
Regulation of the *Escherichia coli* excision repair gene *uvrC*. Overlap between the *uvrC* structural gene and the region coding for a 24 kD protein

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ABSTRACT

The *UvrA*, *UvrB* and *UvrC* proteins of *E. coli* are subunits of a DNA repair enzyme, the ABC exinuclease. In this paper we study the *uvrC* regulatory region. The *uvrC* structural gene is preceded by an open reading frame encoding a 24 kD protein (1). A *uvrC* promoter has been mapped within this gene. The transcription start of a second promoter located 5' of the 24 kD gene is mapped *in vivo*. We show that transcription from both promoters on the chromosome is not inducible by UV damage. The possible translation start codons of the *UvrC* and of the 24 kD protein are determined. Sequences encoding the N-terminal part of the *UvrC* protein overlap with sequences encoding the C-terminal part of the 24 kD protein. To examine a possible function of the 24 kD gene in repair, a 24 kD insertion mutant was created in the chromosome. The mutant however only slightly affects the UV sensitivity of the cell. Transcription of P3 alone provides sufficient *UvrC* protein for the normal repair of UV lesions.

INTRODUCTION

In *Escherichia coli* the *uvrC* gene product in combination with the *uvrA* and *uvrB* subunits constitutes the UVR exinuclease involved in incision of damaged DNA during the excision repair process (for a review see 3) As the three proteins work in concert one might expect that the expression of the *uvr* genes is regulated in a co-ordinated manner. Indeed it has been shown by several laboratories that both the *uvrA* gene and the *uvrB* gene are regulated by the *recA*-*lexA* dependent SOS system (4,5,6,31,32). As far as the *uvrC* component is concerned there have been contradictory reports with respect to the inducibility (2,7).

The chromosomal organization of the *uvrC* gene is more complex than the one of the *uvrA* and *uvrB* genes: a) The control region of the *uvrC* gene is located within the reading frame of a 24 kD protein. Moreover this region is preceded by an open reading frame of 28 kD (1). From the nucleotide sequence Sharma *et al* (1) have indicated three promoters. The P1 promoter (5' to the 28 kD protein) is located approximately 2.3 kb from the *uvrC* gene. The P2a

and P2b promoters (5' to the 24kD protein) are located approximately 0.9 kb from the uvrC gene. It has been suggested that these promoters are required for optimal uvrC expression (1). In other publications two additional promoters have been described. The P3 promoter, located 200 bp from the structural gene, has been shown to be damage inducible (2). The P4 promoter has been located adjacent to the uvrC gene (8).

b) The inducibility of the uvrC gene showed unusual characteristics : the induction of the P3 transcript, which was measured using multi copy plasmids, was observed only two hours after UV or mitomycin C treatment; furthermore DNA sequencing revealed that the P3 promoter did not contain a typical LexA binding site (CTG(N)₁₀ CAG), although a very similar sequence could be found (CTG(N)₁₁ CAG). Moreover uvrC expression was not induced after treatment of the cells with nalidixic acid. Further studies showed that the LexA protein did not bind to this DNA region in vitro, indicating that the postulated LexA box might not be functional (9).

In this paper we studied the uvrC regulatory region containing the P2 and P3 promoters. We determined the transcription start of the P2 promoter in vivo and showed that its transcription proceeds into the uvrC structural gene. The induction of P2 and P3 transcripts from the chromosome is examined by S1 nuclease mapping. No induction of these promoters could be found. These results are discussed in relation to previously obtained results with GalK fusion plasmids.

The translation start codons of the UvrC and of the 24 kD protein were determined. We show that the N-terminal part of the uvrC gene is longer than previously reported (8) and overlaps with sequences encoding the C-terminal part of the 24 kD protein. The properties of a 24 kD deficient mutant are described.

MATERIALS AND METHODS.

Enzymes and reagents.

Restriction endonucleases were from BRL. Alkaline phosphatase (from calf intestine), nuclease Ba131 and polynucleotide kinase were obtained from Boehringer Mannheim and S1 nuclease from Pharmacia. Radiochemicals and in vitro coupled transcription-translation kits were purchased from Amersham. Recombinant DNA techniques were essentially as described (10).

Microbiological procedures

Media, bacterial growth procedures and conjugation experiments are essentially as described by Miller (18). Antibiotics were used in the

following concentrations: 50 mg/l of kanamycin (Kn), 40 mg/l of ampicillin (Ap), 100 mg/l of streptomycin (Sm) and 25 mg/l of chloramphenicol (Cm). A rapid analysis of UV resistance was done by streaking a diluted cell suspension on L-broth agar plates, followed by irradiation of a segment of the plates. Survival curves were obtained from cells grown in minimal medium to a density of 2×10^8 cells/ml. Cells were harvested, resuspended in minimal medium without growth factors and kept at 37°C for one hour to obtain stationary-phase cells. Diluted suspensions (2×10^6 cells/ml) were irradiated with various doses of UV and appropriate dilutions were plated on minimal medium. The plates were incubated overnight at 37°C prior to counting the surviving colonies. Growth conditions of bacteria harbouring *galK* plasmids and the galactokinase assay have been described (2) and are according to McKenny et al (19).

Bacterial strains and plasmids

E. coli K12 strain AB1157 was used as the repair-proficient strain (11) and

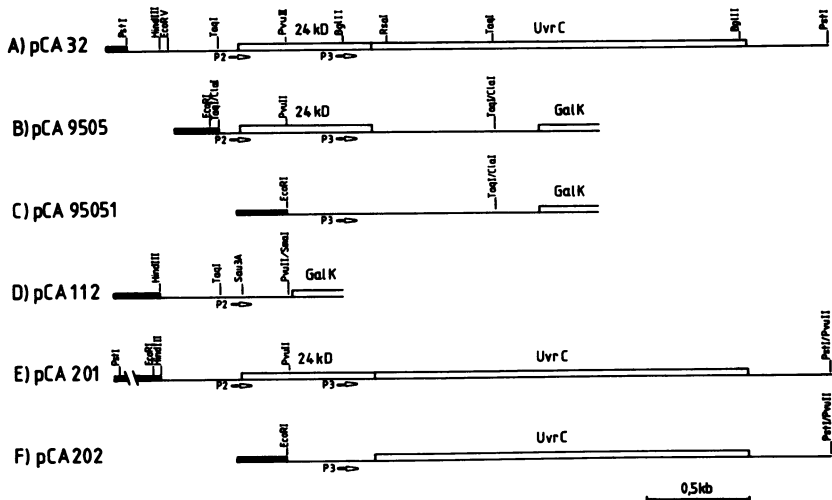


Figure 1. Map of plasmids containing the *uvrC* regulation region.

A) pCA32, carries a chromosomal fragment containing the *uvrC* gene (15). B) pCA9505 (2). C) pCA95051 (2). D) pCA112, containing the 600 bp *Hind*III-*Pvu*II fragment of pCA32 inserted into the *Hind*III and *Sma*I sites of pK01 (19). E) pCA201, was constructed by insertion of the *Hind*III-*Pst*I fragment of pCA32, containing the *uvrC* gene, into the *Hind*III and *Pvu*II sites of pBR322 (the *Pst*I site being digested with *S*I nuclease to create a blunt site). F) pCA202, was constructed by deletion of the *Eco*RI-*Pvu*II fragment of pCA201 (the *Eco*RI site filled in with Klenow polymerase to create a blunt end). The ligation of the blunt *Eco*RI site to the *Pvu*II site has regenerated an *Eco*RI site.

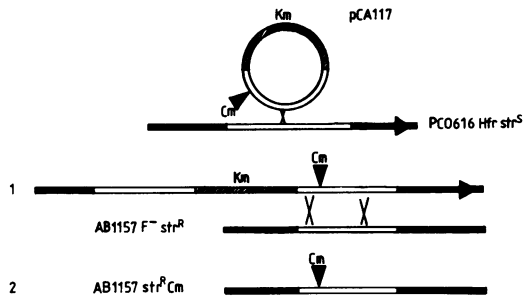


Figure 2. Construction of the 24 kD⁻ strain.

Formation of a plasmid integrate (I) in the donor strain PC0619/pCA117 by a single crossing-over. After conjugational transfer of the plasmid integrate to AB1157, a double crossing-over results in a strain that is Cm Km^S Sm^R.

SR57 (uvrC34 recA56) (12) and AB1884 (uvrC34) (13) as repair-deficient strains. PC0616 (Hfr thi, point of origin:KL96), (K.B. Low strain obtained via Phabagen, Utrecht) was used for the construction of the 24 kD⁻ strain. Strain JM105 (14) was used to harbour the pUD plasmids which carry the tacII promoter, as described in this paper. Plasmid pCA95 is a pK01 derivative (2). The plasmids pCA32, pCA9505, pCA95051, pCA112, pCA201 and pCA202 are described in figure 1. pCA79 (Kn) carries the PstI fragment containing the uvrC gene of pCA32 (fig 1A) inserted into the PstI site of the Ap gene of pACYC177 (15). pCA116 (Ap Cm) was constructed by inserting the 1870 bp PstI fragment (carrying the Cm resistance gene) of Tn9 (16,17) into the PvuII site of pCA9505 (fig 1B). pCA117 (Cm Kn) was constructed by inserting the same Cm fragment into the PvuII site of pCA79.

S1 nuclease mapping and DNA sequencing

Mapping of in vivo uvrC transcripts was carried out according to Berk and Sharp (20). Isolation of DNA fragments from polyacrylamide gels, 5'-labeling with (γ -³²P)dATP and polynucleotide kinase and DNA sequencing were executed according to Maxam and Gilbert (21).

Construction of a 24 kD mutant.

For the construction of a 24 kD⁻ strain the Plasmid Integrate Transfer method (PIT method) was used as described by de Wind et al (22). The PIT method is outlined in figure 2.

PC0616 (Hfr, ori: his anti-clockwise) was transformed with pCA117 (Cm Kn). A transformant was used as a donor in a mating experiment with AB1157 (strA). The conjugation mixture was plated on rich medium containing Sm and Cm. The Sm Cm resistant transconjugants were tested for Kn resistance. About 1% of

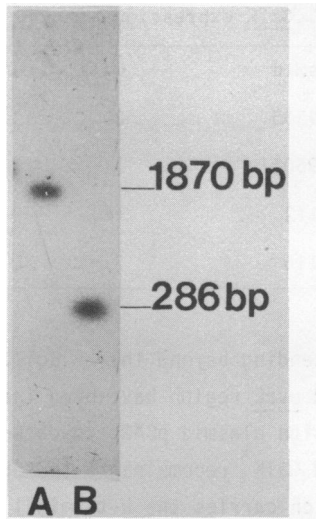


Figure 3. Southern blot analysis of UD1.
 Lane A) UD1, which contains a insertion of approximately 1800 bp.
 B) AB1157, which contains the 286 bp PvuII-BglII fragment.

these transconjugants appeared to have lost the plasmid (are Kn sensitive) indicating that recombination has occurred on both sides of the Cm insert. The Cm resistance could only be transferred by P1 transduction. One Cm^r transductant of AB1157, UD1, was used for further investigations.

Analysis of strain UD1 by Southern blotting

Plasmid pCA9505 was digested with PvuII and BglII (fig 1B). The 286 bp PvuII-BglII fragment was isolated from a low melting 1% agarose gel in Tris-acetate buffer, purified by hot phenol extraction and subsequently by Sephadex G50 column chromatography. Next the fragment was labeled by nick-translation and used as a probe in a hybridization experiment with PvuII-BglII digested chromosomal DNA isolated from strain UD1 and AB1157. Hybridization and blotting conditions were as described by Maniatis (10) and Southern (23). The results are shown in figure 3. UD1 carries the Cm fragment in the chromosome.

RESULTS AND DISCUSSION

Localization of a second uvrC promoter

The uvrC gene was originally cloned on a 3.4 kb PstI fragment from the Clarke-Carbon plasmid pLC13-12 (15). The uvrC encoding region has been located within an open reading frame (ORF) starting 130 bp to the right of

TABLE 1. GalK expression of uvrC promoters.

Plasmid	GalK (units/OD)
pCA9505	20
pCA95051	3 - 5
pCA112	120
pCA116	3 - 5

the 5' BglII site and extending beyond the 3' BglII site (fig 1A) (8). TaqI fragments from the cloned uvrC region have been inserted into the unique ClaI site of the galK expression plasmid pCA95 to detect possible uvrC promoters (2). One of the selected GalK recombinants (pCA9505) was shown to contain a 1300 bp TaqI insert, which carries the N-terminal part of the uvrC gene (fig 1B). Using S1 nuclease mapping a promoter for uvrC expression has been located on this fragment about 200 bp upstream of the structural gene (2) (fig 1B). The presence of this promoter was confirmed by experiments of Sharma *et al* (1), who indicated this promoter as P3. In this paper we will use the same nomenclature.

When the EcoRI-PvuII fragment of pCA9505 was deleted, resulting in pCA95051 (2) (fig 1C), the galactokinase level drops (table 1). This decrease in GalK activity might be due to deletion of a second promoter P2, which was postulated to be present to the left of the PvuII site (1, 7). The presence of P2 was confirmed by the construction of pCA112 in which the HindIII-PvuII fragment of pCA32 is fused to the galK gene (fig 1D, table 1)

As both pCA9505 and pCA95051 carry the N-terminal part of the uvrC gene the observed decrease in GalK activity indicates that transcription from P2 also contributes to uvrC expression. To investigate whether this applies also to the uvrC expression on the chromosome we decided to map transcripts in the uvrC coding region.

Mapping of the uvrC transcripts

For the location of the possible uvrC transcription start sites, the 1076 bp EcoRV-RsaI fragment (fig 1A) was used as a probe in S1 nuclease mapping experiments. The EcoRV site is located 41 bp to the right of the HindIII site, and the RsaI site is located within the uvrC coding region (this paper). This fragment, terminally labeled at the RsaI site, was hybridized to total RNA isolated from strain AB1157. In the autoradiogram shown in figure 4a (lane D) two fragments can be identified of approximately 240 bp and 700

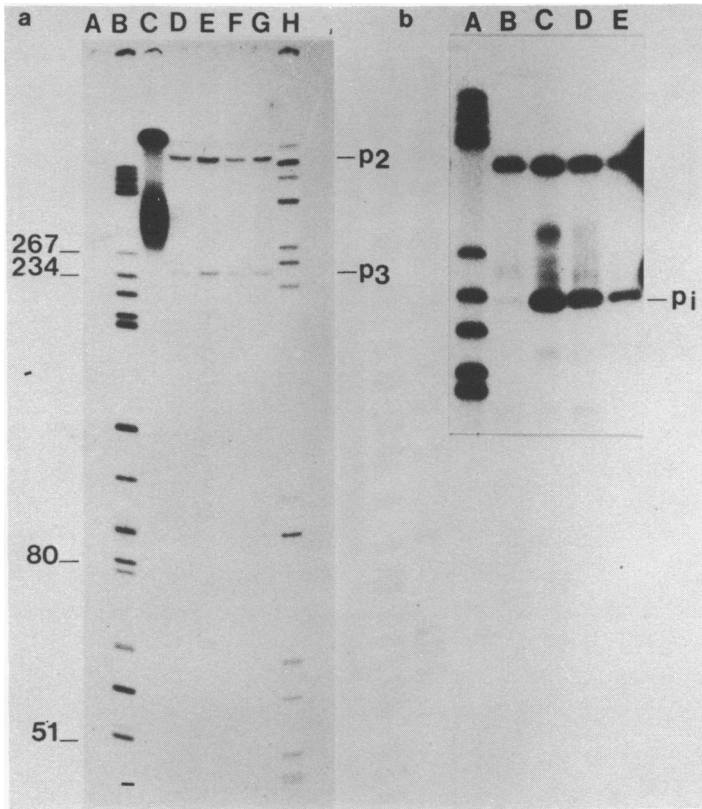


Figure 4a. S1 nuclease mapping of *uvrC* transcripts.

The 1076 bp EcoRV-RsaI fragment, terminally labeled at the RsaI site, was used as a probe (fig 1A). RNA was isolated from AB1157, without UV irradiation and 0.5, 1 and 2.5 hours after UV irradiation. Equal amounts of total RNA were hybridized to the probe and subsequently treated with S1 nuclease. S1 resistant fragments were electrophoresed in parallel with pBR322 marker fragments.

Lane A) probe + S1. B) HaeIII markers. C) probe. D) probe + RNA (-UV).

E,F,G) probe + RNA respectively 0.5, 1 and 2.5 hours after UV irradiation.

H) AluI markers. The positions of the P2 and P3 transcripts are indicated.

Figure 4b. Mapping of the Pi transcript of *ssb*.

The position of the Pi transcript is indicated.

Lane A) HaeIII markers. B) probe + RNA (-UV). C,D,E) probe + RNA respectively 0.5, 1, 2.5 hours after UV irradiation.

bp respectively. The 240 bp transcript corresponds to transcription initiated at the P3 promoter (fig 1B, ref). Only one additional fragment (of about 700 bp) is observed, representing the transcription initiated at P2.

Since the DNA probe used contained the N-terminal part of the *uvrC* coding



Figure 5. Mapping of the transcription start site of the P2 promoter. The 106 bp TaqI-Sau3A fragment (fig 1D), terminally labeled at the Sau3A site, was used as a probe. S1 resistant fragments were electrophoresed in parallel with Maxam and Gilbert sequencing ladders of the same DNA fragment. Lane A) probe + S1. B) probe + RNA. C,D,E,F) sequencing ladder G,A,C,T.

region both transcripts have to proceed into the uvrC gene and are therefore expected to contribute to uvrC expression, which is in agreement with the observed galK expression (table 1).

To determine the start of the P2 promoter more precisely a smaller probe, the 106 bp TaqI-Sau3A fragment (fig 1D) terminally labeled at the Sau3A site, was used. The S1 resistant DNA fragment corresponds to a transcription start site at position 55-58 (GAAT) (fig 5,6). The -35 (TTCAAA) and the -10 (TAATAT) sequences are shown in figure 6.

The transcription start of the P2 promoter represents the proposed P2b consensus of Sharma et al (1). No transcription start representing

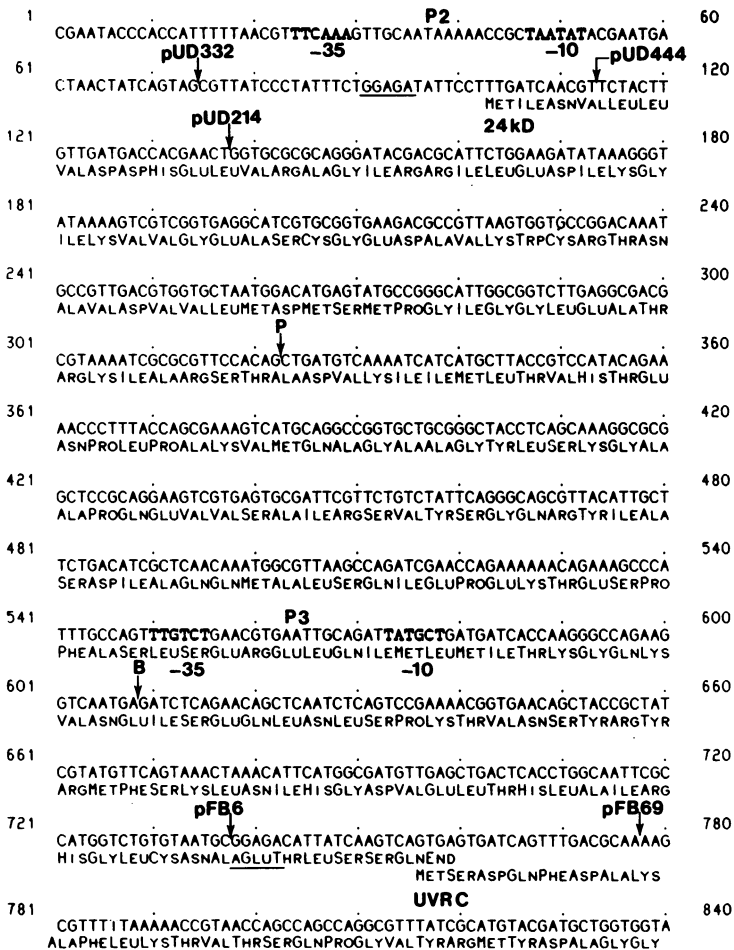


Figure 6. The nucleotide sequence of the *TaqI*-*RsaI* fragment.

The P2 and P3 promoter sequences are indicated. The deletion endpoints of the pCA9505 derivatives (pUD plasmids) and the deletion endpoints of pCA202 derivatives (pFB plasmids) are shown. The SD sequences corresponding to the translation start sites of the 24 kD and UvrC proteins are underlined. P = *PvuII*; B = *BglII*.

transcription initiated at P2a was found, indicating that this consensus probably does not represent a functional promoter. When the *EcoRI*-*RsaI* fragment was used as a probe one also might expect a P4 transcript of approximately 80 nucleotides (fig 6) (8). Even after long exposure this transcript could not be visualised, suggesting that the proposed P4 promoter consensus is also not an *in vivo* functional promoter.

TABLE 2. GalK expression of pCA112 after mitomycin C treatment.

Hours	GalK (units/OD)	
	-	+ mitomycin C (2 µg/ml)
0	229	215
2	225	218

The inducibility of the P2 and P3 transcripts.

All din genes of E.coli are characterized by the presence of a LexA binding site in their promoter region (24, 6). In the DNA region of the P2 promoter no sequence can be indicated which matches the consensus sequence for LexA binding (CTGN₁₀CAG) (1). Therefore the P2 promoter is expected to be independent of LexA regulation.

To investigate the possible inducibility of P2 the intracellular level of galactokinase of cells harbouring pCA112 (containing only the P2 promoter; fig 1D) was measured after mitomycin C treatment as is shown in table 2. Even after two hours no increase of galactokinase activity was observed, indicating that P2 is not inducible.

Van Sluis et al have reported that transcription initiating at P3 is damage inducible, although an abnormal induction profile was found (2). With S1 nuclease mapping it was also shown that the amount of P3-RNA isolated from AB1157 harbouring pCA95051 (fig 1C) increased 2 hours after UV irradiation. In the DNA sequence a putative LexA binding site could be indicated, overlapping the P3 promoter. However no in vitro binding of LexA protein to this region could be detected (9). Moreover with P3 cat-fusion plasmids no induction of Cat synthesis after UV or mitomycin C treatment was found (7).

These results made us reconsider the previous conclusions regarding the inducibility of the P3 promoter. In all experiments concerning the inducibility, galK fusion plasmids were used. An alternative explanation for the observed enhanced galK expression might be that due to the DNA damaging treatment, the supercoiling of the plasmid is temporarily changed, which in turn might influence the strength of the P3 promoter. A similar induction profile also has been observed with the repressor promoter of bacteriophage Mu and the Tc promoter of pBR322, which are independent of LexA regulation. (N. Goosen and J.A. Brandsma, pers. comm.).

We therefore repeated the S1 nuclease mapping experiment with RNA isolated from plasmid free AB1157. The EcoRV-RsaI fragment containing both P2 and P3

promoters was used as a probe (fig 1A). In this way advantage was taken of the non-inducible P2 transcript, which served as an internal standard for hybridization. RNA was isolated from unirradiated cells and from cells 0.5, 1, and 2.5 hours after UV irradiation. From the autoradiogram shown in figure 4a it is evident that the P3 transcript is not inducible. As a control the same RNA was used in hybridization experiments with a probe containing the inducible Pi promoter of the ssb gene of E.coli (25). In this case, as shown in figure 4b, the amount of Pi transcript is strongly increased 0.5 hour after irradiation.

Mapping of the translation start codon of the 24 kD protein

We determined the nucleotide sequence of the TaqI-RsaI fragment containing both P2 and P3 promoters (fig 6). Within this sequence an open reading frame can be indicated starting at position 61 and ending with TGA at position 758-760 (fig 6).

Sharma et al have shown that this DNA region codes for a protein of 24 kD. They proposed a translation start site of this protein at an ATG triplet (position 126-128) (1). However our sequence data indicate that this ATG codon is not in frame with the coding region, as at position 129-130 we find a CC doublet and not as Sharma et al have found a CCC triplet. In the first 100 bp of the ORF no other in frame ATG triplet can be found.

In the majority (>90%) of the E.coli messenger RNAs AUG is used as a translation initiation codon. About 8% of the genes use GUG and about 1% use UUG as a translation start (26). Within the ORF of the 24 kD protein a TTG triplet at position 103 and a GTG triplet at position 140 can be indicated.

Plasmid pCA9505 carries the 1300 bp TaqI fragment containing the P2 promoter and the entire 24 kD ORF (fig 1B). When this plasmid was translated in an in vitro transcription-translation system it produces the 24 kD protein as predicted (fig 7A). For the precise mapping of the translation start we used Bal31 deletion derivatives of pCA9505 as outlined in the legends of figure 7. The tacII promoter (27) was used for 24 kD expression. As the tacII promoter fragment contains stopcodons in all three reading frames no fusion proteins can be synthesized. The deletion derivatives, containing the tacII promoter in the right orientation, were tested for 24 kD production in the in vitro transcription-translation system.

The autoradiogram of the labelled proteins is shown in figure 7. Plasmid pUD332 still encodes the 24 kD protein, whereas no 24 kD protein could be detected from plasmids pUD444 and pUD214. Therefore the translation start codon must be located between the deletion end points of these two plasmids

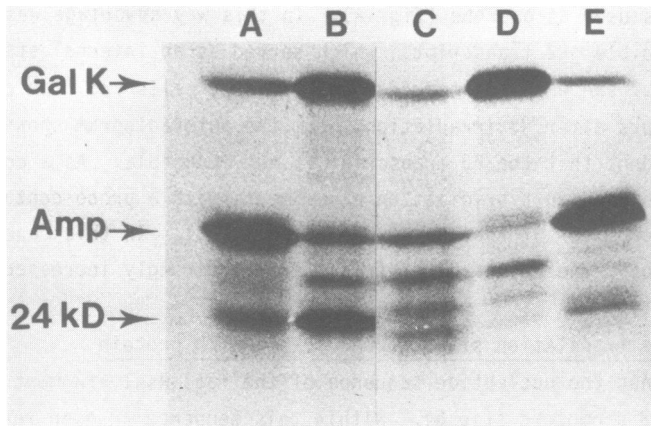


Figure 7. Autoradiogram of (^{35}S)-methionine labeled proteins of pCA9505 deletion derivatives. Deletion derivatives were constructed by digesting pCA9505 with EcoRI (fig 1B) and subsequent treatment with nuclease Bal31. The newly created ends were filled in by Klenow polymerase and EcoRI linkers were joined. Next the tacII promoter, located on an EcoRI fragment of pKM-tacII (27), was cloned into the new EcoRI site. Plasmids having the tacII promoter in the right orientation (pUD plasmids), were tested in a coupled transcription-translation system for 24 kD synthesis. Finally the deletion end points were mapped by Maxam and Gilbert sequencing. Lane A) pCA9505. B) pUD332. C) pUD444. D) pUD214. E) pCA95.

as shown in figure 6. From these results we concluded that the TTG triplet at position 103 is most likely the translation initiation site. A putative Shine & Dalgarno (SD) sequence is located 7 bp upstream of this TTG triplet (fig 6).

Mapping of the translation start codon of the UvrC protein

In the DNA sequence of the uvrC gene an open reading frame can be indicated starting at nucleotide 735 (fig 6) (8). It has been suggested by Sancar et al that the uvrC coding region starts with an ATG triplet at position 822-824 (fig 6). However direct evidence for such a start has not been obtained as the N-terminus of UvrC is blocked, which makes protein sequencing impossible (8). To determine the translation start more precisely we isolated Bal31 deletions.

Plasmid pCA202 was constructed as outlined in figure 1 E,F. For the mapping of the translation start of the UvrC protein Bal31 deletion derivatives (pFB plasmids) were constructed as outlined in figure 8. The pFB plasmids were tested for uvrC complementation using strain SR57 (fig 9). As a control SR57

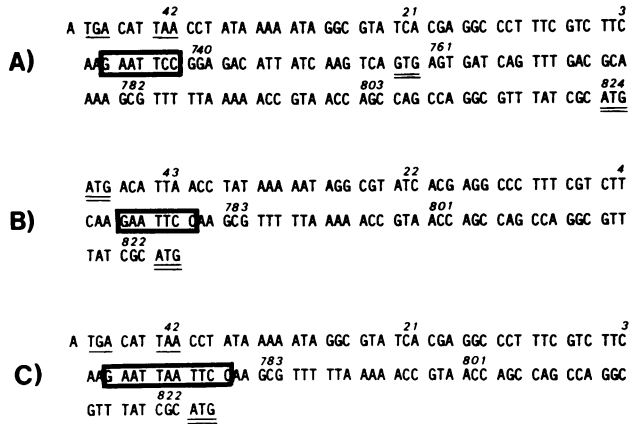


Figure 8. Map of the pFB plasmids.

Deletion derivatives were constructed by digesting pCA202 with *Bal31* from the *EcoRI* site (fig 1F). The ends were filled in with Klenow polymerase and *EcoRI* linkers were joined. The deleted *EcoRI*-*PstI* fragments containing the *uvrC* gene were isolated and rejoined to the small *EcoRI*-*PstI* vector fragment to restore the *Ap* gene. Finally the deletion endpoints were mapped by DNA sequencing. Translation initiation codons and translation stop codons are underlined. *EcoRI* linkers are boxed. *UvrC* sequences are numbered according to figure 6.

A) pFB6. B) pFB69. C) pFB70.

cells transformed with pCA201 were used. pFB6 (fig 8a,9e) complements the *uvrC* mutation. As this plasmid contains no *uvrC* promoter, the observed *uvrC* expression must be directed from a promoter present in pBR322 (28, unpublished results). From the sequence in figure 8a two possible translation starts can be indicated: the ATG at position 822-824 and a GTG at position 756-758. To discriminate between these alternatives plasmid pFB69 was studied, in which the GTG triplet is deleted (fig 8b). This plasmid still complements the *uvrC* mutation (fig 9d) suggesting that the ATG is the translation initiation site. In pFB69 however an additional ATG triplet of the vector pBR322 (at -58) is present, which is in phase with the *uvrC* encoding reading frame and which might serve as a start codon for a fusion protein. Indeed in the transcription-translation system pFB69 produced a protein somewhat larger than the wild type *UvrC* protein (results not shown). To block translation initiated in the vector, the *EcoRI* site of pFB69 was modified with Klenow polymerase resulting in pFB70 (fig 8b). Due to the resulting frameshift the ATG triplet at position -58 is no longer in phase with the *uvrC* reading frame. Moreover in this construction three stop codons

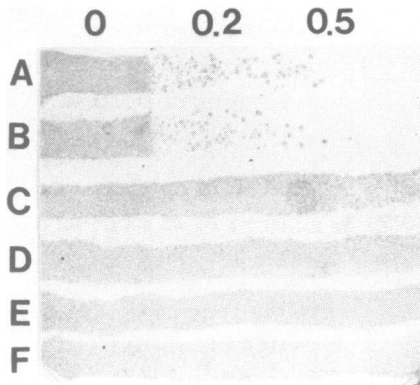


Figure 9. UvrC complementation in SR57. SR57 cells harbouring the pFB plasmids were tested for *uvrC* complementation as described in the Materials and Methods. The UV dose is shown in J/m². a) SR57 + pBR322; b) SR57 + pFB70; c) SR57 + pFB69; d) SR57 + pFB6; e) SR57 + pCA201; f) AB1157.

are created (positions -7, -51 and -57) which prevent translation from any other vector initiation codon. From figure 9c it is clear that pFB70 does not complement for UV resistance, indicating that the ATG triplet at position 822 is not the functional translation initiation codon. From these results we conclude that the translation of the UvrC protein is most probably initiated at the GTG triplet at position 756, which adds 22 aminoacids to the N-terminal part of the UvrC protein.

It is striking that both translation of 24 kD and translation of *uvrC* are not initiated at an AUG triplet but use lower efficiency start codons. It has been shown that the activity ratio of the different initiation codons is UUG:GUG:AUG = 1:2:3 (30). The reason for the use of these low efficiency translation start codons is not clear. From the sequence in figure 6 it is evident that the presumed translation start of the *uvrC* gene is overlapping with the end of the open reading frame of the 24 kD gene. As also the 24 kD transcription initiated at P2 proceeds into the *uvrC* gene, this suggests a strong coupling between the expression of both genes and possibly also the function of both gene products. Therefore we decided to construct a strain mutated in the 24 kD gene and to examine its properties.

The properties of a 24 kD mutant

To examine the role of the 24 kD protein a 24 kD mutant of AB1157 was constructed using the Plasmid Integrate Transfer (PIT) method as described by de Wind *et al* (22). For this purpose plasmids pCA116 and pCA117 were

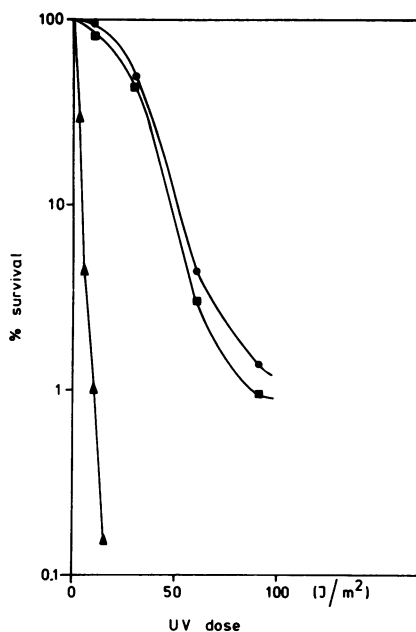


Figure 10. UV survival curves of UD1 (■), AB1157 (●) and AB1884 *uvrC34* (▲).

constructed, in which the 24 kD gene is inactivated by cloning a fragment containing the cat gene of Tn9 into the PvuII site of pCA9505 and pCA79 (Km^r) respectively (Materials and Methods). Plasmids were selected that have the transcription of the cat and 24 kD gene in opposite direction. Plasmid pCA116 shows a reduced level of GalK expression as compared to the isogenic pCA9505 which does not contain the cat insert (table 1), indicating that the insertion exerts a polar effect on P2 transcription. As pCA117 has the most extensive homology with the chromosome this plasmid was used to construct a 24 kD insertion mutant by the PIT method.

The properties of the constructed 24 kD⁻ mutant were examined. No difference in growth rate or colony morphology (either in rich or minimal medium) could be detected. Also no significant difference in UV survival as compared to the isogenic 24 kD⁺ strain could be found (fig 10). This was unexpected since we confirmed by S1 nuclease mapping that transcription from P2 is blocked by the cat insert. Moreover there is no evidence for transcription initiating within the cat fragment that could complement for P2 transcription (results not shown).

From these results two important conclusions can be drawn. First, the 24 kD

protein does not play an important role in repair of UV induced damage. Secondly, transcription of uvrC from P3 alone provides sufficient UvrC protein for the normal repair of UV lesions. To investigate to what extent the P2 promoter contributes to the level of UvrC in the cell it would be necessary to measure this level by means of UvrC antibodies. As it is now there seem to be two possibilities. One is that P2 might hardly contribute to the level of UvrC. This would be the case if the translation of the 24kD gene on the P2 transcript interferes with the translation of uvrC due to the overlap of both reading frames. On the other hand it has been shown that an overlap of reading frames can facilitate the initiation of translation of the second gene (29). If this would be the case for the 24kD and uvrC reading frames P2 would contribute significantly to the level of UvrC, which then might be of importance for processes other than repair of UV lesions.

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