# Regulation of the *Escherichia coli uvrD* Gene In Vivo

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The roles of two putative promoter sequences, P1 and P2, and a potential antiterminator sequence found in the *uvrD* control region were examined in vivo. Constitutive and SOS-induced levels of *uvrD* mRNA were determined by S1 mapping, and it was shown that the majority of *uvrD* transcripts are from P1, while P2 plays only a minor role. A series of increasing deletions from the 5′ end of the *uvrD* gene was used to assay transcription in the promoterless vector pKO-1. Loss of just the −35 region of P1 was sufficient to switch off detectable transcription from both P1 and P2. Disruption of the antiterminator by site-specific mutagenesis had no effect on constitutive levels of transcription, but led to a significant increase over wild-type levels following SOS induction. This suggests that the attenuator comes into play following DNA damage to moderate the increase in UvrD protein synthesis.

The *uvrD* gene of *Escherichia coli* has been cloned (3, 24, 28), and DNA sequence analysis has shown the protein to be of molecular mass 82 kilodaltons (12, 40). Initial studies on the biochemistry of UvrD protein identified it as the ATP-dependent DNA-unwinding enzyme DNA helicase II (1, 2, 14, 19, 29, 35). More recent evidence has shown that the UvrD protein displaces a 12- to 13-nucleotide fragment of single-stranded DNA generated by the action of the UvrABC excision nuclease during repair of UV-damaged DNA (8, 17, 20) and operates in conjunction with other enzymes in the process of methyl-directed mismatch repair (22), which may account, respectively, for the UV sensitivity (18, 30, 38) and high spontaneous mutation rate (32, 34) found in *uvrD* mutants. In addition, *uvrD* mutations affect the frequency of recombination (5, 15, 21) and precise transposon excision (23), although the exact role of UvrD protein in these processes is not clear.

The *uvrD* gene is one of a group of SOS genes regulated by the action of the *lexA* and *recA* gene products (see reference 39 for a review). SOS genes are characterized by increased transcription following DNA damage and by the presence of a binding site for the LexA repressor between the promoter and the ribosome-binding site. UvrD protein synthesis increases two- to threefold in vivo following treatment with DNA-damaging agents (4, 19, 33), and DNA sequence analysis and LexA footprinting have demonstrated the presence of a LexA-binding site in the control region (9, 11). Analysis of the DNA sequence has revealed two possible promoters: P1, upstream of the LexA-binding site and under LexA control, and a second promoter, P2, downstream of this site. Downstream of the LexA-binding site and spanning the −35 region of P2 is a potential stem-loop structure which could act as a transcription terminator or attenuator (9, 11). Studies of these signal sequences in vitro (9, 40) have produced some contradictory data. Easton and Kushner (9) observed an attenuated mRNA from the upstream promoter, P1, which was not detected by Yamamoto et al. (40). However, both groups agreed that all transcription is initiated from P1 and begins at base pair (bp) −76 of *uvrD* (where

bp 1 is the first base to be translated) and that no transcription is detectable in vitro from the second promoter, P2. In this paper we examine the regulation of the *uvrD* gene in vivo: first to determine whether any transcription from P2 occurs, and second to resolve the uncertainty over the role of the attenuator sequence.

## MATERIALS AND METHODS

**Bacterial strains and plasmids.** *E. coli* AB1157 and AB2463 (*recA*) were originally from P. Howard-Flanders and L. Theriot (16). DM1411 (*spr recA*) was from D. Mount, and JM101 was from J. Messing (27). When appropriate, *recA* mutants were used to maximize the stability of plasmids during growth prior to galactokinase assays. For the same reason, fresh transformants were used in experiments in which AB1157 was the host. Strain HMA1354 (*dam* F′ *lac*+ *pro*+) was constructed in this laboratory and was used for preparations of unmethylated M13 DNA. Plasmid pKO-1 was from McKenney et al. (26), and the M13 derivative rp238 was made from rp1 (11) by creating BAL 31 deletions from the unique *Sac*II site within the *uvrD* gene, restriction with *Xma*I, and cloning the deletion fragments into the *Hinc*II-*Xma*I sites of M13mp8 (11). DNA sequence analysis revealed that rp238 contains the entire *uvrD* regulatory region but only 41 bases of the structural gene.

**Enzymes and biochemicals.** Restriction enzymes and T4 DNA ligase were obtained from Boehringer Mannheim Biochemicals and used as specified by the manufacturer. DNA polymerase I (Klenow fragment) was supplied by Pharmacia, and BAL 31 and S1 nuclease were supplied by Bethesda Research Laboratories. Deoxynucleoside and dideoxynucleoside triphosphates were from Sigma Chemical Co. [$^{14}$C]galactose, [$^{35}$S]dATP, and [$\gamma$-$^{32}$P]ATP were purchased from Amersham Corp. Oligonucleotides, phosphorylated at the 5′ end, were supplied by New Brunswick Scientific Co., Inc.

**Galactokinase assay.** Bacteria harboring *galK* plasmids were grown in M9 medium supplemented with 0.2% glucose, 0.1% Casamino Acids (Difco Laboratories), and 50 µg of ampicillin per ml. Cultures were assayed for galactokinase activity as described previously (6), except that the SOS inductions were accomplished by addition of either mitomycin C (1 µg/ml) or nalidixic acid (20 µg/ml).

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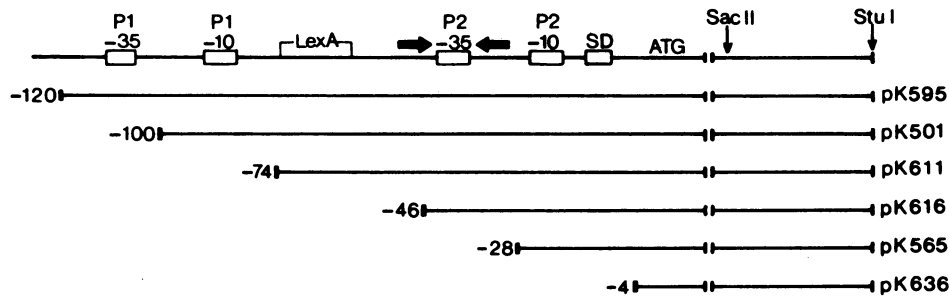† Present address: National Institutes of Health, Bethesda, MD 20892.

FIG. 1. Schematic map of the truncated *uvrD* promoter fragments. Features of the *uvrD* regulatory region are drawn to scale above the series of deleted fragments which were cloned into pKO-1. The base at the 5′ end of each fragment was determined by DNA sequencing and is numbered with respect to the first base of the start codon (bp 1). The −35 and −10 sequences of both promoters, P1 and P2, as well as the ribosome-binding site (SD) are represented by open boxes. The LexA protein-binding site and the start codon (ATG) are illustrated, and the approximate position of the putative terminator is shown by thick horizontal arrows.

**Site-specific mutagenesis.** Unmethylated M13 rp238 DNA was prepared by growth on strain HMA1354. Oligonucleotides (100 pmol) were hybridized to 1 pmol of unmethylated single-stranded DNA preparations of rp238 for 15 min at 57°C in 50 μl of 20 mM Tris (pH 8.5)–10 mM $MgCl_2$–50 mM NaCl–1 mM dithiothreitol. The complementary strand was synthesized onto the oligonucleotide primer by the addition of 2 U of DNA polymerase I (Klenow fragment) and dATP, dGTP, dCTP, and dTTP to a final concentration of 0.5 mM, each, m for 2 h at 20°C. Ligation was catalyzed by 3 U of T4 DNA ligase for 18 h at 20°C. Double-stranded closed-circular DNA was purified from single-stranded M13 in an alkaline sucrose gradient as described previously (41) and then transformed into competent cells of JM101. Mutant bacteriophages were detected by the dideoxy chain termination method of DNA sequencing (31) and occurred at a frequency of approximately 20%.

**S1 mapping of in vivo *uvrD* transcripts.** Total RNA was isolated from AB1157 cells by repeated extraction with phenol at 60°C (37). DNA restriction fragments containing the *uvrD* regulatory region were isolated from polyacryl-amide gels by electroelution (25), and the 5′ end terminus was labeled with [γ-$^{32}$P]ATP by using polynucleotide kinase. Hybrids between end-labeled DNA fragments and total cellular RNA (250 μg) were formed in 80% formamide as described previously (7) and then diluted 10-fold into ice-cold 30 mM sodium acetate (pH 4.6)–50 mM NaCl–1 mM $ZnCl_2$ and incubated for 30 min at 37°C with 50 U of S1 nuclease. After phenol extraction and isopropanol precipitation, the protected DNA fragments were analyzed on 6.0% polyacrylamide–8 M urea sequencing gels.

**Construction of *uvrD-galK* fusion plasmids.** Deletions into the *uvrD* promoter region were generated as described previously (11). Replicative-form DNA of the recombinant M13 phage rp1, which contains a 2.9-kilobase *Pvu*II fragment carrying *uvrD* cloned into the *Hinc*II site of M13mp9, was restricted with *Sma*I, digested with BAL 31, and then restricted a second time with *Hin*dIII and electrophoresed through 0.7% agarose. Fragments of 2.9 kilobases and smaller were purified and cloned into the *Sma*I and *Hin*dIII sites of M13mp9. Dideoxy DNA sequence analysis was used to determine the extent of the deletion in each recombinant.
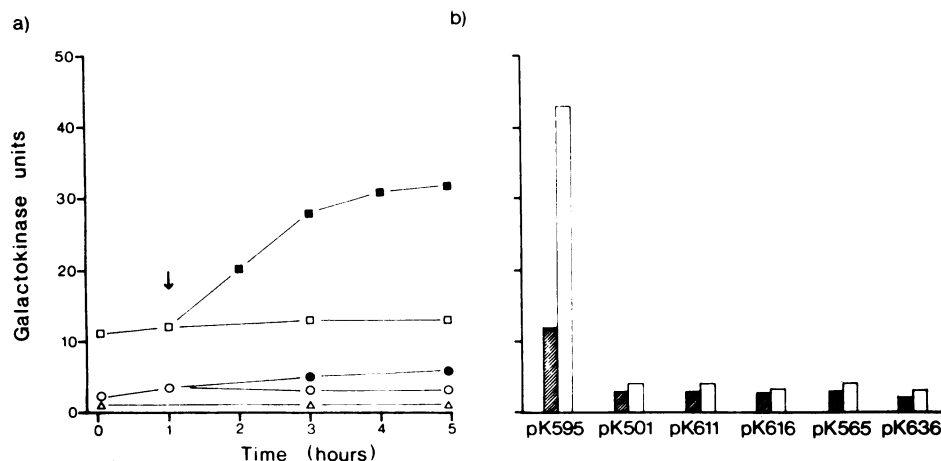


FIG. 2. SOS induction of galactokinase in different strains of *E. coli* carrying *uvrD-galK* fusion plasmids. The data are the average of results of three independent experiments. Galactokinase units were defined as nanomoles of galactose phosphorylated per minute per milliliter of cells at an optical density at 650 nm of 1. (a) Exponential cultures of AB1157 harboring the various plasmids were treated with 1 μg of mitomycin C per ml 1 h after the start of the experiment, as indicated by the arrow. Samples were taken at various times and assayed for galactokinase activity. The plasmids used were pK595 (□, ■) and pKO-1 (△). Plasmids pK501, pK565, pK611, and pK616 are all represented by the same symbols (O, ●), since the data obtained were indistinguishable. Solid symbols represent cells treated with mitomycin C, and open symbols represent untreated cells. (b) Exponential cultures of AB2463 (*recA*) (▨) and DM1411 (*recA spr* [*lexA* defective]) (▭) harboring various *uvrD-galK* fusion plasmids were assayed for constitutive levels of galactokinase activity.

A series of these M13mp9 derivatives carrying the *uvrD* promoter fragments (see Fig. 1) were digested with *Eco*RI and *Stu*I. The promoter fragments were isolated from gels and cloned between the *Eco*RI and *Sma*I sites of pKO-1. M13rp238 and its mutagenized derivatives M13rp117 and M13rp126 were digested with *Eco*RI and *Hind*III, and the fragments containing the entire promoter were cloned between the *Eco*RI and *Hind*III sites of pKO-1 to give pK238, pK117, and pK126, respectively.

## RESULTS

**Transcription from the *uvrD* promoter in vivo.** The plasmid pKO-1 was designed by McKenney et al. (26) for the analysis of DNA fragments which promote transcription. This plasmid was used to measure transcription from the *uvrD* promoter and the series of deleted fragments. Fragments were cloned into one of the unique restriction sites, *Eco*RI, *Hind*III, and *Sma*I, upstream of the promoterless *galK* gene. Translation stop codons in all three reading frames prevent the formation of fused proteins, and the levels of galactokinase synthesized reflect the strength of the promoter on the cloned fragment.

The complete *uvrD* promoter and the series of deleted promoter fragments (Fig. 1) were subcloned into pKO-1 as described in Materials and Methods. The series of plasmids obtained were then transformed into AB1157 and assayed for galactokinase activity. Cultures of AB1157 harboring pK595, which carries the complete *uvrD* control region, had a basal enzyme level of 12 U, which increased two- to threefold following treatment with either mitomycin C (1 μg/ml) (Fig. 2a) or nalidixic acid (20 μg/ml) (results not shown). This is in agreement with results of previous studies of transcription from the *uvrD* promoter, in which production of β-galactosidase from *uvrD*::Mu d(*bla lac*) gene fusions also shows a two- to threefold induction in response to DNA damage (4, 33).

Galactokinase assays of cells harboring the vector pKO-1 with no cloned promoter gave a background level of 1 U. The level of galactokinase in AB1157 cells harboring each of the *galK* plasmids which carried deletions at the 5' end of the *uvrD* promoter was slightly above this at 2 U. As there was no detectable difference between the galactokinase levels in cells harboring pK501 which lacks only the −35 region of P1 and pK636 which is deleted for P1 and P2, it was concluded that the slight increase in activity over background was not due to P1 or P2. The residual activity in cells harboring pK636 may be explained by initiation of secondary transcripts within the *uvrD* structural gene. A slight increase in galactokinase levels occurred after mitomycin C treatment (Fig. 2a) but also occurred to the same degree in cells harboring pK636 or pK501. It therefore appears that P1 and P2 are active only in pK595, which carries the full *uvrD* control region. This conclusion was confirmed by assaying galactokinase production in *recA* mutants which were either *lexA*⁺ (AB2463) or *lexA*-defective (DM1411). Strain AB2463 is SOS repressed, and LexA protein is bound to the operators of all the SOS genes, including *uvrD*, while DM1411 has derepressed SOS genes, since the LexA protein is defective. As expected, cultures of AB2463 harboring pK595, which carries the complete *uvrD* promoter, had a galactokinase content of only 12 U compared with 43 U in cultures of DM1411 harboring pK595. However, none of the deleted *uvrD* promoter fragments gave higher galactokinase levels in either genetic background than was observed for pK636 which is deleted for both promoters (Fig. 2b).
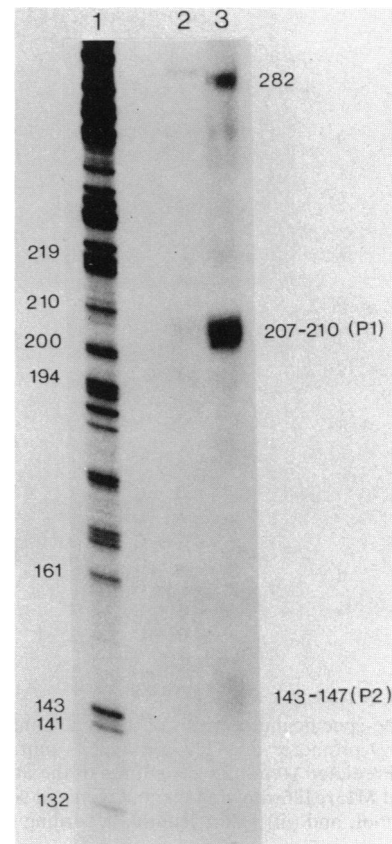


FIG. 3. Mapping of 5' termini of *uvrD* mRNAs. Autoradiographs are shown of S1 nuclease-resistant hybrids formed between a 282-bp *Bst*NI DNA fragment carrying the *uvrD* control region and total cellular RNA extacted from uninduced cells (lane 2) and from cells induced for the SOS response by treatment with 40 μg of nalidixic acid per ml for 4 h (lane 3). Molecular weight markers (lane 1) were the products of a dideoxy DNA sequencing T-track reaction on a 550-bp *Pst*I-*Eco*RI fragment of the *E. coli recA*⁺ gene, cloned from plasmid pPE13 into M13mp9 (10).

The results indicate that the whole of the P1 promoter is required for efficient transcription of *uvrD*. Loss of just the −35 region of P1 (as in pK501) was sufficient to reduce transcription to levels barely above background (Fig. 2). Thus the second putative promoter sequence, P2, appears to play no detectable role in vivo in promoting transcription in the absence of P1.

**S1 mapping of *uvrD* transcripts produced in vivo.** A 282-bp *Bst*NI fragment, which spans the *uvrD* regulatory region, was 5' end labeled with ³²P and used to map transcripts produced from the *uvrD* promoter in vivo. When this fragment was hybridized to total cellular RNA, fragments of approximately 207 to 210 bp were protected against digestion with S1 nuclease (Fig. 3, lane 2). The length of this fragment corresponds to a transcript initiated from P1. When the same fragment was hybridized to total cellular RNA extracted from cells that were induced for the SOS response by treatment with 40 μg of nalidixic acid per ml for 4 h, the intensity of the protected fragments increased severalfold (Fig. 3, lane 3). In addition, a second series of protected fragments of approximately 143 to 147 bp could be seen (Fig. 3, lane 3), and the length of these fragments indicates that
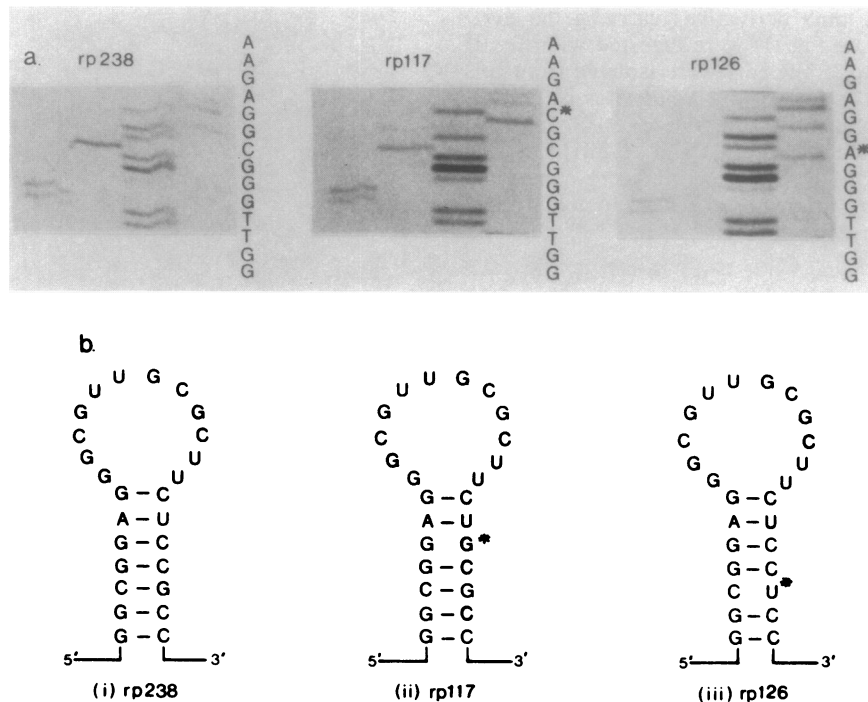
FIG. 4. Site-specific mutagenesis of the *uvrD* control region. (a) Autoradiographs of dideoxy DNA sequencing gels showing a region of the wild-type *uvrD* promoter in M13rp238 and its mutagenized derivatives M13rp117 and M13rp126. The altered bases are marked with an asterisk. (b) Predicted stem-loop structures of the attenuated RNA produced from the wild-type *uvrD* promoter and the altered RNAs from M13rp117 and M13rp126 showing the mutagenized sites. The expected free energies of formation of the three structures are (i) −69.5 kJ/mol, (ii) −31.0 kJ/mol, and (iii) −26.8 kJ/mol, according to the rules of Tinoco et al. (36).

they are protected by transcripts initiated from P2, at approximately 10 to 14 bases upstream from the first base of the start codon. Prolonged exposure of the autoradiograph (not shown) indicated that these fragments are also present in lane 2 but in very small amounts.

**Role of the terminator loop.** The function of the possible attenuator sequences in the *uvrD* control region was investigated by site-specific mutagenesis. Oligonucleotides of 17 bases were made which were complementary to the putative terminator sequence, except for one central mismatched base at the site to be mutated. Mutagenized sites were chosen so as to disrupt the stems of three of the possible hairpin loop structures (9, 11). Following purification of the mutagenized M13 DNA, the expected site of mutation was confirmed by dideoxy DNA sequencing (Fig. 4a). The mutated bases and the expected changes in free energy of the hairpin loop structures (Fig. 4b) show that the mutagenized control region in M13rp117 and M13rp126 would produce attenuated RNA less efficiently than the wild-type control region would.

To test this hypothesis, we constructed three derivatives of pKO-1 containing the wild-type or mutagenized control regions as described in Materials and Methods. Galactokinase synthesis from these plasmids was then assayed by using AB1157. All three plasmids had similar constitutive levels of galactokinase, between 11 and 16 U, and an expected twofold induction following mitomycin C treatment (1 μg/ml) of the wild-type promoter sequence in pK238 (Fig. 5). However, both mutagenized plasmids pK126 and pK117 showed a much more dramatic increase in enzyme production after the addition of mitomycin C (1 μg/ml) (Fig.
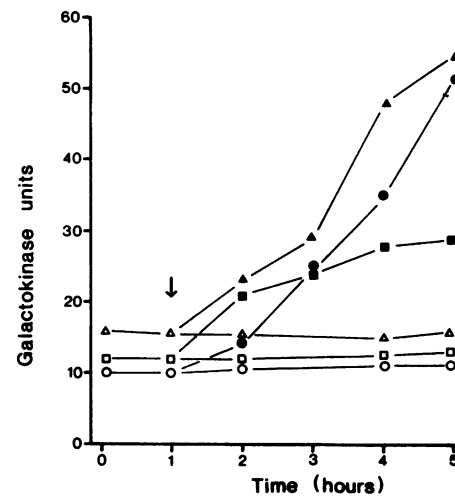


FIG. 5. SOS induction of galactokinase in AB1157 harboring *galK* plasmids with either the wild-type or the mutagenized *uvrD* promoter. Samples were taken from exponential cultures of AB1157 harboring pK238 (□, ■), pK117 (○, ●), or pK126 (△, ▲) and assayed for galactokinase activity. Solid symbols represent cells induced with 1 μg of mitomycin C per ml 1 h after the start of the experiment, as indicated by the arrow. Open symbols represent untreated cells. The values given are the average of results of three independent experiments. Galactokinase units were defined as nanomoles of galactose phosphorylated per minute per milliliter of cells at an optical density at 650 nm of 1.0.

5), suggesting that induced enzyme levels are normally lower owing to attenuation of *uvrD* mRNA.

## DISCUSSION

In vivo transcription from the *uvrD* promoter was studied by using plasmids harboring complete or truncated *uvrD* promoter fragments fused to the *galK* structural gene and assaying galactokinase synthesis. These experiments show that the complete *uvrD* promoter is required for transcription. This is in contrast to the situation reported for the *uvrA* gene, for which loss of the −35 region was not critical and the −10 sequence was sufficient to promote transcription (6). In the *uvrD* control region there are two possible promoter sequences, P1 and P2. Of these, P1 is responsible for initiating the vast majority of transcripts. The second promoter sequence, P2, plays no detectable role in vivo in promoting transcription in the absence of P1 (see Results), nor was its activity seen in vitro (8, 39). This is perhaps not surprising, as the spacing in P2 of only 14 nucleotides between the −35 and the −10 regions is shorter than in other promoters in *E. coli* (13) and the Shine-Dalgarno sequence follows the −10 region too closely. However, a low level of transcription from P2 was detectable following S1 mapping of *uvrD* mRNA made in vivo. This result can be reconciled with those described above if some RNA polymerase molecules binding at P1 move downstream to initiate transcripts at P2. Transcription from P2 would then be dependent on the presence of the full P1 promoter, and because P2 transcripts are normally produced at a very low frequency, they may not be detectable in vitro.

Transcription from the complete *uvrD* promoter fused to *galK* increased two- to threefold following SOS induction, confirming similar observations reported previously (4, 33). S1 mapping of *uvrD* mRNA synthesized in vivo shows that the increased transcription is mainly from P1, although a small increase in the number of P2 transcripts is also seen.

DNA sequence analysis of the *uvrD* control region previously revealed a possible terminator loop (9, 11) which was observed to produce attenuated mRNA in vitro in only one of two studies (9, 40). In the study described in this paper, we investigated the possible attenuator in vivo by disrupting the base pairing of the putative stem-loop structure by site-specific mutagenesis. Plasmids harboring the complete *uvrD* promoter with mutated attenuator sequences produced a much greater increase in transcription of *galK* following SOS induction than the unmutagenized control did. The evidence for attenuation reported here confirms in vivo that of Easton and Kushner (9), who observed attenuated mRNA in vitro. Furthermore, our data suggest that the role of attenuation is not to modify constitutive levels of transcription from the *uvrD* promoter, as this was unchanged by the mutations, but to moderate the number of additional transcripts produced following SOS induction. This attenuation may explain why *uvrD* transcription following DNA damage only increases by two- to threefold. Too much UvrD protein reduces the viability of cells with DNA damage (26, 27), and hence the attenuator may be required to fine-tune UvrD synthesis following SOS induction and thereby maximize the chance of recovery.

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