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Regulation of the *Escherichia coli* K-12 *uvrB* Operon

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The UV light inducibility of the *uvrB* operon of *Escherichia coli* K-12 was previously demonstrated by exploiting a strain in which the gene for the enzyme β-galactosidase was inserted into the *uvrB* operon. This insert is now shown to be located within the structural gene for the *uvrB* enzyme, leaving the regulatory sequences of the operon intact. Analyses to quantitate the induction of this system show that derepression of the operon is first detectable 5 min after UV exposure, with the rate of synthesis increasing to four to six times the uninduced rate during the subsequent 30 min. Induction is unaffected by mutations in other components of nucleotide excision repair. The control of *uvrB* was found to result from direct repression by the *lexA* gene product, with the *recA* gene product playing an indirect role. Nucleotide excision repair thus seems to be part of the SOS response.

The exposure of *Escherichia coli* to UV light leads to a variety of physiological changes (49). These changes, called the SOS response (32, 33), have been attributed to the induction of protein factors which contribute to the repair or processing of UV-induced DNA damage (32, 33, 49). The extent of the SOS response is drastically altered by mutations in two genes, *lexA* and *recA* (5, 8, 27, 30, 49). The role of these gene products in controlling the SOS response has recently been elucidated: the *lexA* gene product is the repressor for the *recA* operon (14), and the *recA* gene product is an activatable protease (34) which can derepress its own synthesis by cleaving the *lexA* product (23) or can derepress other operons by cleaving their repressors (19, 34).

Along with this elegantly controlled set of inducible functions, other UV repair systems have been identified which do not require induction to be detected. One of the important "constitutive" systems is the nucleotide excision repair system. In *E. coli*, this repair process is catalyzed by the combined action of the products of the *uvrA*, *uvrB*, and *uvrC* genes (43, 44). Nucleotide excision repair provides the cell with one of its most powerful defenses against the biological effects of UV light.

Recently we reported the isolation of a strain of *E. coli* K-12 in which Mu-d1(Ap^r *lac*) (3) (hereafter referred to as Mu-d1) was inserted into the *uvrB* operon (10). The mutation produced is called *uvrB*::Mu-d1-48. This insertion event causes the gene for β-galactosidase to be fused to the *uvrB* promoter. When this strain is exposed to UV light, the amount of β-galactosidase synthesized increases dramatically, suggesting that in normal strains the *uvrB* operon is induced after UV treatment. Another report appeared at about the same time showing that the *uvrA* operon is also inducible (20). Thus, at least two of the three components of nucleotide excision repair seem to be controlled.

This paper presents the results of our continuing efforts to understand the regulation of the *uvrB* operon. The gene products involved in its control have been identified, and the kinetics and extent of its induction have been determined. These results, and those already published for the *uvrA* operon (20), suggest that the nucleotide excision repair system is part of the SOS response.

MATERIALS AND METHODS

Chemical reagents. Agar and tryptone were from Difco Laboratories. Ampicillin, tetracycline, and *o*-nitrophenyl-β-D-galactopyranoside, as well as all necessary amino acids, sugars, and vitamins used in the growth of cultures, were purchased from Sigma Chemical Co. L-[^{35}S]methionine and [^{32}P]dTTP were obtained from New England Nuclear Corp. L-[^{14}C]tyrosine was a gift from B. Hall. Restriction enzymes and bovine serum albumin were purchased from Bethesda Research Laboratories. Other chemicals were from J. T. Baker Chemical Co.

Bacterial growth medium. Medium used for the growth of cultures was supplemented M9 salts or L broth as described by Miller (26).

Bacterial strains. The *E. coli* K-12 strains constructed for and used in this study are listed on Table 1. All of the strains used in this study were temperature resistant because they were constructed from CON482R, a temperature-resistant isolate of the original *uvrB*::Mu-d1-48 insert (10).

Determination of the site of insertion of Mu-d1 into

the *uvrB* operon. Strains JC3890, AB1157, and CON484 were grown in L broth at 37°C to a cell density of 5×10^8/ml. The cells were spun down and suspended in 4.5 ml of 20 mM Tris-hydrochloride (pH 7.3)–1 mM EDTA. The cells were lysed and the DNA was extracted as previously described (38). Finally, the DNA was mixed with a solution of CsCl (1.72 g/ml) and spun for 72 h at 110,000 × *g*. The DNA bands were collected and dialyzed extensively against 10 mM Tris-hydrochloride (pH 8.0)–1 mM EDTA. The DNA was then precipitated with ethanol and redissolved in 1.0 ml of water.

The DNA was digested in a 0.15-ml reaction containing 200 nmol of DNA, 20 mM Tris-hydrochloride (pH 7.3), 10 mM $MgCl_2$, 50 mM NaCl, 5 μg of bovine serum albumin per ml, 32 U of restriction endonuclease *Pst*I, and 30 U of restriction endonuclease *Eco*RI. The reaction was incubated for 6 h at 37°C. It then was made 5% in glycerol, 50 mM in EDTA, and 0.125% in sodium dodecyl sulfate. The samples were frozen until gels could be prepared.

About 50 nmol of restricted DNA was added to wells of a 1% agarose slab gel, and electrophoresis was carried out as previously described (45). The DNA in the gels was then denatured and transferred to nitrocellulose paper by the method of Southern (46). To this filter 1.8×10^7 dpm of ^{32}P-labeled pDR1494 DNA was added in Denhardt preincubation mix (9), 4× SSC (1× SSC: 0.15 M NaCl–0.015 M sodium citrate), and 40% formamide. The plasmid pDR1494 was the generous gift of W. D. Rupp. It was constructed by inserting the *uvrB* operon and a portion of the *bio* operon into pBR322. The construction and characterization of this plasmid are described elsewhere (37). This probe was isolated, purified, and labeled in vitro to a specific activity of 10^7 dpm/μg by nick-translation with DNA polymerase I and [^{32}P]dTTP (25). After hybridization for 18 h at 37°C, the filter was washed in 4× SSC–40% formamide and then in 2× SSC. The banding pattern was then determined by autoradiography.

Determination of cellular β-galactosidase levels. The method for determining the amount of β-galactosidase in a sample has been described previously (10). The amount of activity observed was routinely normalized

TABLE 1. Bacterial strain list

Strain	Genotype	Source (reference)
AB1157	F⁻ *thi-1 thr-1 leu-6 proA2 his-4 argE3 lacY1 galK2 ara-14 xyl-5 mtl-1 tsx-33 rpsL31 supE44*	Mount (16)
AB1884	Same as AB1157 plus *uvrC34*	Coli Genetic Stock Center (16)
AB1886	Same as AB1157 plus *uvrA6*	Coli Genetic Stock Center (16)
AB2494	Same as AB1157 plus *lexA1* Met⁻ Arg⁺	Defais (15)
AW518MR	F⁻ *thr leu his tsx gal1 lac xyl ara rpsL tse metF rpoB tonA*	Kort (22)
DM511	Same as AB1157 plus *lexA3 tsl-1*	Mount (29)
DM1420	F⁻ *lexA3 spr sfiA11*	Defais (28)
JC3890	Same as AB1157 plus Δ*uvrB301*	Kato (18)
KL16-99	HfrG6 *recA1 gyrA*	Coli Genetic Stock Center (24)
JC7595	Same as AB1157 plus *tif-1 sfiB103*	Clark (personal communication)
CON482R	AB1157 plus *gal*⁺ *uvrB*::Mu-d1-48	Temperature-resistant derivative of Ampʳ transconjugant from cross of AB1157 and CON48 (10)
CON480A	AB1886 plus *uvrB*::Mu-d1-48	Ampʳ transductant of AB1886 infected with $P1_{cml}$ grown on CON482R
CON480C	AB1884 plus *uvrB*::Mu-d1-48	Ampʳ transductant of AB1884 infected with $P1_{cml}$ grown on CON482R
CON484	AB1157 plus *gal*⁺ *uvrB*::Mu-d1-48	This laboratory (10)
CON48AW	AW518MR plus *uvrB*::Mu-d1-48	Ampʳ transductant of AW518MR infected with $P1_{cml}$ grown on CON482R
CON486	AB2494 plus *uvrB*::Mu-d1-48	Ampʳ transductant of AB2494 infected with $P1_{cml}$ grown on CON482R
CON487	DM511 plus *uvrB*::Mu-d1-48	Ampʳ transductant of DM511 infected with $P1_{cml}$ grown on CON482R
CON488	CON484 plus *his*⁺ *recA1*	*His*⁺ UVˢ transconjugant of cross between CON484 and KL16-99
CON489	JC7595 plus *uvrB*::Mu-d1-48	Ampʳ transductant of JC7595 infected with $P1_{cml}$ grown on CON482R
CON4810	DM1420 plus *uvrB*::Mu-d1-48	Ampʳ transductant of DM1420 infected with $P1_{cml}$ grown on CON482R
CON4811	CON4810 plus *recA1*	Thy⁺ UVˢ transconjugant from a cross between *thyA* derivative of CON4810 and KL16-99
CON48AW(pBR322)	CON48AW carrying pBR322 (1)	Tetʳ transformant of CON48AW
CON48AW(pDR1494)	CON48AW carrying pDR1494 (37)	Tetʳ transductant of CON48AW
CON48AW(pDR1494-1)	CON48AW carrying pDR1494-1 (37)	Tetʳ transductant of CON48AW

to cell number, as reflected in absorbance of 600-nm light.

When the differential rate of synthesis of β-galactosidase was determined relative to total protein synthesized, the procedure was modified slightly. The cells were grown at 37°C in supplemented M9 medium to a cell density of 2×10^8/ml. A 2-ml sample was then removed to a watch glass and exposed to UV light from a Sylvania G30T8 germicidal lamp, maximum output at 254 nm. Samples (1.0 ml) from this irradiated culture and a similar unirradiated control culture were added to 4 ml of growth medium. From these diluted cultures, 0.66-ml samples were removed to separate tubes, and [^{35}S]methionine was added to give a final concentration of 110 μM and 1.2 μCi/ml. Samples (0.5 ml) from the unlabeled culture were taken at intervals and assayed for their ability to hydrolyze *o*-nitrophenyl-β-D-galactopyranoside as described previously (26), and duplicate 0.05-ml samples of the labeled culture were spotted on filter paper and washed three times in 5% trichloroacetic acid and finally in ethanol in preparation for counting in a scintillation counter.

For experiments in which the cells carried a pBR322 plasmid, the procedure was altered somewhat. The radioactive amino acid used for these cells was [^{14}C]tyrosine. Furthermore, a small amount of tryptone (0.2 mg/ml) and tetracycline (15 μg/ml) was added to all cultures to assure maintenance of the plasmid. In this case the final tyrosine concentration during labeling was 60 μM and the radioactivity was 1 μCi/ml.

Determination of differential rates of β-galactosidase synthesis in CON4810 and CON4811 was done by labeling a culture with [^{35}S]methionine and sampling enzyme activity and radioactivity at intervals over the next 4 h.

Kinetics of *uvrB* induction. A culture of CON484 (15 ml) was grown at 37°C in M9 salts supplemented with glucose, necessary amino acids, and 0.2 g of tryptone per liter. At a cell density of 5×10^7/ml, 4.6 ml of culture was placed in a tube containing [^{35}S]methionine to give a final concentration of methionine of 110 μM and 1.2 μCi of radioactivity per ml. Both cultures were allowed to continue growing to a cell density of 10^8/ml. A sample (3 ml) of the unlabeled culture and a sample (1.5 ml) of the labeled cells were placed in watch glasses and exposed to 5 J/m^2 of UV light. The cells were immediately returned to tubes at 37°C and sampled: 0.5 ml of unlabeled cells for β-galactosidase activity, and duplicate 0.05-ml samples of labeled culture for radioactivity. A similar set of tubes was produced for the unirradiated culture. Sampling in this manner was continued at intervals for 30 min. The change in β-galactosidase per micromole of methionine incorporated was determined between each set of adjacent sample points, and this slope was plotted against the median time of the samplings to generate a representation of the change of rate of β-galactosidase production with time after UV exposure.

RESULTS

Gene products involved in control of the *uvrB* operon. There have been indications for some time that certain proteins are induced when *E. coli* is exposed to UV light (32, 33). This induction, known as the SOS response, is substantially reduced in *lexA* and *recA* mutants (49). Although the *uvrB* protein was not thought to participate in the SOS response, the observation that this operon is inducible (10) made it quite plausible that its synthesis is controlled by the *lexA* and *recA* gene products. To test this hypothesis, a strain carrying the *uvrB*::Mu-d1-48 insert and a *lexA1* mutation, designated CON486, and one carrying the insert and a *recA1* mutation, designated CON488, were constructed. Figure 1 shows the comparative levels of β-galactosidase produced in UV-irradiated and control cultures of CON484 (*lexA*$^+$ *recA*$^+$), CON486 (*lexA1 recA*$^+$), and CON488 (*lexA*$^+$ *recA1*). As mentioned in the introduction, the level of β-galactosidase synthesis in these strains is a measure of the amount of transcription of the *uvrB* operon. Neither the *lexA1* nor *recA1* mutation affects the constitutive level of *uvrB* synthesis, but both reduce the extent to which this operon is induced by UV irradiation.

Extent of *uvrB* induction. To measure more accurately the extent of increase in *uvrB* expression after UV exposure, the rate of β-galactosidase synthesis was determined by comparing the amount of β-galactosidase activity to the amount of incorporation of a radioactively labeled amino acid. Figure 2 shows the results of this determination. The slopes of these lines represent the relative rate of β-galactosidase activity in unirradiated cells or cultures exposed to 2.5 J/m^2 of UV light. A comparison of these slopes indicates that this UV exposure produces a 4.6-fold increase in the relative rate of β-galactosidase synthesis. Table 2 gives the results of similar experiments carried out with strains carrying the *lexA1* and *recA1* mutations. These more quantitative results demonstrate that the *lexA1* mutation prevents nearly all UV induction, and the *recA1* allele, while reducing the level of induced synthesis, is somewhat leaky.

To further substantiate the role of the *lexA* and *recA* gene products in control of *uvrB*, a second set of strains was constructed in which the *uvrB*::Mu-d1-48 mutation was placed into cells with either the *tsl-1* allele of *lexA* (29) or the *tif-1* allele of *recA* (21). These mutations have similar effects on the cell, causing increased expression of the SOS response when the cells are grown at 42°C (4, 48). Table 3 shows that both the *tsl-1* and *tif-1* mutations promote the thermal induction of the *uvrB* operon.

Kinetics of *uvrB* induction. Figure 1 indicates that the increase in β-galactosidase activity in a UV-irradiated culture begins soon after treatment. To determine the exact timing of this induction, the differential rate of β-galactosidase synthesis was determined at intervals after UV exposure. Figure 3 is a plot of the relative rate of β-galactosidase synthesis during each of these

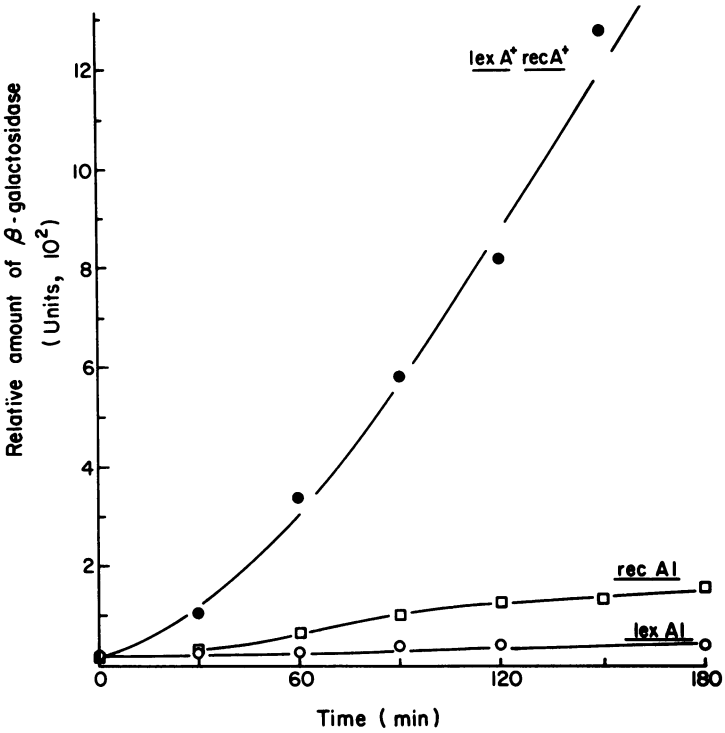

FIG. 1. UV induction of β-galactosidase in CON484 (*lexA*$^+$ *recA*$^+$) (●), CON486 (*lexA1 recA*$^+$) (○), and CON488 (*lexA*$^+$ *recA1*) (□). Cultures were exposed to 2.5 J/m^2 of 254-nm light, and β-galactosidase activity was assayed at the times indicated. Since the shape of these *sfi*$^+$ cells, and thus their absorbance, changes after UV irradiation, units are expressed relative to the absorbance at 600 nm of the culture at the time of irradiation.

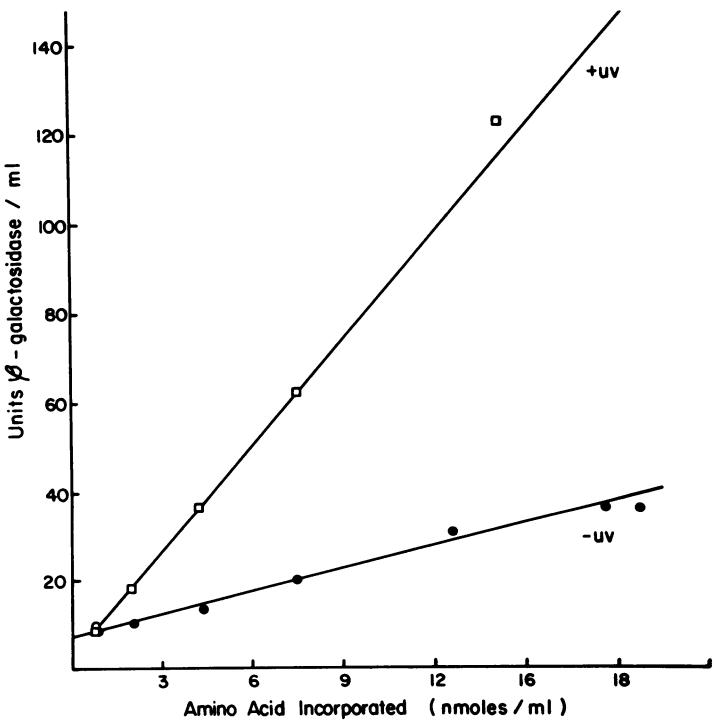

FIG. 2. Differential β-galactosidase synthesis in control (●) or UV-irradiated (2.5 J/m^2) (□) cultures of CON48AW.

TABLE 2. Effect on *uvrB* expression of mutations in other genes involved in DNA repair

Strain	Relevant genotype[a]	Units of β-galactosidase per μmol of methionine incorporated[b]		Fold induction
		Control	+5 J/m² UV	
CON484	*lexA*⁺ *recA*⁺	603	2,922	4.8
CON486	*lexA1 recA*⁺	647	868	1.3
CON488	*lexA*⁺ *recA1*	626	1,287	2.1
CON480A	*uvrA6*	676	3,371	5.0
CON480C	*uvrC34*	599	2,580	4.3
CON4810	*spr recA*⁺	3,661	—	6.1[c]
CON4811	*spr recA1*	3,794	—	6.3[c]

[a] All strains carry the *uvrB*::Mu-d1-48 mutation.
[b] Numbers represent an average of two to five independent determinations. —, Not determined.
[c] Fold induction relative to uninduced CON484.

intervals. An increased rate of synthesis can be seen within 5 min, and full induction is reached within 30 min. Thus derepression of the *uvrB* operon occurs rapidly after UV exposure.

Direct repression of *uvrB* by the *lexA* gene product. The results reported thus far are consistent with two alternative models. Either the *uvrB* operon is directly repressed by the *lexA* gene product, or it has another repressor which is susceptible to *recA* proteolysis. To distinguish between these alternatives, two more strains were constructed. The first strain, called CON4810, was made by transducing *uvrB*::Mu-d1-48 into a strain carrying the *spr* allele of *lexA*. The *spr* mutation destroys *lexA* function, so these cells constitutively produce the SOS response at any temperature (28). Into CON4810, the *recA1* mutation was then introduced to generate a strain called CON4811, in which both *lexA* and *recA* activities were absent. Table 2 shows that the *spr* mutation causes a sixfold increase in the relative rate of β-galactosidase synthesis as compared with a *lexA*⁺ cell. The introduction of the *recA1* allele into these cells did not effect the *spr*-mediated rate of synthesis; thus, in the absence of the *lexA* repressor the proteolytic activity of the *recA* protein is not essential for derepression of *uvrB*. This argues strongly for a direct role for the *lexA* repressor in the control of *uvrB*.

Effect of components of excision repair on control of *uvrB*. Since the insertion of Mu-d1 into the *uvrB* gene prevents the synthesis of active *uvrB* product, all of the *uvrB*::Mu-d1-48 strains are deficient in nucleotide excision repair (10). It could be argued that the *lexA*-controlled induction of this operon results from this deficiency and might not occur in repair-proficient cells. To test this, plasmids carrying a cloned segment of DNA from the *uvrB* region of the chromosome were introduced into cells carrying the *uvrB*::Mu-d1-48 insertion. These plasmids carry either an active or an inactive *uvrB* gene (37). Figure 4 shows a dose-response curve of the relative rate of β-galactosidase synthesis in these strains after various UV doses. The strain without any plasmid, the strain carrying the cloning vector (pBR322), and the strain carrying a plasmid with an inactive *uvrB* clone (pDR1494-1) all show similar dose-response curves. The strain carrying a plasmid with an active *uvrB* gene (pDR1494) requires about 10 times as much UV exposure to respond, but its *uvrB* operon can ultimately be induced to the same extent as its UvrB⁻ counterparts. Thus, the presence of an active nucleotide excision repair system does

TABLE 3. Thermal induction of *uvrB* in strains carrying *tsl* and *tif* mutations

Strain	Relevant genotype[a]	Units of β-galactosidase per μmol of methionine incorporated[b]		Fold induction at 42°C	
		30°C	42°C	Relative to self at 30°C	Relative to CON484 at 42°C
CON484	*lexA*⁺ *recA*⁺	771	695	0.9	1.0
CON487	*tsl-1 recA*⁺	878	2,422[c]	2.8[c]	3.5[c]
CON489	*lexA*⁺ *tif-1*	1,052	5,865	5.6	8.4

[a] All strains carry the *uvrB*::Mu-d1-48 mutation.
[b] Numbers represent an average of two to five independent determinations.
[c] CON487 is *sfi*⁺, thus growth at 42°C kills the cell (12). Methionine incorporation slowed dramatically after a short time at 42°C, preventing complete induction of the *uvrB* operon.

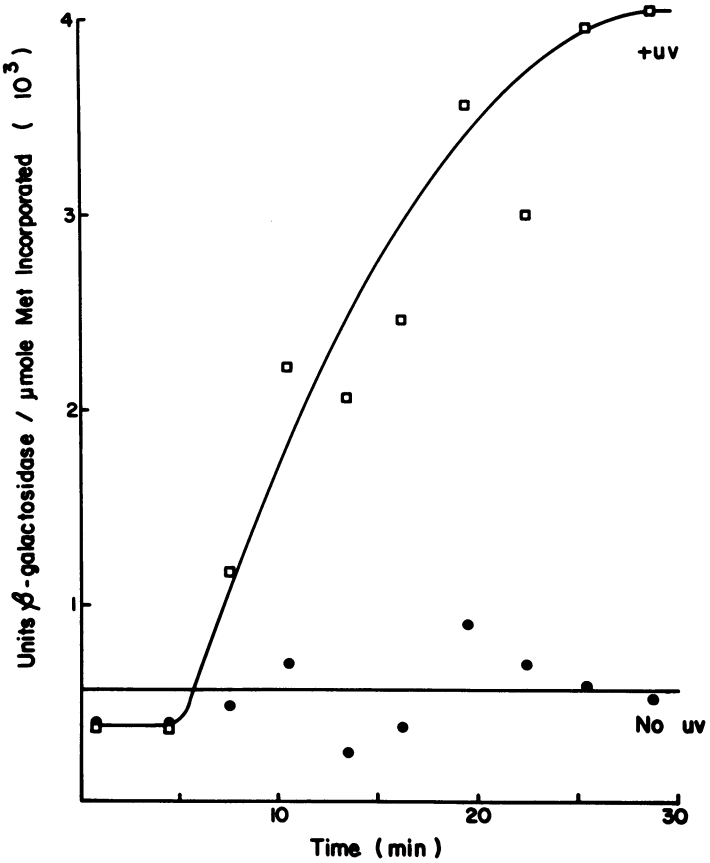

FIG. 3. Rates of induction of the *uvrB* operon after exposure to UV light. A culture of CON484 was split, and half was kept as an unirradiated control (●) while the rest was exposed to a dose of 5 J/m^2 of 254-nm light (□). The amounts of β-galactosidase produced and methionine incorporated were measured at various times, and the rate of β-galactosidase synthesis for each interval was calculated and plotted against the time elapsed since irradiation.

not prevent induction of *uvrB*, although it substantially alters the dose-response curve.

The induction of *uvrB* seems to be unaffected by mutations in the other components of excision repair. Table 2 shows that mutations *uvrA6* and *uvrC34* do not significantly alter the response of the *uvrB* operon to UV damage.

Identification of site of insertion of Mu-d1 into the *uvrB* operon. The results presented show that the β-galactosidase gene inserted within the *uvrB* operon is controlled by the *lexA* protein and is rapidly induced after UV exposure. Whereas the interpretation of these experiments seems straightforward, one possible problem must be considered. The exact location of the Mu-d1 within the *uvrB* operon could be important. If Mu-d1 is inserted within the structural gene for *uvrB*, then the results presented are probably an accurate indication of normal *uvrB* induction. On the other hand, if Mu-d1 is inserted into the regulatory region of the *uvrB* operon somewhere between the promoter and the start of the structural gene, it might have destroyed some important control sequence. If so, the induction of β-galactosidase would not be an accurate representation of what normally occurs with an intact *uvrB* operon.

To determine the site of Mu-d1-48 insertion, DNA was isolated from a strain of *E. coli* which carries the *uvrB*::Mu-d1-48 mutation (CON484), a strain which carries a normal *uvrB* sequence (AB1157), and a strain which is deleted for the *uvrB* gene (JC3890). These DNA samples were digested with *Pst*I and *Eco*RI, the DNA fragments were separated on an agarose gel, and the fragments were transferred to nitrocellulose paper and allowed to hybridize with ^{32}P-labeled pDR1494 DNA. As mentioned before, the plasmid pDR1494 carries the *uvrB* operon and a small portion of the DNA around it (37). The cloned segment is bounded by *Pst*I sites. Within the segment is a single *Eco*RI site, which is

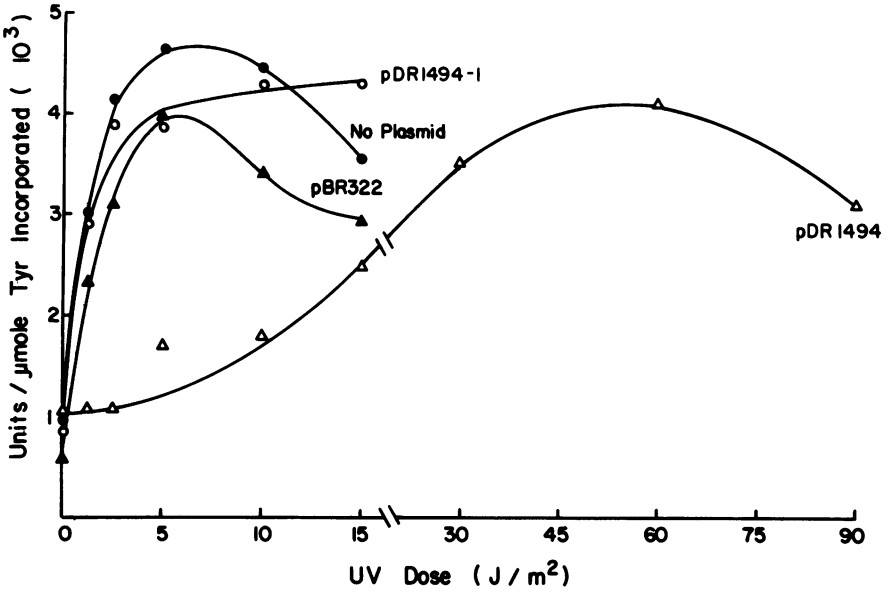

FIG. 4. Differential rate of β-galactosidase synthesis during the 2 h after exposure to various amounts of 254-nm light. The parent strain CON48AW (●), CON48AW(pBR322) (▲), and CON48AW(pDR1494-1) (○) are all phenotypically UvrB$^-$. The strain CON48AW(pDR1494) (△) is phenotypically UvrB$^+$ because it carries a plasmid with an active *uvrB*$^+$ gene.

located within the structural gene but very near the translational start site (W. D. Rupp, personal communication). Thus, the probe will hybridize to two DNA fragments generated by digestion of the *E. coli* genome with *Pst*I and *Eco*RI. The smaller of these fragments (1.1 kilobases) carries the entire regulatory region of the *uvrB* operon plus a few nucleotides of the structural gene. The longer piece (4.7 kilobases) carries most of the structural gene sequences (37).

Figure 5 is a picture of the autoradiogram of the blotted and hybridized *Pst*I-*Eco*RI fragments produced from the DNA of the three strains of *E. coli* described above. In lane A, DNA from AB1157 is shown to contain the two *uvrB* operon restriction fragment bands. In lane B, DNA from CON484 was run. The Mu-d1-48 insertion within this strain left the *uvrB* regulatory sequences intact, while destroying the 4.7-kilobase band which carries the structural gene sequence. In its place, four bands can be seen. Two of these bands represent the *uvrB* structural sequences on either side of the Mu-d1-48 insert. The other two fragments arose from within Mu-d1 itself. They are composed of the sequences which flank the *Pst*I site found within the β-lactamase gene of the Mu-d1 (3). Lane C has DNA from JC3890. This strain is deleted for *uvrB* and shows no *uvrB* bands. These results show that the Mu-d1-48 insertion is located within the structural gene for *uvrB*, and thus the information we have gathered about the control of the *uvrB* operon is probably valid.

DISCUSSION

The results presented in this paper indicate that the *uvrB* protein is one of the inducible *lexA*$^+$ *recA*$^+$-dependent functions which comprise the SOS response (32, 33, 49). When cells are exposed to UV light, the rate of synthesis of the product of the *uvrB* operon increases rapidly, reaching a maximum within 30 min. Given an appropriate UV dose, the induced rate of synthesis can reach five times the basal rate. The rate of production of the *uvrB* gene product is controlled directly by the *lexA* protein, which presumably acts as a repressor for the operon, and indirectly by the *recA* protein, which is instrumental in inactivating the *lexA* protein (23). The other components of nucleotide excision repair, namely, the *uvrA* and *uvrC* gene products, do not seem to be directly involved in the regulation of *uvrB* in that mutations in these genes do not significantly alter the synthesis of *uvrB* either before or after UV exposure. It is possible, of course, that the products of the mutant alleles *uvrA6* and *uvrC34* are deficient in repair but are normal for some hypothetical regulatory function. If so, some other mutant of *uvrA* or *uvrC* might show an effect on *uvrB* induction. Our data cannot exclude this possibility. What is quite clear from our results is that

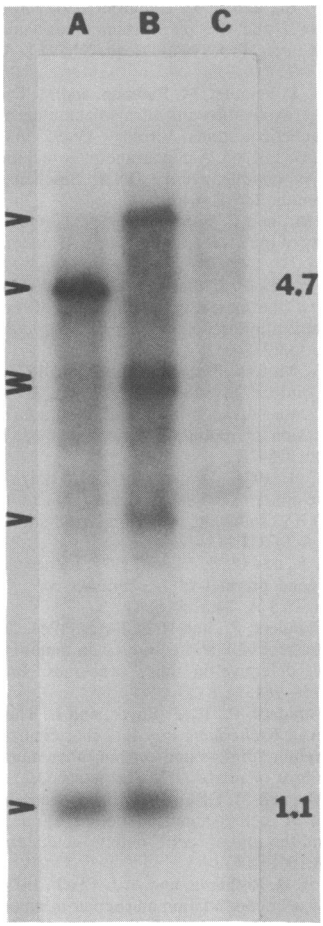

FIG. 5. Autoradiogram of *Pst*I-*Eco*RI fragments of *E. coli* DNA separated on an agarose gel, blotted to nitrocellulose, and hybridized with ^{32}P-labeled pDR1494 DNA. Lane A, AB1157 DNA (*uvrB*$^+$). Lane B, CON484 DNA (*uvrB*::Mu-d1-48). Lane C, JC3890 DNA (Δ*uvrB*). The numbers represent the size of DNA fragments in kilobases.

the *lexA* protein plays an active role in the control of this operon.

The presence of a functional nucleotide excision repair system in *E. coli* increases the dose of UV light necessary to produce maximal induction of *uvrB*, but it does not affect the extent of induction eventually achieved. This shows that the *uvrB* operon can be induced by UV light even in wild-type cells and that the presence of a functional excision repair system effectively reduces, but does not eliminate, the production of DNA degradation products essential for activation of the *recA* protein (17, 31) and induction of the SOS response.

The use of a Mu-d1 insertion to study the regulation of an operon has many advantages over more conventional approaches. One possible problem with this method, however, is that insertions within the regulatory region of an operon (as long as they are not within the promoter) are phenotypically indistinguishable from inserts into the structural gene. If an insertion occurs within the binding site for a regulatory protein, the strain produced will act as if the affected regulatory element is irrelevant to control of the operon. The chances of selecting such a strain are small but not zero; thus, it is important to establish that the insertion has occurred within the structural gene, leaving the control region unaffected. In the case of the *uvrB*::Mu-d1-48 insertion this seems to be the case.

Recently, progress on the sequencing of the regulatory region of the *uvrB* operon has revealed three promoter sequences within this DNA segment (Rupp, personal communication). All of these promoters seem to be active in vitro. Initiation of transcription from at least one of them is apparently unregulated. It is not known which of these sequences is most active in vivo, but in our strains the unregulated promoter appears to be relatively weak, since *lexA* protein can block up to 85% of *uvrB* transcription. It is possible, of course, that the sequence of this region varies among strains of *E. coli* K-12. If strain CA7092, the strain used to generate *uvrB*::Mu-d1-48, were not to carry the unregulated promoter sequence found in strain CS520, from which the Clarke-Carbon bank was derived (6) and from which the sequence data were obtained, then it would be clear why most *uvrB* transcription is regulated in our cells but may not be in some other strains. We have no reason to believe that such variation exists, but since it is possible, we are presently pursuing these questions to try to clarify the situation.

Putting these results together with the report by Kenyon and Walker (20), which showed that the *uvrA* operon is also controlled by the *lexA* gene product, it seems reasonable to anticipate that the *uvrC* operon may also be inducible. If so, then clearly an increase in nucleotide excision repair capacity is part of the SOS response. This notion is supported by a variety of reports which demonstrate the presence of *uvrA*$^+$ *uvrB*$^+$-dependent, inducible functions. These include an inducible form of postreplication repair (39) which may lead to increased resistance to UV light (11, 40–42), and an error-free mechanism of Weigle reactivation (35). The inclusion of nucleotide excision repair in the SOS response is attractive because it simplifies a substantial amount of phenomenology into one unified model. At present, however, few or no hard data are available to substantiate this idea. For the induction of the *uvr* system to have a significant biological impact, the basal level of these

enzymes must normally limit the rate of nucleotide excision repair. Biochemical studies on the rate of pyrimidine dimer excision have found that initially there is a dose-dependent increase in the rate of dimer repair (2). However, experiments designed to detect an increased rate of dimer excision in cells first treated with an inducing dose of UV light were reported to be negative (41). Unfortunately, these experiments made use of large doses of UV which killed most of the cells; thus, the saturation of repair capacity they found may represent the biochemistry of the dead rather than the surviving fraction of cells. Furthermore, the excision of dimers may not be an appropriate criterion upon which to judge whether the *uvrA*, *uvrB*, and *uvrC* operons are induced. These gene products only produce incisions at dimer sites (43, 44). The excision of pyrimidine dimers relies on gene products such as DNA polymerase I (7, 13). Since the *polA* gene is not inducible (47), its gene product may limit the rate of dimer removal. If the incision event is the vital step for producing the biological effects, then the biological phenomenology consistent with *uvr* inducibility may be reconcilable with the biochemical results in the literature. Further work is necessary to clarify this point finally.

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