Genetics and Molecular Biology, 27, 2, 284-290 (2004)

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Research Article

Comparison of base substitutions in response to nitrogen ion implantation and ^{60}Co-gamma ray irradiation in *Escherichia coli*

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Abstract

To identify the specificity of base substitutions, a novel experimental system was established based on rifampicin-resistant (Rif^r) mutant screening and sequencing of the defined region of the *rpoB* gene in *E. coli.* We focused on comparing mutational spectra of base substitutions induced by either low energy nitrogen ion beam implantation or ^{60}Co-gamma rays. The most significant difference in the frequency of specific kinds of mutations induced by low energy nitrogen ion beam was that CG → TA transitions were significantly increased from 32 to 46, AT → TA transversions were doubled from 7 to 15 in 50 mutants, respectively. The preferential base substitutions induced by nitrogen ion beam implantation were CG → TA transitions, AT → GC transitions, AT → TA transversions, which account for 92.13% (82/89) of the total. The mutations induced by ^{60}Co-gamma rays were preferentially GC → AT and AT → GC transitions, which totaled 84.31% (43/51).

Key words: base substitution, low-energy nitrogen ion beam implantation, ^{60}Co-gamma rays, *Escherichia coli*.

Received: July 25, 2003; Accepted: November 14, 2003.

Introduction

The mutational spectrum induced by ionizing radiation has been an issue of long-standing interest in radiation biology (Grosowsky *et al.,* 1988). Southern blotting analysis permits the partitioning of ionizing radiation-induced mutagenesis into detectable deletions and major genomic rearrangements and into point mutations (Grosowsky *et al.,* 1988; (Grosowski *et al.,* 1986). Methods based on specific locus PCR were established to determine the mutational spectrum of fairly large fragments (Hei *et al.,* 1997; Wu *et al.,* 1999). The molecular nature of the point mutations, however, has been left unresolved (Grosowsky *et al.,* 1988). Point mutations comprise base substitutions (transitions and transversions), frameshifts, small deletions and insertions. Among them, base substitutions represent approximately 2/3 of the point mutations analyzed induced by ionizing radiation (Grosowsky *et al.,* 1988). The mechanism by which ionizing radiation produces mutagenicity is not entirely understood at present, which is partially due to little evidence from the mutational spectrum. Assays for transitions, transversions, frameshifts at specific sites and small deletions are based upon *Lac*$^+$ reversion of a specific mutation located within the *lacZ* gene in the F' plasmid. A number of *lacZ* constructs and strains were developed in this experimental system (Cupples *et al.,* 1990; Cupples and Miller 1989; Ohta *et al.,* 1999). In particular, the *lacZ* system has been widely used in mutation spectrum evidence for specific mutations from defined constructs. Yang *et al.* (1997) reported some *in vitro* mutational spectra of low-energy nitrogen ion beam implantation by using the *lacZ* constructs. However, it should be noted that the DNA molecules interact *in vivo* with many molecules and chemicals such as proteins and lipids, indicating that the structure of DNA molecules is more complex than naked DNA. The interaction between an ion beam and DNA is different *in vitro* and *in vivo*. Therefore, it is necessary to establish an experimental system to study how the low-energy nitrogen ion beam induces a mutation *in vivo*.

Rifampicin is an antibiotic that inhibits the function of RNA polymerase in eubacteria. Mutations affecting the beta subunit of RNA polymerase, which is encoded by the *rpoB* gene, can confer resistance to rifampicin (Jin *et al.,* 1988; Jin and Gross 1988). Increased mutagenesis to rifampicin resistance reveals that base substitutions in *rpoB* confer *E. coli* cells this capacity (Jin *et al.,* 1988; Matic *et al.,* 1997). Here, we designed two pairs of primers for specific regions of the *rpoB* gene. The corresponding regions

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were PCR-amplified and sequenced for analyzing the base substitutions. We compared the base substitution mutations induced by nitrogen ion beam implantation with those induced by gamma radiation, in order to analyze the mutagenicity of this procedure.

Materials and Methods

Media and strain

Culture media LB (1% tryptone, 0.5% yeast extract, 1% NaCl) or MM (minimal medium: 0.05% L-asparagine, 0.02% $MgSO_4$, 0.001% $FeSO_4$, 1% glucose) were used, solidified when required with 1.5% agar, and supplemented with rifampicin (150 μg/mL) and streptomycin (30μg/mL), respectively, as appropriate when needed. The *E. coli* K-12 strain AB1157 was kindly provided by M.G. Marinus from the University of Massachusetts Medical School. Its genotype is F^- *thr-1 ara-14 leuB6 (gpt-proA)62 lacY1 tsx-33 supE44*(AS) *galK2*(Oc) *hisG4*(Oc) *rfbD1 mgl-51 rpoS396*(Am) *rpsL31*(Str^R) *kdgK51 xylA5 mtl-1 argE3*(Oc) *thi-1*.

Nitrogen ion beam implantation, ^{60}Co-gamma ray irradiation

Overnight cultures were diluted ~100-fold and grown in LB medium until early log phase (optical density at 600 nm [OD_{600}] = ~0.3). Aliquots of 10 mL were pelleted and resuspended in 10 mL of MM salt solution with 10% glycerol for nitrogen ion treament. The liquid was spread onto the surface of plates. The ion implanted was nitrogen, with an energy of 10 keV, and the dosages (fluence) used were 1.3×10^{14}, 2.6×10^{14}, 3.9×10^{14}, 5.2×10^{14}, 6.5×10^{14}, 7.8×10^{14}, 9.1×10^{14}, 10.4×10^{14}, 11.7×10^{14} ions/cm^2. During implantation, the pressure of the target chamber was ~10^{-3} Pa, and the temperature of the implantation environment was estimated to be approximately 0 °C. The cell-containing liquid was irradiated with ^{60}Co-gamma rays at doses of 0.5 Gy, 1 Gy, 2 Gy, 5 Gy, 10 Gy, 20 Gy, 50 Gy, 80 Gy, 100 Gy, and 120 Gy, respectively.

Mutant screening, mutant frequency, and determination of survivors

The treated and control cells were washed down with 10 mL MM salt solution, and centrifuged for 1 min at 9,000 rpm. The precipitates were resuspended with 5 mL LB medium and incubated at 37 °C for 30 min at 220 rpm. After resuspension in MM salt solution, 50 μL of appropriate dilutions (10^4 ~ 10^6 fold) were spread onto MM plates to determine the total count, and 100 μL of them, with an appropriate cell density, were spread onto LB-rif plates to screen the mutant. The mutant frequencies were calculated by dividing the mean number of mutants by the average number of total cells.

Primer design, oligomer synthesis, PCR amplification and sequencing

The primer pairs for amplification of the regions were designed by the program WEBPRIMER (Stanford University) and reevaluated by the program PRIMER DESIGN. All primers were synthesized in the Expedite™ Nucleic Acid Synthesis System (workstation) in TaKaRa (Dalian Corporation).

After confirmation by streak culture on LB-rif plates, the mutant clones were toothpicked from the plates and suspended in 200 μL of 1*Pfu* PCR buffer, boiled for 10 min in the PCR apparatus (Perkin-Elmer 9600) for lysing the bacteria. Then, the content of each tube was divided into two tubes for two different *rpoB* region amplifications. The PCR mixture was then completed, and 40 cycles (30 s at 94 °C, 30 s at 58 °C, and 45 s at 72 °C) were performed. The final composition of the PCR mixture was 1*Pfu* PCR buffer with Mg^{2+}, 75 pmol of both primers, 200 μM each dNTP (TakaRa, Dalian), and 3 U of cloned *Pfu* polymerase (TakaRa, Dalian). The PCR products were purified with a DNA purification Kit (Wizard® SV Gel and PCR Clean-Up System, Promega) with low-melting agarose gel and sent to sequencing (TaKaRa, Dalian). DNA sequencings were all performed in both directions, using a Perkin-Elmer Applied Biosystems Model 377 DNA Sequencer in TaKaRa (Dalian) Corporation.

Determination of base substitutions

The base substitutions were determined by a run of blastn (blast 2 sequences) between the sense strand sequence of the wild type and mutants on line on the website (http://www.ncbi.nih.gov/blast/). The subject sequence is the sequence of the same region as in *E. coli* K-12 strain MG1655 (GenBank ACCESSION: U00096).

Results

Survival fraction and mutant frequency determination

Figure 1 shows the surviving fraction and mutant frequencies of *E. coli* cells irradiated by either nitrogen ion beam with various dosages or ^{60}Co-gamma rays. As compared to ^{60}Co-gamma rays (Figure 1 B), the 10 keV low-energy nitrogen ion beam induced a different pattern of cytoxicity (Figure 1 A). The moderate decreased pattern of the survival curve showed that low-energy nitrogen ion beam implantation may have a moderate cytotoxic effect on *E. coli* cells. At the dosage of 50% cell killing, the mutant frequency of nitrogen ion beam implantation (Figure 1 C: 6.5×10^{14} ions/cm^2) reached 9.5×10^{-7}, which is ~10-fold higher than that of ^{60}Co-gamma rays (Figure 1 D: 10 Gy mutant frequency = 9.3×10^{-8}). This suggests that low-energy nitrogen ion beam implantation might generate

higher frequency of mutagenicity, specially base substitutions, as they were identified by Rif^r mutant screening.

Primers

To analyze the Rif^r selection mutation and non-selective mutation spectrum, we here chose two regions for determination of the mutation (Figure 2). Region A (492 bp in size) is the highly conserved region, which confers the *E. coli* cell the capacity of Rif^r, when an appropriate base substitution is induced. Region B (530 bp in size) covers a non-conserved region of the *rpoB* coding sequence (419 bp, from 3611 to 4029), 77 bp of non-coding sequence between *rpoB* and *rpoC* genes, 34 bp coding sequence of *rpoC* (from 1 to 34). The primer sequences are listed in Table 1.

Optimization of PCR amplification

Since there is no single set of working conditions fulfilling the requirements of all PCR amplifications, the factors related to these reactions, including reaction component concentrations and procedures (time, temperature parameters and cycles), need to be adjusted within theoretically suggested ranges for efficient amplification of specific targets. In the present study, the bacterial lysate was used directly as PCR template. Briefly, mutant bacteriolysis was achieved in 1*pfu* PCR buffer at 95 °C for 10 min in the PCR apparatus. The content of each tube was divided into two tubes for two different *rpoB* region amplifications in the same mutant. 50 Rif^r mutant clones induced by the 10 keV nitrogen ion beam (three of them shown in

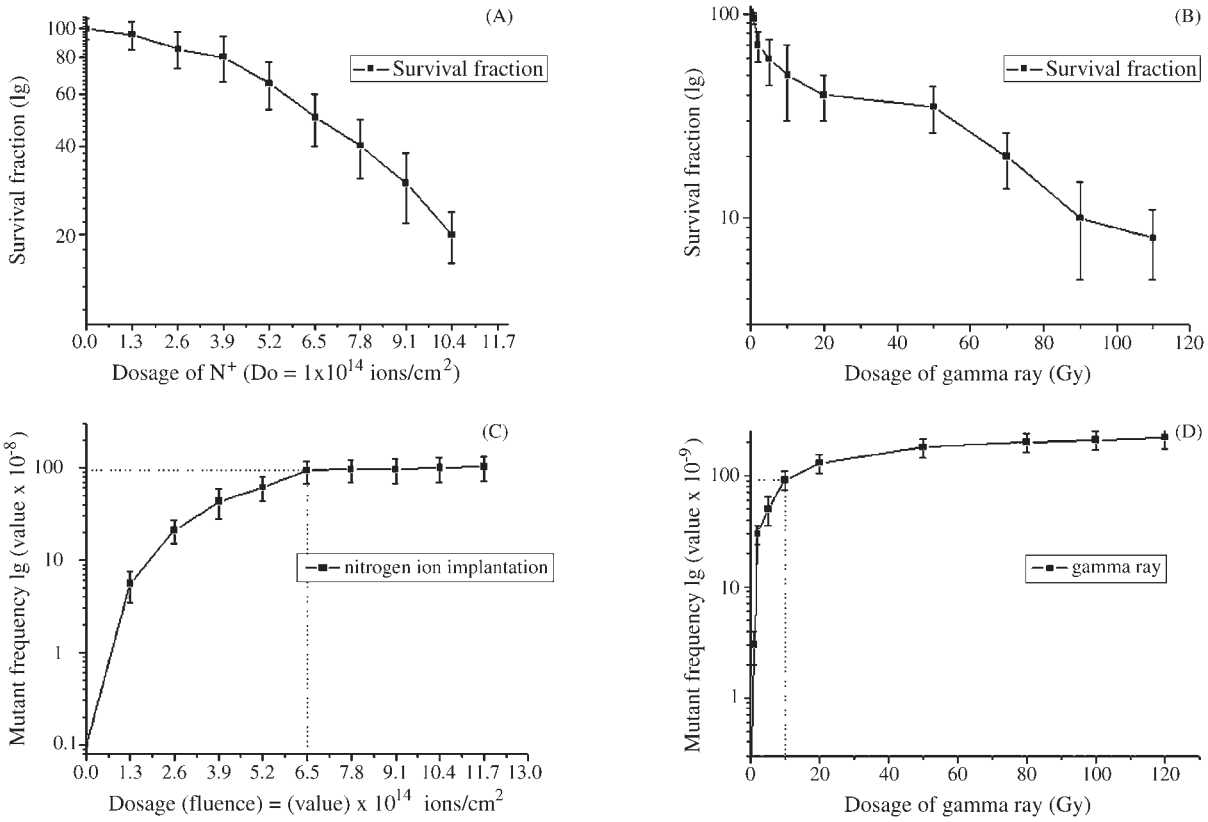

Figure 1 - Cytotoxicity (A, B) and mutagenicity (C, D) induced by low-energy nitrogen ion beam implantation and ^{60}Co-gamma rays.

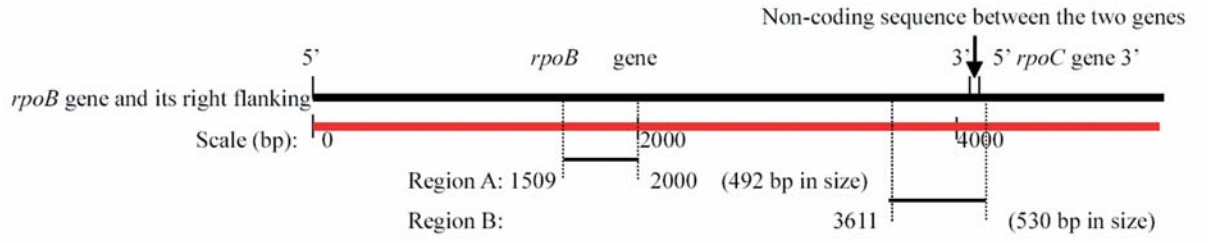

Figure 2 - Sketch map showing the high-fidelity PCR amplification regions.

Table 1 - Primer pairs for amplification of the *rpoB* gene.

Amplification region	Forward primer	Reverse primer
Region A	5'-AGGGACGCACCGACGGATACCA-3'	5'-AGAGTTCTTCGGTTCCAGCCAG-3'
Region B	5'-TGCCGACTTCCGGTCAGAT-3'	5'-GTCTGCGCTTTCAGAAACTT-3'

Figure 3 A) and by 10 Gy ^{60}Co-gamma rays (three of them shown in Figure 3 B) were picked respectively to amplify the two defined regions. The optimized PCR components and procedures are described in Materials and Methods.

rpoB region A is a sensitive region for determination of base substitutions

After amplification and purification, the PCR products were sequenced in both directions. The sequences were compared with the corresponding wild-type sequence by the blastn program (blast the two sequences http://www.nibi.nlm.nih.gov/BLAST.html) to find the mutations. Each mutation site was also verified by comparing the sense strand with its complementary sequence. The distribution and types of mutations are summarized in Table 2. Ninety-seven out of a hundred of the mutants had at least one base substitution mutation in region A. Ninety-nine percent of the mutants had no base changes in region B. This finding suggests that most of the mutations (99%) are derived from the selection of rifampicin resistance, and that region A of the *rpoB* gene is the sensitive region for determining the specificity of base substitutions.

In addition, our observation is consistent with the sequence studies of a number of other laboratories. Severinov *et al.* (1993) analyzed mutations in *rpoB* leading to Rif^r. They found that most of the base substitutions responsible for the Rif^r phenotype lied in two clusters. Even though the full length of this gene is 4029 bp, the Rif^r responsible region was only 177 bp. Previous studies (Jin and Gross 1988; Miller *et al.,* 2002; Phanchaisri *et al.,* 2002) identified 47 single-base substitutions at 29 sites and distributed among 21 coding positions. Most of them (46/47) lie in the region A defined in this research, whereas the mutations of the base substitution type leading to Rif^r in *rpoB* covered all kinds of transitions and transversions.

Comparison of the specificity of base substitutions

To compare the mutagenicity of the two mutagens, a total of 100 independent mutants (50 each) in *rpoB* genes were sequenced. The frequency of total detected nitrogen ion-induced mutation reaches 89, while the ^{60}Co-gamma ray-induced mutation frequency is only 51 (Table 2). The transitions prominently account for 75.28% and 84.31% of base substitutions induced by nitrogen ion beam and ^{60}Co-gamma rays, respectively. On the other hand, the types of base substitutions are different between the two mutagens. Two types of base substitutions (GC → CG transversions, AT → GC transitions) were induced by low-energy nitrogen ion beam, but were not found in cells treated with ^{60}Co-gamma rays, whereas AT → CG transversions were not found in low-energy nitrogen ion beam implantation, but were found in cells treated with ^{60}Co-gamma rays (Table 2).

The treatment was done with double-stranded DNA, and, thus, it was impossible to discriminate from which strand the position of the damage was derived. The types of base substitutions were grouped as CG → TA transitions, AT → GC transitions, AT → TA transversions, and GC → CG transversions. For instance, a CG → TA transition can also be derived from a GC → AT transition on the complementary strand. The specificity of base substitutions derived from low-energy nitrogen ion beam implantation and ^{60}Co-gamma ray-irradiated cells is also summarized in Table 2. The most significant difference in the frequency of specific kinds of mutations induced by low-energy nitrogen ion beam was that CG → TA transitions were significantly increased from 32 to 46, and AT → TA transversions were doubled from 7 to 15, as compared to the frequencies of gamma ray-induced mutations. In summary, the frequencies of nitrogen ion beam implantation-induced mutations

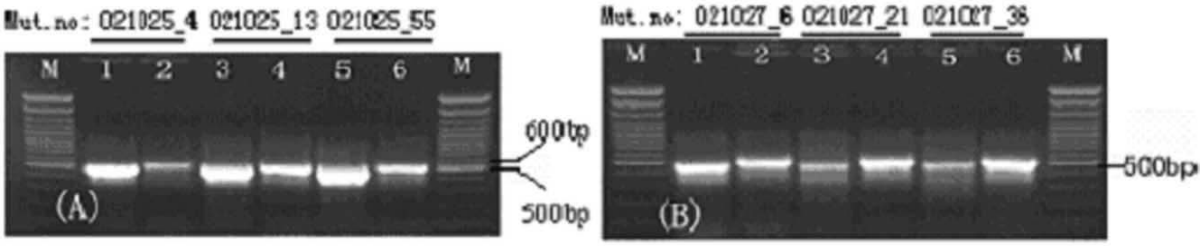

Figure 3 - High-fidelity PCR amplification of two regions of *rpoB* of Rif^r mutants induced by either 10 keV nitrogen ion beam (A) or ^{60}Co-gamma ray (B). M: marker (GeneRuler^{TM} 100bp DNA ladder plus, MBI). Mutant numbers (Mut. no.) are shown above the electrophoresis bands (A) (B) 1,3,5: region A of the corresponding mutant (492 bp in size); (A) (B) 2,4,6: region B of the corresponding mutant (530 bp in size).

Table 2 - Distribution of specific types of base substitutions and relative information.

Type of base substitution	Position	Target sequence (5' → 3')	Amino acid change	Occurrence	Total
I. 10 keV N^+					89
CG → TA transitions	1592	GTATCTCCGCACT	Ser531 Phe	12	
	1576	ATTACGCACAAAC	His526 Tyr	8	
	1565	CGCTGTCTGAGAT	Ser522 Phe	5	
	1692*	AAACCCCTGAAGG	Pro564 Leu	2	
	3921***	TAAAAACATCGTG	No change	1	
	1546	TTTATGGACCAGA	Asp516 Asn	10	
	1600	GCACTCGGCCCAG	Gly534 Ser	7	
	1586	AACGTCGTATCTC	Arg529 His	1	
AT → GC transitions	1552	GACCAGAACAACC	Asn518 Asp	9	
	1547	TTATGGACCAGAA	Asp516 Gly	7	
	1598	CCGCACTCGGCCC	Leu533 Pro	2	
	1598	CCGCACTCGGCCC	Leu533 Pro	3	
AT → TA transversions	1547	TTATGGACCAGAA	Asp516 Val	8	
	1577	TTACGCACAAACG	His526 Leu	5	
	1714	GGTCTGATCAACT	Ile572 Phe	1	
	1598	CCGCACTCGGCCC	Leu533 His	1	
GC → CG Transversions	1551**	GGACCAGAACAAC	Gln517 His	6	
One T insertion	1983_1984	GTGGTA+TCCGTC		1	
II. ^{60}Co-γ ray					51
GC → AT transitions	1546	TTTATGGACCAGA	Asp516 Asn	8	
	1600	GCACTCGGCCCAG	Gly534 Ser	6	
	1586	AACGTCGTATCTC	Arg529 His	3	
	1576	ATTACGCACAAAC	His526 Tyr	7	
	1565	CGCTGTCTGAGAT	Ser522 Phe	5	
	1692*	AAACCCCTGAAGG	Pro564 Leu	3	
AT → GC transitions	1547	TTATGGACCAGAA	Asp516 Gly	5	
	1552	GACCAGAACAACC	Asn518 Asp	3	
	1598	CCGCACTCGGCCC	Leu533 Pro	2	
	1538	TGTCTCAGTTTAT	Gln513 Arg	1	
AT → TA transversions	1577	TTACGCACAAACG	His526 Leu	3	
	1598	CCGCACTCGGCCC	Leu533 His	3	
	1714	GGTCTGATCAACT	Ile572 Phe	1	
AT → CG transversions	1714	GGTCTGATCAACT	Ile572 Leu	1	

showed that the preference of base substitutions induced by nitrogen ion beam were GC → AT transitions, AT → GC transitions, and AT → TA transversions which account for 92.13% (82/89) of the total, while the mutations induced by ^{60}Co-gamma ray were mainly GC → AT transitions, and AT → GC transitions, 84.31% (43/51).

Discussion

Nitrogen ion beam implantation and mutation specificity

During the treatment of *E. coli* cells with 10 keV nitrogen ion beam implantation, the air pressure was 10^{-3} Pa and the temperature was as low as 0 °C in the target chamber. The working conditions of the ion implantation were not suitable for living cells. Even though they were protected with 10% glycerol, 15% of them died during treatment, as compared to a complete control (data not shown). Phanchaisri *et al*. (2002) reported a similar cell-killing effect on *E. coli* cells, when treated with Ar^+ at a working condition of 10^{-4} Pa and approximately 0 °C. We may estimate that the *E. coli* cell has a side surface of about 0.5 to 1 μm^2. Therefore, around $10^5 \sim 10^6$ ions per cell were bombarded on the cell surface when the cells were spread flat. Yu *et al*. (2002) had found evidence that most of Ar^+ with energy of 30 keV can only penetrate the cell wall into ~100

to 200 nm, at a fluence of 1.5×10^{15} ions/cm^2. Accidentally, rare ions could reach a depth of 10 μm in the plant cell wall (Yu *et al.,* 2002). In the present study, 10 keV nitrogen ions at the fluence rank of 10^{14} to 10^{15} were used to implant the *E. coli* cells. We presumed that the cytoplasmic effect might play the most important role in the induction of the mutation, as in the study of Hei *et al.* (1997) in a mammalian system, using exact numbers of alpha particles for the irradiation of cytoplasm.

Since mutational spectra convey only the end point of a complex cascade of events, which includes formation of multiple adducts, repair processing, and polymerase errors, it is difficult to assess the mutational specificity of mutagens directly from them. Exposure to ionizing radiation can damage DNA directly, but the predominant pathway arises from radiolysis of H_2O, which results in the formation of reactive species such as $OH^{\cdot}$. There is evidence indicating that ROS can react with DNA (Wang *et al.,* 1998). The gamma radiation-induced mutations were derived from these direct and/or indirect DNA damages. 8-OH-dG and 5-OH-dC might offer some explanations for the preference of GC → AT transition, according to the summary of potential correlation between mutations observed in oxidant-induced mutational spectra (Hirano *et al.,* 2001; Wang *et al.,* 1998). The greater number of types and higher frequencies of nitrogen ion beam-induced mutations suggest that the mechanisms of nitrogen ion mutagenesis are more complicated than those of gamma-ray radiation. This complexity might result from the complex interactions between nitrogen ions and the target molecules. Low-energy nitrogen ion beams could not only generate ionizing radiation effects similar to gamma-ray radiation, but also the ions themselves could play a role in the formation of adducts. Some *in vitro* studies performed in our laboratory seem to support this point. New amino acids were synthesized in component solutions, and the nitrogen ion itself might also provide a nitrogen group in this reaction (Shi *et al.,* 2001b). As for nucleotides, nitrogen ion implantation might produce an effect of damage and form some adducts (Shi *et al.,* 2001a). As a heavy ion, the high LET irradiation effect (Hendry 1999) might be considered, even though the mutant screening system was aimed at determining base substitution. The cascade effects of high LET irradiation might also play a role in forming the base substitution mutations. We also compared the results with our former naked DNA irradiation studies, in which the transitions were mainly from CG to TA and from AT to GC, and the transversions were mainly from CG to AT and from CG to GC (Yang *et al.,* 1997). This simple mutational spectrum indicated that the interaction between naked DNA *in vitro* and nitrogen ions was also likely to be simple. Therefore, more types and species of reactive adducts could be generated in the process of implantation than in that of gamma ray irradiation and naked DNAs. Apparently, a reactive accelerated nitrogen ion group and its series products could act as component of adducts. Studies about the roles of heavy ions in the formation of the reactive DNA adducts are underway, to explain the detailed mechanisms of the specificity of base substitutions.

Experimental system

It has been deduced that mismatch DNA damages were the most important source of mutations induced by nitrogen ion beam implantation in naked DNA (see review by Yu, 2000). We here constructed a novel experimental system through which the specificity of base substitutions in living cells can be detected and analyzed. The *E. coli* chromosome *rpoB* gene region A contains the two clusters responsible for Rif^r. Previous studies (Jin and Gross 1988; Miller *et al.,* 2002; Severinov *et al.,* 1993) have identified 47 single-base substitutions which cover all kinds of transitions and transversions in region A. Our experiment was time and cost-saving because it was based on a single *E. coli* strain and did not require preparation of chromosome or plasmid DNA. Moreover, it was sensitive and efficient for determining the specificity of base substitutions. We also sequenced the region B of the *rpoB* gene, in order to obtain the nonselective mutation. However, only one T insertion mutation induced by low energy nitrogen ion beam implantation was identified through this screening system, suggesting that it is very hard to identify nonselective mutations based on this system.

Generally, *E. coli* strains have an average mutation rate to rifampicin resistance of about 1×10^{-8}, also called spontaneous mutation rate. Some strains have a spontaneous mutation rate of about 2.0×10^{-9} (Matic *et al.,* 1997). We here found a lower spontaneous mutation rate of about 1×10^{-9} (Figure 1 C and Figure 1 D, dosage = 0). The difference might be due to our protocols, since the cell concentration for mutant screening was around ~10^9 fold higher than the survival-determining one. A different loss in the process of manipulation might lead to this fairly lower spontaneous mutation rate. Theoretically, after irradiated or implanted, the mutant cells have a physiological delay to stabilize the mutant phenotype of up to 4 ~ 5 generations. Routinely, the treated cells are grown overnight (Cupples *et al.,* 1990; Cupples and Miller 1989). Here, the treated cells were grown in an enriched culture medium for only 30 min, and a little longer to wait for spreading. This time period allowed the cells to replicate and divide just one round. Though the mutant phenotype was not quite stable, the homologous mutants could be avoided in cultures, due to cell division. The short culture time might also result in lower mutant frequencies determined in mutagens, because it may have been too short for some mutants to express their mutant phenotype. It would be necessary to have information about how long and how well the culture time was after treatment with mutagens. This work is in progress.

New sites leading to Rif^r

Previous studies (Jin and Gross 1988; Miller *et al.,* 2002; Severinov *et al.*, 1993) have identified 47 single-base substitutions at 29 sites and distributed among 21 coding positions leading to Rif^r in *E. coli*. Here, we identified two new Rif^r-determining sites (Table 2). The complete new site determined in this study is located at nucleotide site 1551 and amino acid site 517. GC → CG transversion at 1551 caused a histidine substitute, Gln517, and led to a fairly high Rif^r capacity. The other new site determined is located at 1692. When dC1692 was replaced by a dT, it resulted in a change of Pro564 into leucine. The synonymous mutation of the second site had been reported previously (Miller *et al.,* 2002)[11], but the nucleotide substitution, dC1692 → dT1692, had not yet been identified. This finding might supplement the Rif^r analysis in *E. coli* and some other pathogenic bacteria.

Acknowledgements

This work was partly supported by grants from the National Natural Science Foundation of China (General Program n. 10375066 & n. 30170234). We thank research assistants Yu, L.X. and Liu, X.H. for their help in nitrogen ion beam implantation.

References

Cupples CG, Cabrera M, Cruz C and Miller JH (1990) A set of lacZ mutations in *Escherichia coli* that allow for rapid detection of specific frameshif mutations. Genetics125:275-280.

Cupples CG and Miller JH (1989) A set of lacZ mutations in *Escherichia coli* that allow for rapid detection of each of the six base substitutions. Proc Natl Acad Sci USA 86:5345-5349.

Grosovsky AJ, de Boer JG, de Jong PJ, Drobetsky EA and Glickman BW (1988) Base substitutions, frameshifts, and small deletions constitute ionizing radiation-induced point mutations in mammalian cells. Proc Natl Acad Sci USA 85(1):185-188.

Grosovsky AJ, Drobetsky EA, deJong PJ and Glickman BW (1986) Southern analysis of genomic alterations in gamma-ray-induced aprt- hamster cell mutants. Genetics 113:405-15.

Hei TK, Wu LJ, Liu SX, Diane V, Waldren CA and Randers-Pehrson G (1997) Mutagenic effects of a single and an exact number of alpha particles in mammalian cells. Proc Natl Acad Sci USA 94:3765-3770.

Hendry JH (1999) Repair of cellular damage after high LET irradiation. J Radiat Res 40, Suppl:60-65.

Hirano T, Hirano H, Yamaguchi R, Asami S, Tsurudome Y and Kasai H (2001) Sequence specificity of the 8-hydroxyguanine repair activity in rat organs. J Radiat Res 42:247-254.

Jin DJ, Cashel M, Friedman DI, Nakamura Y, Walter WA and Gross CA (1988) Effects of rifampicin resistant *rpoB* mutations on antitermination and interaction with nusA in *Escherichia coli*. J Mol Biol 204:247-261.

Jin DJ and Gross CA (1988) Mapping and sequencing of mutations in the *Escherichia coli rpoB* gene that lead to rifampicin resistance. J Mol Biol 202:45-58.

Matic I, Radman M, Taddei F, Picard B, Doit C, Bingen E, Denamur E and Elion J (1997) Highly variable mutation rates in commensal and pathogenic *Escherichia coli*. Science 277:1833-1834.

Miller JH, Funchain P, Clendenin W, Huang T, Nguyen A, Wolff E, Yeung A, Chiang JH, Garibyan L, Slupska MM and Yang HJ (2002) *Escherichia coli s*trains (ndk) lacking nucleoside diphosphate kinase are powerful mutators for base substitutions and frameshifts in mismatch-repair-deficient strains. Genetics 162:5-13.

Ohta T, Watanabe-Akanuma M, Tokishita S and Yamagata H (1999) Mutation spectra of chemical mutagens determined by *Lac*+ reversion assay with *Escherichia coli* WP3101P-WP3106P tester strains. Mutat Res 440(1):59-74

Phanchaisri B, Yu LD, Anuntalabhochai S, Chandej R, Apavatjrut P, Vilaithong T and Brown IG (2002) Characteristics of heavy ion beam-bombarded bacteria *E. coli* and induced direct DNA transfer. Surface and Coatings Technology 158-159:624-629.

Severinov K, Soushko M, Goldfarb A and Nikiforov V (1993) Rifampicin region revisited. New rifampicin-resistant and streptolydigin-resistant mutants in the beta subunit of *Escherichia coli* RNA polymerase. J Biol Chem 268:14820-14825.

Shi HB, Shao CL and Yu ZL (2001a) Dose effect of keV ions irradiation on adenine and cytosine. Acta Biophysica Sinica 17:731-735.

Shi HB, Shao CL and Yu ZL (2001b) Preliminary study on the way of formation of amino acids on primitive earth under non-reducing conditions. Radiation Physics and Chemistry 62:393-397.

Wang D, Kreutzer DA and Essigmann JM (1998) Mutagenecity and repair of oxidative DNA damage: Insight from studies using defined lesions. Mutation Res 400:99-115.

Wu LJ, Randers-Pehrson G, Xu A, Waldren CA, Yu ZL and Hei TK (1999) Targeted cytoplasmic irradiation with alpha particles induces mutations in mammalian cells. Proc Natl Acad Sci USA 96:4959-4964.

Yang JB, Wu LJ, Li L, Yu ZL and Xu ZH (1997) Sequence analysis of *lacZ*¯ mutations induced by ion beam irradiation in double-stranded M13mp18DNA. Science in China (series C) 40:107-112.

Yu LD, Phanchaisri B, Apavatjrut P, Anuntalabhochai S, Vilaithong T and Brown IG (2002). Some investigation of ion bombardment effects on plant cell wall surfaces. Surface and Coatings Technology 158-159:146-150.

Yu ZL (2000) Ion beam application in genetic modification. IEEE Transaction on Plasma Science 28:128-132.

EditorAssociado: Carlos F.M. Menck