IDENTIFICATION OF *uvrA* GENE MUTATION SITES IN TWO MITOMYCIN—SENSITIVE *Deinococcus radiodurans* STRAINS

DU Zeji KONG Xianrong
(*Institute of Radiation Medicine, Suzhou Medical College, Suzhou* 215007)
Issay Narumi Hiroshi Watanabe
(*Biotechnology Laboratory, Japan Atomic Energy Research Institute, Japan*)

ABSTRACT *Deinococcus radiodurans* (*Dr*) possesses a prominent ability to repair the DNA injury induced by various DNA—damaging agents including mitomycin C (MC), ultraviolet light (UV) and ionizing radiation. DNA damage resistance was restored in MC sensitive (MC^s) mutants 2621 and 3021 by transforming with DNAs of four cosmid clones derived from the gene library of strain KD8301 which showed the property of wild type phenotype to DNA—damaging agents. Gene affected by mutation (*mtcA or mtcB*) in both mutants was cloned and its nucleotide sequence was determined. The deduced amino acid (*aa*) sequence of *Dr uvr*A gene product consists of 1016 aa and shares homology with many bacterial UvrA proteins. The mutation sites in both mutants were identified by analyzing the polymerase chain reaction (PCR) fragments derived from the genomicDNA of the mutants. A 144—base pairs (bp) deletion including the start codon for the *uvrA* gene was observed in DNA of the mutant 3021, causing a defect in the gene. On the other hand, an insertion sequence (IS) element intervened in the *uvrA* gene of the mutant 2621, suggesting the insertional inactivation of the gene. The IS element comprises 1322—bp long, flanked by 19—bp inverted terminal repeats (ITR), and generated a 6—bp target duplication (TD). Two open reading frames (ORF) were found in the IS element. The deduced aa sequences of large and small ORF show homology to a putative transposase found in IS4 of *Escherichia coli E. coli*) and to a resolvase found in *IS Xc*5 *of Xanthomonas campestris* (*Xc*), respectively. This is the first discovery of IS element in deinobacteria, and the IS element was designated IS2621.

KEYWORKS DNA repair, *uvrA* gene, Insertion sequence, *Deinococcus radiodurans*

The extraordinary radiation resistance of *Dr* is attributed to its high and accurate capability for repairing DNA damage induced by MC, UV and ionizing radiation. Two types of DNA repair processes, i.e. excision and recombination, have so far been detected in *Dr*[1]. Nucleotide excision repair in this bacteria can proceed through two distinct pathways and the removal of either by mutation does not affect its UV resistance. One pathway requires functional *mtcA* and *mtcB* genes, while the other requires functional *uvsC*, *uvsD* and *uvsE* genes[2]. 5.6— and 2.7—kilobases (kb) *Eco*RI fragments carrying $mtcA^+$ and $mtcB^+$, respectively, were subcloned from a cosmid clone pUE50 containing a DNA fragment carrying both genes[3]. However, no sequ encing analysis of these subclones has been reported. To identify genes affected by mutation in several mutants isolated previously we constructed an unamplified cosmid library of *Dr* strain KD8301, a derivative of strain KD830[4], retaining DNA damage resistance. Four cosmid clones whose target

The project was supported by the Agency of Science and Technique of Japan
Recived: 11 June 1998, Accepted: 4 Sep. 1998

DNAs could transform two MC^S mutants 3021 (*mtcA*) and 2621(*mtcB*) to MC resistance (MC^R) were isolated. Sequencing analysis and transformation experiment revealed that the gene defined by mutations responsible for MC^S of both mutants was a homolog of many bacterial *uvrA* genes. Furthermore, a novel IS element was found in the *uvrA* gene of the mutant 2621.

1 MATERIALS AND METHODS

1.1 Bacterial Strains, Cosmids, Plasmids and Growth Conditions

Bacterial strains, cosmids and plasmids used in this study are listed in Table 1. MR1 and KR1 are wild type strains of *Dr*. However, they are different from each other in several respects including the growth in minimal medium[5], the sensitivity to MC[4] and the plasmid profile[6]. Strains MR1, 262 and 302 were kindly gifted from Dr. Moseley. *Dr* was grown at 30℃ in TGY medium containing 0.5% Bacto—tryptone (Difco), 0.1% glucose, and 0.3% Bacto—yeast extract (Difco) or on TGY plates supplemented with 1.5% Bacto—agar (Difco). Two μg of streptomycin (Sm) per ml was supplemented if necessary. *E. coli* was grown in Luria—Bertine (LB)—Miller medium (1% Bacto—tryptone, 0.5% Bacto—yeast extract, 1%NaCl) or on LB—Lenox plates (1% Bacto—tryptone, 0.5% Bacto—yeast extract, 0.5% NaCl) supplemented with 1.5% Bacto—agar at 37℃. If necessary, 50μg of ampicillin (Ap) per ml and 50μg of kanamycin (Km) per ml were supplemented.

Tab.1 Bacterial strains, cosmids and plasmids (∷:ligate with)

Designation	Relevant description	Source/reference
D radiodurans		
KR_1	wt	Anderson *et al*.[16]
KN101	As KR_1 but Ade^-	Kitayama and Matsuyama[4]
KD830	As KR_1 but Ade^-	Kitayama and Matsuyama[4]
KD8301	As KD830 but Sm^R	This study
MR_1	wt	Moseley[1]
302	As MR_1 but MC^s(*mtcA*)	Moseley and Copland[10]
3021	As 302 but Sm^R	This study
262	As MR_1 but MC^s(*mtcB*)	Moseley and Copland[10]
2621	As 262 but SM^R	This study
E. coli		
XL1—Blue MR	Host for cosmid clones	stratagene
JM109	Host for plasmid subclones	Laboratory stock
cosmid		
SuperCos 1	cosmid vector; 7.6—kb; Ap^R KM^R	Stratagene
pDC111	*Dr* DNA in SuperCos 1; 34.2 kb; *mtcA mtc B*	This study
pDC179	*Dr* DNA in SuperCos 1; 45.8 kb; *mtcA mtc B*	This study
pDC288	*Dr* DNA in SuperCos 1; 44.4 kb; *mtcA mtc B*	This study
pDC288	*Dr* DNA in SuperCos 1; 48.1 kb; *mtcA mtc B*	This study plasmid
pUC19	*EC* cloning vector; 2.7—kb; Ap^R	Fermentas MBI
pGEM—1	*Ec* cloning vector for PCR product; 3.0—kb; AP^R	promega
pKC1	pUC19 *Eco*RI∷2.6—kb *Dr EcoRI* fragment from pDC288; mtc^+	This study
pKC2	pUC19 *Eco*RI∷2.8—kb *Dr EcoRI* fragment from pDC288	This study
pKC3	pUC19 *Eco*RI∷4.2—kb *Dr EcoRI* fragment from pDC288	This study
pKC4	pUC19 *Eco*RI∷4.2—kb *Dr EcoRI* fragment from pDC288; mtc^+	This study
pKC15	pUC19 *Eco*RI∷0.4—kb *Dr EcoRI* fragment from pDC288;	This study
pKC5	pUC19 *Sma*I∷5.2—kb *Dr Bst*PI fragment from pDC288; in vitro blunt—end ligation; $mtcA^+$ $mtcB^+$	This study
pKC6	As pKC5 but teh direction of insertion is opposite to pKC5	This study
pKC7	pGEM—T∷0.9—kb PCR product from *Dr* 3021	This study
pKC8	pGEM—T∷2.4—kb PCR product from *Dr* 2621	This study

1.2 Genomic DNA Preparation

Dr cells from 24h culture were harvested, washed in NE solution(0.5mol/L NaCl/0.1mol/L EDTA; pH 8.0), in butanol—saturated NE solution to facilitate the lysis of the cell walls, and again in NE solution. The washed cells were incubated in NE solution containing 10mg/ml of lysozyme (Sigma) for 1h at 37℃. Subsequently, DNA of *Dr* was isolated and purified by the method of Saito and Miura[7].

1.3 Construction of SuperCos Library

The genomic DNA of *Dr* KD8301 was partially digested with *MboI* (Takara). An unamplified cosmid library was prepared using Super Cos 1 cosmid vector kit (Stratagene) and *In vitro* packaging kit LAMBDA INN (Nippon Gene) according to the manufacturer's instructions. Transfectants were spread onto LB—Lenox plates supplemented with Ap and Km, and 534 colonies grown individually were cultivated in LB—Miller medium supplemented with Ap and Km. The cultures were stored at −80℃ as glycerol stocks.

1.4 Transformation and DNA Manipulations

E. coli JM109 was transformed by electroporation using Gene Pulser (Bio—Rad). Natural transformation of *Dr* was done with 500ng of cosmid or plasmid DNA as previously described[17]. For selecting MC^R transformants, 0.025μg MC per ml was used. Cosmid and plasmid DNAs were isolated by QIAGEN plasmid midi kit and QIAprep spin miniprep kit (Qiagen), respectively. DNA fragment sizing was carried out using Intelligent Quantifier electrophoresis image analysis software package (Bio Image) with a color image scanner model GT—9000ART (Epson). DNA fragments from agarose gels were recovered by electroelution using GeneCAPSULE (Geno Technology). Southern blot hybridization was carried out using digoxigenin (DIG) as previously described[8], except that DIG—ULS labeling kit (Kreatech Diagnostics) was used for labeling probe DNAs and DNAs in agarose gel which were transferred on nylon membranes under alkaline conditions. As a size marker for Southern analysis, 500—bp DNA ladder (GIBCO BRL) was labeled using DIG oligonucleotide 3'—end labeling kit (Boehringer Mannheim) to prevent the increase in size of the marker DNA fragments by labeling. Kilo—deletion kit (Takara) was used to construct a series of deletion plasmids from the cloned DNA. Primers for PCR were synthesized using Oligo—1000 DNA synthesizer (Beckman). Other DNA manipulations were carried out according to standard protocols[9].

1.5 PCR and DNA Sequencing

PCR was done using Ampli *Taq* gold kit and DNA thermal cyclermodel PJ2000 (Perkin—Elmer). *Dr* genomic DNA (50ng) was used as a template DNA. PCR cycling conditions were as follows; 1 cycle of 12min at 95℃, 30 cycle of 1min at 96℃ and 1min at 60℃, and 1cycle of 15min at 60℃. After chilling the reaction mixture, amplified PCR products were purified using Microcon—100 concentrator column (Amicon). Sequence analysis was performed by ABI—PRISM dye terminator cycle sequencing ready reaction kit with Ampli *Taq* DNA polymerase FS and DNA sequencer ABI model 373A system (Perkin—Elmer) using the universal primers and the primers synthesized for PCR. The PCR product (180ng) was used as a template DNA for direct sequencing. Alkaline denaturation of double—stranded plasmid DNA prior to the cycle sequencing step was very efficient to read longer sequence becuse of the high G+C content of *Dr* genomic DNA. One μg of alkaline—denatured DNA was used for sequencing double—stranded plasmid template.

2 RESULTS AND DISCUSSION

2.1 Subcloning of Cosmid Clones Carrying *mtcA*$^+$ and *mtcB*$^+$

DNA of 4 cosmid out of 534 clones (pDC111, pDC179, pDC288 and pDC314) could transform a MC^s mutant 2621 to MC^R. The resistance to MC of another MC^s mutant 3021 was also restored by transformation with DNAs of the same clones, indicating that both *mtcA* and *mtcB* loci were carried in each of these clones. The DNA fragment patterns of the *Eco*RI—digested cosmid DNA shown in Fig. 1A indicate that these clones have 5 fragments of common size. Since a 6.8—kb EcoRI fragment was thought to be derived from cosmid vector, 2.6—, 2.

8—, 4.2— and 5.3—kb *Eco*RI fragments from pDC288 were subcloned to pUC19 and designated pKC1, pKC2, pKC3 and pKC4, respectively. Transformation experiments with the two MC^s mutants demonstrated that pKC4 and pKC1 could transform 3021 and 2621 respectively, indicating that pKC4 contained *mtcA* locus while pKC1 covered *mtcB* locus.

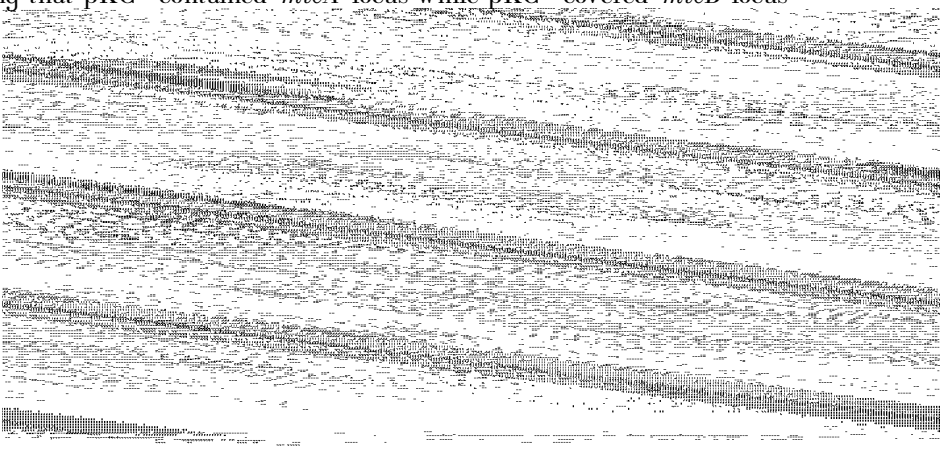


Fig.1 *Eco*RI restriction patterns of the cosmid DNA carrying $mtcA^+$ and $mtcB^+$. Lanes M1, M2 and M3, λ DNA mix marker (Fermentas MBI), 500—bp DNA ladder (GIBCO BRL) and ϕ174/HincII digest (Nippon Gene) respectively; lane 1, pDC111 DNA; lane 2, pDC179 DNA; lane 3, pDC288 DNA; lane 4, pDC314 DNA. (A) Each 200ng of cosmid DNA was completely digested with EcoRI and loaded on 0.8% agarose gel. Five common fragments are indicated by arrows. (B) Each 5μg of cosmid DNA was completely digested with *EcoR*I and loaded on 2% agarose gel. Two common fragments are indicated by arrows

2.2 Identification of the *Dr* urv*A* Gene

Preliminary sequencing analysis of pKC4 and pKC1 with the universal primers indicated that both plasmids contained the nucleotide sequences having homology to many bacterial *uvrA* genes. By comparison with nucleotide positions in sequences of the known *uvrA* genes, it was estimated that pKC4 and pKC1 had the 5′—terminal region and the middle region of *Dr uvrA* gene respectively.

However, it seemed that there was a gap of several hundreds bp between pKC4 and pKC1. By reanalyzing the DNA fragment patterns of 4 cosmids, additional two small common *EcoRI* fragments (0.3 and 0.4kb) were found (Fig.1B), one of which seemed to be located between the 5.3— and 2.6—kb *EcoRI* fragments cloned in pKC4 and pKC1 respectively.

To obtain whole *Dr uvrA* gene, pDC288 was digested with several restriction enzymes and a 5.2—kb *Bst*PI fragment which could hybridize with pKC1 was selected. The fragment was subcloned to pUC19 and two resultants were designated pKC5 and pKC6. Both plasmids have the same 5.2—kb *Bst*PI fragment but the direction of insertion is opposite toeach other. They could transform the mutants 3021 and 2621 to MC^R. A series of deletion plasmids was constructed from pKC5 and pKC6, and used for sequencing and transformation experiment (see section 2.3). The nucleotide sequence of the *Bst*PI fragment contained one large ORF spanning 3096 nucleotide. However, the second ATG seemed to be more plausible as the start codon for *Dr uvrA* (ORF span is 3051 nucleotide), because of an appropriate ribosome—binding site (RBS) existed upstream the ATG and of homology of this ORF to other bacterial *uvrA* genes. The complete nucleotide sequence of this region containing *Dr uvrA* gene has been assigned in the DDBJ/EMBL/GenBank, accession No. AB001610.

2.3 Localization of the 3021 and 2621 Mutations

A series of deletion plasmids constructed from pKC5 and pKC6 was tested for their transforming ability. The results indicated that the 3021 and 2621 mutations were located within 554—bp and 274—bp two regions respectively (Fig. 2A). To identify the mutation sites in 3021 and 2621, these two DNA regions were amplified using two sets of suitable primers (Fig. 2A and B). According to the nucleotide sequence of the *uvrA* region in KD8301, PCR fragments amplified with primer pairs (P1 and P2, P3 and P4) were expected to be 1017 and 1056 bp long respectively. However, a PCR fragment amplified using 3021 DNA with primers P1 and P2 was 0.9kb in size, and a fragment amplified using 2621 DNA with primers P3 and P4 was 2.4kb in size (Fig. 2B). These fragments were cloned in pGEM—T vector and plasmids designated pKC7 (containing the 3021 mutation) and pKC8 (containing the 2621 mutation) were obtained.

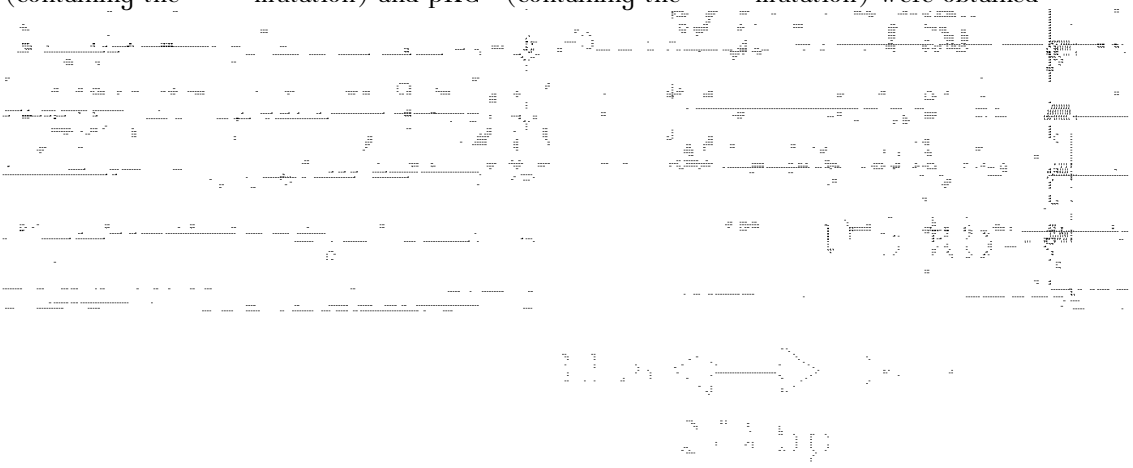


Fig. 2 (A) Localization of the 3021 (*mtcA*) and 2621 (*mtcB*) mutation sites. The open box represents the parent pKC5 or pKC6 DNA from nucleotide positions 1 to 5112. The *Dr* UvrA coding region is indicated by a black bar with start codon ATG and stop codon TAG. The thick lines represent the deletion plasmids derived from the parent plasmids. Results of transforming ability of these deletion plasmids against the mutants 3021 or 2621 are represented as positive (+) or negative (—) on the left or right side, respectively. The regions in which the mutations should be localized are indicated by thick arrows. The nucleotide positions corresponding to both ends of the regions are indicated by the numbers. Primers for amplifying the mutation regions in the mutants are indicated by arrows adjacent to their annealing sites. P1: 5'—CTACAGTTAAGGAAAAGGGCCG—3' (nucleotide positions 632 — 653); P2: 5' — CAGCTTCTTGTACTCGCCTTTG — 3' (positions 1648 — 1627); P3: 5' — CGGCAAAAGCACCCTGATTC—3' (position 3199—3218); P4: 5'—TCGCTCTGCTCGGGTTGAAT—3' (position 4254—4235). (B) Amplified genomic DNAs of the wild type strain and the mutants by PCR with the primers described above. Lane M, 500—bp DNA ladder marker (GIBCO BRL); lanes 1 and 4, KD8301 DNA; lanes 2 and 5, 2621 DNA; lanes 3 and 6, 3021 DNA. Lanes 1 to 3, DNA was amplified with primers P1 and P2; lanes 4 to 6, DNA was amplified with primers P3 and P4

Sequencing analysis of pKC7 revealed that 144—bp including the start codon for the *Dr uvrA* gene had been deleted in DNA of the mutant 3021 (Fig. 3). The accurate ends of the deletion could not be determined because both ends were flanked by CCGGG (nucleotide positions 1036～1040 and 1080～1084). The mutant 3021 is a Sm^R derivative of a MC^s mutant 302 (*mtcA*) which has been isolated through double nitrosoguanidine treatments[10]. It is concluded that the large deletion causes a defect in the *uvrA* gene in the mutant 3021.

The sequence cloned in pKC8 was determined by constructing a series of deletion plasmids. The

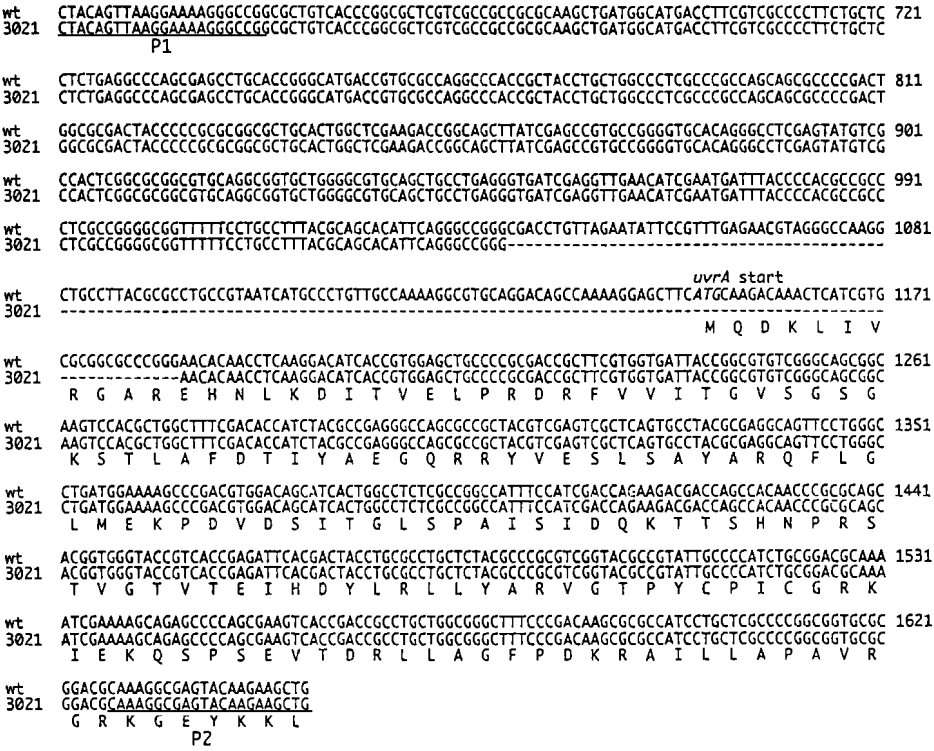
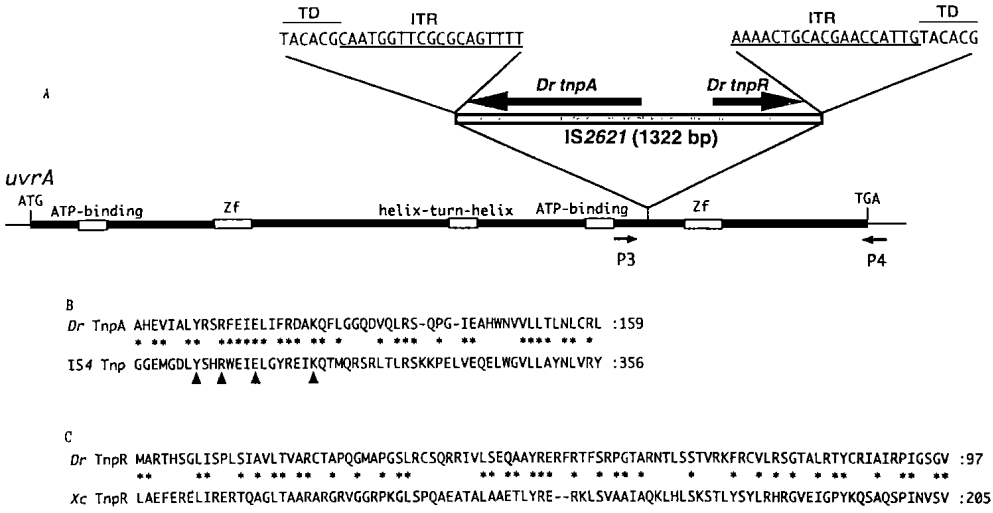


Fig. 3 Nucleotide sequence of the 3021 mutation region and its corresponding sequence of wild type. Deduced aa sequence of the N—terminal region of *Dr* UvrA is also represented by one letter. Numbers on the right of wild type nucleotide sequence indicate the position of the presented sequence relative to the complete sequence. Dashes in the mutant sequence indicate the gaps. The locations of the PCR primers are indicated by underlines

results revealed that the sequence which was 2384 bp in accurate size was completely consistent with that from KD8301 DNA except that an IS element was inserted in the *uvrA* gene of the mutant 2621 (Fig. 4A). The insertion was located upstream C—terminal zinc finger (Zf) domain in which mutations have a profound effect on the *E. coli* UvrA function[11]. A C—terminal 260 aa deletion mutant of *E. coli* UvrA still has some phenotypic properties including UvrA—UvrB interaction and UvrA dimerization, whereas activities of DNA binding, damage recognition and UvrA—DNA complex stabilization properties are missing[12]. Regarding the 2621 mutation, it is concluded that the unique insertion of IS element in the *Dr uvrA* gene of the mutant 2621 prevented the complete expression of the gene and hence interfered the normal function of nucleotide excision repair system in *Dr*. If a truncated UvrA is produced in 2621, the predicted molecular weight of the truncated protein is estimated to be 82.7 kilodaltons (kDa), while that of wild type *Dr* UvrA is 112.1 kDa. The mutant 2621 is a Sm^R derivative of a MC^s mutant 262 (*mtcB*)induced by single nitrosoguanidine treatment followed by transforming with DNA from a recombination—deficient mutant *rec*30[10]. However, the origin of the *mtcB* mutation has still been obscure, and Moseley *et al*[10] has speculated that the *mtcB* mutation might be spontaneous.

2.4 Structural Feature of IS Element

The IS element found in pKC8 was designated IS2621. IS2621 comprises 1322—bp, flanked by 19—bp imperfect ITR, and generated a 6—bp TD (nucleotide positions 3374—3379 of *Dr*



Fig·4 (A) Schematic representation of insertion site of IS 2621 (shaded bar) in *Dr uvrA* (black bar)· Target duplicatins (TD) generated in *uvrA* and inverted terminal repeats (ITR) of IS2621 are indicated by overlines and underlines respectively· Relative positions of functional regions of *Dr* UvrA are indicated by open boxes· The locations of the PCR primers are indicated by arrows· Thick arrows indicate the position and orientation of two putative ORF *Dr tnpA* and (*Dr tnpR*) found in IS2621· (B) The aacomparison of *Dr* TnpA with C1 region of a putative transposase (IS4 Tnp) of *E· coli* IS4· Dashes in sequence indicate the gaps·Asterisks represent identical or functionally related aa residues (G, A, S, T, P; L, I, M, V; D, N, E, Q; K, R, H; Y, W, F)· The position of the Y—X2—R—X3—E—X6—K motif conserved in the C1 region of the IS4 family[13] is indicated by closed triangles· The numbers on the right correspond to the last residue of each sequence· (C) The aa comparison of *Dr* TnpR with *Xc* TnpR of IS*Xc*[14]· Identical or functionally related aa residues are represented by asterisks

uvrA sequence) (Fig·4A)· The G+C content of the IS2621 nucleotide sequence was low as 57·9%, compared with that of *Dr uvrA* coding region (65.7%) and with that of *Dr* total genomic DNA (62～70%)· The complete nucleotide sequence of IS2621 has been assigned in the DDBJ/EMBL/GenBank, accession No·AB001611· Two ORF which consisted of 660 and 327 nucleotide were found in the IS2621 with appropriate RBS· The deduced aa sequence of the large ORF exhibited homology to a putative transposase found in E·*coli* IS4 (Fig· 4B), and this ORF was designated *Dr tnpA*· Although *Dr* TnpA shares the conserved motif found in the C1 region of the IS4family[13], *Dr* TnpA has approximately half size compared to most transposases belonging to the IS4 family, and lacks the Asp—X—Gly/Ala—Tyr/Phe motif found in the N3 region[13]·The deduced aa sequence of the small ORF found in IS2621 exhibited homology to a limited part of a resolvase found in IS*Xc*5 from *Xc*[14](Fig·4C)· Either potential resolvase or repressor activity of this ORF was speculated, thus this ORF was designated *Dr tnpR*· This is the first discovery of IS element in deinobacteria·

2.5 Map of *Dr uvrA* and Its Flanking Region

As described in section 2.2, it was thought that there was a gap of several hundreds bp between pKC4 and pKC1· After sequencing pKC5 and pKC6, it was found that this gap was 315—bp long· A 0.3—kb *Eco*RI fragment shown in Fig·1B seems to be located between pKC4 and pKC1· The relationship of pKC1, pKC4 and pKC5/6 is illustrated in Fig· 5A·Since nucleotide sequence of the right end of pKC5/6 did not match to that of other common *Eco*RI fragment cloned in pKC2 and pKC3, a remaining 0.4—kb *Eco*RI fragment (Fig·1B) was subcloned in pUC19, and designated pKC15· Sequencing analysis of pKC15 indicated that the length of the

fragment was 399bp, and could be located on the right side of *p*KC5/6 (Fig. 5A). A short ORF was found in this region, and designated *orf*288*c* (in detail, see DDBJ/EMBL/GenBank accession No. AB001610).

It has been shown that a cosmid clone pUE50 carrying *mtcA*$^+$ and *mtcB*$^+$ was constructed from MR1 DNA[3]. Furthermore, a subclone pUE58 carrying *mtcA*$^+$ has been obtained from a 5.6—kb *Eco*RI fragment, and another subclone pUE59 carrying *mtcB*$^+$ being from a 2.7—kb *Eco*RI fragment. Using pUE502, a shorten derivative of pUE50, Agostini *et al*[15] constructed pHA15 (5.6—kb insert) carrying *mtcA*$^+$ and pHA16 (2.6—kb insert) carrying *mtcB*$^+$. Plasmid pKC1 (2.6—kb insert) constructed in this study seems tocorrespond to pUE59 and pHA16. However, pKC4 carrying *mtcA*+ has a 5.3—kb insert (Fig. 1A), whereas pUE58 and pHA15 have a 5.6—kb insert. To clarify this discrepancy, Southern blot analysis was carried out using *Eco*RI—digested genomic DNAs from KR1, KD8301, MR1, 2621 and 3021 with DIG—labeled pKC4 as a probe. The results (Fig. 5B) revealed that all signals hybridized with pKC4 were 5.3—kb in size except that a signal in 3021 was slightly smaller than those of others because of the 144—bp deletion. These results imply that the descrepancy in size between pKC4 and the other corresponding subclones decribed above is not due to the difference in strain. Although the reason why pUE58 and pHA15 have a 5.6—kb insert is unknown, the length of an *Eco*RI fragment containing the 5'—region of *Dr uvrA* should be 5.3—kb.

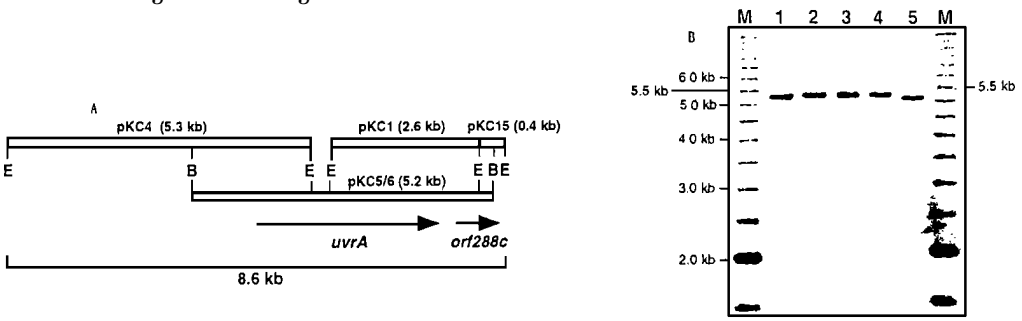


Fig. 5 (A) Map of *Eco*RI and *Bst*PI fragments subcloned from pDC288 with restriction enzyme sites. Each fragment is represented by open box with its plasmid 's name and length (in parenthesis). Letters below or above vertical lines indicate res triction enzyme sites: B, *Bst*PI; E, *Eco*RI. Arrows indicate the position and orientation of *Dr uvrA* and its downstream *orf*288c. (B) Southern blot analysis of *Eco*RI digested genomic DNAs from *Dr* KR1, KD8301, MR1, 2621 and 3021. Each 2.5μg of genomic DNA was completely digested with *Eco*RI and loaded on 1.2% agarose gel. After blotting, the membrane was hybridized with DIG—labeled pKC4 DNA. Lane M, DIG—labeled 500—bp DNA ladder marker (GIBCO BRL), lane 1, KR1 DNA; lane 2, KD8301 DNA; lane 3, MR1 DNA; lane 4, 2621 DNA; lane 5, 3021 DNA

3 CONCLUSIONS

Gene affected by mutation (formerly *mtcA* or *mtcB*) in two *Dr*MC^s mutants was identified as *uvrA*. Its nucleotide sequence was determined. The deduced aa sequence of *Dr* UvrA consisted of 1016 aa. The mutation sites in both two MC^s mutants were identified. A 144—bp deletion including the start codon for the *uvrA* gene was observed in DNA of the MC^s mutant 3021, causing a defect in the gene. An IS element (IS2621) intervened in the *uvrA* gene of another MC^s mutant 2621, suggesting the insertional inactivation of the gene. Two ORF were found in IS2621. It was thought that the large one encoded a putative transposase and small one encoded a putative resolvase or repressorP

Referenecs

1 Moseley B E B·Photochem Photobiol Rev, 1983, **7**:223～269
2 Moseley B E B, Evans D M· J Gen Microbiol, 1983, **192**:2437～2447
3 Al—Bakri G H, Mackay M W, Whittaker P A *et al*·Gene, 1985, **33**:305～311
4 Kitayama S, Matsuyama A· Mutat Res, 1975, **29**:327～33
5 Harsojo, Kitayama S, Matsuyama A· J Biochem, 1981, **90**:877～880
6 Kikuchi M, Kitayama S, Sjarief S H *et al*· Radiat Res, 1994, **139**:123～125
7 Saito H, Miura K· Biochem· Biophys Acta, 1963, **72**:619～629
8 Narumi I· Biotechnol Lett, 1995, **17**:475～479
9 Sambrook J, Fritsch E F, Maniatis T· Molecular Cloning: A Laboratory Manual, 2nd Ed·, Cold Spring Habor Laboratory Press, Cold Spring Harbor, NY
10 Moseley BEB, Copland HJR· Mol Gen Genet, 1978, **160**:331～337
11 Wang J, Mueller K L, Grossman L· J Biol Chem, 1994, **269**:10771～10775
12 Claassen L A, Grossman L· J Biol Chem, 1991, **266**:11388～1139
13 Rezsohazy R, Hallet B, Deicour J *et al*· Mol Microbiol, 1993, **9**:1283～1295
14 Liu C C, Wang H R, Chou M C *et al*· Gene, 1992, **120**:99～103
15 Agostini H J, Carroll J D, Minton K W· J Bacteriol, 1996, **178**:6759～6765
16 Anderson A W, Nordan HC, Cain RF *et al*· Food Technol, 1956, **10**:575～578
17 Kitayama S, Asaka S, Totuka K· J Bacteriol, 1983, **155**:1200～1207

二株丝裂霉素敏感的抗辐射菌 *Deinococcus radiodurans* 的 *uvrA* 基因序列测定及突变位点的确定

杜泽吉　孔向蓉

(苏州医学院放射医学系　苏州 215007)

鸣海一成　渡边宏

(日本原子力研究所生物技术研究室　日本高崎)

摘要　抗辐射菌 *Deinococcus radiodurans* 具有显著的 DNA 损伤修复能力，包括对丝裂霉素(MC)，紫外线(UV)及电离辐射等所致的损伤。在用对 DNA 损伤因子具有抗性的野生型抗辐射菌 KD8301 的基因组 DNA 构建的基因文库中，有四个克隆能够通过其 DNA 转化，使二株对 MC 敏感的 *Deinococcus radiodurans* 的突变体—2621 和 3021 回复对 MC 的抗性。克隆了二株突变体中受突变(*mtcA* 或 *mtcB*)影响的基因，并测定了该基因的核苷酸序列。理论上推定抗辐射菌 *uvrA* 基因产物的氨基酸序列是由 1036 个氨基酸组成，与许多细菌的 UvrA 蛋白质具有同源性。用 PCR 技术扩增二株突变体基因组中相应的 DNA 片段，并对其加以测序分析，确定了突变发生的位点。对于突变体 3021，其 *uvrA* 基因中发生了 144 个碱基对(bp)的缺失突变(缺失部分包括 *uvrA* 的起始密码)，造成了 3021 的 *uvrA* 基因的失活。对于 2621 突变体，其 *uvrA* 基因内却发生了一个插入突变，造成了该基因的插入失活。该插入序列由 1322bp 组成，侧面与 19bp 组成的末端反转重复(Inverted Terminal Repeats，ITR)相连接，并产生了一个由 6bp 组成的靶点复制(Target Duplication，TD)。在该插入序列中，发现二个可读序列，大的可读序列所编码的氨基酸序列与大肠杆菌中插入序列 4 (IS4)推定的转座酶有同源性，小的可读序列与 *Xanthomonas campestris* (*Xc*)中插入序列 5(IS*Xc*5)的解离酶有同源性。本研究是第一次从 *Deino*—菌属中发现插入序列，该插入序列被命名为 IS2621。

关键词　DNA 修复，*uvrA* 基因，插入序列，*Deinococcus radiodurans*

日本科学技术厅资助课题