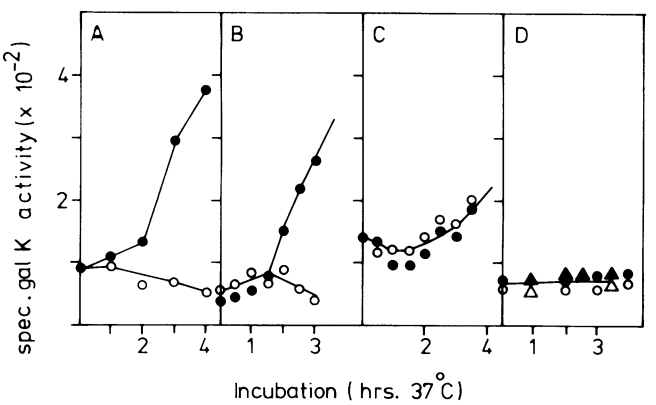

Fig. 2. Kinetics of induction of the *uvrC* promoter on plasmid pCA95051 by various agents. Exponentially growing bacteria were treated with mitomycin C, u.v. or nalidixic acid and incubated at 37°C. Samples were taken at various intervals and the galactokinase level was determined as described in Materials and methods. Open symbols: control cells; closed symbols: treated cells. **(A)** AB1157, mitomycin C (2 μg/ml); **(B)** AB1157, u.v. (60 J/m²); **(C)** AB1157, nalidixic acid (20 μg/ml); **(D)** AB2463 *recA13* (○, ●) and DM49 *lexA*3 (△, ▲), mitomycin C (2 μg/ml).

irradiation. Starting from 90 – 120 min after the inducing treatment, the galactokinase level increases 2- to 3-fold. This is late compared with the derepression of the *recA* gene, which is fully induced by 60 min after treatment (McPartland *et al.*, 1980; Salles and Paoletti, 1983). This phenomenon cannot be ascribed to growth delay as the dose of the various agents employed does not result in a marked retardation of growth.

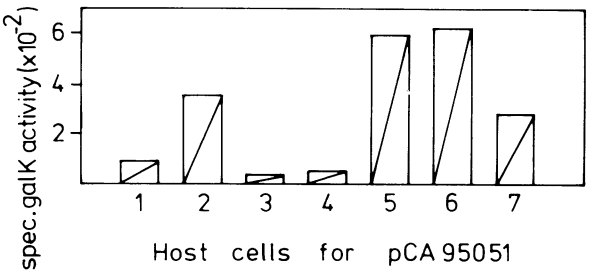

Fig. 3. Galactokinase basal steady state levels in *E. coli* K12 repair-proficient and mutant strains harboring pCA95051. Cultures were grown to mid-exponential phase and galactokinase was determined as described in Materials and methods. **1**: AB1157; **2**: *ibid.* induced with mitomycin C (2 μg/ml) for 3 h; **3**: AB2463 *recA13*; **4**: DM49 *lexA*3; **5**: DM1187 LexA (Def) *spr*-55; **6**: DM1187 harboring pACYC184 vector; **7**: DM1187 harboring pJA33 *lexA*$^+$ (J.A.Bransma, unpublished data).

Since the proteolytic inactivation of LexA repressor is the molecular basis of induction of all *din* genes investigated so far (Little and Mount, 1982), we investigated the influence of RecA and LexA mutations on the induction of the putative *uvrC* promoter. Results given in Figure 2D clearly show that both in RecA13 and LexA3 bacteria no induction of the promoter on pCA95051 by treatment with mitomycin C is observed. Hence host-cell mutations, which affect the damage-inducible cleavage of the LexA repressor, prevent induction of the *uvrC* promoter indicating that, in this respect also, the *uvrC* gene should be classified as a *din* gene.

This assumption was further validated by the experiments depicted in Figure 3. Here we determined the basal expression of the *uvrC* promoter on pCA95051 in various genetic backgrounds. The results show that the *recA* and *lexA* mutations decrease the galactokinase level, in accordance with the increased level of LexA repressor (Little and Mount, 1982). Using a Spr [LexA(Def)] strain we observed a drastic increase in *galK* expression even higher than in induced LexA$^+$ bacteria (see Figure 3.2 and 3.5), which illustrates that the expression of the *uvrC* promoter is inversely related to the intracellular amount of active LexA repressor.

Introduction of a compatible plasmid, pJA33, harboring a functional *lexA* gene (J.A.Brandsma, unpublished data), decreases the level of galactokinase, whereas the pACYC184 vector has no effect. These results confirm the involvement of the LexA repressor in the regulation of the *uvrC* promoter present on pCA95051. As shown in Figure 3.7 the presence of pJA33 in Spr cells does not decrease the galactokinase activity to the wild-type basal level. Possibly the deficient LexA protein in Spr cells interferes with an optimal activity of the plasmid-encoded LexA repressor.

Localization of the inducible promoter

In Figure 4, a detailed restriction map of the *Pvu*II-*Taq*I fragment cloned in pCA95051 is presented. To localize the inducible promoter on this fragment, S1 nuclease mapping was carried out using a series of terminally labelled DNA fragments as hybridization probes. Preliminary experiments with a 600-bp *Hae*III fragment (Figure 4, probe 1) revealed that the probe was fully resistant to S1 nuclease (results not shown). This result indicates that the *uvrC* promoter must be located ahead of the *Hae*III site. Consequently, another experiment was carried out using a 258-bp *Hinf* fragment (Figure 4, probe 2). Here we observed the protection of a 110 – 120 bp fragment from S1 nuclease degradation, indicating that the transcriptional start point is located ~30 bp

ahead of the *Bgl*II site at position 290 (Figure 4).

A precise mapping of *uvrC* transcripts from plasmid pCA95051 was performed using a 330-bp *Hpa*II-*Nco*I fragment (Figure 4, probe 3) uniquely labeled at its *Nco*I terminus. S1-resistant DNA fragments were electrophoresed in parallel with Maxam and Gilbert sequencing ladders of the same *Hpa*II-*Nco*I fragment (Figure 5). From the results presented, we conclude that *uvrC* transcription starts at positions A261, C263 and A264 in the DNA sequence displayed in Figure 6.

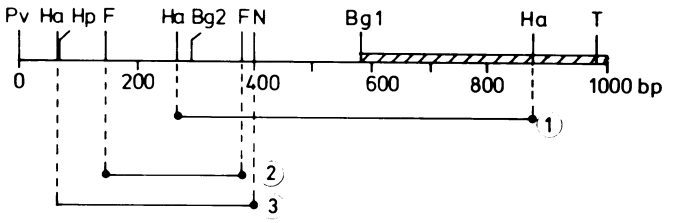

Fig. 4. Strategy of S1 nuclease mapping of *in vivo* transcripts synthesized from the *uvrC* promoter on pCA95051. Relevant restriction sites on the 950-bp *Pvu*II-*Taq*I fragment expressing the *galK* gene on pCA95051 are indicated. Base pair coordinates correspond to the numbering in Figure 1 and the sequence in Figure 6. 5′-^{32}P-labelled DNA probes (1 – 3) are represented by horizontal lines. Only probe 3 was uniquely labeled at the *Nco*I site. For further details see Figure 1 and Materials and methods. Restriction sites: Ha = *Hae*III; F = *Hin*fI; Hp = *Hpa*II; N = *Nco*I; and Figure 1.

Identical transcripts were observed from the chromosomal *uvrC* gene, when RNA isolated from a plasmid-free strain DM1187 (Spr) was analysed (Figure 5, lane 1). Whether the partial protection of the entire *Hpa*II-*Nco*I probe, in this case, has to be ascribed to an additional chromosomal *uvrC* promoter situated upstream from the inducible promoter, is not yet clear. The inducibility of the *uvrC* promoter, as shown by the *galK* fusion experiment in Figure 2 can also be visualized by the S1 mapping technique (Figure 5, lanes 10 – 12). Here also, a major increase in RNA synthesis is only observed 120 min after treatment.

Examination of the 5′-flanking region revealed the presence of promoter consensus sequences (Rosenberg and

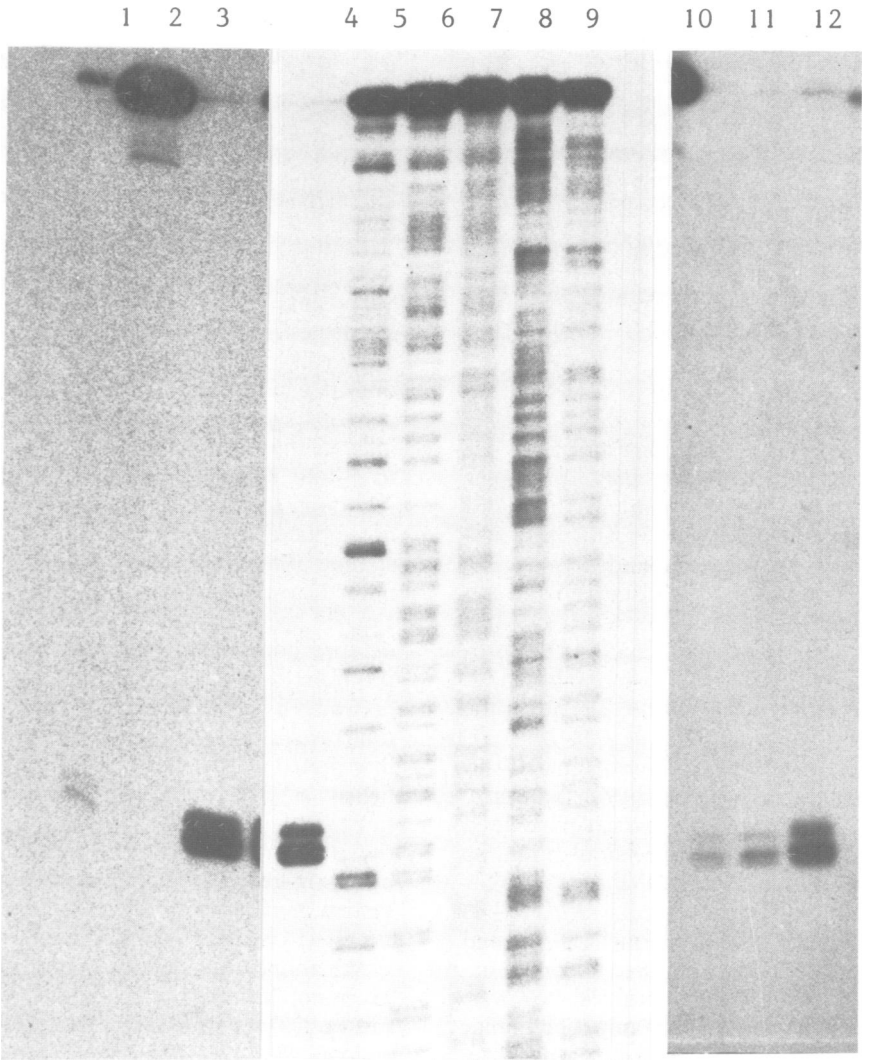

Fig. 5. S1 nuclease mapping of the 5′ terminus of *uvrC*-specific mRNAs. Autoradiogram of nuclease S1-resistant DNA fragments generated from hybrids formed at 46°C with a 330-bp *Hpa*II-*Nco*I fragment 5′-^{32}P-labeled at its *Nco*I terminus (Figure 4, probe 3) and RNA from bacteria carrying the pCA95051 plasmid. Denatured DNA fragments were electrophoresed on polyacrylamide-urea gels as described in Materials and methods. Three separate experiments are shown: **lanes 1 – 3; 4 – 9** and **10 – 12. Lane 1:** strain DM1187 LexA (Def), no plasmid; **lane 2:** probe, no S1 treatment; **lanes 3,4,10:** strain AB1157 + pCA95051: no induction; **lanes 5 – 9**: Maxam-Gilbert degradation: **5**: G; **6**: A + G; **7**: A > C; **8**: C + T; **9**: C; **lanes 11,12**: strain AB1157 + pCA95051 induced with 60 J/m^2 u.v., RNA isolated respectively 30 and 120 min after u.v. treatment.

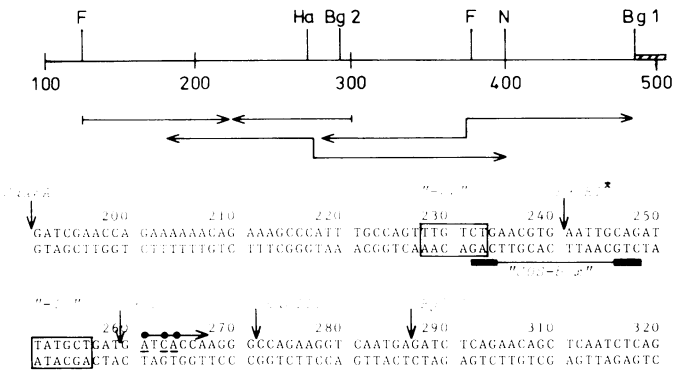

Fig. 6. The regulatory region of the *E. coli uvrC* gene. **(A)** Strategy of Maxam-Gilbert sequencing. **(B)** The DNA sequence from position 190–310 containing the '−35' and '−10' regions (boxed) and the putative LexA binding site (underlined). The starts of *uvrC* transcription (position 261–264) are indicated by a dotted horizontal arrow, whereas vertical arrows denote cleavage sites of restriction enzymes.

Court, 1979): a '−35' sequence 5′ TTGTCT (position 228–233) and a '−10' sequence 5′ TATGCT (position 251–256) which are located at the correct distance from the established transcriptional starts.

From the regulatory regions of other damage-inducible genes, the consensus sequence CTG (N_{10}) CAG for the LexA-binding site has been derived (Little and Mount, 1982). We examined the *uvrC* promoter region for similarities and identified a CTG(N_{11}) CAG sequence (position 232–248) that differs from the consensus only in the spacing of the inverted repeats. This putative LexA-binding site is located between the '−35' and '−10' sequences of the promoter which is similar to the situation of the SOS box in the *recA* gene (Miki *et al.*, 1981a).

Discussion

Using operon fusions, S1 nuclease mapping and DNA sequencing we located and characterized a *uvrC* promoter situated ~220 bp ahead of the putative start of the 68-kd UvrC coding sequence near the *Bgl*I site (W.D.Rupp, personal communication). A '−35' sequence and a '−10' sequence were identified at the appropriate distance from the observed start of the *uvrC* mRNA at position A261, C263 or A264 (see Figure 6). The heterogeneity in length of the protected fragments is frequently found with S1 mapping and it is probably due to a selective breathing of the 3′ end of the DNA fragment in the hybrid (Hentschel *et al.*, 1980).

The location of the *uvrC* promoter as described here, is in agreement with earlier work (Yoakum *et al.*, 1981; Sancar *et al.*, 1981c; Van Sluis and Dubbeld, 1983) which postulated that the DNA region involved in *uvrC* expression should be placed 5′ to the *Bgl*II site at position 290 (Figure 4). Our results, however, differ from those of Sharma *et al.* (1981, 1982), who presented evidence that sequences located >900 bp 5′ to the structural gene are required for *uvrC* expression. Their RNA polymerase binding studies, however, also indicated a secondary binding site which might be similar to the *uvrC* promoter described here. The precise role of sequences preceding the structural gene in modulation of *uvrC* expression is still not fully understood. It is interesting to note that attempts to amplify UvrC protein synthesis by placing strong promoters at various distances from the structural gene have been rather unsuccessful (C.Backendorf, unpublished data). As we have shown earlier, the 3.4-kb *Pst*I fragment originally cloned on pCA32 (Van Sluis and Brandsma, 1981) is fully expressed, independent of the orientation relative to the vector plasmid (Van Sluis and Dubbeld, 1983). This observation indicates that a promoter expressing the *uvrC* gene must be present on the 1.1-kb *Pst*I-*Bgl*II fragment preceding the structural gene. Moreover, insertions of Tn5 into *uvrC*$^+$ plasmids at 300 and 500 bp ahead of the *Bgl*II site do not alleviate the capacity to complement UvrC$^-$ bacteria for u.v. survival (Van Sluis and Brandsma, 1981) indicating that the promoter identified in our studies by itself gives full expression of the *uvrC* gene on multicopy plasmids. At present our results remain at variance with those of Sharma *et al.* (1981, 1982) and of Sharma and Moses (1983).

The results on the inducibility of the *uvrC* promoter clearly establish the role of the cellular SOS response in the expression of the *uvrC* gene. In this respect the *uvrC* gene regulation resembles that of the *uvrA* and *uvrB* genes, which are jointly involved in the synthesis of the UvrABC endonuclease (Sancar and Rupp, 1983; Seeberg *et al.*, 1983).

The use of operon fusions on plasmids to execute regulation studies of these genes was recently also validated for the *uvrA* and *uvrB* genes (Backendorf *et al.*, 1983; and Van den Berg *et al.*, unpublished data) showing that both the *uvrA* and *uvrB* promoters fused to *galK* are inducible in a RecA LexA dependent way. The fact that insertions into the chromosomal *uvrC* gene have not been identified by Kenyon and Walker (1980) might indicate that the *uvrC* gene is required for cell viability.

Both u.v. irradiation and mitomycin C treatment result in increased expression of the *uvrC* promoter on pCA95051. Surprisingly nalidixic acid, which is known to induce all other *din* genes in chromosomal Mud::(Ap,*lac*) fusions (Kenyon and Walker, 1981; Schendel *et al.*, 1982; Bagg *et al.*, 1981), does not induce the *uvrC* promoter. Similar experiments using *recA.kan*^{R} and *uvrA.galK* fusions on multicopy plasmids indicate that nalidixic acid induces the respective promoters 3- to 8-fold (Miki *et al.*, 1981b; Backendorf *et al.*, 1983). Although the SOS-response after nalidixic acid treatment is not induced by chemical damage to DNA but rather by the stalling of the DNA replication fork (Sugino *et al.*, 1977), it is nevertheless evident that nalidixic acid treatment of RecA$^+$ LexA$^+$ bacteria leads to the activation of RecA protease and derepression of LexA regulated genes. Consequently the *uvrC* promoter, harboring a genuine LexA binding site, should be activated by nalidixic acid. It has to be emphasized, however, that results obtained with DNA gyrase inhibitors have to be interpreted with caution, as these agents also affect the superhelicity of DNA and might have unpredictable effects on promoter activities (Smith, 1981). In this respect, recent work on the effect of gyrase B-specific inhibitors indicated that these agents might cause complex responses of the bacterial SOS system, leading to derepression of some *din* genes and having no effect on others (Smith, 1983).

We found that the time course of induction of the *uvrC* promoter is different from the kinetics of derepression of most other *din* genes, as an increase in transcription is observed only 90–120 min after treatment. The response of the *recA* gene is much more rapid, as this gene is fully induced within 60 min after irradiation (MacPartland *et al.*, 1980; Salles and Paoletti, 1983). Also *uvrA* and *uvrB* fusions have a more rapid response both in chromosomal insertions (Schendel *et al.*, 1982) and in multicopy situations (Backen-

dorf *et al.*, 1983). The slow derepression of the *uvrC* promoter could be ascribed to the slight alteration in the structure of the putative LexA binding site where the dyad symmetry is separated by 11 bp instead of the 10-bp distance as is generally observed in other *din* genes (Cole, 1983). It remains, however, to be established whether the consensus sequence observed is involved in the binding of LexA repressor. Alternatively, the results described here are not in disagreement with a model where *uvrC* expression is not directly modulated by LexA but rather by a damage-inducible positive effector.

From the view point of repair it is not easily explained why bacteria undergoing repair have such a late requirement for the UvrC gene product unless UvrC plays a semi-detached, independent role in the detection and processing of DNA damage. Seeberg *et al.* (1983) showed that the UvrABC-catalyzed *in vitro* repair reaction can be separated into two consecutive steps: an ATP-dependent UvrAB-catalyzed binding to the damaged template followed by an ATP-independent UvrC-catalyzed endonucleolytic step. Sharma and Moses (1979) concluded from repair experiments in permeable cells that the UvrC protein might be required late in the incision step and *in vitro* studies on DNA incised by *Micrococcus luteus* pyrimidine dimer-N-glycosylase, revealed that extracts from UvrC$^+$ cells catalyzed repair replication by DNA polymerase I (Sharma *et al.*, 1982). Moreover, Tang *et al.* (1982) found that repair of bacteriophage DNA treated with N-hydroxyamino-fluorene is impaired in *uvrC* mutants but unaffected in UvrA- and UvrB-deficient bacteria. Finally, resident enhanced repair of plasmids, requires both the UvrA and UvrB gene products but is independent of a functional *uvrC* gene (Strike and Roberts, 1982).

In conclusion, the *uvrC* gene resembles in many aspects the cooperative *uvrA* and *uvrB* genes as a damage-inducible repair gene. A more detailed analysis of the various *uvrC* mutants and directed mutagenesis of the regulatory region are required to elucidate the mechanism of the inducible expression of the *E. coli uvrC* gene.

Materials and methods

Bacterial strains and plasmids

The *E. coli* K12 strain AB1157 is used as a repair-proficient strain throughout this study; AB2463 is a RecA13 derivative (Howard-Flanders and Theriot, 1962). Two LexA mutant strains derived from AB1157 have been employed: DM49 *lexA3* harboring a RecA protease-resistant LexA protein and DM1187 *lexA3 spr*-55, which has an inactive LexA (Def) repressor (Mount, 1977). Plasmid pCA95 (Figure 1) is a pK0-1 derivative (McKenney *et al.*, 1981), where a 300-bp *Eco*RI-*Hind*III fragment has been replaced by a 29-bp fragment from pBR322 introducing a *Cla*I site (Sutcliffe, 1979) suitable for insertion of *Taq*I fragments. Derivative plasmids (pCA9505, 9507; Figure 1) were obtained by ligation of a mixture of *Taq*I fragments from the 3.4-kb *Pst*I parental UvrC region with *Cla*I-digested pCA95. Plasmid pCA95051 was obtained after digestion of pCA9505 with *Eco*RI and *Pvu*II and filling in the *Eco*RI terminus with a DNA polymerase I large fragment followed by blunt-end ligation. In this way the *Eco*RI recognition sequence is conserved.

Microbiological procedures

Bacteria harboring *galK* plasmids were routinely grown at 37°C in minimal medium supplemented with 0.4% (w/w) glucose, 0.2% (w/w) casamino acids and 50 μg/ml ampicillin. Galactokinase-positive clones were isolated after transformation of GalK bacteria (Pannekoek *et al.*, 1978) with ligation mixtures, plating on McConkey agar supplemented with 1.2% (w/w) galactose and 50 μg/ml ampicillin and selection for red colonies after overnight incubation at 30°C (McKenney *et al.*, 1981).

Cloning procedures

Plasmid DNA was prepared according to the method of Birnboim and Doly (1979). Protocols for restriction analysis and ligation of DNA fragments have been described in detail elsewhere (Pannekoek *et al.*, 1978); DNA digestion with restriction endonucleases was performed as recommended by the suppliers.

S1 nuclease mapping and DNA sequencing

Mapping of *in vivo* UvrC transcripts was carried out according to Berk and Sharp (1977) with modification as described (Van den Berg *et al.*, 1981). Isolation of DNA fragments from polyacrylamide gels, 5′-labeling with [γ-^{32}P]ATP and polynucleotide kinase, and DNA sequencing was executed according to Maxam and Gilbert (1980).

Galactokinase assay

Overnight cultures of bacteria harboring *galK* plasmids were diluted and grown at 37°C for at least four generations until the absorbance A_{650} reached 0.4. Cells were then washed twice with minimal salts, irradiated with u.v. in 1 mm layers with agitation, resupplied with growth factors and incubated at 37°C with shaking. Throughout the course of the experiment, cultures were kept in the exponential phase by dilution with warm supplemented minimal medium. Samples (1 ml) taken at intervals were centrifuged and pellets were either assayed directly or after overnight storage at −20°C. Measurement of galactokinase activity was carried out as described by McKenney *et al.* (1981) with minor modification. Reactions were terminated by the addition of a stopping mixture [0.1 M galactose, 0.01 M EDTA, 0.05 M Tris-HCl (pH 8)] prior to application of the mixture to DEAE-cellulose paper discs. The specific galactokinase activity is expressed as nmol galactose phosphorylated/min/10^8 bacteria at 32°C.

Enzymes and radiochemicals

Restriction endonucleases employed were from Amersham International, UK; New England Biolabs, USA and Boehringer, Mannheim. Polynucleotide kinase and DNA ligase were obtained from Amersham; DNA polymerase I, mitomycin C and nalidixic acid from Boehringer. [^{14}C]Galactose and [γ-^{32}P]-ATP were purchased from the Radiochemical Centre, UK.

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References

Backendorf,C., Brandsma,J.A., Kartasova,T. and Van de Putte,P. (1983) *Nucleic Acids Res.*, **11**, 5795-5810.
Bagg,A., Kenyon,C.J. and Walker,G.C. (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 5749-5753.
Berk,A.-J. and Sharp,P.A. (1977) *Cell*, **12**, 721-732.
Birnboim,H.C. and Doly,J. (1979) *Nucleic Acids Res.*, **7**, 1513-1523.
Cole,S.T. (1983) *Mol. Gen. Genet.*, **189**, 400-404.
Fogliano,M. and Schendel,P.F. (1981) *Nature*, **289**, 196-198.
Hanawalt,P.C., Cooper,P.K., Ganesan,A.K. and Smith,C.A. (1979) *Annu. Rev. Biochem.*, **48**, 783-836.
Hentschel,C., Irminger,J.-C., Bucher,P. and Birnstiel,M.L. (1980) *Nature*, **285**, 147-151.
Howard-Flanders,P. and Theriot,L. (1962) *Genetics*, **47**, 1219-1224.
Kenyon,C.J. and Walker,G.C. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 2819-2823.
Kenyon,C.J. and Walker,G.C. (1981) *Nature*, **289**, 808-810.
Little,J.W. and Mount,D.W. (1982) *Cell*, **29**, 11-22.
Maxam,A.M. and Gilbert,W. (1980) *Methods Enzymol.*, **65**, 499-559.
McKenney,K., Shimatake,H., Court,D., Schmeissner,U., Brady,C. and Rosenberg,M. (1981) in Chirikjian,J.G. and Papas,T.S. (eds.), *Gene Amplification and Analysis*, Vol. **2**, Elsevier-North-Holland, Amsterdam, pp. 383-415.
McPartland,A., Green,L. and Echols,H. (1980) *Cell*, **20**, 731-737.
Miki,T., Ebina,Y., Kishi,F. and Nakazawa,A. (1981a) *Nucleic Acids Res.*, **9**, 529-543.
Miki,T., Kumahara,H. and Nakazawa,A. (1981b) *Mol. Gen. Genet.*, **183**, 25-31.
Mount,D.W. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 300-304.
Pannekoek,H., Noordermeer,I.A., Van Sluis,C.A. and Van de Putte,P. (1978) *J. Bacteriol.*, **133**, 884-896.
Rosenberg,M. and Court,D. (1979) *Annu. Rev. Genet.*, **13**, 319-353.
Salles,B. and Paoletti,C. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 65-69.

Sancar,A., Clarke,N.D., Griswold,J., Kennedy,W.J. and Rupp,W.D. (1981a) *J. Mol. Biol.*, **148**, 63-70.
Sancar,A., Warton,R.P., Seltzer,S., Kacinski,B.M., Clarke,N.D. and Rupp, W.D. (1981b) *J. Mol. Biol.*, **148**, 45-62.
Sancar,A., Kacinski,B.M., Mott,D.L. and Rupp,W.D. (1981c) *Proc. Natl. Acad. Sci. USA*, **78**, 5450-5454.
Sancar,A., Sancar,G.B., Little,J.W. and Rupp,W.D. (1982a) *Cell*, **28**, 523-530.
Sancar,A., Sancar,G.B., Rupp,W.D., Little,J.W. and Mount,D.W. (1982b) *Nature*, **298**, 96-98.
Sancar,A. and Rupp,W.D. (1983) *Cell*, **33**, 249-260.
Schendel,P.F., Fogliano,M. and Strausbaugh,L.D. (1982) *J. Bacteriol.*, **150**, 676-685.
Seeberg,E. (1981) *Progr. Nucleic Acid Res. Mol. Biol.*, **26**, 217-226.
Seeberg,E., Steinum,A.L. and Linn,S. (1983) *J. Cell. Biochem. Suppl.*, **7B**, 175.
Sharma,S., Ohta,A., Dowhan,W. and Moses,R.E. (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 6033-6037.
Sharma,S., Dowhan,W. and Moses,R.E. (1982) *Nucleic Acids Res.*, **10**, 5209-5221.
Sharma,S. and Moses,R.E. (1979) *J. Bacteriol.*, **137**, 397-408.
Sharma,S. and Moses,R.E. (1983) *J. Cell. Biochem. Suppl.*, **7B**, 176.
Smith,C.L. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 2510-2513.
Smith,G.R. (1981) *Cell*, **24**, 599-600.
Strike,P. and Roberts,R.J. (1982) *J. Bacteriol.*, **150**, 385-388.
Sugino,A., Peebles,C.L., Kreuger,K.N. and Cozzarelli,N.R. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 4767-4771.
Sutcliffe,J.G. (1979) *Cold Spring Harbor Symp. Quant. Biol.*, **43**, 77-90.
Tang,M.-S., Lieberman,M.W. and King,C.M. (1982) *Nature*, **299**, 646-648.
Van den Berg,E., Zwetzloot,J., Noordermeer,I., Pannekoek,H., Dekker,B., Dijkema,R. and Van Ormondt,H. (1981) *Nucleic Acids Res.*, **9**, 5623-5643.
Van Sluis,C.A. and Brandsma,J. (1981) in Seeberg,E. (ed.), *Chromosome Damage and Repair*, Plenum Publishing Corp., NY, pp. 293-302.
Van Sluis,C.A. and Dubbeld,D. (1983) in Friedberg,E.C. and Hanawalt,P.C. (eds.), *DNA Repair*, Vol. **2**, Marcel Dekker, NY, pp. 267-283.
Yoakum,G.H. and Grossmann,L. (1981) *Nature*, **292**, 171-173.