Irradiation-Induced *Deinococcus radiodurans* Genome Fragmentation Triggers Transposition of a Single Resident Insertion Sequence

Cécile Pasternak¹, Bao Ton-Hoang², Geneviève Coste¹, Adriana Bailone¹, Michael Chandler²*¹, Suzanne Sommer¹*¹

¹Université Paris-Sud, Centre National de Recherche Scientifique, Unité Mixte de Recherche 8621, LRC CEA 42V, Institut de Génétique et Microbiologie, Bât. 409, Orsay, France. ²Laboratoire de Microbiologie et Génétique Moléculaires, Centre National de Recherche Scientifique, Unité Mixte de Recherche 5100, Toulouse, France

Abstract

Stress-induced transposition is an attractive notion since it is potentially important in creating diversity to facilitate adaptation of the host to severe environmental conditions. One common major stress is radiation-induced DNA damage. *Deinococcus radiodurans* has an exceptional ability to withstand the lethal effects of DNA-damaging agents (ionizing radiation, UV light, and desiccation). High radiation levels result in genome fragmentation and reassembly in a process which generates significant amounts of single-stranded DNA. This capacity of *D. radiodurans* to withstand irradiation raises important questions concerning its response to radiation-induced mutagenic lesions. A recent study analyzed the mutational profile in the *thyA* gene following irradiation. The majority of *thyA* mutants resulted from transposition of one particular Insertion Sequence (IS), IS Dra2, of the many different ISs in the *D. radiodurans* genome. IS Dra2 is a member of a newly recognized class of ISs, the IS200/IS605 family of insertion sequences.

Introduction

Stress-induced transposition has been an attractive notion for some time since it is potentially important in creating diversity to facilitate adaptation of the host to severe environmental conditions. One common major stress is DNA damage. This induces a variety of responses including changes in expression of numerous genes [1–4], cell cycle arrest [5,6], induction of bacterial prophages [7–8] and, by generating diversity, can also aid development of processes such as bacterial pathogenicity and prophages [7–8]. While some mutants, presumably resulting from point mutations or small insertions or deletions, retained the length of the wild-type gene, the many resident ISs unrelated to IS Dra2 made only small contributions to the mutant pool despite their presence in significant numbers (see www-IS.biotoul.fr) in the *D. radiodurans* R1 genome sequence [21]. The importance of the contribution of IS Dra2 to mutagenesis is further underlined by its low genomic copy number in the standard R1 ATCC 13939 strain used in our studies as judged by a combination of whole genome hybridization and sequencing [19]: 1 complete and 1 inactive degenerate IS Dra2.
Author Summary

Induction of transposition in prokaryotes under cell stress conditions is potentially important in creating diversity facilitating adaptation to severe environments. In Deinococcus radiodurans, the most radiation-resistant organism known, despite abundance of resident insertion sequences (IS), transposition of a single IS, ISdra2, was found to be strongly induced by irradiation. We show that both steps involved in transposition, IS excision, and insertion, increase significantly following host cell irradiation and, using PCR analysis of genomic DNA, that exposure to \(\gamma\)-irradiation stimulates massive excision of the single genomic ISdra2 copy as a DNA circle and reclosure of the empty site. These events are closely correlated with the initiation of the process leading to genome reassembly from chromosomal fragments, which occurs mainly through a mechanism generating long stretches of single-stranded DNA [18]. These events are closely correlated with the initiation of the process leading to genome reassembly from chromosomal fragments, which occurs mainly through a mechanism generating long stretches of single-stranded DNA.

Results

ISdra2 excision and insertion in vivo in D. radiodurans

IS200/IS605 family members often carry 2 genes, \(tnpA\), encoding the transposase and \(tnpB\), a gene of unknown function (Figure 1A). As shown for the related IS608 [23], the transposition cycle occurs in two principal steps: excision from the donor backbone in the form of a single strand circle and subsequent insertion into a suitable DNA target [22]. These steps were monitored for ISdra2 transposition in D. radiodurans using a genetic assay. TnpA and/or TnpB were expressed in trans from a plasmid, pGY11559, under control of the IPTG-inducible \(P_{spac}\) promoter [19] (Table S1). For excision, the unique active ISdra2 copy (ISdra2F; Figure 1A) in D. radiodurans and have identified the mechanism by which ISdra2 transposition is triggered by radiation. Using a genetic system to detect two principal transposition steps, transposon excision and insertion, we show that ISdra2, like other IS200/605 family members, requires TnpA but not TnpB for both and that insertion occurs 3’ to a specific pentanucleotide (as deduced from genome analyses [20]). We demonstrate genetically that both steps are significantly increased following host cell irradiation. We also show that the entire TnpA-catalysed transposition cycle including excision and insertion depends strictly on single strand DNA substrates in vitro. Finally, using a PCR-based approach, we demonstrate that, in vivo, exposure to \(\gamma\)-irradiation stimulates excision of the single genomic copy of ISdra2 from the genome in the form of a DNA circle. These events are closely correlated with the initiation of the process leading to genome reassembly from chromosomal fragments, which occurs mainly through a mechanism generating long stretches of single stranded DNA.

Figure 1. A genetic assay for ISdra2 transposition. (A) Derivatives of ISdra2 (1736 bp). Orfs are indicated as boxes with arrowheads showing the direction of translation; LE and RE, red and blue boxes, respectively. In ISdra2-113 (1509 bp) both \(tnpA\) and \(tnpB\) were replaced by a Cam\(^R\) cassette, while ISdra2-103 (1778 bp) was deleted only of \(tnpB\) and expresses \(tnpA\) from its natural promoter. ISdra2-104 results from replacement of the \(tnpA\) coding region of ISdra2-103 by the lacZ coding region. ISdra2-103Term116 (1889 bp) carries the Deinococcal transcription terminator Term116 [45] downstream of the \(cte\) gene. (B) In vivo genetic assay to measure excision and insertion events of a derivative of ISdra2. The ISdra2F copy was first replaced by a Tet\(^R\) cassette and ISdra2-113 (or ISdra2-103) was inserted at the unique TTGAT target site present in the \(cte\) gene. The second inactive copy, ISdra2\(^\#\), was replaced by the \(sacB\) gene and an accompanying hygromycin (Hyg) resistance cassette as a selective marker. TnpA-dependent ISdra2 excision restores a functional tet\(^R\) gene, giving rise to Tet\(^R\) colonies while insertion into the reporter \(sacB\) gene confers resistance to sucrose. See Figure S1 and its legend. doi:10.1371/journal.pgen.1000799.g001
inserted by homologous recombination at the unique 5’TTGAT3’ target site (see below) present in the \textit{tet} gene. IS excision restores a \textit{tet} \textsuperscript{R} phenotype (Figure 1B). These constructions are described in detail in Figure S1.

Ectopic TnpA expression in this system induced IS\textit{Dra}2-113 excision at a frequency of $2 \times 10^{-3}$ (Table 1). This was not increased when IPTG was added to the medium, to induce TnpA expression. Similar experiments in which \textit{P}_{tnpA} was used to drive a \textit{lacZ} gene in plasmid pGY11556 (Figure S2) showed that addition of IPTG resulted in a 143-fold increase in \beta-galactosidase activity. This suggests that sufficient transposase would be produced by escape synthesis from \textit{P}_{tnpA} to ensure transposition and that activity is limited by the supply of a correct DNA substrate. Activity obtained with ectopic TnpB alone ($<1 \times 10^{-5}$) was similar to the background levels observed with the empty vector plasmid ($<4.8 \times 10^{-9}$) clearly demonstrating that TnpA is absolutely required for IS\textit{Dra}2 excision while TnpB is dispensable. Similar results were obtained with IS\textit{Dra}2-103 (Figure 1A), in which \textit{tnpA} was in its natural location in the IS sequence and expressed from its own promoter (Table 1).

To measure transposon insertion, both non-targeted and targeted approaches were used. In both cases, we measured insertion in the subpopulation of cells in which the transposon IS\textit{Dra}2-113 had excised from its initial locus and had inserted elsewhere in the genome as judged respectively by the reconstitution of the \textit{Tet}\textsuperscript{R} gene and retention of the \textit{Cam}\textsuperscript{R} marker carried by the IS derivative.

In the non-targeted approach, the proportion of \textit{Cam}\textsuperscript{R} among \textit{Tet}\textsuperscript{R} clones, reflecting the frequency of spontaneous insertion, was about $10^{-2}$ in strain GY13120 (Figure 1B, top) expressing TnpA ectopically, compared to $<10^{-9}$ in the absence of TnpA. These insertions were false transposition events since they had occurred 3’ to the pentanucleotide, 5’TCTGGT3’ (Table S2), the sequence preceding the left end of IS\textit{Dra}2 in all genomic loci identified [20].

In the targeted approach, the degenerate IS\textit{Dra}2 copy (IS\textit{Dra}2\textsuperscript{9}) was replaced with the target \textit{sacB} gene from \textit{Bacillus subtilis}, coupled to a hygromycin resistance cassette to assist the construction (Figure 1B, bottom, and Figure S1). As in other bacteria [24,25], \textit{sacB} expression in the presence of sucrose is lethal for \textit{D. radiodurans} (data not shown) and inactivation of \textit{sacB}, for example, by IS insertion, confers a sucrose resistant phenotype. Moreover, \textit{sacB} contains 10 copies of the insertion site 5’TCTGGT3’ (9 on one strand and 1 on the complementary strand; Figure S3A). For this analysis, it was considered prudent to include a transcriptional terminator (Term\textit{116}) downstream of the \textit{cat} gene of IS\textit{Dra}2-113 to avoid possible interference of \textit{sacB} transcription from the strong \textit{P}_{tnpA} promoter with expression of the \textit{cat} gene (Figure 1A and 1B; Figure S1). By imposing a triple selection for \textit{Tet}\textsuperscript{R}, \textit{Cam}\textsuperscript{R} and \textit{Suc}\textsuperscript{R}, we were able to directly collect clones in which IS\textit{Dra}2-103Term\textit{116} had excised from its resident site (\textit{Tet}\textsuperscript{R}) and inserted into \textit{sacB} (\textit{Suc}\textsuperscript{R}). The nucleotide sequence of the \textit{Suc}\textsuperscript{R} mutants confirmed that each had IS\textit{Dra}2-113 inserted into one of the 5’TCTGGT3’ target sequences (Figure S3A and Figure 3B).

Together, these results demonstrate that TnpA alone is sufficient for both transposon excision and insertion.

**Effect of UV and \gamma-radiation on excision and insertion**

Using this genetic system, we then measured the excision frequency of IS\textit{Dra}2-113 and IS\textit{Dra}2-103 following exposure to UV- ($600 \text{ J m}^{-2}$) or \gamma-irradiation (5 kGy). Both treatments increased excision frequencies about 10-fold (Table 1).

To determine whether \gamma-ray irradiation also stimulates the insertion step of transposition, we measured the insertion frequency of IS\textit{Dra}2-103 (Term\textit{116}) expressing TnpA from its own promoter (to remain as close to natural conditions as possible) (Figure 1A) into \textit{sacB}. The frequency of \textit{Tet}\textsuperscript{R} colonies and of \textit{Suc}\textsuperscript{R} \textit{Cam}\textsuperscript{R} \textit{Tet}\textsuperscript{R} colonies in cells irradiated with 5 kGy of \gamma-rays and in non-irradiated cultures was measured in the tester strain GY13174 carrying IS\textit{Dra}2-103 (Term\textit{116}). The \textit{Tet}\textsuperscript{R} frequency, which monitors the excision step, rose from $2.32 \times 10^{-3}$ to $1.85 \times 10^{-2}$ after \gamma-irradiation (Table 1, average values of 10 independent experiments). The frequency of \textit{Suc}\textsuperscript{R} \textit{Cam}\textsuperscript{R} \textit{Tet}\textsuperscript{R} colonies, representing the overall transposition frequency into the reporter gene, also increased from $3.76 \times 10^{-10}$ to $1.8 \times 10^{-8}$ after \gamma-irradiation (Table 2).

**Table 1.** Excision frequencies of IS\textit{Dra}2 derivatives.

| IS\textit{Dra}2 derivative | Plasmid  | Inducing treatment | Excision frequency (\textit{Tet}\textsuperscript{R} / viable cell) (a) |
|---------------------------|----------|--------------------|---------------------------------------------------------------|
| IS\textit{Dra}2-113: \textit{tnpA} \textit{tnpB}\textit{Sac} \textit{Cam} | pGY11559 (empty) | None | <4.8 $\times 10^{-9}$ |
| IS\textit{Dra}2-113: \textit{tnpA} \textit{tnpB}\textit{Sac} \textit{Cam} | pGY13203 (\textit{tnpA}) | None | 2 ($\pm 0.6$) $\times 10^{-3}$ |
| IS\textit{Dra}2-103: \textit{tnpA} \textit{tnpB}\textit{Sac} \textit{Cam} | pGY13204 (\textit{tnpB}) | None | <1 $\times 10^{-9}$ |
| IS\textit{Dra}2-103Term\textit{116}: \textit{tnpA} \textit{tnpB}\textit{Sac} \textit{Cam}\textit{Term}\textit{116} | None | None | 2.62 ($\pm 2.2$) $\times 10^{-3}$ |

\textsuperscript{(a)}Excision frequency of IS\textit{Dra}2 derivatives was calculated as described in Materials and Methods.

\textsuperscript{(b)}5 kGy.

\textsuperscript{(c)}600 J m\textsuperscript{-2}.

\textsuperscript{(d)}doi:10.1371/journal.pgen.1000799.t001

**Table 2.** Insertion frequencies of IS\textit{Dra}2-103Term\textit{116} into \textit{sacB}.

| IS\textit{Dra}2 derivative | Plasmid  | Inducing treatment | \textit{Tet}\textsuperscript{R} \textit{Cam}\textsuperscript{R} \textit{Suc}\textsuperscript{R} / viable cell | \textit{Tet}\textsuperscript{R} \textit{Cam}\textsuperscript{R} \textit{Suc}\textsuperscript{R}/\textit{Tet}\textsuperscript{R} | Insertion stimulation factor |
|---------------------------|----------|--------------------|-------------------------------------------------|-------------------------------|-----------------------------|
| IS\textit{Dra}2-103Term\textit{116}: \textit{tnpA} \textit{tnpB}\textit{Sac} \textit{Cam}\textit{Term}\textit{116} | None | None | 3.76 ($\pm 0.52$) $\times 10^{-10}$ | 1.68 ($\pm 0.54$) $\times 10^{-7}$ | 1 |
| \gamma-rays \textsuperscript{(b)} | 1.8 ($\pm 0.75$) $\times 10^{-8}$ | 1 ($\pm 0.49$) $\times 10^{-6}$ | 5.95 |

\textsuperscript{(b)}5 kGy.

\textsuperscript{(d)}doi:10.1371/journal.pgen.1000799.t002
Thus, both excision and insertion of the \textit{ISDra2} derivatives were stimulated by irradiation. This stimulation is unlikely to result from an increase in \textit{tnpA} expression after irradiation since strain GY14910 in which the coding region of \textit{tnpA} was replaced by the coding region of \textit{lacZ} in \textit{ISDra2-104} (Figure 1A) showed no detectable increase in \textit{β}-galactosidase activity at 0, 30, 60, 120 or 180 min post-irradiation (Figure S2 and data not shown).

\textbf{In vitro cleavage, strand transfer, and insertion of \textit{ISDra2}}

Since the related \textit{TnpA} from IS608 uses obligate single-stranded DNA substrates in \textit{in vitro} transposition reactions [22], we suspected that the stimulation of \textit{ISDra2} excision and insertion might be linked to the formation of single-stranded DNA during repair of DNA damage which would supply the appropriate substrate.

To confirm that \textit{ISDra2} \textit{TnpA} is active only on single-strand DNA substrates, we used an \textit{in vitro} system, developed for the related IS608, to investigate \textit{TnpA}-catalysed cleavage and strand transfer [22,26]. IS608 transposition reactions are strand specific and use, by definition, the top strand. Recombination reactions recapitulating transposon excision and donor joint formation were performed. For this, the \textit{ISDra2} \textit{tnpA} gene was cloned with a C-terminal His\textsubscript{6} tag under control of a \textit{psp} promoter (Materials and Methods) and the protein was purified as described previously [23]. The C-terminal His-tagged \textit{TnpA} from \textit{D. radiodurans} was active since it catalysed \textit{ISDra2-113} excision \textit{in vitro} in a tester strain expressing \textit{TnpA}-His\textsubscript{6} under the control of \textit{psp} promoter in plasmid pGY13505 (data not shown).

The DNA substrate was a 59 nt single strand DNA fragment including the first 39 nt of \textit{ISDra2 LE} carrying a subterminal secondary structure which serves as the \textit{TnpA} binding site (Burgess-Hickman et al., in prep) and a 20 nt 5’ flank with the conserved pentanucleotide target sequence (5’\textit{TTGAT3’}) (Figure 2A). Incubation of the 5’ end-labelled fragment (Figure 2B, lane 1) with purified \textit{TnpA} in the presence of Mg\textsuperscript{2+} generated a cleaved 5’ end-labelled donor flank fragment of 20 nt (Figure 2B, lane 2). When mixed with an unlabelled 63 nt ss DNA fragment composed of the terminal 43 nt of RE (including the secondary structure) and a 3’ 20 nt flank, an additional fragment of 40 nt representing the joined donor flanks was generated (Figure 2B, lane 3). In contrast, double stranded LE (Figure 2B, lane 4) was neither cleaved nor underwent a strand transfer reaction with the unlabelled RE (Figure 2B, lane 5).

Similar results were obtained using 5’ end-labelled RE and unlabelled LE: cleavage and strand transfer were strictly dependent on ss substrates (Figure 2B). We were also able to recapitulate the integration reaction \textit{in vitro} using an end-labelled target DNA and an unlabelled RE-LE junction (Figure 2B).

Thus, the \textit{ISDra2} transposase is active on single- but not double-stranded substrates and is capable of cleavage of both LE and RE and of strand transfer to generate the donor joint and the RE-LE junction.

\textbf{Kinetics of irradiation-induced \textit{ISDra2} excision}

To investigate the relationship between irradiation and induction of \textit{ISDra2} transposition, we analysed the kinetics of γ-irradiation-triggered \textit{ISDra2-113} excision directly from the \textit{D. radiodurans} chromosome. For this, we isolated genomic DNA before and at different times after γ-irradiation and subjected samples to PFGE analysis following \textit{Nod} digestion (Figure 3B) and to PCR analysis (Figure 3A, 3C and 3D). Primer pairs P1+P2, complementary to the \textit{tetA} flanks of \textit{ISDra2-113} (Figure 3A), should generate a 2120 bp fragment when \textit{ISDra2-113} is inserted into \textit{tetA} and a 500 bp fragment when the donor backbone is sealed following \textit{ISDra2-113} excision. IS circle junction formation was monitored using primers P3+P4, complementary to the subterminal IS region (Figure 3A), by the appearance of a 260 bp product representing abutted LE and RE.

Total \textit{Nod}-digested genomic DNA (Figure 3B) showed complete fragmentation immediately following irradiation (compare lanes 1 and 2). Reassembly, evidenced by the gradual reappearance of distinct \textit{Nod} fragments, could be detected at about 2 h post-irradiation while the complete regenerated banding pattern exhibited by the non-irradiated sample (lane 1) occurred after 5 h (lane 6). PCR analysis of these samples using the P1+P2 primer pair revealed the presence of the full length IS together with a low but significant quantity of donor joint prior to irradiation resulting from rejoining of the DNA flanks following IS excision (Figure 3C, lane 1). This indicates that IS excision occurs during normal growth of the host strain as might be expected as \textit{tnpA} expression is under control of \textit{psp} promoter. Importantly, a significant increase in the level of the donor joint (Figure 3C) occurred over the 4.5 h post-irradiation period. We note that the progressive disappearance of the full length IS does not necessarily reflect its excision. In order to visualize the donor joint product, the PCR conditions were adjusted (short extension times; see Materials and Methods) to favour amplification of shorter donor joint fragment. It is probable that the reduction in the intensity of full length IS is due to competition in the reaction mixture from the increasing concentration of the short donor joint. In addition, the P3+P4 primer pair revealed the gradual appearance between 1 and 1.5 h post irradiation of the IS junction species (Figure 3D). The identity of the donor joint and the IS junction was verified by sequencing. The IS junction generated by P3+P4 was cloned into a plasmid carrying Cam\textsuperscript{R} (unable to replicate in \textit{D. radiodurans}) which was then introduced by transformation into a strain expressing ectopic \textit{TnpA}. Ten independent Cam\textsuperscript{R} clones were analysed using inverse PCR and each was shown to carry an insertion 3’ to a 5’\textit{TTGAT3’} target sequence, demonstrating that the IS junction generated by P3+P4 is active in transposition (data not shown).

Although we did not use quantitative PCR, care was taken to include identical quantities of DNA in each reaction and, to a first approximation, the results indicate that both the donor joint and the transposon junction were formed with very similar kinetics. Products started to accumulate at a time which coincided with the end of DNA degradation and probably with the start of the ESDSA pathway generating long stretches of single stranded DNA [18]. These results therefore strongly suggest that the factor which triggers \textit{ISDra2} transposition is the formation of single stranded DNA during \textit{D. radiodurans} genome assembly.

\textbf{Discussion}

\textit{D. radiodurans} has been the object of much interest due to its astonishing capacity to resist high levels of radiation [13,17] and to the genome fragmentation and reassembly processes essential for its survival after irradiation [18]. Clearly such exceptional properties might influence the behavior of mobile genetic elements within the genome and perhaps reveal new and interesting regulatory mechanisms. One hint that this might be the case came from the observation that transposition of one IS, \textit{ISDra2}, into the \textit{thyC} gene was apparently increased by high levels of γ- or UV-irradiation [19]. \textit{ISDra2} was the only insertion sequence of the 12 different IS family members present in the \textit{D. radiodurans} genome to behave in this way.

We show that \textit{ISDra2} transposition is specifically triggered during the process of reassembly of the \textit{D. radiodurans} genome which is associated with recovery from irradiation damage. \textit{ISDra2}
belongs to the IS200/IS605 family (see www.is.biotoul.fr) and the paradigm of this family, IS608, transposes by excision of a single strand circular DNA intermediate which can then insert into a single strand DNA target [22]. Our results demonstrate that ISDra2 also exhibits a strict requirement for single stranded DNA in vitro (Figure 2). Furthermore, we show genetically that both ISDra2 excision (detected by the restoration of an intact tetA gene) and insertion (into the reporter sacB resulting in resistance to sucrose) require TnpA, and that insertion occurs 3' to the specific pentanucleotide sequence, 5' TTGAT3', found adjacent to the left end of all naturally occurring genomic ISDra2 copies [20]. Moreover, we observe a 50- to 60-fold increase in the overall frequency of transposition following UV or γ-irradiation resulting from stimulation of the two transposition steps: a 8-fold increase in excision and a 6-fold increase in insertion of the IS circle transposition intermediate (Table 1). The overall transposition stimulation factor is in accord with the 50- and 100-fold induction of in vivo transposition of wild-type ISDra2 into the thyA gene by UV- and γ-irradiation respectively [19].

Monitoring tnpA promoter activity with the lacZ reporter gene demonstrated that the radiation-triggered ISDra2 transposition was not due to a specific induction of TnpA expression. Moreover, a same stimulatory effect of irradiation was observed whether TnpA was expressed from its natural promoter or from an external IPTG-inducible Pspac promoter.

Importantly, using a physical PCR-based approach, we also demonstrate that ISDra2 excision, reclosure of the chromosomal DNA donor joint and formation of a transposon joint with abutted left and right ends (consistent with a circular form of ISDra2) occur within irradiated D. radiodurans cells. Both rejoined donor DNA flanks and the LE-RE junction begin to accumulate after 90 min of post-irradiation incubation. This correlates with the end of the

Figure 2. ISDra2 TnpA-catalyzed cleavage and strand transfer in vitro. (A) Oligonucleotides used as DNA substrates. Length of cleavage products is indicated. The potential secondary structure in both LE and RE is indicated. Black dotted and black lines: left and right DNA flanks cleavage sites are shown as vertical black arrows. Asterisk (*) indicates radioisotope position. (B) Excision in vitro: donor joint formation and single- versus double-strand substrates. The 5'-32P-labelled oligonucleotide used was the 59-base LE composed of 39 nt of LE and 20 nt 5' to the 5'TTGAT3' and the unlabelled 63-base oligonucleotide RE. Lane 1: no-protein control; lane 2: TnpA alone; lane 3: TnpA and unlabelled RE; lane 4: dsLE, no-protein; lane 5: dsLE, TnpA and ssRE.
degradation of damaged DNA and the start of DNA double strand break repair processes such as extended synthesis dependent strand annealing (ESDSA) that generates high concentration of single stranded DNA [18,27].

Induction of transposition by exposure to environmental stress has often been tacitly assumed. However, there has been only limited supporting evidence for the idea that transposition can be induced efficiently by environmental insults and the sparse data available are generally based on indirect genetic assays [10,12–13,28–32]. These data should be revisited using more powerful technologies now available.

The idea of environmentally-induced transposition has also arisen from analysis of the growing number of available complete prokaryote genome sequences. These have identified many bacterial and archaean species in which the number of ISs is dramatically high. They include different Shigellae species [33], Bordetella pertussis [34], Yersinia pestis [35–36] Lactobacillus [37], Sulfolobus solfataricus [38], among many others. While it is attractive to imagine that this is the result of transposition bursts induced by an environmental trigger, a strong alternative has been to invoke stochastic transposition together with the formation of population bottlenecks produced in small isolated populations to fix such mutational events (insertions) [34].

Irradiation-triggered transposition described here is a response to an extreme set of environmental conditions which transiently generates large quantities of a substrate (single strand DNA) favoring transposition of ISDra2 but not of the other, unrelated, ISs present in the D. radiodurans genome. This response would

Figure 3. Physical evidence for irradiation-induced ISDra2 excision. Strain GY13120 (tetA-ISDra2-113 expressing tnpA in trans) received 5 kGy of γ-irradiation and aliquots were taken to isolate genomic DNA used to prepare DNA agarose plugs and as template for PCR analysis. The time following irradiation is shown in hours above each lane. (A) Schematic representation of IS excision products. The excision products are shown together with the position of primers used in PCR analysis. Note that the transposon circle including the transposon junction is single stranded. (B) Kinetics of double-strand-break repair. DNA agarose plugs were digested with NotI prior to PFGE analyses and loaded onto a 1.06% agarose gel. L: λ Ladder. (C) Kinetics of donor joint appearance. PCR reactions were performed with primer pair P1 and P2 and loaded onto a 1% agarose gel; L: MassRuler DNA Ladder. (D) Kinetics of IS circle junction appearance. PCR reactions were performed with primer pair P3 and P4 and loaded onto a 2% agarose gel; L: O’GeneRuler 100 bp DNA Ladder.

doi:10.1371/journal.pgen.1000799.g003
result in movement of the single “top strand” IS copy generating mutational diversity while retaining the inactive bottom strand copy. Insertion of the single-stranded circle intermediate and replication of the bottom strand would increase the copy number of the IS and disperse it throughout the genome. Moreover, D. radiodurans possesses an efficient natural competence system whose regulation remains to be explored. In view of the fact that such processes in other bacterial species occur using single strand DNA intermediates, transformation may be instrumental in assuring spread of this single strand transposable element [39]. Thus radiation-triggered ISDNA transposition might additionally generate diversity through participation of DNA containing newly transposed ISDNA copies in intercellular transformation.

An increase in the ISDNA copy number exposed to a single gamma irradiation cycle has been observed previously (see Figure S2 in [19]). Moreover, Islam et al [20] characterized the distribution of ISDNA in different laboratory D. radiodurans strains and found that its copy number varied from strain to strain: 7 copies were identified in the published D. radiodurans R1 genome sequence, 6 in the MR1 strain, 21 in the KD8301 strain but only one in KR1, the parent strain of KD8301 and in the reference strain ATCC 13939 used here. Unfortunately, the history and the intermediate strains are not available and no conclusions can be drawn concerning the factors which contributed to the gain or loss of ISDNA copies in the genome of these strains. The expansion of transposon copy number with accompanying ectopic sequence homology raises the question whether this would be a detriment to the extensive chromosome repair process after heavy irradiation. Precise reconstitution of a shattered genome through ESDSA involves annealing of 20–30 kb single-stranded DNA overhangs.

Zahradka et al ([18]) speculated that short blocks, 1–2 kb, of dispersed repetitive sequences present in the genome of D. radiodurans would not compromise the accuracy of repair through ESDSA. These authors pointed out that annealing only a limited repeated sequence block within two long non-complementary single-stranded overhangs could not readily link two fragments together. However, such ectopic sequence homology might stimulate chromosomal rearrangements through other DNA double strand break repair pathway such as single strand annealing (SSA) with a potential of increasing the plasticity of the genome under very adverse conditions.

Since this class of newly recognized transposable elements require single stranded DNA as both substrate and target site, any process which generates single strand DNA such as replication, mismatch repair and transcription, might lead similarly to limited induction of transposition and also provide suitable insertion sites. In view of the extremely widespread occurrence of members of this IS family in nature, this type of mechanism could be important in regulating transposition activity, interfacing transposition with host physiology and cell cycle and in creating genomic diversity as has been recently shown for the IS200/605 family member, IS1541, whose insertion allows the Yersinia pestis host to escape from adaptive immune responses and plague immunity [9].

Materials and Methods

Bacterial strains, media, and growth conditions

Bacterial strains are listed in Table S1. E. coli strain DH5α was the general cloning host and strain SCS110 was used to propagate plasmids prior to introduction into D. radiodurans via transformation [40]. All D. radiodurans strains were derivatives of strain R1 (ATCC 13939). TGY2X liquid medium and TGY plates [41] were used for D. radiodurans and Luria-Bertani (LB) broth for E. coli strains. Media were supplemented with the appropriate antibiotics used at final concentrations of; chloramphenicol 20 μg ml⁻¹ for E. coli and 3 μg ml⁻¹ for D. radiodurans; spectinomycin 40 μg ml⁻¹ for E. coli and 75 μg ml⁻¹ for D. radiodurans; tetracycline 2.5 μg ml⁻¹ for D. radiodurans; hygromycin 50 μg ml⁻¹. 5% (w/v) sucrose was supplemented to isolate the D. radiodurans sacB inactivated mutants.

Transformation of D. radiodurans with genomic DNA, PCR products, or plasmid DNA was performed as described [41].

DNA manipulations

Plasmid DNA was extracted from E. coli using the QiAprep Spin miniprep kit (Qiagen). D. radiodurans chromosomal DNA was isolated as described previously [19]. PCR reactions were carried out with Phusion DNA Polymerase (Abgene).

Inverse PCR was performed as follows: genomic DNA was digested with NdeI, purified by Phase Lock Gel procedure (Eppendorf), and then ligated using T4 DNA ligase. After ethanol precipitation, the ligated circular DNA was used as a template with primers P3 and P4 described in Table S1. The iPCR products were then directly sequenced with LEext and REext by Genome Express (Grenoble, France). Oligonucleotides used are listed in Table S3.

PCR reactions used for analysis of kinetics of irradiation-induced ISDNA excision were performed as follows: PCR was carried out in a final volume of 50 μl with using 0.5 Units of DyNazyme EXT DNA polymerase (Finnzymes) and 200 μM of each dNTP. To detect the donor joint, PCR analysis using P1+P2 primer pair was performed under the following conditions: 94°C for 3 min, 30 cycles of 94°C for 45 s, 60°C for 45 s, and 72°C for 20 s; and finally 72°C for 10 min. To detect the RE-LE junction, PCR analysis using P3+P4 was carried out as follows: the PCR of the first round was performed with 1 μg of genomic DNA under the following conditions: 94°C for 3 min, 30 cycles of 94°C for 45 s, 55°C for 45 s, and 72°C for 10 s, and finally 72°C for 10 min. The second round of PCR used a 15 μl aliquot from round 1 as template and was done under the same conditions than round 1.

Plasmids

For plasmid pGY13224 expressing tnpA and tnpB from a Pspac promoter, the coding sequences of the two genes were amplified by PCR using primers DraF (tagged with EcoRV) and DraR (tagged with XhoI). After cleavage, the PCR fragment was cloned into pGY11559 between the SacI and XhoI sites.

For plasmid pGY13203 expressing tnpA from the Pspac promoter, the D. radiodurans ISDNA2 tnpA gene was amplified by PCR using the primer pair DraF/DraX and D. radiodurans R1 genomic DNA as template. The product was cloned into plasmid pGY11559 between the EcoRV and XhoI sites.

For plasmid pGY13204 expressing tnpB from the Pspac promoter, the N-terminal part of tnpB was amplified using the primer pair 1651F/1651R, digested with NdeI and BsaI and ligated to the 10885-bp NdeI-BsaI fragment from pGY13224.

For plasmid pGY13507 expressing sacB from the Pspac promoter, the B. subtilis sacB gene was amplified with the primer pair NdeUPSacB/XhoDSN sacB and cloned into pGY11559 between the NdeI and XhoI sites.

For plasmid pGY11556 expressing the E. coli lacZ from the Pspac promoter, pGY11559 was digested with BglII and XhoI and ligated to the BglII-XhoI fragment from pGY11540 containing lacZ fused to the Pspac promoter.

Plasmids for production and in vivo analysis of Histagged TnpA

The tnpA coding sequence was amplified with the primer pair NdeUptnpA/TnpAHISph and was cloned at the NdeI and SphI sites of pAPT110 with a C-terminal His₆ tag under the
control of $P_{lac}$, to generate plasmid pGY13503. The plasmid also carried the lacZ gene to regulate $tphA$-HsiC expression. To analyse the ability of C-terminal His-tagged TnpA to catalyse in vivo transposition, the $tphA$-HsiC copy was amplified from pGY13503 with primer UpNde107 (tagged with NdeI) and DwntnpAHISxho (tagged with XhoI) and cloned into pGY11559 at the unique NdeI and XhoI sites to produce plasmid pGY13505, in which $tphA$-HsiC is expressed under the control of $P_{pac}$ promoter.

Pulsed field gel electrophoresis

Irradiated cultures were diluted in TGY2X to an $A_{500} = 0.3$ and incubated at 30°C. At different post-irradiation incubation times, samples (5 ml) were taken to prepare DNA plugs as described [42]. The DNA in the plugs was digested for 16 h at 37°C to produce plasmid pGY11559. Excision of IS

Measurement of in vivo spontaneous, $\gamma$-, and UV-induced excision frequencies of ISDra2 derivatives

Individual Cam$^R$ Tet$^R$ colonies purified from GY1311 or derivatives of GY13113 strain expressing in trans TnpA, or TnpB or no protein were inoculated into 3 ml of TGY2X supplemented with spectinomycin when required and grown to an $A_{500} = 1$–2. The bacterial cultures were washed in 10 mM MgSO$_4$, resuspended in the same buffer to an $A_{500} = 1$. Half of the resuspension was kept on ice, and the second half was exposed to UV light at a dose rate of 3.5 J cm$^{-2}$ s$^{-1}$ in Petri dishes. For $\gamma$-irradiation, the cultures were grown to an $A_{500} = 1$, then concentrated 30-fold in TGY2X and irradiated on ice with a 137Cs irradiation system (Institut Curie, Orsay, France) at a dose rate of 41.8 GY min$^{-1}$.

UV-, $\gamma$- or non-irradiated cells were diluted in TGY2X to an $A_{500} = 0.3$ and grown to stationary phase. Determination of the total number of viable cells was performed on TGY plates and excision of ISDra2 derivatives from tetA gene was selected on TGY plates containing tetracycline. Colonies were counted after 3–4 days of incubation at 30°C. The frequencies of the excision event per viable cell from 10 independent experiments were used to calculate the mean values and the standard deviations.

Measurement of in vivo spontaneous and $\gamma$-induced insertion frequencies of ISDra2-103 into sacB

5 individual Cam$^R$ Tet$^R$ colonies purified from GY13174 strain expressing ISDra2-103 were inoculated into 10 ml of TGY2X supplemented with hygromycin and grown to an $A_{500}$ of 1.5. The bacterial cultures were concentrated 30-fold in TGY2X. 100 µl of the resuspension was kept on ice and the rest was $\gamma$-irradiated as described above. After dilution, $\gamma$- or non-irradiated cells were grown to stationary phase. Excision of ISDra2-103 from tetA and insertion into sacB gene were selected on TGY plates containing tetracycline, or tetracycline, chloramphenicol and sucrose, respectively. Colonies were counted after 3–4 days of incubation at 30°C. The insertion frequencies per viable cell from 5 independent experiments were used to calculate the mean values and the standard deviations.

Measurement of lacZ expression under control of $P_{tphA}$ or $P_{pac}$ promoters

Replacement of the $tphA$ coding region of ISDra2-103 with the lacZ coding region to generate ISDra2-104 (Figure 1A) was performed as follows: the $P_{tphA}$-lacZ fusion was amplified by the joining PCR method [43]; see Table S3. The resulting lacZ fusion, the accompanying chloramphenicol resistance cassette and the right end of ISDra2-103 were then inserted into the tetA gene of strain GY13109 using the tripartite ligation method [44]. The resulting strain GY13110 was selected for Cam$^R$ and the insertion of the lacZ fusion by homologous recombination was confirmed by diagnostic PCR. LacZ gene was expressed under the $P_{pac}$ promoter in strain GY13112 containing plasmid pGY11536. Expression of the lacZ reporter gene was detected in D. radiodurans colonies formed on TGY plates containing 5-bromo-4-chloro-3-indolyl-$\beta$-D-galactoside (X-gal) at 40 µg/ml. $\beta$-galactosidase activity was measured as previously described [41].

TnpA purification, DNA procedures, and oligonucleotide cleavage and strand transfer reactions in vitro

TnpA was purified from E. coli K12 MC1061 endA carrying TnpA-HsiC expression plasmid pGY13503 (Table S1) following induction with 0.5 mM IPTG as previously described [25].

Oligonucleotide substrates for in vitro reactions.

5'-GGGGCTCAATGGCCCATGGTATGTGAGGGCCGACACTCCTGAATCCTAGTACGTTA-3' (LExchange), 5'-CTGCGCCCATGACATCAGGCTGATCGATGTTGTTCAAGAGTCCCTTGGCCCATGA-3' (RE) and 5'-TAACTCATGACTGAAGTCACGAGTGTGCGCCCCTCA-3' (LEcom)

In vitro oligonucleotide cleavage and strand transfer reactions

Reactions were performed by 45 min incubation of 20 fmol of a 5'-32P-labelled oligonucleotide and 1 pmol of the same oligonucleotide unlabelled with or without 10 pmol unlabelled recombining oligonucleotide, 0.5 µg of poly-dIdC and 20 pmol TnpA-HsiC, at 37°C in a final volume of 16 µl in 20 mM HEPES (pH 7.5), 2.5% DMSO, 200 mM NaCl, 5 mM MgCl$_2$, 1 mM TCEP, 20 µg/ml BSA and 10% glycerol. The reactions were terminated by addition of 0.1% SDS followed by 15 min of incubation at 37°C and separated on a 10% denaturing sequencing polyacrylamide gel. The gel was analysed by phosphorimaging.

5'-end labelling.

10 pmol of oligonucleotide was mixed with 16 pmol of [\gamma$^{32}$P] ATP (5000 Ci/mmol, Amersham Inc.) and 1 unit of T4 kinase (NEB Inc) in T4 kinase buffer (70 mM Tris–HCl pH 7.6, 10 mM MgCl$_2$, 5 mM DTT). Incubation was for 1 h at 37°C. Labelled oligonucleotides were purified by filtration through Sephadex G25. Ds substrates were obtained by hybridisation of labelled top-stranded oligonucleotide with cold bottom-stranded oligonucleotide.

Supporting Information

Figure S1 Construction of the D. radiodurans tester strains. All constructions described below were verified by DNA sequencing. The oligonucleotides used for PCR amplification of DNA fragments required for strains or plasmids construction, for diagnostic PCR or for sequencing are described in Table S3. The active ISDra2 genomic copy [loci DR1631-DR1632] was first replaced with a Tet$^R$ cassette expressing the tetA gene from the deinococcal $P_{mesZ}$ promoter using the tripartite ligation method [Mennecker S, Coste G, Servant P, Bailone A, Sommer S: 2004]
Mol Genet Genomics, 272(4):460–469]. The resulting strain, GY13109, was selected for its Tet<sup>R</sup> phenotype and the allelic replacement of ISDra2 by the Tet<sup>R</sup> cassette was confirmed by diagnostic PCR. The ISDra2 derivatives (Figure 1A) were inserted at the unique S<sup>5</sup>-GATC<sup>+</sup> target sequence of the tetA gene by double-crossover events between tripartite ligation products previously amplified by the joining PCR method [Fahret C, Ehrlich SD, Nioro P. 2002. Mol Microbiol, 46(1):25–36.] and chromatographic tetA region. The resulting tester strains (GY13115 with ISDra2-113; GY13111 with ISDra2-103; and GY13173 with ISDra2-103Tetrm16; Figure 1B) were selected for Cam<sup>R</sup> and the insertion of the ISDra2 derivatives into the tetA gene by homologous recombination was confirmed by diagnostic PCR. Transposon insertion into sacB, was studied by first replacing the degenerate ISDra2* copy (loci DR0177-DR0178) with the sacB gene from B. subtilis and the accompanying hygromycin resistance cassette. The strain GY13173 or GY13177 was transformed by the tripartite ligation mixture (see Table S2) and the resulting strains GY13174 and GY13182, respectively, were selected for their Hyg<sup>R</sup> phenotype. The allelic replacement of ISDra2* by the fragment encompassing the Hyg<sup>R</sup> cassette and the sacB gene was confirmed by diagnostic PCR and sequenced. Found at: doi:10.1371/journal.pgen.1000799.s001 (0.46 MB TIF) Schematic representation of the genomic insertions sites. Figure S3 PCR analyses of Sac<sup>R</sup> insertion mutants. (A) Schematic representation of the sacB gene showing potential pentanucleotide insertion sites (S1 - 10) and the position of primers used for PCR analysis. (B) Agarose gel showing the sizes of amplification-products obtained with genomic DNA of 10 Tet<sup>R</sup> Cam<sup>R</sup> Sac<sup>R</sup> mutants (from strain GY13186) as template and the pair of primers Camdwn/RevsacB. Mutants sac1, 3, 4, and 7 are inserted at site S1 of sacB; mutant sac2 is inserted at site S8; mutants sac8 and sac9 at site S9 and mutants sac5, sac6, and sac10 are inserted at site S5.

**Acknowledgments**

We thank I. Narumi for the gift of the pKatHPH1 and pZT29 plasmids. We would like to thank Frederick Dyda, Alison Burgess Hickman, and Oroszlya Barabas for many discussions and also present and past members of the Mobile Genetic Elements group (A. Achard, C. Guynet G, Duval-Valentin, P. Kicheneradja, B. Marty, P. Rousseau, P. Siguer, O. Walisko, E. Gourbeyre).

**Author Contributions**

Conceived and designed the experiments: CP BTH AB MC SS. Performed the experiments: CP BTH GC. Analyzed the data: CP BTH AB MC SS. Wrote the paper: CP BTH AB MC SS.

**References**

1. Walker GC (1984) Mutagenesis and inducible responses to deoxyribonucleic acid damage in Escherichia coli. Microbiol Rev 48(4):492–495.
2. Crissell T, Lesko K, Miyamoto S, Luo G, Boothman DA (2003) Transcription factors activated in mammalian cells after clinically relevant doses of ionizing radiation. Oncogene 22(57): 5613–5627.
3. Gotz D, Payushi S, Munro S, Lundgren M, Bernarder R, White MF (2007) Responses of hyperthermophilic extremophiles to UV irradiation. Genome Biol 8(5): R220.
4. Fu Y, Pastushok I, Xiao W (2008) DNA damage-induced gene expression in Saccharomyces cerevisiae. FEMS Microbiol Rev 32(6): 906–926.
5. Zhou BR, Elledge SJ (2000) The DNA damage response: putting checkpoints in perspective. Nature 408(6811): 1038–1045.
6. Ishikawa K, Ishii H, Saito T (2006) DNA damage-dependent cell cycle checkpoints and genomic stability. DNA Cell Biol 25(7): 60–93.
7. Lwoff A, Siminovitch L, Kjeldgaard N (1950) [Induction of the production of bacteriophages in lysogenic bacteria.]. Ann Inst Pasteur (Paris) 79(6): 815–859.
8. Blasius M, Sommer S, Hubscher U (2008) Deinococcus radiodurans: what belongs to the survival kit? Crit Rev Biochem Mol Biol 43(3): 221–230.
9. Blasius M, Sommer S, Hubscher U (2008) Deinococcus radiodurans: what belongs to the survival kit? Crit Rev Biochem Mol Biol 43(3): 221–230.
10. Blasius M, Sommer S, Hubscher U (2008) Deinococcus radiodurans: what belongs to the survival kit? Crit Rev Biochem Mol Biol 43(3): 221–230.
11. Blasius M, Sommer S, Hubscher U (2008) Deinococcus radiodurans: what belongs to the survival kit? Crit Rev Biochem Mol Biol 43(3): 221–230.
12. Blasius M, Sommer S, Hubscher U (2008) Deinococcus radiodurans: what belongs to the survival kit? Crit Rev Biochem Mol Biol 43(3): 221–230.
13. Blasius M, Sommer S, Hubscher U (2008) Deinococcus radiodurans: what belongs to the survival kit? Crit Rev Biochem Mol Biol 43(3): 221–230.
14. Blasius M, Sommer S, Hubscher U (2008) Deinococcus radiodurans: what belongs to the survival kit? Crit Rev Biochem Mol Biol 43(3): 221–230.
15. Narumi I (2003) Unlocking radiation resistance mechanisms: still a long way to go. Trends Microbiol 11(9): 492–495.
16. Cox MM, Barista JR (2005) Deinococcus radiodurans - the consummate survivor. Nat Rev Microbiol 3(11): 882–892.
17. Blasius M, Sommer S, Hubscher U (2008) Deinococcus radiodurans: what belongs to the survival kit? Crit Rev Biochem Mol Biol 43(3): 221–230.
18. Blasius M, Sommer S, Hubscher U (2008) Deinococcus radiodurans: what belongs to the survival kit? Crit Rev Biochem Mol Biol 43(3): 221–230.
19. Blasius M, Sommer S, Hubscher U (2008) Deinococcus radiodurans: what belongs to the survival kit? Crit Rev Biochem Mol Biol 43(3): 221–230.
20. Blasius M, Sommer S, Hubscher U (2008) Deinococcus radiodurans: what belongs to the survival kit? Crit Rev Biochem Mol Biol 43(3): 221–230.
21. Blasius M, Sommer S, Hubscher U (2008) Deinococcus radiodurans: what belongs to the survival kit? Crit Rev Biochem Mol Biol 43(3): 221–230.
22. Blasius M, Sommer S, Hubscher U (2008) Deinococcus radiodurans: what belongs to the survival kit? Crit Rev Biochem Mol Biol 43(3): 221–230.
23. Blasius M, Sommer S, Hubscher U (2008) Deinococcus radiodurans: what belongs to the survival kit? Crit Rev Biochem Mol Biol 43(3): 221–230.
24. Blasius M, Sommer S, Hubscher U (2008) Deinococcus radiodurans: what belongs to the survival kit? Crit Rev Biochem Mol Biol 43(3): 221–230.
25. Blasius M, Sommer S, Hubscher U (2008) Deinococcus radiodurans: what belongs to the survival kit? Crit Rev Biochem Mol Biol 43(3): 221–230.
27. Slade D, Lindner AB, Paul G, Radman M (2009) Recombination and replication in DNA repair of heavily irradiated Deinococcus radiodurans. Cell 136(6): 1044–1055.
28. Kuan CT, Tesman I (1991) LexA protein of Escherichia coli represses expression of the Tn5 transposase gene. J Bacteriol 173: 6406–6410.
29. Kuan CT, Tesman I (1992) Further evidence that transposition of Tn5 in Escherichia coli is strongly enhanced by constitutively activated RecA proteins. J Bacteriol 174: 6072–6077.
30. Chow KC, Tung WL (2000) Magnetic field exposure stimulates transposition through the induction of DnaK/J synthesis. Biochem Biophys Res Commun 270(3): 745–748.
31. Del Re B, Bersani F, Agostini C, Mesirca P, Giorgi G (2004) Various effects on transposition activity and survival of Escherichia coli cells due to different ELF-MF signals. Radiation and Environmental Biophysics 43(4): 263–270.
32. Del Re B, Garoia F, Mesirca P, Agostini C, Bersani F, Giorgi G (2003) Extremely low frequency magnetic fields affect transposition activity in Escherichia coli. Radiat and Environmental Biophys 42(2): 113–118.
33. Ohnubo H, Nyman K, Doroszkiewicz W, Ohnubo E (1981) Multiple copies of iso-insertion sequences of IS1 in Shigella dysenteriae chromosome. Nature 292(5824): 640–643.
34. Preston A, Parkhill J, Maskell DJ (2004) The Bordetellae: lessons from genomics. Y. Microbiol 2(5): 379–390.
35. Parkhill J, Wren BW, Thomson NR, Titball RW, Prentice MB, Sebaiha M, James KD, Churcher C, Mungall KL, et al. (2001) Genome sequence of Yersinia pestis, the causative agent of plague. Nature 413(6855): 525–527.
36. Chain PS, Carniel E, Larimer FW, Lamerin J, Stuartland PO, Regala WM, Georgescu AM, Vergez LM, Land ML, Motin VL, et al. (2004) Insights into the evolution of Yersinia pestis through whole-genome comparison with Yersinia pseudotuberculosis. Proc Natl Acad Sci U S A x, 101(38): 13826–13831.
37. Callanan M, Kaleta P, O’Callaghan J, O’Sullivan O, Jordan K, McAuliffe O, Sanguador-Vegas A, Slattery L, Fitzgerald GF, Beresford T, et al. (2004) Genome Sequence of Lactobacillus helveticus, an Organism Distinguished by Selective Gene Loss and Insertion Sequence Element Expansion 10.1128/ JRB.01295-07. J Bacteriol 190(2): 727–735.
38. Filee J, Siguer P, Chandler M (2007) Insertion sequence diversity in archaea. Microbiol Mol Biol Rev 71(1): 121–157.
39. Jean-Pierre Claverys, Bernard Martin, Patrice Polard (2009) The genetic transformation machinery: composition, localization, and mechanism. FEMS Microbiology Reviews 33(3): 643–656.
40. Meima R, Rothfuss HM, Gevo L, Lelstrome ME (2001) Promoter cloning in the radioresistant bacterium Deinococcus radiodurans. J Bacteriol 183(10): 3169–3175.
41. Bouacouza de Almeida C, Coste G, Sommer S, Bailone A (2002) Quantification of RecA protein in Deinococcus radiodurans reveals involvement of RecA, but not LexA, in its regulation. Mol Genet Genomics 268(1): 28–41.
42. Mattimore V, Battista JR (1996) Radioresistance of Deinococcus radiodurans: functions necessary to survive ionizing radiation are also necessary to survive prolonged desiccation. J Bacteriol 178(3): 633–637.
43. Fabret C, Ehrlrich SD, Noirot P (2002) A new mutation delivery system for genome-scale approaches in Bacillus subtilis. Mol Microbiol 46(1): 25–36.
44. Menneceier S, Coste G, Servant P, Bailone A, Sommer S (2004) Mismatch repair ensures fidelity of replication and recombination in the radioresistant organism Deinococcus radiodurans. Mol Genet Genomics 272(4): 460–469.
45. Lecointe F, Coste G, Sommer S, Bailone A (2004) Vectors for regulated gene expression in the radioresistant bacterium Deinococcus radiodurans. Gene 336(1): 25–35.