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In vivo transcription of the *E.coli uvrB* gene: both promoters are inducible by UV

Eva A.van den Berg*, Ruud H.Geerse, Hans Pannekoek and Pieter van de Putte

Laboratory of Molecular Genetics, State University of Leiden, 2333 AL Leiden, The Netherlands

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ABSTRACT

The transcriptional activity of the tandem promoters of the *Escherichia coli uvrB* gene was measured *in vivo*. Both promoters are shown to be inducible by UV irradiation. P1, the most proximal promoter, is responsible for the main part of transcription both in uninduced and induced cells. Plasmids have been constructed carrying small deletions in the *lexA* binding site that overlaps with P2, the distal promoter. These deletions result in constitutive transcription from P1. This indicates that the DNA region which contains P2 functions mainly as a target site for regulation of P1 transcription *in vivo*.

INTRODUCTION

When cells of *Escherichia coli* are treated with agents that damage DNA structure or interfere with its synthesis, this leads to activation of a set of genes involved in inhibition of cell division, repair of DNA, mutagenesis, synthesis of bacteriocins and induction of certain prophages. This activation process, termed the SOS response, is coordinated by the RecA and LexA gene products (1,2). The LexA protein binds to the operator-promoter region of the damage inducible genes (*din* genes), thereby preventing efficient transcription initiation. When DNA synthesis is blocked by damaging treatments, the stalled replication machinery generates a signal that converts the RecA protein to a specific protease (3). This protease cleaves the *lexA* repressor, thus allowing expression of the *din* genes (4).

It has been reported that the *uvrA* and *uvrB* genes, both involved in the incision step of the *E.coli* excision repair pathway (5) are inducible by UV irradiation in a *recA-lexA* dependent fashion (6,7).

In a previous report we have shown that the *uvrB* regulatory region harbours two adjacent promoters, which are both active in promoting transcription *in vitro* (8). In P2, the distal promoter, we found a sequence bearing significant homology to the *lexA* binding site in several other *din* genes (9). From this observation we tentatively concluded that P2 was responsible for

the inducible part of *uvrB* expression. Later reports supported this conclusion: it was found that in an *in vitro* system only transcription from P2 was affected by LexA protein (10).

In this paper we report on the regulation of *uvrB* transcription *in vivo*. Our results indicate that P1 and P2 are both regulated by *lexA*.

EXPERIMENTAL PROCEDURES

E.coli K12 bacterial strains and plasmids.

Strain GM1 was originally obtained from J.H. Miller (11). The *uvrB* derivative of this strain was constructed as described (12). Strain AB1157 and the *recA* derivative thereof were originally from Howard Flanders and Theriot (13). The *uvrB* plasmid pNP12 (Fig. 1), used in the S1 mapping experiments, has been described (8).

Molecular cloning procedures.

Digestion of plasmid DNA with restriction endonucleases was performed as prescribed by the enzyme manufacturers. Ligation of DNA fragments and transformation of competent cells was carried out as described (14). Sticky ends of restriction fragments were converted to blunt ends by digestion with S1 nuclease as described (8). Isolation of DNA fragments from low melting agarose was as described (15).

DNA sequencing.

End labeling of restriction fragments, chemical modification and degrada-

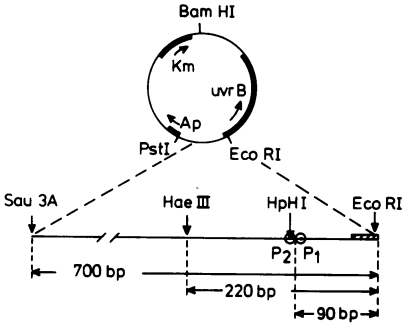

Figure 1. A schematic representation of the recombinant plasmid pNP12 (Km^R), which carries the intact *uvrB* gene and its regulatory elements on a PstI-BamHI fragment, fused to the PstI-BamHI fragment of plasmid pACYC177 (22). The coding regions for the *uvrB* gene, the kanamycin gene (Km) and the C-terminal part of the ampicillin (Ap) gene are indicated. A DNA segment harbouring the regulatory region is enlarged showing the position of promoters P1 and P2, the beginning of the coding region (shaded box) and several relevant restriction sites in this area.

tion of labeled DNA was performed essentially as described (16).

S1 mapping.

RNA isolation was performed as described (18). For isolation of induced transcripts, cells were grown in L broth to an OD of 0,35, irradiated at the same OD in M9 buffer, with 70 J/m^2 (254 nm) and incubated for another 40 min in L broth prior to RNA isolation. End-labeling of DNA, RNA-DNA hybridization and S1 incubation were performed essentially as described (8) except that approximately 50 µg of RNA and 140 units of S1 nuclease were used per incubation. To estimate the level of transcripts before and after induction, equal amounts of RNA (as determined by OD measurements) isolated from either unirradiated or irradiated cells, were used for hybridization with the labeled probe. Since in all experiments only a small fraction of the labeled DNA (< 10%) was protected against S1 nuclease digestion, we assumed that the probe was present in excess over the *uvrB* transcripts. Samples were analysed on a 8% polyacrylamide 7 M urea slabgel followed by autoradiography. Relative intensities were determined by densitometric scanning of the autoradiograms.

Enzymes and radiochemicals.

Restriction enzymes were purchased from the following manufacturers: Miles Lab., Elkhart, Ind. (EcoRI); Biolabs, Beverly, M.A. (HphI, RsaI, Sau3A); Boehringer Mannheim, BRD (BglII). PstI was donated by H.L. Heyneker (Genentech Inc., San Francisco, Cal.). S1 nuclease from *Aspergillus oryzae* was purchased from P-L Biochemicals Inc., Milwaukee, Wis. T4 polynucleotide kinase was from Boehringer Mannheim, BRD. γ-$|^{32}P|$-ATP was from NEN Chemicals.

Determination of β-galactosidase levels.

β-galactosidase activity was monitored essentially as described (19). Cells were grown in supplemented M9 medium to a density of $2x10^8$ cells/ml. 5 ml aliquots were irradiated with 70 J/m^2 (*uvr*$^+$ strain) or 10 J/m^2 (*recA* strain) and diluted 1/5 in M9 medium. Non-irradiated control cells were diluted similarly. At various intervals samples were withdrawn and assayed for β-galactosidase activity. Enzyme activity is expressed as nanomoles of o-nitrophenol per min per OD unit at 600 nm. One enzyme unit equals: $(A_{420} - 1.75 \cdot A_{550})/ t \text{ v } A_{600}$ 100, where t = time in min and v = volume in ml.

RESULTS

P1 and P2 mediated transcription in vivo.

To quantitate the level of the *uvrB* transcripts *in vivo*, we used the S1 mapping technique. A DNA probe overlapping with the *uvrB* regulatory region was hybridized to RNA isolated from either unirradiated or irradiated cells.

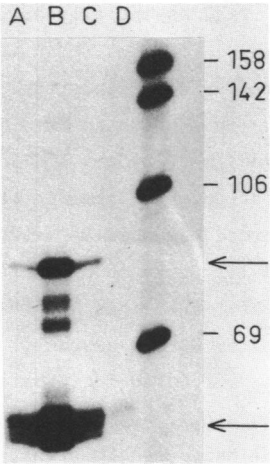

Figure 2. ^{32}P-labeled DNA fragments, protected by *in vivo* synthesized P1 and P2 transcripts against S1 nuclease. A Sau3A-EcoRI fragment containing the regulatory region was end-labeled with ^{32}P and hybridized to equal amounts of RNA (as determined by OD measurements) isolated from normal and irradiated cells. RNA was isolated as described (18) from either strain GM1 (*uvr*$^+$) or a ΔuvrB derivative of this strain (12) carrying pNP12. End-labeling of DNA, RNA-DNA hybridization and S1 incubation were performed as described (8,17). Samples were analysed by electrophoresis on a 8% polyacrylamide 7 M urea slab gel followed by autoradiography. The markers indicated on the photograph are labeled fragments of phage M13 DNA digested with HaeIII (21). Lane A: GM1 Δ*uvrB*/pNP12; lane B: GM1 Δ*uvrB*/pNP12 + UV; lane C: GM1 + UV; lane D: GM1. The arrows indicate the position of P1 (50 nucleotides) and P2 (80 nucleotides).

the hybridization probe was a 700 basepairs Sau3A-EcoRI fragment (Fig. 1) labeled at the RI site situated in the N-terminal part of the structural gene. RNA was isolated from a Δ*uvrB* strain carrying the multicopy plasmid pNP12. As is shown in Fig. 2, P1 transcript is present in 10-20 fold excess over P2 transcript (lane A). After UV irradiation both transcripts are strongly induced, P1 remaining the strongest promoter (lane B). Similar results were found when RNA was used from a strain carrying only the chromosomal copy of the *uvrB* gene, although the levels of both transcripts are much lower in this situation (lane C and D).

Deletion mapping of regulatory sequences.

To identify sequences involved in the regulation of *uvrB* transcription we constructed plasmids in which the region upstream of the HphI site in P2 (Fig. 1) is deleted. The construction is outlined in detail in Fig. 3. Two types of plasmids were isolated which both conferred UV resistance on a *uvrB* strain. Both were further characterized by sequence analysis of which the results are

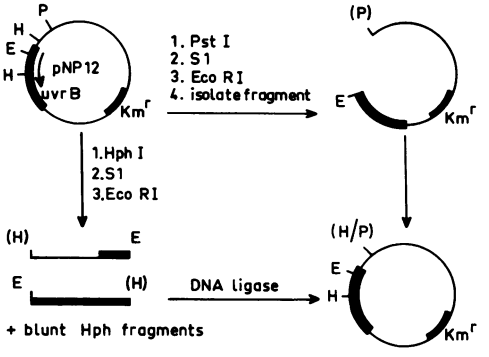

Figure 3. Construction of *uvrB* deletion clones. pNP12 was linearized with PstI, treated with S1 nuclease and digested with EcoRI. The large fragment was isolated from low melting agarose and ligated to a mixture of (blunt) HphI and HpHI-EcoRI fragments; only one of the HphI-EcoRI fragments carries part of the *uvrB* regulatory region. The ligation mixture was used to transform strain GM1 Δ*uvrB* to Km^r, uv^r.

shown in Fig. 4. In plasmid pNP22 the sequence CTGT, which is part of the *lexA* box consensus is replaced by CCAT. The resulting sequence differs only in two bases from the original *lexA* box. In plasmid pNP23 an extra deletion of 80 basepairs had occurred probably by a recombination event between two 7 basepair direct repeats present in the ampicilline gene and the P2 region (indicated in Fig. 3A) resulting in a sequence that has very little homology with the *lexA* box consensus sequence. The *in vivo* transcription pattern of these mutated promoters is shown in Fig. 5, together with that of the wild type promoters on plasmid pNP12. With both mutant plasmids the level of P1

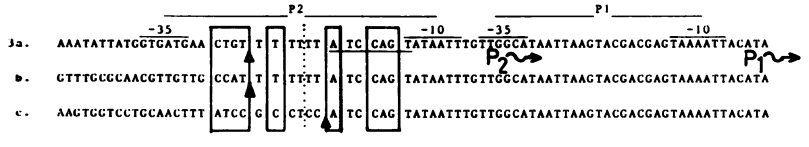

Figure 4. Sequence analysis of deletion mutants in the *uvrB* regulatory region. a. sequence of the intact regulatory region in pNP12. The "-35" and "-10" sequences of P1 and P2 are marked with horizontal lines. The start points of both transcripts are indicated by wavy arrows; bases matching the *lexA* consensus are boxed. The dotted line represents the axis of symmetry of the inverted repeats of the *lexA* box. The arrow indicates the cleavage site for HphI that was used to delete part of P2. The 7 basepair sequence ATCCAGT that is underlined is also present in the Ap gene of the vector; recombination between these direct repeats probably produced plasmid pNP23 (Fig. 3c). b. and c. Analysis of the deletions in pNP22 and pNP23. The site where the vector is fused to the remnants of P2 is indicated by an arrow.

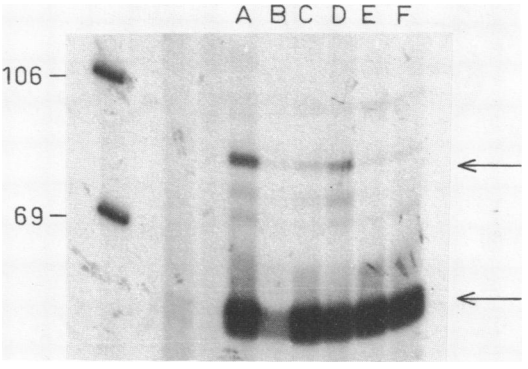

Figure 5. ^{32}P labeled DNA fragments protected against S1 nuclease digestion by *in vivo* transcripts from the mutant plasmids pNP22 and pNP23. All RNA samples were isolated from strain GM1 Δ*uvrB* harbouring the indicated plasmids. Lane A: pNP12 + UV; lane B: pNP12 - UV; lane C: pNP22 + UV; lane D: pNP22 - UV; lane E: pNP23 + UV; lane F: pNP23 - UV. Arrows and markers as in Fig. 2.

transcript in uninduced cells is increased 2-3 fold as compared to pNP12. Apparently the deletions in P2 result in relief of repression of P1. The effect is strongest for pNP23, suggesting that a more extensive deletion in the *lexA* box is reflected in a further increase of P1 transcription. Consequently, only a minor induction is observed upon irradiation. Since both deletions

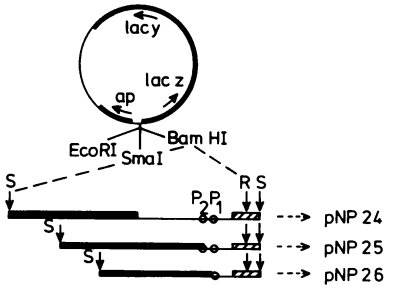

Figure 6. *uvrB* promoter fragments inserted in the BamHI site of pMC1403(17). The fragment inserted in pNP24 was derived from pNP18 (8) a plasmid constructed by deleting the PstI-HaeIII region of pNP12 (see also Fig. 1) pNP25 and pNP26 contain promoter fragments isolated from pNP22 and pNP23 respectively. The position of P1 and P2, the EcoRI (R) and Sau3A (S) sites, the beginning of the *uvrB* coding region (shaded box) and the segment originating from the Ap gene (thick bar) are indicated on the promoter fragments. By EcoRI digestion was shown that the fragments were inserted in the correct orientation, resulting in an in phase fusion of the first 15 *uvrB* codons to the structural *lacZ* gene (results not shown).

greatly alter the "-35 sequence" of P2, it is not surprising that with both mutant plasmids the level of P2 transcript is very low in uninduced as well as in induced cells.

β-galactosidase synthesis under control of the mutant promoter fragments.

We compared the ability of the mutant promoters to direct β-galactosidase synthesis *in vivo* to that of the intact P2-P1 region. Therefore plasmid pNP18 a PNP12 deletion derivative still carrying the P2-P1 region (8), and the mutant plasmids pNP22 and pNP23 were digested with Sau3A. The promoter fragments were isolated and ligated with the promoter-probe *lacZ* plasmid pMC1403 digested with BamHI (Fig. 6). The various ligation mixtures were transformed to strain AB1157 and selected for Ap^r and β-galactosidase activity on Mc Conkey indicator plates yielding pNP24, pNP25 and pNP26 respectively. Digestion with EcoRI revealed that the promoter fragments were in the correct orientation (results not shown) and from the known sequences of the promoter fragments and the *lacZ* gene it can be deduced that this results in an in phase fusion of the first 15 codons of the *uvrB* structural gene to the *lacZ* gene. β-galactosidase levels measured with these plasmids are shown in Table 1.

pNP24 provides AB1157 with a basal level of ± 1500 units β-gal, which increases approximately 5-fold upon irradiation. No induction was found in a $recA^-$ derivative of the same strain, in agreement with earlier reports on the *recA-lexA* dependent inducibility of *uvrB* expression (6). Consistent with the *in vivo* transcription data described above, the mutant promters give rise to a much higher basal level of β-gal synthesis, which is only slightly increased after irradiation.

Table 1. β-galactosidase levels measured with various *uvrB-lacZ* fusion plasmids.

Strain	Plasmid	Units β-galactosidase		ratio +UV/-UV
		-UV	+UV	
AB1157	pNP24	1500	7000	4,6
AB1157 *recA*	pNP24	1100	1600	1,4
AB1157	pNP25	5000	7800	1,6
AB1157	pNP26	6000	9500	1,6

β-galactosidase activity was measured and calculated as described in the section Experimental Procedures. Induction was measured 2.5 hr after irradiation.

DISCUSSION

The *in vivo* transcription data presented here strongly suggest that P1 is responsible for the main part of *uvrB* transcription, and that both P1 and P2 are regulated by *lexA* repression at P2. The results obtained with *uvrB*-β-galactosidase fusion plasmids support this conclusion: UV induction of β-galactosidase synthesis controlled by wild type P2 and P1 is dependent upon the *recA-lexA* control system, whereas the basal level of β-galactosidase synthesis, directed by the plasmids in which part of P2 is deleted, is much higher, and is not significantly induced by UV irradiation.

A discrepancy exists between these *in vivo* results and the *in vitro* regulation of P1 and P2 mediated transcription, since it has been reported that only P2 transcription is repressed by *lexA in vitro*, and that P1 is responsible for constitutive *uvrB* transcription (10). This discrepancy may be caused by the difference in the conformation of the DNA templates used in the *in vivo* and *in vitro* experiments. Linear DNA fragments have been used as templates for *in vitro* transcription, whereas in the *in vivo* experiments transcripts from supercoiled plasmids or from the chromosome are measured.

The region of DNA that interacts with the *lexA* repressor has been determined by DNase footprinting on linear fragments (10), and does not extend beyond P2. It is possible that LexA protein covers a larger stretch of the P2-P1 region when the DNA is in the supercoiled state, thereby preventing efficient transcription from P1. Alternatively, *lexA* may cause steric hindrance for binding of RNA polymerase to P1 only when a supercoiled template is used.

Another explanation for the discrepancy could be found in the existence of an additional repressor acting on P1 transcription *in vivo*, that can only function in cooperation with the *lexA* repressor. A similar difference between *in vitro* and *in vivo* regulation of transcription has been reported for the most proximal promoter of the *E.coli* galactose operon (20). Because P1 is responsible for the main part of *uvrB* transcription *in vivo* either with or without induction, it seems likely that the main function of the DNA region containing P2 is to participate in the regulation of P1.

Our data do not answer the question whether the level of P1 transcription in uninduced cells is due to "off and on" binding of the repressor or to occasional initiation of transcription at P1 despite the presence of the *lexA* repressor at P2.

It has been reported that another strong *in vitro* promoter is located 320 basepairs upstream of P2 (10). We have not been able to detect transcripts deriving from this region *in vivo*, either before or after irradiation. Although

deletion of this promoter has no effect on the basal level of P1 and P2 transcripts (results not shown) it cannot be excluded that sequences upstream of P2 exert some other influence on *uvrB* expression.

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*To whom correspondence should be sent

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