JOURNAL OF BACTERIOLOGY, Feb. 1988, p. 1012–1014
0021-9193/88/021012-03$02.00/0

Vol. 170, No. 2

Identification of the *uvrA6* Mutation of *Escherichia coli*

JOURICA A. BRANDSMA,* MARTINA DE RUIJTER, JAAP BROUWER, AND PIETER VAN DE PUTTE
Laboratory of Molecular Genetics, University of Leiden, P.O. Box 9505, 2300 RA Leiden, The Netherlands

Received 24 August 1987/Accepted 28 October 1987

The *uvrA6* mutation has been cloned on a multicopy plasmid by using a chloramphenicol resistance marker introduced next to the *uvrA* gene in the *Escherichia coli* chromosome. The mutation was shown to reside in the N-terminal part of the *uvrA* gene. Sequencing part of this region of the mutant gene revealed a frameshift mutation at positions 207 to 209, which leads to a stop codon at position 262. A marker rescue experiment showed that this frameshift is the only mutation responsible for the UV-sensitive phenotype of the UvrA6 mutant. The method presented is suitable for the cloning of every chromosomal *uvrA* mutation and can be useful for the study of the functional domains of the UvrA protein.

The mechanism of UvrABC-dependent excision repair, a major DNA repair process in *Escherichia coli*, is being studied extensively (for a review, see reference 5). The UvrA, UvrB, and UvrC proteins form the ABC endonuclease and act in concert to recognize and excise a variety of DNA damages. The eighth phosphodiester bond 5′ and the fourth or fifth phosphodiester bond 3′ to the modified nucleotide(s) are incised (12). A model for the successive steps in this repair process has recently been developed in more detail (11). However, the domains in the proteins which are responsible for their different actions in the process are still unclear. The wild-type *uvr* genes have been cloned, and the complete sequences of the genes have been reported (1, 3, 7, 13). Subsequent analysis of *uvr* mutants can be of great value in studying the individual functions of the *uvr* gene products. We therefore developed a system for a rapid characterization of chromosomal *uvrA* mutations. The method consists of the following steps: (i) introduction of a chloramphenicol (CAM) resistance marker close to the $uvrA^+$ gene on a plasmid; (ii) transformation of a linear DNA fragment containing the $uvrA^+$ and *cam* genes and selection for CAM resistance; (iii) transfer of the resulting CAM insertion in the chromosome, introduced by homologous recombination, by P1 transduction to every strain carrying a *uvrA* mutation to be analyzed; (iv) cloning of the *uvrA* mutation by cocloning with the *cam* gene; (v) rough localization of the mutation by fragment exchange, followed by detailed analysis by DNA sequencing; and (vi) control by marker rescue.

Here we present the cloning and identification of the most widely used *uvrA* mutation, *uvrA6*, isolated many years ago by Howard-Flanders et al. (6).

Cloning of the *uvrA6* mutation. A *uvrA* multicopy plasmid with a *cam* resistance gene upstream of the *uvrA* gene has been constructed (Fig. 1). The *Cla*I fragment of this plasmid (pJA61), containing *cam*, was transformed into strain JC7620, *recB21 recC22 sbcB12* (8). In this genetic background, linear DNA fragments are more persistent, thus allowing homologous recombination events to proceed between the chromosome and the restriction fragment, which may lead to CAM-resistant cells. This technique has also been used by others for insertion and deletion mutagenesis (15). P1 growth on the CAM^r cells and subsequent transduction to the UvrA6 strain AB1886 (6) showed a high cotransducing frequency of the two genes, which confirms that the *cam* gene is adjacent to *uvrA*. In fact, CAM^r cells which are still UV sensitive are found with a frequency of only 1%. Compared with the results with other *uvrA* mutations (unpublished data), this low frequency might indicate that the *uvrA6* mutation is located in the N-terminal part of the *uvrA* gene. The UV^s CAM^r cells were used to isolate chromosomal DNA, which subsequently was digested with *Bam*HI, a restriction enzyme having no recognition sites in the *uvrA* gene or in the *cam* resistance gene. The obtained *Bam*HI fragments were ligated to the *Bam*HI site of vector pUC19 (16), and selection for CAM^r transformants was made. The resulting plasmid, pJA64 (Fig. 2A), has been shown to contain the expected restriction sites of the *uvrA* gene but was not able to confer UV resistance to UvrA6 cells and therefore has to harbor the *uvrA6* mutation.

Mapping of the *uvrA6* mutation. To map the location of the *uvrA6* mutation roughly, the *Bam*HI-*Kpn*I fragment of pJA53 (Fig. 2B), a wild-type *uvrA* plasmid, was replaced by the *Bam*HI-*Kpn*I fragment of the *uvrA6* plasmid pJA64. AB1886 cells containing the modified pJA53 plasmid are UV^s, whereas after exchange of the *Bam*HI-*Nar*I and *Kpn*I-*Pst*I fragments, pJA53 can still confer UV resistance to the UvrA strain. Therefore, the *uvrA6* mutation resides on the *Nar*I-*Kpn*I fragment. DNA sequencing was performed to determine the mutation site precisely. The procedure has

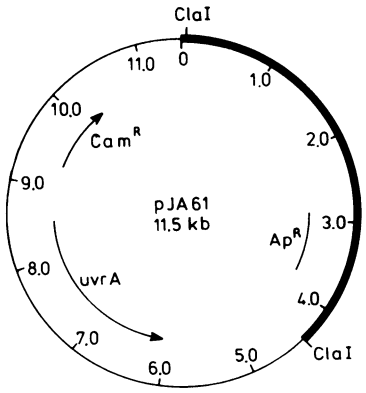

FIG. 1. Plasmid pJA61 consists of a chromosomal DNA fragment containing *uvrA*, cloned into vector pBR322 (black region). The *cam* resistance gene has been inserted upstream of the *uvrA* gene (the details of the construction procedure will be described elsewhere). The position of the *uvrA* gene and the *cam* gene have been indicated. kb, Kilobases.

* Corresponding author.

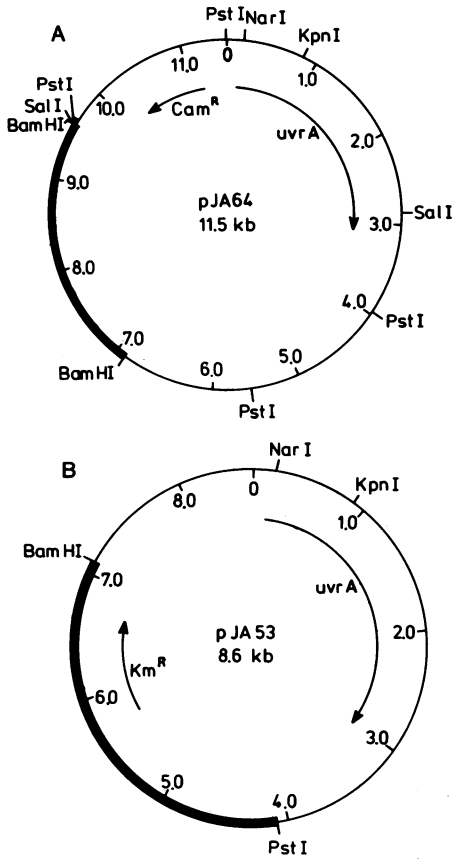

FIG. 2. Schematic maps of plasmids pJA64 and pJA53. The relevant restriction sites have been indicated. (A) Plasmid pJA64 contains a chromosomal *Bam*HI fragment including *cam* and the *uvrA6* mutant gene cloned into vector pUC19 (black area). (B) Plasmid pJA53 is a recombinant plasmid of the wild-type *uvrA* gene in vector pACYC177 (4), constructed by subcloning the *Bam*HI-*Pst*I fragment of plasmid pUvrA7 (2). kb, Kilobases.

been outlined in Fig. 3. The obtained results were compared with the wild-type *uvrA* sequence as published by Husain et al. (7). When the dideoxy-chain-termination method (14) was used, stacking of bands around base pair (bp) 208 was found on the gel and therefore we were unable to read this part of the sequence. Except for this region, however, it was clear that the sequence from *Nar*I to the second *Hpa*II site in Fig. 3 is not different from the *uvrA* wild-type sequence. The DNA sequencing method of Maxam and Gilbert (9) was used to analyze the region around bp 208 (shown schematically in Fig. 3). The result, presented in Fig. 4, clearly shows the absence of one C at positions 207 to 209. We conclude that the *uvrA6* gene contains a 1-bp deletion which results in termination at bp 262.

To exclude the possibility that additional mutations are present further downstream to the *Hpa*II site at position 264 (Fig. 3), we carried out the following experiment. The *Nar*I-*Sau*3A fragment (Fig. 3) from the wild-type *uvrA* gene was cloned in vector pUC09 (10). The resulting plasmid appeared to rescue the *uvrA6* mutation with a frequency of 2×10^{-7}, whereas with the vector alone no rescue was found with a frequency higher than 2×10^{-8}. We conclude, therefore, that the frameshift mutation at positions 207 to 209 is the only mutation responsible for the UV^s phenotype of the UvrA6 mutant.

From the sequencing data, it can be predicted that a

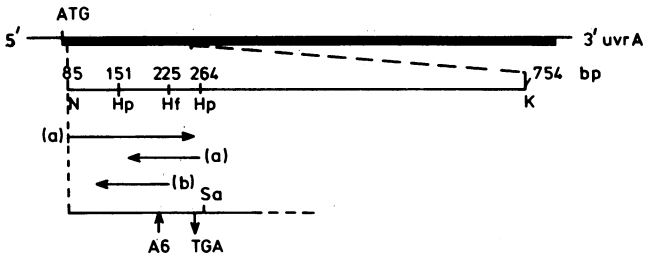

FIG. 3. Sequencing strategy of the *uvrA6* mutation. The *uvrA* gene is shown schematically. For sequencing studies, recombinant M13 phages carrying the *Nar*I-*Kpn*I and *Hpa*II fragments have been isolated. Dideoxy sequencing according to the method of Sanger et al. (14) was performed as shown by arrows marked (a). Both strands were sequenced and showed the abnormality around bp 208 mentioned in the text. Sequencing of a terminally labeled *Hin*fI fragment, indicated by arrow (b), was carried out according to the method of Maxam and Gilbert (9). The positions of the *uvrA6* mutation and the corresponding stop codon have been indicated. The relevant restriction sites have been shown; the base-pair numbering is according to Fig. 3 of reference 7. N, *Nar*I; Hp, *Hpa*II; Hf, *Hin*fI; K, *Kpn*I; Sa, *Sau*3A.

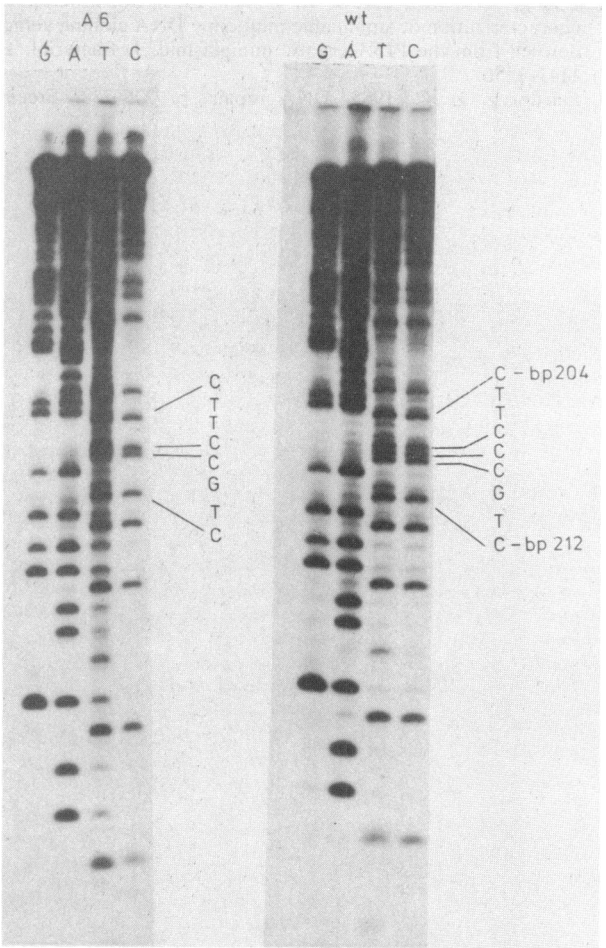

FIG. 4. DNA sequence of the *uvrA6* mutant gene (A6) around position 208. For comparison, the corresponding *uvrA* wild-type sequence (wt) is shown.

mutant UvrA protein of 67 amino acids long can be made which still contains one putative ATP-binding site (7). Since the UvrA6 mutant is used commonly in DNA repair studies as a $UvrA^-$ mutant, one should take into account that a protein with a residual activity may be made.

The system developed to isolate and analyze the *uvrA6* mutation is useful for quick isolation and identification of other chromosomal *uvrA* mutations. The availability of mutant Uvr proteins will be of great value in dissecting the roles of the individual subunits of the endonuclease and the domains within each subunit responsible for the different steps in the complex incision reaction.

This work was supported by the J. A. Cohen Institute for Radiopathology and Radiation Protection (no. 4.2.6).

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