JOURNAL OF BACTERIOLOGY, July 1979, p. 48-53
0021-9193/79/07-0048/06$02.00/0

Vol. 139, No. 1

Expression of the Cloned *uvrB* Gene of *Escherichia coli*: Dependency on Nonsense Suppressors

HANS PANNEKOEK,* INGE NOORDERMEER, AND PIETER VAN DE PUTTE

Laboratory of Molecular Genetics, University of Leiden, The Netherlands

Received for publication 24 April 1979

Recombinant plasmid pNP5, consisting of plasmid pMB9 on which the *uvrB* gene is cloned, fully complements for the defects due to chromosomal *uvrB* mutations in the presence of the amber suppressor *sup-6* or *supF*. Correndonuclease II activity was also completely restored in UvrB strains containing both plasmid pNP5 and amber suppressor *sup-6*, as compared with the parental UvrB$^+$ strain. It is shown that the amber mutation which interferes with the expression of the cloned *uvrB* gene is located outside this gene. Apparently, the amber mutation exerts a polar effect on *uvrB* expression that is relieved by *sup-6* or *supF*. Introduction of a *rho* mutation into suppressor-free UvrB strains, harboring pNP5, did not relieve the polarity caused by the amber mutation.

The *uvrB* gene of *Escherichia coli* encodes a protein which is required for the initial enzymatic reaction(s) of the excision repair process. A mutation in the *uvrB* locus renders the bacterium extremely sensitive to irradiation with UV light. The UvrB$^+$ protein, in concert with the products of the *uvrA* and *uvrC* genes, catalyzes an incision reaction on UV-irradiated DNA, causing a single-strand scission in the direct vicinity of the photoproduct (pyrimidine dimer)(3). It has been shown that *uvrB* mutants indeed lack an enzymatic activity, called correndonuclease II, which is responsible for the incision (2, 10, 15).

Previously, we have reported on the molecular cloning of the *uvrB* gene of *E. coli* (10). In that study we outlined the construction of a recombinant plasmid (pNP5) consisting of the multicopy plasmid pMB9 (13) and an *Eco*RI fragment, derived from phage λ*b2att*^{2}, carrying the *uvrB* gene. It was found that a UvrB deletion strain transformed with plasmid pNP5 was more UV resistant than the untransformed mutant strain, but less UV resistant than the parental UvrB$^+$ strain. Moreover, we could not detect a concomitant increase of correndonuclease II activity upon transformation of UvrB strains with pNP5.

In this paper we have elucidated the reason for the partial restoration of UV resistance caused by plasmid pNP5. The genetic characters were determined which permit complete complementation of chromosomal *uvrB* mutations and provide for a level of correndonuclease II activity equal to that of UvrB$^+$ strains.

MATERIALS AND METHODS

Bacteria and bacteriophages. The bacterial strains used are listed in Table 1. Bacteriophage λ*b2att*^{2} [λ*b2*cI857*intam*6Δ(*bioAB*)*bioFCD*$^+$*uvrB*$^+$] was supplied by M. E. Gottesman. Phage φ80 was obtained from I. E. Mattern. Phage Mu13-4 has been isolated by G. C. Westmaas. Muc$^+$, Mu*Eam*, λ*c*I857*plac5Sam7*, φ80*vir*, and P1 were from our own collection. Phage φ80*supF* was originally from H. Ozeki.

Transformation of competent bacteria. The preparation of competent bacteria and the procedure for transformation with plasmid DNA have been described previously (10).

Survival of bacteria after irradiation with UV light. The survival of bacteria after UV irradiation was determined as described (10). Streaking of single colonies on L-broth agar plates (supplemented with antibiotics if required) followed by irradiation of a segment of the plates was used as a rapid qualitative assay for UV resistance of bacteria.

Construction of λ*b2att*^{2} lysogens. The transduction property of phage λ*b2att*^{2} was employed to isolate lysogens. This phage harbors the *bioFCD* genes which enabled us to select for Bio$^+$ transductants of *bioFCD-uvrB-chlA* deletion strains.

Isolation of UvrB deletion mutants. The UvrB deletion mutants that we constructed all contained the *bioFCD-uvrB-chlA* deletion of strain C261. This deletion was transferred from this strain by P1 transduction. The selection was based on resistance against 0.2% potassium chlorate (8) and checked for a concomitant *bio uvrB* genotype.

Introduction of *rho* mutations in UvrB strains. *Rho* mutations were introduced by P1 transduction. Phage P1 was propagated on strain X60suA *psuA1* and used to select *ile*$^+$ transductants of strain HP3424 Δ*uvrB ile*. Cotransduction of *rho* (approximately 10%) was verified by plating phage Mu13-4, which contains

TABLE 1. *Bacterial strains of E. coli K-12*

Strain[a]	Relevant genotype	Origin
T5-2	Δ(*attλ-bio-uvrB*) *supE*	C. Fuerst
JC3890	Δ(*bio-uvrB*) *supE44*	A. J. Clark
KMBL1121	Δ(*bio-uvrB-chlA*)	Our laboratory
C261	Δ(*bioFCD-uvrB-chlA*)	A. Campbell
HP3412	P678-54 *gal*$^+$ Δ(*uvrBC*)*261 supE*	H. I. Adler; P1 (C261) transduction, gal$^+$ selection
KMBL90	*uvrB501*	Our laboratory
KMBL2838	*uvrB513*	Our laboratory
AB2434	*uvrB5*	A. J. Clark
KMBL566	*sus uvrB*	Our laboratory
S90C	Δ(*lac-pro*)	J. H. Miller via B. W. Glickman
XA101, 102, 103, 105, 106, 10C	Respectively, *supD*, *supE*, *supF*, *sup-5*, *sup-6*, *supC* derivatives of S90C	J. H. Miller via B. W. Glickman
HP3430 to 3437	Δ*uvrB261* into S90C, XA101, 102, 103, 105, 106, 10C	This work
HP3437 to 3444	HP3430 to 3437 containing pNP5	This work
HP3444 and 3445	HP3430 and 3435 lysogenic for λ*b2att*^{2}	This work
HP3446 and 3447	HP3430 and 3435 lysogenic for λ	This work
X60suA	*psuA1*	M. Howe
HP3424	KMBL1121 *ile*	Spontaneous *ile* mutant
HP3464 and 3465	S90C containing, respectively, pNP5 or pMB9	This work
HP3466	HP3430 containing pMB9	This work
HP3467 and 3468	XA106 containing, respectively, pNP5 or pMB9	This work
HP3469	HP3435 containing pMB9	This work
HP3470	KMBL1121 containing plasmid pNP5	This work
HP3471	KMBL1121 lysogenic for φ80*supF*, containing plasmid pNP5	This work
HP3472	KMBL1121 *psuA1*, containing plasmid pNP5	P1 (X60suA) transduction; selection, Mu13-4 titration
HP3473	HP3472 lysogenic for φ80*supF*, containing plasmid pNP5	This work

[a] All bacterial strains are F$^-$.

the insertion element IS1 (16). The *psuA1* allele allows the development of this phage, resulting in plaques of regular size, whereas on Rho$^+$ strains Mu13-4 yields "pinpoint" plaques (P. van de Putte, manuscript in preparation).

Determination of correndonuclease II activity in bacterial extracts. The procedure for the preparation of a lysate from plasmolyzed cells and the determination of the ATP-dependent correndonuclease II activity were according to Seeberg (14). In our assays ^{3}H-labeled DNA of phage PM2 (a gift of G. Veldhuizen, Medical Biological Laboratory, Rijswijk, The Netherlands) was used as a substrate. This DNA preparation was irradiated with a UV dose of 2,000 ergs/mm^2 (2 to 3 pyrimidine dimers per molecule). ^{3}H-labeled PM2 DNA consisted of about 60% covalently closed circles (CCC), a percentage which remained the same after irradiation. Reactions (in duplicate) were carried out in a volume of 0.14 ml for 15 min at 37°C. The reaction mixtures contained 0.1 μg of ^{3}H-labeled PM2 DNA (5×10^3 cpm) and 20 μl of crude extract from plasmolyzed bacteria. Separation of CCC DNA from open circular (OC) DNA was done as described previously (4).

RESULTS

Transformation of UvrB strains with plasmid pNP5. Previously, we have reported that strain JC3890 Δ*uvrB*, containing plasmid pNP5, displays an intermediate Uv^r phenotype (10). Here, we determined whether this property is restricted to this particular UvrB strain. For that purpose various UvrB strains were transformed with plasmid pNP5, and the UV resistance was measured. Our results (Table 2) show that the transformed strains T5-2, JC3890, HP3412, and KMBL90 have an intermediate Uv^r phenotype, whereas others remain as Uv^s as

TABLE 2. *UV resistance of various UvrB strains transformed with plasmid pNP5*[a]

Transformed strain	*uvrB* mutation	UV resistance	Suppressor
T5-2	Deletion	±	*supE*
JC3890	Deletion	±	*supE*
KMBL1121	Deletion	−	−
C261	Deletion	−	−
HP3412	Deletion	±	*supE*
KMBL90	Missense	±	*supE*
KMBL2838	Missense	−	−
AB2434	Missense	−	−
KMBL566	Nonsense	−	−

[a] UvrB strains were transformed with pNP5. Transformants were selected on L-broth agar plates supplemented with 20 μg of tetracycline per ml. Single colonies were tested for Uv^r as described in the text. Intermediate Uv^r strains (indicated by ±) survived a UV dose of 125 ergs/mm^2 in a streaking test, but not one of 250 ergs/mm^2. The presence of *supE* or *supF* was determined by titration of, respectively, Mu*Eam* (amber specifically suppressed by *supE*) and λcI857p*lac*5*Sam*7 (amber specifically suppressed by *supF*).

their untransformed parental strains. Furthermore, there is no relation between the nature of the *uvrB* mutation and an observed partial Uv^r due to the presence of pNP5. Unexpectedly, however, there is a perfect correlation between Uv^r, acquired after transformation, and the presence of a resident nonsense suppressor (*supE*). Apparently, a nonsense mutation on the plasmid interferes with the expression of the cloned *uvrB* gene.

Effect of suppressors on UV resistance mediated by plasmid pNP5. It is conceivable that other nonsense suppressors might also affect the expression of the *uvrB* gene present on pNP5, possibly to a different extent. Therefore, we have constructed a set of isogenic strains, having the same *uvrB* deletion, but harboring different nonsense suppressors, i.e., *supC*, *supD*, *supE*, *supF*, *sup-5*, or *sup-6*. These strains were subsequently transformed with pNP5 and the Uv^r was determined (Fig. 1).

Our data show that in the presence of either amber suppressor *supF* or amber suppressor *sup-6* the pNP5-containing UvrB strains are equally as resistant to UV irradiation as the parental $UvrB^+$ strains. The amber suppressors *supD* and *supE* allow only partial Uv^r, which result confirms our previous observations (10). Strains which contain the ochre suppressors *supC* or *sup-5* are only slightly more Uv^r than an isogenic suppressor-free strain. From these results we conclude that an amber mutation on plasmid pNP5 interferes with the expression of the cloned *uvrB* gene in suppressor-free strains. Such interference can be relieved efficiently by the amber suppressors *supF* and *sup-6*, thereby creating the possibility for optimal expression of the cloned *uvrB* gene.

Correndonuclease II activity mediated by pNP5. We have reported before that no concomitant increase of correndonuclease II activity could be detected after introduction of pNP5 in strain JC3890 Δ*uvrB* (10). This lack of enzymatic activity might be related to the intermediate Uv^r phenotype displayed by this strain. We have applied our findings on the effect of specific amber suppressors on Uv^r of pNP5-containing UvrB strains to determine the correndonuclease II activity in crude extracts of *sup*$^+$ and *sup-6* Δ*uvrB* strains, harboring either plasmid pMB9 or pNP5.

Our data (Table 3) clearly show that an extract of strain HP3442 *sup-6* Δ*uvrB*(pNP5) (line 6) contains the same level of correndonuclease II activity as extracts of $UvrB^+$ strains (lines 1

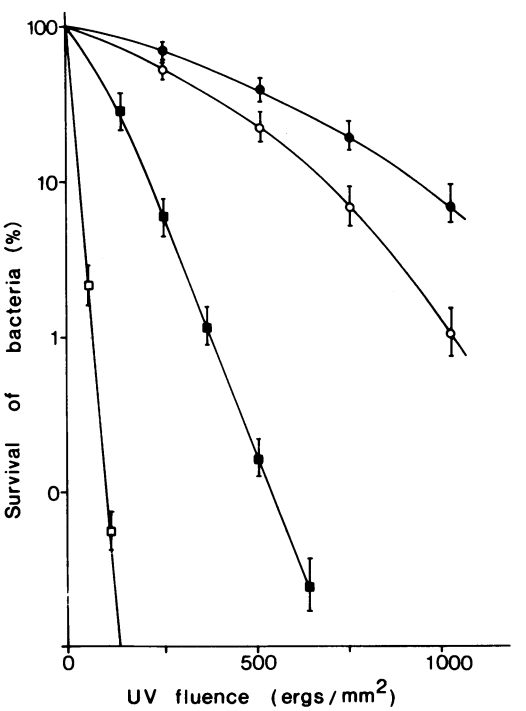

FIG. 1. *Suppression pattern of UV resistance encoded by plasmid pNP5. Strains were grown to the exponential phase, and the survival was determined as described in the text. Symbols:* (●) *S90C sup*$^+$ *uvrB*$^+$, *HP3440 supF uvrB(pNP5), HP3442 sup-6 uvrB(pNP5);* (○) *HP3438 supD uvrB(pNP5), HP3439 supE uvrB(pNP5);* (■) *HP3441 sup-5 uvrB(pNP5), HP3443 supC uvrB(pNP5);* (□) *HP3430 sup*$^+$ *uvrB, HP3435 sup-6 uvrB, HP3437 sup*$^+$ *uvrB(pNP5). The bars indicate the range of Uv*^{r} *of different strains.*

TABLE 3. *Determination of correndonuclease II activity in crude extracts of E. coli strains*[a]

Strain	Suppressor	UvrB	Plasmid	Correndonuclease II
HP3465	–	+	pMB9	29.6
HP3466	–	–	pMB9	−5.9
HP3467	–	–	pNP5	6.7
HP3468	*sup-6*	+	pMB9	30.8
HP3469	*sup-6*	–	pMB9	−2.9
HP3442	*sup-6*	–	pNP5	30.3

[a] The procedure for preparing cell extracts and the conditions for the determination of ATP-dependent correndonuclease II activity are outlined in the text. The characteristics of the strains are given in Table 1. The data given for correndonuclease II are arbitrary values for UV-specific activity calculated after the determination of irradiated and unirradiated ^{3}H-labeled OC DNA (on the filter) and of irradiated and unirradiated ^{3}H-labeled CCC DNA (in the filtrate). The input of ^{3}H-labeled PM2 DNA, consisting of 60% CCC DNA, was 5×10^3 cpm of acid-precipitable material and was identical both for irradiated and unirradiated DNA. UV-specific correndonuclease II activity is given by the following formula: [(cpm of irradiated OC DNA/input cpm) − (cpm of unirradiated OC DNA/input cpm)] × 100%. Each of the four components in this calculation was determined in duplicate reactions. The average value of each component (standard deviation ± 10%) was taken. In a control reaction without extract the result of the calculation was 0%. The recovery of acid-precipitable material, a summation of ^{3}H-labeled CCC DNA and ^{3}H-labeled OC DNA, was approximately 90%. The data given for correndonuclease II activity are not corrected for the amount of OC DNA already present in this preparation of ^{3}H-labeled PM2 DNA. When pNP5 was introduced into either *sup*$^+$ UvrB$^+$ or *sup* UvrB$^+$ strains the same correndonuclease II activity was found as for *sup*$^+$ and *sup* UvrB$^+$ strains containing pMB9 (results not shown).

and 4). Extracts of control strains HP3466 *sup*$^+$ Δ*uvrB*(pMB9) and HP3469 *sup-6* Δ*uvrB*(pMB9) (lines 2 and 5) do not exhibit a significant correndonuclease II activity. In contrast to our previous results (10), we observed a low enzymatic activity in an extract of HP3437 *sup*$^+$ Δ*uvrB*(pNP5) (line 3); this result would account for the slightly higher Uv^r of this strain as compared with the untransformed strain HP3430 *sup*$^+$ Δ*uvrB* (unpublished data).

The results presented so far indicate that the level of Uv^r of pNP5-containing UvrB strains is correlated with the level of correndonuclease II activity in crude extracts. In the presence of amber suppressor *sup-6* both a Uv^r and a correndonuclease II activity are found which are equal to the parameters of UvrB$^+$ strains.

UV resistance of λ*b2att*^{2} lysogens containing various suppressors. We have attempted to locate the amber mutation. Essentially two possibilities can be advanced: (i) the amber codon is located within the cloned *uvrB* gene, or (ii) the mutation is situated outside the *uvrB* gene; in this case the amber must be presented between the promotor and the translation initiator codon of *uvrB* and exerting a polar effect on the expression of the *uvrB* gene.

When the amber mutation is located within the *uvrB* gene one must assume that the transducing phage λ*b2att*^{2} also contains this mutation and has been derived from a strain harboring the amber. Consequently, we have constructed λ*b2att*^{2} lysogens of the isogenic set of UvrB strains which contain different nonsense suppressors. The Uv^r of these UvrB strains lysogenic for phage λ*b2att*^{2} was determined indirectly by measuring the capacity of bacteria to reactivate irradiated φ80 phages. This assay was chosen to avoid induction of the prophage after irradiation with UV. The determination of the so-called "host cell reactivation" (Hcr) character had been shown to result in the same suppression pattern as for the Uv^r of UvrB strains containing plasmid pNP5 (unpublished data).

Our data (Fig. 2) on the Hcr character of *sup*$^+$

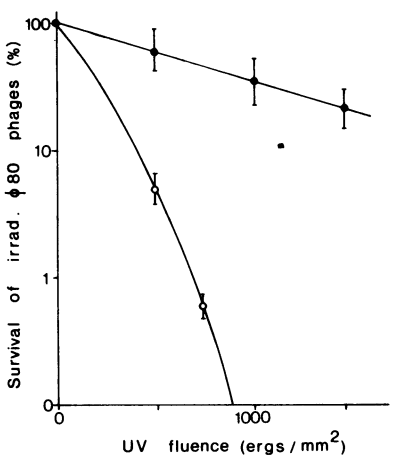

FIG. 2. *UV resistance of UvrB strains lysogenic for phage λb2att*^{2}*, containing different suppressors. Host cell reactivation of irradiated phages was measured. A suspension of φ80 phages (about 10^8 particles per ml) in 10 mM Tris-hydrochloride (pH 7.5) and 10 mM MgSO$_4$ was irradiated as outlined before (14). Phages were titrated in the dark with 2 × 10^8 bacteria of different origins. Symbols: (●) S90C, HP3444 sup*$^+$ *uvrB(λb2att*^{2}*), HP3445 sup-6 uvrB(λb2att*^{2}*); UvrB strains lysogenic for λb2att*^{2}*, containing either supD, supE, supF, sup-5, or supC, have the same host-cell reactivation pattern as the sup*$^+$ *and sup-6 derivatives. (○) HP3430 sup*$^+$ *uvrB, HP3435 sup-6 uvrB, HP3446 sup*$^+$ *uvrB(λ), HP3447 sup-6 uvrB(λ). The bars indicate the range of Hcr of different strains.*

and *sup* UvrB lysogens clearly show that the presence of any of the nonsense suppressors does not alter the Hcr character of these strains. Moreover, the extent of Hcr of these strains is equal to that of the parental nonlysogenic UvrB$^+$ strain. From these results we conclude that the amber mutation is not present within the *uvrB* gene located either on the phage or on the plasmid pNP5. At this point we have to stress that it is unlikely that the amber mutation has been created by a rare event during the cloning experiment. Eight independent clones (containing pNP5-like plasmids) all showed the same suppression pattern (unpublished data). If our conclusion is correct, namely that the amber mutation is not located within the *uvrB* gene, then it follows that the interference on *uvrB* gene expression is due to the polar effect of an amber mutation located outside this gene.

UvrB expression is unaffected by Rho. Polarity is a phenomenon related to the expression of genes arranged in an operon structure. A polar mutation located promotor-proximal with regard to a gene belonging to that operon limits the expression of that gene. Polarity can be exerted at the level of translation (5, 17) or at the level of transcription (1, 12). Obviously, transcriptional polarity also affects the extent of translation. In the accompanying paper we present evidence that the cloned *uvrB* gene is part of an operon-like entity in which the expression of the *uvrB* gene is mediated by a pMB9 promotor (9).

Our findings given in the previous paragraphs indicate that the expression of the cloned *uvrB* gene in suppressor-free strains is prevented by translational polarity, since specific tRNA suppressors can relieve this effect. We have investigated whether the polar mutation also affects the transcription of the *uvrB* gene. Mutations in the *rho* gene, which codes for a transcription termination factor, are known to relieve transcriptional polarity caused by nonsense mutations (1, 11, 12). Consequently, we introduced the *rho* (*psuA1*) mutation into several UvrB strains, resulting in HP3472 *sup*$^+$ *psuA1* Δ*uvrB*(pNP5) and HP3472 *supF psuA1* Δ*uvrB*(pNP5). The Uv^r of these derivatives and of their parental Rho$^+$ strains was determined (Table 4). Our data show that the *rho* mutation does not affect the expression of the cloned *uvrB* gene. Hence, we conclude that the amber mutation does not cause transcriptional polarity, but exerts its effect solely at the translational level. Alternatively, it might be possible that transcriptional polarity provoked by this particular nonsense mutation is independent of Rho factor.

TABLE 4. *Determination of the effect of a rho mutation on the UV resistance of E. coli UvrB strains containing pNP5*[a]

Strain	Relevant genotype	UV resistance
HP3470	*uvrB*(pNP5)	−
HP3471	*supF uvrB*(pNP5)	+
HP3472	*psuA1 uvrB*(pNP5)	−
HP3473	*psuA1 supF uvrB*(pNP5)	+

[a] The strains are derivatives of KMBL1121 *sup*$^+$ Δ(*bio-uvrB-chlA*). Single colonies of these strains were tested for Uv^r by streaking on L-broth agar supplemented with 20 μg of tetracycline per ml. A segment of the plates was irradiated with a UV dose of 500 ergs/mm^2. Uv^s bacteria did not exhibit any growth on the irradiated part, whereas Uv^r bacteria continued growth after this UV dose.

DISCUSSION

Quantitative aspects of the expression of the cloned *uvrB* gene. This study was undertaken to investigate two observations reported previously (10), namely: (i) a relatively low Uv^r displayed by a *uvrB* deletion strain, harboring the *uvrB* gene on a multicopy plasmid, and (ii) the lack of correndonuclease II activity in extracts of this strain. These two observations might be interrelated, but could also have a different cause. Our results demonstrate that in the presence of the nonsense suppressors *sup-6* or *supF* both parameters are altered and now comparable to those of wild-type UvrB$^+$ strains. Consequently, these observations are related to each other. Due to these findings we are now able to study the expression of the cloned *uvrB* gene, starting from a level which is at least equivalent to that in single-copy wild-type UvrB$^+$ strains.

At present we cannot decide whether an excess of the UvrB$^+$ protein is synthesized in cells containing plasmid pNP5. Correndonuclease II activity does not solely rely on a functional UvrB$^+$ gene product. It has been shown by Seeberg et al. (14, 15) that the products of at least three separate genes, i.e., *uvrA*, *uvrB*, and *uvrC*, participate in the interaction of correndonuclease II with irradiated DNA. When these proteins are assembled in an enzymatic complex, then it is conceivable that the UvrB$^+$ component is present in a fixed amount. Consequently, an excess of UvrB$^+$ protein would not result in a higher level of correndonuclease II activity than that of a UvrB$^+$ strain containing only one copy of this gene. Moreover, it would follow that an excess of UvrB$^+$ protein will not lead to a higher Uv^r of pNP5-transformed strains as compared with UvrB$^+$ strains. Experiments are in progress

to determine whether indeed an excess of the UvrB$^+$ protein is synthesized in cells containing plasmid pNP5.

Location of the amber mutation. From the results presented in Fig. 1 and 2 concerning the effect of nonsense suppressors on the expression of the *uvrB* gene located on, respectively, plasmid pNP5 and prophage λ*b2att*^{2}, we have concluded that the amber mutation is not present within the *uvrB* gene. The amber is probably not located on the *uvrB*-containing *Eco*RI fragment at all. This conclusion is reinforced by the observation that the effect of nonsense suppressors on the expression of the cloned *uvrB* gene is limited to UvrB strains harboring pNP5. When, instead of pMB9, other cloning vehicles are used for the insertion of the same *uvrB*-containing *Eco*RI fragment, notably pML21 (6) or pBH20 (7), then the Uv^r of transformed *sup*$^+$ and *sup* UvrB strains does not signficantly differ (mentioned in ref. 9 and 10). A disadvantage, however, of employing the multicopy plasmids pML21 or pBH20 as a vector for the *uvrB* gene is that such recombinant plasmids cause only a low Uv^r in UvrB strains (10).

In the accompanying paper we show that a pMB9 promotor, located adjacent to one of the two *Eco*RI sites of pNP5, mediates the transcription of the *uvrB* gene. This finding offers two possibilities for the location of the amber mutation, namely: (i) it is located on the vector between the promotor and the *Eco*RI site, or (ii) it is generated by "out-of-phase" translation, due to the insertion of the *uvrB*-containing *Eco*RI fragment into pMB9. In this case we assume that translation initiates on the vector pMB9. Recently, we have sequenced an *Eco*RI-*Hin*dIII fragment of pMB9 (approximately 350 base pairs) which contains the relevant promotor (J. Maat and H. Pannekoek, manuscript in preparation). Two of the three translation reading frames harbor numerous nonsense codons, whereas the remaining reading frame does not contain any nonsense codon between its initiator AUG codon and the *Eco*RI site. Based on these results we favor the possibility that the amber mutation is generated by out-of-phase translation.

ACKNOWLEDGMENTS

We thank Barry W. Glickman for the supply of various strains. We appreciate the help of Peter H. Pouwels during the preparation of the manuscript.

This work was supported by Euratom contract 102-72-1 BIAN.

LITERATURE CITED

1. **Adhya, S., M. Gottesman, B. de Crombrugghe, and D. Court.** 1976. Transcription termination regulates gene expression, p. 719–730. *In* R. Losick and M. Chamberlin (ed.), RNA polymerase. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
2. **Braun, A., and L. Grossman.** 1974. An endonuclease from *Escherichia coli* that acts preferentially on UV-irradiated DNA and is absent from the *uvrA* and *uvrB* mutants. Proc. Natl. Acad. Sci. U.S.A. **71**:1838–1842.
3. **Braun, A. G., M. Radman, and L. Grossman.** 1976. Enzymatic repair of DNA: sites of hydrolysis by the *Escherichia coli* endonuclease specific for pyrimidine dimers (correndonuclease II). Biochemistry **18**:4116–4120.
4. **Center, M. S., F. W. Studier, and C. C. Richardson.** 1974. The structural gene for a T7 endonuclease essential for phage DNA synthesis. Proc. Natl. Acad. Sci. U.S.A. **65**:242–248.
5. **Engelhardt, D. L., R. E. Webster, and N. D. Zinder.** 1967. Amber mutants and polarity *in vitro*. J. Mol. Biol. **29**:45–58.
6. **Inselburg, J., and A. Oka.** 1975. Discontinuous replication of colicin E1 plasmid deoxyribonucleic acid. J. Bacteriol. **123**:739–742.
7. **Itakura, K., T. Hirose, R. Crea, A. D. Riggs, H. L. Heyneker, F. Bolivar, and H. W. Boyer.** 1977. Expression in *Escherichia coli* of a chemically synthesized gene for the hormone Somatostatin. Science **198**: 1056–1063.
8. **MacGregor, C. H., and C. A. Schnaitman.** 1971. Alterations in the cytoplasmic membrane proteins of various chlorate-resistant mutants of *Escherichia coli*. J. Bacteriol. **108**:564–570.
9. **Pannekoek, H., I. Noordermeer, and P. van de Putte.** 1979. Expression of the cloned *uvrB* gene of *Escherichia coli:* mode of transcription and orientation. J. Bacteriol. **139**:54–63.
10. **Pannekoek, H., I. A. Noordermeer, C. A. van Sluis, and P. van de Putte.** 1978. Expression of the *uvrB* gene of *Escherichia coli*: in vitro construction of a pMB9 *uvrB* plasmid. J. Bacteriol. **133**:884–896.
11. **Ratner, D.** 1976. Evidence that mutations in the *suA* polarity suppressor gene directly affect termination factor rho. Nature (London) **259**:151–153.
12. **Reyes, O., M. Gottesman, and S. Adhya.** 1976. Suppression of polarity of insertion mutations in the *gal* operon and *N* mutations in bacteriophage lambda. J. Bacteriol. **126**:1108–1112.
13. **Rodriguez, R. L., F. Bolivar, H. M. Goodman, H. W. Boyer, and M. Betlach.** 1976. Construction and characterization of cloning vehicles, p. 471–477. *In* D. P. Nierlich, W. J. Rutter, and C. F. Fox (ed.), ICN-UCLA Symposium on Molecular and Cellular Biology, vol. 5. Academic Press Inc., New York.
14. **Seeberg, E.** 1978. Reconstitution of an *Escherichia coli* repair endonuclease activity from the separated UvrA$^+$ and UvrB$^+$/UvrC$^+$ gene products. Proc. Natl. Acad. Sci. U.S.A. **75**:2569–2573.
15. **Seeberg, E., L. Nissen-Meyer, and P. Strike.** 1976. Incision of ultraviolet-irradiated DNA by extracts of *E. coli* requires three different gene products. Nature (London) **263**:524–526.
16. **van de Putte, P., M. Giphart-Gassler, T. Goosen, A. van Meeteren, and C. Wijffelman.** 1978. Is integration essential for Mu development?, p. 33–40. *In* P. Hofschneider and P. Starlinger (ed.), Integration and excision of DNA molecules. Springer Verlag, Berlin.
17. **Yanofsky, C., and J. Ito.** 1966. Non-sense codons and polarity in the tryptophan operon. J. Mol. Biol. **21**:313–334.