Base Substitution Spectra of Nalidixylate Resistant Mutations Induced by Monochromatic Soft X and \(^{60}\text{Co}\) \(\gamma\)-rays in \textit{Bacillus subtilis} Spores

NOBUHIRO TAKAHASHI\(^1\)\(^†\), KOTARO HIEDA\(^1\)\(*\), FUMIKO MOROHOSHI\(^2\)
and NOBUO MUNAKATA\(^2\)

\(^1\)Biophysics Laboratory, Faculty of Science, Rikkyo (St. Paul’s) University,
Tokyo 171–8501, Japan
\(^2\)Radiobiology Division, National Cancer Center Research Institute,
Tokyo 104–0045, Japan
(Received, January 11, 1999)
(Revision received, March 31, 1999)
(Accepted, April 7, 1999)

Soft X-rays/\(\gamma\)-rays/Mutation/\textit{Bacillus subtilis}/Spore

\textit{Bacillus subtilis} spores were exposed to three types of photons, monochromatic soft X-rays with the energy corresponding to the absorption peak of phosphorus K-shell electron (2,153 eV) and with the slightly lower energy (2,147 eV), and \(^{60}\text{Co}\) \(\gamma\)-rays. From the irradiated spores, 233 mutants exhibiting nalidixic acid resistance were isolated, and together with 94 spontaneous mutants, the sequence changes in the 5'-terminal region of the \textit{gyrA} gene coding for DNA gyrase subunit A were determined. Among eighteen alleles of the \textit{gyrA} mutations, eight were single-base substitutions, nine were tandem double-base substitutions, and one was a double substitution skipping a middle base pair. About 6\% of the radiation-induced mutations were tandem double-base substitutions, whereas none was observed among the spontaneous ones. Among spontaneous mutations, A:T and G:C pairs were equally subjected to mutations, whereas the substitutions from G:C pairs and those to A:T pairs predominated among those induced with soft X-rays. The peak-energy X-rays were more effective in killing and causing mutations than the low-energy X-rays, however, there seemed no base-change events uniquely attributable to phosphorus K-shell absorption.

INTRODUCTION

Monochromatic soft X-rays with the energy corresponding to the phosphorus K-shell absorption peak are selectively absorbed by phosphorus atoms in DNA\(^1\). This absorption leads to Auger events with concentrated energy deposition around the atom\(^2\). Munakata et al\(^3\) reported

\(^*\)Corresponding author: Phone; 81–3–3985–2388, FAX; 81–3–3985–2388, E-mail; hiedak@rikkyo.ac.jp
\(^†\)Present address: Department of Molecular Biology, Institute of Molecular and Cellular Biosciences, University of Tokyo, Tokyo 113–0032, Japan
that the inactivation rate constants of the spores with various genetic constitutions were highest with the photons of the coincident energy to the resonance peak, and argued that this absorption event in spore DNA was responsible for the enhanced inactivation. However, it remained to be answered if this event produce specific types of DNA lesions that could result in unique mutagenic changes. To answer this question, we try to collect and analyze mutations from irradiated spores.

Since an assay system of lac reversion in *Escherichia coli* cells was devised by Miller⁴), a variety of systems have been developed and utilized to catalog mutational spectra. However, most of them are not easily adaptable to different organisms and mutagens. In a previous work⁵), we have established a simple method to isolate and identify many mutants from *Bacillus subtilis* cells. In this system, nalidixic acid resistant mutants growing on the drug-containing medium were selected and analyzed by PCR-SSCP (single-strand conformational polymorphism) and sequencing. Surprisingly, all of more than 600 mutants carried base substitutions in a small portion of *gyrA* gene coding for DNA gyrase A subunit. The changes were limited to three codons, and we identified 13 alleles named from *gyrA1* to *gyrA13*. This system is unique in that only limited types of mutational changes are observed: since the activity of DNA gyrase is indispensable for cellular survival, only base changes leading to appropriate amino-acid substitutions can be identified. Taking advantage of the ease in the quantitative comparisons and the identification of rare alleles, we employed this system for the collection and analysis of mutations induced with soft-X and γ radiations.

**MATERIALS AND METHODS**

**Irradiation of monochromatic soft X-rays and γ-rays**

Purified spores of *Bacillus subtilis* strain, HA101 (*hisH101 metB101 leuA5*) with wild-type repair capability, were diluted with distilled water to a concentration of 4 ¥ 10⁹ spores per ml. The suspension of 50 µl was spotted on a membrane filter (pore size 0.22 µm, Millipore, GSWP 02500) and dried in the air. Spore samples were irradiated in the air with monochromatic soft X-rays obtained from synchrotron radiation at the Beam Line 27A in the Photon Factory, the Institute of Materials Structure Science (Tsukuba, Ibaragi, Japan)⁶). Monochromatic soft X-rays with the energy of 2,153 eV corresponding to the phosphorus K-shell absorption peak and with the energy of 2,147 eV slightly below the peak-energy were employed (abbreviated to peak-energy and low-energy X-rays, respectively). Irradiation of ⁶⁰Co γ-rays was done with Gammacell 220 (Atomic Energy Canada) at a dose rate of 0.7 Gy/s. Absorbed doses of ⁶⁰Co-γ rays were determined with Fricke solution. Exposure (C/kg) of soft X-rays was converted to absorbed dose (Gy) using experimentally determined conversion factors: 32.7 (2,153 eV) and 25.0 (2,147 eV)⁷).

**Survival assay**

After exposure to radiation, each piece of filter was taken into a glass tube. To each tube, 1 ml of water was added, and the extraction of the spores was carried out by sonication. After appropriate dilution, spore suspension was pour-plated in casein enzymatic hydrolysate (50 mg/l,
ICN Pharmaceutical)-supplemented minimal (CMM) agar medium. After one-day incubation at 37°C, the number of colonies was counted with a colony counter, and the fractional survival was calculated.

Isolation of nalidixic acid-resistant mutants

About $1 \times 10^6$ to $1 \times 10^7$ viable spores were suspended in 1 ml water and mixed with 1 ml of the 1.5% molten CMM agar. The mixture was poured in CMM agar medium in a dish of 6-cm diameter. The dish was incubated at 37°C for 4 h, and then overlaid with 1 ml of 0.6% molten agar containing 0.5 mg nalidixic acid. Cells were cultured for five days at 37°C and well-grown colonies were picked up. Each colony was subjected to more than three successive single colony isolations in drug-containing CMM agar (25 μg/ml) and once in Nutrient Agar (Difco) medium. Strains that did not grow well or showed phenotypic abnormalities in single colony isolation were rejected. Since the treated cells were poured on the agar medium without growth in liquid medium, each mutant was ensured to be independently originated.

Cell lysate preparation

Cells of each strain were cultured with shaking overnight at 37°C in 0.5 ml of 1% Nutrient Broth (Difco) supplemented with MgSO₄ (0.2 g/l) and KCl (1.0 g/l) in 12-well culture dishes. The cells collected by centrifugation were stored at -76°C until the following lysis procedure. The cell pellet was suspended in 50 μl mixture of 15% glycerol and M9 buffer (6, 3, 5 and 0.25 g of Na₂HPO₄, KH₂PO₄, NaCl and MgSO₄7H₂O, respectively, per liter). The cell suspension (0.5 μl) mixed with 1 μl of lysis solution (10 mM Tris pH 8.2, 50 mM KCl, 2.5 mM MgCl₂ and 0.45% Tween 20) and Proteinase K (0.1 mg/ml) was frozen for 30 min at -74°C, incubated at 60°C for 1 h, and finally at 95°C for 15 min in a thermal cycler (Perkin Elmer, DNA Thermal Cycler 480).

Polymerase chain reaction

The polymerase chain reaction mixture in 50 μl (1.5 μl of the crude cell lysate, 5 μl of 10X PCR buffer, each 12.5 nmol of dNTP, each 5 pmol of primers and 0.875 unit of Taq DNA polymerase (Boehringer Mannheim)) was subjected to 27 thermal cycles of 94, 55 and 72°C for 1, 1 and 2 min, respectively. A pair of primers, primer 1 (from nucleotide 107 of the \( \text{gyrA} \) coding sequence, 5’-CGGATGTTCGTGACGGTTTA) and primer 2 (from nucleotide 382, 5’-TTCGTGCTTCTGTATAACGC) were employed. The product (276 bp) of the reaction was concentrated to 25 μl with Microcon 30 (Amicon) after subjected to phenol-chloroform and chloroform-isoamyl alcohol extraction.

Terminator cycle sequencing

Sequences of mutants were determined with ABI 373S DNA Sequencing System. Terminator cycle sequencing was performed on 10.35 μl of Ready Reaction Mixture™ (Perkin Elmer) in 30 thermal cycles of 96, 50 and 60°C for 15 sec, 1 sec and 4 min, respectively (Perkin Elmer, GeneAmp PCR System 9600). The product was extracted with phenol-chloroform and precipitated with ethanol twice before being loaded to the sequencing gel.
RESULTS

Survival and mutation induction

The spores were irradiated with three types of radiation. The survivals and frequencies of nalidixic acid resistant mutations are shown as the function of absorbed dose (Gy) in Fig. 1. Irradiation with the peak-energy X-rays was the most effective for killing and mutation induction, followed by that of low-energy X-rays and $^{60}\text{Co} \gamma$-rays. From exponential regressions, the inactivation rate constants (Gy$^{-1}$) of the three types of radiation were 0.0039, 0.0026 and 0.0012, respectively. The values of rate constants for the soft X-rays were similar to those obtained in the previous study of the exposure in vacuum$^3$. This indicates that the lethal process invoked by soft X-rays is similar when the irradiation is performed in air or in vacuum. The mutations were induced exponentially with the rate constants (Gy$^{-1}$) of 0.0021, 0.0015 and 0.0008 for peak-

![Fig. 1. Survival (upper) and mutation frequency (lower) of Bacillus subtilis HA101 spores irradiated with 2,147 eV (squares), 2,153 eV (rhombi) X-rays, and $^{60}\text{Co} \gamma$-rays (triangles).](https://academic.oup.com/jrr/article-abstract/40/2/115/985575)
energy, low-energy X-rays and γ-rays, respectively, though the data scattered due mainly to inhomogeneity of drug-resistant colonies. The ratios of the two rate constants for lethality and mutagenesis were 1.5–1.9, and the relative uniformity of the ratios suggests similarity of the mutational process incurred by three types of radiation.

**Nucleotide substitutions of nalidixic acid-resistant mutations**

We exposed the spores to the doses of 0.76, 1.35 and 1.50 kGy of peak-energy, low-energy X-rays and 60Co γ-rays, respectively, producing the mutations at frequencies of 0.8, 1.6 and 1.4 (×10^−6), respectively. From the exposed spores, we established 233 mutant strains of independent origin. As the controls, 94 spontaneous mutants were collected from the spore stock. The cell lysates from each mutants were subjected to sequence analysis of the gyrA gene coding for DNA gyrase subunit A. In all the mutants, sequence alterations were detected within the amplified fragment of 276 bp and were confined to 7 nucleotides coding for three amino acid residues (Table 1 and Fig. 2). These results are consistent with the previous characterization of this sys-

| Allele | Base change | Number of occurrences of nalidixate resistant alleles |
|--------|-------------|------------------------------------------------------|
| gyrA1  | 262 G A      | 16 29 17 15 77                                        |
| gyrA2  | 251 C T      | 37 25 24 30 116                                      |
| gyrA3  | 263 A C      | 2 3 2 10 17                                         |
| gyrA4  | 263 A G      | 12 7 19 32 70                                        |
| gyrA5  | 263 A T      | 8 4 3 5 20                                          |
| gyrA6  | 262 G C      | 2 2 1 0 5                                           |
| gyrA7  | 262 GA AT    | 0 0 1 0 1                                           |
| gyrA8  | 250 TC AT    | 2 1 0 0 3                                           |
| gyrA9  | 253 G C      | 2 0 0 0 2                                           |
| gyrA10 | 251 CA TG    | 0 0 0 0 0                                           |
| gyrA11 | 250 TCA GTT  | 0 0 0 0 0                                           |
| gyrA12 | 251 CA TT    | 0 0 0 0 0                                           |
| gyrA13 | 250 T G      | 0 2 2 2 6                                           |
| gyrA14 | 262 GAA ACT  | 0 0 0 0 0                                           |
| gyrA15 | 261 TG CA    | 0 0 0 0 0                                           |
| gyrA16 | 249 TTC CTT  | 0 0 0 0 0                                           |
| gyrA17 | 251 CA AT    | 0 0 0 0 0                                           |
| gyrA18 | 251 CA TC    | 0 1 0 0 1                                           |
| gyrA19 | 262 GA TT    | 1 0 1 0 2                                           |
| gyrA20 | 251 CA AC    | 1 1 0 0 2                                           |
| gyrA21 | 263 AA TT    | 1 0 0 0 1                                           |
| gyrA22 | 250 TC AA    | 0 1 0 0 1                                           |
| gyrA23 | 250 TC AG    | 0 0 1 0 1                                           |
| gyrA24 | 250 TC CT    | 0 0 1 0 1                                           |
| gyrA25 | 262 GAA AAG  | 0 1 0 0 1                                           |

|       | Total        | 84 77 72 94 327                                  |

*These alleles detected in the previous studies5,6 were not observed in this study.*
In this study, we found 18 different alleles, of which 11 had been described before (gyrA1 to gyrA9, gyrA13 and gyrA18). Among seven newly identified alleles (gyrA19 to gyrA25), six were tandem double-base substitutions and one was a double-base substitution skipping one nucleotide. Four alleles carried base substitutions in codon 84 (gyrA20, 22, 23 and 24), while three alleles in codon 88 (gyrA19, 21 and 25).

Eight alleles of single base substitutions occur most frequently, and even the rarest allele (gyrA9) has been observed at least six times in this and previous works. No other allele of single-base substitutions has been detected among more than thousand mutants examined to date. This indicates that all possible single-base substitutions that confer the resistance have been detected. We conclude that amino acid changes conferring the resistance to nalidixic acid are restricted to three codons (Ser-84, Ala-85 and Glu-88).

**Spectra of base substitutions**

We classify the mutations into five types of single base substitutions and two types of double-base substitutions. The occurrences of each type are listed in Table 2. Since the mutation frequencies at the doses used to collect these mutants were 3.3–5.1 times the spontaneous one, the contribution of spontaneous mutations could not be totally neglected. However, the correction for this contribution has not been attempted, since it may introduce additional statistical errors. Therefore, any differences between spontaneous and radiation-induced distributions might be underestimated.

---

**Fig. 2.** Region of the *Bacillus subtilis* gyrA gene determining the resistance to nalidixic acid. The alleles obtained among radiation-induced mutants are bracketed.
Generally, the profiles of radiation-induced mutations based on the relative frequency of each substitution type are similar. The most frequent type was one of the two alleles (gyrA1 and gyrA2) of a G:C to A:T transition followed by the gyrA4 allele of an A:T to G:C transition. Two types of the transitions together represented approximately 80% of the radiation-induced mutations. Among transversions, A:T to C:G and A:T to T:A occurred at comparable frequencies, while G:C to C:G was the least frequent. The profile of spontaneous mutations was dissimilar to those of radiation-induced mutations. The most frequent allele was gyrA4 carrying an A:T to G:C transition, and the relative frequency of an A:T to C:G transversion was higher, while none was a G:C to C:G transversion. Some observations concerning single-base substitutions are statistically significant in $\chi^2$-square tests of 2 ¥ 2 contingency tables. When the distribution of mutations induced with soft X-rays are compared to that of spontaneous ones, the relative frequencies of G:C to A:T were higher ($\chi^2 > 8.8$, $p < 0.005$) than A:T to G:C, and the relative frequencies of substitutions from G:C pairs and those of substitutions to A:T pairs were higher ($\chi^2 > 10.5$, $p < 0.002$ and $\chi^2 > 10.8$, $p < 0.002$, respectively). In these cases, the distributions of $\gamma$-ray induced mutations were in the middle of those induced with soft X-rays and spontaneous ones.

Seven alleles of tandem double-base mutations occupied about 6% of the radiation-induced ones, while none was observed among spontaneous mutations. The differences between radiation-induced and spontaneous ones are statistically significant ($\chi^2 > 5.3$, $p < 0.05$). Their occurrences are apparently sporadic without noticeable regularity (Fig. 2). We have tried to find out possible patterns by dissecting each constituent change. All six types of base changes were ob-

| Type of mutation | Peak-X rays | Low-X rays | $\gamma$-rays | Spontaneous |
|------------------|-------------|------------|---------------|-------------|
| Number (%)       | Number (%)  | Number (%) | Number (%)    | Number (%)  |
| Single base transition |           |            |               |             |
| G:C to A:T (2)   | 53 (63)     | 54 (70)    | 41 (57)       | 45 (48)     |
| A:T to G:C (1)   | 12 (14)     | 7 (9)      | 19 (26)       | 32 (34)     |
| Single base transversion |   |            |               |             |
| G:C to C:G (2)   | 4 (5)       | 2 (3)      | 1 (1)         | 0 (0)       |
| A:T to T:A (1)   | 8 (10)      | 4 (5)      | 3 (4)         | 5 (5)       |
| A:T to C:G (2)   | 2 (2)       | 5 (7)      | 4 (6)         | 12 (13)     |
| Double base substitution |   |            |               |             |
| Tandem           | 5 (6)       | 4 (5)      | 4 (6)         | 0 (0)       |
| Non-tandem       | 0 (0)       | 1 (1)      | 0 (0)         | 0 (0)       |
| Total            | 84          | 77         | 72            | 94          |
| Single base substitution |   |            |               |             |
| From G:C         | 57 (68)     | 56 (73)    | 42 (58)       | 45 (48)     |
| From A:T         | 22 (26)     | 16 (21)    | 26 (36)       | 49 (52)     |
| Single base substitution |   |            |               |             |
| To A:T           | 61 (77)     | 58 (81)    | 44 (65)       | 50 (53)     |
| To G:C           | 18 (23)     | 14 (19)    | 24 (35)       | 44 (47)     |
served, and the change to an A:T base pair was five times more frequent than the change to a G:C base pair, though the target base pairs were evenly distributed between A:T and G:C pairs.

DISCUSSION

Monochromatic soft X-rays from synchrotron radiation are useful in probing primary events and subsequent processes of photon absorption to biological materials\(^1\). The enhanced inactivation of dry spores in vacuum caused by the photons with the energy corresponding to the peak of phosphorus absorption was attributable to direct absorption by DNA phosphorus\(^3\). In the present study, the exposure was performed in air to avoid complication due to vacuum-induced mutations, and the above postulate has been extended to mutagenicity. With regard to lethal and mutagenic effects, soft X-rays were more effective than \(^{60}\)Co \(\gamma\)-rays. This is consistent with previous works showing that the irradiation of monochromatic X-rays was more effective than that of \(^{60}\)Co \(\gamma\)-rays for the induction of mitotic gene conversion at the trp-5 locus in yeast\(^6,8\). Also, for the inactivation and mutation induction of cultured hamster cells, 1.5 keV (corresponding to K-shell absorption peak of aluminum) and 0.3 keV (corresponding to K-shell absorption peak of carbon) ultrasoft X-rays were more effective than \(\gamma\)-rays\(^9\).

We have determined the sequence changes of 233 mutations induced with soft X and \(\gamma\) radiations, and compared the base substitution profiles among them and 94 spontaneous mutations. Since there seems no mutational spectra has been presented with \(B.\ \text{subtilis}\), it is necessary to compare them with the works carried out mostly with \(E.\ \text{coli}\) systems to find out the generality and uniqueness of our distributions.

Regarding the base-substitution profile of spontaneous mutations, earlier reports using extrachromosomal \(lacI\) gene showed the predominance of the changes of G:C to A:T transitions\(^{10,11}\). On the other hand, a recent study with chromosomal \(crp\) gene seemed to indicate relatively even distributions covering all types of base substitutions\(^{12}\). Our results showed that spontaneous single-base substitutions were distributed unevenly in 6 alleles, three of which were transitions and together occupied over 80% of total substitutions (Table 1). When seen from the aspects of the target and outcome base pairs of the substitutional changes, G:C and A:T pairs are represented evenly both as the target and the outcome (Table 2).

In contrast, the distributions of base substitutions among mutations induced by the exposure to radiation exhibit that the changes from G:C base pairs and those to A:T base pairs are preponderant. In both regards, these characteristics are more pronounced for the mutations induced by the exposure to soft X-rays than those induced by the exposure to \(^{60}\)Co \(\gamma\)-rays (Table 2). The generality of the pronounced base-substitution specificity shown for the mutations induced by the exposure to soft X-rays needs be investigated in future works with systems such as plasmid DNA in dry or wet conditions, or mammalian cells.

It has been documented in various systems that ionizing radiation induces predominantly the transitional substitutions from G:C to A:T pairs\(^{12-15}\), and this is taken as the evidence that oxidative damage to guanine is involved. These results have been obtained with vegetative cells or phage in solution, and the primary absorption of ionizing radiation takes place to water mol-
ecules producing hydroxyl radicals to attack DNA bases. On the other hand, in dry spores in air of about 50% humidity, most of the absorption occurs directly to DNA molecules. Therefore, we conclude that the preference of G:C pairs as the target takes place in the situation mainly attributable to direct photon absorption.

In certain cases, tandem double-base changes provide unique signatures for particular types of environmental assaults. As examples, unique changes of CC to TT occur about 5 to 10% in the mutations induced by the exposure to ultraviolet radiation\(^\text{16-18}\). We have previously demonstrated that the prolonged exposure of spores to high vacuum produces a unique allele, \textit{gyrA}12, carrying a CA to TT change up to more than 60% of total substitutions.\(^\text{7}\). On the other hand, many reports presented so far indicate that ionizing radiation rarely induces tandem double-base substitutions\(^\text{12-14,18}\). In one study using mammalian chromosomal gene as the target, two tandem double changes were observed.\(^\text{19}\) Our study indicates that soft X and \(\gamma\) radiations are effective in inducing various types of double-base changes with relatively uniform frequencies. All of the double changes occurred at neighboring bases, except \textit{gyrA}25 involving the next-neighboring bases. This raises a possibility that some of DNA lesions induced by ionizing radiation cover two or three contiguous nucleotides and prevent incorporation of proper nucleotides upon replication. As in the case with the lesion in G:C base pairs, the non-instructive base damage often seems to be coped with by the insertions of deoxyadenine residues according to “A-rule”\(^\text{20,21}\) consistent with the results of preferential changes to A:T base pairs.

In conclusion, the occurrence of tandem base changes is characteristics among mutations induced with soft X and \(\gamma\) radiations. The preferences of G:C pairs as targets and A:T pairs as outcomes are pronounced among soft X-rays induced mutations. Distinct differences are not observed in the distributions of base changes among mutations induced with peak-energy and low-energy X-rays of phosphorus K-shell absorption. However, this does not exclude possible differences in the other types of mutations such as frameshifts and deletions, detections of which require different systems of mutation analysis.

ACKNOWLEDGEMENTS

We would like to thank Dr. Katsumi Kobayashi of the Photon Factory, the Institute of Materials Structure Science, for his valuable advice and support and Mr. Masahisa Ohki of Rikkyo University for his kind assistance in irradiation experiments. This work was performed under the approval of the Photon Factory Advisory Committee (Proposal No. 94G088).

REFERENCES

1. Hieda, K., Hirono, T., Azami, A., Suzuki, M., Furusawa, Y., Maezawa, H., Usami, N., Yokoya, A. and Kobayashi, K. (1996) Single- and double-strand breaks in pBR322 plasmid DNA by monochromatic X-rays on and off the K-absorption peak of phosphorus. Int. J. Radiat. Biol. 70: 437–445.
2. Humm, J. L. (1984) The analysis of Auger electrons released following the decay of radioisotopes and photoelectric interactions and their contribution to energy deposition. KFA Report JUL-1932 (KFA, Kernforschungsanlage,
3. Munakata, N., Hieda, K., Usami, N., Yokoya, A. and Kobayashi, K. (1992) Inactivation action spectra of Bacillus subtilis spores with monochromatic soft X-rays (0.1–0.6 nm) of synchrotron radiation. Radiat. Res. 131: 72–80.

4. Miller, J. H. (1983) Mutational specificity in bacteria. Ann. Rev. Genet. 17: 215–238.

5. Munakata, N., Morohoshi, F., Saitou, M., Yamazaki, N. and Hayashi, K. (1994) Molecular characterization of thirteen gyrA mutations conferring nalidixic acid resistance in Bacillus subtilis. Mol. Gen. Genet. 244: 97–103.

6. Kobayashi, K., Hieda, K., Maezawa, H., Ando, M. and Ito, T. (1987) Monochromatic X-ray irradiation system (0.08 nm – 0.4 nm) for radiation biology studies using synchrotron radiation at the Photon Factory. J. Radiat. Res. 28: 243–253.

7. Munakata, N., Saitou, M., Takahashi, N., Hieda, K. and Morohoshi, F. (1997) Induction of unique tandem-base change mutations in bacterial spores exposed to extreme dryness. Mutat. Res. 390: 189–195.

8. Kobayashi, K., Hieda, K., Maezawa, H., Furusawa, Y., Suzuki, M. and Ito, T. (1991) Effects of K-shell X-ray absorption of intracellular phosphorus on yeast cells. Int. J. Radiat. Biol. 59: 643–650.

9. Goodhead, D. T. (1977) Inactivation and mutation of cultured mammalian cells by aluminum characteristic ultrasoft X-rays. III. Implications for theory of dual radiation action. Int. J. Radiat. Biol. 32: 43–70.

10. Yatagai, F. and Glickman, B. W. (1990) Specificity of spontaneous mutation in the lacI gene cloned into bacteriophage M13. Mutat. Res. 243: 21–28.

11. Schaper, R. M. and Dunn, R. L. (1991) Spontaneous mutation in the Escherichia coli lacI gene. Genetics 129: 317–326.

12. Takimoto, K., Tachibana, A., Ayaki, H. and Yamamoto, K. (1997) Spectrum of spontaneous mutations in the cyclic AMP receptor protein gene on chromosomal DNA of Escherichia coli. J. Radiat. Res. 38: 27–36.

13. Tindall, K. R., Stein, J. and Hutchinson, F. (1988) Changes in DNA base sequence induced by γ-ray mutagenesis of lambda phage and prophage. Genetics 118: 551–560.

14. Takimoto, K., Uchino, K., Ishizaki, K. and Ikenaga, M. (1991) Specificity of mutational DNA sequence changes induced by X-rays in the cloned Escherichia coli crp gene. Mutat. Res. 254: 199–206.

15. Bertram, H. and Hagen, U. (1992) Radical effects on mutation spectra in lambda phage. Int. J. Radiat. Biol. 62: 3–8.

16. Schaper, R. M., Dunn, R. L. and Glickman, B. W. (1987) Mechanisms of ultraviolet-induced mutation. J. Mol. Biol. 198: 187–202.

17. Demarini, D. M., Shelton, M. L. and Stankowski Jr., L. F. (1995) Mutational spectra in Salmonella of sunlight, white fluorescent light, and light from tanning salon beds: induction of tandem mutations and role of DNA repair. Mutat. Res. 327: 131–149.

18. Hutchinson, F. (1994) Induction of tandem-base change mutations. Mutat. Res. 309: 11–15.

19. Miles, C. and Meuth, M. (1989) DNA sequence determination of radiation-induced mutations of the hamster aprt locus. Mutat. Res. 227: 97–102.

20. Strauss, B., Rabkin, S., Sagher, D. and Moore, P. (1982) The role of DNA polymerase in base substitution mutagenesis on non-instructional templates. Biochimie 64: 829–838.

21. Tessman, I. (1985) UV-induced mutagenesis of phage S13 can occur in the absence of the RecA and UmuC proteins of Escherichia coli. Proc. Natl. Acad. Sci. USA 82: 6614–6618.